THE SYNTHESIS OF ENANTIOMERICALLY PURE INOSITOL DERIVATIVES: FROM RESOLUTION TO ENZYMATIC DESYMMETRISATION – VOLUME 1



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Abstract

Many naturally occurring inositol derivatives are fundamental regulators of signalling, metabolism, and many other important cellular processes. Synthetic inositols and probes based on such scaffolds are essential to understanding these biological processes. It is thus important to develop efficient regio- and enantioselective syntheses of inositol derivatives. Our research focused on the syntheses of phosphatidylinositol phosphates (PtdIns*P*, Figure 1 A.) and rhizopines (Figure 1 B.).

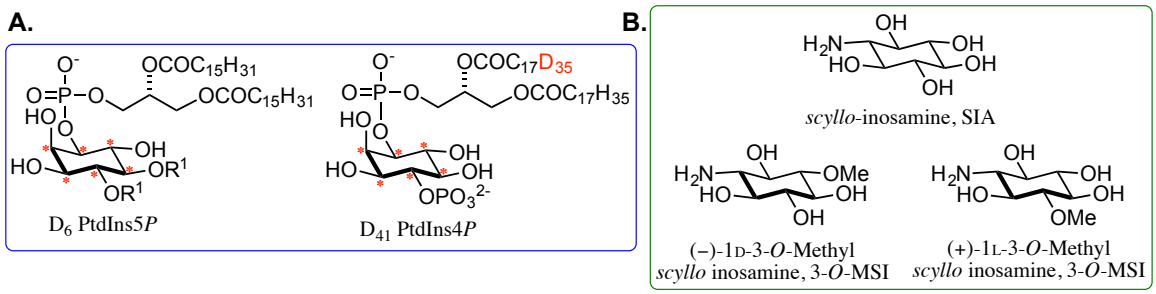


Figure 1 A. Deuterated PtdIns4*P* and PtdIns5*P* probes **B.** Rhizopines *scyllo*-inosamine and 3-*O*-methyl-*scyllo*-inosamine.

PtdInsP_ns possess distinct functions and sub-cellular distributions, which are tightly mediated by kinases and phosphatases. Dysregulation of the phosphoinositide metabolism has been implicated in diseases such as cancer. PtdInsP_n probes that fully retain their biological activity are essential to understanding these processes. We have developed a robust enantioselective synthesis of deuterated PtdIns4*P* and PtdIns5*P*. D_6 4,6-di-*O*-Benzyl-*myo*-inositol was desymmetrised using Lipozyme TL-IM®, to provide the corresponding 1D-1-*O*-acetylated product in 98% yield and >99% *e.e*. From this intermediate, the synthesis of D_{41} PtdIns4*P* (9 steps) and D_6 PtdIns5*P* (4 steps) was achieved.

Rhizopines were identified as ideal signalling molecules for application in biotechnology. *scyllo*-inosamine and 3-*O*-methyl-*scyllo*-inosamine were synthesised from *myo*-inositol, and their biosynthetic pathways proposed. In addition, we have completed the first enantioselective synthesis of (–)-1L- and (+)-1D-3-*O*-methyl-*scyllo*-inosamine, in 13 steps, using a novel diastereoisomeric protection-resolution strategy. This enabled this identification of (–)-1L-3-*O*-methyl-*scyllo*-inosamine as the naturally active enantiomer of 3-*O*-methyl-*scyllo*-inosamine, using a rhizopine catabolism assay.

The synthetic routes developed will be invaluable for the synthesis of unsaturated and metabolically stable PtdInsP_n, but also for the synthesis of similar enantiomerically pure inositol derivatives.

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Abbreviations

3-*O*-MSI	3-*O*-Methyl-*scyllo*-inosamine
Ac	Acetate
Ac_2PIM_1	Diacylated phosphatidylinositol monomannoside
All	Allyl
AM	Acetyl mandeloyl
AMA	Acetyl mandelic acid
aq	aqueous
Ar	Argon
BLAST	Basic local alignment search tool
Bn	Benzyl
Boc	*tert*-Butyloxycarbonyl
BOM	Benzyloxymethyl
br	broad
BsIolG	IolG from *Bacillus subtilis*
Bu	Butyl
Bz	Benzoyl
c	concentration
CA	Chloroacetate
cm^{-1}	Reciprocal centimeters
Cne	Cyanoethyl
COSY	Correlation spectroscopy
CSA	Camphorsulfonic acid
Cy	Cyclohexyl
d	day(s), doublet
DAG	Diacylglycerol
DCC	Dicyclohexylcarbodiimide
DDQ	2,3-Dichloro-5,6-dicyano-1,4-benzoquinone
DFT	Density Functional Theory
DHDPS	Dihydrodipicolinate synthase
DIBAL-H	Diisobutylaluminum hydride
DIPA	Diisopropylamine
DIPEA	*N*,*N*-Diisopropylethylamine
DMAP	*N*,*N*-Dimethyl-4-aminopyridine
DMF	Dimethylformamide

DMSO	Dimethyl sulfoxide
DMT	Dimethoxytritylchloride
DNA	Deoxyribonucleic acid
DNMT1	DNA methyltransferase 1
e.e.	enantiomeric excess
EDC	1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide
EDTA	Ethylenediaminetetraacetic acid
EI/FI	Electron ionisation/field ionisation
ent	enantiomer
EPSRC	Engineering and physical sciences research council
eq	equivalents
ER	Endoplasmic reticulum
ESI	Electronspray ionisation
Et	Ethyl
et. al	and others
FAD	Flavin adenine dinucleotide
FDA	Food and drug administration
g	gram(s)
GC-MS	Gas chromatography-mass spectrometry
GRP1	General receptor of phosphoinositides 1
h	hour(s)
HMBC	Heteronuclear multiple-bond correlation spectroscopy
HPLC	High performance liquid chromatography
HPLC-MS	High-performance liquid chromatography-mass spectrometry
HRMS	High-resolution mass spectrometry
HSQC	Heteronuclear single quantum coherence
IC50	Half maximal inhibitory concentration
Idh	Inositol dehydrogenase
IMG	Integrated microbial genomes
ING2	Inhibitor of growth protein 2
InsP_n	Inositol phosphate
IPA	Isopropyl alcohol
IPGs	Inositol phosphate glycans
IR	Infrared spectroscopy
IUPAC	International union of pure and applied chemistry

kb	kilobase(s)
lit.	literature value
LRMS	Low-resolution mass spectrometry
m	multiplet, medium intensity
M	Molar
M. sativa	*Medicago sativa*
m.p.	melting point
MALDI	Matrix-assisted laser desorption ionization
MAPK	Mitogen-activated protein kinase
mbar	millibar(s)
*m*CPBA	*meta*-Chloroperoxybenzoic acid
MDHP	4-Methoxy-5,6-dihydro-2*H*-pyran
Me	Methyl
MEM	β-Methoxyethoxymethyl
mg	milligram
MHz	Megahertz
min	minute(s)
mL	milliliter(s)
mm	millimeter(s)
MOM	Methoxymethyl
MPA	α-Methoxyphenylacetic acid
Ms	Mesyl
MS	Molecular sieves
MTHP	4-Methoxytetrahydropyran-4-yl
MTPA	α-Methoxy-α-trifluoromethylphenylacetic acid
N/A	Not applicable
NAD	Nicotinamide adenine dinucleotide
NC-IUB	Nomenclature committee of the international union of biochemistry
nm	nanometer(s)
NMO	*N*-Methylmorpholine-*N*-oxide
NMR	Nuclear magnetic resonance
NOE	Nuclear overhauser effect
OD	Optical density
ORF	Open reading frame
PDB	Protein data bank

PDGF	Platelet-derived growth factor
PG	Protecting group
Ph	Phenyl
PH	Pleckstrin homology domain
PHD	Plant homeodomain
Piv	Pivaloyl
PKC	Protein kinase C
PLC	Phospholipase C
PMB	4-Methoxybenzyl
PP-Ins*P*	Diphosphoinositol phosphates
PPL	Porcine pancreatic lipase
ppm	parts per million
PPTS	Pyridinium 4-toluenesulfonate
PtdIns	Phosphatidylinositol
PtdInsK	Phosphatidylinositol kinase
PtdInsP_n	Phosphatidylinositol phosphate
PTSA	4-Toluenesulfonic acid
Pyr.	Pyridine
q	quartet
R_f	Retention factor
RING	Really interesting new gene
R. leguminosarum	*Rhizobium leguminosarum*
RNA	Ribonucleic acid
RPM	Revolutions per minute
RT	Room temperature
s	singlet, strong
S. meliloti	*Sinorhizobium meliloti*
SIA	*scyllo*-Inosamine
t	triplet
TAF3	TATA binding protein associated factor 3
TAS-F	Tris(dimethylamino)sulfonium difluorotrimethylsilicate
TBAF	Tetrabutylammonium fluoride
TBAI	Tetrabutylammonium iodide
TBDMS	*tert*-Butyldimethylsilyl
TBDPS	*tert*-Butyldiphenylsilyl

^{t}Bu	*tert*-Butyl
Tf	Triflate
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
THP	Tetrahydropyranyl
TIPS	Triisopropylsilyl
TLC	Thin layer chromatography
TMS	Tetramethylsilane
TMSBr	Trimethylsilyl bromide
TMSI	*N*-Trimethylsilylimidazole
TPAP	Tetrapropylammonium perruthenate
Troc	2,2,2-Trichloroethyl carbonate
Trt	Trityl
UHRF1	Ubiquitin-like, containing PHD and RING finger domains 1
v/*v*	volume per unit volume (volume to volume ratio)
w	weak
w/*v*	weight per unit volume (weight to volume ratio)
XEPA	*o*-Xylylene *N*,*N*-diethylphosphoramidite

1 Introduction: The synthesis of enantiomerically pure inositol derivatives

1.1. Inositol structure and nomenclature

The *myo*-inositol **1** framework (*cis*-1,2,3,5-*trans*-4,6-cyclohexanehexol), is found in numerous biologically relevant molecules. Indeed, derivatives of this cyclitol, particularly phosphorylated inositols such as polyphosphates (InsP_ns)[1-3] and phosphatidylinositol phosphates (PtdInsP_ns),[4] are fundamental regulators of cell signalling, growth and survival, metabolism, and many other crucial cellular processes.[5]

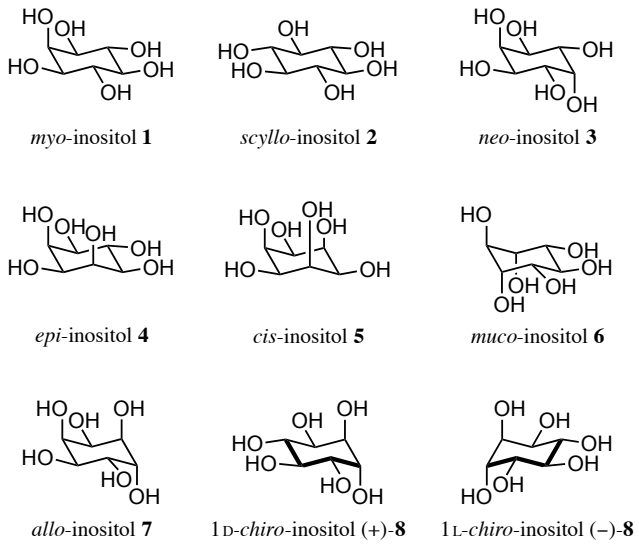


Figure 1.1 The nine stereoisomers of inositol **1–8**, all of which are naturally occurring, except for *cis*-inositol **5**. *myo*-Inositol **1** is the most naturally prevalent.

There are nine possible stereoisomers of inositol **1–8**, of which *myo*-inositol **1** is the most naturally prevalent (Figure 1.1). These isomers arise from the relative spatial arrangement of the six hydroxyl groups above and below the plane of the cyclohexyl ring. Along with *myo*-inositol **1**, seven of these

isomers occur naturally (*scyllo*- **2**, *neo*- **3**, epi- **4**, *muco*- **6**, *allo*- **7**, 1D-(+)-*chiro*- (+)-**8** and 1L-(–)-*chiro*-inositol (–)-**8**) in mammals, non-mammals, bacteria, and plants. These are, however, less widespread and as a result, less studied.[6] The occurrence, roles, and uses of each of these 'other' inositol stereoisomers is described in a comprehensive review by Potter *et al*.[6]

Aside from *chiro*-inositol **8**, which exists in two enantiomeric forms, and racemic *allo*-inositol **7**,[7,8] the remaining cyclitols all feature at least one internal plane of symmetry and are therefore achiral, *meso* molecules. If we consider *myo*-inositol, for example, a plane of symmetry runs through the axial 2- and the equatorial 5-positions of the ring. Whilst substitution at these positions does not alter the symmetry of the molecule, chemical substitution at any of the other, enantiotopic, positions provides two enantiomers (Figure 1.2), unless enantioselective techniques are employed.

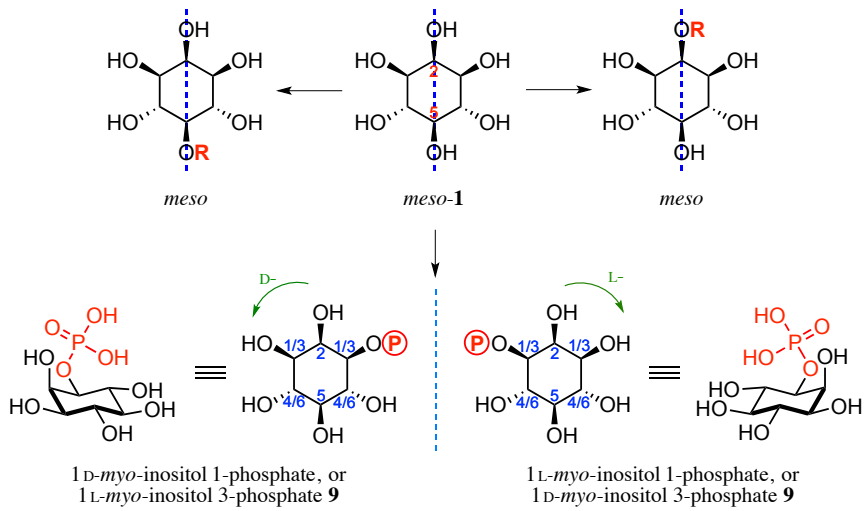


Figure 1.2 Naming and numbering of *myo*-inositol derivatives. When the symmetry of *myo*-inositol is broken, two enantiomers are generated. These can be named using either the 1D- or 1L-nomenclature. Both flat and chair representations are shown. R= functional group.

The symmetrical nature of inositols has led to some confusion in the literature regarding the naming and numbering of their desymmetrised derivatives. Indeed, two descriptors should be assigned to these molecules: the numbering of carbons around the ring, as recommended by the International Union of Pure and Applied Chemistry (IUPAC),[9] and the absolute stereochemistry (prefixes D- and L-). If the ring is numbered clockwise when the substituent at the lowest numbered stereogenic centre (C-1) is pointing towards the viewer, the compound is termed 1L-. Conversely, if the ring is numbered anticlockwise, then the compound is termed 1D- (Figure 1.2).[9] The same inositol derivative can

therefore be designated 1L- or 1D-, depending on which enantiotopic carbon is numbered C-1 (Figure 1.2).

To avoid confusion, the D-numbering system is now used exclusively in the literature for *myo*-inositol derivatives, following the most recent recommendations by the Nomenclature Committee of the International Union of Biochemistry (NC-IUB), which involves a relaxation of the lowest-locant rule.[10,11] In addition, the 'Agranoff turtle' can be used as a useful structural mnemonic for naming *myo*-inositol derivatives.[12] The most accurate representation of these derivatives is probably the 'chair' conformation, which is thought to be the most thermodynamically stable. In this conformation, the axial 2-hydroxyl group has been likened to the head and the remaining equatorial hydroxyl groups to the flippers and tail of the turtle. The turtle is said to be right flippered to represent the 1D- numbering system (Figure 1.3).

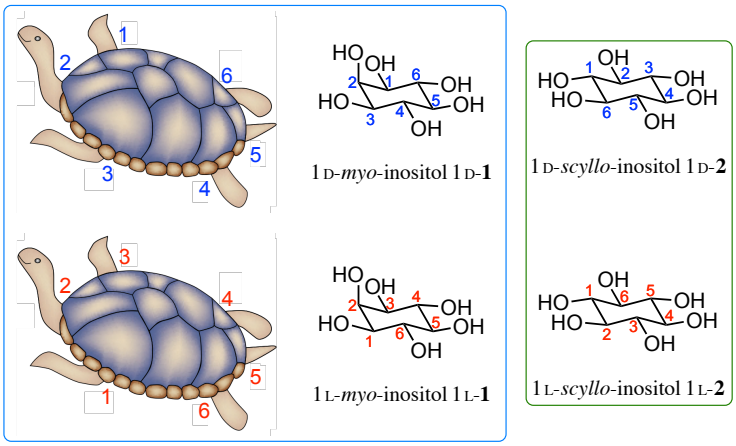
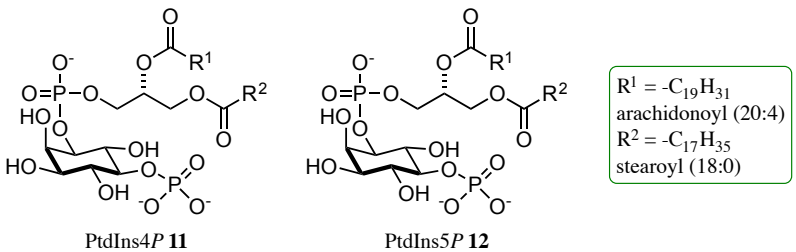


Figure 1.3 The right-flippered 'Agranoff turtle' represents the 1D-nomenclature of *myo*-inositol **1**. On going from *myo*-inositol **1** to *scyllo*-inositol **2**, numbering changes.[12] Figure adapted by permission from Macmillan Publishers Ltd: Nature Reviews Molecular Cell Biology,[2] copyright 2001 (Appendix C).

Similar to *myo*-inositol **1**, *scyllo*-inositol **2** contains a plane of symmetry running through the equatorial 1- and 4-positions. Nomenclature issues therefore also exist for the less studied *scyllo*-inositol derivatives. It is important to note that on going from the *myo*- to the *scyllo*-geometry, numbering changes. The axial 2-position of *myo*-inositol becomes the equatorial 1-position of *scyllo*-inositol (Figure 1.3).

In this dissertation, therefore, the 1D-nomenclature is employed for all *myo*- and *scyllo*-inositol derivatives, except when referring to both enantiomers. In these cases, both D- and L-nomenclatures are used to emphasise that an enantiomeric pair was synthesised (Chapter 5, Experimental).

There exists a plethora of complex, biologically important molecules based on the inositol motif. These include phosphatidylinositols (PtdIns), phosphatidylinositol phosphates (PtdInsP_n), inositol phosphate glycans (IPGs), diphosphoinositol phosphates (PP-Ins*P*s) and many others which have been the subject of numerous books and reviews.[13,14] As a result, inositol derivatives have sparked the interest of synthetic organic chemists.[14-16] Since the original synthesis of the second messenger Ins(1,4,5)P_3 **10** by Ozaki *et al.*,[17] much research has focused on developing efficient regio- and enantioselective syntheses of these molecules, with the aim of minimising the use of multiple protecting groups and inefficient resolution procedures (See Section 1.2).[18,19] The work described in this dissertation focuses on the synthesis of enantiomerically pure PtdInsP_ns **11** and **12** (*myo*-inositol derivatives), and the aminocyclitol compounds rhizopines **13** and **14** (*scyllo*-inositol derivatives), found in leguminous plants (Figure 1.4).



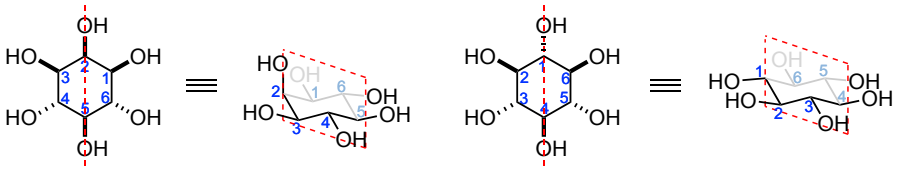
PtdIns4*P* **11** PtdIns5*P* **12**

SIA **13** (–)-1L-3-*O*-MSI (–)-**14** (+)-1D-3-*O*-MSI (+)-**14**

Figure 1.4 Naturally occurring phosphatidylinositol phosphates PtdIns4*P* **11** and PtdIns5*P* **12** and rhizopines SIA **13** and 3-*O*-MSI **14**.

1.2. Chemical synthesis of enantiomerically pure inositol derivatives

The main challenges associated with the synthesis of enantiomerically pure *myo*- and *scyllo*-inositol derivatives stem directly from the structure and *meso* nature of these molecules (Figure 1.5). In the *myo*-inositol configuration, five of the six hydroxyl groups around the ring are equatorial, with the 2-position bearing the only axial hydroxyl group. There is, therefore, a need for regiocontrol in synthesis, as the axial position is the only handle for differentiation between six near-equivalent hydroxyl groups, and stereocontrol, as a result of the plane of symmetry arising from this arrangement. The *scyllo*-inositol configuration features the same requirements, with the added complication that it does not feature any obvious handle for hydroxyl group differentiation (Figure 1.5). In addition, very few reports focus on the synthesis of *scyllo*-inositol derivatives.[6] Synthesis of our rhizopine targets (Figure 1.4; Section 1.4), therefore, was to begin from *myo*-inositol frameworks, building on previous literature in the area.



1 **2**

Figure 1.5 Flat and chair representation of the *myo*- and *scyllo*-inositol configurations, showing the plane of symmetry, in red, running through the axial 2-position and equatorial 5-positions of the *myo*-inositol ring; and the equatorial 1- and 4-positions of the *scyllo*-inositol ring.

Numerous protecting group strategies have been developed, enabling chemists to differentiate between the six hydroxyl groups of the inositol framework.[19] In addition, there are three main general synthetic strategies for the synthesis of enantiomerically pure inositol derivatives.[18] These include:

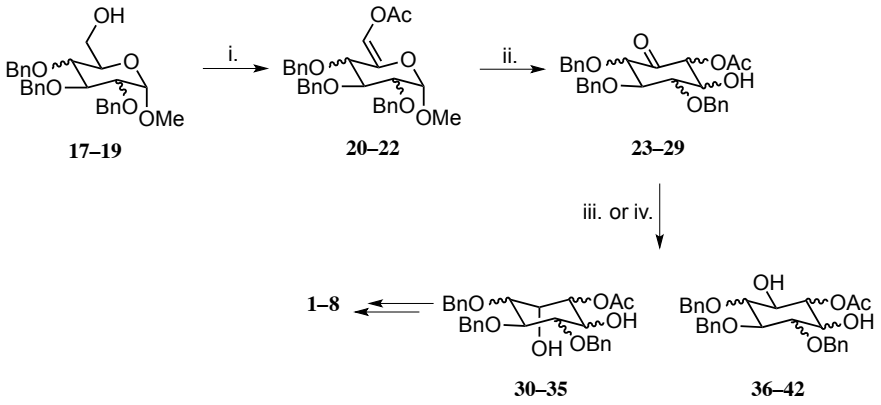
- Starting from chiral pool materials, such as monosaccharides, to give enantiopure inositol intermediates *via* a carbocyclisation step.[20]
- Starting from a conduritol derivative and elaborating this framework *via* an enantioselective dihydroxylation or subsequent epoxidation and hydrolysis of the alkene.[21,22]
- Starting from naturally abundant *myo*-inositol **1** and employing a resolution or desymmetrisation step to generate an optically-pure intermediate.[23,24]

Each of these methodologies are reviewed in the following section of this chapter, providing relevant examples from the literature, particularly those relating to PtdIns5*P* and PtdIns4*P* derivatives. We will discuss the most important protecting group strategies simultaneously and focus on the third synthetic approach. Rhizopines **13–14** are practically absent from the chemistry literature. An overview of previous syntheses of these inositol derivatives is described in Chapter 4, these are all based on the first and third synthetic approach (Section 4.3.1).

1.2.1. Synthesis of inositol derivatives from glucose and other chiral pool materials

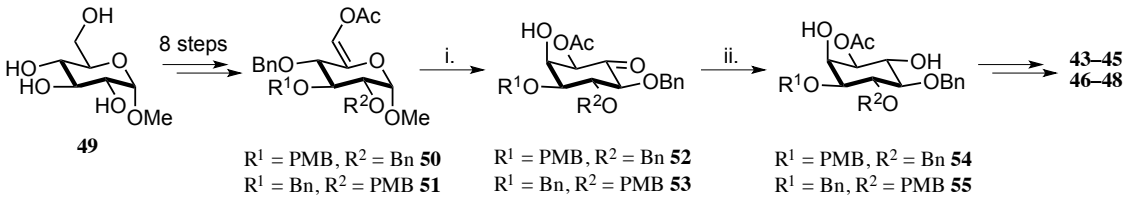
In a biological setting, *myo*-inositol **1** is synthesised from D-glucose 6-phosphate **15**, which is converted into 1D-*myo*-inositol 3-phosphate **16** by D-inositol 3-phosphate synthase, *via* a ring opening and stereospecific intramolecular aldol reaction.[25,26] Taking inspiration from this biological pathway, carbohydrates have been used frequently in the literature as optically pure starting materials for the synthesis of inositol derivatives.[18,27]

Takahashi *et al*. reported the synthesis of all inositol stereoisomers **1–8** from 2,3,4-tri-*O*-benzylglycosides **17–19**. A key $PdCl_2$-mediated Ferrier-II carbocyclisation[20,28] of the enol-acetates **20–22** provided the corresponding cyclohexanones **23–29**. These were subsequently reduced stereoselectively to give a series of precursors **30–42** to all of the inositol stereoisomers **1–8** (Scheme 1.1). This methodology was also applied to the synthesis of InsP_3 and InsP_4 derivatives.[29,30]



Scheme 1.1 Synthesis of all inositol stereoisomers **1–8** starting from 2,3,4-tri-*O*-benzylglycosides **17–19**. The synthesis involves a key $PdCl_2$-mediated Ferrier-II carbocyclisation. *Reagents and conditions:* i. a. DCC, DMSO, pyridine, TFA, benzene, RT, 12 h b. Ac_2O, Et_3N, DMAP, $ClCH_2CH_2Cl$, 100 °C, 2 h, 69–83% ii. $PdCl_2$, dioxane-H_2O, 60 °C, 8 h, 58–81% iii. $Me_4NBH(OAc)_3$, CH_3CN, AcOH, 37–94% iv. $NaBH_4$, MeOH, 84–97%.[29,30] Bn = benzyl; Me = methyl; Ac = acetate.

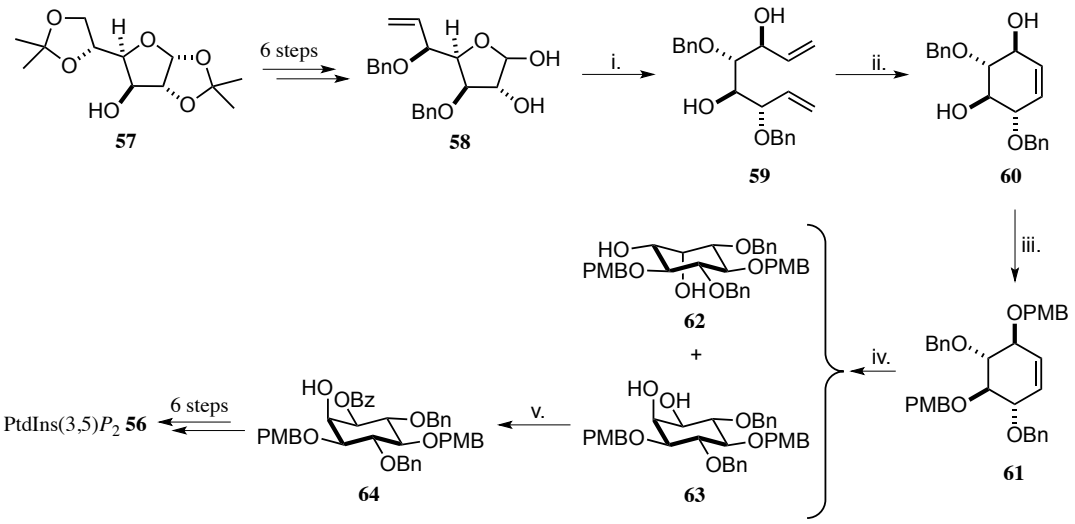
Through a similar approach, Prestwich *et al*. developed a synthesis of PtdIns3*P* **43–45** and PtdIns4*P* **46–48** with short (dibutyryl), medium (dioctanoyl), and long (dipalmitoyl) acyl chains respectively, from optically pure methyl-α-D-gluco-pyranose **49**.[31,32] A $Hg(OAc)_2$-mediated Ferrier-II rearrangement was carried out on intermediates **50–51**, providing ketones **52–53**. Stereoselective reduction of these intermediates with triacetoxyborohydride yielded **54–55** (Scheme 1.2). The main drawback of this strategy is that the key Ferrier-II carbocyclisation is performed with toxic $Hg(OAc)_2$. Nonetheless, this methodology was applied successfully to the synthesis of Ins(1,4,5)P_3 **10** by Dr Neil Keddie in our group.[33,34]



Scheme 1.2 $Hg(OAc)_2$-mediated Ferrier-II rearrangement. *Reagents and conditions*: i. a. $Hg(OAc)_2$, Acetone/H_2O (3:2) 45 min b. $NaCl_{(aq)}$, RT, 18 h, 85–94% ii. $NaBH(OAc)_3$, AcOH, MeCN, 45 min, 81–83%.[31,32] Me = methyl; Ac = acetate; Bn = benzyl; PMB = 4-methoxybenzyl.

Ring-closing metathesis has also been applied to the synthesis of inositol derivatives from carbohydrates.[35,36] The synthesis of 1-butyryl-2-arachidonoyl PtdIns(3,5)P_2 **56** reported by Shirai *et al*. involved a ring-closing metathesis[37] of the 1,7-diene **59** intermediate, derived from 1,2,5,6-*O*-diisopropylidene-D-glucose **57** (Scheme 1.3).[38] The conduritol intermediate **60** obtained was protected, then subjected to a *syn*-dihydroxylation with substoichiometric osmium tetroxide, to generate the diastereoisomeric *cis*-diols **62** and **63**. Selective benzoylation of the equatorial hydroxyl groups generated a mixture of products, which could be separated by column chromatography over silica gel to furnish **64**. Further protecting group manipulation and phosphorylation steps, yielded **56**. This approach was subsequently applied to the synthesis of D- and L-Ins(3,4,5,6)P_4 **65**.[39]

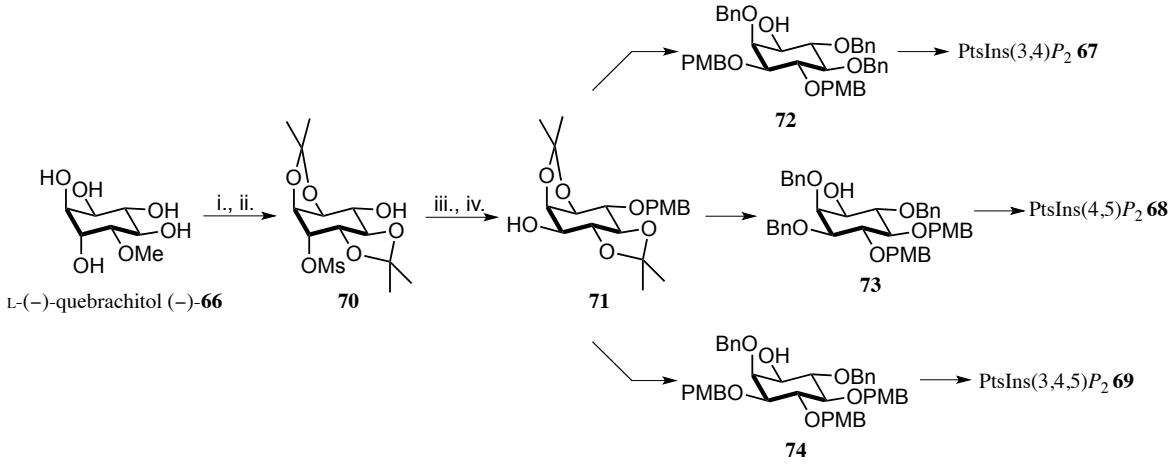
Amongst other optically pure starting materials that have been employed for the synthesis of inositol derivatives is the naturally occurring L-(−)-quebrachitol (−)-**66**, a methylated *chiro*-inositol derivative obtained from rubber trees. Kozikowski *et al*. synthesised dipalmitoyl PtdIns(3,4)P_2 **67**, PtdIns(4,5)P_2 **68**, and PtdIns(3,4,5)P_3 **69**, from L-(−)-quebrachitol (−)-**66**.[40] The key step in this synthetic route was the inversion of stereochemistry at the 3-position mesylate of **70**, through a



Scheme 1.3 Synthesis of PtdIns(3,5)P_2 **56** starting from 1,2,5,6-O-diisopropylidene-D-glucose **57**, *via* a ring-closing metathesis and *cis*-dihydroxylation. *Reagents and conditions*: i. $MePPh_3Br$, *n*BuLi, Et_2O, RT, 18 h, 68% ii. $Cl_2(PCy_2)_2Ru$=CHPh (10 mol%), CH_2Cl_2, RT, 1 h, 85% iii. NaH, PMBCl, DMF, 92% iv. OsO_4, quinuclidine, NMO, CH_2Cl_2, RT, 18 h, 98% (**62**/**63** 1:1) v. BzCl, pyridine, DMAP, 67% (dibenzoates 27%).[40] Bn = benzyl; PMB = 4-methoxybenzyl; Bz = benzoyl.

sequence comprising a Swern oxidation followed by a reduction with $NaBH_4$, to give intermediate **71**. Further protecting group manipulations provided the fully-protected precursors **72**, **73** and **74**, which led to PtdIns(3,4)P_2 **67**, PtdIns(4,5)P_2 **68**, and PtdIns(3,4,5)P_3 **69** respectively (Scheme 1.4).

The advantage of using chiral pool materials towards inositol derivatives is that the stereochemistry is carried through the synthesis and no resolution or desymmetrisation step is required. However, the syntheses based on this strategy are usually lengthy and require numerous protection-deprotection steps to reach the required framework.



Scheme 1.4 Synthesis of PtdIns(3,4)P_2 **67**, PtdIns(4,5)P_2 **68**, and PtdIns(3,4,5)P_3 **69** from L-(−)-quebrachitol (−)-**66**. *Reagents and conditions*: i. a. 2-Methoxypropene, CSA, DMF, 60 °C, 4 h b. MsCl, Et_3N, CH_2Cl_2, 91% ii. a. BBr_3, CH_2Cl_2, 0 °C, 8 h b. 2-Methoxypropene, CSA, DMF, 60 °C, 65% iii. a. NaH, PMBCl, DMF, b. $LiAlH_4$, THF, 63% iv. a. $(COCl)_2$, DMSO, Et_3N b. $NaBH_4$, MeOH, 86%.[40] Me = methyl; Ms = mesyl; PMB = 4-methozybenzyl; Bn = benzyl.

1.2.2. Synthesis of inositol derivatives from conduritol derivatives

Conduritols A–F (cyclohex-5-ene-1,2,3,4-tetraol) **75–80**[41] have been employed as building blocks for the synthesis of inositol derivatives (Figure 1.6).[22,42] Indeed, these molecules possess four hydroxyl groups and an alkene moiety, rendering them amenable to a dihydroxylation to provide a range of inositol derivatives. Both symmetric[22] and non-symmetric[21] conduritols have been employed for this purpose. C_2-symmetric conduritols B **76** and E **79** are particularly valuable as the *cis*-dihydroxylation or epoxidation of the double bond generates a single diastereoisomer. The modular synthesis of all inositol stereoisomers *via* conduritol-B **76**, -C **77**, -E **79** and -F **80** demonstrates the significance of this framework.[42]

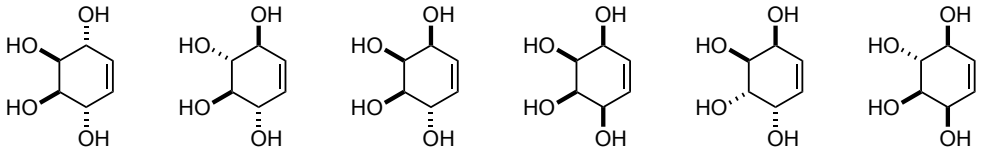
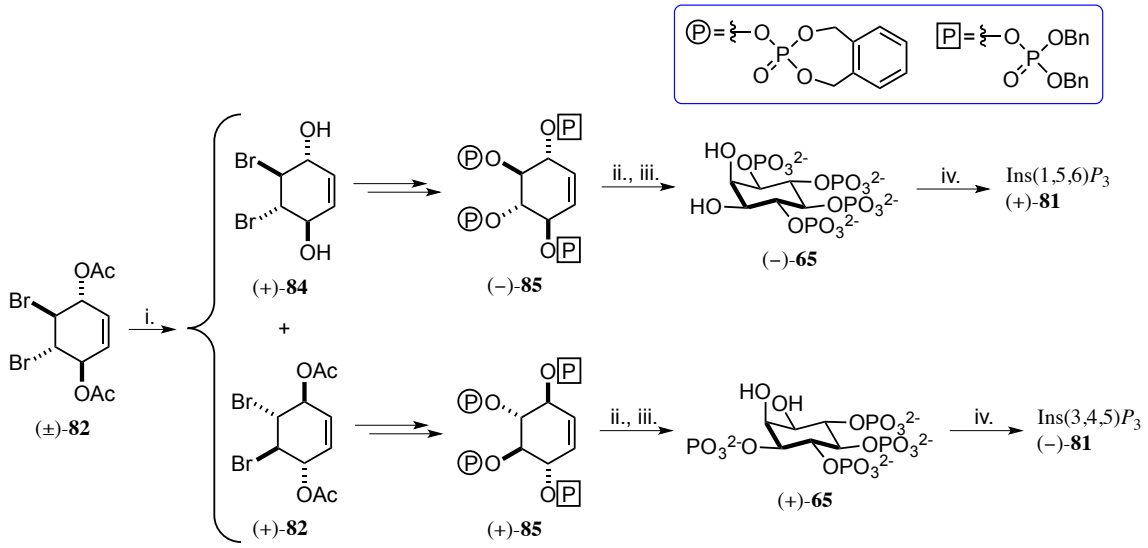


Figure 1.6 Stereoisomers of cyclohex-5-ene-1,2,3,4-tetraol: Conduritols A–F of which conduritol-A **75** and conduritol-B **76** are naturally occurring.

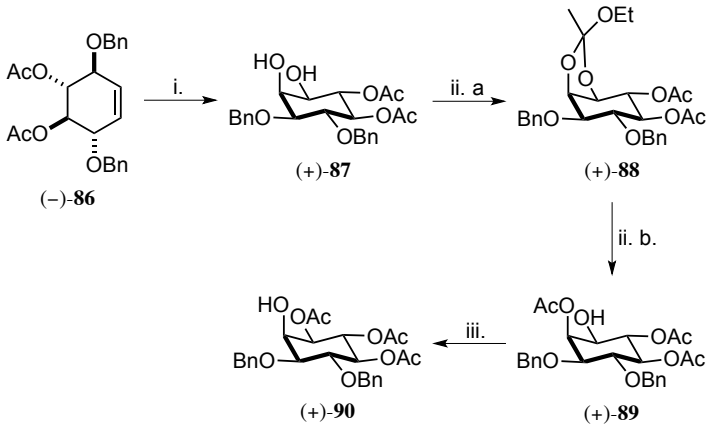
Inositol derivatives have been synthesised *via* conduritol intermediates, starting from chiral-pool materials such as carbohydrates (Scheme 1.3).[38] In addition, *de novo* construction of inositols, has been achieved through the use of enzymatic resolution or dynamic kinetic asymmetric transformation of conduritol-B derivatives.

Through a chemoenzymatic approach, Altenbach *et al*. synthesised the enantiomers Ins(3,4,5)P_3 (–)-**81** and Ins(1,5,6)P_3 (+)-**81** starting from the racemic diacetate (±)-**82**,[43] which was easily obtained from *p*-benzoquinone **83**.[44] An enzymatic resolution step using pig pancreas lipase (PPL)[45] provided the two enantiopure compounds (+)-**84** and (+)-**82**, which were subsequently converted to the tetraphosphorylated conduritol derivatives (–)-**85** and (+)-**85**. A *cis*-dihydroxylation followed by global deprotection provided the enantiomeric pair of InsP_4 (–)-**65** and (+)-**65**. Using the InsP_5/InsP_4 phosphohydrolase from *Dictyostelium discoideum*, these enantiomers were converted to Ins(3,4,5)P_3 (–)-**81** and Ins(1,5,6)P_3 (+)-**81** (Scheme 1.5).



Scheme 1.5 Synthesis of enantiomers Ins(3,4,5)P_3 (−)-**81** and Ins(1,5,6)P_3 (+)-**81** by Altenbach *et al*.[43,45] i. PPL, phosphate buffer (pH 7), RT, 4 days, 38% each ii. $RuCl_3$, $NaIO_4$, EtOAc/MeCN/H_2O, RT, 7 min, 87% iii. Pd/C, $H_{2(g)}$, EtOH/H_2O, 95% iv. InsP_5/InsP_4 phosphohydrolase from *Dictyostelium discoideum*, buffer, RT, 1.5 h, 70–80%. Ac = acetate; Bn = benzyl.

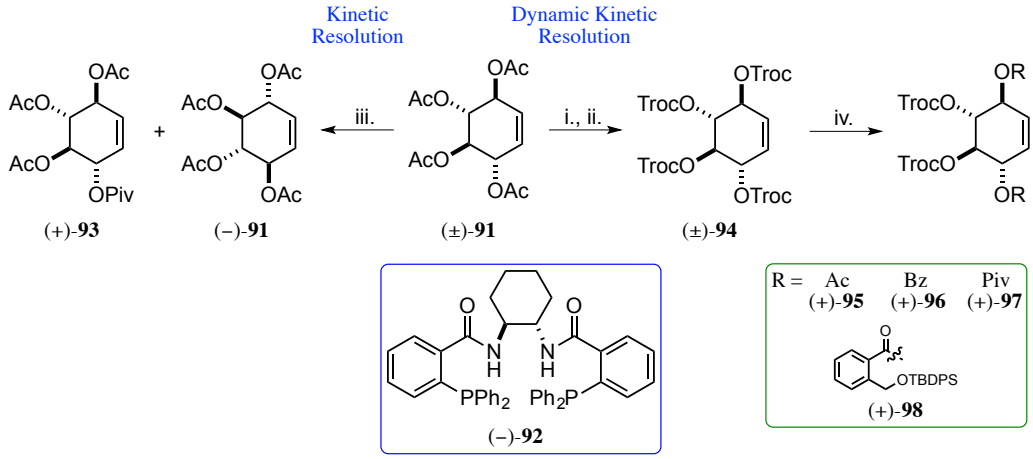
Using this strategy, Altenbach *et al*. further exploited symmetrical and non-symmetrical conduritols to synthesise a library of natural and non-natural InsP_ns.[46] This was mostly enabled by the development of various regioselective transformations on these systems. For example, differentiation between the axial and equatorial hydroxyl groups of (+)-**87**, derived from conduritol (−)-**86**, was achieved by treatment with triethylorthoacetate under acidic conditions. The 1,2-orthoester intermediate (+)-**88** obtained was directly converted to the axial acetate (+)-**89** by treatment with acetic acid,[47] providing an intermediate in the synthesis of Ins1*P* **9** and Ins(1,4,5)P_3 **10** (Scheme 1.6).



Scheme 1.6 Differentiation between the axial 2- and equatorial 1-position on (+)-**87**, employed by Altenbach *et al*. for the synthesis of InsP_ns. *Reagents and conditions*: i. $RuCl_3$, $NaIO_4$, EtOAc/MeCN/H_2O, RT, 7 min, 99% ii. a. MeC$(OEt)_3$, PTSA·H_2O, RT, 24 h b. 80% AcOH, RT, 1 h, 95% iii. TMSOTf (0.2 M), Et_2O, 3Å MS, RT, 48 h, 100%.[21] Ac = acetate; Bn = benzyl.

In addition, forced migration of the acetate group to the equatorial position was possible, giving another synthetically useful, protected inositol derivative (+)-**90** (Scheme 1.6).[22]

Furthermore, Trost *et al*. developed a palladium-catalysed asymmetric allylic alkylation[48] of the conduritol B tetraacetate (±)-**91**. In this kinetic resolution, only one enantiomer reacts to give two products (–)-**91** and (+)-**93**, which are separable by column chromatography.[49,50] When the acetate groups are replaced by 2,2,2-trichloroethylcarbonate (Troc) esters, Trost *et al*. demonstrated that a high yielding, dynamic kinetic resolution results (Scheme 1.7).[49,51] Dr Alex Saunders, in our group, optimised this methodology for the synthesis of a range of optically pure derivatives (+)-**95–**(+)-**98**, and applied it to the synthesis of dipalmitoyl PtdIns*P* **99** and PtdIns(4,5)P_2 **68**.[47]



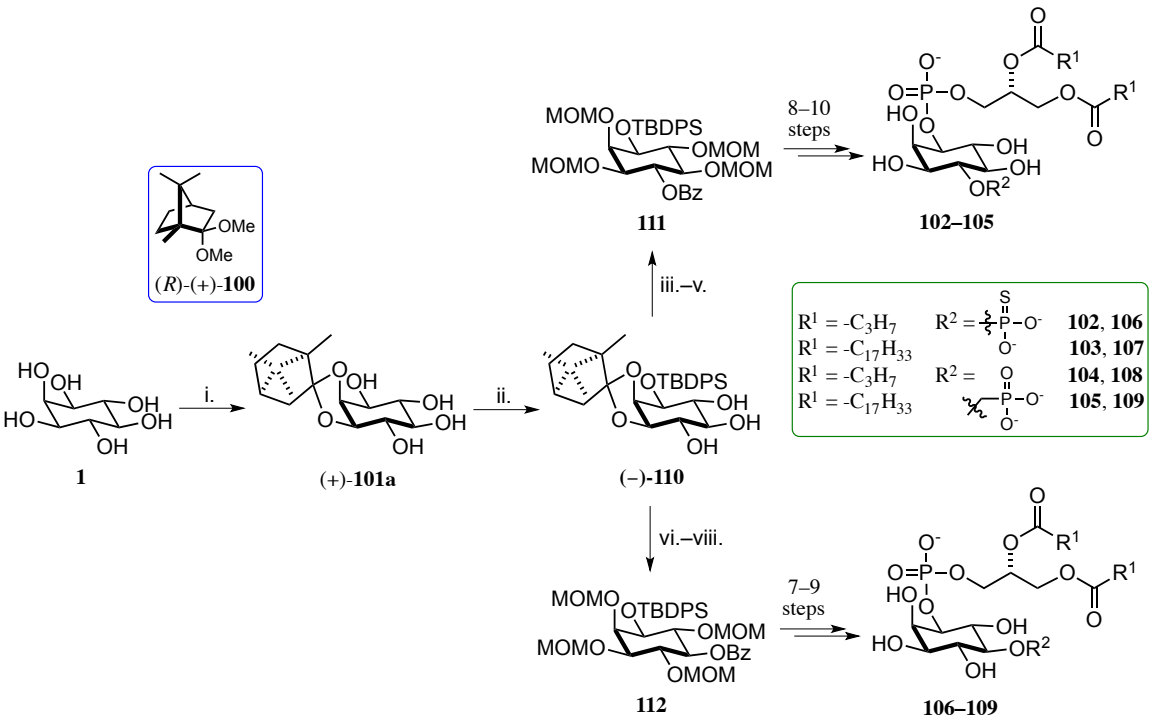
Scheme 1.5 Trost's asymmetric allylic alkylation:[47-50] Comparison of the kinetic resolution and dynamic kinetic resolutions developed for the conduritol-B tetra-acetate (±)-**91**. *Reagents and conditions*: i. Et_3N, H_2O, MeOH, RT, 1 h ii. $ClCO_2CH_2CCl_3$, DMAP, pyridine, CH_2Cl_2, RT, 20 min, 88% over 2 steps iii. PivOH, $(CH_3(CH_2)_5)_4NBr$, $[Pd(\eta^3\text{-allyl})Cl]_2$, (–)-**92**, NaOH (0.50 M), CH_2Cl_2, RT, 18 h, 43%, >96% *e.e.* (+)-**93**, 48%, 90% *e.e.* (–)-**91** iv. ROH, $(CH_3(CH_2)_5)_4NBr$, $[Pd(\eta^3\text{-allyl})Cl]_2$, (–)-**92**, NaOH (1.0 M), CH_2Cl_2, 18 h, 81%, >99% *e.e.* Ph = phenyl; Ac = acetate; Piv = pivaloyl; Troc = 2,2,2 -trichloroethylcarbonate; Bz = benzoyl; TBDPS = *tert*-butyldiphenylsilyl.

Whilst conduritol building blocks have been particularly useful in the synthesis of InsP_n, as demonstrated by Altenbach *et al*., only a few desymmetrisation strategies exist in the literature. These are not well suited to the synthesis of PtdIns4*P* **11**, PtdIns5*P* **12**, and rhizopines **13–14** and hence were not applied in this work.

1.2.3. Synthesis of inositol derivatives, from *myo*-inositol, *via* a resolution step

Although numerous other starting materials have been employed for the synthesis of inositol derivatives,[18,52-54] *myo*-inositol **1** is the most prevalent in the literature. A wealth of literature exists surrounding the resolution or desymmetrisation of *meso*-inositol derivatives.[18]

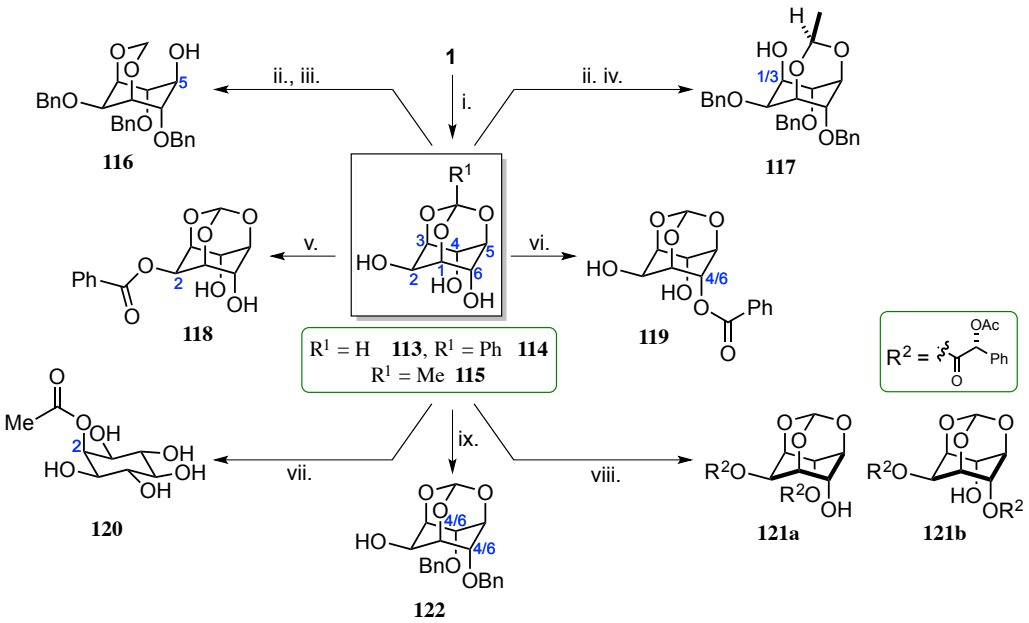
One of the most commonly used synthetic methods towards enantiomerically pure inositol derivatives is the use of chiral auxiliaries. A notable example is the protection-resolution strategy developed by Bruzik and Tsai.[55] Here, *myo*-inositol **1** is reacted with the chiral auxiliary 1-(*R*)-(+)-camphor dimethyl acetal (+)-**100**, followed by mild acidic hydrolysis of the *trans*-acetal products, to give a mixture of four *cis*-1,2-protected diastereoisomers **101a**–**d**.[55-57] One of those diastereoisomers (+)-**101a** can be precipitated selectively over the other three (Scheme 1.8; Section 2.2). Bruzik and Tsai demonstrated that this framework was amenable to regioselective protection at the 1- and 4-positions with bulky protecting groups (Scheme 1.8), enabling the synthesis of all the naturally occurring phosphatidylinositols for the first time.[23]



Scheme 1.6 Synthesis of metabolically stable, methylenephosphonate and phosphorothionate, analogues of PtdIns5*P* and PtdIns4*P* **102–106** by Prestwich *et al*.,[58,59] *via* the protection-resolution methodology developed by Bruzik *et al*.[23] *Reagents and conditions*: i. a. H_2SO_4, DMSO, 70 °C, 3 h b. PTSA·H_2O, $CHCl_3$/MeOH/H_2O (50:5:1), RT, 1.5 h, 25% ii. TBDPSCl, imidazole, –10 °C, 24 h, 88% iii. BzCl, pyridine, –40 °C, 1 h iv. TFA, H_2O, RT, 3 h, 24% over 2 steps v. MOMCl, DIPEA, DMF, 65 °C, 24 h, 82% vi. BzCl, pyridine, –40 °C, 1 h vii. TFA, H_2O, RT, 3 h, 35% over 2 steps viii. MOMCl, DIPEA, DMF, 65 °C, 24 h, 90%. Me = methyl; TBDMS = *tert*-butyldiphenylsilyl; MOM = methoxymethyl; Bz = benzoyl.

This strategy was employed more recently by Prestwich *et al*. for the synthesis of metabolically stable, methylenephosphonate and phosphorothionate, analogues of PtdIns4*P* **102–105**[58] and PtdIns5*P* **106–109** (Scheme 1.8).[59] These derivatives proved invaluable in understanding the cellular roles of PtdIns5*P* and PtdIns4*P* and their interactions with effector proteins (Sections 1.3.2 and 1.3.3). Whilst the synthesis of these complex molecules is achieved stereoselectively, *via* an early resolution, most of the regioselective steps for differentiation of the remaining three hydroxyl groups on (–)-**110** are low yielding.

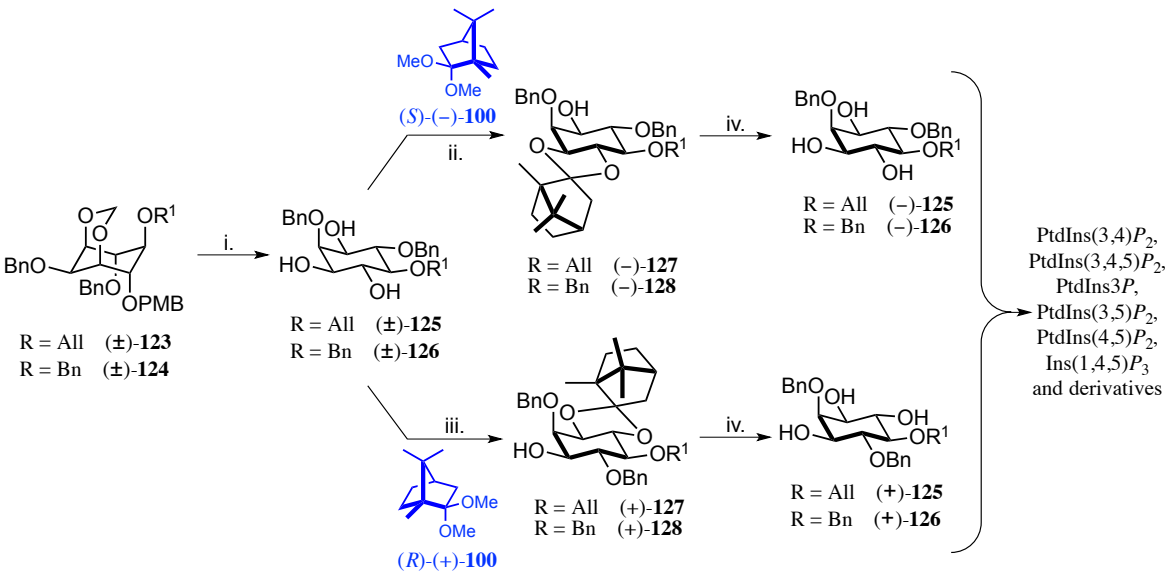
myo-Inositol 1,3,5-orthoformate **113** originally prepared by Kishi and Lee,[60] and used as an alternative protecting group strategy by Billington *et al*.,[61] was an important step forwards in the development of high yielding, regioselective protections of inositols. The adamantane-like mono-orthoesters of *myo*-inositol **113** can be synthesised from *myo*-inositol **1** in a single step, by treatment triethylorthoformate, under acid catalysis.[60,62] Since this pioneering work, various *myo*-inositol orthoformates derivatives, including **113–115**, have been thoroughly investigated, demonstrating that numerous substitution patterns can be generated from this framework (Scheme 1.9).[19] The locked



Scheme 1.7 Examples of regioselective protection of *myo*-inositol orthoformates **113–115**[19,47] *Reagents and conditions*: i. $(MeO)_3CR/(EtO)_3CR$, H^+, DMF/DMSO[60,62] ii. NaH, BnBr, DMF, 0 °C to RT, 18 h, 50% iii. DIBAL-H, CH_2Cl_2/hexane, 0 °C to RT, 24 h, 96% iv. Me_3Al, CH_2Cl_2/hexane, 0 °C to RT, 6 h, 84%[64,72] v. PhCOCl, NaH (2.0 eq), DMF, RT, 24 h, 85% vi. PhCOCl, NaH (1.0 eq), DMF, RT, 24 h, 89%[397] vii. TFA, H_2O, RT, 3 min, 100% viii. (*S*)-(+)-AMCl, pyridine, 0 °C, 2 h, 59% **121a**, 26% **121b**[65,66] ix. LiH, BnBr, DMF, RT, 48 h, 75%.[62,63] Ph = phenyl; Ac = acetate; Bn = benzyl; AMCl = Acetylmandeloyl chloride.

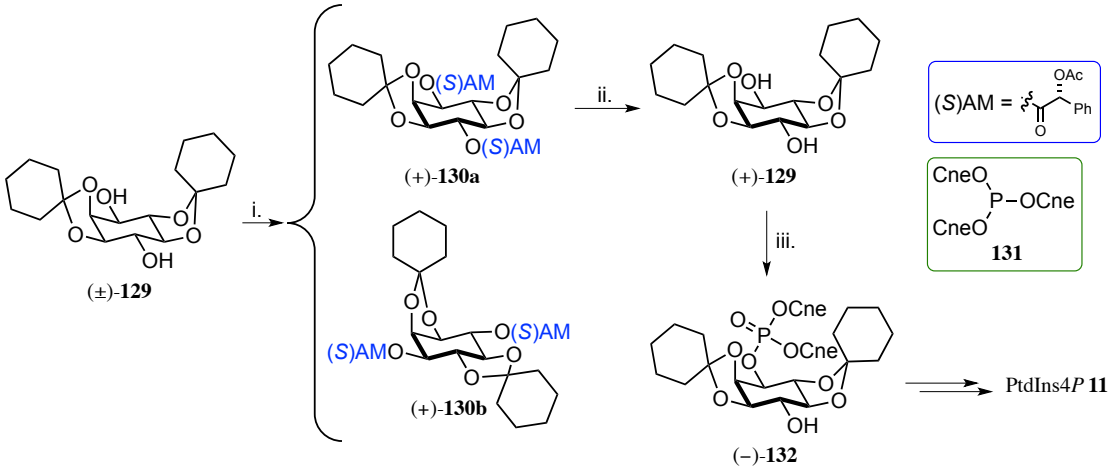
conformation of the orthoformate allows for differentiation between the axial 4- and/or 6-positions and the equatorial 2-position (Scheme 1.9).[62,63] In addition, regioselective functionalisation of the 1-, 3- and 5-positions is possible through opening of the orthoformate under various reaction conditions.[64] Finally, resolution of **113** was also achieved using (*S*)-*O*-acetylmandeloyl chloride (AMCl) or camphor dimethyl acetal **100** as chiral auxiliaries.[65,66] Numerous other inositol derivatives have also been synthesised using **113–115** (Scheme 1.9).[67]

Building on the work by Bruzik *et al*.[23] and Billington *et al*.[61] Holmes developed a route that involves the synthesis of racemic triols (±)-**125**[34,68-70] or (±)-**126**[15,71,72] *via* orthoformates **123** and **124**. Subsequent diastereoisomeric resolution of (±)-**125** and (±)-**126** with 1-(*R*)-(+)-**100** (Section 2.2) or the opposite enantiomer 1-(*S*)-(−)-**100** (Section 4.5.2) generates four diastereoisomers, which can be resolved by column chromatography to give (−)-**127–128** or (+)-**127–128** respectively. Subsequent deprotection provides the resolved triols (−)-**125–126** and (+)-**125–126** (Scheme 1.10). This resolution methodology has been frequently employed in combination with orthoformate strategies to generate versatile protected inositol frameworks for the synthesis of enantiomerically pure InsP_ns, PtdInsP_ns and other derivatives.[34,64,68-70,73,74]



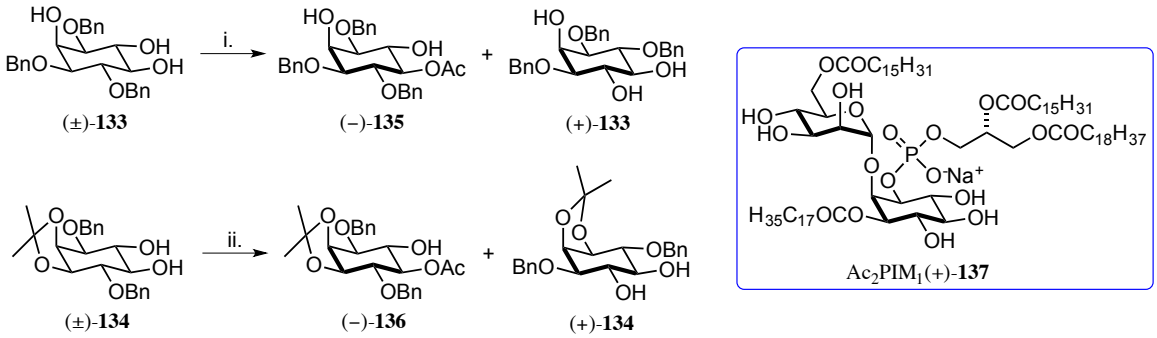
Scheme 1.8 Resolution of triols (±)-**125** and (±)-**126** developed by Holmes *et al*.[15,34,68-72] and later applied to the synthesis of a range of PtdInsP_n and InsP_n derivatives.[34,64,68-70,73,74] *Reagents and conditions*: i. HCl, MeOH, reflux, 78–96% ii. PTSA·H_2O, CH_2Cl_2, reflux, 31% iii. PTSA·H_2O, CH_2Cl_2, reflux, 23% iv. AcCl, CH_2Cl_2/MeOH, 81–93%. All = allyl; Bn = benzyl; PMB = 4-methoxybenzyl.

Amongst other chiral auxiliaries that have been recently used for the synthesis of inositol derivatives[75,76] is acetymandelic acid (AMA). Gaffney *et al*. resolved the inositol building block 2,3:5,6-*O*-dicyclohexylidene *myo*-inositol (±)-**129**, using (*S*)-(+)-*O*-AMA to give the single diastereoisomer (+)-**130a** after selective crystallisation. Deprotection of the acetylmandeloyl ester followed by regioselective phosphorylation at the 1-position with **131** provided the key intermediate (–)-**132** towards a range of PtdIns4*P* derivatives including 1-stearoyl-2-arachidonoyl PtdIns4*P* **11** (Scheme 1.11).[77]



Scheme 1.9 Synthesis of PtdIns4*P* **11** by Gaffney *et al*.,[77] *via* the resolution of (±)-**129** with acetylmadelic acid as a chiral auxiliary. *Reagents and conditions*: i. (*S*)-*O*-AMA, *N*-methyl imidazole, 2,6-dichloro-benzoyl chloride, CH_2Cl_2, 0 °C, 20 min, 48% (+)-**130a**, 46% (+)-**130b** ii. KOH, EtOH, RT, 2 h, 91% iii. **131**, Pyridinium bromide perbromide, CH_2Cl_2/pyridine (9:1), –40 °C to –20 °C, 3 h, 78%. AM = acetylmandeloyl; Cne = cyanoethyl; Ac = acetate.

Chemoenzymatic approaches (Section 1.2.2, Scheme 1.6), have also proven invaluable for the resolution of inositol derivatives. Simas *et al*. in particular, have worked on the development of enzymatic methodologies, including the kinetic resolution of inositol derivatives (±)-**133**[78,79] and



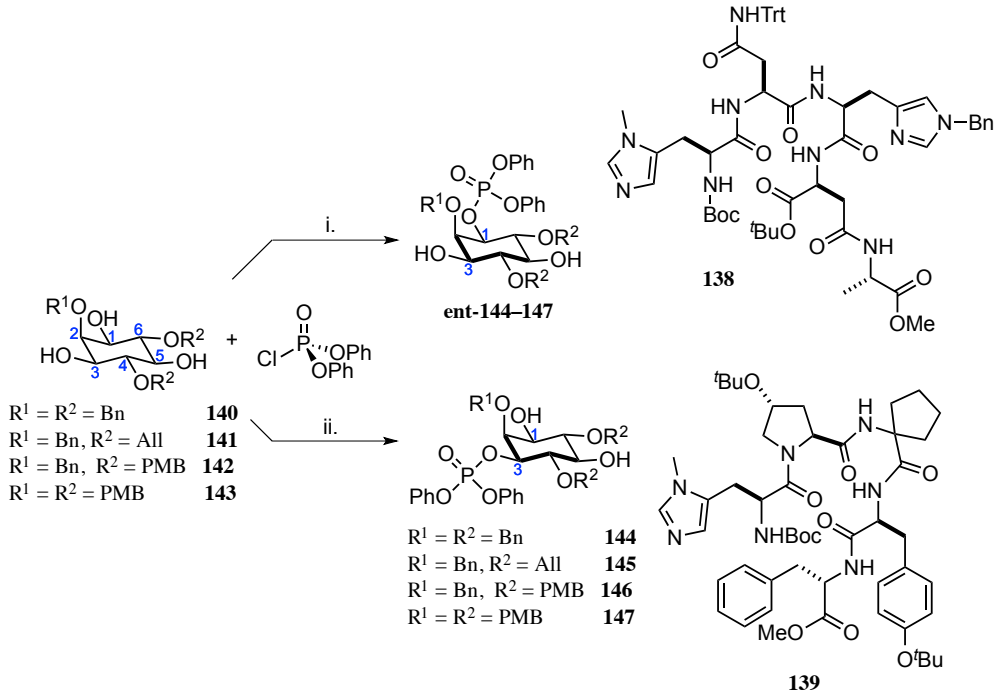
Scheme 1.10 Enzymatic kinetic resolution methodologies developed by Simas *et al*.[78-81] and structure of Ac_2PIM_1 (+)-**137** synthesised by Larsen *et al*.[82] *Reagents and conditions*: i. Novozym® 435, vinyl acetate, hexane, 45 °C, 24 h, 39% (–)-**135**, 43% (+)-**133** ii. Novozym® 435, EtOAc, 30 °C, 48 h, 34% (–)-**136**, 52% (+)-**134**. Bn = benzyl; Ac = acetate.

(±)-**134**[80,81] (Scheme 1.12) with the immobilised enzyme *Candida antarctica* lipase B (Novozym 435®). More recently, Larsen *et al*. reported the synthesis of diacylated phosphatidylinositol monomannoside (Ac_2PIM_1) (+)-**137** using the latter enzymatic resolution (Scheme 1.12).[82]

1.2.4. Synthesis of inositol derivatives, from *myo*-inositol, *via* a desymmetrisation step

Only a handful of enantioselective desymmetrisation strategies exist for *myo*-inositol derivatives.

The state-of-the-art methodology in this area is the enantioselective phosphorylation of *meso* inositol derivatives developed by Miller *et al*. This elegant work is based upon using a library of tetrapetide catalysts such as **138** and **139**,[83-86] for the differentiation of the enantiotopic 1- and 3-positions of 2,4,6-*O*-triprotected *myo*-inositol derivatives **140–143** (Scheme 1.13). Miller *et al*. reported the synthesis of dioctanoyl PtdIns5*P* **148** using this methodology,[87] as well as many other PtdInsP_n[88] and InsP_n derivatives.[24,86]

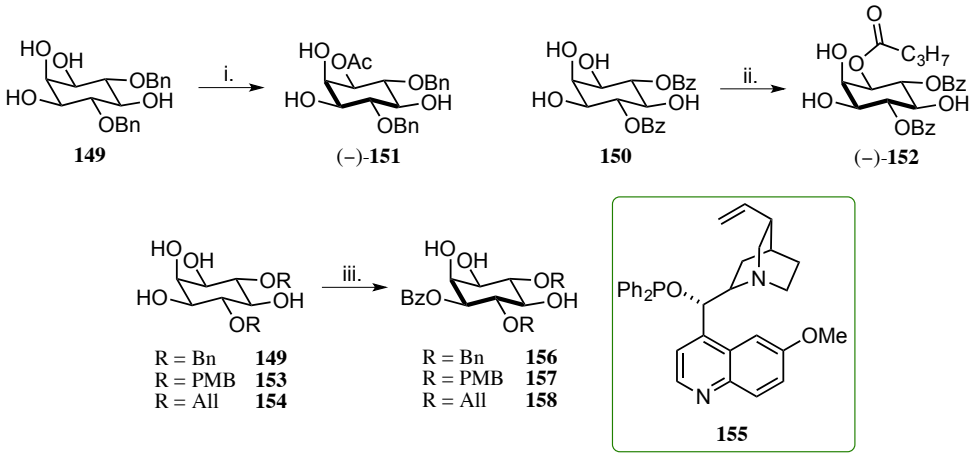


Scheme 1.11 Desymmetrisation developed by Miller *et al*.[24,83-88] involving the enantioselective phosphorylation at the 1- or 3-positions of *meso*-2,4,6-*O*-triprotected *myo*-inositol derivatives. *Reagents and conditions*: i. **138** (2.5 mol%), Et_3N, toluene, 0 °C, 4 h, 46–65%, 94–98% *e.e*. ii. **139** (2.5 mol%), Et_3N, toluene, 0 °C, 4 h, 53–56%, 97–98% *e.e*. Me = methyl; Bn = benzyl; All = allyl; PMB = 4-methoxybenzyl; Bz = benzoyl; Ac = acetate; Ph = phenyl; Trt = trityl; Boc = *tert*-butyloxycarbonyl; ^{t}Bu = *tert*-butyl.

A number of enzymatic methodologies exist for the desymmetrisation[89] of *meso*-4,6-*O*-diprotected *myo*-inositol derivatives such as **149** and **150**. Regio- and enantioselective acylation of the 1-position

provides (–)-**151** and (–)-**152** respectively in high yields and enantiomeric excess (*e.e.*) (Scheme 1.14).[90-92]

Finally, Lauber *et al*. reported the *Cinchona* alkaloid derivative **155** as an efficient organocatalyst for the desymmetrisation of a range of similar frameworks including **149**, **153** and **154** (Scheme 1.14).[93]



Scheme 1.12 Enantioselective desymmetrisations for *meso myo*-inositol derivatives **149**, **150**, **153** and **154** involving enzymes (top)[90-92] and organocatalysis (bottom).[93] *Reagents and conditions*: i. Lipozyme TL-IM, vinyl acetate/hexane (1:1), 45 °C, 12 h, 99%, > 99% *e.e*. ii. Lipoprotein lipase from *Pseudomonas sp*., > 95% *e.e*. iii. **155** (20 mol%), DIPEA, BzCl, propionitrile, –78 °C, 24 h, 65%, >99% *e.e*. Ac = acetate; Bn = benzyl; Bz = benzoyl.

1.3. Phosphatidylinositol phosphates (PtdIns*P*$_n$s)

1.3.1. Structure and biological relevance of phosphatidylinositol phosphates (PtdIns*P*$_n$)

Since the discovery of inositol phospholipids by Folch in the 1940s[94-96] and the pioneering work carried out later by Lowell and Mabel Hokin on the 'phospholipid effect',[97-100] phosphatidylinositol phosphates (PtdIns*P*$_n$s) have come to be recognised[101] as fundamental regulators of a wide range of crucial cellular processes.[102,103]

Structurally, PtdIns **159** comprises a *myo*-inositol headgroup that is linked at the 1-position to the *sn*-3 position of a diacylglycerol backbone *via* a phosphodiester bridge (Figure 1.7).[104] This phospholipid is synthesised by PtdIns synthases[105] in the endoplasmic reticulum (ER), through the condensation of *myo*-inositol with a diacyglycerol (DAG) moiety.[106] PtdIns **159** is then transferred to other cellular membranes by transfer proteins or *via* vesicular transport.[107] Reversible

phosphorylation at the 3-, 4- and/or 5-position of the PtdIns **159** *myo*-inositol headgroup generates a family of seven PtdInsP_ns (Figure 1.7), each with a different phosphorylation pattern.

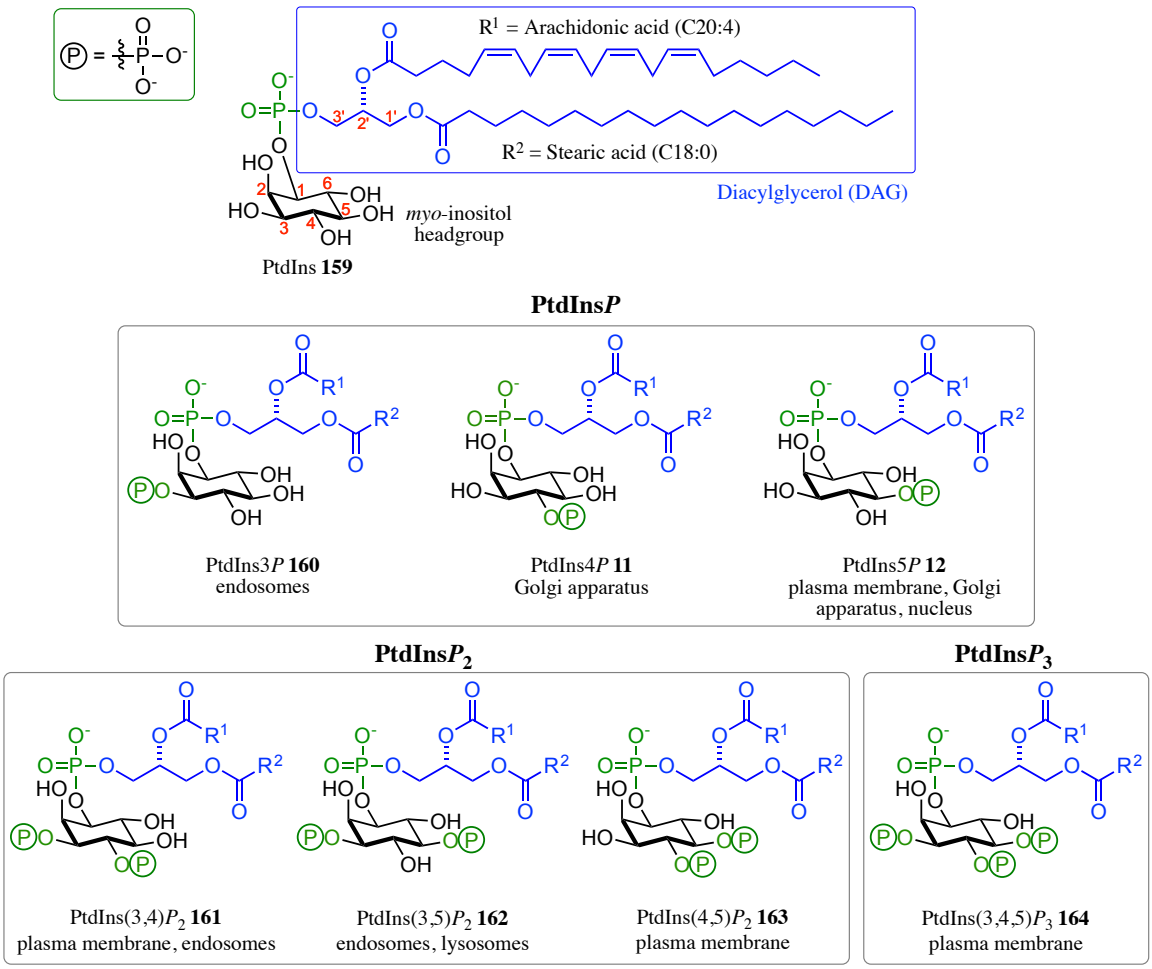
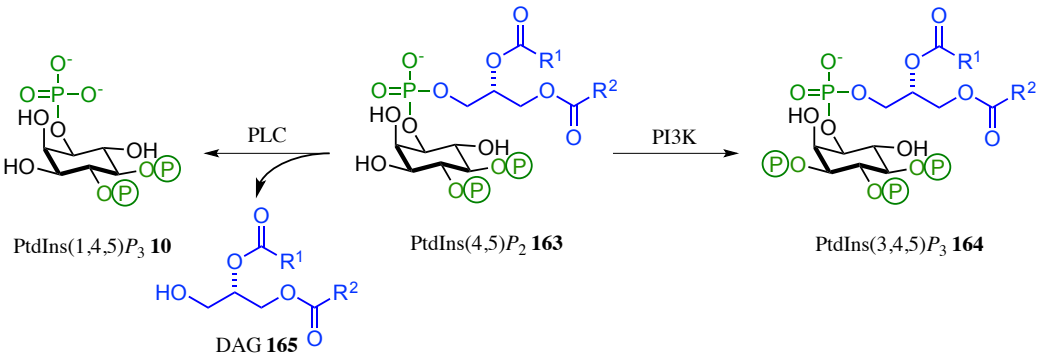


Figure 1.7 Structure of the most abundant, naturally occurring phosphatidylinositol (PtdIns **159**), containing arachidonoyl and stearoyl lipid chains. PtdIns **159** is the precursor of seven PtdInsP_ns which are localised in distinct sub-cellular locations. The main localisation of each PtdInsP_n is indicated.[102,110]

The overall concentration of PtdIns **159** and PtdInsP_ns in eukaryotic cells is relatively small,[108,109] representing only 10-15% of membrane phospholipids, with PtdIns4*P* **11** and PtdIns(4,5)P_2 **163** being the most prevalent members of the PtdInsP_n family (10%).[107] Despite this, each of the seven PtdInsP_ns is characterised by their distinct sub-cellular distribution and biological function.[110] For example, concentrations of PtdIns(4,5)P_2 **163** are high in the plasma membrane, while PtdIns4*P* **11** is mostly located within the Golgi apparatus where a number of PtdIns4*P* effector proteins have been identified (Figure 1.7).[111,112] As a result, this extremely versatile family of membrane phospholipids play an important role in defining organelle identity and mediating organelle function.[113]

These amphiphilic molecules are anchored within membranes and are consequently mainly involved in regulating cellular processes at the membrane-cytosol interface.[107,114] Regulation is effected, directly or indirectly, by interacting specifically with integral membrane proteins or by recruiting specific effector proteins to the membrane such as pleckstrin homology (PH) domains.[115-117] PtdInsP_ns are hence crucial to the regulation of ion channels, pumps, and transporters.[118] They are also key to intracellular signalling pathways, which play a crucial role in vesicle trafficking, cytoskeletal rearrangement, cell proliferation, survival, apoptosis, metabolism, and many other others.[119] A representative example of the importance of PtdInsP_ns in multiple cell-signalling events is PtdIns(4,5)P_2 **163**.[102,120] PtdIns(4,5)P_2 **163** is generated from the phosphorylation of PtdIns4*P* **11** by PtdIns*P*5Ks. Hydrolysis of this signalling molecule by phospholipase C (PLC) generates two second messengers which activate distinct downstream signalling pathways. Ins(1,4,5)P_3 **10** is released in the cytosol and plays an important role in calcium signalling[121-123] while DAG **165** activates the protein kinase C (PKC) pathway, controlling cell proliferation and growth.[124] In addition, **163** can be phosphorylation by PtdIns3K to give PtdIns(3,4,5)P_3 **164** (Scheme 1.13) a second messenger which regulates cell survival *via* binding to protein kinase B.[125]



Scheme 1.13 PtdIns(4,5)P_2 **163** is involved in calcium signalling as well as in the PKC and PtdIns3K signalling pathways. P = phosphate PO_3^{2-}, R^1 = -$C_{19}H_{31}$, R^2 = -$C_{17}H_{35}$.

A series of kinases and phosphatases tightly mediate the interconversion between the various PtdInsP_ns, through phosphorylation and dephosphorylation reactions, respectively (Figure 1.8).[102,126] These enzymes possess a unique structural motif allowing for the recognition of phosphorylation patterns on PtdInsP_n and InsP_n substrates.[127] As a result of this substrate specificity, these lipid kinases and phosphatases can precisely regulate the distinct heterogeneous subcellular distribution

of the PtdInsP_ns within each intracellular compartment, keeping these phospholipids at a steady level within the membranes.

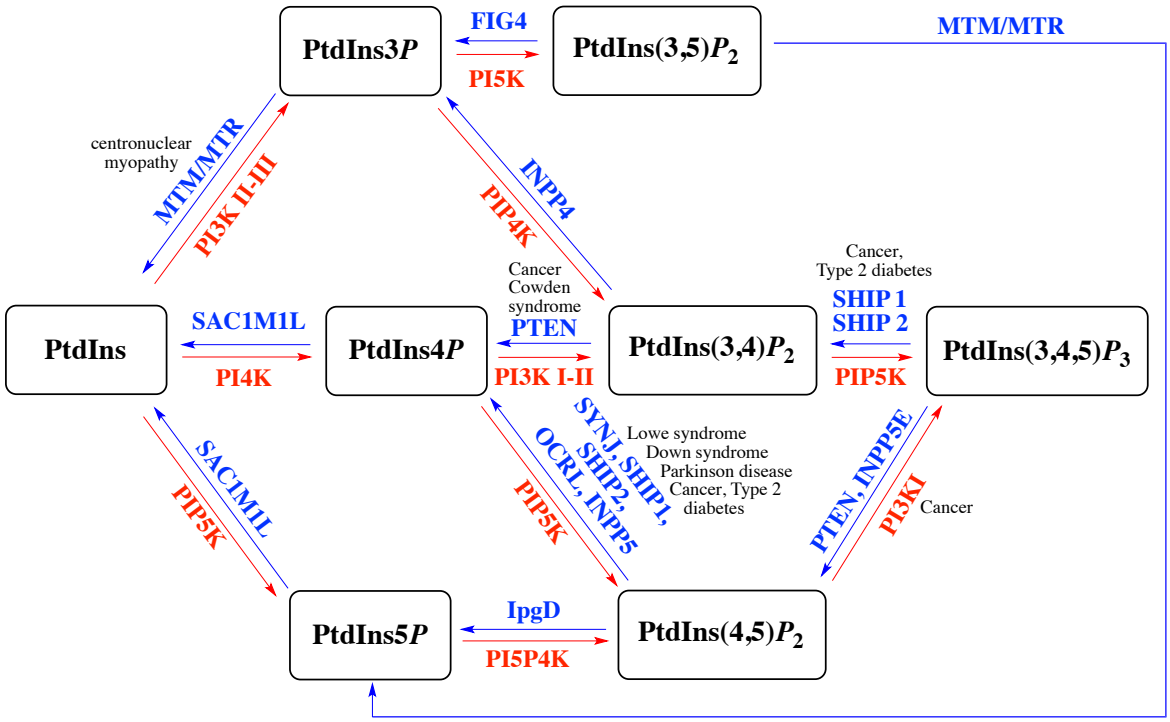


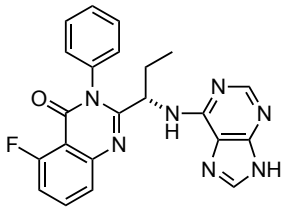
Figure 1.8 Overview of the kinases (red) and phosphatases (blue) that mediate the interconversion between PtdInsP_ns, and a selection of the well-known associated pathologies.[102,110,126]

Whilst the phosphorylation pattern around the cytosolic *myo*-inositol headgroup has been mostly attributed to the differing biological activities of the various PtdInsP_ns, the nature of the lipid chains on the glycerol backbone has been recently shown to be of equal importance.[128] Most naturally occurring PtdInsP_ns in mammals feature a 1-stearoyl-2-arachidonyl glycerol backbone (Figure 1.7).[129-131] This enrichment in particular PtdInsP_n species has been attributed to the specificity of protein targets for PtdInsP_ns.[128,132] A recent study, for example, showed that the isoforms of PtdIns5K (Figure 1.8) can discriminate amongst PtdIns4*P* substrates containing different acyl chains. Interestingly, the 1-stearoyl-2-arachidonyl PtdIns4*P*-derivative **11** was shown to be a much better substrate for all isoforms compared to the fully saturated dipalmitoyl PtdIns4*P* **48**.[133,134] When considering the design of PtdInsP_ns probes therefore, the nature of the lipids on the diacylglycerol backbone should also be considered.[14]

Given the significance of the localisation and function of PtdInsP_ns in numerous cellular processes, it is clear that dysregulation of the phosphoinositide metabolism may lead to the development of

diseases including cancer, diabetes, and neurological disorders such as Alzheimer's disease or Down syndrome (Figure 1.8).[102,110,135-138] Indeed, PtdInsP_n kinases have been recognised as promising therapeutic targets. A number of isoform selective PtdIns3K[139] and PtdIns4K[140,141] inhibitors have been developed, and many are in clinical trials.[142,143] Despite these efforts, only the PtdIns3Kδ inhibitor Idelalisib **166** (Zydelig®, Gilead Sciences) has been approved by the Food and Drug Administration (FDA, 2014) for treatment of haematological malignancies (Figure 1.9).[144,145] This is not surprising, considering the lack of understanding of the roles of each PtdInsK isoforms in signalling and in various disease mechanisms.[146]

It is thus increasingly important to: a. quantify, localise, and understand the roles of PtdInsP_ns intracellularly[147,148] and b. elucidate the specific biological functions, locations and mechanism of action of each of the PtdInsP_n metabolising enzymes.



Idelalisib **166**

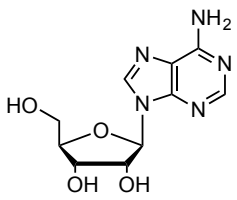
Figure 1.9 Structure of the FDA approved, PtdIns3Kδ inhibitor Idelalisib **166** (Zydelig®, Gilead Sciences).[144, 145]

1.3.2. Understanding the location and biological activities of PtdIns4K isozymes

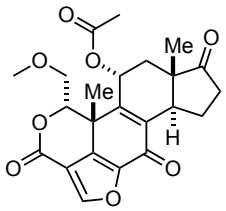
In collaboration with AstraZeneca, we therefore became interested in understanding the specific location and biological activity of the various PtdIns4K isozymes.

The enzyme PtdIns4K catalyses the selective phosphorylation of PtdIns **159** at the 4-position to give PtdIns4*P* **11** (Figure 1.7), which is the most abundant monophosphorylated derivative of PtdIns **159**.[116] PtdIns4*P* **11** was originally regarded as a pivotal synthetic precursor to both PtdIns(3,4)P_2 **161** and PtdIns(3,4,5)P_3 **164** as well as PtdIns(4,5)P_2 **163** a major intermediate in the PKC signalling pathway (Section 1.3.1). However, PtdIns4*P* **11** was subsequently reported to have unique cellular roles of its own, as part of the Golgi membranes,[149-151] but also outside of the Golgi complex.[111,112,152]

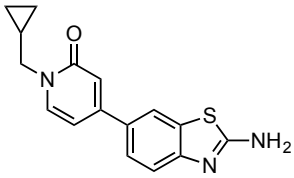
Four PtdIns4Ks (PtdIns4KIIα, PtdIns4KIIβ, PtdIns4KIIIα and PtdIns4KIIIβ) have been identified in mammalian cells.[153,154] Type II and type III PtdIns4K, which can be distinguished by their sensitivity to the inhibitors adenosine **167** and wortmannin **168** (Figure 1.10), respectively, are further subdivided into isoforms α and β. Each of these PtdIns4K isozyme were shown to localise in distinct sub-cellular distributions and to have specific cellular roles.[126,154] PtdIns4IIα, for example, is involved in vesicle trafficking and PtdIns4IIIβ in membrane transport.[102,126] The regulation of these kinases, however, is still unclear.[110]



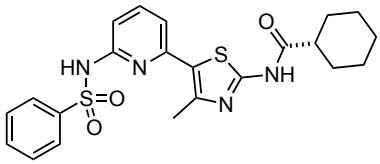
adenosine **167**



wortmannin **168**



169



170

Figure 1.10 Structures of adenosine **167** and wortmannin **168** as well as **169** and **170**, the two PtdIns4KIIIα and PtdIns4KIIIβ selective inhibitors developed by AstraZeneca.[157]

The different PtdIns4K isotypes were shown to be implicated in important human diseases, such as Alzheimer's disease, Gaucher's disease, and RNA viruses.[155,156] In addition, pharmacological control of PtdIns4*P* levels is currently challenging due to the lack of potency and non-specificity of known PtsIns4K inhibitors. Research is therefore focused on understanding the individual cellular roles and location of each PtdIns4K isozyme.[154]

In this context, AstraZeneca recently developed two chemical probes **169** and **170** (Figure 1.10), which were found to be potent, selective inhibitors of each isoform of PtdIns4KIII (Table 1.1).[157] First, the effect of these probes on the cellular concentration of Ins1*P* **9** was investigated. Stimulation of cells with platelet-derived growth factor (PDGF) activates PLC. This activation leads to the hydrolysis of PtdIns(4,5)P_2 **163**, generating Ins(1,4,5)P_3 **10** and subsequently Ins1*P* **9** downstream.

The PI4KIIIα probe **169**, was shown to inhibit the accumulation of Ins1*P* (pIC_{50} 6.3). With the PI4KIIIβ probe **170**, however, inhibition was not observed (Table 1.1).

Table 1.1 Potency and selectivity of inhibitors **169** and **170**, and their effect on the concentration of various signaling molecules involved in the PtdIns(4,5)P_2 pathway.[157]

Entry	PI4KIIIα pIC_{50}[a]	PI4KIIIβ pIC_{50}	Ins1*P* 9 pIC_{50}	PtdIns*P*[b]	PtdInsP_2[b]	PtdInsP_3[b]	PtdIns(4,5)P_2 (basal)[c] [%]
169	8.2	5.9	6.3	+	+	+	19
170	5.1	7.8	< 4.5	–	–	–	0

[a] pIC50 = –log(IC50). IC50 is defined as the molar concentration of the inhibitor required for 50% inhibition [b] Inhibition at 30 μM, cells (NIH3T3 cell line engineered to overexpress the PDGF receptor) treated for 2 h in growth media prior to PDGF stimulation [c] Levels detected in unstimulated cells, inhibition at 30 μM.

Second, the effect of both inhibitors on the cellular concentrations of PtdIns*P*, PtdInsP_2 and PtdInsP_3 was determined qualitatively using methodology based on phosphate methylation coupled to high-performance liquid chromatography-mass spectrometry (HPLC-MS).[158] These studies showed that the PI4KIIIα inhibitor **169** caused a clear reduction in the whole cell levels of PtdIns*P*, PtdInsP_2, and PtdInsP_3. The PI4KIIIβ inhibitor **170**, however, showed no effect (Table 1.1).[157] Finally, using a fluorescence-based method,[159] the PI4KIIIα probe **169** was shown to inhibit the formation of basal PtdIns(4,5)P_2 at the membrane, while **170** did not (Table 1.1).

It was postulated that the PI4KIIIβ inhibitor **170** was not reaching the target kinase as a result of its sub-cellular location. Since the mass spectrometry technique employed in this study was not isomer specific, biological probes based on naturally occurring PtdIns4*P*, and PtdIns(4,5)P_2, which are distinguishable by mass spectrometry, were required to investigate this further.

1.3.3. Understanding the role of PtdIns5*P* in the nucleus

We established a collaboration with Professor Wolfgang Fischle's laboratory (King Abdullah University of Science and Technology, KAUST, Saudi Arabia), to further understand the roles of PtdInsP_n in nuclear signalling events and chromatin remodelling.[160-163] We are interested in PtdIns5*P* derivatives in particular.

PtdIns5*P* **12** is the least characterised of the phosphatidylinositol mono-phosphates, and its biological roles are still unclear.[164] Despite this, PtdIns5*P* derivatives were demonstrated as being crucial in

mediating nuclear stress responses to DNA damage. Indeed, in response to stress the p38 mitogen-activated protein kinase (MAPK) is translocated into the nucleus and inhibits PtdIns*P*5Kβ, leading to a concurrent increase in the levels of nuclear PtdIns5*P*.[165] PtdIns5*P* was then shown to interact with the H3K4me3-binding plant homeodomain (PHD) finger[166] and the adjacent polybasic region of the chromatin-associated inhibitor of growth protein 2 (ING2),[58,167] triggering responses linked the modulation of gene transcription.[168] This includes, for example, apoptosis through acetylation and activation of the tumour suppressor p53. This work represented the first example of a protein domain interacting with PtdIns5*P*. Subsequently, the association of a number of related proteins with chromatin was found to be modulated by PtdIns5*P*. More particularly, both the TAT binding protein associated factor 3 (TAF3)[169] and *Arabidopsis* homolog of trithorax 1 (ATX1)[170] bind PtdIns5*P* and have important roles in transcriptional regulation.

In their recent work on the ubiquitin-like with PHD and RING finger domains 1 (UHRF1), a protein which is involved in maintaining DNA methylation patterns,[171] Fischle *et al*. uncovered a new mode of action for PtsIns5*P*.[172] The propagation of DNA methylation patterns is key to epigenetic inheritance. DNA methyltransferase 1 (DNMT1) copies the methylation pattern during semiconservative replication. During this process, multiple domains of UHRF1 work in concert to enable the correct targeting of DNMT1.[171] Work by Fischle *et al*. suggests that PtdIns5*P* acts as a cofactor that binds UHRF1 allosterically[173,174] and modulates its association with chromatin.[175] The precise mode of binding of PtdIns5*P* and its specific role, however, is still unknown. In addition, it is unclear which species of PtdIns5*P* is preferred in this interaction.

Synthetic PtdIns5*P*s with differing diacylglycerol backbones, as well as biological probes based on this natural signalling molecule are required to further understand whether PtdIns5*P*s does indeed alter UHRF1 chromatin binding.

1.3.4. PtdInsP_n probes in the literature

New synthetic strategies towards PtdInsP_ns have not only provided biologists with synthetic standards, but have led to the development of functionalised derivatives that have proven invaluable

in gaining a better insight into the complex biological activity and sub-cellular localisation of these natural products.[14,15,176]

The synthesis of PtdInsP_ns derivatives containing a range of reporter groups, such as fluorescent tags, have been reported for probing the activity of PtdInsP_ns.[14,176] In addition, photoaffinity-based probes were designed to identify relevant PtdInsP_n binding partners.[16,177]

Photoaffinity probes usually involve a photoreactive moiety that covalently binds to the target protein upon activation with light; and a reporter tag allowing for the detection or isolation of the protein(s) covalently linked to the probe. A range of reporter tags including fluorescent dyes, radioisotopes or biotin (for specific biotin-avidin binding) have been employed for this purpose in the literature.[177]

Prestwich *et al*. developed a series of PtdIns(4,5)P_2, PtdIns(3,4,5)P_3, PtdIns(3,4)P_2, and PtdIns(3)P, tritium labelled photoaffinity probes, such as **171** and **172** (Figure 1.11), containing benzophenone

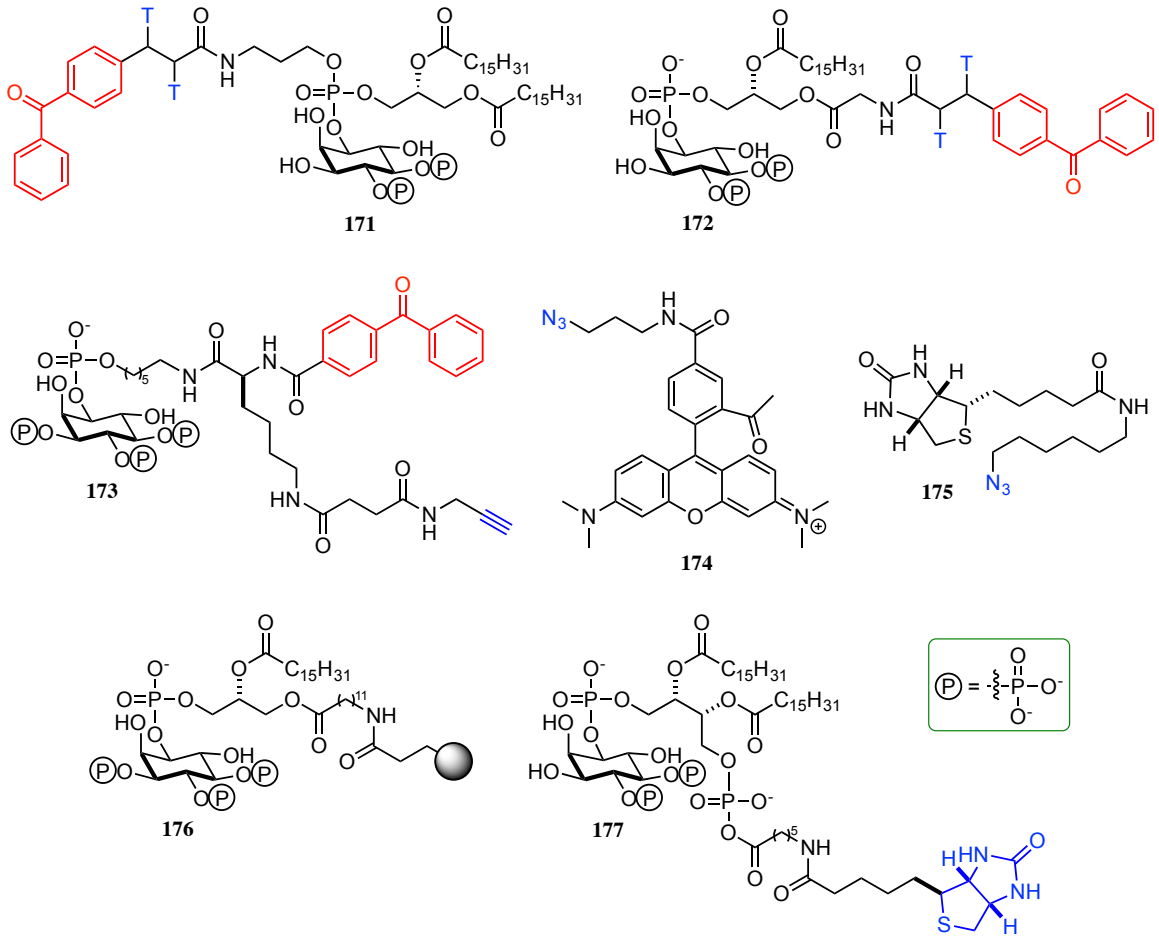


Figure 1.11 Examples of PtdInsP_n probes employed in the literature to understand the biological roles and identify binding partners of various PtdInsP_n.[15,69,178-184]

either on the lipid moiety[178] or on the phosphotriester.[32,179,180] Using these probes in parallel, Prestwich *et al*. were able to show that both the *myo*-inositol headgroup and the diacylglycerol moiety of these molecule are essential for binding the protein profilin.[181]

More recently, Best *et al*. reported the synthesis of a bifunctional PtdIns(3,4,5)P_3 activity probe **173** (Figure 1.11) containing a benzophenone photoaffinity group and a secondary alkyne tag for analysis.[182] This bioorthogonal alkyne tag enabled introduction of rhodamine **174** or biotin **175** *via* click chemistry. Incorporating this tag at a later stage was advantageous as it reduced the overall bulk of the probe. From this study, 265 known and novel PtdIns(3,4,5)P_3 binding proteins were identified. Finally, the synthesis of immobilised PtdInsP_ns, such as immobilised PtdIns(3,4,5)P_3 **176** (Figure 1.11), coupled with affinity chromatography, has enabled the identification of numerous proteins that bind to PtdInsP_n *myo*-inositol headgroups.[15,69]

The probes discussed mostly involve a modification or tethering of the diacylglycerol chain on PtdInsP_ns. The reporter group is thus remote from the *myo*-inositol headgroup, which is known to be the binding site for many PtdInsP_n-binding proteins. Nevertheless, these derivatives have been shown to lose some of their hydrophobic character, interfering with the ability of the diacylglycerol moiety to anchor into lipid bilayers.[16] In addition, as emphasised in Section 1.3.1, the nature of the lipid chains was found to be crucial to the biological activity of PtdInsP_ns. Although Prestwich's phosphotriester probes[183] and 'two-headed' hybrid phospholipids (**177**, Figure 1.11)[184] were a step forwards towards understand the complete biological activity of PtdInsP_n, these probes still feature substantial modification of the PtdInsP_ns framework.

1.3.5. Deuterated PtdInsP_n probes

The main advantage of using isotopically labelled probes is that the alteration to the original framework is structurally minimal. The physicochemical and biological properties of the molecule of interest are therefore, often, mostly retained. A few isotopically labelled PtdInsP_n have been employed in the literature. Klarlund *et al*. identified the general receptor of phosphoinositides 1 (GRP1) as a PtdIns(3,4,5)P_3-binding protein with ^{32}P-labelled PtdInsP_ns.[185,186] ^{11}C and ^{18}F-labelled diacylglycerol were also used to understand aspects of phosphoinositide metabolism.[187,188] These

radioisotopes, however, possess short half lives (~14 days for ^{32}P, ~20 minutes for ^{11}C, and ~110 minutes for ^{18}F) and are difficult to manipulate.[189] In comparison, ^{2}H and ^{13}C are stable isotopes with properties similar to their naturally abundant counterparts. Such labelled PtdInsP_n probes would therefore be stable and observable by mass spectrometry.[190] Although ^{13}C and ^{2}H labelled glucose **181** and **182** are both commercially available, previously developed synthetic strategies starting from chiral pool materials, and which were known to us at the beginning of this project, are lengthy and low yielding (Section 1.2.1). We reasoned, therefore, that the use of *myo*-inositol **1** as a starting material (Sections 1.2.3 and 1.2.4) would be preferable. Considering the high cost of labelled starting materials, Dr Alex Saunders in the group, developed an efficient, large scale and low-cost synthesis of D_6 *myo*-inositol **183**.[47] This synthesis was achieved using well established, rapid and cost effective steps involving the direct exchange of hydrogen atoms with deuterium.[191] This material could thus be used as a starting material for the synthesis of probes which would mimic endogenous PtdInsP_n more closely than those presented in Figure 1.11. For these probes to be distinguishable from their protonated counterparts by mass spectrometry, a minimum of four deuterium atoms needed to be incorporated. These could feature on the lipid moiety and/or on the *myo*-inositol headgroup (Figure 1.12). Such deuterated probes will consequently be directly applicable as internal standards for the quantification of PtdIns*P*s by mass spectrometry.[108,192] Studying metabolic pathways *in vitro* or *in vivo*, however, will require the additional consideration that, in certain cases, the metabolic profile of such deuterated derivatives can be altered as compared to the natural, protonated analogue.[190,193] Indeed, as a result of the greater atomic mass of deuterium (~2.0 AMU), the C–D bond is stronger and more stable than the corresponding C–H bond. C–D bonds therefore have a lower vibrational frequency and consequently a lower zero-point energy than C–H bonds.[194] The slower rate for C–D bond cleavage that results, is described as the primary kinetic isotope effect (KIE, k_H/k_D). Although the probes designed feature deuterium around the *myo*-inositol ring, which is removed from the principal site(s) of metabolism, secondary kinetic isotope effects may be observed. Considering these effects are difficult to predict, it may be necessary, therefore, to conduct additional experiments comparing the *in vitro* metabolism of deuterated and protonated PtdInsP_n analogues.

To answer the biological questions outlined in sections 1.3.2 and 1.3.3, we aimed to synthesise the deuterated PtdIns4*P*, PtdIns5*P*, and PtsIns4,5P_2[47] probes **178–180**, and derivatives thereof, containing the natural 1-stearoyl-2-arachidonyl glycerol backbone (Figure 1.12). Chapters 2 and 3 of this dissertation outline the development of a robust, modular and high yielding route towards enantiomerically pure, deuterated PtdIns4*P* and PtdIns5*P*.

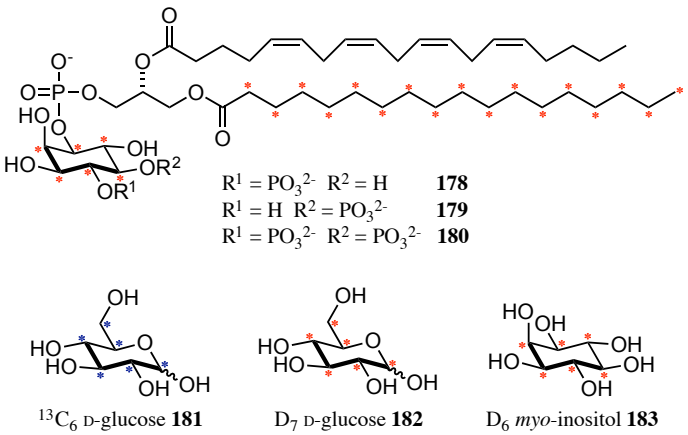


Figure 1.12 The deuterated probes **178–180** designed, containing deuterium around the *myo*-inositol ring and on the diacylglycerol moiety. D_6 *myo*-inositol was chosen as a starting material for the synthesis of these deuterated probes over more expensive ^{13}C and ^{2}H labelled glucose. Dark blue asterisk: ^{13}C; red asterisk: ^{2}H.

1.4. Rhizopines, rare *scyllo*-inositol derivatives with a huge potential

1.4.1. The Rhizosphere

The rhizosphere is a narrow zone of soil surrounding plant roots, comprising a complex and dynamic ecosystem populated by diverse microorganisms and invertebrates. The plant and the rhizomicrobiome communicate and influence each other through the mutual release and detection of signalling molecules, eliciting biological responses.[195] In particular, primary (amino acids and carbohydrates) and secondary (alkaloids and terpenoids) metabolites are released by the plants roots to attract beneficial microorganisms and repel pathogens.[196] As such, organisms present in the rhizosphere directly impact on the growth, nutrition and defence of the plant.[197]

Recent synthetic biology advances currently enable the engineering of rationally designed microorganisms, which could be employed to promote beneficial plant-microbe interactions in the

natural system.[198] Such advances in biotechnology will be essential for addressing crop productivity issues while ensuring the implementation of sustainable agriculture.

1.4.2. Engineering a unique *trans*-kingdom signal from plants to bacteria

In collaboration with Professor Poole's group (Plant Sciences Department, University of Oxford) we set out to engineer the production of a unique *trans*-kingdom signal[I] from plants to bacteria. Plants would release a chemical signal into the rhizosphere, which would lead to the activation of specific gene cassettes in engineered bacteria. This targeted bacterial response would bias the rhizomicrobiome[II] to a chosen phenotype that would promote the growth or survival of a specific plant. This could involve providing essential resources to the plant, such as nitrogen, phosphorus and minerals, modulating hormone production or inhibiting the growth of plant pathogens.[199,200] Such engineered systems would be significant for the progress of agriculture.

Therefore, we aim to engineer the biosynthesis of a signalling metabolite in plants. Considering the diversity of microorganism in the rhizosphere, the competition existing between them, and the complexity of signalling events between plants and rhizomicrobiome,[195] it is important that this signal should be heterogeneous, *i.e.* recognised solely by the engineered bacteria. Common signalling molecules such as carbohydrates, amino acids, alkaloids, and terpenoids can therefore not be employed for this purpose. The added advantage of such a heterogeneous signal, is the 'locking in' of the interaction, avoiding undesired signalling with other plants in the environments. Besides, it is important that: a. engineering of the metabolite's biosynthetic pathway is facile, b. the signal is easily exuded from the plant roots into the rhizosphere, c. the signal can be perceived by the bacteria, triggering a response.

The rhizopines *scyllo*-inosamine **13** (SIA) and 3-*O*-methyl-*scyllo*-inosamine **14** (3-*O*-MSI) were recognised as ideal signals (Figure 1.13). The literature suggests that these rare aminocyclitol compounds, first isolated from the nodules of the alfalfa legume plant *Medicago sativa*,[201-203] would fulfil the requirements outlined above.

[I] *Trans*-kingdom signal describes the signaling between plants and microorganisms through chemical compounds
[II] The rhizomicrobiome describes the community of microorganisms present in the rhizosphere.



SIA **13** (±)-3-*O*-MSI (±)-**14**

Figure 1.14 Structure of rhizopines **13** and (±)-**14**. These aminocyclitol compounds were isolated from *M. sativa* and subsequently shown to be synthesised and catabolised by specific *S. meliloti* and *Rhizobium leguminosarun* bv. *viciae* strains.

Rhizopines **13–14** are completely absent from most plant rhizospheres and were found to exist only in the context of legume-rhizobium symbioses. Legumes release chemical signals that are specifically recognised by strains of beneficial soil sinorhizobia.[III] Signal exchange between the host plant and rhizobia (nod factors) leads to bacterial invasion and curling of the root hair (Figure 1.14, a., b. and c.).[204] This is followed by the formation of infection threads, which extend towards the cortical cells, and that of nodules (Figure 1.14, d., e.).[205-207] Once inside the root nodules, rhizobia are differentiated into non-reproductive bacteroids, which live in symbiosis with the plant.[208-210] In this association, the bacteroid fix nitrogen by converting atmospheric nitrogen into an ammonium salt which legumes can use to grow. In exchange, the bacteroid thrives on nutrients provided by the plant.[211]

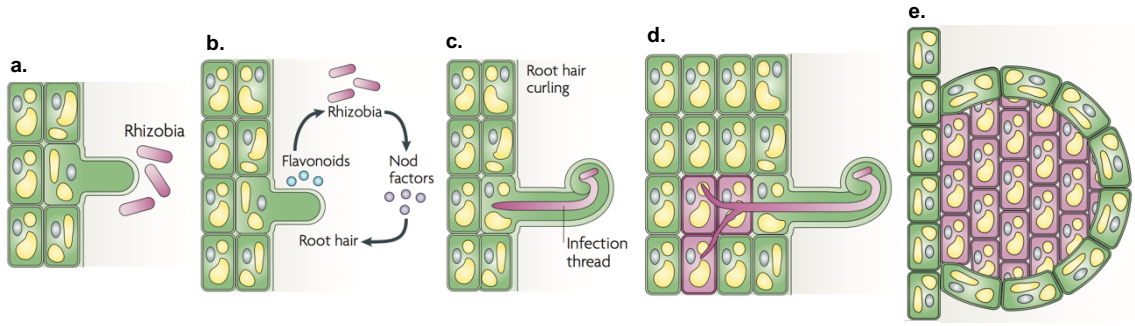


Figure 1.13 Formation of root nodules a. Free-living rhizobia is naturally present in the rhizosphere b. Rhizobia is attracted to a compatible plant host through chemical signals c. Invasion of the root hairs by Rhizobia leads to root hair curling and subsequent formation of an infection threat d. The infection threat extends towards the root's cortical cells e. The infection threads extend to form a nodule. Adapted by permission from Macmillan Publishers Ltd: Nature Reviews Microbiology,[201] copyright 2009 (Appendix C).

Within this context, SIA **13** and 3-O-MSI **14** (Figure 1.13) are thought to be synthesised by nodule bacteroid and catabolised only by the corresponding undifferentiated free-living rhizobial strain.[202,203,212,213] Murphy *et al*. described this as the 'rhizopine concept'.[214] The hypothesis at the core

[III] Nitrogen fixing bacteria

of this concept is that rhizopines synthesised by nodule bacteroid would be catabolised by free-living rhizobia in the nodule, but would also be exuded into the rhizosphere and used as a nutrient (carbon and nitrogen source) only by strains able to catabolise it.215 Indeed, only a small portion of *S. meliloti* (~10%) and *Rhizobium leguminosarun* bv. *viciae* (~12%) strains can synthesise and catabolise rhizopines 13–14.215 These have a competitive advantage for nodulation over mutant strains unable to catabolise rhizopines.[216-218]

In contrast with other plant secondary metabolites, rhizopines **13–14** are synthesised and catabolised by highly specific strains of rhizobia, and are therefore absent from most plant rhizosphere. Besides, the 'rhizopine concept' suggests that rhizopines are exuded out of the legume nodules into the rhizosphere. Finally, the interest for rhizopines in the context of nitrogen fixing symbiosis means that a wealth of literature exists, focusing on the biosynthetic and catabolism pathways of these aminocyclitols. Indeed, the *moc* and *mos* gene loci required for rhizopine catabolism and synthesis were characterised by Murphy *et al*.[202] This provided Dr Barney Geddes with a good platform for engineering rhizopine synthesis in plants.

Synthetic rhizopines are essential to achieving these goals, for use as standards in biochemical studies. In addition, rhizopines are particularly intriguing for organic chemists, as the naturally active enantiomer of 3-*O*-MSI **14** has never been directly identified. Chapter 4 of this dissertation therefore details the development of a synthetic route towards SIA **13** and both enantiomers of 3-*O*-MSI (+)-1D-**14** and (–)-1L-**14**. In addition, the identification of the natural enantiomer of 3-*O*-MSI **14** and the determination of the natural rhizopine biosynthetic pathway is described.

2 Synthesis of PtdIns4*P via* a diastereoisomeric resolution

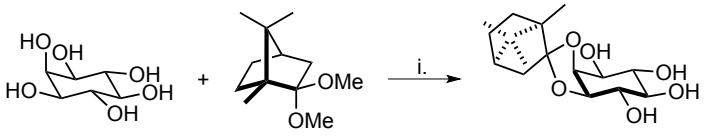
2.1. Aims and introduction

Our research was aimed at answering the biological questions outlined in Chapter 1, through the synthesis of enantiomerically pure, deuterated PtdInsP_n probes **178–180** (Figure 1.12). As organic chemists we were first interested in exploring the various enantioselective methodologies previously developed in the literature for the synthesis of PtdInsP_ns. These include both resolution strategies, that are routinely employed in the literature, and novel enantioselective desymmetrisation strategies. In addition, we sought to develop a synthetic route that fulfilled the following set of requirements:

- Building on previous literature work, the route was to be short, efficient and modular, accommodating for the synthesis of both PtdIns4*P* **11** and PtdIns5*P* **12** derivatives.
- The protecting group strategy developed was to be amenable to the synthesis of natural PtdInsP_ns, containing unsaturated lipid chains such as arachidonic acid (Section 1.3.1, Figure 1.7).
- It was important that this route should be applicable to the synthesis of deuterated PtdIns*P* probes **178–179**, containing deuterium both on the *myo*-inositol core and lipid chains (Section 1.3.5, Figure 1.12).

As outlined in Section 1.4 regio- and enantiocontrol are the two main concerns when designing a synthesis of *myo*-inositol derivatives. This chapter focuses on the synthesis of PtdIns4*P* to explore regiocontrol and to develop novel protecting group strategies.

Amongst the strategies (Section 1.4) that have emerged for the synthesis of PtdIns*P*s, protecting group manipulation on the *myo*-inositol core itself coupled with diastereoisomeric resolution, has been most commonly used in the literature.[65,69,72,77,219,220] In particular, Bruzik *et al*. showed in their pioneering work that with its quaternary, non-enolisable, stereogenic centre camphor dimethyl acetal (+)-**100** can function both as a diol protecting group and a chiral auxiliary for *myo*-inositol **1** (Scheme 2.1).[23,221]



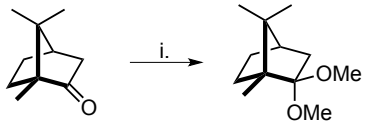
1 (+)-**100** (+)-**101a**

Scheme 2.1 Bruzik's diastereoisomeric resolution using camphor dimethyl acetal as a diol protecting group and a chiral auxiliary. *Reagents and conditions*: i. a. H_2SO_4, DMSO, 70 °C, 3 h b. PTSA·H_2O, $CHCl_3$/MeOH/H_2O (50:5:1), RT, 1.5 h, 25%. Me = methyl.[23, 217]

Initially, designing a synthetic strategy based on Bruzik's work was appealing as this optical resolution is well established in the literature.[23] Adopting this methodology allowed the design of a synthetic route towards unsaturated PtdIns4*P*, which focused on understanding the intricate regioselectivity of the *myo*-inositol framework and on optimising relevant protecting group strategies. This work, therefore, lays the foundation for Chapter 3, in which the optimisation of a desymmetrisation strategy is undertaken.

2.2. Protection-resolution of *myo*-inositol using 1-(*R*)-(+)-camphor dimethyl acetal

First, the chiral auxiliary 1-(*R*)-(+)-camphor dimethyl acetal (+)-**100** was prepared by stirring 1-(*R*)-(+)-camphor (+)-**184** and trimethyl orthoformate for three days, with K-10 Montmorillonite aluminium pillared clay, as the acid catalyst (Scheme 2.2).[222] A mixture of the desired acetal product

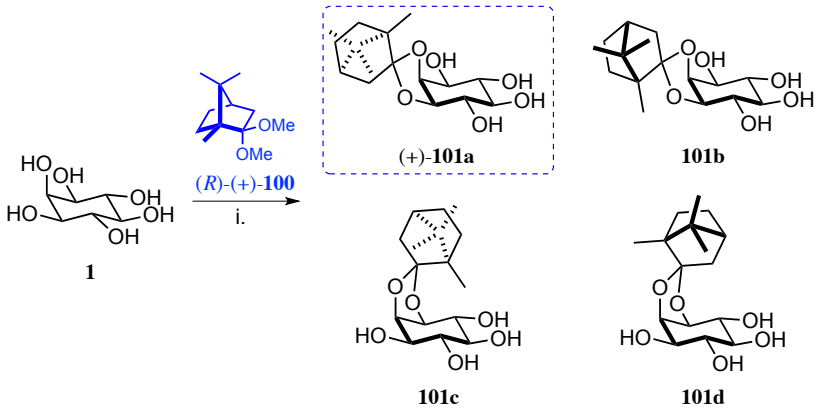


(+)-**184** (+)-**100**

Scheme 2.2 Synthesis of the chiral auxiliary 1-(*R*)-(+)-camphor dimethyl acetal (+)-**100**. *Reagents and conditions*: i. $CH(OMe)_3$, K-10 Montmorillonite clay, hexane, RT, 72 h, 94%. Me = methyl.

(+)-**100** (90–95%) and unreacted 1-(*R*)-(+)-camphor (+)-**184** (5–10%) was obtained, as observed by ^{1}H NMR.

This mixture was then used without further purification in the acid-catalysed (H_2SO_4) acetalisation of the vicinal 2,3-diol of *myo*-inositol **1**, using a modification of the procedure originally developed by Bruzik *et al*.[56,221,223] The reaction mixture was stirred for 3 hours at 70 °C, to give a complex mixture of regio- and stereoisomers of mono-, bis-, *cis* and *trans myo*-inositol camphor acetals. Taking advantage of the greater stability of *cis* 1,2- and 2,3-acetals,[224] mild hydrolysis of the *trans* acetals using 4-toluenesulfonic acid monohydrate (PTSA·H_2O)[225] in $CHCl_3$/MeOH/H_2O (50:5:1), afforded a mixture of four diastereoisomers **101a–d** (Scheme 2.3).[IV, 55-57] These diastereoisomers arise both from the configurations of the *myo*-inositol ring (1D-2,3-*O*- and 1L-2,3-*O*-) and the *endo* or *exo* geometry of the quaternary carbon present on the camphor acetal protecting group (Scheme 2.3). A PTSA-mediated equilibration of the crude mixture then led to the selective precipitation of the favoured, desired diastereoisomer (+)-**101a**. This solid was collected by filtration, but was contaminated with residual *myo*-inositol **1**. We found that sequential washings with $CHCl_3$ and H_2O followed by trituration with MeOH furnished pure tetrol (+)-**101a** in 23% yield.



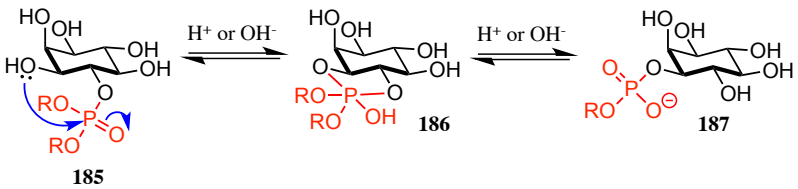
Scheme 2.3 As described by Bruzik *et al*.,[23,55-57,217] protection of *myo*-inositol **1** with the chiral auxiliary 1-(*R*)-(+)-camphor dimethyl acetal (+)-**100**, followed by partial hydrolysis, generated four diastereoisomers **101a–d**. Diastereoisomer (+)-**101a** was selectively precipitated. *Reagents and conditions*: i. a. H_2SO_4, DMSO, 70 °C, 3 h b. PTSA·H_2O, $CHCl_3$/MeOH/H_2O (50:5:1), RT 18 h, 23% (+)-**101a**. Me = methyl.

[IV] The structures of these diastereoisomers were confirmed initially by Bruzik *et al*.[55-57] through NMR and X-ray crystallographic studies. Further studies and isolation of the individual diastereoisomers was then later carried out by Wewers *et al*.[57]

The protected and resolved tetrol (+)-**101a** obtained through this strategy is known to be a versatile optically pure building block, useful in the synthesis of InsP_ns[56,226], PtdInsP_ns[23,227] and various inositol or sugar derivatives.[223,228,229] Similar to other cyclic acetal protected inositols,[230-233] this framework is amenable to a series of regioselective silylation, alkylation, and acylation protection reactions at the neighbouring 1- and 4-positions.[23] The key to designing an efficient synthesis towards unsaturated PtdIns4*P* **11** and PtdIns5*P* **12**, therefore, lay in the choice of orthogonal protecting groups for this system.

2.3. Protecting group strategy and retrosynthesis

One of the most important considerations when designing a synthetic route towards PtdInsP_n derivatives is the nature of the hydroxyl and phosphate protecting groups employed, and their compatibility with the final global deprotection (Figure 2.1). The challenging nature of this deprotection is closely linked to the amphiphilicity and inherent instability of the final PtdInsP_ns. All protecting groups must be removed cleanly, without any degradation or side reactions, as purification of the unprotected lipids is usually best avoided. Notably, as for RNA synthesis,[234,235] migration of protected phosphate esters, such as in **185**, adjacent to free hydroxyls groups, is also a major concern (Scheme 2.4).[62] To circumvent this issue, cleavage of the phosphate esters should be carried out before hydroxyl deprotection. The free phosphates obtained are then less susceptible to migration, although strong basic or acidic conditions should be avoided to limit hydrolysis of the glycerol esters (Figure 2.1).



Scheme 2.4 Phosphate migration is thought to occur *via* a cyclic phosphate ester **186** resulting from the nucleophilic attack of the neighbouring hydroxyl to the phosphotriester on **185**. R= phosphate protecting group.[231]

As such, the stability of the hydroxyl protecting groups present on the inositol ring before global deprotection needs to be finely balanced. They should remain stable throughout the synthesis,

including during protecting group manipulation, phosphorylations, and cleavage of the phosphate esters. They should also be removable under mild conditions in the final step.

Benzyl groups are commonly used for protecting both ring hydroxyls and phosphates on inositol derivatives.[62,64,236] Under hydrogenolysis conditions, deprotection of the benzyl phosphate esters occurs rapidly, allowing for the slower hydroxyl group deprotection to take place without phosphate migration. Furthermore, no subsequent purification of the PtdIns*P* obtained needs to be undertaken. Despite these advantages, hydrogenolysis or other benzyl ether deprotection conditions are not compatible with the polyunsaturated arachidonoyl chain featured in the naturally occuring PtdIns*P*s **11** and **12** (Figure 2.1).

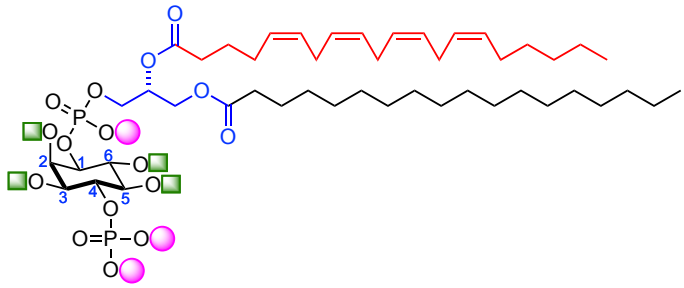
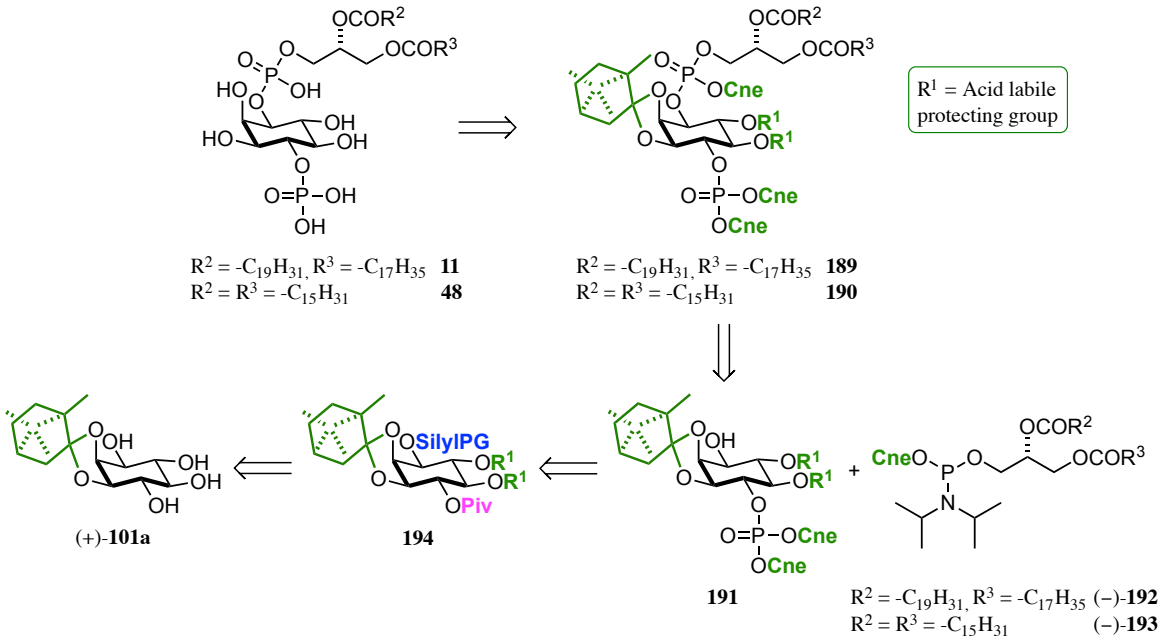


Figure 2.1 Naturally occurring, fully protected PtdIns4*P* before global deprotection. For a successful final deprotection, cleavage of the hydroxyl protecting groups (green squares) around the *myo*-inositol ring should be carried out after deprotection of the phosphate protecting groups (pink circles). Mild conditions should be employed to limit glycerol hydrolysis (blue) and avoid disruption of the polyunsaturated arachidonoyl chain (red).

The base labile phosphate protecting group 2-cyanoethyl (Cne) was therefore selected as an alternative (Scheme 2.5). This protecting group is used extensively in oligonucleotide[237,238] and PtdInsP_n synthesis[77,183,239] for the protection of phosphates. Indeed, in their synthesis of naturally occuring PtdIns(3,4,5)P_3 **164** Gaffney and Reese developed a procedure involving the sequential deprotection of 2-cyanoethyl phosphate esters under basic conditions, followed by that of acid labile groups around the *myo*-inositol ring.[240,241] This was then also later applied to Gaffney's synthesis of 1-stearoyl-2-arachidonoyl PtdIns4*P* **11**[77] and dioleoyl PtdIns(4,5)P_2 **188**.[239]

The choice of the hydroxyl protecting groups on **189** was thus mostly determined by the nature of the 2-cyanoethyl protected phosphates. The 2,3-*O*-camphor acetal, already installed on the starting building block (+)-**101a**, is an easily removable, acid labile protecting group. Its compatibility with the selected global deprotection strategy was advantageous as no extra deprotection-protection steps

had to be undertaken. For blocking of the 5- and 6-positions (R^1 on **189**, **190**, **191**, **194**) it was therefore necessary to investigate various other acid sensitive groups (Scheme 2.5).



Scheme 2.5 Retrosynthesis for naturally-occurring and dipalmitoyl PtdIns4*P* derivatives **11** and **48** starting from optically pure (+)-**101a**. The protecting groups which were to be removed in a final, global deprotection step are highlighted in green (R^1). In blue and pink are the orthogonal protecting groups that allow for the differentiation between the 1- and 4-positions respectively. Cne = cyanoethyl; Piv = pivaloyl; SilylPG = silyl protecting group.

The fully protected lipid precursors **189** and **190** (Scheme 2.5) can be derived from the assembly of two main fragments, the *myo*-inositol core (–)-**191** and the 2-cyanoethyl protected phosphoramidites (–)-**192** or (–)-**193**. For the sake of synthetic simplicity, phosphorylation using the dipalmitoyl (R = $-C_{15}H_{31}$) phosphoramidite **193** was to be attempted first before applying this synthesis to natural, unsaturated derivatives. It is clear that differentiation between the 1- and 4-positions was key to designing an efficient synthesis. We envisioned that a silyl group at the 1-position on **194** would suit the required orthogonality for deprotection in the presence of 2-cyanoethyl groups, as desilylation can be achieved using a source of fluoride.[242] Finally, we anticipated that the sterically congested pivaloyl group would allow for regioselective esterification of the 4-position on (+)-**101a**. Additionally, the base lability of this group would also enable its selective cleavage on **194** (Scheme 2.5).

2.4. Regioselective protection of the 1- and 4-positions of (+)-101a

As established by Bruzik *et al*., the 1- and 4-position hydroxyl groups of (+)-**101a** can be regioselectively protected with orthogonal silyl and acyl groups respectively.[23] Evidence in the literature suggests that bulky silyl protecting groups, such as *tert*-butyldiphenylsilyl (TBDPS), can be placed regioselectively and in high yields at the 1-position of (+)-**101a**. Previous unpublished work by Dr Sonia Diab in the group, however, demonstrated that deprotection of these silyl esters later on in the synthesis was problematic due to their stability to standard desilylation conditions. As a result, the less bulky *tert*-butyldimethylsilyl (TBDMS) group was chosen as an alternative. Reaction of (+)-**101a** with TBDMSCl and imidazole at −10 °C gave (−)-**195** in 57% yield. The regioselectivity of this reaction was unequivocally confirmed using ^{1}H-^{29}Si heteronuclear multiple

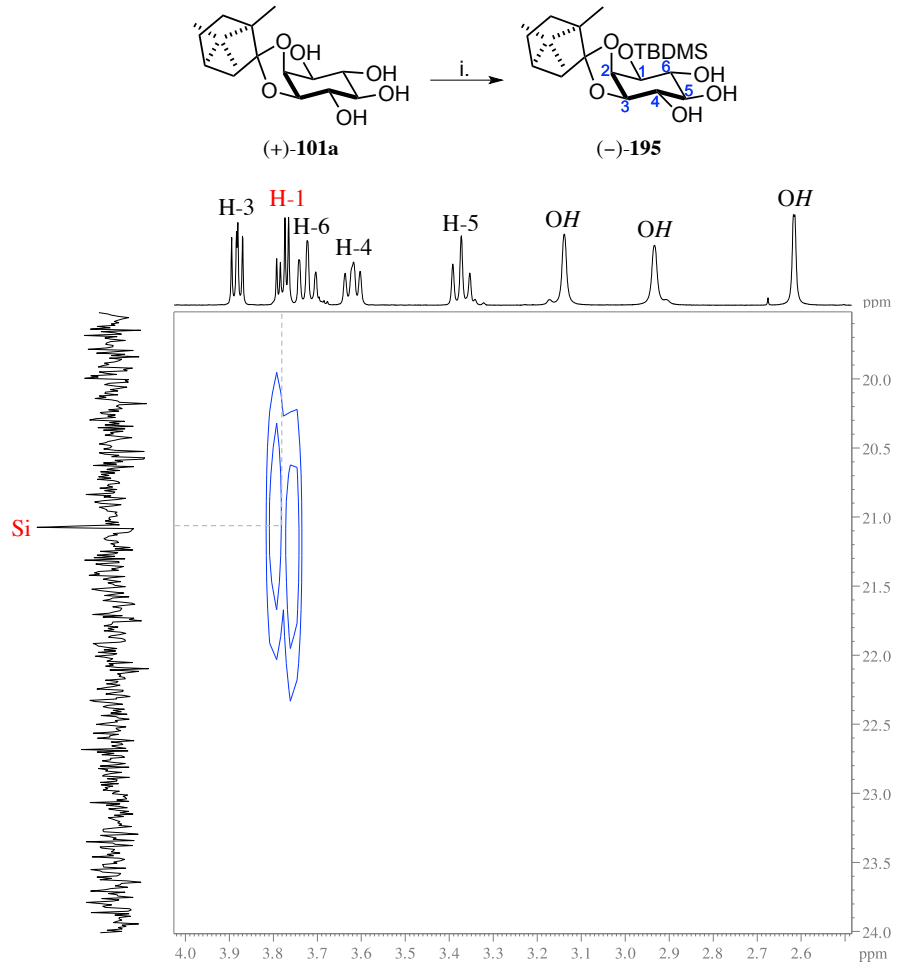


Figure 2.2 Regioselective protection of (+)-**101a** with TBDMS and ^{1}H-^{29}Si HMBC confirming the position of the 1-*O*-TBDMS ether on (−)-**195**. The ^{1}H and ^{29}Si NMR spectra were acquired at 99 MHz and 500 MHz respectively, in $CDCl_3$. *Reagents and conditions*: i. TBDMSCl, imidazole, pyridine, −10 °C, 24 h, 57%. TBDMS = *tert*-butyldimethylsilyl.

bond coherence (HMBC) NMR (Figure 2.2). Following extensive purification by column chromatography, (–)-**195** was found to be only 91% pure by ^{1}H NMR analysis (Appendix A). This compound was contaminated by minor regioisomers which were separated in the subsequent step.

As described in Section 2.3, pivaloyl (Piv) had been chosen as an orthogonal, temporary protecting group for the 4-position of (–)-**195**. Amongst various base labile, acyl protecting groups, pivaloyl is bulky and would therefore fit the criteria for a regioselective protection.[221,226] Moreover, pivalate esters are known to be removable, in the presence of acid labile groups, under a range of reaction conditions.[243] Pivaloylation of (–)-**195** was therefore carried out using pivaloyl chloride (PivCl) in pyridine, at –10 °C, to yield diol (–)-**196** in a 60% yield. Regioselectivity of this transformation was established this time by ^{1}H-^{13}C HMBC NMR (Figure 2.3).

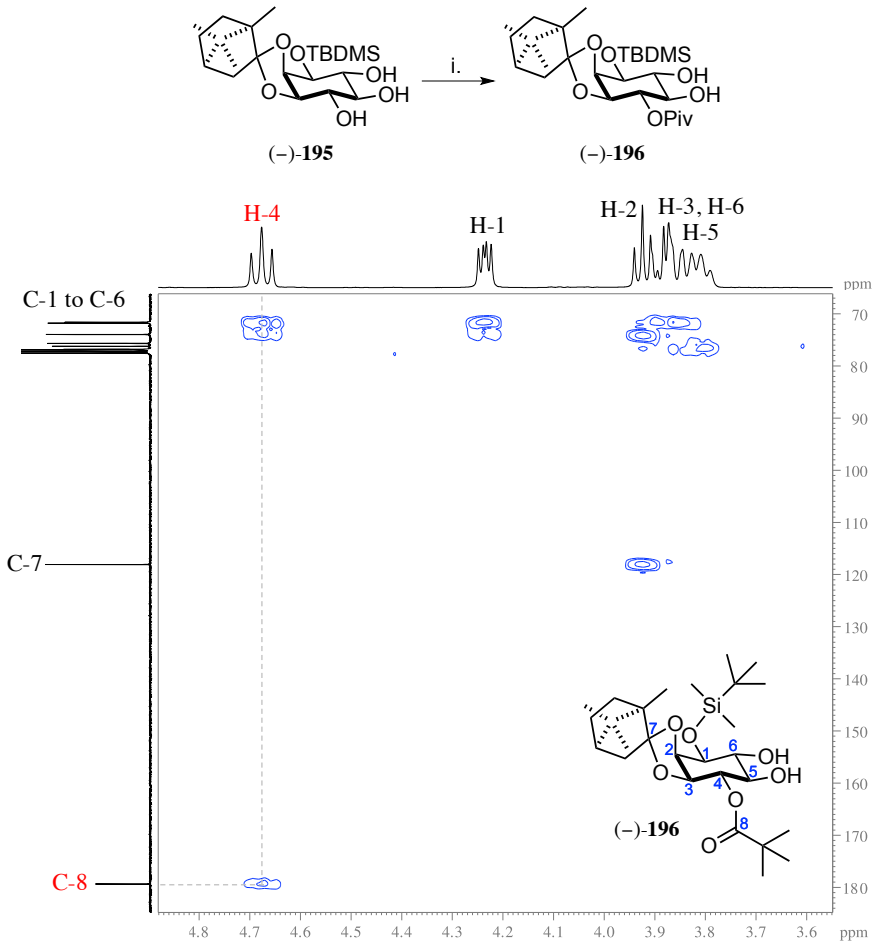


Figure 2.3 Regioselective pivolation of (–)-**195** and ^{1}H-^{13}C HMBC NMR confirming the position of the 4-*O*-pivalate ester on (–)-**196**. The ^{1}H and ^{13}C NMR spectra were acquired at 500 MHz and 12 MHz respectively, in $CDCl_3$. *Reagents and conditions*: i. PivCl, pyridine, –10 °C, 13 h, 60%. TBDMS = *tert*-butyldimethylsilyl; Piv = pivaloyl.

In order to account for the regioselectivity of these transformations, it was essential to think beyond steric factors. Indeed, explaining the enhanced reactivity of the 1-*O* and 4-*O* of (+)-**101a** and (–)-**195** solely through sterics[244] did not seem appropriate. Although it is arguable that steric crowding around the 1-position is alleviated by the neighbouring axial C–O bond, the 4-position does not benefit from such steric relief. Previous work, focusing on carbohydrate regioselectivity, demonstrates that geometrically favourable hydrogen bonding between the hydroxyl protons and the neighbouring ether oxygen of the *cis* acetal is mostly responsible for the enhanced nucleophilicity of the hydroxyl groups observed (Figure 2.4).[245-247] This ether oxygen is possibly a better H-bond acceptor due to the inductive electron donating effect of the camphor group.

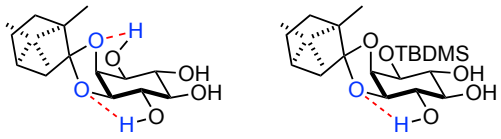


Figure 2.4 Hydrogen bonding interactions between the ether oxygens of the acetal on (+)-**101a** and the neighbouring hydroxyl groups account for the enhanced nucleophilicity of the 1-*O* and 4-*O*. TBDMS = *tert*-butyldimethylsilyl.[241-243]

2.5. Protection of the 5- and 6-position on (–)-196 and pivalate ester cleavage

As described in Section 2.3, it was necessary to block positions 5- and 6- on (–)-**196** with a group that would fulfil the requirements of the final global deprotection. In addition, the conditions used for the installation of this group needed to be tolerated by the remaining protecting groups around the inositol ring. Acetals are ideal acid labile protecting groups for the blocking of two neighbouring hydroxyl groups on polyols. Indeed, simultaneous protection of these positions gives a cyclic acetal, which is entropically favourable. The challenge met with the protection of the 5- and 6-positions on (–)-**196** was their *trans*-diequatorial relationship which would give a particularly unstable cyclic acetal.

Acyclic acetal protecting group such as tetrahydropyran-2-yl (THP), methoxymethyl (MOM) and other related groups (2-methoxyethoxymethyl ether (MEM), benzyloxymethyl acetal (BOM)) have been used in carbohydrate and inositol chemistry for the protection of isolated or neighbouring

equatorial hydroxyl groups (Figure 2.5).[221,242] MOM groups are not suitable in this case as strongly acidic conditions are required for their deprotection. In contrast, the THP group is both stable to a range of reaction conditions, including strong base, and is easily removable with mild acid. The main disadvantage featured by this group is its stereogenic centre, which is known to lead to the undesirable formation of diastereoisomers when protecting stereoisomeric compounds such as those under consideration.[242]

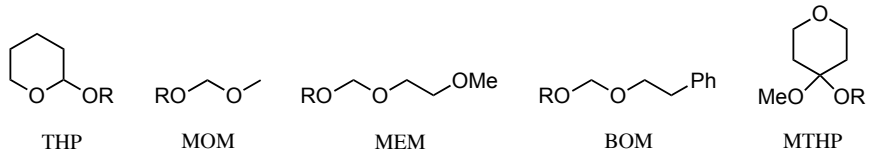
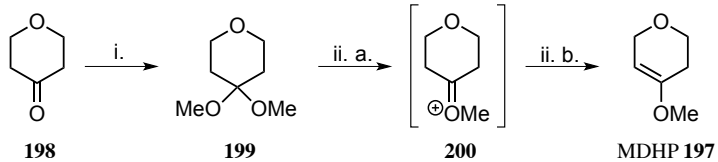


Figure 2.5 Summary of acyclic acetal protecting groups commonly employed in carbohydrate and inositol chemistry. Me = methyl; Ph = phenyl.

The 4-methoxytetrahdropyran (MTHP) group was introduced in the 1960s by Reese *et al*.[248] as an achiral alternative (Figure 2.5).[249] MTHP acetals exhibit a similar range of stability and are cleaved under very mild conditions.[242] As a result, this group has been used frequently by Gaffney *et al*. in their synthesis of PtdInsP_ns.[240,241] Furthermore, the protecting groups on (–)-**196**, including 1-*O*-TBDMS, were found to be stable to triphenylphosphine hydrobromide (PPh_3·HBr),[250,251] the acid catalyst employed for the protection of alcohols with 4-methoxy-5,6-dihydro-2*H*-pyran (MDHP) **197**.[240] Consequently, this group was initially chosen for protecting the 5-*O* and 6-*O* on (–)-**196**.

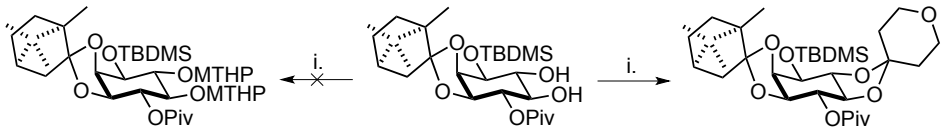
Whilst MTHP acetals present many advantages, the long-standing difficulty in the synthesis and purification of MDHP **197** led Gaffney *et al*. to develop a more efficient synthesis of this reagent (Scheme 2.6).[252] Acetalisation of tetrahydro-4*H*-pyran-4-one **198** using catalytic $TiCl_4$ furnished 4,4-dimethoxytetrahydropyran **199** in 98% purity as determined by ^{1}H NMR analysis.[253] This acetal was then subjected, without further purification, to the conditions developed by Gaffney *et al*. for



Scheme 2.6 Synthesis of the reagent MDHP **197**. *Reagents and conditions*: i. a. $TiCl_4$, CH_2Cl_2, MeOH, RT, 10 min b. Et_3N, RT, 1 h, 82% ii. a. $TiCl_4$, CH_2Cl_2, –78 °C, 1 h b. Pyridine, KOH, –78 °C to RT, 1 h, 82%.[248,249] Me = methyl.

the formation of MDHP **197**.[252] Stoichiometric amounts of $TiCl_4$ at −78 °C generated the presumed oxycarbenium cation **200**. Subsequent addition of excess pyridine and KOH led to the formation of MDHP **197** *via* E1 elimination. The enol ether was obtained in a 82% yield and was free of contaminants including acetal **199** and ketone **198** (Scheme 2.6).

Acetalisation of the 5- and 6-position on (–)-**196** with MDHP **197** was attempted using PPh_3·HBr.[240] After 40 hours of reaction, most of the starting material remained and none of the desired 5,6-di-*O*-MTHP acetal **201** was formed, as judged by mass spectrometry and thin layer chromatography (TLC) analysis. Despite this, mass spectrometry and ^{1}H NMR analysis of the crude reaction mixture did reveal traces of the cyclic acetal **202** (not isolated). This result perhaps suggests that the MTHP groups are too bulky to allow for the masking of both neighbouring 5- and 6-equatorial positions on (–)-**196** (Scheme 2.7).



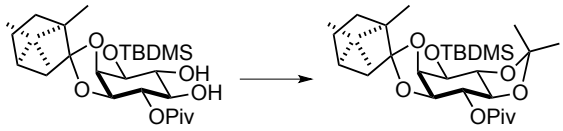
201 (–)-**196** **202**

Scheme 2.7 Acetalisation of the 5-*O* and 6-*O* of (–)-**196** with MDHP **197**. The expected 5,6-di-*O*-MTHP protected inositol **201** was not obtained, however small amounts of the cyclic MTHP acetal **202** was observed by mass spectrometry and ^{1}H NMR analysis. *Reagents and conditions*: i. MDHP **197**, PPh_3·HBr, CH_2Cl_2, RT, 40 h. TBDMS = *tert*-butyldimethylsilyl; Piv = pivaloyl; MTHP = 4-methoxytetrahdropyran.

Encouraged by the formation of a cyclic acetal **202**, acid catalysed transacetalisation of (–)-**196** was attempted using a range of reaction conditions from the literature.[254,255] The expected acetonide **203** was neither observed by mass spectrometry or TLC analysis, nor isolated under any of the reaction conditions outlined in Table 2.1. Whilst the weak acid pyridinium 4-toluenesulfonate (PPTS, pKa ~ 5.2) gave no reaction (Table 2.1, Entry 1), the stronger acid PTSA·H_2O (pKa ~ −3) yielded a complex mixture of products containing deprotected 2,3-*O*- and 1-*O*-derivatives (Table 2.1, Entry 2), but none of the desired acetal.

Having explored acyclic and cyclic acetals for the protection of the 5- and 6-positions of (–)-**196**, without any success, the focus of this investigation shifted towards other protecting groups. Allyl ethers were investigated as they are stable towards a broad range of acid and basic conditions.

Table 2.1 Summary of reaction conditions for the transacetalisation of (–)-**196**.



(–)-**196** **203**

Entry	Reagent (eq)	Catalyst (eq)	Solvent	Time [days]	Yield [%][a]
1	$Me_2C(OMe)_2$ (2.5)	PPTS (0.1)	DMF	6	-[b]
2[c]	$Me_2C(OMe)_2$ (10)	PPTS (0.1)	DMF	5	-[b]
3	$Me_2C(OMe)_2$ (2.0)	PTSA (0.2)	Acetone	1	Complex mixture

[a] Isolated yields [b] None of the product **203** was formed; only the starting material (–)-**196** was isolated [c] This reaction was carried out at 200 mbar. TBDMS = *tert*-butyldimethylsilyl; Piv = pivaloyl

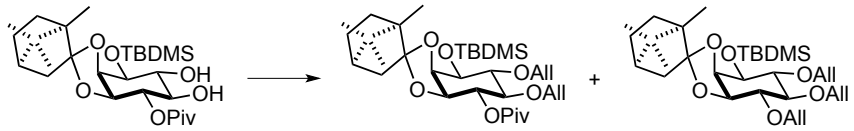
Besides, numerous mild and neutral conditions exist for their deprotection[242] and they have been used for the protection of highly complex systems.[256,257] Allyl ethers are therefore valuable additions to an orthogonal set of protecting groups.

Allyl ethers are commonly deprotected through isomerization of the double bond in conjugation with the oxygen atom of the ether, under mild conditions, followed by hydrolysis.[242] With this in mind, the only concern was whether removal of the allylic ether on framework **190** would be compatible with the polyunsaturated chains in the final global deprotection. Conditions using $PdCl_2$ in MeOH have been developed within the group for the simultaneous deprotection of a camphor acetal and an allyl ether.[68] Palladium catalysts of this type have been employed for various purposes on complex frameworks containing conjugated and skipped alkenes.[258] We hypothesised that these conditions would be applicable to the unsaturated derivatives.

Allylation of (–)-**196** was therefore attempted initially under standard conditions using NaH and allyl bromide in DMF (Table 2.2, Entries 1–2).[259,260] It became quickly apparent that the pivalate ester was not stable to those conditions. We postulated that this occurred as a result of both neighbouring group participation and small amounts of basic alkoxide formed in the reaction. Following work-up and purification by column chromatography, an inseparable mixture of the desired product (–)-**204** and the side product **205** was isolated, as determined by NMR analysis. The order in which the reagents and starting materials were added to the reaction mixture appeared to be important, with a shift from

a 2.9:1.0 to a 12.8:1.0 ratio when NaH was added to (–)-**196** first followed by allyl bromide. Building on this acquired knowledge, cleavage of the pivalate ester and side product formation was limited by adding NaH last and by using only 2.0 equivalents of allyl bromide (Table 2.2, Entry 3). This time, (–)-**204** was isolated free of side product **205** after column chromatography. Despite this, a mixture of other side products was also isolated, accounting for the low yield. The use of catalytic tetrabutylammonium iodide (TBAI) did not improve the isolated yield of this transformation (Table 2.2, Entry 4).

Table 2.2 Summary of reaction conditions for the allylation of (–)-**196**.

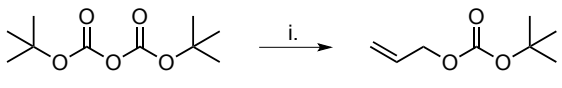


(–)-**196** (–)-**204** **205**

Entry	Reagents [eq][b]	Temperature	Solvent	Time [h]	Ratio of (–)-204/205	Yield of (–)-204[a] [%]
1	(–)-**196** added to NaH (2.2); then AllBr (3.0)	0 °C to RT	DMF	13	2.9:1.0	-
2	NaH (2.2 eq) added to (–)-**196**; then AllBr (3.0)	0 °C to RT	DMF	72	12.8:1.0	-
3	NaH (2.1) added to AllBr (2.0) and (–)-**196**	0 °C to RT	DMF	20	-	5
4	NaH (2.1 eq) added to AllBr (2.0), TBAI (0.10) and (–)-**196**	0 °C to RT	DMF	20	-	7
5	Allyl *tert*-butyl carbonate **207** (5.0), $Pd(PPh_3)_4$ (0.1)	Reflux	THF	4	-	64

[a] Isolated yields [b] Chapter 5: Experimental for more detailed procedures. TBDMS = *tert*-butyldimethylsilyl; Piv = pivaloyl; All = allyl.

In light of these results, we sought milder allylation reaction conditions that would improve the yield of this transformation by eliminating undesired side reactions. Conditions reported by Kraus *et al*.[261] in 2012, describe the selective allylation of hindered alcohols using catalytic $Pd(PPh_3)_4$ and allyl *tert*-butyl carbonate **207**. Synthesis of this allylating agent,[262] following the procedure by Kraus *et*

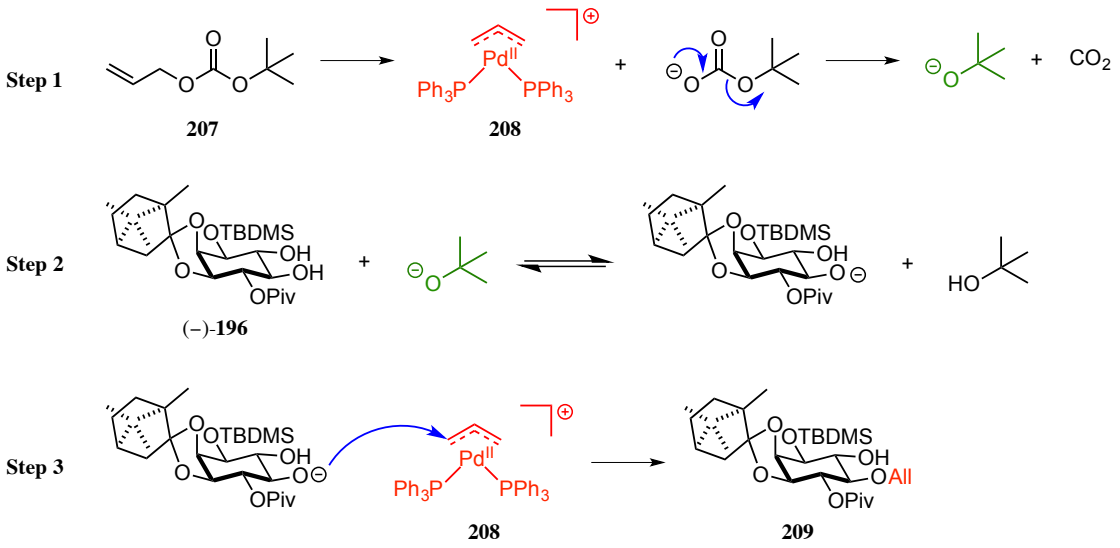


206 **207**

Scheme 2.8 Synthesis of allyl *tert*-butyl carbonate **207** following the procedure developed by Kraus *et al*.[257, 258] *Reagents and conditions*: Allyl alcohol, DMAP, RT, 3 h, 83%.

al., involved reaction of Boc anhydride **206** with excess allyl alcohol and catalytic 4-(dimethylamino)pyridine (DMAP) (Scheme 2.8). When this Pd-catalysed decarboxylative allylation was performed under a strictly inert atmosphere, the fully protected inositol (–)-**204** was isolated in very good yields (Table 2.2, Entry 5).

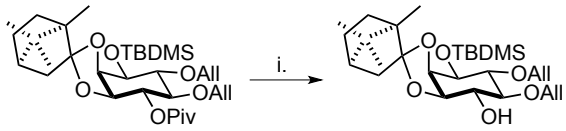
The mechanism for this decarboxylative allylation is divided into three separate steps (Scheme 2.9). First decarboxylation affords the *tert*-butoxide anion (Scheme 2.9, Step 1), which in turn is involved in an acid-base reaction with (–)-**196** (Scheme 2.9, Step 2). Finally, nucleophilic attack by the alkoxide of (–)-**196** onto the Pd η^3 π-allyl complex **208** gives **209** (Scheme 2.9, Step 3). Hence, the success of this allylation relies first on the strict removal of competing nucleophiles such as water, and in the choice of the alkoxide generated. In this case, the bulky *tert*-butoxide anion is slightly less nucleophilic than the alkoxide of (–)-**196**. Nucleophilic competition is therefore reduced during the attack of the π-allyl cation, leading to the preferred formation of **209** (Scheme 2.9, Step 3).



Scheme 2.9 Mechanism for the decarboxylative allylation reaction of (–)-**196** under $Pd(PPh_3)_4$ catalysis.[257] TBDMS = *tert*-butyldimethylsilyl; Piv = pivaloyl; All = allyl.

With the fully protected, differentiated *myo*-inositol derivative (–)-**204** in hand, regioselective deprotection of the 4-*O*-pivalate ester was optimised, leaving the 4-position free for subsequent phosphorylation. Strategically, deprotection and phosphorylation of the 1-*O* position is best left towards the end of the synthesis. This limits the number steps involving intermediates with sensitive saturated and/or unsaturated lipid chains and more importantly, allows for late stage derivatisation.

Pivalate esters are much more stable to hydrolysis than acetates owing to the steric bulk surrounding their carbonyl group. With this in mind, conditions for the unmasking of a pivalate ester in the presence of a TBDMS ether need to be carefully selected. To avoid very basic conditions,[242] diisobutylaluminum hydride (DIBAL-H) has been used frequently in the literature for the selective deprotection of pivalate esters in complex systems.[263-265] As such, reductive cleavage of (–)-**204** was achieved in a 92% yield using 2.5 equivalents of DIBAL-H (1 M in toluene) at –78 °C to give (–)-**210** in 92% yield (Scheme 2.10).



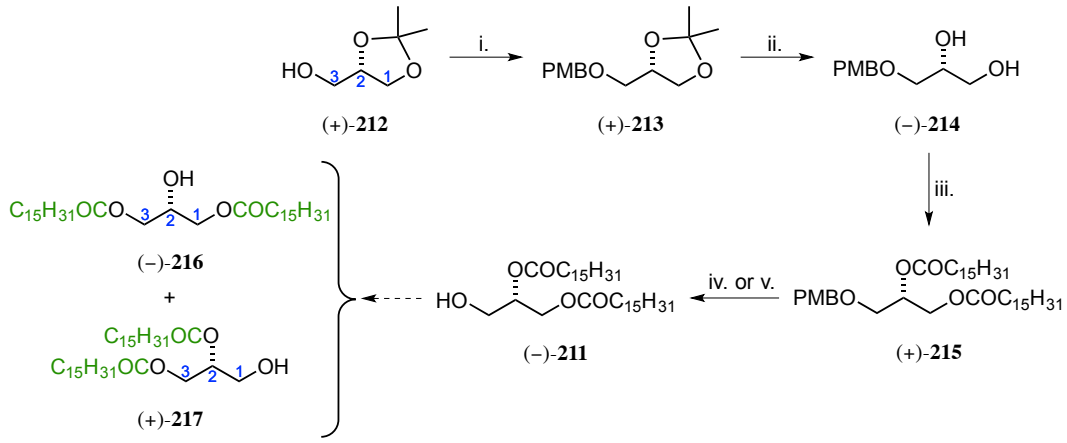
(–)-**204** (–)-**210**

Scheme 2.10 Reductive deprotection of the 4-*O*-pivalate ester on (–)-**204**. *Reagents and conditions*: DIBAL-H, CH_2Cl_2, –78 °C, 4.5 h, 92%. TBDMS = *tert*-butyldimethylsilyl; Piv = pivaloyl; All = allyl.

2.6. Synthesis of (–)-1,2-dipalmitoyl-*sn*-glycerol (–)-211

With the aim to optimise the final stages of the synthesis on a simple saturated system, (–)-1,2-dipalmitoyl-*sn*-glycerol (–)-**211** was prepared according to the reported literature procedures.[64,266] The synthesis convieniently starts with commercially available, enantiomerically pure, (+)-1,2-*O*-isopropylidene-*sn*-glycerol (+)-**212** (Scheme 2.11). Protection of this glycerol had previously been accomplished either with a benzyl[267] or a 4-methoxybenzyl (PMB) group.[64,266] The significance in the choice of this protecting group lies in the conditions used for its cleavage in the last step. As extending this strategy to the synthesis of unsaturated diglyceride derivatives was desirable, the PMB group was selected for its superior lability.

To synthesise (–)-**211**, (+)-1,2-*O*-isopropylidene-*sn*-glycerol (+)-**212** was treated with NaH followed by PMBCl to yield the 3-*O*-PMB-protected acetonide (+)-**213**. Deprotection of the acetonide by acidic methanolysis using substoichiometric PTSA·H_2O provided (–)-**214** (Scheme 2.11). Subsequent acylation of this diol with palmitoyl chloride, 4-DMAP and pyridine generated the desired protected diglyceride (+)-**215** in 91% yield (Scheme 2.11). Difficulties were encountered



Scheme 2.11 Synthesis of (–)-1,2-dipalmitoyl-*sn*-glycerol (–)-**211**. The regioisomers (–)-**216** and (–)-**217** were obtained by silica promoted ester exchange. *Reagents and conditions*: i. a. NaH, DMF, 0 °C to RT, 1 h b. PMBCl, 0 °C to RT, 14 h, 98% ii. PTSA·H_2O, MeOH, RT, 2 h, 81% iii. Palmitoyl chloride, pyridine, DMAP, CH_2Cl_2, 0 °C to RT, 14 h, 91% iv. DDQ, H_2O, CH_2Cl_2, RT, 14 h, 84% v. 10% Pd/C, $H_{2(g)}$, EtOAc, RT, 2 h, 96%. PMB = 4-methoxybenzyl.

when cleavage of the 3-*O*-PMB-ester was attempted. Following the literature procedures, this intermediate was first deprotected under mildly oxidising conditions using 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ).[64] After work-up, however, column chromatography had to be carried out in order to remove any DDQ related contaminants. During this process, silica promoted ester exchange led to the isomerisation of (–)-**211** (migration from the 2-*O* to the 3-*O* to give (–)-**216**, Scheme 2.11) as indicated by thin layer chromatography and ^{1}H NMR. This in turn suggests the possible racemisation of diglyceride (–)-**211** (migration from the 1-*O* to the 3-*O* to give (+)-**217**, Scheme 2.11). Although (–)-**211** could be separated from its regioisomer (–)-**216** through extensive column chromatography, it was not possible to control the amount of concurrent racemisation. This deprotection procedure is therefore not applicable to the synthesis enantiomerically pure glycerol derivatives.

As an alternative, the desired product (–)-1,2-dipalmitoyl-*sn*-glycerol (–)-**211** was obtained through Pd/C-catalysed hydrogenolysis. Purification by column chromatography was not necessary in this case, avoiding migration and racemisation. Indeed, Dr Alex Saunders demonstrated, through α-methoxyphenylacetic acid (MPA) derivatisation and ^{1}H NMR-based methods (See Section 3.6 and 3.15.1 for more detail),[268] that no migration or racemisation occurred during this sequence of steps. The *e.e.* of (–)-**211** determined through this study was shown to be >99%.[47]

Whilst the synthesis of (–)-1,2-dipalmitoyl-*sn*-glycerol (–)-**211** had been accomplished, this synthetic methodology could not be applied later to the synthesis of unsaturated diglyceride derivatives, owing to the incompatible hydrogenolysis step. Further research thus focused on optimising a synthetic route towards these unsaturated diglyceride derivatives (Section 3.15).

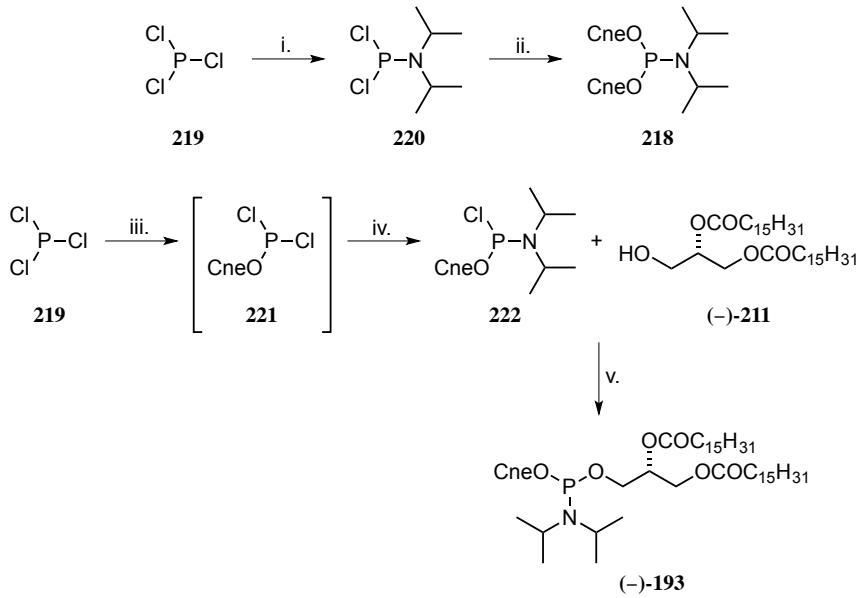
2.7. Synthesis of 2-cyanoethyl protected phosphoramidites 218 and (–)-193

The advancement and development of efficient high yielding synthetic methodologies for oligonucleotide synthesis, has in turn driven the development of organophosphorus chemistry, and in particular hydroxyl group phosphorylation.[269] Within this field of research, the P(III) H-phosphonate and phosphoramidite[270] methodologies have emerged as extremely powerful phosphorylation tools. Consequently, these have been used extensively in the literature for the synthesis of InsP_ns and PtdIndP_ns,[31,64,82,271,272] with some reports of alternative methods.[88,239,273] In particular, phosphoramidite chemistry has been prominent for the synthesis of PtdInsP_ns as the practical difficulties resulting from the instability of the phosphoramidites is outweighed by the high yields obtained from these phosphorylations.[274] This methodology was therefore employed for the phosphorylation of the 4-position on (–)-**210**, and after TBDMS cleavage, for the phosphorylation of the 1-position.

As discussed in section 2.3, the use of base labile, 2-cyanoethyl phosphate protecting groups was a key feature of our synthetic strategy. To install 2-cyanoethyl protected phosphates at the 1- and 4-position of the inositol framework, phosphoramidite chemistry was applied using **218** and (–)-**193**, respectively. Modification of the procedures developed by Uhlmann,[275] Dahl,[276] Johns,[277] and Fraser-Reid[274] were employed for phosphoramidite synthesis and phosphorylation.

The synthesis of **218** started with the preparation of intermediate **220**. Distilled PCl_3 **119** was reacted with two equivalents of diisopropyamine (DIPA) under inert atmosphere (Scheme 2.12). This reaction was followed closely by ^{31}P NMR, showing complete conversion to the mono-substituted phosphoramidite **220**. Filtration of the colourless diisopropylamine hydrochloride salt provided **220**. Analysis by ^{31}P NMR showed that this compound was ~90% pure and was therefore used without

further purification. This unstable intermediate could be stored, under argon and at –20 °C for 8–10 months before significant oxidation was observed. It was important to check **220** by ^{31}P NMR before each use, as purification by vacuum distillation had to be carried out when impurities were observed.



Scheme 2.12 Synthesis of 2-cyanoethyl protected phosphoramidites **218** and (–)-**193**, employed in the synthesis of PtdIns*P*s. *Reagents and conditions*: i. DIPA, Et_2O, 0 °C to RT, 3 h, 77% ii. 3-Hydroxypropionitrile, DIPEA, CH_2Cl_2, 0 °C to RT, 15 min, 69% iii. 3-Hydroxpropionitrile, MeCN, 1 h iv. DIPA, Et_2O, –10 °C to RT, 18 h, 55% over two steps v. DIPEA, CH_2Cl_2, RT, 1 h, 68%. Cne = cyanoethyl.

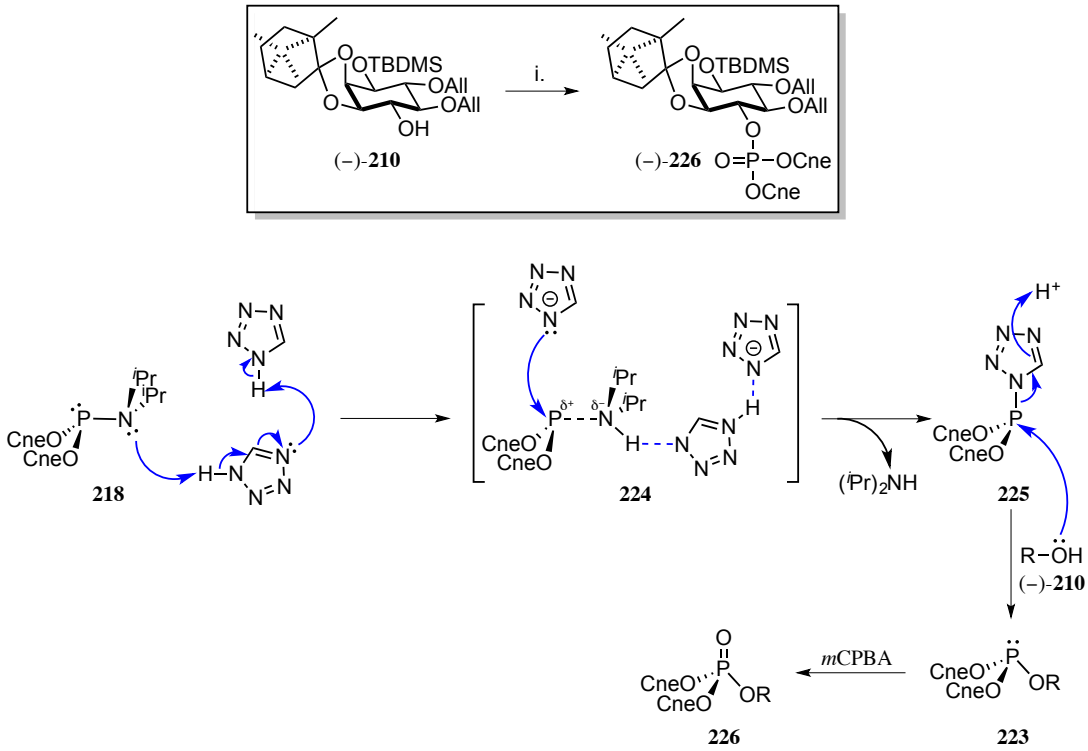
Phosphoramidite **218** was prepared from intermediate **220** using two equivalents of 3-hydroxypropionitrile and excess diisopropylethylamine (DIPEA) followed by rapid purification by column chromatography. This phosphoramidite was found to be unstable, and in order to obtained the best yields in the subsequent phosphorylations, **218** was prepared before each phosphorylation.

The fragment (–)-**193** featuring in the proposed retrosynthesis (Scheme 2.5) was obtained using procedures optimised within the group.[64] To prepare the monosubstituted compound **221**, 3-hydroxypropionitrile was added dropwise to a large excess of PCl_3 **219**. This intermediate **221** was then reacted with two equivalents of DIPA without purification or characterisation. The oil obtained was purified by vacuum distillation to give **222** (Scheme 2.12). This useful precursor was stored under argon, at –20 °C and was handled similarly to **220**. Finally, synthesis of phosphoramidite (–)-**193** was achieved through coupling with the chosen diglyceride, in this case the 1,2-dipalmitoyl-*sn*-glycerol (–)-**211** (Section 2.6), in the presence of excess DIPEA. This phosphoramidite was also

unstable, therefore, purification was performed quickly and (–)-**193** was prepared fresh before each phosphorylation reaction.

2.8. Synthesis of the fully protected lipid precursor (–)-128

This set of phosphoramidites was employed to complete the synthesis of dipalmitoyl PtdIns4*P* **48**. First, the free 4-hydoxyl group on (–)-**210** was phosphitylated under 1*H*-tetrazole catalysis using an excess of phosphoramidite **218** (Scheme 2.13). After stirring overnight, the P(III) phosphite intermediate **223** was observed by TLC and ^{31}P NMR analysis.

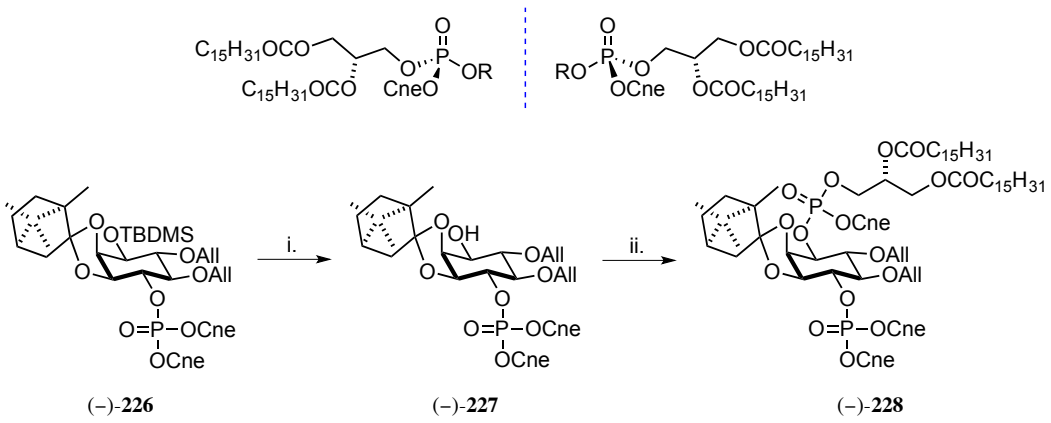


Scheme 2.13 Proposed mechanism for the phosphitylation of (–)-**210** that is consistent with the kinetic data observed by Nurminen *et al*.[274]. Subsequent oxidation to P(V) using *m*CPBA. *Reagents and conditions*: i. a. **218**, 1*H*-tetrazole, CH_2Cl_2, RT, 14 h b. *m*CPBA, –78 °C to RT, 12 h, 82%.[274-277] Cne = cyanoethyl; *i*Pr = isopropyl; TBDMS = *tert*-butyldimethylsilyl; All = allyl.

The mechanism and kinetics for this reaction is well established.[278-281] Acting first as an acid activator, 1*H*-tetrazole protonates phosphoramidite **218** at the nitrogen. The tetrazolide anion generated then engages in the displacement of isopropylamine from the protonated phosphoramidite complex **224** by nucleophilic attack at the phosphorus. This gives a highly reactive intermediate **225**. Alcohol (–)-**210** reacts with this intermediate yielding the phosphite **223**.[278] Oxidation of this intermediate

was achieved with 3-chloroperoxybenzoic acid (*m*CPBA) at –78 °C to give the stable P(V) phosphate (–)-**226**, without affecting the allyl groups (Scheme 2.13).

First we considered performing the cleavage of the 1-*O*-TBDMS ester on (–)-**226** using tetrabutylammonium fluoride (TBAF) as a fluoride source.[242] This reagent, however, is unstable in its anhydrous form,[282] and the tetrabutylammonium hydroxide generated from the presence of water renders TBAF basic.[283] This reagent is therefore not compatible with (–)-**226**. Indeed, the first of the 2-cyanoethyl groups is easily removed under basic conditions by β-elimination as a result of its strong C–H bond acidity. This is particularly true when the neighbouring phosphorus is in its P(V) oxidation state. To circumvent these issues, buffered TBAF has been commonly used in the literature to avoid any side reactions on base sensitive substrates.[284,285] Attempts to remove the TBDMS group on (–)-**226** with TBAF buffered with AcOH at RT was unsuccessful. Cleavage of the 1-*O*-TBDMS ether was not observed, with significant amounts of starting material remaining after 24 hours of reaction. In addition, partial deprotection of the 2-cyanoethyl groups was observed by TLC, mass spectrometry and ^{31}P NMR analysis.



Scheme 2.14 Synthesis of (–)-**228** by cleavage of the 1-*O*-TBDMS ester on to (–)-**226** give (–)-**227**, followed by phosphorylation with phosphoramidite (–)-**193**. Two diastereoisomers of the fully protected PtdIns4*P* precursor (–)-**228** were obtained due to the chirality at phosphorus. *Reagents and conditions*: i. $Et_3N{\cdot}3HF$, THF, 40 °C, 24 h, 72% ii. a. (–)-**193** (5.0 eq), 1*H*-tetrazole, CH_2Cl_2, RT, 25 h b. *m*CPBA, –78 °C to RT, 17 h, 36%. Cne = cyanoethyl; All = allyl; TBDMS = *tert*-butyldimethylsilyl.

In their synthesis of trinucleotides Janczyk *et al*.[237] reported the use of triethylamine trihydrofluoride ($Et_3N{\cdot}3HF$) as an alternative.[286] This mild desilylating agent was shown to be more reliable for the deprotection of the TBDMS group in the presence of base sensitive 2-cyanoethyl groups.[287]

Accordingly, (–)-**226** was treated with excess $Et_3N{\cdot}3HF$ at elevated temperature (40 °C) for 24 hours to give (–)-**227** in good yields (Scheme 2.14).

A first attempt at coupling the fully protected inositol derivative (–)-**227** and phosphoramidite (–)-**193** (Section 2.7) under standard conditions (a. (–)-**193** (3.0 eq), 1*H*-tetrazole, CH_2Cl_2, RT, 25 h b. *m*CPBA, –78 °C to RT, 17 h) only returned starting material. This observation can best be explained by the likely steric clash between the 2,3-*O*-D-camphor dimethyl acetal and the protected phosphorus centre of phosphoramidite (–)-**193**. Increasing the number of equivalents of phosphoramidite (–)-**193** used from three to five, noticeably affected the outcome of the reaction. After 25 hours, partial conversion to the phosphite ester intermediate was observed by TLC and ^{31}P NMR analysis, with some remaining starting material. Oxidation to the phosphate (–)-**228** with *m*CPBA at –78 °C (Scheme 2.14) revealed that the starting material (–)-**227** and the phosphate product (–)-**228** had similar polarities. Extensive purification by column chromatography was therefore undertaken. ^{31}P NMR analysis of the reaction mixture indicated that the product (–)-**228** exists as a 1:1 mixture of diastereoisomers arising from the stereogenic phosphorus centres introduced at the 1-*O* position (Scheme 2.14). Two diastereoisomers were also observed for the P(III) phosphite intermediate. Separation of the diastereoisomers of (–)-**228** by column chromatography is usually viewed unnecessary, as chirality at phosphorus is lost upon final global deprotection. In this case, however, careful purification by column chromatography gave two fractions. The first containing a single diastereoisomer of (–)-**228** (A, Figure 2.6) and the second a mixture of the starting material (–)-**227** and both diastereoisomers of (–)-**228** (A and B, enriched in A, Figure 2.6). The second fraction was subjected once more to the same phosphorylation reaction conditions. Although full conversion was still not achieved, this process led to the isolation of another pure fraction of (–)-**228**, this time containing both diastereoisomers A and B, providing an overall isolated yield of 36%. Although the separation of these diastereoisomers was not useful synthetically, unequivocal assignment of the ^{1}H and ^{13}C NMR spectra for the diastereoisomeric mixture of (–)-**228** and other derivatives was achieved by comparison with that of the single diastereoisomer of (–)-**228** (Figure 2.6).

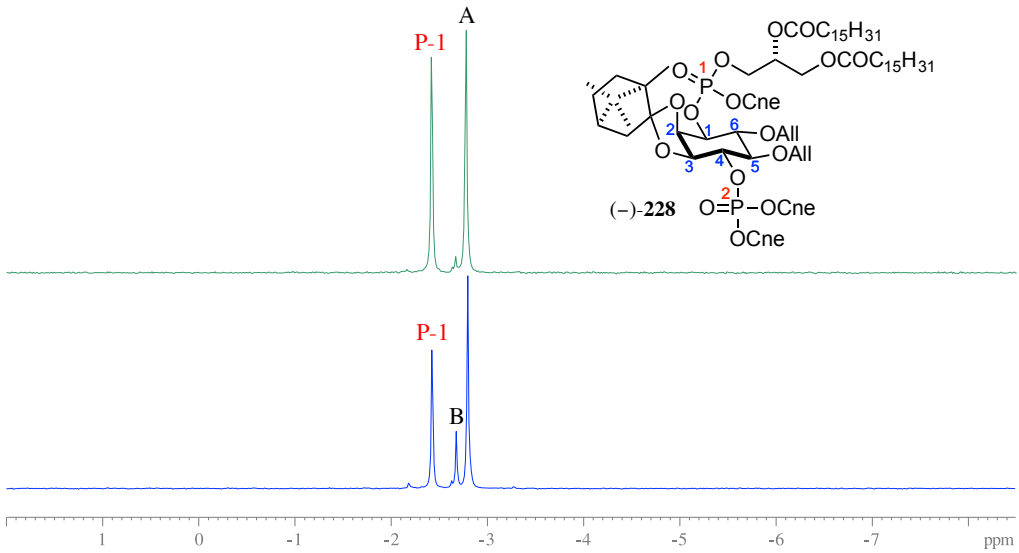
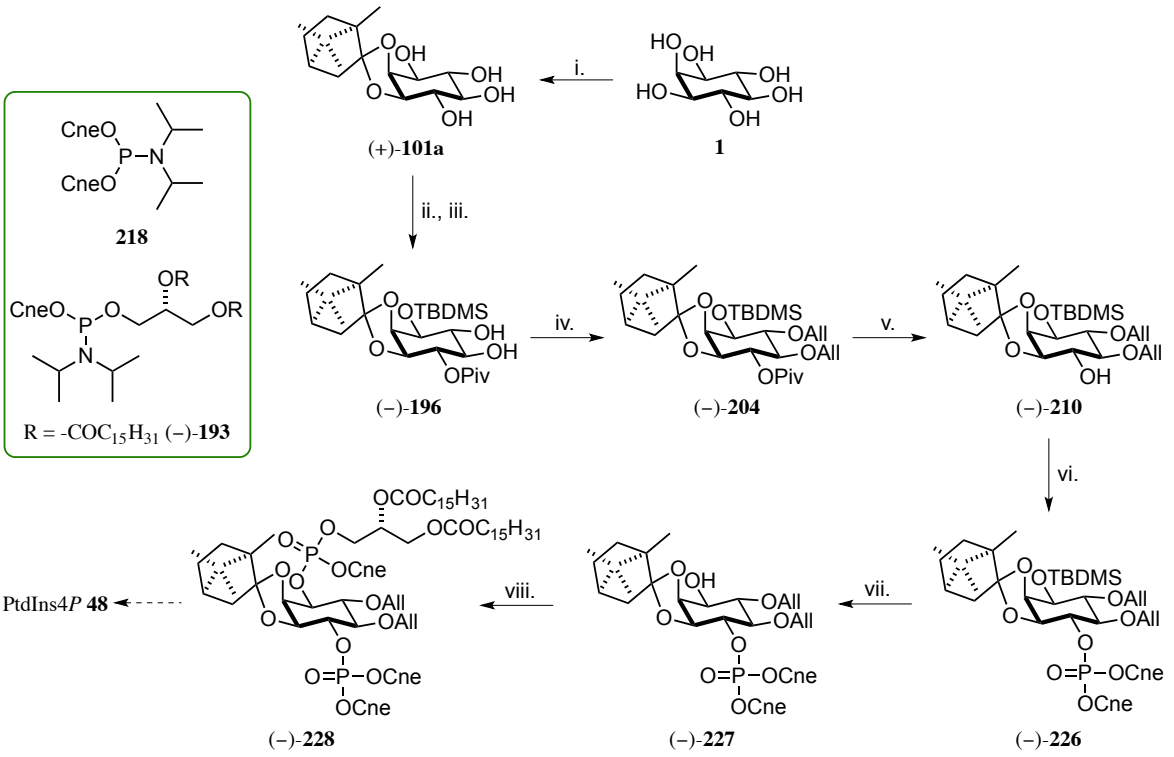


Figure 2.6 ^{31}P NMR spectra of the fractions obtained after extensive column chromatography on (–)-**228**. The first fraction contained diastereoisomer A (green) and the second fraction contained both diastereoisomer A and B (blue). Both NMR spectra were run at 400 MHz, in $CDCl_3$.

Final global deprotection of the fully protected intermediate (–)-**228** obtained was not attempted for a number of reasons. First, a more efficient route, which involved a high yielding enantioselective desymmetrisation step, had been investigated in parallel and was found to be better suited to the synthesis of PtdIns*P*s (Chapter 3). Second, work carried out within the group by Dr Alex Saunders[47] had suggested that multistep deprotections of such complex PtdInsP_n systems, involving the cleavage of 2-cyanoethyl protected phosphates, was challenging. Indeed, these deprotection reactions were difficult to monitor and the final products obtained required purification by ion-exchange column chromatography. If this deprotection was attempted, however, the 2-cyanoethyl groups would be removed first to avoid phosphate migration. These would be initially exchanged for trimethylsilyl (TMS) esters by stirring with 2-*tert*-butyl-1,1,3,3-tetramethyl-guanidine (Barton's base) and TMSCl overnight.[77] Cleavage of the TMS esters would then be achieved by stirring the crude product obtained with MeOH.[77] Simultaneous deprotection of the 5-*O*- and 6-*O*-allyl ethers and 2,3-*O*-camphor acetal groups would be attempted subsequently using $PdCl_2$ in MeOH.[68]

2.9. Summary and conclusions

The key optically pure building block (+)-**101a** was obtained using Bruzik's well established diastereoisomeric protection-resolution methodology.[221] Working within this framework, an efficient protecting group strategy towards dipalmitoyl PtdIns4*P* **48** was developed and optimised. This involved the regioselective installation of a set of three orthogonal groups on the inositol ring hydroxyl groups to give (–)-**204**. Sequential deprotection and phosphorylation of these orthogonal groups, using 2-cyanoethyl protected phosphoramidites **218** and (–)-**193**, yielded the fully protected precursor (–)-**228** over seven steps (Scheme 2.15).



Scheme 2.15 Summary of the optimised synthesis of precursor (–)-**228** *via* a diastereoisomeric optical resolution. *Reagent and conditions*: i. a. (+)-**100**, H_2SO_4, DMSO, 70 °C, 3 h b. PTSA·H_2O, $CHCl_3$/MeOH/H_2O (50:5:1), RT, 18 h, 23% ii. TBDMSCl, imidazole, pyridine, –10 °C, 24 h, 57% iii. PivCl, pyridine, –10 °C, 13 h, 60% iv. **207**, $Pd(PPh_3)_4$, THF, reflux, 4 h, 64% v. DIBAL-H, CH_2Cl_2, –78 °C, 4.5 h, 92% vi. a. **218**, 1*H*-tetrazole, CH_2Cl_2, RT, 14 h b. *m*CPBA, –78 °C to RT, 12 h, 82% vii. Et_3N·3HF, THF, 40 °C, 24 h, 72% viii. a. (–)-**193**, 1*H*-tetrazole, CH_2Cl_2, RT, 25 h b. *m*CPBA, –78 °C to RT, 17 h, 36%. Cne = cyanoethyl; All = allyl; TBDMS = *tert*-butyldimethylsilyl.

This research has highlighted two main limitations associated with such a synthetic route:

- Although the use of an early diastereoisomeric resolution is appropriate when using cheap and readily available *myo*-inositol **1** as a starting material, this methodology does not lend itself to the use of more expensive, deuterated starting materials. Desymmetrisation

strategies will therefore need to be explored in order to improve the efficiency and overall yield of this synthesis.

- Retaining the 2,3-*O*-D-camphor dimethyl acetal protecting group throughout the synthesis led to a sterically congested precursor (–)-**227** which was extremely difficult to phosphorylate. Purification of the fully protected PtdIns4*P* precursor (–)-**228** obtained was also challenging. A revised synthetic plan should therefore involve a less bulky diol protecting group at those positions.

This work therefore laid the foundations for Chapter 3, which builds on the protecting group strategy developed herein and involves the optimisation of a desymmetrisation strategy towards deuterated PtdIns4*P* and PtdIns5*P* derivatives.

3 Synthesis of deuterated PtdIns4*P* and PtdIns5*P* *via* an enzymatic desymmetrisation

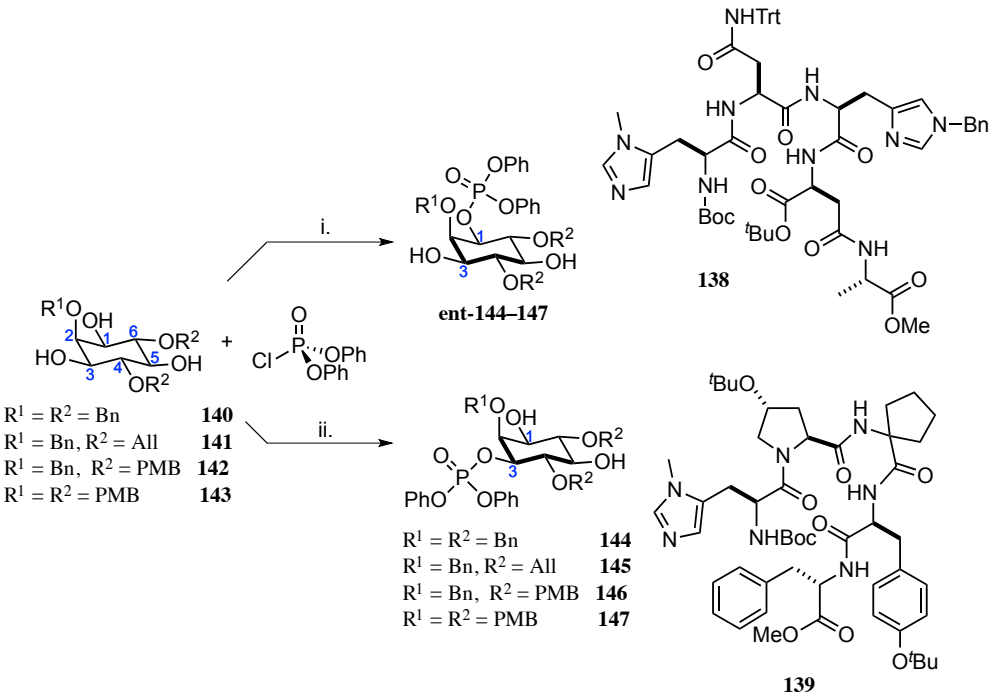
3.1. Aims and introduction

The research described in Chapter 2 led to an understanding of the differing reactivity of the hydroxyl groups around the *myo*-inositol ring and to the development of a set of orthogonal protecting groups for this framework. It is clear, however, that routes towards enantiomerically pure inositols involving optical resolutions or alternative chiral pool materials[34] are typically lengthy and often suffer from poor overall yields. Therefore, the aims of the work described in this chapter were to:

- Optimise an efficient enantioselective desymmetrisation methodology that is applicable to deuterated inositol derivatives.
- Employ this methodology to design and develop a robust, high yielding route towards saturated and unsaturated PtdIns4*P* and PtdIns5*P* derivatives
- Apply this route to the synthesis of deuterated PtdIns*P* analogues.

The enantioselective phosphorylation methodology developed by Miller *et al*. (Scheme 3.1; Section 1.2.4),[83-86] could not be applied efficiently to the synthesis of PtdIns4*P*, as the peptide catalysts available did not accommodate for the required substitution pattern. Although asymmetric organocatalysis has not yet been applied extensively to the synthesis of inositol derivatives,[93] a wealth of literature exists surrounding the desymmetrisation of *meso*-diols and polyols.[288-292] Our aim initially was to investigate whether an organocatalytic approach could be taken to synthesise enantiomerically pure, deuterated PtdIns*P* derivatives. Alternatively, a handful of enzymatic strategies have been developed in the literature. Although enzymatic resolutions have been more

widely explored,[78,82,293,294] this research focused on investigating more efficient and higher yielding enzymatic desymmetrisations, and their application to the synthesis of PtdIns*P* derivatives.[90-92]



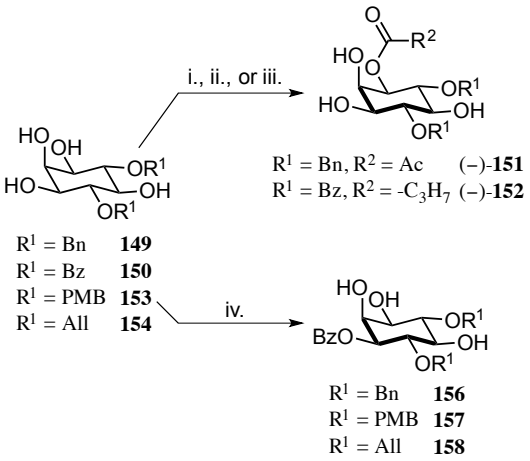
Scheme 3.1 Desymmetrisation developed by Miller *et al.*[24,83-88] involving the enantioselective phosphorylation at the 1- or 3-positions of *meso*-2,4,6-*O*-triprotected *myo*-inositol derivatives. *Reagents and conditions*: i. **138** (2.5 mol%), Et_3N, toluene, 0 °C, 4 h, 46–65%, 94–98% *e.e.* ii. **139** (2.5 mol%), Et_3N, toluene, 0 °C, 4 h, 53–56%, 97–98% *e.e.* Me = methyl; Bn = benzyl; All = allyl; PMB = 4-methoxybenzyl; Bz = benzoyl; Ac = acetate; Ph = phenyl; Trt = trityl; Boc = *tert*-butyloxycarbonyl; ^{t}Bu = *tert*-butyl; ent = enantiomers.

3.2. Protecting group strategy and retrosynthesis

To enable the design of efficient synthetic routes towards differentially phosphorylated enantiopure inositols, it is important to have access to a range of inositol building blocks with varying substitution patterns. In the context of enantioselective desymmetrisation, this means that the *meso* substituted inositols used as a starting point should be versatile and synthetically useful. As such, key desymmetrisation steps can be incorporated into a synthetic route without the need for unnecessary protecting group manipulation.

Starting from the *meso*-2,4,6-*O*-triprotected *myo*-inositol derivatives **140–143**, Miller *et al.* developed a range of tetrapetide-catalysed desymmetrisation phosphorylation reactions allowing for the differentiation of the enantiotopic 1- and 3-positions of the *myo*-inositol ring (Scheme 3.1).[24,83]

meso-4,6-Di-*O*-benzyl *myo*-inositol **149** and other derivatives **150**, **153** and **154** have also been used with a similar mind-set in organocatalytic[93] and enzymatic[90-92] enantioselective acylation reactions[295] to give enantiomerically pure derivatives such as (–)-1D-**151** (Scheme 3.2). Differentiation of the 1- and 3-position hydroxyl groups is known to be promoted by the stereochemical environment of these positions, which comprises both neighbouring axial and equatorial substituents. Contrastingly, the stereochemically identical environments of the 4- and 6-positions render their differentiation more challenging. As a result, fewer methodologies exist for the desymmetrisation of *meso*-1,3,5-*O*-triprotected or 3,5-*O*-diprotected *myo*-inositol derivatives.[86]

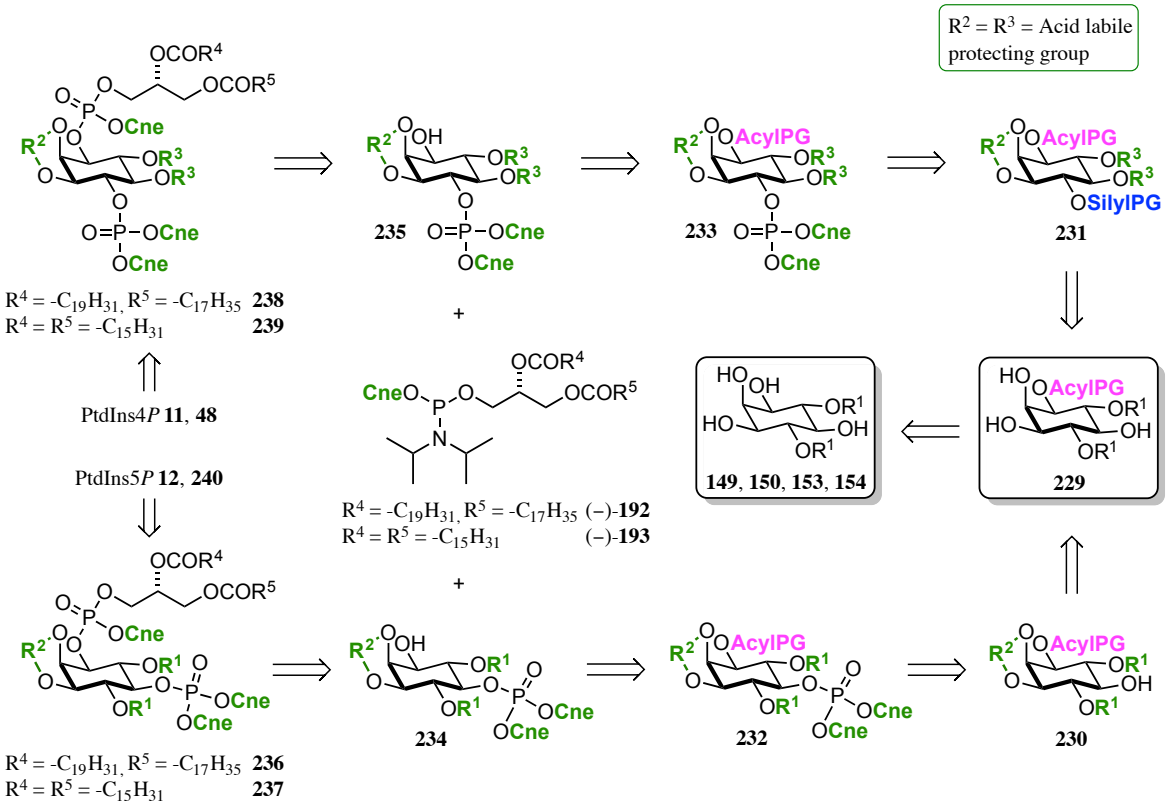


Scheme 3.2 General scheme for the enantioselective acylation of *meso*-4,6-diprotected *myo*-inositol derivatives under organo-[93] or enzymatic catalysis.[90-92] *Reagents and conditions:* i. Lipase amano PS, vinyl acetate, RT, > 99% *e.e.*, (–)-**151** ii. Lipozyme TL-IM, vinyl acetate/hexane (1:1), 45 °C, 12 h, 99%, > 99% *e.e.*, (–)-**151** iii. Lipoprotein lipase from *Pseudomonas sp.*, > 95% *e.e.*, (–)-**152** iv. Phosphinite catalyst **155** (20 mol%), DIPEA, BzCl, propionitrile, –78 °C, 24 h, 65%, >99% *e.e.*, **156–158**. Bn = benzyl; All = allyl; PMB = 4-methoxybenzyl; Bz = benzoyl; Ac = acetate; Ph = phenyl.

We aimed ideally to optimise a desymmetrisation of *meso*-4,6-diprotected *myo*-inositols to give 1-*O*-acylated derivatives such as **229**. The advantage of applying such a methodology to the synthesis of PtdIns*P*s is two fold. Firstly, the enantiomerically pure derivative **229** obtained is already differentiated at the 1-*O*-position, therefore eliminating the need for a low yielding, regioselective protection at that position. Secondly, subsequent protection of the 2,3-*O*-diol directly provide a PtdIns5*P* precursor **230** without the need for further protecting group manipulations (Scheme 3.3). We envisaged that cleavage of the 4,6-*O* ethers, followed by selective protection of the 4-positions with an orthogonal silyl protecting group, would achieve the desired differentiation between the 1- and 4-positions of the inositol ring, to provide intermediate **231** towards saturated and unsaturated

PtdIns4*P* derivatives **11** and **48**. Similar to the route developed in Chapter 2, we hypothesised that protection of the 5- and 6-positions followed by selective silyl deprotection and phosphorylation would give intermediate **233** (Scheme 3.3).

Our main concern with intermediates such as **232** and **233**, was achieving selective cleavage of the 1-*O*-acyl group in the presence of cyanoethyl-protected phosphates. If suitable conditions could not be optimised for this transformation, a revised synthetic plan would need to be developed. This would involve either investigating alternative phosphate protecting groups which are adaptable to the synthesis of unsaturated PtdIns*P* derivatives; or undertaking cleavage of the 1-*O*-acyl and phosphorylation at the 1-position before 4-*O*-silyl deprotection.

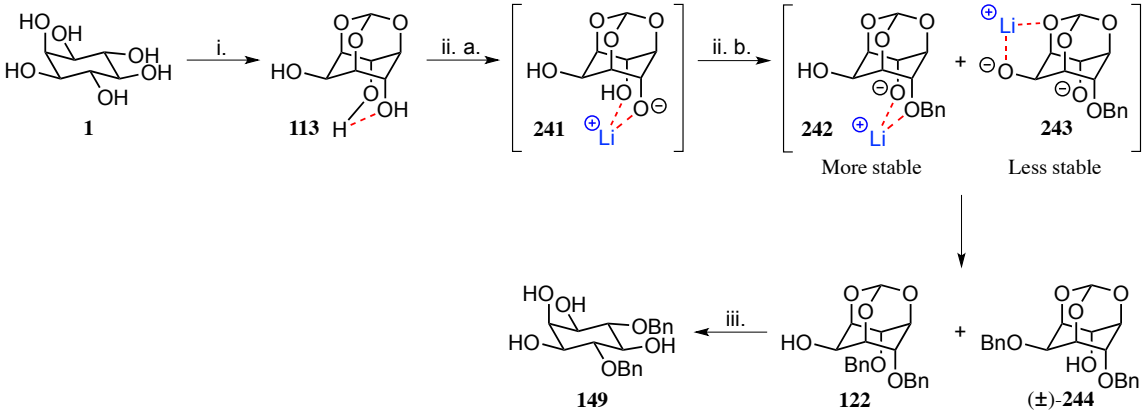


Scheme 3.3 Retrosynthesis for 1-stearoyl-2-arachidonoyl and dipalmitoyl PtdIns4*P* **11**, **48** and PtdIns5*P* **12**, **240** derivatives with **229** as the main, desymmetrised building block. The protecting groups to be removed in a final, global deprotection are highlighted in green. In pink and blue are the orthogonal protecting groups that allow for the differentiation between the 1- and 4-positions respectively. Cne = cyanoethyl; SilylPG = silyl protecting group; AcylPG = Acyl protecting group.

3.3. Synthesis of *meso*-4,6-di-*O*-benzyl *myo*-inositol 149

Amongst the 4,6-diprotected *myo*-inositol derivatives presented in Scheme 3.2, it is clear that 4,6-di-*O*-benzyl-*myo*-inositol **149** has been the most commonly applied to desymmetrisation transformations. This protected inositol was therefore chosen for optimising a novel synthetic route towards PtdIns*P*s.

Synthesis of **149** was accomplished on a large scale following the procedure developed by Billington *et al*. (Scheme 3.4).[296] Reaction of *myo*-inositol **1** with triethylorthoformate and substoichiometric PTSA·H_2O monohydrate in DMF gave the adamantane-like inositol orthoformate **113** in 65% yield.[60] The orthoformate framework is obtained through flipping of the *myo*-inositol ring. Simultaneous protection of the resulting axial 1-, 3- and 5-positions is made possible, giving a structure with one equatorial (C(2)OH) and two axial (C(4)OH and C(6)OH) hydroxyl groups. Regioselective alkylation of these two remaining axial positions is possible. Indeed, formation of a strong intramolecular hydrogen-bond between the 4- and 6-hydroxyl groups of **113** leads to the enhanced acidity of the axial hydroxyl protons.[297] Following the first deprotonation, the axial alkoxide **241** is generated. Stabilisation of this alkoxide is induced by a metal cation chelate bridge to the second neighbouring axial hydroxyl group. Billington *et al*. and other investigators demonstrated that the yield and regioselective outcome of these reaction is dictated by a number of factors including the nature of the solvent, the electrophile and base (cation size) used, but sterics also play a role.[62,298-300]

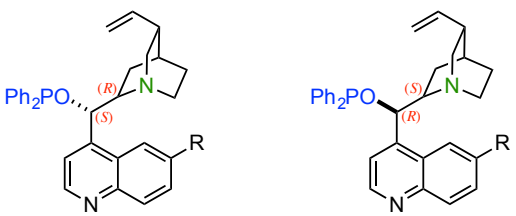


Scheme 3.4 Synthesis of *meso*-4,6-di-*O*-benzyl-*myo*-inositol **149** *via* the regioselective benzylation of *myo*-inositol orthoformate **113**. Regioselectivity stems from the enhanced acidity of the axial hydroxyls on **113** and the stabilisation of the alkoxide **241** through the formation of a lithium cation chelate bridge.[288] *Reagents and conditions*: i. $CH(OEt)_3$, PTSA·H_2O, DMF, 105 °C, 3 d, 65% ii. a. LiH, DMF, RT, 30 min b. BnBr, RT, 48 h, 71% iii. PTSA·H_2O, MeOH, RT, 24 h, 92%. Bn = benzyl.

As such, alkylation with one equivalent of sodium hydride (NaH) has been shown to give nearly exclusively the 4-*O*-substituted orthoformate.[34,62,68] Dialkylation of these triols with two equivalents of sodium hydride and alkyl halide however, provides a mixture of the asymmetric 2,4- and symmetric 4,6-di-*O*-substituted orthoformates **122** and (±)-**244** resulting in low isolated yields of the desired 4,6-di-*O*-substituted orthoformate **122** (~40% yield).[301,302] An extensive study by Sureshan *et al*. [303] showed that greater selectivity for 4,6-di-*O*-alkylated derivatives **122** over 2,4-di-O-alkylated derivatives (±)-**244** could be achieved when using lithium hydride, owing to lithium being a smaller and better coordination ion.[93] The lithium cation was shown to be better at stabilising oxyanion **242** over **243** *via* chelation (Scheme 3.4). This can be attributed the preference for chelation of two axial oxygens as compared to oxygens with an axial-equatorial relationship coupled with the smaller size of Li^+ and stronger coordination ability.[303] The lower basicity of LiH however, renders this reaction slower than that with NaH, and at least 48 hours is required for the reaction to reach completion (Scheme 3.4). The versatile, *meso* intermediate **113** was thus benzylated selectively at the 4- and 6-position using LiH and benzyl bromide in DMF to give the protected orthoformate **122** in 71% yield. Methanolysis of this orthoformate, using stoichiometric PTSA·H_2O, furnished *meso* 4,6-di-*O*-benzyl-*myo*-inositol **149** in 92% yield (Scheme 3.4).

3.4. Asymmetric organocatalytic desymmetrisation approach

The readily available *Cinchona* alkaloid natural products possess a unique stereochemical arrangement which can be harnessed for asymmetric induction. As such, numerous *Cinchona* alkaloid derivatives have been employed as bifunctional organocatalysts in enantioselective

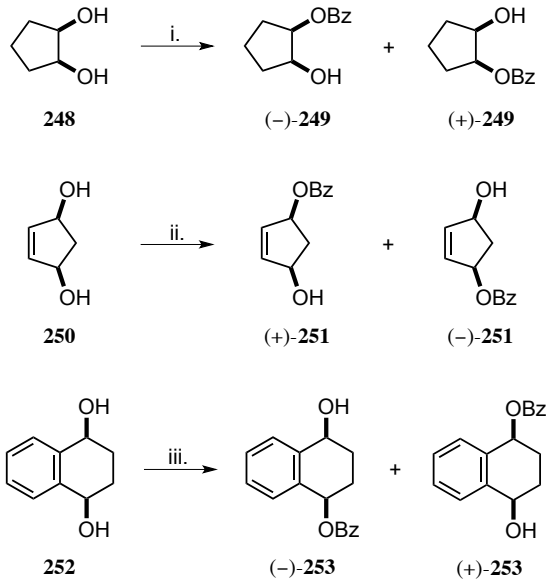


R = OMe **155**
R = H **245**

R = OMe ent-**155**
R = H ent-**245**

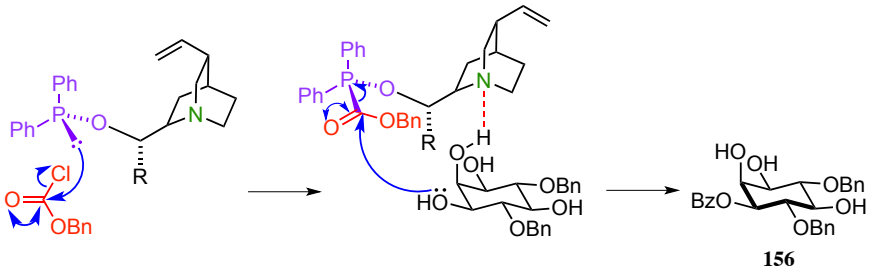
Figure 3.1 Phosphinite derivatives of *Cinchona* alkaloids. **155** and **245** are derived from quinidine (+)-**246** and cinchonine (+)-**247**. ent-**155** and ent-**245** from are derived from quinine (–)-**246** and cinchonidine (–)-**247**. Me = methyl; Ph = phenyl.

reactions.[304] In particular, Mizuta *et al*. reported the asymmetric desymmetrisation of *meso* 1,2-, 1,3- and 1,4-diols, using phosphinite **155** and **245** derived from quinidine (+)-**246** and cinchonine (+)-**247** (Figure 3.1, Scheme 3.5).[289,290,305]



Scheme 3.5 Key examples for the desymmetrisation of *meso*-1,2-, 1,3- and 1,4-diols optimised by Mizuta *et al*.[296-298] *Reagents and conditions*: i. **245** (30 mol%), DIPEA, BzCl, EtCN, −78 °C, 3.5 h, 80%, 93% *e.e*., (−)-**249** ii. **155** (30 mol%), DIPEA, BzCl, CH_2Cl_2, 0 °C, 4 h, 82%, 81% *e.e*., (−)-**251** iii. **155** (30 mol%), DIPEA, BzCl, CH_2Cl_2, 0 °C, 4 h, 55%, 82% *e.e*., (+)-**253**. Bz = benzoyl.

Basing their work on this research, Lauber *et al*. recently developed a procedure for the asymmetric acylation of 4,6-di-*O*-protected *myo*-inositol derivatives **149**, **153** and **154** (Scheme 3.2) using the phosphinite derivative of quinidine **155** (Figure 3.1). These catalysts contain a Lewis-basic trivalent phosphorus centre[306] and a Brønsted basic tertiary amino group, which act cooperatively. The phosphorus centre is postulated to activate the acylating reagent while the quinuclidine moiety (pKa ~11) forms a hydrogen bonding-interaction with the axial hydroxyl group of **149** (Scheme 3.6).[93]

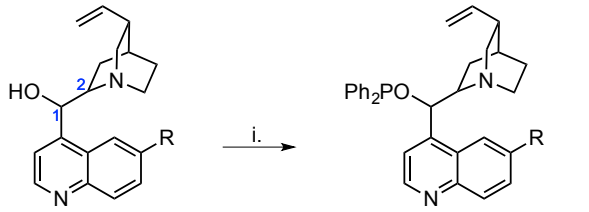


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Scheme 3.6 Postulated mechanism for the acylation of **149** using the phosphinite derivative of quinidine **155**. Benzoyl chloride is activated by the Lewis-basic trivalent phosphorus centre. Differentiation between the enantiotopic 1- and 3-position is achieved as a result of the hydrogen-bonding interaction between the quinuclidine moiety and the axial hydroxyl of **149**. Bn = benzyl; Bz = benzoyl; Ph = phenyl.

Considering the wide scope of this methodology compared to other enzymatic strategies presented in Scheme 3.2, and the excellent yields (70–80%) and enantioselectivities (90–99% *e.e.*) reported by Lauber *et al.*, we attempted to reproduce this work. We then intended to extend this methodology to the 1-*O*-acylation of **149** and other 4,6-di-*O*-protected *myo*-inositol derivatives.

Accordingly, the two bifunctional catalysts **245** and ent-**155** were prepared in one step using an improved procedure developed by Mizuta *et al.*[289,290] Reaction of chlorodiphenylphosphine with cinchonine (+)-**247** or quinine (−)-**246** in the presence of triethylamine, followed by purification over a short pad of silica gel under argon, gave the desired phosphinite derivatives **245** and ent-**155** respectively, contaminated with 5–10% of the corresponding phosphinate, as determined by ^{31}P NMR (Scheme 3.7). These catalysts were found to be extremely difficult to handle due to their susceptibility to oxidation. Not only were the reaction to be kept under strictly anhydrous and oxygen-free conditions, but these catalysts needed to be used immediately following preparation. Finally, although the phophinite derivatives **245** and ent-**155** were obtained in good yields (59–67%), the derivative from quinidine **155** could never be isolated without full oxidation to the phosphinate.



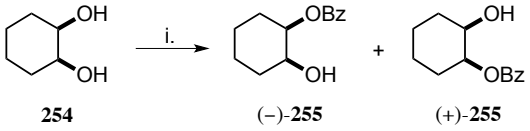
R = OMe (+)-**246** (1*S*, 2*R*)
R = H (+)-**247** (1*S*, 2*R*)
R = OMe (−)-**246** (1*R*, 2*S*)

R = OMe **155** (1*S*, 2*R*) N/A
R = H **245** (1*S*, 2*R*) 59%
R = OMe ent-**155** (1*R*, 2*S*) 67%

Scheme 3.7 Synthesis of phosphinite derivatives of *Cinchona* alkaloids **155**, **245** and ent-**155**.[296-297] *Reagents and conditions*: PPh_2Cl, Et_3N, THF, RT, 2 h, 59–67%. Me = methyl; Ph = phenyl.

Although **155** seemed to be the catalyst of choice for the desymmetrisation of 4,6-di-*O*-protected *myo*-inositols,[93] Mizuta *et al.* had shown that, in some cases, the phosphinite derivative from cinchonine **245** was the most efficient catalyst for the desymmetrisation of a range of *meso*-diols (Scheme 3.5).[289,290] With this in mind, desymmetrisation of a model *meso*-diol system *cis*-1,2-cyclohexane diol **254** with the cinchonine phosphonite catalyst **245** was first undertaken (Scheme 3.8). After 12 hours of stirring at −78 °C, followed by work-up and purification by column chromatography, (−)-**255** was obtained in a 58% yield (Scheme 3.8). Although the enantiomeric

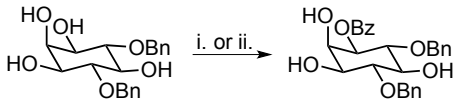
excess (*e.e.*) of (–)-**255** was not determined, comparison of its specific optical rotation ($[\alpha]_D^{20} = -14.0$ (*c* 2.0, $CHCl_3$)) to the value recorded by Mizuta *et al*. ($[\alpha]_D^{23} = -16.5$ (*c* 1.0, $CHCl_3$)], 94% *e.e.*) [289] suggested that the *e.e.* was high.



254 (–)-**255** (+)-**255**

Scheme 3.8 Desymmetrisation of *meso*-**254** using the phosphinite derivative from chinchonine **245**.[296] *Reagents and conditions*: i. **245**, DIPEA, BzCl, EtCN, –78 °C, 12 h, 58%, (–)-**255**. Bz = benzoyl.

Encouraged by these initial results, desymmetrisation of the dibenzylated inositol **149** under the conditions reported by Lauber *et al*. was undertaken using **245**. Unfortunately, after multiple attempts, this reaction was found to be extremely unreliable in our hands. Large amounts or all of the starting material **149** was usually recovered after reaction (Scheme 3.9). When employing the phosphinite derivative from quinine ent-**155** as the catalyst, low yields of racemic product (±)-**156** was isolated (Scheme 3.9) as determined by chiral HPLC (See Appendix A).



149 (±)-**156**

Scheme 3.9 Attempts at the desymmetrisation of **149** using catalysts **245** or ent-**155** were unsuccessful giving small amounts of racemic product (±)-**156**. *Reagents and conditions*: i. **245** (30 mol%), DIPEA, BzCl, EtCN, –78 °C, RT, 24 h, 0% ii. ent-**155** (30 mol %), DIPEA, BzCl, EtCN, –78 °C, RT, 24 h, 23%, <1% *e.e.* Bn = benzyl; Bz = benzoyl.

Considering the difficulties met when synthesising phosphinites **155**, **245** and ent-**155**, and the unreliability of this desymmetrisation procedure in our hands, a different strategy was sought.

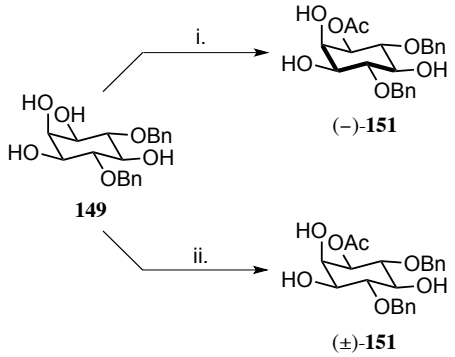
3.5. Chemoenzymatic approach

The use of enzymes in asymmetric organic synthesis has been extensively studied as a result of their efficiency in chemo-, regio- and stereoselective transformations.[307] Alongside other hydrolases and oxidoreductases, lipases (triacylglycerol ester hydrolases, EC 3.1.1.3) are the most commonly used enzymes in organic synthesis.[308] This mostly stems from their ability to operate on a wide range of substrates, in organic solvents,[309] and without cofactors. Although their biological function lies in

catalysing the hydrolysis of water-insoluble substrates such as acyl-glycerols,[310] these ubiquitous enzymes have also been used both for the kinetic resolution of racemic alcohols, acids, esters, and amines[311] and the desymmetrisation of *meso*- or prochiral compounds.[312] This transformation is generally achieved through transesterification with an acyl donor or through hydrolysis.

3.5.1. Desymmetrisation of **149** using Lipozyme TL-IM®

We explored the possibility of using a lipase to catalyse the desymmetrisation of **149**, towards the synthesis of PtdIns*P*s (Section 3.2). Simas *et al*. have recently shown that differentiation between the enantiotopic 1- and 3-positions of **149** can be achieved using the commercially available, immobilised lipase from *Thermomyces lanuginosus* (Lipozyme TL-IM®)[313] to give enantiomerically pure (–)-**151**. Following this procedure, **149** was stirred with Lipozyme TL-IM®[V] in hexane/vinyl acetate at 45 °C for 15 hours. After this time, full consumption of **149** was observed by TLC analysis. Filtration through Celite® followed by purification by column chromatography yielded the desired acetylated product (–)-**151** in 98% yield (Scheme 3.10).



Scheme 3.10 Desymmetrisation of **149** using Lipozyme TL-IM® gave enantiomerically pure (–)-**151**. Racemic (±)-**151** was obtained under standard acylation conditions. *Reagents and conditions*: i. Lipozyme TL-IM®, vinyl acetate/hexane (1:1), 45 °C, 15 h, 98%, >99% *e.e*. ii. Ac_2O, Et_3N, 4-DMAP, CH_2Cl_2, –20 °C to –10 °C, 30 h, 7%. Bn = benzyl; Ac = acetate.

To determine unequivocally that (–)-**151** was formed as a single enantiomer, racemic (±)-**151** was prepared under standard acylation conditions, with acetic anhydride and triethylamine in the presence of substoichiometric 4-DMAP (Scheme 3.10). The mixture of mono- and polyacetylated regioisomers obtained was purified by semi-preparative, reverse phase HPLC to give racemic (±)-**151** (Figure 3.2). The *e.e*. of (–)-**151** was determined by chiral HPLC using a ChiralPak® AD-H

[V] A 100 g sample of this lipase was kindly provided free of charge by Novozymes Biopharma DK A/S.

column, and was found to be greater than 99% (Figure 3.2). Importantly, this reaction can be carried out on gram scale, without concurrent reduction of the yield or *e.e*.

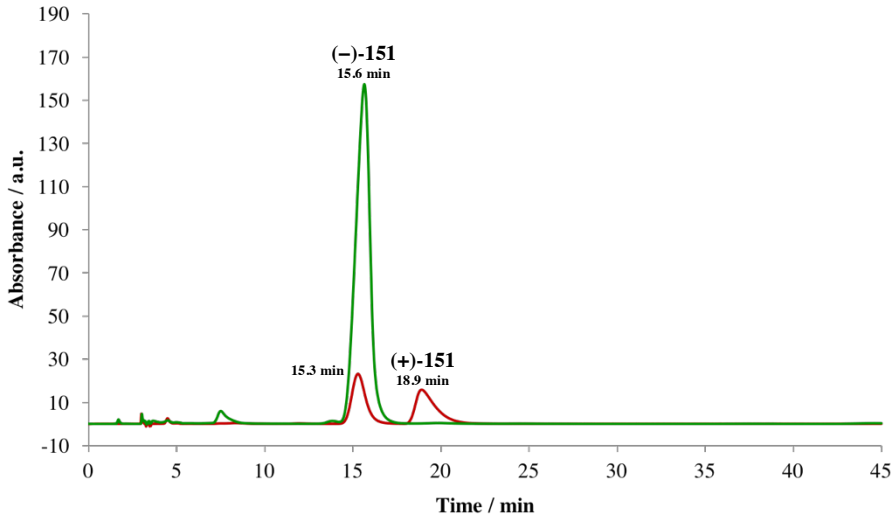


Figure 3.2 Chiral HPLC traces (ChiralPak® AD-H column, heptane/IPA 85:15, 1.0 mL.min^{-1}, 254 nm) of the single enantiomers (–)-**151** (green) obtained from the enzymatic desymmetrisation of **149**, compared to racemic (±)-**151** (red).

A comprehensive review by Fernandez-Lafuente[313] elaborates on the structure, mechanism of action, and interesting industrial applications of Lipozyme TL-IM®. Similar to other lipases, this single chain protein (269 amino acids) exhibits a characteristic α–β hydrolase fold[314] and an alpha-helical, oligopeptide 'lid' which shields the active site (Figure 3.3).[315,316] In water, the lipase adopts a closed

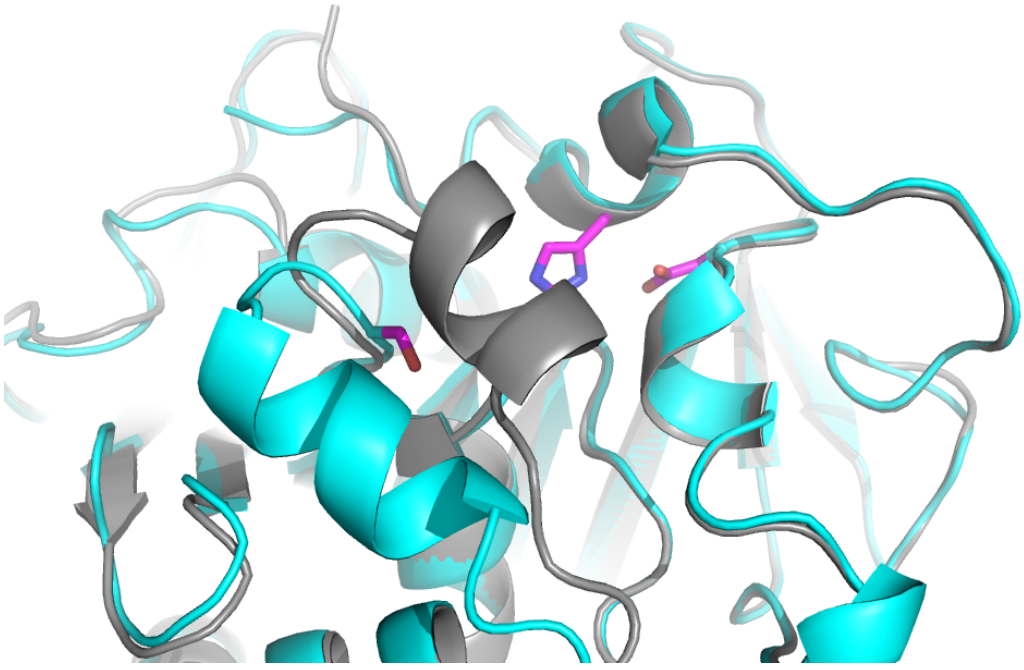
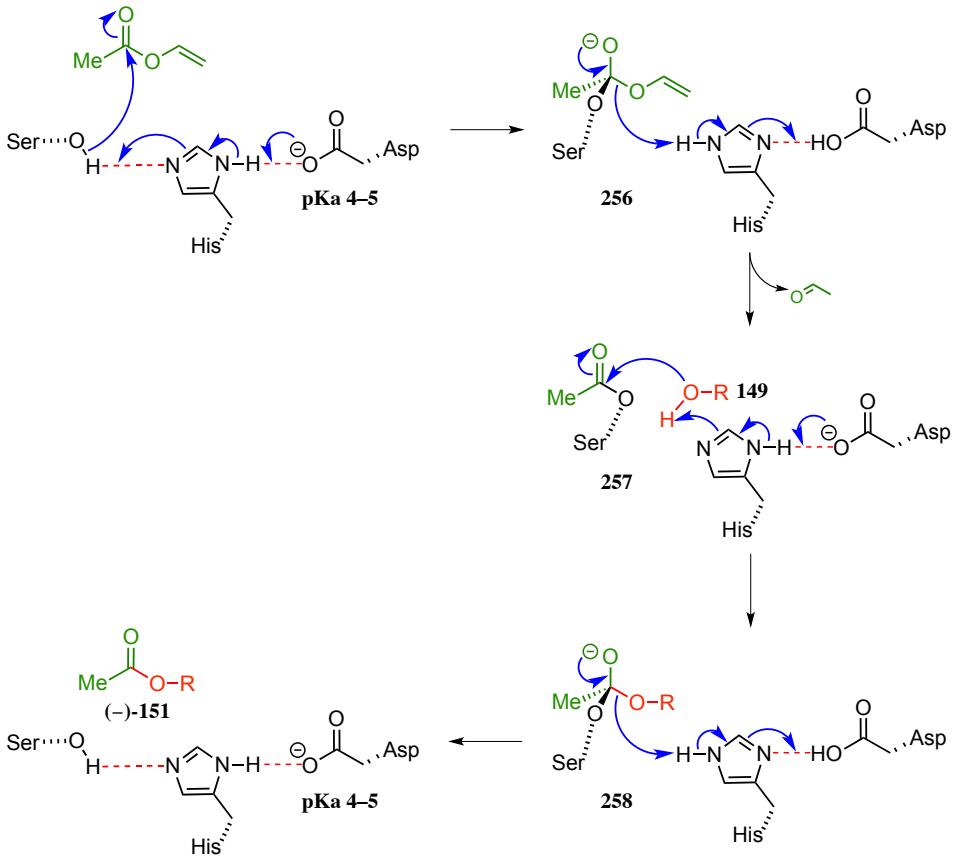


Figure 3.3 Overlay of the X-Ray crystal structure of the open form (turquoise, PDB: 1GT6) and closed form (grey, PDB: 1DT3) of the lipase from *Thermomyces lanuginosus*, showing the catalytic triad serine, histidine, apartic acid. The image was generated by Joe Bluck using PyMol.

conformation, therefore restricting access to the active site. Rearrangement of this lid to an open conformation (Figure 3.3) is postulated to occur upon contact with a hydrophobic interface. Although the precise mechanism for this 'interfacial activation'[317,318] is still unclear,[319] it is known that opening of the lid depends on the intricate nature of the substrate, the environment, and the enzyme itself.[320] In particular, it is promoted by organic solvents,[321] allowing chemoenzymatic reactions to take place.

The active site features a typical catalytic triad which comprises serine, histidine, and aspartic acid, hydrogen bonded to each other (Scheme 3.11). These residues form a charge relay network, allowing for the deprotonation of serine. This activation results in the nucleophilic attack of serine at the carbonyl of vinyl acetate. After collapse of the tetrahedral intermediate **256** obtained and release of the side product, the alcohol nucleophile **149** attacks the acyl-enzyme intermediate **257** generated. Subsequent collapse of the second tetrahedral intermediate **258** releases the product (–)-**151** and regenerates the catalytic site of the lipase (Scheme 3.11).

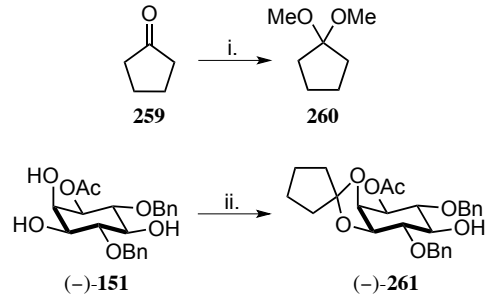


Scheme 3.11 Mechanism of lipase catalysis through the catalytic triad Serine/Histidine/Aspartic acid.

3.5.2. Simultaneous protection of the 2,3-diol of enantiomerically pure (–)-**151**

Having established a key desymmetrisation, the next step in the synthesis towards PtdIns*P*s involved masking the 2,3-diol of enantiomerically pure triol (–)-**151**. The cyclopentylidene acetal group[239,240,322] was chosen for this purpose. This protecting group fulfilled the steric requirements outlined in Section 3.2. In addition, cyclopentylidenes are known to be more easily cleaved compared to acetonides and cyclohexylidenes,[242] which would allow the use of mild acidic conditions in the final global deprotection step. Finally, cyclopentanone **259**, the side product generated from the deprotection of cyclopentylidene acetals, is volatile and can therefore be easily removed at the end of the synthesis without the need for further purification or trituration.

Similar to the synthesis of 1-(*R*)-(+)-camphor dimethyl acetal (+)-**100**, 1,1-dimethoxycyclopentane **260** was prepared by stirring cyclopentanone **259** and trimethyl orthoformate with K-10 Montmorillonite aluminium pillared clay (Scheme 3.12). The mixture of **260** and unreacted cyclopentanone **259** (ratio of 97:3 as observed by ^{1}H NMR) was used without further purification for the acetalisation of (–)-**151** in the presence of PTSA·H_2O, yielding the 2,3-*O*-cyclopentylidene derivative (–)-**261** in 92% yield (Scheme 3.12).



Scheme 3.12 Preparation of 1,1-dimethoxycyclopentane **260** and protection of the 2- and 3-positions of (–)-**151** with a cyclopentylidene acetal group. *Reagents and conditions*: i. $CH(OMe)_3$, K-10 Montmorillonite clay, hexane, RT, 18 h, 88% ii. **260**, PTSA·H_2O, CH_2Cl_2, RT, 18 h, 92%. Me = methyl; Ac = acetate; Bn = benzyl.

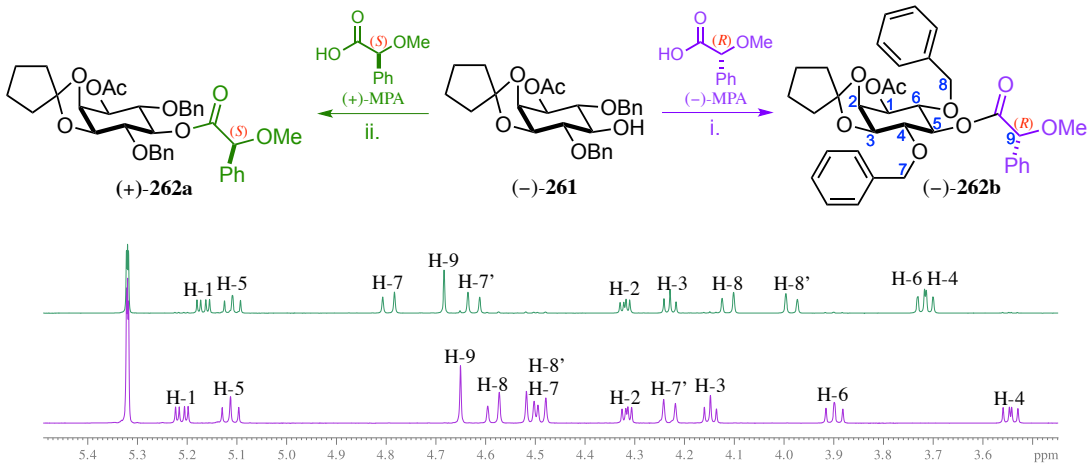
3.6. Confirming the absolute configuration of (–)-261

Although Laumen *et al*. had confirmed the absolute configuration of 1D-(–)-**151** through chemical correlation,[92] no other studies had been conducted since to support this assignment. Various orthogonal methods are now available for assigning the absolute configuration of organic

compounds. X-Ray crystallography[323] (Chapter 4) and NMR[268] are amongst the most prominent. Despite numerous crystallisation trials, however, the crystals obtained for (–)-**151** were of very poor quality, and could not be used for X-Ray crystallographic analysis. However, as described by Wenzel[324] and Seco *et al*.,[268] diastereoisomeric derivatisation coupled with ^{1}H NMR experiments can be used to calculate both the *e.e*. of a compound and assign its absolute stereochemistry. Notably, methods have been developed for the determination of the absolute configuration of inositol derivates[325,326] using the Trost's conformational model.[327] We chose to confirm the stereochemistry of (–)-**261** *via* NMR methods. Assignment of the absolute configuration by NMR was undertaken using the procedures by Seco *et al*.,[268] and following the sequence of steps described below:

1. **Derivatisation of the enantiomerically pure alcohol (–)-261, was carried out with each enantiomer ((*R*)- and (*S*)-) of a chosen chiral anisotropy reagent.**

   Although α-methoxy-α-trifluoromethylphenylacetic acid (MTPA), also called Mosher's acid,[328] is the most well known chiral derivatising agent, α-methoxyphenylacetic acid (MPA) was found to be more reliable for the stereochemical assignment of secondary alcohols.[268] As such, esterification of (–)-**261** with each enantiomer of MPA in the presence of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and 4-DMAP yielded the two diastereoisomeric esters (+)-**162a** and (–)-**162b** (Scheme 3.13). Interestingly, while



Scheme 3.13 Derivatisation of (–)-**261** with each enantiomer of MPA gave two diastereoisomers the (*S*)-MPA-ester (+)-**262a** (green) and the (*R*)-MPA-ester (–)-**262b** (purple). The ^{1}H NMR spectra (500 MHz, CD_2Cl_2) of these diastereoisomers were used for assignment of the absolute configuration. *Reagents and conditions*: i. EDC, 4-DMAP, CH_2Cl_2, RT, 24 h, 72% ii. EDC, 4-DMAP, CH_2Cl_2, RT, 3 d, 52%. Ac = acetate; Bn = benzyl; Me = methyl; Ph = phenyl.

esterification of (–)-**261** with the (*R*)-(–)-MPA proceeded smoothly, reaction with the (*S*)-(+)-MPA was slow, and did not reach completion. As a result, purification of the (*S*)-MPA-ester derivative was found to be extremely difficult as the polarity of the starting material and product were very similar.[VI] Each diastereoisomer gave a different ^{1}H NMR spectrum (Scheme 3.13). These spectra were analysed and compared, to determine the absolute configuration of (–)-**261**.

2. The conformational model for the chiral anisotropy reagent chosen was considered

The stereogenic centre under consideration features the MPA ester, an α-proton, and two substituents L_1 and L_2 (Figures 3.4 and 3.5). The substituent facing the aryl ring of the MPA ester experience an anisotropic shielding effect due to the local induced magnetic field of the aromatic ring. The protons on this substituent therefore appear more upfield (lower δ) by ^{1}H NMR. This shielding effect will be experienced by a different substituent (L_1 or L_2) in each of the two diastereoisomers (+)-**262a** and (–)-**262b**. As such, the difference in the chemical shift value ($\Delta\delta^{RS}$) of the substituent's proton signal in each diastereoisomer is a measure of the anisotropic effect. The sign of $\Delta\delta^{RS}$ is an indication of the relative spacial arrangement of L_1 and L_2 with reference to the phenyl ring on the MPA ester. The protons

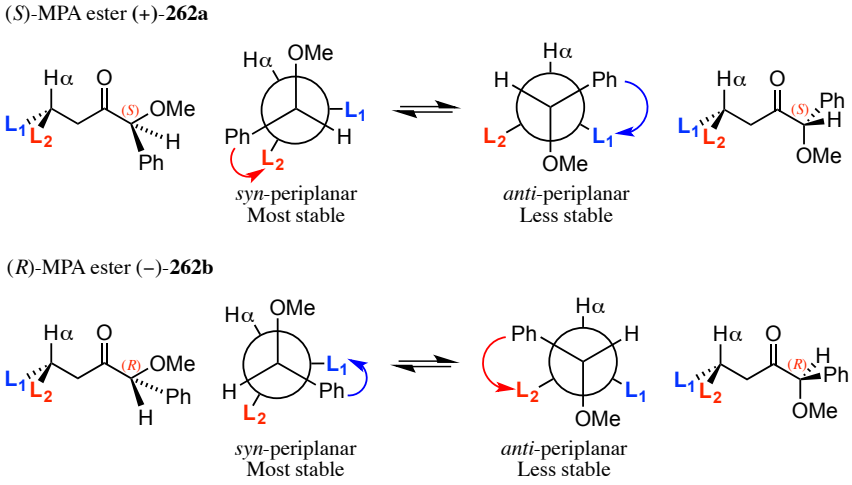


Figure 3.4 Amongst the two main conformations of the (*R*)- and (*S*)-MPA esters, the *syn*-periplanar conformer has been shown to be the most stable. This serves as the basis for the model in Figure 3.5. Me = methyl; Ph = phenyl; Bn = benzyl; MPA = α-methoxyphenylacetic acid.

[VI] It is important to note that when determining the *e.e.*, rather than the absolute configuration using this NMR technique, the products should be kept crude for analysis: See Section 3.15.1.

that are shielded in the diastereoisomer containing (*R*)-MPA (–)-**262b** will give a negative $\Delta\delta^{RS}$ and those that are shielded in (+)-**262a** will give a positive $\Delta\delta^{RS}$. Determination of the absolute configuration can be achieved unequivocally when the system considered is conformationally locked, resulting in large $\Delta\delta$ values.

The conformational model developed by Trost[327] for MPA esters is closely related to the model for MTPA esters developed by Dale and Mosher's.[328] These empirical models were later evaluated and rationalised through computational modelling and dynamic NMR.[329,330] These studies showed that the *syn*-periplanar and *anti*-periplanar conformers are the two main conformers for the MPA esters of secondary alcohols.[VII] The *syn*-periplanar conformation, in which the MPA methoxy, MPA carbonyl and α-proton are placed in the same plane, was found to be the most stable (Figure 3.4).

According to this conformational model, therefore, protons H-1 and H-6 of (+)-**262a** and protons H-3 and H-4 of (–)-**262b** are expected to be shielded, if the configuration of (–)-**261**

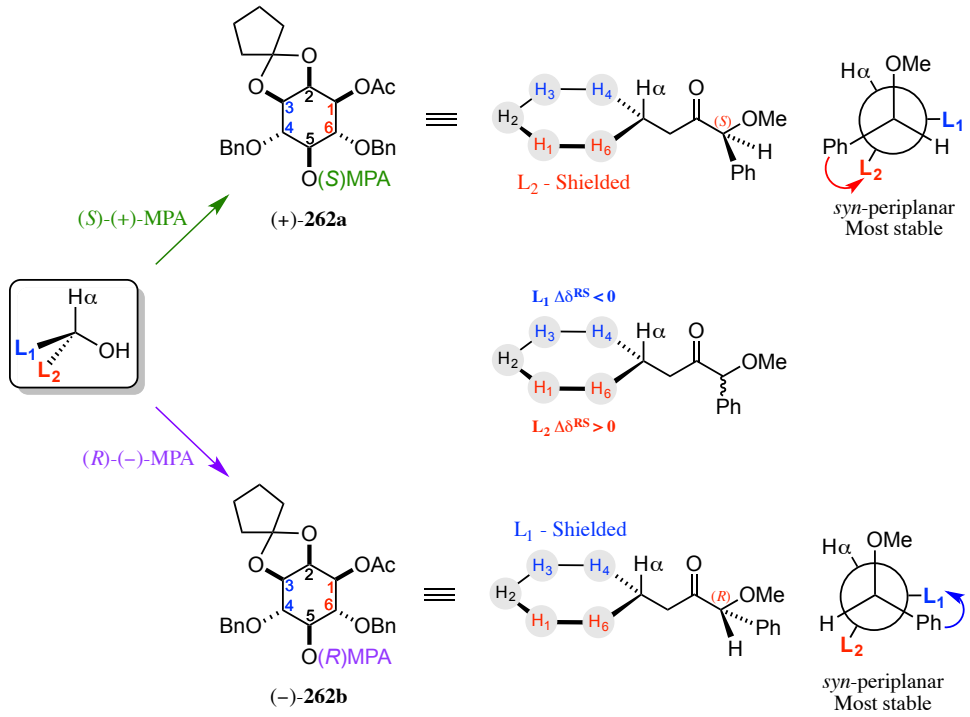


Figure 3.5 Conformational model for MPA esters allowing the determination of the absolute configuration of (–)-**261**. Me = methyl; Ph = phenyl; Ac = acetate; Bn = benzyl; MPA = α-methoxy-phenylacetic acid.

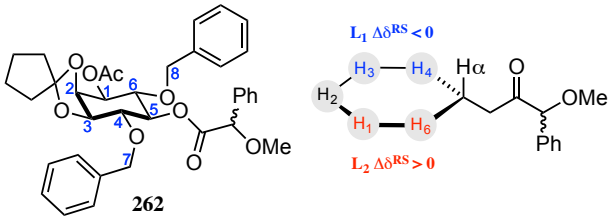
[VII] Contrastingly, MTPA esters of secondary alcohols are known to have three main conformers. This conformational complexity is the reason why MTPA is currently recognised as an unreliable chiral derivatising agent.

is indeed (–)-1D-1-*O*-acetyl-4,6-di-*O*-benzyl-*myo*-inositol. The parameter $\Delta\delta^{RS}$ for this configuration is thus expected to be positive for protons H-1 and H-6 and negative for protons H-3 and H-4 (Figure 3.5).

3. The ^{1}H NMR spectra of (+)-262a and (–)-262b were analysed and the chemical shift differences ($\Delta\delta^{RS}$) were calculated for all relevant protons

As shown in Table 3.1, $\Delta\delta^{RS}$ was indeed negative for protons H-3, H-4 and H-7 and positive for protons H-1, H-6 and H-8. This matched our prediction using the conformational model in Figure 3.5. According to this study, the configuration of (–)-**261** was therefore assigned as (–)-1D-1-*O*-acetyl-4,6-di-*O*-benzyl-*myo*-inositol.[92]

Table 3.1 Calculation of the differences in chemical shifts $\Delta\delta^{RS}$ for relevant protons signals in the ^{1}H NMR spectra of (+)-**262a** and (–)-**262b**. The absolute stereochemistry was confirmed using the model described in Figure 3.5.



262

Position	δ^R (–)-**262b**[a]	δ^S (+)-**262a**[a]	$\Delta\delta^{RS}$	L_1 or L_2
1	5.21	5.17	0.04	L_2
2	4.32	4.32	0.00	N/A
3	4.15	4.23	–0.08	L_1
4	3.54	3.72	–0.18	L_1
5	5.11	5.11	0.00	N/A
6	3.90	3.72	0.18	L_2
7	4.49	4.79	–0.30	L_1
7’	4.23	4.62	–0.39	L_1
8	4.58	4.11	0.47	L_2
8’	4.51	3.98	0.53	L_2

[a]The chemical shift (δ) values quoted were obtained from the ^{1}H NMR spectra shown in scheme 3.13, run at 500 MHz, in CD_2Cl_2. Me = methyl; Ac = acetate; Ph = phenyl.

To investigate the results obtained through this analysis, Density Functional Theory (DFT) calculations were carried out by Joe Bluck and William Glass to predict the theoretical ^{1}H NMR spectrum of each diastereoisomer (+)-**262a** and (–)-**262b**. First, a conformational analysis established that the *syn*-periplanar arrangement is indeed the lowest energy conformation. DFT calculations on this conformation were performed using the B3LYP exchange correlation functional and the basis set 6-31G* (See Appendix D). This analysis was used to predict the ^{1}H NMR chemical shifts for each diastereoisomer (Table 3.1). The results obtained, however, were inconclusive, revealing distinct flaws in the molecular model used for the calculations. Given the complexity of these structures, optimisation of the basis set towards these inositol systems would be needed to generate reliable theoretical data.

The use of Vibrational Circular Dichroism (VCD) to further support the assignment of the absolute stereochemical configuration of (–)-**261** was also considered. This analysis involves the comparison of measured and theoretical VCD spectra for the compounds under consideration.[331,332] The European Centre for Chirality (EC^{2})[VIII] provides this service and will be contacted to carry out these experiments.

[VIII] http://www.chemtech.ugent.be/en/spin-offs/EC2

3.7. Chemoenzymatic approach applied to deuterated derivatives

Having successfully completed the enantioselective desymmetrisation of **149**[90] with Lipozyme TL-IM® and confirmed the absolute configuration of (–)-**261**, it was important to consider the applicability of this methodology to deuterated *myo*-inositol derivatives. Dr Alex Saunders in the group developed a robust and efficient multi-gram synthesis of D_6 *myo*-inositol **183** (deuterium incorporation: 90% D_6, 10% D_5).[47] Hence, we investigated whether D_6 *myo*-inositol **183** could be used as a starting material for the synthesis of D_6 *meso*-4,6-di-*O*-benzyl-*myo*-inositol **263** and whether Lipozyme TL-IM® could tolerate the desymmetrisation of such a deuterated inositol derivative.

3.7.1. Synthesis of D_6 4,6-di-*O*-benzyl-*myo*-inositol **263**

Similar to the synthesis of H_6 **149**, D_6 *myo*-inositol **183** was first treated with triethylorthoformate and substoichiometric PTSA·H_2O in DMF[296] to give D_6 inositol orthoformate **264**. Compared to the preparation of H_6 orthoformate **113**, this reaction was slow and after seven days stirring at 105 °C, significant amounts of starting material remained. The product obtained was purified by column chromatography over silica gel and despite the low yield (31%), any remaining starting material was easily recovered (45%). Mass spectrometry studies showed that there had been no change in the deuterium incorporation during this reaction (89% D_6, 11% D_5). Additionally, the structure of this deuterated orthoformate **264** (Figure 3.6) was established through X-Ray crystal analysis by Dr Amber L. Thompson (Chemical Crystallography Lab, Oxford; Appendix B), and was found to be similar to the protonated analogue **113**.[297]

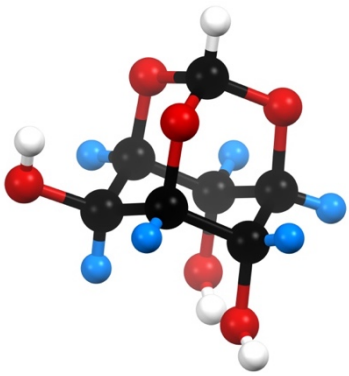
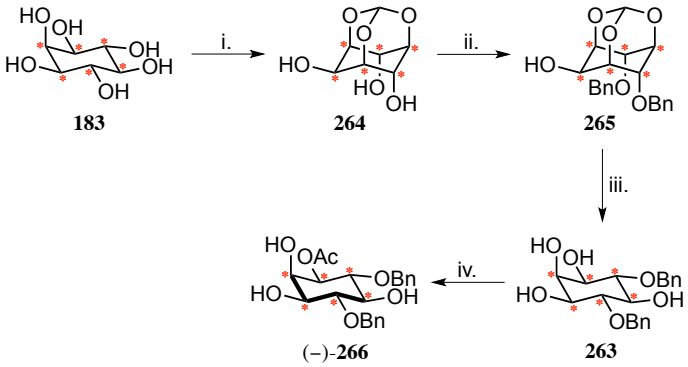


Figure 3.6 X-Ray crystal structure of deuterated orthoformate **264**. This picture was generated through Mercury 3.8. Key: carbon, black; oxygen, red; deuterium, blue; hydrogen, white.

From deuterated orthoformate **264**, regioselective benzylation of the 4- and 6-axial positions followed by acidic methanolysis proceeded smoothly to give *meso* D_6 4,6-*O*-dibenzyl *myo*-inositol **265** in an overall yield of 21%, over three steps, and with a deuterium incorporation of 92% D_6 and 8% D_5, as determined by mass spectrometry (Scheme 3.14).



Scheme 3.14 Synthesis of deuterated (–)-**266** starting from D_6 *myo*-inositol **183**. *Reagents and conditions*: i. $CH(OEt)_3$, PTSA·H_2O, DMF, 105 °C, 7 d, 31%, 89% D_6, 11% D_5 ii. a. LiH, DMF, RT, 30 min b. BnBr, RT, 48 h, 77%, 91% D_6, 9% D_5 iii. PTSA·H_2O, MeOH, RT, 24 h, 86%, 92% D_6, 8% D_5 iv. Lipozyme TL-IM®, vinyl acetate/hexane (1:1), 45 °C, 15 h, 84%, >99% *e.e.*, 89% D_6, 11% D_5. D = deuterium; Ac = acetate; Bn = benzyl.

3.7.2. Desymmetrisation of D_6 4,6-di-*O*-benzyl-*myo*-inositol **263**

Desymmetrisation of the *meso* deuterated derivative **263** using Lipozyme TL-IM® was successful, yielding (–)-**266** in 86% yield, without loss of deuterium incorporation (Scheme 3.14). The *e.e.* of the acetylated product (–)-**266** was determined by chiral HPLC, and was found to be greater than

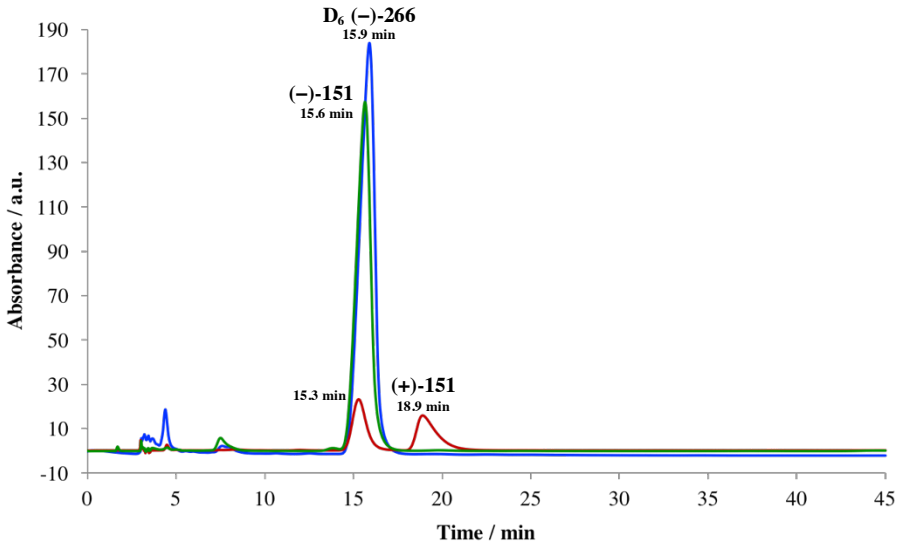


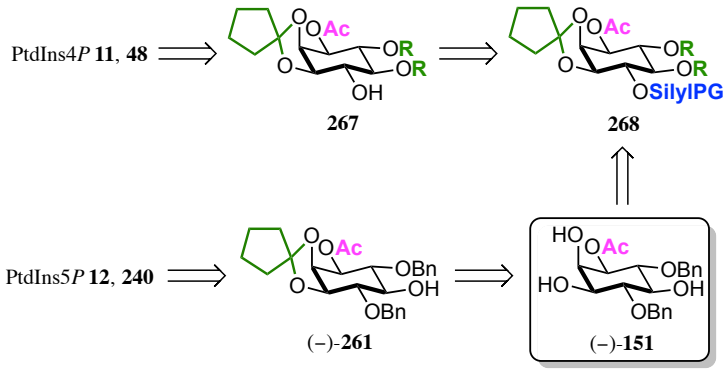
Figure 3.7 Chiral HPLC traces (ChiralPak® AD-H column, heptane/IPA 85:15, 1.0 mL.min^{-1}, 254 nm) of the single enantiomers (–)-**151** (green) and D_6 (–)-**266** (blue), obtained through enzymatic desymmetrisation, compared to the trace for racemic, protonated (±)-**151** (red).

99% (Figure 3.7). The enantioselectivity of this transformation is therefore comparable to that of the desymmetrisation of protonated derivative **149**.

3.8. Synthesis of a fully protected *myo*-inositol derivative towards PtdIns4*P*

Having optimised a robust desymmetrisation strategy for both protonated and deuterated *myo*-inositol analogues **149** and **265**, the focus of this research shifted toward developing an efficient protecting group strategy towards a series of saturated and unsaturated PtdIns4*P* and PtdIns5*P* derivatives (Scheme 3.15).

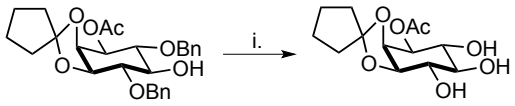
The 2,3-*O*-cyclopentylidene protected derivative (–)-**261** is an ideal precursor towards saturated PtdIns5*P* as it contains orthogonal protecting groups and possesses a free hydroxyl at the 5-position that can be easily phosphorylated. We aimed to synthesise a similar precursor towards PtdIns4*P*, such as **267** (Scheme 3.15), featuring a free hydroxyl group at the 4-position and easily removable protecting groups at the 2-, 3-, 5- and 6-positions.



Scheme 3.15 Retrosynthesis and protecting group strategy towards PtdIns5*P* and PtdIns4*P* featuring the enantiomerically pure derivative (–)-**151**, obtained through enzymatic desymmetrisation. The protecting groups to be removed in a final, global deprotection step are highlighted in green. In pink and blue are the orthogonal protecting groups that allow for the differentiation between the 1- and 4-positions respectively. Silyl = silyl protecting group; Ac = Acetal; Bn = Benzyl. R = acid labile group.

3.8.1. Synthesis of the 4-*O*-silyl-protected diols (–)-**270** and (–)-**273**

Deprotection of the 4- and 6-*O*-benzyl esters on (–)-**261** *via* hydrogenolysis was accomplished smoothly using 10% Pd/C in EtOAc under an atmosphere of $H_{2(g)}$ (Scheme 3.16). Notably, the same transformation in MeOH resulted in the additional, undesired cleavage of the 2,3-*O*-cyclopentylidene acetal, as observed by mass spectrometry and NMR analysis of the crude material.[333]

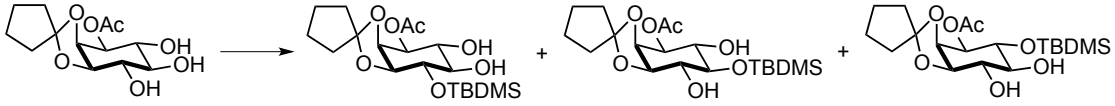


(–)-**261** (–)-**269**

Scheme 3.16 Hydrogenolysis of the 4- and 6-*O*-benzyl ethers on (–)-**261**. *Reagents and conditions*: 10% Pd/C, $H_{2(g)}$, EtOAc, RT, 2 h, 94%. Ac = acetate; Bn = benzyl.

As previously demonstrated in Chapter 2, the 4-position of inositol frameworks such as (–)-**269** can be regioselectively protected as 4-*O*-pivalate esters. We therefore postulated that protection of (–)-**269** with a TBDMS protecting group would be similarly selective for the 4-position hydroxyl group. Attempts to regioselectively silylate (–)-**269** using TBDMSCl, as previously (Section 2.4) proved unsuccessful (Table 3.2; Entries 1 and 2). Even at room temperature and after extended reaction times, only the starting material remained. Contrastingly, the more reactive silylating agent *tert*-butyldimethylsilyl triflate (TBDMSOTf)[334] led to the formation of the expected product (–)-**270** (Table 3.2; Entry 3) in 10% isolated yield, with significant amounts of the starting material (–)-**269** remaining. The low conversion observed in this transformation was later attributed to the the poor solubility of the starting material (–)-**269** in CH_2Cl_2 at –78 °C. In the presence of other bases such as

Table 3.2 Summary of reaction conditions for the silylation of (–)-**269**.



(–)-**269** (–)-**270** (–)-**271** (–)-**272**

Entry	Reagent(s) (eq)	Base	Solvent	Temperature [°C]	Time [hours]	Yield 270:271:272 [%][a]
1	TBDMSCl (1.01) imidazole (1.5)	Pyridine	Pyridine	–10 to RT	52	-[b]
2	TBDMSCl (1.01) imidazole (1.5)	-	DMF	RT	120	-[b]
3[c]	TBDMSOTf (1.0)	2,6-Lutidine (1.5)	CH_2Cl_2	–78 to RT	71	10:0:0
4	TBDMSOTf (1.1)	Et_3N (1.3)	CH_2Cl_2	0 to RT	30	28:27:8
5	TBDMSOTf (1.1)	Pyridine	Pyridine	0 to RT	23	40:36:19
6	TBDMSOTf (1.1)	Pyridine	Pyridine	–30	24	49:36:11
7	TBDMSOTf (1.0)	2,6-Lutidine (1.5)	THF	–78	25	55:33:8

[a] Isolated yields [b] None of the desired product (–)-**270** was formed; only starting was material recovered [c] After 23 hours of reaction at –78 °C TLC analysis indicated that most of the starting material remained, more TBDMSOTf and 2,6-lutidine was therefore

pyridine or triethylamine,[335] and at higher temperatures, formation of the regioisomers (–)-**271** and (–)-**272** was observed (Table 3.2; Entries 4 and 5). The relative ratio of the regioisomers obtained was not significantly altered when the reaction was carried out at –30 °C (Table 3.2; Entry 6). Finally, we found that (–)-**269** could be fully solubilised in THF at –78 °C. As such, treatment with TBDMSOTf in the presence of the preferred milder base, 2,6-lutidine, resulted in the full conversion of (–)-**269**, with (–)-**270** as the major regioisomer (Table 3.2; Entry 7). Difficulties were encountered when purifying the mixture of the three regioisomers, as (–)-**270** and (–)-**272** were found to have very similar polarities. Each regioisomer isolated in this study was fully characterised using 2D NMR experiments including ^{1}H-^{29}Si and ^{1}H-^{13}C HMBC (Figure 3.8).

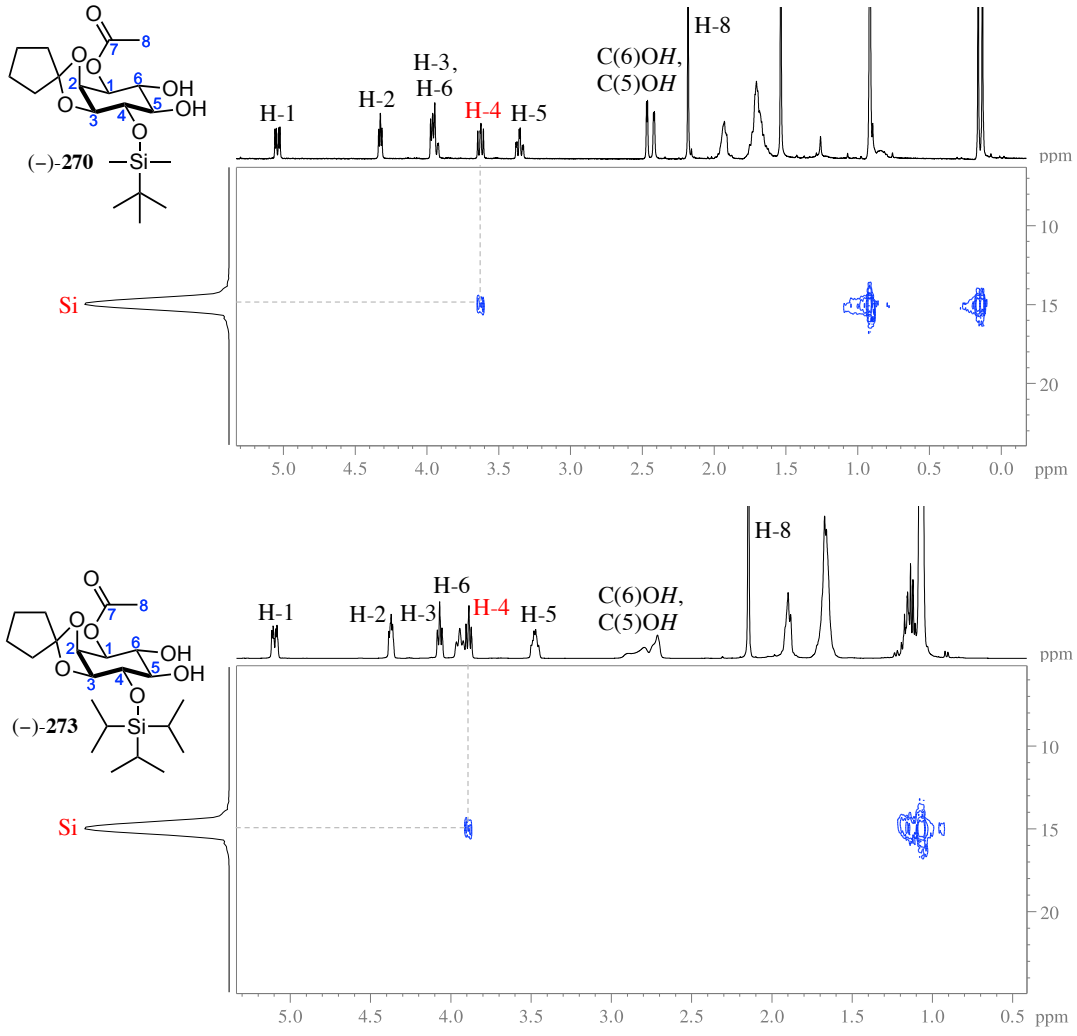
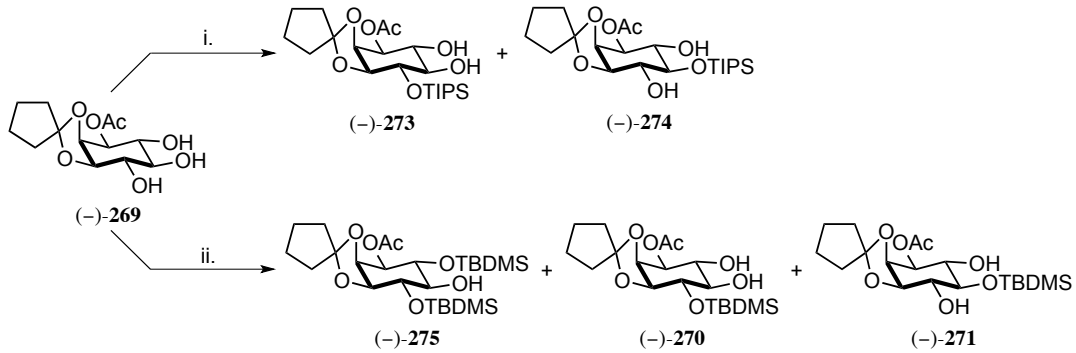


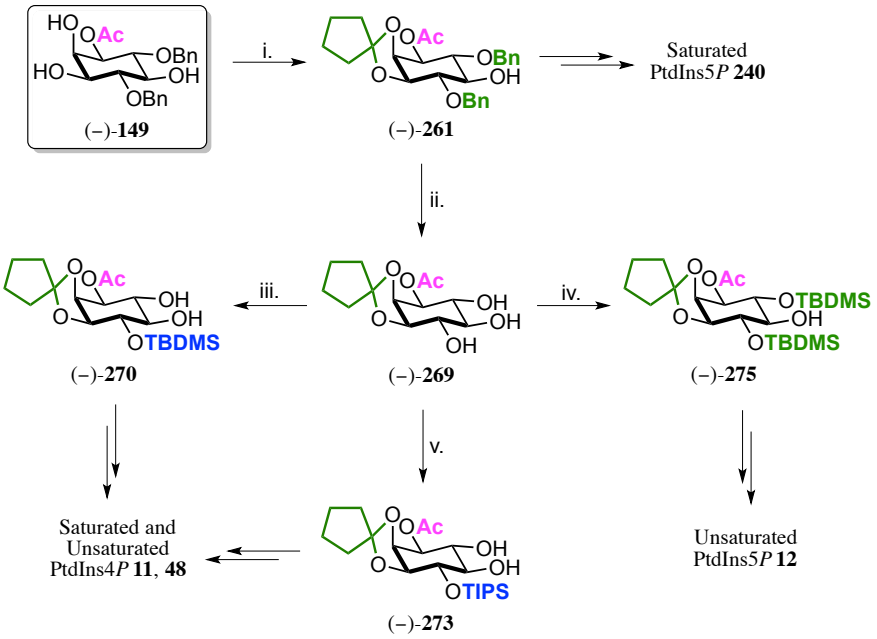
Figure 3.8 ^{1}H-^{29}Si HMBC for (–)-**270** and (–)-**273** accounting for the position of the TBDMS and TIPS ethers on the *myo*-inositol ring. The ^{1}H and ^{29}Si NMR spectra were acquired at 500 MHz and 99 MHz respectively, in $CDCl_3$. TBDMS = *tert*-butyldimethylsilyl; TIPS = triisopropylsilyl.

The regioselectivity of this reaction was significantly improved when (–)-**269** was protected with the bulkier and more stable triisopropylsilyl (TIPS) group. Treatment with up to 2.2 equivalents of triisopropylsilyl triflate (TIPSOTf), under the optimised reaction conditions, yielded the desired regioisomer (–)-**273** in good yields (Scheme 3.17), as determined by ^{1}H-^{29}Si and ^{1}H-^{13}C HMBC NMR (Figure 3.8). Although (–)-**274** was also isolated, the difference in polarity between the two regioisomers greatly facilitated the purification of (–)-**273**. Increasing the number of equivalents used in these silylation transformations led to an interesting observation. Although up to 2.2 equivalents of TIPSOTf was used for the mono-protection of (–)-**269** with TIPS, the disubstituted product 4,6-di-*O*-TBDMS ether (–)-**275** was obtained when an excess of TBDMSOTf was used in the reaction (Scheme 3.17). The difference in steric bulk between the two protecting groups accounts well for this observation.



Scheme 3.17 Silylation of (–)-**269** using an excess of the silylating agents TIPSOTf and TBDMSOTf. *Reagents and conditions*: i. TIPSOTf (2.2 eq), 2,6-lutidine, THF, –78 °C, 16.5 h, 71% (–)-**273**, 20% (–)-**274** ii. TBDMSOTf (2.2 eq), 2,6-lutidine, THF, –78 °C, 17 h, 63% (–)-**275**, 15% (–)-**270**, 17% (–)-**271**. TBDMS = *tert*-butyldimethylsilyl; TIPS = triisopropylsilyl; Ac = acetate.

This study into the silylation of triol (–)-**269**, provided three important building blocks towards the synthesis of PtdIns4*P* and PtdIns5*P*. Although preparation of the 4-*O*-TIPS ether (–)-**273** was higher yielding, TBDMS ethers are usually easier to cleave. As such, both derivatives were considered for optimisation of the subsequent steps towards PtdIns4*P*. Finally, we recognised the 4,6-di-*O*-TBDMS derivative (–)-**275** as a useful precursor to PtdIns5*P*, similarly to (–)-**261**. Protection of the 4- and 6-positions with silyl ethers is favourable, in view of their compatibility with unsaturated chains (Scheme 3.18).



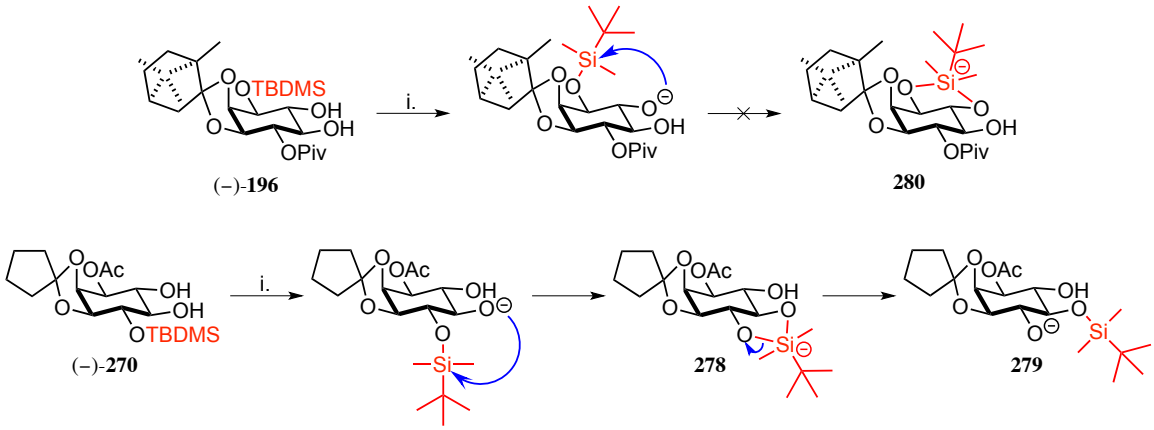
Scheme 3.18 Overview of the synthetic strategy developed towards PtdIns4*P* **11** and **48**, and PtdIns5*P* **12** and **240**. The protecting groups to be removed in a final, global deprotection are highlighted in green. In pink and blue are the orthogonal protecting groups that allow for the differentiation between the 1- and 4-positions respectively. TBDMS = *tert*-butyldimethylsilyl; TIPS = triisopropylsilyl; Ac = acetal; Bn = benzyl.

3.8.2. Protection of the 5- and 6-position of (–)-**270** and (–)-**273**

A thorough investigation into suitable protecting groups for the 5- and 6-positions of inositol frameworks such as (–)-**196** was conducted in Chapter 2. We found that decarboxylative allylation under $Pd(PPh_3)_4$ catalysis gave good yields of the corresponding allylated product.[261] Interestingly, however, reaction of (–)-**270** with the allylating agent **207** under identical conditions, yielded an inseparable mixture of the expected product (–)-**276** and the side product (–)-**277** in a ratio of 1.6:1.0, as determined by ^{1}H NMR analysis (Table 3.3; Entry 1).

We reasoned that these reaction conditions promote TBDMS migration onto the neighbouring 5-hydroxyl group to give alkoxide **279**. Subsequent allylation of the remaining hydroxyl group generates the side product (–)-**277**.[243] It was postulated that migration occurs *via* a five-membered intermediate **278** and is promoted by the formation of an alkoxide in close proximity (Scheme 3.19).[336] The absence of silyl migration for the allylation of (–)-**196** *via* intermediate **280** is difficult to explain definitively, although the electronic and steric environment experienced by the

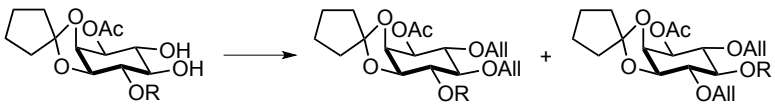
silyl protecting group at the 1-position in (–)-**196** is different to that experienced by a silyl group at the 4-position on (–)-**270** (Scheme 3.19).



Scheme 3.19 Whilst silyl migration was not observed for the allylation of (–)-**196**, migration is thought to occur *via* a pentacoordinate silicon intermediate **278** for (–)-**270**.[327] *Reagents and conditions*: **207**, $Pd(PPh_3)_4$, THF, reflux, 4–6.5 h. Ac = acetate; Piv = pivaloyl.

Reaction of the 4-*O*-TIPS ether derivative (–)-**273** under these allylation conditions led to a similar outcome, giving (–)-**281** and (–)-**282** in a ratio of 1.3:1.0. This indicated that the TIPS group on (–)-**273** is more prone to migration than the TBDMS group (Table 3.3; Entry 2). To identify the

Table 3.3 Summary of reaction conditions for the allylation of (–)-**270** and (–)-**273**.



R = TBDMS (–)-**270** — R = TIPS (–)-**273**

R = TBDMS **276** — R = TIPS **281**

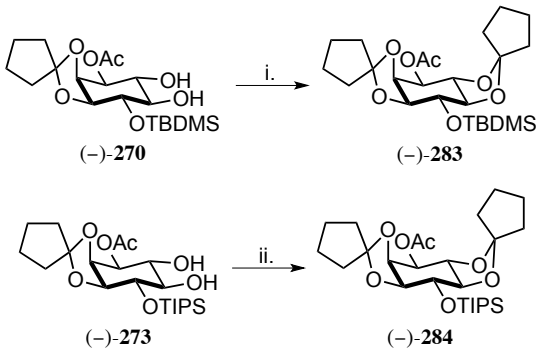
R = TBDMS **277** — R = TIPS **282**

Entry	Substrate	Reagents[a]	Temperature [°C]	Time [hours]	Ratio 276/277, 281/282[b]
1	(–)-**270**	**207**, $Pd(PPh_3)_4$	67	6.5	1.6:1.0
2	(–)-**273**	**207**, $Pd(PPh_3)_4$	67	1.5	1.3:1.0
3	(–)-**270**	**207**, $Pd(PPh_3)_4$	67	1	1.5:1.0
4	(–)-**270**	**207**, $Pd(PPh_3)_4$	RT	48	-[c]
5	(–)-**270**	$Pd(PPh_3)_4$	67	21	No migration

[a] 8.0 eq of **207**, 10% $Pd(PPh_3)_4$ in THF were used in each case [b] Ratio determined by ^{1}H NMR of the crude reaction mixture [c] None of (–)-**276** was formed, only starting material was isolated. Ac = acetate; All = allyl; TBDMS = *tert*-butyldimethylsilyl; TIPS = triisopropylsilyl; Ph = phenyl.

origin of this silyl migration and thus understand whether optimisation of the reaction conditions would circumvent this issue, several test reactions were carried out on (–)-**270**. First, reaction time was found to have little effect on the ratio of expected product to the migrated product obtained (Table 3.3; Entries 1 and 3). Assuming that the reaction is reversible, this suggests that a chemical equilibrium is reached early, making it difficult to control the extent of silyl migration during the reaction. In an attempt to slow down the rate of this transformation, the reaction was performed at RT (Table 3.3; Entry 4), however, after 48 hours, only unreacted starting material was detected by TLC analysis. Finally, migration of the silyl protecting groups on (–)-**270** was not observed when the substrate was heated under reflux in the absence of the allylating agent **207** (Table 3.3; Entry 5). This result confirms that migration does not stem from the catalyst $Pd(PPh_3)_4$ alone, but likely from its ability to generate the *tert*-butoxide anion from **207**, which in turn deprotonates (–)-**270** (Section 2.5, Scheme 2.9).

Considering the allylation of (–)-**270** and (–)-**273** to give **276** and **281** respectively was found to be low yielding and complex to optimise, alternative protecting groups were investigated. In particular, despite previous unsuccessful attempts (Section 2.5), we decided to re-evaluate the use of acetonide protecting groups for the simultaneous protection of the 5,6-diol on (–)-**270**. We hypothesised that protection of the *trans* 5,6-diol with a second cyclopentylidene moiety would be favourable. Although deprotection of the *cis* 2,3-diols would inevitably occur under these conditions, ultimately, both positions would be protected to give the product (–)-**283**. This protecting group is also well suited to the final global deprotection. As such, acetalisation of (–)-**270** with 1,1-dimethoxycyclopentane **260** afforded the 2,3:5,6-di-*O*-cyclopentylidene acetal (–)-**283** in 62% yield (Scheme 3.20). As predicted for a *trans*-diequatorial diol, the rate of protection at these positions was slower than protection of the *cis* 2,3-diol. Indeed, the reaction did not usually reach completion. Small amounts of starting material remained (18%) despite the large excess of reagent. For protection of the 4-*O*-TIPS derivative (–)-**273**, therefore, the reaction was heated to 30 °C for 18 hours. The fully protected inositol (–)-**284** was obtained in 85% yield with minimal amount of starting material remaining (Scheme 3.20).



Scheme 3.20 Protection of the 5,6-diol on (–)-**270** and (–)-**273** using a cyclopentylidene acetal. *Reagents and conditions*: i. **260**, PTSA·H_2O, RT, 4 h, 62% (–)-**283**, 18% (–)-**270** ii. **260**, PTSA·H_2O, 30 °C, 18 h, 85%. TBDMS = *tert*-butyldimethylsilyl; TIPS = triisopropylsilyl; Ac = acetate.

3.8.3. Overview

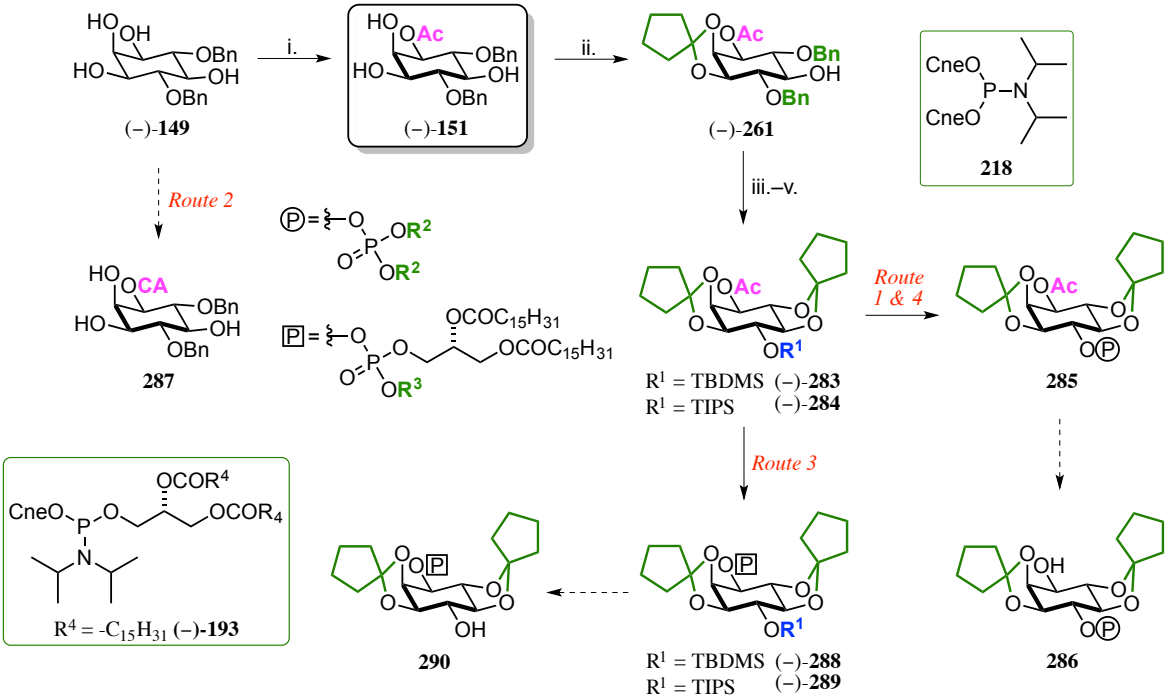
With the fully protected inositol derivatives (–)-**283** and (–)-**284** in hand, a set of parallel routes, briefly described below, were explored towards dipalmitoyl PtdIns4*P* **48** (Scheme 3.21). We aimed to apply the knowledge gained through this research to the synthesis of dipalmitoyl PtdIns5*P* **240**, and subsequently to unsaturated derivatives of PtdIns4*P* and PtdIns5*P* **11** and **12**.

Route 1 – From (–)-**283** or (–)-**284**, deprotection of the 4-*O*-silyl group and phosphorylation with the cyanoethyl-protected phosphoramidite **218** can be undertaken using conditions developed in Chapter 2. We therefore considered optimising the selective deacetylation of the inositol framework **285**, in the presence of cyanoethyl phosphate esters.

Route 2 – Cleavage of a chloroacetate (CA) protecting group is characteristically easier then that of an acetate group. As such we aimed to examine the versatility of Lipozyme TL-IM® for the desymmetrisation of **149** to provide intermediate **287**.

Route 3 – Deprotection of the 1-*O*-acetate group on (–)-**283** and (–)-**284** can be achieved following literature procedures. Success of this route then relied on optimising conditions for the coupling of the dipalmitoyl cyanoethyl-protected phosphoramidite (–)-**193** with (–)-**283** and (–)-**284**, followed by deprotection of the silyl groups without disruption of the diacylglycerol moiety.

Route 4 – We proposed to explore various other phosphate protecting groups on inositol framework **285**, as well as alternative global deprotection strategies.



Scheme 3.21 Overviews of the set of parallel routes explored towards PtdIns4*P*. The protecting groups to be removed in a final, global deprotection step are highlighted in green. *Reagents and conditions*: i. Lipozyme TL-IM®, vinyl acetate/hexane (1:1), 45 °C, 15 h, 98%, >99% *e.e.* ii. **260**, PTSA·H_2O, CH_2Cl_2, RT, 18 h, 92% iii. 10% Pd/C, $H_{2(g)}$, EtOAc, RT, 2 h, 94% iv. TBDMSOTf (1.0 eq), 2,6-lutidine, THF, –78 °C, 16.5 h, 55%, (–)-**270**; or TIPSOTf (2.2 eq), 2,6-lutidine, THF, –78 °C, 16.5 h, 71%, (–)-**273** v. **260**, PTSA·H_2O, RT, 4 h, 62% (–)-**283**; or **260**, PTSA·H_2O, 30 °C, 18 h, 85% (–)-**284**. Ac = acetate; Bn = benzyl; Silyl = *tert*-butyldimethylsilyl or triisopropylsilyl; CA = chloroacetate. R^2 and R^3 are phosphate protecting groups.

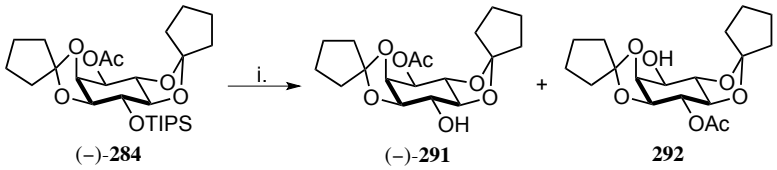
3.9. Route 1 toward PtdIns4*P*

3.9.1. Cleavage of the 4-*O*-TIPS ether on (–)-**284**

We established previously (Section 2.8) that $Et_3N{\cdot}3HF$ could efficiently cleave silyl groups, at the 1-position, on inositol systems featuring both a base-labile acyl group and acid-labile acetal group. The inositol derivative (–)-**284**, however, was found to be unstable in the presence of $Et_3N{\cdot}3HF$. After 41 hours of reaction at 40 °C, the expected product was not observed or isolated and none of the starting material was recovered (Table 3.4; Entry 1). Appropriate mild conditions for the deprotection of TIPS on acid- and base-sensitive substrates such as (–)-**284** were therefore sought.

