Probes for Bacterial Ion Channels



Isabella Diane Swallow

St Hugh's College, University of Oxford

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Supervisor: Prof. Stuart Conway

Abstract: Probes for Bacterial Ion Channels

Miss Isabella Diane Swallow DPhil Organic Chemistry Supervisor: Dr Stuart Conway
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Using three complementary approaches, this work sought to tackle the widespread problem of antibiotic resistance. To circumvent the resistance mechanisms developed by bacteria, it is necessary to establish drug candidates that act on novel therapeutic targets, such as the ion channels used by bacteria to modulate homeostasis. Examples include the potassium efflux channel, Kef, and the mechanosensitive channel of small conductance, MscS, which are not found in humans. How these targets function must be well understood before drug candidates can be developed, as such, their identification and investigation is often accompanied by the evolution of the analytical techniques used to study them.

Membrane protein mass spectrometry is one technique showing potential in the study of ion channels. However, spectra can be clouded by the detergents used to solubilise ion channels from their native membranes. Undertaken herein was the synthesis of some fluorescent glycolipid detergents, which it was hypothesised could be encouraged to dissociate from ion channels *via* laser-induced excitation within the gas phase of a mass spectrometer, thereby improving the clarity with which spectra can be obtained.

For Kef, an unconfirmed mechanism of action had previously been proposed. To explore the suggestion that sterically-demanding central residues are important for channel activation, solid phase peptide synthesis was used to isolate three tripeptide analogues of *N*-ethylsuccinimido glutathione, a known activator with a high affinity for Kef. A competition fluorescence assay suggested these tripeptides bound to Kef with an affinity lower than predicted, allowing the conclusion that a more detailed assessment of the steric bulk required for activation was necessary before a mechanism of action could be confirmed.

Lysophosphatidylcholine has been shown to activate MscS, although it is not known how. Affinity chromatography between MscS and lysophosphatidylcholine was proposed as a means by which specific binding interactions could be investigated. For this technique an amino-derivative of lysophosphatidylcholine was necessary and its challenging synthesis is also detailed herein.

SCHOOL OF CHEMISTRY

DECLARATION OF AUTHORSHIP

Name: ISABELLA DIANE SWALLOW **Candidate number:**

College: ST HUGH'S COLLEGE **Supervisor:** PROF STUART CONWAY

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"These things I have spoken to you, that in Me you may have peace. In the world you have tribulation, but take courage; I have overcome the world." ***John 16:33***

Abbreviations

Ac	Acetyl
ATP	Adenosine triphosphate
Bn	Benzyl
Boc	*t*-Butoxycarbonyl
Bu	Butyl
Bz	Benzoyl
Cbz	Carboxybenzyl
CID	Collision-induced dissociation
CMC	Critical micelle concentration
conc.	Concentrated
CPZ	Chlorpromazine
CuAAC	Copper-catalysed azide-alkyne cycloaddition
Cy	Cyclohexyl
DABCO	1,4-Diazabicyclo[2.2.2]octane
Dansyl	5-(Dimethylamino)naphthalene-1-sulfonyl
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
DCC	*N*,*N*'-Dicyclohexylcarbodiimide
DDM	*n*-Dodecyl-β-D-maltoside
DG	*n*-Dodecyl-β-D-glucopyranoside
DIBAL-H	Di*iso*butylaluminum hydride
DIPEA	*N*,*N*-Di*iso*propylethylamine
DMAP	*N*,*N*-Dimethylaminopyridine
DMF	*N*,*N*-Dimethylformamide
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DNGSH	*S*-(3-[*N*-dansyl]aminopropyl) glutathione
DPC	Dodecyl phosphocholine
DSF	Differential scanning fluorimetry
E. coli	Escherichia coli
EDC	*N*-(3-Dimethylaminopropyl)-*N*'-ethylcarbodiimide
ee	Enantiomeric excess
ENZ	Enzyme
EPR	Electron paramagnetic resonance
eq.	Equivalent(s)
ES(I)	Electrospray (ionisation)
ESG	S-(*N*-ethyl)succinimido glutathione

Et	Ethyl
FA	Formic acid
FGI	Functional group interconversion
Fmoc	9-Fluorenylmethoxycarbonyl
GSH	Glutathione, γ-Glu-Cys-Gly
GSX	Glutathione-*S*-conjugates
H. pylroi	Helicobacter pylori
HMBC	Heteronuclear multiple bond correlation
HOBt	1-Hydroxybenzotriazole
HTS	High-throughput screen(s)
iNKT	Invariant natural killer T (cells)
ITC	Isothermal titration calorimetry
KcsA	Potassium crystallographically-sited activation channel
K_d	Dissociation constant
Kef	Potassium efflux channel
KTN	Potassium transport and nucleotide binding
LG	Leaving group
LPC	Lysophosphatidylcholine
LPL	Lysophospholipid
MALDI	Matrix-assisted laser desorption/ionisation
MC(s)	Mechanosensitive channel(s)
MD	Molecular dynamics
MDR	Multidrug-resistant/resistance
Me	Methyl
Mesyl/Ms	Methanesulfonyl
MS	Mass spectrum/Mass spectrometry
MscK	Potassium-dependant mechanosensitive channel
MscL	Mechanosensitive channel of large conductance
MscM	Mechanosensitive channel of mini conductance
MscS	Mechanosensitive channel of small conductance
MW	Microwave
m/z	mass to charge ratio
NBS	*N*-bromosuccinimide
NHS	*N*-hydroxysuccinimide
NMR	Nuclear magnetic resonance
PDC(s)	Protein-detergent complex(es)
PG	Protecting group
Ph	Phenyl
PMB	4-methoxybenzyl

PTM(s)	Post-translational modification(s)
RNA	Ribonucleic acid
RT	Room temperature
SDS	Sodium dodecyl sulfate
SLG	*S*-lactoyl glutathione
t-/tert-	*tertiary-*
TBAB	*N*,*N*,*N*,*N*-tetrabutylammonium bromide
TBAF	*N*,*N*,*N*,*N*-tetrabutylammonium fluoride
TBAI	*N*,*N*,*N*,*N*-tetrabutylammonium iodide
TBDPS	*tert*-Butyldiphenylsilyl
TBTU	*O*-(Benzotriazol-1-yl)-*N*,*N*,*N*',*N*'-tetramethyluronium tetrafluoroborate
TCEP	*tris*(2-Carboxyethyl)phosphine
Tf	triflyl/trifluoromethanesulfonyl
THF	Tetrahydrofuran
THP	Tetrahydropyranyl
TIPS	Tri*iso*propylsilyl
TLC	Thin layer chromatography
TMS	Trimethylsilyl
TNP	Trinitrophenol
TOF	Time-of-flight
Ts	*para*-toluenesulfonyl
UV	ultra-violet
WT	Wild-type

1. Antibiotic resistance and the subsequent need to develop drug candidates with novel modes of action

The following chapter explains why the development of probes for bacterial ion channels and the pursuit of improvements to the current techniques for investigating their structure and binding interactions are such important approaches in the pursuit of new antibiotics that exhibit novel modes of action.

1.1 Antibiotic Resistance

1.1.1 Emergence of antibiotic resistance

Antibiotic resistance, and the threat it poses to mankind, is finally starting to receive the attention it so desperately warrants. Over the past couple of years experts in the UK, for example, have been petitioning governments to increase awareness and funding for research to combat antibiotic resistance. In her 2013 annual report, the UK's chief medical officer, Prof Dame Sally Davies, described antimicrobial resistance as a '*ticking time bomb not only for the UK but also for the world*' and pressed the need for collaboration to '*ensure the apocalyptic scenario of widespread antimicrobial resistance does not become a reality*'.[1] The current director of The Wellcome Trust, Prof Jeremy Farrar, recently echoed these opinions on UK national radio and, in their proposed budget for 2015, the government of The U.S. has pledged an additional $30 million to detect and prevent antibiotic resistance – more than double the current provision.[2,3]

Broadly speaking, decades of over- and incorrect application of antibiotics have contributed to the increase in multidrug-resistant (MDR) strains of bacteria and microbes. Antibiotics have been termed 'societal drugs' meaning that use by an individual also affects those sharing the same environment. For example, a study by Miller *et al.* showed that the skin flora of those who had been in close contact with a patient undergoing antibiotic treatment for acne contained significantly more antibiotic resistant strains of bacteria compared to those who had not had any contact with such a patient.[4] Additionally, increased levels of antimicrobials observed in waste water supplies, the use of sub-therapeutic amounts of antibiotics to encourage the growth of livestock destined for food, the use of animal manure containing

MDR bacteria to fertilise crops and the dusting of some fruits to prevent prophylaxis have all also been linked to the emergence of antibiotic resistance.[5,6,7,8]

1.1.2 Mechanisms of antibiotic resistance

The antibiotic resistance genes and their precursors in pathogenic and non-pathogenic bacteria, collectively known as 'the resistome', can be transferred between bacteria of different taxonomic and ecological groups by mobile genetic elements such as bacteriophages, plasmids, naked DNA or transposons.[9,10] Such genes are responsible for conferring on bacteria the various mechanisms of antibiotic resistance, some of which are illustrated in **Figure 1.1**.

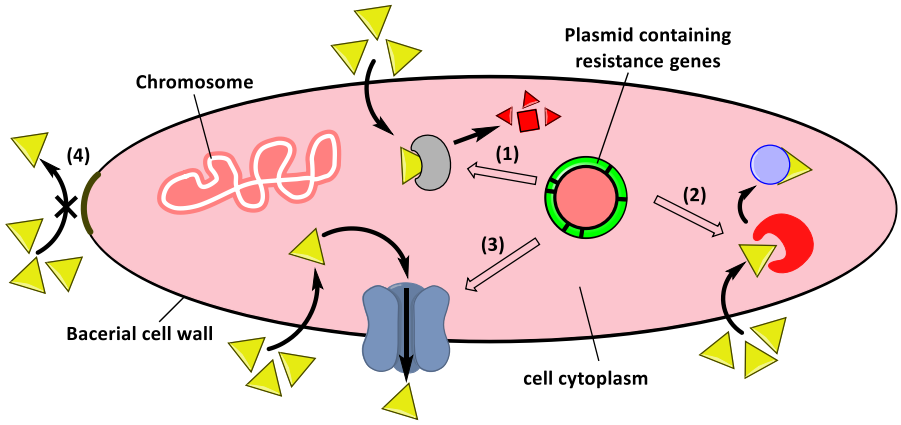


Figure 1.1: Cartoon illustrating some mechanisms of antibiotic resistance. A particular resistance gene may; **(1)** code for an enzyme that breaks down an antibiotic such that it becomes ineffective within the cell; **(2)** code for an enzyme that modifies an antibiotic, again making it ineffective; **(3)** be up-regulated, such that the number of efflux pumps increases allowing rapid removal of antibiotics from within the cell; **(4)** cause mutations of the cell wall (for example in the outer membrane) that block an antibiotic from entering the cell altogether.

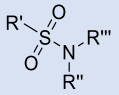
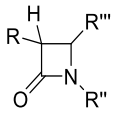
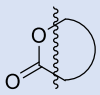
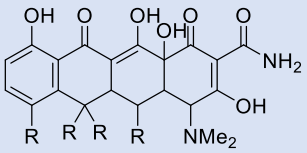
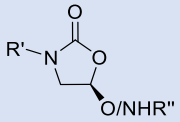
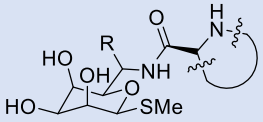
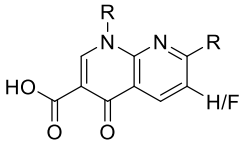
Some mechanisms directly affect the antibiotic. Acquired genes can code for enzymes that recognise the antibiotic and either break it down or modify it such that it becomes ineffective against its original target and is therefore unable to perform its intended function (**1** & **2**, **Figure 1.1**).[11] The most well-known example of this is the ability of β-lactamases, of which

hundreds have now been identified, to breakdown penicillins.[12] Other mechanisms affect antibiotics in a less direct manner. Genes coding for active efflux pumps can be up-regulated. When more efflux pumps are present, antibiotics that have entered a cell can be ejected more rapidly, thereby reducing the concentration of a drug to below the required therapeutic level (**3**, **Figure 1.1**).[13] Mutated genes can give rise to proteins with modified recognition sites, the mutation of the genes coding for enzymes responsible for DNA/RNA synthesis is one such example. The modifications are subtle enough that the enzyme can continue to function normally, yet significant enough to prevent otherwise inhibitory antibiotics from binding (not shown).[14] A further example of this mechanism is the mutation of genes coding for the assembly of cell walls. Cell walls, particularly in gram-negative bacteria, are complex. However, modifications to the outer membrane of the cell wall, which is composed of lipopolysaccharides and an asymmetric bilayer of phospholipid, can alter the permeability of a cell such that an antibiotic is prevented from entering the cell altogether (**4**, **Figure 1.1**).[15,16]

1.1.3 Scaffolds of current antibiotics and their modes of action

There is no doubt that the antibiotics discovered and developed over the past 80 years or so have saved countless lives. However, in most cases, bacterial strains showing resistance to these antibiotics have been reported within the very same year of their approval by the appropriate regulatory bodies.[17,18,19,20] The most well-known antibiotics and their respective modes of action are summarised in **Table 1.1**.

Table 1.1: Summary of the most common antibiotic scaffolds. Examples of each scaffold, in addition to their mechanism of action, are provided (the table is not intended to be an exhaustive list).

Scaffold		Examples (class)	Mode of Action
Sulfonamides		Sulfamethoxazole	Competitive Inhibition of Folic Acid Synthesis
β-Lactams		Amoxicillin (penicillins) Ceftazidime (cephalosporins) Aztreonam (monobactams) Doripenem (carbepenems)	Inhibition of Cell Wall Synthesis
Glycopeptides		Vancomycin (glycopeptides)	
Lipoglycopeptides		Telavancin (lipoglycopeptide)	
Lipopeptides		Daptomycin (lipopeptide)	
Macrolides		Erythromycin Telithromycin (ketolide) Pristinamycin IIA (streptogramin A) Virginiamycin S1 (streptogramin B)	Inhibition of Protein Synthesis
Tetracyclines		Oxytetracycline	
Oxazolidinones		Linezolid	
Lincosamines		Clindamycin	
Aminoglycosides		Tobramycin	
Quinolones		Nalidixic acid (quinolone) Ciprofloxacin (fluoroquinolone)	Inhibition of DNA Synthesis
Rifamycins		Rifampicin	Inhibition of RNA synthesis

Most of the scaffolds rose to prominence as a result of isolated natural products showing good antibacterial activity. The sulfonamides and quinolones, however, were synthetic discoveries. Extensive optimisation of early candidates has provided new antibiotics, but, with the scaffold remaining constant between generations, the effectiveness of candidates developed based on existing scaffolds decreases rapidly with time. Of the new antibiotics launched since 2000,

the majority were fluoroquinolones, while only two compounds, daptomycin and linezolid, represented novel scaffolds.[21] Diversification of the current scaffold pool has been suggested as a method for combatting the rapid emergence of resistance.[22]

1.1.4 What does the future hold?

Diversification of the current scaffold pool, to block or circumvent resistance mechanisms, would be an appropriate approach in the short term.[22] However, in the long term, the development of antibiotics with novel modes of action, such as those which disrupt the bacterial membrane bilayer or the integral proteins contained within, is vital.[23]

Regardless of the approach, in addition to improving diagnostics and stewardship surrounding the prescription and use of antibiotics, it is necessary to encourage research and development into the field.[24] The scientific challenges of developing new broad-spectrum antibiotics were thoroughly outlined by Payne *et al.* of GlaxoSmithKline (GSK).[25] Between 1995 and 2001 they carried out 70 high throughput screens (HTS), which furnished only 5 antibacterial leads. GSK identified the '*acquisition of biologically relevant chemical diversity*' and '*achieving activity across a diverse spectrum of pathogens*' as particular challenges. Additionally, since most courses of antibiotic therapy are short term, a couple of weeks perhaps, the profitability is limited, especially when resistance emerges so rapidly. For these reasons, in recent times, large pharmaceutical companies have regrettably tended to invest their money in more lucrative therapies, such as the development of drugs for the long-term treatment of depression and rheumatoid arthritis.[26] Experts have increasingly pressurised governments to offer incentives to encourage the development of new antibiotics.[1] Under The Generating Antibiotic Incentives Now (GAIN) Act, successfully passed in The U.S. in 2011, qualifying

antibiotics now receive priority review by the Food and Drug Administration (FDA), as well as an additional five years of market exclusively.[27] It is the success of movements like this, coupled with increasing public awareness of the issue, that is slowly but surely causing a resurgence in the development of novel antibiotics.[27]

1.2 Bacterial Ion Channels

In **Section 1.1** the need to develop new antibiotics based on novel scaffolds or with novel therapeutic targets was highlighted. Targeting the bacterial cell membrane, or the essential proteins contained within, may represent a route to antibiotics with novel modes of action, thereby circumventing the issues associated with the rapid emergence of antibiotic resistance.

1.2.1 The role of membrane-spanning ion channels

Ion channels, the macromolecular pores spanning an excitable cell membrane, are essential proteins that link extracellular space with the interior of a cell (**Figure 1.2**). These selectively permeable, gated channels therefore play a vital role in homeostasis. More specifically, they regulate the passage of hydrophilic polar molecules and ions such as sodium, potassium, chloride, calcium and water itself to and from a cell. The rapid movement of such ions (often of the order ~10^7 ions s^{-1}) across a cell membrane in this way generates electrical signals, which promote a multitude of fundamental intracellular events.[28]

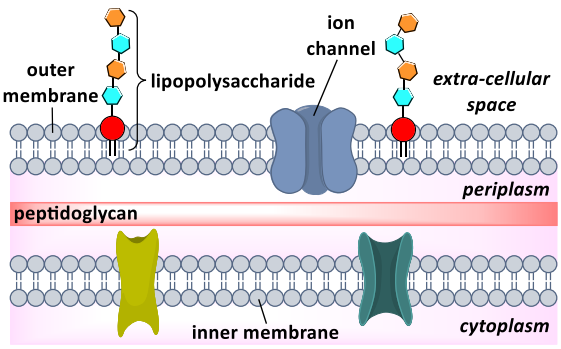


Figure 1.2: Cartoon representation of the cell envelope of a gram-negative bacterium, indicating the location of ion channels.

A vast number of ion channels have been discovered across species. Bacterial ion channels have been particularly well investigated in structural biology (partly because bacteria can be grown so rapidly) and it has been possible to elucidate some of their structures *via* X-ray crystallography.[29,30,31] These structures have not only been useful for determining the composition of specific channels but have also helped facilitate the understanding of basic biophysical and biochemical processes in their eukaryotic analogues.[32,33]

1.2.2 Bacterial ion channels as therapeutic targets

The extensive knowledge gained during investigations into the composition of bacterial cell membranes, and the structures of the many ion channels contained within, offers the opportunity to develop new therapies that selectively target these regions in bacteria.[23]

Indeed, work towards this end has already proved fruitful; the approval of the new antibiotic daptomycin in 2003, a drug that induces membrane permeabilisation and depolarisation, is the most successful example thus far and other agents carrying out similar roles are in development.[23] To date, however, there appears to be no advanced candidates targeting a particular bacterial ion channel, although this is slowly changing. For example, Nguyen *et al.* have published work showing that parabens interact with the bacterial mechanosensitive ion channels MscL and MscS, perhaps *via* a specific gating mechanism (**Section 4.1.3**). This proposed interaction was shown to inhibit the growth of bacteria by opening these channels and causing leakage of cytoplasmic contents.[34] Eukaryotic homologues of MscL and MscS are not currently known, thereby conveying the advantage of targeting these particular channels – agents will be selective for bacterial cells over human cells.[35] MscS will be discussed further in **Section 4.1.2**.

1.3 Techniques for investigating the structure and ligand-binding properties of bacterial ion channels

1.3.1 X-Ray Crystallography

X-Ray crystallography is the 'gold standard' for elucidating the 3D structure of proteins such as enzymes and ion channels. It was first used by Kendrew *et al.* to determine the 3D structure of myoglobin in 1958.[36] Kendrew was later awarded the 1962 Nobel Prize for this work, which he shared with Max Perutz. Thousands of protein X-ray crystal structures followed and, as mentioned previously, even membrane proteins, such as bacterial ion channels, have since been characterised in this way.[37,31] However, working with membrane proteins is notoriously difficult because extracting them from their native membranes, without loss of integrity, can be challenging. They are also difficult to crystallise and X-ray crystallography is, therefore, not always an option.

1.3.2 Protein Mass Spectrometry

With the advent of milder ionisation techniques (electrospray ionisation and matrix-assisted laser desorption/ionisation), mass spectrometry of intact protein complexes has risen to prominence over the past decade or so.[38,39] It was initially viewed as a technique to complement X-ray crystallography and electron microscopy but, with its unique ability to tolerate heterogeneous subunit protein complexes, it is fast becoming an important structural biology tool within its own right.[40] A more in depth discussion of membrane protein mass spectrometry can be found in **Section 2.1.1**.

1.3.3 Other techniques

NMR spectroscopy is another popular technique used to study heterogeneous subunit protein complexes in the solution phase.[41] As with mass spectrometry this technique lends itself to permitting the study of proteins containing weak and transient domain interactions, which may otherwise be lost or distorted during crystallisation for X-ray crystallography. NMR techniques have often been used in combination with *in silico* studies to produce 3D models of dynamic proteins, although fully solved structures are limited to smaller proteins.[42,43,44]

Other techniques include electron microscopy, which has provided valuable insights into the composition and structure of protein complexes.[45,46] Biological techniques, such as co-immunoprecipitation, have also allowed investigation of protein-protein interactions such as those occurring between the subunits of a protein complex.[47] Both of these techniques have been well documented in the literature and are, therefore, beyond the scope of the current discussion.

1.4 Summary

Antibiotic resistance is clearly a growing problem. The development of new antibiotics with novel modes of action, such as those targeting ion channels found exclusively in bacteria, has been proposed as a potential solution. In order to achieve this, it is necessary to learn as much as possible about the structure and function of ion channels, which are transmembrane protein complexes, often comprising numerous weakly-bound subunits. A trinity of approaches to addressing this problem are discussed at length in **Chapters 2**, **3** and **4**.

The first aims to evolve the analytical technique of membrane-protein mass spectrometry. An opportunity arose to collaborate with The Carol Robinson Group at the University of Oxford, who were interested in working with an organic chemist to design and synthesise fluorescent detergents as tools for improving membrane protein mass spectrometry. Advances in this field may one day benefit studies concerning the characterisation and development of probes for bacterial ion channels. This branch of the work will be discussed in **Chapter 2**.

Two bacterial ion channels, KefC and MscS, represent the novel therapeutic targets and will be introduced in more detail in **Chapters 3** and **4** respectively. These targets were selected due to a long-running collaboration with X-ray crystallographers at the University of St Andrew's (The Naismith Group) and microbiologists at the University of Aberdeen (The Booth/Miller Group), all of whom have extensive experience of working on these two targets.

2. Fluorescent Detergents as Tools for Improving Membrane Protein Mass Spectrometry

Protein mass spectrometry was first introduced in **Section 1.3.2**. Herein, its application in the structural proteomics of membrane protein complexes will be discussed, including a brief explanation of the technique and the advantages and disadvantages it affords over its counterparts (such as X-ray crystallography and NMR spectroscopy). The importance of detergents in studying membrane proteins will also be discussed, with a particular focus on the glycolipid detergent *n*-dodecyl-β-D-maltoside (DDM). Following this introduction, work towards the synthesis of novel fluorescent detergents based on DDM, and their possible application in membrane protein mass spectrometry, will be reported.

2.1 Introduction

2.1.1 Mass spectrometry for the characterisation of membrane proteins

Mass spectrometry (MS) is one of a number of techniques that has found substantial application in the field of proteomics, that being the large-scale study of gene and cellular function at the protein level.[48,49] However, since softer ionisation techniques such as electrospray ionisation (ESI) and matrix-assisted laser desorption/ionisation (MALDI) were first adapted to tolerate macromolecular structures, MS has found increasing application in the field of structural proteomics too.[38,39,50,] These techniques, when combined with mechanical innovations and other advances, have allowed proteins to be ionised and transferred into the gas phase within a mass spectrometer, without the loss of key interactions. Preservation of the non-covalent interactions between distinct subunits of a membrane protein complex is just one example. As a result, it has become possible to study the structures, subunit stoichiometry, interaction networks and small molecule ligands of some protein complexes.[51]

A basic depiction of a 'hybrid' tandem mass spectrometer, modified for the running of high molecular weight complexes such as protein complexes, is provided in **Figure 2.1** (adapted from a review by Sharon and Robinson).[40] These machines typically combine a quadrupole mass filter with an orthogonal time-of-flight (TOF) analyser and are separated by a third component, a collisional cell. All of which are explained in further detail below. The term *tandem* MS refers specifically to simultaneous use of the quadrupole mass filter and the collisional cell.[52] The enhanced ability of these machines to select for mass-to-charge ratios (*m/z*) within a defined range reduces the complexity of protein spectra, which would

otherwise contain a substantial degree of overlapping peaks, due to the high molecular mass and the large number oligomeric species that may constitute a protein complex.

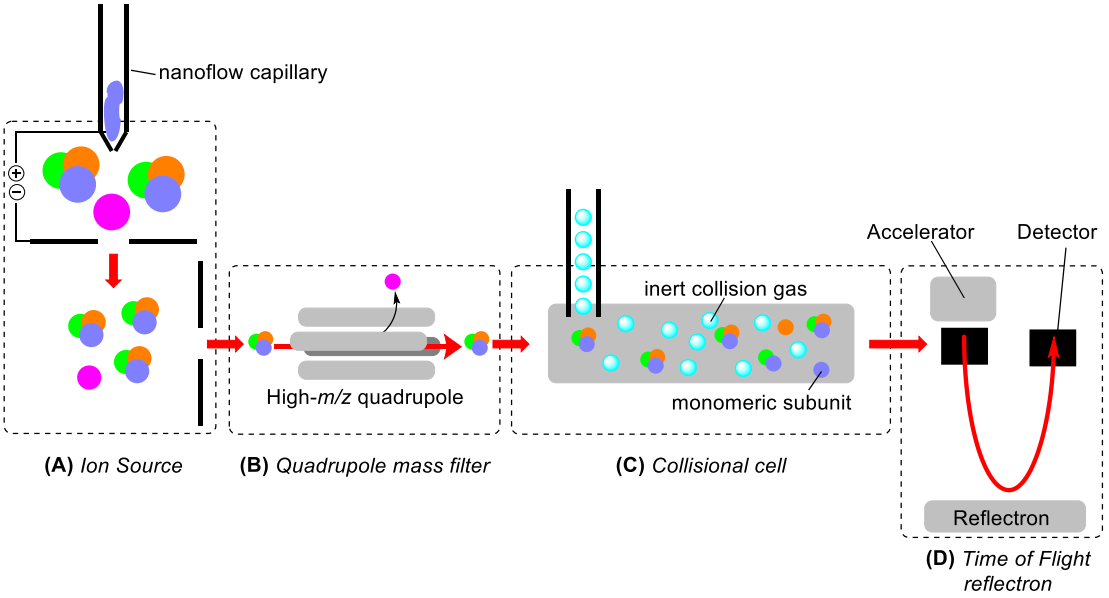


Figure 2.1: A basic depiction of a hybrid tandem mass spectrometer for the study of macromolecules such as protein complexes, adapted from Sharon & Robinson.[40] (**A**) The use of a nanoflow capillary and the application of a high electrostatic charge facilitates the formation of small droplets, which desolvate as they travel down pressure and potential gradients towards the quadrupole. (**B**) A quadrupole mass filter, consisting of 4 parallel rods, provides an oscillating electrical field, which modifies the trajectories of ions depending on their mass-to-charge ratio (*m/z*). (**C**) Collision-induced dissociation (CID) of a charged complex *via* collisions with inert gas molecules. CID results in the formation of lowly-charged 'stripped' complexes and highly-charged subunits. (**D**) Ions adopt a trajectory through the TOF reflectron that is dependent upon their *m/z* value, which directly influences their velocity and therefore the time at which they reach the detector.

A typical experiment begins with a soft ionisation technique, such as nanoflow ESI, a miniaturised form of ESI adopting reduced flow rates (~10-100 nL min^{-1}). Nanoflow ESI vaporises a protein-containing solution by the application of a high positive electrostatic charge to the tip of a narrow capillary (the diameter is of the order 1 µm).[53] The high field causes exposed liquid at the end of the capillary to form small charged droplets, which decrease in size as they traverse down pressure and potential differences. As the droplet size decreases the charge density increases until the Rayleigh limit is reached - the point at which the Coulomb repulsion within the droplet equals its surface tension. The resulting instability manifests itself as a *Coulomb explosion* and is sufficient to cause fission of the droplet, leaving

only desolvated multiply charged protein ions (**A**, **Figure 2.1**).[38,54,55] With nanoflow ESI, when the appropriate sample concentration and needle orifice are applied the aqueous droplets are assumed to contain no greater than one protein complex.

Once in the gas phase, ions pass through a quadrupole mass filter, which possesses a tuneable oscillating electrical field, allowing it to select for ions of a specific *m/z* ratio, or range thereof (**B**, **Figure 2.1**). Ions of the appropriate *m/z* range are permitted to maintain a stable and rapid trajectory through the quadrupole, whilst those outside the defined range are deflected. Once selected by the quadrupole, ions are accelerated into a collisional cell filled with an inert gas such as argon (**C**, **Figure 2.1**). Collisions with the inert gas convert some of an ion's translational kinetic energy into internal vibrational energy. This is known as collision-induced dissociation (CID) because, on attainment of the dissociation threshold, a lowly-charged 'stripped' complex can form through expulsion of an individual, highly-charged subunit.[56] Depending on the conditions within the collisional cell, the stripped complex may undergo further disassociation to afford additional highly-charged subunits.[52]

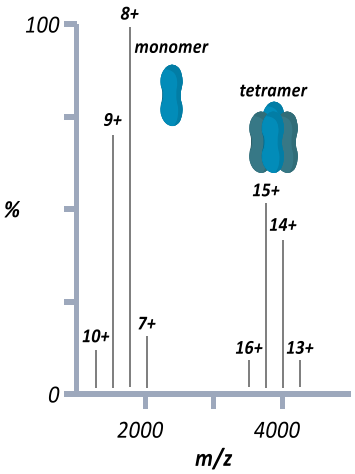


Figure 2.2: Example of a simplified nanoflow ESI MS of the homotetrameric complex transthyretin. Two charge distributions (1400-2000 *m/z* and 3500-4300 *m/z*) are identified. Adapted from Sharon and Robinson.[40]

The stripped complexes and subunits are then accelerated into a TOF reflectron. Ions navigate this chamber over a time related to their *m/z* value (**D**, **Figure 2.1**). Ions with different *m/z* values therefore reach the detector at different times giving rise to the characteristic succession of peaks for each existing component, known as a charge distribution. The ESI spectrum of the homotetrameric complex transthyretin is provided as an example in **Figure 2.2** (adapted from Sharon and Robinson).[40,57]

Protein MS offers a number of advantages over its main rivals. The leading technique for solving high-resolution structures of proteins is X-ray crystallography, however, this technique is often limited to abundant complexes or those that have undergone recombinant assembly *in vitro*.[58] Recombinant proteins may lack naturally occurring isoforms or their post-translational modifications (PTMs) and are often truncated to remove dynamic unstructured regions. As a result, X-ray crystallography provides only a rigid snapshot of a protein, albeit a very useful one. It is unable to capture the dynamic aspects of, for example, the subunits within a membrane protein complex, which is unfortunate because it is often modulation of these dynamic regions that governs function. As has been shown above, soft ionisation techniques, coupled with specially-adapted mass spectrometers, allow non-covalent interactions to be maintained within a spectrometer. The use of CID represents a controlled method of weakening these interactions to reveal constituent subunit combinations. Additionally, MS can be carried out using solutions of wild-type (WT) protein so it is unnecessary for a protein to form crystals for application of this technique. NMR spectroscopy is another solution phase technique that allows the study of dynamic protein complexes in solution.[59,41] However, for NMR, unlike with MS, it is important that a sample be homogeneous. If a protein of interest can't be isolated from a mixture in sufficient purity then it becomes difficult to discern any useful information from an NMR spectrum due to the huge number of overlapping peaks. For this reason, recombinant protein is often used. Additionally, NMR typically requires significantly larger quantities of material than MS. However, with isotopic labelling, increasing magnetic field strengths, the use of cryoprobes and the development of more complex pulse sequences it would be fair to say that protein NMR is evolving as rapidly as protein MS.[60]

Some challenges do remain in protein MS. In particular, there is the prerequisite that intact complexes must be present in sufficient quantities for detection. If the technique could be made more sensitive, thereby reducing the number of complexes necessary for an experiment (currently ~1-2 pmol), then even smaller sample volumes would be tolerated and it would become possible to detect low abundance complexes within a cell. Other challenges include the ambiguity in assigning subunit compositions, especially when non-identical subunits possess similar masses and therefore afford a spectrum with a densely populated region of peaks. Additionally, the measured mass of a complex is often higher than expected from the sum of its components, which can be attributed to the incomplete removal of solvent and buffer prior to detection. This is a particular problem in the MS of membrane protein complexes as they are commonly solubilised in a detergent in order to be extracted from, and stabilised in the absence of, their native membranes (see **Section 2.1.2**). Detergent clusters are commonly visible in spectra and may cause substantial peak broadening or occlusion.[51]

The advances in MS over the past 20 years have allowed the unambiguous determination or confirmation of the stoichiometry of protein complexes.[52,61] An elegant example is provided by Barrera *et al.* who used the technique to confirm the subunit stoichiometry of a heteromeric membrane protein, adenosine 5´-triphosphate (ATP)–binding cassette transporter (or $BtuC_2D_2$).[51] The results agreed well with the previously published X-ray crystal structure and they were even able to extend their experiments to show co-operative binding of ATP to the transporter.[62] More recent work by the same researchers has allowed identification of lipid and nucleotide binding in membrane-associated rotary ATPases/synthases and has been able to probe the effect of these ligands on subunit interactions.[63] Additionally, the technique has advanced so much that it is already being used to monitor real-time reactions, such as the acid-induced denaturation of ferrihaemoglobin.[64]

2.1.2 The importance of detergents in the study of membrane proteins

Biophysical characterisation of proteins by X-ray crystallography, solution NMR and, more recently, MS commonly begins with a solution of monodisperse protein. For membrane proteins, this is not possible without the use of detergents as a mimetic for the phospholipid bilayer of a cell membrane.

Detergents are amphiphilic molecules, possessing both hydrophilic and hydrophobic moieties. The structures of some detergents that have been used in either the crystallisation of or NMR studies of membrane proteins are provided in **Figure 2.3**. Like the phospholipids that constitute cell membranes, all possess a long alkyl chain providing the hydrophobic character and a polar hydrophilic head group, which may be neutral (e.g. the hydroxyl groups on the glycolipid, DDM **1**) or charged (e.g. the *N*-oxide in **5**).

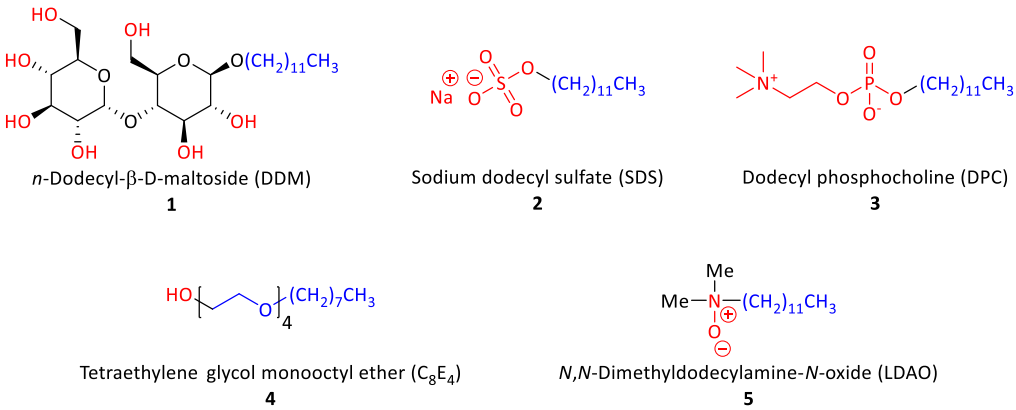


Figure 2.3: The structures of some commonly used detergents for the study of membrane proteins. Hydrophilic components are shown in red and hydrophobic components in blue.

Due to their amphiphilic character, detergents possess self-assembling properties and their behaviour in solution is therefore complex. In aqueous media they are known to form micelles due to the hydrophobic effect. Micelles are spheroid aggregates, where the hydrophilic head groups are exposed on the surface leaving the hydrophobic tails sequestered within the core,

away from the surrounding aqueous environment. A single micelle is composed of many detergent molecules, the specific number of molecules is known as the 'aggregation number' and varies substantially between detergents.[65,66] Detergent monomers have limited solubility in water and only begin to aggregate as micelles when their concentration reaches a specific value, which is known as the critical micelle concentration (CMC). The CMC also varies between detergents but generally, all detergents must be used at a concentration above their CMC to effectively solubilise membrane proteins.

When it comes to membrane protein complexes, detergents are ideal candidates for their solubilisation because their ability to form micelles makes them effective mimics of the phospholipid bilayer membranes where these proteins are normally located. A study by Tulumello and Deber profiled the efficiency of various detergents at maintaining membrane protein structures in their biologically relevant forms and other investigations have revealed that detergent environments allow membrane proteins to retain aspects of their secondary and tertiary structure or even their native quaternary structure and the overall fold the protein.[67,68,69,70]

The ability of detergents to maintain membrane proteins in their native state has been exploited by Barrera *et al*. to improve membrane protein MS.[51] They found that protecting a membrane protein complex within a detergent micelle was a powerful strategy for maintaining these delicate complexes intact within the spectrometer, allowing the determination of subunit stoichiometry and ligand-binding properties for the $BtuC_2D_2$ complex, as discussed previously (**Section 2.1.1**). They used DDM **1** to solubilise $BtuC_2D_2$ as this detergent already had a good reputation for retaining membrane proteins in their native state and was found to stabilise the protein in an active state.[71] Additionally, there was already precedent for the use of DDM **1** in ESI and, as a neutral detergent, it had been shown to be

better tolerated than its charged counterparts during this ionisation process.[72,73] To summarise their work, when the pressure in the collisional cell was low, they observed a spectrum dominated by a very diffuse peak at high *m/z*, attributed to a heterogeneous assembly of $BtuC_2D_2$ associated with upwards of 100 DDM molecules. On increasing the pressure within the collisional cell, DDM molecules began to disassociate, thereby revealing the complex. The diffuse peak at high *m/z* was replaced by a series of sharper peaks, consistent with the intact tetramer alone or the intact tetramer with either small molecules bound or appropriate PTMs. The implications of this were that the tetramer had remained intact inside the micelle, surviving the journey from the initial solution, through ESI and the quadrupole filter, to the collisional cell. In other words, the complex, as well as its bound ligands and PTMs, had been protected and preserved by the micelle. It wasn't until much higher collisional pressures that peaks corresponding to unfolding and the stripping of monomeric subunits were observed. A second interesting observation was that $BtuC_2D_2$ was found to have around a 16% lower average charge than a soluble protein complex with the same accessible surface area. Barrera *et al*. attributed this to a further protective effect of the detergent micelle, suggesting that it was not only able to protect against dissociation and subunit unfolding but that it was also able to shield a complex during the droplet phase of ESI. This methodology is certainly promising, the only issue appearing to be that incomplete dissociation of detergent molecules may lead to the aforementioned 'clouding' of spectra and a potential broadening or occlusion of important peaks. Despite this drawback, this elegant study succeeded in showing that it is indeed possible to use MS to generate useful structural and ligand binding data on delicate membrane protein complexes.

2.1.3 Glycolipids as detergents

Carbohydrates represent a rich collection of water-soluble, hydrophilic building blocks and, when attached to a hydrophobic moiety such as a long alkyl chain, afford amphiphilic species known as glycolipids. One glycolipid, DDM **1**, has already been introduced and its application as a detergent described in **Section 2.1.2**. Before continuing, it seemed appropriate to provide a very brief overview of the wider applicability of such glycolipids, since all applications rely on their exceptional ability to self-assemble into complex supramolecular entities. Their unique properties, including their biocompatibility and biodegradability, have meant they have been widely investigated for a number of applications. In addition to their ability to behave as detergents, their behaviour as hydrogelators (**6**) and liquid crystals (**7**) has also been reported (**Figure 2.4**).[74,75,76] Hydrogelation and liquid crystal formation are exciting prospects in supramolecular chemistry and are currently being exploited in the development of smart materials for bioregenerative medicine and tissue engineering.[77,78] However, these applications are beyond the scope of this discussion.

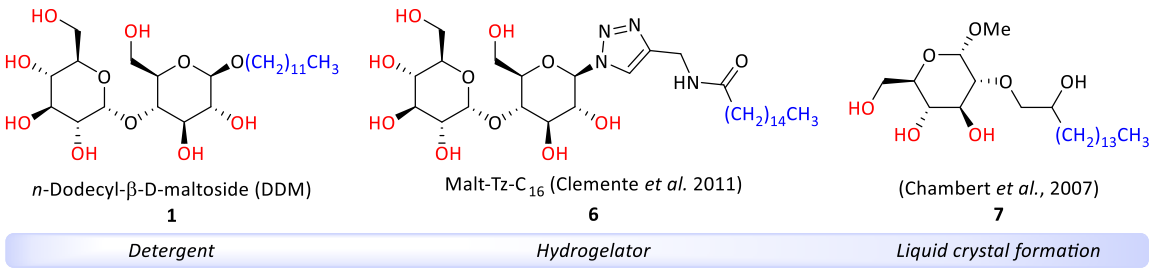


Figure 2.4: The structures of some glycolipids that are known to behave as detergents, hydrogelators or liquid crystals.[76,74,75] Hydrophilic components are shown in red and hydrophobic components in blue.

Herein, the focus will remain on the glycolipid DDM **1**, although other glycolipids, including *n*-decyl-β-D-maltopyranoside and *n*-octyl-β-D-glucopyranoside, are also commonly used in work with membrane proteins.[79] DDM **1** has proved to be an appropriate mild detergent for the

solubilisation of membrane proteins and is already routinely used in membrane protein MS by collaborators in the Robinson group at the University of Oxford.[67,51]

DDM **1** is described as a mild detergent due to its relative propensity to maintain proteins in their native state compared with other detergents. Mild detergents tend to be those with a neutral head group (as opposed to a charged or zwitterionic one, as in SDS **2** and DPC **3** respectively) and a long carbon chain.[76] It is easy to see why DDM **1**, with its neutral yet hydrophilic disaccharide head-group (capable of forming an extensive directional H-bonding network) and its hydrophobic twelve carbon chain, has fared so well in this field. The main limitation of DDM **1** appears to be the formation of relatively large micelles compared to other glycolipids, resulting in large protein-detergent complexes (PDCs) during structural studies.[65,66] As a result, the protein of interest can sometimes be masked since such a large proportion of the mass and volume of the PDC is due to detergent, which can be difficult to completely remove (this was illustrated in **Section 2.1.2** where the observation that DDM clusters can give rise to diffuse peaks in mass spectra was discussed). Evidently, any process which improves the efficacy with which detergents can be removed from a protein would be highly beneficial.

2.1.4 Fluorescent glycolipids

Section 2.2 explains why fluorescent glycolipids as detergents have been proposed as a potential solution for the problem of detergent clusters. It might be envisaged that the modification of a detergent with a fluorophore may adversely affect its properties, including its ability to form micelles. However, a number of fluorescent glycolipids have already been reported and successfully applied in biological stuides.[80]

Madar-Shapiro *et al*. synthesised two fluorescent glycolipids, which were required to assess the intracellular activities of the lysosomal enzymes β-glucocerebrosidase and arylsulfatase A. Mutations of these enzymes have been shown to cause Gaucher's disease and metachromatic leukodystrophy respectively.[81] Despite modification with a relatively large fluorophore (**9**) the two fluorescent probes, Lissamine™ rhodamine dodecanoyl glucocerebroside **8** (**Figure 2.5**) and Lissamine™ rhodamine dodecanoyl sulfatide, were still able to penetrate cells *via* endocytosis and reach the lysosomes where, like the non-fluorescent parent glycolipid, they underwent hydrolysis. This suggested that the addition of the fluorophore had not impacted on the original activity of the substrates and allowed Madar-Shapiro *et al*. to successfully investigate the processes occurring in healthy *vs* diseased cells *in vitro* by real-time monitoring of changes in fluorescence localisation.

A study by Gadella *et al*. used fluorescent glycolipids to follow their redistribution in the sperm plasma membrane during capacitation and zona binding.[82] The glycolipids were synthesised by Marchesini *et al*. and the structure of one of these, galactose(3-sulfate)-β1-1'[*N*-(lissamine™ rhodaminyl)-12-aminododecanoyl]-sphingosine or SGalCer(C12-LRh) **10**, is provided in **Figure 2.5**.[83] Gadella *et al*. had already completed a similar study using immunocytochemical techniques but made sure to outline the main advantages the use of fluorescent probes offered over previous techniques.[84] After incorporation into the membrane, the fluorescent probes were subject to the same restrictions in their lateral diffusion as native membrane lipids and the lateral distribution of the incorporated probe could be monitored in real time as sample fixation and post-incubation labelling procedures were unnecessary. On the negative side, they noted that the introduction of artefacts, due to the modification of the original glycolipid, was a possibility that should be considered when interpreting the experimental data. However, they reasoned that the fluorophore was being

attached away from the polar head group and that its hydrophobic nature would not disrupt the interactions between vicinal lipid chains. This reasoning was substantiated by the fact that their fluorescence data agreed well with the data collected in the previous immunocytochemical studies.[84] Gadella's work is yet another brief illustration of how the addition of a fluorophore to the hydrophobic region of a glycolipid does not necessarily hinder its ability to perform the original function. Other examples exist but will not be discussed here.[85,86]

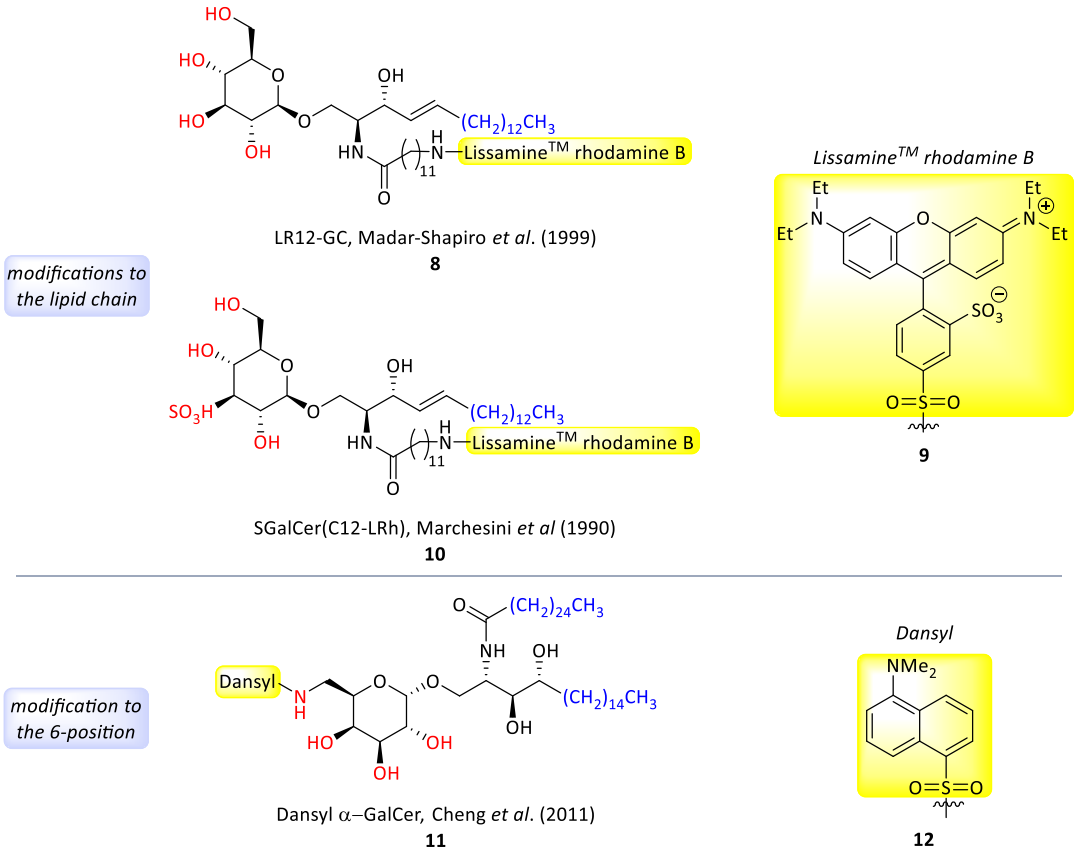


Figure 2.5: The structures of some fluorescent glycolipids. **8** was used in a study by Madar-Shaprio *et al*.[81] **10** was used in a study by Gadella *et al*. but synthesised by Marchesini *et al*.[82,83] **11** was synthesised for a study by Cheng *et al*.[88] Hydrophilic components are shown in red and hydrophobic components in blue. The fluorophores, **9** and **12**, are highlighted in yellow.

Despite the dismissive attitude of Gadella *et al*. to fluorescent modifications of the polar head-group, there are a number of reports where this approach has indeed found success.[87,88,89] A representative example is provided in the form of work by Cheng *et al*.[88] They

synthesised dansyl α-GalCer **11** (**Figure 2.5**), an analogue of the natural product agelasphin-9b that exhibits potent *in vivo* anti-tumour activity in mice. The dansyl fluorophore **12** was attached to the 6-position of the galactose ring because in the underivatised precursor α-GalCer, which also exhibits potent anti-tumour activity, the lipid chains were found to form important binding interactions with the target protein.[90] Like its parent compounds, **11** retained the ability to activate invariant natural killer T (iNKT) cells despite the fluorescent modification.

2.1.5 Carbohydrate chemistry

The manipulation of existing glycolipid detergents, in pursuit of desirable properties for membrane protein MS, requires a knowledge and appreciation of the foundations of carbohydrate chemistry. There are numerous positions within a glycolipid that offer potential for modification. **Section 2.1.4** discusses the addition of fluorophores to either the hydrophobic lipid chain or the 6-position of the hydrophilic sugar head group. Already, one might envisage the need to perform a glycosylation reaction to introduce a fluorescent lipid chain to the parent sugar or the need to selectively functionalise a single hydroxyl of a sugar head-group through the use of protecting group chemistry.

As an aside, it is important to introduce the protocol for the numbering of a sugar ring to aid future discussion and analysis. Numbering begins at the anomeric position of the *reducing end* of the sugar, so called because, in the parent sugar, it is the alcohol at this position that would form an aldehyde *via* isomerism to the open chain form in solution. Whilst commonly referred to as the anomeric position, it also constitutes the 1-position. The numbering then progresses around the carbon backbone until it reaches the *non-reducing end* of the sugar, usually a

primary alcohol, which, in the pictured derivatives of glucose **13** and maltose **14**, are called the 6- or 6'-postion respectively (**Figure 2.6**). The stereochemistry at the 2- to 5-positions is fixed for a given sugar, however, the stereochemistry at the 1-position can vary and each sugar can therefore exist as one of two possible epimers. In carbohydrate chemistry the epimers at the anomeric position are called anomers and are assigned as either the α- or β-anomer, this is discussed further below.

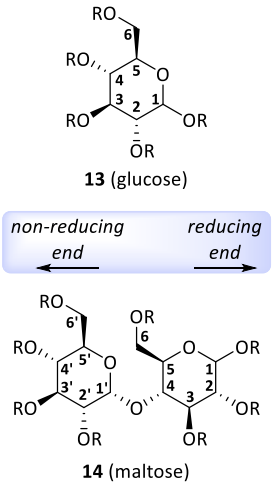
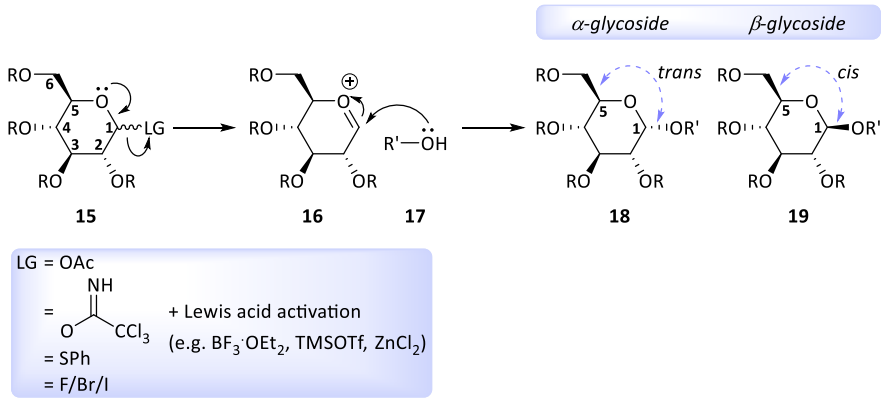


Figure 2.6: The numbering of sugar ring begins at the anomeric centre of the reducing end and progresses around the carbon backbone to the primary alcohol at the non-reducing end.

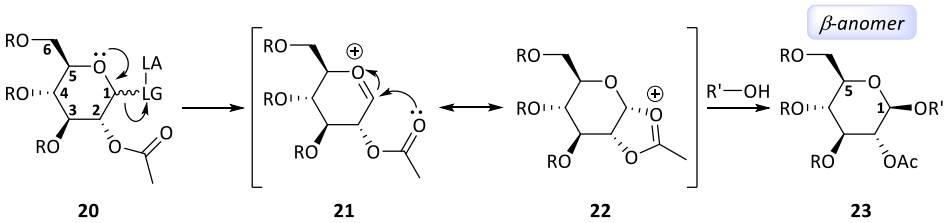
Chemical glycosylation is a vast field, which has been extensively reviewed over the years.[91,92,93,94,95] The process often involves the stereoselective formation of a new bond between a glycosyl donor **15** and a glycosyl acceptor **17**. The donor **15** must be a suitably activated sugar and the acceptor can be any number of nucleophiles, including an alcohol **17** or an azide (**Scheme 2.1**). The anomeric centre within the glycosyl donor **15** contains a pre-installed leaving group (LG), which may be further activated by a Lewis acid during glycosylation. Activation of the anomeric centre as an acetimidate or thioglycoside is a common approach, whilst boron trifluoride diethyletherate and zinc chloride have both found application as Lewis acids.[96,97] The remaining hydroxyl groups in the donor **15**, as well as any appropriate groups in the acceptor **17**, must be suitably protected. Since the anomeric centre is stereogenic, two possible anomers can form during a glycosylation. For a 6-membered ring, the α-anomer **18** is formed when the bonds at the 1- and 5-positions are *trans* to each other. However, when the same bonds are *cis*, the β-anomer **19** is formed. A wealth of conditions

exist for the stereoselective formation of one anomer over the other and details can be found in the aforementioned glycosylation reviews.



Scheme 2.1: A generalised glycosylation reaction with glucose derivative **15**. The glycosyl donor **15** has a leaving group (LG) at the anomeric centre, which may be further activated by Lewis acid catalysis. Loss of this LG affords oxonium **16**, which is susceptible to nucleophilic attack by a glycosyl donor such as alcohol **17**. Since the new bond forms at a stereogenic centre, two anomers can result: the α-anomer **18** (where the bonds at the 1- and 5-position possess a *trans* relationship) and the β-anomer **19** (where the same bonds are *cis*).

The glycolipid detergents that have been used in the study of membrane proteins are all β-anomers of either glucose or maltose. If modifications to the lipid chain were of interest, it might therefore be anticipated that at some point during this project it may be necessary to perform a glycosylation where the β-anomer is the desired product. Amongst other methods, neighbouring group participation in the case of 2-*O*/*N*-acetylated substrates is a well-known approach for the preparation of β-anomers.[91,98] As above, a LG is lost from **20**, encouraged by Lewis acid catalysis, to give oxonium **21** (**Scheme 2.2**). When acetylated, the carbonyl oxygen at the 2-position is able to participate in delocalisation of the resulting positive charge affording intermediate **22**. The stereochemistry of **22** encourages the incoming nucleophile to adopt a trajectory that minimises steric interactions, the result being that the bond formed at the 1-position is *trans* to the existing bond at the 2-position, in this case affording the β-anomer **23**.

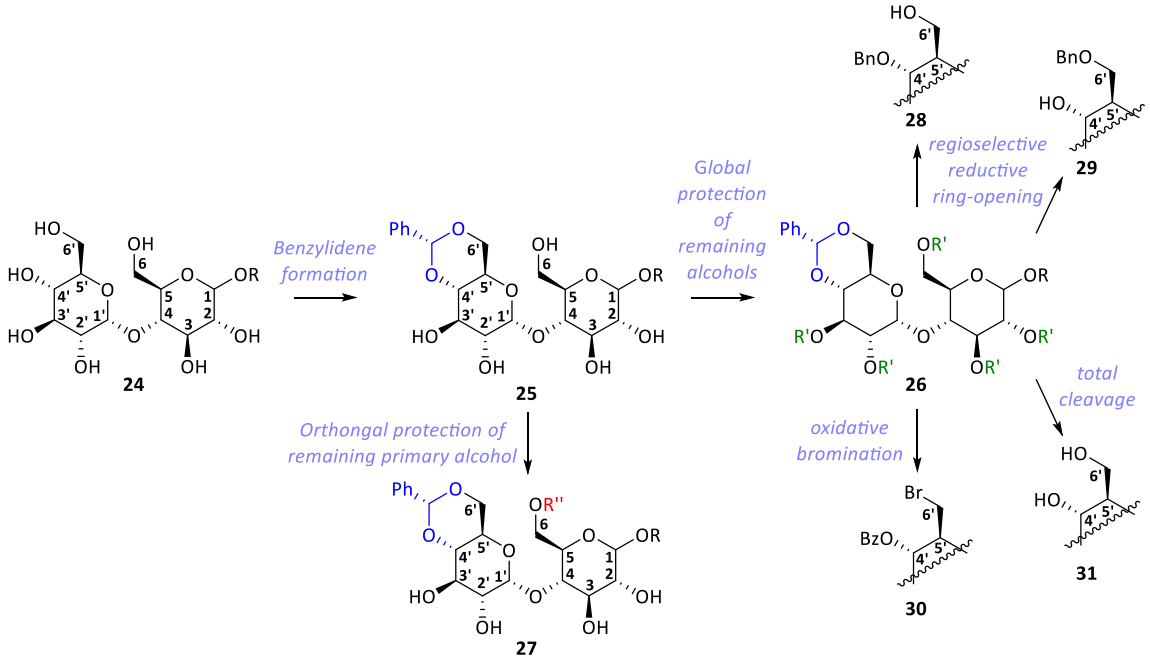


Scheme 2.2: Neighbouring group participation from an acetylated 2-position facilitates the formation of the β-anomer.

With a number of hydroxyl groups to consider, derivatisation of a single position on a sugar ring requires careful consideration. The maltose moiety of DDM **1** represents a particular problem as it contains two sugar rings and therefore two primary alcohols. Glucose, which contains only one, might be expected to be easier to derivatise. However, a wealth of protecting groups are available to allow selective access to one position, of particular use may be those which selectively protect vicinal alcohols as part of a cyclic system.[99] For example, the isopropylidene and benzylidene acetals, which respectively form cyclic 5- and 6-membered acetals with suitably arranged neighbouring alcohols. The formation of a 4',6'-benzylidene acetal on maltose derivative **24**, which has also been reported for various other disaccharides, is provided in **Scheme 2.3**.[100,101,102] The potential for further manipulation of the resulting acetal is also provided, based also on similar examples reported in the literature.

As a result of benzylidene formation, disaccharide **25** contains only one exposed primary alcohol, that at the 6-position. Exploiting the greater inherent nucleophilicity of primary alcohols over secondary alcohols, the 6-position could be selectively protected with a sterically demanding protecting group such as the *t*-butyldiphenylsilyl group, affording **27**.[103] This position could then be further manipulated, perhaps after the remaining hydroxyl groups have been protected with an orthogonal group. The benzylidene itself can undergo a regioselective reductive ring opening, a transformation that has been widely studied due to

its synthetic utility.[104] A wealth of conditions have been reported to afford either the 4'-*O*-benzyl **28** or the 6'-*O*-benzyl **29**, thereby allowing selective access to either of these positions. Access to these positions can also be granted by an oxidative opening of the benzylidene acetal using *N*-bromosuccinimide, which affords bromide **30**.[100,105,106] A final approach might be to remove the benzylidene acetal completely affording diol **31**, which again has only one primary alcohol.[107]



Scheme 2.3: Formation of 4',6'-benzylidene acetal **25** and some methods by which the resulting acetal can be manipulated in order to selectively access various positions on the sugar rings.

2.1.6 Fluorescent detergents as tools for improving membrane protein MS?

Based on this discussion, it is clear that MS is an invaluable technique for the study of membrane protein complexes and advances over recent years mean it has already been used to great effect. The broader aim of this project is to develop new antibiotics with novel modes of action, particularly those that target bacterial ion channels, which are membrane protein complexes. The potential for membrane protein MS to increase understanding of the structure and dynamics of an ion channel, in addition to providing rapid assessment of whether a ligand binds to a channel (and possibly to what subunit) is evident. It would, therefore, be of great benefit to continue evolving this technique such that these ideas can be realised. **Section 2.1.3** illustrated the importance of detergents, such as the glycolipid DDM **1**, in the study of membrane proteins. However, detergents can be difficult to remove from samples, effectively 'masking' a protein and causing diffuse peaks in the MS. An idea arose to investigate whether fluorescent glycolipids could find application in membrane protein MS. A number of fluorescent glycolipids have already been used in biological studies (**Section 2.1.4**) and their fluorescent modifications have not impaired the glycolipid's original function. If an appropriate fluorophore could be attached to a detergent, without impairing its ability to solubilise a protein, could excitation of this fluorophore provide a novel method for promoting detergent disassociation in membrane protein MS? This idea is explored further in the following section.

2.2 Aims

Section 2.1 has shown that membrane protein MS is a valuable emerging technique for elucidating the structure and stoichiometry of membrane-spanning ion channels. The added potential for identification of possible binding partners of these channels is an attractive prospect in the field of drug discovery. It is therefore desirable, where possible, to evolve this technique such that its full potential can be realised.

One proposal is to try and reduce the problems associated with detergent aggregates, which often cloud already complex spectra, by designing and synthesising fluorescent analogues of commonly used detergents. Collaborators in the Robinson group (University of Oxford) have modified their MS machines to include a laser and it was hypothesised that the energy involved in laser-induced excitation of a fluorophore linked to a detergent could encourage it to dissociate from a membrane protein complex (**Figure 2.7**).

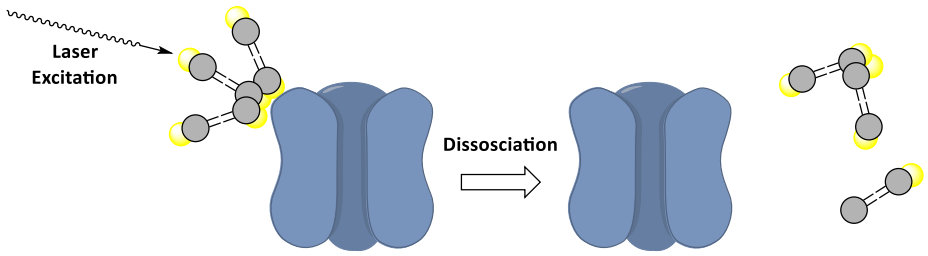


Figure 2.7: Cartoon depicting how laser-induced excitation of a fluorophore could encourage dissociation of detergent from a membrane protein complex.

To investigate this hypothesis, fluorescent analogues of DDM **1** were proposed as synthetic targets (**32** & **33**, **Figure 2.8**). Fluorescent derivatives of the monosaccharide analogue of DDM **1**, *n*-dodecyl-β-D-glucopyranoside (DG) **34**, were also proposed since these analogues were expected to be slightly easier to synthesise by virtue of their simplified structures (**35** & **36**, **Figure 2.8**). A dansyl group **12** was selected as the fluorophore because, in addition to being relatively cheap and easy to attach, it possessed an absorption maximum suitable for the laser

that was expected to be used in the mass spectrometry experiments. It is also a relatively small fluorophore and is therefore less likely to impact on the ability of the detergents to perform their intended functions. The fluorophore was to be tethered to the parent detergent *via* one of three possible connections, henceforth referred to as the linkers (**37**, **38** or **39**, **Figure 2.8**).

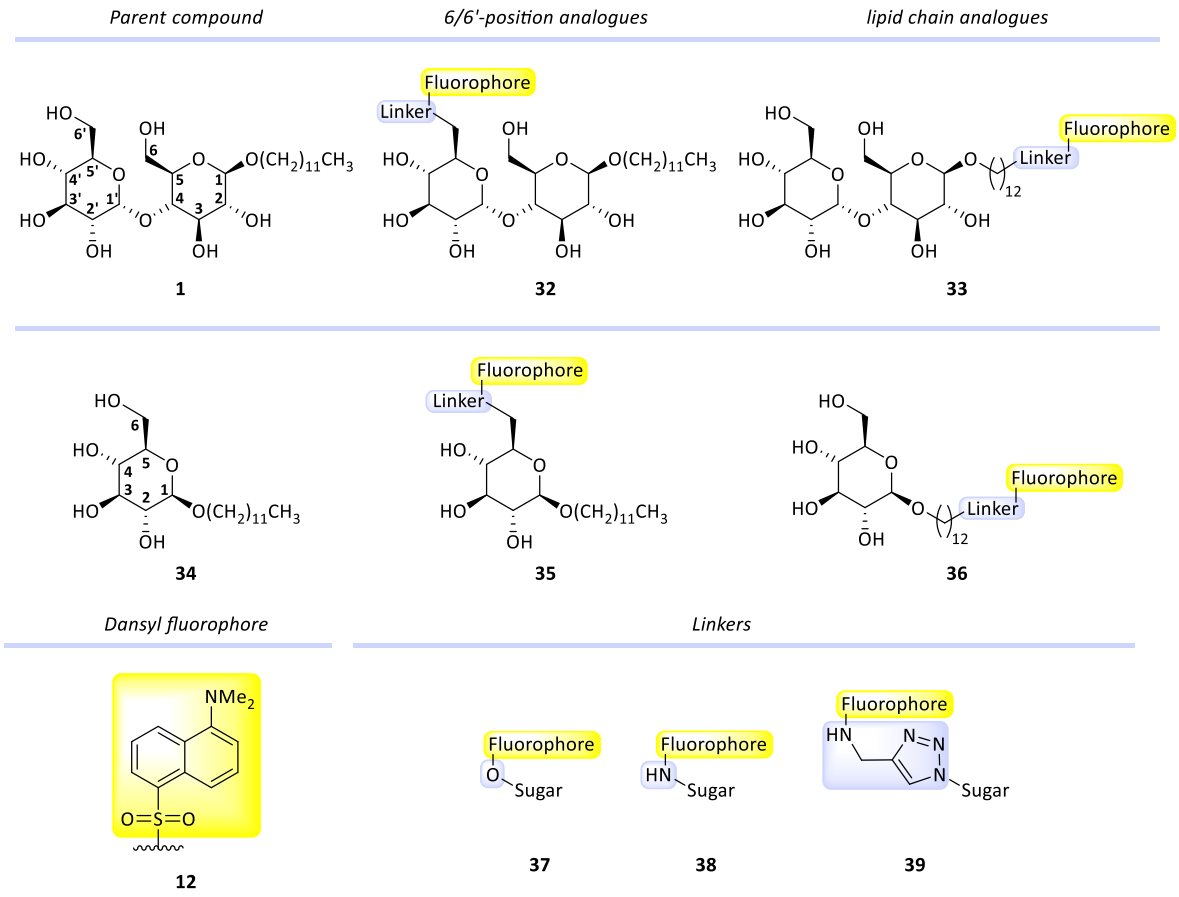


Figure 2.8: The general structures of the fluorescent detergents showing how a fluorophore will be attached to either DDM or DG at the 6/6'-position or at the end of the lipid chain. The dansyl fluorophore **12** and the proposed linkers **37**, **38** and **39** are also shown.

To expand, compounds **40**, **41** and **42** (**Figure 2.9**) are specific examples of the fluorescent DDM derivatives that were to be synthesised with modifications to the 6'-position. Compounds **40** and **41** contain single atom linkers, either an oxygen or a nitrogen atom, tethering the dansyl group to DDM **1** *via* either a sulfonate ester or sulfonamide respectively. Compound **42** contains a larger linker, a 4-(aminomethyl)-1,2,3-triazole, which was expected

to be obtained through a copper-catalysed azide-alkyne cycloaddition (CuAAC) between a dansylated alkyne and a 6'-position azide.[108] The synthesis of **40** requires the least manipulation of DDM **1** and was thus expected to be the easiest to obtain. This compound was therefore targeted first, followed by the sulfonamide **41**. Whilst it was unknown how any of these linkers, in addition to the dansyl group, would affect the ability of DDM to form micelles, a larger linker such as the triazole in **42** might be expected to exert a greater negative effect. It was therefore decided to pursue **42** last of the three.

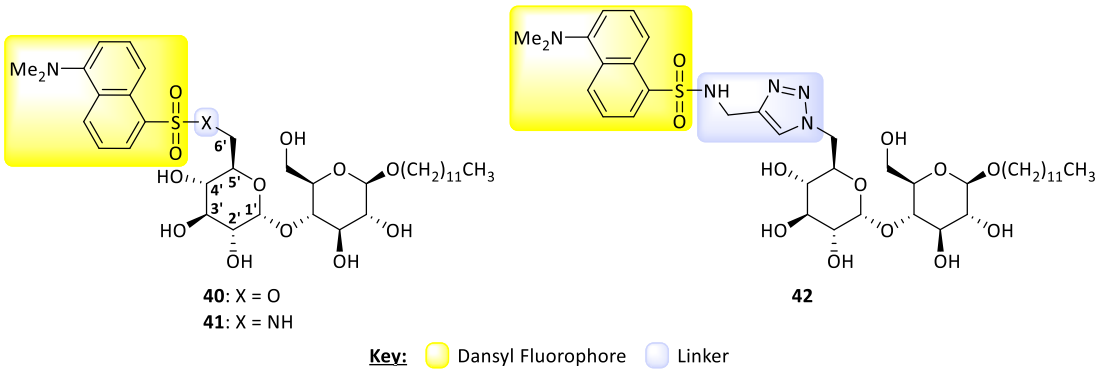


Figure 2.9: DDM analogues **40**, **41** and **42** with the fluorophore at the 6'-position.

Placing the fluorophore at the end of the lipid chain provided a further set of interesting compounds (**Figure 2.10**). Again, **43** and **44** would have the dansyl group attached by either a sulfonamide bond or a 4-(aminomethyl)-1,2,3-triazole moiety, respectively. The sulfonate ester equivalent of compounds **43** and **44** was considered an unsuitable target as there were concerns regarding its stability. A sulfonate ester would lie exposed at the end of a long lipid chain and, with the dansyl moiety being a good leaving group, it was therefore expected to be fairly susceptible to nucleophilic substitution.

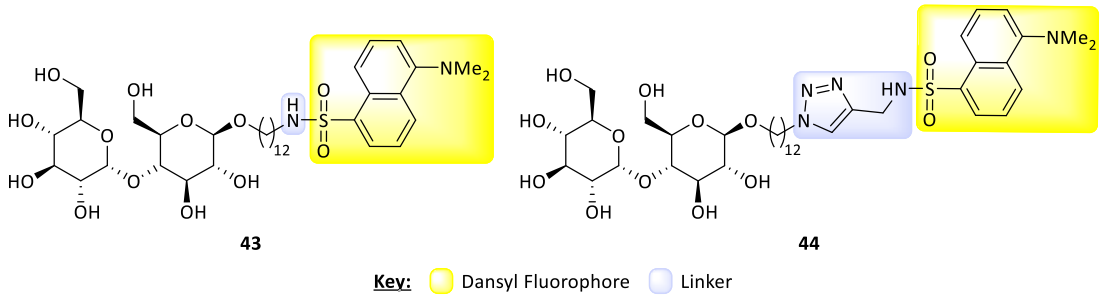


Figure 2.10: DDM analogues **43** and **44** with the fluorophore at the end of the lipid chain.

Figure 2.11 shows the fluorescent DG analogues linked to the dansyl group *via* the 4-(aminomethyl)-1,2,3-triazole linker. These constituted the initial monosaccharide targets and time-permitting, attachment *via* the single atom linkers would also be pursued.

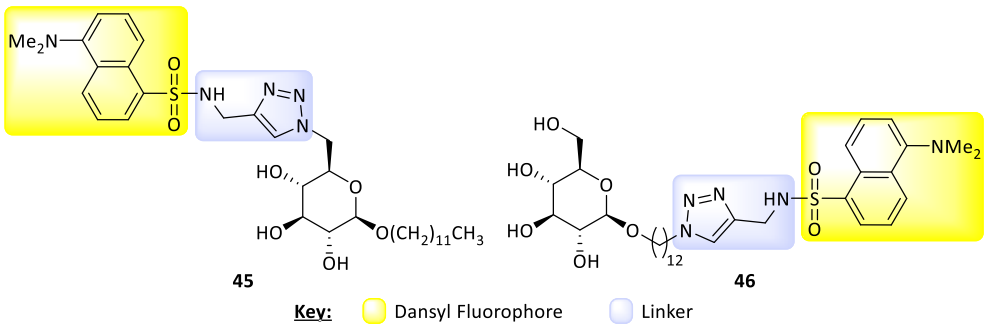


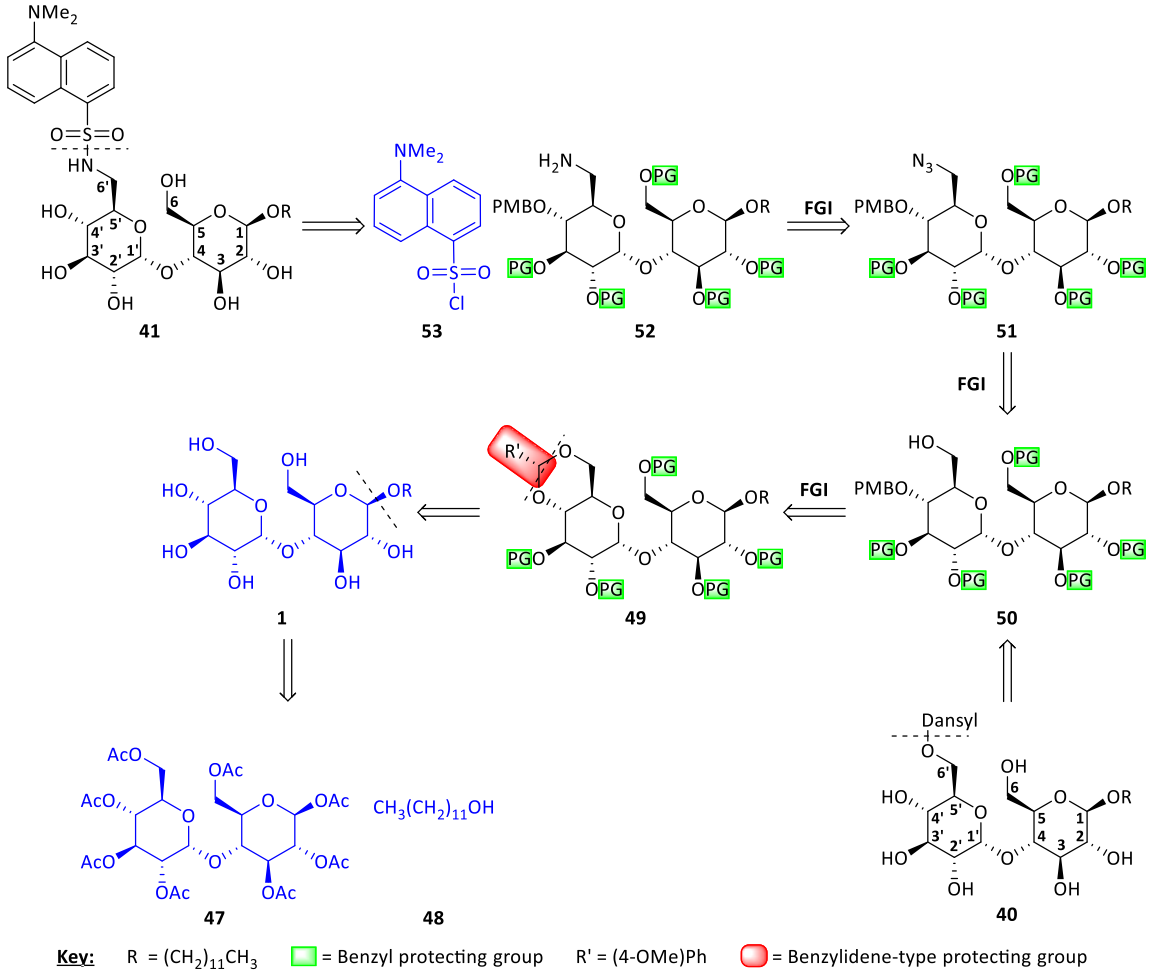
Figure 2.11: DG analogues **45** and **46** linked to the fluorophore *via* a 4-(aminomethyl)-1,2,3-triazole.

With a number of targets identified synthesis could begin. Since DDM **1** was the major detergent of interest initial efforts focused on synthesising the fluorescent analogues **40**, **41** and **42** depicted above. With these compounds in hand, focus shifted to the monosaccharide DG **34** and its fluorescent derivatives, **45** and **46**.

2.3 Results and Discussion

2.3.1 Retrosynthetic analysis of proposed compounds

As previously outlined, DDM **1** analogues with the fluorophore at the 6'-position were to be synthesised as a priority. Retrosynthetic analyses of the sulfonate ester **40** and the sulfonamide-linked compound **41**, which progress through a common intermediate, is provided in **Scheme 2.4**.



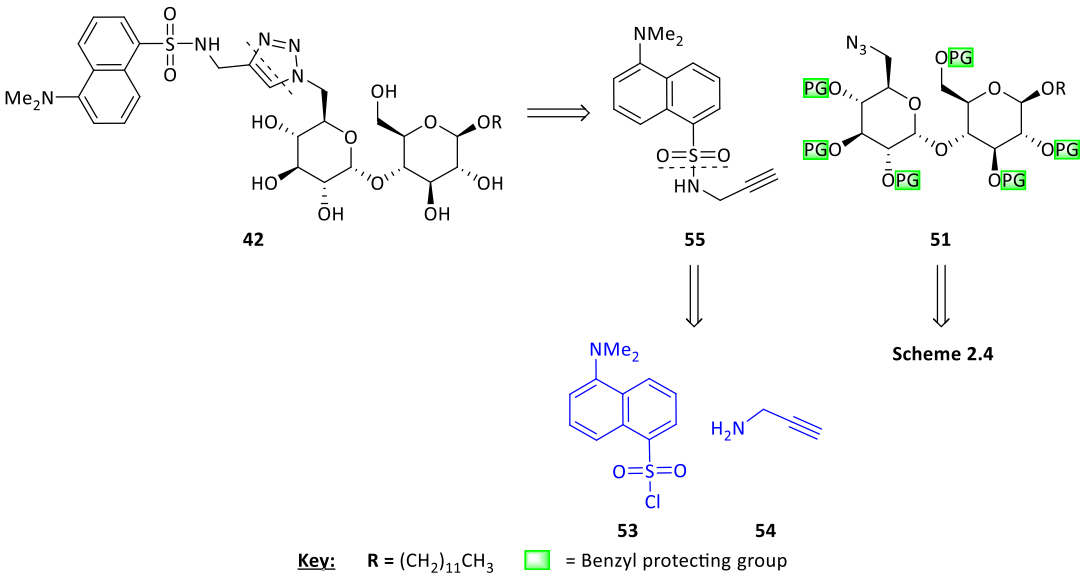
Scheme 2.4: Retrosynthetic analysis of target compounds **40** and **41**. Commercially available starting materials are shown in blue.

The aim was to install the dansyl group in the final step of the synthesis, therefore disconnection across the sulfonamide S-N bond affords amine **52** and commercially available dansyl chloride **53**. A series of functional group interconversions (FGIs) allow amine **52** to be

obtained from the primary alcohol **50** *via* azide **51**. Throughout these interconversions, the other six hydroxyl groups on the maltose rings can be protected as benzyl ethers - a robust, easily-removed protecting group, which can remain in place until the end of the synthesis. The primary alcohol **50** can also be used to obtain target compound **40**, with the sulfonate ester linker, through reaction with dansyl chloride **53**.

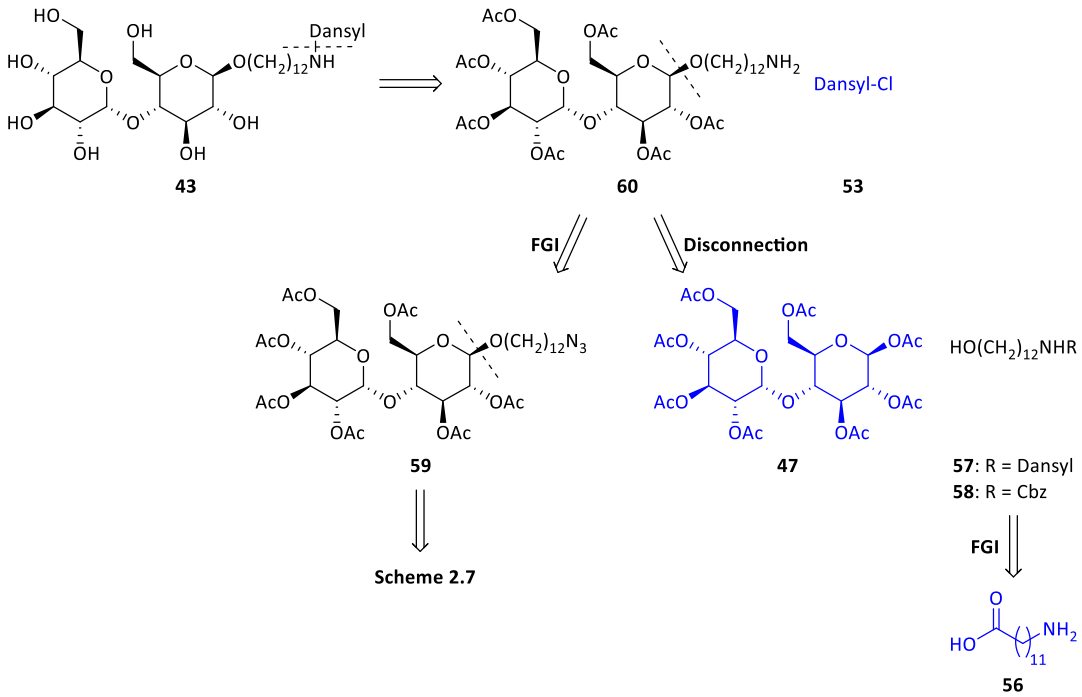
Much literature precedent exists for selectively accessing the 6/6'-position hydroxyl groups in sugars *via* benzylidene precursors.[104,109] A regioselective ring opening of benzylidene **49** (where R' is an aromatic group, commonly phenyl or a 4-methoxyphenyl) was therefore proposed as a viable route to 6'-position alcohol **50**. Removal of the 4',6'-benzylidene and the six benzyl ethers affords a commercially available starting material in the form of the detergent central to this work, DDM **1**. If necessary, it might be possible to synthesise DDM **1** itself from another commercially available compound, β-D-maltose octaacetate **47**, *via* glycosylation of dodecan-1-ol **48**.

In pursuit of the third target compound **42**, disconnection across its 1,2,3-triazole afforded benzylated azide **51**, already discussed in **Scheme 2.4**, and an alkyne **55** (**Scheme 2.5**). Disconnection across the S-N sulfonamide bond of the alkyne **55** suggested dansyl chloride **53** and propargyl amine **54** would be suitable precursors for its preparation. Ample literature precedent exists for the CuAAC of such reagents affording a multitude of options for the forward reaction between the azide **51** and the alkyne **55.**[110,111,112,113,114]



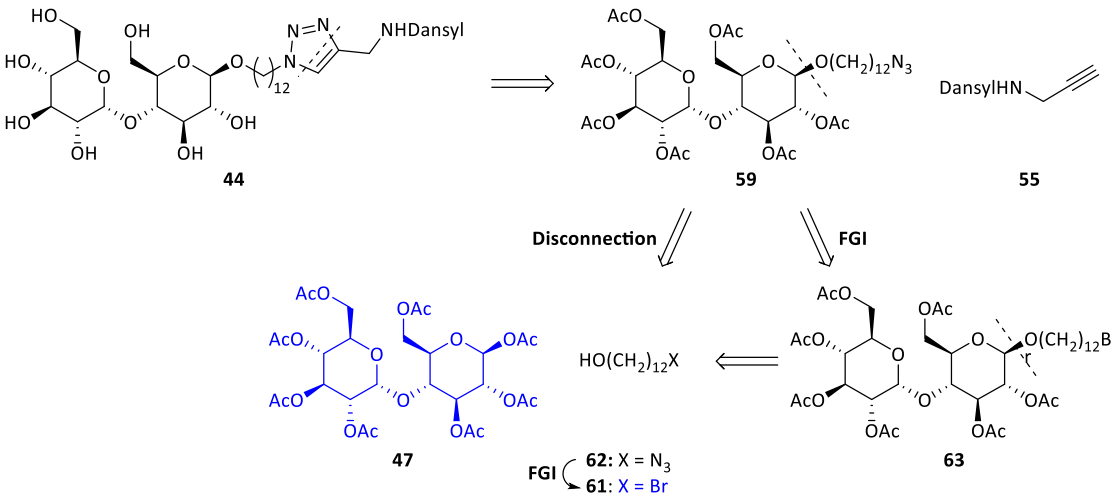
Scheme 2.5: Retrosynthetic analysis of target compound **42**. Commercially available compounds are shown in blue. Further retrosynthetic analysis of the azide **51** can be found in **Scheme 2.4**.

Synthetic routes to the DDM analogues with the fluorophore at the end of the lipid chain were expected to be slightly shorter, since fewer protecting group manipulations and FGIs would be necessary. Retrosynthetic analysis of the fluorescent sulfonamide-linked compound **43** is given in **Scheme 2.6**. Disconnection of the target compound **43** across the S-N bond afforded an amine **60** and dansyl chloride **53**. Two options arose for synthesising this amine. The first, was an FGI from the azide **59**, further disconnections of which are given below in **Scheme 2.7**. The second, was direct installation of the amine through glycosylation of an appropriately *N*-protected 12-aminododecanol, such as **58,** with β-D-maltose octaacetate **59**. Instead of protection, pre-dansylation of the amine in the glycosyl acceptor, to give **57**, was also considered. Whichever alcohol was to be used, all could be synthesised from commercially available 12-aminododecanoic acid **56**.



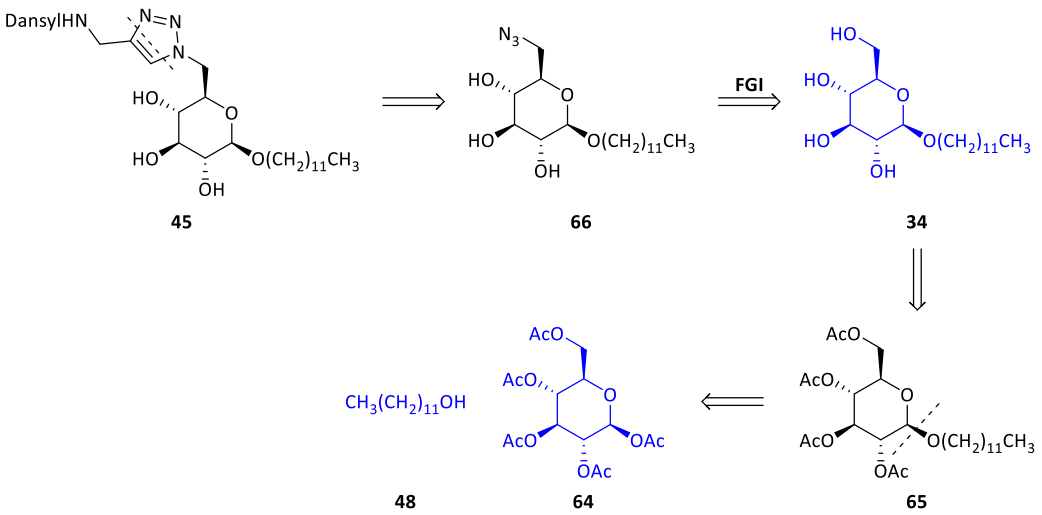
Scheme 2.6: Retrosynthetic analysis of target compound **43**. Commercially available compounds are shown in blue. Further retrosynthetic analysis of azide **59** can be found in **Scheme 2.7**.

As above, the triazole compound **44** was expected to be obtained *via* a CuAAC between azide **59** (**Scheme 2.7**) and the aforementioned dansylated alkyne **55** (**Scheme 2.5**). Glycosylation of 12-azidododecanol **62** with β-D-maltose octaacetate **47** was proposed as a possible route to azide **59**, although many existing procedures first activate the anomeric centre as, for example, the phenylthioglycoside or the trichloroacetimidate.[110,115] A seemingly more common approach was to first glycosylate the peracetate with a halo-alcohol before converting the halogen into an azide.[115,116] Should 12-azidododecanol **62** prove unsuitable for glycosylation, an FGI giving, for example, bromide **63** was a possible alternative. Bromide **63** can also be synthesised *via* a glycosylation, this time of 12-bromododecanol **61** by β-D-maltose octaacetate **47**. Both of these routes are the same length, given that it was first necessary to synthesise 12-azidododecanol **62** from commercially available 12-bromododecanol **61**.



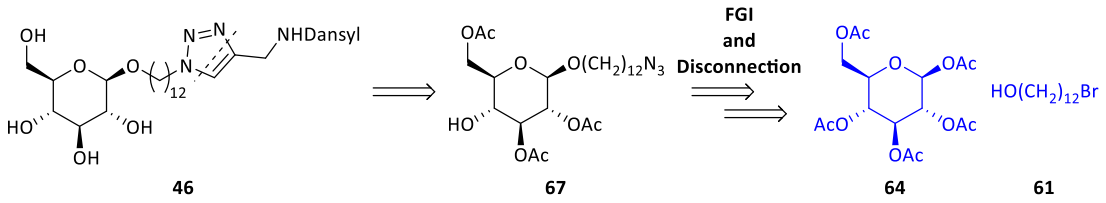
Scheme 2.7: Retrosynthetic analysis of target compound **44**. Commercially available compounds are shown in blue.

Since DG **34** contains only one primary alcohol, as opposed to DDM **1** that contains two, a less demanding protecting group strategy could be adopted to synthesise DG analogues with the fluorophore at the 6-position, so long as reaction conditions were tailored to encourage the desired regioselectivity (**Scheme 2.8**). A now familiar disconnection across the 1,2,3-triazole in **45** gave azide **59** (and alkyne **55**, not shown). An FGI on azide **66** afforded DG **34** itself (with one primary alcohol and three secondary alcohols), which was commercially available, albeit relatively expensive. It was therefore decided to synthesise DG **34** from its pentaacetate **64** by another, now familiar reaction, glycosylation of alcohol **48**.



Scheme 2.8: Retrosynthetic analysis of target compound **45**. Commercially available compounds are shown in blue.

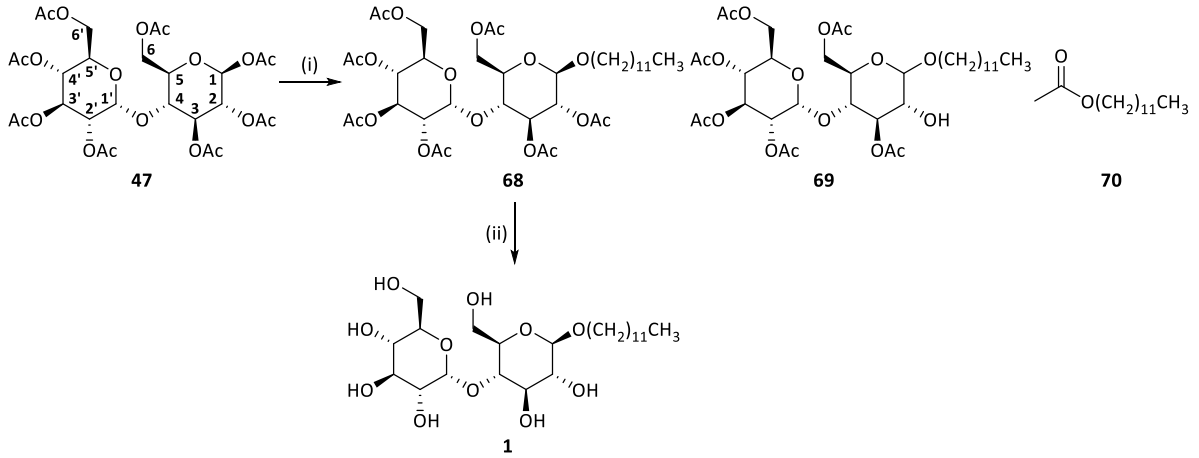
Finally, the proposed synthesis of the DG analogue **46**, with the triazole-linked fluorophore at the end of the lipid chain, was expected to be the same as that of the DDM analogue **44** (**Scheme 2.9** and **Scheme 2.7**, respectively).



Scheme 2.9: Retrosynthetic analysis of target compound **46**. Commercially available compounds are shown in blue.

2.3.2 Synthesis of a fluorescent DDM analogue with the fluorophore attached to the 6'-position *via* a sulfonate ester

As a mild introduction to sugar chemistry, work began with the synthesis of DDM **1**. A glycosylation reaction, using commercially available β-D-maltoseoctaacetate **47** as the glycosyl donor and dodecan-1-ol **48** as the acceptor, was performed employing conditions from a similar reaction in the literature (**Scheme 2.10**, (i)).[117]



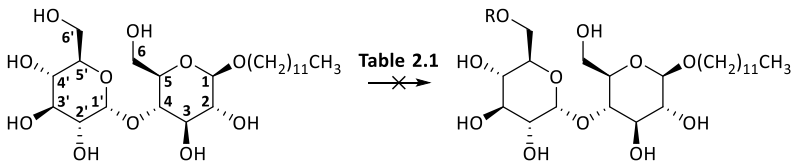
Scheme 2.10: Synthesis of DDM **1**. *Reagents & conditions*: (i) β-D-Maltose octaacetate **47** (1.00 eq.), dodecan-1-ol **48** (1.50 eq.), $ZnCl_2$ (1.50 eq.), toluene, 65 °C, 1 h, 58%; (ii) **68** (1.00 eq.), NaOMe (1.25 eq.), MeOH/CH_2Cl_2, 0 °C → RT, 2 h, 100%.

The glycosylation rarely reached completion, however, lengthening the reaction time is known to increase the risk of anomerisation so it was important to strike a balance. The crude reaction mixture contained by-products identified as acylated dodcan-1-ol **70** (observed *via* MS and TLC) and an α/β-anomeric mixture of **69** (isolated, 8% yield).[117] In spite of these issues, the reaction usually proceeded in acceptable yield and **68** was easily isolated as a single anomer following column chromatography. Due to its severely hygroscopic nature, the Lewis acid, zinc chloride, required rigorous pre-drying in a vacuum oven at 110 °C. This procedure was operationally difficult for small scale reactions and future glycosylations (**Schemes 2.25**, **2.28** & **2.33**) were therefore carried out using a more robust Lewis acid (borontrifluoride

diethyletherate). Following isolation of **68**, a straightforward Zemplén deacetylation provided **1** in excellent yield (**Scheme 2.10**, (ii)).[118] With the ^{1}H NMR spectrum and the optical rotation agreeing well with the literature, the synthesis was continued, although DDM **1** was eventually purchased from a commercial source to increase throughput.

In pursuit of the shortest route to the desired compounds, the next logical step was to investigate whether steric factors alone would be strong enough to direct any group exclusively onto the 6'-position of DDM **1**, without it being necessary to protect the remainder of the hydroxyl groups (**Scheme 2.11**).

Unfortunately, treatment of DDM **1** with the fluorophores dansyl chloride **53** or NHS-activated 5/6-carboxyfluorescein failed to provide either **41** or **71** respectively (**Entries 1** & **2**, **Table 2.1**). Despite significant efforts to maintain anhydrous conditions in these reactions, hydrolysis of the fluorophores was consistently observed, even when molecular sieves were used in the reaction. Treatment of **1** with methanesulfonyl chloride was marginally more successful, the starting material was consumed and the product **72** was visible by MS (**Entry 3**, **Table 2.1**). However, polymesylation was prevalent with significant amounts of a di- and trimesylated species visible by TLC and MS. Attempts to minimise polymesylation were made. Stoichiometric methanesulfonyl chloride was used and the reaction was carried out at −12 °C, however, these efforts failed to supress polymesylation. Based on these results, it was apparent that 6'-position hydroxyl was not sufficiently exposed or reactive to be functionalised in the presence of the six other hydroxyl groups and a protecting group strategy was therefore necessary in order to functionalise this position.



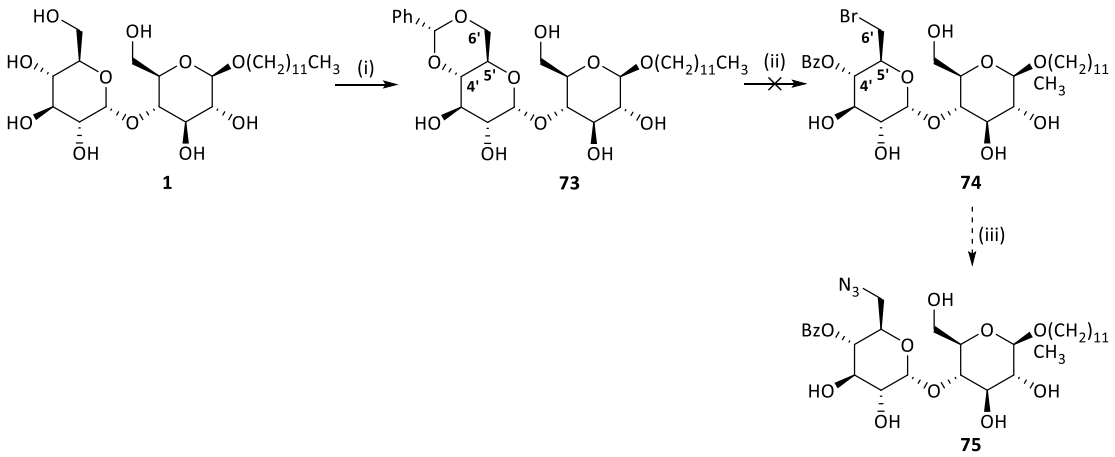
Scheme 2.11: Attempted functionalisation of the 6'-position without protection of the remaining hydroxyl groups. *Reagents & conditions* can be found **Table 2.1**.

Table 2.1: Conditions assessed in pursuit of exclusive 6'-position functionalisation.

Entry	Target	***Reagents & Conditions*** (with **1** (1.00 eq.))	Comments
1	**41**	Dansyl chloride **53** (1.00 eq.) DABCO (0.10 eq.), MeCN, RT, 16 h	• No reaction, starting material recovered • Hydrolysis of dansyl chloride **53**
2	**71**	NHS-5/6-carboxyfluorescein (1.20 eq.) Et_3N (1.00 eq.), DMAP, (0.10 eq.), DMF, RT, 48 h	• No reaction, starting material recovered • Hydrolysis of activated fluorophore
3	**72**	MsCl (1.00 eq.) pyridine, -12 °C, 1 h	• Significant polymesylation observed by TLC and MS

A strategy that would later prove successful on the monosaccharide DG **34** (**Schemes 2.31**) was also tried on DDM **1**. The opening of 4,6-benzylidenes with *N*-bromosuccinimide in carbon tetrachloride under reflux, leaving a bromide at the 6-position and a benzoyl group at the 4-position, was first reported by two different groups in 1966.[109,119] Such a reaction would be ideal as the resultant bromide **74** could be treated with sodium azide to give the key azide intermediate **75** (**Scheme 2.12**).

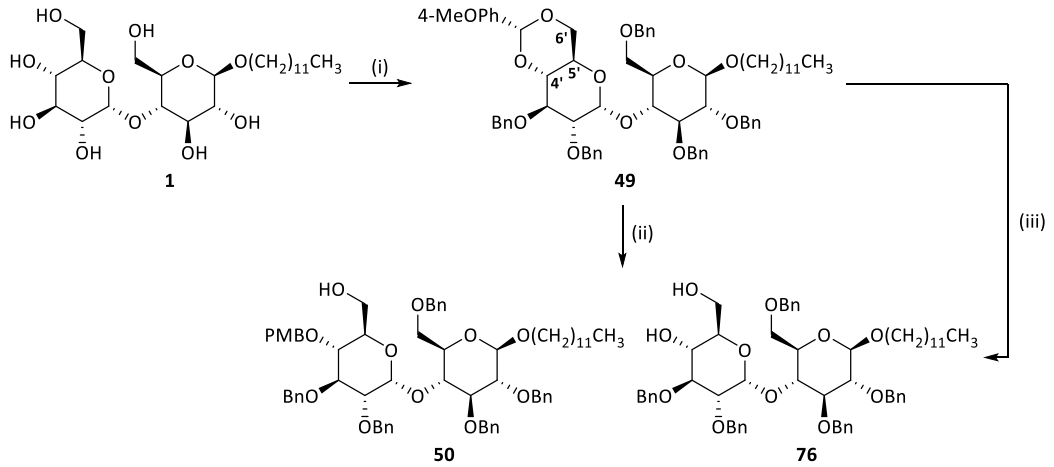
The first step saw formation of benzylidene **73** by reaction of DDM **1** with benzaldehyde dimethylacetal in the presence of a mild acid catalyst (**Scheme 2.12**, (i)). This reversible reaction worked best at reduced pressure, as methanol is produced and its removal helps drive the reaction to completion. An excess of benzaldehyde dimethylacetal was used to account for any evaporation resulting from performing the reaction under vacuum. Under these conditions, the reaction reached completion after between three and six hours, reliably affording **73** in good yield following column chromatography.



Scheme 2.12: Attempted synthesis of azide **75**. *Reagents & conditions*: (i) **1** (1.00 eq.), $PhCH(OMe)_2$ (3.00 eq.), camphorsulfonic acid (0.12 eq.), DMF, 60 °C, 300 mbar, 3 h, 73%; (ii) **73** (1.00 eq.), NBS (1.40 eq.), $BaCO_3$ (2.60 eq.), CCl_4, 80 °C; (iii) **74** (1.00 eq.), NaN_3 (1.10 eq.), DMF, 80 °C, 16 h.

Unfortunately, benzylidene **73** repeatedly failed to react under the bromination conditions, even with the use of fresh reagents and the addition of dichloroethane to aid solubility (**Scheme 2.12**, (ii)). Switching the acid scavenger from barium carbonate to calcium carbonate also failed to improve the reaction.[120] A definitive reason for this failure was not established. Since the monosaccharide DG **34** did react under such conditions, the problem was not the reagents and, with literature precedent for this transformation on disaccharides and even pentasaccharides, the additional anomeric centre was unlikely to have been the problem.[100,121,106] It was noticeable, however, that all literature examples tended to protect the remaining hydroxyl groups (usually as acetates) prior to reaction with *N*-bromosuccinimide suggesting the decreased solubility of **73**, due to the large number of free hydroxyls, might have been hindering the reaction. Due to time constraints, further protection of the free hydroxyl groups was not investigated at this time, and with a second "short" route to functionalising the 6'-position eliminated, it was clear that only a more severe protecting group strategy would suffice. A 4',6'-benzylidene-type compound like **73** was a good starting point as this allowed subsequent protection of the five remaining hydroxyl groups as *O*-benzyl ethers, which could remain intact until the end of the synthesis.

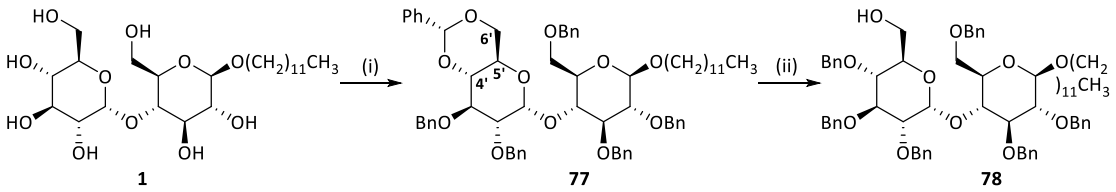
The 4-methoxybenzylidene protecting group was first investigated as this had been successfully used in the group's work with inositols.[122,123] DDM **1** was reacted as above but this time with 4-methoxybenzaldehyde dimethyl acetal. Some literature procedures based on this chemistry reported that it was unnecessary to purify the resulting benzylidene, so the crude reaction mixture was subjected to benzylation conditions affording the fully protected compound **49** (**Scheme 2.13**, (i*a* & *b*)).[124,125,126]



Scheme 2.13. Pursuit of the free 6'-position alcohol **50** *via* 4',6'-(4-methoxybenzylidene) **49**. *Reagents & conditions*: (i) *a*) **1** (1.00 eq.), 4-MeOPhCH(OMe)$_2$ (1.20 eq.), camphorsulfonic acid (0.12 eq.), DMF, 60 °C, 300 mbar, 3 h; *b*) BnBr (5.50 eq.), NaH (10.00 eq.), TBAI (0.05 eq.), DMF, 60 °C, 300 mbar, 66% over 2 steps; (ii) **49** (1.00 eq.), BH_3·THF (3.20 eq.), $CoCl_2$ (3.20 eq.), THF, RT, 18 h, **50** = 23%, **76** = 68%; (iii) **49** (1.00 eq.), ceric ammonium nitrate (1.83 eq.), MeCN/H_2O, RT, 35 min, 68%.