Deprotection of (–)-**284** was attempted using Pyr·HF (Table 3.4; Entry 2).[337] We envisaged that the substrate would be more stable in the presence of a weaker base. After five days stirring at room temperature, purification by column chromatography afforded a mixture of two inseparable

regioisomers. It appeared that following deprotection of the silyl ether, the reaction conditions facilitated partial intermolecular transesterification of the 1-*O*-acetate ester to give the expected product (–)-**291** and the side product **292** in a ratio of 2.9:1.0 (Scheme 3.22). These were identified by NMR analysis. This type of acyl migration has been previously described on furanosides by Chevallier and Migaud.[338] Evidence suggests that optimisation of the reaction concentration would minimise intermolecular acyl migration, however, considering the low overall yield of this transformation, we decided to explore alternative reagents.

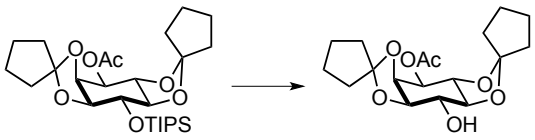


(–)-**284** (–)-**291** **292**

Scheme 3.22 Desilylation of (–)-**284** using Pyr·HF results in the intermolecular transesterification of the 1-*O*-acetate ester to give two inseparable regioisomers (–)-**291** and **292**. *Reagents and conditions*: i. Pyr·HF, THF/Pyridine, RT, 5 d, 39% (–)-**291**/**292** 2.9:1.0. Ac = acetate; TIPS = triisopropylsilyl.

The pentacoordinated silicate tris(dimethylamino)sulfonium difluorotrimethylsilicate, $[(Me_2N)_3S]^+[F_2SiMe_3]^-$ (TAS-F) has been shown to be a soluble, mild and anhydrous source of nucleophilic fluoride.[339] In addition, the electron donating ability of the three $(CH_3)_2N$ substituents on the sulfonium anion in TAS-F renders it non-electrophilic.[283] This reagent has been shown to be successful in the desilylation of highly complex, acid- and base-sensitive substrates,[284,340,341] as well as inositol derivatives.[342] Therefore, (–)-**284** was stirred with three equivalents of TAS-F at RT for

Table 3.4 Screening of reaction conditions for the desilylation of (–)-**284**



(–)-**284** (–)-**291**

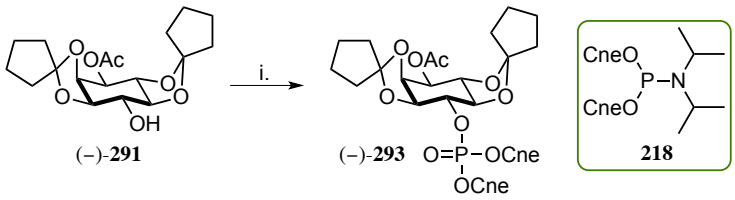
Entry	Reagents (eq)	Temperature [°C]	Solvent	Time [hours]	Yield [%]
1	$Et_3N{\cdot}3HF$ (5)	40	THF	41	-[a]
2	Pyr·HF	RT	THF/Pyridine	120	39% (–)-**291**/**292** 2.9:1.0[b]
3	TAS-F (3)	RT	DMF	1.5	75[c]

[a] The product (–)-**291** was not observed and none of the starting material could be recovered [b] Ratio determined by ^{1}H NMR analysis (See Scheme 3.22) [c] Isolated yield. Ac = acetate; TIPS = triisopropylsilyl; Pyr. = pyridine.

two and a half hours. Work-up and purification by column chromatography over silica gel provided (–)-**291** in 75% yield (Table 3.4; Entry 3).

3.9.2. Phosphorylation of (–)-**291** and selective 1-*O*-acetate cleavage

Phosphorylation of the free 4-*O*-hydroxyl of (–)-**291** was accomplished in 73% yield, under standard conditions, with freshly prepared phosphoramidite **218** (Scheme 3.23). Subsequent removal of the 1-*O*-acetate group was found challenging. Indeed, alkaline conditions could not be used in this instance due to the base-labile cyanoethyl phosphate esters (See Section 2.3 for a discussion about cyanoethyl-protected phosphates). The acidic nature of the protons β to the electron-withdrawing cyano-group allows for facile elimination of the cyanoethyl protecting groups. For this reason, a range of conditions were screened to optimise this selective deprotection.



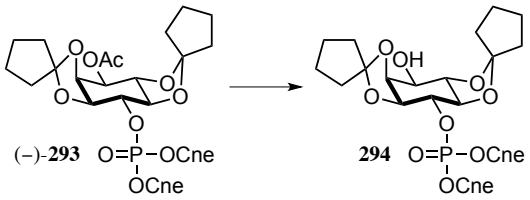
Scheme 3.23 Phosphorylation of (–)-**291** under standard conditions using cyanoethyl-protected phosphoramidite **218**. *Reagents and conditions*: i. a. **218**, 1*H*-tetrazole, CH_2Cl_2, RT, 20.5 h b. *m*CPBA, –78 °C to RT, 2 h, 86%. Ac = acetate; Cne = cyanoethyl.

First, KCN is known as a mild transesterification catalyst that is compatible with acid- and base-sensitive substrates.[343-345] However, when applied to the deacylation of (–)-**293**, cleavage of a cyanoethyl protecting group resulted, as observed by TLC and ^{1}H NMR analysis of the crude reaction mixture (Table 3.5; Entry 1).

Selective and mild cleavage of acetate groups using Bu_2SnO has also been applied to the synthesis of complex,[346] and biologically relevant molecules such as nucleosides[347] and sugars.[348] Deacylation of (–)-**293** with Bu_2SnO in toluene under reflux, gave a complex mixture of products as determined by ^{1}H NMR. In addition, phosphate hydrolysis was observed by ^{31}P NMR analysis of the crude reaction mixture (Table 3.5; Entry 2). When the reaction was performed in anhydrous MeOH, however, the desired product **294** was isolated in 10% yield (Table 3.5; Entry 3). This product was not fully characterised at this stage, as only a very small amount (~3 mg) was isolated.

Finally, considering the extensive use of enzymatic approaches for the mild, regio- and enantioselective deprotection of acetates,[349,350] deprotection of (–)-**293** was attempted using Lipozyme TL-IM®. After 3 days stirring at 40 °C, however, only starting material was recovered (Table 3.5; Entry 4).

Table 3.5 Screening of reaction conditions for the deacetylation of (–)-**294**



Entry	Reagents (eq)	Temperature [°C]	Solvent	Time [hours]	Yield [%]
1	KCN (0.5)	RT	MeOH	3	-[a]
2	Bu_2SnO (0.6)	reflux	Toluene	12	Complex mixture
3	Bu_2SnO (1.5)	reflux	MeOH	6	10[b]
4	Lipozyme TL-IM	40	Toluene/ *n*-butanol	72	-[c]

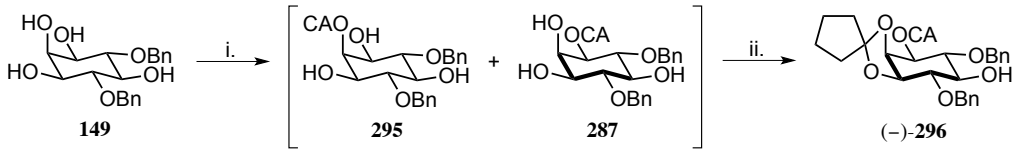
[a] Cleavage of the cyanoethyl phosphate esters was observed by TLC and ^{1}H NMR analysis of the crude reaction mixture [b] Isolated yield on a small scale (10 mg). Not enough of this product was isolated for characterisation [c] None of the product **294** was formed, only starting material was isolated. Ac = acetate; Cne = cyanoethyl.

Although other tin reagents,[351] or lipases such as Amano PS-30[45] and pig pancreas lipase[43] could have been screened for acetate deprotection, these preliminary results did not seem promising and other routes were investigated.

3.10. Route 2 toward PtdIns4*P*

The use of a chloroacetate ester as temporary protection for the 1-position on *meso*-**149** was considered. Cleavage of this protecting group can be accomplished under especially mild conditions, using thiourea,[352] hydrazine dithiocarbonate[353,354] or hydrazine acetate.[355] Therefore, chloroacetate protecting groups are known to be compatible with cyanoethyl phosphate esters[242] and have been used in inositol chemistry.[356] In addition, in their total synthesis of Vineomycin B_2, Kusumi *et al.* showed that chloroacetate groups are compatible with TAS-F for the deprotection of silyl protecting

groups.[341] With this in mind, we investigated the ability of Lipozyme TL-IM®[313] to catalyse the regio- and enantioselective desymmetrisation of *meso*-**149** with a chloroacetate group. Reaction of **149** with Lipozyme TL-IM® in hexane and vinyl chloroacetate did indeed yield the 1-*O*-chloroacetate-protected derivative **287**, however full regioselectivity for the 1-*O*-position was not achieved. An inseparable mixture of **287** and the 2-*O*-chloroacetate regioisomer **295** was obtained in a ratio of 5.0:1.0 as determined by ^{1}H NMR analysis (Scheme 3.24). Separation of this minor regioisomer was achieved in the following step. Acetalisation of the 2,3-diol of **287** with 1,1-dimethoxycyclopentane **260** followed by purification by column chromatography yielded the 1-*O*-chloroacetate-2,3:5,6-di-*O*-cyclopentylidene acetal derivative (–)-**296** in 59% yield over two steps (Scheme 3.24).



Scheme 3.24 Enantioselective, enzymatic desymmetrisation of **149** through protection with a chloroacetate ester. *Reagents and conditions*: i. Lipozyme® TL-IM, vinyl chloroacetate/hexane (1:1), 45 °C, 22 h, **287**/**295** 5.0:1.0 ii. **260**, PTSA·H_2O, RT, 24 h, 59% over two steps. Bn = benzyl; CA = chloroacetate.

Unfortunately, analysis of (–)-**296** by chiral HPLC indicated that the enantioselectivity of the desymmetrisation reaction to give **295** and **287** was low. A mixture of two enantiomers (–)-**296** and (+)-**296** was observed, with a calculated *e.e.* of only 79% (Figure 3.9). In addition, a sample of this

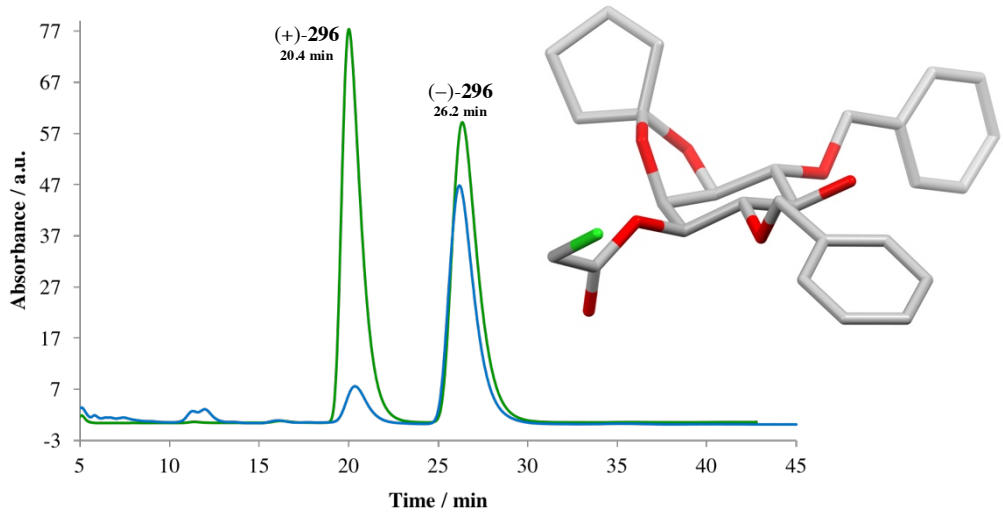


Figure 3.9 Chiral HPLC trace (ChiralPak® AD-H column, heptane/IPA 90:10, 0.8 mL.min^{-1}, 254 nm) of the bulk material of (–)-**296**, with an *e.e.* of 79% (blue), overlaid with that of the racemic crystals obtained (green). X-Ray crystal structure of (±)-**296**. The picture was generated through Mercury 3.8. Key: carbon, silver; oxygen, red; chlorine, green.

material was crystallised. Analysis by X-Ray crystallography (Dr Amber L. Thompson, Chemical Crystallography Lab, Oxford, Appendix B) revealed that the crystals obtained for (–)-**296** possessed a centrosymmetric space group, and were therefore racemic. This was indeed confirmed by chiral HPLC analysis of the crystals (Figure 3.9). Although enantio-enrichement of the bulk material would be possible *via* sequential crystallisations, this would render the synthesis less efficient. We therefore investigated alternative routes.

3.11. Route 3 towards PtdIns4*P*

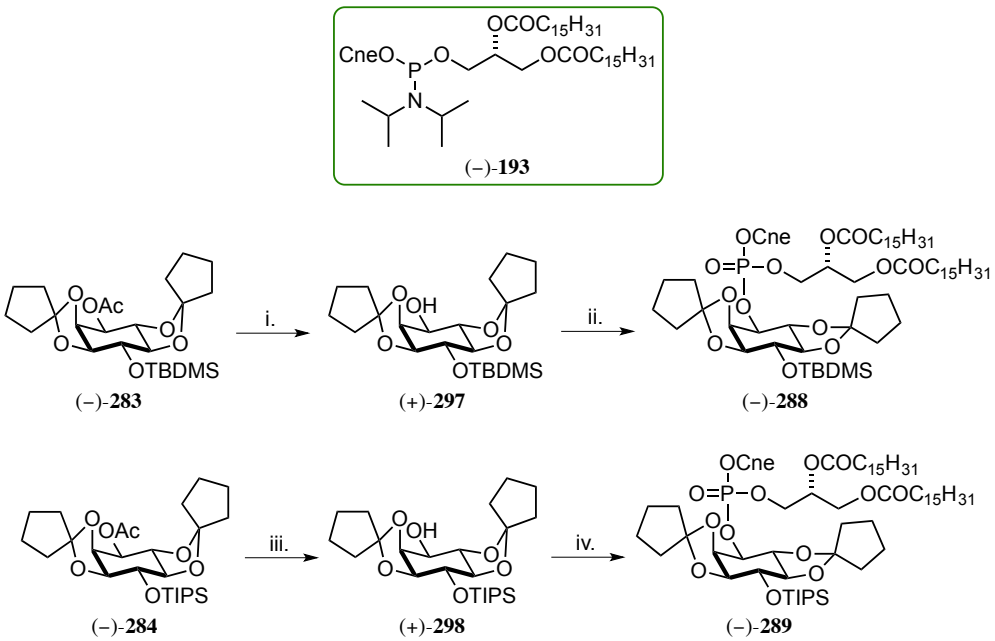
The results of our investigation into Routes 1 and 2 directed our research towards an alternative approach that involved the initial deprotection and phosphorylation of the 1-*O*-position. Although eliminating the need for an ambitious selective deacylation, this strategy required the manipulation of complex lipids over an increased number of synthetic steps.[77] Both fully protected derivatives 4-*O*-TBDMS ether (–)-**283** and 4-*O*-TIPS ether (–)-**284** were considered for this purpose. We reasoned that the difference in stability of the silyl ethers would provide some synthetic flexibility.

3.11.1. Deacetylation and phosphorylation at the 1-*O*-positions of (–)-**283** and (–)-**284**

First, deacetylation of (–)-**283** and (–)-**284** under alkaline conditions[82] yielded (+)-**297** and (+)-**298**, respectively. Installation of the diacylglycerol phosphate was completed using five equivalents of the freshly prepared phosphoramidite (–)-**193**. *In situ* oxidation with *m*CPBA generated both (–)-**288** and (–)-**289** in good yields (Scheme 3.25). Purification by column chromatography was this time not an issue (See Section 2.8). These results support the hypothesis that a less sterically demanding cyclic acetal protecting group at the 2,3-diol, as opposed to a camphor acetal, enables more facile phosphorylation at the neighbouring 1-position (See Section 2.8).

3.11.2. Silyl deprotection in the presence of the 1-*O*-diacylglycerol phosphate

Although the silyl group on (–)-**284** could be deprotected using TAS-F as a fluoride source (Table 3.4), these conditions were unsuccessful for the deprotection of (–)-**289** (Table 3.6; Entry 1). After two and a half hours of reaction the starting material had been fully consumed, as determined by TLC and mass spectrometry analysis. The product isolated, however, was unidentifiable by



Scheme 3.25 Acetate deprotection followed by phosphorylation with phosphoramidite (–)-**193** on both the 4-*O*-TBDMS ether (–)-**283** and 4-*O*-TIPS ether (–)-**284**. *Reagents and conditions*: i. NaOMe (2.6 M), MeOH/CH_2Cl_2, RT, 1.5 h, 92% ii. a. (–)-**193**, 1*H*-tetrazole, CH_2Cl_2, RT, 2.5 h b. *m*CPBA, –78 °C to RT, 16 h, 68% iii. NaOMe (2.6 M), MeOH/CH_2Cl_2, RT, 1 h, 83% iv. a. (–)-**193**, 1*H*-tetrazole, CH_2Cl_2, RT, 5 h b. *m*CPBA, –78 °C to RT, 14 h, 70%. Ac = acetate; Cne = cyanoethyl; TBDMS = *tert*-butyldimethylsilyl; TIPS = triisopropylsilyl.

^{1}H NMR as it featured very broad peaks. It was hypothesised that the basicity of the fluoride in TAS-F led to the undesired deprotection of a cyanoethyl group on the phosphate. Roush *et al*.[339,357,358] demonstrated in their work on the synthesis of (–)-Bafilomycin A1 that including water in silyl deprotections with TAS-F attenuated its basicity and, unlike TBAF, does not lead to undesired side reaction. When applied to the deprotection of (–)-**289**, however, this did not alter the outcome of the reaction (Table 3.6; Entry 2).

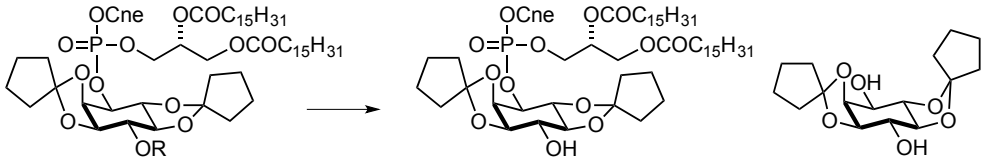
Prestwich *et al*. had previously employed Pyr·HF for the cleavage of various bulky TBDPS ethers in the presence of cyanoethyl-protected phosphorothionates.[58,337] Deprotection of (–)-**289**, using these conditions, however, yielded the hydrolysis product 2,3:5,6-di-*O*-cyclopentylidene-*myo*-inositol (+)-**299** (Table 3.6; Entry 3).[IX]

Finally, ammonium fluoride in MeOH are mildly acidic conditions used for the deprotection of silyl groups.[58,359-361] Whilst neither hydrolysis of the diacylglycerol phosphate nor cyanoethyl deprotection was observed, after three days stirring at elevated temperatures, mostly starting material remained

[IX] (+)-**299** was easily identifiable, as this compound had been synthesised on a large scale prior to this reaction.

(Table 3.6; Entry 4). Attempted deprotection of the less bulky and more labile 4-*O*-TBDMS group on (–)-**288** led to a similar outcome (Table 3.6; Entry 5*)*.

Table 3.6 Screening of reaction conditions for the desilylation of (–)-**288** and (–)-**289**.



Entry	Substrate	Reagents (eq)	Temperature [°C]	Solvent	Time [hours]	Yield [%]
1	TIPS	TAS-F (3)	RT	DMF	2.5	-[a]
2	TIPS	TAS-F (5) H_2O (10)	RT	DMF	4	-[a]
3	TIPS	Pyr·HF	RT	THF/Pyridine	22	-[b]
4	TIPS	NH_4F (18)	40	MeOH	48	-[c]
5	TBDMS	NH_4F (20)	40	MeOH	72	-[c]

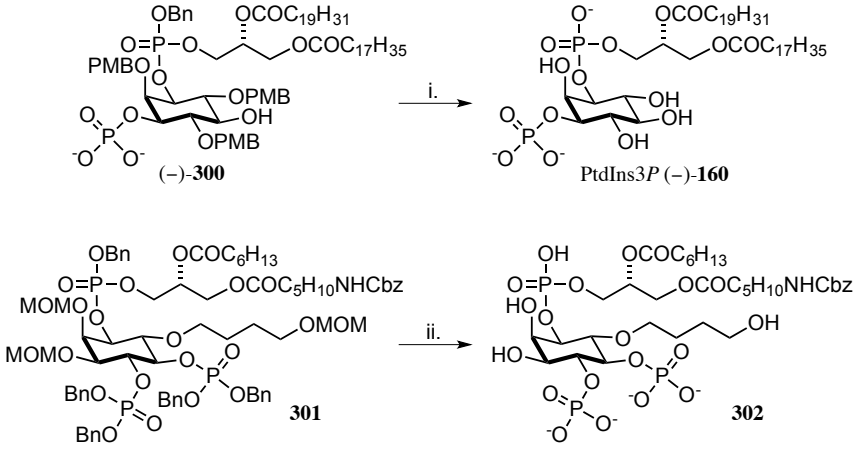
[a] Full consumption of the starting material was observed, but the products were unidentifiable [b] Hydrolysis of the diglyceride phosphate moiety was observed and (+)-**299** was isolated [c] The product **290** was not observed and mostly starting material was recovered. Cne = cyanoethyl; TIPS = triisopropylsilyl; TBDMS = *tert*-butyldimethylsilyl; Pyr = pyridine.

3.12. Route 4 towards PtdIns4*P*

Route 1 was unsuccessful as a result of the difficulties encountered when attempting to cleave an acetate group in the presence of base-labile cyanoethyl phosphate esters. The desymmetrisation of **149** with a chloroacetate group in Route 2 was unsuccessful, leading to low regio- and enantioselectivities. Finally, deprotection of a silyl ether in Route 3 was found to be challenging due to the sensitive nature of the diacylglycerol moiety (Scheme 3.21). As such, we explored the possibility of using alternative, base stable, phosphate ester protecting groups.

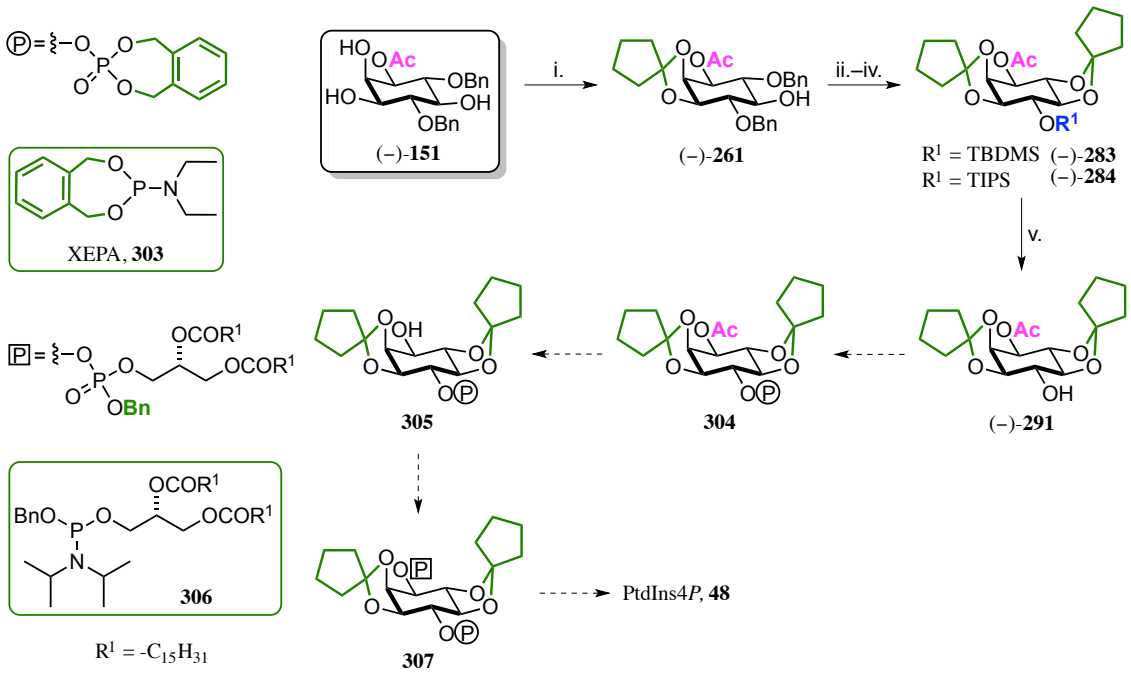
It was crucial to chose an alternative phosphate protecting group that is readily removable in the presence of an unsaturated chain, as was the case for cyanoethyl-protected phosphates (Section 2.3). Deprotection conditions could therefore not involve hydrogenolysis. It is well known in the literature that methyl, ethyl and isopropyl-protected phosphates can be efficiently deprotected under mild

conditions using trimethylsilylbromide (TMSBr).[58,361-365] In addition, a few reports have shown that benzyl-protected phosphates can also be deprotected under those conditions.[88,220,342,366] Finally, simultaneous deprotection of alkyl phosphate esters and acid-labile groups around the inositol ring, such as PMB,[88] MEM[342] or MOM[58,220,361,366] ethers, has been frequently performed as a final, global deprotection step (Scheme 3.26). Considering the steric crowding that the fully protected derivatives (–)-**283** and (–)-**284** experiences as a result of the two cyclopentylidene acetal protecting groups, we envisaged that a less sterically bulky phosphate protecting group would be preferable.[367]



Scheme 3.26 TMSBr has been used by Miller *et al*.[88] and Kohn *et al*.[216] for the deprotection of both benzyl phosphate esters and acid-labile ether protecting groups. *Reagents and conditions*: i. a. TMSBr, toluene, 70 °C, 12 h b. MeOH, 0 °C, 1 h, 61% ii. a. TMSBr, CH_2Cl_2, 0 °C to RT, 1 h b. MeOH, RT, 1 h c. 10% Pd/C, $H_{2(g)}$, MeOH, 1 atm, RT, 90%. PMB = 4-methoxybenzyl; MOM = methoxymethyl; Bn = benzyl; Cbz = carboxybenzyl.

Watanabe *et al*. developed the *o*-xylylene *N*,*N*-diethylphosphoramidite **303** (XEPA, Scheme 3.27) which allows for the efficient phosphitylation of sterically crowded protected inositols.[368-372] This *o*-xylylene-protected phosphoramidite has since been employed fairly frequently for the synthesis of Ins*P*$_n$s,[92,302] PtdIns*P*$_n$s,[373] and other derivatives.[374] We hypothesized that *o*-xylylene-protected phosphates would behave similarly to other alkyl phosphate esters and that deprotection using TMSBr would be achievable in the same way. In addition, this reagent has been previously use for the cleavage of acetals.[76] Therefore, we aimed to phosphorylated the 4-*O*-position of (–)-**291** using XEPA **303** (Scheme 3.27). Acetate deprotection would then be achieved under basic conditions to give intermediate **305**. We postulated that phosphorylation with a benzyl-protected phosphoramidite such as **306** followed by a one step global deprotection using TMSBr would furnish PtdIns4*P* **48** (Scheme 3.27).



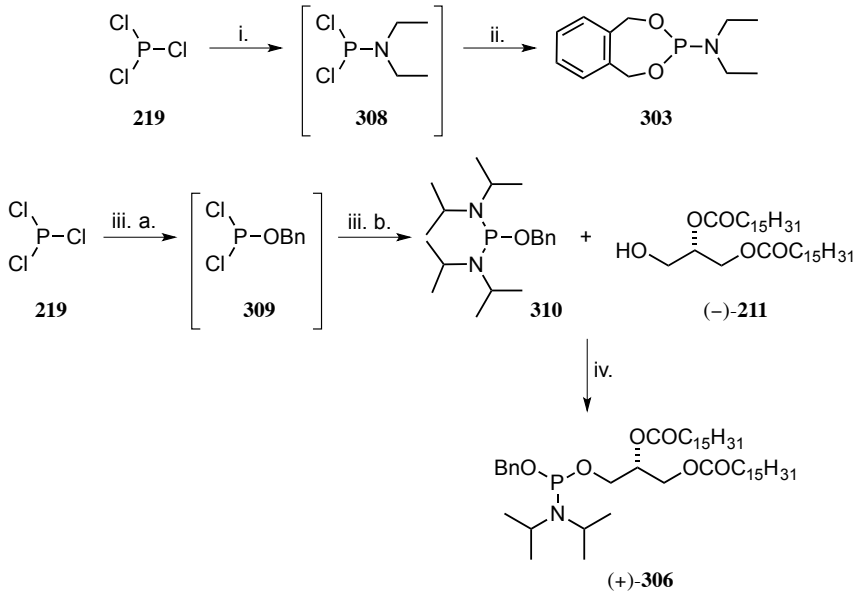
Scheme 3.27 Overview of Route 4. This proposed route involves the use of *o*-xylylene- and benzyl-protected phosphates. The aim was to optimise a final global deprotection using TMSBr that would cleave all remaining protecting groups in one step, and would be compatible with unsaturated lipid chains on R^1 and R^2. *Reagents and conditions*: i. **260**, PTSA·H_2O, CH_2Cl_2, RT, 18 h, 92% ii. 10% Pd/C, $H_{2(g)}$, EtOAc, RT, 2 h, 94% iii. TIPSOTf, 2,6-lutidine, THF, –78 °C, 16.5 h, 71% iv. **260**, PTSA·H_2O, CH_2Cl_2, RT, 4 h, 62% v. TAS-F, DMF, RT, 1.5 h, 75%. Ac = Acetate; Silyl = silyl protecting group; Bn = benzyl. R^1 and R^2 are saturated or unsaturated lipid chains.

3.12.1. Synthesis of XEPA and benzyl-protected phosphoramidites

XEPA **303** was synthesised following procedures first developed by Watanabe *et al.*[369] and improved by Holmes' group.[367] Similarly to the preparation of **218** (Section 2.7), dichloro-*N*,*N*-diethylphosphoramidite **308** was prepared by adding diethylamine dropwise to a solution of PCl_3 **219** in Et_2O at –78 °C. ^{31}P NMR confirmed that this unstable phosphoramidite was ~95% pure. Without further purification, **308** was treated with triethylamine and 1,2-benzenedimethanol. Removal of the precipitate obtained and evaporation *in vacuo* afforded **303** (Scheme 3.28). This phosphoramidite was stored under argon, at –20 °C for long periods of time without oxidation.

The benzyl-protected dipalmitoyl phosphoramidite (+)-**306** was prepared following procedures developed by Bannwarth[375] and optimised by Holmes *et al.*[266] Reaction of PCl_3 **219** with one equivalent of benzyl alcohol at –78 °C in pyridine afforded **309**. Following removal of the resulting pyridinium hydrochloride salt by filtration, and concentration *in vacuo*, this intermediate was treated immediately with DIPA. Similarly, filtration of the DIPA hydrochloride salt and concentration *in*

vacuo yielded benzyloxybis(*N*,*N*-diisopropylamino)phosphine **310** (Scheme 3.28). Before each phosphorylation reaction, this crude phosphine was employed in a 1*H*-tetrazole-catalysed coupling to afford the desired phosphoramidite (+)-**306**. Rapid purification by column chromatography was carried out on (+)-**306** using ~0.5% of triethylamine in the eluent to avoid any phosphoramidite hydrolysis (Scheme 3.28).



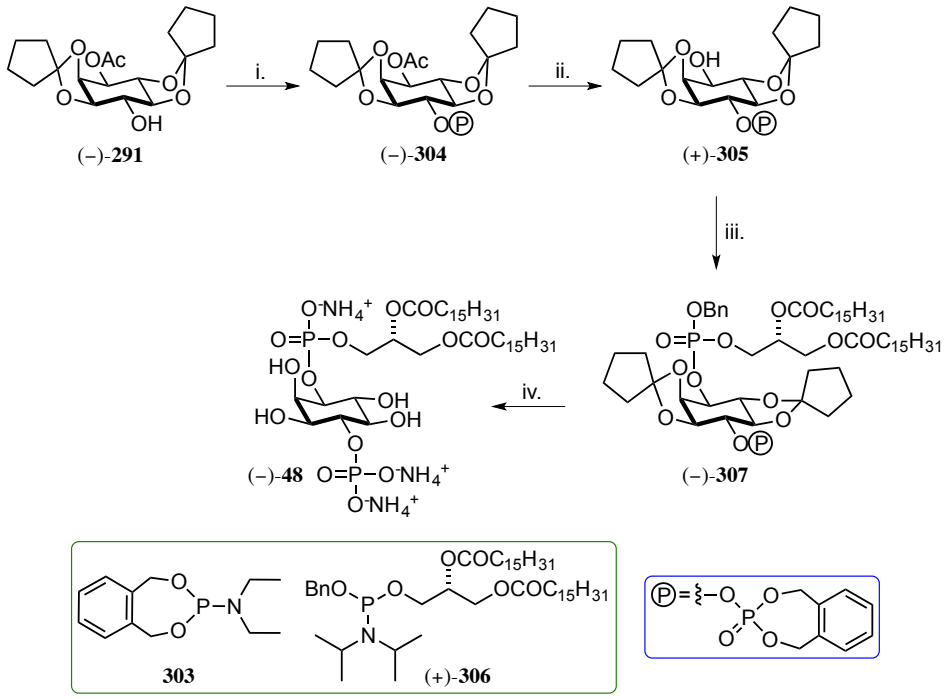
Scheme 3.28 Synthesis of XEPA **303** and the benzyl-protected 1,2-dipalmitoyl-*sn*-glycerol phosphoramidite (+)-**306**. *Reagents and conditions*:* i. Diethylamine, Et_2O, –78 °C to RT, 20 h, 79% ii. 1,2-Benzenedimethanol, Et_3N, THF/Et_2O, –78 °C to RT, 19 h, 75% iii. a. BnOH, Et_2O/Pyridine, –78 °C to RT, 20 h b. DIPA, Et_2O, –10 °C to RT, 51.5 h, 83% iv. 1*H*-tetrazole, CH_2Cl_2, RT, 19 h, 90%. Bn = benzyl. *The yields quoted for unstable phosphoramidites are crude yields, these compounds were used without further purification in the subsequent step.

3.12.2. Synthesis of the fully protected phosphatidylglycerol precursor (–)-**307**

Phosphitylation of (–)-**291** with three equivalents of XEPA **303** in the presence of 1*H*-tetrazole, followed by *m*CPBA oxidation of the intermediate, provided (–)-**304** in excellent yield. The subsequent deacetylation step was achieved under standard basic conditions to furnish (–)-**305** in high yields (Scheme 3.29).

Coupling of the alcohol (–)-**305** with five equivalents of the dipalmitoyl phosphoramidite (+)-**306** and 1*H*-tetrazole gave the corresponding phosphite intermediate. *In situ* oxidation with *m*CPBA afforded crude (–)-**307** (Scheme 3.29). This fully protected precursor was found to be particularly challenging to purify. A set of three consecutive purifications by column chromatography had to be undertaken in order to eliminate any phosphoramidite or *m*CPBA related impurities (See Chapter 5:

Experimental). Although (–)-**307** was obtained in 100% purity as determined by normal phase HPLC, the numerous purification steps had had a significant impact on the yield. We postulated that decreasing the number of equivalents of phosphoramidite employed in the phosphorylation step would facilitate purification. Indeed, reaction of (+)-**305** with just three equivalents of (+)-**306**, followed by a single purification by column chromatography, furnished (–)-**307** in 55% yield (Scheme 3.29).



Scheme 3.29 Synthesis of the fully protected phosphatidylglycerol precursor (–)-**307** and final global deprotection of (–)-**307** using TMSBr. *Reagents and conditions*: i. a. **303**, 1*H*-tetrazole, CH_2Cl_2, RT, 1.5 h b. *m*CPBA, –78 °C to RT, 1.5 h, 79% ii. K_2CO_3, MeOH, RT, 1 h, 74% iii. a. (+)-**306**, 1*H*-tetrazole, CH_2Cl_2, RT, 4 h b. *m*CPBA, –78 °C to RT, 19 h, 55% iv. a. TMSBr, toluene, 70 °C, 12 h b. MeOH, 0 °C, 1 h, 68%. Ac = acetate; Bn = benzyl.

3.12.3. Final global deprotection of (–)-**307**

We reasoned that the conditions optimised by Miller *et al*. for the deprotection of derivative (–)-**300** towards unsaturated PtdIns3*P* (–)-**160** (Scheme 3.26), would be applicable to the deprotection of the inositol framework (–)-**307**.[23,88] The advantage of these conditions stems from their compatibility with unsaturated chains, allowing for the synthesis of natural PtdIns4*P* **11**.

Following Miller's one step procedure, intermediate (–)-**307** was treated with twenty equivalents of TMSBr in toluene and at 70 °C under strictly anhydrous conditions. We found that product formation

could be monitored by TLC analysis using a mixture of CH_3Cl, MeOH, and a 2.2 M solution of NH_4OH (5:3.9:1.1). After 12 hours, the volatile components were evaporated *in vacuo* (0 °C). Methanolysis of the resulting silyl phosphate esters was accomplished by stirring the residue obtained in MeOH at 0 °C for one hour. Although the amphiphilic nature of these compounds makes them difficult to purify by column chromatography, the colourless solid obtained was purified by standard column chromatography over silica gel,[376] using the same eluent employed for TLC analysis, to furnish PtdIns4*P* (–)-**48** as its presumed ammonium salt (Scheme 3.29), in 68% yield.

Considering the complexity of this amphiphilic molecule, NMR analysis had to be carried out at a specific concentration (~6 mg/mL) and in a mixture of CD_3OD, $CDCl_3$ and D_2O (4:3:1). This mixture facilitated complete solvation of both the hydrophilic headgroup and the hydrophobic lipid chains of PtdIns4*P* (–)-**48**. Accordingly, very high quality ^{1}H (Figure 3.10), ^{31}P (Figure 3.11) and 2D NMR (Figure 3.12) data were obtained for this compound. The HSQC NMR spectrum (Figure 3.12) obtained was employed to assign all peaks in the ^{13}C NMR spectrum of PtdIns4*P* (–)-**48**.

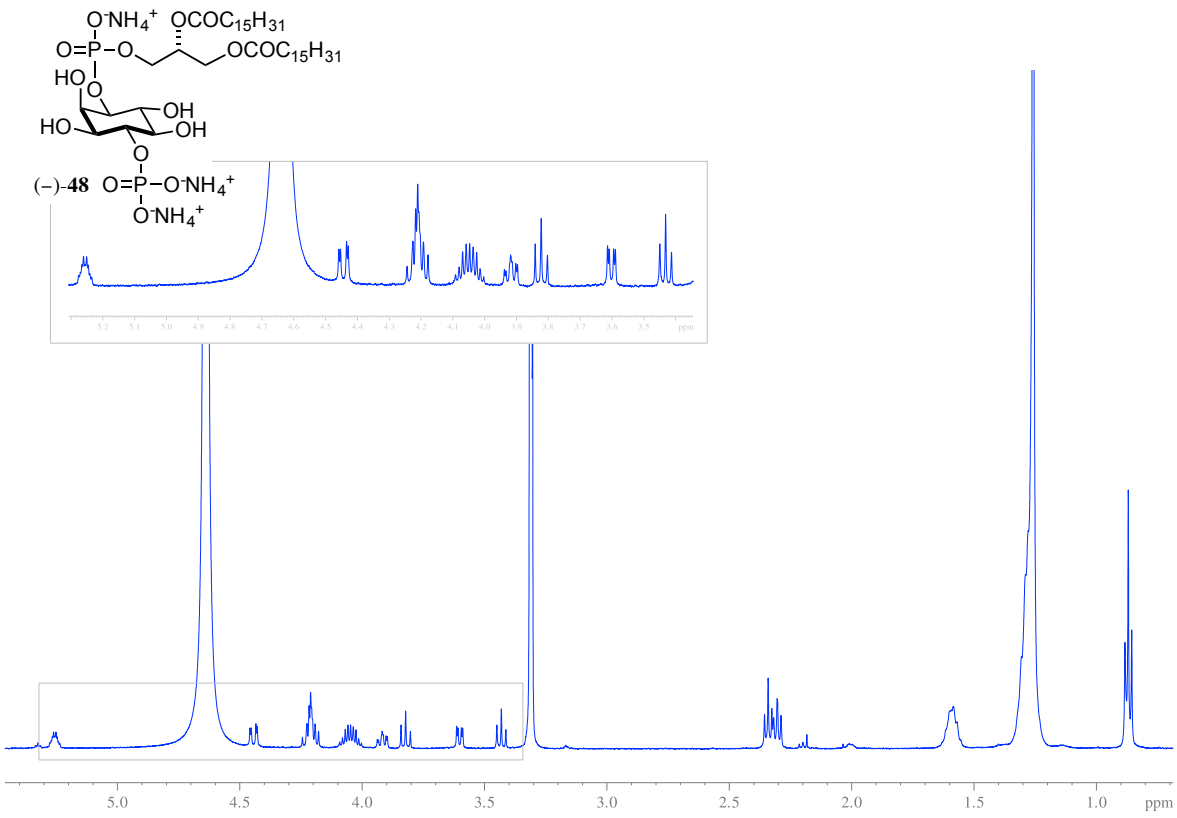


Figure 3.10 ^{1}H NMR spectrum of dipalmitoyl PtdIns4*P* (–)-**48**. NMR spectrum aquired at 500 MHz, in $CD_3OD/CDCl_3/D_2O$ 4:3:1.

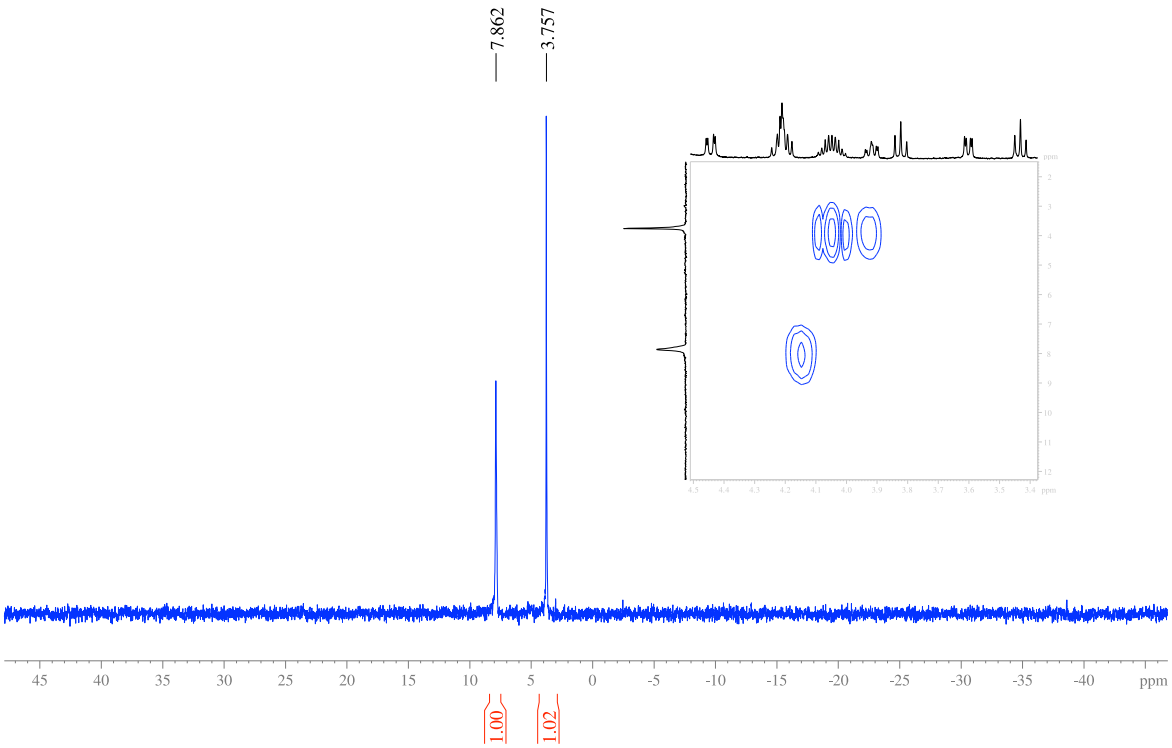


Figure 3.11 ^{31}P NMR and ^{31}P-H HMBC spectra of dipalmitoyl PtdIns4*P* (–)-**48** showing two distinct phosphorus environments. NMR spectrum acquired at 162 MHz, in $CD_3OD/CDCl_3/D_2O$ 4:3:1.

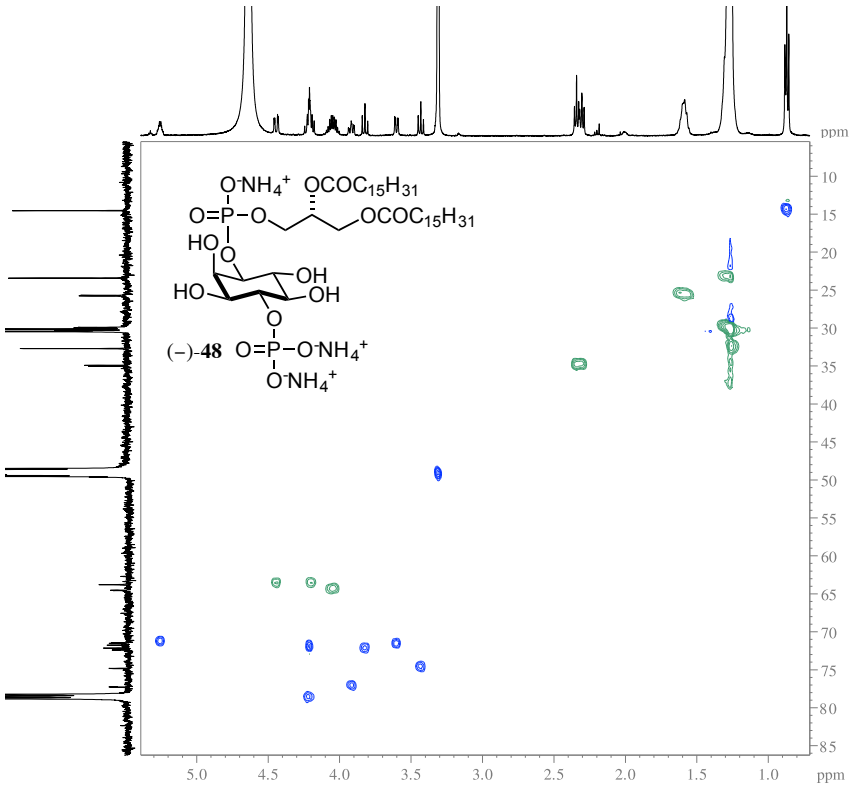
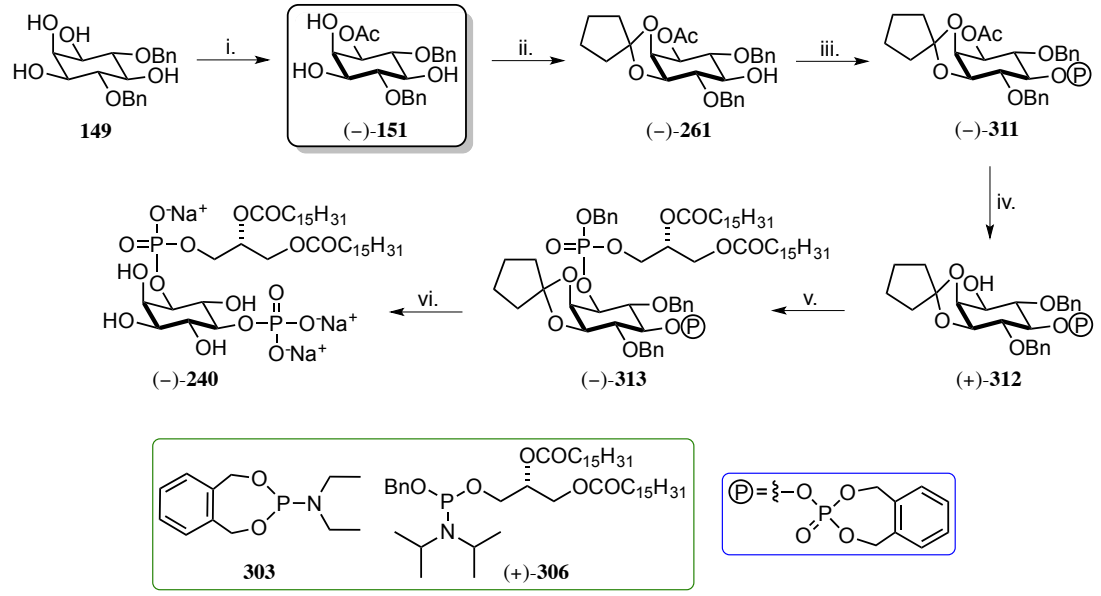


Figure 3.12 HSQC NMR spectrum of dipalmitoyl PtdIns4*P* (–)-**48**. ^{13}C NMR spectrum acquired at 126 MHz, in $CD_3OD/CDCl_3/D_2O$ 4:3:1.

3.13. Synthesis of dipalmitoyl PtdIns5*P* (–)-240

With the key inositol building block (–)-**261** in hand, we reasoned that synthesis of saturated PtdIns5*P* (–)-**240** would be achievable through a sequence of steps similar to those developed for the synthesis of PtdIns4*P* (–)-**48** (Scheme 3.30). Unfortunately, the one step deprotection protocol used for the synthesis of PtdIns4*P*, and involving TMSBr, cannot be applied to the deprotection of this framework. *myo*-Inositol ring benzyl ethers are known to be stable under those conditions. Hydrogenolysis was therefore to be attempted in a final global deprotection step. Considering the 2,3-*O*-cyclopentylidene acetal had been shown to be labile to hydrogenolysis conditions in alcoholic solvents (Section 3.8.1), we reasoned that an acidic step following hydrogenolysis would not be required. The route described herein can only be employed for the synthesis of PtdIns5*P* derivatives bearing saturated chains, such as palmitoyl or stearoyl.

Reaction of the benzylated derivative (–)-**261** with excess XEPA **303** and catalytic 1*H*-tetrazole gave the corresponding phosphite intermediate. *In situ* oxidation with *m*CPBA furnished (–)-**311** in excellent yield. Similar to the synthesis of PtdIns4*P*, removal of the 1-*O*-acetate ester was completed



Scheme 3.30 Synthesis of dipalmitoyl PtdIns5*P* (–)-**240** *Reagents and conditions*: i. Lipozyme TL-IM®, vinyl acetate/hexane (1:1), 45 °C, 15 h, 98%, >99% *e.e.* ii. **260**, PTSA·H_2O, CH_2Cl_2, RT, 18 h, 92% iii. a. **303**, 1*H*-tetrazole, CH_2Cl_2, RT, 1 h b. *m*CPBA, –78 °C to RT, 1.5 h, 92% iv. K_2CO_3, MeOH, RT, 1 h, 74% v. a. (+)-**306**, 1*H*-tetrazole, CH_2Cl_2, RT, 22 h b. *m*CPBA, –78 °C to RT, 4 h, 82% vi. a. Pd black, $H_{2(g)}$, ^{t}BuOH/H_2O, RT, 17 h b. $NaHCO_3$, RT, 4 h, 94%. Bn = benzyl; Ac = acetate.

successfully using potassium carbonate in MeOH. The intermediate (+)-**312** generated was then phosphorylated using phosphoramidite (+)-**306** to give (–)-**313** (Scheme 3.30).

Global deprotection of precursor (–)-**313** was accomplished by palladium black-catalysed hydrogenolysis in a mixture of ^{t}BuOH and H_2O.[33,47,64,68,72] Conveniently, these conditions were found to cleave all of the protecting group, including the acid-labile 2,3-*O*-cyclopentylidene acetal. The protocol for this hydrogenolysis commonly involves adding $NaHCO_3$ at the beginning of the reaction. However, we established that addition of $NaHCO_3$ after 17 hours of reaction, followed by a further 4 hours of stirring at room temperature, limited the amount of phosphate hydrolysis. Finally, careful filtration of the reaction mixture followed by lyophilisation yielded PtdIns5*P* (–)-**240** as its presumed sodium salt (Figures 3.13, 3.14 and 3.15). Replacing Celite® with glass microfiber pads in the final filtration step limited contamination and maximised the overall yield obtained.

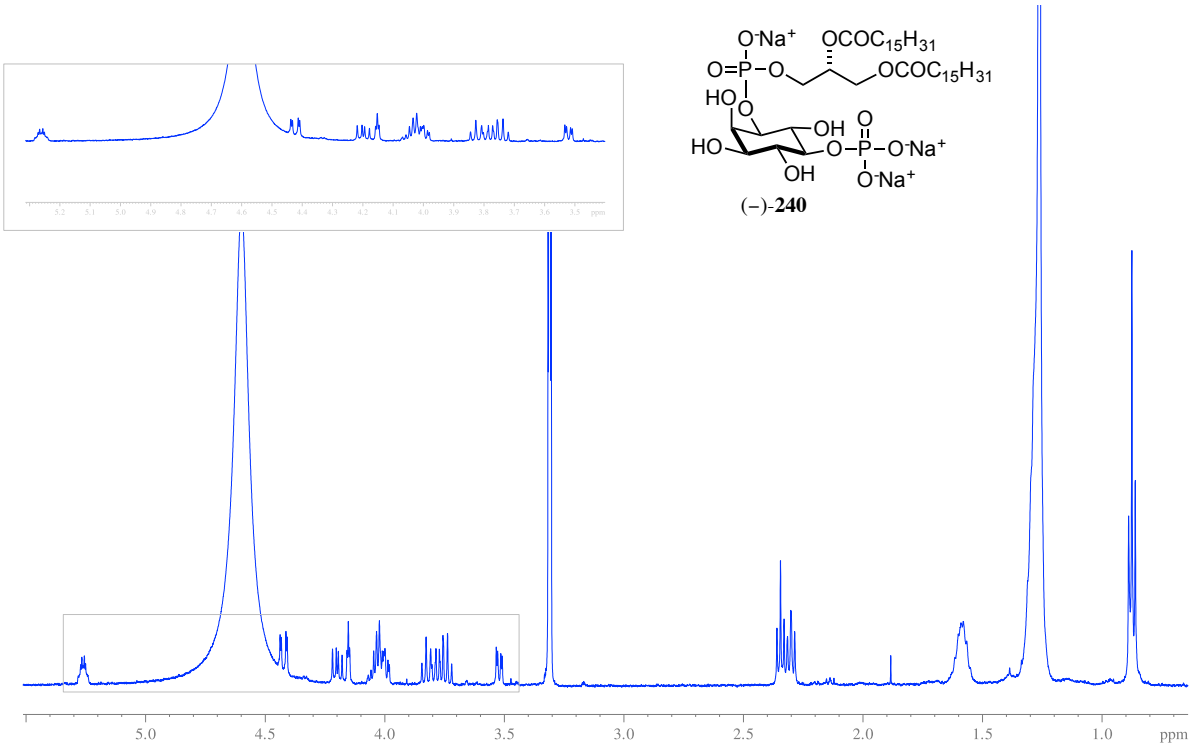


Figure 3.11 ^{1}H NMR spectrum of dipalmitoyl PtdIns5*P* (–)-**240**. NMR spectrum acquired at 500 MHz, in $CD_3OD/CDCl_3/D_2O$ 4:3:1.

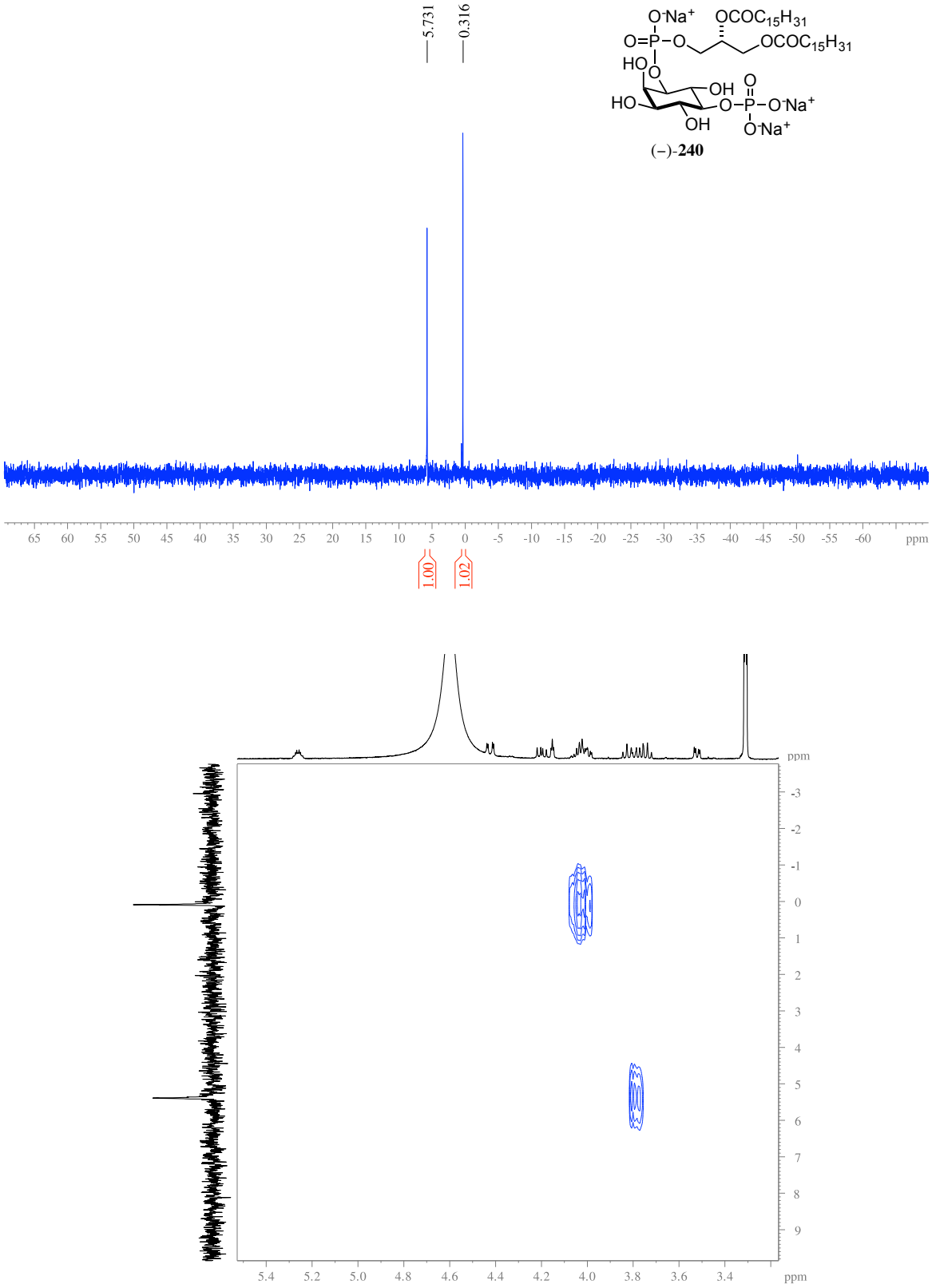


Figure 3.12 ^{31}P NMR and ^{31}P-H HMBC spectra of dipalmitoyl PtdIns5*P* (–)-**240** showing two distinct phosphorus environments. NMR spectrum acquired at 162 MHz, in $CD_3OD/CDCl_3/D_2O$ 4:3:1.

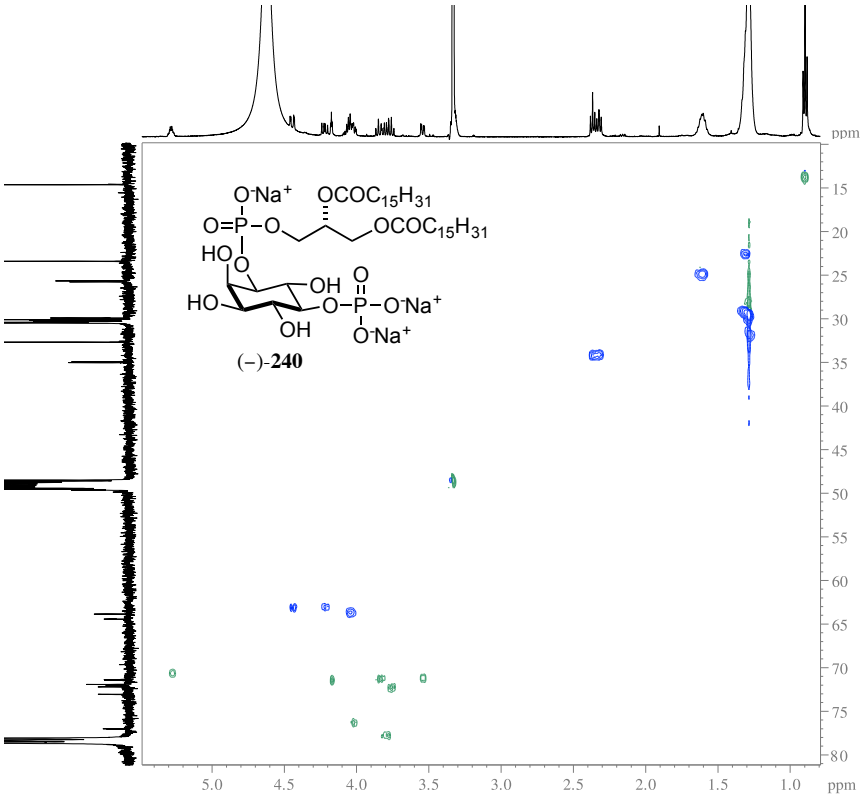
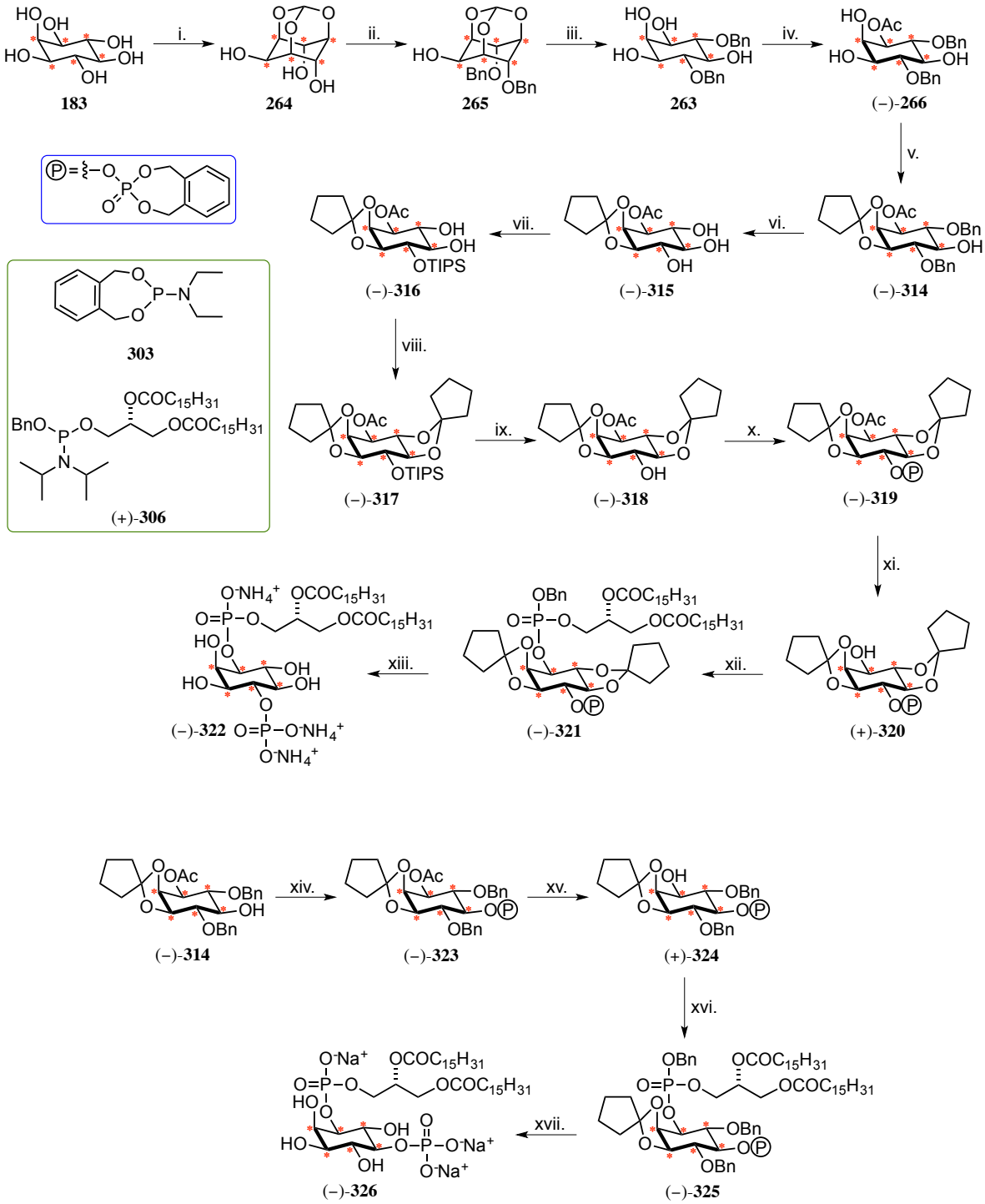


Figure 3.13 HSQC NMR spectrum of dipalmitoyl PtdIns5*P*. ^{13}C NMR spectrum acquired at 126 MHz, in $CD_3OD/CDCl_3/D_2O$ 4:3:1.

3.14. Synthesis of dipalmitoyl D_6 PtdIns4*P* (–)-322 and D_6 PtdIns5*P* (–)-326

Having optimised an efficient and robust synthesis of dipalmitoyl PtdIns4*P* (–)-**48** and PtdIns5*P* (–)-**240**, focus shifted towards applying these synthetic routes to deuterated analogues. As described in Section 3.7, the *meso* derivative **263** was readily prepared from D_6 *myo*-inositol **183** (See Section 3.7.1, provided by Dr Alex Saunders).[47] Enzymatic desymmetrisation of this 4,6-di-*O*-benzylated intermediate using Lipozyme TL-IM® was successful and provided the enantiomerically pure deuterated building block (–)-**266** without loss of deuterium incorporation (>99% *e.e.*, 89% D_6, 11% D_5). Starting from this material, the route developed for the synthesis of protonated PtdIns4*P* (–)-**48** and PtdIns5*P* (–)-**240** analogues was followed step by step (Scheme 3.31). Accordingly, the fully protected derivatives (–)-**322** and (–)-**326** were obtained in similar yields to the protonated analogues.



Scheme 3.31 Synthesis of deuterated PtdIns4*P* and PtdIns5*P*. *Reagents and conditions*: i. $CH(OEt)_3$, PTSA·H_2O, DMF, 105 °C, 7 d, 31%, 89% D_6, 11% D_5 ii. a. LiH, DMF, RT, 30 min b. BnBr, RT, 48 h, 77%, 91% D_6, 9% D_5 iii. PTSA·H_2O, MeOH, RT, 24 h, 86%, 92% D_6, 8% D_5 iv. Lipozyme TL-IM®, vinyl acetate/hexane (1:1), 45 °C, 15 h, 84%, >99% *e.e.*, 89% D_6, 11% D_5 v. **260**, PTSA·H_2O, CH_2Cl_2, RT, 19 h, 87%, 91% D_6, 9% D_5 vi. 10% Pd/C, $H_{2(g)}$, EtOAc, RT, 2 h, 76%, 84% D_6, 16% D_5 vii. TIPSOTf, 2,6-lutidine, THF, –78 °C, 16 h, 74%, 84% D_6, 16% D_5 viii. **260**, PTSA·H_2O, CH_2Cl_2, 30 °C, 16 h, 71%, 85% D_6, 15% D_5 ix. TAS-F, DMF, RT, 3 h, 88%, 84% D_6, 16% D_5 x. a. **303**, 1*H*-tetrazole, CH_2Cl_2, RT, 1 h b. *m*CPBA, –78 °C to RT, 1.5 h, 88%, 84% D_6, 16% D_5 xi. K_2CO_3, MeOH, RT, 1 h, 86%, 84% D_6, 16% D_5 xii. a. (+)-**306**, 1*H*-tetrazole, CH_2Cl_2, RT, 21 h b. *m*CPBA, –78 °C to RT, 4 h, 55%, 87% D_6, 13% D_5 xiii. a. TMSBr, toluene, RT, 12 h b. MeOH, 0 °C, 1 h, 75%, 86% D_6, 14% D_5 xiv. a. **303**, 1*H*-tetrazole, CH_2Cl_2, RT, 1 h b. *m*CPBA, –78 °C to RT, 1.5 h, 61%, 88% D_6, 12% D_5 xv. K_2CO_3, MeOH, RT, 1 h, 89%, 89% D_6, 11% D_5 xvi. a. (+)-**306**, 1*H*-tetrazole, CH_2Cl_2, RT, 20 h b. *m*CPBA, –78 °C to RT, 3 h, 46%, 88% D_6, 12% D_5 xvii. a. Pd black, $H_{2(g)}$, ^{t}BuOH/H_2O, RT, 21 h b. $NaHCO_3$, RT, 2 min, 86%, 90% D_6, 10% D_5. Ac = acetate; Bn = benzyl; TIPS = triisopropylsilyl.

3.14.1. Structural analysis of deuterated derivatives

The deuterium incorporation of each intermediate presented in Scheme 3.31 was verified by mass spectrometry, confirming that the amount of deuterium present initially in the two separate batches of D_6 *myo*-inositol **183** employed was retained throughout the synthesis.

Structural assignment of these deuterated compounds was achieved *via* ^{1}H NMR, ^{2}H NMR, and ^{13}C NMR analysis. As expected, when comparing the ^{1}H NMR spectra of a deuterated derivative (–)-**318** to its protonated analogue (–)-**291**, the signals corresponding to the protons around the *myo*-inositol ring are suppressed (Figure 3.16). Deuterium is a spin-1 (I = 1) quadrupolar nucleus and thus possesses a relatively short spin-lattice relaxation times (T_1).[377] As a result, the peaks observed in ^{2}H NMR spectra are usually very broad and spin-spin coupling cannot be detected. Despite this, the six deuterium atom in their separate environments around the inositol ring can be distinguished by ^{2}H NMR analysis. These signals were assigned by comparison with the chemical shifts of each inositol ring proton in the ^{1}H NMR spectra of the protonated analogue (Figure 3.16).

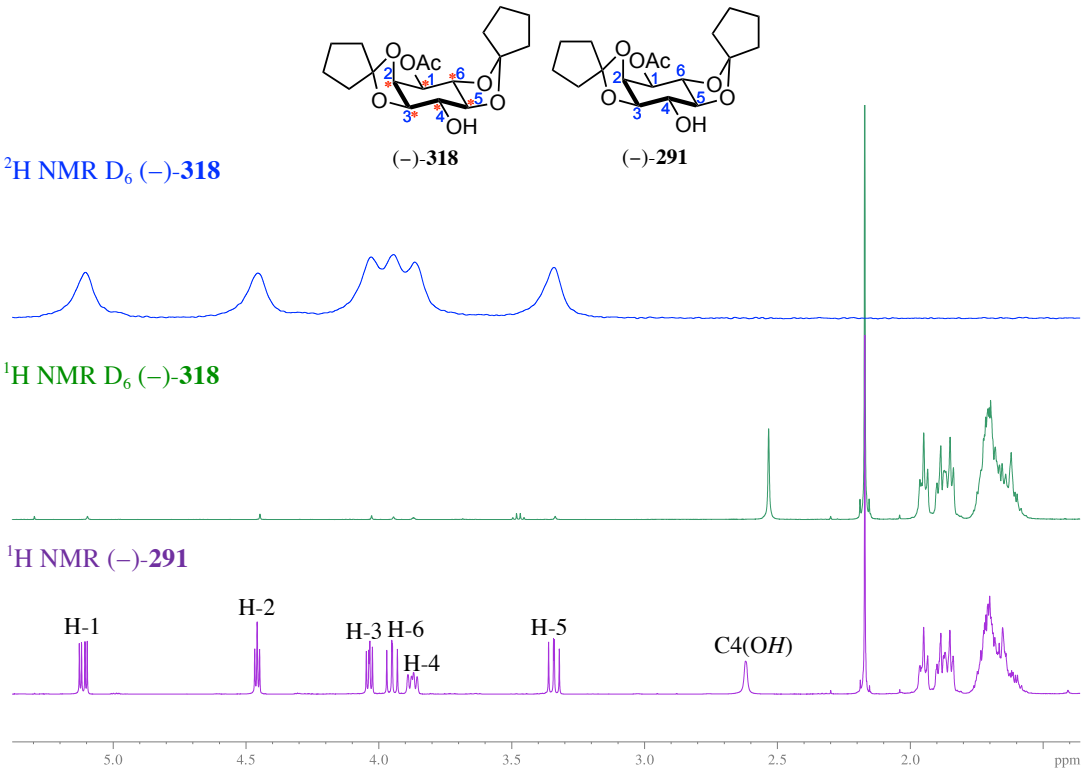


Figure 3.14 Comparison of the ^{1}H NMR of (–)-**291** (purple) with the ^{1}H NMR (green) and ^{2}H NMR (blue) of D_6 (–)-**318**. The ^{2}H NMR spectrum of D_6 (–)-**318** shows six deuterium signals in distinct environments. These can be assigned by comparison with the ^{1}H NMR spectrum of (–)-**291**. The ^{1}H NMR and ^{2}H NMR spectra were acquired at 500 and 96 MHz respectively, in $CDCl_3$. Ac = acetate.

In ^{13}C NMR, the spin-½ ring carbons couple to their neighbouring spin-1 deuterium, resulting in a 1:1:1 triplet (2I +1). Similar to quaternary carbons, these deuterated ring carbons are characterised by their longer relaxation times (T_1) which result from the poor spin-spin coupling of the deuterium nuclei.[47] Smaller signal intensities are therefore observed. Although increasing the number of scans (from ~ 1000 to 3000) was essential to resolving those suppressed signals (Figure 3.17), changes in the relaxation delay (D1 time) did not significantly improve the quality of the spectra acquired. The carbons α- and β- to the deuterium nucleus also experience a small isotope shift upfield due to the more electropositive deuterium atom. A residual singlet for the remaining, partially protonated, D_5 analogues is therefore observed downfield of the suppressed triplet (Figure 3.17). Finally, assignment of ^{13}C NMR spectra for phosphorylated derivatives is further complicated by the presence of spin-½ ^{31}P nuclei which usually give a 1:1 doublet when coupling with ^{13}C. When the deuterated *myo*-inositol ring carbons couple both to the quadrupolar deuterium and to the neighbouring phosphorus, multiplets of weak intensity are observed as shown in Figure 3.18.

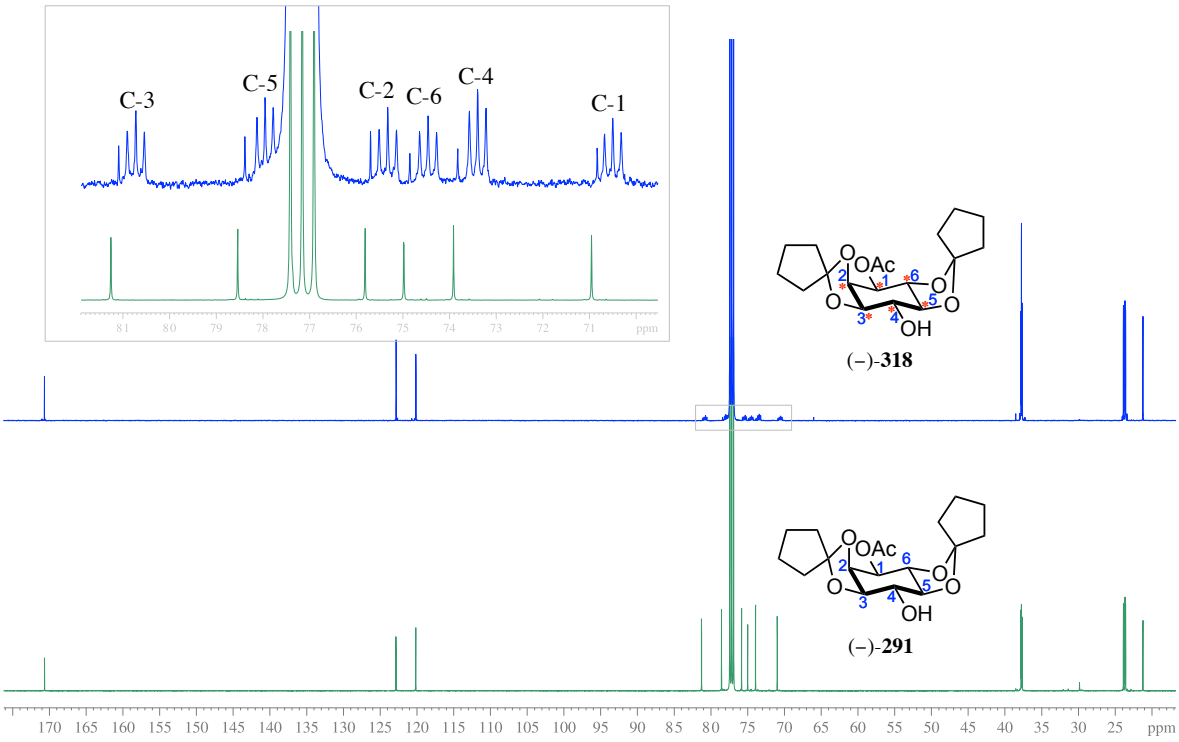


Figure 3.15 ^{13}C NMR spectra of (–)-**291** (green) and of D_6 (–)-**318** (blue). The signals for the deuterated carbons around the *myo*-inositol ring in D_6 (–)-**318** are observed as a triplets of weak intensity with an isotopic shit of ~0.3 ppm upfield as compared to the protonated analogue (–)-**291**. A small signal for the residual protonated D_5 compounds can also be observed. Both ^{13}C NMR spectra were acquired at 126 MHz in $CDCl_3$. Ac = acetate.

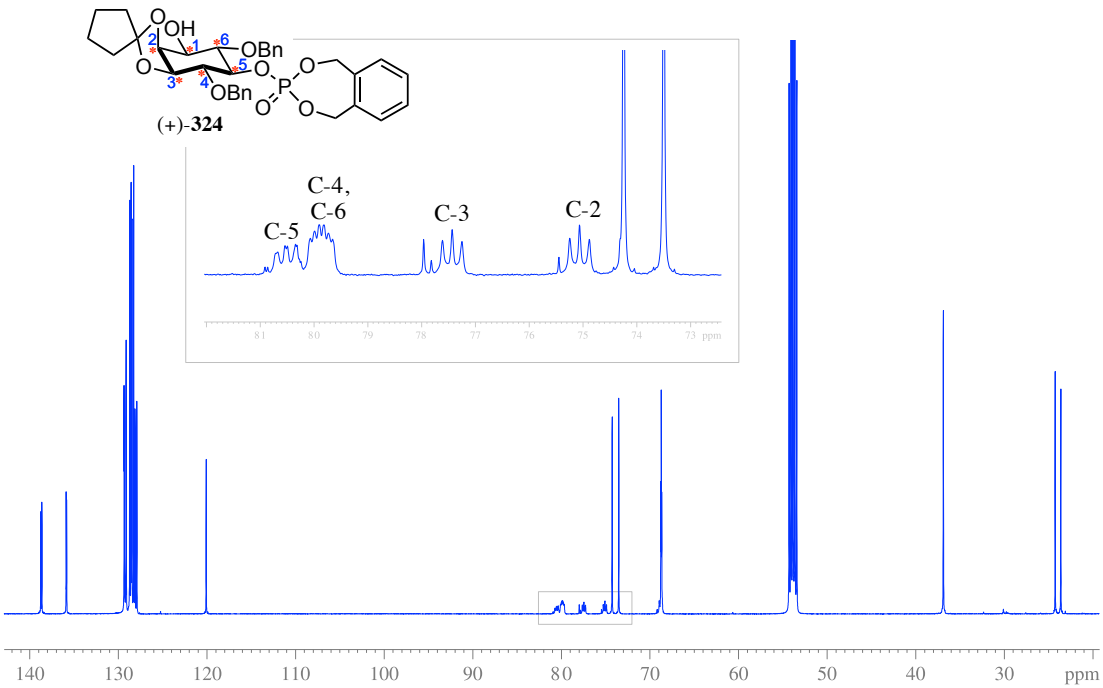


Figure 3.16 ^{13}C NMR of D_6 (+)-**324** showing the complexity of the deuterated carbon signals when coupling to both a quadrupolar deuterium nucleus and a ^{31}P nucleus. This ^{13}C NMR spectra was acquired at 126 MHz in CD_2Cl_2. Bn = benzyl.

3.14.2. Final global deprotection of the deuterated derivatives (–)-**321** and (–)-**325**

Deprotection of the fully protected derivative D_6 (–)-**321** was accomplished as previously outlined for the protonated analogue (–)-**307** (Section 3.12.3), using twenty equivalents of TMSBr in toluene. It was found that the reaction could be performed at room temperature without impacting on the rate of conversion or on the isolated yield. Column chromatography over silica gel[376] using a mixture of CH_3Cl, MeOH and a 2.2 M solution of NH_4OH (9:7:2) provided D_6 PtdIns4*P* (–)-**322** as its presumed ammonium salt in 75% yield and with a deuterium incorporation of 86% D_6 and 14% D_5 (Scheme 3.31).

Analysis of D_6 PtdIns4*P* (–)-**322** by high resolution mass spectrometry showed two distinct peaks at 447.2574 and 895.5215 corresponding to $[(MD_6–H)/2]^-$ and $[MD_6–H]^-$ respectively. A distinct shift of 3.0185 and 6.0361 mass units therefore exists as compared to the peaks observed in the protonated analogue (Figure 3.19).

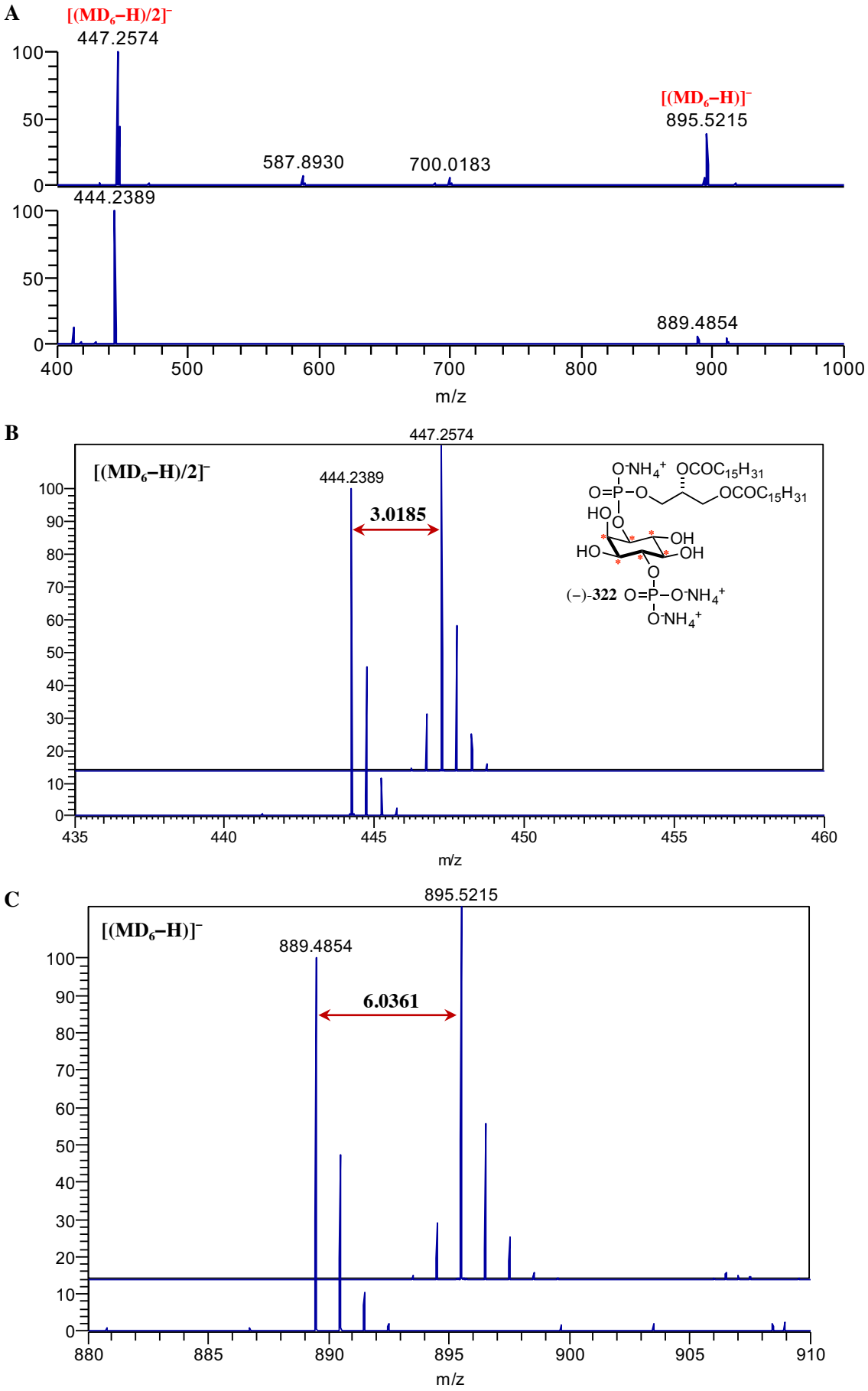


Figure 3.17 A. Comparison of the high resolution mass spectra for PtdIns4*P* (bottom) and D_6 PtdIns4*P* (top) **B.** Expansion of the peaks corresponding to $[(MD_6–H)/2]^-$ for PtdIns4*P* (–)-**48** (bottom) and D_6 PtdIns4*P* (–)-**322** (top) **C.** Expansion of the peaks corresponding to $[(MD_6–H)]^-$ for PtdIns4*P* (bottom) and D_6 PtdIns4*P* (top). The mass differences are marked with a red arrow.

^{1}H, ^{31}P, and 2D NMR spectra were obtained for (–)-**322**. Most importantly, the ^{1}H NMR spectra features the expected suppressed signals accounting for the six deuterium atoms around the ring (Figure 3.20). In addition, the only correlation remaining in the ^{31}P-H HMBC NMR spectra is that between the phosphorus of the phosphate diester and the glycerol protons (Figure 3.22). HSQC NMR (Figure 3.23) was used to assign the ^{13}C NMR spectrum due to the difficulty associated with acquiring high signal to noise spectra on these unstable deuterated compounds (Section 3.13.1). In addition, none of the signals for the deuterated ring carbons are observed, although these can be inferred by comparison with the protonated PtdIns4*P* analogue (–)-**48**. Finally, whilst the ^{2}H NMR spectrum acquired features two distinct deuterium environments, these were found particularly difficult to assign by comparison with the ^{1}H NMR of protonated PtdIns4*P* (–)-**48** (Figure 3.21).

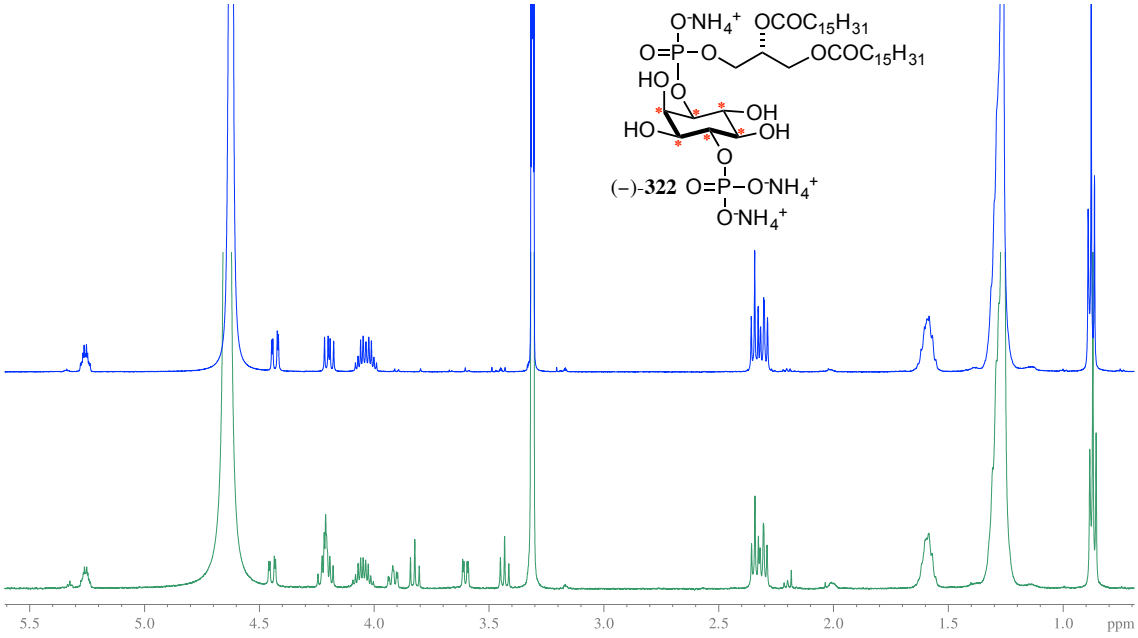


Figure 3.18 Comparison of the ^{1}H NMR spectrum acquired for dipalmitoyl PtdIns4*P* (–)-**48** (green) and dipalmitoyl D_6 PtdIns4P (–)-**322** (blue). Both ^{1}H NMR spectra were acquired at 500 MHz, in $CD_3OD/CDCl_3/D_2O$ 4:3:1.

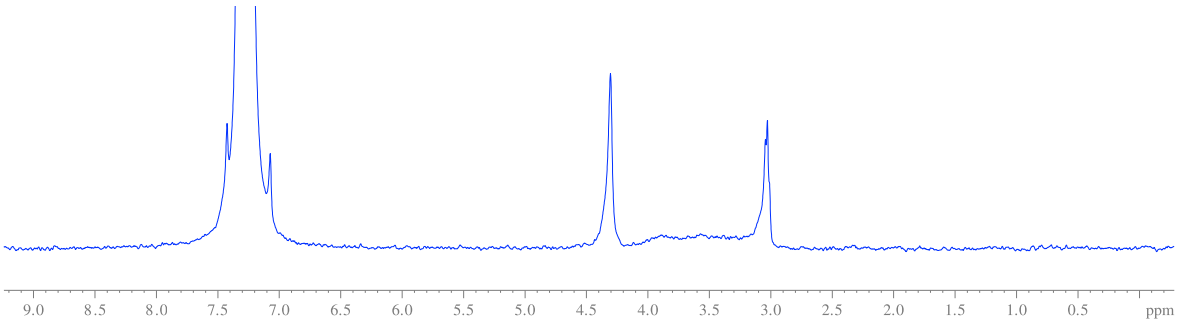
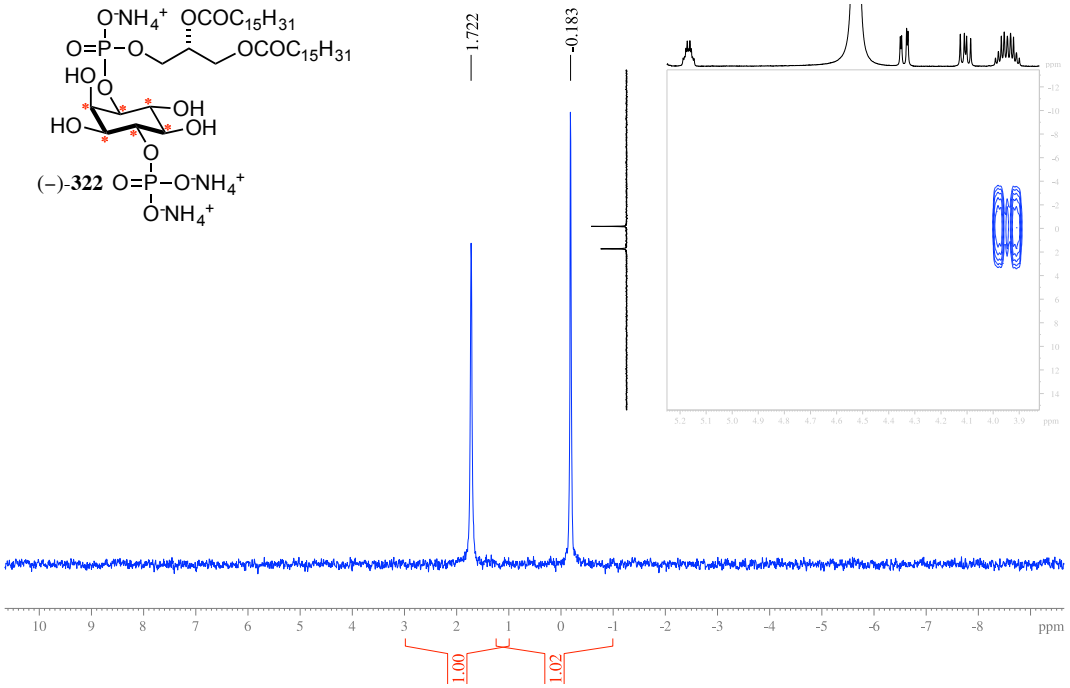


Figure 3.19 ^{2}H NMR spectrum of D_6 PtdIns4*P* (–)-**322** showing two distinct deuterium environments. This spectrum was acquired at 96 MHz in $CHCl_3$ with $CDCl_3$ as a reference.



(–)-**322**

Figure 3.20 ^{31}P and ^{31}P-^{1}H HMBC NMR spectra of D_6 PtdIns4*P* (–)-**322**. The ^{31}P spectrum was acquired at 162 MHz, in $CD_3OD/CDCl_3/D_2O$ 4:3:1.

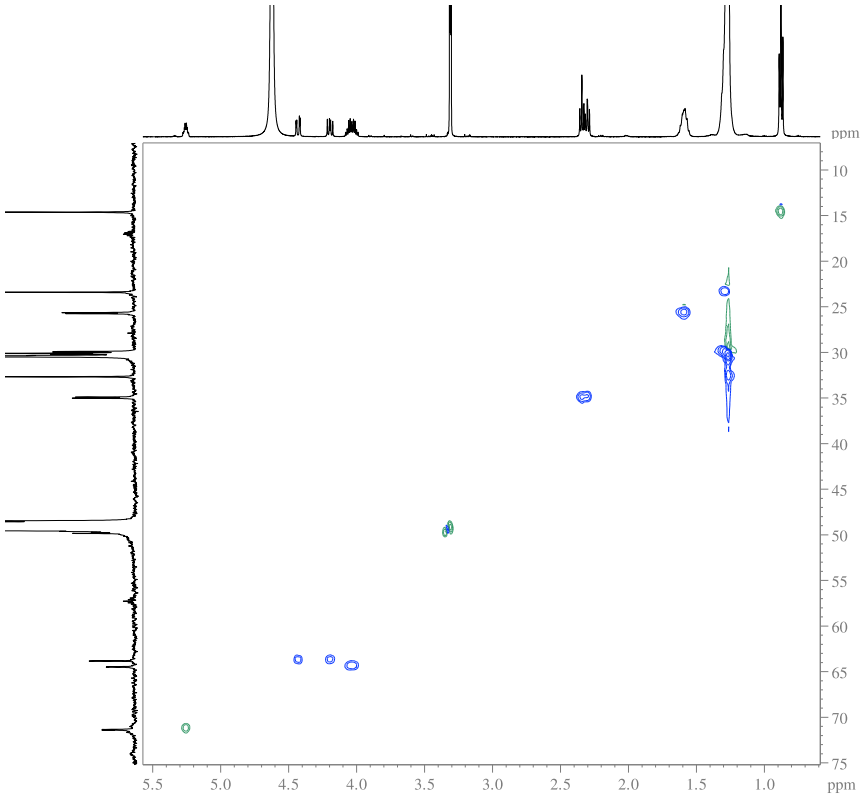


Figure 3.23 HSQC NMR spectrum of D_6 dipalmitoyl PtdIns4*P* (–)-**322**. ^{13}C NMR spectrum acquired at 126 MHz, in $CD_3OD/CDCl_3/D_2O$ 4:3:1.

Deprotection of the deuterated PtdIns5*P* precursor (–)-**325** under hydrogenolysis conditions was more challenging. Indeed, Dr Alex Saunders had previously demonstrated in his research that deprotection of deuterated benzylated PtdInsP_n precursors using palladium black furnished a mixture of hydrolysis products.[47] It is possible that deuterated PtdInsP_ns are more prone to hydrolysis than the corresponding protonated analogues when subjected to hydrogenolysis.[378]

Although such reactions are difficult to monitor, a set of conditions were trialled. At first, we reasoned that short reaction times coupled with the late stage addition of $NaHCO_3$ would limit hydrolysis of the product. Whilst this was the case (Table 3.7; Entry 1) and deprotection of both benzyl ethers and the *o*-xylylene phosphate ester was observed by ^1H and ^{31}P NMR, only partial cleavage of the 2,3-*O*-cyclopentylidene acetal group had occurred (Figure 3.24). The amount of fully deprotected D_6 PtdIns5*P* obtained increased when the reaction was stirred for an additional 11 hours (Table 3.7; Entry 2) although some of the partially protected product (–)-**327** still remained. With 30 equivalents of palladium black and longer reaction times, before and after the addition of $NaHCO_3$, full deprotection was observed by ^1H NMR, however, some hydrolysis products could also be observed (Table 3.7; Entry 3). Replacing the $NaHCO_3$ step with a purification by column chromatography over silica gel as previously described in the synthesis of PtdIns4*P*s (–)-**48** and (–)-**322**, led to a similar outcome (Table 3.7; Entry 4). Finally, stirring with 30 equivalents of palladium black for 19 hours led to the full deprotection of (–)-**325**. $NaHCO_3$ was then added for only two minutes, which limited the amount of hydrolysis product obtained (Table 3.7; Entry 5). D_6 PtdIns5*P* (–)-**326** was thus obtained as its presumed sodium salt in 86% yield and with a deuterium incorporation of 90% D_6 and 10% D_5 (Scheme 3.31).

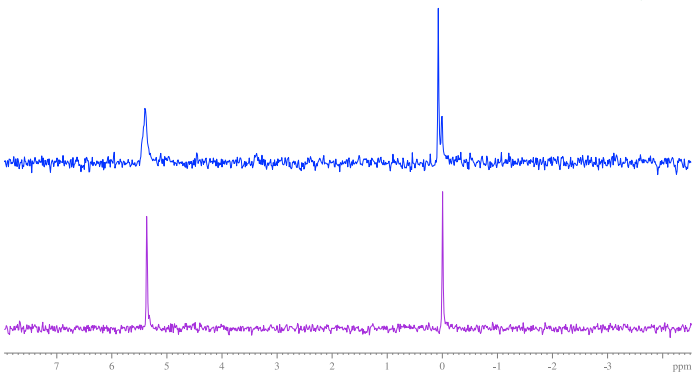
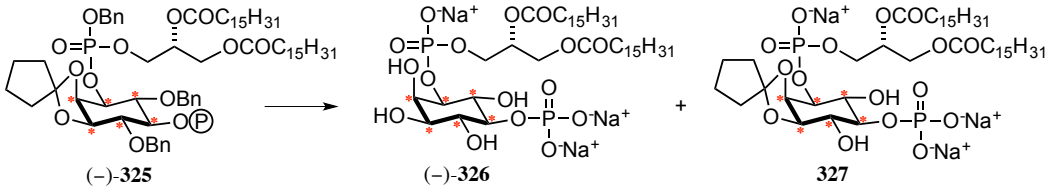


Figure 3.24 Overlay of the ^{31}P NMR (400 MHz, in $CD_3OD/CDCl_3/D_2O$ 4:3:1) spectra obtained for entries 2 (blue) and 5 (purple) of Table 3.7.

Table 3.7 Overview of reaction conditions for the deprotection of the deuterated derivative (–)-**325**



Entry	Pd Black [eq]	Time Step 1[a] [h]	Time Step 2[b] [min]	Work-up / purification	Ratio[c] (–)-326:327	Yield [%]
1	20	3	10	Filtered, lyophilised	1.9:1.0	-
2	20	14	35	Filtered, lyophilised	4.8:1.0	-
3	30	18.5	180	Filtered, lyophilised	-	Hydrolysis[d]
4	30	18.5	-	Filtered, lyophilised Column chromatography[e]	-	Hydrolysis[d]
5	30	21	2	Filtered, lyophilised	-	86%

[a] Step 1: (–)-**325** was stirred at RT with Pd black under $H_{2(g)}$ [b] Step 2: $NaHCO_3$ was added and the reaction was stirred at RT [c] Ratio determined by ^1H NMR analysis. The two products were also distinguishable by ^{31}P NMR [d] (–)-**326** was contaminated with hydrolysis products [e] $CHCl_3$/MeOH/2.2 M NH_4OH 5:3.9:1.1. Bn = benzyl.

Similar to D_6 PtdIns4*P* (–)-**322**, the high resolution mass spectrum of D_6 PtdIns5*P* (–)-**326** revealed two peaks with shifts of 3.0185 and 6.0367 mass units as compared to the peaks observed for the protonated analogue (Figure 3.25 and 3.26). The ^1H, ^{31}P, ^2H and 2D NMR spectra were analysed and compared to protonated PtdIns5*P* (–)-**240**, as described previously (Figures 3.27, 3.28 and 3.29).

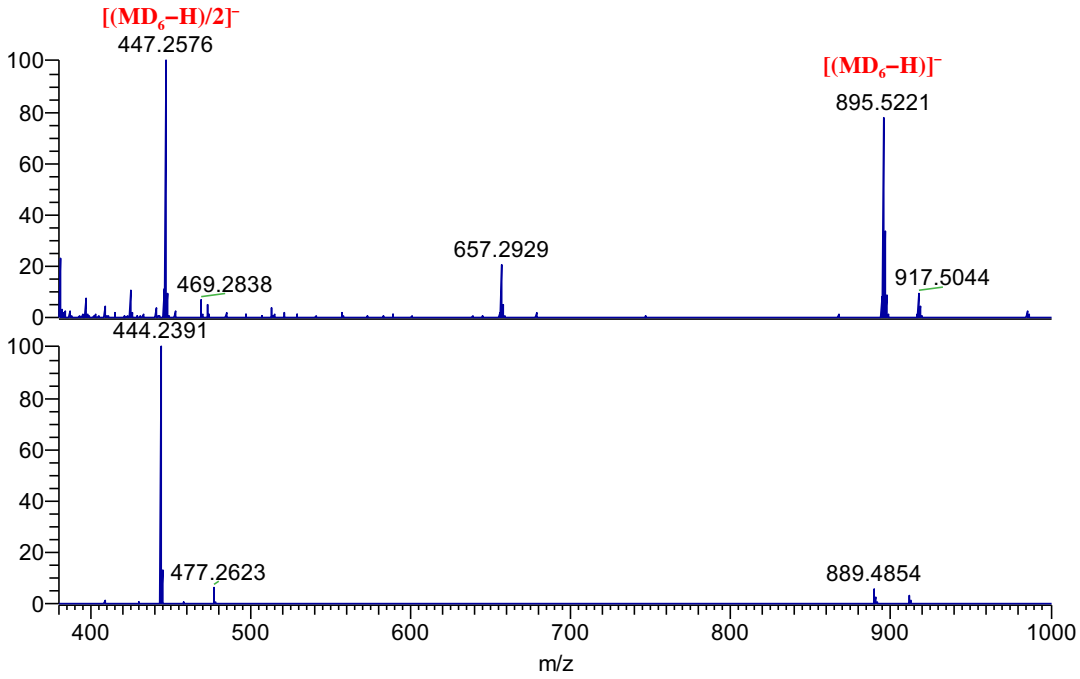


Figure 3.25 High resolution mass spectra for PtdIns5*P* (bottom) and D_6 PtdIns5*P* (top).

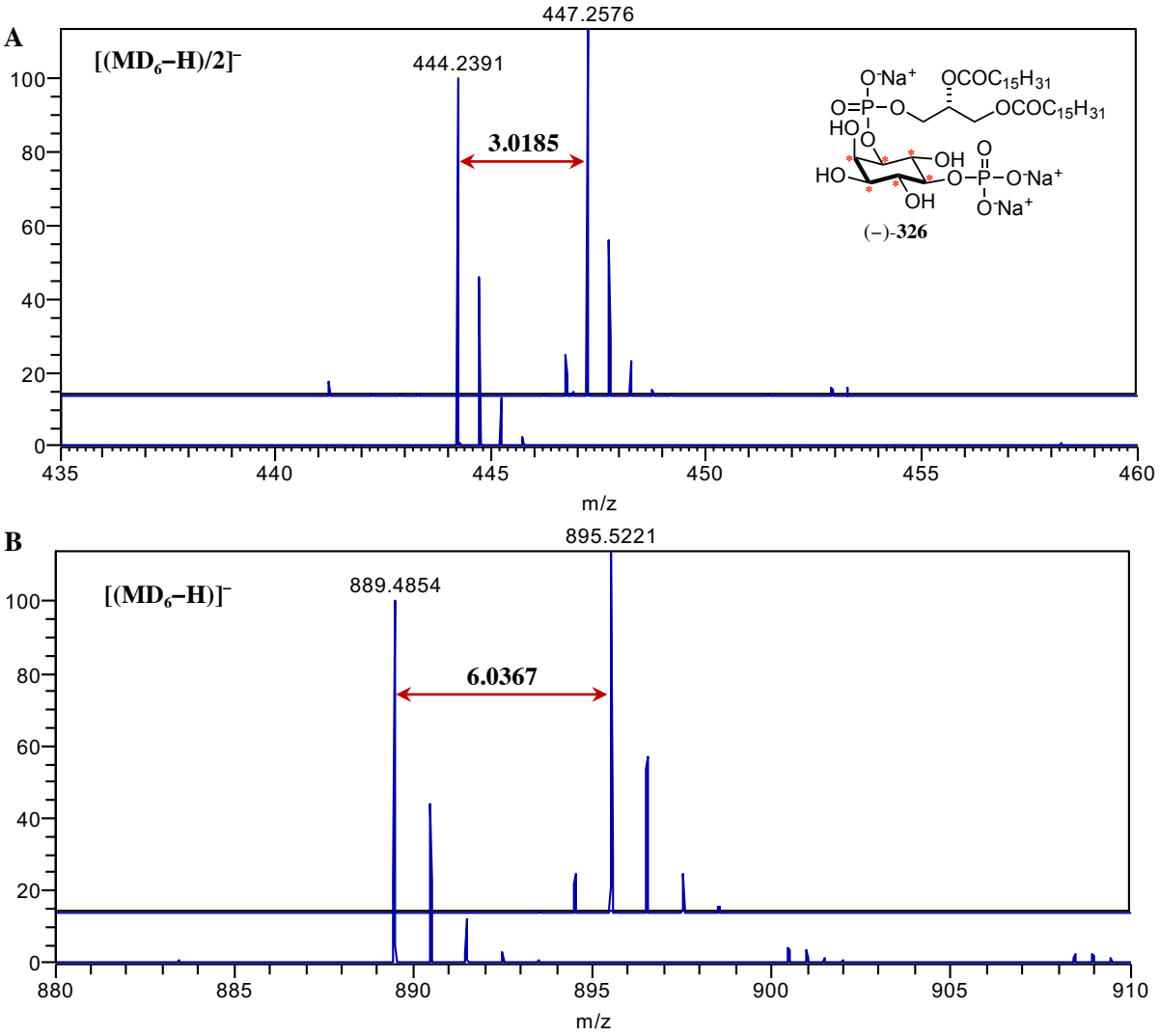


Figure 3.26 Comparison of the high resolution mass spectra for PtdIns5*P* (–)-**326** (bottom) and D_6 PtdIns5*P* (–)-**240** (top) **A.** Expansion of the peaks corresponding to $[(MD_6–H)/2]^-$ **B.** Expansion of the peaks corresponding to $[(MD_6–H)]^-$. The mass differences are marked with a red arrow.

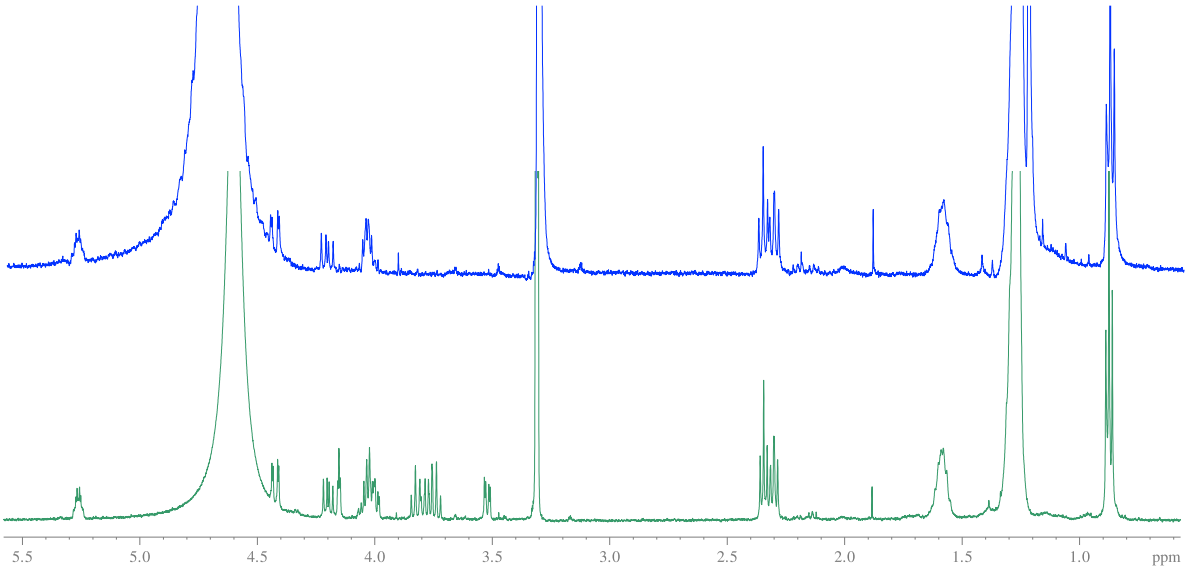


Figure 3.27 Comparison of the ^{1}H NMR obtained for PtdIns5*P* (–)-**240** (green) and D_6 PtdIns5*P* (–)-**326** (blue). Both ^{1}H NMR spectra were acquired at 500 MHz, in $CD_3OD/CDCl_3/D_2O$ 4:3:1.

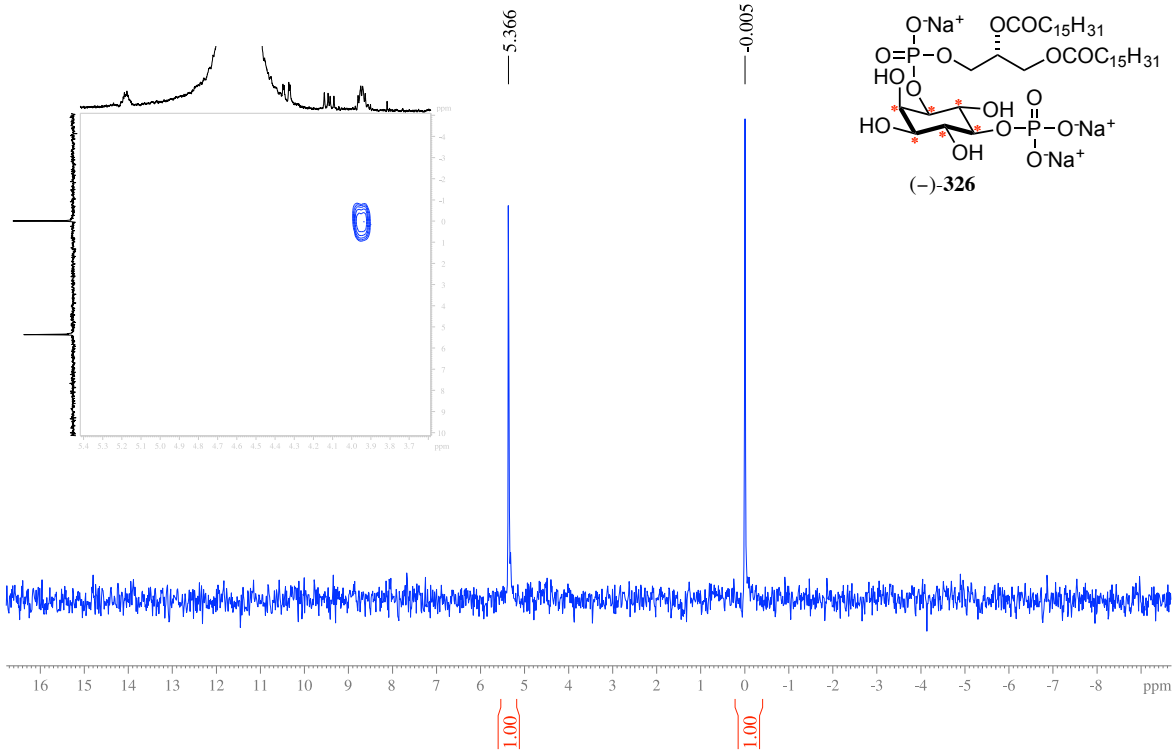


Figure 3.28 ^{31}P and ^{31}P-^{1}H HMBC NMR spectra of D_6 PtdIns5*P* (–)-**326**. The ^{31}P spectrum was acquired at 162 MHz, in $CD_3OD/CDCl_3/D_2O$ 4:3:1.

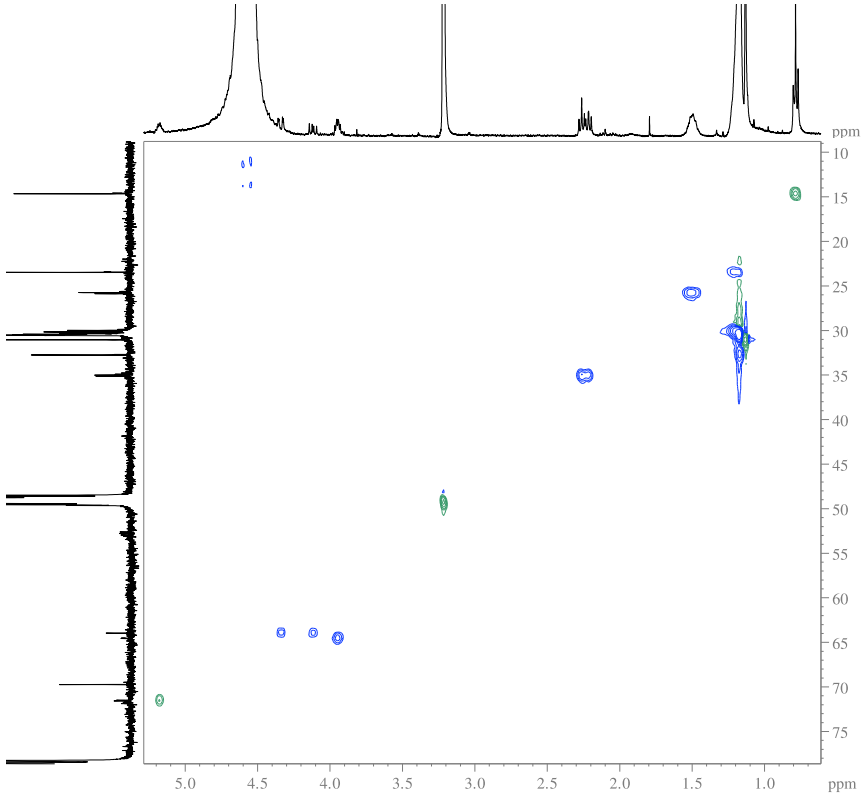


Figure 3.29 HSQC NMR spectrum of D_6 dipalmitoyl PtdIns5*P* (–)-**326**. The ^{13}C NMR spectrum was acquired at 126 MHz, in $CD_3OD/CDCl_3/D_2O$ 4:3:1.

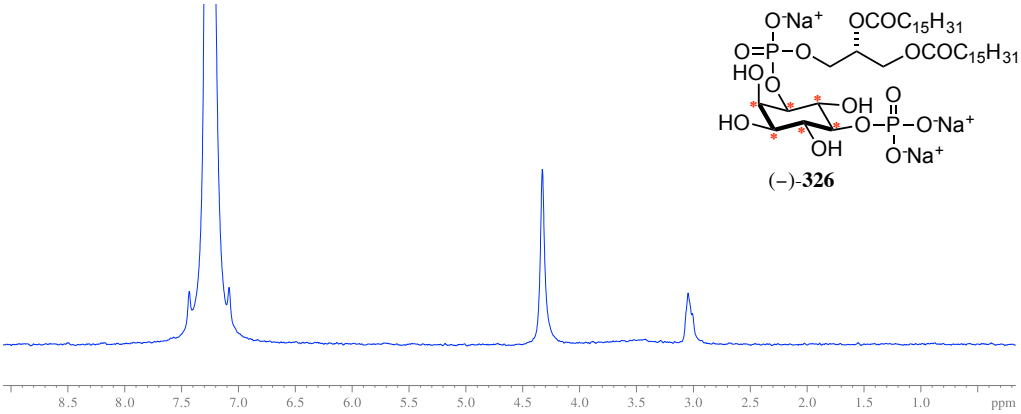


Figure 3.30 ^{2}H NMR spectrum of D_6 PtdIns5*P* (–)-**326** showing two distinct deuterium environments, similarly to the ^{2}H NMR spectrum of D_6 PtdIns4*P*. This spectrum was acquired at 96 MHz in $CHCl_3$ with $CDCl_3$ as a reference.

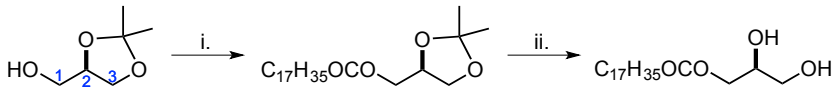
3.15. Synthesis of D_{41} PtdIns4*P*

Having fully optimised the synthesis of saturated dipalmitoyl PtdIns4*P* (–)-**48**, PtdIns5*P* (–)-**240**, D_6 PtdIns4*P* (–)-**322** and D_6 PtdIns5*P* (–)-**326**, we aimed to extend this research to the synthesis of alternative deuterated devatives. In particular, PtdIns*P* probes containing deuterium both around the *myo*-inositol ring and on the lipid chains would be invaluable in establishing their distinct biological roles. Additionally, as described in Chapter 1, the more abundant, naturally occurring 1-stearoyl-2-arachidonoyl PtdIns*P*s **11** and **12** are particularly desirable.[192,379]

3.15.1. Synthesis of D_{35} (*S*)-(–)-1,2-distearoyl-*sn*-glycerol (–)-**328**

A modified synthesis of enantiomerically pure mixed diglycerides was optimised, based on the reported literature.[23,240] In contrast with the synthesis of dipalmitoyl-*sn*-glycerol (–)-**211** described in Chapter 2 (Section 2.6), the glycerol needed to be selectively functionalised at the *sn*-1 and *sn*-2 positions. In addition, due to the incompatibility of unsaturated chains with hydrogenolysis, alternative protecting groups, which are removable under mild conditions, were investigated. Finally, monitoring the *e.e.* of monoacyl and diacylglycerides intermediates during the synthesis was essential, as these are particularly prone to acyl migration under acidic or basic conditions, leading to isomerisation and racemisation (Section 2.6). (*S*)-(–)-1,2-distearoyl-*sn*-glycerol (–)-**328** was chosen as a first target for the optimisation of this chemistry.

Similar to the reported procedures, (*R*)-(−)-2,3-isopropylidene-*sn*-glycerol (−)-**329** was acylated smoothly with stearoyl chloride in the presence of 4-DMAP to provide (−)-**330** in 76% yield (Scheme 3.32). Conditions for the deprotection of an acetonide on frameworks such as (−)-**330** have been thoroughly investigated in the literature.[240,380] Notably, Mori[380] demonstrated that commonly employed conditions such as Amberlyst 15 (H^+ form) in MeOH/CH_2Cl_2,[23,381,382] but also others including $FeCl_3$ on silica[383] or $Zn(NO_3)_2{\cdot}6H_2O$ in MeCN[384] led to acyl migration and racemisation. Mori found that deprotection of (−)-**330** with 80% aqueous acetic acid at 50 °C for 2 hours followed by crystallisation gave (+)-**331** in 94–95% *e.e.* as determined by MTPA/NMR analysis.[380] Deprotection of (−)-**330** under these conditions was also successful in our hands, giving (+)-**331** in a 70% yield after crystallisation from pentane/EtOAc (Scheme 3.32).



(−)-**329** (−)-**330** (+)-**331**

Scheme 3.32 Synthesis of diol (+)-**331** from (*R*)-(−)-2,3-isopropylidene-*sn*-glycerol (−)-**329** *Reagents and conditions:* i. Stearic acid, DCC, 4-DMAP, CH_2Cl_2, 0 °C to RT, 12.5 h, 76% ii. AcOH/H_2O (4:1), 50 °C, 2 h, 70%, >99% *e.e.*

The *e.e.* of (+)-**331** was determined by NMR, as described by Wenzel[324] and Seco *et al.*,[268] by following the sequence of steps outlined below:

1. **Diol (+)-331 was derivatised with each enantiomer ((*R*)- and (*S*)-) of MPA, our chosen chiral anisotropy reagent**

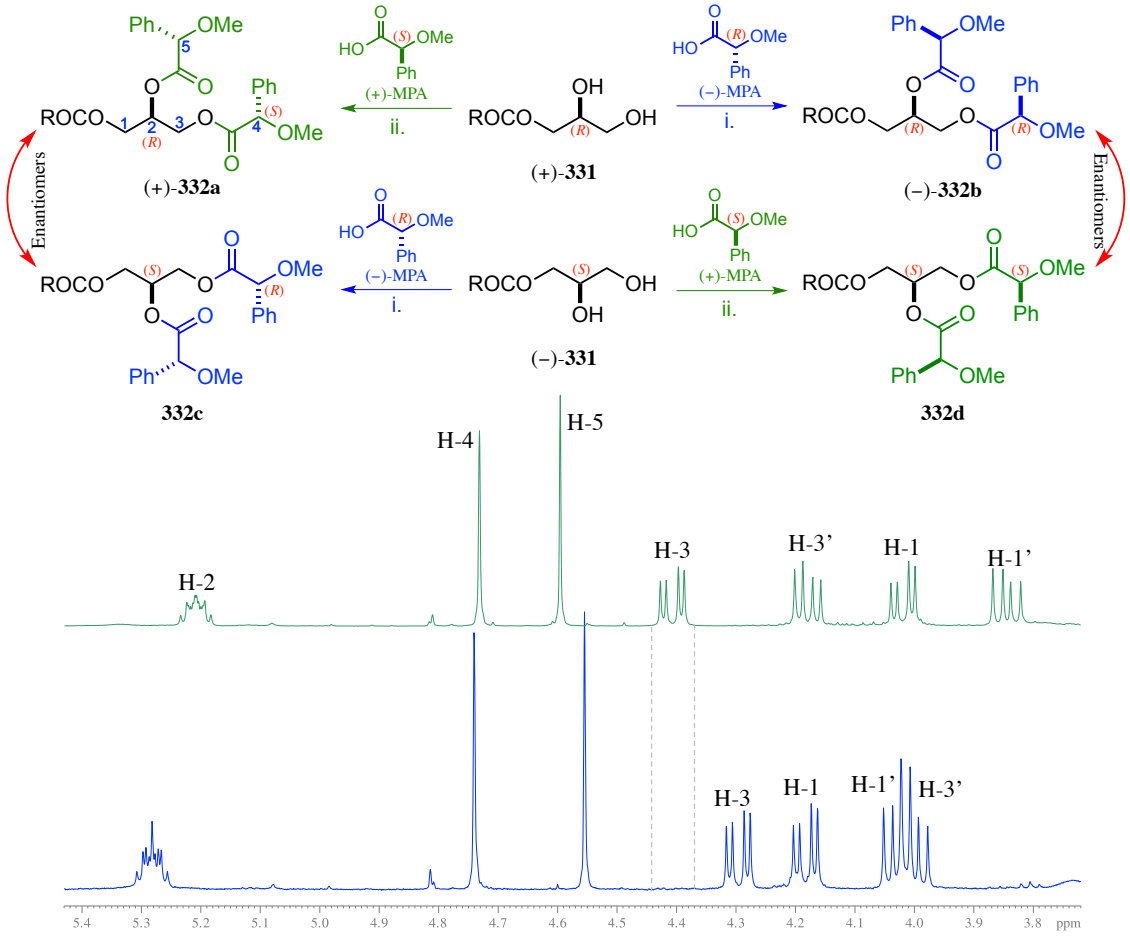
   Condensation of diol (−)-**331** with excess (*S*)-MPA (See Section 3.6 for more detail), in the presence of 4-DMAP and EDC, furnished disatereoisomer (+)-**332a**, whilst reaction with (*R*)-MPA provided disatereoisomer (−)-**332b**.

2. **The enantiomeric excess (*e.e.*) of the substrate was determined by comparing the crude ^{1}H NMR spectra of diastereoisomers** (+)-**332a and** (−)-**332b.**

   Section 3.6 outlines that the absolute configuration of a compound is identified through the ^{1}H NMR spectra of the purified diastereoisomers obtained. In contrast, determination of the *e.e.* is achieved through comparison of crude ^{1}H NMR spectra. This is essential, as

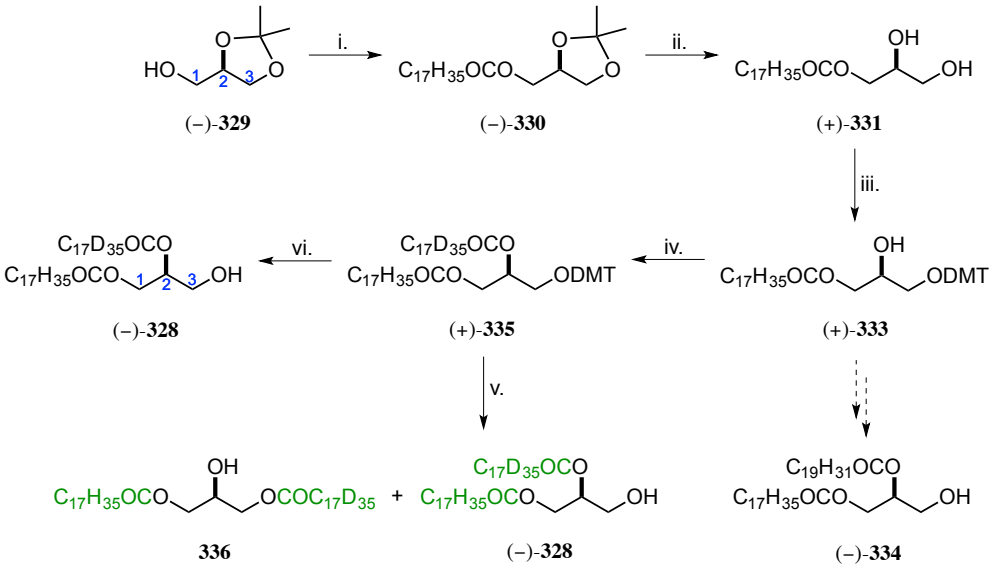
diastereoisomers may be separated during the purification step.

The crude ^{1}H NMR spectra of diastereoisomers (+)-**332a** ((*S*)-MPA) and (–)-**332b** ((*R*)-MPA) feature proton signals with distinct chemical shifts. If the diol (+)-**331** under consideration is not enantiomerically pure, diastereoisomer **332c** will also be formed in the reaction with (*R*)-MPA and diastereoisomer **332d** in the reaction with (*S*)-MPA. As **332c** is the enantiomer of (+)-**332a**, these diastereoisomers will have identical ^{1}H NMR spectra. The same applies for **332d** and (–)-**332b**. As such, if signals for **332c** (equivalent to that of (+)-**332a**) are observed in the crude ^{1}H NMR spectra of (–)-**332b** the *e.e.* can be calculated by integrating the signals corresponding to (–)-**332b** and **332c**. If no residual signals are observed, the *e.e.* is presumed to be greater than 99%. This was indeed the case for (+)-**332a** and (–)-**332b**. The *e.e.* of diol (+)-**331** was therefore greater than 99% (Scheme 3.33).



Scheme 3.33 Comparing the ^{1}H NMR spectra (500 MH, in $CDCl_3$) of the crude diastereoisomers (+)-**332a** and (–)-**332b** obtained from the derivatisation of diol (+)-**331**. Analysis of these spectra shows that the *e.e.* of (+)-**331** is greater than 99%. *Reagents and conditions*: i. (*R*)-MPA, EDC, 4-DMAP, CH_2Cl_2, RT, 24 h, 83% ii. (*S*)-MPA, EDC, 4-DMAP, CH_2Cl_2, RT, 24 h, 89%. R = -$C_{17}H_{35}$; Me = methyl; Ph = phenyl

The primary alcohol of enantiomerically pure diol (–)-**331** was regioselectively protected with a dimethoxytrityl (DMT) group, as described in by Kubiak and Bruzik,[23] using dimethoxytritylchloride (DMTCl) in pyridine. The secondary alcohol (+)-**333** was obtained in good yields (Scheme 3.34). A number of interesting diglycerides, including 1-stearoyl-2-arachidonoyl-*sn*-glycerol (–)-**334**, can be directly prepared from this versatile building block.



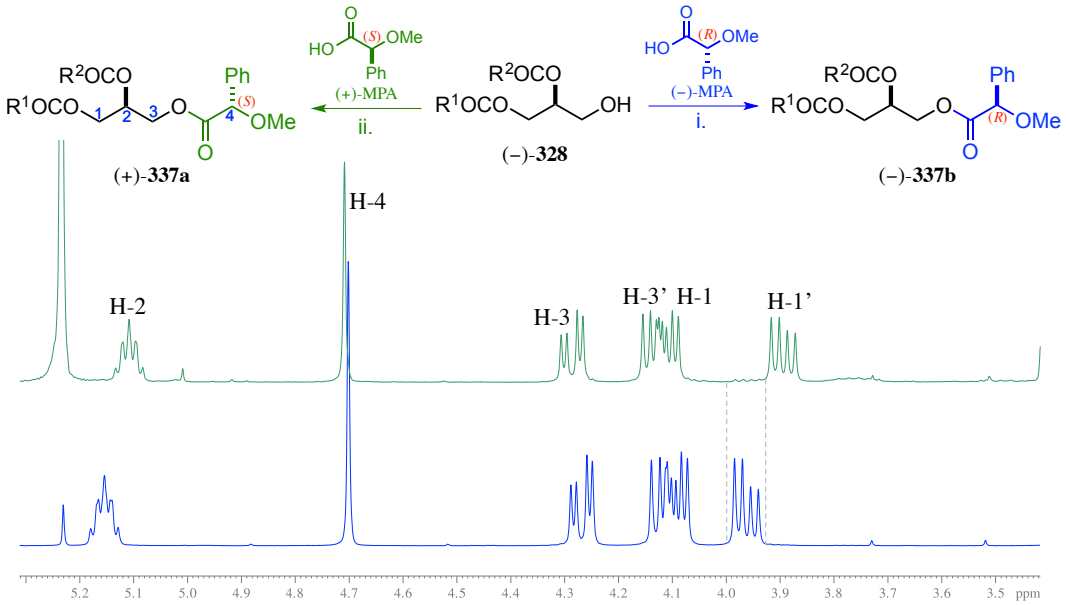
Scheme 3.34 Synthesis of D_{35} (*S*)-(–)-1,2-distearoyl-*sn*-glycerol (–)-**328**. *Reagents and conditions:* i. Stearic acid, DCC, 4-DMAP, CH_2Cl_2, 0 °C to RT, 12.5 h, 76% ii. AcOH/H_2O (4:1), 50 °C, 2 h, 70%, >99% *e.e.* iii. DMTCl, pyridine, RT, 50 min, 67% iv. D_{35} stearic acid, DCC, 4-DMAP, CH_2Cl_2, RT, 24 h, 91%, 70% D_{35}, 30% D_{34} v. TFA, pyrrole, CH_2Cl_2, 3 h, 54%, (–)-**328**/**336** 3.3:1.0 vi. AcOH/H_2O (4:1), 50 °C, 3.5 h, 94%, 72% D_{35}, 28% D_{34}, >96% *e.e.* DMT = dimethoxytrityl.

For the synthesis of D_{35} 1,2-distearoyl-*sn*-glycerol (–)-**328**, condensation of (–)-**333** with commercially available D_{35} stearic acid under standard conditions, afforded the deuterated DMT-protected precursor (+)-**335** in 91% yield (Scheme 3.34). The deuterium incorporation of (+)-**335** was 70% D_{35} and 30% D_{34}, as determined by mass spectrometry.

Deprotection of acid-labile groups at the *sn*-3 position has been accomplished on similar derivatives by Gaffney and Reese,[240] but also Kubiak and Bruzik,[23] with dichloroacetic acid or trifluoroacetic acid (TFA) and pyrrole as a carbocation scavenger. In our hands however, reaction of (+)-**335** with TFA and pyrrole in CH_2Cl_2 followed by crystallisation from hexane and Et_2O yielded a mixture of the expected product (–)-**328** and the migration product **336** (Scheme 3.34). Considering aqueous AcOH is routinely applied to the deprotection of DMT protecting groups in oligonucleotide chemistry, we hypothesised that the conditions used for the cleavage of the acetonide on (–)-**330**

would be applicable to the deprotection of (+)-**335**. Indeed, treatment with 80% aqueous AcOH at 50 °C for 3.5 hours followed by evaporation of the volatile components *in vacuo* and crystallisation from pentane and Et_2O furnished (–)-**328** in 94% yield (Scheme 3.34). This product was not contaminated by any migration regioisomer, and the *e.e.* of (–)-**328** was shown to be greater than 96% by MPA ^{1}H NMR analysis of (+)-**337a** and (–)-**337b** (Scheme 3.35). This diglyceride possessed a deuterium incorporation of 72% D_{35} and 28% D_{34} as determined by mass spectrometry.

We intend on employing this optimised route for the synthesis of other deuterated, unsaturated diglyceride derivatives such as (–)-**334**.



Scheme 3.35 Comparing the ^{1}H NMR spectra of the crude diastereoisomer (+)-**337a** and that of (–)-**337b** obtained from the derivatisation of diglyceride (–)-**328**. Analysis of these spectra shows that the *e.e.* of (–)-**328** is greater than 96%. Multiple recrystallisations of the product has been shown to improve the *e.e.* *Reagents and conditions*: i. (*R*)-MPA, EDC, 4-DMAP, CH_2Cl_2, RT, 24 h, 86%, 70% D_{35}, 30% D_{34} ii. (*S*)-MPA, EDC, 4-DMAP, CH_2Cl_2, RT, 24 h, 94%, 71% D_{35}, 29% D_{34}. $R^1 = -C_{17}H_{35}$; $R^2 = -C_{17}D_{35}$

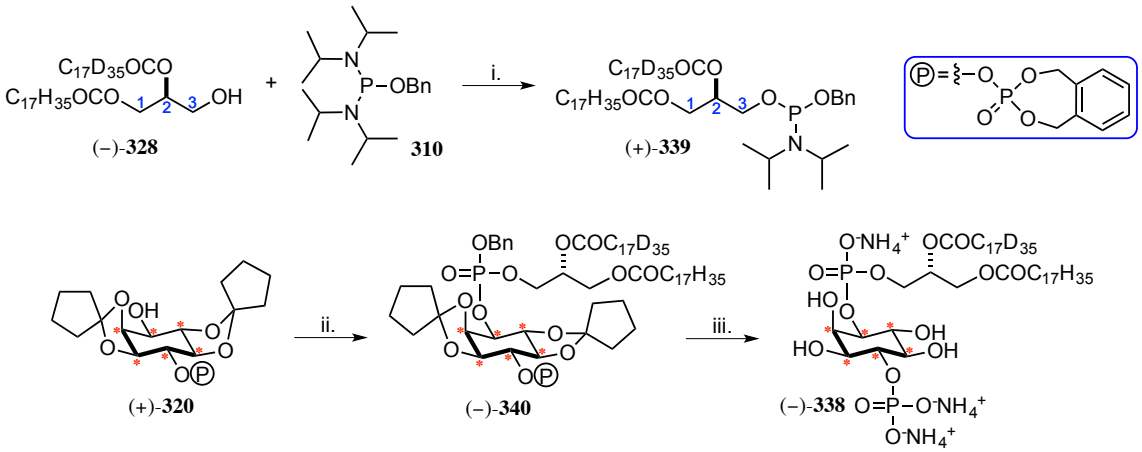
3.15.2. Synthesis of D_{41} PtdIns4*P* (–)-**338**

To demonstrate the robustness and versatility of the route developed towards PtdIns4*P* derivatives, D_{41} PtdIns4*P* (–)-**338** was synthesised using enantiomerically pure D_{35} 1,2-distearoyl-*sn*-glycerol (–)-**328** (Scheme 3.36).

First, phosphoramidite (+)-**339** was prepared fresh by slow addition of the diglyceride (–)-**328** to a solution of benzyloxy-bis(*N*,*N*-diisopropylamino)phosphine **310** and 1*H*-tetrazole in CH_2Cl_2.

Purification by column chromatography, using 0.5% of triethylamine to avoid hydrolysis, gave (+)-**339** as a mixture of two diastereoisomers in 90% yield and without any change in the deuterium incorporation (Scheme 3.36).

Phosphoramidite (+)-**339** was coupled to the deuterated fragment (+)-**320** under 1*H*-tetrazole catalysis to give the corresponding phosphite intermediate. *In situ m*CPBA oxidation provided the fully protected PtdIns4*P* precursor (–)-**340** in 65% yield as a mixture of two diastereoisomers (Scheme 3.36). Although determination of the deuterium incorporation was complicated by the assembly of two deuterated fragments, further high resolution mass spectrometry studies revealed that the overall incorporation was 62% D_{41}, 29% D_{40} and 9% D_{39}.



Scheme 3.36 Synthesis of phosphoramidite (+)-**339**, subsequent coupling with (+)-**320** and deprotection to give D_{41} PtdIns4*P* (–)-**338**. *Reagents and conditions*: i. 1*H*-tetrazole, CH_2Cl_2, RT, 18 h, 90%, 70% D_{35}, 30% D_{34} ii. a. (+)-**339**, 1*H*-tetrazole, CH_2Cl_2, RT, 22 h b. *m*CPBA, –78 °C to RT, 3 h, 65%, 62% D_{41}, 29% D_{40}, 9% D_{39} iii. a. TMSBr, toluene, RT, 12 h b. MeOH, 0 °C, 1 h, 58%, 59% D_{41}, 31% D_{40}, 10% D_{39}. Bn = benzyl.

The ^{1}H NMR spectrum of (–)-**340** features the expected suppressed signals. In addition to the usual signals observed for the deuterated carbons around the *myo*-inositol ring, low intensity multiplets with a distinct upfield isotope shift are found in the ^{13}C NMR spectrum. Finally, the ^{2}H NMR spectrum exhibits both the characteristic signals for the six deuterium atom around the *myo*-inositol ring and four distinct upfield signals corresponding to the deuterated lipid chain (Figure 3.31).

Global deprotection of (–)-**340** was undertaken as previously, with TMSBr in toluene and at room temperature. After column chromatography, D_{41} PtdIns4*P* (–)-**338** was obtained (Figures 3.33 and 3.34) at its presumed ammonium salt in a 58% yield and with a deuterium incorporation of 59% D_{41},

31% D_{40}, 10% D_{39}. Analysis by high resolution mass spectrometry showed a distinct mass corresponding to the product, confirming the value of (–)-**338** as a chemical probe (Figure 3.32).

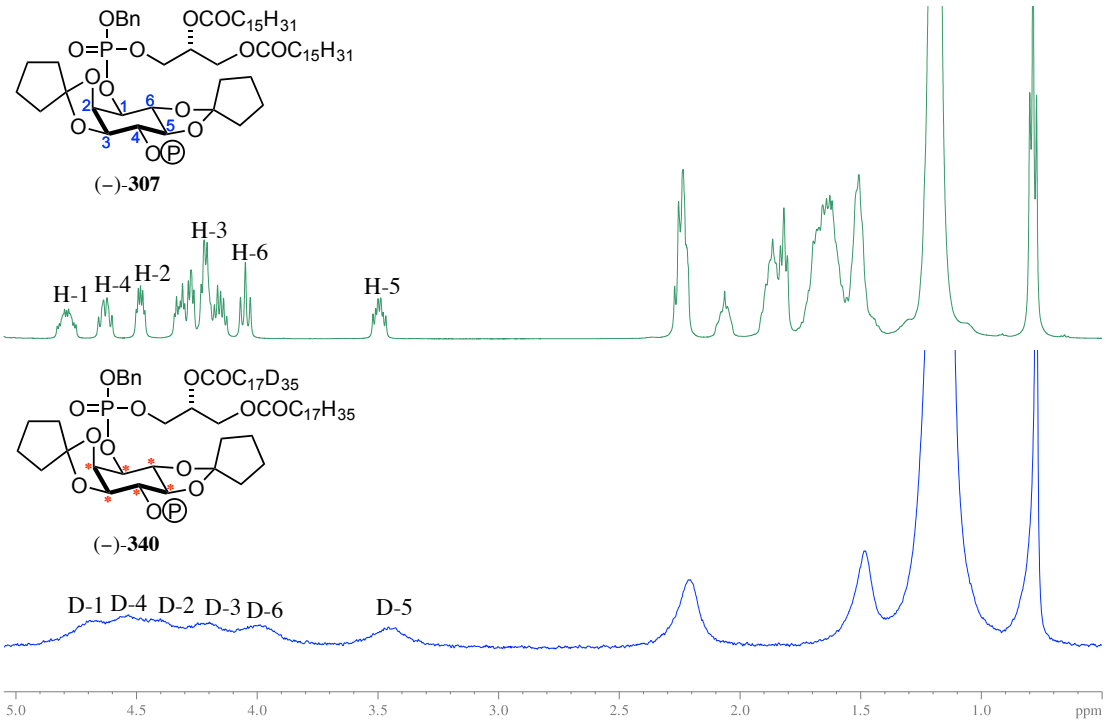


Figure 3.31 Comparison of the ^{1}H NMR spectrum of fully protonated dipalmitoyl (–)-**307** (green) and the ^{2}H NMR spectrum of (–)-**340**, the fully protected precursor to D_{41} PtdIns4*P* (blue). Six distinct deuterium environment can be observed between 5.0 and 3.0 ppm. The signals upfield correspond to the deuterated lipid chain (between 2.5 and 0 ppm). The ^{1}H NMR spectrum was run at 500 MHz in CD_2Cl_2 and the ^{2}H spectrum at 96 MHz in CH_2Cl_3 with $CDCl_3$ as a reference.

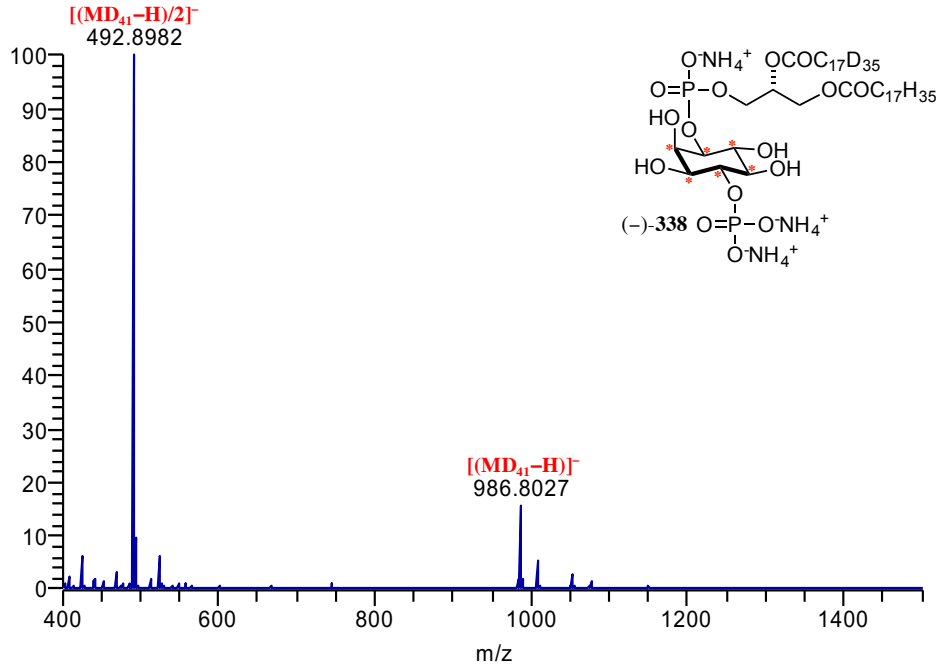


Figure 3.32 High resolution mass spectra of D_{41} PtdIns4*P* (–)-**338**. Two main peaks can be observed, corresponding $[MD_{41}–H]^-$ and $[(MD_{41}–H)/2]^-$.

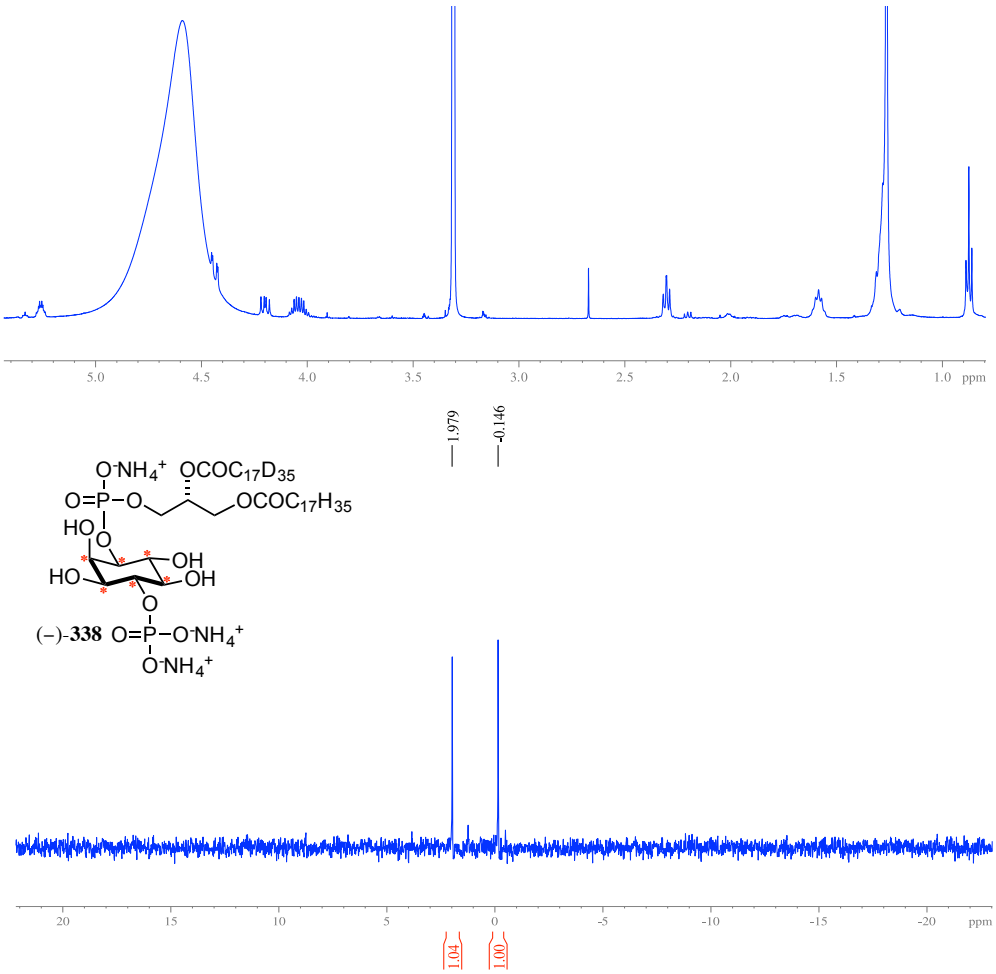


Figure 3.33 ^{1}H NMR (top) and ^{31}P NMR (bottom) NMR data for D_{41} PtdIns4*P* (–)-**338**. The ^{1}H NMR spectrum was run at 400 MHz and the ^{31}P NMR spectrum at 162 MHz, in in $CD_3OD/CDCl_3/D_2O$ 4:3:1.

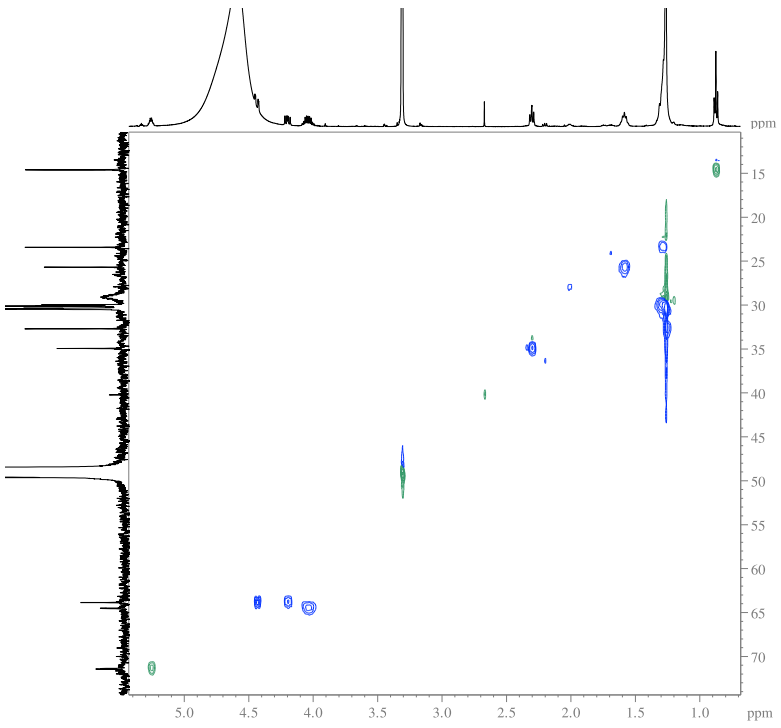


Figure 3.34 HSQC NMR spectrum for D_{41} PtdIns4*P* (–)-**338**. The ^{13}C NMR spectrum was acquired at 126 MHz, in $CD_3OD/CDCl_3/D_2O$ 4:3:1.

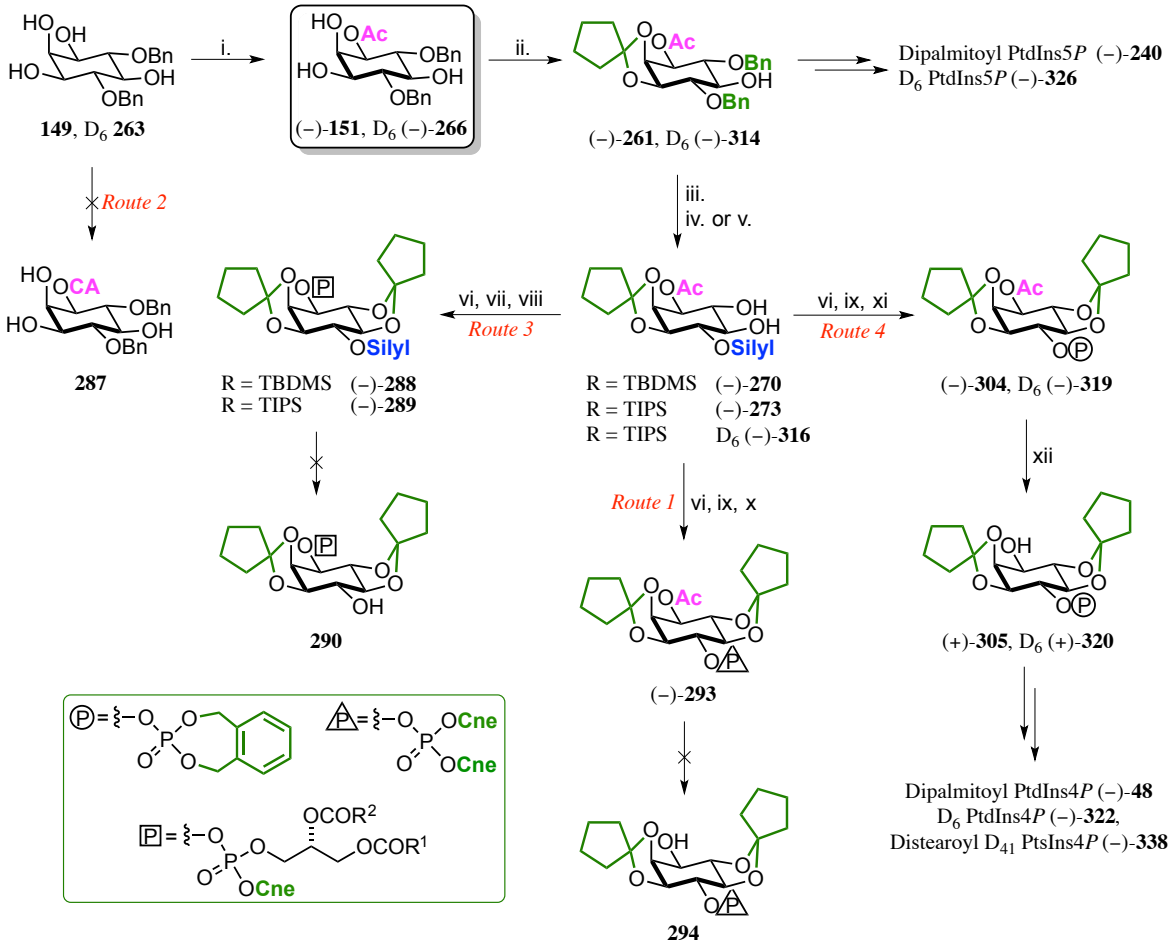
3.16. Summary and conclusions

Building on the knowledge acquired in Chapter 2, our research into the synthesis of PtdIns*P* derivatives led to the following findings (Scheme 3.37):

- After exploring the possibility of using an organocatalytic approach, an enantioselective and high yielding enzymatic methodology was employed for the desymmetrisation of 4,6-di-*O*-benzyl-*myo*-inositol **149** and the deuterated analogue **263** with Lipozyme TL-IM®.
- Enantiomerically pure (–)-**151** was simultaneously protected at the 2- and 3-positions with a cyclopentylidene acetal to generate (–)-**261**, a precursor to PtdIns5*P*. Following hydrogenolysis of the 4,6-di-*O*-benzyl esters, the regioselectivity of triol (–)-**269** was explored. This investigation revealed that 4-*O*-TIPS protection is more selective than 4-*O*-TBDMS protection, giving (–)-**270** and (–)-**273** (Scheme 3.37).
- Although allylation of the 5,6-diol on a framework such as (–)-**270** and (–)-**273** led to undesirable silyl migration, acetalisation of these diols with a second cyclopentylidene acetal was successful.
- A thorough investigation into conditions for the deprotection of the 4-*O*-TIPS ether on (–)-**284** showed that TASF in DMF were the most appropriate.
- Deprotection of: a) an acetate group in the presence of a cyanoethyl-protected phosphate (*Route 1*) or b) a silyl group in the presence of a phosphate diester (*Route 3*) was unsuccessful. In addition, attempts at using a chloroacetate group in the enzymatic desymmetrisation step (*Route 2*) did not provide enantiomerically pure **287** (Scheme 3.37).
- Phosphorylation with XEPA **303** was very high yielding (*Route 4*). The base stability of the *o*-xylylene phosphates esters was the key for a successful deacetylation of (–)-**304**. The framework (+)-**305** thus obtained could subsequently be phosphorylated at the 1-position.
- The dipalmitoyl PtdIns4*P* derivative (–)-**48** was obtained *via* a one step global deprotection using TMSBr in toluene. Column chromatography over silica gel was used to purify the final compound.

- The dipalmitoyl PtdIns5*P* derivative (–)-**240** was obtained *via* hydrogenolysis. All protecting groups were cleaved under those conditions, including the 2,3-*O*-cyclopentlidene acetal protecting group.

The novel route developed through these findings was employed in the synthesis of the first enantiomerically pure deuterated mass spectrometry probes: dipalmitoyl D_6 PtdIns4*P* (–)-**322** and D_6 PtdIns5*P* (–)-**326** as well as distearoyl D_{41} PtdIns4*P* (–)-**338**. These probes will be used in HPLC-MS studies to further understand the specific sub-cellular location and biological function of PtdIns4*P* and PtdIns5*P*.



Scheme 3.37 Overview of synthesis developed towards PtdIns4*P* and PtdIns5*P* derivatives: *Reagents and conditions*: i. Lipozyme TL-IM®, vinyl acetate/hexane (1:1), 45 °C, 15 h, 84–98%, >99% *e.e.* ii. **260**, PTSA·H_2O, CH_2Cl_2, RT, 18–19 h, 87–92%, iii. 10% Pd/C, $H_{2(g)}$, EtOAc, RT, 2 h, 76–94% iv. TIPSOTf, 2,6-lutidine, THF, –78 °C, 16 h, 71–74% v. TBDMSOTf, 2,6-lutidine, THF, –78 °C, 16 h, 55% vi. **260**, PTSA·H_2O, CH_2Cl_2, RT/30 °C, 4–16 h, 62–71% vii. NaOMe (2.6 M), MeOH/CH_2Cl_2, RT, 1–1.5 h, 83–92% viii. a. (–)-**193**, 1*H*-tetrazole, CH_2Cl_2, RT, 2.5–5 h b. *m*CPBA, –78 °C to RT, 14–16 h, 68–70% ix. TAS-F, DMF, RT, 3 h, 75–88% x. a. **218**, 1*H*-tetrazole, CH_2Cl_2, RT, 20.5 h b. *m*CPBA, –78 °C to RT, 2 h, 86% xi. a. XEPA **303**, 1*H*-tetrazole, CH_2Cl_2, RT, 1–1.5 h b. *m*CPBA, –78 °C to RT, 1.5 h, 79–88%, xii. K_2CO_3, MeOH, RT, 1 h, 74–89%. Ac = acetate; Bn = benzyl; Silyl = *tert*-butyldimethylsilyl or triisopropylsilyl; CA = chloroacetate.

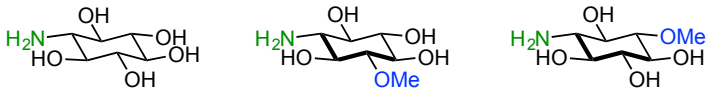
4 Synthesis of rhizopines: Towards establishing a *trans*-kingdom signal between bacteria and plants

4.1. Aims and introduction

In collaboration with Dr Barney Geddes,[X] a postdoctoral associate in Professor Poole's laboratory (Plant Sciences Department, University of Oxford), we became interested in engineering a unique *trans*-kingdom signal from plants to bacteria (Section 1.4).

In this chapter, we aim to demonstrate whether plants can be engineered to produce rhizopines *scyllo*-inosamine **13** (SIA) and/or 3-*O*-methyl-*scyllo*-inosamine **14** (3-*O*-MSI) as unique chemical signals (Figure 4.1). As such, we directed our research towards:

- Understanding whether, in the natural system, rhizopines are indeed efficiently secreted from the plant roots and are subsequently recognised by bacteria in the Rhizosphere;
- Identifying the set of genes involved in rhizopine biosynthesis, basing our work on previous literature.



SIA **13** (−)-1L-3-*O*-MSI (−)-**14** (+)-1D-3-*O*-MSI (+)-**14**

Figure 4.1 Structure of *scyllo*-inosamine **13** (SIA) and 3-*O*-methyl-*scyllo*-inosamine **14** (3-*O*-MSI).

Synthetic rhizopines **13–14** are essential to achieving these goals, as only a limited amount can be isolated from plant nodules.[385] Therefore, using the knowledge gained through the development of a novel synthetic route towards PtdIns*P*s in Chapters 2 and 3, we aimed to optimise an efficient

[X] Dr Barney Geddes carried out all of the biochemical work presented in this Chapter.

synthesis of SIA **13** and both enantiomers of 3-*O*-MSI **14**. We intended on using these synthetic rhizopines not only as standards in biochemical studies, but also for the identification of the natural enantiomer of 3-*O*-MSI for the first time.

4.2. Engineering of a rhizopine biosensor to visualise rhizopine exudation

4.2.1. Development of a Rhizopine biosensor

Dr Barney Geddes from Prof. Philip Pool's laboratory engineered a rhizopine *lux* biosensor (pOPS0046) to uncover whether, in the natural system, rhizopines are released by the plant efficiently and perceived by bacteria in the rhizosphere. The rhizopine biosensor plasmid was constructed by inserting the bacterial luciferase genes (*lux*) under the control of a presumed inducible promoter present in the rhizopine catabolism *moc* locus (Figure 4.3).

In *S. meliloti* L5-30 and *R. leguminosarum* bv. *viciae* strains, the cluster of genes (*moc* locus) responsible for the catabolism of rhizopines[202,386] includes *mocC*, ORF334, ORF293, *mocA*, *mocB*, *mocR*, *mocD*, *mocE* and *mocF* genes (Figure 4.2).[386-388] Schoffers *et al*. recently compared the growth of *S. meliloti* wild-type strain and various *moc* mutant strains with synthetic SIA **13** as sole carbon and nitrogen source.[389] In this catabolism assay, *mocA*, *mocB*, *mocC* and *mocR* mutants did not show any growth, confirming the importance of these genes in the catabolism of SIA **13**. The additional genes *mocDE* were demonstrated to be essential for 3-*O*-MSI **14** catabolism.[387,388] The specific function of the Moc proteins encoded by these genes, however, are still largely unknown.[390] Protein homology studies suggested that MocB is involved in the uptake and transport of rhizopines. MocR is thought to be a regulator protein, and the NAD(H)-dependant *myo*-inositol dehydrogenase MocA may initiate the catabolism process by converting SIA **13** to *scyllo*-inosose **341** (also called 2-keto-*myo*-inositol).[386,388,391] MocC was shown to be important for rhizopine catabolism in mutagenesis

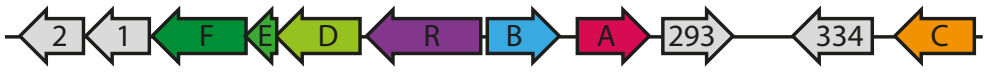
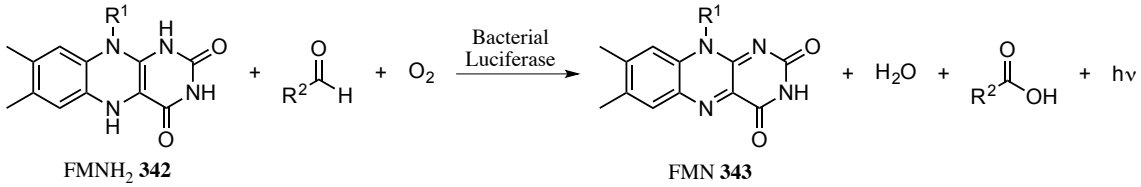


Figure 4.2 Structure of the *moc* gene locus in *S. meliloti* L5-30. The genes *mocABCR* were shown to be essential for rhizopine catabolism. The grey arrows indicate the location of the open reading frames (ORFs) in the moc region.[381,378,383]

studies[386] and to share similarities with the *scyllo*-inosose dehydratase encoded by the *S. meliloti iolE* gene.[390] Finally, *mocDE* are thought to encode a ferredoxin-dependant oxygenase system, which is believed to be involved in the demethylation of 3-*O*-MSI **14**, and MocF shows homologies to ferredoxin reductases. Further catabolism then occurs *via* the inositol catabolism pathway.[387,391]

The bacterial luciferase bioluminescence gene cassette (*lux*) has been commonly employed as a bioreporter to monitor gene expression *in vivo*.[392,393] The *lux* operon comprises five genes: *luxCDABE*. The adjacent *luxA* and *luxB* genes encode the α and β subunits of the luciferase heterodimer. These are flanked by *luxCDE* encoding the fatty acid reductase multienzyme complex that includes the fatty-acid reductase, acyl-transferase, and acyl protein synthetase. This complex is responsible for the biosynthesis of the fatty aldehyde substrates which are necessary for the bioluminescence reaction.[394-396] Green-blue light is emmited when a fatty aldehyde and riboflavin phosphate **342** ($FMNH_2$) are oxidised in the presence of oxygen, under luciferase catalysis (Equation 4.1). The corresponding fatty acid and FMN **343** produced are regenerated by the LuxCDE reductase complex and a flavin reductase (*luxG*) respectively.[397]



Equation 4.1 Bioluminescence reaction in bacteria. Luciferase catalyses the reduction of long chain aldehydes and riboflavin phosphate to emite blue-green light (λ_{max} ~ 490 nm).[389]

As such, Dr Barney Geddes postulated that a rhizopine *lux* biosensor plasmid could be engineered by cloning the genomic region containing divergently transcribed genes *mocB* and *mocR* into the *lux* reporter vector pIJ11268.[398] The cloning was such that *luxCDABE* formed an operon with the presumably highly inducible *mocB* (Figure 4.3).

The design of the biosensor was based on the assumption that MocB is involved in the transport of rhizopines into the cell and that binding of this inducer to the transcriptional regulator protein MocR activates transcription from a rhizopine-inducible promoter PmocB. Since *luxCDABE*, are expressed downstream of *mocB*, PmocB controls *lux* gene expression and therefore bioluminescence.

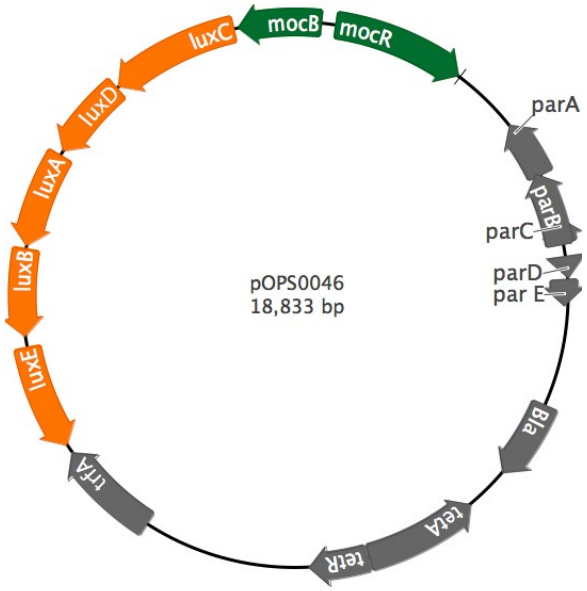


Figure 4.3 Plasmid map for the rhizopine *lux* biosensor (pOPS0046). The *luxCDABE genes* (orange) are expressed downstream of the rhizopine catabolism gene *mocB* (green) which is activated by the divergently transcribed *mocR* (green).

4.2.2. Detection of naturally produced rhizopines exuded into the rhizosphere of *M. sativa*.

Dr Barney Geddes tested the engineered rhizopine biosensor in a plant system to investigate whether the naturally produced rhizopines are exuded into the rhizosphere. Seedlings of the plant *M. sativa* were sterilised with EtOH, sodium hypochlorite, and water then germinated for 3 days on distilled water-agar plates. The roots of the germinated seedlings were co-inoculated with cells of the *S. meliloti* L5-30 rhizobia strain and the Rlv3841/pOPS0046 biosensor (1:1). As a control, a rhizopine synthesis *mosB* mutant was also constructed in *S. meliloti* L5-30 and screened in parallel. The plants obtained were imaged two weeks after inoculation, after formation of nitrogen fixing nodules. Clear bioluminescence of the Rlv3841 biosensor was observed on the surface of those nodules, whilst the control plants showed no background bioluminescence (Figure 4.4).

This experiment suggested that rhizopines **13–14** produced by the wild type *S. meliloti* L5-30 strain are indeed secreted from *M. sativa* root nodules into the rhizosphere and catabolised by free-living rhizobia. To further demonstrate the specificity and sensitivity of this biosensor to rhizopines, a novel synthesis of SIA **13** and 3-*O*-MSI **14** was developed. We proposed to use these synthetic compounds

in combination with the rhizopine biosensor to confirm the exudation of rhizopines into the rhizosphere, and to identify the natural enantiomer of 3-*O*-MSI **14**.

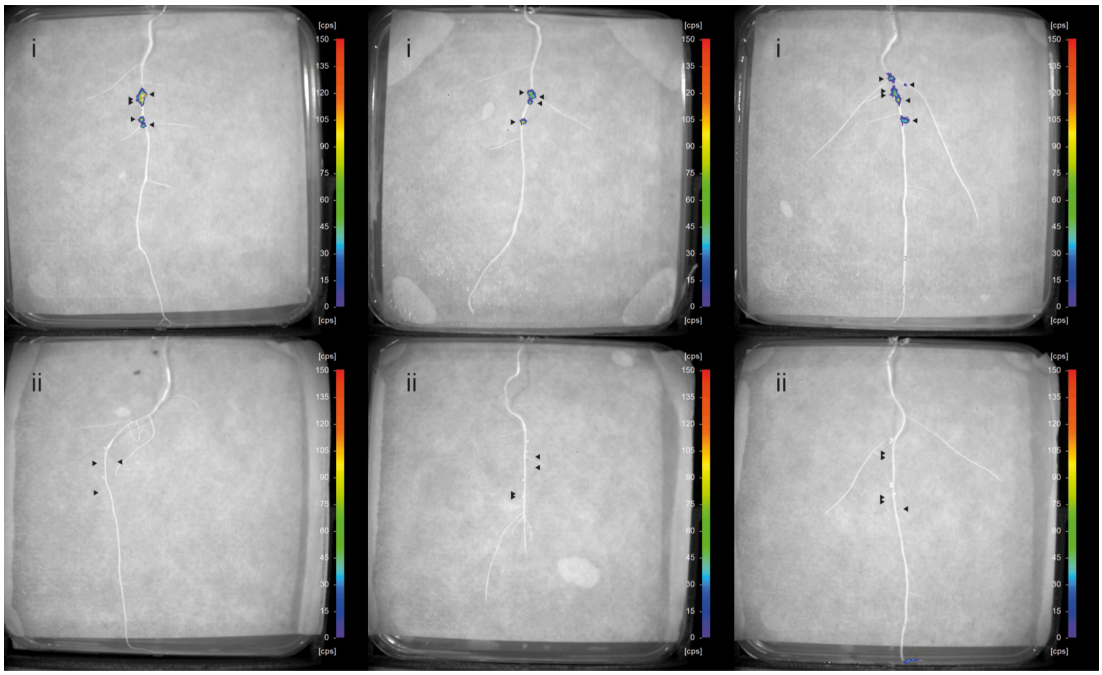
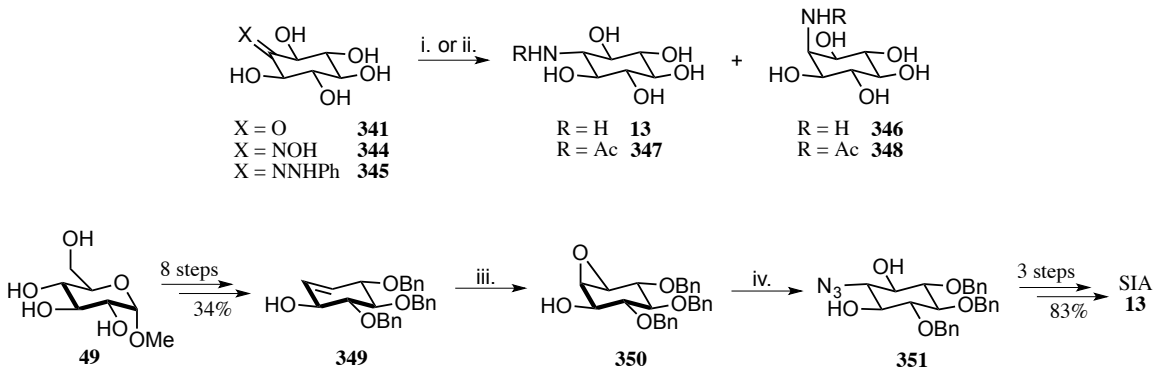


Figure 4.4 Visualisation of rhizopine in the rhizosphere of *M. sativa* nodulated by *S. meliloti* L5-30 (i) *S. meliloti* L5-30 wild type (ii) *S. meliloti* L5-30 *mosB* mutant. Arrows indicate nitrogen fixing nodules.

4.3. Retrosynthetic analysis towards Rhizopines

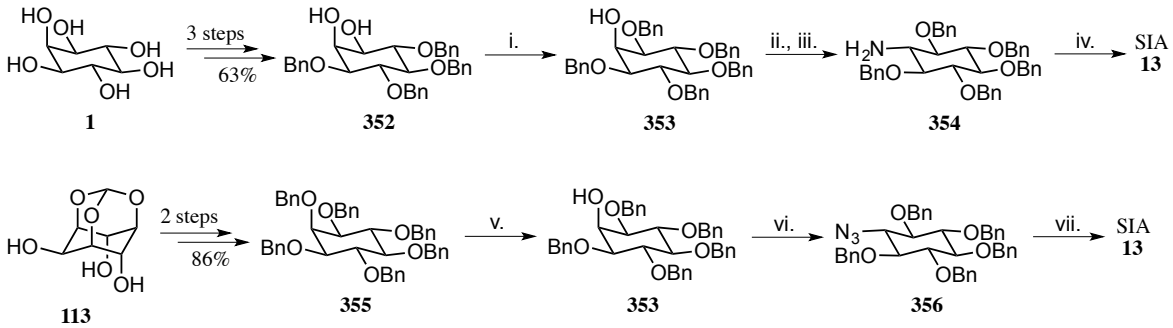
4.3.1. Pevious syntheses of SIA **13** and 3-*O*-MSI **14**

Only a few publications report the chemical synthesis of SIA **13**. Early syntheses involved the reduction of the phenylhydrazone or oxime derivatives **344–345** of *scyllo*-inosose **341**[399] to furnish epimeric mixtures of inosamines **13** and **346** or *N*-acetyl derivatives **347** and **348**. Subsequent crystallisation gave pure SIA **13** in low yields (Scheme 4.1). Other investigators also reported the preparation of *N*-acyl and hexaacetyl derivatives.[400] Although SIA **13**, synthesised *via* these procedures, was used in various biochemical studies, the stereochemistry of the product obtained was ambiguous, and the spectroscopic characterisation data were sparse.[203,387]



Scheme 4.1 Early synthesis of SIA **13** by Anderson *et al*. (top)[391] involving the reduction of phenylhydrazone or oxime derivatives of *scyllo*-inosose; and synthesis of SIA **13** (bottom) developed by Delgado *et al*.[50,393] *via* the opening of epoxide intermediate **350**. *Reagents and conditions*: i. a. Na/Hg b. Ac_2O **347**/**348** c. 1.0 M H_2SO_4 in H_2O, 28% **13** ii. Raney Ni, $H_{2(g)}$, 100–150 atm, 21% **13** iii. *m*CPBA, CH_2Cl_2, RT, 24 h, 93% iv. $LiClO_4$, NaN_3, MeCN, 80 °C, 18 h, 92%.

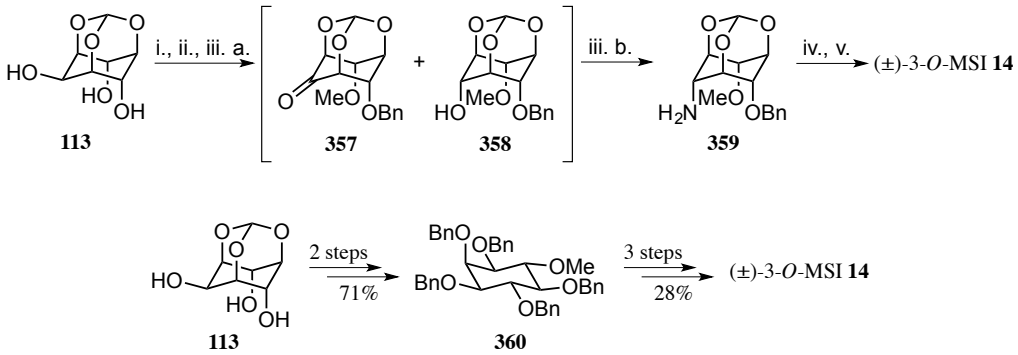
More recently, Delgado *et al*. synthesised a series of aminocyclitol derivatives through a key regio- and stereocontrolled opening of epoxide **350** to give SIA **13** in 24% yield over 13 steps (Scheme 4.1).[50,401] Moreover, Schoffers *et al*. developed an efficient synthesis of SIA **13**, starting from *myo*-inositol **1**, and providing full characterisation of the inosamine.[389] This route relies on a stereoselective substitution reaction at the 2-position to convert **353** from the *myo*- to the *scyllo*-configuration, giving SIA **13** in 31% yield over 7 steps (Scheme 4.2). A similar synthesis was also developed by Phenix *et al*. (23% over 5 steps; Scheme 4.2).[402]



Scheme 4.2 Synthesis of SIA **13** developed by Schoffers *et al*.[381] (top) and Phenix *et al*.[394] (bottom). *Reagents and conditions*: i. Bu_2SnO, Bu_4NI, BnBr, CH_3CN, 83% ii. a. Tf_2O, pyridine, CH_2Cl_2 b. NaN_3, DMF, 81% iii. Ph_3P, THF, H_2O, 96% iv. $H_{2(g)}$, 10% Pd/C, MeOH/CH_2Cl_2/H_2O, HCl, 56 psi, 82% v. $SnCl_4$, CH_2Cl_2, 58% vi. a. MsCl, pyridine b. NaN_3, DMF, 80 °C, 63% vii. a. $^tBoc_2O$, $H_{2(g)}$, 10% Pd/C b. Dowex (H^+), 74% Bn = benzyl; MsCl = mesyl chloride.

The first chemical synthesis of racemic (±)-3-*O*-MSI (±)-**14** was completed by Krief *et al*. in 2004. Regioselective protection of inositol orthoformate **113** and oxidation of the remaining equatorial hydroxyl group, followed by a reductive amination at that position gave intermediate **359**. Cleavage

of the benzyl ether and opening of the orthoformate yielded (±)-3-*O*-MSI (±)-**14** in 10% yield over 7 steps (Scheme 4.3).[403] Structural assignment of this rhizopine, initially isolated from root nodules induced by *S. meliloti* L5-30, was confirmed using synthetic (±)-**14**. Following this work, Phenix *et al*.[402] developed a more efficient synthesis of (±)-3-*O*-MSI (±)-**14** (20% over 5 steps) *via* a similar approach to that employed in their synthesis of SIA **13** (Scheme 4.3).



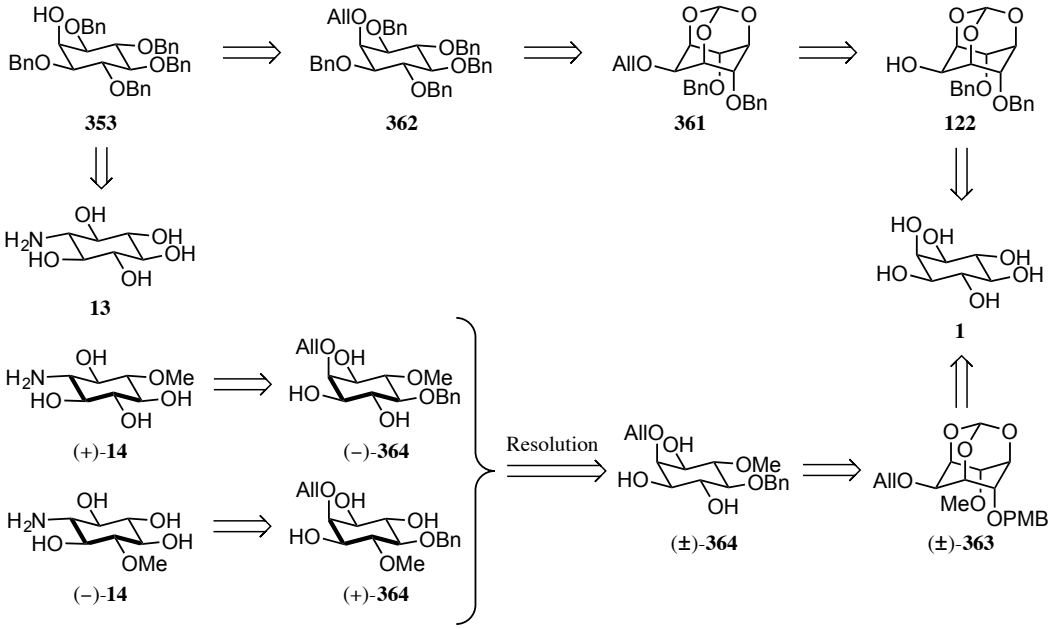
Scheme 4.3 Syntheses of racemic (±)-3-*O*-MSI **14** developed by Krief *et al*.[395] (top) and Schoffers *et al*.[394] (bottom). *Reagents and conditions*: i. a. NaH, DMF, RT, 1 h b. MeI, RT, 15 h, 63% ii. a. NaH, DMF, RT, 2 h b. BnBr, RT, 15 h, 48% iii. a. NMO, TPAP, CH_2Cl_2 b. NH_4OAc, $NaBH_3CN$, RT, 72 h, 45% iv. Na, $NH_{3(l)}$, ^{t}BuOH, THF, –78 °C to RT, 18 h, 86% v. $HCl_{(g)}$, MeOH, RT, 3 h, 90%. Me = methyl; Bn = benzyl.

Although efficient and reasonably high yielding, the syntheses of SIA **13** and racemic (±)-3-*O*-MSI **14** developed by Schoffers *et al*. and Phenix *et al*. comprise low yielding, regioselective steps, on protected *myo*-inositol derivatives, using toxic reagents including Bu_2SnO and $SnCl_4$. Considering these compounds were to be used in biochemical assays with free-living bacteria, the use of toxic metals needed to be avoided.

4.3.2. Protecting group strategy and retrosynthesis

As described in Chapter 3, orthoformate **113** can be regioselectively alkylated at the axial 4- and 6-positions, isolating the 2-position. We reasoned that using **113** as a framework for differentiating the 2-hydroxyl group over the remaining *myo*-inositol positions would eliminate the need for regioselective manipulation later in the synthesis of these rhizopines (Scheme 4.4).

As such, we proposed to employ the 4,6-di-*O*-benzyl orthoformate derivative **122** as a starting point towards the synthesis of SIA **13** (Scheme 4.4). From this intermediates, the 2-position can be readily protected with an orthogonal protecting group such as an allyl ether. This differentially protected orthoformate derivatives **361** can then be deprotected and perbenzylated to give fully protected intermediate **362**. We envisioned that the mild $PdCl_2$ allyl ether cleavage conditions developed within the group[68] would yield **353**. A similar sequence of steps to that developed by Schoffers *et al*. and Phenix *et al*. can then be followed to provide SIA **13** (Scheme 4.2 and 4.4).[389,402]



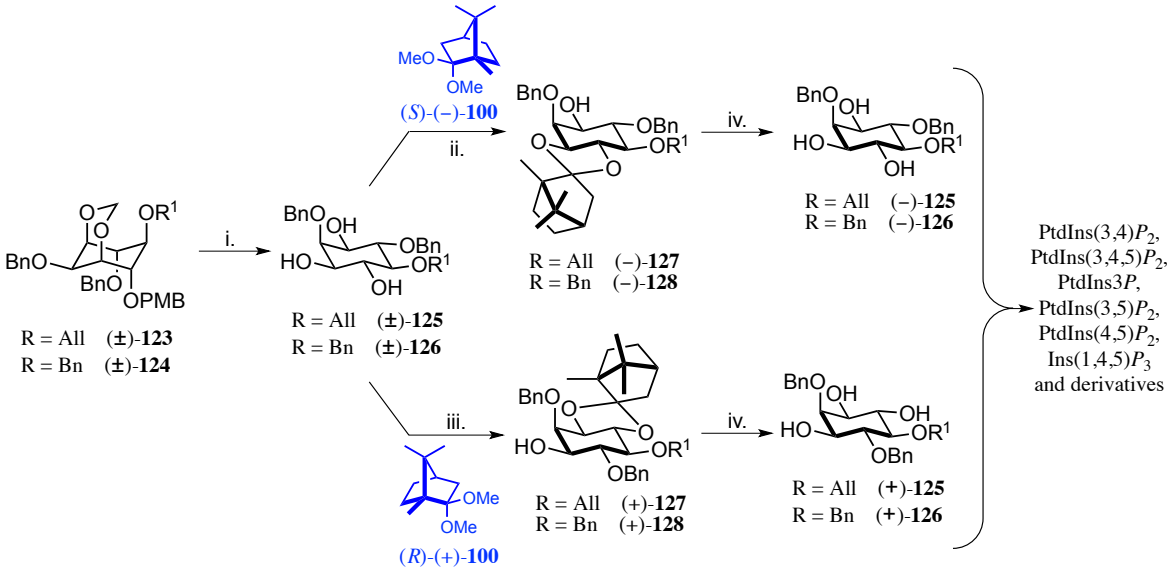
Scheme 4.4 Rhizopines **13** and (±)-**14** can be obtained *via* the orthoformate intermediates **361** and (±)-**363**. We proposed to employ a resolution strategy to synthesised both enantiomers of 3-*O*-MSI (+)-**14** and (−)-**14**. All = Allyl; Bn = benzyl; PMB = 4-methoxybenzyl.

Whilst SIA **13** is a *meso* compound, 3-*O*-MSI **14** exists as two enantiomers but has only been synthesised racemically in the literature. This research, therefore, focused on developing a route towards both enantiomers of 3-*O*-MSI (+)-**14** and (−)-**14**. These synthetic rhizopines would then serve to identify the naturally active enantiomer of 3-*O*-MSI. The rhizopine biosensor developed by Dr Barney Geddes and a catabolism assay with *S. meliloti* L5-30 was employed to achieve this.

We established previously (Chapters 2 & 3) that routes towards enantiomerically pure inositols involving optical resolutions are lengthy and suffer from poor overall yields. In contrast with enantioselective desymmetrisations or syntheses starting form chiral pool materials, however,

resolutions are advantageous when both enantiomers are required. Each enantiomer can be obtained easily through the same process, without the need for further optimisation, and the overal loss of material is reduced. Besides, a chosen resolution step can be incorporated into modular syntheses, without the need for major alterations in the synthetic design.

The diastereoisomeric resolution developed by Holmes *et al*.[72,404] was selected as an ideal strategy for this system (Scheme 4.5; Section 1.2.3).[15,34,68-72]



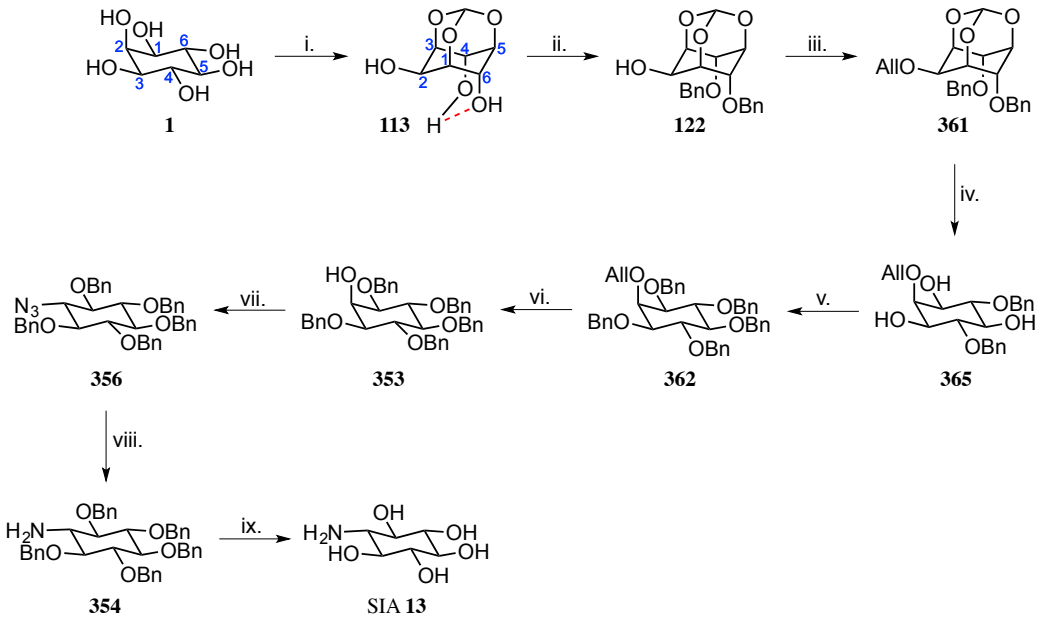
Scheme 4.5 Resolution of triols (±)-**125** and (±)-**126** developed by Holmes *et al*.[15,34,68-72,396] and later applied to the synthesis of a range of PtdInsP_n and InsP_n derivatives. *Reagents and conditions*: i. HCl, MeOH, reflux, 78–96% ii. PTSA·H_2O, CH_2Cl_2, reflux, 31% iii. PTSA·H_2O, CH_2Cl_2, reflux, 23% iv. AcCl, CH_2Cl_2/MeOH, 81–93%.[72] All = allyl; Bn = benzyl; PMB = 4-methoxybenzyl.

The main limitation of this methodology stems for the restricted substitution patterns of the triol substrates (±)-**125** and (±)-**126** employed for the resolution step. Accordingly, we aimed to investigate whether an alternative intermediate such as (±)-**364** could be similarly resolved (Scheme 4.4). This intermediate can be obtained from the desymmetrised racemic orthoformate (±)-**363**, *via* standard orthoformate chemistry. After resolution we envisaged that (+)-**364** and (−)-**364** could be subjected to the same series of steps as for the synthesis of SIA **13** (Scheme 4.4). We planned to synthesise (±)-**364** on a large scale and employ this material as a starting point towards racemic (±)-3-*O*-MSI (±)-**14** and both enantiomers of 3-*O*-MSI (+)-**14** and (−)-**14**.

4.4. Synthesis of *scyllo*-inosamine

4.4.1. Synthesis of the fully protected precursor **354**

Formation of orthoformate **113** from readily available *myo*-inositol **1**, and subsequent regioselective benzylation at the axial 4- and 6-positions, was achieved as described in Chapter 3 (Scheme 4.6, Section 3.3).[19,62,296,405] Allylation of the remaining equatorial 2-hydroxyl group afforded orthoformate **361** which was subsequently opened by acidic methanolysis using concentrated HCl at reflux. The triol **365** provided was perbenzylated to give fully protected *myo*-inositol derivative **362** (Scheme 4.6). Previous work in the group, carried out by Dr Tom Elliot,[68] had shown that $PdCl_2$ in $MeOH/CH_2Cl_2$ can be used as mild reaction conditions for the cleavage of allylic ethers.[406-408] $PdCl_2$ both isomerises the double bond and acts as a mild acid source in the hydrolysis of the vinyl ether intermediate generated. As such, **362** was selectively deprotected by treatment with $PdCl_2$ to yield **353** (Scheme 4.6).



Scheme 4.6 Novel synthesis of SIA **13** *via* the fully protected, differentiated inositol orthoformate **361**. *Reagents and conditions*: i. $CH(OC_2H_5)_3$, PTSA·H_2O, DMF, 150 °C, 96 h, 65% ii. a. LiH, DMF, RT, 30 min b. BnBr, RT, 48 h, 71% iii. a. NaH, DMF, 0 °C to RT, 40 min b. AllBr, 0 °C to RT, 48 h, 92% iv. HCl, MeOH, reflux, 20 h, 94% v. a. NaH, DMF, 0 °C to RT, 1 h b. BnBr, 0 °C to RT, 16 h, 80% vi. $PdCl_2$, $MeOH/CH_2Cl_2$, RT, 19 h, 96% vii. a. CH_3ClO_2S, pyridine, CH_2Cl_2, 0 °C to RT, 21 h b. NaN_3, DMF, 90 °C, 18 h, 88% viii. PPh_3, H_2O, THF, 40 °C, 20 h, 73% ix. Pd/C, $H_{2(g)}$, HCl, $CH_2Cl_2/MeOH/H_2O$, RT, 20.5 h, 80%. All = allyl; Bn = benzyl.

The key S_N2 azidolysis with inversion of configuration on **353**, had been accomplished in the literature either *via* the triflate[389] or *via* the mesylate (Scheme 4.2).[402] In our hands, the procedure

from Schoffer *et al*. *via* the triflate of **353** did not give the desired azide product **356**. Contrastingly, preparation of azide **356** could be achieved *via* the more stable mesylate in high yields, as described by Phenix *et al*. (Scheme 4.6).[402]

Intermediate **356** was shown to have the desired *scyllo* stereochemistry by measurement of the ^{1}H NMR vicinal coupling constants $^{3}J_{H\text{-}H}$ (Figure 4.5). None of the *myo*-epimer was observed. Indeed, the Karplus[409] and Bothner-By[410] equations allow to approximate the value of $^{3}J_{H\text{-}H}$ coupling constants as a function of the dihedral angle between vicinal protons. According to this relationship, vicinal H-H couplings will be maximal for protons with 180° and 0° dihedral angles and minimal for protons with 90° dihedral angles. Hence, when considering an inositol derivative, a proton *cis* to its adjacent protons will exhibit a smaller coupling constant than a proton *trans* to the same neighbouring protons. Accordingly, H-1 on **356** displays a $^{3}J_{H\text{-}H}$ coupling of 10.1 contrasting with its $^{3}J_{H\text{-}H}$ coupling of 2.6 before undergoing inversion of configuration through S_N2 azidolysis (Figure 4.5).

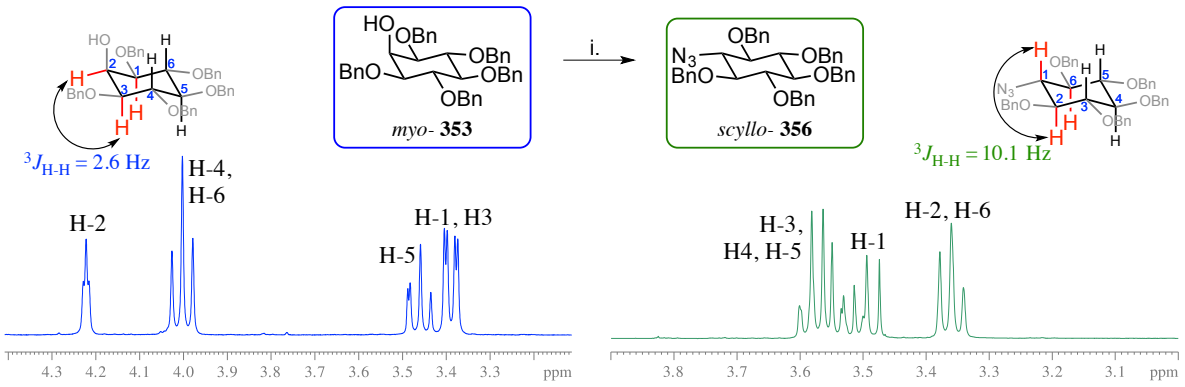
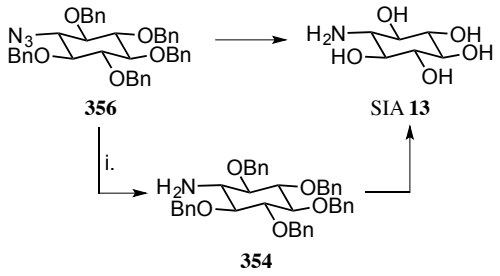


Figure 4.5 Determination of the *myo*- and *scyllo*-stereochemistry of **353** and **356** by ^{1}H NMR. Vicinal coupling $^{3}J_{H\text{-}H}$ is smallest for H-2 on **353** accounting its *myo*-stereochemistry. A larger coupling for H-1 on **356** confirms that inversion of stereochemistry has occurred at that position during S_N2 azidolysis. It is important to note that on going from *myo* to *scyllo*-geometry, inositol numbering changes. *Reagents and conditions*: i. a. CH_3ClO_2S, pyridine, CH_2Cl_2, 0 °C to RT, 21 h b. NaN_3, DMF, 90 °C, 18 h, 88%. Bn = benzyl.

4.4.2. Final global deprotection

The final global deprotection of *scyllo*-azide **356** was found challenging. Initially, simultaneous azide reduction and hydogenolysis of **356** was attempted using the procedure developed by Phenix *et al*.[402] Following reduction of the azide with palladium on carbon and $H_{2(g)}$, the amine **354** obtained was protected *in situ* with $^{t}Boc_2O$ to allow facile cleavage of the benzyl ethers without poisoning of the

Table 4.1 Overview of the reaction conditions employed for the final deprotection towards SIA **13**.



Entry	Substrate	Conditions (eq)	Time [hours]	Purification	Yield [%][a]	Ref.
1	**356**	a. ^{t}Boc$_2$O, 10% Pd/C, $H_{2(g)}$, CH_2Cl_2/MeOH b. Dowex (H^+), H_2O	48 + 18	Filtration celite®, Dowex® (H^+)[b], Amine capture release	Mixture of products	394
2	**356**	10% Pd/C, $H_{2(g)}$ MeOH/CH_2Cl_2/H_2O, HCl	21	Filtration celite®, crystallised EtOH/H_2O	30[c]	381
3	**354**	10% Pd/C, $H_{2(g)}$ MeOH/CH_2Cl_2/H_2O, HCl	24	Filtration celite®, crystallised EtOH/H_2O	66 toxic to *S. meliloti* L5-30	-
4	**354**	10% Pd/C, $H_{2(g)}$ MeOH/CH_2Cl_2/H_2O, HCl	20	Filtration microfiber pad, crystallised EtOH/H_2O	21 toxic to *S. meliloti* L5-30	-
5	**354**	10% Pd/C, $H_{2(g)}$ MeOH/CH_2Cl_2/H_2O, HCl	20	Filtration microfiber pad, reversed phase chromatography	70 toxic to *S. meliloti* L5-30	-
6	**354**	10% Pd/C, $H_{2(g)}$ MeOH/CH_2Cl_2/H_2O, HCl	24	Filtration microfiber pad, Dowex® (H^+)[b]	80	-

[a] Isolated yield [b] DOWEX® 50WX8 cation exchange column: H_2O (10 mL), then 0.1 M HCl (10 mL) and 0.2 M HCl (5 mL)
[c] Contaminated by small amounts of unidentified impurities, as determined by ^{1}H NMR. Bn = benzyl; ^{t}Boc = *tert*-butyloxycarbonyl.

catalyst. Subsequent deprotection of the Boc amine by stirring overnight with Dowex (H^+), followed by loading of the reaction mixture as a column and eluting with a 0.1 M aqueous solution of HCl provided a mixture of products as determined by ^{1}H NMR analysis (Table 4.1; Entry 1). This mixture was purified by amine capture release (Isolute® SCX-2 Cartridge), but SIA **13** was not isolated. In a second attempt, **356** was subjected to standard hydrogenolysis conditions in the presence of concentrated HCl.[389] After 17 hours stirring at room temperature, the suspension was filtered through Celite® to give SIA **13** contaminated with major impurities as determined by ^{1}H NMR (~58% pure). Although a purer sample (~78% pure as determined by ^{1}H NMR) could be isolated after trituration and crystallisation with EtOH the isolated yield of **13** was low (Table 4.1; Entry 2).

To increase the yield and reproducibility of this transformation, **356** was first reduced under mild Staudinger reduction conditions using PPh_3 and H_2O in THF. The protected *scyllo*-inosamine **354** generated was subsequently deprotected under hydrogenolysis conditions using palladium on carbon and $H_{2(g)}$ in the presence of concentrated HCl to avoid poisoning of the palladium catalyst. Filtration through Celite® provided SIA **13** in good yields, as the presumed HCl salt (Table 4.1; Entry 3). We established by ^{1}H and ^{13}C NMR analysis that **13** did not require any further purification. The overall yields obtained from these transformations, however, were found to be unusually high (>>100%).

We aimed to optimise a catabolism assay with *S. meliloti* L5-30 that could be employed for determining the natural enantiomer of 3-*O*-MSI **14**. In a first trial, Dr Barney Geddes used synthetic SIA **13** as sole carbon and nitrogen souce for the growth of *S. meliloti*.[389] As a positive control, the growth of rhizobia was also monitored using *myo*-inositol **1** and ammonium chloride (Figure 4.6; Orange). Unfortunately, SIA **13** was found to be toxic to *S. meliloti* L5-30 and no growth was observed after 70 hours (Figure 4.6; Green). Sequential washings and crystallisation with H_2O/EtOH of **13** did not alter the toxicity observed (Table 4.1; Entry 3).

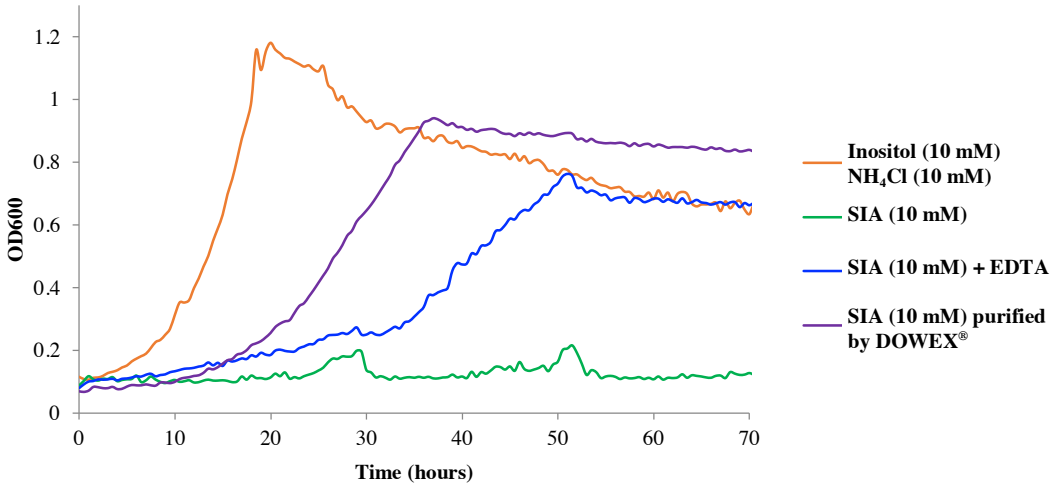


Figure 4.6 Optimisation of the rhizopine catabolism assay. Synthetic SIA **13** was found to be toxic to *S. meliloti* L5-30, no growth was observed after 70 hours (green). When EDTA was added, SIA **13** was able to act as a sole C and N source for the growth of wild type *S. meliloti* L5-30 (blue). Purification of SIA by DOWEX® 50WX8 ion exchange eliminated the toxicity observed (purple).

We postulated that the type of Celite® employed (Aldrich Celite® 545) may be dissolved by HCl, leading to the unusually high yields and the toxicity observed during the catabolism assay. The use of glass microfibre pads for the final filtration circumvented the problem caused by the Celite® and reasonable yields were obtained. The compound isolated, however, remained toxic to *S. meliloti*

L5-30. Neither crystallisation of *scyllo*-inosamine **13** from H_2O/EtOH (Table 4.1; Entry 4) nor purification by reversed-phase chromatography (Table 4.1; Entry 5) altered the toxicity observed. We reasoned that trace metals may be inhibiting the growth of *S. meliloti* L5-30. Indeed, in the presence of ethylenediaminetetraacetic acid (EDTA), *S. meliloti* L5-30 could efficiently use SIA **13** as a growth substrate (Figure 4.6; Blue). Purification of SIA **13** by DOWEX® 50WX8 ion exchange chromatography (Table 4.1; Entry 6) completely eliminated the toxicity observed (Figure 4.6).

Synthetic SIA **13** was therefore obtained from othoformate **122** in 22% yield, over 8 steps. The efficiency of this synthetic route is comparable to the literature,[389,402] but is advantageous as it does not involve the use of toxic tin reagents.

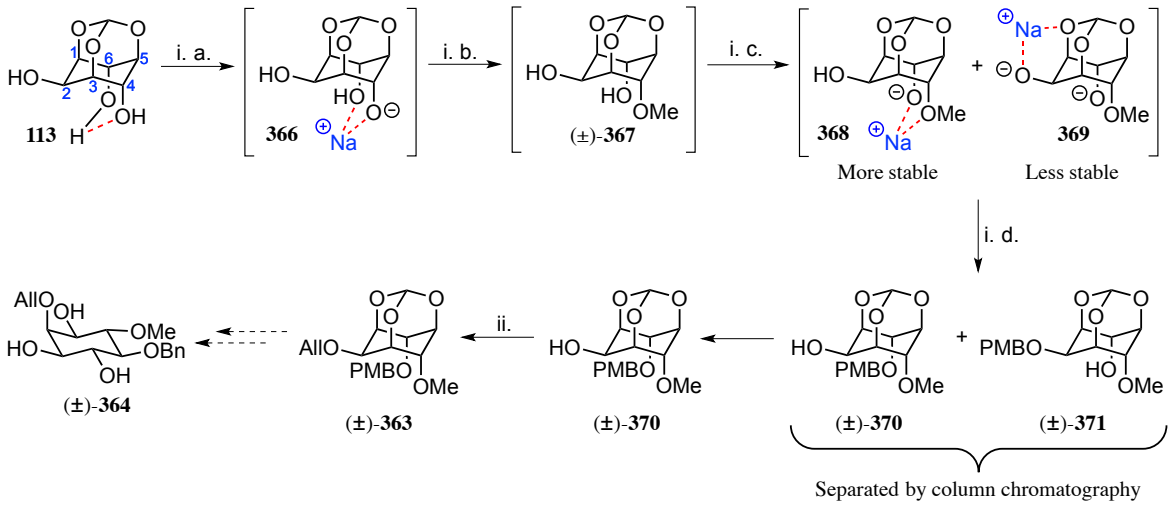
4.5. Synthesis of 3-*O*-methyl-*scyllo*-inosamine 14

4.5.1. Synthesis of the key triol (±)-**364** *via* orthoformate chemistry

As outlined in Section 4.3, our synthesis of racemic and enantiomerically pure 3-*O*-MSI **14** relies on the synthesis of the key intermediate (±)-**364** (Scheme 4.7). Accordingly, *myo*-inositol orthoformate **113**[60,62] was prepared in a single step by reaction with triethylorthoformate under PTSA catalysis. Orthoformate **113** is a symmetrical structure with one equatorial (C(2)OH) and two axial positions (C(4)OH and (C(6)OH). This framework can be regioselectively monoprotected at the axial 4- (or 6-) position. Indeed, the strong hydrogen bond existing between these positions enhances the acidity of the axial hydroxyl protons and further stabilisation of the axial alkoxide is achieved through formation of a chelate bridge (Section 3.3). Although LiH is known to give better selectivity for the 4,6-di-*O*-substituted orthoformates over the 2,4-regioisomer (Section 3.3), we decided to perform the 4-*O*-methylation and 6-*O*-PMB-protection of **113** in one pot. We reasoned that the stronger base NaH would be more suited to such a transformation and that any undesired regioisomers would be readily separated by column chromatography.

Accordingly, treatment of orthoformate **113** with a slight excess of NaH (1.02 eq) led to the selective deprotonation of one acidic axial hydroxyl. The sodium cation stabilises the alkoxide obtained *via* a presumed chelate bridge in **366**. Quenching of this alkoxide **366** with methyl iodide (1.04 eq)

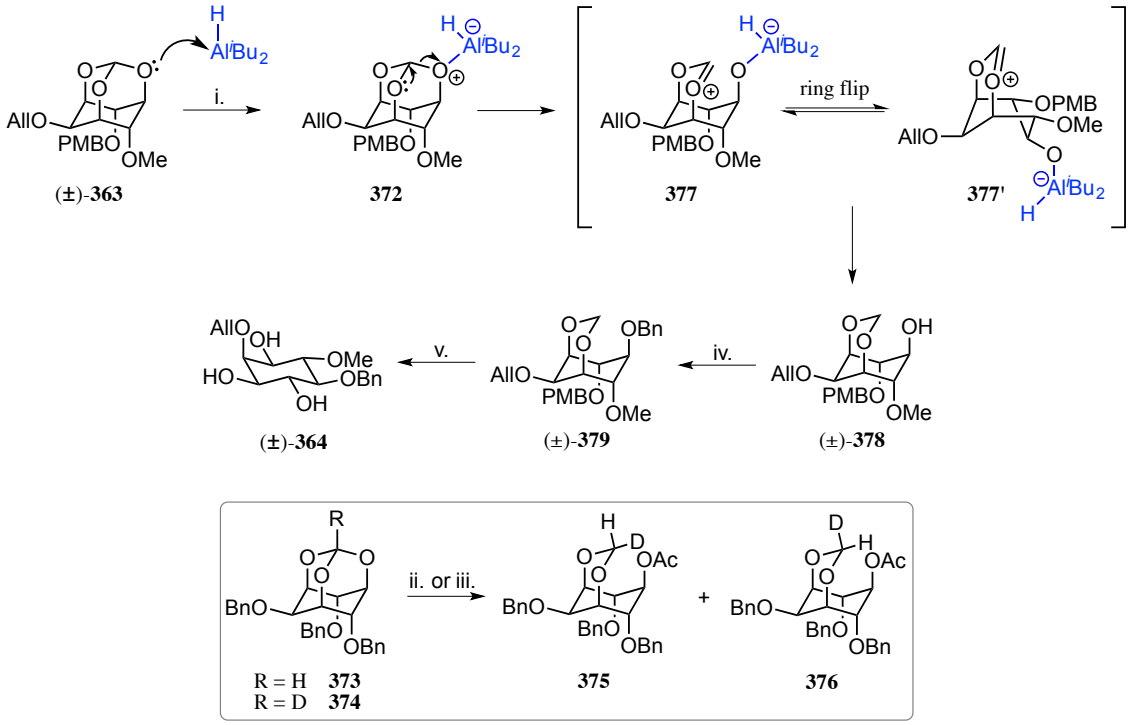
generated the mono-methylated orthoformate (±)-**367** (Scheme 4.7). Through this process, the *meso*-orthoformate **113** is desymmetrised non-stereoselectively, to give (±)-**367** as a racemic mixture (50:50 of the 4-*O* and 6-*O*-substituted derivatives). Once TLC analysis indicated full conversion of the starting material to the mono-methylated product (±)-**367**, further NaH (1.02 eq) was added to the reaction mixture. Deprotonation of the second axial hydroxyl group to give alkoxide **368** is preferred, although deprotonation of the equatorial hydroxyl also occurs to provide **369**. Subsequent quenching of these alkoxides with PMBCl afforded the racemic 4,6-di-*O*-substituted orthoformate (±)-**370**, contaminated with other regioisomers including 4-*O*-(4-methoxybenzyl)-2-*O*-methyl orthoformate (±)-**371** (Scheme 4.7). Purification by column chromatography gave (±)-**370** in 46% yield. This reaction was carried out on a 20 gram scale. Analysis by reverse phase HPLC revealed that this material was only 86% pure. Despite this, we envisaged that any remaining side products would be removed in the subsequent step. Indeed, allylation of the equatorial 2-hydroxyl groups with excess NaH and allyl bromide, followed by purification by column chromatography yielded (±)-**363** in 97% purity, as determined by reverse phase HPLC (Scheme 4.7).



Scheme 4.7 Synthesis of fully protected orthoformate (±)-**363**. *Reagents and conditions*: i. a. NaH, DMF, RT, 30 min b. MeI, RT, 16 h, c. NaH, RT, 1 h d. PMBCl, RT, 16 h, 46% ii. a. NaH, DMF, 0 °C to RT, 40 min b. AllBr, 0 °C to RT, 48 h, 55%. Me = methyl; All = allyl; PMB = 4-methoxybenzyl.

The next step towards the key racemic intermediate (±)-**364** involves differentiation of the 5-position. This was achieved by regioselective opening of orthoformate (±)-**363** with five equivalents of DIBAL-H to provide the 1,3-*O*-acetal (±)-**374**. Mechanistic explanation for the regio- and stereoselectivity observed in these reactions was investigated by Holmes *et al*, through

deuterium-labelling experiments.[64,411] First, it was found that at least two equivalents of DIBAL-H are required for full consumption of the starting material. It was therefore suggested that the first equivalent of DIBAL-H acts as a Lewis acid and coordinates to the least sterically hindered 5-position oxygen to give **372**. Second, Holmes *et al*. reported that whilst treatment of orthoformate **373** with DIBAL-D, followed by acetylation, generates predominently the 1,3-*O*-acetal **375**, selectivity is inversed to give **376** when reacting deuterated orthoformate **374** with DIBAL-H (Scheme 4.8). This experimental observation suggests that reduction occurs with retention of stereochemistry, on the *endo* face. This indicates that the C-O bond is cleaved prior to hydride delivery. Consequently, in the proposed mechanism, cleavage of the orthoformate generates the oxocarbenium ion **377**. Relief of the unfavourable 1,3-diaxial steric interactions can be achieved by ring flipping to a boat conformation **377'** (Scheme 4.8). Accordingly, reduction of the oxocarbenium ion is thought to occur intermolecularly *via* the boat conformation **377'**, with a second equivalent of



Scheme 4.8 Proposed mechanism for the DIBAL-H mediated, regioselective cleavage of orthoformate (±)-**363**. Deuterium-labelling experiments established that opening of the orthoformate occurs first, followed by an inter- or intramolecular, stereoselective reduction of oxocarbenium ions **377** or **377'**.[64,403] *Reagents and conditions*: i. DIBAL-H, CH_2Cl_2, 0 °C to RT, 24 h, 90% (±)-**378** ii. From **373**: a. DIBAL-D, CH_2Cl_2, 0 °C b. Ac_2O, Et_3N, DMAP, CH_2Cl_2, RT, 93%, **375**/**376** 92:8 iii. From **374**: a. DIBAL-H, CH_2Cl_2, 0 °C to RT b. Ac_2O, Et_3N, DMAP, CH_2Cl_2, RT, 83%, **375**/**376** 10:90 iv. a. NaH, imidazole, DMF, 0 °C to RT, 45 min b. BnBr, 0 °C to RT, 20 h, 93% v. HCl, MeOH, 0 °C to reflux, 2 h, 88%. Ac = acetate; ^{t}Bu = *tert*-butyl; Bn = benzyl.

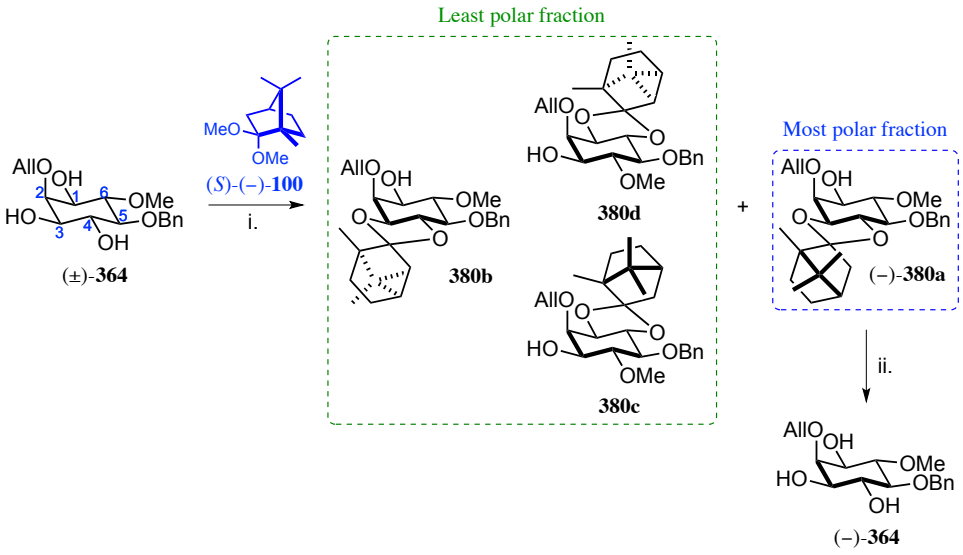
DIBAL-H. Nonetheless, no experiment has discounted the possibility of an intramolecular pathway, involving reduction of oxocarbenium ion **377**.

With alcohol (±)-**378** in hand, the 5-position was protected as the benzyl ether by reaction with NaH and benzyl bromide to give the fully protected 1,3-*O*-acetal (±)-**379** in 80% yield. Simultaneous cleavage of the acetal and PMB ether by acidic methanolysis yielded the desired triol (±)-**364** (Scheme 4.8). Up to 10 grams of this triol could be synthesised, providing enough material for the preparation of both enantiomers of 3-*O*-MSI (+)-**14** and (−)-**14**.

4.5.2. Resolution of triol (±)-**364**

Having optimised the orthoformate chemistry to (±)-**364**, we investigated whether the diastereoisomeric resolution methodology developed by Holmes *et al*.[72,404] could be extended beyond the resolution of triols (±)-**125** and (±)-**126** (Scheme 4.5).[34,64,68-70]

Resolution of the differently substituted, racemic intermediate (±)-**364** was thus attempted. First, protection of the 3,4-diol with 1-(*S*)-(−)-camphordimethyl acetal (−)-**100** was achieved following the original procedures by Bruzik *et al*.[23] and Holmes *et al*.[72,404] The chiral auxiliary (−)-**100** was prepared, as described in Section 2.2 for 1-(*R*)-(+)-**100**, by stirring 1-(*S*)-(−)-camphor (−)-**184** with trimethylorthoformate and K-10 Montmorillonite clay for three days at room temperature. Triol



Scheme 4.9 Protection-resolution of (±)-**364** with the chiral auxiliary 1-(*S*)-(−)-camphor dimethyl acetal (−)-**100**, generated four diastereoisomers **380a–d**. Extensive purification by column chromatography provided diastereoisomer (−)-**380a** (blue) and a diastereoisomeric mixture of **380b–d** (green). *Reagents and conditions*: i. a PTSA·H_2O, reflux, 24 h, 28% (−)-**380a**, 61% **380b–d**. See Figure 4.6 for ^{1}H NMR spectra. Me = methyl; All = allyl; Bn = benzyl.

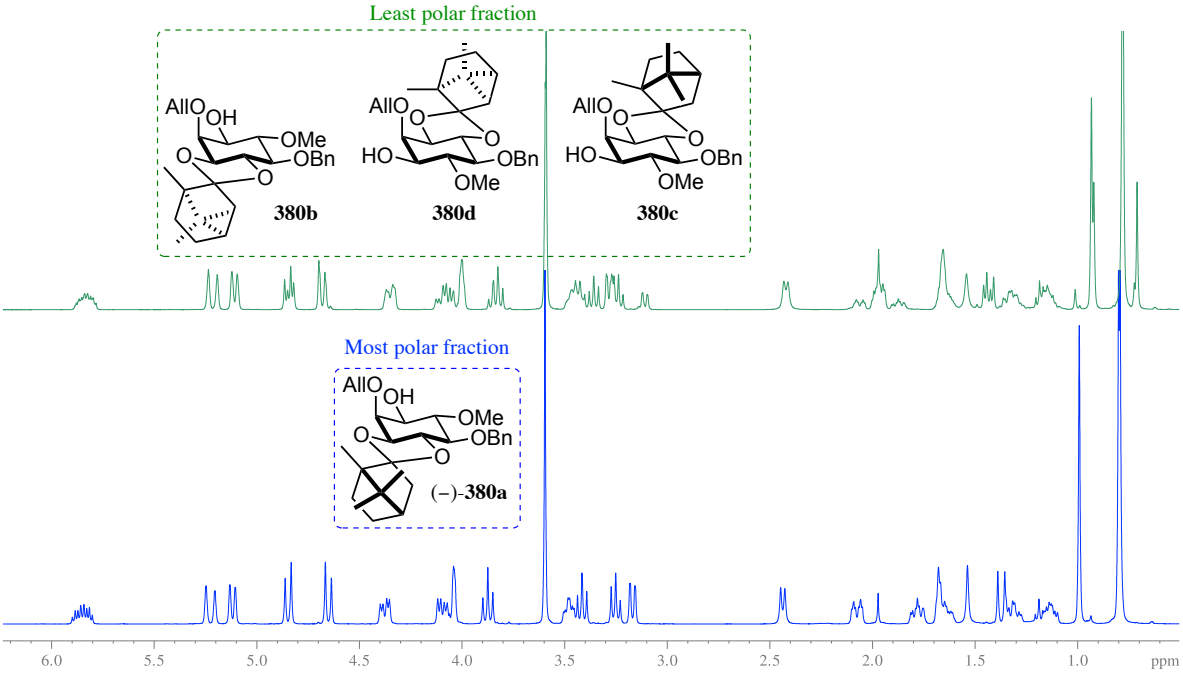


Figure 4.7 Comparison of the ^{1}H NMR spectra of the two fractions obtained through resolution by column chromatography over silica gel. Whilst the ^{1}H NMR spectrum for (–)-**380a** (blue) clearly shows one set of peaks, multiple diastereoisomers can be observed in the spectrum for the least polar fraction containing **380b–d** (green). The ^{1}H NMR spectra were both acquired at 500 MHz, in $CDCl_3$.

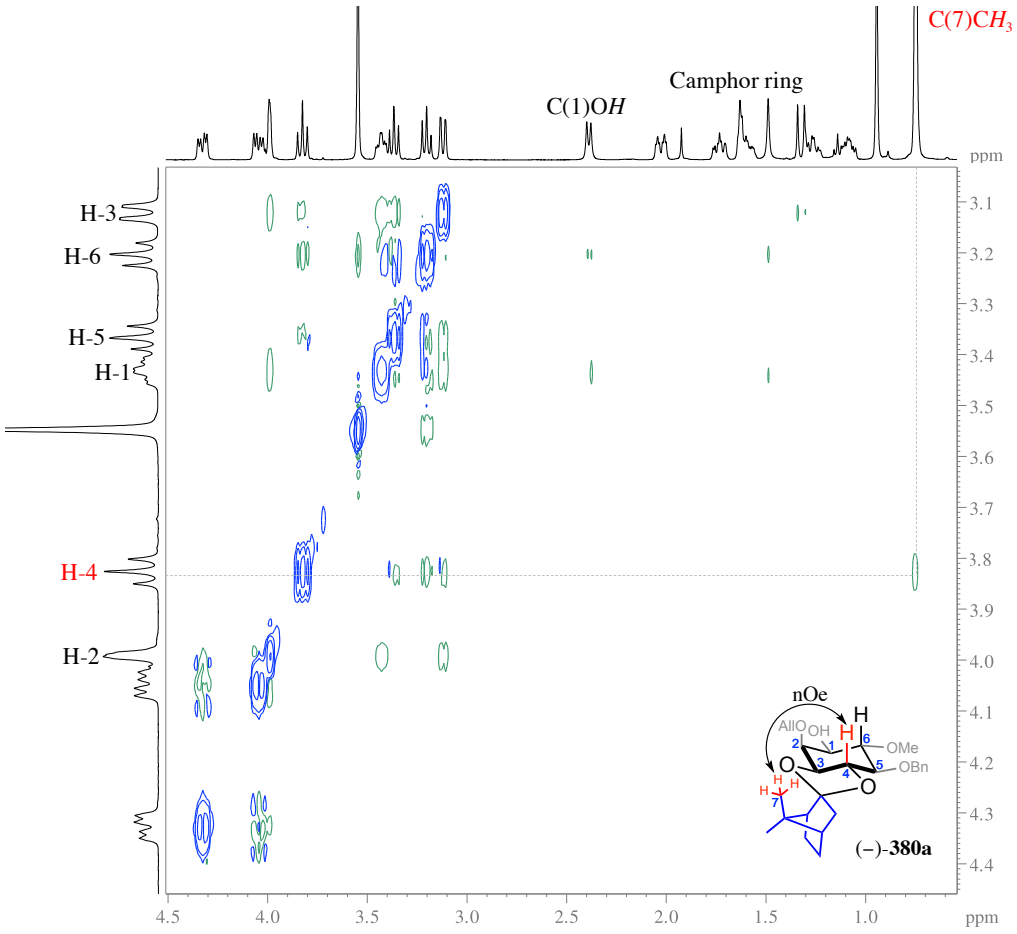


Figure 4.8 The 2D Nuclear Overhauser Spectrocopy (NOESY) spectrum for (–)-**380a**, showing a through space correlation between the axial proton on the 4-position of the inositol ring and the protons on the camphor chiral auxiliary. The ^{1}H NMR spectrum was acquired at 500 MHz, in $CDCl_3$.

(±)-**364** was reacted with the crude product (–)-**100** obtained (90–95% pure as determined by ^{1}H NMR), in CH_2Cl_2 and in the presence of substoichiometric PTSA·H_2O for 24 hours under reflux. Work-up of this reaction provided a diastereoisomeric mixture of four 3,4-acetals **380a–d** (Scheme 4.9). TLC analysis of this mixture revealed that, as reported in the literature for triols (±)-**125** and (±)-**126**,[72] one diastereoisomer was more polar than the other three. As such, after resolution through extensive purification by column chromatography over silica gel, two fractions were isolated. A first fraction was identified as the single diastereoisomer (–)-**380a** through ^{1}H NMR (Figure 4.7), nOe experiments (Figure 4.8) and comparison with the literature.[72] A second fraction comprised a mixture of the other three diastereoisomers **380b–c** which were found to be chromatographically inseparable.

These results were so far consistent with those published by Holmes *et al*.[72] for the resolution of triols (±)-**125** and (±)-**126** (Scheme 4.5). Although the stereochemistry of the resolved products obtained from this methodology, had been determined by chemical correlation and NMR experiments,[404] these had never been analysed by chiral HPLC. Therefore, following mild acid hydrolysis of (–)-**380a**, the *e.e*. of the presumed resolved triol (–)-**364** obtained was determined by chiral HPLC using a ChiralPak® AD-H column (Figure 4.9). Unfortunately, triol (–)-**364** was found to have an *e.e*. of 45% which indicated that (–)-**380a** was contaminated with its enantiomer **380c**[XI] (Figure 4.9). This result

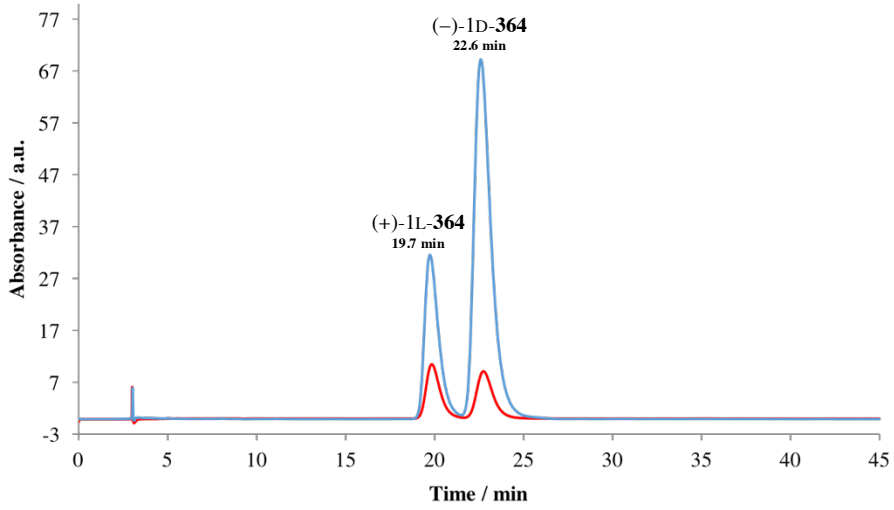
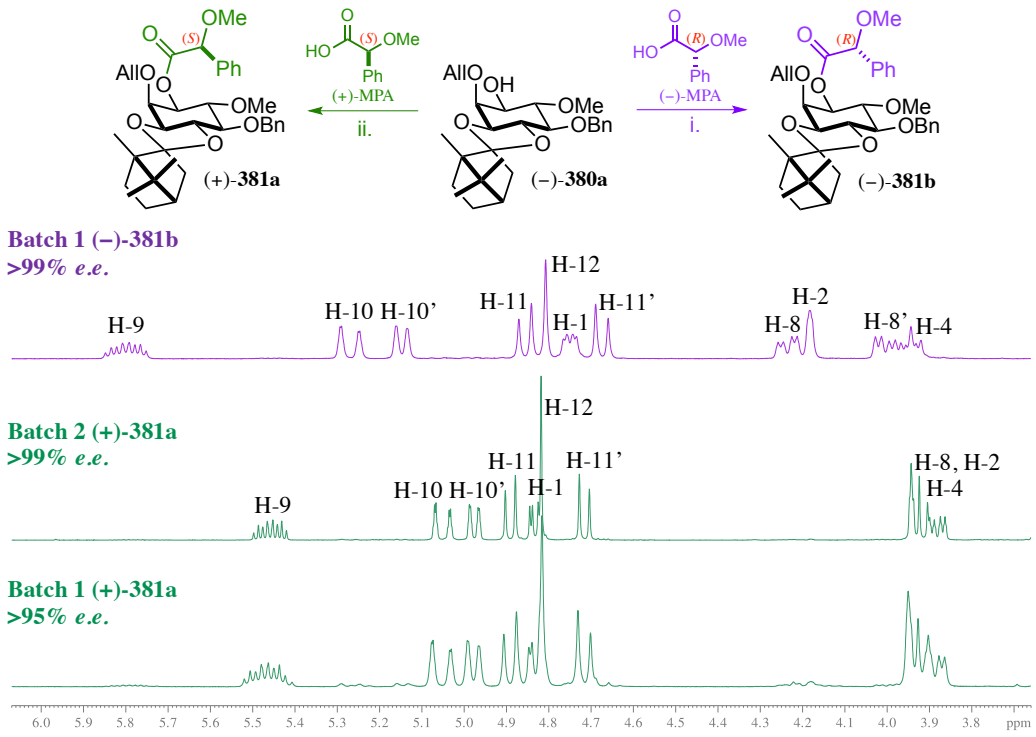


Figure 4.9 Chiral HPLC traces (ChiralPak® AD-H column, heptane/IPA 90:10, 0.8 mL.min^{-1}, 254 nm) of racemic (±)-**364** (red) and partially resolved triol (–)-**364** (blue) with a calculated *e.e*. of 45%.

[XI] If (–)-1D-**380a** had been contaminated with diastereoisomers **380b** or **380d**, this would have been visible by ^{1}H NMR.

suggests that the scope of this resolution strategy can in fact not be extended beyond the original inositol systems (±)-**125** and (±)-**126** (Scheme 4.5).

With the intention to further resolve (–)-**364**, we selected a reagent that would not only serve as a chiral auxiliary, but would simultaneously allow for the direct determination of the *e.e.* and absolute configuration of (–)-**380a** (arbitrarily assigned as (–)-1D-**380a** by comparison with the literature). We had previously employed MPA[268] as a chiral anisotropy reagent for the determination of the absolute configuration and *e.e.* of protected inositol and diglyceride derivatives (See Sections 3.6 and 3.14 for a detailed description). Diastereoisomer (–)-**380a** was therefore esterified with each enantiomer of MPA in the presence of EDC and 4-DMAP to provide two diastereoisomeric esters (+)-**381a** and (–)-**381b** (Scheme 4.10). In both cases, further resolution by column chromatography over silica gel could be achieved.



Scheme 4.10 Esterification of (–)-**380a** with each enantiomer of MPA gave two diastereosiomers: the (*S*)-MPA ester (+)-**381a** (green) and the (*R*)-MPA ester (–)-**381b** (purple). The ^{1}H NMR spectra (500 MHz, $CDCl_3$) of these diastereosiomers were used for determining the *e.e.* of triol (–)-**364**. *Reagents and conditions*: i. EDC, 4-DMAP, CH_2Cl_2, RT, 17 h, 74% ii. EDC, 4-DMAP, CH_2Cl_2, RT, 18 h, 81%. All = allyl; Bn = benzyl; Me = methyl; Ph = phenyl.

As both (+)-**381a** and (–)-**381b** feature a chiral anisotropy group (MPA), it was possible to predict, *via* NMR analysis, the *e.e.* that (–)-**364** would possess after cleavage of the chiral auxiliaries

(Scheme 4.10). Determination of the *e.e.* by NMR was thus accomplished following the sequence of steps outlined in Chapter 3 (See Sections 3.6 and 3.14 for a detailed description), demonstrating that complete resolution (–)-**364** had indeed been achieved. The *e.e.* calculated for triol (–)-**364** was shown to range between 95 and 99%, depending on the quality of the resolution for each particular batch of material (Scheme 4.10).

The absolute configuration of these derivatives was also assigned by analysing the ^{1}H NMR of diastereoisomers (+)-**381a** and (–)-**381b**, following the sequence of steps described in Section 3.6. According to the conformational model[327-330] shown in Figure 4.10, protons H-2 and H-3 of (+)-**381a** and protons H-5 and H-6 of (–)-**381b** are expected to be shielded if the configuration of (–)-**380a** is indeed (–)-1D-2-*O*-allyl-3,4-*O*-(L-1,7,7-trimethylbicyclo[2.2.1]hept-2'-ylidene)-5-*O*-benzyl-6-*O*-methyl-*myo*-inositol. The parameter $\Delta\delta^{RS}$ (difference in chemical shifts of the substituents) for this configuration is therefore expected to be positive for protons H-2 and H-3 and negative for protons H-5 and H-6 (Figure 4.10). Table 4.2 clearly shows that $\Delta\delta^{RS}$ is indeed negative for protons H-5, H-6 and H-7 and positive for protons H-2, H-3, H-8, H-9 and H-10. The absolute configuration of (–)-**380a** was therefore proved to be (–)-1D-**380a**.

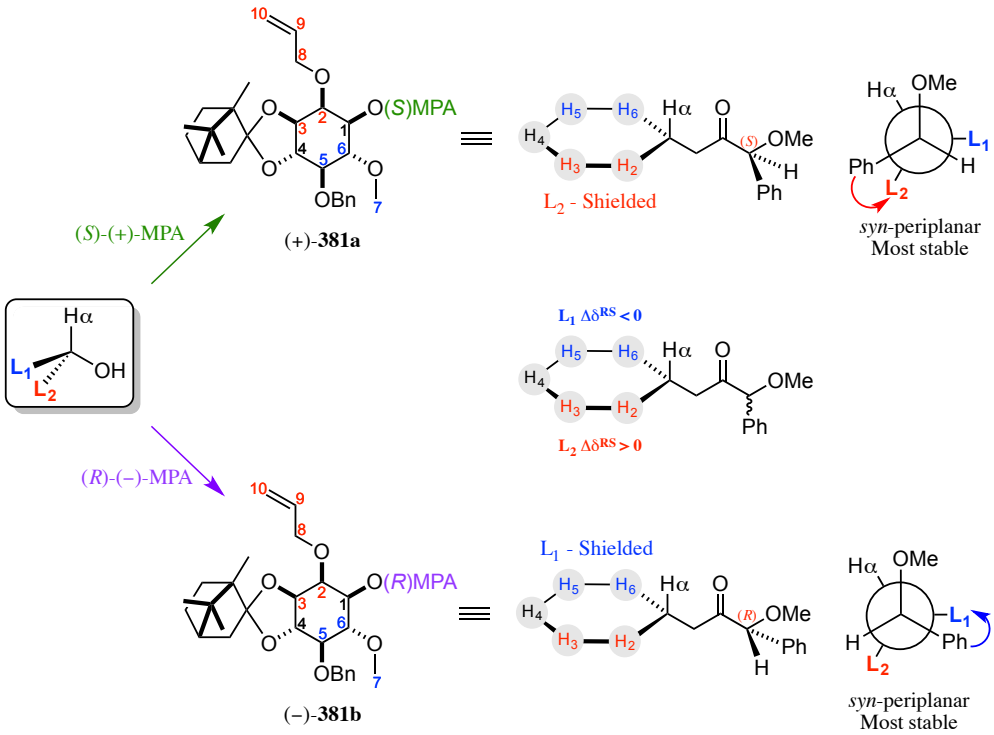
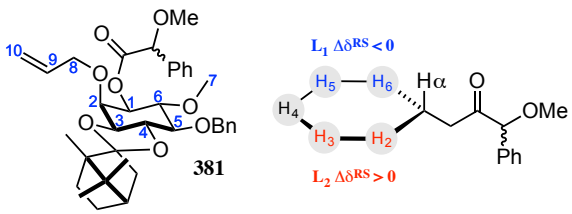


Figure 4.10 Conformational model for MPA esters allowing the determination of the absolute configuration of (–)-**380a**. Me = methyl; Ph = phenyl; Ac = acetate; Bn = benzyl; MPA = α-methoxy-phenylacetic acid.

Table 4.2 Calculation of the differences in chemical shifts $\Delta\delta^{RS}$ for relevant protons signals in the ^{1}H NMR spectra of (+)-**381a** and (–)-**381b**. The absolute stereochemistry was confirmed using the model described in Figure 4.8.



Position	δ^R (–)-**381b**	δ^S (+)-**381a**[a]	$\Delta\delta^{RS}$	L_1 or L_2
1	4.75	4.83	–0.08	N/A
2	4.18	3.95	0.23	L_2
3	3.26	3.22	0.04	L_2
4	3.94	3.93	0.01	N/A
5	3.45	3.51	–0.06	L_1
6	3.46	3.59	–0.13	L_1
7	3.06	3.52	–0.46	L_1
8[b]	4.23	3.89	0.34	L_2
8'[b]	4.00	3.59	0.41	L_2
9	5.80	5.46	0.34	L_2
10[b]	5.27	5.05	0.22	L_2
10'[b]	5.15	4.98	0.17	L_2

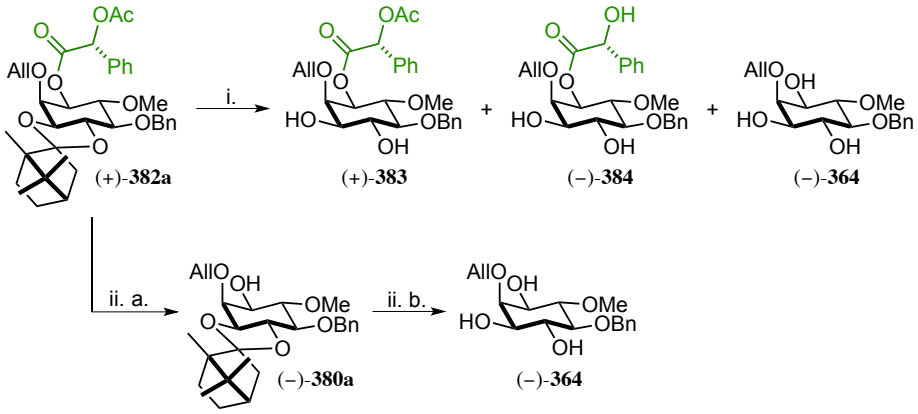
[a] The chemical shift (δ) values quoted were obtained from a ^{1}H NMR spectra, run at 400 MHz, in CH_3Cl [b]For multiplets, the middle of the multiplet is quoted, if the peaks were not distinguishable, HSQC was employed to assign each chemical shift. Me = methyl; Ph = phenyl

4.5.3. Replacing MPA for a more affordable chiral auxiliary

The main limitation of the strategy outlined above is the cost of MPA. Hence, we investigated whether a similar, cheaper chiral auxiliary could be employed for a larger scale resolution of (±)-**364**. Notably, both (*S*)-(+)- and (*R*)-(–)-*O*-AMA are inexpensive and have been used frequently for the resolution of racemic inositol frameworks[77,230,236,325,326,362,412] More recently, Sureshan *et al.* demonstrated that AMA can also be employed as a chiral anisotropy reagent for determining the absolute configuration of resolved diastereoisomers by ^{1}H NMR.[325,326]

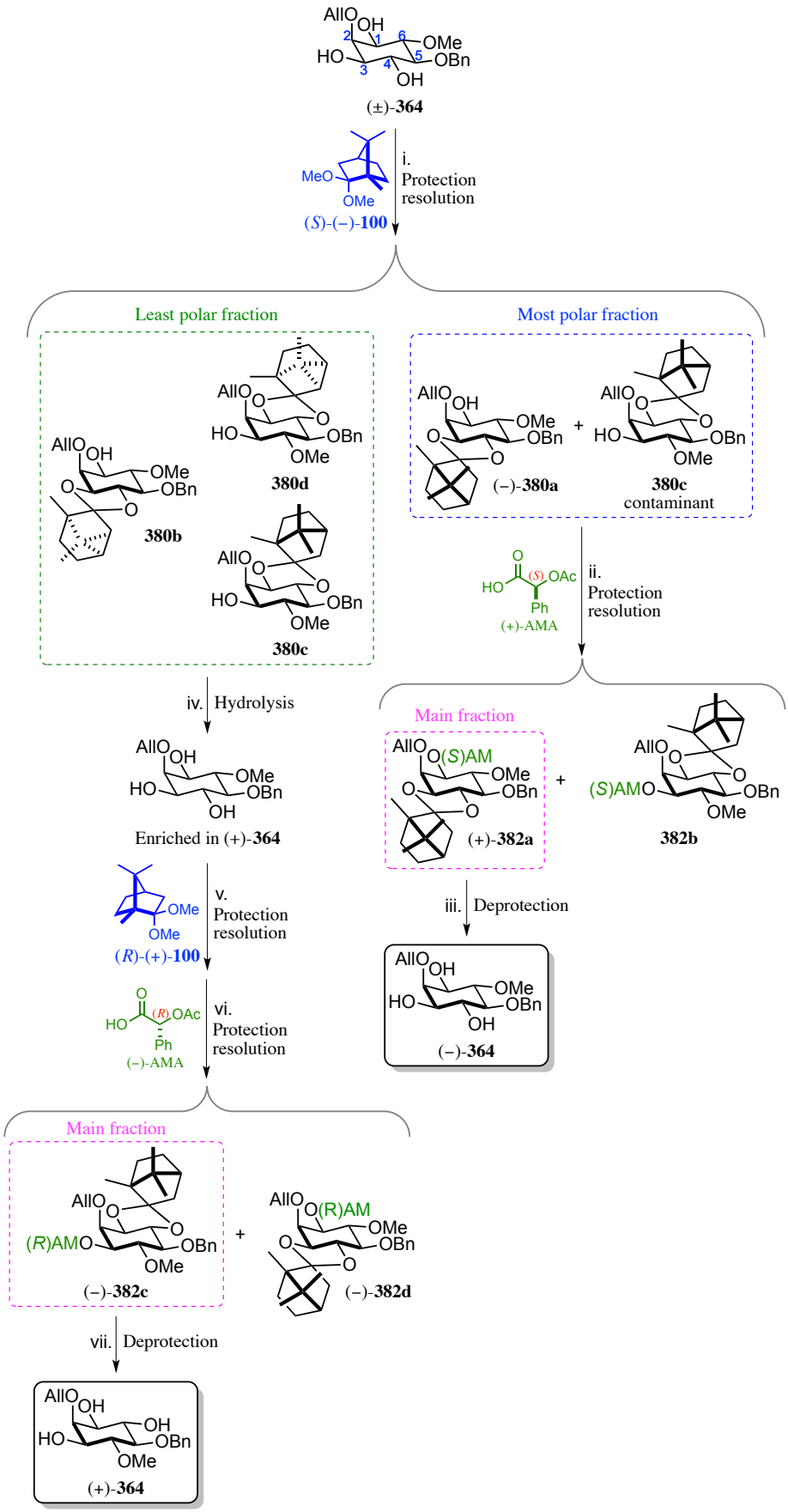
Accordingly, protection-resolution of racemic triol (±)-**364** with 1-(*S*)-(−)-camphordimethyl acetal (−)-**100** provided partially resolved (−)-**380a** (contaminated with (−)-**380c**) and a mixture of the other three diastereoisomers (−)-**380b–d**, as before (Scheme 4.9 and 4.12). This alcohol was esterified with (*S*)-(+)-AMA in the presence of EDC and 4-DMPA, then further resolved by column chromatography to give the single diastereoisomer (+)-1D-**382a** (Scheme 4.12).

Two routes were explored for the deprotection of this diastereoisomer towards triol (−)-1D-**364**. First, hydrolysis of the 2,3-*O*-camphor acetal was attempted by treatment with acetyl chloride in a mixture of MeOH and CH_2Cl_2. The expected product (+)-1D-**383** was obtained, but two other side products, including the fully deprotected triol (−)-1D-**364** and the deacetylated derivative **384** were also isolated after column chromatography over silica gel (Scheme 4.11). Contrastingly, initial cleavage of the 1-*O*-acetylmandelic acid ester under basic conditions (NaOH/MeOH), provided (−)-1D-**380a** in excellent yield. Without further purification,[XII] this material was subjected to acidic hydrolysis to give an off-white solid. Crystallisation from EtOAc yielded (−)-1D-**364** in 79% yield, over 2 steps (Scheme 4.11).



Scheme 4.11 Deprotection of diastereoisomer (+)-**382**. *Reagents and conditions*: i. AcCl, MeOH/CH_2Cl_2, RT, 19 h, 17% (+)-**383**, 32% (−)-**384**, 37% (−)-**364** ii. a. NaOH, MeOH, reflux, 30 min b. AcCl, MeOH/CH_2Cl_2, RT, 16.5 h, 84%. All = allyl, Bn = benzyl; Ac = acetate; Me = methyl; Ph = phenyl.

[XII] This material was purified once for characterisation purposes, as described in the Experimental Section.



Scheme 4.12 Strategy for the resolution of triol (±)-**364**. *Reagents and conditions*: PTSA·H_2O, CH_2Cl_2, reflux, 24 h, 16% **380a,c**, 67% **380b–d** ii. EDC, 4-DMAP, CH_2Cl_2, RT, 17 h, 74% (+)-**382a** iii. a. NaOH, MeOH, reflux, 30 min b. AcCl, MeOH/CH_2Cl_2, RT, 16.5 h, 79% iv. AcCl, MeOH/CH_2Cl_2, RT, 21 h, 94% v. PTSA·H_2O, CH_2Cl_2, reflux, 24 h, 37% vi. EDC, 4-DMAP, CH_2Cl_2, RT, 17 h, 74% (−)-**382c** vii. a. NaOH, MeOH, reflux, 30 min b. AcCl, MeOH/CH_2Cl_2, RT, 18.5 h, 84%. All = allyl, Bn = benzyl; Me = methyl; Ac = acetate; Ph = phenyl; AMA = acetylmandelic acid.

As similar protocol was followed to generate the opposite enantiomer of the triol intermediate (+)-1L-**364**. Although racemic (±)-**364** can be employed again as a starting substrate, the remaining diastereoisomeric mixture of **380b–d**, obtained from the synthesis of (–)-1D-**364**, can be hydrolysed to regenerate **364**. This triol is now enriched in the desired opposite enantiomer. Starting from this material, protection-resolution was achieved as before, but this time with the enantiomeric chiral auxiliaries: 1-(*R*)-(+)-camphor dimethyl acetal (+)-**100** and (*R*)-(–)-*O*-acetylmandelic acid. The diastereoisomer (–)-1L-**382c** obtained was deprotected as before by sequential cleavage of the AM group and acid hydrolysis, to provide the opposite enantiomer (+)-1L-**364**, after crystallisation from EtOAc (Scheme 4.12).

Both enantiomers (–)-1D-**364** and (+)-1L-**364** were analysed by chiral HPLC (Figure 4.11) as compared to their racemic counterpart (Figure 4.9). Through this study, we were able to confirm the *e.e.*s predicted previously *via* NMR analysis (>95–99% *e.e.*, dependant on the batch).

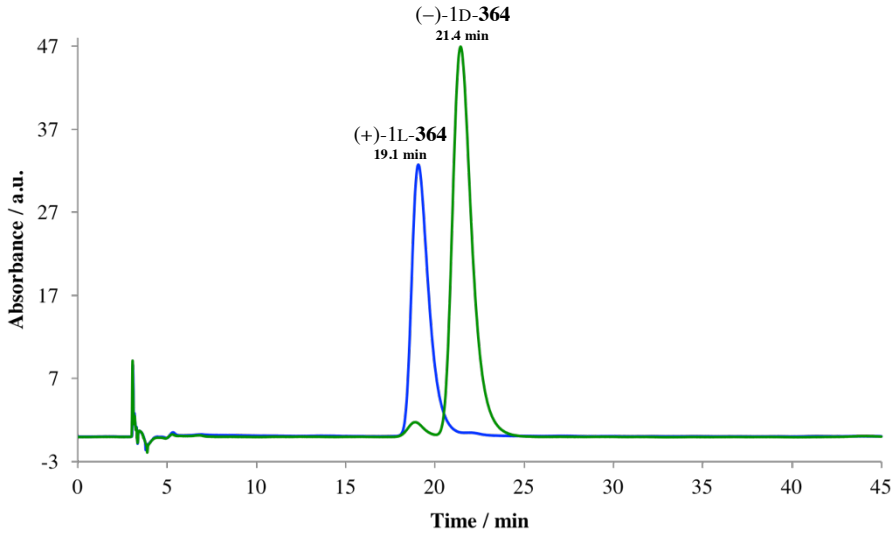


Figure 4.11 Chiral HPLC traces (ChiralPak® AD-H column, heptane/IPA 90:10, 0.8 mL.min^{-1}, 254 nm) of the triols (–)-1D-**364** (blue, >99% *e.e.*) and (+)-1L-**364** (green, >95% *e.e.*), obtained after resolution.

Furthermore, single crystals of each enantiomer were obtained and analysed by X-ray crystallography (Dr Amber L. Thompson and Dr Kirsten Christensen, Chemical Crystallography Lab, Oxford; Appendix B). Although determing the absolute configuration of chiral molecules can be achieved without the need to derivatise the compounds,[323] good quality crystals are usually required. Both (–)-1D-**364** and (+)-1L-**364**, however, were found to give poor single crystal X-ray

diffraction data. In both cases, data were collected on several crystals at low temperature and room temperature. Despite this, the results remained poor and the structure exhibited a large amount of apparent disorder. On close inspection of the raw diffraction images there was some evidence of diffuse scattering and weak superlattice reflections. Despite these difficulties, the best structures for each enantiomer (–)-1D-**364** and (+)-1L-**364** were analysed for assignment of their absolute configuration (Figure 4.12). Whilst the Flack x [0.0(3) and 0.0(3)][413,414] and Hooft y [0.3(1) and 0.0(1)][415] parameters calculated for both enantiomers were close to 0, these values could not be taken into consideration due to their large standard uncertainties. However, the enantiopurity of the bulk material had been established to be >95% for both compounds. Bayesian analysis allows the calculation of conditional probabilities. The (P2) probability that the absolute configuration of (–)-**364** is indeed (–)-1D-**364**, assuming that the structure is enantiopure, is >99.99%. When allowing the additional (P3) probability that (–)-**364** could be racemic that probability becomes 94.66%. Similar results were obtained for (+)-**364**, with a P2 of >99.99% and P3 of 99.99%. Although the absolute configuration of the bulk sample cannot be deduced directly from this analysis, these data strongly support the results obtained *via* NMR and chiral HPLC analysis.

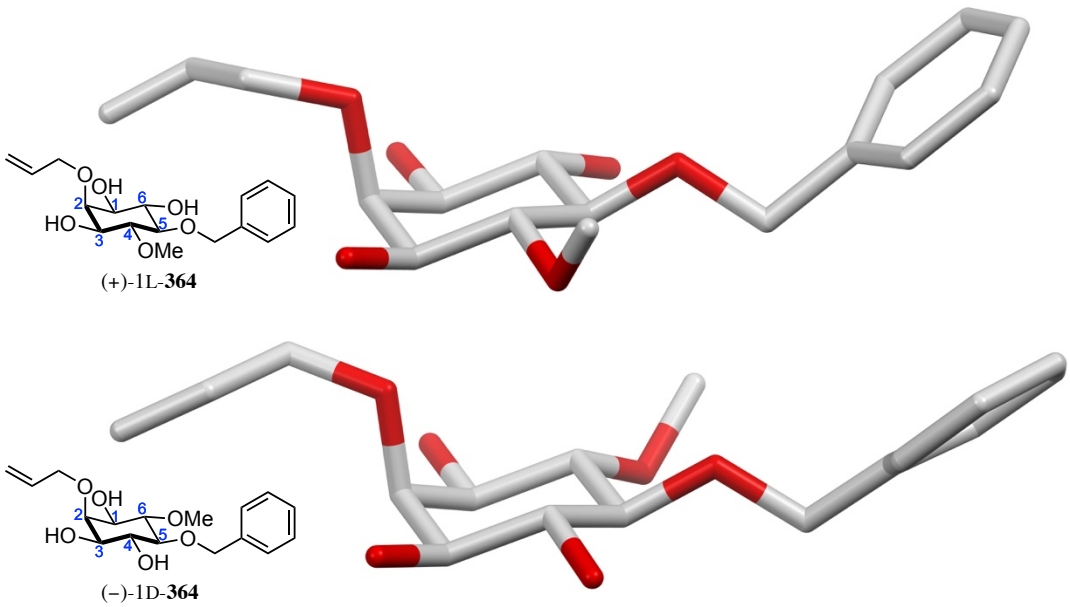
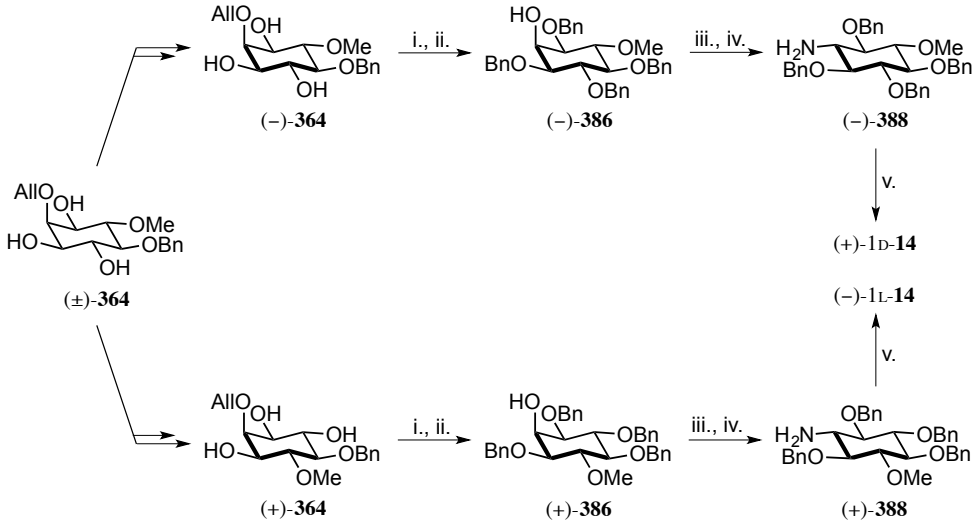


Figure 4.12 Stick representation of the X-ray crystal structure of triols (–)-1D-**364** and (+)-1L-**364**. Key: carbon, silver; oxygen, red.

4.5.4. Synthesis of (+)-1D-3-*O*-MSI (+)-1D-**14** and (–)-1L-3-*O*-MSI (–)-1L-**14**

Similar to our synthesis of SIA **13**, both enantiomers (+)-1L-**364** and (–)-1D-**364** were perbenzylated and selectively deallylated with $PdCl_2$ in MeOH. Mesylation and S_N2 azidolysis yielded the protected *scyllo*-amines (–)-**386** and (+)-**386**. As for **356**, the *scyllo*-stereochemistry was confirmed through ^{1}H NMR by calculation of the $^3J_{H\text{-}H}$ couplings (Figure 4.5). Staudinger reduction and final, global deprotection afforded the enantiomers (+)-1D-**14** and (–)-1L-**14** for the first time. (Scheme 4.13). Racemic 3-*O*-MSI (±)-**14** was also synthesised, starting from triol (±)-**364**, through the same sequence of steps (Scheme 4.13).



Scheme 4.13 Overview of the synthesis of (+)-1D-**14** and (–)-1L-**14** from the resolved triols (–)-1D-**364** and (+)-1L-**364**. *Reagents and conditions*: i. a. NaH, DMF, 0 °C to RT, 40 min b. BnBr, 0 °C to RT, 20 h, 90–94% (–)-**385** and (+)-**385** ii. $PdCl_2$, MeOH/CH_2Cl_2, RT, 19 h, 93–94% (–)-**386** and (+)-**386** iii. a. MsCl, pyridine, CH_2Cl_2, 0 °C to RT, 20 h b. NaN_3, DMF, 90 °C, 18 h, 89–95% (–)-**387** and (+)-**387** iv. PPh_3, H_2O, THF, 40 °C, 28 h, 60–63% (–)-**388** and (+)-**388** v. 10% Pd/C, $H_{2(g)}$, HCl, CH_2Cl_2/MeOH/H_2O, 20 h, 72–77%. Bn = benzyl; All = allyl; Me = methyl.

4.6. Identification of the natural enantiomer of 3-*O*-MSI 14

4.6.1. Biosensor studies

The specificity and sensitivity of the engineered rhizopine biosensor was investigated using synthetic SIA **13**, (±)-**14**, (+)-1D-**14** and (–)-1L-**14**.

The biosensor showed a clear response to **13** and (±)-**14** (Figure 4.14), as opposed to similar naturally abundant metabolites such as (+)-1D-ononitol (+)-**389** or (+)-1D- and (–)-1L-pinitol **390**

(Figure 4.13). In the natural system, rhizopines exuded into the Rhizosphere are thus specifically detected by the biosensor. It is clear, therefore, that rhizopines can be perceived by bacteria and elicite a response. In addition, the specificity of the biosensor towards (–)-1L-**14** over (+)-1D-**14** (Figure 4.14) demonstrates that the regulator protein for catabolism, MocR, is able to distinguish between the two enantiomers. It is therefore clear from this experiment that (–)-1L-**14** is the naturally active enantiomer of this rhizopine.

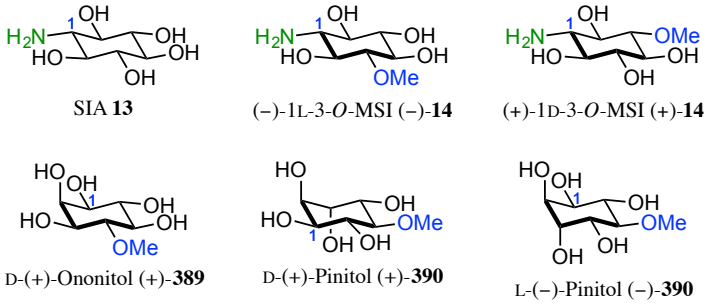


Figure 4.14 Structure of SIA **13**, both enantiomers of 3-*O*-MSI **14**, and methylated cyclitols commonly found in plants, including (+)-1D-ononitol (+)-**389** as well as (+)-1D- and (–)-1L-pinitol **389**.

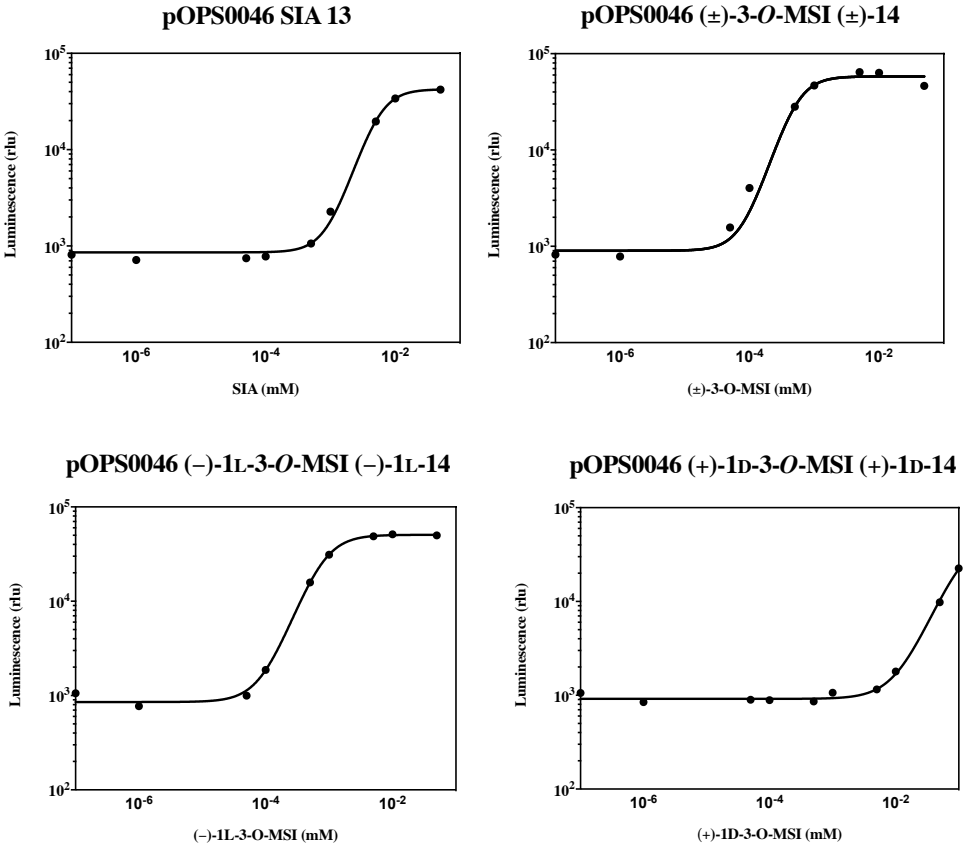


Figure 4.13 Induction curves of Rlv3841/pOPS0046 rhizopine biosensor in response to **13**, (±)-**14**, (–)-1L-**14** and (+)-1D-**14**.

4.6.2. Catabolism assay

As described in Section 4.3.2, we optimised an *in vitro* catabolism assay[389] to show that chemically synthesised SIA **13** can be utilised by wild-type *S. meliloti* L5-30 as a sole carbon and nitrogen source. To further demonstrate that (–)-1L-**14** is indeed the natural enantiomer of rhizopine 3-*O*-MSI, synthetic (–)-1L-**14** and (+)-1D-**14** were similarly used as substrates for the growth of *S. meliloti* L5-30. The results clearly show that only (–)-1L-**14** is able to serve as a sole carbon and nitrogen source for *S. meliloti* L5-30 (Figure 4.15). In addition, racemic (±)-**14** can only promote about half of the growth observed with (–)-1L-**14** (Figure 4.15). This provides further evidence that (–)-1L-**14** is the naturally active enantiomer produced and catabolised by *S. meliloti* L5-30. This finding is in accordance with recent data generated *via* chiral supercriticial fluid chromatography and vibrational circular dichroism.[416]

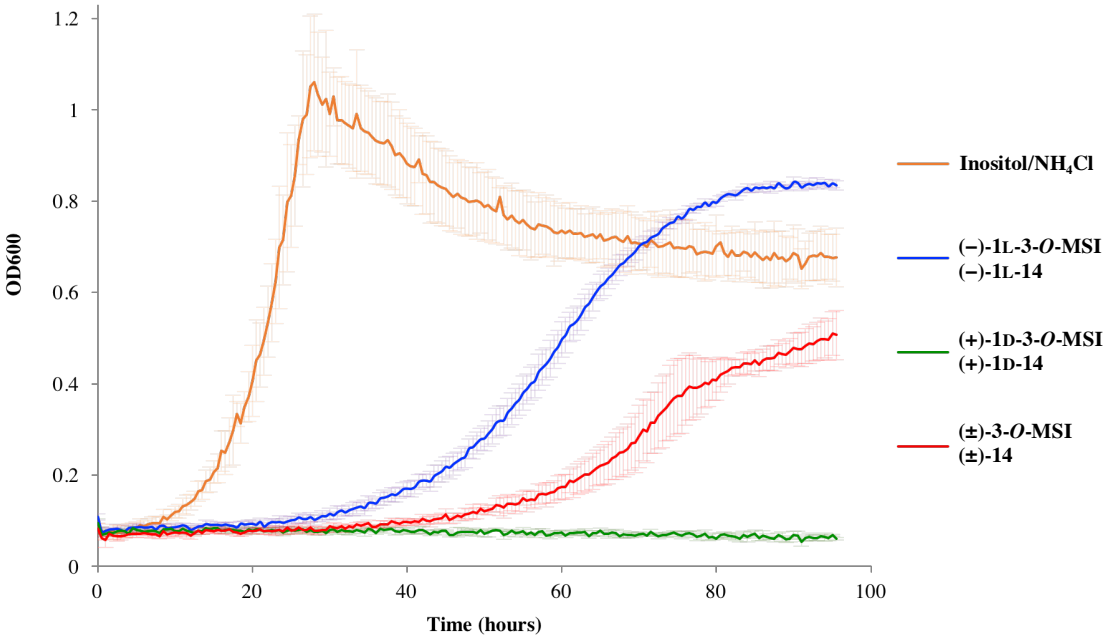


Figure 4.15 Growth curves showing enantiomer (–)-1L-**14** (blue) acting as a sole C and N source for the growth of wild type *S. meliloti* L5-30. Enantiomer (+)-1D-**14** (green) does not promote the growth of *S. meliloti* L5-30 and racemic (±)-**14** (red) only promotes about half of the growth observed for (–)-1L-**14**. Assay showing three independent biological replicates inoculated at OD600 of 0.1 and grown in UMS defined medium at 28 °C while shaking at 500 RPM for >100 hours. The OD600 was monitored every 30 minutes. The error bars represent the standard deviation of the OD600 readings for the three independent replicates, and the line represents the average value.

4.7. Determination of the natural rhizopine biosynthetic pathway

Having established that rhizopines are indeed exuded into the Rhizosphere, the next step was to engineer rhizopine biosynthesis in plants.

4.7.1. Previous research into the rhizopine biosynthetic pathway

The bacterial *mos* genes, required for rhizopines biosynthesis, were originally isolated and studied by Murphy *et al*.[202,203] This genetic locus was shown to be closely linked (~4 kb apart) to the catabolism *moc* genes (See section 4.2.1) as part of the large symbiotic Sym plasmid of *S. Meliloti*.[202] This plasmid also features the nitrogen-fixation (*nif* and *fix*) and nodulation (*nod* and *hsn*) genes. Mutation studies suggested that the *mos* locus is directly controlled by the symbiotic nitrogen-fixation NifA/NtrA-regulated promoter.[212,213] Rhizopines are therefore only produced when the symbiotic genes are expressed. It is these findings that led Murphy *et al*. to propose the 'rhizopine concept'. Whilst the *moc* genes are expressed in free-living bacteria, the synthesis *mos* genes are thought to be mainly expressed by the differentiated bacteroids, in symbiosis with the plants. Although rhizopines are not essential for establishing a symbiotic association, the strains of rhizobia able to catabolise rhizopines[215] have a lasting competitive advantage in nodulation over those that lack the *moc* loci.[202,217,218]

Murphy *et al*. demonstrated that the *S. meliloti* L5-30 *mos* locus involved in producing 3-*O*-MSI **14** in nodules consists of ORF1, *mosA*, *mosB* and *mosC* in an operon (Figure 4.16; A.).[417] First, MosC was identified as a transmembrane transporter protein. Murphy *et al*. also reported that MosB showed homology with regulatory proteins involved in carbohydrate metabolism. Later, it was found that the N-terminal region of this protein shares similaritied to the NifT/FixU nitrogen fixation protein.[390] More interestingly, its central domain shows homology to an aminotransferase family.[390] This family includes L-glutamine:*scyllo*-inosose aminotransferases such as StsC, which catalyse the amino transfer to *scyllo*-inosose **341** to give SIA **13**, an intermediate in streptomycin biosynthesis.[418] It is likely, therefore, that MosB catalyses the amino-transfer in the biosynthetic pathway. Comparatively, the *S. meliloti* Rm220-3 *mos* locus producing SIA **13** lacks a large genetic region which included the

entire *mosA* gene (*mosBC*).[203] These observations led to the hypothesis that 3-*O*-MSI **14** is synthesised *via* SIA **13**, with MosA acting as a methyltransferase (Figure 4.16; B).[419] However, MosA was recently shown to function as a dihydrodipicolinate synthase (DHDPS) *in vivo* and *in vitro*.[417,420] Palmer *et al*. reported the structure of this enzyme (PDB: 2VC6; 1.95 Å) and found no evidence supporting the involvement of MosA in rhizopine biosynthesis.[402] The precise rhizopine biosynthetic pathway was therefore unclear although MosB could potentially act as an aminotransferase (Figure 4.16; B).

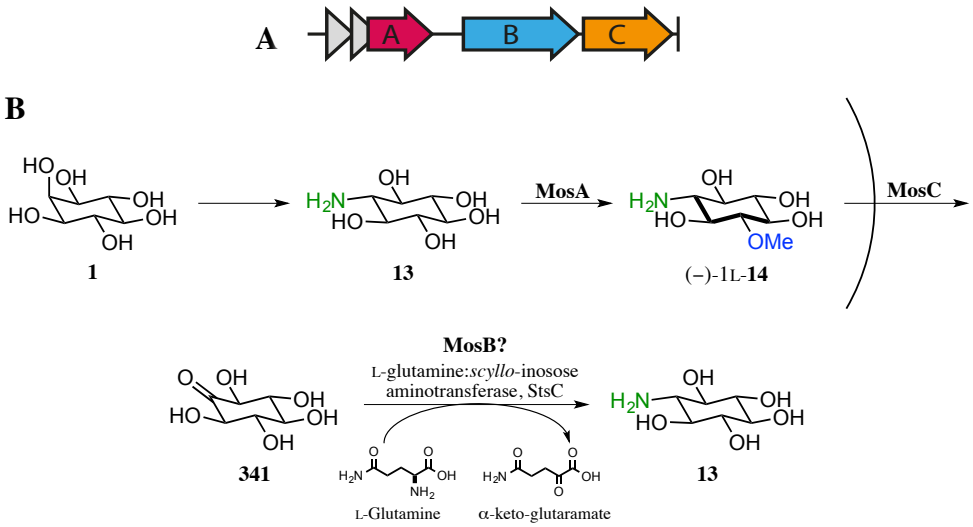


Figure 4.16 *mosABC* as rhizopine synthesis genes **A.** *Mos* gene locus **B.** Rhizopine biosynthetic pathway proposed by Murphy *et. al*. in which 3-*O*-MSI **14** is synthesised *via* SIA **13** with MosA acting as a methyltransferase. MosB was later proposed to act as an aminotransferase in the biosynthetic pathway, maybe similarly to L-glutamine:*scyllo*-inosose aminotransferases

4.7.2. Identification of novel 3-*O*-MSI synthesis genes: *mosDEF*

Dr Barney Geddes investigated further the biosynthetic pathway of **13** and **14** as a starting point towards engineering a *trans*-kingdom signal. Considering that previous attempts to transfer of rhizopine synthesis in plants using *mosABC* had been unsuccessful,[390] we hypothesised that other unidentified genes may be required for the synthesis of 3-*O*-MSI in *S. meliloti*. A bioinformatics analysis (BLAST query of the IMG database)[421] of presumed rhizopine synthesis loci, from recently sequences rhizobia genomes, was carried out by Dr Barney Geddes. Through this analysis, three previously uncharacterised genes, named *mosDEF*, were identified. These were found to be immediately downstream of *mosABC* in an operon arrangement and were predicted by homology to

encode an flavin adenine dinucleotide (FAD) dependent dehydrogenase complex. Two main loci arrangements were observed in other *S. meliloti* and *R. leguminosarum* strains containing MosB homologs; either *mosBCDEF* (lacking the *mosA*), or *mosAB* (lacking *mosCDEF*) (Figure 4.17). To verify if these loci encode 3-*O*-MSI synthesis, the nodules of several representative strains were

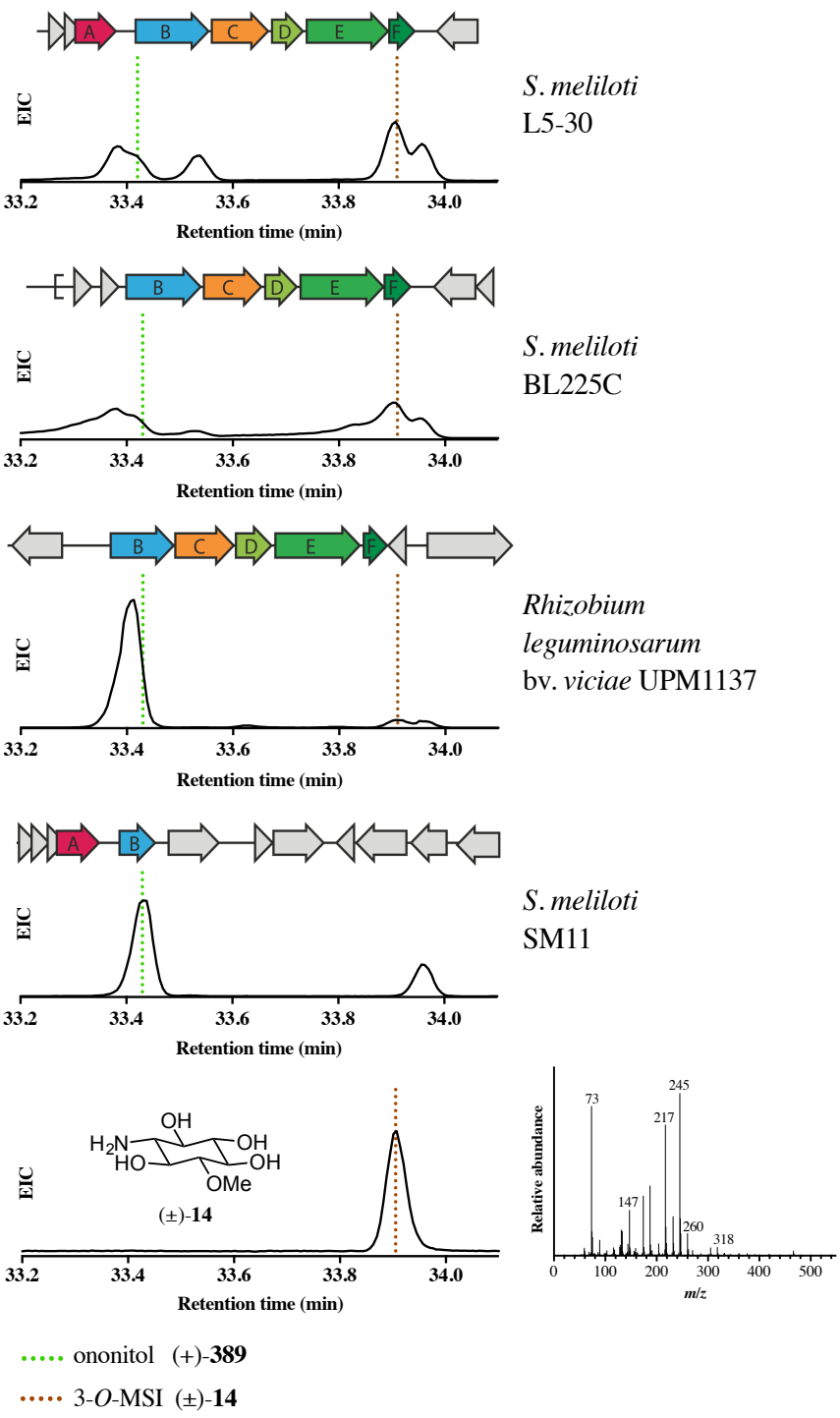


Figure 4.17 Genetic loci and GC-MS chromatograms of nodule extracts from homologues to *mosB* and other presumed rhizopine synthesis genes. Synthetic 3-*O*-MSI (±)-**14** was used as a standard. Derivatisation was carried out with TMSI.

analysed *via* GC-MS. The nodule extracts were derivatised with *N*-trimethylsilylimidazole (TMSI) and compared with synthetic (±)-**14** as an analytical standards (Figure 4.17). All strains containing *mosBCDEF* showed the presence of 3-*O*-MSI **14** in their nodules, while no rhizopine was detected in a strain carrying *mosAB* alone (Figure 4.17). These results further confirm that *mosA* is indeed not involved in rhizopine biosynthesis.

To further understand the role of these novel *mos* genes both a *mosE* and *mosB* mutant was constructed in *S. meliloti* L5-30. The carbohydrate fraction of the nodules induced by these mutant

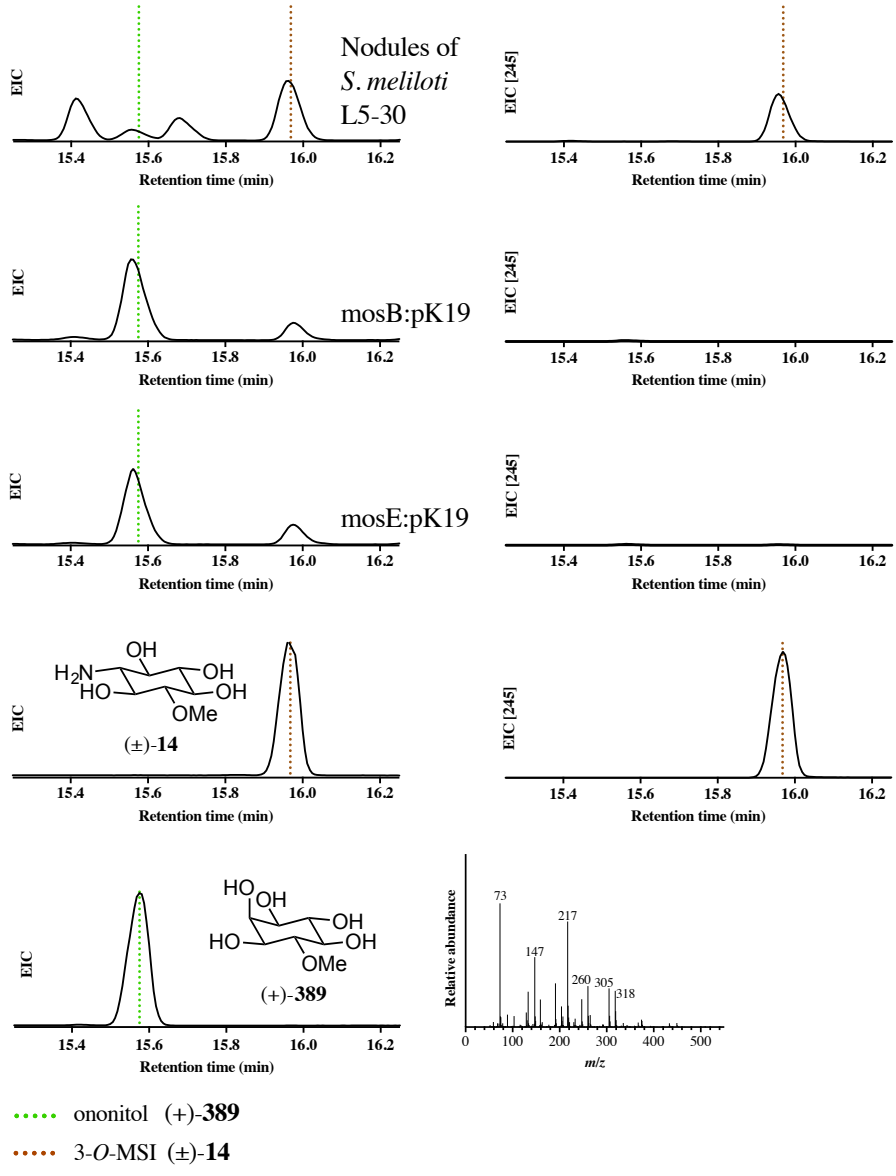


Figure 4.18 GC-MS chromatograms of the nodule extracts from *S. meliloti* L5-30 wild-type, *mosB* and *mosE* mutant strains. The GC-MS chromatograms of the 254 ion, which is a unique rhizopine ion fragment (See mass spectrum Figure 4.17), clearly demonstrates that 3-*O*-MSI **14** is not produced in the mutant strains. Synthetic 3-*O*-MSI (±)-**14** and ononitol (+)-**389** were used as chemical standards. Derivatisation was carried out with TMSI.

strains was analysed by GC-MS and compared to that of *S. meliloti* L5-30 wild type, using synthetic 3-*O*-MSI (±)-**14** as a standard. Both mutants possessed a similar GC-MS profile, which clearly showed the lack of 3-*O*-MSI **14** production (Figure 4.18). This result further confirmed the importance of *mosDEF* and *mosB* in rhizopine synthesis. Furthermore, the levels of carbohydrates present in the nodules formed by *S. meliloti* L5-30 wild-type and *S. meliloti* L5-30 *mosB* and *mosE* mutants were compared. A significant increase in the levels of pinitol **390** and ononitol (+)-**389** was observed in the mutants (Figures 4.18 and 4.19).

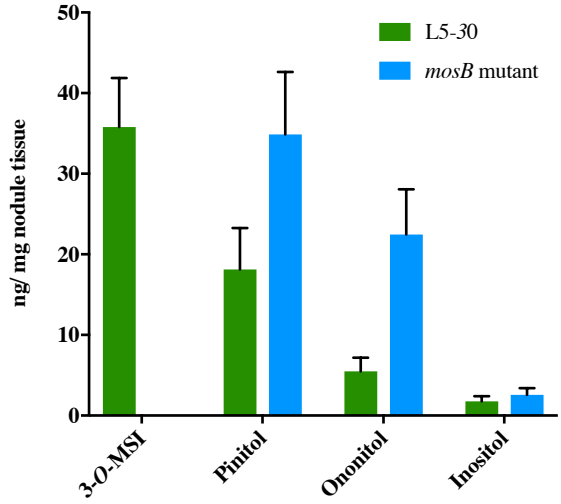
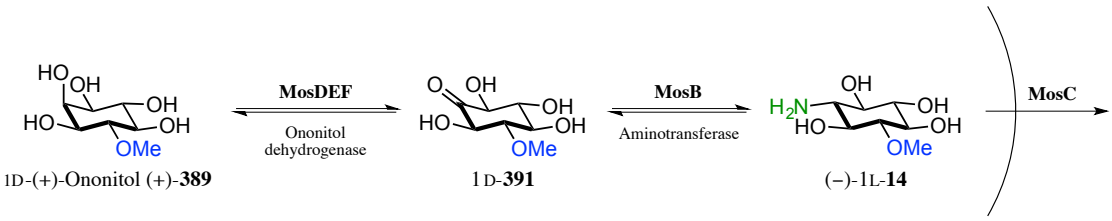


Figure 4.19 Quantification of the relative abundance of relevant carbohydrates in the nodules of *S. meliloti* L5-30 wild-type and *mosB* mutant. A clear increase in the levels of pinitol **390** and ononitol (+)-**389** is observed in the mutant strain.

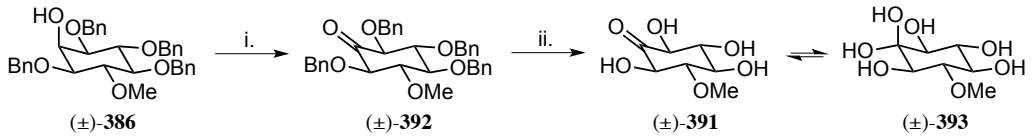
4.7.3. Novel biosynthetic pathway for 3-*O*-MSI **14** synthesis in *S. meliloti* L5-30

Depletion of ononitol (+)-**389** pools in nodules induced by wild type *S. meliloti* L5-30, the stereochemistry of this known plant metabolite[422] and previous research in this area, led us to hypothesise a novel pathway for rhizopine synthesis in *S. meliloti* L5-30. The dehydrogenase complex *mosDEF* could oxidise (+)-1D-ononitol (+)-1D-**389** at the axial 2-position to form 1D-3-*O*-methyl-*scyllo*-inosose 1D-**391**. This oxidised product could then undergo an aminotransferase reaction mediated by MosB, as suggested by the literature,[390] to provide (–)-1L-**14**, the naturally active enantiomer of 3-*O*-MSI (Scheme 4.14).



Scheme 4.14 Hypothesised biosynthetic pathway to (−)-1L-**14**. MosDEF serves as a dehydrogenase to give 1D-**391** and the aminotransferase MosB then mediates an amino transfer to provide (−)-1L-**14**.

To investigate the first step of this hypothesised pathway, Dr Barney Geddes expressed *mosDEF* from its presumed native promoter in *S. meliloti* Rm1021, a strain which does not naturally synthesise rhizopines. Racemic (±)-3-*O*-methyl *scyllo*-inosose (±)-**391** was then synthesised, using the versatile route previously developed (See section 4.5). Alcohol (±)-**386** was subjected to a Swern oxidation to give the fully protected ketone (±)-**392**. Deprotection under hydrogenolysis conditions afforded a mixture of (±)-3-*O*-methyl *scyllo*-inosose (±)-**391** and its hydrate form (±)-**393**[423,424] in a 1.3:1.0 ratio, as determined by ^{1}H NMR (Scheme 4.15).



Scheme 4.15 Synthesis of (±)-**391** from the racemic alcohol intermediate (±)-**386**. *Reagents and conditions*: i. a. $(COCl)_2$, DMSO, CH_2Cl_2, –60 °C, 2.5 h b. Et_3N, –60 °C to RT, 1 h, 46% ii. 10% Pd/C, $H_{2(g)}$, MeOH/CH_2Cl_2/H_2O/AcOH, RT, 17 h, 84% (±)-**391**/(±)-**393** 1.3:1.0.

A number of peaks where observed in the GC-MS chromatogram after derivatisation of (±)-**391** with TMSI (Figure 4.20). It was difficult to determine whether these peaks corresponded to derivatised (±)-**391** and its hydrate form (±)-**393**, or to other isomers generated during the derivatisation step. Whilst one of these peaks had the same retention time and mass spectrum as ononitol-5-TMS by GC-MS, another was found to be unique and characteristic of (±)-**391**. The carbohydrate fraction from the nodules induced by *S. meliloti* Rm1021 expressing *mosDEF*, was therefore analysed by GC-MS and compared to the synthetic standard containing (±)-**391**. The GC-MS chromatogram showed that 1D-**391** had been produced. This GC-MS study therefore provides evidence that *mosDEF* is an ononitol dehydrogenase and 1D-**391** an intermediate in the biosynthetic pathway to (−)-1L-**14** (Figure 4.20).

Furthermore, an *in vitro* biochemical assay was developed to determine whether MosB functions indeed as an aminotransferase, converting synthetic (±)-3-*O*-methyl-*scyllo*-inosose (±)-**391** into 3-*O*-MSI **14** in the second step of the proposed biosynthetic pathway. First, Dr Barney Geddes purified MosB from Rlv3841 by affinity chromatography using a maltose binding protein N-terminal tag. MosB was then incubated with chemically synthesised (±)-**391**, pyridoxal 5' phosphate and L-glutamate as an amino donor. Significant production of 3-*O*-MSI **14** *in vitro* was observed by

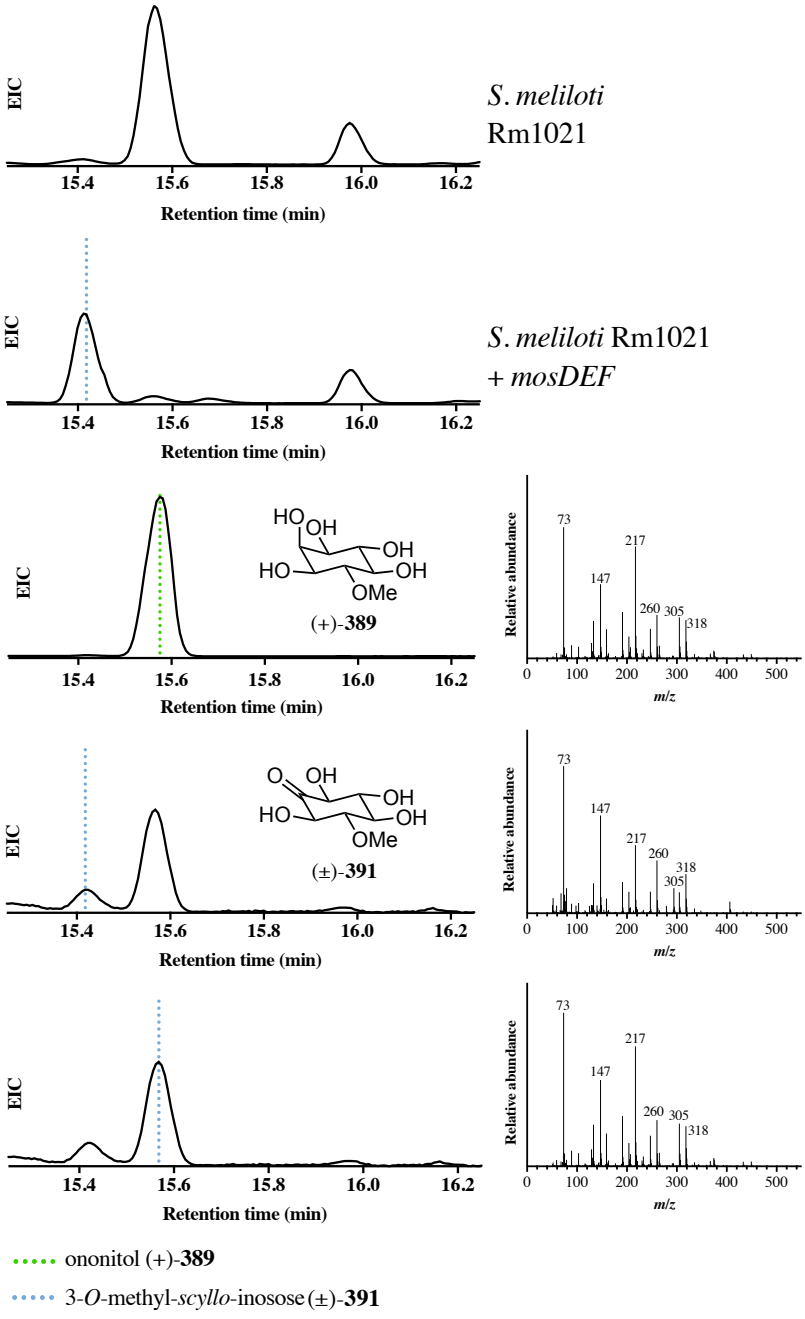


Figure 4.20 GC-MS chromatograms of the nodule extract from *S. meliloti* Rm1021 and *mosDEF* expressed in *S. meliloti* Rm1021 as well as synthetic ononitol (+)-**389** and 3-*O*-methyl *scyllo*-inosose (±)-**392** as standards. Derivatisation was carried out with TMSI.

GC-MS analysis (Figure 4.21). Although L-lysine, β-alanine, L-glutamine, L-aspartate and L-asparagine were all tested as potential amino donors, only L-glutamate could serve as an amino donor for the reaction (Figure 4.21, B.). These results confirm the role of MosB as a 3-*O*-methyl-*scyllo*-inosose:L-glutamate aminotransferase to generate 3-*O*-MSI **14** and presumably α-keto-glutarate.

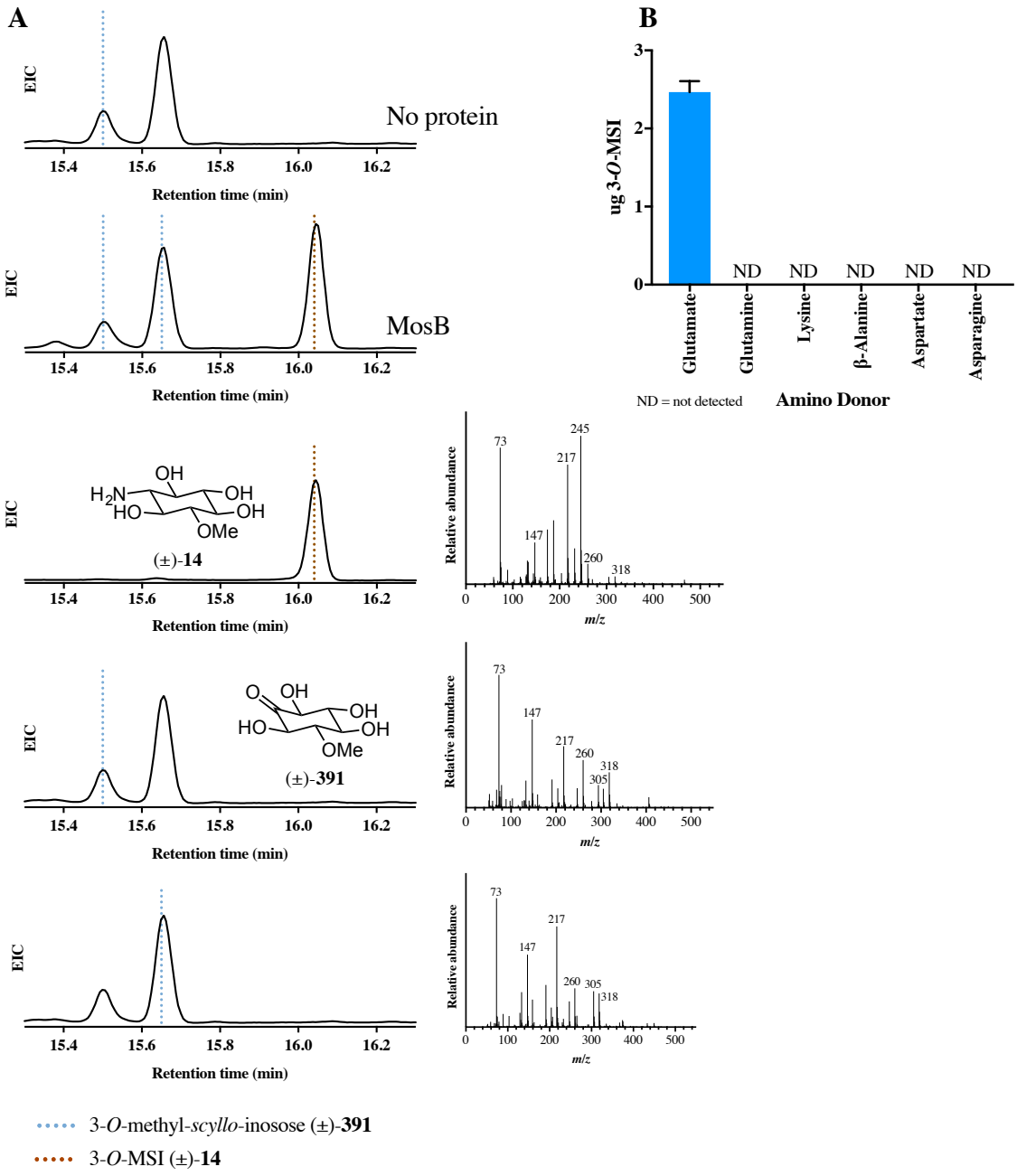
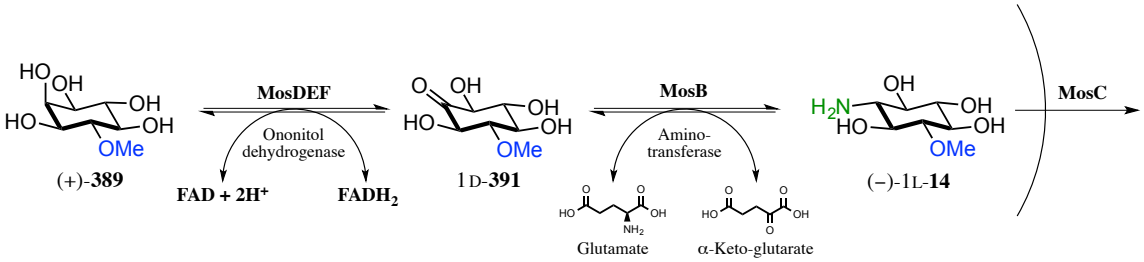


Figure 4.21 A. GC-MS chromatograms of the *in vitro* assay for 3-*O*-MSI biosynthesis from 3-*O*-methyl-*scyllo*-inosose (±)-**391** by MosB as compared to chemical standards **B.** 3-*O*-MSI **14** production by MosB *in vitro* with various amino donors. Derivatisation was carried out with TMSI.

A novel biosynthetic pathway to 3-*O*-MSI **14** is therefore proposed: Ononitol (+)-**389** is oxidised to 3-*O*-methyl-*scyllo*-inosose (±)-**391** by MosDEF, which serves as a substrate for transamination with L-glutamate by MosB to generate (–)-1L-**14** (Scheme 4.16). It is thought that this rhizopine is then exported by MosC, as suggested by the literature. [417]



Scheme 4.14 Proposed novel 3-*O*-MSI biosynthetic pathway in *S. meliloti* L5-30 nodules.

4.7.4. Novel biosynthetic pathway for SIA **13** synthesis in *S. meliloti* L5-30

Building on the knowledge that MosB functions as a 3-*O*-methyl-*scyllo*-inosose:L-glutamate aminotransferase, we hypothesised that MosB may also be involved in the synthesis of SIA **13**, through an analogous reaction with *scyllo*-inosose **341**. Indeed, the first step of *myo*-inositol catabolism is oxidation to *scyllo*-inosose **341** by inositol dehydrogenase (Idh).[425]

To validate this pathway *in vitro*, Dr Barney Geddes purified the well-characterised inositol dehydrogenase IolG from *Bacillus subtilis* (BsIolG) using N-terminal His-tags and affinity chromatography.[426] This soluble bacterial inositol dehydrogenase was chosen, as kinetic studies showed superior rates of inositol oxidation in *in vitro* experiments. Accordingly, in an *in vitro* assay, IolG and its cofactor NAD^+ as well as MosB and its cofactor pyridoxal 5' phosphate with L-glutamate as an amino donor, were incubated with *myo*-inositol **1**. In the presence of both MosB and IolG, production of SIA **13** was observed by GC-MS analysis (Figure 4.22). However, when one or both proteins were excluded, SIA synthesis was not observed (Figure 4.22). As such, MosB also acts as a *scyllo*-inosose:L-glutamate aminotransferase *in vitro* to generate SIA **13**.

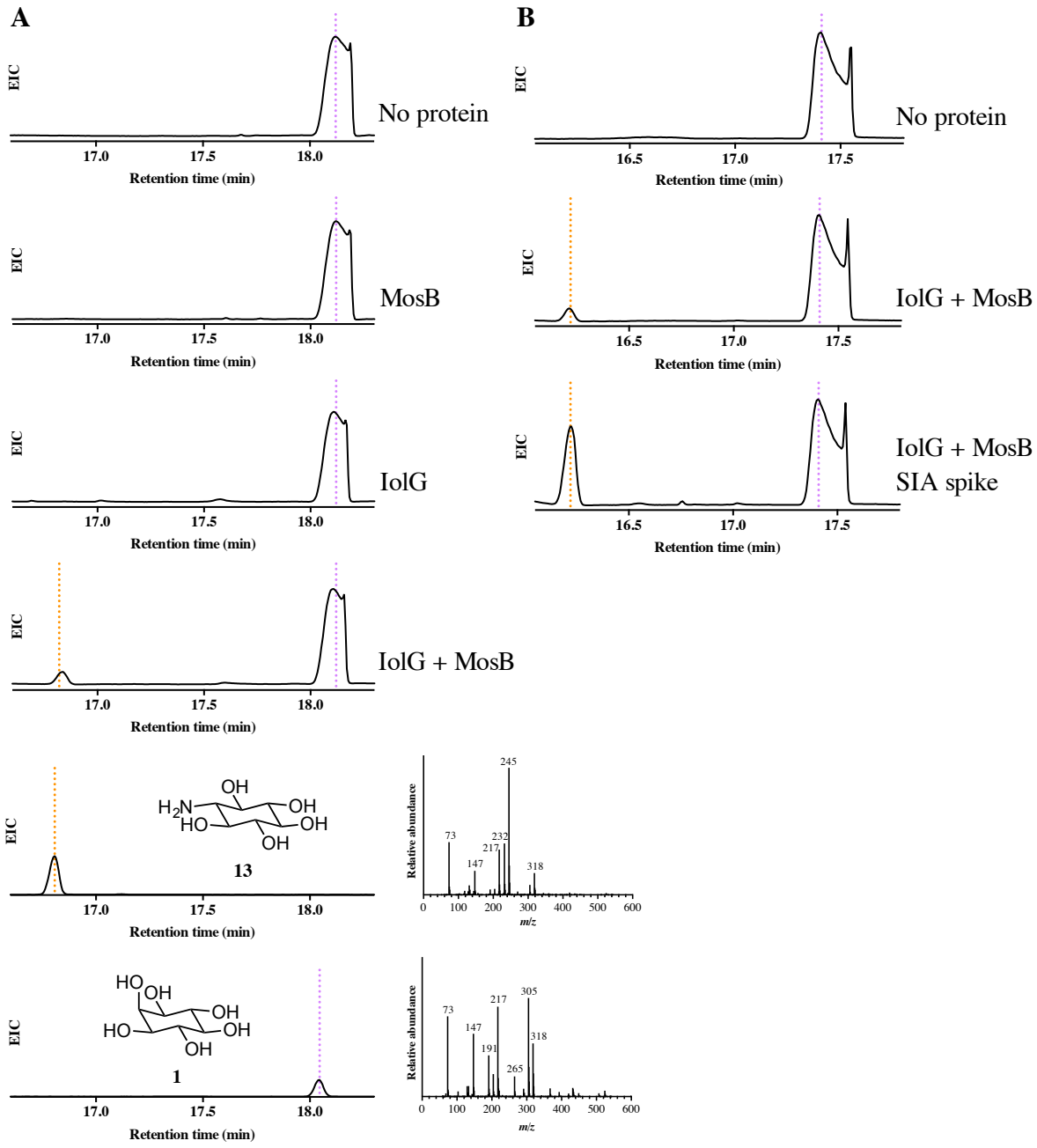
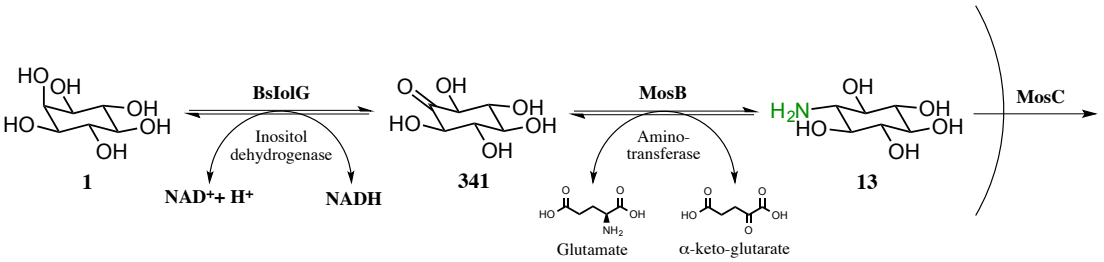


Figure 4.22 A. GC-MS chromatograms of the *in vitro* assay for SIA **13** biosynthesis from *myo*-inositol **1** as compared to chemical standards. SIA is produced when incubated with both IolG and MosB. **B.** Spiking experiment is consistent with the peak at 16.2 min being assigned as SIA. Derivatisation was carried out with TMSI.

Dr Barney Geddes therefore characterised a novel biosynthetic pathway for the synthesis of SIA **13** using *B. subtilis* inositol dehydrogenase (BsIolG) to oxidize *myo*-inositol **1** to *scyllo*-inosose **341**, *S. meliloti* L5-30 MosB to generate SIA **13** from *scyllo*-inosose **341** and L-glutamate as an amino-donor (Scheme 4.17). This simple pathway required only two genes and therefore was thought to be amenable to a broad range of plant host transfers.



Scheme 4.15 Novel synthetic pathway for the synthesis of SIA **13** using *B. subtilis* inositol dehydrogenase (BsIolG) and the aminotransferase MosB, with L-glutamate as an amino-donor.

4.7.5. Transfer of rhizopine biosynthetic pathways into plants

Although transfer of the new 3-*O*-MSI **14** pathway to the tabacco plant *Nicotiana benthamiana* was unsuccessful, most probably due to the complex nature of the ononitol dehydrogenase MosDEF, SIA **13** biosynthesis was found to be amenable to plant transfer. Transfer of the SIA biosynthetic pathway in *Nicotiana benthamiana* and in the legume plant *Medicago truncatula* was successful. Importantly, activation of the rhizopine *lux* biosensor in the rhizosphere of the engineered roots was detected, demonstrating that SIA **13** is produced in sufficient quantities to elicit a bacterial response.

4.8. Summary and Conclusions

The research presented in this chapter focused on demonstrating that rhizopines are ideal signals for engineering *trans*-kingdom signalling between plants and bacteria. The biosynthetic pathway of rhizopines SIA **13** and 3-*O*-MSI **14** was then investigated, towards transferring this pathway to plants. This work therefore led to the following findings:

- Using a rhizopine *lux* biosensor, engineered by Dr Barney Geddes, we were able to demonstrate that, in the natural system, rhizopines are efficiently secreted from the plant roots into the Rhizosphere.
- A modified synthesis of SIA **13** was optimised.
- Basing our research on the knowledge gained in Chapters 2 and 3, a resolution strategy towards enantiomerically pure 3-*O*-MSI was developed. The enantiomers (–)-1L-**14** and (+)-1D-**14** of this rhizopine were synthesised for the first time, and their absolute configuration determined using NMR methods and X-ray crystallography.

- The specificity and sensitivity of the biosensor to rhizopines was demonstrated using synthetic SIA **13** and 3-*O*-MSI **14**. This clearly demonstrated that rhizopines can be perceived by bacteria to elicit a response.
- The naturally active enantiomer of 3-*O*-MSI was identified as being (–)-1L-3-*O*-MSI (–)-1L-**14** . This was achieved by using the engineered rhizopine *lux* biosensor and through a catabolism assay with *S. meliloti* L5-30.
- The mutagenesis studies and *in vitro* assays, carried out by Dr Barney Geddes, led to the proposal of novel SIA and 3-*O*-MSI biosynthetic pathways.
- Transfer of the SIA biosynthetic pathway to plants was successful, with clear activation of the rhizopine *lux* biosensor in the rhizosphere.

These findings are invaluable to engineering a specific *trans*-kingdom signal allowing for the activation of a targeted bacterial response, promoting the growth or survival of plants. In addition, this research represents the first step towards creating a biased rhizosphere, in which rhizopines behave not only as signals, but also as selective growth substrate for engineered bacteria.[390]

5 Conclusions and Future Work: The synthesis of enantiomerically pure inositol derivatives

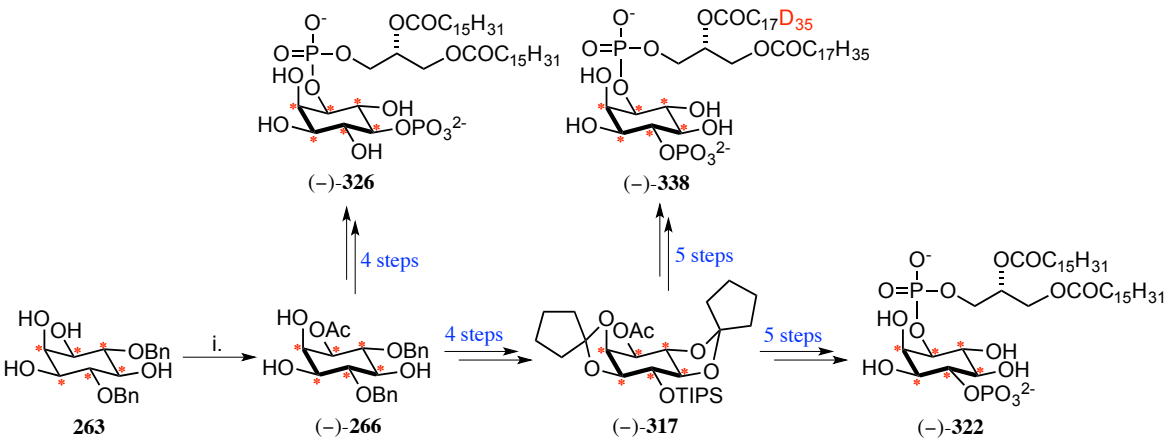
5.1. Conclusions

The aim of this work was to develop efficient enantioselective synthetic routes to biologically relevant inositol derivative, more particularly PtdIns*P*s and rhizopines. This was achieved by exploring various regioselective and enantioselective methodologies (Chapter 1).

Firstly, to understand the intracellular roles and location of PtdIns4*P* **11** and PtdIns5*P* **12**, we focused on the synthesis of deuterated PtdIns*P* probes (Chapters 2 and 3), in collaboration with AstraZeneca and Professor Wolfgang Fischle's laboratory (King Abdullah University of Science and Technology, KAUST, Saudi Arabia). As such, Chapter 2 detailed the design of a protecting group strategy to dipalmitoyl saturated PtdIns4*P* (–)-**48** which relied on a well established, early stage resolution developed by Bruzik *et al*. [23] This initial work led to an understanding of the intricacies linked with differentiating between the six near equivalent hydroxyl groups of the inositol framework.

Building on this work, in Chapter 3, we developed a novel enzyme-mediated approach for the synthesis of enantiomerically pure dipalmitoyl PtdIns4*P* (–)-**48** and PtdIns5*P* (–)-**240**. The synthetic route to PtdIns5*P* (–)-**240** developed involves 8 steps, compared to 11–12 in the literature.[87,118] Whilst the route to PtdIns4*P* (–)-**48** involves 13 steps, which is comparable to the literature,[23,59,77] previous routes featured less efficient resolution strategies. Additionally, this approach was shown to be compatible with deuterated material, allowing for the synthesis of dipalmitoyl D_6 PtdIns5*P* (–)-**326**, dipalmitoyl D_6 PtdIns4*P* (–)-**322**, and distearoyl D_{41} PtdIns4*P* (–)-**338** for the first time (Scheme 5.1).

The latter probe contained deuterium both around the *myo*-inositol ring and on the diacylglycerol chains, which is invaluable for detection of both moieties by mass-spectrometry.



Scheme 5.1 Overview of the route developed towards dipalmitoyl D_6 PtdIns5*P* (–)-**326**, dipalmitoyl D_6 PtdIns4*P* (–)-**322**, and distearoyl D_{41} PtdIns4*P* (–)-**338**. *Reagents and conditions*: i. Lipozyme TL-IM®, vinyl acetate/hexane (1:1), 45 °C, 15 h, 84–98%, >99% *e.e.*

Secondly, in Chapter 4, we demonstrated that rhizopines fulfil all of the requirements for engineering a specific *trans*-kingdom signal between plants and engineered bacteria. The *meso* rhizopine SIA **13** was synthesised using orthoformate chemistry. Besides, both enantiomers of 3-*O*-MSI (–)-1L-**14** and (+)-1D-**14** were synthesised for the first time, *via* a novel resolution route and (–)-1L-**14** was identified as the naturally active enantiomer of this rhizopine. Finally, a novel biosynthetic pathway for both SIA **13** and 3-*O*-MSI **14** was determined, allowing for the transfer of SIA biosynthesis into plants.

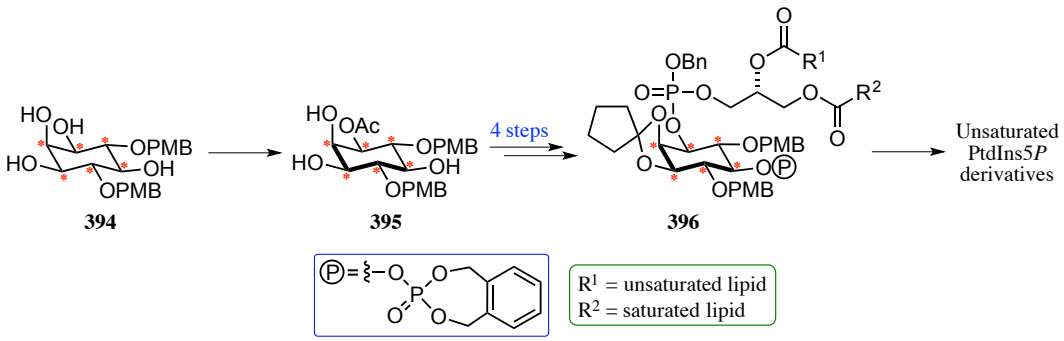
Although PtdInsP_ns and rhizopine are biologically unrelated, and the motivation behind their synthesis was distinct, their common inositol framework allowed us to study in detail the value of various resolution and desymmetrisation strategies for the synthesis of inositol derivatives. In particular, we established that chemoenzymatic transformations can be powerful tools in enantioselective synthesis.[89,312] Although efficient and practically easy, the use of enzymes in desymmetrisations restricts the chemist to particular starting inositol derivatives, providing a unique enantiomer. Whilst resolutions are seen as being inefficient and low-yielding, these can become useful when both enantiomers of the same derivative are desired.

5.2. Future work: Synthesis of unsaturated and metabolically stable PtdIns*P*$_n$s

Having developed a robust synthesis of PtdIns4*P* and PtdIns5*P*, we propose to extend this work to the synthesis of unsaturated and metabolically stable PtdIns*P*$_n$s for use in biological studies.

5.2.1. Synthesis of unsaturated deuterated PtdIns*P*$_n$s

The final global deprotection (TMSBr, Toluene, RT, 12 h) of the fully protected PtdIns4*P* precursor (–)-**321**, was shown to be compatible with unsaturated lipid chains.[88] We will therefore follow the same sequence of steps outlined in Scheme 5.1 for the synthesis of unsaturated, deuterated PtdIns4*P* **178**. Contrastingly, the final hydrogenolysis employed for the synthesis of D_6 PtdIns5*P* (–)-**326** cannot be applied to unsaturated derivatives. We propose to modify the current protecting group strategy, and explore whether the key enzymatic transformation can be extended to the 4,6-di-*O*-PMB derivative **394** (Scheme 5.2). The fully protected PtdIns5*P* framework **396** generated would thus be amenable to deprotection with TMSBr in toluene, as previously demonstrated.



Scheme 5.2 Proposed synthetic route towards unsaturated, deuterated PtdIns5*P* derivatives. Optimisation of the enzymatic desymmetrisation with the 4,6-di-*O*-PMB derivative **394**, would provide precursor **396**, which we envisage could be deprotected using TMSBr in toluene. PMB = 4-methozybenzyl; Bn = benzyl.

5.2.2. Synthesis of metabolically stable PtdIns4*P* and PtdIns5*P* derivatives

We propose to apply our synthetic route to the preparation of complementary, deuterated, phospholipase- and phosphatase-resistant analogues of PtdIns4*P* and PtdIns5*P*. Indeed, molecular and cell biology studies using phosphorylated inositol derivatives have previously been shown to be challenging as these molecules are quickly metabolised by kinases and phosphatases. In particular, methylene-phosphonate, phosphorothioate analogues[59,337,366,427] and non-hydrolysable analogues[428,429] have been used successfully in biological studies. Deuterated 4- and 5-phosphorothioate derivatives

397 and **398**, as well as 4- and 5-methylene-phosphonates **399** and **400**, will therefore be synthesised using our synthetic route in combination with literature procedures (Figure 5.1).

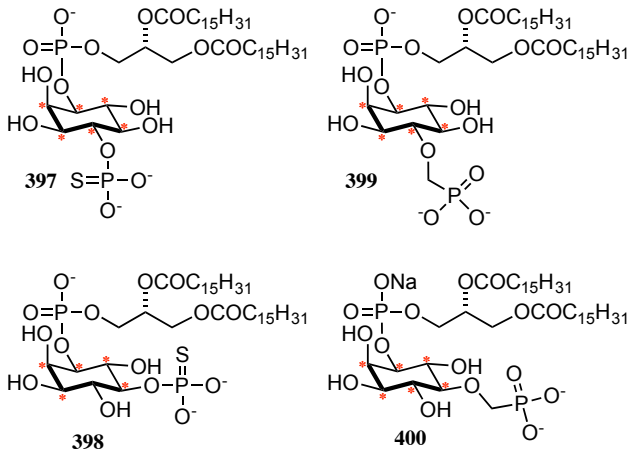


Figure 5.1 Metabolically stable PtdIns4*P* and PtdIns5*P* derivatives, containing either a methylene-phosphonate or a phosphorothionate moiety.

5.3. Future work: Using deuterated PtdIns*P*$_n$s to solve biological questions

5.3.1. Deuterated PtdIns*P*$_n$ as internal standards for the quantification of endogenous PtdIns*P*$_n$ in plants by LC-MS

Having been awarded the 2016 EPSRC Doctoral Prize, we have established a new collaboration with the Dolan laboratory at the Department of Plant Sciences (University of Oxford).

PtdIns kinases (PtdInsK) control a variety of cell growth processes in plants where polar localisation of PtdIns4*P* and PtdIns(4,5)P_2 determines the site of growth in expanding cells.[430,431] The Dolan laboratory has recently discovered that PtdIns4K is not only required for plant cell growth, but is also required to repress cell division in differentiating plant cells. During wild-type development of the liverwort *Marchantia polymorpha*, asymmetric division of unicellular spores produces a basal cell that undergoes immediate terminal differentiation without further cell division (rooting structure). In contrast, the apical cell becomes a stem cell and divides multiple times to form the future plant body. The basal cells of PtdIns4K mutants, with decreased PtdIns4K activity, divide instead of differentiating and the development of the plant is defective. The occurrence of supernumerary cell divisions in a PtdIns4K mutant indicates that PtdIns4K activity represses cell

division during the differentiation process of normal basal cells. This is the first discovery of a role for PtdIns4K in cell division repression in plants. We propose to quantify the precise levels of endogenous PtdIns4*P* in PtdIns4K mutants and in plants that overexpress PtdIns4K, using LC-MS. The use of deuterated internal standards for quantification of PtdInsP_ns is advantageous as these exhibit the same retention and ionisation behaviour, but can be recognised by their characteristic mass. More importantly, matrix effects[XIII] can be excluded considering the similar physicochemical properties of the standard and the analyte under consideration. These studies will allow to establish a quantitative relationship between the levels of PtdInsP_n *in vivo* and cell division repression in plants.

5.3.2. Understanding the metabolism of PtdInsP_n by mass-spectrometry

Hawkins et *al*. have previously developed an HPLC-MS based method allowing for the absolute and relative measurements of the concentration of PtdInsP_ns in cells.[158] This method, amongst other mass spectrometry protocols, is limited as the various PtdInsP_n positional isomers cannot be distinguished. The deuterated PtdInsP_n probes synthesised herein can thus be used to reveal isomer-specific details of the phospholipid metabolism. First, delivery of the deuterated PtdIns5*P* and PtdIns4*P* probes into MCF7 and MCF10a breast cancer cells was achieved using a set of commercially available polyamine carriers. Following the previously developed Nature Methods protocol,[158] the lipids were extracted, derivatised *via* phosphate methylation using TMS-diazomethane, and analysed by HPLC-MS. Initial results have revealed that D_6 PtdIns4*P* was more effectively converted to PtdIns(4,5)P_2 and PtdIns(3,4,5)P_3 than D_6 PtdIns5*P* in MCF7 and MCF10a cells. These data will provide further insights into the cellular roles of these phospholipids. Future experiments will include: 1) investigating the effect of these deuterated probes on the cellular levels of endogenous PtdInsP_n; 2) including a cell fractionation step in the protocol to quantitatively compare PtdInsP_n concentration in various subcellular locations; 3) as previously mentioned (Chapter 1), additional experiments should be conducted comparing the *in vitro* metabolism of deuterated and protonated PtdInsP_n analogues.

[XIII] Change in HPLC-MS response obtained for an analyte in a biological matrix relative to that for the pure analyte in solution. These effects are due to matrix components altering the chromatographic response and ionisation efficiency of the target analyte.

5.3.3. Deuterated PtdInsP_n as Raman probes for bioorthogonal chemical imaging

Stimulated Raman Scattering (SRS)[432,433] and hyperspectral SRS (hsSRS) microscopy are highly sensitive techniques that could be used to quantitatively image deuterated PtdInsP_n *in vitro* and *in vivo* with subcellular resolution.[434,435] Indeed, deuterium exhibits a specific Raman signal, at ~2200 cm^{-1}, which is in the cell silent spectral region (1800–2800 cm^{-1}). The C–D bond is therefore an ideal bioorthogonal chemical label.[436] This technique is advantageous as it allows: "*real-time dynamic analysis […] to track the intracellular fates of small biomolecules in live systems*".[436] Important information regarding the dynamics and intracellular spatial localisation of PtdInsP_n in living cells and organisms could therefore be obtained using this technique.

5.4. Concluding remark

From medicinal chemistry to plant sciences, inositol derivatives are crucial to a myriad of signalling processes, only a fraction of which are currently understood. The research presented in this dissertation was driven principally by interdisciplinary and interdepartmental collaborations. This has made us aware of the ever growing importance of bridging the gaps between disciplines, within institutions, nationally, and internationally.

6 Experimental Section: The synthesis of enantiomerically pure inositol derivatives

6.1. General experimental protocols

NMR spectroscopy

^{1}H NMR spectra were recorded on Bruker DPX 200 (200 MHz), Bruker AVIIIHD 400 nanobay (400 MHz), Bruker AVII 500 (500 MHz) with dual ^{13}C(^{1}H) cryoprobe, or Bruker AVIIIHD 500 (500 MHz) spectrometer in the stated solvents as a reference for the internal deuterium lock. The chemical shift data for each signal are given as δ_H in units of parts per million (ppm) relative to tetramethylsilane (TMS) where δ_H (TMS) = 0.00 ppm. The spectra are calibrated using the solvent peak with the data provided by Fulmer *et al*.[437] The multiplicity of each signal is indicated by s (singlet); br s (broad singlet); d (doublet); dd (doublet of doublets), ddd (doublet of doublet of doublets), t (triplet), q (quartet), dq (double of quartet) or m (multiplet). The number of protons (n) for a given resonance signal is indicated by nH. Where appropriate, coupling constants (*J*) are quoted in Hz and are recorded to the nearest 0.1 Hz. Identical proton coupling constants (*J*) are averaged in each spectrum and reported to the nearest 0.1 Hz. The coupling constants are determined by analysis using Bruker TopSpin version 3.2 software. ^{1}H spectra were assigned using 2D NMR experiments including COSY, HMBC, HSQC, ^{29}Si-^{1}H HMBC and ^{31}P-^{1}H HMBC.

^{13}C NMR spectra were recorded on a Bruker AVIIIHD 400 nanobay (101 MHz), or Bruker AVII 500 (126 MHz) spectrometer, with dual ^{13}C(^{1}H) cryoprobe, in the stated solvents, with broadband proton decoupling and an internal deuterium lock. The chemical shift data for each signal are given as δ_C in units of parts per million (ppm) relative to tetramethylsilane (TMS) where δ_C (TMS) = 0.00 ppm.

The spectra are calibrated using the solvent peak with the data provided by *Fulmer et al*.[437] The shift values of resonances are quoted to 1 decimal place unless peaks have similar chemical shifts, in which case 2 decimal places are used. Where appropriate, the multiplicity of each signal is indicated by d (doublet), t (triplet) or m (multiplet). A subscript D (e.g. t_D) indicates splitting caused by deuterium (I = 1); and a subscript P (e.g. d_P) indicated splitting caused by phosphorus (I = 1/2). Coupling constants (J_P and J_D) are quoted in Hz and are recorded to the nearest 0.1 Hz. These were determined using Bruker TopSpin version 3.2 software. ^{13}C spectra were assigned using 2D NMR experiments including HMBC and HSQC.

^{31}P NMR spectra were recorded on a Bruker AVIIIHD 400 nanobay (162 MHz), or Bruker AVIIIHD 500 (202 MHz) spectrometer in the stated solvents as a reference for the internal deuterium lock, using a broadband proton decoupling pulse sequence. The chemical shift for each signal are given as δ_P in units of parts per million (ppm) relative to 85% phosphoric acid as an external reference where δ_P (H_3PO_4) = 0.00 ppm. Signals are singlets unless otherwise stated. ^{31}P spectra were assigned using ^{1}H-^{31}P NMR experiments as necessary.

^{2}H NMR spectra were recorded on a Bruker AVII 500 (77 MHz) or Bruker AVIIIHD 600 (92 MHz) spectrometer in the stated solvents using a drop of relevant deuterated solvent as a reference for the internal deuterium lock. Signals are typically broad singlets. The chemical shift data for each signal are given as δ_D in units of parts per million (ppm) relative to tetramethylsilane (TMS) where δ_D (TMS) = 0.00 ppm. The spectra are calibrated using the solvent peak with the data provided by *Fulmer et al*.[437] The spectra are assigned by matching the signals to those obtained in the ^{1}H spectra of the protonated counterparts.

When two diastereoisomers are present in the sample, A and B denotes each of the two diastereoisomers without distinguishing between them. A is arbitrarily assigned to the diastereoisomer with the highest ppm shift and B to the diastereoisomer with the lowest ppm shift, in ^{1}H NMR, ^{13}C NMR and ^{31}P NMR spectra. In cases of multiplets which are assigned to multiple protons, where possible resonances are quoted in the order in which they appear as assigned by 2D NMR experiments.

Mass spectrometry

Electrospray ionisation spectra were acquired on a Waters LCT Premier spectrometer or Agilent 6120 Quadrupole spectrometer (low resolution) or a Bruker MicroTOF spectrometer (high resolution), operating in positive or negative mode, as indicated, from solutions of MeOH, MeCN or H_2O. *m/z* values are reported in Daltons and followed by their percentage abundance in parentheses. Electron ionisation/field ionisation (EI/FI) was carried out on a Waters GCT with a temperature programmed solids probe inlet. MALDI was carried out on a Waters MALDI Micro MX. When a compound was not observed by LRMS, only HRMS is quoted.

Melting points

Melting points were determined using a Leica Galen III hot stage microscope and are uncorrected. The solvents of crystallisation are shown in parentheses.

Infrared spectra

Infrared (IR) spectra were obtained from neat samples, either as liquids or solids using a diamond ATR module. The spectra were recorded on a Bruker Tensor 27 spectrometer. Absorption maxima are reported in wavenumbers (cm^{-1}) and reported as s (strong), m (medium), w (weak) or br (broad). Only the main, relevant peaks have been assigned.

Specific optical rotation

Specific optical rotations were measured using either a Perkin Elmer Model 241 polarimeter or Schmidt + Haensch UniPol L2000 polarimeter, in cells with a path length of 1 dm, using a sodium lamp at 589 nm. The concentration (*c*) is expressed in g/100 mL (equivalent to g/0.1 dm^3) Specific rotations are denoted $[\alpha]_D^T$ and are given in implied units of $10^{-1}degcm^2g^{-1}$ at the temperature stated.

Analytical thin layer chromatography

Thin layer chromatography (TLC) was carried out on normal phase Merck silica gel 60 F_{254} aluminium-supported chromatography sheets. Visualisation was by absorption of UV light (λ_{max} 254 nm), exposure to iodine vapour or thermal development after dipping in either an ethanolic solution of ninhydrin or an aqueous solution of potassium permanganate.

Silica gel flash column chromatography

Normal phase silica gel flash column chromatography was performed either manually using VWR Prolabo silica gel 60 (240–400 mesh) under a positive pressure of nitrogen or on a Biotage SP1 automated column chromatography system using KP-Sil® SNAP Flash Silica Cartridges. Reversed phase silica gel column chromatography was carried out using C18 Fluka silica gel, eluting with the appropriate solvents under a positive pressure of compressed nitrogen.

Concentration *in vacuo* and vacuum transfer

In vacuo refers to the removal of solvents under reduced pressure using a Büchi™ rotary evaporator in a water bath at 40 °C, unless otherwise stated. Vacuum transfer refers to the removal of solvents on a manifold linked to a high vacuum pump at RT.

Chemicals

Chemicals were purchased from Acros UK, Apollo Scientific, Enamine, Sigma Aldrich UK, Alfa Aesar UK, Fisher Scientific UK, Fluka UK, Fluorochem, Merck, Argo International Limited and TCI-Europe. All reagents were purified, when necessary, by standard techniques.[438] In particular, Et_3N, pyridine and DIPEA were dried by stirring over solid KOH pellets overnight followed by fractional distillation. DIPA was distilled from NaH. These were stored under Ar and over 3 Å molecular sieves. PCl_3 was heated under reflux to expel dissolved HCl, then distilled and stored under Ar over 4 Å molecular sieves. Anhydrous solvents were obtained under the following conditions: Et_2O, toluene and CH_2Cl_2 were dried by passing through a column of activated basic alumina according to the Grubbs' procedure.[439] Anhydrous DMF, DMSO, MeOH and MeCN were purchased from Sigma Aldrich UK in SureSeal™ bottles and used without further purification. Anhydrous THF was distilled from sodium metal, using benzophenone as an indicator.[438] All other solvents were used as supplied (analytical or HPLC grade) without purification. Where appropriate and if not otherwise stated, all non-aqueous reactions were performed in a flame dried flask under an inert atmosphere. Hexane refers to a mixture of hexane isomers and petroleum ether refers to the fraction of light petroleum ether boiling within the range of 40–60 °C. Brine refers to a saturated

aqueous solution of sodium chloride. Rochelle's salt refers to an aqueous solution of potassium sodium tartrate tetrahydrate.

Compound purity

Compound purity was determined by analytical high-performance liquid chromatography (HPLC) on a PerkinElmer Flexar system with a Binary LC Pump and UV/VIS LC Detector using: a) a reversed phase Dionex Acclaim® 120 column (C18, 5 μm, 4.6 × 150 mm) with H_2O/MeCN/TFA 95:5:0.1 (A) and MeCN/ H_2O/TFA 95:5:0.1 (B) or H_2O/MeCN 95:5 (A) and MeCN/H_2O 95:5 (B) as eluents; or b) a normal phase HyperSil GOLD™ Silica column (5 μm, 4.6 × 150 mm) with heptane or hexane (A) and IPA (B) as eluents. Gradient methods of 19 to 25 minutes were employed with a constant flow rate, and detection at 254 or 220 nm (Methods 1, 2, 3, 5, 6 and 7). In certain cases, isocratic methods were employed (Methods 4 and 8). Samples were injected by dissolving in the relevant solvent system. The methods used are described below:

Method 1: A = H_2O/MeCN/TFA 95:5:0.1; B = MeCN/ H_2O/TFA 95:5:0.1; 1.0 mL.min^{-1}; 254 nm.

Method 2: A = H_2O/MeCN 95:5; B = MeCN/ H_2O 95:5; 1.0 mL.min^{-1}; 254 nm.

Method 3: A = H_2O/MeCN 95:5; B = MeCN/ H_2O 95:5; 1.0 mL.min^{-1}; 220 nm.

Method 4: Isocratic; MeCN; 1.5 mL.min^{-1}; 254 nm.

Method 5: Normal Phase; A = Heptane; B = IPA; 1.0 mL.min^{-1}; 254 nm.

Method 6: Normal Phase; A = Hexane; B = IPA; 2.0 mL.min^{-1}; 254 nm.

Method 7: Normal Phase; A = Hexane; B = IPA; 1.5 mL.min^{-1}; 254 nm.

Method 8: Normal Phase; Isocratic; Hexane; 1.0 mL.min^{-1}; 254 nm.

Table A Reverse Phase HPLC gradient for Method 1, Method 2 and Method 3

Step length (min)	Elapsed time (min)	%A	%B
1	1	100	0
10	11	0	100
3	14	0	100
1	15	100	0
5	20	100	0

Table B Normal phase HPLC gradient for Method 5

Step length (min)	Elapsed time (min)	%A	%B
1	1	95	5
12	13	5	95
5	18	5	95
1	19	95	5
6	25	95	5

Table C Normal phase HPLC gradient for Method 6

Step length (min)	Elapsed time (min)	%A	%B
1	1	98	2
5	6	90	10
13	19	90	10

Table D Normal phase HPLC gradient for Method 7

Step length (min)	Elapsed time (min)	%A	%B
1	1	98	2
15	16	90	10
3	19	90	10

Compounds which did not contain a chromophore, could not be analysed by analytical HPLC, and their purity was therefore not determined *via* this method.

Determination of enantiomeric purity

Enantiomeric purity was determined by chiral analytical high-performance liquid chromatography (HPLC) on a PerkinElmer Flexar system with a Binary LC Pump and UV/VIS LC Detector using a ChiralPak® AD-H column (5 μ m, 4.6 × 150 mm) with IPA (A) and heptane (B) as eluents. Isocratic methods of 45 minutes were employed with a constant flow rate of 1.0 mL.min^{-1} or 0.8 mL.min^{-1} and detection at 220 or 254 nm as indicated. Samples were injected by dissolving in the relevant solvent system. The enantiomeric excess (*e.e*) was determined using the following equation: *e.e*. = ((R–S)/R+S)) × 100 where R and S stand for the individual enantiomers and $R + S = 1$.

Semi-preparative HPLC

Semi preparative HPLC was performed on a Dionex P680 HPLC pump using a reversed phase Agilent ZORBAX 300SB-C18 column (5 μm, 9.4 × 250 mm) with H_2O/MeCN/TFA 95:5:0.1 (A) and MeCN/ H_2O/TFA 95:5:0.1 (B) as eluents. A gradient method of 29 minutes was employed with a constant flow rate of 3.0 mL.min^{-1} and detection at 254 nm. Samples were injected by dissolving in MeCN/H_2O. The method used is described below:

Method 1: A = H_2O/MeCN/TFA 95:5:0.1; B = MeCN/ H_2O/TFA 95:5:0.1; 3.0 mL/min^{-1}; 254 nm.

Table E Semi-preparative HPLC gradient for Method 1

Step length (min)	Elapsed time (min)	%A	%B
2	2	100	0
3	5	74	26
20	25	74	26
2	27	0	100
2	29	100	0

GC-MS analysis

For GC-MS analysis, samples were dried overnight in a vacuum centrifuge then derivatised by reaction with 25 μL of pyridine and 35 μL of TMSI for 60 minutes at 37 °C with shaking at 950 RPM. GC-MS analysis was performed using an Agilent 79890 GC coupled to an Agilent 5975 quadrupole

MS detector, electron 702 impact ionisation (70 eV) equipped with an Agilent CP9013 column (30 mm, 0.25 mm inner diameter) at the 703 facility, in the Department of Plant Sciences, University of Oxford, UK.

Lyophilisation

Lyophilisation refers to the removal of H_2O from aqueous solutions by freeze drying using a CHRIST Alpha 1-2 LD lyophiliser.

Reaction monitoring

Reaction progress was monitored at appropriate times either by TLC or by ^{31}P NMR.

Celite®

Celite® refers to Celite® 545 filter aid, treated with sodium carbonate, flux-calcined which was purchased from Sigma Aldrich.

Glass microfiber filter

Glass microfiber filter refers to Whatman® borosilicate glass microfiber filters, Grade GF/B.

Deuterium atoms

Where not explicitly stated in a chemical structure, deuterium atoms are indicated by a red asterisk next to the relevant carbon.

Deuterium Incorporation

The deuterium incorporation of deuterated compounds is shown after the yield. Mass spectrometry techniques were used to calculate the incorporation shown and are ^{13}C corrected.

Deuterated *myo*-inositol 183

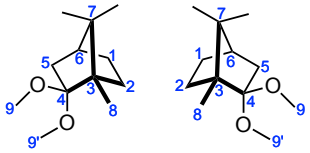
Deuterated *myo*-inositol was prepared by Dr Alex Saunders as described in his DPhil Dissertation: *Synthesis of Deuterated Phosphatidylinositol Phosphates* (2016).[47]

DFT Methods

For the DFT calculations, all molecules were protonated and then subjected to a low energy conformational search with the MMFF94 force field in InstantJChem, Marvin 17.13.0, 2017, ChemAxon (http://www.chemaxon.com). The lowest energy conformation was selected and subjected to further geometry optimisation through density functional theory (DFT) calculations using the 6-31G basis set, in Guassian16 (Gaussian Inc., Wallingford CT, 2016). These calculations used the B3LYP exchange correlation functional. The NMR spectrum for all atoms was predicted, again using the 6-31G basis set in Gaussian16, and visualised in GaussView, version 5.0.9. We would like to acknowledge the use of the University of Oxford Advanced Research Computing (ARC) facility in carrying out this work. http://dx.doi.org/10.5281/zenodo.22558

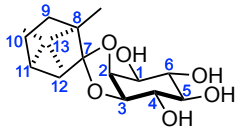
6.2. Experimental procedures and compound characterisation: Chapter 2

1-(*S*)-(−)-Camphor dimethylacetal (−)-100 & 1-(*R*)-(+)-Camphor dimethylacetal (+)-100



To a solution of (*S*)-(−)-camphor (−)-**184** or (*R*)-(+)-camphor (+)-**184** (20.0 g, 131 mmol, 1.0 eq) in hexane (200 mL) was added K-10 montmorillonite clay (36.0 g) and trimethylorthoformate (55.6 g, 524 mmol, 4.0 eq). The suspension was stirred for 3 days at RT. In the case when the reaction only reached 50% conversion after 48 hours of stirring, concentrated H_2SO_4 (280 μL, 0.525 mmol, 0.0040 eq) was added. The reaction mixture was then left to stir for another 2 days. When the reaction was shown to be complete by ^{1}H NMR analysis, the mixture was filtered through a pad of Celite®, washed with hexane (4 × 100 mL) and the combined filtrates were concentrated *in vacuo* to give (−)-**100** (24.7 g, 95%) or (+)-**100** (24.4 g, 94%) as a crude, yellow oil. Analysis by ^{1}H NMR showed that the product was 90-95% pure and the compound was therefore used in subsequent steps without further purification or characterisation: R_f 0.90 (Petroleum ether/Et_2O 8:2); ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 3.22 (3H, s, H-9 or H-9'), 3.16 (3H, s, H-9 or H-9'), 2.22–2.15 (1H, m, H_{eq}-5), 1.78-1.68 (2H, m, H_{eq}-1 and H_{eq}-2), 1.66–1.62 (1H, m, H-6), 1.40-1.30 (1H, m, H_{ax}-2), 1.27–1.18 (1H, m, H_{ax}-1), 1.14 (1H, d, *J* 12.9, H_{ax}-5), 0.96 (3H, s, C(7)CH_3), 0.90 (3H, s, H-8), 0.82 (3H, s, C(7)CH_3). These data are in good agreement with the literature values.[55]

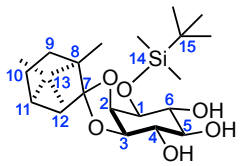
(+)-1D-2,3-*O*-(D-1,7,7-Trimethyl[2.2.1]bicyclohept-2-ylidene)-*myo*-inositol (+)-101a



1-(*R*)-(+)-**100** (41.6 g, 209 mmol, 2.2 eq) and concentrated H_2SO_4 (950 μL, 17.8 mmol, 0.19 eq) were added to a heterogeneous solution of *myo*-inositol **1** (16.9 g, 93.6 mmol, 1.0 eq) in anhydrous DMSO

(184 mL). The reaction mixture was stirred for 3 hours at 75 °C, cooled to RT, neutralised with Et_3N (6.3 mL) and concentrated *in vacuo* until the total weight reached 110 g. $CHCl_3$/MeOH/H_2O (50:5:1 *v*/*v*/*v*, 309 mL) and PTSA·H_2O (63 mg) were added and the mixture was stirred for 18 hours and then neutralised with Et_3N (2.1 mL). The colourless precipitate obtained was collected then washed with $CHCl_3$ (2 × 200 mL) and H_2O (3 × 200 mL). Subsequent trituration with MeOH (0.1% Et_3N) gave pure tetrol (+)-**101a** (6.80 g, 23%) as a colourless solid. To prevent acetal migration or deprotection, (+)-**101a** was stored at −20 °C: R_f 0.62 ($CHCl_3$/MeOH 8:2); $[\alpha]_D^{20}$ = +43.2 (*c* 2.0, Pyridine) [lit.[221] $[\alpha]_D$ = +44.3 (*c* 1.90, Pyridine)]; m.p. 236–237 °C (MeOH (0.1% Et_3N) then H_2O) [lit.[221] 231–232 °C (MeOH)]; ^{1}H NMR (400 MHz; D_6-DMSO) δ_H 4.79–4.74 (3H, m, C(1)O*H*, C(4)O*H* and C(6)O*H*), 4.68 (1H, d, *J* 4.1, C(5)O*H*), 4.08 (1H, dd, *J* 5.6, 4.5, H-2), 3.65 (1H, dd, *J* 6.7, 5.6, H-3), 3.51 (1H, ddd, *J* 9.2, 4.6, 4.5, H-1), 3.27 (1H, ddd, *J* 9.3, 9.2, 4.3, H-6), 3.21 (1H, ddd, *J* 9.7, 6.7, 5.0, H-4), 2.92 (1H, ddd, *J* 9.7, 9.3, 4.1, H-5), 1.98–1.83 (2H, m, H_{eq}-9 and H_{eq}-12), 1.72–1.60 (2H, m, H-11 and H_{eq}-10), 1.39 (1H, d, *J* 13.0, H_{ax}-12), 1.35–1.25 (1H, m, H_{ax}-9), 1.19–1.07 (1H, m, H_{ax}-10), 0.95 (3H, s, C(13)CH_3), 0.81 (3H, s, C(8)CH_3), 0.76 (3H, s, C(13)CH_3); LRMS *m*/*z* (ESI$^+$) 359.1 ([M+2Na−H]$^+$, 100%). These data are in good agreement with the literature values.[56,221,223]

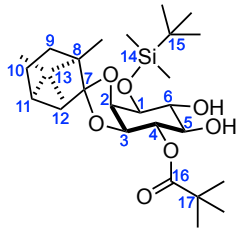
(−)-1D-1-*O*-(*tert*-Butyldimethylsilyl)-2,3-*O*-(D-1,7,7-trimethyl[2.2.1]bicyclohept-2-ylidene)-*myo*-inositol (−)-195



A solution of tetrol (+)-**101a** (6.50 g, 20.7 mmol, 1.0 eq) and imidazole (2.11 g, 31.0 mmol, 1.5 eq) in anhydrous pyridine (68 mL) was treated with TBDMSCl (3.25 g, 21.6 mmol, 1.04 eq) at −10 °C. After 24 hours the mixture was concentrated *in vacuo* and H_2O (20 mL) was added. The aqueous layer was extracted with EtOAc (4 × 20 mL). The combined organic layers were washed with H_2O (20 mL) and brine (2 × 20 mL), dried ($MgSO_4$), filtered, and concentrated *in vacuo*. The residue was purified twice by column chromatography over silica gel ($CHCl_3$/MeOH 10:0, 9.5:0.5, 9:1) to give

(–)-**195** (5.05 g, 57%) as a colourless, glassy solid. Compound (–)-**195** was contaminated by other minor isomers, which could only be separated in the subsequent step. Analysis by ^{1}H NMR showed that the product was 91% pure: R_f 0.26 ($CHCl_3$/MeOH 8:2); $[\alpha]_D^{20} = -12.6$ (*c* 1.0, $CHCl_3$); m.p. 172–173 °C ($CHCl_3$/MeOH); ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 4.17 (1H, dd, *J* 5.3, 4.2, H-2), 3.83 (1H, dd, *J* 7.0, 5.3, H-3), 3.73 (1H, dd, *J* 9.3, 4.2, H-1), 3.67 (1H, dd, *J* 9.4, 9.3, H-6), 3.58 (1H, dd, *J* 9.8, 7.0, H-4), 3.32 (1H, dd, *J* 9.8, 9.4, H-5), 2.77 (2H, br s, C(4)O*H* and C(6)O*H*), 2.48 (1H, br s, C(5)O*H*), 2.03–1.87 (2H, m, H_{eq}-9 and H_{eq}-12), 1.77–1.66 (2H, m, H-11 and H_{eq}-10), 1.49 (1H, d, *J* 12.8, H_{ax}-12), 1.45–1.34 (1H, m, H_{ax}-9), 1.28–1.17 (1H, m, H_{ax}-10), 0.99 (3H, s, C(8)CH_3), 0.91 (9H, s, C(15)CH_3), 0.85 (3H, s, C(13)CH_3), 0.83 (3H, s, C(13)CH_3), 0.13 (3H, s, Si(14)CH_3), 0.13 (3H, s, Si(14)CH_3); ^{13}C NMR (101 MHz; $CDCl_3$) δ_c 118.4 (C-7), 76.7 (C-2), 76.5 (C-4), 76.2 (C-3), 73.2 (C-5), 72.6 (C-6), 72.4 (C-1), 51.7 (C-8), 48.2 (C-13), 45.5 (C-12), 45.4 (C-11), 29.7 (C-9), 27.2 (C-10), 25.8 (C(15)CH_3), 20.6 (C(13)*C*H_3), 20.4 (C(8)*C*H_3), 18.2 (C-15), 10.0 (C(13)*C*H_3), –4.3 (Si(14)*C*H_3), –4.5 (Si(14)*C*H_3); $\bar{\nu}_{max}$(solid)/cm^{-1} 3363 (O-H) (br), 2930 (m), 1473 (w), 1389 (w), 1250 (Si-CH_3) (m), 1119 (s), 1041 (Si-O) (s), 1001 (s), 968 (m), 907 (m), 863 (s), 836 (s), 776 (s), 735 (m), 668 (m); LRMS *m/z* (ESI$^+$) 429.3 ([M+H]$^+$, 100%); HRMS *m/z* (ESI$^+$) found 429.26546 [M+H]$^+$ ($C_{22}H_{41}O_6Si$ requires 429.26669 [M+H]$^+$).

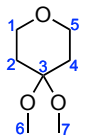
(–)-1D-1-*O*-(*tert*-Butyldimethylsilyl)-2,3-*O*-(D-1,7,7-trimethyl[2.2.1]bicyclohept-2-ylidene)-4-*O*-(trimethylacetyl)-*myo*-inositol (–)-196



Triol (–)-**195** (5.00 g, 11.7 mmol, 1.0 eq) was dissolved in anhydrous pyridine (20 mL) and PivCl (1.57 mL, 12.8 mmol, 1.1 eq) was added at –10 °C. After 13 hours of stirring at this temperature the mixture was quenched by addition of H_2O (20 mL), extracted with EtOAc (4 × 15 mL), then washed with H_2O (20 mL) and brine (2 × 20 mL). The combined organic layers were dried ($MgSO_4$), filtered,

and concentrated *in vacuo*. The residue was purified by column chromatography over silica gel ($CHCl_3$/Et_2O 9:1) to give the diol (–)-**196** (3.61 g, 60%) as a colourless, amorphous foam: R_f 0.39 (Hexane/Acetone 8:2); $[\alpha]_D^{20} = -13.2$ (*c* 1.0, $CHCl_3$); m.p. 69–71 °C (pyridine); ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 4.68 (1H, dd, *J* 8.2, 8.2 H-4), 4.24 (1H, dd, *J* 6.3, 3.8, H-1), 3.92 (1H, dd, *J* 6.4, 6.3, H-2), 3.88–3.77 (3H, m, H-3, H-6 and H-5), 2.47 (1H, br s, C(5)O*H*), 2.26 (1H, br s, C(6)O*H*), 1.99–1.89 (2H, m, H_{eq}-9 and H_{eq}-12), 1.75–1.66 (2H, m, H-11 and H_{eq}-10), 1.46 (1H, d, *J* 12.8, H_{ax}-12), 1.40–1.34 (1H, m, H_{ax}-9), 1.25–1.21 (10H, m, C(17)CH_3 and H_{ax}-10), 0.99 (3H, s, C(8)CH_3), 0.90 (9H, s, C(15)CH_3), 0.87 (3H, s, C(13)CH_3), 0.86 (3H, s, C(13)CH_3), 0.12 (3H, s, Si(14)CH_3), 0.11 (3H, s, Si(14)CH_3); ^{13}C NMR (101 MHz; $CDCl_3$) δ_C 179.3 (C-16), 118.0 (C-7), 76.8 (C-4), 76.2 (C-2), 75.6 (C-1), 73.9 (C-5), 71.7 (C-6), 71.6 (C-3), 51.6 (C-8), 48.1 (C-13), 45.3 (C-11), 44.5 (C-12), 39.1 (C-17), 29.8 (C-9), 27.3 (C(17)*C*H$_3$), 27.2 (C-10), 25.8 (C(15)*C*H$_3$), 20.5 (C(13)*C*H$_3$), 20.4 (C(8)*C*H$_3$), 18.2 (C-15), 10.1 (C(13)*C*H$_3$), –4.3 (Si(14)*C*H$_3$), –4.6 (Si(14)*C*H$_3$); $\bar{\nu}_{max}$(solid)/cm^{-1} 3491 (O-H) (br), 2931 (m), 1714 (C=O) (m), 1479 (w), 1391 (w), 1287 (m), 1252 (Si-CH_3) (m), 1173 (s), 1111 (Si-O) (s), 1037 (m), 970 (m), 898 (m), 837 (s), 777 (s), 669 (w); LRMS *m/z* (ESI$^+$) 513.3 ([M+H]$^+$, 100%); HRMS *m/z* (ESI$^+$) found 513.32272 [M+H]$^+$ ($C_{27}H_{49}O_7Si$ requires 513.32421 [M+H]$^+$).

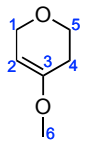
4,4-Dimethoxytetrahydro-2*H*-pyran 199



A 1.0 M solution of $TiCl_4$ in anhydrous CH_2Cl_2 (20 μL, 0.0200 mmol, 0.010 eq) was added to a stirred solution of tetrahydro-4*H*-pyran-4-one **198** (200 mg, 2.00 mmol, 1.0 eq) in MeOH (4.0 mL) at RT. After 10 minutes, Et_3N (33 μL, 0.240 mmol, 0.12 eq) was added and the resulting solution was stirred for an additional hour. The reaction was quenched with H_2O (2 mL) and the aqueous layer was then extracted with Et_2O (4 × 4 mL). The combined organic layers were washed with H_2O (2 mL), dried ($MgSO_4$), filtered, and concentrated *in vacuo* at RT to give **199** (239 mg, 82%) as an

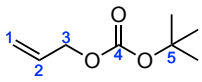
orange oil. Analysis by ^{1}H NMR showed that the product was 98% pure and this compound was used in the subsequent step without further purification: R_f 0.47 (Hexane/EtOAc 5:5); ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 3.67 (4H, t, *J* 5.2, H-1 and H-5), 3.19 (3H, s, H-6 or H-7), 3.18 (3H, s, H-6 or H-7), 1.76 (4H, t, *J* 5.2, H-2 and H-4); LRMS *m/z* (ESI$^+$) 169.1 ([M+Na]$^+$, 100%). These data are in good agreement with the literature values.[252]

4-Methoxy-3,6-dihydro-2*H*-pyran 197



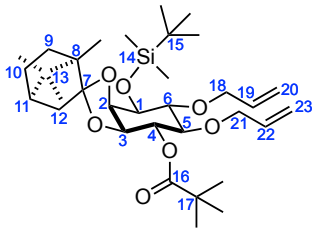
$TiCl_4$ (160 μL, 1.51 mmol, 1.1 eq) was added dropwise to a solution of **199** (200 mg, 1.37 mmol, 1.0 eq) in anhydrous CH_2Cl_2 (2.0 mL) at –78 °C. After 1 hour, anhydrous pyridine (550 μL, 6.77 mmol, 5.0 eq) was added followed by ground KOH (430 mg, 7.66 mmol, 5.6 eq, dried under vacuum at 100 °C for 14 hours). After 20 minutes the reaction was warmed to RT and was stirred for a further hour. In the case when TLC analysis indicated the presence of tetrahydro-4*H*-pyran-4-one [R_f 0.29 (Petroleum ether/EtOAc 5:5)], more KOH (77 mg, 1.37 mmol, 1.0 eq) was added and the reaction mixture was stirred until a single spot was observed. The reaction mixture was then poured into Et_2O (4 mL), filtered through Celite® and washed with Et_2O (3 × 2 mL). The filtrate was washed with H_2O (2 mL) and brine (2 mL), dried ($MgSO_4$), filtered, and concentrated *in vacuo* at RT. Et_3N (20 μL) was added and the residue was purified by column chromatography (Hexane/Et_2O 5:5, Et_3N 0.02%) and was subsequently washed with an aqueous solution of $CuSO_4$ (10% *w/v*, 2 × 2 mL) to yield **197** (128 mg, 82%) as a yellow oil: R_f 0.78 (Hexane/EtOAc 5:5); ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 4.62–4.55 (1H, m, H-2), 4.20 (2H, q, *J* 2.4, H-1), 3.83 (2H, t, *J* 5.6, H-5), 3.54 (3H, s, H-6), 2.24–2.13 (2H, m, H-4); LRMS *m/z* (ESI$^+$) 115.1 ([M+H]$^+$, 100%). These data are in good agreement with the literature values.[252]

Allyl *tert*-butyl carbonate 207



To a flask fitted with a condenser and a calcium chloride drying tube were added di-*tert*-butyl-dicarbonate **206** (5.43 mL, 22.9 mmol, 1.0 eq) and anhydrous allyl alcohol (4.98 mL, 73.3 mmol, 3.2 eq). 4-DMAP (138 mg, 1.14 mmol, 0.050 eq) was then added in one portion. After 3 hours of stirring at RT, the crude mixture obtained was purified by column chromatography over silica gel (Hexane/EtOAc 9.5:0.5) to give **207** (3.62 g, 83%) as a colourless oil: R_f 0.40 (Hexane/EtOAc 9.5:0.5); ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 5.99–5.87 (1H, m, H-2), 5.37–5.30 (1H, m, H-1), 5.27–5.22 (1H, m, H-1'), 4.57–4.53 (2H, m, H-3), 1.49 (9H, s, C(5)CH_3); LRMS *m/z* (ESI$^+$) 181.2 ([M+Na]$^+$, 26%), 197.2 ([M+K]$^+$, 100%). These data are in agreement with the literature values.[261,440]

(–)-1D-1-*O*-(*tert*-Butyldimethylsilyl)-2,3-*O*-(D-1,7,7-trimethyl[2.2.1]bicyclohept-2-ylidene)-4-*O*-(trimethylacetyl)-5,6-di-*O*-allyl-*myo*-inositol (–)-204



Method 1 A solution of the diol (–)-**196** (100 mg, 0.195 mmol, 1.0 eq) in anhydrous DMF (1.0 ml) was cooled to 0 °C. NaH (17 mg, 0.429 mmol, 2.2 eq, 60% dispersion in mineral oil) was added portionwise and the reaction mixture was stirred at 0 °C for 30 minutes. Allyl bromide (51 μL, 0.585 mmol, 3.0 eq) was added dropwise at 0 °C. The reaction mixture was then warmed to RT and stirred for a further 72 hours. After quenching with H_2O (1 mL), the aqueous phase was extracted with EtOAc (3 × 5 mL). The combined organic layers were washed with H_2O (3 × 5 mL) and brine (3 × 5 mL), dried ($MgSO_4$), filtered, and concentrated *in vacuo*. The residue obtained was purified by column chromatography over silica gel (Petroleum ether/Et_2O 10:0, 9.5:0.5) to give a mixture of

the product (–)-**204** and the inseparable side product **205** (14 mg) as a colourless oil, in a ratio of 12.8:1.0 as determined by ^{1}H NMR. The side products could only be separated in the subsequent step.

Method 2 A solution of diol (–)-**196** (600 mg, 1.17 mmol, 1.0 eq) in anhydrous DMF (3.0 mL) was added dropwise *via* cannula, over 15 minutes, to a suspension of NaH (103 mg, 2.57 mmol, 2.2 eq, 60% dispersion in mineral oil) in anhydrous DMF (3.0 mL) at 0 °C. The mixture was stirred for 1 hour at 0 °C, warmed to RT and stirred for a further 30 minutes. The mixture was then cooled to 0 °C and allyl bromide (304 μL, 3.51 mmol, 3.0 eq) was added dropwise over 10 minutes. The mixture was allowed to warm to RT and stirred for a further 13 hours. After quenching with H_2O (5 mL), the aqueous phase was extracted with EtOAc (3 × 10 mL). The combined organic layers were washed with H_2O (3 × 10 mL) and brine (3 × 10 mL), dried ($MgSO_4$), filtered, and concentrated *in vacuo*. The residue was purified by column chromatography over silica gel (Hexane/Et_2O 10:0, 9.5:0.5) to give a mixture of the desired product (–)-**204** and an inseparable side product **205** (305 mg) as a colourless oil, in a ratio of 2.9:1.0 as determined by ^{1}H NMR. This side product could only be separated in the subsequent step.

Method 3 Diol (–)-**196** (50 mg, 0.0975 mmol, 1.0 eq) and allyl bromide (17 μL, 0.195 mmol, 2.0 eq) were dissolved in anhydrous DMF (0.20 mL) and the solution was cooled to 0 °C. NaH (8 mg, 0.205 mmol, 2.1 eq, 60% dispersion in mineral oil) was added and the reaction mixture was stirred at 0 °C for 30 minutes. The reaction was then warmed to RT, stirred for a further 20 hours, and quenched by addition of a saturated aqueous solution of NH_4Cl (1.0 mL). The aqueous phase was extracted with EtOAc (3 × 1 mL), and the combined organic layers were washed with H_2O (3 × 1 mL), and brine (3 × 1 mL), dried ($MgSO_4$), filtered, and concentrated *in vacuo*. The residue was purified by column chromatography over silica gel (Hexane/Et_2O 10:0, 9.5:0.5) to give (–)-**204** (3 mg, 5%) as a colourless oil and a mixture of side products: R_f 0.80 (Petroleum ether/EtOAc 8:2); $[\alpha]_D^{20}$ = –1.8 (*c* 0.49, $CHCl_3$); ^{1}H NMR (500 MHz; $CDCl_3$) δ_H 5.92–5.77 (2H, m, H-19 and H-22), 5.26–5.16 (2H, m, H-20 and H-23), 5.14–5.07 (2H, m, H-20' and H-23'), 4.87 (1H, dd, *J* 7.8, 6.9, H-4), 4.30–4.24 (1H, m, H-21), 4.23–4.16 (2H, m, H-1 and H-18), 4.16–4.07 (2H, m, H-21' and

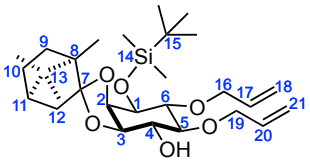
H-18'), 4.00 (1H, dd, *J* 8.4, 4.1, H-2), 3.97 (1H, dd, *J* 6.6, 6.6, H-6), 3.65–3.56 (2H, m, H-5 and H-3), 1.97–1.89 (2H, m, H_{eq}-9 and H_{eq}-12), 1.76–1.66 (2H, m, H-11 and H_{eq}-10), 1.43 (1H, d, *J* 12.8, H_{ax}-12), 1.45–1.34 (1H, m, H_{ax}-9), 1.25–1.17 (10H, m, C(17)CH_3 and H_{ax}-10), 1.01 (3H, s, C(8)CH_3), 0.92 (3H, s, C(13)CH_3), 0.90 (9H, s, C(15)CH_3), 0.86 (3H, s, C(13)CH_3), 0.09 (3H, s, Si(14)CH_3), 0.08 (3H, s, Si(14)CH_3); ^{13}C NMR (126 MHz; $CDCl_3$) δ_C 177.7 (C-16), 135.1, 135.0 (C-19, C-22), 117.4 (C-7), 116.68, 116.67 (C-20, C-23), 79.8 (C-5), 78.9 (C-3), 75.9 (C-1), 75.3 (C-6), 74.7 (C-4), 73.2 (C-18), 72.0 (C-21), 70.6 (C-2), 51.6 (C-13), 48.0 (C-8), 45.3 (C-11), 44.1 (C-12), 38.8 (C-17), 30.0 (C-9), 27.3 (C(17)*C*H_3), 27.2 (C-10), 26.0 (C(15)*C*H_3), 20.53 (C(8)*C*H_3), 20.49 (C(13)*C*H_3), 18.2 (C-15), 10.5 (C(13)*C*H_3), –4.6 (Si(14)*C*H_3), –4.7 (Si(14)*C*H_3); $\bar{\nu}_{max}$(thin film)/cm^{-1} 2953 (w), 2927(w), 2890 (w), 2855 (w), 1734 (C=O) (m), 1476 (w), 1460 (w), 1281 (w), 1249 (Si-CH_3) (w), 1183 (m), 1167 (m), 1151 (m), 1124 (s), 1110 (Si-O) (s), 1095 (m), 1086 (m), 1039 (m), 984 (m), 969 (m), 940 (m), 919 (m), 863 (s), 837 (s), 776 (s); LRMS *m/z* (ESI$^+$) 615.4 ([M+Na]$^+$, 100%); HRMS *m/z* (ESI$^+$) found 593.38598 [M+H]$^+$ ($C_{33}H_{57}O_7Si$ requires 593.38736 [M+H]$^+$).

Method 4 Diol (–)-**196** (100 mg, 0.195 mmol, 1.0 eq), allyl bromide (34 μL, 0.390 mmol, 2.0 eq) and TBAI (7 mg, 0.0195 mmol, 0.10 eq) were dissolved in anhydrous DMF (1.2 mL) and the solution was cooled to 0 °C. NaH (16 mg, 0.410 mmol, 2.1 eq, 60% dispersion in mineral oil) was added portionwise and the reaction mixture was stirred at 0 °C for 30 minutes. The reaction was then warmed to RT, stirred for a further 20 hours, and quenched by addition of a saturated aqueous solution of NH_4Cl (1.0 mL). The aqueous phase was extracted with EtOAc (3 × 2 mL), and the combined organic layers were washed with H_2O (3 × 2 mL) and brine (3 × 2 mL), dried ($MgSO_4$), filtered, and concentrated *in vacuo*. The residue was purified by column chromatography over silica gel (Hexane/Et_2O 10:0, 9.5:0.5) to give (–)-**204** (8 mg, 7%) as a colourless oil and a mixture of side products. The data are identical to those reported above.

Method 5 A solution of the diol (–)-**196** (200 mg, 0.390 mmol, 1.0 eq) and allylating agent **207** (308 mg, 1.95 mmol, 5.0 eq) in freshly distilled, degassed THF (22 mL) and under a flow of Ar was heated under reflux for 10 minutes. $Pd(PPh_3)_4$ (45 mg, 0.0390 mmol, 0.10 eq) was then added to the solution. After 1.5 hours, another portion of **207** (154 mg, 0.975 mmol, 2.5 eq) was added dropwise

to the reaction mixture. After 4 hours of heating at reflux, the reaction mixture was concentrated *in vacuo* and the residue obtained was purified by column chromatography over silica gel (Petroleum ether/Et_2O 10:0, 9.5:0.5, 9:1, 8.5:1.5, 8:2) to give (–)-**204** (149 mg, 64%) as a colourless oil. The data are identical to those reported above.

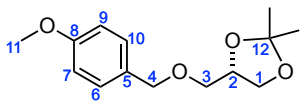
(–)-1D-1-*O*-(*tert*-Butyldimethylsilyl)-2,3-*O*-(D-1,7,7-trimethyl[2.2.1]bicyclohept-2-ylidene)-5,6-di-*O*-allyl-*myo*-inositol (–)-210



DIBAL-H (423 μL, 0.423 mmol, 2.5 eq, 1.0 M in toluene) was added dropwise to a solution of (–)-**204** (100 mg, 0.169 mmol, 1.0 eq) in anhydrous CH_2Cl_2 (0.90 mL) at –78 °C. The mixture was stirred for 4.5 hours at –78 °C. A saturated aqueous solution of Rochelle salt (3 mL) was gradually added. The mixture warmed to RT and stirred for a further 30 minutes. The aqueous phase was extracted with EtOAc (3 × 5 mL) and the combined organic layers were washed with H_2O (5 mL), and brine (5 mL), then dried ($MgSO_4$), filtered, and concentrated *in vacuo*. Purification by column chromatography over silica gel (Hexane/Et_2O 9:1) afforded (–)-**210** (79.1 mg, 92%) as a colourless oil: R_f 0.14 (Hexane/Et_2O 8:2); $[\alpha]_D^{20}$ = –0.77 (*c* 0.65, $CHCl_3$); ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 6.02–5.89 (2H, m, H-17 and H-20), 5.34–5.23 (2H, m, H-18 and H-21), 5.21–5.13 (2H, m, H-18' and H-21'), 4.43-4.35 (1H, m, H-19), 4.35–4.27 (1H, m, H-16), 4.27–4.16 (2H, m, H-16' and H-19'), 4.13 (1H, dd, *J* 5.4, 4.8, H-1), 3.90–3.82 (2H, m, H-2 and H-6), 3.53–3.33 (3H, m, H-3, H-5 and H-4), 2.54 (1H, br s, C(4)O*H*), 2.00–1.87 (2H, m, H_{eq}-9 and H_{eq}-12), 1.78–1.67 (2H, m, H-11 and H_{eq}-10), 1.46 (1H, d, *J* 12.9, H_{ax}-12), 1.44–1.34 (1H, m, H_{ax}-9), 1.33–1.18 (1H, m, H_{ax}-10), 1.01 (3H, s, C(8)CH_3), 0.91 (9H, s, C(15)CH_3), 0.88 (3H, s, C(13)CH_3), 0.87 (3H, s, C(13)CH_3), 0.11 (3H, s, Si(14)CH_3), 0.09 (3H, s, Si(14)CH_3); ^{13}C NMR (101 MHz; $CDCl_3$) δ_C 135.3, 135.2 (C-17, C-20), 117.7 (C-7), 117.3, 117.0 (C-18, C-21), 82.9, 80.7 (C-5, C-3), 77.0 (C-1), 76.1 (C-6), 74.0 (C-16), 73.4 (C-4), 72.3 (C-19), 71.4 (C-2), 51.7, 48.1 (C-8, C-13), 45.4 (C-11), 45.1 (C-12), 29.8 (C-9),

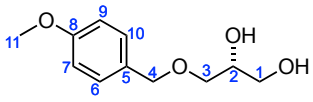
27.2 (C-10), 25.9 (C(15)*C*H$_3$), 20.6 (C(13)*C*H$_3$), 20.5 (C(8)*C*H$_3$), 18.1 (C-15), 10.4 (C(13)*C*H$_3$), –4.4, –4.6 (Si(14)*C*H$_3$); $\bar{\nu}_{max}$(thin film)/cm^{-1} 3417 (O-H) (br), 3015 (m), 2933 (m), 2857 (m), 1474 (w), 1461 (w), 1359 (w), 1311 (w), 1249 (Si-CH$_3$) (m), 1205 (w), 1118 (s), 1107 (Si-O) (s), 1081 (m), 1045 (s), 999 (m), 967 (m), 920 (m), 860 (m), 837 (s), 774 (s); LRMS *m/z* (ESI$^+$) 509.3 ([M+H]$^+$, 100%); HRMS *m/z* (ESI$^+$) found 509.32783 [M+H]$^+$ (C$_{28}$H$_{49}$O$_6$Si requires 509.32929 [M+H]$^+$).

(*S*)-(+)-1,2-*O*-Isopropylidene-3-*O*-(4-methoxybenzyl)-*sn*-glycerol (+)-213



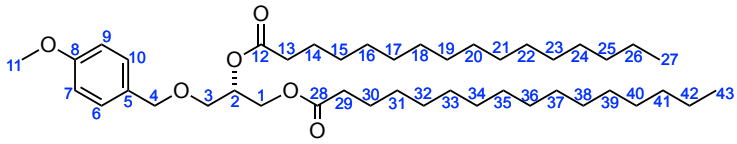
(*S*)-(+)-1,2-Isopropylidene-*sn*-glycerol (+)-**212** (935 μL, 7.56 mmol, 1.0 eq) was added dropwise to a suspension of NaH (480 mg, 12.0 mmol, 1.6 eq, 60% dispersion in mineral oil) in anhydrous DMF (5.0 mL) and at 0 °C. The solution obtained was warmed to RT, stirred for 1 hour, then cooled to 0 °C. PMBCl (1.53 mL, 11.0 mmol, 1.5 eq) was added dropwise, and the solution was warmed to RT and stirred for a further 14 hours. The reaction was then quenched by addition of H$_2$O (10 mL) and the aqueous layer was extracted with Et$_2$O (3 × 15 mL). The combined organic layers were washed with H$_2$O (3 × 15 mL), then brine (2 × 15 mL), dried (MgSO$_4$), filtered, and concentrated *in vacuo*. Purification by column chromatography over silica gel (Petroleum ether/EtOAc 8:2) afforded (+)-**213** (1.87 g, 98%) as a colourless oil: R$_f$ 2.3 (Petroleum ether/EtOAc 8:2); $[\alpha]_D^{20}$ = +21.1 (*c* 1.0, CHCl$_3$) [lit.[441] $[\alpha]_D^{21}$ = +21.8 (*c* 1.0, CHCl$_3$)]; ^{1}H NMR (200 MHz; CDCl$_3$) δ_H 7.32–7.20 (2H, m, H-6 and H-10), 6.92–6.83 (2H, m, H-7 and H-9), 4.54 (1H, d, J_{AB} 11.7, H-4), 4.47 (1H, d, J_{AB} 11.7, H-4'), 4.35–4.21 (1H, m, H-2), 4.04 (1H, dd, J_{XY} 8.2, 6.4, H-3), 3.81 (3H, s, H-11), 3.72 (1H, dd, J_{XY} 8.2, 6.4, H-3'), 3.53 (1H, dd, J_{XY} 9.7, 5.7, H-1), 3.43 (1H, dd, J_{XY} 9.7, 6.3, H-1'), 1.42 (3H, s, C(12)C*H*$_3$), 1.36 (3H, s, C(12)C*H*$_3$); LRMS *m/z* (ESI$^+$) 275.0 ([M+Na]$^+$, 100%). These data are in good agreement with the literature values.[69,441]

(*R*)-(−)-3-*O*-(4-Methoxybenzyl)-*sn*-glycerol (−)-214



PTSA·H_2O (500 mg, 2.62 mmol, 0.22 eq) was added to solution of acetal (+)-**213** (3.00 g, 11.9 mmol, 1.0 eq) in MeOH (83 mL). After stirring at RT for 2 hours the reaction was neutralised with Et_3N (360 μL, 2.62 mmol, 0.22 eq) and the solvent was removed *in vacuo*. Purification by column chromatography over silica gel (Hexane/EtOAc 4:6, 2:8) gave the diol (−)-**214** (6.80 g, 81%) as a colourless solid: R_f 0.35 (EtOAc); $[\alpha]_D^{20}$ = −2.2 (*c* 2.0, $CHCl_3$) [lit.[69] $[\alpha]_D^{22}$ = −1.1 (*c* 3.04, $CHCl_3$)]; m.p. 40–41 °C (EtOAc) [lit.[69] 41–43 °C (EtOAc)]; ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 7.27–7.22 (2H, m, H-6 and H-10), 6.91–6.87 (2H, m, H-7 and H-9), 4.49 (2H, s, H-4), 3.92–3.84 (1H, m, H-2), 3.81 (3H, s, H-11), 3.71 (1H, dd, J_{XY} 11.4, 3.9, H-1), 3.63 (1H, dd, J_{XY} 11.4, 5.4, H-1'), 3.56 (1H, dd, J_{XY} 9.6, 3.9, H-3), 3.52 (1H, dd, J_{XY} 9.6, 6.2, H-3'); LRMS *m/z* (ESI$^+$) 235.1 ([M+Na]$^+$, 100%). These data are in good agreement with the literature values.[69]

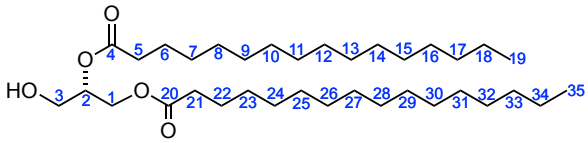
(*S*)-(+)-1,2-Di-*O*-palmitoyl-3-*O*-(4-methoxybenzyl)-*sn*-glycerol (+)-215



To a stirred solution of the protected diol (−)-**214** (200 mg, 0.942 mmol, 1.0 eq) in anhydrous CH_2Cl_2 (4.0 mL) was added anhydrous pyridine (190 μL, 2.36 mmol, 2.5 eq) and 4-DMAP (6 mg, 0.0470 mmol, 0.050 eq). The solution was cooled to 0 °C and palmitoyl chloride (629 μL, 2.07 mmol, 2.2 eq) was added dropwise over 10 minutes. After stirring for 15 minutes at this temperature, the solution was allowed to warm to RT and was stirred for a further 14 hours. The reaction was quenched with H_2O (2 mL). The aqueous phase was extracted with CH_2Cl_2 (4 × 4 mL) and the combined organic layers were washed successively with a 2.0 M aqueous solution of HCl (2.5 mL), H_2O (3 mL) and brine (3 mL). The combined organic layers were then dried ($MgSO_4$), filtered, and

concentrated *in vacuo*. Purification by column chromatography over silica gel (Petroleum ether/CH_2Cl_2 5:5) afforded (+)-**215** (592 mg, 91%) as a colourless solid: R_f 0.78 (EtOAc); $[\alpha]_D^{20}$ = +10.2 (*c* 2.0, $CHCl_3$) [lit.[442] $[\alpha]_D^{27}$ = +11.3 (*c* 6.8, $CHCl_3$)]; m.p. 62–63 °C (CH_2Cl_2) [lit.[442] 63.5–64 °C]; ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 7.25–7.19 (2H, m, H-6 and H-10), 6.89–6.83 (2H, m, H-7 and H-9), 5.26–5.18 (1H, m, H-2), 4.48 (1H, d, J_{AB} 11.7, H-4), 4.44 (1H, d, J_{AB} 11.7, H-4'), 4.33 (1H, dd, J_{XY} 11.9, 3.6, H-1), 4.17 (1H, dd, J_{XY} 11.9, 6.4, H-1'), 3.79 (3H, s, H-11), 3.59–3.51 (2H, m, H-3), 2.34–2.23 (4H, m, H-13 and H-29), 1.59–1.45 (4H, m, H-14 and H-30), 1.26 (48H, br s, H-15 to H-26 and H-31 to H-42), 0.88 (6H, t, *J* 6.5, H-27 and H-43); ^{13}C NMR (101 MHz; $CDCl_3$) δ_C 173.6, 173.3 (C-12, C-28), 159.5 (C-8) 130.0 (C-5), 129.4 (C-6, C-10), 114.0 (C-7, C-9), 73.1 (C-4), 70.2 (C-2), 68.1 (C-3), 62.8 (C-1), 55.4 (C-11), 34.5, 34.3 (C-13, C-29), 32.1 (C-25, C-41), 29.9–29.8, 29.7, 29.51, 29.49, 29.29, 29.26 (C-15 to C-24 and C-31 to C-40), 25.12, 25.05 (C-14, C-30), 22.8 (C-26, C-42), 14.3 (C-27, C-43); $\bar{\nu}_{max}$(thin film)/cm^{-1} 2955 (m), 2916 (s), 2849 (s), 1730 (C=O) (s), 1612 (w), 1514 (m), 1472 (m), 1443 (m), 1358 (w), 1263 (m), 1244 (m), 1197 (m), 1171 (s), 1109 (s), 1084 (m), 1030 (m), 1016 (m), 947 (w), 831 (m), 816 (m), 718 (m); HRMS *m/z* (EI/FI) found 688.5432 $[M]^+$ ($C_{43}H_{76}O_6$ requires 688.5642 $[M]^+$). These data are in good agreement with the literature values.[47,442]

(*S*)-(−)-1,2-Di-*O*-palmitoyl-*sn*-glycerol (−)-211

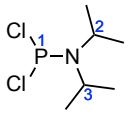


Method 1 DDQ (182 mg, 0.802 mmol, 2.05 eq) was added to a stirred solution of (+)-**215** (270 mg, 0.391 mmol, 1.0 eq) in CH_2Cl_2 (16 mL) and H_2O (1.0 mL). The reaction mixture was stirred for 14 hours at RT, then quenched with a saturated aqueous solution of $NaHCO_3$ (18 mL). The aqueous layer was extracted with CH_2Cl_2 (4 × 9 mL). The combined organic phases were washed with a saturated aqueous solution of $NaHCO_3$ (13 mL), and brine (2 × 13 mL), then dried ($MgSO_4$), filtered, and concentrated *in vacuo*. Purification by column chromatography over silica gel (Petroleum

ether/Et_2O 6:4) afforded the alcohol (–)-**211** (186 mg, 84%) as a colourless solid. Formation of the small amounts of the regioisomer (–)-**216** was observed by ^1H NMR, suggesting that concurrent racemisation may be taking place.

Method 2 To a solution of (+)-**215** (1.48 g, 2.15 mmol, 1.0 eq) in EtOAc (155 mL) under $N_{2(g)}$, was added 10% Pd/C (389 mg, 0.362 mmol, 0.17 eq). The fask was purged with 3 balloons of $H_{2(g)}$. After stirring at RT for 2 hours under $H_{2(g)}$, the reaction mixture was filtered through a pad of Celite®, washed with EtOAc (3 × 20 mL), and the combined filtrates were concentrated *in vacuo* to give the alcohol (–)-**211** (1.17 g, 96%) as a colourless solid: R_f 0.71 (Hexane/EtOAc 6:4); $[\alpha]_D^{20}$ = –2.4 (*c* 1.0, $CHCl_3$) [lit.[443] $[\alpha]_D^{25}$ = –2.6 (*c* 8.0, $CHCl_3$); lit.[442] $[\alpha]_D^{25}$ = –2.69 (*c* 3.9, $CHCl_3$);]; m.p. 67–68 °C (Pentane/EtOAc) [lit.[443] 67–69 °C (Hexane/Et_2O); lit.[442] 66–67 °C (Pentane/EtOAc)]; ^1H NMR (400 MHz; $CDCl_3$) δ_H 5.12–5.05 (1H, m, H-2), 4.32 (1H, dd, J_{XY} 11.9, 5.1, H-1), 4.23 (1H, dd, J_{XY} 11.9, 5.1, H-1'), 3.76–3.70 (2H, m, H-3), 2.34 (2H, t, *J* 7.5, H-5 or H-21), 2.32 (2H, t, *J* 7.5, H-5 or H-21), 2.06 (1H, t, *J* 6.3, C(3)O*H*), 1.63–1.57 (4H, m, H-6 and H-22), 1.26 (48H, br s, H-7 to H-18 and H-23 to H-34), 0.88 (6H, t, *J* 6.6, H-19 and H-35); LRMS *m/z* (ESI^+) 551.1 ($[M–OH]^+$, 100%). These data are in good agreement with the literature values.[443]

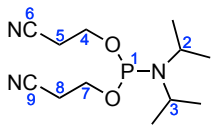
Dichloro(diisopropylamino)phosphine 220



DIPA (64.3 mL, 0.458 mol, 2.0 eq) was added dropwise to a solution of PCl_3 **219** (20.0 mL, 0.229 mol, 1.0 eq) in anhydrous Et_2O (400 mL), over 45 minutes and at 0 °C. After a further 15 minutes stirring at 0 °C, a colourless precipitate formed. The reaction mixture was diluted with Et_2O (200 mL) and stirred at RT for 2 hours. The precipitate (DIPA hydrochloride salt) was removed by Schlenk filtration and washed with Et_2O (3 × 100 mL). The combined filtrates were then concentrated *in vacuo* to give **220** (32.3 mL, 77%) as a colourless oil. Analysis by ^{31}P NMR showed that the product was ~90% pure and this phosphoramidite was therefore used in subsequent steps

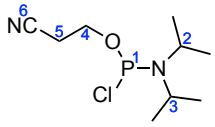
without further purification. The product was stored under Ar at –20 °C and was checked by ^{31}P NMR before each use. In the case when impurities were observed by ^{31}P NMR, the product was purified by vacuum distillation (b.p. 41–43 °C, 0.13 mbar); ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 3.93 (2H, sept, *J* 6.6, H-2 and H-3), 1.28 (12H, d, *J* 6.6, C(2)C*H*$_3$ and C(3)C*H*$_3$); ^{31}P NMR (162 MHz; $CDCl_3$) δ_P 169.6 (P-1); LRMS *m/z* (ESI$^+$) 239.3 ([M+K]$^+$, 100%). These data are in good agreement with the literature values.[444,445]

Bis-(2-cyanoethyoxy)-*N*,*N*-diisopropylphosphoramidite 218



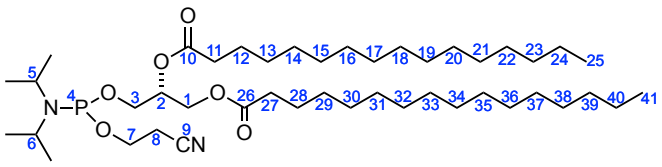
To a solution of 3-hydroxypropionitrile (270 μL, 3.97 mmol, 2.0 eq) and distilled DIPEA (1.04 mL, 5.94 mmol, 3.0 eq) in anhydrous CH_2Cl_2 (2.0 mL) and at 0 °C, was added **220** (0.365 mL, 1.98 mmol, 1.0 eq) dropwise over 5 minutes. The reaction mixture was warmed to RT and stirred for a further 10 minutes. The solvent was removed *in vacuo* until a thick oil was obtained (if too much solvent was removed, the solid obtained could not be easily loaded onto the column). This oil was quickly purified by column chromatography over silica gel (Petroleum ether/Et_2O 6:4, 5:5) to give **218** (371 mg, 69%) as a colourless oil. The product was synthesised fresh before each use: R_f 0.49 (Petroleum ether/Et_2O 7:3); ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 3.95–3.77 (4H, m, H-4 and H-7), 3.70–3.55 (2H, m, H-2 and H-3), 2.65 (4H, t, *J* 6.3, H-5 and H-8), 1.19 (12H, d, *J* 6.8, C(2)C*H*$_3$ and C(3)C*H*$_3$); ^{31}P NMR (162 MHz; $CDCl_3$) δ_P 149.2 (P-1); LRMS *m/z* (ESI$^+$) 294.2 ([M+Na]$^+$, 100%). These data are in good agreement with the literature values.[446]

Chloro-(2-cyanoethyl)-*N*,*N*-diisopropylphosphoramidite 222



To a solution of PCl_3 (26.3 mL, 0.301 mol, 5.0 eq) in anhydrous MeCN (30 mL) was added 3-hydroxypropionitrile (4.11 mL, 0.0602 mol, 1.0 eq) dropwise. After stirring at RT for 1 hour the cloudy reaction mixture was concentrated *in vacuo* to give **221**. The resulting oil was re-dissolved in anhydrous Et_2O (45 mL) and DIPA (16.9 mL, 0.120 mol, 2.0 eq) was added dropwise at –10 °C over 10 minutes, to give a colourless precipitate. The reaction was warmed to RT. After stirring for 18 hours, the precipitate was removed by Schlenk filtration and washed with Et_2O (3 × 100 mL). The combined filtrates were concentrated *in vacuo* to give a yellow oil which was distilled by vacuum distillation (b.p. 114–116 °C, 0.2 mbar) yielding **222** (7.80 g, 55%) as a colourless oil. The product was stored under Ar at –20 °C and was checked by ^{31}P NMR before each use: ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 4.09–3.99 (2H, m, H-4), 3.86–3.72 (2H, m, H-2 and H-3), 2.78–2.69 (2H, m, H-5), 1.32–1.20 (12H, m, C(2)CH_3 and C(3)CH_3); ^{31}P NMR (126 MHz; $CDCl_3$) δ_P 180.0 (P-1). These data are in good agreement with the literature values.[447]

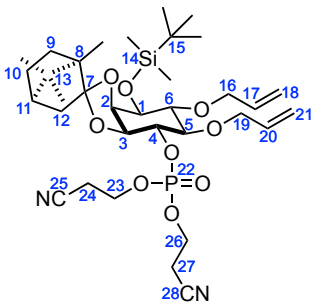
(*R*)-(+)-(2-Cyanoethyl)-*N*,*N*-diisopropyl-(1,2-di-*O*-palmitoyl-*sn*-glycer-3-yl)phosphoramidite (–)-193



DIPEA (100 μL, 0.576 mmol, 4.0 eq) and **222** (64.0 μL, 0.288 mmol, 2.0 eq) were added to a solution of (–)-**211** (82 mg, 0.144 mmol, 1.0 eq) in CH_2Cl_2 (1.0 mL). After 1 hour of stirring at RT the solvent was concentrated *in vacuo* until a thick oil was obtained. The residue was then purified by column chromatography over silica gel (Petroleum ether/EtOAc 8:2) to give (–)-**193** (75 mg, 68%) as a

colourless oil and containing a mixture of two diastereoisomers. This phosphoramidite was synthesised fresh before each use: R_f 0.67 (Petroleum ether/EtOAc 8:2); ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 5.24–5.15 (2H, m, H-2 Diast. A and B), 4.29 (1H, dd, J_{XY} 11.5, 3.5, H-1 Diast. A), 4.25 (1H, dd, J_{XY} 11.7, 3.5, H-1 Diast. B), 4.11 (1H, dd, J_{XY} 11.5, 6.2, H-1' Diast. A), 4.08 (1H, dd, J_{XY} 11.7, 6.5, H-1' Diast. B), 3.89–3.75 (6H, m, H-3 and H-7 Diast. A and B), 3.74–3.66 (2H, m, H-3' Diast. A and B), 3.64–3.53 (4H, m, H-5 and H-6 Diast. A and B), 2.63 (4H, t, *J* 6.4, H-8 Diast. A and B), 2.34–2.28 (8H, m, H-11 and H-27 Diast. A and B), 1.66–1.56 (8H, m, H-12 and H-28 Diast. A and B), 1.29–1.24 (96H, m, H-13 to H-24 and H-29 to H-40 Diast. A and B), 1.20–1.16 (24H, m, C(5)CH_3 and C(6)CH_3 Diast. A and B), 0.81 (12H, m, H-25 and H-41 Diast. A and B); ^{31}P NMR (126 MHz; $CDCl_3$) δ_P 149.6 (P-4, Diast. A), 149.5 (P-4, Diast. B); LRMS *m/z* (ESI$^+$) 807.6 ([M+Na]$^+$, 100%). These data are in good agreement with the literature values.[448]

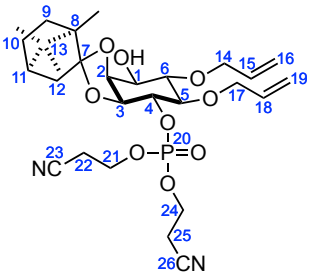
(–)-1D-1-*O*-(*tert*-Butyldimethylsilyl)-2,3-*O*-(D-1,7,7-trimethyl[2.2.1]bicyclohept-2-ylidene)-4-*O*-(bis(2-cyanoethyl)phosphate)-5,6-di-*O*-allyl-*myo*-inositol (–)-226



A solution of phosphoramidite **218** (67.0 mg, 0.248 mmol, 2.8 eq) and 1*H*-tetrazole (550 μL, 0.248 mmol, 2.8 eq, 0.45 M in MeCN) in anhydrous CH_2Cl_2 was stirred at RT for 30 minutes. Alcohol (–)-**210** (45.0 mg, 0.0885 mmol, 1.0 eq) in CH_2Cl_2 was added dropwise to the cloudy reaction mixture and the resulting solution was stirred at RT for 14 hours. The reaction mixture was then cooled to –78 °C and *m*CPBA (55.6 mg, 0.248 mmol, 2.8 eq, 77%) was added in a single portion. The mixture was stirred for 2 hours at –78 °C, warmed to RT, and stirred for a further 12 hours. After quenching with a saturated aqueous solution of $NaHSO_3$ (5 mL) the aqueous layer was extracted with CH_2Cl_2 (3 × 5 mL). The combined organic layers were washed with a saturated aqueous solution of $NaHSO_3$

(2 × 5 mL), and brine (5 mL), then dried ($MgSO_4$) filtered, and concentrated *in vacuo*. Purification by column chromatography over silica gel (Hexane/EtOAc 7:3) gave (–)-**226** (47.0 mg, 82%) as a colourless solid: R_f 0.28 (Hexane/EtOAc 7:3); $[\alpha]_D^{20}$ = –4.4 (*c* 1.16, $CHCl_3$); m.p. 93–94 °C (Et_2O); ^{1}H NMR (500 MHz; $CDCl_3$) δ_H 6.00–5.85 (2H, m, H-17 and H-20), 5.35–5.25 (2H, m, H-18 and H-21), 5.20–5.13 (2H, m, H-18' and H-21'), 4.44–4.38 (1H, m, H-16 or H-19), 4.35–4.11 (9H, m, H-16 or H-19, H-16', H-19', H-23, H-26, H-4 and H-1) 3.96–3.88 (2H, m, H-2 and H-6), 3.66–3.59 (2H, m, H-3 and H-5), 2.81–2.70 (4H, m, H-24 and H-27), 1.94–1.86 (2H, m, H_{eq}-9 and H_{eq}-12), 1.78–1.67 (2H, m, H-11 and H_{eq}-10), 1.44 (1H, d, *J* 12.7, H_{ax}-12), 1.46–1.36 (1H, m, H_{ax}-9), 1.33–1.17 (1H, m, H_{ax}-10), 1.00 (3H, s, C(8)CH_3), 0.90 (9H, s, C(15)CH_3), 0.88 (3H, s, C(13)CH_3), 0.87 (3H, s, C(13)CH_3), 0.10 (6H, s, Si(14)CH_3); ^{13}C NMR (126 MHz; $CDCl_3$) δ_C 135.1, 135.0 (C-17, C-20), 118.0 (C-7), 117.0, 116.4 (C-18, C-21), 116.63, 116.58 (C-25, C-28), 81.1 (d_P, J_P 3.5, C-3), 80.5 (d_P, J_P 6.9, C-4), 78.7 (C-5), 76.4 (C-1), 75.6 (C-6), 73.5, 71.8 (C-16, C-19), 70.8 (C-2), 62.2 (d_P, J_P 1.9, C-23 or C-26), 62.2 (d_P, J_P 1.9, C-23 or C-26), 51.7 (C-13), 48.1 (C-8), 45.3 (C-11), 44.7 (C-12), 29.9 (C-9), 27.2 (C-10), 25.8 (C(15)*C*H_3), 20.5 (C(13)*C*H_3), 20.4 (C(8)*C*H_3), 19.74, 19.68 (C-24, C-27), 18.1 (C-15), 10.4 (C(13)*C*H_3), –4.4, –4.7 (Si(14)*C*H_3); ^{31}P NMR (162 MHz; $CDCl_3$) δ_P –2.48 (P-22); $\bar{\nu}_{max}$(solid)/cm^{-1} 2956 (w), 2886 (w), 2255 (C≡N) (w), 1473 (w), 1463 (w), 1391 (w), 1259 (Si-CH_3) (m), 1118 (Si-O) (m), 1075 (m), 1035 (s), 1019 (P-O) (s), 1006 (s), 908 (s), 837 (m), 778 (m), 730 (s); LRMS *m/z* (ESI$^+$) 717.3 ([M+Na]$^+$, 100%); HRMS *m/z* (ESI$^+$) found 717.32783 [M+H]$^+$ ($C_{34}H_{55}O_9N_2NaPSi$ requires 717.33067 [M+H]$^+$).

(–)-1D-2,3-*O*-(D-1,7,7-Trimethyl[2.2.1]bicyclohept-2-ylidene)-4-*O*-(bis(2-cyanoethyl)-phosphate)-5,6-di-*O*-allyl-*myo*-inositol (–)-227

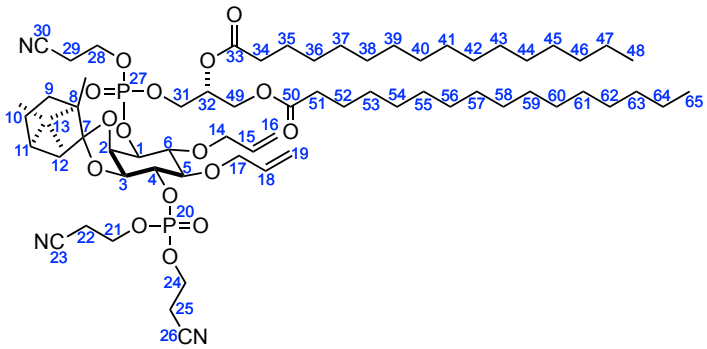


Method 1 A 1:1 (*mol*/*mol*) solution of TBAF/AcOH (18 μL, 0.0172 mmol, 1.2 eq, pH 6.5–7.0) was added to a solution of (–)-**226** (10 mg, 0.0143 mmol, 1.0 eq) in anhydrous THF (0.20 mL). After 2 hours stirring at RT, (–)-**226** could still be observed by TLC and another portion of 1:1 (*mol*/*mol*) TBAF/AcOH solution (18 μL, 0.0172 mmol, 1.2 eq, pH 6.5–7.0) was added. After 24 hours, the reaction was quenched by addition of a saturated aqueous solution of NH_4Cl (0.1 mL) and the aqueous phase was extracted with EtOAc (3 × 1 mL). The combined organic layers were washed with a saturated aqueous solution of NH_4Cl (0.5 mL), then dried ($MgSO_4$), filtered, and concentrated *in vacuo*. The residue obtained was purified by column chromatography over silica gel (Hexane/EtOAc 8:2, 10:0), but none of the desired product (–)-**227** was isolated.

Method 2 To a solution of (–)-**226** (50 mg, 0.0720 mmol, 1.0 eq) in anhydrous THF (0.60 mL) was added $Et_3N{\cdot}3HF$ (59 μL, 0.300 mmol, 6.0 eq) dropwise. The reaction mixture was heated to 40 °C for 24 hours. The solvent was concentrated using a stream of $N_{2(g)}$ and the residue obtained was purified by column chromatography over silica gel (Petroleum ether/EtOAc 5:5), to give (–)-**227** (29 mg, 72%) as a colourless oil: R_f 0.23 (Petroleum ether/EtOAc 8:2); $[\alpha]_D^{20} = -1.3$ (*c* 0.80, $CHCl_3$); ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 6.02–5.87 (2H, m, H-15 and H-18), 5.38–5.27 (2H, m, H-16 and H-19), 5.24–5.15 (2H, m, H-16' and H-19'), 4.43–4.20 (10H, m, H-1, H-4, H-21, H-24, H-14, H-17, H-14' and H-17), 4.07–4.00 (2H, m, H-2 and H-6), 3.88 (1H, dd, *J* 8.5, 7.3, H-3 or H-5), 3.78 (1H, dd, *J* 5.7, 5.7, H-3 or H-5), 2.81–2.69 (4H, m, H-22 and H-25), 2.39 (1H, d, *J* 3.4, C(1)O*H*), 2.00–1.87 (2H, m, H_{eq}-9 and H_{eq}-12), 1.82–1.69 (2H, m, H-11 and H_{eq}-10), 1.45 (1H, d, *J* 12.9, H_{ax}-12),

1.46–1.38 (1H, m, H_{ax}-9), 1.33–1.17 (1H, m, H_{ax}-10), 1.01 (3H, s, C(8)CH_3), 0.88 (6H, s, C(13)CH_3); ^{13}C NMR (101 MHz; $CDCl_3$) δ_C 135.0, 134.5 (C-15, C-18), 118.1 (C-7), 117.5, 117.2 (C-16, C-19), 116.5 (C-23, C-26), 80.9 (d_P, J_P 6.9, C-4), 80.0 (d, J_P 3.4, C-3 or C-5), 79.8 (d_P, J_P 4.4, C-3 or C-5), 75.8 (C-6), 74.5 (C-1), 72.3, 71.9 (C-14, C-17), 69.2 (C-2), 62.4 (d_P, J_P 5.1, C-21 or C-24), 62.3 (d_P, J_P 5.1, C-21 or C-24), 51.7 (C-13), 48.1 (C-8), 45.3 (C-11), 43.8 (C-12), 30.1 (C-9), 27.1 (C-10), 20.6 (C(8)*C*H$_3$), 20.3 (C(13)*C*H$_3$), 19.8, 19.7 (C-22, C-25), 10.1 (C(13)*C*H$_3$); ^{31}P NMR (162 MHz; $CDCl_3$) δ_P –2.82 (P-20); $\bar{\nu}_{max}$(solid)/cm^{-1} 3437 (O-H) (br), 2936 (w), 2876 (w), 2256 (C≡N) (w), 1475 (w), 1453 (w), 1418 (w), 1391 (w), 1371 (w), 1266 (m), 1074 (m), 1008 (m), 1003 (P-O) (s), 967 (m), 928 (m), 790 (m), 736 (m); LRMS *m/z* (ESI$^+$) 603.2 ([M+Na]$^+$, 100%); HRMS *m/z* (ESI$^+$) found 603.24233 [M+H]$^+$ ($C_{28}H_{41}O_9N_2NaP$ requires 603.24419 [M+H]$^+$).

(–)-1D-1-*O*-(1,2-Di-*O*-palmitoyl-*sn*-glycer-3-yl)-(2-cyanoethyl)phosphate)-5,6-di-*O*-allyl-2,3-*O*-(D-1,7,7-trimethyl[2.2.1]bicyclohept-2-ylidene)-4-*O*-(bis(2-cyanoethyl)phosphate)-*myo*-inositol (–)-228



Method 1 A solution of phosphoramidite (–)-**193** (79 mg, 0.103 mmol, 3.0 eq) and 1*H*-tetrazole (229 μL, 0.103 mmol, 3.0 eq, 0.45 M in MeCN) in anhydrous CH_2Cl_2 (1.8 mL) was stirred for 1 hour. A solution of the alcohol (–)-**227** (20 mg, 0.0344 mmol, 1.0 eq) in anhydrous CH_2Cl_2 (0.50 mL) was subsequently added dropwise and the reaction mixture was stirred for 24 hours at RT. *m*CPBA (23 mg, 0.103 mmol, 3.0 eq, 77%) was added at –78 °C. The reaction mixture was stirred for 1 hour, warmed to RT, and stirred for a further 16 hours. The reaction was quenched by addition of a saturated aqueous solution of $NaHSO_3$ (5 mL). The aqueous phase was extracted with CH_2Cl_2 (4 ×

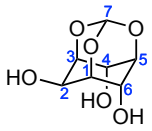
2 mL) and the combined organic layers were washed with a saturated aqueous solution of $NaHSO_3$ (2 mL), and brine (2 mL), then dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The residue obtained was purified by column chromatography over silica gel (Petroleum ether/EtOAc 7:3, 9:1, 10:0), however none of the desired product was isolated.

Method 2 To a solution of (–)-**227** (50 mg, 0.0861 mmol, 1.0 eq) and phosphoramidite (–)-**193** (340 mg, 0.431 mmol, 5.0 eq) in anhydrous CH_2Cl_2 (9.0 mL) was added 1*H*-tetrazole (957 μL, 0.431 mmol, 5.0 eq, 0.45 M in MeCN). The cloudy reaction mixture was stirred at RT for 25 hours, then cooled to –78 °C and *m*CPBA (97 mg, 0.431 mmol, 5.0 eq, 77%) was added in one portion. The mixture was stirred for 1 hour, warmed to RT and stirred for a further 16 hours. The reaction was quenched by addition of an aqueous solution of $Na_2S_2O_3$ (10% *w*/*v*, 5 mL). The aqueous phase was extracted with CH_2Cl_2 (4 × 10 mL), and the combined organic layers were washed with an aqueous solution of $Na_2S_2O_3$ (10% *w*/*v*, 3 × 5 mL), and brine (5 mL), then dried (Na_2SO_4), filtered, and concentrated *in vacuo*. Purification by column chromatography over silica gel (Petroleum ether/EtOAc 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, 0:10) afforded two fractions: First, an inseparable mixture of the two diastereoisomers A and B of (–)-**228** and the starting material (–)-**227** (93.6 mg) in a ratio of 2:5:2.5:1.0 as determined by ^{31}P NMR. Second, (–)-**228** (29 mg, 26%) as a single diastereoisomer. The first fraction, containing (–)-**227** (15 mg, 0.0250 mmol, 1.0 eq), was redissolved in anhydrous CH_2Cl_2 (1.4 mL). Phosphoramidite (–)-**193** (108 mg, 0.137 mmol, 5.5 eq) and 1*H*-tetrazole (305 μL, 0.137 mmol, 5.5 eq, 0.45 M in MeCN) were added subsequently. After 1.5 hours of stirring at RT the reaction mixture was cooled to –78 °C and *m*CPBA (31 mg, 0.137 mmol, 5.5 eq, 77%) was added in one portion. The reaction mixture was stirred for 1 hour, warmed to RT, stirred for a further 16 hours then quenched by addition of an aqueous solution of $Na_2S_2O_3$ (10% *w*/*v*, 5 mL). The aqueous phase was extracted with CH_2Cl_2 (4 × 10 mL), and the combined organic layers were washed with an aqueous solution of $Na_2S_2O_3$ (10% *w*/*v*, 3 × 5 mL), and brine (5 mL), then dried (Na_2SO_4), filtered, and concentrated *in vacuo*. Purification by column chromatography over silica gel (Petroleum ether/EtOAc 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, 0:10) gave another batch of (–)-**228** this time as a mixture of the two diastereoisomers A and B which, when combined with the second fraction, afforded (–)-**228** (39 mg, 36%) as a colourless, glassy solid. ^{1}H and ^{13}C data was collected on a sample of the single

diastereoisomer B, for ease of analysis. These data were employed to unequivocally assign the ^{1}H and ^{13}C data of similar compounds containing a mixture of two diastereoisomers: Diast. A R_f 0.37 ($CHCl_3$/EtOH 9.6:0.4); Diast. B R_f 0.31 ($CHCl_3$/EtOH 9.6:0.4); Diast. B $[\alpha]_D^{20} = -7.3$ (*c* 1.0, $CHCl_3$); Diast. B ^{1}H NMR (500 MHz; $CDCl_3$) δ_H 5.98–5.87 (2H, m, H-15 and H-18), 5.37–5.28 (2H, m, H-16 and H-19), 5.25–5.15 (3H, m, H-16', H-19' and H-32), 4.61 (1H, ddd, *J* 8.7, 8.6, 4.1, H-1), 4.52 (1H, dd, *J* 6.5, 4.1, H-2), 4.38–4.21 (12H, m, H-5, H-14, H-17, H-17', H-21, H-24, H-28, H-31 and H-49), 4.20–4.12 (3H, m, H-14', H-31' and H-49'), 4.05 (1H, dd, *J* 6.5, 6.1, H-3), 3.89 (1H, dd, *J* 8.7, 7.3, H-6), 3.68 (1H, dd, *J* 7.6, 6.1, H-4), 2.81–2.69 (6H, m, H-22, H-25 and H-29), 2.32 (2H, t, *J* 7.6, H-34), 2.30 (2H, t, *J* 7.6, H-51), 1.98–1.92 (1H, m, H_{eq}-12), 1.90–1.81 (1H, m, H_{eq}-9), 1.78–1.68 (2H, m, H-11 and H_{eq}-10), 1.64–1.56 (4H, m, H-35 and H-52), 1.45 (1H, d, *J* 13.1, H_{ax}-12), 1.46–1.38 (1H, m, H_{ax}-9), 1.34–1.17 (49H, m, H-36 to H-47, H-53 to H-64 and H_{ax}-10), 1.00–0.95 (3H, m, C(8)CH_3), 0.90–0.84 (12H, m, H-48, H-65, C(13)CH_3); Diast B. ^{13}C NMR (126 MHz; $CDCl_3$) δ_C 173.3 (C-50), 172.9 (C-33), 134.6 (C-15), 134.5 (C-18), 118.6 (C-7), 117.6, 117.3 (C-16, C-19), 116.6, 116.5, 116.4 (C-23, C-26, C-30), 80.3 (d_P, J_P 6.7, C-5), 79.8 (d_P, J_P 2.7, C-4), 76.9 (C-6), 75.5 (d_P, J_P 6.6, C-1), 75.0 (C-3), 73.9 (C-2), 73.4 (C-17), 71.8 (C-14), 69.2 (d_P, J_P 8.1, C-32), 66.0 (d_P, J_P 5.3, C-31), 62.50, 62.45, 62.41, 62.40 (C-21, C-24, C-28), 61.7 (C-49), 51.7 (C-13), 48.1 (C-8), 45.2 (C-11), 44.2 (C-12), 34.2, 34.1 (C-34, C-51), 32.0 (C-46, C-63), 29.9 (C-9), 29.83, 29.80, 29.78, 29.6, 29.5, 29.44, 29.43, 29.3, 29.2 (C-36 to C-45, C-53 to C-62), 27.0 (C-10), 25.0 (C-35, C-52), 22.8 (C-47, C-64), 20.5 (C(8)*C*H_3), 20.4 (C(13)*C*H_3), 19.8, 19.75, 19.71 (C-22, C-25, C-29), 14.2 (C-48, C-65), 10.2 (C(13)*C*H_3); ^{31}P NMR (162 MHz; $CDCl_3$) δ_P –2.41 (P-20), –2.66 (P-27, Diast. A), –2.77 (P-27, Diast. B); $\bar{\nu}_{max}$(thin film)/cm^{-1} 3980 (m), 2922 (m), 2853 (m), 2257 (C≡N) (w), 1743 (C=O) (m), 1465 (w), 1390 (w), 1265 (m), 1204 (m), 1110 (m), 1073 (m), 1020 (P-O) (s), 1005 (P-O) (s), 968 (m), 953 (m), 869 (w), 791 (w), 711 (w); MS *m/z* (MALDI) found 1285.19 [M–H+Na], 1301.19 [M–H+K] ($C_{66}H_{111}N_3O_{16}P_2$ requires 1263.74 $[M]^+$).

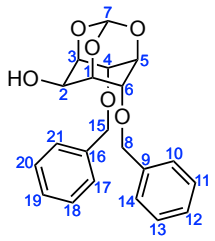
6.3. Experimental procedures and compound characterisation: Chapter 3

myo-Inositol 1,3,5-orthoformate 113



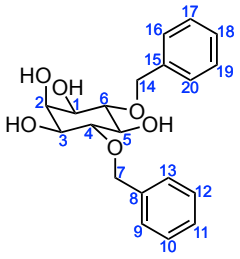
To a solution of *myo*-inositol **1** (20.0 g, 111 mmol, 1.0 eq) in DMF (80 mL) were added triethylorthoformate (82.5 g, 555 mmol, 5.0 eq) and PTSA·H_2O (8.45 g, 44.5 mmol, 0.40 eq). The reaction mixture was stirred at 105 °C for 3 days, then allowed to cool to RT. The solution was neutralised by addition of solid $NaHCO_3$ (10 g). The volatile components were removed *in vacuo* to give a yellow paste which was taken up in MeOH (300 mL). The mixture was cooled to –20 °C for 13 hours, then filtered to remove excess solid $NaHCO_3$ and sodium tosylate by-products. The filtrate was concentrated *in vacuo* to give a brown oil which was taken up in MeOH (50 mL) and cooled to –20 °C for 2 days. The resulting colourless crystalline solid was isolated by filtration, washed with $CHCl_3$ (25 mL), and dried. The remaining filtrate was concentrated *in vacuo* and purified by column chromatography over silica gel (CH_2Cl_2/MeOH 9.5:0.5) to give another batch of the crystalline solid. The combined materials provided **133** (14.9 g, 65%): R_f 0.40 (MeCN/EtOAc 8:2); m.p. 300–302 °C (MeOH, sealed tube) [lit.[60,61] 300–302 °C (MeOH, sealed tube)]; ^{1}H NMR (400 MHz; D_6-DMSO) δ_H 5.48 (1H, br s, C(2)O*H*), 5.44–5.36 (2H, m, C(4)O*H* and C(6)O*H*), 5.31 (1H, d, *J* 6.2, H-7) 4.31–4.24 (2H, dd, *J* 3.9, 3.9 H-1 and H-3), 4.09–4.04 (1H, m, H-5), 4.02–3.98 (1H, m, H-2), 3.97–3.92 (2H, m, H-4 and H-6); ^{13}C NMR (101 MHz; D_6-DMSO) δ 101.9 (C-7), 74.4 (C-1, C-3), 69.3 (C-5), 67.5 (C-4, C-6), 58.6 (C-2); LRMS *m/z* (ESI^+) 191.2 ($[M+H]^+$, 100%). These data are in good agreement with the literature values.[60,61]

4,6-Di-*O*-benzyl-*myo*-inositol 1,3,5-orthoformate 122



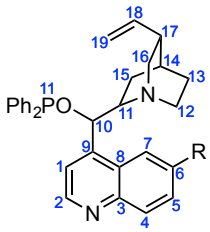
LiH (167 mg, 21.0 mmol, 4.0 eq) was gradually added to a solution of **113** (1.00 g, 5.26 mmol, 1.0 eq) in anhydrous DMF (15 mL), and the resulting solution was stirred at RT for 30 minutes. Benzyl bromide (1.44 mL, 12.1 mmol, 2.3 eq) was then added dropwise and the solution was stirred for a further 48 hours. After quenching the reaction with H_2O (30 mL), the aqueous phase was extracted with EtOAc (3 × 30 mL). The combined organic layers were washed with H_2O (4 × 30 mL), and brine (2 × 30 mL), then dried ($MgSO_4$), filtered, and concentrated *in vacuo*. The residue obtained was filtered through a short plug of silica gel (Petroleum ether/EtOAc 6:4) then crystallised (EtOAc) to yield **122** (1.37 g, 71%) as a colourless solid: R_f 0.48 (Petroleum ether/EtOAc 5:5); m.p. 123–124 °C (EtOAc) [lit.[449] 122–124 °C (EtOAc); lit.[62] 124–125 °C (EtOAc)]; ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 7.31–7.26 (10H, m, H-10 to H-14 and H-17 to H-21), 5.47 (1H, d, *J* 1.0, H-7), 4.67 (2H, d, J_{AB} 11.5, H-8 and H-15), 4.59 (2H, d, J_{AB} 11.5, H-8' and H-15'), 4.48–4.45 (1H, m, H-2), 4.38 (2H, dd, *J* 3.7, 3.7, H-4 and H-6), 4.25–4.18 (3H, m, H-1, H-3 and H-5), 3.04 (1H, d, *J* 11.5, C(2)O*H*); LRMS *m/z* (ESI$^+$) 393.1 ([M+Na]$^+$, 100%). These data are in good agreement with the literature values.[60,62,93,449]

4,6-Di-*O*-benzyl-*myo*-inositol 149



The protected orthoformate **122** (1.00 g, 2.70 mmol, 1.0 eq) was dissolved in MeOH (2.7 ml) and PTSA·H_2O (514 mg, 2.70 mmol, 1.0 eq) was added. The solution was stirred for 24 hours, then concentrated *in vacuo*. The residue was filtered through a short plug of silica gel (EtOAc) then crystallised (Hexane/CH_2Cl_2) to yield **149** (890 mg, 92%) as a colourless, fluffy, crystalline solid: R_f 0.40 (EtOAc); m.p. 140–141 °C (Hexane/CH_2Cl_2) [lit.[92] 138.5–139 °C (Hexane/CH_2Cl_2)]; ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 7.40–7.28 (10H, m, H-9 to H-13 and H-16 to H-20), 4.90 (2H, d, J_{AB} 11.4, H-7 and H-14), 4.84 (2H, d, J_{AB} 11.4, H-7' and H-14'), 4.13 (1H, dd, *J* 2.9, 2.9, H-5), 3.70–3.64 (2H, m, H-4 and H-6), 3.58–3.52 (3H, m, H-1, H-2 and H-3), 2.37 (4H, br s, C(1)O*H*, C(2)O*H*, C(3)O*H* and C(5)O*H*); LRMS *m/z* (ESI$^+$) 383.1 ([M+Na]$^+$, 100%). These data are in good agreement with the literature values.[90]

General procedure: Preparation of phosphinite catalysts derived from cinchona alkaloids 155, 245 and *ent*-155.



A solution of distilled Et_3N (170 μL, 1.23 mmol, 2.0 eq) in anhydrous, degassed THF (0.40 mL) and a solution of PPh_2Cl (136 mg, 0.617 mmol, 1.0 eq) in THF (0.40 mL) were consecutively added dropwise to a suspension of the cinchona alkaloid (0.617 mmol, 1.0 eq) in anhydrous, degassed THF

(1.6 mL). The reaction was monitored by ^{31}P NMR. After 2 hours stirring at RT, the residue was filtered through a pad of Celite® under Ar and the filtrate was concentrated *in vacuo*. The residue obtained was quickly purified by column chromatography over a short pad of silica gel (EtOAc) under Ar to give the corresponding phosphinite derivative as a colourless amorphous foam. Due to their susceptibility to oxidation, these compounds were characterised only by ^{1}H, ^{31}P NMR and in certain cases LRMS:

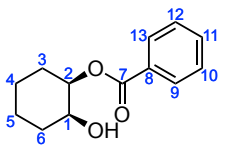
Phosphinite derived from quinidine (11*R*, 10*S*, R = OMe) **155** could not be obtained without full oxidation to the phosphinate.

Phosphinite derived from cinchonine (11*R*, 10*S*, R = H) **245** (174 mg, 59%) was obtained as a colourless amorphous solid, containing ~6% of the oxidised phosphinate side product as determined by ^{31}P NMR: R_f 0.14 (EtOAc); ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 8.74 (1H, d, *J* 4.4, H-2), 8.13 (1H, d, *J* 7.6, H-7), 8.06 (1H, d, *J* 8.4, H-4), 7.69–7.05 (13H, m, H-1, H-5, H-6 and P(20)(C_6H_5)$_2$), 5.97–5.85 (1H, m, H-18), 5.57 (1H, br s, H-10), 5.09–4.95 (2H, m, H-19), 3.29 (1H, br s, H-11), 2.93–2.54 (4H, m, H-12 and H-16), 2.23–1.46 (6H, m, H-13, H-14, H-15 and H-17); ^{31}P NMR (162 MHz; $CDCl_3$) δ_P 115.1 (P-20), 32.7 (phosphinate). These data are in good agreement with the literature values.[289]

Phosphinite derived from quinine (11*S*, 10*R*, R = OMe) ent-**155** (211 mg, 67%) was obtained as a colourless amorphous solid, containing ~3% of the oxidised, phosphinate side product as determined by ^{31}P NMR: R_f 0.11 (EtOAc); ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 8.63–8.55 (1H, m, H-2), 7.96 (1H, d, *J* 9.0, H-4), 7.58–7.00 (13H, m, H-1, H-5, H-7 and P(20)(C_6H_5)$_2$), 5.93–5.73 (1H, m, H-18), 5.64 (1H, br s, H-10), 5.06–4.93 (2H, m, H-19), 3.87 (3H, br s, C(6)OCH_3), 3.30 (1H, br s, H-11), 3.13–2.45 (4H, m, H-16 and H-12), 2.30–1.39 (6H, m, H-13, H-14, H-15 and H-17);[XIV] ^{31}P NMR (162 MHz; $CDCl_3$) δ_P 114.0 (P-20), 32.7 (phosphinate); LRMS *m/z* (ESI$^+$) 509.2 ([M+H]$^+$, 100%). These data are in good agreement with the literature values.[289]

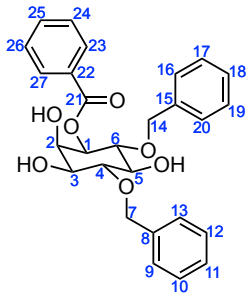
[XIV] The ^{1}H NMR spectrum obtained was found to be very broad and therefore difficult to assign.

(–)-(1*R*,2*S*)-2-Hydroxycyclohexyl benzoate (–)-255



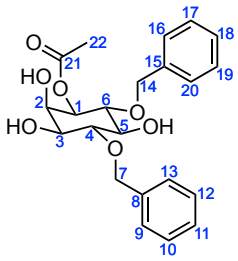
A solution of cis-1,2-cyclohexane diol **254** (58 mg, 0.500 mmol, 1.0 eq) and the cinchonine phosphonite catalyst **245** (96 mg, 0.200 mmol, 1.0 eq) in extra pure, degassed EtCN (2.5 mL) was cooled to –78 °C. Anhydrous, degassed DIPEA (87 μL, 0.500 mmol, 1.0 eq) and benzoyl chloride (87 μL, 0.750 mmol, 1.5 eq) were added dropwise to the solution. The solution was stirred at –78 °C for 12 hours and was then quenched with H_2O (2 mL). The aqueous phase was extracted with CH_2Cl_2 (4 × 3 mL) and the combined organic layers were washed with brine (2 × 2 mL), then dried ($MgSO_4$), filtered, and concentrated *in vacuo*. The residue obtained was purified by column chromatography over silica gel (Hexane/EtOAc 6:2) to give (–)-**255** (63 mg, 58%) as a colourless oil: R_f 0.63 (Petroleum ether/EtOAc 5:5); $[\alpha]_D^{20}$ = –14.0 (*c* 2.0, $CHCl_3$) [lit.[289] $[\alpha]_D^{23}$ = –16.5 (*c* 1.0, $CHCl_3$)]; ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 8.08–8.02 (2H, m, H-9 and H-13), 7.59–7.53 (1H, m, H-11), 7.47–7.40 (2H, m, H-10 and H-12), 5.25–5.17 (1H, m, H-2), 3.99–3.90 (1H, m, H-1), 2.19 (1H, br s, C(1)O*H*), 2.06–1.95 (1H, m, H-3), 1.91–1.60 (5H, m, H-3', H-6, H-4 and H-5), 1.53–1.33 (2H, m, H-4' and H-5'); LRMS *m/z* (ESI$^+$) 243.2 ([M+Na]$^+$, 100%).[289]. These data are in good agreement with the literature values.[289]

(±)-1-*O*-Benzoyl-4,6-di-*O*-benzyl-*myo*-inositol (±)-156



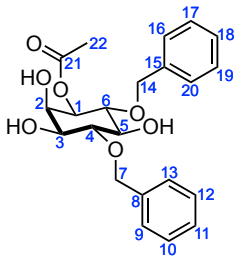
The quinine derived phosphonite catalyst ent-**155** (42 mg, 0.0832 mmol, 0.30 eq) and **149** (100 mg, 0.277 mmol, 1.0 eq) were dissolved in extra pure, degassed EtCN (1.4 mL) and cooled to –78 °C. Anhydrous, degassed DIPEA (36 mg, 0.277 mmol, 1.0 eq) and benzoyl chloride (59 mg, 0.416 mmol, 1.5 eq) were added successively. After stirring at RT for 24 hours, H_2O (10 mL) was added. The aqueous phase was extracted with CH_2Cl_2 (2 × 10 mL) and the combined organic layers were dried ($MgSO_4$), filtered, and concentrated *in vacuo*. The residue obtained was purified by column chromatography over silica gel (Cyclohexane/EtOAc 7:3, 6:4) to yield **156** (29 mg, 23%, <1% *e.e.*) as a colourless solid: R_f 0.11 (Cyclohexane/EtOAc 5:5); $[\alpha]_D^{20}$ = 0.0 (*c* 1.0, MeOH) [lit.[450] for D-(–)-**156**: $[\alpha]_D^{30}$ = –71.0 (*c* 1.0, MeOH)]; ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 8.15–8.08 (2H, m, H-23 and H-27), 7.65–7.56 (1H, m, H-25), 7.52–7.19 (12H, m, H-24, H-26, H-9 to H-13 and H-16 to H-20), 5.13 (1H, dd, *J* 9.8, 2.7, H-1), 5.01 (1H, d, J_{AB} 11.4, H-7), 4.83 (1H, d, J_{AB} 11.2, H-14), 4.79 (1H, d, J_{AB} 11.4, H-7'), 4.73 (1H, d, J_{AB} 11.2, H-14'), 4.36 (1H, dd, *J* 2.7, 2.7, H-2), 4.10 (1H, dd, *J* 9.8, 9.8, H-6), 3.81–3.66 (3H, m, H-4, H-3 and H-5); LRMS *m/z* (ESI$^+$) 487.0 ([M+Na]$^+$, 100%); Chiral HPLC (Heptane/IPA 85:15, 1.0 mL.min^{-1}, 220 nm) t_R = 22.22 min, 48.01%, t_R = 24.62 min, 51.99% [lit.[93] (Chiralpak OD column, Heptane/IPA 85:15, 1.0 mL. min^{-1}) Major: t_R = 19.8 min, Minor: t_R = 30.6 min]. These data are in good agreement with the literature values.[93]

(±)-1-*O*-Acetyl-4,6-di-*O*-benzyl-*myo*-inositol (±)-151



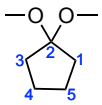
To a solution of **149** (150 mg, 0.416 mmol, 1.0 eq) in anhydrous CH_2Cl_2 (4.0 mL) and at –20 °C were added acetic anhydride (39 μL, 0.416 mmol, 1.0 eq), Et_3N (64 μL, 0.458 mmol, 1.1 eq) and 4-DMAP (3 mg, 0.0208 mmol, 0.050 eq). The reaction mixture was stirred at –20 °C for 18 hours, then warmed to –15 °C, and stirred for a further 12 hours. The solution was diluted in CH_2Cl_2 (5 mL) and the organic layer was washed with a saturated aqueous solution of $NaHCO_3$ (2 mL), and brine (2 mL), then dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The residue obtained was purified by column chromatography over silica gel (Petroleum ether/EtOAc 9:1, 8:2, 7:3, 4:6) to give a mixture of regioisomers containing (±)-**151**. Separation of this mixture was achieved by semi-preparative, reverse phase HPLC (Method 1) to give (±)-**151** (11 mg, 7%) as a colourless solid: R_f 0.67 (EtOAc); m.p. 97–98 °C (EtOAc) [lit.[90] 96–97 °C]; ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 7.39–7.27 (10H, m, H-16 to H-20 and H-9 to H-13), 4.99 (1H, d, J_{AB} 11.4, H-7), 4.88 (1H, dd, *J* 10.0, 2.7, H-1), 4.80 (1H, d, J_{AB} 11.4, H-14), 4.75 (1H, d, J_{AB} 11.4, H-7'), 4.74 (1H, d, J_{AB} 11.4, H-14'), 4.22 (1H, dd, *J* 2.8, 2.7, H-2), 3.92 (1H, dd, *J* 10.0, 9.6, H-6), 3.71 (1H, dd, *J* 9.6, 9.6, H-4), 3.66–3.57 (2H, m, H-5 and H-3), 2.49 (1H, d, *J* 2.1, C(5)O*H*), 2.44 (1H, d, *J* 3.2, C(3)O*H*), 2.39 (1H, s, C(2)O*H*), 2.08 (3H, s, H-22); ^{13}C NMR (126 MHz; $CDCl_3$) δ_C 170.3 (C-21), 138.6, 138.5 (C-8, C-15), 128.9, 128.8 (C-10, C-12, C-17, C-19), 128.21, 128.1 (C-11, C-18), 128.18, 127.8 (C-9, C-13, C16, C-20), 80.8 (C-4), 79.7 (C-6), 75.5 (C-14), 75.3 (C-7), 75.1 (C-5), 73.5 (C-1), 71.4 (C-3), 70.2 (C-2), 21.2 (C-22); LRMS *m/z* (ESI$^+$) 425.1 ([M+Na]$^+$, 100%); Chiral HPLC (Heptane/IPA 85:15, 1.0 mL.min^{-1}, 254 nm) t_R = 15.29 min, 50.90% (–)-**151**, t_R = 18.89 min, 49.07% (+)-**151** [lit.[90] (Chiralcel OD-H column, Hexane/IPA 70:30, 0.8 mL.min^{-1}) t_R = 43.6 min (+)-**151**; t_R = 50.5 min (–)-**151**]. These data are in good agreement with the literature values.[90,92]

(–)-1D-1-*O*-Acetyl-4,6-di-*O*-benzyl-*myo*-inositol (–)-151



The immobilised Lipozyme® TL-IM (150 mg) was added to a solution of **149** (50 mg, 0.139 mmol, 1.0 eq) in vinyl acetate (12.5 mL) and hexane (12.5 mL). After stirring at 45 °C for 15 hours the reaction mixture was filtered through a pad of Celite®, washed with hexane (3 × 2 mL) and the combined filtrates were concentrated *in vacuo*. The residue obtained was purified by column chromatography over silica gel (Petroleum ether/EtOAc 8:2) to give (–)-**151** (55 mg, 98%, >99% *ee*) as a colourless solid: $[\alpha]_D^{25}$ = –39.1 (*c* 1.0, MeOH) [lit.[90] $[\alpha]_D^{20}$ = –39.3 (*c* 1.0, MeOH); lit.[92] $[\alpha]_D^{20}$ = –39.1 (*c* 1.0, MeOH)]; Chiral HPLC (Heptane/IPA 85:15, 1.0 mL.min^{-1}, 254 nm) t_R = 15.65 min, 99.45% (–)-**151**, >99% *e.e.* (other enantiomer not observed, t_R = 18.89 min). All other data (R_f, m.p., ^{1}H NMR, ^{13}C NMR, IR) matched those of the racemic product (±)-**151** and that of the literature values.[90,92]

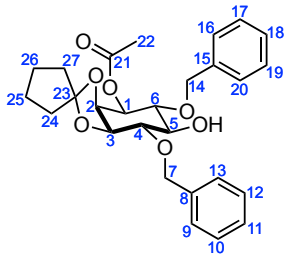
1,1-Dimethoxycyclopentane 260



To a solution of cyclopentanone **259** (6.31 mL, 71.3 mmol, 1.0 eq) in hexane (48 mL) were added K-10 montmorillonite clay (10.8 g) and trimethylorthoformate (17.9 mL, 159 mmol, 2.3 eq). The suspension was stirred rapidly for 18 hours at RT. The dark brown reaction mixture was then filtered through a pad of Celite® and washed with hexane (2 × 10 mL) and EtO_2 (2 × 10 mL). The combined filtrates were concentrated *in vacuo* to give **260** (7.08 g, 88%) as a yellowish oil. Analysis by ^{1}H NMR showed that the product was ~97% pure. This compound was therefore used in subsequent steps

without further purification: R_f 0.51 (Petroleum ether/EtOAc 8:2); ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 3.20 (6H, s, C(2)OCH_3), 1.80–1.71 (4H, m, H-3 and H-1), 1.69–1.61 (4H, m, H-4 and H-5); ^{13}C NMR (126 MHz; $CDCl_3$) δ_C 112.3 (C-2), 49.4 (C(2)O*C*H$_3$), 34.3 (C-3, C-1), 23.3 (C-4, C-5); HRMS *m/z* (EI/FI$^+$) found 130.0997 $[M]^+$ ($C_7H_{14}O_2$ requires 130.0994 $[M]^+$). These data are in good agreement with the literature values.[240,451]

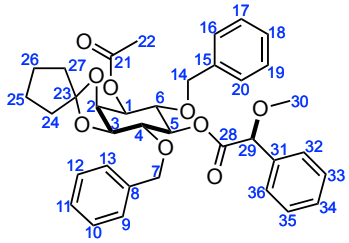
(–)-1D-1-*O*-Acetyl-2,3-*O*-cyclopentylidene-4,6-di-*O*-benzyl-*myo*-inositol (–)-261



To a clear solution of (–)-**151** (800 mg, 1.99 mmol, 1.0 eq) and **260** (4.55 mL, 33.2 mmol, 17 eq) in CH_2Cl_2 (5.0 mL) was added PTSA·H_2O (30 mg, 0.159 mmol, 0.080 eq). The resulting green solution was stirred at RT for 18 hours. The dark purple solution was then quenched with Et_3N (22 μL, 0.159 mmol, 0.080 eq) and concentrated *in vacuo*. The residue obtained was purified by column chromatography over silica gel (Petroleum ether/EtOAc 9:1, 8:2, 7:3, 6:4, 5:5) to give (–)-**261** (857 mg, 92%) as a yellow-brown oil: R_f 0.86 (Petroleum ether/EtOAc 2:8); $[\alpha]_D^{25}$ = –23.2 (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 7.41–7.26 (10H, m, H-9 to H-13 and H-16 to H-20), 5.19 (1H, dd, *J* 8.3, 3.8, H-1), 4.93 (1H, d, J_{AB} 11.6, H-7), 4.82 (1H, d, J_{AB} 11.6, H-14), 4.76 (1H, d, J_{AB} 11.6, H-14'), 4.72 (1H, d, J_{AB} 11.6, H-7'), 4.33 (1H, dd, *J* 5.7, 3.8, H-2), 4.20–4.14 (1H, m, H-3), 3.85–3.76 (1H, m, H-6), 3.68–3.60 (2H, m, H-5 and H-4), 2.66 (1H, s, C(5)O*H*), 2.08 (3H, s, H-22), 1.98–1.84 (2H, m, H-24 or H-27), 1.79–1.62 (6H, m, H-25, H-26 and H-24 or H-27); ^{13}C NMR (126 MHz; $CDCl_3$) δ_C 170.3 (C-21), 138.5, 138.3 (C-8, C-15), 128.6, 128.2, 128.0, 127.9, 127.7 (C-9 to C-13, C-16 to C-20), 120.1 (C-23), 81.1 (C-4), 79.5 (C-6), 78.5 (C-3), 74.5 (C-14), 74.4 (C-2), 73.7 (C-5), 73.5 (C-7), 71.3 (C-1), 37.4, 37.2 (C-24, C-27), 24.0, 23.6 (C-25, C-26), 21.2 (C-22); $\bar{\nu}_{max}$(thin film)/cm^{-1} 3471 (O-H) (br), 2972 (w), 2874 (w), 1742 (C=O) (m), 1454 (m), 1370 (m),

1335 (m), 1234 (s), 1103 (s), 1070 (s), 1028 (s), 972 (m), 911 (m), 732 (s), 697 (s); LRMS *m/z* (ESI^+) 469.2 ($[M+H]^+$, 38%), 491.2 ($[M+Na]^+$, 100%); HRMS *m/z* (ESI^+) found 491.2032 $[M+Na]^+$ ($C_{27}H_{32}O_7Na$ requires 491.2040 $[M+Na]^+$); NP-HPLC (Method 5) t_R = 6.36 min, 90.41%; Chiral HPLC (Heptane/IPA 85:15, 1.0 mL.min^{-1}, 254 nm) t_R = 16.75 min, 99% (–)-**261**, >99% *e.e.* (other enantiomer not observed).[239]

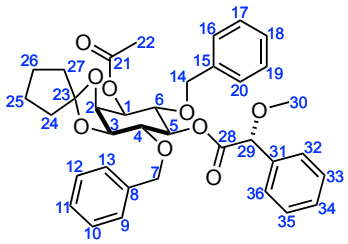
(+)-1D-1-*O*-Acetyl-2,3-*O*-cyclopentylidene-4,6-di-*O*-benzyl-5-*O*-((*S*)-α-methoxyphenyl-acetoxy)-*myo*-inositol (+)-262a



(*R*)-(–)-α-methoxyphenylacetic acid (13 mg, 0.0768 mmol, 2.0 eq), 1-ethyl-3-(3-dimethylamino-propyl)carbodiimide (20 mg, 0.104 mmol, 2.7 eq) and DMAP (2.3 mg, 0.0192 mmol, 0.50 eq) were added to a solution of (–)-**261** (18 mg, 0.0384 mmol, 1.0 eq) in CH_2Cl_2 (0.4 mL). After 48 hours, the reaction mixture was diluted with CH_2Cl_2 (3 mL), and the organic layer was washed with a saturated aqueous solution of $NaHCO_3$ (2 × 5 mL), brine (5 mL), then dried ($MgSO_4$), filtered, and concentrated *in vacuo*. Purification by column chromatography over silica gel (Petroleum ether/EtOAc 10:0, 9:1, 8:2) afforded (+)-**262a** (16 mg, 68%) as a colourless solid: R_f 0.26 (Petroleum ether/EtOAc 8:2); $[\alpha]_D^{25}$ = +4.8 (*c* 0.55, $CHCl_3$); m.p. 105–107 °C (Hexane/EtOAc); ^{1}H NMR (500 MHz, CD_2Cl_2) δ_H 7.42–7.37 (2H, m, H-32 and H-36), 7.36–7.19 (11H, m, H-33 to H-35, H-17 to H-19 and H-9 to H-13), 7.10–7.05 (2H, m, H-16 and H-20), 5.17 (1H, dd, *J* 8.9, 3.7, H-1), 5.11 (1H, dd, *J* 8.2, 8.2, H-5), 4.79 (1H, d, J_{AB} 11.8, H-7), 4.68 (1H, s, H-29), 4.62 (1H, d, J_{AB} 11.8, H-7'), 4.32 (1H, dd, *J* 6.0, 3.7, H-2), 4.23 (1H, dd, *J* 6.1, 6.0, H-3), 4.11 (1H, d, J_{AB} 11.4, H-14), 3.98 (1H, d, J_{AB} 11.4, H-14), 3.74–3.69 (1H, m, H-4 and H-6), 3.30 (3H, s, H-30), 1.94 (3H, s, H-22), 1.96–1.91 (2H, m, H-24 and H-27), 1.74–1.58 (6H, m, H-24', H-27', H-25 and H-26); ^{13}C NMR (126 MHz, CD_2Cl_2) δ_C 170.1 (C-21), 169.9 (C-28), 138.57, 138.42 (C-8, C-15), 136.6 (C-31), 129.2, 129.0,

128.7, 128.5 (C-10 to C-12, C-17 to C-19 and C-33 to C-35), 128.2, 128.1, 127.8, 127.7 (C-9, C-13, C-16, C-20, C-32, C-36), 120.3 (C-23), 82.9 (C-29), 79.1 (C-4), 78.1 (C-3), 77.3 (C-6), 75.2 (C-5), 74.3 (C-2), 73.9 (C-14), 73.3 (C-7), 70.7 (C-1), 57.6 (C-30), 37.23, 37.17 (C-24, C-27), 24.2, 23.7 (C-25, C-27), 21.1 (C-22); $\bar{\nu}_{max}$(thin film)/cm^{-1} 3089 (w), 3063 (w), 3032 (w), 2956 (m), 2829 (w), 2359 (w), 2337 (w), 1748 (C=O) (s), 1497 (w), 1455 (m), 1370 (m), 1337 (m), 1234 (s), 1200 (C-O) (m), 1177 (m), 1109 (s), 1073 (m), 1029 (m), 1005 (m), 973 (m), 737 (m), 698 (m); LRMS *m/z* (ESI$^+$) 639.2 ([M+Na]$^+$, 100%); HRMS *m/z* (ESI$^+$) found 639.25568 [M+Na]$^+$ ($C_{36}H_{40}O_9Na$ requires 639.25645 [M+Na]$^+$).

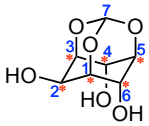
(–)-1D-1-*O*-Acetyl-2,3-*O*-cyclopentylidene-4,6-di-*O*-benzyl-5-*O*-((*R*)-α-methoxyphenyl-acetoxy)-*myo*-inositol (–)-262b



(*R*)-(–)-α-methoxyphenylacetic acid (13 mg, 0.0768 mmol, 2.0 eq), 1-ethyl-3-(3-dimethylamino-propyl)carbodiimide (20 mg, 0.104 mmol, 2.7 eq) and DMAP (2.3 mg, 0.0192 mmol, 0.50 eq) were added to a solution of (–)-**261** (18 mg, 0.0384 mmol, 1.0 eq) in CH_2Cl_2 (0.4 mL). After 24 hours stirring at RT, the reaction mixture was diluted with CH_2Cl_2 (3 mL), and the organic layer was washed with a saturated aqueous solution of $NaHCO_3$ (2 × 5 mL), brine (5 mL), then dried ($MgSO_4$), filtered, and concentrated *in vacuo*. Purification by column chromatography over silica gel (Petroleum ether/EtOAc 9:1, 8:2, 7:3) afforded (–)-**262b** (17 mg, 72%) as a colourless solid: R_f 0.26 (Petroleum ether/EtOAc 8:2); $[\alpha]_D^{25}$ = –5.5 (*c* 0.34, $CHCl_3$); m.p. 105–107 °C (Hexane/EtOAc); ^{1}H NMR (500 MHz, CD_2Cl_2) δ_H 7.41–7.36 (2H, m, H-32 and H-36), 7.35–7.18 (11H, m, H-33 to H-35, H-10 to H-12 and H-16 to H-20), 7.10–7.05 (2H, m, H-9 and H-13), 5.21 (1H, dd, *J* 8.7, 3.8, H-1), 5.11 (1H, dd, *J* 8.5, 8.4, H-5), 4.65 (1H, s, H-29), 4.58 (1H, d, J_{AB} 11.6, H-14), 4.51 (1H, d,

J_{AB} 11.6, H-14'), 4.49 (1H, d, J_{AB} 12.0, H-7), 4.32 (1H, dd, *J* 6.0, 3.8, H-2), 4.23 (1H, d, J_{AB} 12.0, H-7'), 4.15 (1H, dd, *J* 6.1, 6.0, H-3), 3.90 (1H, dd, *J* 8.7, 8.4, H-6), 3.54 (1H, dd, *J* 8.5, 6.1, H-4), 3.30 (3H, s, H-30), 2.03 (3H, s, H-22), 1.90–1.83 (1H, m, H-24 or H-27), 1.82–1.75 (1H, m, H-24 or H-27), 1.69–1.58 (6H, m, H-24', H-27', H-25 and H-26); ^{13}C NMR (126 MHz, CD_2Cl_2) δ_C 170.2 (C-21), 170.0 (C-28), 138.52, 138.47 (C-8, C-15), 136.5 (C-31), 129.1, 129.0, 128.7, 128.4 (C-10 to C-12, C-17 to C-19 and C-33 to C-35), 128.1, 128.0, 127.9, 127.73, 127.67 (C-9, C-13, C-16, C-20, C-32, C-36), 120.3 (C-23), 83.0 (C-29), 79.2 (C-4), 78.1 (C-3), 77.3 (C-6), 75.2 (C-5), 74.6 (C-14), 74.3 (C-2), 73.0 (C-7), 71.0 (C-1), 57.7 (C-30), 37.3, 37.1 (C-24, C-27), 24.2, 23.7 (C-25, C-27), 21.2 (C-22); $\bar{\nu}_{max}$(thin film)/cm^{-1} 3089 (w), 3063 (w), 3032 (w), 2956 (m), 2829 (w), 2360 (w), 2337 (w), 1748 (C=O) (s), 1497 (w), 1455 (m), 1434 (w), 1370 (m), 1337 (m), 1234 (s), 1200 (C-O) (s), 1177 (C-O) (s), 1109 (s), 1073 (s), 1029 (m), 1005 (m), 973 (m), 913 (m), 873 (w), 737 (m), 698 (m); LRMS *m/z* (ESI$^+$) 639.2 ([M+Na]$^+$, 100%); HRMS *m/z* (ESI$^+$) found 639.25598 [M+Na]$^+$ ($C_{36}H_{40}O_9Na$ requires 639.25645 [M+Na]$^+$).[XV]

D_6 *myo*-Inositol orthoformate 264



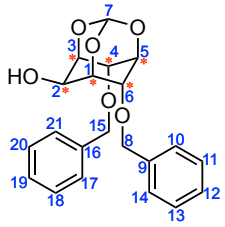
To a solution of *myo*-inositol **183** (608 mg, 3.27 mmol, 1.0 eq) in DMF (2.8 mL) were added triethylorthoformate (2.72 mL, 16.3 mmol, 5.0 eq) and PTSA·H_2O (248 mg, 1.31 mmol, 0.40 eq). The reaction mixture was stirred at 105 °C for 3 days, then allowed to cool to RT. The solvent was then concentrated *in vacuo* and the residue obtained was purified by column chromatography over silica gel (CH_2Cl_2/MeOH 10:0, 9.9:0.1, 9.8:0.2, 9.7:0.3, 9.6:0.4, 9.5:0.5) to afford **264** (200 mg, 31%, 89% D_6, 11% D_5) as a colourless solid: R_f 0.40 (MeCN/EtOAc 8:2); m.p. 299–301 °C (MeOH, sealed tube); ^{1}H NMR (500 MHz; D_6-DMSO) δ_H 5.45 (1H, s, C(2)O*H*), 5.43 (2H, br s, C(4)O*H*, C(6)O*H*),

[XV] HPLC data could not be acquired on this compound as it seemed to be breaking down on both normal phase and reversed phase columns.

5.27 (1H, s, H-7); ^{13}C NMR (126 MHz; D_6-DMSO) δ_C 101.8 (C-7), 73.8 (t_D, J_D 24.0, C-1, C-3), 68.6 (t_D, J_D 23.7, C-5), 66.8 (t_D, J_D 22.9, C-4, C-6), 58.0 (t_D, J_D 21.9, C-2); ^{2}H NMR (77 MHz; DMSO; D_6-DMSO) δ_D 4.24 (2D, br s, D-1 and D-3), 4.12–3.74 (4D, br s, D-5, D-2, D-4 and D-6); $\bar{\nu}_{max}$(thin film)/cm^{-1} 3385 (O-H) (br), 3291 (O-H) (br), 3013 (w), 2360 (w), 2179 (w), 1410 (m), 1335 (m), 1258 (m), 1155 (s), 1096 (s), 1069 (s), 1044 (s), 995 (s), 955 (s), 935 (s); LRMS *m/z* (ESI$^+$) 218.0 ([MD_5+Na]$^+$, 11%), 219.0 ([MD_6+Na]$^+$, 100%); HRMS *m/z* (ESI$^-$) found 194.07178 [MD_5–H]$^-$, 195.07791 [MD_6–H]$^-$ ($C_7H_4D_6O_6Na$ requires 195.07812 [MD_6–H]$^-$).

* *X-ray crystallographic data for this compound can be found in Appendix B*

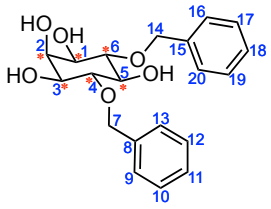
D_6 4,6-Di-*O*-benzyl-*myo*-inositol 1,3,5-orthoformate 265



LiH (22 mg, 2.55 mmol, 4.0 eq) was gradually added to a solution of the orthoformate **264** (125 mg, 0.637 mmol, 1.0 eq, 89% D_6, 11% D_5) in anhydrous DMF (1.8 mL), and the resulting solution was stirred at RT for 30 minutes. Benzyl bromide (174 μL, 1.47 mmol, 2.3 eq) was then added dropwise and the solution was stirred for a further 48 hours. The reaction was quenched by addition of H_2O (3 mL) and the aqueous phase was extracted with EtOAc (4 × 5 mL). The combined organic layers were washed with H_2O (4 × 5 mL) and brine (4 × 5 mL), then dried ($MgSO_4$), filtered, and concentrated *in vacuo*. The residue was purified by column chromatography over silica gel (Petroleum ether/EtOAc 9:1, 8:2, 7:3, 6:4) to yield **265** (185 mg, 77%, 91% D_6, 9% D_5) as a colourless solid: R_f 0.48 (Petroleum ether/EtOAc 5:5); m.p. 125–127 °C (MeOH); ^{1}H NMR (500 MHz; $CDCl_3$) δ_H 7.29–7.22 (10H, m, H-10 to H-14 and H-17 to H-21), 5.44 (1H, s, H-7), 4.64 (2H, d, J_{AB} 11.5, H-8 and H-15), 4.56 (2H, d, J_{AB} 11.5, H-8' and H-15'); ^{13}C NMR (126 MHz; $CDCl_3$) δ_C 137.6 (C-9, C-16), 128.6 (C-11, C-13, C-18, C-20), 128.0 (C-12, C-19), 127.8 (C-10, C-14, C-17, C-21), 103.4 (C-7), 73.2 (t_D, J_D 22.6, C-4, C-6), 72.5 (t_D, J_D 24.2, C-1, C-3), 71.8 (C-8, C-15), 67.4 (t_D, J_D 23.7,

C-2), 61.0 (t_D, J_D 22.7, C-5); ^{2}H NMR (77 MHz; $CHCl_3$; $CDCl_3$) δ_D 4.44 (1D, br s, D-2), 4.36 (2D, br s, D-4 and D-6), 4.22 (3D, br s, D-1, D-3 and D-5); $\bar{\nu}_{max}$(thin film)/cm^{-1} 3503 (m), 3020 (w), 2981 (w), 2360 (w), 2342 (w), 1498 (m), 1452 (m), 1390 (m), 1316 (m), 1245 (m), 1208 (m), 1165 (s), 1117 (m), 1107 (m), 1082 (s), 1021 (m), 996 (m), 968 (m), 936 (m), 866 (s), 783 (m), 746 (s), 730 (s), 695 (s); LRMS *m/z* (ESI$^+$) 398.2 ([MD_5+Na]$^+$, 9%), 399.2 ([MD_6+Na]$^+$, 100%); HRMS *m/z* (ESI$^+$) found 398.16238 [MD_5+Na]$^+$, 399.16829 [MD_6+Na]$^+$ ($C_{21}H_{16}D_6O_6Na$ requires 399.16852 [MD_6+Na]$^+$); RP-HPLC (Method 2) t_R = 12.59 min, 99.96%.

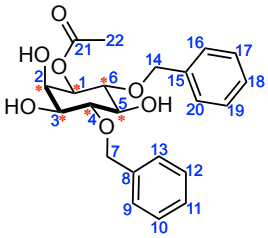
D_6 4,6-Di-*O*-benzyl-*myo*-inositol 263



The protected orthoformate **265** (179 mg, 0.476 mmol, 1.0 eq, 91% D_6, 9% D_5) was dissolved in MeOH (0.50 mL) and PTSA·H_2O (90 mg, 0.476 mmol, 1.0 eq) was added. The solution was stirred for 24 hours then concentrated *in vacuo*. The residue obtained was filtered through a short pad of silica gel (EtOAc) then crystallised (Hexane/CH_2Cl_2) to yield **263** (150 mg, 86%, 92% D_6, 8% D_5) as a colourless, fluffy, crystalline solid: R_f 0.40 (EtOAc); m.p. 144–145 °C (Hexane/CH_2Cl_2); ^{1}H NMR (500 MHz; $CDCl_3$) δ_H 7.41–7.28 (10H, m, H-9 to H-13 and H-16 to H-20), 4.90 (2H, d, J_{AB} 11.5, H-7 and H-14), 4.84 (2H, d, J_{AB} 11.5, H-7' and H-14'), 2.69 (1H, br s, C(5)O*H*), 2.52 (1H, br s, C(2)O*H*), 2.51 (2H, br s, C(1)O*H* and C(3)O*H*); ^{13}C NMR (126 MHz; $CDCl_3$) δ_C 138.6 (C-8, C-15), 128.8 (C-10, C-12, C-17, C-19), 128.2 (C-9, C-11, C-13, C-16, C-18, C-20), 81.1 (t_D, J_D 21.9, C-4, C-6), 75.2 (C-7, C-14), 74.3 (t_D, J_D 21.7, C-2), 71.4 (t_D, J_D 21.7, C-1, C-3), 71.1 (t_D, J_D 22.7, C-5); ^{2}H NMR (77 MHz; $CHCl_3$; $CDCl_3$) δ_D 4.10 (1D, br s, D-5), 3.65 (2D, br s, D-4 and D-6), 3.53 (3D, br s, D-1, D-2 and D-3); $\bar{\nu}_{max}$(thin film)/cm^{-1} 3658 (O-H) (br), 3529 (O-H) (br), 3435 (O-H) (br), 3354 (O-H) (br), 2981 (s), 2885 (m), 1496 (w), 1473 (w), 1455 (w), 1384 (m), 1306 (w), 1251 (w), 1206 (m), 1176 (m), 1139 (m), 1083 (m), 1060 (m), 1038 (m), 1027 (m), 1013 (m), 992 (m),

980 (m), 966 (m), 952 (m), 936 (m), 754 (m), 704 (s), 696 (s); LRMS *m/z* (ESI$^+$) 388.2 ([MD_5+Na]$^+$, 9%), 389.2 ([MD_6+Na]$^+$, 100%); HRMS *m/z* (ESI$^+$) found 388.17803 [MD_5+Na]$^+$, 389.18402 [MD_6+Na]$^+$ ($C_{20}H_{18}D_6O_6Na$ requires 389.18417 [MD_6+Na]$^+$); RP-HPLC (Method 2) t_R = 9.66 min, 97.15%.

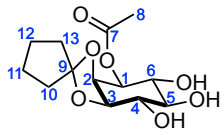
D_6 (–)-1D-1-*O*-Acetyl-4,6-di-*O*-benzyl-*myo*-inositol (–)-266



The immobilised Lipozyme® TL-IM (403 mg) was added to a solution of **263** (140 mg, 0.382 mmol, 1.0 eq, 92% D_6, 8% D_5) in vinyl acetate (33 mL) and hexane (33 mL). After stirring at 45 °C for 15 hours, the reaction mixture was filtered through a pad of Celite®, washed with hexane (3 × 10 mL), and the combined filtrates were concentrated *in vacuo*. The residue obtained was purified by column chromatography over silica gel (Petroleum ether/EtOAc 8:2) to give (–)-**266** (128 mg, 84%, >99% *e.e.*, 89% D_6, 11% D_5) as a colourless solid: R_f 0.64 (EtOAc); $[\alpha]_D^{25}$ = –38.8 (*c* 1.0, MeOH); m.p. 97–98 °C (EtOAc); ^{1}H NMR (500 MHz; $CDCl_3$) δ_H 7.39–7.27 (10H, m, H-9 to H-13 and H-16 to H-20), 4.98 (1H, d, J_{AB} 11.4, H-7), 4.80 (1H, d, J_{AB} 11.7, H-14), 4.75 (1H, d, J_{AB} 11.4, H-7'), 4.74 (1H, d, J_{AB} 11.7, H-14'), 2.53 (1H, br s, C(5)O*H*), 2.50 (1H, br s, C(2)O*H*), 2.48 (1H, br s, C(3)O*H*), 2.08 (3H, s, H-22); ^{13}C NMR (126 MHz; $CDCl_3$) δ_C 170.3 (C-21), 138.6, 138.5 (C-8, C-15), 128.8, 128.7 (C-10, C-12, C-17, C-19), 128.19, 128.0 (C-11, C-18), 128.17, 127.7 (C-9, C-13, C-16, C-20), 80.3 (t_D, J_D 22.0, C-4), 79.1 (t_D, J_D 22.0, C-6), 75.4 (C-14), 75.2 (C-7), 74.4 (t_D, J_D 21.8, C-5), 73.0 (t_D, J_D 22.2, C-1), 70.8 (t_D, J_D 21.5, C-3), 69.6 (t_D, J_D 23.0, C-2), 21.2 (C-22); ^{2}H NMR (77 MHz; $CHCl_3$; $CDCl_3$) δ_D 4.85 (1D, br s, D-1), 4.19 (1D, br s, D-2), 3.91 (1D, br s, D-6), 3.68 (1D, br s, D-4), 3.59 (2D, br s, D-3 and D-5); $\bar{\nu}_{max}$(thin film)/cm^{-1} 3658 (O-H) (br), 2981 (s), 2888 (m), 1737 (C=O) (w), 1494 (w), 1473 (w), 1462 (w), 1381 (m), 1253 (m), 1209 (w), 1155 (m), 1090 (m), 1072 (m), 1031 (m), 1011 (m), 953 (m); LRMS *m/z* (ESI$^+$) 430.2 ([MD_5+Na]$^+$, 12%), 431.2

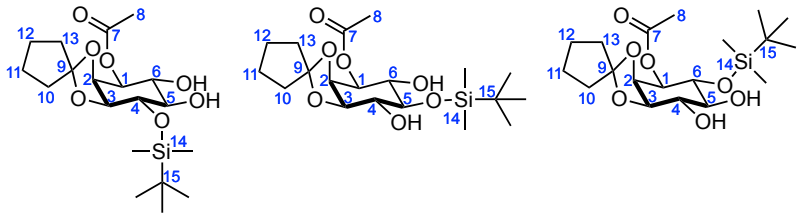
([MD_6+Na]$^+$, 100%); HRMS *m/z* (ESI$^+$) found 430.18850 [MD_5+Na]$^+$, 431.19456 [MD_6+Na]$^+$ ($C_{22}H_{20}D_6O_7Na$ requires 431.19473 [MD_6+Na]$^+$); RP-HPLC (Method 2) t_R = 10.71 min, 98.70%; Chiral HPLC (Heptane/IPA 85:15, 1.0 mL.min^{-1}, 254 nm) t_R = 15.89 min, 99.97% (–)-**266**, >99% *e.e.* (other enantiomer not observed, t_R = 18.89 min).

(–)-1D-1-*O*-Acetyl-2,3-*O*-cyclopentylidene-*myo*-inositol (–)-269



To a solution of (–)-**261** (3.06 g, 6.53 mmol, 1.0 eq) in EtOAc (124 mL) was added 10% Pd/C (1.04 g, 0.979 mmol, 0.15 eq) in one portion. After bubbling two balloons of $H_{2(g)}$ through the reaction, the mixture was stirred vigorously for 2 hours under an atmosphere of $H_{2(g)}$, then filtered through a pad of Celite® and concentrated *in vacuo* to give (–)-**269** (1.78 g, 94%) as a colourless solid: R_f 0.087 (Petroleum ether/EtOAc 2:8); $[\alpha]_D^{25}$ = –36.9 (*c* 0.68, $CHCl_3$); m.p. 156–158 °C (EtOAc); ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 5.00 (1H, dd, *J* 9.9, 4.1, H-1), 4.31 (1H, dd, *J* 4.6, 4.1, H-2), 4.05 (1H, dd, *J* 7.5, 4.6, H-3), 3.92 (1H, ddd, *J* 9.9, 9.9, 3.3, H-6), 3.65 (1H, ddd, *J* 10.1, 7.5, 2.6, H-4), 3.39 (1H, ddd, *J* 10.1, 9.9, 2.0, H-5), 3.05 (1H, s, C(5)O*H*), 2.78 (1H, s, C(4)O*H*), 2.67 (1H, s, C(6)O*H*), 2.20 (3H, s, H-8), 2.01–1.89 (2H, m, H-10 or H-13), 1.78–1.61 (6H, m, H-11, H-12 and H-10 or H-13); ^{13}C NMR (126 MHz; $CDCl_3$) δ_C 171.1 (C-7), 120.4 (C-9), 78.4 (C-3), 74.7 (C-4), 74.5 (C-2), 73.6 (C-5), 72.1 (C-1), 70.7 (C-6), 37.99, 37.97 (C-10, C-13), 23.9, 23.7 (C-11, C-12), 21.2 (C-8); $\bar{\nu}_{max}$(thin film)/cm^{-1} 3656 (O-H) (m), 3537 (O-H) (br), 2980 (w), 2965 (w), 2936 (w), 2886 (w), 1721 (C=O) (s), 1376 (m), 1332 (m), 1261 (s), 1236 (m), 1186 (m), 1111 (s), 1100 (s), 1069 (m), 1035 (m), 973 (s), 871 (m); LRMS *m/z* (ESI$^-$) 333.1 ([M+FA–H]$^-$, 100%), 323.0 ([M+Cl]$^-$, 7%); HRMS *m/z* (ESI$^+$) found 289.12819 [M+H]$^+$ ($C_{13}H_{20}O_7$ requires 289.12818 [M+H]$^+$); RP-HPLC (Method 3) t_R = 15.04 min, 98.99%.[452]

(–)-1D-1-*O*-Acetyl-2,3-*O*-cyclopentylidene-4-*O*-(*tert*-butyldimethylsilyl)-*myo*-inositol (–)-270; (–)-1D-1-*O*-acetyl-2,3-*O*-cyclopentylidene-5-*O*-(*tert*-butyldimethylsilyl)-*myo*-inositol (–)-271; (–)-1D-1-*O*-acetyl-2,3-*O*-cyclopentylidene-6-*O*-(*tert*-butyldimethylsilyl)-*myo*-inositol (–)-272



Method 1 To a solution of triol (–)-**269** (50 mg, 0.173 mmol, 1.0 eq) and imidazole (18 mg, 0.260 mmol, 1.5 eq) in anhydrous pyridine (0.80 mL) was added TBDMSCl (26 mg, 0.175 mmol, 1.01 eq) at –10 °C. After 12 hours of stirring at –10 °C the reaction mixture was warmed to RT. After 52 hours of stirring at RT, no product could be observed.

Method 2 To a solution of triol (–)-**269** (50 mg, 0.173 mmol, 1.0 eq) and imidazole (18 mg, 0.260 mmol, 1.5 eq) in anhydrous DMF (0.60 mL) was added TBDMSCl (26 mg, 0.173 mmol, 1.0 eq). After stirring at RT for 5 days, no product could be observed.

Method 3 To a solution of triol (–)-**269** (25 mg, 0.0867 mmol, 1.0 eq) and 2,6-lutidine (15 μL, 0.130 mmol, 1.5 eq) in anhydrous CH_2Cl_2 (0.80 mL) was added TBDMSOTf (20 μL, 0.0867 mmol, 1.0 eq) at –78 °C. The reaction mixture was stirred for 21 hours and warmed to RT. After 2 hours of stirring, 2,6-lutidine (15 μL, 0.130 mmol, 1.5 eq) and TBDMSOTf (20 μL, 0.0867 mmol, 1.0 eq) were added sequentially and the reaction mixture was stirred at RT for 48 hours. The reaction mixture was concentrated *in vacuo* and the residue obtained was purified by column chromatography over silica gel (Petroleum ether/EtOAc 6:4, 5:5) to give (–)-**270** (3 mg, 10%) as a colourless solid: R_f 0.55 (Petroleum ether/EtOAc 5:5); $[\alpha]_D^{25}$ = –25.0 (*c* 1.0, $CHCl_3$); m.p. 108–112 °C (Hexane/EtOAc); ^{1}H NMR (500 MHz; $CDCl_3$) δ_H 5.04 (1H, dd, *J* 9.9, 4.3, H-1), 4.32 (1H, dd, *J* 4.5, 4.3, H-2), 3.99–3.90 (2H, m, H-3 and H-6), 3.63 (1H, dd, *J* 8.9, 6.8, H-4), 3.34 (1H, ddd, *J* 8.9, 8.9, 2.7, H-5), 2.47 (1H, d, *J* 2.8, C(6)O*H*), 2.42 (1H, d, *J* 2.7, C(5)O*H*), 2.18 (3H, s, H-8), 1.98–1.88 (2H, m, H-10 or H-13), 1.76–1.62 (6H, m, H-11, H-12 and H-10 or H-13), 0.91 (9H, s, C(15)C*H*$_3$), 0.16 (3H, s,

Si(14)CH_3), 0.13 (3H, s, Si(14)CH_3); ^{13}C NMR (126 MHz; $CDCl_3$) δ_C 171.0 (C-7), 120.0 (C-9), 79.0 (C-3), 76.1 (C-4), 75.0 (C-5), 74.4 (C-2), 72.1 (C-1), 70.4 (C-6), 37.32, 37.27 (C-10, C-13), 26.0 (C(15)CH$_3$), 23.6, 23.3 (C-11, C-12), 21.2 (C-8), 18.3 (C-15), –4.1, –4.7 (Si(14)CH$_3$); $\bar{\nu}_{max}$(thin film)/cm^{-1} 3561 (O-H) (br), 2961 (w), 2930 (w), 2855 (w), 1725 (C=O) (s), 1430 (w), 1376 (w), 1331 (m), 1254 (Si-CH$_3$) (s), 1201 (m), 1163 (m), 1130 (m), 1100 (Si-O) (m), 1047 (m), 994 (m), 968 (m), 912 (m), 872 (m), 855 (s), 834 (s), 780 (s), 730 (m), 672 (m); LRMS *m/z* (ESI$^+$) 425.2 ([M+Na]$^+$, 100%); HRMS *m/z* (ESI$^+$) found 403.21494 [M+H]$^+$ ($C_{19}H_{35}O_7Si$ requires 403.21466 [M+H]$^+$).

Method 4 To a solution of triol (–)-**269** (15 mg, 0.0520 mmol, 1.0 eq) and Et_3N (9 μL, 0.0676 mmol, 1.3 eq) in anhydrous CH_2Cl_2 (0.30 mL) was added TBDMSOTf (13 μL, 0.0572 mmol, 1.1 eq) at 0 °C. The reaction mixture was stirred for 2 hours and warmed to RT. After 28 hours of stirring, the solvent was concentrated *in vacuo* and the residue obtained was purified by column chromatography over silica gel (Petroleum ether/EtOAc 9.5:0.5, 9:1, 8:2, 7:3, 6:4, 5:5) to give (–)-**270** (6 mg, 28%) as a colourless solid as well as (–)-**272** (2 mg, 8%) and (–)-**271** (6 mg, 27%) as colourless oils: (–)-**271** R_f 0.79 (Petroleum ether/EtOAc 5:5); $[\alpha]_D^{25}$ = –23.5 (*c* 2.0, $CHCl_3$); ^{1}H NMR (500 MHz; $CDCl_3$) δ_H 5.00 (1H, dd, *J* 9.6, 4.1, H-1), 4.29 (1H, dd, *J* 4.8, 4.1, H-2), 3.97 (1H, dd, J 7.6, 4.8, H-3), 3.85 (1H, ddd, *J* 9.6, 9.2, 2.8, H-6), 3.57 (1H, ddd, *J* 9.8, 7.6, 2.6, H-4), 3.33 (1H, dd, *J* 9.8, 9.2, H-5), 2.27 (1H, d, *J* 2.6, C(4)O*H*), 2.22 (1H, d, *J* 2.8, C(6)O*H*), 2.18 (3H, s, H-8), 2.02–1.90 (2H, m, H-10 or H-13), 1.82–1.60 (6H, m, H-11, H-12 and H-10 or H-13), 0.92 (9H, s, C(15)CH_3), 0.15 (6H, Si(14)CH_3); ^{13}C NMR (126 MHz; $CDCl_3$) δ_C 170.9 (C-7), 120.2 (C-9), 78.3 (C-3), 75.6 (C-5), 75.1 (C-4), 74.3 (C-2), 71.9 (C-1), 71.1 (C-6), 38.0, 37.9 (C-10, C-13), 26.1 (C(15)CH$_3$), 23.9, 23.7 (C-11, C-12), 21.2 (C-8), 18.5 (C-15), –4.2, –4.4 (Si(14)CH$_3$); $\bar{\nu}_{max}$(thin film)/cm^{-1} 3479 (O-H) (br), 2954 (m), 2930 (m), 2895 (w), 2856 (w), 2361 (w), 2341 (w), 1728 (C=O) (m), 1373 (m), 1333 (m), 1247 (Si-CH$_3$) (s), 1143 (s), 1109 (s), 1035 (s), 972 (m), 838 (s), 780 (s); LRMS *m/z* (ESI$^+$) 425.2 ([M+Na]$^+$, 100%); HRMS *m/z* (ESI$^+$) found 425.19632 [M+Na]$^+$ ($C_{19}H_{34}O_7NaSi$ requires 425.19660 [M+Na]$^+$); (–)-**272**: R_f 0.61 (Petroleum ether/EtOAc 5:5); $[\alpha]_D^{25}$ = –25.2 (*c* 0.30, $CHCl_3$); ^{1}H NMR (500 MHz; CD_2Cl_2) δ_H 4.94 (1H, dd, *J* 9.1, 4.0, H-1), 4.27 (1H, dd, *J* 5.0, 4.0, H-2), 3.95 (1H, dd, *J* 7.5, 5.0, H-3), 3.87 (1H, dd, *J* 9.1, 8.8, H-6), 3.61 (1H, ddd, *J* 10.2, 7.5, 2.8, H-4), 3.29 (1H, ddd,

J 10.2, 8.8, 2.9, H-5), 2.54 (1H, d, *J* 2.8, C(4)O*H*), 2.44 (1H, d, *J* 2.9, C(5)O*H*), 2.11 (3H, s, H-8), 2.99–1.87 (2H, m, H-10 or H-13), 1.76–1.56 (6H, m, H-11, H-12 and H-10 or H-13), 0.89 (9H, s, C(15)CH_3), 0.14 (3H, s, Si(14)CH_3), 0.13 (3H, s, Si(14)CH_3); ^{13}C NMR (126 MHz; CD_2Cl_2) δ_C 170.4 (C-7), 120.1 (C-9), 78.4 (C-3), 74.8 (C-5), 74.6 (C-2), 74.4 (C-4), 72.8 (C-1), 72.3 (C-6), 38.1, 38.0 (C-10, C-13), 25.9 (C(15)*C*H$_3$), 24.2, 23.9 (C-11, C-12), 21.4 (C-8), 18.4 (C-15), –4.2, –4.4 (Si(CH_3); $\bar{\nu}_{max}$(thin film)/cm^{-1} 3445 (O-H) (br), 2980 (s), 2971 (s), 2930 (m), 2890 (m), 2858 (m), 2360 (w), 2341 (w), 1745 (C=O) (m), 1473 (w), 1462 (w), 1380 (m), 1249 (m), 1144 (m), 1117 (m), 1072 (m), 1036 (m), 967 (m), 871 (w), 838 (m), 780 (m); LRMS *m/z* (ESI$^+$) 425.2 ([M+Na]$^+$, 100%); HRMS *m/z* (ESI$^+$) found 425.19632 [M+Na]$^+$ ($C_{19}H_{34}O_7NaSi$ requires 425.19660 [M+Na]$^+$). Spectral data for (–)-**270** are identical to those reported above.

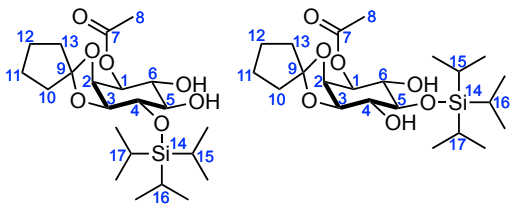
Method 5 To a solution of triol (–)-**269** (12 mg, 0.0416 mmol, 1.0 eq) in anhydrous pyridine (0.30 mL) was added TBDMSOTf (11 μL, 0.0458 mmol, 1.1 eq) at 0 °C. The reaction mixture was stirred for 2 hours and warmed to RT. After 23 hours of stirring, the solvent was concentrated *in vacuo* and the residue obtained was purified by column chromatography over silica gel (Petroleum ether/EtOAc 9.5:0.5, 9:1, 8:2, 7:3, 6:4, 5:5) to give (–)-**270** (7 mg, 40%) as a colourless solid as well as (–)-**272** (3 mg, 19%) and (–)-**271** (6 mg, 36%) as colourless oils. Spectral data are identical to those reported above.

Method 6 To a solution of triol (–)-**269** (100 mg, 0.347 mmol, 1.0 eq) in anhydrous pyridine (1.2 mL) was added TBDMSOTf (88 μL, 0.382 mmol, 1.1 eq) at –30 °C. The reaction mixture was stirred for 24 hours and the solvent was then concentrated *in vacuo*. The residue obtained was purified by column chromatography over silica gel (Petroleum ether/EtOAc 9:1, 8:2, 7:3, 6:4, 5:5) to give (–)-**270** (68 mg, 49%) as a colourless solid as well as (–)-**272** (15 mg, 11%) and (–)-**271** (51 mg, 36%) as colourless oils. Spectral data are identical to those reported above.

Method 7 To a solution of triol (–)-**269** (100 mg, 0.347 mmol, 1.0 eq) and 2,6-lutidine (61 μL, 0.520 mmol, 1.5 eq) in anhydrous THF (3.6 mL) was added TBDMSOTf (81 μL, 0.347 mmol, 1.0 eq) at –78 °C. After stirring for 24 hours, TBDMSOTf (40 μL, 0.175 mmol, 0.505 eq) and 2,6-lutidine (31 μL, 0.267 mmol, 0.77 eq) were added sequentially and the solution was allowed to stir for another

2 hours. H_2O (5 mL) was then added and the aqueous layer was extracted with EtOAc (4 × 5 mL). The combined organic layers were washed with H_2O (5 mL) and brine (5 mL), then dried ($MgSO_4$), filtered, and concentrated *in vacuo*. Purification by column chromatography over silica gel (Petroleum ether/EtOAc 6:4, 5:5) afforded (–)-**270** (76 mg, 55%) as a colourless solid as well as (–)-**272** (46 mg, 33%) and (–)-**271** (11 mg, 8%) as colourless oils. Spectral data are identical to those reported above.

(–)-1D-1-*O*-Acetyl-2,3-*O*-cyclopentylidene-4-*O*-(triisopropylsilyl)-*myo*-inositol (–)-273 & (–)-1D-1-*O*-acetyl-2,3-*O*-cyclopentylidene-5-*O*-(triisopropylsilyl)-*myo*-inositol (–)-274

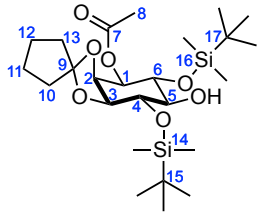


To a solution of triol (–)-**269** (350 mg, 1.21 mmol, 1.0 eq) and 2,6-lutidine (435 μL, 3.74 mmol, 3.08 eq) in anhydrous THF (13 mL) was added TIPSOTf (718 μL, 2.67 mmol, 2.2 eq) dropwise at –78 °C. After 16.5 hours of stirring, H_2O (10 mL) was added and the aqueous layer was extracted with EtOAc (4 × 15 mL). The combined organic layers were washed with H_2O (15 mL) and brine (15 mL), then dried ($MgSO_4$), filtered, and concentrated *in vacuo*. Purification by column chromatography over silica gel (Petroleum ether/EtOAc 6:4, 5:5) afforded (–)-**273** (384 mg, 71%) as a colourless solid and the regioisomer (–)-**274** (109 mg, 20%) as a colourless oil: (–)-**273** R_f 0.76 (Petroleum ether/EtOAc 5:5); $[\alpha]_D^{25} = -40.5$ (*c* 1.0, $CHCl_3$); m.p. 80–83 °C (Hexane/EtOAc); ^{1}H NMR (500 MHz; $CDCl_3$) δ_H 5.12 (1H, dd, *J* 9.8, 3.9, H-1), 4.40 (1H, dd, *J* 5.9, 3.9, H-2), 4.09 (1H, dd, *J* 5.9, 5.8, H-3), 3.97 (1H, dd, *J* 9.8, 7.5, H-6), 3.90 (1H, dd, *J* 7.3, 5.8, H-4), 3.50 (1H, dd, *J* 7.5, 7.3, H-5), 2.17 (3H, s, H-8), 1.96–1.88 (2H, m, H-10 or H-13), 1.73–1.63 (6H, m, H-11, H-12 and H-10 or H-13), 1.21–1.12 (3H, m, H-15, H-16 and H-17), 1.09 (12H, s, C(15)CH_3 and C(17)CH_3), 1.08 (6H, s, C(16)CH_3); ^{13}C NMR (126 MHz; $CDCl_3$) δ_C 171.0 (C-7), 119.9 (C-9), 78.9 (C-3), 76.6 (C-5), 75.4 (C-4), 73.9 (C-2), 72.2 (C-1), 71.3 (C-6), 36.62, 36.61 (C-10, C-13), 23.8,

23.2 (C-11, C-12), 21.3 (C-8), 18.3, 18.2 (C(15)*C*H$_3$, C(16)*C*H$_3$, C(17)*C*H$_3$), 12.6 (C-15, C-16, C-17); $\bar{\nu}_{max}$(thin film)/cm^{-1} 3495 (O-H) (br), 3274 (O-H) (br), 2943 (m), 2892 (m), 2866 (m), 1720 (C=O) (s), 1464 (m), 1433 (w), 1374 (m), 1332 (m), 1242 (s), 1142 (s), 1117 (s), 1101 (Si-O) (s), 1040 (s), 998 (s), 988 (s), 919 (m), 882 (s), 829 (m), 730 (m), 680 (s), 655 (s); LRMS *m/z* (ESI$^+$) 467.2 ([M+Na]$^+$, 100%); HRMS *m/z* (ESI$^+$) found 445.26144 [M+H]$^+$ ($C_{22}H_{41}O_7Si$ requires 445.26161 [M+H]$^+$); (–)-**274** R_f 0.83 (Petroleum ether/EtOAc 5:5); $[\alpha]_D^{25} = -15.3$ (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz; $CDCl_3$) δ_H 4.99 (1H, dd, *J* 9.4, 4.0, H-1), 4.30 (1H, dd, *J* 5.0, 4.0, H-2), 3.98 (1H, dd, *J* 7.3, 5.0, H-3), 3.86 (1H, dd, *J* 9.4, 9.3, H-6), 3.59 (1H, dd, *J* 9.6, 7.3, H-4), 3.52 (1H, dd, *J* 9.6, 9.3, H-5), 2.18 (3H, s, H-8), 2.01–1.91 (2H, m, H-10 or H-13), 1.77–1.59 (6H, m, H-11, H-12 and H-10 or H-13), 1.23–1.15 (3H, m, H-15, H-16 and H-17), 1.10 (12H, s, C(15)C*H*$_3$ and C(17)C*H*$_3$), 1.08 (6H, s, C(16)C*H*$_3$); ^{13}C NMR (126 MHz; $CDCl_3$) δ_C 170.9 (C-7), 120.2 (C-9), 78.3 (C-3), 76.1 (C-5), 75.4 (C-4), 74.1 (C-2), 72.2 (C-1), 71.4 (C-6), 37.9, 37.8 (C-10, C-13), 23.9, 23.6 (C-11, C-12), 21.2 (C-8), 18.4 (C(15)*C*H$_3$, C(16)*C*H$_3$, C(17)*C*H$_3$), 13.0 (C-15, C-16, C-17); $\bar{\nu}_{max}$(thin film)/cm^{-1} 3477 (O-H) (br), 2943 (m), 2893 (m), 2866 (m), 1728 (C=O) (m), 1465 (w), 1372 (m), 1333 (m), 1239 (s), 1146 (s), 1106 (Si-O) (s), 1034 (s), 972 (m), 918 (m), 882 (s), 819 (m), 756 (s), 734 (s), 717 (m), 678 (s); LRMS *m/z* (ESI$^+$) 467.2 ([M+Na]$^+$, 100%); HRMS *m/z* (ESI$^+$) found 467.24342 [M+Na]$^+$ ($C_{22}H_{40}O_7NaSi$ requires 467.24355 [M+Na]$^+$).

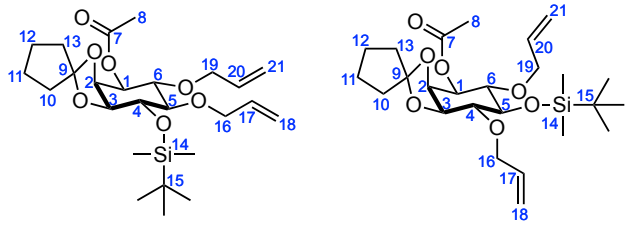
(–)-1D-1-*O*-Acetyl-2,3-*O*-cyclopentylidene-4,6-di-*O*-(*tert*-butyldimethylsilyl)-*myo*-inositol

(–)-275



To a solution of triol (–)-**269** (250 mg, 0.867 mmol, 1.0 eq) and 2,6-lutidine (311 μL, 286 mg, 2.67 mmol, 3.1 eq) in anhydrous THF (9.1 mL) was added TBDMSOTf (438 μL, 504 mg, 1.91 mmol, 2.2 eq) dropwise at –78 °C. The reaction mixture was stirred for 17 hours. H_2O (10 mL) was added and the aqueous layer was extracted with EtOAc (4 × 10 mL). The combined organic layers were washed with H_2O (10 mL) and brine (10 mL), then dried ($MgSO_4$), filtered, and concentrated *in vacuo*. Purification by column chromatography over silica gel (Petroleum ether/EtOAc 7:3, 6:4, 5:5) afforded (–)-**275** (282 mg, 63%) as a colourless oil as well as (–)-**270** (52 mg, 15%) and (–)-**271** (59 mg, 17%) as colourless oils: (–)-**275** R_f 0.64 (Petroleum ether/EtOAc 8:2); $[\alpha]_D^{25}$ = –30.6 (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz; $CDCl_3$) δ_H 5.06 (1H, dd, *J* 8.6, 3.8, H-1), 4.33 (1H, dd, *J* 6.0, 3.8, H-2), 3.98 (1H, dd, *J* 6.2, 6.0, H-3), 3.90 (1H, dd, *J* 8.6, 6.9, H-6), 3.72 (1H, dd, *J* 8.3, 6.2, H-4), 3.37 (1H, ddd, *J* 8.3, 6.9, 4.1, H-5), 2.37 (1H, d, *J* 4.1, C(5)O*H*), 2.12 (3H, s, H-8), 1.96–1.88 (2H, m, H-10 or H-13), 1.75–1.62 (6H, m, H-11, H-12 and H-10 or H-13), 0.91 (9H, s, C(15)CH_3 or C(17)CH_3), 0.87 (9H, s, C(15)CH_3 or C(17)CH_3), 0.14 (6H, s, Si(14)CH_3 or Si(16)CH_3), 0.12 (3H, s, Si(14)CH_3 or Si(16)CH_3), 0.11 (3H, s, Si(14)CH_3 or Si(16)CH_3); ^{13}C NMR (126 MHz; $CDCl_3$) δ_C 170.5 (C-7), 119.6 (C-9), 78.8 (C-3), 76.6 (C-5), 74.9 (C-4), 74.1 (C-2), 72.9 (C-1), 72.7 (C-6), 36.8, 36.7 (C-10, C-13), 26.0, 25.9 (C(15)*C*H$_3$, C(17)*C*H$_3$), 23.9, 23.3 (C-11, C-12), 21.4 (C-8), 18.25, 18.20 (C-15, C-17), –4.2, –4.3, –4.6, –4.7 (Si(14)*C*H$_3$, Si(16)*C*H$_3$); $\bar{\nu}_{max}$(thin film)/cm^{-1} 3658 (O-H) (br), 2980 (s), 2931 (m), 2889 (m), 2858 (m), 1744 (C=O) (m), 1473 (m), 1463 (m), 1379 (br), 1336 (m), 1250 (Si-CH$_3$) (s), 1149 (m), 1106 (Si-O) (s), 1030 (m), 967 (m), 910 (w), 837 (s), 779 (s), 669 (w); LRMS *m/z* (ESI$^+$) 517.3 ([M+H]$^+$, 70%), 539.3 ([M+Na]$^+$, 100%); HRMS *m/z* (ESI$^+$) found 517.30109 [M+H]$^+$ ($C_{25}H_{49}O_7Si_2$ requires 517.30113 [M+H]$^+$).

1D-1-*O*-Acetyl-2,3-*O*-cyclopentylidene-4-*O*-(*tert*-butyldimethylsilyl)-5,6-di-*O*-allyl-*myo*-inositol 276 & 1D-1-*O*-acetyl-2,3-*O*-cyclopentylidene-5-*O*-(*tert*-butyldimethylsilyl)-4,6-di-*O*-allyl-*myo*-inositol 277

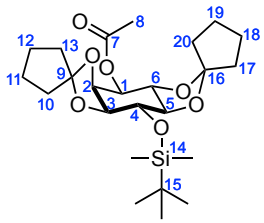


Method 1 A solution of diol (–)-**270** (35 mg, 0.0869 mmol, 1.0 eq) and the allylating agent **207** (110 mg, 0.696 mmol, 8.0 eq) in freshly distilled, degassed THF (4.2 mL) and under a constant flow of Ar was heated under reflux for 15 minutes. $Pd(PPh_3)_4$ (10 mg, 0.00869 mmol, 0.10 eq) was then added to the solution in one portion. After 6.5 hours, the reaction mixture was concentrated *in vacuo* and the residue obtained was purified by column chromatography over silica gel (Petroleum ether/Et_2O 10:0, 9.9:0.1, 9.8:0.2, 9.7:0.3, 9.6:0.4, 9.5:0.5, 9.4:0.6, 9.2:0.8, 8.8:1.2) to give an inseparable mixture of two regioisomers (–)-**276** (Isomer A) and (–)-**277** (Isomer B) (35 mg, 52% (–)-**276**, 31% (–)-**277**) as a colourless oil, in a ratio of 1.6:1.0 as determined by ^{1}H NMR: R_f 0.47 (Petroleum ether/Et_2O 8:2); ^{1}H NMR (500 MHz; $CDCl_3$) δ_H 5.99–5.83 (4H, m, H-17 and H-20 Isomers A and B), 5.30–5.18 (4H, m, H-18 and H-21 Isomers A and B), 5.16–5.09 (5H, m, H-1 Isomer A, H-18' and H-21' Isomers A and B), 5.07 (1H, dd, *J* 8.1, 3.7, H-1 Isomer B), 4.33 (1H, dddd, *J* 12.3, 5.6, 1.4, 1.4, H-16 or H-19 Isomer B), 4.27–4.10 (9H, m, H-2, H-16' and H-19' Isomer A and B, H-16 and H-19 Isomer A, H-16 or H-19 Isomer B), 4.04 (1H, dd, *J* 6.6, 6.4, H-3 Isomer B), 3.95 (1H, dd, *J* 6.1, 6.0, H-3 Isomer A), 3.75 (1H, dd, *J* 8.6, 6.1, H-4 Isomer A), 3.68 (1H, dd, *J* 8.5, 8.5, H-6 Isomer A), 3.56 (1H, dd, *J* 8.1, 8.0, H-6 Isomer B), 3.48 (1H, dd, *J* 9.2, 8.0, H-5 Isomer B), 3.41 (1H, dd, *J* 9.2, 6.6, H-4 Isomer B), 3.19 (1H, dd, *J* 8.6, 8.5, H-5 Isomer A), 2.12 (3H, s, H-8 Isomer A), 2.11 (3H, s, H-8 Isomer B), 1.99–1.86 (4H, m, H-10 or H-13 Isomers A and B), 1.75–1.59 (12H, m, H-11, H-12 and H-10 or H-13 Isomers A and B), 0.90 (18H, s, C(15)CH_3 Isomers A and B), 0.12 (3H, s, Si(14)CH_3 Isomer A), 0.09 (3H, s, Si(14)CH_3 Isomer A), 0.083 (3H, s, Si(14)CH_3 Isomer B), 0.079 (3H, s, Si(14)CH_3 Isomer B); ^{13}C NMR (126 MHz; $CDCl_3$) δ_C 170.5

(C-7 Isomer A), 170.4 (C-7 Isomer B), 135.4, 134.8 (C-17, C-20 Isomer B), 135.1, 135.0 (C-17, C-20 Isomer A), 119.9 (C-9 Isomer B), 119.8 (C-9 Isomer A), 116.9, 116.74, 116.68 (C-18, C-21 Isomers A and B), 82.7 (C-5 Isomer A), 82.0 (C-4 Isomer B), 79.9 (C-6 Isomer B), 79.5 (C-3 Isomer A), 79.1 (C-3 Isomer B), 79.0 (C-6 Isomer A), 75.1 (C-4 Isomer A), 74.6 (C-5 Isomer B), 74.2 (C-2 Isomer A), 74.1, 73.9, 73.7, 73.6, 72.9 (C-16, C-19 Isomers A and B, C-2 Isomer B), 71.2 (C-1 Isomer B), 71.1 (C-1 Isomer A), 37.4, 37.2 (C-10, C-13 Isomer B), 37.1, 36.9 (C-10, C-13 Isomer A), 26.1 (C(15)*C*H_3 Isomer B), 26.0 (C(15)*C*H_3 Isomer A), 24.0, 23.5 (C-11, C-12 Isomer B), 23.7, 23.3 (C-11, C-12 Isomer A), 21.30 (C-8 Isomer B), 21.29 (C-8 Isomer A), 18.29 (C-15 Isomer B), 18.26 (C-15 Isomer A), –4.1, –4.3 (Si(14)*C*H_3 Isomer B), –4.3, –4.4 (Si(14)*C*H_3 Isomer A); $\bar{\nu}_{max}$(thin film)/cm^{-1} 2955 (w), 2929 (w), 2895 (w), 2856 (w), 1746 (C=O) (m), 1472 (w), 1462 (w), 1371 (m), 1335 (m), 1235 (Si-CH_3) (s), 1153 (m), 1105 (Si-O) (s), 1075 (s), 1039 (s), 921 (m), 856 (s), 836 (s), 778 (s), 726 (w); LRMS *m/z* (ESI^+) 483.3 ($[M+H]^+$, 59%), 505.3 ($[M+Na]^+$, 100%); HRMS *m/z* (ESI^+) found 505.25887 $[M+Na]^+$ ($C_{25}H_{42}O_7NaSi$ requires 505.25920 $[M+Na]^+$).

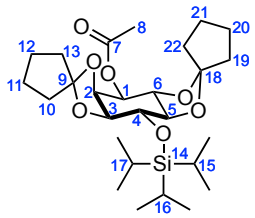
Method 2 A solution of the allylating agent **207** (73 mg, 0.463 mmol, 8.0 eq) and $Pd(PPh_3)_4$ (45 mg, 0.0390 mmol, 0.10 eq) in freshly distilled, degassed THF (3 mL) and under a flow of Ar was heated under reflux for 5 minutes. A solution of the diol (–)-**270** (23 mg, 0.579 mmol, 1.0 eq) was then added dropwise to the reaction mixture over 15 minutes. After 1.5 hours, the reaction mixture was concentrated *in vacuo* and the residue obtained was purified by column chromatography over silica gel (Petroleum ether/Et_2O 10:0, 9.5:0.5, 9:1, 8.5:1.5, 8:2) to give an inseparable mixture of isomers (–)-**276** and (–)-**277** (30 mg, 51% (–)-**276**, 35% (–)-**277**) as a colourless oil, in a ratio of 1.5:1.0 as determined by ^1H NMR. Spectral data are identical to those reported above.

(–)-1D-1-*O*-Acetate-2,3-*O*-cyclopentylidene-4-*O*-(*tert*-butyldimethylsilyl)-5,6-*O*-cyclopentylidene-*myo*-inositol (–)-283



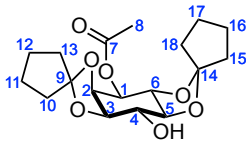
To a clear solution of (–)-**270** (51 mg, 0.128 mmol, 1.0 eq) and **260** (297 μL, 2.17 mmol, 17 eq) in CH_2Cl_2 (0.30 mL) was added PTSA·H_2O (1 mg, 0.00638 mmol 0.05 eq). The resulting yellow solution was allowed to stir at RT for 4 hours. The brown reaction mixture obtained was then quenched with Et_3N (0.9 μL, 0.00638 mmol, 0.05 eq) and concentrated *in vacuo*. The residue obtained was purified by column chromatography over silica gel (Petroleum ether/Et_2O 10:0, 9.5:0.5, 9:1) to give (–)-**283** (37 mg, 62%) as a glassy solid and some recovered starting material (–)-**270** (9 mg, 18%): R_f 0.30 (Petroleum ether/EtOAc 9:1); $[\alpha]_D^{25}$ = –39.9 (*c* 1.0, $CHCl_3$); m.p. 93–95 °C (Hexane/EtOAc); ^{1}H NMR (500 MHz; $CDCl_3$) δ_H 5.06 (1H, dd, *J* 10.6, 4.4, H-1), 4.42 (1H, dd, *J* 4.7, 4.4, H-2), 3.98 (1H, dd, *J* 6.2, 4.7, H-3), 3.90 (1H, dd, *J* 10.6, 9.5, H-6), 3.78 (1H, dd, *J* 10.4, 6.2, H-4), 3.26 (1H, dd, *J* 10.4, 9.5, H-5), 2.16 (3H, s, H-8), 1.95–1.57 (16H, m, H-10 to H-13 and H-17 to H-20), 0.91 (9H, s, C(15)CH_3), 0.11 (3H, s, Si(14)CH_3), 0.10 (3H, s, Si(14)CH_3); ^{13}C NMR (126 MHz; $CDCl_3$) δ_C 170.7 (C-7), 121.9 (C-16), 119.6 (C-9), 82.3 (C-3), 79.3 (C-5), 75.8 (C-2), 74.81 (C-4), 74.75 (C-6), 71.3 (C-1), 37.7, 37.5 (C-17, C-20), 37.5, 37.4 (C-10, C-13), 25.9 (C(15)CH$_3$), 23.6, 23.50, 23.47, 23.4 (C-11, C-12, C-18, C-19), 21.3 (C-8), 18.4 (C-15), –4.4 (Si(14)CH$_3$), –4.5 (Si(14)CH$_3$); $\bar{\nu}_{max}$(thin film)/cm^{-1} 2958 (m), 2935 (m), 2891 (m), 2857 (m), 2360 (w), 1745 (C=O) (s), 1473 (w), 1463 (w), 1434 (w), 1371 (m), 1330 (m), 1239 (Si-CH_3) (s), 1201 (m), 1171 (m), 1115 (Si-O) (s), 1104 (s), 1041 (m), 1005 (m), 955 (m), 858 (s), 838 (s), 780 (s); LRMS *m/z* (ESI$^+$) 469.3 ([M+H]$^+$, 15%), 491.2 ([M+Na]$^+$, 100%); HRMS *m/z* (ESI$^+$) found 491.27963 [M+Na]$^+$ ($C_{25}H_{44}O_6NaSi$ requires 491.27994 [M+Na]$^+$).

(–)-1D-1-*O*-Acetyl-2,3-*O*-cyclopentylidene-4-*O*-(triisopropylsilyl)-5,6-*O*-cyclopentylidene-*myo*-inositol (–)-284



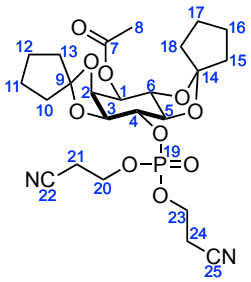
To a clear solution of (–)-**273** (310 mg, 0.697 mmol, 1.0 eq) and **260** (1.62 mL, 11.9 mmol, 17 eq) in CH_2Cl_2 (1.6 mL) was added PTSA·H_2O (7 mg, 0.0349 mmol 0.050 eq). The resulting yellow solution was stirred at 30 °C for 18 hours. The brown reaction mixture obtained was then quenched with Et_3N (5 μL, 0.0349 mmol, 0.050 eq) and concentrated *in vacuo*. The residue obtained was purified by column chromatography over silica gel (Petroleum ether/Et_2O 10:0, 9.5:0.5, 9:1) to give (–)-**284** (303 mg, 85%) as a colourless oil: R_f 0.20 (Petroleum ether/EtOAc 7:3); $[\alpha]_D^{25} = -26.7$ (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz; $CDCl_3$) δ_H 5.06 (1H, dd, *J* 10.6, 4.6, H-1), 4.44 (1H, dd, *J* 5.3, 4.6, H-2), 4.00 (1H, dd, *J* 5.8, 5.3, H-3), 3.95 (1H, dd, *J* 10.0, 5.8, H-4), 3.91 (1H, dd, *J* 10.6, 9.8, H-6), 3.29 (1H, dd, *J* 10.0, 9.8, H-5), 2.16 (3H, s, H-8), 1.94–1.76 (6H, m, H-19, H-22 and H-10 or H-13), 1.75–1.55 (10H, m, H-11, H-12, H-20, H-21 and H-10 or H-13), 1.17–1.09 (3H, m, H-15, H-16 and H-17), 1.08 (12H, s, C(15)C*H*$_3$ and C(17)C*H*$_3$), 1.07 (6H, s, C(16)C*H*$_3$); ^{13}C NMR (126 MHz; $CDCl_3$) δ_C 170.7 (C-7), 121.8 (C-18), 119.5 (C-9), 82.9 (C-3), 79.7 (C-5), 75.6 (C-2), 75.0 (C-4), 74.6 (C-6), 71.4 (C-1), 37.51, 37.47 (C-19, C-22), 37.28, 37.21 (C-10, C-13), 23.5, 23.4, 23.3 (C-11, C-12, C-20, C-21), 21.2 (C-8), 18.07, 18.06 (C(15)*C*H$_3$, C(17)*C*H$_3$, C(16)*C*H$_3$), 12.5 (C-15, C-16, C-17); $\bar{\nu}_{max}$(thin film)/cm^{-1} 2944 (m), 2893 (m), 2867 (m), 1745 (C=O) (m), 1464 (w), 1433 (w), 1370 (m), 1330 (m), 1237 (s), 1172 (m), 1115 (s), 1040 (Si-O) (m), 974 (m), 909 (m), 882 (m), 829 (m), 757 (m), 741 (m), 680 (s); LRMS *m/z* (ESI$^+$) 511.4 ([M+H]$^+$, 4%), 533.3 ([M+Na]$^+$, 82%); HRMS *m/z* (ESI$^+$) found 533.29045 [M+Na]$^+$ ($C_{27}H_{46}O_7NaSi$ requires 533.29050 [M+Na]$^+$).

(–)-1D-1-*O*-Acetyl-2,3-*O*-cyclopentylidene-5,6-*O*-cyclopentylidene-*myo*-inositol (–)-291



Method 1 To a solution of (–)-**284** (56 mg, 0.110 mmol, 1.0 eq) in DMF (5 mL) was added TAS-F (91 mg, 0.329 mmol, 3.0 eq) in one portion. After stirring at RT for 2.5 hours the reaction was quenched with phosphate buffer (5 mL, pH 7.4, 50 mM NaH_2PO_4/Na_2HPO_4, 150 mM NaCl) and dissolved with EtOAc (5 mL). The aqueous layer was extracted with EtOAc (4 × 10 mL). The combined organic layers were washed with H_2O (2 × 10 mL), brine (2 × 10 mL) and an aqueous solution of LiCl (10% *w*/*v*, 10 mL), then dried ($MgSO_4$), filtered, and concentrated *in vacuo*. Purification by column chromatography over silica gel (Petroleum ether/EtOAc 10:0, 9:1, 8:2, 7:3, 6:4) afforded (–)-**291** (30 mg, 77%) as a colourless solid: R_f 0.24 (Petroleum ether/EtOAc 6:4); $[\alpha]_D^{25} = -53.2$ (*c* 0.70, $CHCl_3$); m.p. 157–159 °C (Hexane/EtOAc); ^{1}H NMR (500 MHz; $CDCl_3$) δ_H 5.11 (1H, dd, *J* 10.6, 4.5, H-1), 4.46 (1H, dd, *J* 4.7, 4.5, H-2), 4.04 (1H, dd, *J* 6.5, 4.7, H-3), 3.95 (1H, dd, *J* 10.6, 9.3, H-6), 3.87 (1H, dd, *J* 10.9, 6.5, H-4), 3.34 (1H, dd, *J* 10.9, 9.3, H-5), 2.62 (1H, br s, C(4)O*H*), 2.17 (3H, s, H-8), 1.99–1.82 (6H, m, H-15 and H-18, H-10 or H-13), 1.76–1.58 (10H, m, H-11, H-12, H-16, H-17 and H-10 or H-13); ^{13}C NMR (126 MHz; $CDCl_3$) δ_C 170.7 (C-7), 122.8 (C-14), 120.1 (C-9), 81.3 (C-3), 78.5 (C-5), 75.8 (C-2), 75.0 (C-6), 73.9 (C-4), 71.0 (C-1), 37.8, 37.73 (C-15, C-18), 37.72, 37.6 (C-10, C-13), 23.9, 23.64, 23.62, 23.55 (C-11, C-12, C-16 and C-17), 21.2 (C-8); $\bar{\nu}_{max}$(thin film)/cm^{-1} 3460 (O-H) (br), 2970 (m), 2876 (w), 1741 (C=O) (m), 1433 (w), 1372 (m), 1332 (m), 1236 (s), 1201 (m), 1154 (m), 1118 (s), 1091 (s), 1040 (s), 971 (m), 907 (m), 730 (s); LRMS *m*/*z* (ESI$^+$) 377.2 ([M+Na]$^+$, 100%); HRMS *m*/*z* (ESI$^+$) found 377.15693 [M+Na]$^+$ ($C_{18}H_{26}O_7Na$ requires 377.15707 [M+Na]$^+$).

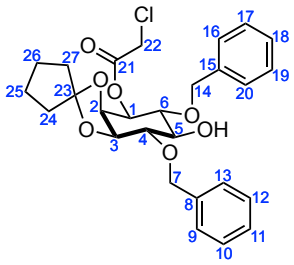
(–)-1D-1-*O*-Acetate-2,3-*O*-cyclopentylidene-4-*O*-(bis(2-cyanoethyl)phosphate)-5,6-*O*-cyclopentylidene-*myo*-inositol (–)-293



To a solution of (–)-**291** (80 mg, 0.226 mmol, 1.0 eq) and phosphoramidite **218** (184 mg, 0.677 mmol, 3.0 eq) in CH_2Cl_2 (4.0 mL) was added 1*H*-tetrazole (1.50 mL, 0.677 mmol, 3.0 eq, 0.45 M in MeCN). After stirring at RT for 20.5 hours, the cloudy reaction mixture was cooled to –78 °C and *m*CPBA (152 mg, 0.677 mmol, 3.0 eq, 77%) was added in a single portion. The reaction mixture was then gradually warmed to RT over 10 minutes, stirred for 2 hours and quenched with an aqueous solution of $Na_2S_2O_3$ (10% *w*/*v*, 5 mL). The aqueous layer was extracted with CH_2Cl_2 (4 × 5 mL) and the combined organic layers were washed with an aqueous solution of $Na_2S_2O_3$ (10% *w*/*v*, 3 × 5 mL), brine (5 mL), and a saturated aqueous solution of $NaHCO_3$ (5 mL), then dried ($MgSO_4$), filtered, and concentrated *in vacuo*. Purification by column chromatography over silica gel (Petroleum ether/EtOAc gradient 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, 0:10) afforded (–)-**293** (105 mg, 86%) as a colourless oil: R_f 0.31 (Petroleum ether/Et_2O 2:8); $[\alpha]_D^{25} = -54.3$ (*c* 2.0, $CHCl_3$); ^{1}H NMR (400 MHz; CD_2Cl_2) δ_H 5.15 (1H, dd, *J* 10.7, 4.2, H-1), 4.57 (1H, ddd, *J* 11.0, 9.0, 6.7, H-4), 4.44 (1H, dd, *J* 4.6, 4.2, H-2), 4.37–4.24 (4H, m, H-20 and H-23), 4.19 (1H, dd, *J* 6.7, 4.6, H-3), 3.99 (1H, dd, *J* 10.7, 9.7, H-6), 3.49 (1H, dd, *J* 9.7, 9.0, H-5), 2.83–2.75 (4H, m, H-21 and H-24), 2.13 (3H, s, H-8), 2.11–2.04 (1H, m, H-10 or H-13), 1.99–1.91 (1H, m, H-10 or H-13), 1.91–1.81 (4H, m, H-15 and H-18), 1.79–1.55 (10H, m, H-10', H-13', H-11, H-12, H-16 and H-17); ^{13}C NMR (101 MHz; CD_2Cl_2) δ_C 170.4 (C-7), 123.3 (C-14), 120.8 (C-9), 116.95, 116.87 (C-22, C-25), 80.3 (d_P, J_P 5.7, C-4), 79.6 (d_P, J_P 3.7, C-3), 77.0 (d_P, J_P 3.7, C-5), 75.9 (C-2), 75.3 (C-6), 70.3 (C-1), 62.74 (d_P, J_P 4.9, C-20 or C-23), 62.67 (d_P, J_P 4.9, C-20 or C-23), 37.91, 37.87, 37.7 (C-10, C-13, C-15, C-18), 24.1, 24.0, 23.8, 23.7 (C-11, C-12, C-16, C-17), 21.1 (C-8), 19.94, 19.87, 19.8 (C-21, C-24); ^{31}P NMR (162 MHz;

CD_2Cl_2) δ_P –0.50 (P-19); $\bar{\nu}_{max}$(thin film)/cm^{-1} 2967 (w), 2361 (C≡N) (m), 2342 (C≡N) (w), 1741 (C=O) (m), 1433 (w), 1372 (w), 1334 (w), 1239 (P=O) (m), 1039 (P-O) (s), 964 (w); LRMS *m/z* (ESI$^+$) 563.2 ([M+Na]$^+$, 100%); HRMS *m/z* (ESI$^+$) found 563.17559 [M+Na]$^+$ ($C_{24}H_{33}O_{10}N_2NaP$ requires 563.17650 [M+Na]$^+$).

(±)-1D-1-*O*-Chloroacetate-2,3-*O*-cyclopentylidene-4,6-di-*O*-benzyl-*myo*-inositol (±)-296

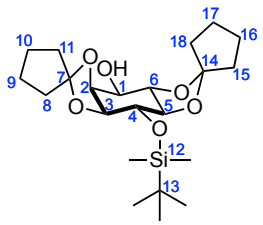


To a solution of **149** (50 mg, 0.139 mmol, 1.0 eq) in vinyl chloroacetate (12.5 mL) and hexane (12.5 mL) was added the immobilised Lipozyme® TL-IM (150 mg). The reaction mixture was stirred at 45 °C for 22 hours. The volatile components were removed *in vacuo* and the residue obtained was purified by column chromatography over silica gel (Petroleum ether/EtOAc 8:2, 7:3, 6:4, 5:5) to afford an inseparable mixture of regioisomers **287** and **295** in a ratio of 5.0:1.0 as determined by ^{1}H NMR (48 mg, 79%). To a clear solution of this inseparable mixture, containing **287** (48 mg, 0.110 mmol, 1.0 eq), and **260** (256 μL, 1.87 mmol, 17 eq) in CH_2Cl_2 (0.30 mL) was added PTSA·H_2O (1 mg, 0.00549 mmol 0.050 eq). The resulting green solution was allowed to stir at RT for 24 hours. The black reaction mixture was then quenched with Et_3N (0.6 μL, 0.00549 mmol, 0.050 eq) and concentrated *in vacuo*. The residue obtained was purified by column chromatography over silica gel (Petroleum ether/EtOAc 9:1, 8:2, 7:3, 6:4, 5:5) to give (–)-**296** (41 mg, 59% over two steps, 79% *e.e.*). Part of this material was crystallised from EtOAc/Hexane (1:1) to afford as a colourless crystalline solid (0% *e.e.*): R_f 0.66 (EtOAc); $[\alpha]_D^{25}$ = –33.7 (*c* 1.0, $CHCl_3$); m.p. 101–102 °C (Hexane/EtOAc); ^{1}H NMR (500 MHz; $CDCl_3$) δ_H 7.40–7.26 (10H, m, H-9 to H-13 and H-16 to H-20), 5.23 (1H, dd, *J* 8.1, 3.7, H-1), 4.91 (1H, d, J_{AB} 11.5, H-7), 4.84 (1H, d, J_{AB} 11.7, H-14), 4.73 (1H, d, J_{AB} 11.7, H-14'), 4.70 (1H, d, J_{AB} 11.5, H-7'), 4.36 (1H, dd, *J* 5.8, 3.7, H-2), 4.21–4.18 (1H,

m, H-3), 4.06 (1H, d, J_{AB} 15.1, H-22), 3.94 (1H, d, J_{AB} 15.1, H-22'), 3.83–3.79 (1H, m, H-6), 3.70–3.62 (2H, m, H-5 and H-4), 2.67 (1H, d, *J* 2.25, C(5)O*H*), 1.96–1.84 (2H, m, H-24 or H-27), 1.76–1.61 (6H, H-25, H-26 and H-24 or H-27); ^{13}C NMR (126 MHz; $CDCl_3$) δ_C 166.8 (C-21), 138.3, 138.1 (C-8, C-15), 128.6, 128.2, 128.05, 127.96, 127.7 (C-9 to C-13 and C-16 to C-20), 120.2 (C-23), 80.7 (C-4), 79.4 (C-6), 78.3 (C-3), 74.4 (C-14), 73.9 (C-2), 73.7 (C-5), 73.4 (C-7), 73.1 (C-1), 40.9 (C-22), 37.2, 37.0 (C-24, C-27), 24.0, 23.5 (C-25, C-26); $\bar{\nu}_{max}$(thin film)/cm^{-1} 3484 (O-H) (br), 3064 (w), 3031 (w), 2955 (m), 2875 (m), 2362 (w), 2342 (w), 1765 (C=O) (s), 1454 (w), 1364 (w), 1310 (m), 1186 (m), 1166 (m), 1107 (s), 1070 (s), 1028 (m), 1002 (s), 974 (m), 737 (s), 698 (s); LRMS *m/z* (ESI$^+$) 504.1 ([M+H]$^+$, 8%), 525.2 ([M+Na]$^+$, 100%); HRMS *m/z* (ESI$^+$) found 525.16465 [M+Na]$^+$ ($C_{27}H_{31}O_7ClNa$ requires 525.16505 [M+Na]$^+$); RP-HPLC (Method 2) t_R = 14.14 min, 96.52%; Chiral HPLC of bulk material (Heptane/IPA 90:10, 0.8 mL.min^{-1}, 254 nm) t_R = 20.36 min, 10.36% (+)-**296**, t_R = 26.18 min, 89.64% (–)-**296**, 79% *e.e.*; Chiral HPLC of crystals (Heptane/IPA 90:10, 0.8 mL.min^{-1}, 254 nm) t_R = 22.24 min, 50.03% (+)-**296**, t_R = 26.18 min, 49.97% (–)-**296**, racemic.*

* *X-ray crystallographic data for this compound can be found in Appendix B*

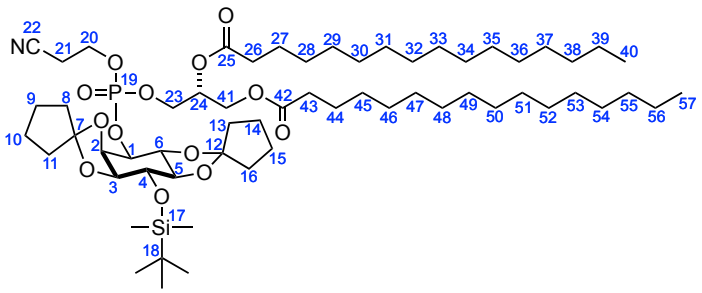
(+)-1D-2,3-*O*-cyclopentylidene-4-*O*-(*tert*-butyldimethylsilyl)-5,6-*O*-cyclopentylidene-*myo*-inositol (+)-297



To a solution of (–)-**283** (25 mg, 0.0527 mmol, 1.0 eq) in MeOH/CH_2Cl_2 (4:1 *v/v*, 2.0 mL) was added a 2.6 M solution of NaOMe (17 μL, 0.0448 mmol, 0.85 eq). The solution was stirred at RT for 1.5 hours, then quenched by addition of H_2O (2 mL) and brine (2 mL). The aqueous layer was extracted with EtOAc (4 × 5 mL) and the combined organic layers were washed with H_2O (5 mL) and brine (2 × 5 mL), then dried ($MgSO_4$) and concentration *in vacuo*. The residue obtained was purified by column chromatography over silica gel (Petroleum ether/EtOAc 9:1, 8.5:1.5, 8:2, 7.5:2.5)

to give (+)-**297** (21 mg, 92%) as a colourless solid: R_f 0.18 (Petroleum ether/EtOAc 8:2); m.p. 126–127 °C; $[\alpha]_D^{25}$ = +21.8 (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz; $CHCl_3$) δ_H 4.28 (1H, dd, *J* 4.9, 4.7, H-2), 3.99 (1H, dd, *J* 6.0, 4.9, H-3), 4.00–3.93 (1H, m, H-1), 3.76 (1H, dd, *J* 10.6, 6.0, H-4), 3.68 (1H, dd, *J* 9.8, 9.7, H-6), 3.18 (1H, dd, *J* 10.6, 9.7, H-5), 2.32 (1H, d, *J* 9.1, C(1)O*H*), 1.98–1.59 (16H, m, H-8 to H-11 and H-15 to H-18), 0.91 (9H, s, C(13)CH_3), 0.11 (3H, s, Si(12)CH_3), 0.10 (3H, s, Si(12)CH_3); ^{13}C NMR (126 MHz; $CHCl_3$) δ_C 121.8 (C-14), 119.5 (C-7), 82.5 (C-3), 79.0 (C-5), 78.25, 78.23 (C-2, C-6), 75.3 (C-4), 70.2 (C-1), 37.3, 37.64, 37.60, 37.2 (C-8, C-11, C-15, C-18), 25.9 (C(13)*C*H_3), 23.48, 23.46, 23.45, 23.39 (C-9, C-10, C-16, C-17), 18.4 (C-13), –4.4 (Si(12)CH_3), –4.5 (Si(12)CH_3); $\bar{\nu}_{max}$(thin film)/cm^{-1} 3427 (O-H) (br), 2957 (m), 2931 (m), 2883 (m), 2857 (m), 2361 (w), 2342 (w), 1473 (w), 1333 (m), 1251 (Si-CH_3) (m), 1188 (m), 1110 (Si-O) (s), 862 (m), 838 (m), 779 (m); LRMS *m/z* (ESI$^+$) 449.2 ([M+Na]$^+$, 100%); HRMS *m/z* (ESI$^+$) found 449.23268 [M+Na]$^+$ ($C_{22}H_{38}O_6NaSi$ requires 449.23299 [M+Na]$^+$).

(–)-1D-1-*O*-(1,2-Di-*O*-palmitoyl-*sn*-glycer-3-yl-(2-cyanoethyl)phosphate)-2,3-*O*-cyclopentylidene-4-*O*-(*tert*-butyldimethylsilyl)-5,6-*O*-cyclopentylidene-*myo*-inositol (–)-288

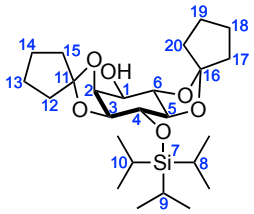


To a solution of (+)-**297** (23 mg, 0.0539 mmol, 1.0 eq) and phosphoramidite (–)-**193** (212 mg, 0.270 mmol, 5.0 eq) in anhydrous CH_2Cl_2 (2.8 mL) was added 1*H*-tetrazole (600 μL, 0.270 mmol, 5.0 eq, 0.45 M in MeCN) dropwise. The cloudy reaction mixture was stirred at RT for 2.5 hours, then cooled to –78 °C and *m*CPBA (61 mg, 0.270 mmol, 5.0 eq, 77%) was added in one portion. The reaction mixture was stirred for 1 hour, warmed to RT and stirred for a further 15 hours. The reaction was quenched by addition of an aqueous solution of $Na_2S_2O_3$ (10% *w/v*, 2 mL). The aqueous phase was extracted with CH_2Cl_2 (4 × 3 mL) and the combined organic layers were washed with an aqueous

solution of $Na_2S_2O_3$ (10% *w*/*v*, 3 mL), and brine (2 × 3 mL), then dried (Na_2SO_4), filtered, and concentrated *in vacuo*. Purification twice by column chromatography over silica gel (Petroleum ether/EtOAc 9.5:0.5, 8:2, 7:3, 6:4) afforded (–)-**288** (41 mg, 68%) as a gummy solid: R_f 0.33 (Petroleum ether/EtOAc 7:3); $[\alpha]_D^{25} = -2.7$ (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz; $CDCl_3$) δ_H 5.25 (2H, m, H-24 Diast. A and B), 4.72–4.65 (2H, m, H-1 Diast. A and B), 4.39 (2H, dd, *J* 10.5, 4.7, H-2 Diast. A and B), 4.37–4.19 (10H, m, H-41, H-20, H-23 and H-23' Diast. A and B), 4.17 (1H, dd, *J* 6.0, 4.4, H-41' Diast. A), 4.15 (1H, dd, *J* 6.0, 4.4, H-41' Diast. B), 4.00–3.96 (2H, m, H-3 Diast. A and B), 3.87 (2H, dd, *J* 9.9, 9.9, 4.0, H-6 Diast. A and B), 3.76 (2H, dd, *J* 10.7, 6.5, H-4 Diast. A and B), 3.24–3.18 (2H, m, H-5 Diast. A and B), 2.82–2.76 (4H, m, H-21 Diast. A and B), 2.35–2.27 (8H, m, H-26 and H-43 Diast. A and B), 1.98–1.55 (40H, m, H-8 to H-11, H-13 to H-16, H-27 and H-44 Diast. A and B), 1.34–1.20 (96H, m, H-28 to H-39 and H-45 to H-56 Diast. A and B), 0.90 (18H, s, C(18)CH_3 Diast. A and B), 0.88 (12H, m, H-40 and H-57), 0.10 (6H, s, Si(17)CH_3), 0.09 (6H, s, Si(17)CH_3); ^{13}C NMR (126 MHz; $CDCl_3$) δ_C 173.4 (C-42 Diast. A and B), 173.0, 172.9 (C-25 Diast. A and B), 122.18, 122.16 (C-12 Diast. A and B), 119.9 (C-7 Diast. A and B), 116.3, 116.2 (C-22 Diast. A and B), 82.4 (d_P, J_P 2.8, C-3 Diast. A and B), 78.9 (d_P, J_P 6.6, C-5 Diast. A and B), 77.0 (d_P, J_P 5.0, C-2 Diast. A and B), 75.7 (d_P, J_P 5.2, C-1 Diast. A and B), 75.4 (d_P, J_P 6.2, C-6 Diast. A and B), 74.7 (d_P, J_P 2.1, C-4 Diast. A and B), 69.4 (d_P, J_P 8.1, C-24 Diast. A), 69.3 (d_P, J_P 7.9, C-24 Diast. B), 66.3 (d_P, J_P 5.6, C-23 Diast. A), 65.9 (d_P, J_P 5.4, C-23 Diast. B), 62.4 (d_P, J_P 4.8, C-20 Diast. A), 62.2 (d_P, J_P 5.2, C-20 Diast. B), 61.9, 61.7 (C-41 Diast. A and B), 37.6, 37.5, 37.4, 37.3 (C-8, C-11, C-13, C-16 Diast. A and B), 34.32, 34.26, 34.1 (C-26, C-43 Diast. A and B), 32.1 (C-38, C-55 Diast. A and B), 29.84, 29.81, 29.6, 29.5, 29.4, 29.28, 29.25 (C-28 to C-37 and C-45 to C-54 Diast. A and B), 25.8 (C(18)*C*H$_3$ Diast. A and B), 25.08, 24.98 (C-27, C-44 Diast. A and B), 23.53, 23.49, 23.45, 23.43, 23.39, 23.36, 23.33 (C-9, C-10, C-14, C-15 Diast. A and B), 22.8 (C-39, C-56 Diast. A and B), 19.5 (d_P, J_P 4.1, C-21 Diast. A), 19.4 (d_P, J_P 3.4, C-21 Diast. B), 18.4 (C-18 Diast. A and B), 14.3 (C-40, C-57 Diast. A and B), –4.4, –4.6 (Si(17)*C*H$_3$); ^{31}P NMR (162 MHz; $CDCl_3$) δ_P –2.67 (P-19 Diast. A), –2.85 (P-19 Diast. B); $\bar{\nu}_{max}$(thin film)/cm^{-1} 2980 (m), 2958 (m), 2924 (s), 2854 (s), 2360 (w), 2341 (w), 1743 (C=O) (m), 1465 (w), 1380 (w), 1331 (w), 1254 (Si-CH$_3$) (m), 1170 (m), 1116

(m), 1071 (Si-O) (m), 1031 (m), 971 (m), 954 (m), 912 (w), 858 (w), 838 (w), 780 (w); HRMS *m/z* (ESI$^+$) found 1110.73653 $[M+H]^+$ ($C_{60}H_{109}O_{13}NPSi$ requires 1110.74003 $[M+H]^+$).

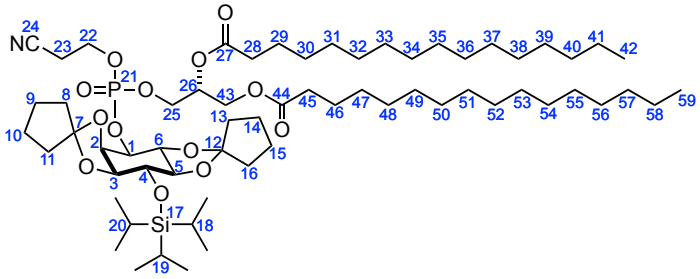
(+)-1D-2,3-*O*-cyclopentylidene-4-*O*-(triisopropylsilyl)-5,6-*O*-cyclopentylidene-*myo*-inositol (+)-298



To a solution of (–)-**284** (150 mg, 0.294 mmol, 1.0 eq) in MeOH/CH_2Cl_2 (4:1 *v/v*, 11.3 mL) was added a 2.6 M solution of NaOMe (96 μL, 0.250 mmol, 0.85 eq). The solution was stirred at RT for 1 hour and then quenched by addition of H_2O (10 mL) and brine (5 mL). The aqueous layer was extracted with EtOAc (4 × 10 mL) and the combined organic layers were successively washed with H_2O (10 mL) and brine (2 × 10 mL), then dried ($MgSO_4$), filtered, and concentrated *in vacuo*. The residue obtained was purified by column chromatography over silica gel (Petroleum ether/EtOAc 9:1, 8.5:1.5, 8:2, 7.5:2.5) to give (+)-**298** (115 mg, 83%) as a colourless oil: R_f 0.35 (Petroleum ether/EtOAc 8:2); $[\alpha]_D^{25}$ = +18.4 (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz; $CDCl_3$) δ_H 4.30 (1H, dd, *J* 5.2, 4.7, H-2), 4.02 (1H, dd, *J* 5.7. 5.2, H-3), 3.97 (1H, ddd, *J* 9.8, 9.5, 4.7, H-1), 3.92 (1H, dd, *J* 10.3, 5.7, H-4), 3.69 (1H, dd, *J* 9.8, 9.7, H-6), 3.21 (1H, dd, *J* 10.3, 9.7, H-5), 2.36 (1H, d, *J* 9.5, C(1)O*H*), 1.95–1.61 (16H, m, H-12 to H-15 and H-17 to H-20), 1.16–1.10 (3H, m, H-8, H-9 and H-10), 1.08 (12H, s, C(8)CH_3 and C(10)CH_3), 1.07 (6H, s, C(9)CH_3); ^{13}C NMR (126 MHz; $CDCl_3$) δ_C 121.7 (C-16), 119.4 (C-11), 83.1 (C-3), 79.3 (C-5), 78.13 (C-2), 78.08 (C-6), 75.6 (C-4), 70.3 (C-1), 37.6, 37.5 (C-17, C-20), 37.4, 37.1 (C-12, C-15), 23.41, 23.39, 23.3 (C-13, C-14, C-18, C-19), 18.08, 18.07 (C(8)*C*H$_3$, C(9)*C*H$_3$, C(10)*C*H$_3$), 12.5 (C-8, C-9, C-10); $\bar{\nu}_{max}$(thin film)/cm^{-1} 3447 (O-H) (br), 2943 (m), 2894 (w), 2867 (m), 1466 (w), 1433 (w), 1332 (m), 1183 (w), 1117 (C=O) (s), 1084 (m), 1017 (Si-O) (m), 998 (m), 882 (m), 835 (m), 734 (m), 681 (m); LRMS *m/z* (ESI$^+$) 491.3 ([M+Na]$^+$,

100%); HRMS *m/z* (ESI$^+$) found 491.27963 $[M+Na]^+$ ($C_{25}H_{44}O_6NaSi$ requires 491.27994 $[M+Na]^+$).

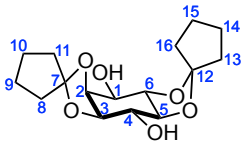
(–)-1D-1-*O*-(1,2-Di-*O*-palmitoyl-*sn*-glycer-3-yl-(2-cyanoethyl)phosphate)-2,3-*O*-cyclopentylidene-4-*O*-(triisopropylsilyl)-5,6-*O*-cyclopentylidene-*myo*-inositol (–)-289



To a solution of (+)-**298** (20 mg, 0.0427 mmol, 1.0 eq) and phosphoramidite (–)-**193** (169 mg, 0.213 mmol, 5.0 eq) in anhydrous CH_2Cl_2 (2.7 mL) was added 1*H*-tetrazole (0.470 mL, 0.213 mmol, 5.0 eq, 0.45 M in MeCN) dropwise. The cloudy reaction mixture was stirred at RT for 5 hours, then cooled to –78 °C and *m*CPBA (48 mg, 0.213 mmol, 5.0 eq, 77%) was added in one portion. The reaction mixture was stirred for 1 hour, warmed to RT and stirred for a further 14 hours. The reaction was quenched by addition of an aqueous solution of $Na_2S_2O_3$ (10% *w*/*v*, 2 mL). The aqueous phase was extracted with CH_2Cl_2 (4 × 3 mL) and the combined organic layers were washed with an aqueous solution of $Na_2S_2O_3$ (10% *w*/*v*, 3 mL), and brine (2 × 3 mL), then dried (Na_2SO_4), filtered, and concentrated *in vacuo*. Purification by column chromatography over silica gel (Petroleum ether/EtOAc 9.5:0.5, 9:1, 8.5:1.5, 8:2, 7.5:2.5, 7:3, 6.5:3.5, 6:4) yielded (–)-**289** (34 mg, 70%) as a gummy solid; R_f 0.77 (Petroleum ether/EtOAc 6:4); $[\alpha]_D^{25} = -1.6$ (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz; $CDCl_3$) δ_H 5.29–5.21 (2H, m, H-26 Diast. A and B), 4.69 (1H, ddd, *J* 18.4, 9.0, 4.6, H-1 Diast. A), 4.69 (1H, ddd, *J* 16.4, 10.0, 4.6, H-1 Diast. B), 4.40 (2H, dd, *J* 10.2, 4.6, H-2 Diast. A and B), 4.37–4.19 (10H, m, H-43, H-22, H-25 and H-25' Diast. A and B), 4.17 (1H, dd, J_{XY} 5.9, 5.9, H-43' Diast. A), 4.15 (1H, dd, J_{XY} 5.9, 5.9, H-43' Diast. B), 4.02–3.98 (2H, m, H-3 Diast. A and B), 3.92 (2H, dd, *J* 10.2, 6.0, H-4 Diast. A and B), 3.88 (2H, ddd, *J* 9.9, 9.0, 3.7, H-6 Diast. A, *J* 10.0, 9.9, 3.7 H-6 Diast. B), 3.23 (2H, ddd, *J* 10.2, 9.9, 1.5, H-5 Diast. A and B), 2.84–2.73 (4H, m, H-23

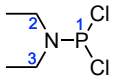
Diast. A and B), 2.33 (2H, t, *J* 7.4, H-28 Diast. A), 2.32 (2H, t, *J* 7.6, H-28 Diast. B), 2.30 (4H, t, *J* 7.4, H-45 Diast. A and B), 1.97–1.54 (40H, m, H-8 to H-11, H-13 to H-16, H-29 and H-46 Diast. A and B), 1.35–1.18 (96H, m, H-30 to H-41 and H-47 to H-58 Diast. A and B), 1.16–1.09 (6H, m, H-18, H-19 and H-20 Diast. A and B), 1.09–1.03 (36H, m, C(18)CH_3, C(19)CH_3 and C(20)CH_3 Diast. A and B), 8.87 (12H, t, *J* 7.0, H-42 and H-59 Diast. A and B); ^{13}C NMR (126 MHz; $CDCl_3$) δ_C 173.37, 173.35 (C-44 Diast. A and B), 173.0, 172.9 (C-27 Diast. A and B), 122.03, 122.01 (C-12 Diast. A and B), 119.8 (C-7 Diast. A and B), 116.27, 116.18 (C-24 Diast. A and B), 82.9 (d_P, J_P 2.6, C-3 Diast. A and B), 79.2 (d_P, J_P 6.4, C-5 Diast. A and B), 76.9 (C-2 Diast. A and B), 75.7 (d_P, J_P 7.6, C-1 Diast. A and B), 75.3 (d_P, J_P 6.2, C-6 Diast. A and B), 74.9 (d_P, J_P 1.9, C-4 Diast. A and B), 69.4 (d_P, J_P 7.8, C-26 Diast. A), 69.3 (d_P, J_P 7.8, C-26 Diast. B), 66.3 (d_P, J_P 5.6, C-25 Diast. A), 65.9 (d_P, J_P 5.4, C-25 Diast. B), 62.4 (d_P, J_P 5.3, C-22 Diast. A), 62.2 (d_P, J_P 5.0, C-22 Diast. B), 61.9, 61.7 (C-43 Diast. A and B), 37.5, 37.47, 37.46, 37.39, 37.37, 37.30, 37.29, 37.2 (C-8, C-11, C-13, C-16 Diast. A and B), 34.31, 34.26, 34.1 (C-28, C-45 Diast. A and B), 32.1 (C-40, C-57 Diast. A and B), 29.84, 29.80, 29.6, 29.5, 29.4, 29.27, 29.25 (C-30 to C-39 and C-47 to C-56 Diast. A and B), 25.0, 24.97 (C-29, C-46 Diast. A and B), 23.5, 23.4, 23.35, 23.33, 23.30, 23.28 (C-9, C-10, C-14, C-15 Diast. A and B), 22.8 (C-41, C-58 Diast. A and B), 19.5 (d_P, J_P 4.5, C-23 Diast. A) 19.4 (d_P, J_P 4.2, C-23 Diast. B), 18.05, 18.04 (C(18)*C*H_3, C(19)*C*H_3, C(20)*C*H_3 Diast. A and B), 14.3 (C-42, C-59 Diast. A and B), 12.5 (C-18, C-19, C-20 Diast. A and B); ^{31}P NMR (162 MHz; $CDCl_3$) δ_P –2.72 (P-21 Diast. A), –2.89 (P-21 Diast. B); $\bar{\nu}_{max}$(thin film)/cm^{-1} 2923 (C-H) (s), 2854 (C-H) (s), 2360 (w), 2342 (w), 1743 (C=O) (s), 1465 (m), 1331 (m), 1281 (m), 1261 (m), 1173 (m), 1117 (Si-O) (s), 1029 (P-O) (s), 911 (m), 883 (m), 737 (w), 724 (w), 682 (w); HRMS *m/z* (ESI$^+$) found 1152.78466 $[M+H]^+$ ($C_{63}H_{115}O_{13}NPSi$ requires 1152.78698 $[M+H]^+$).

(+)-1D-2,3:5,6-di-*O*-Cyclopentylidene-*myo*-inositol (+)-299



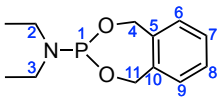
To a solution of (+)-**298** (120 mg, 0.256 mmol, 1.0 eq) in DMF (11 mL) was added TAS-F (212 mg, 0.768 mmol, 3.0 eq) in one portion. After stirring at RT for 1.5 hours, the reaction was quenched with phosphate buffer (5 mL, pH 7.4, 50 mM NaH_2PO_4/Na_2HPO_4, 150 mM NaCl) and dissolved with EtOAc (5 mL). The aqueous layer was extracted with EtOAc (3 × 10 mL). The combined organic layers were washed with H_2O (3 × 10 mL), brine (3 × 10 mL) and an aqueous solution of LiCl (10% *w*/*v*, 10 mL), then dried ($MgSO_4$), filtered, and concentrated *in vacuo*. Purification by column chromatography over silica gel (Petroleum ether/EtOAc gradient 6:4, 5:5, 4:6) yielded (+)-**299** (65 mg, 81%) as a colourless solid: R_f 0.18 (Petroleum ether/EtOAc 5:5); $[\alpha]_D^{25} = +13.4$ (*c* 0.69, $CHCl_3$); m.p. 133–135 °C (Hexane/EtOAc); ^{1}H NMR (500 MHz; $CDCl_3$) δ_H 4.33 (1H, dd, *J* 4.7, 4.7, H-2), 4.06–4.00 (2H, m, H-1 and H-3), 3.86 (1H, ddd, *J* 10.7, 6.6, 2.6, H-4), 2.74 (1H, dd, *J* 9.8, 9.6, H-6), 3.26 (1H, dd, *J* 10.7, 9.6, H-5), 2.60 (1H, d, *J* 2.6, C(4)O*H*), 2.42 (1H, d, *J* 9.0, C(1)O*H*), 2.01–1.58 (16H, m, H-8 to H-11 and H-13 to H-16); ^{13}C NMR (126 MHz; $CDCl_3$) δ_C 122.7 (C-12), 120.1 (C-7), 81.4 (C-3), 78.4 (C-6), 78.3 (C-2, C-5), 74.4 (C-4), 70.0 (C-1), 37.8, 37.5 (C-8, C-11, C-13, C-16), 23.8, 23.61, 23.55 (C-9, C-10, C-14, C-15); $\bar{\nu}_{max}$(thin film)/cm^{-1} 3657 (O-H) (br), 3449 (O-H) (br), 2980 (s), 2972 (s), 2885 (s), 2359 (w), 2342 (w), 1383 (m), 1333 (m), 1251 (w), 1153 (s), 1120 (s), 1079 (s), 1042 (m), 993 (m), 968 (m), 955 (m), 827 (w), 730 (w); LRMS *m*/*z* (ESI$^+$) 313.2 ([M+H]$^+$, 29%), 335.1 ([M+Na]$^+$, 100%); HRMS *m*/*z* (ESI$^+$) found 335.14670 [M+Na]$^+$ ($C_{16}H_{24}O_6Na$ requires 335.14651 [M+Na]$^+$).

Dichloro-*N*,*N*-diethylphosphoramidite 308



A solution of freshly distilled diethylamine (6.03 mL, 58.3 mmol, 2.0 eq) in anhydrous Et_2O (58 mL) was added *via* cannula, over 45 minutes, to a solution of PCl_3 **219** (2.54 mL, 29.1 mmol, 1.0 eq) in anhydrous Et_2O (174 mL) at –78 °C. After addition was complete, the reaction mixture was stirred at –78 °C for 1.5 hour, then warmed to RT, and stirred for a further 18 hours. The resulting precipitate was removed by Schlenk filtration under Ar and washed with Et_2O (2 × 50 mL). The filtrate was concentrated *in vacuo* to give **308** (4.00 g, crude) as a colourless oil that was used without further purification or characterisation. Analysis by ^{31}P NMR showed that this compound was ~95% pure. The product was stored under Ar at –20 °C and was checked by ^{31}P NMR before each use: ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 3.32 (4H, dq, *J* 14.2, 6.8, H-2 and H-3), 1.18 (6H, t, *J* 7.1, C(2)CH_3 and C(3)CH_3); ^{31}P NMR (162 MHz; $CDCl_3$) δ_P 162.43 (P-1). These data are in good agreement with the literature values.[367]

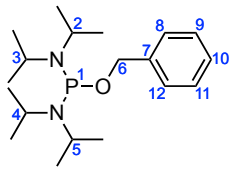
(1,5-Dihydro-2,4,3-benzodioxaphosphepin-3-yl)diethylamine 303



A solution of triethylamine (8.11 mL, 58.3 mmol, 2.0 eq) and 1,2-benzenedimethanol (4.02 g, 29.1 mmol, 1.0 eq) in a mixture of THF (40 mL) and Et_2O (160 mL) was added to a solution of **308** (5.06 g, 29.1 mmol, 1.0 eq) in anhydrous Et_2O (200 mL) at –78 °C, *via* cannula, over 1 hour. The reaction mixture was warmed to RT and stirred for a further 18 hours. The resulting precipitate was removed by Schlenk filtration under Ar and washed with Et_2O (2 × 50 mL). The filtrate was concentrated *in vacuo* to give **303** (5.23 g, crude) as a colourless oil. Analysis by ^{31}P NMR showed that the product was ~87% pure. This unstable phosphoramidite was therefore used in subsequent steps without further purification or characterisation. The product was stored under Ar at –20 °C and

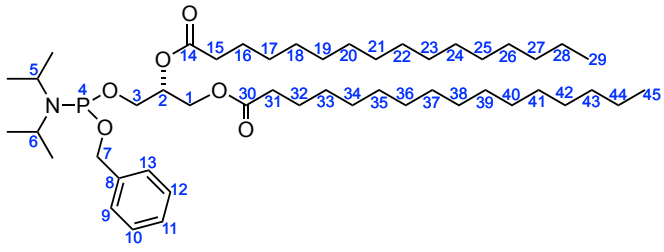
was checked by ^{31}P NMR before each use: ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 7.30–7.16 (4H, m, H-6 to H-9), 5.18 (2H, dd, J_{ABX} 13.8, 7.0, H-4 and H-11), 4.91 (2H, dd, J_{ABX} 19.4, 13.8, H-4' and H-11'), 3.19 (4H, dq, J 9.9, 7.1, H-2 and H-3), 1.11 (6H, t, J 7.1, C(2)CH_3 and C(3)CH_3); ^{31}P NMR (162 MHz; $CDCl_3$) δ_P 145.32 (P-1). These data are in good agreement with the literature values.[367]

Benzyloxy bis(*N*,*N*-diisopropylamino)phosphine 310



PCl_3 **219** (9.55 mL, 109 mmol, 1.0 eq) was dissolved in anhydrous Et_2O (200 mL) and anhydrous pyridine (7.06 mL, 87.4 mmol, 0.80 eq). The resulting mixture was cooled to –78 °C and benzyl alcohol (11.3 mL, 109 mmol, 1.0 eq) in anhydrous Et_2O (50 mL) was added dropwise, *via* cannula, over 1 hour. The mixture was warmed to RT and stirred for a further 19 hours. The resulting colourless precipitate (pyridinium hydrochloride) was removed by Schlenk filtration under Ar and the remaining solid was washed further with anhydrous Et_2O (2 × 25 mL). The supernatant containing **309** was placed under Ar and cooled to –10 °C. DIPA (84.2 mL, 0.601 mmol, 5.5 eq) was added dropwise, *via* cannula, over 2.5 hours so as to keep the temperature below –5 °C. The mixture was stirred at –10 °C for 1 hour then warmed to RT and stirred for 48 hours. The resulting colourless precipitate (DIPA hydrochloride salt) was removed by Schlenk filtration under Ar and washed with anhydrous Et_2O (2 × 25 mL). The supernatant was concentrated *in vacuo* to yield **310** (30.5 g, crude) as a yellow oil. This unstable phosphine was used in subsequent steps without further purification or characterisation. The product was stored under Ar at –20 °C and was checked by ^{31}P NMR before each use: ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 7.42–7.20 (5H, m, H-8 to H-12), 4.65 (2H, d, J 7.3, H-6), 3.65–3.52 (4H, m, H-2, H-3, H-4 and H-5), 1.21–1.16 (24H, m, C(2)CH_3, C(3)CH_3, C(4)CH_3 and C(5)CH_3); ^{31}P NMR (162 MHz; $CDCl_3$) δ_P 123.6 (P-1). These data are in good agreement with the literature values.[375,453,454]

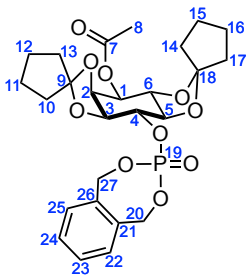
(*R*)-(+)-Benzyloxy-*N*,*N*-diisopropyl-(1,2-di-*O*-palmitoyl-*sn*-glycer-3-yl)phosphoramidite (+)-306



A solution of alcohol (–)-**211** (521 mg, 1.54 mmol, 2.5 eq) in anhydrous CH_2Cl_2 (17 mL) was added dropwise, over 45 minutes, *via* cannula, to a solution of benzyloxy-bis(*N*,*N*-diisopropyl-amino)phosphine **310** (350 mg, 0.615 mmol, 1.0 eq) and 1*H*-tetrazole (4.1 mL, 1.85 mmol, 3.0 eq, 0.45 M in MeCN) in anhydrous CH_2Cl_2 (28 mL). The reaction mixture was stirred at RT for 18 hours, then diluted with CH_2Cl_2 (20 mL) and quenched by addition of a saturated aqueous solution of $NaHCO_3$ (20 mL). The organic phase was extracted with CH_2Cl_2 (3 × 20 mL). The combined organic layers were washed with brine (20 mL), dried ($MgSO_4$), filtered, and concentrated *in vacuo*. The residue was purified by column chromatography over silica gel (Hexane/EtOAc/Et_3N 8:1.5:0.5) to give phosphoramidite (+)-**306** (446 mg, 90%) as a colourless waxy solid, and containing a mixture of two diastereoisomers. This phosphoramidite was synthesised fresh before each use: R_f 0.72 (Hexane/EtOAc 8:2); $[\alpha]_D^{25}$ = +6.8 (*c* 3.0, $CHCl_3$) [lit. [266] $[\alpha]_D^{24}$ = +6.2 (*c* 1.21, $CHCl_3$)]; ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 7.37–7.23 (10H, m, H-9 to H-13 Diast. A and B), 5.22–5.15 (2H, m, H-2 Diast. A and B), 4.79–4.62 (4H, m, H-7, Diast. A and B), 4.35 (1H, dd, J_{XY} 8.3, 3.7, H-1 Diast. A), 4.32 (1H, dd, J_{XY} 8.2, 3.8, H-1 Diast. B), 4.20–4.13 (2H, m, H-1' Diast. A and B), 3.83–3.58 (8H, m, H-5, H-6 and H-3 Diast. A and B), 2.29 (8H, t, *J* 7.6, H-15 and H-31 Diast. A and B), 1.64–1.55 (8H, m, H-16 and H-32), 1.32–1.16 (96H, m, C-17 to C-28 and C-33 to C-44 Diast. A and B), 1.20–1.15 (24H, m, C(5)C*H*$_3$ and C(6)C*H*$_3$ Diast. A and B), 0.88 (12H, t, *J* 6.8, H-29 and H-45 Diast. A and B); ^{31}P NMR (400 MHz; $CDCl_3$) δ_P 148.7 (P-4, Diast. A), 148.5 (P-4, Diast. B); $\bar{\nu}_{max}$(thin film)/cm^{-1} 2962 (m), 2924 (s), 2854 (m), 2360 (w), 2341 (w), 1744 (C=O) (s), 1464 (w), 1310 (w), 1184 (m),

1158 (m), 1125 (w), 1024 (P-O) (m), 976 (m), 771 (w), 731 (w). These data are in good agreement with the literature values.[266,455]

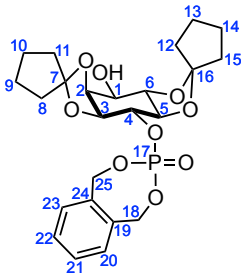
(–)-1D-1-*O*-Acetate-2,3-*O*-cyclopentylidene-4-*O*-(2-oxo-5.6-benzo-1,3,2-dioxaphosphep-2-yl)-5,6-*O*-cyclopentylidene-*myo*-inositol (–)-304



To a solution of (–)-**291** (48 mg, 0.135 mmol, 1.0 eq) and phosphoramidite **303** (97 mg, 0.406 mmol, 3.0 eq) in CH_2Cl_2 (2.3 mL) was added 1*H*-tetrazole (903 μL, 0.406 mmol, 3.0 eq, 0.45 M in MeCN). After stirring at RT for 1.5 hour, the cloudy reaction mixture was cooled to –78 °C and *m*CPBA (91 mg, 0.406 mmol, 3.0 eq, 77%) was added in a single portion. The reaction mixture was then gradually warmed to RT over 20 minutes, stirred for 1 hour and quenched with an aqueous solution of $Na_2S_2O_3$ (10% *w*/*v*, 3 mL). The aqueous layer was extracted with CH_2Cl_2 (4 × 3 mL) and the combined organic layers were washed with an aqueous solution of $Na_2S_2O_3$ (10% *w*/*v*, 3 × 3 mL), brine (3 × 3 mL), and a saturated aqueous solution of $NaHCO_3$ (3 mL), then dried (Na_2SO_4), filtered, and concentrated *in vacuo*. Purification by column chromatography over silica gel (Petroleum ether/EtOAc 9:1, 8:2, 7:3, 6:4, 5:5) afforded (–)-**304** (57 mg, 79%) as an amorphous solid: R_f 0.60 (Petroleum ether/Et_2O 4:6); $[\alpha]_D^{25}$ = –26.4 (*c* 1.0, $CHCl_3$); m.p. 193–195 °C (Hexane/EtOAc); ^{1}H NMR (500 MHz; $CDCl_3$) δ_H 7.39–7.31 (2H, m, H-22 and H-25), 7.31–7.24 (2H, m, H-23 and H-24), 5.34–5.25 (2H, m, H-27 and H-20), 5.20–5.05 (3H, m, H-20', H-27' and H-1), 4.63 (1H, ddd, *J* 10.8, 9.8, 6.6, H-4), 4.46 (1H, dd, *J* 4.5, 4.3, H-2), 4.26 (1H, dd, *J* 6.6, 4.5, H-3), 4.01 (1H, dd, *J* 10.4, 9.5, H-6), 3.51 (1H, dd, *J* 10.8, 9.5, H-5), 2.17 (3H, s, H-8), 2.19–2.11 (1H, m, H-10 or H-13), 2.00–1.50 (15H, m, H-11, H12, H-14 to H-17, H-10', H-13' and H-10 or H-13); ^{13}C NMR (126 MHz; $CDCl_3$) δ_C 170.6 (C-7), 135.5, 135.4 (C-21, C-26), 129.20, 129.17 (C-22, C-25), 129.0, 128.9 (C-23,

C-24), 123.0 (C-18), 120.6 (C-9), 79.4 (d_P, J_P 3.8, C-3), 79.3 (d_P, J_P 5.8, C-4), 76.1 (C-5), 75.9 (C-2), 74.9 (C-6), 70.4 (C-1), 68.80 (d_P, J_P 5.8, C-20 or C-27), 68.75 (d_P, J_P 6.1, C-20 or C-27), 37.8, 37.7 (C-14, C-17), 37.7, 37.5 (C-10, C-13), 24.0, 23.8, 23.63, 23.60 (C-11, C-12, C-15, C-16), 21.2 (C-8); ^{31}P NMR (162 MHz; $CDCl_3$) δ_P –2.10 (P-19); $\bar{\nu}_{max}$(thin film)/cm^{-1} 2961 (w), 2895 (w), 1742 (C=O) (m), 1453 (w), 1371 (w), 1332 (m), 1289 (m), 1226 (m), 1200 (m), 1119 (m), 1090 (m), 1013 (P-O) (s), 973 (m), 917 (m), 849 (m), 777 (w), 729 (s); LRMS *m/z* (ESI$^+$) 559.2 ([M+Na]$^+$, 100%); HRMS *m/z* (ESI$^+$) found 559.17018 [M+Na]$^+$ ($C_{26}H_{33}O_{10}NaP$ requires 559.17035 [M+Na]$^+$); RP-HPLC (Method 2) t_R = 12.67 min, 100%.

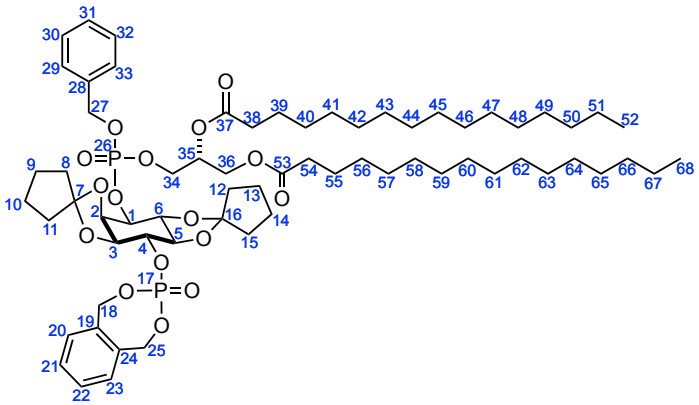
(+)-1D-2,3-*O*-cyclopentylidene-4-*O*-(2-oxo-5.6-benzo-1,3,2-dioxaphosphep-2-yl)-5,6-*O*-cyclopentylidene-*myo*-inositol (+)-305



To a solution of (–)-**304** (53 mg, 0.0984 mmol, 1.0 eq) in MeOH (9 mL) was added ground K_2CO_3 (27 mg, 0.197 mmol, 2.0 eq). The reaction mixture was stirred at RT for 1 hour. The solvent was then partially removed *in vacuo* at RT and H_2O (5 mL) was added to the resulting residue. The aqueous layer was extracted with EtOAc (4 × 6 mL) and the combined organic layers were washed with H_2O (6 mL) and brine (2 × 6 mL), then dried (Na_2SO_4), filtered, and concentrated *in vacuo*. Purification by column chromatography over silica gel (Petroleum ether/EtOAc 9:1, 8:2, 7:3, 6:4, 5:5) afforded (+)-**305** (36 mg, 74%) as an amorphous, colourless foam: R_f 0.32 (Petroleum ether/EtOAc 8:3); $[\alpha]_D^{25}$ = +15.9 (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz; CD_2Cl_2) δ_H 7.43–7.36 (2H, m, H-20 and H-23), 7.36–7.30 (2H, m, H-21 and H-22), 5.37–5.29 (2H, m, H-18 and H-25), 5.09 (1H, dd, J_{ABX} 20.6, 11.4, H-18' or H-25'), 5.05 (1H, dd, J_{ABX} 20.6, 11.5, H-18' or H-25'), 4.56 (1H, ddd, *J* 10.9, 9.6, 6.5, H-4), 4.33 (1H, dd, *J* 4.7, 4.5, H-2), 4.26 (1H, dd, *J* 6.5, 4.7, H-3), 4.00 (1H, ddd,

J 9.3, 9.7, 4.5, H-1), 3.79 (1H, dd, *J* 10.3, 9.7, H-6), 3.43 (1H, dd, *J* 10.3, 9.6, H-5), 2.40 (1H, d, *J* 9.3, C(1)O*H*), 2.14–2.04 (1H, m, H-8 or H-11), 1.96–1.83 (5H, m, H-8 or H-11, H-8', H-11' and H-12 or H-15), 1.82–1.61 (10H, m, H-9, H-10, H-13, H-14 and H-12 or H-15); ^{13}C NMR (126 MHz; CD_2Cl_2) δ_C 136.14, 136.05 (C-19, C-24), 129.61, 129.58 (C-20, C-23), 129.43, 129.40 (C-21, C-22), 122.9 (C-16), 120.6 (C-7), 80.1 (d_P, J_P 5.2, C-4), 80.0 (d_P, J_P 3.4, C-3), 78.9 (C-6), 78.7 (C-2), 77.0 (d_P, J_P 4.5, C-5), 69.8 (C-1), 69.24 (d_P, J_P 3.3, C-18 or C-25), 69.17 (d_P, J_P 3.0, C-18 or C-25), 38.0, 37.90 (C-12, C-15), 37.89, 37.7 (C-8, C-11), 24.1, 24.0, 23.84, 23.81 (C-9, C-10, C-13, C-14); ^{31}P NMR (162 MHz; CD_2Cl_2) δ_P –1.47 (P-17); $\bar{\nu}_{max}$(thin film)/cm^{-1} 3398 (O-H) (br), 2959 (w), 2894 (w), 2874 (w), 1333 (m), 1289 (m), 1224 (w), 1197 (w), 1156 (w), 1121 (m), 1056 (m), 1014 (P-O) (s), 975 (m), 887 (w), 860 (w), 850 (w), 777 (w), 732 (w), 701 (w); LRMS *m/z* (ESI$^+$) 517.2 ([M+Na]$^+$, 100%); HRMS *m/z* (ESI$^+$) found 517.15925 [M+Na]$^+$ ($C_{24}H_{31}O_9NaP$ requires 517.15979 [M+Na]$^+$); RP-HPLC (Method 2) t_R = 11.53 min, 99.33%.

(–)-1D-1-*O*-(1,2-Di-*O*-palmitoyl-*sn*-glycer-3-yl-benzylphosphate)-2,3-*O*-cyclopentylidene-4-*O*-(2-oxo-5.6-benzo-1,3,2-dioxaphosphep-2-yl)-5,6-*O*-cyclopentylidene-*myo*-inositol (–)-307



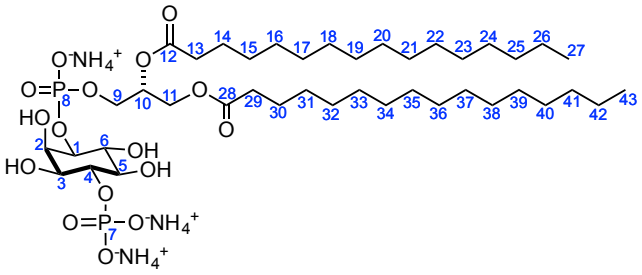
Method 1 To a solution of (+)-**305** (34 mg, 0.0688 mmol, 1.0 eq) and phosphoramidite (+)-**306** (277 mg, 0.344 mmol, 5.0 eq) in anhydrous CH_2Cl_2 (3.5 mL) was added 1*H*-tetrazole (764 μL, 0.344 mmol, 5.0 eq, 0.45 M in MeCN) dropwise. The cloudy reaction mixture was stirred at RT for 4 hours, then cooled to –78 °C and *m*CPBA (77 mg, 0.344 mmol, 5.0 eq, 77%) was added in one portion. The reaction mixture was stirred for 1 hour, warmed to RT, and stirred for a further 18 hours.

The reaction was quenched by addition of an aqueous solution of $Na_2S_2O_3$ (10% *w*/*v*, 3 mL). The aqueous phase was extracted with CH_2Cl_2 (4 × 3 mL) and the combined organic layers were washed with an aqueous solution of $Na_2S_2O_3$ (10% *w*/*v*, 2 × 3 mL), and brine (2 × 3 mL), then dried (Na_2SO_4), filtered, and concentrated *in vacuo*. Three consecutive purifications by column chromatography over silica gel were then carried out (Purification 1 - Petroleum ether/EtOAc 10:0, 9:1, 8:2, 7:3, 6:4; Purification 2 – CH_2Cl_2/MeOH 10:0, 9.5:0.5; Purification 3 - Petroleum ether/EtOAc 10:0, 9:1, 8:2, 7:3, 6:4) yielding (–)-**307** (11 mg, 13%) as a colourless, gummy solid, and containing a mixture of two diastereoisomers: R_f 0.34 (Petroleum ether/EtOAc 5:5); $[\alpha]_D^{25} = -1.9$ (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz; CD_2Cl_2) δ_H 7.46–7.30 (18H, m, H-20 to H-23 and H-29 to H-33 Diast. A and B), 5.38–5.29 (4H, m, H-18 and H-25, Diast. A and B), 5.26–5.18 (2H, m, H-35 Diast. A and B), 5.17–5.06 (6H, m, H-27, H-27' and H-18' or H-25' Diast. A and B), 5.05 (2H, dd, J_{ABX} 13.6, 11.4, H-18' or H-25' Diast. A and B), 4.81–4.70 (2H, m, H-1 Diast. A and B), 4.60 (2H, ddd, *J* 10.8, 9.4, 6.7, H-4 Diast. A and B), 4.46 (2H, ddd, *J* 6.9, 4.4, 4.3, H-2 Diast A and B), 4.30 (2H, ddd, *J* 12.0, 4.0, 4.0, H-36 Diast. A and B), 4.25 (2H, dd, *J* 6.7, 4.4, H-3 Diast. A and B), 4.23–4.10 (6H, m, H-34, H-34' and H-36' Diast. A and B), 4.04 (2H, dd, *J* 9.8, 9.7, H-6 Diast. A and B), 3.50 (2H, ddd, *J* 10.8, 9.7, 4.3, H-5 Diast. A and B), 2.34–2.24 (8H, m, H-38 and H-54 Diast. A and B), 2.18–2.07 (2H, m, H-8 or H-11 Diast A and B), 2.01–1.68 (30H, m, H-8', H-11', H-8 or H-11, H-12, H-15, H-9, H-10, H-13, H-14 Diast. A and B), 1.63–1.50 (8H, m, H-39 and H-55 Diast. A and B), 1.37–1.19 (96H, m, H-40 to H-51 and H-56 to H-67 Diast. A and B), 0.88 (12H, t, *J* 6.8, H-52 and H-68 Diast. A and B); ^{13}C NMR (126 MHz; CD_2Cl_2) δ_C 173.43, 173.41 (C-53 Diast. A and B), 173.05, 173.03 (C-37 Diast. A and B), 136.17 (d_P, J_P 7.6, C-28 Diast. A), 136.1–136.0 (m_P, C-28 Diast. B, C-19 or C-24 Diast. A and B), 135.96 (C-19 or C-24 Diast. A and B), 129.61, 129.60, 129.43, 129.40, 129.05, 129.02, 129.0, 128.96 (C-20 to C-23, C-30 to C-32 Diast. A and B), 128.4, 128.3 (C-29, C-33 Diast. A and B), 123.4 (C-16 Diast. A), 123.3 (C-16 Diast. B), 120.9 (C-7 Diast. A and B), 79.9 (d_P, J_P 3.4, C-3 Diast. A and B), 79.2 (d_P, J_P 5.0, C-4 Diast. A and B), 77.6 (C-2 Diast. A and B), 76.9 (d_P, J_P 3.6, C-5 Diast. A and B), 76.1 (d_P, J_P 6.5, C-6 Diast. A and B), 74.6 (d_P, J_P 4.8, C-1 Diast. A), 74.5 (d_P, J_P 4.8, C-1 Diast. B), 70.2 (d_P, J_P 5.7, C-27 Diast. A), 70.0 (d_P, J_P 5.6, C-27 Diast. B), 69.7 (d_P, J_P 7.3, C-35 Diast. A), 69.6 (d_P, J_P 7.2, C-35 Diast. B), 69.23 (d_P, J_P 4.6, C-18 or C-25 Diast. A and B), 69.17

(d_P, J_P 4.7, C-18 or C-25 Diast. A and B), 66.2 (d_P, J_P 5.7, C-34 Diast. A), 65.9 (d_P, J_P 5.1, C-34 Diast. B), 62.05, 61.96 (C-36 Diast. A and B), 38.0, 37.92, 37.87, 37.84, 37.7 (C-12, C-15, C-8, C-11 Diast. A and B), 34.5, 34.3 (C-38, C-54 Diast. A and B), 32.3 (C-50, C-66 Diast. A and B), 30.11, 30.09, 30.07, 29.9, 29.8, 29.7, 29.52, 29.49 (C-40 to C-49, C-56 to C-65 Diast. A and B), 25.2 (C-39, C-55 Diast. A and B), 24.20, 24.18, 24.1, 23.8 (C-9, C-10, C-13, C-14 Diast. A and B), 23.1 (C-51, C-67 Diast. A and B), 14.3 (C-52, C-68 Diast. A and B); ^{31}P NMR (162 MHz; CD_2Cl_2) δ_P –2.17 (P-26 Diast. A), –2.22 (P-26 Diast. B), –3.32 (P-17); $\bar{\nu}_{max}$(thin film)/cm^{-1} 2955 (m), 2918 (s), 2850 (s), 2361 (w), 2340 (w), 1743 (C=O) (m), 1435 (w), 1419 (w), 1332 (w), 1285 (m), 1171 (m), 1118 (m), 1015 (P-O) (s), 922 (w), 907 (w), 861 (w), 741 (m); LRMS *m/z* (ESI$^+$) 1237.6 ([M+Na]$^+$, 100%), 1238.6 ([M+Na+H]$^+$, 57%); HRMS *m/z* (ESI$^+$) found 1215.68389 [M+H]$^+$ ($C_{66}H_{105}O_{16}P_2$ requires 1215.68724 [M+H]$^+$); NP-HPLC (Method 7) t_R = 4.86 min, 100%.

Method 2 To a solution of (+)-**305** (50 mg, 0.101 mmol, 1.0 eq) and phosphoramidite (+)-**306** (244 mg, 0.303 mmol, 3.0 eq) in anhydrous CH_2Cl_2 (5.0 mL) was added 1*H*-tetrazole (674 μL, 0.303 mmol, 3.0 eq, 0.45 M in MeCN) dropwise. The cloudy reaction mixture was stirred at RT for 19.5 hours, then cooled to –78 °C and *m*CPBA (68 mg, 0.303 mmol, 3.0 eq, 77%) was added in one portion. The reaction mixture was stirred for 1 hour, warmed to RT, stirred for a further 3 hours, then quenched by addition of an aqueous solution of $Na_2S_2O_3$ (10% *w/v*, 5 mL). After stirring for 15 minutes, the aqueous phase was extracted with CH_2Cl_2 (4 × 5 mL) and the combined organic layers were washed with an aqueous solution of $Na_2S_2O_3$ (10% *w/v*, 2 × 3 mL), and brine (2 × 3 mL), then dried (Na_2SO_4), filtered, and concentrated *in vacuo*. Purification by column chromatography over silica gel (Petroleum ether/EtOAc 10:0, 9:1, 8:2, 7:3, 6:4, 5:5) yielded (–)-**307** (68 mg, 55%) as a colourless, gummy solid, and containing a mixture of two diastereoisomers. Spectral data are identical to those reported above.

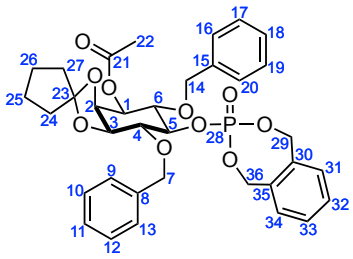
(–)-1D-Dipalmitoyl-phosphatidylinositol-4-phosphate triammonium salt (–)-48



To a solution of (–)-**307** (10 mg, 0.00823 mmol, 1.0 eq) in toluene (0.5 mL) was added TMSBr (21 μL, 0.165 mmol, 20 eq). The solution was heated to 70 °C for 12 hours, then cooled to 0 °C and concentrated by vacuum transfer. The residue was dissolved in toluene (0.75 mL) and concentrated again by vacuum transfer. This was repeated three times. The residue was then dissolved in MeOH (0.50 mL), stirred at 0 °C for 1 hour and the solution was concentrated *in vacuo* at 0 °C. The residue obtained was dissolved in the minimal amount of MeOH/CH_2Cl_2 (1:1, *v*/*v*) and purified by column chromatography over silica gel ($CHCl_3$/MeOH/2.2 M NH_4OH 5:3.9:1.1). [376] The combined fractions were partially concentrated *in vacuo* at 0 °C, then H_2O (30 mL) was added. Lyophilising this solution gave (–)-**48** (6 mg, 68%) as a colourless, fluffy solid.[88] The compound was stored at −80 °C as the ammonium salt: R_f 0.60 ($CHCl_3$/MeOH/2.2 M NH_4OH 5:3.9:1.1); $[\alpha]_D^{25} = -11.7$ (*c* 0.050, MeOH/$CHCl_3$/H_2O 4:3:1); ^{1}H NMR (500 MHz; CD_3OD/$CDCl_3$/D_2O 4:3:1) δ_H 5.28–5.23 (1H, m, H-10), 4.44 (1H, dd, *J* 12.1, 2.7, H-11), 4.25–4.17 (3H, m, H-11', H-4 and H-2), 4.10–4.00 (2H, m, H-9), 3.95–3.89 (1H, m, H-1), 3.82 (1H, dd, *J* 9.6, 9.4, H-6), 3.60 (1H, dd, *J* 9.6, 2.7, H-3), 3.43 (1H, dd, *J* 9.4, 9.2, H-5), 2.37–2.28 (4H, m, H-13 and H-29), 1.65–1.54 (4H, m, H-14 and H-30), 1.36–1.21 (48H, m, H-15 to H-26 and H-31 to H-42), 0.87 (6H, t, *J* 6.9, H-27 and H-43); ^{13}C NMR (126 MHz; CD_3OD/$CDCl_3$/D_2O 4:3:1) δ_C 175.2, 174.9 (C-12, C-28), 78.4 (C-4), 77.3 (d_P, J_P 6.1, C-1), 74.8 (C-5), 72.4 (d_P, J_P 5.7, C-6), 72.1 (C-2), 71.7 (d_P, J_P 2.9, C-3), 71.5 (d_P, J_P 8.7, C-10), 64.5 (d_P, J_P 6.0, C-9), 63.8 (C-11), 35.0, 34.9 (C-13, C-29), 32.7 (C-25, C-41), 30.43, 30.39, 30.35, 30.30, 30.17, 30.10, 29.92, 29.88 (C-15 to C-24 and C-31 to C-40), 25.74, 25.66 (C-14, C-30), 23.4 (C-26, C-42), 14.5 (C-27, C-43); ^{31}P NMR (162 MHz; CD_3OD/$CDCl_3$/D_2O 4:3:1) δ_P 7.86 (P-7), 3.76 (P-8); $\bar{\nu}_{max}$(thin film)/cm^{-1} 3660 (O-H) (w), 3221 (O-H) (br), 2981 (s), 2917 (m), 2890 (m), 2850 (m), 1737

(C=O) (m), 1464 (w), 1382 (m), 1250 (m), 1152 (s), 1061 (s), 953 (P-O) (s), 805 (w); HRMS *m/z* (ESI$^-$) found 889.48442 $[M-H]^-$ ($C_{41}H_{79}O_{16}P_2$ requires 889.48488 $[M-H]^-$). These data are in good agreement with the literature values.[31,64]

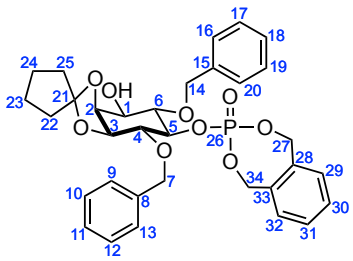
(–)-1D-1-*O*-Acetate-2,3-*O*-cyclopentylidene-4,6-di-*O*-benzyl-5-*O*-(2-oxo-5.6-benzo-1,3,2-dioxaphosphep-2-yl)-*myo*-inositol (–)-311



To a solution of (–)-**261** (400 mg, 0.854 mmol, 1.0 eq) and phosphoramidite **303** (613 mg, 2.56 mmol, 3.0 eq) in CH_2Cl_2 (15 mL) was added 1*H*-tetrazole (5.70 mL, 2.56 mmol, 3.0 eq, 0.45 M in MeCN). After stirring at RT for 1 hour, the cloudy reaction mixture was cooled to –78 °C and *m*CPBA (574 mg, 2.56 mmol, 3.0 eq, 77%) was added in a single portion. The reaction mixture was gradually warmed to RT over 20 minutes, then stirred for 1 hour and finally quenched with an aqueous solution of $Na_2S_2O_3$ (10% *w/v*, 10 mL). The aqueous layer was extracted with CH_2Cl_2 (4 × 10 mL) and the combined organic layers were washed with an aqueous solution of $Na_2S_2O_3$ (10% *w/v*, 3 × 10 mL), brine (3 × 10 mL), and a saturated aqueous solution of $NaHCO_3$ (10 mL), then dried (Na_2SO_4), filtered, and concentrated *in vacuo*. Purification by column chromatography over silica gel (Petroleum ether/EtOAc 9:1, 8:2, 7:3, 6:4, 5:5) afforded (–)-**311** (509 mg, 92%) as an amorphous, colourless foam: R_f 0.66 (Petroleum ether/Et_2O 4:6); $[\alpha]_D^{25}$ = –8.1 (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz; $CDCl_3$) δ_H 7.44–7.39 (2H, m, H-9 and H-13), 7.37–7.24 (10H, m, H-10 to H-12, H-16 to H-20, H-32 and H-33), 7.20–7.14 (2H, m, H-31 and H-34), 5.25 (1H, dd, *J* 8.6, 3.7, H-1), 5.22–5.07 (2H, m, H-29 and H-36), 5.06–4.93 (2H, m, H-29' and H-36'), 4.86 (1H, d J_{AB} 11.3, H-14), 4.85 (1H, d J_{AB} 11.6, H-7), 4.76 (1H, dd J_{AB} 11.6, H-7'), 4.69 (1H, dd, J_{AB} 11.3, H-14'), 4.68–4.61 (1H, m, H-5), 4.39 (1H, dd, *J* 6.2, 3.7, H-2), 4.23 (1H, dd, *J* 6.2, 6.1, H-3), 4.04 (1H, dd, *J* 8.6, 7.0, H-6), 3.90

(1H, dd, *J* 7.8, 6.1, H-4), 2.05 (3H, s, H-22), 1.98–1.84 (2H, m, H-24 or H-27), 1.75–1.61 (6H, m, H-25, H-26 and H-24 or H-27); ^{13}C NMR (126 MHz; $CDCl_3$) δ_C 170.2 (C-21), 138.2, 138.1 (C-8, C-15), 135.42, 135.39 (C-30, C-35), 129.1, 129.0, 128.43, 128.35, 128.1, 127.9, 127.7 (C-32, C-33, C-9 to C-13 and C-16 to C-20), 128.8 (C-31, C-34), 120.2 (C-23), 80.7 (d_P, J_P 6.7, C-5), 79.3 (d_P, J_P 2.9, C-4), 78.0 (d_P, J_P 3.8, C-6), 77.6 (C-3), 74.4 (C-14), 73.8 (C-2), 73.3 (C-7), 70.5 (C-1), 68.4 (d_P, J_P 5.7, C-29, C-36), 36.9, 36.7 (C-24, C-27), 24.1, 23.5 (C-25, C-26), 21.2 (C-22); ^{31}P NMR (162 MHz; $CDCl_3$) δ_P –0.27 (P-28); $\bar{\nu}_{max}$(thin film)/cm^{-1} 2981 (s), 2888 (m), 2363 (w), 2337 (w), 1745 (C=O) (m), 1497 (w), 1374 (m), 1290 (m), 1237 (m), 1152 (m), 1108 (m), 1073 (m), 1015 (P-O) (s), 977 (m), 860 (w), 735 (m), 699 (m); LRMS *m/z* (ESI$^+$) 673.2 ([M+Na]$^+$, 100%); HRMS *m/z* (ESI$^+$) found 673.21543 [M+Na]$^+$ ($C_{35}H_{39}O_{10}NaP$ requires 673.21730 [M+Na]$^+$); RP-HPLC (Method 2) t_R = 14.73 min, 96.87%.