Unfortunately, rapid breakdown of **49** was consistently observed, making purification and characterisation difficult. Additionally, the regioselective reductive ring-opening (discussed later in reference to **78**, **Scheme 2.14**), using the optimised conditions of borane·tetrahydrofuran complex and cobalt chloride, afforded only minimal amounts of the desired 6'-position alcohol **50** (**Scheme 2.13**, (ii)). The major product was identified as diol **76**, believed to result from decomposition of the benzylidene accelerated by the reaction conditions. Eventually, the supply of **49** was intentionally converted to diol **76** using ceric ammonium nitrate (**Scheme 2.13**, (iii)) such that it could be incorporated into the synthesis of the unsubstituted benzylidene **77**, discussed below.

With the 4-methoxybenzylidene proving too unstable for purpose, focus was switched to the unsubstituted benzylidene, already seen in **Scheme 2.12**. As above, DDM **1** was reacted with benzaldehyde dimethyl acetal, then benzylated with benzyl bromide, affording the fully-protected benzylidene **77** in excellent yield over two steps (**Scheme 2.14**, (i*a* & *b*)). The greater two-step yield for the benzylidene **77** compared to the 4-methoxybenzylidene **49** (84% versus 66% respectively) suggested the unsubstituted benzylidene possessed the desired stability.



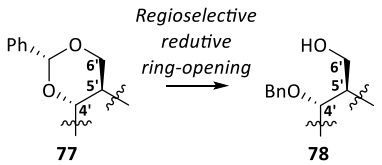
Scheme 2.14: Synthesis of the 6'-alcohol **78**. *Reagents & conditions*: (i) *a*) **1** (1.00 eq.), $PhCH(OMe)_2$ (1.50 eq.), camphorsulfonic acid (0.12 eq.), DMF, 60 °C, 300 mbar, 2 h; *b*) BnBr (5.50 eq.), NaH (10.00 eq.), TBAI (0.05 eq.), DMF, RT, 6.5 h, 82% over 2 steps; (ii) **77** (1.00 eq.), BH_3·THF (3.50 eq.), $CoCl_2$ (3.50 eq.), THF, RT, 20 h, 76%.

With the fully-protected benzylidene **77** in hand, it was necessary to establish conditions that would effect a regioselective reductive ring-opening of the benzylidene to give the 6'-position alcohol and the 4'-*O*-benzyl moiety (**Scheme 2.14**, (ii)). A plethora of conditions that achieve this transformation have been reported, those screened herein are reported in **Table 2.2**.[104]

Efforts were initially focused on the use of di*iso*butylaluminum hydride, which has been successfully used to effect the same transformation in the group's inositol chemistry, however, this reagent proved too harsh for **77** (**Entry 1**, **Table 2.2**).[122,127,123] Although some of the desired product was obtained, a complex mixture of products was visible by TLC, presumably a result of hydride attack at the anomeric centres, in addition to that at the benzylic carbon of the benzylidene. When the reaction was attempted at lower temperatures,

namely −78 °C and −30 °C, in an effort to reduce these undesired side reactions, it completely stalled and only starting material was recovered.

Table 2.2: Screen of conditions to effect the regioselective reductive ring opening of **77**.



77 **78**

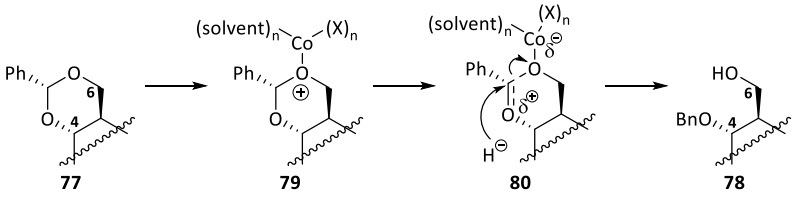
Entry[a]	***Reagents & Conditions***[b] (with **77** (1.00 eq.))	**Yield**[b]
1 (n = 5)	DIBAL-H (2.00 eq.) CH_2Cl_2, RT, 24 h	15% (0-15%)
2 (n = 10)	Et_3SiH (3.02 eq.), $PhBCl_2$ (3.42 eq.) CH_2Cl_2, 4 Å MS, −78 °C, 1 h	52% (0-52%)
3 (n = 4)	BH_3·THF (3.00 eq.), $CoCl_2$ (3.00 eq.) THF, RT, 18 min	46% (43-46%)
4 (n = 2)	BH_3·THF (3.00 eq.), $CoCl_2$ (3.00 eq.) *freshly distilled* THF, RT, 20 h	76% (71-76%)

[a]: n = The number of times the transformation was attempted for a given reagent combination but with slight modifications to the reaction conditions. [b]: Only the 'optimum' conditions and the best yield are reported, the range of yields for a particular reagent combination is provided in parentheses.

A combination of dichlorophenylborane (as the Lewis acid) and triethylsilane (as the reducing agent) was then tried (**Entry 2**, **Table 2.2**) and a 52% yield early on was promising.[128,129] However, these conditions provided hugely unreliable results often affording no product at all or, as with di*iso*butylaluminum hydride, a complex mixture of products.

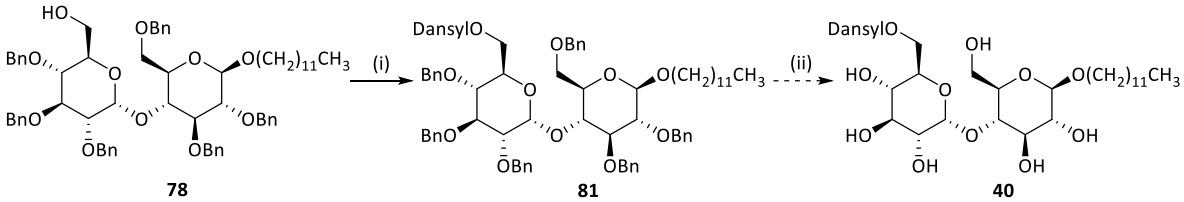
A more modern combination of reagents came in the form of borane·tetrahydrofuran complex and cobalt chloride in tetrahydrofuran (**Entry 3**, **Table 2.2**).[130] From the outset reliable, albeit low yields, of around 46% were obtainable. Pleasingly, however, when freshly distilled solvent was used the yield rose to 76% (**Entry 4**, **Table 2.2**). Under these conditions, the polar solvent (tetrahydrofuran) modulates the reactivity of the Lewis acid ($CoCl_2$) by ensuring it is well solvated, thereby slowing the reaction rate.[104] This favours the desired regioselectivity by

encouraging preferential coordination of the Lewis acid to the more nucleophilic 6-position oxygen (as opposed to the 4-position oxygen) in the proposed rate-determining step, affording **79** (**Scheme 2.15**). Hydride attack on the resultant intermediate **80**, which is believed to be more like an intimate ion pair than a discrete oxocarbenium ion, opens the benzylidene giving the desired 6-position alcohol **78**. When the reducing agent is a more activated borane, such as borane·dimethylamine complex, the regioselectivity is inversed because the borane reagent is now more electrophilic than the Lewis acid. The reducing agent therefore out-competes the Lewis acid for coordination of the 6-position oxygen, leaving the Lewis acid to coordinate to the 4-position. Nevertheless, with borane·tetrahydrofuran complex, a reliable route to the 6' alcohol **78** had been established and the synthesis could therefore progress to functionalising this position.



Scheme 2.15: Basic mechanistic explanation for the regioselective reductive ring-opening of a 4,6-benzylidene to give a free 6-position alcohol in the presence of a reducing agent, a Lewis acid and polar solvent.

The dansylation, which had been unsuccessful with free DDM **1** (**Scheme 2.11**), was repeated on the 6' alcohol **77** and the desired product **81** was isolated in 38% (**Scheme 2.16**, (i)). Unfortunately, **81** was found to be fairly unstable with decomposition observed in the NMR tube and after storage under argon at −18 °C.



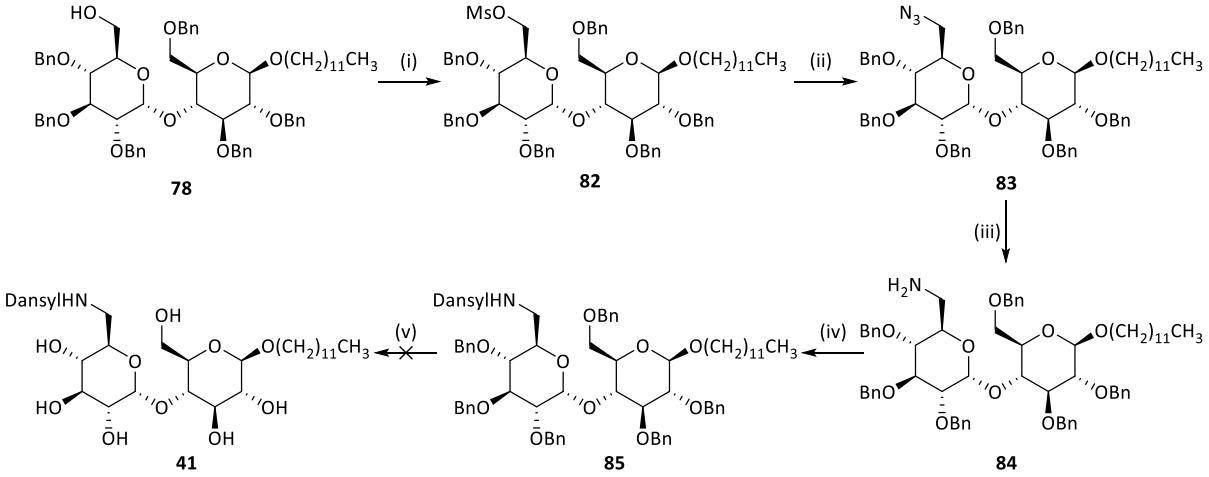
Scheme 2.16: Attempted synthesis of target compound **40**. *Reagents & conditions*: (i) **78** (1.00 eq.), dansyl chloride **53** (3.00 eq.), Et_3N (3.00 eq.), CH_2Cl_2, RT, 4 h, 38%; (ii) Global debenzylation, not attempted due to the instability of **81**.

A study by Miller profiled the promiscuous lability of various sulfonate esters so it seemed appropriate to attribute the low yield to instability.[131] More worryingly, it raised concerns about the stability of the target compound **40** as it was hydrolysis of the sulfonate ester that appeared to be causing the instability. Since the free hydroxyl groups in the target compound were expected to make it fairly polar, it follows that polar solvents such as methanol or water were likely to be required for its synthesis and certainly for its characterisation. This suggested that **81** would be unlikely to survive the debenzylation conditions (**Scheme 2.16**, (ii)) and any product isolated was predicted to be unstable too. With this in mind, focus switched to synthesising target compounds **41** and **42** with the sulfonamide **38** and 4-aminomethyl-1,2,3-triazole **39** linkers respectively. These linkers were expected to be more stable to hydrolysis.

2.3.3 Synthesis of a fluorescent DDM analogue with the fluorophore attached to the 6'-position *via* a sulfonamide

In order to form the sulfonamide linkage it was necessary to convert the primary alcohol of **78** into an amine **84**. Commonly, this requires activation of the alcohol by attachment of a good leaving group. The leaving group (and the oxygen atom) are subsequently displaced by a nucleophilic amine precursor such as azide and a Staudinger reduction can then convert the azide into the desired amine.[132]

The conversion of **78** to azide **83** was straightforward. The alcohol **78** was activated *via* mesylation with methanesulfonyl chloride, providing **82** in excellent yield, and subsequent reaction with sodium azide provided azide **83**, again in excellent yield (**Scheme 2.17**, (i) & (ii)). A Staudinger reduction with triphenylphosphine and water occurred in modest yield (**Scheme 2.17**, (iii)). A reaction with polymer-bound triphenylphosphine was investigated in an attempt to increase the yield, however, this appeared to decrease the reaction rate and the yield.



Scheme 2.17: Conversion of primary alcohol **78** to amine **84** followed by subsequent dansylation and attempted debenzylation in pursuit of target compound **41**. *Reagents & conditions*: (i) **78** (1.00 eq.), MsCl (1.20 eq.), Et_3N (2.20 eq.), CH_2Cl_2, RT, 1 h, 100%; (ii) **82** (1.00 eq.), NaN_3 (3.00 eq.), DMF, 60 °C, 24 h, 98%; (iii) **83** (1.00 eq.), PPh_3 (1.50 eq.), H_2O, THF, RT, 18 h, 53%; (iv) **84** (1.00 eq.), dansyl chloride **53** (1.50 eq.), Et_3N (1.50 eq.), CH_2Cl_2, RT, 24 h, 35%; (v) see **Section 2.3.5**.

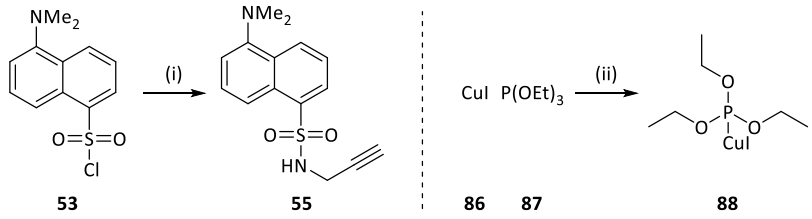
As with alcohol **78**, amine **84** was reacted with dansyl chloride **53** in the presence of base affording compound **85** with the desired sulfonamide linker (**Scheme 2.17**, (iv)). Interestingly, like the earlier dansylation of alcohol **78** (**Scheme 2.16**), this transformation also occurred in disappointing yield, yet the product **85** appeared to be more stable than its sulfonate ester counterpart **81**. An additional explanation for these poor yielding dansylations was therefore required. Literature yields for such transformations, even within the same paper, appeared variable, although a trend was never identified.[133,134] Ultimately, it was suggested that the steric bulk conferred upon substrates **81** and **85** by the six benzyl protecting groups, which it

must be remembered are similar in size to the central sugar rings, was hindering the ability of the 6'-position to perform as a nucleophile.

Despite the low yield, sufficient amounts of **85** were isolated to attempt the final debenzylation. Unfortunately, despite some literature precedent for debenzylation in the presence of a sulfonamide-linked dansyl group,[88] various debenzylation conditions caused either cleavage of the sulfonamide linker or decomposition of the aromatic dansyl moiety as detailed further in **Section 2.3.5**.

2.3.4 Synthesis of a fluorescent DDM analogue with the fluorophore attached to the 6'-position *via* a 4-aminomethyl-1,2,3-triazole linker

After synthesising azide **83** *en route* to amine **84**, a logical diversion was to use this azide and a fluorescent alkyne to create a 1,2,3-triazole-linked fluorescent compound *via* a CuAAC.[108] For this to be realised a dansylated alkyne was necessary and this was synthesised from dansyl chloride **53** and propargyl amine in excellent yield (**Scheme 2.18**, (i)).[135]



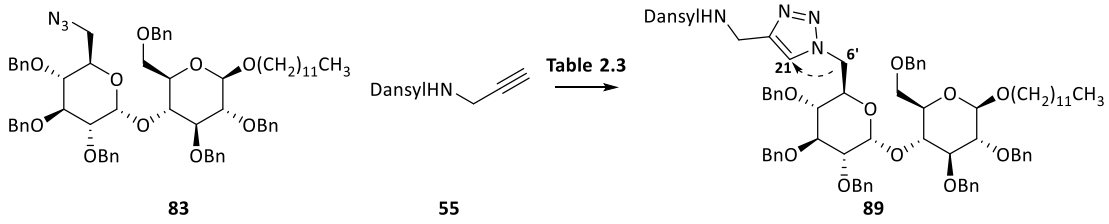
53 **55** **86** **87** **88**

Scheme 2.18: Synthesis of dansyl alkyne **55** and copper catalyst **88** for CuAAC. *Reagents & conditions*: (i) **53** (1.00 eq.), propargyl amine (1.50 eq.), Et_3N (1.50 eq.), CH_2Cl_2, RT, 30 min, 100%, (ii) CuI **86** (1.00 eq.), $P(OEt)_3$ **87** (1.00 eq.), toluene, 80 °C, 18 h, 66%.

With the reactants established, work progressed to assessing conditions for a CuAAC (**A**, **Scheme 2.19** & **Table 2.3**). The most traditional conditions were employed first, however, reactivity was low due to the poor solubility of the relatively apolar reactants in *t*-butanol/water (**Entry 1**, **Table 2.3**).[108] Heating this reaction to 80 °C, which would be

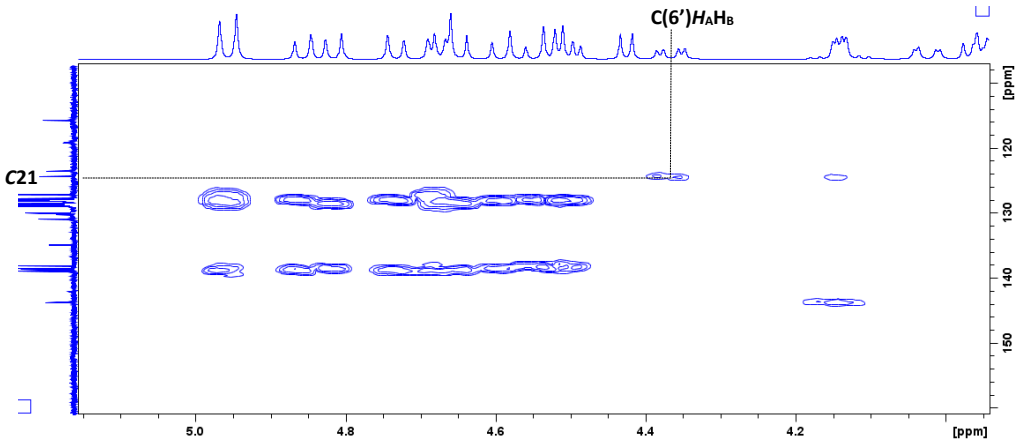
undesirable for large scale reactions with azides, vastly improved the solubility and provided product **89**, albeit in a low yield of 38%. The regiochemistry of the CuAAC was confirmed using 2D HMBC analysis. A signal was observed indicating a long range coupling between one of the protons on the 6'-position of the sugar ring and 'carbon-21' of the triazole ring (**B**, **Scheme 2.19**).

A



83 **55** **89**

B



Scheme 2.19: **A**: A CuAAC between **83** and **55**. A screen of conditions can be found in Table **2.3**. **B**: HMBC analysis of compound **89** confirmed the regiochemistry of the CuAAC.

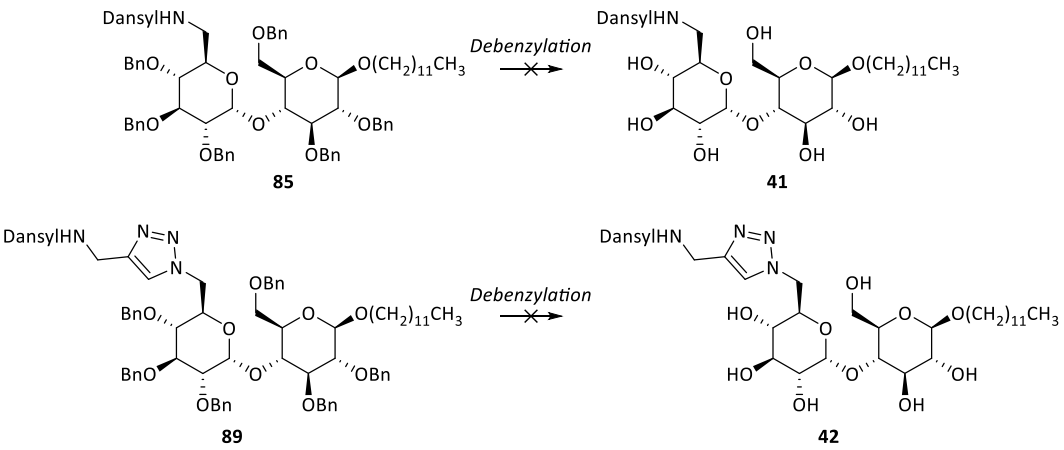
Table 2.3: Screen to establish suitable conditions for the CuAAC. The arrows indicate how certain conditions were altered over the course of a single reaction in attempts to drive the reaction to completion.

Entry	***Reagents & Conditions*** with **83**, (1.00 eq.) & **55** (1.00 eq.)*		**Yield**
1	$CuSO_4$ (0.01 → 0.10 eq.), Sodium ascorbate (0.10 → 1.00 eq.) *t*-BuOH/H_2O	RT → 80 °C, 24 h → 48 h	38%
2	Cu(I)I($PO(Et)_3$) **88** (0.20 → 0.40 eq.), Et_3N (3.00 eq.) toluene	MW 300 W 10 × 2 min	61%
3	Cu(I)I($PO(Et)_3$) **88** (0.20 eq.), Et_3N (3.00 eq.) toluene	RT 18 h →24 h	63%
4	Cu(I)I($PO(Et)_3$) **88** (0.20 eq.), DIPEA (0.10 eq.) MeOH *__55__ (1.10 eq.)	RT 18 h	76%

Entry 2 (**Table 2.3**) employs reagents and conditions more suited to apolar reactants.[136] As an alternative to copper sulfate, catalyst **88** was used. **88** was synthesised from copper iodide **86** and triethylphosphite **87** (**Scheme 2.18**, (ii)) and stored *in vacuo* due to its hygroscopic nature.[137] With these conditions solubility was improved and the yield increased by over 20%. Microwave irradiation was deemed unnecessary when the same set of conditions gave a similar yield at room temperature (**Entry 3**, **Table 2.3**), although the lower temperature may have had a small negative effect on solubility. With the yield moving in the right direction the solvent was considered in more detail. Pleasingly, modifying the literature procedure by switching the solvent from toluene to methanol increased the yield of **89** to a reliable 76% (**Entry 4**, **Table 2.3**).[136] The reaction did not need to be rigorously anhydrous and these conditions became the conditions of choice for all future CuAAC reactions.

2.3.5 Establishing debenzylation conditions to afford target compounds 41 and 42

Having synthesised the fully-protected fluorescent DDM analogues **85** and **89**, the final step of the synthesis was expected to be a straightforward debenzylation (**Scheme 2.20**). The benzyl groups were originally chosen for their ease of removal - a hydrogenolysis with catalytic palladium under an atmosphere of hydrogen usually affords a product that requires little, if any, purification. However, this deprotection proved much more complicated and compounds **85** and **89** exhibited a remarkable resistance to palladium-catalysed hydrogenolysis. A number of conditions were screened for their ability to debenzylate these compounds and are summarised in **Table 2.4**.



Scheme 2.20: Debenzylation to give target compounds **41** and **42**. A screen of conditions is provided in **Table 2.4**.

Table 2.4: Screen of debenzylation conditions for the synthesis of **41** and **42**. The arrows indicate how certain conditions were altered over the course of a single reaction in attempts to drive the reaction to completion.

Entry	Substrate	*Reagents & Conditions*	Comments
1	**89**	H_2, Pd/C (0.60 → 1.00 eq.) MeOH/EtOAc/AcOH, RT, 36 h	• Little reaction • Some mono-debenzylation (MS & TLC) • Starting material recovered
2	**85**	H_2, Pd black (3.00 eq.) MeOH, RT, 48 h	• Little reaction • Some mono-debenzylation (MS & TLC) • Starting material recovered
3	**89**	H_2, $Pd(OH)_2$, (0.60 → 2.40 eq.) MeOH/EtOAc, RT, 16 h → 36 h	• Little reaction • Some mono-debenzylation (MS & TLC) • Starting material recovered
4	**89**	H_2, Raney® Nickel (excess) EtOH/H_2O, RT, 24 h	• No reaction • Starting material recovered
5	**89**	H-Cube®, Pd/C cartridge MeOH/EtOAc, 40 °C, 1 mL min^{-1}	• No reaction • Starting material recovered
6	**85**	H_2, $Pd(OH)_2$ (20% w/benzyl group) MeOH/EtOAc, 5 atm., 24 h	• Signs of decomposition after 24 h • Amine **84** visible (MS & TLC)
7	**89**	H_2, Pd/C (20% w/benzyl group) MeOH/EtOAc, 5 atm., 24 h → 72 h	• No reaction • Starting material recovered
8	**89**	BBr_3 (6.10 eq.) CH_2Cl_2, −78 °C, 3 min	• Starting material consumed • Evidence of significant debenzylation • Over-reaction, complex crude mixture • **42** visible (MS) but not isolated
9	**85** **89**	$NaBrO_3$ (15.60 eq.), $Na_2S_2O_3$ (14.00 eq.) EtOAc/H_2O, RT, 3 h	• Starting material consumed • Fluorescence lost (TLC) • Loss of dansyl and benzyls • Starting material consumed • Fluorescence lost (TLC) • Loss of dansyl and benzyls

The simplest conditions for debenzylation were assessed first – the substrates were stirred under an atmosphere of hydrogen in the presence of either palladium on carbon, palladium black or palladium hydroxide (**Entries 1**, **2** & **3** respectively, **Table 2.4**). In all of these cases minimal reactivity was observed despite literature precedent for similar debenzylations on sugars containing lipid side chains and even triazoles.[138,139,140,141] Increasing the reaction time, the equivalents of catalyst or applying heat (not shown) had no effect on the progress of the reaction. Solvent changes were also investigated, taking into account the huge difference in polarity between the starting materials and the products. An atmospheric hydrogenolysis with a non-palladium based catalyst, namely Raney® Nickel, was also tried without success (**Entry 4**, **Table 2.4**).

There was some concern that the exposed amines in either the dansyl group, the triazole or the sulfonamide were poisoning the catalyst so the use of a flow system, in the form of an H-Cube®, was investigated as a potential solution (**Entry 5**, **Table 2.4**). However, no reaction at all was observed and only starting material was recovered.

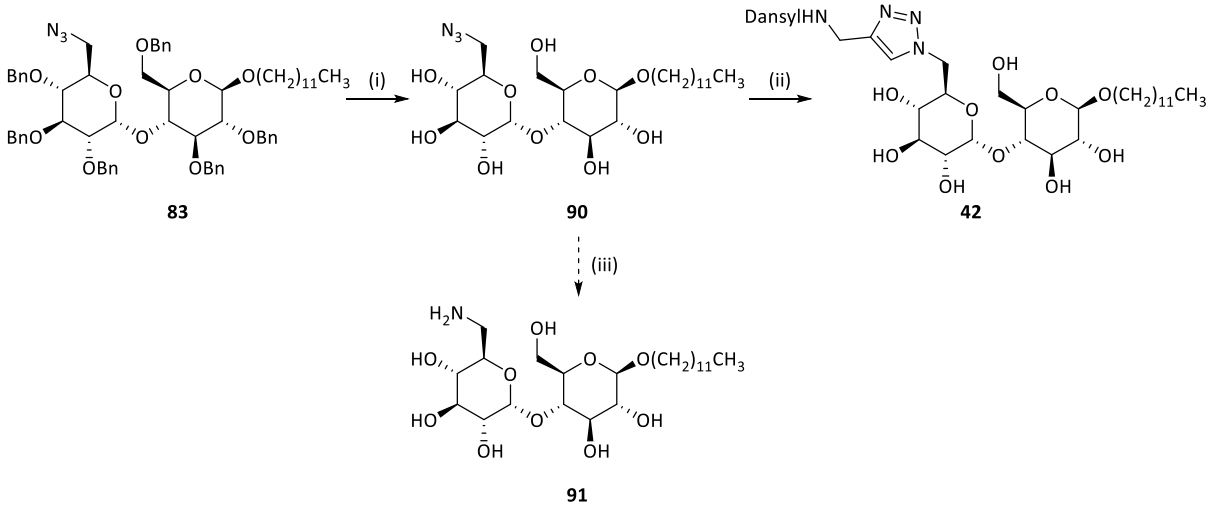
High pressure conditions were employed in the search for harsher hydrogenolysis conditions (**Entries 6** & **7**, **Table 2.4**). **85** and more equivalents of palladium hydroxide were stirred under five atmospheres of hydrogen, however, this proved too harsh for the sulfonamide. Cleavage of the S-N bond was observed, with amine **84** and an unidentified dansyl adduct readily visible by MS and TLC. No debenzylation was observed. In contrast, **89** appeared to be stable at high pressure to the extent that no reaction was observed at all and once again starting material was recovered.

With metal-catalysed hydrogenolyses consistently proving unfruitful, a diversion was required. Boron tribromide had previously been used to rapidly remove benzyl groups so this seemed like a promising alternative (**Entry 8**, **Table 2.4**).[142] Pleasingly, **89** appeared consumed after three minutes when stirred with boron tribromide at −78 °C and **42** was visible in the MS. Unfortunately, TLC analysis indicated that a substantial number of polar by-products were present in the reaction mixture and it was therefore impossible to ascertain which spot might be the desired product. Attempted isolation of **42** *via* column chromatography was unsuccessful suggesting the presence of only a trace amount of this material. It was clear that boron tribromide had reacted with other parts of the substrate in addition to cleaving the benzyl groups. Despite failing to isolate **42**, it was encouraging to see **89** react at all. Two extremes for the debenzylation had therefore been established, metal-catalysed hydrogenolysis was too mild and reaction with boron tribromide too harsh.

An intermediate methodology was required and a paper by Iadonisi *et al*. looked like it might provide a solution.[143] The work pioneered the use of sodium bromate and sodium dithionite to cleave benzyl ethers on monosaccharaides in the presence of a variety of protecting groups,[143,144] capitalising on the observation by Ishii *et al*. that sodium bromate in the presence of a reducing agent can be used to alpha-brominate alkyl benzenes.[145] Further work by Kong *et al*. used Iadonisi's method to debenzylate a monobenzylated trisaccharide with a lipid chain at the 1-position in good yield.[146] Both substrates reacted under these conditions with the starting material being consumed. However, following UV analysis of the crude TLC, it was apparent that there were no fluorescent products present in the crude reaction mixture suggesting that the dansyl group had undergone decomposition (**Entry 9**, **Table 2.4**).

The additional aromatic environments in substrates **85** (dansyl) and **89** (dansyl and triazole) were potentially causing problems during the debenzylation as evidenced by the loss of the

characteristic fluorescence of the dansyl groups by TLC analysis. It therefore became necessary to consider the possibility of carrying out the bromate/dithionite debenzylation before addition of the dansyl group. Of course, focusing on target **42**, this could only be after functionalisation of the 6' alcohol leaving mesylate **82** and azide **83** as potential substrates. A paper by Unverzagt *et al*. provided the confidence required to opt for the azide.[147] So, retreating to azide **83**, it was pleasing to see that it reacted with sodium bromate and sodium dithionite, finally affording the debenzylated compound **90** in 49% yield (**Scheme 2.21**, (i)). Whilst lower yielding than literature reports, the yield was deemed acceptable given the previous failures and the dubious solubility of the product. No oxidation of the primary 6-position alcohol was observed by MS, sodium bromate and sodium hydrogen sulfate had been shown to oxidise primary alcohols in the original work by Ishii *et al*.[148] With azide **90** in hand, the CuAAC was performed. **90** dissolved well in methanol and target compound **42** was isolated in 70% yield (**Scheme 2.21**, (ii)).



Scheme 2.21: Debenzylation of azide **83**, in advance of dansylation. *Reagents & conditions*: (i) **83** (1.00 eq.) $NaBrO_3$ (15.60 eq.), $Na_2S_2O_4$ (14.00 eq.), EtOAc/H_2O, RT, 3 h, 49%; (ii) **90** (1.00 eq.), **55** (1.10 eq.), Cu(I)IP(OEt)$_3$ **88** (0.05 eq.), DIPEA (0.10 eq.), MeOH, RT, 18 h, 70%; (iii) **90** (1.00 eq.), PPh_3 (1.50 eq.), H_2O, THF, RT.

Time constraints and the limited amount of material available meant that azide **90** was not converted into amine **91** *via* a Staudinger reaction in order to pursue target compound **41**. It

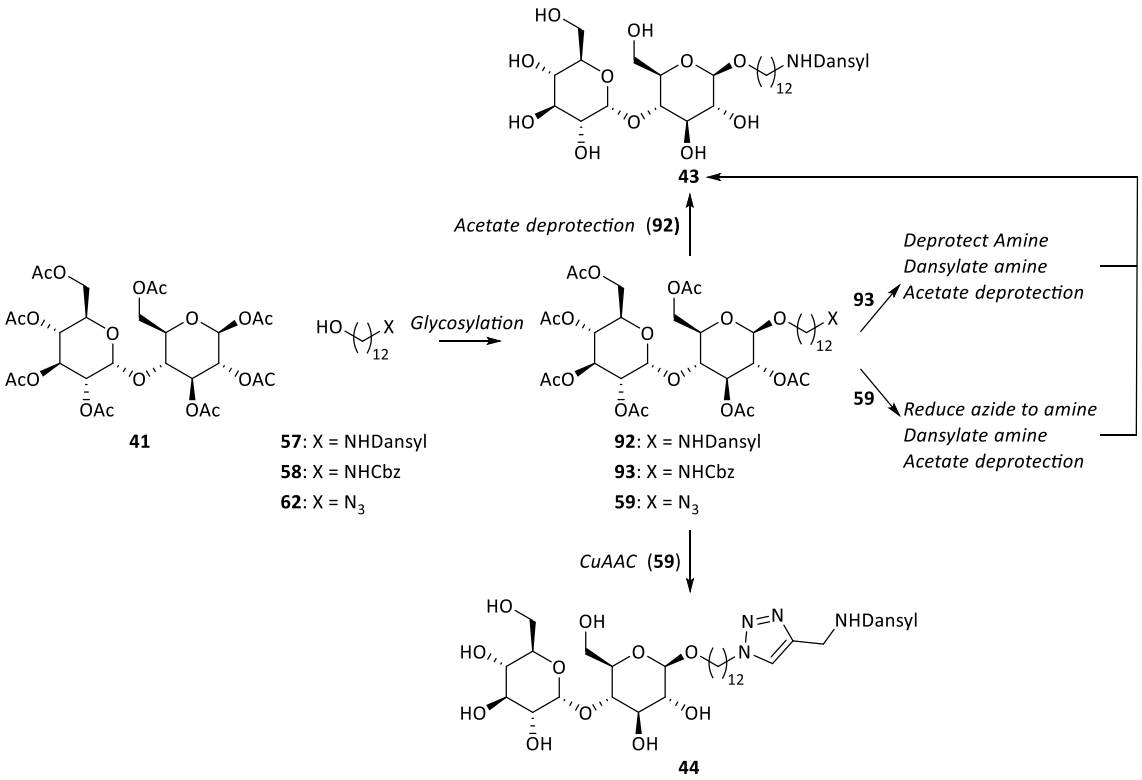
was also anticipated that the dansylation reaction between the amine **91** and dansyl chloride **53**, which was routinely carried out in dichloromethane, would be complicated by the reduced solubility conferred upon **91** by the amine and the six hydroxyl groups. A suitable solvent would need to be found for a reaction that already had a reputation for being low yielding, efforts therefore focused on the 1,2,3-triazole linked compounds, the synthesis of which had already proved more reliable.

2.3.6 Synthesis of fluorescent DDM analogues with the fluorophore at the end of the lipid chain

Attaching a fluorophore at the end of the lipid chain of DDM **1** required glycosylation of a dodecan-1-ol (with some form of desirable functionality at the 12-position) by β-D-maltose octaacetate **41** (**Scheme 2.22**). The alcohols chosen herein were **57**, **58** and **62**, possessing a dansyl group, a Cbz-protected amine or an azide respectively. The Cbz-protected species **58** was chosen simply because it also found application in some of the work discussed in **Chapter 4**. The three products of such a glycosylation **92**, **93** and **59** could be further manipulated, according to **Scheme 2.22**, to afford the sulfonamide-linked target compound **43** and, in the case of azide **59**, also the 1,2,3-triazole-linked target compound **44**.

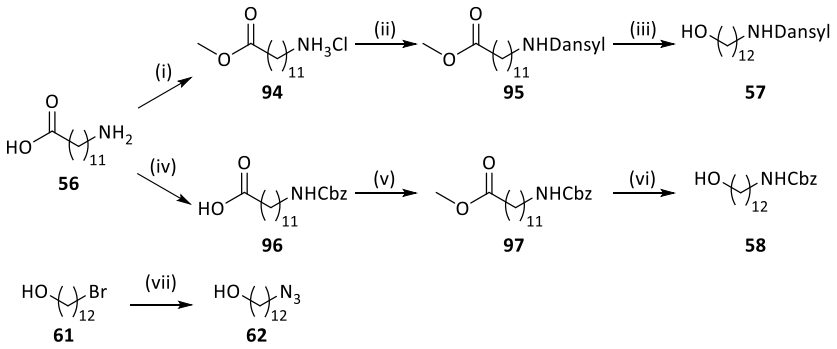
In order that these approaches be possible, it was necessary to synthesise the glycosyl acceptors **57**, **58** and **62** (**Scheme 2.23**). A combination of literature procedures allowed the three-step syntheses of **58** and **59** from 12-aminododecanoic acid **56**.[149,150] All steps proceeded in good yields with the exception of the Cbz protection of **56** (**Scheme 2.23**, (iv)). The poor solubility of **56** was deemed to be the problem in this aqueous reaction, however, the conditions were not optimized as the reaction had been carried out on a large enough

scale that plenty of **96** was isolated. The alcohol functionality was achieved *via* a hydride reduction of the relevant methyl esters **95** and **97** and proceeded without affecting either the dansyl group or the Cbz protecting group (**Scheme 2.23**, (iii) & (vi) respectively).[151]



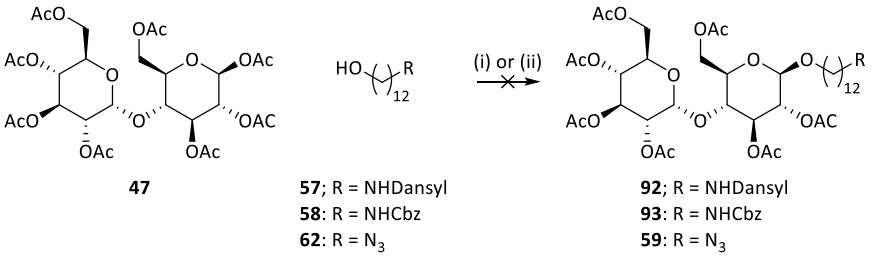
Scheme 2.22: Approaches to functionalisation of the end of the lipid chain in pursuit of target compounds **43** and **44**.

The third alcohol, 12-azidododecanol **62**, was synthesised from 12-bromododecanol **61** *via* straightforward displacement of the bromide with sodium azide (**Scheme 2.23**, (viii)), as in the synthesis of azide **83** (**Scheme 2.17**). This reaction also proceeded in good yield but was difficult to monitor due to the starting material and product possessing the same R_f and exhibiting poor sensitivity to ESI MS.



Scheme 2.23: Synthesis of glycosyl acceptors **57**, **58** and **62**. *Reagents & conditions*: (i) **56** (1.00 eq.), $SOCl_2$ (2.22 eq.), MeOH, 0 °C → RT, 10 h, 89%; (ii) **94** (1.00 eq.), DABCO (2.20 eq.), dansyl chloride **53** (1.20 eq.), CH_2Cl_2/DMF, 0 °C → RT, 18 h, 81%; (iii) **95** (1.00 eq.), $LiAlH_4$ (4.00 eq.), THF, 0 °C → RT, 4 h, 90%; (iv) **56** (1.00 eq.), benzyl chloroformate (1.10 eq.), $NaHCO_3$ (1.50 eq.) H_2O, RT, 18 h, 23%; (v) **96** (1.00 eq.), $SOCl_2$ (2.20 eq.), MeOH, 0 °C → RT, 1.5 h, 75%; (vi) **97** (1.00 eq.), $LiAlH_4$ (4.00 eq.), THF, 0 °C → RT, 1 h, 90%; (vii) **61** (1.00 eq.), NaN_3 (1.20 eq.), DMF, 60 °C, 8 h, 86%.

Despite the efforts expended synthesising the glycosyl acceptors, they all failed to react with maltose octaacetate **47** under glycosylating conditions (**Scheme 2.24**, (i) or (ii)). Both of the Lewis acids, zinc chloride (seen in **Scheme 2.10**) and boron trifluoride diethyletherate, failed to promote the reaction.



47

57; R = NHDansyl
58: R = NHCbz
62: R = N_3

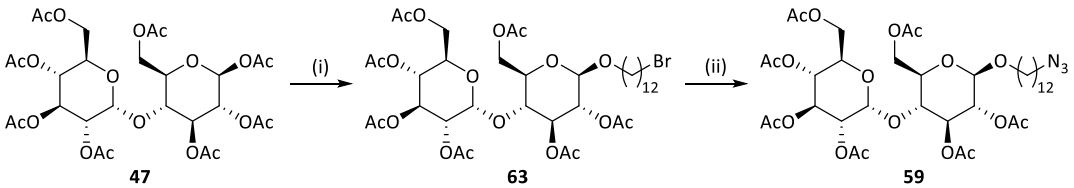
92; R = NHDansyl
93: R = NHCbz
59: R = N_3

Scheme 2.24: Alcohols **57**, **58** and **62** were unreactive towards glycosylation. *Reagents & conditions*: (i) **47** (1.00 eq.), **57**/**58**/**62** (1.50 eq.), $ZnCl_2$ (1.50 eq.), toluene, 60 °C, 18 h; (ii) **47** (1.00 eq.), **57**/**58**/**62** (2.00 eq.), $BF_3{\cdot}OEt_2$ (1.50 eq.), CH_2Cl_2, RT, 18 h.

Despite some literature precedent for successful glycosylations in the presence of Cbz-protected amines and exposed azides,[152,153] TLC and MS showed that only small amounts of acylated alcohol (as observed in **Scheme 2.10** with dodecanol **48**) and starting material were present. It is difficult to explain the failures. Many of the literature examples use alcohols with shorter carbon chains so perhaps the lipophilicity of a twelve carbon chain has implications on solubility and thus reactivity. Further factors could include coordination of nitrogen atom lone pairs in the substrates to the Lewis acid catalyst or the poor nucleophilicity of the alcohols as

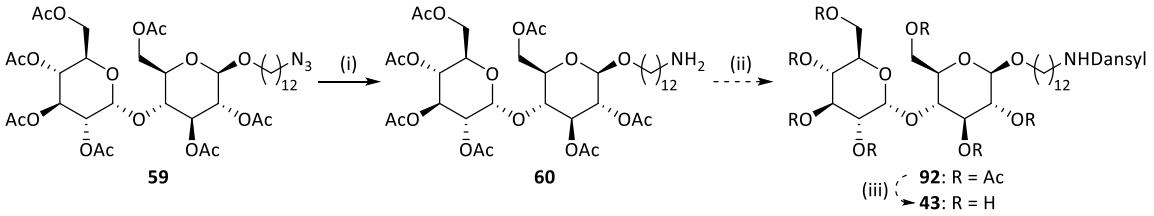
a result of functionalising the 12-position with electron-withdrawing groups. Indeed, some literature examples that use glycosyl acceptors with exposed azides first activate the glycosyl donor as either a trichloroacetimidate or a phenylthioglycoside.[110,115] It is, however, debatable that the electron-withdrawing groups here could exert a significant effect along a chain of twelve carbon atoms.

Scans of the literature found examples of glycosylations where chloro- or bromoalcohols constituted the glycosyl acceptors, many of which were subsequently treated with azide to substitute the halide atoms.[116,115] 12-Bromododecanol **61** was available having been used to synthesise azide **62** and was therefore employed as a forth glycosyl acceptor. In fact, this particular glycosylation had already been reported and occurred successfully, affording **63** in 48% yield (**Scheme 2.25**, (i)).[117] 12-Bromododecanol **61** was thought to be slightly unstable, appearing to breakdown on TLC and turning increasingly orange and "clumpy", despite being stored in the fridge under argon. The slightly poor, but acceptable yield, was attributed to this instability. The bromide **63** was converted to an azide **59** in excellent yield *via* reaction with sodium azide (**Scheme 2.25**, (ii)). Fortunately, since the azide **59** can also be converted into an amine **60**, in addition to being a suitable substrate for CuAAC, it is a common intermediate in the syntheses of both target compounds **43** and **44**.



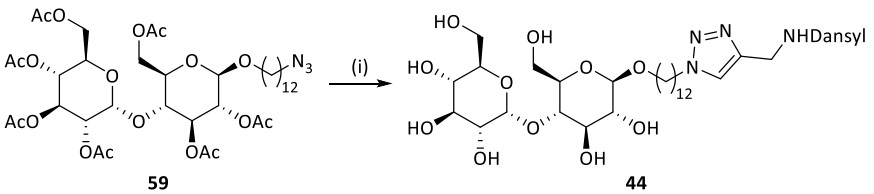
Scheme 2.25: Synthesis of azide **59** using 12-bromododecanol **61**. *Reagents & conditions*: (i) **47** (1.00 eq.), 12-bromododecanol **61** (2.00 eq.), $BF_3{\cdot}OEt_2$ (1.50 eq.), CH_2Cl_2, RT, 1 h, 48%; (ii) **63** (1.00 eq.), NaN_3 (3.00 eq.), DMF, 60 °C, 18 h, 89%.

As above, in order to obtain the fluorescent sulfonamide-linked species **43** it was necessary to convert the azide **59** to an amine **60**, again using Staudinger conditions (**Scheme 2.26**, (i)). This reaction proceeded in good yield, however, subsequent dansylation to give **92** was unsuccessful. The reasons behind this, third, poor dansylation were frustratingly unclear, however, the proposed steric hindrance of the amine (put forward in **Section 2.3.3**) could not be responsible.



Scheme 2.26: Attempted synthesis of target compound **43** *via* conversion of azide **59** into amine **60**. *Reagents & conditions*: (i) **59** (1.00 eq.), PPh_3 (1.50 eq.), H_2O/THF, RT, 16 h, 71%; (ii) **60** (1.00 eq.), dansyl chloride (1.50 eq.), DABCO (1.50 eq.), CH_2Cl_2, RT, 24 h, no product observed; (iii) **92** (1.00 eq.), NaOMe (1.25 eq.), CH_2Cl_2/MeOH, RT, 10 min.

The CuAAC conditions established in **Section 2.3.4** were applied to azide **59** in order to attach the 1,2,3-triazole-linked dansyl group (**Scheme 2.27**, (i*a*)). The reaction proceeded smoothly once it was realised that the basic methanolic conditions of the CuAAC were causing partial acetate deprotection. Since the subsequent and final step was a global acetate deprotection, this unexpected reactivity was in fact beneficial. Addition of sodium methoxide to the crude reaction solution thus allowed *in situ* completion of the acetate deprotection affording target compound **44** in 73% yield over two steps (**Scheme 2.27**, (i*b*)).

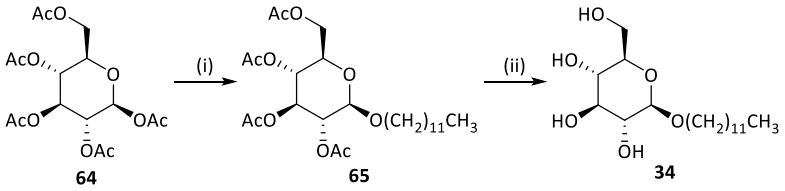


Scheme 2.27: Synthesis of target compound **44** *via* a CuAAC. *Reagents & conditions*: (i) *a*) **59** (1.00 eq.), **55** (1.10 eq.), **88** (0.05 eq.), DIPEA (0.10 eq.), MeOH, RT, 2 h; *b*) NaOMe (1.50 eq.), MeOH, RT, 6 h, 73% over 2 steps.

2.3.7 Synthesis of a fluorescent DG analogue with the fluorophore at the 6-position

As proposed in **Section 2.2**, only fluorescent DG analogues with 1,2,3-triazole-linked fluorophores were synthesised in the first instance. Despite not being the main detergent of interest these targets were expected to provide useful data in relation to whether these compounds might still form micelles. They were also expected to be slightly easier to synthesise than their DDM counterparts. Attachment of the fluorophore to the 6-position of DG **34** was actually achieved *via* two routes, both of which had been unachievable with DDM **1**. The first step, however, was to synthesise DG **34** itself, since it happens to be more expensive and less widely available than DDM **1** (**Scheme 2.28**).

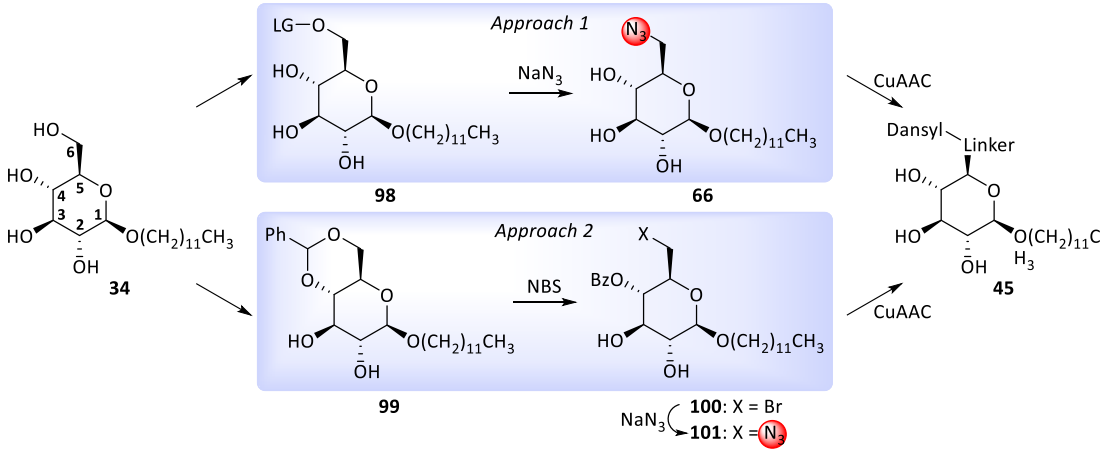
As with DDM **1**, the synthesis began by using β-D-glucose pentaacetate **64** to glycosylate dodecanol-1-ol **48**, affording **65** in a yield that is poor, yet comparable to that achieved in the literature (**Scheme 2.28**, (i)).[154] The poor yield can again be attributed to formation of side products such as acylated dodecan-1-ol **70**. **65** was deacetylated using standard Zemplén conditions affording DG **34** in excellent yield as a single anomer (**Scheme 2.28**, (ii)).



Scheme 2.28: Synthesis of DG **34**. *Reagents & conditions*: (i) **64** (1.00 eq.), dodecan-1-ol (1.20 eq.), $BF_3{\cdot}OEt_2$ (1.50 eq.), CH_2Cl_2, RT, 3 h, 38%; (ii) **65** (1.00 eq.), NaOMe (0.70 eq.), MeOH/CH_2Cl_2, RT, 50 min, 97%.

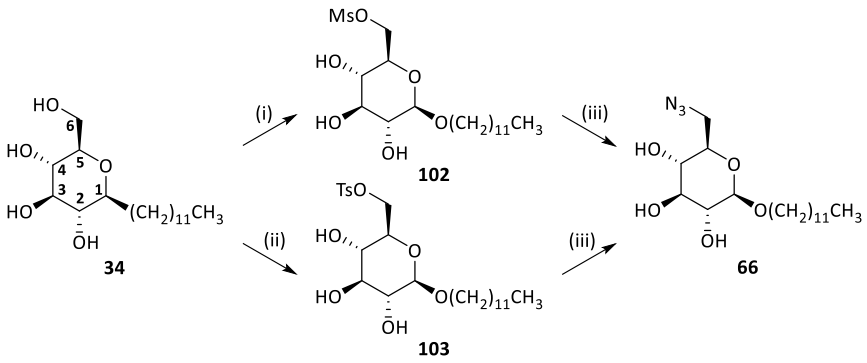
With a stock of DG **34** in hand, functionalisation of the 6-position as an azide, the prerequisite species for a CuAAC, commenced. Two potential approaches were considered and these are summarised in **Scheme 2.29**. This first approach involved activation of the 6-position alcohol with a leaving group to give **98**, followed by its displacement with azide to give **66** (*Approach 1*, **Scheme 2.29**). This had failed with DDM **1** due to its poor solubility and the presence of two

primary alcohols. It was hoped that, in this case, the greater inherent nucleophilicity of the primary 6-position alcohol over the three secondary alcohols at the 2-, 3- and 4-positions would be sufficient to govern the regioselectivity. Were this not to be the case, indirect functionalisation of the 6-position *via* the 4,6-benzylidene to give **99** formed the basis of the second approach (*Approach 2*, **Scheme 2.29**). This would allow regioselective access to bromide **100** and subsequently azide **101**.



Scheme 2.29: Outline of the two approaches in pursuit of target compound **45**. *Approach 1* includes direct functionalisation of the 6-position with a leaving group (**98**), whilst *approach 2* employs a 4,6-benzylidene (**99**) to regioselectively access the 6-position. LG = leaving group.

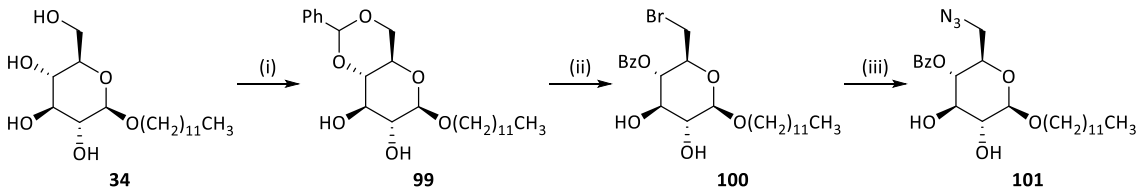
Approach 1, the shorter of the two, was investigated first. It was hoped that the 6-position alcohol could be activated with a leaving group *via* treatment with one of two electrophiles, either methanesulfonyl chloride or the more sterically demanding 4-toluenesulfonyl chloride. It was anticipated that the greater inherent nucleophilicity of the 6-position alcohol relative to the remaining three secondary alcohols would be sufficient to govern the regioselectivity, affording either **102** or **103**. A literature procedure for the facile conversion of DG **34** to the 6-*O*-tosyl species **103** in biphasic conditions provided a promising starting point in pursuit of azide **66**.[154] However, despite multiple attempts, no reactivity was observed under these conditions and it was therefore decided to revert to the conditions first used in **Section 2.3.3** to activate alcohol **78** through mesylation or tosylation (**Scheme 2.30**).



Scheme 2.30: Direct functionalisation of the 6-position of DG **34**. *Reagents & conditions*: (i) **34** (1.00 eq.), MsCl (1.00 eq.), Et_3N (1.10 eq.), CH_2Cl_2, RT, 1 h, 31% by ^1H NMR; (ii) **103** (1.00 eq.), TsCl (1.10 eq.), Et_3N (1.10 eq.), CH_2Cl_2, RT, 6 h, 37%; (iii) **102** or **103** (1.00 eq.), NaN_3 (1.10 eq.), DMF, 60 °C, 18 h, 61% from **102** or 52% from **103**.

Unfortunately, these conditions were less successful with DG **34**. **102** and **103** were isolated but yields were low and the reactions themselves unreliable, with most attempts affording no desired product at all (**Scheme 2.30**, (i) & (ii)). Taking the mesylation as an example (**Scheme 30**, (i)), it was clear from MS and TLC that the regioselectivity was poor with mono-, di- and tri-mesylated species visible. Each of these components showed the expected decrease in polarity as more alcohols were masked, however, the differences between their R_f values were small making purification of the desired product difficult and further impacting on the yield. The yield for the mesylation (with the smaller electrophile) was slightly lower than that for the tosylation and mesylate **102** could not isolated entirely cleanly from the close-running by-products. Attempts to improve regioselectivity by decreasing the temperature failed as the reactions stalled at 0 °C. The subsequent reaction of **102** or **103** with sodium azide proceeded in reasonable yield, affording azide **66** (**Scheme 2.30**, step (iii)).

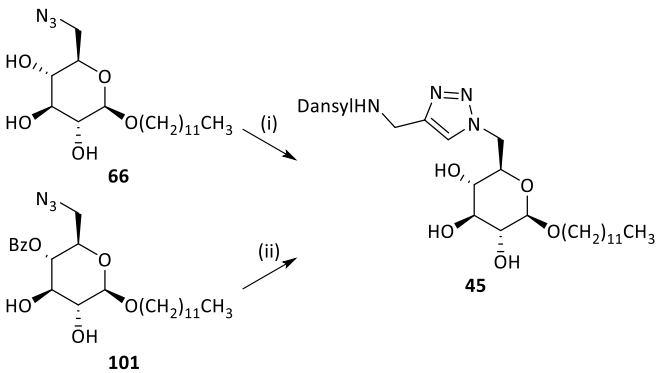
With yields for direct functionalisation as either the mesylate or the tosylate fairly low, *approach 2*, involving initial formation of the 4'6'-benzylidene **99**, was also investigated (**Scheme 2.31**). This three-step approach (that had also failed with DDM **1** (**Section 2.3.2**)) was expected to eliminate problems relating to regioselectivity, which had arisen in the direct functionalisation of *approach 1*.



Scheme 2.31: Synthesis of azide **101** *via* benzylidene **99**. *Reagents & conditions*: (i) **34** (1.00 eq.), $PhCH(OMe)_2$ (3.00 eq.), camphorsulfonic acid (0.12 eq.), DMF, 60 °C, 300 mbar, 4 h, 68%; (ii) **99** (1.00 eq.), NBS (1.40 eq.), $BaCO_3$ (2.60 eq.), CCl_4, reflux, 4 h, 51%; (iii) **100** (1.00 eq.), NaN_3 (1.10 eq.), DMF, 60 °C, 18 h, 89%.

DG **34** was reacted with benzaldehyde dimethyl acetal to afford 4,6-benzylidene **99** in good yield (**Scheme 2.31**, (i)). The benzylidene was then treated with *N*-bromosuccinimide in the presence of barium carbonate, which successfully opened the 6-membered ring to give **100**, converting the 6-position exclusively to a bromide and leaving a benzoyl protecting group on the 4-position alcohol (**Scheme 2.31**, (ii)). The yield for this transformation was reasonable yet pleasing, given the previous failures with DDM **1**. As with the previous approach, the 6-position was converted to an azide *via* reaction with sodium azide, giving **101** in excellent yield (**Scheme 2.31**, (iii)). Of course, a benzoyl group remained on the 4-position alcohol, however, with similar properties to that of an acetate protecting group, it was hoped that this group would also be cleaved *in situ* during the CuAAC.

66 and **101** were subsequently subjected to the now familiar CuAAC conditions affording target compound **45** in 100% and 42% yields respectively (**Scheme 2.32**). **Table 2.5** provides the overall yields for the isolation of target compound **45** *via* the two approaches starting from DG **34**.



Scheme 2.32: A CuAAC of **66** and **101** to afford target compound **45**. *Reagents & conditions*: (i) **66** (1.00 eq.), **55** (1.10 eq.), **88** (0.05 eq.), DIPEA, MeOH, RT, 2-4 h; 100%; (ii) *a*) **101** (1.00 eq.), **55** (1.10 eq.), **88** (0.05 eq.), DIPEA, MeOH, RT, 2-4 h; *b*) NaOMe (1.50 eq.), MeOH, RT, 1 h, 42% over 2 steps.

According to **Table 2.5** neither approach appears particularly better yielding than the other for the synthesis of target compound **45**. However, for unknown reasons, the second approach suffered from an uncharacteristically low yielding CuAAC. Had this not been the case, this approach would undoubtedly have prevailed as the more efficient of the two considering the unreliability and poor regioselectivity observed during the reactions of DG **34** with either methanesulfonyl or 4-toluenesulfonyl chloride.

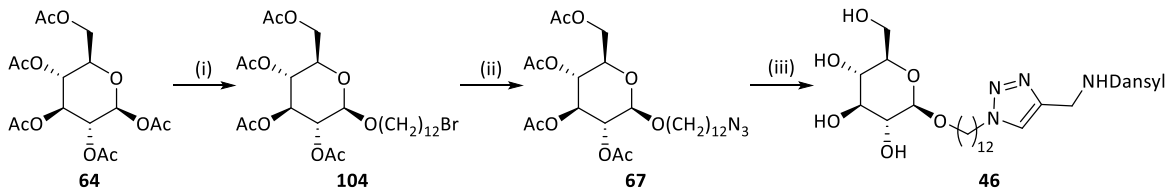
Table 2.5: Overall yields for the two approaches (*via* intermediates **102**, **103** and **99**) in pursuit of target compound **45**.

Approach	Yield	Steps
1 (**34** → **102** → **66** → **45**)	18%	3
1 (**34** → **103** → **66** → **45**)	18%	3
2 (**34** → **99** → **100** → **101** → **45**)	16%	5

2.3.8 Synthesis of a fluorescent DG analogue with the fluorophore at the end of the lipid chain

With the experience gained from the synthesis of the other target compounds (particularly that of **44** in **Section 2.3.6**), the synthesis of the final target **46**, DG with a 1,2,3-triazole-linked dansyl group at the end of the lipid chain, was the most straightforward of all. The route is provided in **Scheme 2.33** and is identical to that of target compound **44**.

A glycosylation of 12-bromododecanol **61** with β-D-glucopyranoside **64** afforded **104** in 43% yield as a single anomer. The yield was low but comparable to that observed for the same reaction in the literature.[117] The bromide was displaced by azide to give **67** in 90% yield and a subsequent CuAAC, accompanied by *in situ* acetate deprotection, gave target compound **46** in excellent yield over two steps.



Scheme 2.33: Synthesis of target compound **46**. *Reagents & conditions*: (i) **64** (1.00 eq.), 12-Bromododecanol **61** (2.00 eq.), $BF_3{\cdot}OEt_2$ (1.10 eq.), CH_2Cl_2, RT, 5 h, 43%; (ii) **104** (1.00 eq.), NaN_3 (3.00 eq.), DMF, 60 °C, 18 h, 90%; (iii) *a*) **67** (1.00 eq.), **55** (1.10 eq.), **88** (0.05 eq.), DIPEA (0.10 eq.), MeOH, RT, 6 h; *b*) NaOMe (2.00 eq.), MeOH, RT, 2 h, 93% over 2 steps.

2.4 Biophysical data

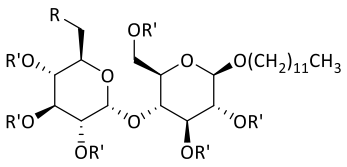
Compounds **42**, **44**, **45** and **46** were submitted to the Robinson group for initial biophysical testing, however, the results are yet to be obtained because of problems associated with the functioning of the laser. When the facilities allow, the compounds will be dissolved in a buffer solution (usually sodium acetate in water) and subjected to ESI within the MS machine in order to assess whether micelle formation is possible. Positive results from this initial screen would see the compounds progress to MS studies with membrane proteins in order to investigate the initial hypothesis of whether fluorescent detergents can give clearer MS data by laser-induced dissociation from the proteins of interest.

2.5 Conclusions

This chapter describes the successful syntheses of a number of novel fluorescent detergents, compounds **42**, **44**, **45** and **46**, which may find application in membrane protein mass spectrometry. All of these compounds possess a dansyl fluorophore **12** attached to the detergent at either the 6'- or 6-position of the sugar ring (in DDM **1** or DG **34** respectively) or at the end of the lipid chain. In all cases, the fluorophore was attached *via* a 4-aminomethyl-1,2,3-triazole linker **39**, which was achieved through a CuAAC. This linker was

in fact one of three originally proposed linkers, however, the above discussion details how it became the main linker when efforts to attach the dansyl group *via* sulfonate ester **37** or sulfonamide **38** linkers were consistently low yielding.

A number of challenges were met during the pursuit of the novel 6'-position DDM derivatives in particular. After screening a number of conditions, detailed in **Section 2.3.2**, it was particularly pleasing to establish reliable conditions for the regioselective reductive ring-opening of benzylidene **77** to give alcohol **78** (Figure 2.12). The search for debenzylation conditions in **Section 2.3.5** also proved a challenge, however, when the bromate/dithionite conditions provided the required breakthrough, a reliable method for the debenzylation of functionalised DDM derivatives was established. Unexpectedly, azide **90** became a key intermediate in the synthesis of target compound **42**. Although there was neither time nor enough material to convert azide **90** into another key intermediate, amine **91**, it was believed that this conversion would be straightforward providing opportunities for further derivatisation.



78: R = OH, R' = Bn
90: R = N_3, R' = H
91: R = NH_2, R' = H

Figure 2.12: Milestone compounds in the synthesis of 6'-position DDM derivatives.

2.6 Future Work

Of course, immediate future work will pursue the important initial biophysical data. Once the required data has been obtained it will be possible to assess whether the properties of the detergents have been adversely affected by addition of the dansyl fluorophore. Should the dansyl fluorophore prove unsuitable, milestone compounds such as azide **90** and amine **91**

represent key intermediates to which alternative fluorophores (and perhaps linkers) could be attached in order to tune the activity for future investigations (**Figure 2.12**).

3. Small molecule probes targeting the Potassium Efflux Channel, Kef

Kef has been identified as a promising therapeutic target for the development of new antibiotics with novel modes of action. Herein, the history of Kef, including its structure, proposed mechanism of action and existing probes, will be discussed in support of this claim (**Section 3.1**). Work towards new probes of Kef activity will be based on this discussion. The synthesis of these probes, as well as some initial work evaluating biological activity, will be discussed in **Section 3.3**.

3.1 Introduction

3.1.1 The importance of potassium ion transport within bacteria

In bacteria, potassium ions (K^+) play a vital role in regulating cell turgor and cytoplasmic pH.[155,156] As such, their transport back and forth across the cell membrane is tightly regulated by a number of complementary transmembrane and cytoplasmic proteins. Central to the regulation of intracellular K^+ is a common protein fold called the potassium transport and nucleotide-binding (KTN) domain, linking uptake systems, channels and efflux systems.[37] Many bacterial K^+ efflux channels have been identified and characterised in the literature.[157] The most well studied is probably KcsA, the existence of which was first reported by Schrempf *et al.* in 1995. Its X-ray crystal structure, the first of a K^+ channel, was subsequently reported by Doyle *et al*. in 1998.[158,159] KcsA has since become an important tool for studying gating mechanisms and provides information on how these channels selectively conduct K^+ through the membrane.[160,161]

3.1.2 The function of Kef within *E. coli*

Another well-studied K^+ channel, which is part of a KTN-regulated system, is the bacterial potassium efflux channel, Kef. KefC, found with its ancillary subunit KefF in *E. coli*, is one such example.[37,162] Kef is a ligand-gated K^+/H^+ antiporter with homologues in the majority of Gram negative pathogens. It is implicated in the protection of bacterial pathogens against toxic electrophiles, such as methylglyoxal **105**, through modulation of intracellular pH, as outlined in **Figure 3.1**.[163,164,165,166]

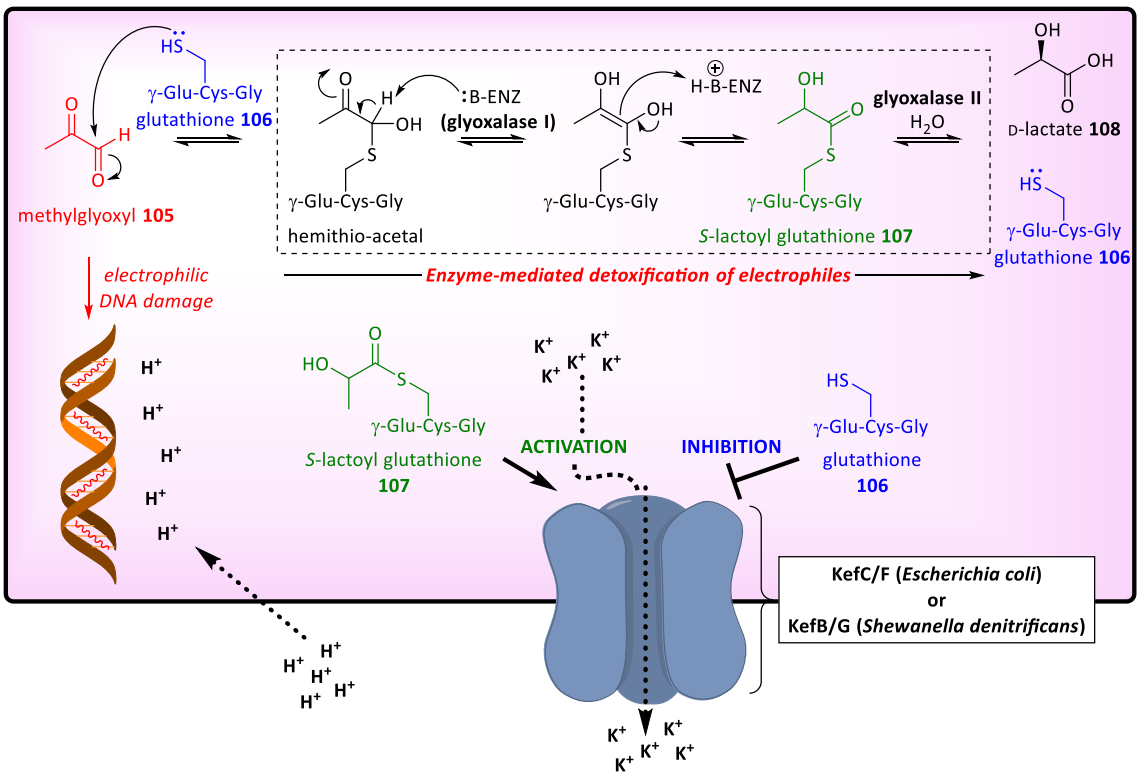
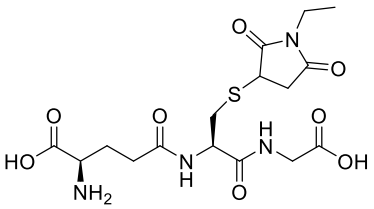


Figure 3.1: Cartoon representation of how Kef activity contributes to the acid-mediated detoxification of DNA-damaging electrophiles *via* potassium efflux and concomitant proton influx, which culminates in acidification of the cell cytoplasm.[169]

Kef is inhibited by the ubiquitous tripeptide glutathione (GSH) **106**, which binds in a mode that holds the channel in a closed conformation, thereby preventing K^+ efflux. GSH **106** also behaves as a scavenger of toxic electrophiles (including methylglyoxal **105**) forming glutathione-*S*-conjugates (GSXs), such as *S*-lactoyl glutathione (SLG) **107**, which are subsequently degraded *via* enzyme-mediated catalysis. GSXs, even at low concentrations in comparison to GSH **106**, have been shown to interact with Kef in a manner that causes channel activation. The outcome is rapid K^+ efflux and concomitant acidification of the cell cytoplasm by proton influx.[164,166] The resultant decrease in pH is thought to cause protonation of nucleophiles contained within the DNA, thereby blocking sites that would otherwise react with those toxic electrophiles that are yet to be degraded.[165,163]

3.1.3 A proposed mechanism of activation for Kef

An *apo* structure of the soluble C-terminal KTN-domain of KefC and its ancillary subunit KefF was first published by Roosild *et al*. in 2009.[37] Subsequently, the GSX, *S*-(*N*-ethyl)succinimido glutathione (ESG) **108**, which had already been identified as a strong activator of KefC (**Figure 3.2**), was put into crystallisation trials in collaboration with Roosild *et al*. and an X-ray crystal structure of this compound bound to KefC was obtained. It provided a rigid snap-shot of the channel in its open, activated form (**B, Figure 3.3**).[162] An analogous structure of KefC in complex with GSH **106**, which could only be achieved by *in situ* TCEP-promoted reduction of ESG **108** within the binding site, depicted the channel in its closed form (**A**, **Figure 3.3**). It should be noted that since the time of this publication, the accuracy regarding the positioning of the (*N*-ethyl)succinimido ring of ESG **108** within the reported crystal structure has been questioned, it is therefore interpreted with caution in the following discussion.[167] The mutational studies, which appear in the same paper, were not subject to such debate. They can therefore be interpreted with greater confidence and support the proposed gating mechanism.



108

Figure 3.2: The structure of ESG **108**, a GSX and activator of KefC.

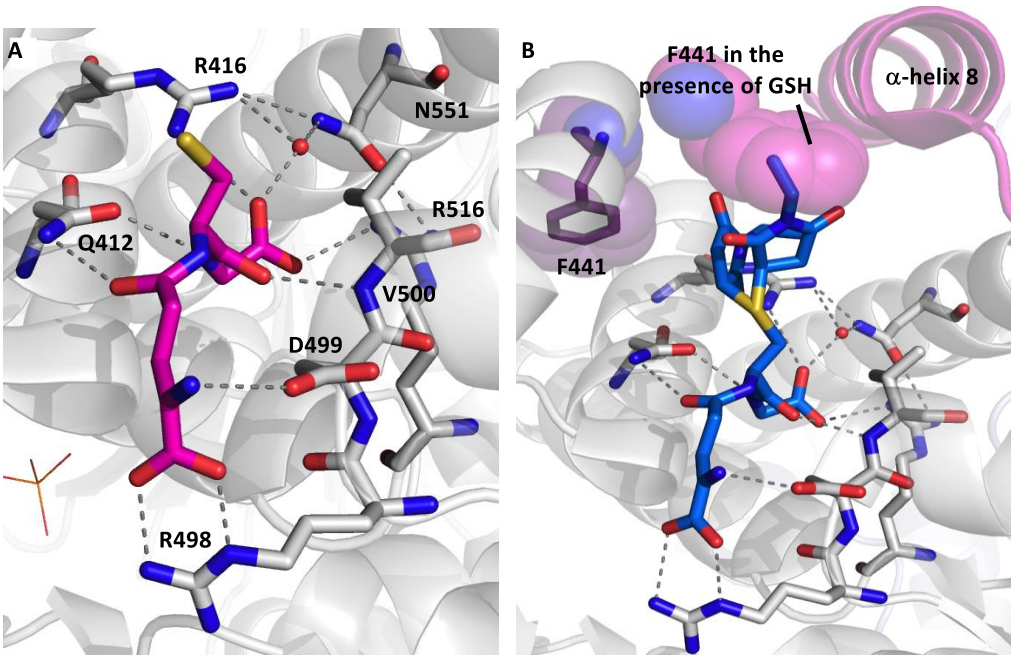


Figure 3.3: X-Ray crystal structures of ligand-bound KefC from *E. coli*.[162] **A**: GSH **106**, an inhibitory ligand, bound to KefC with the key contacts highlighted. **B**: ESG **108**, an activatory ligand, bound to KefC, the same key contacts observed with GSH **106** are apparent and a large displacement of the F441 residue is highlighted.

ESG **108** contains the same peptidic backbone as GSH **106** and this backbone can be seen to form the same interactions with KefC in each of the crystal structures. In both cases, the *α*-carboxylic acid of *γ*-glutamic acid can been seen to be interacting with an arginine residue (R498), whilst the *α*-amine interacts with an aspartic residue (D499). At the glycine end of the tripeptides, the carboxyl interacts with two further arginine residues (R416 & R516) and a water-mediated interaction with an asparagine residue (N551) is also visible. Serial mutation and functional analysis of the D499 and Q412 residues helped to support the accuracy with which these binding interactions are portrayed in the *in vivo* binding of GSH **106** to KefC. In advance of the discussed crystal structures, previous mutational analysis had also alluded to the importance of the R416, R516 and N551 residues within the binding site.[168,37]

With the binding interactions of the terminal amino acids established, and appearing to overlap, the X-ray crystal structure of ESG **108** bound to KefC allowed a mechanism of channel activation to be postulated. The thiol of GSH **106** does not appear to be involved in binding.

However, a steric clash between the large (*N*-ethyl)succinimido substituent of ESG **108** and F441, the phenylalanine residue capping the hydrophobic pocket at the binding site, is thought to cause F441 to reposition. This movement is proposed to evoke a significant conformational change within the protein. It is this change that may be the root of channel activation, allowing F441 to be likened to a single amino acid 'switch' helping to gate Kef. Of course, with the recent debate, it must be stressed that this is merely a proposed mechanism.

Since an X-ray crystal structure provides only a static picture of a ligand-bound protein and considering the recent debate, it was fortunate that the published work also included some mutagenesis studies, which assessed the importance of the F441 residue in gating. Results were gathered by observing the effect of certain mutations on ESG-promoted K^+ efflux through KefC (**Figure 3.4**).[162] Switching the phenylalanine for similarly-sized hydrophobic residues such as tryptophan (F441W) and tyrosine (F441Y) was well tolerated with no significant loss of activity. However, gating was progressively reduced for leucine and aspartic acid mutations (F441L and F441D respectively) suggesting the flexibility of these residues limits their effectiveness as triggers for conformational change. These results support the proposal that F441 plays a critical role as a sensor for GSXs and as a trigger for channel activation, although more work is necessary before a definitive mechanism can be put forward.

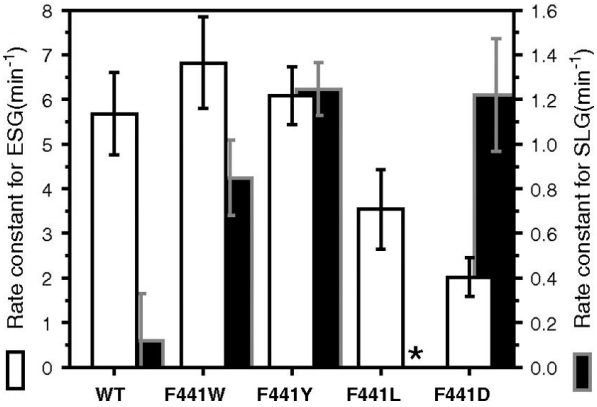


Figure 3.4: Reproduced from Rooslid *et al*.[162] Rate constants (mean and standard deviation) for K^+ efflux of WT KefC and a series of mutants in the presence of the activating GSXs, ESG **108** and SLG **107**.

3.1.4 Investigating the structural requirements for molecular activation of the Kef system

With the knowledge that GSH **106** and ESG **108** bind at overlapping sites, recently published collaborative work by the Conway group sought to further investigate the structural requirements necessary for activation of Kef.[169] At this time, the affinities of GSH **106** and ESG **108** were unknown, however, it was important to acknowledge that the high intracellular abundance of GSH **106** (~10-20 mM) could potentially hinder the binding of any potential drug candidate in the future. It would therefore be necessary to pursue activatory ligands with a much higher affinity for Kef than GSH **106**.

With a view to developing a competition fluorescence assay capable of affording both qualitative and quantitative detection of binding to Kef, a novel solvatochromic fluorescent probe and GSX, *S*-(3-[*N*-dansyl]aminopropyl) glutathione (DNGSH) **109**, was designed and synthesised by Healy *et al*.[169] For this study, it is important to note that KefB from *Shewanella dentrificans* (SdKefB) was used instead of KefC from *E. coli*. The latter could only be isolated from *E. coli.* as the KefF/C dimer, which was found to be unstable in extracts thereby interfering with previous biochemical assays.[37] Homologues were sought by Healy *et al*. and it was found that KefB from *Shewanella dentrificans* had high sequence homology with KefC from *E. coli* at the binding site, remained stable without its ancillary protein KefG and was, therefore, better suited for use in biophysical assays.[169] Across the full length of the protein, SdKefB was found to have 64% sequence homology with KefC from *E. coli*.[169]

The fluorescence properties of DNGSH **109** were shown to reflect changes in the hydrophobicity of its experienced environment (**Figure 3.5**). In aqueous solution, free DNGSH **109** was found to exhibit a low intensity fluorescence with a λ_{max} at 570 nm but, when bound to Kef, the fluorescence increased in intensity and shifted to a λ_{max} of 535 nm. Healy

et al. were therefore able to develop an assay whereby the ability of a ligand to compete DNGSH **109** out of the binding site could be quantified by the observed change in the fluorescence intensity. When DNGSH **109** is displaced from the binding site, by a ligand with a higher affinity for Kef, its fluorescence is quenched by exposure to the surrounding aqueous media.

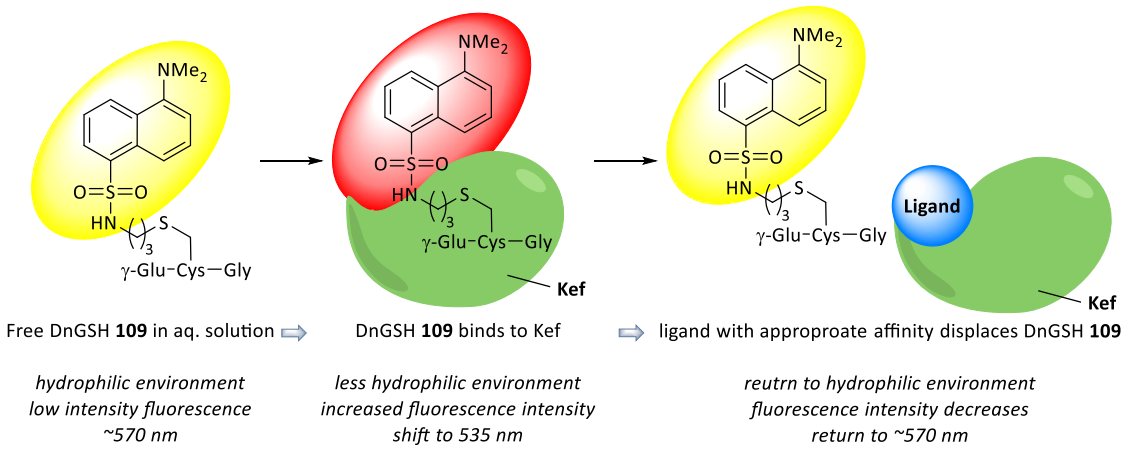


Figure 3.5: Cartoon representation of the novel competition fluorescence assay developed by Healy *et al*.[169]

As a result of this behaviour, a decrease in fluorescence intensity is observed, as shown in **Figure 3.6** for an assay with the high affinity ligand ESG **108**. The magnitude of this decrease can provide quantitative information regarding the affinity of a variety of structurally diverse ligands and therefore, in combination with isothermal titration calorimetry (ITC) and differential scanning fluorimetry (DSF), the structural requirements for activation of Kef. ITC and DSF are explained in greater detail below (**Section 3.1.5**).

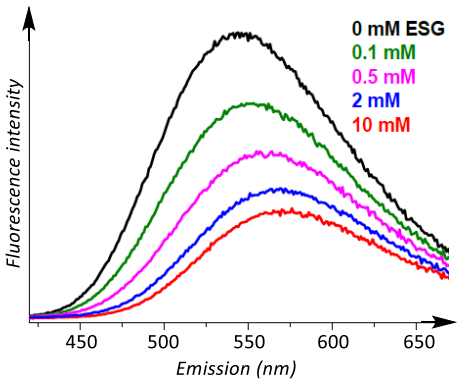


Figure 3.6: Changes in the intensity of the emission spectra of DNGSH **109** following the addition of different concentrations of ESG **108**. [Kef] = 47 µM and [DNGSH **109**] = 112 µM.

Analysis of competition fluorescence assays with DNGSH **109** and ESG **108**, GSH **106** or SLG **107** allowed their dissociation constants, K_d, to be determined. K_d is a measure of how tightly a ligand binds to a protein and is given in molar units, which correspond to the concentration of the ligand required such that 50% of binding sites are occupied. A lower K_d is thus indicative of a higher affinity. The results confirmed that, as predicted, ESG **108** (K_d = 12 ± 3 µM) has a much higher affinity for Kef than GSH **106** (K_d = 900 ± 200 µM) allowing it to bind to Kef in the

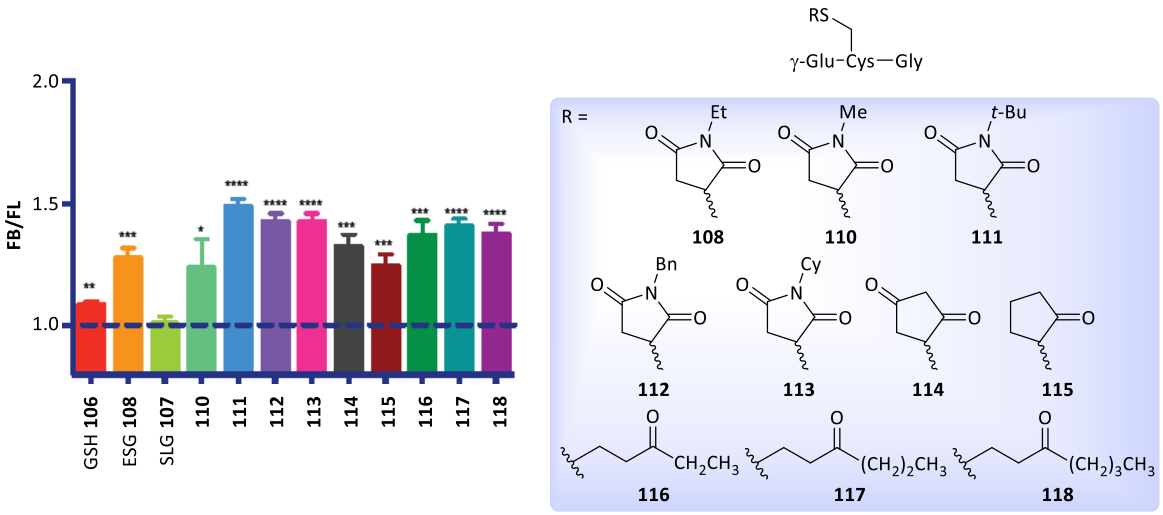


Figure 3.7: A: Modified from Healy *et al*.[169] Competition fluorescence data for ESG analogues **110**-**118**, performed with 1 mM of the competing ligand. B: Structures of ESG analogues **110**-**118**.

presence of a high intracellular concentration of GSH **106**. SLG **107** (K_d = 900 ± 200 µM), a mild activator of Kef, was found to have a similar affinity to GSH **106**.

Since established activatory and inhibitory ligands provided the expected results in the competition fluorescence assay, the binding of a series of ESG analogues, compounds **110**-**118**, was investigated. The results from Healy *et al*. are reproduced in **Figure 3.7**, where the binding of a particular ligand is represented by $DNGSH_{bound}/DNGSH_{ligand}$ or FB/FL, which is the ratio of the fluorescence intensity of DNGSH **109** bound to Kef before ($DNGSH_{bound}$) and after ($DNGSH_{ligand}$) the addition of a competing ligand. A larger ratio, resulting from a significant decrease in fluorescence intensity following addition of a ligand, therefore

indicates a ligand has a higher affinity than DNGSH **109** for Kef.[169] All of the ESG analogues afforded high FB/FL ratios consistent with a higher affinity than GSH **106** for Kef, *S*-(*N*-*t*-butyl)succinimido glutathione **111** showed the highest affinity of all and thermal shift data (using DSF) correlated well with the results for all analogues.

To assess whether the compounds were able to activate Kef, a whole cell K^+ efflux assay, using the necessary electrophiles for *in vitro* formation of compounds **110**-**118**, followed. In comparison to ESG **108**, adducts containing rigid bulky side chains such as **111** and **112** caused significant K^+ efflux, compounds with rigid but substantially less bulky side chains such as **114** and **115** caused little or no K^+ efflux and those with flexible side chains such as **116** and **117** caused significantly impaired K^+ efflux (data not shown).

The paper reached two main conclusions, that; (i) alone, a high affinity for Kef is insufficient for activation and (ii) there must be a simultaneous requirement for steric bulk *and* structural rigidity within the side chain for efficient activation. Coupled with the mutagenesis studies mentioned previously, this certainly seemed to suggest that rigid, steric bulk in close proximity to the F441 residue within the binding site is closely linked with channel activation.

3.1.5 Methods for assessing ligand binding and activity of KefC

Section 3.1.4 has already detailed the competition fluorescence assay, developed by Healy *et al*., which provides both a quantitative and qualitative measure of the affinity of a particular ligand for Kef.[169] The straightforward whole cell K^+ efflux assay, which provides an indication of Kef activation, has also been mentioned.[162] However, it is important to corroborate data with more than one method.

A thermal shift assay, also known as differential scanning fluorimetry (DSF), was used above to support the results from the competition fluorescence assay. DSF assess the ability of a ligand to stabilise a protein, which can be an indication of how well a ligand binds and is popular because it requires little protein and can provide results rapidly. Appropriately folded soluble proteins gradually unfold with increasing temperature, exposing otherwise hidden hydrophobic regions and eventually resulting in protein aggregation. When a ligand binds to a protein, it can potentially stabilise the protein's native state resulting in an increased melting temperature (T_m) (**Figure 3.8**).

The difference in melting temperature between the native and ligand-bound protein, ΔT_m, can allow determination of ligand binding affinity constants. The assay is commonly run with SYPRO® orange dye, although other dyes can be used. The fluorescence properties of SYPRO® orange change as it interacts with an unfolding protein.[170] Its fluorescence is quenched in the aqueous environment of a correctly folded solubilised protein, however, as the protein unfolds, exposing its hydrophobic core, the fluorescence is no longer quenched. The midpoint of the protein melting curve (the T_m value) increases in the presence of ligands that bind more tightly to the protein's native state, indeed, similar assays have shown that the stabilising effect of a bound ligand is proportional to the concentration and affinity of the ligand.[171]

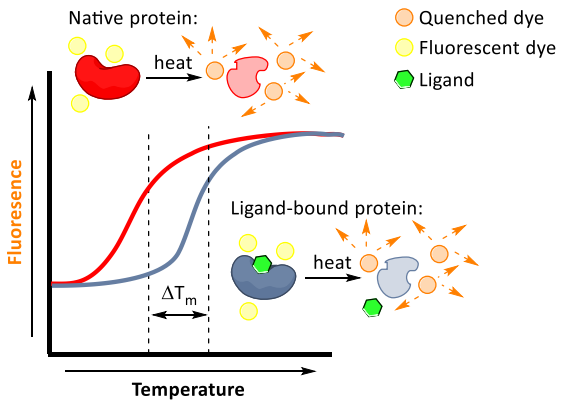


Figure 3.8: Cartoon representation of a thermal shift assay. The magnitude of ΔT_m, the difference in melting temperatures between a native protein and its ligand-bound form, can give an indication of ligand affinity.[170]

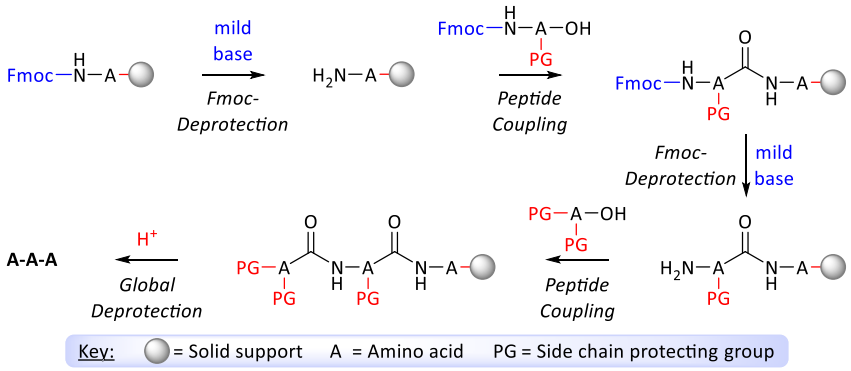
Isothermal titration calorimetry (ITC), which assesses binding through detection of small temperature changes on addition of increasing amounts of ligand to a protein, has already been used by Healy *et al*. to provide association constants for the binding of ligands to Kef. [169] The results from an ITC experiment tend to correlate with those from techniques such as DSF. ITC is useful because it relies only on detection of the heat changes that occur on addition of a ligand to a protein, that is to say, it does not require the development of a specialised substrate for detection. The ligand scope is therefore broad, provided the heat change is large enough to be measured. The problem with ITC is that it requires substantial amounts of protein and is therefore not always used to assess binding in the first instance.

Other techniques which may be appropriate for assessing the efficiency of protein-ligand binding are surface plasmon resonance (SPR), fluorescence polarisation (FP), alpha screen and NMR techniques such as saturation transfer difference NMR (STD-NMR), some of which are compared in a paper by Rossi and Taylor.[172] Optimisation of the protocols for these techniques in the specific case of Kef is still underway by other members of the Conway group, as such they are therefore beyond the scope of this project, for now.

3.1.6 Peptide synthesis

Due to the pivotal role they play in biological and pharmaceutical research, improving the synthesis of peptides (of which, GSH **106** and ESG **108** are examples) has been a major focus for organic chemists since the first successful coupling of two amino acids was reported by Fischer and Otto over a century ago.[173] Peptide synthesis had made huge advances since their acid chloride-mediated coupling reactions.

For a long time, the huge number of necessary couplings, deprotections, isolations and purifications meant the solution-phase assembly of long peptides or small proteins was a laborious process, often made more difficult by the solubility issues surrounding the synthesis of such structures. A revolutionary approach, pioneered by Merrifield and first published in 1963, soon changed this.[174] His idea to assemble peptides on a solid support, 'Merrifield Resin', was so successful that he was awarded the Nobel Prize for his work in 1984 and consequently, the field of solid phase peptide synthesis (SPPS) was born. A number of different resins have since been developed, offering better solvation behaviour and higher mechanical



Scheme 3.1: The key principles of Fmoc-SPPS, illustrated for the synthesis of the generic tripeptide, **A**-**A**-**A**.

stability.[175,176] Today, commercially-available resins are generally modified by appropriate handles allowing C-terminal amino acid residues to be tethered to a solid support *via* either an ester or amide bond, which is cleaved at the end of the synthesis.

The development of protecting groups compatible with milder chemistry also afforded significant advances in SPPS. With the advent of the Fmoc group, cleavable under mildly basic conditions, Fmoc-SPPS gained in popularity (**Scheme 3.1**). [177,178] Switching the dominant *α*-amine protecting group from an acid-labile one to a base-labile one, such as Fmoc, allowed a rearrangement of the overall protecting group strategy. All side chain protecting groups and the handle anchoring the peptide to the resin were incorporated as acid-labile moieties,

thereby limiting exposure to strongly acidic reagents (or hydrogen fluoride in the case of cleavage from Merrifield resin) to a single, global deprotection at the end of the synthesis. There are now many books and reviews on Fmoc-SPPS.[179,180]

In addition to the developments in SPPS, there have been huge advances in the chemistry of peptide bond formation itself. Traditional coupling methods such as diimide-based activation with DCC **119** and CDI **120,** or anhydride-mediated couplings, have largely been replaced by a plethora of complex but remarkably efficient phosphonium-, uronium- and pyridinium-based coupling reagents (**Figure 3.9**).[181,182,183,184,185,186,187] The scope of these coupling reagents, and others, has been discussed at length in a number of reviews.[188,189]

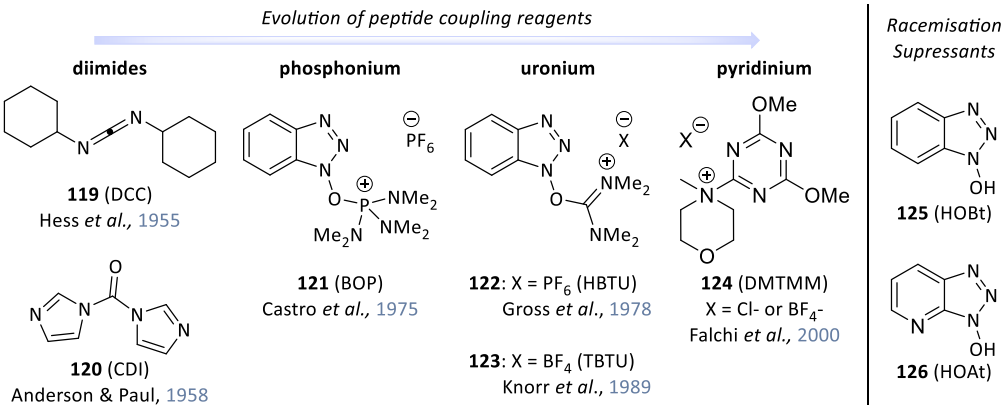


Figure 3.9: Some of the common peptide coupling reagents discovered over the past 60 years, illustrating the increasing complexity of the structures.[181,183,184,185,186,187] The structures of two common racemisation suppressants are also provided.

Many of the modern coupling reagents help limit racemisation of the α-carbon centres during a peptide coupling. However, it is also common to add a racemisation suppressant such as HOBt **125** or HOAt **126** to maintain chiral integrity and such additives have also been found to enhance reactivity (**Figure 3.9**).[190,191] The choice of base to accompany a coupling has also been well studied with Carpino *et al*. publishing work that illustrates how switching from

di*iso*propylethylamine to a more sterically hindered base such as collidine reduces the degree of racemisation in certain peptide couplings.[192,193]

3.1.7 Kef as a promising therapeutic target?

To conclude, the importance of Kef in protecting bacteria against the effects of toxic electrophiles has been discussed. Various mutagenic studies have alluded to the importance of both a ligand containing a sterically demanding, rigid side chain and the F441 residue within the binding site in channel activation and work towards elucidation of the precise mechanism of activation is ongoing.[162,168,169] X-Ray crystal structures of KefC bound to either the inhibitory ligand GSH **106** or the activating ligand ESG **108** have also been discussed, although it has since been reported that these structures should be interpreted with caution.[162,167]

Solid phase peptide synthesis of GSH **106** and ESG **108** analogues may provide the means with which to further investigate the requirements for channel activation and, once a mechanism is established, it may be possible to design novel drug candidates targeting Kef. Agonists of Kef activity will promote prolonged K^+ efflux, which will adversely affect both the pH of the cytoplasm and cell turgor. Alternatively, antagonists will hold channels closed, preventing K^+ efflux and the concomitant H^+ efflux that is vital for the detoxification of DNA-damaging electrophiles within bacteria. Regardless of how far along this path this project progresses, the discussed biological assays will provide a means through which proposed ligands can be assessed for binding and activity against Kef.[169] The proposed ligands and the reasons for their selection are discussed in more detail in the following section.

3.2 Aims

To further support the observations that the F441 residue within the ligand binding site and ligands with bulky side chains are important for activation of Kef, three tripeptides were identified as the initial synthetic targets. These tripeptides were broadly similar to GSH **106**, but the central cysteine residue was substituted for the more sterically demanding amino acid residues of phenylalanine, tryptophan and tyrosine affording tripeptides **127**, **128** and **129** respectively (**Figure 3.10**).

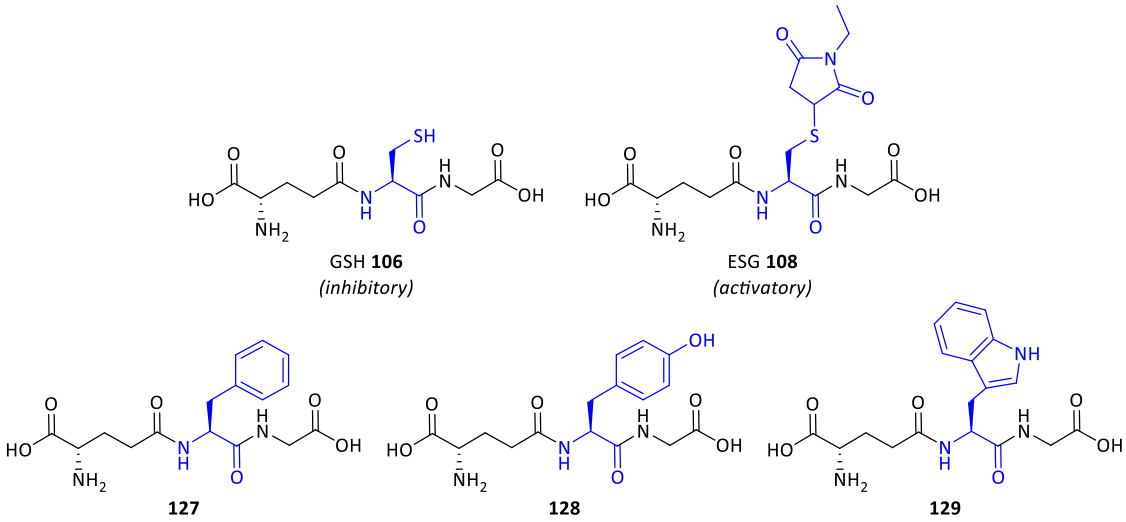


Figure 3.10: Structure of the tripeptides **127**, **128** and **129** proposed as GSH mutants. The sterically demanding central amino acid residues are highlighted in blue.

The abilities of GSH **106** and ESG **108** to behave respectively as inhibitory or activatory ligands of Kef activity was outlined in the previous section. Additionally, mutational analysis of the F441 residue within the ligand binding site (of KefC from *E. coli*) suggested that a sterically demanding residue at this position was important for channel activation, substitution of F441 for smaller residues saw decreased ESG-evoked K^+ efflux (**Section 3.1.3**). Herein, the structural requirements for channel activation would be further investigated by adopting an inverse, but complimentary, approach to the functional mutagenic studies. 'Chemical mutations' to the inhibitory ligand GSH **106**, namely the switching of the central cysteine residue to the more

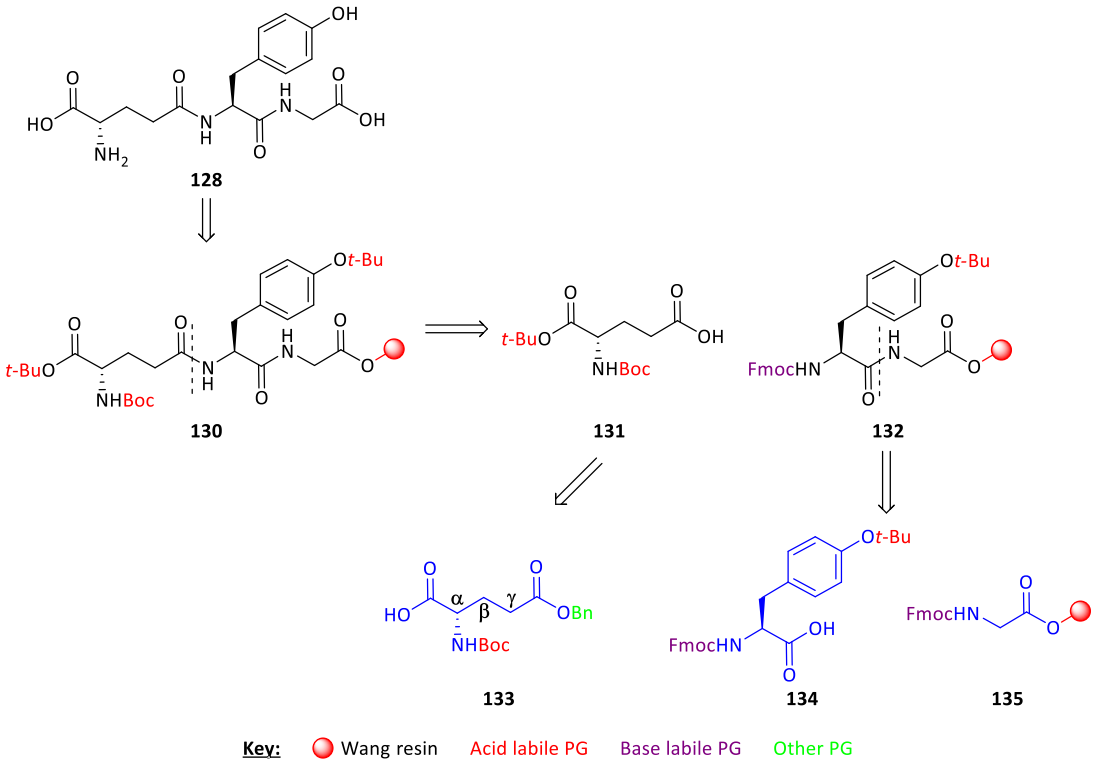
sterically demanding residues outlined above, were proposed in order to investigate whether this affected the ability of a ligand to bind to and inhibit Kef. If the hypothesis that channel activation is promoted by a steric clash between F441 and a bulky side chain is correct, then these compounds might be expected to evoke the same steric clash with the single amino acid 'switch' of F441 as the (*N*-ethyl)succinimido moiety of ESG **108** and subsequently cause channel activation. More specifically, it might be envisioned that switching the central cysteine residue of GSH **106** to, for example, phenylalanine, might afford a tripeptide (**127**) that is able to activate Kef.

The tripeptides were to be synthesised *via* Fmoc-SPPS as this represented a rapid, robust and well-established approach to such compounds, as described in **Section 3.1.6**. Subsequently, it would be necessary to determine whether the three tripeptides possessed the ability to bind to Kef. Thermal shift would provide an indication of the affinity with which these compounds bind by allowing the ability of **127**, **128** and **129** to stabilise KefB from *Shewanella dentrificans* to be compared relative to the established ligands GSH **106** and ESG **108** ($K_{d\ (GSH)}$ = 900 ± 200 µM and $K_{d\ (ESG)}$ = 12 ± 3 µM, by fluorescence).[169] The competition fluorescence assay described in **Section 3.1.4** is a further option for establishing how well these ligands bind Kef and would allow K_d values to be determined.[169]

3.3 Results and Discussion

3.3.1 Retrosynthetic analysis of proposed compounds

The tripeptides were synthesised *via* Fmoc-SPPS as this method represented a robust and established technique for the rapid synthesis of such frameworks.[194,195] A retrosynthetic analysis of **128** (*γ*-Glu-Tyr-Gly) is provided in **Scheme 3.2**.