(+)-1D-2,3-*O*-Cyclopentylidene-4,6-di-*O*-benzyl-5-*O*-(2-oxo-5.6-benzo-1,3,2-dioxaphosphep-2-yl)-*myo*-inositol (+)-312

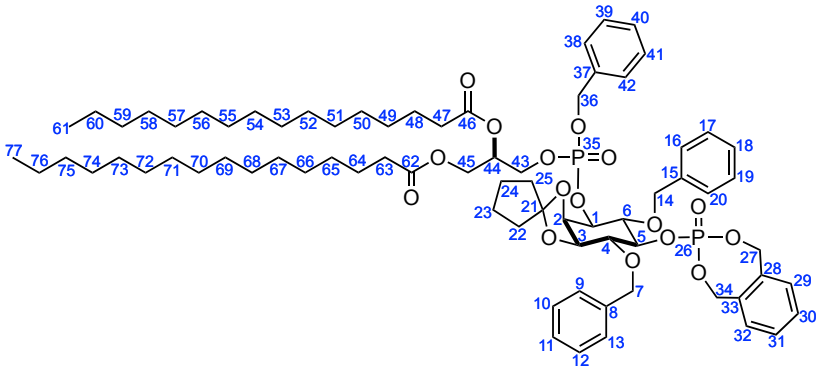


To a solution of (–)-**311** (200 mg, 0.307 mmol, 1.0 eq) in MeOH (28 mL) was added ground K_2CO_3 (85 mg, 0.615 mmol, 2.0 eq). The reaction mixture was stirred at RT for 1 hour. The solvent was then partially removed *in vacuo* at RT and H_2O (10 mL) was added to the resulting residue. The aqueous layer was extracted with EtOAc (4 × 10 mL) and the combined organic layers were washed successively with H_2O (10 mL) and brine (2 × 10 mL), then dried (Na_2SO_4), filtered, and concentrated *in vacuo*. Purification by column chromatography over silica gel (Petroleum ether/EtOAc 9:1, 8:2, 7:3, 6:4, 5:5) afforded (+)-**312** (139 mg, 74%) as a colourless oil: R_f 0.59 (Petroleum ether/EtOAc

2:8); $[\alpha]_D^{25}$ = +3.1 (*c* 2.0, $CHCl_3$); ^{1}H NMR (400 MHz; CD_2Cl_2)[XVI] δ_H 7.45–7.38 (4H, m, H-9, H-13, H-16 and H-20), 7.38–7.25 (8H, m, H-10 to H-12, H-17 to H-19, H-30 and H-31), 7.25–7.18 (2H, m, H-29 and H-32), 5.16–5.06 (3H, m, H-27 or H-34, H-27' and H-34'), 5.00 (1H, dd, J_{ABX} 17.8, 13.8, H-27 or H-34), 4.88 (1H, d, J_{AB} 11.1, H-7 or H-14), 4.87 (1H, d, J_{AB} 11.6, H-7 or H-14), 4.723 (1H, d, J_{AB} 11.6, H-7' or H-14'), 4.719 (1H, d, J_{AB} 11.1, H-7' or H-14'), 4.55 (1H, ddd, *J* 8.4, 8.4, 6.5, H-5), 4.33 (1H, dd, *J* 6.5, 3.7, H-2), 4.23 (1H, dd, *J* 6.5, 6.5, H-3), 4.00 (1H, ddd, *J* 7.7, 4.1, 3.7, H-1), 3.98–3.90 (2H, m, H-4 and H-6), 2.50 (1H, d, *J* 4.1, C(1)O*H*), 2.00–1.91 (2H, m, H-22 or H-25), 1.81–1.62 (6H, m, H-23, H-24 and H-22 or H-25); ^{13}C NMR (101 MHz; CD_2Cl_2) δ_C 138.8, 138.6 (C-8, C-15), 135.9, 135.8 (C-28, C-33), 129.35, 129.32, 129.12, 129.11 (C-29 to C-32), 128.7, 128.6, 128.5, 128.3, 128.1, 127.9 (C-9 to C-13, C-16 to C-20), 120.1 (C-21), 81.0 (d_P, J_P 6.9, C-5), 80.5 (d_P, J_P 3.7, C-4 or C-6), 80.4 (d_P, J_P 3.7, C-4 or C-6), 78.0 (C-3), 75.6 (C-2), 74.3 (C-7 or C-14), 73.6 (C-7 or C-14), 69.4 (C-1), 68.74 (d_P, J_P 6.1, C-27 or C-34), 68.68 (d_P, J_P 6.1, C-27 or C-34), 36.88, 36.87 (C-22, C-25), 24.2, 23.6 (C-23, C-24); ^{31}P NMR (162 MHz; CD_2Cl_2) δ_P –0.31 (P-26); $\bar{\nu}_{max}$(thin film)/cm^{-1} 3398 (O-H) (br), 2981 (m), 2972 (m), 2889 (m), 2361 (w), 2341 (w), 1497 (w), 1454 (w), 1380 (w), 1335 (w), 1278 (m), 1265 (m), 1150 (m), 1103 (m), 1089 (m), 1014 (s), 1001 (P-O) (s), 980 (m), 868 (m), 734 (m), 698 (m), 677 (m), 624 (m); LRMS *m/z* (ESI$^+$) 631.2 ([M+Na]$^+$, 100%); HRMS *m/z* (ESI$^+$) found 631.20654 [M+Na]$^+$ ($C_{33}H_{37}O_9NaP$ requires 631.20674 [M+Na]$^+$); RP-HPLC (Method 2) t_R = 13.95 min, 100%.

[XVI] This compound was found to be unstable in $CDCl_3$. When left in solution for more than 30 minutes, deprotection of the cyclopentylidene protecting group was observed. Characterisation was therefore carried out in CD_2Cl_2 as well as is $CDCl_3$. This is true for any sterically congested, cyclopentylidene containing compounds in this series.

(–)-1D-1-*O*-(1,2-Di-*O*-palmitoyl-*sn*-glycer-3-yl-benzylphosphate)-2,3-*O*-cyclopentylidene-4,6-di-*O*-benzyl-5-*O*-(2-oxo-5.6-benzo-1,3,2-dioxaphosphep-2-yl)-*myo*-inositol (–)-313

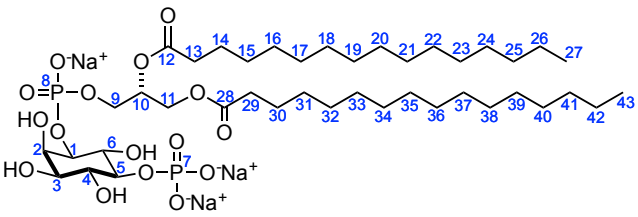


Method 1 To a solution of (+)-**312** (64 mg, 0.104 mmol, 1.0 eq) and phosphoramidite (+)-**306** (423 mg, 0.522 mmol, 5.0 eq) in anhydrous CH_2Cl_2 (5.4 mL) was added 1*H*-tetrazole (470 μL, 0.522 mmol, 5.0 eq, 0.45 M in MeCN) dropwise. The cloudy reaction mixture was stirred at RT for 4 hours, then cooled to –78 °C and *m*CPBA (117 mg, 0.522 mmol, 5.0 eq, 77%) was added in one portion. The reaction mixture was stirred for 1 hour, warmed to RT, and stirred for a further 20.5 hours. The reaction was quenched by addition of an aqueous solution of $Na_2S_2O_3$ (10% *w*/*v*, 5 mL). The aqueous phase was extracted with CH_2Cl_2 (4 × 5 mL) and the combined organic layers were washed with an aqueous solution of $Na_2S_2O_3$ (10% *w*/*v*, 2 × 3 mL), brine (2 × 3 mL), then dried (Na_2SO_4), filtered, and concentrated *in vacuo*. Three consecutive purifications by column chromatography over silica gel were then carried out (Purification 1 - Petroleum ether/EtOAc 10:0, 9:1, 8:2, 7:3, 6:4, 5:5; Purification 2 - CH_2Cl_2/MeOH 10:0, 9.5:0.5; Purification 3 - Petroleum ether/EtOAc 10:0, 9:1, 8:2, 7:3, 6:4) to give (–)-**313** (58 mg, 42%) as a colourless, gummy solid, and containing a mixture of two diastereoisomers: R_f 0.34 (Petroleum ether/EtOAc 5:5); $[\alpha]_D^{25} = -1.8$ (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz; CD_2Cl_2) δ_H 7.43–7.24 (34H, m, H-9 to H-13, H-16 to H-20, H-38 to H-42, H-30 and H-31 Diast. A and B), 7.24–7.19 (4H, m, H-29 and H-32 Diast. A and B), 5.20–5.15 (1H, m, H-44 Diast. A), 5.15–4.92 (13H, m, H-44 Diast. B, H-27, H-34 and H-36 Diast. A and B), 4.87–4.79 (4H, m, H-7 and H-14 Diast. A and B), 4.76–4.70 (4H, m, H-7' and H-14' Diast. A and B), 4.69 (1H, ddd, *J* 9.7, 8.4, 2.0, H-1 Diast. A), 4.68 (1H, ddd, *J* 9.7, 8.5, 1.9, H-1 Diast. B), 4.63–4.56 (2H, m, H-5 Diast. A and B), 4.48 (2H, ddd, *J* 9.7, 6.3, 3.8, H-2 Diast. A and B), 4.27–4.21 (3H, m, H-3 Diast. A and B,

H-45 Diast. A), 4.19–4.04 (7H, m, H-45' Diast. A, H-45 Diast. B, H-43 Diast. A and B, H-43' Diast. A, H-6 Diast. A and B), 4.04–3.98 (1H, m, H-43' Diast. B), 3.94 (1H, dd, J_{XY} 12.0, 6.1, H-45' Diast. B), 3.89 (2H, ddd, J 7.8, 5.8, 2.1, H-4 Diast. A and B), 2.30–2.19 (8H, m, H-47 and H-63 Diast. A and B), 2.00–1.88 (4H, m, H-22 and H-25 Diast. A and B), 1.76–1.63 (12H, m, H-22', H-25', H-23 and H-24 Diast. A and B), 1.63–1.48 (8H, m, H-48 and H-64 Diast. A and B), 1.35–1.19 (96H, m, H-49 to H-60 and H-65 to H-76 Diast. A and B), 0.88 (12H, t, J 6.9, H-61 and H-77 Diast. A and B); ^{13}C NMR (126 MHz; CD_2Cl_2) δ_C 173.41, 173.36 (C-62 Diast. A and B), 173.1, 173.0 (C-46 Diast. A and B), 138.45, 138.37, 138.32 (C-8, C-15 Diast. A and B), 136.24 (d_P, J_P 7.8, C-37 Diast. A), 136.17 (d_P, J_P 7.9, C-37 Diast. B), 135.81, 135.76 (C-28, C-33 Diast. A and B), 129.36, 129.34, 129.11, 128.97, 128.95, 128.92, 128.85, 128.7, 128.6, 128.4, 128.33, 128.29, 128.25, 128.11, 128.06, 128.04 (C-9 to C-13, C-16 to C-20, C-29 to C-32, C-38 to C-42 Diast. A and B), 120.4 (C-21 Diast. A and B), 80.4 (d_P, J_P 6.1, C-5 Diast A and B), 79.5 (d_P, J_P 2.8, C-4 Diast A and B), 78.7–78.4 (m_P, C-6 Diast. A and B), 77.6 (C-3 Diast. A and B), 75.63 (d_P, J_P 4.8, C-1 Diast. A), 75.59 (d_P, J_P 4.6, C-1 Diast. B), 74.6 (C-2, C-7 Diast. A and B), 73.5 (C-14 Diast. A and B), 69.9 (d_P, J_P 2.6, C-36 Diast. A), 69.8 (d_P, J_P 2.3, C-36 Diast. B), 69.7 (d_P, J_P 4.8, C-44 Diast. A), 69.6 (d_P, J_P 5.6, C-44 Diast. B), 68.7 (d_P, J_P 7.0, C-27 and C-34 Diast. A and B), 66.0 (d_P, J_P 5.2, C-43 Diast. A), 65.9 (d_P, J_P 4.9, C-43 Diast. B), 62.0 (C-45 Diast. A), 61.9 (C-45 Diast. B), 36.9, 36.78, 36.76 (C-22, C-25 Diast. A and B), 34.5, 34.4, 34.33, 34.30 (C-47, C-63 Diast. A and B), 32.3 (C-59, C-75 Diast. A and B), 30.12, 30.07, 29.9, 29.8, 29.7, 29.52, 29.49, 29.48 (C-49 to C-58 and C-65 to C-74 Diast. A and B), 25.23, 25.20 (C-48, C-64 Diast. A and B), 24.23, 24.20, 23.6 (C-23, C-24 Diast. A and B), 23.1 (C-60, C-76 Diast. A and B), 14.3 (C-61, C-77 Diast. A and B); ^{31}P NMR (162 MHz; CD_2Cl_2) δ_P –0.90 (P-26), –1.80 (P-35 Diast. A), –1.94 (P-35 Diast. B); $\bar{\nu}_{max}$(thin film)/cm^{-1} 2924 (s), 2853 (m), 1743 (C=O) (m), 1456 (w), 1285 (m), 1266 (m), 1173 (m), 1156 (m), 1112 (m), 1073 (m), 1012 (P-O) (s), 866 (w), 735 (m), 697 (m); LRMS *m/z* (ESI$^+$) 1351.5 ([M+Na]$^+$, 9%); HRMS *m/z* (ESI$^+$) found 1351.70985 [M+Na]$^+$ ($C_{75}H_{110}O_{16}NaP_2$ requires 1351.71613 [M+Na]$^+$); NP-HPLC (Method 7) t_R = 4.07 min, 48.36% Diast. A, t_R = 4.42 min, 49.32% Diast. B.

Method 2 To a solution of (+)-**312** (30 mg, 0.0493 mmol, 1.0 eq) and phosphoramidite (+)-**306** (423 mg, 0.148 mmol, 3.0 eq) in anhydrous CH_2Cl_2 (4.0 mL) was added 1*H*-tetrazole (329 μL, 0.148 mmol, 3.0 eq, 0.45 M in MeCN) dropwise. The cloudy reaction mixture was stirred at RT for 22 hours, then cooled to −78 °C and *m*CPBA (33 mg, 0.148 mmol, 3.0 eq, 77%) was added in one portion. The reaction mixture was stirred for 1 hour, warmed to RT, stirred for a further 3 hours, then quenched by addition of an aqueous solution of $Na_2S_2O_3$ (10% *w*/*v*, 5 mL). After stirring for 15 minutes, the aqueous phase was extracted with CH_2Cl_2 (4 × 5 mL) and the combined organic layers were washed with an aqueous solution of $Na_2S_2O_3$ (10% *w*/*v*, 2 × 3 mL), and brine (2 × 3 mL), then dried (Na_2SO_4), filtered, and concentrated *in vacuo*. Purification by column chromatography over silica gel (Petroleum ether/EtOAc 10:0, 9:1, 8:2, 7:3, 6:4, 5:5) yielded (−)-**313** (54 mg, 82%) as a colourless, gummy solid, and containing a mixture of two diastereoisomers. Spectral data are identical to those reported above.

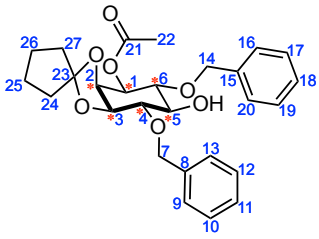
(−)-1D-Dipalmitoyl-phosphatidylinositol 5-phosphate trisodium salt (−)-240



To a solution of (−)-**313** (34 mg, 0.0256 mmol, 1.0 eq) in a mixtue of ^{t}BuOH (1.36 mL) and H_2O (0.24 mL) was added palladium black (55 mg, 0.512 mmol, 20 eq) under $N_{2(g)}$. The flask was then purged with 3 balloons of $H_{2(g)}$, and stirred at RT under $H_{2(g)}$ for 17 hours. $NaHCO_3$ (6 mg, 0.0767 mmol, 3.0 eq) was then added in one portion and the reaction mixture was stirred for a further 4 hours under $H_{2(g)}$. The reaction mixture was filtered through a glass microfiber filter and washed with H_2O (4 × 20 mL). The combined aqueous filtrates were lyophilised to give (−)-**240** (23 mg, 94%) as a fluffy, colourless solid. The compound was stored at −80 °C as the sodium salt: $[\alpha]_D^{25}$ =

–18.1 (*c* 0.076, MeOH/$CHCl_3$/H_2O 4:3:1)[XVII] [lit.[64] $[\alpha]_D^{28}$ = –4.7 (*c* 0.58, $CHCl_3$)]; ^{1}H NMR (500 MHz; CD_3OD/$CDCl_3$/D_2O 4:3:1)[XVIII] δ_H 5.29–5.23 (1H, m, H-10), 4.42 (1H, dd, J_{XY} 12.2, 2.5, H-11), 4.20 (1H, dd, J_{XY} 12.2, 7.8, H-11'), 4.15 (1H, dd, *J* 2.9, 2.8, H-2), 4.08–3.98 (3H, m, H-9 and H-1), 3.85–3.71 (3H, m, H-6, H-5 and H-4), 3.52 (1H, dd, *J* 9.6, 2.9, H-3), 2.37–2.27 (4H, m, H-13 and H-29), 1.65–1.53 (4H, m, H-14 and H-30), 1.34–1.21 (48H, m, H-15 to H-26 and H-31 to H-42), 0.87 (6H, t, *J* 6.9, H-27 and H-43); ^{13}C NMR (126 MHz; CD_3OD/$CDCl_3$/D_2O 4:3:1) δ_C 175.3, 175.0 (C-12, C-28), 78.5 (C-5), 77.0 (d_P, J_P 6.3, C-1), 73.0 (d_P, J_P 2.3, C-4), 72.2 (C-2), 72.1–71.9 (m_P, C-6), 71.9 (C-3), 71.4 (d_P, J_P 8.7, C-10), 64.4 (d_P, J_P 4.6, C-9), 63.8 (C-11), 35.0, 34.9 (C-13, C-29), 32.7 (C-25, C-41), 30.5, 30.43, 30.37, 30.3, 30.2, 30.11, 30.08, 29.94, 29.89 (C-15 to C-24, C-31 to C-40), 25.7, 25.6 (C-14, C-30), 23.4 (C-26, C-42), 14.6 (C-27, C-43); ^{31}P NMR (162 MHz; CD_3OD/$CDCl_3$/D_2O 4:3:1) δ_P 5.39 (P-7), 0.08 (P-8); $\bar{\nu}_{max}$(thin film)/cm^{-1} 3216 (O-H) (br), 2957 (m), 2917 (s), 2850 (s), 1737 (C=O) (m), 1631 (m), 1468 (m), 1379 (m), 1244 (m), 1199 (m), 1097 (P-O) (s), 1041 (P-O) (s), 971 (s), 865 (m), 821 (m), 721 (m); HRMS *m/z* (ESI^-) found 889.48524 $[M–H]^-$ ($C_{41}H_{79}O_{16}P_2$ requires 889.48488 $[M–H]^-$). These data are in good agreement with the literature values.[58,64,87]

D_6 (–)-1D-1-*O*-Acetyl-2,3-*O*-cyclopentylidene-4,6-di-*O*-benzyl-*myo*-inositol (–)-314

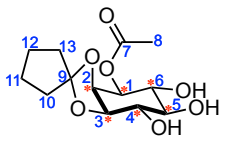


To a clear solution of (–)-**266** (99 mg, 0.242 mmol, 1.0 eq, 89% D_6, 11% D_5) and **260** (565 μL, 4.11 mmol, 17 eq) in CH_2Cl_2 (0.60 mL) was added PTSA·H_2O (4 mg, 0.0194 mmol, 0.080 eq). The resulting green solution was allowed to stir at RT for 19 hours. The dark purple solution was then

[XVII] The optical rotation of this compound could not be obtained in $CHCl_3$ or H_2O due to solubility issues.
[XVIII] The spectra were calibrated to CD_3OD

quenched with Et_3N (3 μL, 0.0194 mmol, 0.080 eq) and concentrated *in vacuo*. The residue obtained was purified by column chromatography over silica gel (Petroleum ether/EtOAc 9:1, 8:2, 7:3, 6:4, 5:5) to give (–)-**314** (100 mg, 87%, 91% D_6, 9% D_5) as a yellow-brown oil: R_f 0.86 (Petroleum ether/EtOAc 2:8); $[\alpha]_D^{25}$ = –29.4 (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz; $CDCl_3$) δ_H 7.41–7.26 (10H, m, H-9 to H-13 and H-16 to H-20), 4.92 (1H, d, J_{AB} 11.6, H-7), 4.82 (1H, d, J_{AB} 11.5, H-14), 4.76 (1H, d, J_{AB} 11.5, H-14'), 4.72 (1H, d, J_{AB} 11.6, H-7'), 2.70 (1H, br s, C(5)O*H*), 2.09 (3H, s, H-22), 1.98–1.84 (2H, m, H-24 or H-27), 1.79–1.58 (6H, m, H-25, H-26 and H-24 or H-27); ^{13}C NMR (126 MHz; $CDCl_3$) δ_C 170.3 (C-21), 138.4, 138.2 (C-8, C-15), 128.5, 128.1, 127.7 (C-9, C-10, C-12, C-13, C-16, C-17, C-19, C-20), 127.9, 127.8 (C-11, C-18), 120.0 (C-23), 80.4 (t_D, J_D 21.7, C-4), 78.9 (t_D, J_D 21.8, C-6), 77.8 (t_D, J_D 22.8, C-3), 74.4 (C-14), 73.9 (t_D, J_D 22.9, C-2), 73.4 (C-7), 73.1 (t_D, J_D 21.6, C-5), 70.8 (t_D, J_D 22.1, C-1), 37.4, 37.2 (C-24, C-27), 24.0, 23.5 (C-25, C-26), 21.2 (C-22); ^{2}H NMR (92 MHz; $CHCl_3$; $CDCl_3$) δ_D 5.19 (1D, br s, D-1), 4.34 (1D, br s, D-2), 4.19 (1D, br s, D-3), 3.81 (1D, br s, D-6), 3.64 (2H, br s, D-5 and D-4); $\bar{\nu}_{max}$(thin film)/cm^{-1} 3486 (O-H) (br), 2962 (w), 2873 (w), 1738 (C=O) (m), 1370 (w), 1337 (m), 1309 (m), 1232 (m), 1211 (m), 1101 (s), 1070 (s), 1026 (s), 995 (s), 911 (m), 733 (s), 697 (s); LRMS *m/z* (ESI$^+$) 496.2 ([MD_5+Na]$^+$, 10%), 497.2 ([MD_6+Na]$^+$, 100%); HRMS *m/z* (ESI$^+$) found 496.23569 [MD_5+Na]$^+$, 497.24162 [MD_6+Na]$^+$ ($C_{27}H_{26}D_6O_7Na$ requires 497.24168 [MD_6+Na]$^+$); RP-HPLC (Method 2) t_R = 14.21 min, 100%.

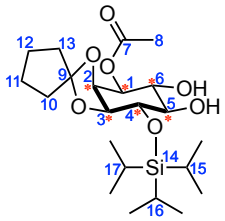
D_6 (–)-1D-1-*O*-Acetyl-2,3-*O*-cyclopentylidene-*myo*-inositol (–)-315



To a solution of (–)-**314** (430 mg, 0.906 mmol, 1.0 eq, 84% D_6, 16% D_5) in EtOAc (17 mL) was added 10% Pd/C (145 mg, 0.136 mmol, 0.15 eq) in one portion. After bubbling two balloons of $H_{2(g)}$ through the reaction, the mixture was stirred vigorously for 2 hours under an atmosphere of $H_{2(g)}$, then filtered through a pad of Celite® and concentrated *in vacuo* to give (–)-**315** (204 mg, 76%, 84% D_6, 16% D_5) as a colourless solid: R_f 0.087 (Petroleum ether/EtOAc 2:8); $[\alpha]_D^{25}$ = –39.0 (*c* 0.70,

$CHCl_3$); m.p. 156–159 °C (EtOAc); ^{1}H NMR (500 MHz; $CDCl_3$) δ_H 4.50 (1H, s, C(5)O*H*), 4.08 (1H, s, C(4)O*H*), 3.89 (1H, br s, C(6)O*H*), 2.18 (3H, s, H-8), 2.01–1.88 (2H, m, H-10 or H-13), 1.77–1.56 (6H, m, H-11, H-12 and H-10 or H-13); ^{13}C NMR (126 MHz; $CDCl_3$) δ_C 171.2 (C-7), 120.2 (C-9), 78.1–77.5 (m_D, C-3), 74.4–73.7 (m_D, C-4, C-2), 73.3–72.7 (m_D, C-5), 71.5–71.0 (m_D, C-1), 70.5–69.9 (m_D, C-6), 37.93, 37.89 (C-10, C-13), 23.9, 23.7 (C-11, C-12), 21.2 (C-8); ^{2}H NMR (92 MHz; $CHCl_3$; $CDCl_3$) δ_D 5.04 (1D, br s, D-1), 4.24 (1D, br s, D-2), 3.92 (2D, br s, D-3 and D-6), 3.60 (1D, br s, D-4), 3.40 (1D, br s, D-5); $\bar{\nu}_{max}$(thin film)/cm^{-1} 3659 (O-H) (br), 3508 (O-H) (br), 3332 (O-H) (br), 2980 (s), 2888 (m), 1723 (C=O) (w), 1473 (w), 1461 (w), 1381 (m), 1337 (w), 1253 (m), 1150 (m), 1101 (m), 1044 (m), 989 (m), 955 (m), 877 (w), 799 (w); LRMS *m/z* (ESI$^+$) 316.2 ([MD_5+Na]$^+$, 18%), 317.2 ([MD_6+Na]$^+$, 100%); HRMS *m/z* (ESI$^+$) found 316.14147 [MD_5+Na]$^+$, 317.14751 [MD_6+Na]$^+$ ($C_{13}H_{14}D_6O_7Na$ requires 317.14778 [MD_6+Na]$^+$).

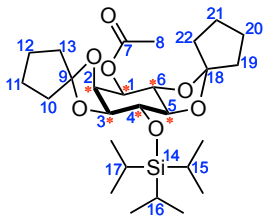
D_6 (–)-1D-1-*O*-Acetyl-2,3-*O*-cyclopentylidene-4-*O*-(triisopropylsilyl)-*myo*-inositol (–)-316



To a solution of triol (–)-**315** (190 mg, 0.646 mmol, 1.0 eq, 84% D_6, 16% D_5) and 2,6-lutidine (232 μL, 1.99 mmol, 3.08 eq) in anhydrous THF (7.0 mL) was added TIPS triflate (382 μL, 1.42 mmol, 2.2 eq) dropwise at –78 °C. After 16 hours of stirring at –78 °C, H_2O (5 mL) was added and the aqueous layer was extracted with EtOAc (4 × 8 mL). The combined organic layers were washed with H_2O (5 mL) and brine (5 mL), then dried ($MgSO_4$), filtered, and concentrated *in vacuo*. Purification by column chromatography over silica gel (Petroleum ether/EtOAc 7:3, 6:4, 5:5) gave (–)-**316** (214 mg, 74%, 84% D_6, 16% D_5) as a colourless solid: R_f 0.76 (Petroleum ether/EtOAc 5:5); $[\alpha]_D^{25}$ = –58.3 (*c* 0.58, $CHCl_3$); m.p. 82–83 °C (Hexane/EtOAc); ^{1}H NMR (500 MHz; $CDCl_3$) δ_H 2.57 (1H, br s, C(5)O*H*), 2.42 (1H, br s, C(6)O*H*), 2.17 (3H, s, H-8), 1.97-1.87 (2H, m, H-10 or H-13), 1.77–1.63 (6H, m, H-11, H-12 and H-10 or H-13), 1.21–1.12 (3H, m, H-15, H-16 and H-17), 1.09

(12H, s, C(15)CH_3 and C(17)CH_3), 1.08 (6H, s, C(16)CH_3); ^{13}C NMR (126 MHz; $CDCl_3$) δ_C 171.1 (C-7), 119.9 (C-9), 78.3 (t_D, J_D 22.9, C-3), 76.2 (t_D, J_D 22.0, C-5), 74.7 (t_D, J_D 22.1, C-4), 73.4 (t_D, J_D 23.4, C-2), 71.3 (t_D, J_D 22.2, C-1), 70.9 (t_D, J_D 22.3, C-6), 36.59, 36.55 (C-10, C-13), 23.8, 23.2 (C-11, C-12), 21.3 (C-8), 18.3, 18.2 (C(15)*C*H$_3$, C(16)*C*H$_3$, C(17)*C*H$_3$), 12.6 (C-15, C-16, C-17); ^{2}H NMR (92 MHz; $CHCl_3$; $CDCl_3$) δ_D 5.11 (1D, br s, D-1), 4.40 (1D, br s, D-2), 4.08 (1D, br s, D-3), 3.94 (1D, br s, D-6), 3.91 (1D, br s, D-4), 3.49 (1D, br s, D-5); $\bar{\nu}_{max}$(thin film)/cm^{-1} 3655 (O-H) (w), 3501 (O-H) (w), 2980 (s), 2972 (s), 2891 (w), 2867 (w), 2184 (w), 2162 (w), 2049 (w), 2036 (w), 2019 (w), 1995 (w), 1719 (C=O) (m), 1463 (m), 1433 (m), 1377 (m), 1337 (m), 1243 (m), 1201 (s), 1153 (s), 1125 (s), 1105 (s), 1062 (s), 1043 (Si-O) (s), 1010 (s), 985 (m), 960 (m), 920 (s), 882 (m), 799 (m), 680 (s); LRMS *m/z* (ESI$^+$) 472.2 ([MD_5+Na]$^+$, 18%), 473.2 ([MD_6+Na]$^+$, 100%); HRMS *m/z* (ES$^+$) found 472.27366 [MD_5+Na]$^+$, 473.27972 [MD_6+Na]$^+$ ($C_{22}H_{34}D_6O_7NaSi$ requires 473.28121 [MD_6+Na]$^+$).

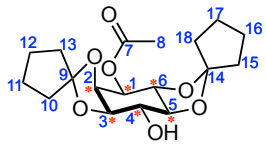
D_6 (–)-1D-1-*O*-Acetyl-2,3-*O*-cyclopentylidene-4-*O*-(triisopropylsilyl)-5,6-*O*-cyclopentylidene-*myo*-inositol (–)-317



To a clear solution of (–)-**316** (204 mg, 0.453 mmol, 1.0 eq, 84% D_6, 16% D_5) and **260** (1.10 mL, 7.70 mmol, 17 eq) in CH_2Cl_2 (1.05 mL) was added PTSA·H_2O (4 mg, 0.0226 mmol 0.050 eq). The resulting yellow solution was then stirred at 30 °C for 16 hours. The brown reaction obtained was then quenched with Et_3N (3 μL, 0.0226 mmol, 0.050 eq) and concentrated *in vacuo*. The residue obtained was purified by column chromatography over silica gel (Petroleum ether/Et_2O 10:0, 9.5:0.5, 9:1) to give (–)-**317** (167 mg, 71%, 85% D_6, 15% D_5) as a colourless oil: R_f 0.20 (Petroleum ether/EtOAc 7:3); $[\alpha]_D^{25}$ = –35.8 (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz; $CDCl_3$) δ_H 2.16 (3H, s, H-8), 1.95–1.75 (6H, m, H-19, H-22 and H-10 or H-13), 1.75–1.56 (10H, m, H-11, H-12, H-20, H-21 and

H-10 or H-13), 1.17–1.09 (3H, m, H-15, H-16 and H-17), 1.08 (12H, s, C(15)CH_3 and C(17)CH_3), 1.07 (6H, s, C(16)CH_3); ^{13}C NMR (126 MHz; $CDCl_3$) δ_C 170.7 (C-7), 121.8 (C-18), 119.4 (C-9), 82.3 (t_D, J_D 22.9, C-3), 79.1 (t_D, J_D 21.6, C-5), 75.1 (t_D, J_D 24.5, C-2), 74.5 (t_D, J_D 21.7, C-4), 74.1 (t_D, J_D 22.9, C-6), 71.0 (t_D, J_D 22.5, C-1), 37.52, 37.48 (C-19, C-22) 37.3, 37.2 (C-10, C-13), 23.5, 23.4, 23.3 (C-11, C-12, C-20, C-21), 21.2 (C-8), 18.07, 18.06 (C(15)CH$_3$, C(17)CH$_3$, C(16)CH$_3$), 12.5 (C-15, C-16 and C-17); ^{2}H NMR (92 MHz; $CHCl_3$; $CDCl_3$) δ_D 5.04 (1D, br s, D-1), 4.43 (1D, br s, D-2), 3.93 (3D, br s, D-3, D-4 and D-6), 3.29 (1D, br s, D-5); $\bar{\nu}_{max}$(thin film)/cm^{-1} 2980 (s), 2971 (s), 2890 (m), 2867 (m), 1741 (C=O) (m), 1463 (m), 1433 (w), 1372 (m), 1337 (m), 1249 (m), 1204 (m), 1180 (m), 1105 (s), 1080 (m), 1053 (m), 1026 (Si-O) (s), 1001 (m), 971 (m), 920 (m), 882 (m), 801 (m), 775 (m), 678 (m); LRMS *m/z* (ESI$^+$) 538.4 ([MD_5+Na]$^+$, 17%), 539.4 ([MD_6+Na]$^+$, 100%); HRMS *m/z* (ESI$^+$) found 538.32239 [MD_5+Na]$^+$, 539.32819 [MD_6+Na]$^+$ ($C_{27}H_{40}D_6O_7NaSi$ requires 539.32816 [MD_6+Na]$^+$).

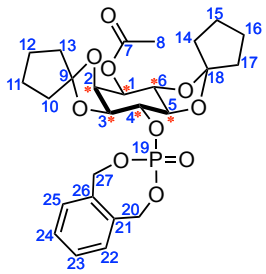
D_6 (–)-1D-1-*O*-Acetyl-2,3-*O*-cyclopentylidene-5,6-*O*-cyclopentylidene-*myo*-inositol (–)-318



To a solution of (–)-**317** (157 mg, 0.304 mmol, 1.0 eq, 85% D_6, 15% D_5) in DMF (14 mL) was added TAS-F (251 mg, 0.911 mmol, 3.0 eq) in one portion. After stirring at RT for 3 hours, the reaction was quenched with phosphate buffer (10 mL, pH 7.4, 50 mM NaH_2PO_4/Na_2HPO_4, 150 mM NaCl) and dissolved with EtOAc (15 mL). The aqueous layer was extracted with EtOAc (4 × 15 mL). The combined organic layers were washed with H_2O (2 × 15 mL), brine (2 × 15 mL) and an aqueous solution of LiCl (10% *w/v*, 15 mL), then dried ($MgSO_4$), filtered, and concentrated *in vacuo*. Purification by column chromatography over silica gel (Petroleum ether/EtOAc 10:0, 9:1, 8:2, 7:3, 6:4) afforded (–)-**318** (96 mg, 88%, 84% D_6, 16% D_5) as a colourless solid: R_f 0.24 (Petroleum ether/EtOAc 6:4); $[\alpha]_D^{25}$ = –55.6 (*c* 0.79, $CHCl_3$); m.p. 160–162 °C (Hexane/EtOAc); ^{1}H NMR (500 MHz; $CDCl_3$) δ_H 2.53 (1H, br s, C(4)O*H*), 2.17 (3H, s, H-8), 1.99–1.82 (6H, m, H-15 and H-18,

H-10 or H-13), 1.76–1.58 (10H, m, H-11, H-12, H-16, H-17 and H-10 or H-13); ^{13}C NMR (126 MHz; $CDCl_3$) δ_C 170.7 (C-7), 122.8 (C-14), 120.1 (C-9), 80.7 (t_D, J_D 23.0, C-3), 78.0 (t_D, J_D 21.8, C-5), 75.3 (t_D, J_D 23.4, C-2), 74.5 (t_D, J_D 22.9, C-6), 73.4 (t_D, J_D 22.4, C-4), 70.5 (t_D, J_D 22.5, C-1), 37.8, 37.7, 37.6 (C-15, C-18, C-10, C-13), 23.9, 23.65, 23.63, 23.56 (C-11, C-12, C-16 and C-17), 21.2 (C-8); ^{2}H NMR (92 MHz; $CHCl_3$; $CDCl_3$) δ_D 5.11 (1D, br s, D-1), 4.46 (1D, br s, D-2), 4.03 (1D, br s, D-3), 3.94 (1D, br s, D-6), 3.86 (1D, br s, D-4), 3.34 (1D, br s, D-5); $\bar{\nu}_{max}$(thin film)/cm^{-1} 3504 (O-H) (br), 2941 (m), 2185 (w), 2162 (w), 1737 (C=O) (m), 1433 (w), 1370 (m), 1337 (m), 1307 (w), 1250 (m), 1227 (m), 1197 (m), 1157 (w), 1131 (m), 1104 (s), 1062 (m), 1014 (s), 991 (s), 969 (m), 873 (m), 864 (m), 797 (m), 733 (m); LRMS *m/z* (ESI$^+$) 382.2 ([MD_5+Na]$^+$, 18%), 383.2 ([MD_6+Na]$^+$, 100%); HRMS *m/z* (ESI$^+$) found 382.18863 [MD_5+Na]$^+$, 383.19459 [MD_6+Na]$^+$ ($C_{18}H_{20}D_6O_7Na$ requires 383.19473 [MD_6+Na]$^+$).

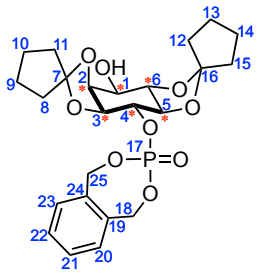
D_6 (–)-1D-1-*O*-Acetate-2,3-*O*-cyclopentylidene-4-*O*-(2-oxo-5.6-benzo-1,3,2-dioxaphosphep-2-yl)-5,6-*O*-cyclopentylidene-*myo*-inositol (–)-319



To a solution of (–)-**318** (90 mg, 0.250 mmol, 1.0 eq, 85% D_6, 15% D_5) and phosphoramidite **303** (179 mg, 0.749 mmol, 3.0 eq) in CH_2Cl_2 (4.3 mL) was added 1*H*-tetrazole (1.66 mL, 0.749 mmol, 3.0 eq, 0.45 M in MeCN). After stirring at RT for 1 hour, the cloudy reaction mixture was cooled to –78 °C and *m*CPBA (168 mg, 0.749 mmol, 3.0 eq, 77%) was added in a single portion. The reaction mixture was gradually warmed to RT over 20 minutes, stirred for 1 hour, and quenched with an aqueous solution of $Na_2S_2O_3$ (10% *w/v*, 5 mL). The aqueous layer was extracted with CH_2Cl_2 (4 × 10 mL) and the combined organic layers were washed with an aqueous solution of $Na_2S_2O_3$ (10% *w/v*, 3 × 10 mL), brine (3 × 10 mL), and a saturated aqueous solution of $NaHCO_3$ (10 mL), then

dried (Na_2SO_4), filtered, and concentrated *in vacuo*. Purification by column chromatography over silica gel (Petroleum ether/EtOAc 9:1, 8:2, 7:3, 6:4, 5:5) afforded (–)-**319** (120 mg, 88%, 84% D_6, 16% D_5) as an amorphous solid: R_f 0.60 (Petroleum ether/EtOAc 4:6); $[\alpha]_D^{25} = -27.2$ (*c* 0.88, $CHCl_3$); m.p. 197–199 °C (Hexane/EtOAc); ^{1}H NMR (500 MHz; CD_2Cl_2) δ_H 7.47–7.42 (2H, m, H-22 and H-25), 7.37–7.31 (2H, m, H-23 and H-24), 5.37–5.29 (2H, m, H-20 and H-27), 5.13–5.01 (2H, m, H-20' and H-27'), 2.14 (3H, s, H-8), 2.13–2.06 (1H, m, H-10 or H-13), 1.97–1.55 (15H, m, H-11, H12, H-14 to H-17, H-10', H-13' and H-10 or H-13); ^{13}C NMR (126 MHz; CD_2Cl_2) δ_C 170.5 (C-7), 136.1, 136.0 (C-21, C-26), 129.60, 129.59 (C-22, C-25), 129.43, 129.40 (C-23, C-24), 123.1 (C-18), 120.7 (C-9), 79.6–78.6 ($m_{D,P}$, C-3, C-4), 77.0–76.4 ($m_{D,P}$, C-5), 75.9 (t_D, J_D 23.3, C-2), 74.8 (t_D, J_D 22.7, C-6), 70.0 (t_D, J_D 21.9, C-1), 69.22 (d_P, J_P 5.3, C-20 or C-27), 69.17 (d_P, J_P 5.2, C-20 or C-27), 38.03, 37.96 (C-14, C-17), 37.91, 37.7 (C-10, C-13), 24.2, 24.1, 23.9, 23.8 (C-11, C-12, C-15, C-16), 21.1 (C-8); ^{31}P NMR (162 MHz; CD_2Cl_2) δ_C –1.41 (P-19); ^{2}H NMR (92 MHz; $CHCl_3$; $CDCl_3$) δ_D 5.12 (1D, br s, D-1), 4.63 (1D, br s, D-4), 4.47 (1D, br s, D-2), 4.26 (1D, br s, D-3), 4.02 (1D, br s, D-6), 3.52 (1D, br s, D-5); $\bar{\nu}_{max}$(thin film)/cm^{-1} 2958 (w), 2899 (w), 2875 (w), 2162 (w), 1738 (C=O) (m), 1433 (w), 1365 (w), 1338 (w), 1289 (m), 1245 (m), 1225 (m), 1203 (m), 1100 (m), 1054 (m), 999 (P-O) (s), 973 (P-O) (s), 953 (m), 877 (m), 849 (m), 786 (m), 736 (m); LRMS *m/z* (ESI$^+$) 564.2 ([MD_5+Na]$^+$, 19%), 565.2 ([MD_6+Na]$^+$, 100%); HRMS m/z (ESI$^+$) found 542.21897 [MD_5+H]$^+$, 543.22511 [MD_6+H]$^+$ ($C_{26}H_{28}D_6O_{10}P$ requires 543.22607 [MD_6+H]$^+$); RP-HPLC (Method 2) t_R = 12.01 min, 97.45%.

D_6 (+)-1D-2,3-*O*-Cyclopentylidene-4-*O*-(2-oxo-5.6-benzo-1,3,2-dioxaphosphep-2-yl)-5,6-*O*-cyclopentylidene-*myo*-inositol (+)-320

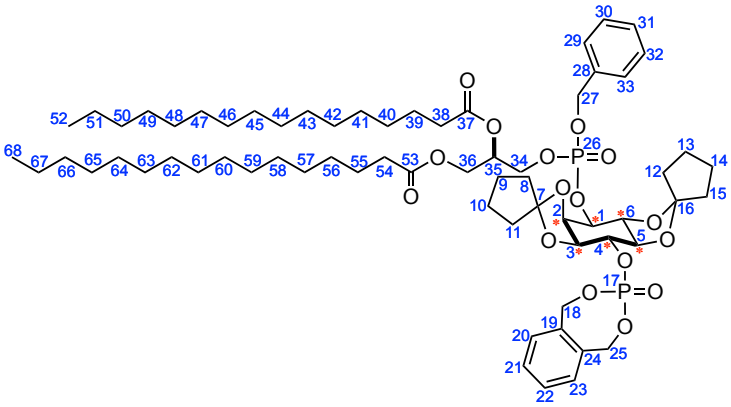


To a solution of (–)-**319** (110 mg, 0.203 mmol, 1.0 eq, 84% D_6, 16% D_5) in MeOH (19 mL) was added ground K_2CO_3 (56 mg, 0.405 mmol, 2.0 eq). The reaction mixture was stirred at RT for 1 hour. The solvent was then partially removed *in vacuo* at RT and H_2O (10 mL) was added. The aqueous layer was extracted with EtOAc (4 × 10 mL) and the combined organic layers were then washed with H_2O (10 mL) and brine (2 × 10 mL), then dried (Na_2SO_4), filtered, and concentrated *in vacuo*. Purification by column chromatography over silica gel (Petroleum ether/EtOAc 9:1, 8:2, 7:3, 6:4, 5:5) yielded (+)-**320** (87 mg, 86%, 84% D_6, 16% D_5) as an amorphous, colourless foam: R_f 0.32 (Petroleum ether/EtOAc 8:3); $[\alpha]_D^{25}$ = +20.3 (*c* 0.46 in $CHCl_3$); ^{1}H NMR (500 MHz; CD_2Cl_2) δ_H 7.42–7.37 (2H, m, H-20 and H-23), 7.36–7.31 (2H, m, H-21 and H-22), 5.38–5.29 (2H, m, H-18 and H-25), 5.08 (1H, dd, J_{ABX} 20.7, 13.6, H-18' or H-25'), 5.05 (1H, dd, J_{ABX} 20.8, 13.9, H-18' or H-25'), 2.37 (1H, s, C(1)O*H*), 2.12–2.04 (1H, m, H-8 or H-11), 1.96–1.83 (5H, m, H-8 or H-11, H-8', H-11' and H-12 or H-15), 1.81–1.59 (10H, m, H-9, H-10, H-13, H-14 and H-12 or H-15); ^{13}C NMR (126 MHz; CD_2Cl_2) δ_C 136.1, 136.0 (C-19, C-24), 129.60, 129.58 (C-20, C-23), 129.43, 129.41 (C-21, C-22), 122.9 (C-16), 120.6 (C-7), 80.1–79.0 ($m_{D,P}$, C-4, C-3), 78.3 (t_D, J_D 25.5, C-6), 78.1 (t_D, J_D 23.9, C-2), 76.7–76.1 ($m_{D,P}$, C-5), 69.4–68.9 ($m_{D,P}$, C-1), 69.22 (d_P, J_P 3.7, C-18 or C-25), 69.16 (d_P, J_P 3.7, C-18 or C-25), 38.0, 37.9 (C-12, C-15), 37.9,[XIX] 37.7 (C-8, C-11), 24.1, 24.0, 23.82, 23.78 (C-9, C-10, C-13, C-14); ^{31}P NMR (162 MHz; CD_2Cl_2) δ_C –2.28 (P-17); ^{2}H NMR (92 MHz; $CHCl_3$; $CDCl_3$) δ_D 4.59 (1D, br s, D-4), 4.32 (2D, br s, D-2 and D-3), 4.03 (1D, br s, D-1), 3.80 (1D, br s, D-6), 3.46 (1D, br s, D-5); $\bar{\nu}_{max}$(thin film)/cm^{-1} 3407 (O-H) (br), 2962 (w), 2874 (w), 2361 (w),

[XIX] Overlapping peaks

2342 (w), 1454 (w), 1433 (w), 1374 (m), 1338 (m), 1290 (m), 1224 (m), 1201 (m), 1121 (m), 1103 (m), 1056 (m), 1016 (P-O) (s), 999 (s), 979 (m), 928 (m), 851 (m), 753 (m), 734 (m); LRMS *m/z* (ESI$^+$) 522.2 ([MD_5+Na]$^+$, 19%), 523.2 ([MD_6+Na]$^+$, 100%); HRMS *m/z* (ESI$^+$) found 500.20862 [MD_5+Na]$^+$, 501.21477 [MD_6+H]$^+$ ($C_{24}H_{26}D_6O_9P$ requires 501.21551 [MD_6+H]$^+$); RP-HPLC (Method 2) t_R = 10.67 min, 98.00%.

D_6 (–)-1D-1-*O*-(1,2-Di-*O*-palmitoyl-*sn*-glycer-3-yl-benzylphosphate)-2,3-*O*-cyclopentylidene-4-*O*-(2-oxo-5.6-benzo-1,3,2-dioxaphosphep-2-yl)-5,6-*O*-cyclopentylidene-*myo*-inositol (–)-321

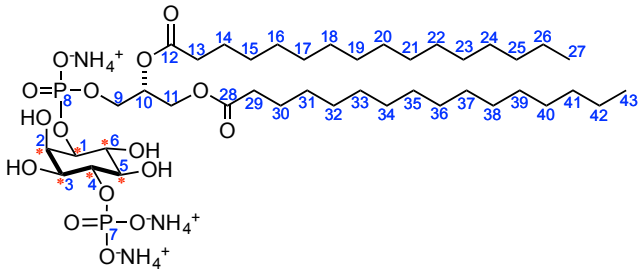


To a solution of (+)-**320** (20 mg, 0.0400 mmol, 1.0 eq, 84% D_6, 16% D_5) and phosphoramidite (+)-**306** (97 mg, 0.120 mmol, 3.0 eq) in anhydrous CH_2Cl_2 (2.0 mL) was added 1*H*-tetrazole (266 μL, 0.120 mmol, 3.0 eq, 0.45 M in MeCN) dropwise. The cloudy reaction mixture was stirred at RT for 21 hours, then cooled to –78 °C and *m*CPBA (27 mg, 0.120 mmol, 3.0 eq, 77%) was added in one portion. The reaction mixture was stirred for 1 hour, warmed to RT, then stirred for a further 3 hours. The reaction was quenched by addition of an aqueous solution of $Na_2S_2O_3$ (10% *w/v*, 3 mL) and stirred for 15 minutes. The aqueous phase was extracted with CH_2Cl_2 (4 × 3 mL) and the combined organic layers were washed with an aqueous solution of $Na_2S_2O_3$ (10% *w/v*, 2 × 3 mL), and brine (2 × 3 mL), then dried (Na_2SO_4), filtered, and concentrated *in vacuo*. Purifications by column chromatography over silica gel (Petroleum ether/EtOAc 10:0, 9:1, 8:2, 7:3, 6:4, 5:5) yielded (–)-**321** (27 mg, 55%, 87% D_6, 13% D_5) as a colourless, gummy solid: R_f 0.34 (Petroleum ether/EtOAc 5:5); $[\alpha]_D^{25}$ = –1.9 (*c* 0.52, $CHCl_3$); ^{1}H NMR (400 MHz; CD_2Cl_2) δ_H 7.43–7.31 (18H, m, H-20 to H-23 and

H-29 to H-33 Diast. A and B), 5.36–5.29 (4H, m, H-18 and H-25 Diast. A and B), 5.25–5.18 (2H, m, H-35 Diast. A and B), 5.16–5.06 (6H, m, H-27 and H-18' or H-25' Diast. A and B), 5.05 (2H, dd, J_{ABX} 14.0, 14.0, H-18' or H-25' Diast. A and B), 4.30 (2H, ddd, J_{XY} 12.0, 4.4, 4.4, H-36 Diast. A and B), 4.22–4.09 (6H, m, H-34 and H-36' Diast. A and B), 2.33–2.25 (8H, m, H-38 and H-54 Diast. A and B), 2.16–2.08 (2H, m, H-8 or H-11 Diast A and B), 1.98–1.63 (30H, m, H-8 or H-11, H-8', H-11', H-12, H-15, H-9, H-10, H-13, H-14 Diast. A and B), 1.63–1.49 (8H, m, H-39 and H-55 Diast. A and B), 1.34–1.19 (96H, m, H-40 to H-51 and H-56 to H-67 Diast. A and B), 0.88 (12H, t, *J* 6.9, H-52 and H-68 Diast. A and B); ^{13}C NMR (126 MHz; CD_2Cl_2) δ_C 173.45, 173.43 (C-53 Diast. A and B), 173.06, 173.05 (C-37 Diast. A and B), 136.2 (d_P, J_P 7.3, C-28 Diast. A), 136.12–136.00 (m_P, C-28 Diast. B, C-19 or C-24 Diast. A and B), 135.96 (C-19 or C-24 Diast. A and B), 129.6, 129.43, 129.40, 120.05, 129.02, 128.99, 128.96 (C-20 to C-23, C-30 to C-32 Diast. A and B), 128.4, 128.3 (C-29, C-33 Diast. A and B), 123.35 (C-16 Diast. A), 123.32 (C-16 Diast. B), 120.9 (C-7 Diast. A and B), 79.8–79.1 ($m_{D,P}$, C-3 Diast. A and B), 79.1–78.5 ($m_{D,P}$, C-4 Diast. A and B), 77.4–76.8 ($m_{D,P}$, C-2 Diast. A and B), 76.6–76.1 ($m_{D,P}$, C-5 Diast. A and B), 75.8–75.2 ($m_{D,P}$, C-6 Diast. A and B), 74.5–73.8 ($m_{D,P}$, C-1 Diast. A and B), 70.2 (d_P, J_P 5.7, C-27 Diast. A), 70.0 (d_P, J_P 5.7, C-27 Diast. B), 69.7 (d_P, J_P 6.7, C-35 Diast. A), 69.6 (d_P, J_P 6.7, C-35 Diast. B), 69.23 (d_P, J_P 4.8, C-18 or C-25 Diast. A and B), 69.18 (d_P, J_P 4.8, C-18 or C-25 Diast. A and B), 66.2 (d_P, J_P 5.7, C-34 Diast. A), 65.9 (d_P, J_P 5.1, C-34 Diast. B), 62.04, 61.96 (C-36 Diast. A and B), 38.0, 37.93, 37.88, 37.85, 37.7 (C-12, C-15, C-8, C-11 Diast. A and B), 34.5, 34.3 (C-38, C-54 Diast. A and B), 32.3 (C-50, C-66 Diast. A and B), 30.11, 30.09, 30.06, 29.9, 29.8, 29.7, 29.52, 29.49 (C-40 to C-49, C-56 to C-65 Diast. A and B), 25.2 (C-39, C-55 Diast. A and B), 24.20, 24.17, 24.08, 23.8 (C-9, C-10, C-13, C-14 Diast. A and B), 23.1 (C-51, C-67 Diast. A and B), 14.3 (C-52, C-68 Diast. A and B); ^{31}P NMR (162 MHz; CD_2Cl_2) δ_P –2.15 (P-26 Diast. A), –2.20 (P-26 Diast. B), –3.32 (P-17); ^{2}H NMR (92 MHz; $CHCl_3$; $CDCl_3$) δ_D 4.62 (2D, br s, D-1 and D-4), 4.46 (1D, br s, D-2), 4.25 (1D, br s, D-3), 4.02 (1D, br s, D-6), 3.45 (1D, br s, D-5); $\bar{\nu}_{max}$(thin film)/cm^{-1} 2955 (m), 2924 (s), 2853 (m), 2359 (w), 2339 (w), 1743 (C=O) (m), 1435 (w), 1418 (w), 1377 (w), 1339 (w), 1292 (m), 1207 (m), 1176 (m), 1156 (m), 1104 (m), 1020 (P-O) (s), 885 (w), 851 (w), 775 (w), 733 (w); LRMS *m/z* (ESI$^+$) 1242.6 ([MD_5+Na]$^+$, 4%), 1243.6 ([MD_6+Na]$^+$, 13%); HRMS *m/z* (ESI$^+$) found 1242.69965 [MD_5+Na]$^+$, 1243.70607

$[MD_6+Na]^+$ ($C_{66}H_{98}D_6O_{16}NaP_2$ requires 1243.70684 $[MD_6+Na]^+$); NP-HPLC (Method 6) t_R = 4.82 min, 100%.

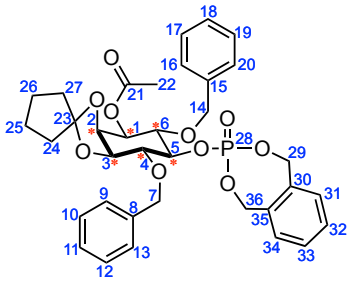
D_6 (–)-1D-Dipalmitoyl-phosphatidylinositol-4-phosphate triammonium salt (–)-322



To a solution of (–)-**321** (12 mg, 0.00982 mmol, 1.0 eq, 87% D_6, 13% D_5) in toluene (0.50 mL) was added TMSBr (20 μL, 0.147 mmol, 15 eq). The solution was stirred at RT for 12 hours, then cooled to 0 °C and concentrated by vacuum transfer. The residue was dissolved in toluene (0.6 mL) and concentrated again by vacuum transfer. This was repeated three times. The residue was then dissolved in MeOH (0.5 mL), stirred at 0 °C for 1 hour and the solution was concentrated *in vacuo* at 0 °C. The residue obtained was dissolved in the minimal amount of MeOH/CH_2Cl_2 (1:1 *v*/*v*) and purified by column chromatography over silica gel ($CHCl_3$/MeOH/2.2 M NH_4OH 5:3.9:1.1). The combined fractions were partially concentrated *in vacuo* at 0 °C, then H_2O (30 mL) was added. Lyophilising this solution yielded (–)-**322** (7 mg, 75%, 86% D_6, 14% D_5) as a colourless, fluffy solid. The compound was stored at –80 °C as the ammonium salt: R_f 0.60 ($CHCl_3$/MeOH/2.2 M NH_4OH 5:3.9:1.1); $[\alpha]_D^{25}$ = –9.9 (*c* 0.06, MeOH/$CHCl_3$/H_2O 4:3:1); ^{1}H NMR (500 MHz; CD_3OD/$CDCl_3$/D_2O 4:3:1) δ_H 5.29–5.23 (1H, m, H-10), 4.43 (1H, dd, J_{XY} 12.1, 2.5, H-11), 4.20 (1H, dd, J_{XY} 12.1, 7.8, H-11'), 4.09–3.98 (2H, m, H-9), 2.37–2.27 (4H, m, H-13 and H-29), 1.65–1.54 (4H, m, H-14 and H-30), 1.36-1.21 (48H, m, H-15 to H-26 and H-31 to H-42), 0.88 (6H, t, *J* 6.9, H-27 and H-43); ^{13}C NMR (126 MHz; CD_3OD/$CDCl_3$/D_2O 4:3:1) δ_C 175.2, 174.9 (C-12, C-28), 71.4 (d_P, J_P 8.5, C-10), 64.5 (d_P, J_P 4.8, C-9), 63.8 (C-11), 35.0, 34.9 (C-13, C-29), 32.7 (C-25, C-41), 30.43, 30.38, 30.31, 30.2, 30.1, 29.93, 29.88 (C-15 to C-24 and C-31 to C-40), 25.7, 25.6 (C-14, C-30), 23.4 (C-26, C-42),

14.6 (C-27, C-43);[XX] ^{31}P NMR (162 MHz; $CD_3OD/CDCl_3/D_2O$ 4:3:1) δ_P 1.72 (P-7), –0.18 (P-8); ^{2}H NMR (92 MHz; $CH_3OH/CHCl_3/H_2O$ 4:3:1; $CDCl_3$) δ_D 4.30 (br s, inositol ring), 3.04 (br s, inositol ring), 3.03 (br s, inositol ring); $\bar{\nu}_{max}$(thin film)/cm^{-1} 3211 (O-H) (br), 3070 (O-H) (br), 2956 (w), 2917 (m), 2850 (m), 1738 (C=O) (m), 1464 (m), 1417 (m), 1196 (m), 1169 (m), 1089 (s), 1064 (s), 1033 (s), 1013 (P-O) (s), 993 (s), 897 (m), 873 (m), 720 (m), 681 (m), 615 (s); HRMS *m/z* (ESI$^-$) found 894.51613 ([MD_5–H]$^-$, 16%), 895.52186 ([MD_6–H]$^-$, 100%) ($C_{41}H_{73}D_6O_{16}P_2$ requires 895.52254 [MD_6–H]$^-$).

D_6 (–)-1D-1-*O*-Acetate-2,3-*O*-cyclopentylidene-4,6-di-*O*-benzyl-5-*O*-(2-oxo-5.6-benzo-1,3,2-dioxaphosphep-2-yl)-*myo*-inositol (–)-323



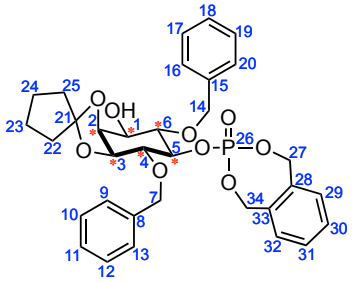
To a solution of (–)-**314** (100 mg, 0.211 mmol, 1.0 eq, 91% D_6, 9% D_5) and phosphoramidite **303** (151 mg, 0.632 mmol, 3.0 eq) in CH_2Cl_2 (3.7 mL) was added 1*H*-tetrazole (1.40 mL, 0.632 mmol, 3.0 eq, 0.45 M in MeCN). After stirring at RT for 1 hour, the cloudy reaction mixture was cooled to –78 °C and *m*CPBA (142 mg, 0.632 mmol, 3.0 eq, 77%) was added in a single portion. The reaction mixture was gradually warmed to RT over 20 minutes, stirred for 1 hour and quenched with an aqueous solution of $Na_2S_2O_3$ (10% *w*/*v*, 10 mL). The aqueous layer was extracted with CH_2Cl_2 (4 × 2 mL) and the combined organic layers were washed with an aqueous solution of $Na_2S_2O_3$ (10% *w*/*v*, 3 × 2 mL), brine (3 × 2 mL), and a saturated aqueous solution of $NaHCO_3$ (2 mL), then dried (Na_2SO_4), filtered, and concentrated *in vacuo*. Purification by column chromatography over silica gel (Petroleum ether/EtOAc 9:1, 8:2, 7:3, 6:4, 5:5) afforded (–)-**323** (85 mg, 61%, 88% D_6, 12% D_5)

[XX] Due to the instability of this compound in solution, only a minimal number of scans could be used for acquiring the ^{13}C NMR spectrum, this was not enough to observe the peaks corresponding to C-1, C-2, C-3, C-4, C-5 and C-6. Those can be inferred however by comparison with the ^{13}C NMR spectrum of the equivalent non-deuterated compounds.

as an amorphous, colourless foam: R_f 0.66 (Petroleum ether/Et_2O 4:6); $[\alpha]_D^{25} = -6.6$ (*c* 2.0, $CHCl_3$); ^{1}H NMR (500 MHz; $CDCl_3$) δ_H 7.44–7.38 (2H, m, H-9 and H-13), 7.38–7.24 (10H, m, H-10 to H-12, H-16 to H-10, H-32 and H-33), 7.21–7.14 (2H, m, H-31 and H-34), 5.22–5.07 (2H, m, H-29 or H-36), 5.06–4.94 (2H, m, H-29 or H-36), 4.85 (1H, d, J_{AB} 11.2, H-14), 4.84 (1H, d, J_{AB} 11.6, H-7), 4.76 (1H, d, J_{AB} 11.6, H-7'), 4.69 (1H, d, J_{AB} 11.2, H-14'), 2.05 (3H, s, H-22), 1.98–1.84 (2H, m, H-24 and H-27), 1.74–1.62 (6H, m, H-25, H-26, H-24' and H-27'); ^{13}C NMR (126 MHz; $CDCl_3$) δ_C 170.2 (C-21), 138.2, 138.1 (C-8, C-15), 135.43, 135.39 (C-30, C-35), 128.8 (C-31, C-34), 129.1, 129.0, 128.43, 128.36, 128.1, 127.9, 127.74, 127.73 (C-32, C-33, C-9 to C-13 and C-16 to C-20), 120.1 (C-23), 80.6–79.9 ($m_{D,P}$, C-5), 79.0–78.4 ($m_{D,P}$, C-4), 78.1–76.8 ($m_{D,P}$, C-6, C-3), 74.3 (C-14), 73.5–72.9 (m_D, C-2), 73.2 (C-7), 70.3–69.7 (m_D, C-1), 68.4 (d_P, J_P 5.6, C-29, C-36), 36.9, 36.7 (C-24, C-27), 24.1, 23.5 (C-25, C-26), 21.2 (C-22); ^{13}C NMR (126 MHz; CD_2Cl_2)[XXI] δ_C 170.2 (C-21), 138.58, 138.55 (C-8, C-15), 135.83, 135.79 (C-30, C-35), 129.1 (C-31, C-34), 129.34, 129.31, 128.7, 128.6, 128.34, 128.27, 128.05, 127.99 (C-32, C-33, C-9 to C-13 and C-16 to C-20), 120.4 (C-23), 80.5–79.9 ($m_{D,P}$, C-5), 79.6–79.0 ($m_{D,P}$, C-4), 77.9–77.1 ($m_{D,P}$, C-6, C-3), 74.6 (C-14), 73.9–73.4 (m_D, C-2), 73.5 (C-7), 70.4–69.8 (m_D, C-1), 68.7 (d_P, J_P 7.7, C-29, C-36), 37.2, 37.0 (C-24, C-27), 24.2, 23.7 (C-25, C-26), 21.2 (C-22); ^{31}P NMR (162 MHz; $CDCl_3$) δ_P 0.25 (P-28); ^{2}H NMR (92 MHz; $CHCl_3$; $CDCl_3$) δ_D 5.18 (1D, br s, D-1), 4.53 (1D, br s, D-5), 4.30 (1D, br s, D-2), 4.20 (1D, br s, D-3), 3.96 (1D, br s, D-6), 3.83 (1D, br s, D-4); $\bar{\nu}_{max}$(thin film)/cm^{-1} 2981 (s), 2972 (s), 2888 (m), 1742 (C=O) (w), 1473 (w), 1461 (s), 1382 (m), 1339 (w), 1252 (m), 1153 (m), 1074 (m), 1015 (P-O) (m), 967 (m), 955 (m), 850 (w), 777 (w), 735 (w), 699 (w); LRMS *m/z* (ESI$^+$) 678.2 ([MD_5+Na]$^+$, 13%), 679.2 ([MD_6+Na]$^+$, 100%); HRMS *m/z* (ESI$^+$) found 678.24795 [MD_5+Na]$^+$, 679.25368 [MD_6+Na]$^+$ ($C_{35}H_{33}D_6O_{10}NaP$ requires 679.25497 [MD_6+Na]$^+$); RP-HPLC (Method 2) t_R = 14.20 min, 100%.

[XXI] ^{13}C NMR data was run both in $CDCl_3$ and CD_2Cl_2 as some of the peaks were obscured under the solvent peak in $CDCl_3$

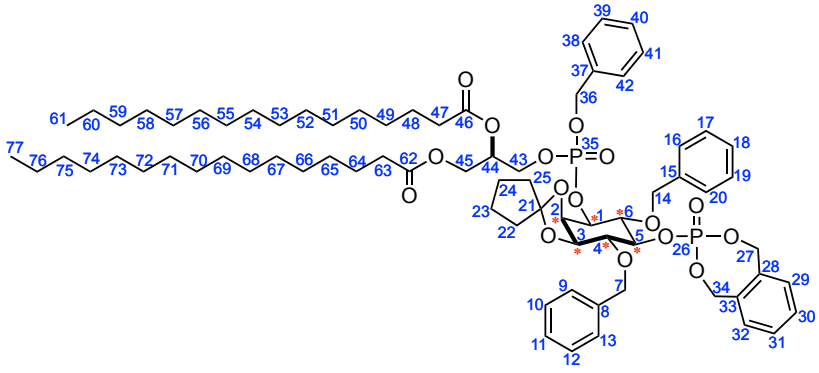
D_6 (+)-1D-2,3-*O*-Cyclopentylidene-4,6-di-*O*-benzyl-5-*O*-(2-oxo-5.6-benzo-1,3,2-dioxaphosphep-2-yl)-*myo*-inositol (+)-324



To a solution of (–)-**323** (80 mg, 0.122 mmol, 1.0 eq, 88% D_6, 12% D_5) in MeOH (11 mL) was added ground K_2CO_3 (34 mg, 0.244 mmol, 2.0 eq). The reaction mixture was stirred at RT for 1 hour. The solvent was then partially removed *in vacuo* at RT and H_2O (5 mL) was added to the resulting residue. The aqueous layer was extracted with EtOAc (4 × 10 mL) and the combined organic layers were then washed with H_2O (5 mL) and brine (2 × 5 mL), then dried (Na_2SO_4), filtered, and concentrated *in vacuo*. Purification by column chromatography over silica gel (Petroleum ether/EtOAc 9:1, 8:2, 7:3, 6:4, 5:5) afforded (+)-**324** (66 mg, 89%, 89% D_6, 11% D_5) as a colourless oil: R_f 0.28 (Petroleum ether/EtOAc 4:6); $[\alpha]_D^{25}$ = +2.8 (*c* 2.0, $CHCl_3$); ^{1}H NMR (500 MHz; CD_2Cl_2) δ_H 7.44–7.38 (4H, m, H-9, H-13, H-16 and H-20), 7.38–7.25 (8H, m, H-10 to H-12, H-17 to H-19, H-30 and H-31), 7.25–7.19 (2H, m, H-29 and H-32), 5.16–5.07 (3H, m, H-27 or H-34, H-27' and H-34'), 5.00 (1H, dd, J_{ABX} 17.9, 13.8, H-27 or H-34), 4.88 (1H, d, J_{AB} 11.3, H-7 or H-14), 4.87 (1H, d, J_{AB} 11.3, H-7 or H-14), 4.73 (2H, d, J_{AB} 11.3, H-7' and H-14'), 2.56 (1H, br s, C(1)O*H*), 2.00–1.91 (2H, m, H-22 or H-25), 1.81–1.62 (6H, m, H-23, H-24 and H-22 or H-25); ^{13}C NMR (126 MHz; CD_2Cl_2) δ_C 138.7, 138.6 (C-8, C-15), 135.9, 135.8 (C-28, C-33), 129.34, 129.31, 129.12, 129.11, 128.7, 128.6, 128.5, 128.3, 128.1, 127.9 (C-9 to C-13, C-16 to C-20, C-29 to C-32), 120.1 (C-21), 81.0–80.2 ($m_{P,D}$, C-5), 80.2-79.5 ($m_{P,D}$, C-4, C-6), 77.4 (t_D, J_D 22.8, C-3), 75.1 (t_D, J_D 22.9, C-2), 74.2, 73.5 (C-7, C-14), 68.9 (t_D, J_D 28.4, C-1), 68.72 (d_P, J_P 7.8, C-27 or C-34), 68.67 (d_P, J_P 6.6, C-27 or C-34), 24.2 (C-22, C-25), 23.6 (C-23, C-24), 2.00–1.91 (2H, m, H-22 or H-25), 1.81–1.62 (6H, m, H-23, H-24 and H-22 or H-25); ^{31}P NMR (162 MHz; CD_2Cl_2) δ_C –1.02 (P-26); ^{2}H NMR (77 MHz; CH_2Cl_2; CD_2Cl_2) δ_D 4.53 (1D, br s, D-5), 4.32 (1D, br s, D-2), 4.23 (1D, br s, D-3), 3.96 (3D, br s, D-1, D-4 and D-6); $\bar{\nu}_{max}$(thin

film)/cm^{-1} 3398 (O-H) (br), 3063 (w), 3030 (w), 2959 (w), 2873 (w), 1497 (w), 1454 (w), 1433 (w), 1385 (w), 1337 (w), 1284 (m), 1208 (m), 1155 (m), 1097 (m), 1073 (m), 1050 (m), 1016 (P-O) (s), 860 (m), 777 (m), 735 (m), 699 (m); LRMS *m/z* (ESI$^+$) 636.2 ([MD$_5$+Na]$^+$, 12%), 637.2 ([MD$_6$+Na]$^+$, 100%); HRMS *m/z* (ESI$^+$) found 614.25673 [MD$_5$+H]$^+$, 615.26287 [MD$_6$+H]$^+$ ($C_{33}H_{32}D_6O_9P$ requires 615.26246 [MD$_6$+H]$^+$); RP-HPLC (Method 2) t_R = 13.34 min, 96.87%.

D$_6$ (–)-1D-1-*O*-(1,2-Di-*O*-palmitoyl-*sn*-glycer-3-yl-benzylphosphate)-2,3-*O*-cyclopentylidene-4,6-di-*O*-benzyl-5-*O*-(2-oxo-5.6-benzo-1,3,2-dioxaphosphep-2-yl)-*myo*-inositol (–)-325

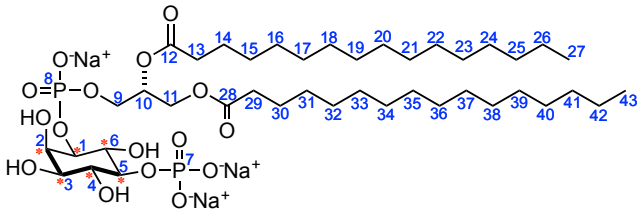


To a solution of (+)-**324** (49 mg, 0.0797 mmol, 1.0 eq, 89% D$_6$, 11% D$_5$) and phosphoramidite (+)-**306** (193 mg, 0.239 mmol, 3.0 eq) in anhydrous CH_2Cl_2 (4.1 mL) was added 1*H*-tetrazole (531 μL, 0.239 mmol, 3.0 eq, 0.45 M in MeCN) dropwise. The cloudy reaction mixture was stirred at RT for 20 hours, then cooled to –78 °C and *m*CPBA (54 mg, 0.239 mmol, 3.0 eq, 77%) was added in one portion. The reaction mixture was stirred for 1 hour, warmed to RT, and stirred for a further 2 hours. The reaction was quenched by addition of an aqueous solution of $Na_2S_2O_3$ (10% *w/v*, 5 mL), then stirred for 15 minutes. The aqueous phase was extracted with CH_2Cl_2 (4 × 5 mL) and the combined organic layers were washed with an aqueous solution of $Na_2S_2O_3$ (10% *w/v*, 2 × 3 mL), and brine (2 × 3 mL), then dried (Na_2SO_4), filtered, and concentrated *in vacuo*. Purification by column chromatography over silica gel (Petroleum ether/EtOAc 10:0, 9:1, 8:2, 7:3, 6:4, 5:5) yielded (–)-**325** (50 mg, 46%, 88% D$_6$, 12% D$_5$) as a colourless, waxy solid: R_f 0.34 (Petroleum ether/EtOAc 5:5); $[\alpha]_D^{25}$ = –2.0 (*c* 0.83, $CHCl_3$); ^{1}H NMR (500 MHz; CD_2Cl_2) δ_H 7.43–7.24 (34H, m, H-9 to H-13, H-16 to H-20, H-38 to H-42, H-30 and H-31 Diast. A and B), 7.24–7.19 (4H, m, H-29 and H-32

Diast. A and B), 5.21–5.14 (1H, m, H-44 Diast. A), 5.13–4.93 (13H, m, H-44 Diast. B, H-27, H-34 and H-36 Diast. A and B), 4.86–4.79 (4H, m, H-7 and H-14 Diast. A and B), 4.77–4.70 (4H, m, H-7' and H-14' Diast. A and B), 4.26 (1H, dd, J_{ABX} 11.9, 4.0, H-45 Diast. A), 4.20–4.04 (5H, m, H-45' Diast. A, H-45 Diast. B, H-43 Diast. A and B, H-43' Diast. A), 4.04–3.99 (1H, m, H-43' Diast. B), 3.95 (1H, dd, J_{ABX} 11.9, 5.9, H-45' Diast. B), 2.32–2.19 (8H, m, H-47 and H-63 Diast. A and B), 2.01–1.89 (4H, m, H-22 and H-25 Diast. A and B), 1.76–1.63 (12H, m, H-22', H-25', H-23 and H-24 Diast. A and B), 1.62–1.49 (8H, m, H-48 and H-64 Diast. A and B), 1.35–1.18 (96H, m, H-49 to H-60 and H-65 to H-76 Diast. A and B), 0.89 (12H, t, *J* 6.9, H-61 and H-77 Diast. A and B); ^{13}C NMR (126 MHz; CD_2Cl_2) δ_C 173.41, 173.37 (C-62 Diast. A and B), 173.07, 173.05 (C-46 Diast. A and B), 138.5, 138.41, 138.36 (C-8, C-15 Diast. A and B), 136.3 (d_P, J_P 7.5, C-37 Diast. A), 136.2 (d_P, J_P 7.5, C-37 Diast. B), 135.85, 135.80 (C-28, C-33 Diast. A and B), 129.39, 129.36, 129.1, 128.99, 128.96, 128.94, 128.87, 128.7, 128.6, 128.38, 128.36, 128.33, 128.27, 128.13, 128.06, 128.04 (C-9 to C-13, C-16 to C-20, C-29 to C-32, C-38 to C-42 Diast. A and B), 120.4 (C-21 Diast. A and B), 80.3–79.7 ($m_{D,P}$, C-5 Diast. A and B), 79.3–78.7 ($m_{D,P}$, C-4 Diast A and B), 78.4–77.7 ($m_{D,P}$, C-6 Diast. A and B), 77.4–76.8 ($m_{D,P}$, C-3 Diast. A and B), 75.5–74.9 ($m_{D,P}$, C-1 Diast. A and B), 74.52, 74.51 (C-7 Diast. A and B), 74.3–73.8 ($m_{D,P}$, C-2 Diast. A and B), 73.4 (C-14 Diast. A and B), 69.90 (d_P, J_P 2.2, C-36 Diast. A), 69.86 (d_P, J_P 2.5, C-36 Diast. B), 69.71 (d_P, J_P 6.6, C-44 Diast. A), 69.67 (d_P, J_P 6.4, C-44 Diast. B), 68.7 (d_P, J_P 6.2, C-27 and C-34 Diast. A and B), 66.1 (d_P, J_P 5.0, C-43 Diast. A), 65.9 (d_P, J_P 5.3, C-43 Diast. B), 62.0 (C-45 Diast. A), 61.9 (C-45 Diast. B), 36.9, 36.75, 36.73 (C-22, C-25 Diast. A and B), 34.50, 34.45, 34.35, 34.33 (C-47, C-63 Diast. A and B), 32.4 (C-59, C-75 Diast. A and B), 30.13, 30.11, 30.09, 29.9, 29.8, 29.7, 29.55, 29.51 (C-49 to C-58 and C-65 to C-74 Diast. A and B), 25.27, 25.26, 25.22 (C-48, C-64 Diast. A and B), 24.3, 24.2, 23.6 (C-23, C-24 Diast. A and B), 23.1 (C-60, C-76 Diast. A and B), 14.3 (C-61, C-77 Diast. A and B); ^{31}P NMR (162 MHz; CD_2Cl_2) δ_P –0.93 (P-26), –1.78 (P-35 Diast. A), –1.92 (P-35 Diast. B); ^{2}H NMR (96 MHz; $CHCl_3$; $CDCl_3$) δ_D 4.63 (3D, br s, D-1, D-5 and D-2), 4.11 (3D, br s, D-3, D-6 and D-4); $\bar{\nu}_{max}$(thin film)/cm^{-1} 2918 (m), 2850 (m), 1739 (C=O) (m), 1467 (w), 1455 (w), 1417 (w), 1382 (w), 1338 (m), 1210 (m), 1170 (m), 1099 (m), 1072 (m), 1050 (m), 1015 (P-O) (s), 1000 (P-O) (s), 865 (m), 733 (m), 696 (m); LRMS *m/z* (ESI$^+$) 1357.6 ([MD_6+Na]$^+$, 0.7%); HRMS *m/z* (ESI$^+$) found 1356.74890 [MD_5+Na]$^+$,

1357.75281 $[MD_6+Na]^+$ ($C_{75}H_{104}D_6O_{16}NaP_2$ requires 1357.75379 $[MD_6+Na]^+$); NP-HPLC (Method 7) t_R = 3.92 min, 47.13% Diast. A, t_R = 4.27 min, 50.47% Diast. B.

D_6 (–)-1D-Dipalmitoyl-phosphatidylinositol-5-phosphate trisodium salt (–)-326



Method 1 To a solution of (–)-**325** (20 mg, 0.0150 mmol, 1.0 eq, 88% D_6, 12% D_5) in a mixture of ^{t}BuOH (800 μL) and H_2O (140 μL) was added palladium black (32 mg, 0.301 mmol, 20 eq) under $N_{2(g)}$. The flask was purged with 3 balloons of $H_{2(g)}$. After stirring at RT under $H_{2(g)}$ for 3 hours, $NaHCO_3$ (4 mg, 0.0451 mmol, 3.0 eq) was added in one portion and the reaction mixture was stirred for a further 10 minutes under $H_{2(g)}$. The reaction mixture was then filtered through a glass microfiber pad and washed with H_2O (2 × 5 mL). The combined aqueous filtrates were lyophilised to give (–)-**326** (14 mg) as a fluffy, colourless solid, contaminated with partially deprotected **327** in a ratio of 1.9:1.0 as determined by ^{1}H NMR.

Method 2 To a solution of (–)-**325** (8 mg, 0.00599 mmol, 1.0 eq, 88% D_6, 12% D_5) in a mixture of ^{t}BuOH (320 μL) and H_2O (60 μL) was added palladium black (13 mg, 0.120 mmol, 20 eq) under $N_{2(g)}$. The flask was purged with 3 balloons of $H_{2(g)}$. After stirring at RT under $H_{2(g)}$ for 14 hours, $NaHCO_3$ (2 mg, 0.0180 mmol, 3.0 eq) was added in one portion and the reaction mixture was stirred for a further 35 minutes under $H_{2(g)}$. The reaction mixture was then filtered through a glass microfiber pad and washed with H_2O (2 × 5 mL). The combined aqueous filtrates were lyophilised to give (–)-**326** (4 mg) as a fluffy, colourless solid, contaminated with partially deprotected **327** in a ratio of 4.8:1.0 as determined by ^{1}H NMR.

Method 3 To a solution of (–)-**325** (8 mg, 0.00599 mmol, 1.0 eq, 88% D_6, 12% D_5) in a mixture of ^{t}BuOH (320 μL) and H_2O (60 μL) was added palladium black (19 mg, 0.180 mmol, 30 eq) under $N_{2(g)}$. The flask was purged with 3 balloons of $H_{2(g)}$. After stirring at RT under $H_{2(g)}$ for 18.5 hours,

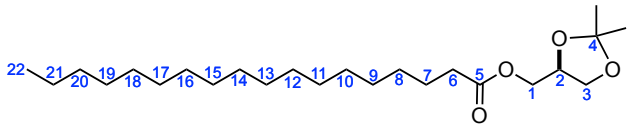
$NaHCO_3$ (2 mg, 0.0180 mmol, 3.0 eq) was added in one portion and the reaction mixture was stirred for a further 3 hours under $H_{2(g)}$. The reaction mixture was then filtered through a glass microfiber pad and washed with H_2O (2 × 5 mL). The combined aqueous filtrates were lyophilised to give (–)-**326** (3 mg) as a fluffy, colourless solid, contaminated with hydrolysis product.

Method 4 To a solution of (–)-**325** (10 mg, 0.00749 mmol, 1.0 eq, 88% D_6, 12% D_5) in a mixture of ^{t}BuOH (400 μL) and H_2O (70 μL) was added palladium black (24 mg, 0.225 mmol, 30 eq) under $N_{2(g)}$. The flask was purged with 3 balloons of $H_{2(g)}$. After stirring at RT under $H_{2(g)}$ for 18.5 hours, the reaction mixture was filtered through a glass microfiber pad and washed with H_2O (2 × 5 mL). The combined aqueous filtrates were lyophilised. The colourless, fluffy solid obtained was dissolved in the minimal amount of MeOH/CH_2Cl_2 (1:1 *v*/*v*) and purified by column chromatography over silica gel ($CHCl_3$/MeOH/2.2 M NH_4OH 5:3.9:1.1) [376] The combined fractions were partially concentrated *in vacuo* at 0 °C, then H_2O (30 mL) was added. Lyophilising this solution yielded (–)-**326** (2 mg) as a fluffy, colourless solid, contaminated with hydrolysis product.

Method 5 To a solution of (–)-**325** (10 mg, 0.00749 mmol, 1.0 eq, 88% D_6, 12% D_5) in a mixture of ^{t}BuOH (400 μL) and H_2O (70 μL) was added palladium black (24 mg, 0.225 mmol, 30 eq) under $N_{2(g)}$. The flask was purged with 3 balloons of $H_{2(g)}$. After stirring at RT under $H_{2(g)}$ for 21 hours, $NaHCO_3$ (6 mg, 0.0767 mmol, 3.0 eq) was added in one portion and the reaction mixture was stirred for a further 2 minutes under $H_{2(g)}$. The reaction mixture was then filtered through a microfiber pad and washed with H_2O (2 × 5 mL). The combined aqueous filtrates were lyophilised to give (–)-**326** (6 mg, 86%, 90% D_6, 10% D_5) as a fluffy, colourless solid. The compound was stored at –80 °C as the sodium salt: R_f 0.60 ($CHCl_3$/MeOH/2.2 M NH_4OH 5:3.9:1.1); $[\alpha]_D^{25}$ = –16.2 (*c* 0.054, MeOH/$CHCl_3$/H_2O 4:3:1); ^{1}H NMR (500 MHz; CD_3CD/$CDCl_3$/D_2O 4:3:1) δ_H 5.30–5.25 (1H, m, H-10), 4.43 (1H, dd, J_{XY} 12.2, 2.5, H-11), 4.21 (1H, dd, J_{XY} 12.2, 8.0, H-11'), 4.09–3.99 (2H, m, H-9), 2.38–2.28 (4H, m, H-13 and H-29), 1.65–1.53 (4H, m, H-14 and H-30), 1.34–1.21 (48H, m, H-15 to H-26 and H-31 to H-42), 0.87 (6H, t, *J* 6.9, H-27 and H-43); ^{13}C NMR (126 MHz; CD_3CD/$CDCl_3$/D_2O 4:3:1) δ_C 175.4, 175.2 (C-12, C-28), 71.5 (d_P, J_P 8.8, C-10), 64.5 (d_P, J_P 4.7, C-9), 64.0 (C-11), 35.1, 35.0 (C-13, C-29), 32.7 (C-25, C-41), 30.55, 30.52, 30.50, 30.48, 30.45,

30.43, 30.39, 30.37, 30.3, 30.2, 30.1, 30.0, 29.7 (C-15 to C-24, C-31 to C-40), 25.8, 25.7 (C-14, C-30), 23.4 (C-26, C-42), 14.6 (C-27, C-43);[XXII] ^{31}P NMR (162 MHz; $CD_3CD/CDCl_3/D_2O$ 4:3:1) δ_P 5.37 (P-7), 0.005 (P-8); ^{2}H NMR (96 MHz; $MeOH/CHCl_3/H_2O$ 4:3:1; $CDCl_3$) δ_D 4.33 (br s, inositol ring), 3.04 (br s, inositol ring), 3.01 (br s, inositol ring); $\bar{\nu}_{max}$(thin film)/cm^{-1} 3301 (O-H) (br), 2957 (w), 2917 (m), 2850 (m), 1735 (C=O) (m), 1627 (m), 1467 (m), 1380 (s), 1355 (s), 1244 (m), 1199 (m), 1101 (s), 1022 (P-O) (s), 970 (P-O) (s), 833 (s), 682 (s), 648 (s), 638 (s); HRMS *m/z* (ESI$^-$) found 894.51615 ([MD_5–H]$^-$, 11%), 895.52192 ([MD_6–H]$^-$, 100%) ($C_{41}H_{73}D_6O_{16}P_2$ requires 895.52254 [MD_6–H]$^-$).

(*S*)-(–)-1-*O*-Stearoyl-2,3-*O*-isopropylidene-*sn*-glycerol (–)-330

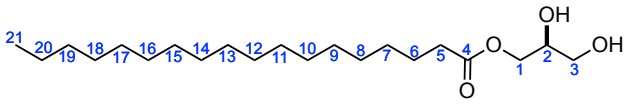


A solution of *N*,*N*'-dicyclohexylcarbodiimide (3.12 g, 15.1 mmol, 2.0 eq) in anhydrous CH_2Cl_2 (27 mL) was added dropwise to a stirred solution of (*R*)-(–)-2,3-isopropylidene-*sn*-glycerol (–)-**329** (1.00 g, 7.57 mmol, 1.0 eq), stearic acid (2.15 g, 7.57 mmol, 1.0 eq) and 4-DMAP (55 mg, 0.454 mmol, 0.060 eq) in anhydrous CH_2Cl_2 (20 mL) over 30 minutes, at 0 °C. The reaction mixture was gradually warmed to RT, and was stirred for a further 12 hours. The mixture was then diluted with hexane (10 mL), filtered through Celite® and washed with hexane (3 × 10 mL). The combined filtrate and washings were concentrated *in vacuo*. The residue obtained was purified by column chromatography over silica gel (Hexane/EtOAc 20:1) to give (–)-**330** (2.29 g, 76%) as a colourless solid: R_f 0.43 (Hexane/EtOAc 20:1); $[\alpha]_D^{25}$ = –7.9 (*c* 4.0, Hexane) [lit. [380] $[\alpha]_D^{23}$ = –7.38 (*c* 3.95, Hexane)]; m.p. 40–41 °C (Pentane/EtOAc) [lit. [380] 40–41 °C]; ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 4.35–4.28 (1H, m, H-2), 4.17 (1H, dd, J_{XY} 11.7, 4.6, H-1), 4.09 (1H, dd, J_{XY} 11.7, 5.6, H-1'), 4.08 (1H, dd, J_{XY} 8.3, 6.1, H-3), 3.74 (1H, dd, J_{XY} 8.3, 6.2, H-3'), 2.34 (2H, t, *J* 7.6, H-6), 1.67–1.56 (2H, m, H-7),

[XXII] Due to the instability of this compound in solution, only a minimal number of scans could be used for acquiring the ^{13}C NMR spectrum, this was not enough to observe the peaks corresponding to C-1, C-2, C-3, C-4, C-5 and C-6. Those can be inferred however by comparison with the ^{13}C NMR spectrum of the equivalent non-deuterated compounds.

1.44 (3H, s, C(4)CH_3), 1.37 (3H, s, C(4)CH_3), 1.33–1.23 (28H, m, H-8 to H-21), 0.88 (3H, t, *J* 6.8, H-22); ^{13}C NMR (126 MHz; $CDCl_3$) δ_C 173.5 (C-5), 109.8 (C-4), 73.7 (C-2), 66.4 (C-3), 64.5 (C-1), 34.1 (C-6), 32.0 (C-20), 29.75, 29.72, 29.69, 29.6, 29.5, 29.4, 29.3, 29.2 (C-8 to C-19), 26.7 (C(4)CH_3), 25.4 (C(4)CH_3), 24.9 (C-7), 22.7 (C-21), 14.1 (C-22); LRMS *m/z* (ESI$^-$) 415.1 ([M–OH]$^-$, 100%). These data are in good agreement with the literature values.[23,240,380]

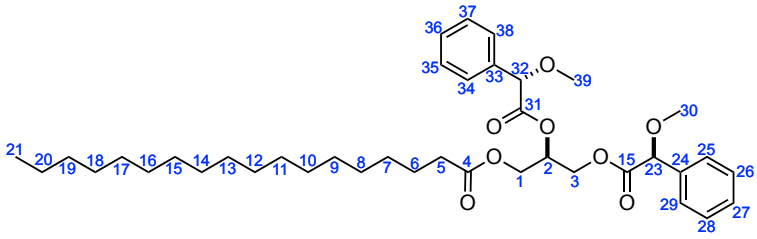
(*S*)-(+)-1-*O*-Stearoyl-*sn*-glycerol (+)-331



Aqueous acetic acid (AcOH/H_2O 4:1 *v/v*, 5 mL) was added to solid (–)-**330** (500 mg, 1.25 mmol, 1.0 eq), and the mixture was stirred at 50 °C for 2 hours. The reaction mixture was cooled to RT and diluted in Et_2O (10 mL). The organic layer was washed with H_2O (8 mL), a saturated aqueous solution of $NaHCO_3$ (8 mL), and brine (8 mL), then dried ($MgSO_4$), filtered, and concentrated *in vacuo*. The colourless solid obtained was crystallised from Pentane/EtOAc to give (+)-**331** (314 mg, 70%, >99% *e.e.*)[XXIII] as silver, shiny crystals: R_f 0.25 (Petroleum ether/EtOAc 7:3); $[\alpha]_D^{25}$ = +3.9 (*c* 2.9, Pyridine) [lit.[380] $[\alpha]_D^{23}$ = +4.10 (*c* 2.58, Pyridine)]; m.p. 74–75 °C (Pentane/EtOAc) [lit.[380] 73–74 °C]; ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 4.21 (1H, dd, J_{XY} 11.7, 4.5, H-1), 4.15 (1H, dd, J_{XY} 11.7, 6.1, H-1'), 3.98–3.89 (1H, m, H-2), 3.70 (1H, ddd, J_{XY} 11.4, 6.6, 4.0, H-3), 3.60 (1H, ddd, J_{XY} 11.4, 5.7, 5.7, H-3'), 2.53 (1H, br s, C(2)O*H*), 2.35 (2H, t, *J* 7.7, H-5), 2.10 (1H, br s, C(3)O*H*), 1.67–1.58 (2H, m, H-6), 1.36–1.19 (28H, m, H-7 to H-20), 0.87 (3H, t, *J* 6.8, H-21); ^{13}C NMR (126 MHz; $CDCl_3$) δ_C 174.5 (C-4), 70.4 (C-2), 65.3 (C-1), 63.5 (C-3), 34.3 (C-5), 32.1 (C-19), 29.83, 29.79, 29.74, 29.6, 29.5, 29.4, 29.3 (C-7 to C-18), 25.0 (C-6), 22.8 (C-20), 14.3 (C-21); LRMS *m/z* (ESI$^+$) 381.3 ([M+Na]$^+$, 100%). These data are in good agreement with the literature values.[23,240,380]

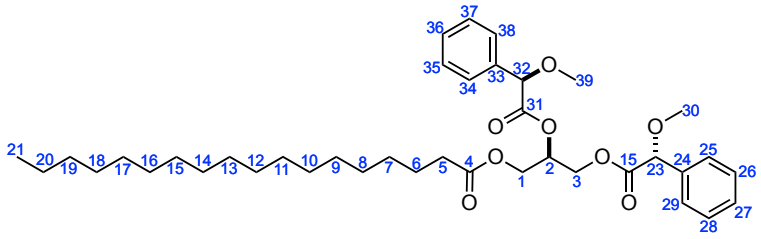
[XXIII] The enantiomeric excess was determined by MPA ^{1}H NMR analysis

(*R*)-(+)-1-*O*-Stearoyl-2,3-di-*O*-((*S*)-α-methoxyphenylacetoxy)-*sn*-glycerol (+)-332a



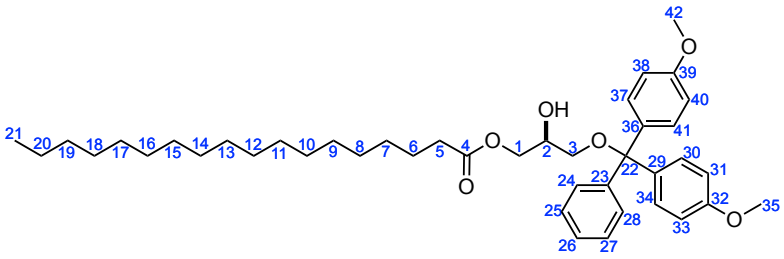
(*S*)-(+)-α-methoxyphenylacetic acid (37 mg, 0.223 mmol, 4.0 eq), 1-ethyl-3-(3-dimethylamino propyl)carbodiimide (58 mg, 0.301 mmol, 5.4 eq) and 4-DMAP (3 mg, 0.0278 mmol, 0.50 eq) were added to a solution of (+)-**331** (20 mg, 0.0558 mmol, 1.0 eq) in CH_2Cl_2 (0.50 mL). After 24 hours stirring at RT, the reaction mixture was diluted with CH_2Cl_2 (3 mL), and the organic layer was washed with a saturated aqueous solution of $NaHCO_3$ (2 × 5 mL), and brine (5 mL), then dried ($MgSO_4$), filtered, and concentrated *in vacuo*. Purification by column chromatography over silica gel (Petroleum ether/EtOAc 9:1, 8:2, 7:3) afforded (+)-**332a** (32 mg, 89%) as a colourless solid: R_f 0.59 (Petroleum ether/EtOAc 6:4); $[\alpha]_D^{25}$ = +51.7 (*c* 1.0, $CHCl_3$); m.p. 50–51 °C; ^{1}H NMR (500 MHz; $CDCl_3$) δ_H 7.44–7.29 (10H, m, H-25 to H-29 and H-34 to H-38), 5.24–5.18 (1H, m, H-2), 4.73 (1H, s, H-23), 4.60 (1H, s, H-32), 4.41 (1H, dd, J_{XY} 12.1, 3.9, H-3), 4.18 (1H, dd, J_{XY} 12.1, 5.4, H-3'), 4.02 (1H, dd, J_{XY} 11.9, 4.3, H-1), 3.85 (1H, dd, J_{XY} 11.9, 6.7, H-1'), 3.40 (3H, s, H-30 or H-39), 3.38 (3H, s, H-30 or H-39), 2.07–1.95 (2H, m, H-5), 1.47–1.39 (2H, m, H-6), 1.32–1.18 (28H, m, H-7 to H-20), 0.88 (3H, t, *J* 6.9, H-21); ^{13}C NMR (126 MHz; $CDCl_3$) δ_C 173.1 (C-4), 170.3 (C-22), 169.9 (C-31), 136.02, 136.00 (C-24, C-33), 129.1, 128.9, 128.7 (C-26 to C-28, C-35 to C-37), 127.3 (C-25, C-29), 127.2 (C-34, C-38), 82.3 (C-23), 82.2 (C-32), 69.7 (C-2), 62.58 (C-3), 62.57 (C-1), 57.52, 57.50 (C-30, C-39), 33.8 (C-5), 32.1 (C-19), 29.84, 29.80, 29.76, 29.6, 29.5, 29.4, 29.2 (C-7 to C-18), 24.7 (C-6), 22.8 (C-20), 14.3 (C-21); $\bar{\nu}_{max}$(thin film)/cm^{-1} 2980 (m), 2971 (m), 2923 (s), 2853 (m), 1744 (C=O) (s), 1494 (w), 1456 (m), 1380 (m), 1251 (m), 1198 (m), 1166 (C-O) (s), 1116 (C-O) (s), 1076 (m), 1029 (w), 998 (w), 967 (w), 731 (m), 697 (m); LRMS *m/z* (ESI$^+$) 677.4 ([M+Na]$^+$, 100%); HRMS *m/z* (ESI$^+$) found 677.40230 [M+Na]$^+$ ($C_{39}H_{58}O_8Na$ requires 677.40239 [M+Na]$^+$); RP-HPLC (Method 4) t_R = 8.27 min, 98.61%.

(*R*)-(−)-1-*O*-Stearoyl-2,3-di-*O*-((*R*)-α-methoxyphenylacetoxy)-*sn*-glycerol (−)-332b



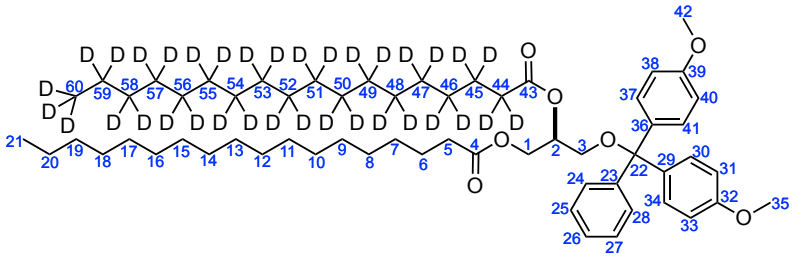
(*R*)-(−)-α-methoxyphenylacetic acid (37 mg, 0.223 mmol, 4.0 eq), 1-ethyl-3-(3-dimethylamino propyl)carbodiimide (58 mg, 0.301 mmol, 5.4 eq) and 4-DMAP (3 mg, 0.0278 mmol, 0.50 eq) were added to a solution of (+)-**331** (20 mg, 0.0558 mmol, 1.0 eq) in CH_2Cl_2 (0.50 mL). After 24 hours stirring at RT, the reaction mixture was diluted with CH_2Cl_2 (3 mL), and the organic layer was washed with a saturated aqueous solution of $NaHCO_3$ (2 × 5 mL), and brine (5 mL), then dried ($MgSO_4$), filtered, and concentrated *in vacuo*. Purification by column chromatography over silica gel (Petroleum ether/EtOAc 9:1, 8:2, 7:3) afforded (−)-**332b** (30 mg, 83%) as a colourless solid: R_f 0.63 (Petroleum ether/EtOAc 6:4); $[\alpha]_D^{25}$ = –47.7 (*c* 0.97, $CHCl_3$); m.p. 44–45 °C (Hexane/EtOAc); ^{1}H NMR (500 MHz; $CDCl_3$) δ_H 7.45–7.29 (10H, m, H-25 to H-29 and H-34 to H-38), 5.31–5.25 (1H, m, H-2), 4.74 (1H, s, H-23), 4.56 (1H, s, H-32), 4.30 (1H, dd, J_{XY} 12.0, 4.1, H-3), 4.18 (1H, dd, J_{XY} 12.1, 4.2, H-1), 4.03 (1H, dd, J_{XY} 12.1, 6.1, H-1'), 4.00 (1H, dd, J_{XY} 12.0, 6.3, H-3'), 3.37 (3H, s, H-30 or H-39), 3.34 (3H, s, H-30 or H-39), 2.20 (2H, t, *J* 8.0, H-5), 1.59–1.50 (2H, m, H-6), 1.33–1.21 (28H, m, H-7 to H-20), 0.88 (3H, t, *J* 6.9, H-21); ^{13}C NMR (126 MHz; $CDCl_3$) δ_C 173.2 (C-4), 170.2, 170.1 (C-22, C-31), 136.1, 136.0 (C-24, C-33), 129.02, 129.00, 128.8 (C-26 to C-28, C-35 to C-37), 127.28, 127.26 (C-25, C-29, C-34, C-38), 82.5 (C-23), 82.2 (C-32), 69.8 (C-2), 62.6 (C-3), 61.7 (C-1), 57.5 (C-30, C-39), 34.0 (C-5), 32.1 (C-19), 29.84, 29.80, 29.76, 29.6, 29.5, 29.4, 29.2 (C-7 to C-18), 24.9 (C-6), 22.8 (C-20), 14.3 (C-21); $\bar{\nu}_{max}$(thin film)/cm^{-1} 2923 (s), 2853 (m), 1742 (C=O) (s), 1495 (w), 1455 (m), 1349 (w), 1318 (w), 1245 (m), 1198 (C-O) (s), 1166 (C-O) (s), 1113 (C-O) (s), 1076 (m), 1029 (m), 997 (m), 730 (s), 697 (s); LRMS *m/z* (ESI$^+$) 677.4 ([M+Na]$^+$, 100%); HRMS *m/z* (ESI$^+$) found 677.40268 [M+Na]$^+$ ($C_{39}H_{58}O_8Na$ requires 677.40239 [M+Na]$^+$); RP-HPLC (Method 4) t_R = 7.93 min, 98.76%.

(*S*)-(+)-1-*O*-Stearoyl-3-*O*-(dimethoxytrityl)-*sn*-glycerol (+)-333



To a solution of (+)-**331** (200 mg, 0.558 mmol, 1.0 eq) in anydrous pyridine (5 mL) was added 4,4′-dimethoxytrityl chloride (227 mg, 0.669 mmol, 1.2 eq) in one portion. After 50 minutes stirring at RT, the yellow solution was concentrated *in vacuo* at RT. The residue obtained was purified by column chromatography over silica gel (Petroleum ether/EtOAc 9.5:0.5, 9:1, 8.5:1.5, 8:2, 7:3) to give (+)-**333** (249 mg, 67%) as a colourless oil: R_f 0.54 (Petroleum ether/EtOAc 7:3); $[\alpha]_D^{25}$ = +3.0 (*c* 2.0, $CHCl_3$); ^{1}H NMR (500 MHz; $CDCl_3$) δ_H 7.43–7.39 (2H, m, H-24 and H-28), 7.33–7.26 (6H, m, H-30, H-34, H-37, H-41, H-25 and H-27), 7.24–7.20 (1H, m, H-26), 6.85–6.81 (4H, m, H-31, H-33, H-38 and H-40), 4.20 (1H, dd, J_{XY} 11.5, 4.3, H-1), 4.16 (1H, dd, J_{XY} 11.5, 6.2, H-1'), 4.01–3.95 (1H, m, H-2), 3.79 (6H, s, H-35 and H-42), 3.22 (1H, dd, J_{XY} 9.5, 5.0, H-3), 3.19 (1H, dd, J_{XY} 9.5, 5.5, H-3'), 2.42 (1H, d, *J* 5.2, C(2)O*H*), 2.28 (2H, t, *J* 7.6, H-5), 1.62–1.54 (2H, m, H-6), 1.34–1.20 (28H, m, H-7 to H-20), 0.88 (3H, t, *J* 6.9, H-21); ^{13}C NMR (126 MHz; $CDCl_3$) δ_C 174.1 (C-4), 158.7 (C-32, C-39), 144.7 (C-23), 135.9 (C-29, C-36), 130.1 (C-30, C-34, C-37, C-41), 128.2 (C-24, C-28), 128.0 (C-25, C-27), 127.0 (C-26), 113.3 (C-31, C-33, C-38, C-40), 86.4 (C-22), 69.5 (C-2), 65.8 (C-1), 64.1 (C-3), 55.3 (C-35, C-42), 34.3 (C-5), 32.1 (C-19), 29.84, 29.80, 29.76, 29.6, 29.5, 29.4, 29.3 (C-7 to C-18), 25.0 (C-6), 22.8 (C-20), 14.3 (C-21); $\bar{\nu}_{max}$(thin film)/cm^{-1} 3480 (O-H) (br), 2980 (m), 2922 (s), 2852 (m), 1737 (C=O) (m), 1608 (m), 1582 (w), 1509 (s), 1463 (m), 1445 (m), 1415 (w), 1379 (w), 1300 (m), 1249 (s), 1175 (C-O) (s), 1155 (m), 1116 (m), 1076 (m), 1035 (m), 982 (m), 902 (w), 828 (m), 791 (w), 771 (w), 754 (w), 726 (w), 702 (m); HRMS *m/z* (ESI$^+$) found 683.42851 $[M+Na]^+$ ($C_{42}H_{60}O_6Na$ requires 683.42821 $[M+Na]^+$); RP-HPLC (Method 2) t_R = 6.88 min, 96.13%. These data are in good agreement with the literature values.[23]

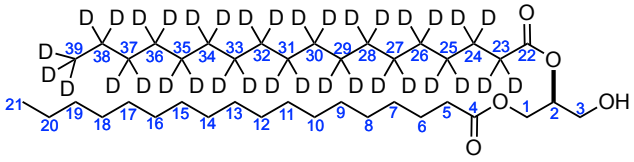
D_{35} (*S*)-(+)-1-*O*-Stearoyl-2-*O*-(D_{35}-stearoyl)-3-*O*-(dimethoxytrityl)-*sn*-glycerol (+)-335



To a stirred solution of (+)-**333** (600 mg, 0.908 mmol, 1.0 eq), D_{35} stearic acid (290 mg, 0.908 mmol, 1.0 eq) and 4-DMAP (30 mg, 0.245 mmol, 0.27 eq) in anhydrous CH_2Cl_2 (14 mL) was added *N*,*N*'-dicyclohexylcarbodiimide (227 mg, 1.10 mmol, 1.21 eq). The reaction mixture was stirred at RT for 24 hours and the solvent was concentrated *in vacuo*. The residue obtained was purified by column chromatography over silica gel (Petroleum ether/Et_2O 10:0, 9.5:0.5, 9:1, 8.5:1.5, 8:2) to give (+)-**335** (795 mg, 91%, 70% D_{35}, 30% D_{34}) as a colourless solid: R_f 0.88 (Petroleum ether/EtOAc 7:3); $[\alpha]_D^{25}$ = +11.3 (*c* 1.0, $CHCl_3$); m.p. 31–32 °C (Hexane/EtOAc); ^{1}H NMR (500 MHz; $CDCl_3$) δ_H 7.43–7.39 (2H, m, H-24 and H-28), 7.33–7.27 (6H, m, H-25, H-27, H-30, H-34, H-37 and H-41), 7.24–7.19 (1H, m, H-26), 6.85–6.79 (4H, m, H-31, H-33, H-38 and H-40), 5.28–5.22 (1H, m, H-2), 4.34 (1H, dd, J_{XY} 11.7, 3.8, H-1), 4.23 (1H, dd, J_{XY} 11.7, 6.8, H-1'), 3.79 (6H, s, H-35 and H-42), 3.23 (1H, dd, J_{XY} 9.9, 4.8, H-3), 3.20 (1H, dd, J_{XY} 9.9, 5.2, H-3'), 2.28 (2H, t, *J* 7.6, H-5), 1.59–1.50 (2H, m, H-6), 1.34–1.18 (28H, m, H-7 to H-20), 0.88 (3H, t, *J* 6.9, H-21); ^{13}C NMR (126 MHz; $CDCl_3$) δ_C 173.6 (C-4), 173.3 (C-43), 158.6 (C-39, C-32), 144.7 (C-23), 135.91, 135.88 (C-29, C-36), 130.16, 130.13 (C-30, C-34, C-37, C-41), 128.2 (C-24, C-28), 128.0 (C-25, C-27), 127.0 (C-26), 113.3 (C-31, C-33, C-38, C-40), 86.2 (C-22), 70.6 (C-2), 63.1 (C-1), 62.2 (C-3), 55.3 (C-35, C-42), 34.3 (C-5), 34.2–33.4 (m_D, C-44), 32.1 (C-19), 31.1–30.2 (m_D, C-58), 29.9, 29.83, 29.81, 29.79, 29.7, 29.5, 29.4, 29.3 (C-7 to C-18), 28.9–27.7 (m_D, C-46 to C-57), 25.0 (C-6), 24.4–23.7 (m_D, C-45), 22.8 (C-20), 21.9–21.1 (m_D, C-59), 14.3 (C-21), 13.5–12.6 (m_D, C-60); ^{2}H NMR (96 MHz; $CDCl_3$) δ_D 2.29 (2D, br s, D-44), 1.57 (2D, br s, D-45), 1.19 (28D, br s, D-46 to D-59), 0.83 (3D, br s, D-60); $\bar{\nu}_{max}$(thin film)/cm^{-1} 2981 (s), 2919 (m), 2851 (m), 2194 (w), 2088 (w), 1739 (C=O) (m), 1608 (w), 1509 (m), 1468 (w), 1446 (w), 1381 (w), 1298 (w), 1250 (s), 1174 (s), 1088 (m), 1034 (m), 953 (w), 828 (m),

724 (w), 702 (w); HRMS *m/z* (ESI$^+$) found 983.90219 ([MD_{34}+Na]$^+$, 33%), 984.90804 ([MD_{35}+Na]$^+$, 100%) ($C_{60}H_{59}D_{35}O_7Na$ requires 984.90886 [MD_{35}+Na]$^+$); NP-HPLC (Method 8) t_R = 1.39 min, 100%.

D_{35} (*S*)-(–)-1-*O*-Stearoyl-2-*O*-(D_{35}-stearoyl)-*sn*-glycerol (–)-328



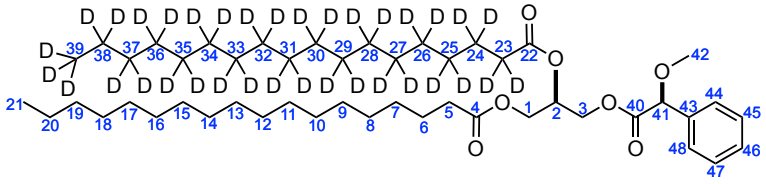
Method 1 TFA (0.3 μL) was added to a solution of (+)-**335** (30 mg, 0.0312 mmol, 1.0 eq, 70% D_{35}, 30% D_{34}) and pyrrole (4 mg, 0.0596 mmol, 1.9 eq) in anhydrous CH_2Cl_2 (0.20 mL). After stirring at RT for 2 hours, further TFA (0.3 μL) was added. The solution was then stirred for a further hour, and the solvent was concentrated *in vacuo*. The solid obtained was crystallised from Hexane/Et_2O to give (–)-**328** (10 mg, 54%) as a colourless solid. Analysis by ^{1}H NMR revealed that (–)-**328** was contaminated with some migration product **336** in a ratio of 3.3:1.0.

Method 2 Aqueous acetic acid (AcOH/H_2O 4:1 *v/v*, 5 mL) was added to solid (+)-**335** (923 mg, 0.959 mmol, 1.0 eq, 70% D_{35}, 30% D_{34}), and the mixture was stirred at 50 °C for 3.5 hours. The solution was then cooled to RT and concentrated *in vacuo*. The colourless solid obtained was crystallised from Pentane/Et_2O[XXIV] to give (–)-**328** (596 mg, 94%, 72% D_{35}, 28% D_{34}, >96% *e.e.*)[XXV] as a colourless solid: R_f 0.19 (Petroleum ether/Et_2O 7:3); $[\alpha]_D^{25}$ = –2.9 (*c* 1.0, $CHCl_3$); m.p. 72–73 °C (Pentane/Et_2O); ^{1}H NMR (500 MHz; $CDCl_3$) δ_H 5.11–5.06 (1H, m, H-2), 4.32 (1H, dd, J_{XY} 11.9, 4.5, H-1), 4.24 (1H, dd, J_{XY} 11.9, 5.7, H-1'), 3.77–3.68 (2H, m, H-3), 2.32 (2H, t, *J* 7.6, H-5), 2.03 (1H, dd, *J* 6.5, 6.5, C(3)O*H*), 1.65–1.58 (2H, m, H-6), 1.34–1.20 (28H, m, H-7 to H-20), 0.88 (3H, t, *J* 7.0, H-21); ^{13}C NMR (126 MHz; $CDCl_3$) δ_C 174.0 (C-4), 173.7 (C-22), 72.2 (C-2), 62.1 (C-1), 61.7 (C-3), 34.3 (C-5), 34.1–33.3 (m_D, C-23), 32.1 (C-19), 31.1–30.2 (m_D, C-37), 29.85, 29.81, 29.77, 29.6, 29.5,

[XXIV] Purification by column chromatography over silica gel was attempted, however this led to migration of the lipid chain and racemisation.
[XXV] The enantiomeric excess was determined by MPA ^{1}H NMR analysis.

29.4, 29.3 (C-7 to C-18), 28.9–27.7 (m_D, C-25 to C-36), 25.0 (C-6), 24.4–23.6 (m_D, C-24), 22.8 (C-20), 21.9–21.1 (m_D, C-38), 14.3 (C-21), 13.5–12.7 (m_D, C-39); ^{2}H NMR (96 MHz; $CDCl_3$) δ_D 2.31 (2D, br s, D-23), 1.58 (2D, br s, D-24), 1.20 (28D, br s, D-25 to D-38), 0.83 (3D, br s, D-39); $\bar{\nu}_{max}$(thin film)/cm^{-1} 3501 (O-H) (w), 2955 (w), 2918 (s), 2850 (s), 2193 (m), 2088 (m), 1729 (C=O) (s), 1709 (C=O) (s), 1600 (w), 1468 (m), 1451 (m), 1389 (m), 1367 (m), 1328 (m), 1317 (m), 1276 (s), 1256 (m), 1241 (m), 1222 (m), 1164 (m), 1147 (m), 1089 (s), 1062 (s), 1039 (m), 1027 (m), 1010 (m), 724 (m); HRMS *m/z* (ESI$^+$) found 681.77168 ([MD_{34}+Na]$^+$, 34%), 682.77748 ([MD_{35}+Na]$^+$, 100%) ($C_{39}H_{41}D_{35}O_5Na$ requires 682.77818 [MD_{35}+Na]$^+$).

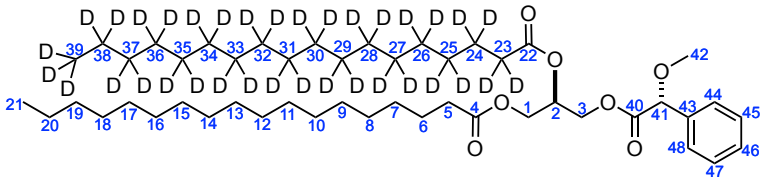
D_{35} (*R*)-(+)-1-*O*-Stearoyl-2-*O*-(D_{35}-stearoyl)-3-*O*-((*S*)-α-methoxyphenylacetoxy)-*sn*-glycerol (+)-337a



(*S*)-(+)-α-methoxyphenylacetic acid (19 mg, 0.115 mmol, 3.8 eq), 1-ethyl-3-(3-dimethylamino propyl)carbodiimide (30 mg, 0.158 mmol, 5.2 eq) and 4-DMAP (3 mg, 0.0242 mmol, 0.80 eq) were added to a solution of (–)-**328** (20 mg, 0.0303 mmol, 1.0 eq, 72% D_{35}, 28% D_{34}) in CH_2Cl_2 (1 mL). After stirring at RT for 24 hours, the reaction mixture was diluted with CH_2Cl_2 (3 mL), and the organic layer was washed with a saturated aqueous solution of $NaHCO_3$ (2 × 5 mL), and brine (5 mL), then dried ($MgSO_4$), filtered, and concentrated *in vacuo*. Purification by column chromatography over silica gel (Petroleum ether/Et_2O 10:0, 9:1, 8:2, 7:3) provided (+)-**337a** (23 mg, 94%, 71% D_{35}, 29% D_{34}) as a colourless solid: R_f 0.44 (Petroleum ether/Et_2O 7:3); $[\alpha]_D^{25}$ = +20.7 (*c* 0.79, $CHCl_3$); m.p. 58–60 °C (Pentane/Et_2O); ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 7.44–7.40 (2H, m, H-45 and H-47), 7.38–7.30 (3H, m, H-44, H-46 and H-48), 5.20–5.14 (1H, m, H-2), 4.77 (1H, s, H-41), 4.35 (1H, dd, J_{XY} 11.9, 4.1, H-3), 4.20 (1H, dd, J_{XY} 11.9, 5.7, H-3'), 4.28 (1H, dd, J_{XY} 11.9, 4.4, H-1), 3.96 (1H, dd, J_{XY} 11.9, 5.9, H-1'), 3.41 (3H, s, H-42), 2.26 (2H, t, *J* 7.5, H-5), 1.61–1.52 (2H, m, H-6), 1.33–

1.20 (28H, m, H-7 to H-20), 0.88 (3H, t, *J* 6.9, H-21); ^{13}C NMR (126 MHz; $CDCl_3$) δ_C 173.3 (C-4), 172.9 (C-22), 170.3 (C-40), 136.0 (C-43), 129.0 (C-46), 128.8 (C-44, C-48), 127.3 (C-45, C-47), 82.5 (C-41), 68.7 (C-2), 62.7 (C-3), 61.9 (C-1), 57.5 (C-42), 34.1 (C-5), 33.9–33.0 (m_D, C-23), 32.1 (C-19), 31.1–30.3 (m_D, C-37), 29.85, 29.81, 29.77, 29.6, 29.5, 29.4, 29.2 (C-7 to C-18), 28.9–27.7 (m_D, C-25 to C-36), 25.0 (C-6), 24.2–23.4 (m_D, C-24), 22.8 (C-20), 21.9–21.1 (m_D, C-38), 14.3 (C-21), 13.5–12.7 (m_D, C-39); ^{2}H NMR (96 MHz; $CDCl_3$) δ_D 2.15 (2D, br s, D-23), 1.48 (2D, br s, D-24), 1.20 (28D, br s, D-25 to D-38), 0.83 (3D, br s, D-39); $\bar{\nu}_{max}$(thin film)/cm^{-1} 2919 (s), 2851 (m), 2193 (m), 2087 (m), 1737 (C=O) (s), 1469 (m), 1385 (w), 1261 (m), 1250 (m), 1213 (m), 1191 (m), 1166 (s), 1149 (s), 1117 (s), 1088 (s), 1032 (m), 981 (m), 947 (m), 932 (m), 726 (s), 695 (m); HRMS *m/z* (ESI$^+$) found 829.82428 ([MD_{34}+H]$^+$, 34%), 830.82988 ([MD_{35}+H]$^+$, 100%) ($C_{48}H_{49}D_{35}O_7Na$ requires 830.83061 [MD_{35}+Na]$^+$); RP-HPLC (Method 8) t_R = 1.45 min, 96.86%.

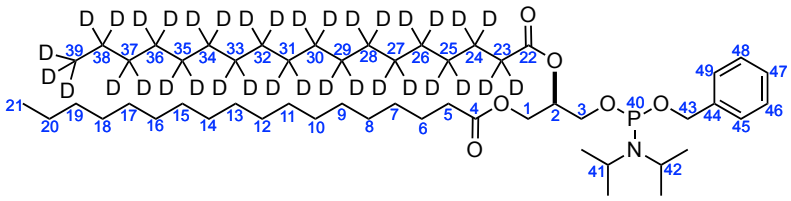
D_{35} (*R*)-(–)-1-*O*-Stearoyl-2-*O*-(D_{35}-stearoyl)-3-*O*-((*R*)-α-methoxyphenylacetoxy)-*sn*-glycerol (–)-337b



(*R*)-(–)-α-Methoxyphenylacetic acid (19 mg, 0.115 mmol, 3.8 eq), 1-ethyl-3-(3-dimethylamino propyl)carbodiimide (30 mg, 0.158 mmol, 5.2 eq) and 4-DMAP (3 mg, 0.0242 mmol, 0.80 eq) were added to a solution of (–)-**328** (20 mg, 0.0303 mmol, 1.0 eq, 72% D_{35}, 28% D_{34}) in CH_2Cl_2 (1 mL). After stirring at RT for 24 hours, the reaction mixture was diluted with CH_2Cl_2 (3 mL), and the organic layer was washed with a saturated aqueous solution of $NaHCO_3$ (2 × 5 mL), and brine (5 mL), then dried ($MgSO_4$), filtered, and concentrated *in vacuo*. Purification by column chromatography over silica gel (Petroleum ether/Et_2O 10:0, 9:1, 8:2, 7:3) gave (–)-**337b** (21 mg, 86%, 70% D_{35}, 30% D_{34}) as a colourless solid: R_f 0.44 (Petroleum ether/Et_2O 7:3); $[\alpha]_D^{25}$ = –19.6 (*c* 1.0, $CHCl_3$); m.p. 58–60 °C (Pentane/Et_2O); ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 7.44–7.39 (2H, m, H-45 and H-47), 7.38–7.30 (3H, m, H-44, H-46 and H-48), 5.25–5.19 (1H, m, H-2), 4.77 (1H, s, H-41), 4.34 (1H, dd, J_{XY} 12.1,

4.0, H-3), 4.18 (1H, dd, J_{XY} 12.1, 6.1, H-3'), 4.16 (1H, dd, J_{XY} 12.0, 4.9, H-1), 4.03 (1H, dd, J_{XY} 12.0, 5.7, H-1'), 3.41 (3H, s, H-42), 2.26 (2H, t, *J* 7.6, H-5), 1.61–1.52 (2H, m, H-6), 1.33–1.20 (28H, m, H-7 to H-20), 0.88 (3H, t, *J* 6.9, H-21); ^{13}C NMR (126 MHz; $CDCl_3$) δ_C 173.3 (C-4), 172.9 (C-22), 170.4 (C-40), 136.1 (C-43), 129.0 (C-46), 128.8 (C-44, C-48), 127.3 (C-45, C-47), 82.4 (C-41), 68.6 (C-2), 63.0 (C-3), 61.9 (C-1), 57.5 (C-42), 34.1 (C-5), 33.9–32.9 (m_D, C-23), 32.1 (C-19), 31.1–30.2 (m_D, C-37), 29.85, 29.81, 29.77, 29.6, 29.5, 29.4, 29.2 (C-7 to C-18), 28.9–27.6 (m_D, C-25 to C-36), 25.0 (C-6), 24.2–23.4 (m_D, C-24), 22.8 (C-20), 21.9-21.1 (m_D, C-38), 14.3 (C-21), 13.5–12.7 (m_D, C-39); ^{2}H NMR (96 MHz; $CDCl_3$) δ_D 2.13 (2D, br s, D-23), 1.48 (2D, br s, D-24), 1.19 (28D, br s, D-25 to D-38), 0.83 (3D, br s, D-39); $\bar{\nu}_{max}$(thin film)/cm^{-1} 2918 (s), 2850 (m), 2193 (m), 2088 (m), 1736 (C=O) (s), 1469 (m), 1286 (m), 1274 (m), 1211 (m),1177 (s), 1115 (s), 1089 (m), 1058 (m), 744 (m), 724 (m), 698 (m); HRMS *m/z* (ESI$^+$) found 829.82412 ([MD_{34}+Na]$^+$, 35%), 830.82983 ([MD_{35}+Na]$^+$, 100%) ($C_{48}H_{49}D_{35}O_7Na$ requires 830.83061 [MD_{35}+Na]$^+$); RP-HPLC (Method 8) t_R = 1.45 min, 96.86%.

D_{35} (*R*)-(+)-Benzyloxy-*N*,*N*-diisopropyl-(1-*O*-Stearoyl-2-*O*-(D_{35}-stearoyl)-*sn*-glyceryl) phosphoramidite (+)-339



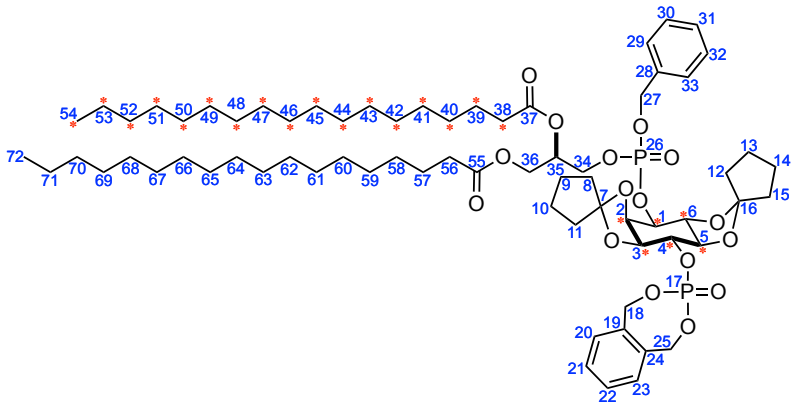
A solution of alcohol (–)-**328** (200 mg, 0.303 mmol, 1.0 eq, 72% D_{35}, 28% D_{34}) in anhydrous CH_2Cl_2 (9 mL) was added dropwise, over 45 minutes and *via* cannula, to a solution of benzyloxy bis(*N*,*N*-diisopropylamino)phosphine **310** (256 mg, 0.757 mmol, 2.5 eq) and 1*H*-tetrazole (2.02 mL, 0.909 mmol, 3.0 eq, 0.45 M in MeCN) in anhydrous CH_2Cl_2 (15 mL). The reaction mixture was stirred at RT for 18 hours, diluted with CH_2Cl_2 (10 mL), and quenched by addition of a saturated aqueous solution of $NaHCO_3$ (10 mL). The organic phase was extracted with CH_2Cl_2 (3 × 10 mL). The combined organic layers were washed with brine (10 mL), then dried ($MgSO_4$), filtered, and

concentrated *in vacuo*. The residue obtained was purified by column chromatography over silica gel (Hexane/EtOAc/Et_3N 8:1.5:0.5) to give phosphoramidite (+)-**339** (143 mg, 90%, 70% D_{35}, 30% D_{34}) as a colourless waxy solid, containing a mixture of two diastereoisomers. This phosphoramidite was synthesised fresh before each use: R_f 0.84 (Petroleum ether/EtOAc 8:2); $[\alpha]_D^{25}$ = +4.5 (*c* 2.0, $CHCl_3$); ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 7.39–7.22 (10H, m, C-45 to C-49 Diast. A and B), 5.23–5.15 (2H, m, H-2 Diast. A and B), 4.82–4.62 (4H, m, H-43), 4.36 (1H, dd, *J* 11.7, 3.7, H-1 Diast. A), 4.34 (1H, dd, *J* 11.9, 3.7, H-1 Diast. B), 4.18 (1H, dd, *J* 11.7, 5.2, H-1' Diast. A), 4.17 (1H, dd, *J* 11.9, 5.0, H-1' Diast. B), 3.83–3.69 (4H, m, H-3), 3.69–3.56 (4H, m, H-41 and H-42), 2.29 (4H, t, *J* 7.8, H-5 Diast. A and B), 1.66–1.54 (4H, m, H-6 Diast. A and B), 1.35–1.23 (56H, m, H-7 to H-20 Diast. A and B), 1.20 (3H, s, C(41)CH_3 or C(42)CH_3 Diast. A), 1.19 (3H, s, C(41)CH_3 or C(42)CH_3 Diast. A), 1.18 (3H, s, C(41)CH_3 or C(42)CH_3 Diast. B), 1.17 (3H, s, C(41)CH_3 or C(42)CH_3 Diast. B), 0.88 (3H, t, *J* 6.8, H-21); ^{13}C NMR (126 MHz; $CDCl_3$) δ_C 173.5 (C-4 Diast A and B), 173.2 (C-22 Diast. A and B), 139.5 (d_P, J_P 3.5, C-44 Diast. A), 139.4 (d_P, J_P 3.4, C-44 Diast. B), 128.40, 128.36 (C-46, C-48 Diast. A and B), 127.4, 127.3 (C-47 Diast. A and B), 127.12, 127.08 (C-45, C-49 Diast. A and B), 70.95 (d_P, J_P 4.4, C-2 Diast. A), 70.87 (d_P, J_P 5.1, C-2 Diast. B), 65.6 (d_P, J_P 3.5, C-43 Diast. A), 65.4 (d_P, J_P 3.6, C-43 Diast. B), 62.6 (d_P, J_P 4.7, C-1 Diast. A and B), 61.9 (d_P, J_P 16.9, C-3 Diast. A), 61.7 (d_P, J_P 16.7, C-3 Diast. B), 43.3, 43.2 (C-41, C-42 Diast. A and B), 34.3 (C-5 Diast. A and B), 32.1 (C-19 Diast. A and B), 29.85, 29.82, 29.79, 29.6, 29.5, 29.4, 29.3 (C-7 to C-18 Diast. A and B), 28.9–27.7 (m_D, C-25 to C-36 Diast. A and B), 25.1 (C-6 Diast. A and B), 24.83, 24.75, 24.72, 24.67, 24.65 (C(41)*C*H_3 and C(42)*C*H_3 Diast. A and B), 22.8 (C-20 Diast. A and B), 14.3 (C-21 Diast. A and B)[XXVI]; ^{31}P NMR (162 MHz; $CDCl_3$) δ_P 148.8 (P-40 Diast. A), 148.6 (P-40 Diast. B); $\bar{\nu}_{max}$(thin film)/cm^{-1} 2964 (m), 2924 (s), 2854 (m), 2197 (m), 2095 (m), 1742 (C=O) (s), 1456 (m), 1396 (w), 1364 (m), 1255 (m), 1200 (m), 1184 (m), 1127 (m), 1080 (m), 1059 (m), 1024 (P-O) (s), 975 (P-O) (s), 877 (w), 830 (w), 760 (m), 730 (s), 695 (m), 641 (w); HRMS *m/z* (ESI$^+$)

[XXVI] Due to the instability of this compound in solution, only a minimal number of scans could be used for acquiring the ^{13}C NMR spectrum, this was not enough to observe the peaks corresponding to C-23, C-24, C-37, C-38 and C-39. These, however, can be inferred by comparison with the ^{13}C NMR of the equivalent non-deuterated compound. ^{2}H NMR was also not acquired for the same reasons.

found 896.91706 ([MD_{34}+H]$^+$, 35%), 897.92254 ([MD_{35}+H]$^+$, 100%) ($C_{52}H_{62}D_{35}O_6NP$ requires 897.92449 [MD_{35}+Na]$^+$).

D_{41} (−)-1D-1-*O*-(1-*O*-Stearoyl-2-*O*-(D_{35}-stearoyl)-*sn*-glycer-3-yl-benzylphosphate)-2,3-*O*-cyclopentylidene-4-*O*-(2-oxo-5.6-benzo-1,3,2-dioxaphosphep-2-yl)-5,6-*O*-cyclopentylidene-*myo*-inositol (−)-340

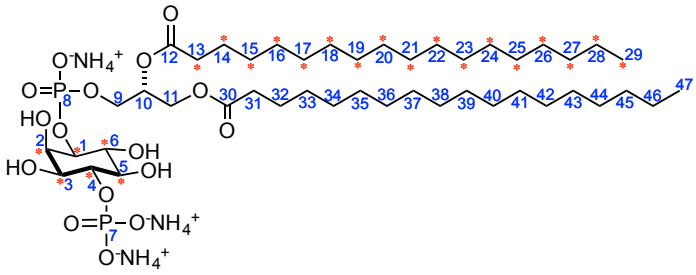


To a solution of (+)-**320** (20 mg, 0.0400 mmol, 1.0 eq, 84% D_6, 16% D_5) and phosphoramidite (+)-**339** (108 mg, 0.120 mmol, 3.0 eq) in anhydrous CH_2Cl_2 (2.0 mL) was added 1*H*-tetrazole (266 μL, 0.120 mmol, 3.0 eq, 0.45 M in MeCN) dropwise. The cloudy reaction mixture was stirred at RT for 22 hours, then cooled to −78 °C and *m*CPBA (27 mg, 0.120 mmol, 3.0 eq, 77%) was added in one portion. The reaction mixture was stirred for 1 hour, warmed to RT, and stirred for a further 2 hours. The reaction was quenched by addition of an aqueous solution of $Na_2S_2O_3$ (10% *w*/*v*, 3 mL) and stirred for a further 15 minutes. The aqueous phase was extracted with CH_2Cl_2 (4 × 3 mL) and the combined organic layers were washed with an aqueous solution of $Na_2S_2O_3$ (10% *w*/*v*, 2 × 3 mL), brine (2 × 3 mL), then dried (Na_2SO_4), filtered, and concentrated *in vacuo*. Purification by column chromatography over silica gel (Petroleum ether/EtOAc 10:0, 9:1, 8:2, 7:3, 6:4, 5:5) gave (−)-**340** (34 mg, 65%, 62% D_{41}, 29% D_{40}, 9% D_{39}) as a colourless, gummy solid: R_f 0.34 (Petroleum ether/EtOAc 5:5); $[\alpha]_D^{25}$ = −5.9 (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz; CD_2Cl_2) δ_H 7.44–7.31 (18H, m, H-20 to H-23 and H-29 to H-33 Diast. A and B), 5.37–5.29 (4H, m, H-18 and H-25, Diast. A and B), 5.25–5.18 (2H, m, H-35 Diast. A and B), 5.16–5.06 (6H, m, H-27 and H-18' or H-25' Diast. A and

B), 5.05 (2H, dd, J_{ABX} 13.8, 13.8, H-18' or H-25' Diast. A and B), 4.30 (2H, ddd, J_{XY} 12.0, 4.3, 4.3, H-36 Diast. A and B), 4.22–4.10 (6H, m, H-34 and H-36' Diast. A and B), 2.28 (2H, t, *J* 7.6, H-56 Diast. A), 2.27 (2H, t, *J* 7.6, H-56 Diast. B), 2.16–2.08 (2H, m, H-8 or H-11 Diast A and B), 1.98–1.63 (30H, m, H-8 or H-11, H-8', H-11', H-12, H-15, H-9, H-10, H-13, H-14 Diast. A and B), 1.63–1.50 (4H, m, H-57 Diast. A and B), 1.34–1.21 (56H, m, H-58 to H-71 Diast. A and B), 0.88 (6H, t, *J* 6.9, H-71 Diast. A and B); ^{13}C NMR (126 MHz; CD_2Cl_2) δ_C 173.45, 173.43 (C-55 Diast. A and B), 173.1 (C-37 Diast. A and B), 136.2 (d_P, J_P 7.4, C-28 Diast. A), 136.11–136.00 (m, C-28 Diast. B, C-19 or C-24 Diast. A and B), 135.96 (C-19 or C-24 Diast. A and B), 129.61, 129.60, 129.44, 129.40, 129.05, 129.02, 129.00 (C-20 to C-23, C-30 to C-32 Diast. A and B), 128.4, 128.3 (C-29, C-33 Diast. A and B), 123.35 (C-16 Diast. A), 123.33 (C-16 Diast. B), 120.9 (C-7 Diast. A and B), 79.8–79.1 ($m_{D,P}$, C-3 Diast. A and B), 79.1–78.5 ($m_{D,P}$, C-4 Diast. A and B), 77.4–76.8 ($m_{D,P}$, C-2 Diast. A and B), 76.6–76.1 ($m_{D,P}$, C-5 Diast. A and B), 75.8–75.2 ($m_{D,P}$, C-6 Diast. A and B), 74.5–73.8 ($m_{D,P}$, C-1 Diast. A and B), 70.2 (d_P, J_P 5.7, C-27 Diast. A), 70.0 (d_P, J_P 5.7, C-27 Diast. B), 69.64 (d_P, J_P 6.7, C-35 Diast. A), 69.58 (d_P, J_P 6.8, C-35 Diast. B), 69.24 (d_P, J_P 4.8, C-18 or C-25 Diast. A and B), 69.19 (d_P, J_P 4.8, C-18 or C-25 Diast. A and B), 66.2 (d_P, J_P 5.5, C-34 Diast. A), 65.9 (d_P, J_P 5.2, C-34 Diast. B), 62.05, 61.97 (C-36 Diast. A and B), 38.0, 37.94, 37.88, 37.85, 37.7 (C-12, C-15, C-8, C-11 Diast. A and B), 34.3 (C-56 Diast. A and B), 34.2–33.3 (m_D, C-38 Diast. A and B), 32.3 (C-70 Diast. A and B), 31.3–30.6 (m_D, C-52 Diast. A and B), 30.11, 30.06, 29.9, 29.8, 29.7, 29.5 (C-58 to C-69 Diast. A and B), 29.2–28.0 (m_D, C-40 to C-51 Diast. A and B), 25.2 (C-57 Diast. A and B), 24.4–23.6 (m_D, C-39 Diast. A and B), 24.19, 24.17, 24.1, 23.8 (C-9, C-10, C-13, C-14 Diast. A and B), 23.1 (C-71 Diast. A and B), 22.2–21.4 (m_D, C-53 Diast. A and B), 14.3 (C-72 Diast. A and B), 13.6–12.7 (m_D, C-54 Diast. A and B); ^{31}P NMR (162 MHz; CD_2Cl_2) δ_P –2.17 (P-26 Diast. A), –2.22 (P-26 Diast. B), –3.30 (P-17); ^{2}H NMR (92 MHz; $CHCl_3$; $CDCl_3$) δ_D 4.69 (1D, br s, D-1), 4.53 (1D, br s, D-4), 4.40 (1D, br s, D-2), 4.20 (1D, br s, D-3), 3.99 (1D, br s, D-6), 3.45 (1D, br s, D-5), 2.21 (2D, br s, D-38), 1.49 (2D, br s, D-39), 1.15 (28D, br s, D-40 to D-53), 0.78 (3D, br s, D-54); $\bar{\nu}_{max}$(thin film)/cm^{-1} 2920 (s), 2851 (m), 2359 (w), 2339 (w), 2194 (w), 2088 (w), 1743 (C=O) (m), 1468 (w), 1339 (w), 1289 (m), 1208 (m), 1175 (m), 1143 (m), 1104 (m), 1019 (P-O) (s), 885 (w), 851 (w), 733 (w), 698 (w); HRMS *m/z* (ESI$^+$) found 1310.9918 ([MD_{39}+H]$^+$, 11%), 1312.00179 ([MD_{40}+H]$^+$,

43%), 1313.00682 ([MD_{41}+H]$^+$, 100%) ($C_{70}H_{72}D_{41}O_{16}P_2$ requires 1313.00718 [MD_{41}+H]$^+$); NP-HPLC (Method 6) 4.63 min, 99.80%.

D_{41} (–)-1D-Distearoyl-phosphatidylinositol-4-phosphate triammonium salt (–)-338

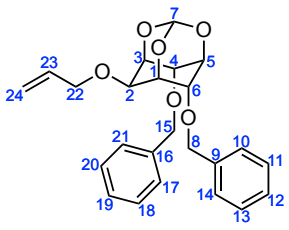


To a solution of (–)-**340** (13 mg, 0.00990 mmol, 1.0 eq, 62% D_{41}, 29% D_{40}, 9% D_{39}) in toluene (0.30 mL) was added TMSBr (20 μL, 0.148 mmol, 15 eq). The solution was stirred at RT for 12 hours, heated to 70 °C for 45 minutes, then cooled to 0 °C and concentrated by vacuum transfer. The residue was dissolved in toluene (0.5 mL) and concentrated again by vacuum transfer. This was repeated three times. The residue was then dissolved in MeOH (0.4 mL), stirred at 0 °C for 1 hour and the solution was concentrated *in vacuo* at 0 °C. The residue obtained was dissolved in the minimal amount of MeOH/CH_2Cl_2 (1:1 *v*/*v*) and purified by column chromatography over silica gel ($CHCl_3$/MeOH/2.2 M NH_4OH 5:3.9:1.1). The combined fractions were partially concentrated *in vacuo* at 0 °C, then H_2O (30 mL) was added. Lyophilising this solution gave (–)-**338** (6 mg, 58%, 59% D_{41}, 31% D_{40}, 10% D_{39}) as a colourless, fluffy solid. The compound was stored at –80 °C as the ammonium salt: R_f 0.60 ($CHCl_3$/MeOH/2.2 M aqueous solution of NH_4OH 5:3.9:1.1); $[\alpha]_D^{25}$ = –26.1 (*c* 0.04, MeOH/$CHCl_3$/H_2O 4:3:1); ^{1}H NMR (500 MHz; CD_3OD/$CDCl_3$/D_2O 4:3:1) δ_H 5.30–5.22 (1H, m, H-10), 4.44 (1H, dd, J_{XY} 12.2, 2.5, H-11), 4.20 (1H, dd, J_{XY} 12.2, 7.8, H-11'), 4.09–3.98 (2H, m, H-9), 2.33–2.27 (2H, m, H-31), 1.63–1.55 (2H, m, H-32), 1.34–1.21 (28H, m, H-33 to H-46), 0.87 (3H, t, *J* 6.9, H-47); ^{13}C NMR (126 MHz; CD_3OD/$CDCl_3$/D_2O 4:3:1) δ_C 175.2, 175.1 (C-12, C-30), 71.4 (d_P, J_P 8.5, C-10), 64.5 (d_P, J_P 4.8, C-9), 63.8 (C-11), 34.9 (C-31), 32.7 (C-45), 30.43, 30.38, 30.3, 30.12, 30.09, 30.0 (C-33 to C-44), 29.6–28.4 (m_D, C-15 to C-26), 25.7 (C-32), 23.4

(C-46), 14.6 (C-47);[XXVII] ^{31}P NMR (162 MHz; $CD_3OD/CDCl_3/D_2O$ 4:3:1) δ_P 1.98 (P-7), –0.15 (P-8); ^{2}H NMR (92 MHz; $CH_3OH/CHCl_3/H_2O$ 4:3:1; $CDCl_3$) δ_H 4.35 (br s, inositol ring), 3.04 (br s, inositol ring), 2.14–1.76 (2D, m, D-13), 1.42–1.07 (2D, m, D-14), 1.08–0.64 (28D, m, D-15 to D-28), 0.50 (3D, br s, D-29); $\bar{\nu}_{max}$(thin film)/cm^{-1} 3219 (O-H) (br), 2920 (m), 2851 (m), 2361 (m), 2341 (m), 2194 (w), 2162 (w), 2087 (w), 2035 (w), 1979 (w), 1774 (C=O) (w), 1737 (C=O) (m), 1468 (m), 1419 (m), 1262 (w), 1170 (m), 1067 (P-O) (s), 873 (w), 721 (m), 694 (m), 615 (m); HRMS *m/z* (ESI$^-$) found 984.79065 ([MD_{39}–H]$^-$, 13%), 985.79674 ([MD_{40}–H]$^-$, 45%), 986.80280 ([MD_{41}–H]$^-$, 100%) ($C_{45}H_{46}D_{41}O_{16}P_2$ requires 986.80373 [MD_{41}–H]$^-$).

6.4. Experimental procedures and compound characterisation: Chapter 4

2-*O*-Allyl-4,6-di-*O*-benzyl-*myo*-inositol 1,3,5-orthoformate 361

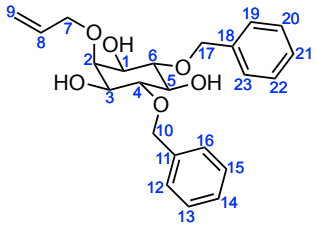


A solution of **122** (800 mg, 2.36 mmol, 1.0 eq) in anhydrous DMF (14 mL) was cooled to 0 °C and NaH (170 mg, 7.09 mmol, 3.0 eq, 60% dispersion in mineral oil) was added portionwise over 5 minutes. The reaction mixture was left to stir at 0 °C for 10 minutes, then warmed to RT, and stirred for a further 30 minutes. After addition of allyl bromide (0.614 mL, 7.09 mmol, 3.0 eq) at 0 °C, followed by stirring for 10 minutes, the reaction mixture was warmed to RT, and left to stir for a further 17 hours. The reaction was then quenched with H_2O (5 ml) and diluted in EtOAc (5 mL). The aqueous phase was extracted with EtOAc (4 × 10 mL), and the combined organic layers were washed with H_2O (2 × 10 mL), and brine (2 × 10 mL), then dried ($MgSO_4$), filtered, and concentrated *in*

[XXVII] Due to the instability of this compound in solution, only a minimal number of scans could be used for acquiring the ^{13}C NMR spectrum, this was not enough to observe the peaks corresponding to C-1, C-2, C-3, C-4, C-5 and C-6 as well as C-13, C-14, C-27, C-28 and C-29. These, however, can be inferred by comparison with the ^{13}C NMR of the equivalent non-deuterated compound.

vacuo. Purification by column chromatography over silica gel (Petroleum ether/EtOAc 9:1, 8:2, 7:3, 6:4, 5:5) afforded **361** (893 mg, 92%) as a thick colourless oil: R_f 0.73 (Petroleum ether/EtOAc 5:5); ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 7.35–7.27 (10H, m, H-10 to H-14 and H-17 to H-21), 6.04–5.91 (1H, m, H-23), 5.53 (1H, s, H-7), 5.35–5.27 (1H, m, H-24), 5.24–5.18 (1H, m, H-24'), 4.69 (2H, d, J_{AB} 11.7, H-8 and H-15), 4.57 (2H, d, J_{AB} 11.7, H-8' and H-15'), 4.49–4.43 (1H, m, H-5), 4.40–4.35 (2H, m, H-4 and H-6), 4.35–4.31 (2H, m, H-1 and H-3), 4.14–4.09 (2H, ddd, J_{XY} 5.6, 1.3, 1.3, H-22 and H-22'), 3.98–4.02 (1H, m, H-2); $\bar{\nu}_{max}$(thin film)/cm^{-1} 3384 (O-H) (br), 3064 (w), 3030 (w), 2970 (br), 2961 (w), 1497 (w), 1454 (w), 1377 (w), 1306 (w), 1207 (w), 1164 (s), 1139 (m), 1098 (s), 996 (s), 950 (m), 933 (m), 896 (m), 820 (w), 739 (m), 699 (m); LRMS *m/z* (ESI$^+$) 411.2 ([M+H]$^+$, 100%), 433.2 ([M+Na]$^+$, 73%). These data are in good agreement with the literature values.[456]

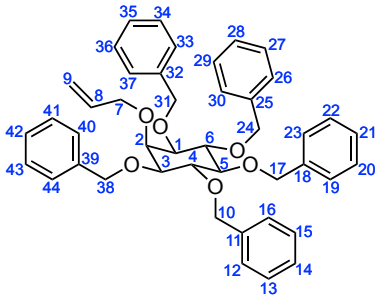
2-*O*-Allyl-4,6-di-*O*-benzyl-*myo*-inositol 365



To a solution of **361** (893 mg, 2.18 mmol, 1.0 eq) in MeOH (2.2 mL) was added PTSA·H_2O (414 mg, 2.18 mmol, 1.0 eq). The reaction mixture was stirred at RT for 16 hours and quenched with Et_3N (304 μL, 2.18 mmol, 1.0 eq). The solution was concentrated *in vacuo*, and the residue obtained was purified by column chromatography over silica gel (Petroleum ether/EtOAc 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8) to give **365** (822 mg, 94%) as a colourless oil: R_f 0.34 (EtOAc); ^{1}H NMR (500 MHz; $CDCl_3$) δ_H 7.40–7.35 (8H, m, H-12, H-13, H-15, H-16, H-19, H-20, H-22 and H-23), 7.34–7.29 (2H, m, H-14 and H-21), 5.94 (1H, dddd, *J* 17.2, 10.4, 5.5, 5.5, H-8), 5.28 (1H, dddd, J_{XY} 17.2, 1.8, 1.7, 1.7, H-9), 5.20 (1H, dddd, J_{XY} 10.4, 1.8, 1.4, 1.4, H-9'), 4.89 (2H, d, J_{AB} 11.4, H-10 and H-17), 4.86 (2H, d, J_{AB} 11.4, H-10' and H-17'), 4.33 (2H, ddd, J_{XY} 5.5, 1.7, 1.4, H-7 and H-7'), 3.90 (1H, dd, *J* 2.8, 2.8, H-2), 3.64 (2H, dd, *J* 9.4, 9.4, H-4 and H-6), 3.57–3.50 (3H, m, H-1, H-3 and H-5), 2.53 (1H, d, *J* 1.9, C(5)O*H*), 2.42 (1H, s, C(1)O*H*), 2.41 (1H, s, C(3)O*H*); ^{13}C NMR (126 MHz; $CDCl_3$) δ_C 138.6

(C-11, C-18), 135.0 (C-8), 128.8, 128.2, 128.1 (C-12 to C-16 and C-19 to C-23), 117.2 (C-9), 82.2 (C-4, C-6), 79.3 (C-2), 75.2 (C-10, C-17), 75.1 (C-5), 74.4 (C-7), 72.6 (C-1, C-3); $\bar{\nu}_{max}$(thin film)/cm^{-1} 3545 (O-H) (br), 3444 (O-H) (br), 3031 (w), 2981 (w), 2884 (w), 1497 (w), 1398 (w), 1364 (w), 1251 (w), 1211 (w), 1115 (s), 1061 (s), 1030 (s), 1002 (s), 930 (m), 732 (s), 697 (s); LRMS *m/z* (ESI$^+$) 423.2 ([M+Na]$^+$, 100%); HRMS *m/z* (ESI$^+$) found 401.19523 [M+H]$^+$ ($C_{23}H_{29}O_6$ requires 401.19587 [M+H]$^+$). These data are in good agreement with the literature values.[93,457]

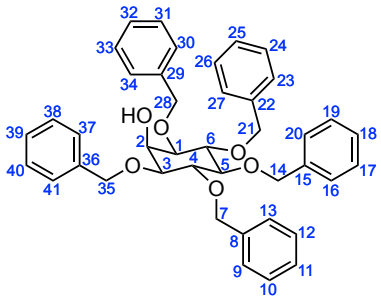
2-*O*-Allyl-1,3,4,5,6-penta-*O*-benzyl-*myo*-inositol 362



A solution of **365** (786 mg, 1.96 mmol, 1.0 eq) in DMF (16 mL) was cooled to 0 °C and NaH (471 mg, 11.8 mmol, 6.0 eq, 60% dispersion in mineral oil) was added portionwise. The reaction mixture was stirred at this temperature for 10 minutes, warmed to RT, and stirred for a further 30 minutes. The reaction mixture was then once again cooled to 0 °C. BnBr (1.38 mL, 11.6 mmol, 5.9 eq) was added dropwise. The reaction mixture was stirred at 0 °C for 10 minutes, warmed to RT, and stirred for a further 15 hours. After quenching with H_2O (8 mL), the aqueous phase was extracted with EtOAc (4 × 10 mL) and the combined organic layers were washed with H_2O (10 mL), and brine (10 mL), then dried ($MgSO_4$), filtered, and concentrated *in vacuo*. The residue obtained was purified by column chromatography over silica gel (Petroleum ether/EtOAc 9.5:0.5, 9:1, 8.5:1.5, 8:2, 8:3) to give **362** (1.04 g, 80%) as a colourless solid: R_f 0.66 (Petroleum ether/EtOAc 7:3); m.p. 102–103 °C (Hexane/EtOAc); ^{1}H NMR (500 MHz; $CDCl_3$) δ_H 7.36–7.23 (25H, m, H-12 to H-16, H-19 to H-23, H-26 to H-30, H-33 to H-37 and H-40 to H-44), 5.98 (1H, dddd, *J* 17.4, 10.3, 6.0, 6.0 H-8), 5.30 (1H, dddd, J_{XY} 17.4, 1.8, 1.7, 1.7, H-9), 5.18 (1H, dddd, J_{XY} 10.3, 1.8, 1.4, 1.4, H-9'), 4.91 (2H, d, J_{AB} 10.6, H-10 and H-24), 4.87 (2H, s, H-17), 4.82 (2H, d, J_{AB} 10.6, H-10' and H-24'), 4.68 (2H, d,

J_{AB} 12.2, H-31 and H-38), 4.66 (2H, d, J_{AB} 12.2, H-31' and H-38'), 4.34 (2H, ddd, J_{XY} 6.0, 1.7, 1.4, H-7 and H-7'), 4.03 (2H, dd, *J* 9.7, 9.4, H-4 and H-6), 3.97 (1H, dd, *J* 2.4, 2.4, H-2), 3.46 (1H, dd, *J* 9.4, 9.4, H-5), 3.33 (2H, dd, *J* 9.7, 2.4, H-1 and H-3); ^{13}C NMR (126 MHz; $CDCl_3$) δ_C 139.00 (C-11, C-25), 138.97 (C-18), 138.5 (C-32, C-39), 135.9 (C-8), 128.53, 128.49, 128.46, 128.2, 128.0, 127.8, 127.6 (C-12 to C-16, C-19 to C-23, C-26 to C-30, C-33 to C-37 and C-40 to C-44), 117.0 (C-9), 83.8 (C-5), 81.8 (C-4, C-6), 80.9 (C-1, C-3), 76.1 (C-17), 76.0 (C-10, C-24), 74.0 (C-2), 73.6 (C-7), 72.9 (C-31, C-38); $\bar{\nu}_{max}$(thin film)/cm^{-1} 3065 (w), 3029 (w), 2921 (w), 2874 (w), 1497 (w), 1454 (w), 1358 (m), 1215 (w), 1159 (w), 1130 (m), 1090 (s), 1066 (s), 1039 (s), 1029 (s), 998 (m), 930 (w), 749 (m), 730 (s), 694 (s); LRMS *m/z* (ESI$^+$) 693.3 ([M+Na]$^+$, 100%); HRMS *m/z* (ESI$^+$) found 671.33641 [M+H]$^+$ ($C_{44}H_{47}O_6$ requires 671.33672 [M+H]$^+$); Anal. Calcd. for $C_{44}H_{46}O_6$: C, 78.78; H, 6.91; Found: C, 78.49; H, 7.13.[XXVIII] These data are in good agreement with the literature values.[457]

1,3,4,5,6-Penta-*O*-benzyl-*myo*-inositol 353

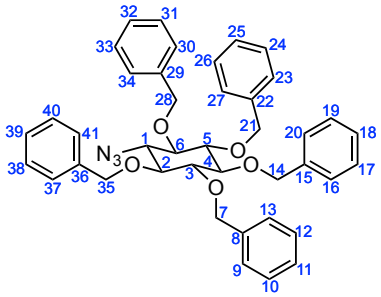


To a solution of **362** (820 mg, 1.22 mmol, 1.0 eq) in anhydrous MeOH/CH_2CH_2 (2.5:1.0 *v/v*, 8.8 mL) was added $PdCl_2$ (24 mg, 0.133 mol, 0.11 eq). The reaction mixture was stirred at RT for 18 hours, then diluted with CH_2Cl_2 (5 mL), filtered through a pad of Celite® and washed with CH_2Cl_2 (3 × 5 mL). The filtrate was concentrated *in vacuo*, and the residue obtained was purified by column chromatography over silica gel (Petroleum ether/EtOAc 9:1, 8:2, 7:3), to give **353** (739 mg, 96%) as a colourless solid: R_f 0.43 (Petroleum ether/EtOAc 7:3); m.p. 129–130 °C (Hexane/EtOAc) [lit.[458]

[XXVIII] Purity data acquired within the group by Dr Gavin Miller.

128–129 °C; lit.[459] 128–130 °C]; ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 7.37–7.24 (25H, m, H-9 to H-13, H-16 to H-20, H-23 to H-27, H-30 to H-34 and H-37 to H-41), 4.93–4.82 (6H, m, H-7, H-14 and H-21), 4.73 (2H, d, J_{AB} 12.3, H-28 and H-35), 4.70 (2H, d, J_{AB} 12.3, H-28' and H-35'), 4.22 (1H, dd, *J* 2.6, 2.6, H-2), 4.00 (2H, dd, *J* 9.5, 9.5, H-4 and H-6), 3.46 (1H, dd, *J* 9.5, 9.5, H-5), 3.39 (2H, dd, *J* 9.5, 2.6, H-1 and H-3), 2.45 (1H, br s, C(2)O*H*); ^{13}C NMR (101 MHz; $CDCl_3$) δ_C 138.91 (C-8, C-22), 138.85 (C-15), 138.1 (C-29, C-36), 128.6, 128.5, 128.1, 128.02, 127.98, 127.71, 127.68 (C-9 to C-13, C-16 to C-20, C-23 to C-27, C-30 to C-34 and C-37 to C-41), 83.3 (C-5), 81.4 (C-4, C-6), 80.0 (C-1, C-3), 76.1 (C-7, C-14, C-21), 72.9 (C-28, C-35), 67.7 (C-2); $\bar{\nu}_{max}$(thin film)/cm^{-1} 3449 (O-H) (br), 3065 (w), 3032 (w), 2882 (w), 1497 (w), 1454 (m), 1358 (m), 1134 (m), 1086 (m), 1064 (s), 1030 (s), 914 (w), 753 (s), 726 (s), 695 (s); LRMS *m/z* (ESI$^+$) 631.3 ([M+H]$^+$, 5%), 653.3 ([M+Na]$^+$, 100%); HRMS *m/z* (ESI$^+$) found 631.30512 [M+H]$^+$ ($C_{41}H_{43}O_6$ requires 631.30542 [M+H]$^+$). The data are in good agreement with the literature values.[402,458,459]

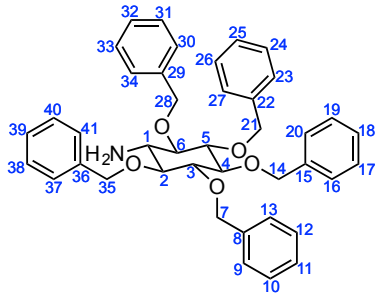
2,3,4,5,6-Penta-*O*-benzyl-1-azido-1-deoxy-*scyllo*-inositol 356



A solution of **353** (100 mg, 0.159 mmol, 1.0 eq) in anhydrous pyridine (1.4 mL) was cooled to 0 °C. Methanesulfonyl chloride (288 μL, 3.73 mmol, 23.5 eq) was added dropwise. The cloudy solution obtained was gradually warmed to RT and was stirred for a further 24 hours. The reaction was then quenched by the addition of H_2O (1 mL) and diluted with CH_2Cl_2 (2 mL). The aqueous phase was extracted with CH_2Cl_2 (3 × 2 mL) and the combined organic layers were washed with H_2O (2 × 1 mL), and brine (2 × 1 mL), then dried (Na_2SO_4), filtered, and concentrated *in vacuo*. Without any further purification the presumed crude mesyl inositol intermediate obtained was dissolved in anhydrous DMF (1.4 mL). NaN_3 (75 mg, 1.16 mmol, 7.3 eq) was added in one portion and the

reaction mixture was stirred at 90 °C for 15 hours. After cooling to RT, H_2O (2 mL) was added. The aqueous phase was extracted with CH_2Cl_2 (3 × 2 mL), and the combined organic layers were washed with H_2O (2 × 1 mL), a saturated aqueous solution of $NaHCO_3$ (1 mL), and brine (2 × 1 mL), then dried ($MgSO_4$), filtered, and concentrated *in vacuo*. Purification by column chromatography over silica gel (Petroleum ether/EtOAc 9.5:0.5, 9:1, 8:2) yielded **356** (91 mg, 88%) as a yellow solid: R_f 0.65 (Petroleum ether/EtOAc 8:2); m.p. 96–97 °C (Hexane/EtOAc) [lit.[402] 95–96 °C]; ^{1}H NMR (500 MHz; $CDCl_3$) δ_H 7.41–7.25 (25H, m, H-9 to H-13, H-16 to H-20, H-23 to H-27, H-30 to H-34 and H-37 to H-41), 4.93–4.84 (10H, m, H-7, H-14, H-21, H-28 and H-35), 3.62–3.52 (3H, m, H-3, H-4 and H-5), 3.49 (1H, dd, *J* 10.1, 10.1, H-1), 3.36 (2H, dd, *J* 9.0, 9.0, H-2 and H-6); LRMS *m/z* (ESI$^+$) 678.3 ([M+Na]$^+$, 100%); HRMS *m/z* (ESI$^+$) found 678.29340 [M+Na]$^+$ ($C_{41}H_{41}O_5N_3Na$ requires 678.29384 [M+Na]$^+$). The data are in good agreement with the literature values.[389,402]

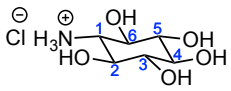
2,3,4,5,6-Tetra-*O*-benzyl-*scyllo*-inosamine 354



To a solution of **356** (2.00 g, 3.05 mmol, 1.0 eq) in THF (49 mL) was added PPh_3 (1.60 g, 6.10 mmol, 2.0 eq) and H_2O (2.1 mL). The solution was stirred at 40 °C for 30 hours, then cooled to RT. The solvents were concentrated *in vacuo* and the residue obtained was redissolved in CH_2Cl_2 (10 mL) and H_2O (5 mL) was added. The aqueous layer was extracted with CH_2Cl_2 (4 × 10 mL) and the combined organic layers were washed with H_2O (10 mL), and brine (10 mL), then dried (Na_2SO_4), filtered, and concentrated *in vacuo*. Purification by column chromatography over silica gel (Petroleum ether/EtOAc/Et_3N 9.5:0.45:0.05, 9:0.95:0.05, 8:1.95:0.05, 7:2.95:0.05, 6:3.95:0.05, 5:4.95:0.05) gave **354** (1.40 g, 73%) as a colourless glassy solid; R_f 0.59 (Petroleum ether/EtOAc 5:5); m.p. 122–123 °C (EtOAc); ^{1}H NMR (500 MHz; $CDCl_3$) δ_H 7.37–7.27 (25H, m, H-9 to H-13,

H-16 to H-20, H-23 to H-27, H-30 to H-34 and H-37 to H-41), 4.99 (2H, d, J_{AB} 10.9, H-28 and H-35), 4.93 (2H, d, J_{AB} 10.8, H-7 and H-21), 4.91 (2H, s, H-14), 4.86 (2H, d, J_{AB} 10.8, H-7' and H-21'), 4.70 (2H, d, J_{AB} 10.9, H-28' and H-35'), 3.65–3.59 (3H, m, H-3, H-4 and H-5), 3.40–3.31 (2H, m, H-2 and H-6), 2.94 (1H, dd, *J* 9.9, 9.9, H-1); ^{13}C NMR (126 MHz; $CDCl_3$) δ_C 138.55 (C-15), 138.51, 138.48 (C-8, C-22, C-29, C-36), 128.7, 128.61 (C-10, C-12, C-24, C-26, C-31, C-33, C-38, C-40), 128.58 (C-17, C-19), 128.04, 128.02, 127.97, 127.94, 127.85, 127.82 (C-9, C-11, C-13, C-16, C-18, C-20, C-23, C-25, C-27, C-30, C-32, C-34, C-37, C-39, C-41), 84.5 (C-2, C-6), 83.7 (C-4), 83.1 (C-3, C-5), 76.2 (C-14), 76.0, 75.9 (C-7, C-21, C-28, C-35), 55.5 (C-1); $\bar{\nu}_{max}$(thin film)/cm^{-1} 3067 (N-H) (w), 3030 (N-H) (w), 2908 (w), 2870 (w), 1574 (w), 1496 (w), 1454 (w), 1351 (m), 1215 (w), 1151 (w), 1126 (m), 1094 (m), 1062 (s), 1040 (s), 1026 (s), 1011 (s), 906 (w), 830 (w), 751 (s), 694 (s), 661 (m), 624 (m); LRMS *m/z* (ESI$^+$) 630.3 ([M+H]$^+$, 100%); HRMS *m/z* (ESI$^+$) found 630.32066 [M+H]$^+$ ($C_{41}H_{44}NO_5$ requires 630.32140 [M+H]$^+$); RP-HPLC (Method 1) t_R = 11.46 min, 95.25%.

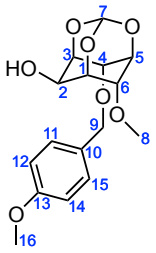
scyllo-Inosamine hydrochloride 13



To a solution of **354** (304 mg, 0.483 mmol, 1.0 eq) in CH_2Cl_2/MeOH/H_2O (3:2:1 *v/v/v*, 7.2 mL) was added 10% Pd/C (82 mg, 0.0773 mmol, 0.16 eq) and molecular biology grade concentrated HCl (200 μL) under $N_{2(g)}$. The flask was purged with 3 balloons of $H_{2(g)}$, and the suspension was stirred at RT under $H_{2(g)}$ for 24 hours. The reaction mixture was filtered through a glass microfiber filter and washed with MeOH/H_2O (4:1 *v/v*, 20 mL). The filtrate was partially concentrated *in vacuo*, then lyophilised. The colourless solid obtained was then dissolved in the minimal amount of H_2O (0.3 mL) and loaded onto a pre-washed DOWEX® 50WX8 cation exchange column (~3 mL of resin). The column was eluted with H_2O (10 mL), then 0.1 M HCl (10 mL) and 0.2 M HCl (5 mL). The fractions were combined and lyophilised to give the hydrochloride salt **13** (83 mg, 80%) as a colourless solid: decomp. 290–291 °C (EtOH/H_2O) [lit.[389] 290 °C]; ^{1}H NMR (400 MHz; D_2O) δ_H 3.49 (2H, dd, *J* 10.5, 9.0, H-2 and H-6), 3.42 (2H, dd, *J* 9.2, 9.0, H-3 and H-5), 3.35–3.29 (1H, m, H-4), 3.04 (1H, dd,

J 10.5, 10.5, H-1); ^{13}C NMR (126 MHz; D_2O) δ_C 74.2 (C-3, C-5), 73.1 (C-4), 70.1 (C-2, C-6), 55.7 (C-1); LRMS *m/z* (ESI$^+$) 180.1 ([M+H]$^+$, 100%); GC-MS (derivatised with TMSI) t_R = 16.81 min, 100%. The data are in good agreement with the literature values.[389,402]

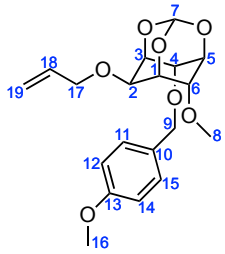
(±)-4-*O*-(4-Methoxybenzyl)-6-*O*-methyl-*myo*-inositol 1,3,5-orthoformate (±)-370



To a solution of **113** (19.7 g, 104 mmol, 1.0 eq) in anhydrous DMF (150 mL) was added NaH (4.24 g, 106 mmol, 1.02 eq, 60% dispersion in mineral oil) portionwise over 10 minutes. The solution was stirred at RT for 30 minutes, then MeI (6.73 mL, 108 mmol, 1.04 eq) was added dropwise. After 16 hours stirring at RT, NaH (4.24 g, 106 mmol, 1.02 eq, 60% dispersion in mineral oil) was added portionwise and the reaction mixture was stirred for 1 hour. PMBCl (14.9 mL, 108 mmol, 1.04 eq) was then added dropwise over 10 minutes, and the solution was stirred at RT for 16 hours. The reaction was quenched by addition of H_2O (2 mL) and diluted in CH_2Cl_2 (20 mL). The aqueous layer was extracted with CH_2Cl_2 (3 × 10 mL). The combined organic phases were washed with H_2O (2 × 10 mL), brine (2 × 10 mL), and an aqueous solution of LiCl (10% *w/v*, 2 × 10 mL), then dried ($MgSO_4$), filtered, and concentrated *in vacuo*. The residue obtained was purified twice by column chromatography over silica gel (Petroleum ether/EtOAc 8:2, 7:3, 6:4, 5:5) to give (±)-**370** (1.56 g, 46%) as a yellow oil. This compound could not be purified further due to contamination by other regioisomers, which could only be separated in subsequent steps: R_f 0.27 (Petroleum ether/EtOAc 5:5); ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 7.28–7.22 (2H, m, H-11 and H-15), 6.91–6.85 (2H, m, H-12 and H-14), 5.45 (1H, s, H-7), 4.59 (1H, d, J_{AB} 11.5, H-9), 4.51 (1H, d, J_{AB} 11.5, H-9'), 4.42–4.38 (1H, m, H-5), 4.32–4.27 (1H, m, H-4), 4.25–4.21 (1H, m, H-1), 4.18–4.10 (2H, m, H-6 and H-3), 4.09–4.06 (1H, m, H-2), 3.80 (3H, s, H-16), 3.44 (3H, s, H-8), 3.09–2.97 (1H, m, C(2)O*H*); ^{13}C NMR

(101 MHz; $CDCl_3$) δ_C 159.6 (C-13), 129.8 (C-10), 129.4 (C-11, C-15), 114.1 (C-12, C-14), 103.5 (C-7), 76.0 (C-6), 73.3 (C-4), 73.1 (C-3), 72.7 (C-1), 71.5 (C-9), 67.7 (C-5), 61.5 (C-2), 57.7 (C-8), 55.4 (C-16); $\bar{\nu}_{max}$(thin film)/cm^{-1} 3476 (O-H) (br), 2980 (w), 2884 (w), 1734 (m), 1612 (w), 1514 (m), 1373 (w), 1302 (w), 1242 (s), 1144 (s); LRMS *m/z* (ESI^+) 347.1 ($[M+Na]^+$, 100%); HRMS *m/z* (ESI^+) found 347.10932 $[M+Na]^+$ ($C_{17}H_{24}O_6Na$ requires 347.1107 $[M+H]^+$); RP-HPLC (Method 1) t_R = 10.62 min, 85.85%.

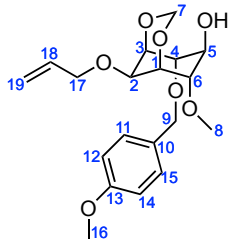
(±)-2-*O*-Allyl-4-*O*-(4-methoxybenzyl)-6-*O*-methyl-*myo*-inositol 1,3,5-orthoformate (±)-363



A solution of **370** (14.8 g, 45.6 mmol, 1.0 eq) in anhydrous DMF (250 mL) was cooled to 0 °C. NaH (4.56 g, 114 mmol, 2.5 eq, 60% dispersion in mineral oil) was added portionwise over 10 minutes. The reaction mixture was warmed to RT and stirred for 2 hours. Allyl bromide (7.90 mL, 91.3 mmol, 2.0 eq) was subsequently added dropwise at 0 °C. The solution was warmed to RT and stirred for a further 17 hours. The reaction was quenched with H_2O (60 mL) and the aqueous layer was extracted with EtOAc (3 × 40 mL). The combined organic layers were washed with H_2O (2 × 30 mL), and brine (2 × 30 mL), then dried ($MgSO_4$), filtered, and concentrated *in vacuo*. Purification twice by column chromatography over silica gel (Petroleum ether/EtOAc 9:1, 8:2, 7:3, 6:4, 5:5) gave (±)-**363** (9.08 g, 55%) as a colourless oil. ^{1}H NMR analysis indicated that this compound was contaminated with ~3% of regioisomers: R_f 0.67 (Petroleum ether/EtOAc 5:5); ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 7.28–7.23 (2H, m, H-11 and H-15), 6.92–6.86 (2H, m, H-12 and H-14), 5.98 (1H, dddd, J_{XY} 17.1, 10.7, 5.6, 5.4, H-18), 5.50 (1H, s, H-7), 5.36–5.28 (1H, m, H-19), 5.24–5.18 (1H, m, H-19'), 4.61 (1H, d, J_{AB} 11.7, H-9), 4.50 (1H, d, J_{AB} 11.7, H-9'), 4.41–4.37 (1H, m, H-5), 4.36–4.31 (1H, m, H-1), 4.31–4.27 (1H, m, H-4), 4.24–4.19 (1H, m, H-3), 4.17–4.10 (3H, m, H-6 and H-17), 3.88–3.85 (1H,

m, H-2), 3.81 (3H, s, H-16), 3.44 (3H, s, H-8); ^{13}C NMR (101 MHz; $CDCl_3$) δ_C 159.6 (C-13), 134.8 (C-18), 129.9 (C-10), 129.4 (C-11, C-15), 117.8 (C-19), 114.1 (C-12, C-14), 103.4 (C-7), 76.2 (C-6), 73.8 (C-4), 71.7 (C-9), 70.8 (C-17), 70.7 (C-3), 70.4 (C-1), 68.0 (C-5), 67.6 (C-2), 57.6 (C-8), 55.4 (C-16); $\bar{\nu}_{max}$(thin film)/cm^{-1} 3564 (w), 2995 (w), 2916 (m), 2837 (w), 1612 (m), 1514 (s), 1464 (w), 1302 (m), 1250 (s), 1175 (s), 1138 (s); LRMS *m/z* (ESI$^+$) 387.2 ([M+Na]$^+$, 100%); HRMS *m/z* (ESI$^+$) found 365.15893 [M+H]$^+$ ($C_{19}H_{25}O_7$ requires 365.16003 [M+H]$^+$); RP-HPLC (Method 1) t_R = 13.45 min, 97.11%.

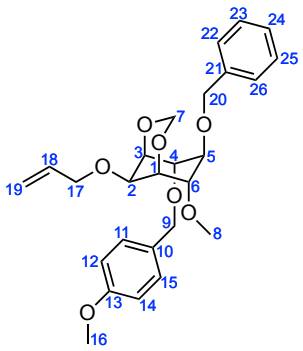
(±)-2-*O*-Allyl-4-*O*-(4-methoxybenzyl)-6-*O*-methyl-*myo*-inositol-1,3-*O*-methylene (±)-378



To a solution of (±)-**363** (10.3 g, 28.2 mmol, 1.0 eq) in anhydrous CH_2Cl_2 (140 mL) at 0 °C was added DIBAL-H (141 mL, 141 mmol, 5.0 eq, 1.0 M in hexane) dropwise over 10 minutes. After stirring at 0 °C for 10 minutes, the solution was warmed to RT and stirred for 24 hours. The reaction was then quenched by slow addition of a 1.0 M aqueous solution of Rochelle's salt (20 mL) and a saturated aqueous solution of NH_4Cl (20 mL) at 0 °C. After 30 minutes, the solution was warmed to RT, stirred for a further hour and then diluted in CH_2Cl_2 (20 mL). The aqueous phase was extracted with CH_2Cl_2 (3 × 20 mL), and the combined organic layers were washed with brine (15 mL), then dried ($MgSO_4$), filtered, and concentrated in *vacuo*. Purification by column chromatography over silica gel (Petroleum ether/EtOAc 7:3) afforded a yellowish oil which was cooled to –20 °C for 48 hours to give a colourless solid which was crystallised from Et_2O to yield (±)-**378** (3.05 g, 90%) as a colourless crystalline solid: R_f 0.42 (Petroleum ether/EtOAc 5:5); m.p. 63–65 °C (Et_2O); ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 7.30–7.23 (2H, m, H-11 and H-15), 6.92–6.84 (2H, m, H-12 and H-14), 5.94 (1H, dddd, *J* 17.1, 10.5, 5.6, 5.4, H-18), 5.47 (1H, d, J_{AB} 5.0, H-7), 5.35–5.26 (1H, m, H-19), 5.24–5.17 (1H, m, H-19'), 4.66 (1H, d, J_{AB} 5.0, H-7'), 4.62 (1H, d, J_{AB} 11.8, H-9), 4.55 (1H,

d, J_{AB} 11.8, H-9'), 4.41–4.37 (1H, m, H-1), 4.33–4.29 (1H, m, H-3), 4.12–4.07 (2H, m, H-17), 4.06–4.03 (1H, m, H-2), 3.97–3.93 (1H, m, H-4), 3.88–3.83 (1H, m, H-5), 3.81 (3H, s, H-16), 3.79–3.76 (1H, m, H-6), 3.46 (3H, s, H-8), 2.91 (1H, br s, C(5)O*H*); ^{13}C NMR (101 MHz; $CDCl_3$) δ_C 159.5 (C-13) 134.4 (C-18), 130.1 (C-10), 129.5 (C-11, C-15), 117.4 (C-19), 114.1 (C-12, C-14), 85.1 (C-7), 83.7 (C-6), 80.8 (C-4), 72.8 (C-3), 72.3 (C-1), 72.0 (C-9), 70.4 (C-2), 69.83 (C-17), 69.77 (C-5), 58.3 (C-8), 55.4 (C-16); $\bar{\nu}_{max}$(thin film)/cm^{-1} 3545 (O-H) (br), 2933 (w), 2920 (w), 1611 (w), 1514 (m), 1408 (w), 1250 (m), 1184 (m), 1134 (s), 1094 (s), 1060 (s), 1028 (s); LRMS *m/z* (ESI$^+$) 389.2 ([M+Na]$^+$, 100%); HRMS *m/z* (ESI$^+$) found 389.15637 [M+Na]$^+$ ($C_{19}H_{26}O_7Na$ requires 389.15762 [M+Na]$^+$); RP-HPLC (Method 1) t_R = 11.62 min, 96.99%.

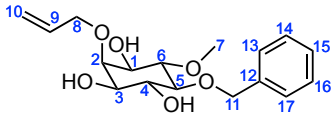
(±)-2-*O*-Allyl-5-*O*-benzyl-4-*O*-(4-methoxybenzyl)-6-*O*-methyl-*myo*-inositol-1,3-*O*-methylene (±)-379



A solution of (±)-**378** (5.19 g, 14.2 mmol, 1.0 eq) in anhydrous DMF (40 mL) was cooled to 0 °C. Imidazole (675 mg, 9.91 mmol, 0.70 eq) was added to the solution, followed by NaH (1.70 g, 42.5 mmol, 3.0 eq, 60% dispersion in mineral oil). The reaction mixture was stirred at 0 °C for 10 minutes, warmed to RT, and stirred for a further 45 minutes. BnBr (5.05 mL, 42.5 mmol, 3.0 eq) was subsequently added dropwise at 0 °C. The reaction mixture was stirred for 25 minutes, warmed to RT, and stirred for 20 hours. The reaction was quenched with H_2O (25 ml) and diluted in EtOAc (25 mL). The aqueous phase was extracted with EtOAc (5 × 20 mL), and the combined organic layers were washed with brine (2 × 20 mL), H_2O (2 × 20 ml), and an aqueous solution of LiCl (10% *w/v*, 20 mL). The organic phase was then dried ($MgSO_4$), filtered, and concentrated in *vacuo*. Purification

by column chromatography over silica gel (Petroleum ether/EtOAc 9:1, 8.5:1.5, 8.2) afforded (±)-**379** (6.03 g, 93%) as a thick colourless oil: R_f 0.75 (Petroleum ether/EtOAc 5:5); ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 7.29–7.14 (7H, m, H-11, H-15 and H-22 to H-26), 6.82–6.76 (2H, m, H-12 and H-14), 5.90 (1H, dddd, *J* 17.2, 10.5, 5.9, 5.5, H-18), 5.30–5.21 (1H, m, H-19), 5.18–5.11 (1H, m, H-19'), 5.05 (1H, d, J_{AB} 5.6, H-7), 4.78 (1H, d, J_{AB} 5.6 H-7'), 4.62 (2H, s, H-20), 4.52 (1H, d, J_{AB} 11.4, H-9), 4.52 (1H, d, J_{AB} 11.4 H-9'), 4.16–4.12 (1H, m, H-1), 4.11–4.03 (3H, m, H-3 and H-17), 3.89–3.85 (1H, m, H-4), 3.73 (3H, s, H-16), 3.65–3.60 (1H, m, H-6), 3.60–3.56 (1H, m, H-2), 3.46 (1H, dd, *J* 5.9, 5.9, H-5), 3.35 (3H, s, H-8); ^{13}C NMR (101 MHz; $CDCl_3$) δ_C 159.6 (C-13), 138.6 (C-21), 134.5 (C-18), 129.9 (C-10), 129.7 (C-11, C-15), 128.5 (C-23, C-25), 127.9 (C-22, C-26), 127.8 (C-24), 117.7 (C-19), 114.0 (C-12, C-14), 85.6 (C-7), 84.8 (C-6), 81.8 (C-4), 80.8 (C-5), 73.6 (C-20), 72.3 (C-1), 71.8 (C-3), 71.7 (C-9), 70.1 (C-2, C-17), 57.7 (C-8), 55.4 (C-16); $\bar{\nu}_{max}$(thin film)/cm^{-1} 2864 (w), 2836 (w), 1612 (w), 1514 (m), 1455 (w), 1366 (w), 1248 (m), 1176 (m), 1084 (s), 1029 (s), 1012 (s), 821 (m), 733 (s), 698 (s); LRMS *m/z* (ESI$^+$) 479.2 ([M+Na]$^+$, 100%); HRMS *m/z* (ESI$^+$) found 479.20308 [M+Na]$^+$ ($C_{26}H_{32}O_7Na$ requires 479.20402 [M+Na]$^+$); RP-HPLC (Method 1) t_R = 14.56 min, 94.32%.

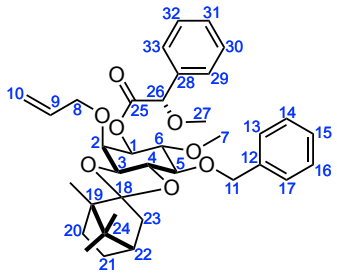
(±)-2-*O*-Allyl-5-*O*-benzyl-6-*O*-methyl-*myo*-inositol (±)-364



To a solution of (±)-**379** (832 mg, 1.82 mmol, 1.0 eq) in MeOH (10 mL) at 0 °C was added concentrated HCl (1.30 mL). The solution was warmed to RT and subsequently heated under reflux for 2 hours. After cooling to RT, the reaction was quenched with a saturated aqueous solution of $NaHCO_3$ until the pH reached 7. The volatile components were concentrated *in vacuo* and the residue obtained was purified by column chromatography over silica gel (Petroleum ether/EtOAc 3:7, 2:8, 1:9). The colourless solid obtained was then crystallised from EtOAc to give (±)-**364** (518 mg, 88%) as a colourless crystalline solid: R_f 0.32 (Petroleum ether/EtOAc 5:5); m.p. 141–143 °C (EtOAc); ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 4.41–7.28 (5H, m, H-13 to H-17), 5.93 (1H, dddd, *J* 17.2, 10.4, 5.6,

5.6, H-9), 5.30 (1H, dddd, J_{XY} 17.2, 1.9, 1.7, 1.5, H-10), 5.20 (1H, dddd, J_{XY} 10.4, 1.9, 1.6, 1.4 H-10'), 4.90 (1H, d, J_{AB} 11.4, H-11), 4.78 (1H, d, J_{AB} 11.4, H-11'), 4.36 (1H, dddd, J_{XY} 12.9, 5.6, 1.7, 1.6, H-8), 4.31 (1H, dddd, J_{XY} 12.9, 5.6, 1.5, 1.4, H-8'), 3.92 (1H, dd, *J* 2.6, 2.6, H-2), 3.82–3.75 (1H, m, H-4), 3.66 (3H, s, H-7), 3.54–3.48 (1H, m, H-1 or H-3), 3.48–3.40 (2H, m, H-1 or H-3 and H-6), 3.23 (1H, dd, *J* 9.0, 9.0, H-5), 2.45 (1H, d, *J* 1.6, C(4)O*H*), 2.42 (1H, d, *J* 5.5, C(1)O*H* or C(3)O*H*), 2.38 (1H, d, *J* 6.5, C(1)O*H* or C(3)O*H*); ^{13}C NMR (101 MHz; $CDCl_3$) δ_C 138.7 (C-12), 135.0 (C-9), 128.7 (C-14, C-16), 128.1 (C-13, C-17), 128.0 (C-15), 117.1 (C-10), 83.9 (C-6), 83.0 (C-5), 78.7 (C-2), 75.1 (C-11), 74.4 (C-8), 74.1 (C-4), 72.9 (C-1 or C-3), 72.6 (C-1 or C-3), 61.4 (C-7); $\bar{\nu}_{max}$(thin film)/cm^{-1} 3360 (O-H) (br), 2918 (w), 1498 (w), 1362 (w), 1192 (m), 1156 (s), 1053 (s), 1033 (s), 923 (m), 913 (m), 733 (s), 695 (s); LRMS *m/z* (ESI$^+$) 347.1 ([M+Na]$^+$, 100%)**;** HRMS *m/z* (ESI$^+$) found 347.14600 [M+Na]$^+$ ($C_{17}H_{24}O_6Na$ requires 347.14651 [M+Na]$^+$); RP-HPLC (Method 1) t_R = 9.09 min, 98.22%; Chiral HPLC (Heptane/IPA 90:10, 0.8 mL.min^{-1}, 254 nm) t_R = 19.59 min, 50.49% (+)-1L-**364**, t_R = 22.39 min, 49.51% (–)-1D-**364**.

(+)-1D-1-*O*-((*S*)-α-methoxyphenylacetoxy)-2-*O*-allyl-3,4-*O*-(L-1,7,7-trimethyl[2.2.1]bicyclo hept-2-ylidene)-5-*O*-benzyl-6-*O*-methyl-*myo*-inositol (+)-1D-381a



To a solution of (±)-**364** (2.00 g, 6.17 mmol, 1.0 eq) in anhydrous CH_2Cl_2 (39 mL) were added (–)-**100** (3.67 g, 18.5 mmol, 3.0 eq) and PTSA·H_2O (59 mg, 0.308 mmol, 0.050 eq). The solution was heated under reflux for 24 hours, allowed to cool to RT and quenched with Et_3N (43 µL, 0.308 mmol, 0.050 eq). The solvent was removed *in vacuo*. The residue obtained, containing a mixture the four diastereoisomers **380a–d**, was then purified and resolved by column chromatography over silica gel (Petroleum ether/EtOAc 9.7:0.3, 9.6:0.4, 9.5:0.5, 9.4:0.6, 9.3:0.7,

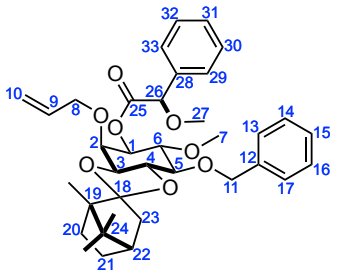
9.2:0.8, 9.1:0.9, 9:1, 8.8:1.2, 8.5:1.5, 8:2, 7.5:2.5, 7:3, 6.5:3.5, 6:4) to give a mixture of diastereoisomers of **380b–d** (1.90 g, 67%) and (–)-1D-**380a** (466 mg, 16%) as colourless oils.

Deprotection of of small sample of (–)-1D-**380a**, with acetyl chloride (2.48 mmol, 0.60 eq) in MeOH/CH_2Cl_2 (1.0:1.8 *v/v*) for 21 hours, as described below, and analysis of the triol (–)-**364** obtained by chiral HPLC revealed that this material was only partially resolved: Chiral HPLC (Heptane/IPA 90:10, 0.8 mL.min^{-1}, 254 nm) t_R = 19.74 min, 72.40% (+)-1L-**364**, t_R = 22.60 min, 27.60% (–)-1D-**364**, 45% *e.e.*

Intermediate (–)-1D-**380a** was thus further resolved, and its *e.e.* was simultaneous determined, using α-methoxyphenylacetic acid: To a solution of (–)-1D-**380a** (15 mg, 0.0327 mmol, 1.0 eq) in CH_2Cl_2 (0.30 mL) were added (*S*)-(+)-α-methoxyphenylacetic acid (14 mg, 0.0818 mmol, 2.5 eq), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (14 mg, 0.0883 mmol, 2.7 eq) and 4-DMAP (2 mg, 0.0164 mmol, 0.50 eq). After 42 hours stirring at RT, the reaction mixture was diluted with CH_2Cl_2 (3 mL), and the organic layer was washed with a saturated aqueous solution of $NaHCO_3$ (2 × 5 mL), and brine (5 mL), then dried ($MgSO_4$), filtered, and concentrated *in vacuo*. Purification by column chromatography over silica gel (Petroleum ether/EtOAc 9:1, 8:2) yielded (+)-1D-**381a** (18 mg, 90%) as a colourless oil: R_f 0.43 (Petroleum ether/EtOAc 8:2); $[\alpha]_D^{25}$ = +27.2 (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz; $CDCl_3$) δ_H 7.51–7.45 (2H, m, H-29 and H-33), 7.40–7.24 (8H, m, H-13 to H-17 and H-30 to H-32), 5.46 (1H, dddd, *J* 17.4, 10.6, 5.5, 5.4, H-9), 5.08–5.02 (1H, m, H-10), 5.01–4.95 (1H, m, H-10'), 4.89 (1H, d, J_{AB} 11.8, H-11), 4.83 (1H, dd, *J* 9.9, 2.8, H-1), 4.82 (1H, s, H-26), 4.72 (1H, d, J_{AB} 11.8, H-11'), 3.97–3.85 (3H, m, H-8, H-2 and H-4), 3.63–3.55 (2H, m, H-8' and H-6), 3.52 (3H, s, H-7), 3.54–4.48 (1H, m, H-5), 3.44 (3H, s, H-27), 3.22 (1H, dd, *J* 9.8, 1.0, H-3), 2.11 (1H, dt, *J* 13.3, 3.5, H_{eq}-23), 1.84–1.75 (1H, m, H_{eq}-20), 1.74–1.64 (2H, m, H-22 and H_{eq}-21), 1.40 (1H, d, *J* 13.3, H_{ax}-23), 1.32 (1H, td, *J* 12.2, 4.2, H_{ax}-20), 1.20–1.11 (1H, m, H_{ax}-21), 1.03 (3H, s, C(24)CH_3), 0.84 (3H, s, C(19)CH_3 or C(24)CH_3), 0.82 (3H, s, C(19)CH_3 or C(24)CH_3); ^{13}C NMR (126 MHz; $CDCl_3$) δ_C 170.2 (C-25), 138.8 (C-12), 136.2 (C-28), 134.6 (C-9), 128.9, 128.8, 128.4, 127.9, 127.6, 127.4 (C-13 to C-17 and C-29 to C-33), 120.8 (C-18), 116.8 (C-10), 82.6 (C-26), 81.9 (C-6), 80.4 (C-5), 77.2 (C-4), 76.0 (C-3), 75.0 (C-1), 72.9 (C-8), 72.6 (C-11), 71.9 (C-2), 61.5 (C-7), 57.6 (C-27),

53.0 (C-19), 48.4 (C-24), 46.2 (C-23), 45.0 (C-22), 28.9 (C-20), 26.7 (C-21), 20.4 (C(24)*C*H$_3$), 20.3 (C(24)*C*H$_3$), 9.7 (C(19)*C*H$_3$); $\bar{\nu}_{max}$(thin film)/cm^{-1} 2975 (m), 2932 (m), 2871 (m), 1754 (C=O) (m), 1496 (w), 1454 (m), 1371 (w), 1349 (w), 1310 (w), 1262 (w), 1202 (m), 1165 (m), 1153 (m), 1115 (s), 1089 (s), 1067 (s), 1046 (m), 1028 (m), 1016 (m), 926 (m), 844 (w), 823 (w), 736 (m), 697 (s); LRMS *m/z* (ESI$^+$) 607.3 ([M+H]$^+$, 100%); HRMS *m/z* (ESI$^+$) found 607.32603 [M+H]$^+$ (C$_{36}$H$_{47}$O$_8$ requires 607.32654 [M+H]$^+$).

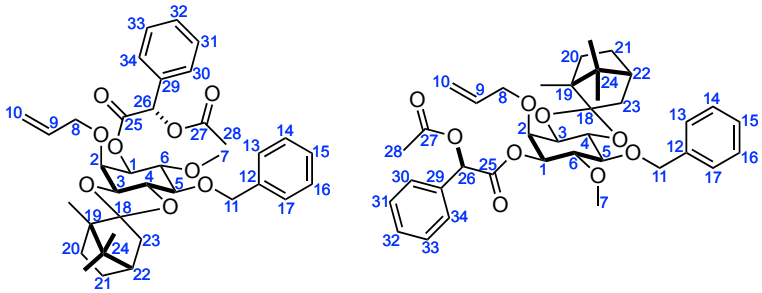
(–)-1D-1-*O*-((*R*)-α-methoxyphenylacetoxy)-2-*O*-allyl-3,4-*O*-(L-1,7,7-trimethyl[2.2.1]bicyclo hept-2-ylidene)-5-*O*-benzyl-6-*O*-methyl-*myo*-inositol (–)-1D-381b



The partially resolved intermediate (–)-1D-**380a** obtained as described above, was further resolved, and its *e.e*. determined, using α-methoxyphenylacetic acid: To a solution of (–)-1D-**380a** (15 mg, 0.0327 mmol, 1.0 eq) in CH_2Cl_2 (0.30 mL) were added (*R*)-(–)-α-methoxyphenylacetic acid (14 mg, 0.0818 mmol, 2.5 eq), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (14 mg, 0.0883 mmol, 2.7 eq) and 4-DMAP (2 mg, 0.0164 mmol, 0.50 eq). After 42 hours stirring at RT, the reaction mixture was diluted with CH_2Cl_2 (3 mL), and the organic layer was washed with a saturated aqueous solution of $NaHCO_3$ (2 × 5 mL), and brine (5 mL), then dried ($MgSO_4$), filtered, and concentrated *in vacuo*. Purification by column chromatography over silica gel (Petroleum ether/EtOAc 9:1, 8:2) yielded (–)-1D-**381b** (7 mg, 57%) as a colourless oil: R_f 0.43 (Petroleum ether/EtOAc 8:2); $[\alpha]_D^{25}$ = –29.6 (*c* 0.56, $CHCl_3$); ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 7.50–7.45 (2H, m, H-29 and H-33), 7.40–7.23 (8H, m, H-13 to H-17 and H-30 to H-32), 5.80 (1H, dddd, *J* 17.4, 10.6, 5.5, 5.4, H-9), 5.32–5.23 (1H, m, H-10), 5.19–5.12 (1H, m, H-10'), 4.86 (1H, d, J_{AB} 11.8, H-11), 4.81 (1H, s, H-26), 4.75 (1H, dd, *J* 9.0, 3.3, H-1), 4.68 (1H, d, J_{AB} 11.8, H-11'), 4.27–4.20 (1H, m, H-8), 4.20–4.16 (1H, m, H-2),

4.04–3.97 (1H, m, H-8'), 3.98–3.91 (1H, m, H-4), 3.48–3.44 (2H, m, H-5 and H-6), 3.43 (3H, s, H-27), 3.25 (1H, dd, *J* 9.9, 1.0, H-3), 3.05 (3H, s, H-7), 2.12 (1H, dt, *J* 13.3, 3.5, H_{eq}-23), 1.89–1.78 (1H, m, H_{eq}-20), 1.77–1.64 (2H, m, H-22 and H_{eq}-21), 1.43 (1H, d, *J* 13.3, H_{ax}-23), 1.36 (1H, td, *J* 12.2, 4.2, H_{ax}-20), 1.23–1.14 (1H, m, H_{ax}-21), 1.05 (3H, s, C(24)CH_3), 0.85 (6H, s, C(19)CH_3 and C(24)CH_3); ^{13}C NMR (101 MHz; $CDCl_3$) δ_C 170.3 (C-25), 138.8 (C-12), 136.1 (C-28), 134.7 (C-9), 129.0, 128.8, 128.4, 127.9, 127.7, 127.6 (C-13 to C-17 and C-29 to C-33), 120.8 (C-18), 116.9 (C-10), 82.8 (C-26), 81.9 (C-6), 80.7 (C-5), 77.4 (C-4), 76.2 (C-3), 75.2 (C-1), 73.0 (C-8), 72.7 (C-11), 72.1 (C-2), 61.2 (C-7), 57.4 (C-27), 53.1 (C-19), 48.4 (C-24), 46.1 (C-23), 45.1 (C-22), 29.0 (C-20), 26.8 (C-21), 20.4 (C(24)*C*H_3), 20.3 (C(24)*C*H_3), 9.76 (C(19)*C*H_3); $\bar{\nu}_{max}$(thin film)/cm^{-1} 2930 (br), 2874 (w), 1753 (w), 1735 (C=O) (w), 1454 (w), 1261 (m), 1202 (m), 1167 (m), 1109 (s), 1088 (s), 1066 (s), 1028 (s), 1015 (s), 926 (w), 737 (m), 697 (s); LRMS *m/z* (ESI$^+$) 607.3 ([M+H]$^+$, 100%); HRMS *m/z* (ESI$^+$) found 629.30679 [M+Na]$^+$ ($C_{36}H_{46}O_8Na$ requires 629.30849 [M+Na]$^+$); NP-HPLC (Method 5) t_R = 5.69 min, 100%.

(+)-1D-1-*O*-((*S*)-Acetylmandelyl)-3,4-*O*-(L-1,7,7-trimethylbicyclo[2.2.1]hept-2-ylidene)-5-*O*-benzyl-6-*O*-methyl-*myo*-inositol (+)-1D-382a & (–)-1L-1-*O*-((*R*)-acetylmandelyl)-2-*O*-allyl-3,4-*O*-(L-1,7,7-trimethylbicyclo[2.2.1]hept-2-ylidene)-5-*O*-benzyl-6-*O*-methyl-*myo*-inositol (–)-1L-382c



To a solution of (±)-**364** (2.00 g, 6.17 mmol, 1.0 eq) in anhydrous CH_2Cl_2 (39 mL) were added (–)-**100** (3.67 g, 18.5 mmol, 3.0 eq) and PTSA·H_2O (59 mg, 0.308 mmol, 0.050 eq). The solution was heated under reflux for 24 hours, allowed to cool to RT and quenched with Et_3N (43 μL, 0.308 mmol, 0.050 eq). The solvent was removed *in vacuo*. The residue obtained, containing a

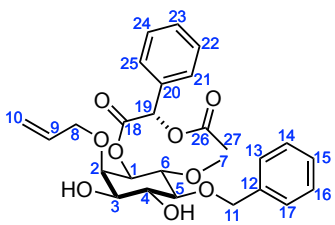
mixture of four diastereoisomers, was then purified and resolved by column chromatography over silica gel (Petroleum ether/EtOAc 9.7:0.3, 9.6:0.4, 9.5:0.5, 9.4:0.6, 9.3:0.7, 9.2:0.8, 9.1:0.9, 9:1, 8.8:1.2, 8.5:1.5, 8:2, 7.5:2.5, 7:3, 6.5:3.5, 6:4) to give a mixture of diastereoisomers of **380b–d** (1.90 g, 67%) and partially resolved (–)-1D-**380a** (466 mg, 16%) as colourless oils. Intermediate (–)-1D-**380a** was then further resolved using (*S*)-(+)-*O*-acetylmandelic acid as a chiral auxiliary: To a solution of (–)-1D-**380a** (450 mg, 0.981 mmol, 1.0 eq) in CH_2Cl_2 (9.3 mL) were added (*S*)-(+)-*O*-acetylmandelic acid (381 mg, 1.96 mmol, 2.0 eq), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (508 mg, 2.65 mmol, 2.7 eq) and 4-DMAP (60 mg, 0.491 mmol, 0.5 eq). After 17 hours stirring at RT the yellow solution was diluted with CH_2Cl_2 (4 mL), and washed with a saturated aqueous solution of $NaHCO_3$ (2 × 10 mL). The aqueous layer was then back-extracted with CH_2Cl_2 (2 × 10 mL). The combined organic layers were dried ($MgSO_4$), filtered, and concentrated *in vacuo*. Purification and further resolution by column chromatography over silica gel (Petroleum ether/EtOAc 9.9:0.1, 9.8:0.2, 9.7:0.3, 9.6:0.4, 9.5:0.5, 9:1, 8.5:1.5, 8:2) yielded (+)-1D-**382a** (458 mg, 74%) as an amorphous, colourless foam: R_f 0.43 (Petroleum ether/EtOAc 8:2); $[\alpha]_D^{25}$ = +11.9 (*c* 2.0, CH_3Cl); ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 7.54–7.48 (2H, m, H-30 and H-34), 7.44–7.26 (8H, m, H-13 to H-17 and H-31 to H-33), 5.96 (1H, s, H-26), 5.49 (1H, dddd, *J* 17.2, 10.3, 5.5, 5.4, H-9), 5.10–5.03 (1H, m, H-10), 5.01–4.95 (1H, m, H-10'), 4.89 (1H, d, J_{AB} 12.0, H-11), 4.86 (1H, dd, *J* 9.2, 3.2, H-1), 4.73 (1H, d, J_{AB} 12.0, H-11'), 3.98–3.87 (3H, m, H-8, H-4 and H-2), 3.70–3.63 (1H, m, H-8'), 3.60 (1H, dd, *J* 9.2, 9.2, H-6), 3.57 (3H, s, H-7), 3.50 (1H, dd, *J* 9.2, 8.6, H-5), 3.20 (1H, dd, *J* 9.8, 1.4, H-3), 2.19 (3H, s, H-28), 2.10 (1H, dt, *J* 13.6, 3.8, H_{eq}-23), 1.82–1.74 (1H, m, H_{eq}-20), 1.74–1.63 (2H, m, H-22 and H_{eq}-21), 1.39 (1H, d, *J* 13.6, H_{ax}-23), 1.29 (1H, td, *J* 12.0, 4.3, H_{ax}-20), 1.19–1.10 (1H, m, H_{ax}-21), 1.04 (3H, s, C(19)CH_3), 0.83 (3H, s, C(24)CH_3), 0.82 (3H, s, C(24)CH_3); ^{13}C NMR (101 MHz; $CDCl_3$) δ_C 170.3 (C-25), 168.4 (C-27), 138.8 (C-12), 134.6 (C-9), 133.8 (C-29), 129.4, 128.9, 128.4, 127.9, 127.6 (C-13 to C-17 and C-30 to C-34), 120.8 (C-18), 116.6 (C-10), 82.1 (C-6), 80.4 (C-5), 77.4 (C-4), 76.1 (C-3), 75.4 (C-1), 74.6 (C-26), 72.9 (C-8), 72.7 (C-11), 72.0 (C-2), 61.7 (C-7), 53.1 (C-19), 48.4 (C-24), 46.2 (C-23), 45.1 (C-22), 29.0 (C-20), 26.7 (C-21), 20.8 (C-28), 20.4 (C(24)*C*H$_3$), 20.3 (C(19)*C*H$_3$), 9.69 (C(24)*C*H$_3$); $\bar{\nu}_{max}$(thin film)/cm^{-1} 2934 (br), 2873 (w), 2360 (w), 1749 (C=O) (s), 1454 (w), 1373 (m), 1263 (m), 1229 (s), 1206 (s), 1166 (m), 1110 (s), 1087 (s), 1064

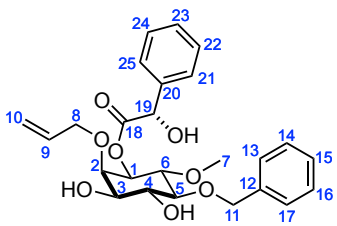
(s), 1047 (s), 918 (m), 734 (s), 696 (s); LRMS *m/z* (ESI$^+$) 635.3 ([M+H]$^+$, 100%), 657.3 ([M+Na]$^+$, 16%); HRMS *m/z* (ESI$^+$) found 635.3209 [M+H]$^+$ ($C_{37}H_{47}O_9$ requires 635.32146 [M+H]$^+$); NP-HPLC (Method 5) t_R = 6.56 min, 99.73%.

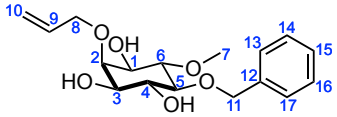
Acetyl chloride (177 μL, 2.48 mmol, 0.60 eq) was added to a solution of the diastereoisomeric mixture **380b–d** (1.90 g, 4.14 mmol, 1.0 eq) in MeOH/CH_2Cl_2 (1.0:1.8 *v/v*, 27 mL). After stirring at RT for 21 hours, the reaction was quenched with Et_3N (346 μL, 2.48 mmol, 0.60 eq) and the solvents were removed *in vacuo*. The off-white solid obtained was then crystallised from EtOAc to give triol (+)-**364** (1.34 g, 94%) enriched in the opposite enantiomer.

Similarly therefore, from enriched (+)-**364** or racemic (±)-**364** (1.14 g, 3.51 mmol, 1.0 eq), (+)-**100** (3.48 g, 17.6 mmol, 5.0 eq) and PTSA·H_2O (33 mg, 0.176 mmol, 0.050 eq) in CH_2Cl_2 (18 mL), (+)-1L-**380c** (175 mg, 11% from racemic (±)-**364**, 589 mg, 37% from enriched (+)-**364**) was obtained as a colourless oil. Reaction of (+)-1L-**380c** (170 mg, 0.371 mmol, 1.0 eq) with (*R*)-(–)-*O*-acetylmandelic acid (144 mg, 0.741 mmol, 2.0 eq), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (192 mg, 1.00 mmol, 2.7 eq) and 4-DMAP (23 mg, 0.185 mmol, 0.50 eq) yielded (–)-1L-**382c** (191 mg, 74–81%) as a colourless oil: $[\alpha]_D^{25}$ = –11.2 (*c* 1.0, CH_3Cl). All other data (R_f, ^{1}H NMR, ^{13}C NMR, IR, LRMS, HRMS, HPLC) match those of (+)-1D-**382a**.

(+)-1D-1-*O*-((*S*)-Acetylmandelyl)-2-*O*-allyl-5-*O*-benzyl-6-*O*-methyl-*myo*-inositol (+)-1D-383; (–)-1D-1-*O*-((*S*)-2-hydroxy-2-phenylacetoxy)-2-*O*-allyl-5-*O*-benzyl-6-*O*-methyl-*myo*-inositol (–)-1D-384 & (–)-1D-2-*O*-allyl-5-*O*-benzyl-6-*O*-methyl-*myo*-inositol (–)-1D-364





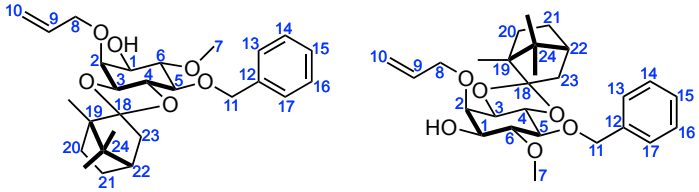


To a solution of (+)-1D-**382a** (30 mg, 0.0473 mmol, 1.0 eq) in MeOH/CH_2Cl_2 (1:2 *v/v*) was added acetyl chloride (2 μL, 0.0284 mmol, 0.60 eq). The solution was stirred at RT for 19 hours, then neutralised with Et_3N (4 μL, 0.0284 mmol, 0.60 eq) and the solvents were concentrated *in vacuo*.

Purification by column chromatography over silica gel (Petroleum ether/EtOAc, 5:5, 4:6, 3:7, 2:8) afforded the expected product (+)-1D-**383** (4 mg, 17%) as a colourless gum, but also the deacetylated product (–)-1D-**384** (11 mg, 32%), and the fully deprotected product (–)-1D-**364** (6 mg, 37%) as colourless solids: (+)-1D-**383** R_f 0.22 (Petroleum ether/EtOAc 5:5); $[\alpha]_D^{25}$ = +17.7 (*c* 0.27, CH_3Cl); ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 7.54–7.48 (2H, m, H-21, H-25), 7.43–7.27 (8H, m, H-22 to H-24 and H-13 to H-17), 5.97 (1H, s, H-19), 5.57 (1H, dddd, *J* 17.1, 10.5, 5.7, 5.5, H-9), 5.02–4.94 (2H, m, H-10 and H-10'), 4.92 (1H, d, J_{AB} 11.4, H-11), 4.89 (1H, dd, *J* 9.9, 2.6, H-1), 4.73 (1H, d, J_{AB} 11.4, H-11'), 3.70 (1H, ddd, *J* 9.3, 9.3, 1.5, H-4), 3.68–3.57 (7H, m, H-7, H-6, H-2, H-8 and H-8'), 3.42 (1H, ddd, *J* 9.3, 6.8, 2.9, H-3), 3.25 (1H, dd, *J* 9.3, 9.3, H-5), 2.39 (1H, d, *J* 1.5, C(4)O*H*), 2.22–2.19 (4H, m, C(3)O*H* and H-27); ^{13}C NMR (101 MHz; $CDCl_3$) δ_C 170.4 (C-18), 168.4 (C-26), 138.7 (C-12) 134.6 (C-9), 133.6 (C-20), 129.7, 129.1, 128.7, 128.2, 128.1, 128.0 (C-13 to C-17 and C-21 to C-25), 116.8 (C-10), 82.6 (C-5), 81.4 (C-6), 77.4 (C-2), 75.4 (C-11), 75.3 (C-1), 74.7 (C-19), 73.9 (C-8), 73.5 (C-4), 71.9 (C-3), 61.3 (C-7), 20.8 (C-27); $\bar{\nu}_{max}$(thin film)/cm^{-1} 3426 (O-H) (br), 2930 (w), 1744 (C=O) (s), 1497 (w), 1455 (w), 1372 (m), 1229 (s), 1210 (s), 1180 (m), 1118 (m), 1048 (s), 924 (m), 738 (m), 698 (s); LRMS *m/z* (ESI$^+$) 501.2 ([M+H]$^+$, 3%), 523.2 ([M+Na]$^+$, 100%); HRMS *m/z* (ESI$^+$) found 523.1936 [M+Na]$^+$ ($C_{27}H_{32}O_9Na$ requires 523.19385 [M+Na]$^+$); NP-HPLC (Method 5) t_R = 13.21 min, 88.75%; (–)-1D-**384** R_f 0.11 (Petroleum ether/EtOAc 5:5); m.p. 145–146 °C; $[\alpha]_D^{25}$ = –5.16 (*c* 0.95, $CHCl_3$); ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 7.46–7.42 (2H, m, H-21, H-25), 7.40–7.28 (8H, m, H-22 to H-24 and H-13 to H-17), 5.47 (1H, dddd, *J* 17.1, 10.5, 5.9, 5.5, H-9), 5.21 (1H, d, *J* 5.1, H-19), 5.00–4.95 (1H, m, H-10), 4.95–4.88 (1H, m, H-10'), 4.90 (1H, d, J_{AB} 11.3, H-11), 4.86 (1H, dd, *J* 10.3, 2.6, H-1), 4.76 (1H, d, J_{AB} 11.3, H-11'), 3.70 (1H, dd, *J* 9.4, 9.4, H-4), 3.67–3.60 (2H, m, H-6 and H-2), 3.58 (3H, s, H-7), 3.52 (1H, d, *J* 5.1, C(19)O*H*), 3.45–3.38 (3H, m, H-3, H-8 and H-8'), 3.25 (1H, dd, *J* 9.4, 9.4, H-5), 2.43 (1H, d, *J* 1.5, C(4)O*H*), 2.22 (1H, d, *J* 7.1, C(3)O*H*); ^{13}C NMR (101 MHz; $CDCl_3$) δ_C 173.3 (C-18), 138.6 (C-12), 138.0 (C-20), 134.5 (C-9), 129.0, 128.9, 128.7, 128.08, 128.05 (C-13 to C-17 and C-22 to C-24), 127.0 (C-21, C-25), 116.8 (C-10), 82.7 (C-5), 81.2 (C-6), 77.3 (C-2), 76.1 (C-1), 75.4 (C-11), 73.70 (C-8), 73.65 (C-4), 73.4 (C-19), 71.8 (C-3), 61.4 (C-7); $\bar{\nu}_{max}$(thin film)/cm^{-1} 3395 (O-H) (br), 2934 (w), 2874 (w), 1739 (m), 1714 (w), 1453 (w), 1421 (w), 1262 (w), 1206 (w), 1182 (w), 1156 (m), 1143 (m), 1123 (m), 1053

(s), 1030 (s), 933 (m), 735 (m), 696 (s); LRMS *m/z* (ESI$^+$) 481.2 ([M+Na]$^+$, 100%); HRMS *m/z* (ESI$^+$) found 481.1831 [M+Na]$^+$ ($C_{25}H_{30}O_8Na$ requires 481.18329 [M+Na]$^+$); NP-HPLC (Method 5) t_R = 11.36 min, 99.51%. The data for (–)-1D-**364** are identical to those reported below.

(–)-1D-2-*O*-Allyl-3,4-*O*-(L-1,7,7-trimethylbicyclo[2.2.1]hept-2-ylidene)-5-*O*-benzyl-6-*O*-methyl-*myo* inositol (–)-1D-380a & (+)-1L-2-*O*-allyl-3,4-*O*-(L-1,7,7-trimethylbicyclo[2.2.1]hept-2-ylidene)-4-*O*-methyl-5-*O*-benzyl-*myo* inositol (+)-1L-380c

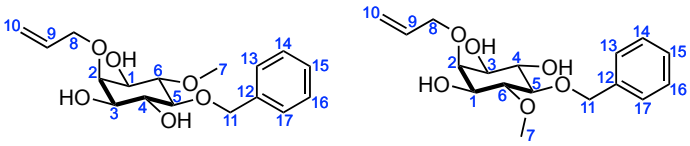


Ground solid NaOH (135 mg, 3.37 mmol, 8.1 eq) was added to a solution of (+)-1D-**382a** (263 mg, 0.414 mmol, 1.0 eq) in MeOH (35 mL). The reaction mixture was heated under reflux for 30 minutes, then allowed to cool to RT and neutralised by slow addition of solid CO_2. The solvent was concentrated *in vacuo* and the residue obtained was purified by column chromatography over silica gel (Petroleum ether/EtOAc 8:2, 7:3, 6:4, 5:5) to afford (–)-1-D-**380a** (187 mg, 99%) as a colourless oil: R_f 0.44 (Petroleum ether/EtOAc 5:5); $[\alpha]_D^{25}$ = –11.7 (*c* 0.74, $CHCl_3$); ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 7.34–7.17 (5H, m, H-13 to H-17), 5.84 (1H, dddd, *J* 17.2, 10.6, 5.4, 5.2, H-9), 5.26–5.18 (1H, m, H-10), 5.14–5.08 (1H, m, H-10'), 4.84 (1H, d, J_{AB} 11.7, H-11), 4.64 (1H, d, J_{AB} 11.7, H-11'), 4.40–4.33 (1H, m, H-8), 4.13–4.04 (1H, m, H-8'), 4.05–4.00 (1H, m, H-2), 3.87 (1H, dd, *J* 9.7, 9.4, H-4), 3.59 (3H, s, H-7), 3.51–3.44 (1H, m, H-1), 3.41 (1H, dd, *J* 9.4, 8.9, H-5), 3.24 (1H, dd, *J* 8.9, 8.7, H-6), 3.16 (1H, dd, *J* 9.7, 1.2, H-3), 2.43 (1H, d, *J* 6.8, C(1)O*H*), 2.11–2.02 (1H, m, H_{eq}-23), 1.82–1.73 (1H, m, H_{ax}-20), 1.70–1.58 (2H, m, H-22 and H_{eq}-21), 1.36 (1H, d, *J* 13.6, H_{ax}-23), 1.34–1.25 (1H, m, H_{ax}-20), 1.18–1.08 (1H, m, H_{ax}-21), 0.98 (3H, s, C(24)CH_3), 0.792 (3H, s, C(27)CH_3), 0.785 (3H, s, C(24)CH_3); ^{13}C NMR (101 MHz; $CDCl_3$) δ_C 138.8 (C-12), 134.4 (C-9), 128.3 (C-14, C-16), 127.8 (C-13, C-17), 127.5 (C-15), 120.4 (C-18), 117.2 (C-10), 85.4 (C-6), 80.6 (C-5), 77.3 (C-4), 76.8 (C-3), 73.8 (C-2), 73.3 (C-1), 73.1 (C-8), 72.5 (C-11), 61.7 (C-7), 52.9 (C-19), 48.3 (C-24), 46.1

(C-23), 45.0 (C-22), 28.9 (C-20), 26.7 (C-21), 20.3, 20.2 (C-25, C-26), 9.7 (C-27); $\bar{\nu}_{max}$(thin film)/cm^{-1} 3426 (O-H) (br), 2933 (m), 1454 (w), 1312 (w), 1262 (w), 1202 (m), 1163 (m), 1088 (s), 1069 (s), 1032 (s), 949 (m), 926 (m), 777 (w), 740 (m), 698 (m); LRMS *m/z* (ESI$^+$) 459.3 ([M+H]$^+$, 100%); HRMS *m/z* (ESI$^+$) found 481.25495 [M+Na]$^+$ ($C_{27}H_{38}O_6Na$ requires 481.25606 [M+Na]$^+$); RP-HPLC (Method 2) t_R = 29.92 min, 97.42%.

Similarly, from (–)-1L-**382c** (253 mg, 0.399 mmol, 1.0 eq) and ground NaOH (129 mg, 3.23 mmol, 8.1 eq) in MeOH (34 mL), (+)-1L-**380c** (190 mg, 89%) was obtained as a colourless oil: $[\alpha]_D^{25}$ = +11.9 (*c* 0.70, $CHCl_3$). All other data (R_f, ^{1}H NMR, ^{13}C NMR, IR, LRMS, HRMS, HPLC) matched those of the opposite enantiomer (–)-1D-**XX**.

(–)-1D-2-*O*-Allyl-5-*O*-benzyl-6-*O*-methyl-*myo*-inositol (–)-1D-364 & (+)-1L-2-*O*-allyl-5-*O*-benzyl-6-*O*-methyl-*myo*-inositol (+)-1L-364



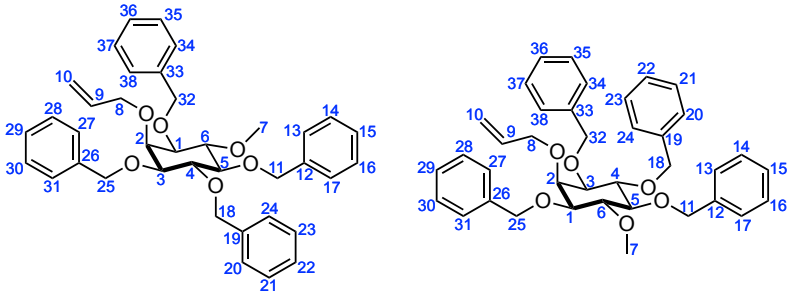
Acetyl chloride (13 µL, 0.192 mmol, 0.60 eq) was added to a solution of (–)-1D-**380a** (147 mg, 0.321 mmol, 1.0 eq) in MeOH/CH_2Cl_2 (1.0:1.8 *v/v*, 2.2 mL). After stirring at RT for 16.5 hours, the reaction was quenched with Et_3N (27 µL, 0.192 mmol, 0.60 eq) and the solvents were removed *in vacuo*. The off-white solid obtained was then crystallised from EtOAc to give (–)-1D-**364** (99 mg, 95%, >95% *e.e.*) as a colourless crystalline solid: $[\alpha]_D^{25}$ = –15.4 (*c* 1.0, $CHCl_3$); RP-HPLC (Method 1) t_R = 9.05 min, 100%; Chiral HPLC (Heptane/IPA 90:10, 0.8 mL.min^{-1}, 254 nm) t_R = 18.88 min, 2.39% (+)-1L-**364**, t_R = 21.43 min, 97.61% (–)-1D-**364**, >95% *e.e.*[XXIX] All other data (R_f, m.p., ^{1}H NMR, ^{13}C NMR, IR, LRMS, HRMS, Purity) match those of the racemic product (±)-**364**.

[XXIX] Other batches of this resolved compound were found to have an *e.e.* > 99%.

Similarly, by treating (+)-1L-**380c** (158 mg, 0.345 mmol, 1.0 eq) with acetyl chloride (15.0 µL, 0.207 mmol, 0.60 eq) in MeOH/CH_2Cl_2 (0.57:1.0 *v*/*v*, 2.4 mL), the opposite enantiomer (+)-1L-**364** (103 mg, 93%, >99% *e.e.*) was obtained as a colourless crystalline solid: $[\alpha]_D^{25}$ = +15.9 (*c* 1.0, $CHCl_3$); Chiral HPLC (Heptane/IPA 90:10, 0.8 mL.min^{-1}, 254 nm) t_R = 19.08 min, 99.99% (+)-1L-**364**, >99% *e.e.* (other enantiomer not observed, t_R = 21.43 min). All other data (R_f, m.p., ^{1}H NMR, ^{13}C NMR, IR, HRMS, Purity) match those of the racemic product (±)-**364**.

* *X-ray crystallographic data for these compounds can be found in Appendix B.*

(–)-1D-2-*O*-Allyl-1,3,4,5-tetra-*O*-benzyl-6-*O*-methyl-*myo*-inositol (–)-1D-385 & (+)-1L-2-O-allyl-1,3,4,5-tetra-*O*-benzyl-6-*O*-methyl-*myo*-inositol (+)-1L-385



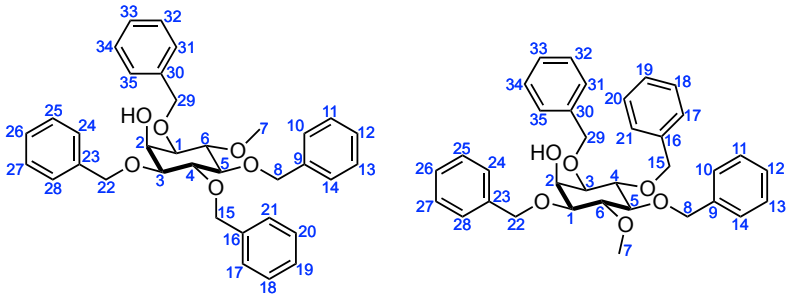
To a solution of (–)-1D-**364** (116 mg, 0.358 mmol, 1.0 eq) in DMF (3.0 mL) at 0 °C was added NaH (86 mg, 2.15 mmol, 6.0 eq, 60% dispersion in mineral oil) portionwise. The reaction mixture was stirred at 0 °C for 10 minutes, warmed to RT and stirred for a further 30 minutes. After cooling once again to 0 °C, BnBr (251 µL, 2.11 mmol, 5.9 eq) was added dropwise. The reaction mixture was stirred for 10 minutes at 0 °C, then warmed to RT and stirred for 20 hours. The reaction was quenched by addition of H_2O (2.0 mL), then diluted in EtOAc (5 mL). The layers were separated and the aqueous phase was extracted with EtOAc (4 × 10 mL). The combined organic layers were washed with H_2O (2 × 10 mL), brine (2 × 10 mL), and an aqueous solution of LiCl (10% *w*/*v*, 2 × 10 mL), then dried ($MgSO_4$), filtered, and concentrated *in vacuo*. The residue obtained was purified by column chromatography over silica gel (Petroleum ether/EtOAc 9.5:0.5, 9:1, 8.5:1.5, 8:2) to give (–)-1D-**385** (200 mg, 94%) as a colourless solid: R_f 0.90 (Petroleum ether/EtOAc 5:5); $[\alpha]_D^{25}$ = –13.4 (*c* 1.0, $CHCl_3$); m.p. 70–72 °C (CH_3Cl); ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 7.42–7.29 (20H, m, H-13 to

H-17, H-20 to H-24, H-27 to H-31 and H-34 to H-38), 5.98 (1H, dddd, *J* 17.2, 10.2, 5.5, 5.6, H-9), 5.34–5.26 (1H, m, H-10), 5.22–5.16 (1H, m, H-10'), 4.90 (1H, d, J_{AB} 10.6, H-11), 4.86 (2H, s, H-18), 4.82 (1H, d, J_{AB} 10.6, H-11'), 4.65–4.54 (4H, m, H-25 and H-32), 4.34–4.30 (2H, m, H-8), 3.99 (1H, dd, *J* 9.5, 9.5, H-4), 3.95 (1H, dd, *J* 2.2, 2.2, H-2), 3.74 (1H, dd, *J* 9.5, 9.5, H-6), 3.68 (3H, s, H-7), 3.36 (1H, dd, *J* 9.5, 9.5, H-5), 3.31 (1H, dd, *J* 9.5, 2.2, H-3), 3.22 (1H, dd, *J* 9.5, 2.2, H-1); ^{13}C NMR (101 MHz; $CDCl_3$) δ_C 139.1 (C-12, C-19), 138.7, 138.5 (C-26, C-33), 136.0 (C-9), 128.53, 128.48, 128.4, 128.2, 128.1, 127.8, 127.73, 127.65, 127.6 (C-13 to C-17, C-20 to C-24, C-27 to C-31, C-34 to C-38), 116.8 (C-10), 84.0 (C-5), 83.7 (C-6), 81.7 (C-4), 80.9 (C-3), 80.8 (C-1), 75.99 (C-11), 75.97 (C-18), 74.2 (C-2), 73.5 (C-8), 72.9 (C-32, C-25), 61.5 (C-7); $\bar{\nu}_{max}$(thin film)/cm^{-1} 2956 (m), 2919 (s), 2851 (m), 2362 (m), 2338 (m), 1457 (w), 1361 (w), 1261 (w), 1159 (w), 1130 (m), 1090 (s), 1043 (m), 801 (w), 735 (m), 698 (m); LRMS *m/z* (ESI$^+$) 595.3 ([M+H]$^+$, 16%), 617.3 ([M+Na]$^+$, 100%); HRMS *m/z* (ESI$^+$) found 617.28706 [M+Na]$^+$ ($C_{38}H_{42}O_6Na$ requires 617.28736 [M+Na]$^+$); Chiral HPLC (Heptane/IPA 95:5, 1.0 mL.min^{-1}, 254 nm) t_R = 11.94 min, 99.85%.[xxx]

Similarly, (+)-1L-**364** (116 mg, 0.357 mmol, 1.0 eq), NaH (86.0 mg, 2.15 mmol, 6.0 eq, 60% dispersion in mineral oil), and BnBr (251 μL, 2.11 mmol, 5.9 eq) in DMF (3 mL) afforded (+)-1-L-**385** (191 mg, 90%) as a colourless solid: $[\alpha]_D^{25}$ = +12.3 (*c* 1.0, $CHCl_3$). All other data (R_f, m.p., ^{1}H NMR, ^{13}C NMR, IR, LRMS, HRMS, Purity) match those of the opposite enantiomer (–)-1D-**385**.

[xxx] The enantiomers could not be separated by chiral HPLC. This value was therefore used as a mesure of purity for this compound.

(–)-1D-1,3,4,5-Tetra-*O*-benzyl-6-*O*-methyl-*myo*-inositol (–)-1D-386 & (+)-1L-1,3,4,5-tetra-*O*-benzyl-6-*O*-methyl-*myo*-inositol (+)-1L-386

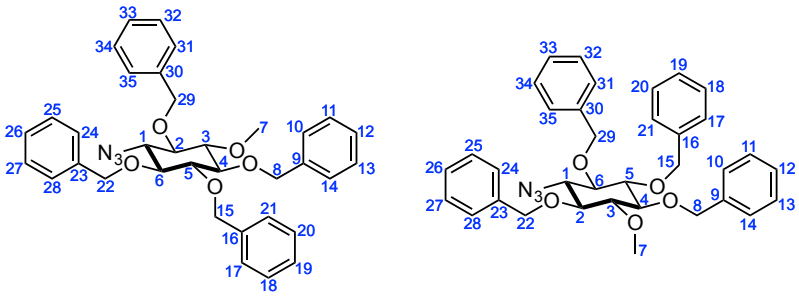


To a solution of (–)-1D-**385** (182 mg, 0.306 mmol, 1.0 eq) in MeOH/CH_2Cl_2 (2.5:1.0 *v*/*v*, 2.2 mL) was added $PdCl_2$ (6.0 mg, 0.0337 mmol, 0.11 eq). The reaction mixture was stirred at RT for 19 hours, then diluted in CH_2Cl_2 (5 mL). The solution was filtered through a short pad of Celite®, washed with CH_2Cl_2 (5 × 10 mL), and the filtrate was concentrated *in vacuo*. Purification by column chromatography over silica gel (Petroleum ether/EtOAc 8:2, 7:3) afforded (–)-1D-**386** (160 mg, 94%) as a colourless oil: R_f 0.32 (Petroleum ether/EtOAc 7:3); $[\alpha]_D^{25}$ = –19.4 (*c* 0.81, $CHCl_3$); ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 7.41–7.26 (20H, m, H-10 to H-14, H-17 to H-21, H-24 to H-28 and H-31 to H-35), 4.91–4.81 (4H, m, H-8 and H-15), 4.77–4.66 (4H, m, H-22 and H-29), 4.19 (1H, dd, *J* 2.7, 2.7, H-2), 3.95 (1H, dd, *J* 9.6, 9.6, H-5), 3.70 (1H, dd, *J* 9.6, 9.6, H-6), 3.68 (3H, s, H-7), 3.39–3.32 (2H, m, H-3 and H-4), 3.28 (1H, dd, *J* 9.6, 2.7, H-1), 2.43 (1H, s, C(2)O*H*); ^{13}C NMR (101 MHz; $CDCl_3$) δ_C 139.0, 138.9 (C-9, C-16), 138.3, 138.1 (C-23, C-30), 128.6, 128.49, 128.48, 128.12, 128.11, 128.01, 127.98, 127.96, 127.92, 127.72, 127.69 (C-10 to C-14, C-17 to C-21, C-24 to C-28 and C-31 to C-35), 83.4 (C-4), 83.2 (C-6), 81.2 (C-5), 79.9 (C-3), 79.8 (C-1), 76.1, 76.0 (C-8, C-15), 72.92, 72.85 (C-22, C-29), 67.9 (C-2), 61.5 (C-7); $\bar{\nu}_{max}$(thin film)/cm^{-1} 3465 (O-H) (w), 3063 (w), 3030 (w), 2924 (w), 2878 (w),1497 (w), 1454 (w), 1360 (w), 1128 (m), 1085 (s), 1069 (s), 1028 (m), 732 (s), 696 (s); LRMS *m*/*z* (ESI$^+$) 572.3 ([M+NH_4]$^+$, 73%), 577.3 ([M+Na]$^+$, 100%); HRMS *m*/*z* (ESI$^+$) found 577.25541 [M+Na]$^+$ ($C_{35}H_{38}O_6Na$ requires 577.25606 [M+Na]$^+$); RP-HPLC (Method 1) t_R = 15.95 min, 98.57%.

Similarly, from (+)-1L-**385** (173 mg, 0.291 mmol, 1.0 eq) and $PdCl_2$ (6 mg, 0.0320 mmol, 0.11 eq) in MeOH/CH_2Cl_2 (2.5:1.0 *v*/*v*, 2.0 mL), (+)-L-**386** (150 mg, 93%) was obtained as a colourless oil:

$[\alpha]_D^{25}$ = +19.6 (*c* 1.0, $CHCl_3$). All other data (R_f, m.p., ^1H NMR, ^{13}C NMR, IR, LRMS, HRMS, Purity) match those of the opposite enantiomer (–)-1D-**386**.

(+)-1D-2,4,5,6-Tetra-*O*-benzyl-3-*O*-methyl-1-azido-1-deoxy-*scyllo*-inositol (+)-1D-387 & (–)-1-L-2,4,5,6-tetra-*O*-benzyl-3-*O*-methyl-1-azido-1-deoxy-*scyllo*-inositol (–)-1L-387

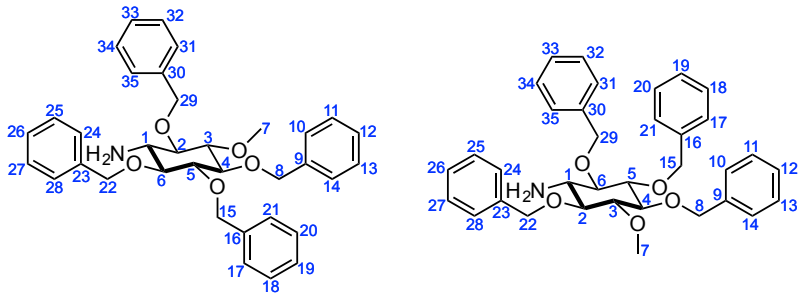


A solution of (–)-1D-**386** (250 mg, 0.451 mmol, 1.0 eq) in pyridine (4.0 mL) was cooled to 0 °C. Methanesulfonyl chloride (837 µL, 10.8 mmol, 24 eq) was added dropwise and the reaction mixture was gradually warmed to RT. After stirring for 20 hours, the reaction was quenched with H_2O (3 mL) and diluted with CH_2Cl_2 (3 mL). The aqueous layer was extracted with CH_2Cl_2 (3 × 5 mL) and the combined organic layers were washed with H_2O (2 × 3 mL) and brine (2 × 3 mL), then dried (Na_2SO_4), filtered, and concentrated *in vacuo*. Without any further purification, the yellowish oil obtained was dissolved in DMF (4.0 mL). NaN_3 (214 mg, 3.29 mmol, 7.3 eq) was added and the reaction mixture was heated at 90 °C for 18 hours. H_2O (3 mL) was added and the aqueous phase was extracted with EtOAc (2 × 5 mL). The combined organic layers were washed with H_2O (5 mL) and brine (5 mL), then dried ($MgSO_4$), filtered, and concentrated *in vacuo*. The yellow oil obtained was purified by column chromatography over silica gel (Hexane/EtOAc 10:2) to give (+)-1D-**387** (248 mg, 95%) as a gummy solid: R_f 0.77 (Petroleum ether/EtOAc 7:3); $[\alpha]_D^{25}$ = +11.6 (*c* 0.82, $CHCl_3$); ^1H NMR (500 MHz; $CDCl_3$) δ_H 7.47–7.27 (20H, m, H-10 to H-14, H-17 to H-21, H-24 to H-28 and H-31 to H-35), 4.90–4.81 (8H, m, H-8, H-15, H-22 and H-29), 3.68 (3H, s, H-7), 3.55–3.47 (1H, m, H-4), 3.47–3.38 (2H, m, H-1 and H-5), 3.32 (1H, dd, *J* 9.7, 9.7, H-2 or H-6), 3.28–3.22 (2H, m, H-3 and H-2 or H-6); ^{13}C NMR (126 MHz; $CDCl_3$) δ_C 138.5, 138.05, 137.97 (C-9, C-16,

C-23, C-30), 128.63, 128.60, 128.57, 128.5, 128.4, 128.2, 128.11, 128.06, 127.9, 127.8 (C-10 to C-14, C-17 to C-21, C-24 to C-28 and C-31 to C-35), 85.5 (C-3), 83.2 (C-4), 82.8 (C-5), 81.3 (C-2 or C-6), 81.2 (C-2 or C-6), 76.11, 76.08, 76.03, 76.02 (C-8, C-15, C-22, C-29), 66.9 (C-1), 61.7 (C-7); $\bar{\nu}_{max}$(thin film)/cm^{-1} 3031 (w), 2919 (br), 2105 (N_3) (m), 1497 (w), 1454 (w), 1359 (w), 1264 (w), 1137 (m), 1063 (s), 1027 (m), 735 (m), 697 (s); LRMS *m/z* (ESI$^+$) 602.3 ([M+Na]$^+$, 76%), 618.3 ([M+K]$^+$, 100%); HRMS *m/z* (ESI$^+$) found 602.26233 [M+Na]$^+$ ($C_{35}H_{37}O_5N_3Na$ requires 602.26254 [M+Na]$^+$). The data are in good agreement with the literature values for racemic (±)-**387**.[389,402]

Similarly, the reaction of (+)-1-L-**386** (300 mg, 0.541 mmol, 1.0 eq) and methanesulfonyl chloride (1.00 mL, 13.0 mmol, 24 eq) in pyridine (4.8 mL), followed by aqueous work-up, gave a residue which was dissolved in anhydrous DMF (4.8 mL). NaN_3 (257 mg, 3.95 mmol, 7.3 eq) was added and the reaction mixture was heated at 90 °C for 18 hours. Aqueous work-up and purification by column chromatography over silica gel (Hexane/EtOAc 10:2) yielded (–)-1L-**387** (280 mg, 89%) as a gummy solid: $[\alpha]_D^{25}$ = –12.5 (*c* 1.0 in $CHCl_3$). All other data (R_f, ^{1}H NMR, ^{13}C NMR, IR, LRMS, HRMS) match those of the opposite enantiomer (+)-1D-**387** and those of the literature values for racemic racemic (±)-**387**.[389,402]

(–)-1D-2,4,5,6-Tetra-*O*-benzyl-3-*O*-methyl-s*cyllo*-inosamine (–)-1D-388 & (+)-1L-2,4,5,6-tetra-*O*-benzyl-3-*O*-methyl-s*cyllo*-inosamine (+)-1L-388

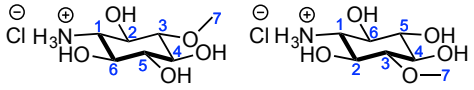


To a solution of the azide (+)-1D-**387** (232 mg, 0.400 mmol, 1.0 eq) in THF (6.3 mL) were added PPh_3 (210 mg, 0.801 mmol, 2.0 eq) and H_2O (272 μL). The solution was stirred at 40 °C for 28 hours then cooled to RT. The volatile components were concentrated *in vacuo*. EtOAc (10 mL) and H_2O

(5 mL) were added to the residue obtained. The aqueous layer was extracted with EtOAc (3 × 8 mL) and the combined organic layers were washed with H_2O (8 mL), and brine (8 mL), then dried (Na_2SO_4), filtered, and concentrated *in vacuo*. Purification by column chromatography over silica gel (Petroleum ether/EtOAc/Et_3N 9:0.95:0.05, 8:1.95:0.05, 7:2.95:0.05, 6:3.95:0.05) yielded (−)-1D-**388** (134 mg, 60%) as a colourless, glassy solid: R_f 0.36 (Petroleum ether/EtOAc 5:5); m.p. 85–88 °C (Hexane/EtOAc); $[\alpha]_D^{25}$ = −5.2 (*c* 2.0, $CHCl_3$); ^{1}H NMR (500 MHz; $CDCl_3$) δ_H 7.51–7.05 (20H, m, H-10 to H-14, H-17 to H-21, H-24 to H-28 and H-31 to H-35), 4.943 (1H, d, J_{AB} 10.9, H-22 or H-29), 4.940 (1H, d, J_{AB} 10.9, H-22 or H-29), 4.88 (1H, d, J_{AB} 10.8, H-15), 4.85 (1H, d, J_{AB} 10.6, H-8), 4.82 (1H, d, J_{AB} 10.6, H-8'), 4.79 (1H, d, J_{AB} 10.8, H-15'), 4.66 (1H, d, *J* 10.9, H-22' or H-29'), 4.65 (1H, d, *J* 10.9, H-22' or H-29'), 3.65 (3H, s, H-7), 3.53 (1H, dd, *J* 9.5, 9.1, H-5), 3.47 (1H, dd, *J* 9.1, 8.9, H-4), 3.30 (1H, dd, *J* 9.7, 9.5, H-6), 3.27 (1H, dd, *J* 9.1, 8.9, H-3), 3.23 (1H, dd, *J* 9.5, 9.1, H-2), 2.85 (1H, dd, *J* 9.7, 9.5, H-1), 1.81 (3H, br s, C(1)N*H*$_2$); ^{13}C NMR (126 MHz; $CDCl_3$) δ_C 138.6, 138.55, 138.45 (C-9, C-16, C-23, C-30), 128.71, 128.68, 128.6 (C-11, C-13, C-18, C-20, C-25, C-27, C-32, C-34), 128.2, 128.1, 128.04, 128.00, 127.95, 127.86, 127.8 (C-10, C-12, C-14, C-17, C-19, C-21, C-24, C-26, C-28, C-31, C-33, C-35), 86.5 (C-3), 84.2 (C-5), 83.7 (C-4), 83.00, 82.98 (C-2, C-6), 76.1 (C-8), 75.94 (C-15), 75.86, 75.85 (C-22, C-29), 61.4 (C-7), 55.3 (C-1); $\bar{\nu}_{max}$(thin film)/cm^{-1} 3378 (N-H) (w), 3064 (w), 3030 (w), 2903 (w), 2360 (w), 2341 (w), 1581 (N-H) (w), 1497 (m), 1453 (m), 1353 (m), 1213 (w), 1150 (m), 1133 (m), 1065 (s), 1047 (s), 1027 (s), 940 (w), 803 (s), 753 (m), 735 (s), 695 (s), 661 (m), 626 (m); LRMS *m/z* (ESI$^+$) 554.3 ([M+H]$^+$, 100%); HRMS *m/z* (ESI$^+$) found 554.28939 [M+H]$^+$ ($C_{35}H_{40}O_5N$ requires 554.29010 [M+H]$^+$); RP-HPLC (Method 1) t_R = 13.14 min, 95.81%.

Similarly, from the azide (−)-1L-**387** (261 mg, 0.450 mmol, 1.0 eq), PPh_3 (236 mg, 0.900 mmol, 2.0 eq) and H_2O (306 μL) in THF (7.1 mL), (+)-1L-**388** (136 mg, 63%) was obtained as a colourless, glassy solid: $[\alpha]_D^{25}$ = +4.7 (*c* 1.7, $CHCl_3$). All other data (R_f, ^{1}H NMR, ^{13}C NMR, IR, LRMS, HRMS) match those of the opposite enantiomer (−)-1D-**388**.

(+)-1D-3-*O*-Methyl-s*cyllo*-inosamine hydrochloride (+)-1D-14 & (–)-1L-3-*O*-methyl-s*cyllo*-inosamine hydrochloride (–)-1L-14[XXXI]

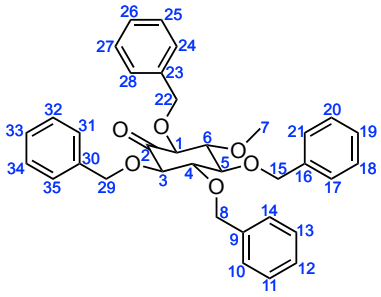


To a solution of the protected amine (–)-1D-**388** (136 mg, 0.246 mmol, 1.0 eq) in CH_2Cl_2/MeOH/H_2O (3:2:1 *v*/*v*/*v*, 3.6 mL) was added 10% Pd/C (42 mg, 0.0393 mmol, 0.16 eq) and molecular biology grade concentrated HCl (23 μL) under $N_{2(g)}$. The flask was purged with 3 balloons of $H_{2(g)}$, and the suspension was stirred at RT, under $H_{2(g)}$ for 20 hours. The reaction mixture was then filtered through a glass microfiber filter and washed with H_2O (5 × 5 mL). The filtrate was lyophilised to give a yellowish amorphous solid. The colourless solid obtained was dissolved in the minimal amount of H_2O (0.5 mL) and loaded onto a pre-washed DOWEX® 50WX8 cation ion exchange column (~0.9 mL of resin). The column was eluted with H_2O (10 mL), then with a 0.10 M aqueous solution of HCl (10 mL). The fractions were combined and lyophilised to give the hydrochloride salt (+)-1D-**14** (43 mg, 72%) as a colourless solid: decomp. 290–291 °C (EtOH) [lit.[402] 290 °C]; $[\alpha]_D^{25}$ = +2.6 (*c* 2.0 in $CHCl_3$); ^{1}H NMR (500 MHz; D_2O) δ_H 3.67–3.59 (4H, m, H-2 and H-7), 3.55–3.49 (1H, m, H-4), 3.47-3.40 (2H, m, H-5 and H-6), 3.22 (1H, dd, *J* 8.4, 8.4, H-3), 3.13 (1H, dd, *J* 10.5, 10.5, H-1); ^{13}C NMR (126 MHz; D_2O) δ_C 84.0 (C-3), 74.1 (C-5), 72.5 (C-6), 69.7 (C-4), 69.3 (C-2), 60.3 (C-7), 55.6 (C-1); LRMS *m*/*z* (ESI$^+$) 194.2 ([M+H]$^+$, 100%); HRMS *m*/*z* (ESI$^+$) found 194.10254 [M+Na]$^+$ ($C_7H_{16}O_5N$ requires 194.10230 [M+Na]$^+$); GC-MS (derivatised with TMSI) t_R = 15.97 min, 97.27%. The data are in good agreement with the literature values for (±)-**14**.[389,402]

Similarly, reaction of (+)-1L-**388** (158 mg, 0.285 mmol, 1.0 eq) and 10% Pd/C (49 mg, 0.0457 mmol, 0.16 eq) in CH_2Cl_2/MeOH/H_2O (3:2:1 *v*/*v*/*v*, 4.3 mL) and under $H_{2(g)}$, yielded (–)-1L-**14** (53.6 mg, 77%) as a colourless solid: $[\alpha]_D^{25}$ = –2.2 (*c* 2.0, $CHCl_3$). All other data (m.p., ^{1}H NMR, ^{13}C NMR, LRMS, HRMS) match those of the opposite enantiomer (+)-1D-**14**.

[XXXI] Racemic (±)-1D-3-*O*-Methyl-s*cyllo*-inosamine hydrochloride was synthesed in the same way, as described.

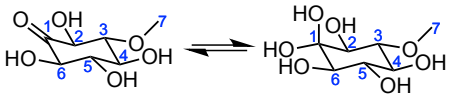
(±)-2-Keto-1,3,4,5-tetra-*O*-benzyl-6-*O*-methyl-*myo*-inositol (±)-392



A solution of anhydrous DMSO (196 μL, 2.76 mmol, 2.2 eq) in anhydrous CH_2Cl_2 (0.40 mL) was added dropwise over 5 minutes to a solution of oxalyl chloride (117 μL, 1.38 mmol, 1.1 eq) in CH_2Cl_2 (2.0 mL) at –60 °C. A solution of (±)-**386** (695 mg, 1.25 mmol, 1.0 eq) in anhydrous CH_2Cl_2 (1.6 mL) was then added dropwise to the reaction mixture and the solution was stirred for a further 2.5 hours, maintaining the temperature between –60 °C and –55 °C. Anhydrous Et_3N (803 μL, 5.76 mmol, 4.6 eq) was then added and the reaction was stirred for a further 5 minutes before warming up to RT. After 1 hour, H_2O (3 mL) was added and the reaction mixture was diluted in CH_2Cl_2 (6 mL). The organic phase was separated and the aqueous layer was extracted with CH_2Cl_2 (3 × 6 mL). The combined organic layers were washed with brine (6 mL) and H_2O (6 mL), then dried ($MgSO_4$), filtered, and concentrated *in vacuo*. Purification by column chromatography over silica gel (Petroleum ether/EtOAc 9.5:0.5, 9:1, 8:2, 7:3) followed by crystallisation from Et_2O afforded (±)-**392** (320 mg, 46%) as a colourless solid: R_f 0.86 (Petroleum ether/EtOAc 7:3); m.p. 97–99 °C (Et_2O); ^{1}H NMR (500 MHz; $CDCl_3$) δ_H 7.43–7.27 (20H, m, H-10 to H-14, H-17 to H-21, H-24 to H-28 and H-31 to H-35), 4.91–4.83 (5H, m, H-8, H-15, H-22 and H-29), 4.76 (1H, d, J_{AB} 10.6, H-8'), 4.58 (1H, d, J_{AB} 11.7, H-29'), 4.53 (1H, d, J_{AB} 11.5, H-22'), 4.12 (1H, dd, *J* 9.8, 1.5, H-3), 4.04 (1H, dd, *J* 9.9, 1.5, H-1), 3.77 (1H, dd, *J* 9.4, 9.3, H-5), 3.65 (3H, s, H-7), 3.57 (1H, dd, *J* 9.8, 9.4, H-4), 3.32 (1H, dd, *J* 9.9, 9.3, H-6); ^{13}C NMR (126 MHz; $CDCl_3$) δ_C 202.2 (C-2), 138.4, 138.3 (C-9, C-16), 137.6 (C-30), 137.5 (C-23), 128.6, 128.55, 128.52, 128.3, 128.21, 128.19, 128.11, 128.08, 127.93, 127.91 (C-10 to C-14, C-17 to C-21, C-24 to C-28, C-31 to C-35), 83.8 (C-3), 83.7 (C-1), 83.5 (C-6), 82.4 (C-5), 81.5 (C-4), 76.25 (C-15), 76.16 (C-8), 73.5 (C-29), 73.4 (C-22), 61.8 (C-7); $\bar{\nu}_{max}$(thin film)/cm^{-1} 3063 (w), 3031 (w), 2909 (w), 2867 (w), 1731 (C=O) (m), 1497 (w), 1454 (w), 1360 (w),

1214 (w), 1133 (m), 1065 (s), 1026 (s), 970 (m), 921 (w), 754 (m), 732 (m), 695 (s); LRMS *m/z* (ESI$^+$) 575.2 ([M+Na]$^+$, 100%); HRMS *m/z* (ESI$^+$) found 575.24020 [M+Na]$^+$ ($C_{35}H_{36}O_6Na$ requires 575.24041 [M+Na]$^+$); RP-HPLC (Method 2) 16.01 min, 99.80%.

(±)-3-*O*-Methyl-*scyllo*-inososе (±)-391



To a solution of (±)-**392** (27 mg, 0.0494 mmol, 1.0 eq) in MeOH/CH_2Cl_2/H_2O/AcOH (5:2:1:1 *v/v/v/v* 3.6 mL) was added 10% Pd/C (7 mg, 0.00658 mmol, 0.13 eq) under $N_{2(g)}$. The flask was purged with 3 balloons of $H_{2(g)}$, and the suspension was stirred at RT, under $H_{2(g)}$ for 17 hours. The reaction mixture was then filtered through a microfibre glass filter and washed with H_2O (3 × 2 mL). The filtrate was lyophilised to give (±)-**391** (8 mg, 84%) as a colourless solid. This compound was found to be very unstable, and in equilibrium with the hydrate form (±)-**393** as observed by ^{1}H NMR ((±)-**391** (Ket.)/(±)-**393** (Diol) 1.3:1.0). As a result, clean ^{1}H NMR and ^{13}C NMR data for this compounds could not be obtained. This compounds was made fresh before use in *in vitro* assays: R_f 0.55 (EtOAc/H_2O/IPA 1:1:1); m.p. 95–97 °C (H_2O); ^{1}H NMR (500 MHz; D_2O) δ_H 4.51 (1H, dd, *J* 10.2, 1.6, H-2 Ket.), 4.37 (1H, dd, *J* 10.3, 1.6, H-6 Ket.), 3.89 (1H, dd, *J* 9.7, 9.5, H-4 Ket.), 3.62 (3H, s, H-7 Ket.), 3.59 (3H, s, H-7 Diol), 3.50–3.34 (5H, m, H-5 Ket., H-6, H-5, H-4 and H-2 Diol), 3.23 (1H, dd, *J* 10.2, 9.7, H-3 Ket.), 3.24–3.16 (1H, m, H-3 Diol); ^{13}C NMR (126 MHz; D_2O) δ_C 205.6 (C-1 Ket.), 94.1 (C-1 Diol), 83.3 (C-3 Ket.), 83.0 (C-3 Diol), 75.9 (C-6 Ket.), 75.5 (C-2 Ket.), 74.1 (C-6 Diol), 73.7 (C-2 Diol), 73.6 (C-5 Ket.), 73.14, 73.07 (C-5, C-4 Diol), 72.3 (C-4 Ket.), 59.8 (C-7 Diol), 59.7 (C-7 Ket.); $\bar{\nu}_{max}$(thin film)/cm^{-1} 3089 (w), 3063 (w), 3030 (w), 2911 (w), 2867 (w), 1731 (C=O) (m), 1497 (w), 1454 (m), 1361 (m), 1215 (w), 1133 (s), 1065 (s), 1035 (s), 1026 (s), 970 (m), 921 (m), 754 (s), 734 (s), 695 (s); LRMS *m/z* (ESI$^+$) 407.2 ([2M+Na]$^+$, 100%, Ket.), 421.0 ([2M+H]$^+$, 29%, Diol);[XXXII] HRMS (Ket.) *m/z* (ESI$^-$) found 227.03279 [M+Cl]$^-$ ($C_7H_{12}O_6Cl$ requires 227.03279

[XXXII] A few, weak intensity, unidentifiable peaks were also observed by ^{13}C NMR (See Appendix A)

$[M+Cl]^-$); HRMS (Diol) *m/z* (ESI^-) found 245.04314 $[M+Cl]^-$ ($C_7H_{14}O_7Cl$ requires 245.04335 $[M+Cl]^-$). The data are in good agreement with the literature values.[424]

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Appendices

Appendix A – Selected Spectra

Selected NMR spectra (^{1}H, ^{13}C, and for relevant compounds: ^{31}P, ^{2}H, ^{1}H-^{13}C HMBC, H-^{13}C HSQC, ^{1}H-^{31}P HMBC and ^{1}H-^{29}Si HMBC) are presented for all novel and final compounds. For penultimate and final compounds purity data has also been included. Mass spectrometry data is included for deuterated compounds. Red asterisks indicate deuterium atoms attached to the indicated carbons.

Appendix B – X-Ray Crystallography Data

Crystallographic data for **264** (6558), (±)-**296** (6607), (+)-1L-**364** (6552) and (–)-1L-**364** (6002) is included.

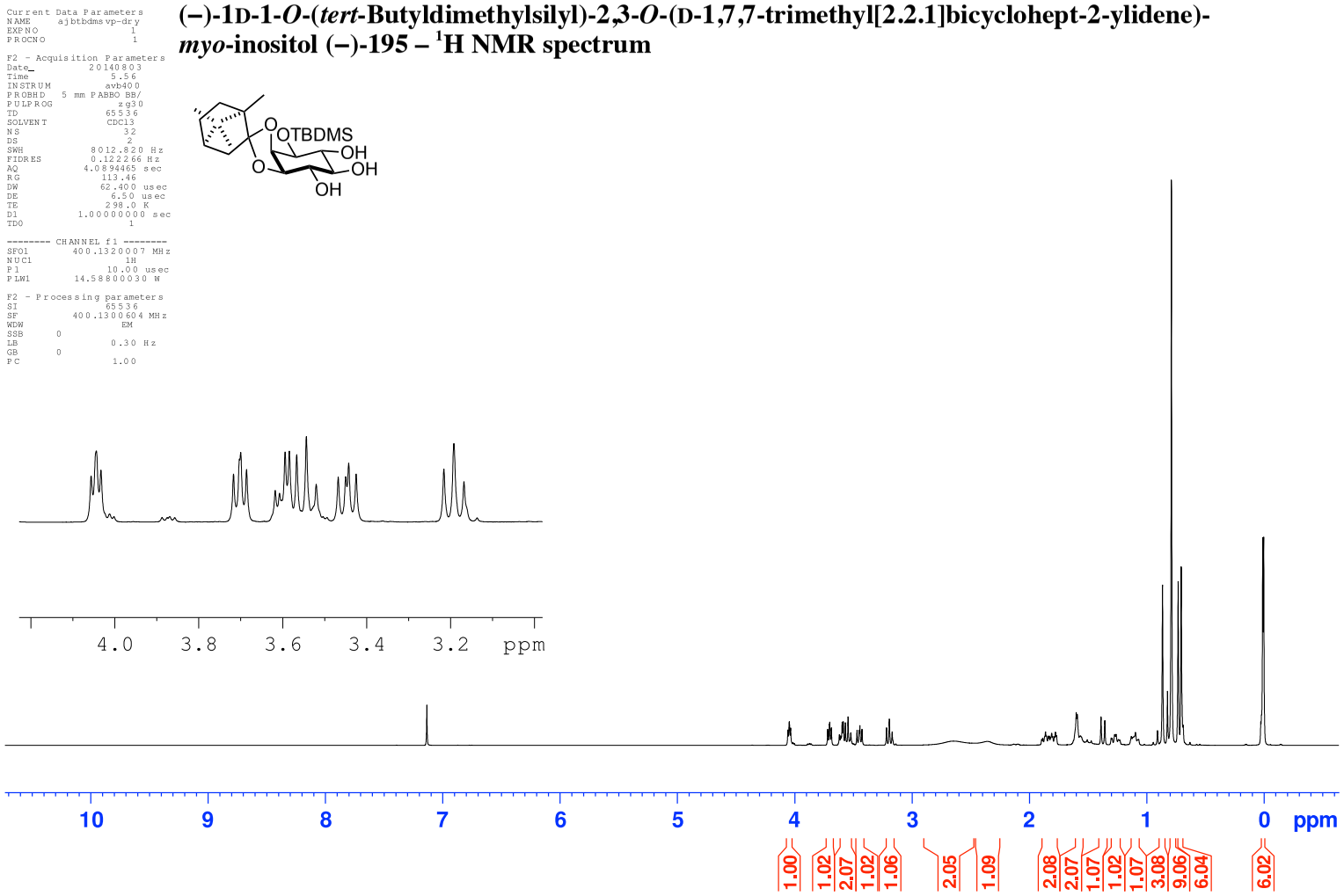
Appendix C – Image Permissions

Permissions for the images featured in Chapter 1 of this dissertation (p. 3 and p. 30) can be found at the end of the appendices.

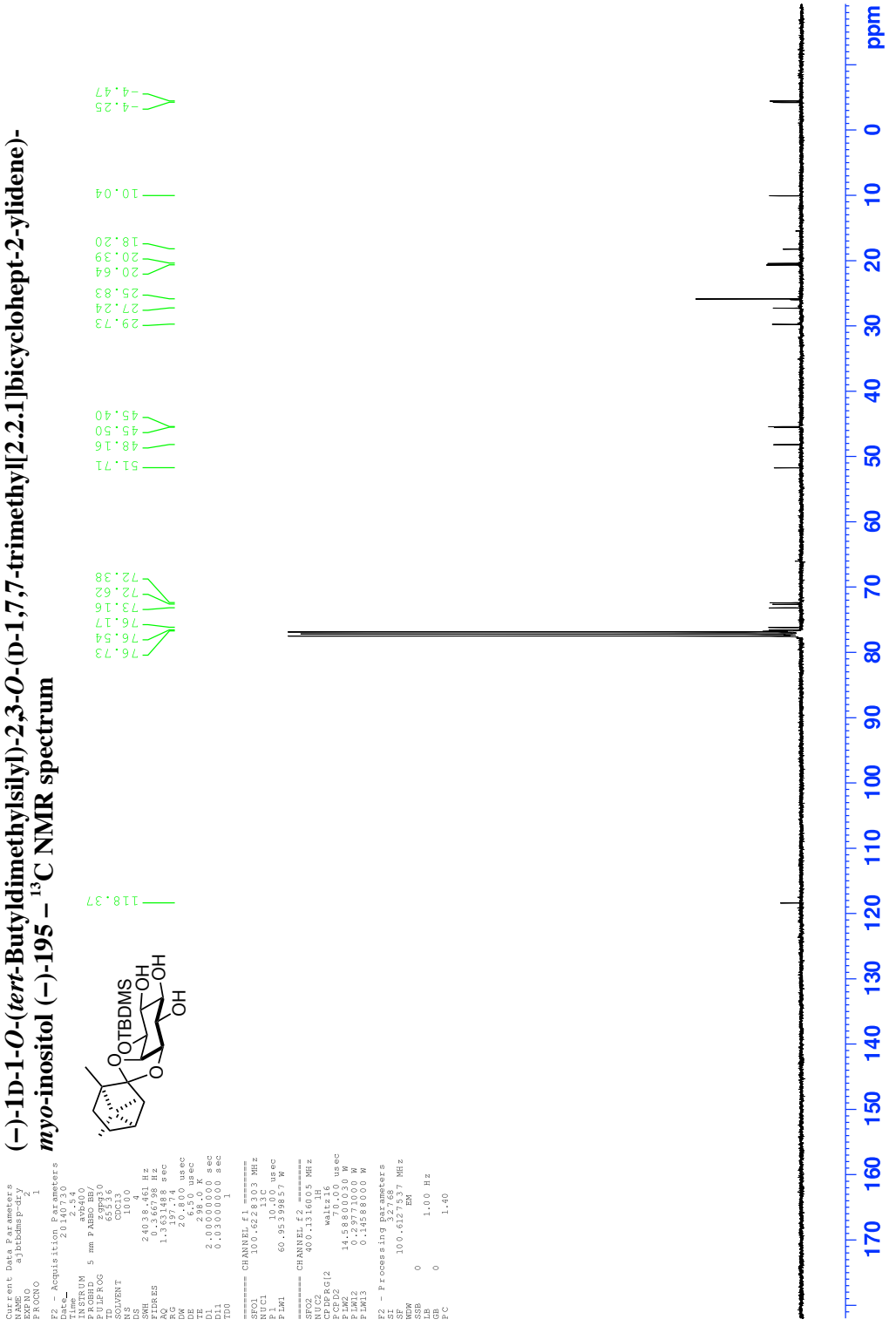
Appendix D – DFT Calculations

Please contact the author for this information

(–)-1D-1-*O*-(*tert*-Butyldimethylsilyl)-2,3-*O*-(D-1,7,7-trimethyl[2.2.1]bicyclohept-2-ylidene)-*myo*-inositol (–)-195 – ^{1}H NMR spectrum



(−)-1D-1-*O*-(*tert*-Butyldimethylsilyl)-2,3-*O*-(D-1,7,7-trimethyl[2.2.1]bicycloheptan-2-ylidene)-*myo*-inositol (−)-195 – ^{13}C NMR spectrum



(–)-1D-1-*O*-(*tert*-Butyldimethylsilyl)-2,3-*O*-(D-1,7,7-trimethyl[2.2.1]bicyclohept-2-ylidene)-*myo*-inositol (–)-195 – ^{1}H-^{29}Si HMBC NMR spectrum

```
Current Data Parameters
NAME      ajbtbdms-vp-dry-Si-Hcorr
EXPNO                 3
PROCNO                1

F2 - Acquisition Parameters
Date_          20131121
Time              15.33
INSTRUM         drx500
PROBHD    5 mm PABBO BB/
PULPROG      hmbcgpndqf
TD                2048
SOLVENT           DMSO
NS                  32
DS                   8
SWH            8012.820 Hz
FIDRES         3.912510 Hz
AQ             0.1277952 sec
RG               14596.5
DW                62.400 usec
DE                11.00 usec
TE                297.7 K
CNST13         6.0000000
d0             0.00000300 sec
D1             2.00000000 sec
d6             0.08333334 sec
D16            0.00020000 sec
IN0            0.00005030 sec

======== CHANNEL f1 ========
NUC1                 1H
P1                12.50 usec
p2                25.00 usec
PL1       0 dB
SFO1         500.1320005 MHz

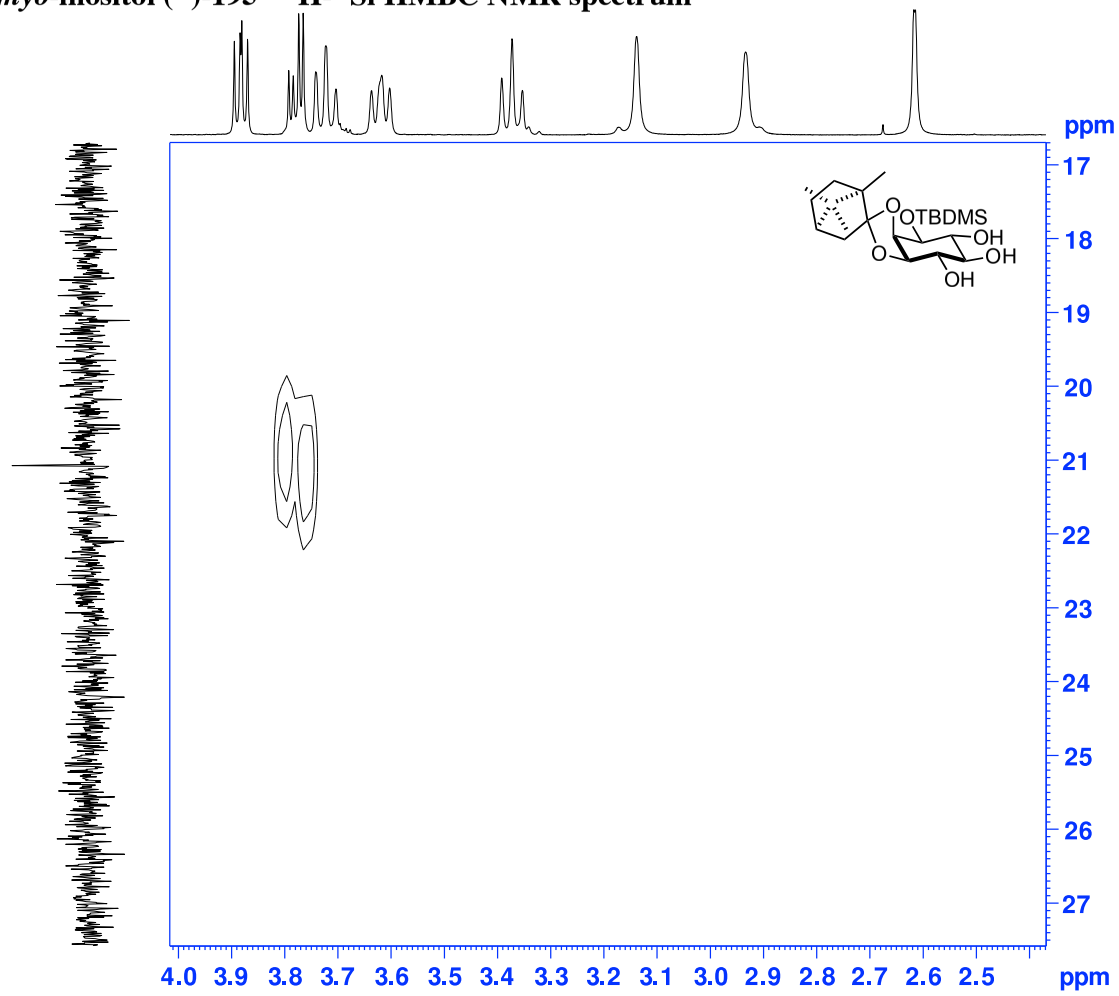
======== CHANNEL f2 ========
NUC2                29Si
P3                12.00 usec
PL2                6.00 dB
SFO2          99.3638486 MHz

====== GRADIENT CHANNEL =====
GPNAM[1]        SINE.100
GPNAM[2]        SINE.100
GPNAM[3]        SINE.100
GPZ1               60.00 %
GPZ2               20.00 %
GPZ3               55.89 %
P16              1000.00 usec

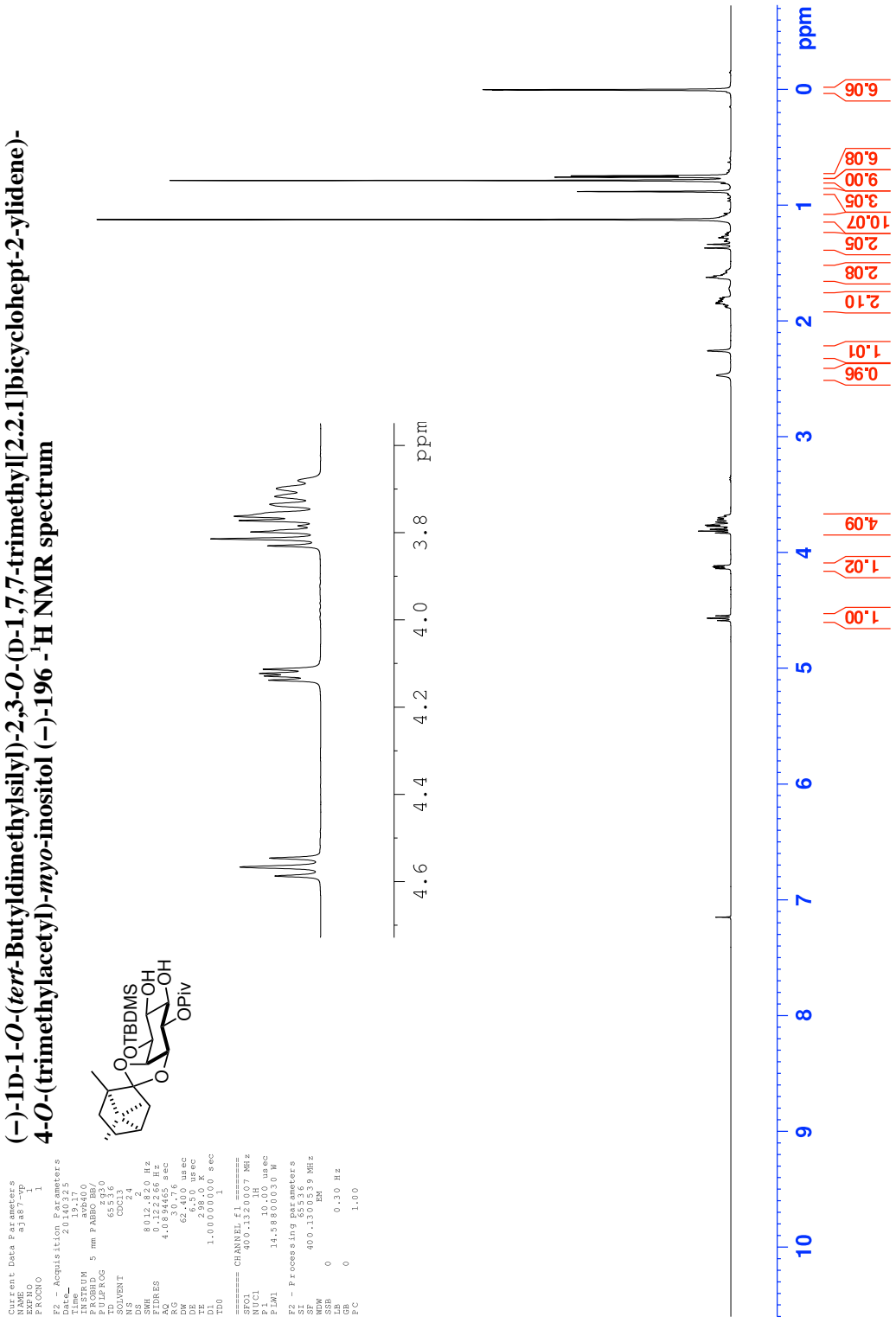
F1 - Acquisition parameters
TD                   57
SFO1           99.36385 MHz
FIDRES        174.392242 Hz
SW               100.040 ppm
FnMODE              QF

F2 - Processing parameters
SI                 1024
SF           500.1300000 MHz
WDW                QSINE
SSB       0
LB        0 Hz
GB        0
PC                 1.40

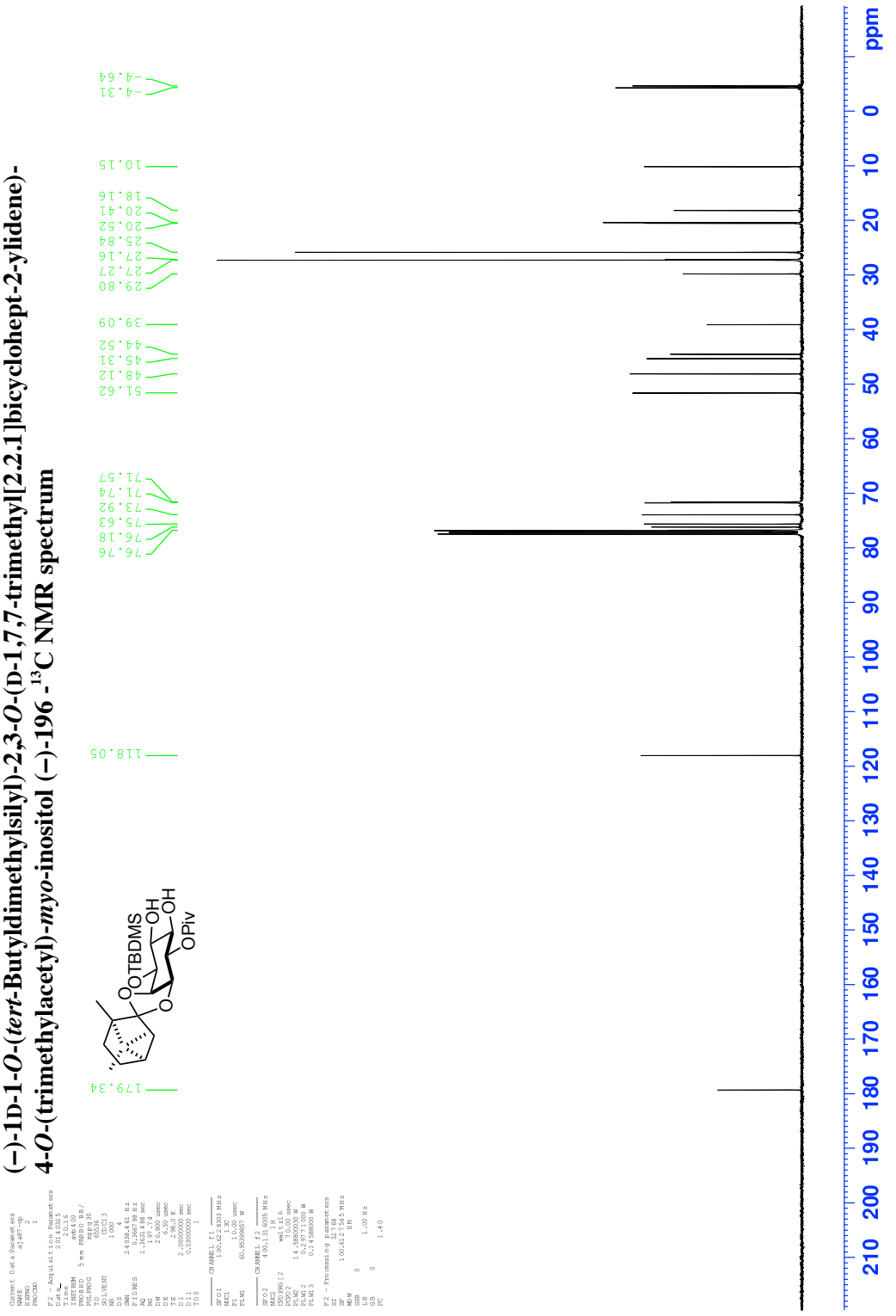
F1 - Processing parameters
SI                  512
MC2                 QF
SF           99.3617620 MHz
WDW                QSINE
SSB       0
LB        0 Hz
GB        0
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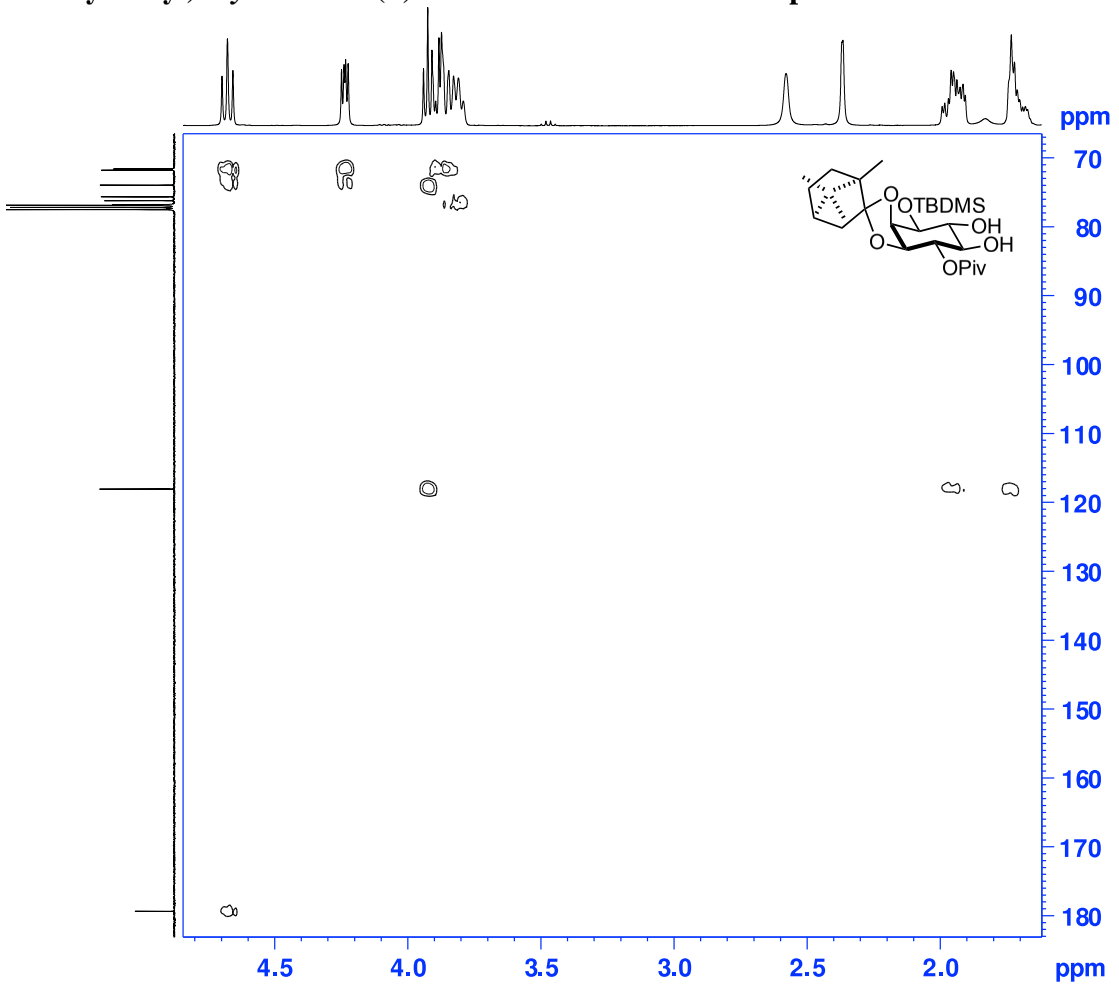
(−)-1D-1-*O*-(*tert*-Butyldimethylsilyl)-2,3-*O*-(D-1,7,7-trimethyl[2.2.1]bicycloheptt-2-ylidene)-4-*O*-(trimethylacetyl)-*myo*-inositol (−)-196 -^{1}H NMR spectrum



(–)-1D-1-*O*-(*tert*-Butyldimethylsilyl)-2,3-*O*-(D-1,7,7-trimethyl[2.2.1]bicyclohept-2-ylidene)-4-*O*-(trimethylacetyl)-*myo*-inositol (–)-196 -^{13}C NMR spectrum



(–)-1D-1-*O*-(*tert*-Butyldimethylsilyl)-2,3-*O*-(D-1,7,7-trimethyl[2.2.1]bicyclohept-2-ylidene)-4-*O*-(trimethylacetyl)-*myo*-inositol (–)-196 – ^{1}H-^{13}C HMBC NMR spectrum




```
Current Data Parameters
NAME                  AJ4-HMBC
EXPNO                        1
PROCNO                       1

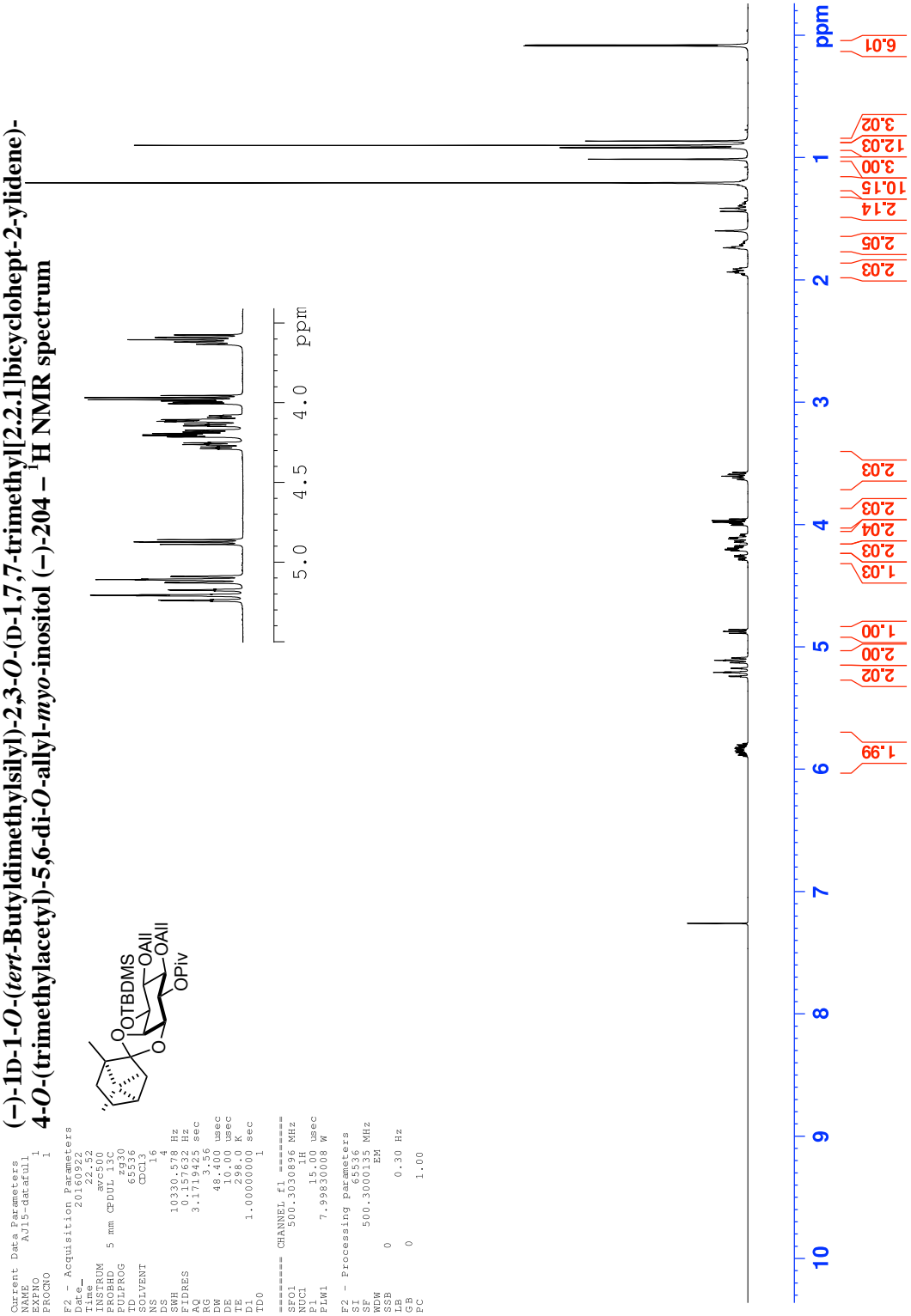
F2 - Acquisition Parameters
Date_              20161114
Time                   18.24
INSTRUM                avb400
PROBHD    Z116098_0219 (
PULPROG     hmbcgpl2ndqf
TD                     4096
SOLVENT                CDCl3
NS                        32
DS                        16
SWH                47953.396 Hz
FIDRES              1.170751 Hz
AQ                  0.427077 sec
RG                     197.74
DW                    104.267 usec
DE                      6.50 usec
TE                    298.0 K
CNST6             125.0000000
CNST7             165.0000000
CNST13              8.0000000
d0                  0.00000300 sec
D1                  1.50000000 sec
d6                  0.06250000 sec
D16                 0.00020000 sec
DELTA1              0.00280000 sec
DELTA2              0.00183030 sec
DELTA3              0.06129600 sec
in0          0 sec
ST1CNT                       0
d0orig              0.00000300 sec
phlloop                      0
tlloop                       0
SFO1              400.1320007 MHz
NUC1                        1H
P1                       10.00 usec
p2                       20.00 usec
PLW1              14.58800030 W
SFO2              100.6228303 MHz
NUC2                       13C
P3                       10.00 usec
PLW2              65.01300049 W
GPNAM[1]          SMSQ10.100
GPNAM[2]          SMSQ10.100
GPNAM[3]          SMSQ10.100
GPNAM[4]          SMSQ10.100
GPNAM[5]          SMSQ10.100
GPNAM[6]          SMSQ10.100
GPZ1                    50.00 %
GPZ2                    30.00 %
GPZ3                    40.10 %
GPZ4                    15.00 %
GPZ5                   -10.00 %
GPZ6                    -5.00 %
P16                  1000.00 usec

F1 - Acquisition parameters
TD                         128
SFO1              100.6228 MHz
FIDRES            174.386154 Hz
SW                  221.833 ppm
FnMODE                     QF

F2 - Processing parameters
SI                        1024
SF                400.1300092 MHz
WDW                       SINE
SSB          0
LB           0 Hz
GB           0
PC                        1.40

F1 - Processing parameters
SI                        1024
MC2                         QF
SF                100.6127565 MHz
WDW                       SINE
SSB          0
LB           0 Hz
GB           0
```

(−)-1D-1-*O*-(*tert*-Butyldimethylsilyl)-2,3-*O*-(D-1,7,7-trimethyl[2.2.1]bicycloheptan-2-ylidene)-4-*O*-(trimethylacetyl)-5,6-di-*O*-allyl-*myo*-inositol (−)-204 – ^{1}H NMR spectrum



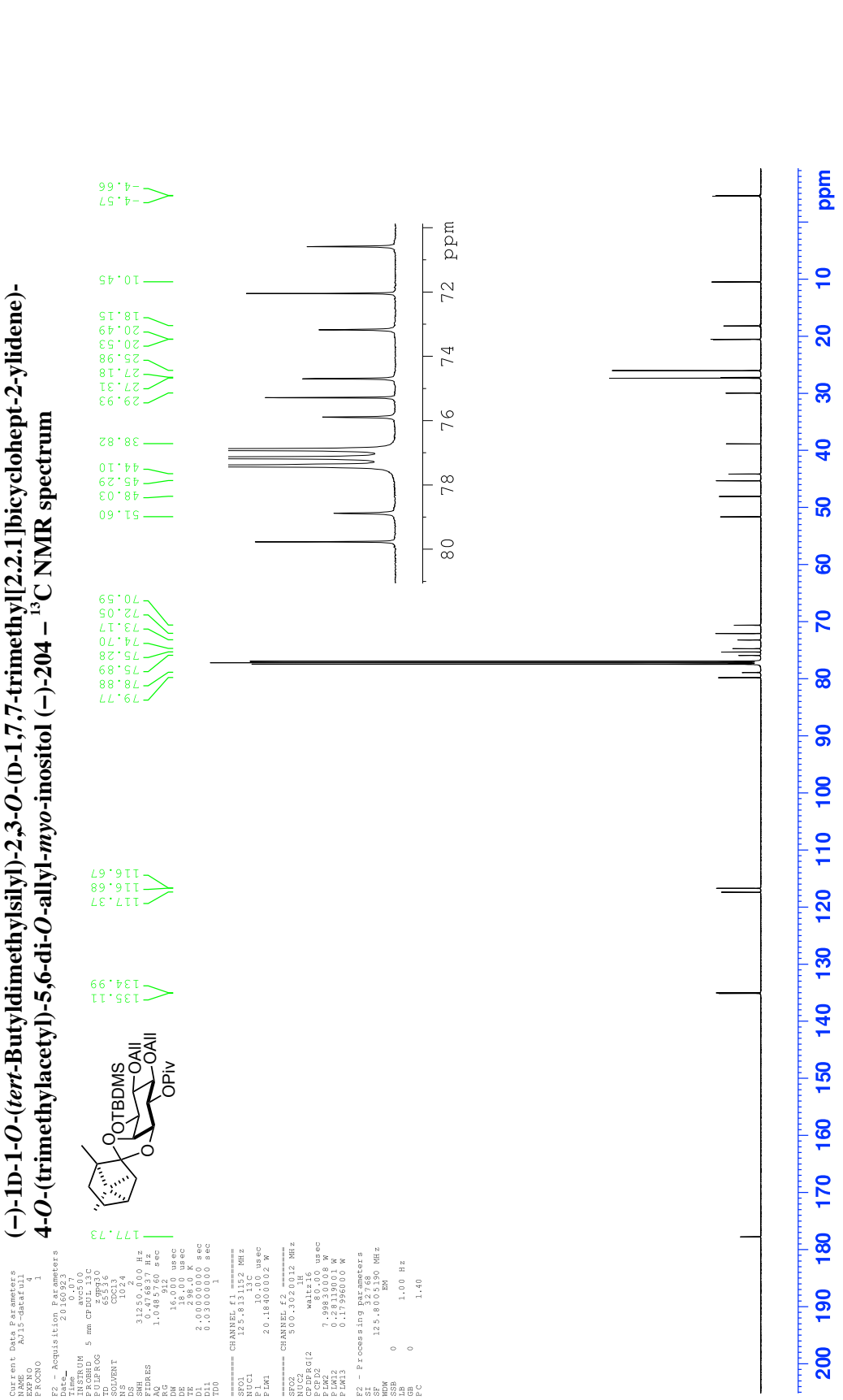

```
Current Data Parameters
NAME                  AJ15-dataful
EXPNO                          1
PROCNO                         1

F2 - Acquisition Parameters
Date_              20160222
Time               19.22
INSTRUM            awc500
PROBHD     5 mm CPDUL 13C
PULPROG            zg30
TD                 65536
SOLVENT            CDCl3
NS                    16
DS                     4
SWH            10330.578 Hz
FIDRES         0.157633 Hz
AQ             3.1719425 sec
RG                  3.56
DW                 48.400 usec
DE                 10.00 usec
TE                 298.0 K
D1             1.00000000 sec
TD0                    1

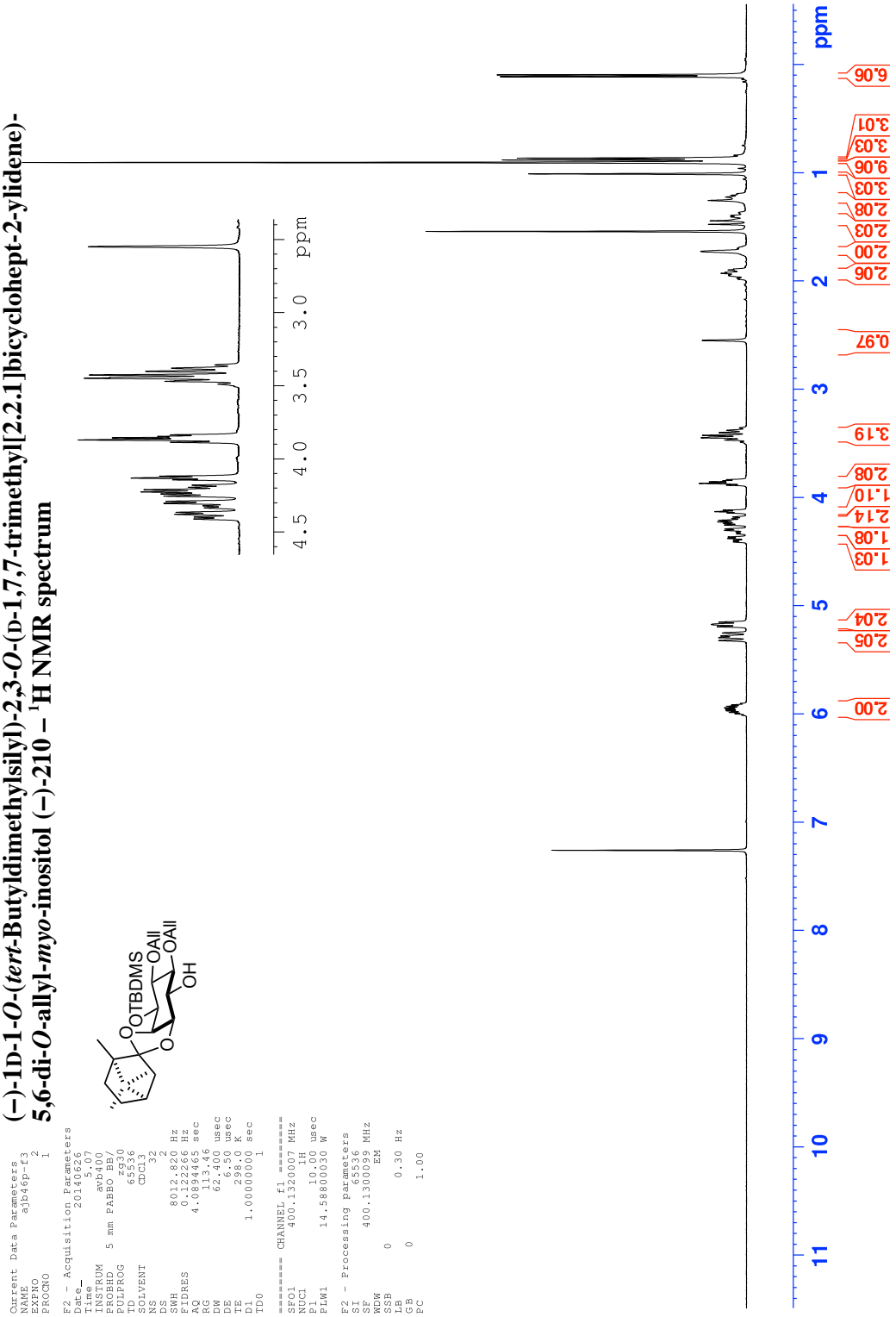
======== CHANNEL f1 ========
SFO1           500.3030896 MHz
NUC1                   1H
P1                 15.00 usec
PLW1           7.99830008 W

F2 - Processing parameters
SI                 65536
SF             500.3000136 MHz
WDW                    EM
SSB                 0
LB                  0.30 Hz
GB                  0
PC                  1.00
```

(–)-1D-1-*O*-(*tert*-Butyldimethylsilyl)-2,3-*O*-(D-1,7,7-trimethyl[2.2.1]bicyclohept-2-ylidene)-4-*O*-(trimethylacetyl)-5,6-di-*O*-allyl-*myo*-inositol (–)-204 – ^{13}C NMR spectrum



(–)-1D-1-*O*-(*tert*-Butyldimethylsilyl)-2,3-*O*-(D-1,7,7-trimethyl[2.2.1]bicyclohept-2-ylidene)-5,6-di-*O*-allyl-*myo*-inositol (–)-210 – ^{1}H NMR spectrum



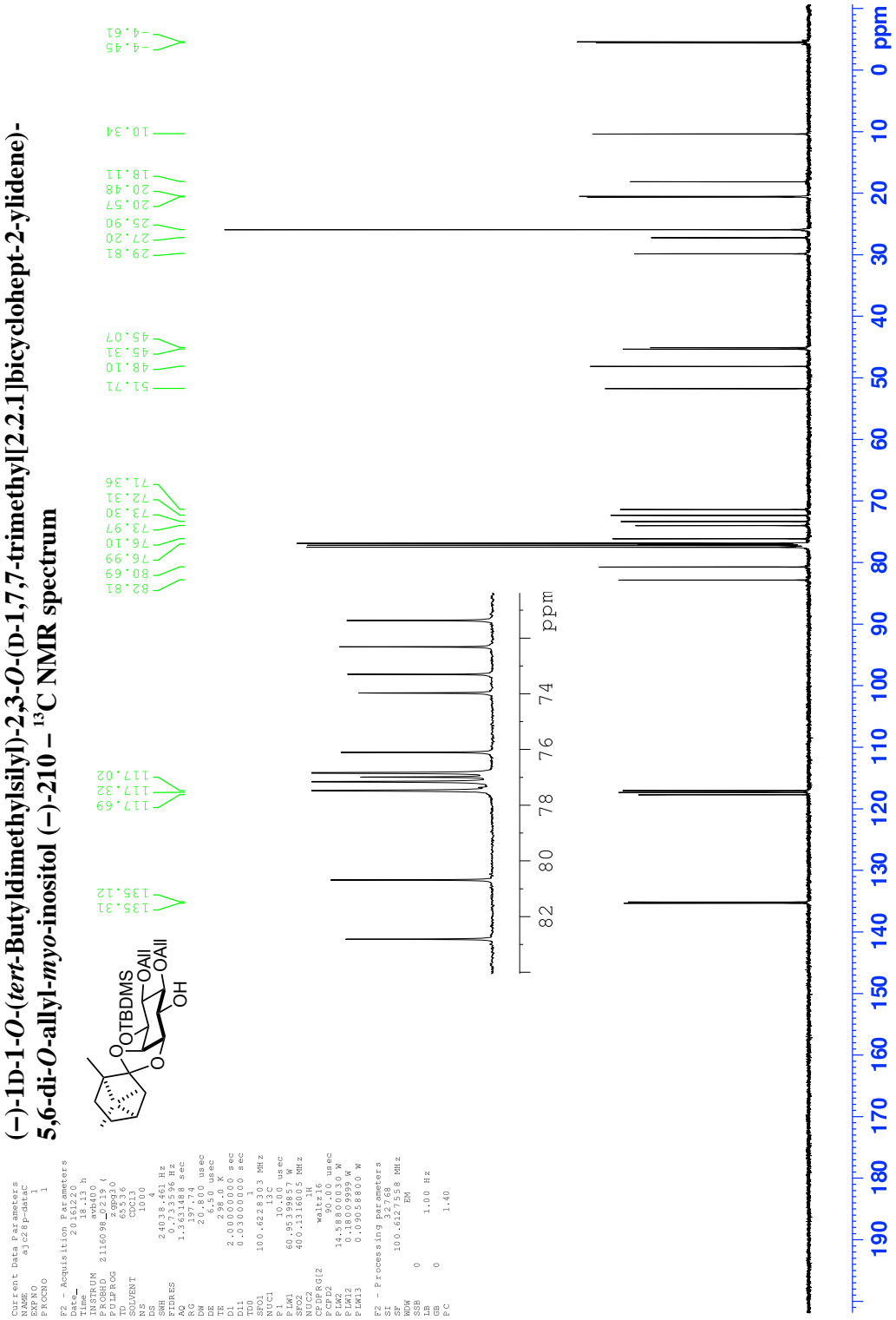

```
Current Data Parameters
NAME          ajb46p-f3
EXPNO                 2
PROCNO                1

F2 - Acquisition Parameters
Date_          20140626
Time           15.07
INSTRUM          av400
PROBHD  5 mm PABBO BB/
PULPROG          zg30
TD              65536
SOLVENT         CDCl3
NS                 32
DS                  2
SWH          8012.820 Hz
FIDRES       0.122266 Hz
AQ           4.0894465 sec
RG              113.46
DW             62.400 usec
DE              6.50 usec
TE             298.0 K
D1             1.00000000 sec
TD0                 1

======== CHANNEL f1 ========
SFO1         400.1320007 MHz
NUC1               1H
P1               10.00 usec
PLW1         14.58800030 W

F2 - Processing parameters
SI              65536
SF         400.1300000 MHz
WDW                EM
SSB             0
LB              0.30 Hz
GB              0
PC              1.00
```

(–)-1D-1-*O*-(*tert*-Butyldimethylsilyl)-2,3-*O*-(D-1,7,7-trimethyl[2.2.1]bicycloheept-2-ylidene)-5,6-di-*O*-allyl-*myo*-inositol (–)-210 – ^{13}C NMR spectrum



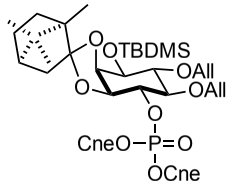
(–)-1D-1-*O*-(*tert*-Butyldimethylsilyl)-2,3-*O*-(D-1,7,7-trimethyl[2.2.1]bicyclohept-2-ylidene)-4-*O*-(bis(2-cyanoethyl)phosphate)-5,6-di-*O*-allyl-*myo*-inositol (–)-226 – ^{1}H NMR spectrum

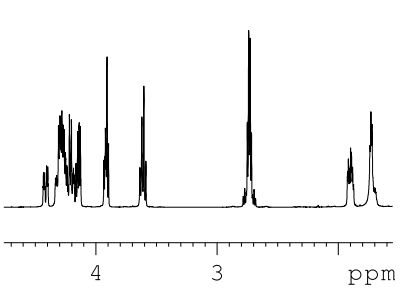
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Current Data Parameters
NAME       AJ19-datafull
EXPNO                 1
PROCNO                1

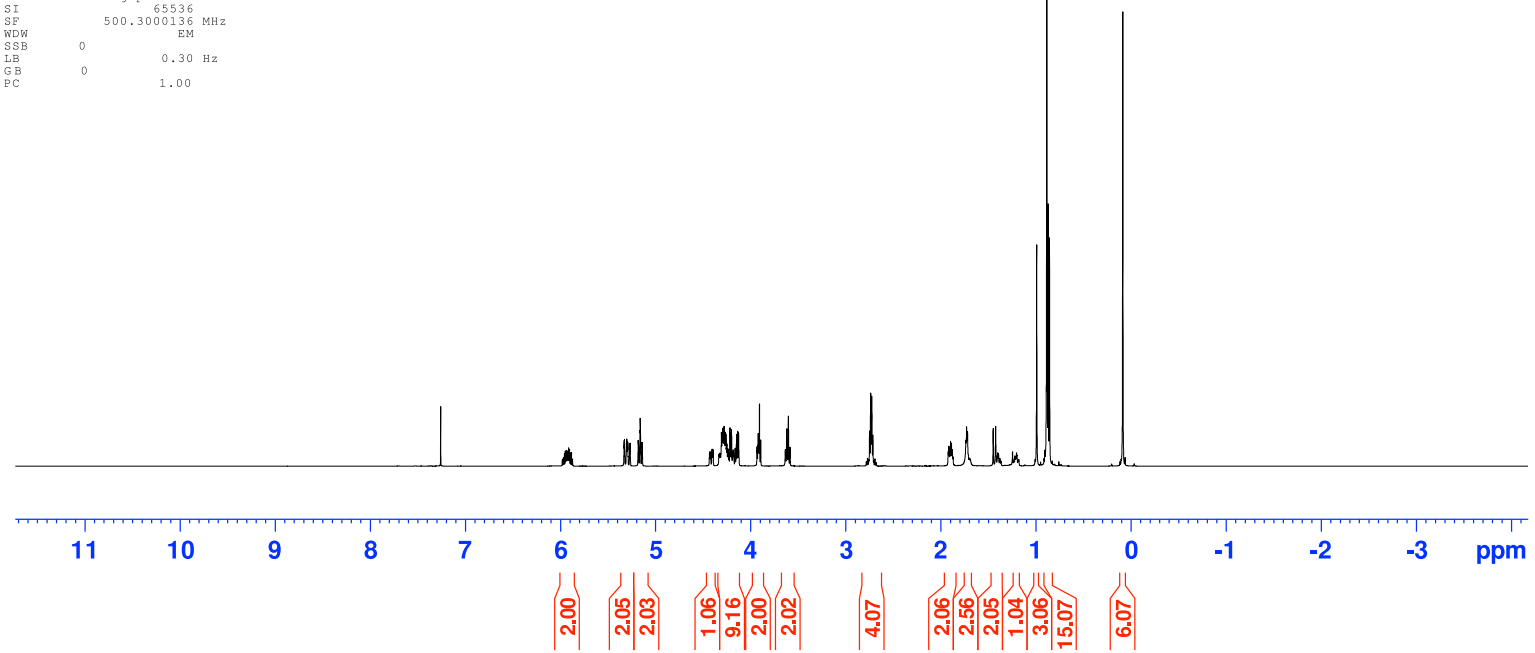
F2 - Acquisition Parameters
Date_          20160923
Time              2.36
INSTRUM         avc500
PROBHD   5 mm CPDUL 13C
PULPROG           zg30
TD               65536
SOLVENT          CDCl3
NS                  16
DS                   4
SWH          10330.578 Hz
FIDRES        0.157632 Hz
AQ           3.1719425 sec
RG                3.56
DW              48.400 usec
DE              10.00 usec
TE              298.0 K
D1          1.00000000 sec
TD0                  1

======== CHANNEL f1 ========
SFO1        500.3030896 MHz
NUC1               1H
P1              15.00 usec
PLW1         7.99830008 W

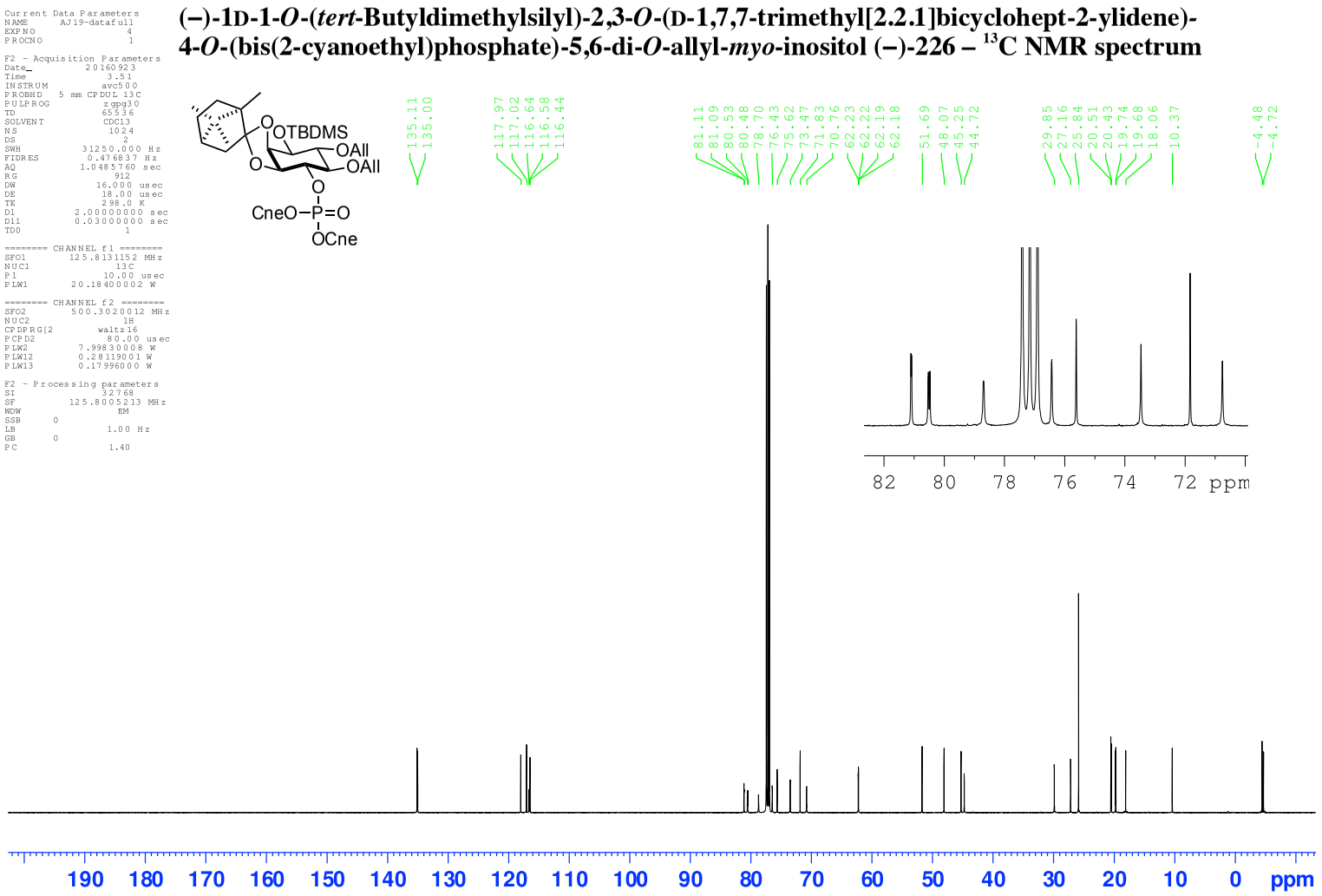
F2 - Processing parameters
SI               65536
SF          500.3000136 MHz
WDW                EM
SSB       0
LB              0.30 Hz
GB        0
PC              1.00
```







(–)-1D-1-*O*-(*tert*-Butyldimethylsilyl)-2,3-*O*-(D-1,7,7-trimethyl[2.2.1]bicyclohept-2-ylidene)-4-*O*-(bis(2-cyanoethyl)phosphate)-5,6-di-*O*-allyl-*myo*-inositol (–)-226 – ^{13}C NMR spectrum




```
Current Data Parameters
NAME       AJ19-datafull
EXPNO                  4
PROCNO                 1

F2 - Acquisition Parameters
Date_           20160923
Time                3.51
INSTRUM           avc500
PROBHD   5 mm CPDUL 13C
PULPROG           zgpg30
TD                65536
SOLVENT           CDCl3
NS                 1024
DS                    2
SWH           31250.000 Hz
FIDRES         0.476837 Hz
AQ             1.0485760 sec
RG                  912
DW               16.000 usec
DE               18.00 usec
TE                298.0 K
D1           2.00000000 sec
D11          0.03000000 sec
TD0                   1

======== CHANNEL f1 ========
SFO1         125.8131152 MHz
NUC1               13C
P1               10.00 usec
PLW1         20.18400002 W

======== CHANNEL f2 ========
SFO2         500.3020012 MHz
NUC2               1H
CPDPRG[2         waltz16
PCPD2             80.00 usec
PLW2          7.99830008 W
PLW12         0.28119001 W
PLW13         0.17996000 W

F2 - Processing parameters
SI                 32768
SF           125.8005213 MHz
WDW                  EM
SSB       0
LB                1.00 Hz
GB        0
PC                1.40
```

(–)-1D-1-*O*-(*tert*-Butyldimethylsilyl)-2,3-*O*-(D-1,7,7-trimethyl[2.2.1]bicyclohept-2-ylidene)-4-*O*-(bis(2-cyanoethyl)phosphate)-5,6-di-*O*-allyl-*myo*-inositol (–)-226 – ^{31}P NMR spectrum

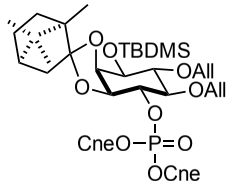
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Current Data Parameters
NAME          ajb48bp-dry
EXPNO                   2
PROCNO                  1

F2 - Acquisition Parameters
Date_          20140623
Time              14.57
INSTRUM           avb400
PROBHD   5 mm PABBO BB/
PULPROG           zgpg30
TD                65536
SOLVENT           CDCl3
NS                  150
DS                    4
SWH           64102.562 Hz
FIDRES        0.978127 Hz
AQ            0.5111808 sec
RG               197.74
DW               7.800 usec
DE               6.50 usec
TE               298.0 K
D1            2.00000000 sec
D11           0.03000000 sec
TD0                   1

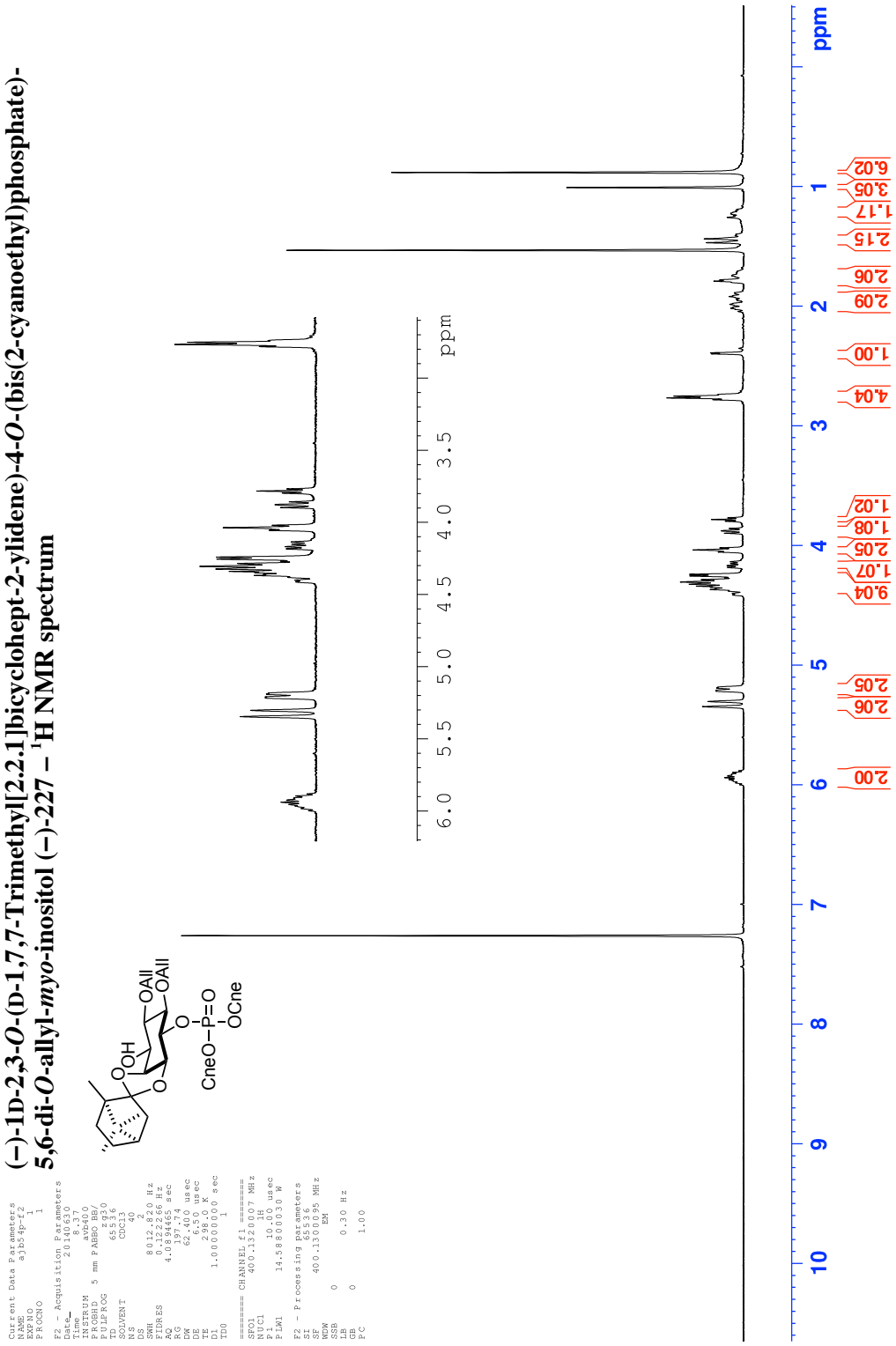
======== CHANNEL f1 ========
SFO1         161.9674942 MHz
NUC1                31P
P1                8.00 usec
PLW1         54.00000000 W

======== CHANNEL f2 ========
SFO2         400.1316005 MHz
NUC2                1H
CPDPRG[2          waltz16
PCPD2              70.00 usec
PLW2         14.58800030 W
PLW12         0.29771000 W
PLW13         0.14588000 W

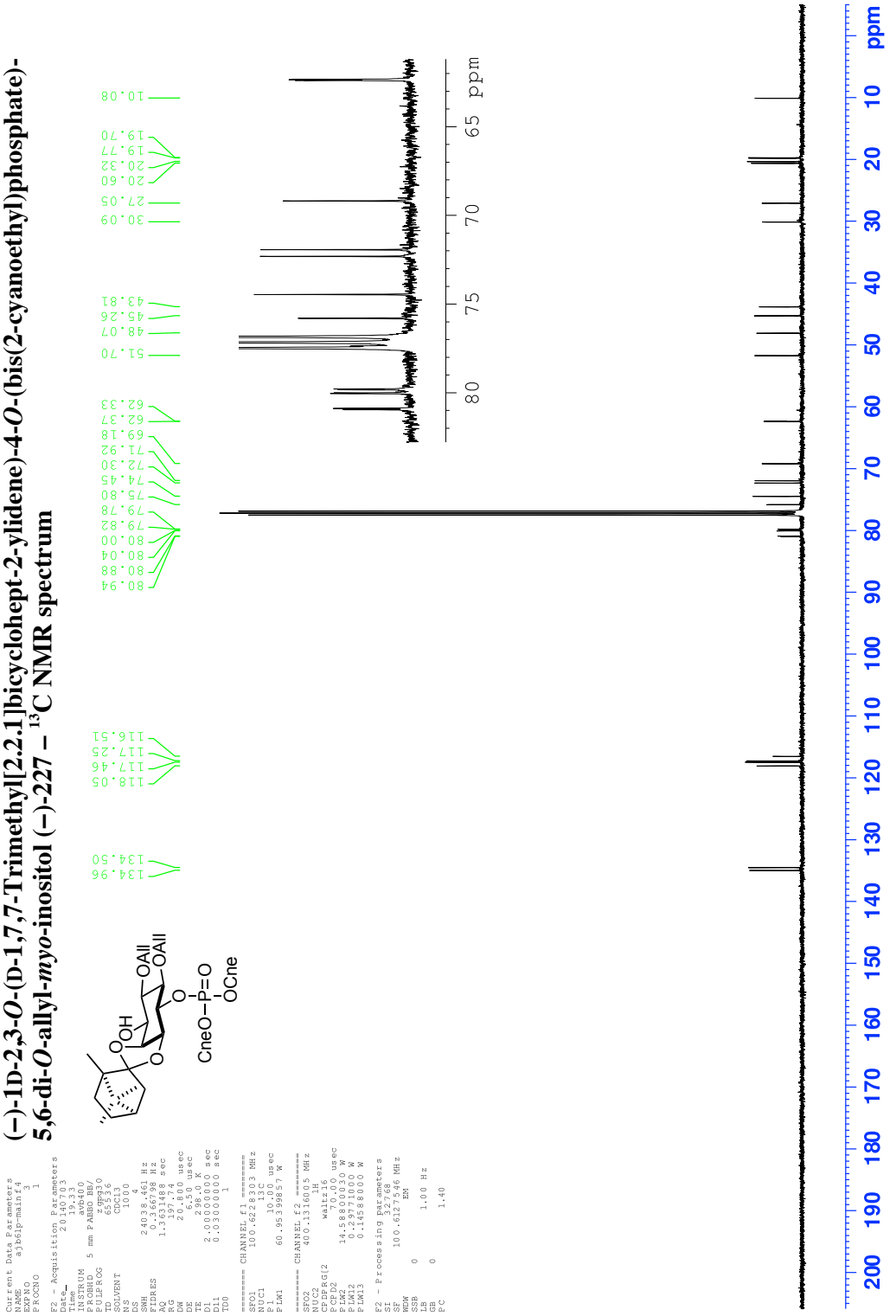
F2 - Processing parameters
SI                 32768
SF           161.9755930 MHz
WDW                  EM
SSB       0
LB                1.00 Hz
GB        0
PC                1.40
```



(–)-1D-2,3-*O*-(D-1,7,7-Trimethyl[2.2.1]bicyclohept-2-ylidene)-4-*O*-(bis(2-cyanoethyl)phosphate)-5,6-di-*O*-allyl-*myo*-inositol (–)-227 – ^{1}H NMR spectrum



(–)-1D-2,3-*O*-(D-1,7,7-Trimethyl[2.2.1]bicycloheptan-2-ylidene)-4-*O*-(bis(2-cyanoethyl)phosphate)-5,6-di-*O*-allyl-*myo*-inositol (–)-227 – ^{13}C NMR spectrum



(–)-1D-2,3-*O*-(D-1,7,7-Trimethyl[2.2.1]bicyclohept-2-ylidene)-4-*O*-(bis(2-cyanoethyl)phosphate)-5,6-di-*O*-allyl-*myo*-inositol (–)-227 – ^{31}P NMR spectrum

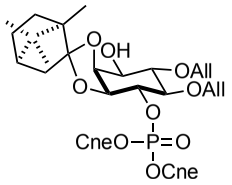
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Current Data Parameters
NAME       ajb61p-main f4
EXPNO                   2
PROCNO                  1

F2 - Acquisition Parameters
Date_          20140703
Time              18.35
INSTRUM           avb400
PROBHD   5 mm PABBO BB/
PULPROG           zgpg30
TD                65536
SOLVENT           CDCl3
NS                  100
DS                    4
SWH           64102.562 Hz
FIDRES        0.978127 Hz
AQ            0.5111808 sec
RG               197.74
DW               7.800 usec
DE               6.50 usec
TE               298.0 K
D1            2.00000000 sec
D11           0.03000000 sec
TD0                   1

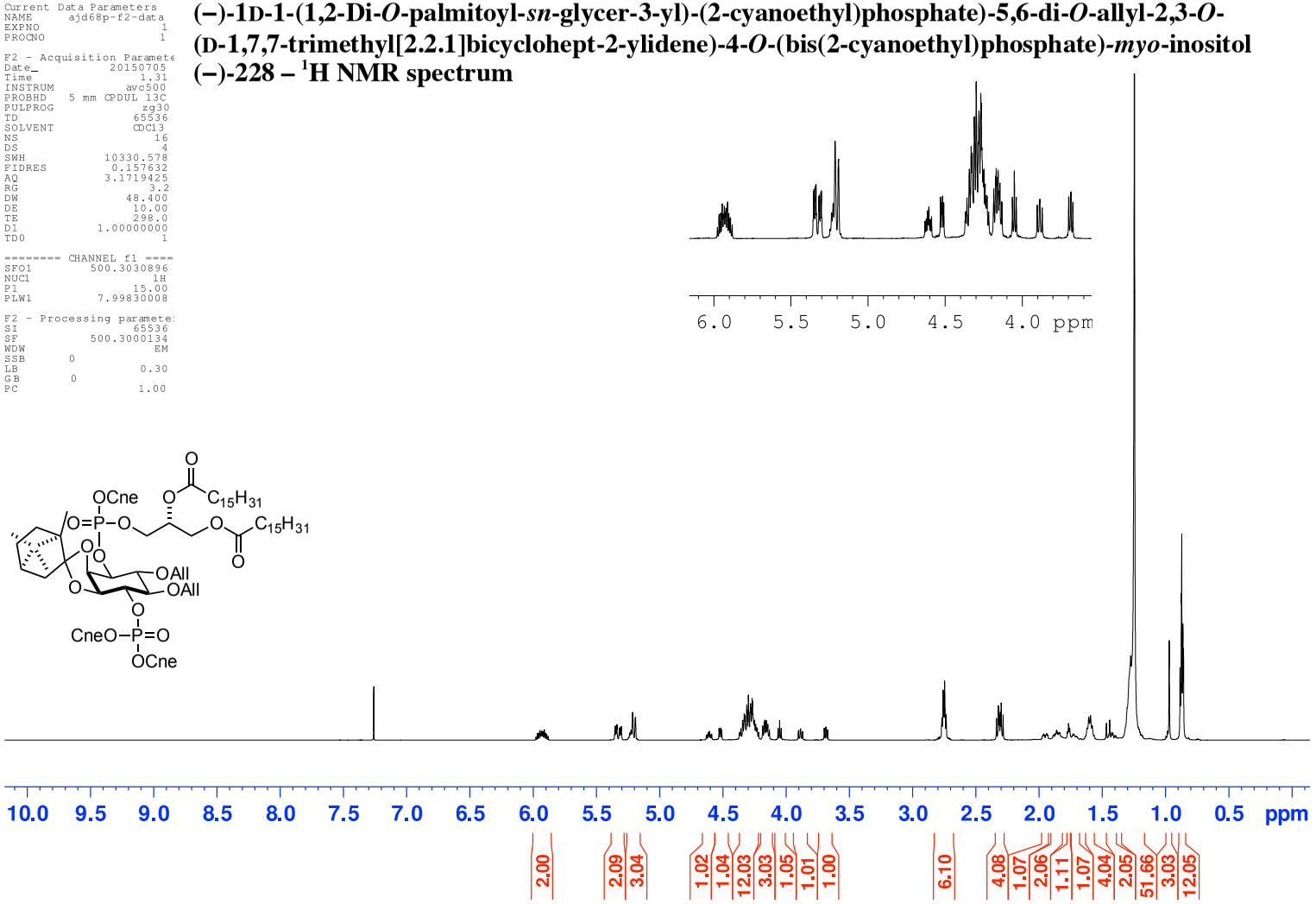
======== CHANNEL f1 ========
SFO1         161.9755930 MHz
NUC1                 31P
P1                8.00 usec
PLW1         54.00000000 W

======== CHANNEL f2 ========
SFO2         400.1316005 MHz
NUC2                 1H
CPDPRG[2          waltz16
PCPD2             70.00 usec
PLW2         14.58800030 W
PLW12         0.29771000 W
PLW13         0.14588000 W

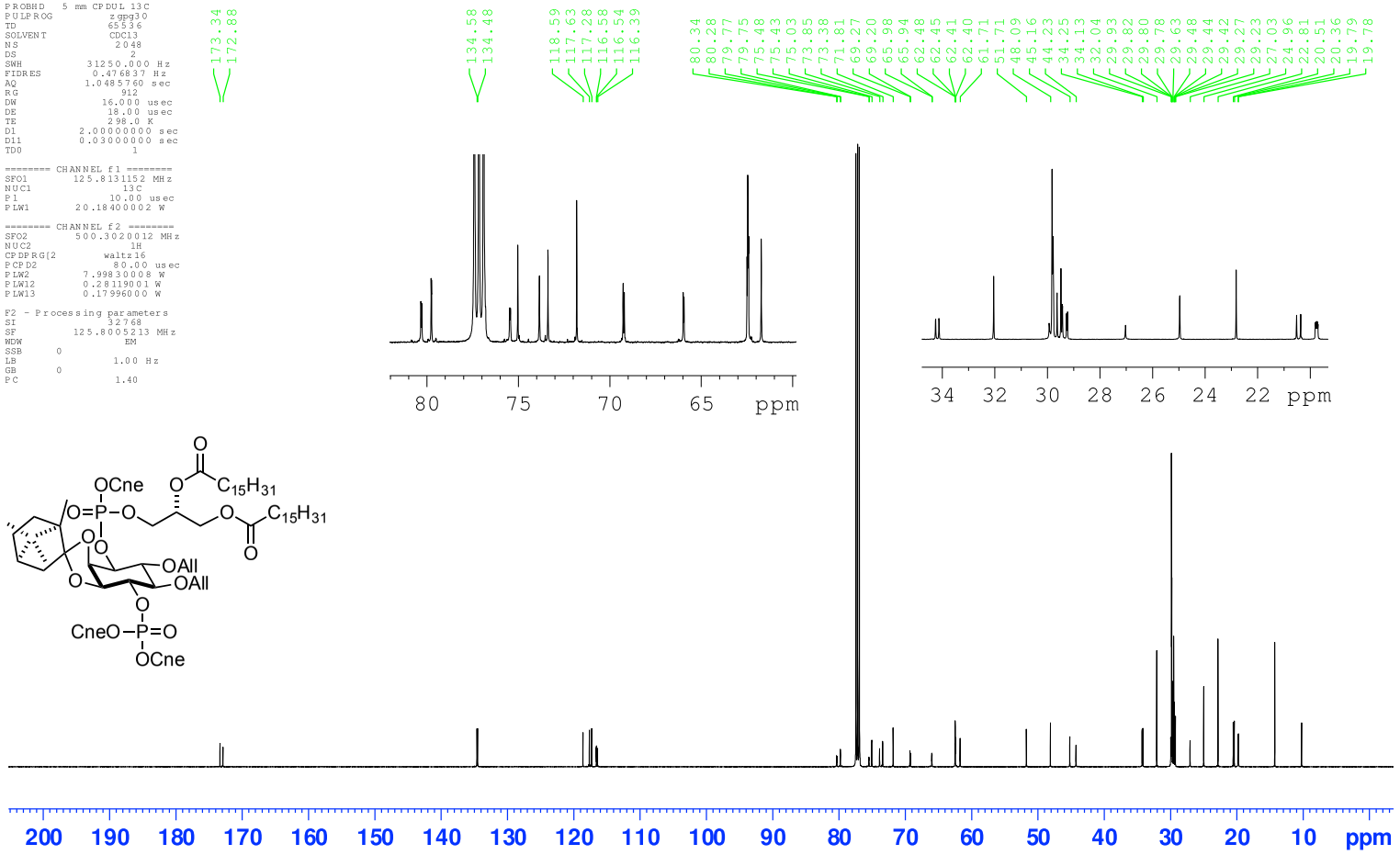
F2 - Processing parameters
SI                  32768
SF           161.9755930 MHz
WDW                    EM
SSB      0
LB                 1.00 Hz
GB       0
PC                 1.40
```



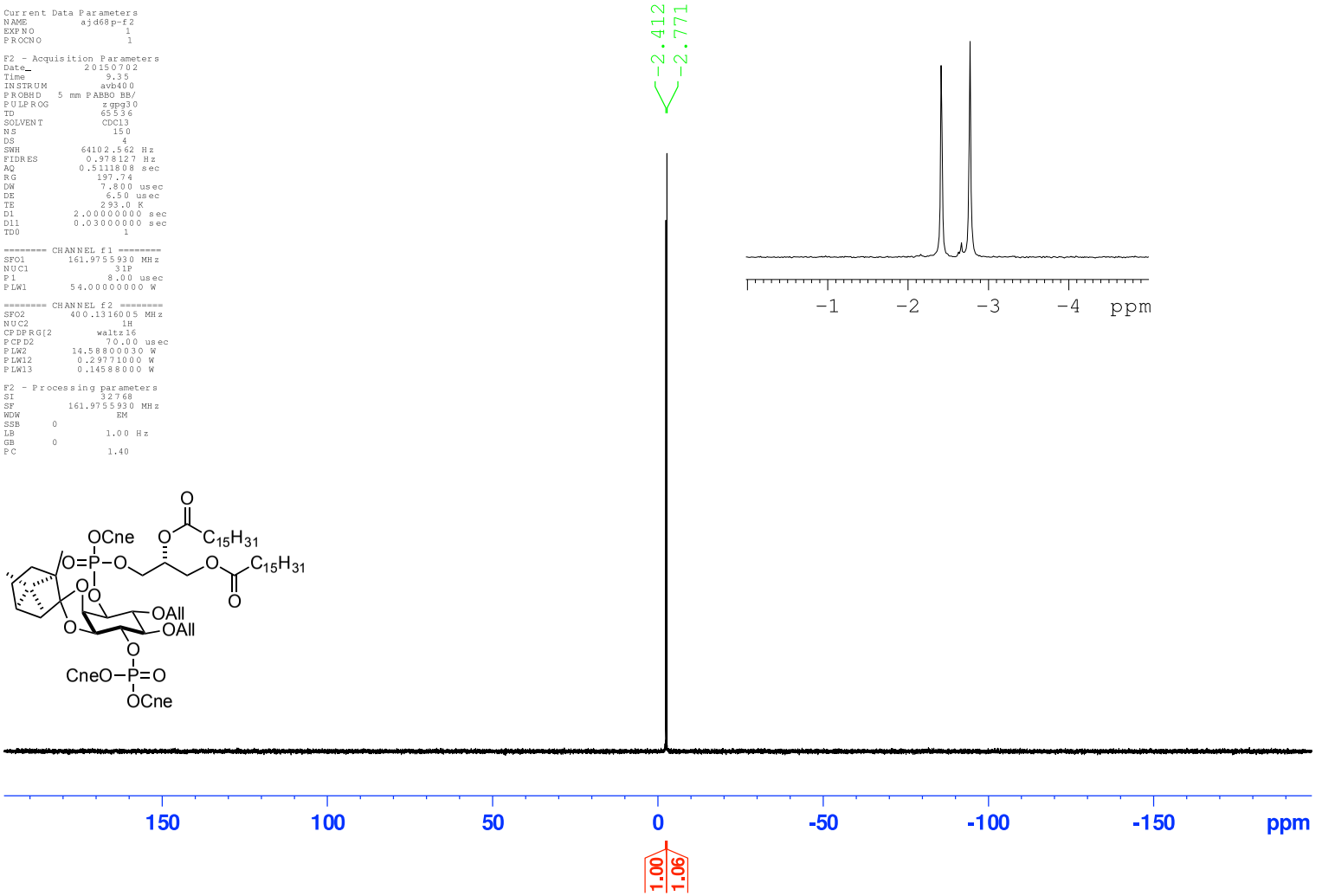
(–)-1D-1-(1,2-Di-*O*-palmitoyl-*sn*-glycer-3-yl)-(2-cyanoethyl)phosphate)-5,6-di-*O*-allyl-2,3-*O*-(D-1,7,7-trimethyl[2.2.1]bicyclohept-2-ylidene)-4-*O*-(bis(2-cyanoethyl)phosphate)-*myo*-inositol (–)-228 – ^{1}H NMR spectrum



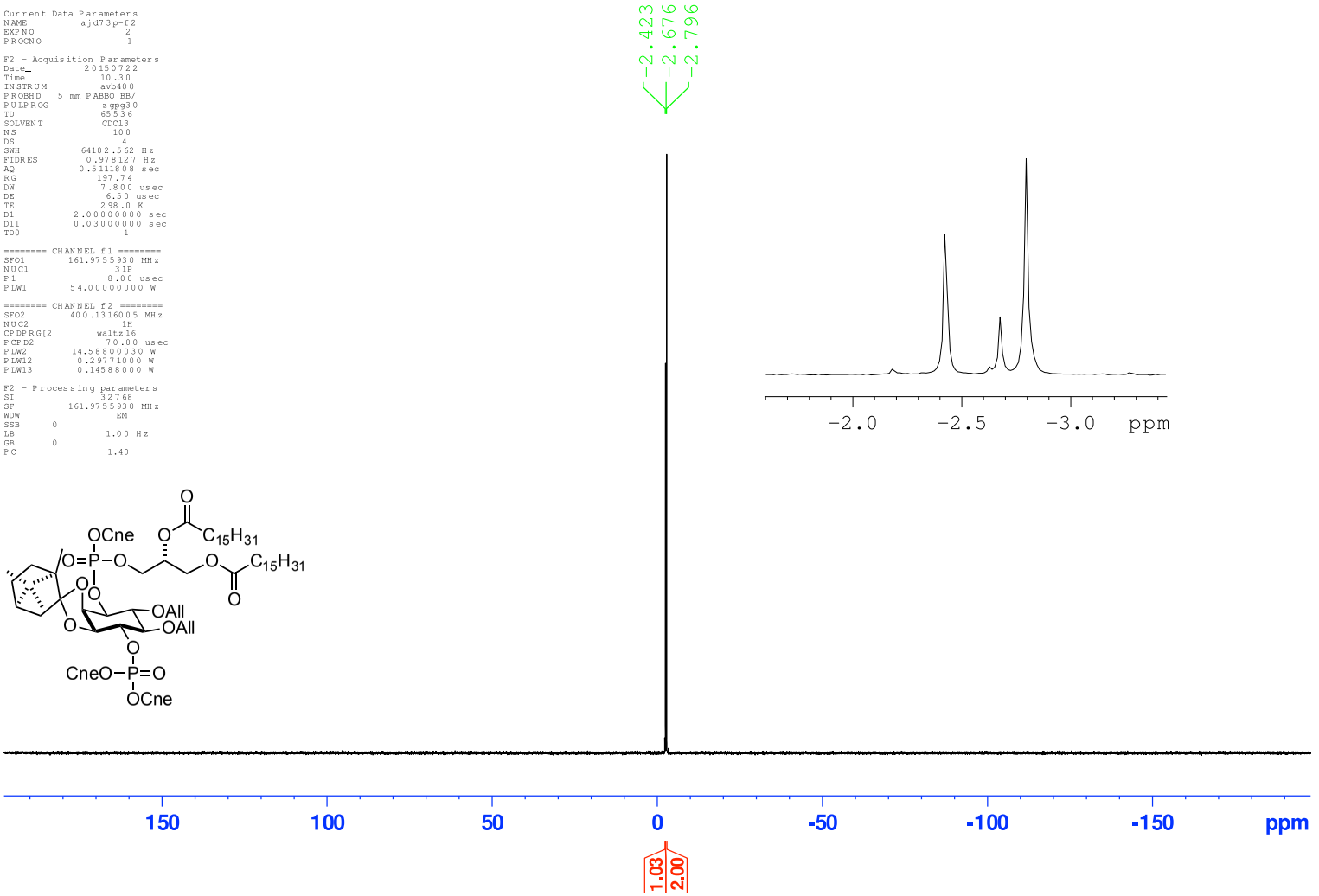
(–)-1D-1-*O*-(1,2-Di-*O*-palmitoyl-*sn*-glycer-3-yl)-(2-cyanoethyl)phosphate)-5,6-di-*O*-allyl-2,3-*O*-(D-1,7,7-trimethyl[2.2.1]bicyclohept-2-ylidene)-4-*O*-(bis(2-cyanoethyl)phosphate)-*myo*-inositol (–)-228 – ^{13}C NMR spectrum



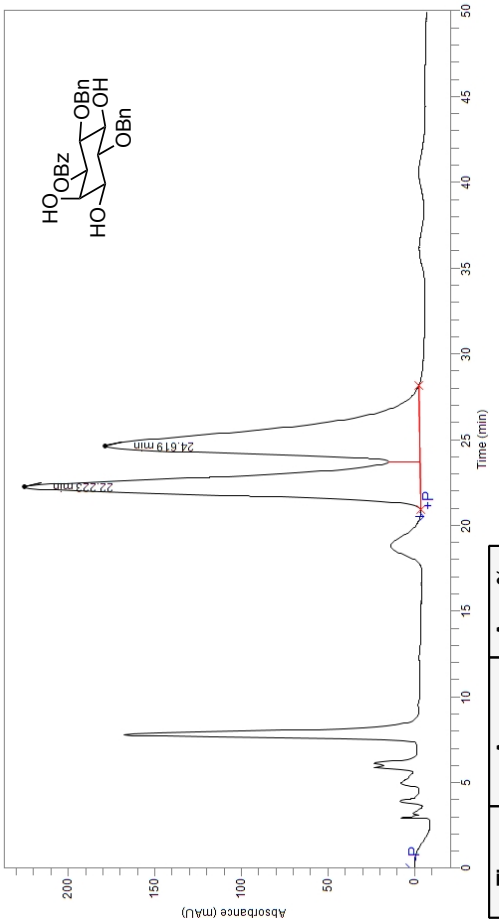
(–)-1D-1-*O*-(1,2-Di-*O*-palmitoyl-*sn*-glycer-3-yl)-(2-cyanoethyl)phosphate)-5,6-di-*O*-allyl-2,3-*O*-(D-1,7,7-trimethyl [2.2.1]bicyclohept-2-ylidene)-4-*O*-(bis(2-cyanoethyl)phosphate)-*myo*-inositol (–)-228 – ^{31}P NMR spectrum



(–)-1D-1-*O*-(1,2-Di-*O*-palmitoyl-*sn*-glycer-3-yl-(2-cyanoethyl)phosphate)-5,6-di-*O*-allyl-2,3-*O*-(D-1,7,7-trimethyl [2.2.1]bicyclohept-2-ylidene)-4-*O*-(bis(2-cyanoethyl)phosphate)-*myo*-inositol (–)-228 – ^{31}P NMR spectrum

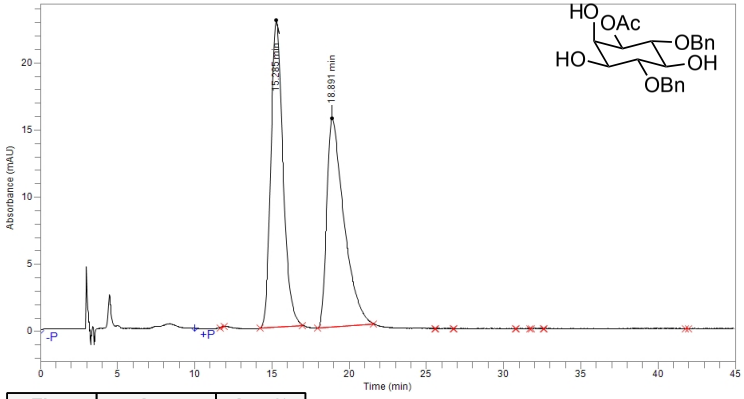


(±)-1-*O*-Benzoyl-4,6-di-*O*-benzyl-*myo*-inositol (±)-156 – Chiral HPLC (Heptane/IPA 85:15, 1.0 mL.min^{-1}, 220 nm)



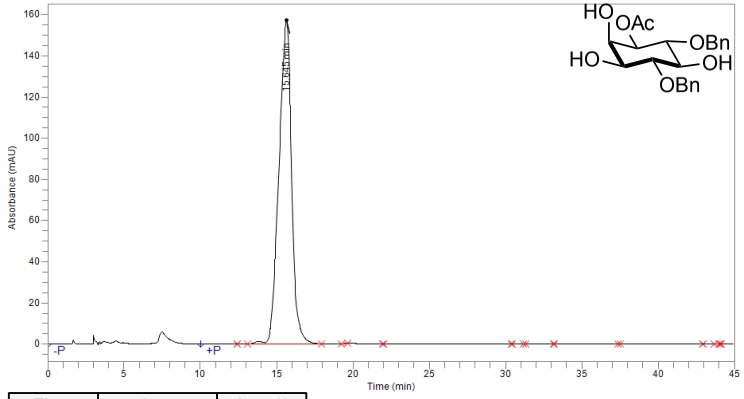
Time	Area	Area %
22.223	16,154,197	48.01
24.619	17,495,981	51.99
Total	33,650,178	100.00

(±)-1D-1-*O*-Acetyl-4,6-di-*O*-benzyl-*myo*-inositol (±)-151 – Chiral HPLC (Heptane/IPA 85:15, 1.0 mL.min^{-1}, 254 nm)



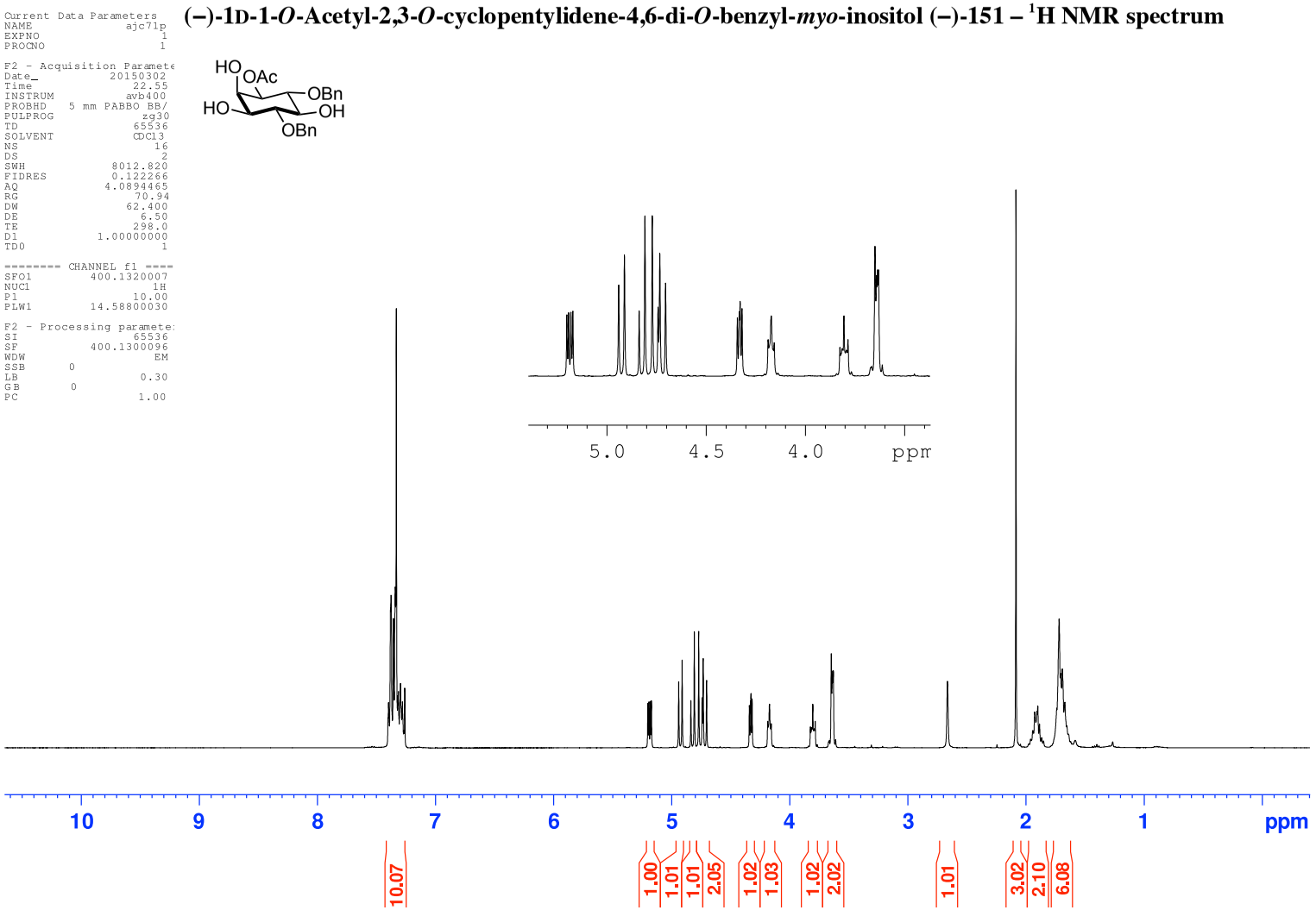
Time	Area	Area %
11.895	372	0.02
15.285	1,253,208	50.90
18.891	1,208,110	49.07
25.571	28	0.00
26.768	33	0.00
30.783	39	0.00
31.730	59	0.00
32.605	28	0.00
41.809	162	0.01
Total	2,462,039	100.00

(–)-1D-1-*O*-Acetyl-4,6-di-*O*-benzyl-*myo*-inositol (–)-151 – Chiral HPLC (Heptane/IPA 85:15, 1.0 mL.min^{-1}, 254 nm)

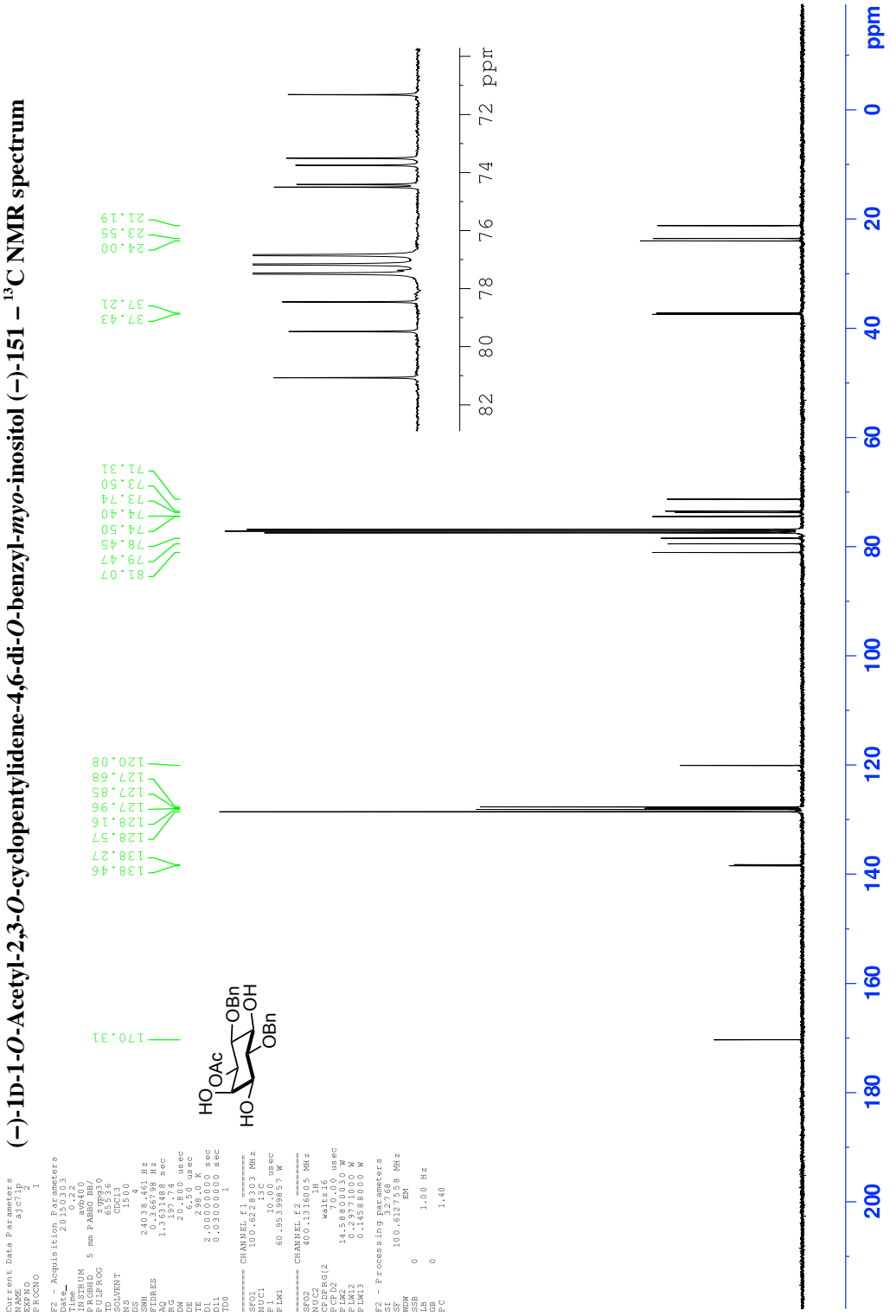


Time	Area	Area %
12.421	34	0.00
13.812	48,653	0.54
15.645	9,026,166	99.45
19.634	425	0.00
21.959	26	0.00
30.416	47	0.00
31.206	126	0.00
33.152	42	0.00
37.415	137	0.00
42.948	28	0.00
43.951	249	0.00
44.147	76	0.00
Total	9,076,008	100.00

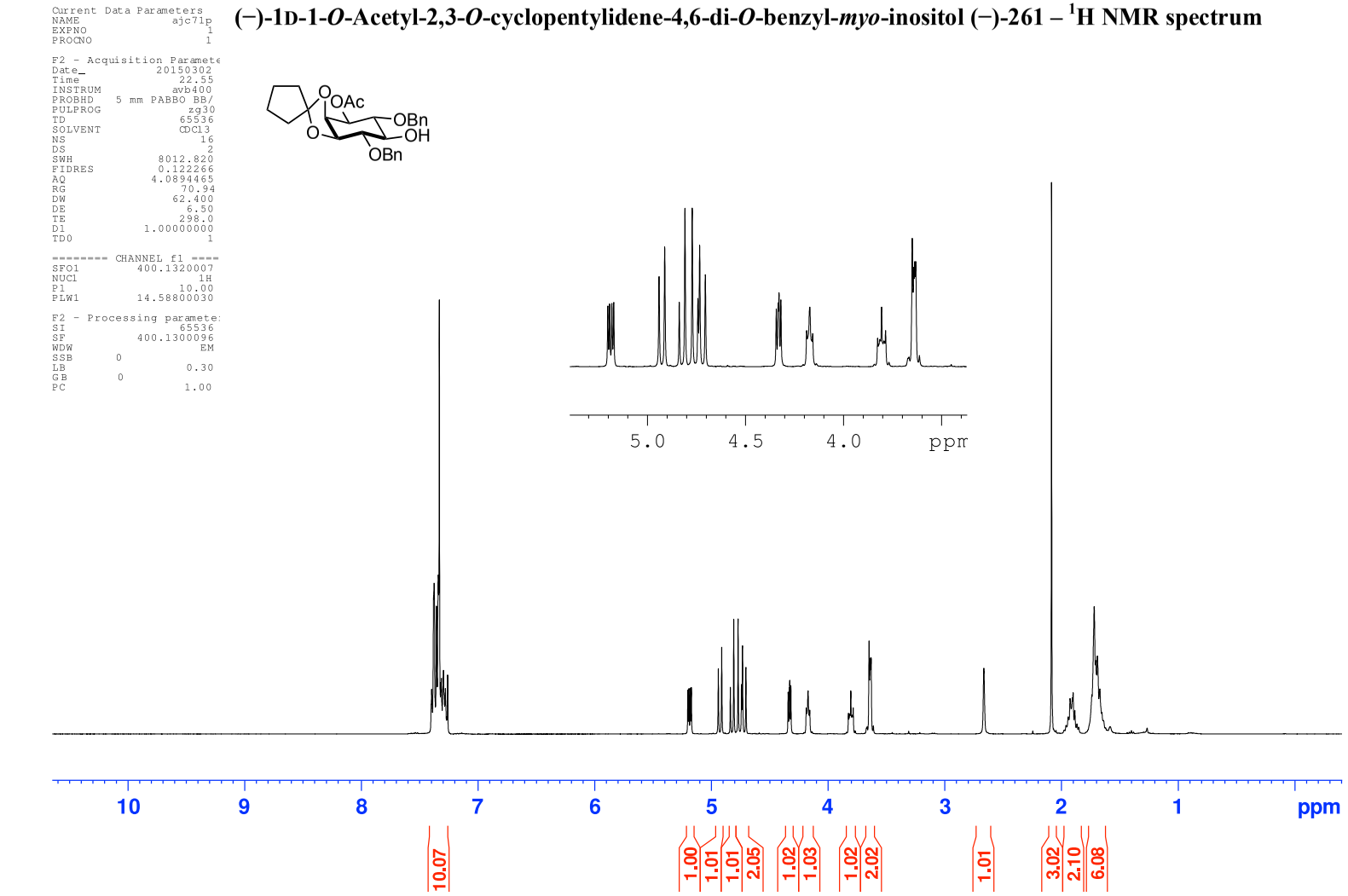
(–)-1D-1-*O*-Acetyl-2,3-*O*-cyclopentylidene-4,6-di-*O*-benzyl-*myo*-inositol (–)-151 – ^{1}H NMR spectrum



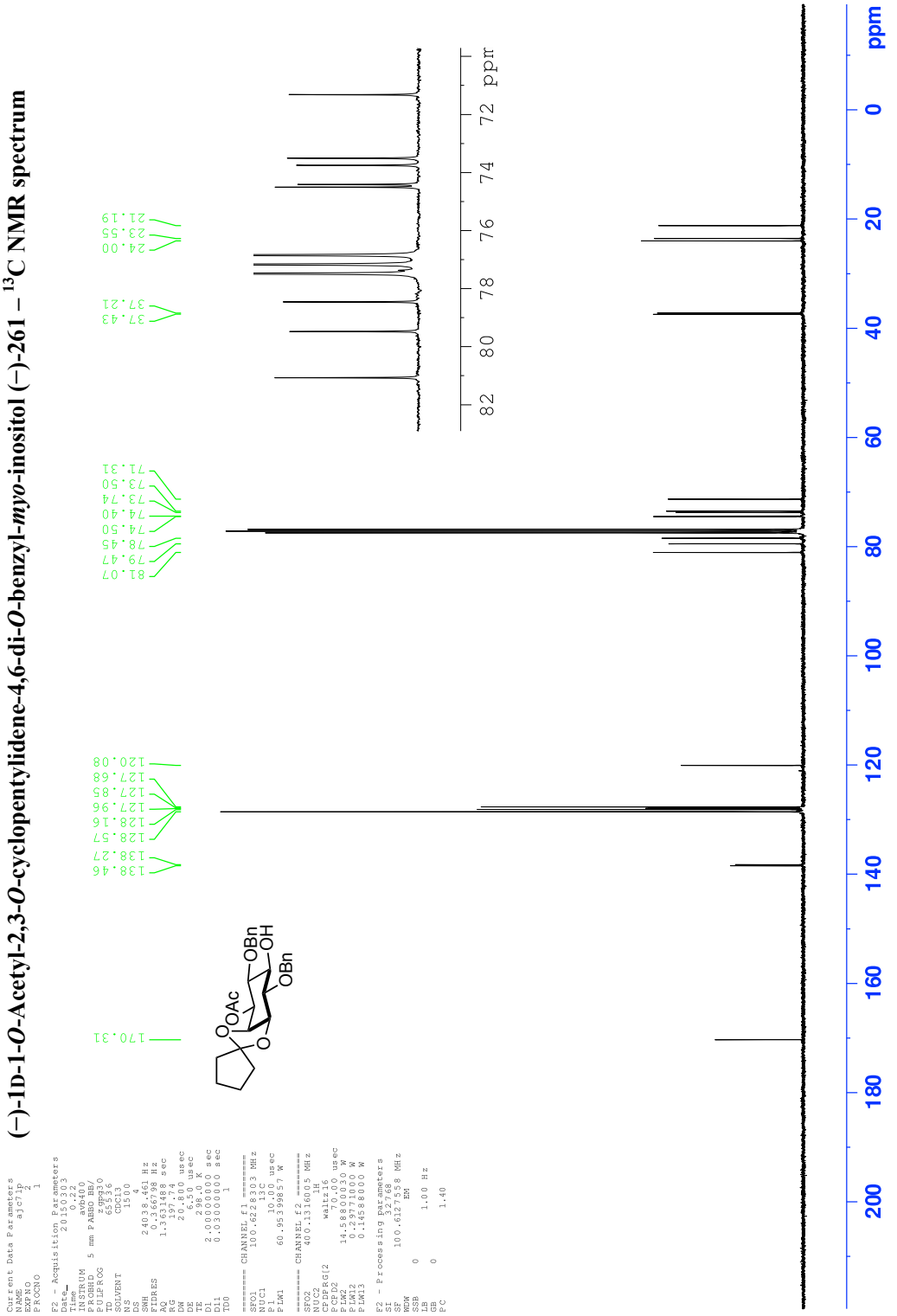
(–)-1D-1-*O*-Acetyl-2,3-*O*-cyclopentylidene-4,6-di-*O*-benzyl-*myo*-inositol (–)-151 – ^{13}C NMR spectrum



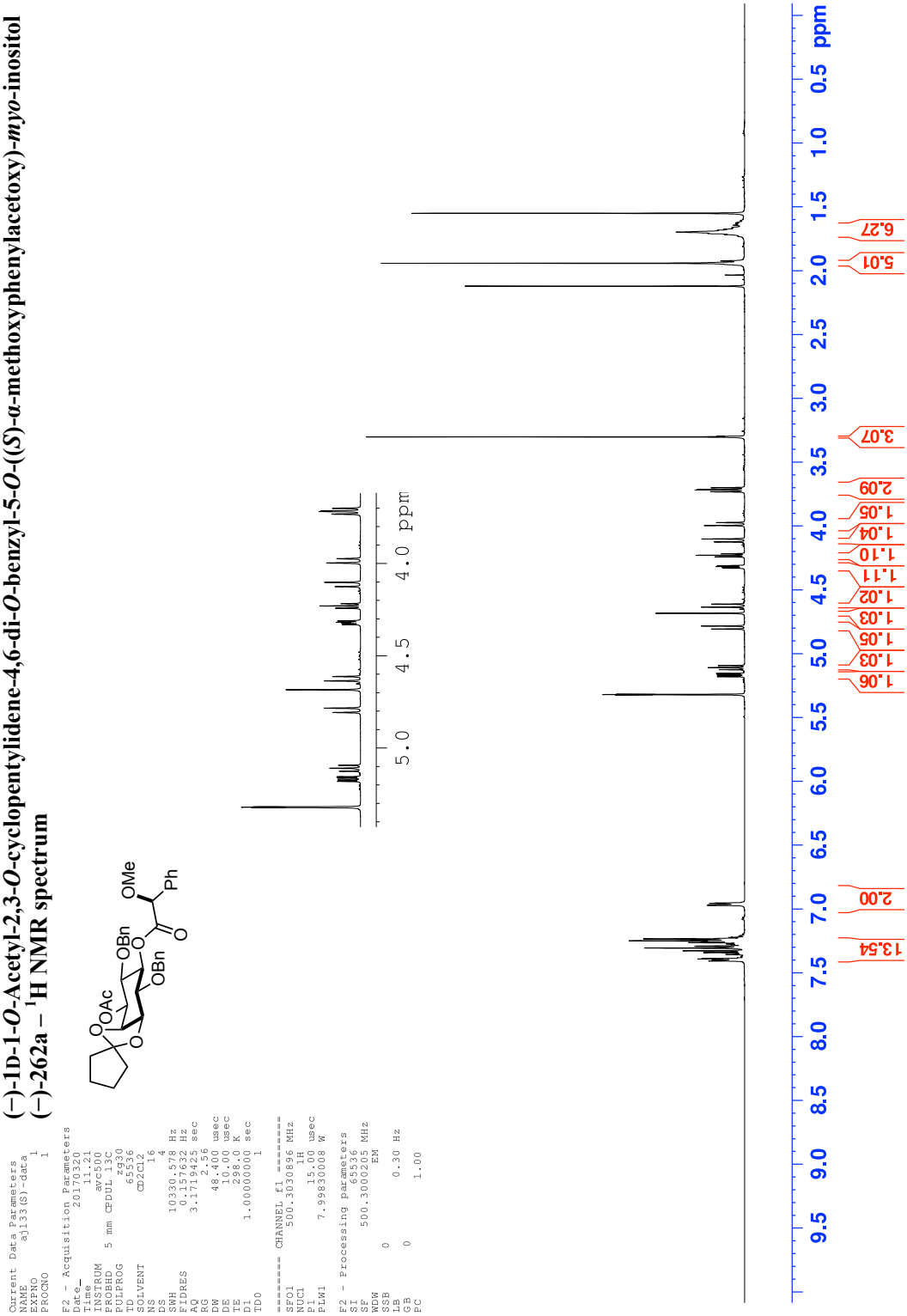
(−)-1D-1-*O*-Acetyl-2,3-*O*-cyclopentylidene-4,6-di-*O*-benzyl-*myo*-inositol (−)-261 – ^{1}H NMR spectrum



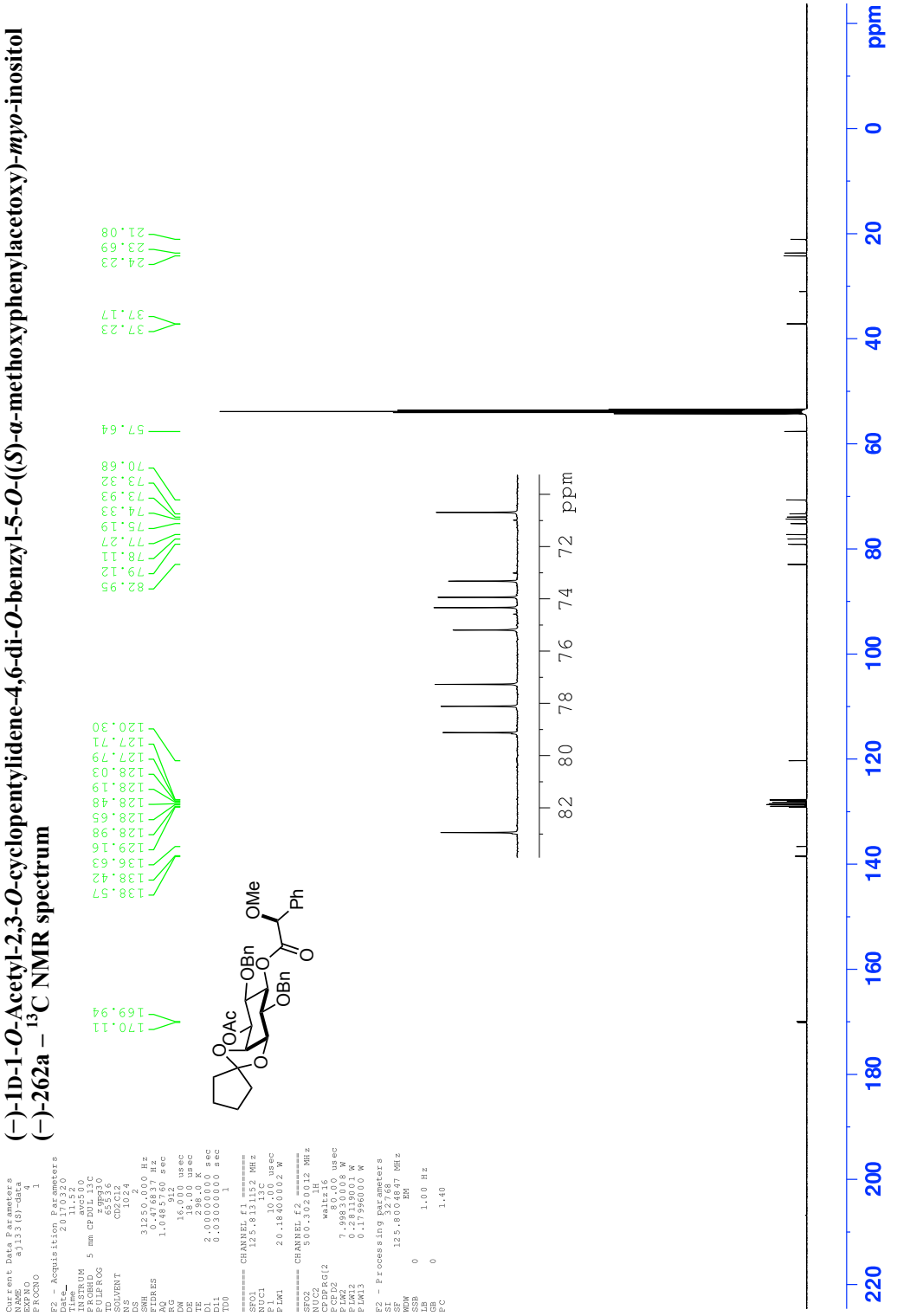
(−)-1D-1-*O*-Acetyl-2,3-*O*-cyclopentylidene-4,6-di-*O*-benzyl-*myo*-inositol (−)-261 – ^{13}C NMR spectrum



(−)-1D-1-*O*-Acetyl-2,3-*O*-cyclopentylidene-4,6-di-*O*-benzyl-5-*O*-((*S*)-α-methoxyphenylacetoxy)-*myo*-inositol
(−)-262a – ^{1}H NMR spectrum



(−)-1D-1-*O*-Acetyl-2,3-*O*-cyclopentylidene-4,6-di-*O*-benzyl-5-*O*-((*S*)-α-methoxyphenylacetoxy)-*myo*-inositol
(−)-262a – ^{13}C NMR spectrum



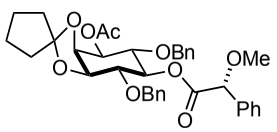
(−)-1D-1-*O*-Acetyl-2,3-*O*-cyclopentylidene-4,6-di-*O*-benzyl-5-*O*-((*R*)-α-methoxyphenylacetoxy)-*myo*-inositol
(−)-262b – ^{1}H NMR spectrum

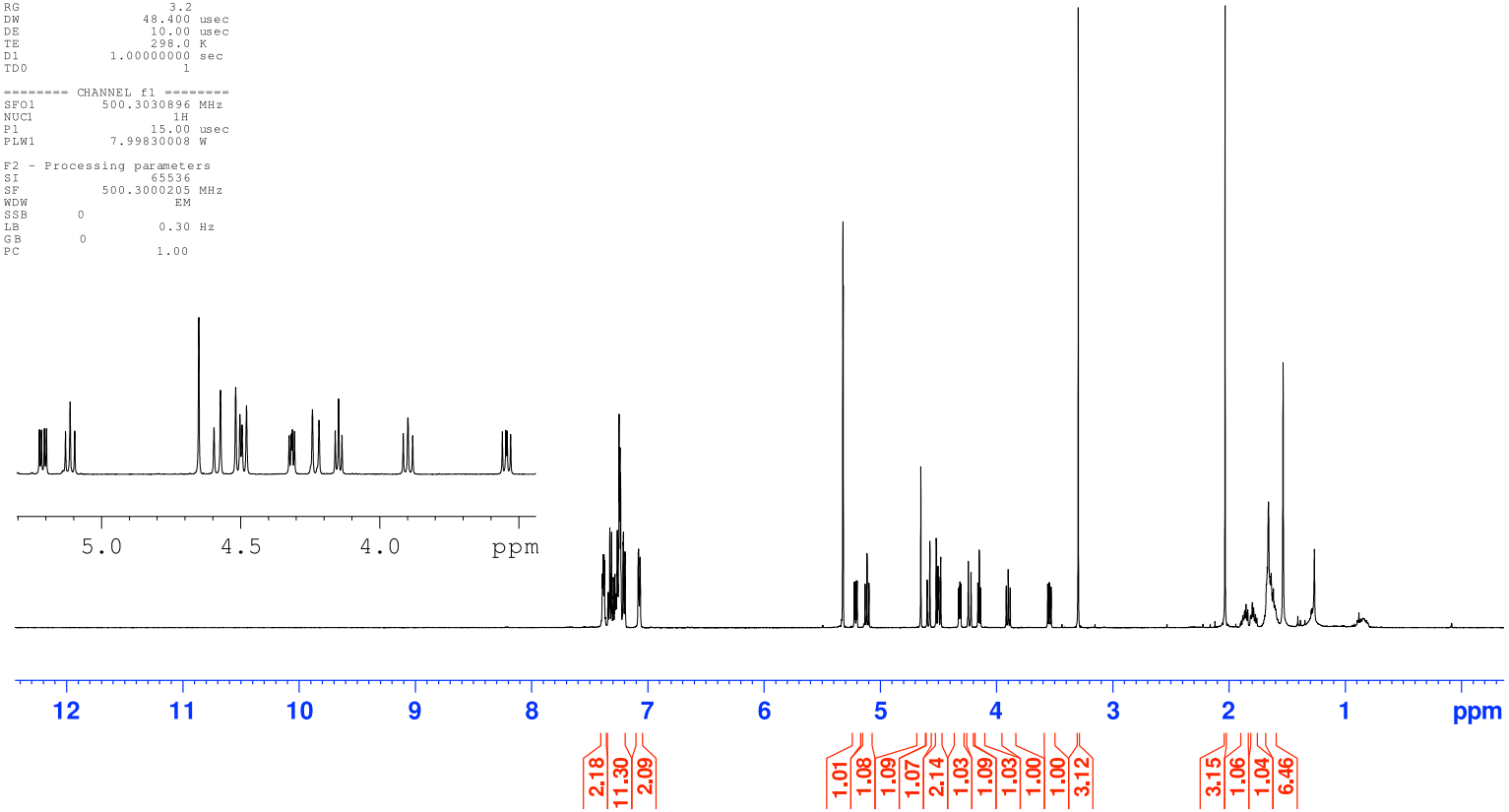
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Current Data Parameters
NAME      ajf70p(R)-data
EXPNO                  1
PROCNO                 1

F2 - Acquisition Parameters
Date_           20160930
Time              19.28
INSTRUM           avc500
PROBHD   5 mm CPDUL 13C
PULPROG             zg30
TD                65536
SOLVENT           CD2Cl2
NS                    16
DS                     4
SWH           10330.578 Hz
FIDRES         0.157632 Hz
AQ            3.1719425 sec
RG                  3.2
DW                48.400 usec
DE                10.00 usec
TE                298.0 K
D1           1.00000000 sec
TD0                    1

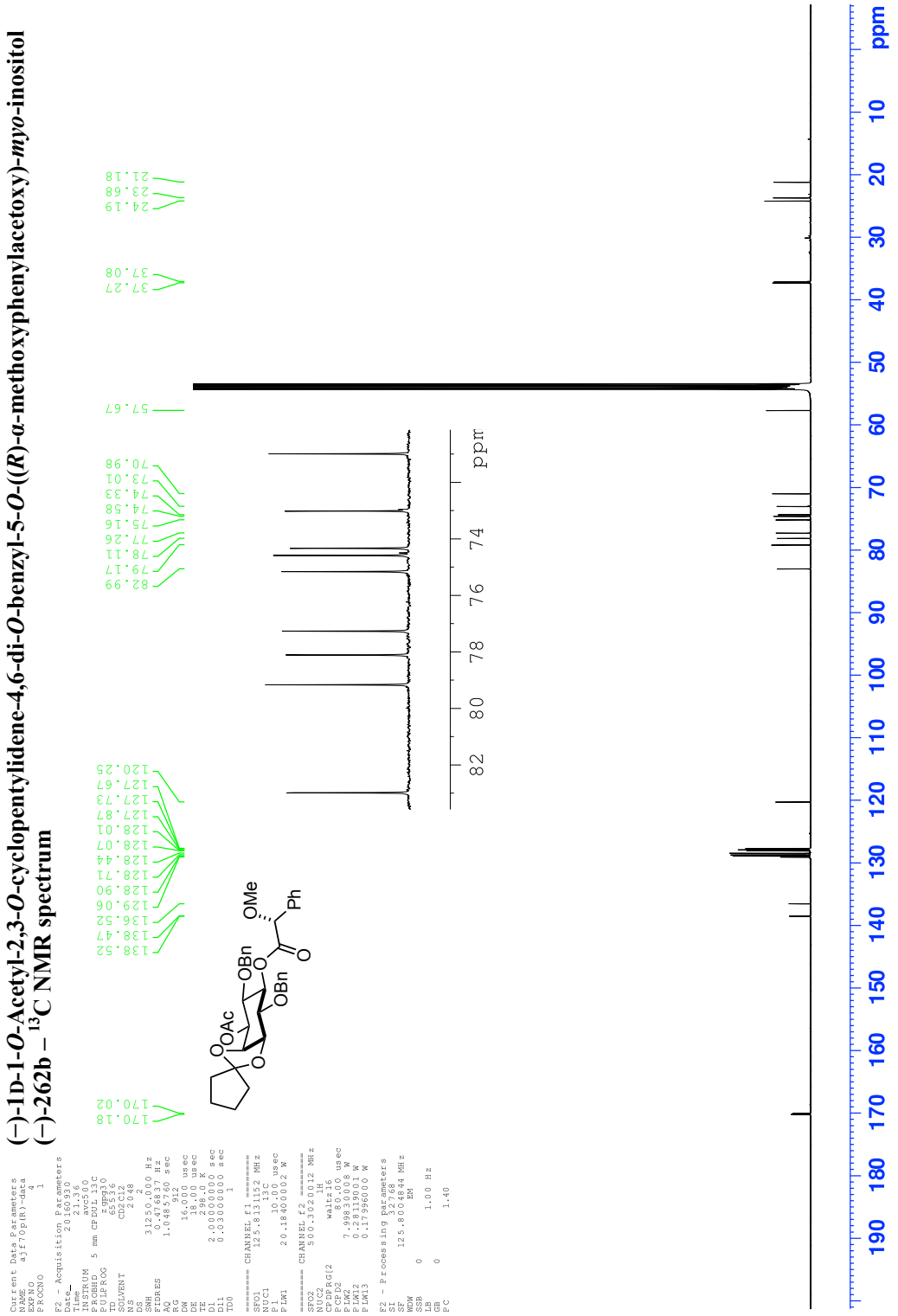
======== CHANNEL f1 ========
SFO1         500.3030896 MHz
NUC1                1H
P1                15.00 usec
PLW1          7.99830008 W

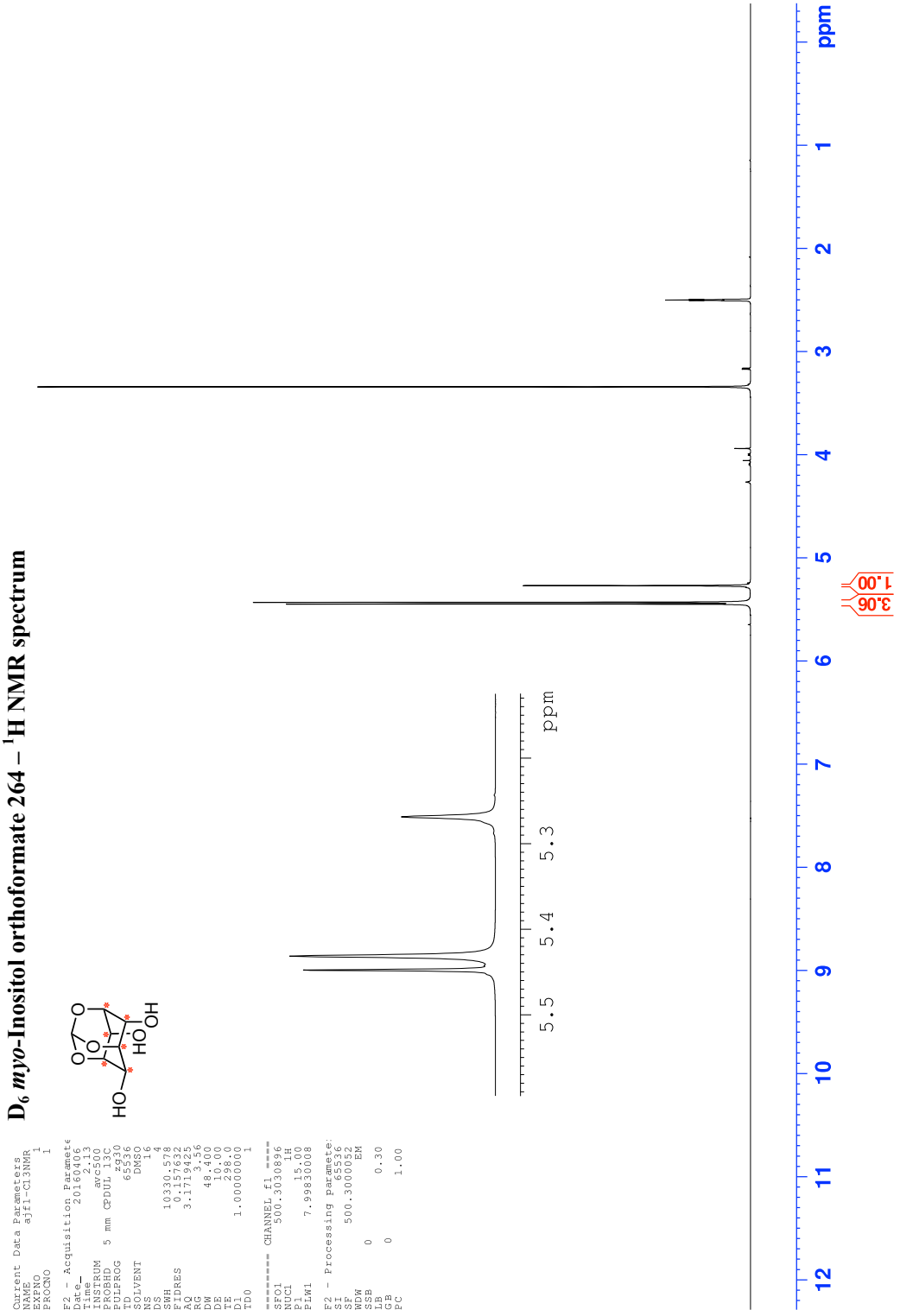
F2 - Processing parameters
SI                 65536
SF           500.3000205 MHz
WDW                EM
SSB       0
LB                0.30 Hz
GB        0
PC                1.00
```



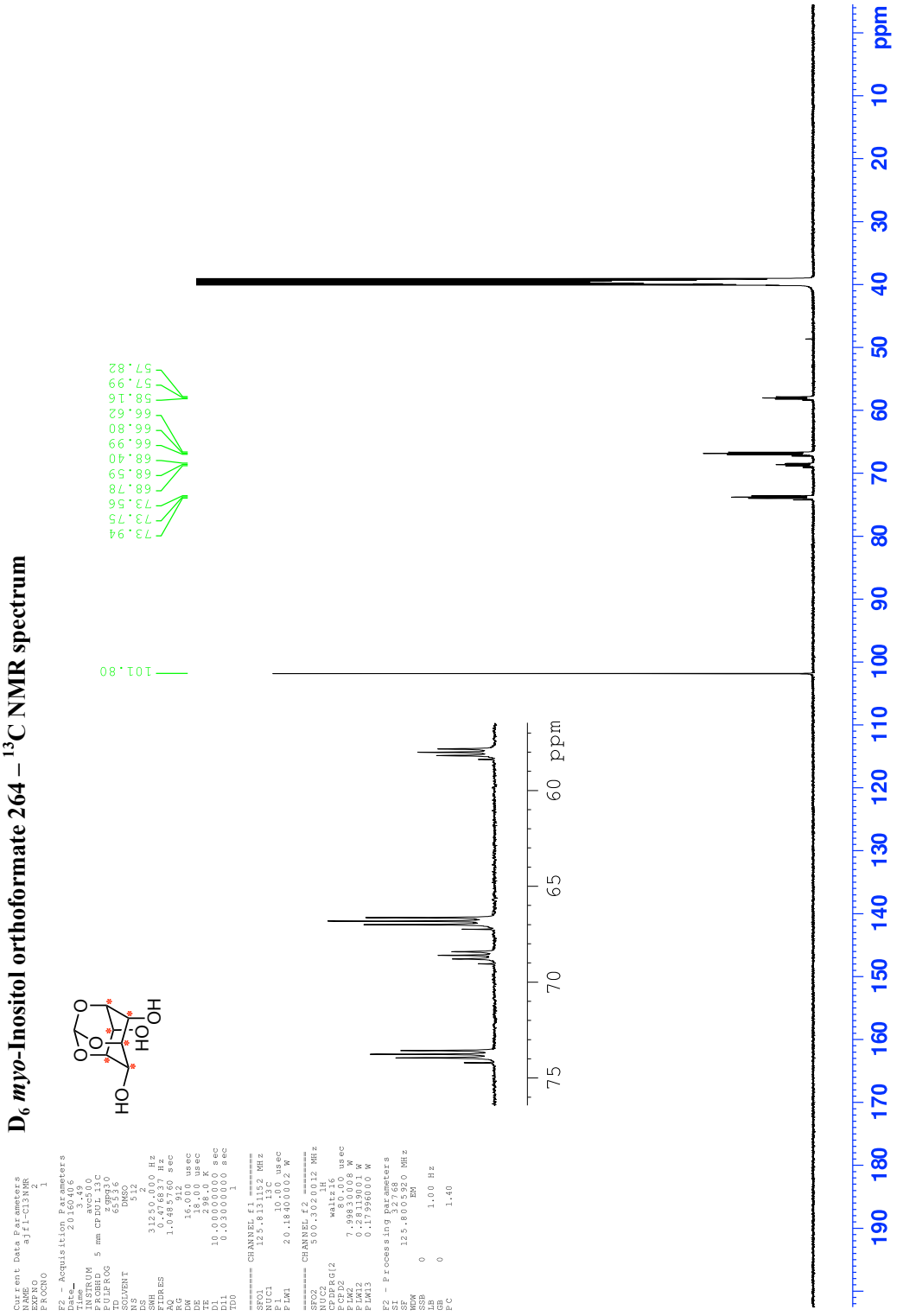


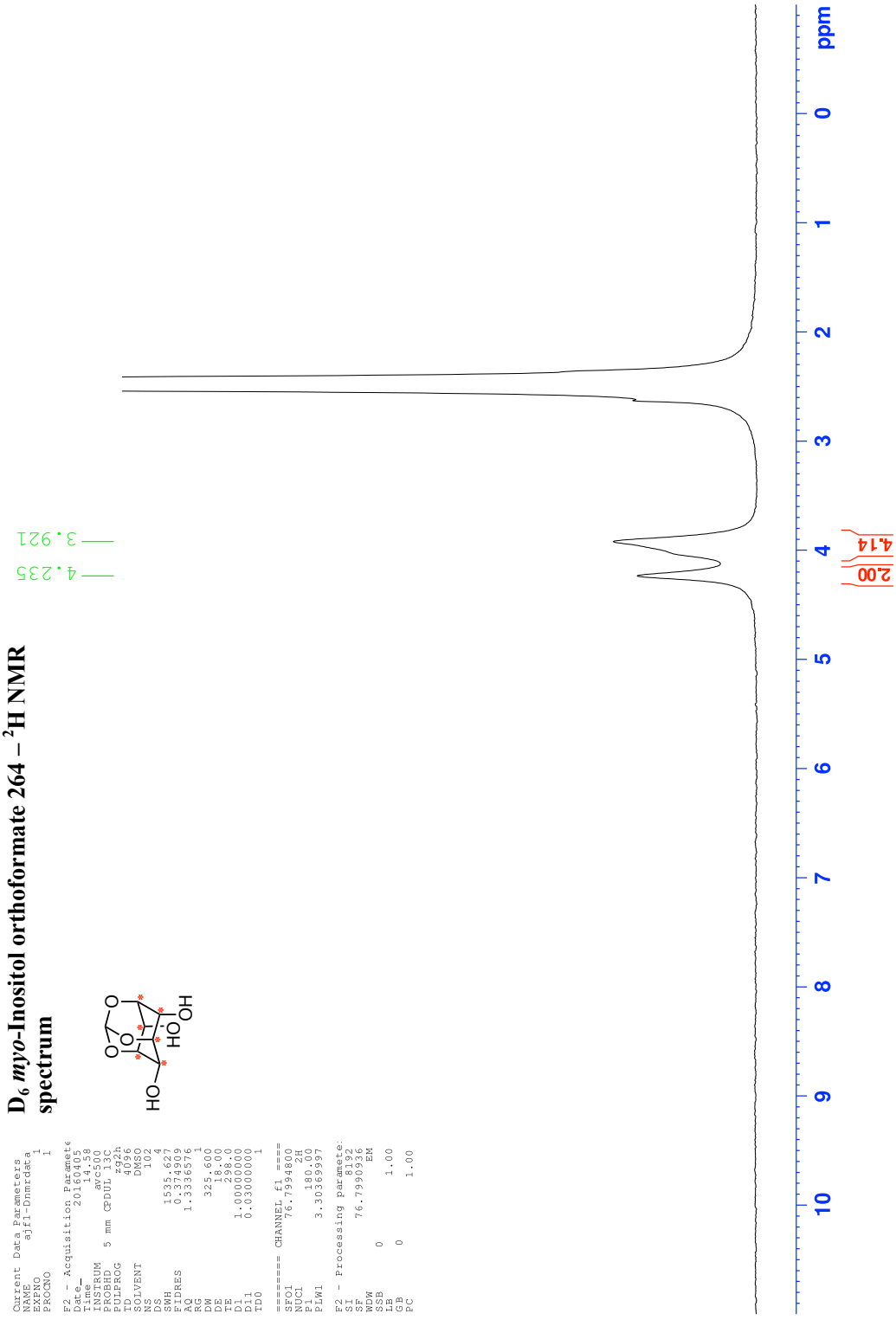
(−)-1D-1-*O*-Acetyl-2,3-*O*-cyclopentylidene-4,6-di-*O*-benzyl-5-*O*-((*R*)-α-methoxyphenylacetoxy)-*myo*-inositol
(−)-262b – ^{13}C NMR spectrum



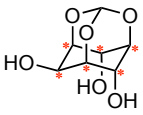

D6 myo-Inositol orthoformate 264 – 1H NMR spectrum
Current Data Parameters
NAME aji1-C13NMR
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20160416
Time 12.13
INSTRUM avc500
PROBHD 5 mm CPDUL 13C
PULPROG zg30
TD 65536
SOLVENT DMSO
NS 4
DS 4
SWH 10330.578
FIDRES 0.157632
AQ 3.1719425
RG 3.55
DW 48.400
DE 10.00
TE 298.0
D1 1.00000000
TD0 1
======== CHANNEL f1 ========
SFO1 500.3030896
NUC1 1H
P1 15.00
PLW1 7.99830008
F2 - Processing parameters
SI 65536
SF 500.3000052
WDW EM
SSB 0
LB 0.30
GB 0
PC 1.00
HO
OH
HO
5.5
5.4
5.3
ppm
3.06
1.00
12
11
10
9
8
7
6
5
4
3
2
1
ppm

D_6 *myo*-Inositol orthoformate 264 – ^{13}C NMR spectrum

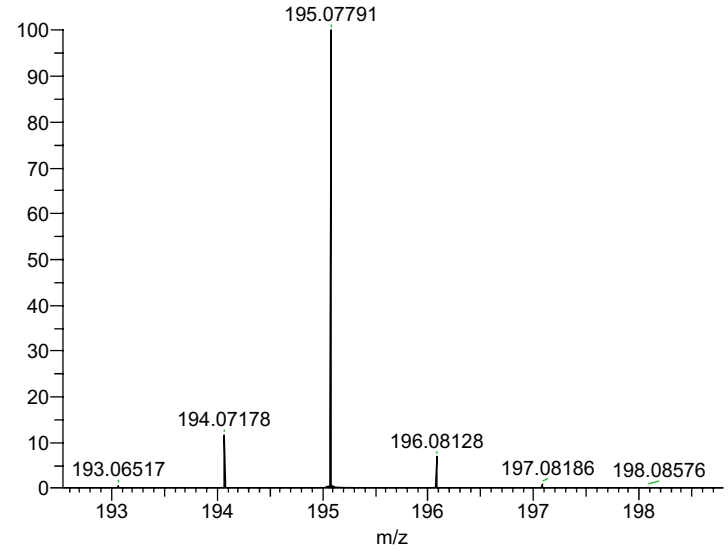



Current Data Parameters
NAME ajf-Dmcdaa
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20140415
Time 14.58
INSTRUM avc500
PROBHD 5 mm CPDUL 13C
PULPROG zg2h
TD 4096
SOLVENT DMSO
NS 4
DS 2
SWH 1535.627
FIDRES 0.374909
AQ 1.3336576
RG 1
DW 325.600
DE 18.00
TE 298.0
D1 1.00000000
D11 0.03000000
TD0 1
======== CHANNEL f1 ========
SFO1 76.7994800
NUC1 2H
P1 180.00
PLW1 3.30369997
F2 - Processing parameters
SI 8192
SF 76.7990936
WDW EM
SSB 0
LB 1.00
GB 0
PC 1.00
D6 myo-Inositol orthoformate 264 – 2H NMR spectrum
HO
OH
HO
4.235
3.921
2.00
4.14
10
9
8
7
6
5
4
3
2
1
0
ppm

D_6 *myo*-Inositol orthoformate 264 – Mass spectrum

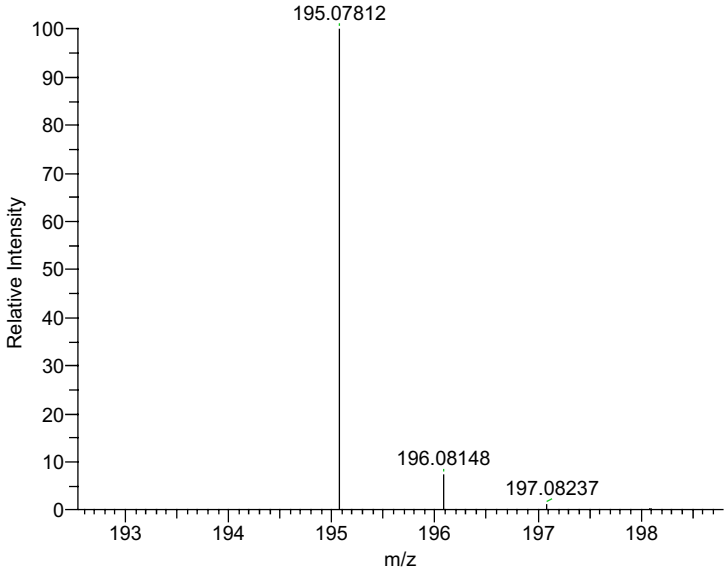


S:\data\June 16\ESI57609.raw 07/06/2016 10:45 am



NL: 4.32E7
ESI57609 #10-25 RT: 0.11-0.27 AV: 8 NL: 4.32E7
T: FTMS {1,2} - p ESI Full ms [80.00-1600.00]

Measured Spectrum

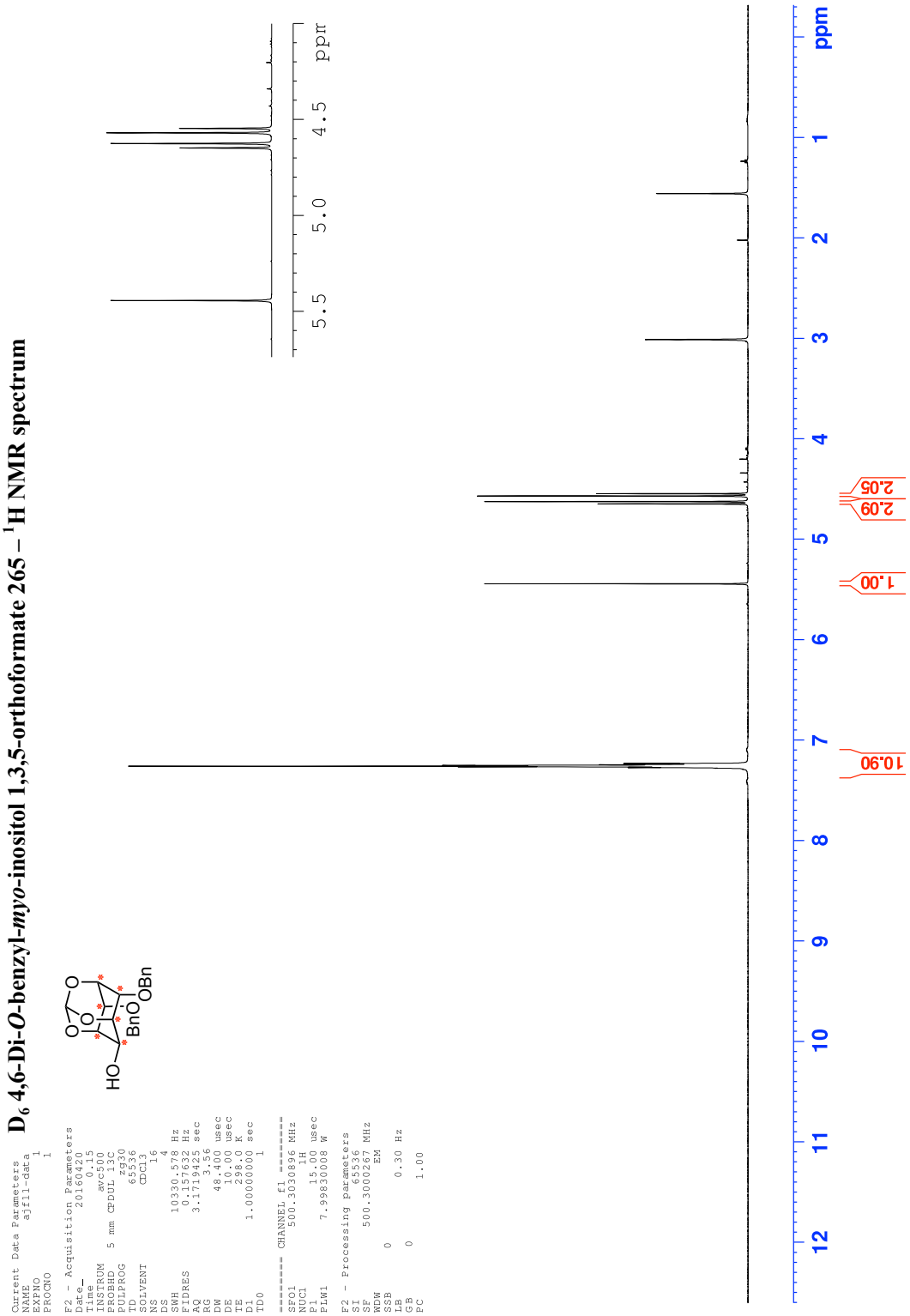


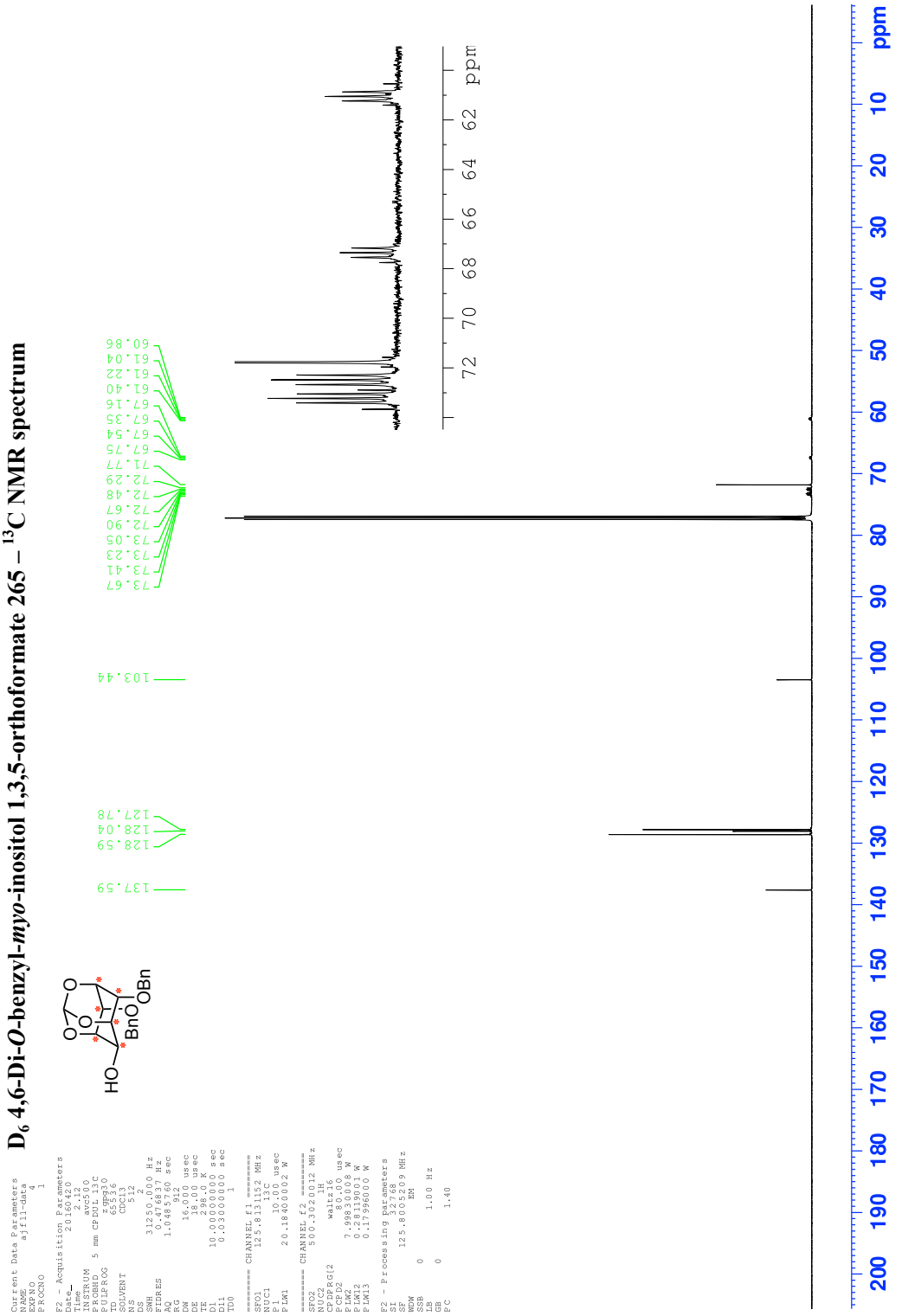
NL: 9.14E5
C7H3[2]H6O6: C_7 H_3 $^2H_6$ O_6 Chrg -1 R: 1000000 Res. Pwr. @FWHM

Theoretical Spectrum

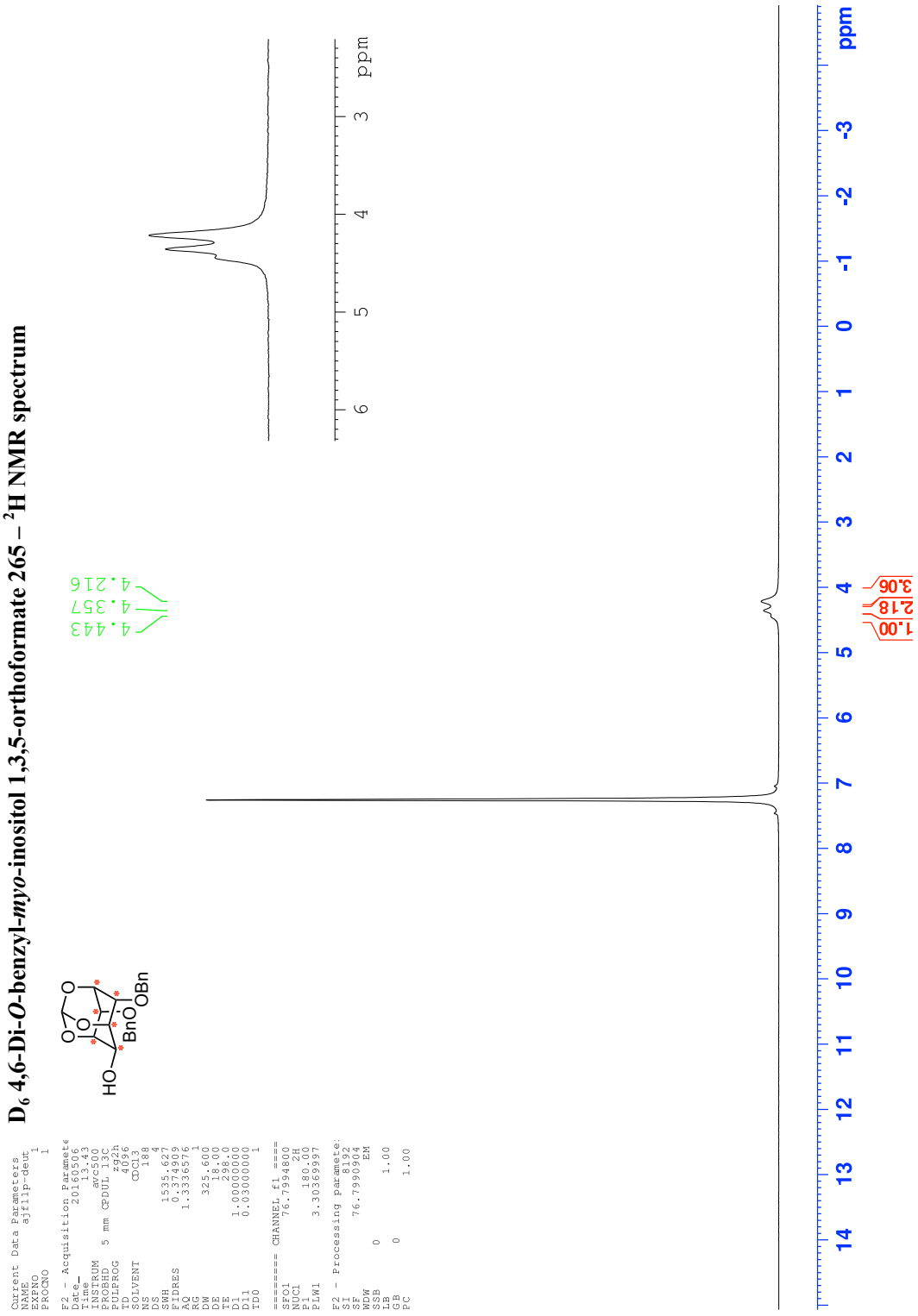
m/z	Formula	RDB	Delta ppm	Theo. Mass
195.07791	$C_7H_3{}^2H_6O_6$	3.5	-1.11	195.07812

D$_6$ 4,6-Di-*O*-benzyl-*myo*-inositol 1,3,5-orthoformate 265 – ^{1}H NMR spectrum

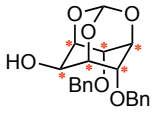



D6 4,6-Di-O-benzyl-myo-inositol 1,3,5-orthoformate 265 – 13C NMR spectrum
Current Data Parameters
NAME ajf11-data 4
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20160420
Time 22.20
INSTRUM avc500
PROBHD 5 mm CPDUL 13C
PULPROG zgpg30
TD 65536
SOLVENT CDCl3
NS 512
DS 4
SWH 31250.000 Hz
FIDRES 0.476837 Hz
AQ 1.0485760 sec
RG 912
DW 16.000 usec
DE 18.00 usec
TE 298.0 K
D1 10.00000000 sec
D11 0.03000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 125.8131152 MHz
NUC1 13C
P1 10.00 usec
PLW1 20.18400002 W
======== CHANNEL f2 ========
SFO2 500.3020012 MHz
NUC2 1H
CPDPRG[2 waltz16
PCPD2 80.00 usec
PLW2 7.99830008 W
PLW12 0.42313001 W
PLW13 0.11796000 W
F2 - Processing parameters
SI 32768
SF 125.8005209 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
103.44
137.59
128.59
128.04
127.78
73.67
73.41
73.23
73.05
72.90
72.67
72.48
72.29
71.77
67.75
67.54
67.35
67.16
61.40
61.22
61.04
60.86
72 70 68 66 64 62 ppm
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 ppm

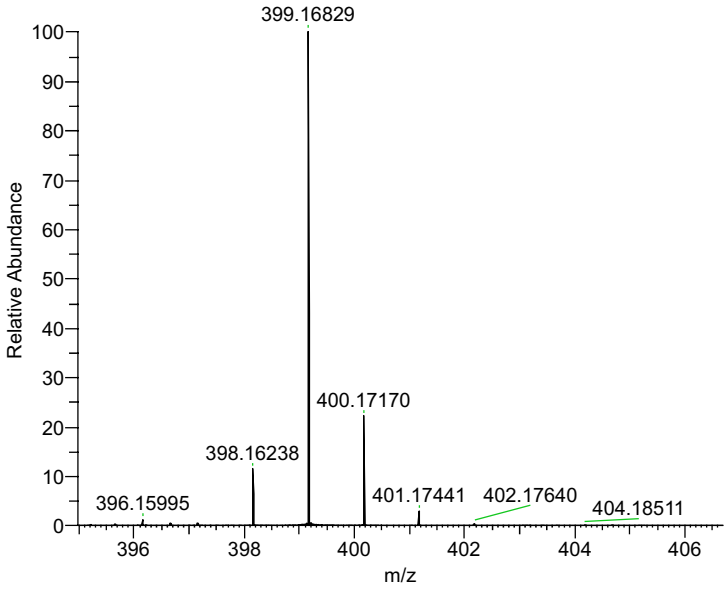
D_6 4,6-Di-*O*-benzyl-*myo*-inositol 1,3,5-orthoformate 265 – ^{2}H NMR spectrum



D_6 4,6-Di-*O*-benzyl-*myo*-inositol 1,3,5-orthoformate 265 – Mass spectrum

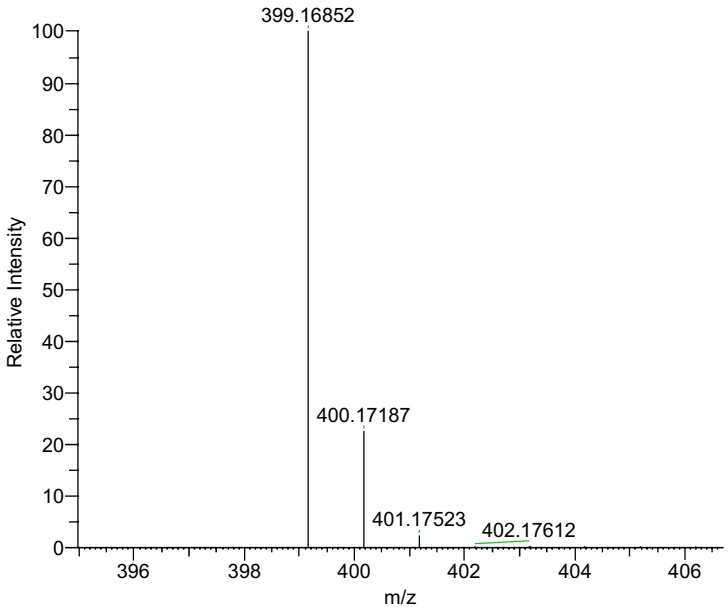


W:\data\May 16\ESI56916.raw 09/05/2016 10:46 am



NL: 1.20E8
ESI56916 #12-26 RT: 0.15-0.28 AV: 7 NL: 1.20E+008
T: FTMS {1,1} + p ESI Full lock ms [80.00-1600.00]

Measured Spectrum

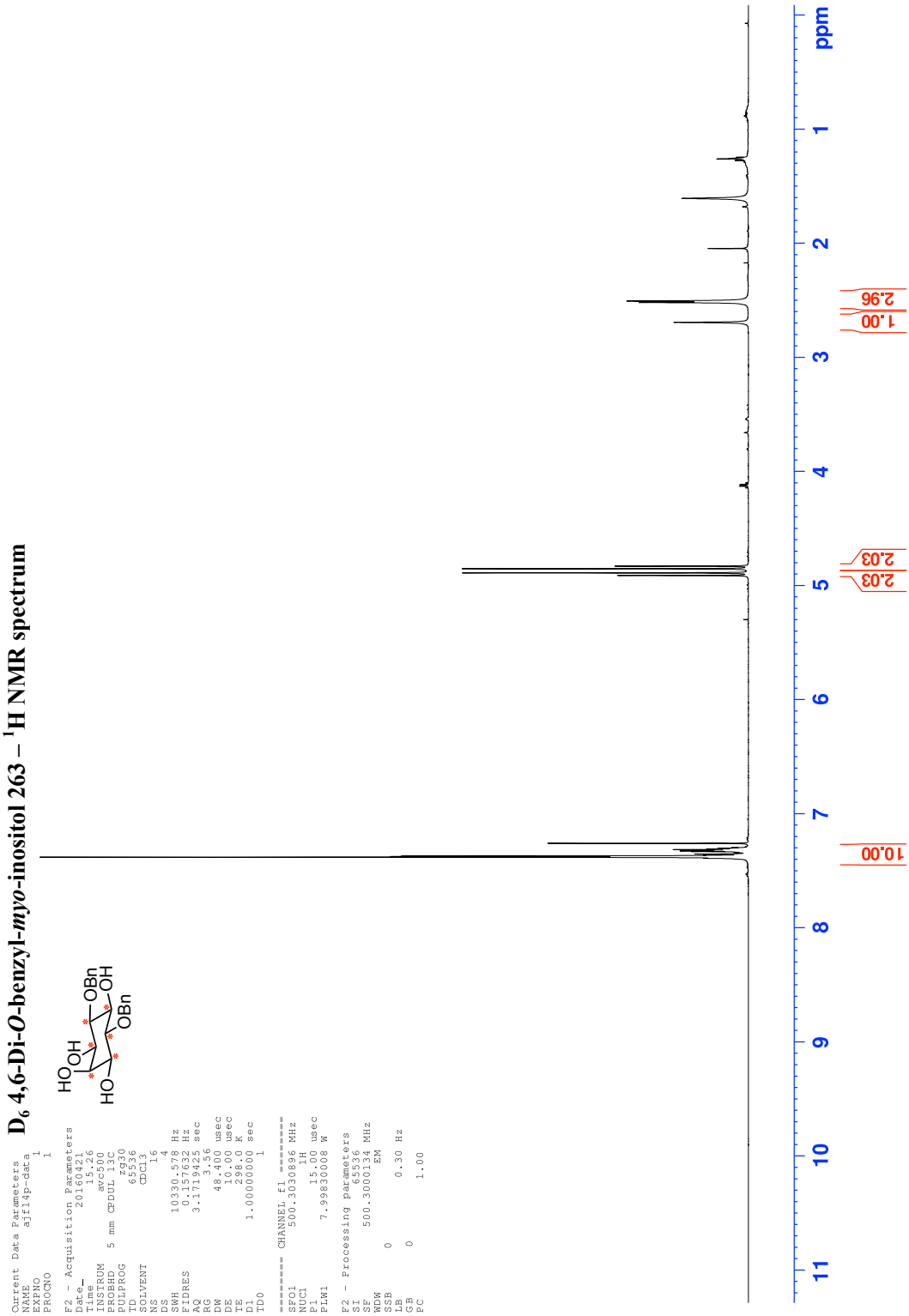


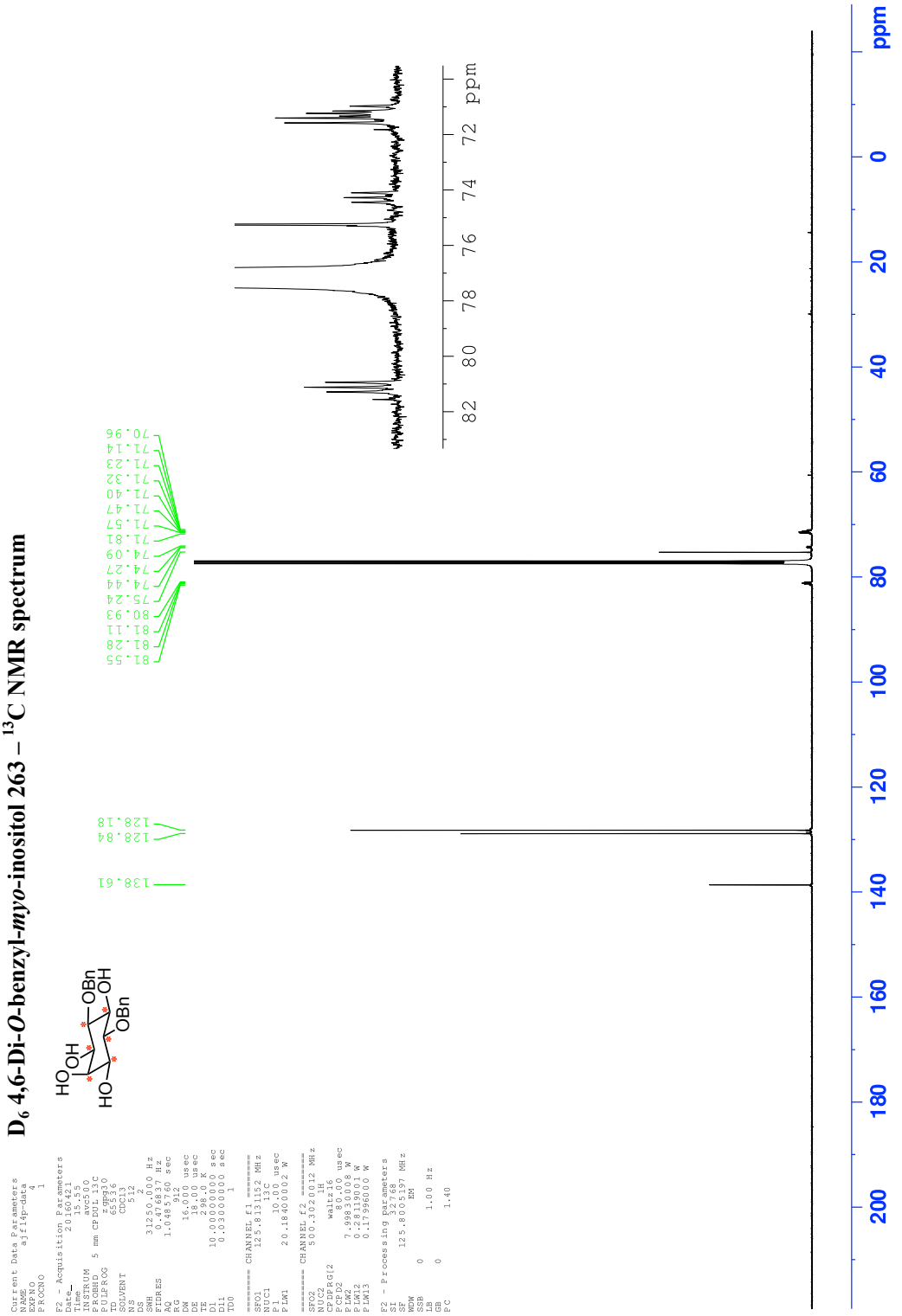
NL: 7.85E5
C21H16[2]H6O6Na1: $C_{21}H_{16}{}^{2}H_{6}O_{6}Na$
Chrg 1 R: 1000000 Res. Pwr. @FWHM

Theoretical Spectrum

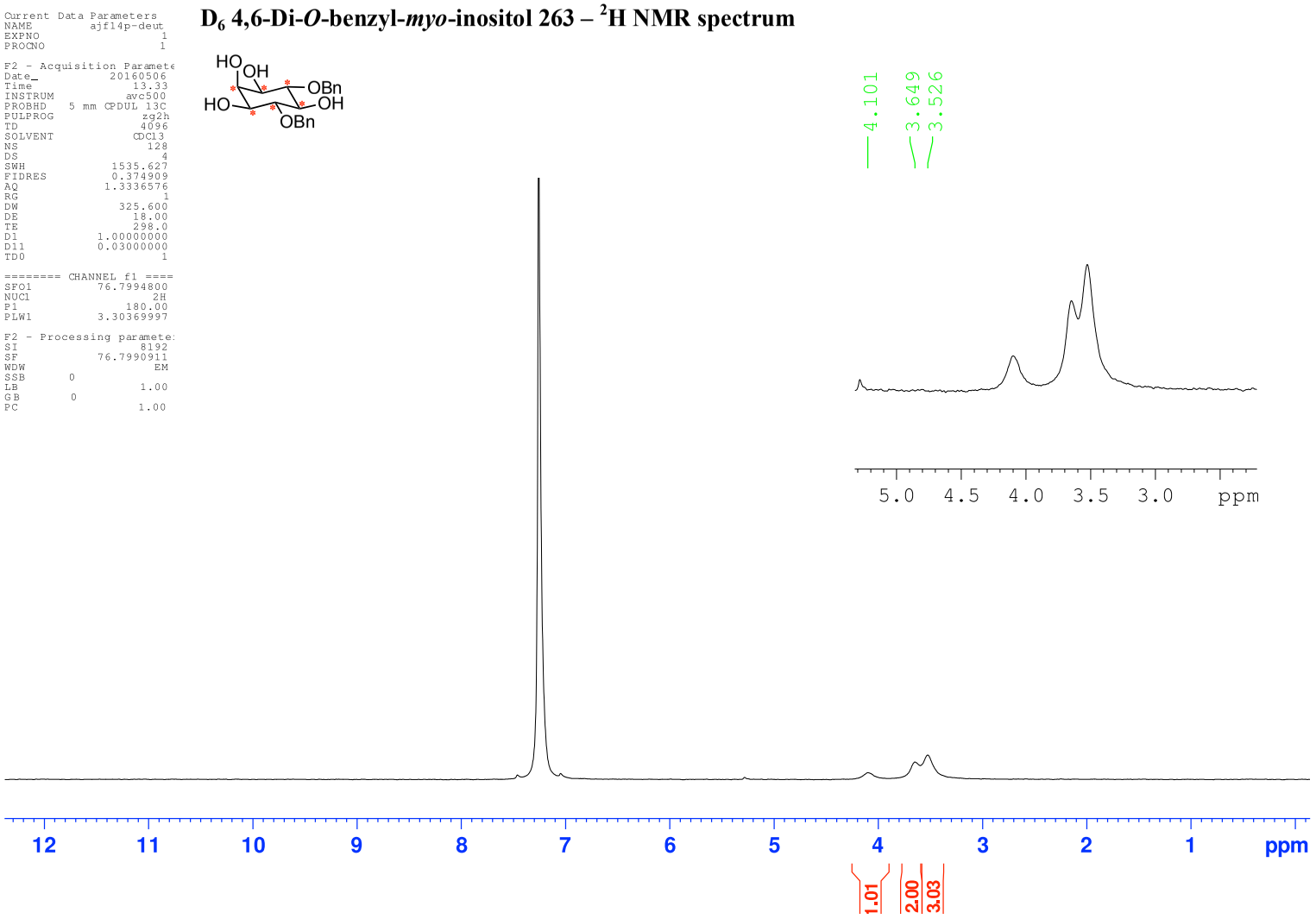
m/z	Formula	RDB	Delta ppm	Theo. Mass
399.16827	$C_{21}H_{16}{}^{2}H_{6}O_{6}{}^{23}Na$	10.5	-0.62	399.16852

D_6 4,6-Di-*O*-benzyl-*myo*-inositol 263 – ^{1}H NMR spectrum

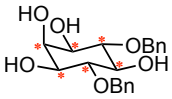



D6 4,6-Di-O-benzyl-myo-inositol 263 – 13C NMR spectrum
HO
OH
OBn
OH
OBn
138.61
128.84
128.18
81.55
81.28
81.11
80.93
75.24
74.44
74.27
74.09
71.81
71.57
71.47
71.40
71.32
71.23
71.14
70.96
82 80 78 76 74 72 ppm
200 180 160 140 120 100 80 60 40 20 0 ppm

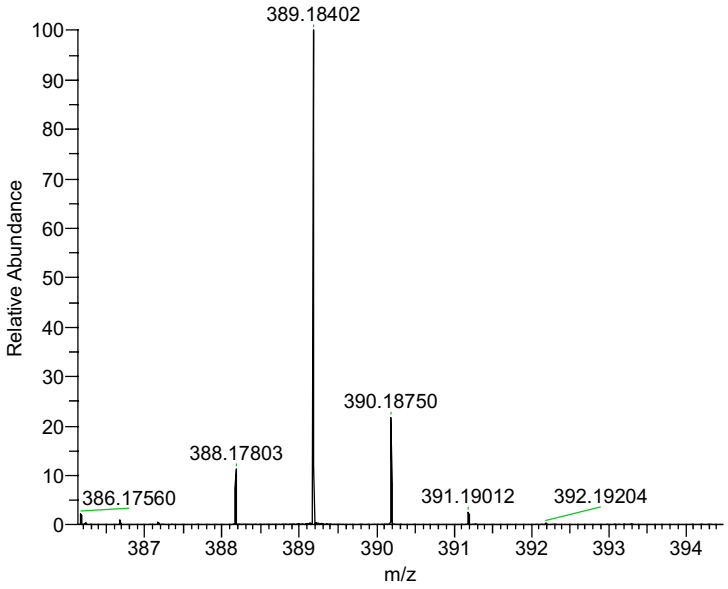
D_6 4,6-Di-*O*-benzyl-*myo*-inositol 263 – ^2H NMR spectrum



D_6 4,6-Di-*O*-benzyl-*myo*-inositol 263 – Mass spectrum

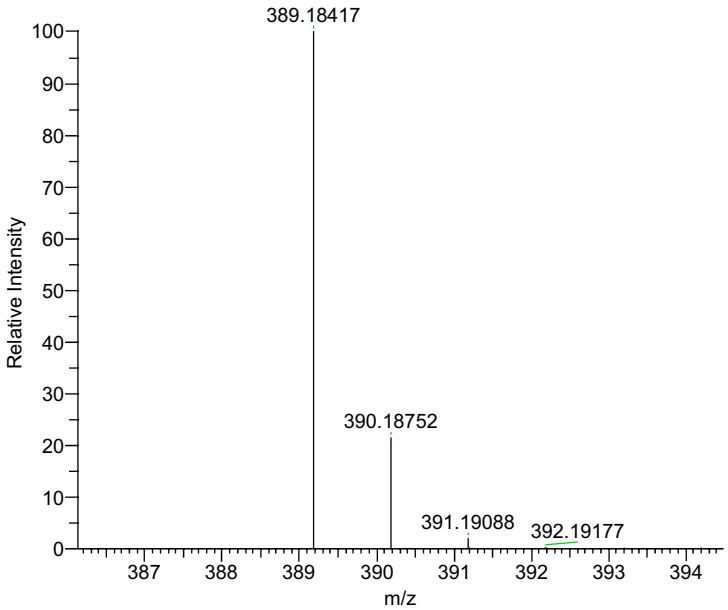


W:\data\May 16\ESI56917.raw 09/05/2016 10:54 am



NL: 3.94E7
ESI56917 #12-27 RT: 0.14-0.3 AV: 8 NL: 3.94E+007
T: FTMS {1,1} + p ESI Full lock ms [80.00-1600.00]

Measured Spectrum

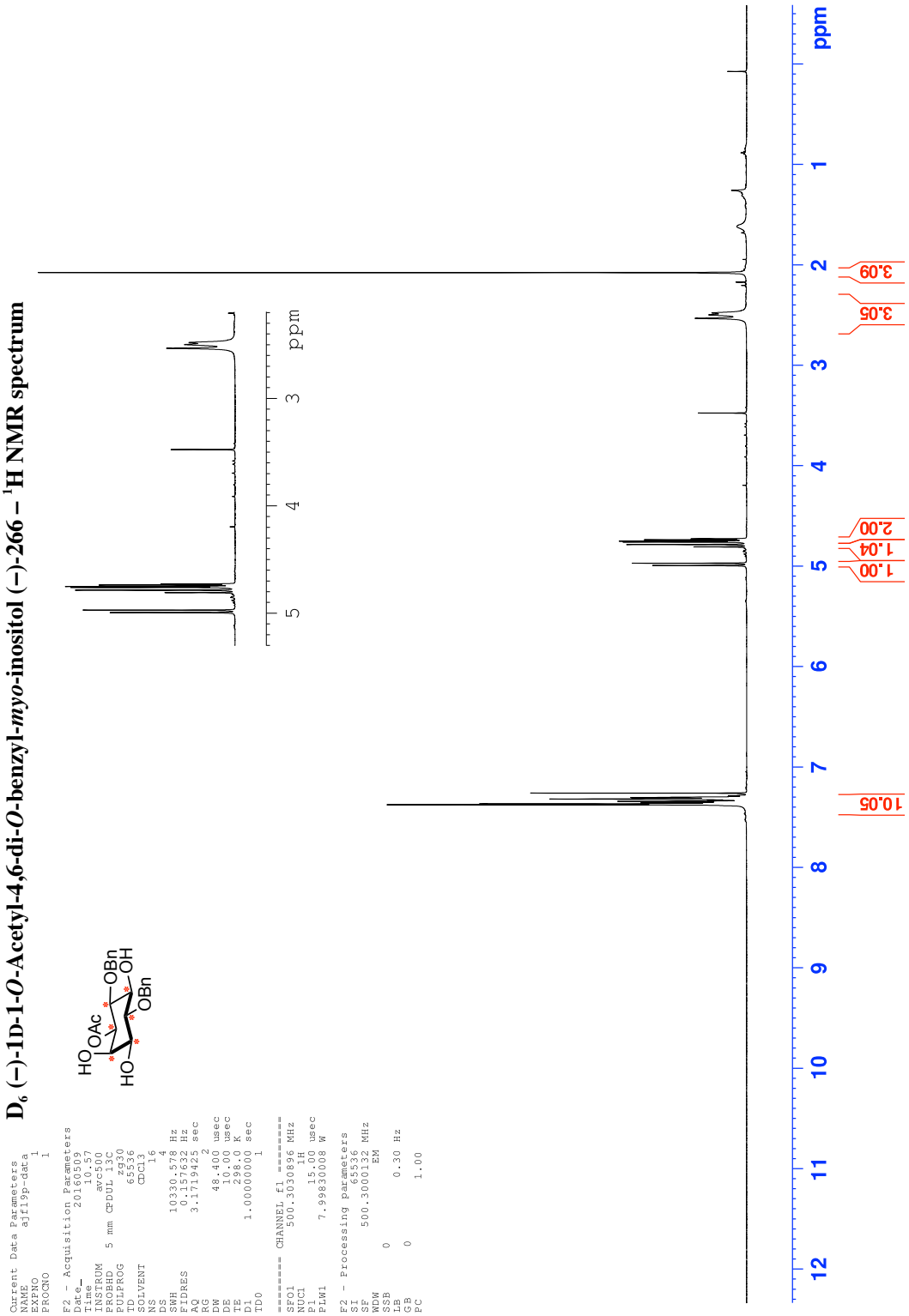


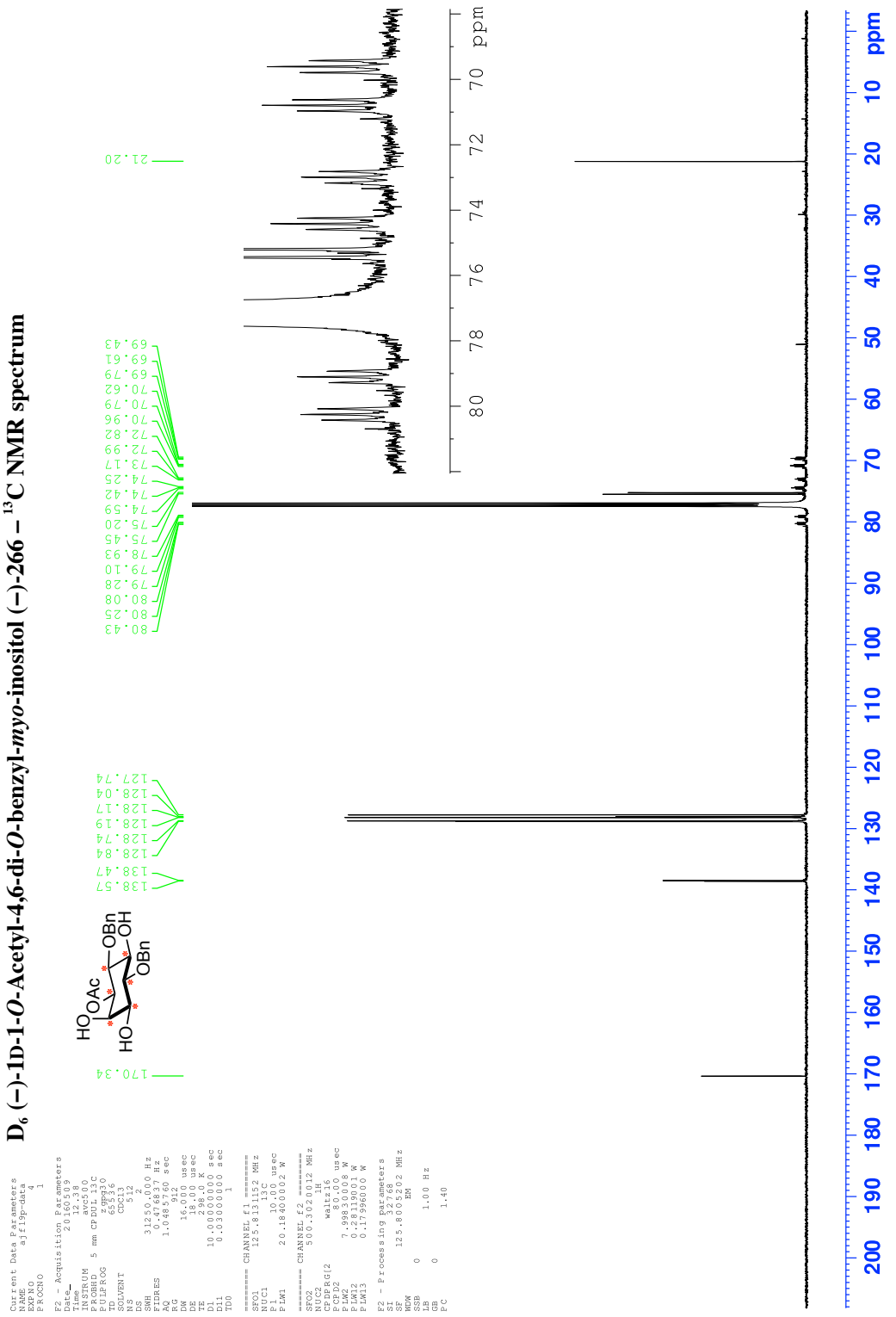
NL: 7.93E5
C20H18[2]H6O6Na1: $C_{20}H_{18}{}^{2}H_{6}O_{6}Na$
Chrg 1 R: 1000000 Res. Pwr. @FWHM

Theoretical Spectrum

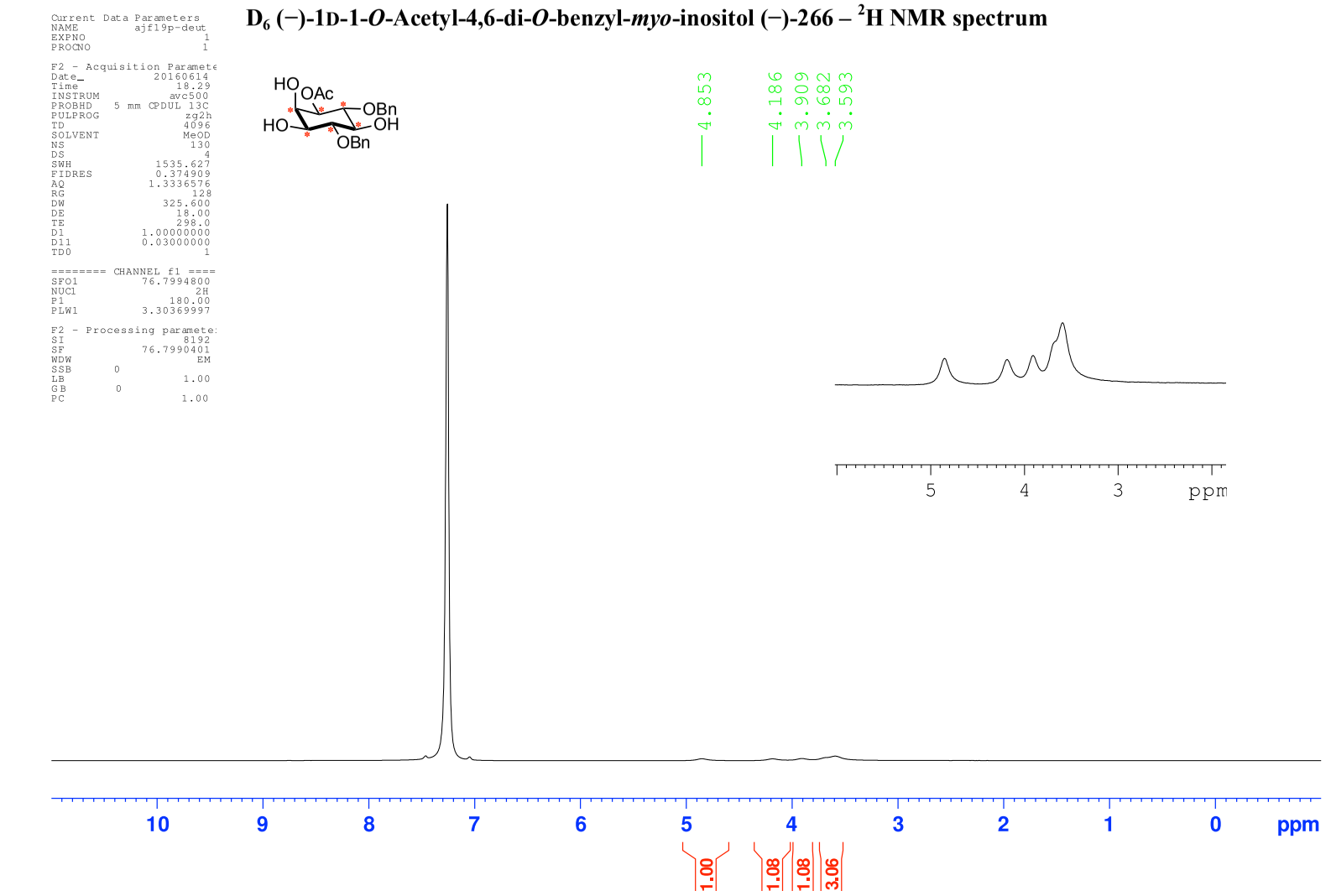
m/z	Formula	RDB	Delta ppm	Theo. Mass
389.18402	$C_{20}H_{18}{}^{2}H_{6}O_{6}{}^{23}Na$	8.5	-0.38	389.18417

D_6 (–)-1D-1-*O*-Acetyl-4,6-di-*O*-benzyl-*myo*-inositol (–)-266 – ^{1}H NMR spectrum

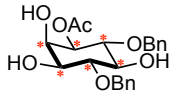



D6 (−)-1D-1-O-Acetyl-4,6-di-O-benzyl-myo-inositol (−)-266 – 13C NMR spectrum
Current Data Parameters
NAME ajf19p-data
EXPNO 4
PROCNO 1
F2 - Acquisition Parameters
Date_ 20160509
Time 22.38
INSTRUM avc500
PROBHD 5 mm CPDUL 13C
PULPROG zgpg30
TD 65536
SOLVENT CDCl3
NS 512
DS 4
SWH 31250.000 Hz
FIDRES 0.476837 Hz
AQ 1.0485760 sec
RG 912
DW 16.000 usec
DE 18.00 usec
TE 298.0 K
D1 10.00000000 sec
D11 0.03000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 125.8131152 MHz
NUC1 13C
P1 10.00 usec
PLW1 20.18400002 W
======== CHANNEL f2 ========
SFO2 500.3020012 MHz
NUC2 1H
CPDPRG2 waltz16
PCPD2 80.00 usec
PLW2 7.99830008 W
PLW12 0.42313001 W
PLW13 0.11796001 W
F2 - Processing parameters
SI 32768
SF 125.8052002 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
170.34
138.57
138.47
128.84
128.74
128.19
128.17
128.04
127.74
80.43
80.25
80.08
79.28
79.10
78.93
75.45
75.20
74.59
74.42
74.25
73.17
72.99
72.82
70.96
70.79
70.62
69.79
69.61
69.43
21.20
80
78
76
74
72
70 ppm
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10 ppm

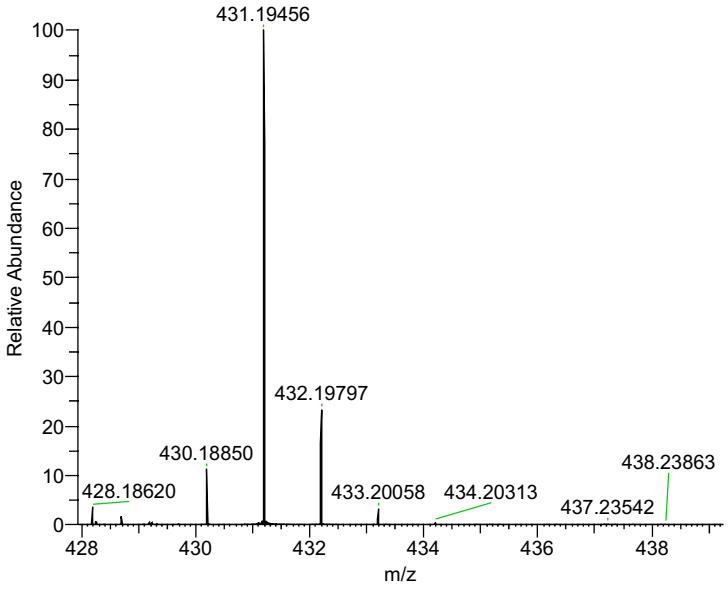
D_6 (−)-1D-1-*O*-Acetyl-4,6-di-*O*-benzyl-*myo*-inositol (−)-266 – ^{2}H NMR spectrum



D_6 (−)-1D-1-*O*-Acetyl-4,6-di-*O*-benzyl-*myo*-inositol (−)-266 – Mass spectrum

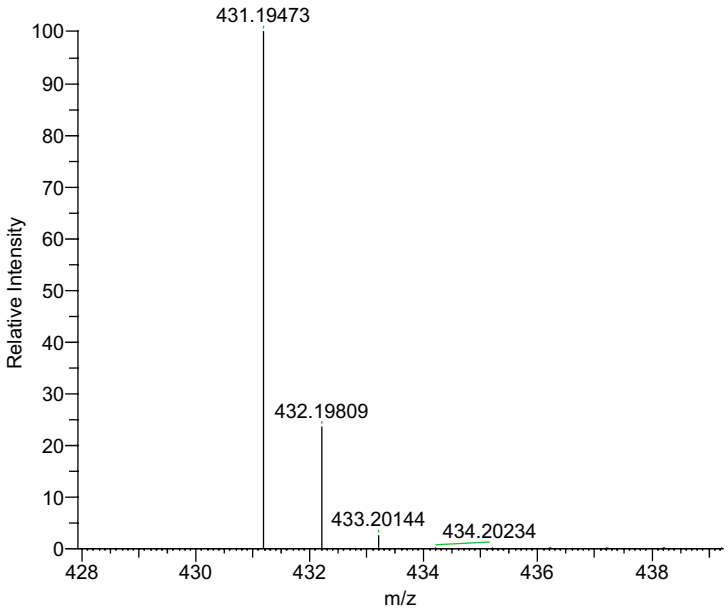


W:\data\May 16\ESI56918.raw 09/05/2016 10:55 am



NL: 3.53E7
ESI56918 #12-27 RT: 0.14-0.3 AV: 8 NL: 3.53E+007
T: FTMS {1,1} + p ESI Full lock ms [80.00-1600.00]

Measured Spectrum

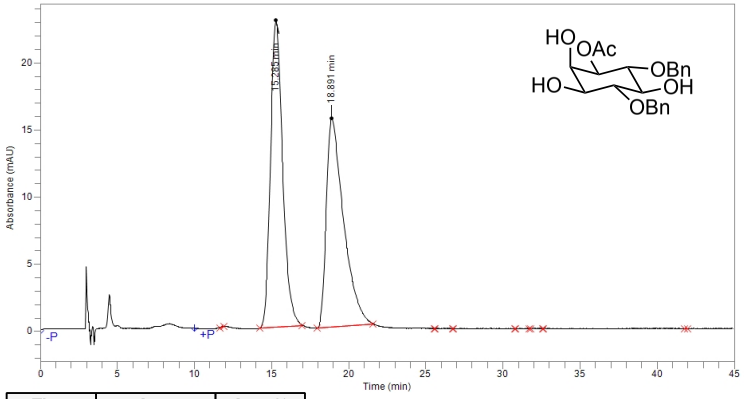


NL: 7.74E5
C22H20[2]H6O7Na1: $C_{22}H_{20}{}^{2}H_{6}O_{7}Na$
Chrg 1 R: 1000000 Res. Pwr. @FWHM

Theoretical Spectrum

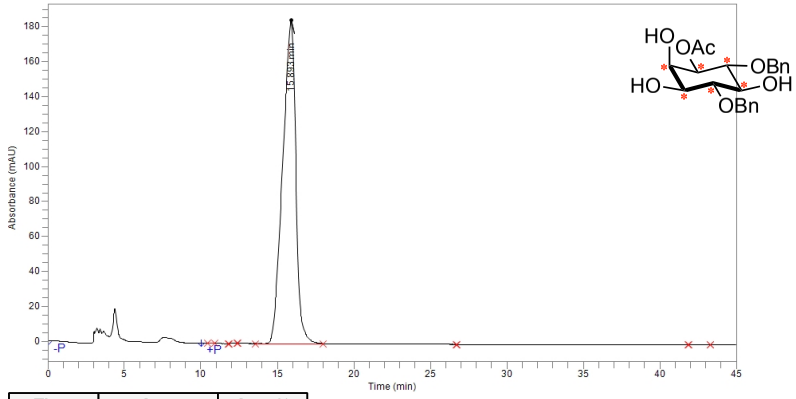
m/z	Formula	RDB	Delta ppm	Theo. Mass
431.19455	$C_{22}H_{20}{}^{2}H_{6}O_{7}{}^{23}Na$	9.5	-0.43	431.19473

(±)-1D-1-*O*-Acetyl-4,6-di-*O*-benzyl-*myo*-inositol (±)-151 – Chiral HPLC (Heptane/IPA 85:15, 1.0 mL.min^{-1}, 254 nm)



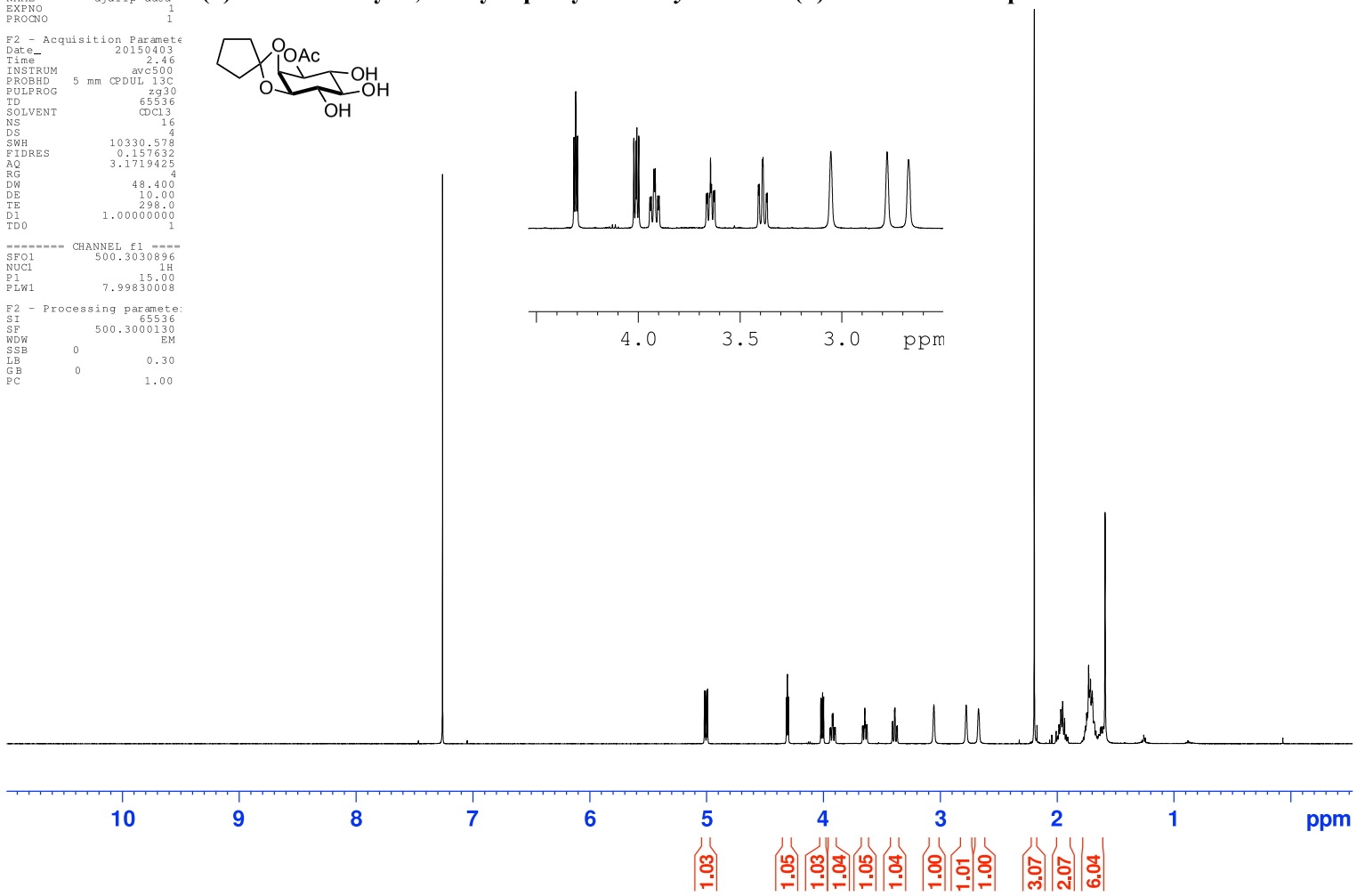
Time	Area	Area %
11.895	372	0.02
15.285	1,253,208	50.90
18.891	1,208,110	49.07
25.571	28	0.00
26.768	33	0.00
30.783	39	0.00
31.730	59	0.00
32.605	28	0.00
41.809	162	0.01
Total	2,462,039	100.00

D_6 (–)-1D-1-*O*-Acetyl-4,6-di-*O*-benzyl-*myo*-inositol (–)-266 – Chiral HPLC (Heptane/IPA 85:15, 1.0 mL.min^{-1}, 254 nm)

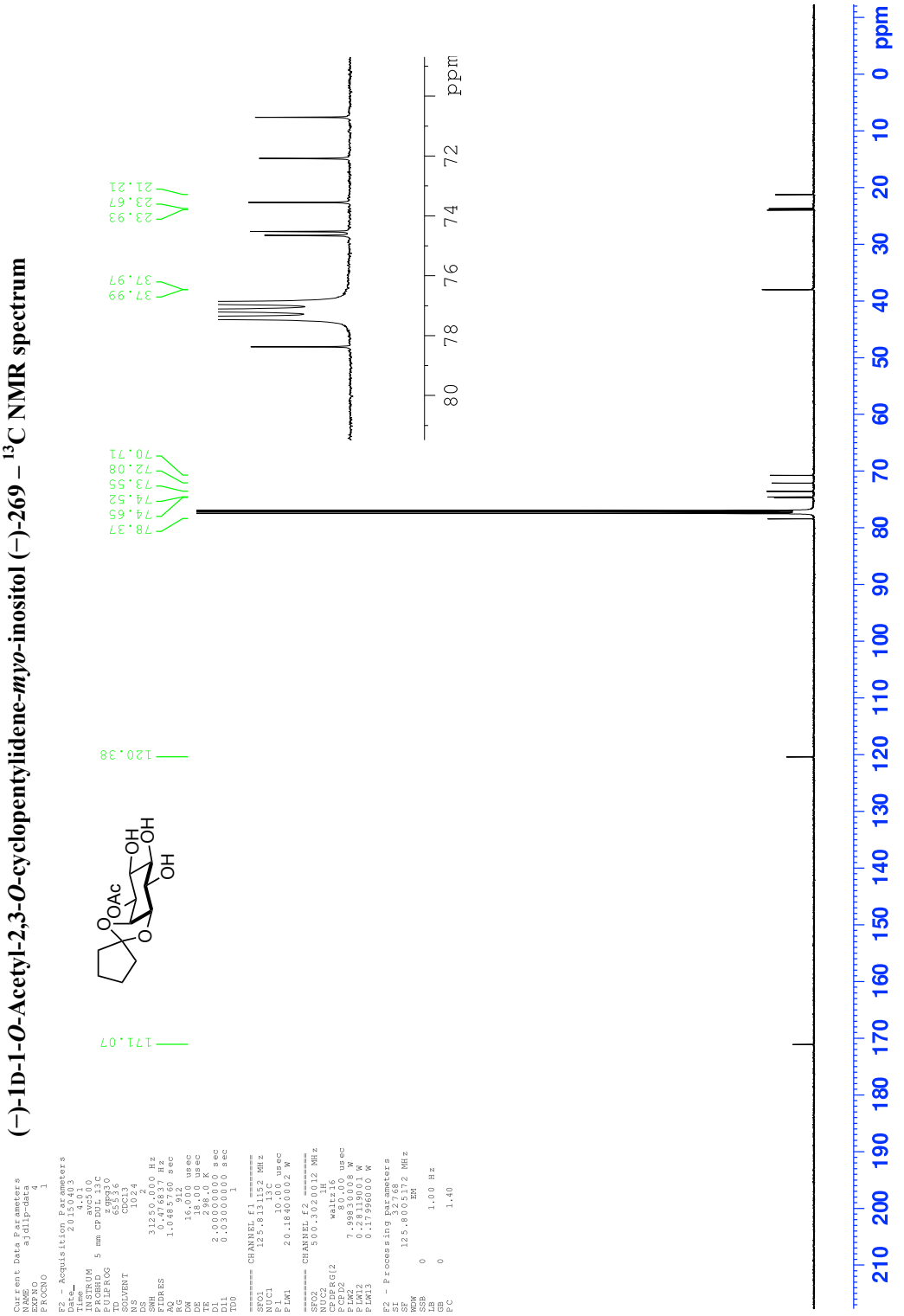


Time	Area	Area %
10.642	2,451	0.02
11.845	39	0.00
12.405	31	0.00
13.608	775	0.01
15.893	11,038,336	99.97
26.708	30	0.00
41.897	42	0.00
43.301	6	0.00
Total	11,041,710	100.00

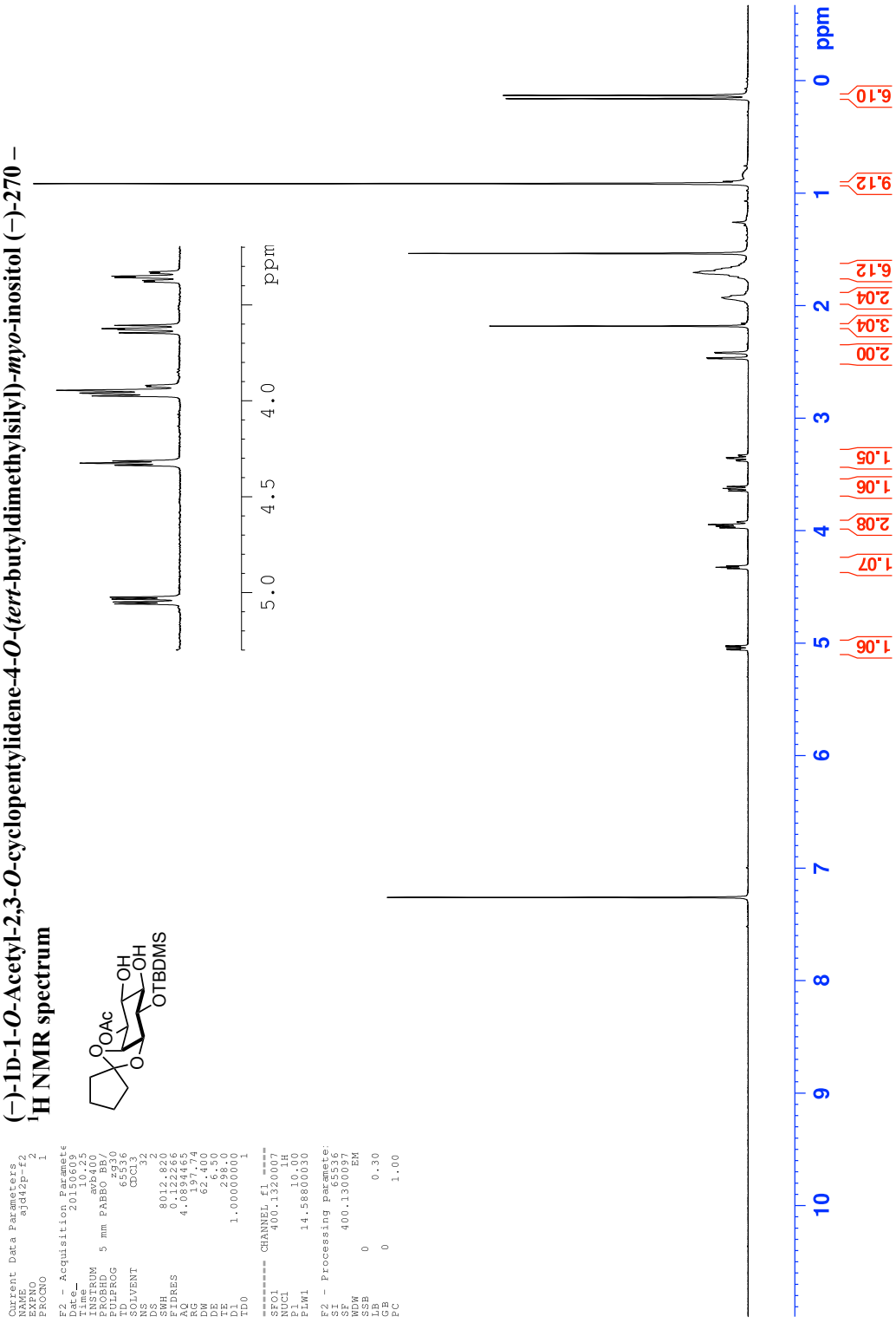
(−)-1D-1-*O*-Acetyl-2,3-*O*-cyclopentylidene-*myo*-inositol (−)-269 – ^{1}H NMR spectrum



(–)-1D-1-*O*-Acetyl-2,3-*O*-cyclopentylidene-*myo*-inositol (–)-269 – ^{13}C NMR spectrum



(−)-1D-1-*O*-Acetyl-2,3-*O*-cyclopentylidene-4-*O*-(*tert*-butyldimethylsilyl)-*myo*-inositol (−)-270 – ^{1}H NMR spectrum



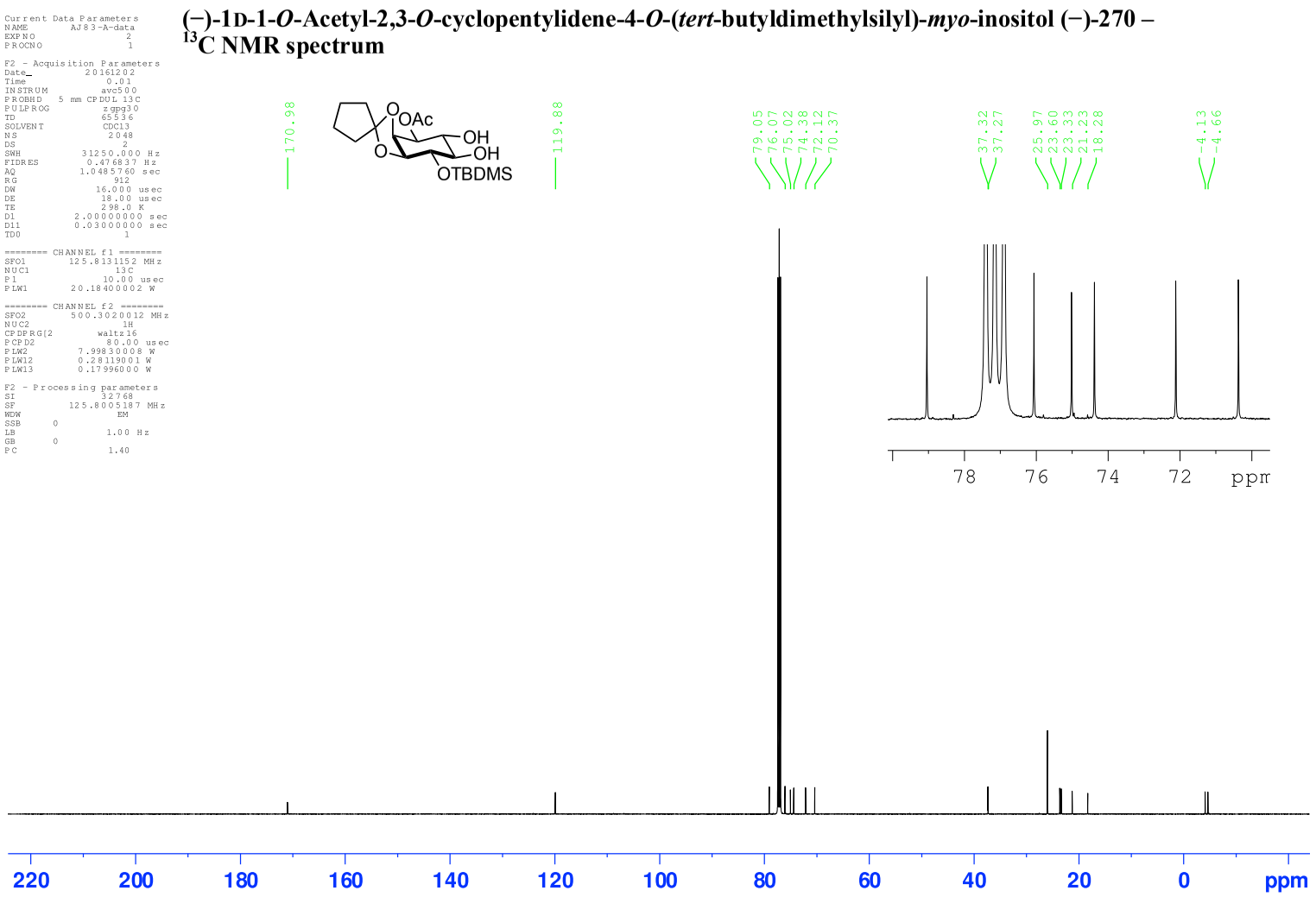

```
Current Data Parameters
NAME                  ajd42p-f2
EXPNO                         2
PROCNO                        1

F2 - Acquisition Parameters
Date_               20150609
Time                   13.25
INSTRUM                avb400
PROBHD     5 mm PABBO BB/
PULPROG                zg30
TD                    65536
SOLVENT               CDCl3
NS                        32
DS                         2
SWH                8012.820
FIDRES             0.122266
AQ                 4.089946
RG                    181.74
DW                    62.400
DE                      6.50
TE                     298.0
D1                  1.00000000
TD0                        1

======== CHANNEL f1 ========
SFO1               400.1320007
NUC1                        1H
P1                         10.00
PLW1               14.58800030

F2 - Processing parameters
SI                        65536
SF                 400.1300097
WDW                        EM
SSB                 0
LB                      0.30
GB                  0
PC                      1.00
```

(−)-1D-1-*O*-Acetyl-2,3-*O*-cyclopentylidene-4-*O*-(*tert*-butyldimethylsilyl)-*myo*-inositol (−)-270 – ^{13}C NMR spectrum



(−)-1D-1-*O*-Acetyl-2,3-*O*-cyclopentylidene-4-*O*-(*tert*-butyldimethylsilyl)-*myo*-inositol (−)-270 – ^{1}H-^{29}Si HMBC NMR spectrum


```
Current Data Parameters
NAME      ajd29p-f3-si-H
EXPNO              2
PROCNO             1

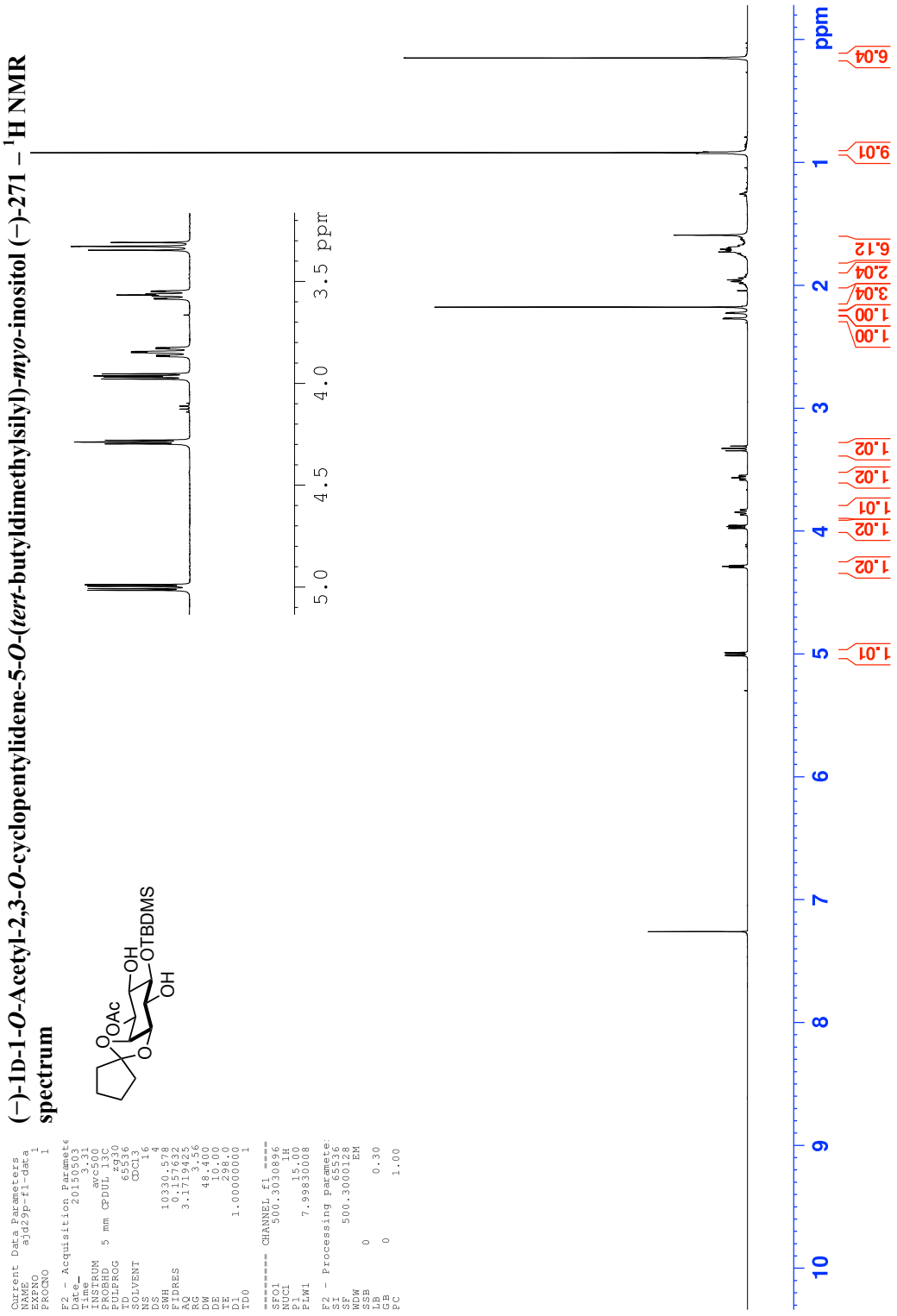
F2 - Acquisition Parameters
Date_        20150505
Time              11.25
INSTRUM          avx500
PROBHD   Z113652_0208 (
PULPROG   hmbcgpndqf
TD                4096
SOLVENT          CDCl3
NS                   2
DS                  16
SWH          6009.615 Hz
FIDRES       1.467191 Hz
AQ           0.3407872 sec
RG                 191.37
DW              83.200 usec
DE               6.50 usec
TE                298.0 K
CNST13       6.0000000
d0           0.00000300 sec
D1           2.00000000 sec
d6           0.08333334 sec
D16          0.00020000 sec
in0                  0 sec
STlCNT               0
d0orig       0.00000300 sec
philoop              0
tiloop               0
SFO1         500.1323506 MHz
NUC1                 1H
P1                10.00 usec
p2                20.00 usec
PLW1         20.50000000 W
SFO2         99.3617620 MHz
NUC2                 29Si
P3                12.50 usec
PLW2         80.00000000 W
GPNAM[1]         SMSQ10.100
GPNAM[2]         SMSQ10.100
GPNAM[3]         SMSQ10.100
GPZ1                70.00 %
GPZ2                30.00 %
GPZ3                59.90 %
P16              1000.00 usec

F1 - Acquisition parameters
TD                   128
SFO1         99.36176 MHz
FIDRES       77.659042 Hz
SW           100.042 ppm
FnMODE               QF

F2 - Processing parameters
SI                  2048
SF         500.1300096 MHz
WDW                SINE
SSB                   4
LB          0 Hz
GB          0
PC             1.40

F1 - Processing parameters
SI                  1024
MC2                   QF
SF         99.3624076 MHz
WDW              QSINE
SSB          0
LB           0 Hz
GB           0
```

(−)-1D-1-*O*-Acetyl-2,3-*O*-cyclopentylidene-5-*O*-(*tert*-butyldimethylsilyl)-*myo*-inositol (−)-271 – ^{1}H NMR spectrum



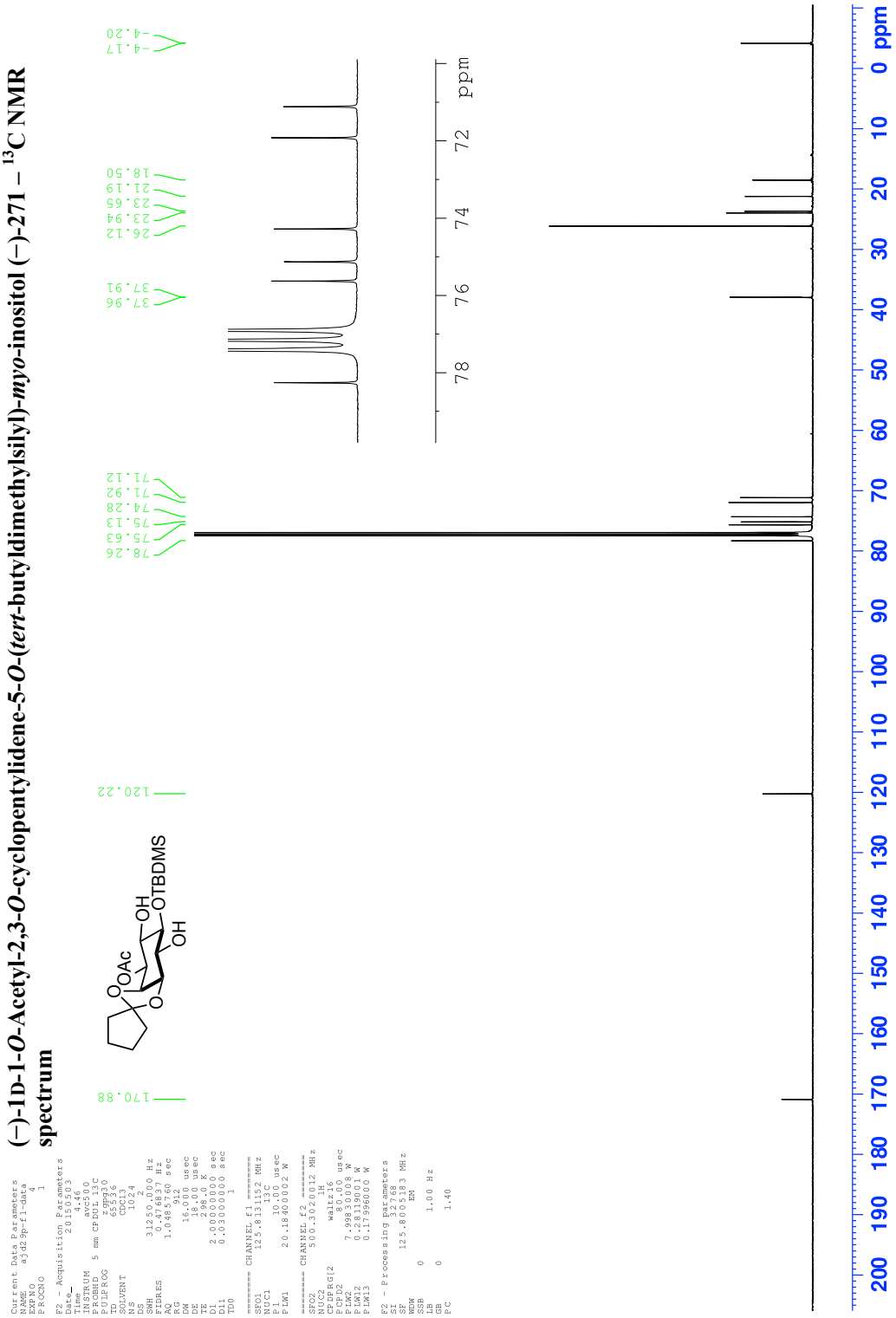

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Current Data Parameters
NAME                 sja29pr-f1-dea
EXPNO                             1
PROCNO                            1

F2 - Acquisition Parameters
Date_               20150513
Time                    3.31
INSTRUM                 avc500
PROBHD     5 mm CPDUL 13C
PULPROG                 zg30
TD                     65536
SOLVENT                CDCl3
NS                        16
DS                         4
SWH                10330.578
FIDRES              0.157632
AQ                  3.1719425
RG                       8.5
DW                      48.400
DE                      10.00
TE                      298.0
D1                 1.00000000
TD0                        1

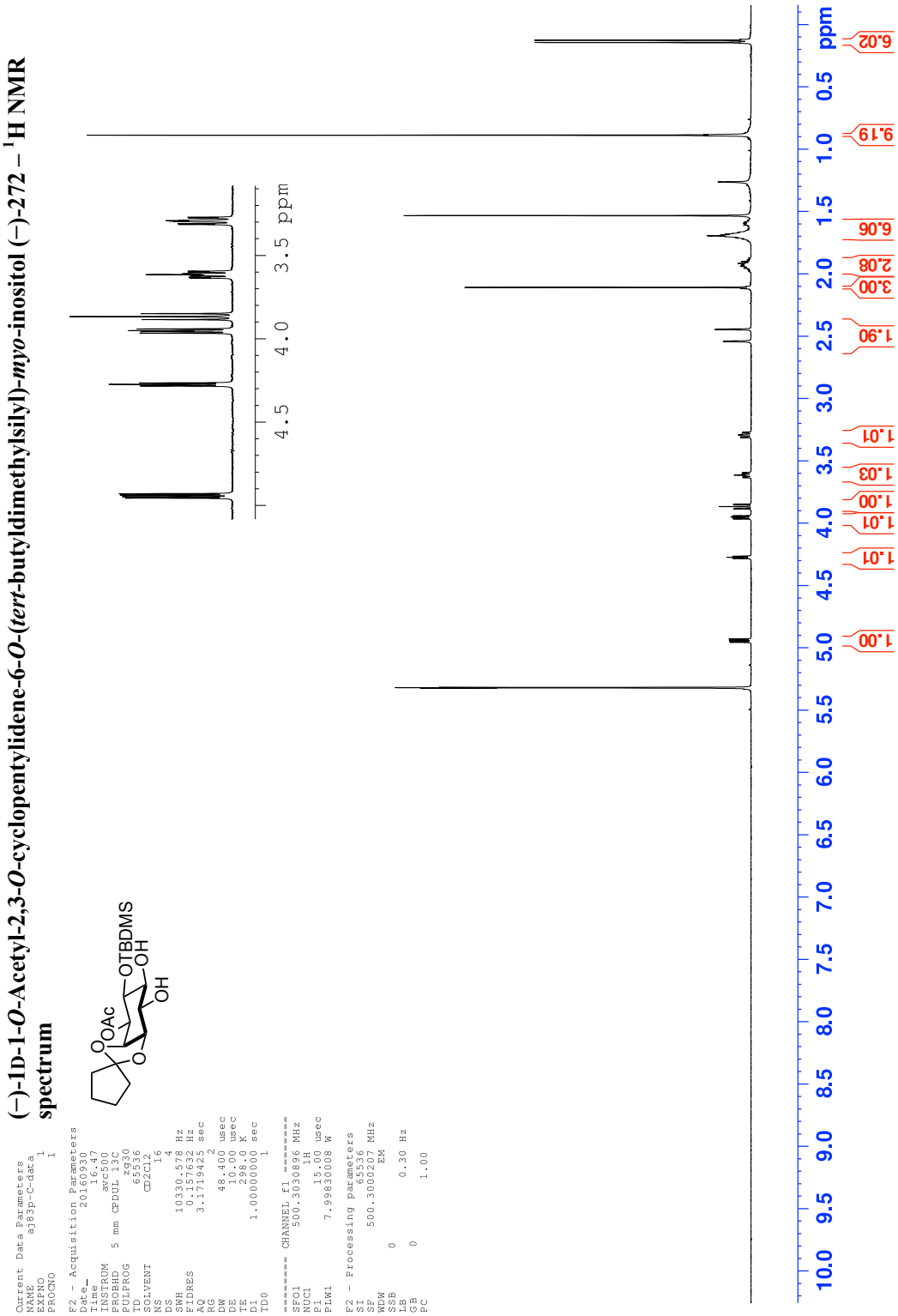
======== CHANNEL f1 ========
SFO1               500.3030896
NUC1                        1H
P1                       15.00
PLW1               7.99830008

F2 - Processing parameters
SI                         65536
SF                 500.3000128
WDW                           EM
SSB                 0
LB                      0.30
GB                  0
PC                      1.00
```

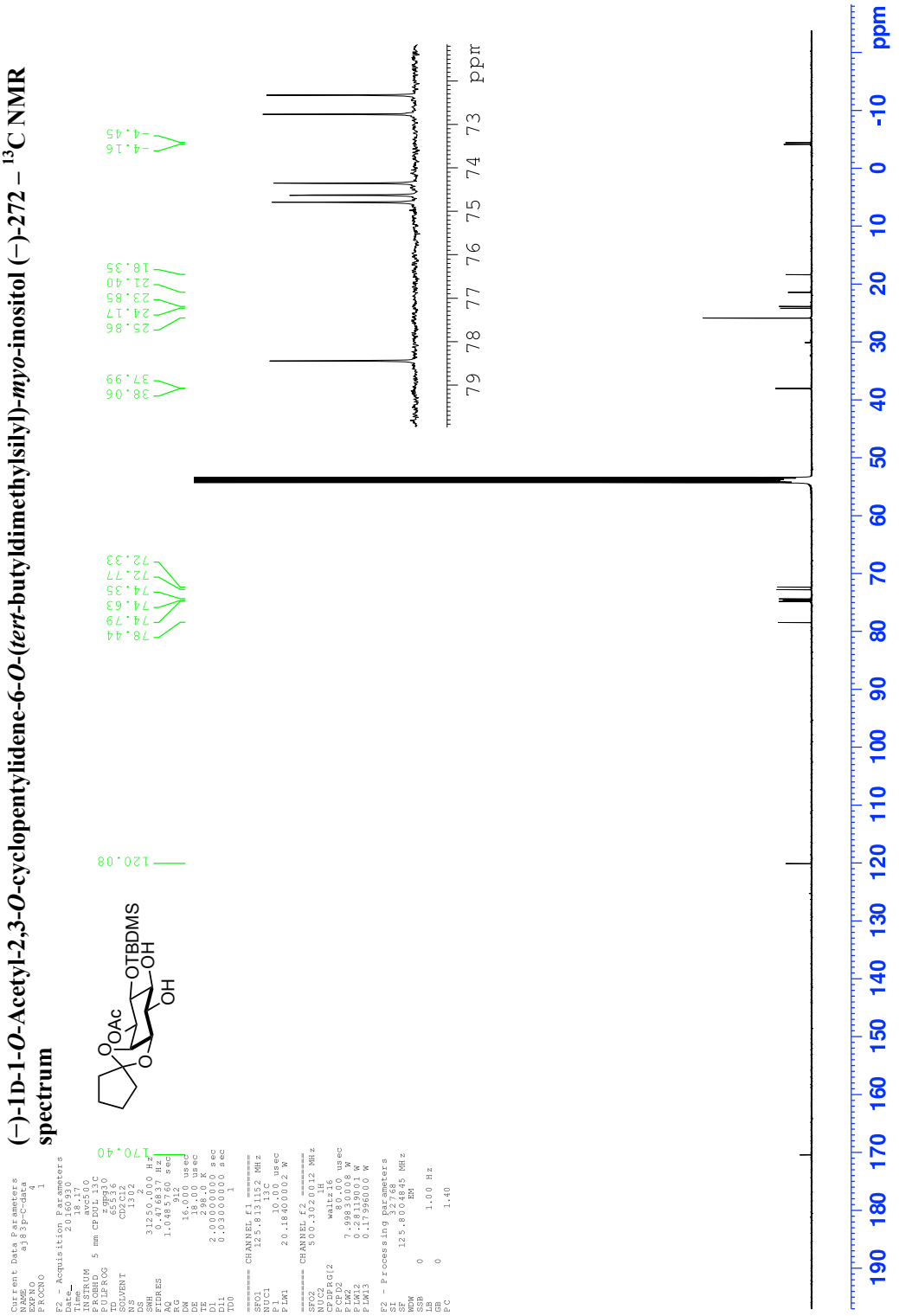
(−)-1D-1-*O*-Acetyl-2,3-*O*-cyclopentylidene-5-*O*-(*tert*-butyldimethylsilyl)-*myo*-inositol (−)-271 – ^{13}C NMR spectrum



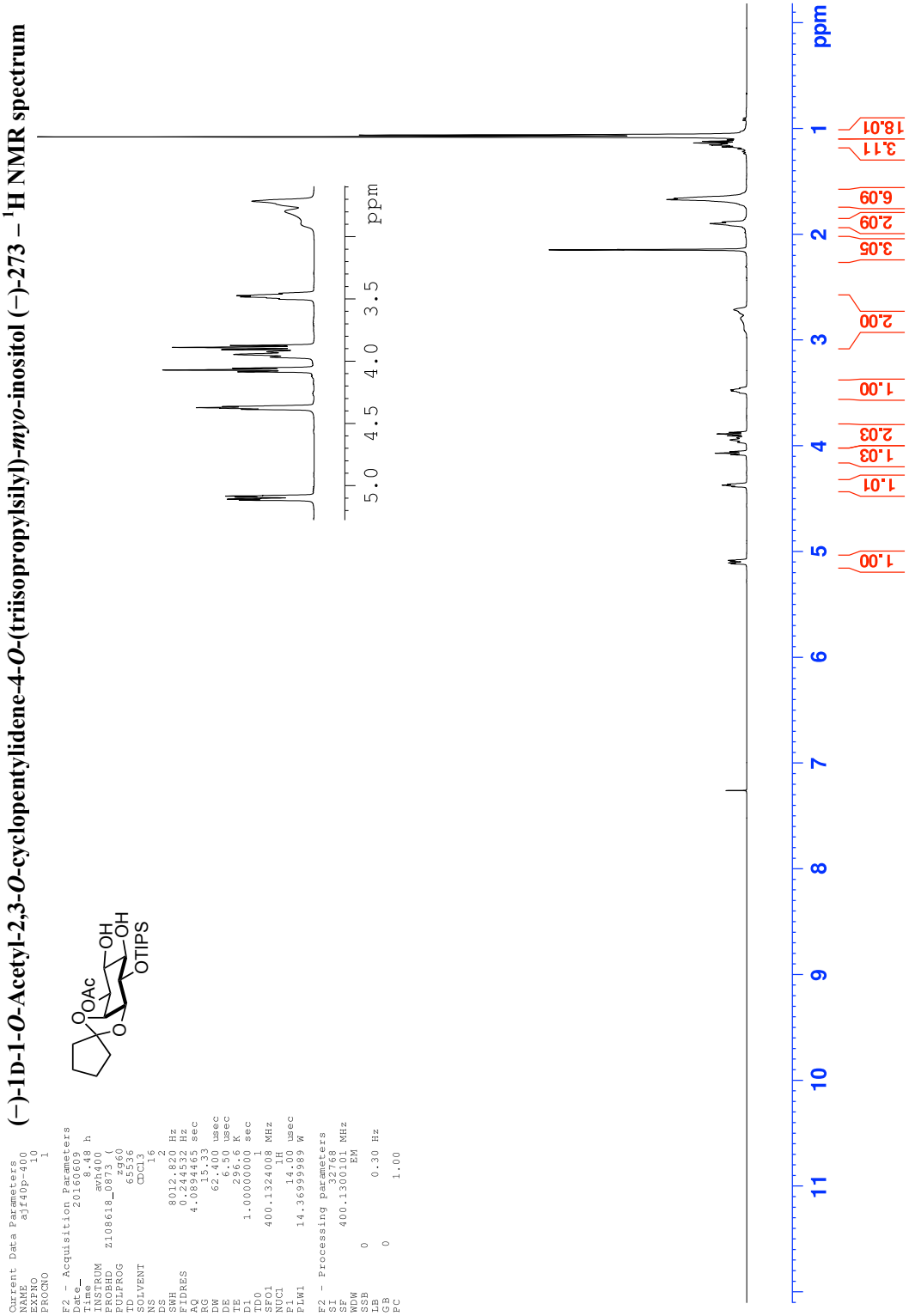
(–)-1D-1-*O*-Acetyl-2,3-*O*-cyclopentylidene-6-*O*-(*tert*-butyldimethylsilyl)-*myo*-inositol (–)-272 – ^{1}H NMR spectrum



(–)-1D-1-*O*-Acetyl-2,3-*O*-cyclopentylidene-6-*O*-(*tert*-butyldimethylsilyl)-*myo*-inositol (–)-272 – ^{13}C NMR spectrum



(−)-1D-1-*O*-Acetyl-2,3-*O*-cyclopentylidene-4-*O*-(triisopropylsilyl)-*myo*-inositol (−)-273 – ^{1}H NMR spectrum

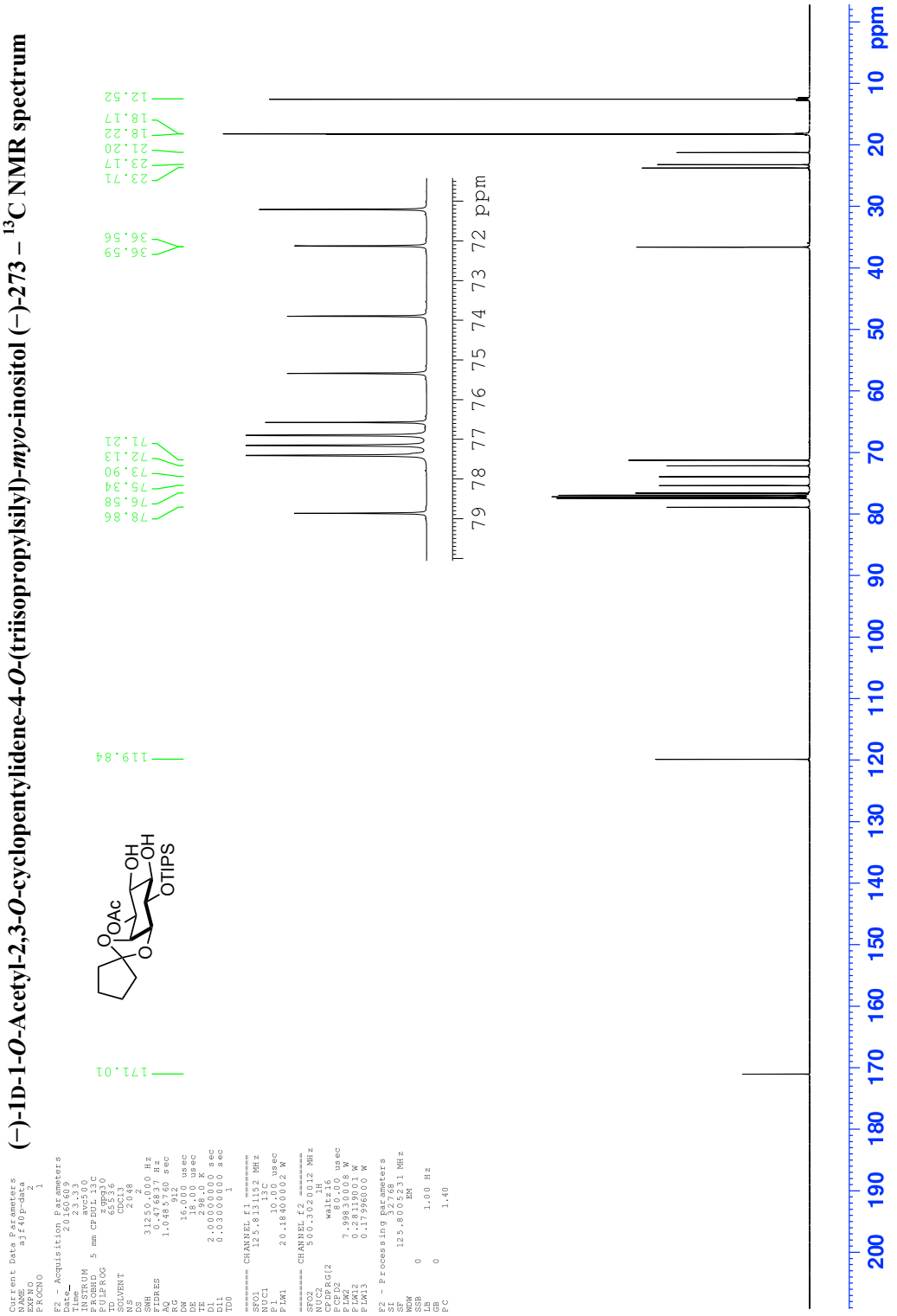



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Current Data Parameters
NAME          ajf40p-400
EXPNO                 10
PROCNO                 1

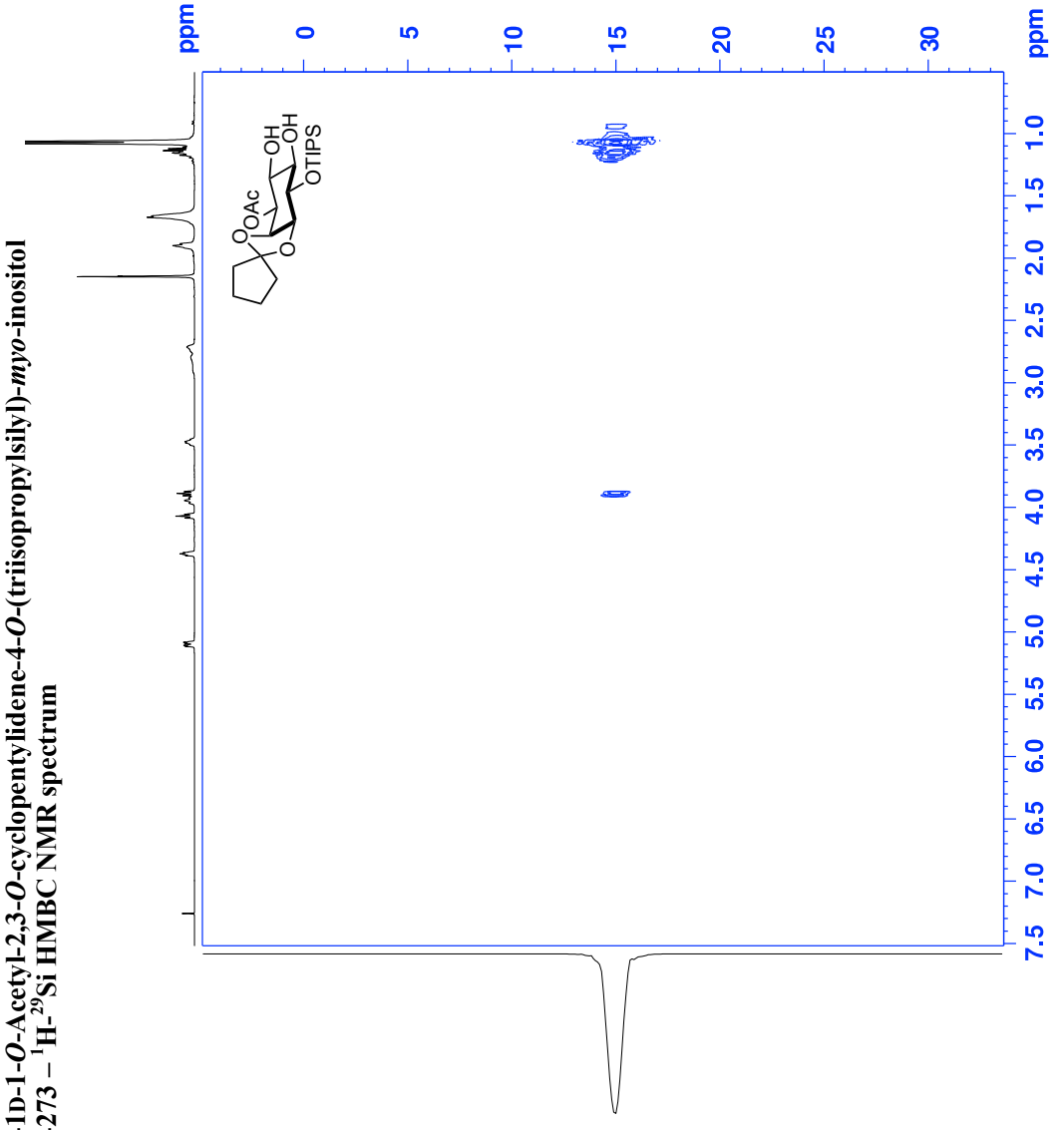
F2 - Acquisition Parameters
Date_          20160609
Time           9.48 h
INSTRUM           av400
PROBHD     Z108618_0873 (
PULPROG           zg60
TD                65536
SOLVENT           CDCl3
NS                   16
DS                    2
SWH            8012.820 Hz
FIDRES         0.244533 Hz
AQ             4.0894465 sec
RG                15.33
DW               62.400 usec
DE                6.50 usec
TE                296.6 K
D1             1.00000000 sec
TD0                   1
SFO1           400.1324008 MHz
NUC1                  1H
P1                14.00 usec
PLW1          14.36999989 W

F2 - Processing parameters
SI                32768
SF             400.1300101 MHz
WDW                   EM
SSB                   0
LB                 0.30 Hz
GB                    0
PC                 1.00
```

(−)-1D-1-*O*-Acetyl-2,3-*O*-cyclopentylidene-4-*O*-(triisopropylsilyl)-*myo*-inositol (−)-273 – ^{13}C NMR spectrum



(−)-1D-1-*O*-Acetyl-2,3-*O*-cyclopentylidene-4-*O*-(triisopropylsilyl)-*myo*-inositol
(−)-273 – ^{1}H-^{29}Si HMBC NMR spectrum




```
Current Data Parameters
NAME          ajd65p-f2-Si-H
EXPNO                      2
PROCNO                     1

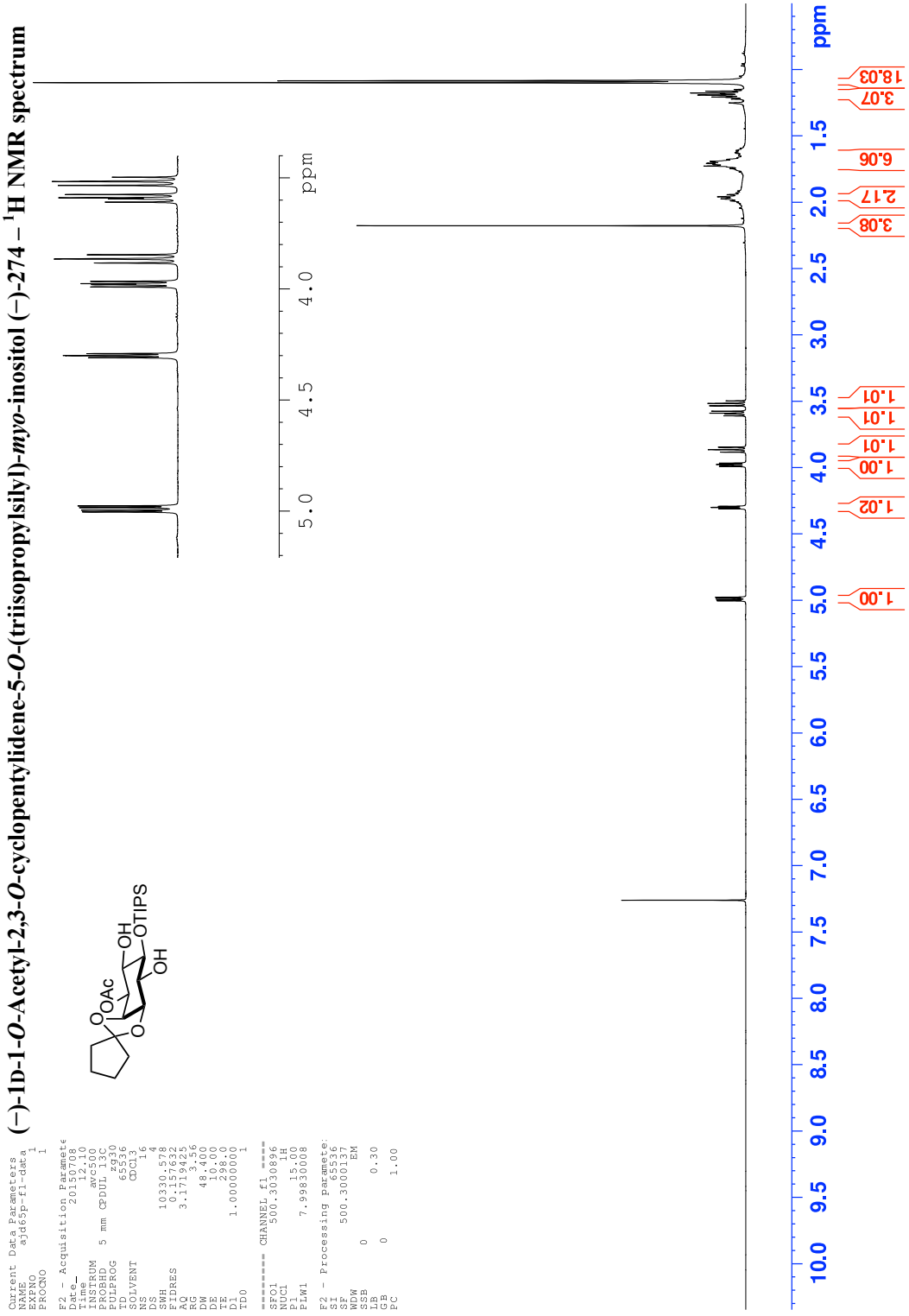
F2 - Acquisition Parameters
Date_           20150709
Time              11.46
INSTRUM             avx500
PROBHD   Z113652_0208 (
PULPROG   inmbcgpndqf
TD                 4096
SOLVENT           CDCl3
NS                   16
DS                   16
SWH          6009.615 Hz
FIDRES          1.467191 Hz
AQ           0.3407872 sec
RG                191.37
DW               83.200 usec
DE                6.50 usec
TE                298.0 K
CNST13       6.0000000
d0           0.00000300 sec
D1           2.00000000 sec
d6           0.08333334 sec
D16          0.00020000 sec
in0                  0 sec
STICNT               0
d0orig       0.00000300 sec
philoop              0
tiloop               0
SFO1         500.1323506 MHz
NUC1                 1H
P1                10.00 usec
p2                20.00 usec
PLW1         20.50000000 W
SFO2         99.3617620 MHz
NUC2                 29Si
P3                12.50 usec
PLW2         80.00000000 W
GPNAM[1]          SMSQ10.100
GPNAM[2]          SMSQ10.100
GPNAM[3]          SMSQ10.100
GP21                 70.00 %
GP22                 30.00 %
GP23                 59.90 %
P16               1000.00 usec

F1 - Acquisition parameters
TD                    128
SFO1         99.36176 MHz
FIDRES       77.659042 Hz
SW           100.042 ppm
FnMODE               QF

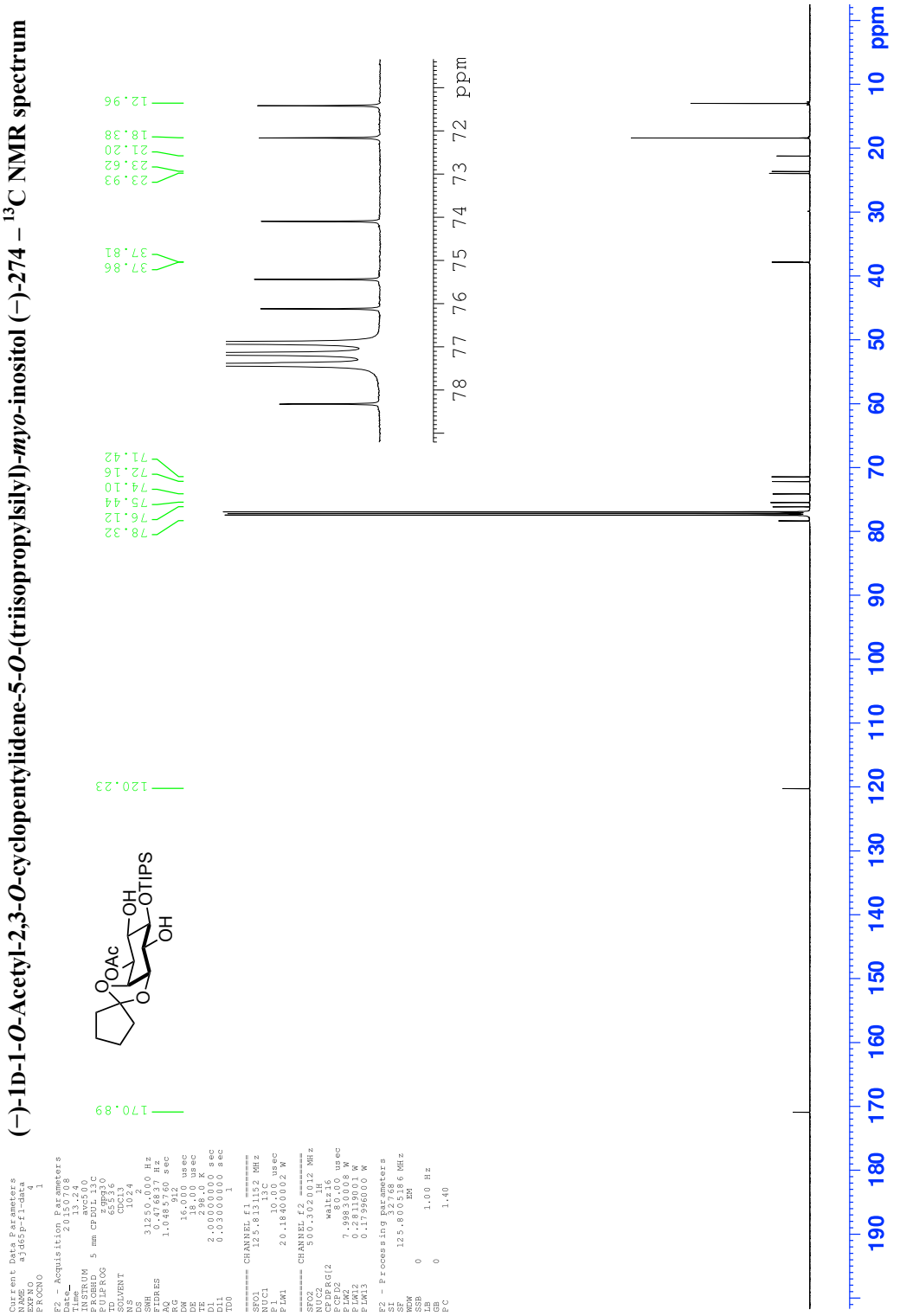
F2 - Processing parameters
SI                   2048
SF           500.1300197 MHz
WDW                  SINE
SSB                     4
LB          0 Hz
GB          0
PC                   1.40

F1 - Processing parameters
SI                   1024
MC2                  QF
SF           99.3617619 MHz
WDW                  QSINE
SSB          0
LB           0 Hz
GB           0
```

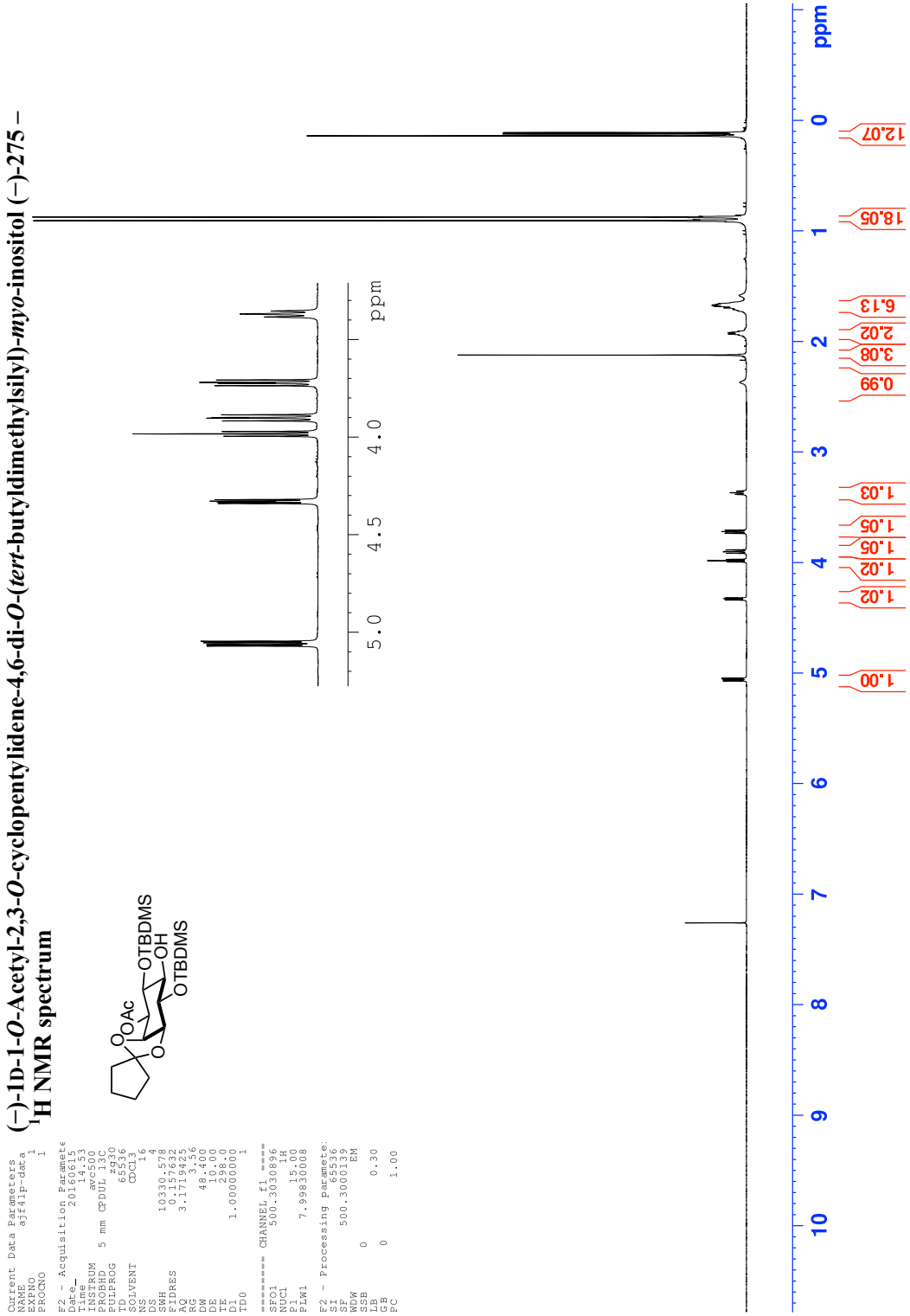
(−)-1D-1-*O*-Acetyl-2,3-*O*-cyclopentylidene-5-*O*-(triisopropylsilyl)-*myo*-inositol (−)-274 – ^{1}H NMR spectrum



(−)-1D-1-*O*-Acetyl-2,3-*O*-cyclopentylidene-5-*O*-(triisopropylsilyl)-*myo*-inositol (−)-274 – ^{13}C NMR spectrum



(−)-1D-1-*O*-Acetyl-2,3-*O*-cyclopentylidene-4,6-di-*O*-(*tert*-butyldimethylsilyl)-*myo*-inositol (−)-275 – ^{1}H NMR spectrum



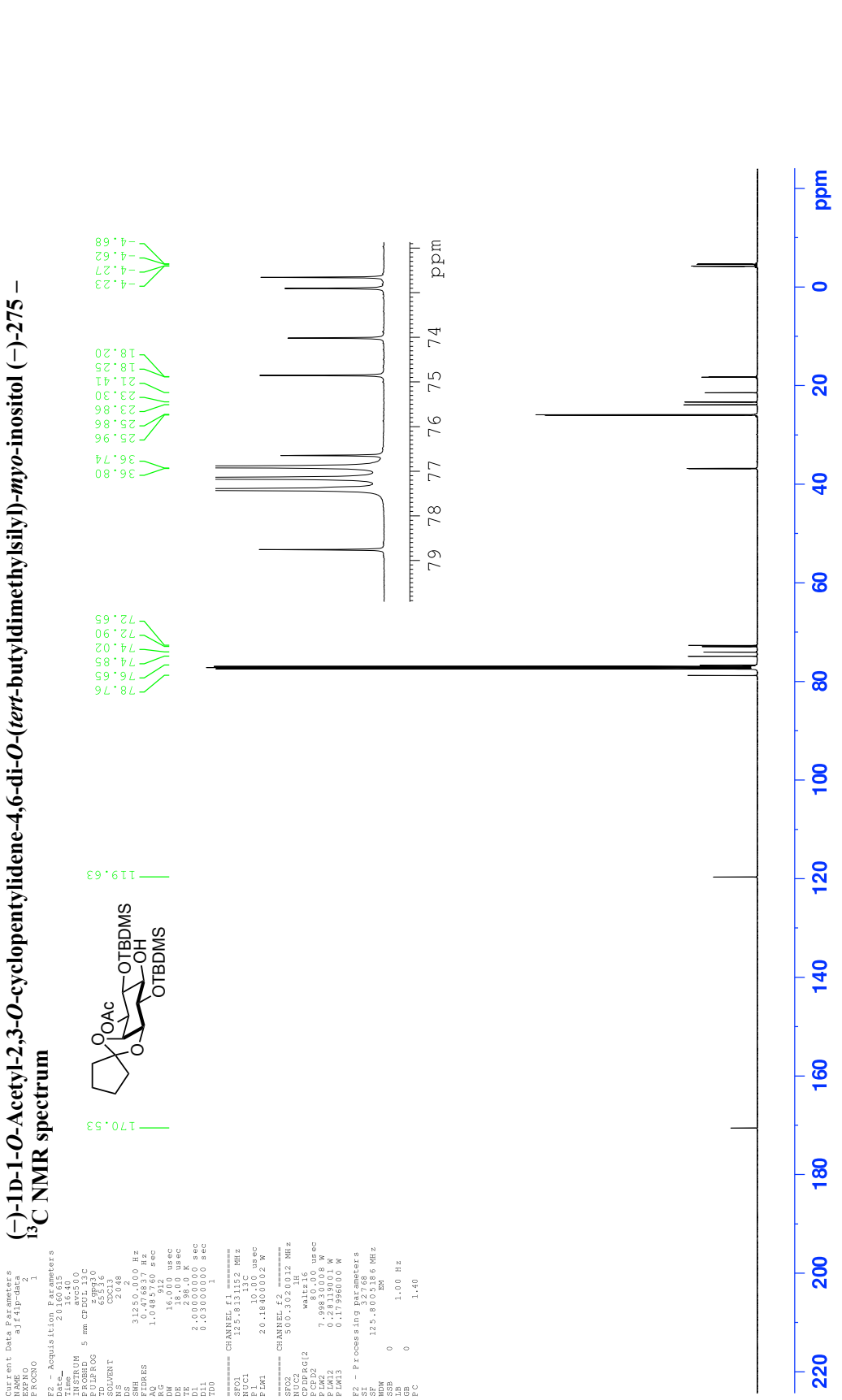

```
Current Data Parameters
NAME      ajf41p-data
EXPNO              1
PROCNO             1

F2 - Acquisition Parameters
Date_         20160715
Time              14.53
INSTRUM         avc500
PROBHD  5 mm CPDUL 13C
PULPROG          zg30
TD              65536
SOLVENT         CDCl3
NS                 16
DS                  4
SWH         10330.578
FIDRES      0.157632
AQ          3.1719425
RG                256
DW             48.400
DE             10.00
TE             298.0
D1          1.00000000
TD0                1

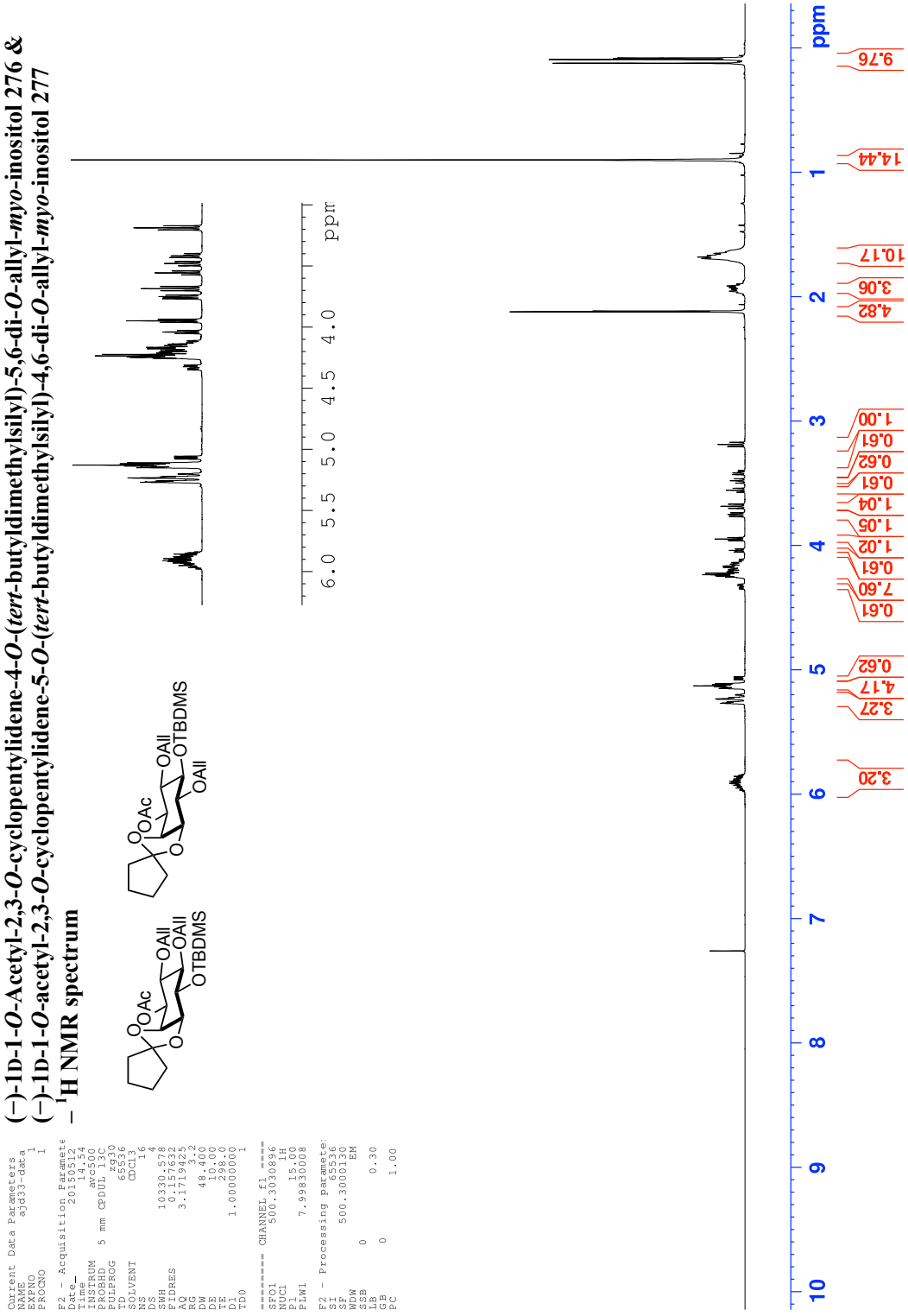
======== CHANNEL f1 ========
SFO1       500.3030896
NUC1            1H
P1             15.00
PLW1        7.99830008

F2 - Processing parameters
SI              65536
SF         500.3000139
WDW                EM
SSB             0
LB              0.30
GB              0
PC              1.00
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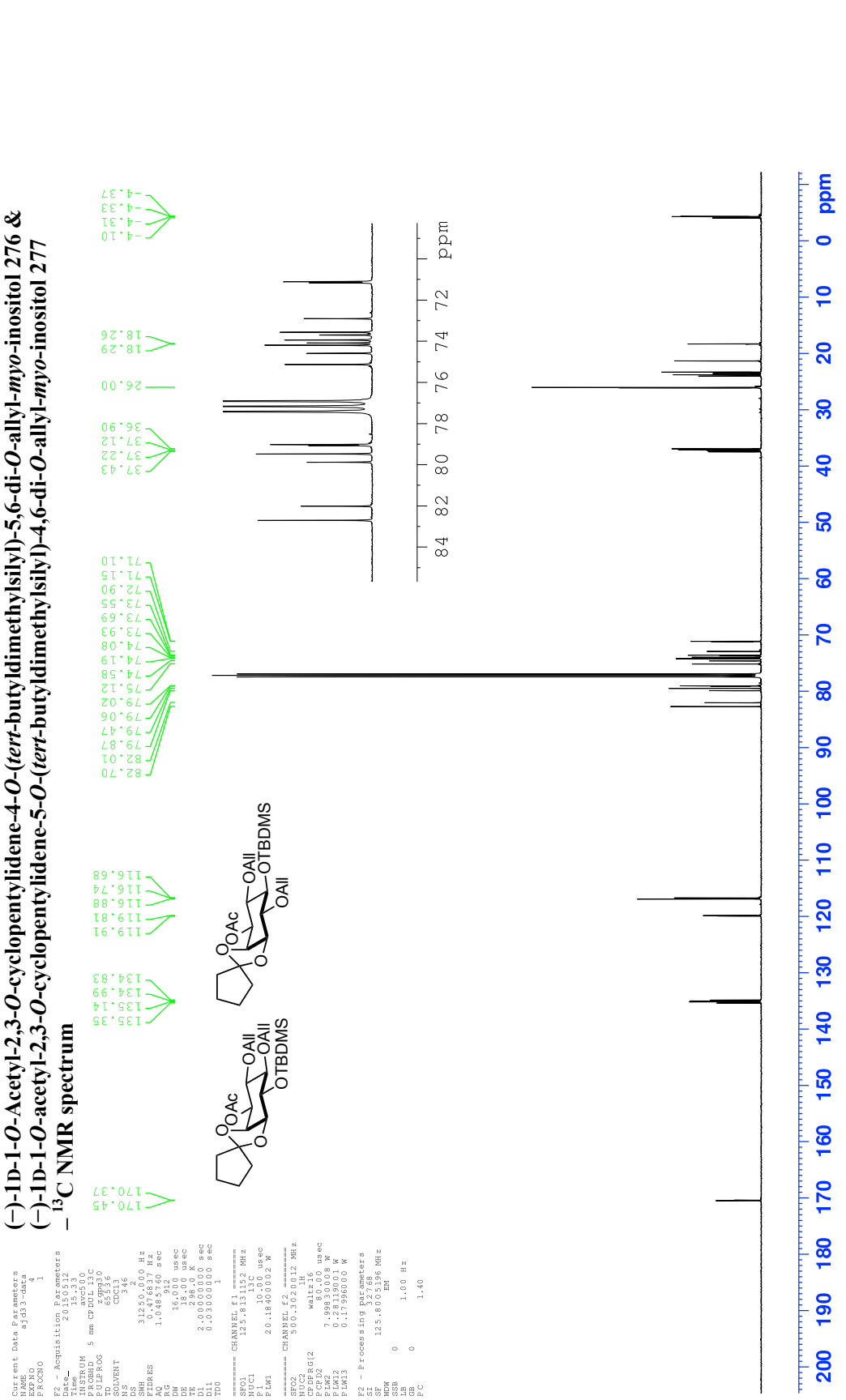
(–)-1D-1-*O*-Acetyl-2,3-*O*-cyclopentylidene-4,6-di-*O*-(*tert*-butyldimethylsilyl)-*myo*-inositol (–)-275 – ^{13}C NMR spectrum



(−)-1D-1-*O*-Acetyl-2,3-*O*-cyclopentylidene-4-*O*-(*tert*-butyldimethylsilyl)-5,6-di-*O*-allyl-*myo*-inositol 276 &
(−)-1D-1-*O*-acetyl-2,3-*O*-cyclopentylidene-5-*O*-(*tert*-butyldimethylsilyl)-4,6-di-*O*-allyl-*myo*-inositol 277
– ^{1}H NMR spectrum



(–)-1D-1-*O*-Acetyl-2,3-*O*-cyclopentylidene-4-*O*-(*tert*-butyldimethylsilyl)-5,6-di-*O*-allyl-*myo*-inositol 276 &
(–)-1D-1-*O*-acetyl-2,3-*O*-cyclopentylidene-5-*O*-(*tert*-butyldimethylsilyl)-4,6-di-*O*-allyl-*myo*-inositol 277
– ^{13}C NMR spectrum



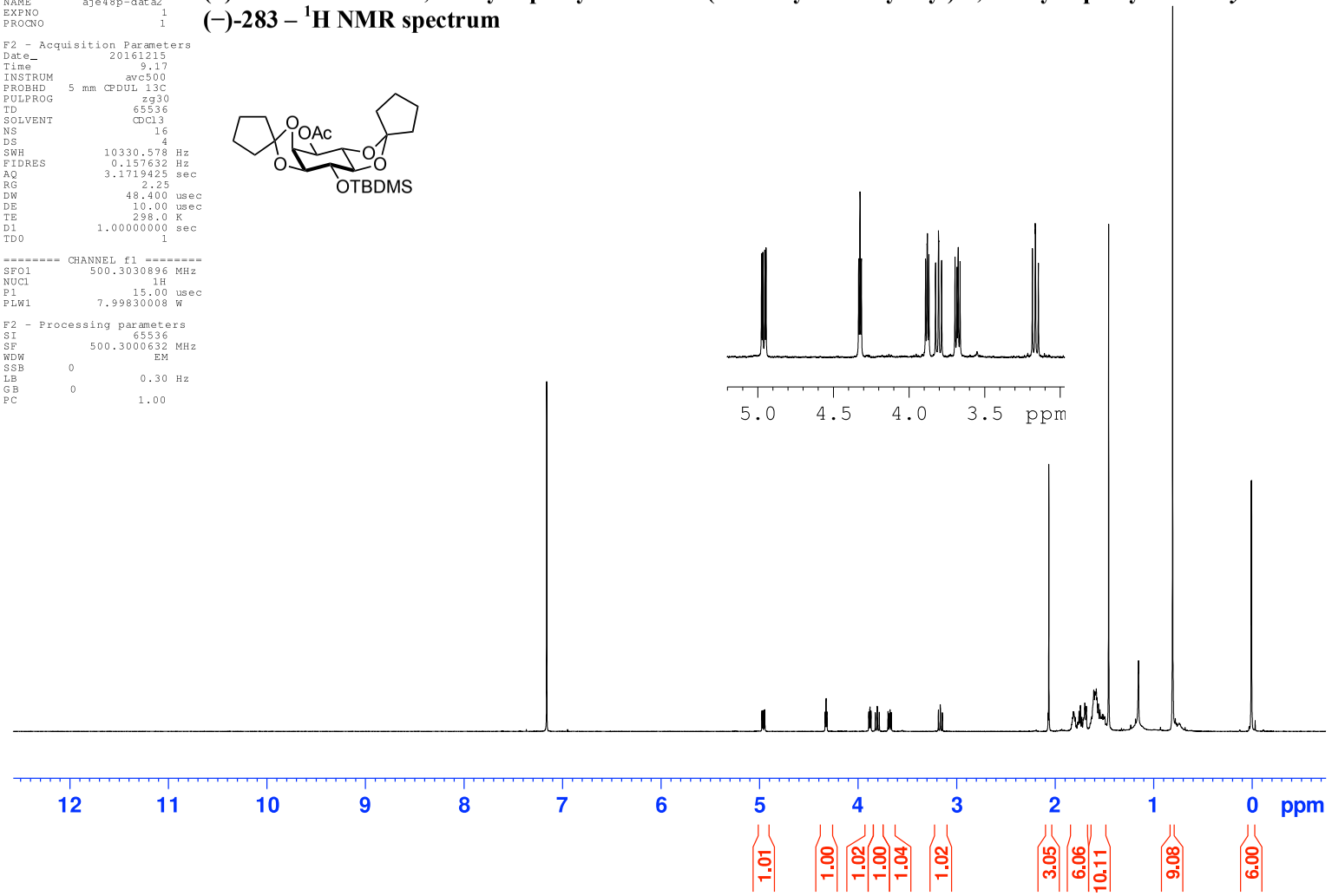
(−)-1D-1-*O*-Acetate-2,3-*O*-cyclopentylidene-4-*O*-(*tert*-butyldimethylsilyl)-5,6-*O*-cyclopentylidene-*myo*-inositol
(−)-283 – ^{1}H NMR spectrum

```
Current Data Parameters
NAME         aje48p-data2
EXPNO                   1
PROCNO                  1

F2 - Acquisition Parameters
Date_           20161215
Time               9.17
INSTRUM          avc500
PROBHD   5 mm CPDUL 13C
PULPROG            zg30
TD                65536
SOLVENT           CDCl3
NS                   16
DS                    4
SWH           10330.578 Hz
FIDRES        0.157632 Hz
AQ            3.1719425 sec
RG                 2.25
DW                48.400 usec
DE                10.00 usec
TE                298.0 K
D1           1.00000000 sec
TD0                   1

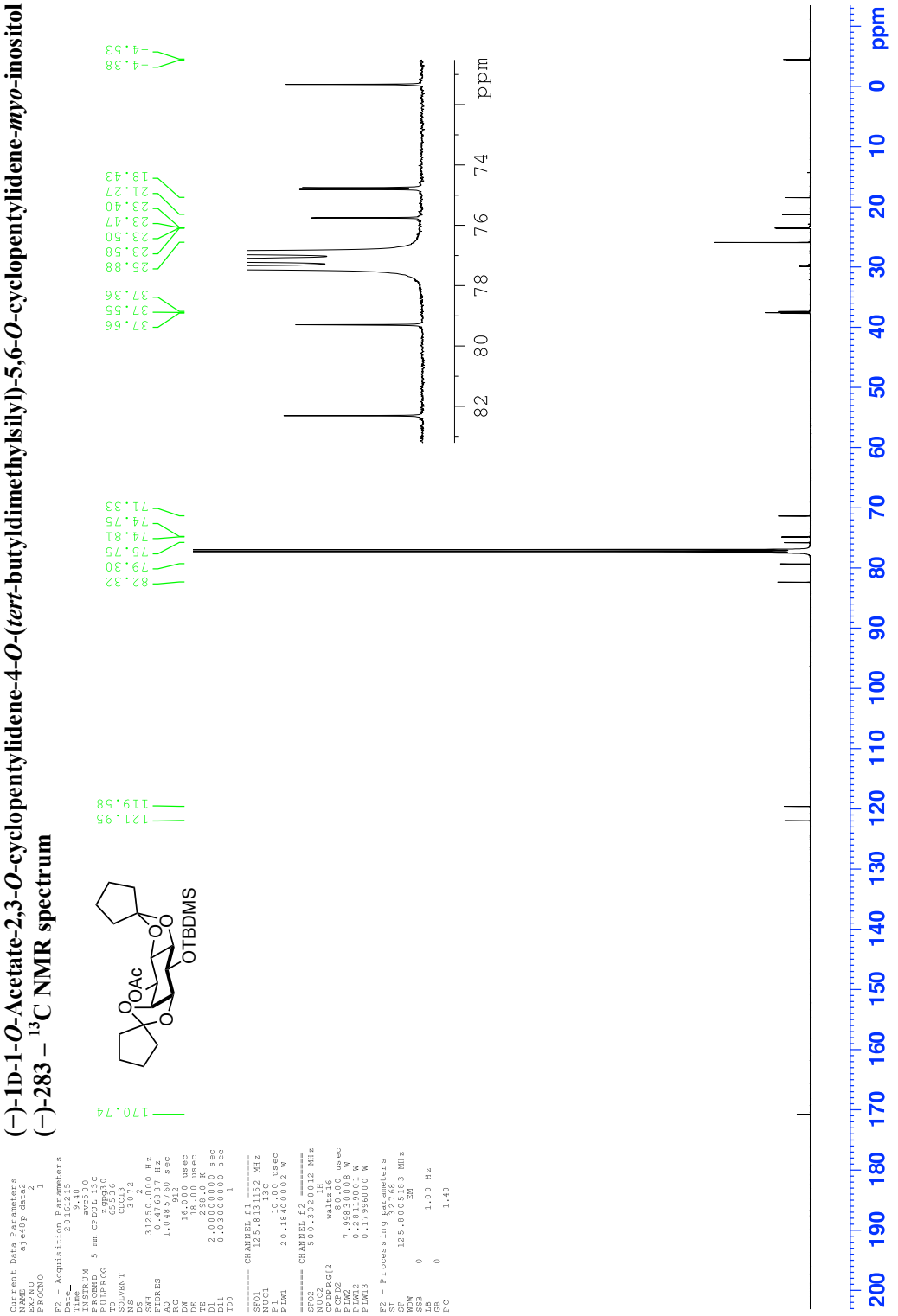
======== CHANNEL f1 ========
SFO1        500.3030896 MHz
NUC1                1H
P1                15.00 usec
PLW1         7.99830008 W

F2 - Processing parameters
SI                65536
SF          500.3000632 MHz
WDW                 EM
SSB       0
LB                0.30 Hz
GB        0
PC                1.00
```

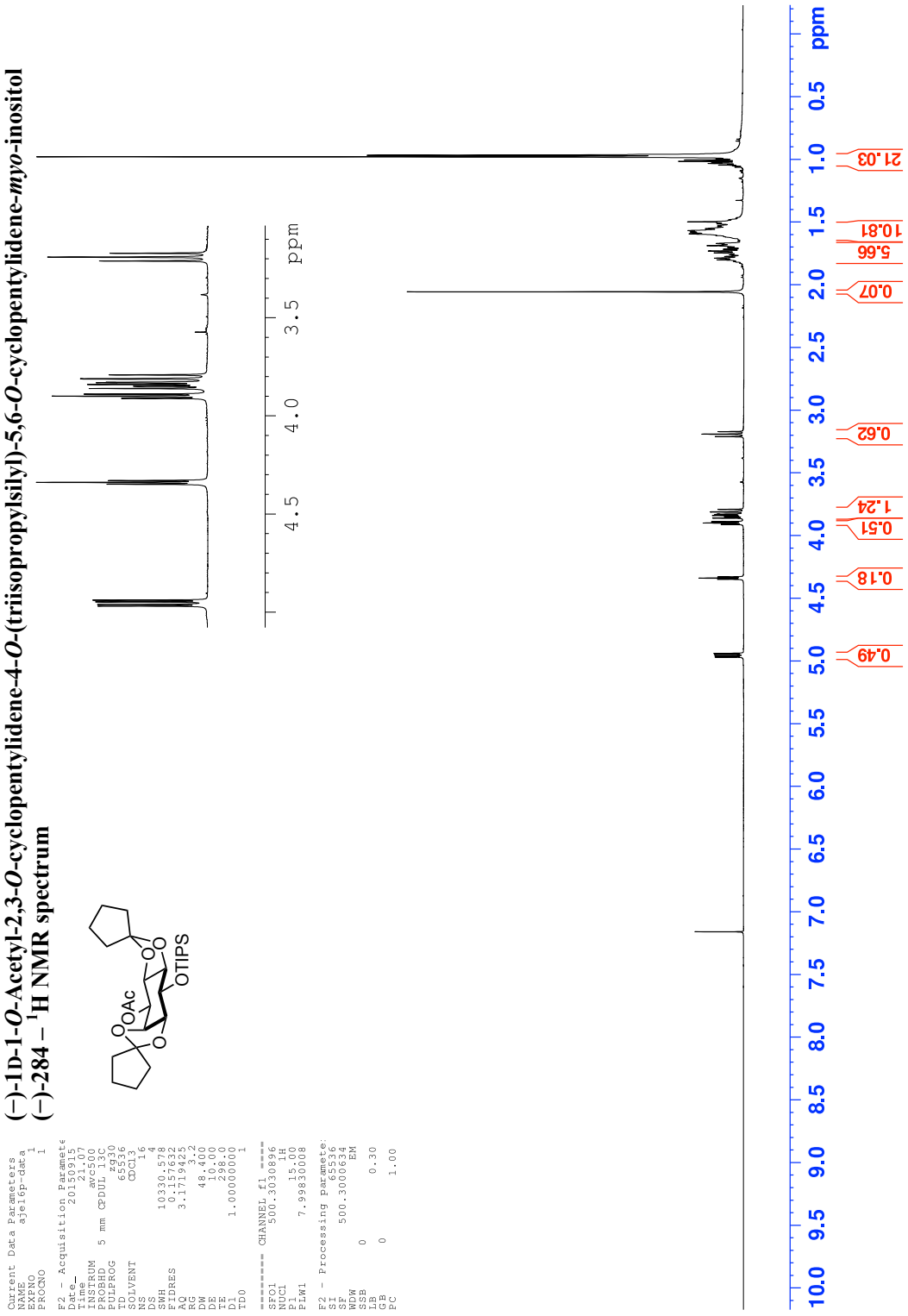


(–)-1D-1-*O*-Acetate-2,3-*O*-cyclopentylidene-4-*O*-(*tert*-butyldimethylsilyl)-5,6-*O*-cyclopentylidene-*myo*-inositol

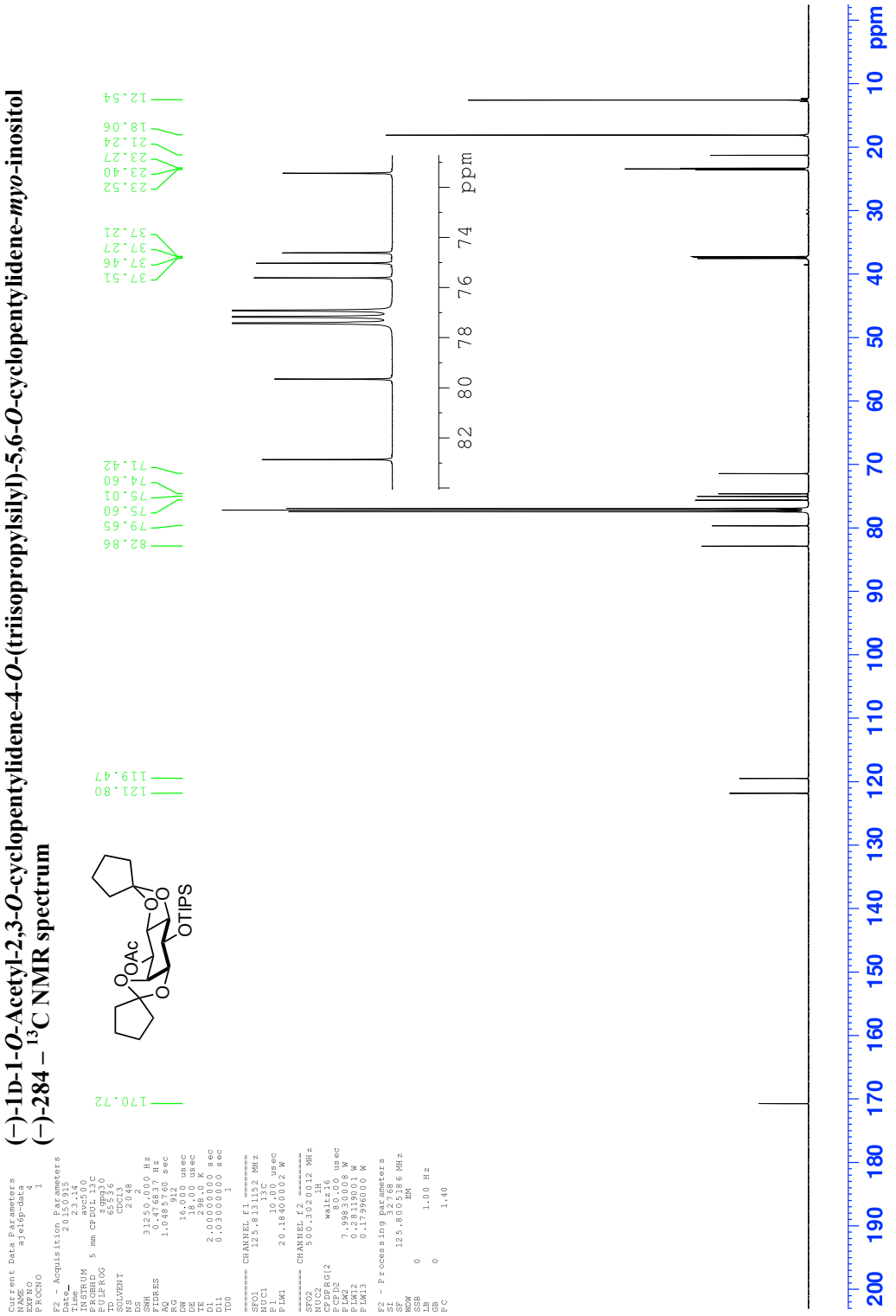
(–)-283 – ^{13}C NMR spectrum



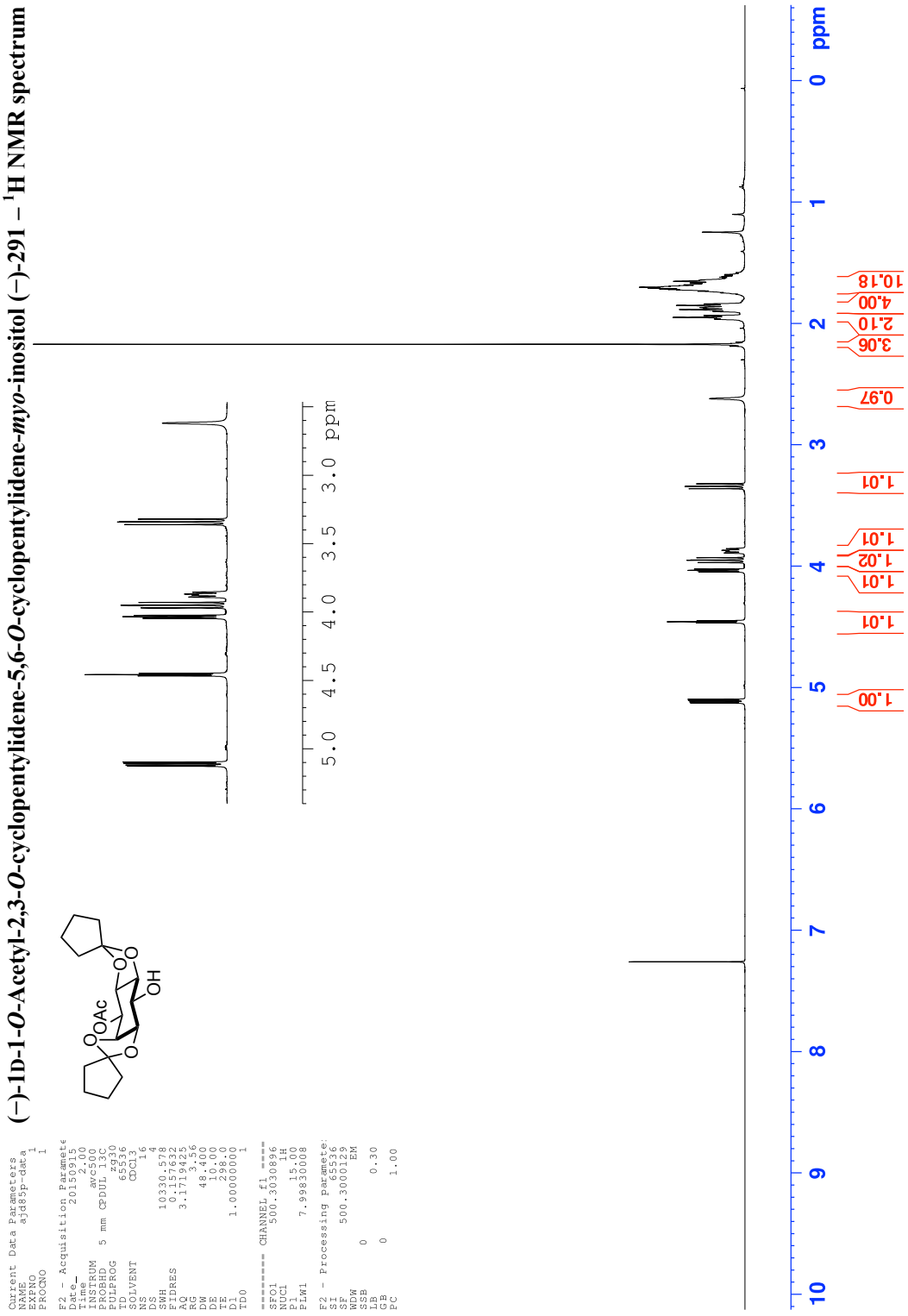
(−)-1D-1-*O*-Acetyl-2,3-*O*-cyclopentylidene-4-*O*-(triisopropylsilyl)-5,6-*O*-cyclopentylidene-*myo*-inositol
(−)-284 – ^{1}H NMR spectrum



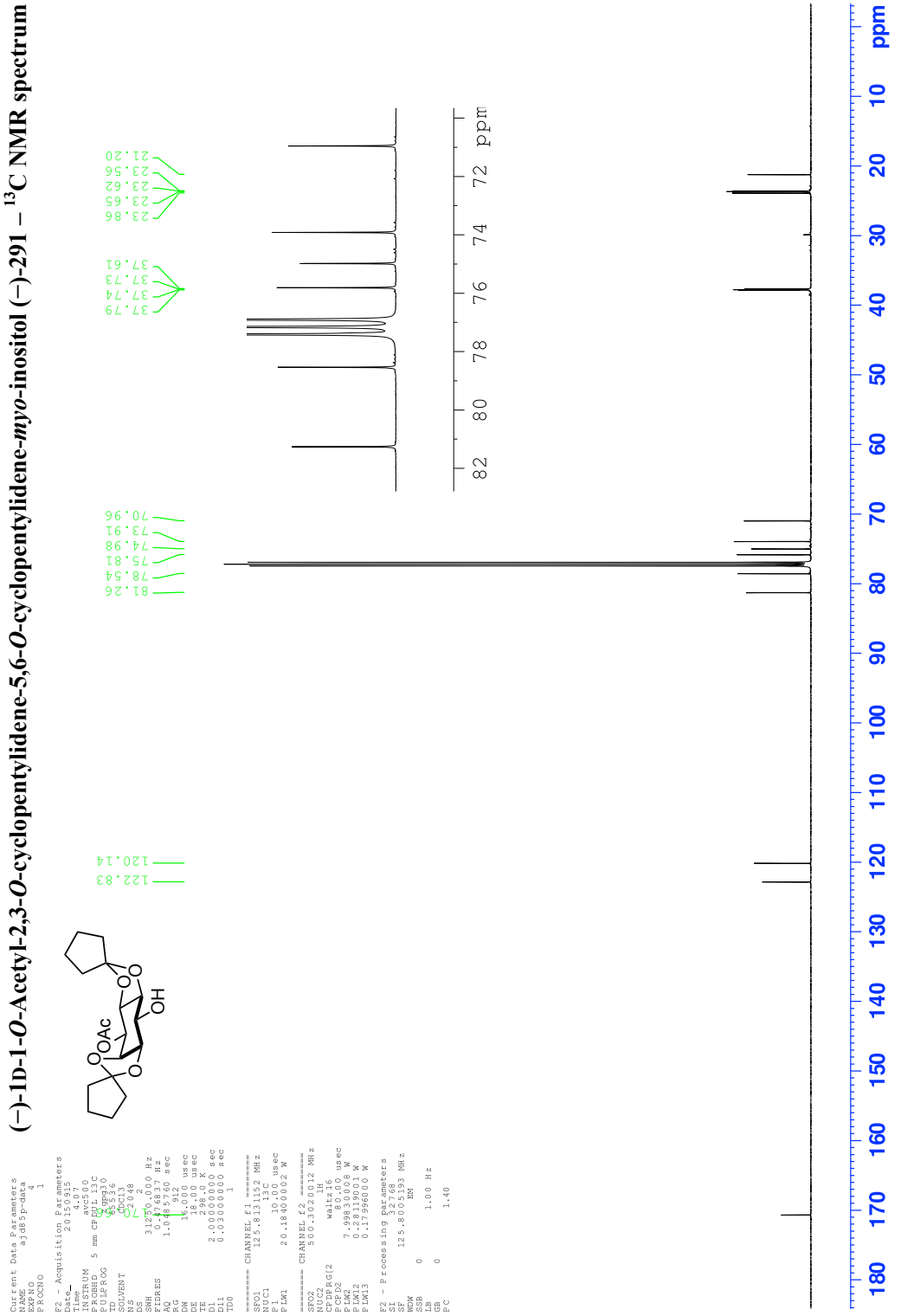
(–)-1D-1-*O*-Acetyl-2,3-*O*-cyclopentylidene-4-*O*-(triisopropylsilyl)-5,6-*O*-cyclopentylidene-*myo*-inositol
(–)-284 – ^{13}C NMR spectrum



(−)-1D-1-*O*-Acetyl-2,3-*O*-cyclopentylidene-5,6-*O*-cyclopentylidene-*myo*-inositol (−)-291 – ^{1}H NMR spectrum



(−)-1D-1-*O*-Acetyl-2,3-*O*-cyclopentylidene-5,6-*O*-cyclopentylidene-*myo*-inositol (−)-291 – ^{13}C NMR spectrum