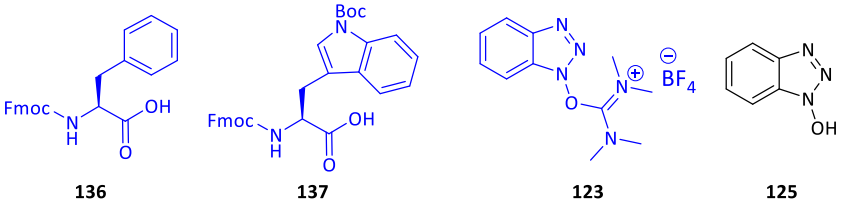


Scheme 3.2: Retrosynthetic analysis of *γ*-Glu-Tyr-Gly **128**. Commercially available compounds are shown in blue.

In accordance with traditional Fmoc-SPPS the final immobilised compound **130** possessed only acid labile protecting groups. These were present on the glutamic acid residue and, if appropriate, the side chain of the central amino acid residue. Additionally, **130** was immobilised onto the solid support, here Wang resin, *via* an acid labile bond.[196] Disconnecting **130** at the *γ*-Glu-Tyr peptide bond revealed the appropriate glutamic acid fragment, Boc-L-glutamic acid 1-*tert*-butyl ester **131**, and the Fmoc-protected immobilised dipeptide **132**.

Boc-L-glutamic acid 1-*tert*-butyl ester **131** could be synthesised from commercially available **133** – a relatively cheap glutamic acid derivative with an acid labile Boc group on the amine and a benzyl group, which is neither acid nor base labile, on the *γ*-carboxylic acid. In the forward synthesis, addition of the acid labile protecting group to the *α*-carboxylic acid followed by removal of the protecting group on the *γ*-carboxylic acid would give the desired fragment in two straightforward steps, provided the reaction conditions were tailored to minimise racemisation of the stereogenic *α*-carbon centre.

The Fmoc-protected immobilised dipeptide **132** could be disconnected at the Tyr-Gly peptide bond affording the two remaining amino acid fragments. Again, in accordance with Fmoc-SPPS, these were the commercially available Fmoc-(*O*-*tert*-butyl)-tyrosine **134,** with an acid labile side chain protecting group, and Fmoc-glycine immobilised onto Wang resin **135**. For the other two tripeptides, *γ*-Glu-Phe-Gly **127** and *γ*-Glu-Trp-Gly **129**, the central amino acids required were commercially available Fmoc-phenylalanine **136** and Fmoc-($N_{(ind)}$-Boc)-tryptophan **137** respectively (**Figure 3.11**).



136 **137** **123** **125**

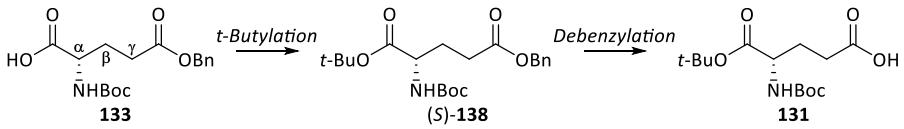
Figure 3.11: The commercially available central amino acid fragments required for the synthesis of *γ*-Glu-Phe-Gly **127** (**136**) and *γ*-Glu-Trp-Gly **129** (**137**) and the structures of the coupling reagent TBTU **123** and racemisation suppressor HOBt **125**.

With the identity of the amino acid fragments established, the syntheses became straightforward. Piperidine in DMF removes Fmoc-protecting groups and a plethora of conditions could be called upon to effect a peptide coupling.[188,189] Commonly, a combination of the two amino acids, in the presence of a coupling reagent (e.g. TBTU **123**), a racemisation suppressor (such as HOBt, **125**) and a mild base (e.g. di*iso*propylethylamine) in DMF, is

sufficient (**Figure 3.11**). With the immobilised tripeptides assembled, an acidic cleavage cocktail would release the crude compounds, which could then be purified, to afford the desired target compounds.[197,198]

3.3.2 Synthesis of the required glutamic acid fragment

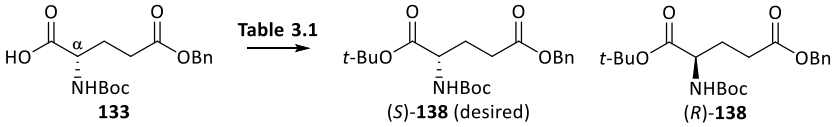
Boc-L-glutamic acid 1-*tert*-butyl ester **131** was identified above as the necessary glutamic acid fragment to form a peptide bond to phenylalanine (or tyrosine/tryptophan) through its *γ*-carboxylic acid. Whilst **131** was commercially available, it was expensive, so it was decided to instead synthesise it from an analogue **133** (**Scheme 3.3**). The debenzylation step (hydrogenolysis in the presence of palladium on carbon) was expected to be trivial. However, the *t*-butylation required some consideration as it was desirable to establish conditions that minimised racemisation at the stereogenic *α*-carbon centre, giving the desired (*S*)-enantiomer of compound **138**.



Scheme 3.3: A *t*-butylation and subsequent debenzylation were proposed as a route to the required glutamic acid fragment **131**.

A number of conditions were assessed for their ability to add a *t*-butyl group to the *α*-carboxylic acid without causing racemisation at the *α*-carbon (**Scheme 3.4**). Chiral HPLC was used as a means of visualising racemisation and therefore required a racemic form of **138** to be synthesised (compound **142**, **Section 3.3.3**). The results of the chiral HPLC investigation are summarised in **Table 3.1**. It should be noted that complete separation of the two enantiomers was very difficult to achieve on the available columns, a large number of conditions were

screened on various chiral columns before settling on a final method. It was also necessary to inject the samples at a larger concentration than usual because of their low UV activity, further compromising the less than ideal separation.

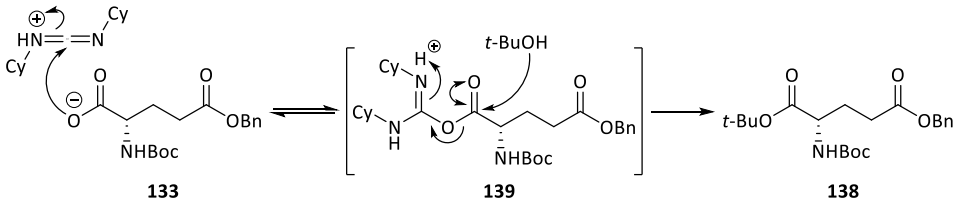


Scheme 3.4: Racemisation at the *α*-carbon during *t*-Butylation of **133** could result in the synthesis of two enantiomers. A screen of *reagents & conditions* in pursuit of a reaction that gives only the desired (*S*)-enantiomer of **138** is provided in **Table 3.1**.

Table 3.1: Conditions assessed in pursuit of a *t*-butylation that minimises racemisation at the *α*-carbon of **133**. The degree of racemisation was determined by chiral HPLC. To achieve the traces, a 20 min isocratic gradient of 5% *iso*propanol in hexane was run on a Chiralpak® AD-H column and samples were injected at a concentration of ~3 mg/mL in *iso*propanol. Further details of the chiral HPLC protocol and the traces themselves can be found in **Appendix I.II**.

Entry	*Reagents & conditions*	Yield	Ratio of (*S*):(*R*) from chiral HPLC and comments on racemisation
1	**133** (1.00 eq.), *t*-BuOH (5.00 eq.), EDC.HCl (1.10 eq.) DMAP (0.25 eq.), CH_2Cl_2, 0 °C → RT, 16 h	57%	• (*S*):(*R*) 65:35 (%) • Significant racemisation, in accordance with literature.[199]
2	**133** (1.00 eq.), *t*-BuOH (3.00 eq.), DCC (1.15 eq.) DMAP (0.10 eq.), CH_2Cl_2, 0 °C → RT, 18 h	60%	• (*S*):(*R*) 52:48 (%) • Significant racemisation.
3	**133** (1.00 eq.), *Iso*butene (10.00 eq.) *conc*. H_2SO_4 (0.10 eq.), CH_2Cl_2, -10 °C → RT, 16 h	19%	• (*S*):(*R*) 99.8:0.2 (%) • Minimal racemisation.
4	**133** (1.00 eq.), Boc_2O (1.20 eq.) DMAP (0.30 eq.), *t*-BuOH, RT, 2 h	78%	• (*S*):(*R*) 98:2 (%) • Slight racemisation.

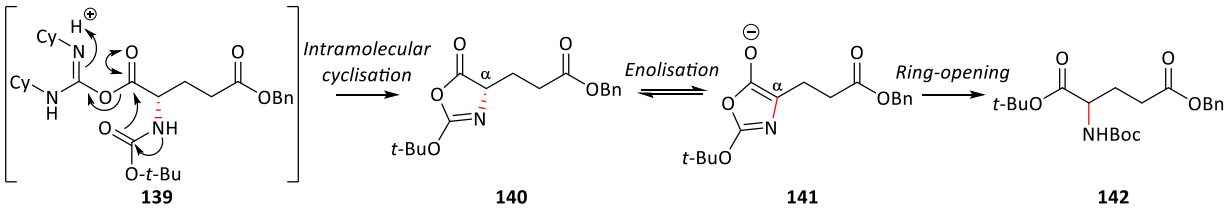
It was clear that *t*-butylation with the carbodiimides EDC or DCC (**Entries 1** & **2**, **Table 3.1**), was causing significant racemisation of the *α*-carbon centre.[199] The mechanism for this type of coupling is well established (**Scheme 3.5**), with active species **139** forming from nucleophilic attack of the carboxylate of **131** at the electron-deficient carbon of the carbodiimide. This constitutes activation of the carboxylic acid (which can be further encouraged by the use of catalytic DMAP) making **131** susceptible to nucleophilic attack, in this case, by *t*-butanol. An ester bond is formed, affording the desired product **138**.



133 139 138

Scheme 3.5: Mechanism of a carbodiimide esterification.

Direct enolisation of **138**, *via* abstraction of the α-proton, is believed to be one cause of racemisation. A further cause is that this type of carboxylic acid activation also renders the active species **139** susceptible to intramolecular nucleophilic attack by the adjacent amide group (**Scheme 3.6**).[188] This 'over-activation' facilitates formation of oxazolone **140** and enolisation of this species, at the α-carbon (pKa of ~9), destroys the integrity of the stereogenic centre.[200] Oxazolone **141** is then ring-opened, in the presence of catalytic DMAP, to give the desired product **142**, but with altered enantiopurity.



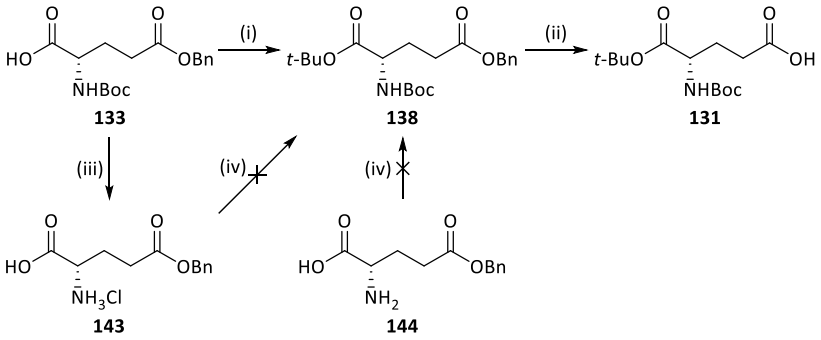
139 140 141 142

Scheme 3.6: One proposed mechanism for racemisation at the α-carbon of Boc-protected amino acids.

It is evident that, whilst carbodiimide couplings are robust and reliable, generally proceeding in good yields, in this case they were unable to provide compounds with the desired enantiopurity for future biological testing. Alternative *t*-butylation conditions were therefore pursued.

*Iso*butene in the presence of catalytic sulfuric acid had been used to *t*-butylate amino acid derivatives on many occasions so it seemed logical to investigate these conditions next (**Entry 3**, **Table 3.1** and **Scheme 3.7**, (i)).[201,202,203] Whilst chiral HPLC suggested minimal racemisation under these conditions, the yield was consistently very low (at best, 19%). Presumably, this was due to acid-mediated cleavage of the Boc group protecting the α-amine,

a theory which was supported by the appearance of a polar ninhydrin-staining baseline spot when monitoring the reaction *via* TLC.



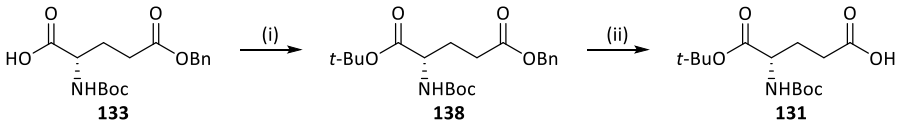
Scheme 3.7: Routes to **131** using *iso*butene as the *t*-butylating agent. *Reagents & Conditions*: (i) **133** (1.00 eq.), *Iso*butene (10.00 eq.), *conc*. H_2SO_4 (0.10 eq.), CH_2Cl_2, 0 °C → RT, 8 h, 19%; (ii) **138** (1.00 eq.) H_2, Pd/C (0.01 eq.), MeOH, RT, 16 h, 76%; (iii) **133** (1.00 eq.), HCl in dioxane (excess), 24 h, 100%; (iv) *a*) **143** or **144** (1.00 eq.), *iso*butene (10.00 eq.), conc. H_2SO_4 (0.10 eq.), dioxane, 5 °C → RT, 4 h; *b*) Boc_2O (1.20 eq.), $NaHCO_3$ (2.50 eq.), dioxane/H_2O, RT, 16 h.

In attempts to improve this reaction, the Boc group was removed from **131** to give the hydrochloride salt **143** (**Scheme 3.7**, (iii)). Unfortunately, *t*-butylation of **143** with *iso*butene, followed by replacement of the Boc group (**Scheme 3.7**, (iv)) was unsuccessful, perhaps due to the poor solubility of **143** in dioxane. Commercially available **144** was acquired, alleviating the need for the Boc deprotection, but this also repeatedly failed to react with *iso*butene.

Given that the target fragment **131** was required in large quantities for Fmoc-SPPS, it was decided that optimising this slightly longer route was undesirable. There were also concerns that the basic conditions required for reattaching the Boc group might cause racemisation, thereby cancelling out the benefit of using *iso*butene in the first place. Despite its excellent ability to *t*-butylate **133** without causing racemisation, the yields of the reactions with *iso*butene were simply too low to economically synthesise large quantities of **131**. Additionally, residual sulfuric acid catalyst was found to promote esterification of the carbonyl group in **131** with the reaction solvent methanol during hydrogenolysis (**Scheme 3.7**, (ii)). This

impacted further on the quantity of **131** that could be isolated and the search for appropriate *t*-butylation conditions therefore continued.

The final set of conditions assessed for *t*-butylation came in the form of di-*tert*-butyl dicarbonate and catalytic DMAP in *t*-butanol (**Entry 4**, **Table 3.1**).[204] Chiral HPLC suggested a small amount of racemisation under these conditions, however, the consistently good yield, the operational simplicity of the reaction and the quick reaction time made the conditions an appropriate compromise. Takeda *et al.* state that these reaction conditions are near neutral which, in combination with the fast reaction rate, is believed to be the reason for the minimal racemisation.[204] These conditions were applied on a multi-gram scale and, following hydrogenolysis with catalytic palladium on carbon, yielded a stock of the desired fragment **131** (**Scheme 3.8**). The optical rotation of **138** agreed well with a literature value, obtained from a compound that had been successfully *t*-butylated using isobutene.[205] Additionally, Chiral HPLC of **131** alongside the racemic analogue **148** confirmed that, as expected, no additional racemisation had occurred during the hydrogenolysis (**Appendix I.II**) and that **131** was isolated with an enantiomeric excess (ee) of 96%.

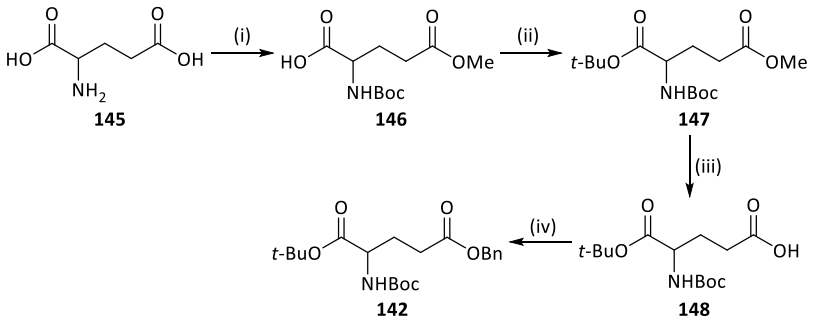


Observed:	$[\alpha]_D^{22}$ = +9.0 (*c* 1.0, MeOH)	$[\alpha]_D^{22}$ = −31.4 (*c* 1.0, MeOH)
Lit.[205]	$[\alpha]_D^{22}$ = +9.0 (*c* 1.0, MeOH)	$[\alpha]_D^{22}$ = −27.5 (*c* 0.6, MeOH)
		ee = 96%

Scheme 3.8: Final route to glutamic acid fragment **131** and optical rotation values. *Reagents & conditions*: (i) **133** (1.00 eq.), di-*tert*-butyl dicarbonate (1.20 eq.) DMAP (0.30 eq.), *t*-BuOH, RT, 2 h, 78%; (ii) **138** (1.00 eq.), H_2, Pd/C (0.01 eq.), MeOH, RT, 16 h, 98%.

3.3.3 Synthesis of a racemic glutamic acid fragment as a standard for HPLC

Use of chiral HPLC to assess racemisation required the synthesis of the racemic analogue of **138**. With literature precedent for each step, the synthesis of **142** was fairly straightforward and is provided in **Scheme 3.9**.

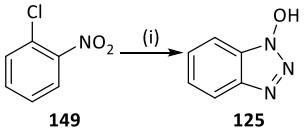


Scheme 3.9: Synthesis of the racemic glutamic acid fragment **142** for use in Chiral HPLC. *Reagents & conditions*: (i) a) D/L-Glutamic acid·H_2O (1.00 eq.), Me_3SiCl (1.50 eq.), MeOH, 1 h, RT; b) Boc_2O (1.20 eq.), $NaHCO_3$ (2.50 eq.), 1,4-dioxane, 0 °C → RT, 18 h, 72% over 2 steps; (ii) **146** (1.00 eq.), *t*-BuOH (2.00 eq.), DCC (1.15 eq.), DMAP (0.10 eq.), CH_2Cl_2, RT, 18 h, 33%; (iii) **147** (1.00 eq.), LiOH (2.00 eq.), THF/H_2O, RT, 16 h, 36%; (iv) **148** (1.00 eq.), benzyl chloroformate (1.20 eq.), DMAP (0.20 eq.), Et_3N (1.00 eq.), CH_2Cl_2, RT, 16 h, 41%.

D/L-Glutamic acid **145** was selectively mono-esterified at the *γ*-carboxylic acid using chlorotrimethylsilane in anhydrous methanol.[206] The reaction conditions provide an anhydrous source of hydrochloric acid, which protonates the *α*-amine. The proximity of this protonated amine to the *α*-carboxylic acid explains why methyl ester formation occurs predominantly at the more distant *γ*-carboxylic acid. In a similar fashion, acidic conditions have been used to selectively benzylate the *γ*-carboxylic acid.[207] Subsequent Boc protection afforded **146** in a pleasing 72% yield, over two steps.[206] With racemisation of no concern, standard DCC coupling conditions were employed to introduce the *t*-butyl protecting group at the *α*-carboxylic acid, giving **147**.[208] The base-labile methyl ester was hydrolysed using aqueous lithium hydroxide affording **148**, which was then reacted with benzyl chloroformate in the presence of catalytic DMAP to yield the desired racemic compound **142** *via* a mixed anhydride mechanism.[209]

3.3.4 Synthesis of a peptide coupling reagent

A simple combination of HOBt **125**, TBTU **123** and di*iso*propylethylamine was selected as the reagent combination for the solid phase peptide couplings. A stock of HOBt **125** was synthesised from 1-chloro-2-nitrobenzene **149** according to a modified literature procedure (**Scheme 3.10**).[210] An excess of hydrazine was used to account for its evaporation during the reaction since it has a similar boiling point to the toluene solvent.



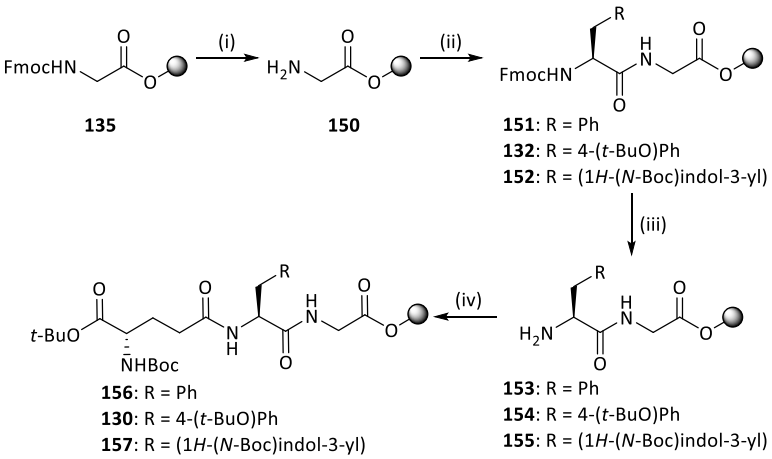
Scheme 3.10: Synthesis of HOBt **125**. *Reagents & conditions*: (i) **149** (1.00 eq.), hydrazine hydrate (4.00 eq.), toluene, reflux, 24 h, 38%.

3.3.5 Solid phase peptide synthesis

With the required amino acid fragments (particularly the glutamic acid fragment **131**) and HOBt **125** in hand, Fmoc-SPPS began. It was convenient to perform the syntheses of the three tripeptides **127**, **128** and **129** in parallel since, apart from coupling with the three different central residues (Fmoc-Phe-OH **136**, Fmoc-(*O*-*t*-butyl)-Tyr-OH **132** and Fmoc-($N_{(ind)}$-Boc)-Trp-OH **137**) to give the immobilised dipeptides, all other reactions required the same reagents.

Fmoc-SPPS began with commercially available Fmoc-Gly-Wang resin **135** (**Scheme 3.11**).[196] The Fmoc group was removed under standard conditions using piperidine in DMF affording Wang-immobilised glycine **150** (**Scheme 3.11**, (i)). A positive Kaiser test result indicated that the Fmoc deprotection had been successful.[211] At the end of each step thorough washing of the resin with DMF was important to avoid influencing the result of the Kaiser test and,

although this test is merely a qualitative measure of the progress of a reaction, it was generally fit for purpose. An undesired result in the Kaiser test simply meant running more cycles of a given step until the desired result was obtained.



Scheme 3.11: Fmoc SPPS. *Reagents & conditions*: (i) **135** (1.00 eq.), 20% piperidine in DMF (1 mL/1.00 g resin) [3 × cycles]; (ii) **150** (1.00 eq.), **136**/**134**/**137** (5.00 eq.), HOBt **125** (5.00 eq.), TBTU **123** (4.90 eq.), di*iso*propylethylamine (10.00 eq.), DMF (1 mL/1.00 g resin) [2 × cycles]; (iii) as in (i); (iv) **151**/**132**/**152** (1.00 eq.), Boc-L-glutamic acid 1-*tert*-butyl ester **131** (5.00 eq.), HOBt **125** (5.00 eq.), TBTU **123** (4.90 eq.), di*iso*propylethylamine (10.00 eq.), DMF (1 mL/1.00 g resin) [2 × cycles]. A Kaiser test was performed between each step.[211]

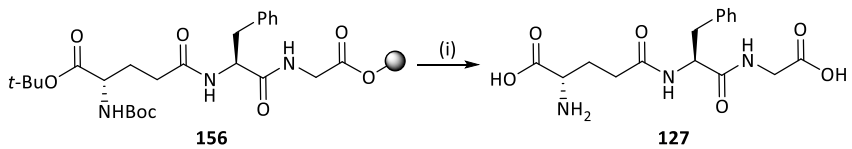
Following Fmoc deprotection, **150** was coupled to the central amino acid residues, either Fmoc-phenylalanine **136**, Fmoc-(*O*-*tert*-butyl)-tyrosine **134** or Fmoc-($N_{(ind)}$-Boc)-tryptophan **137**, to give the immobilised dipeptides **151**, **132** or **152**, respectively (**Scheme 3.11**, (ii)). A negative result in the Kaiser test was used as an indication that the peptide coupling had been successful, which was usually the case following two cycles of coupling. Occasionally, batches required more than two cycles of coupling before the desired Kaiser test result was obtained, this was carried out reluctantly, due to the large excesses of reactants and reagents required. The more cycles the greater the final yield was likely to be, however, it was important to remember that only small quantities of materials were required for initial biological testing.

A second Fmoc deprotection of the immobilised dipeptides **151**, **132** and **152** gave **153**, **154** and **155** respectively (**Scheme 3.11**, (iii)). A further peptide coupling with Boc-L-glutamic acid

1-*tert*-butyl ester **131**, completed the peptide chain, affording the immobilised tripeptides **156**, **130** and **157** respectively (**Scheme 3.11**, (iv)). To conclude the syntheses, the immobilised tripeptides were thoroughly washed with DMF and dichloromethane then dried *in vacuo* overnight, in preparation for the final global deprotection and cleavage from the solid support resin.

3.3.6 Isolation and purification of the tripeptides

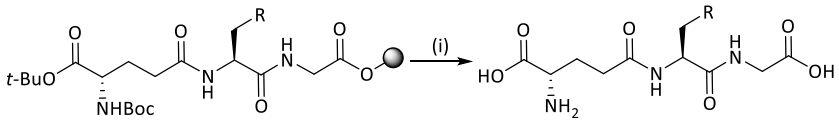
The syntheses of the immobilised tripeptides eventually became fairly straightforward and it was possible to prepare these compounds in under two days. The isolation and purification, however, was more protracted. Naively, initial batches of immobilised *γ*-Glu-Phe-Gly **156**, were globally deprotected with a solution of 80% trifluoroacetic acid in dichloromethane (**Scheme 3.12**). A disregard for the effect these conditions might have on the central amino acid side chains of the other two immobilised tripeptides **130** and **157**, as well as a lack of cation scavengers, is apparent. The work up for this initial global deprotection saw the solution concentrated *in vacuo* and the trifluoroacetic acid azeotroped with cyclohexane. Reverse phase column chromatography followed by way of purification and, despite the simplicity of this process, ^{1}H NMR spectra suggested that some of the desired tripeptide **127** had been isolated in this manner. However, the material was never sufficiently clean and it was decided that applying more consideration to the composition of the cleavage cocktail, as well as carrying out a more thorough work-up, would no doubt be beneficial.



156 **127**

Scheme 3.12: A basic global deprotection of **156** afforded some of the desired tripeptide **127**, however, it wasn't sufficiently pure for biological testing. *Reagents & conditions*: (i) **156** (1.00 eq.), trifluoroacetic acid (80% in CH_2Cl_2, 1 mL/1.00 g of resin), RT, 18 h.

A more thorough global deprotection procedure was established (**Scheme 3.13**). Cleavage cocktails were selected based on the needs of each individual tripeptide (**Table 3.2**). A solution of trifluoroacetic acid, phenol, water and tri*iso*-propylsilane (commonly known as 'reagent B') was used for the deprotection of **156**, the main change from initial work being the use of the latter three components as cation scavengers.[212,198] **130** and **157** were both deprotected using 'reagent K', which replaces the tri*iso*-propylsilane in reagent B with ethanedithiol.[197] Ethanedithiol is particularly useful for preventing reduction of the indole ring of Trp.[213]



156: R = Ph
130: R = 4-(*t*-BuO)Ph
157: R = (1*H*-(*N*-Boc)indol-3-yl)

127: R = Ph
128: R = (4-OH)Ph
129: R = (1*H*-indol-3-yl)

Scheme 3.13: Acid-mediated global deprotection of immobilised tripeptides **156**, **130** and **157**. *Reagents & conditions*: (i) **156**, **130** or **157** (1.00 eq.), cleavage cocktail (10 mL/1.00 g of resin), RT. See **Table 3.2** for cleavage cocktails. Approximate yields following work-up and purification are reported in **Table 3.2**.

Table 3.2: Cleavage cocktail compositions, reaction time and approximate yield according to substrate.
*Yields were calculated based on an average resin loading of 0.6 mmol g^{-1}, as per the manufacturer's instruction.

Substrate	Cleavage Cocktail	Time	Approximate Yield*
156	TFA:PhOH:H_2O:TIPS (88:5:5:2)	18 h	34%
130	TFA:PhOH:H_2O:$HS(CH_2)_2SH$ (88:5:5:2)	18 h	43%
157	TFA:PhOH:H_2O:$HS(CH_2)_2SH$ (88:5:5:2)	2 h	18%

Following implementation of these cleavage conditions, a modified work-up procedure was applied. Under a stream of nitrogen, the cleavage solution containing the crude tripeptides was reduced to a small volume and transferred to a number of small Eppendorf® vials. Ice-cold diethyl ether was added, the vials centrifuged and the supernatant removed. This process was repeated (keeping the vials cold) until precipitation of a colourless to pale yellow solid had

occurred. Reverse phase column chromatography of these precipitates allowed pure samples of tripeptides **127**, **128** and **129** to be isolated with yields of 34%, 43% and 18% respectively, over five steps based on an average resin loading of 0.60 mmol g^{-1}.

3.3.7 Biological testing

Unfortunately, the sensitivity of thermal shift analysis was insufficient to distinguish between the affinities of tripeptides **127**, **128** and **129**, which fell in the range of GSH **106**, and the data is, therefore, not presented herein. More promising results were achieved from the complementary competition fluorescence assay, which was kindly performed by Sam Grayer, a DPhil student from the Conway group at the University of Oxford. KefB from *Shewanella dentrificans* (SdKefB) was used instead of KefC from *E. coli*, the reasons for which were explained in **Section 3.1.4**.

Figure 3.12 portrays the control experiments, confirming the suitability of the assay for assessing the affinity of new ligands for Kef. The graph shows that DNGSH **109** and GSH **106** possess similar affinities for Kef as there is minimal change in the intensity of the fluorescence (Fluorescence [A. U.]) when GSH **106** is added to a solution of Kef pre-incubated with DNGSH **109**. As mentioned previously, the work was keen to establish ligands that bind to Kef with a stronger affinity than that of GSH **106** due to its ubiquitous nature and relatively

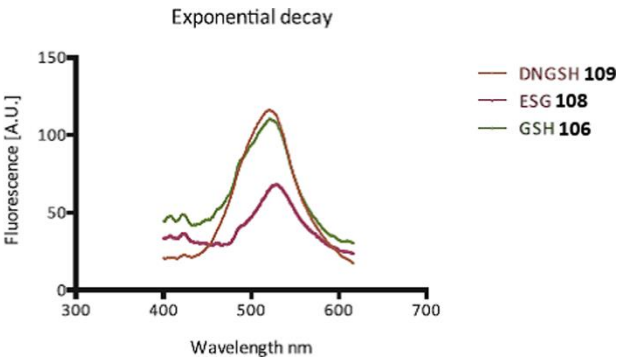


Figure 3.12: Emission spectra at 340 nm excitation for: (i) SdKefB (6 µM) and DNGSH **109** (5 µM) alone, representing the background measurement [brown]; (ii) SdKefB/ DNGSH **109** following the addition of GSH **106** (1 mM) [green]; (iii) SdKefB/DNGSH **109** following the addition of ESG **108** (1 mM) [purple]. The data shows that, as predicted, ESG **108** binds Kef with a stronger affinity than either GSH **106**or DNGSH **109**.

high concentration within bacteria. When the established activatory ligand ESG **108** was added to Kef pre-incubated with DNGSH **109** a drop in the intensity of the fluorescence was observed, which confirmed that ESG **108** was successfully outcompeting DNGSH **109** for Kef binding. The fluorescence of the displaced DNGSH **109** was quenched by exposure to the aqueous environment used to solubilise and stabilise the protein during the assay, thereby accounting for the observed decreased in fluorescence intensity.

The data for the synthesised tripeptides **127**, **128** and **129** are detailed in **Figure 3.13**. A comparison of the data for all of the tripeptides is provided in a single graph (**A**) followed, for clarity, by the observations for each individual tripeptide (**B**, **C** & **D**).

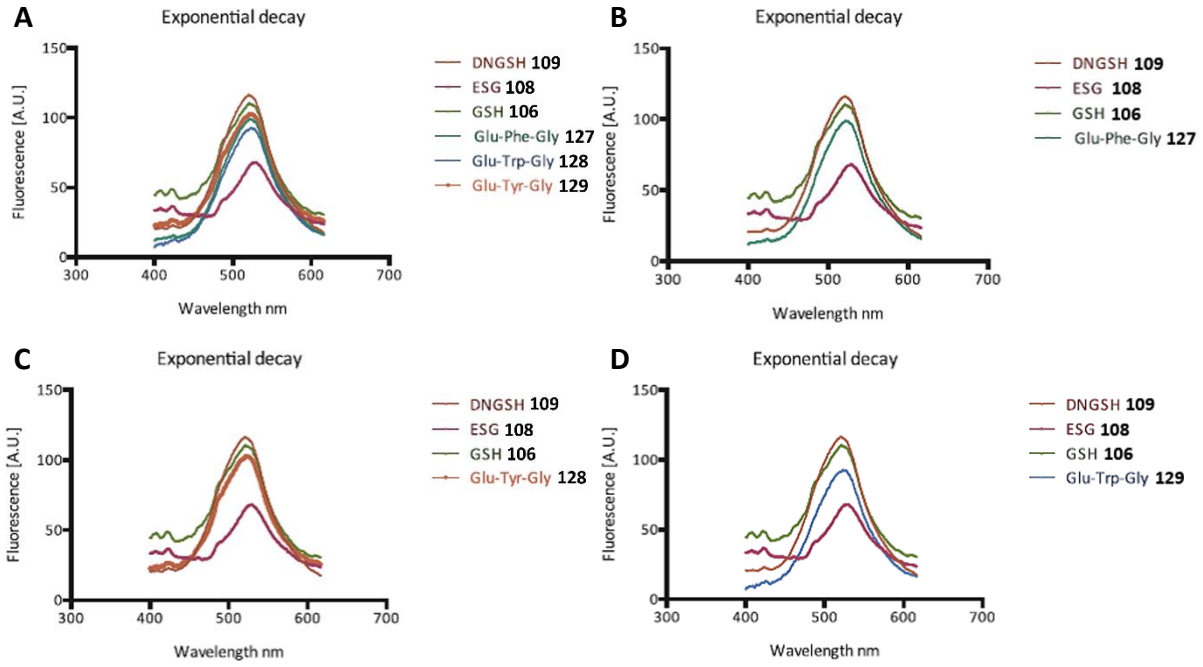


Figure 3.13: Emission spectra at 340 nm excitation for tripeptides **127**, **128** and **129**. SdKefB (6 µM) was pre-incubated with DNGSH **109** (5 µM) before the addition of tripeptide (1 mM). **A**: Complete data for all compounds; **B**: Data for *γ*-Glu-Phe-Gly **127** and the controls GSH **106** and ESG **108**; **C**: Data for *γ*-Glu-Tyr-Gly **128**; **D**: *γ*-Glu-Trp-Gly **129**.

It is immediately evident that the tripeptides do not possess as high an affinity for Kef as ESG **108**, in fact, they seem to bind with an affinity more closely resembling that of GSH **106**. *γ*-Glu-Tyr-Gly **128** [orange] appeared to have the lowest affinity for Kef (**A** & **C**, **Figure 3.13**),

whilst a slightly larger decrease in intensity, and therefore a slightly higher affinity, was observed for *γ*-Glu-Phe-Gly **127** [teal] (**A** & **B**, **Figure 3.13**). The most promising result was the larger decrease in fluorescence intensity observed when *γ*-Glu-Trp-Gly **129** was added to the SdKefB/DNGSH **109** mixture, indicating an affinity midway between GSH **106** and ESG **108** (**A & D**, **Figure 3.13**). The results are summarised more clearly in graph **A** of **Figure 3.14**, for which the ratio of the fluorescence intensity (at 525.5 nm) of DNGSH **109** bound to Kef before (FB) and after (FL) the addition of a competing ligand, or FB/FL, has been calculated for each tripeptide (this was originally explained in **Section 3.1.4**). A control experiment, where DNGSH **109** and either **127**, **128** or **129** were incubated together in the absence of protein, confirmed that the addition of the tripeptides alone was not sufficient to cause quenching of DNGSH **109** (**B**, **Figure 3.14**). Changes in fluorescence intensity occur only in the presence of SdKefB.

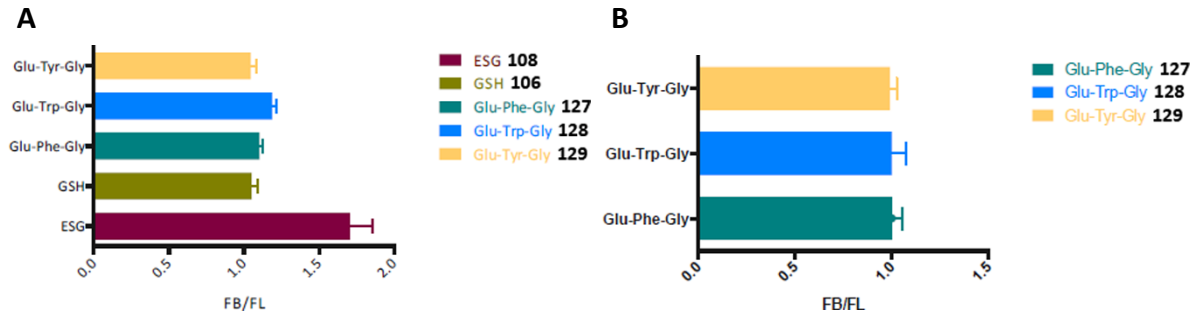
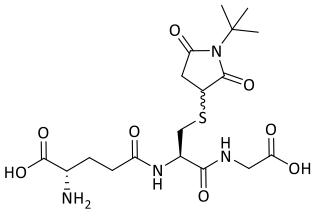


Figure 3.14: **A**: FB/FL ratios for GSH **106**, ESG **108** and tripeptides **127**, **128** and **129**, calculated using the fluorescence intensity for each species at 525.5 nm; **B**: A control experiment, the tripeptides were incubated with DNGSH **109** in the absence of protein, the ratios are all 1, confirming that the addition of the tripeptides alone is not sufficient to cause quenching of DNGSH **109**.

It was originally hypothesised that replacement of the central cysteine residue of GSH **106** by a sterically demanding amino acid may change the behaviour of the resulting tripeptide such that it switches from an inhibitor of Kef activity to an activator. Such tripeptides might be expected to possess an affinity for Kef more closely resembling that of the activatory tripeptide ESG **108**, which also contains a sterically demanding central residue. Whilst it is true

that the biggest tripeptide synthesised, *γ*-Glu-Trp-Gly **129**, possessed the highest affinity for Kef, the competition fluorescence results as a whole may suggest that sterically demanding substrates alone may not be the key to high affinity ligands and, therefore, that the proposed mechanism of activation may require elaboration. Compounds **127**, **128** and **129** represent the first examples of tripeptide probes of Kef activity that do not contain a central thiol or thioether, allowing investigation of how important this functionality might be in affording ligands with a high affinity for Kef. Previous work within the Conway group, both published and unpublished, has tended to conjugate the cysteine thiol to a large side chain rather than switching it for a completely different residue altogether and generally, these compounds have shown the expected increase in affinity. Compound **111** in **Figure 3.15** is a representative example.[169] Following the uncertainty surrounding the ligand-bound crystal structures, it is possible that some key binding interactions between the sulfur atom and the active site of Kef remain to be discovered, which would explain the relatively poor affinities of tripeptides **127**, **128** and **129** compared to thioether-containing tripeptides.



111

Compound	FB/FL
DnGSH **109**	1.00
GSH **106**	1.10
ESG **108**	1.30
111	1.50

Figure 3.15: **111**, A tripeptide that out-performed ESG **108** in a competition fluorescence assay reported by Healy *et al*.[169] FB/FL ratios were calculated using the fluorescence intensity for each species at 525.5 nm.

Alternatively, it may be that the side chains of the new tripeptides are simply too short to reach into the region of the binding site where the interactions that cause channel activation occur. In tripeptides **127**, **128** and **129**, the sterically demanding side chains are connected to the *α*-carbon of the central amino acid residue *via* a single methylene group. In the thioether-containing compounds, such as **111**, a methylene group and a sulfur atom link the *t*-butylsuccinimido side chain to the *α*-carbon of the central amino acid residue. Being in the second row of the periodic table, sulfur atoms can be considered relatively large linkers. With

this in mind, it seems reasonable to suggest that the side chains of **127**, **128** and **129** may not be able to reach as far into a binding site as their thioether-linked counterparts. Consideration of side chain size may also explain why *γ*-Glu-Trp-Gly **129** exhibited a slightly greater affinity for Kef than either **127** or **128** – its indole side chain is larger than either a phenyl or phenol side chain.

3.4 Conclusions

The target tripeptides **127**, **128** and **129** were successfully synthesised and purified in reasonable overall yields of 34%, 43% and 18% respectively, according to established Fmoc-SPPS procedures. In the absence of thermal shift data, a competition fluorescence assay provided the first evidence that these compounds do bind to Kef, albeit with affinities comparable to that of GSH **106** rather than ESG **108**. The proposed mechanism of action appears to be more complicated than first thought, with a suggestion that the thioether is important for binding affinity. Whether this is because of a specific binding interaction formed by the sulfur atom or simply because its large size projects the side chain further into the site of interest is yet to be realised. Further work, including potassium efflux assays, to distinguish between activatory and inhibitory behaviour, is now required to investigate whether they also activate KefC. Such assays generally require membrane permeability such that it would be necessary to convert tripeptides to the corresponding ethyl esters.

The synthesis of glutamate fragment **131** provided an unanticipated foray in the field of enantiopurity and a significant amount of time was spent establishing conditions that would provide a large amount of **131** in high enantiopurity. *t*-Butylation of the *α*-carboxylic acid of **133** was found to proceed rapidly, in good yield and high enantiopurity using the optimised conditions of di-*tert*-butyl dicarbonate, DMAP and *t*-BuOH. These conditions have now been used by many members of the Conway group who have required a method of esterifying amino acids, which does not result in racemisation of the *α*-position. Although the chiral HPLC protocol could be improved, it was pleasing to establish a method that could assess enantiopurity to a useful level.

3.5 Future work

Future work will include establishing whether the tripeptides cause activation of Kef, this would allow further interpretation of the importance of sterically demanding ligands and the required nature of the side chains, for activation of Kef.[162] Of course, it would also be hugely gratifying to obtain a new crystal structure of a modified tripeptide bound to Kef. It should be reiterated that the assays were carried out using SdKefB yet all previous crystal structures were obtained using KefC from *E. coli*. A KefB X-ray crystal structure with a new ligand bound, in combination with the appropriate assays, would provide further vital information for establishing the elusive mode of action.

Tripeptides **127**, **128** and **129** have furnished interesting data concerning the mechanism of action of Kef, which appears to be more complicated than expected. Other members of the Conway group are now pursuing further peptidic and non-peptidic modulators of Kef activity. *In silico* screening has been a useful tool for suggesting scaffolds that may be appropriate for development as modulators of Kef activity.

4. A Small Molecule Probe for the Mechanosensitive Channel of Small Conductance, MscS

In this final chapter a second type of bacterial ion channel will be introduced. First, a general introduction to mechanosensitive ion channels will be provided, followed by a more in-depth look at the mechanosensitive channel of small conductance, MscS, including structural details and a proposed gating mechanism (**Section 4.1.2**). Subsequently, a new probe for investigating the mechanism of action of MscS will be presented (**Section 4.2**) and work towards the synthesis of this probe will be discussed in **Section 4.3**.

4.1 Introduction

4.1.1 Mechanosensitive ion channels (MCs) in microbes

The invention and evolution of the patch clamp technique by Neher and Sakmann nearly forty years ago, for which they were later awarded The Nobel Prize, has been deemed largely responsible for the ensuing boom in the study of ion channels in microbes.[214,215,216] The patch clamp is a small electrophysiological device that is able to hold constant the voltage across an excitable membrane. Its discovery allowed ion currents through individual membrane proteins to be examined for the first time. The technique enabled the study of much smaller systems, literally patches of a membrane, than was previously possible with voltage clamp and microelectrodes. Patch clamp was used by Martinac *et al*. to study the activities of mechanosensitive ion channels (MCs) in microbes.[217] The results from this work, later followed by the elucidation of the gene sequences for various MCs, generated substantial interest from the microbiological community and bacterial MCs have since been intensely studied due to the simplicity they offer in comparison to animal cells.[216,218,219]

The fact that water is essential for life is a globally acknowledged fact. The cells of all organisms, including bacteria, are composed of around 80% water and the osmotic forces governing water content are therefore of extreme importance. Indeed, Kung stated that only a 10 milli-osmolar difference across a barrier was sufficient to produce an osmotic pressure of 180 mmHg (or 2.5×10^5 dyn cm^{-1}), which generates a 12 dyn cm^{-1} stretch force on the surface of a sphere just 2 microns in diameter.[220] In bacteria, the ability to cope with osmotic stress is not only important to avoid the high pressures that would lead to cell lysis. Bacteria must also maintain an internal osmotic pressure above that of a growth medium in order to generate cell turgor, which is believed to be a necessary driving force for cell growth and division.[221]

Bacteria, like all organisms, have developed various mechanisms to deal with osmotic stress. The most immediate approach to such stress is the use of MCs.

Since they were first discovered using patch clamp in *E. coli*, MCs have been established as the emergency valves in times of osmotic stress.[217] These channels were reported to open in response to stretching of the surrounding membrane, by application of a small positive or negative pressure, releasing cytoplasmic osmolytes and reducing the turgor pressure, thereby preventing a membrane from rupturing. In the original study, it was initially concluded that a single type of MC had been discovered. Additionally, the channels' proposed location was the outer membrane of *E. coli* because they exhibited similar properties to porins - the largely non-specific water-filled pores, also found in the outer membrane, that allow the movement of small hydrophilic molecules *via* passive diffusion. Both of these proposals were later dispelled when a number of different types of MCs, opening at different conductances, were reported.[222] They were also found to be localised within the inner membrane of bacteria when subsequent studies reported that; (i) pressure-activated, high conductance channels were present in Gram positive bacteria, which lack outer membranes and (ii) inner and outer membranes of Gram negative bacteria could be isolated and studied independently and the pressure-activated channels were present, exclusively, in the inner membrane portions.[223,224]

By the early 1990s, different MCs had been discovered, all localised within the inner membrane of bacteria. The different MCs are characterised by their conductance, which is the measured current through a channel under constant voltage (in Siemens (S)). MscL, the mechanosensitive channel of large conductance, opens at tensions close to the lytic limit of a cell and has a relatively large conductance of 3 nS coupled with low substrate specificity.[222] Sieving experiments have indicated that the pore within MscL expands to around 30 Å in

diameter, an increase of 15-16 Å upon opening, which is the largest reported conformational change within a channel protein.[225,226] The mechanosensitive channel of small conductance, MscS, is a second MC and was reported at the same time as MscL.[222] It is characterised by a smaller conductance of approximately 1 nS, around a third that of MscL. MscS appears to be the most abundant of the MCs, and is therefore expected to mediate the majority of turgor adjustments.[227] MscM, the mini-conductance channel, has the lowest threshold and a conductance of 0.3-0.4 nS, therefore opening first in response to osmotic downshock.[228,229] The final reported MC is MscK, the K^+-dependent mechanosensitive channel, formerly known as KefA. MscK appears to have a similar conductance to MscS but shows increased sensitivity to membrane tension and a highly regulated nature suggesting a more specialised physiological role than the other MCs.[230]

Bacterial MCs channels represent promising therapeutic targets for the development of new antibiotics as database searches have revealed homologs of *E. coli* MscS and MscK exist in virtually all walled organisms, including in many other bacteria.[231] MscL was also found to be widespread amongst bacteria, albeit with decreased homology. Membrane-embedded portions of all of these channels appear to be highly conserved between bacteria. Minor conservation of MCs is observed in Eukarya, some MscS-like proteins have been identified in plants and yeasts, however, there appear to be no reported homologs of MscS in animals.[232] With this in mind, the potential to develop drug candidates selective for bacterial MCs is evident.

4.1.2 The structure of MscS and a proposed gating mechanism

MscS, a stretch-activated channel that has been most well characterised in the inner membrane of *E. coli*, opens in response to changes in membrane tension, allowing small osmotically-active molecules to exit the cell.[219] As discussed above, MscS channels form part of a wider family of MCs that bacteria use as an emergency survival mechanism on exposure to hypo-osmotic stress. With no MscS reported in animals, any molecule that modulates the activity of MscS may pave the way for the development of antibiotics with novel modes of action. For this to be realised, a significant understanding of the structure of MscS and how it is gated (i.e. what changes occur on going from a closed to open form) is necessary. Extensive work from various research groups has already furnished a number of X-ray crystal structures of MscS at varying resolutions, although not all can be discussed here.[31,233,234] EPR spectroscopy has also been used in structural studies of MscS.[235] A significant proportion of these structural studies have been carried out by collaborators in the Booth and Naismith groups, hence the particular focus on MscS.

Bass *et al.* were the first to publish an X-ray crystal structure of MscS, which was believed to show the channel in its open form (**Figure 4.1**).[31] The lateral pressure supplied by the detergent used to solubilise MscS (foscholine-14) was believed to be insufficient to maintain the channel in the closed form. However, MD simulations later reassigned the structure as a non-conducting or closed state when a number of glycine and alanine residues found in the TM3a helix were deemed important for gating.[236] Their hydrophobic nature was found to prevent wetting of the pore, thereby blocking the passage of ions, meaning this structure could not be an open form. Nevertheless, the 3.9 Å resolution structure revealed MscS to be

a membrane-spanning homoheptamer with a large additional cytoplasmic domain (**A**, **Figure 4.1**).

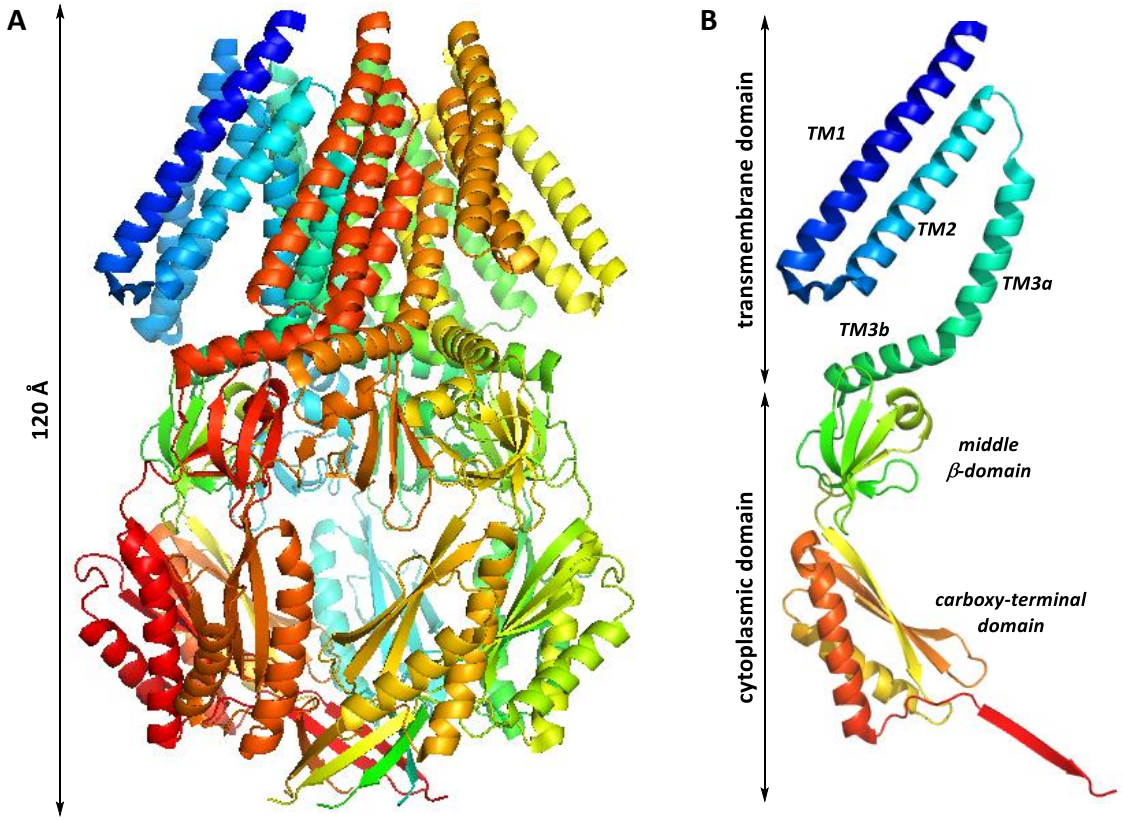


Figure 4.1: X-ray crystal structure of MscS at 3.9 Å resolution, reported by *Bass et al.* (PDB code: 20AU).[31] **A**: MscS is a heptameric channel protein. The transmembrane domain forms a near-cylindrical pore that exits into a large water-filled chamber formed by the cytoplasmic domain (each subunit is depicted in a different colour). **B**: A detailed look at a single heptamer. The three helices, TM1, TM2 and TM3a and TM3b constitute the transmembrane domain, whilst the middle β-domain and the carboxy-terminal domain constitute the cytoplasmic region of the protein.

Each subunit contains 286 residues and reaches a length of approximately 120 Å (**B**, **Figure 4.1**). The N-terminal transmembrane subunits pack tightly to form a near cylindrical 11 Å channel through the membrane, which opens into the cytoplasmic domain. The cytoplasmic domain can be further divided into a barrel-like assembly of β-sheets and a large carboxy-terminal. The carboxy terminal forms a fenestrated chamber, which may serve as a filter for screening out impermeant molecules based on size and electrostatic factors. This has been observed for similarly structured chambers in the acetylcholine receptor and the Shaker receptor.[237,238] The transmembrane region of each heptamer was shown to be composed of

three transmembrane helices named TM1 (residues 29-57), TM2 (residues 68-91) and TM3 (residues 96-127), a kink at Gly113 of TM3 marks the likely position of the membrane boundary and separates this helix into two parts, TM3a and TM3b. Intriguingly, the TM1 and TM2 helices were reported to be tightly packed together but slightly displaced from TM3, and their density appears less well defined suggesting they may be more conformationally mobile than the rest of the protein.

A slightly higher resolution open form structure (3.45 Å) was reported by Wang *et al.* in 2008.[233] They identified A106V as one of a series of mutants that provided unique electrophysiological properties and was fully functional *in vivo*; it assembled in the membrane, protected against hypo-osmotic shock and gated at pressures intermediate between WT MscS and MscL. [239] This A106V variant of MscS was successfully crystallised using the same procedure reported for the WT protein and, like the WT, was found to be homoheptameric with the same cytoplasmic and transmembrane domains. The cytoplasmic domain was largely identical to that of the WT, however, substantial differences in the transmembrane domain led to this new structure being assigned as an open state of MscS (**A**, **Figure 4.2**). Substantial rotational rearrangement of the helices in the transmembrane domain was observed. TM1 and TM2 had undergone a 45° clockwise rotation and their tilt with respect to the seven-fold axis had increased by 15°, the rotation increased the buried volume of these helices within the surrounding membrane (**B**, **Figure 4.2**). Changes in the TM3a helix were also apparent, with the hydrophobic side chains of the L105 and L109 residues, in particular, seen to withdraw from the central pore. When considered as a whole, the movement of the helices in each heptamer can be likened to an iris-like expansion of the channel pore affording a diameter of around 13 Å (compared to 4.8 Å for the closed form). That these changes occurred

remotely from the position of the A106V mutation gave the authors confidence that the observed structure was not merely an artifactual open state.

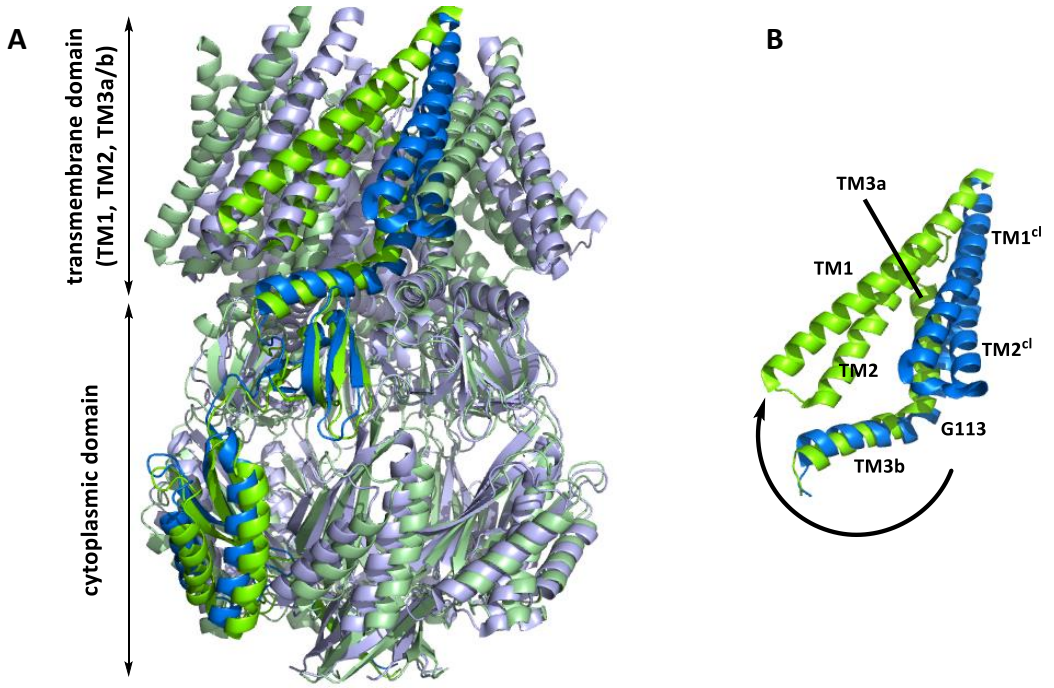


Figure 4.2: A second X-ray crystal structure of MscS (A106V mutant) at 3.45 Å resolution, was reported by Wang *et al.* (PDB code: 2VV5).[233] **A**: The A106V structure (green) overlaid with the original WT structure (blue). The cytoplasmic domain is largely conserved, however, in the A106V variant the transmembrane helices are noticeably displaced. **B**: An overlay of the cytoplasmic domains of a single subunit, further illustrating the displacement of the TM1, TM2 and TM3a helices relative to the WT structure.

The observed rearrangement of the three transmembrane helices, in addition to some mutational analysis, formed the basis of a proposal for a gating mechanism of MscS. At high turgor pressure, the density of the surrounding phospholipid membrane decreases as the cell bulges. TM1 and TM2 respond to this stress by rotating about the seven-fold axis, increasing their buried volume. The rotations of these two helices may serve as a lever for TM3a, which then pivots around residues Q112 and G113, withdrawing hydrophobic side chains from the central pore and thereby allowing solvated ion transport. This proposal was supported by Vasquez *et al.* who, at the same time, published work that had used functional measurements, EPR spectroscopy and computational analysis to investigate the structural rearrangements associated with gating of MscS.[235]

In **Chapter 2** the exceptional ability of DDM **1** to maintain membrane proteins in their native state was discussed. In 2013, two further crystal structures, *E. coli* MscS at 4.4 Å resolution and *H. pylori* MscS at 4.2 Å resolution, achieved by initial solubilisation in DDM **1**, were reported by Lai *et al*.[234] This work was exciting because it was of interest to see whether there were any conformational variations between different bacteria and whether a change of detergent (the two previously discussed structures were obtained *via* solubilisation in foscholine-14) affected which form of MscS was adopted. The cytoplasmic domains were again found to be unchanged, but distinct conformations of the transmembrane domains were observed. The DDM solubilised WT *E. coli* MscS was found to adopt an open conformation like that of the A106V variant, but interestingly the DDM solubilised WT *H. pylori* MscS was found to adopt a non-conducting state, like that of the foscholine-14 solubilised WT MscS.[31,233] The similarities between these structures and previous structures emphasised the mechanistic significance of the two established conformational states. Overall, this work illustrated the sensitivity of membrane protein conformation to variations in detergent, crystallisation conditions and protein sequence. They warned that future studies on MCs should be undertaken with caution, using an approach that samples conformational space by screening different detergents, constructs, homologs and crystallisation conditions.

4.1.3 Existing modulators of MC activity

Note that the title of this Section refers to 'modulators' of activity rather than 'ligands' since most of the reported compounds are believed to cause gating of MCs through interaction with the surrounding membrane, rather than binding directly to the channel at a specific ligand binding site.

The first major work to discuss compounds that activate MCs was published by Martinac *et al*. in 1990.[240] It preceded the reports revealing the existence of different types of MCs and therefore incorrectly assumed that only a single type of MC existed and that they were located in the outer membrane of bacteria (see **Section 4.1.1**). Nevertheless, the work was hugely relevant, showing that amphipathic molecules slowly activate MCs with an effectiveness proportional to their lipophilicity and that cationic and anionic amphipaths possessed the ability to compensate for each other's effect, presumably through the bilayer couple hypothesis (**Figure 4.3**). This hypothesis suggests that the two halves of a membrane respond differently to perturbation due to the asymmetric distribution of proteins and polar lipids throughout the bilayer.[241] Accordingly, cationic amphipaths (e.g. chlorpromazine (CPZ) **158**) preferentially intercalate into the more negatively charged inner half of the membrane causing cells to form cups, while anionic amphipaths (e.g. trinitrophenol (TNP) **159**) cause cells to crenate *via* preferential insertion into the more positively charged outer half of the membrane. The observations in this work supported the view that mechanical gating forces could come from surrounding lipids and the results paved the way for further investigation into the physics and biology of mechanosensitisation.

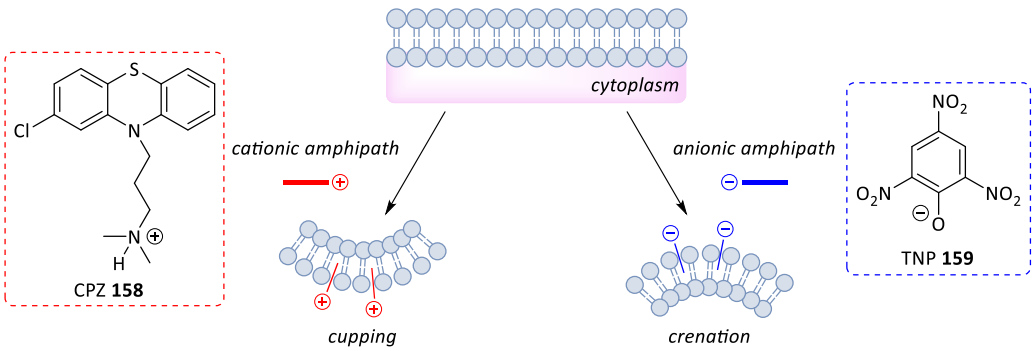


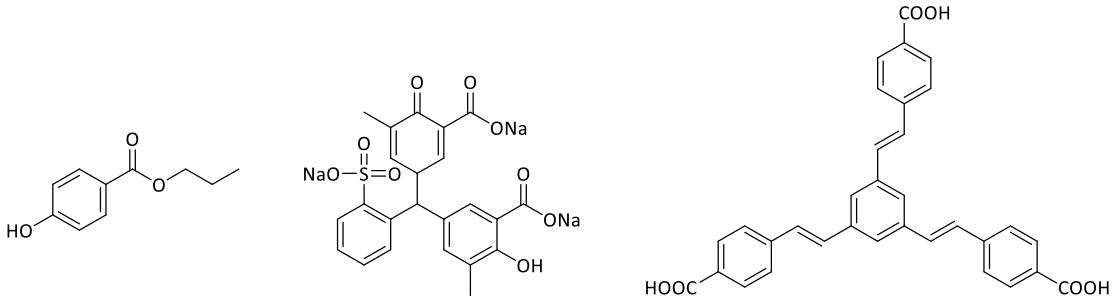
Figure 4.3: Illustration of the bilayer couple hypothesis. Cationic amphipaths (e.g. **158**) preferentially insert into the more negatively charged inner half of the membrane whilst anionic amphipaths (e.g. **159**) prefer the more positively charged outer half.[240] Insertion into the inner half results in cupping and insertion into the outer half results in crenations.

Lysophosphatidylcholine (or LPC) **160** has been used as an activator in gating studies of both MscL and MscS (for the structure of LPC **160** see **Section 4.1.4**, **Figure 4.5**). The aforementioned work by Vasquez *et al*. used LPC **160** to elicit spontaneous opening of MscS, such that they could record the channel in its open form (**Section 4.1.2**).[235] A 2002 paper by Perozo *et al*. also used LPC **160** to trap MscL in its open form, in order to investigate the physical principles underlying the transduction of bilayer deformation during the gating of MCs.[242] The latter of these two studies again assumed that the lipid-mediated gating of MCs occurs by an indirect mechanism, the lipids are believed to insert into the surrounding membrane and the resulting distortion is detected by the MCs, which respond by activating. As an aside, the gadolinium ion (Gd^{3+}), which is clearly very different in structure from the aforementioned amphipaths, was found to block the release of metabolites in large stretch-activated channels suggesting it may be an inhibitor of such channels. However, as a heavy atom that precipitates in phosphate media, it was expected to have limited use in *in vivo* experiments.[243]

It is clear that most studies to date have demonstrated that gating of MCs is likely mediated by largely hydrophobic additives, which cause perturbations of the surrounding lipid bilayer. Due to the complexity of these interactions within a bilayer they will not be discussed further here and they have already been well reviewed.[244,245]

Examples of compounds that bind directly to MscS or MscL are limited. In 2005, Nguyen *et al.* published work suggesting that parabens bind directly to both MscS and MscL and they used *in silico* methods to suggest a binding mode for propyl paraben **161** (**Figure 4.4**) with MscL.[34] In the same year, these researchers also presented work reporting that eriochrome® cyanine R (ECR) **162** binds MscL with a greater affinity than the aforementioned parabens, however,

this work does not appear to have been published.[246] Later PhD work by Boulos, at the University of Western Australia, used *in silico* modelling of parabens and ECR **162** to develop the ligand Ramizol™ **163**, which, in its ionised form, is reported to show unprecedented binding to MscL.[247] The work is unpublished as the researcher has since set up 'Boulos & Cooper Pharmaceuticals', a company '*focused on the design and development of novel antibiotics'* through '*targeting the highly conserved mechanosensitive ion channel of large conductance*', a feat that surely highlights the potential of this field in the development of novel antibiotics.[248]



161 **162** **163**

Figure 4.4: The structures of some compounds proposed to bind specifically to MscS and/or MscL.

If specific ligands could be established, it may become possible to selectively target a particular MC, with both selective agonists and antagonists having a potential therapeutic application. Prolonged opening of MscS, promoted by an agonist, would lead to excessive leakage of vital intracellular components and conversely, an antagonist would hold a channel closed, leading to the rupturing of bacterial cell membranes in times of osmotic downshock.

4.1.4 Structure and syntheses of lysophosphatidylcholine (LPC) and its analogues

Section 4.1.3 mentioned a number of studies where LPC **160** had been used to activate MscS or MscL. Later in this chapter, a derivative of LPC **160**, is proposed as the synthetic target (**Section 4.2**). This Section therefore introduces LPC **160**, a type of lysophospholipid (LPL), in greater detail and outlines some of its reported syntheses with a view to providing a basis for the development of a synthetic route to a particular LPC analogue.

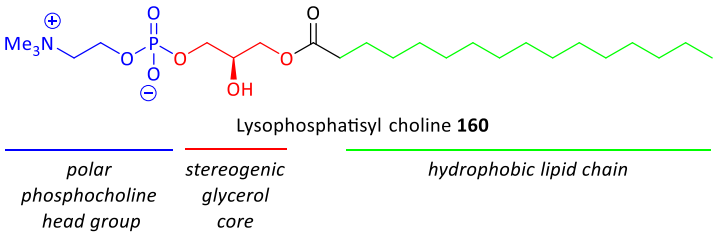
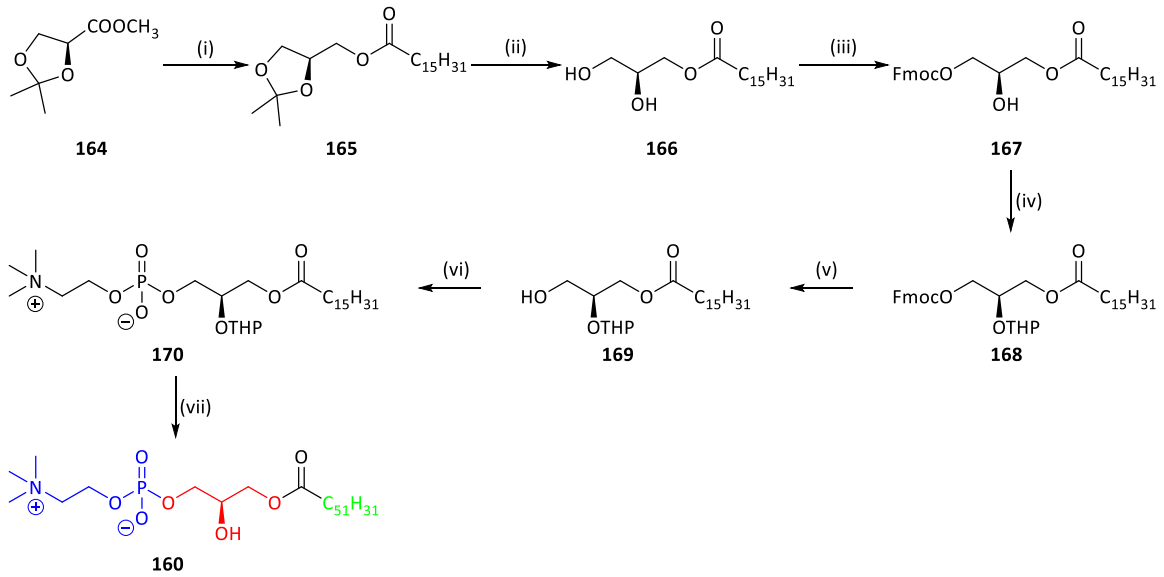


Figure 4.5: The structural features of lysophosphatidylcholine, LPC **160**.

Despite the widespread use of LPC **160** in biology, to a synthetic organic chemist, this amphipathic molecule is complex (**Figure 4.5**). Its structure can be divided into three main components; a polar phosphocholine head group, a glycerol core and a lipid chain. The phosphocholine head group contains a quaternary amine linked to a negatively charged phosphate group *via* an ethyl chain, making LPC neutral overall. The central glycerol core contains a stereocentre and, with three alcohols in close proximity, the possibility of substituent migration during derivatisation of these alcohols is an immediate concern for an organic chemist. The lipid chain, which is linked to the glycerol core *via* an ester bond, is relatively straightforward, however, the hydrophobicity of this moiety may make compounds containing it poor candidates for procedures such as aqueous work ups. The 'lyso' in LPL refers to the fact that these molecules, of which LPC **160** is an example, contain just one *O*-acyl lipid chain and therefore a single free alcohol on the central glycerol core.

Regardless of the apparent structural complexity, numerous syntheses of LPC **160** have been reported in the literature. The Hajdu group are responsible for a number of these, in all of which they adopt a substantial protecting group strategy to reach the target.[249,250,251] Their high-yielding synthesis from the initial paper is outlined in **Scheme 4.1**, their subsequent reports switch the Fmoc group for either a 4-nitrophenyl group or a tosyl group.

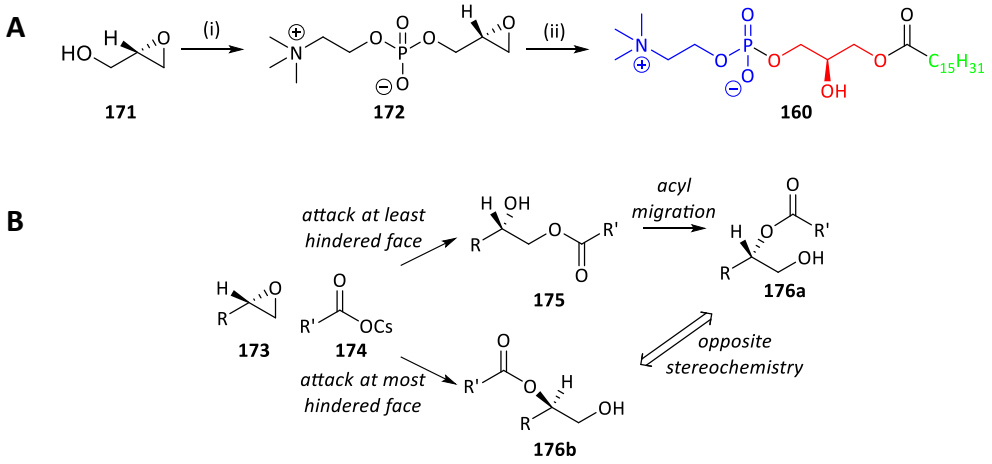


Scheme 4.1: The preparation of LPC **160** according to the Hajdu group.[249] *Reagents & conditions*: (i) *a*) $LiBH_4$, Et_2O, 95%; *b*) $C_{15}H_{31}COOH$, DCC, DMAP, CH_2Cl_2, 95%; (ii) 0.4 N HCl, dioxane/H_2O, 84%; (iii) Fmoc-Cl, DMAP, $CHCl_3$, 58%; (iv) 3,4-Dihydro-2*H*-pyran, pyridinium *p*-toluenesulfonate; CH_2Cl_2, 86%; (v) Piperidine, CH_2Cl_2, 87%; (vi) *a*) 2-Chloro-2-oxo-1,3,2-dioxaphospholane, Et_3N, benzene; *b*) Me_3N, MeCN, 61% over 2 steps; (vii) 0.15 N HCl, dioxane/H_2O, 98%.

The synthesis began with a masked form of the central glycerol core, the isopropylidene ester **164**. This was reduced to a primary alcohol before the lipid chain was installed *via* a DCC-mediated esterification affording **165** (**Scheme 4.1**, (i)). Removal of the isopropylidene acetal afforded diol **166** and a series of protecting group manipulations eventually afforded alcohol **169** (**Scheme 4.1**, (ii)-(v)). NMR data were reported to confirm that there were no regioselectivity issues during these steps. Addition of the phosphocholine head group, for which there is ample literature precedent, gave **170** and in the final step the THP protecting group was removed under mild conditions furnishing LPC **160** (**Scheme 4.1**, (vi)-(vii)).[252,253]

Apart from the reported high yields, the route also offers potential for diversification of the lipid chain and preliminary evidence was provided to suggest that fatty acids substituted with fluorescent reporter groups could be incorporated into the synthesis.

Earlier work by Lindberg *et al*. provided a shorter route to LPC **160**, starting from the commercially available epoxide **171** (**A**, **Scheme 4.2**).[254] The phosphocholine head group was added in three steps without isolation of any of the intermediates and the resulting epoxide **172** was isolated in 53% yield (**A**, **Scheme 4.2**, (i)). A subsequent epoxide ring opening with the caesium salt of the desired fatty acid, in this case palmitic acid, afforded LPC **160** in good yield (**A**, **Scheme 4.2**, (ii)). The optical rotation of **160** was found to be equal and of opposite sign to that published for the enantiomer and no regioselectivity issues were reported for this particular substrate under these conditions. However, regioisomers were observed under the same reaction conditions for a similar substrate. It was initially unclear whether the regioisomers were a result of acyl migration from the primary position to the secondary within product **175**, or direct nucleophilic attack of the carboxylate **174** at the more hindered carbon of the epoxide **173** during the reaction (**B**, **Scheme 4.2**). Respectively, the two mechanisms afford minor regioisomers with opposite stereochemistry, either **176a** or **176b**, so the optical purity of the regioisomers was examined by derivatisation with Mosher's acid and this suggested that acyl migration within the product **175** was responsible. In addition to being short, this route again offered scope for diversification of the lipid chain and the more detailed experimental data and characterisation, compared to that reported in the Hadju route, may make this route more accessible to those wishing to reproduce it.



Scheme 4.2: (**A**) The preparation of LPC **160** according to Lindberg *et al*.[254] *Reagents & conditions*: (i) *a*) $POCl_3$, DIPEA, $CHCl_3$, 0 °C, 2 h; *b*) Choline tosylate, pyridine, RT, 5 h; *c*) H_2O, RT, 1 h, 53% over 3 steps; (ii) $C_{15}H_{31}COOH$, $C_{15}H_{31}COOCs$, DMF, 80 °C, 26 h, 65%. (**B**) The stereochemistry of the minor regioisomer **176a/b** was used to determine the mechanism by which it had formed.

LPLs more generally have been found to mediate a wide variety of responses in biology, including those relating to cell signalling, cell survival, proliferation, invasion, wound healing and angiogenesis.[255,256,257] LPLs are also being investigated in oncology where they have been found to stimulate the growth, survival and migration of tumour cells from various sources.[258] The increasing frequency of these reports led D'Arrigo and Servi to publish a review on the various preparations of LPLs, both enzymatic and chemical, which included the two aforementioned syntheses as well as others that may be adapted for the synthesis of LPC **160**.[259]

4.1.5 Could an LPC derivative be used to investigate whether LPC forms a specific binding interaction with MscS?

Thus far, **Section 4.1** has introduced MCs with a particular focus on MscS, which has homologs across bacteria, fungi, yeasts and some plants but not animals. There are currently no reports to suggest that gating of MscS, which is necessary for the protection of bacteria in times of

osmotic stress, is a result of a specific binding interaction between MscS and a ligand. Most of those working in the field believe that gating of MCs in general is a result of perturbations within the surrounding membrane bilayer. However, for MscS to be realised as a therapeutic target in the development of antibiotics with novel modes of action, specificity would be necessary. That is to say, a drug candidate would need to act only on MscS, thereby reducing undesired side effects.

Affinity chromatography offers a method through which specific binding interactions can be investigated.[260] This high resolution technique is able to separate an individual protein (or group thereof) from a biochemical mixture based on its reversible interaction with a specific ligand that has been pre-immobilised onto a chromatography matrix (e.g. Affi-Gel®). An advantage of affinity chromatography is the unique opportunity it provides to separate a protein based purely on biological function or chemical structure. LPC **160** is one of only a few compounds that have been shown to activate MscS (as discussed above), although it is not known how this activation occurs. If it were possible to synthesise a form of LPC **160** that could be immobilised onto a chromatography matrix, it would become possible to investigate whether it participates in a specific binding interaction with MscS. If this was found to be the case, a binding site could be identified paving the way for the design of substrates that interact specifically with this site. This is explored further in the following Section.

4.2 Aims

An amino-derivative of LPC **160**, amino-LPC **177**, was identified as the target compound on the basis that it constitutes a good candidate for assessing whether LPC **160** binds specifically to MscS *via* affinity chromatography. The structure of amino-LPC **177**, alongside the parent compound LPC **160**, is shown in **Figure 4.6**. LPC **160** is commercially available, however, it was decided to synthesise it along amino-LPC **177** because the progress of the reactions, in particular the yields and characterisation data, could be compared directly to those in literature procedures. The terminal amine of amino-LPC **177** provides the necessary handle *via* which the compound can be immobilised for affinity chromatography. Positive results from such an assay would indicate the presence of a specific LPC binding site, consequently paving the way for elucidation of the mechanism of action of MscS and/or the design of small molecules that modulate its activity.

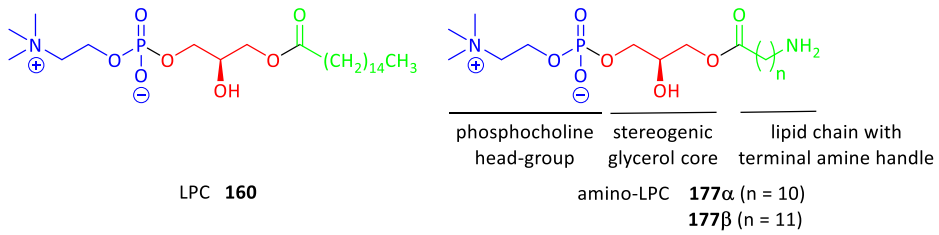


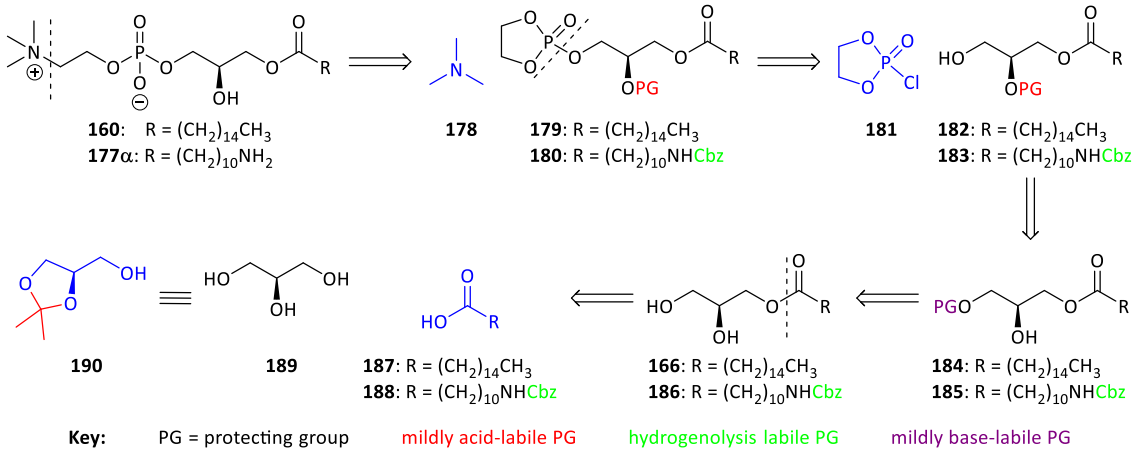
Figure 4.6: The structures of target compounds LPC **160** and amino-LPC **177**. The length of the lipid chain in amino-LPC **177** varies between n = 10 (**177α**) and n = 11 (**177β**) depending simply on what was available at the time of each synthesis, see **Schemes 4.12**, **4.16** & **4.17**.

4.3 Results and Discussion

4.3.1 Retrosynthesis of proposed compounds

Despite literature precedent for the synthesis of LPC **160**, syntheses of the target compounds **160** and **177** were expected to be challenging given their complex structural features.[259] A number of routes were therefore considered in anticipation of a difficult synthesis. Three retrosyntheses are outlined herein, named *route A*, *route B* and *route C*. *Routes A* and *B* were largely based on existing literature procedures for the synthesis of LPC **160** whilst *route C* combined aspects from both *A* and *B*.[249,254]

Route A builds on an existing literature procedure by Bibak *et al*., which adopted a fairly severe protecting group strategy in pursuit of lysophosphatidylcholines.[249,251] The choline moiety was added towards the end of the synthesis, an attractive prospect given that the undesirable solubility of this moiety was expected to make the compounds containing it difficult to purify. A retrosynthetic analysis of LPC **160** and amino-LPC **177α** in accordance with *route A* is provided in **Scheme 4.3**.



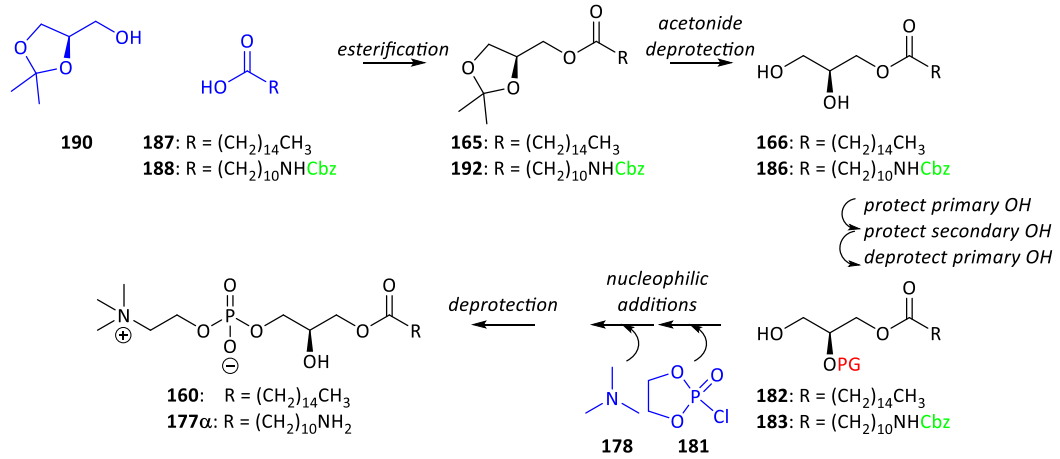
Scheme 4.3: A retrosynthetic analysis of target compounds **160** and **177α** in accordance with *route A*. Commercially available compounds are shown in blue.

Taking amino-LPC **177α** as the example, if the choline moiety was to be added last it followed that the first disconnection of **177α** was across the C-N bond shown, affording the nucleophile trimethylamine **178** (commercially available in solution or as the hydrochloride salt) and a dioxaphospholane **180**. Disconnection of the P-O bond on the glycerol side of this dioxaphospholane afforded the electrophilic species 2-chloro-2-oxo-1,3,2-dioxaphospholane **181** and a deceptively simple looking mono-esterified alcohol **183**. However, protecting group chemistry came into play at this point. Differential protection of the two alcohols in **186** alleviates concerns regarding regioselectivity during formation of dioxaphospholane **180** – whether this be during the nucleophilic addition of **183** to **181** or possible phosphate migration within **180** itself. Of course, with an ester present in **186** it was important that the two protecting groups be cleavable under mild conditions to avoid ester hydrolysis. In Bibak's work a THP group protected the secondary alcohol (**169**) and an Fmoc group protected the primary (**167**). Regardless of the protecting groups, the starting point for this differential protection was deemed to be diol **186**.

A final disconnection across the C-O bond of the ester in diol **186** afforded the primary alcohol-containing **189** and carboxylic acid **188**, in the forward synthesis a straightforward DCC coupling would be sufficient to esterify these two components. However, in order that only the desired alcohol in **189** reacted under DCC coupling conditions it would be necessary to have the other two protected. A suitable, commercially available, starting material was therefore **190**, a masked chiral glycerol, in which two of the alcohols were simultaneously protected as an acetonide. After the DCC coupling the acetonide could be removed under mildly acidic conditions affording the diol **186**, which was then predisposed for the differential protection outlined above to give **183**. For amino-LPC **177** the ideal carboxylic acid was 15-aminopentadecanoic acid, this was not, however, commercially available and **Scheme 4.3**

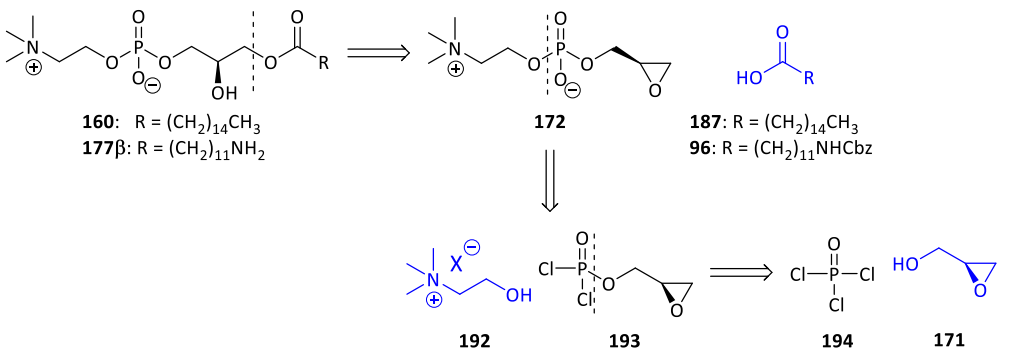
was therefore constructed with Cbz-protected 11-aminoundecanoic acid **188**, which was easily synthesised. To prevent side reactions throughout the synthesis, as well as to aid purification, it was logical to protect the amine in the parent compound, 11-aminoundecanoic acid. With acid or base labile groups already present throughout the synthesis, a group that could be removed under orthogonal conditions was necessary. A Cbz group seemed appropriate for two reasons; it could be cleaved under hydrogenolysis conditions as the end of the synthesis and it conferred UV activity on all intermediates, thereby facilitating reaction monitoring and purification.

In summary, a somewhat complex *route A* begins with a DCC coupling between a masked chiral glycerol, acetonide **190**, and the carboxylic acid of the desired lipid chain **188** (**Scheme 4.4**). Removal of the acetonide from the resultant ester **192** gives diol **186**. Differential protection of the two alcohols allows the synthesis of primary alcohol **183**. Two nucleophilic additions install the phosphocholine head-group, followed by the removal of the remaining protecting groups to afford target compound **177α**. *Route A* is long yet thorough, taking care to account for possible phosphate migration. In the first instance, it was attempted racemically in order to avoid the consumption of expensive starting materials.



Scheme 4.4: Summary of *route A* from Bibak et al.[249]

Route B, a literature procedure by Lindberg *et al.*, is the shortest of the three potentially requiring only two purification steps (**Scheme 4.5**).[254] In the case of amino-LPC **177**, *Route B* not only sees the phosphocholine head group attached before the ester harbouring the lipid chain, setting it apart from routes A and C, it also uses just one protecting group – the Cbz group required to protect the amine on the lipid chain.



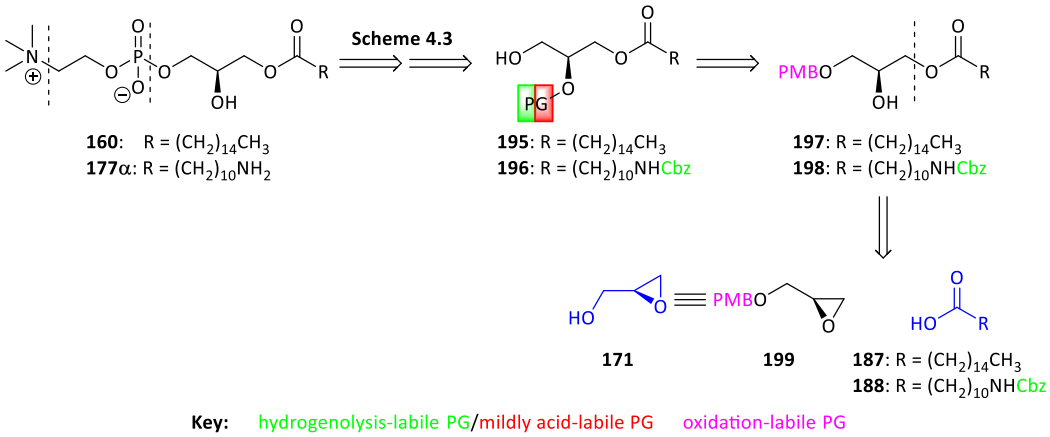
Scheme 4.5: A retrosynthetic analysis of target compounds **160** and **177β** in accordance with *route B*.[254] Commercially available compounds are shown in blue.

Disconnection of amino-LPC **177β** across the C-O bond adjacent to the ester afforded epoxide **172** and carboxylic acid **96** (**Scheme 4.5**). Ester formation in the forward direction was possible *via* nucleophilic attack of **96** on **172**, such that both of the oxygen atoms constituting the ester are derived from the parent carboxylic acid. A disconnection across the P-O bond on the choline side of **172** furnished choline **192** itself (commercially available as a variety of salts) and the phosphorus species **193**. The phosphorus species was not isolated in the literature procedure, presumably because it is a fairly reactive intermediate, so disconnection at the P-O bond shown gave rise to the necessary starting materials, phosphorus oxychloride **194** and an epoxy alcohol **171**.

Route B is attractive because it is short, with few protecting groups and little purification. However, there are a number of steps between the starting epoxy alcohol **171** and the phosphocholine epoxide **172** where no intermediates are isolated. It was anticipated that

these steps might be difficult to monitor since the intermediates were expected to be highly reactive with unpredictable polarities and little UV activity. It was also unclear whether the epoxide opening would occur with high regioselectivity.

A final route, *route C* (**Scheme 4.6**), combined aspects from both *routes A* and *B*. In the same manner as *route A*, the phosphocholine head group was added last following the same epoxide opening found in *route B*. *Route C* is able to circumvent the need for the differential diol protection found in *route A via* adoption of an alternative protecting group strategy.



Scheme 4.6: A retrosynthetic analysis of target compounds **160** and **177α** in accordance with *route C*. Commercially available compounds are shown in blue.

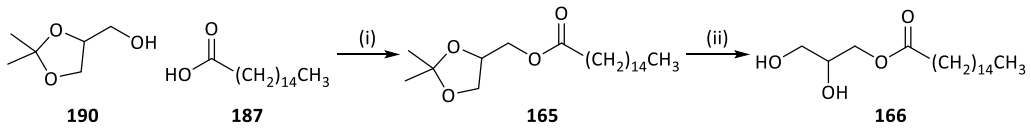
Compounds **160** and **177α** can be disconnected as in *route A*, first across the C-N bond and then across the P-O bond to give **195** and **196**, respectively (**Scheme 4.6**). Again using amino-LPC **177α** as the example, the secondary alcohol in **196** is protected such that regioselectivity problems and phosphate migration are avoided during the phosphocholination. Retreating one step further gives the protected primary alcohol **198**. This time, however, the protecting group is installed much earlier in the synthesis. A disconnection across the C-O bond adjacent to the ester bond gives rise to carboxylic acid **188** and epoxide **199**, in a similar fashion to *route B*. Conveniently, epoxide **199** already has the

protecting group for the primary alcohol in place thanks to a straightforward protection of the solitary primary alcohol in commercially available epoxide **171**.

Route C is marginally shorter than *route A* and does not require differential protection of a diol. Although the diols in question should contain alcohols of differing reactivity by virtue of one being primary and one being secondary, as shown by Bibak *et al.*, it seemed sensible to have *route C* as a backup since any regioselectivity issues would adversely affect purification and yields. *Route C* deals with regioselectivity early on, thereby avoiding the potential loss of precious material. Additionally, coupling the phosphocholine head group towards the end of the synthesis limits potential solubility problems, which would otherwise impact on purification and yields.

4.3.2 Towards the synthesis of LPC and amino-LPC *via* Route A

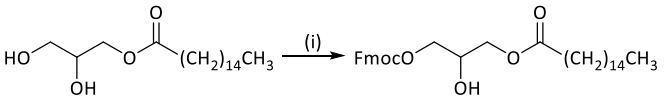
As discussed in **Section 4.2**, *route A*, with its thorough protecting group strategy, constituted the first attempts at the synthesis of target compounds **160** and **177**. In order to assess the routes suitability, it was first performed racemically, in pursuit of the known target compound LPC **160**. The synthesis began with a DCC-mediated esterification between (±)-1,2-isopropylideneglycerol **190** and palmitic acid **187**, which reliably afforded ester **165** in good yield (**Scheme 4.7**, (i)). A subsequent acetonide deprotection, in mild acid to avoid ester hydrolysis, provided diol **166** following lyophilisation and crystallisation, in accordance with the literature (**Scheme 4.7**, (ii)).[249]



190 **187** **165** **166**

Scheme 4.7: Synthesis of diol **166**, a key intermediate in *route A*. *Reagents & conditions*: (i) (±)-1,2-Isopropylideneglycerol **190** (1.50 eq.), $CH_3(CH_2)_{14}COOH$ **187** (1.00 eq.), DCC (1.15 eq.), DMAP (0.10 eq.), CH_2Cl_2, RT, 24 h, 83%; (ii) **165** (1.00 eq.), 0.4 M HCl in 90 % (v/v) dioxane/distilled H_2O, RT, 18 h, 70%.

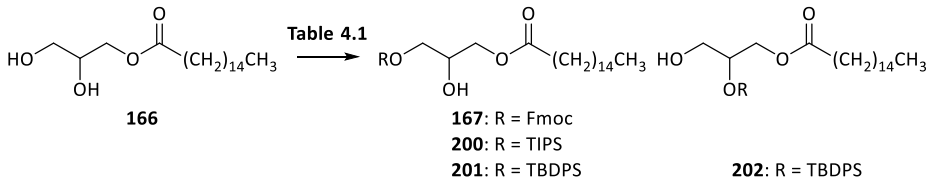
Problems arose at the next stage when it was necessary to protect the primary alcohol of **166**. The literature procedure obtained a 58% yield of the Fmoc carbonate **167** following reaction of Fmoc chloride with a two-fold molar excess of diol **166** (**Scheme 4.8**). Unfortunately, after two attempts, it was clear that this transformation was not going to work. TLC and MS analysis indicated some form of decomposition since neither the Fmoc carbonate **167** nor the starting diol **166** were visible when monitoring the reaction (**Scheme 4.9** and **Entry 1**, **Table 4.1**). With attempts at Fmoc protection failing it was decided to evaluate alternative protecting groups



166 **167**

Scheme 4.8: Literature procedure for the selective Fmoc protection of **166** using Fmoc-Cl.[249] *Reagents & conditions*: (i) Fmoc-Cl (1.00 eq.), **167** (2.00 eq.), DMAP (1.00 eq.), CH_2Cl_2, −10 °C, 30 min, 58%.

and ‘bulky’ silyl groups were identified as the leading candidates.



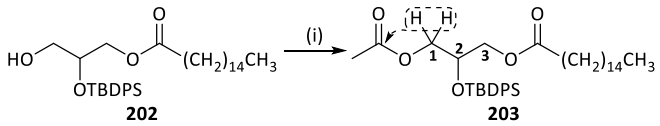
Scheme 4.9: Selective protection of primary alcohol **166**. A screen of protecting groups and conditions are given in **Table 4.1**.

Table 4.1: A screen of protecting groups in pursuit of a selective protection of the primary alcohol within diol **166**. n = the number of times a transformation was attempted with slight modifications to the conditions provided. Only the conditions affording the best yield or observation are reported.

Entry	Reactants	*Reagents & Conditions*	Yield/Comments
1 (n = 2)	**166** (2.00 eq.) Fmoc-Cl (1.00 eq.)	DMAP (1.00 eq.) CH_2Cl_2, −10 °C, 30 min → 18 h	• Neither **167** nor **166** visible by TLC and MS. • Decomposition assumed.
2 (n = 1)	**166** (1.00 eq.) TIPSCl (1.20 eq.)	Imidazole (2.50 eq.) DMF, 0 °C → RT, 30 min → 3h	• Complex mixture of products observed.
3 (n = 10)	**166** (1.00 eq.) TBDPSCl (1.25 → 1.65 eq.)	DMAP (0.10 eq. → 0.20 eq.) Et_3N (1.25 eq. → 1.65 eq.) CH_2Cl_2, RT, 10 h → 48 h	• **201**: 26% **202**: 8% (TBDPS on secondary OH) • Poor yield • Regioselectivity problem

Unfortunately, the reaction of the diol **166** with chloro(tri*iso*-propyl)silane provided only a complex mixture of products (**Entry 2**, **Table 4.1**). Rather than trying to optimise this reaction, the silylating agent was immediately switched to the much bulkier chloro(*t*-butyl)diphenylsilane (**Entry 3**, **Table 4.1**). This reaction was unreliable, the first couple of attempts afforded no product at all (including one attempt at 0 °C to encourage the desired regioselectivity). However, with persistence, a further attempt, carried out using fresh chloro(*t*-butyl)diphenylsilane and freshly distilled triethylamine, provided some of the desired product **201** after two days and a second addition of silylating agent. Frustratingly, the yield was low and ^{1}H NMR analysis suggested the minor product, which ran very close to the major

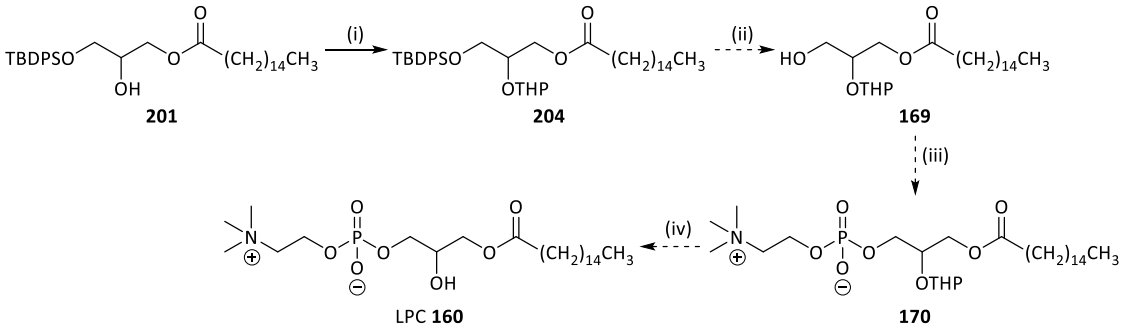
on TLC, was the regioisomer **202** – that with a silyl group on the secondary alcohol. **202** was acylated and HMBC analysis of the product **203**, confirmed the regiochemistry (**Scheme 4.10**). Attempts at this silylation using DMAP and the base imidazole, in place of triethylamine, returned only starting material.



Scheme 4.10: Acylation of **202** to confirm the regiochemistry. As expected, a HMBC signal from C(1)$\mathbf{H}_2$ → **C**=O was observed. *Reagents & conditions*: (i) **203** (1.00 eq.), Ac_2O (50.00 eq.), Pyridine, 0 °C → RT, 6 h, 81%.

It was unclear whether the secondary silylated species **202** formed as a result of direct silylation of the secondary alcohol or *via* silyl migration from the primary position. However, given the substantial 'bulk' of the TBDPS group, the presence of base in the reaction and in line with some investigations in the literature, it was presumed to be the latter.[261] Attempts to reduce silyl migration by limiting exposure to increased temperatures during rotary evaporation had no effect and, as noted above, reducing the temperature to 0 °C stalled the reaction completely.

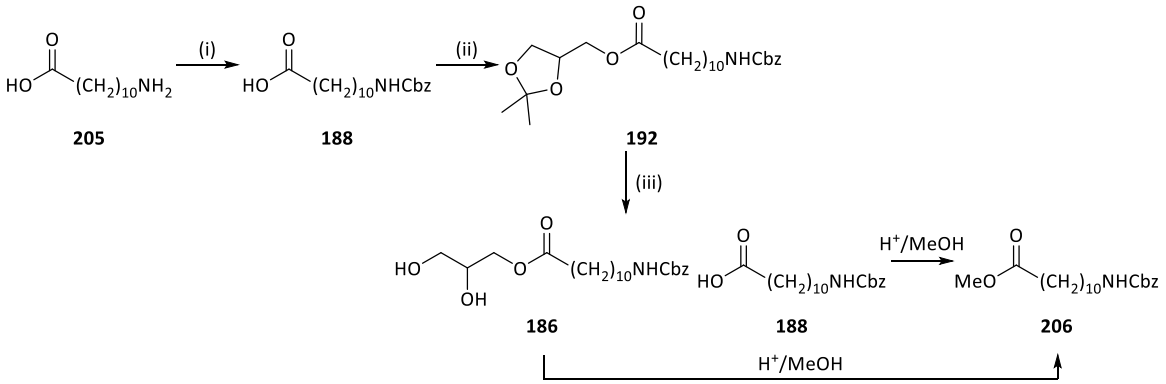
While attempts at optimising the silylation continued, the small amount of material that had been isolated was progressed to the next stage of the synthesis. Returning to the literature procedure, the next step was to perform a THP protection of the remaining secondary alcohol in **201** (**Scheme 4.11**, (i)). Again, this transformation proved problematic, affording the desired fully-protected compound **204** in only 18% yield. There appeared to be a close-running impurity by TLC, which was assumed to be the fully-protected regioisomer forming as a result of incomplete removal of the regioisomer **202** from the previous silylation step.



Scheme 4.11: Latter stages of the synthesis of LPC **160** according to *route A*. *Reagents & conditions*: (i) **201** (1.00 eq.), 3,4-dihydro-[2*H*]-pyran (1.50 eq.), pyridinium 4-toluenesulfonate (0.10 eq.), CH_2Cl_2, RT, 24 h, 18%; (ii) **204** (1.00 eq.), TBAF (0.50 eq.), THF, pH 7, 25 °C, 5 min → 24 h; (iii) *a*) **169** (1.00 eq.), 2-chloro-2-oxo-1,3,2-dioxaphospholane (1.20 eq.); triethylamine (1.40 eq.), benzene, 5 °C, 24 h; *b*) Me_3N (excess), MeCN, 65 °C, 24 h; (iv) **170** (1.00 eq.), 0.15 M HCl in dioxane (50% aq.), RT, 2 h.

Following two consecutive low-yielding reactions, regioselectivity issues and with only a small amount of material in hand for the three remaining steps of the synthesis (**Scheme 4.11**, (ii)-(iv)) *route A*, selected because of its thoroughness, was beginning to lose its appeal. However, it seemed sensible to evaluate the efficiency of these steps in the context of amino-LPC **177** since it would be useful to know whether the presence of the amine at the end of the lipid chain altered the reactivity of the various intermediates.

Running in parallel to the *route A* synthesis of LPC **160**, was therefore the synthesis of amino-LPC **177α** *via* the same route. Since the lipid chain of amino-LPC **177α** contained an amine at the 11-position, the synthesis began with 11-aminoundecanoic acid **205**. It was necessary to protect the amine, and reaction with Cbz chloride afforded the Cbz-protected amine **188** in 38% yield (**Scheme 4.12**, (i)). The low yield was a result of the poor solubility of 11-aminoundecanoic acid **205** in aqueous sodium hydroxide, however, the reaction had been performed on a large enough scale that plenty of material had been isolated, so time was not spent optimising this step.