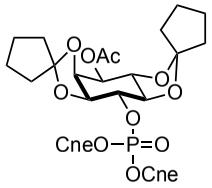


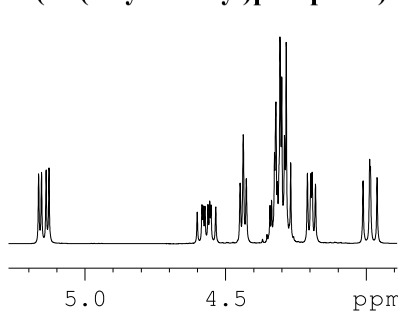
(−)-1D-1-*O*-Acetate-2,3-*O*-cyclopentylidene-4-*O*-(bis(2-cyanoethyl)phosphate)-5,6-*O*-cyclopentylidene-*myo*-inositol (−)-293 – ^{1}H NMR spectrum

```
Current Data Parameters
NAME      aje71p2-datanoEtOAc
EXPNO                       1
PROCNO                      1

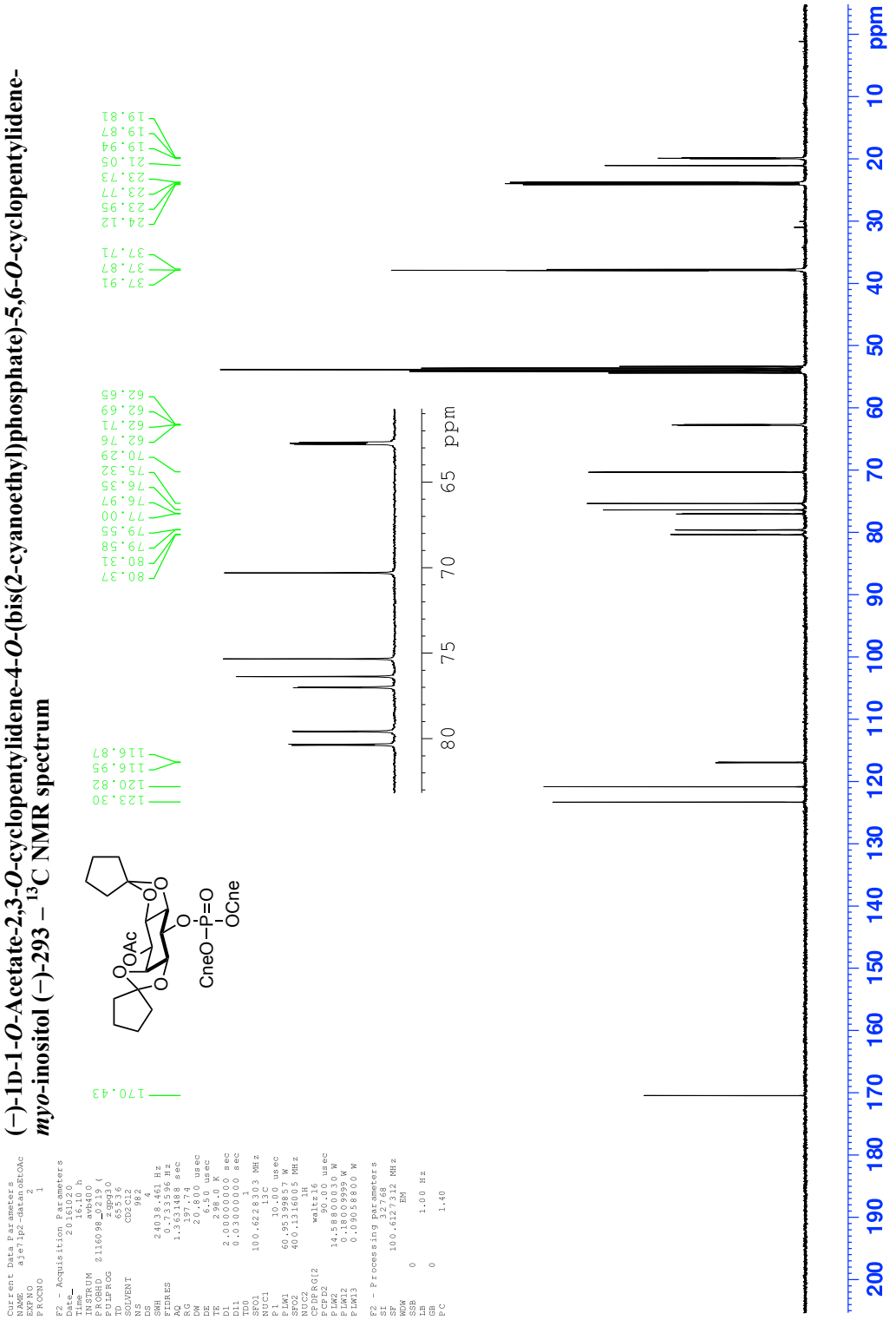
F2 - Acquisition Parameters
Date_            20161020
Time                15.13 h
INSTRUM            avb400
PROBHD    Z116098_0219 (
PULPROG              zg30
TD                  65536
SOLVENT           CD2Cl2
NS                    16
DS                     2
SWH              8012.820 Hz
FIDRES           0.244532 Hz
AQ               4.0894465 sec
RG                  30.76
DW                 62.400 usec
DE                  6.50 usec
TE                298.0 K
D1             1.00000000 sec
TD0                    1
SFO1           400.1320007 MHz
NUC1                  1H
P1                 10.00 usec
PLW1           14.58800030 W

F2 - Processing parameters
SI                  65536
SF             400.1300155 MHz
WDW                   EM
SSB       0
LB                 0.30 Hz
GB        0
PC                 1.00
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(−)-1D-1-*O*-Acetate-2,3-*O*-cyclopentylidene-4-*O*-(bis(2-cyanoethyl)phosphate)-5,6-*O*-cyclopentylidene-*myo*-inositol (−)-293 – ^{13}C NMR spectrum

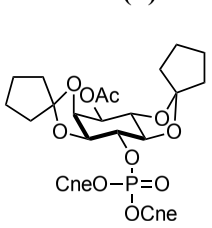


(−)-1D-1-*O*-Acetate-2,3-*O*-cyclopentylidene-4-*O*-(bis(2-cyanoethyl)phosphate)-5,6-*O*-cyclopentylidene-*myo*-inositol (−)-293 – ^{31}P NMR spectrum

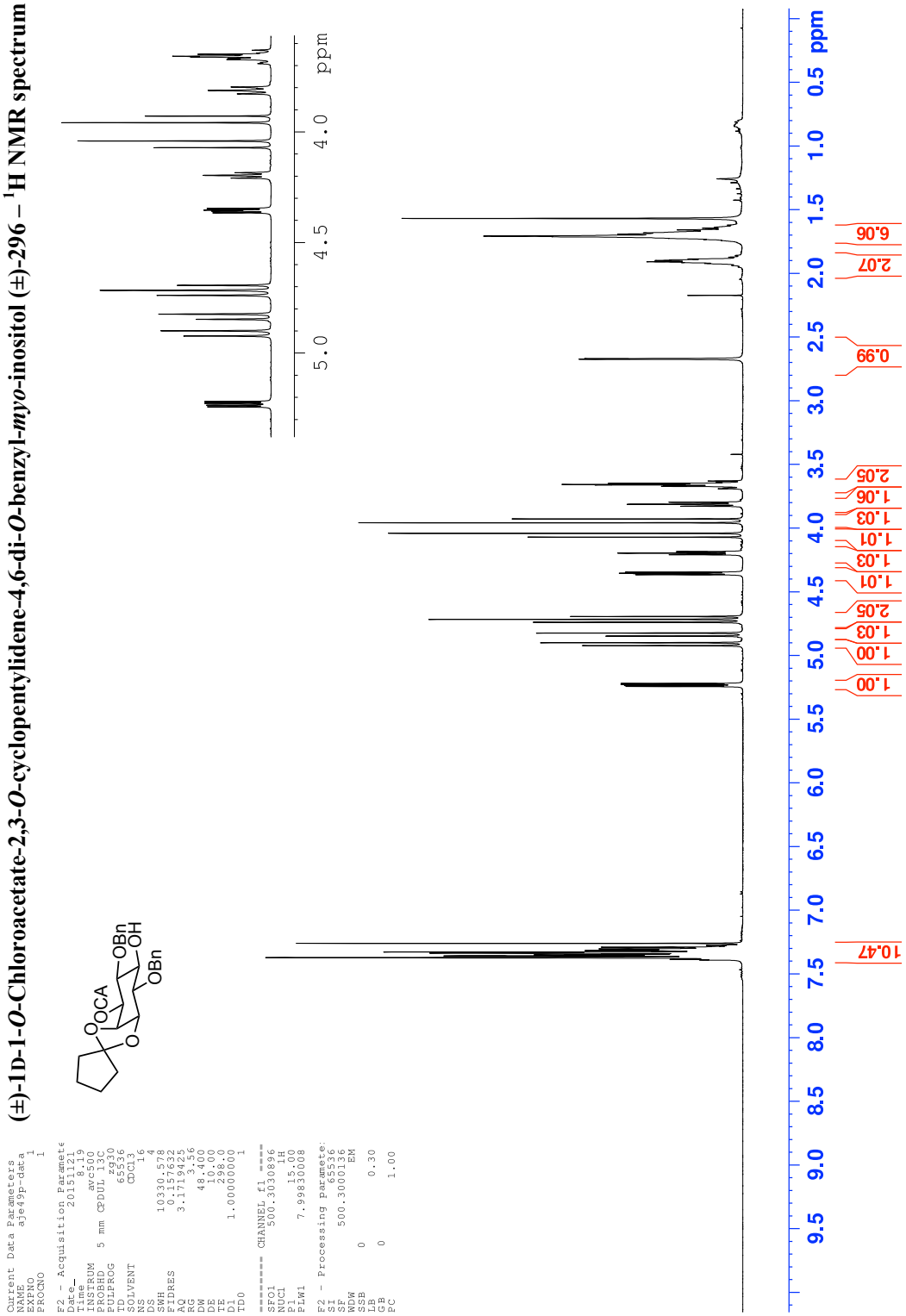
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Current Data Parameters
NAME      aje71-PNMRinDCM
EXPNO                   2
PROCNO                  1

F2 - Acquisition Paramete
Date_           20161130
Time              14.46
INSTRUM          avb400
PROBHD    Z116098_0219 (
PULPROG         zgpg30
TD               65536
SOLVENT          CDCl3
NS                  19
DS                   4
SWH           64102.562
FIDRES        1.9562255
AQ            0.5111808
RG               197.74
DW                7.800
DE                6.50
TE              298.1
D1           2.00000000
D11          0.03000000
TD0                  1
SFO1       161.9755930
NUC1              31P
P1               8.00
PLW1       54.00000000
SFO2       400.1316005
NUC2              1H
CPDPRG[2        waltz16
PCPD2            90.00
PLW2       14.58800030
PLW12       0.18009999
PLW13       0.09058800

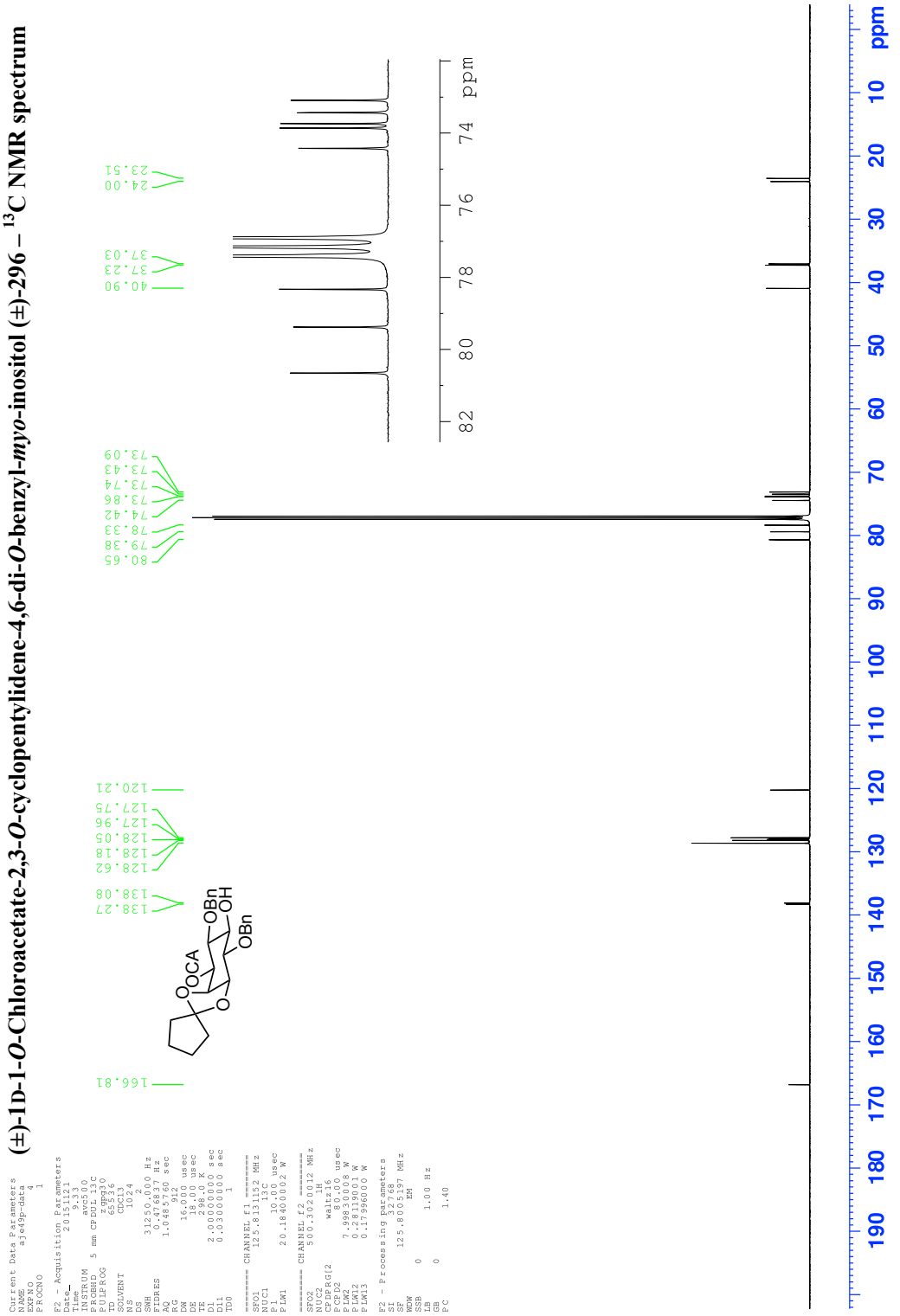
F2 - Processing paramete
SI               32768
SF         161.9755930
WDW                 EM
SSB       0
LB                1.00
GB        0
PC                1.40
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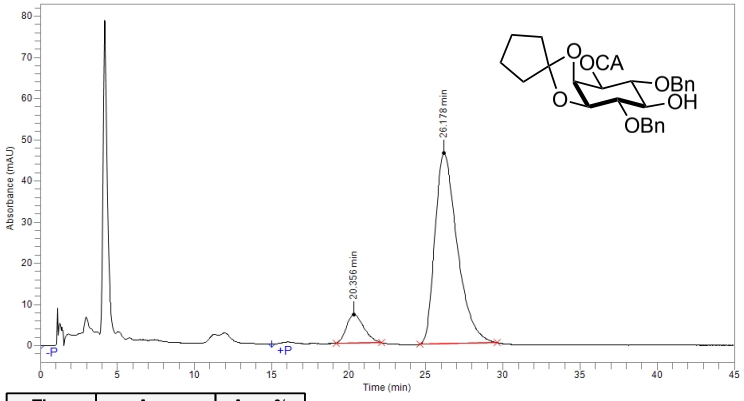
(±)-1D-1-*O*-Chloroacetate-2,3-*O*-cyclopentylidene-4,6-di-*O*-benzyl-*myo*-inositol (±)-296 – ^{1}H NMR spectrum



(±)-1D-1-*O*-Chloroacetate-2,3-*O*-cyclopentylidene-4,6-di-*O*-benzyl-*myo*-inositol (±)-296 – ^{13}C NMR spectrum

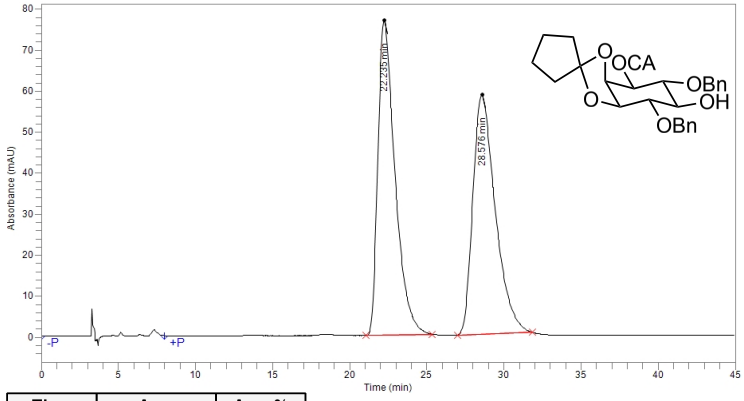


(–)-1D-1-*O*-Chloroacetate-2,3-*O*-cyclopentylidene-4,6-di-*O*-benzyl-*myo*-inositol (–)-296 – Chiral HPLC bulk material (Heptane/IPA 90:10, 0.8 mL.min^{-1}, 254 nm)



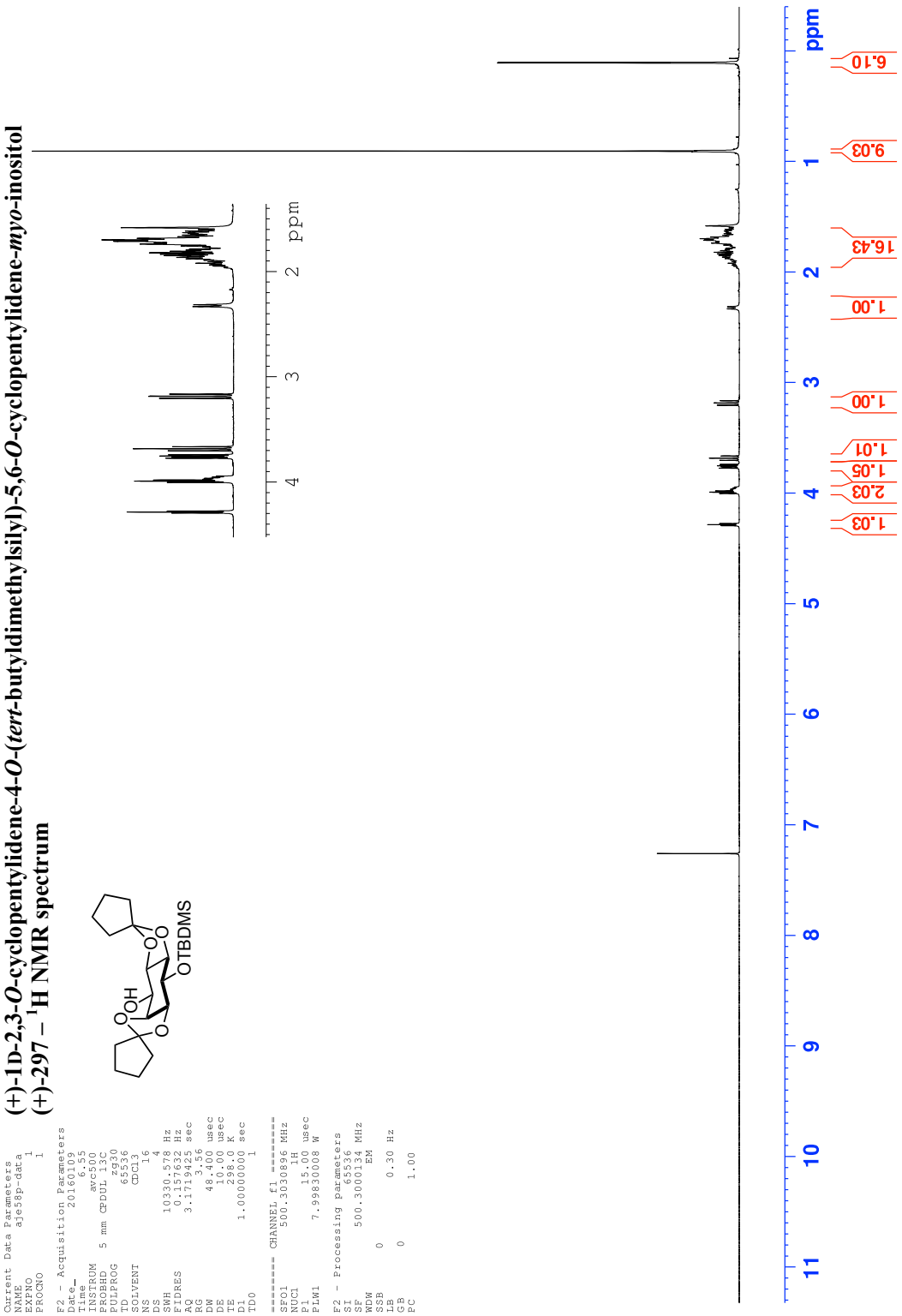
Time	Area	Area %
20.356	539,337	10.36
26.178	4,668,579	89.64
Total	5,207,916	100.00

(±)-1D-1-*O*-Chloroacetate-2,3-*O*-cyclopentylidene-4,6-di-*O*-benzyl-*myo*-inositol (±)-296 – Chiral HPLC crystals (Heptane/IPA 90:10, 0.8 mL.min^{-1}, 254 nm)

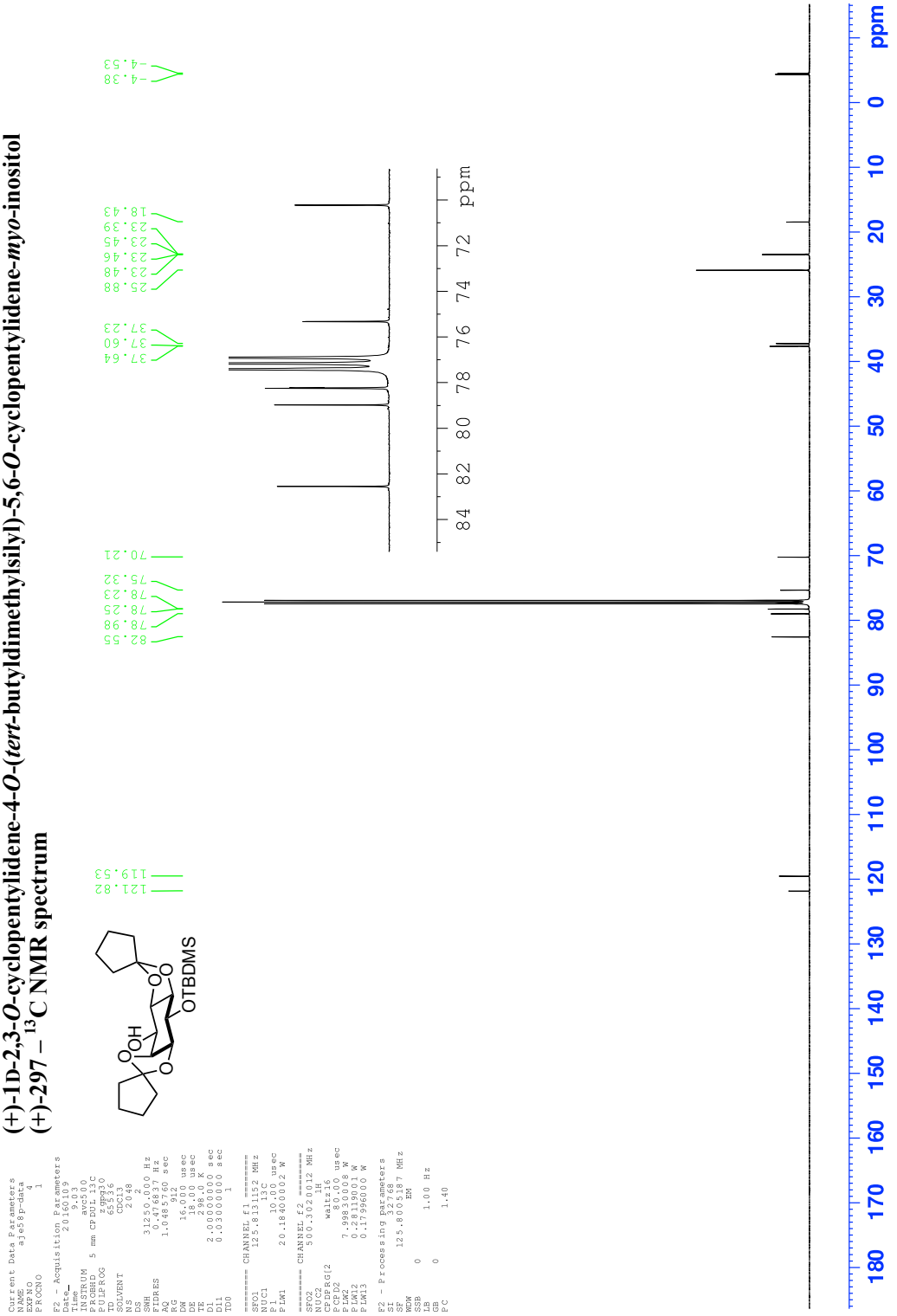


Time	Area	Area %
22.235	5,917,198	50.03
28.576	5,909,600	49.97
Total	11,826,798	100.00

(+)-1D-2,3-*O*-cyclopentylidene-4-*O*-(*tert*-butyldimethylsilyl)-5,6-*O*-cyclopentylidene-*myo*-inositol
(+)-297 – ^{1}H NMR spectrum



(+)-1D-2,3-*O*-cyclopentylidene-4-*O*-(*tert*-butyldimethylsilyl)-5,6-*O*-cyclopentylidene-*myo*-inositol
(+)-297 – ^{13}C NMR spectrum



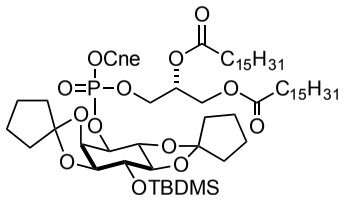
(−)-1D-1-*O*-(1,2-Di-*O*-palmitoyl-*sn*-glycer-3-yl-(2-cyanoethyl)phosphate)-2,3-*O*-cyclopentylidene-4-*O*-(*tert*-butyldimethylsilyl)-5,6-*O*-cyclopentylidene-*myo*-inositol (−)-288 – ^{1}H NMR spectrum

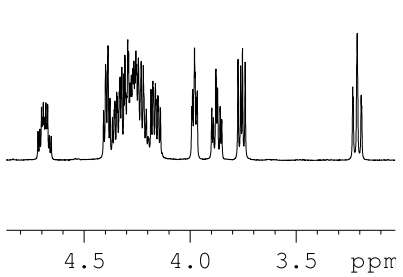
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Current Data Parameters
NAME          ajf10p-data
EXPNO                   1
PROCNO                  1

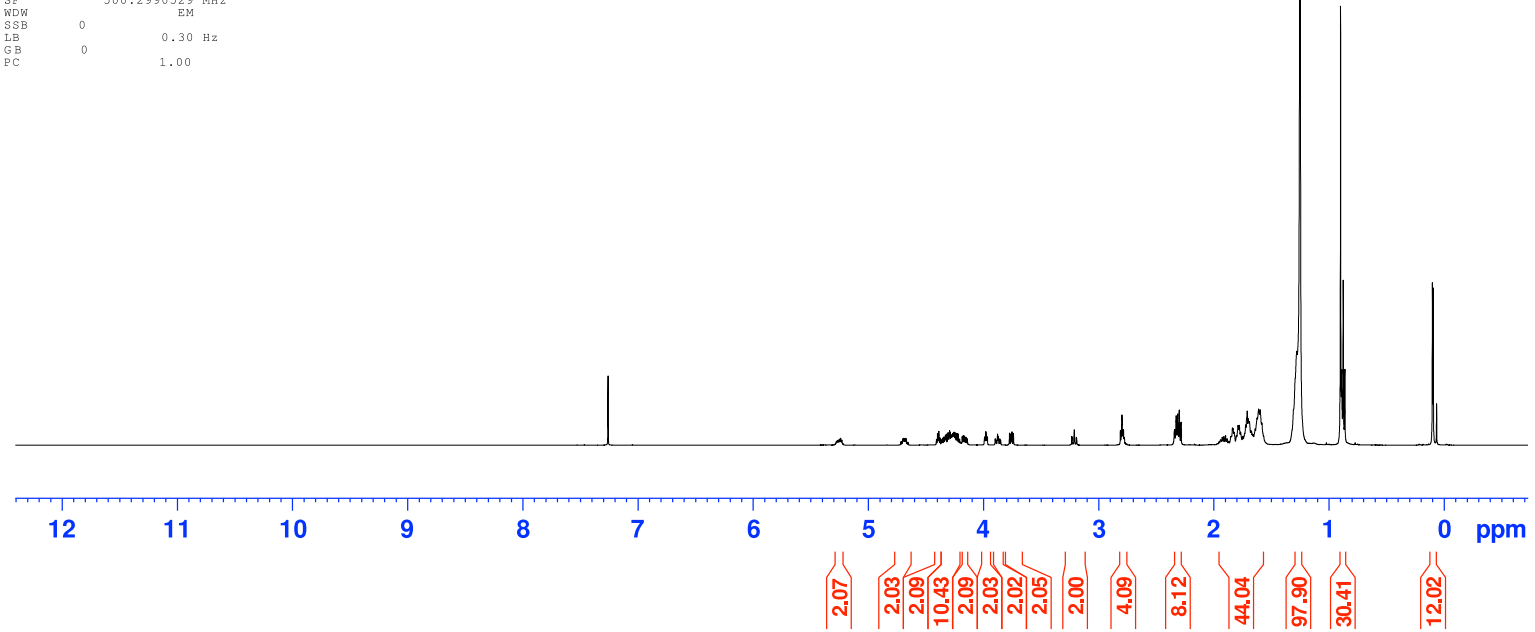
F2 - Acquisition Parameters
Date_            20160414
Time                11.15
INSTRUM           avc500
PROBHD   5 mm CPDUL 13C
PULPROG             zg30
TD                 65536
SOLVENT           CD2Cl2
NS                    16
DS                     4
SWH            10330.578 Hz
FIDRES         0.157632 Hz
AQ             3.1719425 sec
RG                  3.56
DW                48.400 usec
DE                10.00 usec
TE                298.0 K
D1            1.00000000 sec
TD0                    1

======== CHANNEL f1 ========
SFO1          500.3030896 MHz
NUC1                  1H
P1                 15.00 usec
PLW1           7.99830008 W

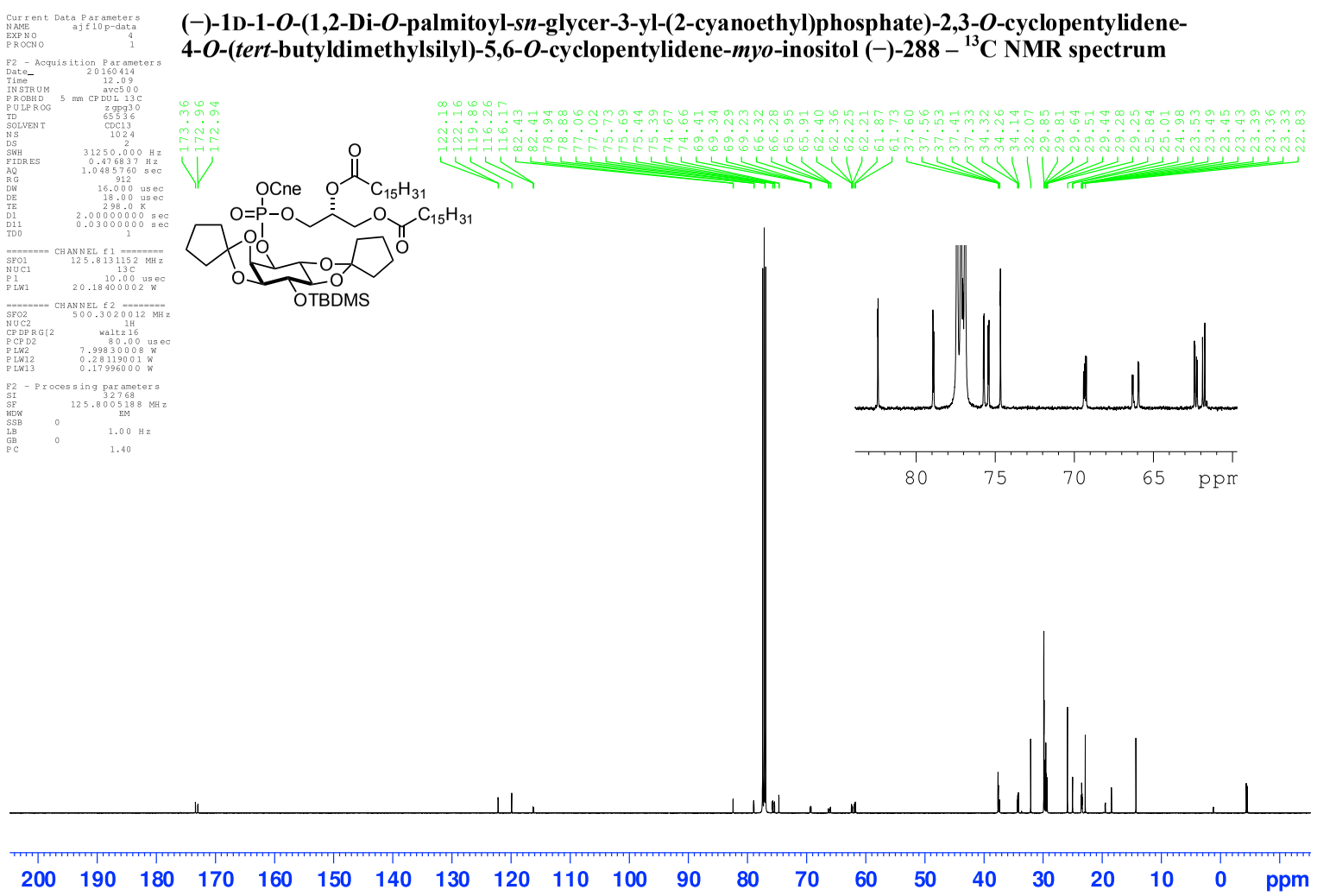
F2 - Processing parameters
SI                 65536
SF            500.2990529 MHz
WDW                   EM
SSB       0
LB                 0.30 Hz
GB        0
PC                 1.00
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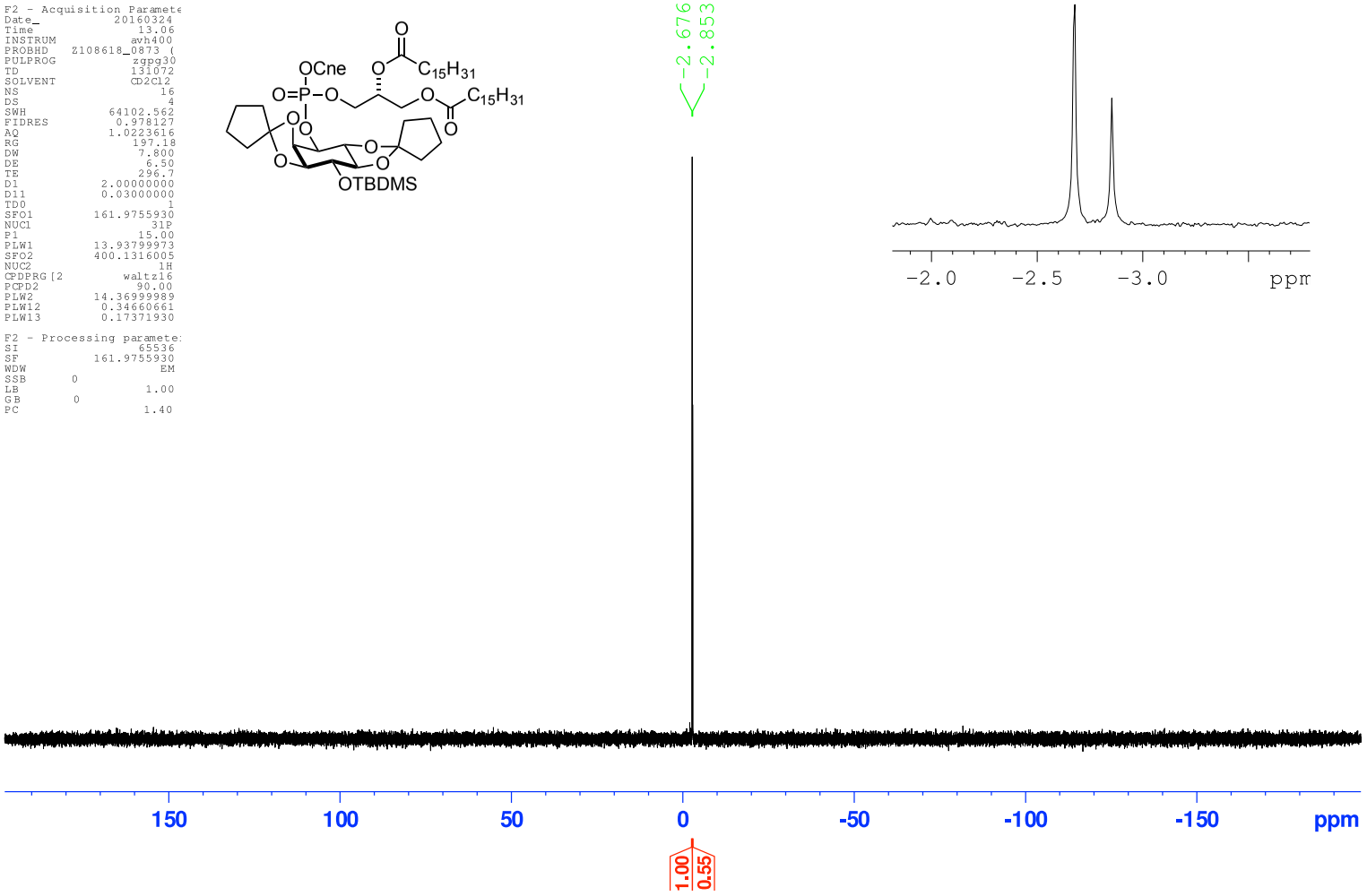




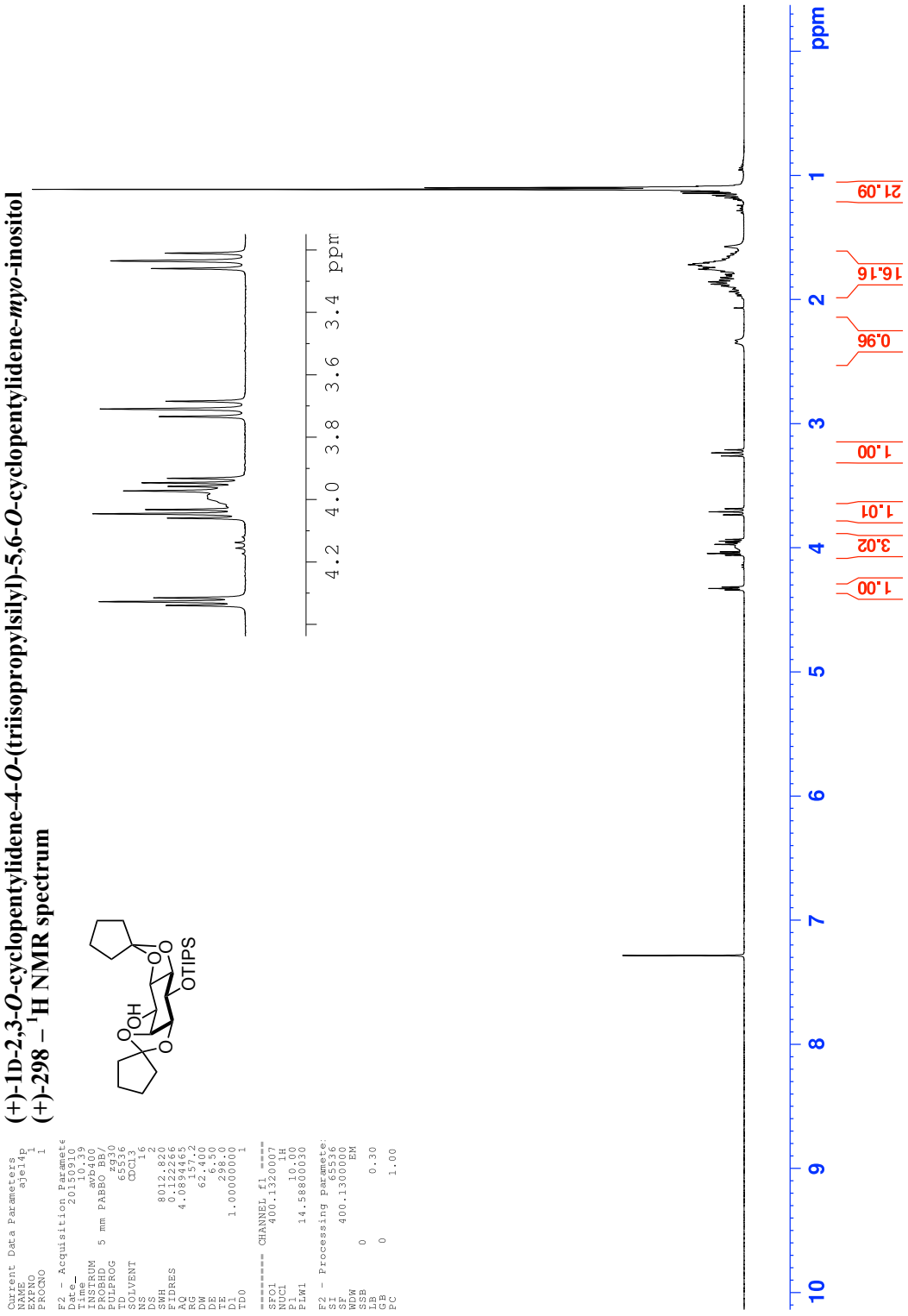
(−)-1D-1-*O*-(1,2-Di-*O*-palmitoyl-*sn*-glycer-3-yl-(2-cyanoethyl)phosphate)-2,3-*O*-cyclopentylidene-4-*O*-(*tert*-butyldimethylsilyl)-5,6-*O*-cyclopentylidene-*myo*-inositol (−)-288 – ^{13}C NMR spectrum



(−)-1D-1-*O*-(1,2-Di-*O*-palmitoyl-*sn*-glycer-3-yl-(2-cyanoethyl)phosphate)-2,3-*O*-cyclopentylidene-4-*O*-(*tert*-butyldimethylsilyl)-5,6-*O*-cyclopentylidene-*myo*-inositol (−)-288 – ^{31}P NMR spectrum



(+)-1D-2,3-*O*-cyclopentylidene-4-*O*-(triisopropylsilyl)-5,6-*O*-cyclopentylidene-*myo*-inositol
(+)-298 – ^{1}H NMR spectrum

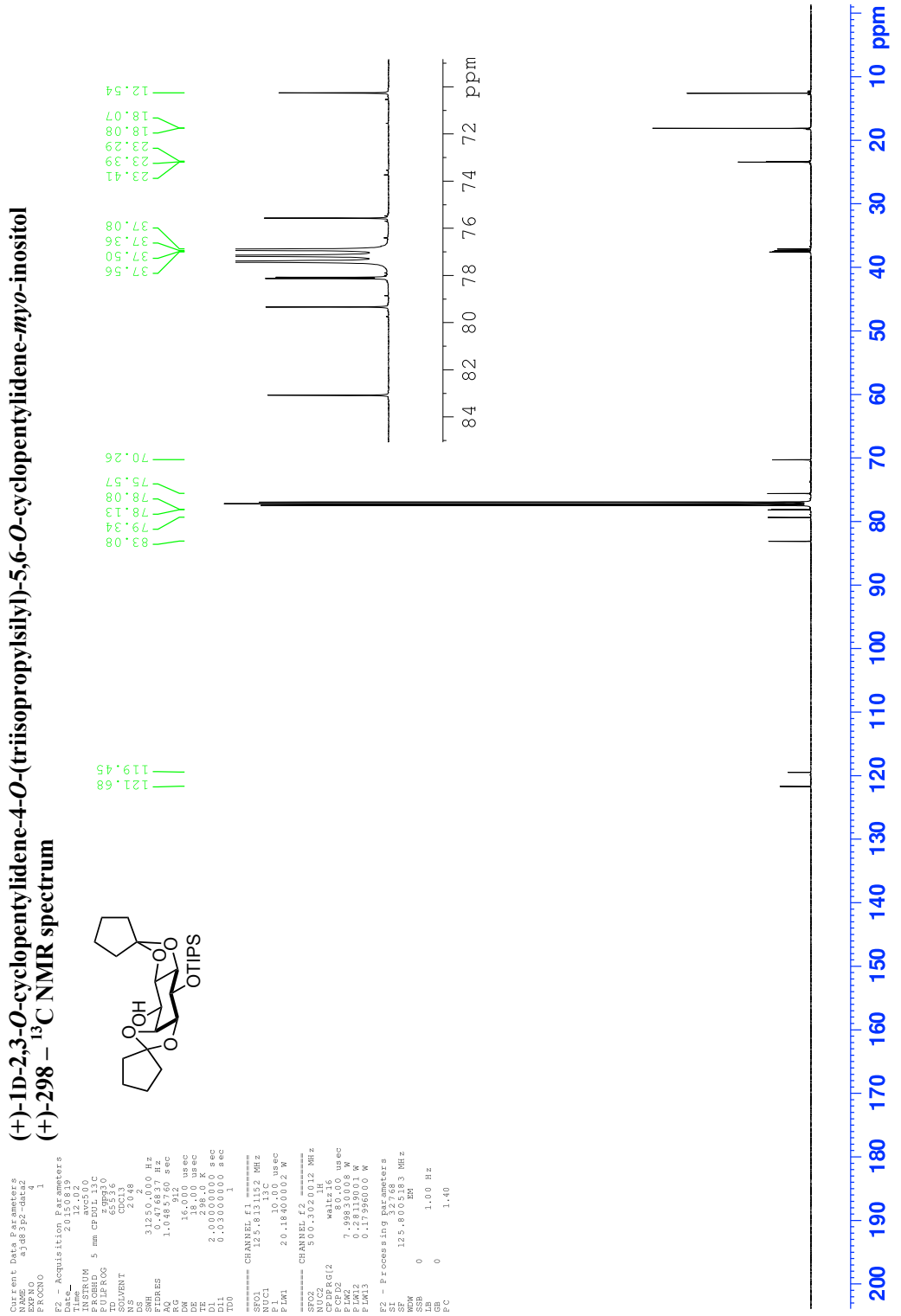



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Current Data Parameters
NAME                 ajel14p
EXPNO                      1
PROCNO                     1

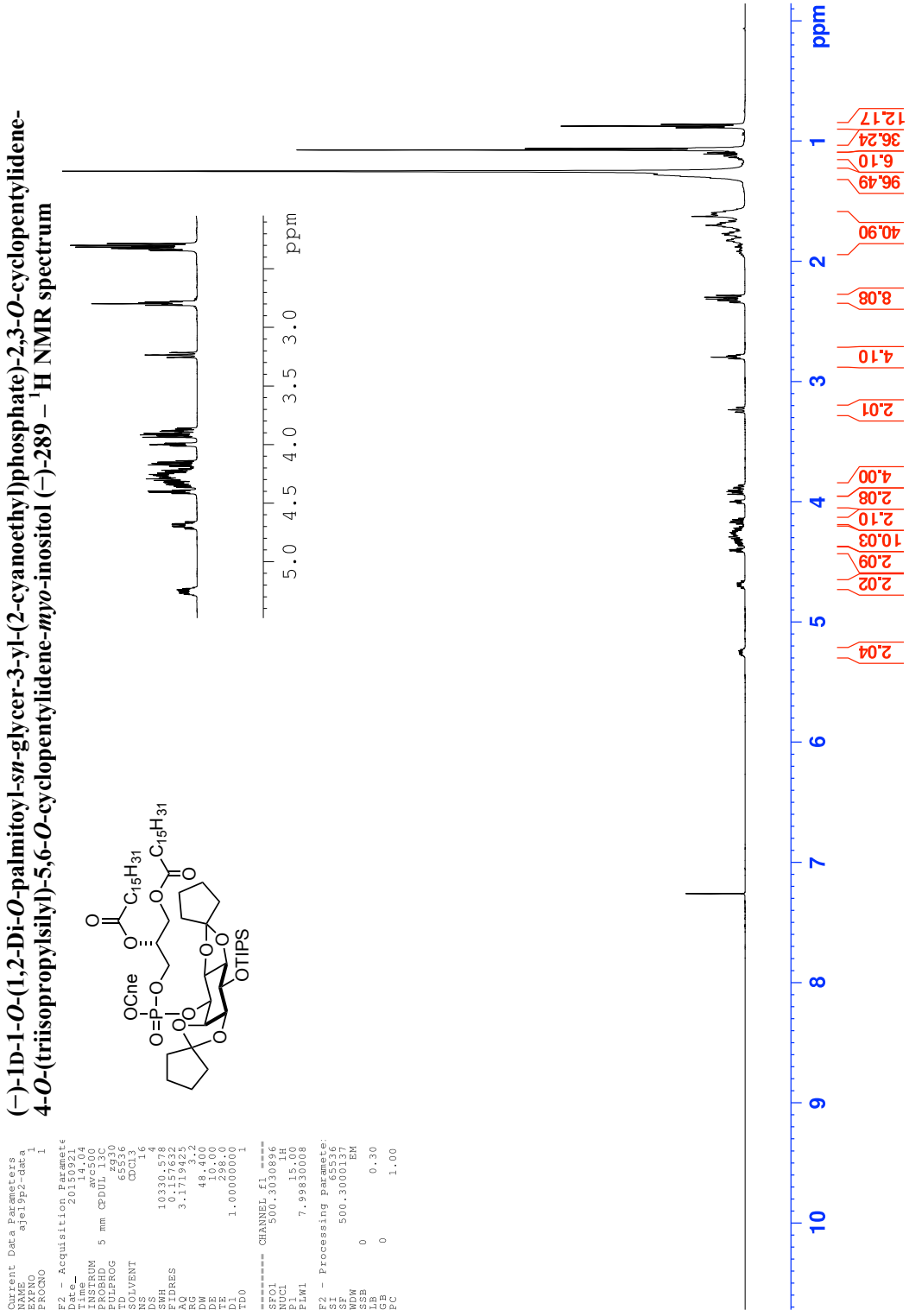
F2 - Acquisition Parameters
Date_              20150910
Time               10.39
INSTRUM                avb400
PROBHD     5 mm PABBO BB/
PULPROG                zg30
TD                     65536
SOLVENT                CDCl3
NS                        16
DS                         2
SWH                8012.820
FIDRES             0.122266
AQ                 4.0894465
RG                    57.2
DW                    62.400
DE                     6.50
TE                    298.0
D1                 1.00000000
TD0                        1

======== CHANNEL f1 ========
SFO1               400.1320007
NUC1                       1H
P1                        10.00
PLW1               14.58800030

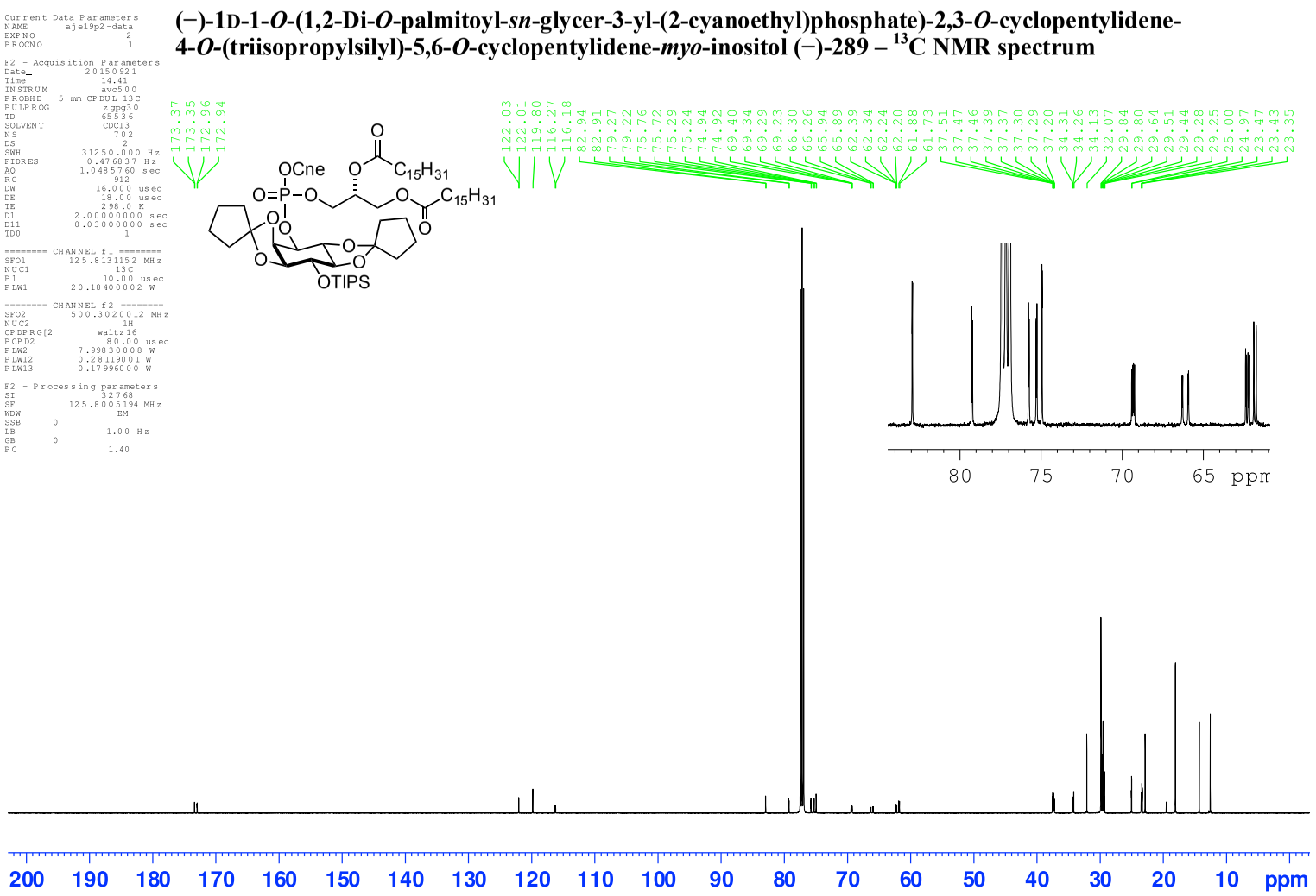
F2 - Processing parameters
SI                        65536
SF                 400.1300000
WDW                           EM
SSB                0
LB                         0.30
GB                 0
PC                         1.00
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(+)-1D-2,3-O-cyclopentylidene-4-O-(triisopropylsilyl)-5,6-O-cyclopentylidene-myo-inositol
(+)-298 – 13C NMR spectrum
OTIPS
OH
121.68
119.45
83.08
79.34
78.13
78.08
75.57
70.26
37.56
37.50
37.36
37.08
23.41
23.39
23.29
18.08
18.07
12.54
84 82 80 78 76 74 72 ppm
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 ppm

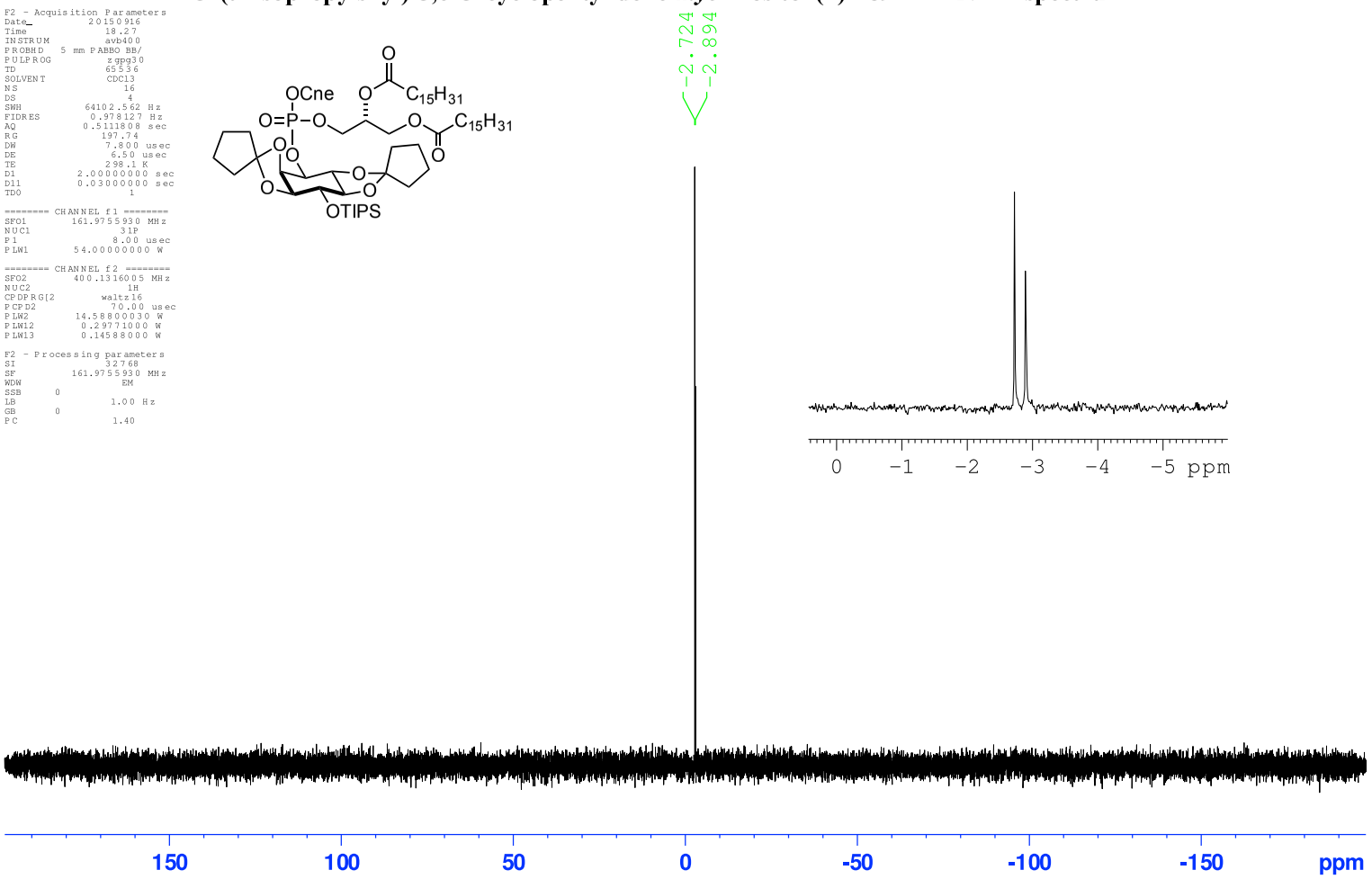
(−)-1D-1-*O*-(1,2-Di-*O*-palmitoyl-*sn*-glycer-3-yl-(2-cyanoethyl)phosphate)-2,3-*O*-cyclopentylidene-4-*O*-(triisopropylsilyl)-5,6-*O*-cyclopentylidene-*myo*-inositol (−)-289 – ^{1}H NMR spectrum



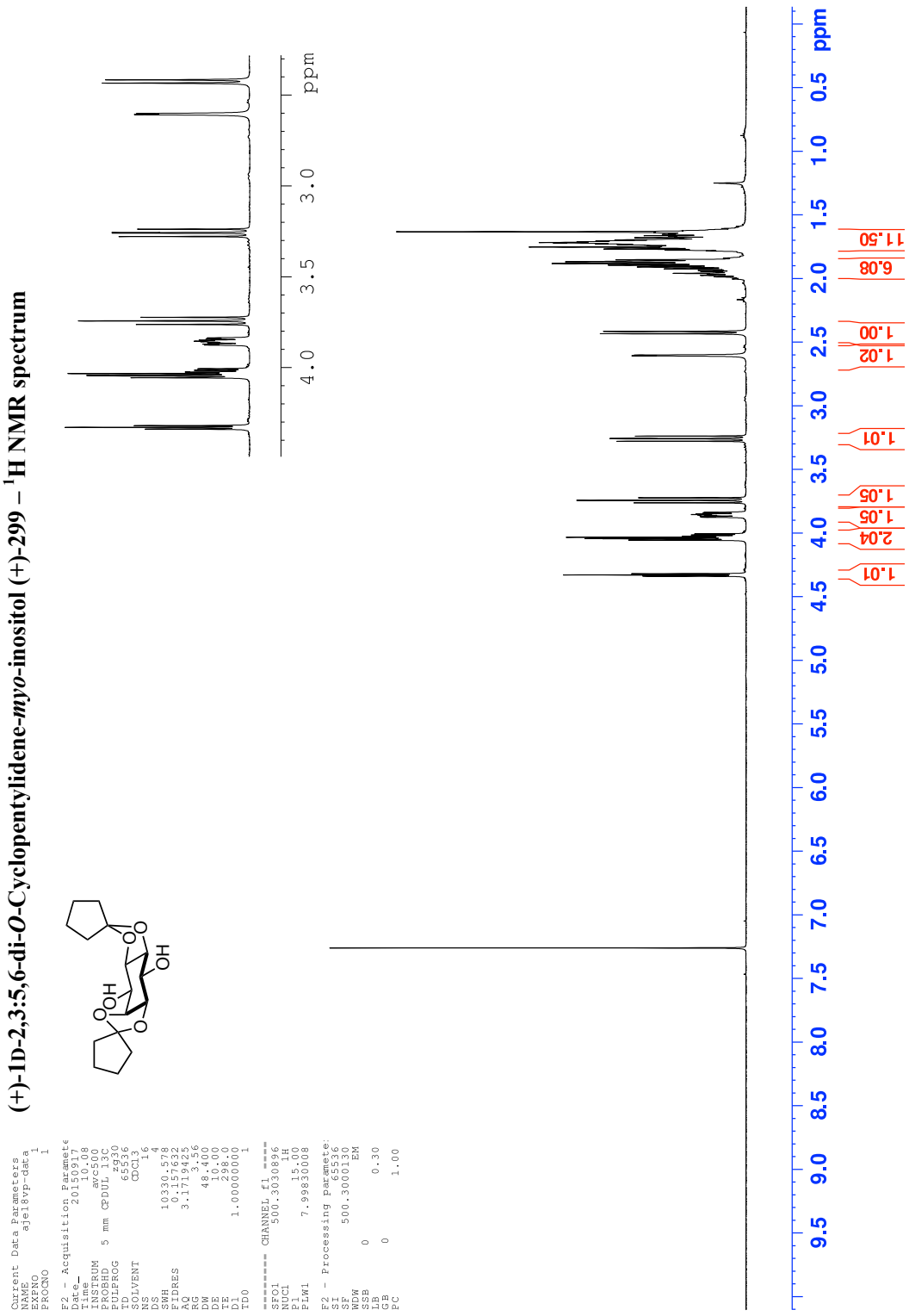
(−)-1D-1-*O*-(1,2-Di-*O*-palmitoyl-*sn*-glycer-3-yl-(2-cyanoethyl)phosphate)-2,3-*O*-cyclopentylidene-4-*O*-(triisopropylsilyl)-5,6-*O*-cyclopentylidene-*myo*-inositol (−)-289 – ^{13}C NMR spectrum

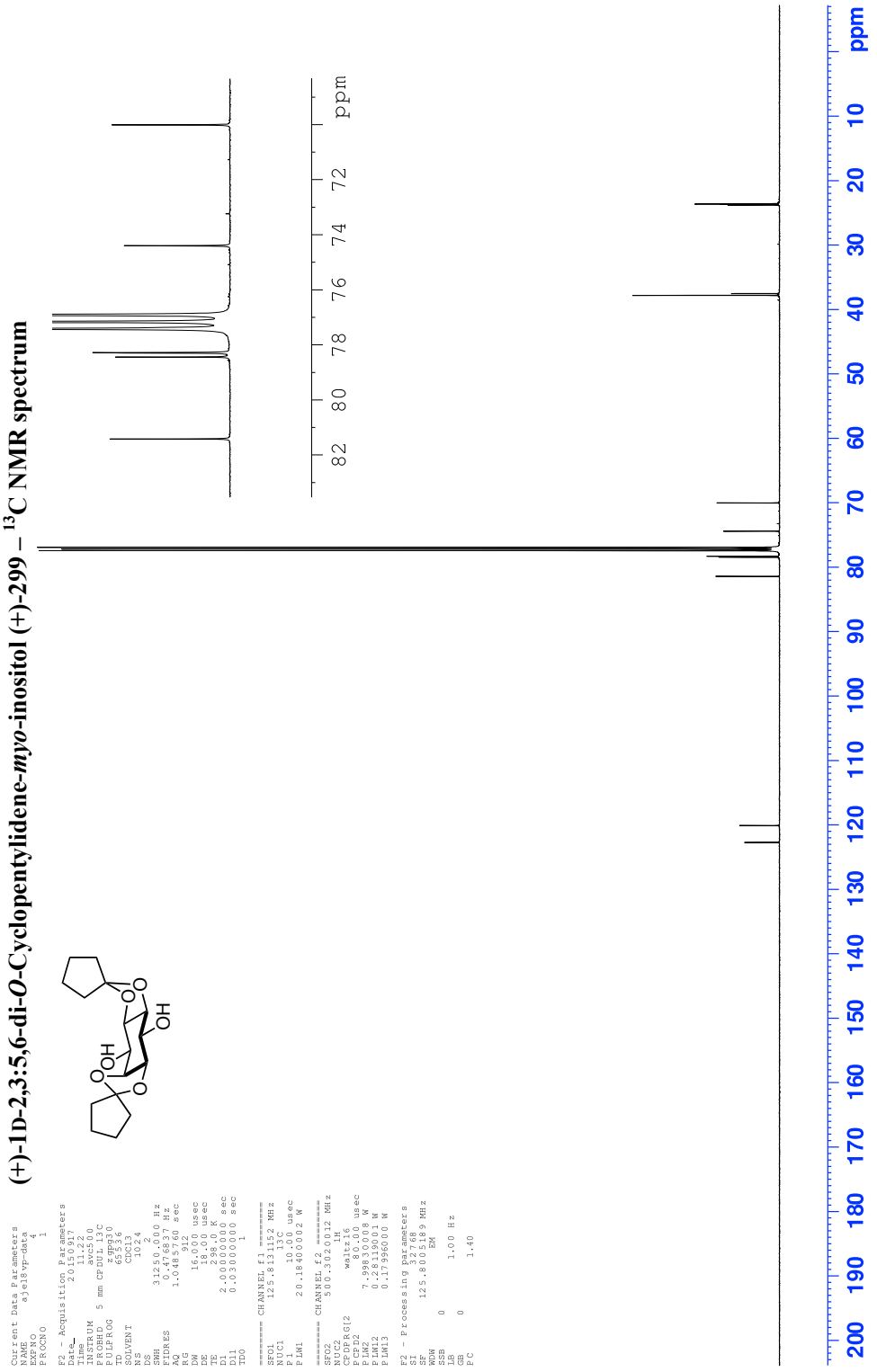


(−)-1D-1-*O*-(1,2-Di-*O*-palmitoyl-*sn*-glycer-3-yl-(2-cyanoethyl)phosphate)-2,3-*O*-cyclopentylidene-4-*O*-(triisopropylsilyl)-5,6-*O*-cyclopentylidene-*myo*-inositol (−)-289 – ^{31}P NMR spectrum

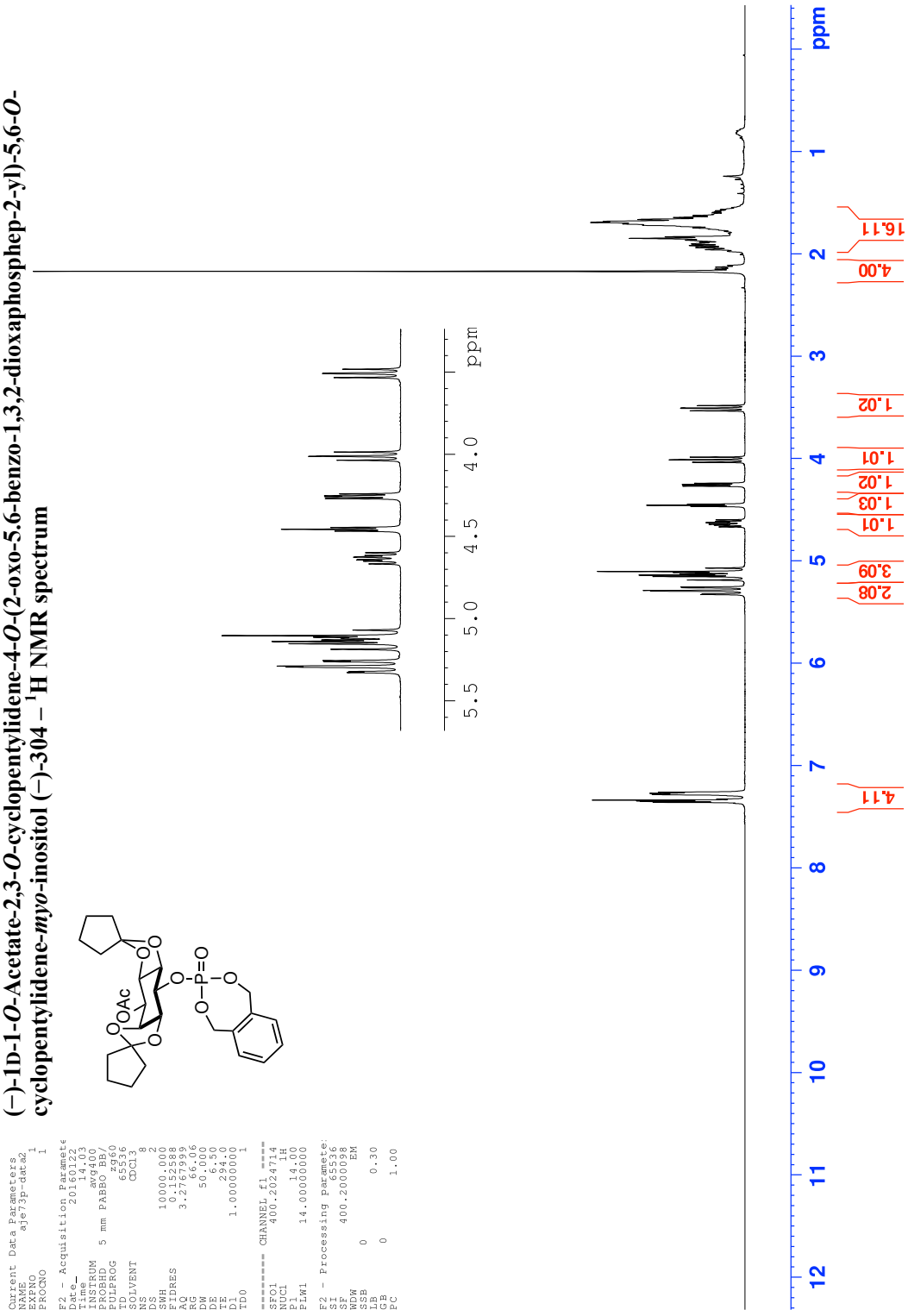


(+)-1D-2,3:5,6-di-*O*-Cyclopentylidene-*myo*-inositol (+)-299 – ^{1}H NMR spectrum




(+)-1D-2,3:5,6-di-O-Cyclopentylidene-myo-inositol (+)-299 – 13C NMR spectrum
Current Data Parameters
NAME ajs18vp-data
EXPNO 4
PROCNO 1
F2 - Acquisition Parameters
Date_ 20150917
Time 11.22
INSTRUM av400.0
PROBHD 5 mm CPDUL 13C
PULPROG zgpg30
TD 65536
SOLVENT CDCl3
NS 1024
DS 2
SWH 31250.000 Hz
FIDRES 0.476837 Hz
AQ 1.0485760 sec
RG 912
DW 16.000 usec
DE 6.00 usec
TE 298.0 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 125.8131152 MHz
NUC1 13C
P1 10.00 usec
PLW1 20.18400002 W
======== CHANNEL f2 ========
SFO2 500.3020012 MHz
NUC2 1H
CPDPRG[2 waltz16
PCPD2 100.00 usec
PLW2 7.99830018 W
PLW12 0.28119001 W
PLW13 0.17996000 W
F2 - Processing parameters
SI 32768
SF 125.8005189 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
82 80 78 76 74 72 ppm
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 ppm

(−)-1D-1-*O*-Acetate-2,3-*O*-cyclopentylidene-4-*O*-(2-oxo-5,6-benzo-1,3,2-dioxaphosphep-2-yl)-5,6-*O*-cyclopentylidene-*myo*-inositol (−)-304 – ^{1}H NMR spectrum



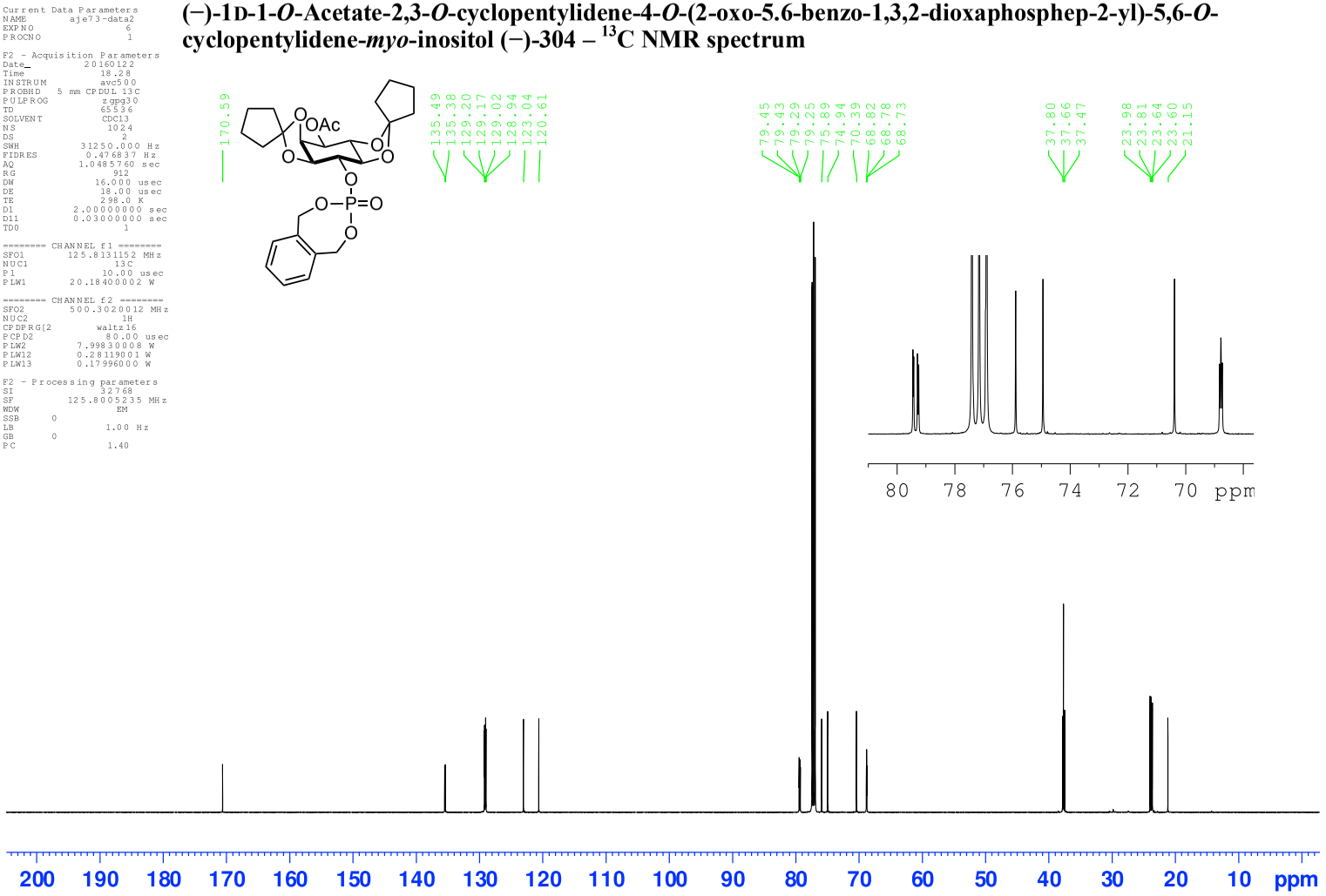

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Current Data Parameters
NAME        ajel3p-data2
EXPNO                  1
PROCNO                 1

F2 - Acquisition Parameters
Date_          20142212
Time           14.10.12
INSTRUM            av400
PROBHD  5 mm PABBO BB/
PULPROG          zg960
TD              65536
SOLVENT         CDCl3
NS                 16
DS                  2
SWH         10000.000
FIDRES      0.152588
AQ          3.2767939
RG                 64
DW             50.000
DE                6.50
TE              294.0
D1          1.00000000
TD0                 1

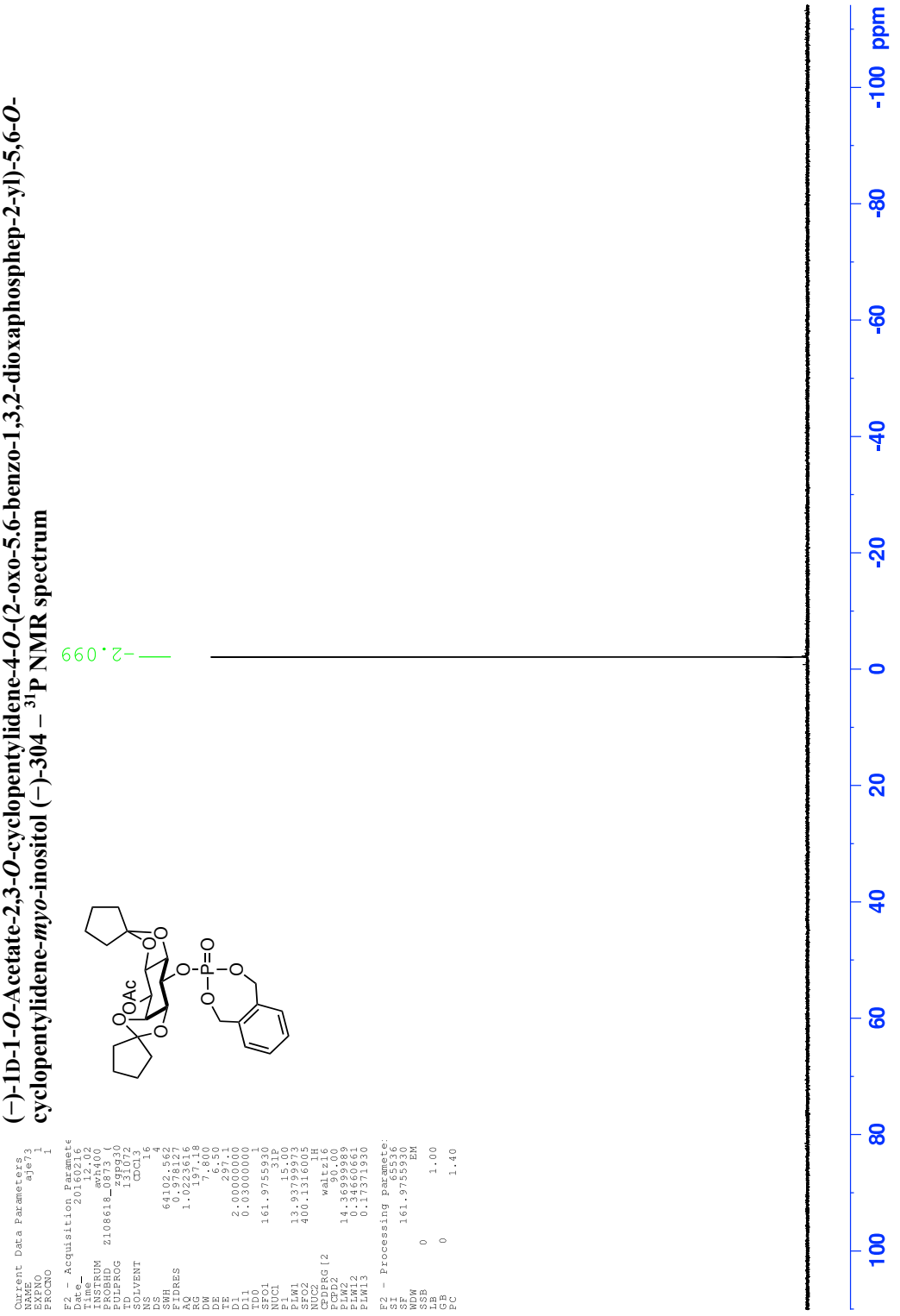
======== CHANNEL f1 ========
SFO1        400.2024714
NUC1             1H
P1              14.00
PLW1        14.00000000

F2 - Processing parameters
SI              65536
SF          400.2000098
WDW                EM
SSB                 0
LB                0.30
GB                  0
PC                1.00
```

(−)-1D-1-*O*-Acetate-2,3-*O*-cyclopentylidene-4-*O*-(2-oxo-5.6-benzo-1,3,2-dioxaphosphep-2-yl)-5,6-*O*-cyclopentylidene-*myo*-inositol (−)-304 – ^{13}C NMR spectrum



(−)-1D-1-*O*-Acetate-2,3-*O*-cyclopentylidene-4-*O*-(2-oxo-5,6-benzo-1,3,2-dioxaphosphep-2-yl)-5,6-*O*-cyclopentylidene-*myo*-inositol (−)-304 – ^{31}P NMR spectrum

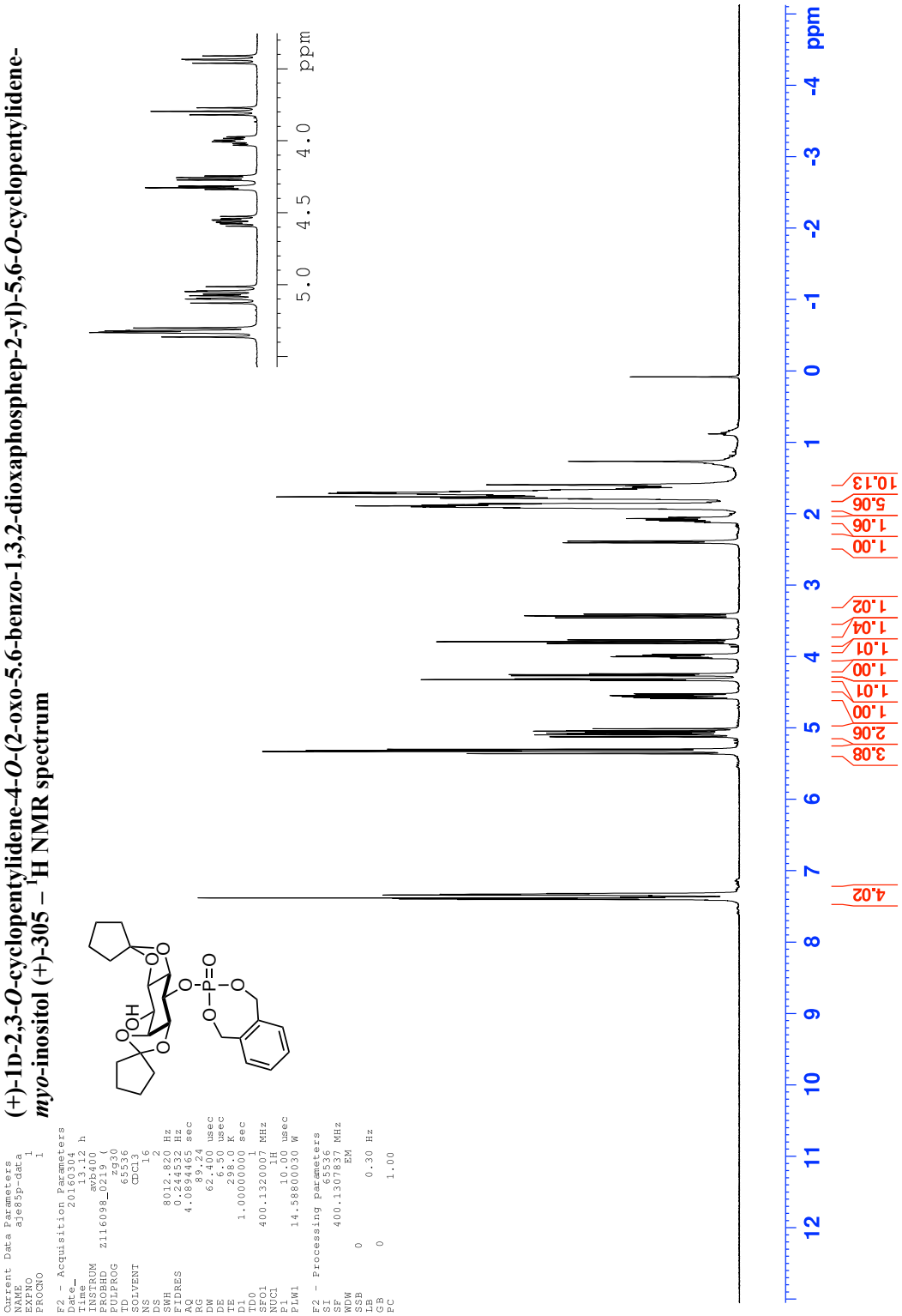



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Current Data Parameters
NAME                  ejm73
EXPNO                     1
PROCNO                    1

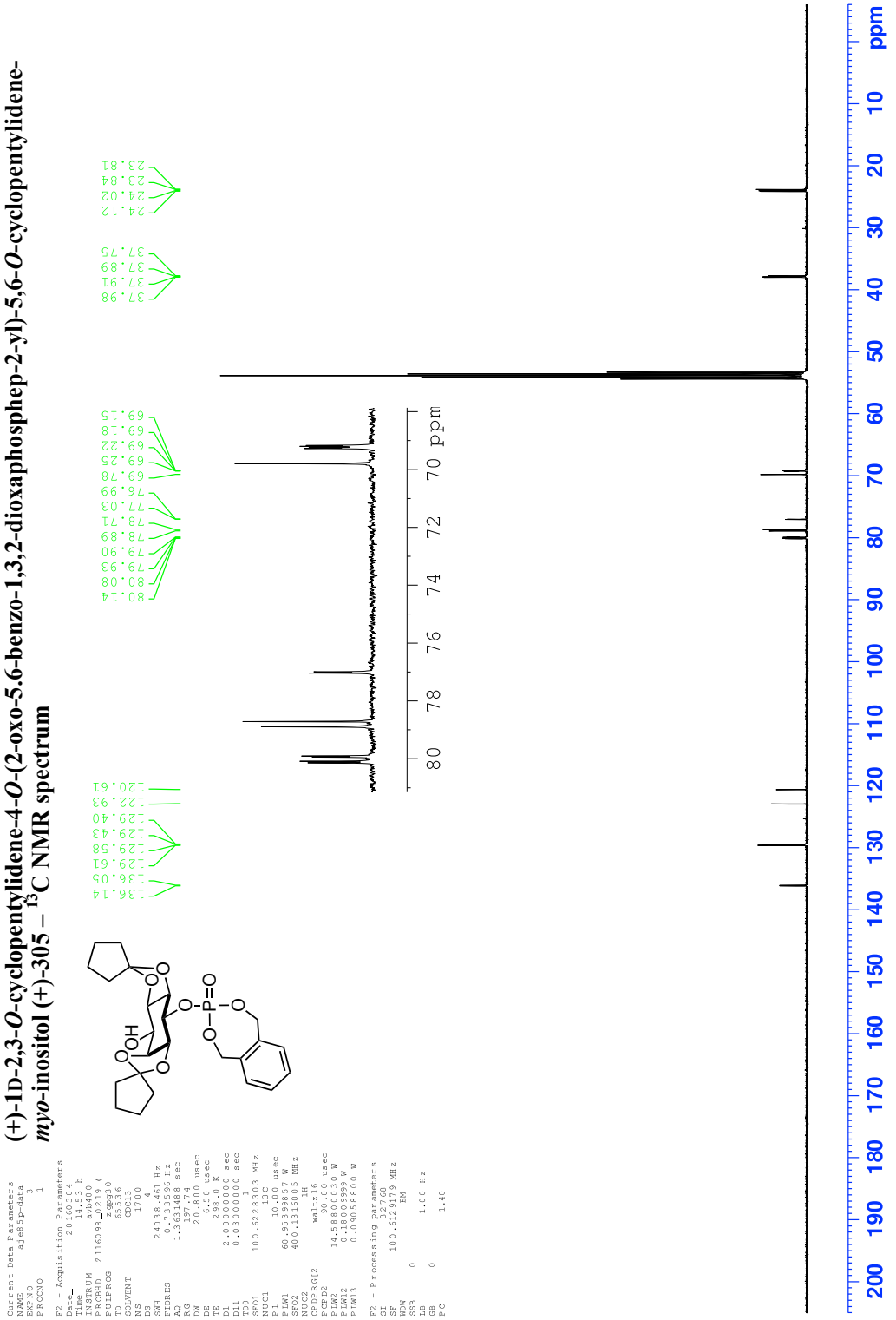
F2 - Acquisition Parameters
Date_           20162215
Time                12.02
INSTRUM           avh400
PROBHD     2108618_0873 (
PULPROG          zgpg30
TD                 131072
SOLVENT              CDCl3
NS                      16
DS                       4
SWH               64102.562
FIDRES            0.978827
AQ                1.022815
RG                    197.18
DW                   7.800
DE                    6.50
TE                   295.1
D1                2.0000000
D11               0.0300000
TD0                       1
SFO1         161.9755930
NUC1                   31P
P1                   15.00
PL1               13.93799973
SFO2         400.1316005
NUC2                   1H
CPDPRG[2             waltz16
PCPD2                90.00
PLW2              14.36960869
PLW12              1.34660683
PLW13              0.17371930

F2 - Processing parameters
SI                   65536
SF         161.9755930
WDW                      EM
SSB                   0
LB                 1.00
GB                    0
PC                 1.40
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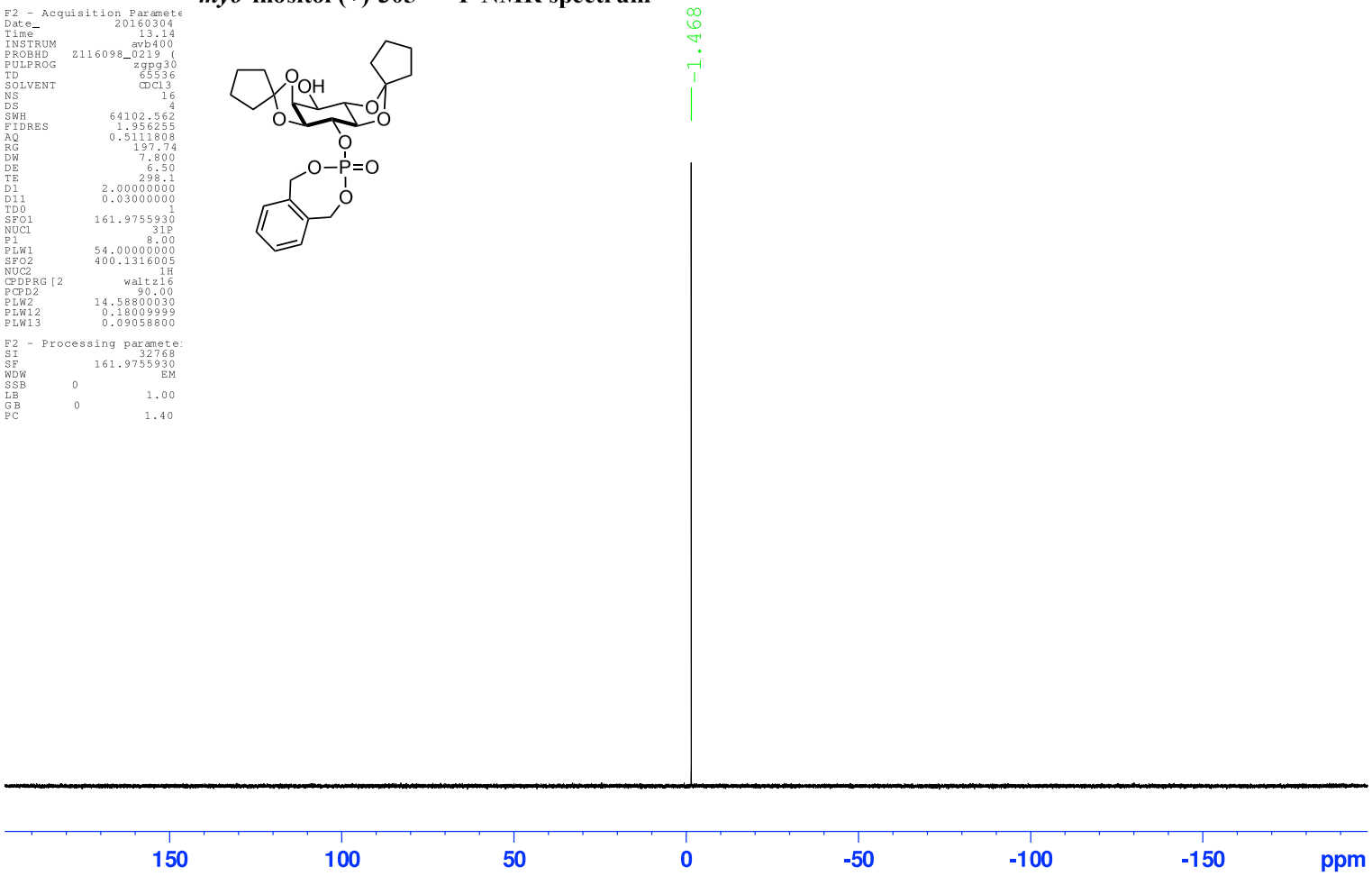
(+)-1D-2,3-*O*-cyclopentylidene-4-*O*-(2-oxo-5.6-benzo-1,3,2-dioxaphosphep-2-yl)-5,6-*O*-cyclopentylidene-*myo*-inositol (+)-305 – ^{1}H NMR spectrum



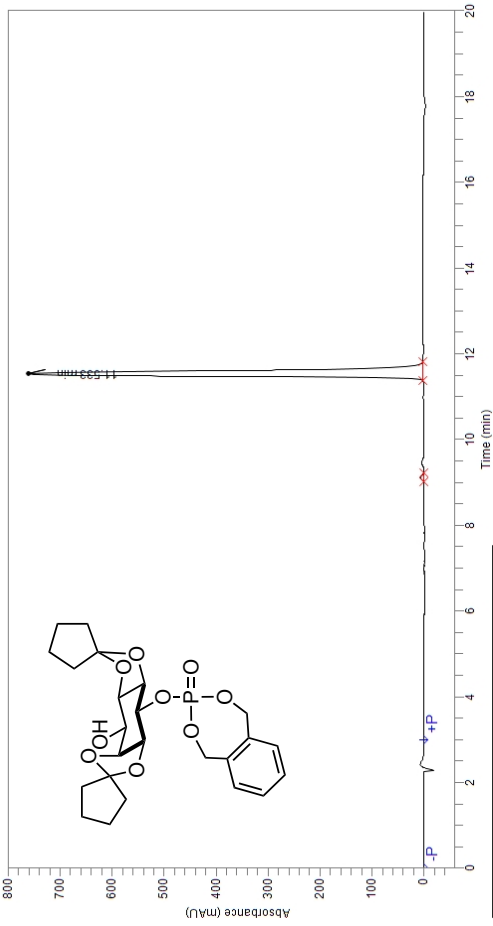
(+)-1D-2,3-*O*-cyclopentylidene-4-*O*-(2-oxo-5.6-benzo-1,3,2-dioxaphosphep-2-yl)-5,6-*O*-cyclopentylidene-*myo*-inositol (+)-305 – ^{13}C NMR spectrum



(+)-1D-2,3-*O*-cyclopentylidene-4-*O*-(2-oxo-5.6-benzo-1,3,2-dioxaphosphep-2-yl)-5,6-*O*-cyclopentylidene-*myo*-inositol (+)-305 – ^{33}P NMR spectrum



(+)-1D-2,3-*O*-cyclopentylidene-4-*O*-(2-oxo-5.6-benzo-1,3,2-dioxaphosphep-2-yl)-5,6-*O*-cyclopentylidene-*myo*-inositol (+)-305 – RP-HPLC (Method 2)



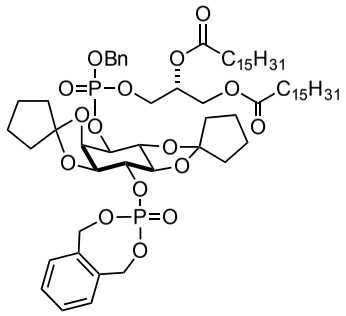
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11.533	6,576,972	99.33
Total	6,621,399	100.00

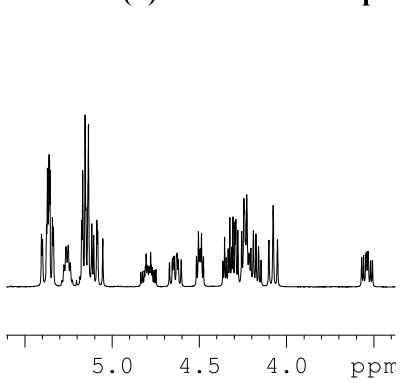
(−)-1D-1-*O*-(1,2-Di-*O*-palmitoyl-*sn*-glycer-3-yl-benzylphosphate)-2,3-*O*-cyclopentylidene-4-*O*-(2-oxo-5.6-benzo-1,3,2-dioxaphosphep-2-yl)-5,6-*O*-cyclopentylidene-*myo*-inositol (−)-307 – ^{1}H NMR spectrum

```
Current Data Parameters
NAME                 ajf80p
EXPNO                    10
PROCNO                    1

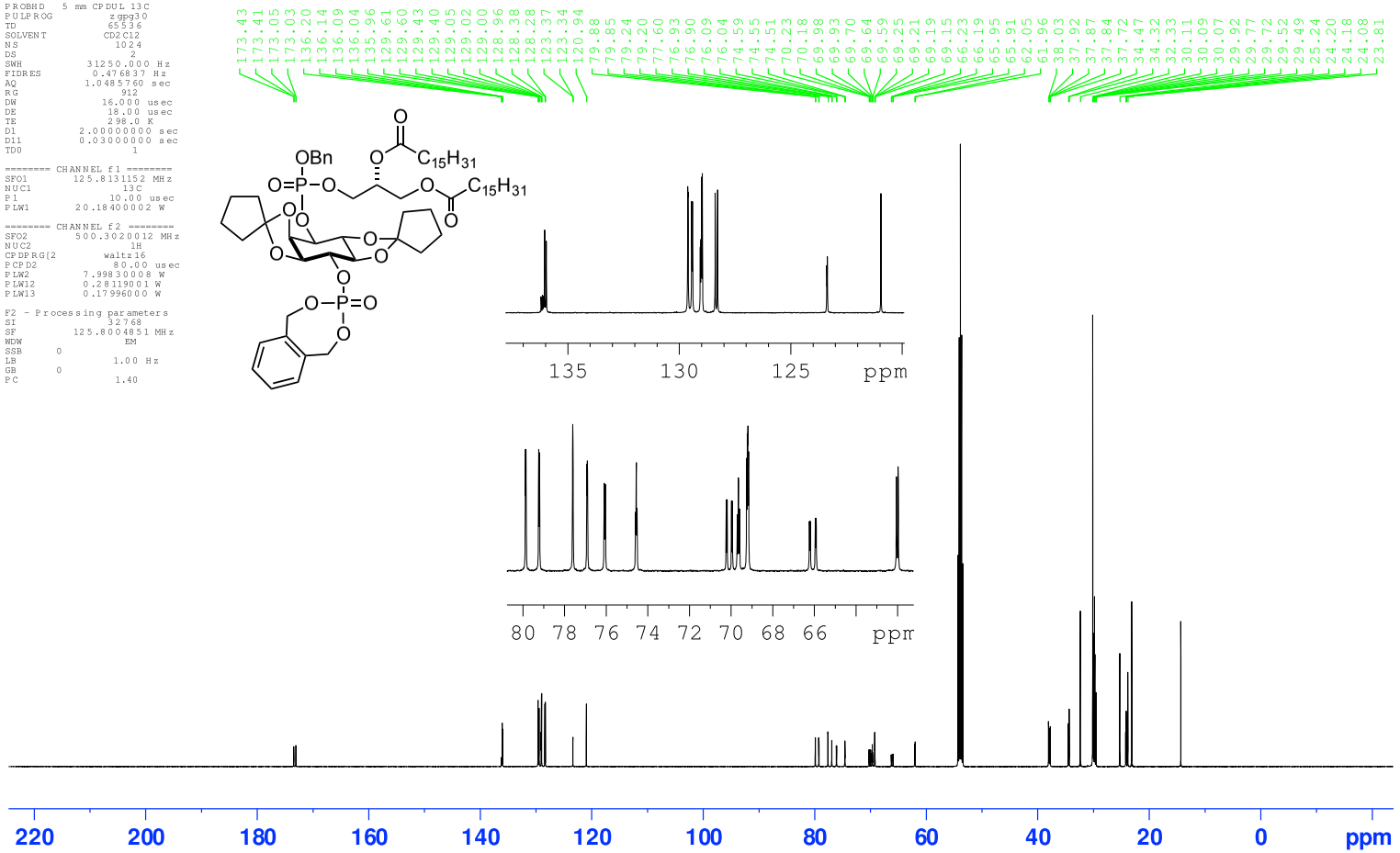
F2 - Acquisition Parameters
Date_             20161010
Time                 14.01 h
INSTRUM              avh400
PROBHD    Z108618_0873 (
PULPROG              zg60
TD                  65536
SOLVENT             CD2Cl2
NS                      16
DS                       2
SWH                8012.820 Hz
FIDRES             0.244532 Hz
AQ                 4.0894465 sec
RG                    30.75
DW                   62.400 usec
DE                    6.50 usec
TE                   296.3 K
D1              1.00000000 sec
TD0                      1
SFO1           400.1324008 MHz
NUC1                    1H
P1                   14.00 usec
PLW1           14.36999989 W

F2 - Processing parameters
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SF              400.1300000 MHz
WDW                     EM
SSB       0
LB                    0.30 Hz
GB        0
PC                    1.00
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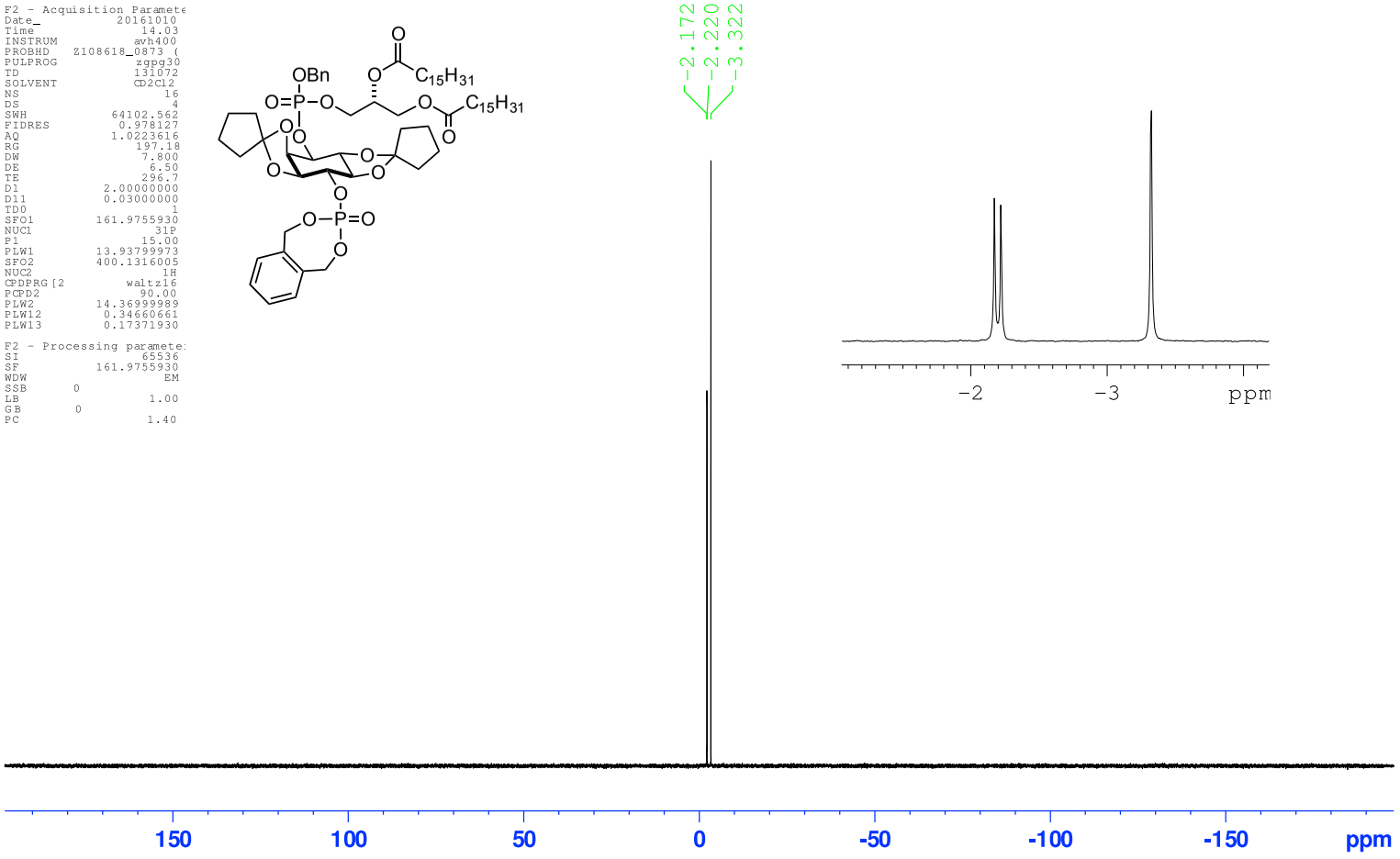




(−)-1D-1-*O*-(1,2-Di-*O*-palmitoyl-*sn*-glycer-3-yl-benzylphosphate)-2,3-*O*-cyclopentylidene-4-*O*-(2-oxo-5.6-benzo-1,3,2-dioxaphosphep-2-yl)-5,6-*O*-cyclopentylidene-*myo*-inositol (−)-307 – ^{13}C NMR spectrum

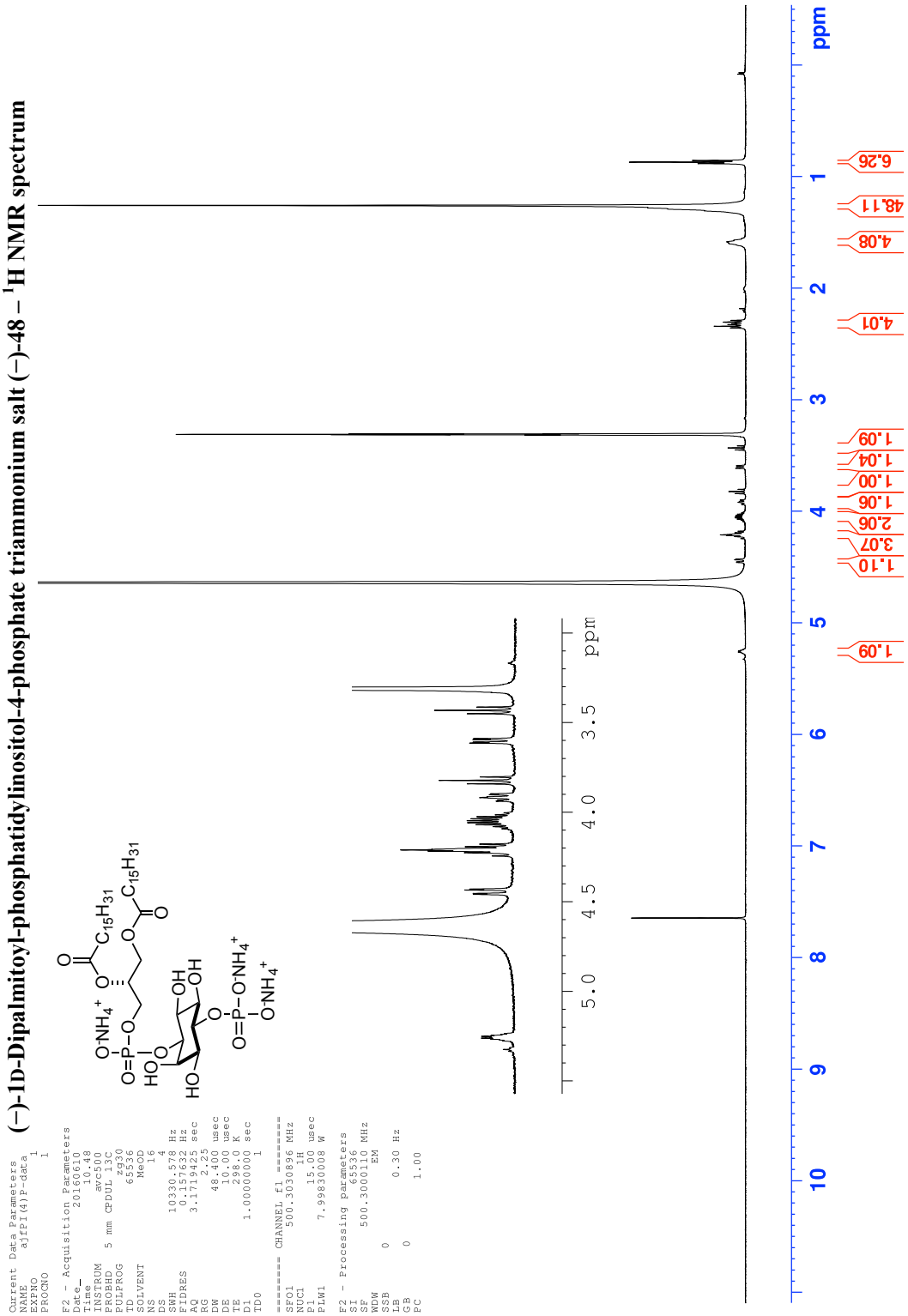


(−)-1D-1-*O*-(1,2-Di-*O*-palmitoyl-*sn*-glycer-3-yl-benzylphosphate)-2,3-*O*-cyclopentylidene-4-*O*-(2-oxo-5.6-benzo-1,3,2-dioxaphosphep-2-yl)-5,6-*O*-cyclopentylidene-*myo*-inositol (−)-307 – ^{31}P NMR spectrum

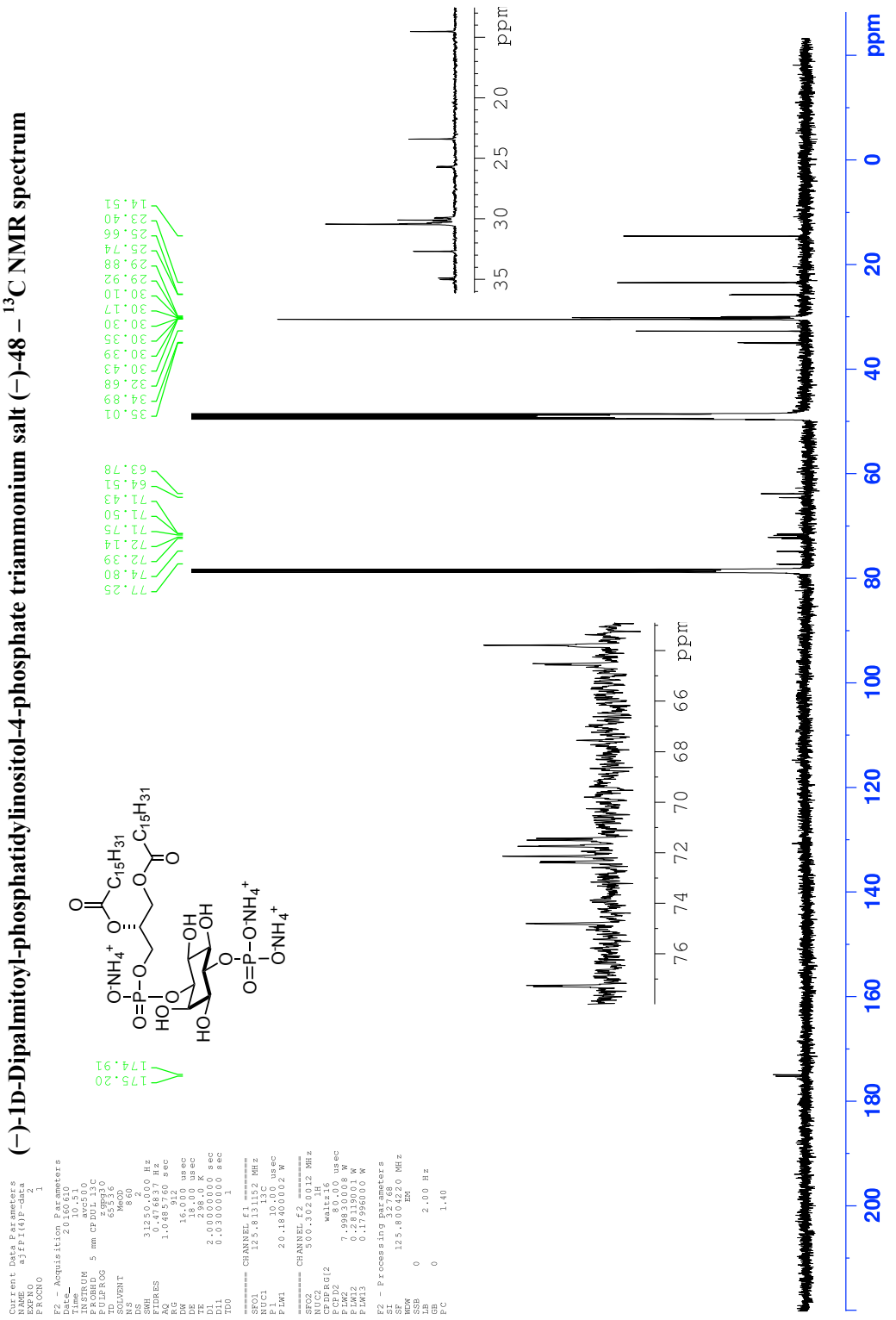


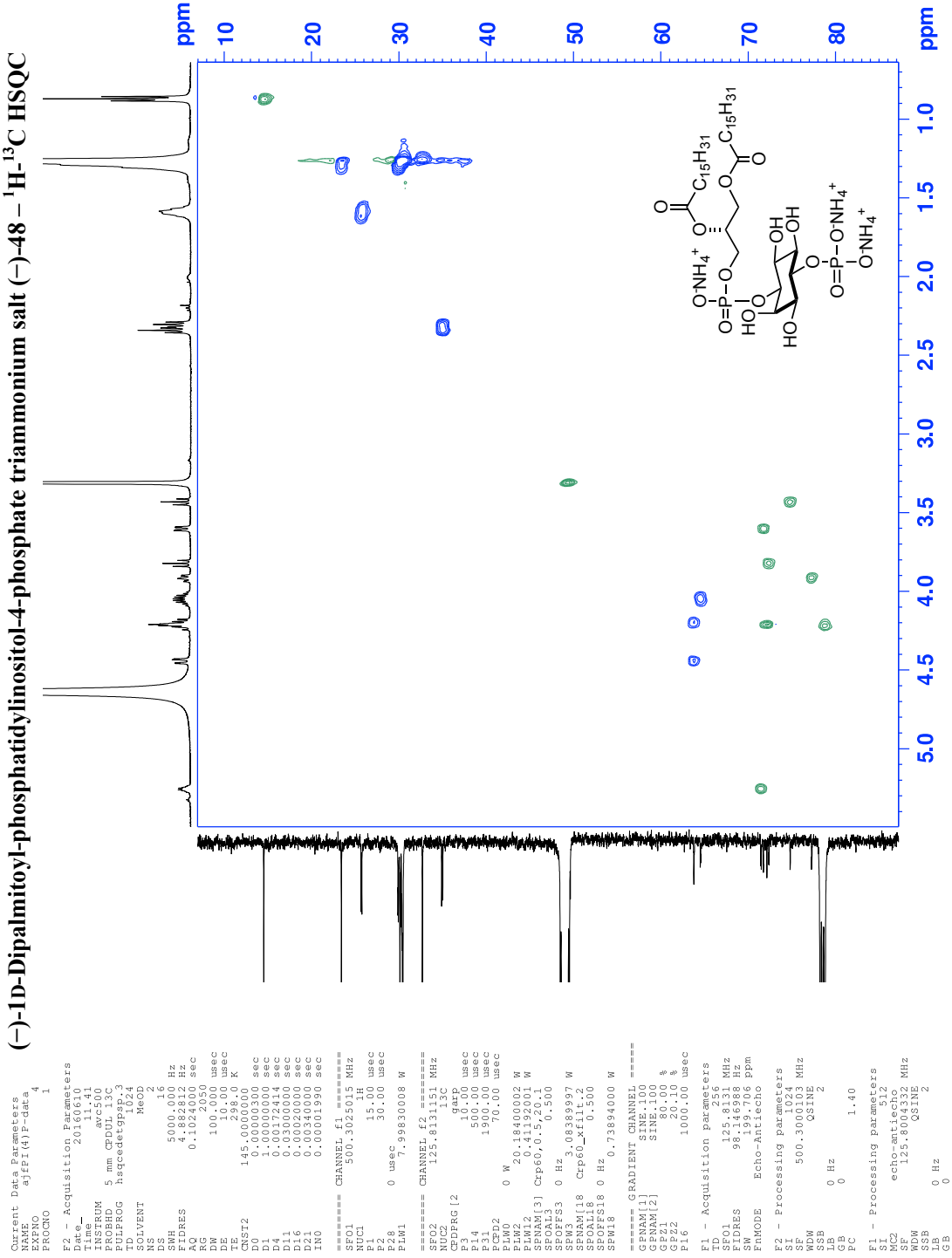
(−)-1D-1-*O*-(1,2-Di-*O*-palmitoyl-*sn*-glycer-3-yl-benzylphosphate)-2,3-*O*-cyclopentylidene-4-*O*-(2-oxo-5.6-benzo-1,3,2-dioxaphosphep-2-yl)-5,6-*O*-cyclopentylidene-*myo*-inositol (−)-307 – NP-HPLC (Method 7)

Time	Area	Area %
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Total	1,050,011	100.00

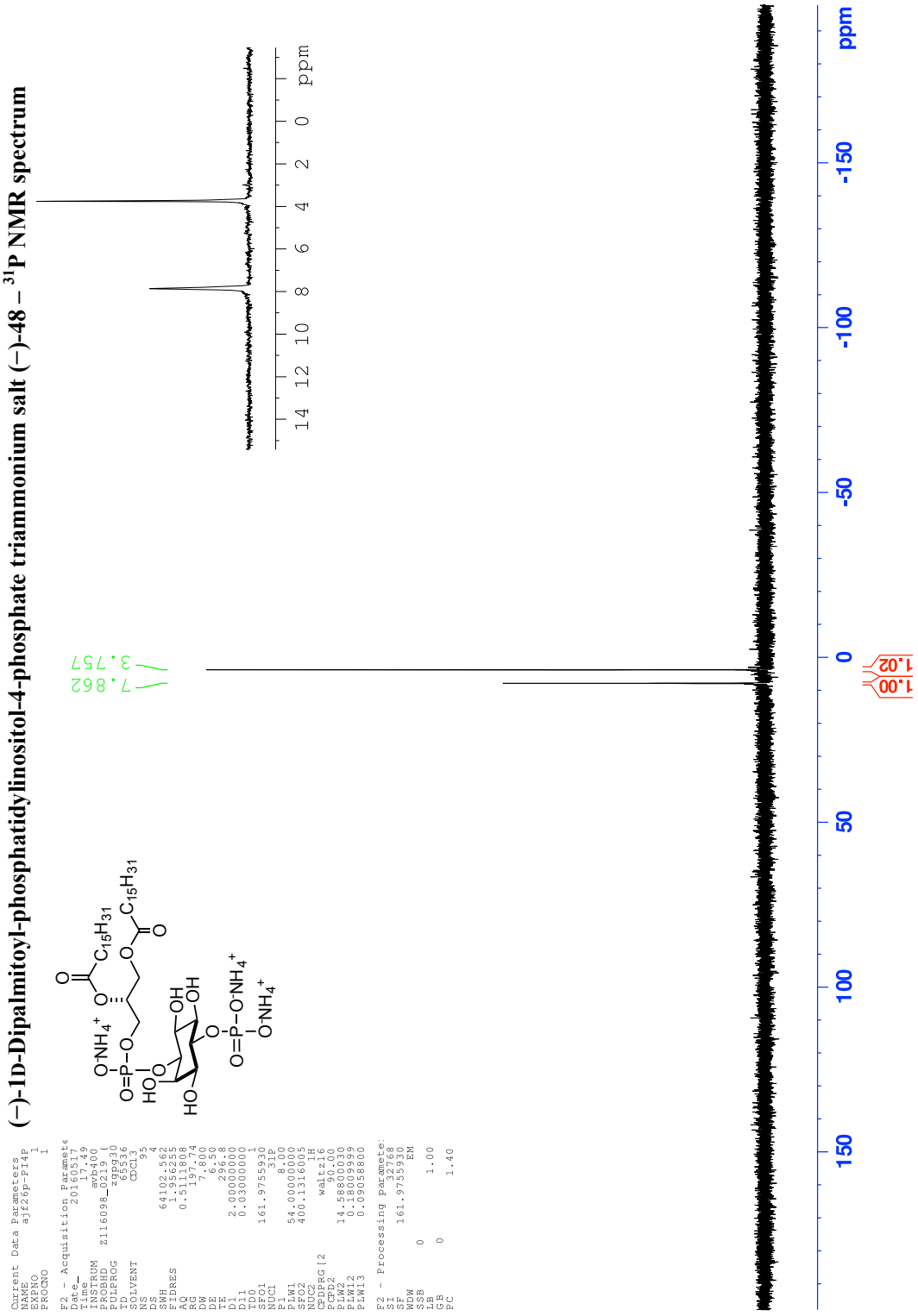

(−)-1D-Dipalmitoyl-phosphatidylinositol-4-phosphate triammonium salt (−)-48 – 1H NMR spectrum
Current Data Parameters
NAME ajfPI(4)P-data
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20160610
Time 10.48
INSTRUM avc500
PROBHD 5 mm CPDUL 13C
PULPROG zg30
TD 65536
SOLVENT MeOD
NS 16
DS 4
SWH 10330.578 Hz
FIDRES 0.157632 Hz
AQ 3.1719425 sec
RG 2.25
DW 48.400 usec
DE 10.00 usec
TE 298.0 K
D1 1.00000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 500.3030896 MHz
NUC1 1H
P1 15.00 usec
PLW1 7.99830008 W
F2 - Processing parameters
SI 65536
SF 500.3000110 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
5.0
4.5
4.0
3.5
ppm
1.09
1.10
3.07
2.06
1.06
1.00
1.04
1.09
4.01
4.08
48.11
6.26
ppm

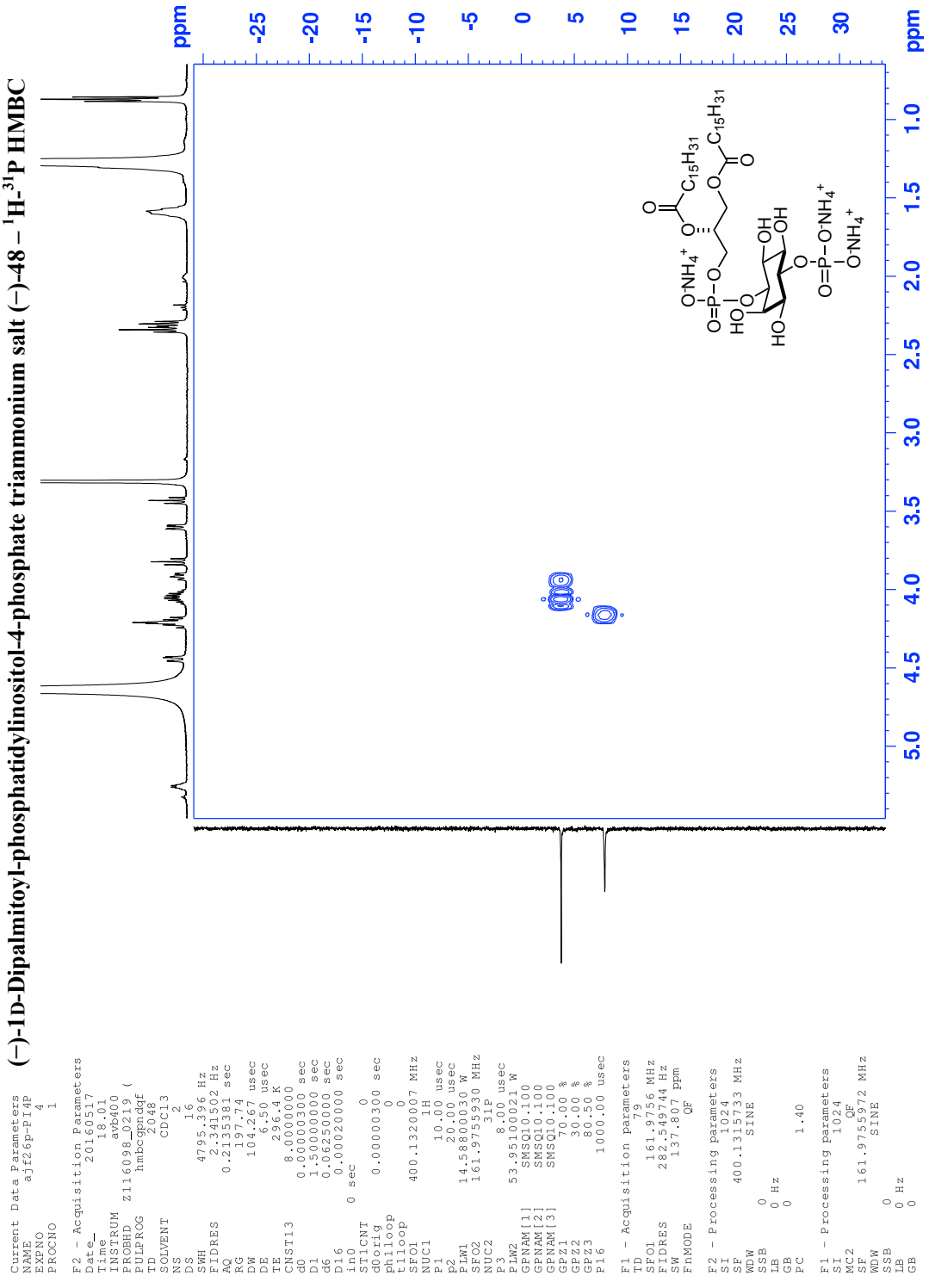
(−)-1D-Dipalmitoyl-phosphatidylinositol-4-phosphate triammonium salt (−)-48 – ^{13}C NMR spectrum

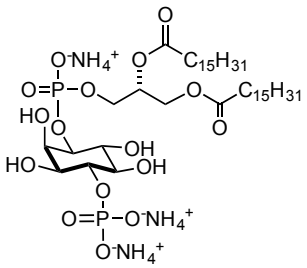



(−)-1D-Dipalmitoyl-phosphatidylinositol-4-phosphate triammonium salt (−)-48 – ^{1}H-^{13}C HSQC
ppm
10
20
30
40
50
60
70
80
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm
$C_{15}H_{31}$
$C_{15}H_{31}$
$O^-NH_4^+$
$O^-NH_4^+$
$O^-NH_4^+$
O=P–O
O=P–O
HO
HO
OH
OH
O
O
O
O
O
O
Current Data Parameters
NAME ajfPI(4)P-data
EXPNO 4
PROCNO 1
F2 - Acquisition Parameters
Date_ 20160610
Time 11.41
INSTRUM avc500
PROBHD 5 mm CPDUL 13C
PULPROG hsqcedetgpsp.3
TD 1024
SOLVENT MeOD
NS 2
DS 16
SWH 5000.000 Hz
FIDRES 4.882812 Hz
AQ 0.1024000 sec
RG 2050
DW 100.000 usec
DE 10.00 usec
TE 298.0 K
CNST2 145.0000000
D0 0.0000030 sec
D1 1.0000000 sec
D4 0.0017241 sec
D11 0.0300000 sec
D16 0.0002000 sec
D21 0.0034000 sec
IN0 0.0000199 sec
======== CHANNEL f1 ========
SFO1 500.3025015 MHz
NUC1 1H
P1 15.00 usec
P2 30.00 usec
P28 0 usec
PLW1 7.99830008 W
======== CHANNEL f2 ========
SFO2 125.8131151 MHz
NUC2 13C
CPDPRG[2 garp
P3 10.00 usec
P14 500.00 usec
P31 1900.00 usec
PCPD2 70.00 usec
PLW0 0 W
PLW2 20.18400002 W
PLW12 0.41192001 W
SPNAM[3] Crp60,0.5,20.1
SPOAL3 0.500
SPOFFS3 0 Hz
SPW3 3.08389997 W
SPNAM[18 Crp60_xfilt.2
SPOAL18 0.500
SPOFFS18 0 Hz
SPW18 0.73894000 W
===== GRADIENT CHANNEL =====
GPNAM[1] SINE.100
GPNAM[2] SINE.100
GPZ1 80.00 %
GPZ2 20.10 %
P16 1000.00 usec
F1 - Acquisition parameters
TD 256
SFO1 125.8131 MHz
FIDRES 98.146988 Hz
SW 199.706 ppm
FnMODE Echo-Antiecho
F2 - Processing parameters
SI 1024
SF 500.3000103 MHz
WDW QSINE
SSB 2
LB 0 Hz
GB 0
PC 1.40
F1 - Processing parameters
SI 512
MC2 echo-antiecho
SF 125.8004332 MHz
WDW QSINE
SSB 2
LB 0 Hz
GB 0

(−)-1D-Dipalmitoyl-phosphatidylinositol-4-phosphate triammonium salt (−)-48 – ^{31}P NMR spectrum

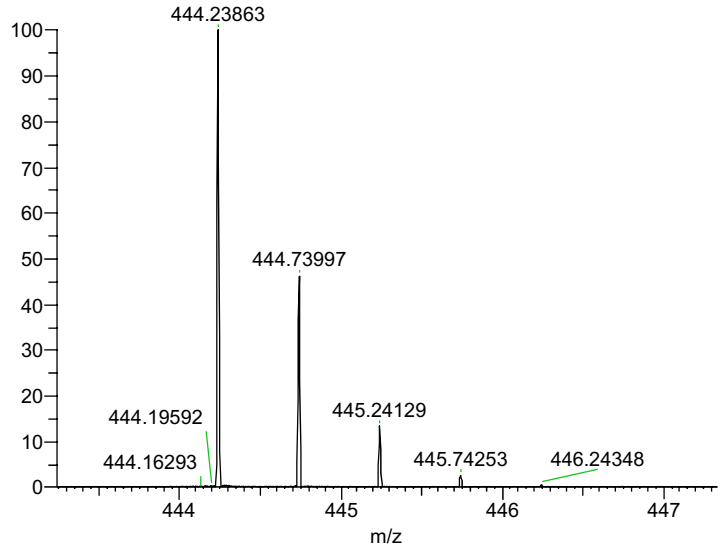



(–)-1D-Dipalmitoyl-phosphatidylinositol-4-phosphate triammonium salt (–)-48 – ^{1}H-^{31}P HMBC
ppm
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
-25
-20
-15
-10
-5
0
5
10
15
20
25
30
$C_{15}H_{31}$
$O^-NH_4^+$
O=P–O
HO
OH
O=P–O$^-NH_4^+$
$O^-NH_4^+$



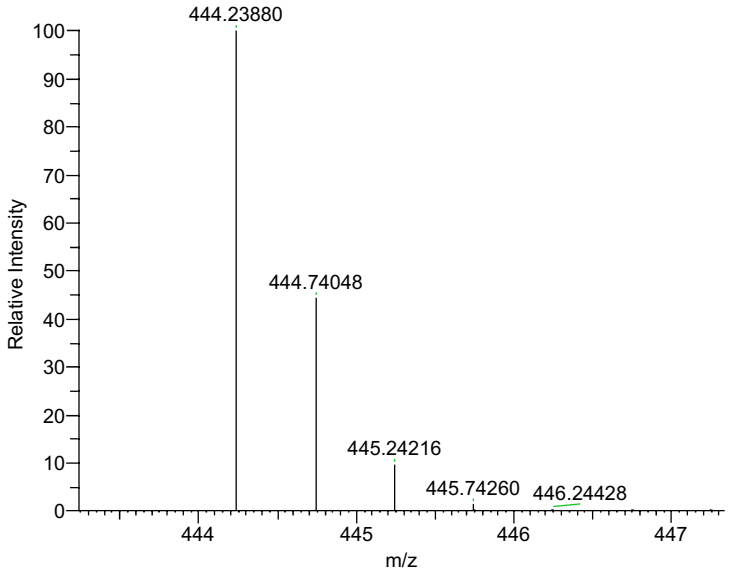
(−)-1D-Dipalmitoyl-phosphatidylinositol-4-phosphate triammonium salt (−)-48 – Mass Spectrum

S:\data\June 16\ESI57677.raw 13/06/2016 10:29 am



NL: 4.48E7
ESI57677 #12-20 RT: 0.13-0.22 AV: 5 NL: 4.48E7
T: FTMS {1,2} - p ESI Full ms [80.00-1600.00]

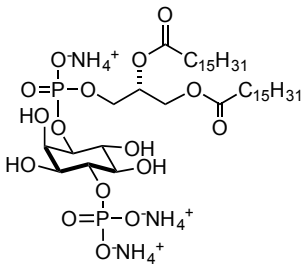
Measured Spectrum



NL: 6.13E5
C41H78O16P2: $C_{41}H_{78}O_{16}P_2$ Chrg -2 R: 1000000 Res. Pwr. @FWHM

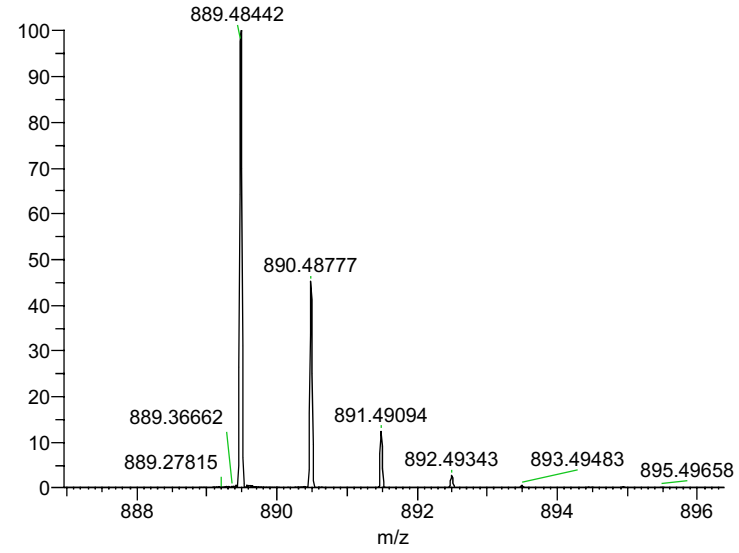
Theoretical Spectrum

m/z	Formula	RDB	Delta ppm	Theo. Mass
444.23862	$C_{41}H_{78}O_{16}P_2$	4	-0.42	444.23880



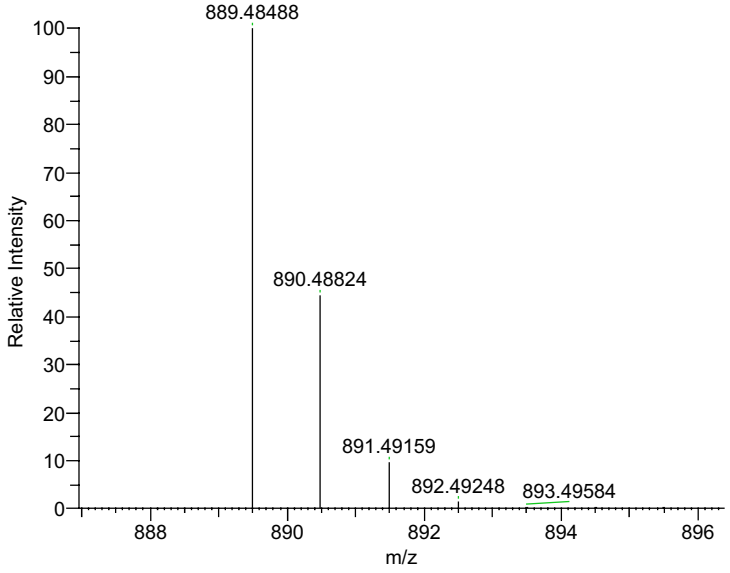
(−)-1D-Dipalmitoyl-phosphatidylinositol-4-phosphate triammonium salt (−)-48 – Mass Spectrum

Q:\MassStore-Research Groups\STUART CONWAY GROUP\Amelie...06/12/2016 12:37 pm



NL: 7.78E6
ESI57677 #13-24 RT: 0.16-0.27 AV: 6 NL: 3.86E7
T: FTMS {1,2} - p ESI Full ms [80.00-1600.00]

Measured Spectrum

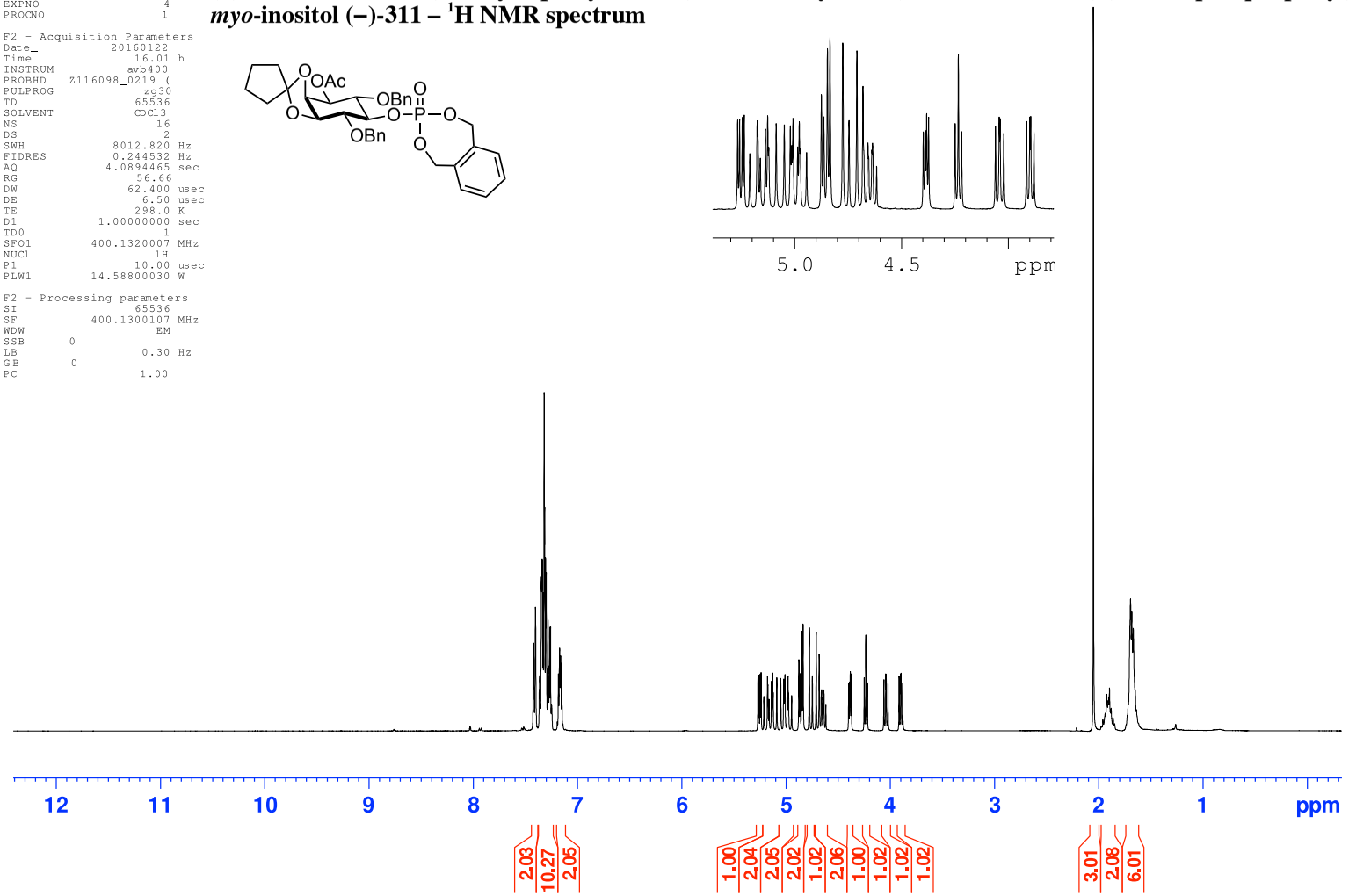


NL: 6.13E5
C41H79O16P2: $C_{41}H_{79}O_{16}P_2$ Chrg -1 R: 1000000 Res. Pwr. @FWHM

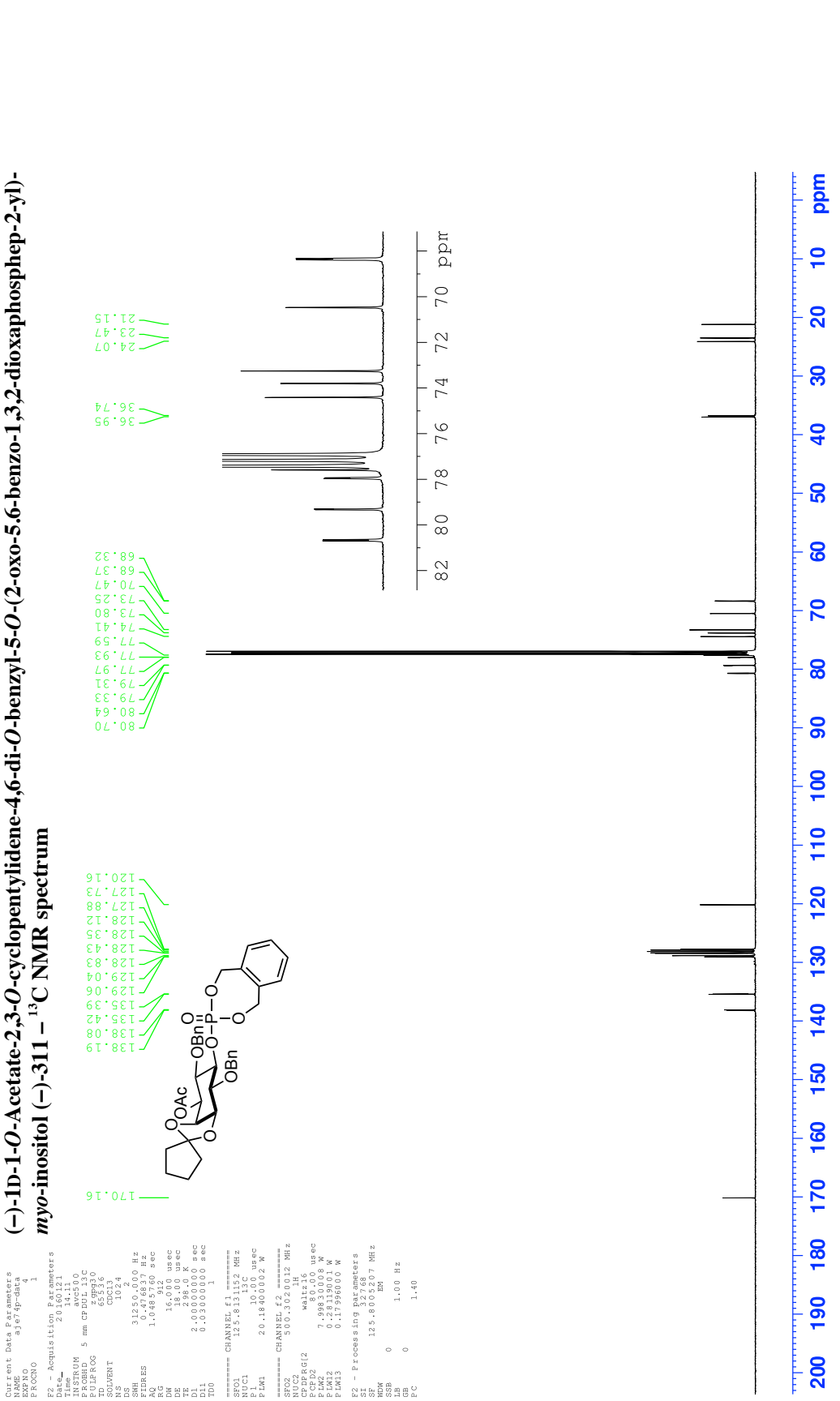
Theoretical Spectrum

m/z	Formula	RDB	Delta ppm	Theo. Mass
889.48444	$C_{41}H_{79}O_{16}P_2$	3.5	-0.5	889.48488

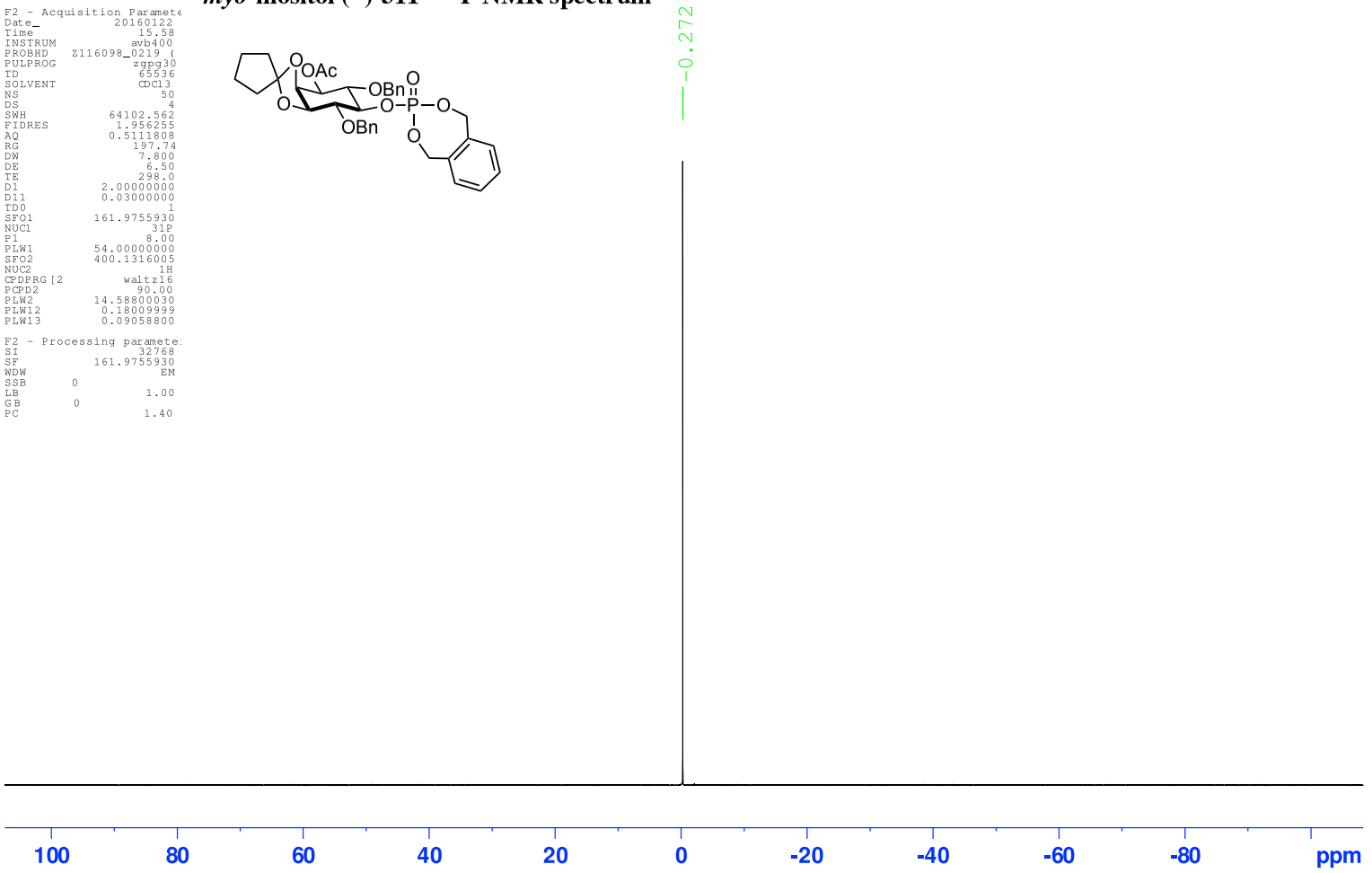
(–)-1D-1-*O*-Acetate-2,3-*O*-cyclopentylidene-4,6-di-*O*-benzyl-5-*O*-(2-oxo-5.6-benzo-1,3,2-dioxaphosphep-2-yl)-*myo*-inositol (–)-311 – ^{1}H NMR spectrum



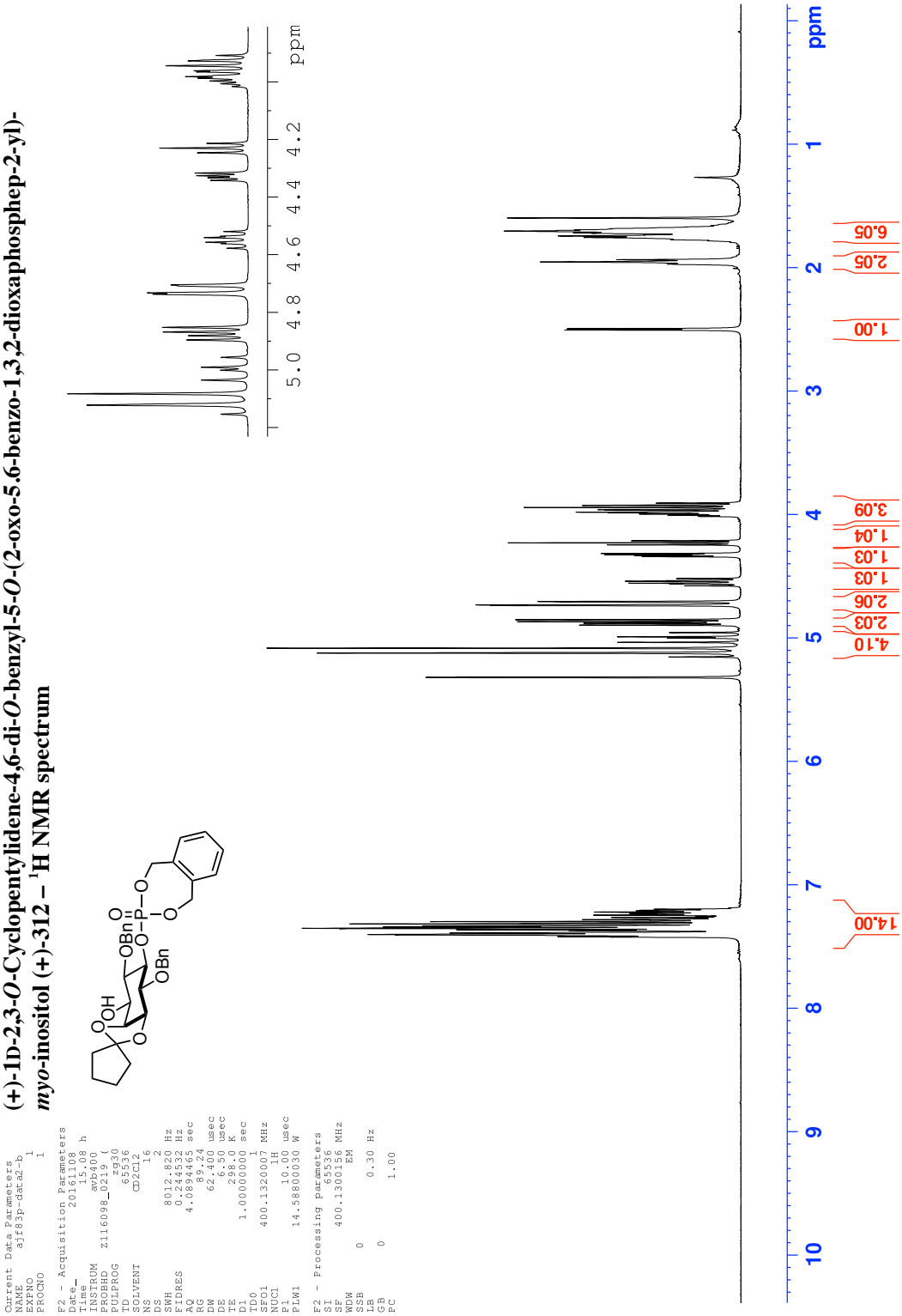
(–)-1D-1-*O*-Acetate-2,3-*O*-cyclopentylidene-4,6-di-*O*-benzyl-5-*O*-(2-oxo-5.6-benzo-1,3,2-dioxaphosphep-2-yl)-*myo*-inositol (–)-311 – ^{13}C NMR spectrum



(–)-1D-1-*O*-Acetate-2,3-*O*-cyclopentylidene-4,6-di-*O*-benzyl-5-*O*-(2-oxo-5.6-benzo-1,3,2-dioxaphosphep-2-yl)-*myo*-inositol (–)-311 – ^{31}P NMR spectrum



(+)-1D-2,3-*O*-Cyclopentylidene-4,6-di-*O*-benzyl-5-*O*-(2-oxo-5.6-benzo-1,3,2-dioxaphosphep-2-yl)-*myo*-inositol (+)-312 – ^{1}H NMR spectrum

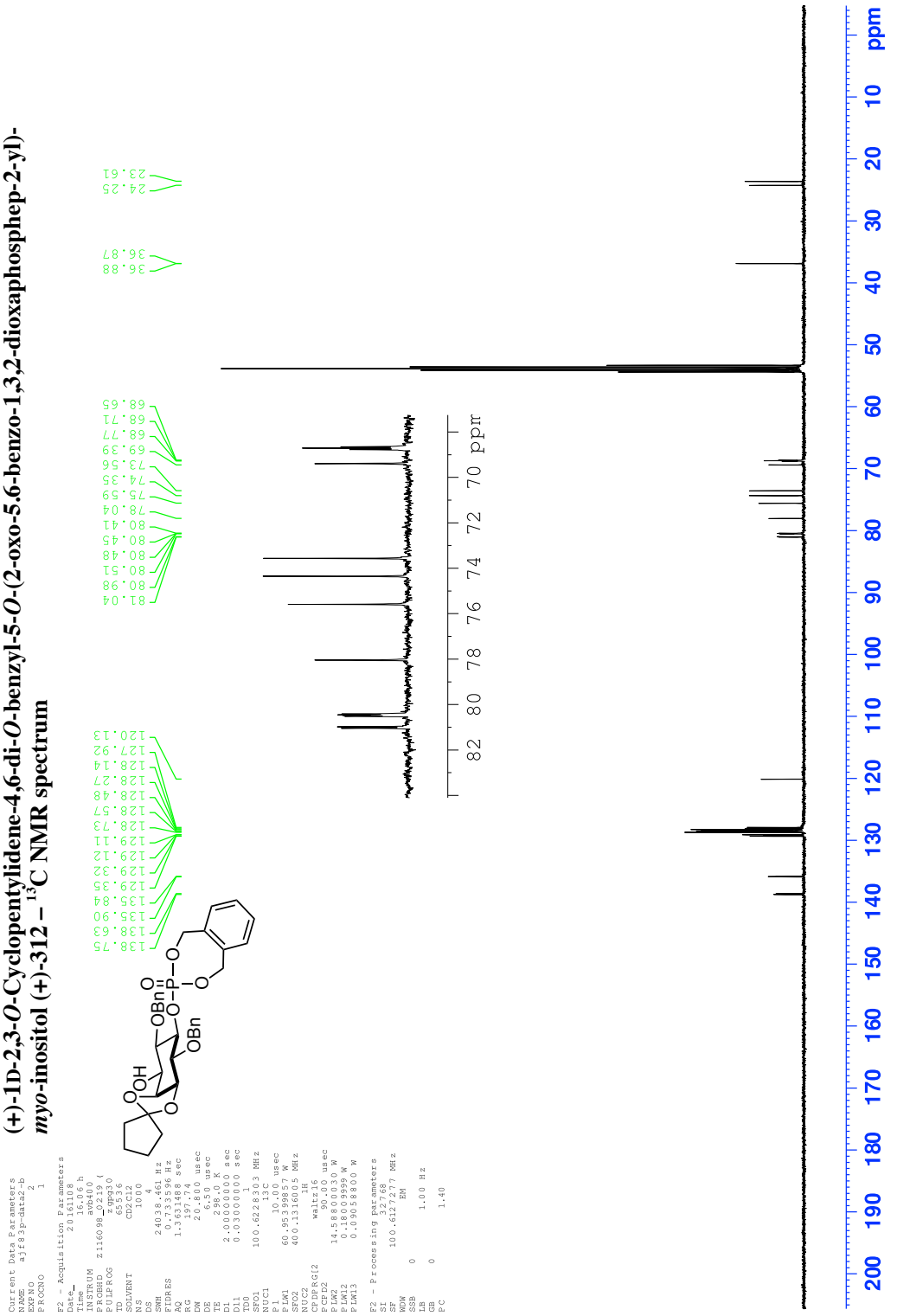



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Current Data Parameters
NAME          ajf83p-data2-b
EXPNO                      1
PROCNO                     1

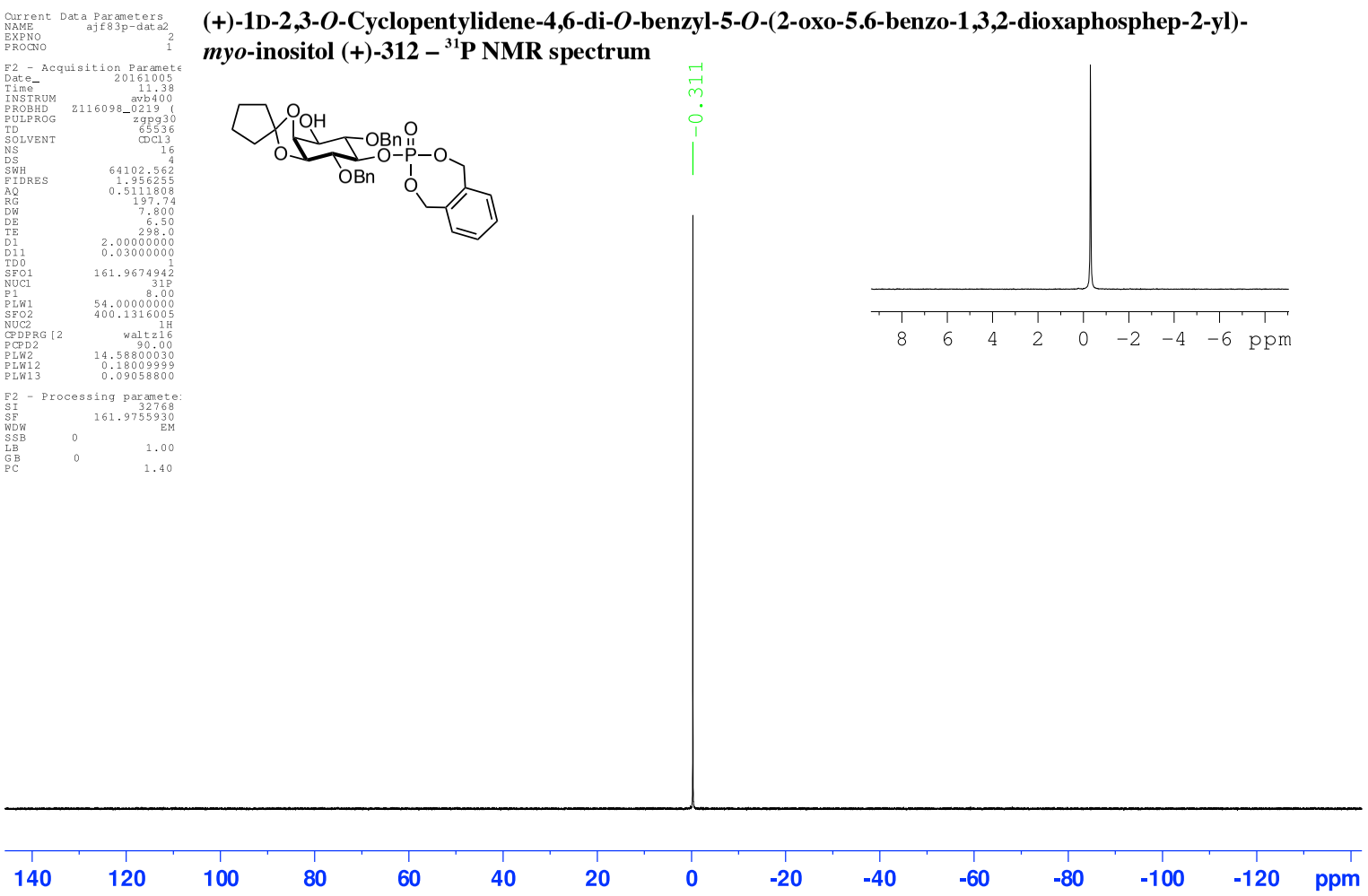
F2 - Acquisition Parameters
Date_          20161108
Time_              15.08 h
INSTRUM            avb400
PROBHD     Z116098_0219 (
PULPROG            zg30
TD                 65536
SOLVENT            CD2Cl2
NS                    16
DS                     2
SWH            8012.820 Hz
FIDRES         0.244533 Hz
AQ             4.0894465 sec
RG                89.24
DW                62.400 usec
DE                 6.50 usec
TE                298.0 K
D1             1.00000000 sec
TD0                    1
SFO1           400.1320007 MHz
NUC1                  1H
P1                 10.00 usec
PLW1          14.58800030 W

F2 - Processing parameters
SI                 65536
SF             400.1300156 MHz
WDW                   EM
SSB                    0
LB                  0.30 Hz
GB                     0
PC                  1.00
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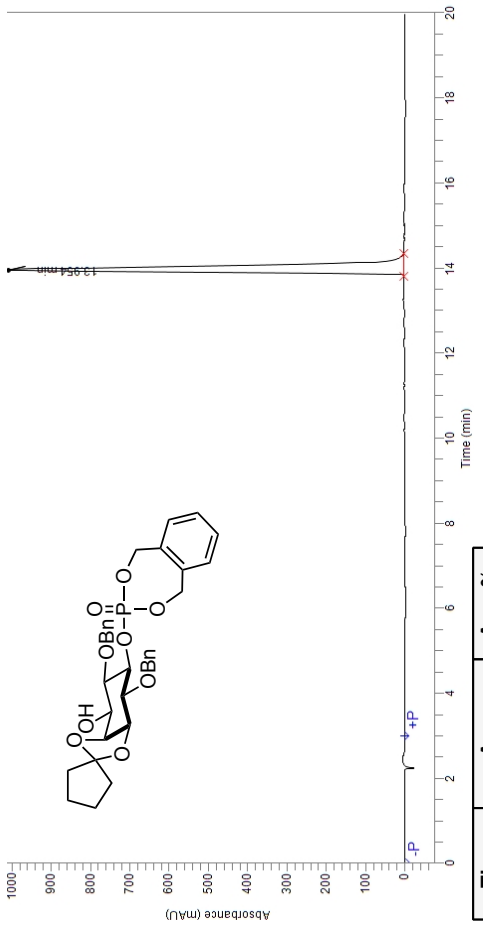
(+)-1D-2,3-*O*-Cyclopentylidene-4,6-di-*O*-benzyl-5-*O*-(2-oxo-5.6-benzo-1,3,2-dioxaphosphep-2-yl)-*myo*-inositol (+)-312 – ^{13}C NMR spectrum



(+)-1D-2,3-*O*-Cyclopentylidene-4,6-di-*O*-benzyl-5-*O*-(2-oxo-5.6-benzo-1,3,2-dioxaphosphep-2-yl)-*myo*-inositol (+)-312 – ^{31}P NMR spectrum



(+)-1D-2,3-*O*-Cyclopentylidene-4,6-di-*O*-benzyl-5-*O*-(2-oxo-5.6-benzo-1,3,2-dioxaphosphep-2-yl)-*myo*-inositol (+)-312 – RP-HPLC (Method 2)



Time	Area	Area %
13.954	9,287,838	100.00
Total	9,287,838	100.00

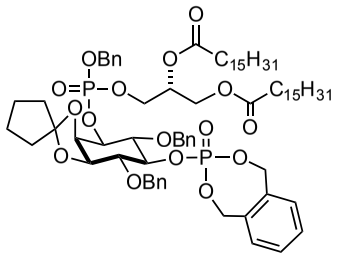
(–)-1D-1-*O*-(1,2-Di-*O*-palmitoyl-*sn*-glycer-3-yl-benzylphosphate)-2,3-*O*-cyclopentylidene-4,6-di-*O*-benzyl-5-*O*-(2-oxo-5.6-benzo-1,3,2-dioxaphosphep-2-yl)-*myo*-inositol (–)-313 – ^{1}H NMR spectrum

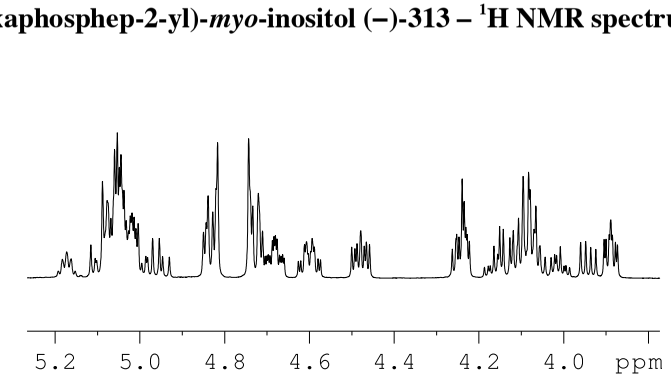
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Current Data Parameters
NAME          aje83p-data
EXPNO                   1
PROCNO                  1

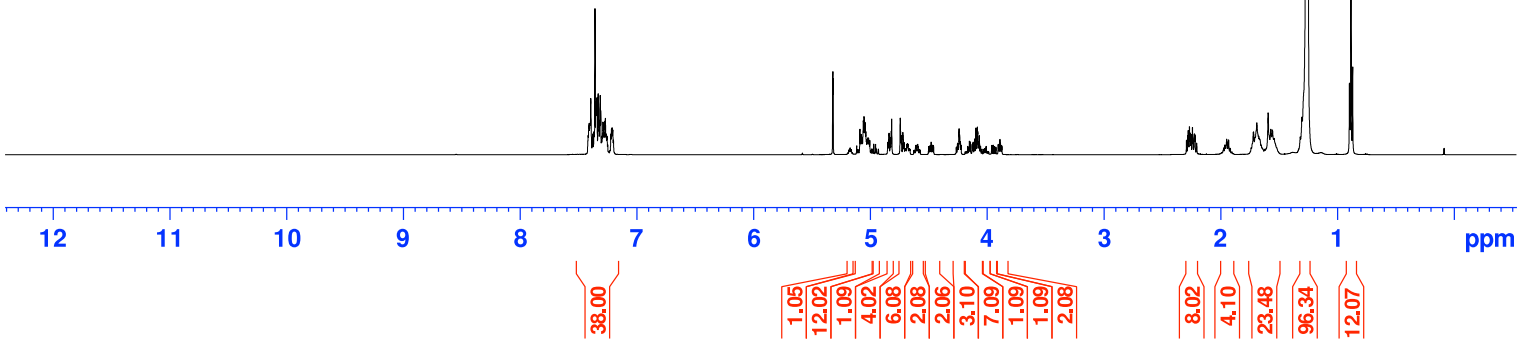
F2 - Acquisition Parameters
Date_           20160316
Time              12.27
INSTRUM          avc500
PROBHD   5 mm CPDUL 13C
PULPROG           zg30
TD               65536
SOLVENT          CD2Cl2
NS                  16
DS                   4
SWH          10330.578 Hz
FIDRES        0.157632 Hz
AQ           3.1719425 sec
RG                3.56
DW              48.400 usec
DE              10.00 usec
TE              298.0 K
D1          1.00000000 sec
TD0                 1

======== CHANNEL f1 ========
SFO1       500.3030896 MHz
NUC1               1H
P1              15.00 usec
PLW1        7.99830008 W

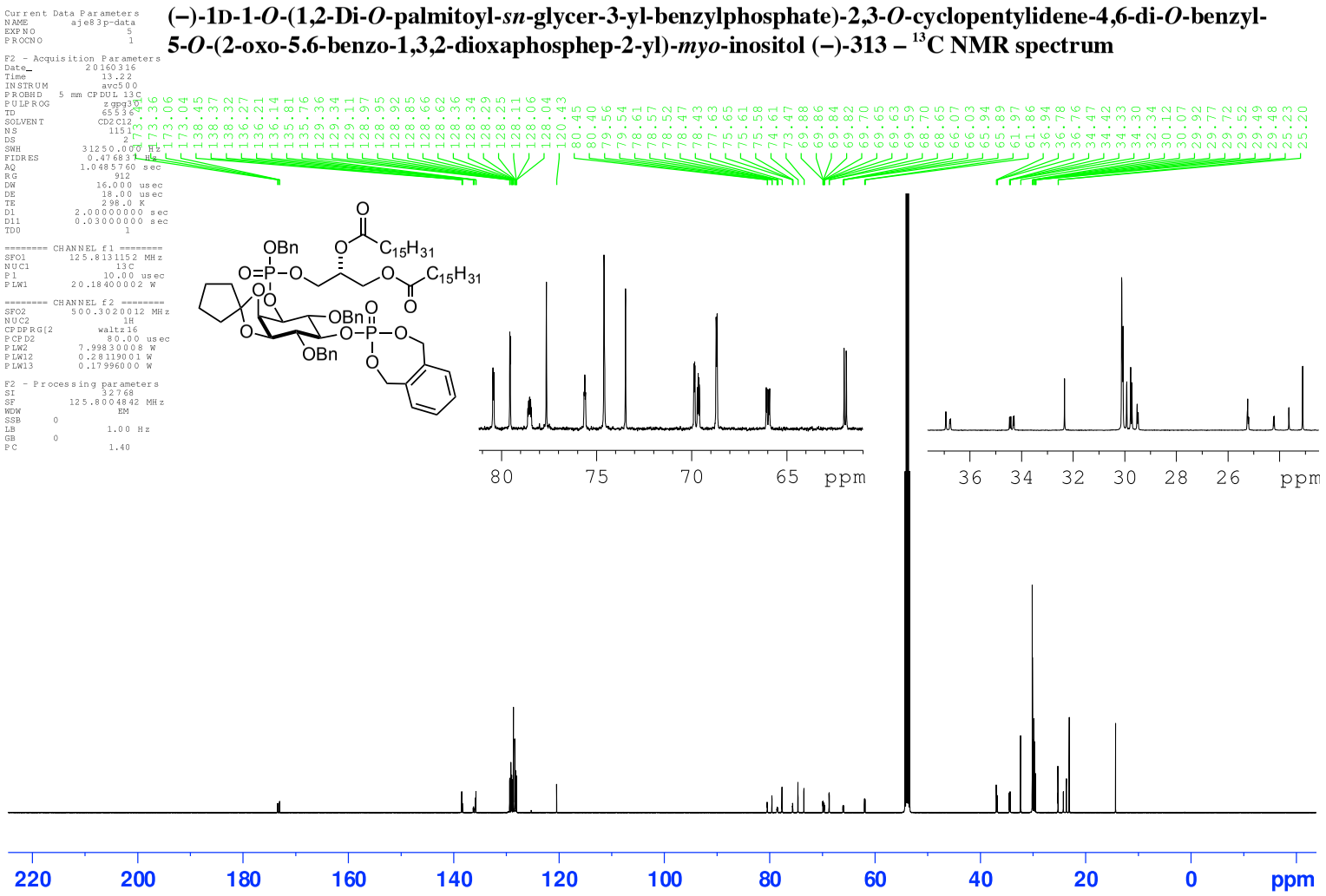
F2 - Processing parameters
SI               65536
SF          500.3000205 MHz
WDW                EM
SSB       0
LB              0.30 Hz
GB        0
PC              1.00
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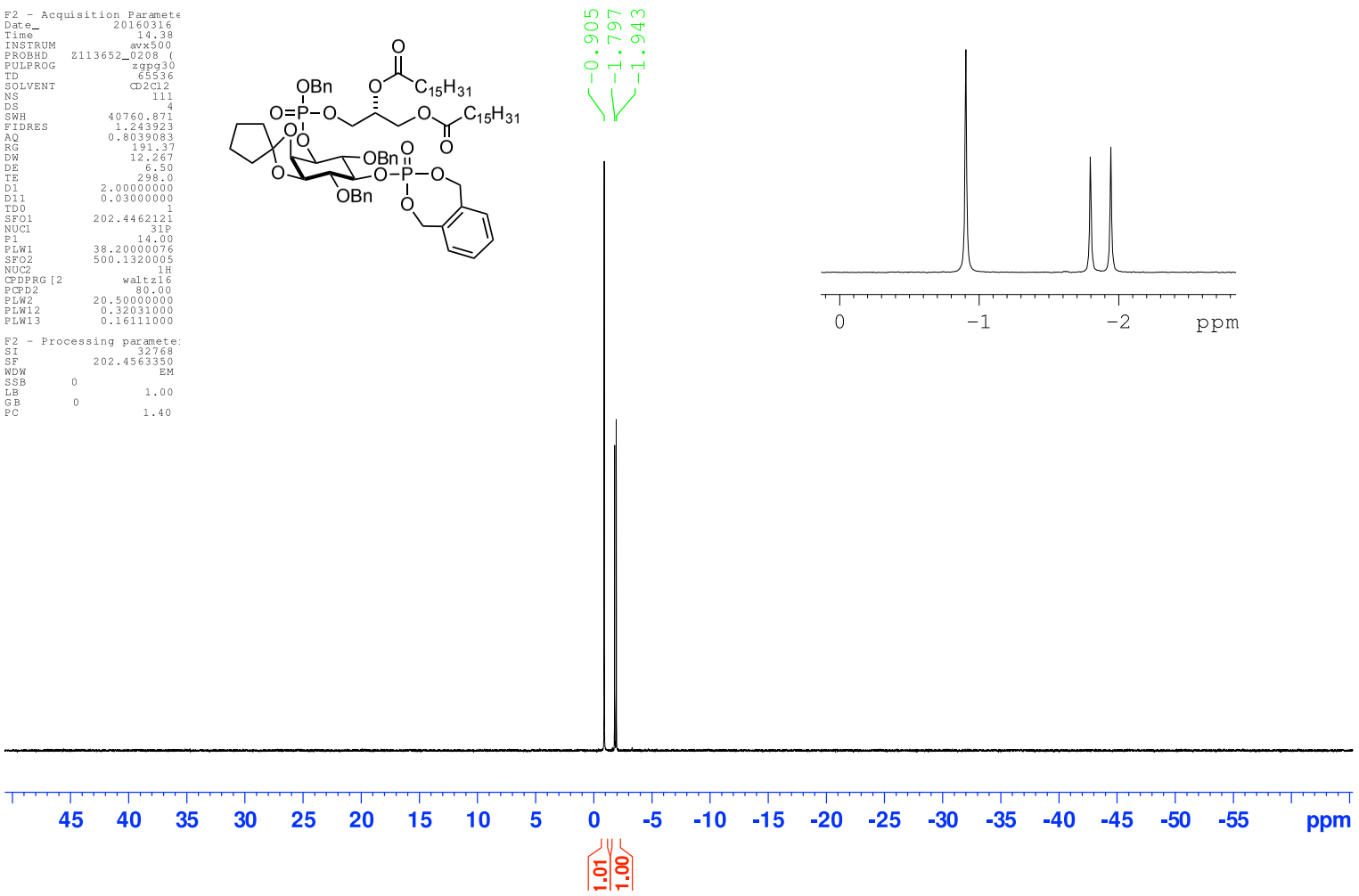




(–)-1D-1-*O*-(1,2-Di-*O*-palmitoyl-*sn*-glycer-3-yl-benzylphosphate)-2,3-*O*-cyclopentylidene-4,6-di-*O*-benzyl-5-*O*-(2-oxo-5.6-benzo-1,3,2-dioxaphosphep-2-yl)-*myo*-inositol (–)-313 – ^{13}C NMR spectrum

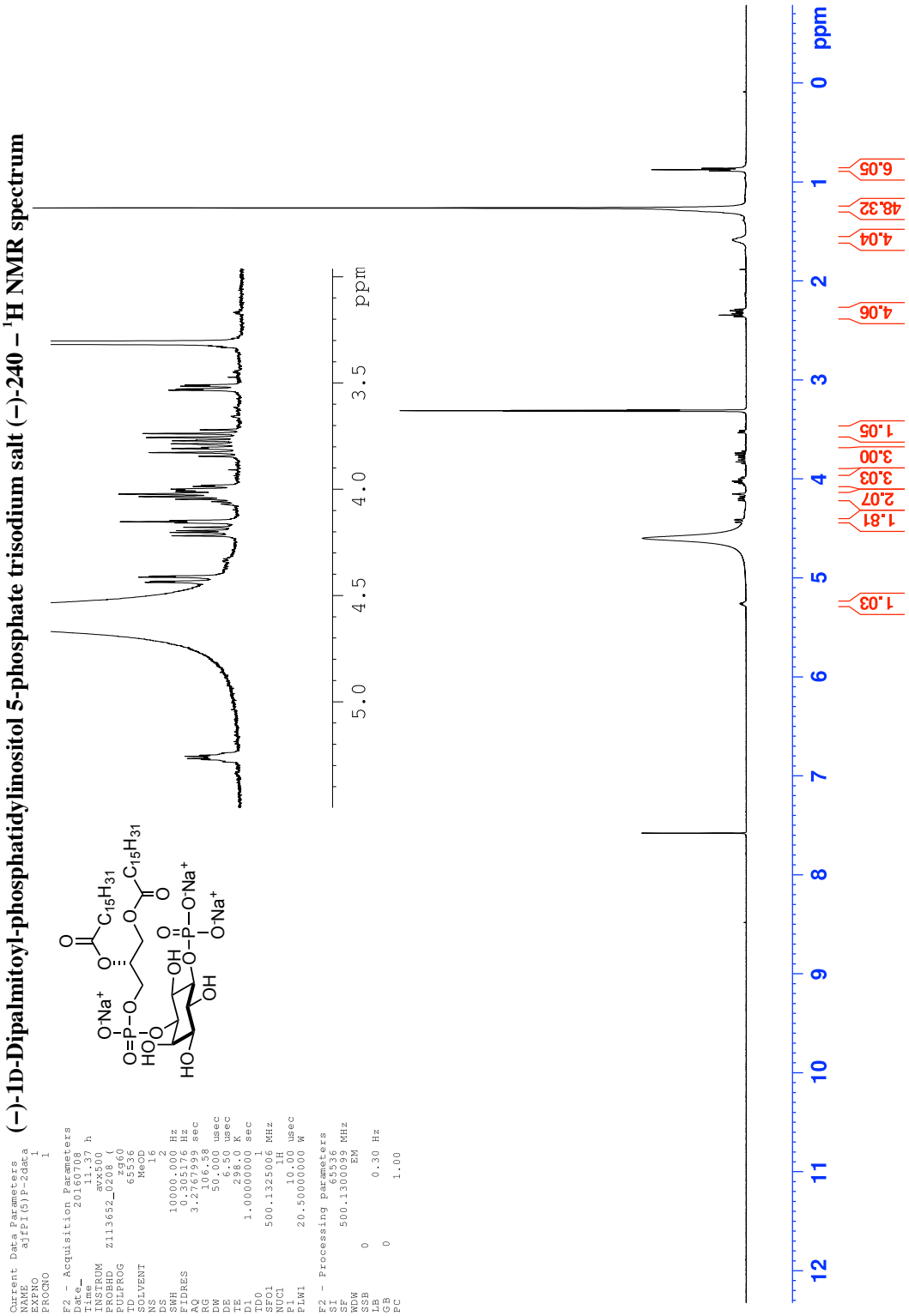


(–)-1D-1-*O*-(1,2-Di-*O*-palmitoyl-*sn*-glycer-3-yl-benzylphosphate)-2,3-*O*-cyclopentylidene-4,6-di-*O*-benzyl-5-*O*-(2-oxo-5.6-benzo-1,3,2-dioxaphosphep-2-yl)-*myo*-inositol (–)-313 – ^{13}C NMR spectrum

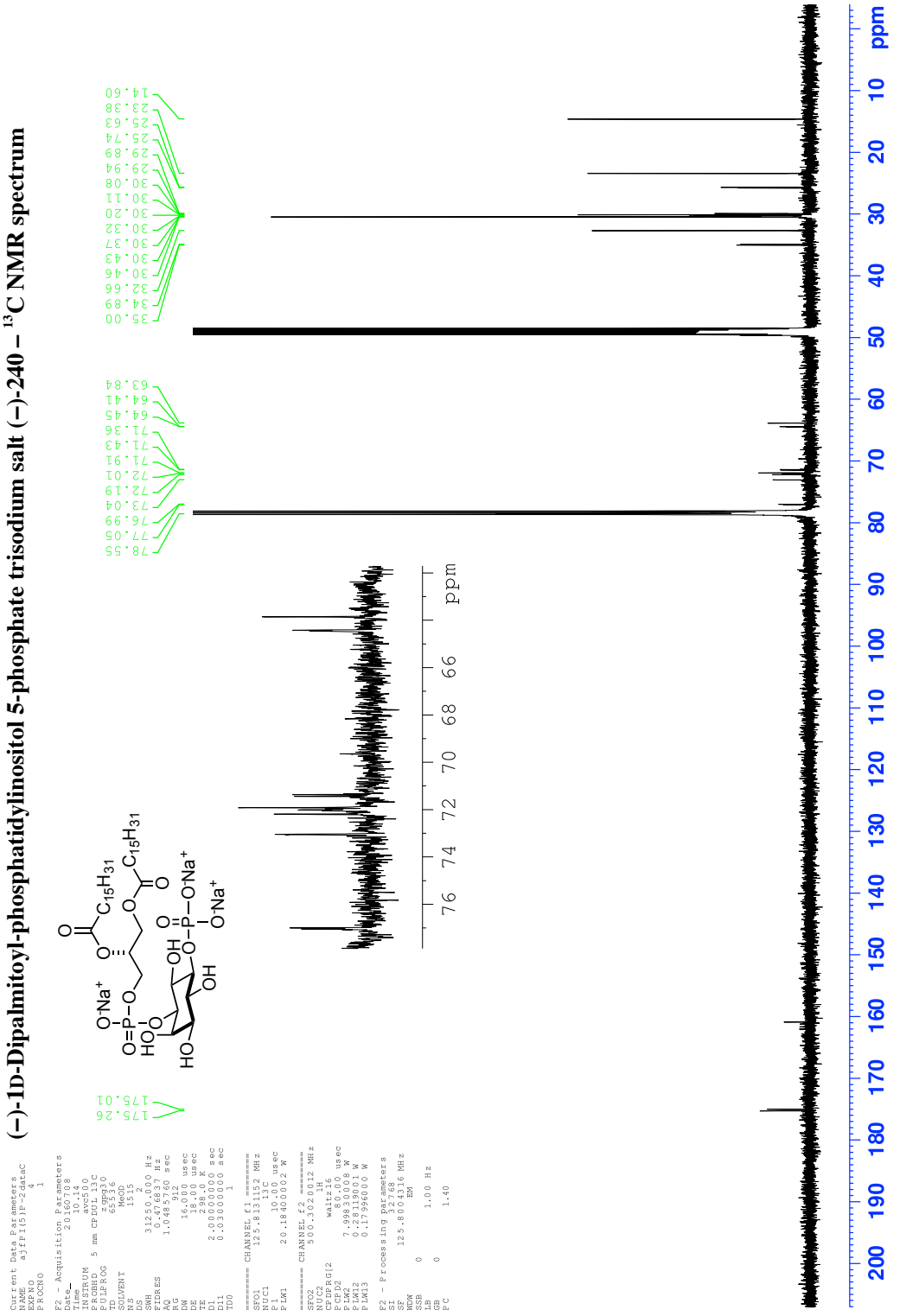


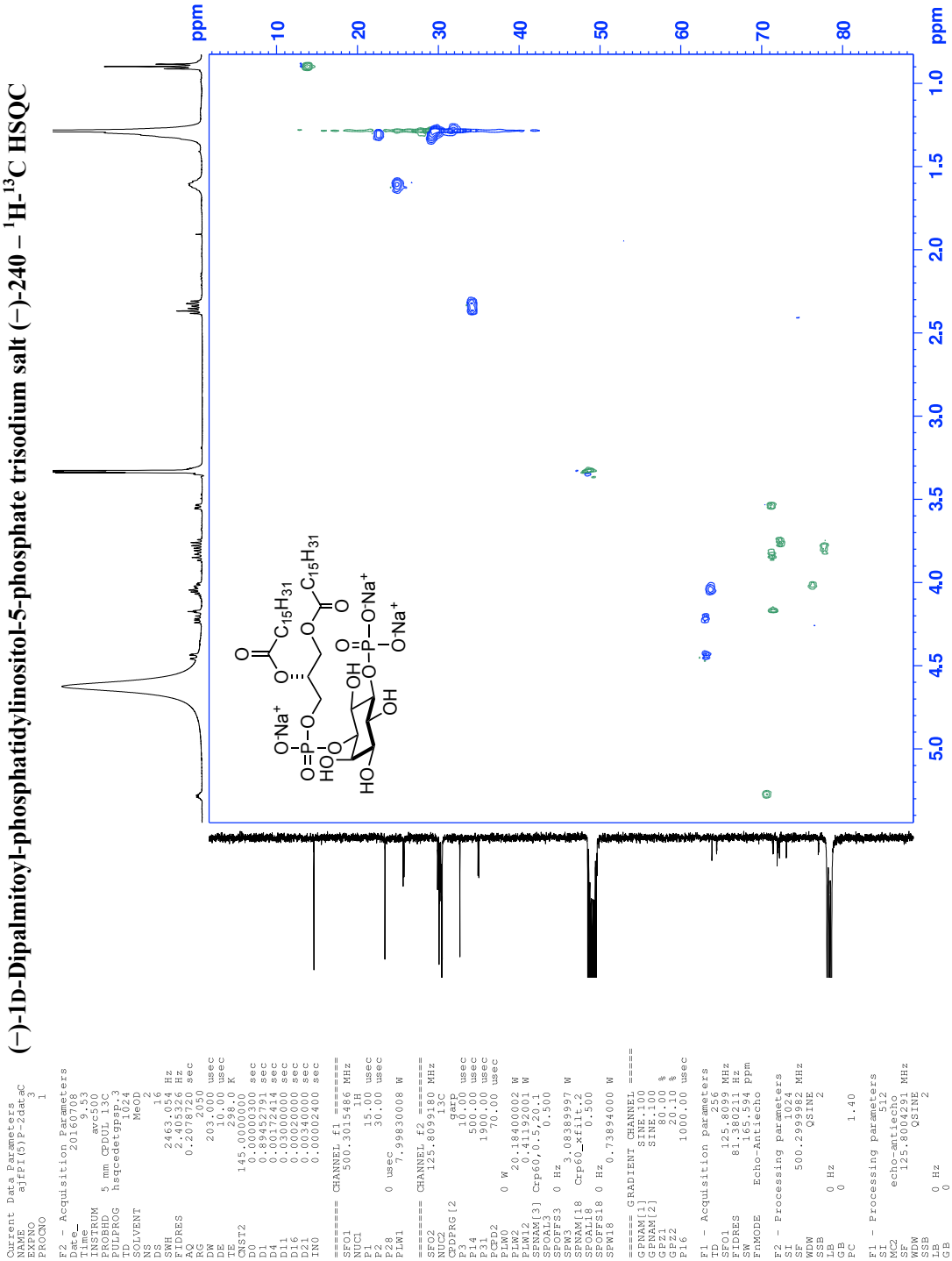
(–)-1D-1-*O*-(2,3-Di-*O*-palmitoyl-*sn*-glyceryl-1-benzylphosphate)-2,3-*O*-cyclopentylidene-4,6-di-*O*-benzyl-5-*O*-(2-oxo-5,6-benzo-1,3,2-dioxaphosphep-2-yl)-*myo*-inositol (–)-313 – NP-HPLC (Method 7)

Time	Area	Area %
2.529	29,723	1.09
2.589	33,437	1.23
4.065	1,314,178	48.36
4.416	1,340,319	49.32
Total	2,717,656	100.00

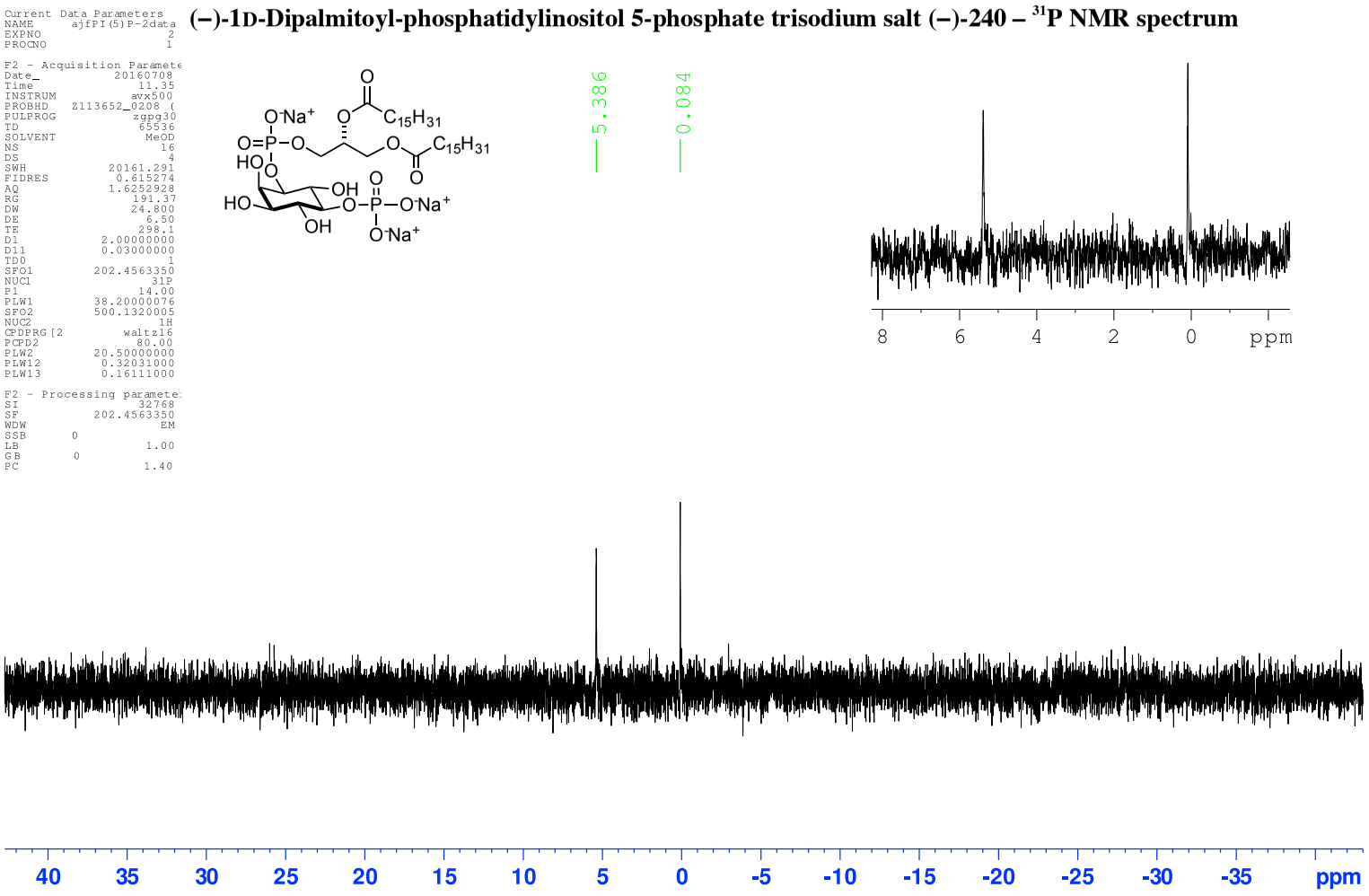

(–)-1D-Dipalmitoyl-phosphatidylinositol 5-phosphate trisodium salt (–)-240 – 1H NMR spectrum
Current Data Parameters
NAME ajfPI(5)P-2data
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20161703
Time 11.37 h
INSTRUM avx500
PROBHD Z113652_0208 (
PULPROG zg60
TD 65536
SOLVENT MeOD
NS 16
DS 2
SWH 10000.000 Hz
FIDRES 0.305176 Hz
AQ 3.2767999 sec
RG 106.58
DW 50.000 usec
DE 6.50 usec
TE 298.0 K
D1 1.00000000 sec
TD0 1
SFO1 500.1325006 MHz
NUC1 1H
P1 10.00 usec
PLW1 20.50000000 W
F2 - Processing parameters
SI 65536
SF 500.1300099 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
5.0
4.5
4.0
3.5
ppm
12
11
10
9
8
7
6
5
4
3
2
1
0
ppm
6.05
48.32
4.04
4.06
1.05
3.00
3.03
2.07
1.81
1.03

(–)-1D-Dipalmitoyl-phosphatidylinositol 5-phosphate trisodium salt (–)-240 – ^{13}C NMR spectrum

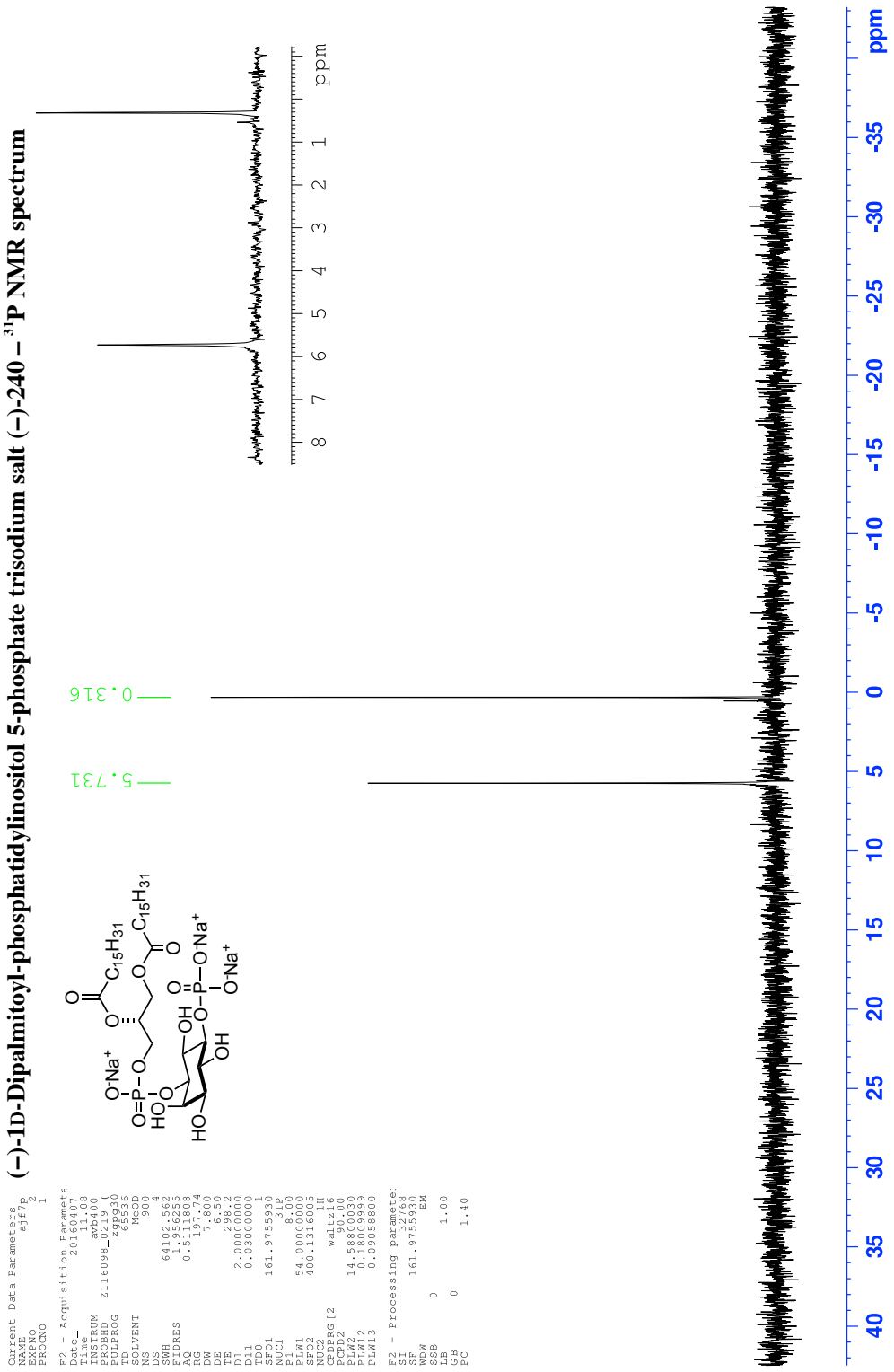


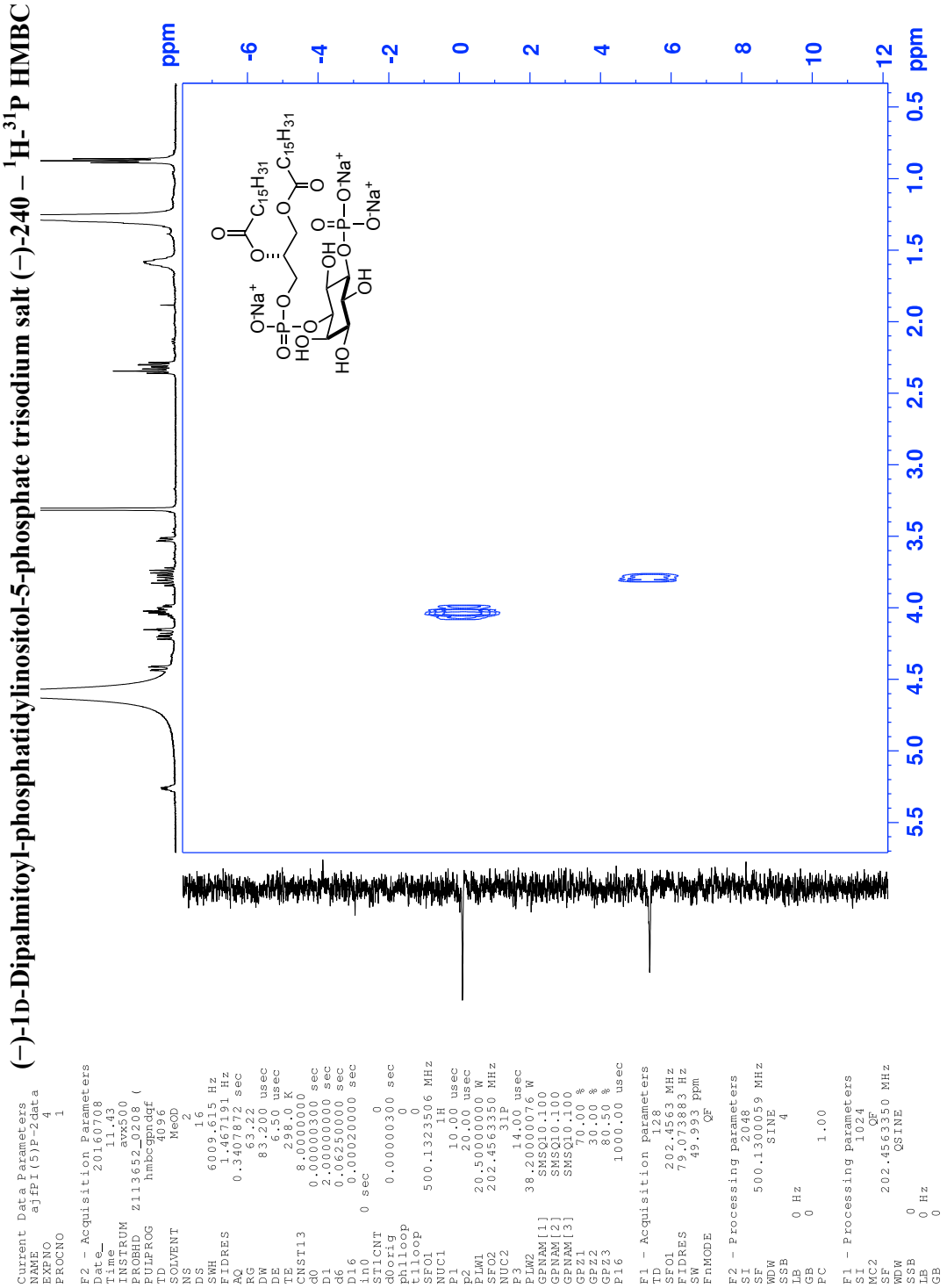

(−)-1D-Dipalmitoyl-phosphatidylinositol-5-phosphate trisodium salt (−)-240 – ^{1}H-^{13}C HSQC
ppm
10
20
30
40
50
60
70
80
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm
$C_{15}H_{31}$
$C_{15}H_{31}$
O⁻Na⁺
O=P–O
HO
O
OH
O=P–O⁻Na⁺
OH
HO
O⁻Na⁺

(–)-1D-Dipalmitoyl-phosphatidylinositol 5-phosphate trisodium salt (–)-240 – ^{31}P NMR spectrum

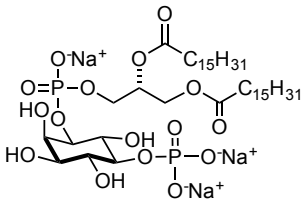


(–)-1D-Dipalmitoyl-phosphatidylinositol 5-phosphate trisodium salt (–)-240 – ^{31}P NMR spectrum

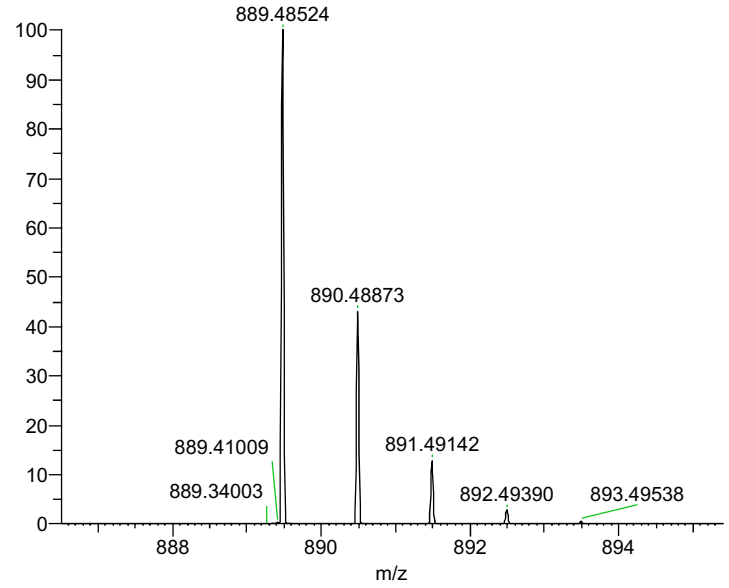



(−)-1D-Dipalmitoyl-phosphatidylinositol-5-phosphate trisodium salt (−)-240 – ^{1}H-^{31}P HMBC
ppm
-6
-4
-2
0
2
4
6
8
10
12
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm
$C_{15}H_{31}$
$C_{15}H_{31}$
O⁻Na⁺
O⁻Na⁺
O⁻Na⁺
O=P–O
HO
HO
OH
OH
O=P–O
Current Data Parameters
NAME ajfpi(5)p-2data
EXPNO 4
PROCNO 1
F2 - Acquisition Parameters
Date_ 20160708
Time 11.43
INSTRUM avx500
PROBHD Z113652_0208 (
PULPROG hmbcgpndqf
TD 4096
SOLVENT MeOD
NS 2
DS 16
SWH 6009.615 Hz
FIDRES 1.467191 Hz
AQ 0.3407872 sec
RG 63.22
DW 83.200 usec
DE 6.50 usec
TE 298.0 K
CNST13 8.0000000
d0 0.00000300 sec
D1 2.00000000 sec
d6 0.06250000 sec
D16 0.00020000 sec
in0 0 sec
STICNT 0
d0orig 0.00000300 sec
philoop 0
tiloop 0
SFO1 500.1323506 MHz
NUC1 1H
P1 10.00 usec
p2 20.00 usec
PLW1 20.50000000 W
SFO2 202.4563350 MHz
NUC2 31P
P3 14.00 usec
PLW2 38.20000076 W
GPNAM[1] SMSQ10.100
GPNAM[2] SMSQ10.100
GPNAM[3] SMSQ10.100
GPZ1 70.00 %
GPZ2 30.00 %
GPZ3 80.50 %
P16 1000.00 usec
F1 - Acquisition parameters
TD 128
SFO1 202.4563 MHz
FIDRES 79.073883 Hz
SW 49.993 ppm
FnMODE QF
F2 - Processing parameters
SI 2048
SF 500.1300059 MHz
WDW SINE
SSB 4
LB 0 Hz
GB 0
PC 1.00
F1 - Processing parameters
SI 1024
MC2 QF
SF 202.4563350 MHz
WDW QSINE
SSB 0
LB 0 Hz
GB 0

(−)-1D-Dipalmitoyl-phosphatidylinositol-5-phosphate trisodium salt (−)-240 – Mass HMBC

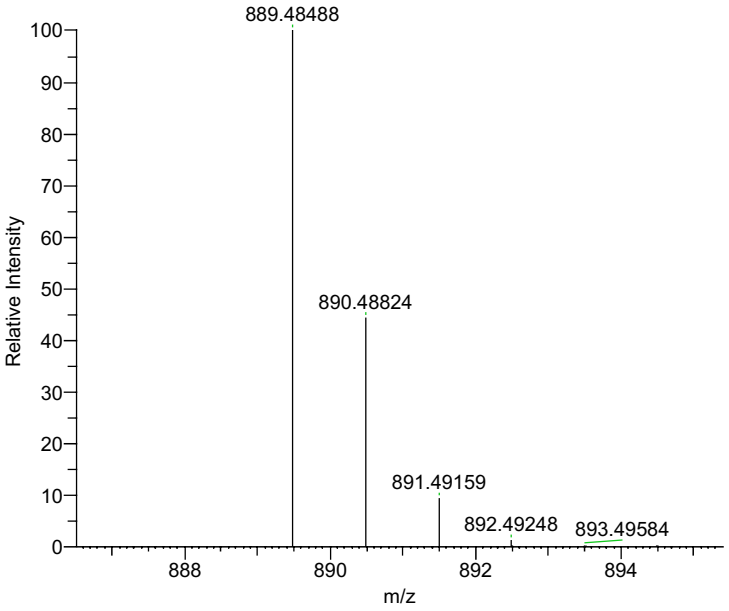


W:\data\July 16\MSS182.raw 21/07/2016 12:30 pm



NL: 1.65E6
MSS182 #13-28 RT: 0.16-0.32 AV: 8 NL: 2.57E7
T: FTMS {1,2} - p ESI Full ms [80.00-1600.00]

Measured Spectrum

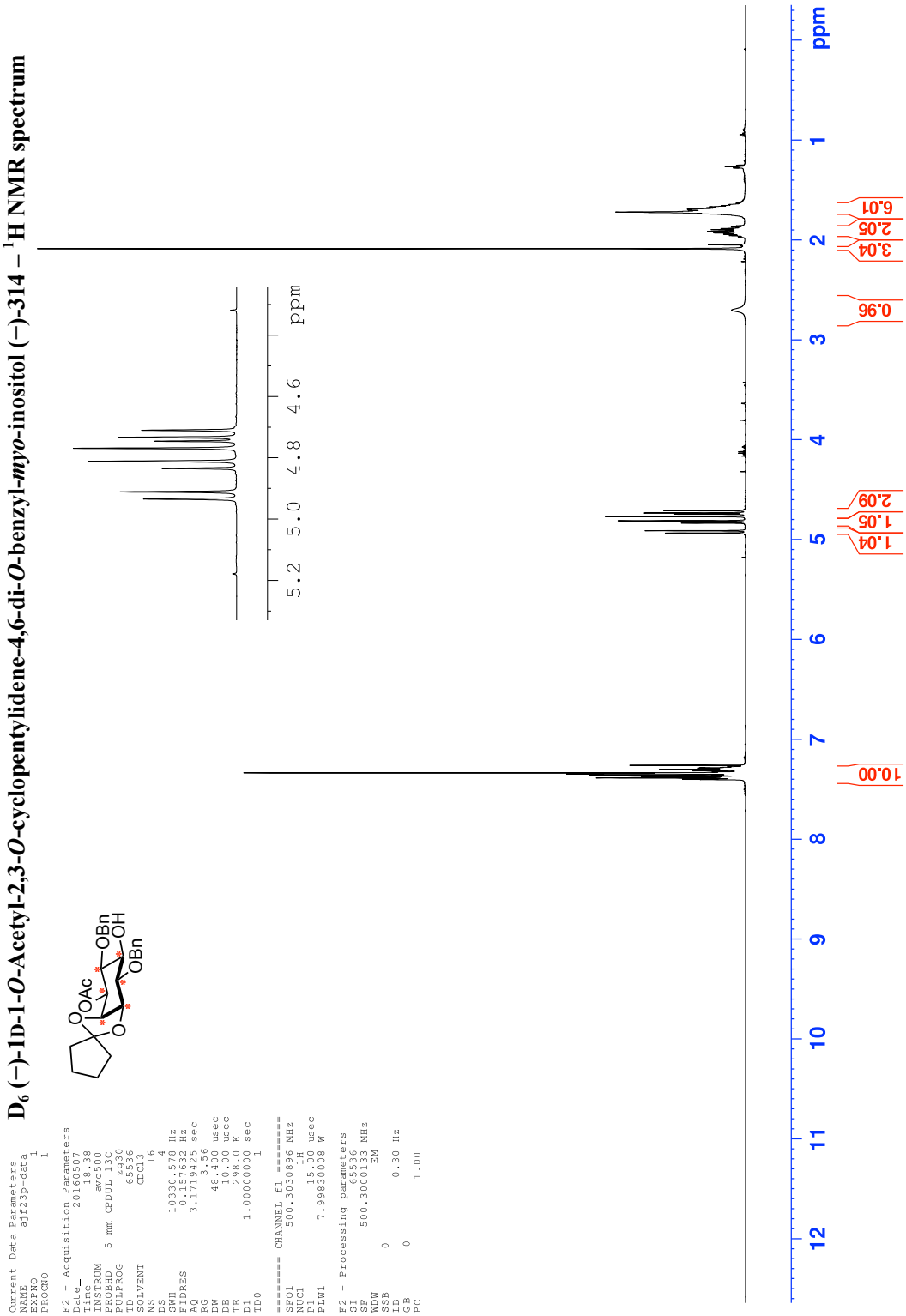


NL: 6.13E5
C41H79O16P2: $C_{41}H_{79}O_{16}P_2$ Chrg -1 R: 1000000 Res. Pwr. @FWHM

Theoretical Spectrum

m/z	Formula	RDB	Delta ppm	Theo. Mass
889.48523	$C_{41}H_{79}O_{16}P_2$	3.5	0.39	889.48488

D_6 (−)-1D-1-*O*-Acetyl-2,3-*O*-cyclopentylidene-4,6-di-*O*-benzyl-*myo*-inositol (−)-314 – ^{1}H NMR spectrum

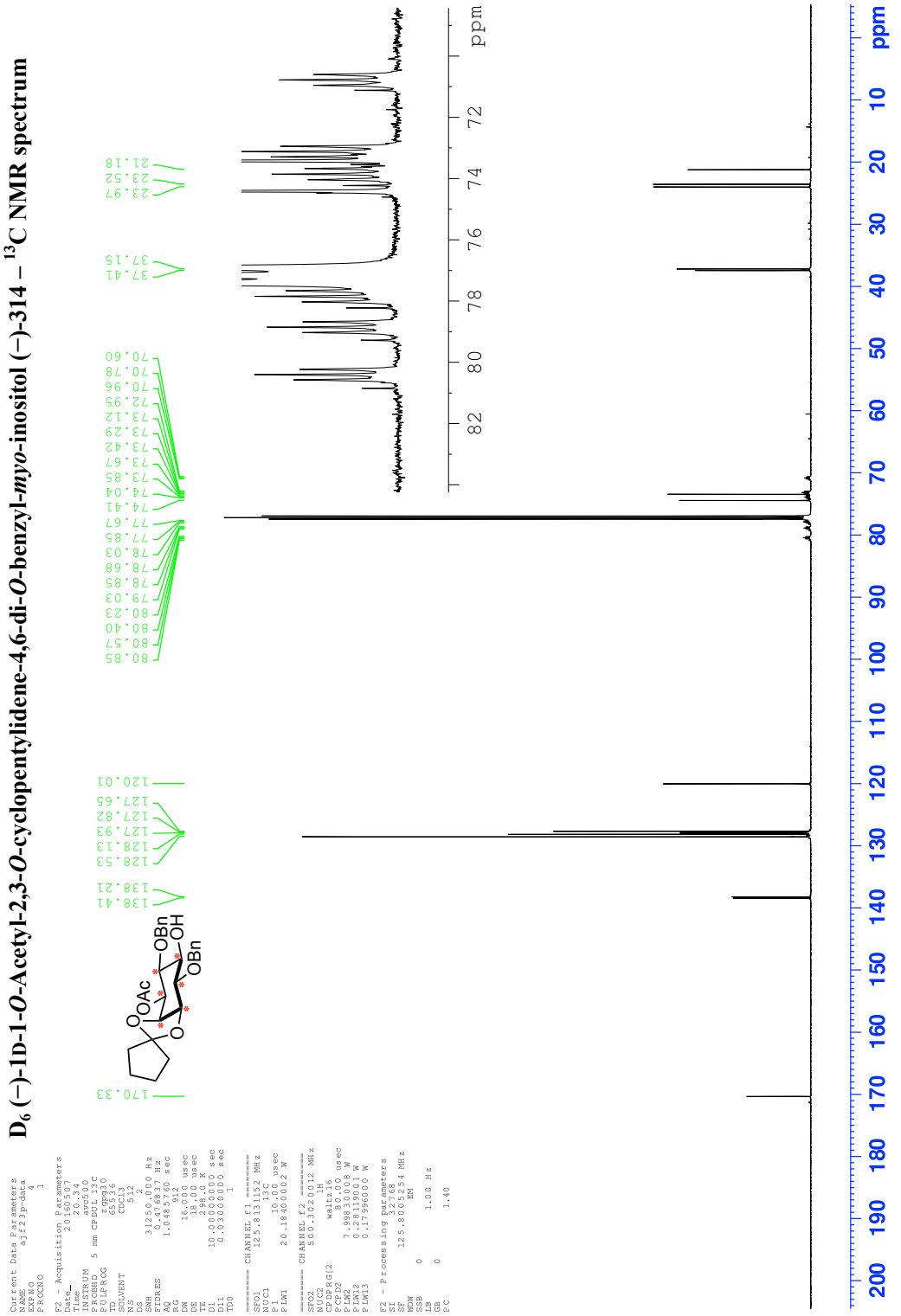



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NAME                  ajf23p-data
EXPNO                           1
PROCNO                          1

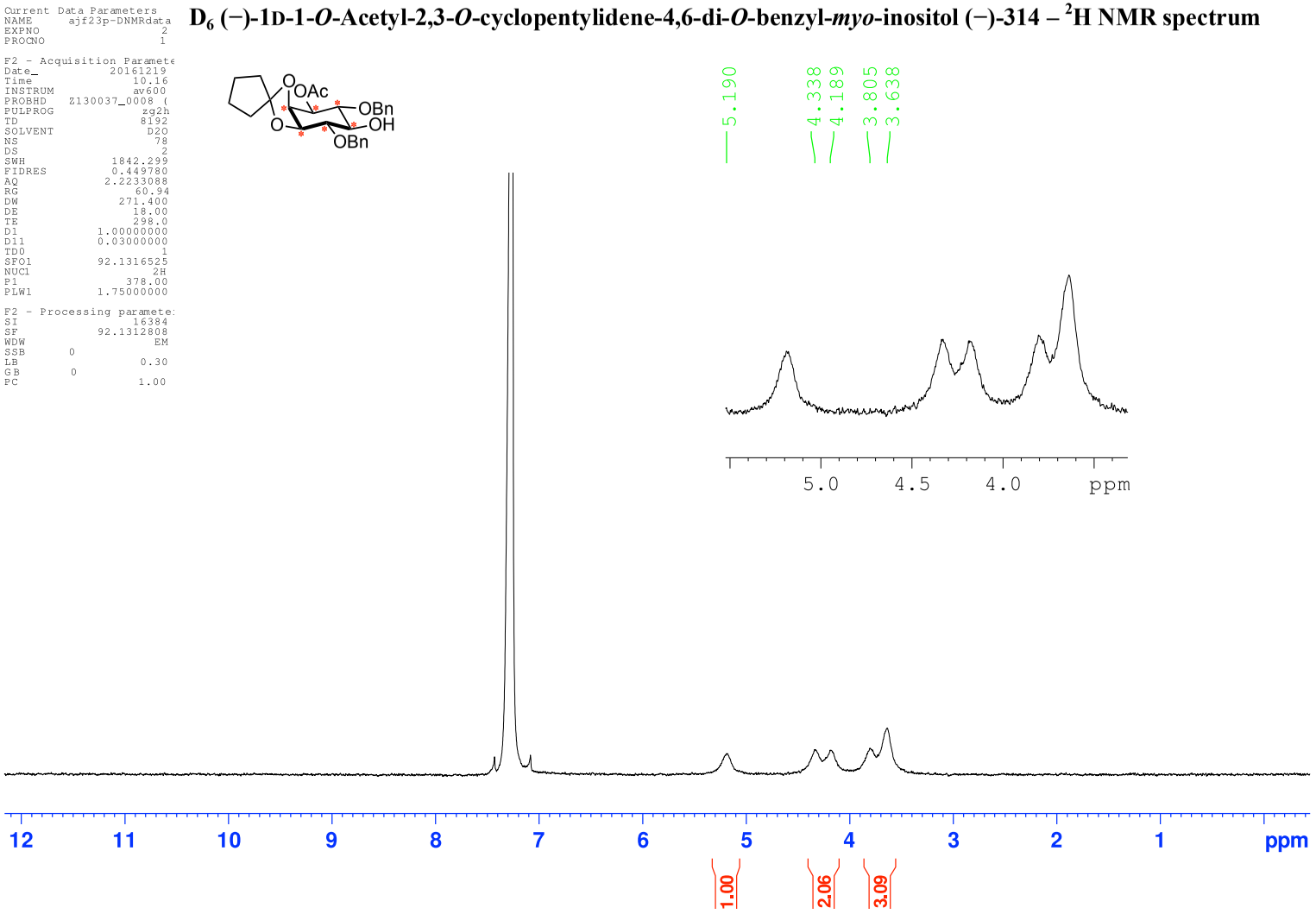
F2 - Acquisition Parameters
Date_              20161027
Time               13.38
INSTRUM                 avc500
PROBHD     5 mm CPDUL 13C
PULPROG                 zg30
TD                     65536
SOLVENT                CDCl3
NS                        16
DS                         4
SWH               10330.578 Hz
FIDRES            0.157633 Hz
AQ                3.1719425 sec
RG                      3.56
DW                   48.400 usec
DE                   10.00 usec
TE                   298.0 K
D1                1.00000000 sec
TD0                        1

======== CHANNEL f1 ========
SFO1              500.3030896 MHz
NUC1                        1H
P1                       15.00 usec
PLW1              7.99830008 W

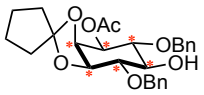
F2 - Processing parameters
SI                        65536
SF                500.3000136 MHz
WDW                         EM
SSB                  0
LB                       0.30 Hz
GB                   0
PC                       1.00
```


D6 (−)-1D-1-O-Acetyl-2,3-O-cyclopentylidene-4,6-di-O-benzyl-myo-inositol (−)-314 – 13C NMR spectrum
OAc
OBn
OH
OBn
170.33
138.41
138.21
128.53
128.13
127.93
127.82
127.65
120.01
80.85
80.57
80.40
80.23
79.03
78.85
78.68
78.03
77.85
77.67
74.41
74.04
73.85
73.67
73.42
73.29
73.12
72.95
70.96
70.78
70.60
37.41
37.15
23.97
23.52
21.18
ppm
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 ppm

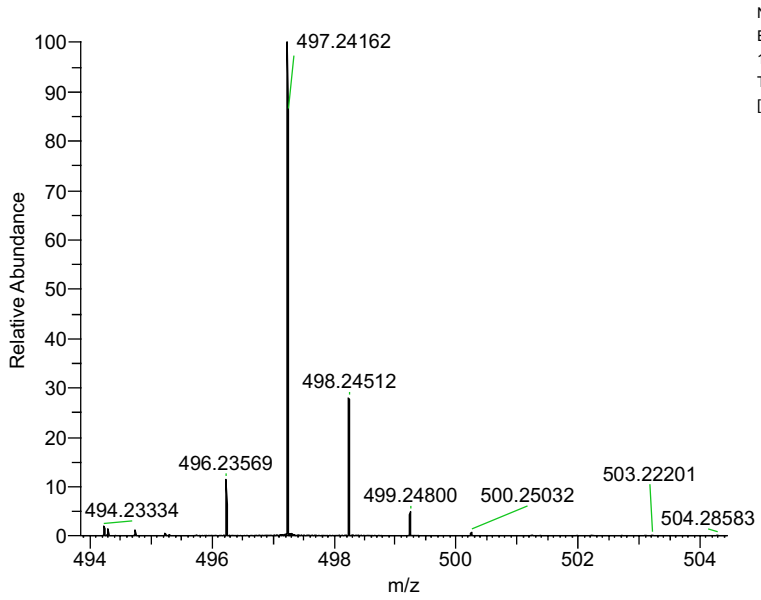
D_6 (−)-1D-1-*O*-Acetyl-2,3-*O*-cyclopentylidene-4,6-di-*O*-benzyl-*myo*-inositol (−)-314 – ^{2}H NMR spectrum

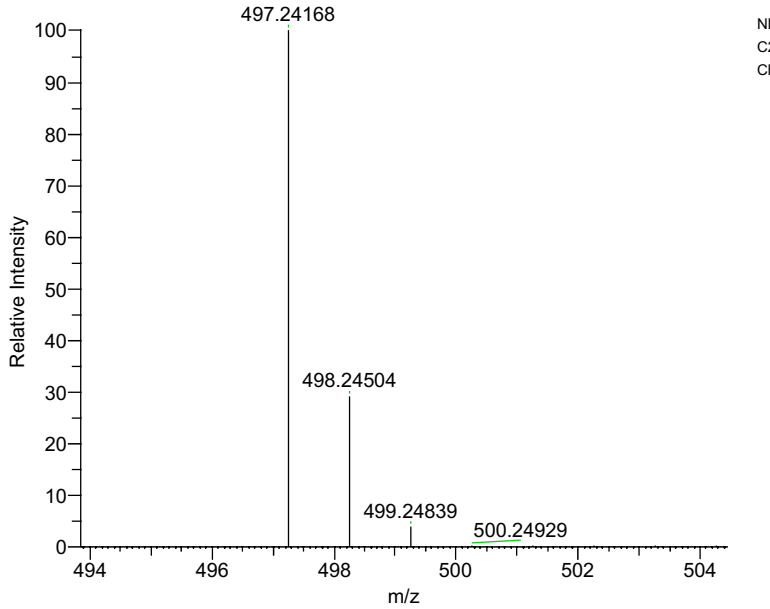


D_6 (−)-1D-1-*O*-Acetyl-2,3-*O*-cyclopentylidene-4,6-di-*O*-benzyl-*myo*-inositol (−)-314 – Mass spectrum



W:\data\May 16\ESI56919.raw 09/05/2016 10:56 am





m/z	Formula	RDB	Delta ppm	Theo. Mass
497.24161	$C_{27}H_{26}{}^{2}H_{6}O_{7}{}^{23}Na$	11.5	-0.16	497.24168

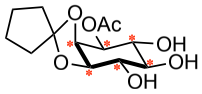
D_6 (–)-1D-1-*O*-Acetyl-2,3-*O*-cyclopentylidene-*myo*-inositol (–)-315 – ^{1}H NMR spectrum

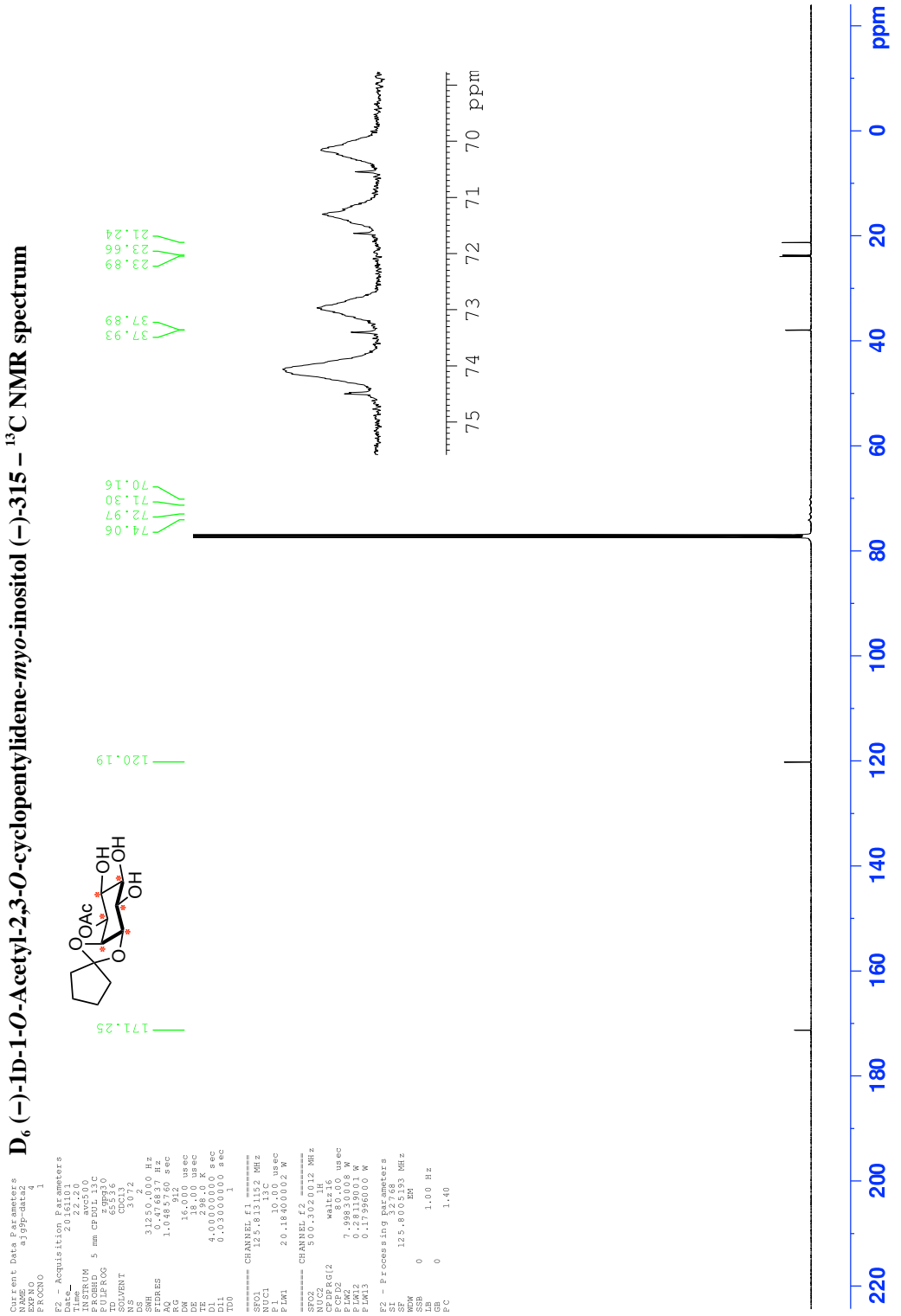
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Current Data Parameters
NAME          ajg9p-data2
EXPNO                   1
PROCNO                  1

F2 - Acquisition Parameters
Date_           20161101
Time              17.08
INSTRUM          avc500
PROBHD   5 mm CPDUL 13C
PULPROG            zg30
TD                65536
SOLVENT           CDCl3
NS                   16
DS                    4
SWH           10330.578 Hz
FIDRES         0.157632 Hz
AQ            3.1719425 sec
RG                    4
DW               48.400 usec
DE               10.00 usec
TE               298.0 K
D1           1.00000000 sec
TD0                   1

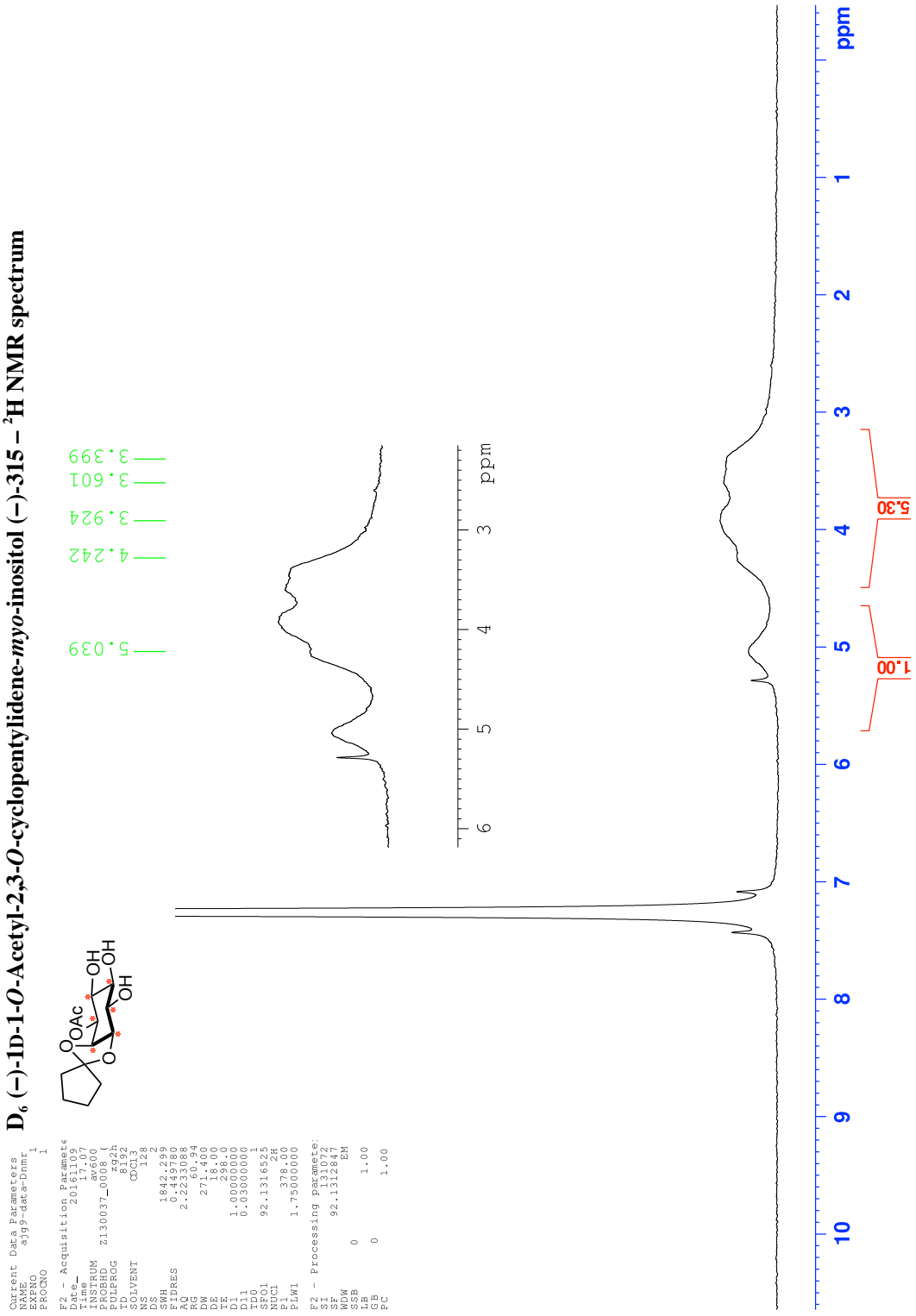
======== CHANNEL f1 ========
SFO1        500.3030896 MHz
NUC1               1H
P1               15.00 usec
PLW1          7.99830008 W

F2 - Processing parameters
SI                65536
SF          500.3000135 MHz
WDW                EM
SSB       0
LB              0.30 Hz
GB        0
PC              1.00
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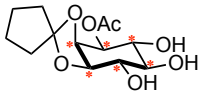



D6 (−)-1D-1-O-Acetyl-2,3-O-cyclopentylidene-myo-inositol (−)-315 – 13C NMR spectrum
Current Data Parameters
NAME ajg99r-data2
EXPNO 4
PROCNO 1
F2 - Acquisition Parameters
Date_ 20161101
Time 22.20 h
INSTRUM avc500
PROBHD 5 mm CPDUL 13C
PULPROG zgpg30
TD 65536
SOLVENT CDCl3
NS 3072
DS 4
SWH 31250.000 Hz
FIDRES 0.476837 Hz
AQ 1.0485760 sec
RG 912
DW 16.000 usec
DE 18.00 usec
TE 298.0 K
D1 4.00000000 sec
D11 0.03000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 125.8131152 MHz
NUC1 13C
P1 10.00 usec
PLW1 20.18400002 W
======== CHANNEL f2 ========
SFO2 500.3020012 MHz
NUC2 1H
CPDPRG2 waltz16
PCPD2 80.00 usec
PLW2 7.99830008 W
PLW12 0.42313001 W
PLW13 0.11796001 W
F2 - Processing parameters
SI 32768
SF 125.8053193 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
120.19
171.25
74.06
72.97
71.30
70.16
37.93
37.89
23.89
23.66
21.24
75 74 73 72 71 70 ppm
220 200 180 160 140 120 100 80 60 40 20 0 ppm

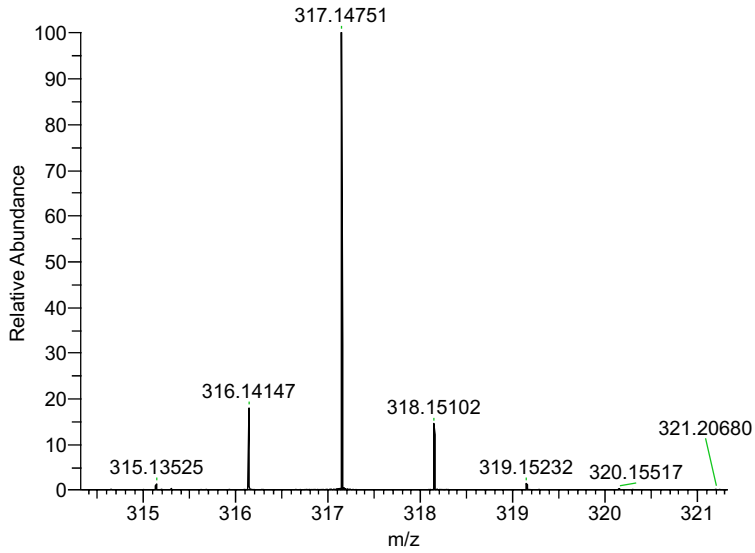
D_6 (–)-1D-1-*O*-Acetyl-2,3-*O*-cyclopentylidene-*myo*-inositol (–)-315 – ^{2}H NMR spectrum



D_6 (–)-1D-1-*O*-Acetyl-2,3-*O*-cyclopentylidene-*myo*-inositol (–)-315 – Mass spectrum

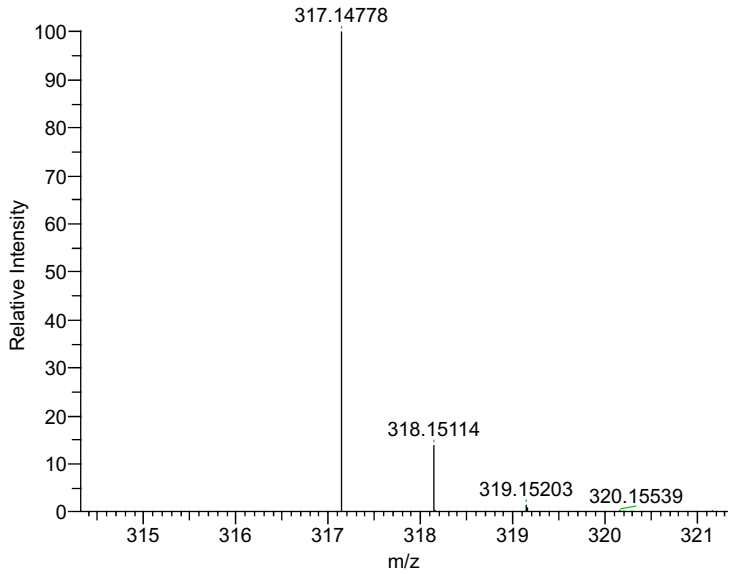


S:\data\Nov 16\ESI59700.raw 08/11/2016 2:59 pm



NL: 5.87E7
ESI59700 #15-25 RT: 0.17-0.28 AV: 6 NL: 5.87E+007
T: FTMS {1,1} + p ESI Full lock ms [80.00-1600.00]

Measured Spectrum



NL: 8.53E5
C13H14[2]H6O7Na1: C_{13} H_{14} $^{2}H_{6}$ O_{7} Na
Chrg 1 R: 1000000 Res. Pwr. @FWHM

Theoretical Spectrum

m/z	Formula	RDB	Delta ppm	Theo. Mass
317.14752	$C_{13}H_{14}{}^{2}H_{6}O_{7}{}^{23}Na$	3.5	-0.83	317.14778

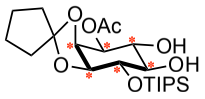
D_6 (−)-1D-1-*O*-Acetyl-2,3-*O*-cyclopentylidene-4-*O*-(triisopropylsilyl)-*myo*-inositol (−)-316 – ^{1}H NMR spectrum

```
Current Data Parameters
NAME         ajg11-p-data
EXPNO                   1
PROCNO                  1

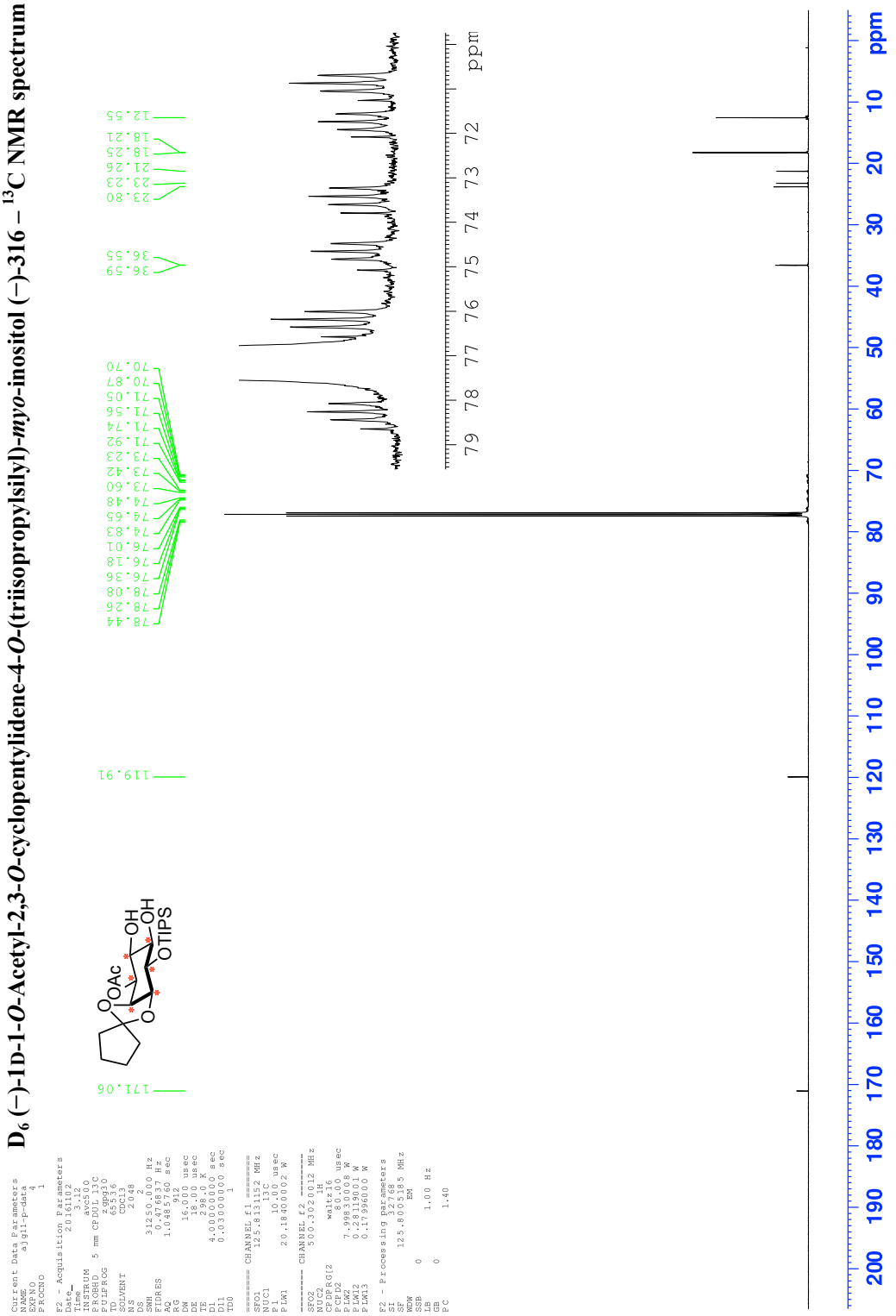
F2 - Acquisition Parameters
Date_           20161101
Time              23.28
INSTRUM          avc500
PROBHD   5 mm CPDUL 13C
PULPROG           zg30
TD               65536
SOLVENT          CDCl3
NS                  16
DS                   4
SWH          10330.578 Hz
FIDRES        0.157632 Hz
AQ           3.1719425 sec
RG                3.56
DW               48.400 usec
DE               10.00 usec
TE               298.0 K
D1          1.00000000 sec
TD0                  1

======== CHANNEL f1 ========
SFO1        500.3030896 MHz
NUC1               1H
P1               15.00 usec
PLW1         7.99830008 W

F2 - Processing parameters
SI               65536
SF          500.3000133 MHz
WDW                EM
SSB       0
LB              0.30 Hz
GB        0
PC              1.00
```



D$_6$ (−)-1D-1-*O*-Acetyl-2,3-*O*-cyclopentylidene-4-*O*-(triisopropylsilyl)-*myo*-inositol (−)-316 – ^{13}C NMR spectrum

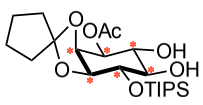


D_6 (−)-1D-1-*O*-Acetyl-2,3-*O*-cyclopentylidene-4-*O*-(triisopropylsilyl)-*myo*-inositol (−)-316 – ^{2}H NMR spectrum

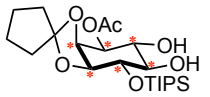
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Current Data Parameters
NAME       ajg11-data-DNMR
EXPNO                   11
PROCNO                   1

F2 - Acquisition Parameter
Date_             20161109
Time                 16.41
INSTRUM              av600
PROBHD    Z130037_0008 (
PULPROG              zg2h
TD                   8192
SOLVENT             CDCl3
NS                     69
DS                      2
SWH              1842.299
FIDRES           0.449780
AQ               2.2233088
RG                   50.94
DW                 271.400
DE                   18.00
TE                   298.0
D1              1.00000000
D11             0.03000000
TD0                     1
SFO1            92.1316525
NUC1                   2H
P1                  378.00
PLW1            1.75000000

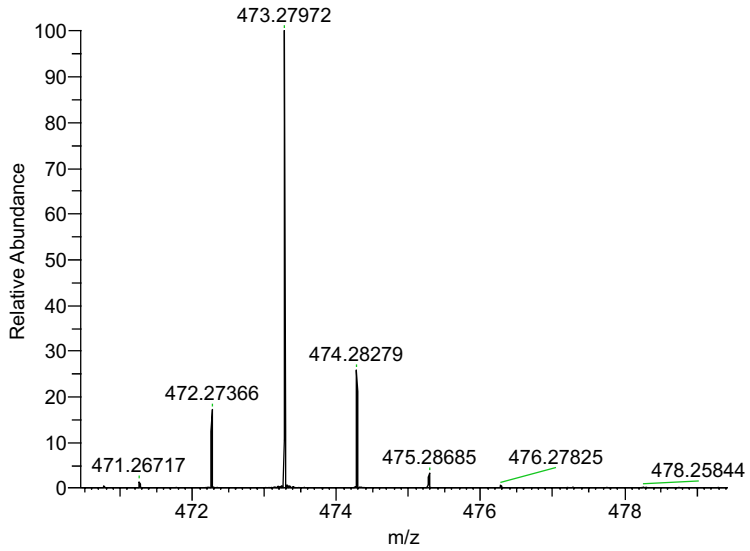
F2 - Processing parameters
SI                   16384
SF              92.1312840
WDW                     EM
SSB       0
LB                    0.30
GB        0
PC                    1.00
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D_6 (−)-1D-1-*O*-Acetyl-2,3-*O*-cyclopentylidene-4-*O*-(triisopropylsilyl)-*myo*-inositol (−)-316 – Mass spectrum

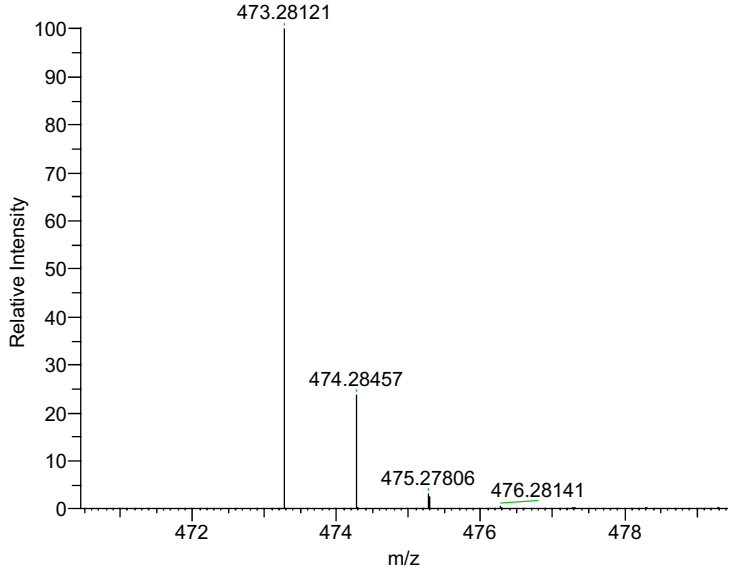


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NL: 5.97E7
ESI59716 #15-25 RT: 0.17-0.28 AV: 6 NL: 5.97E+007
T: FTMS {1,1} + p ESI Full ms [80.00-1600.00]

Measured Spectrum



NL: 7.13E5
C22H34[2]H6O7Na1Si1: C_{22} H_{34} $^{2}H_{6}$ O_{7} Na Si Chrg 1 R: 1000000 Res. Pwr. @FWHM

Theoretical Spectrum

m/z	Formula	RDB	Delta ppm	Theo. Mass
473.27972	$C_{22}H_{34}{}^{2}H_{6}O_{7}{}^{23}Na^{28}Si$	3.5	-3.14	473.28121