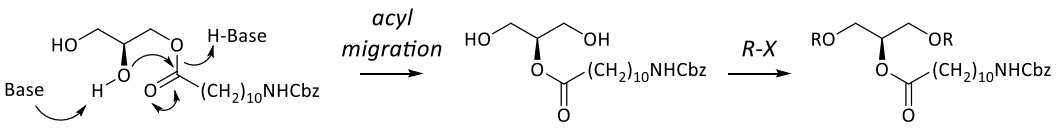


Scheme 4.12: Synthesis of diol **186** *en route* to amino-LPC **177α**. *Reagents & conditions*: (i) 11-Aminoundecanoic acid **205** (1.00 eq.), benzyl chloroformate (1.00 eq.), 2 M NaOH aq., RT, 48 h, 23%; (ii) **192** (1.00 eq.), (±)-1,2-isopropylideneglycerol **190** (1.50 eq.), DCC (1.15 eq.), DMAP (0.10 eq.), CH_2Cl_2, RT, 24 h, 76% ; (iii) **192** (1.00 eq.), 0.4 M HCl in 90% (v/v) dioxane/distilled H_2O, RT, 18 h, 38%.

As above, a DCC-mediated esterification between **188** and (±)-1,2-isopropylideneglycerol **190** afforded ester **192** in good yield (**Scheme 4.12,** (ii)). The acetonide deprotection proved more problematic than that of the analogous species **165** (**Scheme 4.12**, (iii)). Unlike in the synthesis of diol **166**, it was noticed that significant ester hydrolysis occurred, with acid **188** clearly visible by TLC and MS. Following lyophilisation, crystallisation of the crude material from methanol afforded significant amounts of methyl ester **206.** Unlike previously, evaporation and lyophilisation had been ineffective at removing all of the acid used to effect the deprotection. In addition to the potential for transesterification of the desired product **186**, the residual acid and the elevated temperature of the crystallisation were believed to be responsible for the esterification of the by-product **188**, affording significant amounts of the corresponding methyl ester **206** (**Scheme 4.12**). A modified work up and purification protocol was investigated involving all rotary evaporation being carried out at room temperature, an aqueous workup to remove the acid and reluctantly, since lysophosphatidylcholines have a reputation for undergoing acyl migration on silica supports, column chromatography.[262,263,264] This allowed isolation of diol **186**, albeit in low yield. It was subsequently concluded that most of the ester hydrolysis was happening during the reaction rather than during the work up. The

ester contained within the diol **186** was seemingly much more susceptible to acid-catalysed hydrolysis and transesterification than the free lipid analogue **166** and attempts to increase the yield by limiting this undesired reactivity were futile.

Despite the problems, with a small amount of the diol **186** in hand, the next step was again to pursue differential protection of the primary alcohol. The greater lability of the ester within diol **186** compared to diol **166** raised concerns about possible diprotection in this already troublesome transformation. All of the potential protections required the presence of base to quench the evolved hydrogen chloride (**Scheme 4.14** and **Table 4.2**). Base-induced acyl migration of this seemingly labile ester was therefore a tenable possibility.[173,265] Migration of the acyl group from the primary alcohol to the secondary alcohol affords a regioisomer of **186** with two primary alcohols **207**. In fact, the molecule becomes symmetrical and both of the primary alcohols possess the same reactivity thereby making diprotection, to give a compound of general structure **208**, a real possibility (**Scheme 4.13**).

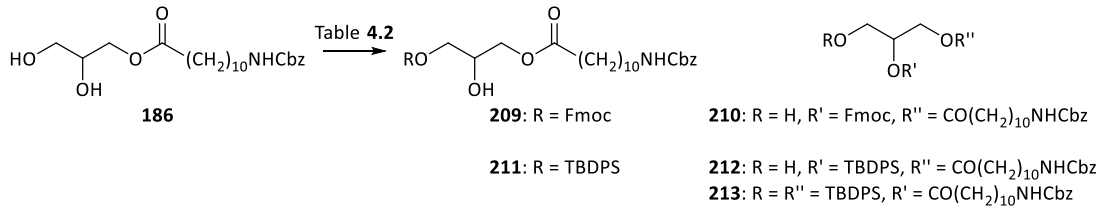


186 **207** **208**

Scheme 4.13: Base-induced acyl migration from the primary alcohol to the secondary alcohol in diol **186**, affording the symmetrical diol **207**. Both alcohols in **207** possess equal reactivity and can therefore react to form a species of the general structure in **208**.

Unlike with the free lipid diol **166**, reaction of **186** with Fmoc chloride succeeded in affording some of the primary protected carbonate **209** (**Scheme 4.14** and **Entry 1**, **Table 4.2**). The yield, however, was low as the reaction did not go to completion but, with concerns about acyl migration and/or diprotection, increasing the equivalents of Fmoc chloride and base was undesirable. A small amount of what was presumed to be the secondary Fmoc-protected

alcohol **210**, because of an appropriate mass spectrum and its proximity to **209** on TLC, was isolated but not characterised.



186

209: R = Fmoc

211: R = TBDPS

210: R = H, R' = Fmoc, R'' = $CO(CH_2)_{10}NHCbz$

212: R = H, R' = TBDPS, R'' = $CO(CH_2)_{10}NHCbz$

213: R = R'' = TBDPS, R' = $CO(CH_2)_{10}NHCbz$

Scheme 4.14: Differential protection of diol **186**. *Reagents & conditions* are provided in **Table 4.2**.

Table 4.2: Summary of conditions used to effect a differential protection of the primary alcohol in **186**.

Entry	Reactants	*Reagents & conditions*	Yields
1	**186** (1.00 eq.) Fmoc-Cl (1.10 eq.)	DMAP (0.10 eq.), Pyridine (1.10 eq.) CH_2Cl_2, RT, 20 h	**209**: 25% **210**: ~5%
2	**186** (1.00 eq.) TBDPS-Cl (1.20 eq.)	Imidazole (1.20 eq.) CH_2Cl_2, 0 °C → RT, 16 h	**211** 14% (desired) **212** 22% **213** 10%

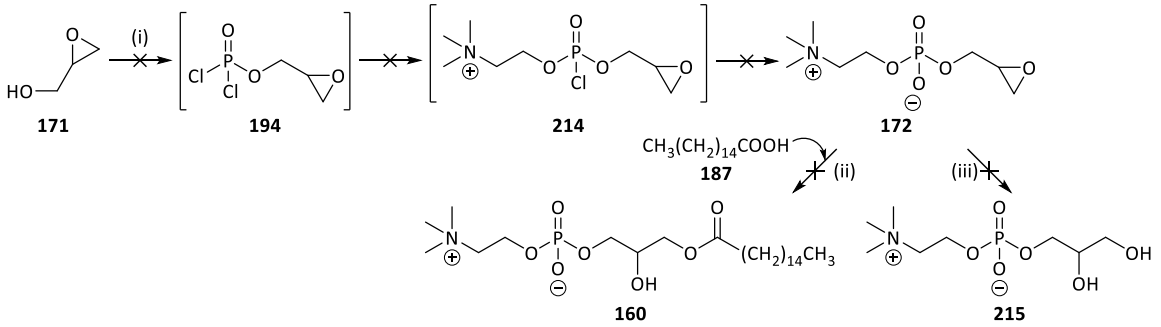
Despite the relative success of the Fmoc protection when compared to the LPC analogue **167**, it was decided to also try the silyl protection, which had been mildly successful on diol **166**. The yield was worse in the case of diol **186**, most of the material was isolated as the secondary silylated species **212**, indicating significant silyl migration. The anticipated diprotection, as a result of acyl-migration affording two primary alcohols, was also observed (**Entry 2**, **Table 4.2**). The symmetry in the ^{1}H NMR spectrum of compound **213**, particularly the overlapping peaks for the two methylene groups of the glycerol core, provided confirmation of the regiochemistry. It should be noted that compounds **211**, **212** and **213** were isolated in such small amounts that full characterisation could not be completed.

With the differential protections now proving difficult for both the LPC **160** and amino-LPC **177** analogues, a change of route was looking increasingly necessary. Additionally, the unfortunate

susceptibility of compound **186** and its successors to acyl migration, as well as the lability of the ester, were likely to be a continual cause of problems in future steps of the synthesis.

4.3.3 Towards the synthesis of LPC and amino-LPC *via* Route B

With *route A* failing it was decided to switch to *route B*. With so few steps, it was possible to determine quickly whether *route B* was going to provide the desired results. Unfortunately, *route B* also proved problematic (**Scheme 4.15**).



Scheme 4.15: Attempted synthesis of LPC **160** *via route B*. *Reagents & conditions*: (i) *a*) (*S*)-(±)-Glycidol **171** (1.00 eq.); $POCl_3$ (1.10 eq.), DIPEA (1.10 eq.), 0 °C, 2 h; *b*) Choline tosylate (1.20 eq.), pyridine, 0 °C → RT, 6-12 h; *c*) H_2O, RT, 1 h; (ii) **172** (1.00 eq.), **187** (2.20 → 1.50 eq.), $CsCO_3$ (1.00 eq.), DMF, 80 °C, 24 h; (iii) **172** (1.00 eq.), K_2CO_3 (0.62 eq.), H_2O, reflux, 5 h.

A first racemic attempt at this literature procedure for the synthesis of intermediate **172** from **171** looked promising until the purification stage (**Scheme 4.15**, (i)).[254] (±)-Glycidol was treated with phosphorus oxychloride to give **194**, followed by *in situ* treatment with choline tosylate to give species **214**. As highly reactive intermediates neither **194** nor **214** were isolated. Surprisingly, however, compound **214** was actually observed in the MS (*m*/z (ES)$^+$ 258 ([M]$^+$, 65%)). Compound **214** was subsequently stirred with water for one hour to displace the final chloride with a hydroxyl group supposedly affording compound **172**. Purification of **172**, *via* column chromatography with the vaguely described "70% ethanol" suggested by the literature (assumed to be 70% ethanol in water on normal phase silica) afforded none of the

desired product. All fractions were isolated and the column flushed yet still none of the desired product, even in crude form, was found. The same purification conditions were also attempted using reverse-phase silica to no avail. The tosyl counterion accompanying the choline swamped mass spectra, ^{1}H NMR spectra and TLC (because of its strong UV activity). Step (i) (**Scheme 4.15**) was repeated using a fresh bottle of phosphorus oxychloride, distilled di*iso*propylethylamine and distilled chloroform. After two hours of stirring with phosphorus oxychloride, glycidol **171** appeared consumed by TLC although the location of the product **194** on the TLC was unclear. The dimethyl phosphate ester of **194** (where the two chlorides had been displaced by the MS solvent methanol) was visible by MS (*m*/z (ES)$^{+}$ 205 ([M+Na]$^{+}$, 30%)) so choline tosylate was added to effect step (i*b*) (**Scheme 4.15**). After the suggested six hours there had been little change by TLC, however, **214** was visible in the MS with a stronger intensity than observed previously (*m*/z (ES)$^{+}$ 258 ([M]$^{+}$, 92%)). Step (i*b*) was left to run for up to twenty four hours in the hope that some changes would be visible by TLC. Little change occurred and the aforementioned intense sodium adduct peak disappeared from the MS, although a small peak potentially representing the ammonium adduct was visible. Appreciating that mass spectrometry can sometimes give unreliable spectra step (i*c*) (**Scheme 4.15**) was carried out anyway. No signs of **172** were visible by TLC although a very small peak for the acetonitrile adduct was visible *via* MS. Following thorough column chromatography it was again the case that no product was found. Two further attempts at these three steps saw the equivalents of the reagents increased and the base in step (i*a*) switched to triethylamine (as per another literature procedure) but unfortunately the reactions did not improve.[266] Reverse phase column chromatography was also attempted. Compound **172** was expected to fly well by mass spectrometry due to its charged nature, which may explain why small peaks pertaining to this compound were visible. In reality the

failure to isolate **172** *via* column chromatography suggested than only trace amounts were actually present. To confirm that **172** was not simply being lost, perhaps through decomposition during column chromatography, a crude batch was split and subjected to the reaction conditions required to synthesise compounds **160** and **215** (**Scheme 4.15**, steps (ii) & (iii) respectively).

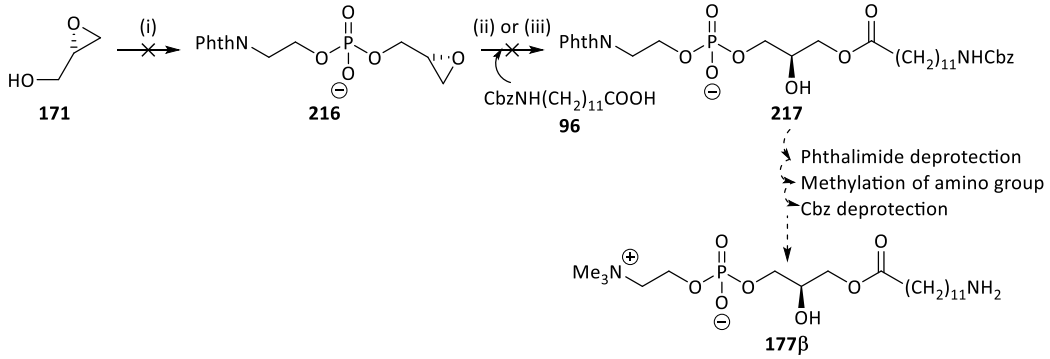
Ring opening of epoxide **172** was attempted with the caesium salt of carboxylic acid **187**, which was pre-formed by stirring the free acid with caesium carbonate.[254] The reactants were heated to 80 °C in DMF but even at this temperature there appeared to be solubility issues. It was therefore unsurprising to find that various attempts at this reaction did not afford the desired product **160**, returning only the carboxylic acid **187**.

When the ring opening of the epoxide **172** was unsuccessful, it was decided to see if base would open the epoxide to give diol **215** (**Scheme 4.15**, (iii)).[266] A second crude batch of the epoxide (the literature procedure also used the crude compound) was heated under reflux in the presence of aqueous potassium carbonate. The protocol then neutralised the reaction through the addition of 1% hydrogen chloride, however, the crude reaction solution already appeared to be neutral and again, the desired product **215** was not isolated.

It was clear that little progress was being accomplished with *route B* but, as with *route A*, the analogous reactions in pursuit of amino-LPC **177** were still investigated. It should be added that 12-aminododecanoic acid **56** became available at this point and its Cbz-protected form **96** was henceforth used in all *route B* attempts at synthesising amino-LPC **177β** (details of the Cbz protection can be found in **Section 2.3.6**).

Before the pursuit of amino-LPC **177β** *via route B*, a modification was proposed in an attempt to make the first three transformations easier to monitor (**Scheme 4.16**, (i*a-c*)). The choline

reagent (a tosylate salt) normally used in step (i*b*) was switched for *N*-phthalimide-protected ethanolamine. The reasons behind this were three-fold; the phthalimide possessed UV activity potentially allowing easier visualisation of intermediates by TLC, without the tosylate anion sensitivity in mass spectrometry was expected to improve since the tosyl group was previously found to be 'swamping' spectra and the phthalimide moiety was expected to confer solubility on, for example, species **216**, which it was hoped would aid purification.



Scheme 4.16: Attempted synthesis of amino-LPC **177** *via route B*. *Reagents & conditions*: (i) *a*) (*S*)-(−)-Glycidol **171** (1.00 eq.); $POCl_3$ (1.01 eq.), Et_3N (1.05 eq.), 0 °C, 50 min; *b*) *N*-(2-Hydroxyethyl)phthalimide (1.16 eq.), pyridine (1.57 eq.), RT, 12 h; *c*) H_2O, RT, 1 h; (ii) **216** (1.00 eq.), **96** (1.20 eq.), $CsCO_3$ (0.60 eq.), DMF, 60 °C, 16 h; (iii) **216** (1.00 eq.), **96** (1.10 eq.), TBABr (1.10 eq.), MeCN, 60 °C, 16 h.

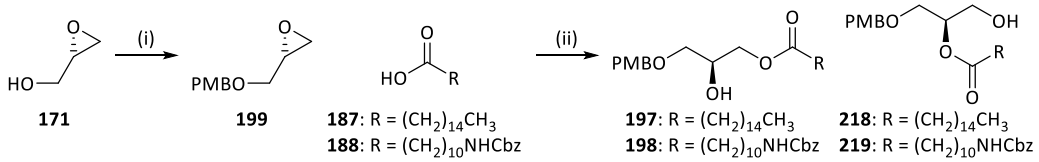
As in **Scheme 4.15**, the synthesis began with (*S*)-glycidol **171** (not racemic now in the interests of time). Reaction of **171** with phosphorus oxychloride, followed by *in situ* treatment with *N*-(2-hydroxyethyl)phthalimide (**Scheme 4.16**, (i*a*-*b*)) and subsequently water (i*c*), was supposed to afford epoxide **216**. Unfortunately, despite the modification, the synthesis did not improve and again **216** was not isolated after column chromatography. As above, to confirm that the epoxide was not simply being lost during purification, crude samples were carried through to the epoxide opening step.

The original conditions, namely treatment of epoxide **216** with the caesium salt of carboxylic acid **96**, were tested first but to no avail (**Scheme 4.16**, (ii)). A second set of conditions, used later in *route C*, were also screened. Epoxide **216** was treated with carboxylic acid **96** in the

presence of tetrabutylammonium bromide (**Scheme 4.16**, (iii)).[267] The outcome was unchanged and disappointingly, **217** was not isolated *via* either of the conditions. Discouraged by the lack of results in relation to *route B* it was again decided to pursue an alternative route.

4.3.4 Towards the synthesis of LPC and amino-LPC *via* Route C

Route C combines aspects of both *routes A* and *B*. As in *route A*, the strategy was to install the phosphocholine head group towards the end of the synthesis using the same proposed conditions (**Scheme 4.17**). However, instead of starting with acetonide **190** and arriving at diols **166** or **186** (which were troublesome to differentially protect), the synthesis would begin with an epoxide, which would eventually become the glycerol core. Ring-opening of this epoxide would be attempted using the same conditions screened in *route B*. To summarise, the lipid chain would be attached to the glycerol core first, using the conditions from *route B*, and the phosphocholine head group installed last, as in *route A*. In the interests of time it was decided that attempts at this synthesis not be carried out racemically.



171 **199** **187**: R = $(CH_2)_{14}CH_3$; **188**: R = $(CH_2)_{10}NHCbz$ **197**: R = $(CH_2)_{14}CH_3$; **198**: R = $(CH_2)_{10}NHCbz$ **218**: R = $(CH_2)_{14}CH_3$; **219**: R = $(CH_2)_{10}NHCbz$

Scheme 4.17: Ring-opening of epoxide **199**. *Reagents & conditions*: (i) **171** (1.00 eq.), 4-methoxybenzyl chloride (1.60 eq.), NaH (1.60 eq.), DMF, 0 °C → rt, 16 h, 80%; (ii) **199** (1.00 eq.), **187** (1.30 eq.)/**188** (1.50 eq.), TBAB (1.10 eq.); MeCN, 60-80 °C, 18-24 h, 82% by ^{1}H NMR for **197** or 88% by ^{1}H NMR for **198** (both contained minor amounts of regioisomer **218** or **219** respectively).

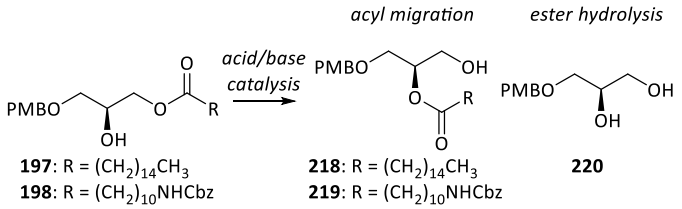
A transformation common to both LPC **160** and amino-LPC **177α** commenced the route. A PMB protection of the alcohol in (*S*)-(−)-glycidol **171** gave **199** in good yield (**Scheme 4.17**, (i)). No opening of the epoxide was observed during this reaction and, as a precaution, all rotary evaporation was carried out at room temperature. For both targets, the next step was to open

the epoxide in **199** *via* nucleophilic attack with a carboxylic acid possessing the appropriate lipid chain, **187** for LPC **160** and **188** for amino-LPC **177α**. Use of the caesium salt of the carboxylic acid to effect this transformation, as attempted in *route B*, was avoided because of the reported solubility issues. Instead, the second set of conditions screened in *route B*, namely treatment of epoxide **199** with the appropriate carboxylic acid (**187** or **188**) in the presence of the phase transfer catalyst tetrabutylammonium bromide, were assessed for their suitability (**Scheme 4.17**, (ii)).[267] It was pleasing to see that epoxide **199** reacted with both carboxylic acids.

The known compound **197** was synthesised first so that characterisation data could be compared.[268] Whilst the original literature reported that 0.31 equivalents of this catalyst were appropriate for the transformation, it was found here that larger amounts were necessary, in the end 1.10 equivalents were used. After twenty four hours, and a second addition of palmitic acid **187**, the starting material appeared to be consumed by TLC and the desired product **197** was visible by MS. A small peak pertaining to be the epoxide opened with bromide instead of the carboxylic acid was also apparent in the MS however, this compound was never isolated. Frustratingly, the crude TLC showed an impurity running just below the product spot which, after the problems experienced in *route A* and a similar observation in the literature, was presumed to be the regioisomer **218**.[269,270] Column chromatography confirmed these suspicions as it was very difficult to separate the two spots, yet the MS only ever contained a peak for the desired product **197**. Repeating column chromatography slowly afforded just enough of the pure product for characterisation and HMBC analysis confirmed the regiochemistry. The rest of the material was isolated and subsequently used as a 5:1 regioisomeric mixture of **197**:**218**, the combined yield of the two being 99%.

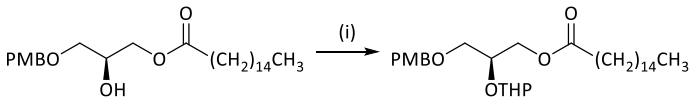
Using the optimised amount of “catalyst” (now 1.1 equivalents), synthesis of the amino-LPC intermediate **198** proceeded in much the same way (**Scheme 4.17**, (ii)). In a slight modification, the reaction temperature was decreased in the hope that this would supress the formation of the undesired regioisomer. As a result, a slightly larger excess of carboxylic acid **188** was used in an attempt to shorten the reaction time. These alterations made no difference as a 5:1 regioisomeric mixture of **198**:**219** was again isolated, in another combined yield of 99%. It is notable that in this case separation of the two regioisomers by column chromatography was much more difficult and isolation of even a small analytical sample of **198** was not possible. Attempts at preferentially crystallising one regioisomer over the other also failed.

Considering the difficulty in isolating large quantities of pure alcohol **198** it was decided that in the meantime the regioisomeric mixture could be used to begin screening conditions for protection of the secondary alcohol. As discussed in **Section 4.3.2**, compounds **197** and **198** are both susceptible to ester hydrolysis or acyl migration in conditions that are either too basic or too acidic (**Scheme 4.18**). It was therefore vital to establish conditions that would limit these two side reactions. **197** and **198** resemble intermediates **201** and **211** respectively, which had been difficult to isolate *via route A*. They are all secondary alcohols containing the central glycerol core, the lipid chain and a protecting group on the primary alcohol. Due to this resemblance, the first approach was therefore to remain with *route A* and attempt a THP protection of the secondary alcohol, which could be carried out in mild acid.



Scheme 4.18: **197** and **198** can undergo acyl migration and ester hydrolysis.

Reaction of **197** with 3,4-dihydro-[2*H*]-pyran was poor and increasing the equivalents of 3,4-dihydro-[2*H*]-pyran over a twenty four hour period failed to cause consumption of starting material (**Scheme 4.19**). Some of the desired product **221** was observed by MS, however, the crude TLC was complex and the ^{1}H NMR spectrum even more so. It was difficult to determine the success of this reaction due to the presence of the contaminating regioisomer, which, being a less hindered primary alcohol, was also expected to have reacted with 3,4-dihydro-[2*H*]-pyran. The presence of two stereogenic regioisomers that are each attached to a THP group possessing its own stereogenic centre meant that each regioisomer would exist as a pair of diastereoisomers. So, even before considering other possible by-products, there were already four compounds visible in this ^{1}H NMR spectrum. Given the complexity, optimisation of this reaction was halted until **197** could be exclusively isolated without the minor regioisomer **219**.



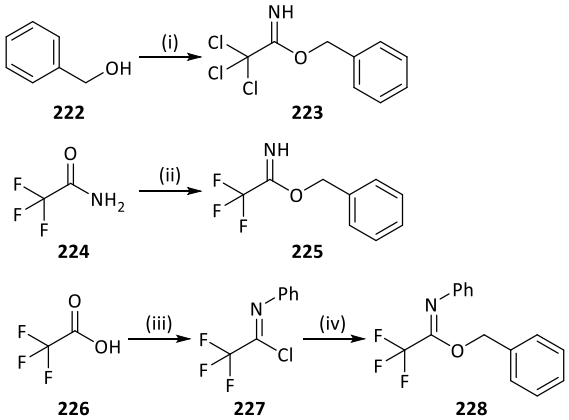
197 **221**

Scheme 4.19: THP protection of the secondary alcohol in **197**. *Reagents & conditions*: (i) **197**:**219** (1.00 eq.), 3,4-dihydro-[2*H*]-pyran (1.20 → 2.40 eq.), pyridinium 4-toluenesulfonate (0.20 eq.), CH_2Cl_2, RT, 4 h → 24 h. The reaction was carried out using the 5:1 regioisomeric mixture of **197**:**219**

Switching from a THP group to a benzyl group was an attractive prospect for a number of reasons. In the case of LPC **160**, once the PMB protecting group was removed all subsequent intermediates were to be left without UV activity, making the phosphocholination difficult to follow (as was shown in *route B*). A benzyl group on the secondary alcohol would provide a means of maintaining the UV activity. In the case of amino-LPC **177**, the lipid chain had a Cbz-protected amine at its terminus, which required removal at the end of the synthesis, likely by hydrogenolysis in the presence of catalytic palladium. If a benzyl group was attached to the

secondary alcohol of **197** then these two groups could be removed simultaneously with the same conditions, thereby reducing the number of steps in the synthesis.

It was apparent that traditional benzylation conditions, such as the use of a benzyl halide and sodium hydride in DMF, would be unsuitable as they would likely promote acyl migration. Alternative conditions were therefore necessary and an idea arrived to use benzyl groups activated as various acetimidates.[271,272,273] With these highly electrophilic reactants it was hoped that it would be possible to perform the benzylation under milder conditions and it was encouraging to see reports of benzyl acetimidates successfully benzylating ester-containing substrates where other conditions had failed.[274,275] To this end, three benzyl acetimidates, **223**, **225** and **228**, were synthesised from existing procedures, according to **Scheme 4.20**.[276,272,273]



Scheme 4.20: Synthesis of benzylacetimidates **223**, **224** and **225**. *Reagents & conditions*: (i) $PhCH_2OH$ **222** (1.00 eq.), CCl_3CN (10.00 eq.), DBU (0.40 eq.), CH_2Cl_2, 0 °C → RT, 3 h, >99%.; (ii) Trifluoroacetamide **224** (3.30 eq.), $(COCl)_2$ (3.00 eq.), $(CH_3)_2SO$ (10.00 eq.), CH_2Cl_2, −78 °C, 10 min then Et_3N (10.00 eq.), −78 °C, 30 min then $PhCH_2OH$ (1.00 eq.), DBU, −78 °C → RT, 16 h, 66%; (iii) CF_3COOH **226** (1.00 eq.), $PhNH_2$ (1.20 eq.), PPh_3 (3.00 eq.), Et_3N (1.20 eq.), CCl_4, 0 °C → RT, 34%; (iv) $PhCH_2OH$ **222** (1.00 eq.), **227** (1.10 eq.), NaH (1.50 eq.), CH_2Cl_2, 0 °C → RT, 2 h, 81%.

Naively, the first benzylation of **197**, with benzyl 2,2,2-trichloracetimidate **223**, was attempted in the presence of triflic acid (**Scheme 4.21** and **Entry 1**, **Table 4.3**).[277] Decomposition of **197** was instantaneous, the reaction changing rapidly from a pale yellow solution to a deep purple suspension. Evidently, triflic acid was far too strong an acid for a molecule as delicate as **197**

and, whilst the ester managed to remain intact in the anhydrous conditions, MS and TLC indicated loss of the PMB group.



197 **229**

Scheme 4.21: Attempted benzylation of alcohol **197**. A screen of reagents & conditions is provided in **Table 4.3**.

Table 4.3: *Reagents & conditions* assessed in pursuit of compound **229**.

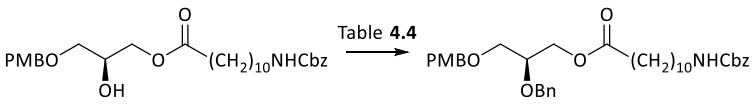
Entry	*Reagents & conditions*	Comments
1	**197**:**219** (1.00 eq.) Benzyl 2,2,2-trichloroacetimidate **223** (2.44 eq.) Triflic acid (1.22 eq.) CH_2Cl_2/Petrol, 0 °C → RT, 5 h	• Too acidic, immediate decomposition • Loss of PMB group evident in MS; *m/z* $(ES)^+$ 353 ($[M+Na]^+$, 100%)
2	**197**:**219** (1.00 eq.) Benzyl 2,2,2-trichloroacetimidate **223** (2.50 → 7.50 eq.) $Y(OTf)_3$ (1.20 eq.) Toluene, 4 Å molecular sieves, −78 °C → RT, 16 h → 32 h	• Reaction did not run to completion • Crude TLC indicated complex mixture • Complex ^1H NMR from 7 mg material,
3	**197**:**219** (1.00 eq.) Benzyl 2,2,2-trichloroacetimidate **223** (3.00 → 6.00 eq.) $Y(OTf)_3$ (1.20 → 1.50 eq.) CH_2Cl_2, 4 Å molecular sieves, −78 °C → RT, 1.5 → 22 h	• Changing the solvent had little effect
4	**197**:**219** (1.00 eq.) Benzyl 2,2,2-trichloroacetimidate **223** (2.00 → 4.00 eq.) $BF_3{\cdot}OEt_2$ (0.40 → 0.80 eq.) CH_2Cl_2, −78 °C → RT, 16 → 24 h	• No reaction, starting material recovered

In subsequent attempts, triflic acid was switched for a milder Lewis acid since a number of metal-based Lewis acids had been reported to be effective at promoting this transformation.[273] Initially, only yttrium triflate was available so investigations began there. A first attempt at benzylation of **197** with benzyl 2,2,2-trichloroacetimidate **223** and yttrium triflate was inconclusive (**Entry 2**, **Table 4.3**). TLC showed little, except breakdown of the acetimidate **223**. To account for this, a large excess of **223** was eventually added to try to push the reaction to completion. However, consumption of the starting material was never achieved and the little material that was recovered following column chromatography was disappointing. Mass spectrometry provided no evidence that the desired product **229** was

present, the aromatic region of the ^{1}H NMR spectrum over-integrated and the usually distinguishable protons of the glycerol core were difficult to identify. The over integration of the aromatics was possibly due to the large excess of acetimidate **223**, it seemed that its decomposition products, such as benzyl alcohol **222**, (which itself could react with acetimidate **223**) were difficult to remove from other products of interest. An identical reaction but with only two equivalents of acetimidate **223** throughout (not shown in **Table 4.3**) contained some of the desired product **229** in the crude MS. After column chromatography, the small amount of isolated material was again heavily contaminated with aromatic impurities possessing similar polarities to **223**, as indicated by TLC analysis.

Unsure as to what was happening during the benzylation, the solvent was switched from toluene to dichloromethane to investigate the effect (**Entry 3**, **Table 4.3**). After four hours, the MS showed mainly starting material and some product so, additional acetimidate **223** was added to account for the breakdown visible by TLC and after eight hours extra yttrium triflate was also added. The reaction failed to go to completion after a day but nevertheless the prolonged presence of **229** in the MS was encouraging. Following work up and purification, material recovery was poor and the problem of over representation of the aromatic region in the ^{1}H NMR spectrum persisted. It could only be assumed that what was happening during the benzylation was complex. The regioisomeric substrate and its potential to undergo acyl migration or ester hydrolysis gave rise to a number of possible alcohols all of which could undergo benzylation affording a number of species all with similar polarities. This would explain the complexity of the ^{1}H NMR and the difficulties faced when attempting to purify the crude reaction mixture.

Employing boron trifluoride diethyletherate as the Lewis acid failed to promote any reaction whatsoever and, after a day which included increasing the equivalents of Lewis acid and acetimidate **223**, both **197** and **229** were the only visible components by TLC (**Entry 4**, **Table 4.3**). In fact, it was interesting to note that, unlike in the previous cases, little breakdown of acetimidate **223** was observed over the reaction period.



198 **230**

Scheme 4.22: Attempted benzylation of **198**. A screen of *reagents & conditions* is provided in **Table 4.4**.

Table 4.4: *Reagents & conditions* assessed in pursuit of target compound **230**.

Entry	*Reagents & conditions*	Comments
1	**198**:**219** (1.00 eq.) Benzyl 2,2,2-trichloroacetimidate **223** (6.00 eq.) $Y(OTf)_3$ (1.50 eq.) CH_2Cl_2, −78 °C → RT → 40 °C, 21 → 40 h	• Reaction did not run to completion • Crude TLC was complex
2	**198**:**219** (1.00 eq.) Benzyl 2,2,2-trifluoroacetimidate **225** (1.50 → 3.00 eq.) $Y(OTf)_3$ (1.20 eq.) CH_2Cl_2, RT → 40 °C, 1 → 24 h	• No reaction, starting material recovered
3	**198**:**219** (1.00 eq.) Benzyl-(*N*-phenyl)-2,2,2-trifluoroacetimidate **228** (1.10 eq.) $Y(OTf)_3$ (0.10 eq.) Dioxane, 5 Å molecular sieves, RT, 20 min → 6 h	• No reaction, starting material recovered
4	**198**:**219** (1.00 eq.) Benzyl-(*N*-phenyl)-2,2,2-trifluoroacetimidate **228** (1.10 eq.) $Yb(OTf)_3$ (0.10 eq.) Dioxane, 5 Å molecular sieves, RT, 20 min → 6 h	• Reaction did not run to completion • Breakdown of acetimidate observed • Integration in ^{1}H NMR reasonable

Attempted benzylation of the amino-LPC intermediate **198** afforded much the same story (**Scheme 4.22**). The reagents and conditions assessed are provided in **Table 4.4**. As above, the first approach was to treat **198** with benzyl 2,2,2-trichloracetimidate **223** in the presence of yttrium triflate (**Entry 1**, **Table 4.4**). From the outset excess acetimidate was added, thereby allowing better maintenance of the anhydrous environment and hopefully reducing its hydrolysis. After nearly 24 hours a promising product peak was visible in the MS, although the

main component was still starting material **198**. Rather than adding more acetimidate **223**, the reaction was heated to 40 °C but after 40 hours, TLC indicated little change in the proportions of starting material **198** and product **230**. The columned material again gave rise to a complex ^{1}H NMR spectrum with a 'busy' glycerol region. The MS of the NMR sample contained peaks pertaining to the product and a species where the PMB group was, surprisingly, replaced by a benzyl. The PMB group was not expected to be unstable under the mild reaction conditions although the application of heat may have been a factor in its demise.

Wanting to avoid the application of heat it was decided to try reactions between **198** and either benzyl 2,2,2-trifluoracetimidate **225** or (*N*-phenyl)-2,2,2-trifluoroacetimidate **228**, which are expected to be more reactive than **223** (**Entries 2** & **3**, **Table 4.4**). Unfortunately, little reaction occurred in both cases and despite the addition of more acetimidate only its breakdown was observed after 24 hours. The starting material was recovered from each reaction.

In a final attempt at benzylation, acetimidate **228**, first synthesised and assessed in a paper by Okada *et al.*, was again selected.[273] This acetimidate was reported to be more stable than benzyl-2,2,2-trichloracetimidate **223** suggesting that it should be less susceptible to hydrolysis during the timescale of the reaction. The catalyst was switched to ytterbium triflate, which had performed well in the original paper (**Entry 4**, **Table 4.4**). Decomposition of acetimidate **228** was observed by TLC and the reaction didn't go to completion, however, following work up and purification by column chromatography, a very small amount of material was obtained for ^{1}H NMR analysis. The NMR spectrum was not clean but it was very encouraging to see that the aromatic region integrated well and the relative integration of the three benzyl groups (from the PMB, benzyl and Cbz groups) was reasonable. This was certainly the best ^{1}H NMR

spectrum from all the attempts thus far and it was hoped that it would be possible to optimise these conditions further when purer samples of alcohols **197** and **198** were available.

4.4 Conclusions

The attempted syntheses of LPC **160** and amino-LPC **177** had been blighted by problems with acyl migration, ester hydrolysis and poor reproduction of literature procedures. Whilst some progress had been made, and *route C* appeared promising, it was apparent that even when the regioselectivity issues were resolved there was still the phosphocholination to contend with. In the remaining time it was therefore regrettably decided to focus on areas of the project that were delivering results more rapidly, namely the work detailed in **Chapters 2** & **3**.

4.5 Future Work

Amino-LPC **177** is clearly a difficult target compound. However, it remains a highly desirable probe for the investigation of the mechanism of action of MscS and future work will certainly build on the foundations in this chapter.

Aside from the amino-LPC **177** target herein, Dr Corrina Kaul, a post-doctoral research assistant within the Conway group, has synthesised LPC analogues lacking some of the characteristic features, such as the polar choline head-group, for SAR work. Collaborators at the Universities of Aberdeen and St Andrew's had also suggested that analogues of LPC **160** with bromide atoms at various points along the lipid chain could be used to probe MscS activity, these brominated lipids can interact with tryptophan residues mutated into MscS at strategic positions and cause a transfer of fluorescence. It was hoped that visualisation of this

fluorescence would provide information regarding the location of the interaction within MscS, however, the results gained since have proved inconclusive.

A further branch of the project moved away from LPC analogues altogether. Collaborators had also been able to perform a compound library screen in search of small molecule modulators of MscS activity. Compounds that performed well in this study also became candidates for SAR work.

5. Summary

This dissertation brings together 3 projects, all tailored towards the pursuit of antibiotics with novel modes of action, which are necessary to combat increasing antibiotic resistance worldwide. The modulation of bacterial ion channels was identified as a focus for developing these new antibiotics.

Chapter 2 detailed the successful syntheses of a number of fluorescent detergents that had been suggested as possible tools for improving membrane protein MS – a useful technique for studying membrane proteins such as bacterial ion channels. A number of novel, fluorescent compounds were synthesised, however, they have yet to be applied in MS studies.

Chapters 3 and **4** discussed the syntheses of some small molecule probes for the ion channels Kef and MscS respectively. In **Chapter 3** three tripeptides were successfully synthesised by SPPS and their biological activity was investigated using a competition fluorescence assay. The tripeptides were found to bind to Kef with a lower affinity than expected, suggesting the mechanism of activation proposed at the start of the project required further investigation.

In **Chapter 4** an analogue of LPC, predisposed for immobilisation and affinity chromatography, was required to investigate whether any specific binding interactions occur between MscS and LPC. Such information could be used to design modulators of MScS activity. To this end, the synthesis of amino-LPC was attempted. Despite assessing a number of different routes, the challenging structure of amino-LPC meant it was not possible to isolate it at this time.

6. Experimental

6.1 General Experimental

^{1}H NMR spectra were recorded on Bruker DPX300, Bruker AVIII400, Bruker AVII400, Bruker DRX500, Bruker AVII500 (with cryoprobe) and Bruker AVII700 spectrometers using the deuterated solvent specified as a reference for the internal deuterium lock. The chemical shift data for each signal are given as δ X.XX in units of parts per million (ppm) relative to tetramethylsilane (TMS) where δ (TMS) = 0.00 ppm. The multiplicity of each signal is indicated by: s (singlet); d (doublet); t (triplet); q (quartet); qn (quintet); dd (doublet of doublets); ddd (doublet of doublet of doublets); dt (doublet of triplets) or m (multiplet). A broadened signal is denoted by the prefix br (broad) where appropriate. The number of protons (n) for a given resonance signal is indicated by nH. 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.

^{13}C NMR spectra were recorded on Bruker DPX300 (76 MHz), Bruker AVIII400 (101 MHz), Bruker AVII400 (101 MHz), Bruker DRX500 (126 MHz), Bruker AVII500 (126 MHz, with cryoprobe) and Bruker AVII700 (176 MHz) spectrometers using a DEPT Q pulse sequence with broadband proton decoupling and internal deuterium lock. The chemical shift data for each signal are given as δ XX.X in units of parts per million (ppm) relative to tetramethylsilane (TMS) where δ (TMS) = 0.00 ppm. Where appropriate, coupling constants (*J*) are quoted in Hz and are recorded to the nearest 0.1 Hz.

^{19}F NMR spectra were recorded on Bruker AVIII400 (377 MHz) and Bruker AVII500 (470 MHz) spectrometers using a deuterated solvent as a reference for internal deuterium lock. The

spectra are decoupled and the chemical shift data for each signal are given as δ XX.X in units of parts per million (ppm) relative to trichlorofluoromethane (TCFM) where δ (TCFM) = 0.00 ppm.

NMR spectra were assigned using 2D NMR experiments including COSY, HSQC, HMBC and TOCSY and analysed using Bruker TopSpin software.

Mass spectra were acquired on either a Waters LCT Premier or an Agilent 6120 Quadruple spectrometer (low resolution) using electrospray ionization operating in positive or negative mode, from solutions of methanol, MeCN or water. *m/z* values are reported in Daltons and followed by their percentage abundance in parentheses. **High Resolution Mass Spectra** were acquired on a Bruker µTOF spectrometer using electrospray ionization as above.

Melting points were determined using a Leica Galen III hot stage microscope and are uncorrected.

Infrared Spectra were obtained from neat samples as solids or from solutions as thin films, using a diamond ATR module. The spectra were recorded on a Bruker Tensor 27 spectrometer. Absorption maxima (ν_{max}) are reported in wavenumbers (cm^{-1}) and assigned as s (strong), m (medium) or w (weak) and, where appropriate, br (broad).

Specific Optical Rotations were measured using a Perkin Elmer Model 341 polarimeter, in cells with a path length of 1 dm. The light source was maintained 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 deg cm^2 g^{-1} (T = ambient temperature in °C).

High-Performance Liquid Chromatography was carried out using a PerkinElmer Flexar system with a Binary LC pump and UV/VIS LC detector. For determination of compound purity a

Dionex Acclaim 120 column (C18, 5 µm, 120 Å, 4.6 × 150 mm) or a Supelcosil LC-Si (5 µm, 120 Å, 4.6 × 250 mm) were used with the protocols described in **Appendix I.I**. Determination of enantiopurity used a Daicel ChiralPak® (AD-H, 5 µm, 4.6 × 250 mm) column with the protocol described in **Appendix I.II**. Chromera software was used to determine purity and, where possible, enantiomeric excess from relative peak areas of UV/VIS absorbance at either 254 or 220 nm. When possible, samples were injected in a solution identical to that of the mobile phase, otherwise, $(CH_3)_2SO$ was used.

Microanalyses were obtained from the Elemental Analysis Service, London Metropolitan University, London.

Analytical thin layer chromatography (TLC) was carried out on normal phase Merck silica gel 60 F_{254} or reverse phase Merck silica gel 60 RP-18 $F_{254}S$ aluminium-supported thin layer chromatography sheets, unless otherwise stated. Visualisation was by absorption of UV light (λ_{max} 254 or 365 nm), exposure to iodine vapour or thermal development after dipping in one of: **a** ethanolic solutions of: phosphomolybdic acid (PMA), sulfuric acid or ninhydrin; **b** aqueous solution of potassium permanganate and hydrochloric acid.

Flash column chromatography was performed on a Biotage SP4® System using KP-Sil® cartridges (Biotage®) or carried out Manually on VWR Prolabo silica gel 60 (240-400 mesh), eluting with solvents as supplied, under a positive pressure of compressed air (Manual).

Anhydrous solvents were obtained under the following conditions: CH_2Cl_2 and toluene were dried by passing through a column of activated basic alumina according to the Grubbs procedure.[278] Anhydrous THF obtained by the same method was always distilled from sodium and stored over activated 3 Å molecular sieves before use. Anhydrous DMF, MeOH, *tert*-BuOH, pyridine and DMSO were purchased from SigmaAldrich UK in SureSeal™ bottles. With the

exception of *tert*-BuOH, all anhydrous solvents were decanted into a flame-dried flask containing activated 3 Å molecular sieves, and stored under argon for at least 12 h before use. Unless otherwise stated, all other solvents were used as supplied (analytical or HPLC grade) without purification. **Chemicals** were purchased from Apollo, Acros, Sigma Aldrich, Alfa Aesar, Fisher, Fluka, Fluorochem, Merck or TCI-Europe. **Purification** of reactants, reagents and solvents was, where necessary, achieved *via* standard techniques.[279]

Non-aqueous reactions were performed in a flame-dried flask or microwave vial under an atmosphere of argon, unless otherwise stated.

In vacuo refers to removal of volatile components under reduced pressure using Buchi™ rotary evaporators, with the water bath at a temperature of 40 °C unless otherwise stated.

Brine refers to a saturated aqueous solution of sodium chloride.

Petrol refers to the fraction of light petroleum ether boiling in the range 40-60 °C.

Lyophilisation refers to the removal of water from aqueous solutions by freeze drying and sublimation under high vacuum using a Christ Alpha 2-4-LD lyophilizer.

Solid Phase Synthesis Rotation was carried out on a SP2 Rotator (Stuart®) at a speed of 20 rpm. A Preppy™ 12-port vacuum manifold (Supelco) was used to remove the spent reaction solution from the resin.

The Kaiser test is a qualitative technique, based on the ninhydrin test, used to assess the presence of free amines.[211] During SPPS, the test was carried out after each Fmoc deprotection and each peptide coupling to follow the progress of the reactions. The reagents required for carrying out this test are; 5% ninhydrin in ethanol (w/v), 80% phenol in ethanol (w/v) and 2% 0.001 M KCN aq. in pyridine (v/v).[179] The method is as follows: a small sample of

resin beads (~5 mg) were removed from the reaction tube and washed thoroughly with ethanol. Following this, the beads were placed into an Eppendorf® vial and 100 µL of each of the above solutions were added to the beads using a micropipette. The vial was placed into a heating block at 120 °C for 5 min. After this time, a colour change from pale green to navy blue indicated the presence of free amines. No colour change suggested the absence of free amines.

Protein expression and purification was kindly performed by Sam Grayer, Conway Group, University of Oxford. Obtained from collaborators at the University of Aberdeen, the expression construct used was pTrc*Sd*KefH6, which contained the Kef gene from *Shewanella denitrificans* OS217 (accession number NC007954.1), encoding 608 amino acids with an additional C-terminal LEH6-tag. Sequencing by the Geneservice DNA sequencing facility in the department of Biochemistry, University of Oxford using the pBAD reverse sequence primer was performed to confirm the construct. pTrc*Sd*KefQCTD was transformed for expression into *E. coli* strain MJF373, which lacks the gene for the catabolite activator protein (CAP); a common contaminant in immobilized metal ion affinity chromatography. The protein was purified using a nickel affinity purification column and eluted protein was buffer exchanged using a PD-10 column (GE Healthcare) into 50 mM phosphate buffer, 150 mM NaCl, pH 7.2. The purity of protein was analysed by SDS-PAGE.

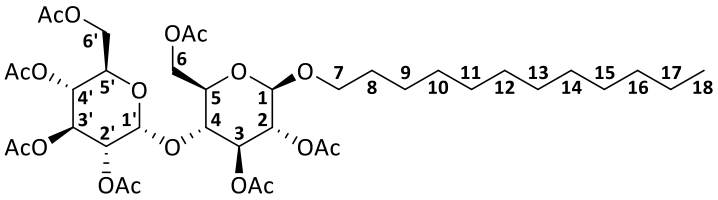
Competition fluorescence measurements were also kindly performed by Sam Grayer, Conway Group, University of Oxford. Measurements were obtained using an Edinburgh Instruments FLS920 Spectrometer in a 100 µL cuvette of 3 mm excitation and emission path lengths (Hellma GmbH & Co.). 150 µL samples were prepared containing *Sd*KefB (6 µM) and DNGSH (5 µM) in 50 mM phosphate buffer pH 7.4 and 150 mM NaCl. To each sample, the desired

competing ligand (1 mM) was added. Samples were excited with light of 340 nm and the emission spectra measured from 375-670 nm (the step was 1 nm, the dwell time 0.3 s, 3 repeats, the excitation slit was 1 nm, the emission slit 2 nm and the temperature was held at 20 °C). A decrease in the fluorescence intensity after the addition of the desired competing ligand, at a final concentration of 1 mM, was interpreted as an indication of binding. The data are represented as ratios of the fluorescence intensities before and after the addition of the tested compound at 525.5 nm (FB/FL).

6.2 Synthesis of fluorescent probes as tools for membrane protein mass spectrometry

6.2.1 Synthesis of fluorescent DDM analogues

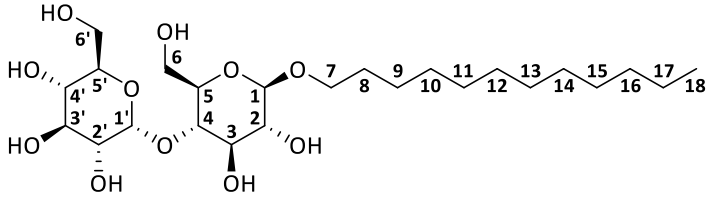
_n_-Dodecyl-β-D-maltose octaacetate (68)



β-D-maltose octaacetate **47** (500 mg, 0.74 mmol, 1.00 eq.) and 1-dodecanol **48** (247 µL, 206 mg, 1.11 mmol, 1.50 eq.) were dissolved in anhydrous toluene (10 mL) under an atmosphere of argon in a microwave vial. Zinc chloride (151 mg, 1.11 mmol, 1.50 eq.; pre-dried *in vacuo* at 110 °C for 10 min) was added, the microwave vial was sealed under argon and the resulting suspension heated to 65 °C in an oil bath for 1 h. After this time, the reaction mixture was quenched *via* the dropwise addition of sat. $NaHCO_3$ aq. (1.0 mL) then diluted with EtOAc (20 mL). The resulting suspension was stirred at RT for 10 min then filtered through Celite®, eluting with EtOAc (20 mL). The organic filtrate was washed with distilled H_2O (3 × 50 mL) and brine (3 × 50 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo* to give the crude product. The crude material was adsorbed onto Celite® and purified *via* flash column chromatography on silica gel (Manual; 2:3 EtOAc:Petrol) affording **68** (344 mg, 58%) as a colourless solid. $\mathbf{R_f}$: 0.63 (2:3 EtOAc:Petrol); $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\boldsymbol{20}}$: +28.0 (*c* 1.0, $CHCl_3$); **mp**: 78-80 °C ($CHCl_3$); **IR** (thin film) ν_{max} cm^{-1}: 2926 (w), 1753 (s), 1369 (w), 1233 (s); **^{1}H NMR** (500 MHz, $CDCl_3$): δ 5.40 (1H, d, *J* = 4.1 Hz, C(1')*H*), 5.34 (1H, dd, *J* = 10.1, 10.1 Hz, C(3')*H*), 5.23 (1H, dd, *J* = 9.3, 9.3 Hz, C(3)*H*), 5.03 (1H, dd, *J* = 10.1, 10.1 Hz, C(4')*H*), 4.84 (1H, dd, *J* = 10.1, 4.1 Hz, C(2')*H*), 4.79 (1H, dd, *J* = 9.3,

7.9 Hz, C(2)*H*), 4.49 (1H, d, *J* = 7.9 Hz, C(1)*H*), 4.45 (1H, dd, *J* = 12.1, 2.9 Hz, C(6)H_AH_B), 4.26-4.19 (2H, m, C(6)H_AH_B & C(6')H_AH_B), 4.02 (1H, dd, *J* = 12.4, 2.1 Hz, C(6')H_AH_B), 3.98 (1H, dd, *J* = 9.4, 9.3 Hz, C(4)*H*), 3.96-3.90 (1H, m, C(5')*H*), 3.82 (1H, dt, *J* = 9.6, 6.6 Hz, C(7)H_AH_B), 3.65 (1H, ddd, *J* = 9.4, 4.2, 2.9 Hz, C(5)*H*), 3.44 (1H, dt, *J* = 9.6, 6.6 Hz, C(7)H_AH_B), 2.12 (3H, s, C(O)CH_3), 2.08 (3H, s, C(O)CH_3), 2.02 (3H, s, C(O)CH_3), 2.00 (3H, s, C(O)CH_3), 1.99 (3H, s, C(O)CH_3), 1.98 (6H, s, 2 × C(O)CH_3), 1.58-1.46 (2H, m, C(8)H_2), 1.32-1.16 (18H, m, C(9-17)H_2), 0.86 (3H, t, *J* = 6.8 Hz, C(18)H_3); **^{13}C NMR** (126 MHz, $CDCl_3$): δ 170.65 (2 × *C*=O), 170.61 (*C*=O), 170.4 (*C*=O), 170.1 (*C*=O), 169.7 (*C*=O), 169.5 (*C*=O), 100.4 (*C*1), 95.6 (*C*1'), 75.6 (*C*3), 72.9 (*C*4), 72.3 (*C*2), 72.2 (*C*5), 70.3 (*C*7), 70.1 (*C*2'), 69.5 (*C*3'), 68.6 (*C*5'), 68.1 (*C*4'), 63.0 (*C*6), 61.6 (*C*6'), 32.0 (1 × *C*(8-17)), 29.8-29.4 (8 × *C*(8-17)), 25.9 (1 × *C*(8-17)), 21.03 (C(O)*C*H_3), 20.96 (C(O)*C*H_3), 20.8 (C(O)*C*H_3), 20.73 (C(O)*C*H_3), 20.72 (C(O)*C*H_3), 20.69 (C(O)*C*H_3), 20.68 (C(O)*C*H_3), 14.2 (*C*18); **HRMS**: *m/z* (ES)$^+$ Found [M+Na]$^+$ 827.3663, $C_{38}H_{60}O_{18}Na^+$ requires [M]$^+$ 827.3672; **LRMS**: *m/z* (ES)$^+$ 827 ([M+Na]$^+$, 100%); **Anal. Calcd.** for $C_{38}H_{60}O_{18}$: *C*, 56.71; *H*, 7.51; *N*, 0.00. Found: *C*, 56.58; *H*, 7.54; *N*, 0.00.

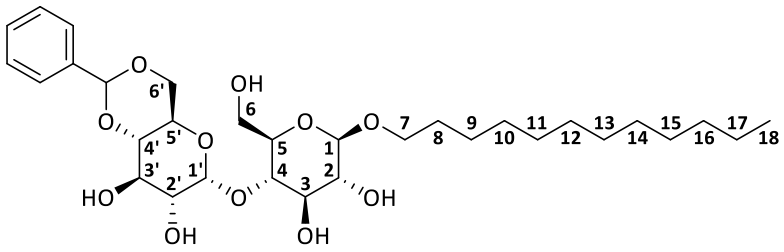
***n*-Dodecyl-β-D-maltoside (1)**



68 (150 mg, 0.19 mmol, 1.00 eq.) was dissolved in anhydrous CH_2Cl_2 (1.8 mL) and anhydrous MeOH (0.9 mL). The resulting solution was cooled to 0 °C and sodium methoxide (13 mg, 0.23 mmol, 1.25 eq.) was added. The reaction was allowed to warm to RT and stirred for 2 h. After this time, the reaction solution was cooled to 0 °C and quenched *via* the dropwise

addition of acetic acid (52 µL). The resulting mixture was concentrated *in vacuo*, the acetic acid removed *via* azeotropic distillation (toluene), to give the crude product. The crude material was adsorbed onto Celite® and purified *via* flash column chromatography on silica gel (Manual; 1:5 → 1:4, MeOH:CH_2Cl_2) affording **1** (104 mg, 100%) as a colourless solid. **R_f**: 0.19 (1:5 MeOH:CH_2Cl_2); $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\boldsymbol{20}}$: +45.2 (*c* 1.0, MeOH) [Lit.[280] $[\alpha]_D^{20}$: +46.7 (*c* 1.0, MeOH)]; **mp**: 102-104 °C (MeOH) [Lit.[281] value 104-105 °C (MeOH)]; **^{1}H NMR** (400 MHz, CD_3OD): δ 5.17 (1H, d, *J* = 3.8 Hz, C(1')*H*), 4.27 (1H, d, *J* = 7.8 Hz, C(1)*H*), 3.93-3.77 (4H, m, C(6)H_AH_B, C(6')H_AH_B & C(7)H_AH_B,), 3.72-3.58 (4H, m, C(3)*H*, C(3')*H*, C(5')*H* & C(6')H_AH_B), 3.58-3.49 (2H, m, C(4)*H* & C(7)H_AH_B), 3.46 (1H, dd, *J* = 9.4, 3.8 Hz, C(2')*H*), 3.37 (1H, ddd, *J* = 9.4, 4.3, 1.9 Hz, C(5)*H*), 3.27 (1H, dd, *J* = 9.3, 9.3 Hz, C(4')*H*), 3.23 (1H, dd, *J* = 9.2, 7.8 Hz, C(2)*H*), 1.67-1.56 (2H, m, (C(8)H_2), 1.45-1.22 (18H, m, C(9-17)H_2), 0.90 (3H, t, *J* = 6.8 Hz, C(18)H_3); **LRMS**: *m/z* $(ES)^+$ 533 ($[M+Na]^+$, 100%). The data are in good agreement with literature values.[282]

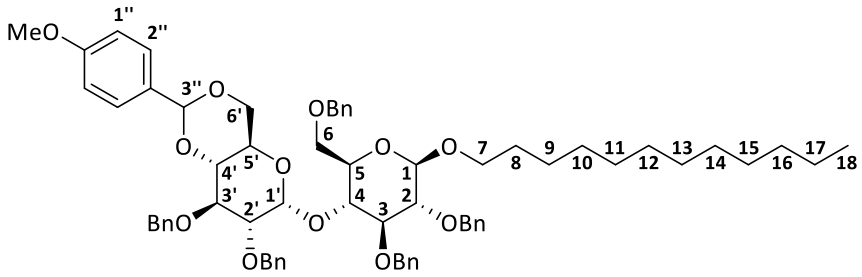
***n*-Dodecyl-(4',6'-*O*-benzylidene)-β-D-maltoside (73)**



1 (500 mg, 0.98 mmol, 1.00 eq.) and camphorsulfonic acid (26 mg, 0.11 mmol, 0.12 eq.) were dissolved in anhydrous DMF (5 mL) and sealed under argon in a microwave vial. Benzaldehyde dimethylacetal (441 µL, 447 mg, 2.94 mmol, 3.00 eq.) was added and the resulting solution was heated to 60 °C at 300 mbar for 3 h. After this time, the reaction solution was diluted with EtOAc (100 mL). The organic layer was washed with distilled H_2O water (3 × 100 mL) and brine

(3 × 100 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo* to give the crude product. The crude material was adsorbed onto Celite® and purified *via* flash column chromatography on pre-washed (1% triethylamine in $CHCl_3$) silica gel (Biotage®; gradient elution: 1% → 10% MeOH in $CHCl_3$) affording **73** (429 mg, 73%) as a colourless solid. $\mathbf{R_f}$: 0.28 (1:19 MeOH:$CHCl_3$); $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\boldsymbol{20}}$: +27.5 (*c* 0.5, $CHCl_3$); **mp**: 71-73 °C ($(CH_3)_2O$); **IR** (thin film) ν_{max} cm^{-1}: 3383 (br, m), 2924 (m), 2854 (m), 2361 (w), 2341 (w); **^{1}H NMR** (500 MHz, $CDCl_3$): δ 7.51-7.45 (2H, m, Ar C*H*), 7.38-7.32 (3H, m, Ar C*H*), 5.57 (1H, br s, C(3)HO*H*), 5.49 (1H, s, PhC*H*), 5.31 (1H, d, *J* = 5.2 Hz, C(2')HO*H*), 5.15 (1H, d, *J* = 3.7 Hz, C(1')*H*), 4.30 (1H, br s, C(3')HO*H*), 4.27 (1H, d, *J* = 8.1 Hz, C(1)*H*), 4.23 (1H, dd, *J* = 10.3, 4.7 Hz, C(6')H_AH_B), 4.01 (1H, br s, C(2)HO*H*), 3.93 (1H, dd, *J* = 9.4, 9.3 Hz, C(3')*H*), 3.92-3.79 (3H, m, C(5')*H*, C(6)H_AH_B & C(7)H_AH_B), 3.76 (1H, dd, *J* = 12.2, 3.4 Hz, C(6)H_AH_B), 3.73-3.60 (4H, m, C(2')*H*, C(3)*H*, C(5)*H* & C(6')H_AH_B), 3.54-3.46 (1H, m, C(7)H_AH_B), 3.45 (1H, dd, *J* = 9.4, 9.4 Hz, C(4')*H*), 3.38 (1H, dd, *J* = 8.5, 8.1 Hz, C(2)*H*), 3.34-3.28 (1H, m, C(4)*H*), 2.61 (1H, t, *J* = 6.4 Hz, C(6)H_2O*H*), 1.66-1.55 (2H, m, C(8)H_2), 1.34-1.19 (18H, m, C(9-17)H_2), 0.88 (3H, t, *J* = 6.9 Hz, CH_3); **^{13}C NMR** (126 MHz, $CDCl_3$): δ 137.3 (Ar *C*), 129.3 (Ar *C*H), 128.4 (Ar *C*H), 126.5 (Ar *C*H), 102.8 (*C*1), 102.3 (*C*1'), 101.9 (Ph*C*H), 80.8 (*C*4'), 80.4 (*C*5), 76.1 (*C*3), 74.8 (*C*4), 73.5 (*C*2'), 73.4 (*C*2), 70.9 (*C*3'), 70.6 (*C*7), 68.9 (*C*6'), 63.9 (*C*5'), 61.5 (*C*6), 32.1 (1 × *C*(9-17)), 29.84-29.75 (5 × *C*8 & C3''*C*(9-17)), 29.7 (1 × *C*(9-17)), 29.5 (1 × *C*(9-17)), 26.0 (1 × *C*(9-17)), 22.8 (1 × *C*(9-17)), 14.3 (*C*H_3); **HRMS**: *m/z* (ES)$^+$ Found $[M+Na]^+$ 621.3259, $C_{31}H_{50}NaO_{11}^+$ requires $[M]^+$ 621.3245; **LRMS**: *m/z* (ES)$^+$ 511 (100%), 599 ($[M+H]^+$, 97%); **HPLC** (Protocol A): ret. time = 9.39 min, purity = 93%.

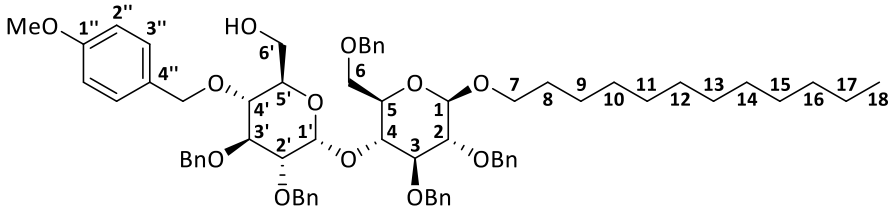
***n*-Dodecyl-(2,3,6,2',3'-*O*-pentabenzyl-4',6'-*O*-[4-methoxybenzylidene])-β-D-maltoside (49)**



1 (500 mg, 0.98 mmol, 1.00 eq.) and camphorsulfonic acid (26 mg, 0.11 mmol, 0.12 eq.) were dissolved in anhydrous DMF (5 mL) and sealed under argon in a microwave vial. 4-Methoxybenzaldehyde dimethylacetal (220 µL, 224 mg, 1.23 mmol, 1.20 eq.) was added and the resulting solution was heated to 60 °C at 300 mbar for 3 h. After this time, the reaction solution was transferred *via* cannula to a suspension of NaH (60% in oil, 392 mg, 9.80 mmol, 10.00 eq.) and TBAI (18 mg, 0.05 mmol, 0.05 eq.) in anhydrous DMF (25 mL) at 0 °C. The resulting suspension was stirred at 0 °C for 10 min then benzyl bromide (802 µL, 921 mg, 5.39 mmol, 5.50 eq.) was added dropwise. The reaction was stirred for a further 10 min at 0 °C then allowed to warm to RT and stir for 6 h. After this time, the reaction solution was cooled to 0 °C and quenched *via* the dropwise addition of distilled H_2O (5 mL). The resulting mixture was diluted with EtOAc (100 mL). The organic layer was washed with distilled H_2O (3 × 100 mL) and brine (3 × 100 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo* to give the crude product. The crude material was adsorbed onto Celite® and purified *via* flash column chromatography on pre-washed (1% triethylamine in petrol) silica gel (Biotage®; gradient elution: 5% → 10% EtOAc in petrol) affording **49** (814 mg, 66% over 2 steps) as a colourless oil. The resulting material was found to be unstable and used rapidly without full characterisation. $\mathbf{R_f}$: 0.15 (1:19 EtOAc:Petrol); **^{1}H NMR** (400 MHz, $CDCl_3$): δ 7.44 (2H, d, *J* = 8.7 Hz, C(2'')*H*), 7.41-7.13 (25H, m, Ar C*H*), 6.92 (2H, d, *J* = 8.7 Hz, C(1'')*H*), 5.72 (1H, d,

J = 3.8 Hz, C(1')*H*), 5.51 (1H, m, C(3'')*H*), 4.98 (1H, d, *J* = 11.2 Hz, PhC(α)H_AH_B), 4.94 (1H, d, *J* = 10.8 Hz, PhC(β)H_AH_B), 4.89 (1H, d, *J* = 11.2 Hz, PhC(γ)H_AH_B), 4.80-4.68 (3H, m, PhC(α)H_AH_B, PhC(γ)H_AH_B & PhC(δ)H_AH_B), 4.68 (1H, d, *J* = 11.8 Hz, *PhC*(ε)H_AH_B), 4.62 (1H, d, *J* = 10.8 Hz, PhC(β)H_AH_B), 4.61 (1H, d, *J* = 12.6 Hz, PhC(δ)H_AH_B), 4.56 (1H, d, *J* = 11.8 Hz, PhC(ε)H_AH_B), 4.43 (1H, d, *J* = 7.7 Hz, C(1)*H*), 4.17-3.45 (17H, m, C(2-5)*H*, C(6)H_AH_B, C(2'-5')*H*, C(6')H_AH_B, C(7)H_AH_B and OCH_3), 1.66-1.52 (2H, m, C(8)H_2), 1.50-1.18 (18H, m, C(9-17)H_2), 0.81 (3H, t, *J* = 6.9 Hz, C(18)H_3); **HRMS**: *m/z* (ES)$^+$ Found $[M+H]^+$ 1101.5801, $C_{67}H_{82}O_{12}Na^+$ requires $[M]^+$ 1101.5698; **LRMS**: *m/z* (ES)$^+$ 804 (100%), 1080 ($[M+H]^+$, 10%).

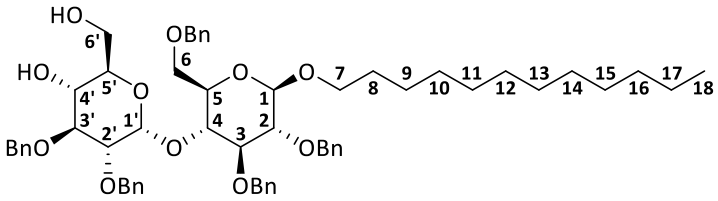
_n_-Dodecyl-(2,3,6,2',3'-*O*-pentabenzyl-4'-*O*-[4-methoxybenzyl])-β-D-maltoside (50)



49 (112 mg, 0.10 mmol, 1.00 eq.) was dissolved in freshly distilled anhydrous THF (0.9 mL). Borane·THF complex (0.34 mL, 29 mg, 0.34 mmol, 3.20 eq.) was added dropwise followed by cobalt(II) chloride (44 mg, 0.34 mmol, 3.20 eq.). The resulting bright blue suspension was stirred at RT for 18 h. After this time, the reaction mixture was diluted with EtOAc (20 mL) and filtered through Celite®. The filtrate was treated with $NaBH_4$ (625 µL, 0.1% w/v in distilled H_2O) and filtered again through Celite® eluting with EtOAc (20 mL). The organic filtrate was then washed with sat. $NaHCO_3$ aq. (3 × 50 mL) and distilled H_2O (3 × 50 mL) dried ($MgSO_4$), filtered and concentrated *in vacuo* to give the crude product. The crude material was adsorbed onto Celite® and purified *via* flash column chromatography on silica gel (Biotage®; gradient elution: 5% → 40% EtOAc in petrol) affording **50** (26 mg, 23%) as a colourless oil. Further elution

afforded **76** (45 mg, 45%) as a colourless solid, see below for characterisation. $\mathbf{R_f}$: 0.10 (1:9 EtOAc:Petrol); $[\alpha]_D^{20}$: +17.7 (*c* 1.0, $CHCl_3$); **IR** (thin film) ν_{max} cm^{-1}: 3032 (w), 2926 (m), 2854 (w), 2247 (w); **^{1}H NMR** (500 MHz, $CDCl_3$): δ 7.36-7.15 (27H, m, Ar CH & C(3'')*H*), 6.87 (2H, d, *J* = 8.5 Hz, C(2'')*H*), 5.65 (1H, d, *J* = 3.6 Hz, C(1')*H*), 4.98 (1H, d, *J* = 11.7 Hz, PhC(α)H_AH_B), 4.95 (1H, d, *J* = 11.0 Hz, PhC(β)H_AH_B), 4.90 (1H, d, *J* = 11.1 Hz, PhC(γ)H_AH_B), 4.82 (1H, d, *J* = 11.1 Hz, PhC(γ)H_AH_B), 4.79 (1H, d, *J* = 10.9 Hz, PhC(δ)H_AH_B), 4.76 (1H, d, *J* = 11.7 Hz, PhC(α)H_AH_B), 4.63 (1H, d, *J* = 11.0 Hz, PhC(β)H_AH_B), 4.63-4.55 (4H, m, PhC(ε)H_AH_B, PhC(ζ)H_AH_B & PhC(δ)H_AH_B), 4.53 (1H, d, *J* = 11.9 Hz, PhC(ζ)H_AH_B), 4.43 (1H, d, *J* = 7.8 Hz, C(1)*H*), 4.05 (1H, dd, *J* = 9.1, 9.1 Hz, C(4)*H*), 3.96 (1H, dt, *J* = 9.9, 9.2 Hz, C(7)H_AH_B), 3.91 (1H, dd, *J* = 9.6, 9.4 Hz, C(3')*H*), 3.84-3.74 (6H, m, C(3)*H*, C(6')H_AH_B & CH_3O), 3.72-3.64 (2H, m, C(5')*H* & C(6)H_AH_B), 3.59-3.49 (4H, m, C(2)*H*, C(5)*H*, C(6)H_AH_B & C(7)H_AH_B), 3.47 (1H, dd, *J* = 9.4, 9.4 Hz, C(4')*H*), 3.42 (1H, dd, *J* = 9.6, 3.6 Hz, C(2')*H*), 1.82-1.60 (2H, m, C(8)H_2), 1.48-1.37 (2H, m, C(9)H_2), 1.37-1.22 (16H, m, C(10-17)H_2), 0.90 (3H, t, *J* = 6.8 Hz, C(18)H_3); **^{13}C NMR** (126 MHz, $CDCl_3$): δ 159.5 (*C*1''), 138.93 (Ar *C*), 138.86 (Ar *C*), 138.4 (Ar *C*), 138.3 (Ar *C*), 138.0 (Ar *C*), 130.5 (*C*4''), 129.9 (*C*3''), 128.5-128.3 (multiple Ar *C*H), 127.9 (Ar *C*H), 127.8-127.7 (multiple Ar *C*H), 127.3 (Ar *C*H), 126.7 (Ar *C*H), 114.0 (*C*2''), 103.6 (*C*1), 96.6 (*C*1'), 84.9 (*C*3), 82.4 (*C*2), 81.9 (*C*3'), 79.5 (*C*2'), 77.5 (*C*4'), 75.6 (Ph*C*H_2), 74.9 (Ph*C*H_2), 74.7 (Ph*C*H_2), 74.6 (*C*5), 74.0 (Ph*C*H_2), 73.6 (Ph*C*H_2), 73.4 (Ph*C*H_2), 72.6 (*C*4), 71.9 (*C*5'), 70.1 (*C*7), 68.8 (*C*6'), 61.9 (*C*6), 55.4 (*C*H_3O), 32.1 (1 × *C*8 or *C*(10-17)), 30.0 (1 × *C*8 or *C*(10-17)), 29.82 (1 × *C*8 or *C*(10-17)), 29.78 (1 × *C*8 or *C*(10-17)), 29.76 (1 × *C*8 or *C*(10-17)), 29.6 (1 × *C*8 or *C*(10-17)), 29.5 (1 × *C*8 or *C*(10-17)), 26.4 (*C*9), 22.8 (1 × *C*8 or *C*(10-17)), 14.3 (*C*18); **HRMS**: *m/z* (ES)$^+$ Found $[M+Na]^+$ 1103.5842, $C_{67}H_{84}NaO_{12}^+$ requires $[M]^+$ 1103.5855; **LRMS**: *m/z* (ES)$^+$ 1104 ($[M+Na]^+$, 100%); **Anal. Calcd.** for $C_{67}H_{84}O_{12}$: *C*, 74.42; *H*, 7.83; *N*, 0.00. Found: *C*, 74.59; *H*, 7.71; *N*, 0.00.

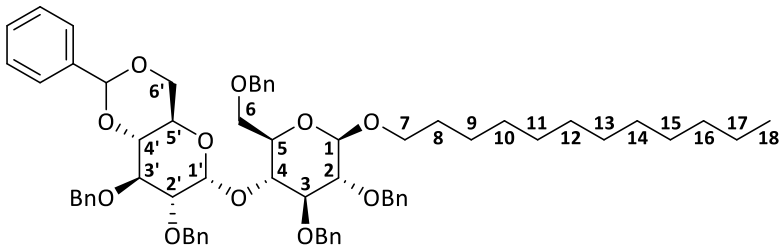
***n*-Dodecyl-(2,3,6,2',3'-*O*-hexabenzyl)-β-D-maltoside (76)**



49 (644 mg, 0.60 mmol, 1.00 eq.) was dissolved in MeCN (4.4 mL) and distilled H_2O (4.4 mL). Ceric ammonium nitrate (603 mg, 1.10 mmol, 1.83 eq.) was added and the resulting suspension was stirred at RT for 35 min under an atmosphere of nitrogen. After this time, the reaction mixture was diluted with EtOAc (40 mL) and stirred at RT for 10 min. The organic layer was then washed with distilled H_2O (3 × 50 mL) and brine (3 × 50 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo* to give the crude product. The crude material was adsorbed onto Celite® and purified *via* flash column chromatography on silica gel (Biotage®; gradient elution: 30% → 40% EtOAc in petrol) affording **76** (392 mg, 68%) as a colourless solid. $\mathbf{R_f}$: 0.16 (3:7 EtOAc:Petrol); $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\boldsymbol{20}}$: +7.5 (*c* 1.0, $CHCl_3$); **IR** (neat) ν_{max} cm^{-1}: 3405 (w), 2853 (m), 2803 (m), 1497 (m); **^{1}H NMR** (500 MHz, $CDCl_3$): δ 7.41-7.16 (25H, m, Ar C*H*), 5.69 (1H, d, *J* = 3.6 Hz, C(1')*H*), 5.02 (1H, d, *J* = 11.7 Hz, PhC(α)H_AH_B), 4.96 (1H, d, *J* = 14.9 Hz, PhC(β)H_AH_B), 4.94 (1H, d, *J* = 15.5 Hz, PhC(γ)H_AH_B), 4.75 (1H, d, *J* = 11.7 Hz, PhC(α)H_AH_B), 4.70-4.63 (4H, m, PhC(β)H_AH_B, PhC(γ)H_AH_B & PhC(δ)H_2), 4.55 (2H, s, PhC(ε)H_2), 4.46 (1H, d, *J* = 7.8 Hz, C(1)*H*), 4.08 (1H, dd, *J* = 9.1, 9.1 Hz, C(4)*H*), 4.02-3.95 (1H, m, C(7)H_AH_B), 3.87-3.77 (3H, m, C(3)*H* & C(6)H_AH_B), 3.74 (1H, dd, *J* = 9.4, 9.2 Hz, C(3')*H*), 3.72-3.47 (7H, m, C(6')H_AH_B, C(5')*H*, C(7)H_AH_B, C(5)*H*, C(2)*H* & C(4')*H*), 3.42 (1H, dd, *J* = 9.4, 3.6 Hz, C(2')*H*), 1.74-1.64 (2H, m, C(8)H_2), 1.50-1.21 (18H, m, C(9-17)H_2), 0.91 (3H, t, *J* = 6.9 Hz, C(18)H_3); **^{13}C NMR** (126 MHz, $CDCl_3$): δ 137.9 (Ar *C*), 137.7 (Ar *C*), 137.4 (Ar *C*), 137.3 (Ar *C*), 136.9 (Ar *C*), 127.7 (Ar *C*H), 127.5-127.4 (multiple Ar *C*H), 127.3 (Ar *C*H), 126.9-126.7 (multiple Ar *C*H), 126.3 (Ar *C*H), 125,7 (Ar *C*H), 102.6 (*C*1), 95.5 (*C*1'),

83.9 (*C*3), 81.3 (*C*2), 80.3 (C3'), 78.2 (*C*2'), 74.3 (Ph*C*H_2), 73.7 (Ph*C*H_2), 73.6 (*C*5), 72.9 (Ph*C*H_2), 72.6 (Ph*C*H_2), 72.1 (Ph*C*H_2), 71.4 (*C*4) 71.0 (*C*5'), 69.6 (*C*4'), 69.1 (*C*7), 67.8 (*C*6), 61.4 (*C*6'), 31.0 (1 × *C*(8) or *C*(10-17)), 28.9 (1 × *C*(8) or *C*(10-17)), 28.81-28.75 (4 × *C*(8) or *C*(10-17)), 28.6 (1 × *C*(8) or *C*(10-17)), 28.5 (1 × *C*(8) or *C*(10-17)), 25.4 (*C*9), 21.8 (1 × *C*(8) or *C*(10-17)), 12.3 (*C*18); **HRMS**: *m/z* (ES)$^+$ Found [M+Na]$^+$ 983.5281; $C_{59}H_{76}NaO_{11}^+$ requires [M]$^+$ 983.5280; **LRMS**: *m/z* (ES)$^+$ 984 ([M+Na]$^+$, 100%); **Anal. Calcd.** for $C_{59}H_{76}O_{11}$: *C*, 73.72; *H*, 7.97; *N*, 0.00. Found: *C*, 73.81; *H*, 8.13; *N*, 0.00.

***n*-Dodecyl-(2,3,6,2',3'-*O*-pentabenzyl-4',6'-*O*-benzylidene)-β-D-maltoside (77)**

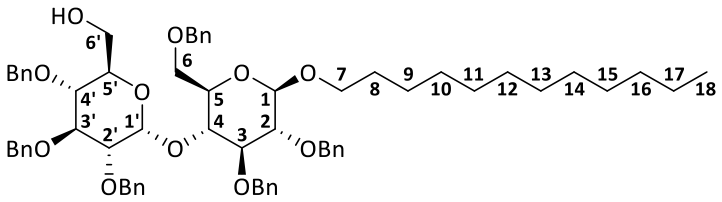


1 (500 mg, 0.98 mmol, 1.00 eq.) and camphorsulfonic acid (26 mg, 0.11 mmol, 0.12 eq.) were dissolved in anhydrous DMF (5 mL) and sealed under argon in a microwave vial. Benzaldehyde dimethylacetal (220 µL, 223 mg, 1.47 mmol, 1.50 eq.) was added and the resulting solution was heated to 60 °C at 300 mbar for 2 h. After this time, the reaction solution was transferred *via* cannula to a suspension of NaH (60% in oil, 392 mg, 9.80 mmol, 10.00 eq.) and TBAI (18 mg, 0.05 mmol, 0.05 eq.) in DMF (20 mL) at 0 °C. The resulting suspension was stirred at 0 °C for 10 min then benzyl bromide (922 µL, 921 mg, 5.39 mmol, 5.50 eq.) was added dropwise. The reaction was stirred at 0 °C for a further 10 min then allowed to warm to RT and stir for 6.5 h. After this time, the reaction solution was cooled to 0 °C and quenched *via* the dropwise addition of distilled H_2O (5 mL). The resulting mixture was diluted with EtOAc (100 mL). The

organic layer was washed with distilled H_2O (3 × 100 mL) and brine (3 × 100 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo* to give the crude product. The crude material was adsorbed onto Celite® and purified *via* flash column chromatography on pre-washed (1% triethylamine in petrol) silica gel (Biotage®; gradient elution: 5% → 10% EtOAc in petrol) affording **77** (862 mg, 84% over 2 steps) as a colourless oil. $\mathbf{R_f}$: 0.43 (1:9 EtOAc:Petrol); $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\mathbf{20}}$: +3.4 (*c* 1.0 in $CHCl_3$); **IR** (thin film) ν_{max} cm^{-1}: 3040 (w), 2924 (m), 2854 (m), 1496 (m), 1453 (m), 1365 (m), 1087 (s), 1027 (s); **^{1}H NMR** (700 MHz, $CDCl_3$): δ 7.53-7.51 (2H, m, Ar C*H*), 7.43-7.36 (6H, m, Ar C*H*), 7.36-7.24 (15H, m, Ar C*H*), 7.22-7.16 (7H, m, Ar C*H*), 5.73 (1H, d, *J* = 3.9 Hz, C(1')*H*), 5.56 (1H, s, PhC*H*), 4.99 (1H, d, *J* = 12.0 Hz, PhC(α)H_AH_B), 4.95 (1H, d, *J* = 10.8 Hz, PhC(β)H_AH_B), 4.91 (1H, d, *J* = 11.3 Hz, PhC(γ)H_AH_B), 4.76 (1H, d, *J* = 11.3 Hz, PhC(γ)H_AH_B), 4.74 (1H, d, *J* = 12.0 Hz, PhC(α)H_AH_B), 4.71 (1H, d, *J* = 12.2 Hz, PhC(δ)H_AH_B), 4.68 (1H, d, *J* = 11.7 Hz, PhC(ε)H_AH_B), 4.63 (1H, d, *J* = 10.8 Hz, PhC(β)H_AH_B), 4.62 (1H, d, *J* = 12.2 Hz, PhC(δ)H_AH_B), 4.57 (1H, d, *J* = 11.7 Hz, PhC(ε)H_AH_B), 4.44 (1H, d, *J* = 7.8 Hz, C(1)*H*), 4.19 (1H, dd, *J* = 10.3, 4.9 Hz, C(6')H_AH_B), 4.12 (1H, dd, *J* = 9.2, 9.2 Hz, C(4)*H*), 4.00 (1H, dd, *J* = 9.4, 9.3 Hz, C(3')*H*), 3.98-3.95 (1H, m, C(7)H_AH_B), 3.88 (1H, ddd, *J* = 10.1, 9.6, 4.9 Hz, C(5')*H*), 3.85 (1H, dd, *J* = 10.9, 4.0 Hz, C(6)H_AH_B), 3.83-3.77 (2H, m, C(3)*H* & C(6)H_AH_B), 3.66 (1H, dd, *J* = 10.3, 10.1 Hz, C(6')H_AH_B), 3.62 (1H, dd, *J* = 9.6, 9.4 Hz, C(4')*H*), 3.60-3.50 (4H, m, C(2)*H*, C(2')*H*, C(5)*H* & C(7)H_AH_B), 1.73-1.64 (2H, m, C(8)H_2), 1.48-1.38 (2H, m, C(9)H_2), 1.36-1.25 (16H, m, C(10-17)H_2), 0.91 (3H, t, *J* = 7.1 Hz, C(18)H_3); **^{13}C NMR** (101 MHz, $CDCl_3$); δ 138.9 (Ar *C*), 138.8 (Ar *C*), 138.39 (Ar *C*), 138.37 (Ar *C*), 137.9 (Ar *C*), 137.7 (Ar *C*), 128.9 (Ar *C*H), 128.4-128.3 (8 × Ar *C*H), 128.1 (Ar *C*H), 127.9 (Ar *C*H), 127.8 (Ar *C*H), 127.71 (Ar *C*H), 127.67 (Ar *C*H), 127.5 (Ar *C*H), 127.2 (Ar *C*H), 126.7 (Ar *C*H), 126.1 (Ar *C*H), 103.6 (*C*(1)H), 101.2 (Ph*C*H), 97.3 (*C*1'), 85.0 (*C*3), 82.42 (*C*5), 82.35 (*C*4'), 78.83-78.78 (*C*2' & *C*3'), 75.4 (Ph*C*H_2), 74.7 (Ph*C*H_2), 74.3 (*C*2), 73.9 (Ph*C*H_2), 73.8 (Ph*C*H_2), 73.5 (Ph*C*H_2), 72.0 (*C*4), 70.1 (*C*7), 69.0 (*C*6'), 68.9 (*C*6), 63.3 (*C*5'), 32.0 (1 × *C*8 & *C*(10-17)),

29.9 (1 × *C*8 & *C*(10-17)), 29.8-29.7 (4 × *C*8 & *C*(10-17)), 29.6 (1 × *C*8 & *C*(10-17)), 29.5 (1 × *C*8 & *C*(10-17)), 26.3 (*C*9), 22.8 (1 × *C*8 & *C*(10-17)), 14.3 (*C*18); **HRMS**: *m/z* (ES)$^+$ Found [M+Na]$^+$ 1071.5590. $C_{66}H_{80}O_{11}Na^+$ requires [M]$^+$ 1071.5593; **LRMS**: *m/z* (ES)$^+$ 1050 ([M+H]$^+$, 100%); **Anal. Calcd.** for $C_{66}H_{80}O_{11}$: *C*, 75.54; *H*, 7.68; *N*, 0.00. Found: *C*, 75.38; *H*, 7.42; *N*, 0.00.

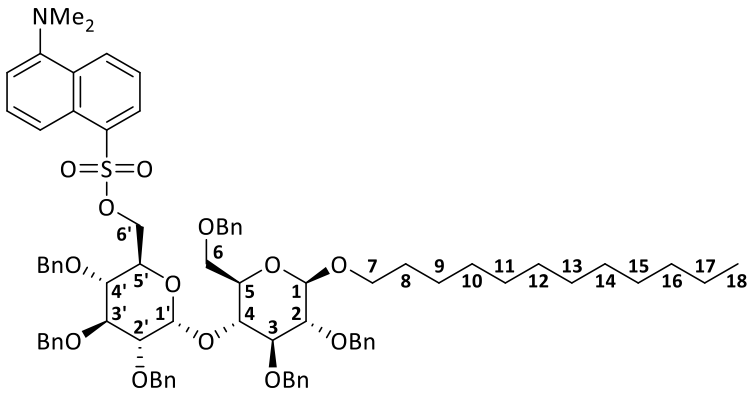
***n*-Dodecyl-(2,3,6,2',3',4'-*O*-hexabenzyl)-β-D-maltoside (78)**



77 (862 mg, 0.82 mmol, 1.00 eq.) was dissolved in freshly distilled anhydrous THF (7.7 mL). Borane·THF complex (2.88 mL, 247 mg, 2.89 mmol, 3.50 eq.) was added dropwise followed by cobalt(II) chloride (374 mg, 2.89 mmol, 3.50 eq.). The resulting bright blue suspension was stirred at RT for 20 h. After this time, the reaction mixture was diluted with EtOAc (25 mL) and filtered through Celite®. The filtrate was treated with $NaBH_4$ (5.1 mL, 0.1% w/v in distilled H_2O) and filtered again through Celite® eluting with EtOAc (25 mL). The organic filtrate was then washed with sat. $NaHCO_3$ aq. (3 × 40 mL) and distilled H_2O (3 × 40 mL) dried ($MgSO_4$), filtered and concentrated *in vacuo* to give the crude product. The crude material was adsorbed onto Celite® and purified *via* flash column chromatography on silica gel (Biotage®; gradient elution: 5% → 40% EtOAc in petrol) affording **78** (660 mg, 76%) as a colourless oil. $\mathbf{R_f}$: 0.09 (1:9 EtOAc:Petrol); $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\mathbf{20}}$: +22.9 (*c* 1.0, $CHCl_3$); **IR** (thin film) ν_{max} cm^{-1}: 3030 (w), 2925 (m), 2855 (m), 1454 (m), 1362 (m), 1091 (s), 1062 (s), 1027 (s); **^{1}H NMR** (700 MHz, $CDCl_3$): δ 7.36-7.23 (23H, m, Ar C*H*), 7.23-7.16 (7H, m, Ar C*H*), 5.64 (2H, d, *J* = 2.1 Hz, C(1')*H*), 4.98 (1H, d, *J* = 11.6 Hz, PhC(α)H_AH_B), 4.94 (1H, d, *J* = 10.9 Hz, PhC(β)H_AH_B), 4.89 (1H, d, *J* = 10.8 Hz, PhC(γ)H_AH_B), 4.86

(1H, d, *J* = 11.1 Hz, PhC(δ)H_AH_B), 4.80 (1H, d, *J* = 10.8 Hz, PhC(γ)H_AH_B), 4.75 (1H, d, *J* = 10.9 Hz, PhC(α)H_AH_B), 4.66-4.60 (4H, m, PhC(β)H_AH_B, PhC(δ)H_AH_B & PhC(ε)H_2), 4.59 (1H, d, *J* = 11.8 Hz, PhC(ζ)H_AH_B), 4.53 (1H, d, *J* = 11.8 Hz, PhC(ζ)H_AH_B), 4.42 (1H, d, *J* = 7.8 Hz, C(1)*H*), 4.05 (1H, dd, *J* = 9.1, 9.1 Hz, C(4)*H*), 3.99-3.93 (1H, m, C(7)H_AH_B), 3.92 (1H, dd, *J* = 9.6, 9.4 Hz, C(3')*H*), 3.83-3.74 (3H, m, C(3)*H* & C(6)H_AH_B), 3.73-3.64 (2H, m, C(5')*H* & C(6')H_AH_B), 3.60-3.46 (5H, m, C(2)*H*, C(5)*H*, C(4')*H*, C(6')H_AH_B & C(7)H_AH_B), 3.42 (1H, dd, *J* = 9.6, 2.1 Hz, C(2')*H*), 1.76 (1H, br s, O*H*), 1.72-1.64 (2H, m, C(8)H_2), 1.46-1.37 (2H, m, C(9)H_2), 1.36-1.21 (16H, m, C(10-17)H_2), 0.89 (3H, t, *J* = 7.1 Hz, C(18)H_3); **^{13}C NMR** (176 MHz, $CDCl_3$) δ 138.9 (Ar *C*), 138.8 (Ar *C*), 138.4 (Ar *C*), 138.33 (Ar *C*), 138.25 (Ar *C*), 138.0 (Ar *C*), 128.6 (Ar *C*H), 128.5-128.4 (multiple Ar *C*H), 128.3 (Ar *C*H), 128.2 (Ar *C*H), 128.0 (Ar *C*H), 127.8 (multiple Ar *C*H), 127.7 (Ar *C*H), 127.9-127.8 (multiple Ar *C*H), 127.74 (Ar *C*H), 127.67 (Ar *C*H), 127.3 (Ar CH), 126.7 (Ar *C*H), 103.6 (*C*1), 96.5 (*C*1'), 84.9 (*C*3), 82.3 (*C*2), 81.9 (*C*3'), 79.5 (*C*2'), 77.7 (*C*4'), 75.6 (Ph*C*H_2), 75.2 (Ph*C*H_2), 74.7 (Ph*C*H_2), 74.6 (*C*5), 74.0 (Ph*C*H_2), 73.6 (Ph*C*H_2), 73.4 (Ph*C*H_2), 72.5 (*C*4), 71.8 (*C*5'), 70.1 (*C*7), 68.8 (*C*6), 61.8 (*C*6') 32.0 (1 × *C*(10-17)), 30.0 (*C*8), 29.9 (1 × *C*(10-17)), 29.82-29.76 (3 × *C*(10-17)), 29.6 (1 × *C*(10-17)), 29.5 (1 × *C*(10-17)), 26.4 (*C*9), 22.8 (1 × *C*(10-17)), 14.3 (*C*18); **HRMS**: *m/z* (ES)$^+$ Found [M+Na]$^+$ 1073.5710, $C_{66}H_{82}O_{11}Na^+$ requires [M]$^+$ 1073.5749); **LRMS**: *m/z* (ES)$^+$ 1052 ([M+H]$^+$, 100%); **Anal. Calcd.** for $C_{66}H_{82}O_{11}$: *C*, 75.40; *H*, 7.86; *N*, 0.00. Found: *C*, 75.39; *H*, 7.76; *N*, 0.00.

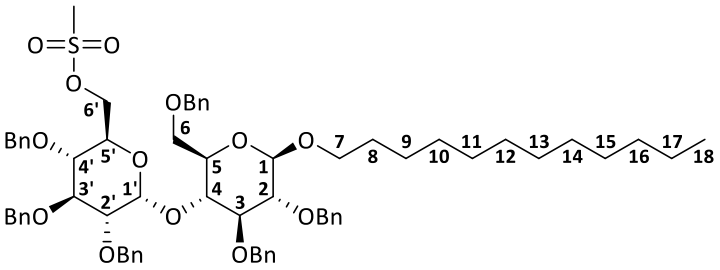
***n*-Dodecyl-(2,3,6,2',3',4'-*O*-hexabenzyl-6'-*O*-dansyl)-β-D-maltoside (81)**



78 (72 mg, 0.07 mmol, 1.00 eq.), dansyl chloride **53** (56 mg, 0.21 mmol, 3.00 eq.) and anhydrous triethylamine (29 µL, 21 mg, 0.21 mmol, 3.00 eq.) were dissolved in anhydrous CH_2Cl_2 (0.7 mL). The resulting solution was stirred at RT for 4 h. After this time, the reaction solution was concentrated *in vacuo* to give the crude product. The crude material was adsorbed onto Celite® and purified *via* flash column chromatography on pre-washed (1% triethylamine in petrol) silica gel (Biotage®; gradient elution: 2% → 20% EtOAc in petrol) affording **81** (34 mg, 38%) as a yellow oil. $\mathbf{R_f}$: 0.78 (1:4 EtOAc:Petrol); $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\mathbf{20}}$: +19.4 (*c* 1.0, $CHCl_3$); **IR** (thin film) ν_{max} cm^{-1}: 3602 (w), 3352 (w), 3285 (w), 3089 (w), 3064 (w), 3031 (w), 2925 (m), 2854 (m), 2360 (w), 2342 (w), 2161 (w), 2029 (w), 1980 (w), 1884 (m), 1718 (s); **^{1}H NMR** (500 MHz, $CDCl_3$): δ 8.53 (1H, d, *J* = 8.3 Hz, Napth. C*H*), 8.30 (1H, d, *J* = 8.6 Hz, Napth. C*H*), 8.18 (1H, dd, *J* = 7.5, 1.1 Hz, Napth. C*H*), 7.50 (1H, dd, *J* = 8.6, 7.5 Hz, Napth. C*H*), 7.45 (1H, dd, *J* = 8.3, 7.5 Hz, Napth. C*H*), 7.33-7.17 (26H, m, Ar C*H*), 7.16-7.12 (2H, m, Ar C*H*), 7.12 (1H, d, *J* = 7.6 Hz, Napth. C*H*), 6.99-6.94 (2H, m, Ar C*H*), 5.44 (1H, d, *J* = 3.5 Hz, C(1')*H*), 4.93 (1H, d, *J* = 10.9 Hz, PhC(α)H_AH_B), 4.92 (1H, d, *J* = 12.0 Hz, PhC(β)H_AH_B), 4.84 (1H, d, *J* = 11.1 Hz, PhC(γ)H_AH_B), 4.71 (1H, d, *J* = 12.0 Hz, PhC(β)H_AH_B), 4.69 (1H, d, *J* = 11.1 Hz, PhC(γ)H_AH_B), 4.65 (1H, d, *J* = 10.7 Hz, PhC(δ)H_AH_B), 4.63 (1H, d, *J* = 10.9 Hz, PhC(α)H_AH_B), 4.50 (1H, d, *J* = 12.2 Hz, PhC(ε)H_AH_B), 4.48-4.44 (3H, m, PhC(ε)H_AH_B & PhC(ζ)CH_AH_B), 4.39 (1H, d, *J* = 7.8 Hz, C(1)*H*), 4.21

(1H, d, *J* = 10.7 Hz, PhC(δ)H_AH_B), 4.08 (1H, dd, *J* = 10.7, 1.0 Hz, C(6')H_AH_B), 3.98-3.91 (3H, m, C(4)*H*, C(6')H_AH_B & C(7)H_AH_B), 3.81 (1H, dd, *J* = 9.6, 9.4 Hz, C(3')*H*), 3.78-3.69 (3H, m, C(3)*H*, C(6)H_AH_B & C(5')*H*), 3.65 (1H, dd, *J* = 11.2, 1.8 Hz, C(6)H_AH_B), 3.54 (1H, dt, *J* = 9.5, 7.1 Hz, C(7)H_AH_B) 3.52-3.43 (2H, m, C(2)*H* & C(5)*H*), 3.39 (1H, dd, *J* = 9.5, 9.4 Hz, C(4')*H*), 3.19 (1H, dd, *J* = 9.6, 3.5 Hz, C(2')*H*), 2.82 (6H, s, N(CH_3)$_2$), 1.71-1.62 (2H, m, C(8)H_2), 1.46-1.36 (2H, m, C(9)H_2), 1.36-1.22 (16H, m, C(10-17)H_2), 0.90 (3H, t, *J* = 6.9 Hz, C(18)H_3); **^{13}C NMR** (126 MHz, $CDCl_3$): δ 151.9 (Napth. *C*), 138.9 (Ar *C*), 138.7 (Ar *C*), 138.5 (Ar *C*), 138.3 (Ar *C*), 138.0 (Ar *C*), 137.9 (Ar *C*), 131.6 (Napth. *C*), 131.5 (Napth. *C*H), 130.6 (Napth. *C*H), 130.0 (Napth. *C*), 129.9 (Napth. *C*), 128.9 (Napth. *C*H), 128.5-128.3 (multiple Ar *C*H), 128.0 (Ar *C*H), 127.8-127.7 (multiple Ar *C*H), 127.64-127.57 (multiple Ar *C*H), 127.3 (Ar *C*H), 126.8 (Ar *C*H), 123.2 (Napth. *C*H), 119.8 (Napth. *C*H), 115.7 (Napth. *C*H), 103.7 (*C*1), 96.2 (*C*1'), 84.8 (*C*3), 82.2 (*C*5), 81.6 (*C*3'), 79.3 (*C*2'), 77.0 (*C*4'), 75.4 (Ph*C*H_2), 74.9 (Ph*C*H_2), 74.7 (Ph*C*H_2), 74.5 (*C*2), 73.8 (Ph*C*H_2), 73.4 (Ph*C*H_2), 73.2 (Ph*C*H_2), 72.6 (*C*4), 70.1 (*C*7), 69.4 (*C*5'), 69.03 (*C*6), 68.99 (*C*6'), 45.5 (N(*C*H_3)$_2$), 32.1 (1 × *C*8 or *C*(10-17)), 30.0 (1 × *C*8 or *C*(10-17)), 29.8-29.7 (3 × *C*8 or *C*(10-17)), 29.6 (1 × *C*8 or *C*(10-17)), 29.5 (1 × *C*8 or *C*(10-17)), 26.4 (*C*9), 22.8 (1 × *C*8 or *C*(10-17)), 14.3 (*C*18); **HRMS**: *m/z* (ES)$^+$ Found [M+Na]$^+$ 1306.6272; $C_{78}H_{93}NNaO_{13}S^+$ requires [M]$^+$ 1306.6260; **LRMS**: *m/z* (ES)$^+$ 1285 ([M+H]$^+$, 100%); **HPLC** (Protocol B): ret. time = 9.16 min, purity = 95.4%.

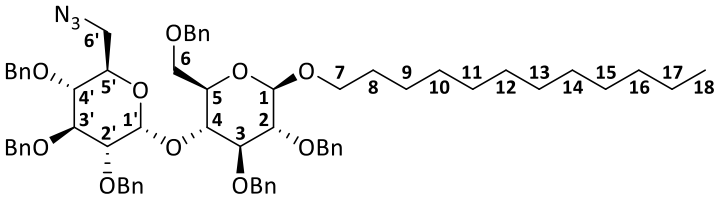
***n*-Dodecyl-(2,3,6,2',3',4'-*O*-hexabenzyl-6'-*O*-mesyl)-β-D-maltoside (82)**



78 (749 mg, 0.71 mmol, 1.00 eq.) and anhydrous triethylamine (218 µL, 159 mg, 1.57 mmol, 2.20 eq.) were dissolved in anhydrous CH_2Cl_2 (18 mL). Mesyl chloride (66 µL, 98 mg, 0.86 mmol, 1.20 eq.) was added and the resulting solution was stirred at RT for 1 h. After this time, the reaction solution was passed through a silica plug, eluting with 1:1 EtOAc:Petrol. The solution was concentrated *in vacuo* affording **82** (805 mg, 100%) as a colourless oil that was used without further purification. $\mathbf{R_f}$: 0.63 (1:4 EtOAc:Petrol); $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\boldsymbol{20}}$: +26.7 (*c* 1.0, $CHCl_3$); **IR** (thin film) ν_{max} cm^{-1}: 2925 (m), 2854 (m), 1360 (m), 1177 (m); **^{1}H NMR** (500 MHz, $CDCl_3$): δ 7.37-7.23 (22H, m, Ar C*H*), 7.22-7.12 (8H, m, Ar C*H*), 5.67 (1H, d, *J* = 3.6 Hz, C(1')*H*), 4.99 (1H, d, *J* = 11.7 Hz, PhC(α)H_AH_B), 4.95 (1H, d, *J* = 10.9 Hz, PhC(β)H_AH_B), 4.91 (1H, d, *J* = 10.9 Hz, PhC(γ)H_AH_B), 4.87 (1H, d, *J* = 10.9 Hz, PhC(δ)H_AH_B), 4.80 (1H, d, *J* = 10.9 Hz, PhC(γ)H_AH_B), 4.67 (1H, d, *J* = 10.9 Hz, PhC(α)H_AH_B), 4.65-4.56 (4H, m, PhC(β)H_AH_B, PhC(δ)H_AH_B, PhC(ε)H_AH_B & PhC(ζ)H_AH_B), 4.53 (1H, d, J = 11.8 Hz, PhC(ε)H_AH_B), 4.53 (1H, d, PhC(ζ)H_AH_B), 4.42 (1H, d, *J* = 7.8 Hz, C(1)*H*), 4.17 (1H, dd, *J* = 11.1, 3.1 Hz, C(6')H_AH_B), 4.07 (1H, dd, *J* = 9.2, 9.0 Hz, C(4)*H*), 4.99-4.03 (1H, m, C(6')H_AH_B), 3.99-3.93 (1H, m, C(7)H_AH_B), 3.91 (1H, dd, *J* = 9.6, 9.4 Hz, C(3')*H*), 3.88-3.82 (2H, m, C(5')*H* & C(6)H_AH_B), 3.79 (1H, dd, *J* = 9.0, 8.8 Hz, C(3)*H*), 3.76-3.71 (1H, m, C(6)H_AH_B), 3.59-3.48 (4H, m, C(2)*H*, C(5)*H*, C(4')*H* & C(7)H_AH_B), 3.43 (1H, dd, *J* = 9.6, 3.6 Hz, C(2')*H*), 2.78 (3H, s, SO_2CH_3), 1.72-1.62 (2H, m, C(8)H_2), 1.47-1.18 (18H, m, C(9-17)H_2), 0.88 (3H, t, *J* = 6.9 Hz, C(18)H_3); **^{13}C NMR** (126 MHz, $CDCl_3$): δ 138.7 (Ar *C*), 138.6 (Ar *C*), 138.4 (Ar *C*), 138.3 (Ar *C*), 138.0 (Ar *C*), 137.8 (Ar *C*), 128.6 (Ar *C*H), 128.51-128.48 (multiple Ar *C*H), 128.4

(Ar *C*H), 128.3 (Ar *C*H), 128.1 (Ar *C*H), 127.9-127.7 (multiple Ar *C*H), 127.4 (Ar *C*H), 126.8 (Ar *C*H), 103.7 (*C*1), 96.1 (*C*1'), 84.9 (*C*3), 82.4 (*C*5), 81.8 (*C*3'), 79.4 (*C*2'), 77.1 (*C*4'), 75.6 (Ph*C*H_2), 75.3 (Ph*C*H_2), 74.7 (Ph*C*H_2), 74.5 (*C*2), 73.9 (Ph*C*H_2), 73.7 (Ph*C*H_2), 73.5 (Ph*C*H_2), 71.7 (*C*4), 70.2 (*C*7), 69.4 (*C*5'), 68.9 (*C*6), 68.7 (*C*6'), 37.2 (SO_2CH_3), 32.0 (1 × *C*8 or *C*(10-17)), 29.9 (1 × *C*8 or *C*(10-17)), 29.82 (1 × *C*8 or *C*(10-17)), 29.80-29.7 (3 × *C*8 or *C*(10-17)), 29.6 (1 × *C*8 or *C*(10-17)), 29.5 (1 × *C*8 or *C*(10-17)), 26.4 (*C*9), 22.8 (1 × *C*8 or *C*(10-17)), 14.3 (*C*18); **HRMS**: *m/z* (ES)$^+$ Found $[M+Na]^+$ 1151.5521, $C_{67}H_{84}NaO_{13}S^+$ requires $[M]^+$ 1151.5525; **LRMS**: *m/z* (ES)$^+$ 1129 ($[M+H]^+$, 100%); **Anal. Calcd.** for $C_{67}H_{84}O_{13}S$: *C*, 71.25; *H*, 7.50; *N*, 0.00. Found: *C*, 71.32; *H*, 7.50; *N*, 0.00.