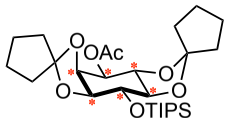
D_6 (−)-1D-1-*O*-Acetyl-2,3-*O*-cyclopentylidene-4-*O*-(triisopropylsilyl)-5,6-*O*-cyclopentylidene-*myo*-inositol (−)-317 – ^{1}H NMR spectrum

```
Current Data Parameters
NAME          ajg12p-data
EXPNO                   1
PROCNO                  1

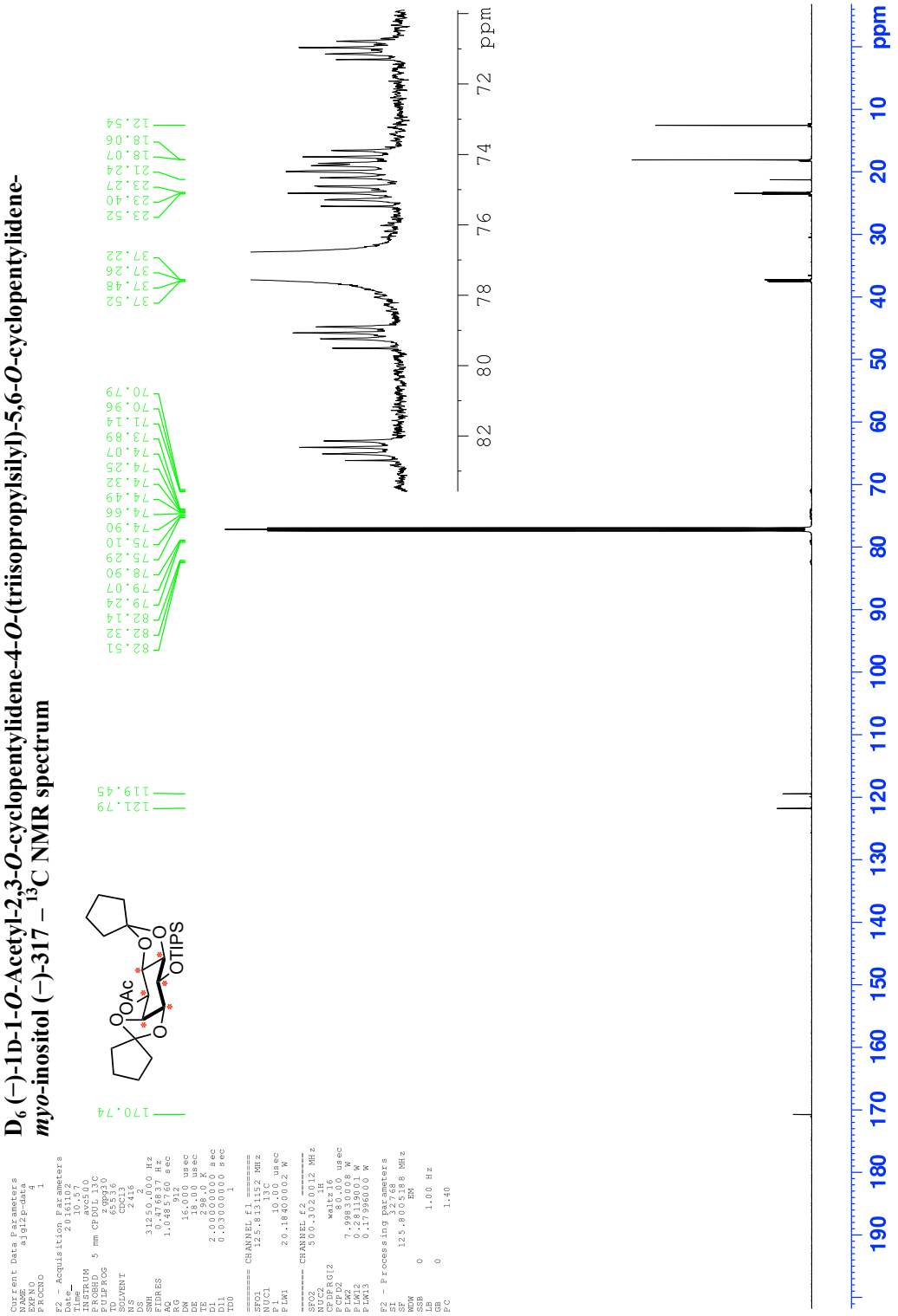
F2 - Acquisition Parameters
Date_           20161102
Time               9.53
INSTRUM          avc500
PROBHD   5 mm CPDUL 13C
PULPROG            zg30
TD                65536
SOLVENT           CDCl3
NS                   16
DS                    4
SWH           10330.578 Hz
FIDRES        0.157632 Hz
AQ            3.1719425 sec
RG                 2.56
DW               48.400 usec
DE               10.00 usec
TE               298.0 K
D1            1.00000000 sec
TD0                   1

======== CHANNEL f1 ========
SFO1        500.3030896 MHz
NUC1                1H
P1                15.00 usec
PLW1         7.99830008 W

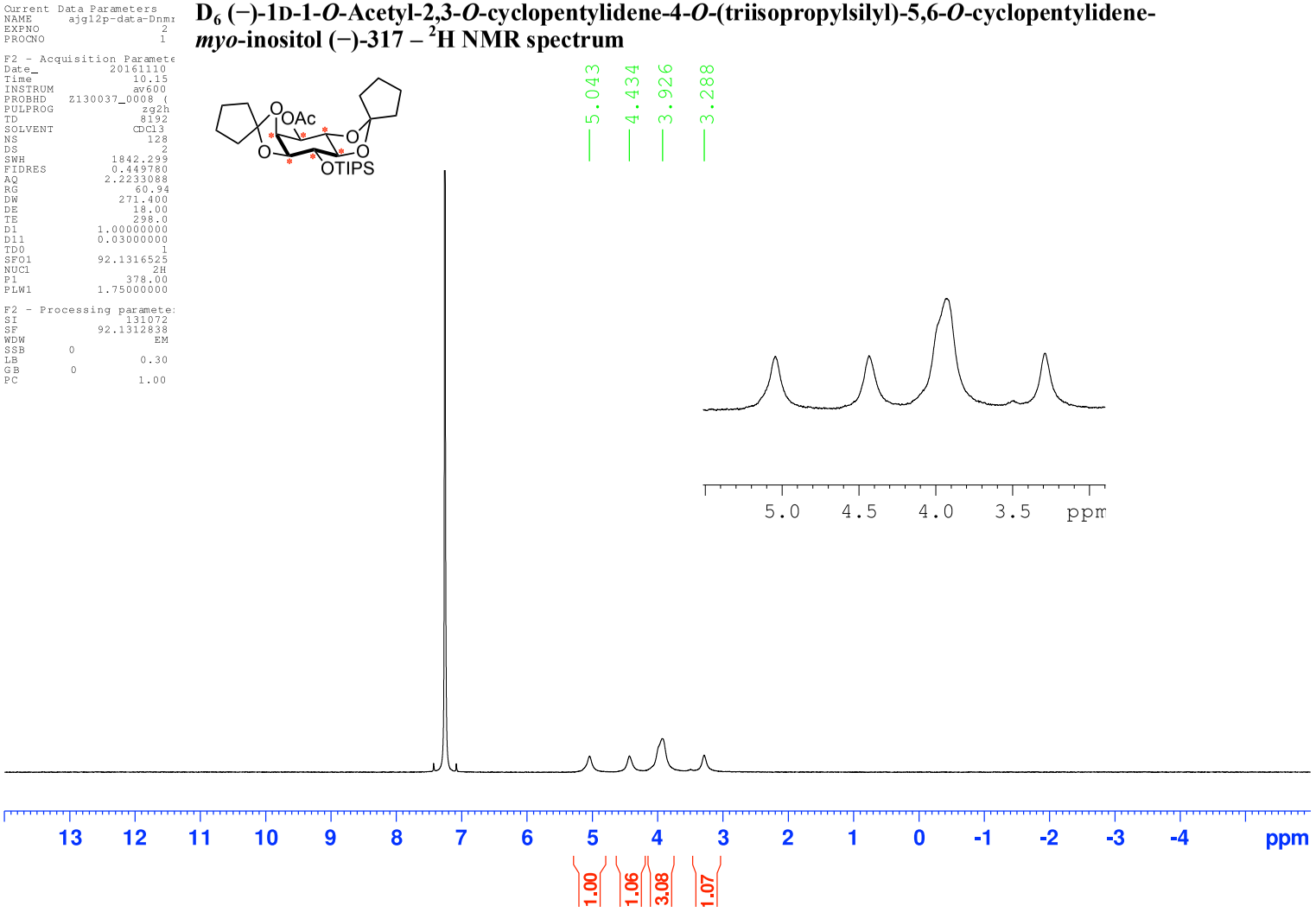
F2 - Processing parameters
SI                65536
SF          500.3000135 MHz
WDW                 EM
SSB       0
LB                0.30 Hz
GB        0
PC                1.00
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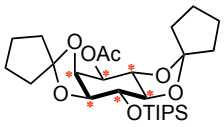
D_6 (−)-1D-1-*O*-Acetyl-2,3-*O*-cyclopentylidene-4-*O*-(triisopropylsilyl)-5,6-*O*-cyclopentylidene-*myo*-inositol (−)-317 – ^{13}C NMR spectrum



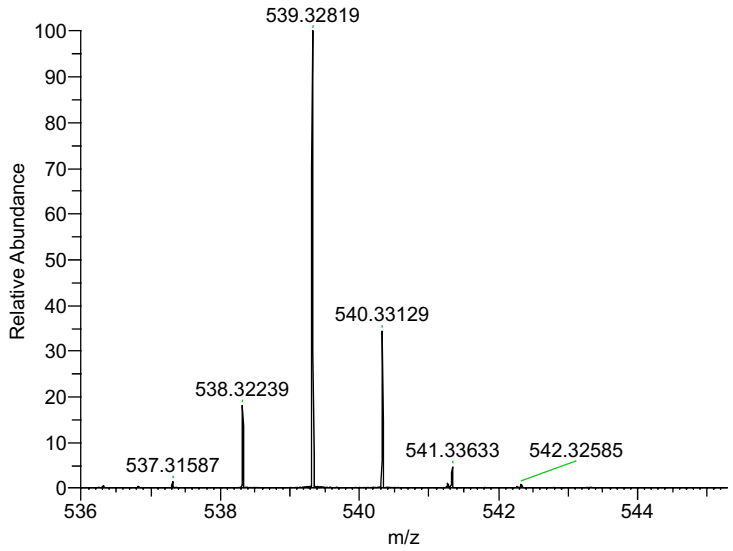
D_6 (−)-1D-1-*O*-Acetyl-2,3-*O*-cyclopentylidene-4-*O*-(triisopropylsilyl)-5,6-*O*-cyclopentylidene-*myo*-inositol (−)-317 – ^{2}H NMR spectrum



D_6 (−)-1D-1-*O*-Acetyl-2,3-*O*-cyclopentylidene-4-*O*-(triisopropyl-silyl)-5,6-*O*-cyclopentylidene-*myo*-inositol (−)-317 – Mass spectrum

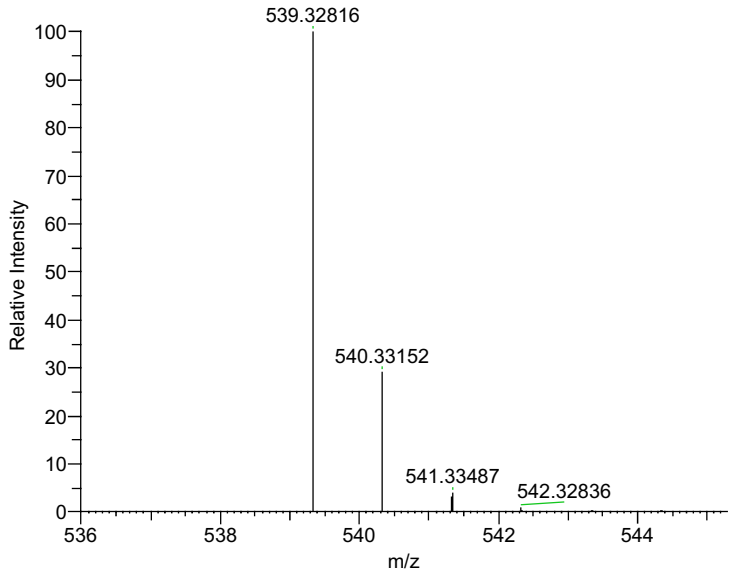


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NL: 4.94E7
ESI59717 #15-25 RT: 0.17-0.28 AV: 6 NL: 4.94E+007
T: FTMS {1,1} + p ESI Full lock ms [80.00-1600.00]

Measured Spectrum



NL: 6.75E5
C27H40[2]H6O7Na1Si1: C_{27} H_{40} $^{2}H_{6}$ O_{7} Na Si Chrg 1 R: 1000000 Res. Pwr. @FWHM

Theoretical Spectrum

m/z	Formula	RDB	Delta ppm	Theo. Mass
539.32819	$C_{27}H_{40}{}^{2}H_{6}O_{7}{}^{23}Na{}^{28}Si$	5.5	0.04	539.32816

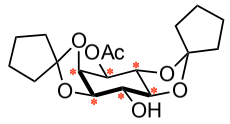
D_6 (–)-1D-1-*O*-Acetyl-2,3-*O*-cyclopentylidene-5,6-*O*-cyclopentylidene-*myo*-inositol (–)-318 – ^{1}H NMR spectrum

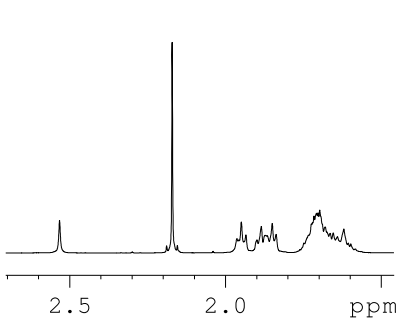
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Current Data Parameters
NAME          ajg13p-data
EXPNO                   1
PROCNO                  1

F2 - Acquisition Parameters
Date_           20161102
Time              13.32
INSTRUM          avc500
PROBHD   5 mm CPDUL 13C
PULPROG           zg30
TD               65536
SOLVENT          CDCl3
NS                  16
DS                   4
SWH          10330.578 Hz
FIDRES        0.157632 Hz
AQ           3.1719425 sec
RG                2.25
DW              48.400 usec
DE              10.00 usec
TE              298.0 K
D1          1.00000000 sec
TD0                  1

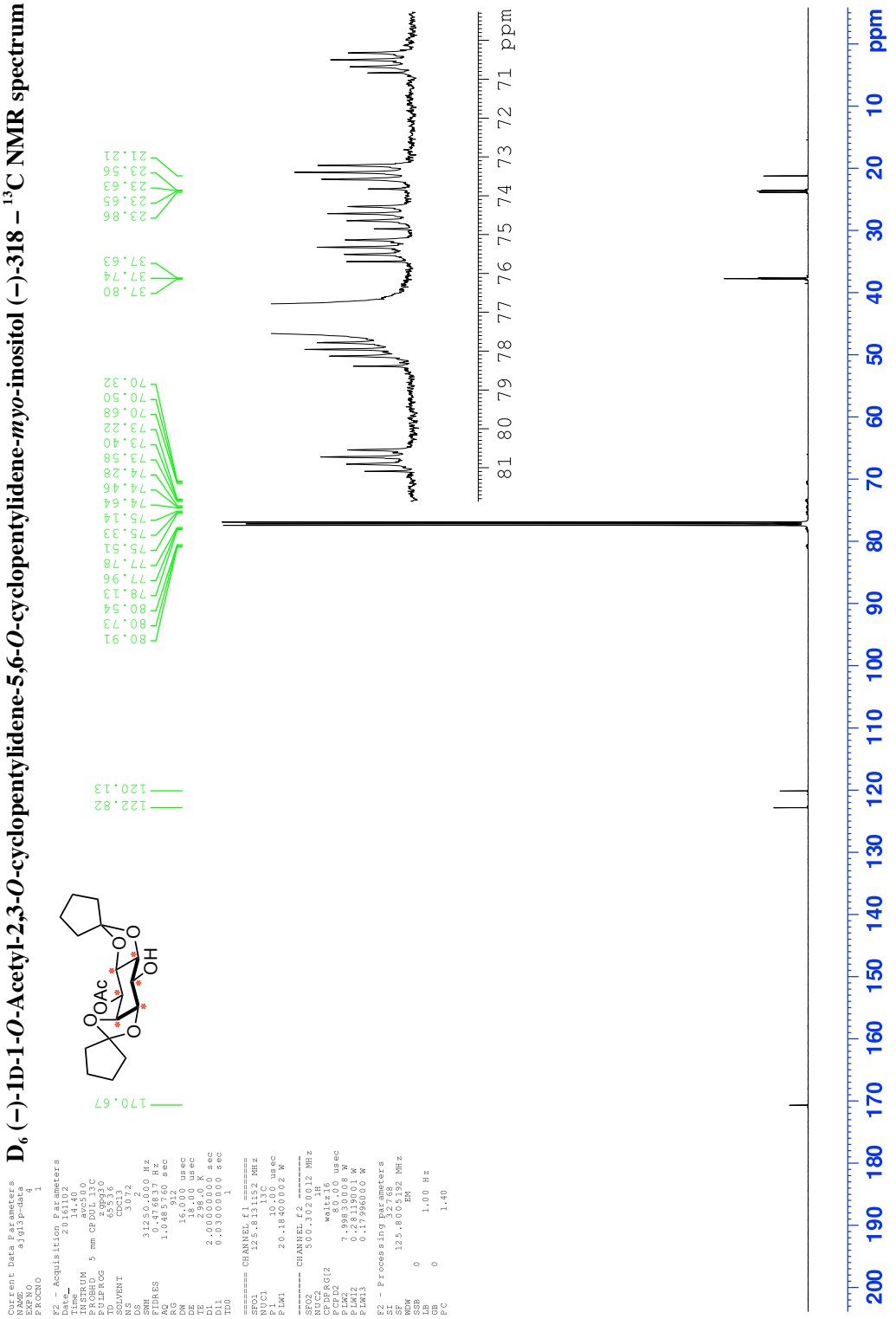
======== CHANNEL f1 ========
SFO1       500.3030896 MHz
NUC1               1H
P1              15.00 usec
PLW1        7.99830008 W

F2 - Processing parameters
SI               65536
SF         500.3000137 MHz
WDW                EM
SSB       0
LB              0.30 Hz
GB        0
PC              1.00
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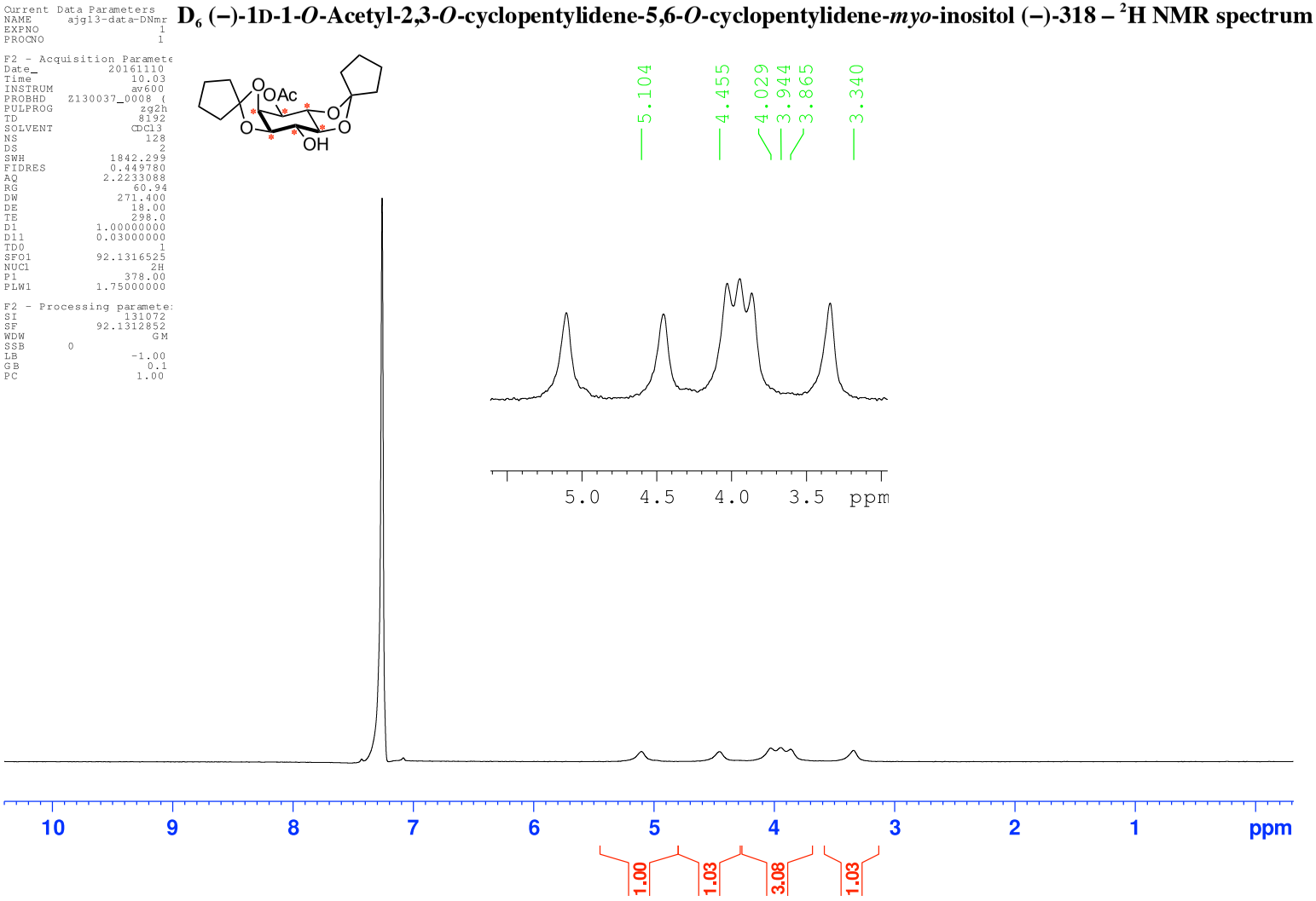




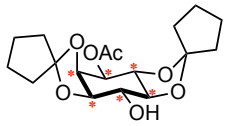
D$_6$ (–)-1D-1-*O*-Acetyl-2,3-*O*-cyclopentylidene-5,6-*O*-cyclopentylidene-*myo*-inositol (–)-318 – ^{13}C NMR spectrum



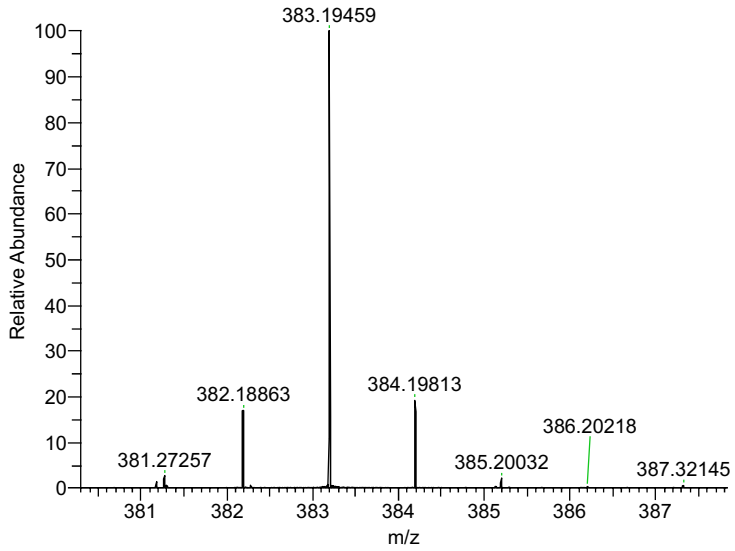
D_6 (–)-1D-1-*O*-Acetyl-2,3-*O*-cyclopentylidene-5,6-*O*-cyclopentylidene-*myo*-inositol (–)-318 – ^{2}H NMR spectrum



D_6 (–)-1D-1-*O*-Acetyl-2,3-*O*-cyclopentylidene-5,6-*O*-cyclopentylidene-*myo*-inositol (–)-318 – ^{2}H NMR spectrum

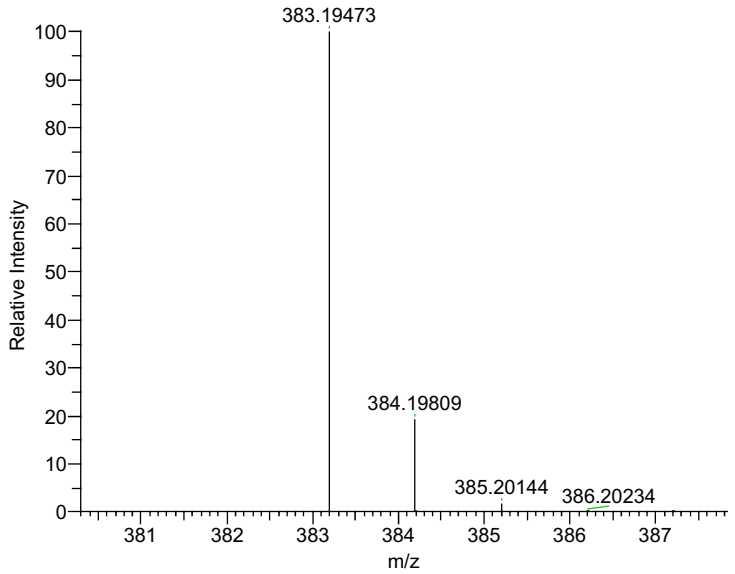


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NL: 5.73E7
ESI59718 #15-24 RT: 0.17-0.26 AV: 5 NL: 5.73E+007
T: FTMS {1,1} + p ESI Full lock ms [80.00-1600.00]

Measured Spectrum

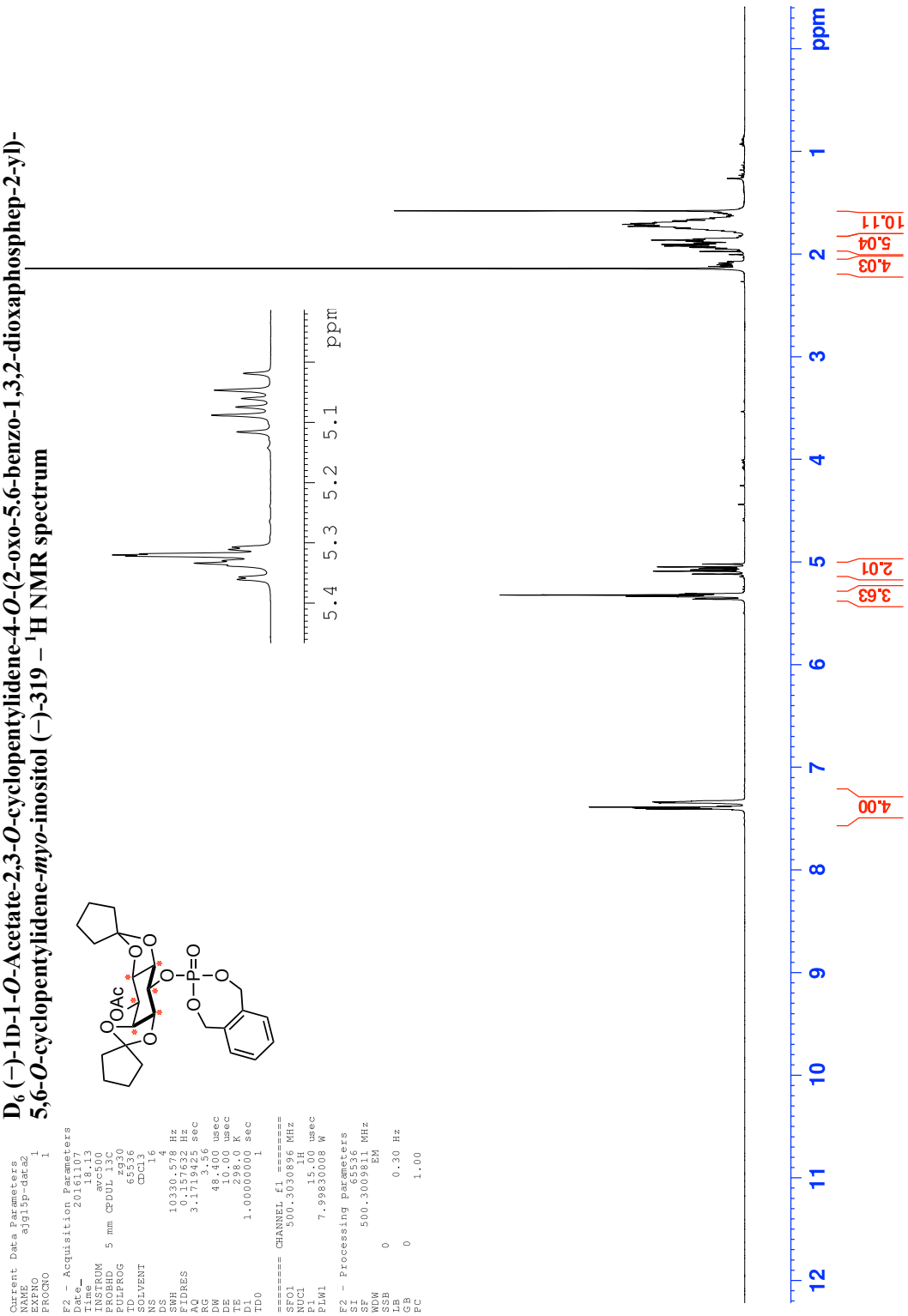


NL: 8.08E5
C18H20[2]H6O7Na1: C_{18} H_{20} $^2H_6$ O_7 Na
Chrg 1 R: 1000000 Res. Pwr. @FWHM

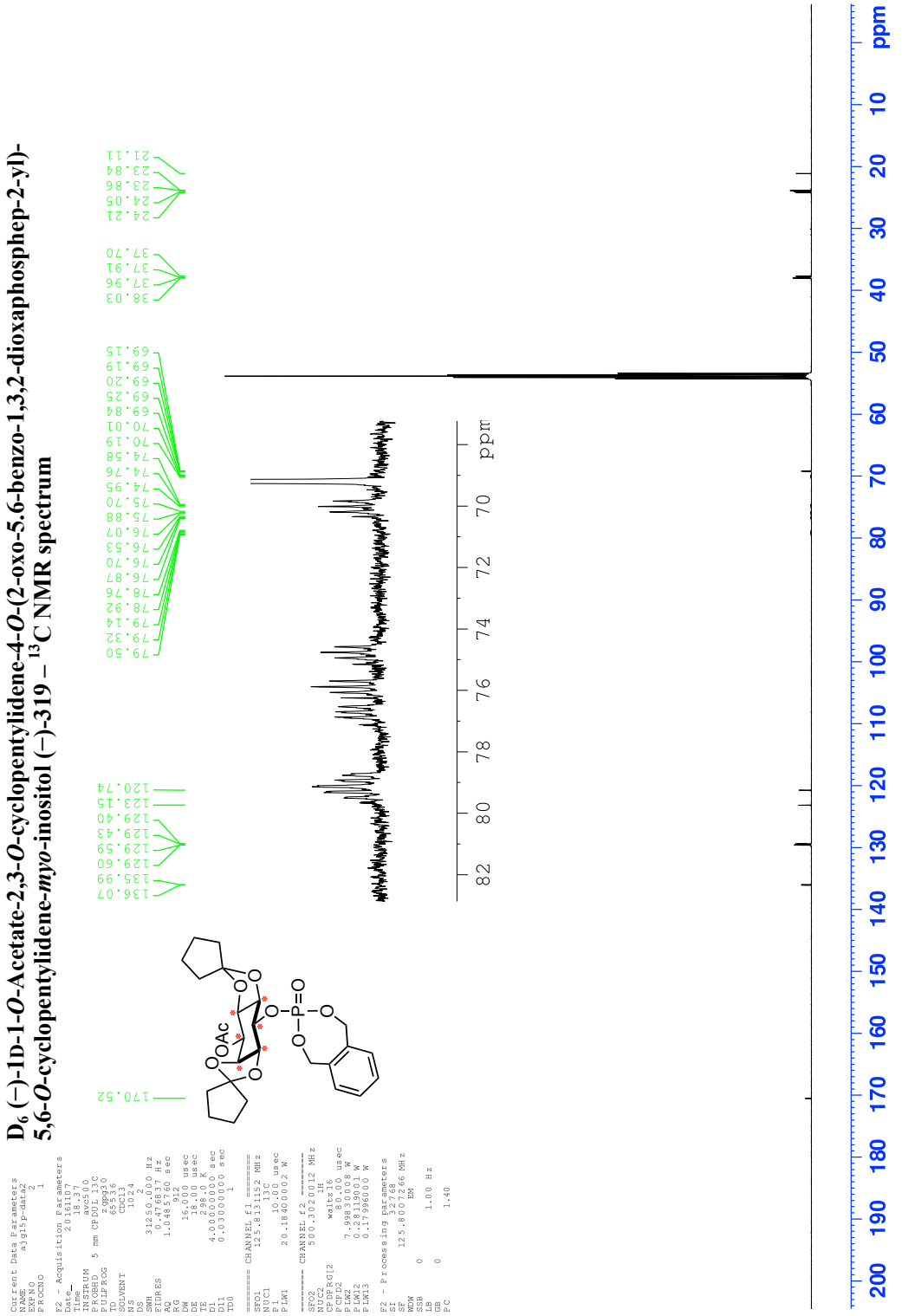
Theoretical Spectrum

m/z	Formula	RDB	Delta ppm	Theo. Mass
383.19458	$C_{18}H_{20}{}^2H_6O_7{}^{23}Na$	5.5	-0.4	383.19473

D_6 (−)-1D-1-*O*-Acetate-2,3-*O*-cyclopentylidene-4-*O*-(2-oxo-5.6-benzo-1,3,2-dioxaphosphep-2-yl)-5,6-*O*-cyclopentylidene-*myo*-inositol (−)-319 – ^{1}H NMR spectrum



D_6 (−)-1D-1-*O*-Acetate-2,3-*O*-cyclopentylidene-4-*O*-(2-oxo-5,6-benzo-1,3,2-dioxaphosphep-2-yl)-5,6-*O*-cyclopentylidene-*myo*-inositol (−)-319 – ^{13}C NMR spectrum

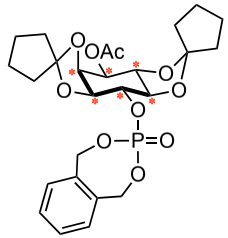


D_6 (−)-1D-1-*O*-Acetate-2,3-*O*-cyclopentylidene-4-*O*-(2-oxo-5.6-benzo-1,3,2-dioxaphosphep-2-yl)-5,6-*O*-cyclopentylidene-*myo*-inositol (−)-319 – ^{13}P NMR spectrum

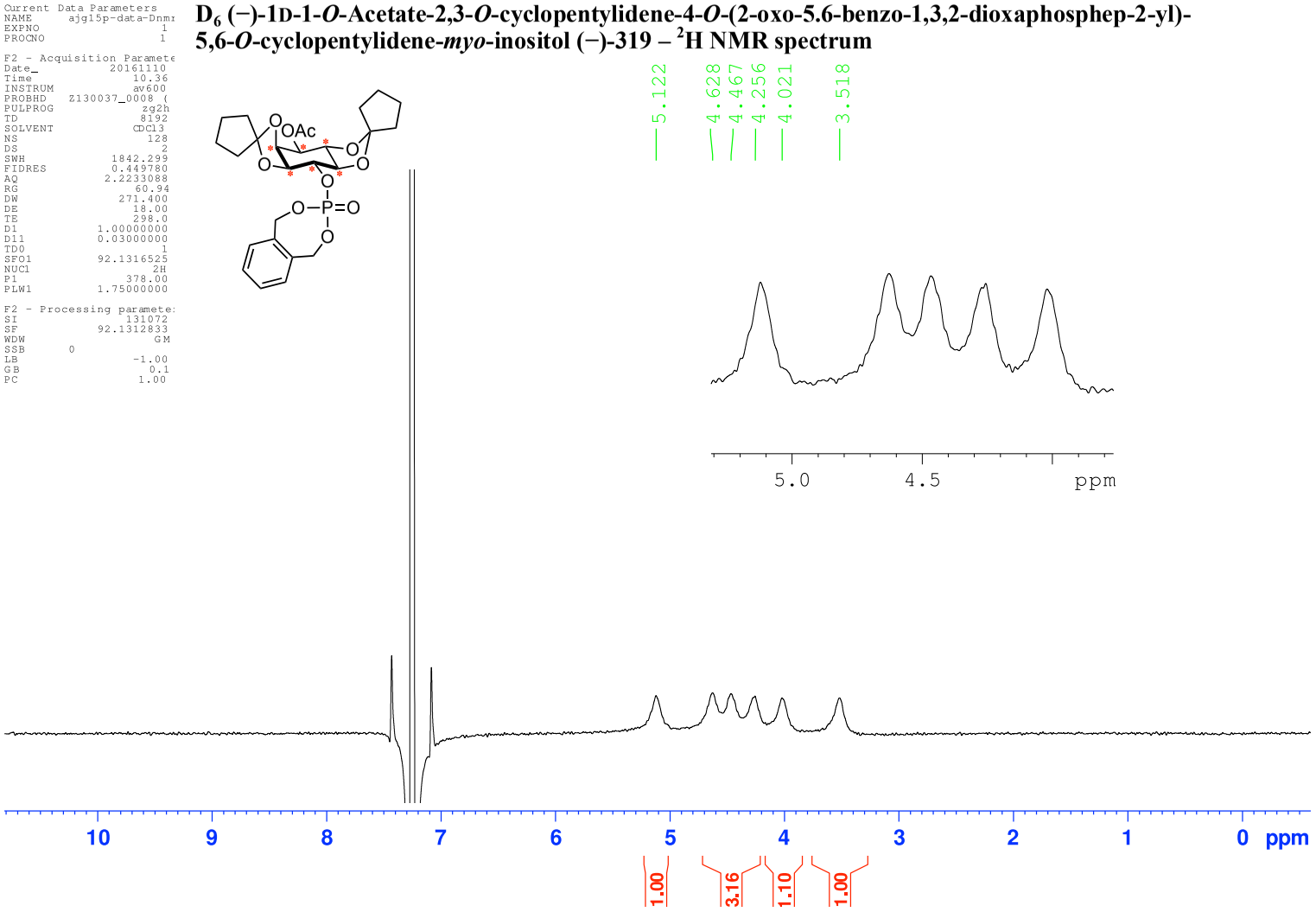
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Current Data Parameters
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EXPNO                      1
PROCNO                     1

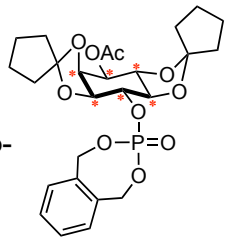
F2 - Acquisition Parameter
Date_              20161102
Time                  17.37
INSTRUM              avb400
PROBHD    Z116098_0219 (
PULPROG              zgpg30
TD                    65536
SOLVENT               CDCl3
NS                       16
DS                        4
SWH                64102.562
FIDRES             1.956255
AQ                 0.5111808
RG                    197.74
DW                     7.800
DE                      6.50
TE                    298.1
D1                 2.00000000
D11                0.03000000
TD0                        1
SFO1           161.9755930
NUC1                     31P
P1                      8.00
PLW1           54.00000000
SFO2           400.1316005
NUC2                     1H
CPDPRG[2              waltz16
PCPD2                 90.00
PLW2           14.58800030
PLW12           0.18009999
PLW13           0.09058800

F2 - Processing parameter
SI                    32768
SF             161.9755930
WDW                      EM
SSB       0
LB                     1.00
GB        0
PC                     1.40
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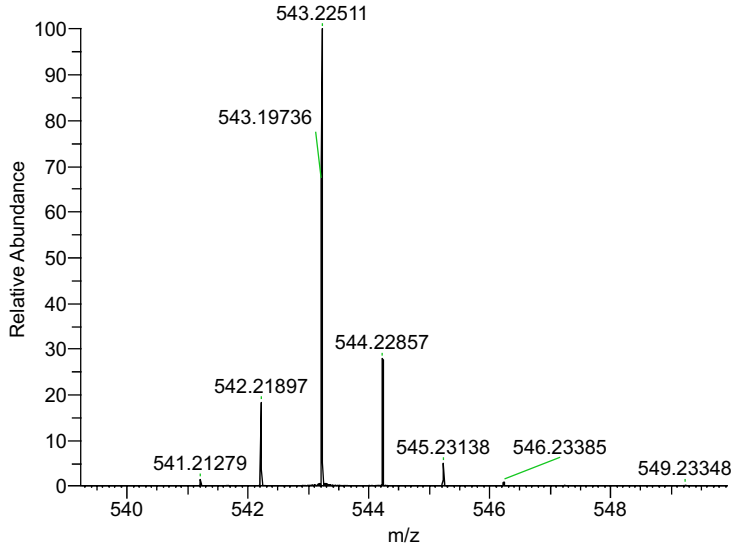
D_6 (−)-1D-1-*O*-Acetate-2,3-*O*-cyclopentylidene-4-*O*-(2-oxo-5.6-benzo-1,3,2-dioxaphosphep-2-yl)-5,6-*O*-cyclopentylidene-*myo*-inositol (−)-319 – ^{2}H NMR spectrum





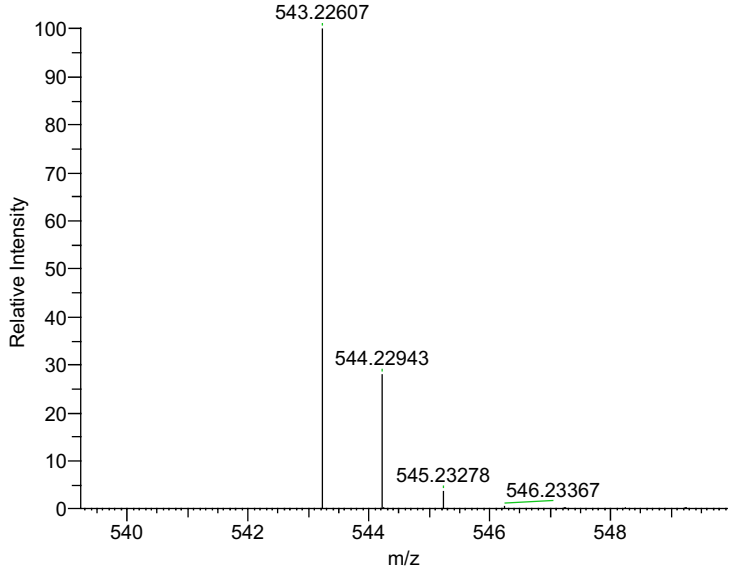
D_6 (−)-1D-1-*O*-Acetate-2,3-*O*-cyclopentylidene-4-*O*-(2-oxo-5.6-benzo-1,3,2-dioxaphosphep-2-yl)-5,6-*O*-cyclopentylidene-*myo*-inositol (−)-319 – Mass spectrum

S:\data\Nov 16\ESI59703.raw 08/11/2016 3:10 pm



NL: 3.81E7
ESI59703 #15-25 RT: 0.17-0.28 AV: 6 NL: 5.29E+007
T: FTMS {1,1} + p ESI Full ms [80.00-1600.00]

Measured Spectrum



NL: 7.35E5
C26H28[2]H6O10P1: C_{26} H_{28} $^{2}H_{6}$ O_{10} P
Chrg 1 R: 1000000 Res. Pwr. @FWHM

Theoretical Spectrum

m/z	Formula	RDB	Delta ppm	Theo. Mass
543.22510	$C_{26}H_{28}{}^{2}H_{6}O_{10}P$	10.5	-1.79	543.22607

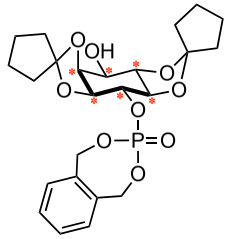
D_6 (+)-1D-2,3-*O*-Cyclopentylidene-4-*O*-(2-oxo-5.6-benzo-1,3,2-dioxaphosphep-2-yl)-5,6-*O*-cyclopentylidene-*myo*-inositol (+)-320 – ^{1}H NMR spectrum

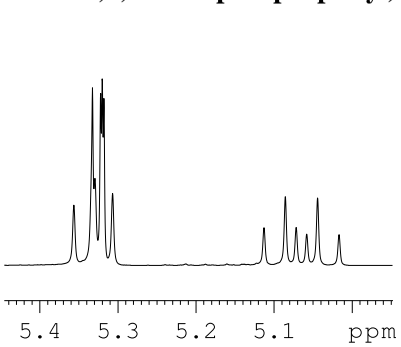
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Current Data Parameters
NAME          ajg17p-data
EXPNO                   1
PROCNO                  1

F2 - Acquisition Parameters
Date_           20161109
Time               14.16
INSTRUM          avc500
PROBHD   5 mm CPDUL 13C
PULPROG            zg30
TD                65536
SOLVENT          CD2Cl2
NS                    16
DS                     4
SWH            10330.578 Hz
FIDRES         0.157632 Hz
AQ             3.1719425 sec
RG                   3.2
DW                48.400 usec
DE                10.00 usec
TE                298.0 K
D1            1.00000000 sec
TD0                    1

======== CHANNEL f1 ========
SFO1         500.3030896 MHz
NUC1                1H
P1                15.00 usec
PLW1          7.99830008 W

F2 - Processing parameters
SI                 65536
SF           500.3000205 MHz
WDW                  EM
SSB       0
LB                0.30 Hz
GB        0
PC                1.00
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D_6 (+)-1D-2,3-*O*-Cyclopentylidene-4-*O*-(2-oxo-5.6-benzo-1,3,2-dioxaphosphep-2-yl)-5,6-*O*-cyclopentylidene-*myo*-inositol (+)-320 – ^{13}C NMR spectrum

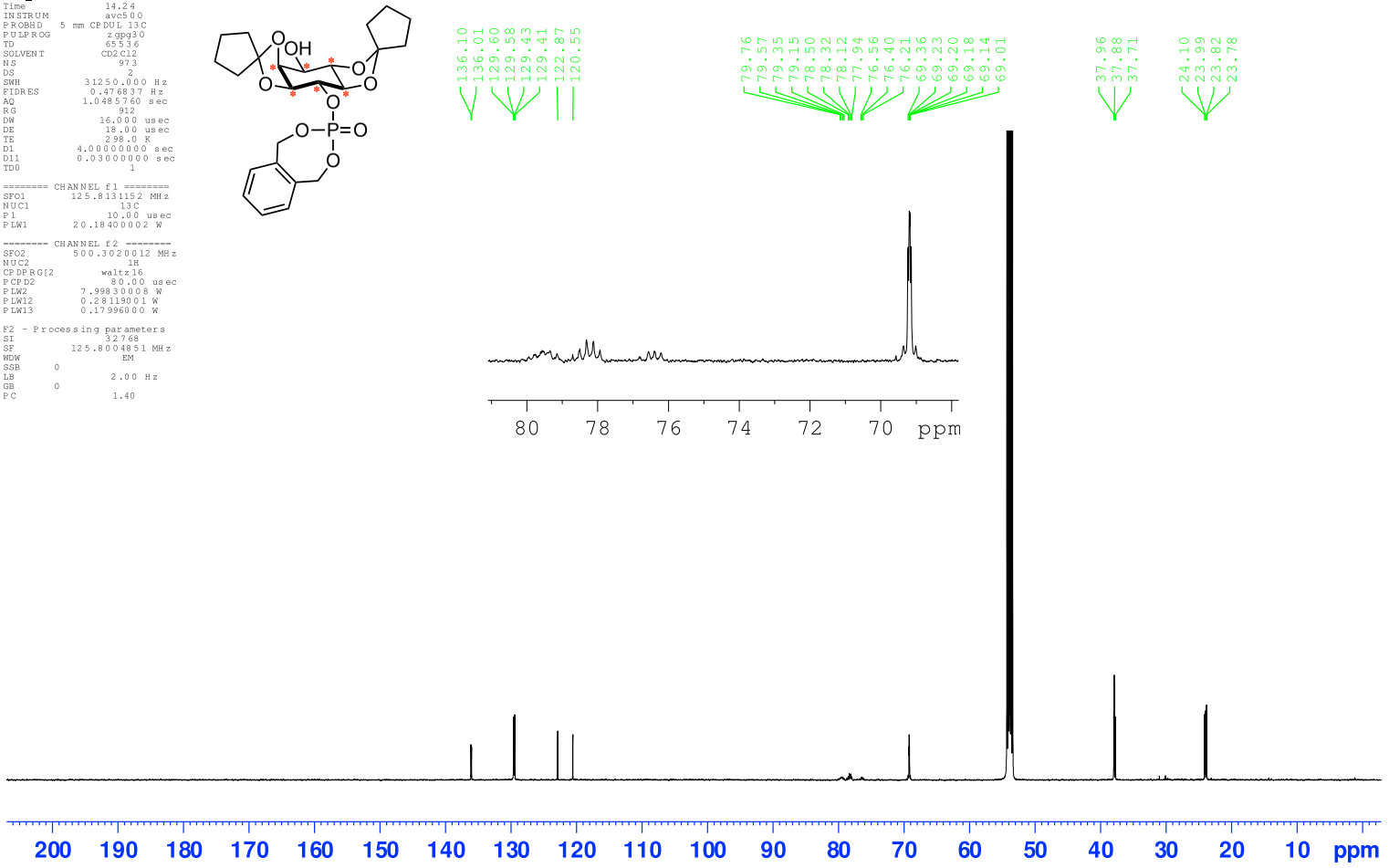
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Current Data Parameters
NAME          ajgl7p-data
EXPNO                   2
PROCNO                  1

F2 - Acquisition Parameters
Date_          20161109
Time              14.24
INSTRUM          avc500
PROBHD   5 mm CPDUL 13C
PULPROG          zgpg30
TD               65536
SOLVENT          CD2Cl2
NS                  973
DS                    2
SWH           31250.000 Hz
FIDRES         0.476837 Hz
AQ            1.0485760 sec
RG                  912
DW               16.000 usec
DE               18.00 usec
TE              298.0 K
D1            4.00000000 sec
D11           0.03000000 sec
TD0                   1

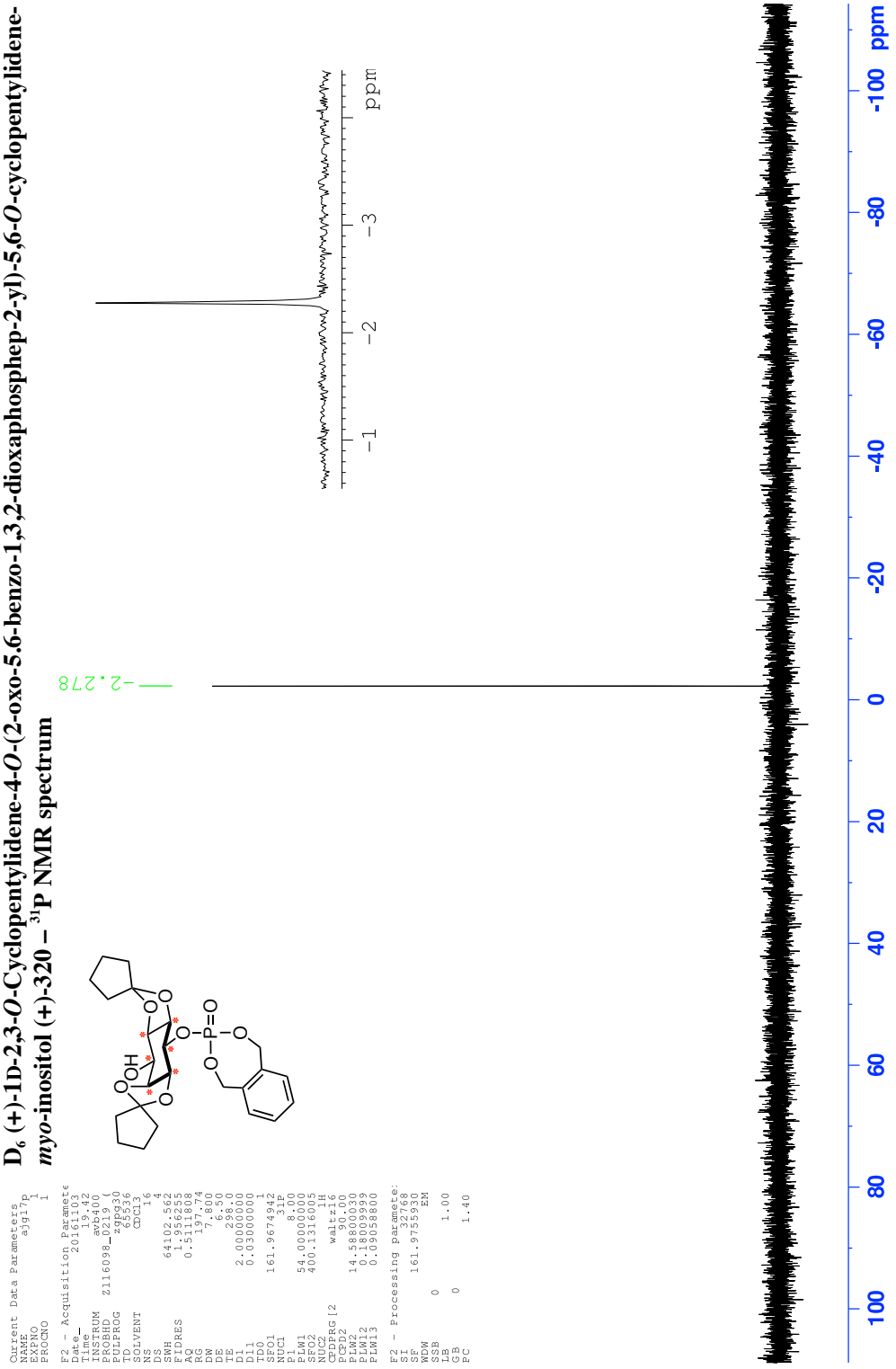
======== CHANNEL f1 ========
SFO1         125.8131152 MHz
NUC1              13C
P1               10.00 usec
PLW1         20.18400002 W

======== CHANNEL f2 ========
SFO2         500.3020012 MHz
NUC2              1H
CPDPRG[2         waltz16
PCPD2             80.00 usec
PLW2          7.99830008 W
PLW12         0.28119001 W
PLW13         0.17996000 W

F2 - Processing parameters
SI                32768
SF           125.8004851 MHz
WDW               EM
SSB      0
LB                2.00 Hz
GB       0
PC                1.40
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D_6 (+)-1D-2,3-*O*-Cyclopentylidene-4-*O*-(2-oxo-5,6-benzo-1,3,2-dioxaphosphep-2-yl)-5,6-*O*-cyclopentylidene-*myo*-inositol (+)-320 – ^{31}P NMR spectrum

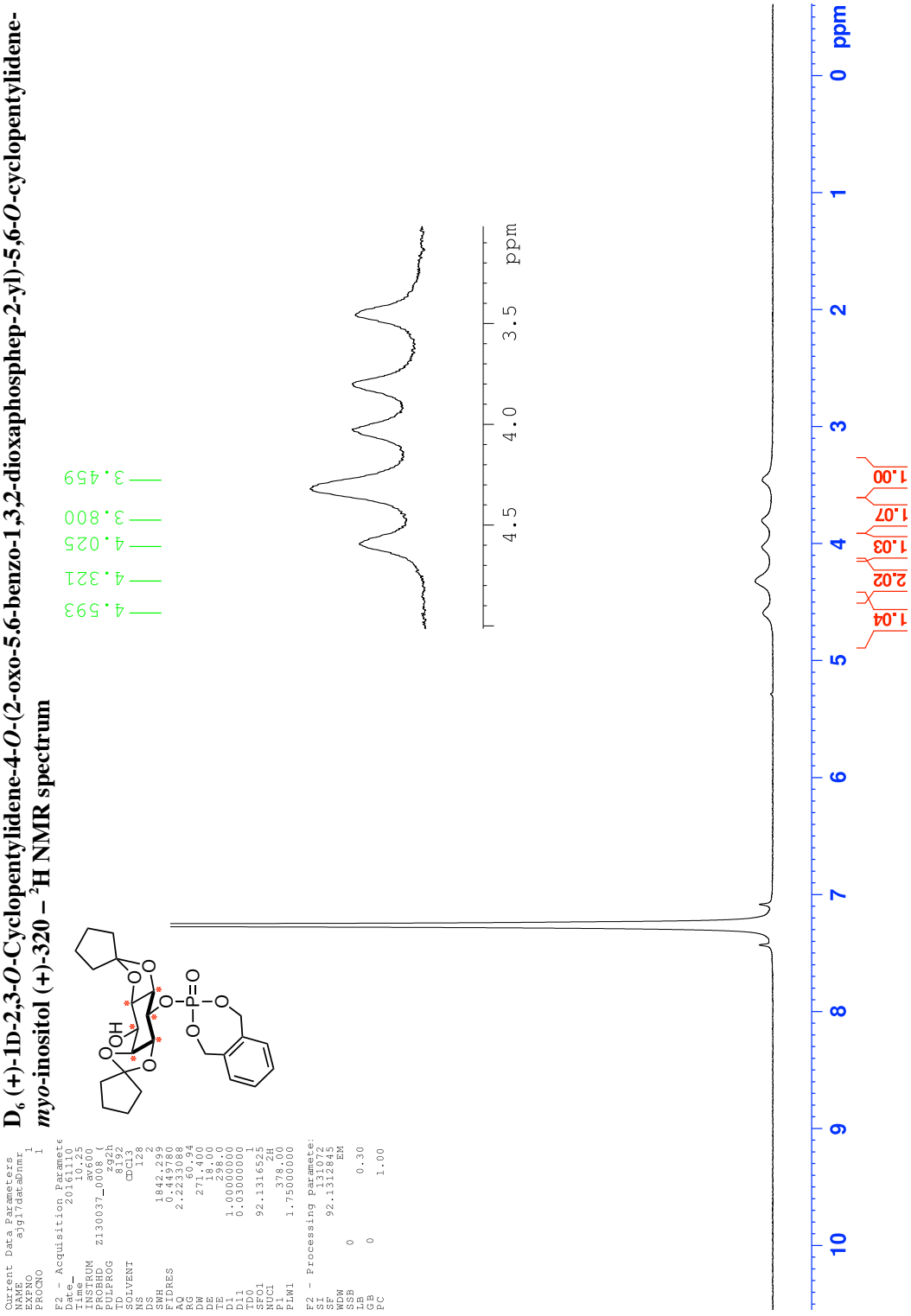


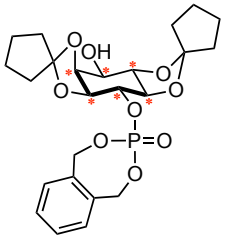
Current Data Parameters
NAME ajg17p
EXPNO 1
PROCNO 1

F2 - Acquisition Parameters
Date_ 20161213
Time 19.42
INSTRUM avb400
PROBHD 2116098_0219 (
PULPROG zgpg30
TD 65536
SOLVENT CDCl3
NS 16
DS 4
SWH 64102.562
FIDRES 1.956255
AQ 0.5111808
RG 197.74
DW 7.800
DE 6.50
TE 298.0
D1 2.00000000
D11 0.03000000
TD0 1
SFO1 161.9674942
NUC1 31P
P1 8.00
PLW1 54.00000000
SFO2 400.1316005
NUC2 1H
CPDPRG[2 waltz16
PCPD2 90.00
PLW2 14.58800928
PLW12 0.18000939
PLW13 0.09058800

F2 - Processing parameters
SI 32768
SF 161.9753920
WDW EM
SSB 0
LB 1.00
GB 0
PC 1.40

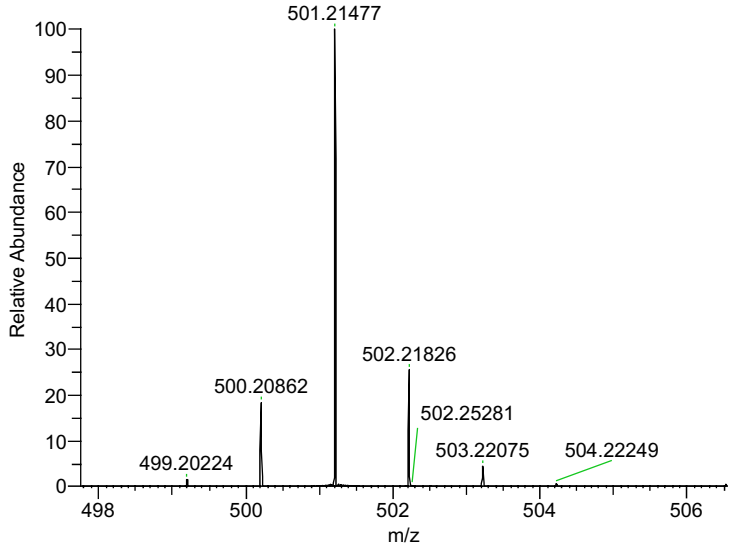
D_6 (+)-1D-2,3-*O*-Cyclopentylidene-4-*O*-(2-oxo-5.6-benzo-1,3,2-dioxaphosphep-2-yl)-5,6-*O*-cyclopentylidene-*myo*-inositol (+)-320 – ^{2}H NMR spectrum





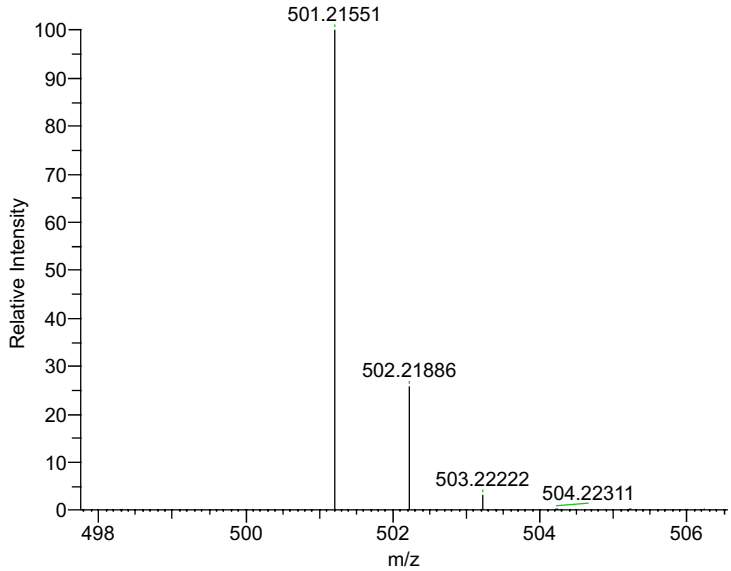
D_6 (+)-1D-2,3-*O*-Cyclopentylidene-4-*O*-(2-oxo-5.6-benzo-1,3,2-dioxaphosphep-2-yl)-5,6-*O*-cyclopentylidene-*myo*-inositol (+)-320 – Mass spectrum

S:\data\Nov 16\ESI59702.raw 08/11/2016 3:07 pm



NL: 4.16E7
ESI59702 #15-25 RT: 0.17-0.28 AV: 6 NL: 5.29E+007
T: FTMS {1,1} + p ESI Full ms [80.00-1600.00]

Measured Spectrum



NL: 7.53E5
C24H26[2]H6O9P1: $C_{24}H_{26}{}^{2}H_{6}O_{9}P$ Chrg 1 R: 1000000 Res. Pwr. @FWHM

Theoretical Spectrum

m/z	Formula	RDB	Delta ppm	Theo. Mass
501.21475	$C_{24}H_{26}{}^{2}H_{6}O_{9}P$	9.5	-1.5	501.21551

D_6 (+)-1D-2,3-*O*-Cyclopentylidene-4-*O*-(2-oxo-5.6-benzo-1,3,2-dioxaphosphep-2-yl)-5,6-*O*-cyclopentylidene-*myo*-inositol (+)-320 – RP-HPLC (Method 2)

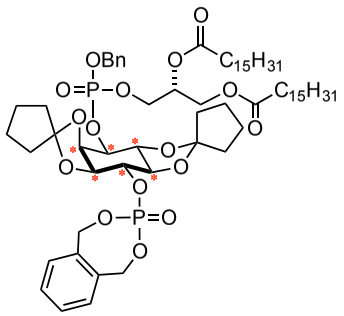
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11.703	15863	0.26
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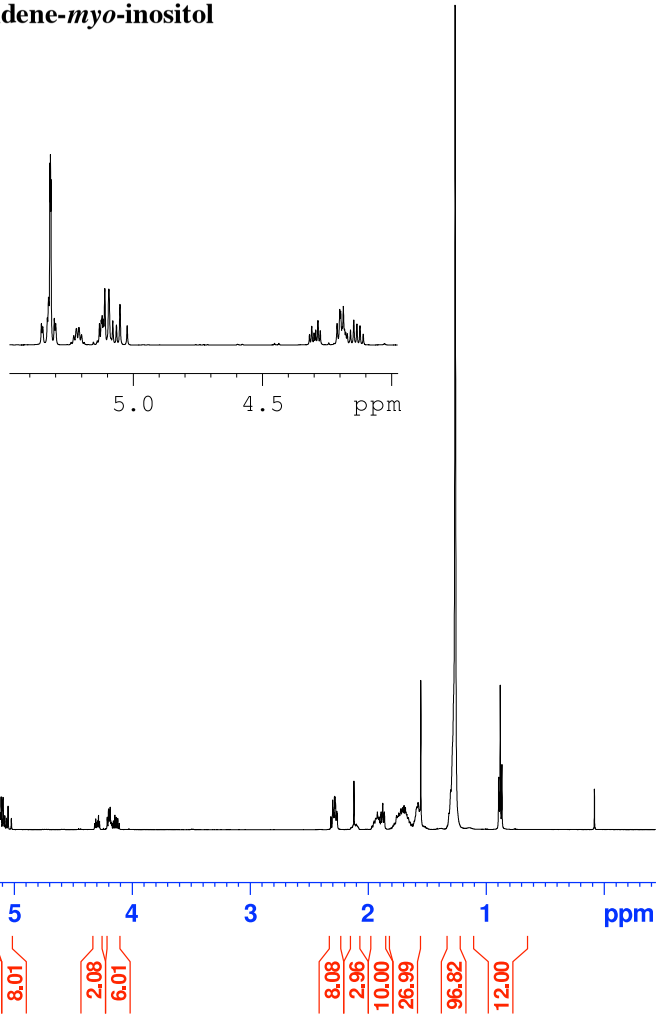
D_6 (–)-1D-1-*O*-(1,2-Di-*O*-palmitoyl-*sn*-glycer-3-yl-benzylphosphate)-2,3-*O*-cyclopentylidene-4-*O*-(2-oxo-5.6-benzo-1,3,2-dioxaphosphep-2-yl)-5,6-*O*-cyclopentylidene-*myo*-inositol
(–)-321 – ^{1}H NMR spectrum

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PROCNO                  1

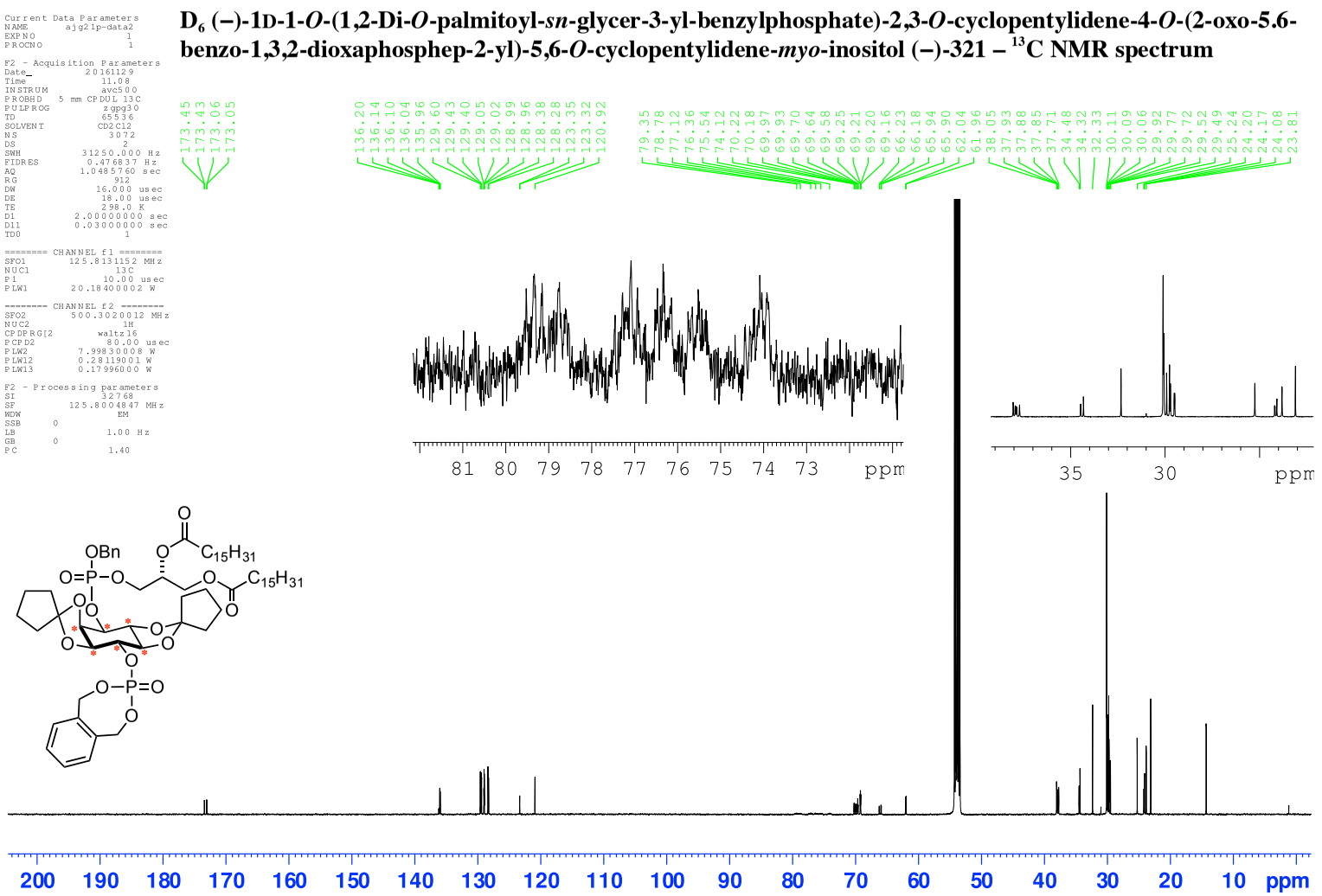
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NS                    16
DS                     2
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FIDRES            0.305176 Hz
AQ            3.2767999 sec
RG                 44.54
DW                 50.000 usec
DE                  6.50 usec
TE                298.0 K
D1            1.00000000 sec
TD0                    1
SFO1          500.1325006 MHz
NUC1                  1H
P1                 10.00 usec
PLW1          20.50000000 W

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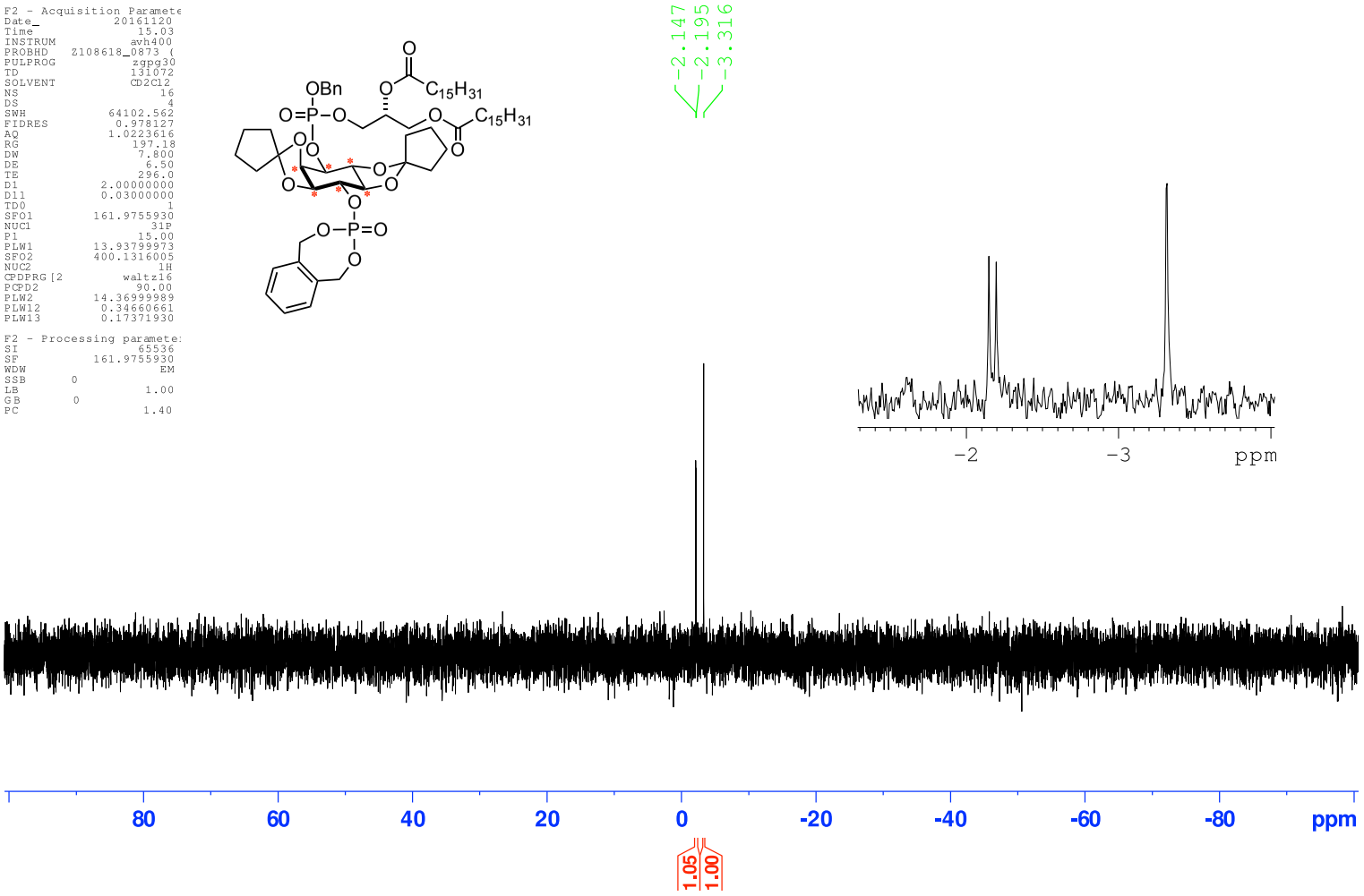




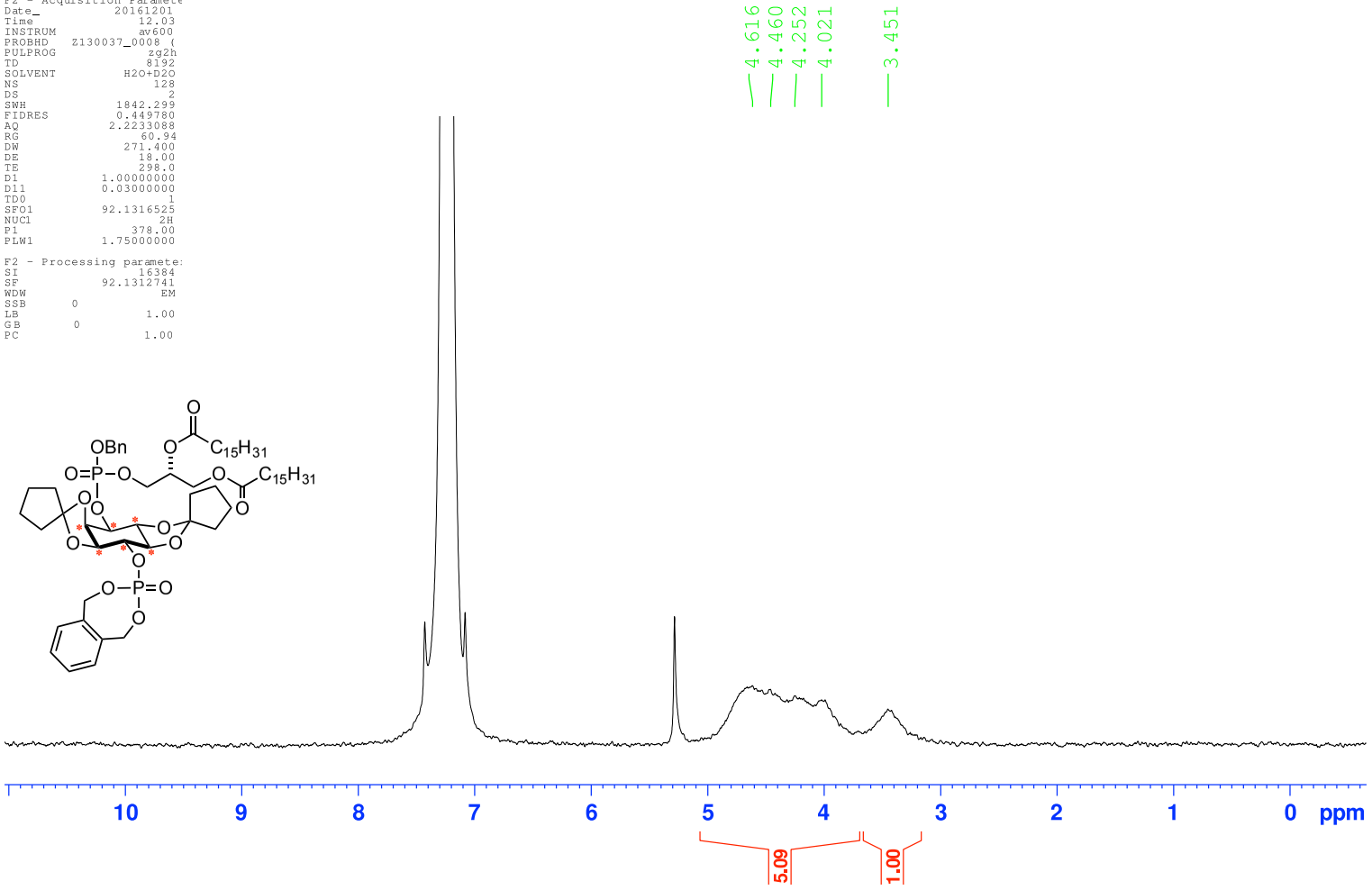
D_6 (–)-1D-1-*O*-(1,2-Di-*O*-palmitoyl-*sn*-glycer-3-yl-benzylphosphate)-2,3-*O*-cyclopentylidene-4-*O*-(2-oxo-5.6-benzo-1,3,2-dioxaphosphep-2-yl)-5,6-*O*-cyclopentylidene-*myo*-inositol (–)-321 – ^{13}C NMR spectrum



D_6 (–)-1D-1-*O*-(1,2-Di-*O*-palmitoyl-*sn*-glycer-3-yl-benzylphosphate)-2,3-*O*-cyclopentylidene-4-*O*-(2-oxo-5.6-benzo-1,3,2-dioxaphosphep-2-yl)-5,6-*O*-cyclopentylidene-*myo*-inositol (–)-321 – ^{31}P NMR spectrum

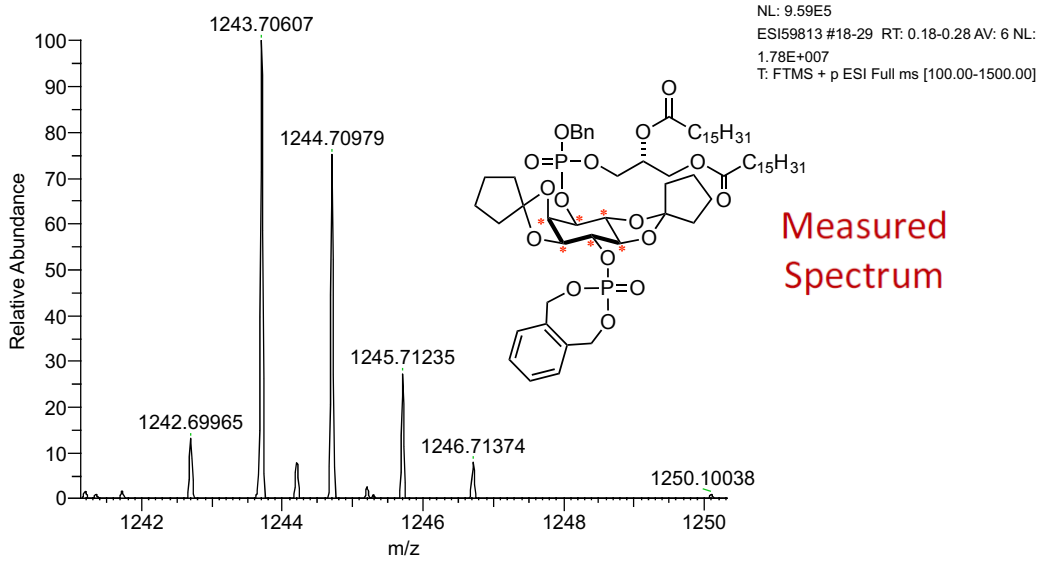


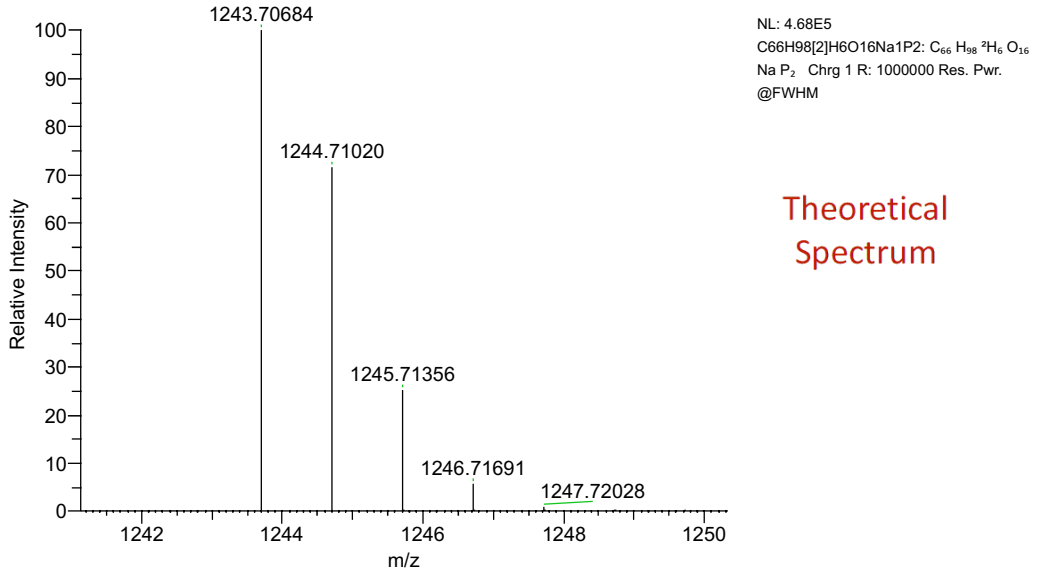
D_6 (–)-1D-1-*O*-(1,2-Di-*O*-palmitoyl-*sn*-glycer-3-yl-benzylphosphate)-2,3-*O*-cyclopentylidene-4-*O*-(2-oxo-5.6-benzo-1,3,2-dioxaphosphep-2-yl)-5,6-*O*-cyclopentylidene-*myo*-inositol (–)-321 – ^{2}H NMR spectrum



D_6 (−)-1D-1-*O*-(1,2-Di-*O*-palmitoyl-*sn*-glycer-3-yl-benzylphosphate)-2,3-*O*-cyclopentylidene-4-*O*-(2-oxo-5.6-benzo-1,3,2-dioxaphosphep-2-yl)-5,6-*O*-cyclopentylidene-*myo*-inositol (−)-321 – Mass spectrum

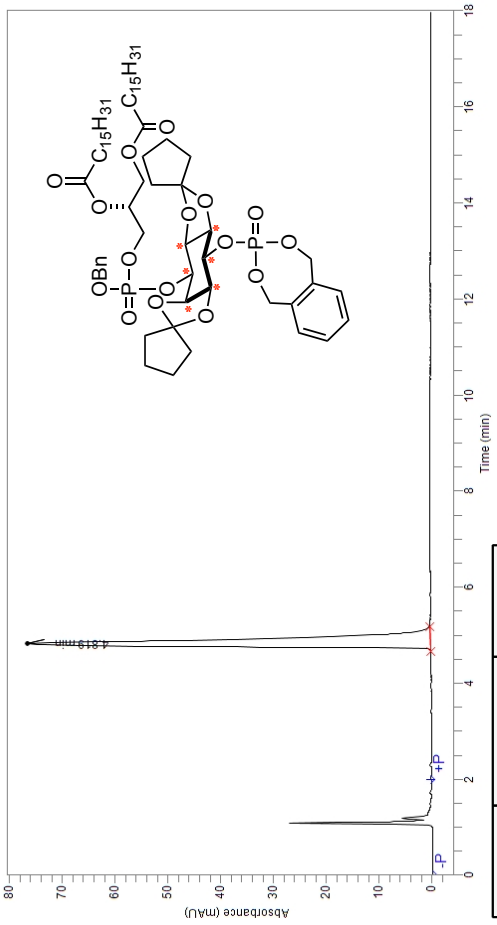
V:\data\MSservice\Nov 16\ESI59813.raw 15/11/2016 4:25 pm





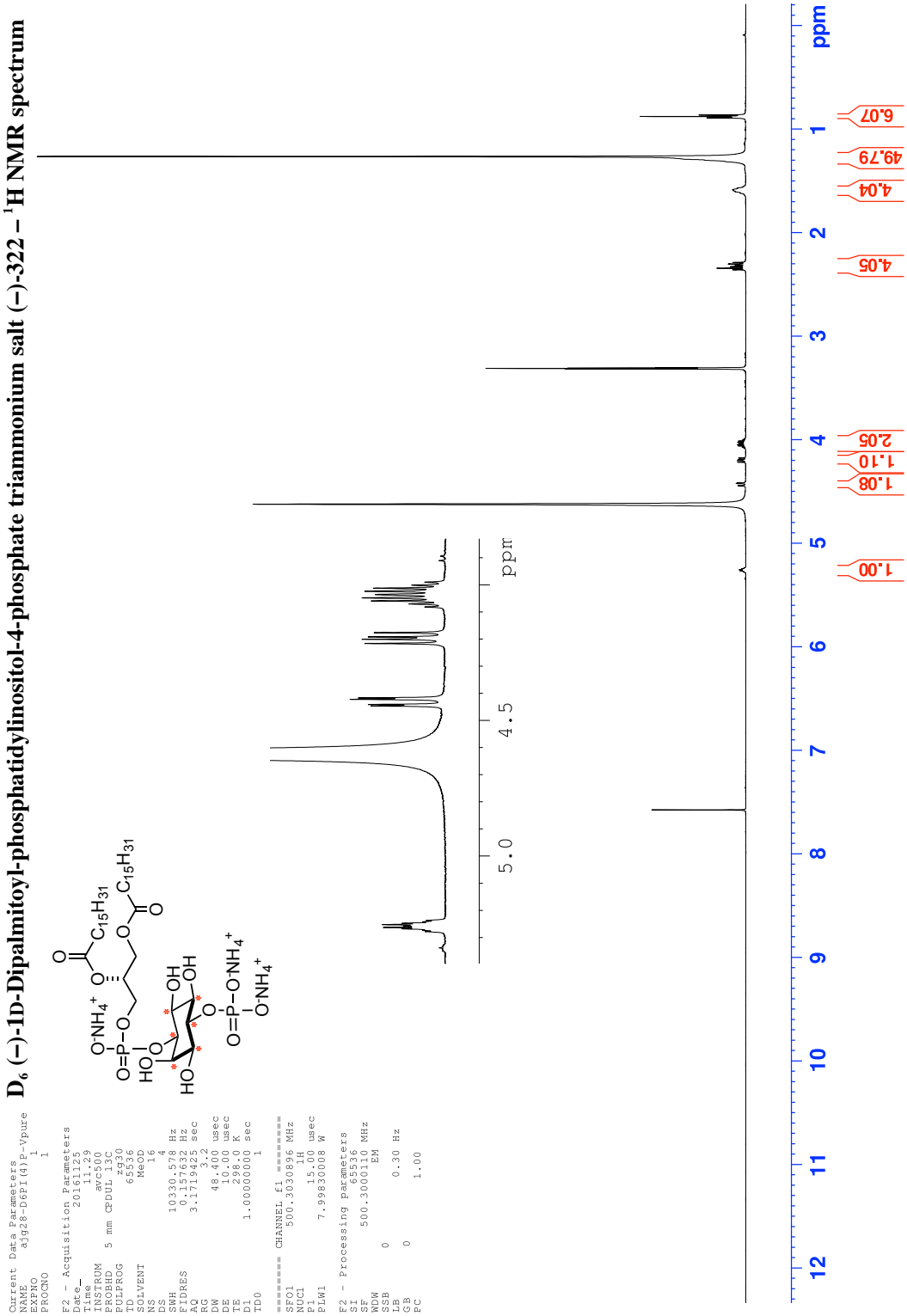
m/z	Formula	RDB	Delta ppm	Theo. Mass
1243.70605	$C_{66}H_{98}{}^{2}H_6O_{16}{}^{23}NaP_2$	15.5	-0.63	1243.70684

D_6 (−)-1D-1-*O*-(1,2-Di-*O*-palmitoyl-*sn*-glycer-3-yl-benzylphosphate)-2,3-*O*-cyclopentylidene-4-*O*-(2-oxo-5.6-benzo-1,3,2-dioxaphosphep-2-yl)-5,6-*O*-cyclopentylidene-*myo*-inositol (−)-321 – NP-HPLC (Method 6)

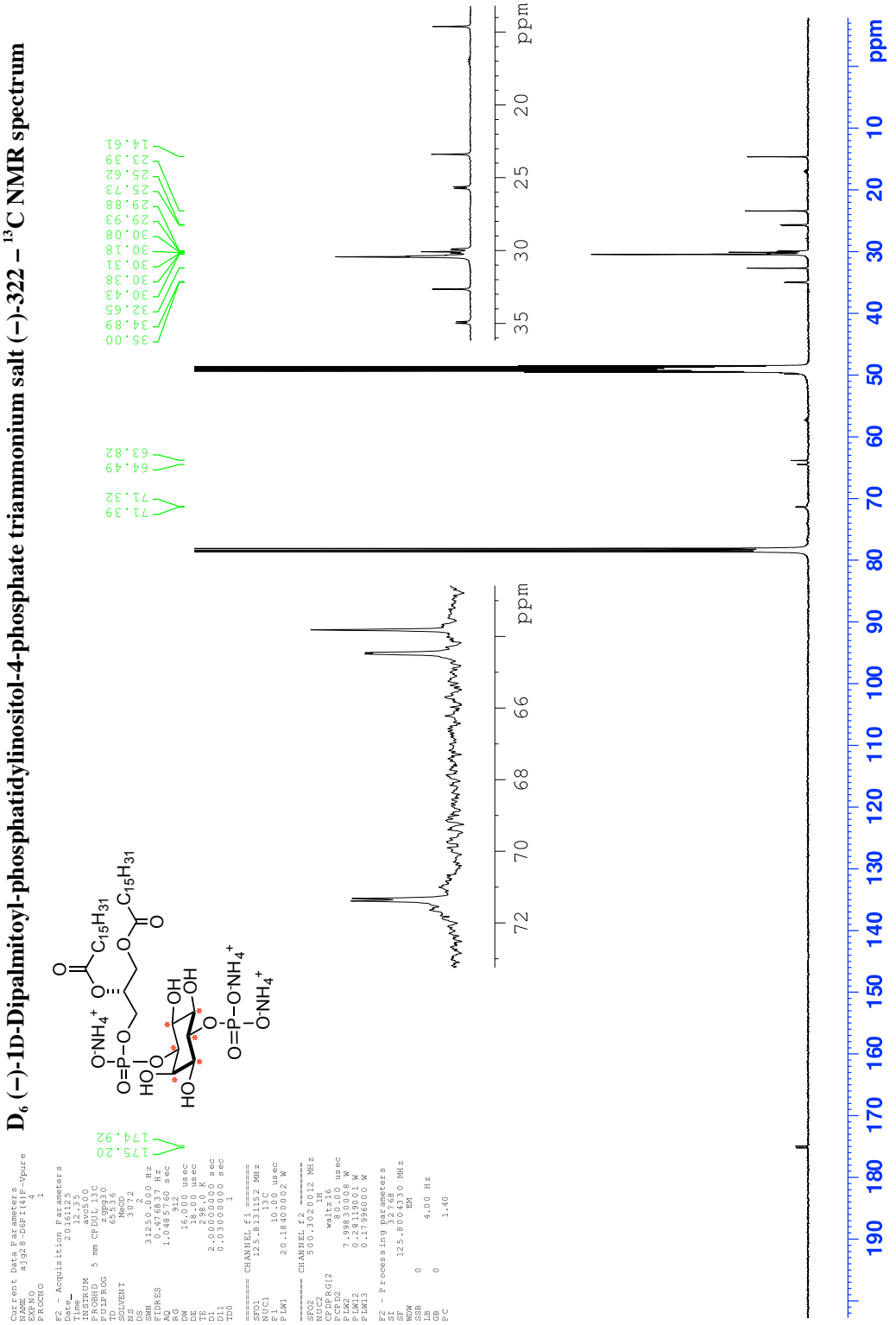


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Total	765,265	100.00

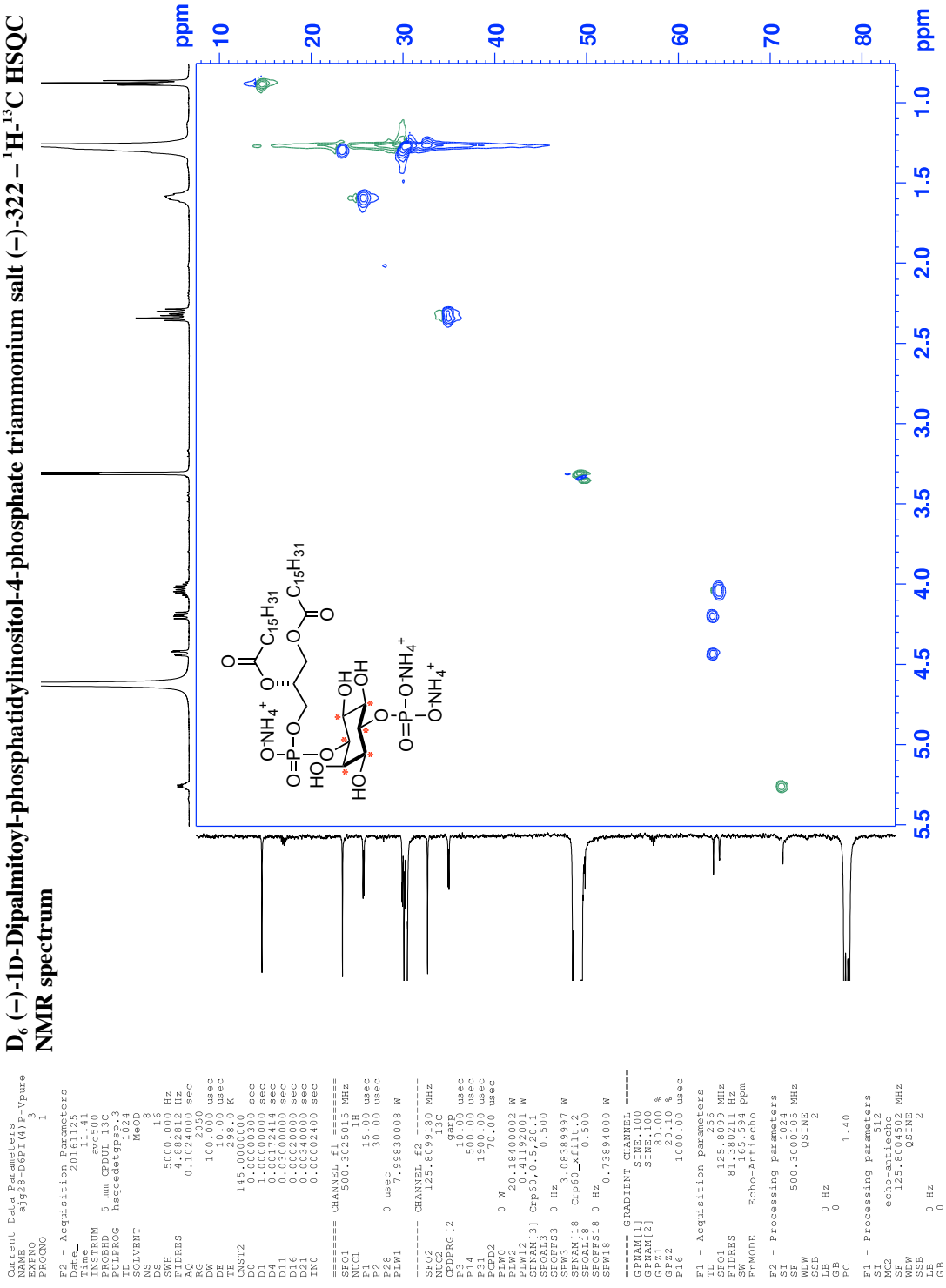
D$_6$ (–)-1D-Dipalmitoyl-phosphatidylinositol-4-phosphate triammonium salt (–)-322 – ^{1}H NMR spectrum



D_6 (–)-1D-Dipalmitoyl-phosphatidylinositol-4-phosphate triammonium salt (–)-322 – ^{13}C NMR spectrum



D_6 (–)-1D-Dipalmitoyl-phosphatidylinositol-4-phosphate triammonium salt (–)-322 – ^{1}H-^{13}C HSQC NMR spectrum




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Current Data Parameters
NAME      ajg28-D6PI(4)P-Vpure
EXPNO                        3
PROCNO                       1

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TD                  1024
SOLVENT             MeOD
NS                    16
DS                     4
SWH           5000.000 Hz
FIDRES        4.882812 Hz
AQ            0.1024000 sec
RG                 2050
DW             100.000 usec
DE                 6.00 usec
TE                298.0 K
CNST2         145.0000000
D0            0.00000300 sec
D1            1.00000000 sec
D4            0.00172414 sec
D11           0.03000000 sec
D16           0.00200000 sec
D21           0.00340000 sec
IN0           0.00002400 sec

======== CHANNEL f1 ========
SFO1          500.3025015 MHz
NUC1                  1H
P1                 15.00 usec
P2                 30.00 usec
P28            0 usec
PLW1          7.99830008 W

======== CHANNEL f2 ========
SFO2          125.8093180 MHz
NUC2                 13C
CPDPRG[2              garp
P3                 10.00 usec
P14               500.00 usec
P31              1900.00 usec
PCPD2              70.00 usec
PLW0          0 W
PLW1          20.18400002 W
PLW2          0.41192001 W
SPNAM[3]      Crp60,0.5,20.1
SPOAL3             0.500
SPOFFS3       0 Hz
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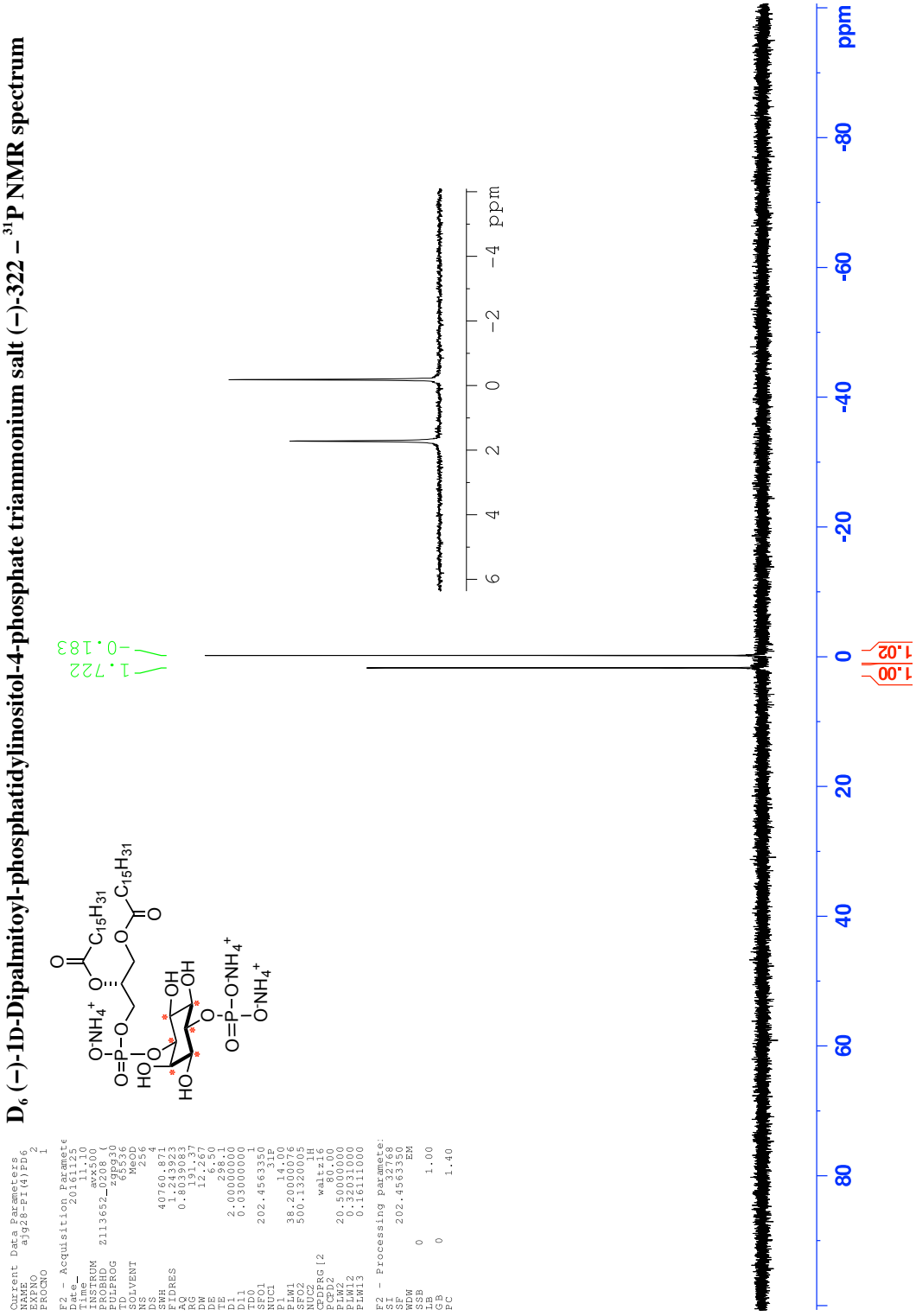
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GPZ2               1000.00 usec
P16

F1 - Acquisition parameters
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FIDRES        81.380211 Hz
SW            1.65.594 ppm
FnMODE     Echo-Antiecho

F2 - Processing parameters
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SSB                      2
LB                0 Hz
GB                   0
PC                   1.40

F1 - Processing parameters
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MC2          echo-antiecho
SF            125.8004502 MHz
WDW                  QSINE
SSB                      2
LB                0 Hz
GB                   0
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D_6 (−)-1D-Dipalmitoyl-phosphatidylinositol-4-phosphate triammonium salt (−)-322 – ^{31}P NMR spectrum



D_6 (−)-1D-Dipalmitoyl-phosphatidylinositol-4-phosphate triammonium salt
(−)-322 – ^{1}H-^{31}P HMBC NMR spectrum

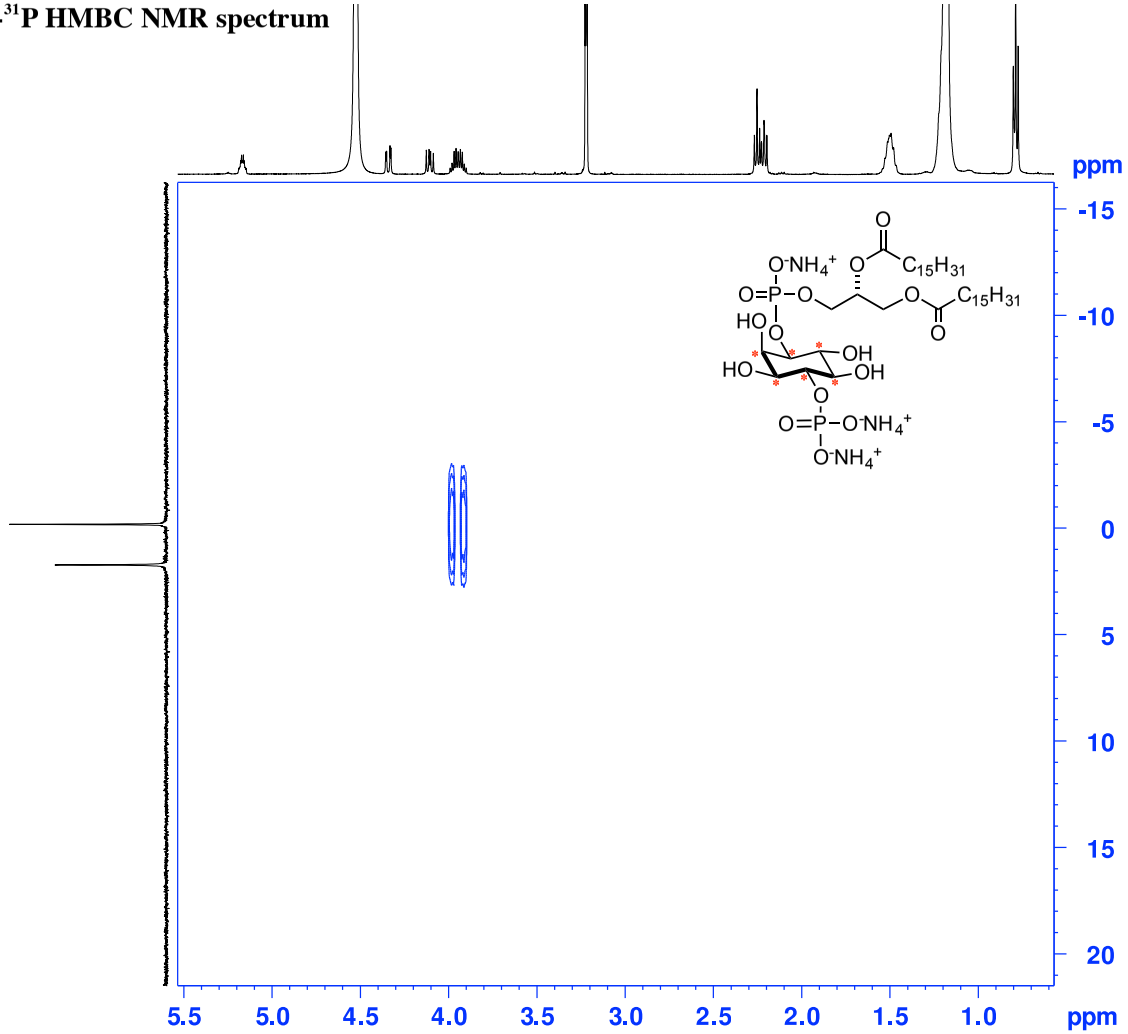
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PROCNO                 1

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TD                 4096
SOLVENT            MeOD
NS                    2
DS                   16
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FIDRES         1.467191 Hz
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RG               191.37
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DE                6.50 usec
TE                298.0 K
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D1            2.00000000 sec
d6            0.06250000 sec
D16           0.00020000 sec
in0      0 sec
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d0orig        0.00000300 sec
ph1loop                0
t1loop                 0
SFO1         500.1323506 MHz
NUC1                  1H
P1                10.00 usec
p2                20.00 usec
PLW1         20.50000000 W
SFO2         202.4563350 MHz
NUC2                 31P
P3                14.00 usec
PLW2         38.20000076 W
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GPZ3              80.50 %
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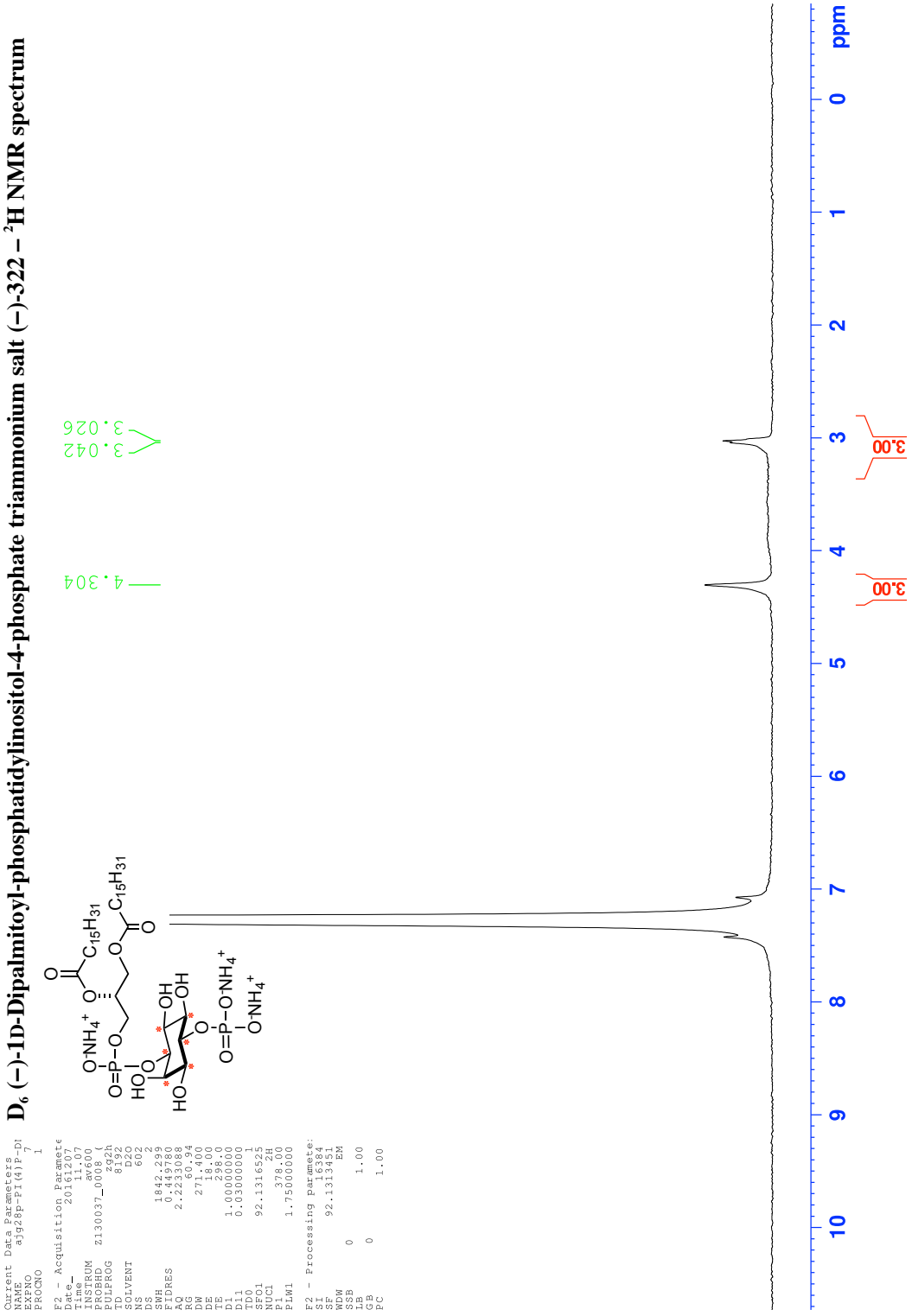
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FnMODE              QF

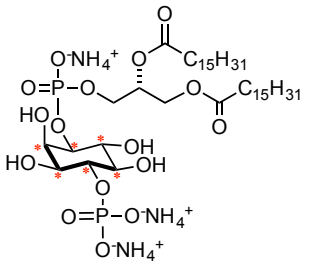
F2 - Processing parameters
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SF          500.1300534 MHz
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SSB                   4
LB       0 Hz
GB       0
PC                1.40

F1 - Processing parameters
SI                 1024
MC2                  QF
SF          202.4563278 MHz
WDW              QSINE
SSB      0
LB       0 Hz
GB       0
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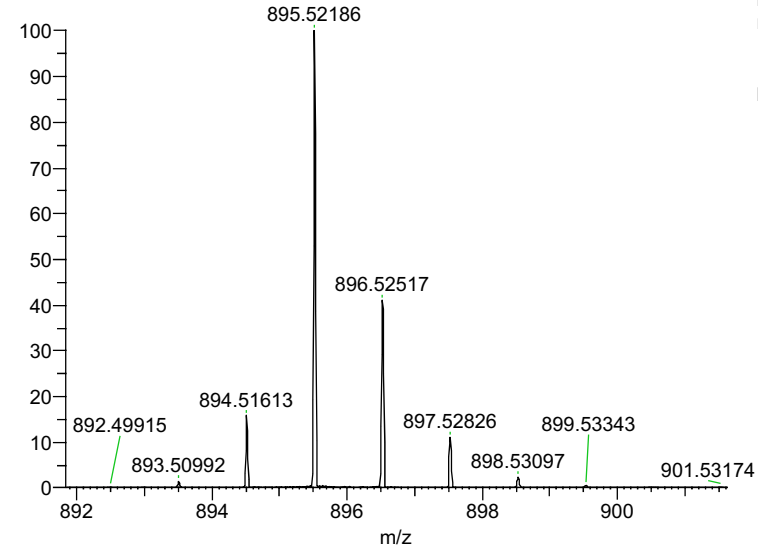
D_6 (–)-1D-Dipalmitoyl-phosphatidylinositol-4-phosphate triammonium salt (–)-322 – ^{2}H NMR spectrum





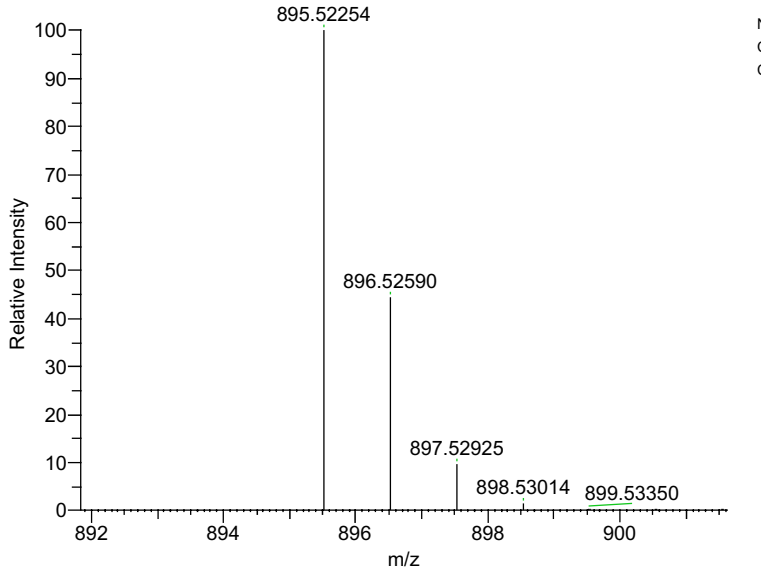
D_6 (–)-1D-Dipalmitoyl-phosphatidylinositol-4-phosphate triammonium salt (–)-322 – Mass spectrum

S:\data\Dec 16\ESI59994.raw 06/12/2016 12:35 pm



NL: 5.23E6
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T: FTMS {1,2} - p ESI Full ms [80.00-1600.00]

Measured Spectrum

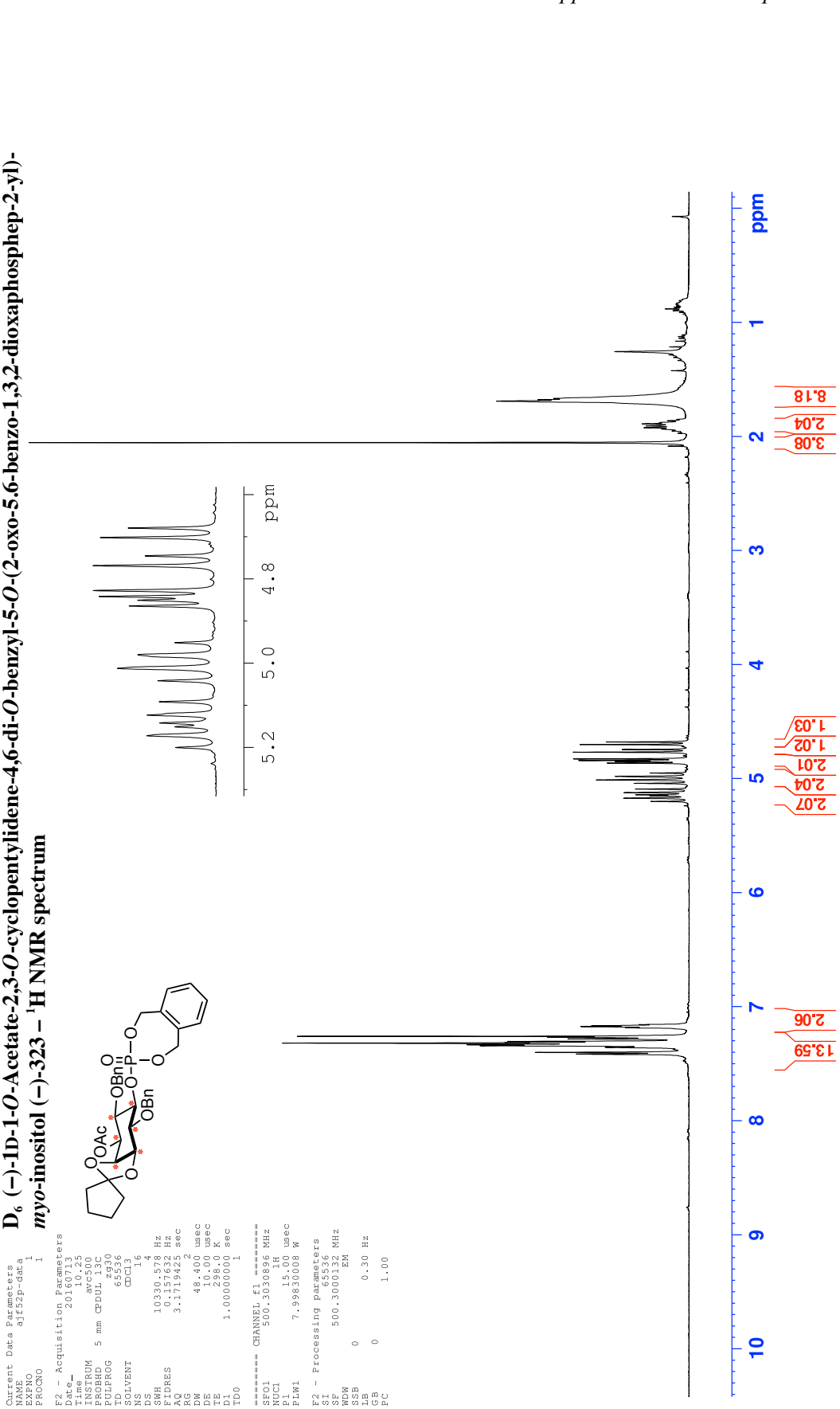


NL: 6.14E5
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Chrg -1 R: 1000000 Res. Pwr. @FWHM

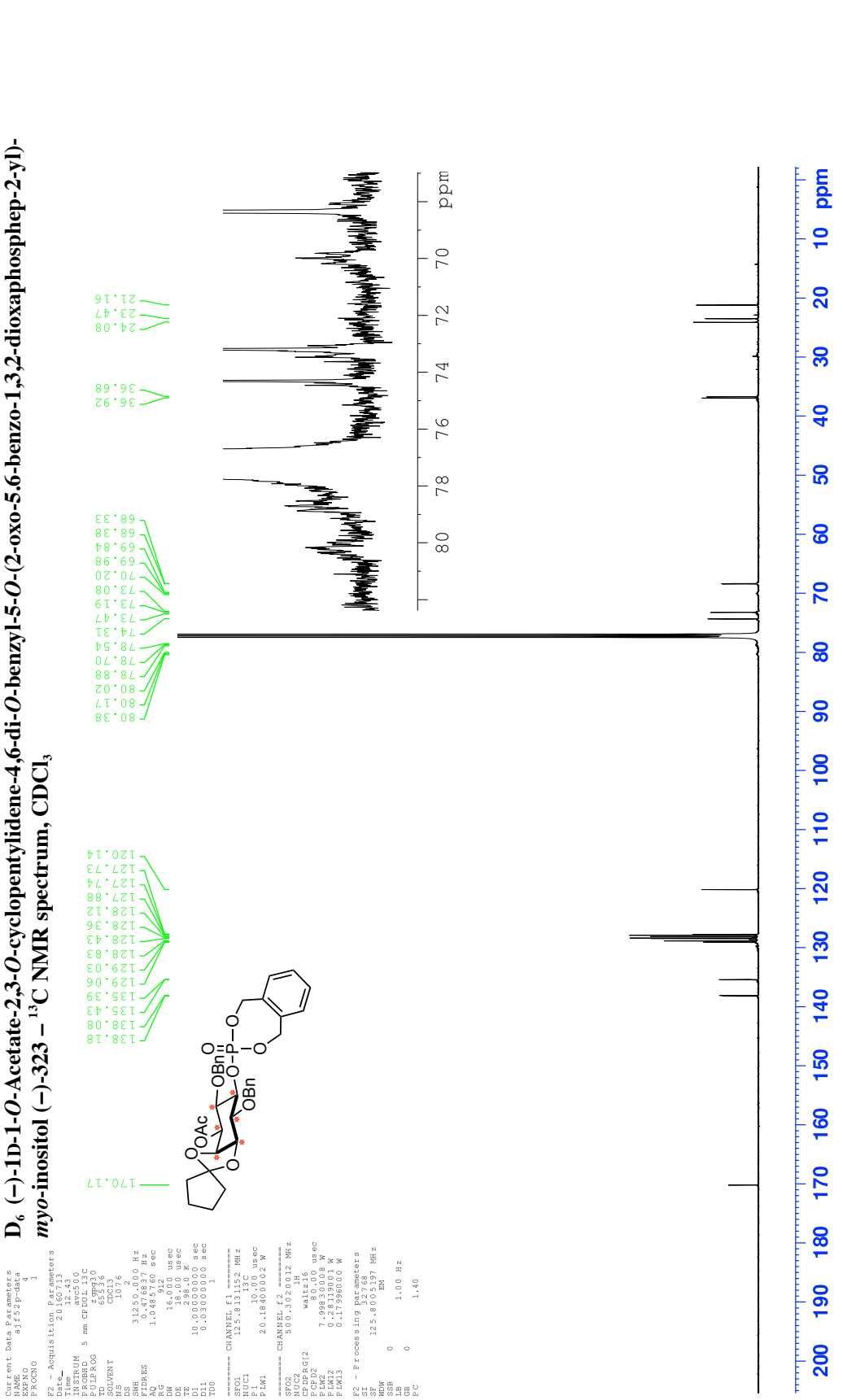
Theoretical Spectrum

m/z	Formula	RDB	Delta ppm	Theo. Mass
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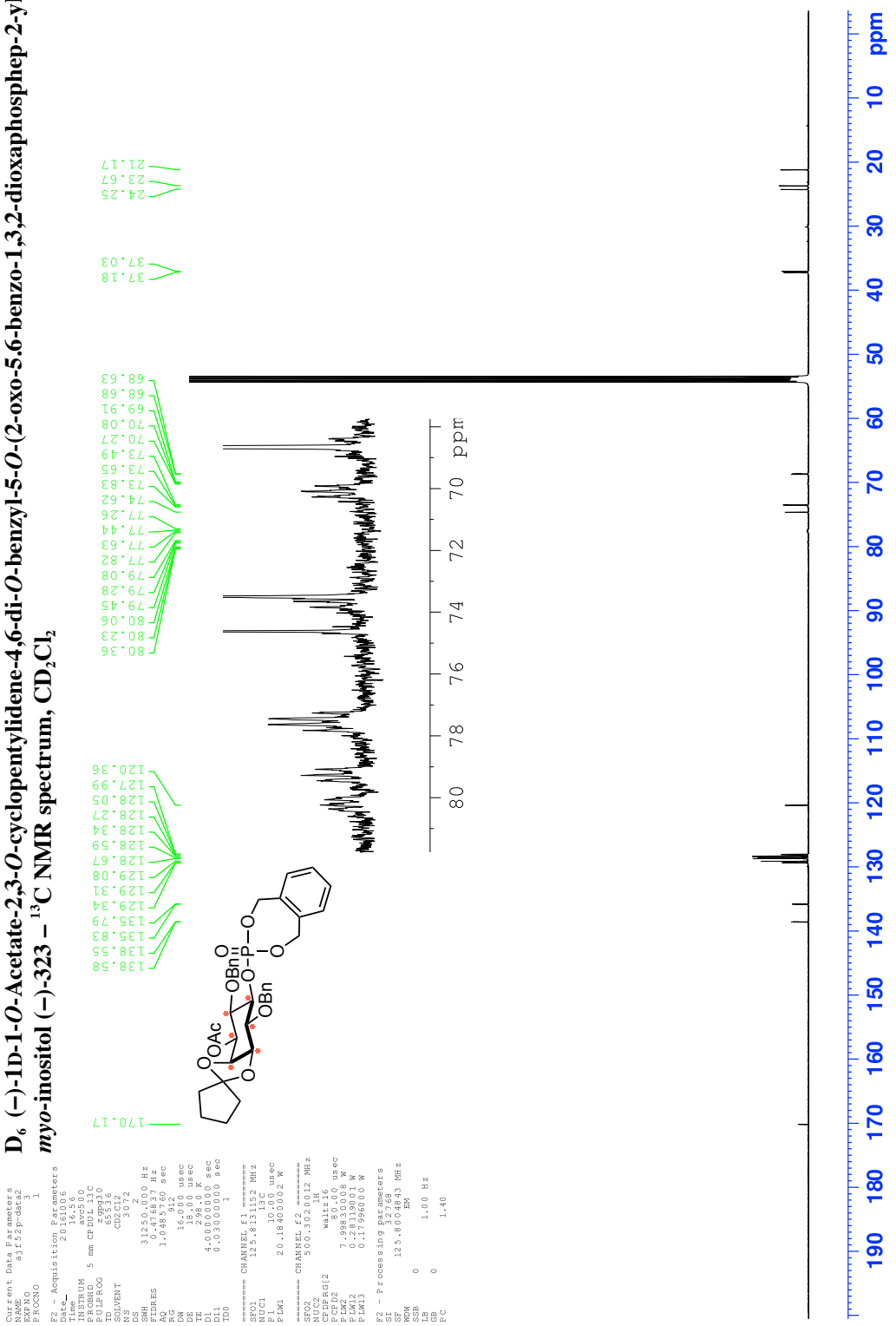
D$_6$ (−)-1D-1-*O*-Acetate-2,3-*O*-cyclopentylidene-4,6-di-*O*-benzyl-5-*O*-(2-oxo-5.6-benzo-1,3,2-dioxaphosphep-2-yl)-*myo*-inositol (−)-323 – ^{1}H NMR spectrum



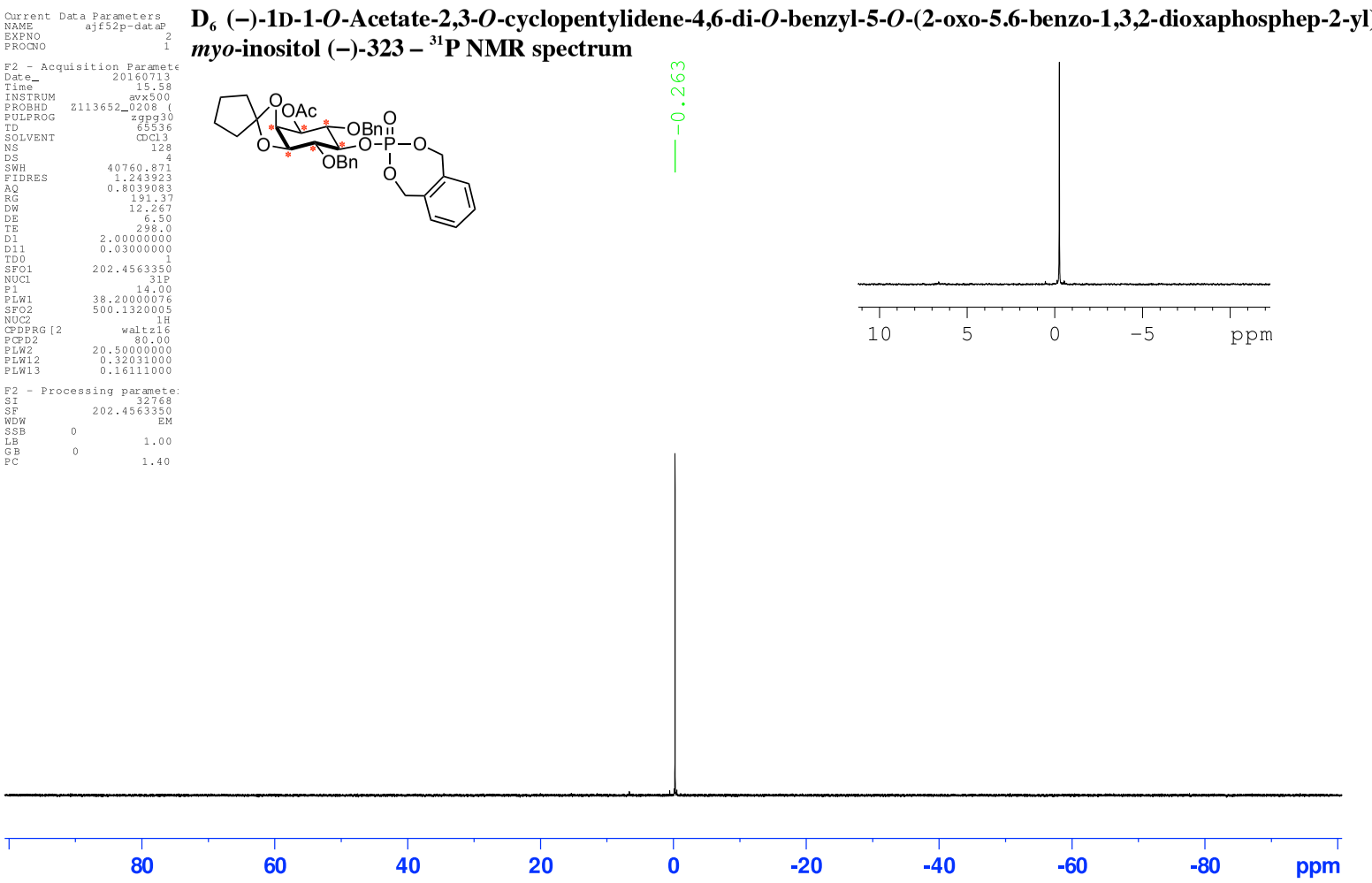
D$_6$ (–)-1D-1-*O*-Acetate-2,3-*O*-cyclopentylidene-4,6-di-*O*-benzyl-5-*O*-(2-oxo-5.6-benzo-1,3,2-dioxaphosphep-2-yl)-*myo*-inositol (–)-323 – ^{13}C NMR spectrum, CDCl$_3$



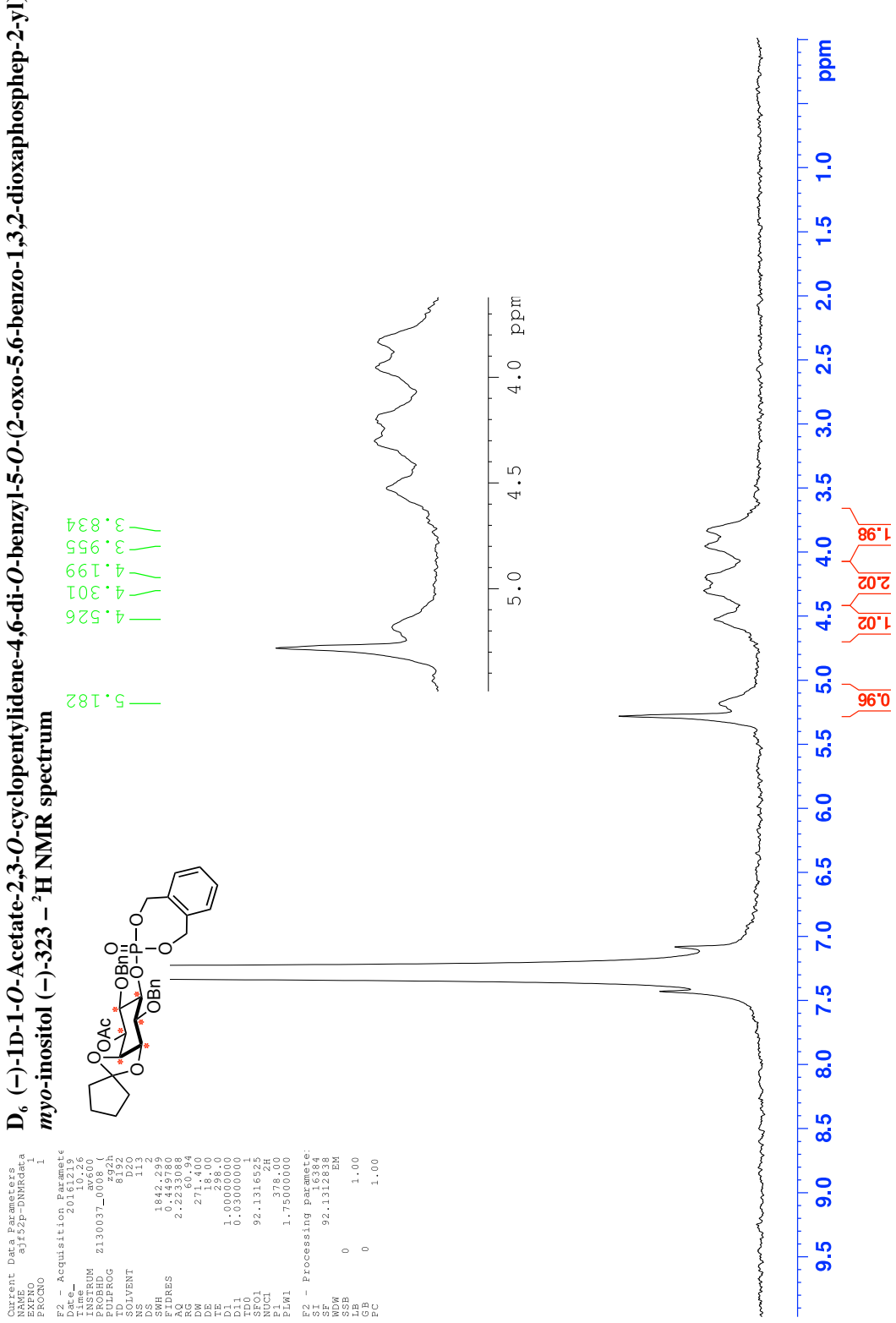
D_6 (–)-1D-1-*O*-Acetate-2,3-*O*-cyclopentylidene-4,6-di-*O*-benzyl-5-*O*-(2-oxo-5.6-benzo-1,3,2-dioxaphosphep-2-yl)-*myo*-inositol (–)-323 – ^{13}C NMR spectrum, CD_2Cl_2



D_6 (–)-1D-1-*O*-Acetate-2,3-*O*-cyclopentylidene-4,6-di-*O*-benzyl-5-*O*-(2-oxo-5.6-benzo-1,3,2-dioxaphosphep-2-yl)-*myo*-inositol (–)-323 – ^{31}P NMR spectrum



D$_6$ (−)-1D-1-*O*-Acetate-2,3-*O*-cyclopentylidene-4,6-di-*O*-benzyl-5-*O*-(2-oxo-5.6-benzo-1,3,2-dioxaphosphep-2-yl)-*myo*-inositol (−)-323 – ^{2}H NMR spectrum

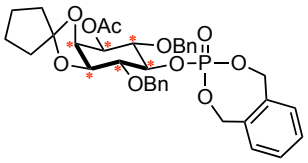


Current Data Parameters
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EXPNO 1
PROCNO 1

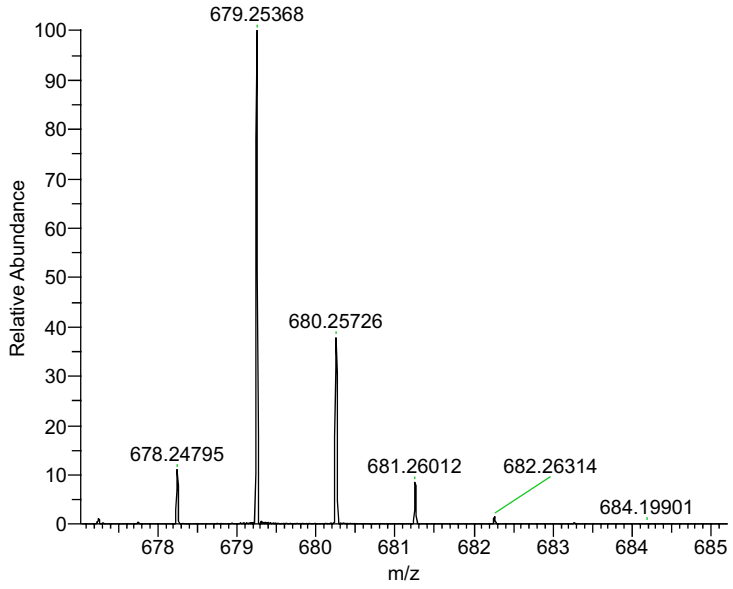
F2 - Acquisition Parameters
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Time 10.26
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TD 8192
SOLVENT CD3OD
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SWH 1842.299
FIDRES 0.449780
AQ 2.2223088
RG 60.344
DW 271.400
DE 18.00
TE 298.0
D1 1.00000000
D11 0.03000000
TD0 1
SFO1 92.1311525
NUC1 2H
P1 378.00
PLW1 1.75000000

F2 - Processing parameters
SI 16384
SF 92.1312838
WDW EM
SSB 0
LB 1.00
GB 0
PC 1.00

D_6 (−)-1D-1-*O*-Acetate-2,3-*O*-cyclopentylidene-4,6-di-*O*-benzyl-5-*O*-(2-oxo-5.6-benzo-1,3,2-dioxaphosphep-2-yl)-*myo*-inositol (−)-323 – Mass spectrum

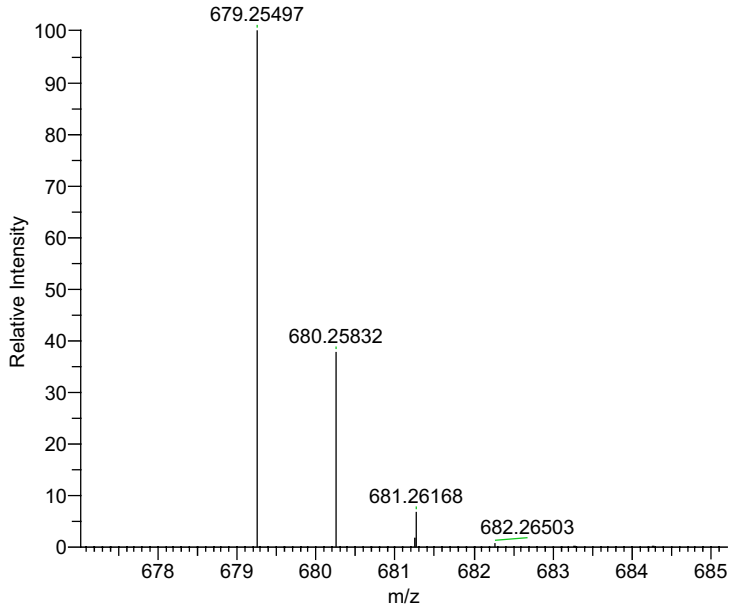


W:\data\July 16\ESI58013.raw 06/07/2016 8:30 am



NL: 7.40E7
ESI58013 #12-27 RT: 0.15-0.3 AV: 8 NL: 8.67E+007
T: FTMS {1,1} + p ESI Full ms [80.00-1600.00]

Measured Spectrum



NL: 6.67E5
C35H33[2]H6O10Na1P1: $C_{35}H_{33}{}^{2}H_{6}O_{10}$ Na P Chrg 1 R: 1000000 Res. Pwr. @FWHM

Theoretical Spectrum

m/z	Formula	RDB	Delta ppm	Theo. Mass
679.25366	$C_{35}H_{33}{}^{2}H_{6}O_{10}{}^{23}NaP$	16.5	-1.92	679.25497

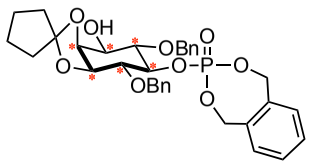
D_6 (+)-1D-2,3-*O*-Cyclopentylidene-4,6-di-*O*-benzyl-5-*O*-(2-oxo-5.6-benzo-1,3,2-dioxaphosphep -2-yl)-*myo*-inositol (+)-324 – ^{1}H NMR spectrum

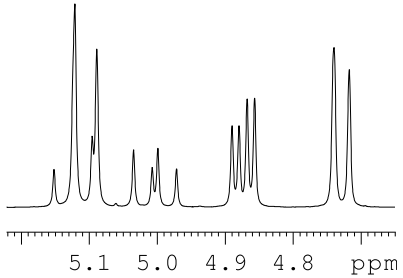
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Current Data Parameters
NAME          ajf59p-data
EXPNO                   1
PROCNO                  1

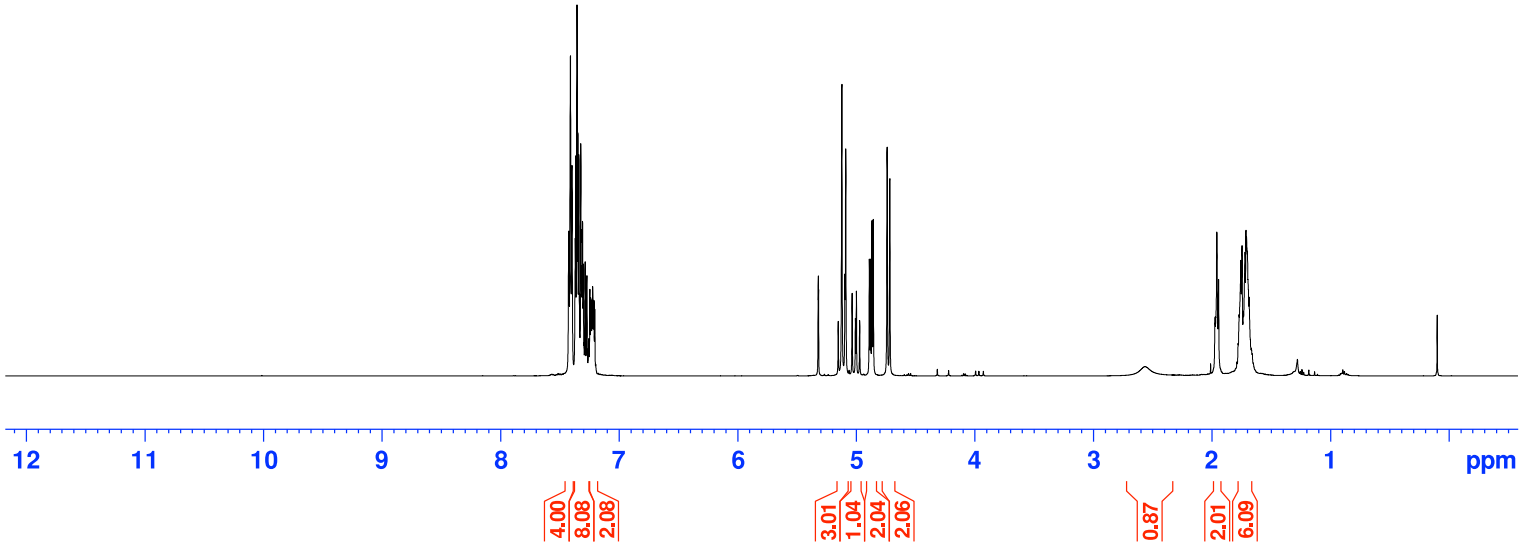
F2 - Acquisition Parameters
Date_           20160718
Time              11.58
INSTRUM           avc500
PROBHD   5 mm CPDUL 13C
PULPROG             zg30
TD                65536
SOLVENT           CD2Cl2
NS                    16
DS                     4
SWH           10330.578 Hz
FIDRES        0.157632 Hz
AQ            3.1719425 sec
RG                   3.2
DW                48.400 usec
DE                10.00 usec
TE                298.0 K
D1           1.00000000 sec
TD0                    1

======== CHANNEL f1 ========
SFO1        500.3030896 MHz
NUC1                1H
P1                15.00 usec
PLW1         7.99830008 W

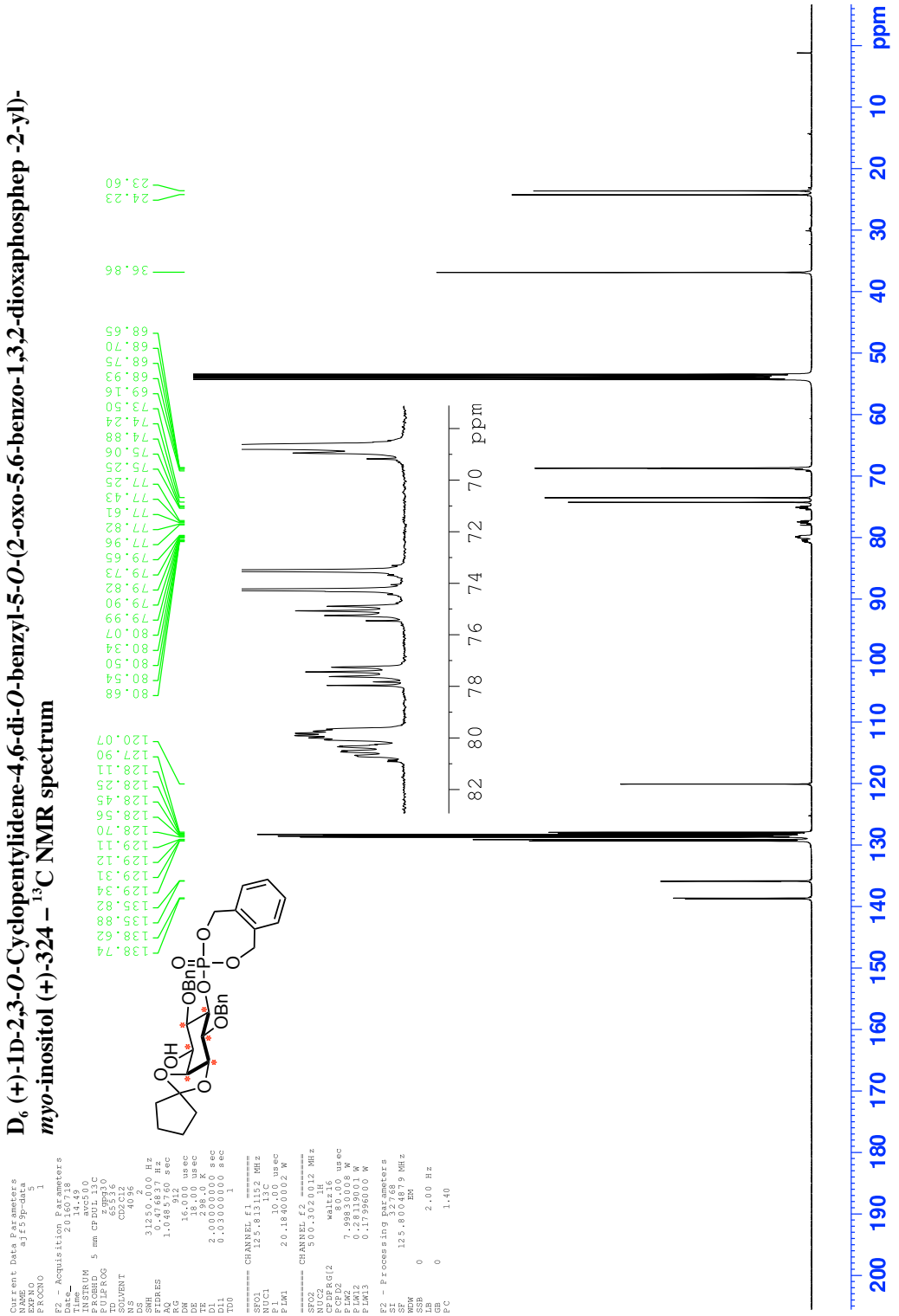
F2 - Processing parameters
SI                 65536
SF          500.3000206 MHz
WDW                   EM
SSB       0
LB                 0.30 Hz
GB        0
PC                 1.00
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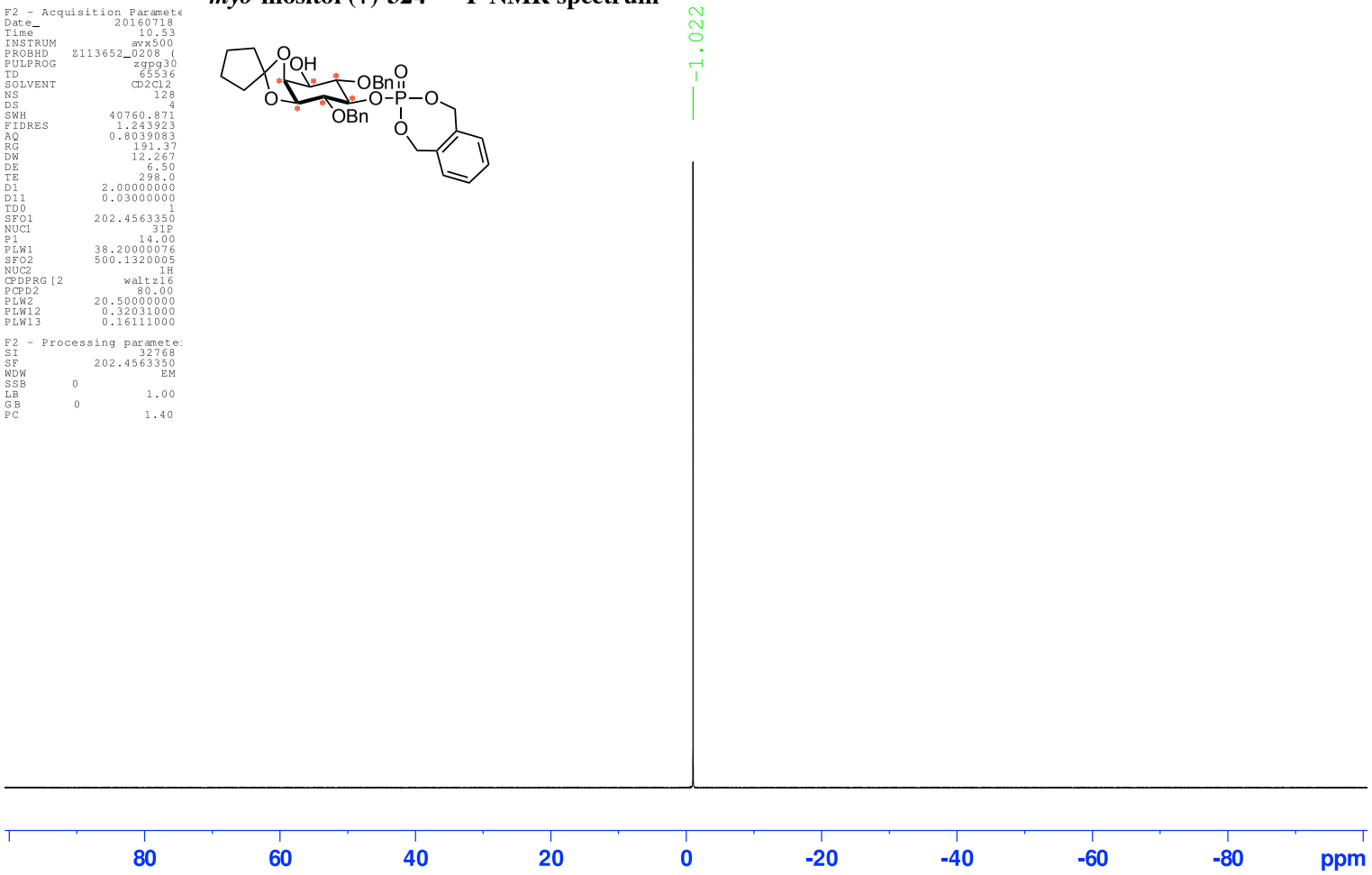




D_6 (+)-1D-2,3-*O*-Cyclopentylidene-4,6-di-*O*-benzyl-5-*O*-(2-oxo-5.6-benzo-1,3,2-dioxaphosphep -2-yl)-*myo*-inositol (+)-324 – ^{13}C NMR spectrum



D_6 (+)-1D-2,3-*O*-Cyclopentylidene-4,6-di-*O*-benzyl-5-*O*-(2-oxo-5.6-benzo-1,3,2-dioxaphosphep -2-yl)-*myo*-inositol (+)-324 – ^{31}P NMR spectrum



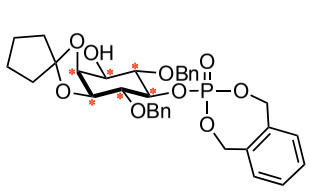
D_6 (+)-1D-2,3-*O*-Cyclopentylidene-4,6-di-*O*-benzyl-5-*O*-(2-oxo-5.6-benzo-1,3,2-dioxaphosphep -2-yl)-*myo*-inositol (+)-324 – ^{2}H NMR spectrum

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Current Data Parameters
NAME          ajf59p-DNMR
EXPNO                   1
PROCNO                  1

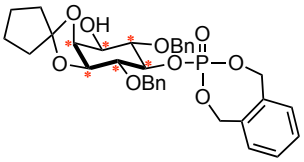
F2 - Acquisition Parameter
Date_            20161007
Time                15.14
INSTRUM           avc500
PROBHD   5 mm CPDUL 13C
PULPROG             zg2h
TD                  4096
SOLVENT             CDCl3
NS                    555
DS                      4
SWH              1535.627
FIDRES           0.374909
AQ               1.3336576
RG                      1
DW                325.600
DE                  18.00
TE                  298.0
D1               1.00000000
D11              0.03000000
TD0                     1

======== CHANNEL f1 ====
SFO1            76.7994800
NUC1                    2H
P1                  180.00
PLW1            3.30369997

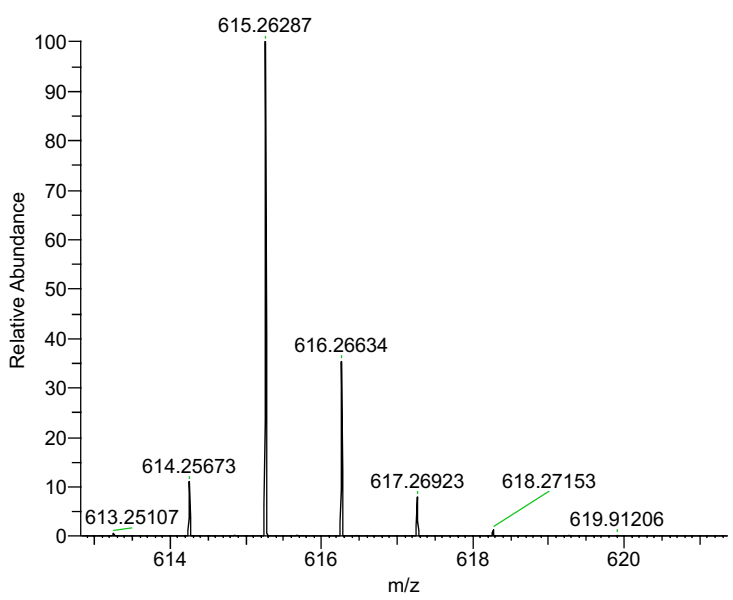
F2 - Processing parameters
SI                      8192
SF              76.7990912
WDW                     EM
SSB       0
LB                   1.00
GB        0
PC                   1.00
```



D_6 (+)-1D-2,3-*O*-Cyclopentylidene-4,6-di-*O*-benzyl-5-*O*-(2-oxo-5.6-benzo-1,3,2-dioxaphosphep -2-yl)-*myo*-inositol (+)-324 – Mass spectrum

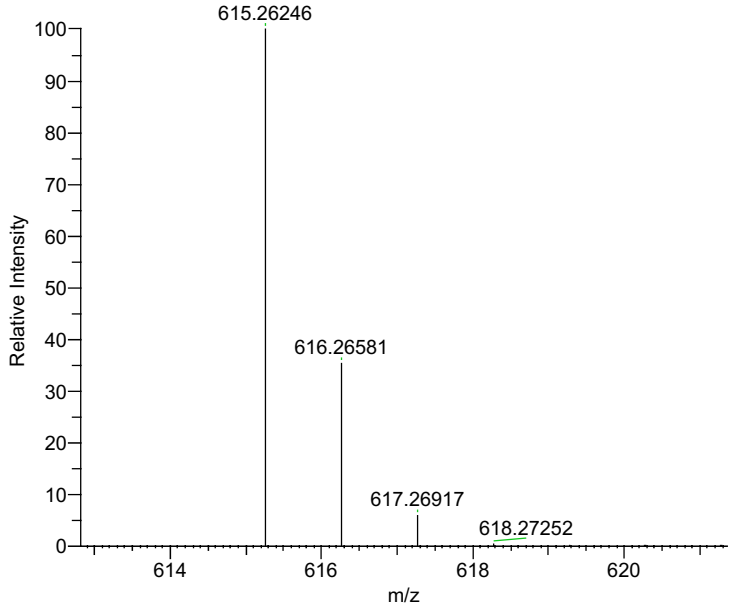


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NL: 7.87E7
ESI59268 #14-31 RT: 0.14-0.29 AV: 9 NL: 1.27E+008
T: FTMS + p ESI Full ms [100.00-1500.00]

Measured Spectrum



NL: 6.83E5
C33H32[2]H6O9P1: $C_{33}H_{32}{}^{2}H_{6}O_{9}P$ Chrg 1 R: 1000000 Res. Pwr. @FWHM

Theoretical Spectrum

m/z	Formula	RDB	Delta ppm	Theo. Mass
615.26288	$C_{33}H_{32}{}^{2}H_{6}O_{9}P$	15.5	0.69	615.26246

D_6 (+)-1D-2,3-*O*-Cyclopentylidene-4,6-di-*O*-benzyl-5-*O*-(2-oxo-5.6-benzo-1,3,2-dioxaphosphep -2-yl)-*myo*-inositol (+)-324 – RP-HPLC (Method 2)

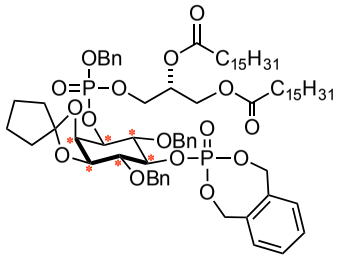
Time	Area	Area %
9.857	141,121	2.59
12.399	3,681	0.07
12.815	25,784	0.47
13.342	5,275,487	96.87
Total	5,446,073	100.00

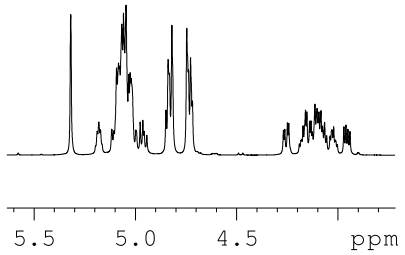
D_6 (–)-1D-1-*O*-(1,2-Di-*O*-palmitoyl-*sn*-glycer-3-yl-benzylphosphate)-2,3-*O*-cyclopentylidene-4,6-di-*O*-benzyl-5-*O*-(2-oxo-5.6-benzo-1,3,2-dioxaphosphep-2-yl)-*myo*-inositol (–)-325 – ^{1}H NMR spectrum

```
Current Data Parameters
NAME          ajf81p-data
EXPNO                   1
PROCNO                  1

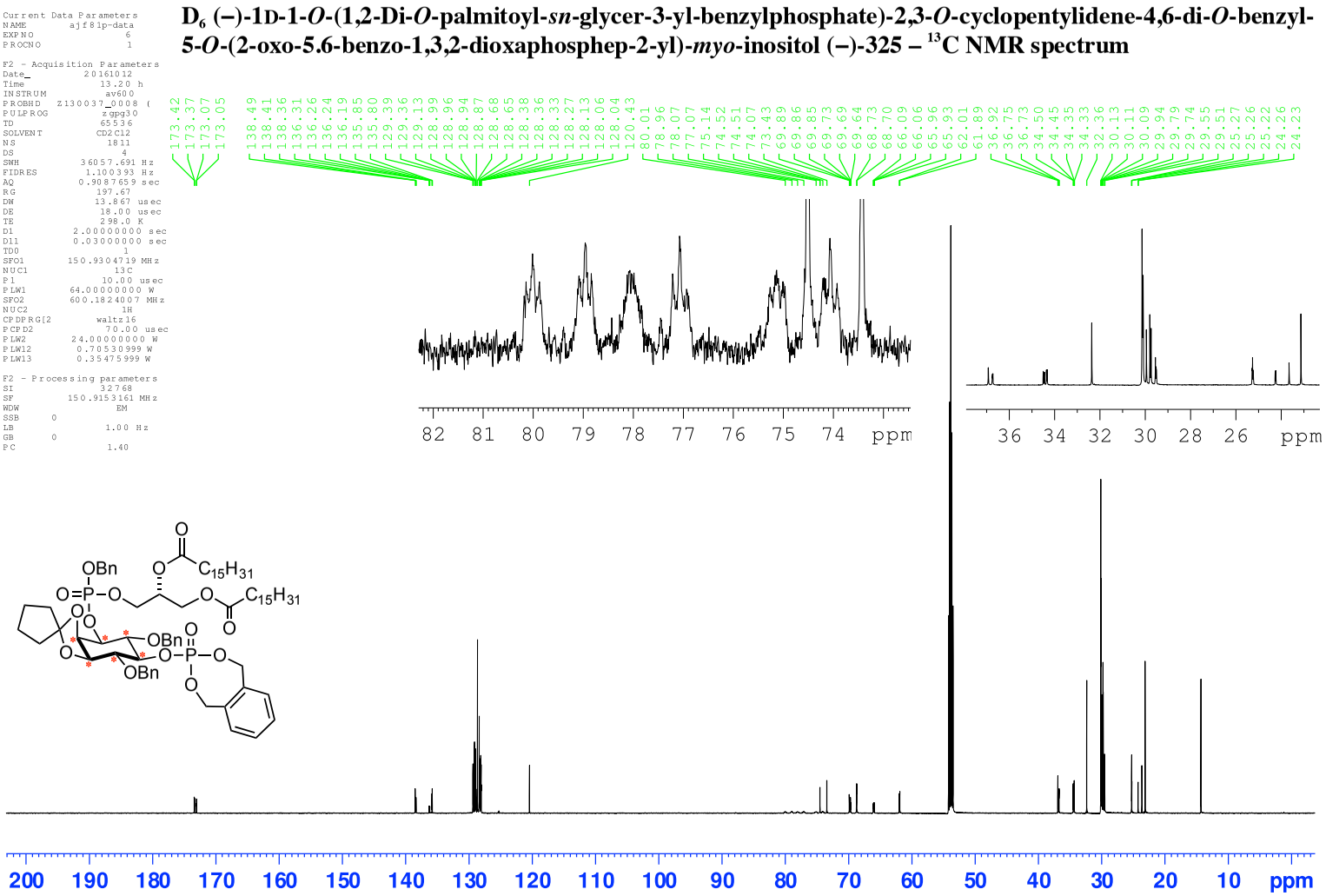
F2 - Acquisition Parameters
Date_           20161012
Time              11.04 h
INSTRUM             av600
PROBHD    Z130037_0008 (
PULPROG             zg30
TD                 65536
SOLVENT           CD2Cl2
NS                    16
DS                     2
SWH           12019.230 Hz
FIDRES            0.366798 Hz
AQ             2.7262976 sec
RG                 22.67
DW                41.600 usec
DE                10.00 usec
TE                298.0 K
D1            1.00000000 sec
TD0                    1
SFO1          600.1830009 MHz
NUC1                  1H
P1                12.00 usec
PLW1          24.00000000 W

F2 - Processing parameters
SI                 65536
SF            600.1800237 MHz
WDW                   EM
SSB       0
LB                 0.30 Hz
GB        0
PC                 1.00
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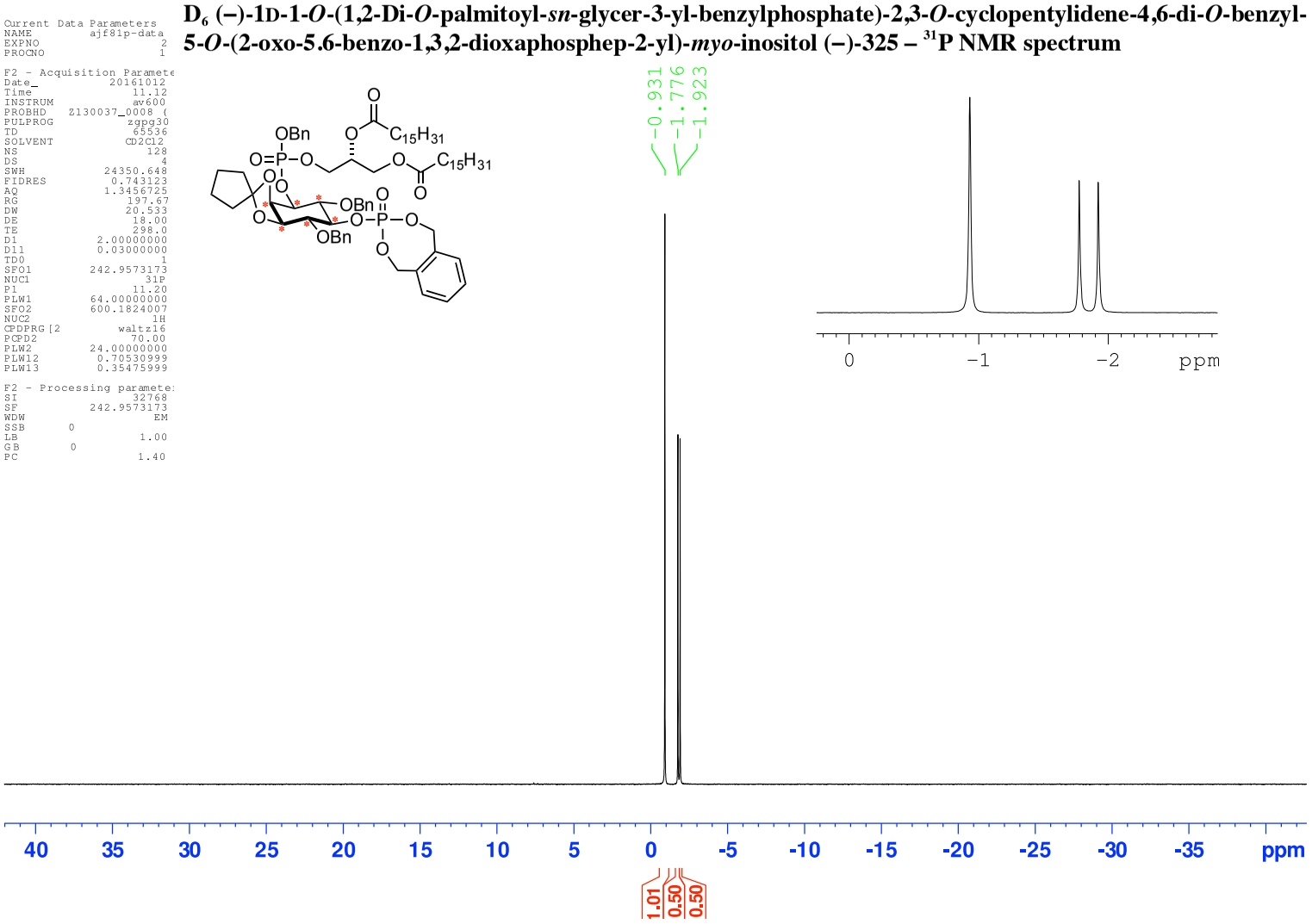




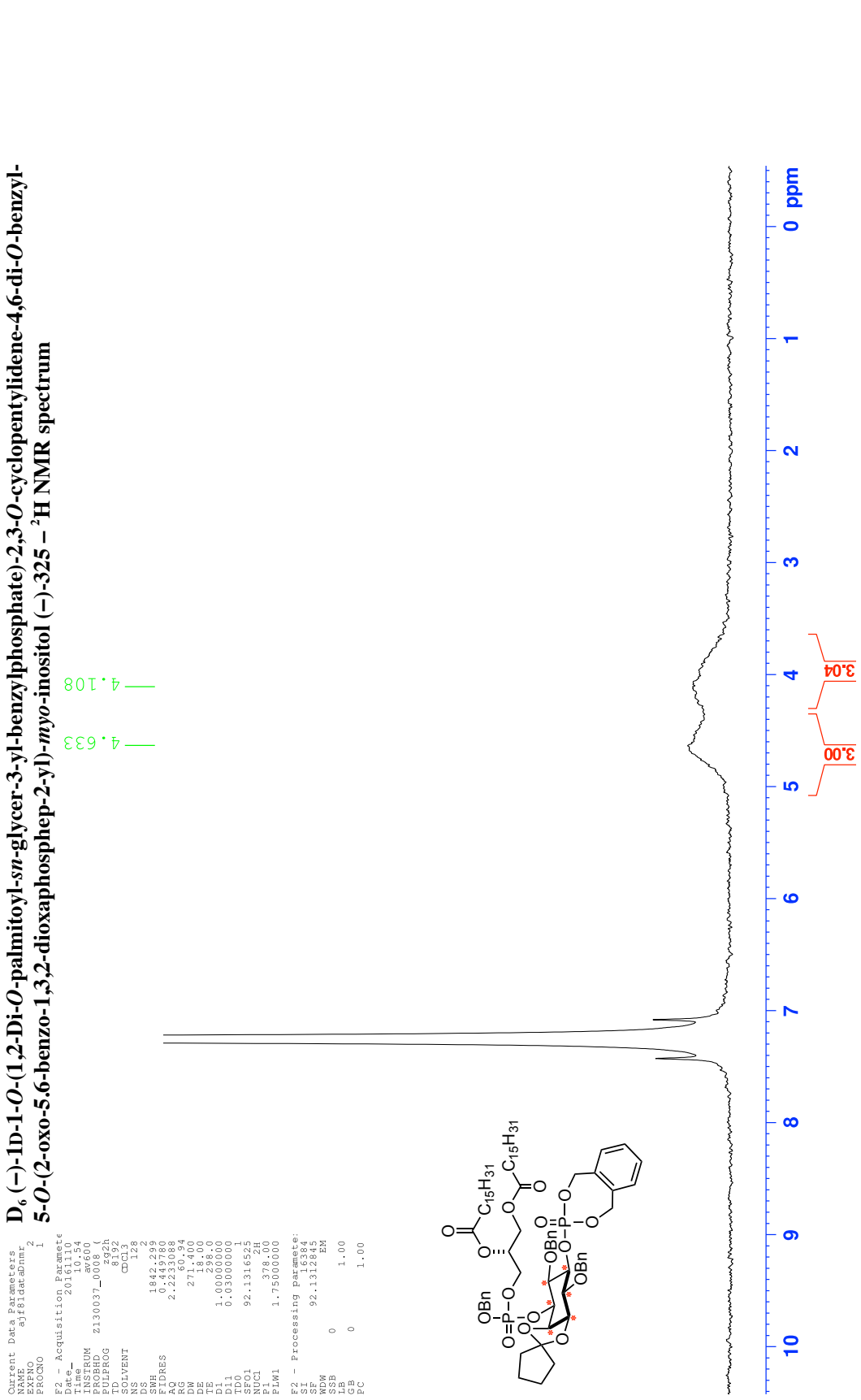
D_6 (–)-1D-1-*O*-(1,2-Di-*O*-palmitoyl-*sn*-glycer-3-yl-benzylphosphate)-2,3-*O*-cyclopentylidene-4,6-di-*O*-benzyl-5-*O*-(2-oxo-5.6-benzo-1,3,2-dioxaphosphep-2-yl)-*myo*-inositol (–)-325 – ^{13}C NMR spectrum



D_6 (–)-1D-1-*O*-(1,2-Di-*O*-palmitoyl-*sn*-glycer-3-yl-benzylphosphate)-2,3-*O*-cyclopentylidene-4,6-di-*O*-benzyl-5-*O*-(2-oxo-5.6-benzo-1,3,2-dioxaphosphep-2-yl)-*myo*-inositol (–)-325 – ^{31}P NMR spectrum

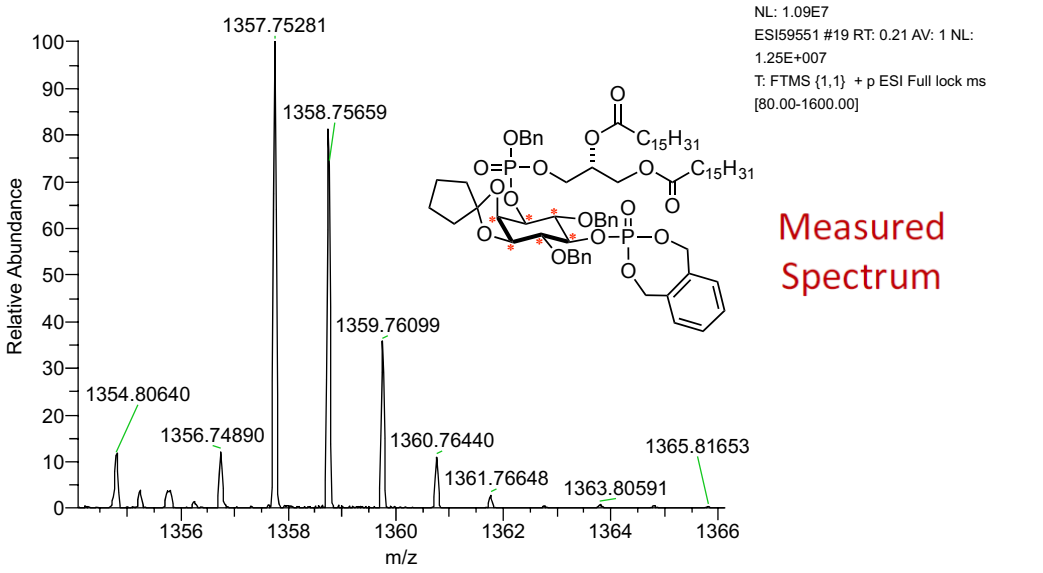


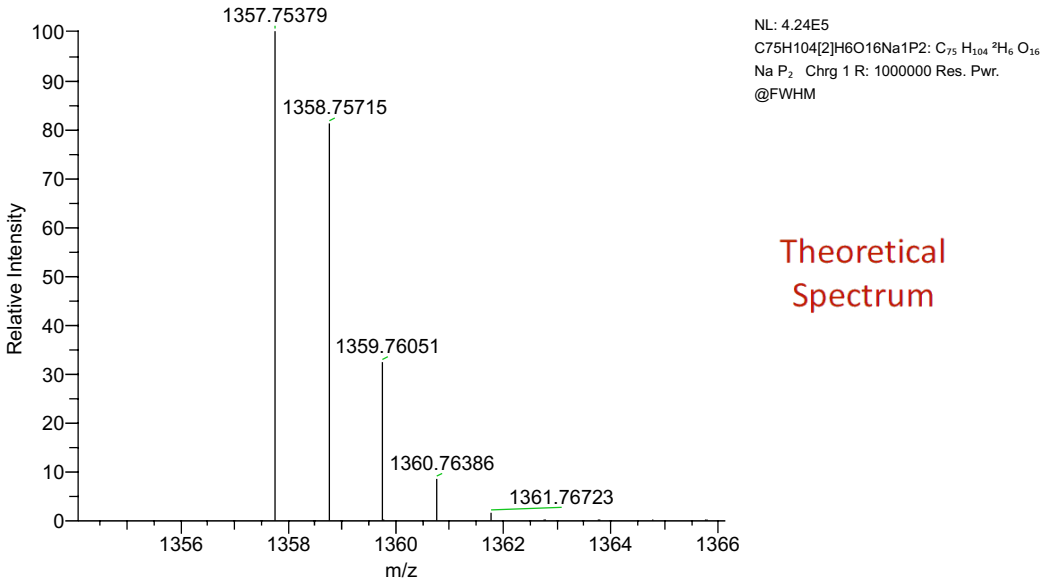
D_6 (−)-1D-1-*O*-(1,2-Di-*O*-palmitoyl-*sn*-glycer-3-yl-benzylphosphate)-2,3-*O*-cyclopentylidene-4,6-di-*O*-benzyl-5-*O*-(2-oxo-5.6-benzo-1,3,2-dioxaphosphep-2-yl)-*myo*-inositol (−)-325 – ^{2}H NMR spectrum



D_6 (–)-1D-1-*O*-(1,2-Di-*O*-palmitoyl-*sn*-glycer-3-yl-benzylphosphate)-4,6-di-*O*-benzyl-5-*O*-(2-oxo-5.6-benzo-1,3,2-dioxaphosphep-2-yl)-*myo*-inositol (–)-325 – Mass spectrum

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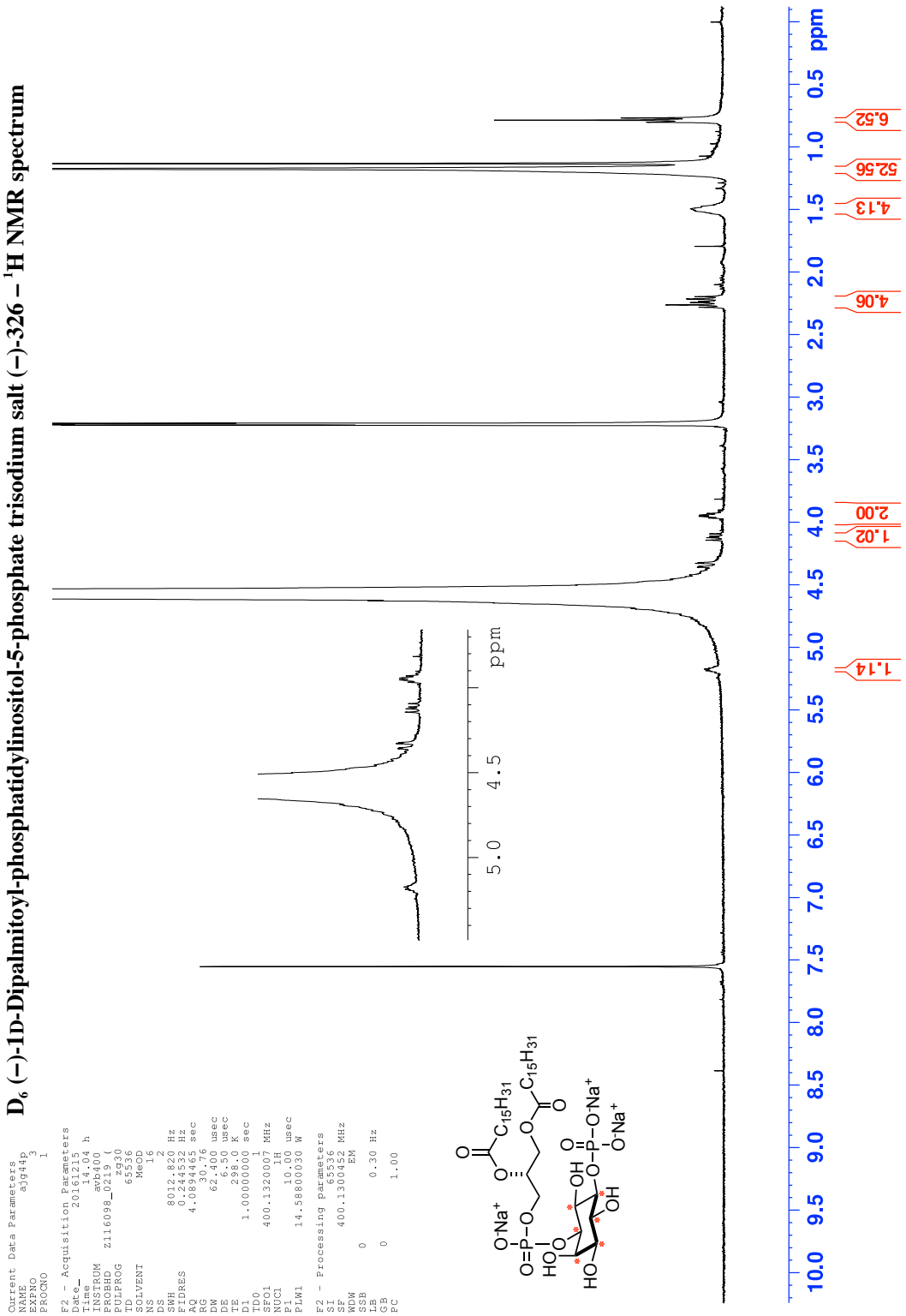


m/z	Formula	RDB	Delta ppm	Theo. Mass
1357.75281	$C_{75}H_{104}{}^{2}H_{6}O_{16}{}^{23}NaP_2$	21.5	-0.72	1357.75379

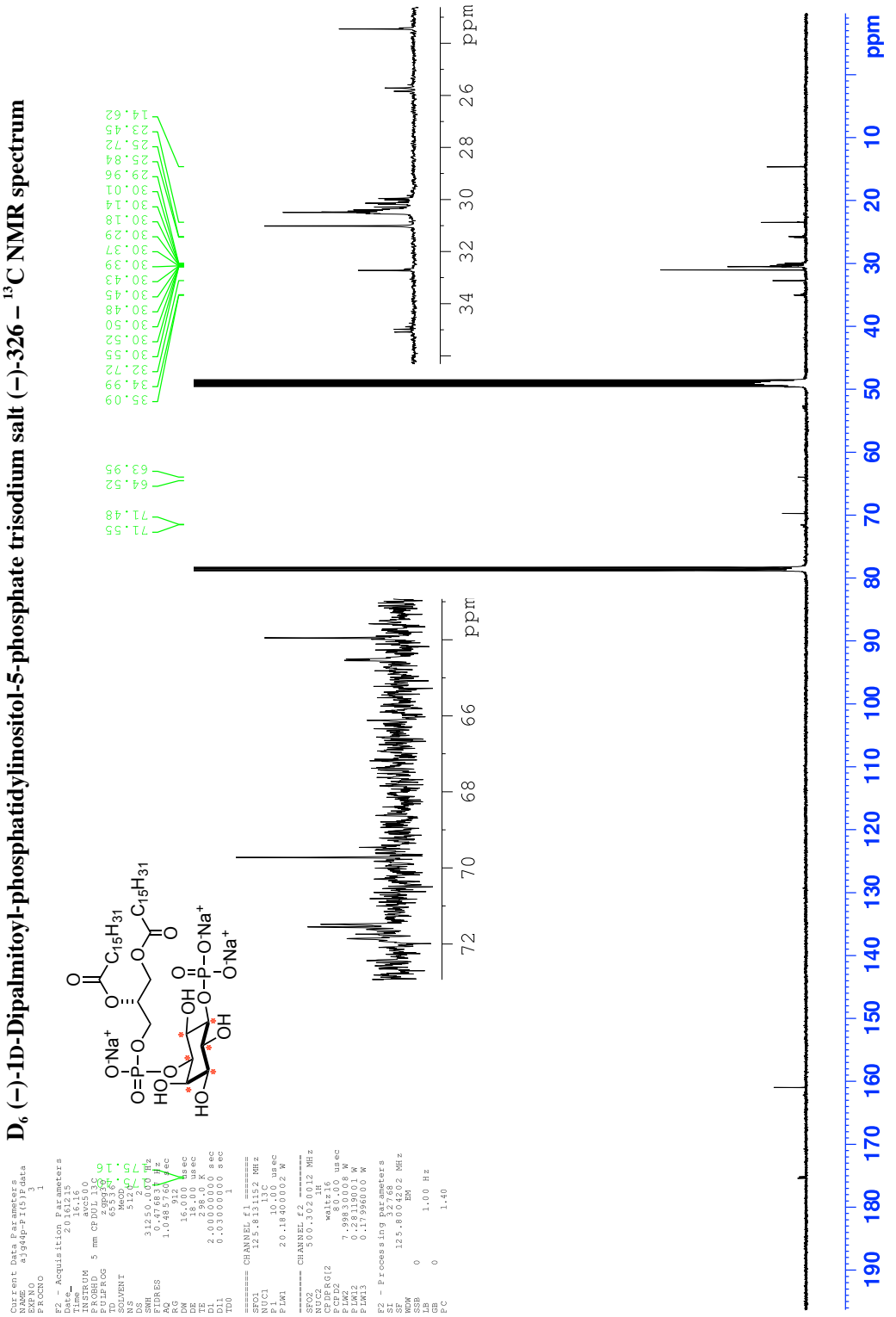
D_6 (−)-1D-1-*O*-(1,2-Di-*O*-palmitoyl-*sn*-glycer-3-yl-benzylphosphate)-2,3-*O*-cyclopentylidene-4,6-di-*O*-benzyl-5-*O*-(2-oxo-5.6-benzo-1,3,2-dioxaphosphep-2-yl)-*myo*-inositol (−)-325 – NP-HPLC (Method 7)

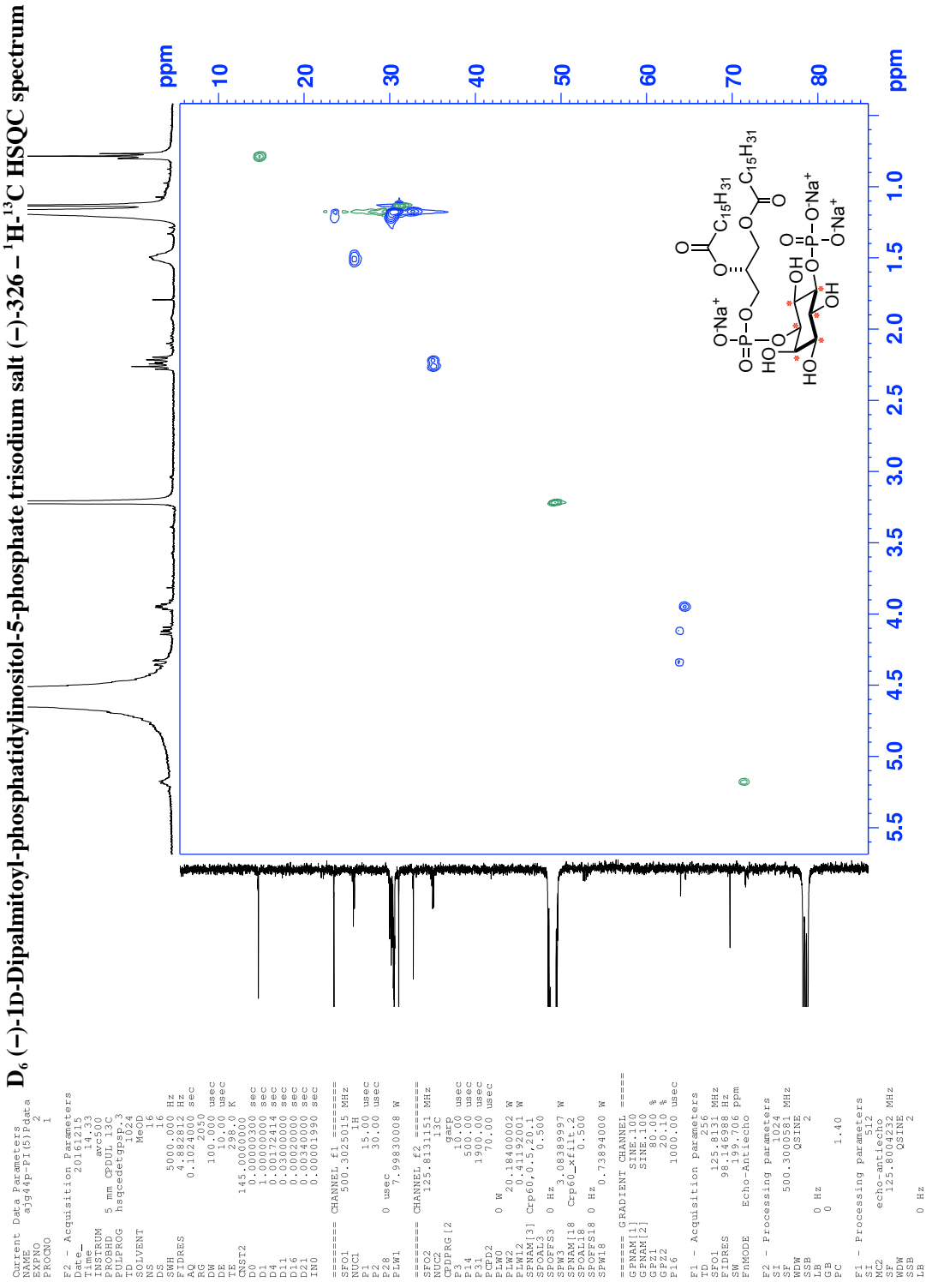
Time	Area	Area %
2.576	163,765	2.40
3.923	3,217,843	47.13
4.268	3,445,914	50.47
Total	6,827,522	100.00

D_6 (–)-1D-Dipalmitoyl-phosphatidylinositol-5-phosphate trisodium salt (–)-326 – ^{1}H NMR spectrum

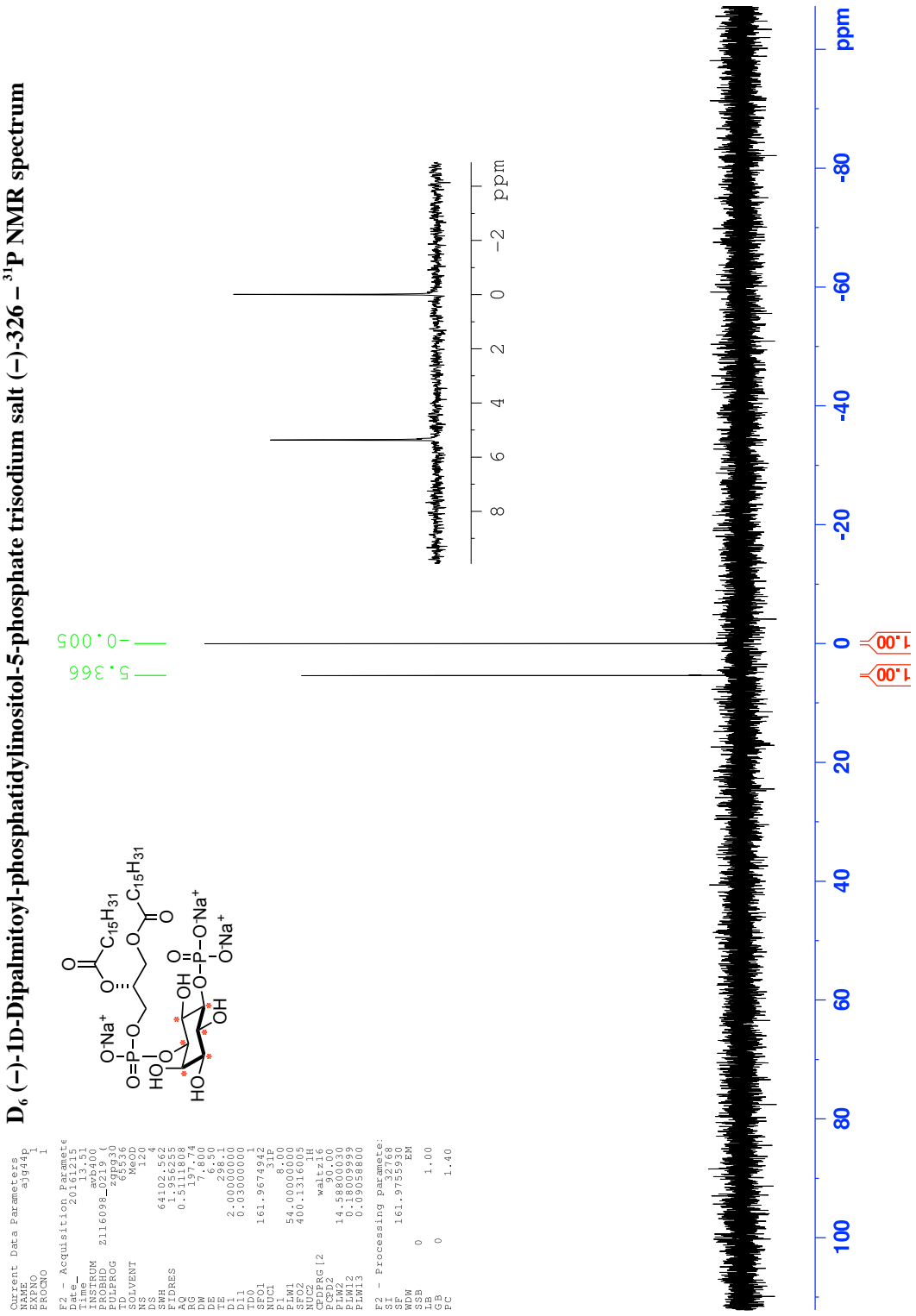


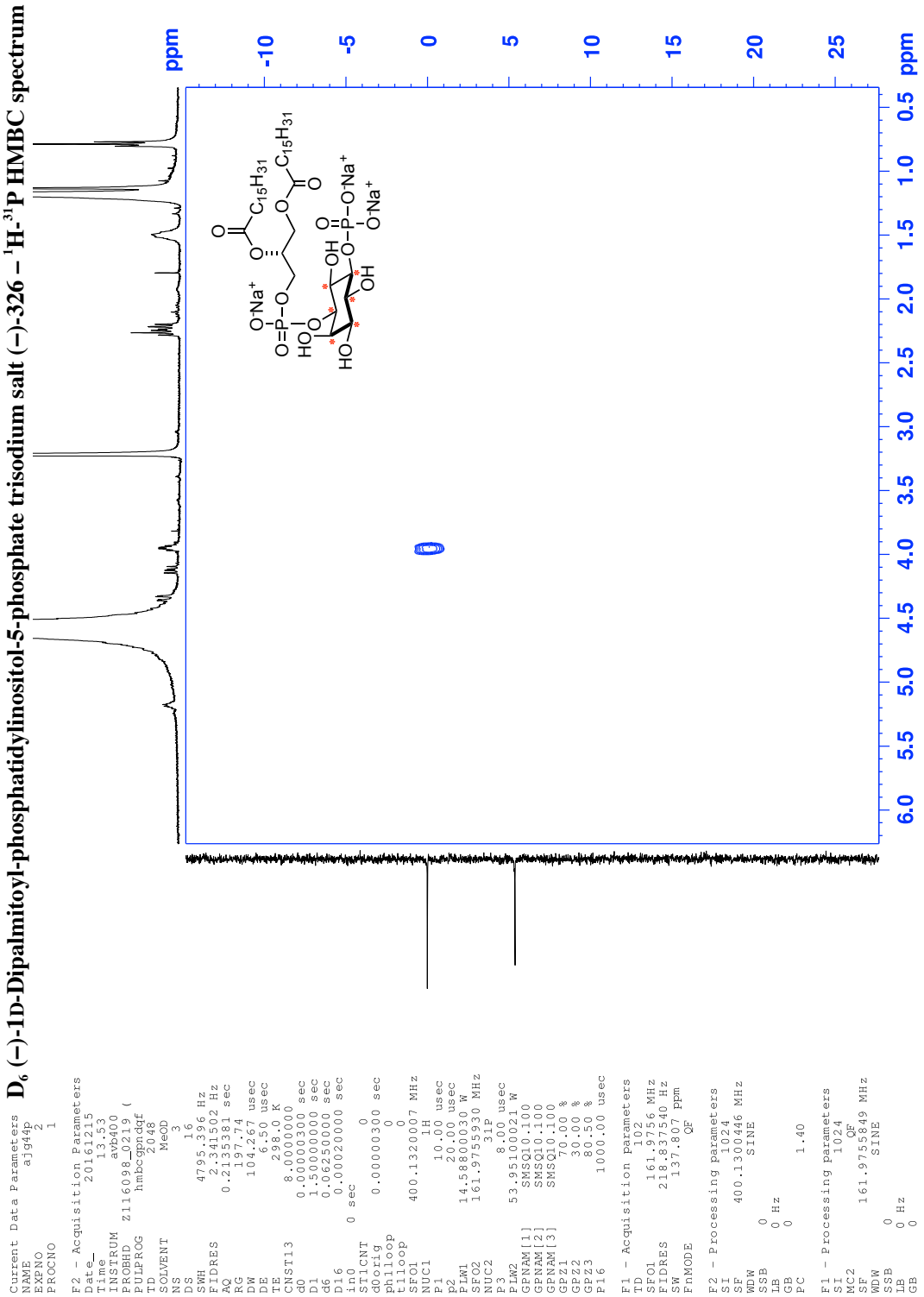
D_6 (−)-1D-Dipalmitoyl-phosphatidylinositol-5-phosphate trisodium salt (−)-326 – ^{13}C NMR spectrum



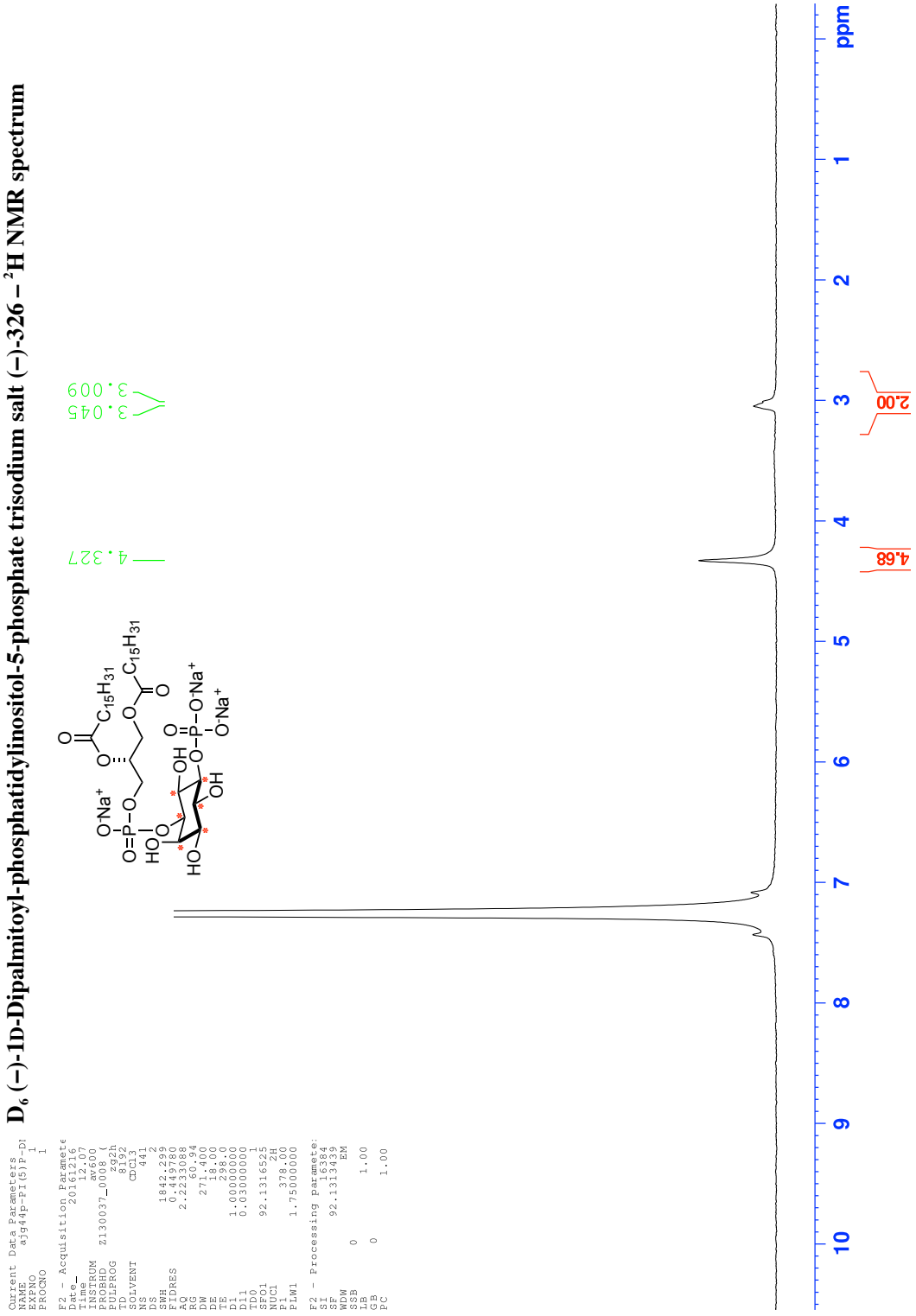

D6 (–)-1D-Dipalmitoyl-phosphatidylinositol-5-phosphate trisodium salt (–)-326 – 1H-13C HSQC spectrum
Current Data Parameters
NAME ajg44p-PI(5)Pdata
EXPNO 2
PROCNO 1
F2 - Acquisition Parameters
Date_ 20151215
Time 14.33
INSTRUM avc500
PROBHD 5 mm CPDUL 13C
PULPROG hsqcedetgpsp.3
TD 1024
SOLVENT MeOD
NS 16
DS 16
SWH 5000.000 Hz
FIDRES 4.882812 Hz
AQ 0.1024000 sec
RG 2050
DW 100.000 usec
DE 10.00 usec
TE 298.0 K
CNST2 145.0000000
D0 0.00000300 sec
D1 1.00000000 sec
D4 0.00172414 sec
D11 0.03000000 sec
D16 0.00200000 sec
D21 0.00340000 sec
IN0 0.00001990 sec
======== CHANNEL f1 ========
SFO1 500.3025015 MHz
NUC1 1H
P1 15.00 usec
P2 30.00 usec
P28 0 usec
PLW1 7.99830008 W
======== CHANNEL f2 ========
SFO2 125.8131151 MHz
NUC2 13C
CPDPRG[2 garp
P3 10.00 usec
P14 500.00 usec
P31 1900.00 usec
PCPD2 70.00 usec
PLW0 0 W
PLW2 20.18400002 W
PLW12 0.41192001 W
SPNAM[3] Crp60,0.5,20.1
SPOAL3 0.500
SPOFFS3 0 Hz
SPW3 3.08389997 W
SPNAM[18 Crp60_xfilt.2
SPOAL18 0.500
SPOFFS18 0 Hz
SPW18 0.73894000 W
====== GRADIENT CHANNEL =====
GPNAM[1] SINE.100
GPNAM[2] SINE.100
GPZ1 50.00 %
GPZ2 20.10 %
P16 1000.00 usec
F1 - Acquisition parameters
TD 256
SFO1 125.8431 MHz
FIDRES 98.14838 Hz
SW 199.706 ppm
FnMODE Echo-Antiecho
F2 - Processing parameters
SI 1024
SF 500.3000581 MHz
WDW QSINE 2
SSB 0
LB 0 Hz
GB 0
PC 1.40
F1 - Processing parameters
SI 512
MC2 echo-antiecho
SF 125.8004232 MHz
WDW QSINE 2
SSB 0
LB 0 Hz
GB 0
ppm
10
20
30
40
50
60
70
80
ppm
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0

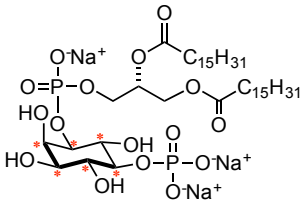
D_6 (–)-1D-Dipalmitoyl-phosphatidylinositol-5-phosphate trisodium salt (–)-326 – ^{31}P NMR spectrum




D6 (–)-1D-Dipalmitoyl-phosphatidylinositol-5-phosphate trisodium salt (–)-326 – 1H-31P HMBC spectrum
Current Data Parameters
NAME ajg44p
EXPNO 2
PROCNO 1
F2 - Acquisition Parameters
Date_ 20161215
Time 13.53
INSTRUM avb400
PROBHD Z116098_0219 (
PULPROG hmbcgpndqf
TD 2048
SOLVENT MeOD
NS 16
DS 4
SWH 4795.396 Hz
FIDRES 2.341502 Hz
AQ 0.21335381 sec
RG 197.74
DW 104.267 usec
DE 6.50 usec
TE 298.0 K
CNST13 8.00000000
d0 0.00000300 sec
D1 1.50000000 sec
d6 0.06250000 sec
D16 0.00020000 sec
in0 0 sec
ST1CNT 0
d0orig 0.00000300 sec
phlloop 0
tlloop 0
SFO1 400.1320007 MHz
NUC1 1H
P1 10.00 usec
p2 20.00 usec
PLW1 14.58800030 W
SFO2 161.9755930 MHz
NUC2 31P
P3 8.00 usec
PLW2 53.95100021 W
GPNAM[1] SMSQ10.100
GPNAM[2] SMSQ10.100
GPNAM[3] SMSQ10.100
GPZ1 70.00 %
GPZ2 30.00 %
GPZ3 80.50 %
P16 1000.00 usec
F1 - Acquisition parameters
TD 102
SFO1 161.9756 MHz
FIDRES 218.837540 Hz
SW 137.807 ppm
FnMODE QF
F2 - Processing parameters
SI 1024
SF 400.1300446 MHz
WDW SINE
SSB 0
LB 0 Hz
GB 0
PC 1.40
F1 - Processing parameters
SI 1024
MC2 QF
SF 161.9755849 MHz
WDW SINE
SSB 0
LB 0 Hz
GB 0
ppm
6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 ppm
-10 -5 0 5 10 15 20 25 ppm

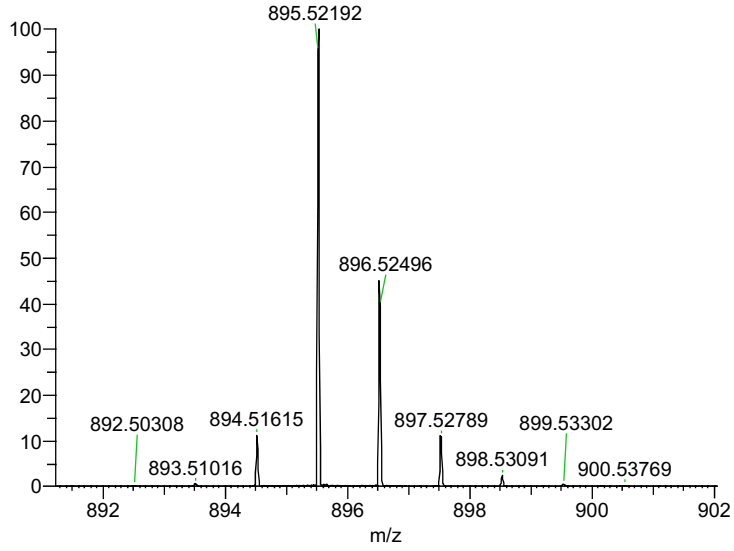
D_6 (–)-1D-Dipalmitoyl-phosphatidylinositol-5-phosphate trisodium salt (–)-326 – ^{2}H NMR spectrum





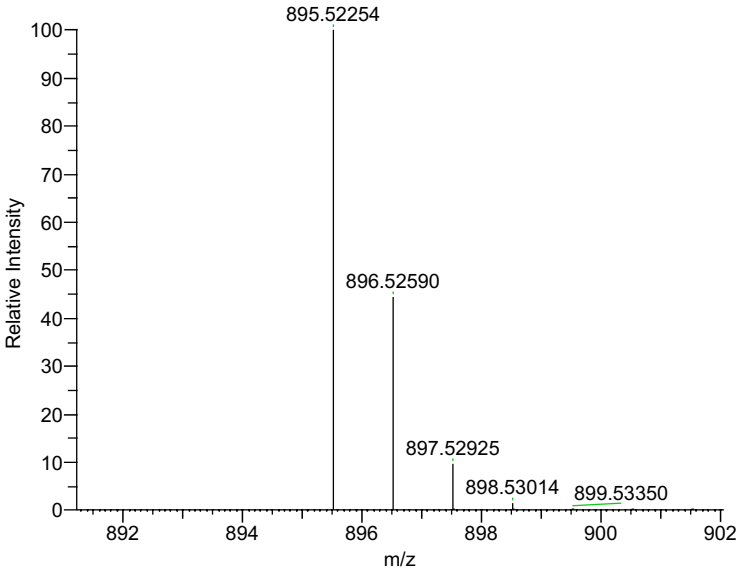
D_6 (–)-1D-Dipalmitoyl-phosphatidylinositol-5-phosphate trisodium salt (–)-326 – Mass spectrum

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NL: 6.92E6
ESI59993 #11-24 RT: 0.13-0.27 AV: 7 NL: 6.92E6
T: FTMS {1,2} - p ESI Full ms [80.00-1600.00]

Measured Spectrum



NL: 6.14E5
C41H73[2]H6O16P2: $C_{41}H_{73}{}^{2}H_{6}O_{16}P_{2}$
Chrg -1 R: 1000000 Res. Pwr. @FWHM

Theoretical Spectrum

m/z	Formula	RDB	Delta ppm	Theo. Mass
895.52191	$C_{41}H_{73}{}^{2}H_{6}O_{16}P_{2}$	3.5	-0.7	895.52254

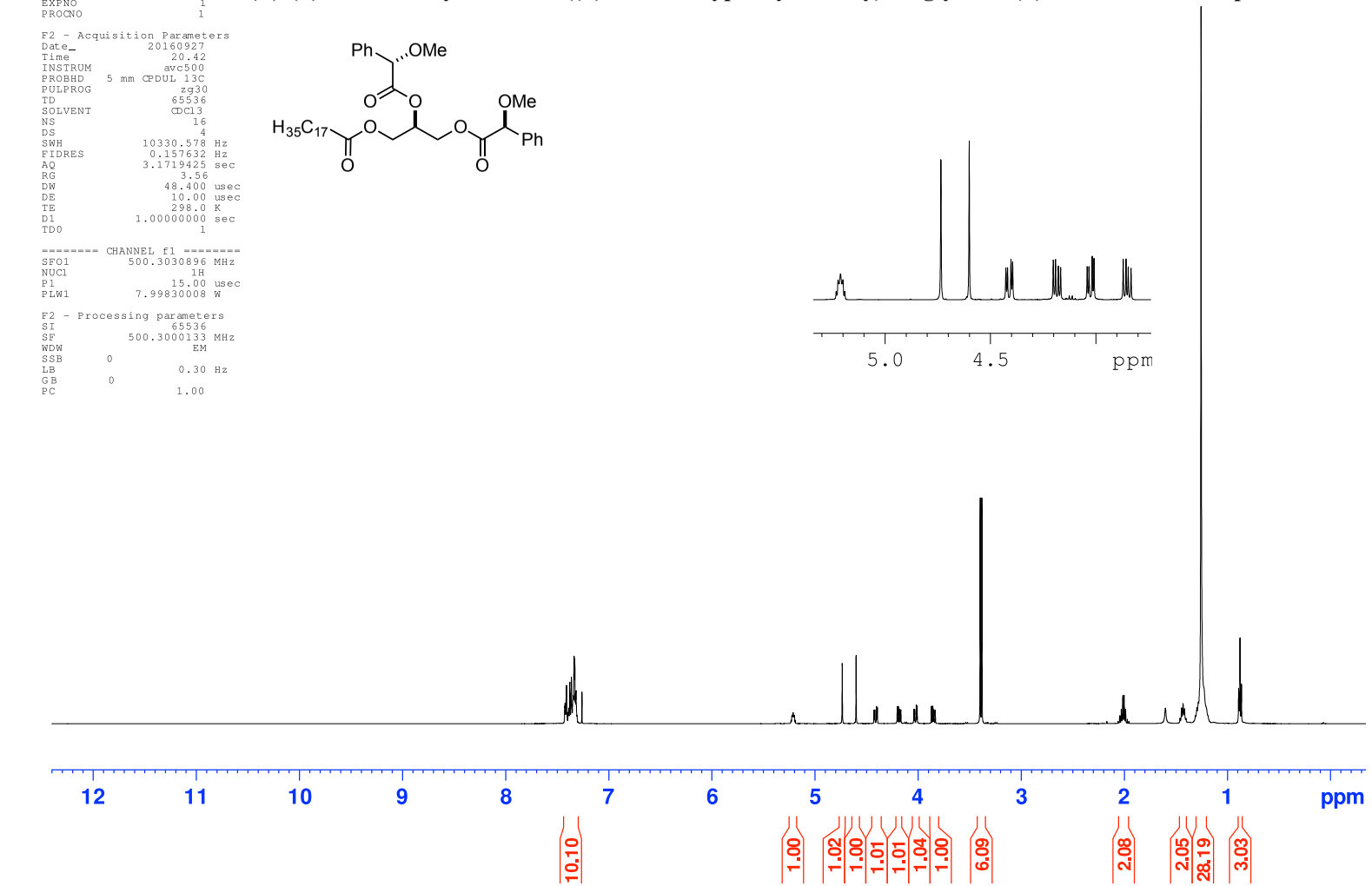
(*R*)-(+)-1-*O*-Stearoyl-2,3-di-*O*-((*S*)-α-methoxyphenylacetoxy)-*sn*-glycerol (+)-332a – ^{1}H NMR spectrum

```
Current Data Parameters
NAME      ajf76(S)-data-Hnmr
EXPNO                    1
PROCNO                   1

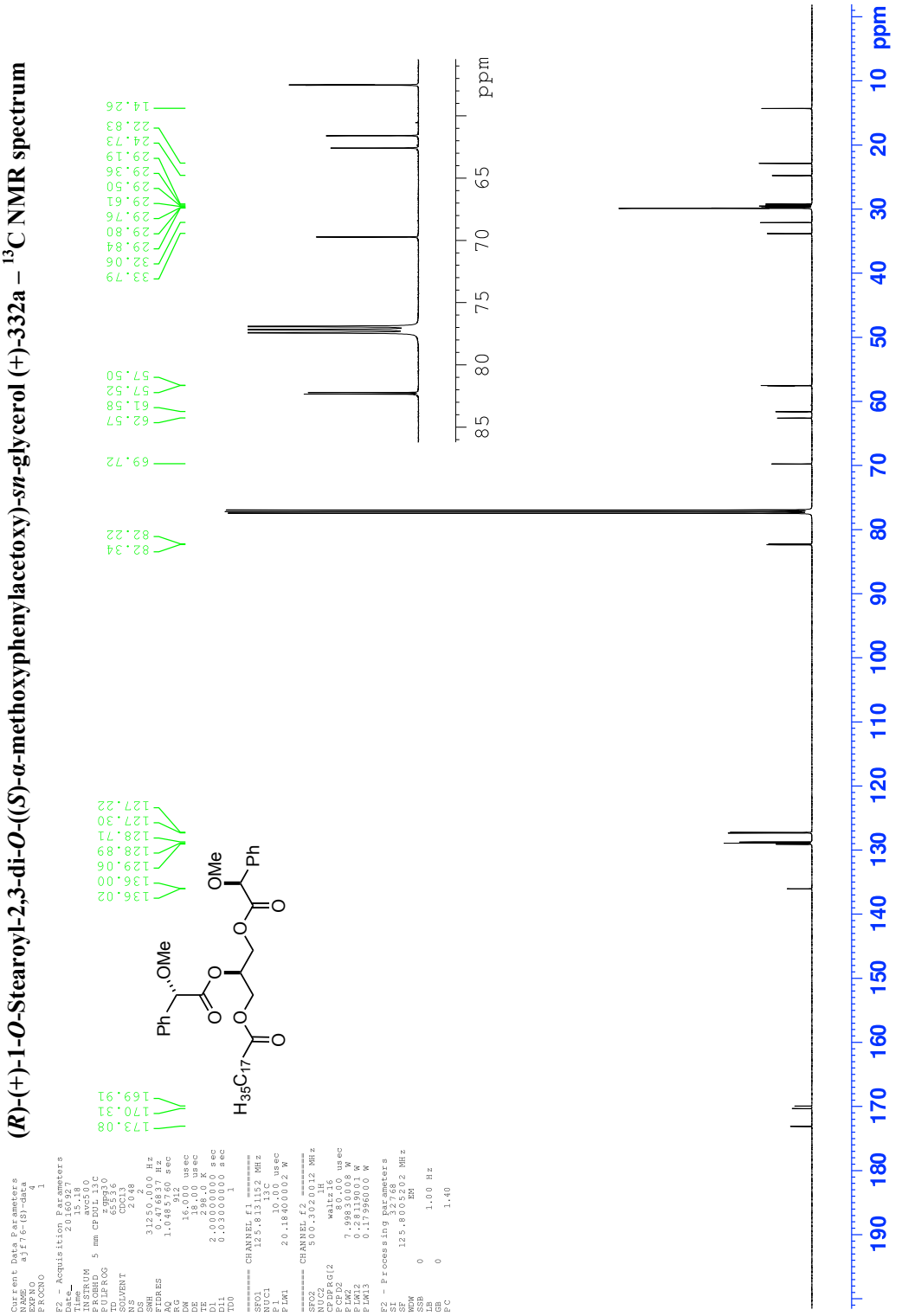
F2 - Acquisition Parameters
Date_           20160927
Time              20.42
INSTRUM          avc500
PROBHD   5 mm CPDUL 13C
PULPROG            zg30
TD                65536
SOLVENT           CDCl3
NS                   16
DS                    4
SWH           10330.578 Hz
FIDRES         0.157632 Hz
AQ            3.1719425 sec
RG                 3.56
DW                48.400 usec
DE                10.00 usec
TE                298.0 K
D1           1.00000000 sec
TD0                  1

======== CHANNEL f1 ========
SFO1         500.3030896 MHz
NUC1               1H
P1                15.00 usec
PLW1          7.99830008 W

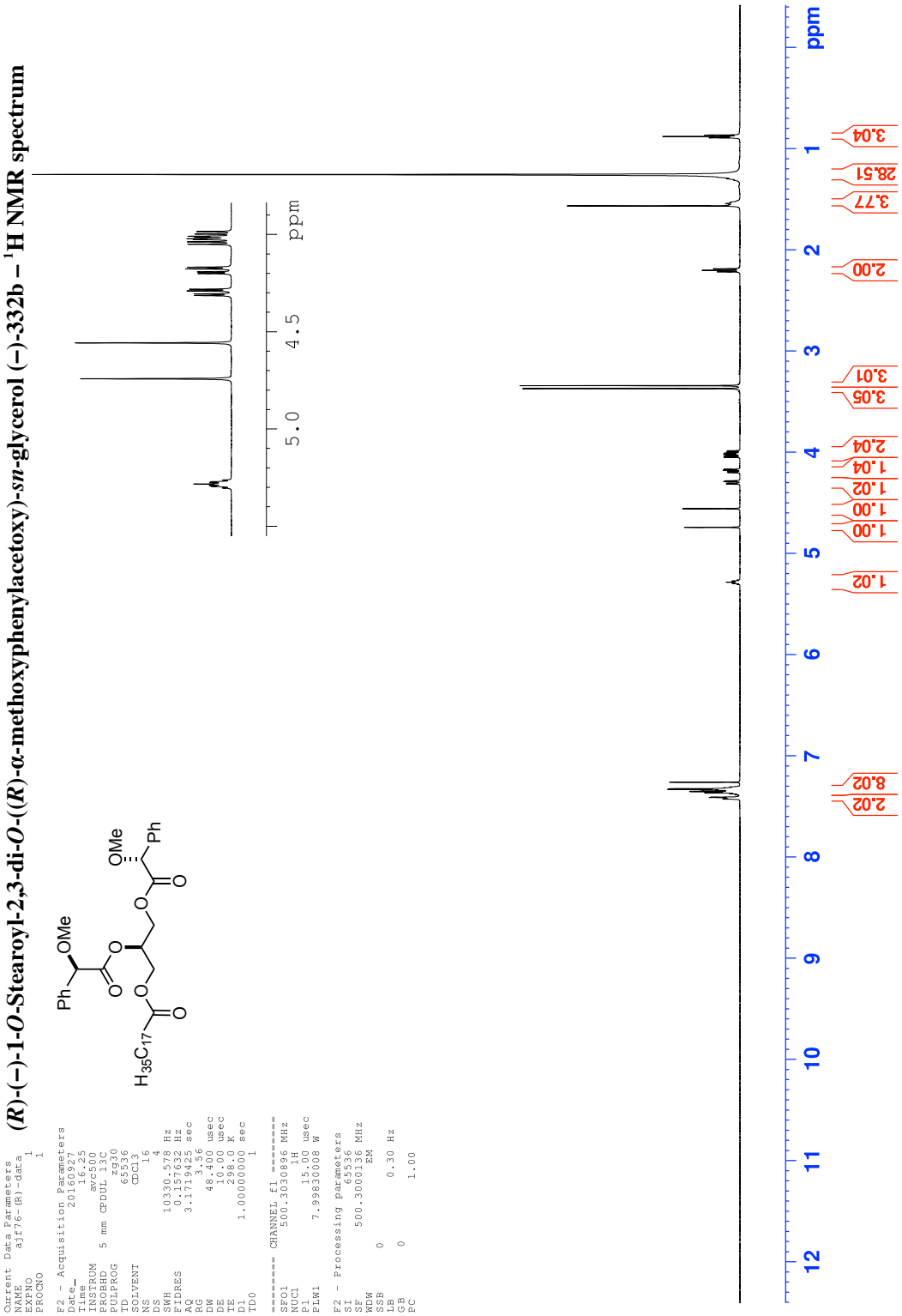
F2 - Processing parameters
SI                65536
SF           500.3000133 MHz
WDW                EM
SSB       0
LB              0.30 Hz
GB        0
PC              1.00
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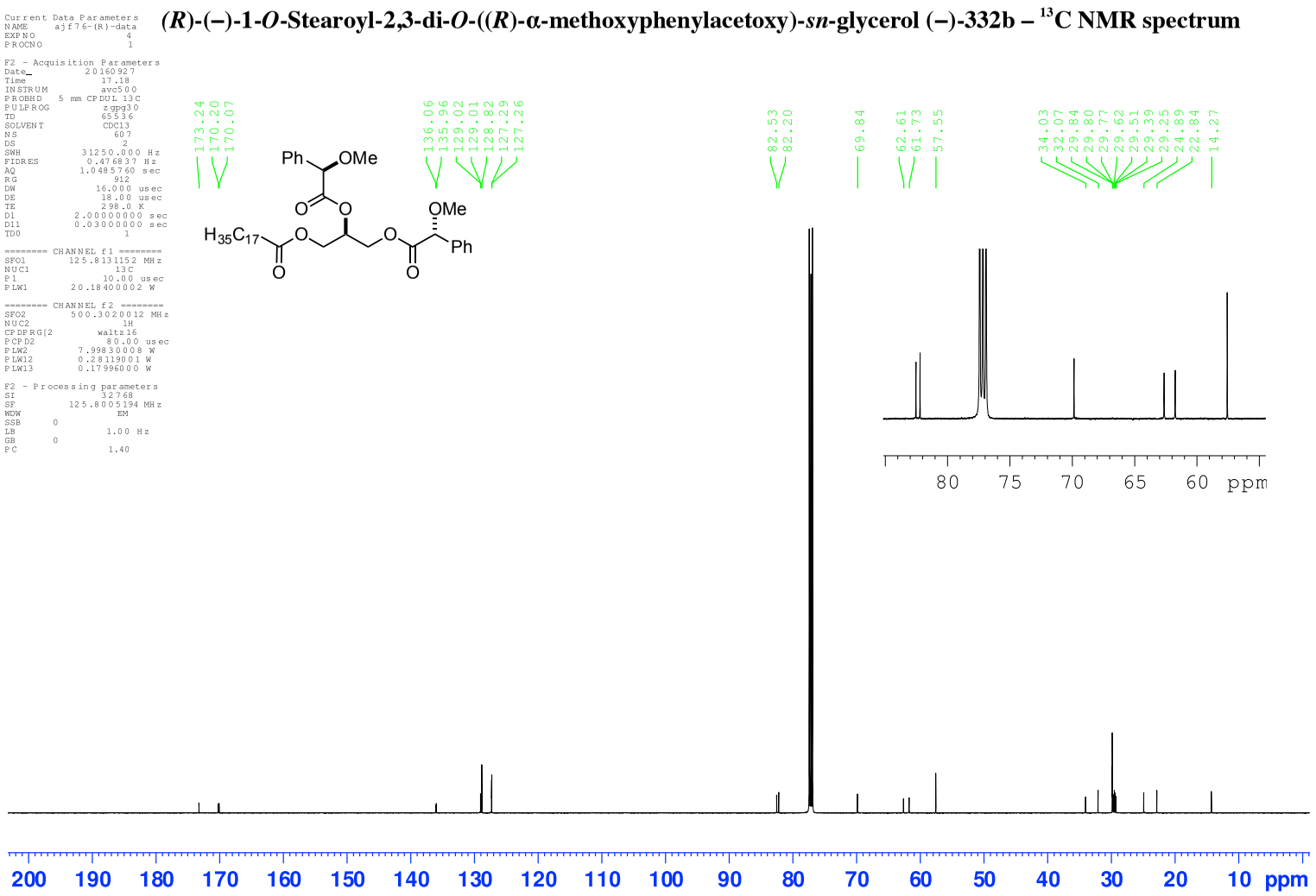
(*R*)-(+)-1-*O*-Stearoyl-2,3-di-*O*-((*S*)-α-methoxyphenylacetoxy)-*sn*-glycerol (+)-332a – ^{13}C NMR spectrum



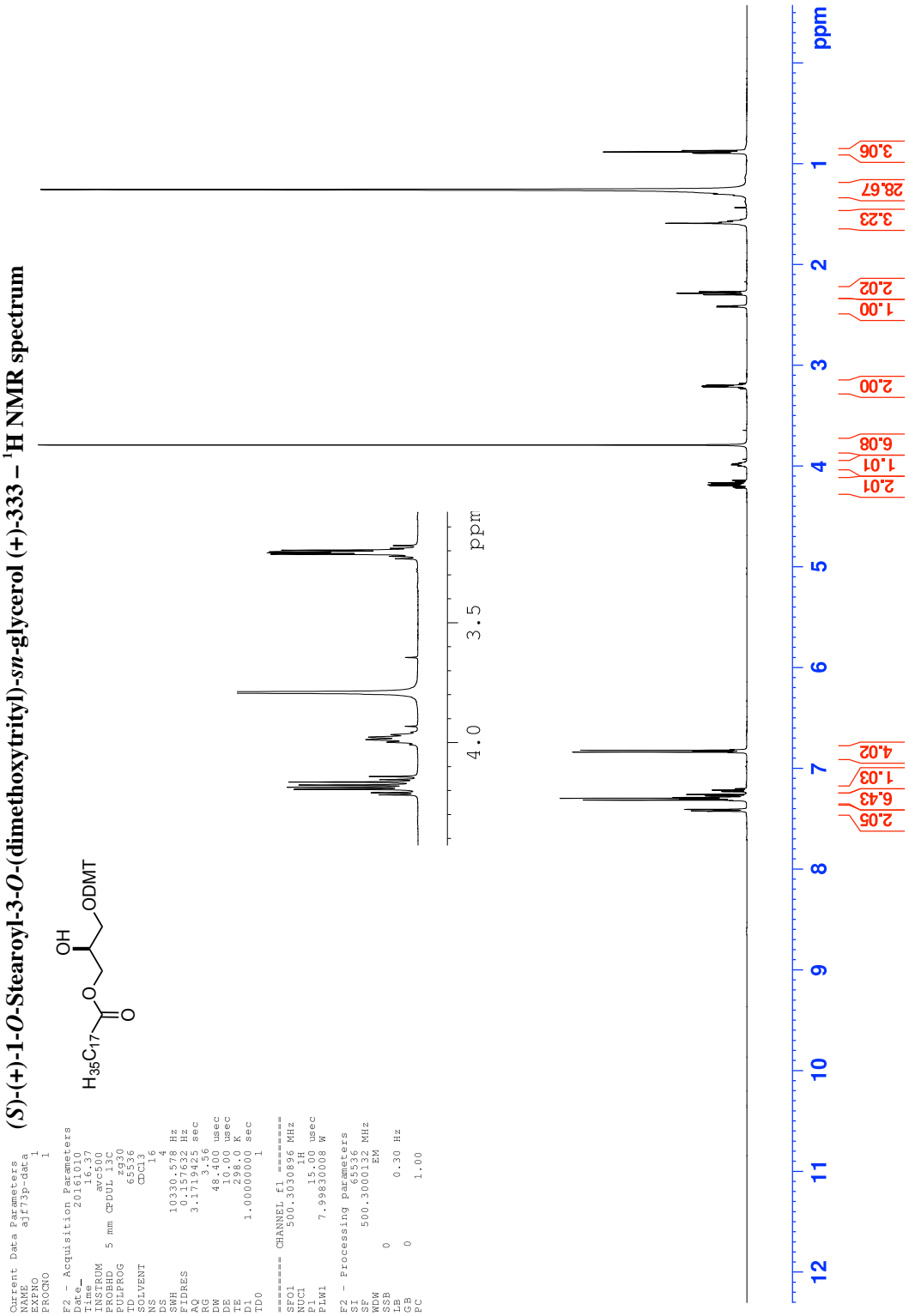
(*R*)-(−)-1-*O*-Stearoyl-2,3-di-*O*-((*R*)-α-methoxyphenylacetoxy)-*sn*-glycerol (−)-332b – ^{1}H NMR spectrum



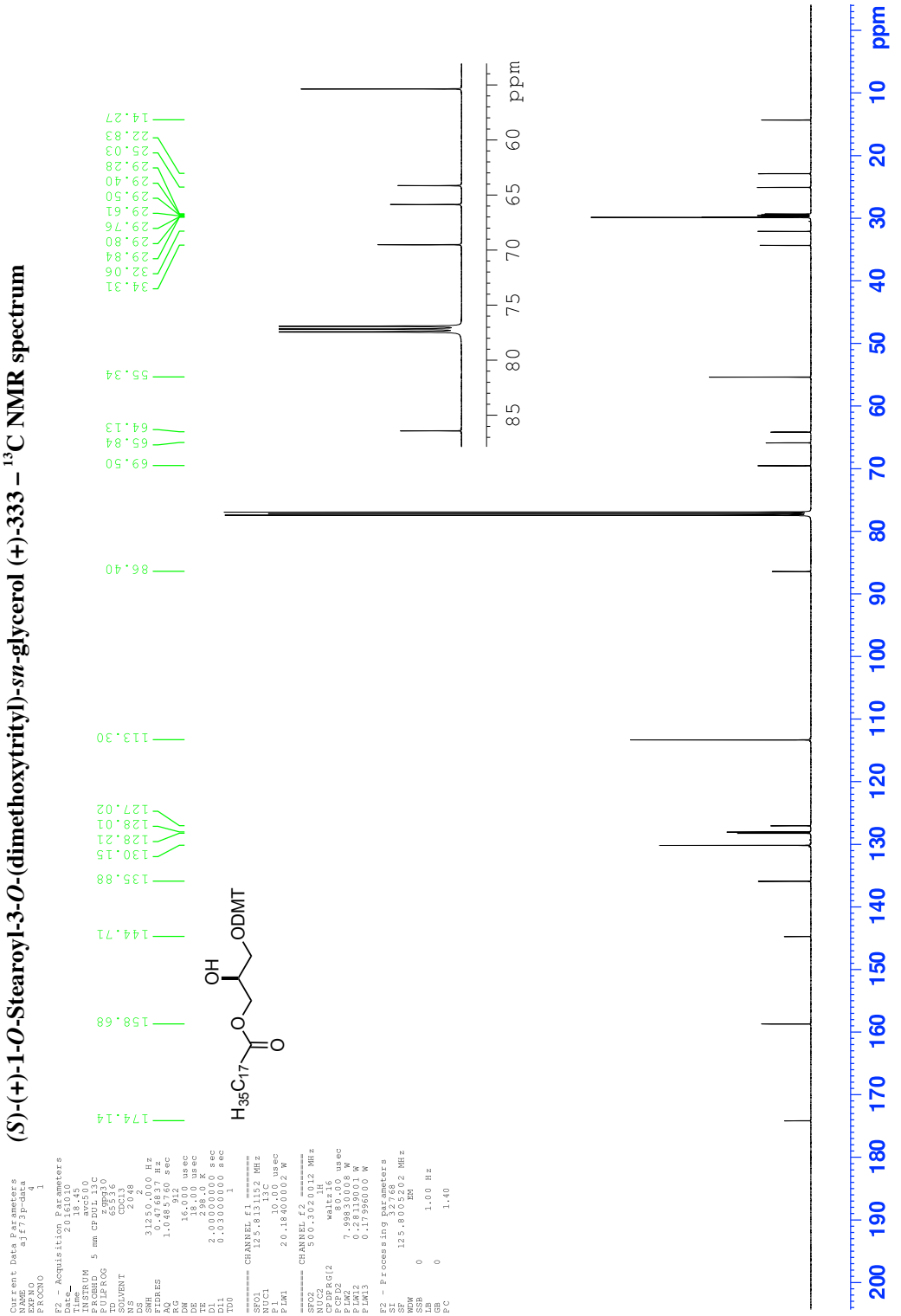
(*R*)-(–)-1-*O*-Stearoyl-2,3-di-*O*-((*R*)-α-methoxyphenylacetoxy)-*sn*-glycerol (–)-332b – ^{13}C NMR spectrum



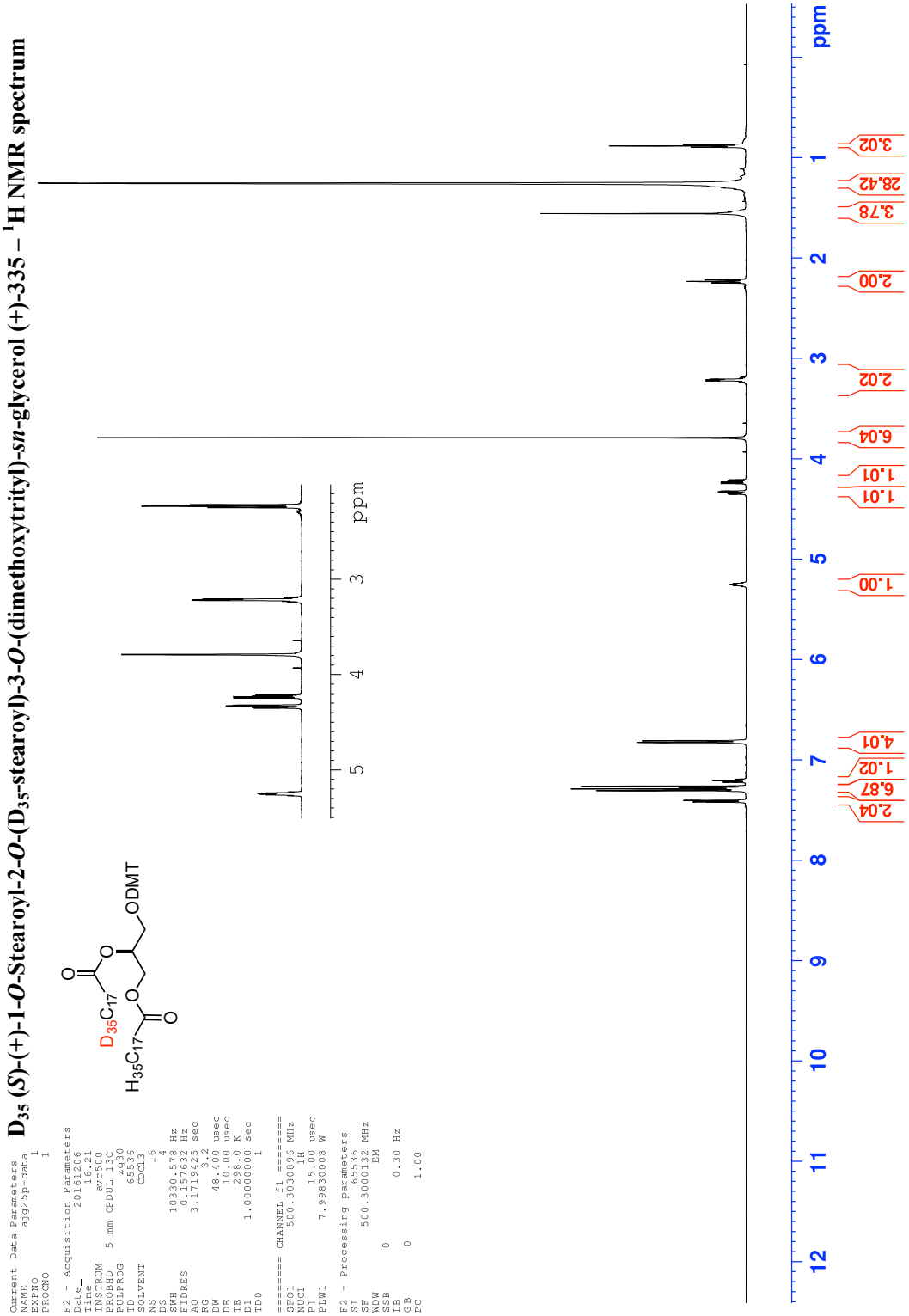
(*S*)-(+)-1-*O*-Stearoyl-3-*O*-(dimethoxytrityl)-*sn*-glycerol (+)-333 – ^{1}H NMR spectrum



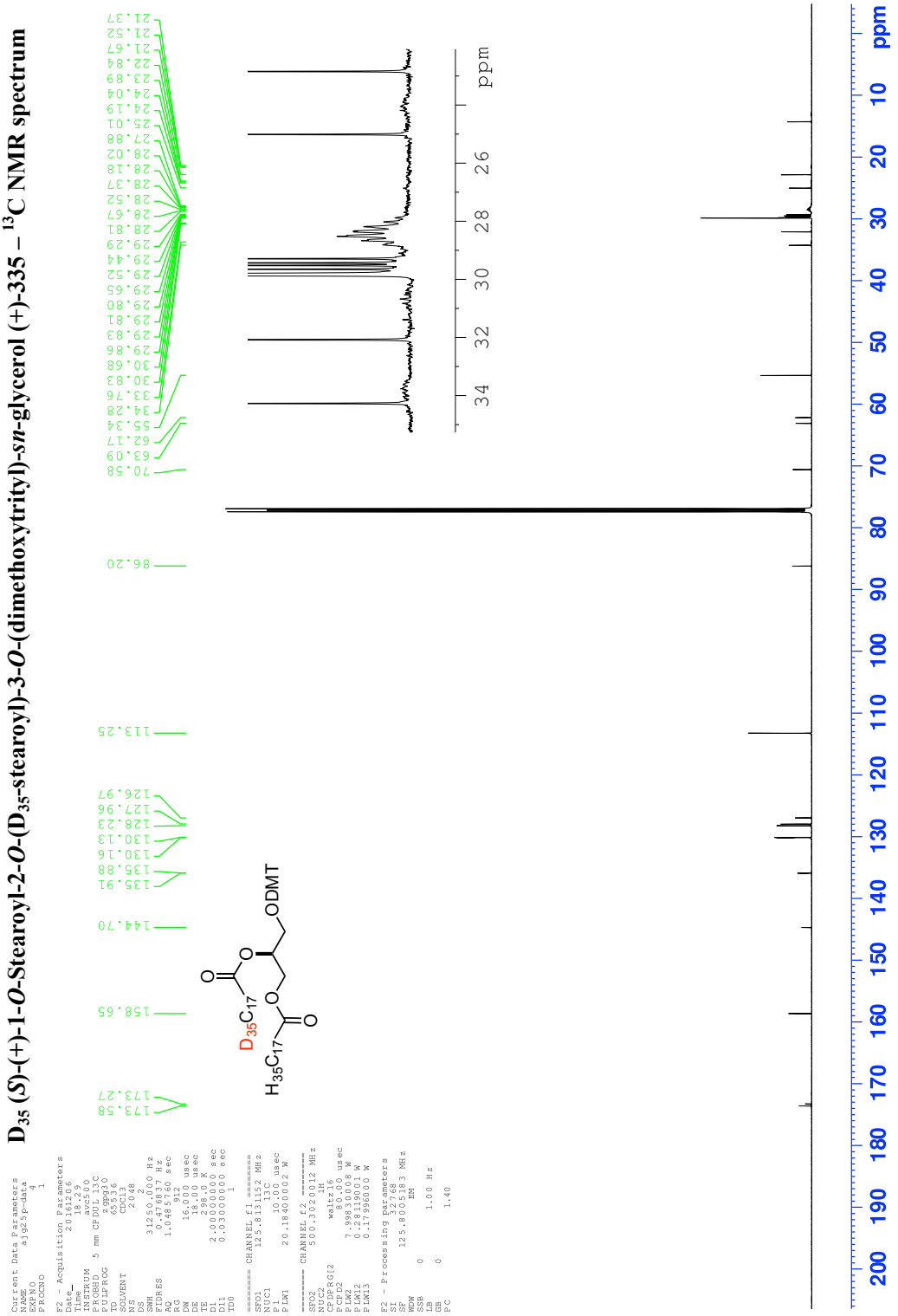
(*S*)-(+)-1-*O*-Stearoyl-3-*O*-(dimethoxytrityl)-*sn*-glycerol (+)-333 – ^{13}C NMR spectrum



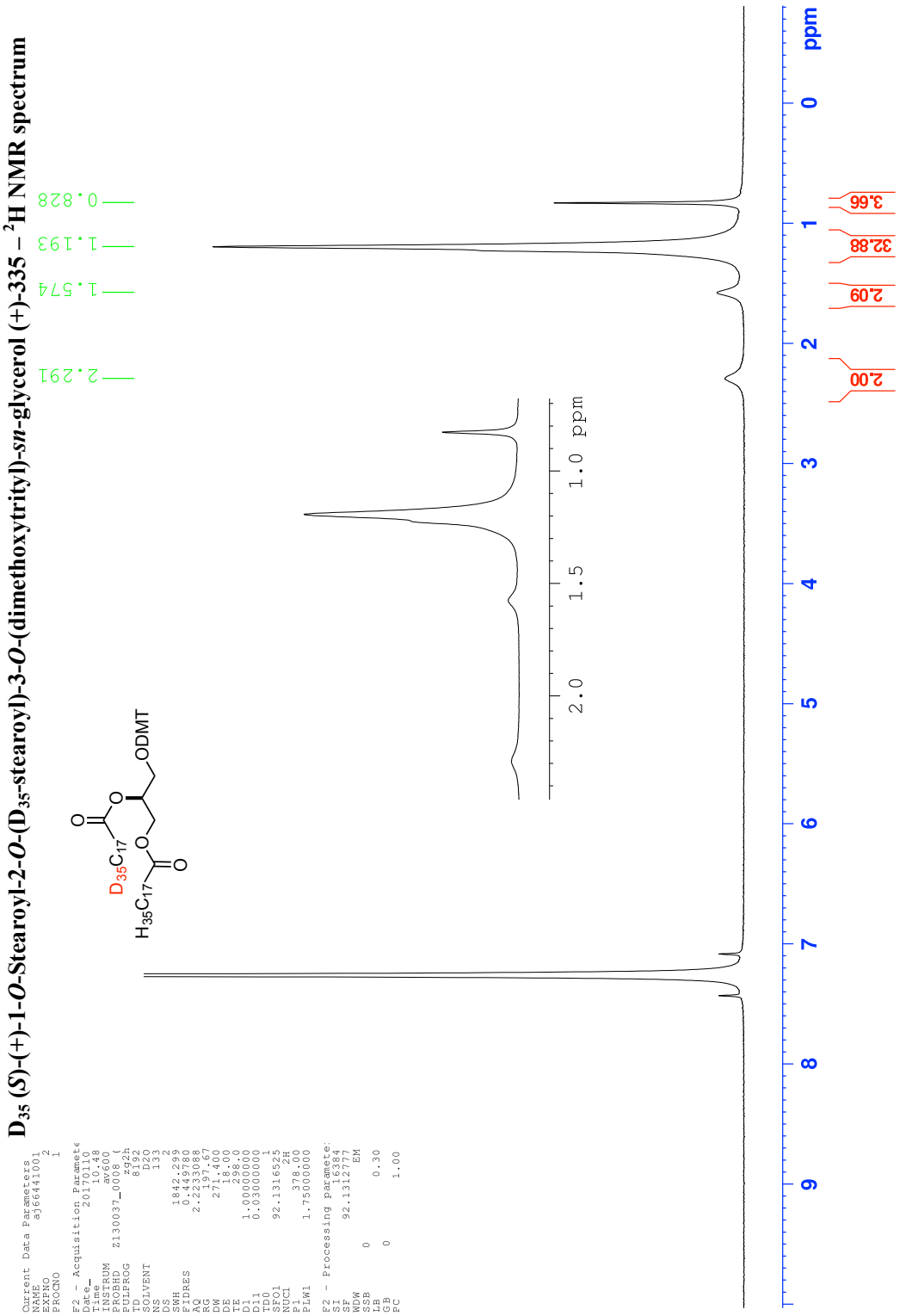
D_{35} (*S*)-(+)-1-*O*-Stearoyl-2-*O*-(D_{35}-stearoyl)-3-*O*-(dimethoxytrityl)-*sn*-glycerol (+)-335 – ^{1}H NMR spectrum



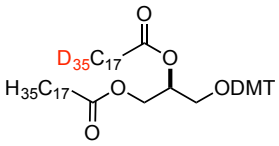
D_{35} (*S*)-(+)-1-*O*-Stearoyl-2-*O*-(D_{35}-stearoyl)-3-*O*-(dimethoxytrityl)-*sn*-glycerol (+)-335 – ^{13}C NMR spectrum



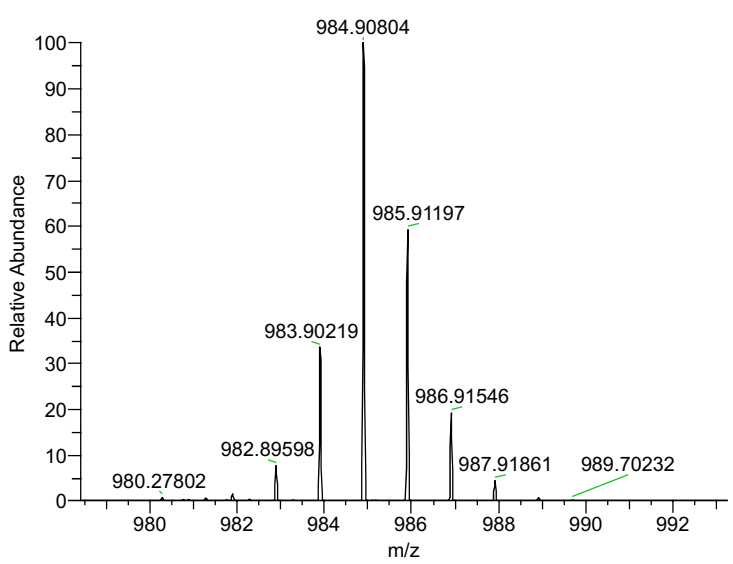
D_{35} (*S*)-(+)-1-*O*-Stearoyl-2-*O*-(D_{35}-stearoyl)-3-*O*-(dimethoxytrityl)-*sn*-glycerol (+)-335 – ^{2}H NMR spectrum



D_{35} (*S*)-(+)-1-*O*-Stearoyl-2-*O*-(D_{35}-stearoyl)-3-*O*-(dimethoxytrityl)-*sn*-glycerol (+)-335 – Mass spectrum

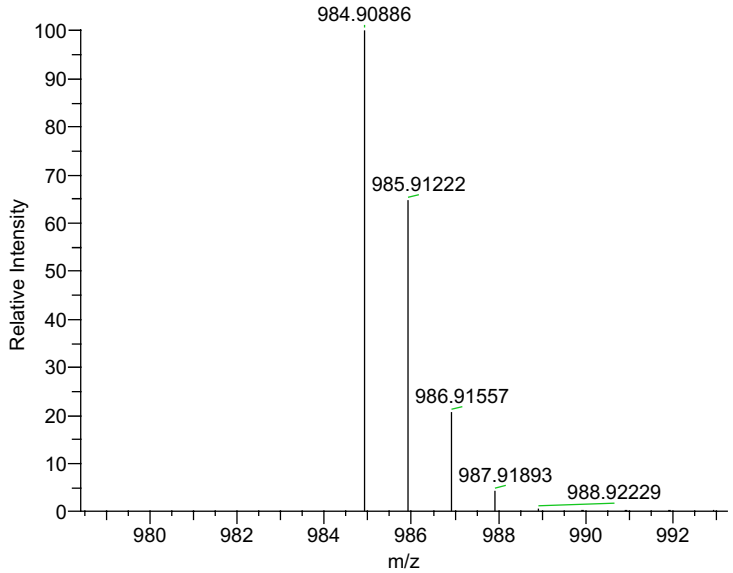


S:\data\Nov 16\ESI59933.raw 23/11/2016 10:46 am



NL: 4.07E6
ESI59933 #15-25 RT: 0.17-0.28 AV: 6 NL: 6.53E+007
T: FTMS {1,1} + p ESI Full lock ms [80.00-1600.00]

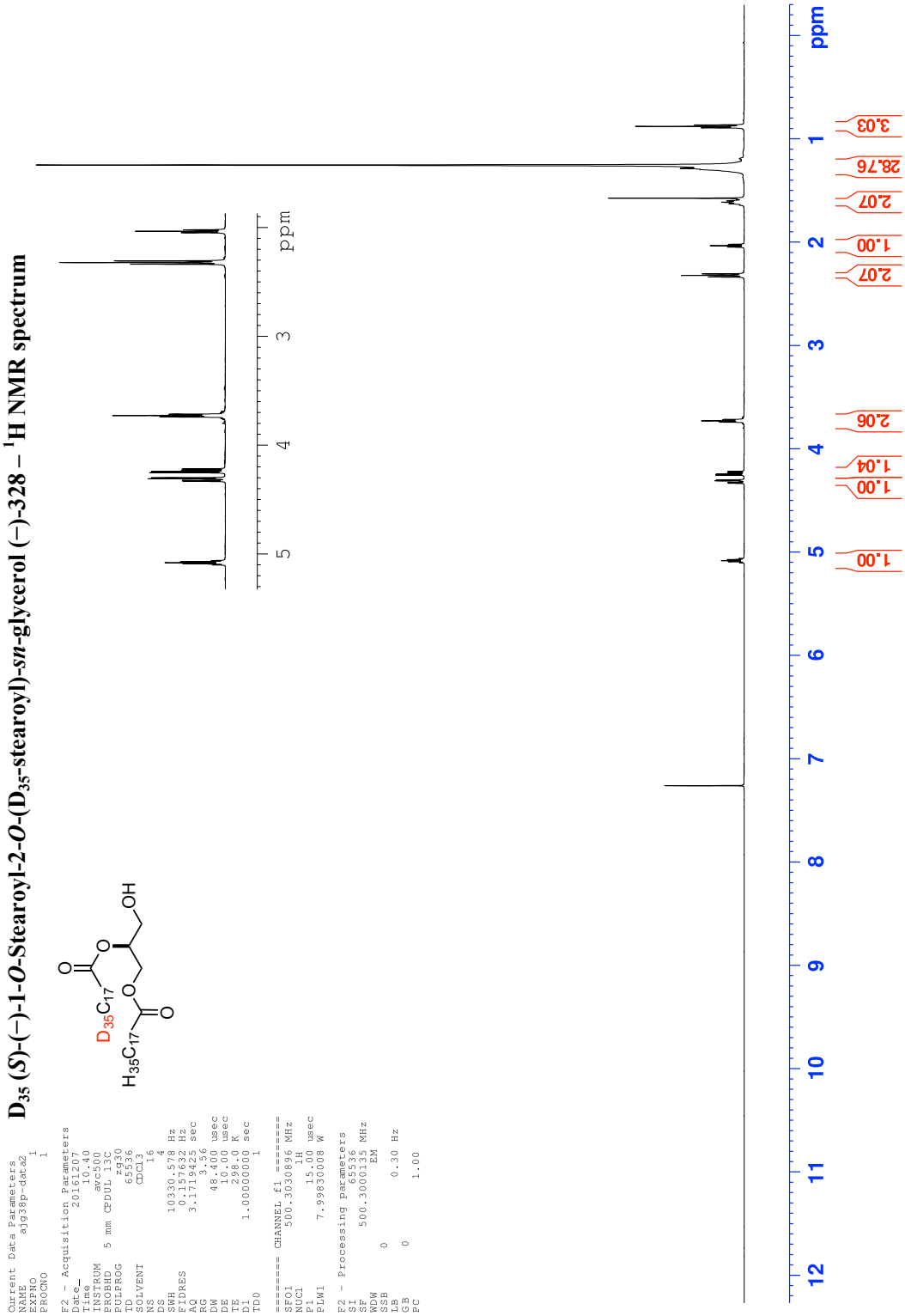
Measured Spectrum



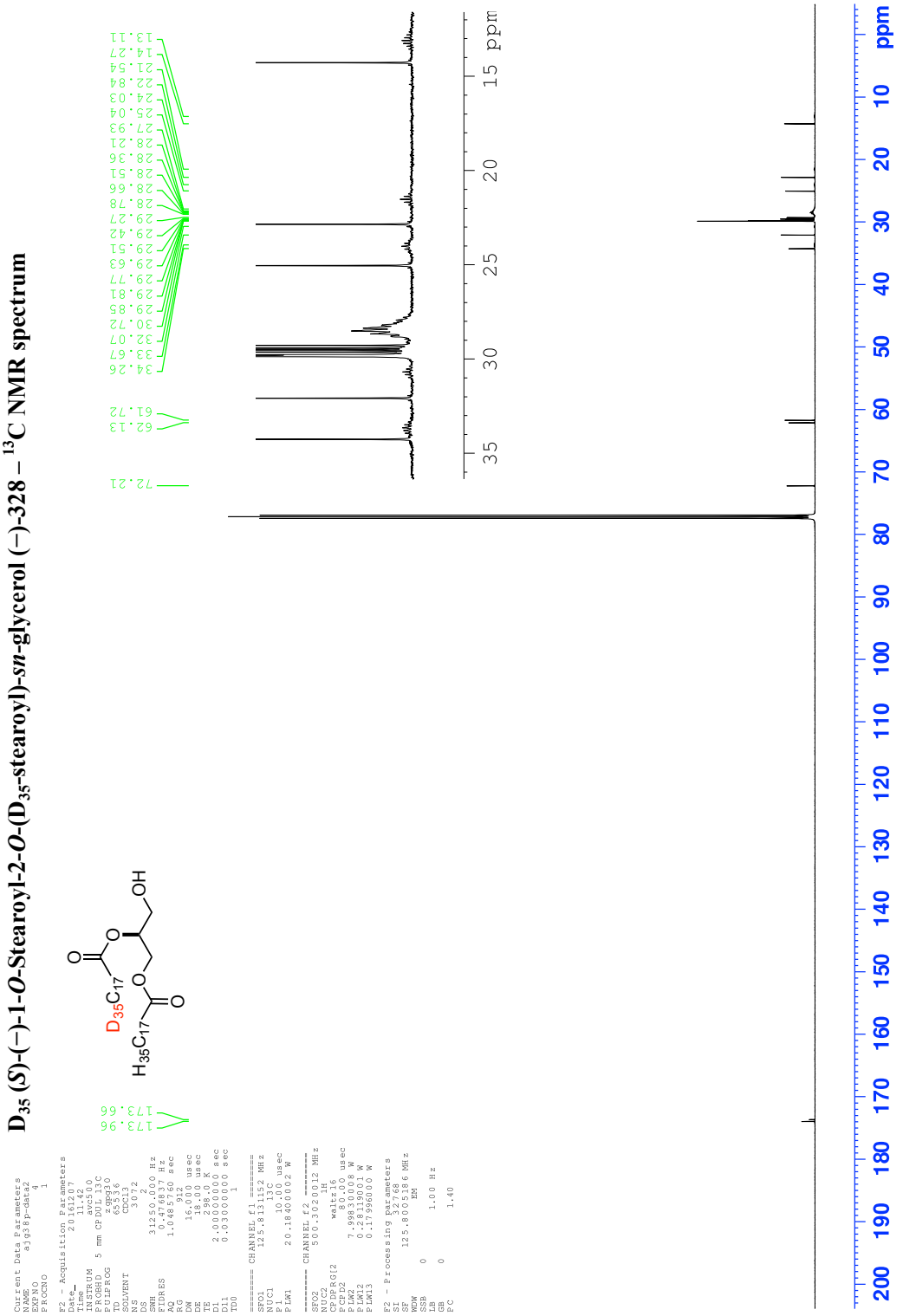
NL: 5.12E5
C60H59[2]H35O7Na1: C_{60} H_{59} $^{2}H_{35}$ O_{7} Na
Chrg 1 R: 1000000 Res. Pwr. @FWHM

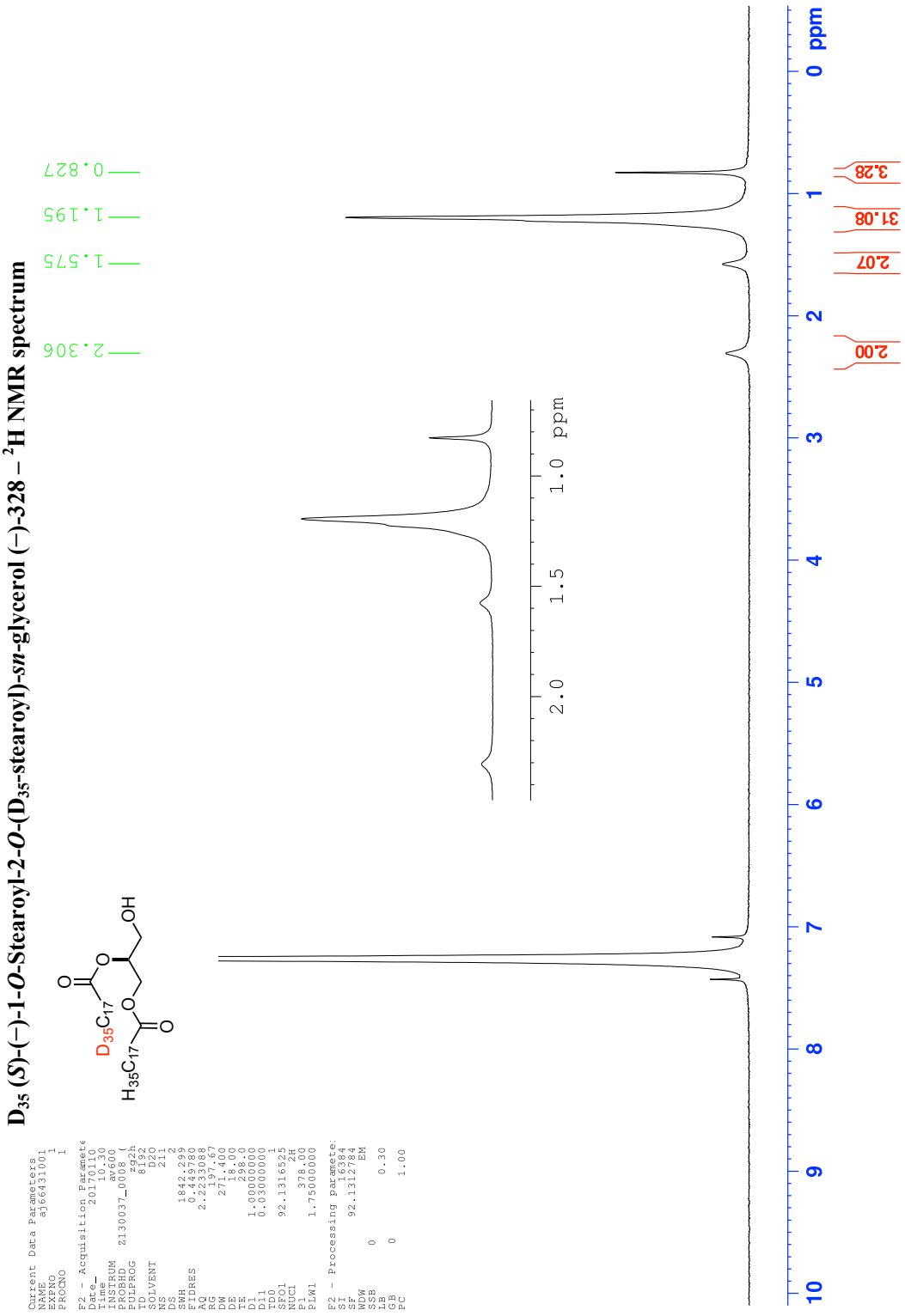
Theoretical Spectrum

m/z	Formula	RDB	Delta ppm	Theo. Mass
984.90802	$C_{60}H_{59}{}^{2}H_{35}O_{7}{}^{23}Na$	13.5	-0.86	984.90886

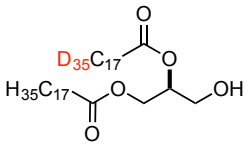

Current Data Parameters
NAME ajg38p-data2
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20161207
Time 10.40
INSTRUM avc500
PROBHD 5 mm CPDUL 13C
PULPROG zg30
TD 65536
SOLVENT CDCl3
NS 16
DS 4
SWH 10330.578 Hz
FIDRES 0.157672 Hz
AQ 3.1719425 sec
RG 3.56
DW 48.400 usec
DE 10.00 usec
TE 298.0 K
D1 1.00000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 500.3030896 MHz
NUC1 1H
P1 15.00 usec
PLW1 7.99830008 W
F2 - Processing parameters
SI 65536
SF 500.3000135 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
D35 (S)-(−)-1-O-Stearoyl-2-O-(D35-stearoyl)-sn-glycerol (−)-328 – 1H NMR spectrum
D35C17
H35C17
OH
O
O
O
O
O
ppm
1.00
1.04
2.06
1.00
2.07
1.00
2.07
28.76
3.03

D_{35} (*S*)-(−)-1-*O*-Stearoyl-2-*O*-(D_{35}-stearoyl)-*sn*-glycerol (−)-328 – ^{13}C NMR spectrum

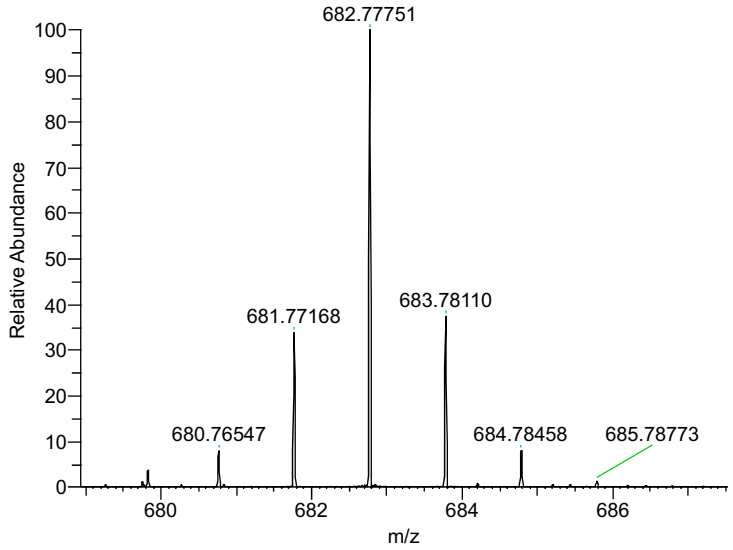



D35 (S)-(−)-1-O-Stearoyl-2-O-(D35-stearoyl)-sn-glycerol (−)-328 – 2H NMR spectrum
Current Data Parameters
NAME a166431001
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20170110
Time 10.30
INSTRUM av600
PROBHD Z130037_0008 (
PULPROG zg2h
TD 8192
SOLVENT CHCl3
NS 211
DS 2
SWH 1842.299
FIDRES 0.449780
AQ 2.2233088
RG 197.67
DW 271.400
DE 18.00
TE 298.0
D1 1.00000000
D11 0.03000000
TD0 1
SFO1 92.1311525
NUC1 2H
P1 378.00
PLW1 1.75000000
F2 - Processing parameters
SI 16384
SF 92.1312784
WDW EM
SSB 0
LB 0.30
GB 0
PC 1.00
D35C17
H35C17
OH
2.306
1.575
1.195
0.827
2.0
1.5
1.0 ppm
2.00
2.07
31.08
3.28
10
9
8
7
6
5
4
3
2
1
0 ppm

D_{35} (*S*)-(−)-1-*O*-Stearoyl-2-*O*-(D_{35}-stearoyl)-*sn*-glycerol (−)-328 – Mass spectrum

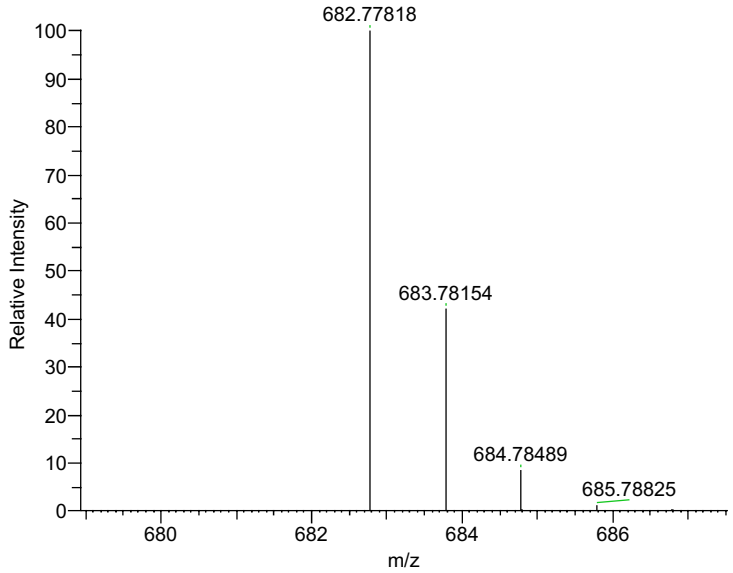


S:\data\Dec 16\ESI60072.raw 01/12/2016 5:40 pm



NL: 1.06E7
ESI60072 #15-25 RT: 0.17-0.28 AV: 6 NL: 1.06E+007
T: FTMS {1,1} + p ESI Full lock ms [80.00-1600.00]

Measured Spectrum



NL: 6.46E5
C39H41[2]H35O5Na1: C_{39} H_{41} $^{2}H_{35}$ O_5 Na
Chrg 1 R: 1000000 Res. Pwr. @FWHM

Theoretical Spectrum

m/z	Formula	RDB	Delta ppm	Theo. Mass
682.77753	$C_{39}H_{41}{}^{2}H_{35}O_5{}^{23}Na$	1.5	-0.96	682.77818

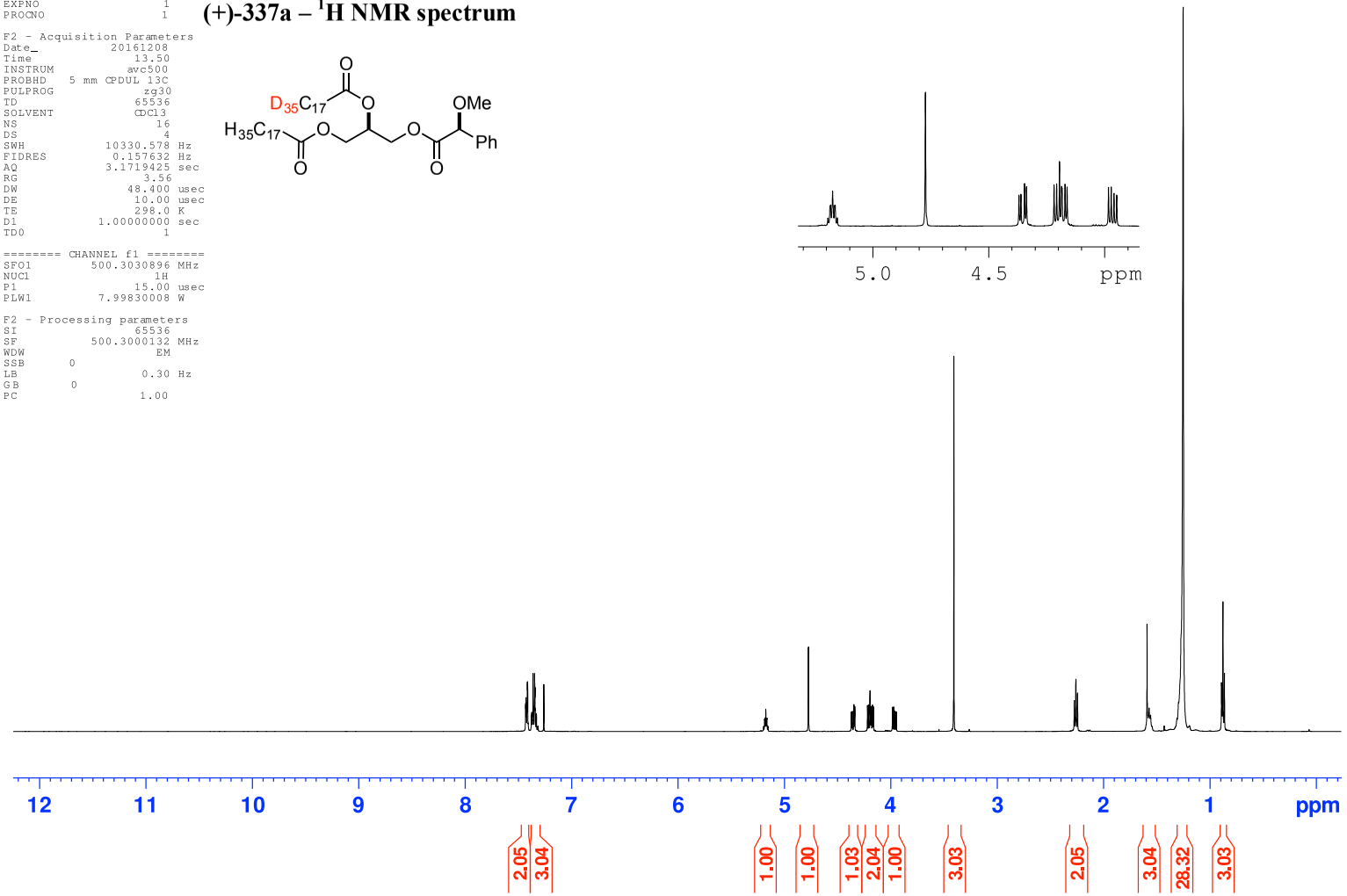
D_{35} (*R*)-(+)-1-*O*-Stearoyl-2-*O*-(D_{35}-stearoyl)-3-*O*-((*S*)-α-methoxyphenylacetoxy)-*sn*-glycerol (+)-337a – ^{1}H NMR spectrum

```
Current Data Parameters
NAME      ajg43p(S)-data
EXPNO                  1
PROCNO                 1

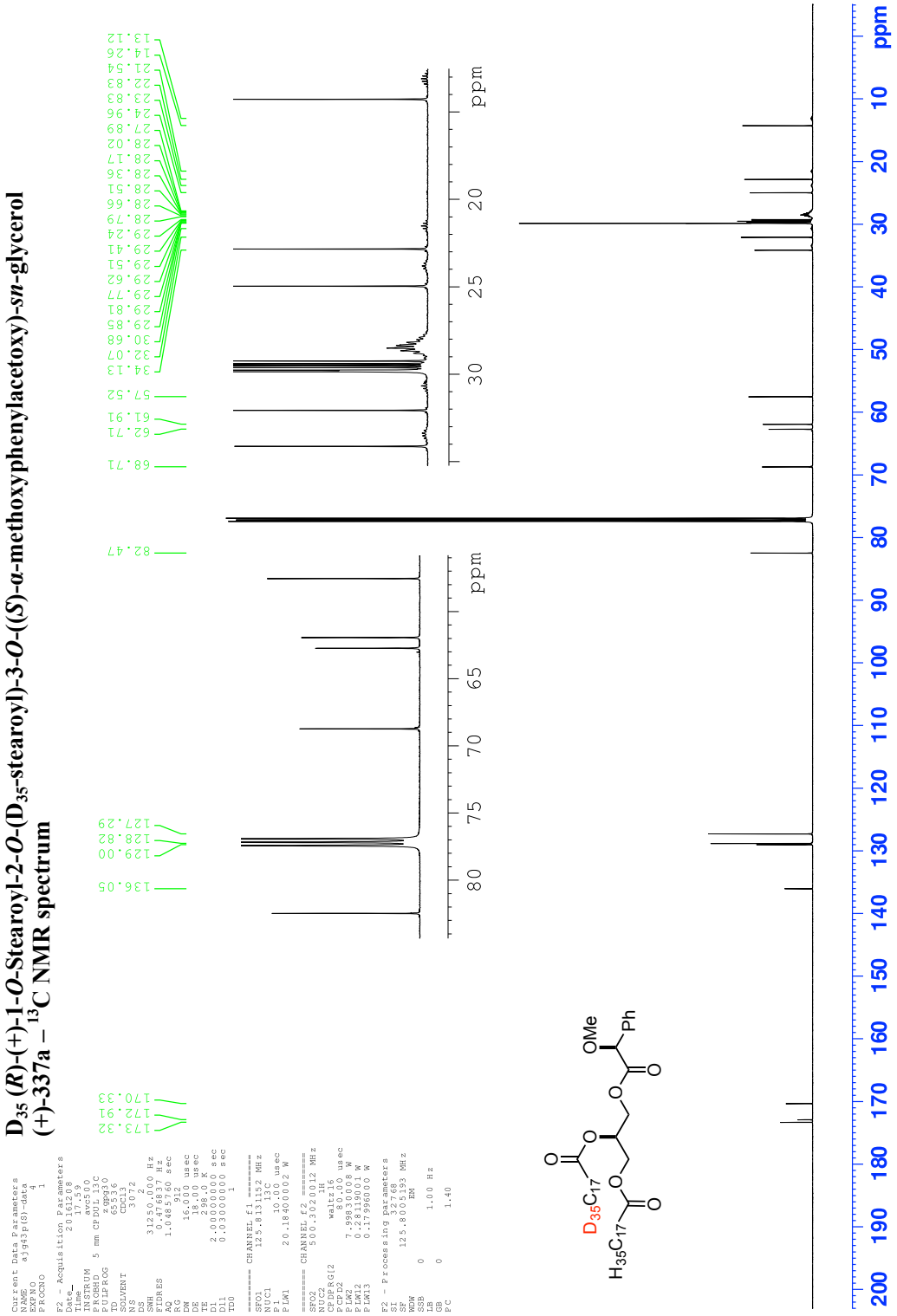
F2 - Acquisition Parameters
Date_           20161208
Time               13.50
INSTRUM           avc500
PROBHD   5 mm CPDUL 13C
PULPROG             zg30
TD                 65536
SOLVENT            CDCl3
NS                    16
DS                     4
SWH            10330.578 Hz
FIDRES             0.157632 Hz
AQ             3.1719425 sec
RG                  3.56
DW                 48.400 usec
DE                 10.00 usec
TE                 298.0 K
D1             1.00000000 sec
TD0                    1

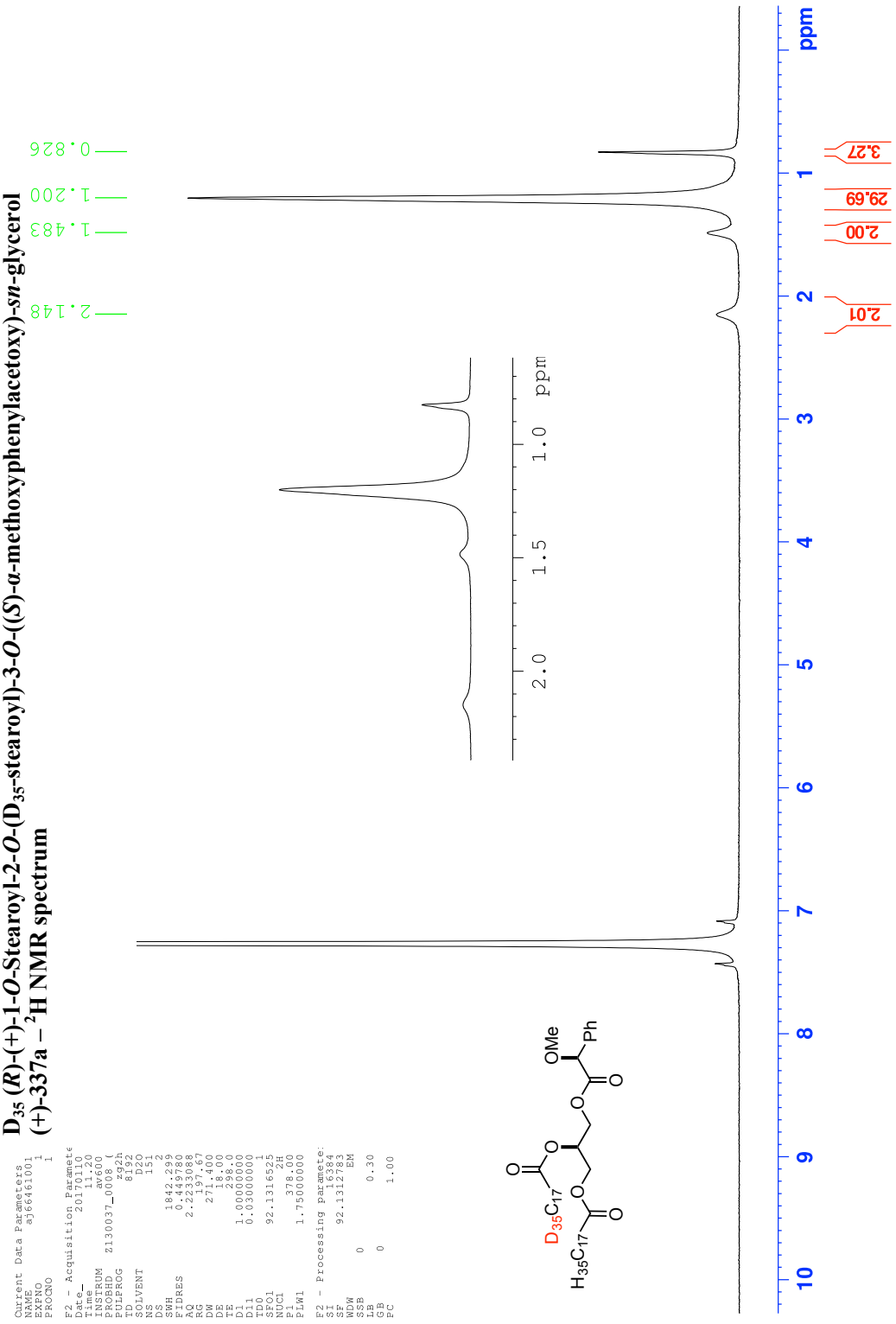
======== CHANNEL f1 ========
SFO1          500.3030896 MHz
NUC1                  1H
P1                 15.00 usec
PLW1           7.99830008 W

F2 - Processing parameters
SI                 65536
SF            500.3000132 MHz
WDW                   EM
SSB       0
LB                 0.30 Hz
GB        0
PC                 1.00
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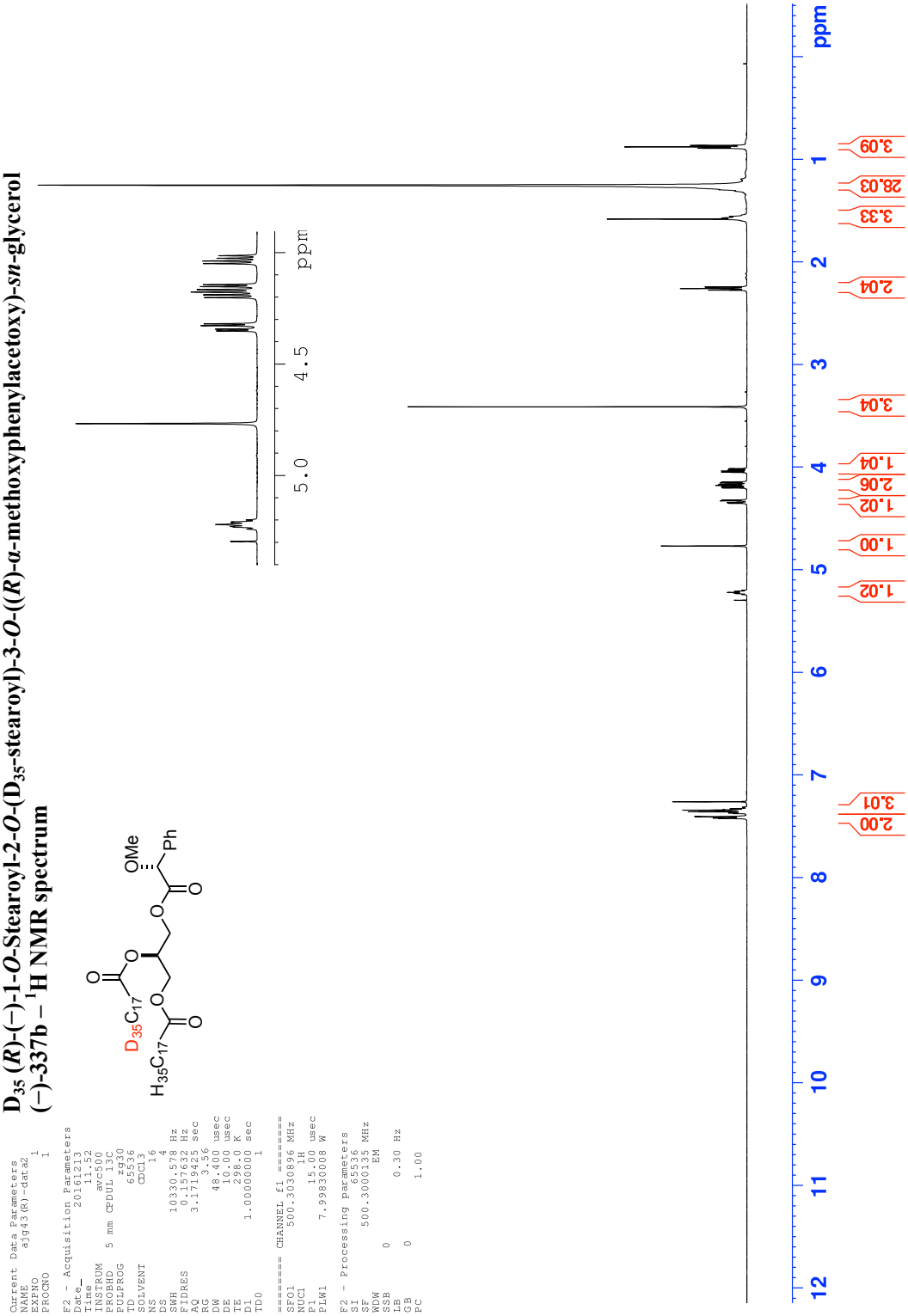


D_{35} (*R*)-(+)-1-*O*-Stearoyl-2-*O*-(D_{35}-stearoyl)-3-*O*-((*S*)-α-methoxyphenylacetoxy)-*sn*-glycerol
(+)-337a – ^{13}C NMR spectrum

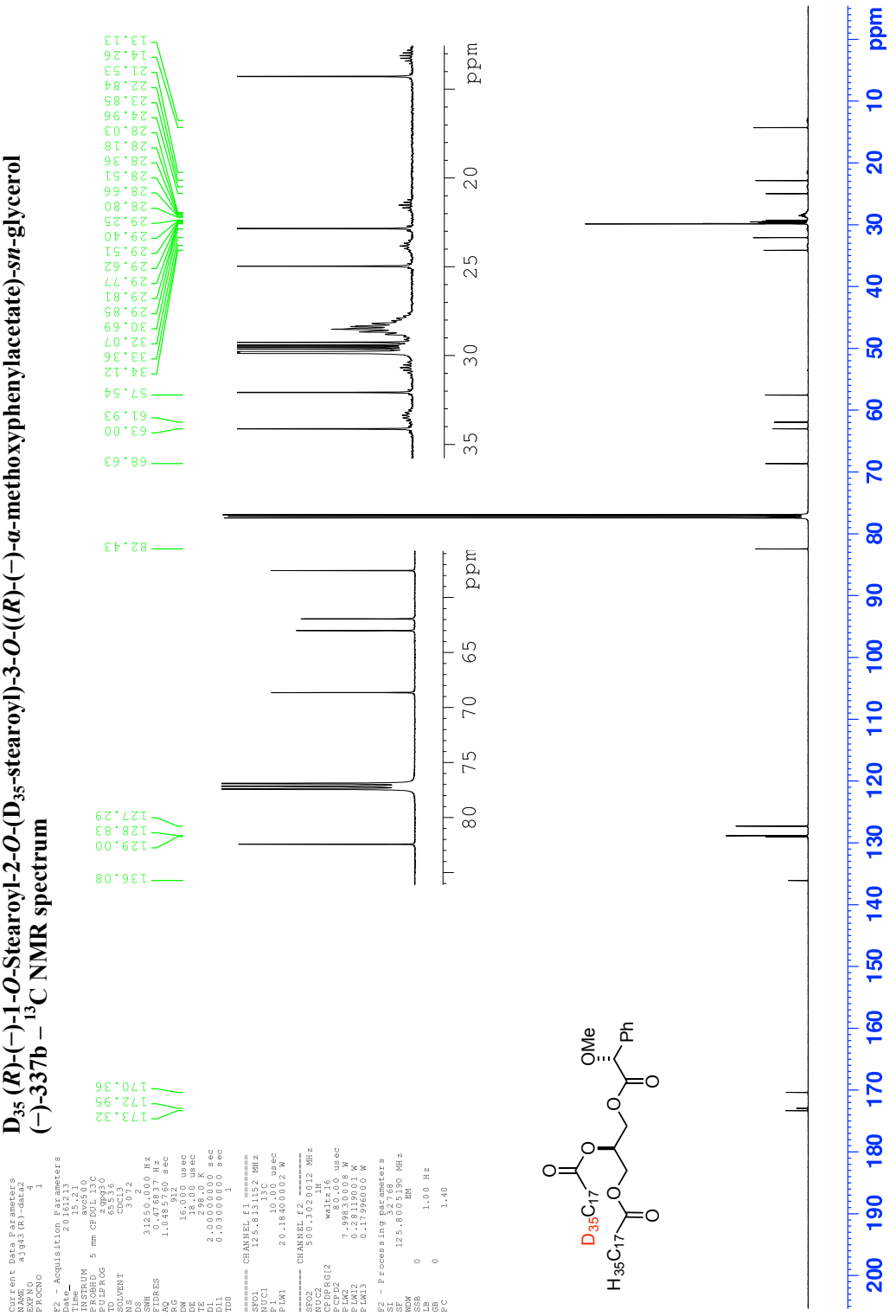



D35 (R)-(+)-1-O-Stearoyl-2-O-(D35-stearoyl)-3-O-((S)-α-methoxyphenylacetoxy)-sn-glycerol
(+)-337a – 2H NMR spectrum
Current Data Parameters
NAME 336461001
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20171110
Time 11.20
INSTRUM av600
PROBHD Z130037_0008 (
PULPROG zg2h
TD 8192
SOLVENT CHCl3
NS 151
DS 2
SWH 1842.299
FIDRES 0.449780
AQ 2.2233088
RG 197.67
DW 271.400
DE 18.00
TE 298.0
D1 1.00000000
D11 0.03000000
TD0 1
SFO1 92.1316525
NUC1 2H
P1 378.00
PLW1 1.75000000
F2 - Processing parameters
SI 16384
SF 92.1312783
WDW EM
SSB 0
LB 0.30
GB 0
PC 1.00
2.148
1.483
1.200
0.826
2.0
1.5
1.0
ppm
OMe
Ph
O
D35C17
H35C17
10
9
8
7
6
5
4
3
2
1
ppm
2.01
2.00
29.69
3.27

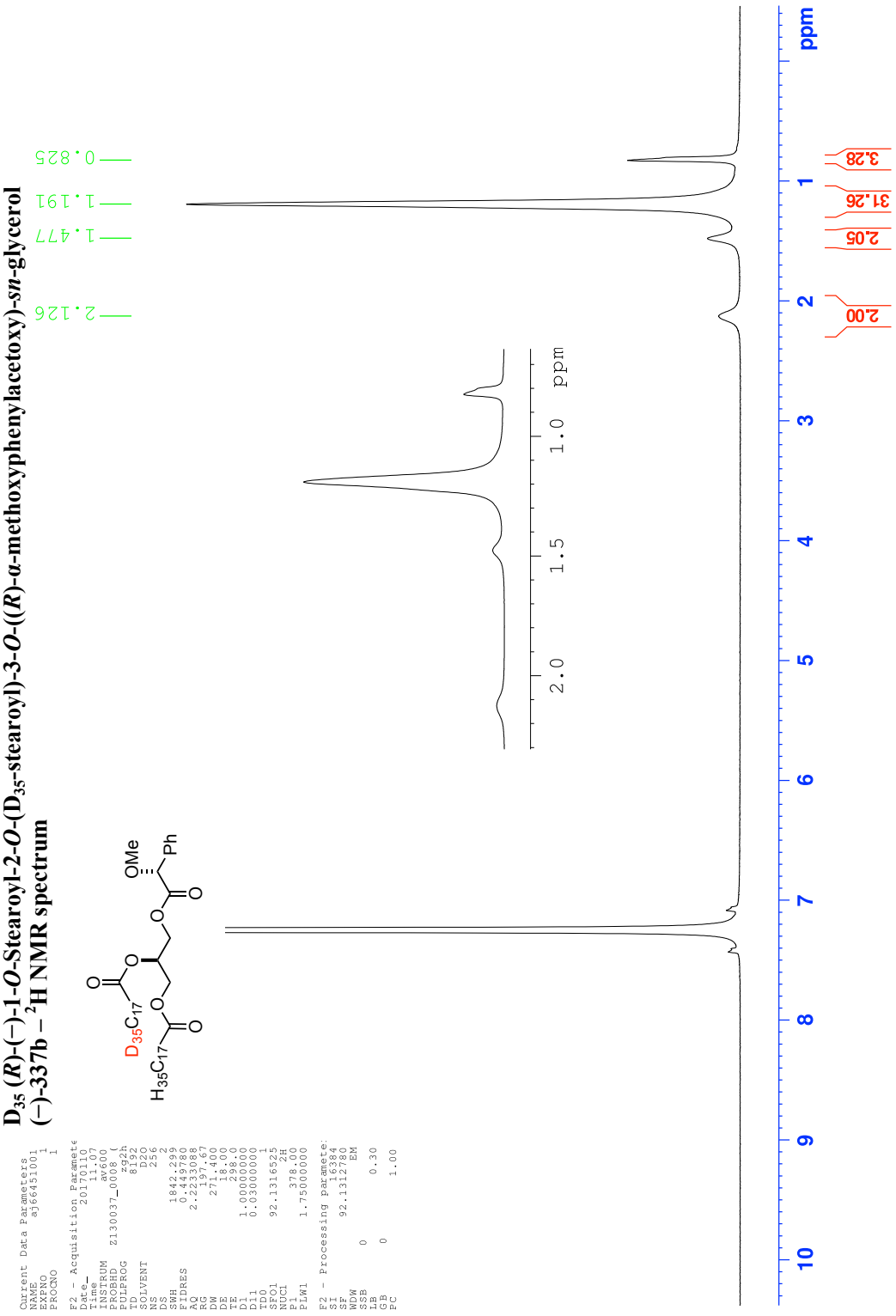
D_{35} (*R*)-(−)-1-*O*-Stearoyl-2-*O*-(D_{35}-stearoyl)-3-*O*-((*R*)-α-methoxyphenylacetoxy)-*sn*-glycerol
(−)-337b – ^{1}H NMR spectrum



D_{35} (*R*)-(−)-1-*O*-Stearoyl-2-*O*-(D_{35}-stearoyl)-3-*O*-((*R*)-(−)-α-methoxyphenylacetate)-*sn*-glycerol
(−)-337b – ^{13}C NMR spectrum



D_{35} (*R*)-(−)-1-*O*-Stearoyl-2-*O*-(D_{35}-stearoyl)-3-*O*-((*R*)-α-methoxyphenylacetoxy)-*sn*-glycerol
(−)-337b – ^{2}H NMR spectrum

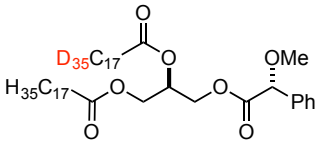



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Current Data Parameters
NAME          sj66451001
EXPNO                  1
PROCNO                 1

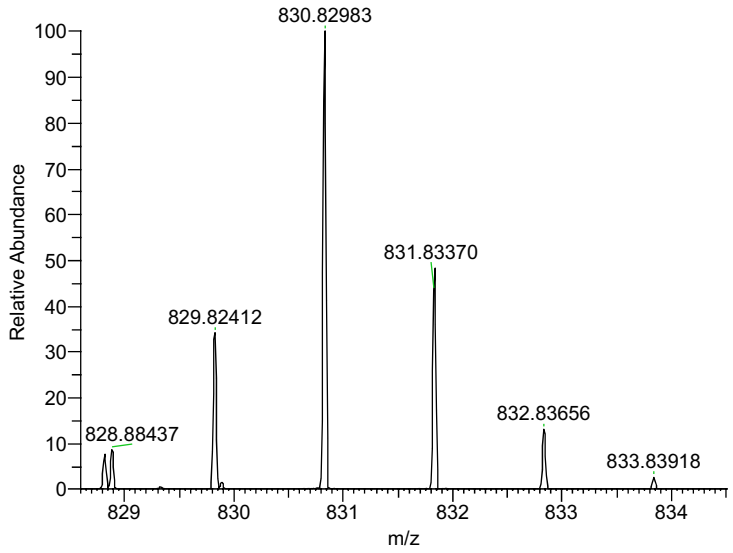
F2 - Acquisition Parameters
Date_         20171107
Time          11.07
INSTRUM           av600
PROBHD    Z130037_0008 (
PULPROG           zg2h
TD               81920
SOLVENT            CDCl3
NS                   256
DS                     2
SWH            1842.299
FIDRES         0.449780
AQ             2.2233088
RG                 197.67
DW                 271.400
DE                  18.00
TE                 298.0
D1             1.00000000
D11            0.03000000
TD0                    1
SFO1           92.1311525
NUC1                  2H
P1                 378.00
PLW1           1.75000000

F2 - Processing parameters
SI                 16384
SF             92.1312780
WDW                   EM
SSB                    0
LB                  0.30
GB                     0
PC                  1.00
```

D_{35} (*R*)-(−)-1-*O*-Stearoyl-2-*O*-(D_{35}-stearoyl)-3-*O*-((*R*)-α-methoxyphenylacetoxy)-*sn*-glycerol (−)-337b – Mass spectrum

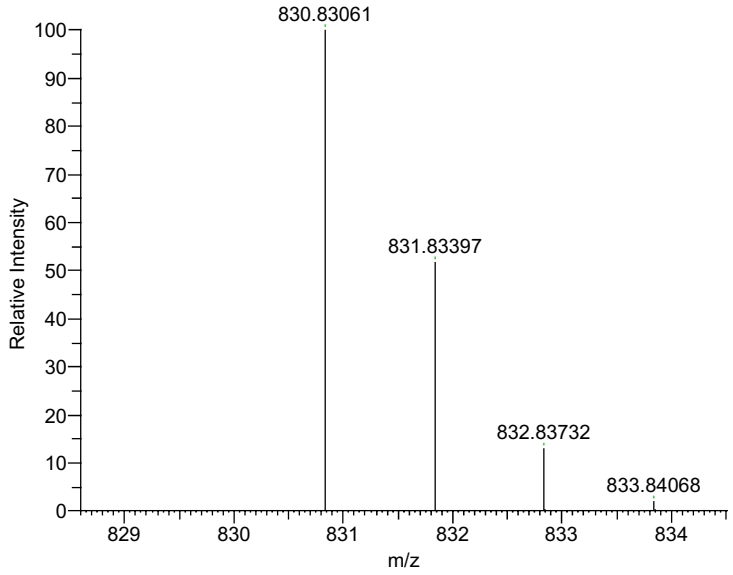


X:\data\Dec 16\ESI60219.raw 14/12/2016 3:19 pm



NL: 4.96E6
ESI60219 #13-27 RT: 0.15-0.31 AV: 8 NL: 3.11E+007
T: FTMS {1,1} + p ESI Full lock ms [80.00-1600.00]

Measured Spectrum



NL: 5.83E5
C48H49[2]H35O7Na1: C_{48} H_{49} $^{2}H_{35}$ O_{7} Na
Chrg 1 R: 1000000 Res. Pwr. @FWHM

Theoretical Spectrum

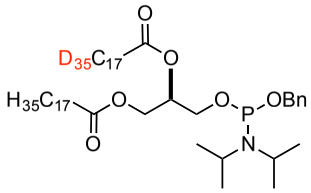
m/z	Formula	RDB	Delta ppm	Theo. Mass
830.82983	$C_{48}H_{49}{}^{2}H_{35}O_{7}{}^{23}Na$	6.5	-0.94	830.83061

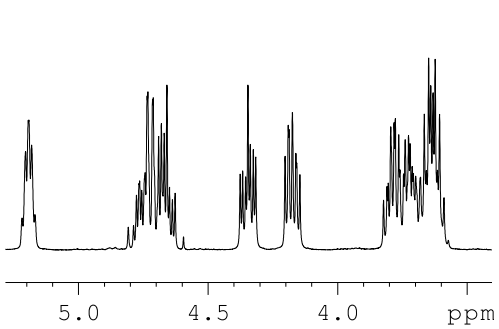
D_{35} (*R*)-(+)-Benzyloxy-*N*,*N*-diisopropylamino-(1-*O*-Stearoyl-2-*O*-(D_{35}-stearoyl)-*sn*-glyceryl)phosphoramidite (+)-339 – ^{1}H NMR spectrum

```
Current Data Parameters
NAME                 ajg40p2
EXPNO                      1
PROCNO                     1

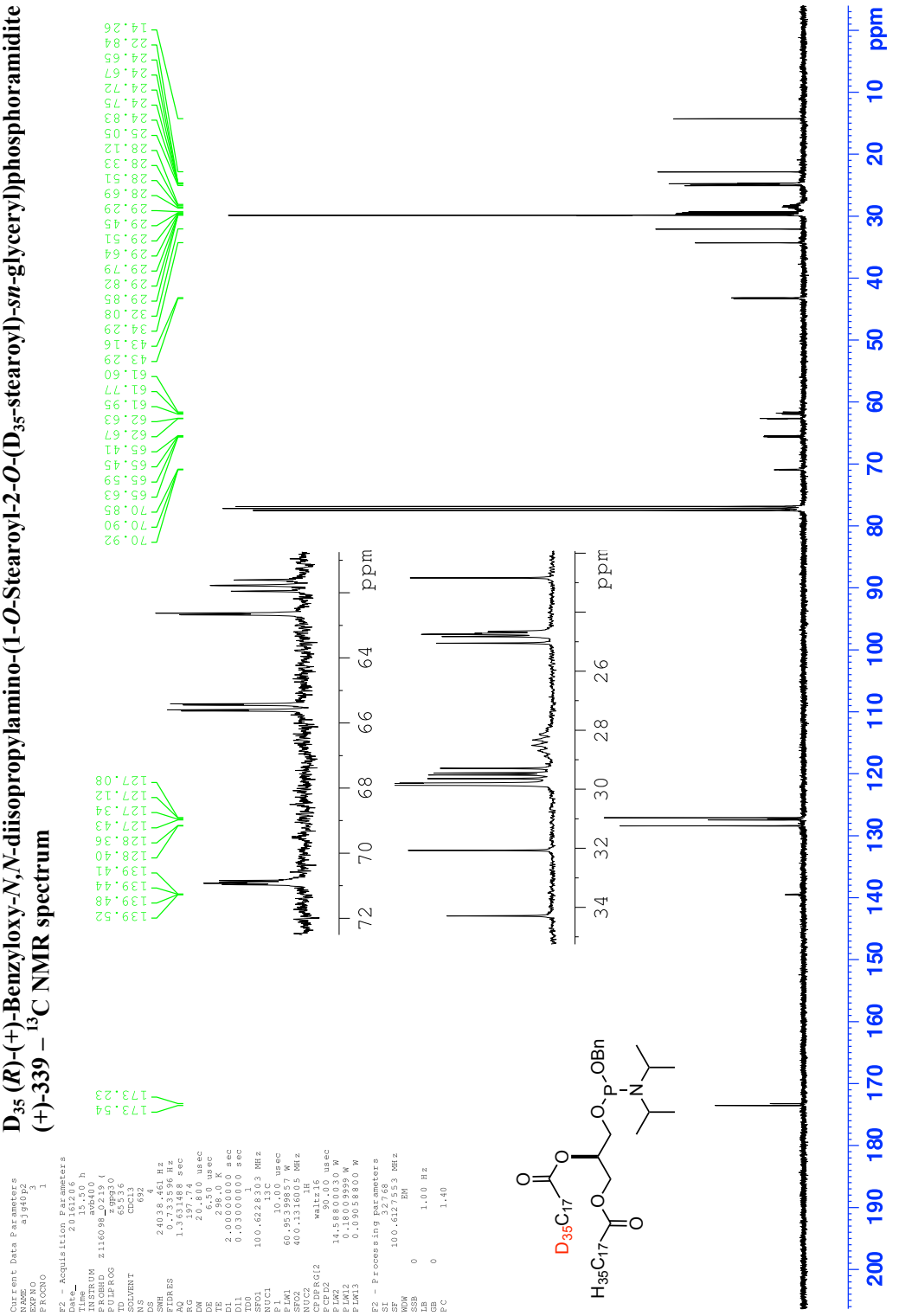
F2 - Acquisition Parameters
Date_              20161206
Time                 15.07 h
INSTRUM              avb400
PROBHD    Z116098_0219 (
PULPROG              zg30
TD                   65536
SOLVENT              CDCl3
NS                      16
DS                       2
SWH                8012.820 Hz
FIDRES             0.244532 Hz
AQ                 4.0894465 sec
RG                    30.76
DW                   62.400 usec
DE                    6.50 usec
TE                   298.0 K
D1              1.00000000 sec
TD0                        1
SFO1           400.1320007 MHz
NUC1                    1H
P1                   10.00 usec
PLW1           14.58800030 W

F2 - Processing parameters
SI                   65536
SF              400.1300099 MHz
WDW                     EM
SSB       0
LB                    0.30 Hz
GB        0
PC                    1.00
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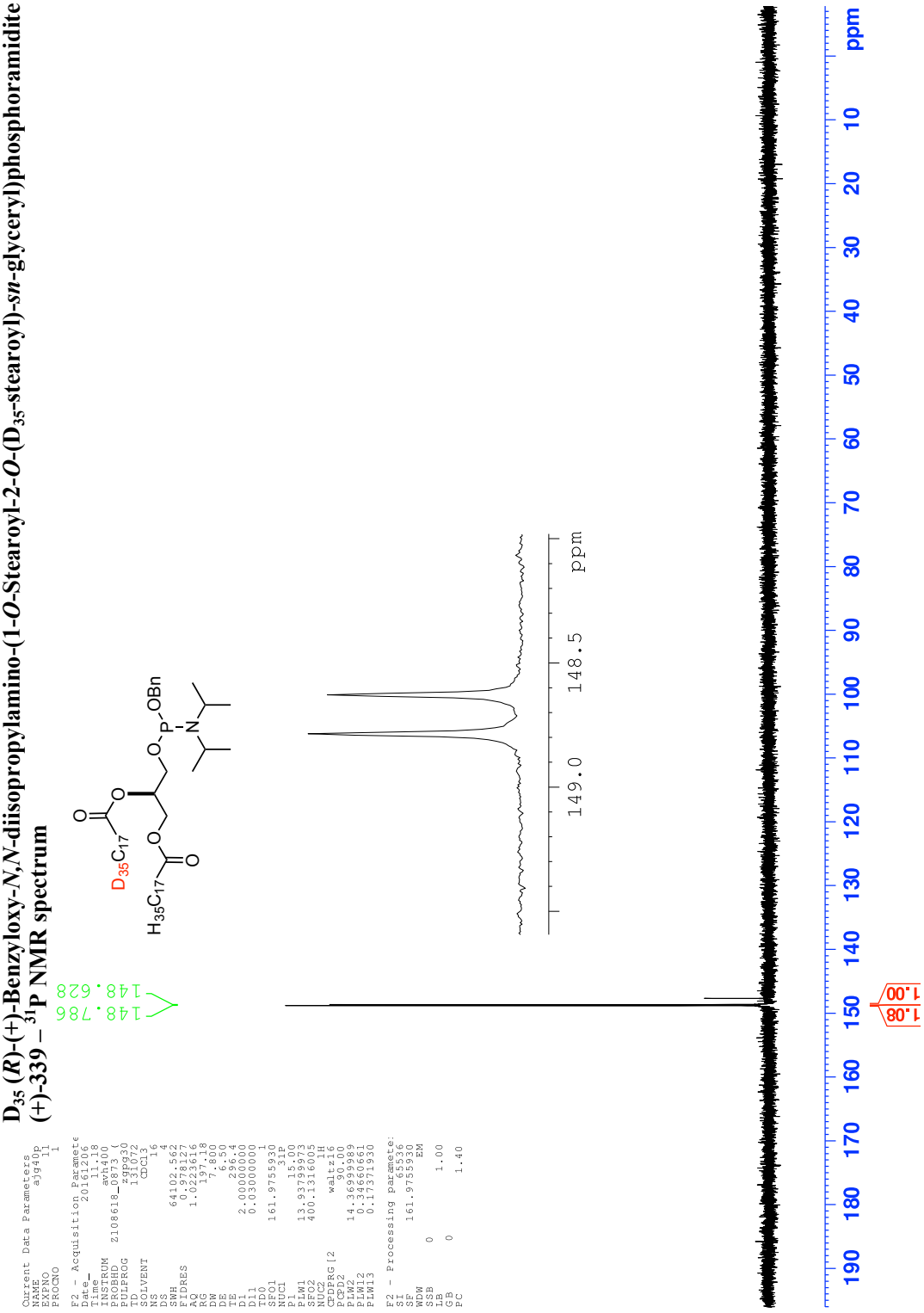


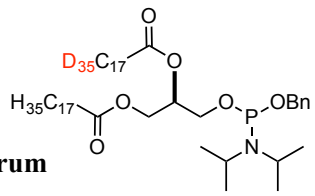


D_{35} (*R*)-(+)-Benzyloxy-*N*,*N*-diisopropylamino-(1-*O*-Stearoyl-2-*O*-(D_{35}-stearoyl)-*sn*-glyceryl)phosphoramidite (+)-339 – ^{13}C NMR spectrum



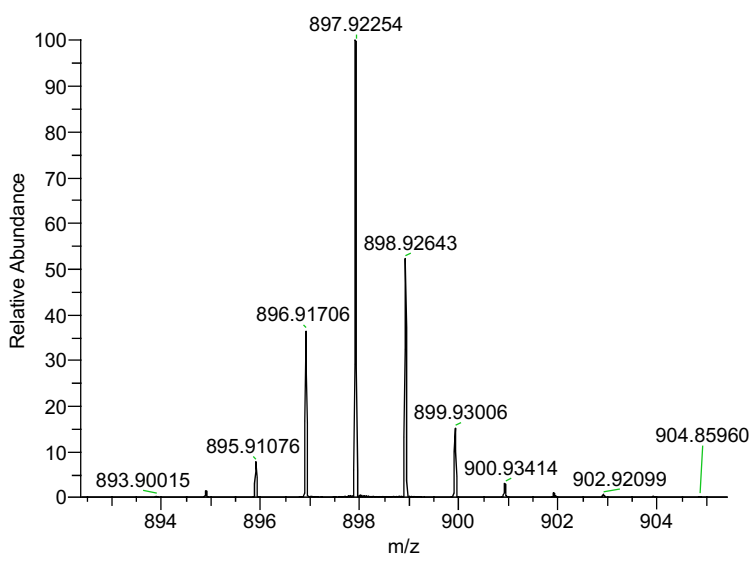
D_{35} (*R*)-(+)-Benzyloxy-*N*,*N*-diisopropylamino-(1-*O*-Stearoyl-2-*O*-(D_{35}-stearoyl)-*sn*-glyceryl)phosphoramidite
(+)-339 – ^{31}P NMR spectrum





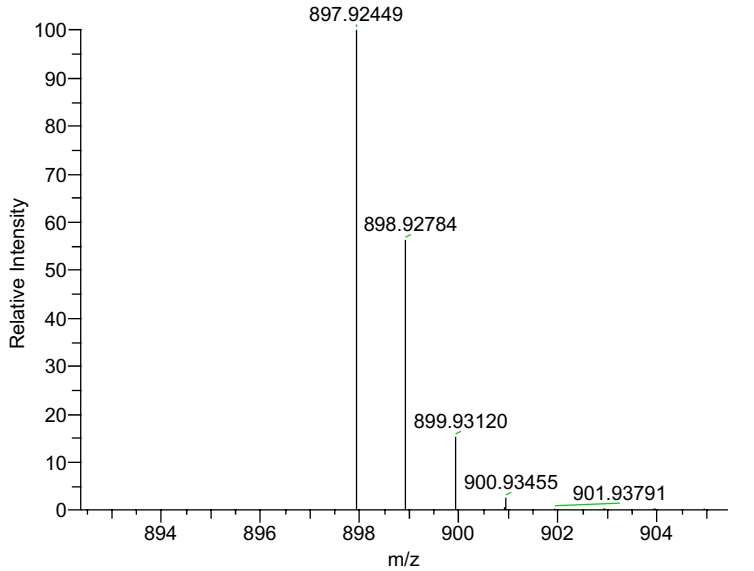
D_{35} (*R*)-(+)-Benzyloxy-*N*,*N*-diisopropylamino-(1-*O*-Stearoyl-2-*O*-(D_{35}-stearoyl)-*sn*-glyceryl)phosphoramidite (+)-339 – Mass spectrum

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NL: 2.18E7
ESI60145 #13-27 RT: 0.15-0.31 AV: 8 NL: 7.00E+007
T: FTMS {1,1} + p ESI Full ms [80.00-1600.00]

Measured Spectrum



NL: 5.57E5
C52H62[2]H35O6N1P1: C_{52} H_{62} $^{2}H_{35}$ O_{6} N P Chrg 1 R: 1000000 Res. Pwr. @FWHM

Theoretical Spectrum

m/z	Formula	RDB	Delta ppm	Theo. Mass
897.92255	$C_{52}H_{62}{}^{2}H_{35}O_{6}NP$	5.5	-2.16	897.92449

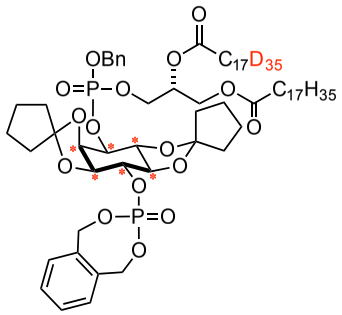
D_{41} (−)-1D-1-*O*-(1-*O*-Stearoyl-2-*O*-(D_{35}-stearoyl)-*sn*-glycer-3-yl-benzylphosphate)-2,3-*O*-cyclopentylidene-4-*O*-(2-oxo-5.6-benzo-1,3,2-dioxaphosphep-2-yl)-5,6-*O*-cyclopentylidene-*myo*-inositol (−)-340 – ^{1}H NMR spectrum

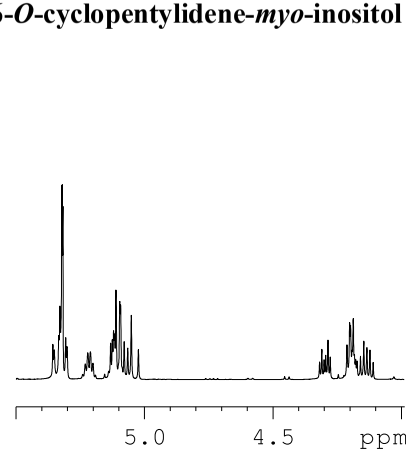
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NAME          ajg41p-data
EXPNO                   1
PROCNO                  1

F2 - Acquisition Parameters
Date_          20161216
Time              11.31
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PROBHD   5 mm CPDUL 13C
PULPROG           zg30
TD               65536
SOLVENT          CD2Cl2
NS                 256
DS                   4
SWH          10330.578 Hz
FIDRES        0.157632 Hz
AQ           3.1719425 sec
RG                2.25
DW              48.400 usec
DE              10.00 usec
TE              298.0 K
D1           1.00000000 sec
TD0                  1

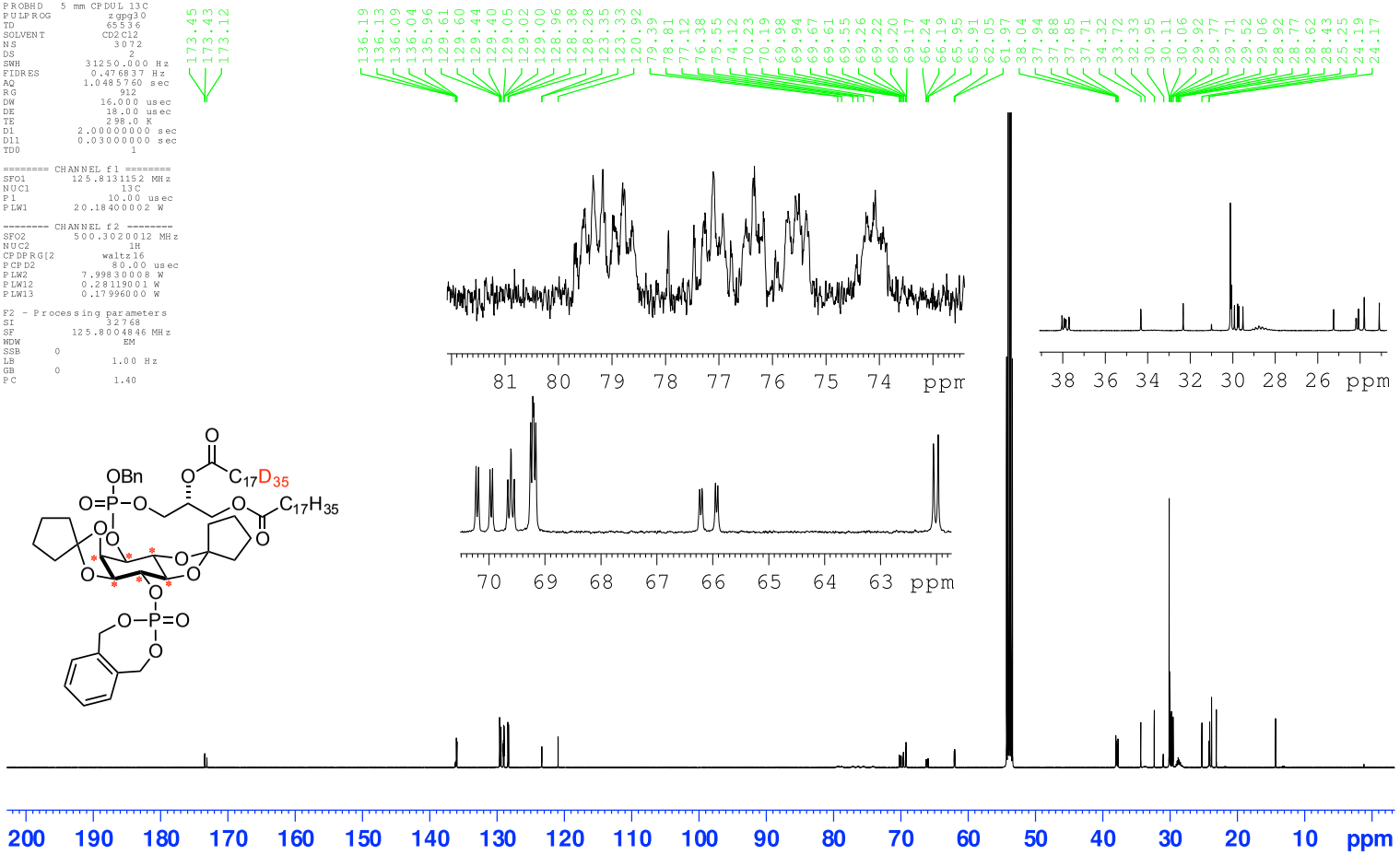
======== CHANNEL f1 ========
SFO1        500.3030896 MHz
NUC1               1H
P1              15.00 usec
PLW1         7.99830008 W

F2 - Processing parameters
SI               65536
SF          500.3000206 MHz
WDW                EM
SSB      0
LB              0.30 Hz
GB       0
PC              1.00
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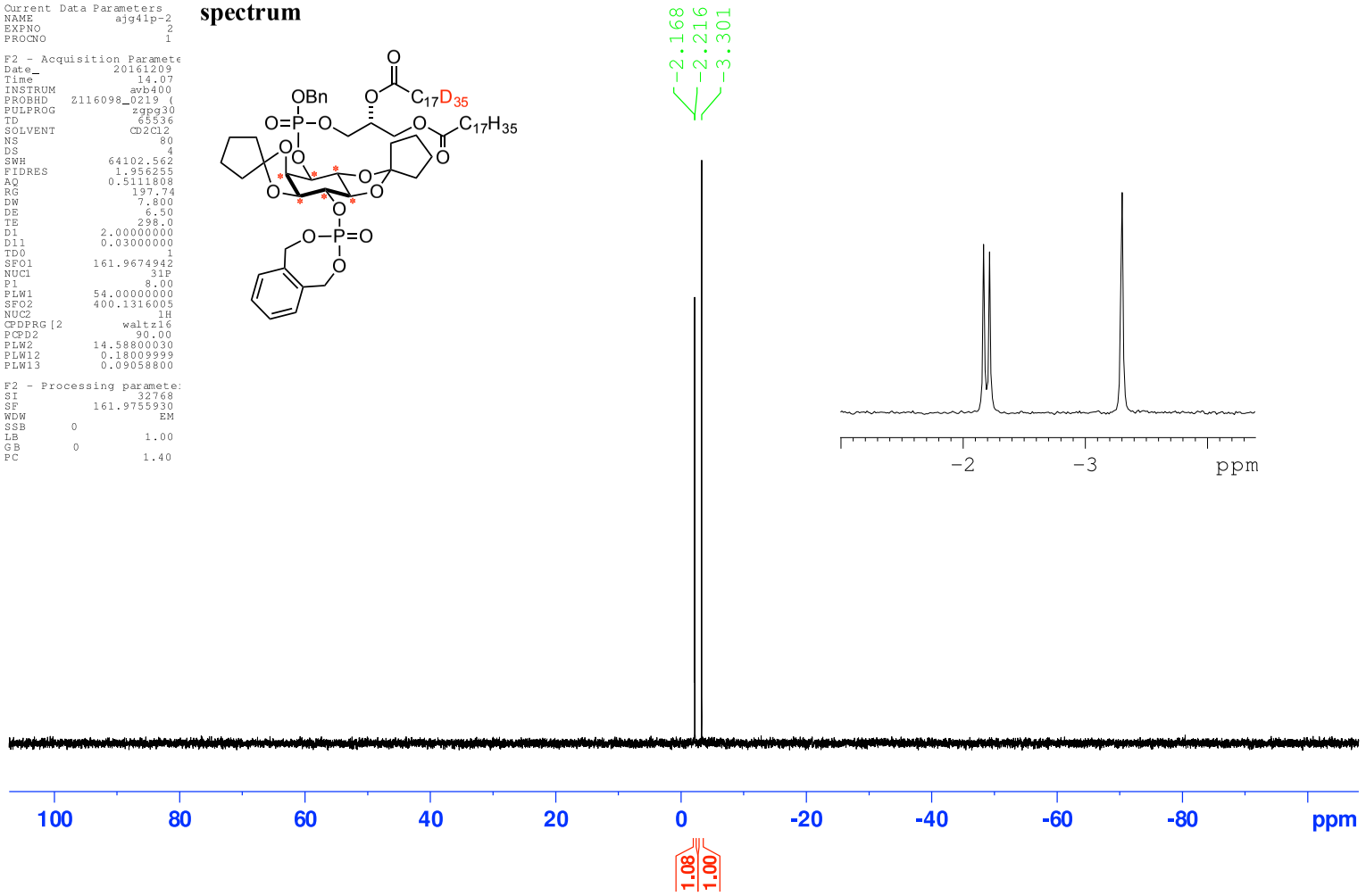




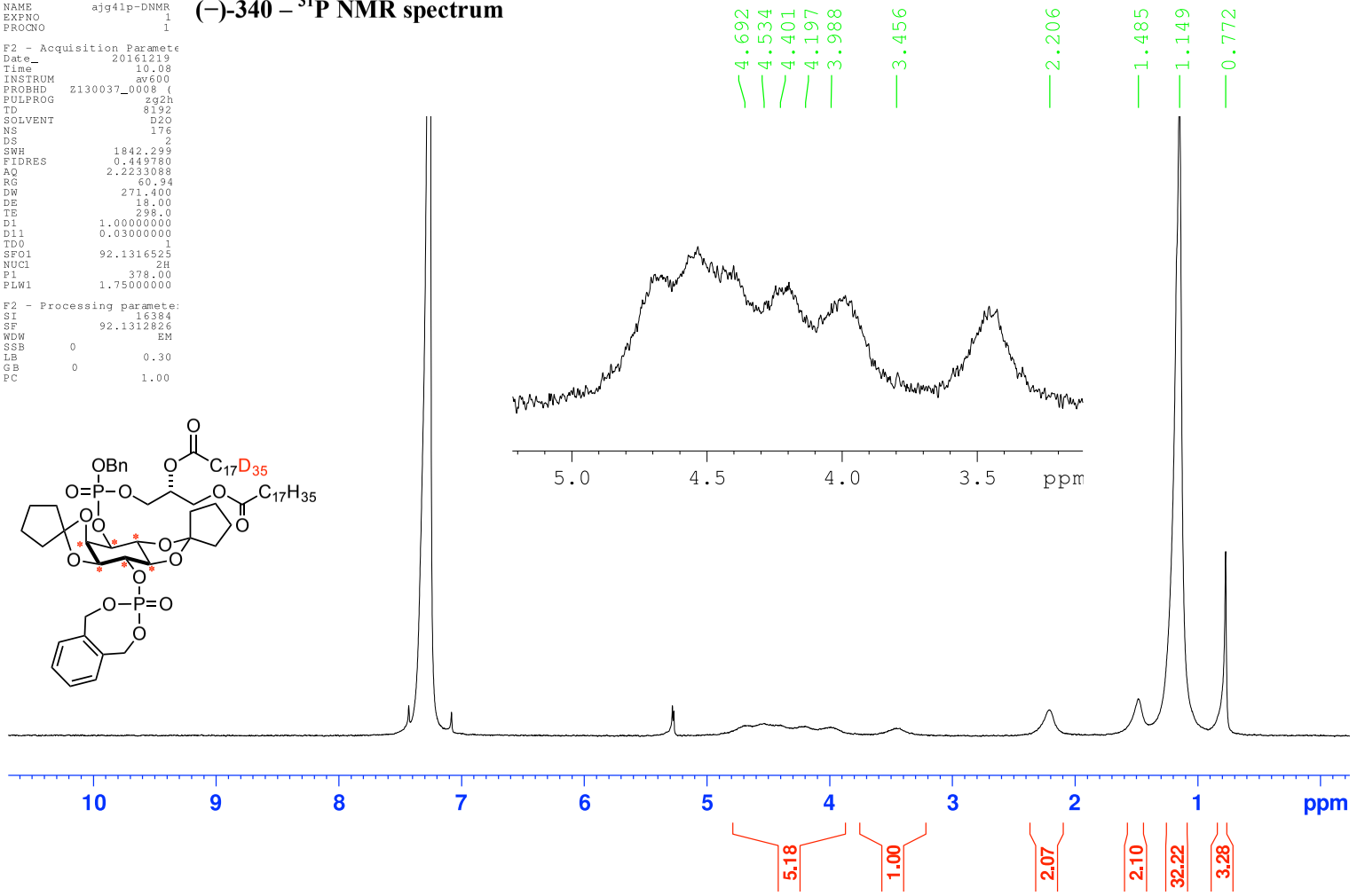
D_{41} (−)-1D-1-*O*-(1-*O*-Stearoyl-2-*O*-(D_{35}-stearoyl)-*sn*-glycer-3-yl-benzylphosphate)-2,3-*O*-cyclopentylidene-4-*O*-(2-oxo-5.6-benzo-1,3,2-dioxaphosphep-2-yl)-5,6-*O*-cyclopentylidene-*myo*-inositol (−)-340 – ^{13}C NMR spectrum



D_{41} (−)-1D-1-*O*-(1-*O*-Stearoyl-2-*O*-(D_{35}-stearoyl)-*sn*-glycer-3-yl-benzylphosphate)-2,3-*O*-cyclopentylidene-4-*O*-(2-oxo-5.6-benzo-1,3,2-dioxaphosphep-2-yl)-5,6-*O*-cyclopentylidene-*myo*-inositol (−)-340 – ^{31}P NMR spectrum

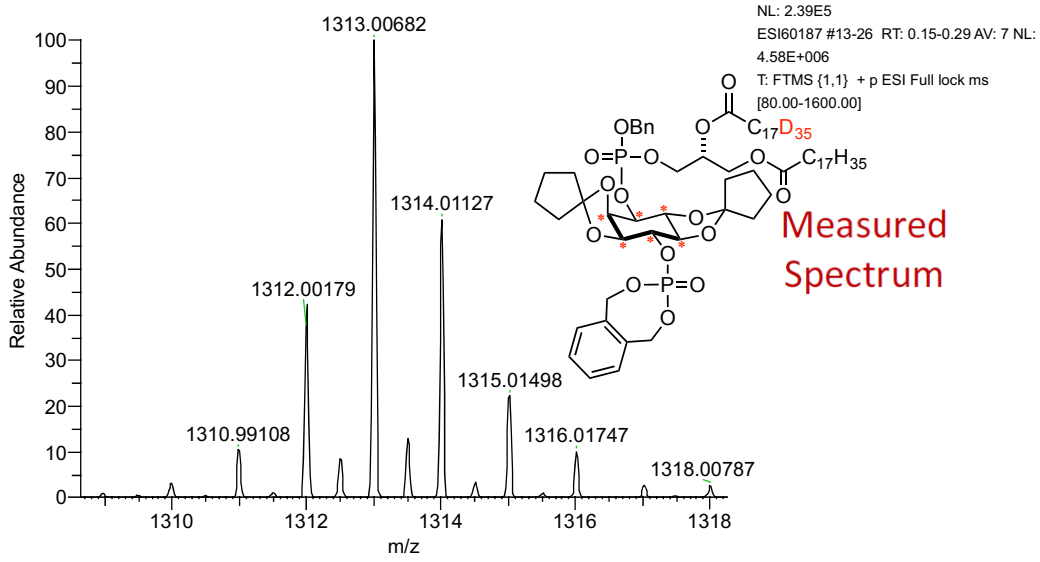


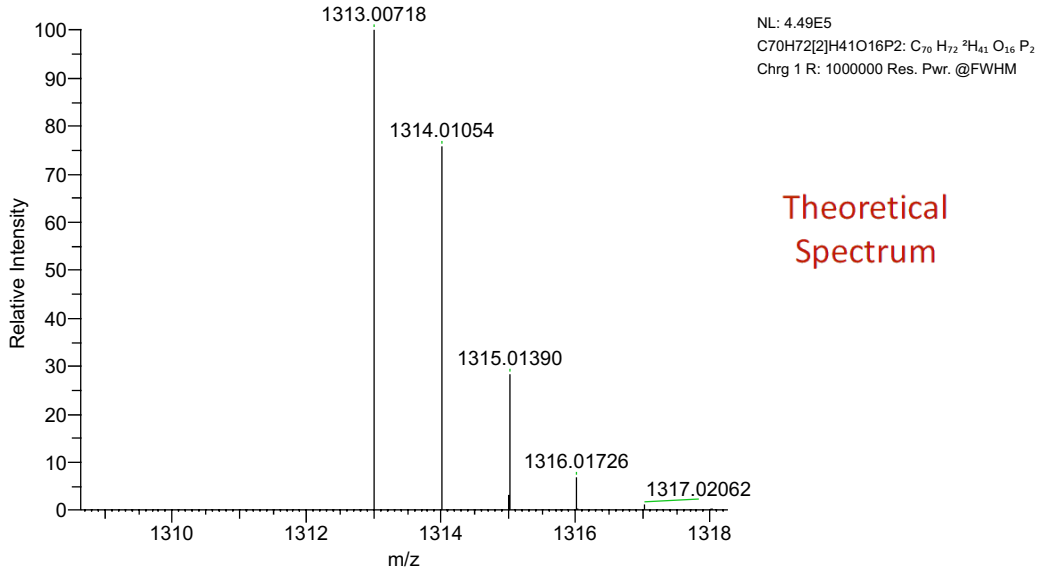
D_{41} (−)-1D-1-*O*-(1-*O*-Stearoyl-2-*O*-(D_{35}-stearoyl)-*sn*-glycer-3-yl-benzylphosphate)-2,3-*O*-cyclopentylidene-4-*O*-(2-oxo-5.6-benzo-1,3,2-dioxaphosphep-2-yl)-5,6-*O*-cyclopentylidene-*myo*-inositol (−)-340 – ^{31}P NMR spectrum



D_{41} (−)-1D-1-*O*-(1-*O*-Stearoyl-2-*O*-(D_{35}-stearoyl)-*sn*-glycer-3-yl-benzylphosphate)-2,3-*O*-cyclopentylidene-4-*O*-(2-oxo-5.6-benzo-1,3,2-dioxaphosphep-2-yl)-5,6-*O*-cyclopentylidene-*myo*-inositol (−)-340 – Mass spectrum

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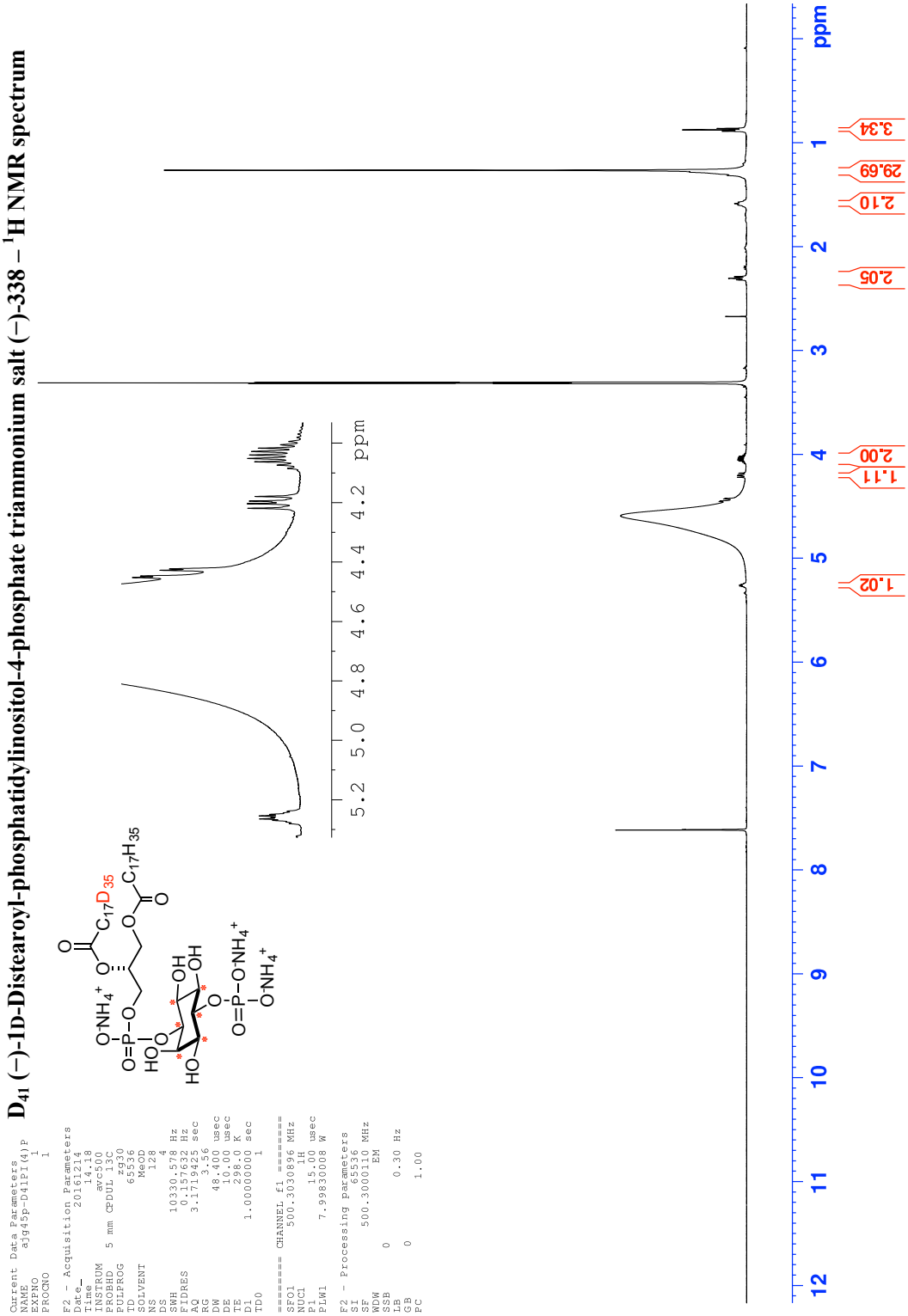


m/z	Formula	RDB	Delta ppm	Theo. Mass
1313.00684	$C_{70}H_{72}{}^{2}H_{41}O_{16}P_2$	15.5	-0.26	1313.00718

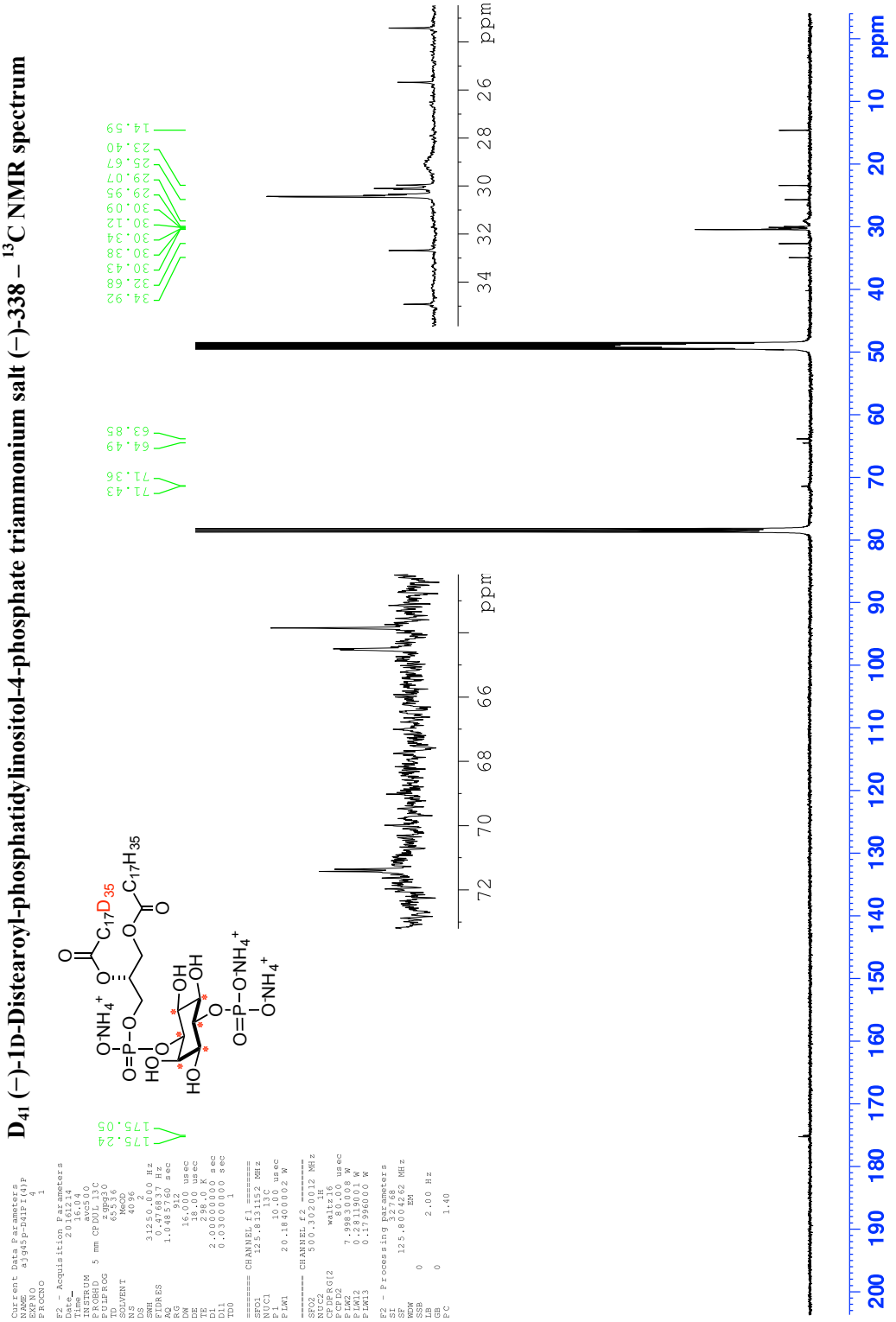
D_{41} (−)-1D-1-*O*-(1-*O*-Stearoyl-2-*O*-(D_{35}-stearoyl)-*sn*-glycer-3-yl-benzylphosphate)-2,3-*O*-cyclopentylidene-4-*O*-(2-oxo-5.6-benzo-1,3,2-dioxaphosphep-2-yl)-5,6-*O*-cyclopentylidene-*myo*-inositol (−)-340 – NP-HPLC (Method 6)

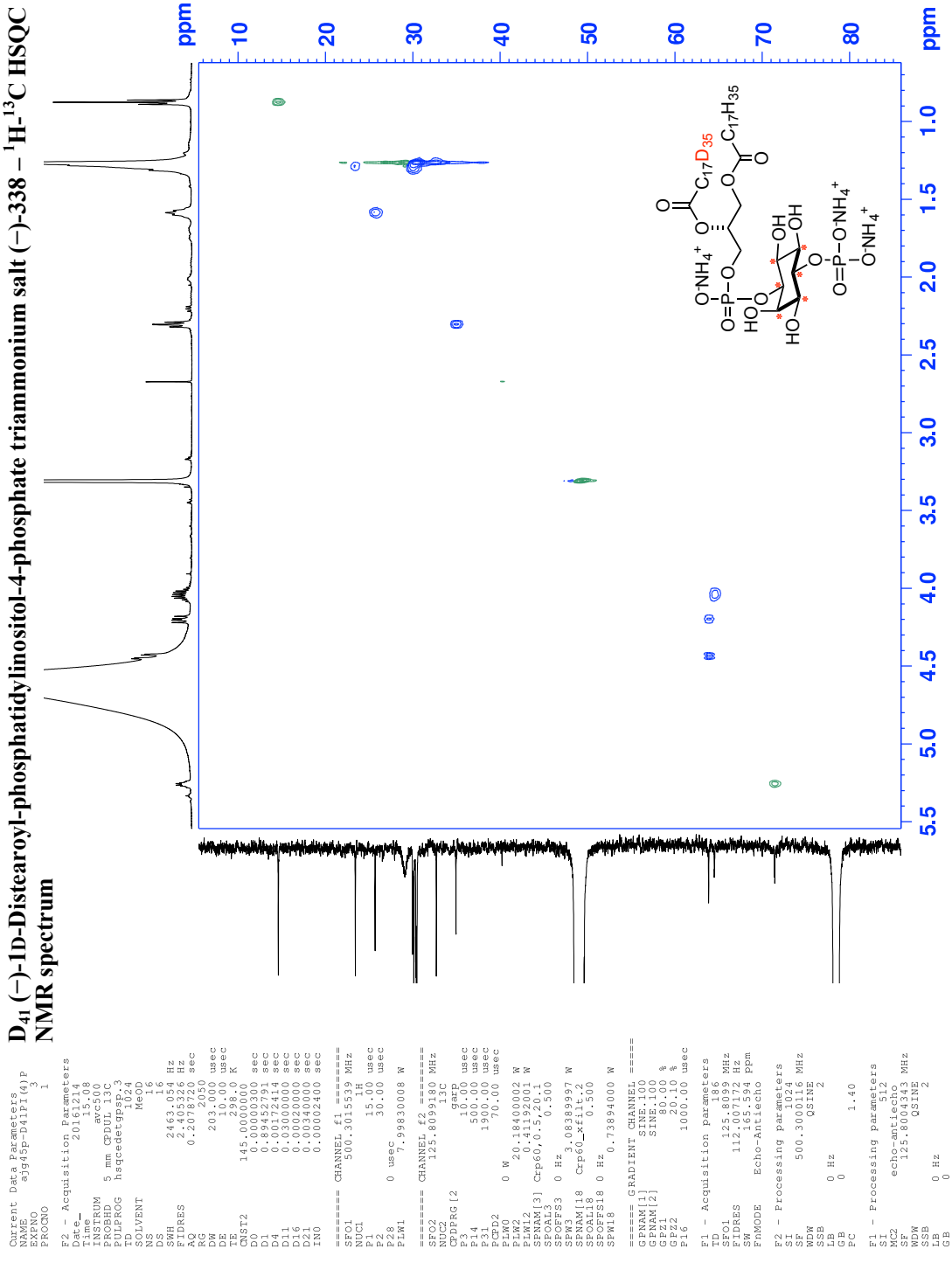
Time	Area	Area %
2.310	4,666	0.20
4.634	2,290,414	99.80
Total	2,295,080	100.00

D_{41} (−)-1D-Distearoyl-phosphatidylinositol-4-phosphate triammonium salt (−)-338 – ^{1}H NMR spectrum

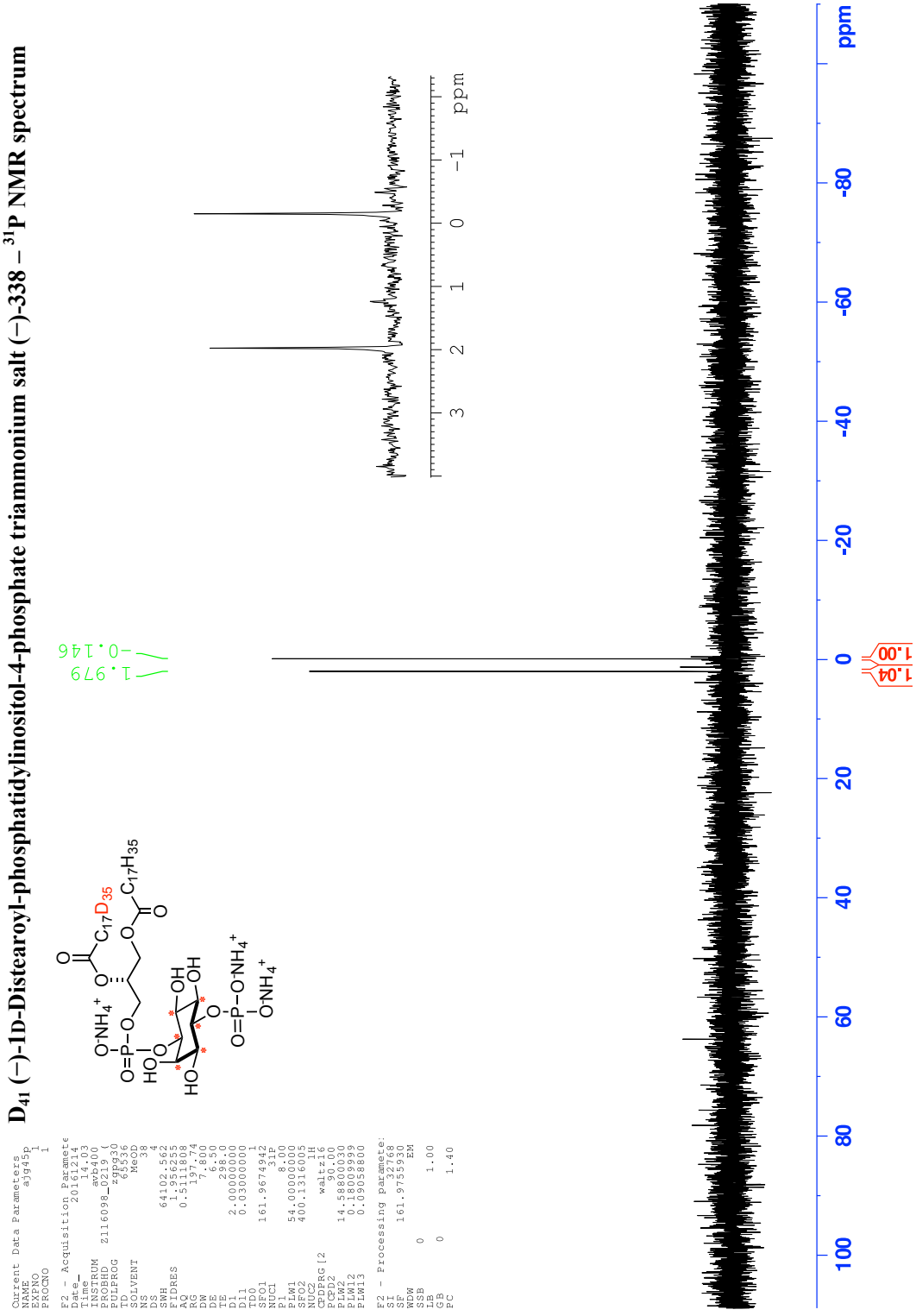


D_{41} (−)-1D-Distearoyl-phosphatidylinositol-4-phosphate triammonium salt (−)-338 – ^{13}C NMR spectrum

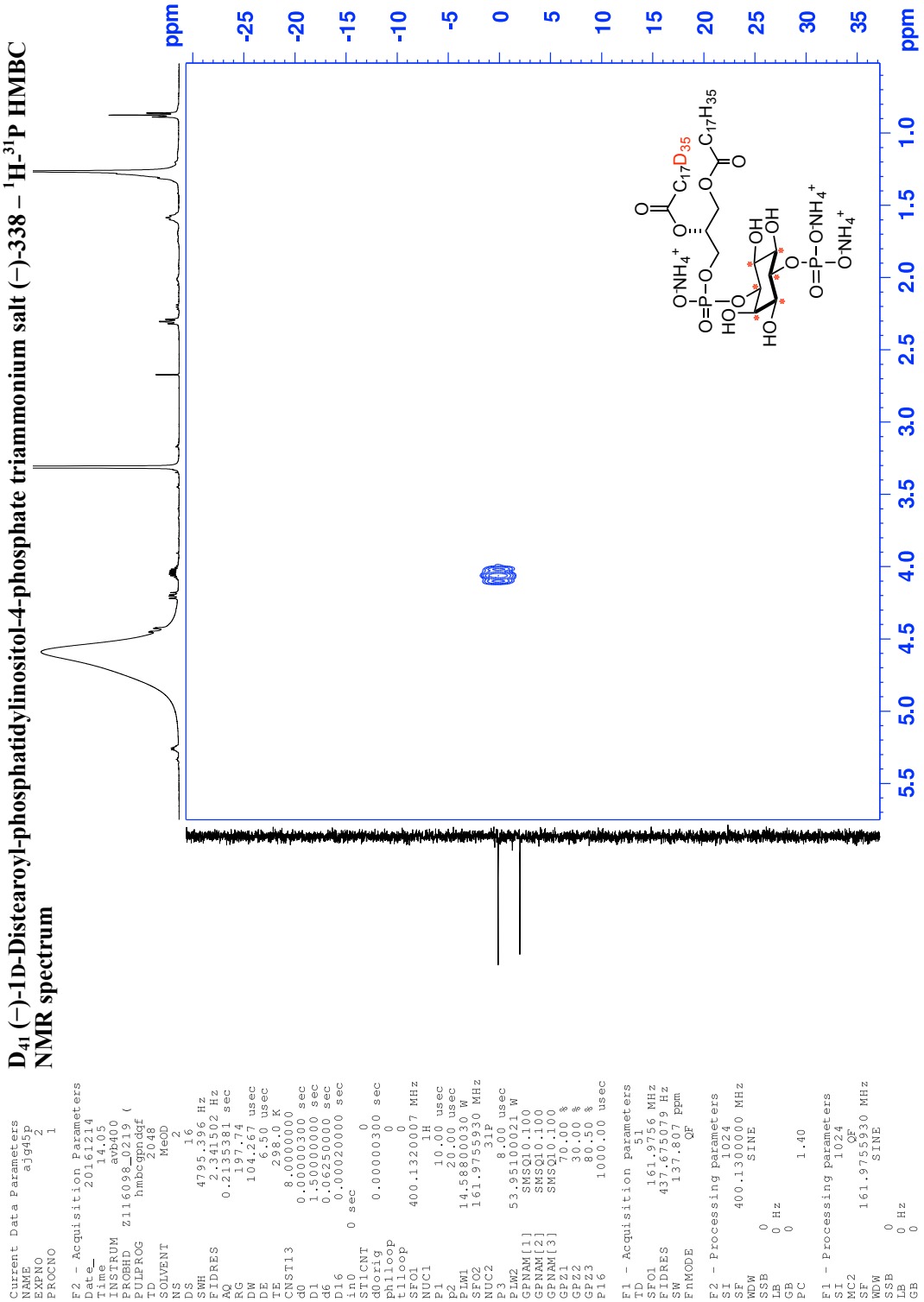



D41 (−)-1D-Distearoyl-phosphatidylinositol-4-phosphate triammonium salt (−)-338 – 1H-13C HSQC
NMR spectrum
ppm
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
10
20
30
40
50
60
70
80
C17D35
C17H35
O-NH4+
O=P-O
HO
OH
O
O=P-O-NH4+

D_{41} (−)-1D-Distearoyl-phosphatidylinositol-4-phosphate triammonium salt (−)-338 – ^{31}P NMR spectrum



D_{41} (−)-1D-Distearoyl-phosphatidylinositol-4-phosphate triammonium salt (−)-338 – ^{1}H-^{31}P HMBC NMR spectrum