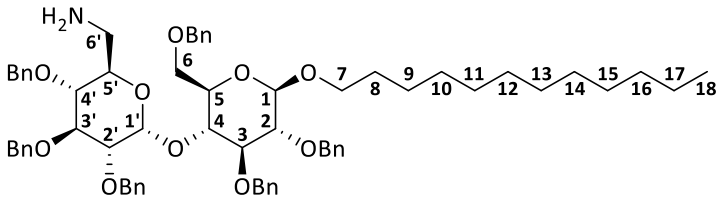
***n*-Dodecyl-(2,3,6,2',3',4'-*O*-hexabenzyl-6'-deoxy-6'-azido)-β-D-maltoside (83)**



82 (801 mg, 0.71 mmol, 1.00 eq.) was dissolved in anhydrous DMF (18 mL). Sodium azide (138 mg, 2.13 mmol, 3.00 eq.) was added and the resulting suspension was heated to 60 °C for 24 h. After this time, the reaction solution was diluted with EtOAc (40 mL). The organic layer was washed with distilled H_2O (3 × 50 mL) and brine (3 × 50 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo* to give the crude product. The crude material was adsorbed onto Celite® and purified *via* flash column chromatography on silica gel (Biotage®; gradient elution: 2% → 20% EtOAc in petrol) affording **83** (745 mg, 98%) as a pale yellow oil. $\mathbf{R_f}$: 0.54 (1:9 EtOAc:Petrol); $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\mathbf{20}}$: +42.3 (*c* 1.0, $CHCl_3$); **IR** (thin film) ν_{max} cm^{-1}: 2924 (m), 2854 (m), 2100 (m); **^{1}H NMR** (700 MHz, $CDCl_3$): δ 7.40-7.22 (23H, m, Ar C*H*), 7.21-7.12 (7H, m, Ar C*H*), 5.67

(1H, d, *J* = 3.6 Hz, C(1')*H*), 4.96 (1H, d, *J* = 11.7 Hz, PhC(α)H_AH_B), 4.94 (1H, d, *J* = 10.4 Hz, PhC(β)H_AH_B), 4.89 (1H, d, *J* = 10.9 Hz, PhC(γ)H_AH_B), 4.87 (1H, d, *J* = 11.1 Hz, PhC(δ)H_AH_B), 4.76 (1H, d, *J* = 10.9 Hz, PhC(γ)H_AH_B), 4.72 (1H, d, *J* = 11.7 Hz, PhC(α)H_AH_B), 4.62 (1H, d, *J* = 10.4 Hz, PhC(β)H_AH_B), 4.61-4.56 (2H, m, PhC(ε)H_AH_B), 4.56 (1H, d, *J* = 11.1 Hz, PhC(δ)H_AH_B), 4.56-4.51 (2H, m, PhC(ζ)H_AH_B), 4.43 (1H, d, *J* = 7.8 Hz, C(1)*H*), 4.07 (1H, dd, *J* = 9.1, 9.1 Hz, C(4)*H*), 3.96 (1H, dt, *J* = 9.2, 6.6 Hz, C(7)H_AH_B), 3.89 (1H, dd, *J* = 9.3, 9.3 Hz, C(3')*H*), 3.84-3.75 (4H, m, C(3)*H*, C(6)H_AH_B & C(5')*H*), 3.59-3.51 (3H, m, C(2)*H*, C(5)*H* & C(7)H_AH_B), 3.49-3.43 (2H, m, C(2')*H* & C(4')*H*), 3.29 (1H, dd, *J* = 13.4, 2.9 Hz, C(6')H_AH_B), 3.15 (1H, dd, *J* = 13.2, 2.9 Hz, C(6')H_AH_B), 1.72-1.62 (2H, m, C(8)H_2), 1.46-1.20 (2H, m, C(9-17)H_2), 0.89 (3H, t, *J* = 7.1 Hz, CH_3); **^{13}C NMR** (126 MHz, $CDCl_3$): δ 138.8 (Ar *C*), 138.7 (Ar *C*), 138.43 (Ar *C*), 138.37 (Ar *C*), 138.2 (Ar *C*), 137.9 (Ar *C*), 128.6 (Ar *C*H), 128.5-128.4 (multiple Ar *C*H), 128.3 (Ar *C*H), 128.1 (Ar *C*H), 128.0 (Ar *C*H), 127.9-127.7 (multiple Ar *C*H), 127.3 (Ar *C*H), 126.7 (Ar *C*H), 103.7 (*C*1), 96.1 (*C*1'), 84.8 (*C*3), 82.3 (*C*2), 81.7 (*C*3'), 79.6 (*C*2'), 78.3 (*C*4'), 75.6 (Ph*C*H_2), 75.3 (Ph*C*H_2), 74.7 (Ph*C*H_2), 74.6 (*C*5), 73.7 (Ph*C*H_2), 73.6 (Ph*C*H_2), 73.3 (Ph*C*H_2), 72.2 (*C*4), 70.8 (*C*5'), 70.2 (*C*7), 69.2 (*C*6), 51.2 (*C*6'), 32.1 (1 × *C*(8) or *C*(10-17)), 29.9 (1 × *C*(8) or *C*(10-17)), 29.82-29.76 (3 × *C*(8) or *C*(10-17)), 29.6 (1 × *C*(8) or *C*(10-17)), 29.5 (1 × *C*(8) or *C*(10-17)), 26.4 (*C*9), 22.8 (1 × *C*(8) or *C*(10-17)), 14.3 (*C*18); **HRMS**: *m/z* $(ES)^+$ Found $[M+Na]^+$ 1098.5845, $C_{66}H_{81}N_3NaO_{10}^+$ requires $[M]^+$ 1098.5814; **LRMS**: *m/z* $(ES)^+$ 1099 ($[M+Na]^+$, 100%); **HPLC** (Protocol B): ret. time = 3.10 min, purity = 95.5%.

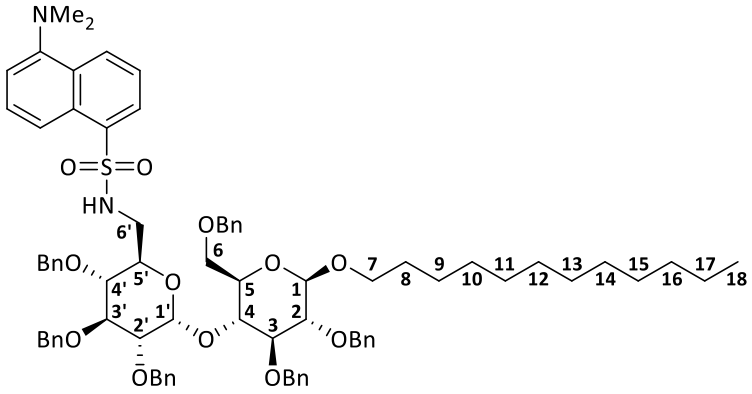
***n*-Dodecyl-(2,3,6,2',3',4'-*O*-hexabenzyl-6'-deoxy-6'-amino)-β-D-maltoside (84)**



83 (103 mg, 0.10 mmol, 1.00 eq.) and powdered triphenylphosphine (38 mg, 0.14 mmol, 1.50 eq.) were dissolved in THF (1.4 mL) and distilled H_2O (0.29 mL). The resulting solution was stirred at RT for 18 h under an atmosphere of nitrogen. After this time, the reaction mixture was concentrated *in vacuo* to give the crude product. The crude material was adsorbed onto Celite® and purified *via* flash column chromatography on pre-washed (1% triethylamine in petrol) silica gel (Biotage®; gradient elution: 30% → 70% EtOAc in petrol) affording **83** (53 mg, 53%) as a colourless oil. **R_f**: 0.08 (3:2 EtOAc:Petrol); $[\alpha]_D^{20}$: +29.7 (*c* 1.0, $CHCl_3$); **IR** (thin film) ν_{max} cm^{-1}: 3089 (w), 3064 (w), 3031 (w), 2924 (m), 2854 (m), 1060 (s); **^{1}H NMR** (400 MHz, $CDCl_3$): δ 7.42-7.07 (30H, m, Ar C*H*), 5.63 (1H, d, *J* = 3.6 Hz, C(1')*H*), 5.02-4.84 (4H, m, C(α)H_AH_B, C(β)H_AH_B, C(γ)H_AH_B & C(δ)H_AH_B), 4.79 (1H, d, *J* = 10.5 Hz, C(γ)H$_A$$H_B$), 4.73 (1H, d, *J* = 11.0 Hz, C(α)H$_A$$H_B$), 4.67-4.53 (6H, m, C(β)H$_A$$H_B$, C(δ)H$_A$$H_B$, C(ε)$H_AH_B$ & C(ζ)H_AH_B), 4.43 (1H, d, *J* = 7.7 Hz, C(1)*H*), 4.07-3.86 (3H, m, C(4)*H*, C(3')*H* & C(7)H_AH_B), 3.84-3.73 (2H, m, C(3)*H* & C(6)H_AH_B), 3.67-3.46 (4H, m, C(2)*H*, C(5)*H*, C(5')*H* & C(7)H$_A$$H_B$), 3.41 (1H, dd, *J* = 9.8, 3.6 Hz, C(2')*H*), 3.32 (1H, dd, *J* = 9.3, 9.3 Hz, C(4')*H*), 2.88 (1H, d, *J* = 12.9 Hz, C(6')H_AH_B), 2.61 (1H, dd, *J* = 12.9, 5.9 Hz, C(6')H$_A$$H_B$), 1.81-1.58 (2H, m, C(8)H_2), 1.48-1.16 (18H, m, C(9-17)H_2), 0.89 (3H, t, *J* = 6.7 Hz, C(18)H_3); **^{13}C NMR** (75 MHz, $CDCl_3$) δ 138.9 (Ar *C*),138.8 (Ar *C*), 138.4 (Ar *C*), 138.3 (Ar *C*), 138.2 (Ar *C*), 138.0 (Ar *C*), 128.6-128.3 (multiple Ar *C*H), 128.0-127.6 (multiple Ar *C*H), 127.3 (Ar *C*H), 126.8 (Ar *C*H), 103.6 (*C*1), 96.0 (*C*1'), 85.0 (*C*3), 82.3 (*C*2), 82.0 (*C*3'), 79.8 (*C*2'), 78.8 (*C*4'), 75.6 (Ph*C*H$_2$), 75.1 (Ph*C*H$_2$), 74.7 (Ph*C*H$_2$), 74.6 (*C*5), 73.8 (Ph*C*H$_2$), 73.7 (Ph*C*H$_2$), 73.3 (Ph*C*H$_2$), 72.3

(*C*5'), 72.0 (*C*4), 70.1 (*C*7), 69.0 (*C*6), 42.9 (*C*6'), 32.0 (1 × *C*(8-17)), 30.0 (1 × *C*(8-17)), 29.81-29.76 (4 × *C*(8-17)), 29.6 (1 × *C*(8-17)), 29.5 (1 × *C*(8-17)), 26.4 (1 × *C*(8-17)), 22.8 (1 × *C*(8-17)), 14.3 (*C*18); **HRMS**: *m/z* (ES)$^+$ Found $[M+H]^+$ 1050.6112, $C_{66}H_{84}NO_{10}^+$ requires $[M]^+$ 1050.6090; **LRMS**: *m/z* (ES)$^+$ 1050.67 ($[M+H]^+$, 100%); **Anal. Calcd.** for $C_{66}H_{83}NO_{10}$: *C*, 75.47; *H*, 7.96; *N*, 1.33. Found: *C*, 75.57; *H*, 8.02; *N*, 1.25.

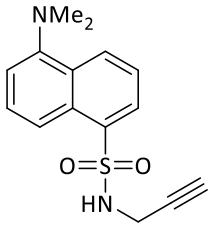
_n_-Dodecyl-(2,3,6,2',3',4'-*O*-hexabenzyl-6'-deoxy-6'-[*N*-dansyl]amino)-β-D-maltoside (85)



84 (75 mg, 0.07 mmol, 1.00 eq.), dansyl chloride **53** (29 mg, 0.11 mmol, 1.50 eq.) and anhydrous triethylamine (15 µL, 11 mg, 0.11 mmol, 1.50 eq.) were dissolved in anhydrous CH_2Cl_2 (2 mL) in a microwave vial. The microwave vial was sealed under an atmosphere of nitrogen and the resulting solution was stirred at RT for 24 h. After this time, the reaction solution was diluted with CH_2Cl_2 (18 mL). The organic layer was washed with distilled H_2O (3 × 20 mL) and brine (3 × 20 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo* to give the crude product. The crude material was adsorbed onto Celite® and purified *via* flash column chromatography on silica gel (Biotage®; gradient elution: 2% → 20% EtOAc in petrol) affording **85** (32 mg, 35%) as a yellow oil. $\mathbf{R_f}$: 0.14 (1:9 EtOAc:Petrol); $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\mathbf{20}}$: +16.3 (*c* 1.0, $CHCl_3$); **IR** (thin film) ν_{max} cm^{-1}: 3285 (w), 3063 (w), 3030 (w), 2925 (m), 2854 (m), 2159 (w), 2029 (w),

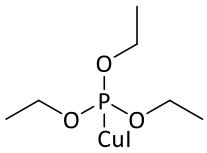
1976 (w), 1739 (m), 1497 (w), 1454 (w), 1364 (m); **^{1}H NMR** (400 MHz, $CDCl_3$): δ 8.51 (1H, d, *J* = 8.1 Hz, Napth. C*H*), 8.28 (1H, d, *J* = 8.6 Hz, Napth. C*H*), 8.16 (1H, dd, *J* = 7.6, 1.1 Hz, Napth. C*H*), 7.52 (1H, dd, *J* = 8.6, 7.8 Hz, Napth. C*H*), 7.48 (1H, dd, *J* = 8.1, 7.6 Hz, Napth. C*H*), 7.37-7.11 (31H, m, Ar C*H* & Napth. C*H*), 5.50 (1H, d, *J* = 3.6 Hz, C(1')*H*), 5.06 (1H, dd, *J* = 8.5, 3.1 Hz, N*H*), 4.94 (1H, d, *J* = 10.9 Hz, PhC(α)H_AH_B), 4.93 (1H, d, *J* = 11.7 Hz, PhC(β)H_AH_B), 4.84 (1H, d, *J* = 11.0 Hz, PhC(γ)H_AH_B), 4.72 (1H, d, *J* = 11.0 Hz, PhC(γ)H_AH_B), 4.70-4.64 (2H, m, PhC(β)H_AH_B & PhC(δ)H_AH_B), 4.62 (1H, d, *J* = 10.9 Hz, PhC(α)H_AH_B), 4.57-4.46 (4H, m, PhC(ε)H_2 & PhC(ζ)H_2), 4.38 (1H, d, *J* = 7.8 Hz, C(1)*H*), 4.36 (1H, d, *J* = 10.7 Hz, PhC(δ)H_AH_B), 3.98-3.90 (2H, m, C(4)*H* & C(7)H_AH_B), 3.82 (1H, dd, *J* = 9.6, 9.4 Hz, C(3')*H*), 3.73 (1H, dd, *J* = 8.9, 8.9 Hz, C(3)*H*), 3.72-3.62 (3H, m, C(5')*H* & C(6)H_AH_B), 3.54 (1H, dt, *J* = 9.4, 6.9 Hz, C(7)H_AH_B), 3.49-3.44 (2H, m, C(2)*H* & C(5)*H*), 3.35 (1H, dd, *J* = 9.4, 9.4 Hz, C(4')*H*), 3.28 (1H, dd, *J* = 9.6, 3.6 Hz, C(2')*H*), 3.09-2.99 (1H, m, C(6')H_AH_B), 2.96-2.88 (1H, m, C(6')H_AH_B), 2.84 (6H, s, N(CH_3)$_2$), 1.71-1.61 (2H, m, C(8)H_2), 1.46-1.21 (18H, m, C(9-17)H_2), 0.89 (3H, t, *J* = 6.8 Hz, C(18)H_3); **^{13}C NMR** (126 MHz, $CDCl_3$): δ 152.0 (Napth. *C*), 138.8 (Ar *C*), 138.7 (Ar *C*), 138.4 (Ar *C*), 138.3 (Ar *C*), 138.1 (Ar *C*), 137.9 (Ar *C*), 134.8 (Napth. *C*), 130.5 (Napth. *C*H), 130.0 (Napth. *C*), 129.8 (Napth. *C*), 129.7 (Napth. *C*H), 128.53 (Napth. *C*H), 128.4-128.4 (multiple Ar *C*H), 128.4-128.2 (multiple Ar *C*H), 128.0-127.6 (multiple Ar *C*H), 127.4 (Ar *C*H), 126.8 (Ar *C*H), 123.3 (Napth. *C*H), 119.1 (Napth. *C*H), 115.4 (Napth. *C*H), 103.6 (*C*1), 96.2 (*C*1'), 84.9 (*C*3), 82.3 (*C*2), 81.6 (*C*3'), 79.3 (*C*2'), 78.0 (*C*4'), 75.4 (Ph*C*H_2), 75.0 (Ph*C*H_2), 74.7 (Ph*C*H_2), 74.5 (*C*5), 73.9 (Ph*C*H_2), 73.41 (Ph*C*H_2), 73.37 (Ph*C*H_2), 72.2 (*C*4), 70.1 (*C*7), 69.9 (*C*5'),68.3 (*C*6), 45.5 (N(*C*H_3)$_2$), 43.2 (*C*6'), 32.1 (1 × *C*8 or *C*(10-17)), 30.0 (1 × *C*8 or *C*(10-17)), 29.83-29.77 (4 × *C*8 or *C*(10-17)), 29.6 (1 × *C*8 or *C*(10-17)), 29.5 (1 × *C*8 or *C*(10-17)), 26.4 (*C*9), 22.8 (1 × *C*8 or *C*(10-17)),14.3 (*C*18); **HRMS**: *m/z* (ES)$^+$ Found $[M+Na]^+$ 1305.6431, $C_{68}H_{94}N_2NaO_{12}S^+$ requires $[M]^+$ 1305.6420); **LRMS**: *m/z* (ES)$^+$ 1284 ($[M+H]^+$, 100%).

***N*-(Dansyl)prop-2-yn-1-amine (55)**



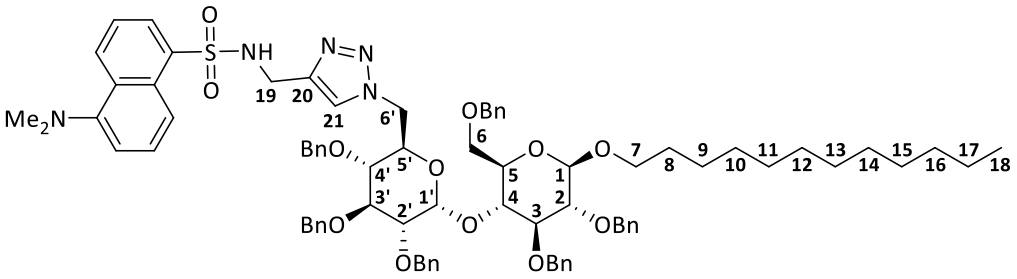
Dansyl chloride **53** (509 mg, 1.89 mmol, 1.00 eq.) and triethylamine (395 µL, 286 mg, 2.83 mmol, 1.50 eq.; distilled and stored in the dark over 3 Å molecular sieves) were dissolved in anhydrous CH_2Cl_2 (26 mL). Propargyl amine (181 µL, 156 mg, 2.83 mmol, 1.50 eq.) was added dropwise and the resulting solution was stirred at RT for 30 min. After this time, the reaction solution was concentrated *in vacuo*. The residue was dissolved in a small amount of EtOAc/petrol (3:2) and passed through a plug of silica (eluting with 3:2 EtOAc:Petrol). The solution was concentrated *in vacuo* affording **55** (571 mg, 100%) as a yellow solid that was used without further purification. $\mathbf{R_f}$: 0.52 (3:7 EtOAc:Petrol); **mp**: 91-93 °C (EtOAc/petrol) [Lit.[135] mp: 93-95 °C]; **^{1}H NMR** (400 MHz, $CDCl_3$): δ 8.57 (1H, d, *J* = 8.5 Hz, Napth. C*H*), 8.30-8.25 (2H, m, 2 × Napth. C*H*), 7.59 (1H, dd, *J* = 8.6, 7.6 Hz, Napth. C*H*), 7.54 (1H, dd, *J* = 8.5, 7.4 Hz, Napth. C*H*), 7.21 (1H, d, *J* = 7.6 Hz, Napth. C*H*), 4.87 (1H, t, *J* = 6.0 Hz, N*H*), 3.78 (2H, dd, *J* = 6.0, 2.5 Hz, CH_2), 2.90 (6H, s, N(CH_3)$_2$), 1.92 (1H, t, *J* = 2.5 Hz, C*H*); **LRMS**: *m/z* (ES)$^+$ 289 ([M+H]$^+$, 100%). The data are in good agreement with literature values.[283]

Iodo(triethylphosphite)copper(I) (88)



Copper iodide **86** (1.00 g, 5.25 mmol, 1.00 eq.) was suspended in anhydrous toluene (10.5 mL). Triethylphosphite **87** (0.90 mL, 872 mg, 2.25 mmol, 1.00 eq.) was added dropwise and the resulting suspension was heated to 80 °C for 18 h. After this time, the reaction mixture was concentrated *in vacuo* to give the crude product. The resulting material was crystallised from hexane affording **88** (1.24 g, 66%) as a colourless crystalline solid. **mp**: 112-114 °C (Hexane) [Lit.[284] mp: 110-111 °C]; **^{1}H NMR** (250 MHz, $CDCl_3$): δ 4.11 (6H, qn, *J* = 7.2 Hz, CH_2), 1.29 (9H, t, *J* =7.1 Hz, CH_3). The data are in good agreement with literature values.[137]

***n*-Dodecyl-(2,3,6,2',3',4'-*O*-hexabenzyl-6'-deoxy-6'-[4-{*N*-dansyl}-aminomethyl-1*H*-1,2,3-triazol-1-yl])-β-D-maltoside (89)**

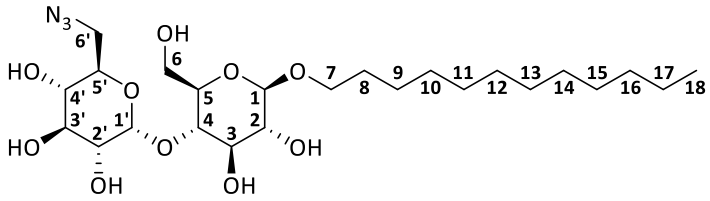


83 (217 mg, 0.20 mmol, 1.00 eq.), **55** (64 mg, 0.22 mmol, 1.10 eq.), **88** (14 mg, 0.04 mmol, 0.20 eq.) and DIPEA (105 µL, 78 mg, 0.61 mmol, 0.10 eq.) were dissolved in anhydrous MeOH (5 mL) and stirred for 18 h. After this time, the reaction solution was concentrated *in vacuo* and the residue was diluted with EtOAc (20 mL). The organic layer was washed with distilled H_2O (3 × 20 mL) and brine (3 × 20 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo* to

give the crude product. The crude material was adsorbed onto Celite® and purified *via* flash column chromatography on silica gel (Biotage®; gradient elution: 20% → 40% EtOAc in petrol) affording **89** (210 mg, 76%) as a yellow oil. $\mathbf{R_f}$: 0.16 (3:7 EtOAc:Petrol); $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\mathbf{20}}$: +16.7 (*c* 1.0, $CHCl_3$); **IR** (thin film) ν_{max} cm^{-1}: 2924 (m), 2855 (w), 1454 (m); **^{1}H NMR** (500 MHz, $CDCl_3$): δ 8.53 (1H, d, *J* = 7.8 Hz, Napth. C*H*), 8.29 (1H, d, *J* = 7.5 Hz, Napth. C*H*), 8.25 (1H, d, *J* = 7.0 Hz, Napth. C*H*), 7.54 (1H, dd, *J* = 7.6, 7.5 Hz, Napth. C*H*), 7.47 (1H, dd, *J* = 7.8, 7.0 Hz, Napth. C*H*), 7.39-7.13 (32H, m, Ar C*H*, Napth. C*H* & C(21)*H*), 5.57 (1H, d, *J* = 3.4 Hz, C(1')*H*), 5.34 (1H, br s, N*H*), 4.98-4.93 (2H, m, PhC(α)H_AH_B & PhC(β)H_AH_B), 4.86 (1H, d, *J* = 10.9 Hz, PhC(γ)H_AH_B), 4.82 (1H, d, *J* = 10.7 Hz, PhC(δ)H_AH_B), 4.73 (1H, d, *J* = 10.9 Hz, PhC(γ)H_AH_B), 4.70-4.63 (3H, m, PhC(α)H_AH_B, PhC(β)H_AH_B & PhC(δ)H_AH_B), 4.59 (1H, d, *J* = 11.9 Hz, PhC(ε)H_AH_B), 4.55 (1H, d, *J* = 11.8 Hz, PhC(ζ)H_AH_B), 4.51 (1H, d, *J* = 11.9 Hz, PhC(ε)H_AH_B), 4.50 (1H, d, *J* = 11.8 Hz, PhC(ζ)H_AH_B), 4.43 (1H, d, *J* = 7.8 Hz, C(1)*H*), 4.37 (1H, dd, *J* = 14.4, 4.3 Hz, C(6')H_AH_B), 4.19-4.10 (2H, m, C(19)H_2), 4.02 (1H, dd, *J* = 14.4, 2.8 Hz, C(6')H_AH_B), 3.98-3.90 (3H, m, C(5')*H*, C(4)*H* & C(7)H_AH_B), 3.87 (1H, dd, *J* = 9.5, 9.3 Hz, C(3')*H*), 3.77 (1H, dd, *J* = 8.8, 8.8 Hz, C(3)*H*), 3.63 (2H, d, *J* = 2.8 Hz, C(6)H_AH_B), 3.59-3.48 (3H, m, C(2)*H*, C(5)*H* & C(7)H_AH_B), 3.26 (1H, dd, *J* = 9.5, 3.4 Hz, C(2')*H*), 2.95 (1H, dd, *J* = 9.4, 9.3 Hz, C(4')*H*), 2.87 (6H, s, N(CH_3)$_2$), 1.71-1.60 (2H, m, C(8)H_2), 1.45-1.20 (18H, m, C(9-17)H_2), 0.88 (3H, t, *J* = 7.0 Hz, C(18)H_3); **^{13}C NMR** (126 MHz, $CDCl_3$): δ 152.2 (Napth. *C*), 143.5 (*C*20), 138.7 (Ar *C*), 138.5 (Ar *C*), 138.4 (Ar *C*), 138.32 (Ar *C*), 138.26 (Ar *C*), 137.9 (Ar *C*), 134.7 (Napth. *C*), 130.7 (Napth. *C*H), 130.0 (Napth. *C*), 129.8 (Napth. *C*), 129.7 (Napth. *C*H), 128.7 (Napth. *C*H), 128.6-128.5 (multiple Ar *C*H), 128.4 (Ar *C*H), 128.3 (Ar *C*H), 128.0 (Ar *C*H), 127.9-127.7 (multiple Ar *C*H), 127.5 (Ar *C*H), 126.9 (Ar *C*H), 124.1 (*C*21), 123.3 (Napth. *C*H), 118.9 (Napth. *C*H), 115.5 (Napth. *C*H), 103.7 (*C*1), 96.0 (*C*1'), 84.8 (*C*3), 82.3 (*C*2), 81.8 (*C*3'), 79.6 (*C*2'), 77.6 (*C*4'), 75.6 (Ph*C*H_2), 75.1 (Ph*C*H_2), 74.7 (Ph*C*H_2), 74.4 (*C*5), 73.9 (Ph*C*H_2), 73.7 (Ph*C*H_2), 73.5 (Ph*C*H_2), 72.1 (*C*5'), 70.2 (*C*7), 69.6 (*C*4), 68.9 (*C*6), 50.1 (*C*6'), 45.6

(N(CH_3)$_2$), 39.0 (*C*19), 32.0 (1 × *C*(8-17)), 30.0 (1 × *C*(8-17)), 29.82-29.76 (4 × *C*(8-17)), 29.6 (1 × *C*(8-17)), 29.5 (1 × *C*(8-17)), 26.3 (1 × *C*(8-17)), 22.8 (1 × *C*(8-17)), 14.3 (*C*18); **HRMS**: *m/z* (ES)$^+$ Found [M+Na]$^+$ 1386.6683, $C_{81}H_{97}N_5NaO_{12}S^+$ requires [M]$^+$ 1386.6747; **LRMS**: *m/z* (ES)$^+$ 1365 ([M+H]$^+$, 100%); **Anal. Calcd.** for $C_{81}H_{97}N_5O_{12}S$: *C*, 71.29; *H*, 7.16; *N*, 5.13. Found: *C*, 71.15; *H*, 7.03; *N*, 5.19.

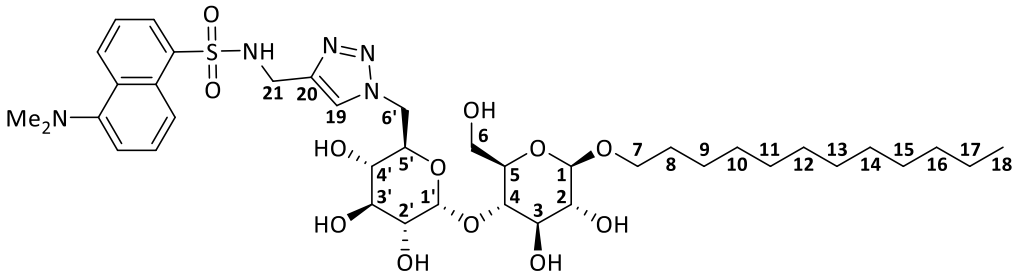
***n*-Dodecyl-(6'-deoxy-6'-azido)- β-D-maltoside (90)**



83 (98 mg, 0.09 mmol, 1.00 eq.) was dissolved in a biphasic mixture of EtOAc (3.9 mL) and distilled H_2O (2.6 mL). Subsequently, $NaBrO_3$ (214 mg, 1.42 mmol, 15.60 eq.) in distilled H_2O (1.0 mL) was added, followed by the dropwise addition of $Na_2S_2O_3$ (222 mg, 1.27 mmol, 14.00 eq.) in distilled H_2O (2.1 mL) over 3 min. The resulting mixture was stirred vigorously at RT for 3 h under an atmosphere of nitrogen. After this time, the reaction mixture was diluted with EtOAc (20 mL). The organic layer was washed with NaS_2O_4 aq. (3 × 20 mL, 10% w/v), distilled H_2O (1 × 20 mL) and brine (3 × 20 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo* to give the crude product. The crude material was adsorbed onto Celite® and purified *via* flash column chromatography on silica gel (Biotage®; gradient elution: 1% → 10% MeOH in $CHCl_3$) affording **90** (24 mg, 49%) as a colourless oil. $\mathbf{R_f}$: 0.05 (1:19 EtOAc:Petrol); $[\alpha]_D^{20}$: +23.2 (*c* 0.25, MeOH); **IR** (thin film) ν_{max} cm^{-1}: 3365 (w), 2970 (w), 2854 (w), 2102 (w), 1739 (m); **^{1}H NMR** (400 MHz, CD_3OD): δ 5.18 (1H, d, *J* = 3.8 Hz, C(1')*H*), 4.27 (1H, d, *J* = 7.8 Hz, C(1)*H*), 3.94 (1H, dd, *J* = 12.0, 1.9 Hz, C(6')H_AH_B), 3.90 (1H, dt, *J* = 9.5, 6.9 Hz, C(7)H_AH_B), 3.83-3.76 (2H, m,

C(5)*H* & C(6')H_AH_B), 3.65-3.52 (4H, m, C(3)*H*, C(3')*H*, C(7)H_AH_B & C(6)H_AH_B), 3.50 (1H, dd, *J* = 9.2, 9.2 Hz, C(4')*H*), 3.45 (1H, dd, *J* = 9.6, 3.8 Hz, C(2')*H*), 3.46-3.43 (2H, m, C(6)H_AH_B & C(5')*H*), 3.26 (1H, dd, *J* = 9.4, 9.4 Hz, C(4)*H*), 3.22 (1H, dd, *J* = 9.3, 7.8 Hz, C(2)*H*), 1.67-1.58 (2H, m, C(8)H_2), 1.43-1.23 (18H, m, C(9-17)H_2), 0.90 (3H, t, *J* = 6.8 Hz, C(18)H_3); **^{13}C NMR** (126 MHz, CD_3OD): δ 104.3 (*C*1), 102.8 (*C*1'), 81.6 (*C*4'), 77.9 (*C*3), 76.6 (*C*5'), 74.8 (*C*3'), 74.7 (*C*2), 74.1 (*C*2'), 73.7 (*C*5), 72.2 (*C*4), 71.0 (*C*7), 62.4 (*C*6'), 53.0 (*C*6), 33.1 (1 × *C*(8-17)), 30.80-30.76 (5 × *C*(8-17)), 30.6 (1 × *C*(8-17)), 30.5 (1 × *C*(8-17)), 27.1 (1 × *C*(8-17)), 23.7 (1 × *C*(8-17)), 14.4 (*C*18); **HRMS**: *m/z* $(ES)^+$ Found $[M+Na]^+$ 558.3002, $C_{24}H_{45}N_3NaO_{10}^+$ requires $[M]^+$ 558.2997; **LRMS**: *m/z* $(ES)^+$ 130 (100%), 558 ($[M+Na]^+$, 40%).

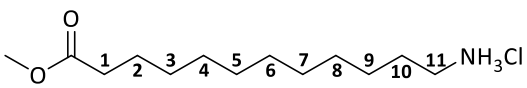
_n_-Dodecyl-(6'-deoxy-6'-[4-{_N_-Dansyl}-aminomethyl-1_H_-1,2,3-triazol-1-yl])-β-D-maltoside (42)



90 (35 mg, 0.06 mmol, 1.00 eq.), **55** (21 mg, 0.07 mmol, 1.10 eq.), **88** (1 mg, 3.23 µmol, 0.05 eq.) and DIPEA (1 µL, 1 mg, 6.46 µmol, 0.10 eq.) were dissolved in anhydrous MeOH (1.5 mL) and stirred for 18 h. After this time, the reaction solution was concentrated *in vacuo* and the residue was taken up in EtOAc (20 mL). The organic layer was washed with distilled H_2O (3 × 20 mL) and brine (3 × 20 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo* to give the crude product. The crude material was adsorbed onto Celite® and purified *via* flash column chromatography on silica gel (Biotage®; gradient elution: 2% → 10% MeOH in $CHCl_3$)

affording **42** (37 mg, 70%) as a yellow foam. $\mathbf{R_f}$: 0.22 (1:9 MeOH:$CHCl_3$); $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\mathbf{20}}$: +14.9 (*c* 1.0, MeOH); **IR** (thin film) ν_{max} cm^{-1}: 3352 (br, s), 2924 (s), 2853 (m), 2789 (w), 2362 (w), 2099 (w); **^{1}H NMR** (500 MHz, CD_3OD): δ 8.56 (1H, d, *J* = 8.7 Hz, Napth. C*H*), 8.32 (1H, d, *J* = 8.7 Hz, Napth. C*H*), 8.22 (1H, dd, *J* = 8.1, 1.0 Hz, Napth. C*H*), 7.59 (1H, dd, *J* = 8.7, 8.1 Hz, Napth. C*H*), 7.58 (1H, dd, *J* = 8.7, 8.1 Hz, Napth. C*H*), 7.45 (1H, s, C(21)*H*), 7.28 (1H, d, *J* = 8.1 Hz, Napth. C*H*), 5.06 (1H, d, *J* = 3.7 Hz, C(1')*H*), 4.61 (1H, dd, *J* = 14.2, 2.1 Hz, C(6')H_AH_B), 4.26 (1H, dd, *J* = 14.2, 8.2 Hz, C(6')H_AH_B), 4.22 (1H, d, *J* = 7.8 Hz, C(1)*H*), 4.16 (2H, s, C(19)H_2), 3.90-3.82 (2H, m, C(7)H_AH_B & C(5')*H*), 3.57 (1H, dd, *J* = 9.3, 9.3 Hz, C(3')*H*), 3.54 (1H, dd, *J* = 9.1, 9.1 Hz, C(3)*H*), 3.53-3.48 (1H, m, C(7)H_AH_B), 3.40-3.33 (3H, m, C(2')*H*, C(4)*H* & C(6)H_AH_B), 3.21-3.16 (2H, m, C(5)*H* & C(2)*H*), 3.09-3.00 (2H, m, C(6)H_AH_B & C(4')*H*), 2.90 (6H, s, N(CH_3)$_2$), 1.65-1.57 (2H, m, C(8)H_2), 1.42-1.21 (16H, m, C(9-17)H_2), 0.89 (3H, t, *J* = 7.0 Hz, C(18)H_3); **^{13}C NMR** (500 MHz, CD_3OD): δ 153.2 (Napth. *C*), 145.5 (*C*20), 136.8 (Napth. *C*), 131.3 (Napth. *C*H), 131.1 (Napth. *C*), 130.9 (Napth. *C*), 103.5 (Napth. *C*H), 129.3 (Napth. *C*H), 125.8 (*C*21), 124.4 (Napth. *C*H), 120.5 (Napth. *C*H), 116.4 (Napth. *C*H), 104.1 (*C*1), 102.7 (*C*1'), 81.6 (*C*4), 77.7 (*C*3), 76.2 (*C*2), 74.7-74.6 (*C*3' & *C*5), 74.0 (*C*2'), 73.0 (*C*5'), 72.5 (*C*4'), 71.0 (*C*7), 61.8 (*C*6), 52.4 (*C*6'), 45.9 (N(*C*H_3)$_2$)), 39.1 (*C*19), 33.1 (1 × *C*(8-17)), 30.8-30.7 (5 × *C*(8-17)), 30.6 (1 × *C*(8-17)), 30.5 (1 × *C*(8-17)), 27.1 (1 × *C*(8-17)), 23.7 (1 × *C*(8-17)), 14.1 (*C*18); **HRMS**: *m/z* (ES)$^+$ Found [M+Na]$^+$ 846.3933, $C_{39}H_{61}N_5NaO_{12}S$ requires [M]$^+$ 846.3930; **LRMS**: *m/z* (ES)$^+$ 824 ([M+H]$^+$, 100%); **HPLC** (Protocol A): ret. time = 6.36 min, purity = 98.0%.

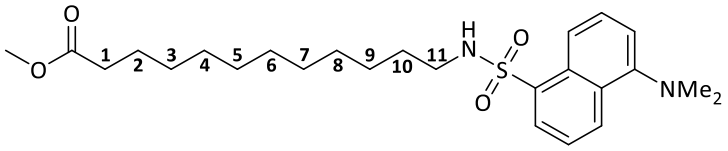
Methyl 12-aminododecanoate·hydrogen chloride (94)



Anhydrous MeOH (3 mL) was cooled to 0 °C and thionyl chloride (150 µL, 245 mg, 2.06 mmol, 2.22 eq.) was added dropwise. The resulting solution was stirred at 0 °C for 20 min.

12-Aminododecanoic acid **56** (200 mg, 0.93 mmol, 1.00 eq.) was added portion-wise and the resulting suspension was stirred at RT for 10 h. After this time, the reaction mixture was concentrated *in vacuo* to give the crude product. The crude material was triturated (hexane) and the solid isolated *via* gravity filtration affording **94** (220 mg, 89%) as a colourless solid that was used without further purification. $\mathbf{R_f}$: 0.45 (19:1 EtOAc:Petrol); **mp**: 156-158 °C (MeOH) [Lit.[285] mp: 159-160 °C (MeOH)]; **^{1}H NMR** (300 MHz, $(CD_3)_2SO$): δ 7.78 (3H, br s, NH_3), 3.58 (3H, s, CH_3), 2.75 (2H, t, *J* = 7.5 Hz, C(11)H_2), 2.29 (2H, t, *J* = 7.4 Hz, C(1)H_2), 1.60-1.42 (2H, m, C(2)H_2 & C(10)H_2), 1.37-1.18 (14H, m, C(3-9)H_2); **LRMS**: *m/z* $(ES)^+$ 230 ($[M\text{-}Cl]^+$, 100%). The data are in good agreement with literature values.[286]

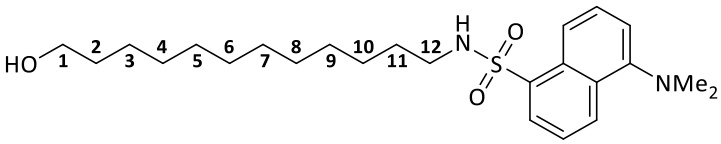
Methyl 12-(*N*-dansyl)aminododecanoate (95)



94 (50 mg, 0.19 mmol, 1.00 eq.) and DABCO (47 mg, 0.41 mmol, 2.20 eq.) were dissolved in anhydrous CH_2Cl_2 (500 µL) and anhydrous DMF (500 µL). Dansyl chloride **53** (61 mg, 0.23 mmol, 1.20 eq.) was added and the resulting solution was stirred at RT for 18 h. After this time, the reaction solution was diluted with CH_2Cl_2 (25 mL). The organic layer was washed with distilled H_2O (3 × 25 mL) and brine (3 × 25 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo* to give the crude product. The crude material was adsorbed onto Celite® and purified *via* flash column chromatography on silica gel (Biotage®; gradient elution: 5% → 40% EtOAc in petrol) affording **95** (70 mg, 81%) as a yellow oil. $\mathbf{R_f}$: 0.19 (1:9 EtOAc:Petrol); **IR** (thin film) ν_{max} cm^{-1}: 2928 (w), 1739 (s), 1366 (m), 1217 (m); **^{1}H NMR** (400 MHz, $CDCl_3$): δ 8.52 (1H, d,

J = 8.4 Hz, Napth. C*H*), 8.30 (1H, d, *J* = 8.5 Hz, Napth. C*H*), 8.23 (1H, dd, *J* = 7.3, 1.1 Hz, Napth. C*H*), 7.53 (1H, dd, *J* = 8.5, 7.6 Hz, Napth. C*H*), 7.50 (1H, dd, *J* = 8.4, 7.3 Hz, Napth. C*H*), 7.16 (1H, d, *J* = 7.6 Hz, Napth. C*H*), 4.89 (1H, t, *J* = 6.1 Hz, N*H*), 3.65 (3H, s, OCH_3), 2.92-2.82 (8H, m, C(11)H_2 & N(CH_3)$_2$), 2.27 (2H, t, *J* = 7.5 Hz, C(1)H_2), 1.66-1.53 (2H, m, C(2)H_2), 1.39-1.00 (16H, m, C(3-10)H_2); **^{13}C NMR** (101 MHz, $CDCl_3$): δ 174.4 (*C*=O), 152.0 (Napth. *C*), 134.9 (Napth. *C*), 130.4 (Napth. *C*H), 130.0 (Napth. *C*), 129.72 (Napth. *C*), 129.65 (Napth. *C*H), 128.4 (Napth. *C*H), 123.3 (Napth. *C*H), 118.9 (Napth. *C*H), 115.2 (Napth. *C*H), 51.5 (O*C*H_3), 45.5 (N(*C*H_3)$_2$), 43.4 (*C*11), 34.2 (*C*1), 29.5 (1 × *C*(3-10)), 29.41 (1 × *C*(3-10)), 29.38 (1 × *C*(3-10)), 29.35 (1 × *C*(3-10)), 29.3 (1 × *C*(3-10)), 29.2 (1 × *C*(3-10)), 29.0 (1 × *C*(3-10)), 26.4 (1 × *C*(3-10)), 25.0 (*C*2); **HRMS**: *m/z* (ES)$^+$ Found [M+Na]$^+$ 485.2443, $C_{25}H_{38}N_2NaO_4S^+$ requires [M]$^+$ 485.2444); **LRMS**: *m/z* (ES)$^+$ 463 ([M+H]$^+$, 100%); **Anal. Calcd.** for $C_{25}H_{38}N_2O_4S$: *C*, 64.90; *H*, 8.28; *N*, 6.06. Found: *C*, 64.66; *H*, 8.18; *N*, 6.16.

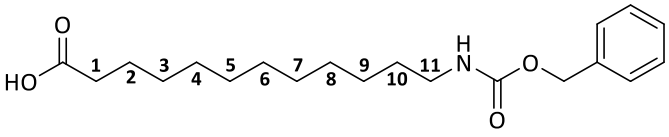
12-(*N*-Dansyl)aminododecanol (57)



95 (496 mg, 1.07 mmol, 1.00 eq.) was dissolved in anhydrous THF (4.5 mL) and cooled to 0 °C. $LiAlH_4$ (4.3 mL, 163 mg, 4.29 mmol, 4.00 eq.; 1 M in THF) was added and the resulting solution was stirred at RT for 4 h. After this time, the reaction solution was cooled to 0 °C and quenched *via* the dropwise addition of distilled H_2O (0.5 mL). The resulting mixture was concentrated *in vacuo* and the residue was taken up in EtOAc (50 mL). The organic layer was washed with distilled H_2O (3 × 40 mL) and brine (3 × 40 mL), dried ($MgSO_4$), filtered and concentrated *in*

vacuo to give the crude product. The crude material was adsorbed onto Celite® and purified *via* flash column chromatography on silica gel (Manual; 3:7 → 2:3 EtOAc:Petrol) affording **57** (422 mg, 90%) as a yellow solid. **R_f**: 0.09 (1:4 EtOAc:Petrol); **mp**: 66-68 °C (EtOAc/Petrol); **IR** (neat) ν_{max} cm^{-1}: 3505 (w), 2978 (w), 2850 (w), 1321 (w), 1140 (w); **^{1}H NMR** (400 MHz, $CDCl_3$): δ 8.52 (1H, d, *J* = 8.5 Hz, Napth. C*H*), 8.30 (1H, d, *J* = 8.6 Hz, Napth. C*H*), 8.23 (1H, dd, *J* = 7.3, 1.2 Hz, Napth. C*H*), 7.54 (1H, dd, *J* = 8.6, 7.6 Hz, Napth. C*H*), 7.51 (1H, dd, *J* = 8.5, 7.3 Hz, Napth. C*H*), 7.17 (1H, d, *J* = 7.6 Hz, Napth. C*H*), 4.92 (1H, t, *J* = 6.1 Hz, N*H*), 3.61 (2H, t, *J* = 6.7 Hz, C(1)H_2), 2.91-2.82 (8H, m, C(12)H_2 & N(CH_3)$_2$), 1.58-1.49 (2H, m, C(2)H_2), 1.39-0.99 (18H, m, C(3-11)H_2); **^{13}C NMR** (101 MHz, $CDCl_3$): δ 152.0 (Napth. *C*), 134.9 (Napth. *C*), 130.4 (Napth. *C*), 130.0 (Napth. *C*), 129.75 (Napth. *C*), 129.67 (Napth. *C*H), 128.4 (Napth. *C*H), 123.3 (Napth. *C*H), 118.9 (Napth. *C*H), 115.2 (Napth. *C*H), 63.1 (*C*1), 45.5 (N(*C*H_3)$_2$), 43.4 (*C*12), 32.8 (*C*2), 29.6-29.5 (5 × *C*(3-11)), 29.4 (1 × *C*(3-11)), 29.0 (1 × *C*(3-11)), 26.5 (1 × *C*(3-11)), 25.8 (1 × *C*(3-11)); **HRMS**: *m/z* (ES)$^+$ Found [M+Na]$^+$ 457.2503. $C_{24}H_{38}N_2NaO_3S^+$ requires [M]$^+$ 457.2495; **LRMS**: *m/z* (ES)$^+$ 435 ([M+H]$^+$, 100%); **Anal. Calcd.** for $C_{24}H_{38}N_2O_3S$: *C*, 66.32; *H*, 8.81; *N*, 6.45. Found: *C*, 66.42; *H*, 8.89; *N*, 6.39.

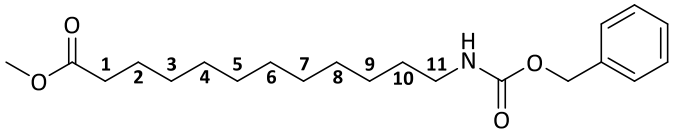
12-(*N*-Benzyloxycarbonyl)aminododecanoic acid (96)



12-Aminododecanoic acid **56** (1.00 g, 4.64 mmol, 1.00 eq.) was suspended in $NaHCO_3$ aq. (8.7 mL, 585 mg, 1.50 eq., 0.8 M) and benzyl chloroformate (729 µL, 871 mg, 5.11 mmol, 1.10 eq.) was added dropwise. The resulting suspension was stirred at RT for 18 h under an

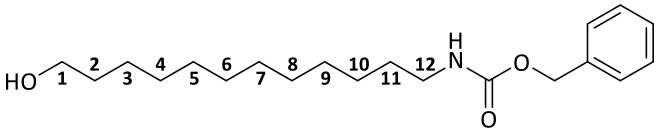
atmosphere of nitrogen. After this time, the pH was adjusted to pH < 3 and Et_2O (100 mL) was added dissolving the precipitate. The organic layer was isolated and washed with distilled H_2O (3 × 50 mL) and brine (3 × 50 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo* to give the crude product. The crude material was crystallised from EtOAc affording **96** (376 mg, 23%) as a colourless crystalline solid. **R_f**: 0.19 (3:7 EtOAc:Petrol); **mp**: 80-82 °C (EtOAc) [Lit.[285] mp: 86-87 °C (EtOAc)]; **^{1}H NMR** (400 MHz, $CDCl_3$): δ 7.42-7.25 (5H, m, Ar C*H*), 5.15-5.10 (2H, m, PhCH_2), 4.75 (1H, br s, N*H*), 3.18 (2H, dt, *J* = 6.7, 6.6 Hz, C(11)H_2), 2.35 (2H, t, *J* = 7.3 Hz, C(1)H_2), 1.70-1.57 (2H, m, C(2)H_2), 1.55-1.41 (2H, m, C(10)H_2), 1.39-1.20 (14H, m, C(3-9)H_2); **LRMS**: *m/z* $(ES)^-$ 348 ($[M-H]^-$, 100%). The data are in good agreement with literature values.[287]

Methyl 12-(*N*-benzyloxycarbonyl)aminododecanoate (97)



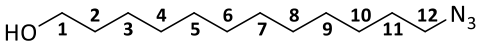
Anhydrous MeOH (1 mL) was cooled to 0 °C and thionyl chloride (46 µL, 75 mg, 0.63 mmol, 2.20 eq.) was added dropwise. The resulting solution was stirred at 0 °C for 20 min. **96** (100 mg, 0.29 mmol, 1.00 eq.) was added and the resulting suspension was stirred at RT for 1.5 h. After this time, the reaction mixture was concentrated *in vacuo* and the residue was taken up in EtOAc (50 mL). The organic layer was washed with distilled H_2O (3 × 40 mL) and brine (3 × 40 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo* affording **97** (79 mg, 76%) as a colourless oil that was used without further purification. **R_f**: 0.27 (1:9 EtOAc:Petrol); **^{1}H NMR** (300 MHz, $CDCl_3$): δ 7.38-7.29 (5H, m, Ar C*H*), 5.10 (2H, s, PhCH_2), 4.71 (1H, br s, N*H*), 3.67 (3H, s, OCH_3), 3.22-3.15 (2H, m, C(11)H_2), 2.30 (2H, t, *J* = 7.5 Hz, C(1)H_2), 1.68-1.57 (2H, m, C(2)H_2), 1.53-1.42 (2H, m, C(10)H_2), 1.36-1.19 (14H, m, C(3-9)H_2); **LRMS**: *m/z* $(ES)^+$ 364 ($[M+H]^+$, 100%). The data are in good agreement with literature values.[150]

12-(*N*-Benzyloxycarbonyl)aminododecanol (58)



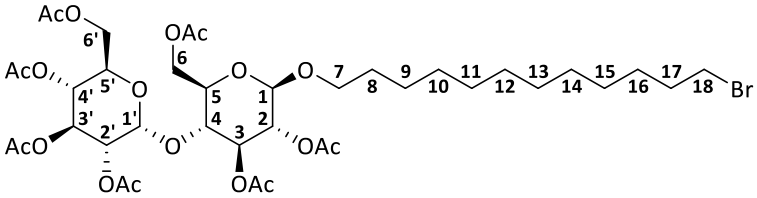
97 (79 mg, 0.22 mmol, 1.00 eq.) was dissolved in anhydrous THF (0.8 mL) and cooled to 0 °C. $LiAlH_4$ (871 µL, 33 mg, 0.87 mmol, 4.00 eq., 1 M in THF) was added and the resulting solution was stirred at RT for 1 h. After this time, the reaction solution was cooled to 0 °C and quenched *via* the dropwise addition of distilled H_2O (0.5 mL). The resulting mixture was concentrated *in vacuo* and the residue was taken up in EtOAc (25 mL). The organic layer was washed with distilled H_2O (3 × 25 mL) and brine (3 × 25 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo* to give the crude product. The crude material was adsorbed onto Celite® and purified *via* flash column chromatography on silica gel (Manual; 3:7 → 2:3 EtOAc:Petrol) affording **58** (422 mg, 90%) as a colourless solid. **R_f**: 0.21 (3:7 EtOAc:Petrol); **mp**: 87-88 °C (EtOAc); **IR** (neat) ν_{max} cm^{-1}: 3397 (w), 3346 (m), 2924 (m), 2849 (m), 1687 (s), 1530 (m); **^{1}H NMR** (400 MHz, $CDCl_3$): δ 7.39-4.28 (5H, m, Ar C*H*), 5.09 (2H, s, PhCH_2), 4.75 (1H, br s, N*H*), 3.63 (2H, t, *J* = 6.6 Hz, C(1)H_2), 3.25-3.10 (2H, m, C(12)H_2), 1.61-1.41 (4H, m, C(2)H_2 & C(11)H_2), 1.64-1.16 (16H, m, C(3-10)H_2); **^{13}C NMR** (101 MHz, $CDCl_3$): δ 156.7 (*C*=O), 136.8 (Ar *C*H), 128.6 (Ar *C*H), 128.2 (Ar *C*H), 66.7 (Ph*C*H_2), 63.2 (*C*1), 41.2 (*C*12), 32.9 (*C*2), 30.1 (*C*11), 29.7-29.5 (5 × *C*(3-10)), 29.4 (1 × *C*(3-10)), 26.8 (1 × *C*(3-10)), 25.8 (1 × *C*(3-10)); **HRMS**: *m/z* $(ES)^+$ Found $[M+Na]^+$ 358.2354, $C_{20}H_{33}NNaO_3^+$ requires $[M]^+$ 358.2353; **LRMS**: *m/z* $(ES)^+$ 358 ($[M+Na]^+$, 100%); **Anal. Calcd.** for $C_{20}H_{33}NO_3$: *C*, 71.60; *H*, 9.91; *N*, 4.18. Found: *C*, 71.52; *H*, 9.78; *N*, 4.25.

12-Azidododecanol (62)



12-Bromododecanol **61** (100 mg, 0.38 mmol, 1.00 eq.) was dissolved in anhydrous DMF (5 mL) in a microwave vial. Sodium azide (29 mg, 0.80 mmol, 1.20 eq.) was added, the microwave vial sealed, and the resulting suspension heated to 60 °C for 8 h. After this time, the reaction solution was diluted with EtOAc (50 mL). The organic layer was washed with distilled H_2O (3 × 40 mL) and brine (3 × 40 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo* to give the crude product. The crude material was adsorbed onto Celite® and purified *via* flash column chromatography on silica gel (Biotage®; gradient elution: 5% → 40% EtOAc in petrol) affording **62** (201 mg, 89%) as a colourless oil. $\mathbf{R_f}$: 0.49 (2:8 EtOAc:Petrol); **^{1}H NMR** (500 MHz, $CDCl_3$): δ 3.62 (2H, t, *J* = 6.6 Hz, C(1/12)H_2), 3.24 (2H, t, *J* = 7.0 Hz, C(1/12)H_2), 1.62-1.52 (4H, m, C(2)H_2 & C(11)H_2), 1.39-1.22 (m, 16H, C(3-10)H_2); **LRMS**: *m/z* (ES)$^+$ 130 (100%), 250 ([M+Na]$^+$, 86%). The data are in good agreement with literature values.[288]

***n*-12-Bromododecyl-β-D-maltose heptaacetate (63)**

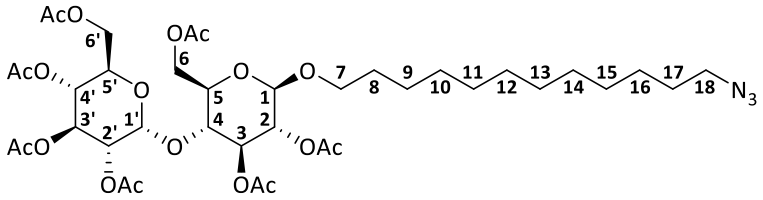


β-D-Maltose octaacetate **47** (200 mg, 0.29 mmol, 1.00 eq.) was dissolved in anhydrous CH_2Cl_2 (2.1 mL) and cooled to 0 °C. $BF_3{\cdot}OEt_2$ (55 µL, 63 mg, 0.44 mmol, 1.50 eq.) was added dropwise and the resulting solution was stirred at 0 °C for 10 min. After this time, 12-bromododecanol (156 mg, 0.59 mmol, 2.00 eq.) in anhydrous CH_2Cl_2 (1.0 mL) was added and the resulting

solution was stirred at RT for 1 h. After this time, the reaction solution was quenched *via* the dropwise addition of distilled H_2O and the resulting mixture diluted with CH_2Cl_2 (50 mL). The organic layer was washed with distilled H_2O (3 × 40 mL) and brine (3 × 40 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo* to give the crude product. The crude material was adsorbed onto Celite® and purified *via* flash column chromatography on silica gel (Biotage®; gradient elution: 7% → 60% EtOAc in petrol) affording **63** (120 mg, 48%) as a colourless waxy solid. **R_f**: 0.24 (3:7 EtOAc:Petrol); $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\boldsymbol{20}}$: +27.4 (*c* 1.0 in $CHCl_3$) [Lit.[117] value $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\boldsymbol{20}}$: +37.5 (*c* 1.6 in $CHCl_3$)]; **mp**: 64-66 °C (EtOAc/Petrol) [Lit.[117] value mp: 82-85 °C]; **IR** (neat) ν_{max} cm^{-1}: 2927 (w), 2855 (w), 1756 (s), 1209 (s); **^{1}H NMR** (500 MHz, $CDCl_3$): δ 5.36 (1H, d, *J* = 4.0 Hz, C(1')*H*), 5.30 (1H, dd, *J* = 10.3, 9.9 Hz, C(3')*H*), 5.19 (1H, dd, *J* = 9.2, 8.7 Hz, C(3)*H*), 4.99 (1H, dd, *J* = 9.9, 9.8 Hz, C(4')*H*), 4.80 (1H, dd, *J* = 10.3, 4.0 Hz, C(2')*H*), 4.75 (1H, dd, *J* = 8.7, 8.0 Hz, C(2)*H*), 4.46 (1H, d, *J* = 8.0 Hz, C(1)*H*), 4.41 (1H, dd, *J* = 12.1, 2.6 Hz, C(6)H_AH_B), 4.23-4.16 (2H, m, C(6)H_AH_B & C(6')H_AH_B), 3.98 (1H, dd, *J* = 12.4, 1.9 Hz, C(6')H_AH_B), 3.94 (1H, dd, *J* = 9.3, 9.2 Hz, C(4)*H*), 3.93-3.88 (1H, m, C(5')*H*), 3.79 (1H, dt, *J* = 9.6, 9.5 Hz, C(7)H_AH_B), 3.68-3.59 (1H, m, C(5)*H*), 3.41 (1H, dt, *J* = 9.5, 6.9 Hz, C(7)H_AH_B), 3.35 (2H, t, *J* = 7.0 Hz, C(18)H_2), 2.08 (3H, s, C(O)CH_3), 2.04 (3H, s, C(O)CH_3), 1.98 (3H, s, C(O)CH_3), 1.97 (3H, s, C(O)CH_3), 1.95 (3H, s, C(O)CH_3), 1.94 (6H, s, 2 × C(O)CH_3), 1.83-1.75 (2H, m, C(17)H_2), 1.53-1.44 (2H, m, C(8)H_2), 1.40-1.32 (2H, m, C(16)H_2), 1.29-1.16 (14H, m, C(9-15)H_2); **^{13}C NMR** (126 MHz, $CDCl_3$): δ 170.51 (*C*=O), 170.49 (*C*=O), 170.46 (*C*=O), 170.3 (*C*=O), 169.9 (*C*=O), 169.6 (*C*=O), 169.4 (*C*=O), 100.3 (*C*1), 95.5 (*C*1'), 75.5 (*C*3), 72.8 (*C*4), 72.2 (*C*2), 72.1 (*C*5), 70.2 (*C*7), 70.0 (*C*2'), 69.3 (*C*3'), 68.5 (*C*5'), 68.0 (*C*4'), 62.9 (*C*6), 61.5 (*C*6'), 34.0 (*C*18), 32.8 (*C*17), 29.6 (1 × *C*8 or *C*(10-15)), 29.51 (1 × *C*8 or *C*(10-15)), 29.50 (1 × *C*8 or *C*(10-15)), 29.41 (1 × *C*8 or *C*(10-15)), 29.37 (1 × *C*8 or *C*(10-15)), 29.3 (1 × *C*8 or *C*(10-15)), 28.7 (1 × *C*8 or *C*(10-15)), 28.2 (*C*16), 25.8 (*C*9), 20.93 (C(O)*C*H_3), 20.86 (C(O)*C*H_3), 20.69 (C(O)*C*H_3), 20.66-20.5 (4 × C(O)*C*H_3); **HRMS**: *m/z* (ES)$^+$ Found [^{79}M+Na]$^+$ 905.2767,

[^{81}M+Na]$^+$ 907.2756; $C_{38}H_{59}{}^{79}BrO_{18}Na^+$ requires [M]$^+$ 905.2782, $C_{38}H_{59}{}^{81}BrO_{18}Na^+$ requires [M]$^+$ 907.2762; **LRMS**: *m/z* (ES)$^+$ 905 ([^{79}M+Na]$^+$, 95%), 907 ([^{81}M+Na]$^+$, 100%); **Anal. Calcd.** for $C_{38}H_{59}BrO_{18}$: *C*, 51.64; *H*, 6.73; *N*, 0.00. Found: *C*, 51.67; *H*, 6.82; *N*, 0.00. The NMR data are in agreement with literature values.[117]

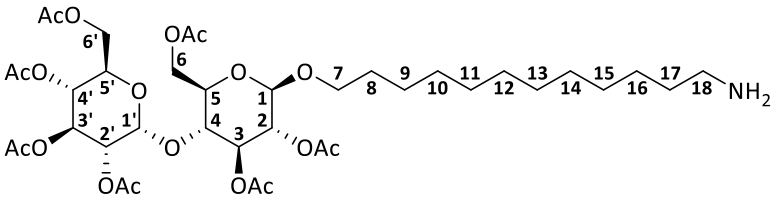
***n*-12-Azidododecyl-β-D-maltose heptaacetate (59)**



63 (236 mg, 0.27 mmol, 1.00 eq.) was dissolved in anhydrous DMF (6.6 mL) in a microwave vial. Sodium azide (52 mg, 0.80 mmol, 3.00 eq.) was added, the microwave vial sealed, and the resulting suspension heated to 60 °C for 18 h. After this time, the reaction solution was diluted with EtOAc (50 mL). The organic layer was washed with distilled H_2O (3 × 40 mL) and brine (3 × 40 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo* to give the crude product. The crude material was adsorbed onto Celite® and purified *via* flash column chromatography on silica gel (Biotage®; gradient elution: 5% → 40% EtOAc in petrol) affording **59** (201 mg, 89%) as a colourless oil. $\mathbf{R_f}$: 0.24 (3:7 EtOAc:Petrol); $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\mathbf{20}}$: +28.2 (*c* 1.0, $CHCl_3$); **mp**: 62-64 °C (EtOAc/petrol); **IR** (thin film) ν_{max} cm^{-1}: 2927 (m), 2955 (w), 2557 (m), 1740 (s); **^{1}H NMR** (500 MHz, $CDCl_3$): δ 5.42 (1H, d, *J* = 4.0 Hz, C(1')*H*), 5.36 (1H, dd, *J* = 10.3, 9.9 Hz, C(3')*H*), 5.25 (1H, dd, *J* = 9.2, 9.2 Hz, C(3)*H*), 5.05 (1H, dd, *J* = 9.9, 9.9 Hz, C(4')*H*), 4.86 (1H, dd, *J* = 10.3, 4.0 Hz, C(2')*H*), 4.81 (1H, dd, *J* = 9.2, 8.0 Hz, C(2)*H*), 4.51 (1H, d, *J* = 8.0 Hz, C(1)*H*), 4.47 (1H, dd, *J* = 12.1, 2.6 Hz, C(6)H_AH_B), 4.28-4.21 (2H, m, C(6)H_AH_B & C(6')H_AH_B), 4.04 (1H, dd, *J* = 12.5, 1.9 Hz, C(6')H_AH_B), 4.00 (1H, dd, *J* = 9.3, 9.2 Hz, C(4)*H*), 3.99-3.94 (1H, m, C(5')*H*), 3.84 (1H, dt,

J = 9.6, 6.7 Hz, C(7)H_AH_B), 3.70-3.64 (1H, m, C(5)*H*), 3.47 (1H, dt, *J* = 9.4, 6.7 Hz, C(7)H_AH_B), 3.26 (2H, t, *J* = 7.0 Hz, C(18)H_2), 2.14 (3H, s, C(O)CH_3), 2.10 (3H, s, C(O)CH_3), 2.07 (3H, s, C(O)CH_3), 2.05 (3H, s, C(O)CH_3), 2.03 (3H, s, C(O)CH_3), 2.02 (3H, s, C(O)CH_3), 2.00 (6H, s, 2 × C(O)CH_3), 1.64-1.50 (4H, m, C(8)H_2 & C(17)H_2), 1.40-1.22 (16H, m, C(9-16)H_2); **^{13}C NMR** (126 MHz, $CDCl_3$): δ 170.7 (*C*=O), 170.6 (*C*=O), 170.4 (*C*=O), 170.1 (*C*=O), 169.7 (*C*=O), 169.6 (*C*=O), 100.4 (*C*1), 95.6 (*C*1'), 75.6 (*C*3), 72.9 (*C*4), 72.4 (*C*2), 72.2 (*C*5), 70.4 (*C*7), 70.1 (*C*2'), 69.5 (*C*3'), 68.6 (*C*5'), 68.2 (*C*4'), 63.0 (*C*6), 61.6 (*C*6'), 51.6 (*C*18), 29.7-29.6 (4 × *C*(8-16)), 29.5 (1 × *C*(8-16)), 29.4 (1 × *C*(8-16)), 29.3 (1 × *C*(8-16)), 29.0 (*C*17), 26.8 (1 × *C*(8-16)), 25.9 (1 × *C*(8-16)), 21.1 (C(O)*C*H_3), 21.0 (C(O)*C*H_3), 20.82 (C(O)*C*H_3), 20.77 (C(O)*C*H_3), 20.75 (C(O)*C*H_3), 20.7 (C(O)*C*H_3); **HRMS**: *m/z* (ES)$^+$ Found [M+Na]$^+$ 868.3675, $C_{38}H_{59}N_3NaO_{18}^+$ requires [M]$^+$ 868.3686; **LRMS**: *m/z* (ES)$^+$ 868 ([M+Na]$^+$, 100%).

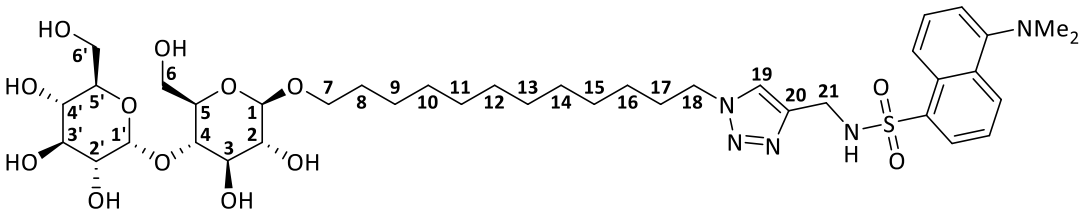
***n*-12-Aminododecyl-β-D-maltose heptaacetate (60)**



59 (29 mg, 0.03 mmol, 1.00 eq.) and powdered triphenylphosphine (13 mg, 0.05 mmol, 1.50 eq.) were dissolved in THF (1.4 mL) and distilled H_2O (0.29 mL). The resulting solution was stirred at RT for 16 h under an atmosphere of nitrogen. After this time, the reaction mixture was concentrated *in vacuo* and the residue taken up in EtOAc (25 mL). The organic layer was washed with distilled H_2O (3 × 25 mL) and brine (3 × 25 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo* to give the crude product. The crude material was adsorbed onto Celite® and purified *via* flash column chromatography on pre-washed (1% triethylamine in

petrol) silica gel (Biotage®; gradient elution: 40% → 100% EtOAc in petrol) affording **60** (20 mg, 71%) as a colourless oil. $\mathbf{R_f}$: 0.41 (3:1 EtOAc:Petrol); $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\boldsymbol{20}}$: +33.1 (*c* 1.0, $CHCl_3$); **IR** (thin film) ν_{max} cm^{-1}: 3297 (br, w), 2927 (w), 2855 (w), 1749 (s); **^{1}H NMR** (400 MHz, $CDCl_3$): δ 5.48 (2H, br s, NH_2), 5.40 (1H, d, *J* = 4.0 Hz, C(1')*H*), 5.35 (1H, dd, *J* = 10.4, 10.1 Hz, C(3')*H*), 5.24 (1H, dd, *J* = 9.3, 9.3 Hz, C(3)*H*), 5.04 (1H, dd, *J* = 10.1, 9.9 Hz, C(4')*H*), 4.84 (1H, dd, *J* = 10.4, 4.0 Hz, C(2')*H*), 4.80 (1H, dd, *J* = 9.3, 8.0 Hz, C(2)*H*), 4.50 (1H, d, *J* = 8.0 Hz, C(1)*H*), 4.46 (1H, dd, *J* = 12.1, 2.8 Hz, C(6)H_AH_B), 4.27-4.17 (2H, m, C(6)H_AH_B & C(6')H_AH_B), 4.06-3.90 (3H, m, C(4)*H*, C(5')*H* & C(6')H_AH_B), 3.83 (1H, dt, *J* = 9.6, 6.3 Hz, C(7)H_AH_B), 3.66 (1H, ddd, *J* = 9.5, 4.1, 2.8 Hz, C(5)*H*), 3.45 (1H, dt, *J* = 9.6, 6.3 Hz, C(7)H_AH_B), 3.25-3.17 (2H, m, C(18)H_2), 2.13 (3H, s, C(O)CH_3), 2.09 (3H, s, C(O)CH_3), 2.03 (3H, s, C(O)CH_3), 2.01 (3H, s, C(O)CH_3), 2.00 (3H, s, C(O)CH_3), 1.99 (6H, s, 2 × C(O)CH_3), 1.96 (3H, s, C(O)CH_3), 1.59-1.42 (4H, m, C(8)H_2 & C(17)H_2), 1.34-1.17 (16H, m, C(9-16)H_2); **^{13}C NMR** (101 MHz, $CDCl_3$): δ 170.7 (*C*=O), 170.6 (*C*=O), 170.4 (*C*=O), 170.08 (*C*=O), 170.07 (*C*=O), 169.7 (*C*=O), 169.5 (*C*=O), 100.4 (*C*1), 95.7 (*C*1'), 75.6 (*C*3), 73.0 (*C*4), 72.4(*C*2), 72.2 (*C*5), 70.3 (*C*7), 70.2 (*C*2'), 69.5 (*C*3'), 68.6 (*C*5'), 68.2 (*C*4'), 63.1 (*C*6), 61.7 (*C*6'), 39.8 (*C*18), 29.8-29.4 (7 × *C*(8-17)), 27.0 (1 × *C*(8-17)), 25.9 (1 × *C*(8-17)), 23.5 (1 × *C*(8-17)), 21.1 (C(O)*C*H_3), 21.0 (C(O)*C*H_3), 20.8-20.7 (5 × C(O)*C*H_3); **HRMS**: *m/z* (ES)$^+$ Found $[M+Na]^+$ 842.3817, $C_{38}H_{61}NNaO_{18}^+$ requires $[M]^+$ 842.3781; **LRMS**: *m/z* (ES)$^+$ 864 ($[M+2Na-H]^+$, 100%).

***n*-12-(4-[*N*-Dansyl]-aminomethyl-1*H*-1,2,3-triazol-1-yl)-dodecyl-β-D-maltoside (44)**

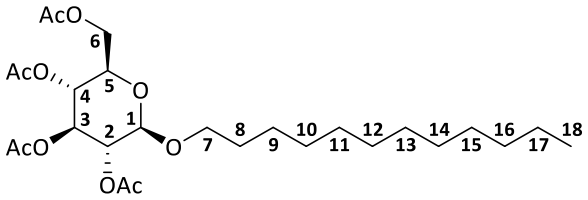


59 (38 mg, 0.04 mmol, 1.00 eq.), **55** (14 mg, 0.05 mmol, 1.10 eq.), **88** (1 mg, 2.23 µmol, 0.05 eq.) and DIPEA (1 µL, 1 mg, 4.46 µmol, 0.10 eq.) were dissolved in anhydrous MeOH (1.5 mL) and stirred for 6 h. After this time, sodium methoxide (4 mg, 0.07 mmol, 1.50 eq.) was added and the reaction was stirred at RT for a further 2 h. After this time, the reaction solution was quenched *via* the dropwise addition of distilled H_2O (1 mL) and diluted with EtOAc (20 mL). The organic layer was washed with distilled H_2O (3 × 20 mL) and brine (3 × 20 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo* to give the crude product. The crude material was adsorbed onto Celite® and purified *via* flash column chromatography on silica gel (Biotage®; gradient elution: 2% → 20% MeOH in $CHCl_3$) affording **44** (27 mg, 73% over 2 steps) as a yellow foam. **R_f**: 0.26 (1:9 MeOH:CH_2Cl_2); $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\boldsymbol{20}}$: +26.2 (*c* 1.0, MeOH); **IR** (thin film) ν_{max} cm^{-1}: 3343 (br, m), 2924 (m), 2853 (m), 1574 (w), 1504 (w); **^{1}H NMR** (500 MHz, CD_3OD): δ 8.53 (1H, d, *J* = 8.2 Hz, Napth. C*H*), 8.29 (1H, d, *J* = 8.4 Hz, Napth. C*H*), 8.19 (1H, d, *J* = 7.6 Hz, Napth. C*H*), 7.57 (1H, dd, *J* = 8.4, 7.8 Hz, Napth. C*H*), 7.54 (1H, dd, *J* = 8.2, 7.6 Hz, Napth. C*H*), 7.32 (1H, s, C(19)*H*), 7.26 (1H, d, *J* = 7.8 Hz, Napth. C*H*), 5.16 (1H, d, *J* = 3.7 Hz, C(1')*H*), 4.26 (1H, d, *J* = 7.9 Hz, C(1)*H*), 4.18 (2H, s, C(21)H_2), 4.12 (1H, t, *J* = 6.8 Hz, C(18)H_2), 3.92-3.78 (4H, m, C(6)H_AH_B, C(6')H_AH_B & C(7)H_AH_B), 3.72-3.64 (2H, m, C(5')*H* & C(6')H_AH_B), 3.64-3.58 (2H, m, C(3)*H* & C(3')*H*), 3.57-3.49 (2H, m, C(4)*H* & C(7)H_AH_B), 3.44 (1H, dd, *J* = 9.7, 3.7 Hz, C(2')*H*), 3.38-3.33 (1H, m, C(5)*H*), 3.27 (1H, dd, *J* = 9.3, 9.3 Hz, C(4')*H*), 3.22 (1H, dd, *J* = 9.1, 7.9 Hz, C(2)*H*), 2.89 (6H, s, N(CH_3)$_2$), 1.71-1.56 (4H, m, C(8)H_2 & C(17)H_2), 1.43-1.22 (14H, m,

7 × C(9-16)H_2), 1.21-1.08 (2H, m, 1 × C(9-16)H_2); **^{13}C NMR** (126 MHz, CD_3OD): δ 153.2 (Napth. *C*), 145.4 (*C*20), 137.0 (Napth. *C*), 131.3 (Napth. *C*H), 131.0 (Napth. *C*), 130.9 (Napth. *C*), 130.4 (Napth. *C*H), 129.2 (Napth. *C*H), 124.3 (Napth. *C*H), 120.5 (Napth. *C*H), 116.4 (Napth. *C*H), 104.3 (*C*1), 102.9 (*C*1'), 81.3 (*C*4), 77.9 (*C*3), 76.6 (*C*5), 75.1 (*C*3'), 74.8 & 74.7 (*C*5' & *C*2), 74.2 (*C*2'), 71.5 (*C*4'), 70.9 (*C*7), 62.8 (*C*6'), 62.2 (*C*6), 51.1 (*C*21), 45.8 (N(CH_3)$_2$), 38.9 (*C*18), 31.0 (1 × *C*(8-17)), 30.8 (1 × *C*(8-17)), 30.7-30.6 (4 × *C*(8-17)), 30.5 (1 × *C*(8-17)), 30.0 (1 × *C*(8-17)), 27.3 (1 × *C*(8-17)), 27.1 (1 × *C*(8-17)); **HRMS**: *m/z* (ES)$^+$ Found $[M+Na]^+$ 862.3844, $C_{39}H_{61}N_5NaO_{13}^+$ requires $[M]^+$ 862.3879; **LRMS**: *m/z* (ES)$^+$ 840 ($[M+H]^+$, 100%); **HPLC** (Protocol C): ret. time = 8.26 min, purity = 96.5%.

6.2.2 Synthesis of fluorescent DG analogues

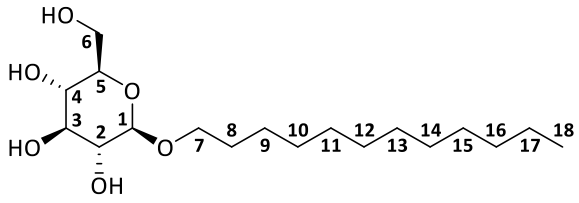
_n_-Dodecyl-(2,3,4,6-_O_-tetraacetyl)-β-D-glucopyranoside (65)



β-D-Glucose pentaacetate **64** (250 mg, 0.64 mmol, 1.00 eq.) and 1-dodecanol (172 µL, 143 mg, 0.77 mmol, 1.20 eq.) were dissolved in anhydrous CH_2Cl_2 (4.6 mL). The resulting solution was cooled to 0 °C and $BF_3{\cdot}OEt_2$ (119 µL, 136 mg, 0.96 mmol, 1.50 eq.) was added dropwise. The reaction was stirred at RT for 3 h. After this time, the reaction solution was quenched *via* the dropwise addition of H_2O (1 mL) then diluted with CH_2Cl_2 (45 mL). The organic layer was washed with distilled H_2O (3 × 25 mL) and brine (3 × 25 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo* to give the crude product. The crude material was adsorbed onto

Celite® and purified *via* flash column chromatography on silica gel (Biotage®; gradient elution: 5% → 40% EtOAc in petrol) affording **65** (125 mg, 38%) as a colourless solid. $\mathbf{R_f}$: 0.53 (3:7 EtOAc:Petrol); $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\mathbf{20}}$: −11.6 (*c* 1.0, $CHCl_3$) [Lit.[289] $[\alpha]_D^{20}$: −14 (*c* 1.1, $CHCl_3$)]; **mp**: 51-53 °C (EtOAc/Hexane) [Lit.[289] mp: 53-55 °C]; **^{1}H NMR** (400 MHz, $CDCl_3$): δ 5.20 (1H, dd, *J* = 9.6, 9.6 Hz, C(3)*H*), 5.08 (1H, dd, *J* = 9.8, 9.6 Hz, C(4)*H*), 4.98 (1H, dd, *J* = 9.6, 8.0 Hz, C(2)*H*), 4.48 (1H, d, *J* = 8.0 Hz, C(1)*H*), 4.26 (1H, dd, *J* = 12.3, 4.5 Hz, C(6)H_AH_B), 4.12 (1H, dd, *J* = 12.3, 2.5 Hz, C(6)H_AH_B), 3.86 (1H, dt, *J* = 9.6, 6.3 Hz, C(7)H_AH_B), 3.68 (1H, ddd, *J* = 9.8, 4.5, 2.5 Hz, C(5)*H*), 3.46 (1H, dt, *J* = 9.6, 6.8 Hz, C(7)H_AH_B), 2.08 (3H, s, C(O)CH_3), 2.03 (3H, s, C(O)CH_3), 2.02 (3H, s, C(O)CH_3), 2.00 (3H, s, C(O)CH_3), 1.64-1.47 (2H, m, C(8)H_2), 1.35-1.19 (18H, m, C(9-17)H_2), 0.87 (3H, t, *J* = 6.9 Hz, C(18)H_3); **LRMS**: *m/z* (ES)$^+$ 131 (100%), 539 ([M+Na]$^+$, 72%). The data are in good agreement with literature values.[154]

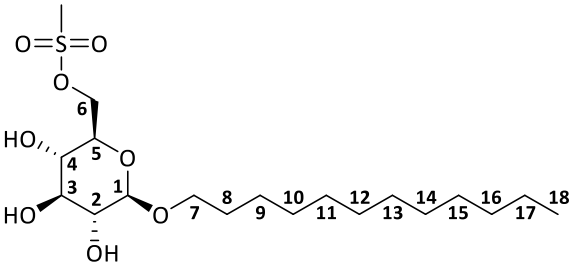
***n*-Dodecyl-β-D-glucopyranoside (34)**



65 (228 mg, 0.44 mmol, 1.00 eq.) was dissolved in anhydrous CH_2Cl_2 (4.3 mL) and anhydrous MeOH (2.2 mL). The resulting solution was cooled to 0 °C and sodium methoxide (17 mg, 0.31 mmol, 0.70 eq.) was added. The reaction solution was allowed to warm to RT and stirred for 50 min. After this time, the reaction solution was quenched *via* the dropwise addition of acetic acid (68 µL). The resulting mixture was concentrated *in vacuo* and the acetic acid removed *in vacuo via* azeotropic distillation (toluene) to give the crude product. The crude material was adsorbed onto Celite® and purified *via* flash column chromatography on silica

gel (Biotage®; gradient elution: 1% → 10% MeOH in $CHCl_3$) affording **34** (149 mg, 97%) as a colourless solid. **R_f**: 0.08 (1:19 MeOH:CH_2Cl_2); $[\alpha]_D^{20}$: −21.4 (*c* 1.0, $CHCl_3$) [Lit.[290] $[\alpha]_D^{20}$: −24.1 (*c* 1.0, MeOH)]; **mp**: 142-143 °C (isotropic, MeOH) [Lit.[154] mp: 143 °C]; **^{1}H NMR** (400 MHz, CD_3OD): δ 4.20 (1H, d, *J* = 7.8 Hz, C(1)*H*), 3.86 (1H, dt, *J* = 9.5, 6.9 Hz, C(7)H_AH_B), 3.82 (1H, dd, *J* = 11.9, 1.9 Hz, C(6)H_AH_B), 3.63 (1H, dd, *J* = 11.9, 5.3 Hz, C(6)H_AH_B), 3.49 (1H, dt, *J* = 9.5, 6.8 Hz, C(7)H_AH_B), 3.34-3.18 (3H, m, C(3)*H*, C(4)*H*, C(5)*H*), 3.13 (1H, dd, *J* = 8.9, 7.8 Hz, C(2)*H*), 1.64-1.54 (2H, m, C(8)H_2), 1.40-1.20 (18H, m, C(9-17)H_2), 0.86 (3H, t, *J* = 6.9 Hz, CH_3); **LRMS**: *m/z* $(ES)^-$ 393 ($[M+FA-H]^-$, 100%). The data are in good agreement with literature values.[154]

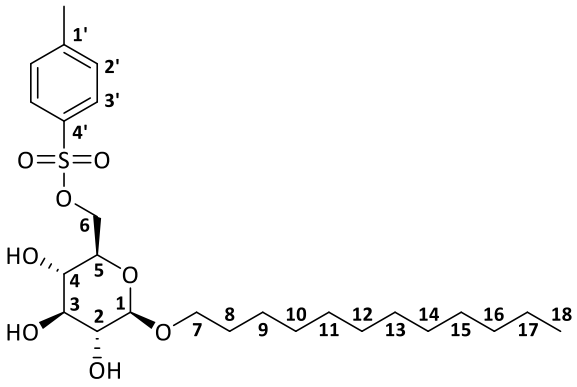
***n*-Dodecyl-(6-*O*-mesyl)-β-D-glucopyranoside (102)**



34 (48 mg, 0.14 mmol, 1.00 eq.) and anhydrous triethylamine (21 µL, 15 mg, 0.15 mmol, 1.10 eq.) were dissolved in anhydrous CH_2Cl_2 (3.6 mL). Mesyl chloride (11 µL, 16 mg, 0.14 mmol, 1.00 eq.) was added and the resulting solution was stirred at RT for 1 h. After this time, the reaction solution was concentrated *in vacuo* to give the crude product. The crude material was adsorbed onto Celite® and purified *via* flash column chromatography on silica gel (Biotage®; gradient elution: 1% → 14% MeOH in $CHCl_3$) affording **102** (18 mg, 31% by NMR) as a colourless solid. **R_f**: 0.23 (7:93 MeOH:$CHCl_3$); $[\alpha]_D^{20}$: −17.1 (*c* 0.2, MeOH); **mp**: 86-88 °C (MeOH); **^{1}H NMR** (400 MHz, CD_3OD): δ 4.53 (1H, dd, *J* = 11.3, 1.5 Hz, C(6)H_AH_B), 4.38 (1H, dd, *J* = 11.3, 5.4 Hz, C(6)H_AH_B), 4.29 (1H, d, *J* = 8.1 Hz, C(1)*H*), 3.84 (1H, dt, *J* = 9.5, 6.8 Hz, C(7)H_AH_B),

3.56 (1H, dt, *J* = 9.5, 6.6 Hz, C(7)H_AH_B), 3.50 (1H, ddd, *J* = 9.5, 5.4, 1.5 Hz, C(5)*H*), 3.38-3.28 (2H, m, C(3)*H* & C(4)*H*), 3.17 (1H, dd, *J* = 8.4, 8.1 Hz, C(2)*H*), 3.10 (3H, s, SO_2CH_3), 1.66-1.59 (2H, m, C(8)H_2), 1.44-1.23 (18H, m, C(9-17)H_2), 0.90 (3H, t, *J* = 6.9 Hz, C(18)H_3); **^{13}C NMR** (126 MHz, CD_3OD): δ 104.4 (*C*1), 77.8 (*C*3), 75.2 (*C*5), 75.0 (*C*2), 71.1 (*C*7), 71.0 (*C*4), 70.6 (*C*6), 37.4 (SO_2CH_3), 33.1 (1 × *C*(8-17)), 30.84-30.76 (5 × *C*(8-17)), 30.6 (1 × *C*(8-17)), 30.5 (1 × *C*(8-17)), 27.1 (1 × *C*(8-17)), 23.7 (1 × *C*(8-17)), 14.4 (*C*18); **HRMS**: *m/z* (ES)$^+$ Found $[M+H]^+$ 449.2176, $C_{19}H_{38}NaO_8S^+$ requires $[M]^+$ 449.2180; **LRMS**: *m/z* (ES)$^+$ 449 ($[M+Na]^+$, 100%).

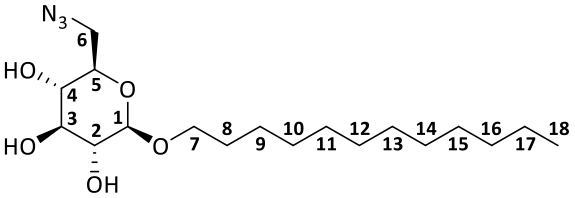
***n*-Dodecyl-(6-*O*-tosyl)-β-D-glucopyranoside (103)**



34 (38 mg, 0.11 mmol, 1.00 eq.) and anhydrous triethylamine (17 µL, 12 mg, 0.12 mmol, 1.10 eq.) were dissolved in anhydrous CH_2Cl_2 (2.8 mL). Tosyl chloride (23 mg, 0.12 mmol, 1.10 eq., recrystalised from $(CH_3)_2CO$) was added and the resulting solution was stirred at RT for 6 h. After this time, the reaction solution was concentrated *in vacuo* to give the crude product. The crude material was adsorbed onto Celite® and purified *via* flash column chromatography on silica gel (Biotage®; gradient elution: 0% → 10% MeOH in $CHCl_3$) affording impure **103** (32 mg, 42% from NMR) as a colourless oil. $\mathbf{R_f}$: 0.29 (1:19 MeOH:$CHCl_3$); $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\boldsymbol{20}}$: −24.5 (*c* 1.0, $CHCl_3$); **IR** (thin film) ν_{max} cm^{-1}: 3375 (br, m), 2924 (s), 2854 (s), 2361 (w), 2341

(w), 1642 (w); **^{1}H NMR** (400 MHz, $CDCl_3$): δ 7.78 (d, 2H, *J* = 8.2 Hz, Ar C*H*), 7.31 (d, 2H, *J* = 8.2 Hz, Ar C*H*), 4.80 (1H, br s, O*H*), 4.42-4.26 (2H, m, O*H* & C(6)H_AH_B), 4.25-4.17 (2H, m, C(1)*H* & C(6)H_AH_B), 3.81-3.68 (2H, m, C(7)H_AH_B & O*H*), 3.56-3.36 (4H, m, C(3)*H*, C(4)*H*, C(5)*H*, C(7)H_AH_B), 3.30 (1H, dd, *J* = 8.3, 8.3 Hz, C(2)*H*), 2.42 (3H, s, CH_3Ph), 1.63-1.50 (2H, m, C(8)H_2), 1.33-1.17 (18H, m, C(9-17)H_2), 0.87 (3H, t, *J* = 6.7 Hz, C(18)H_3); **^{13}C NMR** (101 MHz, $CDCl_3$): δ 144.9 (*C*1'), 133.0 (*C*4'), 130.0 (*C*2'), 128.1 (*C*3'), 102.6 (*C*1), 76.4 (*C*3), 73.6 (*C*2 & *C*5), 70.4 (*C*7), 69.9 (*C*4), 69.4 (*C*6), 32.1 (1 × *C*(8-17)), 29.84-29.80 (5 × *C*(8-17)), 29.6 (1 × *C*(8-17)), 29.5 (1 × *C*(8-17)), 26.1 (1 × *C*(8-17)), 22.8 (1 × *C*(8-17)), 21.8 (*C*H_3Ph), 14.2 (*C*18); **HRMS**: *m/z* $(ES)^+$ Found $[M+Na]^+$ 525.2488, $C_{25}H_{42}NaO_8S^+$ requires $[M]^+$ 525.2493; **LRMS**: *m/z* $(ES)^+$ 503 ($[M+H]^+$, 100%); **HPLC** (Protocol A): ret. time = 10.75 min, purity = 98.1%.

***n*-Dodecyl-(6-deoxy-6-azido)-β-D-glucopyranoside (66)**



Route A

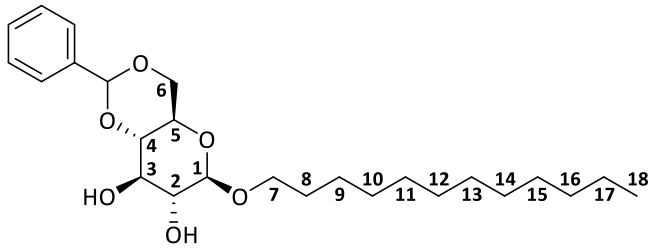
102 (20 mg, 0.05 mmol, 1.00 eq.) was dissolved in anhydrous DMF (0.5 mL) in a microwave vial. Sodium azide (3 mg, 0.05 mmol, 1.10 eq.), was added, the microwave vial sealed, and the resulting suspension heated to 60 °C for 18 h. After this time, the reaction solution was diluted with EtOAc (50 mL). The organic layer was washed with distilled H_2O (3 × 40 mL) and brine (3 × 40 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo* to give the crude product. The crude material was adsorbed onto Celite® and purified *via* flash column chromatography on

silica gel (Biotage®; gradient elution: 1% → 10% EtOAc in petrol) affording **66** (11 mg, 61%) as a colourless oil.

Route B

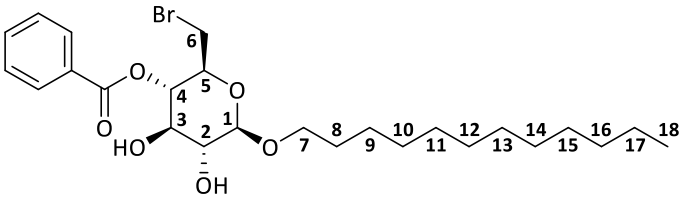
103 (28 mg, 0.06 mmol, 1.00 eq.) and sodium azide (4 mg, 0.06 mmol, 1.10 eq.) were dissolved in anhydrous DMF (0.7 mL) and were reacted and purified as in Route A affording **66** (11 mg, 52%) as a colourless oily solid. $\mathbf{R_f}$: 0.17 (1:19 MeOH:$CHCl_3$); $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\boldsymbol{20}}$: −40.1 (*c* 0.25, CH_3OH); **IR** (thin film) ν_{max} cm^{-1}: 3402 (m), 3298 (m), 2954 (m), 2924 (s), 2872 (m), 2851 (s), 2360 (m), 2341 (m), 2164 (w), 2121 (m), 2096 (m); **^{1}H NMR** (500 MHz, CD_3OD): δ 4.28 (1H, d, *J* = 7.8 Hz, C(1)*H*), 3.87 (1H, dt, *J* = 9.5, 6.9 Hz, C(7)H_AH_B), 3.56 (1H, dt, *J* = 9.5, 6.6 Hz, C(7)H_AH_B), 3.48-3.37 (3H, m, C(5)*H*, C(6)H_AH_B, C(6)H_AH_B), 3.36-3.29 (1H, m, C(3)*H*), 3.32 (1H, dd, *J* = 9.0, 9.0 Hz, C(4)*H*), 3.19 (1H, dd, *J* = 9.2, 7.8 Hz, C(2)*H*), 1.67-1.58 (2H, m, C(8)H_2), 1.46-1.23 (18H, m, C(9--17)H_2), 0.90 (3H, t, *J* = 6.9 Hz, CH_3); **^{13}C NMR** (126 MHz, CD_3OD): δ 104.3 (*C*1), 77.8 (*C*3), 77.1 (*C*5), 75.1 (*C*2), 72.6 (*C*4), 70.9 (*C*7), 52.8 (*C*6), 33.1 (1 × *C*(8-17)), 30.8-30.7 (5 × *C*(8-17)), 30.52 (1 × *C*(8-17)), 30.48 (1 × *C*(8-17)), 27.1 (1 × *C*(8-17)), 23.7 (1 × *C*(8-17)), 14.4 (*C*H_3); **HRMS**: *m/z* $(ES)^+$ Found $[M+Na]^+$ 396.2478, $C_{18}H_{35}N_3NaO_5^+$ requires $[M]^+$ 396.2469); **LRMS**: *m/z* $(ES)^-$ 418 ($[M+FA-H]^-$, 100%).

n-Dodecyl-(4,6-*O*-bezylidene)-β-D-glucopyranoside (99)



34 (165 mg, 0.47 mmol, 1.00 eq.) and camphorsulfonic acid (13 mg, 0.05 mmol, 0.12 eq.) were dissolved in anhydrous DMF (2.4 mL) and sealed under argon in a microwave vial. Benzaldehyde dimethylacetal (213 µL, 216 mg, 1.42 mmol, 3.00 eq.) was added and the resulting solution was heated to 60 °C at 300 mbar for 4 h. After this time, the reaction solution was diluted with EtOAc (75 mL).The organic layer was washed with distilled H_2O water (3 × 25 mL) and brine (3 × 25 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo* to give the crude product. The crude material was adsorbed onto Celite® and purified *via* flash column chromatography on pre-washed (1% triethylamine in $CHCl_3$) silica gel (Biotage®; gradient elution: 0% → 10% MeOH in $CHCl_3$) affording **99** (140 mg, 68%) as a pale yellow solid. **R_f**: 0.43 (1:19 MeOH:$CHCl_3$); $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\boldsymbol{20}}$: −41.1 (*c* 1.0, $CHCl_3$) [Lit.[291] $[\alpha]_D$: −39.0 (*c* 0.2, $CHCl_3$)]; **mp**: 122-124 °C (EtOAc); **^{1}H NMR** (400 MHz, $CDCl_3$): δ 7.56-7.45 (2H, m, Ar C*H*), 7.40-7.33 (3H, m, Ar C*H*), 5.53 (1H, s, PhC*H*), 4.38 (1H, d, *J* = 7.7 Hz, C(1)*H*), 4.34 (1H, dd, *J* = 10.4, 4.8 Hz, C(6)H_AH_B), 3.94-3.85 (1H, m, C(7)H_AH_B), 3.85-3.74 (2H, m, C(3)*H* & C(6)H_AH_B), 3.60-3.41 (4H, m, C(2)*H*, C(4)*H*, C(5)*H* & C(7)H_AH_B), 2.89 (1H, br s, C(3)O*H*), 2.68 (1H, br s, C(2)O*H*), 1.70-1.59 (2H, m, C(8)H_2), 1.42-1.78 (18H, m, C(9-17)H_2), 0.88 (3H, t, *J* = 6.7 Hz, C(18)H_3); **LRMS**: *m/z* (ES)$^+$ 437 ([M+H]$^+$, 100%). The data are in good agreement with literature values.[291]

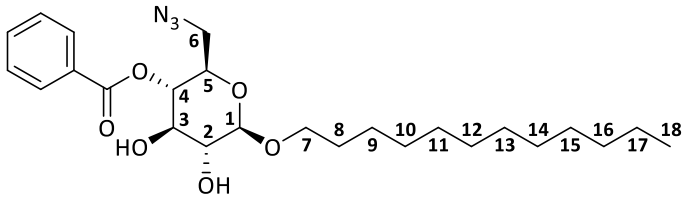
***n*-Dodecyl-(4-*O*-benzoyl-6-deoxy-6-bromo)-β-D-glucopyranoside (100)**



99 (50 mg, 0.11 mmol, 1.00 eq.), *N*-bromosuccinimide (29 mg, 0.16 mmol, 1.40 eq.) and $BaCO_3$ (59 mg, 0.30 mmol, 2.60 eq.) were sealed in a microwave vial, which was evacuated and back-filled with argon (× 3). CCl_4 (3.1 mL; distilled and stored in the dark over 3 Å molecular sieves) was added and the resulting suspension was heated under reflux for 4 h. After this time, the suspension was allowed to cool to RT and filtered through Celite®, eluting with CH_2Cl_2 (50 mL). The filtrate was concentrated *in vacuo* to give the crude product. The crude material was adsorbed onto Celite® and purified *via* flash column chromatography on silica gel (Biotage®; gradient elution: 1% → 10% MeOH in $CHCl_3$) affording **100** (30 mg, 51%) as a colourless solid. **R_f**: 0.38 (1:19 MeOH:$CHCl_3$); $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\boldsymbol{20}}$: +27.4 (*c* 0.5, $CHCl_3$); **mp**: 71-73 °C ($(CH_3)_2O$); **IR** (thin film) ν_{max} cm^{-1}: 3383 (br, m), 2924 (m), 2854 (m), 2361 (w), 2341 (w); **^{1}H NMR** (400 MHz, $CDCl_3$): δ 8.07-8.02 (2H, m, Ar C*H*), 7.63-7.57 (1H, m, Ar C*H*), 7.50-7.43 (2H, m, Ar C*H*), 5.06 (1H, dd, *J* = 9.7, 9.4 Hz, C(4)*H*), 4.38 (1H, d, *J* = 7.8 Hz, C(1)*H*), 3.95 (1H, dt, *J* = 9.5, 6.9 Hz, C(7)H_AH_B), 3.84 (1H, ddd, *J* = 9.4, 9.3, 3.4 Hz, C(3)*H*), 3.77 (1H, ddd, *J* = 9.7, 7.8, 2.4 Hz, C(5)*H*), 3.63-3.51 (2H, m, C(7)H_AH_B & C(2)*H*), 3.51 (1H, dd, *J* = 11.1, 2.4 Hz, C(6)H_AH_B), 3.44 (1H, dd, *J* = 11.1, 7.8 Hz, C(6)H_AH_B), 3.00 (1H, d, *J* = 3.4 Hz, C(3)HO*H*), 2.88 (1H, d, *J* = 1.9 Hz, C(2)HO*H*), 1.71-1.62 (2H, m, C(8)H_2), 1.45-1.16 (18H, C(9-17)H_2), 0.88 (3H, t, *J* = 6.8 Hz, CH_3); **^{13}C NMR** (101 MHz, $CDCl_3$): δ 166.3 (*C*=O), 133.9 (Ar *C*H), 130.1 (Ar *C*H), 129.1 (Ar *C*), 128.7 (Ar *C*H), 102.5 (*C*1), 74.6 (*C*3), 74.5 (*C*5), 73.94 (*C*2), 73.85 (*C*4), 70.7 (*C*7), 32.1 (1 × *C*(8-17)), 31.5 (*C*6), 29.81 (1 × *C*(8-17)), 29.78 (1 × *C*(8-17)), 29.76 (1 × *C*(8-17)), 29.73 (1 × *C*(8-17)), 29.72

(1 × *C*(8-17)), 29.55 (1 × *C*(8-17)), 29.49 (1 × *C*(8-17)), 26.1 (1 × *C*(8-17)), 22.8 (1 × *C*(8-17)), 14.3 (*C*H$_3$); **HRMS:** *m/z* (ES)$^+$ Found [^{79}M+Na]$^+$ 537.1832, [^{81}M+Na]$^+$ 539.1814; $C_{25}H_{39}{}^{79}BrNaO_6{}^+$ requires [M]$^+$ 537.1822, $C_{25}H_{39}{}^{81}BrNaO_6{}^+$ requires [M]$^+$ 539.1803); **LRMS**: (ES)$^+$ 559 ([^{79}M+FA−H]$^-$, 96%), 561 ([^{81}M+FA−H]$^-$, 100%); **HPLC** (Protocol A): ret. time = 13.56 min, purity = 97.9%.

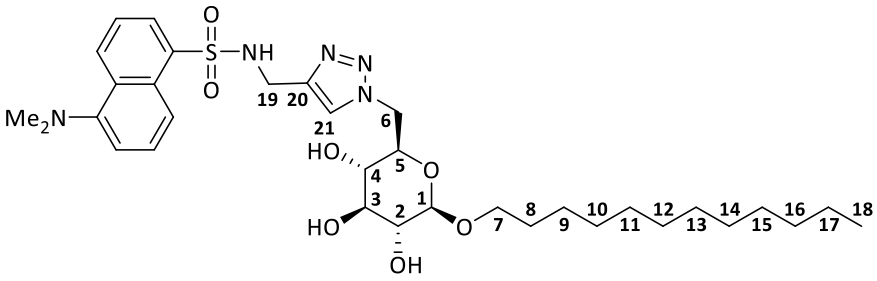
***n*-Dodecyl-(4-*O*-benzoyl-6-deoxy-6-azido)-β-D- glucopyranoside (101)**



100 (24 mg, 0.05 mmol, 1.00 eq.) was dissolved in anhydrous DMF (1.0 mL) in a microwave vial. Sodium azide (3 mg, 0.05 mmol, 1.10 eq.) was added, the microwave vial sealed, and the resulting suspension heated to 60 °C for 18 h. After this time, the reaction solution was diluted with EtOAc (50 mL). The organic layer was washed with distilled H_2O (3 × 40 mL) and brine (3 × 40 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo* to give the crude product. The crude material was adsorbed onto Celite® and purified *via* flash column chromatography on silica gel (Biotage®; gradient elution: 5% → 40% EtOAc in petrol) affording **101** (201 mg, 89%) as a colourless oil. $\mathbf{R_f}$: 0.35 (1:19 MeOH:$CHCl_3$); $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\boldsymbol{20}}$: −27.0 (*c* 0.5, $CHCl_3$); **IR** (thin film) ν_{max} cm^{-1}: 3427 (br, m), 2924 (s), 2854 (m), 2361 (m), 2342 (m), 2169 (w), 2099 (s), 1725 (s), 1603 (w), 1585 (w); **^{1}H NMR** (400 MHz, $CDCl_3$): δ 8.10-7.99 (2H, m, Ar C*H*), 7.64-7.57 (1H, m, Ar C*H*), 7.50-7.42 (2H, m, Ar C*H*), 5.08 (1H, dd, *J* = 9.5, 9.4 Hz, C(4)*H*), 4.40 (1H, d, *J* = 7.7 Hz, C(1)*H*), 3.95 (1H, dt, *J* = 9.5, 6.8 Hz, C(7)H_AH_B), 3.85 (1H, dd, *J* = 9.4, 9.2 Hz, C(3)*H*), 3.81-3.74 (1H, m, C(5)*H*), 3.65-3.41 (3H, m, C(7)H_AH_B, C(2)*H* & C(6)H_AH_B), 3.21 (1H, dd, *J* = 13.3, 2.1 Hz,

C(6)H_AH_B), 2.88 (1H, br s, O*H*), 2.75 (1H, br s, O*H*), 1.82-1.54 (4H, m, C(8-9)H_2), 1.46-1.15 (16H, m, C(10-17)H_2), 0.88 (3H, t, *J* = 6.7 Hz, CH_3); **^{13}C NMR** (126 MHz, $CDCl_3$): δ 169.3 (Ar *C*), 133.9 (Ar *C*H), 130.0 (Ar *C*H), 128.7 (Ar *C*H), 102.4 (*C*1), 74.6 (*C*3), 74.5 (*C*2), 74.3 (*C*5), 72.4 (*C*4), 70.5 (*C*7), 51.6 (*C*6), 32.1 (1 × *C*(8-17)), 29.81-29.7 (5 × *C*(8-17)), 29.54 (1 × *C*(8-17)), 29.50 (1 × *C*(8-17)), 26.1 (1 × *C*(8-17)), 22.8 (1 × *C*(8-17)), 14.3 (*C*H_3); **HRMS**: *m/z* (ES)$^+$ Found $[M+Na]^+$ 500.2739, $C_{25}H_{39}N_3NaO_6^+$ requires $[M]^+$ 500.2731; **LRMS**: *m/z* (ES)$^+$ 500 ($[M+Na]^+$, 100%).

_n_-Dodecyl-(6-deoxy-6-[4-{*N*-dansyl}aminomethyl-1*H*-1,2,3-triazol-1-yl]-β-D-glucopyranoside (45)



Route A

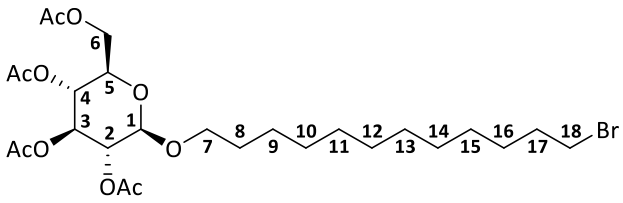
66 (9 mg, 0.02 mmol, 1.00 eq.), **55** (8 mg, 0.03 mmol, 1.10 eq.), **88** (429 µg, 1.20 µmol, 0.05 eq.) and DIPEA (0.42 µL, 311 µg, 2.51 µmol, 0.10 eq.) were dissolved in anhydrous MeOH (0.5 mL) and stirred at RT for 2 h. After this time, the reaction solution was concentrated *in vacuo* and the residue was diluted with EtOAc (20 mL). The organic layer was washed with distilled H_2O (3 × 20 mL) and brine (3 × 20 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo* to give the crude product. The crude material was adsorbed onto Celite® and purified *via* flash column chromatography on silica gel (Biotage®; gradient elution: 1% → 10% MeOH in $CHCl_3$) affording **45** (16 mg, 100%) as a pale yellow solid.

Route B

101 (21 mg, 0.06 mmol, 1.00 eq.), **55** (24 mg, 0.08 mmol, 1.50 eq.), **88** (1 mg, 2.78 µmol, 0.05 eq.) and DIPEA (1 µL, 1 mg, 5.571 µmol, 0.10 eq.) in anhydrous MeOH (1.5 mL) were reacted as in Route A for 4 h. After this time, sodium methoxide (5 mg, 0.08 mmol, 1.50 eq.) was added and the reaction stirred at RT for a further 1 h. The reaction solution was then quenched *via* the dropwise addition of distilled H_2O (1 mL) and concentrated *in vacuo*. The residue was taken up in EtOAc (20 mL). The organic layer was washed with distilled H_2O (3 × 20 mL) and brine (3 × 20 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo* to give the crude product. The crude material was adsorbed onto Celite® and purified *via* flash column chromatography on silica gel (Biotage®; gradient elution: 1% → 10% MeOH in $CHCl_3$) affording **45** (15 mg, 42% over 2 steps) as a pale yellow solid. $\mathbf{R_f}$: 0.33 (1:9 MeOH:$CHCl_3$); $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\boldsymbol{20}}$: −9.9 (*c* 0.75, $(CH_3)_2SO$); **mp**: 182-184 °C ($(CH_3)_2CO$); **IR** (neat) ν_{max} cm^{-1}: 3295 (br, m), 2923 (s), 2852 (m), 2789 (w), 1613 (m); **^{1}H NMR** (300 MHz, $(CD_3)_2SO$): δ 8.44 (1H, d, *J* = 7.9 Hz, Napth. C*H*), 8.27 (1H, d, *J* = 7.9 Hz, Napth. C*H*), 8.08 (1H, d, *J* = 7.9 Hz, Napth. C*H*), 7.64 (1H, s, C(21)*H*), 7.59 (1H, dd, *J* = 7.9, 7.9 Hz, Napth. C*H*), 7.56 (1H, dd, *J* = 7.9, 7.9 Hz, Napth. C*H*), 7.25 (1H, d, *J* = 7.9 Hz, Napth. C*H*), 5.36 (1H, d, *J* = 5.5 Hz, C(4)HO*H*), 5.09 (1H, d, *J* = 4.9 Hz, C(3)HO*H*), 5.09 (1H, d, *J* = 5.0 Hz, C(2)HO*H*), 4.62 (1H, dd, *J* = 14.3, 1.8 Hz, C(6)H_AH_B), 4.24 (1H, dd, *J* = 14.3, 8.8 Hz, C(6)H_AH_B), 4.06 (2H, s, C(19)H_2), 4.01 (1H, d, *J* = 7.8 Hz, C(1)*H*), 3.52 (1H, dt, *J* = 9.7, 6.7 Hz, C(7)H_AH_B), 3.40-3.29 (1H, m, C(5)*H*), 3.24 (1H, dt, *J* = 9.7, 6.5 Hz, C(7)H_AH_B), 3.20-3.10 (1H, m, C(3)*H*), 3.03-2.89 (2H, m, C(2)*H* & C(4)*H*), 2.83 (6H, s, N(CH_3)$_2$), 1.48-1.35 (2H, m, C(8)H_2), 1.30-1.11 (18H, m, C(9-17)H_2), 0.83 (3H, t, *J* = 6.7 Hz, C(18)H_3); **^{13}C NMR** (101 MHz, $(CD_3)_2SO$): δ 151.3 (Napth. *C*), 143.6 (*C*19), 136.0 (Napth. *C*), 129.3 (Napth. *C*H), 129.0 (Napth. *C*), 128.0 (Napth. *C*H), 127.7 (Napth. *C*H), 123.9 (*C*20), 123.4 (Napth. *C*H), 119.2 (Napth. *C*H),

115.1 (Napth. *C*H), 102.7 (*C*1), 76.2(*C*3), 74.2 (*C*5), 73.2 (*C*4), 71.3 (*C*2), 68.7 (*C*7), 50.8 (*C*6), 45.0 (N(*C*H_3)$_2$), 37.8 (*C*18), 31.2 (1 × *C*(8-17)), 29.2 (1 × *C*(8-17)), 29.01-28.95 (4 × *C*(8-17)), 28.8 (1 × *C*(8-17)), 28.7 (1 × *C*(8-17)), 25.4 (1 × *C*(8-17)), 22.0 (1 × *C*(8-17)), 13.9 (*C*18); **HRMS**: *m/z* (ES)$^+$ Found [M+Na]$^+$ 684.3396, $C_{33}H_{51}NaN_5O_7S^+$ requires [M]$^+$ 684.3401); **LRMS**: *m/z* (ES)$^+$ 662 ([M+H]$^+$, 100%); **HPLC** (Protocol A): ret. time = 7.26 min, purity = 98.4%.

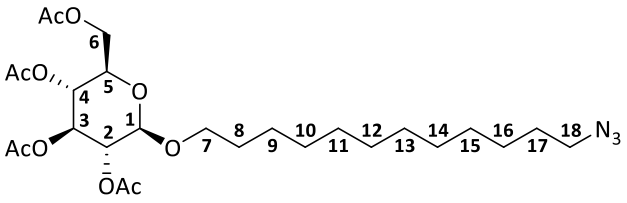
_n_-12-Bromododecyl-(2,3,4,6-_O_-tetraacetyl)-β-D-glucopyranoside (104)



β-D-Glucose pentaacetate **64** (250 mg, 0.64 mmol, 1.00 eq.) and 12-bromododecanol **61** (340 mg, 1.28 mmol, 2.00 eq.) were dissolved in anhydrous CH_2Cl_2 (4.6 mL). The resulting solution was cooled to 0 °C and $BF_3{\cdot}OEt_2$ (87 µL, 100 mg, 0.70 mmol, 1.10 eq.) was added dropwise. The reaction was stirred at RT for 5 h. After this time, the reaction solution was quenched *via* the dropwise addition of H_2O (1 mL) then diluted with CH_2Cl_2 (45 mL). The organic layer was washed with distilled H_2O (3 × 25 mL) and brine (3 × 25 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo* to give the crude product. The crude material was adsorbed onto Celite® and purified *via* flash column chromatography on silica gel (Biotage®; gradient elution: 5% → 40% EtOAc in petrol) affording **104** (165 mg, 43%) as a colourless solid. $\mathbf{R_f}$: 0.49 (3:7 EtOAc:Petrol); $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\mathbf{20}}$: −12.0 (*c* 1.1, $CHCl_3$) [Lit.[117] $[\alpha]_D^{24}$: −12.7 (*c* 2.2, $CHCl_3$)]; **mp**: 45-46 °C (EtOAc/Hexane) [Lit.[117] mp: 50-52 °C]; **^{1}H NMR** (400 MHz, $CDCl_3$): δ 5.20 (1H, dd, *J* = 9.6, 9.5 Hz, C(3)*H*), 5.09 (1H, dd, *J* = 9.9, 9.5 Hz, C(4)*H*), 4.98 (1H, dd, *J* = 9.6, 7.8 Hz, C(2)*H*), 4.48 (1H, d, *J* = 7.8 Hz, C(1)*H*), 4.26 (1H, dd, *J* = 12.3, 4.7 Hz, C(6)H_AH_B), 4.13 (1H, dd, *J* = 12.3, 2.4 Hz,

C(6)H_AH_B), 3.87 (1H, dt, *J* = 9.6, 6.6 Hz, C(7)H_AH_B), 3.68 (1H, ddd, *J* = 10.0, 4.7, 2.4 Hz, C(5)*H*), 3.46 (1H, dt, *J* = 9.6, 6.6 Hz, C(7)H_AH_B), 3.41 (2H, t, *J* = 6.9 Hz, C(18)H_2), 2.08 (3H, s, C(O)CH_3), 2.04 (3H, s, C(O)CH_3), 2.02 (3H, s, C(O)CH_3), 2.01 (3H, s, C(O)CH_3), 1.89-1.80 (2H, m, C(17)H_2), 1.64-1.49 (2H, m, C(8)H_2), 1.46-1.36 (2H, m, C(16)H_2), 1.35-1.20 (14H, m, C(9-15)H_2); **LRMS**: *m/z* (ES)$^+$ 612 ([^{79}M+NH_4]$^+$, 98%), 614 ([^{81}M+NH_4]$^+$, 100%). The data are in good agreement with literature values.[117]

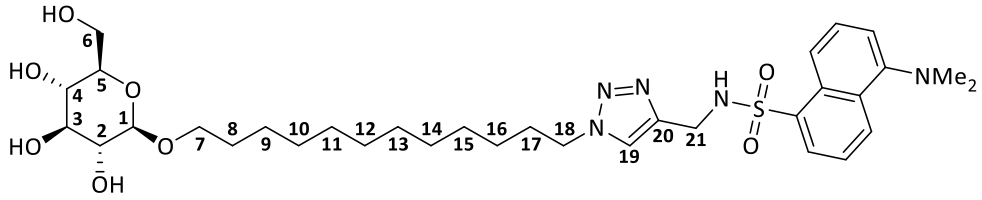
***n*-12-Azidododecyl-(2,3,4,6-*O*-tetraacetyl)-β-D-glucopyranoside (67)**



104 (265 mg, 0.45 mmol, 1.00 eq.) was dissolved in anhydrous DMF (1.1 mL) in a microwave vial. Sodium azide (87 mg, 1.34 mmol, 3.00 eq.) was added, the microwave vial sealed, and the resulting suspension heated to 60 °C for 18 h. After this time, additional sodium azide (87 mg, 1.34 mmol, 3.00 eq.) was added and the reaction heated to 60 °C for a further 6 h. After this time, the reaction solution was diluted with EtOAc (75 mL). The organic layer was washed with distilled H_2O (3 × 50 mL) and brine (3 × 50 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo* to give the crude product. The crude material was adsorbed onto Celite® and purified *via* flash column chromatography on silica gel (Biotage®; gradient elution: 5% → 40% EtOAc in petrol) affording **67** (224 mg, 90%) as a colourless oil. $\mathbf{R_f}$: 0.24 (2:8 EtOAc:Petrol); $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\boldsymbol{20}}$ −8.7 (*c* 1.0, $CHCl_3$); **IR** (thin film) ν_{max} cm^{-1}: 2926 (w), 2854 (w), 2160 (m), 1757 (s); **^{1}H NMR** (400 MHz, $CDCl_3$): δ 5.19 (1H, dd, *J* = 9.6, 9.6 Hz, C(3)*H*), 5.08 (1H, dd, *J* = 9.8, 9.6 Hz, C(4)*H*), 4.97 (1H, dd, *J* = 9.6, 8.0 Hz, C(2)*H*), 4.48 (1H, d, *J* = 8.0 Hz, C(1)*H*), 4.25 (1H, dd,

J = 12.3, 4.7 Hz, C(6)H_AH_B), 4.12 (1H, dd, *J* = 12.3, 2.4 Hz, C(6)H_AH_B), 3.86 (1H, dd, *J* = 9.6, 6.36 Hz, C(7)H_AH_B), 3.67 (1H, ddd, *J* = 9.8, 4.7, 2.4 Hz, C(5)*H*), 3.45 (1H, dd, *J* = 9.6, 6.8 Hz, C(7)H_AH_B), 3.24 (3H, t, *J* = 7.0 Hz, C(18)H_2), 2.07 (3H, s, C(O)CH_3), 2.03 (3H, s, C(O)CH_3), 2.01 (3H, s, C(O)CH_3), 2.00 (3H, s, C(O)CH_3), 1.64-1.47 (4H, m, C(8)H_2 & C(17)H_2) 1.43-1.16 (16H, m, C(9-16)H_2); **^{13}C NMR** (101 MHz, $CDCl_3$): δ 170.8 (*C*=O), 170.5 (*C*=O), 169.5 (*C*=O), 169.4 (*C*=O), 101.0 (*C*1), 73.0 (*C*3), 71.8 (*C*5), 71.5 (*C*2), 70.4 (*C*7), 68.6 (*C*4), 62.1 (*C*6), 51.6 (*C*18), 29.68 (1 × *C*(8-17)), 29.65 (1 × *C*(8-17)), 29.62 (1 × *C*(8-17)), 29.58 (1 × *C*(8-17)), 29.5 (1 × *C*(8-17)), 29.4 (1 × *C*(8-17)), 29.3 (1 × *C*(8-17)), 28.9 (1 × *C*(8-17)), 26.8 (1 × *C*(8-17)), 25.9 (1 × *C*(8-17)), 20.9 (C(O)*C*H_3), 20.77 (C(O)*C*H_3), 20.76 (C(O)*C*H_3), 20.7 (C(O)*C*H_3); **HRMS**: *m/z* (ES)$^+$ Found [M+Na]$^+$ 580.2834, $C_{26}H_{43}N_3NaO_{10}^+$ requires [M]$^+$ 580.2841; **LRMS**: *m/z* (ES)$^+$ 596 ([M+K]$^+$, 100%).

***n*-12-(4-[*N*-Dansyl]-aminomethyl-1*H*-1,2,3-triazol-1-yl)-dodecyl-β-D-glucopyranoside (46)**



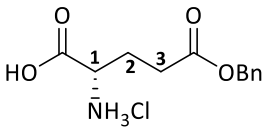
67 (181 mg, 0.32 mmol, 1.00 eq.), **55** (103 mg, 0.36 mmol, 1.10 eq.), **88** (6 mg, 0.02 mmol, 0.05 eq.) and DIPEA (6 µL, 4 mg, 0.03 mmol, 0.10 eq.) were dissolved in anhydrous MeOH (8.0 mL) and stirred at RT for 6 h. After this time, sodium methoxide (35 mg, 0.65 mmol, 2.00 eq.) was added and the reaction was stirred at RT for a further 2 h. After this time, the reaction solution was quenched *via* the dropwise addition of distilled H_2O (1 mL) and concentrated *in vacuo*. The residue was taken up in EtOAc (50 mL). The organic layer was washed with distilled H_2O (3 × 20 mL) and brine (3 × 20 mL), dried ($MgSO_4$), filtered and

concentrated *in vacuo* to give the crude product. The crude material was adsorbed onto Celite® and purified *via* flash column chromatography on silica gel (Biotage®; gradient elution: 0% → 10% MeOH in CH_2Cl_2) affording **46** (205 mg, 93% over 2 steps) as a yellow foam. $\mathbf{R_f}$: 0.26 (1:19 MeOH:CH_2Cl_2); $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\mathbf{20}}$: −10.8 (*c* 1.0, MeOH); **IR** (thin film) ν_{max} cm^{-1}: 2920 (s), 2851(m), 2360 (w), 2160 (m), 2034 (m), 1977 (m), 1616 (m); **^{1}H NMR** (400 MHz, CD_3OD): δ 8.53 (1H, d, *J* = 8.2 Hz, Napth. C*H*), 8.30 (1H, d, *J* = 8.4 Hz, Napth. C*H*), 8.19 (1H, d, *J* = 7.5 Hz, Napth. C*H*), 7.57 (1H, dd, *J* = 8.4, 7.8 Hz, Napth. C*H*) 7.55 (1H, dd, *J* = 8.2, 7.5 Hz, Napth. C*H*), 7.34 (1H, s, C(19)*H*), 7.27 (1H, d, *J* = 7.8 Hz, Napth. C*H*), 4.24 (1H, d, *J* = 7.9 Hz, C(1)*H*), 4.18 (2H, s, C(21)H_2), 4.12 (1H, t, *J* = 7.1 Hz, C(18)H_2), 3.90 (1H, dt, *J* = 9.4, 6.8 Hz, C(7)H_AH_B), 3.86 (1H, dd, *J* = 11.9, 1.9 Hz, C(6)H_AH_B), 3.66 (1H, dd, *J* = 11.9, 5.3 Hz, C(6)H_AH_B), 3.53 (1H, dt, *J* = 9.4, 6.8 Hz, C(7)H_AH_B), 3.38-3.22 (3H, m, C(3)*H*, C(4)*H* & C(5)*H*), 3.16 (1H, dd, *J* = 8.9, 7.9 Hz, C(2)*H*), 2.89 (6H, s, N(CH_3)$_2$), 1.72-1.56 (4H, m, C(8)H_2 & C(17)H_2), 1.44-1.22 (14H, m, 7 × C(9-16)H_2), 1.22-1.11 (2H, m, 1 × C(9-16)H_2); **^{13}C NMR** (126 MHz, CD_3OD): δ 153.2 (Napth. *C*), 145.3 (*C*20), 137.0 (Napth. *C*), 131.2 (Napth. *C*H), 131.1 (Napth. *C*), 130.9 (Napth. *C*), 130.4 (Napth. *C*H), 129.2 (Napth. *C*H), 124.3 (Napth. *C*H), 120.5 (Napth. *C*H), 116.4 (Napth. *C*H), 104.4 (*C*1), 78.1 (*C*3), 77.9 (*C*5), 75.1 (*C*2), 71.7 (*C*4), 70.9 (*C*7), 62.8 (*C*6), 51.1 (*C*18), 45.8 (N(CH_3)$_2$), 38.9 (*C*21), 31.0 (1 × *C*(8-17)), 30.8 (1 × *C*(8-17)), 30.70 (1 × *C*(8-17)), 30.65 (1 × *C*(8-17)), 30.6 (3 × *C*(8-17)), 30.5 (1 × *C*(8-17)), 30.0 (1 × *C*(8-17)), 27.4 (1 × *C*(8-17)), 27.1 (1 × *C*(8-17)); **HRMS**: *m/z* $(ES)^+$ Found $[M+Na]^+$ 700.3375, $C_{33}H_{51}N_5NaO_8S^+$ requires $[M]^+$ 700.3351; **LRMS**: *m/z* $(ES)^+$ 678 ($[M+H]^+$, 100%); **HPLC** (Protocol C): ret. time = 8.57 min, purity = 98.6%.

6.3 Synthesis of GSH analogues with sterically-demanding side chains

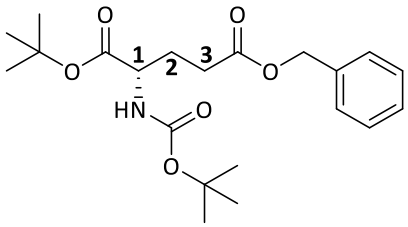
6.3.1 Synthesis of amino acid fragments & coupling reagent

Glutamic acid 5-benzyl ester hydrochloride salt (143)



Boc-L-glutamic acid 5-benzyl ester **133** (5.00 g, 14.82 mmol, 1.00 eq.) was dissolved in HCl in dioxane (4 M, 25 mL) and stirred at RT for 24 h under an atmosphere of nitrogen. After this time, the solution was concentrated *in vacuo*, suspended in distilled H_2O and lyophilised to give **143** (4.05 g, 100%) as a colourless solid that was used without further purification. $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\boldsymbol{20}}$: +16.3 (*c* 1.0, $(CH_3)_2SO$); **mp**: 160-162 °C [Lit.[292] mp: 163-164 °C]; **^{1}H NMR** (400 MHz, CD_3OD): δ 7.42-7.27 (5H, m, Ar C*H*), 5.16 (2H, s, PhCH_2), 4.10-4.00 (1H, m, C(1)*H*), 2.74-2.54 (2H, m, C(3)H_2), 2.33-2.09 (2H, m, C(2)H_AH_B); **LRMS**: *m/z* (ES)$^+$ 260 ([M+Na]$^+$, 10%), 471 (100%).

1-(*tert*-Butyl) 5-benzyl Boc-L-glutamate (138)



Method A

Boc-L-glutamic acid 5-benzyl ester **133** (500 mg, 1.48 mmol, 1.00 eq.) and DMAP (45 mg, 0.37 mmol, 0.25 eq.) were dissolved in anhydrous CH_2Cl_2 (10 mL) and cooled to 0 °C. EDC·HCl (313 mg, 1.63 mmol, 1.10 eq.) was added and the resulting solution was stirred at 0 °C for 15 min under an atmosphere of nitrogen. Anhydrous *tert*-BuOH (709 µL, 549 mg, 7.41 mmol,

5.00 eq.) was added dropwise and the resulting solution was allowed warm to RT and stirred for 6 h. After this time, the reaction solution was concentrated *in vacuo* and the residue taken up in EtOAc (50 mL). The organic layer was washed with 10% $NaHCO_3$ aq. (3 × 25 mL), 10% citric acid aq. (3 × 25 mL) and distilled H_2O (3 × 25 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo* to give the crude product. The crude material was adsorbed onto Celite® and purified *via* flash column chromatography on pre-washed (1% triethylamine in petrol) silica gel (Biotage®; gradient elution: 2% → 20% EtOAc in petrol) affording **138** (331 mg, 57%) as a colourless solid.

Method B

Boc-L-glutamic acid 5-benzyl ester **133** (2.11 g, 6.25 mmol, 1.00 eq.) and DMAP (76 mg, 0.63 mmol, 0.10 eq.) were dissolved in anhydrous CH_2Cl_2 (42 mL). DCC (1.48 g, 7.19 mmol, 1.15 eq.) was added and the resulting mixture was stirred at RT for 15 min under an atmosphere of nitrogen. Anhydrous *tert*-BuOH (1.79 mL, 1.39 g, 18.76 mmol, 3.00 eq.) was added and the reaction mixture was stirred at RT for a further 18 h. After this time, the reaction mixture was filtered under vacuum and the residue washed with CH_2Cl_2 (100 mL). The organic filtrate was washed with sat. $NaHCO_3$ aq. (2 × 75 mL), 10% citric acid aq. (2 × 75 mL) and distilled H_2O (2 × 75 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo* to give the crude product. The crude material was adsorbed onto Celite® and purified *via* flash column chromatography on pre-washed (1% triethylamine in petrol) silica gel (Biotage®; gradient elution: 2% → 20% EtOAc in petrol) affording **138** (1.47 g, 60%) as a colourless solid.

Method C

Boc-L-glutamic acid 5-benzyl ester **133** (10.00 g, 29.64 mmol, 1.00 eq.) was dissolved in anhydrous CH_2Cl_2 (200 mL) and conc. H_2SO_4 (158 µL, 291 mg, 2.96 mmol, 0.10 eq.) was added

slowly *via* syringe. The resulting solution was cooled to −10 °C and *iso*butene (28 mL, 16.63 g, 296.41 mmol, 10.00 eq.) was added using a dry ice/$(CH_3)_2CO$ cold finger. A deflated balloon was attached to the reaction flask which was then sealed and stirred at −10 °C for 1 h before being allowed to warm to RT and stir for a further 16 h. After this time, nitrogen was bubbled through the reaction solution for 10 min to aid the removal of residual *iso*butene. The remaining solution was poured slowly into a beaker of cold sat. $NaHCO_3$ aq. (100 mL) and the resulting mixture filtered through Celite®. The organic layer was separated, washed CAREFULLY* with sat. $NaHCO_3$ aq. (3 × 100 mL), water (3 × 100 mL) and brine (3 × 50 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo* to give the crude product. The crude material was adsorbed onto Celite® and purified *via* flash column chromatography on pre-washed (1% triethylamine in petrol) silica gel (Biotage®; gradient elution: 2% → 20% EtOAc in petrol) affording **138** (2.26 g, 19%) as a colourless solid.

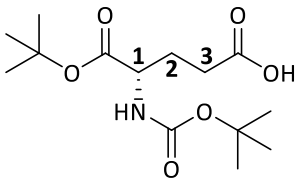
*It was important to be aware of possible pressure build up, caused by the evolution of excess *iso*butene, during the work-up.

Method D

Boc-L-glutamic acid 5-benzyl ester **133** (20.00 g, 59.28 mmol, 1.00 eq.) and DMAP (2.17 g, 17.78 mmol, 0.30 eq.) were dissolved in anhydrous *tert*-BuOH (400 mL) at 40 °C. Boc_2O (15.53 g, 71.14 mmol, 1.20 eq.) was added and the resulting solution stirred at RT for 2 h. After this time, the solution was concentrated *in vacuo* and the residue taken up in EtOAc (100 mL). The organic layer was washed with water (3 × 75 mL) and brine (3 × 75 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo* to give the crude product. The crude material was adsorbed onto Celite® and purified *via* flash column chromatography on pre-washed (1% triethylamine in petrol) silica gel (Biotage®; gradient elution: 5% → 20% EtOAc in petrol) affording **138**

(18.08 g, 78%) as a colourless solid. **R_f**: 0.11 (1:19 EtOAc:Petrol); $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\boldsymbol{20}}$: +9.0 (*c* 1.0, $CHCl_3$) [Lit.[205] $[\alpha]_D^{22}$: +9.0 (*c* 1.0, $CHCl_3$)]; **mp**: 34-36 °C (EtOAc/Hexane) [Lit.[205] mp: 35-37 °C]; **^{1}H NMR** (400 MHz, $CDCl_3$): δ 7.40-7.30 (5H, m, Ar C*H*), 5.14-5.12 (2H, m, PhCH_2), 5.07 (1H, br s, N*H*), 4.27-4.17 (1H, m, C(1)*H*), 2.54-2.36 (2H, m, C(3)H_2), 2.25-2.09 (1H, m, C(2)H_AH_B), 1.99-1.87 (1H, m, C(2)H_AH_B), 1.46 (9H, s, C(CH_3)$_3$), 1.44 (9H, s, C(CH_3)$_3$); **LRMS**: *m/z* (ES)$^+$ 809 ([2M+Na]$^+$, 100%). The data are in good agreement with literature values.[205]

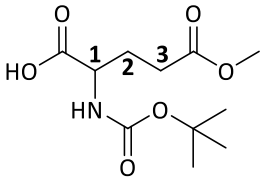
Boc-L-glutamic acid 1-tert-butyl ester (131)



138 (18.40 g, 46.76 mmol, 1.00 eq.) was dissolved in anhydrous methanol (250 mL) and the reaction flask purged with argon (× 3). Carefully, palladium on activated charcoal (496 mg, 0.47 mmol, 0.01 eq.; 10% Pd basis) was added and the reaction flask was purged with argon (× 3). Subsequently, the reaction flask was purged with hydrogen (× 1) then stirred under an atmosphere of hydrogen for 16 h. After this time, the reaction mixture was filtered through Celite® and the residue washed with MeOH (100 mL). The filtrate was concentrated *in vacuo* to give a colourless oil which was suspended in distilled H_2O (with sonication) and lyophilised affording **131** (13.98 g, 98%) as a colourless solid that was used without further purification. **R_f**: 0.37 (3:7 EtOAc:Petrol); $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\boldsymbol{20}}$: −31.4 (*c* 1.0, MeOH) [Lit.[205] $[\alpha]_D^{22}$: −27.5 (*c* 0.6, MeOH)]; **mp**: 105-107 °C (Et_2O/petrol) [Lit.[205] mp: 108-110 °C (Et_2O/petrol)]; **^{1}H NMR** (400 MHz, $CDCl_3$): δ 5.25-5.11 (1H, m, N*H*), 4.28-4.18 (1H, m, C(1)*H*), 2.55-2.35 (2H, m, C(3)H_2), 1.93-2.10 (1H, m,

C(2)H_AH_B), 1.74-1.88 (1H, m, C(2)H_AH_B), 1.47 (9H, s, C(CH_3)$_3$), 1.44 (9H, s, C(CH_3)$_3$); **LRMS**: *m/z* (ES)$^-$ 605 ([2M−H]$^-$, 100%). The data are in good agreement with literature values.[293]

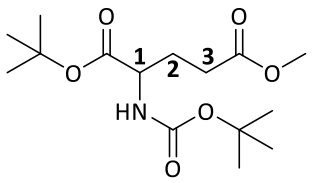
Boc-(DL)-glutamic acid 5-methyl ester (146)



DL-Glutamic acid·H_2O **145** (2.00 g, 12.11 mmol, 1.00 eq.) was suspended in anhydrous MeOH (25 mL) and chlorotrimethylsilane (2.31 mL, 1.97 g, 18.17 mmol, 1.50 eq.) was added dropwise over the course of 5 min. The resulting solution was stirred at RT for 1 h. After this time, the solution was concentrated *in vacuo* to give a pale yellow oil, which dissolved in distilled H_2O (10 mL) and 1,4-dioxane (20 mL). The resulting solution was cooled to 0 °C. Boc_2O (3.17 g, 14.53 mmol, 1.20 eq.) and $NaHCO_3$ aq. (2.54 g, 30.28 mmol, 2.50 eq.) were added and the solution was allowed to warm to RT and stirred for 18 h under an atmosphere of nitrogen. After this time, the reaction mixture was concentrated *in vacuo* and the resulting residue taken up in 10% $NaHCO_3$ aq. (30 mL). The aqueous phase was washed with Et_2O (3 × 20 mL) and acidified to pH 4 *via* the portion-wise addition of solid citric acid. The aqueous phase was then extracted with Et_2O (3 × 25 mL). The combined organic layers were washed with distilled H_2O (3 × 50 mL) and brine (3 × 50 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo* affording **146** (2.28 g, 72% over 2 steps) as a colourless oil that was used without further purification. **R_f**: 0.25 (1:1 EtOAc:Petrol); **mp**: 72-73 °C (EtOAc/petrol); **^{1}H NMR** (400 MHz, $CDCl_3$): δ 10.63 (1H, br s, COO*H*), 5.22 (1H, d, *J* = 7.8 Hz, N*H*), 4.42-4.30 (1H, m, C(1)*H*), 3.68 (3H, s, OCH_3), 2.58-2.37 (2H, m, C(3)H_2), 2.31-2.18 (1H, m, C(2)H_AH_B), 2.11-1.96 (1H, m,

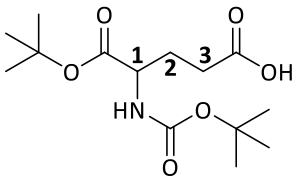
C(2)H_AH_B), 1.44 (9H, s, OC(CH_3)$_3$); **LRMS**: *m/z* (ES)$^-$ 260 ([M−H]$^-$, 100%). The data are in good agreement with literature values for the (*S*)-enantiomer.[294]

1-(*tert*-Butyl) 5-methyl Boc-(DL)-glutamate (147)



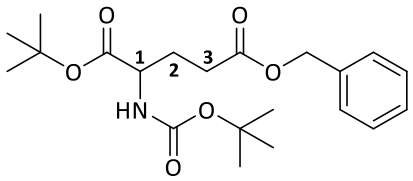
146 (2.00 g, 7.66 mmol, 1.00 eq.) and DMAP (95 mg, 0.78 mmol, 0.10 eq.) were dissolved in anhydrous CH_2Cl_2 (40 mL). DCC (1.82 g, 8.81 mmol, 1.15 eq.) was added and the resulting mixture was stirred at RT for 15 min under an atmosphere of nitrogen. Anhydrous *tert*-BuOH (1.47 mL, 1.14 g, 15.32 mmol, 2.00 eq.) was added and the reaction mixture was stirred at RT for a further 18 h. After this time, the reaction mixture was filtered under vacuum and the residue washed with CH_2Cl_2 (100 mL). The organic filtrate was washed with sat. $NaHCO_3$ aq. (2 × 75 mL), 10% citric acid aq. (2 × 75 mL) and distilled H_2O (2 × 75 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo* to give the crude product. The crude material was adsorbed onto Celite® and purified *via* flash column chromatography on pre-washed (1% triethylamine in petrol) silica gel (Biotage®; gradient elution: 2% → 20% EtOAc in petrol) affording **147** (810 mg, 33%) as a colourless oil. $\mathbf{R_f}$: 0.63 (1:4 EtOAc:Petrol); **^{1}H NMR** (400 MHz, $CDCl_3$): δ 5.07 (1H, d, *J* = 7.4 Hz, N*H*), 4.26-4.13 (1H, m, C(1)*H*), 3.67 (3H, s, OCH_3), 2.49-2.29 (2H, m, C(3)H_2), 2.23-2.05 (1H, m, C(2)H_AH_B), 1.99-1.83 (1H, m, C(2)H_AH_B), 1.47 (9H, s, C(CH_3)$_3$), 1.43 (9H, s, C(CH_3)$_3$); **LRMS**: *m/z* (ES)$^+$ 657 ([2M+H]$^+$, 100%); The data are in good agreement with literature values for the (*S*)-enantiomer.[206]

Boc-(DL)-glutamic acid 1-tert-butyl ester (148)



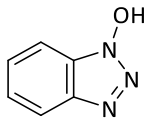
147 (810 mg, 2.55 mmol, 1.00 eq.) was dissolved in THF (8 mL) and LiOH (5.10 mL, 122 mg, 5.10 mmol, 2.00 eq.; 1 M in distilled H_2O) was added. The reaction mixture was stirred for 16 h under an atmosphere of nitrogen. After this time, the reaction mixture was concentrated *in vacuo* and the residue taken up in distilled H_2O (20 mL). The aqueous phase was washed with diethyl ether (3 × 20 mL) and acidified to pH 4 *via* the portion-wise addition of solid citric acid. The precipitate was taken up in EtOAc (60 mL). The organic layer was separated, washed with distilled H_2O (3 × 25 mL) and brine (3 × 25 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo* affording **148** (280 mg, 36%) as a colourless solid that was used without further purification. $\mathbf{R_f}$: 0.11 (1:9 EtOAc:Petrol); **mp**: 101-102 °C (Et_2O/petrol); **^{1}H NMR** (400 MHz, $CDCl_3$): δ 10.10 (1H, br s, COO*H*), 5.21 (1H, d, *J* = 8.2 Hz, N*H*), 4.24-4.13 (1H, m, C(1)*H*), 2.51-2.28 (2H, m, C(3)H_2), 2.19-2.02 (1H, m, C(2)H_AH_B), 1.97-1.79 (1H, m, C(2)H_AH_B), 1.43 (9H, s, C(CH_3)$_3$), 1.40 (9H, s, C(CH_3)$_3$); **LRMS**: *m/z* (ES)$^-$ 302 ([M−H]$^-$, 100%). The data are in accordance with the (*S*)-enantiomer, compound **131**.

1-(*tert*-Butyl) 5-benzyl Boc-(DL)-glutamate (142)



148 (110 mg, 0.36 mmol, 1.00 eq.), anhydrous triethylamine (51 µL, 37 mg, 0.36 mmol, 1.00 eq.) and DMAP (9 mg, 0.07 mmol, 0.20 eq.) were dissolved in anhydrous CH_2Cl_2 (5 mL). Benzyl chloroformate (62 µL, 75 mg, 0.44 mmol, 1.20 eq.) was added dropwise and the reaction was stirred at RT for 16 h under an atmosphere of nitrogen. After this time, the solution was concentrated *in vacuo* and the residue taken up in CH_2Cl_2 (20 mL). The organic layer was and washed with 10% citric acid aq. (3 × 20 mL), distilled H_2O (3 × 20 mL) and brine (3 × 20 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo* to give the crude product. The crude material was adsorbed onto Celite® and purified *via* flash column chromatography on pre-washed (1% triethylamine in petrol) silica gel (Biotage®; gradient elution: 1% → 5% EtOAc in petrol) affording **142** (57 mg, 40%) as a colourless solid. **R_f**: 0.13 (1:19 EtOAc:Petrol); **mp**: 33-34 °C (EtOAc/Hexane); **^{1}H NMR** (400 MHz, $CDCl_3$): δ 7.39-7.29 (5H, m, Ar C*H*), 5.22-5.04 (3H, m, N*H* and PhCH_2), 4.26-4.15 (1H, m, C(1)*H*), 2.53-2.35 (2H, m, C(3)H_2), 2.23-2.08 (1H, m, C(2)H_AH$_B$), 1.98-1.85 (1H, m, C(2)H$_A$$H_B$), 1.45 (9H, s, C(C$H_3$)$_3$), 1.43 (9H, s, C(C$H_3$)$_3$); **LRMS**: *m/z* (ES)$^+$ 809 ([2M+Na]$^+$, 100%). The data are in accordance with the (*S*)-enantiomer, compound **138**.

1*H*-Benzotriazol-1-ol (125)



1-Chloro-2-nitrobenzene **149** (10.00 g, 63.47 mmol, 1.00 eq.) was dissolved in toluene (150 mL) and hydrazine hydrate (25.42 mL, 12.71 g, 253.89 mmol, 4.00 eq.; 50% aq. solution) was added. The resulting solution was heated under reflux for 24 h under an atmosphere of nitrogen. After this time, the reaction solution was carefully concentrated *in vacuo* and the orange residue taken up in 10% Na_2CO_3 aq. (25 mL). The aqueous phase was washed with diethyl ether (3 × 25 mL) and acidified to pH 2 *via* the dropwise addition of conc. HCl. The resulting precipitate was isolated *via* vacuum filtration, washed with distilled H_2O and dried, affording **125** (3.29 g, 38%) as a colourless crystalline solid that was used without further purification. **R_f**: 0.00 (1:1 EtOAc:Petrol); **mp**: 153-155 °C (CH_2Cl_2/MeOH) [Lit.[295] mp: 155-157 °C (CH_2Cl_2/MeOH)]; **^{1}H NMR** (400 MHz, $(CD_3)_2SO$): δ 13.61 (1H, br s, O*H*), 7.99 (1H, d, *J* = 8.5 Hz, Ar C*H*,), 7.75-7.69 (1H, m, Ar C*H*), 7.58-7.51 (1H, m, Ar C*H*), 7.44-7.38 (1H, m, Ar C*H*); **LRMS**: *m/z* (ES)$^+$ 136 ([M+H]$^+$, 100%). The data are in good agreement with literature values.[296]

6.3.2 Solid phase peptide synthesis

Tripeptide synthesis involved: preparation of Fmoc-Gly-Wang resin **135**, Fmoc-deprotection, peptide coupling with the desired Fmoc-protected amino acid giving an immobilised dipeptide, a further Fmoc-deprotection and a final peptide coupling with Boc-L-glutamic acid 1-*tert*-butyl ester (**131**) to give an immobilised appropriately protected tripeptide (**Scheme 3.11**). All solid phase reactions were monitored using the Kaiser test (**Section 5.1**),

which qualitatively indicates the presence (or absence) of a free amine. A global deprotection, with a cleavage cocktail specific to the each tripeptide removed the remaining protecting groups and cleaved the tripeptide from the resin giving the crude product (**Scheme 3.13**). This is detailed in General procedure A.

General procedure A

Step 1: Fmoc-Glycine-Wang resin **135** (assumed 0.8 mmol/g, 1.00 eq.) was swollen in CH_2Cl_2 (1 mL/100 mg) for 30 min at RT in a SPPS tube. After this time, the CH_2Cl_2 was allowed to drain under vacuum without drying out the resin.

Step 2: The resin was washed *via* rotation with anhydrous DMF (1 mL/1.00 g) in a sealed SPPS tube, followed by subsequent draining of the DMF under vacuum without drying out the resin (5 × cycles).

Step 3: The prepared, washed resin was rotated with 20% piperidine in DMF (1 mL/1.00 g, pre-dried over 3 Å molecular sieves) in a sealed SPPS tube, followed by subsequent draining of the solution under vacuum (5 × cycles). Following a positive Kaiser test, **150** was washed with DMF (5 × 1 mL/1.00 g) as in **step 2**.

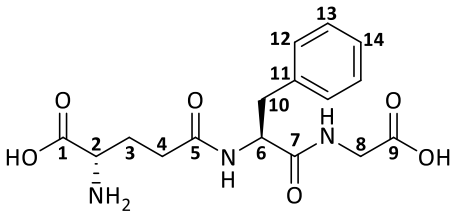
Step 4: An Fmoc-protected amino acid **136**/**134**/**137** (5.00 eq.), HOBt **125** (5.00 eq.) and TBTU **123** (4.90 eq.) were dissolved in anhydrous DMF (1 mL/1.00 g of resin) and subsequently, DIPEA (10.00 eq.) was added. The resulting solution was added to the Fmoc-deprotected resin **150** (assumed 0.8 mmol/g, 1.00 eq.) and the suspension rotated at RT for 2 h in a sealed SPPS tube, followed by subsequent draining of the solution under vacuum (2 × cycles). Following a negative Kaiser test, the resin was washed with DMF (5 × 1 mL/1.00 g) affording an immobilised Fmoc-protected dipeptide **151**/**132**/**152** (assumed 0.8 mmol/g, 1.00 eq.).

Step 5: The immobilised dipeptide **151**/**132**/**152** underwent an Fmoc-deprotection as in **step 3** affording an immobilised Fmoc-deprotected dipeptide **153**/**154**/**155**.

Step 6: **153**/**154**/**155** (5.00 eq.), HOBt **125** (5.00 eq.) and TBTU **123** (4.90 eq.) were dissolved in anhydrous DMF (1 mL/1.00 g of resin) and subsequently, DIPEA (10.00 eq.) was added. The resulting solution was added to the Fmoc-deprotected dipeptide (assumed 0.8 mmol/g, 1.00 eq.) and the suspension rotated at RT for 2 h in a sealed SPPS tube, followed by subsequent draining of the solution under vacuum (2 × cycles). Following a negative Kaiser test, the resin was washed with DMF (5 × 1 mL/1.00 g) as in **step 2** and dried *in vacuo* for 18 h affording an immobilised tripeptide **156**/**130**/**157** (assumed 0.8 mmol/g, 1.00 eq.).

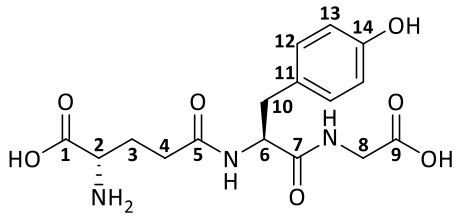
Step 7: The immobilised tripeptide **156**/**130**/**157** was transferred to a round bottomed flask and stirred with an acidic cleavage cocktail under an atmosphere of nitrogen (**Table 3.2**). Subsequently, the resin was removed *via* vacuum filtration and washed with trifluoroacetic acid (5 mL). The filtrate was concentrated under a stream of nitrogen until < 1 mL remained and transferred into a number of Eppendorf® vials. A precipitate was collected from the remaining filtrate *via* the addition of ice cold Et_2O. The resulting precipitate was isolated *via* centrifugation, suspended in distilled H_2O and lyophilised to give the crude tripeptide **127**/**128**/**129**.

***γ*-Glu-Phe-Gly (127)**



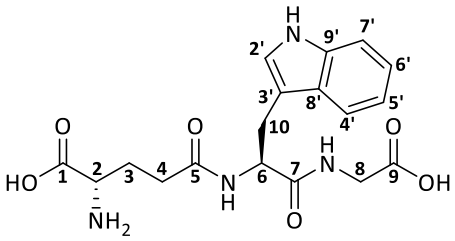
127 was synthesised according to general procedure A from Fmoc-Glycine-Wang **135** (700 mg, 0.56 mmol, 1.00 eq.), Fmoc-Phenylalanine **136** (1.08 g, 2.80 mmol, 5.00 eq.) and **131** (849 mg, 2.80 mmol, 5.00 eq.). The immobilised tripeptide **156** was cleaved from the Wang resin by stirring in a cleavage cocktail of trifluoroacetic acid:phenol:water:tri*iso*propylsilane (88:5:5:2, 7 mL). Following isolation of the crude product, the material was purified *via* flash column chromatography on RP silica gel (Manual; 19:1 H_2O:MeCN) affording **127** (64 mg, approx. 43% over 5 steps) as a hygroscopic colourless solid. $\mathbf{R_f}$: 0.85 (RP; 4:1 H_2O:MeOH); $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\mathbf{20}}$: +3.3 (*c* 0.18, $(CH_3)_2SO$); **IR** (neat) ν_{max} cm^{-1}: 3287 (w), 1756 (m), 1636 (s), 1548 (s); **^{1}H NMR** (500 MHz, $(CD_3)_2SO$): δ 8.64 (1H, br s, N*H*), 8.51 (1H, br s, N*H*), 7.39-7.22 (4H, m, C(12)*H* and C(13)*H*), 7.21-7.14 (1H, m, C(14)*H*), 4.51-4.42 (1H, m, C(6)*H*), 3.78-3.67 (2H, m, C(8)H_2), 3.32 (1H, m, C(2)*H*), 3.08 (1H, dd, *J* = 13.8, 3.6 Hz, C(10)H_AH_B), 2.75 (1H, dd, *J* = 13.8, 11.0 Hz, C(10)H_AH_B), 2.34-2.22 (1H, dd, C(4)H_AH_B), 2.18-2.08 (1H, m, C(4)H_AH_B), 1.89-1.72 (2H, m, C(3)H_2); **^{13}C NMR** (126 MHz, $(CD_3)_2SO$): δ 171.8 (*C*7), 171.6 (*C*5), 171.2 (*C*9), 170.5 (*C*1), 138.4 (*C*11), 129.1 (*C*12), 128.1 (*C*13), 126.2 (*C*14), 54.2 (*C*6), 53.0 (*C*2), 41.2 (*C*8), 37.3 (*C*10), 31.3 (*C*4), 26.8 (*C*3); **HRMS**: *m/z* $(ES)^-$ Found $[M-H]^-$ 350.1355, $C_{16}H_{20}N_3O_6^-$ requires $[M]^-$ 350.1358; **LRMS**: *m/z* $(ES)^-$ 350 ($[M-H]^-$, 100%); **HPLC** (Protocol D): ret. time = 2.36 min, purity = 96.4%.

***γ*-Glu-Tyr-Gly (128)**



128 was synthesised according to general procedure A from Fmoc-Glycine-Wang **135** (1.00 g, 0.80 mmol, 1.00 eq.), Fmoc-*O*-*tert*-butyl-L-tyrosine **134** (1.84 g, 4.00 mmol, 5.00 eq.) and **131** (1.21 g, 4.00 mmol, 5.00 eq.). The immobilised tripeptide **130** was cleaved from the Wang resin by stirring in a cleavage cocktail of trifluoroacetic acid:phenol:water:ethanedithiol (88:5:5:2, 10 mL). Following isolation of the crude product, the material was purified *via* flash column chromatography on RP silica gel (Manual; 1:0 → 39:1 H_2O:MeCN) affording **128** (101 mg, approx. 34% over 5 steps) as a hygroscopic colourless solid. $\mathbf{R_f}$: 0.91 (RP 3:2 H_2O:MeOH); $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\mathbf{20}}$: +12.9 (*c* 0.75, H_2O); **IR** (neat) ν_{max} cm^{-1}: 2971 (br, w), 1727 (m), 1639 (s); **^{1}H NMR** (500 MHz, D_2O): δ 7.17-7.11 (2H, m, C(13)*H*), 6.86-6.81 (2H, m, C(12)*H*), 4.63-4.68 (1H, m, C(6)*H*), 4.00-3.90 (2H, m, C(8)H_2), 3.88-3.83 (1H, m, C(2)*H*), 3.14-3.07 (1H, m, C(10)H_AH_B), 2.91-2.84 (1H, m, C(10)H_AH_B), 2.46-2.39 (2H, m, C(4)H_2), 2.08-1.99 (2H, m, C(3)H_2); **^{13}C NMR** (126 MHz, D_2O): δ 174.1 (*C*5), 173.7 (*C*7), 173.0 (*C*9), 172.1 (*C*1), 154.4 (*C*14), 130.5 (*C*13), 128.3 (*C*11), 115.4 (*C*12), 55.0 (*C*6), 52.6 (*C*2), 41.0 (*C*8), 36.3 (*C*10), 31.0 (*C*4), 25.7 (*C*3); **HRMS**: *m/z* $(ES)^+$ Found $[M+Na]^+$ 390.1263, $C_{16}H_{21}N_3NaO_7^+$ requires $[M+Na]^+$ 390.1272; **LRMS**: *m/z* $(ES)^+$ 390 ($[M+Na]^+$, 58%), 416 (100%).

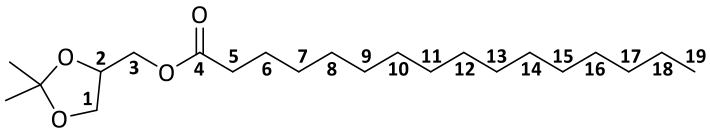
***γ*-Glu-Trp-Gly (129)**



129 was synthesised according to general procedure A from Fmoc-Glycine-Wang **135** (700 mg, 0.56 mmol, 1.00 eq.), Fmoc-($N_{(ind)}$-Boc)-tryptophan **137** (1.47 g, 2.80 mmol, 5.00 eq.) and **131** (849 mg, 2.80 mmol, 5.00 eq.). The immobilised tripeptide **157** was cleaved from the Wang resin by stirring in a cleavage cocktail of trifluoroacetic acid:phenol:water:ethanedithiol (88:5:5:2, 7 mL). Following isolation of the crude product, the material was purified *via* flash column chromatography on RP silica gel (Manual; 19:1 → 9:1 H_2O:MeCN) affording **129** (29 mg, approx. 18% over 5 steps) as a hygroscopic colourless solid. $\mathbf{R_f}$: 0.95 (RP 9:1 H_2O:MeOH); $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\boldsymbol{20}}$: +1.9 (*c* 0.60, $(CH_3)_2SO$); **IR** (neat) ν_{max} cm^{-1}: 3387 (w), 3177 (w), 2548 (w), 2929 (w), 1669 (m) 1638 (s), 1517 (m); **^{1}H NMR** (500 MHz, $(CD_3)_2SO$): δ 10.81 (d, 1H, *J* = 1.7 Hz, N(1')*H*), 8.39 (t, 1H, *J* = 6.0 Hz, N*H*C(8)H_2), 8.28-8.15 (m, 3H, NH_2C(2)H & N*H*C(6)H), 7.61 (d, 1H, *J* = 7.5 Hz, C(4')*H*), 7.32 (d, 1H, *J* = 7.6 Hz, C(7')*H*), 7.13 (d, 1H, *J* = 1.7 Hz, C(2')*H*), 7.05 (dd, 1H, *J* = 7.6, 7.2 Hz, C(6')*H*), 6.97 (dd, 1H, *J* = 7.5, 7.2 Hz, C(5')*H*), 4.56 (ddd, 1H, *J* = 9.1, 8.8, 4.4 Hz, C(6)*H*), 3.91-3.84 (m, 2H, C(2)*H*), 3.77 (d, 2H, *J* = 6.0 Hz, C(8)H_2), 3.14 (dd, 1H, *J* = 14.7, 4.4 Hz, C(10)H_AH_B), 2.91 (dd, 1H, *J* = 14.7, 9.1 Hz, C(10)H_AH_B), 2.26 (t, 2H, *J* = 7.8 Hz, C(4)H_2), 1.98-1.84 (m, 2H, C(3)H_2); **^{13}C NMR** (126 MHz, $(CD_3)_2SO$): δ 172.0 (*C*7), 171.0 (*C*9), 170.8 (*C*1/5), 170.7 (*C*1/5), 136.1 (*C*9'), 127.3 (*C*8'), 123.6 (*C*2'), 120.8 (*C*6'), 118.4 (*C*4'), 118.2 (*C*5'), 111.3 (*C*7'), 110.2 (*C*3'), 53.3 (*C*6), 51.6 (*C*2), 40.7 (*C*8), 30.5 (*C*4), 27.9 (*C*10), 25.8 (*C*3); **HRMS**: *m/z* $(ES)^-$ Found $[M-H]^-$ 389.1456, $C_{16}H_{21}N_3O_7^-$ requires $[M]^-$ 389.1467; **LRMS**: *m/z* $(ES)^-$ 389 ($[M-H]^-$, 89%).

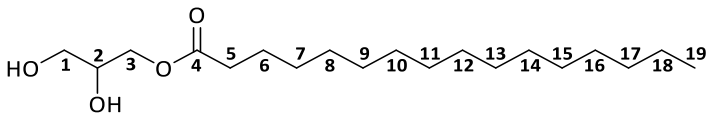
6.4 Towards the synthesis of amino-LPC, a candidate for immobilisation

(±)-(2,2-Dimethyl-1,3-dioxolan-4-yl)methyl palmitate (165)



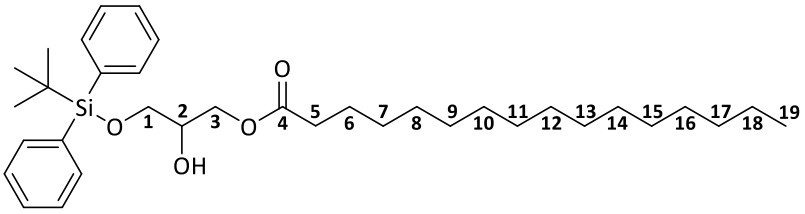
Palmitic acid **187** (5.00 g, 19.51 mmol, 1.00 eq.) and DMAP (238 mg, 1.95 mmol, 0.10 eq.) were dissolved in anhydrous CH_2Cl_2 (50 mL). DCC (4.63 g, 22.44 mmol, 1.15 eq.) was added and the resulting mixture was stirred at RT for 15 min under an atmosphere of nitrogen. (±)-1,2-*iso*propylideneglycerol **190** (3.64 mL, 3.87 g, 29.27 mmol, 1.50 eq.) was added and the reaction mixture was stirred at RT for a further 24 h. After this time, the reaction mixture was filtered under vacuum and the residue washed with CH_2Cl_2 (50 mL). The organic filtrate was washed with sat. $NaHCO_3$ aq. (2 × 50 mL), 10% citric acid aq. (2 × 50 mL) and distilled H_2O (2 × 50 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo* to give the crude product. The crude material was adsorbed onto Celite® and purified *via* flash column chromatography on pre-washed (1% triethylamine in petrol) silica gel (Manual; 1:4 EtOAc:Petrol) affording **165** (5.99 g, 83%) as a colourless waxy solid. **R_f**: 0.27 (1:4 EtOAc:Petrol); **mp**: 31-32 °C (EtOH) [Lit.[297] mp: 29-30 °C]; **^{1}H NMR** (400 MHz, $CDCl_3$): δ 4.34-4.28 (1H, m, C(2)*H*), 4.16 (1H, dd, *J* = 11.5, 4.7 Hz, C(3)H_AH_B), 4.11-4.05 (2H, m, C(1)H_AH_B and C(3)H_AH_B), 3.73 (1H, dd, *J* = 8.4, 6.2 Hz, C(1)H_AH_B), 2.33 (2H, t, *J* = 7.6 Hz, C(5)H_2), 1.66-1.59 (2H, m, C(6)H_2), 1.43 (3H, s, CH_3), 1.37 (3H, s, CH_3), 1.34-1.19 (24H, m, C(7-18)H_2), 0.88 (3H, t, *J* = 6.8 Hz, C(19)H_3); **LRMS**: *m/z* (ES)$^+$ 764 ([2M+Na]$^+$, 100%). The data are in good agreement with literature values.[298]

(±)-2,3-Dihydroxypropyl palmitate (166)



165 (2.50 g, 6.75 mmol, 1.00 eq.) was dissolved in 0.4 M HCl in 90% (v/v) dioxane in distilled H_2O (50 mL; 5 mL 4 M HCl in dioxane, 40 mL dioxane, 5 mL distilled H_2O). The resulting solution was stirred for 18 h under an atmosphere of nitrogen. After this time, the solution was concentrated *in vacuo*. The resulting residue was taken up in distilled H_2O and lyophilised to give the crude product. The crude material was crystallised from methanol affording **166** (1.56 g, 70%) as a colourless crystalline solid. **R_f**: 0.39 (1:1 EtOAc:Petrol); **mp**: 69-71 °C (EtOAc/Hexane) [Lit.[297] mp: 72 °C]; **^{1}H NMR** (400 MHz, $CDCl_3$): δ 4.22 (1H, dd, *J* = 11.6, 4.7 Hz, C(3)H_AH_B); 4.15 (1H, dd, *J* = 11.5, 6.1 Hz, C(3)H_AH_B), 3.99-3.91 (1H, m, C(2)*H*), 3.71 (1H, dd, *J* = 11.5, 3.9 Hz, C(1)H_AH_B), 3.61 (1H, dd, *J* = 11.6, 5.7 Hz, C(1)H_AH_B), 2.35 (2H, t, *J* = 7.6 Hz, C(5)H_2), 1.68-1.56 (2H, m, C(6)H_2), 1.37-1.18 (24H, m, C(7-18)H_2), 0.88 (2H, t, *J* = 6.7 Hz, C(19)H_3); **LRMS**: *m/z* (ES)$^+$ 353 ([M+Na]$^+$, 100%). The data are in good agreement with literature values.[297]

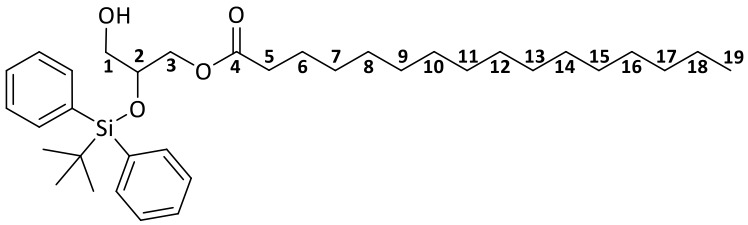
(±)-2-Hydroxy-3-(*tert*-butyldiphenylsilyl)oxypropyl palmitate (201)



166 (0.50 g, 1.45 mmol, 1.00 eq.), DMAP (0.04 g, 0.3 mmol, 0.10 eq.) and anhydrous triethylamine (210 µL, 153 mg, 1.51 mmol, 1.25 eq.) were dissolved in anhydrous CH_2Cl_2 (10 mL) over 3 Å molecular sieves. *tert*-Butyl(chloro)diphenylsilane (472 µL, 499 mg, 1.81 mmol, 1.25 eq.) was added dropwise and the resulting solution was stirred for 21 h. After this time, further *tert*-butyl(chloro)diphenylsilane (151 µL, 160 mg, 0.58 mmol, 0.40 eq.), DMAP (18 mg, 0.15 mmol, 0.10 eq.) and distilled triethylamine (81 µL, 59 mg, 0.58 mmol, 0.40 eq.) were added and the reaction solution was stirred for a further 27 h. After this time, the molecular sieves were removed *via* gravity filtration and washed with CH_2Cl_2. The filtrate was concentrated *in vacuo* (with the water bath at RT) and the resulting residue taken up in CH_2Cl_2 (25 mL). The organic layer was washed with 10% citric acid aq. (2 × 20 mL), sat. $NaHCO_3$ aq. (2 × 20 mL) and distilled water (3 × 20 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo* (with the water bath at RT) to give the crude product. The crude material was adsorbed onto Celite® and purified *via* flash column chromatography on silica gel (Manual; 0:1 → 1:1 EtOAc:Petrol) affording **201** (211 mg, 26%) as a colourless oil. Further elution afforded **202** (see below). **R_f**: 0.11 (1:9 EtOAc:Petrol); **IR** (thin film) ν_{max} cm^{-1}: 3449 (w), 2924 (s), 2854 (m), 1740 (s); **^{1}H NMR** (500 MHz, $CDCl_3$): δ 7.77-7.63 (4H, m, Ar C*H*), 7.48-7.37 (6H, m, Ar C*H*), 4.20 (1H, dd, *J* = 11.6, 5.0 Hz, C(3)H_AH_B), 4.16 (1H, dd, *J* = 11.6, 6.1 Hz, C(3)H_AH_B), 3.97-3.91 (1H, m, C(2)*H*), 3.72 (1H, dd, *J* = 10.3, 5.6 Hz, C(1)H_AH_B), 3.67 (1H, dd, *J* = 10.3, 4.8 Hz, C(1)H_AH_B), 2.52 (1H, d, *J* = 6.5 Hz, O*H*), 2.30 (2H, t, *J* = 7.6 Hz, C(5)H_2), 1.63-1.57 (2H, m, C(6)H_2),

1.37-1.21 (24H, m, C(7-18)H_2), 1.08 (9H, s, C(CH_3)$_3$), 0.89 (3H, t, *J* = 6.8 Hz, C(19)H_3); **^{13}C NMR** (126 MHz, $CDCl_3$): δ 174.1 (*C*4), 135.7 (Ar *C*H), 133.0 (Ar *C*), 130.0 (Ar *C*H), 128.0 (Ar *C*H), 70.2 (*C*2), 65.1 (*C*3), 64.6 (*C*1), 34.3 (*C*5), 32.1 (1 × *C*(7-18)), 29.8-29.7 (3 × *C*(7-18)), 29.6 (1 × *C*(7-18)), 29.5 (1 × *C*(7-18)), 29.4 (1 × *C*(7-18)), 29.3 (1 × *C*(7-18)), 27.0 (C(*C*H_3)$_3$), 25.0 (*C*6), 22.8 (1 × *C*(7-18)), 19.4 (1 × *C*(7-18)), 14.3 (*C*19); **HRMS**: *m/z* (ES)$^+$ Found [M+Na]$^+$ 591.3842, $C_{35}H_{56}NaO_4Si^+$ requires [M]$^+$ 591.3840; **LRMS**: *m/z* (ES)$^+$ 591 ([M+Na]$^+$, 100%); **Anal. Calcd.** for $C_{35}H_{56}O_4Si$: *C*, 73.89; *H*, 9.92; *N*, 0.00. Found: *C*, 74.01; *H*, 9.84; *N*, 0.00.

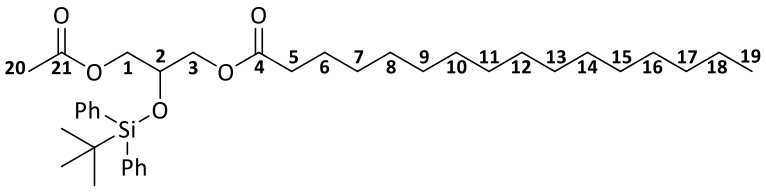
(±)-2-(*tert*-Butyldiphenylsilyl)oxy-3-hydroxypropyl palmitate (202)



Further elution afforded **202** (66 mg, 8%) as a colourless oil. **R_f**: 0.11 (1:9 EtOAc:Petrol); **IR** (thin film) ν_{max} cm^{-1}: 3441 (w), 1738 (s); **^{1}H NMR** (400 MHz, $CDCl_3$): δ 7.71-7.64 (4H, m, Ar C*H*), 7.48-7.43 (2H, m, Ar C*H*), 7.43-7.37 (4H, m, Ar C*H*), 5.02-4.96 (1H, m, C(2)*H*), 3.91-3.78 (4H, m, C(1)H_AH_B & C(3)H_AH_B), 2.04 (1H, d, *J* = 6.3 Hz, O*H*), 2.30 (2H, t, *J* = 7.6 Hz, C(5)H_2), 1.66-1.58 (2H, m, C(6)H_2), 1.35-1.22 (24H, m, C(7-18)H_2), 1.07 (9H, s, C(CH_3)$_3$), 0.89 (3H, t, *J* = 6.7 Hz, C(19)H_3); **^{13}C NMR** (126 MHz, $CDCl_3$): δ 173.9 (*C*4), 135.73 (Ar *C*H), 135.66 (Ar *C*H), 133.13 (Ar *C*), 133.07 (Ar *C*), 130.0 (Ar *C*H), 127.94 (Ar *C*H), 127.91 (Ar *C*H), 74.6 (*C*2), 63.2 & 62.8 (*C*1 & *C*3), 34.5 (*C*5), 32.1 (C(*C*H_3)$_3$), 29.84-29.76 (6 × *C*(7-18)), 29.6 (1 × *C*(7-18)), 29.5 (1 × *C*(7-18)), 29.4 (1 × *C*(7-18)), 29.3 (1 × *C*(7-18)), 26.9 (1 × *C*(7-18)), 25.1 (*C*6), 22.8 (1 × *C*(7-18)), 14.3 (*C*19);

HRMS: *m/z* (ES)$^+$ Found [M+Na]$^+$ 591.3848, $C_{35}H_{56}NaO_4Si^+$ requires [M]$^+$ 591.3840; **LRMS**: *m/z* (ES)$^+$ 591 ([M+Na]$^+$, 100%).

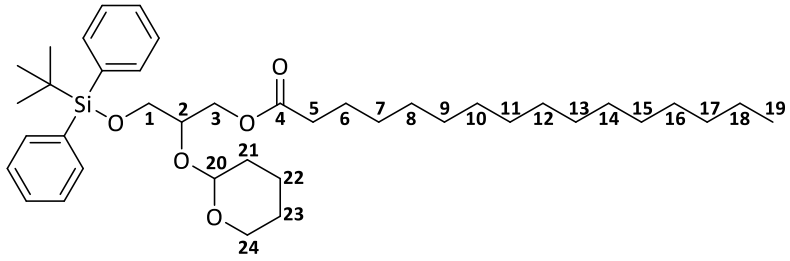
(±)-2-(*tert*-Butyldiphenylsilyl)oxy-3-acetoxypropyl palmitate (203)



202 (50 mg, 0.09 mmol, 1.00 eq.) was dissolved in anhydrous pyridine (2 mL) under an atmosphere of nitrogen. The resulting solution was cooled to 0 °C and acetic anhydride (415 µL, 449 mg, 4.39 mmol, 50.00 eq.) was added dropwise over the course of 1 min. The reaction solution was allowed to warm to RT and stirred for 6 h. After this time, the solution was concentrated *in vacuo* and the residue taken up in $CHCl_3$. The organic layer was washed with 10% citric acid aq. (3 × 10 mL), distilled H_2O (3 × 10 mL) and brine (3 × 10 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo* affording **203** (43 mg, 81%) as a colourless oil, which was characterised without further purification. $\mathbf{R_f}$: 0.68 (1:9 EtOAc:Petrol); **IR** (thin film) ν_{max} cm^{-1}: 2925 (m), 2854 (m), 1743 (s), 1112 (s); **^{1}H NMR** (500 MHz, $CDCl_3$): δ 7.74-7.61 (2H, m, Ar C*H*), 7.48-7.42 (2H, m, Ar C*H*), 7.42-7.34 (4H, m, Ar C*H*), 5.21-5.15 (1H, m, C(2)*H*), 4.39 (1H, dd, *J* = 11.8, 3.8 Hz, C(1)H_AH_B), 4.22 (1H, dd, *J* = 11.8, 6.3 Hz, C(1)H_AH_B), 3.78 (1H, dd, *J* = 11.0, 5.2 Hz, C(3)H_AH_B), 3.75 (1H, dd, *J* = 11.0, 5.3 Hz, C(3)H_AH_B), 2.35-2.23 (2H, m, C(5)H_2), 2.03 (3H, s, C(20)H_3), 1.65-1.56 (2H, m, C(6)H_2), 1.35-1.21 (24H, m, C(7-18)H_2), 1.06 (9H, s, C(CH_3)$_3$), 0.89 (3H, t, *J* = 6.9 Hz, C(19)H_3); **^{13}C NMR** (126 MHz, $CDCl_3$): δ 173.2 (*C*4), 170.8 (*C*20), 135.72 (Ar *C*H), 135.66 (Ar *C*H), 133.2 (Ar *C*), 133.1 (Ar *C*) 130.0 (Ar *C*H), 127.90 (Ar *C*H), 127.88 (Ar *C*H), 71.5 (*C*2), 62.8 (*C*1), 62.4 (*C*3), 34.5 (*C*5 & 1 × *C*(7-18)), 32.1 (1 × *C*(7-18)), 29.84-29.76

(4 × *C*(7-18)), 29.6 (1 × *C*(7-18)), 29.5 (1 × *C*(7-18)), 29.4 (1 × *C*(7-18)), 29.2 (1 × *C*(7-18)), 26.8 (C(*C*H$_3$)$_3$), 25.1 (*C*6 & 1 × *C*(7-18)), 22.8 (1 × *C*(7-18)), 20.9 (*C*20), 14.3 (*C*19); **HRMS**: *m/z* (ES)$^+$ Found [M+Na]$^+$ 633.3949, $C_{37}H_{58}NaO_5Si^+$ requires [M]$^+$ 633.3951; **LRMS**: *m/z* (ES)$^+$ 633 ([M+Na]$^+$, 100%); **Anal. Calcd.** for $C_{37}H_{58}O_5Si$: *C*, 72.74; *H*, 9.57; *N*, 0.00. Found: *C*, 72.81; *H*, 9.45; *N*, 0.00.

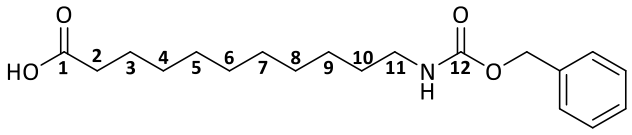
(±)-2-(Tetrahydro-2*H*-pyran-2-yl)oxy-3-(*tert*-butyldiphenylsilyl)oxypropyl palmitate (204)



201 (50 mg, 0.09 mmol, 1.00 eq.) and pyridinium 4-toluenesulfonate (2 mg, 8.79 µmol, 0.10 eq.) were dissolved in anhydrous CH_2Cl_2 (0.5 mL). 3,4-Dihydro-[2*H*]-pyran (12 µL, 11 mg, 0.13 mmol, 1.50 eq.) was added and the resulting solution was stirred at RT for 24 h. After this time, the solution was concentrated *in vacuo* and the residue taken up in EtOAc (20 mL). The organic layer was washed with 10% Na_2CO_3 aq. (3 × 10 mL) distilled H_2O (3 × 10 mL) and brine (3 × 10 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo* to give the crude product. The crude material was adsorbed onto Celite® and purified *via* flash column chromatography on pre-washed (1% triethylamine in petrol) silica gel (Biotage®; gradient elution: 0% → 10% EtOAc in petrol) affording a diastereoisomeric mixture of **204** (11 mg, 18%) as a colourless oil. **R$_f$**: 0.16 (1:9 EtOAc:Petrol); **IR** (thin film) ν_{max} cm^{-1}: 2924 (s), 2854 (m), 1739 (m); **^{1}H NMR** (400 MHz, $CDCl_3$): δ 7.75-7.60 (8H, m, Ar C*H*), 7.49-7.35 (12H, m, Ar C*H*), 4.77 (1H, dd, *J* = 3.2, 3.2 Hz, C(20)*H*), 4.74 (1H, dd, *J* = 3.4, 3.4 Hz, C(20')*H*), 4.47 (1H, dd, *J* = 11.7, 3.7 Hz, C(3)H_AH_B),

4.30 (1H, dd, *J* = 11.4, 4.6 Hz, C(3')H_AH_B), 4.21 (1H, dd, *J* = 11.4, 5.6 Hz, C(3')H_AH_B), 4.15 (1H, dd, *J* = 11.7, 5.5 Hz, C(3)H_AH_B), 4.03-3.89 (3H, m, C(2)*H*, C(2')*H* & C(24)H_AH_B), 3.82 (1H, dd, *J* = 10.2, 4.9 Hz, C(1)H_AH_B), 3.82-3.68 (4H, m, C(24')H_AH_B, C(1)H_AH_B & C(1')H_AH_B), 3.52-3.44 (1H, m, C(24)H_AH_B), 3.42-3.34 (1H, m, C(24')H_AH_B), 2.32-2.25 (4H, m, C(5)H_2 & C(5')H_2), 1.87-1.15 (64H, m, C(6-18)H_2, C(6'-18')H_2, C(21-23)H_2 & C(21'-23')H_2), 1.05 (9H, s, C(CH_3)$_3$), 1.04 (9H, s, C(C'H_3)$_3$), 0.89 (6H, t, *J* = 6.8 Hz, C(19)H_3 & C(19')H_3); **^{13}C NMR** (126 MHz, $CDCl_3$): δ 173.9 & 173.8 (*C*4 & *C*4'), 135.8-135.7 (Ar *C*H & Ar *C*'H) 133.6-133.4 (Ar *C* & Ar *C*'), 129.9-129.8 (Ar *C*H & Ar *C*'H), 128.0 (*C*$(CH_3)_3$ & *C*'(C'H_3)$_3$), 127.84-127.79 (Ar *C*H & Ar *C*'H), 98.3 (*C*20'), 97.7 (*C*20), 74.9 (*C*2'), 74.3 (*C*2), 64.3 (*C*3'), 63.7 (*C*1), 63.3 & 63.2 (*C*3 & *C*1'), 62.19 & 62.17 (*C*24 & *C*24'), 34.5 (*C*5), 34.4 (*C*5'), 32.1-29.3 (*C*(6-18) & *C*(6'-18')), 26.93 & 26.88 (C(*C*H_3)$_3$ & C'(*C*'H_3)$_3$), 19.33 & 19.28 (*C*23 & *C*23'), 14.3 (*C*19 & *C*19'); **HRMS**: *m/z* (ES)$^+$ Found $[M+Na]^+$ 675.4434, $C_{40}H_{64}NaO_5Si^+$ requires $[M]^+$ 675.4415; **LRMS**: *m/z* (ES)$^+$ 675 ($[M+Na]^+$, 100%).

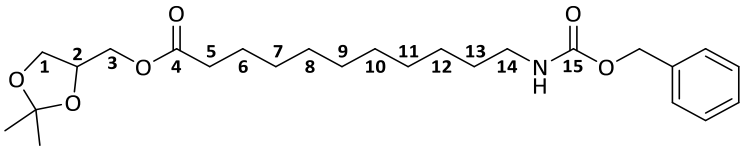
11-(*N*-Benzyloxycarbonyl)aminoundecanoic acid (188)



11-Aminoundecanoic acid **205** (1.00 g, 4.97 mmol, 1.00 eq.) was suspended in 2 M NaOH aq. (10 mL) and benzyl chloroformate (709 µL, 847 mg, 4.97 mmol, 1.00 eq.) was added dropwise. The resulting suspension was stirred at RT for 48 h under an atmosphere of nitrogen. After this time, the reaction mixture was washed with Et_2O (3 × 75 mL). The aqueous layer was retained and the pH adjusted to pH < 3 *via* the dropwise addition of conc. HCl. The resulting precipitate was isolated *via* vacuum filtration, washed with distilled H_2O (50 mL) and dried to

give the crude product. The crude material was recrystallised from EtOAc affording **188** (459 mg, 23%) as a colourless crystalline solid. **R_f**: 0.21 (3:7 EtOAc:Petrol); **mp** 90-93 °C (EtOAc) [Lit.[299] mp: 96 °C]; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 10.7 (1H, br s, COO*H*), 7.39-7.28 (5H, m, Ar C*H*), 5.09 (2H, s, PhCH_2), 4.74 (1H, br s, N*H*), 3.24-3.11 (2H, m, C(11)H_2), 2.34 (2H, t, *J* = 7.4 Hz, C(2)H_2), 1.68-1.57 (2H, m, C(3)H_2), 1.54-1.43 (2H, m, C(10)H_2), 1.39-1.18 (12H, m, C(4-9)H_2); **LRMS**: *m/z* $(ES)^-$ 334 ($[M-H]^-$, 100%). The data are in good agreement with literature values.[86]

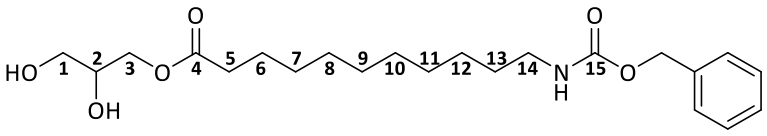
(±)-(2,2-Dimethyl-1,3-dioxolan-4-yl)methyl 11-(*N*-benzyloxycarbonyl)aminoundecanoate (192)



188 (1.00 g, 2.98 mmol, 1.00 eq.), DCC (708 mg, 3.43 mmol, 1.15 eq.) and DMAP (36 mg, 0.30 mmol, 0.10 eq.) were dissolved in anhydrous CH_2Cl_2 (20 mL). The resulting solution was stirred for 15 min under an atmosphere of nitrogen. (±)-1,2-*iso*propylideneglycerol **190** (557 µL, 591 mg, 4.47 mmol, 1.50 eq.) was added and the reaction was stirred at RT for a further 24 h. After this time, the precipitate was removed *via* vacuum filtration and the filtrate concentrated *in vacuo*. The residue was taken up in CH_2Cl_2 (50 mL). The organic layer was washed with distilled H_2O (3 × 30 mL) and brine (3 × 30 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo* to give the crude product. The crude material was adsorbed onto Celite® and purified *via* flash column chromatography on pre-washed (1% triethylamine in petrol) silica gel (Biotage®; gradient elution: 1% → 10% EtOAc in petrol) affording **192** (1.02 g, 76%) as a pale yellow waxy solid. **R_f**: 0.64 (3:7 EtOAc:Petrol); **mp**: 41-43 °C (EtOAc/petrol); **IR**

(thin film) ν_{max} cm^{-1}: 3340 (m), 2918 (m), 2857 (m), 1724 (m), 1685 (s); **^{1}H NMR** (400 MHz, $CDCl_3$): δ 7.39-7.28 (5H, m, Ar C*H*), 5.08 (2H, s, PhCH_2), 4.77 (1H, br s, N*H*), 4.34-4.27 (1H, m, C(2)*H*), 4.16 (1H, dd, *J* = 11.5, 4.7 Hz, C(3)H_AH_B), 4.11-4.04 (2H, m, C(1)H_AH_B & C(3)H_AH_B), 3.73 (dd, 1H, *J* = 8.4, 6.2 Hz, C(1)H_AH_B), 3.22-3.13 (2H, m, C(14)H_2), 2.33 (2H, t, *J* = 7.6 Hz, C(5)H_2), 1.67-1.55 (2H, m, C(6)H_2), 1.53-1.43 (2H, m, C(13)H_2), 1.43 (3H, s, CH_3), 1.36 (3H, s, CH_3), 1.34-1.21 (12H, m, C(7-12)H_2); **^{13}C NMR** (126 MHz, $CDCl_3$): 173.7 (*C*4), 156.5 (*C*15), 136.8 (Ar *C*), 128.6 (Ar *C*H), 128.23 (Ar *C*H), 128.19 (Ar *C*H), 109.9 (*C*$(CH_3)_2$), 73.8 (*C*2), 66.7 (Ph*C*H_2), 66.6 (*C*1), 64.6 (*C*4), 41.2 (*C*14), 34.2 (*C*5), 30.1 (*C*13), 29.5-29.0 (6 × *C*(7-12)), 26.8 (*C*H_3), 25.5 (*C*H_3), 25.0 (*C*6); **HRMS**: *m/z* $(ES)^+$ Found $[M+Na]^+$ 472.2659, $C_{25}H_{39}NaNO_6^+$ requires $[M]^+$ 472.2675; **LRMS**: *m/z* $(ES)^+$ 922 ($[2M+Na]^+$, 100%); **Anal. Calcd.** for $C_{25}H_{39}NO_6$: *C*, 66.79; *H*, 8.74; *N*, 3.12. Found: *C*, 66.63; *H*, 8.85; *N*, 3.14.

(±)-2,3-Dihydroxypropyl 11-(*N*-benzyloxycarbonyl)aminoundecanoate (186)

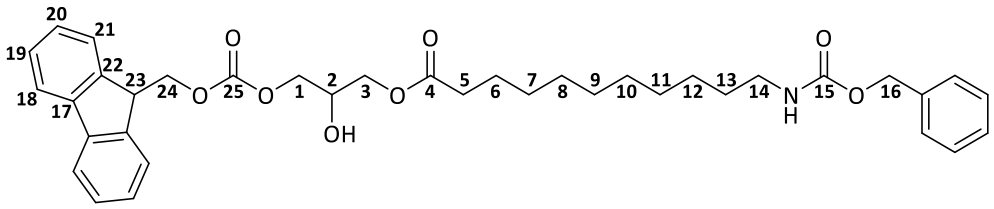


192 (2.10 g, 4.67 mmol, 1.00 eq.) was dissolved in 0.4 M HCl in dioxane (32 mL; 3.2 mL 4 M HCl in dioxane, 28.8 mL dioxane). Distilled H_2O (84 µL, 84 mg, 4.67 mmol, 1.00 eq.) was added and the resulting solution stirred for 6 h under an atmosphere of nitrogen. After this time, further distilled H_2O (50 µL, 50 mg, 2.78 mmol, 0.50 eq.) was added and the solution stirred for a further 12 h. The reaction solution was concentrated *in vacuo* (with the water bath at RT) and the resulting residue was taken up in EtOAc (75 mL). The organic layer was washed with distilled H_2O (3 × 50 mL) and brine (3 × 50 mL), dried ($MgSO_4$), filtered and concentrated

in vacuo (with the water bath at RT) to give the crude product. The crude material was adsorbed onto Celite® and purified *via* flash column chromatography on silica gel (Manual; 2:3 → 1:0 EtOAc:Petrol) affording **186** (729 mg, 38%) as a colourless solid. **R_f**: 0.22 (1:4 EtOAc:Petrol); **mp**: 78-81 °C (EtOAc); **IR** (thin film) ν_{max} cm^{-1}: 3330 (m), 2922 (m), 2850 (m), 1727 (m), 1689 (s); **^{1}H NMR** (500 MHz, $CDCl_3$): δ 7.38-7.38 (5H, m, Ar C*H*), 5.09 (2H, s, PhCH_2), 4.78 (1H, br s, N*H*), 4.19 (1H, dd, *J* = 11.7, 4.7 Hz, C(3)H_AH_B), 4.14 (1H, dd, *J* = 11.7, 6.1 Hz, C(3)H_AH_B), 3.96-3.89 (1H, m, C(2)*H*), 3.72-3.65 (1H, m, C(1)H_AH_B), 3.59 (1H, dd, *J* = 11.2, 5.9 Hz, C(1)H_AH_B), 3.21-3.12 (2H, m, C(14)H_2), 2.70 (1H, br s, O*H*), 2.34 (2H, t, *J* = 7.5 Hz, C(5)H_2), 2.30 (1H, br s, O*H*), 1.66-1.58 (2H, m, C(6)H_2), 1.53-1.43 (2H, m, C(13)H_2), 1.35-1.19 (12H, m, C(7-12)H_2); **^{13}C NMR** (126 MHz, $CDCl_3$): δ 174.4 (*C*4), 156.6 (*C*15), 136.7 (Ar *C*), 128.7 (Ar *C*H), 128.2 (Ar *C*H), 70.4 (*C*2), 66.8 (Ph*C*H_2), 65.3 (*C*3), 63.5 (*C*1), 41.2 (*C*14), 34.3 (*C*5), 30.0 (*C*13), 29.5 (1 × *C*(7-12)), 29.4 (1 × *C*(7-12)), 29.3 (1 × *C*(7-12)), 29.2 (1 × *C*(7-12)), 29.1 (1 × *C*(7-12)), 26.8 (1 × *C*(7-12)), 25.0 (*C*6); **HRMS**: *m/z* (ES)$^+$ Found $[M+Na]^+$ 432.2351, $C_{22}H_{35}NNaO_6^+$ requires $[M]^+$ 432.2357; **LRMS**: *m/z* (ES)$^+$ 432 ($[M+Na]^+$, 100%); **Anal. Calcd.** for $C_{22}H_{35}NO_6$: *C*, 64.52; *H*, 8.61; *N*, 3.42. Found: *C*, 64.61; *H*, 8.73; *N*, 3.47.

(±)-2-Hydroxy-3-([9*H*-fluoren-9-yl]methoxycarbonyl)oxypropyl

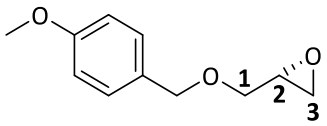
11-(*N*-benzyloxycarbonyl)aminoundecanoate (209)



186 (50 mg, 0.12 mmol, 1.00 eq.), DMAP (2 mg, 0.01 mmol, 0.10 eq.) and anhydrous pyridine (11 µL, 11 mg, 0.13 mmol, 1.10 eq.) were dissolved in anhydrous CH_2Cl_2 (1 mL). 9-Fluorenylmethoxycarbonyl chloride (35 mg, 0.13 mmol, 1.10 eq.) was added and the resulting solution was stirred at RT for 20 h. After this time, the reaction solution was concentrated *in vacuo* (with the water bath at RT) and the residue taken up in EtOAc (25 mL). The organic layer was washed with distilled H_2O (3 × 20 mL) and brine (3 × 20 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo* (with the water bath at RT) to give the crude product. The crude material was adsorbed onto Celite® and purified *via* flash column chromatography on silica gel (Manual; 1:9 → 2:3 EtOAc:Petrol) affording **209** (17 mg, 25%) as a colourless oil. **R_f**: 0.18 (2:3 EtOAc:Petrol); **IR** (thin film) ν_{max} cm^{-1}: 3383 (w), 2927 (m), 2854 (m), 1742 (s), 1527 (w), 1451 (m), 1258 (s); **^{1}H NMR** (500 MHz, $CDCl_3$): δ 7.77 (2H, d, *J* = 7.6 Hz, C(18)*H*), 7.61 (2H, d, *J* = 7.5 Hz, C(21)*H*), 7.42 (2H, dd, *J* = 7.6, 7.6 Hz, C(19)*H*), 7.38-7.28 (7H, m, C(20)*H* & 5 × Ar C*H*), 5.20-5.09 (2H, m, C(16)H_2), 4.68-4.78 (1H, m, N*H*), 4.44 (2H, d, *J* = 7.3 Hz, C(24)H_2), 4.30-4.10 (6H, m, C(23)*H*, C(1)H_AH_B, C(2)*H* & C(3)H_AH_B), 3.22-3.11 (2H, m, C(14)H_2), 2.60 (1H, d, *J* = 4.8 Hz, O*H*), 2.35 (2H, t, *J* = 7.5 Hz, C(5)H_2), 1.70-1.56 (2H, m, C(6)H_2), 1.53-1.41 (2H, m, C(13)H_2), 1.36-1.19 (12H, m, C(7-12)H_2); **^{13}C NMR** (126 MHz, $CDCl_3$): δ 174.0 (*C*4), 156.7 (*C*15), 155.3 (*C*25), 143.3 (*C*22), 141.4 (*C*17), 136.8 (Ar *C*), 128.6 (Ar *C*H), 128.3 (Ar *C*H), 128.2 (Ar *C*H), 128.1 (*C*19), 127.3 (*C*20), 125.2 (*C*21), 120.2 (*C*18), 70.3 (*C*24), 68.7 (*C*1),

68.2 (*C*2), 66.7 (*C*16), 64.9 (*C*3), 46.8 (*C*23), 41.2 (*C*14), 34.2 (*C*5), 30.1 (*C*13), 29.5 (1 × *C*(7-12)), 29.4 (1 × *C*(7-12)), 29.30 (1 × *C*(7-12)), 29.26 (1 × *C*(7-12)), 29.2 (1 × *C*(7-12)), 26.8 (1 × *C*(7-12)), 25.0 (*C*6); **HRMS**: *m/z* (ES)$^+$ Found [M+Na]$^+$ 654.3033, $C_{37}H_{45}NNaO_8^+$ requires [M]$^+$ 654.3037); **LRMS**: *m/z* (ES)$^+$ 654 ([M+Na]$^+$, 100%); **Anal. Calcd.** for $C_{37}H_{45}NO_8$: *C*, 70.34; *H*, 7.18; *N*, 2.22. Found: *C*, 70.40; *H*, 7.10; *N*, 2.38.

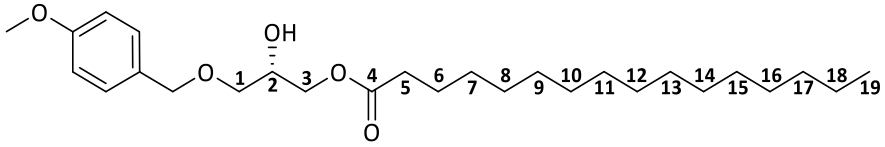
(*R*)-2-([{4-Methoxybenzyl}oxy]methyl)oxirane (199)



A suspension of NaH (1.93 g, 48.21 mmol, 1.60 eq.; 60% dispersion in oil) in anhydrous DMF (45 mL) was cooled to 0 °C. (*S*)-(−)-Glycidol **171** (2.00 mL, 2.23 g, 30.13 mmol, 1.00 eq.) was added dropwise and the resulting mixture was stirred for 30 min. At 0 °C, 4-methoxybenzyl chloride (6.54 mL, 7.55 g, 48.21 mmol, 1.60 eq.) was added dropwise. The resulting solution was allowed to warm to RT and stirred for 16 h. After this time, the solution was cooled to 0 °C, quenched *via* the dropwise addition of distilled H_2O and concentrated *in vacuo*. The residue was taken up in EtOAc (50 mL). The organic layer was washed with water (3 × 50 mL) and brine (3 × 50 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo* to give the crude product. The crude material was adsorbed onto Celite® and purified *via* flash column chromatography on silica gel (Manual; 2:3 EtOAc:Petrol) affording **199** (4.69 g, 80%) as an opaque oil. $\mathbf{R_f}$: 0.71 (2:3 EtOAc:Petrol); $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\mathbf{20}}$: +4.1 (*c* 1.2, $CHCl_3$) [Lit.[300] $[\alpha]_D^{22}$: +3.6 (*c* 1.24, $CHCl_3$)]; **^{1}H NMR** (400 MHz, $CDCl_3$): δ 7.28 (2H, d, *J* = 8.5 Hz, Ar C*H*), 6.89 (2H, d, *J* = 8.5 Hz, Ar C*H*), 4.55 (1H, d, *J* = 11.5 Hz, PhCH_AH$_B$), 4.49 (1H, d, *J* = 11.5 Hz, PhCH$_A$$H_B$), 3.81 (3H, s, OC$H_3$), 3.74 (1H, dd, *J* = 11.4, 3.0 Hz, C(1)H_AH$_B$), 3.42 (1H, dd, *J* = 11.4, 5.9 Hz, C(1)H$_A$$H_B$), 3.16-3.12 (1H, m, C(2)*H*), 2.80 (1H, dd, *J* = 4.6, 4.6 Hz, C(3)H_AH$_B$), 2.62 (1H, dd, *J* = 5.0, 2.7 Hz, C(3)H$_A$$H_B$);

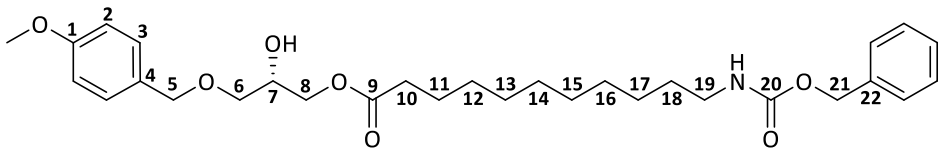
LRMS: *m/z* (ES)$^+$ 217 ([M+Na]$^+$, 100%). The data are in good agreement with literature values.[300]

(*S*)-2-Hydroxy-3-(4-methoxybenzyl)oxypropyl palmitate (197)



199 (210 mg, 1.08 mmol, 1.00 eq.), TBABr (384 mg, 1.19 mmol, 1.10 eq.) and palmitic acid **187** (305 mg, 1.19 mmol, 1.10 eq.) were dissolved in anhydrous MeCN (10 mL). The resulting solution was heated under reflux for 16 h. Additional palmitic acid (55 mg, 0.22 mmol, 0.20 eq.) was added and the solution heated to reflux for a further 8 h. After this time, the reaction solution was concentrated *in vacuo* to give the crude product. The crude material was adsorbed onto Celite® and purified *via* flash column chromatography on silica gel (Manual; 1:19 → 1:4 EtOAc:Petrol) affording a 5:1 regioisomeric mixture of **197** (~398 mg, 82% by NMR):**218** (~82 mg, 17% by NMR) as a colourless solid. An analytical sample of **197** was obtained by repeating column chromatography and increasing the gradient more slowly. $\mathbf{R_f}$: 0.10 (1:9 EtOAc:Petrol); $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\boldsymbol{20}}$: +1.4 (*c* 1.0, $CHCl_3$); **mp**: 38-39 °C (EtOAc/petrol) [Lit.[268] mp: 40-41 °C]; **^{1}H NMR** (400 MHz, $CDCl_3$): δ 7.26 (2H, d, *J* = 8.5 Hz, Ar C*H*), 6.89 (2H, d, *J* = 8.5 Hz, Ar C*H*), 4.49 (2H, s, PhCH_2), 4.18 (1H, dd, *J* = 11.5, 4.4 Hz, C(3)H_AH_B), 4.13 (1H, dd, *J* = 11.5, 6.1 Hz, C(3)H_AH_B), 4.06-3.97 (1H, m, C(2)*H*), 3.82 (3H, s, OCH_3), 3.53 (1H, dd, *J* = 9.6, 4.3 Hz, C(1)H_AH_B), 3.46 (1H, dd, *J* = 9.6, 6.3 Hz, C(1)H_AH_B), 2.49 (1H, br s, O*H*), 2.33 (2H, t, *J* = 7.6 Hz, C(5)H_2), 1.67-1.53 (26H, m, C(6-18)H_2), 0.89 (3H, t, *J* = 6.8 Hz, C(19)H_3); **LRMS**: *m/z* (ES)$^+$ 924 ([2M+Na]$^+$, 100%). The data are in good agreement with literature values.[268]

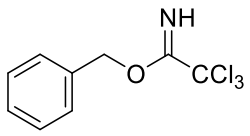
(*S*)-2-Hydroxy-3-(4-methoxybenzyl)oxypropyl 11-(*N*-benzyloxycarbonyl)aminoundecanoate (198)



199 (200 mg, 1.03 mmol, 1.00 eq.), TBABr (365 mg, 1.13 mmol, 1.10 eq.) and **188** (518 mg, 1.55 mmol, 1.50 eq.) were dissolved in anhydrous MeCN (10 mL). The resulting solution was heated to 60 °C for 18 h. After this time, the reaction solution was concentrated *in vacuo* to give the crude product. The crude material was adsorbed onto Celite® and purified *via* flash column chromatography on silica gel (Manual; 1:9 → 3:2 EtOAc:Petrol) affording a 5:1 regioisomeric mixture of **198** (~472 mg, 88% by NMR):**219** (~59 mg, 11% by NMR) as a pale yellow odorous solid. The total combined yield was 99%. $\mathbf{R_f}$: 0.16 (3:7 EtOAc:Petrol); $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\boldsymbol{20}}$: +1.7 (*c* 1.0 in $CHCl_3$); **mp**: 40-42 °C ($CHCl_3$/Hexane); **IR** (thin film) ν_{max} cm^{-1}: 3430 (br, m), 3320 (br, m), 2919 (m), 2852 (m), 1705 (s), 1684 (s); **^{1}H NMR** (400 MHz, $CDCl_3$): δ 7.32-7.19 (5H, m, Ar C*H*), 7.16 (2H, d, *J* = 8.5 Hz, C(2)*H*), 6.79 (2H, d, *J* = 8.5 Hz, C(3)*H*), 5.00 (2H, s, C(21)H_2), 4.68 (1H, br s, N*H*), 4.39 (2H, s, C(5)H_2), 4.09 (1H, dd, *J* = 11.5, 4.3 Hz, C(8)H_AH_B), 4.03 (1H, dd, *J* = 11.5, 6.1 Hz, C(8)H_AH_B), 3.97-3.88 (1H, m, C(7)*H*), 3.71 (3H, s, OCH_3), 3.43 (1H, dd, *J* = 9.5, 4.2 Hz, C(6)H_AH_B), 3.37 (1H, dd, *J* = 9.5, 6.2 Hz, C(6)H_AH_B), 3.14-3.01 (2H, m, C(19)H_2), 2.30-2.08 (2H, t, *J* = 8.6 Hz, C(10)H_2 & 1H, br s, O*H*), 1.59-1.45 (2H, m, C(11)H_2), 1.45-1.32 (2H, m, C(18)H_2), 1.26-1.11 (12H, m, C(12-17)H_2); **^{13}C NMR** (101 MHz, $CDCl_3$): δ 174.0 (*C*9), 159.4 (*C*1), 156.5 (*C*20), 136.7 (*C*22), 129.8 (*C*4), 129.5 (*C*2), 128.6 (Ar *C*H), 128.2 (Ar *C*H), 128.1 (Ar *C*H), 113.9 (*C*3), 73.2 (*C*21), 70.7 (*C*6), 68.9 (*C*5), 66.6 (*C*7), 65.5 (*C*8), 55.3 (O*C*H_3), 41.2 (*C*19), 34.2

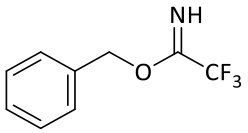
(*C*10), 30.0 (*C*18), 29.4 (1 × *C*(12-17)), 29.3 (1 × *C*(12-17)), 29.2 (1 × *C*(12-17)), 29.2 (1 × *C*(12-17)), 29.1 (1 × *C*(12-17)), 26.7 (1 × *C*(12-17)), 24.9 (*C*11); **HRMS**: *m/z* (ES)$^+$ Found $[M+Na]^+$ 552.2934, $C_{38}H_{60}O_{18}Na^+$ requires $[M]^+$ 552.2932; **LRMS**: *m/z* (ES)$^+$ 530 ($[M+H]^+$, 100%); **Anal. Calcd.** for $C_{38}H_{60}O_{18}$: *C*, 68.03; *H*, 8.18; *N*, 2.64. Found: *C*, 67.93; *H*, 8.16; *N*, 2.73.