```
Current Data Parameters
NAME            ajg45p
EXPNO                2
PROCNO               1

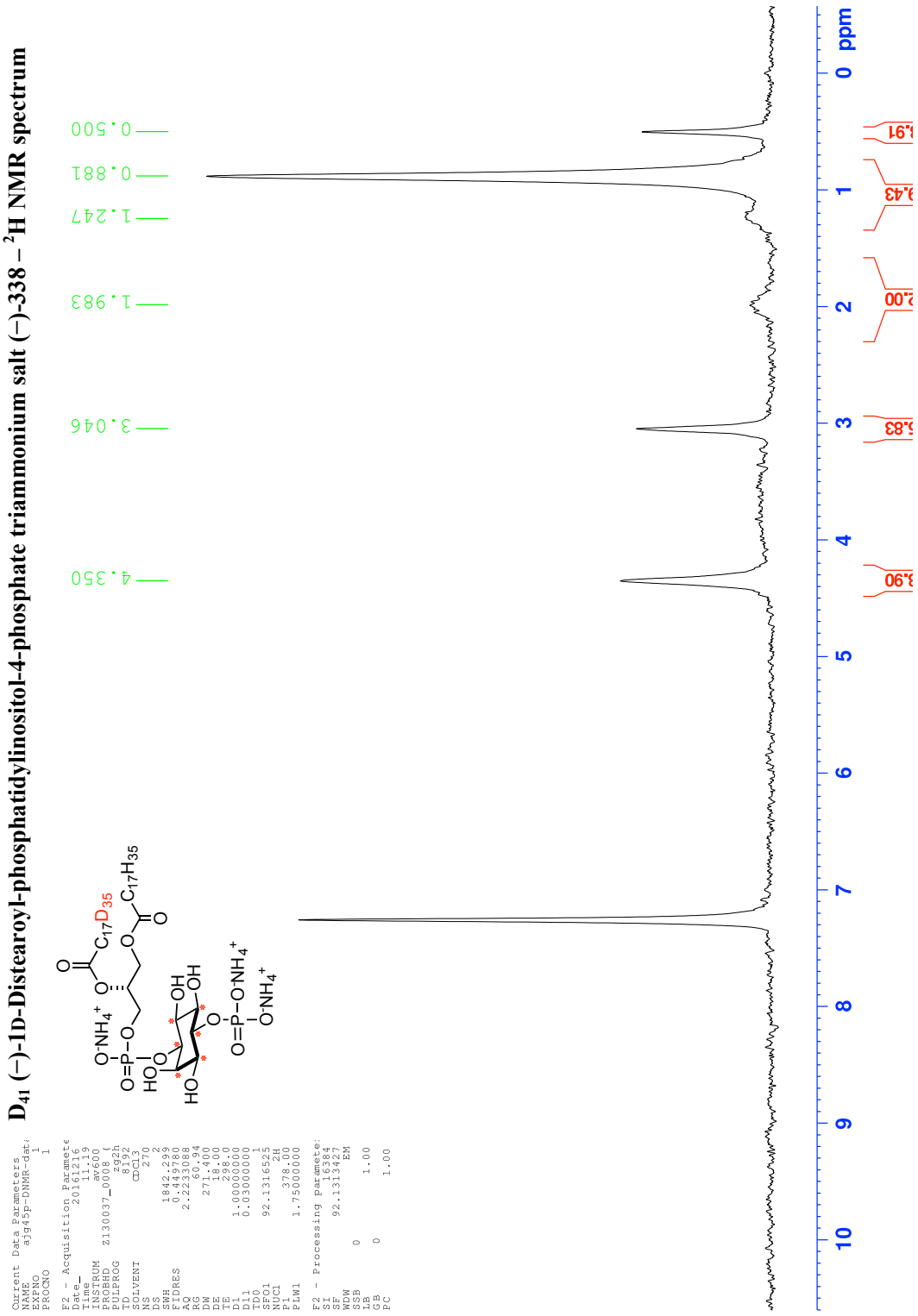
F2 - Acquisition Parameters
Date_         20161214
Time               14.05
INSTRUM            avb400
PROBHD     Z116098_0219 (
PULPROG      hmbcgpndqf
TD                 2048
SOLVENT            MeOD
NS                    2
DS                   16
SWH          4795.396 Hz
FIDRES          2.341502 Hz
AQ           0.21351381 sec
RG                 197.74
DW              104.267 usec
DE                 6.50 usec
TE                298.0 K
CNST13          8.0000000
d0              0.00000300 sec
D1              1.50000000 sec
d6              0.06250000 sec
D16             0.00020000 sec
in0                0 sec
ST1CNT                0
d0orig          0.00000300 sec
phlloop               0
tlloop                0
SFO1         400.1320007 MHz
NUC1                 1H
P1                10.00 usec
p2                20.00 usec
PLW1         14.58800030 W
SFO2         161.9755930 MHz
NUC2                31P
P3                 8.00 usec
PLW2         53.95100021 W
GPNAM[1]       SMSQ10.100
GPNAM[2]       SMSQ10.100
GPNAM[3]       SMSQ10.100
GPZ1              70.00 %
GPZ2              30.00 %
GPZ3              80.50 %
P16            1000.00 usec

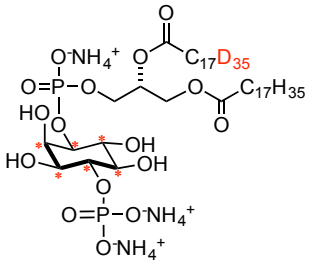
F1 - Acquisition parameters
TD                   512
SFO1         161.9756 MHz
FIDRES       437.675079 Hz
SW           137.807 ppm
FnMODE               QF

F2 - Processing parameters
SI                  1024
SF           400.1300000 MHz
WDW                 SINE
SSB                    0
LB                 0 Hz
GB                     0
PC                  1.40

F1 - Processing parameters
SI                  1024
MC2                   QF
SF           161.9755930 MHz
WDW                 SINE
SSB                    0
LB                 0 Hz
GB                     0
```

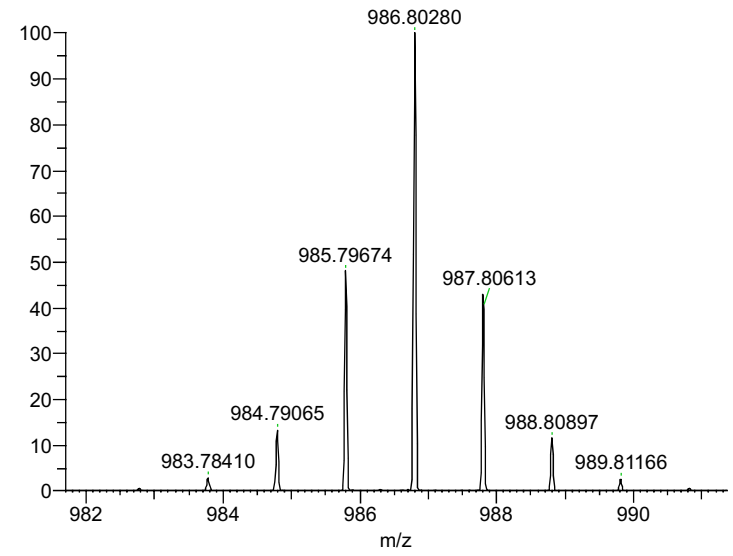
D_{41} (–)-1D-Distearoyl-phosphatidylinositol-4-phosphate triammonium salt (–)-338 – ^{2}H NMR spectrum





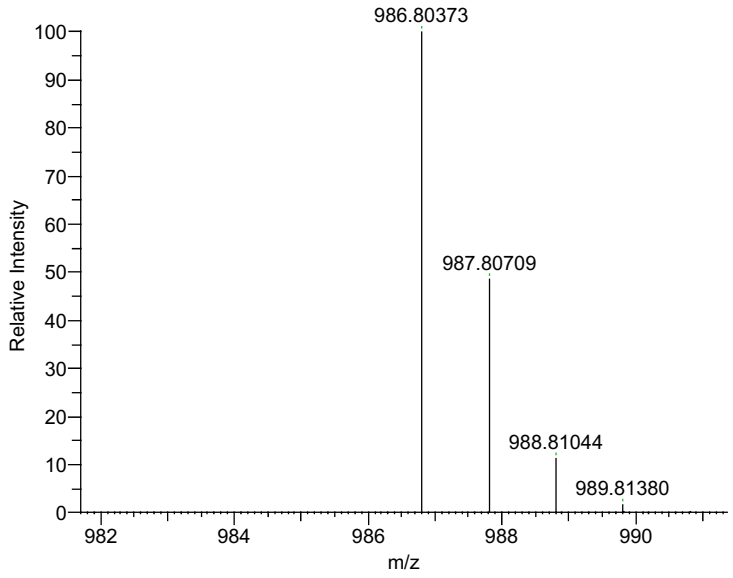
D_{41} (−)-1D-Distearoyl-phosphatidylinositol-4-phosphate triammonium salt (−)-338 – ^{2}H NMR spectrum

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NL: 7.71E5
ESI60247 #10-34 RT: 0.11-0.39 AV: 13 NL: 7.95E6
T: FTMS {1,2} - p ESI Full ms [80.00-1600.00]

Measured Spectrum

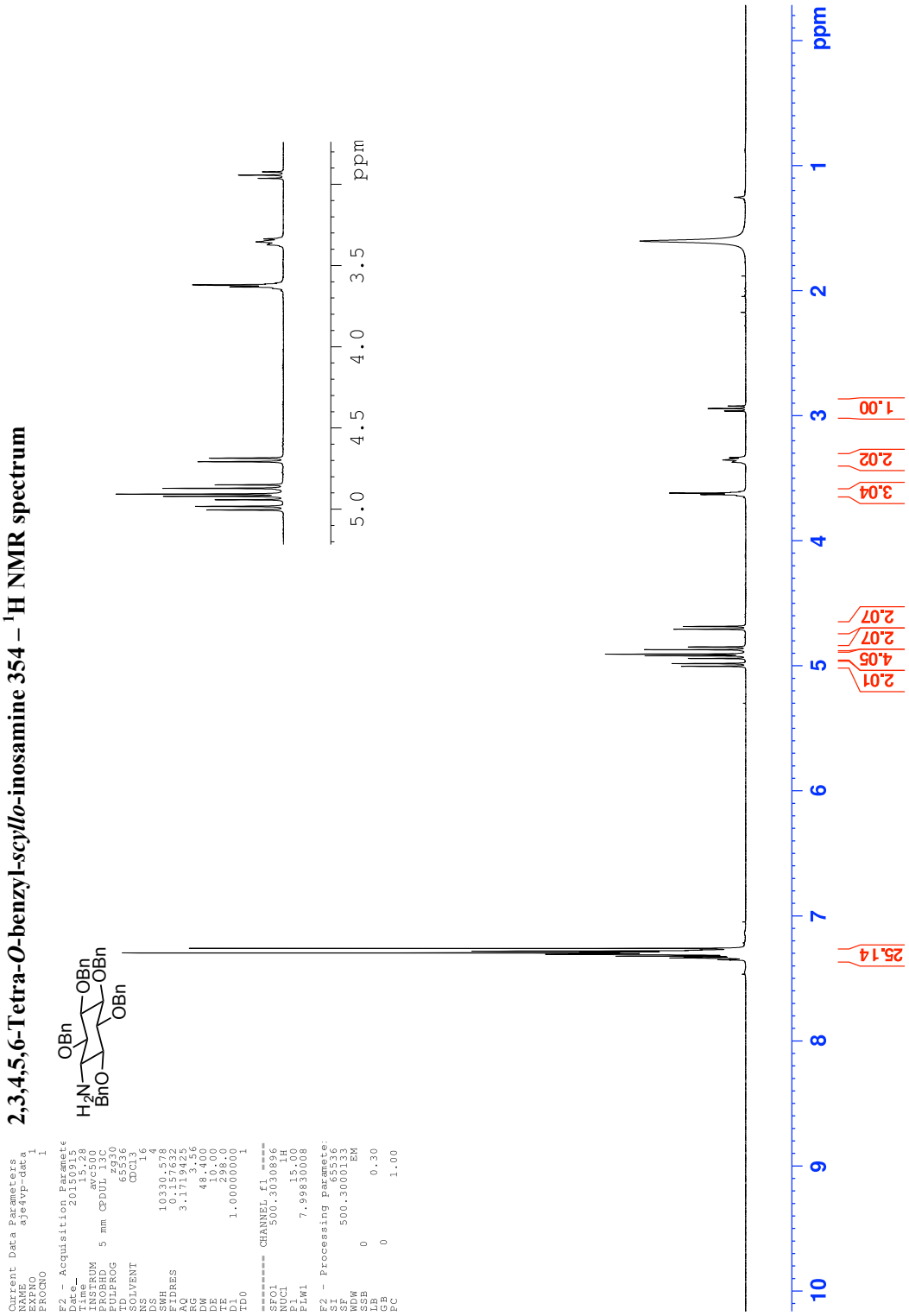


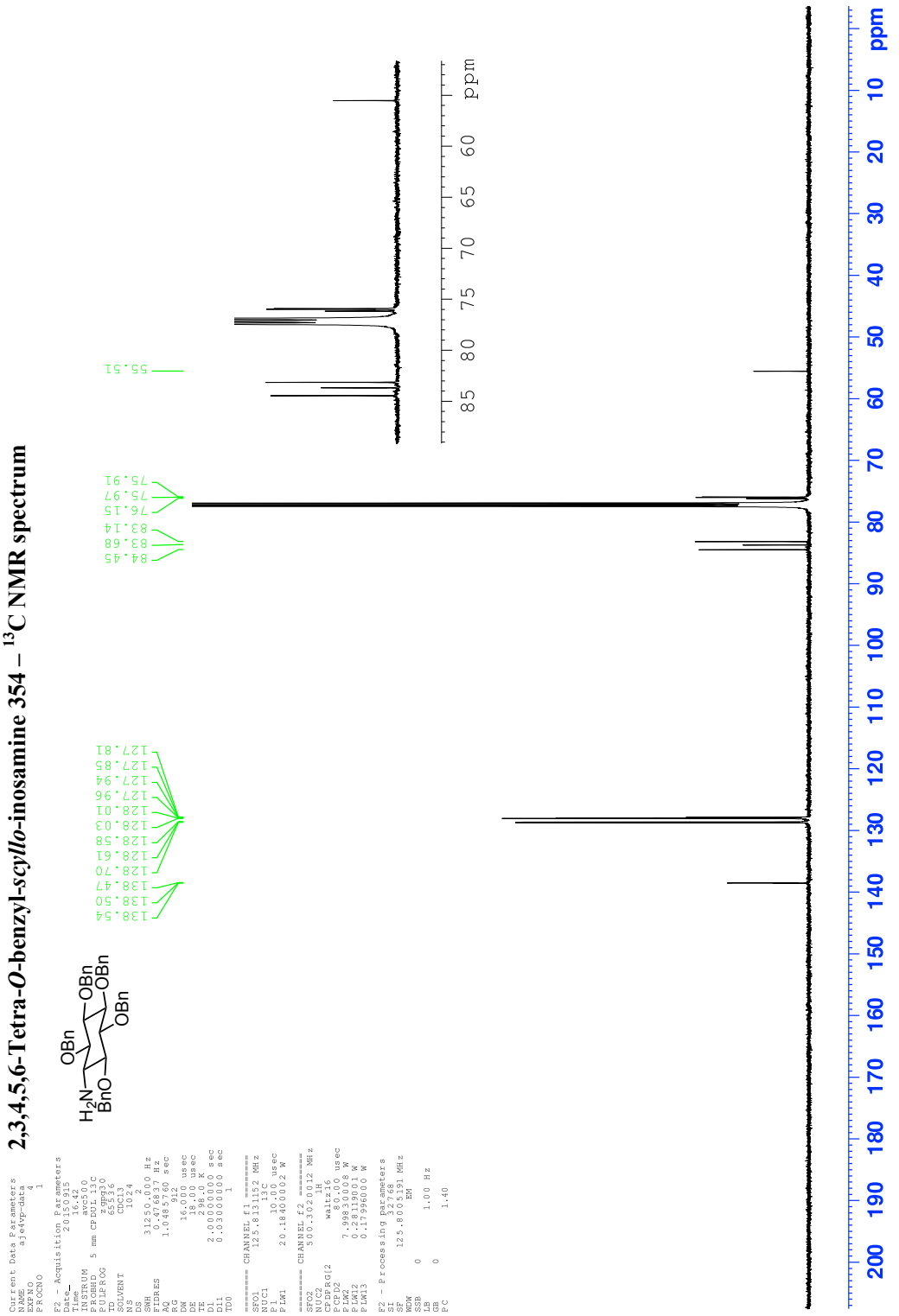
NL: 5.90E5
c45h46D41O16P2: $C_{45}H_{46}D_{41}O_{16}P_2$ pa
Chrg 1 R: 1000000 Res. Pwr. @FWHM

Theoretical Spectrum

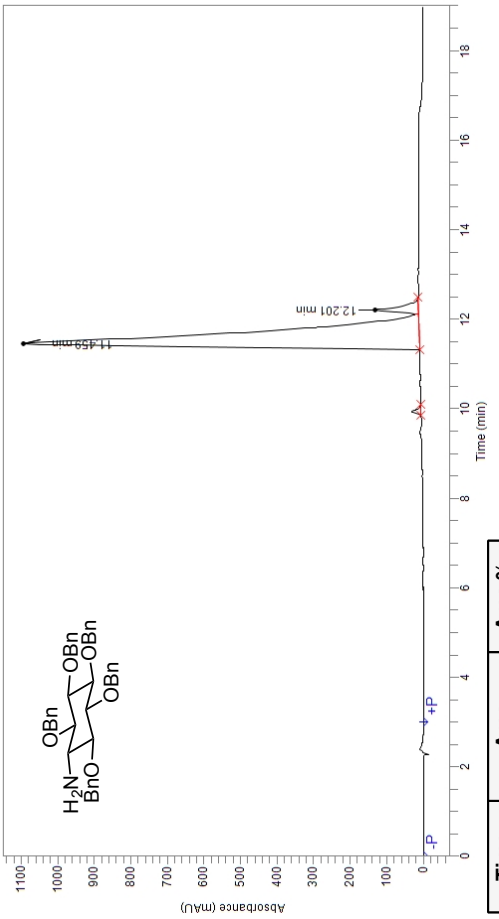
m/z	Formula	RDB	Delta ppm	Theo. Mass
986.80280	$C_{45}H_{46}{}^{2}H_{41}O_{16}P_2$	3.5	-0.95	986.80373

2,3,4,5,6-Tetra-*O*-benzyl-*scyllo*-inosamine 354 – ^{1}H NMR spectrum




2,3,4,5,6-Tetra-O-benzyl-scyllo-inosamine 354 – 13C NMR spectrum
H2N
BnO
OBn
OBn
OBn
OBn
138.54
138.50
138.47
128.70
128.61
128.58
128.03
128.01
127.96
127.94
127.85
127.81
84.45
83.68
83.14
76.15
75.97
75.91
55.51
85 80 75 70 65 60 ppm
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 ppm

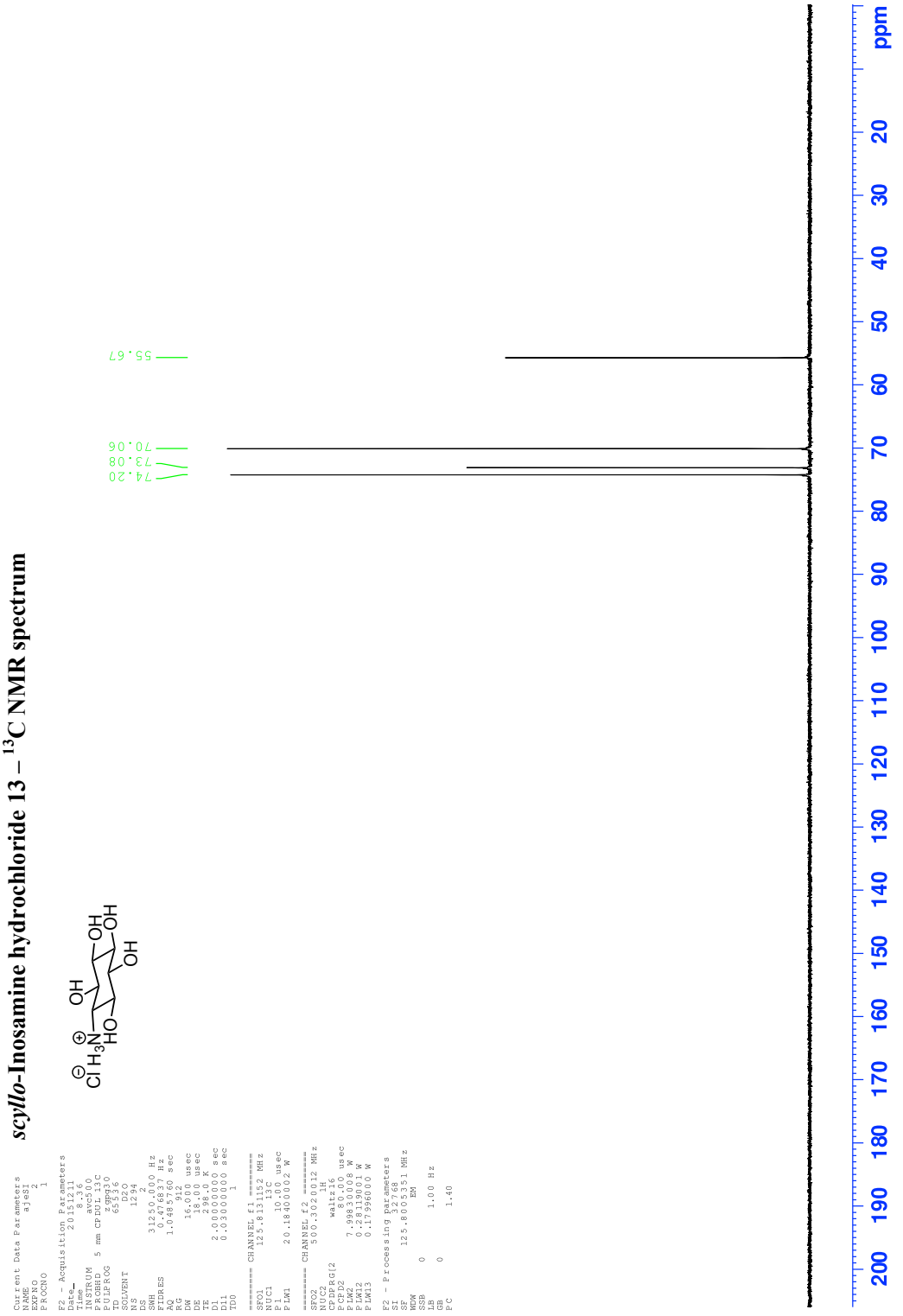
2,3,4,5,6-Tetra-*O*-benzyl-*scyllo*-inosamine 354 – RP-HPLC (Method 1)



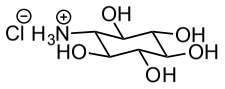
Time	Area	Area %
9.932	138,738	0.62
11.459	21,350,728	95.25
12.201	924,866	4.13
Total	22,414,332	100.00

scyllo-Inosamine hydrochloride 13 – ^{1}H NMR spectrum

scyllo-Inosamine hydrochloride 13 – ^{13}C NMR spectrum



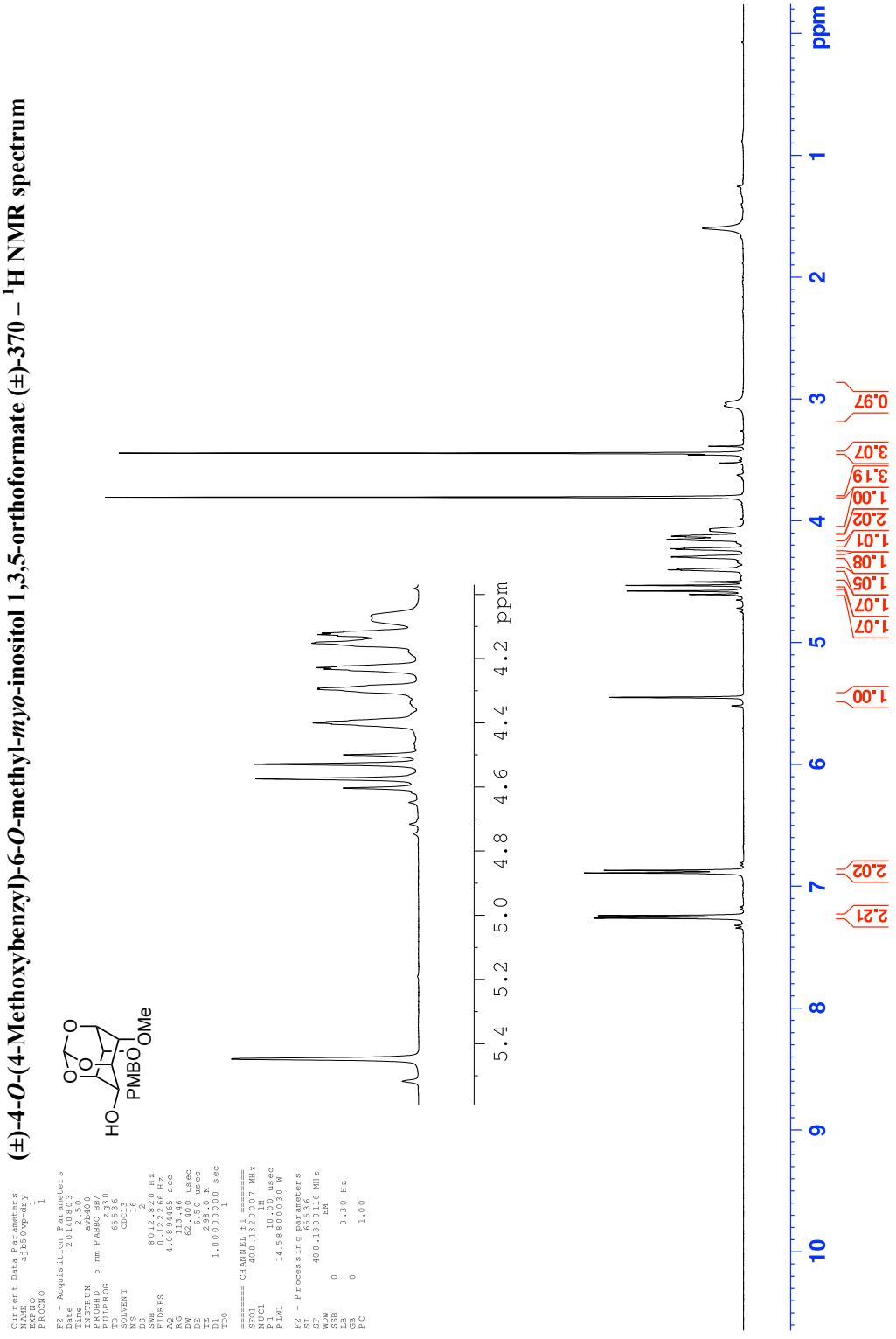
***scyllo*-Inosamine hydrochloride 13 – GC-MS chromatogram (derivatised with TMSI)**



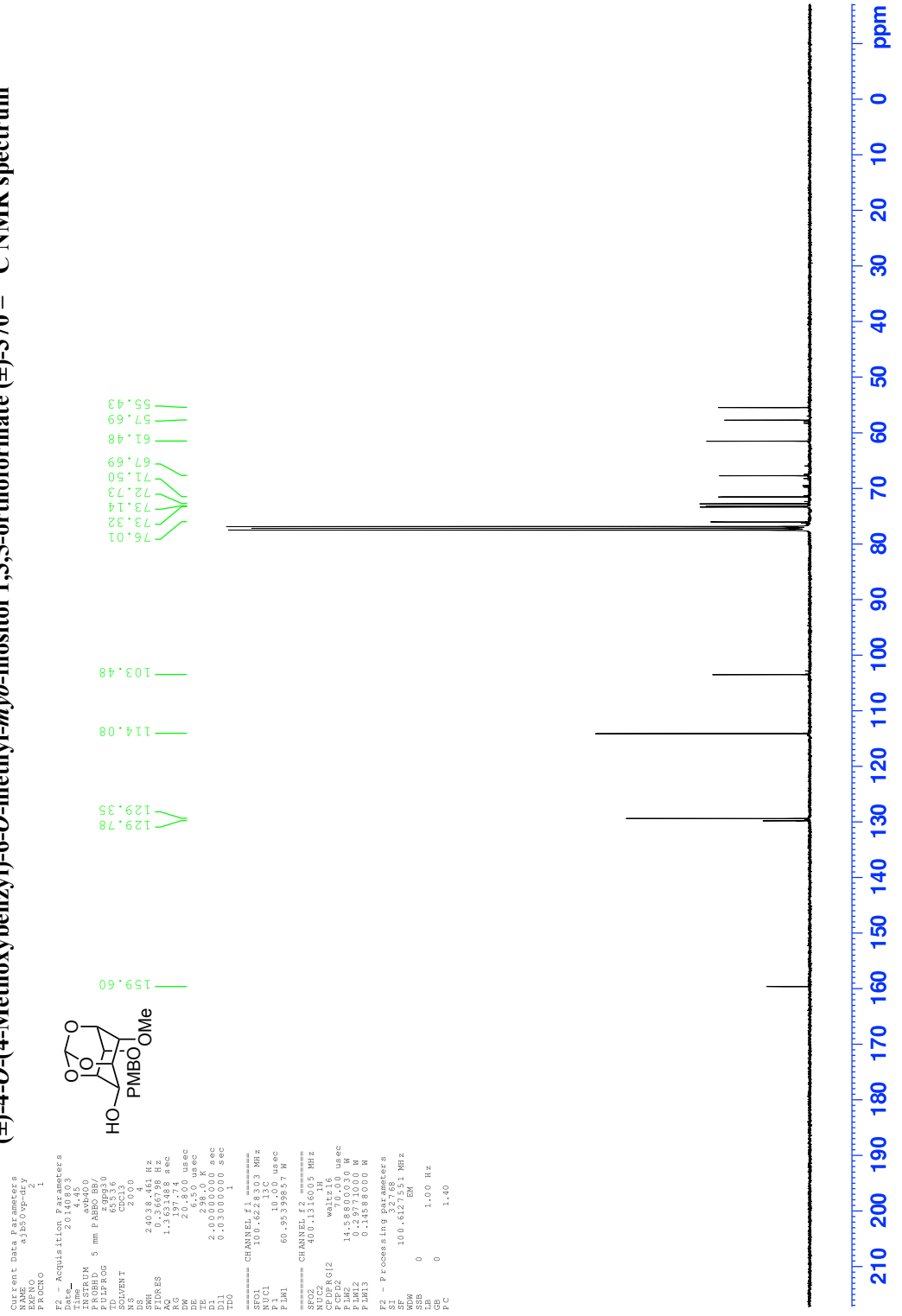
Retention time (min)	Area
11.204	98949704
11.248	197465628
16.809	**685934003**

Total area = 685934003 **Percentage Purity** = 100%

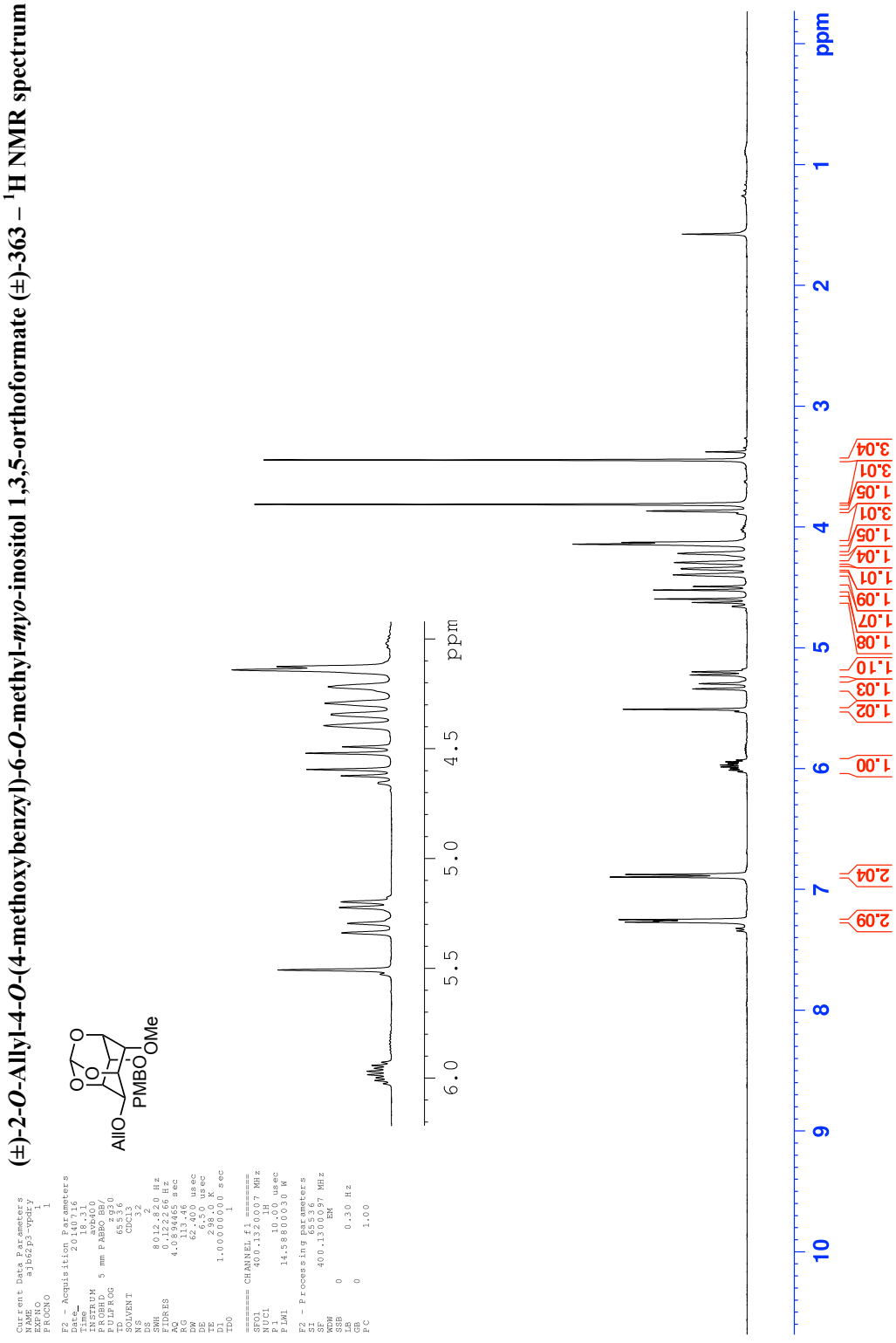
(±)-4-*O*-(4-Methoxybenzyl)-6-*O*-methyl-*myo*-inositol 1,3,5-orthoformate (±)-370 – ^{1}H NMR spectrum



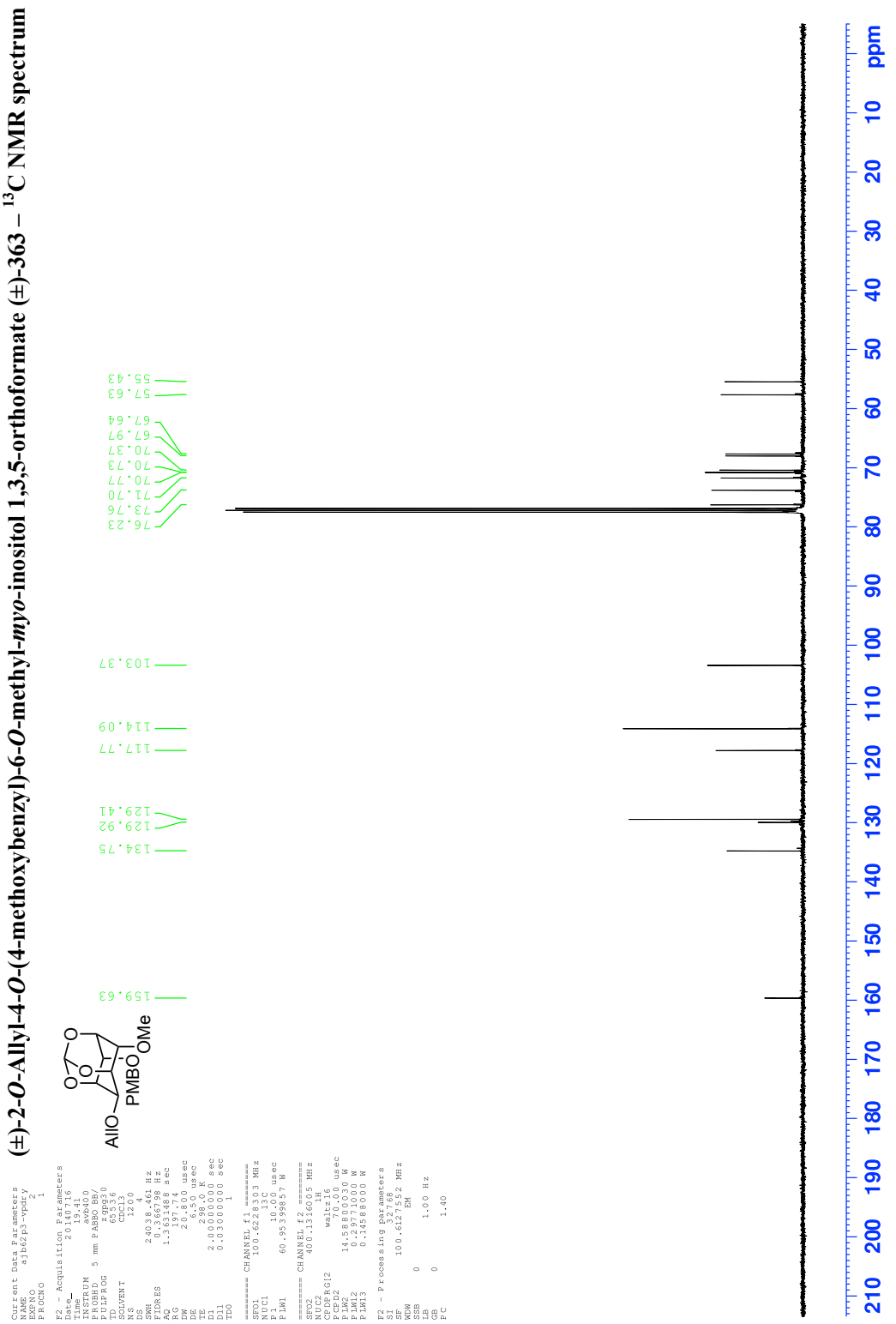
(±)-4-*O*-(4-Methoxybenzyl)-6-*O*-methyl-*myo*-inositol 1,3,5-orthoformate (±)-370 – ^{13}C NMR spectrum



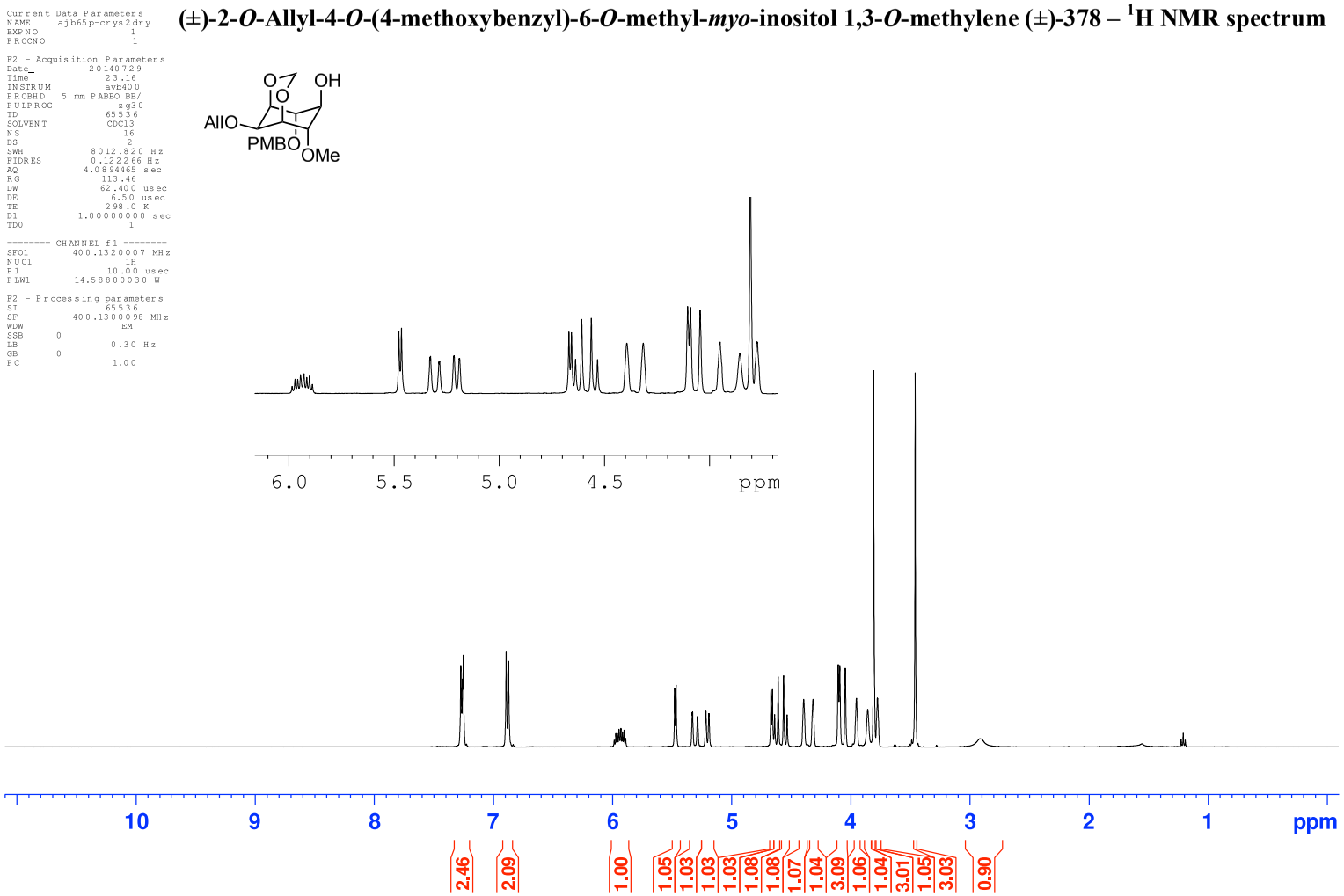
(±)-2-*O*-Allyl-4-*O*-(4-methoxybenzyl)-6-*O*-methyl-*myo*-inositol 1,3,5-orthoformate (±)-363 – ^{1}H NMR spectrum



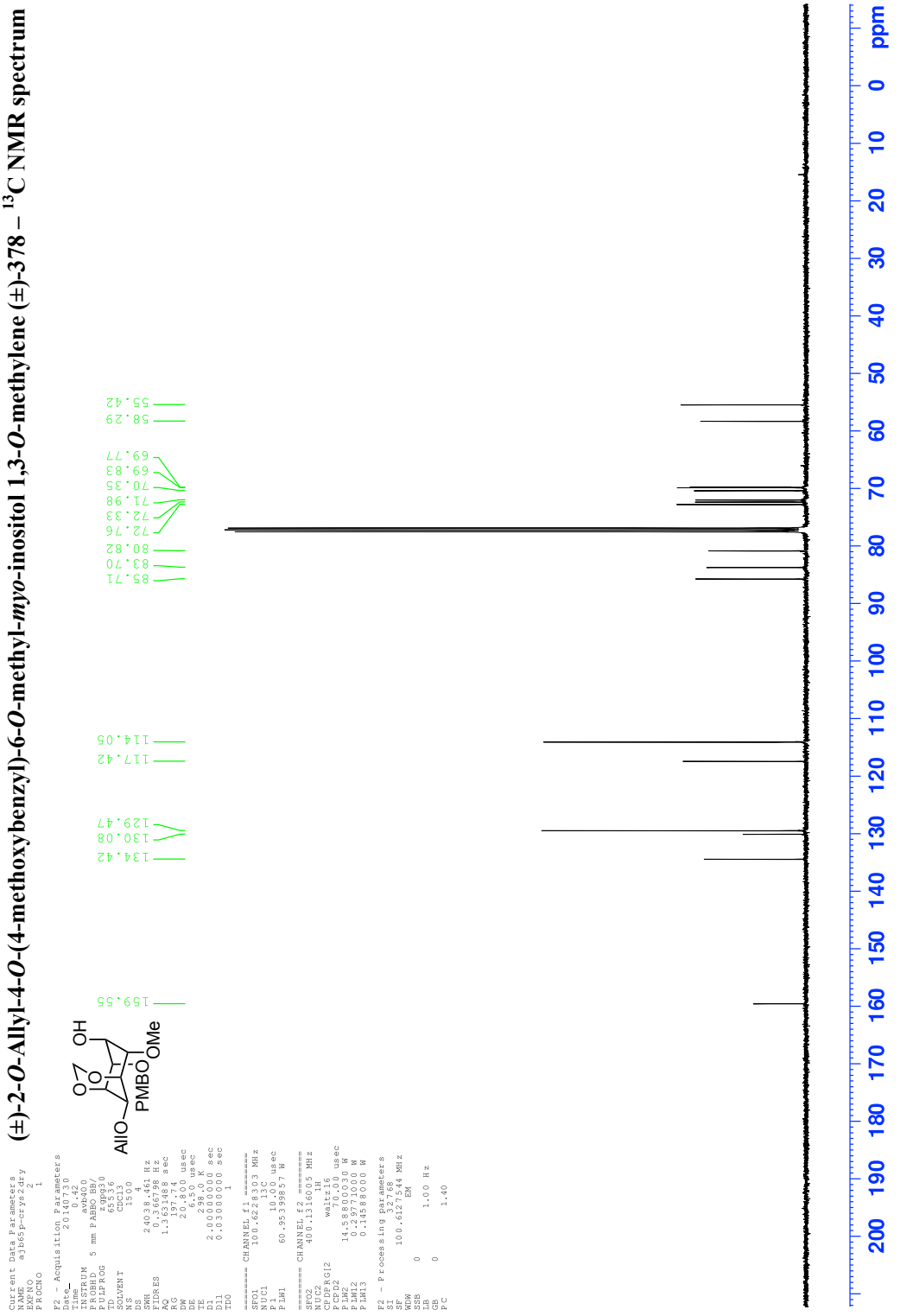
(±)-2-*O*-Allyl-4-*O*-(4-methoxybenzyl)-6-*O*-methyl-*myo*-inositol 1,3,5-orthoformate (±)-363 – ^{13}C NMR spectrum



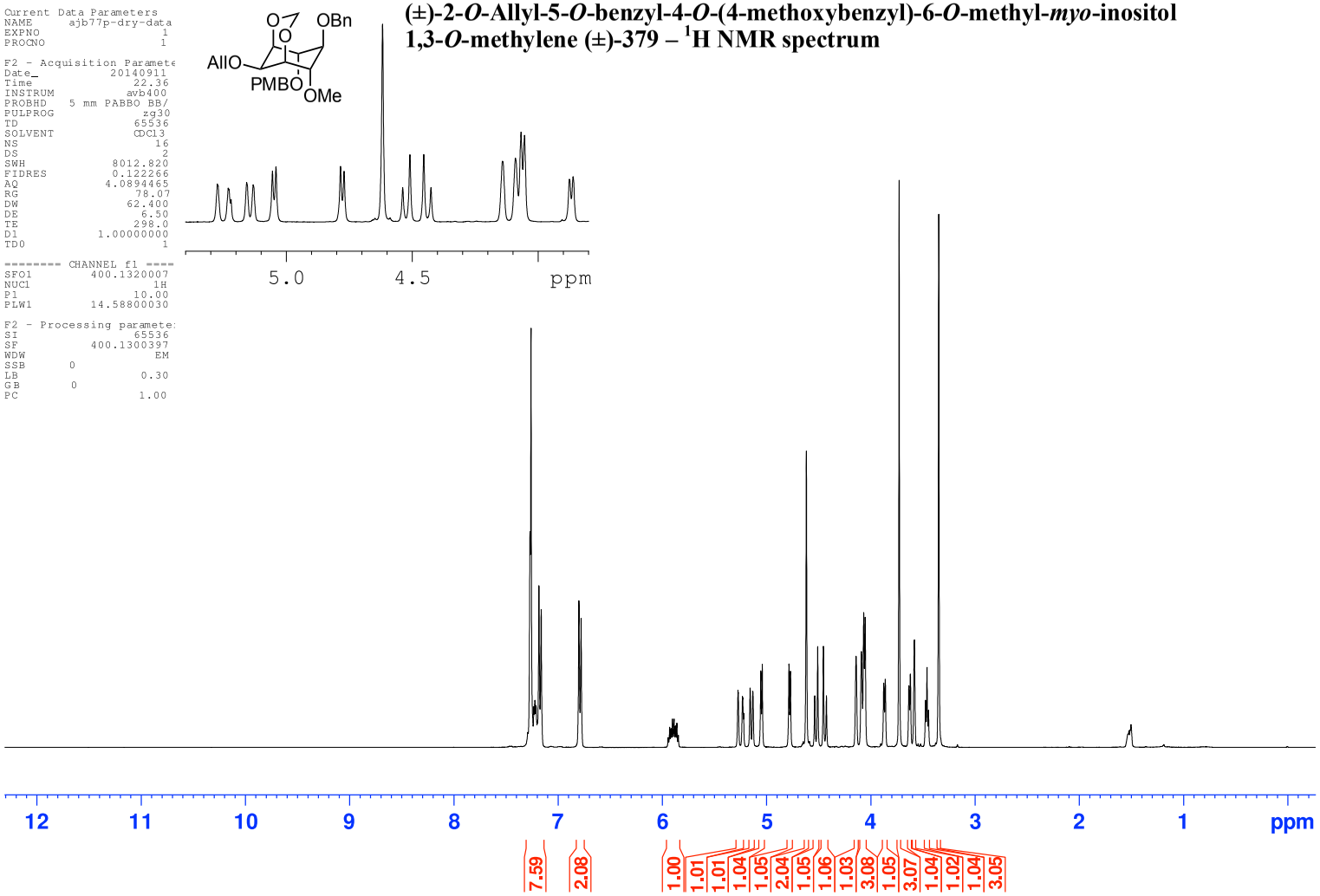
(±)-2-*O*-Allyl-4-*O*-(4-methoxybenzyl)-6-*O*-methyl-*myo*-inositol 1,3-*O*-methylene (±)-378 – ^{1}H NMR spectrum



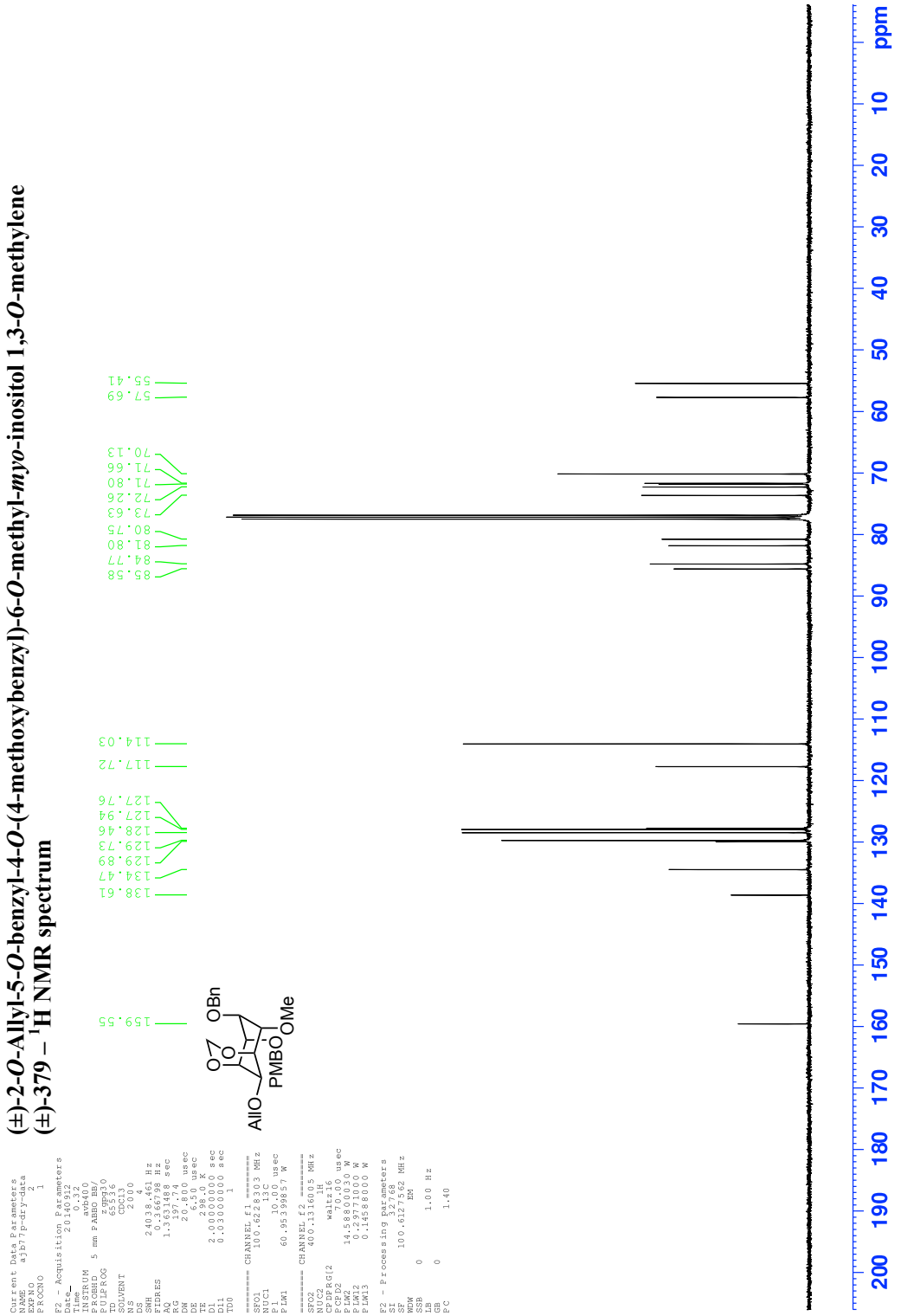
(±)-2-*O*-Allyl-4-*O*-(4-methoxybenzyl)-6-*O*-methyl-*myo*-inositol 1,3-*O*-methylene (±)-378 – ^{13}C NMR spectrum



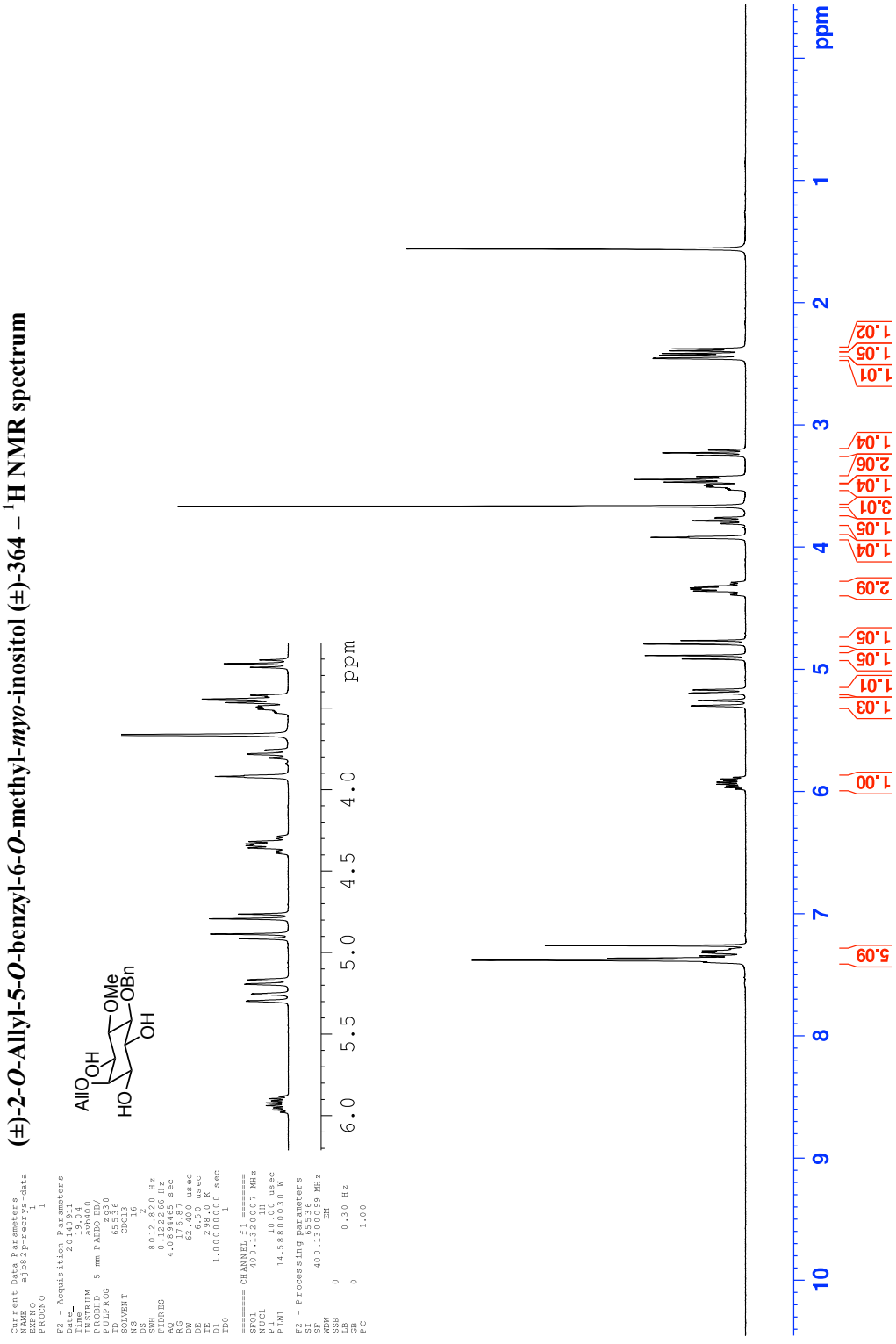
(±)-2-*O*-Allyl-5-*O*-benzyl-4-*O*-(4-methoxybenzyl)-6-*O*-methyl-*myo*-inositol 1,3-*O*-methylene (±)-379 – ^{1}H NMR spectrum



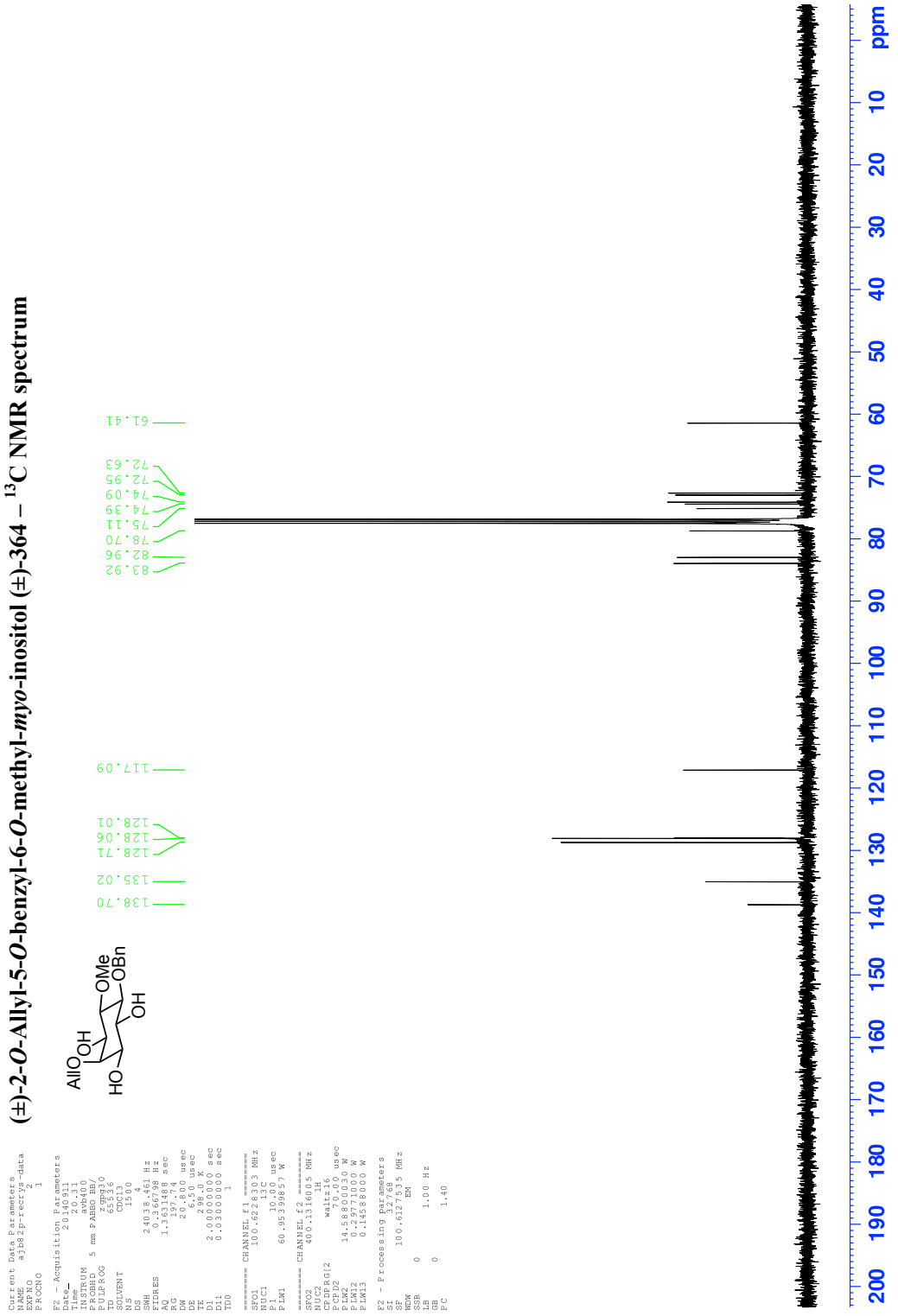
(±)-2-*O*-Allyl-5-*O*-benzyl-4-*O*-(4-methoxybenzyl)-6-*O*-methyl-*myo*-inositol 1,3-*O*-methylene
(±)-379 – ^{1}H NMR spectrum



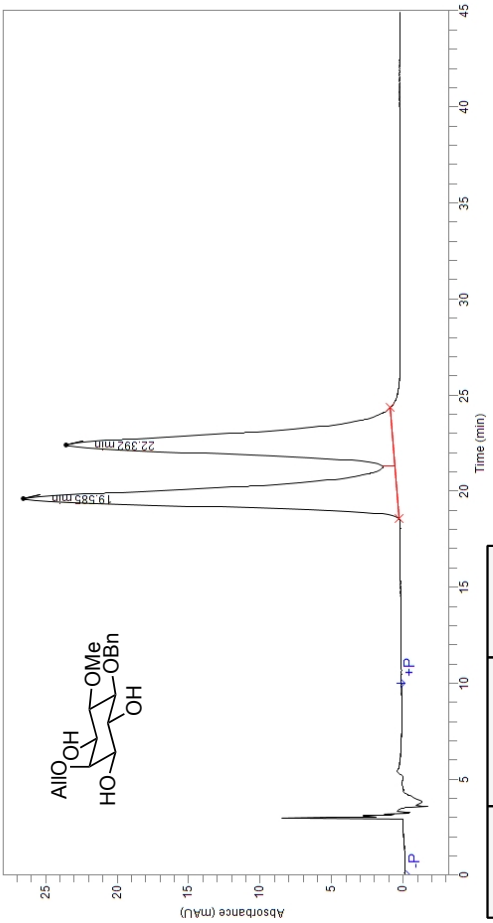
(±)-2-*O*-Allyl-5-*O*-benzyl-6-*O*-methyl-*myo*-inositol (±)-364 – ^{1}H NMR spectrum



(±)-2-*O*-Allyl-5-*O*-benzyl-6-*O*-methyl-*myo*-inositol (±)-364 – ^{13}C NMR spectrum

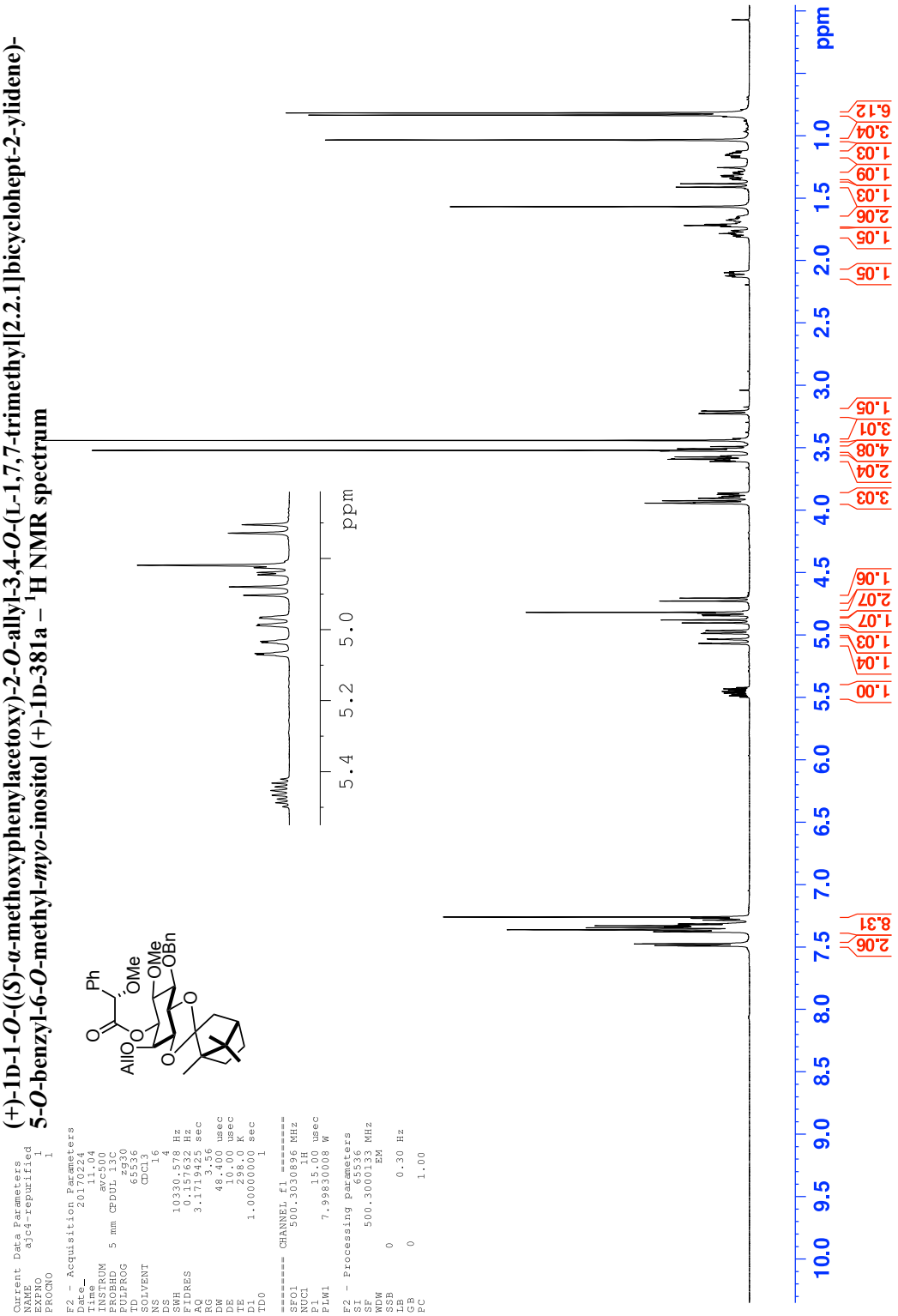


(±)-2-*O*-Allyl-5-*O*-benzyl-6-*O*-methyl-*myo*-inositol (±)-364 – Chiral HPLC (Heptane/IPA 90:10, 0.8 mL.min^{-1}, 254 nm)

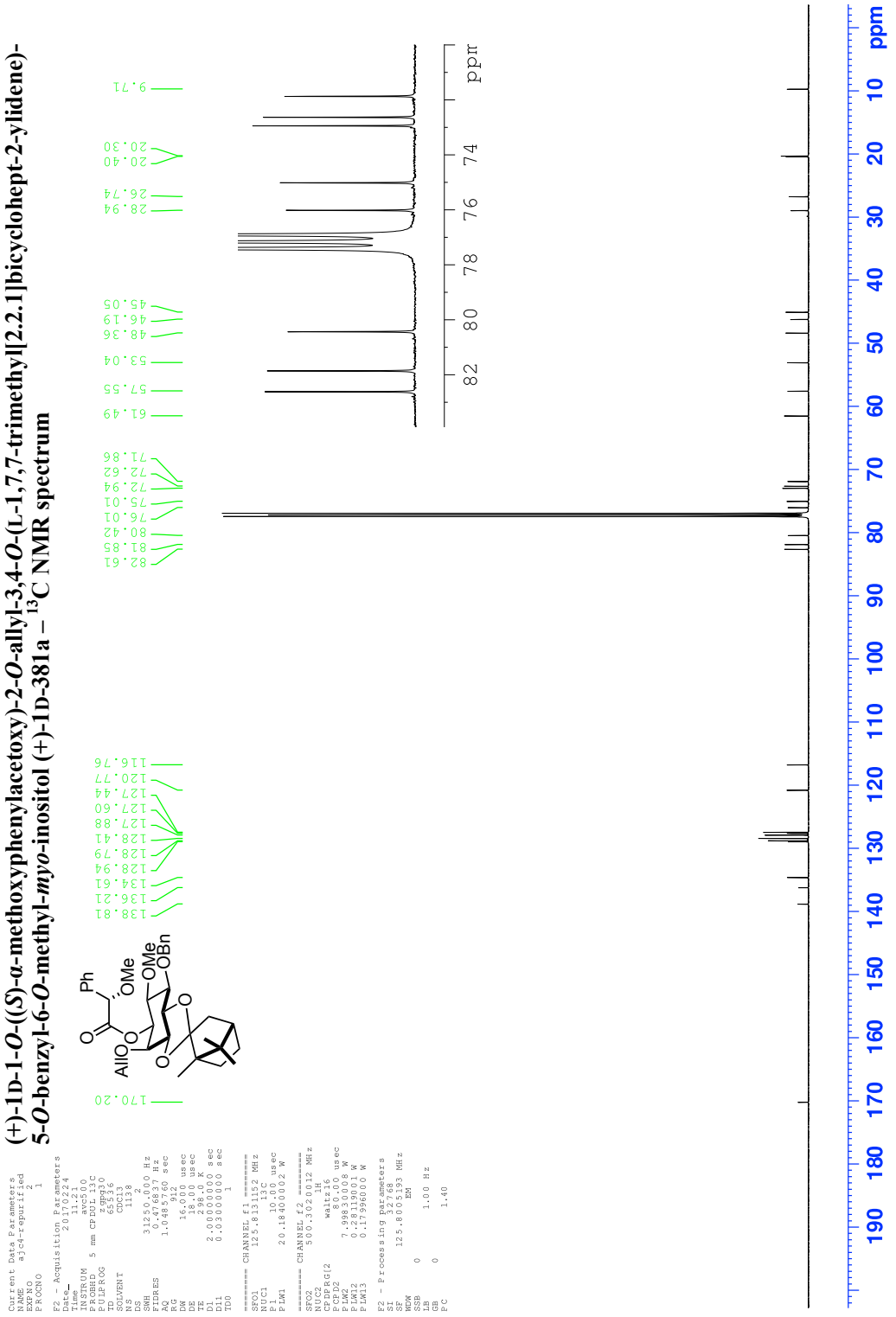


Time	Area	Area %
19.585	1,655,418	50.49
22.392	1,623,458	49.51
Total	3,278,876	100.00

(+)-1D-1-*O*-((*S*)-α-methoxyphenylacetoxy)-2-*O*-allyl-3,4-*O*-(L-1,7,7-trimethyl[2.2.1]bicycloheptan-2-ylidene)-5-*O*-benzyl-6-*O*-methyl-*myo*-inositol (+)-1D-381a – ^{1}H NMR spectrum



(+)-1D-1-*O*-((*S*)-α-methoxyphenylacetoxy)-2-*O*-allyl-3,4-*O*-(L-1,7,7-trimethyl[2.2.1]bicycloheptan-2-ylidene)-5-*O*-benzyl-6-*O*-methyl-*myo*-inositol (+)-1D-381a – ^{13}C NMR spectrum




```
Current Data Parameters
NAME          ajc4-repurified
EXPNO                       2
PROCNO                      1

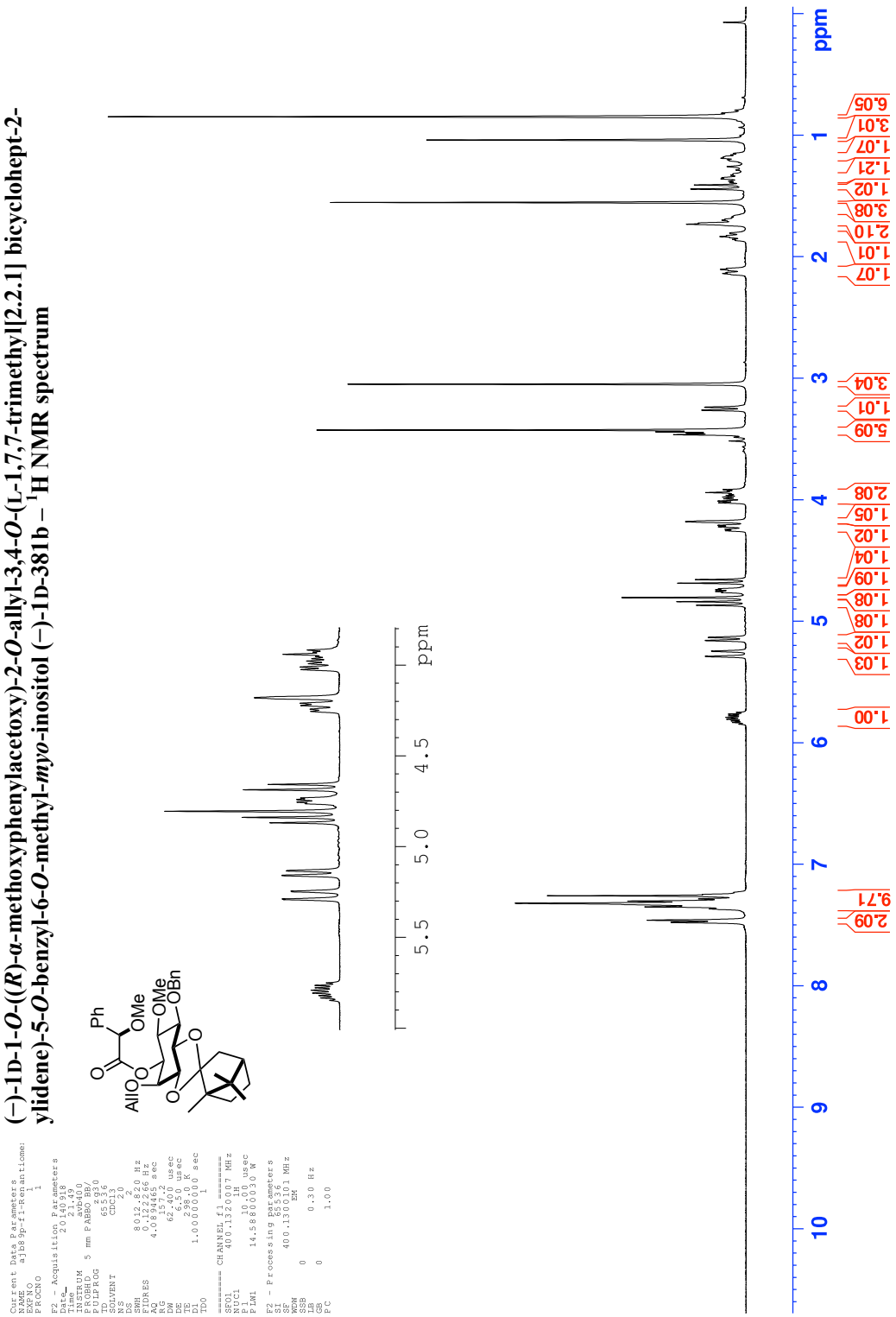
F2 - Acquisition Parameters
Date_          20170224
Time              11:21
INSTRUM         avc500
PROBHD   5 mm CPDUL 13C
PULPROG          zgpg30
TD              65536
SOLVENT         CDCl3
NS               1138
DS                  4
SWH          31250.000 Hz
FIDRES       0.476837 Hz
AQ           1.048576 sec
RG                912
DW            16.000 usec
DE             18.00 usec
TE             298.0 K
D1          2.00000000 sec
D11         0.03000000 sec
TD0                 1

======== CHANNEL f1 ========
SFO1       125.8131152 MHz
NUC1              13C
P1              10.00 usec
PLW1        20.18400002 W

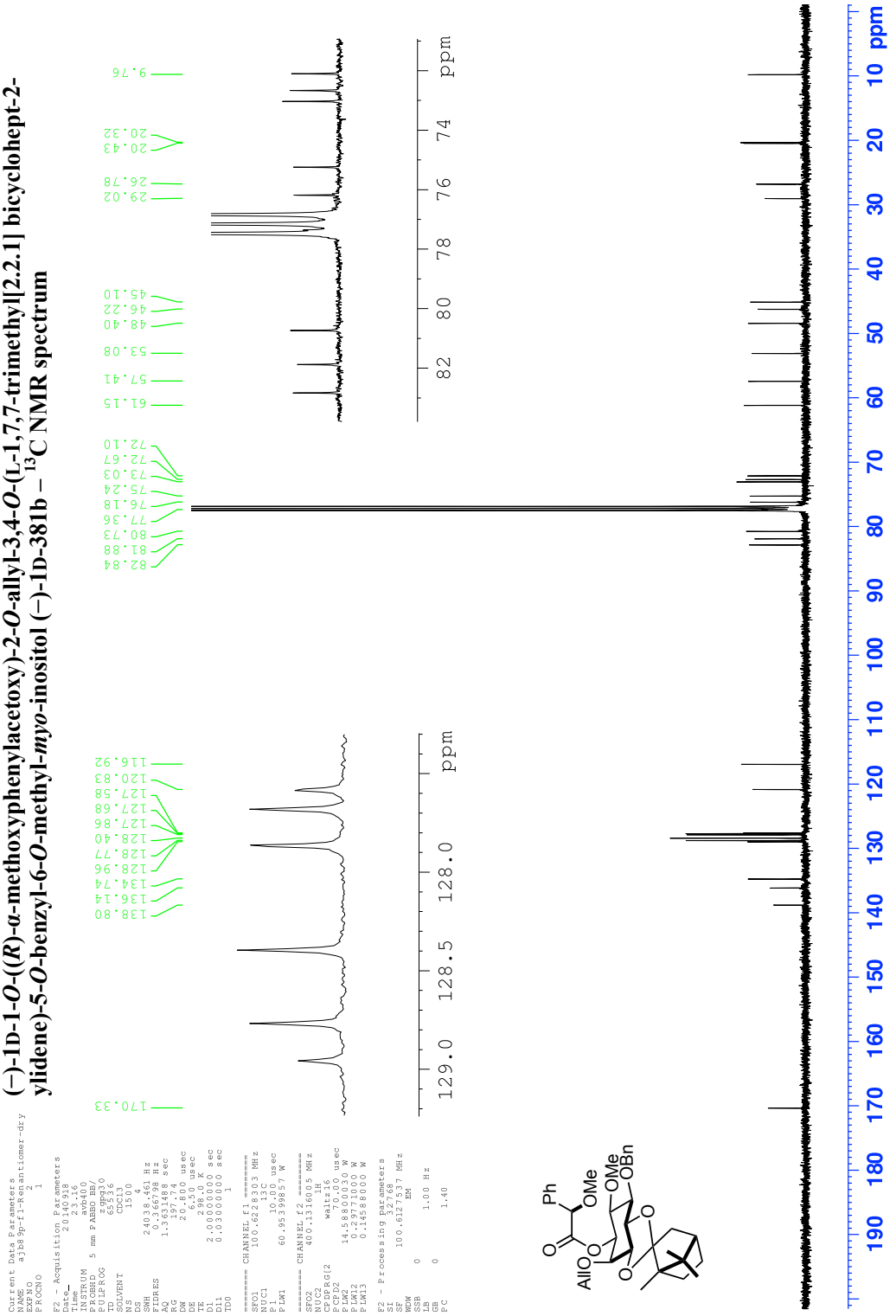
======== CHANNEL f2 ========
SFO2       500.3020012 MHz
NUC2               1H
CPDPRG2        waltz16
PCPD2          80.00 usec
PLW2        7.99830008 W
PLW12       0.42313001 W
PLW13       0.17396000 W

F2 - Processing parameters
SI              131072
SF         125.8005193 MHz
WDW                EM
SSB                 0
LB               1.00 Hz
GB                  0
PC               1.40
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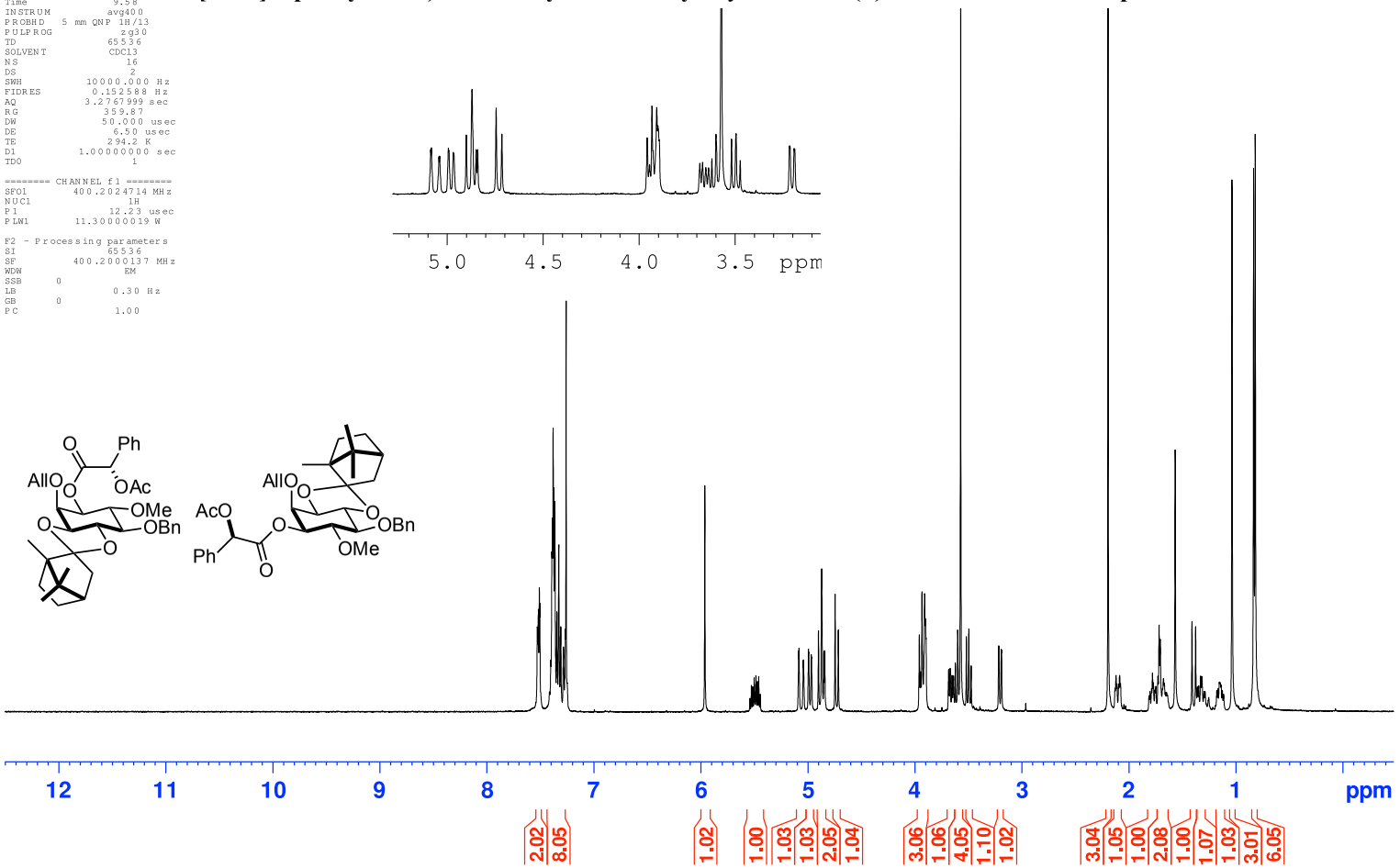
(–)-1D-1-*O*-((*R*)-α-methoxyphenylacetoxy)-2-*O*-allyl-3,4-*O*-(L-1,7,7-trimethyl[2.2.1] bicycloheptyl-2-ylidene)-5-*O*-benzyl-6-*O*-methyl-*myo*-inositol (–)-1D-381b – ^{1}H NMR spectrum



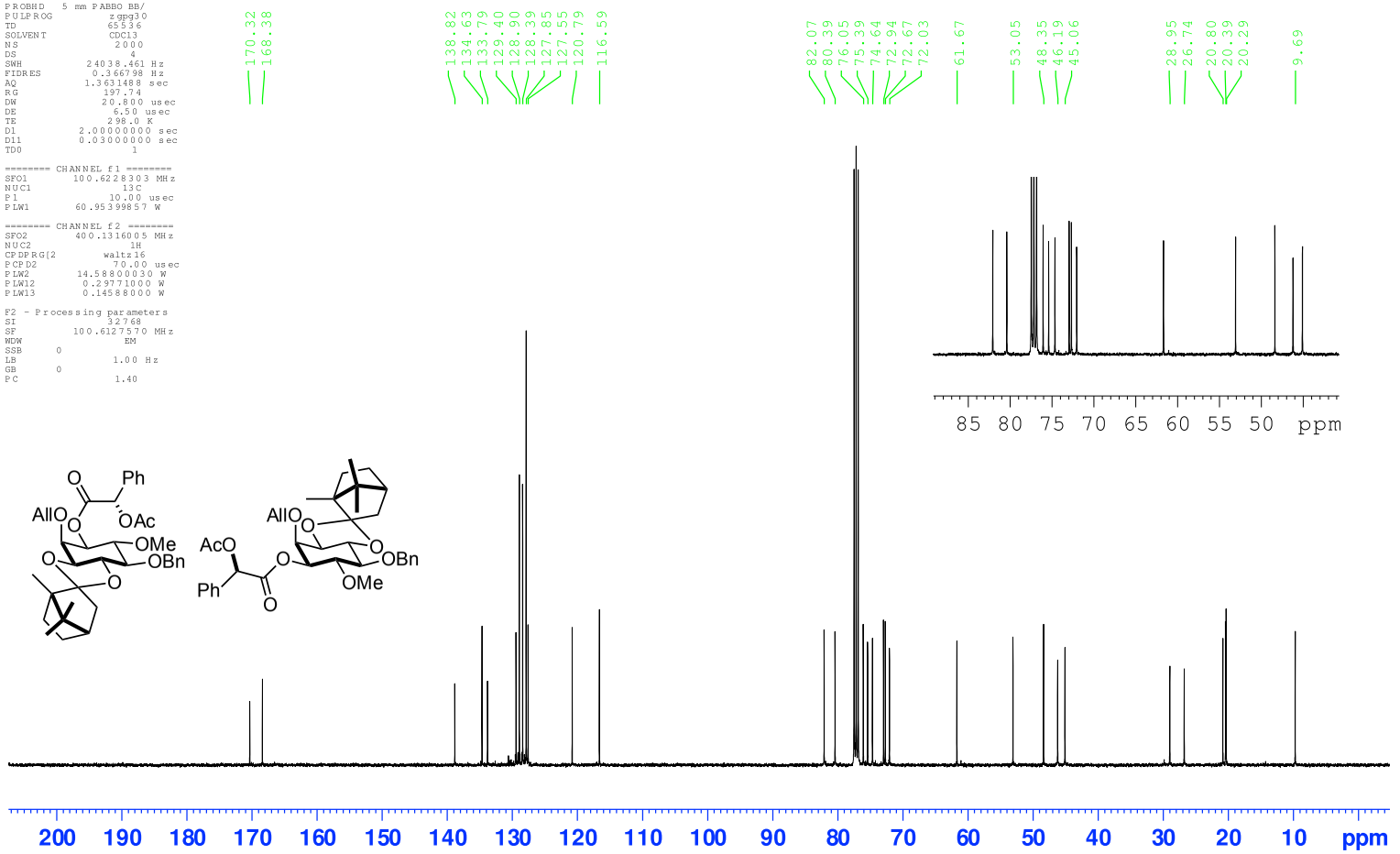
(−)-1D-1-*O*-((*R*)-α-methoxyphenylacetoxy)-2-*O*-allyl-3,4-*O*-(L-1,7,7-trimethyl[2.2.1] bicycloheptan-2-ylidene)-5-*O*-benzyl-6-*O*-methyl-*myo*-inositol (−)-1D-381b – ^{13}C NMR spectrum

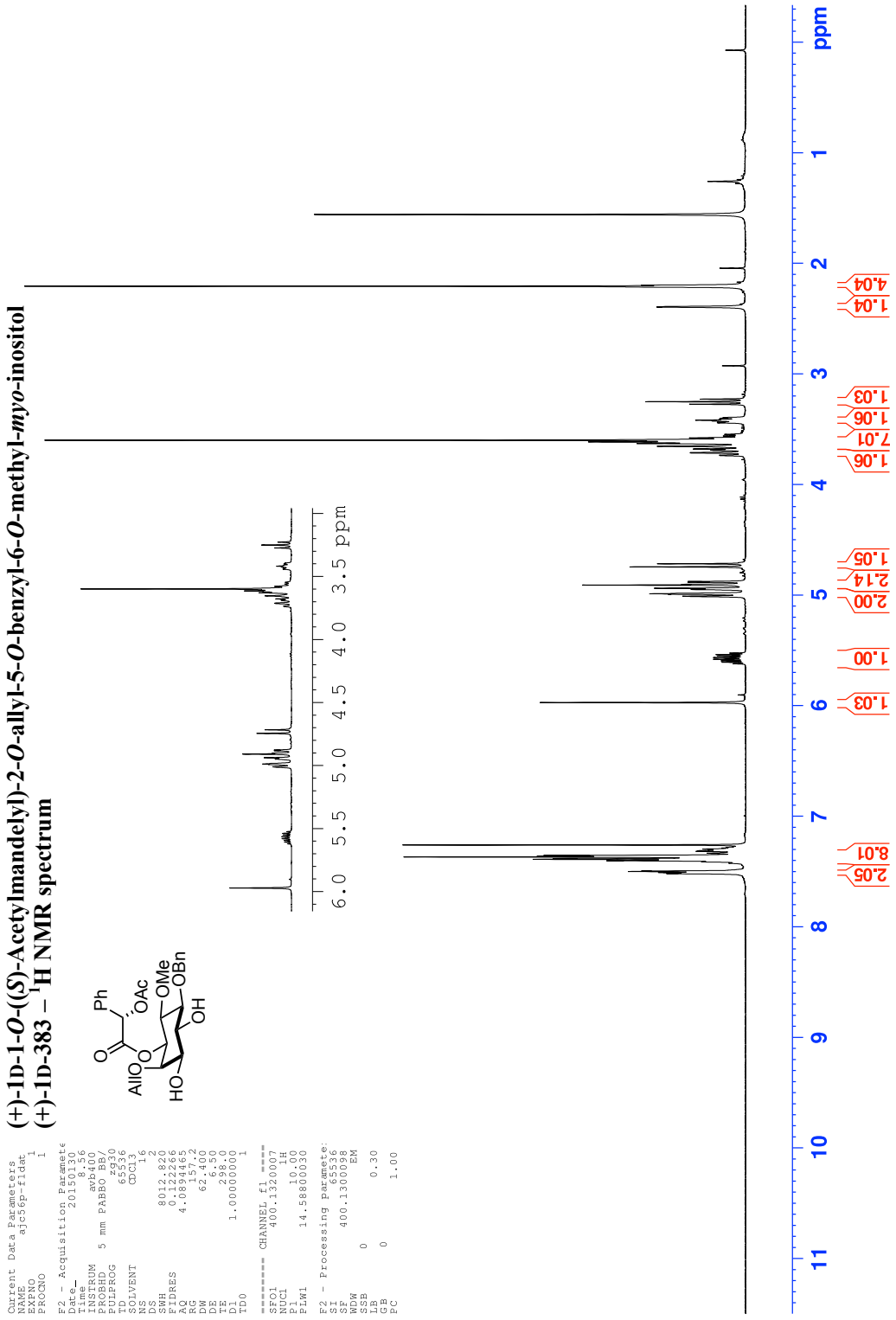


(+)-1D-1-*O*-((*S*)-Acetylmandelyl)-3,4-*O*-(L-1,7,7-trimethylbicyclo[2.2.1]hept-2-ylidene)-5-*O*-benzyl-6-*O*-methyl-*myo*-inositol (+)-1D-382a & (−)-1L-1-*O*-((*R*)-acetylmandelyl)-2-*O*-allyl-3,4-*O*-(L-1,7,7-trimethylbicyclo[2.2.1]hept-2-ylidene)-5-*O*-benzyl-6-*O*-methyl-*myo*-inositol (−)-1L-382c – ^{1}H NMR spectrum

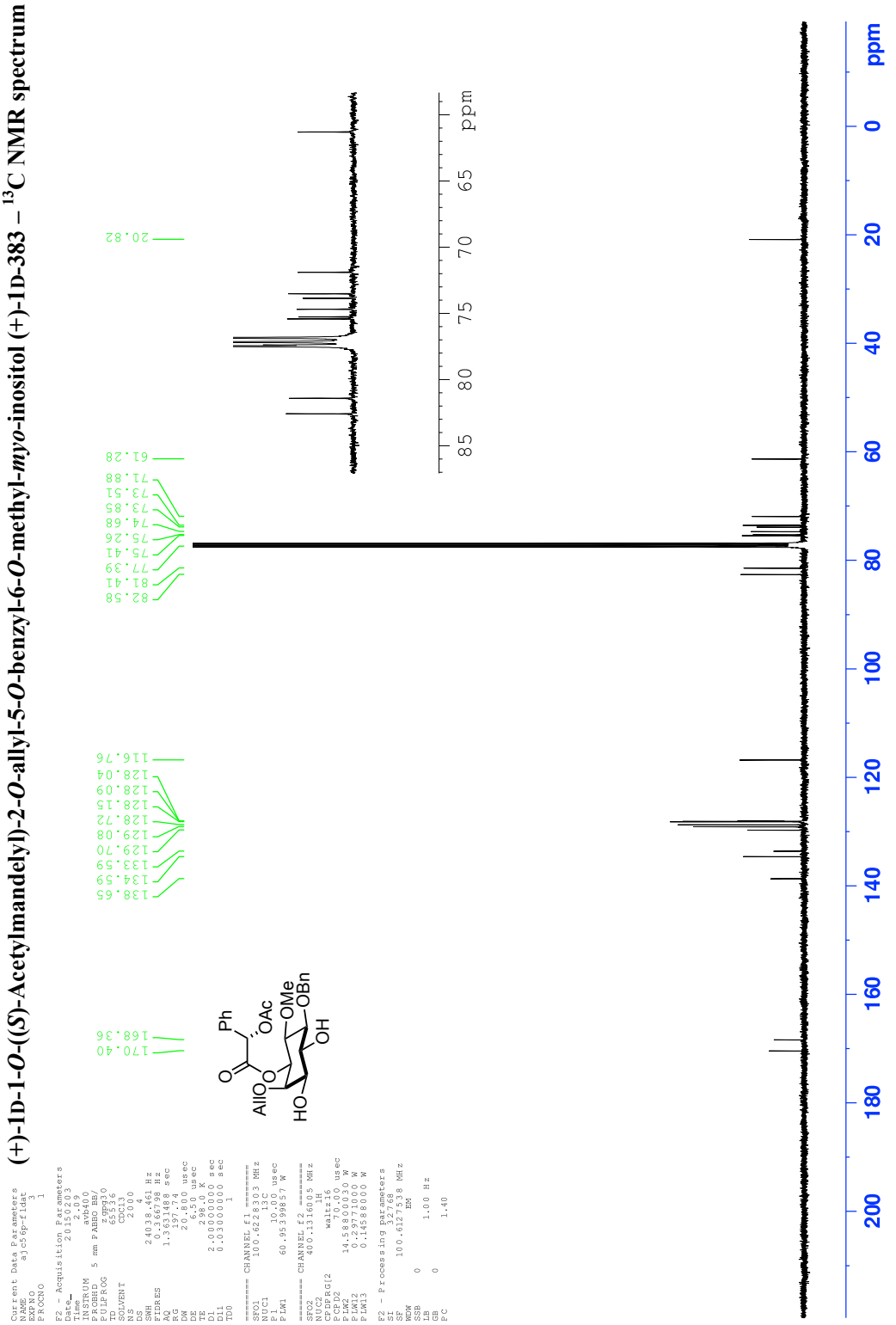


(+)-1D-1-*O*-((*S*)-Acetylmandelyl)-3,4-*O*-(L-1,7,7-trimethylbicyclo[2.2.1]hept-2-ylidene)-5-*O*-benzyl-6-*O*-methyl-*myo*-inositol (+)-1D-382a & (−)-1L-1-*O*-((*R*)-acetylmandelyl)-2-*O*-allyl-3,4-*O*-(L-1,7,7-trimethylbicyclo-[2.2.1]hept-2-ylidene)-5-*O*-benzyl-6-*O*-methyl-*myo*-inositol (−)-1L-382c – ^{13}C NMR spectrum

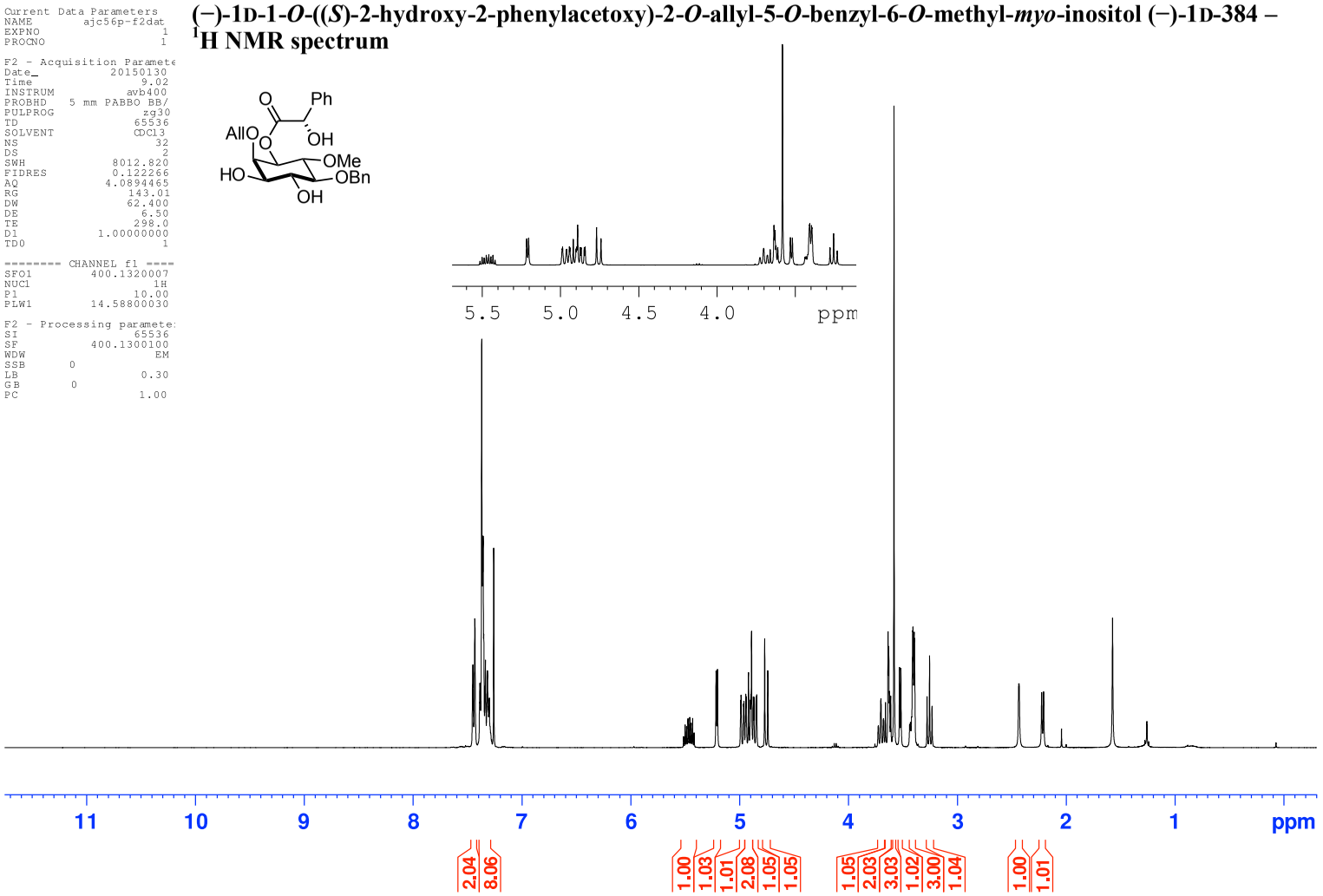



(+)-1D-1-O-((S)-Acetylmandelyl)-2-O-allyl-5-O-benzyl-6-O-methyl-myo-inositol
(+)-1D-383 – 1H NMR spectrum
Ph
OAc
OMe
OBn
OH
AllO
HO
6.0 5.5 5.0 4.5 4.0 3.5 ppm
ppm
2.05
8.01
1.03
1.00
2.00
2.14
1.05
1.06
7.01
1.06
1.03
1.04
4.04

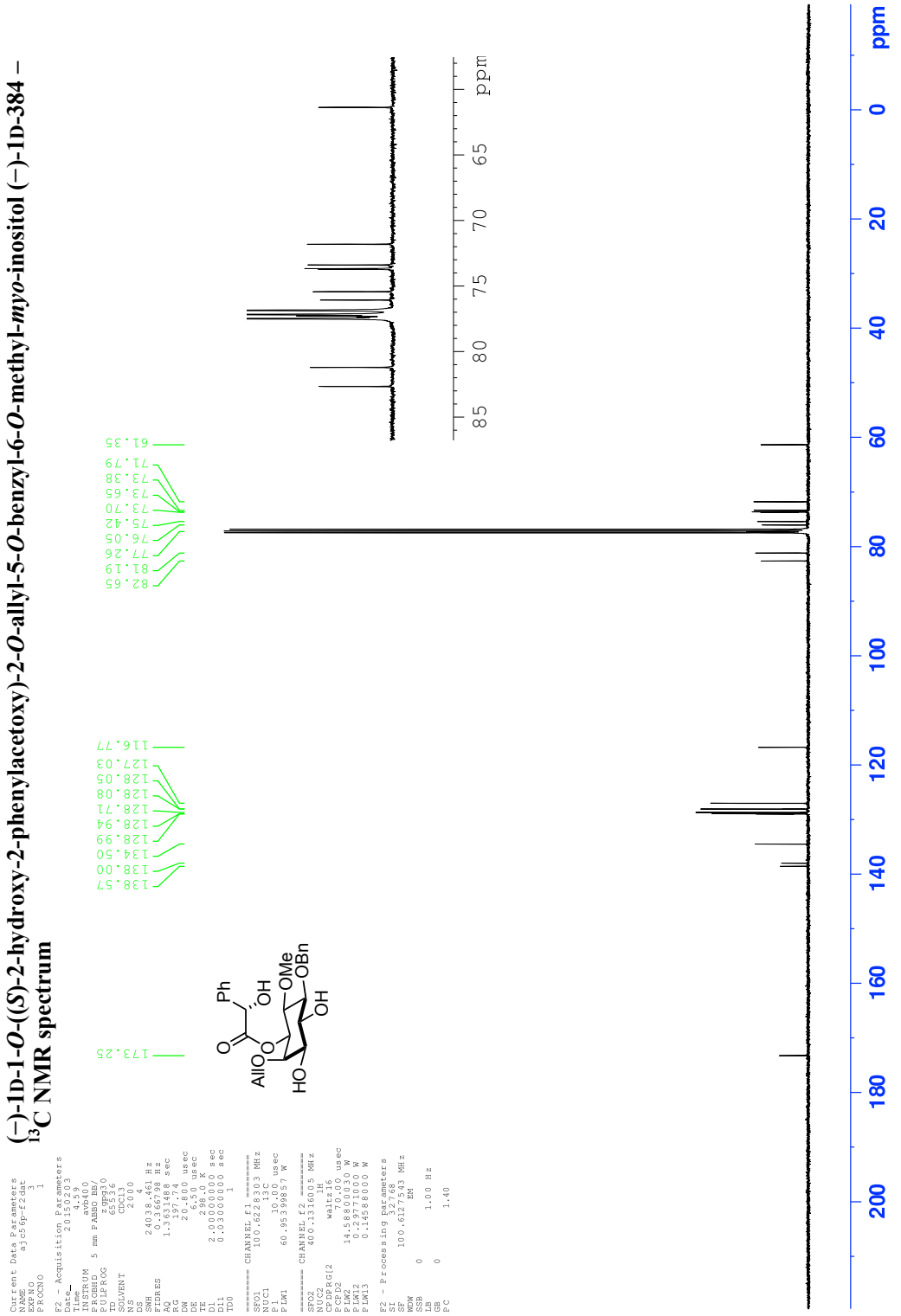
(+)-1D-1-*O*-((*S*)-Acetylmandelyl)-2-*O*-allyl-5-*O*-benzyl-6-*O*-methyl-*myo*-inositol (+)-1D-383 – ^{13}C NMR spectrum



(−)-1D-1-*O*-((*S*)-2-hydroxy-2-phenylacetoxy)-2-*O*-allyl-5-*O*-benzyl-6-*O*-methyl-*myo*-inositol (−)-1D-384 – ^{1}H NMR spectrum



(−)-1D-1-*O*-((*S*)-2-hydroxy-2-phenylacetoxy)-2-*O*-allyl-5-*O*-benzyl-6-*O*-methyl-*myo*-inositol (−)-1D-384 – ^{13}C NMR spectrum



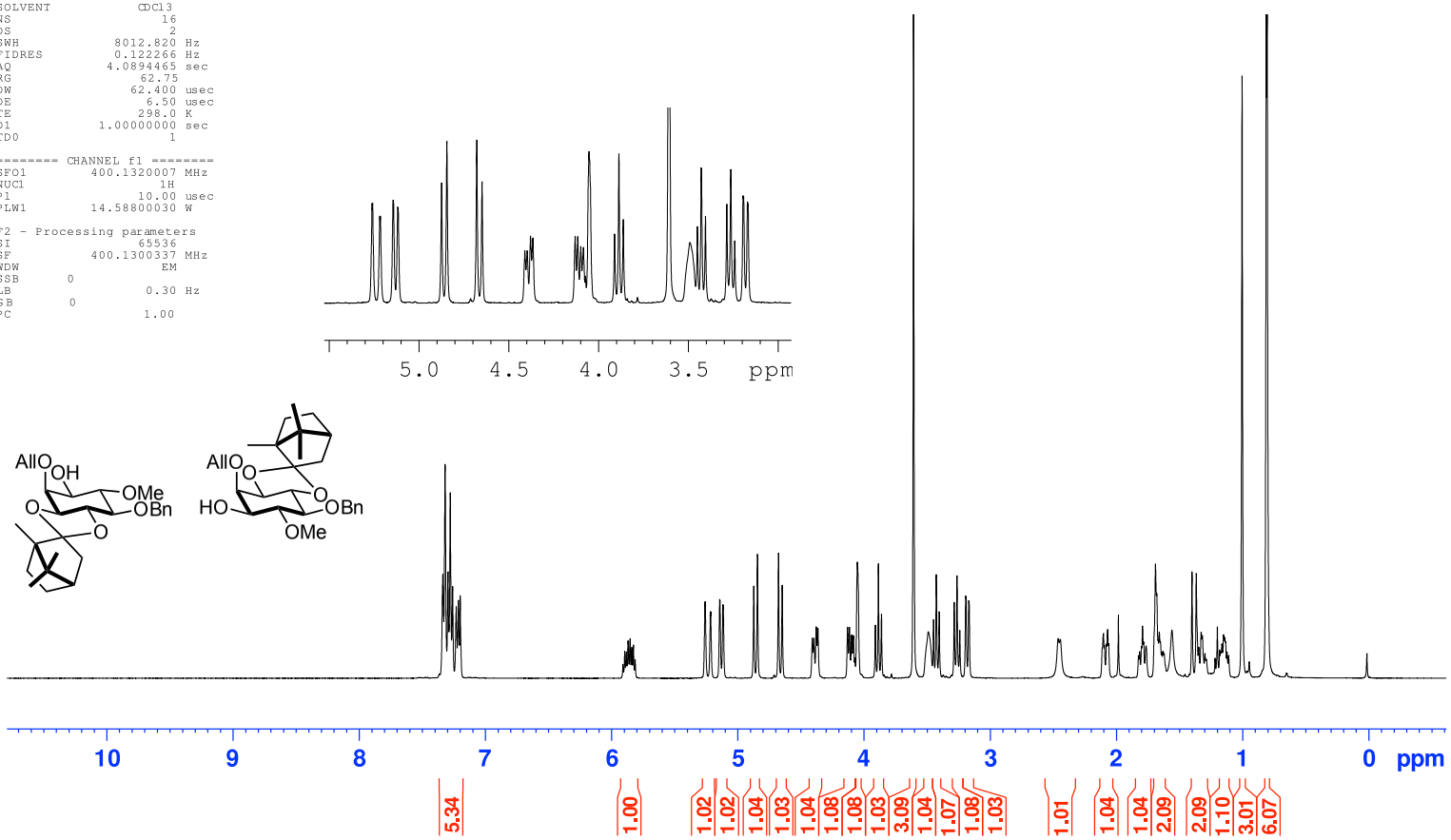
(−)-1D-2-*O*-Allyl-3,4-*O*-(L-1,7,7-trimethylbicyclo[2.2.1]hept-2-ylidene)-5-*O*-benzyl-6-*O*-methyl-*myo* inositol
(−)-1D-380a & (+)-1L-2-*O*-allyl-3,4-*O*-(L-1,7,7-trimethylbicyclo[2.2.1]hept-2-ylidene)-4-*O*-methyl-5-*O*-benzyl-*myo* inositol (+)-1L-380c – ^{1}H NMR spectrum

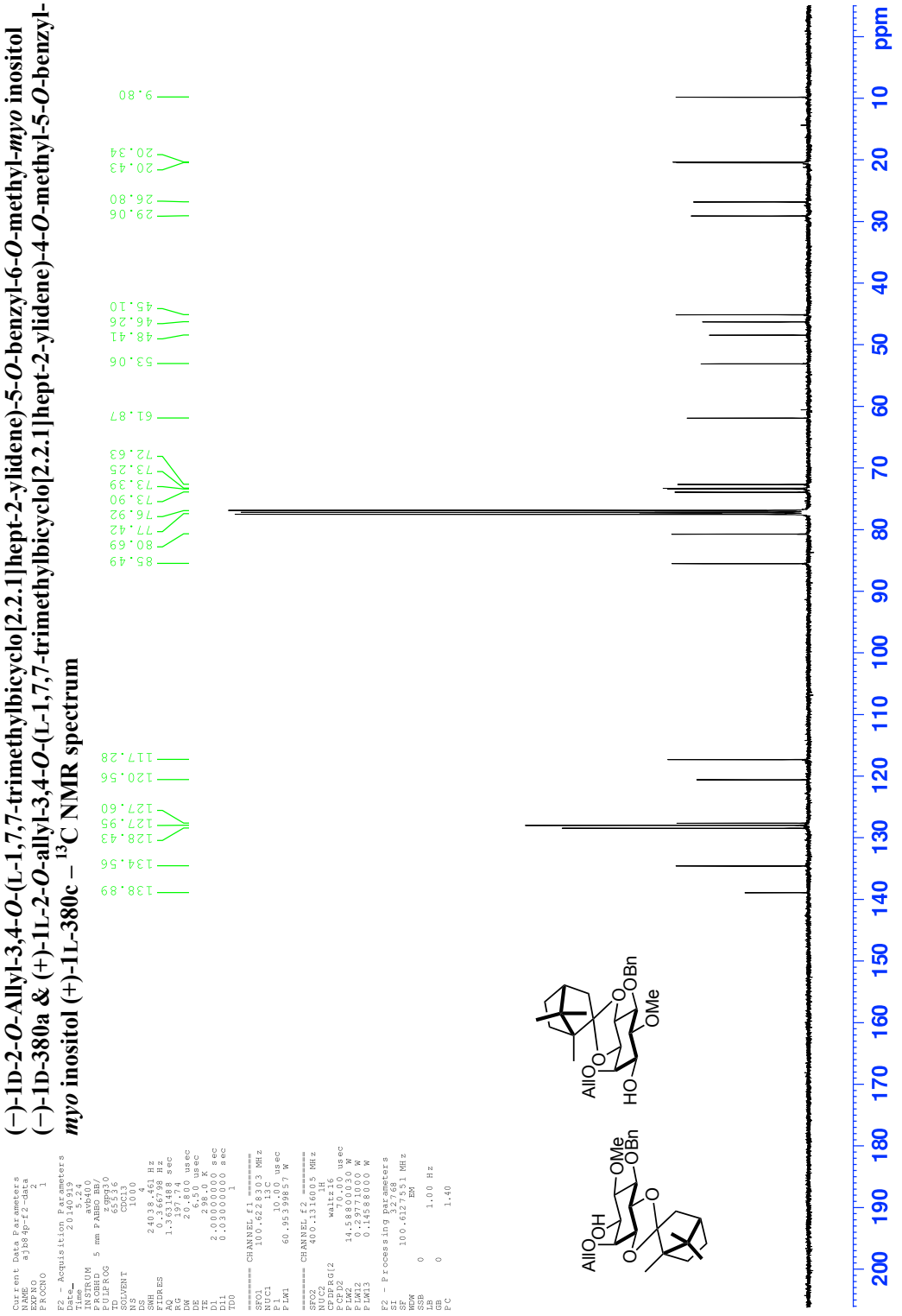
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EXPNO                 1
PROCNO                1

F2 - Acquisition Parameters
Date_          20140919
Time               4.26
INSTRUM          avb400
PROBHD   5 mm PABBO BB/
PULPROG            zg30
TD                65536
SOLVENT           CDCl3
NS                   16
DS                    2
SWH            8012.820 Hz
FIDRES         0.122266 Hz
AQ             4.0894465 sec
RG                62.75
DW                62.400 usec
DE                 6.50 usec
TE               298.0 K
D1            1.00000000 sec
TD0                   1

======== CHANNEL f1 ========
SFO1         400.1320007 MHz
NUC1                 1H
P1                10.00 usec
PLW1         14.58800030 W

F2 - Processing parameters
SI                65536
SF           400.1300337 MHz
WDW                  EM
SSB       0
LB                0.30 Hz
GB        0
PC                1.00
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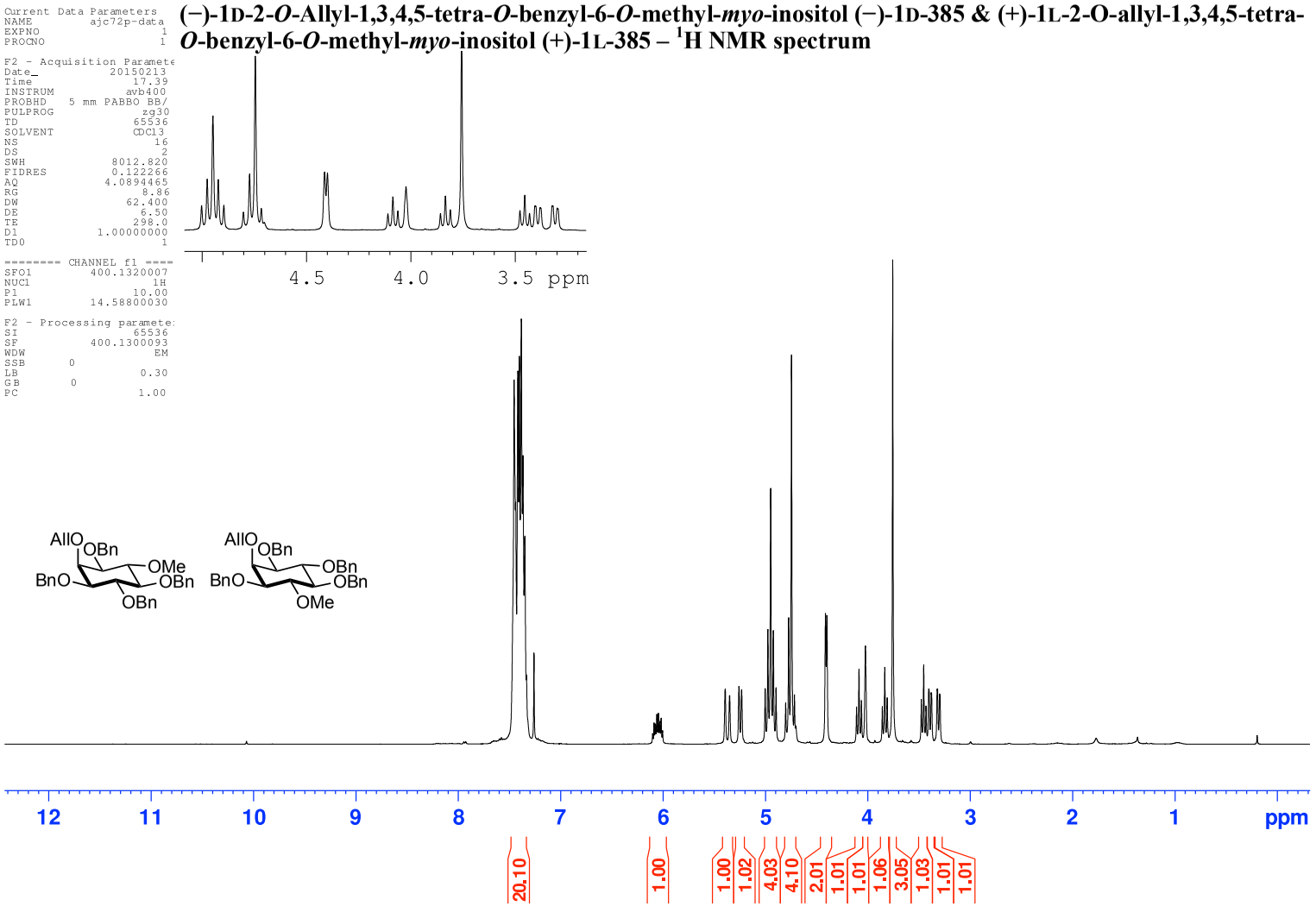

(−)-1D-2-O-Allyl-3,4-O-(L-1,7,7-trimethylbicyclo[2.2.1]hept-2-ylidene)-5-O-benzyl-6-O-methyl-myo inositol
(−)-1D-380a & (+)-1L-2-O-allyl-3,4-O-(L-1,7,7-trimethylbicyclo[2.2.1]hept-2-ylidene)-4-O-methyl-5-O-benzyl-myo inositol (+)-1L-380c – 13C NMR spectrum
138.89
134.56
128.43
127.95
127.60
120.56
117.28
85.49
80.69
77.42
76.92
73.90
73.39
73.25
72.63
61.87
53.06
48.41
46.26
45.10
29.06
26.80
20.43
20.34
9.80
AllO
OH
OMe
OBn
AllO
O
HO
OBn
OMe
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm

(−)-1D-2-*O*-Allyl-5-*O*-benzyl-6-*O*-methyl-*myo*-inositol (−)-1D-364 & (+)-1L-2-*O*-allyl-5-*O*-benzyl-6-*O*-methyl-*myo*-inositol (+)-1L-364 – Chiral HPLC (Heptane/IPA 90:10, 0.8 mL.min^{-1}, 254 nm)

Time	Area	Area %
12.940	51	0.00
13.062	40	0.00
19.075	2,180,129	99.99
30.681	105	0.00
Total	2,180,325	100.00

Time	Area	Area %
18.878	86,506	2.39
21.434	3,532,172	97.61
Total	3,618,678	100.00

(−)-1D-2-*O*-Allyl-1,3,4,5-tetra-*O*-benzyl-6-*O*-methyl-*myo*-inositol (−)-1D-385 & (+)-1L-2-O-allyl-1,3,4,5-tetra-*O*-benzyl-6-*O*-methyl-*myo*-inositol (+)-1L-385 – ^{1}H NMR spectrum



(−)-1D-2-*O*-Allyl-1,3,4,5-tetra-*O*-benzyl-6-*O*-methyl-*myo*-inositol (−)-1D-385 & (+)-1L-2-O-allyl-1,3,4,5-tetra-*O*-benzyl-6-*O*-methyl-*myo*-inositol (+)-1L-385 – ^{13}C NMR spectrum

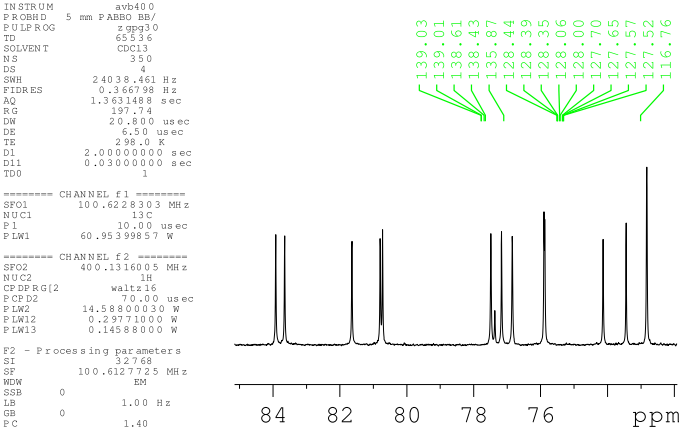
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Current Data Parameters
NAME          ajc72p-data
EXPNO                   2
PROCNO                  1

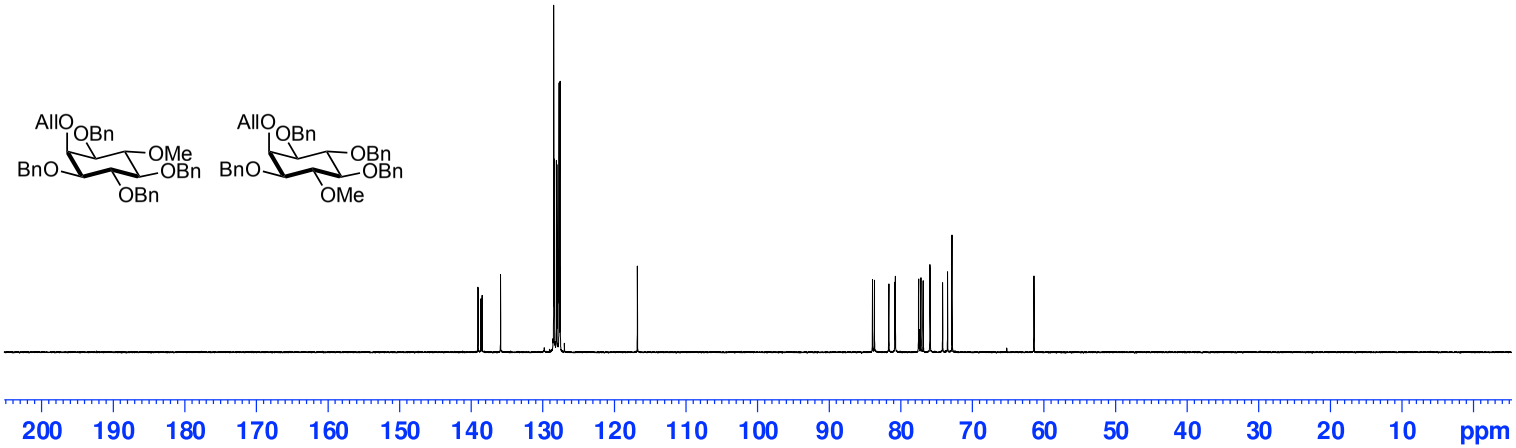
F2 - Acquisition Parameters
Date_          20150213
Time              17.55
INSTRUM           avb400
PROBHD   5 mm PABBO BB/
PULPROG           zgpg30
TD                65536
SOLVENT           CDCl3
NS                  350
DS                    4
SWH           24038.461 Hz
FIDRES         0.366798 Hz
AQ             1.3631488 sec
RG                197.74
DW               20.800 usec
DE                6.50 usec
TE                298.0 K
D1            2.00000000 sec
D11           0.03000000 sec
TD0                   1

======== CHANNEL f1 ========
SFO1          100.6228303 MHz
NUC1                  13C
P1                 10.00 usec
PLW1          60.95399857 W

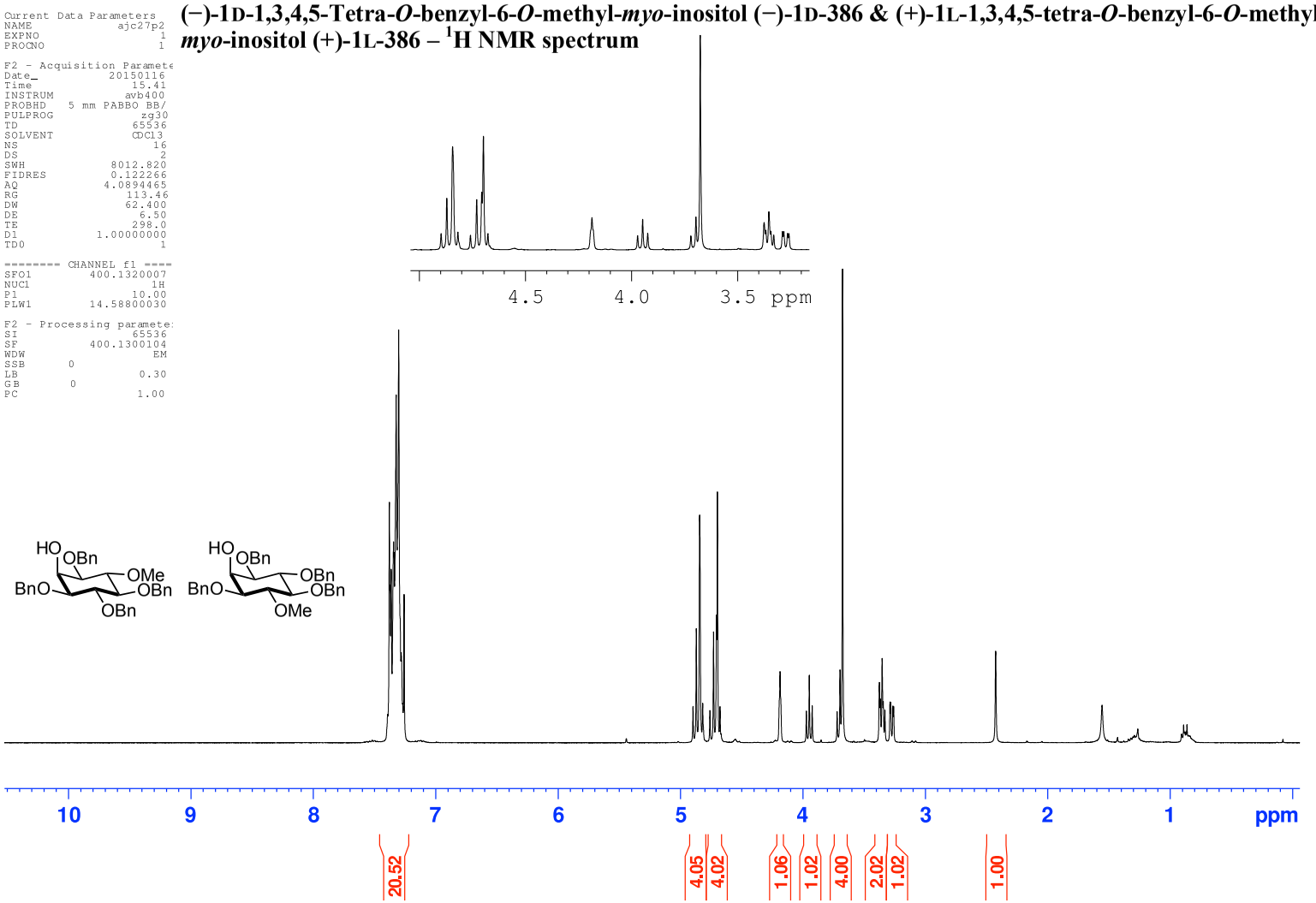
======== CHANNEL f2 ========
SFO2          400.1316005 MHz
NUC2                  1H
CPDPRG[2           waltz16
PCPD2              70.00 usec
PLW2          14.58800030 W
PLW12          0.29771000 W
PLW13          0.14588000 W

F2 - Processing parameters
SI                 32768
SF            100.6127725 MHz
WDW                   EM
SSB       0
LB                 1.00 Hz
GB        0
PC                 1.40
```





(−)-1D-1,3,4,5-Tetra-*O*-benzyl-6-*O*-methyl-*myo*-inositol (−)-1D-386 & (+)-1L-1,3,4,5-tetra-*O*-benzyl-6-*O*-methyl-*myo*-inositol (+)-1L-386 – ^{1}H NMR spectrum



(−)-1D-1,3,4,5-Tetra-*O*-benzyl-6-*O*-methyl-*myo*-inositol (−)-1D-386 & (+)-1L-1,3,4,5-tetra-*O*-benzyl-6-*O*-methyl-*myo*-inositol (+)-1L-386 – ^{13}C NMR spectrum

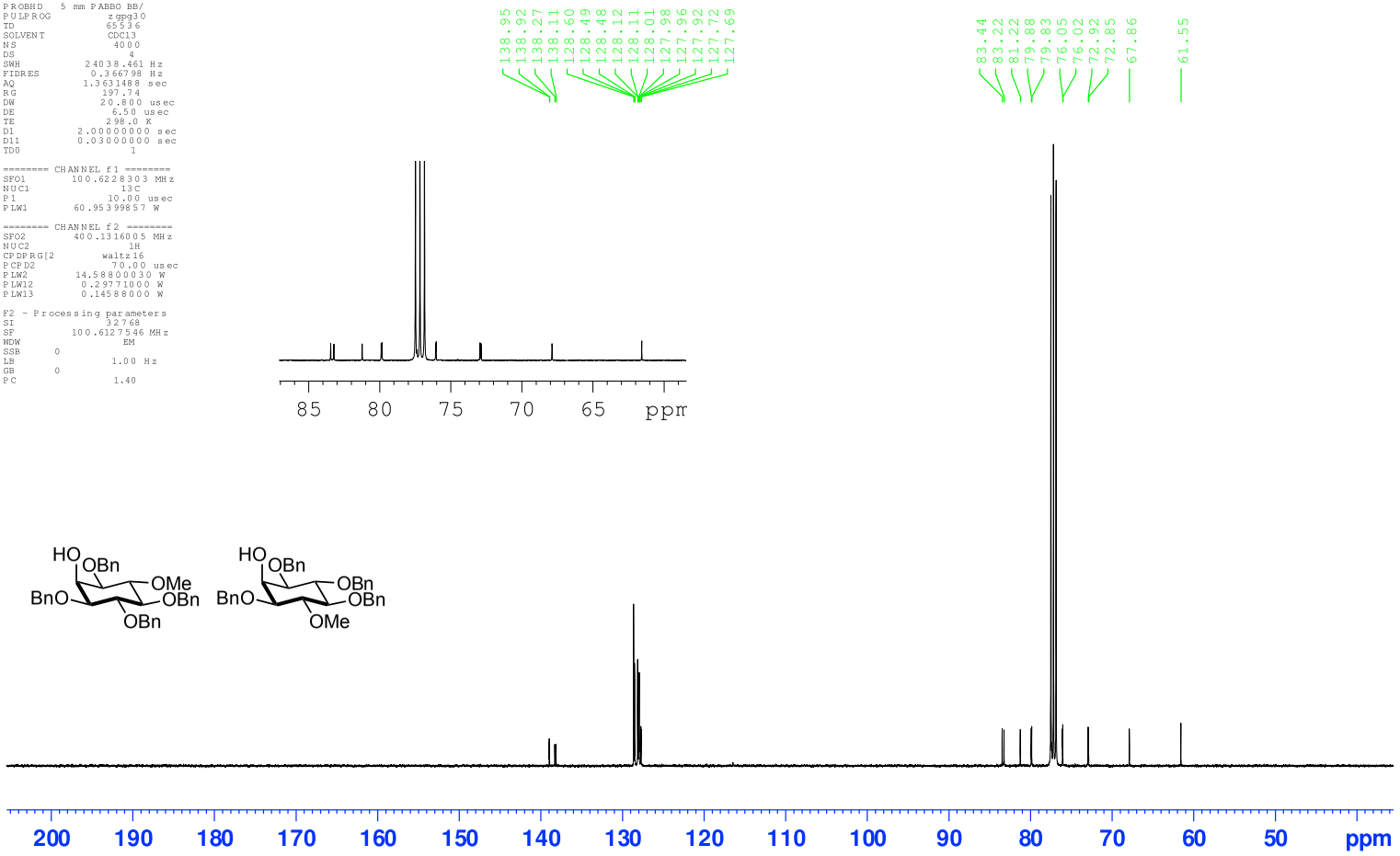
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Current Data Parameters
NAME      ajc27p2-greaswash
EXPNO                     2
PROCNO                    1

F2 - Acquisition Parameters
Date_           20150124
Time                5.43
INSTRUM           avb400
PROBHD   5 mm PABBO BB/
PULPROG           zgpg30
TD                65536
SOLVENT             CDCl3
NS                   4000
DS                      4
SWH           24038.461 Hz
FIDRES         0.366798 Hz
AQ             1.3631488 sec
RG                 197.74
DW                20.800 usec
DE                 6.50 usec
TE                298.0 K
D1           2.00000000 sec
D11          0.03000000 sec
TD0                     1

======== CHANNEL f1 ========
SFO1         100.6228303 MHz
NUC1               13C
P1                10.00 usec
PLW1         60.95399857 W

======== CHANNEL f2 ========
SFO2         400.1316005 MHz
NUC2               1H
CPDPRG[2         waltz16
PCPD2             70.00 usec
PLW2         14.58800030 W
PLW12         0.29771000 W
PLW13         0.14588000 W

F2 - Processing parameters
SI                32768
SF           100.6127546 MHz
WDW                EM
SSB       0
LB                 1.00 Hz
GB        0
PC                 1.40
```

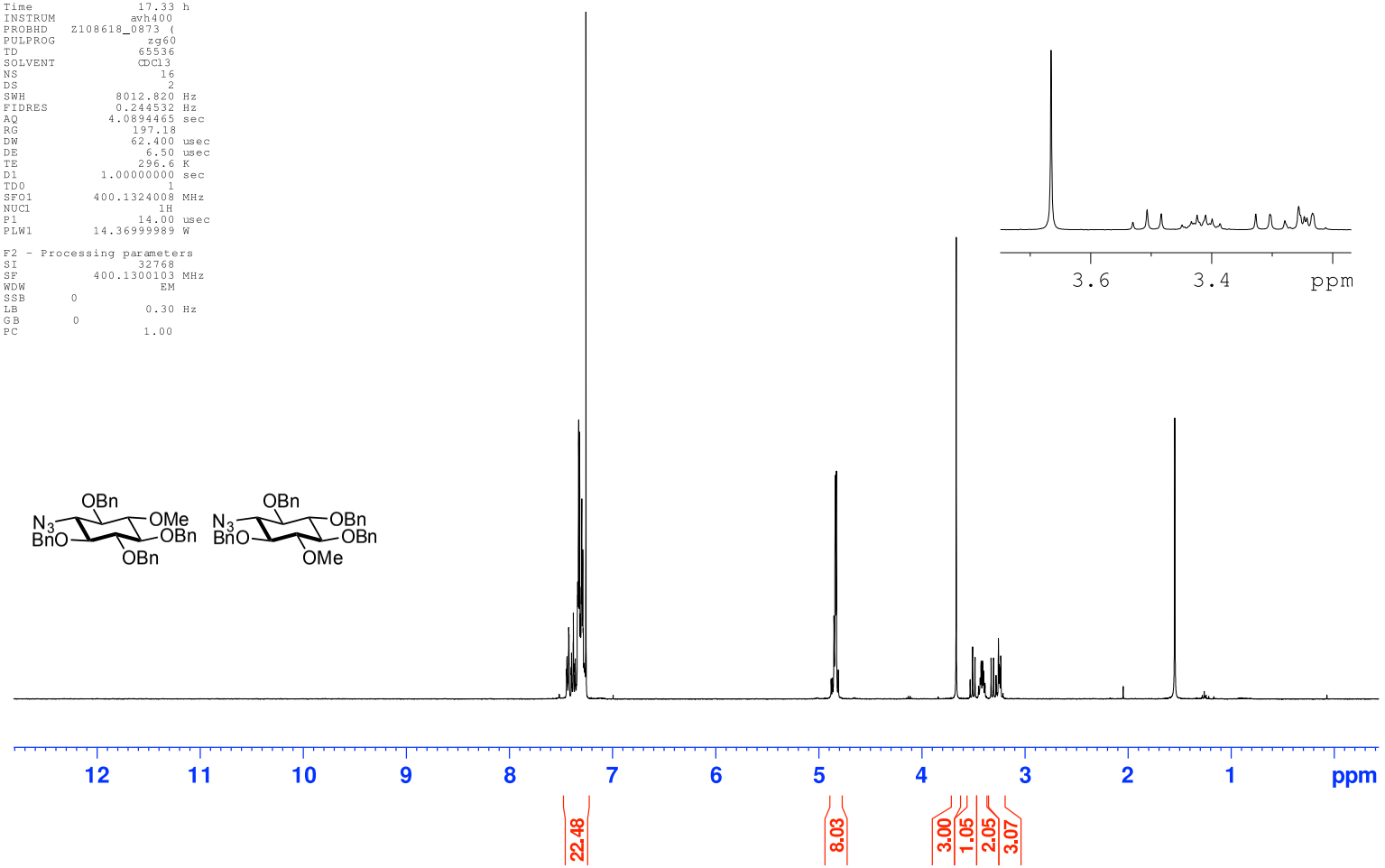


(+)-1D-2,4,5,6-Tetra-*O*-benzyl-3-*O*-methyl-1-azido-1-deoxy-*scyllo*-inositol (–)-1D-387 & (–)-1-L-2,4,5,6-tetra-*O*-benzyl-3-*O*-methyl-1-azido-1-deoxy-*scyllo*-inositol (–)-1L-387 – ^{1}H NMR spectrum

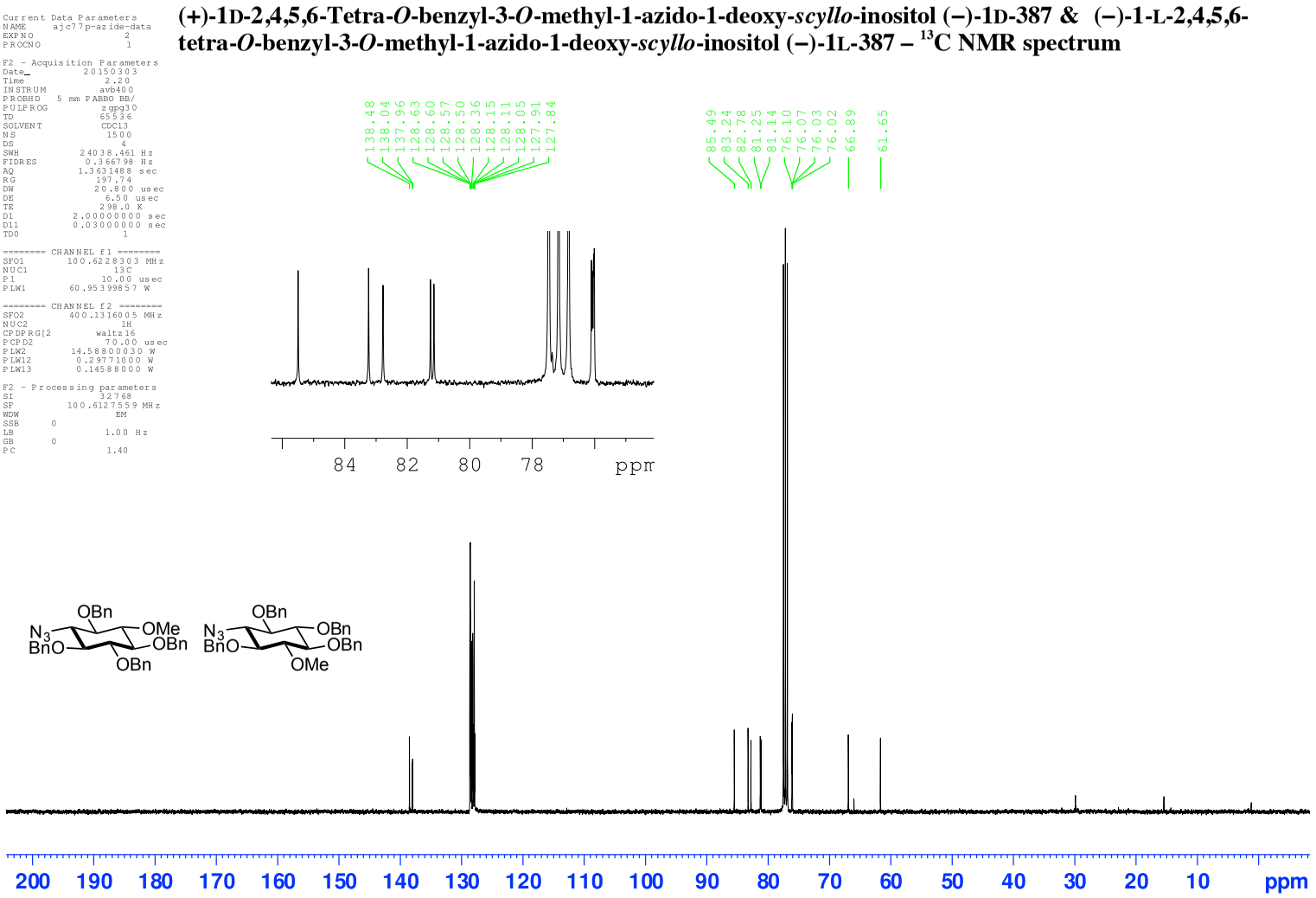
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Current Data Parameters
NAME                 ajf65p
EXPNO                    10
PROCNO                    1

F2 - Acquisition Parameters
Date_              20160720
Time                 17.33 h
INSTRUM              avh400
PROBHD    Z108618_0873 (
PULPROG              zg60
TD                  65536
SOLVENT             CDCl3
NS                     16
DS                      2
SWH               8012.820 Hz
FIDRES               0.244532 Hz
AQ                4.0894465 sec
RG                   197.18
DW                   62.400 usec
DE                    6.50 usec
TE                   296.6 K
D1             1.00000000 sec
TD0                     1
SFO1           400.1324008 MHz
NUC1                   1H
P1                   14.00 usec
PLW1           14.36999989 W

F2 - Processing parameters
SI                   32768
SF             400.1300103 MHz
WDW                    EM
SSB       0
LB                    0.30 Hz
GB        0
PC                    1.00
```



(+)-1D-2,4,5,6-Tetra-*O*-benzyl-3-*O*-methyl-1-azido-1-deoxy-*scyllo*-inositol (–)-1D-387 & (–)-1-L-2,4,5,6-tetra-*O*-benzyl-3-*O*-methyl-1-azido-1-deoxy-*scyllo*-inositol (–)-1L-387 – ^{13}C NMR spectrum



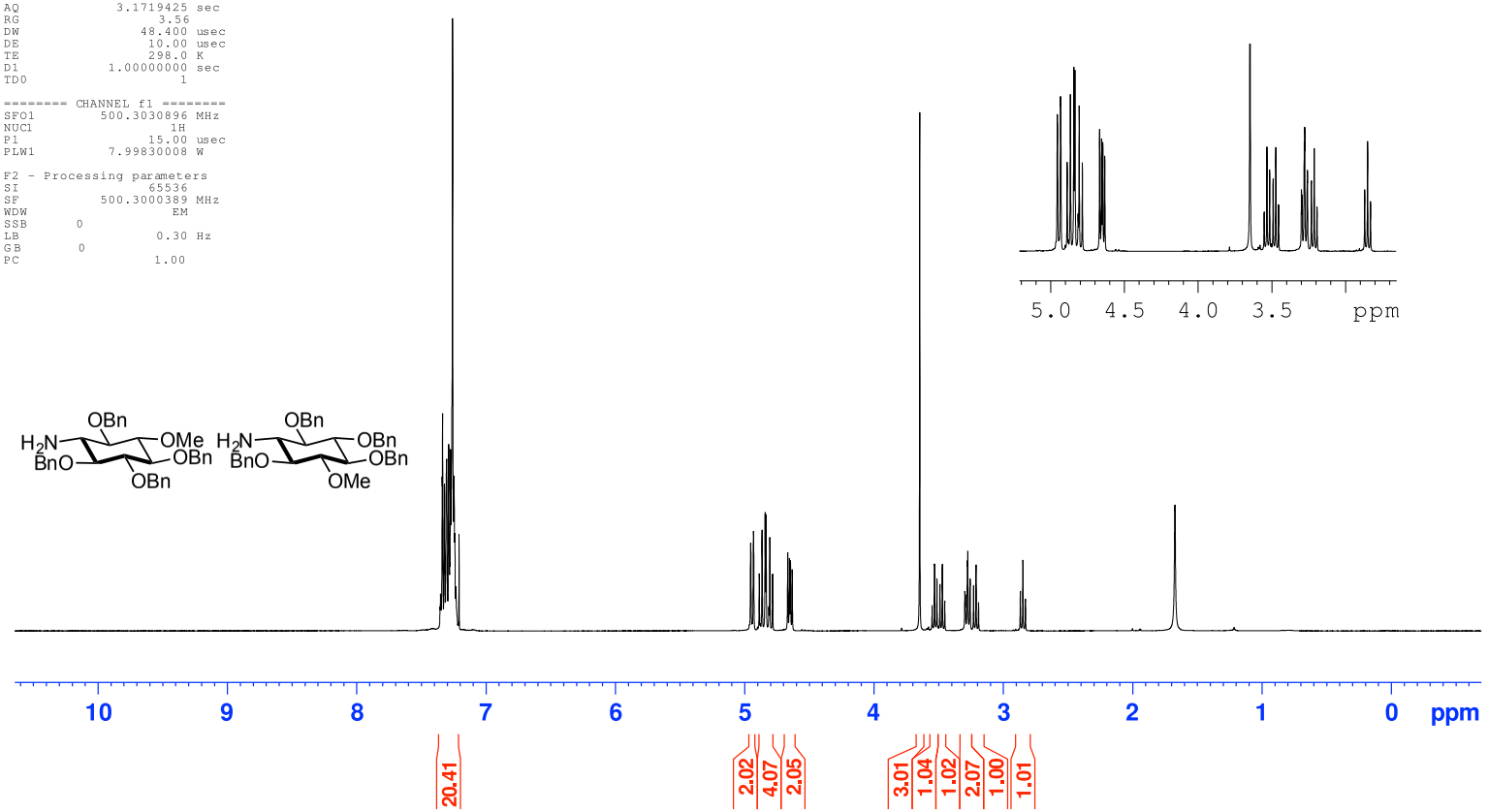
(–)-1D-2,4,5,6-Tetra-*O*-benzyl-3-*O*-methyl-s*cyllo*-inosamine (–)-1D-388 & (+)-1L-2,4,5,6-tetra-*O*-benzyl-3-*O*-methyl-s*cyllo*-inosamine (+)-1L-388 – ^{1}H NMR spectrum

```
Current Data Parameters
NAME          ajf6p-data
EXPNO                  1
PROCNO                 1

F2 - Acquisition Parameters
Date_           20160406
Time               6.37
INSTRUM          avc500
PROBHD   5 mm CPDUL 13C
PULPROG            zg30
TD                65536
SOLVENT           CDCl3
NS                   16
DS                    4
SWH           10330.578 Hz
FIDRES         0.157632 Hz
AQ            3.1719425 sec
RG                 3.56
DW               48.400 usec
DE               10.00 usec
TE               298.0 K
D1            1.00000000 sec
TD0                    1

======== CHANNEL f1 ========
SFO1        500.3030896 MHz
NUC1                   1H
P1                15.00 usec
PLW1         7.99830008 W

F2 - Processing parameters
SI                 65536
SF          500.3000389 MHz
WDW                   EM
SSB       0
LB                0.30 Hz
GB        0
PC                1.00
```



(–)-1D-2,4,5,6-Tetra-*O*-benzyl-3-*O*-methyl-*scyllo*-inosamine (–)-1D-388 & (+)-1L-2,4,5,6-tetra-*O*-benzyl-3-*O*-methyl-*scyllo*-inosamine (+)-1L-388 – ^{13}C NMR spectrum

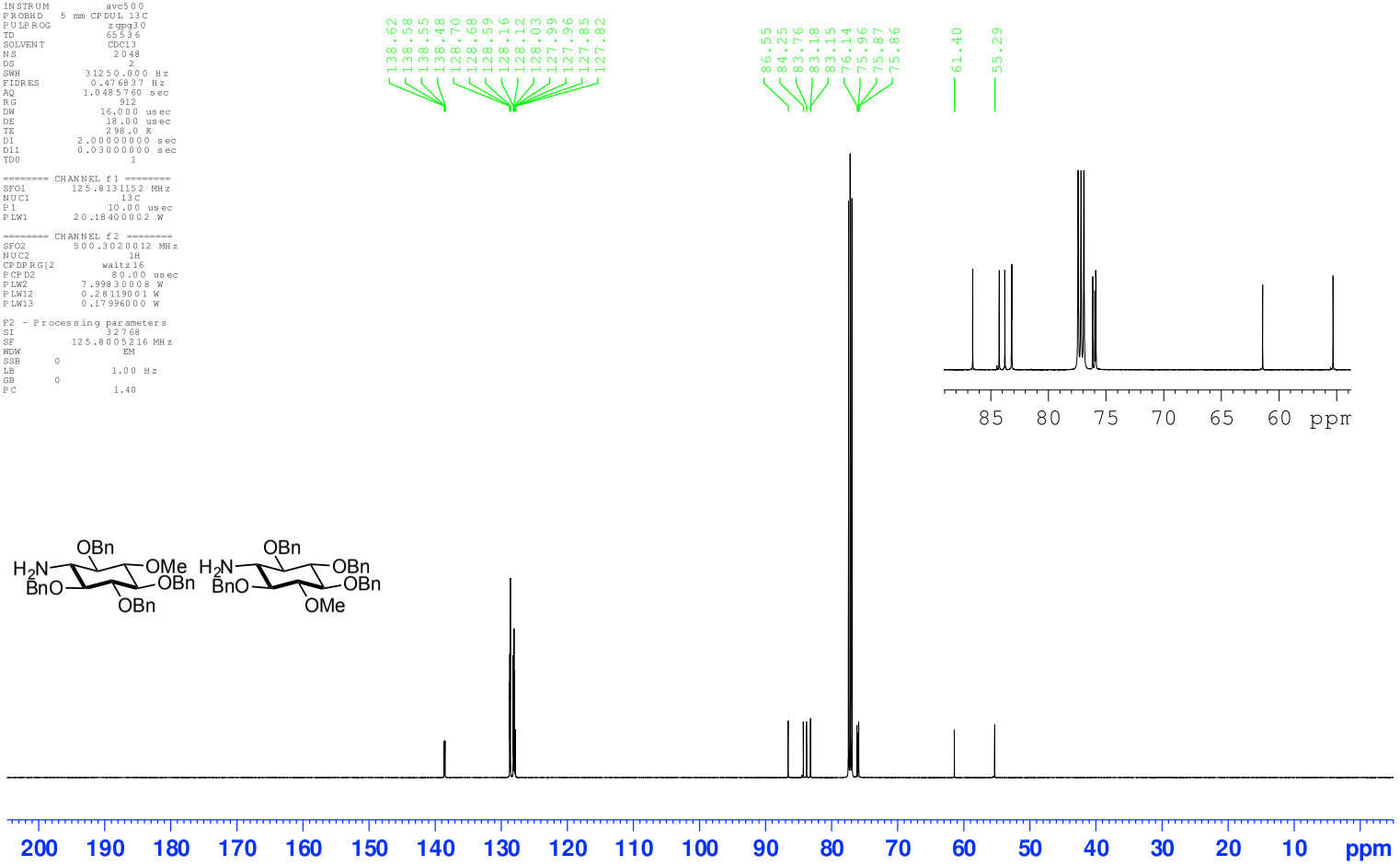
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Current Data Parameters
NAME          ajf6p-data
EXPNO                  2
PROCNO                 1

F2 - Acquisition Parameters
Date_          20160406
Time               8.24
INSTRUM          avc500
PROBHD   5 mm CPDUL 13C
PULPROG          zgpg30
TD               65536
SOLVENT          CDCl3
NS                  2048
DS                     2
SWH          31250.000 Hz
FIDRES        0.476837 Hz
AQ            1.0485760 sec
RG                   912
DW               16.000 usec
DE               18.00 usec
TE               298.0 K
D1            2.00000000 sec
D11           0.03000000 sec
TD0                    1

======== CHANNEL f1 ========
SFO1         125.8131152 MHz
NUC1               13C
P1               10.00 usec
PLW1         20.18400002 W

======== CHANNEL f2 ========
SFO2         500.3020012 MHz
NUC2               1H
CPDPRG[2         waltz16
PCPD2             80.00 usec
PLW2          7.99830008 W
PLW12         0.28119001 W
PLW13         0.17996000 W

F2 - Processing parameters
SI                 32768
SF           125.8005216 MHz
WDW                EM
SSB      0
LB                1.00 Hz
GB       0
PC                1.40
```



(–)-1D-2,4,5,6-Tetra-*O*-benzyl-3-*O*-methyl-*scyllo*-inosamine (–)-1D-388
& (+)-1L-2,4,5,6-tetra-*O*-benzyl-3-*O*-methyl-*scyllo*-inosamine (+)-1L-388
– RP-HPLC (Method 1)

Time	Area	Area %
10.477	111,715	1.33
13.136	8,063,880	95.81
14.376	104,809	1.25
14.740	135,855	1.61
Total	8,416,259	100.00

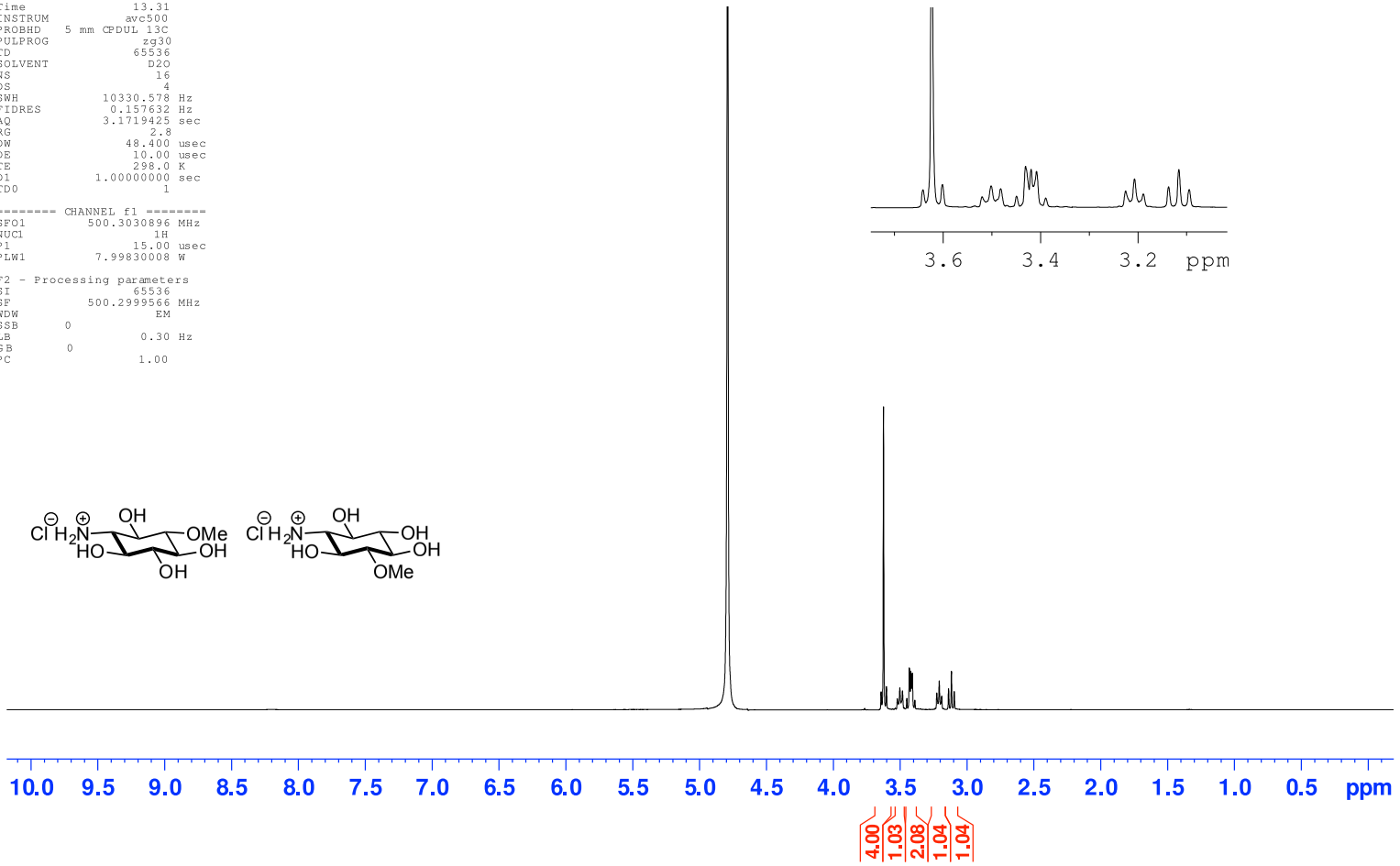
(+)-1D-3-*O*-Methyl-s*cyllo*-inosamine hydrochloride (+)-1D-14 & (−)-1L-3-*O*-methyl-s*cyllo*-inosamine hydrochloride (−)-1L-14– ^{1}H NMR spectrum

```
Current Data Parameters
NAME      (+)-3-O-MSI data
EXPNO                    1
PROCNO                   1

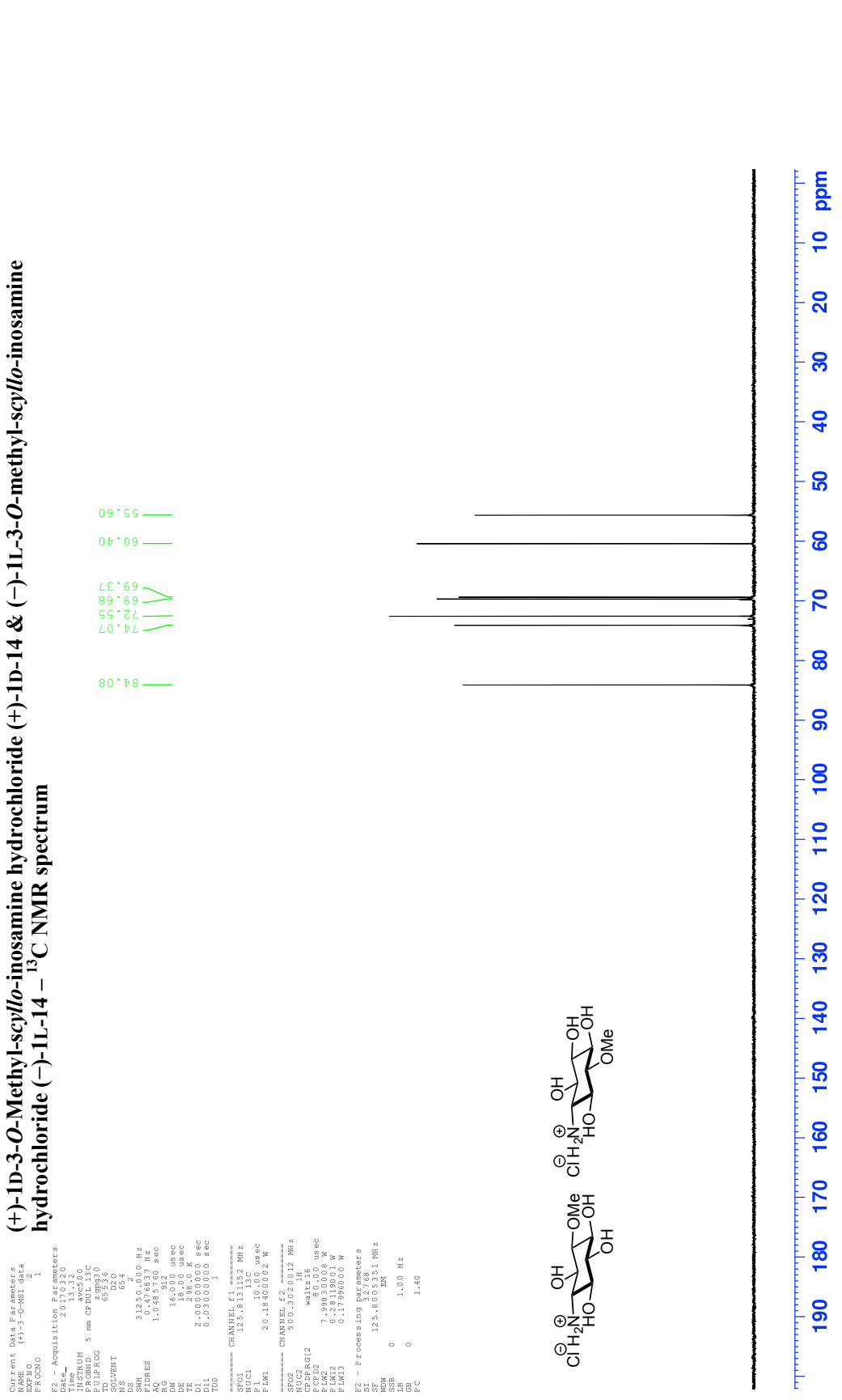
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TD                65536
SOLVENT             D2O
NS                   16
DS                    4
SWH           10330.578 Hz
FIDRES         0.157632 Hz
AQ            3.1719425 sec
RG                  2.8
DW                48.400 usec
DE                10.00 usec
TE                298.0 K
D1           1.00000000 sec
TD0                  1

======== CHANNEL f1 ========
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NUC1               1H
P1                15.00 usec
PLW1          7.99830008 W

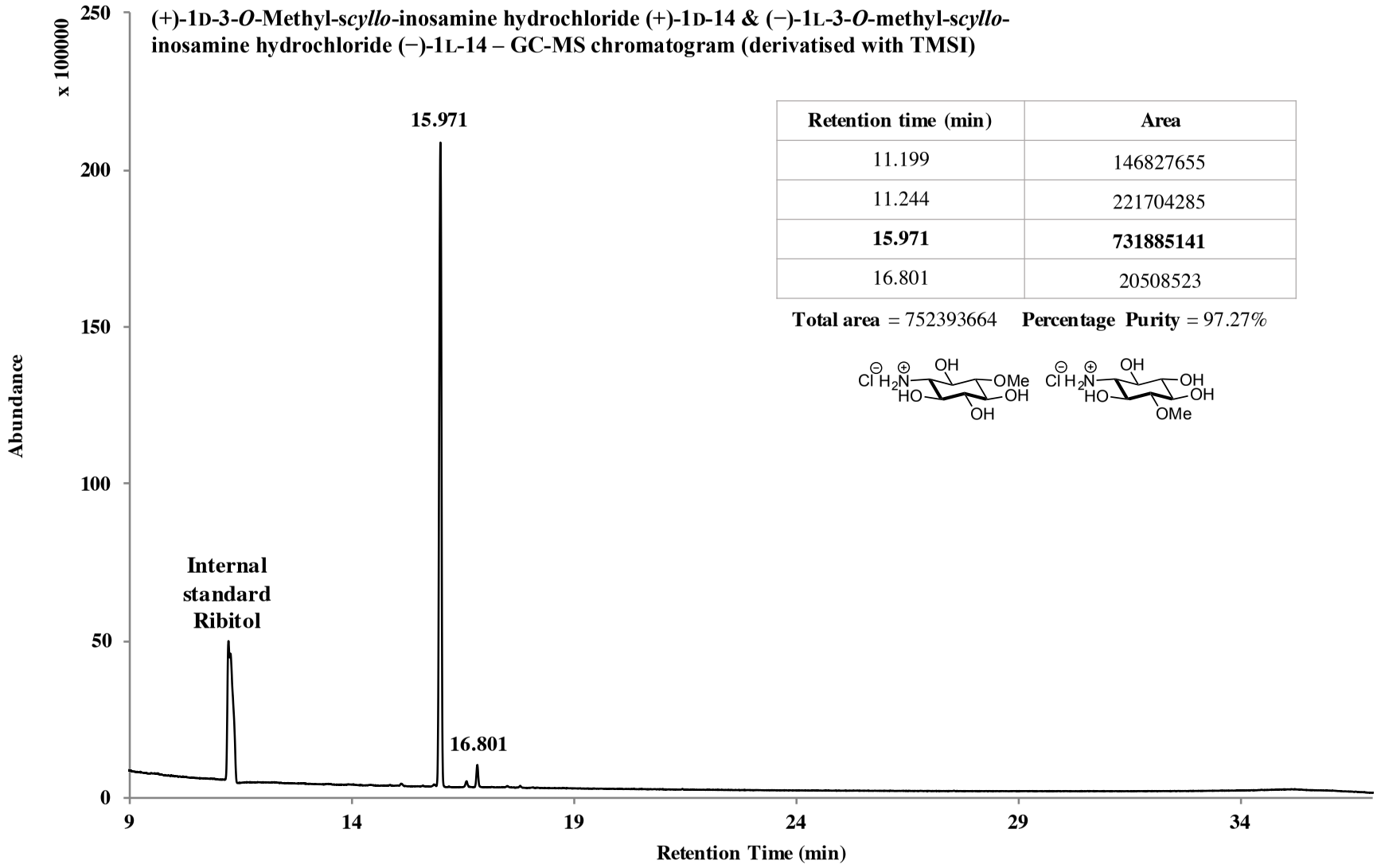
F2 - Processing parameters
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SF           500.2999566 MHz
WDW                EM
SSB       0
LB                0.30 Hz
GB        0
PC                1.00
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(+)-1D-3-*O*-Methyl-*scyllo*-inosamine hydrochloride (+)-1D-14 & (−)-1L-3-*O*-methyl-*scyllo*-inosamine hydrochloride (−)-1L-14 – ^{13}C NMR spectrum



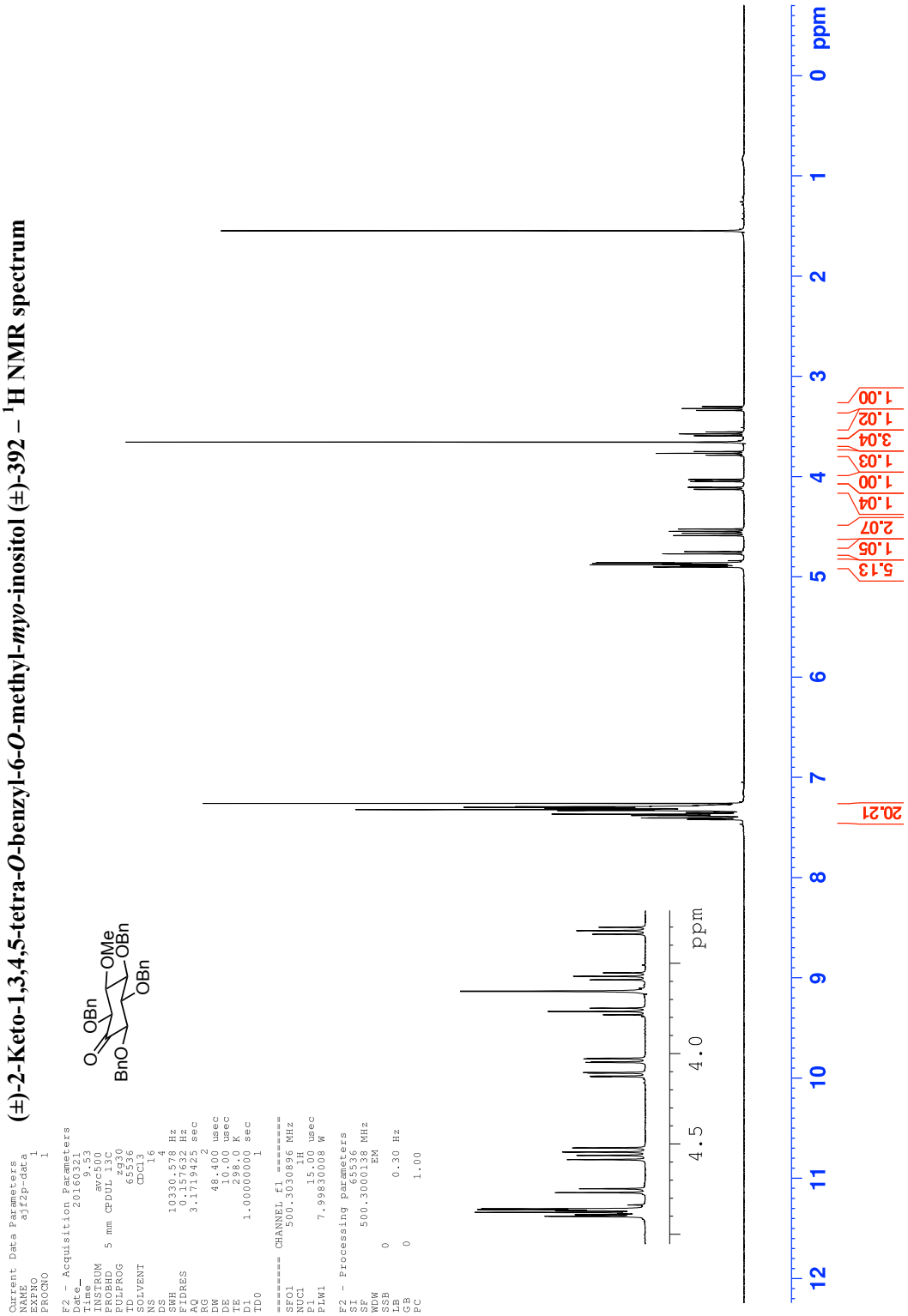
(+)-1D-3-*O*-Methyl-s*cyllo*-inosamine hydrochloride (+)-1D-14 & (−)-1L-3-*O*-methyl-s*cyllo*-inosamine hydrochloride (−)-1L-14 – GC-MS chromatogram (derivatised with TMSI)



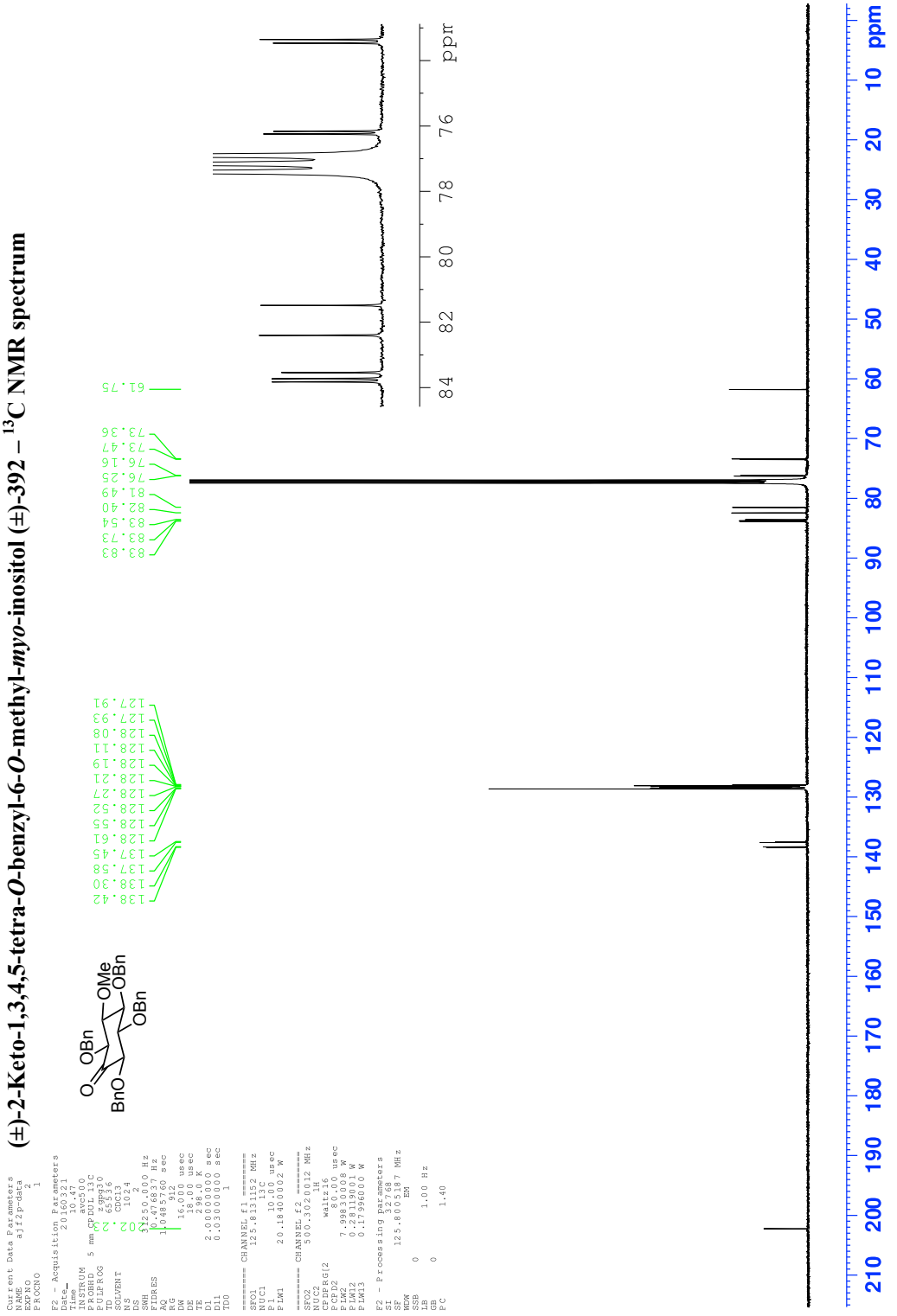
Retention time (min)	Area
11.199	146827655
11.244	221704285
15.971	**731885141**
16.801	20508523

Total area = 752393664 **Percentage Purity** = 97.27%

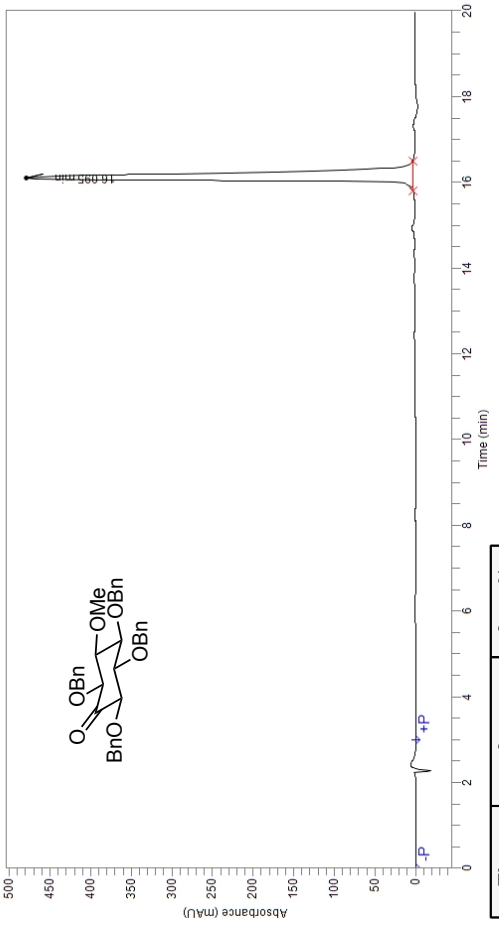
(±)-2-Keto-1,3,4,5-tetra-*O*-benzyl-6-*O*-methyl-*myo*-inositol (±)-392 – ^{1}H NMR spectrum



(±)-2-Keto-1,3,4,5-tetra-*O*-benzyl-6-*O*-methyl-*myo*-inositol (±)-392 – ^{13}C NMR spectrum



(±)-2-Keto-1,3,4,5-tetra-*O*-benzyl-6-*O*-methyl-*myo*-inositol (±)-392 – RP-HPLC (Method 2)



Time	Area	Area %
15.863	10,430	0.20
16.095	5,298,550	99.80
Total	5,308,979	100.00

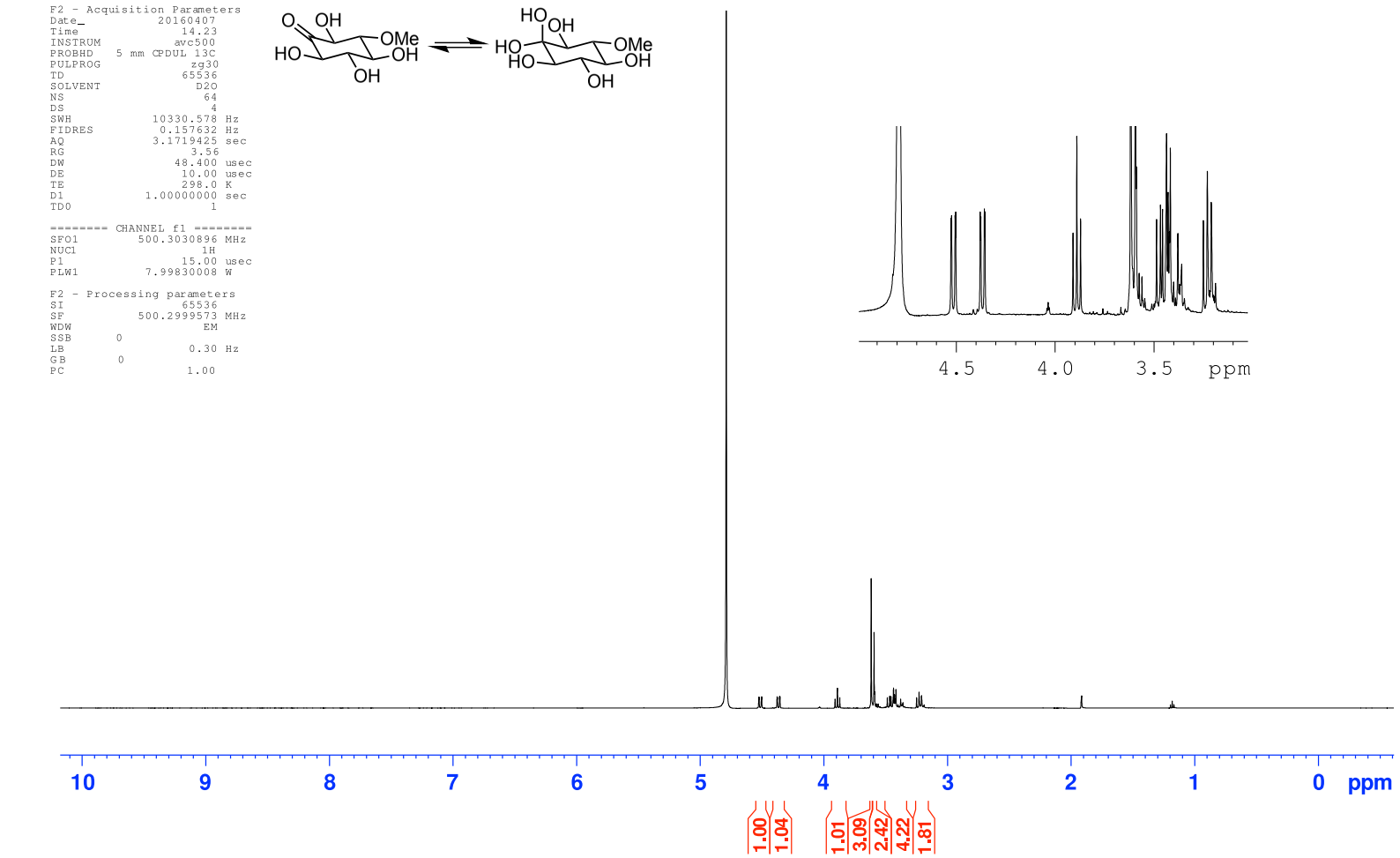
(±)-3-*O*-Methyl-*scyllo*-inososе (±)-391 – ^{1}H NMR spectrum

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Current Data Parameters
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EXPNO                        1
PROCNO                       1

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TD                     65536
SOLVENT                  D2O
NS                        64
DS                         4
SWH                10330.578 Hz
FIDRES             0.157632 Hz
AQ                 3.1719425 sec
RG                      3.56
DW                    48.400 usec
DE                    10.00 usec
TE                    298.0 K
D1                 1.00000000 sec
TD0                          1

======== CHANNEL f1 ========
SFO1              500.3030896 MHz
NUC1                       1H
P1                      15.00 usec
PLW1               7.99830008 W

F2 - Processing parameters
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SF                500.2999573 MHz
WDW                        EM
SSB       0
LB                      0.30 Hz
GB        0
PC                      1.00
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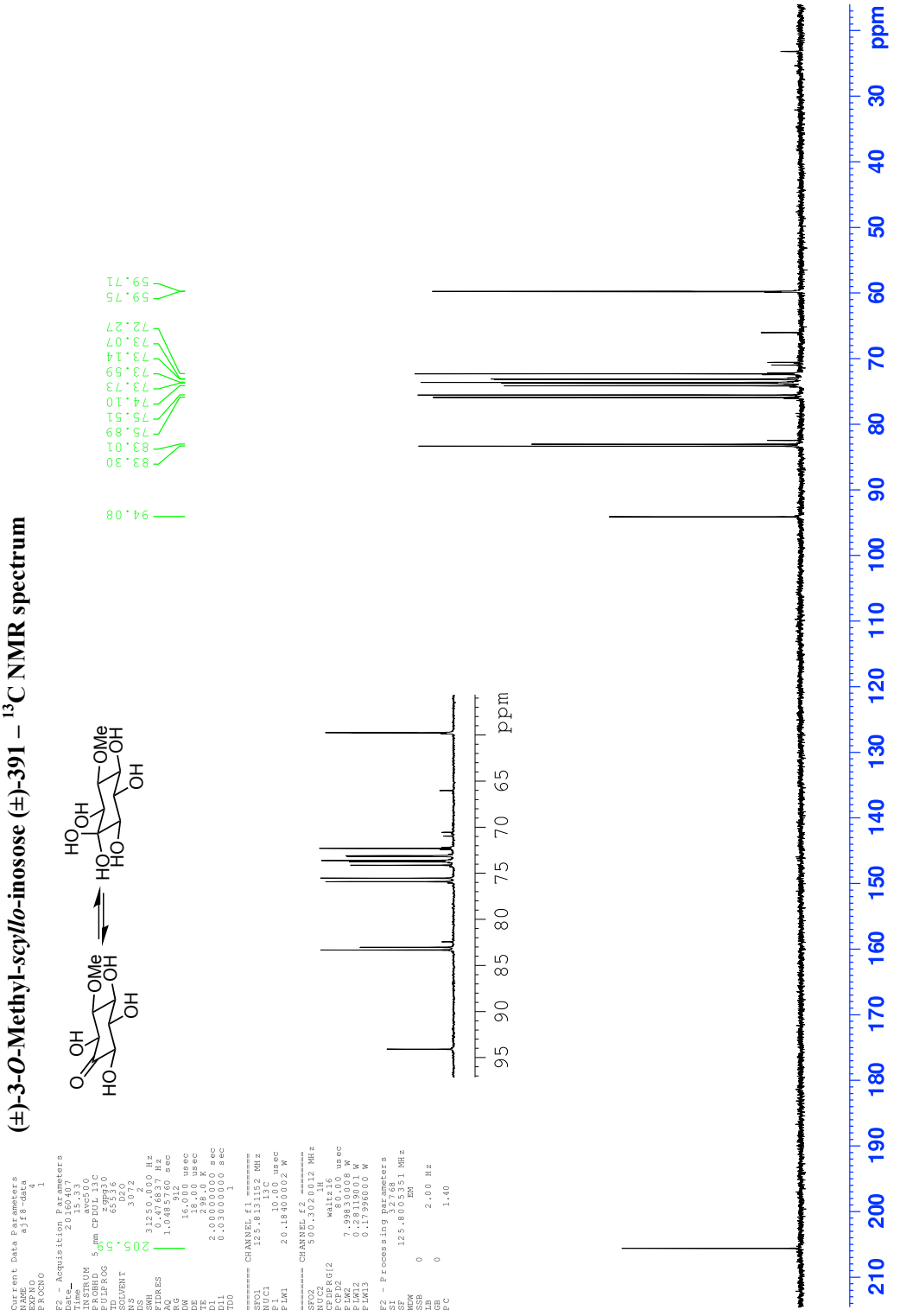

(±)-3-O-Methyl-scyllo-inosose (±)-391 – 13C NMR spectrum
205.59
94.08
83.30
83.01
75.89
75.51
74.10
73.73
73.59
73.14
73.07
72.27
59.75
59.71
ppm

Table 1. Crystal data and structure refinement for 6558.

Identification code	6558	
Empirical formula	C7 H4 D6 O6	
Formula weight	196.19	
Temperature	150 K	
Wavelength	1.54184 Å	
Crystal system	Orthorhombic	
Space group	P n a 21	
Unit cell dimensions	a = 12.1157(4) Å	α= 90°.
	b = 6.1134(2) Å	β= 90°.
	c = 9.9700(3) Å	γ = 90°.
Volume	738.46(4) $Å^3$	
Z	4	
Density (calculated)	1.765 Mg/m^3	
Absorption coefficient	1.332 mm^{-1}	
F(000)	400	
Crystal size	0.38 x 0.20 x 0.12 mm^3	
Theta range for data collection	7.311 to 76.931°.	
Index ranges	-14<=h<=15, -7<=k<=7, -11<=l<=12	
Reflections collected	6213	
Independent reflections	1449 [R(int) = 0.021]	
Completeness to theta = 76.931°	99.6 %	
Absorption correction	Semi-empirical from equivalents	
Max. and min. transmission	0.85 and 0.77	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	1447 / 1 / 119	
Goodness-of-fit on F^2	1.0267	
Final R indices [I>2sigma(I)]	R1 = 0.0309, wR2 = 0.0816	
R indices (all data)	R1 = 0.0310, wR2 = 0.0816	
Absolute structure parameter	0.06(15)	
Largest diff. peak and hole	0.30 and -0.20 $e.Å^{-3}$	

Table 2. Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters ($Å^2$x 10^3) for 6558. U(eq) is defined as one third of the trace of the orthogonalized U^{ij} tensor.

	x	y	z	U(eq)
C(1)	5497(1)	-661(2)	2772(2)	15
C(2)	6389(1)	-625(2)	3848(2)	17
C(3)	7459(1)	391(2)	3318(2)	17
C(4)	7196(1)	2716(2)	2872(2)	17
C(5)	6308(1)	2770(2)	1787(2)	17
C(6)	5273(1)	1707(2)	2367(2)	16
O(7)	6008(1)	728(2)	4950(1)	19
O(8)	6761(1)	3868(2)	4025(2)	22
O(9)	4956(1)	2911(2)	3551(2)	21
C(10)	5800(1)	2866(2)	4510(2)	20
O(11)	4523(1)	-1724(2)	3215(2)	25
O(12)	7865(1)	-810(2)	2200(2)	25
O(13)	6640(1)	1737(2)	570(2)	20
D(11)	5781	-1486	1996	15
D(21)	6524	-2121	4177	18
D(31)	8009	438	4025	21
D(41)	7867	3479	2583	22
D(51)	6136	4324	1578	20
D(61)	4656	1767	1729	19

Table 3. Bond lengths [Å] and angles [°] for 6558.

C(1)-C(2)	1.5223(19)	C(3)-C(2)-O(7)	107.40(10)
C(1)-C(6)	1.5273(18)	C(1)-C(2)-D(21)	109.814
C(1)-O(11)	1.4174(16)	C(3)-C(2)-D(21)	110.576
C(1)-D(11)	0.986	O(7)-C(2)-D(21)	109.234
C(2)-C(3)	1.5309(18)	C(2)-C(3)-C(4)	107.62(10)
C(2)-O(7)	1.4509(16)	C(2)-C(3)-O(12)	110.69(11)
C(2)-D(21)	0.985	C(4)-C(3)-O(12)	108.97(11)
C(3)-C(4)	1.5229(19)	C(2)-C(3)-D(31)	110.073
C(3)-O(12)	1.4231(17)	C(4)-C(3)-D(31)	109.178
C(3)-D(31)	0.971	O(12)-C(3)-D(31)	110.242
C(4)-C(5)	1.5263(18)	C(3)-C(4)-C(5)	111.98(11)
C(4)-O(8)	1.4476(17)	C(3)-C(4)-O(8)	107.33(11)
C(4)-D(41)	0.980	C(5)-C(4)-O(8)	107.23(11)
C(5)-C(6)	1.5265(18)	C(3)-C(4)-D(41)	110.894
C(5)-O(13)	1.4260(18)	C(5)-C(4)-D(41)	111.493
C(5)-D(51)	0.995	O(8)-C(4)-D(41)	107.669
C(6)-O(9)	1.4435(17)	C(4)-C(5)-C(6)	107.57(11)
C(6)-D(61)	0.981	C(4)-C(5)-O(13)	113.25(11)
O(7)-C(10)	1.4014(18)	C(6)-C(5)-O(13)	111.46(11)
O(8)-C(10)	1.4018(17)	C(4)-C(5)-D(51)	108.440
O(9)-C(10)	1.4007(19)	C(6)-C(5)-D(51)	108.312
C(10)-H(101)	0.975	O(13)-C(5)-D(51)	107.676
O(11)-H(111)	0.831	C(1)-C(6)-C(5)	110.92(11)
O(12)-H(121)	0.826	C(1)-C(6)-O(9)	108.29(11)
O(13)-H(131)	0.823	C(5)-C(6)-O(9)	108.14(11)
		C(1)-C(6)-D(61)	110.031
C(2)-C(1)-C(6)	107.43(11)	C(5)-C(6)-D(61)	111.362
C(2)-C(1)-O(11)	112.24(11)	O(9)-C(6)-D(61)	107.975
C(6)-C(1)-O(11)	111.62(11)	C(2)-O(7)-C(10)	110.61(11)
C(2)-C(1)-D(11)	108.220	C(4)-O(8)-C(10)	111.34(10)
C(6)-C(1)-D(11)	109.836	C(6)-O(9)-C(10)	110.76(10)
O(11)-C(1)-D(11)	107.447	O(8)-C(10)-O(7)	111.47(11)
C(1)-C(2)-C(3)	111.34(11)	O(8)-C(10)-O(9)	111.27(13)
C(1)-C(2)-O(7)	108.40(11)	O(7)-C(10)-O(9)	111.29(11)

O(8)-C(10)-H(101)	107.6	C(1)-O(11)-H(111)	109.5
O(7)-C(10)-H(101)	107.4	C(3)-O(12)-H(121)	109.2
O(9)-C(10)-H(101)	107.6	C(5)-O(13)-H(131)	113.4

Symmetry transformations used to generate equivalent atoms:

Table 4. Anisotropic displacement parameters ($Å^2 x\ 10^3$) for 6558. The anisotropic displacement factor exponent takes the form: $-2\pi^2[\ h^2\ a^{*2}U^{11} + ...\ + 2\ h\ k\ a^*\ b^*\ U^{12}\ ]$

	U^{11}	U^{22}	U^{33}	U^{23}	U^{13}	U^{12}
C(1)	13(1)	17(1)	16(1)	-2(1)	3(1)	-5(1)
C(2)	17(1)	18(1)	15(1)	0(1)	1(1)	1(1)
C(3)	12(1)	25(1)	15(1)	0(1)	-2(1)	4(1)
C(4)	10(1)	22(1)	20(1)	-1(1)	0(1)	-3(1)
C(5)	14(1)	17(1)	20(1)	3(1)	0(1)	0(1)
C(6)	9(1)	22(1)	18(1)	-2(1)	0(1)	1(1)
O(7)	20(1)	24(1)	14(1)	-3(1)	3(1)	-3(1)
O(8)	18(1)	21(1)	25(1)	-8(1)	3(1)	-7(1)
O(9)	13(1)	24(1)	26(1)	-6(1)	2(1)	5(1)
C(10)	15(1)	22(1)	22(1)	-7(1)	3(1)	-2(1)
O(11)	25(1)	30(1)	20(1)	-8(1)	7(1)	-16(1)
O(12)	21(1)	35(1)	21(1)	-1(1)	1(1)	16(1)
O(13)	16(1)	29(1)	15(1)	4(1)	0(1)	2(1)

Table 5. Hydrogen coordinates (x 10^4) and isotropic displacement parameters (Å^{2}x 10^{-3}) for 6558.

	x	y	z	U(eq)
H(101)	5550	3704	5284	24
H(111)	4367	-1300	3984	37
H(121)	8344	-1673	2458	39
H(131)	7088	741	682	31

Table 6. Hydrogen bonds for 6558 [Å and °].

D-H...A	d(D-H)	d(H...A)	d(D...A)	<(DHA)
O(11)-H(111)...O(13)#1	0.83	2.01	2.739(2)	145
O(12)-H(121)...O(11)#2	0.83	1.89	2.708(2)	171
O(13)-H(131)...O(8)#3	0.82	2.45	3.033(2)	129
O(13)-H(131)...O(12)	0.82	2.02	2.696(2)	139

Symmetry transformations used to generate equivalent atoms:

#1 -x+1,-y,z+1/2 #2 x+1/2,-y-1/2,z #3 -x+3/2,y-1/2,z-1/2

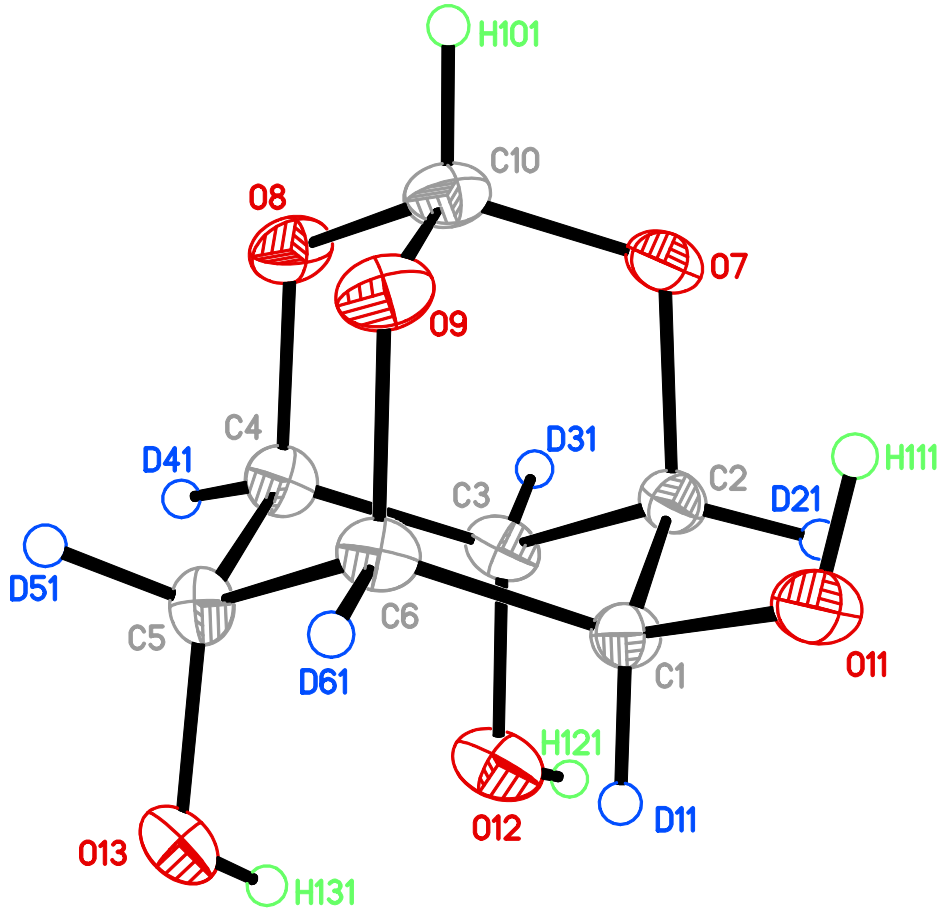


Table 1. Crystal data and structure refinement for 6607.

Identification code	6607	
Empirical formula	C17 H24 O6	
Formula weight	324.37	
Temperature	293 K	
Wavelength	1.54184 Å	
Crystal system	Orthorhombic	
Space group	P 21 21 21	
Unit cell dimensions	a = 4.8687(1) Å	α= 90°.
	b = 12.5438(4) Å	β= 90°.
	c = 28.9905(12) Å	γ = 90°.
Volume	1770.51(10) $Å^3$	
Z	4	
Density (calculated)	1.217 Mg/m^3	
Absorption coefficient	0.761 mm^{-1}	
F(000)	696.000	
Crystal size	0.42 x 0.10 x 0.02 mm^3	
Theta range for data collection	3.840 to 76.533°.	
Index ranges	-4<=h<=6, -15<=k<=15, -36<=l<=35	
Reflections collected	23601	
Independent reflections	3671 [R(int) = 0.051]	
Completeness to theta = 75.003°	99.9 %	
Absorption correction	Semi-empirical from equivalents	
Max. and min. transmission	0.98 and 0.58	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	3669 / 372 / 301	
Goodness-of-fit on F^2	0.9955	
Final R indices [I>2sigma(I)]	R1 = 0.0502, wR2 = 0.1316	
R indices (all data)	R1 = 0.0616, wR2 = 0.1452	
Absolute structure parameter	0.0(3)	
Largest diff. peak and hole	0.17 and -0.20 e.$Å^{-3}$	

Table 2. Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters ($Å^2$x 10^3) for 6607. U(eq) is defined as one third of the trace of the orthogonalized U^{ij} tensor.

	x	y	z	U(eq)
C(1)	1772(4)	4492(2)	5729(1)	65
C(2)	2601(4)	5627(2)	5600(1)	71
C(3)	1119(4)	5982(2)	5166(1)	76
C(4)	1565(4)	5217(2)	4774(1)	68
C(5)	604(4)	4108(2)	4906(1)	63
C(6)	2070(4)	3719(2)	5335(1)	57
O(7)	3502(4)	4147(2)	6100(1)	76
C(8)	2193(14)	3958(11)	6524(3)	107
C(9)	4260(20)	3476(6)	6849(2)	110
C(10)	5250(30)	2466(6)	6783(4)	129
C(11)	7150(30)	1995(8)	7087(5)	156
C(12)	7940(30)	2568(14)	7461(4)	156
C(13)	7080(30)	3579(15)	7538(4)	160
C(14)	5140(30)	4011(10)	7231(4)	139
O(15)	1958(5)	6327(2)	5968(1)	105
C(16)	4238(11)	6759(4)	6194(2)	138
O(17)	1990(3)	7031(1)	5044(1)	97
O(18)	4403(3)	5205(1)	4650(1)	69
C(19)	4961(6)	5768(4)	4238(2)	91
C(20)	4120(20)	5150(6)	3838(2)	134
C(21)	2620(30)	5481(11)	3496(4)	191
O(22)	843(3)	3386(1)	4529(1)	72
O(23)	1038(3)	2698(1)	5461(1)	67
C(108)	2046(18)	3664(17)	6463(4)	117
C(109)	4000(30)	3480(9)	6853(3)	122
C(110)	5010(40)	2487(10)	6946(7)	158
C(111)	6860(40)	2299(15)	7308(9)	175
C(112)	7840(40)	3150(20)	7540(7)	162
C(113)	6940(40)	4159(19)	7460(5)	150
C(114)	5030(40)	4310(11)	7104(6)	138
C(119)	4916(7)	5030(3)	4141(2)	71

C(120)	4577(18)	6016(5)	3887(2)	100
C(121)	3220(30)	6144(8)	3505(3)	149

Table 3. Bond lengths [Å] and angles [°] for 6607.

C(1)-C(2)	1.526(3)	O(15)-C(16)	1.399(5)
C(1)-C(6)	1.504(3)	C(16)-H(161)	0.971
C(1)-O(7)	1.434(3)	C(16)-H(162)	0.965
C(1)-H(11)	0.971	C(16)-H(163)	0.956
C(2)-C(3)	1.519(4)	O(17)-H(171)	0.804
C(2)-O(15)	1.416(3)	O(18)-C(19)	1.414(4)
C(2)-H(21)	0.991	O(18)-C(119)	1.513(5)
C(3)-C(4)	1.503(4)	C(19)-C(20)	1.454(7)
C(3)-O(17)	1.426(2)	C(19)-H(191)	0.960
C(3)-H(31)	0.982	C(19)-H(192)	0.977
C(4)-C(5)	1.518(3)	C(20)-C(21)	1.299(8)
C(4)-O(18)	1.427(2)	C(20)-H(201)	0.938
C(4)-H(41)	0.967	C(21)-H(211)	0.941
C(5)-C(6)	1.515(3)	C(21)-H(212)	0.937
C(5)-O(22)	1.425(3)	O(22)-H(221)	0.837
C(5)-H(51)	0.970	O(23)-H(231)	0.795
C(6)-O(23)	1.423(2)	C(108)-C(109)	1.497(6)
C(6)-H(61)	0.982	C(108)-H(1081)	0.978
O(7)-C(8)	1.405(5)	C(108)-H(1082)	0.979
O(7)-C(108)	1.405(6)	C(109)-C(110)	1.365(6)
C(8)-C(9)	1.505(5)	C(109)-C(114)	1.366(5)
C(8)-H(81)	0.983	C(110)-C(111)	1.402(6)
C(8)-H(82)	0.981	C(110)-H(1101)	0.932
C(9)-C(10)	1.369(5)	C(111)-C(112)	1.352(5)
C(9)-C(14)	1.365(5)	C(111)-H(1111)	0.939
C(10)-C(11)	1.405(5)	C(112)-C(113)	1.357(6)
C(10)-H(101)	0.937	C(112)-H(1121)	0.931
C(11)-C(12)	1.357(5)	C(113)-C(114)	1.402(5)
C(11)-H(111)	0.939	C(113)-H(1131)	0.925
C(12)-C(13)	1.354(5)	C(114)-H(1141)	0.930
C(12)-H(121)	0.927	C(119)-C(120)	1.448(7)
C(13)-C(14)	1.406(5)	C(119)-H(1191)	0.968
C(13)-H(131)	0.929	C(119)-H(1192)	0.956
C(14)-H(141)	0.937	C(120)-C(121)	1.299(8)

C(120)-H(1201)	0.932	C(1)-C(6)-O(23)	110.60(18)
C(121)-H(1211)	0.939	C(5)-C(6)-H(61)	108.8
C(121)-H(1212)	0.939	C(1)-C(6)-H(61)	107.0
		O(23)-C(6)-H(61)	109.1
C(2)-C(1)-C(6)	113.0(2)	C(1)-O(7)-C(8)	116.2(3)
C(2)-C(1)-O(7)	108.0(2)	C(1)-O(7)-C(108)	113.3(4)
C(6)-C(1)-O(7)	108.52(17)	O(7)-C(8)-C(9)	108.09(7)
C(2)-C(1)-H(11)	108.9	O(7)-C(8)-H(81)	110.4
C(6)-C(1)-H(11)	110.2	C(9)-C(8)-H(81)	110.9
O(7)-C(1)-H(11)	108.0	O(7)-C(8)-H(82)	109.4
C(1)-C(2)-C(3)	110.5(2)	C(9)-C(8)-H(82)	110.3
C(1)-C(2)-O(15)	109.7(2)	H(81)-C(8)-H(82)	107.7
C(3)-C(2)-O(15)	109.7(2)	C(8)-C(9)-C(10)	121.33(6)
C(1)-C(2)-H(21)	108.6	C(8)-C(9)-C(14)	121.31(6)
C(3)-C(2)-H(21)	108.8	C(10)-C(9)-C(14)	117.30(6)
O(15)-C(2)-H(21)	109.5	C(9)-C(10)-C(11)	122.18(7)
C(2)-C(3)-C(4)	111.81(17)	C(9)-C(10)-H(101)	118.2
C(2)-C(3)-O(17)	109.6(2)	C(11)-C(10)-H(101)	119.6
C(4)-C(3)-O(17)	111.0(2)	C(10)-C(11)-C(12)	117.77(7)
C(2)-C(3)-H(31)	108.6	C(10)-C(11)-H(111)	121.5
C(4)-C(3)-H(31)	106.6	C(12)-C(11)-H(111)	120.7
O(17)-C(3)-H(31)	109.2	C(11)-C(12)-C(13)	122.55(7)
C(3)-C(4)-C(5)	110.4(2)	C(11)-C(12)-H(121)	119.0
C(3)-C(4)-O(18)	109.69(18)	C(13)-C(12)-H(121)	118.4
C(5)-C(4)-O(18)	110.59(17)	C(12)-C(13)-C(14)	117.80(7)
C(3)-C(4)-H(41)	109.2	C(12)-C(13)-H(131)	120.6
C(5)-C(4)-H(41)	108.3	C(14)-C(13)-H(131)	121.6
O(18)-C(4)-H(41)	108.6	C(13)-C(14)-C(9)	122.22(7)
C(4)-C(5)-C(6)	110.98(18)	C(13)-C(14)-H(141)	118.3
C(4)-C(5)-O(22)	111.26(19)	C(9)-C(14)-H(141)	119.4
C(6)-C(5)-O(22)	112.78(17)	C(2)-O(15)-C(16)	114.7(3)
C(4)-C(5)-H(51)	105.8	O(15)-C(16)-H(161)	110.1
C(6)-C(5)-H(51)	108.0	O(15)-C(16)-H(162)	110.3
O(22)-C(5)-H(51)	107.7	H(161)-C(16)-H(162)	109.8
C(5)-C(6)-C(1)	111.66(17)	O(15)-C(16)-H(163)	109.4
C(5)-C(6)-O(23)	109.50(17)	H(161)-C(16)-H(163)	108.8

H(162)-C(16)-H(163)	108.5	C(109)-C(110)-C(111)	122.19(7)
C(3)-O(17)-H(171)	107.5	C(109)-C(110)-H(1101)	118.5
C(4)-O(18)-C(19)	113.11(9)	C(111)-C(110)-H(1101)	119.3
C(4)-O(18)-C(119)	113.99(9)	C(110)-C(111)-C(112)	117.76(7)
O(18)-C(19)-C(20)	110.64(10)	C(110)-C(111)-H(1111)	120.5
O(18)-C(19)-H(191)	108.0	C(112)-C(111)-H(1111)	121.7
C(20)-C(19)-H(191)	109.0	C(111)-C(112)-C(113)	122.53(7)
O(18)-C(19)-H(192)	110.1	C(111)-C(112)-H(1121)	118.9
C(20)-C(19)-H(192)	110.9	C(113)-C(112)-H(1121)	118.6
H(191)-C(19)-H(192)	108.3	C(112)-C(113)-C(114)	117.77(7)
C(19)-C(20)-C(21)	126.63(10)	C(112)-C(113)-H(1131)	121.0
C(19)-C(20)-H(201)	117.8	C(114)-C(113)-H(1131)	121.2
C(21)-C(20)-H(201)	115.5	C(113)-C(114)-C(109)	122.19(7)
C(20)-C(21)-H(211)	120.1	C(113)-C(114)-H(1141)	118.2
C(20)-C(21)-H(212)	122.0	C(109)-C(114)-H(1141)	119.6
H(211)-C(21)-H(212)	118.0	O(18)-C(119)-C(120)	110.63(10)
C(5)-O(22)-H(221)	105.3	O(18)-C(119)-H(1191)	109.7
C(6)-O(23)-H(231)	106.4	C(120)-C(119)-H(1191)	109.6
O(7)-C(108)-C(109)	108.08(7)	O(18)-C(119)-H(1192)	110.3
O(7)-C(108)-H(1081)	110.8	C(120)-C(119)-H(1192)	106.2
C(109)-C(108)-H(1081)	110.0	H(1191)-C(119)-H(1192)	110.2
O(7)-C(108)-H(1082)	110.0	C(119)-C(120)-C(121)	126.61(10)
C(109)-C(108)-H(1082)	109.2	C(119)-C(120)-H(1201)	116.3
H(1081)-C(108)-H(1082)	108.8	C(121)-C(120)-H(1201)	117.1
C(108)-C(109)-C(110)	121.27(7)	C(120)-C(121)-H(1211)	122.3
C(108)-C(109)-C(114)	121.27(7)	C(120)-C(121)-H(1212)	120.5
C(110)-C(109)-C(114)	117.24(7)	H(1211)-C(121)-H(1212)	117.2

Symmetry transformations used to generate equivalent atoms:

Table 4. Anisotropic displacement parameters ($Å^2 x\ 10^3$) for 6607. The anisotropic displacement factor exponent takes the form: $-2\pi^2[\ h^2\ a^{*2}U^{11} + ... + 2\ h\ k\ a^*\ b^*\ U^{12}\ ]$

	U^{11}	U^{22}	U^{33}	U^{23}	U^{13}	U^{12}
C(1)	39(1)	53(1)	101(1)	0(1)	13(1)	0(1)
C(2)	45(1)	49(1)	120(1)	-7(1)	16(1)	0(1)
C(3)	32(1)	43(1)	152(1)	14(1)	6(1)	2(1)
C(4)	32(1)	56(1)	116(1)	23(1)	-9(1)	-2(1)
C(5)	33(1)	50(1)	105(1)	11(1)	-2(1)	-1(1)
C(6)	35(1)	44(1)	93(1)	8(1)	8(1)	2(1)
O(7)	59(1)	81(1)	87(1)	3(1)	11(1)	-5(1)
C(8)	77(4)	152(6)	93(2)	3(4)	24(2)	3(3)
C(9)	95(5)	149(5)	87(3)	20(3)	21(3)	-7(4)
C(10)	136(6)	142(5)	109(6)	32(4)	5(5)	-2(4)
C(11)	162(7)	177(7)	128(7)	38(5)	-6(6)	19(6)
C(12)	142(7)	206(10)	120(6)	43(6)	-7(5)	3(7)
C(13)	158(8)	223(10)	99(6)	12(6)	-9(5)	15(8)
C(14)	139(7)	199(7)	79(5)	2(4)	13(4)	21(6)
O(15)	91(1)	67(1)	158(2)	-33(1)	37(1)	-3(1)
C(16)	145(3)	115(3)	152(3)	-53(3)	15(3)	-30(3)
O(17)	45(1)	44(1)	201(2)	25(1)	8(1)	1(1)
O(18)	35(1)	77(1)	96(1)	24(1)	-5(1)	-5(1)
C(19)	56(2)	103(4)	115(3)	48(3)	-12(2)	-19(3)
C(20)	153(7)	151(7)	98(2)	20(4)	10(4)	-7(6)
C(21)	225(14)	242(16)	106(6)	15(8)	-39(6)	-17(12)
O(22)	49(1)	66(1)	101(1)	5(1)	-7(1)	-6(1)
O(23)	50(1)	43(1)	108(1)	11(1)	7(1)	0(1)
C(108)	100(7)	156(9)	96(5)	23(5)	14(3)	-36(6)
C(109)	103(8)	185(7)	78(4)	17(5)	26(4)	-14(6)
C(110)	165(11)	195(8)	115(10)	42(7)	2(8)	-6(8)
C(111)	176(12)	207(10)	142(12)	32(9)	-19(9)	4(10)
C(112)	146(11)	221(13)	120(10)	32(9)	12(7)	-13(10)
C(113)	141(9)	216(11)	92(7)	14(8)	0(5)	-15(10)
C(114)	121(8)	210(9)	84(7)	8(6)	11(5)	-23(7)
C(119)	49(2)	63(3)	100(2)	9(3)	-6(2)	-12(2)

C(120)	122(6)	82(4)	95(3)	23(3)	31(4)	13(4)
C(121)	165(11)	185(12)	97(5)	48(6)	33(5)	85(10)

Table 5. Hydrogen coordinates (x 10^4) and isotropic displacement parameters (Å^{2}x 10^{-3}) for 6607.

	x	y	z	U(eq)
H(11)	-111	4497	5839	75
H(21)	4606	5642	5543	82
H(31)	-864	5990	5227	90
H(41)	509	5450	4509	82
H(51)	-1333	4178	4979	74
H(61)	4041	3660	5268	67
H(81)	614	3479	6482	128
H(82)	1498	4633	6649	128
H(101)	4624	2080	6527	154
H(111)	7838	1307	7036	186
H(121)	9124	2258	7673	187
H(131)	7759	3969	7785	192
H(141)	4436	4692	7294	166
H(161)	5241	7228	5987	206
H(162)	5432	6198	6302	206
H(163)	3633	7163	6454	206
H(171)	658	7410	5050	144
H(191)	3933	6421	4245	110
H(192)	6912	5946	4221	110
H(201)	4770	4449	3814	161
H(211)	2196	5016	3251	229
H(212)	1937	6178	3483	229
H(221)	2431	3134	4548	107
H(231)	2340	2335	5514	100
H(1081)	1247	2986	6363	141
H(1082)	566	4134	6567	141
H(1101)	4420	1918	6765	190
H(1111)	7386	1601	7384	211
H(1121)	9178	3047	7765	195
H(1131)	7575	4727	7634	180

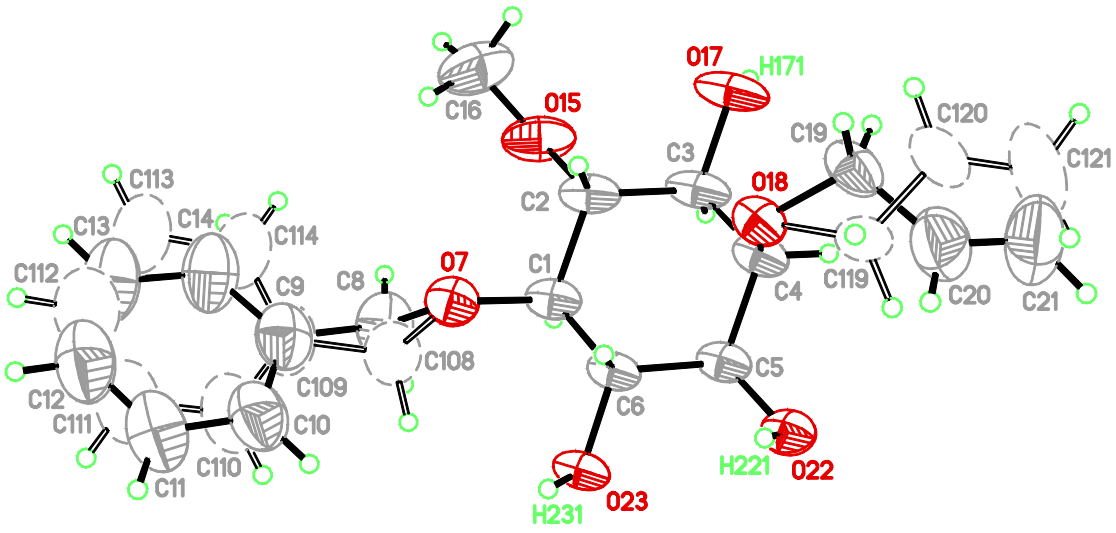
H(1141)	4453	5002	7040	166
H(1191)	3653	4500	4023	85
H(1192)	6770	4807	4091	85
H(1201)	5394	6619	4015	120
H(1211)	2348	5575	3354	178
H(1212)	3084	6820	3369	178

Table 6. Hydrogen bonds for 6607 [Å and °].

D-H...A	d(D-H)	d(H...A)	d(D...A)	<(DHA)
O(17)-H(171)...O(17)#1	0.80	1.94	2.716(14)	163
O(22)-H(221)...O(23)#2	0.84	2.04	2.872(14)	170
O(23)-H(231)...O(22)#2	0.80	1.93	2.706(14)	163
C(20)-H(1191)...O(22)	1.00	2.44	3.383(14)	156
C(119)-H(1191)...O(22)	0.97	2.44	3.074(14)	123

Symmetry transformations used to generate equivalent atoms:

#1 x-1/2,-y+3/2,-z+1 #2 x+1/2,-y+1/2,-z+1



Displacement ellipsoids drawn at 30% for clarity.

Table 1. Crystal data and structure refinement for 6552.

Identification code	6552	
Empirical formula	C27 H31 Cl1 O7	
Formula weight	502.99	
Temperature	150 K	
Wavelength	1.54184 Å	
Crystal system	Monoclinic	
Space group	P 1 21/c 1	
Unit cell dimensions	a = 21.6782(4) Å	α= 90°.
	b = 9.4770(2) Å	β= 91.7088(15)°.
	c = 12.0900(2) Å	γ = 90°.
Volume	2482.72(8) $Å^3$	
Z	4	
Density (calculated)	1.346 Mg/m^3	
Absorption coefficient	1.743 mm^{-1}	
F(000)	1064	
Crystal size	0.38 x 0.28 x 0.20 mm^3	
Theta range for data collection	4.080 to 77.165°.	
Index ranges	-27<=h<=27, -11<=k<=10, -15<=l<=15	
Reflections collected	54113	
Independent reflections	5162 [R(int) = 0.059]	
Completeness to theta = 74.850°	99.9 %	
Absorption correction	Semi-empirical from equivalents	
Max. and min. transmission	0.71 and 0.49	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	5174 / 0 / 316	
Goodness-of-fit on F^2	0.9389	
Final R indices [I>2sigma(I)]	R1 = 0.0659, wR2 = 0.1790	
R indices (all data)	R1 = 0.0695, wR2 = 0.1817	
Largest diff. peak and hole	0.84 and -0.52 $e.Å^{-3}$	

Table 2. Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters ($Å^2$x 10^3) for 6552. U(eq) is defined as one third of the trace of the orthogonalized U^{ij} tensor.

	x	y	z	U(eq)
C(1)	3260(1)	6322(3)	3011(2)	25
C(2)	2829(2)	5069(3)	3032(2)	27
C(3)	2397(2)	4925(3)	2021(3)	30
C(4)	2127(2)	6328(4)	1621(3)	29
C(5)	2623(2)	7450(3)	1514(3)	29
C(6)	2950(1)	7681(3)	2632(3)	26
O(7)	3519(1)	6596(2)	4111(2)	27
C(8)	4061(1)	5991(3)	4365(3)	29
O(9)	4301(1)	5105(3)	3818(2)	46
C(10)	4330(2)	6495(3)	5457(3)	32
Cl(11)	4295(1)	8373(1)	5578(1)	36
O(12)	2393(1)	5206(2)	3890(2)	29
C(13)	1907(2)	4226(3)	3616(3)	31
C(14)	1995(2)	2791(4)	4187(3)	37
C(15)	1554(2)	2818(4)	5143(3)	44
C(16)	1000(2)	3600(4)	4633(3)	42
C(17)	1295(2)	4814(4)	4004(3)	36
O(18)	1925(1)	4038(3)	2435(2)	38
O(19)	1823(1)	6109(3)	573(2)	38
C(20)	1187(2)	6366(4)	562(3)	38
C(21)	907(2)	6167(4)	-577(3)	31
C(22)	284(2)	6450(5)	-766(3)	47
C(23)	9(2)	6280(5)	-1801(4)	51
C(24)	352(2)	5786(5)	-2662(3)	45
C(25)	970(2)	5510(4)	-2492(3)	41
C(26)	1248(2)	5700(4)	-1453(3)	32
O(27)	2351(1)	8749(3)	1164(2)	37
O(28)	3418(1)	8718(2)	2529(2)	32
C(29)	3282(2)	10069(4)	2997(3)	39
C(30)	3637(2)	11151(3)	2374(3)	34
C(31)	4227(2)	11537(4)	2697(3)	46

C(32)	4563(2)	12471(5)	2070(4)	52
C(33)	4313(2)	12995(4)	1103(4)	49
C(34)	3728(2)	12602(5)	761(4)	61
C(35)	3387(2)	11687(5)	1395(4)	51

Table 3. Bond lengths [Å] and angles [°] for 6552.

C(1)-C(2)	1.511(4)	C(16)-C(17)	1.530(5)
C(1)-C(6)	1.517(4)	C(16)-H(162)	0.972
C(1)-O(7)	1.451(3)	C(16)-H(161)	0.977
C(1)-H(11)	0.986	C(17)-H(171)	0.989
C(2)-C(3)	1.524(4)	C(17)-H(172)	0.965
C(2)-O(12)	1.430(4)	O(19)-C(20)	1.399(4)
C(2)-H(21)	0.975	C(20)-C(21)	1.501(5)
C(3)-C(4)	1.525(5)	C(20)-H(201)	0.970
C(3)-O(18)	1.427(4)	C(20)-H(202)	0.954
C(3)-H(31)	0.981	C(21)-C(22)	1.388(5)
C(4)-C(5)	1.520(4)	C(21)-C(26)	1.383(5)
C(4)-O(19)	1.427(4)	C(22)-C(23)	1.379(5)
C(4)-H(41)	0.985	C(22)-H(221)	0.925
C(5)-C(6)	1.523(4)	C(23)-C(24)	1.379(6)
C(5)-O(27)	1.425(4)	C(23)-H(231)	0.944
C(5)-H(51)	1.004	C(24)-C(25)	1.374(5)
C(6)-O(28)	1.420(4)	C(24)-H(241)	0.937
C(6)-H(61)	0.999	C(25)-C(26)	1.388(5)
O(7)-C(8)	1.335(4)	C(25)-H(251)	0.935
C(8)-O(9)	1.198(4)	C(26)-H(261)	0.944
C(8)-C(10)	1.505(4)	O(27)-H(271)	0.834
C(10)-Cl(11)	1.788(3)	O(28)-C(29)	1.435(4)
C(10)-H(101)	0.988	C(29)-C(30)	1.499(5)
C(10)-H(102)	0.990	C(29)-H(292)	0.989
O(12)-C(13)	1.435(4)	C(29)-H(291)	1.002
C(13)-C(14)	1.535(5)	C(30)-C(31)	1.376(5)
C(13)-C(17)	1.527(5)	C(30)-C(35)	1.384(5)
C(13)-O(18)	1.440(4)	C(31)-C(32)	1.385(6)
C(14)-C(15)	1.522(5)	C(31)-H(311)	0.928
C(14)-H(141)	0.963	C(32)-C(33)	1.367(6)
C(14)-H(142)	0.988	C(32)-H(321)	0.949
C(15)-C(16)	1.526(5)	C(33)-C(34)	1.375(6)
C(15)-H(151)	0.972	C(33)-H(331)	0.935
C(15)-H(152)	0.974	C(34)-C(35)	1.386(6)

C(34)-H(341)	0.931	C(5)-C(6)-H(61)	109.1
C(35)-H(351)	0.943	C(1)-C(6)-H(61)	110.2
		O(28)-C(6)-H(61)	110.5
C(2)-C(1)-C(6)	113.9(2)	C(1)-O(7)-C(8)	116.6(2)
C(2)-C(1)-O(7)	110.4(2)	O(7)-C(8)-O(9)	124.6(3)
C(6)-C(1)-O(7)	106.3(2)	O(7)-C(8)-C(10)	112.4(3)
C(2)-C(1)-H(11)	110.8	O(9)-C(8)-C(10)	123.0(3)
C(6)-C(1)-H(11)	109.4	C(8)-C(10)-Cl(11)	111.8(2)
O(7)-C(1)-H(11)	105.6	C(8)-C(10)-H(101)	109.1
C(1)-C(2)-C(3)	115.0(3)	Cl(11)-C(10)-H(101)	106.8
C(1)-C(2)-O(12)	111.3(2)	C(8)-C(10)-H(102)	110.3
C(3)-C(2)-O(12)	100.7(2)	Cl(11)-C(10)-H(102)	107.1
C(1)-C(2)-H(21)	109.2	H(101)-C(10)-H(102)	111.6
C(3)-C(2)-H(21)	110.5	C(2)-O(12)-C(13)	105.7(2)
O(12)-C(2)-H(21)	109.9	O(12)-C(13)-C(14)	112.9(3)
C(2)-C(3)-C(4)	113.4(3)	O(12)-C(13)-C(17)	109.4(3)
C(2)-C(3)-O(18)	101.7(2)	C(14)-C(13)-C(17)	106.4(3)
C(4)-C(3)-O(18)	110.6(3)	O(12)-C(13)-O(18)	105.5(2)
C(2)-C(3)-H(31)	110.9	C(14)-C(13)-O(18)	109.3(3)
C(4)-C(3)-H(31)	109.5	C(17)-C(13)-O(18)	113.5(3)
O(18)-C(3)-H(31)	110.5	C(13)-C(14)-C(15)	104.8(3)
C(3)-C(4)-C(5)	111.8(3)	C(13)-C(14)-H(141)	108.7
C(3)-C(4)-O(19)	108.6(3)	C(15)-C(14)-H(141)	109.9
C(5)-C(4)-O(19)	109.6(3)	C(13)-C(14)-H(142)	110.3
C(3)-C(4)-H(41)	108.5	C(15)-C(14)-H(142)	113.6
C(5)-C(4)-H(41)	110.3	H(141)-C(14)-H(142)	109.4
O(19)-C(4)-H(41)	108.0	C(14)-C(15)-C(16)	101.9(3)
C(4)-C(5)-C(6)	109.7(2)	C(14)-C(15)-H(151)	113.5
C(4)-C(5)-O(27)	110.0(3)	C(16)-C(15)-H(151)	112.8
C(6)-C(5)-O(27)	108.5(3)	C(14)-C(15)-H(152)	110.7
C(4)-C(5)-H(51)	109.8	C(16)-C(15)-H(152)	111.4
C(6)-C(5)-H(51)	109.2	H(151)-C(15)-H(152)	106.6
O(27)-C(5)-H(51)	109.6	C(15)-C(16)-C(17)	103.3(3)
C(5)-C(6)-C(1)	109.6(2)	C(15)-C(16)-H(162)	111.1
C(5)-C(6)-O(28)	109.7(2)	C(17)-C(16)-H(162)	108.7
C(1)-C(6)-O(28)	107.6(2)	C(15)-C(16)-H(161)	113.5

C(17)-C(16)-H(161)	113.6	C(26)-C(25)-H(251)	119.7
H(162)-C(16)-H(161)	106.6	C(25)-C(26)-C(21)	120.5(3)
C(16)-C(17)-C(13)	105.0(3)	C(25)-C(26)-H(261)	120.3
C(16)-C(17)-H(171)	111.1	C(21)-C(26)-H(261)	119.2
C(13)-C(17)-H(171)	109.8	C(5)-O(27)-H(271)	108.1
C(16)-C(17)-H(172)	110.4	C(6)-O(28)-C(29)	115.2(2)
C(13)-C(17)-H(172)	108.8	O(28)-C(29)-C(30)	107.3(3)
H(171)-C(17)-H(172)	111.5	O(28)-C(29)-H(292)	109.4
C(13)-O(18)-C(3)	108.4(2)	C(30)-C(29)-H(292)	111.4
C(4)-O(19)-C(20)	114.4(2)	O(28)-C(29)-H(291)	109.7
O(19)-C(20)-C(21)	111.0(3)	C(30)-C(29)-H(291)	110.7
O(19)-C(20)-H(201)	110.0	H(292)-C(29)-H(291)	108.4
C(21)-C(20)-H(201)	110.7	C(29)-C(30)-C(31)	121.8(3)
O(19)-C(20)-H(202)	109.8	C(29)-C(30)-C(35)	119.1(3)
C(21)-C(20)-H(202)	110.1	C(31)-C(30)-C(35)	118.8(3)
H(201)-C(20)-H(202)	105.1	C(30)-C(31)-C(32)	120.9(4)
C(20)-C(21)-C(22)	119.5(3)	C(30)-C(31)-H(311)	119.9
C(20)-C(21)-C(26)	122.1(3)	C(32)-C(31)-H(311)	119.2
C(22)-C(21)-C(26)	118.4(3)	C(31)-C(32)-C(33)	119.9(4)
C(21)-C(22)-C(23)	121.3(4)	C(31)-C(32)-H(321)	119.6
C(21)-C(22)-H(221)	119.1	C(33)-C(32)-H(321)	120.4
C(23)-C(22)-H(221)	119.6	C(32)-C(33)-C(34)	119.9(4)
C(22)-C(23)-C(24)	119.7(4)	C(32)-C(33)-H(331)	118.8
C(22)-C(23)-H(231)	119.4	C(34)-C(33)-H(331)	121.2
C(24)-C(23)-H(231)	120.9	C(33)-C(34)-C(35)	120.4(4)
C(23)-C(24)-C(25)	119.8(3)	C(33)-C(34)-H(341)	120.7
C(23)-C(24)-H(241)	120.0	C(35)-C(34)-H(341)	118.9
C(25)-C(24)-H(241)	120.2	C(34)-C(35)-C(30)	120.1(4)
C(24)-C(25)-C(26)	120.3(3)	C(34)-C(35)-H(351)	119.5
C(24)-C(25)-H(251)	119.9	C(30)-C(35)-H(351)	120.5

Symmetry transformations used to generate equivalent atoms:

Table 4. Anisotropic displacement parameters ($Å^2 x\ 10^3$) for 6552. The anisotropic displacement factor exponent takes the form: $-2\pi^2[\ h^2\ a^{*2}U^{11} + ... + 2\ h\ k\ a^*\ b^*\ U^{12}\ ]$

	U^{11}	U^{22}	U^{33}	U^{23}	U^{13}	U^{12}
C(1)	27(2)	24(2)	23(1)	-1(1)	-1(1)	-1(1)
C(2)	33(2)	23(1)	25(1)	-1(1)	0(1)	-1(1)
C(3)	35(2)	29(2)	27(2)	-3(1)	0(1)	-4(1)
C(4)	31(2)	34(2)	23(1)	-1(1)	-1(1)	-1(1)
C(5)	31(2)	27(2)	28(2)	5(1)	1(1)	4(1)
C(6)	30(2)	22(1)	28(2)	2(1)	3(1)	0(1)
O(7)	29(1)	28(1)	24(1)	-3(1)	-3(1)	2(1)
C(8)	27(2)	25(2)	34(2)	-1(1)	-1(1)	1(1)
O(9)	48(2)	43(2)	48(2)	-16(1)	-10(1)	20(1)
C(10)	34(2)	28(2)	36(2)	-1(1)	-6(1)	3(1)
Cl(11)	36(1)	31(1)	42(1)	-8(1)	0(1)	-3(1)
O(12)	35(1)	26(1)	24(1)	-2(1)	0(1)	-8(1)
C(13)	35(2)	26(2)	30(2)	2(1)	-5(1)	-9(1)
C(14)	47(2)	26(2)	39(2)	3(1)	-4(2)	-5(2)
C(15)	57(2)	40(2)	35(2)	10(2)	-5(2)	-9(2)
C(16)	40(2)	48(2)	37(2)	4(2)	1(2)	-11(2)
C(17)	35(2)	33(2)	40(2)	2(2)	-4(1)	-4(1)
O(18)	49(1)	35(1)	29(1)	-3(1)	-3(1)	-18(1)
O(19)	32(1)	55(2)	26(1)	-5(1)	-4(1)	3(1)
C(20)	34(2)	50(2)	32(2)	-8(2)	-1(1)	2(2)
C(21)	31(2)	28(2)	35(2)	-2(1)	-2(1)	-1(1)
C(22)	34(2)	62(3)	45(2)	-11(2)	-1(2)	10(2)
C(23)	32(2)	69(3)	51(2)	-5(2)	-10(2)	7(2)
C(24)	43(2)	55(2)	36(2)	-3(2)	-10(2)	-2(2)
C(25)	43(2)	48(2)	32(2)	-5(2)	-2(2)	1(2)
C(26)	31(2)	31(2)	34(2)	0(1)	-2(1)	2(1)
O(27)	44(1)	34(1)	33(1)	11(1)	3(1)	10(1)
O(28)	35(1)	19(1)	43(1)	-1(1)	6(1)	-3(1)
C(29)	59(2)	23(2)	36(2)	-4(1)	10(2)	-2(2)
C(30)	46(2)	20(2)	36(2)	-2(1)	6(1)	1(1)
C(31)	58(2)	46(2)	34(2)	-5(2)	0(2)	-9(2)

C(32)	53(2)	49(2)	55(2)	-15(2)	6(2)	-16(2)
C(33)	58(2)	25(2)	65(3)	5(2)	19(2)	0(2)
C(34)	56(3)	51(3)	76(3)	38(2)	3(2)	6(2)
C(35)	42(2)	46(2)	65(3)	23(2)	-3(2)	0(2)

Table 5. Hydrogen coordinates (x 10^4) and isotropic displacement parameters ($Å^2$x 10^{-3}) for 6552.

	x	y	z	U(eq)
H(11)	3616	6127	2545	30
H(21)	3071	4210	3142	32
H(31)	2606	4460	1411	36
H(41)	1816	6636	2147	35
H(51)	2933	7138	963	34
H(61)	2642	7988	3180	32
H(101)	4086	6102	6062	39
H(102)	4771	6224	5534	38
H(141)	1876	2059	3672	45
H(142)	2432	2659	4423	45
H(151)	1449	1885	5413	53
H(152)	1734	3332	5771	53
H(162)	773	3005	4107	50
H(161)	704	3911	5177	50
H(171)	1033	5098	3358	43
H(172)	1377	5600	4494	43
H(201)	991	5753	1089	46
H(202)	1111	7301	818	47
H(221)	50	6741	-182	56
H(231)	-417	6468	-1902	59
H(241)	166	5662	-3365	54
H(251)	1207	5219	-3083	49
H(261)	1676	5552	-1346	37
H(292)	2832	10244	2931	46
H(291)	3407	10078	3802	47
H(311)	4404	11177	3346	55
H(321)	4972	12710	2299	62
H(331)	4557	13568	662	59
H(341)	3553	12943	100	72
H(351)	2984	11431	1152	61

H(271)	2422	8861	496	55

Table 6. Hydrogen bonds for 6552 [Å and °].

D-H...A	d(D-H)	d(H...A)	d(D...A)	<(DHA)
C(10)-H(101)...O(28)#1	0.99	2.33	3.244(5)	153
C(10)-H(102)...O(9)#2	0.99	2.48	3.422(5)	159
O(27)-H(271)...O(12)#3	0.83	2.13	2.927(5)	159

Symmetry transformations used to generate equivalent atoms:

#1 x,-y+3/2,z+1/2 #2 -x+1,-y+1,-z+1 #3 x,-y+3/2,z-1/2

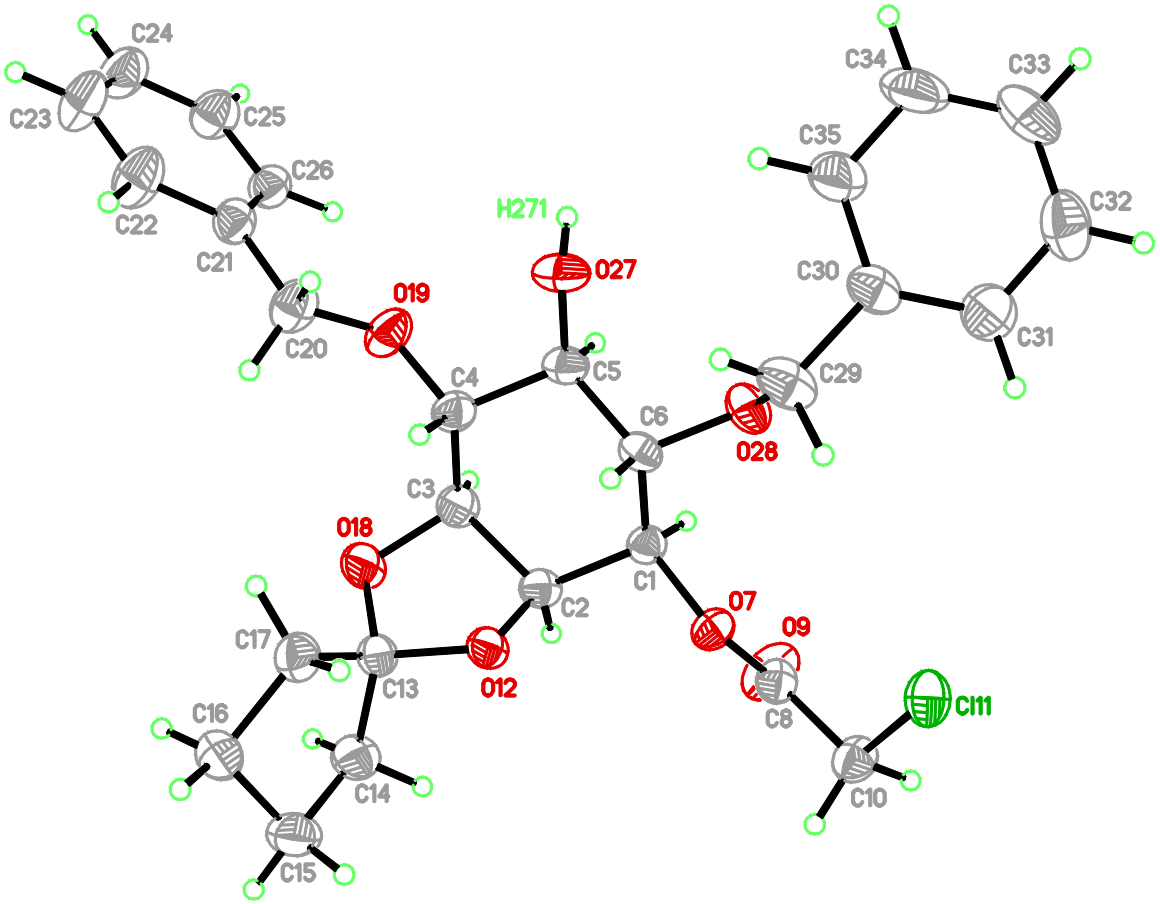


Table 1. Crystal data and structure refinement for 6602.

Identification code	6602b	
Empirical formula	C17 H24 O6	
Formula weight	324.37	
Temperature	293 K	
Wavelength	1.54184 Å	
Crystal system	Orthorhombic	
Space group	P 21 21 21	
Unit cell dimensions	a = 4.8658(2) Å	α= 90°.
	b = 12.5495(6) Å	β= 90°.
	c = 28.980(2) Å	γ = 90°.
Volume	1769.62(17) $Å^3$	
Z	4	
Density (calculated)	1.217 Mg/m^3	
Absorption coefficient	0.761 mm^{-1}	
F(000)	696.000	
Crystal size	0.60 x 0.02 x 0.02 mm^3	
Theta range for data collection	3.838 to 76.552°.	
Index ranges	-6<=h<=5, -15<=k<=13, -35<=l<=34	
Reflections collected	22068	
Independent reflections	3657 [R(int) = 0.062]	
Completeness to theta = 74.256°	99.6 %	
Absorption correction	Semi-empirical from equivalents	
Max. and min. transmission	0.98 and 0.44	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	3657 / 372 / 301	
Goodness-of-fit on F^2	0.9873	
Final R indices [I>2sigma(I)]	R1 = 0.0515, wR2 = 0.1347	
R indices (all data)	R1 = 0.0619, wR2 = 0.1485	
Absolute structure parameter	0.0(3)	
Largest diff. peak and hole	0.21 and -0.19 $e.Å^{-3}$	

Table 2. Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters ($Å^2$x 10^3) for 6602. U(eq) is defined as one third of the trace of the orthogonalized U^{ij} tensor.

	x	y	z	U(eq)
C(1)	8244(5)	5508(2)	4270(1)	62
C(2)	7407(5)	4376(2)	4400(1)	69
C(3)	8891(4)	4016(2)	4833(1)	74
C(4)	8441(4)	4780(2)	5229(1)	67
C(5)	9413(5)	5893(2)	5093(1)	62
C(6)	7939(4)	6280(2)	4667(1)	56
O(7)	6501(4)	5856(2)	3900(1)	73
C(8)	7796(16)	5972(13)	3466(3)	105
C(9)	5770(20)	6504(6)	3144(2)	119
C(10)	5060(40)	7545(7)	3200(4)	149
C(11)	3080(40)	8043(10)	2916(5)	163
C(12)	2170(40)	7488(15)	2543(5)	158
C(13)	2940(40)	6474(15)	2453(5)	158
C(14)	4890(40)	6002(9)	2753(4)	145
O(15)	8052(5)	3673(2)	4030(1)	103
C(16)	5744(12)	3242(4)	3804(2)	135
O(17)	8019(4)	2965(1)	4956(1)	96
O(18)	5609(3)	4794(1)	5350(1)	69
C(19)	5058(7)	4222(4)	5763(2)	89
C(20)	5910(20)	4834(6)	6168(2)	135
C(21)	7350(40)	4484(11)	6512(4)	189
O(22)	9170(4)	6612(1)	5471(1)	69
O(23)	8982(3)	7303(1)	4538(1)	65
C(108)	7932(12)	6337(12)	3528(3)	100
C(109)	5879(19)	6548(7)	3148(2)	109
C(110)	4630(30)	7517(8)	3107(5)	145
C(111)	2800(40)	7744(13)	2745(7)	162
C(112)	2100(30)	6932(18)	2462(5)	156
C(113)	2990(30)	5917(16)	2526(4)	149
C(114)	4920(30)	5740(9)	2879(5)	135
C(119)	5113(8)	4964(3)	5862(2)	71

C(120)	5451(19)	3974(5)	6116(2)	97
C(121)	6820(30)	3849(8)	6492(4)	148

Table 3. Bond lengths [Å] and angles [°] for 6602.

C(1)-C(2)	1.526(3)	O(15)-C(16)	1.409(5)
C(1)-C(6)	1.512(3)	C(16)-H(161)	0.989
C(1)-O(7)	1.434(3)	C(16)-H(162)	0.963
C(1)-H(11)	0.981	C(16)-H(163)	0.952
C(2)-C(3)	1.516(4)	O(17)-H(171)	0.813
C(2)-O(15)	1.424(3)	O(18)-C(19)	1.420(5)
C(2)-H(21)	0.975	O(18)-C(119)	1.516(5)
C(3)-C(4)	1.510(4)	C(19)-C(20)	1.464(7)
C(3)-O(17)	1.430(2)	C(19)-H(191)	0.971
C(3)-H(31)	0.986	C(19)-H(192)	0.984
C(4)-C(5)	1.526(3)	C(20)-C(21)	1.292(8)
C(4)-O(18)	1.422(3)	C(20)-H(201)	0.929
C(4)-H(41)	0.984	C(21)-H(211)	0.939
C(5)-C(6)	1.508(3)	C(21)-H(212)	0.941
C(5)-O(22)	1.426(3)	O(22)-H(221)	0.833
C(5)-H(51)	0.970	O(23)-H(231)	0.807
C(6)-O(23)	1.431(2)	C(108)-C(109)	1.510(5)
C(6)-H(61)	0.963	C(108)-H(1081)	0.972
O(7)-C(8)	1.413(5)	C(108)-H(1082)	0.988
O(7)-C(108)	1.418(6)	C(109)-C(110)	1.364(6)
C(8)-C(9)	1.514(5)	C(109)-C(114)	1.361(5)
C(8)-H(81)	0.978	C(110)-C(111)	1.407(5)
C(8)-H(82)	0.986	C(110)-H(1101)	0.934
C(9)-C(10)	1.362(5)	C(111)-C(112)	1.351(5)
C(9)-C(14)	1.364(5)	C(111)-H(1111)	0.939
C(10)-C(11)	1.412(5)	C(112)-C(113)	1.357(6)
C(10)-H(101)	0.938	C(112)-H(1121)	0.932
C(11)-C(12)	1.360(5)	C(113)-C(114)	1.408(5)
C(11)-H(111)	0.939	C(113)-H(1131)	0.925
C(12)-C(13)	1.351(5)	C(114)-H(1141)	0.933
C(12)-H(121)	0.931	C(119)-C(120)	1.453(7)
C(13)-C(14)	1.416(5)	C(119)-H(1191)	0.972
C(13)-H(131)	0.932	C(119)-H(1192)	0.952
C(14)-H(141)	0.936	C(120)-C(121)	1.288(8)

C(120)-H(1201)	0.934	C(5)-C(6)-O(23)	109.57(18)
C(121)-H(1211)	0.938	C(1)-C(6)-H(61)	106.0
C(121)-H(1212)	0.941	C(5)-C(6)-H(61)	108.8
		O(23)-C(6)-H(61)	110.8
C(2)-C(1)-C(6)	112.4(2)	C(1)-O(7)-C(8)	115.6(3)
C(2)-C(1)-O(7)	108.1(2)	C(1)-O(7)-C(108)	114.0(3)
C(6)-C(1)-O(7)	108.42(18)	O(7)-C(8)-C(9)	107.68(7)
C(2)-C(1)-H(11)	108.5	O(7)-C(8)-H(81)	109.8
C(6)-C(1)-H(11)	110.2	C(9)-C(8)-H(81)	110.4
O(7)-C(1)-H(11)	109.1	O(7)-C(8)-H(82)	109.8
C(1)-C(2)-C(3)	110.8(2)	C(9)-C(8)-H(82)	111.3
C(1)-C(2)-O(15)	109.3(2)	H(81)-C(8)-H(82)	107.9
C(3)-C(2)-O(15)	109.5(2)	C(8)-C(9)-C(10)	120.94(7)
C(1)-C(2)-H(21)	110.0	C(8)-C(9)-C(14)	120.94(7)
C(3)-C(2)-H(21)	108.1	C(10)-C(9)-C(14)	117.54(7)
O(15)-C(2)-H(21)	109.0	C(9)-C(10)-C(11)	121.80(7)
C(2)-C(3)-C(4)	111.69(18)	C(9)-C(10)-H(101)	119.2
C(2)-C(3)-O(17)	109.8(2)	C(11)-C(10)-H(101)	119.0
C(4)-C(3)-O(17)	110.7(2)	C(10)-C(11)-C(12)	117.38(7)
C(2)-C(3)-H(31)	109.3	C(10)-C(11)-H(111)	121.5
C(4)-C(3)-H(31)	106.5	C(12)-C(11)-H(111)	121.1
O(17)-C(3)-H(31)	108.7	C(11)-C(12)-C(13)	123.01(7)
C(3)-C(4)-C(5)	109.9(2)	C(11)-C(12)-H(121)	118.6
C(3)-C(4)-O(18)	109.67(19)	C(13)-C(12)-H(121)	118.4
C(5)-C(4)-O(18)	110.67(18)	C(12)-C(13)-C(14)	117.41(7)
C(3)-C(4)-H(41)	108.9	C(12)-C(13)-H(131)	120.8
C(5)-C(4)-H(41)	110.1	C(14)-C(13)-H(131)	121.8
O(18)-C(4)-H(41)	107.6	C(13)-C(14)-C(9)	121.84(7)
C(4)-C(5)-C(6)	111.01(19)	C(13)-C(14)-H(141)	119.0
C(4)-C(5)-O(22)	110.8(2)	C(9)-C(14)-H(141)	119.2
C(6)-C(5)-O(22)	112.71(18)	C(2)-O(15)-C(16)	114.4(3)
C(4)-C(5)-H(51)	105.6	O(15)-C(16)-H(161)	110.7
C(6)-C(5)-H(51)	109.1	O(15)-C(16)-H(162)	110.8
O(22)-C(5)-H(51)	107.3	H(161)-C(16)-H(162)	110.7
C(1)-C(6)-C(5)	111.72(18)	O(15)-C(16)-H(163)	107.1
C(1)-C(6)-O(23)	109.9(2)	H(161)-C(16)-H(163)	109.1

H(162)-C(16)-H(163)	108.4	C(109)-C(110)-C(111)	121.82(7)
C(3)-O(17)-H(171)	105.4	C(109)-C(110)-H(1101)	119.0
C(4)-O(18)-C(19)	112.68(9)	C(111)-C(110)-H(1101)	119.1
C(4)-O(18)-C(119)	113.49(9)	C(110)-C(111)-C(112)	117.38(7)
O(18)-C(19)-C(20)	110.94(10)	C(110)-C(111)-H(1111)	121.4
O(18)-C(19)-H(191)	107.5	C(112)-C(111)-H(1111)	121.2
C(20)-C(19)-H(191)	109.1	C(111)-C(112)-C(113)	123.01(7)
O(18)-C(19)-H(192)	109.5	C(111)-C(112)-H(1121)	118.9
C(20)-C(19)-H(192)	110.5	C(113)-C(112)-H(1121)	118.1
H(191)-C(19)-H(192)	109.2	C(112)-C(113)-C(114)	117.38(7)
C(19)-C(20)-C(21)	126.35(10)	C(112)-C(113)-H(1131)	121.2
C(19)-C(20)-H(201)	117.0	C(114)-C(113)-H(1131)	121.4
C(21)-C(20)-H(201)	116.6	C(113)-C(114)-C(109)	121.82(7)
C(20)-C(21)-H(211)	120.1	C(113)-C(114)-H(1141)	118.7
C(20)-C(21)-H(212)	121.0	C(109)-C(114)-H(1141)	119.5
H(211)-C(21)-H(212)	118.9	O(18)-C(119)-C(120)	110.94(10)
C(5)-O(22)-H(221)	107.3	O(18)-C(119)-H(1191)	110.7
C(6)-O(23)-H(231)	98.1	C(120)-C(119)-H(1191)	109.9
O(7)-C(108)-C(109)	107.68(7)	O(18)-C(119)-H(1192)	109.5
O(7)-C(108)-H(1081)	110.7	C(120)-C(119)-H(1192)	106.4
C(109)-C(108)-H(1081)	111.1	H(1191)-C(119)-H(1192)	109.3
O(7)-C(108)-H(1082)	109.8	C(119)-C(120)-C(121)	126.32(10)
C(109)-C(108)-H(1082)	109.2	C(119)-C(120)-H(1201)	117.3
H(1081)-C(108)-H(1082)	108.4	C(121)-C(120)-H(1201)	116.4
C(108)-C(109)-C(110)	120.91(6)	C(120)-C(121)-H(1211)	121.2
C(108)-C(109)-C(114)	120.91(6)	C(120)-C(121)-H(1212)	120.4
C(110)-C(109)-C(114)	117.51(7)	H(1211)-C(121)-H(1212)	118.4

Symmetry transformations used to generate equivalent atoms:

Table 4. Anisotropic displacement parameters ($Å^2 x\ 10^3$) for 6602. The anisotropic displacement factor exponent takes the form: $-2\pi^2[\ h^2\ a^{*2}U^{11} + ... + 2\ h\ k\ a^*\ b^*\ U^{12}\ ]$

	U^{11}	U^{22}	U^{33}	U^{23}	U^{13}	U^{12}
C(1)	42(1)	45(1)	99(1)	-1(1)	10(1)	0(1)
C(2)	49(1)	41(1)	118(1)	-8(1)	15(1)	0(1)
C(3)	38(1)	35(1)	148(2)	13(1)	6(1)	0(1)
C(4)	36(1)	49(1)	115(1)	22(1)	-7(1)	-3(1)
C(5)	40(1)	43(1)	101(1)	9(1)	-1(1)	-1(1)
C(6)	38(1)	37(1)	92(1)	5(1)	6(1)	-1(1)
O(7)	60(1)	74(1)	85(1)	3(1)	11(1)	-5(1)
C(8)	95(5)	130(7)	90(2)	-1(4)	27(2)	7(4)
C(9)	132(8)	147(6)	79(3)	11(4)	19(5)	11(6)
C(10)	191(11)	134(6)	122(7)	25(5)	-32(7)	9(7)
C(11)	209(12)	156(7)	124(8)	25(6)	-42(8)	18(8)
C(12)	190(12)	187(10)	97(7)	24(7)	-15(6)	13(9)
C(13)	187(11)	197(11)	90(7)	6(7)	-8(6)	16(9)
C(14)	170(10)	189(8)	75(6)	-4(5)	12(5)	27(7)
O(15)	95(1)	60(1)	153(2)	-33(1)	36(1)	-4(1)
C(16)	150(3)	108(3)	148(4)	-51(3)	15(3)	-35(3)
O(17)	49(1)	36(1)	201(2)	25(1)	9(1)	-1(1)
O(18)	40(1)	72(1)	95(1)	23(1)	-3(1)	-5(1)
C(19)	56(3)	101(5)	111(3)	47(3)	-13(2)	-19(3)
C(20)	166(8)	141(7)	97(2)	25(4)	0(5)	-13(6)
C(21)	240(15)	224(16)	103(7)	14(8)	-46(7)	-6(13)
O(22)	51(1)	61(1)	96(1)	3(1)	-6(1)	-6(1)
O(23)	52(1)	37(1)	107(1)	10(1)	6(1)	-1(1)
C(108)	78(5)	128(8)	93(4)	14(4)	15(2)	-25(4)
C(109)	81(6)	162(6)	84(4)	24(4)	19(4)	-30(5)
C(110)	105(7)	174(7)	155(9)	39(7)	-20(6)	-18(6)
C(111)	146(10)	195(9)	146(12)	43(8)	-32(8)	-18(8)
C(112)	135(9)	222(12)	110(8)	37(8)	8(6)	-41(9)
C(113)	142(8)	223(10)	82(6)	2(7)	1(4)	-27(9)
C(114)	120(7)	208(8)	79(6)	-8(6)	12(4)	-25(6)
C(119)	56(3)	60(3)	98(2)	9(3)	-5(2)	-13(2)

C(120)	129(6)	75(4)	89(3)	18(3)	22(4)	8(4)
C(121)	172(12)	180(13)	93(6)	37(6)	20(5)	89(11)

Table 5. Hydrogen coordinates (x 10^4) and isotropic displacement parameters ($Å^2$x 10^{-3}) for 6602.

	x	y	z	U(eq)
H(11)	10156	5496	4162	73
H(21)	5437	4345	4460	80
H(31)	10887	4004	4774	87
H(41)	9476	4525	5499	80
H(51)	11355	5821	5024	72
H(61)	6000	6318	4732	65
H(81)	9449	6409	3499	126
H(82)	8358	5268	3349	126
H(101)	5906	7944	3434	179
H(111)	2418	8729	2981	196
H(121)	970	7822	2339	189
H(131)	2214	6107	2202	190
H(141)	5571	5324	2683	174
H(161)	4707	2766	4013	203
H(162)	4577	3800	3687	203
H(163)	6412	2838	3549	203
H(171)	9406	2603	4954	142
H(191)	6101	3563	5751	107
H(192)	3085	4054	5780	107
H(201)	5326	5537	6186	162
H(211)	7784	4938	6758	228
H(212)	7977	3775	6519	228
H(221)	7593	6870	5462	103
H(231)	7532	7591	4484	97
H(1081)	8814	6993	3627	119
H(1082)	9364	5847	3413	119
H(1101)	5027	8047	3323	173
H(1111)	2119	8435	2696	195
H(1121)	926	7067	2215	187
H(1131)	2327	5363	2347	178

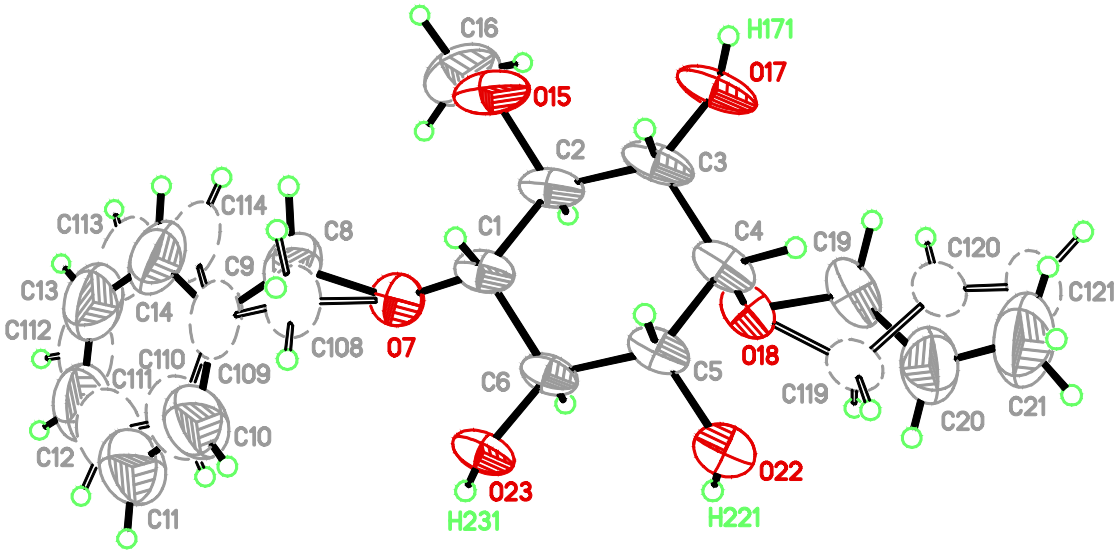
H(1141)	5562	5048	2928	162
H(1191)	6352	5503	5983	86
H(1192)	3263	5185	5909	86
H(1201)	4624	3365	5994	117
H(1211)	7692	4426	6637	179
H(1212)	6973	3171	6628	179

Table 6. Hydrogen bonds for 6602 [Å and °].

D-H...A	d(D-H)	d(H...A)	d(D...A)	<(DHA)
O(17)-H(171)...O(17)#1	0.81	1.91	2.710(14)	166
O(22)-H(221)...O(23)#2	0.83	2.04	2.868(14)	172
O(23)-H(231)...O(22)#2	0.81	1.92	2.709(14)	164
C(20)-H(1191)...O(22)	1.02	2.45	3.402(14)	155
C(119)-H(1191)...O(22)	0.97	2.45	3.075(14)	122

Symmetry transformations used to generate equivalent atoms:

#1 x+1/2,-y+1/2,-z+1 #2 x-1/2,-y+3/2,-z+1



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