Benzyl-2,2,2-trichloroacetimidate (223)



Benzyl alcohol **222** (480 µL, 502 mg, 4.64 mmol, 1.00 eq.) and DBU (277 µL, 282 mg, 1.85 mmol, 0.40 eq.) were dissolved in anhydrous CH_2Cl_2 (25 mL) and cooled to 0 °C. Trichloroacetonitrile (4.64 mL, 6.68 g, 46.24 mmol, 10.00 eq.) was added dropwise and the resulting solution was allowed to warm to RT and stirred for 3 h. After this time, the solution was concentrated *in vacuo* (with the water bath at RT) to give the crude product. The crude material was purified *via* flash column chromatography on silica gel (Manual; 1:19 → 1:9 EtOAc:Petrol) affording **223** (1.15 g, 100%) as a pale yellow oil. $\mathbf{R_f}$: 0.69 (1:9 EtOAc:Petrol); ^{1}**H NMR** (400 MHz, $CDCl_3$): δ 8.40 (1H, br s, N*H*), 7.48-7.31 (5H, m, Ar C*H*), 5.35 (2H, s, PhCH_2); **LRMS**: *m/z* (ES)$^+$ 239 (100%), 251 ($[M+H]^+$, 82%). The data are in good agreement with literature values.[301]

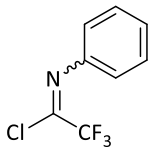
Benzyl-2,2,2-trifluoroacetimidate (225)



Trifluoroacetamide **224** (373 mg, 3.30 mmol, 3.30 eq.) and anhydrous DMSO (710 µL, 781 mg, 10.00 mmol, 10.00 eq.) were dissolved in anhydrous CH_2Cl_2 (10 mL) and the resulting solution was cooled to −78 °C. Freshly distilled anhydrous oxalyl chloride (254 µL, 381 mg, 3.00 mmol, 3.00 eq.) was slowly added dropwise, ensuring the reaction solution remained at −78 °C. The resulting solution was stirred at −78 °C for 10 min. After this time, anhydrous triethylamine (1.39 mL, 1.01 g, 10.00 mmol, 10.00 eq.) was slowly added dropwise and the reaction solution stirred at −78 °C for a further 30 min. After this time, a solution of freshly distilled anhydrous benzyl alcohol (100 µL, 105 mg, 0.97 mmol, 1.00 eq.) and DBU (300 µL, 305 mg, 2.01 mmol, 2.0 eq.) in anhydrous CH_2Cl_2 (5 mL) was added dropwise and the reaction solution stirred at −78 °C for 15 min. Following this, the reaction solution was allowed to warm to RT and stirred for 16 h. After this time, the reaction was then quenched *via* the dropwise addition of distilled H_2O (1 mL) and diluted with CH_2Cl_2 (50 mL). The organic layer was washed with water (2 × 50 mL) and brine (2 × 50 mL), dried (Na_2SO_4), filtered and concentrated *in vacuo* (with pressure no lower than 200 mbar) to give the crude product. The crude material was first purified *via* flash column chromatography on pre-washed (1% triethylamine in petrol) silica gel (Manual; 1:9 EtOAc:Petrol) and then by Kugelrohr distillation (bp: 50 °C/6 mbar) to give **225** (130 mg, 66%) as a colourless oil. **R_f**: 0.67 (1:10 EtOAc:Petrol); **bp**: 50 °C/4.5 mm Hg [Lit.[272] bp: 20 °C/1 mbar]; **^{1}H NMR** (400 MHz; $CDCl_3$): δ 8.29 (1H, br s, N*H*), 7.44-7.33 (5H, m, Ar C*H*), 5.32 (2H, s, PhCH_2); **^{13}C NMR** (76 MHz, $CDCl_3$): δ 157.9 (q, *J* = 37.9 Hz, *C*=NH), 134.9 (Ar *C*), 128.6 (Ar *C*H), 128.5 (Ar *C*H), 128.1 (Ar *C*H), 115.4 (q, *J* = 288.4 Hz, *C*F_3), 69.3 (Ph*C*H_2); **^{19}F NMR**

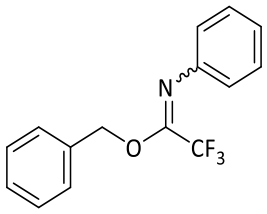
(470 MHz; $CDCl_3$): δ −74.4; **HRMS**: *m/z* (EI/FI)$^+$ Found $[M]^+$ 203.0559, $C_9H_8F_3NO^+$ requires $[M]^+$ 203.0558. The data are in good agreement with literature values.[272]

2,2,2-Trifluoro-*N*-phenylacetimidoyl chloride (227)



Triphenylphosphine (20.55 g, 78.35 mmol, 3.00 eq.) and anhydrous triethylamine (4.37 mL, 3.17 g, 31.34 mmol, 1.20 eq.) were dissolved in CCl_4 (25 mL) and the resulting solution was cooled to 0 °C. Trifluoroacetic acid **226** (2.00 mL, 2.98 g, 26.12 mmol, 1.00 eq.) was added slowly *via* syringe and the resulting mixture was stirred at 0 °C for 10 min. Following this, a solution of aniline (2.87 mL, 2.93 g, 31.34 mmol, 1.20 eq.) in CCl_4 (12.6 mL; pre-dried over 3 Å molecular sieves) was added slowly *via* syringe and the resulting foamy mixture was heated under reflux for 3 h. After this time, the reaction mixture was concentrated *in vacuo* and the resulting residue purified *via* fractional distillation (bp: 52 °C/11 mbar) affording **227** (2.49 g, 34%) as a colourless oil. **R_f**: 1.00 (100% petrol); **bp**: 52 °C/11 mbar [Lit.[302] bp: 55-56 °C/13 mbar]; **^{1}H NMR** (400 MHz, $CDCl_3$): δ 7.37-7.26 (2H, m, Ar C*H*), 7.23-7.13 (1H, m, Ar C*H*), 7.02-6.93 (2H, m, Ar C*H*); **^{13}C NMR** (76 MHz, $CDCl_3$): δ 143.5 (Ar *C*), 132.0 (q, *J* = 43.1 Hz, *C*=NPh), 129.2 (Ar *C*H), 127.4 (Ar *C*H), 120.7 (Ar *C*H), 116.7 (q, *J* = 277.4 Hz, *C*F_3); **^{19}F NMR** (470 MHz, $CDCl_3$) δ −71.6; **HRMS**: *m/z* (EI/FI)$^+$ Found $[M]^+$ 207.0067, $C_8H_5ClF_3N^+$ requires $[M]^+$ 207.0063. The data are in good agreement with literature values.[302]

Benzyl-2,2,2-trifluoro-*N*-phenylacetimidate (228)



NaH (253 mg, 7.25 mmol, 1.50 eq. 60% in oil) was suspended in anhydrous CH_2Cl_2 (24 mL) and cooled to 0 °C. Benzyl alcohol **222** (454 µL, 475 mg, 4.39 mmol, 1.00 eq.) was slowly added dropwise and the resulting mixture stirred at 0 °C for 10 min and then at RT for 20 min. The reaction mixture was then cooled to 0 °C and **227** (1.00 g, 4.83 mmol, 1.10 eq.) was added dropwise. The resulting mixture was allowed to warm to RT and stirred for 2 h. After this time, the reaction was quenched *via* the dropwise addition of distilled H_2O (1 mL) and extracted with CH_2Cl_2 (25 mL). The organic layer was washed with brine (2 × 50 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo* to give the crude product. The crude material was purified *via* flash column chromatography on pre-washed (1% triethylamine in petrol) silica gel (Manual; 1:49 Et_2O:Petrol) affording **228** (1.10 mg, 81%) as a pale yellow oil that solidified during storage at −18 °C. **R_f**: 0.66 (1:49 Et_2O:Petrol); **mp**: 18-20 °C [Lit.[273] mp: 19-20 °C]; **^{1}H NMR** (300 MHz, $CDCl_3$): δ 7.37-7.13 (7H, m, Ar C*H*), 7.03-6.94 (1H, m, Ar C*H*), 6.75-6.66 (2H, m, Ar C*H*), 5.20 (2H, s, PhCH_2); **^{13}C NMR** (76 MHz, $CDCl_3$): δ 145.1 (q, *J* = 35.0 Hz, *C*=NPh), 144.3 (Ar *C*), 135.1 (Ar *C*), 128.8 (Ar *C*H), 128.7 (Ar *C*H), 128.5 (Ar *C*H), 128.4 (Ar *C*H), 128.3 (Ar *C*H), 124.0 (Ar *C*H), 119.6 (Ar *C*H), 119.6 (Ar *C*H), 116.2 (q, *J* = 286.8 Hz, *C*F_3), 69.7 (Ph*C*H_2); **^{19}F NMR** (470 MHz, $CDCl_3$): δ −65.3; **HRMS**: *m/z* (EI/FI)$^+$ Found $[M]^+$ 279.0871, $C_9H_8F_3NO^+$ requires $[M]^+$ 279.0866. The data are in good agreement with literature values.[273]

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Appendices

Appendix I – HPLC Data

I.I HPLC methods

Protocol A

Column: RP, Dionex Acclaim 120 column (C18, 5 µm, 120 Å, 4.6 × 150 mm)

	A	B	Time
Equil:	50%	50%	0.1 min
Run:	50%	50%	1.0 min
	0%	100%	5.0 min
	0%	100%	8.0 min
	50%	50%	1.0 min
	50%	50%	5.0 min
Total time (-Equil.)			20 min

Solvent A: 95% H_2O, 5% MeCN, 0.1% TFA

Solvent B: 95% MeCN, 5% H_2O, 0.1% TFA

Protocol B

Column: NP, Supelcosil LC-Si (5 µm, 120 Å, 4.6 × 250 mm)

	A	B	Time
Equil:	100%	0%	0.1 min
Run:	100%	0%	3.0 min
	50%	50%	3.0 min
	50%	50%	8.0 min
	100%	0%	1.0 min
	100%	0%	5.0 min
Total time (-Equil.)			20 min

Solvent A: Hexane

Solvent B: *iso*-Propanol

Protocol C

Column: RP, Dionex Acclaim 120 column (C18, 5 µm, 120 Å, 4.6 × 150 mm)

	A	B	Time
Equil:	90%	10%	0.1 min
Run:	90%	10%	1.0 min
	0%	100%	8.0 min
	0%	100%	5.0 min
	90%	10%	1.0 min
	90%	10%	5.0 min
Total time (-Equil.)			20 min

Solvent A: 95% H_2O, 5% MeCN, 0.1% TFA

Solvent B: 95% MeCN, 5% H_2O, 0.1% TFA

Protocol D

Column: RP, Dionex Acclaim 120 column (C18, 5 µm, 120 Å, 4.6 × 150 mm)

	A	B	Time
Equil:	90%	10%	0.1 min
Run:	90%	10%	3.0 min
	5%	95%	3.0 min
	5%	95%	9.0 min
	90%	10%	1.0 min
	90%	10%	4.0 min
Total time (-Equil.)			20 min

Solvent A: 97% H_2O, 3% MeCN, 0.25 mM TEAA

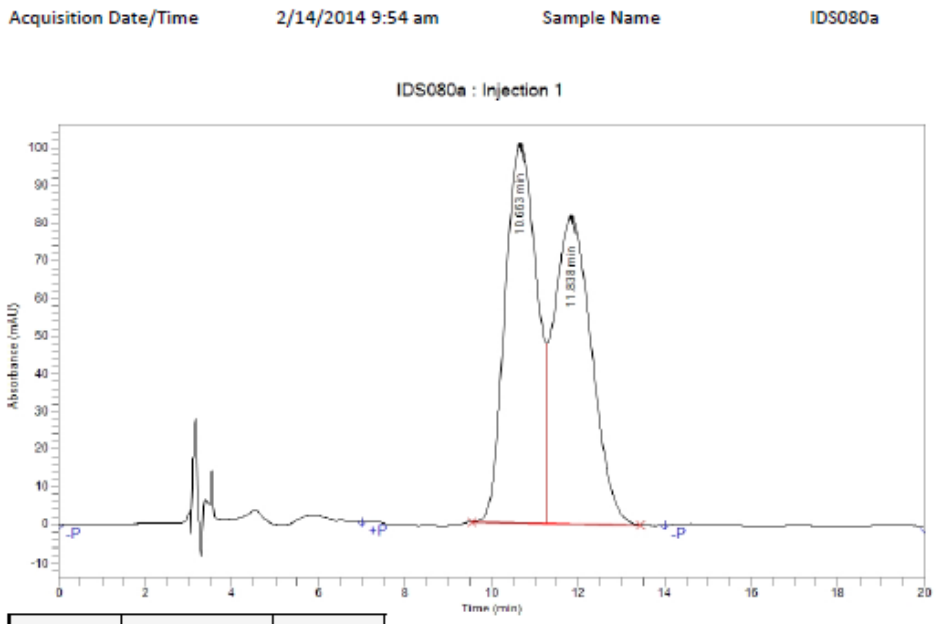
Solvent B: 97% MeCN, 3% H_2O, 0.25 mM TEA

I.II Determination of enantiopurity by Chiral HPLC

In **Section 3.3.2** the pursuit of a *t*-butylation that proceeds with minimal racemisation of the α-carbon of compound **133** was discussed. The chiral HPLC traces, achieved using a 20 min isocratic gradient of 5% *iso*-propanol in hexane on a Chiralpak® AD-H column, are presented herein. Samples were injected at a concentration of ~3 mg/mL in *iso*-propanol.

For clarity only peaks occurring within the retention time range of 9-14 min (the range where the enantiomers of interest elute) are considered in the Area % analysis.

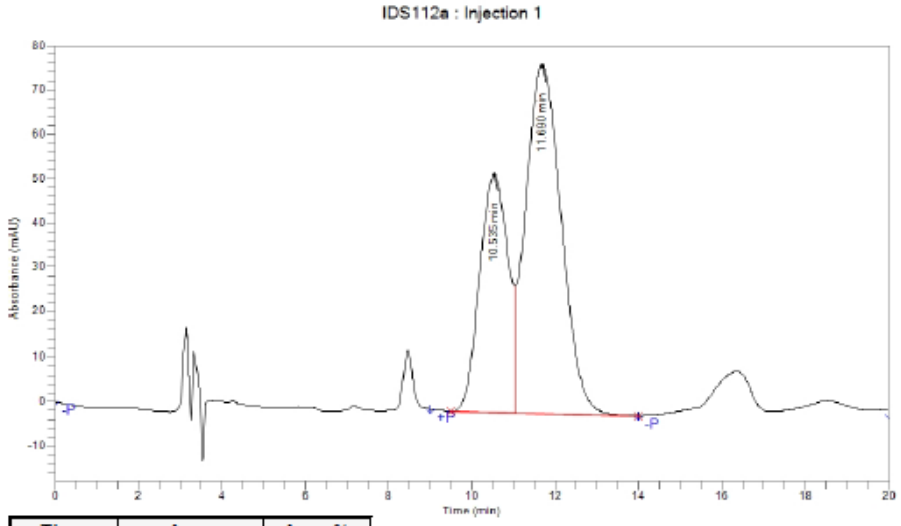
(*RS*)- 5-benzyl 1-(*t*-butyl) (*t*-butoxycarbonyl)glutamate (142)



Time	Area	Area %
10.663	5,159,248	51.19
11.838	4,919,677	48.81
Total	10,078,925	100.00

1-(*tert*-Butyl) 5-benzyl Boc-L-glutamate (138) [Synthesised *via* Method A – EDC, DMAP]

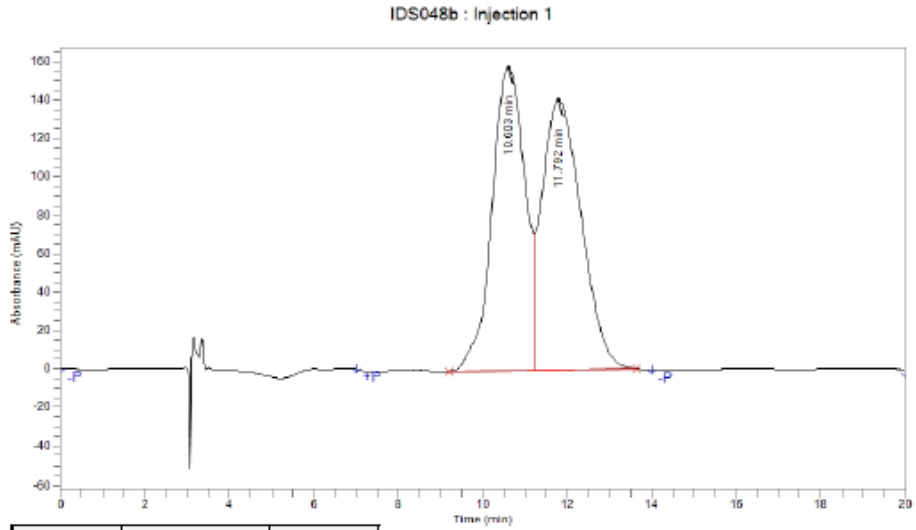
Acquisition Date/Time 2/14/2014 5:04 pm Sample Name IDS112a



Time	Area	Area %
10.535	2,496,463	34.99
11.690	4,637,330	65.01
Total	7,133,793	100.00

1-(*tert*-Butyl) 5-benzyl Boc-L-glutamate (138) [Synthesised *via* Method B – DCC, DMAP]

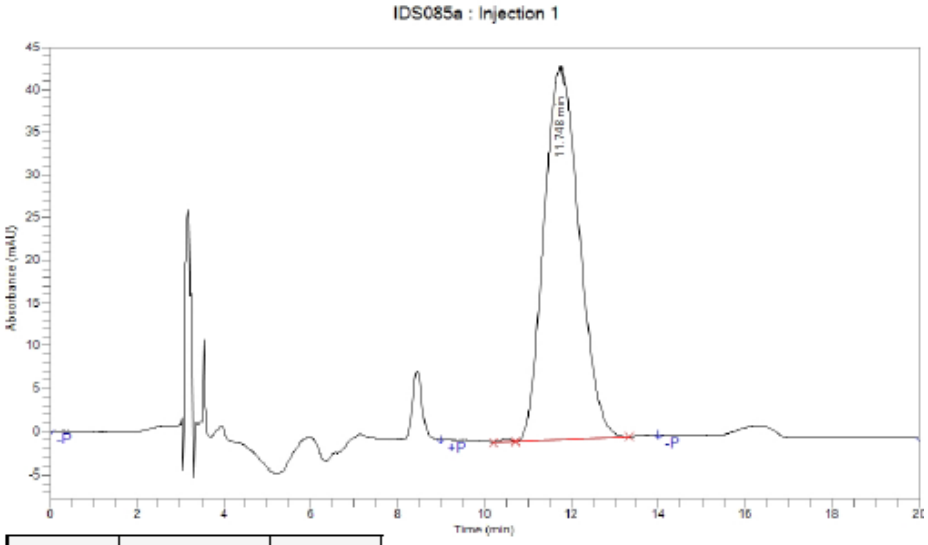
Acquisition Date/Time 2/14/2014 11:21 am Sample Name IDS048b



Time	Area	Area %
10.603	8,508,885	48.34
11.792	9,094,942	51.66
Total	17,603,827	100.00

1-(*tert*-Butyl) 5-benzyl Boc-L-glutamate (138) [Synthesised *via* Method C – Isobutene, H_2SO_4]

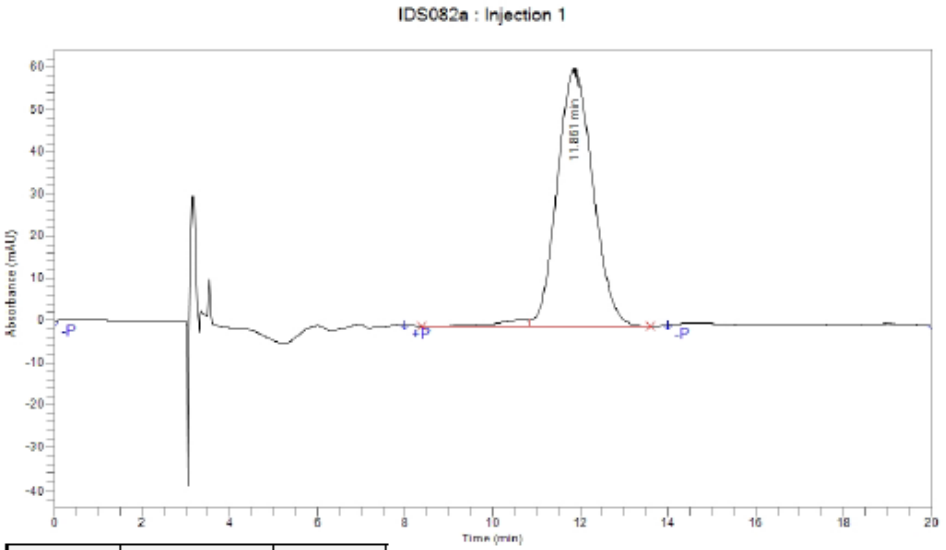
Acquisition Date/Time	2/14/2014 1:07 pm	Sample Name	IDS085a



Time	Area	Area %
10.504	5,894	0.24
11.748	2,454,422	99.76
Total	2,460,316	100.00

1-(*tert*-Butyl) 5-benzyl Boc-L-glutamate (138) [Synthesised *via* Method D – Boc_2O, DMAP]

Acquisition Date/Time	2/14/2014 11:41 am	Sample Name	IDS082a

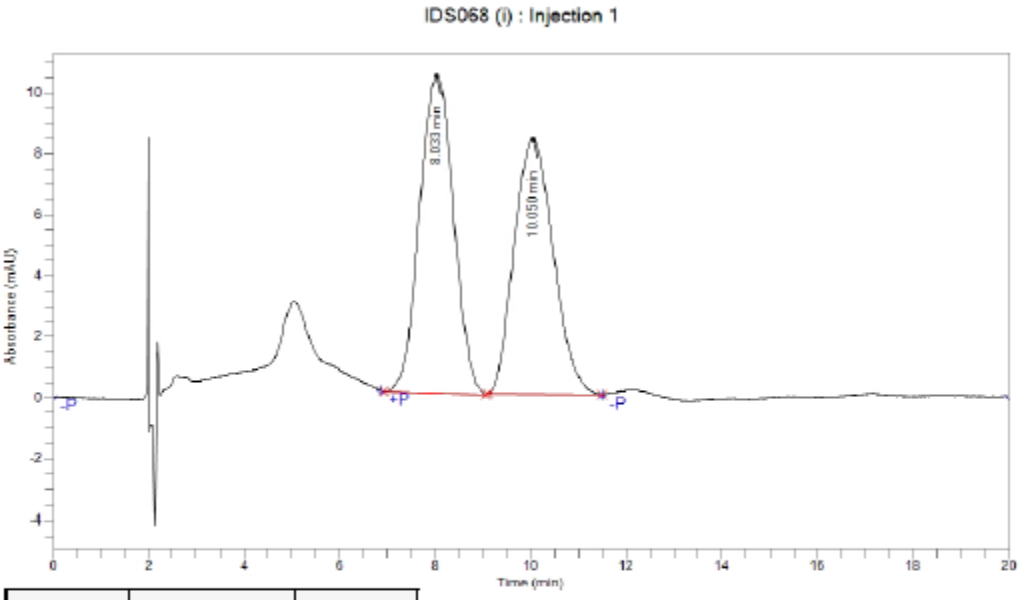


Time	Area	Area %
10.648	72,351	2.06
11.861	3,437,208	97.94
Total	3,509,559	100.00

Compound **138** (Method D) was subjected to hydrogenolysis conditions to afford the necessary glutamic acid fragment **131** for the SPPS of tripeptides **127**, **128** and **129**. Chiral HPLC confirmed that no additional racemisation occurred during the hydrogenolysis. The racemic analogue is depicted first.

Boc-(DL)-glutamic acid 1-tert-butyl ester (148)

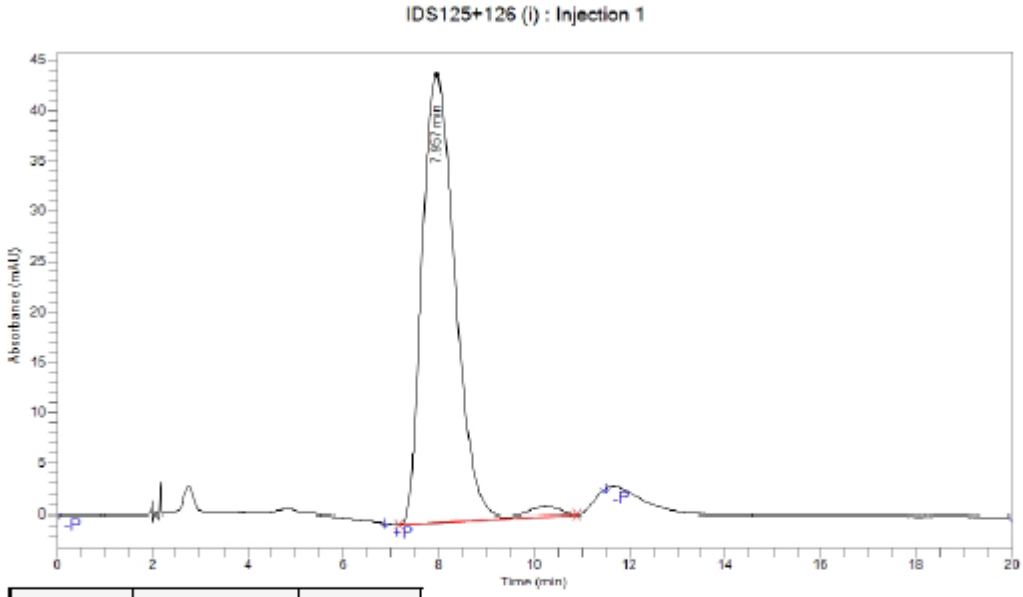
Acquisition Date/Time	2/9/2014 8:32 pm	Sample Name	IDS068 (i)



Time	Area	Area %
8.033	516,164	52.03
10.050	475,808	47.97
Total	991,972	100.00

Boc-L-glutamic acid 1-tert-butyl ester (131) [Synthesised from **138**, Method D]

Acquisition Date/Time	2/9/2014 9:18 pm	Sample Name	IDS125+126 (i)

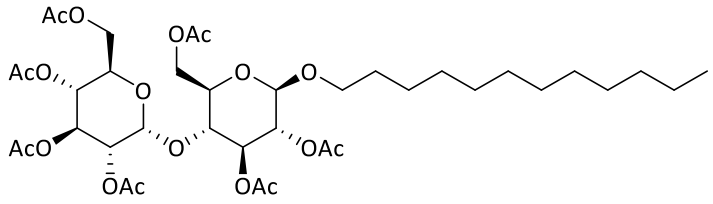


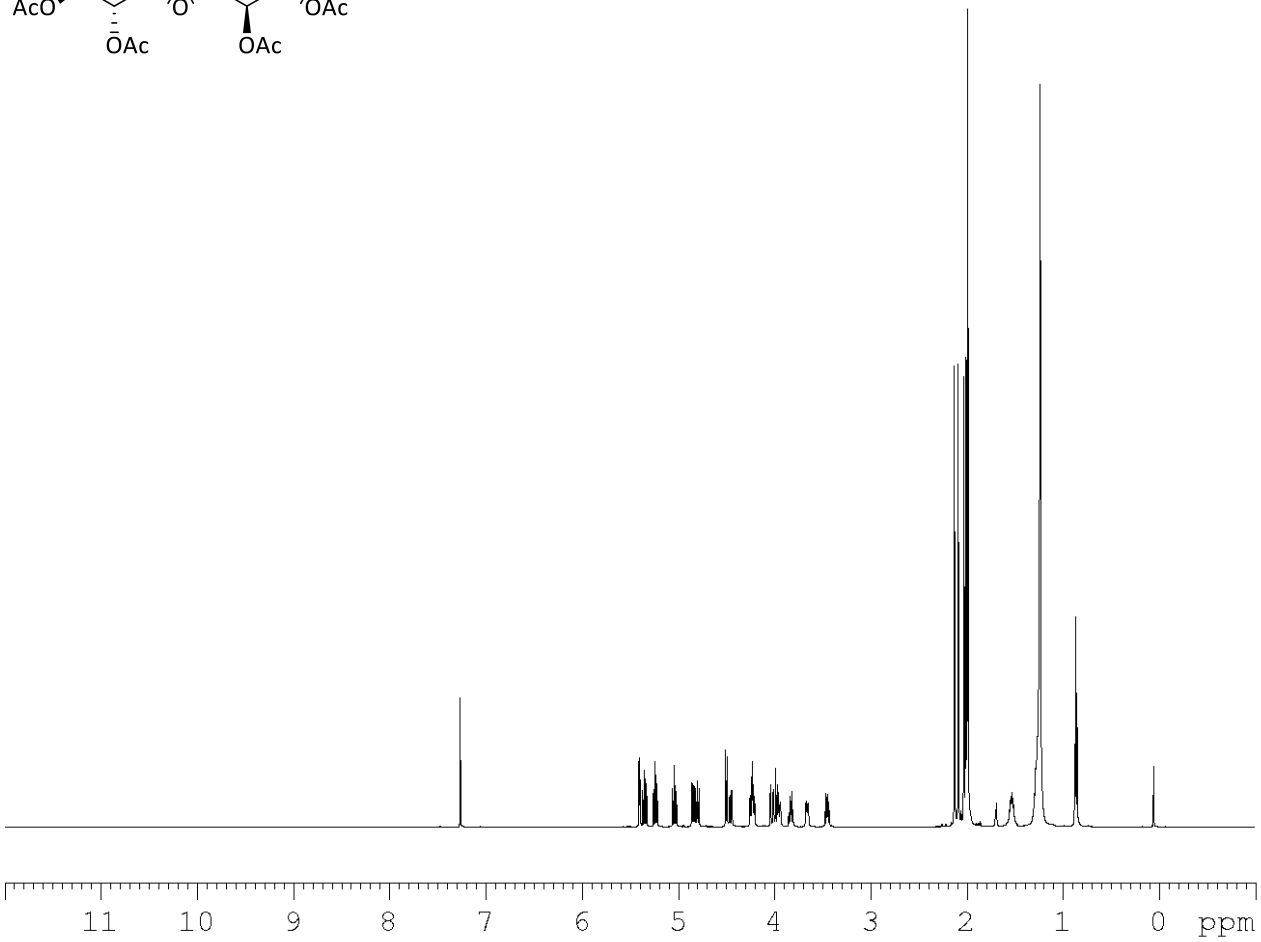
Time	Area	Area %
7.957	2,110,218	97.99
10.229	43,212	2.01
Total	2,153,430	100.00

Appendix II – NMR Data

NMR data for the synthesis of fluorescent detergents

n-Dodecyl-β-D-maltose octaacetate **68**






```
Instrument AVC500
Group SJC
8192 Diane Swallow 22/2/12


Current Data Parameters
NAME      IDS159 - ds81922202
EXPNO                    1
PROCNO                   1

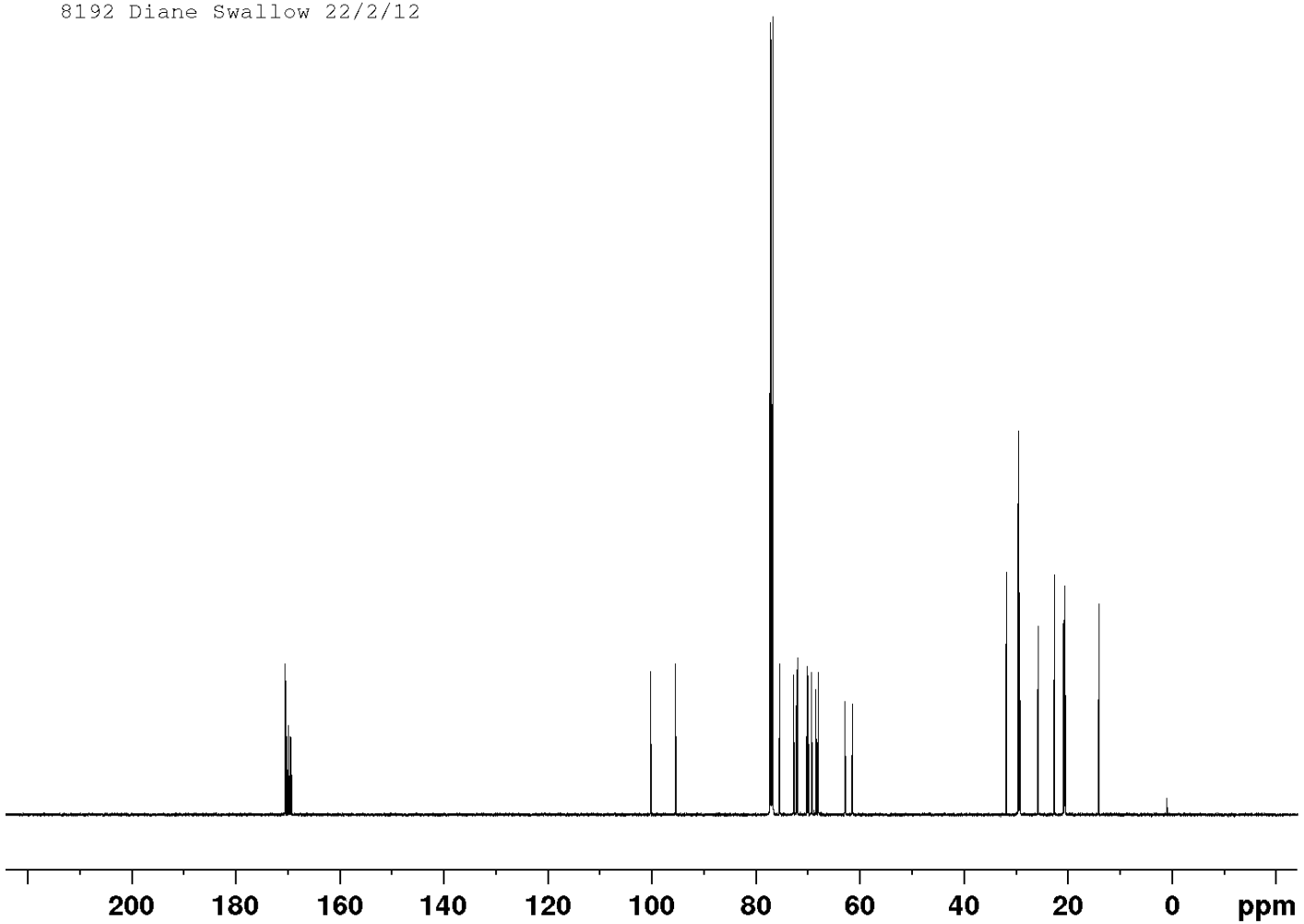
F2 - Acquisition Parameters
Date_           20120224
Time                5.38
INSTRUM           avc500
PROBHD   5 mm CPDUL 13C
PULPROG             zg30
TD                 65536
SOLVENT            CDCl3
NS                    16
DS                     2
SWH            10330.578 Hz
FIDRES          0.157632 Hz
AQ             3.1719425 sec
RG                     4
DW                48.400 usec
DE                 6.00 usec
TE                298.0 K
D1           1.00000000 sec
TD0                    1

======== CHANNEL f1 ========
NUC1                  1H
P1                 9.60 usec
PL1               -6.00 dB
PL1W        15.19999981 W
SFO1        500.3030896 MHz

F2 - Processing parameters
SI                 32768
SF          500.3000279 MHz
WDW                   EM
SSB       0
LB                 0.30 Hz
GB        0
PC                 1.00
```

n-Dodecyl-β-D-maltose octaacetate **68**

Instrument AVC500
Group SJC
8192 Diane Swallow 22/2/12




```
Current Data Parameters
NAME      IDS159 - ds81922202
EXPNO                       4
PROCNO                      1

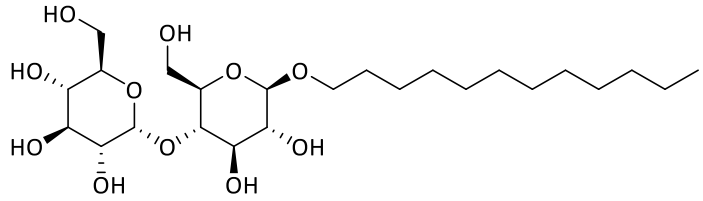
F2 - Acquisition Parameters
Date_              20120224
Time                   6.14
INSTRUM              avc500
PROBHD    5 mm CPDUL 13C
PULPROG              zgpg30
TD                    65536
SOLVENT               CDCl3
NS                      256
DS                        2
SWH              31250.000 Hz
FIDRES            0.476837 Hz
AQ                1.0485760 sec
RG                      812
DW                 16.000 usec
DE                 20.00 usec
TE                   298.0 K
D1              2.00000000 sec
D11             0.03000000 sec
TD0                       1

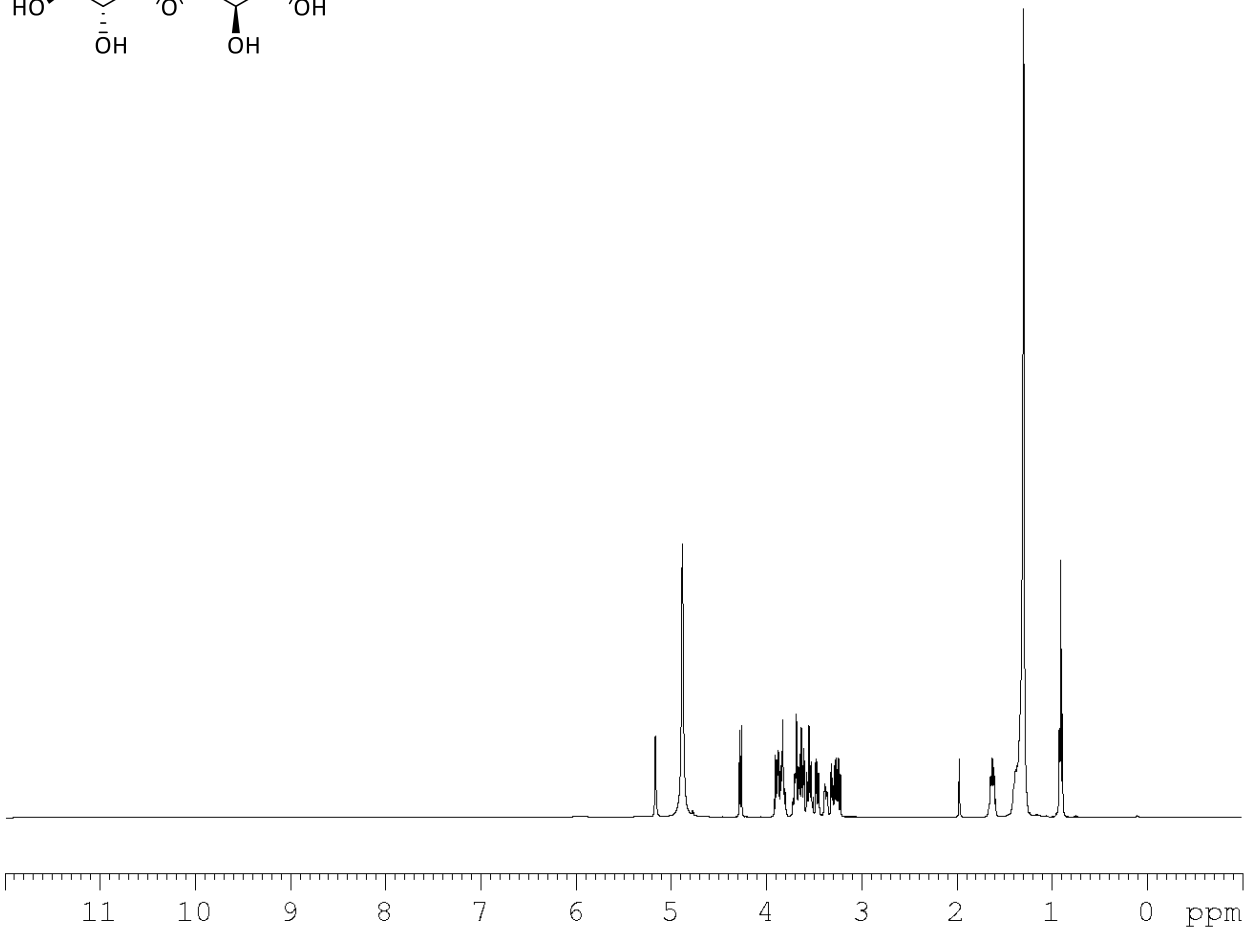
======== CHANNEL f1 ========
NUC1                    13C
P1                  10.00 usec
PL1                 -4.40 dB
PL1W          28.15752029 W
SFO1          125.8131151 MHz

======== CHANNEL f2 ========
CPDPRG[2           waltz16
NUC2                    1H
PCPD2               80.00 usec
PL2                 -6.00 dB
PL12                12.42 dB
PL13                18.42 dB
PL2W          15.19999981 W
PL12W          0.21869738 W
PL13W          0.05493430 W
SFO2          500.3020012 MHz

F2 - Processing parameters
SI                      32768
SF            125.8005245 MHz
WDW                        EM
SSB       0
LB                   1.00 Hz
GB        0
PC                      1.40
```

n-Dodecyl-β-D-maltoside **1**






```
Instrument DPX400
Chemist ds
Group sjc
IDS163
Qh1acq.au MeOD {C:\NMR} sjcgrp 3

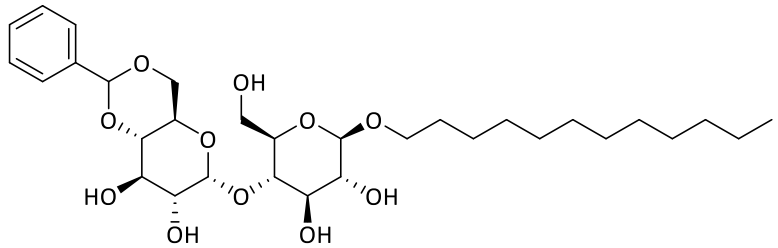
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EXPNO                         3
PROCNO                        1

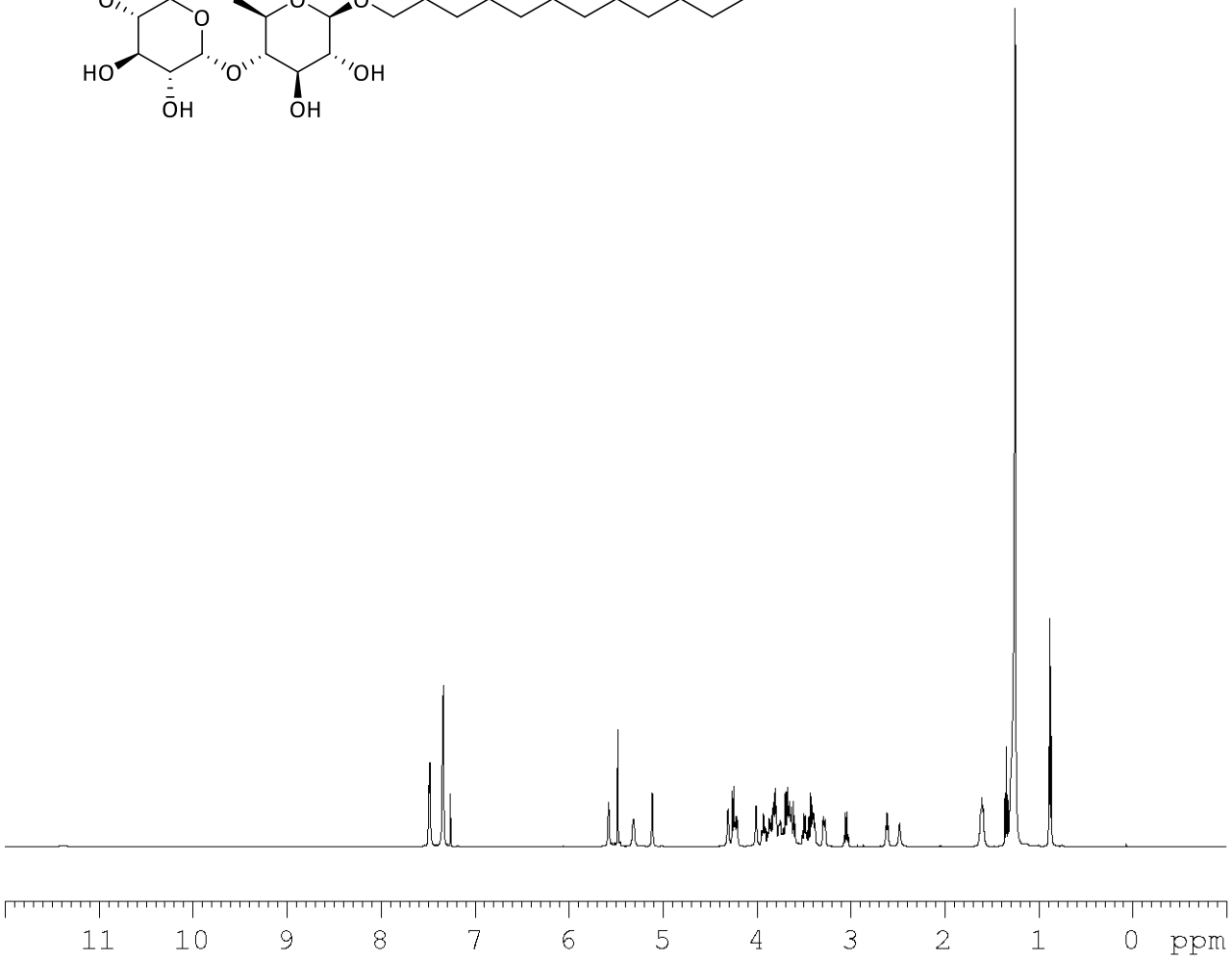
F2 - Acquisition Parameters
Date_               20120308
Time                    0.55
INSTRUM                dpx400
PROBHD    5 mm Dual 1H/1
PULPROG                 zg60
TD                     32768
SOLVENT                 MeOD
NS                        16
DS                         2
SWH                 5592.841 Hz
FIDRES               0.170680 Hz
AQ                  2.9294591 sec
RG                       45.3
DW                     89.400 usec
DE                     17.00 usec
TE                     300.0 K
D1                1.00000000 sec

======== CHANNEL f1 ========
NUC1                       1H
P1                      7.30 usec
PL1       0 dB
SFO1           400.1320007 MHz

F2 - Processing parameters
SI                         32768
SF              400.1300074 MHz
WDW                           EM
SSB       0
LB                      0.30 Hz
GB        0
PC                      0.60
```

n-Dodecyl-(4',6'-*O*-benzylidene)-β-D-maltoside **73**






```
DRX500 BBO Probe IDS360
h1acqbbo CDCl3 {C:\NMR} sjcogroup 11

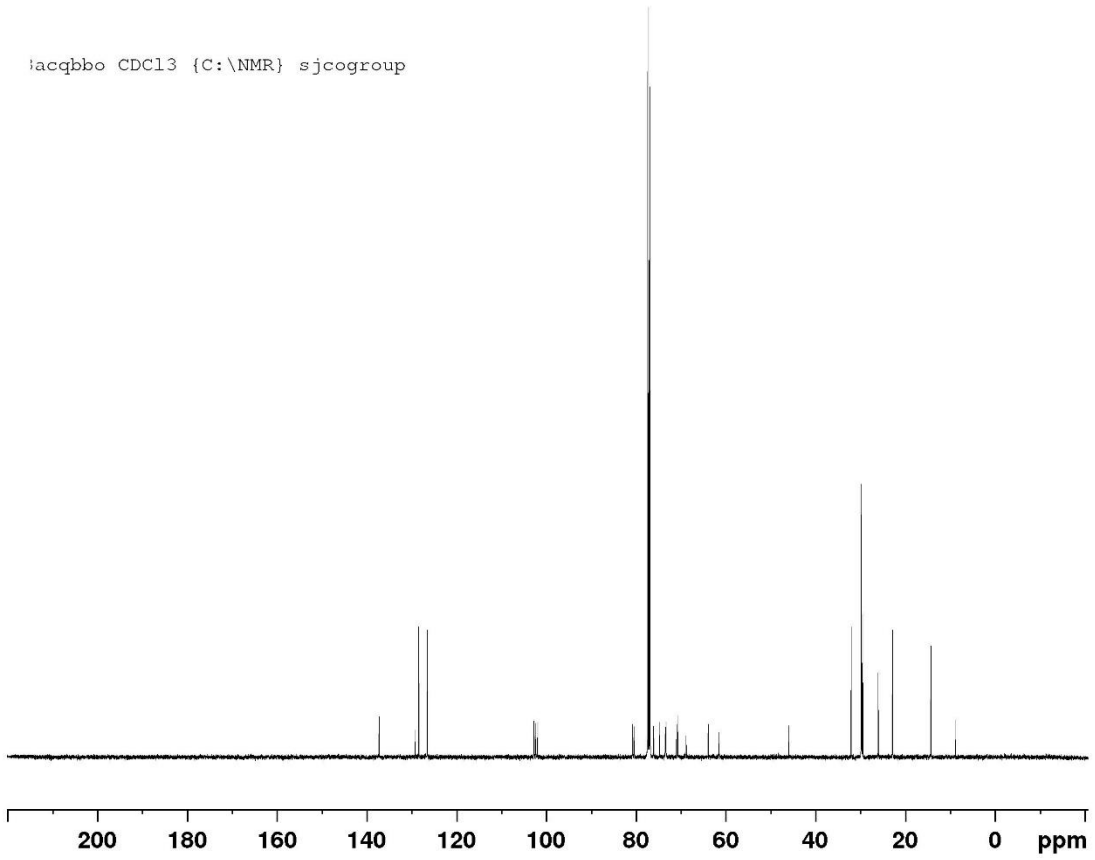
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NAME          IDS360(II)
EXPNO                  1
PROCNO                 1

F2 - Acquisition Parameters
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Time               18.38
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PROBHD   5 mm PABBO BB/
PULPROG             zg60
TD                 65536
SOLVENT            CDCl3
NS                    16
DS                     2
SWH           10330.578 Hz
FIDRES         0.157632 Hz
AQ            3.1719425 sec
RG                  45.3
DW                48.400 usec
DE                17.00 usec
TE                297.7 K
D1           1.00000000 sec

======== CHANNEL f1 ========
NUC1                   1H
P1                 11.00 usec
PL1       0 dB
SFO1        500.1325007 MHz

F2 - Processing parameters
SI                 32768
SF          500.1300284 MHz
WDW                   EM
SSB       0
LB                  0.30 Hz
GB        0
PC                  1.00
```

n-Dodecyl-(4',6'-*O*-benzylidene)-β-D-maltoside **73**




```
Current Data Parameters
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PROCNO                 1

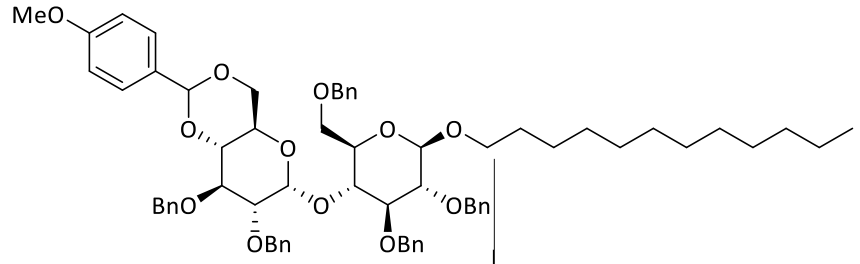
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PULPROG          zgpg30
TD                65536
SOLVENT           CDCl3
NS                 1024
DS                    4
SWH           30303.031 Hz
FIDRES         0.462388 Hz
AQ            1.0813440 sec
RG               4597.6
DW               16.500 usec
DE               11.00 usec
TE               297.7 K
D1            2.00000000 sec
d11           0.03000000 sec
DELTA         1.89999998 sec
TD0                    1

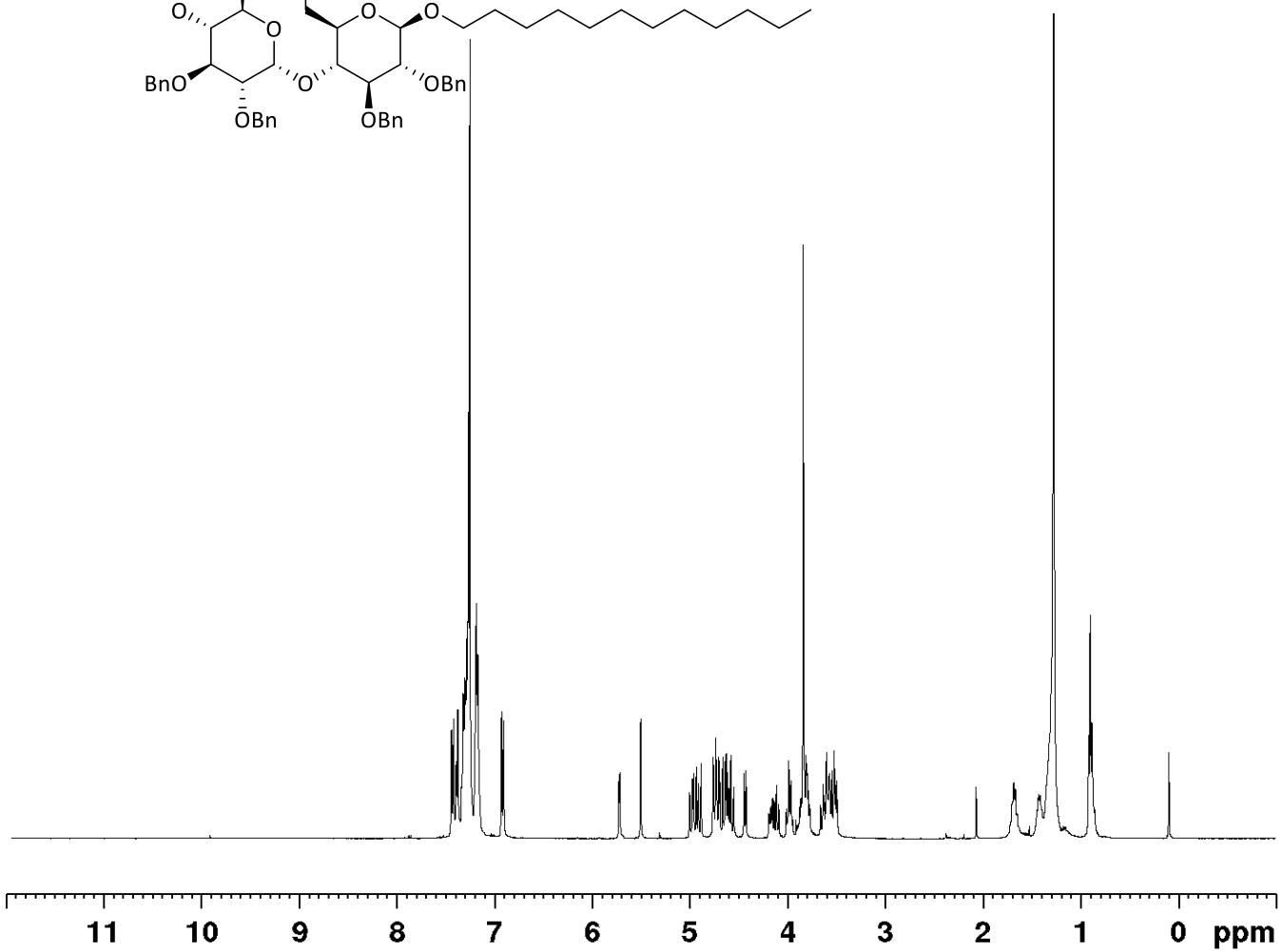
======== CHANNEL f1 ========
NUC1                  13C
P1                   6.80 usec
PL1                  5.00 dB
SFO1        125.7703148 MHz

======== CHANNEL f2 ========
CPDPRG[2          waltz16
NUC2                  1H
PCPD2              100.00 usec
PL2       0 dB
PL12                19.00 dB
PL13                23.00 dB
SFO2        500.1320005 MHz

F2 - Processing parameters
SI                 32768
SF          125.7577783 MHz
WDW                   EM
SSB       0
LB                   1.00 Hz
GB        0
PC                   1.40
```

n-Dodecyl-(2,3,6,2’,3’-*O*-pentabenzyl-4’,6’-*O*-[4-methoxybenzylidene])-β-D-maltoside **49**






```
Instrument DPX400
    Chemist ds
    Group sjc
      IDS187
Qh1acq.au CDCl3 {C:\NMR} sjcgrp 34

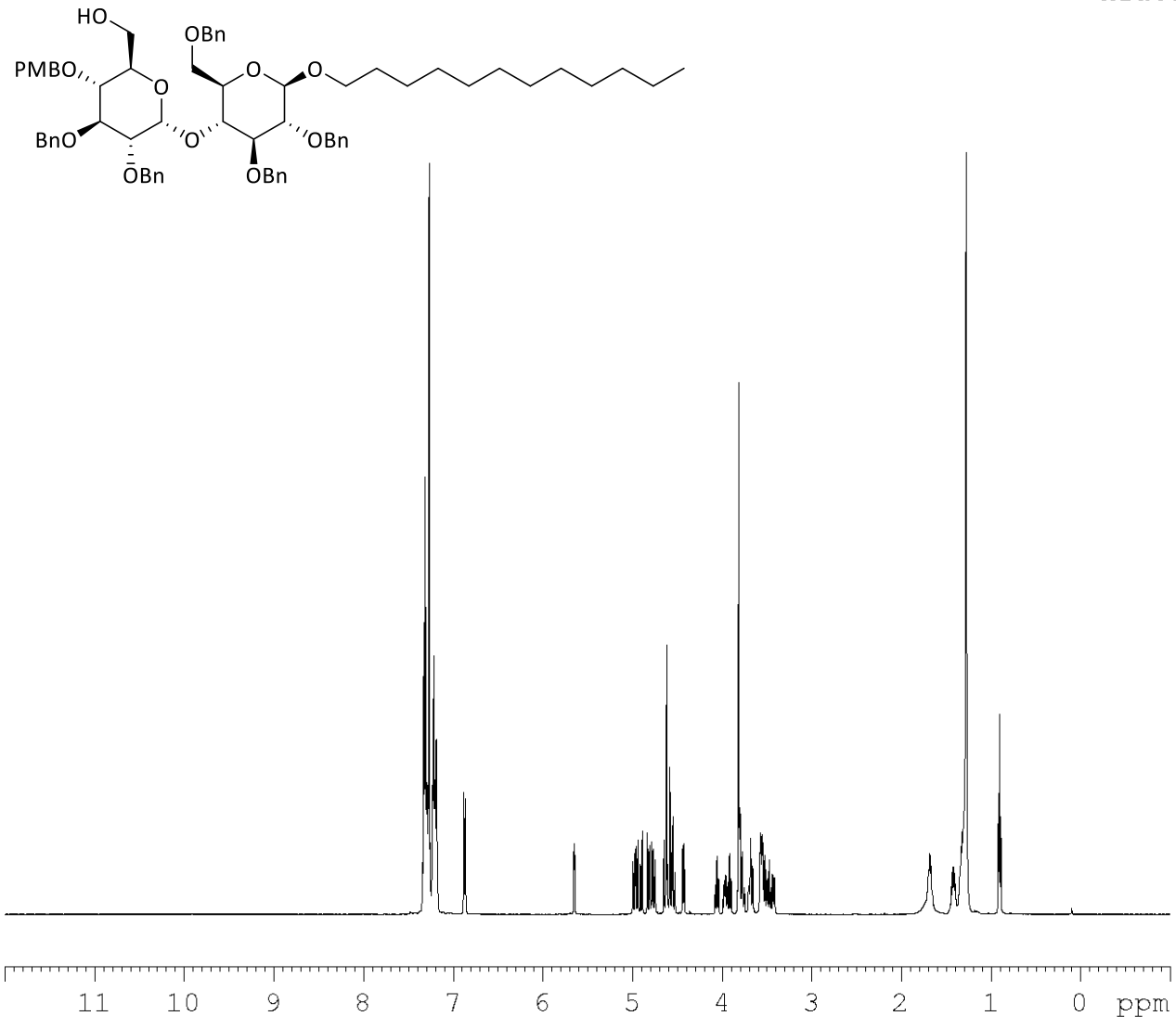
Current Data Parameters
NAME      IDS187 - Jun04-2012-34
EXPNO                      1
PROCNO                     1

F2 - Acquisition Parameters
Date_            20120604
Time                15.32
INSTRUM           dpx400
PROBHD   5 mm Dual 1H/1
PULPROG              zg60
TD                  32768
SOLVENT             CDCl3
NS                     16
DS                      2
SWH              5592.841 Hz
FIDRES           0.170680 Hz
AQ               2.9294591 sec
RG                   45.3
DW                 89.400 usec
DE                 17.00 usec
TE                 300.0 K
D1             1.00000000 sec

======== CHANNEL f1 ========
NUC1                   1H
P1                  7.30 usec
PL1       0 dB
SFO1         400.1320007 MHz

F2 - Processing parameters
SI                  32768
SF            400.1300173 MHz
WDW                    EM
SSB       0
LB                  0.30 Hz
GB        0
PC                  0.60
```

n-Dodecyl-(2,3,6,2',3'-*O*-pentabenzyl-4'-*O*-[4-methoxybenzyl])-β-D-maltoside **50**




```
DRX500 BBO Probe IDS277II
h1acqbbo CDCl3 {C:\NMR} sjcogroup 3

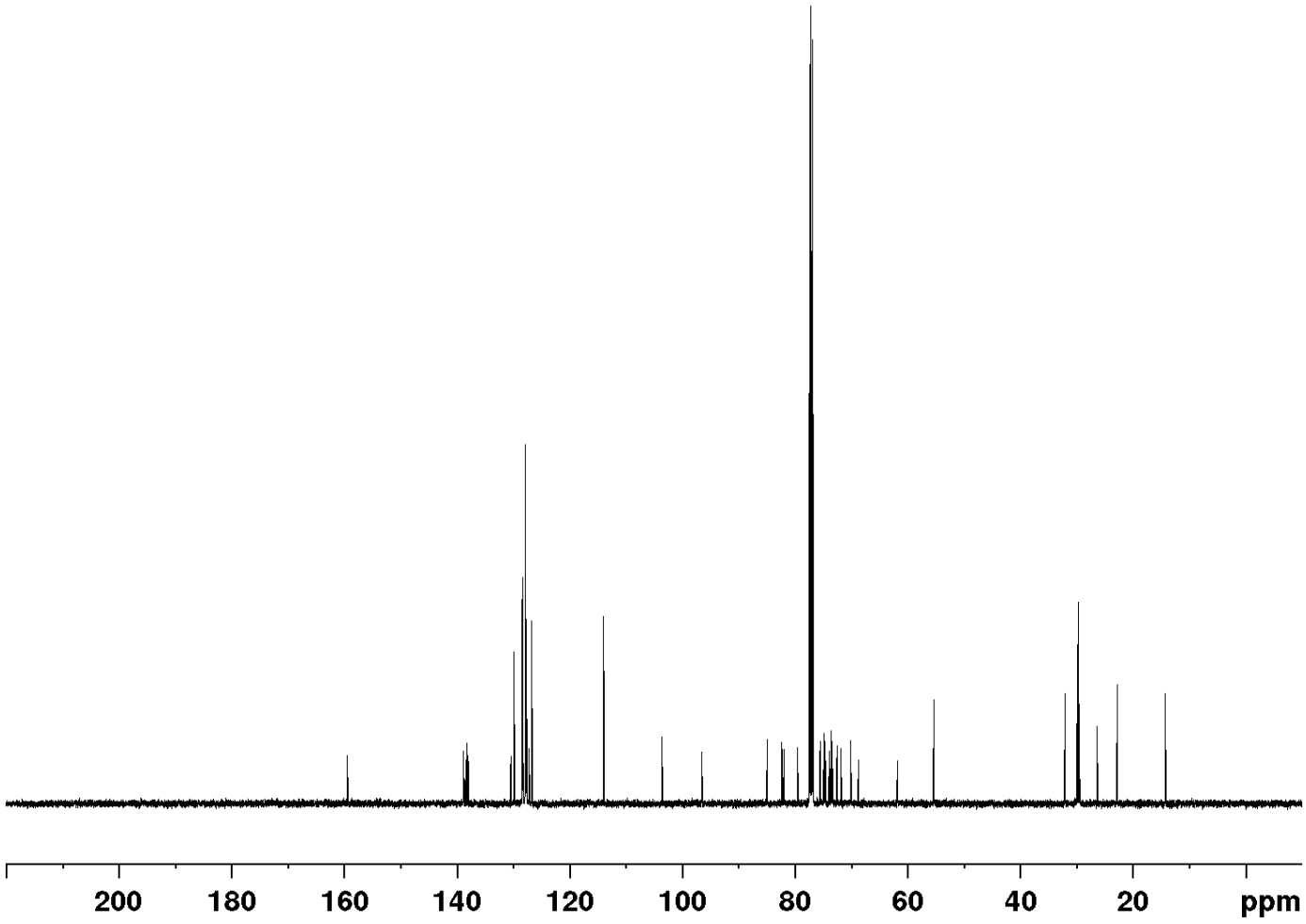
Current Data Parameters
NAME      IDS277II - R1 F18-24
EXPNO                    1
PROCNO                   1

F2 - Acquisition Parameters
Date_           20130314
Time               22.10
INSTRUM           drx500
PROBHD   5 mm PABBO BB/
PULPROG             zg60
TD                 65536
SOLVENT            CDCl3
NS                    16
DS                     2
SWH           10330.578 Hz
FIDRES         0.157632 Hz
AQ             3.1719425 sec
RG                  50.8
DW                48.400 usec
DE                17.00 usec
TE                298.0 K
D1           1.00000000 sec

======== CHANNEL f1 ========
NUC1                   1H
P1                 11.00 usec
PL1       0 dB
SFO1        500.1325007 MHz

F2 - Processing parameters
SI                 32768
SF          500.1300237 MHz
WDW                   EM
SSB       0
LB                 0.30 Hz
GB        0
PC                 1.00
```

n-Dodecyl-(2,3,6,2',3'-*O*-pentabenzyl-4'-*O*-[4-methoxybenzyl])-β-D-maltoside **50**




```
Current Data Parameters
NAME      IDS277II - R1 F18-24
EXPNO                    2
PROCNO                   1

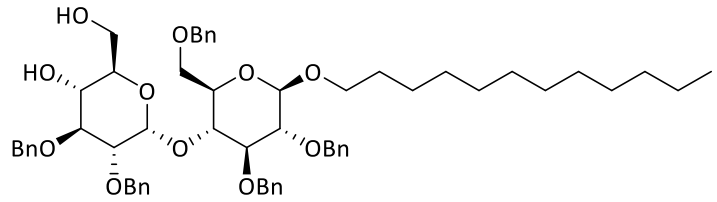
F2 - Acquisition Parameters
Date_           20130314
Time               22.51
INSTRUM           drx500
PROBHD   5 mm PABBO BB/
PULPROG           zgpg30
TD                65536
SOLVENT           CDCl3
NS                  750
DS                    4
SWH           30303.031 Hz
FIDRES         0.462388 Hz
AQ             1.0813440 sec
RG                2580.3
DW                16.500 usec
DE                11.00 usec
TE                297.9 K
D1            2.00000000 sec
d11           0.03000000 sec
DELTA         1.89999998 sec
TD0                   1

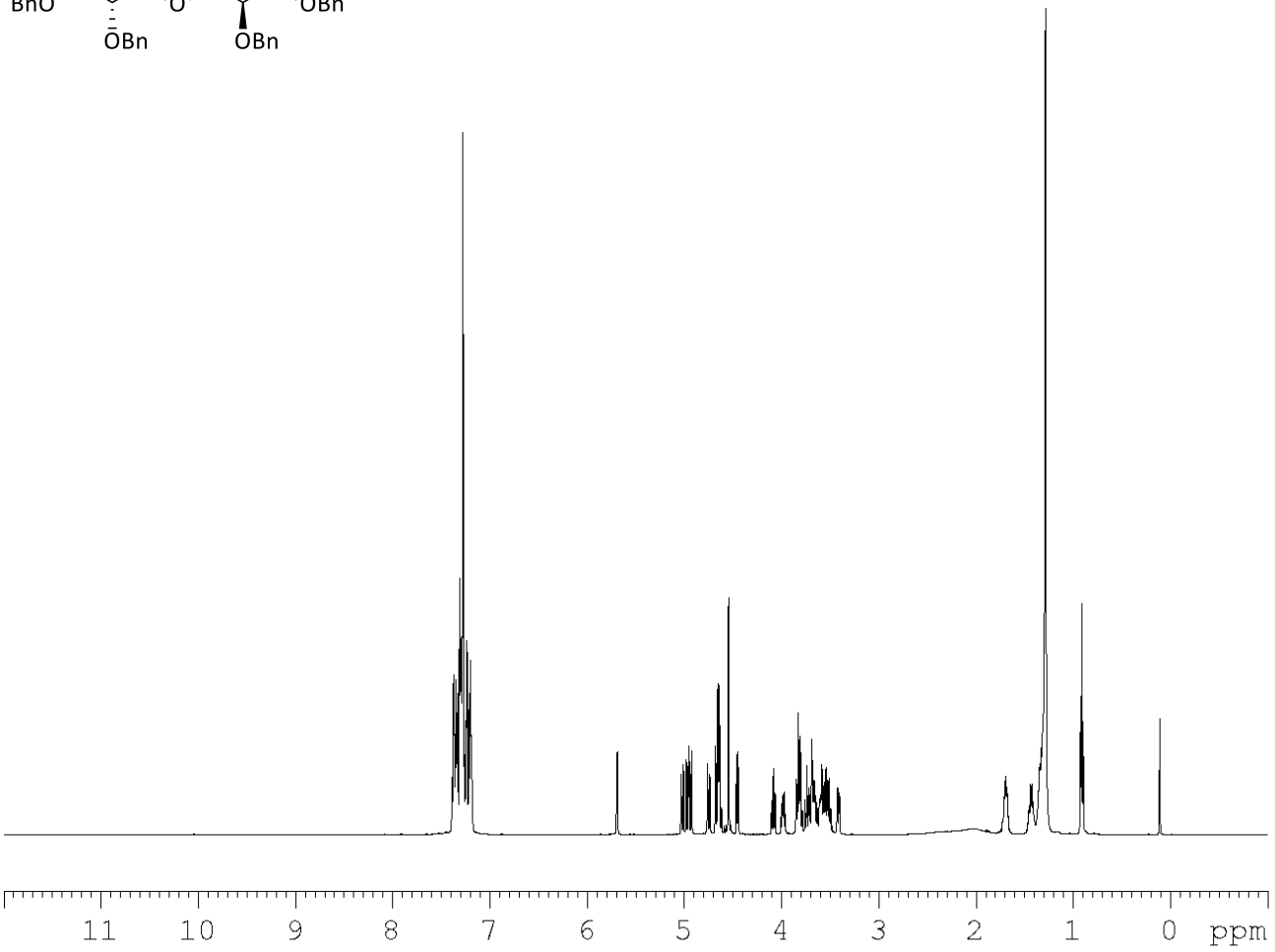
======== CHANNEL f1 ========
NUC1                 13C
P1                  6.80 usec
PL1                 5.00 dB
SFO1         125.7703148 MHz

======== CHANNEL f2 ========
CPDPRG[2         waltz16
NUC2                 1H
PCPD2             100.00 usec
PL2       0 dB
PL12                19.00 dB
PL13                23.00 dB
SFO2         500.1320005 MHz

F2 - Processing parameters
SI                  32768
SF           125.7577791 MHz
WDW                  EM
SSB       0
LB                  1.00 Hz
GB        0
PC                  1.40
```

n-Dodecyl-(2,3,6,2’,3’-*O*-hexabenzyl)-β-D-maltoside **76**






```
DRX500 BBO Probe IDS277 - R2 F5-13 (diol)
h1acqbbo CDCl3 {C:\NMR} sjcogroup 5

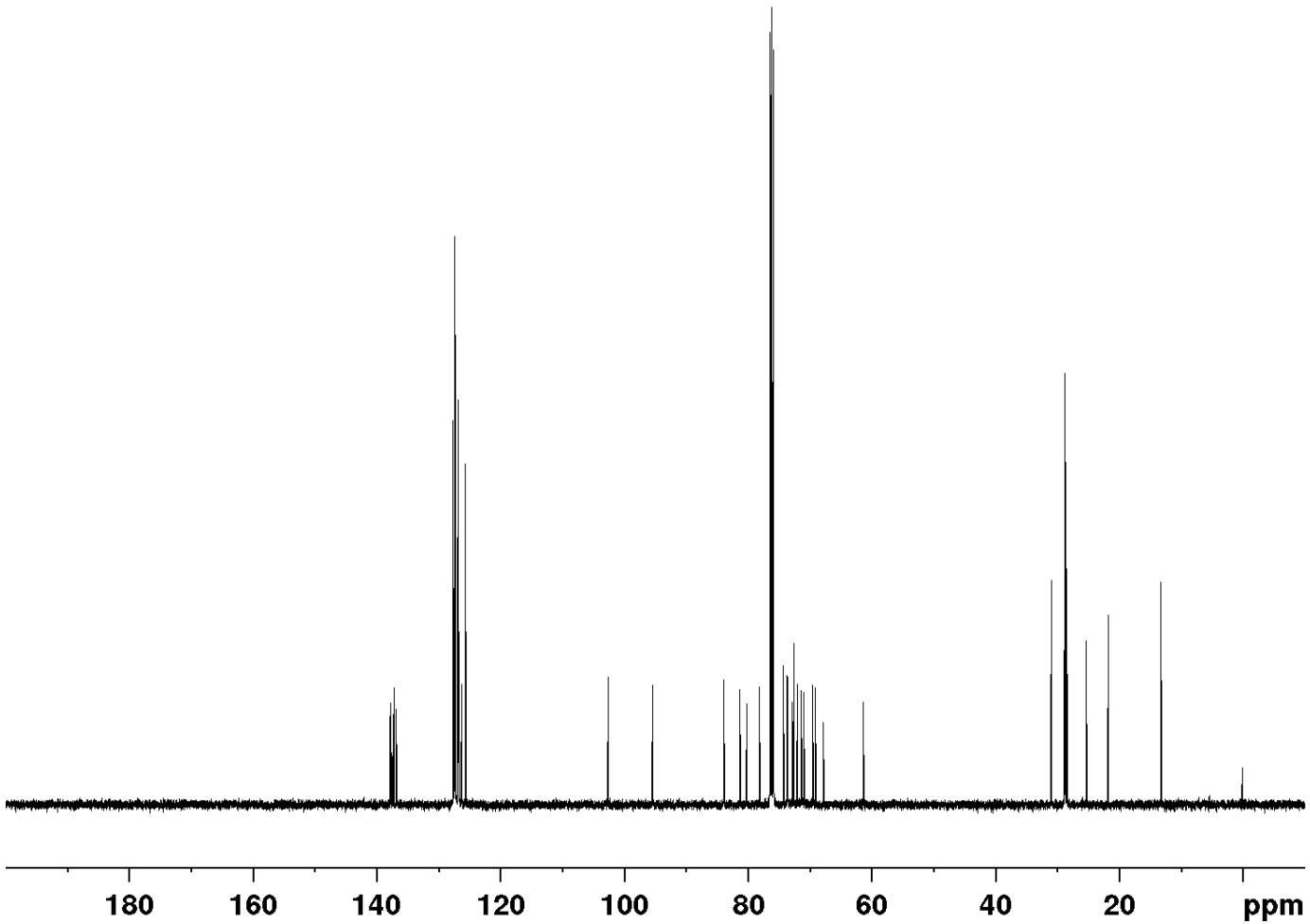
Current Data Parameters
NAME      IDS277 - R2 F5-13 diol
EXPNO                  1
PROCNO                 1

F2 - Acquisition Parameters
Date_           20130311
Time              22.49
INSTRUM           drx500
PROBHD   5 mm PABBO BB/
PULPROG             zg60
TD                65536
SOLVENT           CDCl3
NS                   16
DS                    2
SWH           10330.578 Hz
FIDRES         0.157632 Hz
AQ            3.1719425 sec
RG                   32
DW                48.400 usec
DE                17.00 usec
TE                298.0 K
D1           1.00000000 sec

======== CHANNEL f1 ========
NUC1                 1H
P1                11.00 usec
PL1       0 dB
SFO1         500.1325007 MHz

F2 - Processing parameters
SI                32768
SF          500.1300237 MHz
WDW                  EM
SSB       0
LB                0.30 Hz
GB        0
PC                1.00
```

n-Dodecyl-(2,3,6,2’,3’-*O*-hexabenzyl)-β-D-maltoside **76**




```
Current Data Parameters
NAME      IDS277 - R2 F5-13 diol
EXPNO                  2
PROCNO                 1

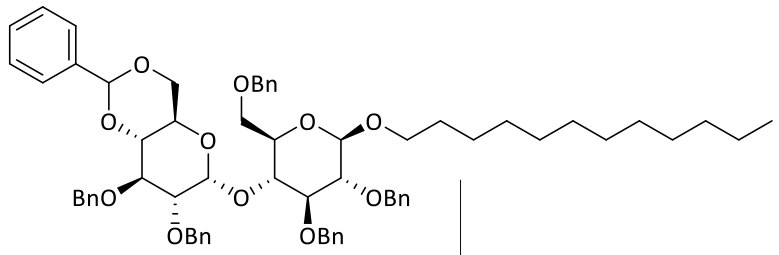
F2 - Acquisition Parameters
Date_           20130311
Time              23.18
INSTRUM           drx500
PROBHD    5 mm PABBO BB/
PULPROG           zgpg30
TD                65536
SOLVENT           CDCl3
NS                  512
DS                    4
SWH           30303.031 Hz
FIDRES         0.462388 Hz
AQ             1.0813440 sec
RG                9195.2
DW                16.500 usec
DE                11.00 usec
TE                298.0 K
D1            2.00000000 sec
d11           0.03000000 sec
DELTA         1.89999998 sec
TD0                   1

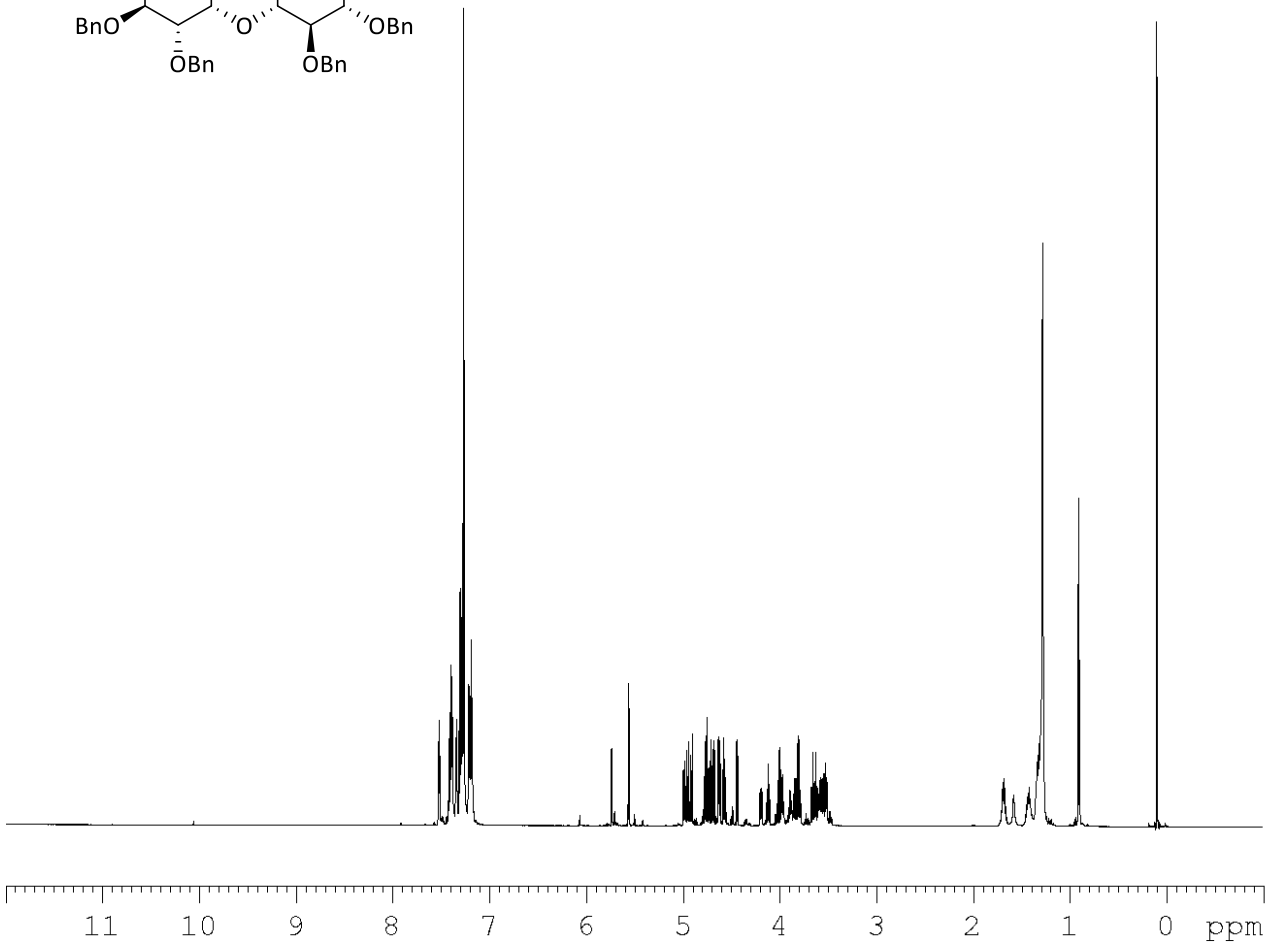
======== CHANNEL f1 ========
NUC1                 13C
P1                  6.80 usec
PL1                 5.00 dB
SFO1         125.7703148 MHz

======== CHANNEL f2 ========
CPDPRG[2          waltz16
NUC2                 1H
PCPD2             100.00 usec
PL2       0 dB
PL12                19.00 dB
PL13                23.00 dB
SFO2         500.1320005 MHz

F2 - Processing parameters
SI                  32768
SF           125.7579078 MHz
WDW                  EM
SSB       0
LB                  1.00 Hz
GB        0
PC                  1.40
```

n-Dodecyl-(2,3,6,2’,3’-*O*-pentabenzyl-4’,6’-*O*-benzylidene)-β-D-maltoside **77**





AV700 1H
Diane Swallow SJC 30664 20/7/12

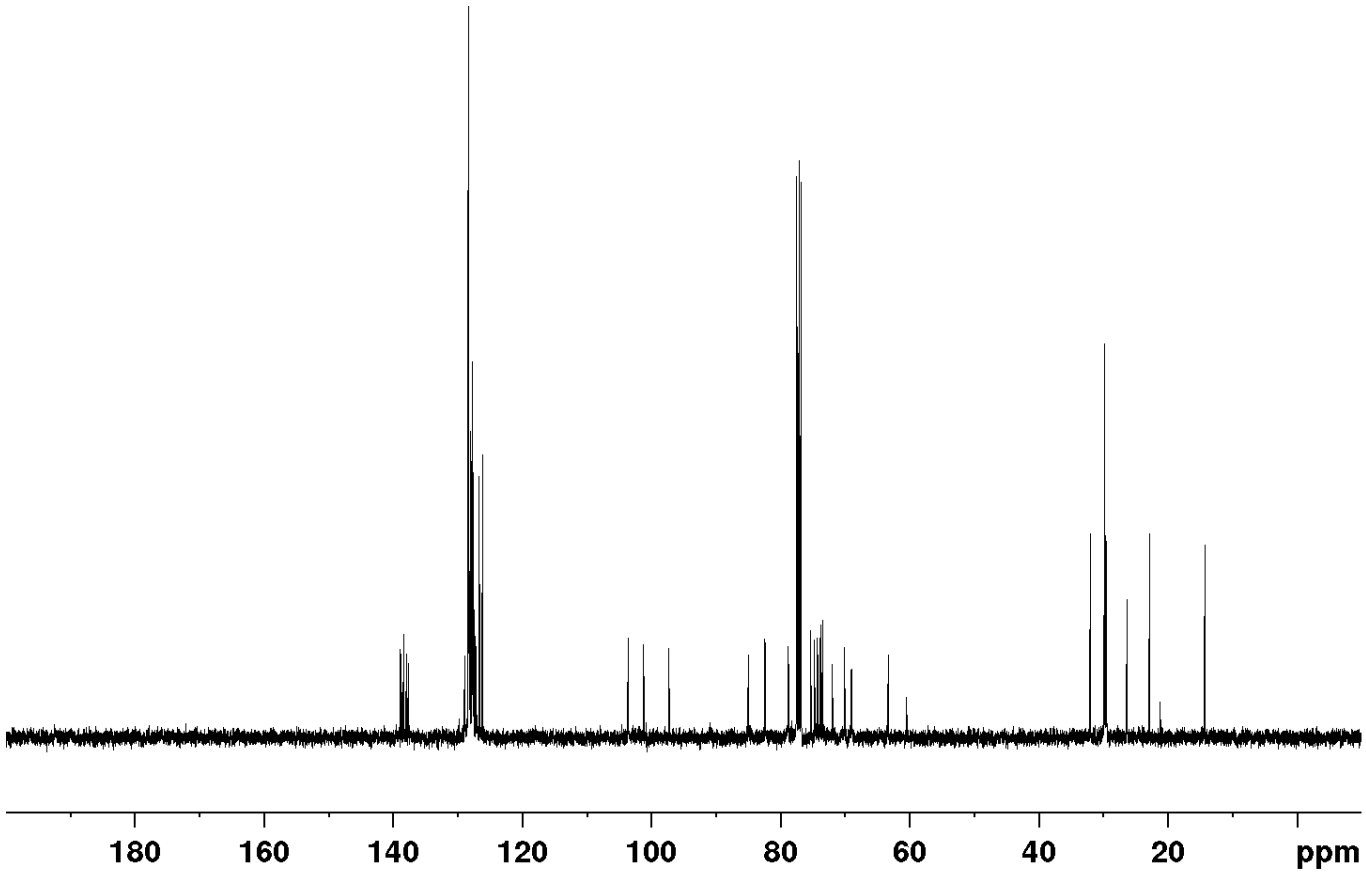
```
Current Data Parameters
NAME      IDS201 - ds06642007
EXPNO                    1
PROCNO                   1

F2 - Acquisition Parameters
Date_           20120720
Time               15.41
INSTRUM            av700
PROBHD   5 mm CPTCI 1H-
PULPROG             zg60
TD                 65536
SOLVENT            CDCl3
NS                    16
DS                     2
SWH           11160.714 Hz
FIDRES         0.170299 Hz
AQ            2.9360127 sec
RG                    64
DW               44.800 usec
DE                6.50 usec
TE                298.0 K
D1           1.00000000 sec
TD0                    1

======== CHANNEL f1 ========
NUC1                  1H
P1                 8.00 usec
PL1                6.65 dB
PL1W         5.48682833 W
SFO1        699.9935000 MHz

F2 - Processing parameters
SI                 65536
SF          699.9900044 MHz
WDW                   no
SSB       0
LB        0 Hz
GB        0
PC                  1.00
```

n-Dodecyl-(2,3,6,2’,3’-*O*-pentabenzyl-4’,6’-*O*-benzylidene)-β-D-maltoside **77**




```
Current Data Parameters
NAME      IDS201 - Jul15-2012-36
EXPNO                          3
PROCNO                         1

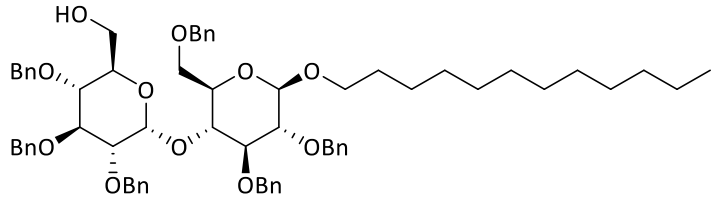
F2 - Acquisition Parameters
Date_               20120715
Time                   16.23
INSTRUM                av400
PROBHD    5 mm QNP 1H/13
PULPROG              zgpg30
TD                     32768
SOLVENT                CDCl3
NS                       256
DS                         4
SWH                26178.010 Hz
FIDRES              0.798889 Hz
AQ                  0.6258688 sec
RG                     32768
DW                    19.100 usec
DE                     7.50 usec
TE                     300.0 K
D1                 1.00000000 sec
D11                0.03000000 sec
TD0                        1

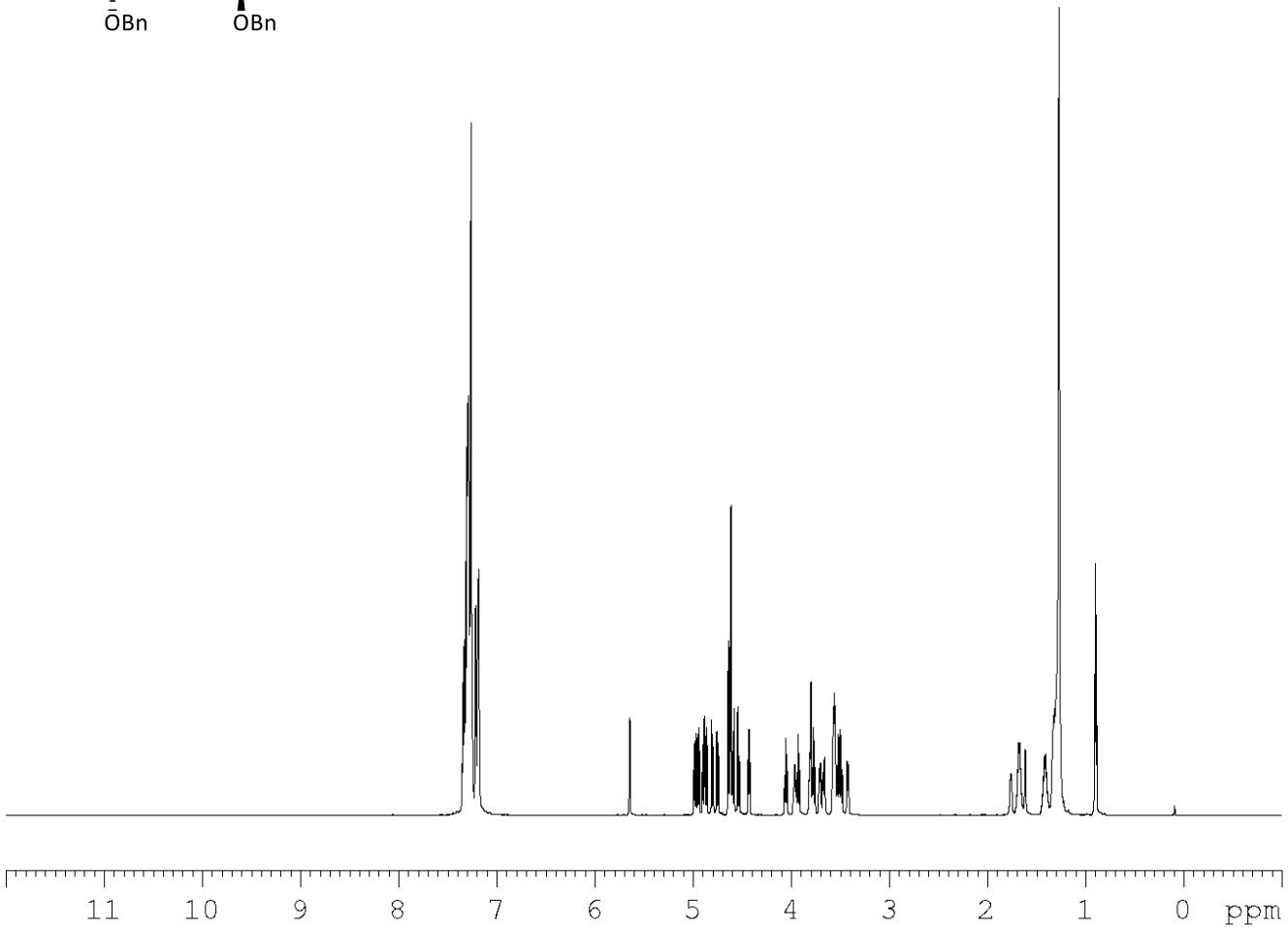
======== CHANNEL f1 ========
NUC1                      13C
P1                       9.50 usec
PL1       0 dB
SFO1           100.6403931 MHz

======== CHANNEL f2 ========
CPDPRG[2              waltz16
NUC2                      1H
PCPD2                  80.00 usec
PL2       0 dB
PL12                   19.00 dB
PL13                   25.00 dB
SFO2           400.2016008 MHz

F2 - Processing parameters
SI                     32768
SF                100.6303647 MHz
WDW                       EM
SSB       0
LB                      1.00 Hz
GB        0
PC                      1.40
```

n-Dodecyl-(2,3,6,2’,3’,4’-*O*-hexabenzyl)-β-D-maltoside **78**





AV700 1H
Diane Swallow SJC 30850 16/8/12

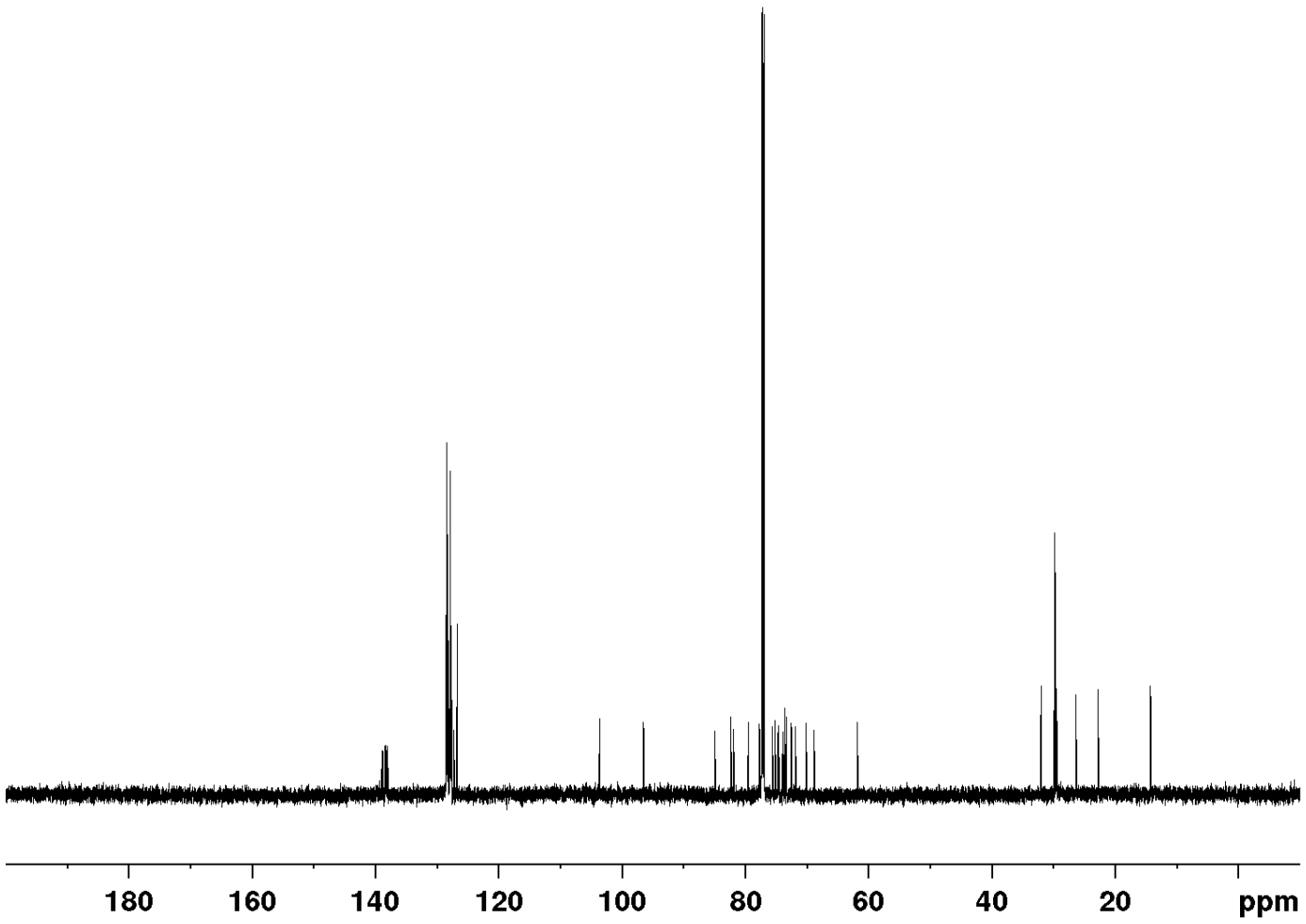
```
Current Data Parameters
NAME      IDS207 - ds08501608
EXPNO                    1
PROCNO                   1

F2 - Acquisition Parameters
Date_             20120816
Time                 17.32
INSTRUM              av700
PROBHD    5 mm CPTCI 1H-
PULPROG                zg60
TD                    65536
SOLVENT               CDCl3
NS                        8
DS                        2
SWH              11160.714 Hz
FIDRES            0.170299 Hz
AQ               2.9360127 sec
RG                        8
DW                  44.800 usec
DE                   6.50 usec
TE                  298.0 K
D1             1.00000000 sec
TD0                       1

======== CHANNEL f1 ========
NUC1                     1H
P1                    8.00 usec
PL1                   6.65 dB
PL1W           5.48682833 W
SFO1          699.9935000 MHz

F2 - Processing parameters
SI                    65536
SF            699.9900155 MHz
WDW                      no
SSB       0
LB        0 Hz
GB        0
PC                    1.00
```

n-Dodecyl-(2,3,6,2’,3’,4’-*O*-hexabenzyl)-β-D-maltoside **78**




```
Current Data Parameters
NAME      IDS207 - ds08501608
EXPNO                      13
PROCNO                      1

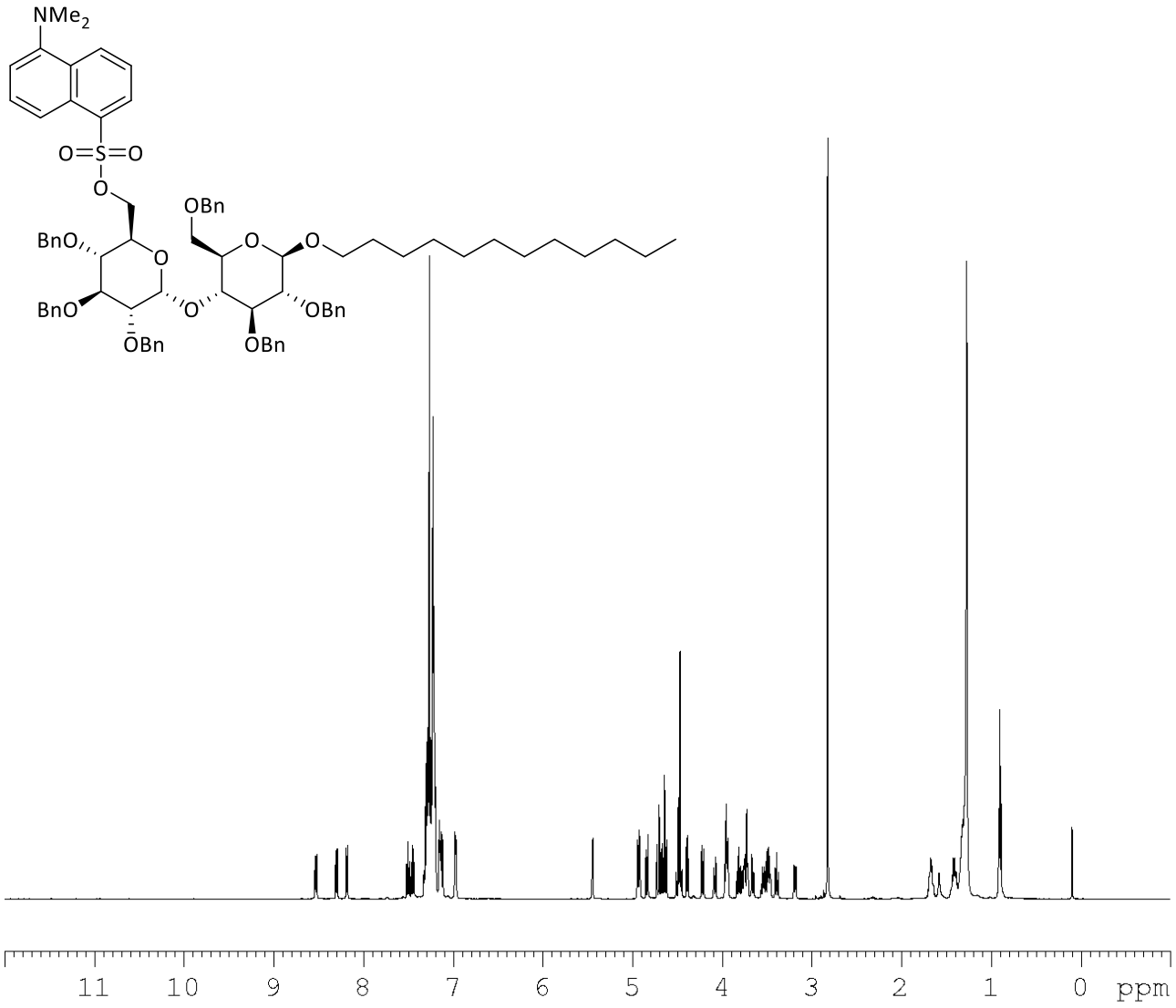
F2 - Acquisition Parameters
Date_              20120816
Time                  18.54
INSTRUM               av700
PROBHD    5 mm CPTCI 1H-
PULPROG             zgpg30
TD                    65536
SOLVENT               CDCl3
NS                      256
DS                        2
SWH              41666.668 Hz
FIDRES            0.635783 Hz
AQ                0.7864320 sec
RG                        2
DW                   12.000 usec
DE                    6.50 usec
TE                   298.0 K
D1              2.00000000 sec
D11             0.03000000 sec
TD0                       1

======== CHANNEL f1 ========
NUC1                    13C
P1                   15.00 usec
PL1                   2.70 dB
PL1W          56.11685562 W
SFO1         176.0302268 MHz

======== CHANNEL f2 ========
CPDPRG[2           waltz16
NUC2                    1H
PCPD2                65.00 usec
PL2                   6.65 dB
PL12                 23.35 dB
PL13                 29.35 dB
PL2W           5.48682833 W
PL12W          0.11730630 W
PL13W          0.02946601 W
SFO2          699.9928000 MHz

F2 - Processing parameters
SI                    32768
SF           176.0126066 MHz
WDW                      EM
SSB       0
LB                    1.00 Hz
GB        0
PC                    1.40
```

n-Dodecyl-(2,3,6,2',3',4'-*O*-hexabenzyl-6'-*O*-dansyl)-β-D-maltoside **81**




```
h1acqbbo CDCl3 {C:\NMR} sjcogroup 3

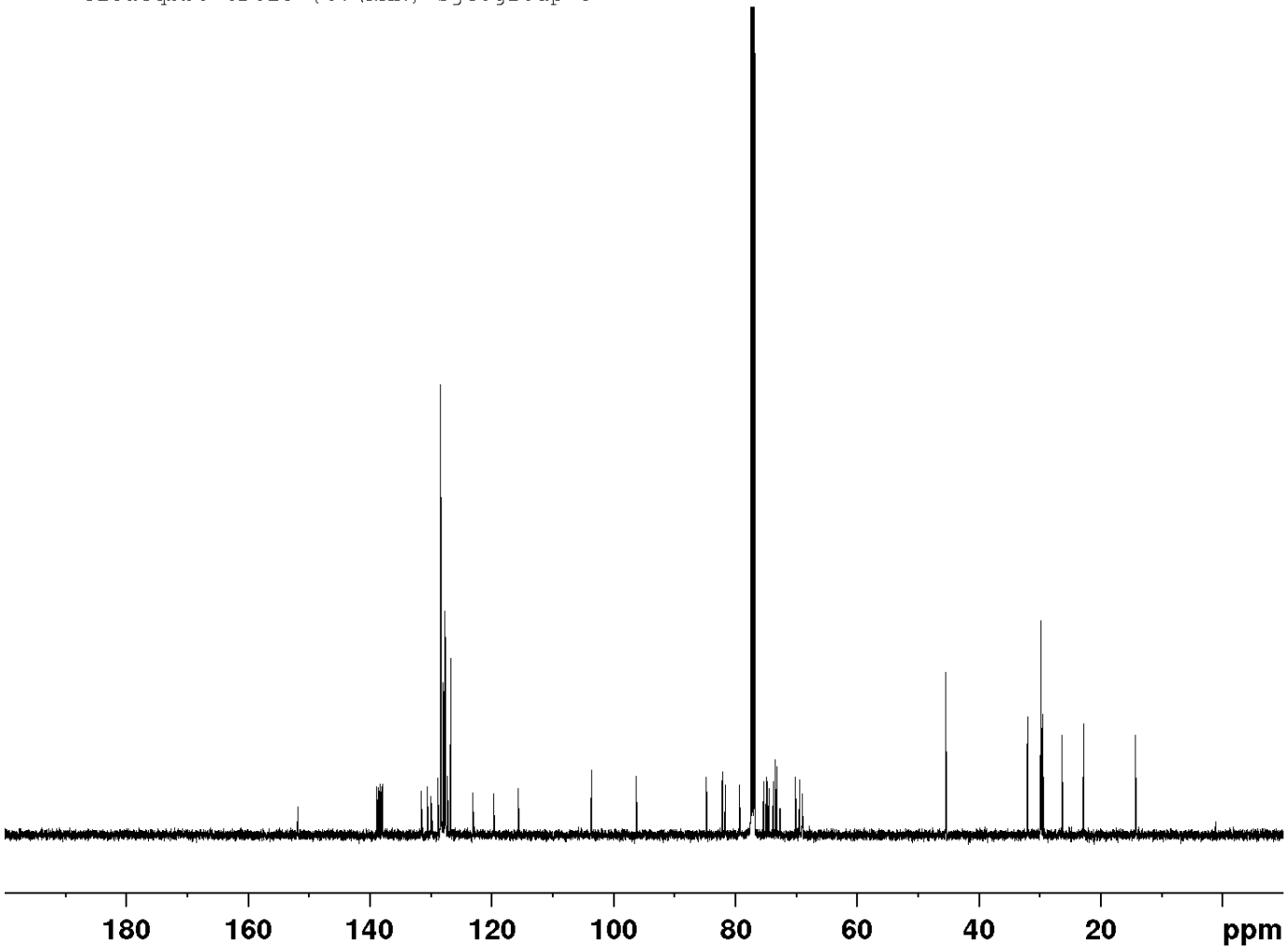
Current Data Parameters
NAME             IDS260b
EXPNO                  1
PROCNO                 1

F2 - Acquisition Parameters
Date_           20131028
Time               18.15
INSTRUM          drx500
PROBHD   5 mm PABBO BB/
PULPROG             zg60
TD                 65536
SOLVENT            CDCl3
NS                    16
DS                     2
SWH           10330.578 Hz
FIDRES         0.157632 Hz
AQ            3.1719425 sec
RG                  71.8
DW                48.400 usec
DE                17.00 usec
TE                298.7 K
D1           1.00000000 sec

======== CHANNEL f1 ========
NUC1                   1H
P1                 11.00 usec
PL1       0 dB
SFO1        500.1325007 MHz

F2 - Processing parameters
SI                 32768
SF          500.1300237 MHz
WDW                   EM
SSB       0
LB                  0.30 Hz
GB        0
PC                  1.00
```

n-Dodecyl-(2,3,6,2',3',4'-*O*-hexabenzyl-6'-*O*-dansyl)-β-D-maltoside **81**




```
Current Data Parameters
NAME             IDS260b
EXPNO                  2
PROCNO                 1

F2 - Acquisition Parameters
Date_           20131028
Time               19.11
INSTRUM           drx500
PROBHD   5 mm PABBO BB/
PULPROG          zgpg30
TD                65536
SOLVENT            CDCl3
NS                  1024
DS                     4
SWH           30303.031 Hz
FIDRES         0.462388 Hz
AQ            1.0813440 sec
RG               3649.1
DW               16.500 usec
DE               11.00 usec
TE                298.7 K
D1           2.00000000 sec
d11          0.03000000 sec
DELTA        1.89999998 sec
TD0                    1

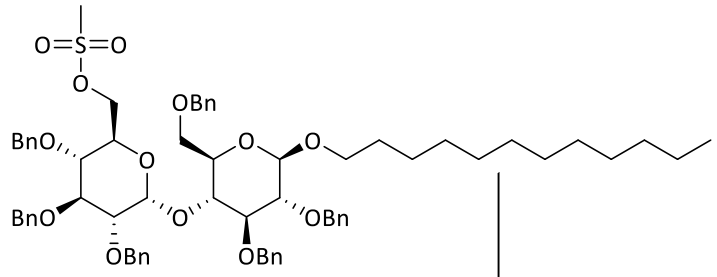
======== CHANNEL f1 ========
NUC1                  13C
P1                   6.80 usec
PL1                  5.00 dB
SFO1        125.7703148 MHz

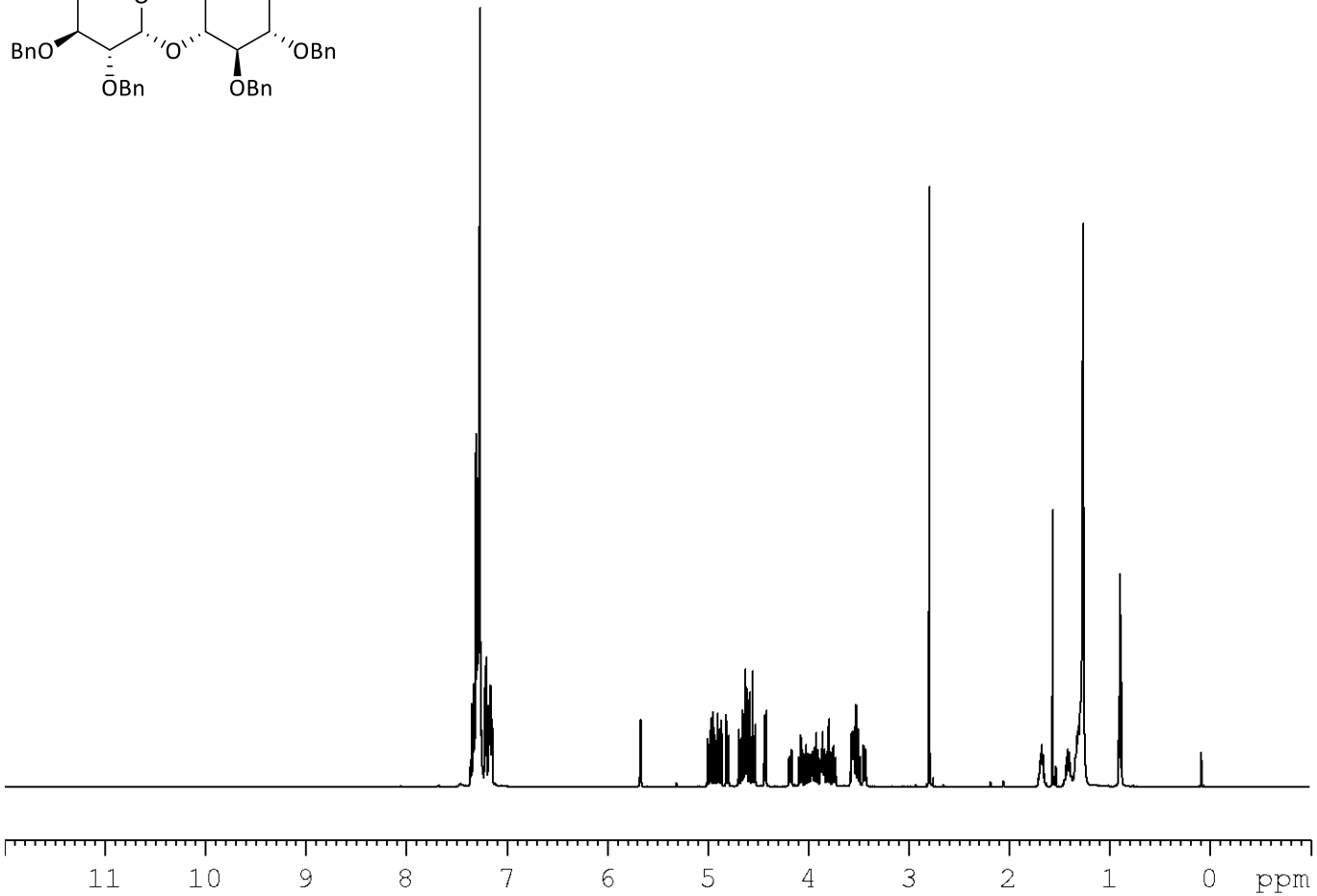
======== CHANNEL f2 ========
CPDPRG[2         waltz16
NUC2                  1H
PCPD2             100.00 usec
PL2       0 dB
PL12                19.00 dB
PL13                23.00 dB
SFO2        500.1320005 MHz

F2 - Processing parameters
SI                 32768
SF          125.7577781 MHz
WDW                   EM
SSB       0
LB                  1.00 Hz
GB        0
PC                  1.40
```

n-Dodecyl-(2,3,6,2',3',4'-*O*-hexabenzyl-6'-*O*-mesyl)-β-D-maltoside **82**

h1acqbbo CDCl3 {C:\NMR} sjcogroup 4





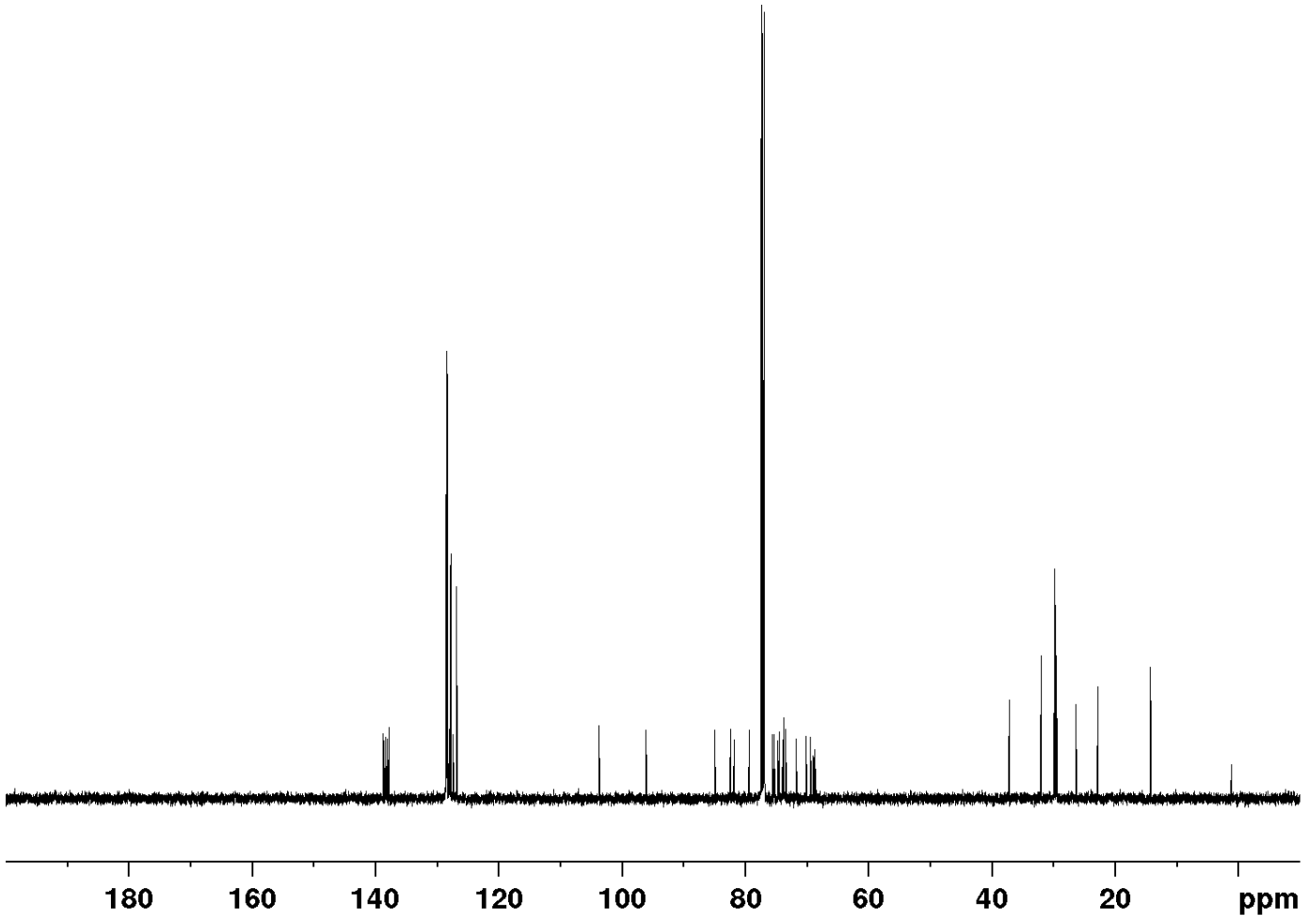

```
Current Data Parameters
NAME       IDS238 DRX500
EXPNO                 1
PROCNO                1

F2 - Acquisition Parameters
Date_          20121102
Time              13.58
INSTRUM          drx500
PROBHD   5 mm PABBO BB/
PULPROG            zg60
TD                65536
SOLVENT           CDCl3
NS                  128
DS                    2
SWH           10330.578 Hz
FIDRES         0.157632 Hz
AQ            3.1719425 sec
RG                  128
DW               48.400 usec
DE               17.00 usec
TE               298.0 K
D1           1.00000000 sec

======== CHANNEL f1 ========
NUC1                1H
P1              11.00 usec
PL1      0 dB
SFO1        500.1325007 MHz

F2 - Processing parameters
SI                32768
SF          500.1300237 MHz
WDW                 EM
SSB      0
LB                0.30 Hz
GB       0
PC                1.00
```

n-Dodecyl-(2,3,6,2',3',4'-*O*-hexabenzyl-6'-*O*-mesyl)-β-D-maltoside **82**




```
Current Data Parameters
NAME                  IDS246
EXPNO                      3
PROCNO                     1

F2 - Acquisition Parameters
Date_              20121203
Time                 14.14
INSTRUM              drx500
PROBHD    5 mm PABBO BB/
PULPROG              zgpg30
TD                    65536
SOLVENT               CDCl3
NS                      256
DS                        4
SWH              30303.031 Hz
FIDRES            0.462388 Hz
AQ               1.0813440 sec
RG                     2048
DW                  16.500 usec
DE                  11.00 usec
TE                  298.0 K
D1              2.00000000 sec
d11             0.03000000 sec
DELTA           1.89999998 sec
TD0                        1

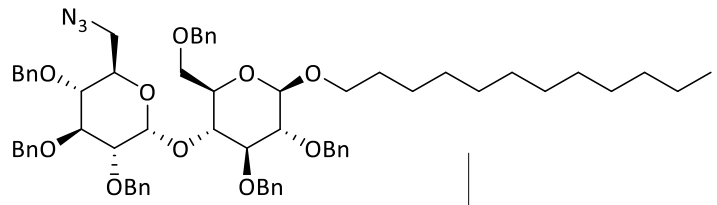
======== CHANNEL f1 ========
NUC1                    13C
P1                   6.80 usec
PL1                  5.00 dB
SFO1          125.7703148 MHz

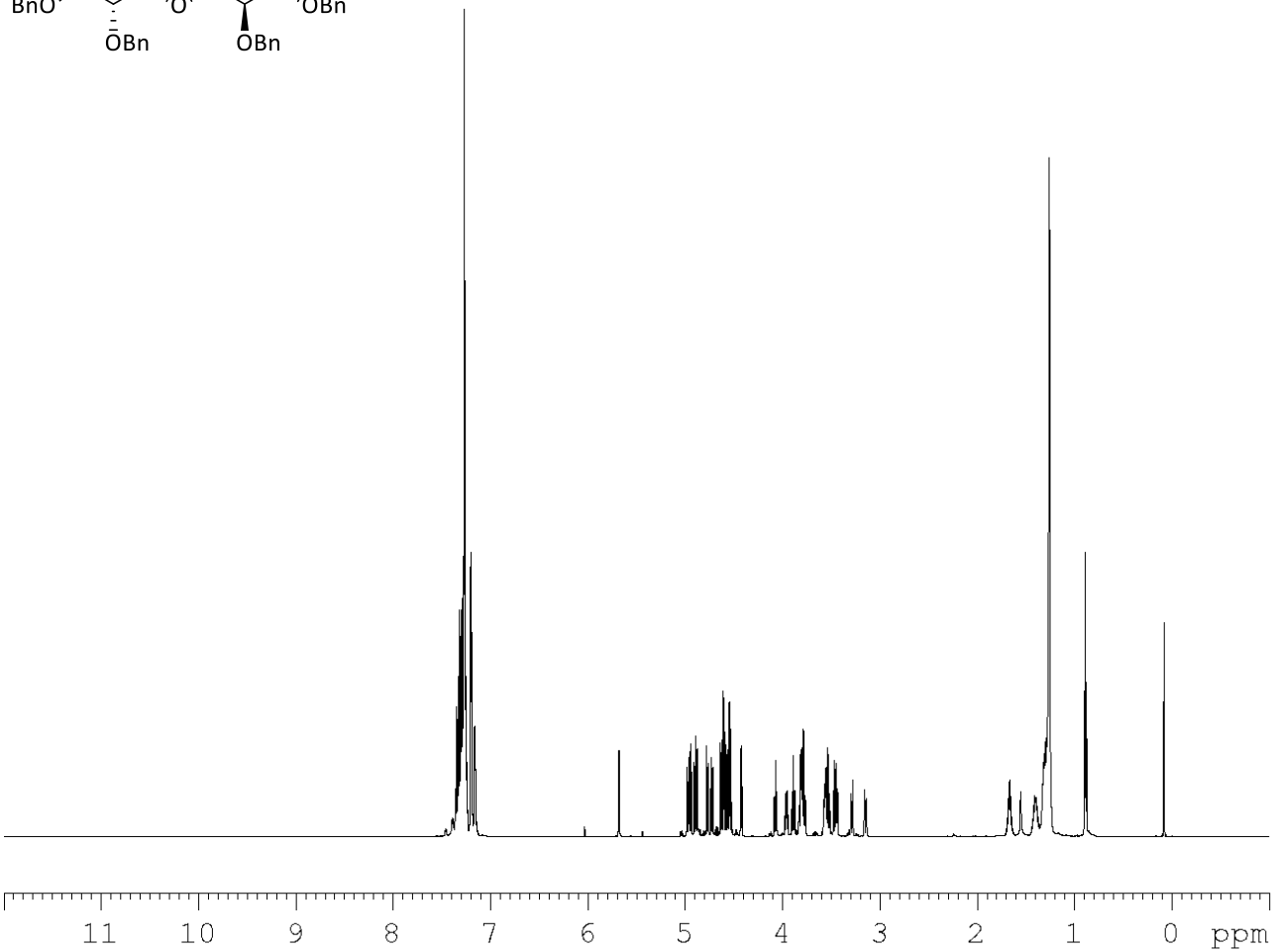
======== CHANNEL f2 ========
CPDPRG[2           waltz16
NUC2                    1H
PCPD2               100.00 usec
PL2       0 dB
PL12                19.00 dB
PL13                23.00 dB
SFO2          500.1320005 MHz

F2 - Processing parameters
SI                     32768
SF            125.7577804 MHz
WDW                       EM
SSB       0
LB                   1.00 Hz
GB        0
PC                   1.40
```

n-Dodecyl-(2,3,6,2',3',4'-*O*-hexabenzyl-6'-deoxy-6'-azido)-β-D-maltoside **83**

AV700 1H DIANE SWALLOW 32645 24/1/13 SJC





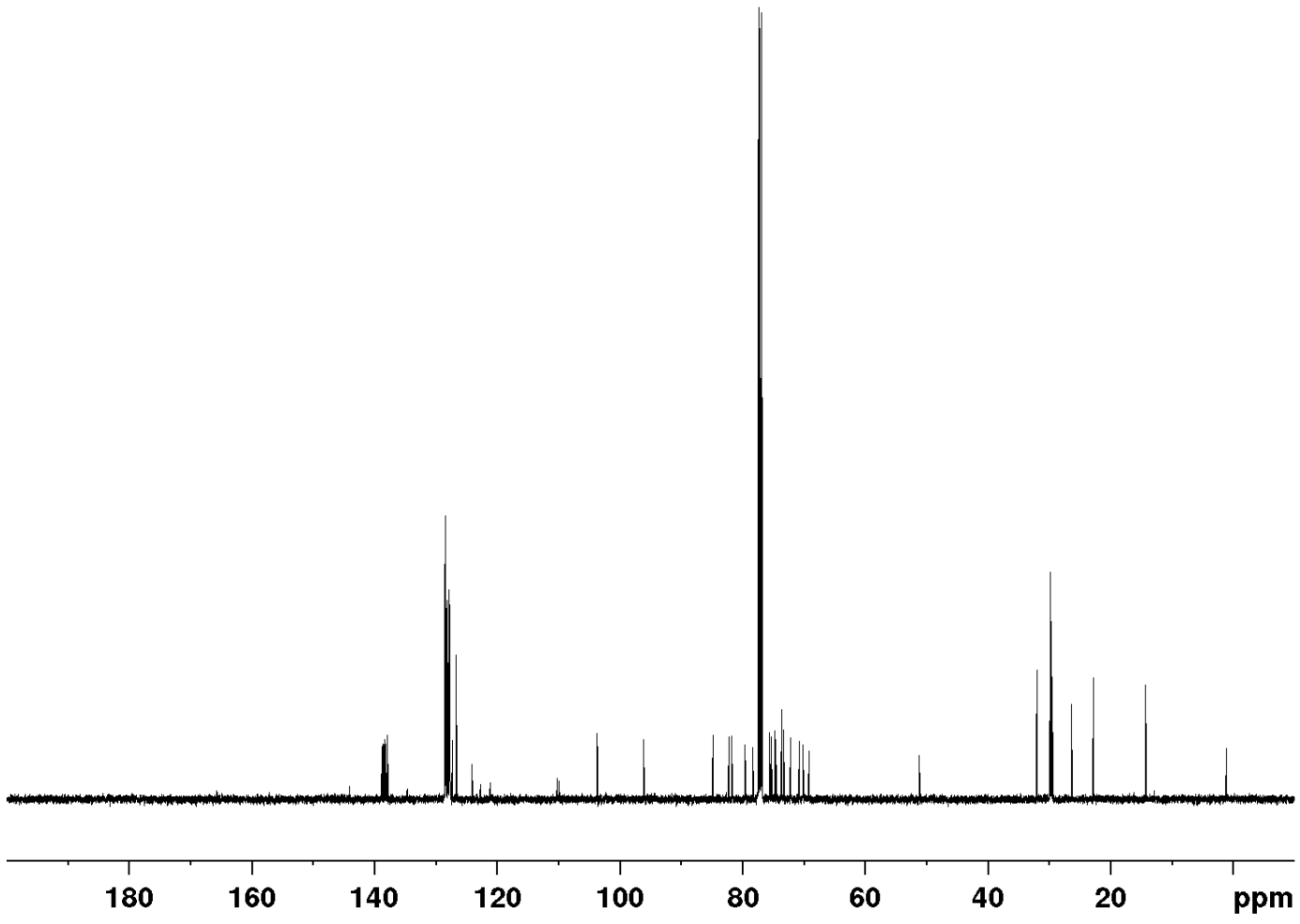

```
Current Data Parameters
NAME      IDS250 - ds26452401
EXPNO                    1
PROCNO                   1

F2 - Acquisition Parameters
Date_           20130124
Time               10.36
INSTRUM            av700
PROBHD   5 mm CPTCI 1H-
PULPROG             zg60
TD                 65536
SOLVENT            CDCl3
NS                    16
DS                     2
SWH            11160.714 Hz
FIDRES          0.170299 Hz
AQ             2.9360127 sec
RG                   4.5
DW                44.800 usec
DE                 6.50 usec
TE                298.0 K
D1             1.00000000 sec
TD0                    1

======== CHANNEL f1 ========
NUC1                  1H
P1                 8.00 usec
PL1                6.65 dB
PL1W          5.48682833 W
SFO1        699.9935000 MHz

F2 - Processing parameters
SI                 65536
SF          699.9900131 MHz
WDW                   EM
SSB       0
LB                 0.30 Hz
GB        0
PC                 1.00
```

n-Dodecyl-(2,3,6,2',3',4'-*O*-hexabenzyl-6'-deoxy-6'-azido)-β-D-maltoside **83**




```
Current Data Parameters
NAME      IDS250 - DRX500
EXPNO                  4
PROCNO                 1

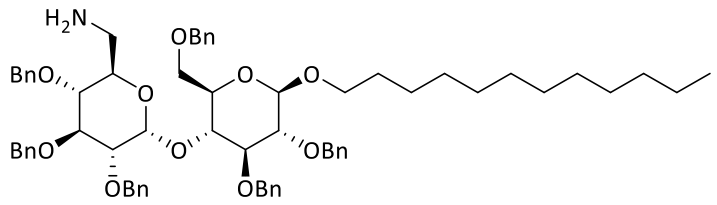
F2 - Acquisition Parameters
Date_           20121207
Time               11.56
INSTRUM           drx500
PROBHD   5 mm PABBO BB/
PULPROG          zgpg30
TD                65536
SOLVENT           CDCl3
NS                  530
DS                    4
SWH           30303.031 Hz
FIDRES         0.462388 Hz
AQ             1.0813440 sec
RG                 4096
DW                16.500 usec
DE                11.00 usec
TE                298.0 K
D1            2.00000000 sec
d11           0.03000000 sec
DELTA         1.89999998 sec
TD0                    1

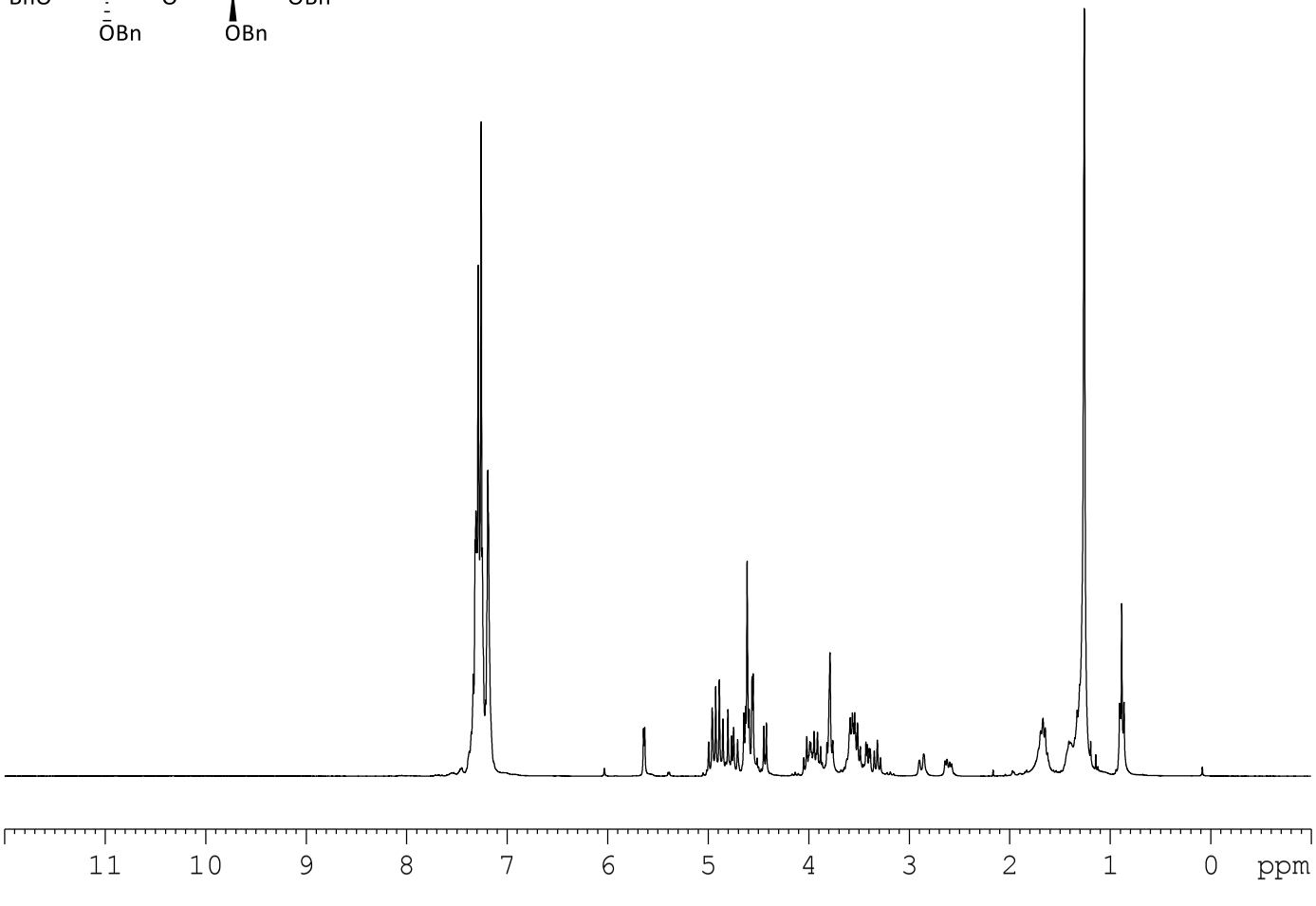
======== CHANNEL f1 ========
NUC1                  13C
P1                   6.80 usec
PL1                  5.00 dB
SFO1         125.7703148 MHz

======== CHANNEL f2 ========
CPDPRG[2         waltz16
NUC2                  1H
PCPD2             100.00 usec
PL2       0 dB
PL12                19.00 dB
PL13                23.00 dB
SFO2         500.1320005 MHz

F2 - Processing parameters
SI                 32768
SF           125.7577795 MHz
WDW                   EM
SSB       0
LB                  1.00 Hz
GB        0
PC                  1.40
```

n-Dodecyl-(2,3,6,2',3',4'-*O*-hexabenzyl-6'-deoxy-6'-amino)-β-D-maltoside **84**






```
Instrument DPX300
Chemist Name ds
Research Group SJC
IDS166

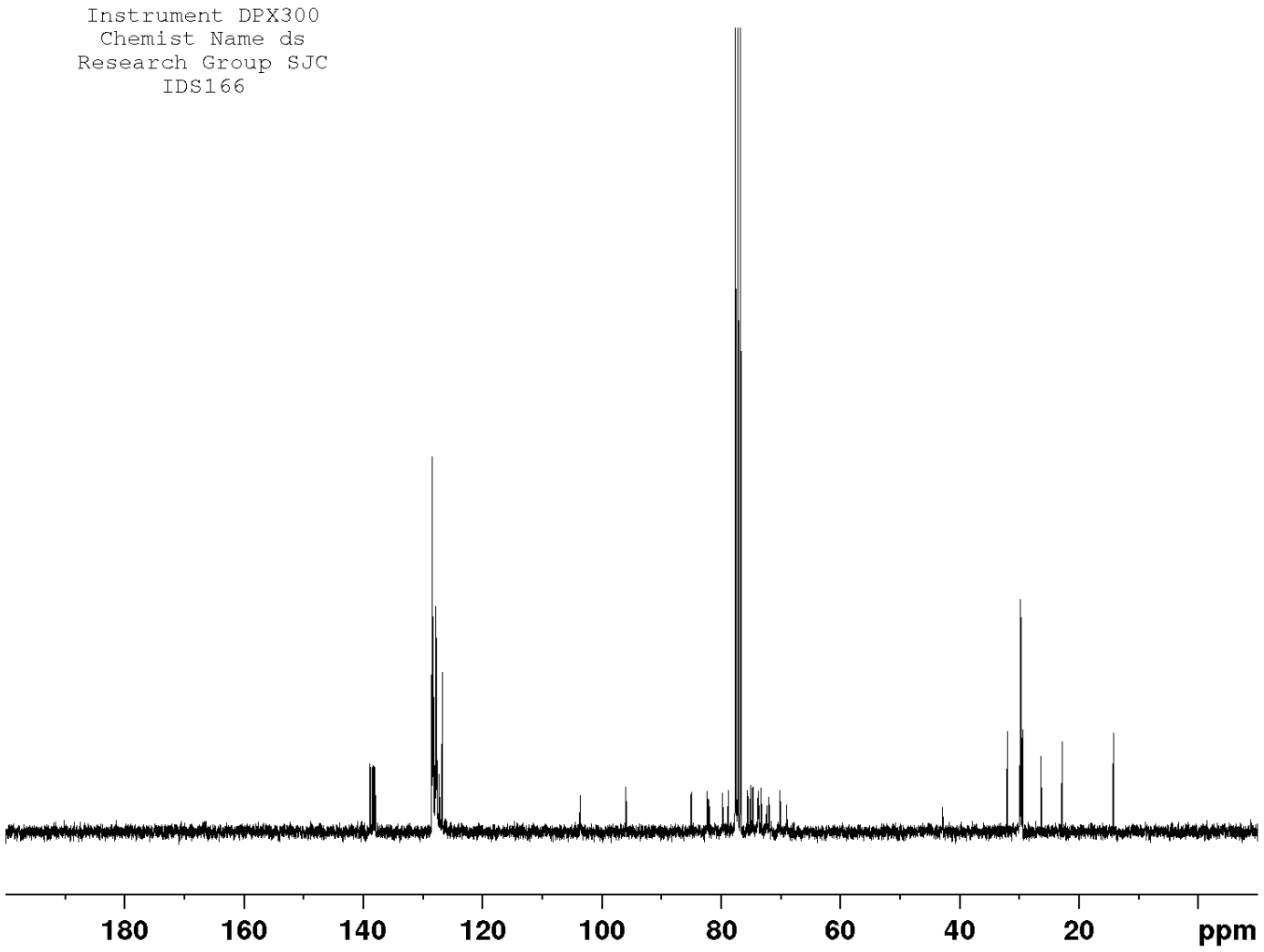
Current Data Parameters
NAME      IDS266 - Jan31-2013-29
EXPNO                  1
PROCNO                 1

F2 - Acquisition Parameters
Date_           20130131
Time               8.42
INSTRUM          DPX300
PROBHD   5 mm DUL 13C-1
PULPROG            zg60
TD                32768
SOLVENT           CDCl3
NS                   16
DS                    2
SWH            4789.272 Hz
FIDRES         0.146157 Hz
AQ             3.4209793 sec
RG                 80.6
DW              104.400 usec
DE                6.00 usec
TE                300.0 K
D1           1.00000000 sec
TD0                   1

======== CHANNEL f1 ========
NUC1                  1H
P1                16.50 usec
PL1               -6.00 dB
SFO1        300.1315007 MHz

F2 - Processing parameters
SI                32768
SF          300.1300105 MHz
WDW                  EM
SSB      0
LB                0.30 Hz
GB       0
PC                1.00
```

n-Dodecyl-(2,3,6,2',3',4'-*O*-hexabenzyl-6'-deoxy-6'-amino)-β-D-maltoside **84**




```
Current Data Parameters
NAME      IDS266 - Jan31-2013-29
EXPNO                  5
PROCNO                 1

F2 - Acquisition Parameters
Date_           20130131
Time               9.16
INSTRUM           DPX300
PROBHD   5 mm DUL 13C-1
PULPROG           zgpg30
TD                65536
SOLVENT           CDCl3
NS                  400
DS                    4
SWH           17985.611 Hz
FIDRES         0.274439 Hz
AQ             1.8219008 sec
RG                9195.2
DW                27.800 usec
DE                 6.00 usec
TE                300.0 K
D1            2.00000000 sec
d11           0.03000000 sec
DELTA         1.89999998 sec
TD0                    1

======== CHANNEL f1 ========
NUC1                  13C
P1                   8.25 usec
PL1                 -2.40 dB
SFO1          75.4752953 MHz

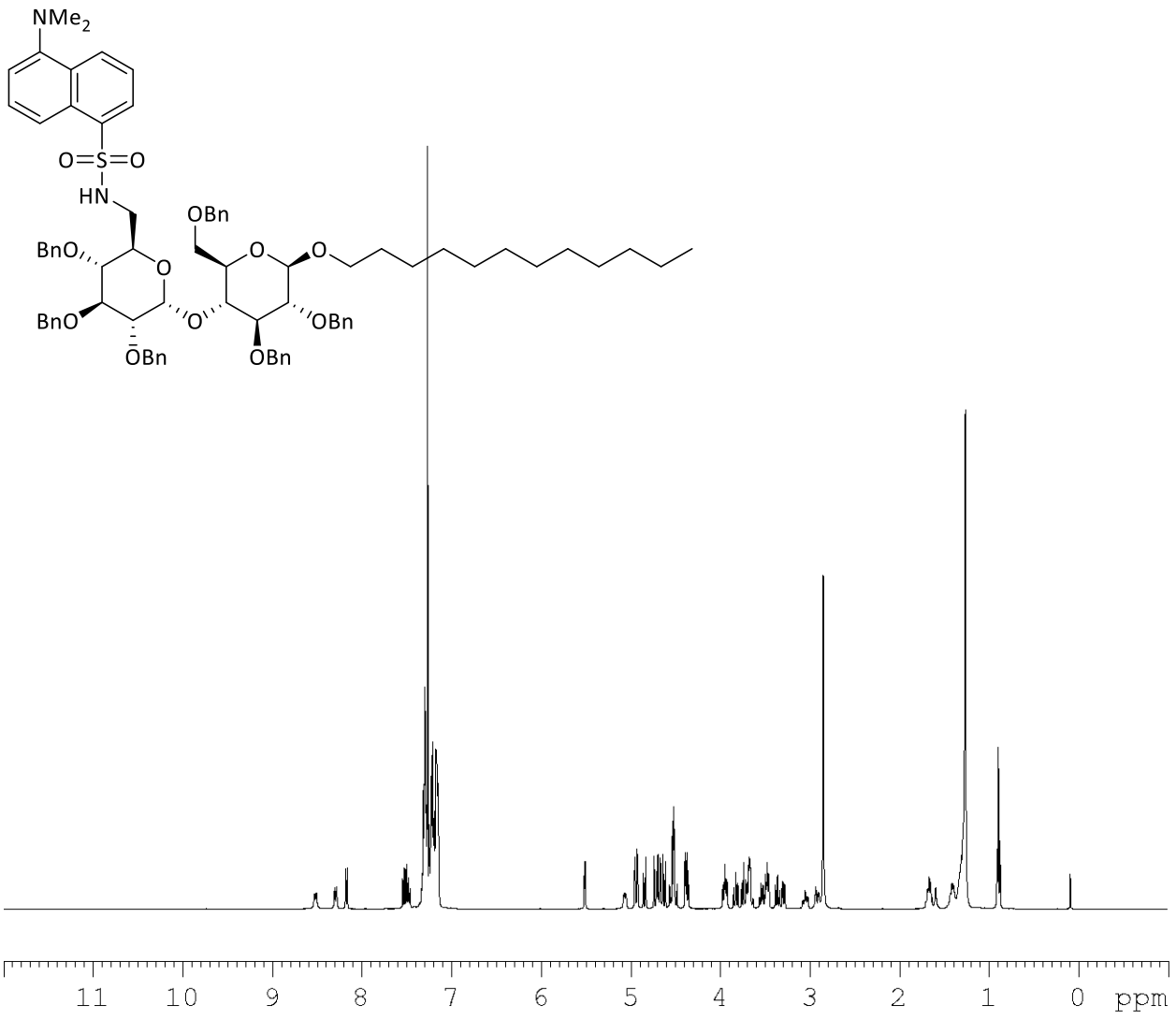
======== CHANNEL f2 ========
CPDPRG[2         waltz16
NUC2                  1H
PCPD2              80.00 usec
PL2                -6.00 dB
PL12               11.00 dB
PL13               11.00 dB
SFO2         300.1312005 MHz

F1 - Acquisition parameters
TD                   128
SFO1           75.47528 MHz
FIDRES        131.082214 Hz
SW               222.305 ppm
FnMODE                QF

F2 - Processing parameters
SI                 32768
SF            75.4677422 MHz
WDW                   EM
SSB       0
LB                 1.00 Hz
GB        0
PC                 1.40

F1 - Processing parameters
SI                 1024
MC2                  QF
SF            75.4677490 MHz
WDW                SINE
SSB       0
LB        0 Hz
GB        0
```

n-Dodecyl-(2,3,6,2',3',4'-*O*-hexabenzyl-6'-deoxy-6'-[*N*-dansyl]amino)-β-D-maltoside **85**




```
        Instrument AVN400
           Chemist ds
           Group SJC
            IDS269
h1acq.crl CDCl3 {C:\NMR} sjcgrp 48

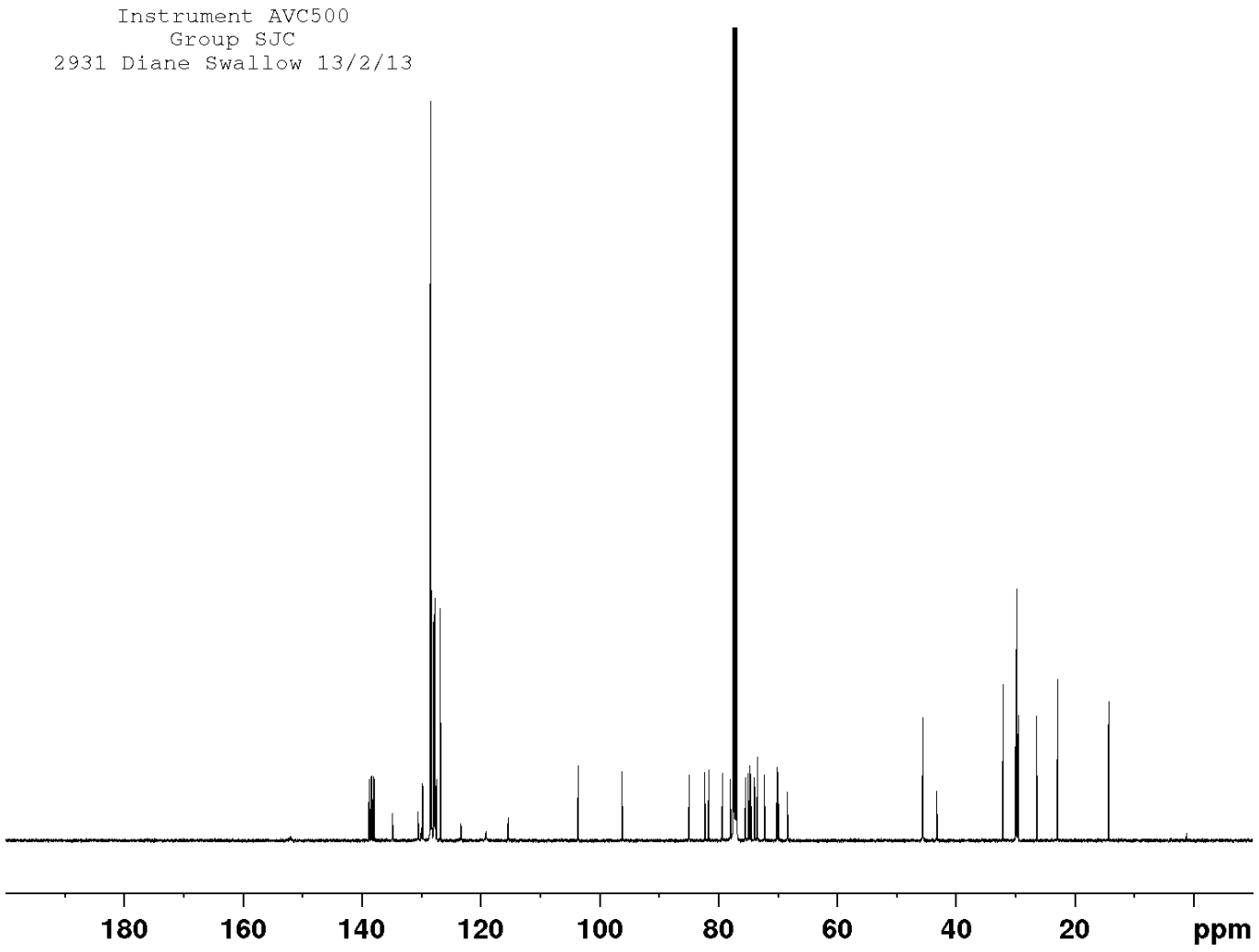
Current Data Parameters
NAME      IDS269 - Feb12-2013-48
EXPNO                  1
PROCNO                 1

F2 - Acquisition Parameters
Date_           20130212
Time              13.31
INSTRUM           avn400
PROBHD   5 mm PABBO BB/
PULPROG             zg60
TD                65536
SOLVENT           CDCl3
NS                   16
DS                    2
SWH            8012.820 Hz
FIDRES         0.122266 Hz
AQ             4.0894465 sec
RG                45.32
DW               62.400 usec
DE                6.50 usec
TE               294.5 K
D1            1.00000000 sec
TD0                   1

======== CHANNEL f1 ========
SFO1         400.2524015 MHz
NUC1                 1H
P1                12.65 usec
PLW1         16.70000076 W

F2 - Processing parameters
SI                32768
SF           400.2500109 MHz
WDW                  EM
SSB       0
LB                0.30 Hz
GB        0
PC                1.00
```

n-Dodecyl-(2,3,6,2',3',4'-*O*-hexabenzyl-6'-deoxy-6'-[*N*-dansyl]amino)-β-D-maltoside **85**




```
Current Data Parameters
NAME      IDS269 - ds29311302
EXPNO                       4
PROCNO                      1

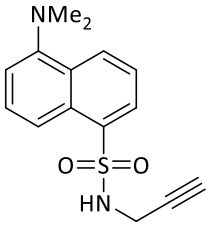
F2 - Acquisition Parameters
Date_            20130214
Time                11.13
INSTRUM           avc500
PROBHD   5 mm CPDUL 13C
PULPROG           zgpg30
TD                65536
SOLVENT           CDCl3
NS                  256
DS                    2
SWH           31250.000 Hz
FIDRES         0.476837 Hz
AQ             1.0485760 sec
RG                  912
DW               16.000 usec
DE               20.00 usec
TE               298.0 K
D1           2.00000000 sec
D11          0.03000000 sec
TD0                   1

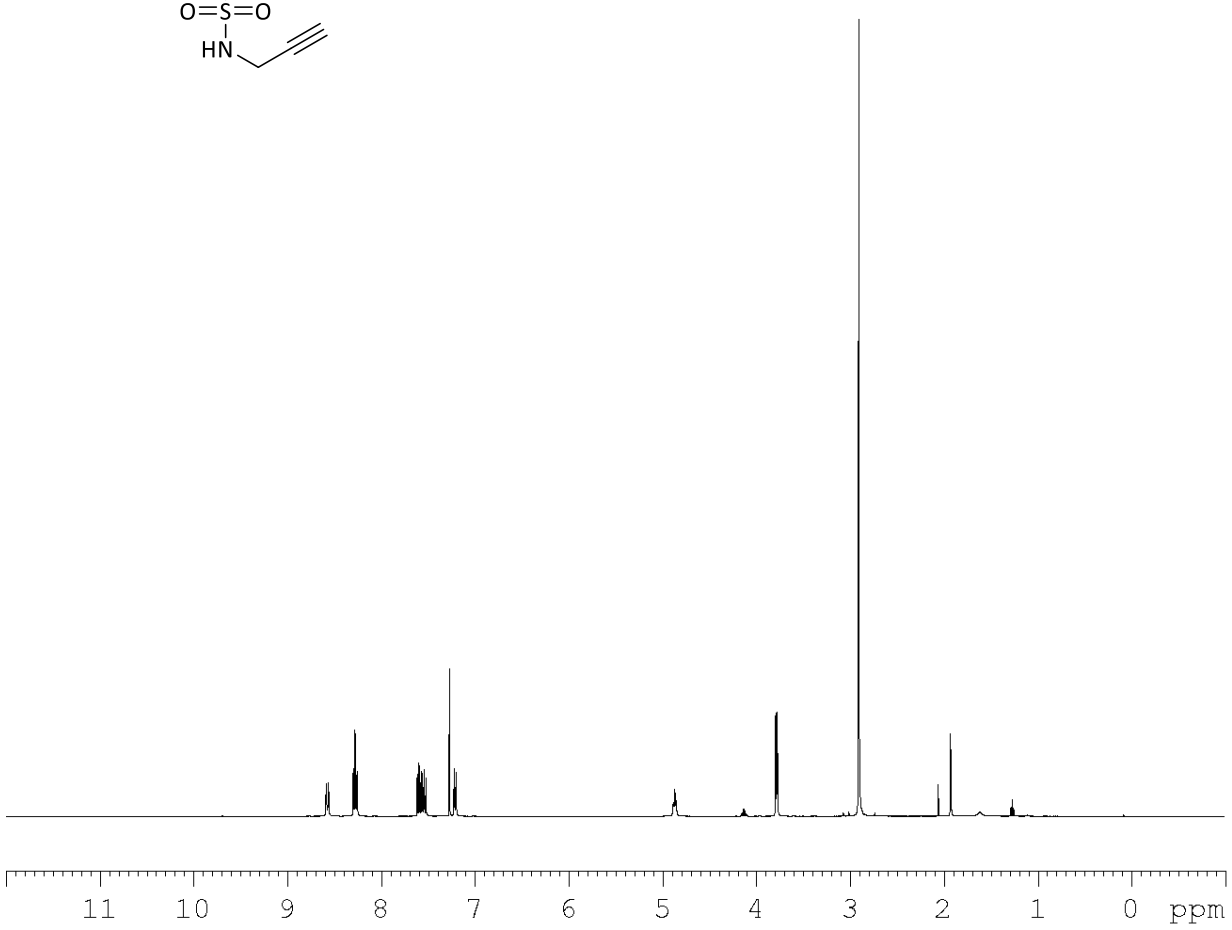
======== CHANNEL f1 ========
NUC1                 13C
P1                10.25 usec
PL1                8.00 dB
PL1W         1.62029624 W
SFO1        125.8131151 MHz

======== CHANNEL f2 ========
CPDPRG[2         waltz16
NUC2                 1H
PCPD2             80.00 usec
PL2                6.00 dB
PL12               23.56 dB
PL13               29.56 dB
PL2W          0.95905519 W
PL12W         0.01682068 W
PL13W         0.00422516 W
SFO2        500.3020012 MHz

F2 - Processing parameters
SI                32768
SF          125.8005251 MHz
WDW                  EM
SSB       0
LB                 1.00 Hz
GB        0
PC                 1.40
```

N-(Dansyl)prop-2-yn-1-amine **55**






```
          Instrument DQX400
             Chemist ds
             Group SJC
          IDS293 - columned
h1acq.au CDCl3 {E:\NMR2013} sjcgrp 16

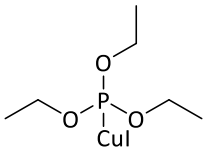

 Current Data Parameters
 NAME      IDS293 columned
           Apr11-2013-16
 EXPNO                   1
 PROCNO                  1

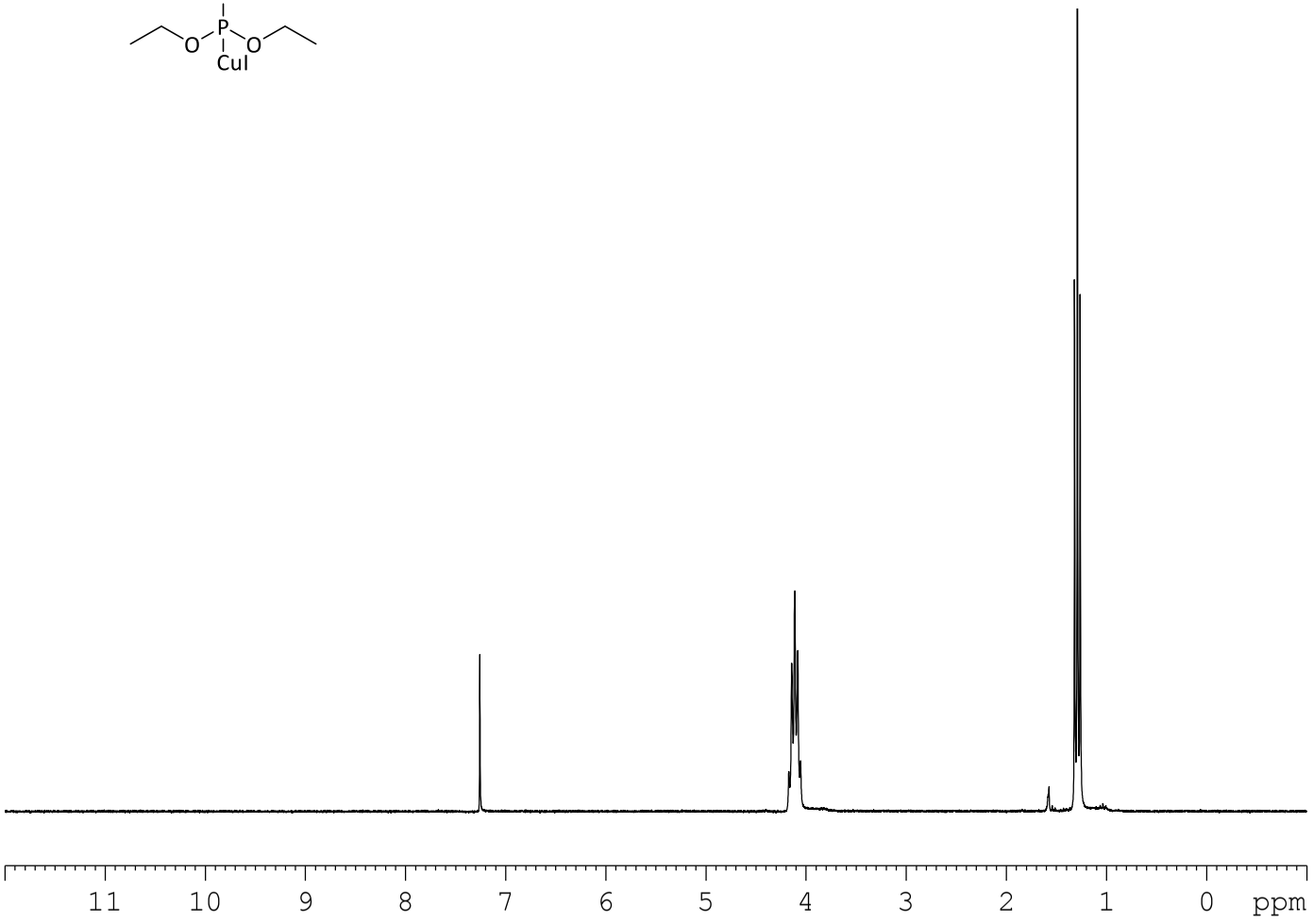
 F2 - Acquisition Parameters
 Date_            20130411
 Time                10.12
 INSTRUM             av400
 PROBHD   5 mm QNP 1H/13
 PULPROG              zg60
 TD                  65536
 SOLVENT             CDCl3
 NS                     16
 DS                      2
 SWH              8278.146 Hz
 FIDRES           0.126314 Hz
 AQ               3.9583745 sec
 RG                    128
 DW                 60.400 usec
 DE                   7.50 usec
 TE                  300.0 K
 D1             1.00000000 sec

 ========= CHANNEL f1 ========
 NUC1                   1H
 P1                  10.10 usec
 PL1       0 dB
 SFO1         400.2024714 MHz

 F2 - Processing parameters
 SI                  32768
 SF           400.2000028 MHz
 WDW                    EM
 SSB       0
 LB                  0.30 Hz
 GB        0
 PC                  1.00
```

Iodo(triethylphosphite)copper(I) **88**





DPX250 1H Acquisition

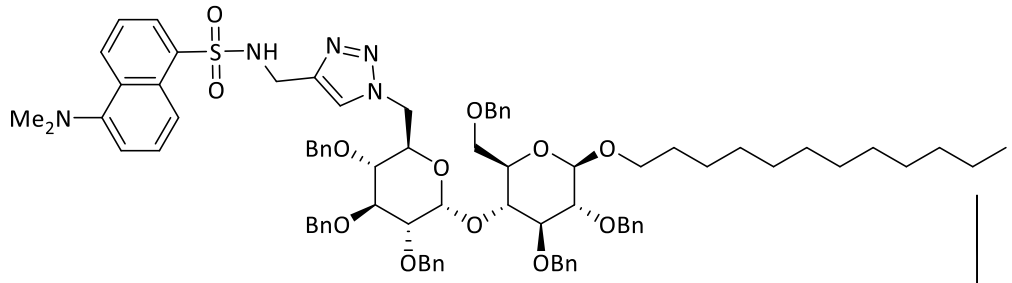
```
Current Data Parameters
NAME      IDS294 product
EXPNO                 1
PROCNO                1

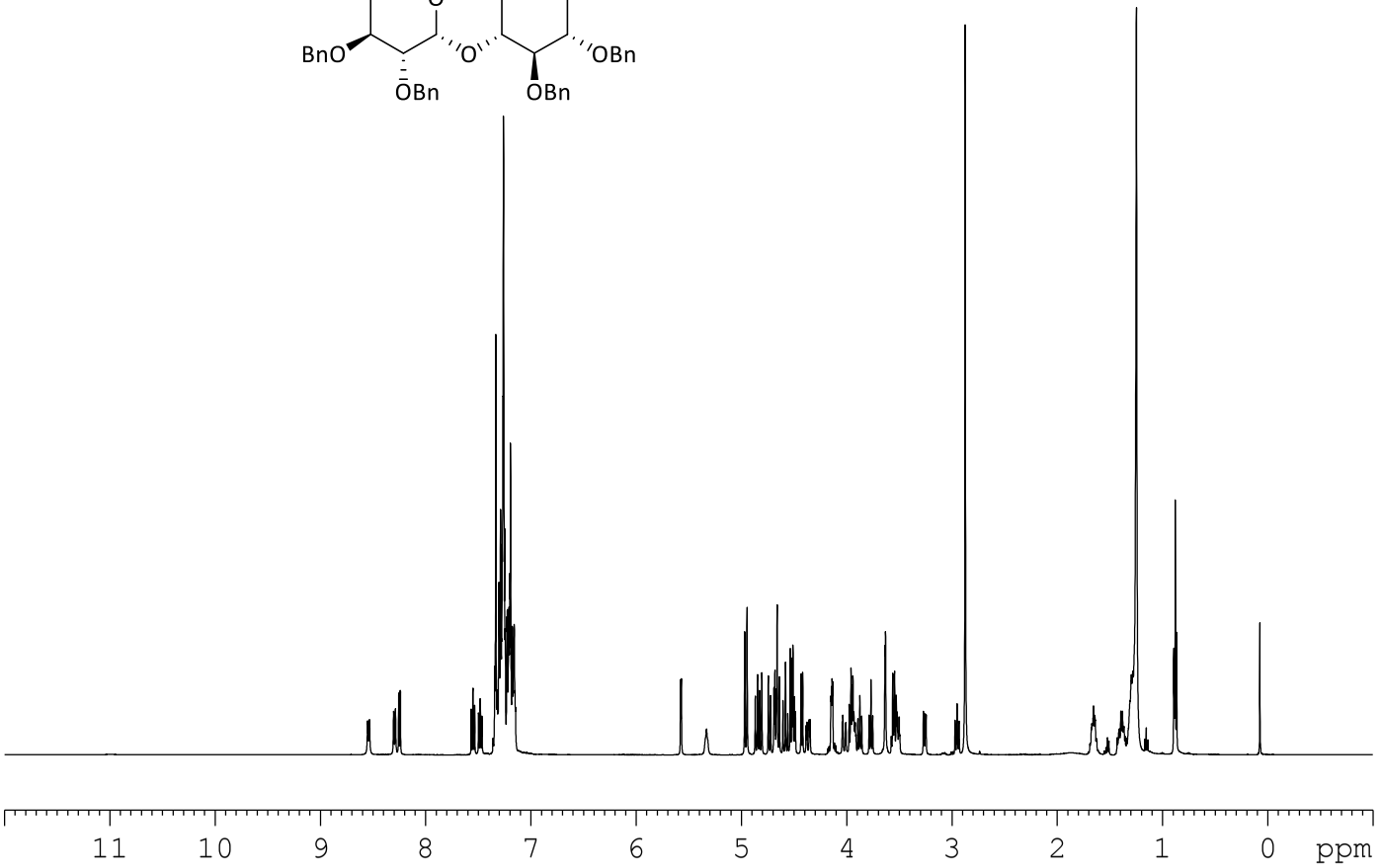
F2 - Acquisition Parameters
Date_          20130603
Time               13.15
INSTRUM          dpx250
PROBHD   5 mm Multinucl
PULPROG             zg60
TD                 32768
SOLVENT            CDCl3
NS                    22
DS                     2
SWH             4990.020 Hz
FIDRES          0.152283 Hz
AQ             3.2833536 sec
RG                   512
DW               100.200 usec
DE                 6.00 usec
TE                295.1 K
D1           1.00000000 sec

======== CHANNEL f1 ========
NUC1                  1H
P1                10.00 usec
PL1                3.00 dB
SFO1        250.1312507 MHz

F2 - Processing parameters
SI                 16384
SF          250.1300151 MHz
WDW                   no
SSB       0
LB        0 Hz
GB        0
PC                  1.00
```

n-Dodecyl-(2,3,6,2',3',4'-*O*-hexabenzyl-6'-deoxy-6'-[4-{*N*-dansyl}-aminomethyl-1*H*-1,2,3-triazol-1-yl])-β-D-maltoside **89**






```
DRX500 BBO Probe IDS295+6
acqbbo CDCl3 {C:\NMR} sjcogroup

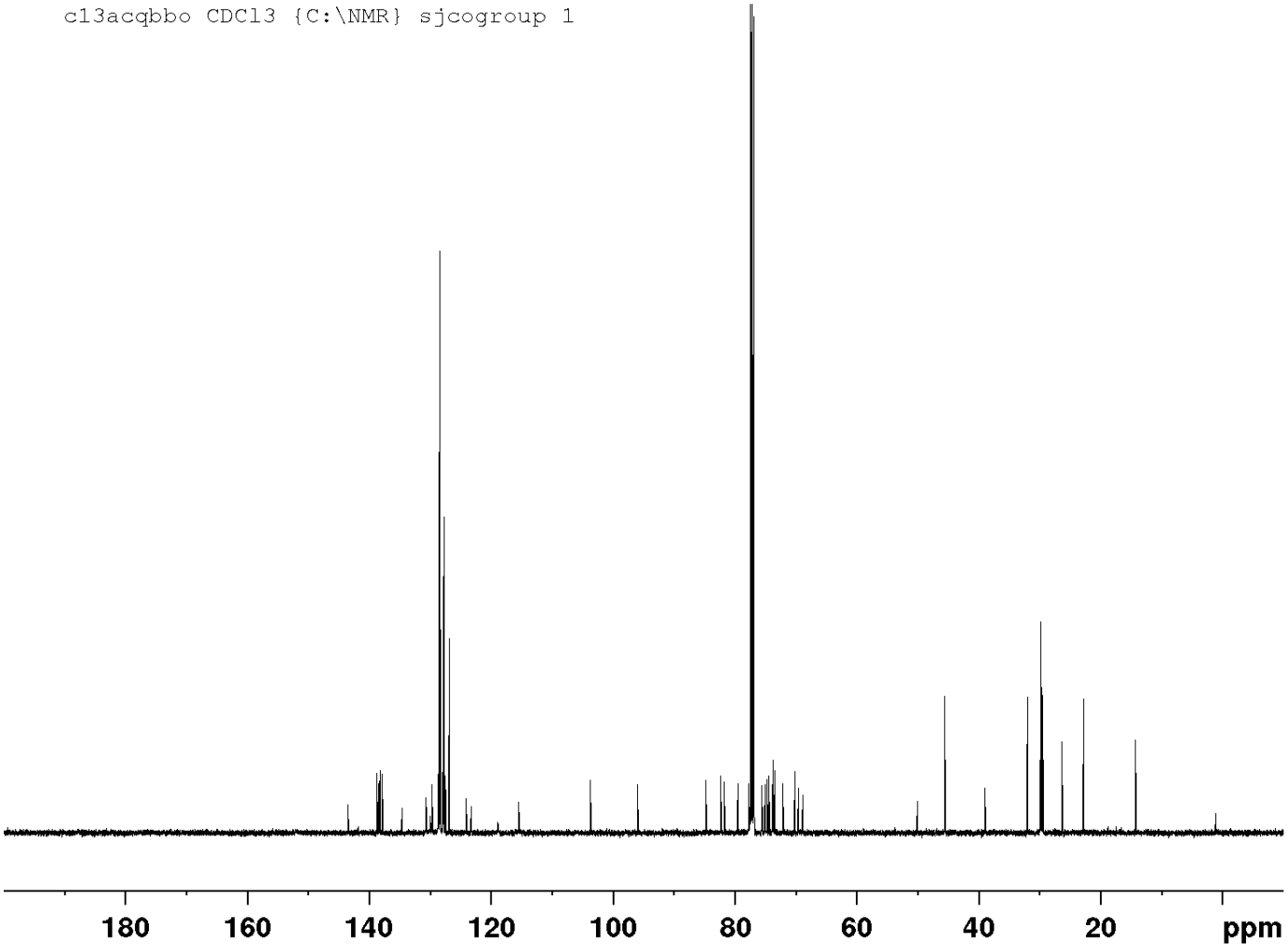
Current Data Parameters
NAME              IDS295+6
EXPNO                    1
PROCNO                   1

F2 - Acquisition Parameters
Date_              20130422
Time                 18.21
INSTRUM              drx500
PROBHD   5 mm PABBO BB/
PULPROG                zg60
TD                    65536
SOLVENT               CDCl3
NS                       16
DS                        2
SWH              10330.578 Hz
FIDRES            0.157632 Hz
AQ                3.1719425 sec
RG                     50.8
DW                   48.400 usec
DE                   17.00 usec
TE                   297.7 K
D1              1.00000000 sec

======== CHANNEL f1 ========
NUC1                     1H
P1                   11.00 usec
PL1       0 dB
SFO1          500.1325007 MHz

F2 - Processing parameters
SI                    32768
SF            500.1300339 MHz
WDW                      EM
SSB       0
LB                    0.30 Hz
GB        0
PC                    1.00
```

n-Dodecyl-(2,3,6,2',3',4'-*O*-hexabenzyl-6'-deoxy-6'-[4-{*N*-dansyl}-aminomethyl-1*H*-1,2,3-triazol-1-yl])-β-D-maltoside **89**




```
Current Data Parameters
NAME            IDS295+6
EXPNO                  3
PROCNO                 1

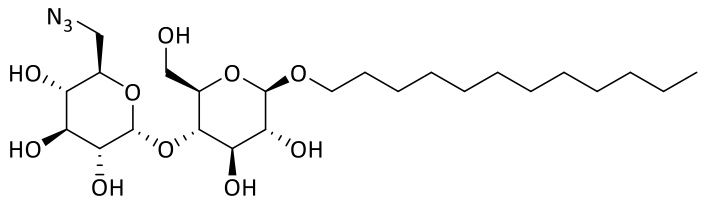
F2 - Acquisition Parameters
Date_           20130422
Time               19.53
INSTRUM           drx500
PROBHD   5 mm PABBO BB/
PULPROG           zgpg30
TD                 65536
SOLVENT            CDCl3
NS                  1500
DS                     4
SWH           30303.031 Hz
FIDRES         0.462388 Hz
AQ            1.0813440 sec
RG               4597.6
DW                16.500 usec
DE                11.00 usec
TE                297.7 K
D1           2.00000000 sec
d11          0.03000000 sec
DELTA        1.89999998 sec
TD0                    1

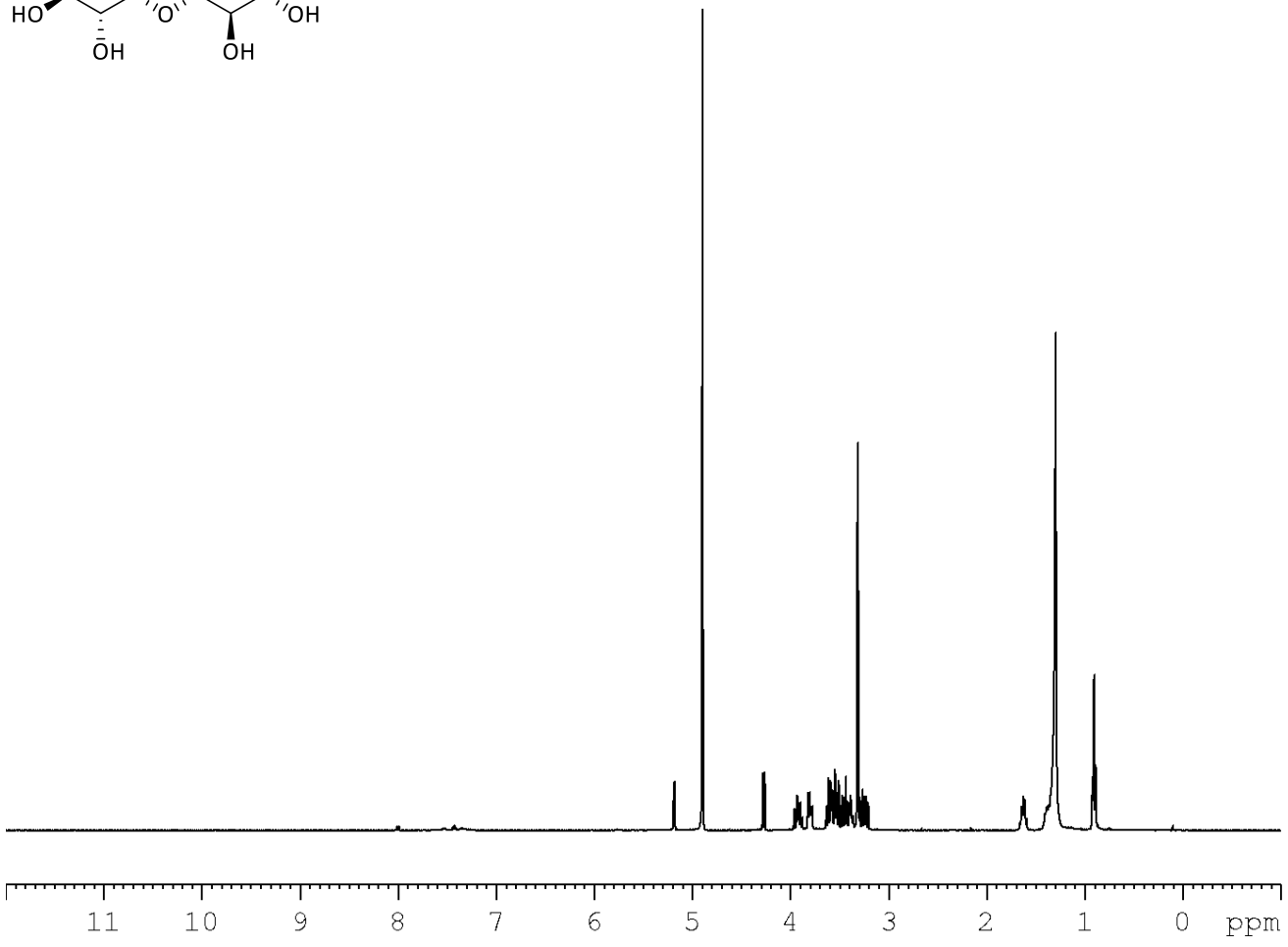
======== CHANNEL f1 ========
NUC1                  13C
P1                   6.80 usec
PL1                  5.00 dB
SFO1        125.7703148 MHz

======== CHANNEL f2 ========
CPDPRG[2         waltz16
NUC2                  1H
PCPD2              100.00 usec
PL2       0 dB
PL12                19.00 dB
PL13                23.00 dB
SFO2        500.1320005 MHz

F2 - Processing parameters
SI                 32768
SF          125.7577798 MHz
WDW                   EM
SSB       0
LB                  1.00 Hz
GB        0
PC                  1.40
```

n-Dodecyl-(6'-deoxy-6'-azido)- β-D-maltoside **90**






```
Instrument AVG400
    Group SJC
   Chemist ds
  IDS367 - columned
h1acq.crl MeOD {C:\NMR} sjcgrp 22

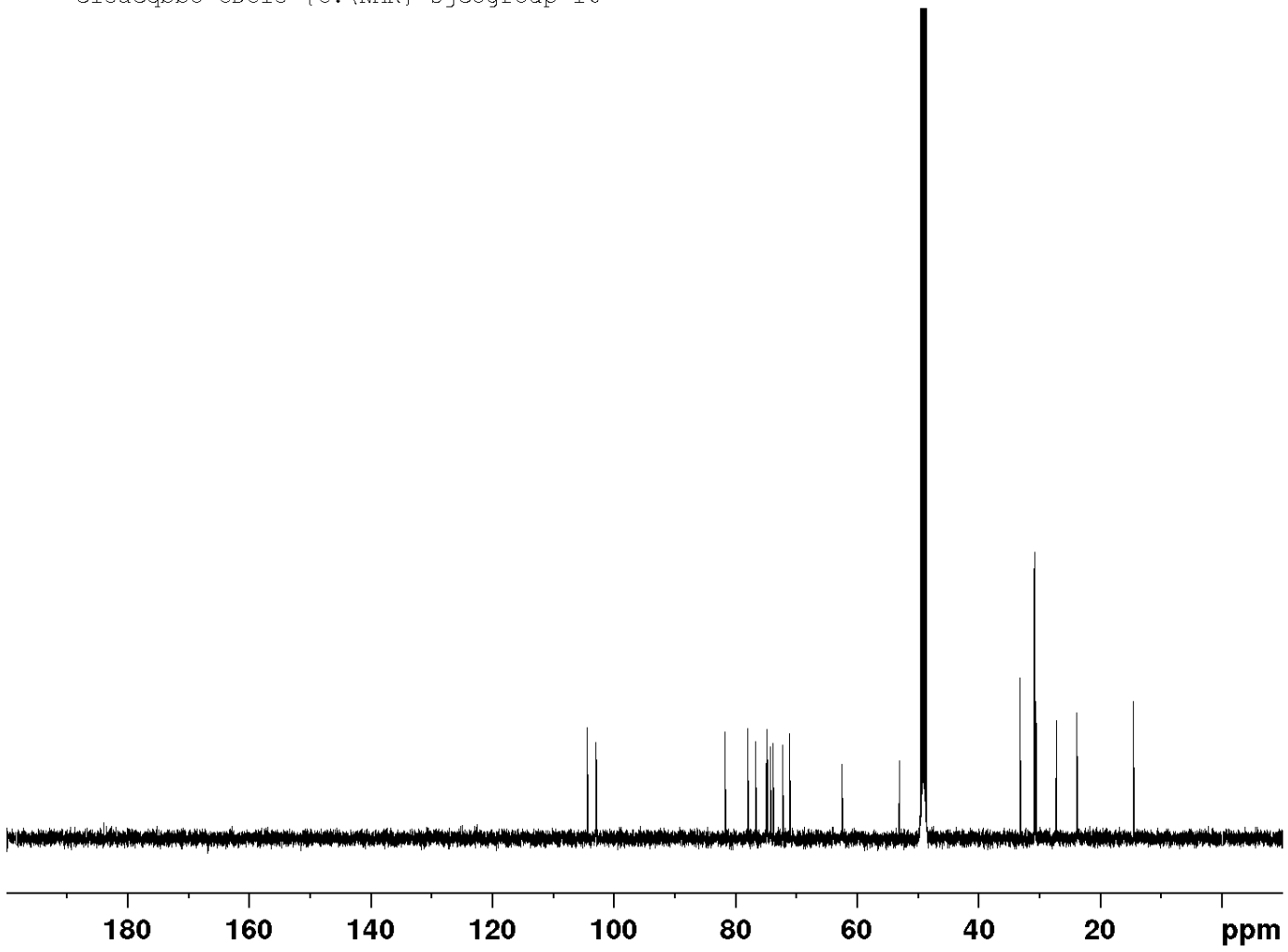
Current Data Parameters
NAME      IDS367 columned
           Nov25-2013-22
EXPNO                   1
PROCNO                  1

F2 - Acquisition Parameters
Date_           20131125
Time               11.50
INSTRUM           avg400
PROBHD   5 mm QNP 1H/13
PULPROG             zg30
TD                 65536
SOLVENT             MeOD
NS                    16
DS                     2
SWH           10000.000 Hz
FIDRES         0.152588 Hz
AQ            3.2767999 sec
RG               411.88
DW               50.000 usec
DE                6.50 usec
TE               294.1 K
D1          1.00000000 sec
TD0                    1

======== CHANNEL f1 ========
SFO1         400.2024714 MHz
NUC1                   1H
P1                12.23 usec
PLW1         11.30000019 W

F2 - Processing parameters
SI                 65536
SF           400.2000113 MHz
WDW                   EM
SSB       0
LB                0.30 Hz
GB        0
PC                1.00
```

n-Dodecyl-(6'-deoxy-6'-azido)- β-D-maltoside **90**




```
Current Data Parameters
NAME            IDS367i
EXPNO                 4
PROCNO                1

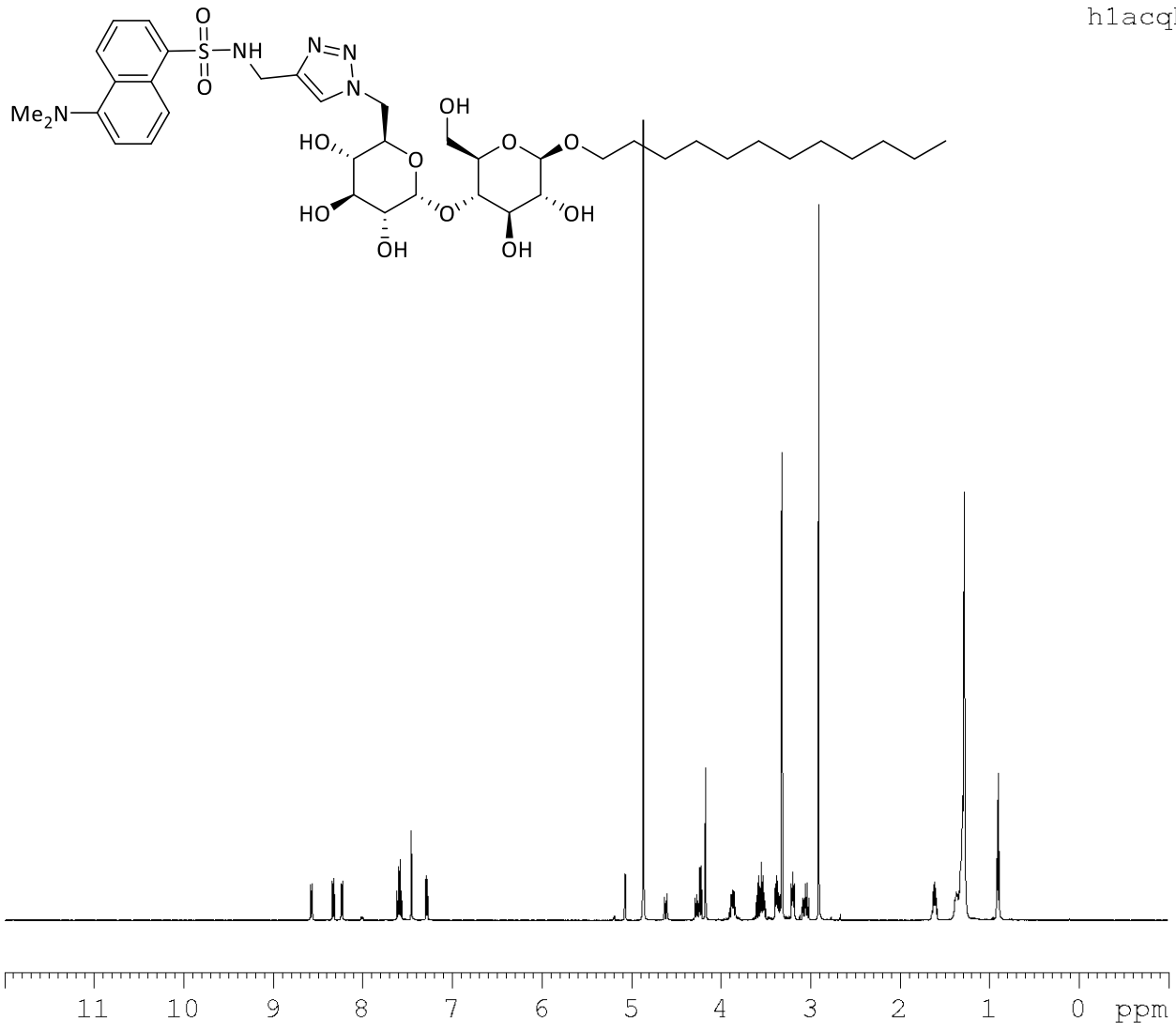
F2 - Acquisition Parameters
Date_          20131126
Time               5.39
INSTRUM          drx500
PROBHD   5 mm PABBO BB/
PULPROG          zgpg30
TD                65536
SOLVENT           CDCl3
NS                 3072
DS                    4
SWH           30303.031 Hz
FIDRES         0.462388 Hz
AQ            1.0813440 sec
RG                 4096
DW               16.500 usec
DE               11.00 usec
TE                297.7 K
D1           2.00000000 sec
d11          0.03000000 sec
DELTA        1.89999998 sec
TD0                   1

======== CHANNEL f1 ========
NUC1                 13C
P1                6.80 usec
PL1               5.00 dB
SFO1        125.7703148 MHz

======== CHANNEL f2 ========
CPDPRG[2         waltz16
NUC2                 1H
PCPD2            100.00 usec
PL2       0 dB
PL12              19.00 dB
PL13              23.00 dB
SFO2        500.1320005 MHz

F2 - Processing parameters
SI                32768
SF          125.7581114 MHz
WDW                  EM
SSB       0
LB                1.00 Hz
GB        0
PC                1.40
```

n-Dodecyl-(6'-deoxy-6'-[4-{*N*-Dansyl}-aminomethyl-1*H*-1,2,3-triazol-1-yl])-β-D-maltoside **42**




```
DRX500 BBO Probe IDS371v
  dilute sample for 1H
h1acqbbo MeOD {C:\NMR} sjcogroup 1

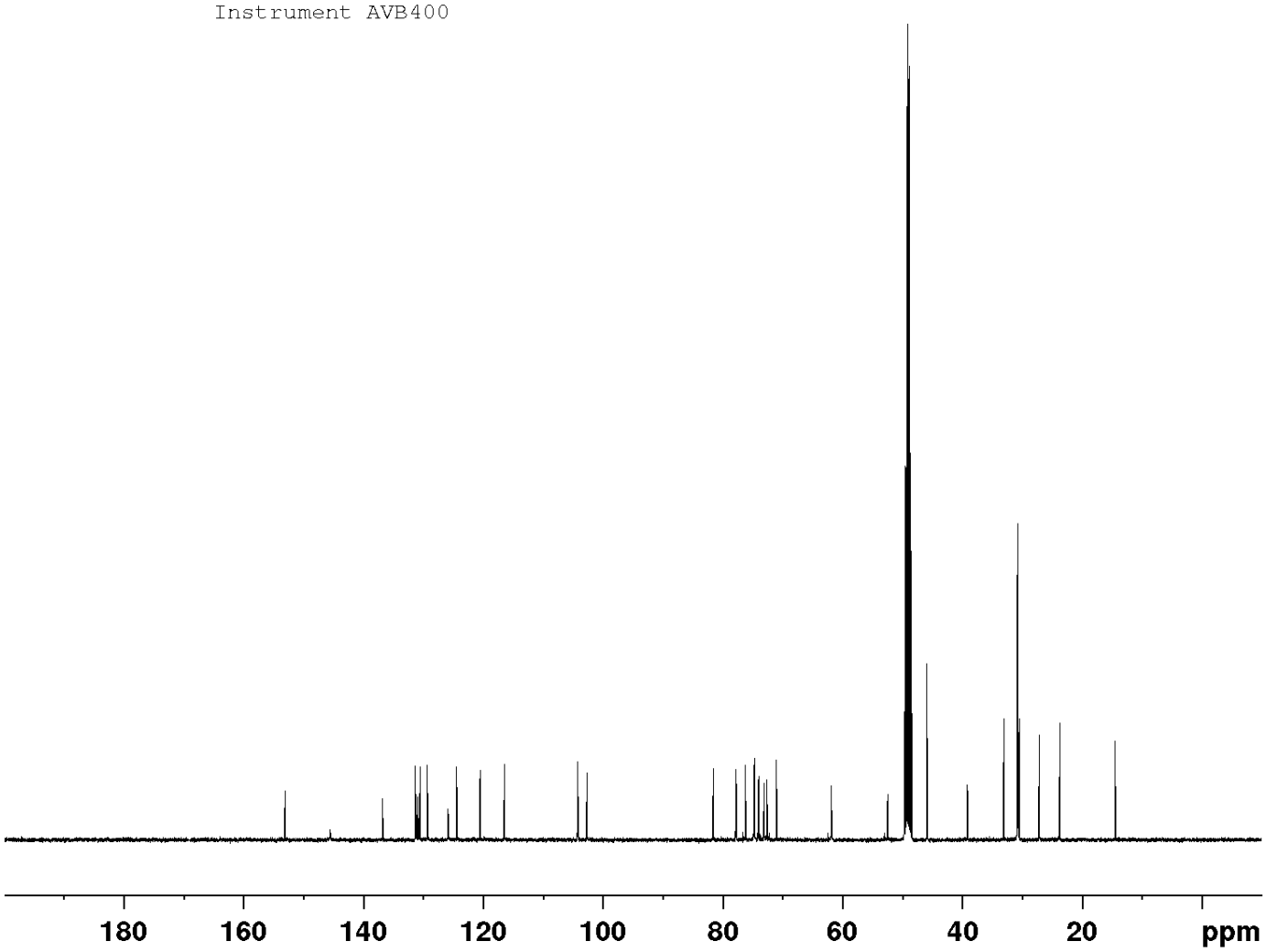
Current Data Parameters
NAME              IDS371v
EXPNO                   1
PROCNO                  1

F2 - Acquisition Parameters
Date_            20131210
Time                20.56
INSTRUM           drx500
PROBHD   5 mm PABBO BB/
PULPROG              zg60
TD                  65536
SOLVENT              MeOD
NS                     16
DS                      2
SWH            10330.578 Hz
FIDRES          0.157632 Hz
AQ             3.1719425 sec
RG                  203.2
DW                 48.400 usec
DE                 17.00 usec
TE                 300.0 K
D1           1.00000000 sec

======== CHANNEL f1 ========
NUC1                   1H
P1                  11.00 usec
PL1       0 dB
SFO1         500.1325007 MHz

F2 - Processing parameters
SI                  32768
SF           500.1300108 MHz
WDW                    EM
SSB       0
LB                  0.30 Hz
GB        0
PC                  1.00
```

n-Dodecyl-(6'-deoxy-6'-[4-{*N*-Dansyl}-aminomethyl-1*H*-1,2,3-triazol-1-yl])-β-D-maltoside **42**




```
Current Data Parameters
NAME           IDS371vii
EXPNO                  2
PROCNO                 1

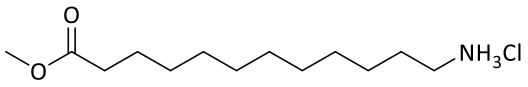
F2 - Acquisition Parameters
Date_           20131211
Time               21.31
INSTRUM          avb400
PROBHD   5 mm PABBO BB/
PULPROG          zgpg30
TD                65536
SOLVENT             MeOD
NS                 3072
DS                    4
SWH           24038.461 Hz
FIDRES         0.366798 Hz
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

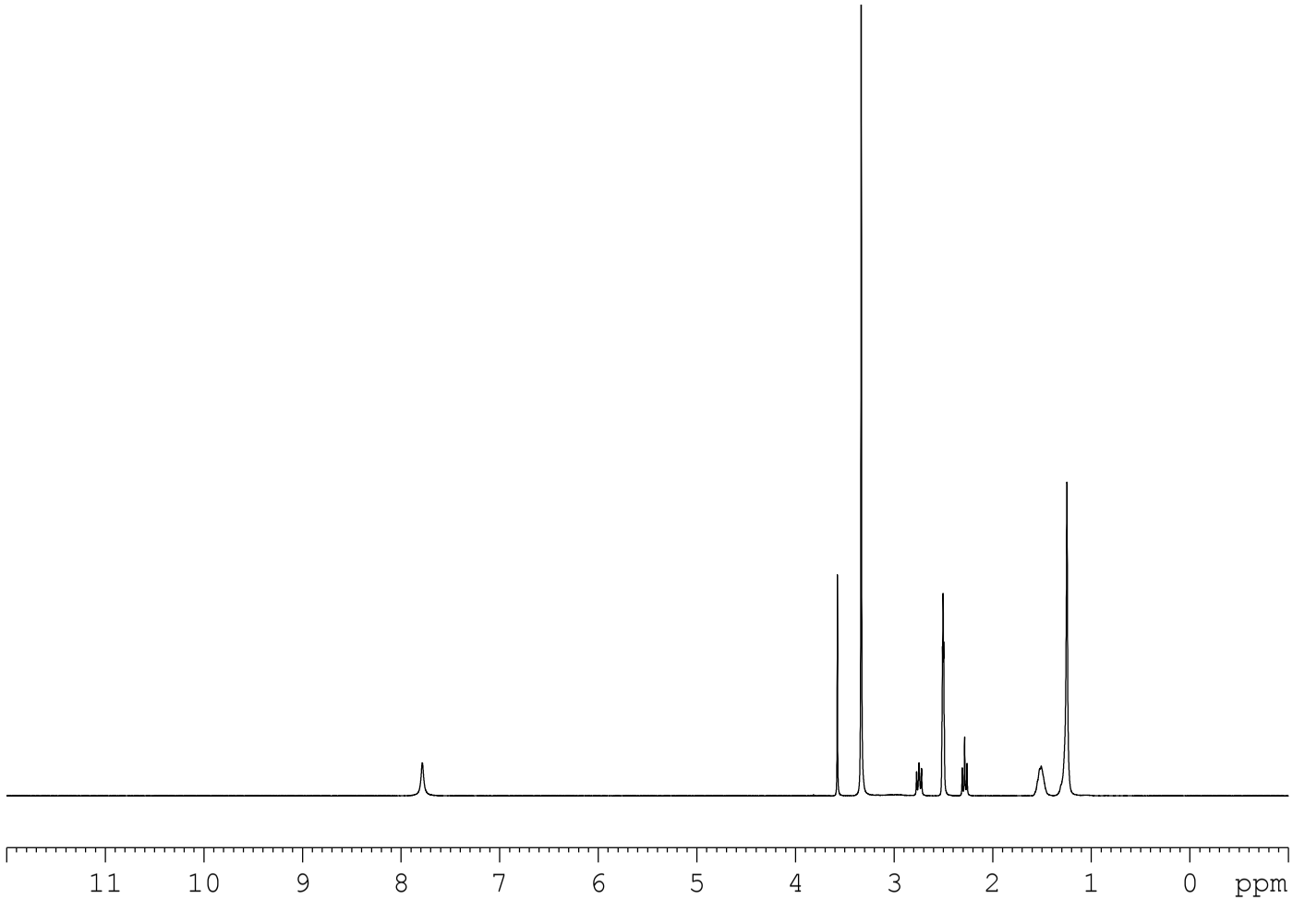
======== CHANNEL f1 ========
SFO1         100.6228303 MHz
NUC1                13C
P1                10.00 usec
PLW1         60.95399857 W

======== 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           100.6126299 MHz
WDW                   EM
SSB       0
LB                 1.00 Hz
GB        0
PC                 1.40
```

Methyl 12-aminododecanoate·hydrogen chloride **94**






```
Instrument DPX300
Chemist Name ds
Research Group SJC
IDS233

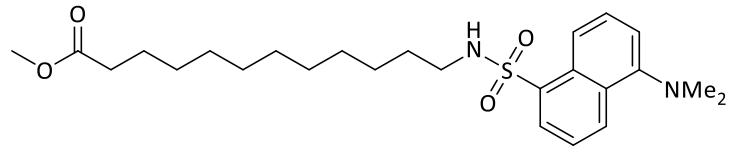
Current Data Parameters
NAME      IDS233 Oct16-2012-58
EXPNO                      1
PROCNO                     1

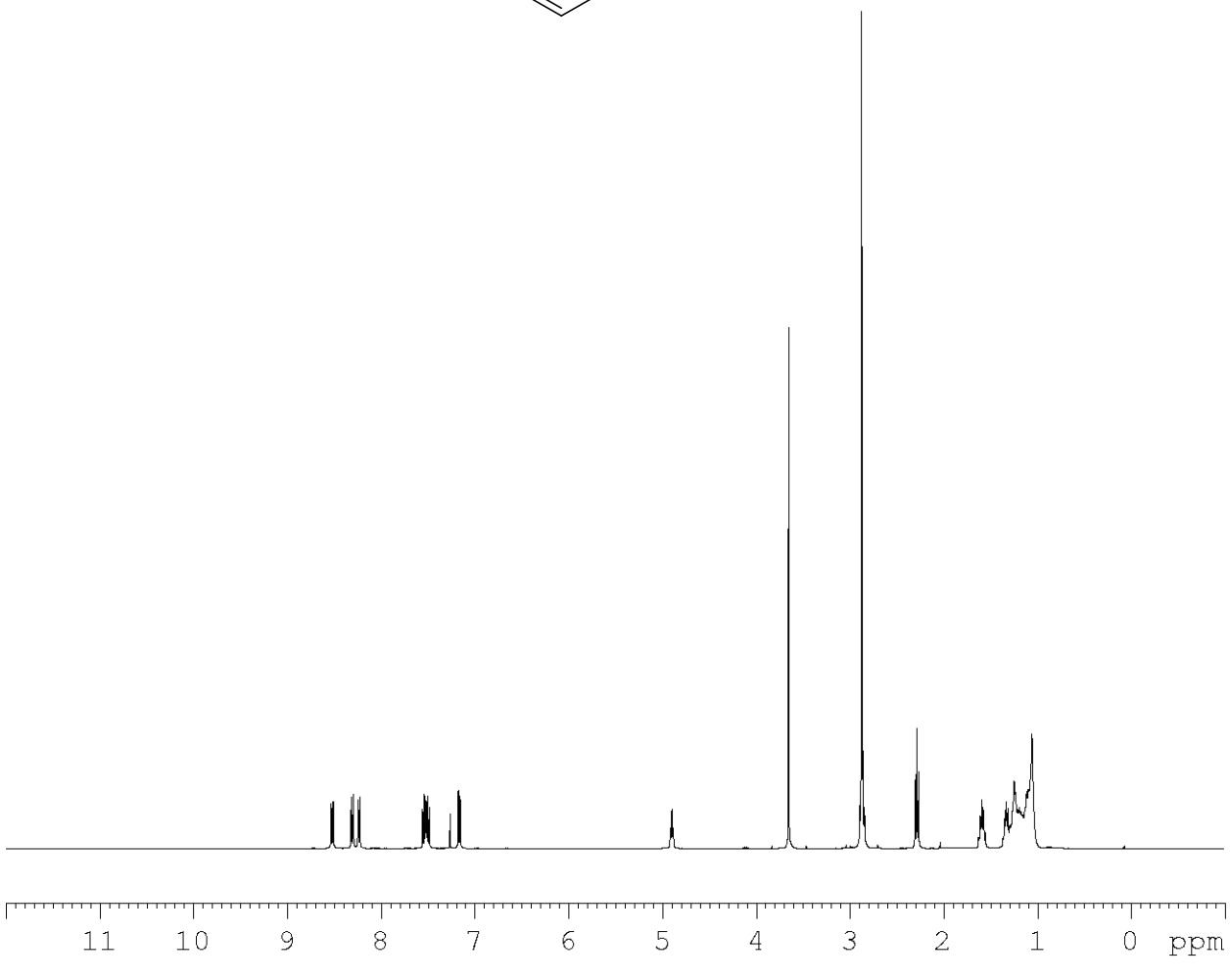
F2 - Acquisition Parameters
Date_              20121016
Time                   9.17
INSTRUM              DPX300
PROBHD    5 mm DUL 13C-1
PULPROG                zg60
TD                    32768
SOLVENT                DMSO
NS                       16
DS                        2
SWH               4789.272 Hz
FIDRES            0.146157 Hz
AQ                3.4209793 sec
RG                      512
DW                  104.400 usec
DE                    6.00 usec
TE                   300.0 K
D1             1.00000000 sec
TD0                       1

======== CHANNEL f1 ========
NUC1                      1H
P1                    16.50 usec
PL1                   -6.00 dB
SFO1          300.1315007 MHz

F2 - Processing parameters
SI                    32768
SF            300.1300000 MHz
WDW                      EM
SSB       0
LB                    0.30 Hz
GB        0
PC                    1.00
```

Methyl 12-(*N*-dansyl)aminododecanoate **95**






```
          Instrument AVN400
            Chemist ds
            Group SJC
              IDS448
 h1acq.crl CDCl3 {C:\NMR} sjcgrp 9

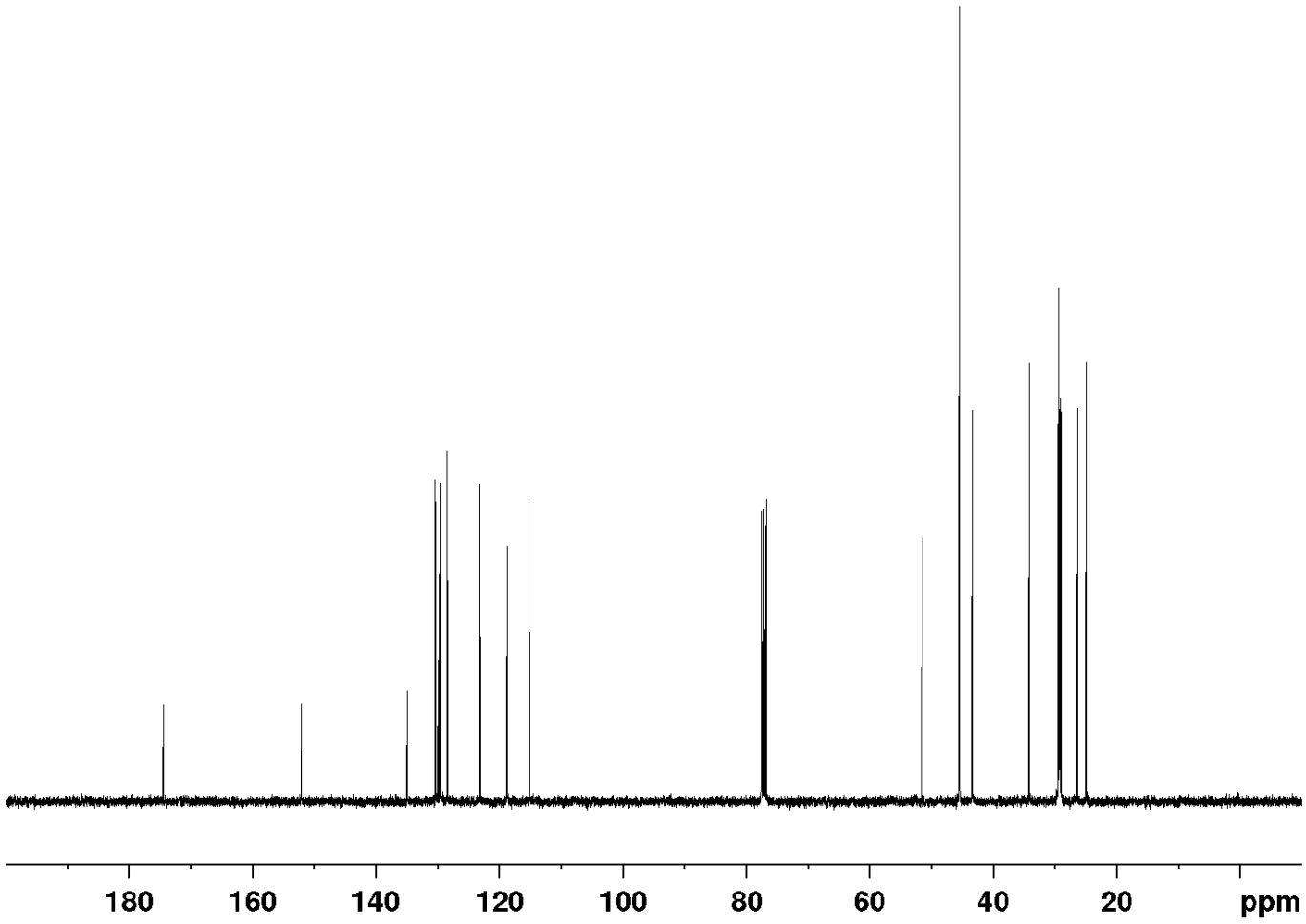
Current Data Parameters
NAME      IDS248 - Nov26-2012-9
EXPNO                     1
PROCNO                    1

F2 - Acquisition Parameters
Date_           20121126
Time                9.32
INSTRUM           avn400
PROBHD   5 mm PABBO BB/
PULPROG             zg60
TD                 65536
SOLVENT            CDCl3
NS                    16
DS                     2
SWH             8012.820 Hz
FIDRES          0.122266 Hz
AQ             4.0894465 sec
RG                 13.23
DW                62.400 usec
DE                 6.50 usec
TE                294.3 K
D1           1.00000000 sec
TD0                    1

======== CHANNEL f1 ========
SFO1         400.2524015 MHz
NUC1                   1H
P1                 12.65 usec
PLW1         16.70000076 W

F2 - Processing parameters
SI                 32768
SF           400.2500121 MHz
WDW                   EM
SSB       0
LB                 0.30 Hz
GB        0
PC                 1.00
```

Methyl 12-(*N*-dansyl)aminododecanoate **95**




```
Current Data Parameters
NAME      IDS248 - Nov26-2012-9
EXPNO                         5
PROCNO                        1

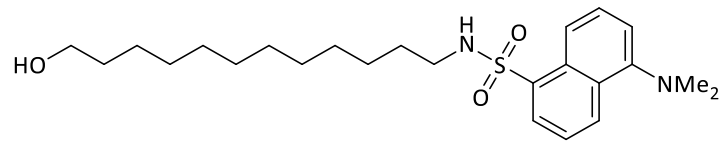
F2 - Acquisition Parameters
Date_              20121126
Time                  10.05
INSTRUM              avn400
PROBHD    5 mm PABBO BB/
PULPROG              zgpg30
TD                    32768
SOLVENT               CDCl3
NS                      256
DS                        4
SWH              26041.666 Hz
FIDRES            0.794729 Hz
AQ                0.6291456 sec
RG                   205.43
DW                   19.200 usec
DE                    6.50 usec
TE                   295.6 K
D1              1.00000000 sec
D11             0.03000000 sec
TD0                       1

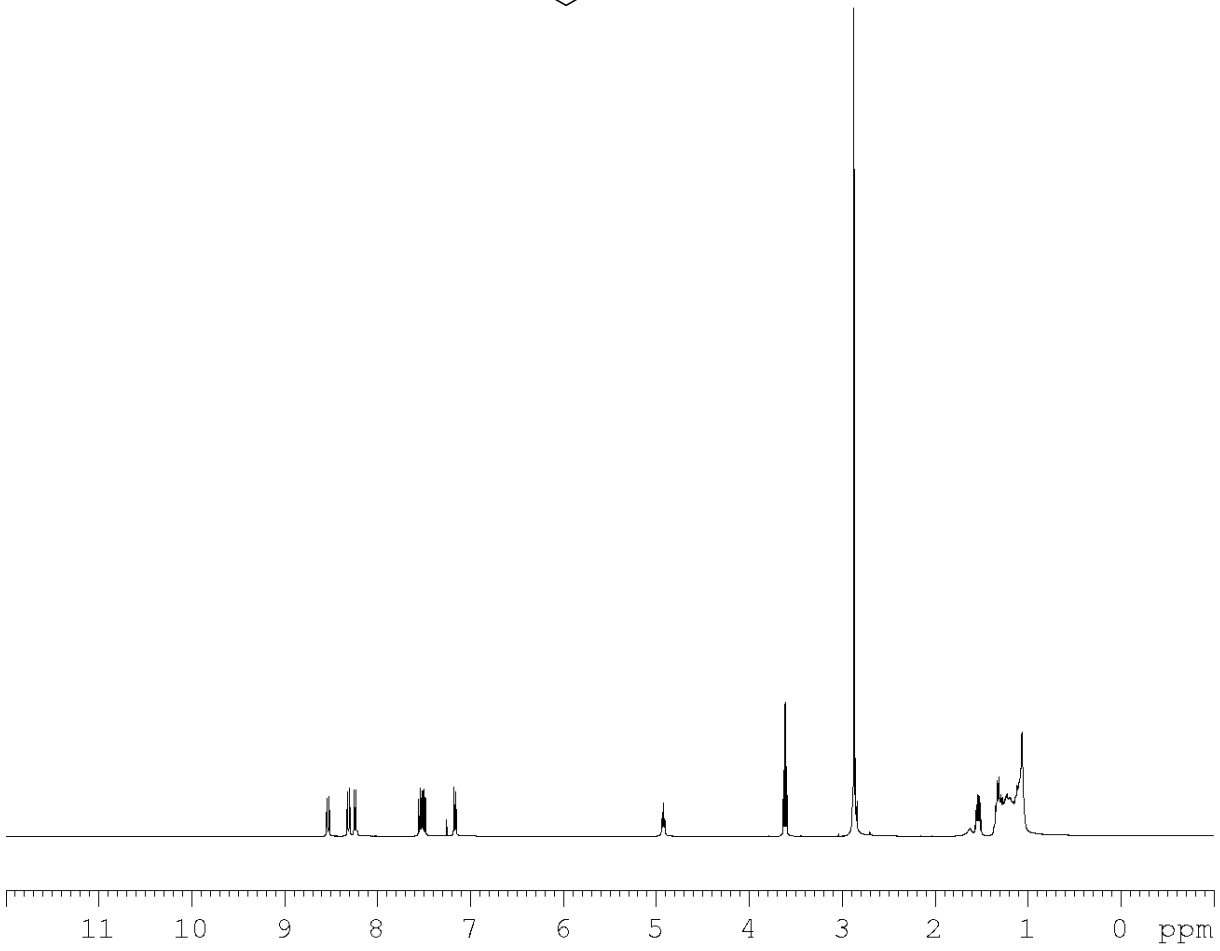
======== CHANNEL f1 ========
SFO1           100.6530073 MHz
NUC1                    13C
P1                     9.00 usec
PLW1           58.70000076 W

======== CHANNEL f2 ========
SFO2           400.2516010 MHz
NUC2                    1H
CPDPRG[2           waltz16
PCPD2                90.00 usec
PLW2           16.70000076 W
PLW12           0.32991999 W
PLW13           0.26723999 W

F2 - Processing parameters
SI                    32768
SF             100.6429369 MHz
WDW                      EM
SSB       0
LB                    1.00 Hz
GB        0
PC                    1.40
```

12-(*N*-Dansyl)aminododecanol **57**






```
          Instrument AVN400
             Chemist ds
             Group SJC
               IDS255
 h1acq.crl CDCl3 {C:\NMR} sjcgrp 22

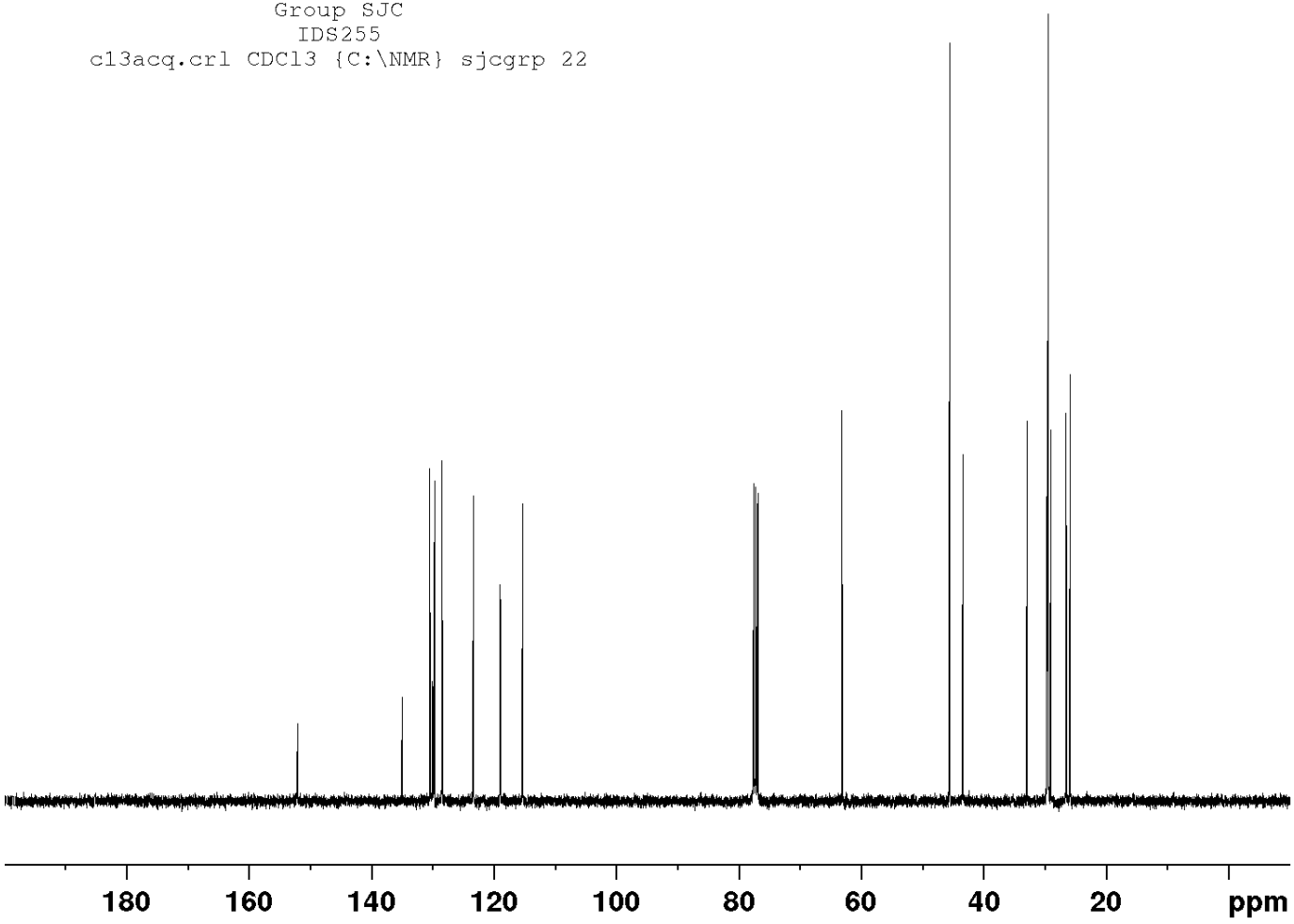
Current Data Parameters
NAME      IDS255 - Dec19-2012-22
EXPNO                  1
PROCNO                 1

F2 - Acquisition Parameters
Date_           20121220
Time                1.10
INSTRUM           avn400
PROBHD   5 mm PABBO BB/
PULPROG             zg60
TD                 65536
SOLVENT            CDCl3
NS                    16
DS                     2
SWH             8012.820 Hz
FIDRES          0.122266 Hz
AQ             4.0894465 sec
RG                 23.04
DW                62.400 usec
DE                 6.50 usec
TE                296.1 K
D1           1.00000000 sec
TD0                    1

======== CHANNEL f1 ========
SFO1         400.2524015 MHz
NUC1                  1H
P1                 12.65 usec
PLW1         16.70000076 W

F2 - Processing parameters
SI                 32768
SF          400.2500112 MHz
WDW                   EM
SSB       0
LB                 0.30 Hz
GB        0
PC                 1.00
```

12-(*N*-Dansyl)aminododecanol **57**




```
Current Data Parameters
NAME      IDS255 - Dec19-2012-22
EXPNO                  3
PROCNO                 1

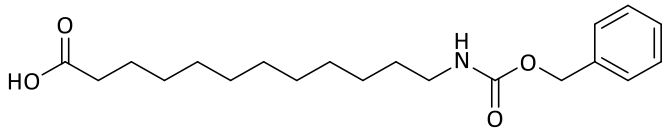
F2 - Acquisition Parameters
Date_           20121220
Time                1.23
INSTRUM           avn400
PROBHD   5 mm PABBO BB/
PULPROG           zgpg30
TD                32768
SOLVENT           CDCl3
NS                  256
DS                    4
SWH           26041.666 Hz
FIDRES         0.794729 Hz
AQ            0.6291456 sec
RG               205.43
DW               19.200 usec
DE                 6.50 usec
TE                297.2 K
D1           1.00000000 sec
D11          0.03000000 sec
TD0                   1

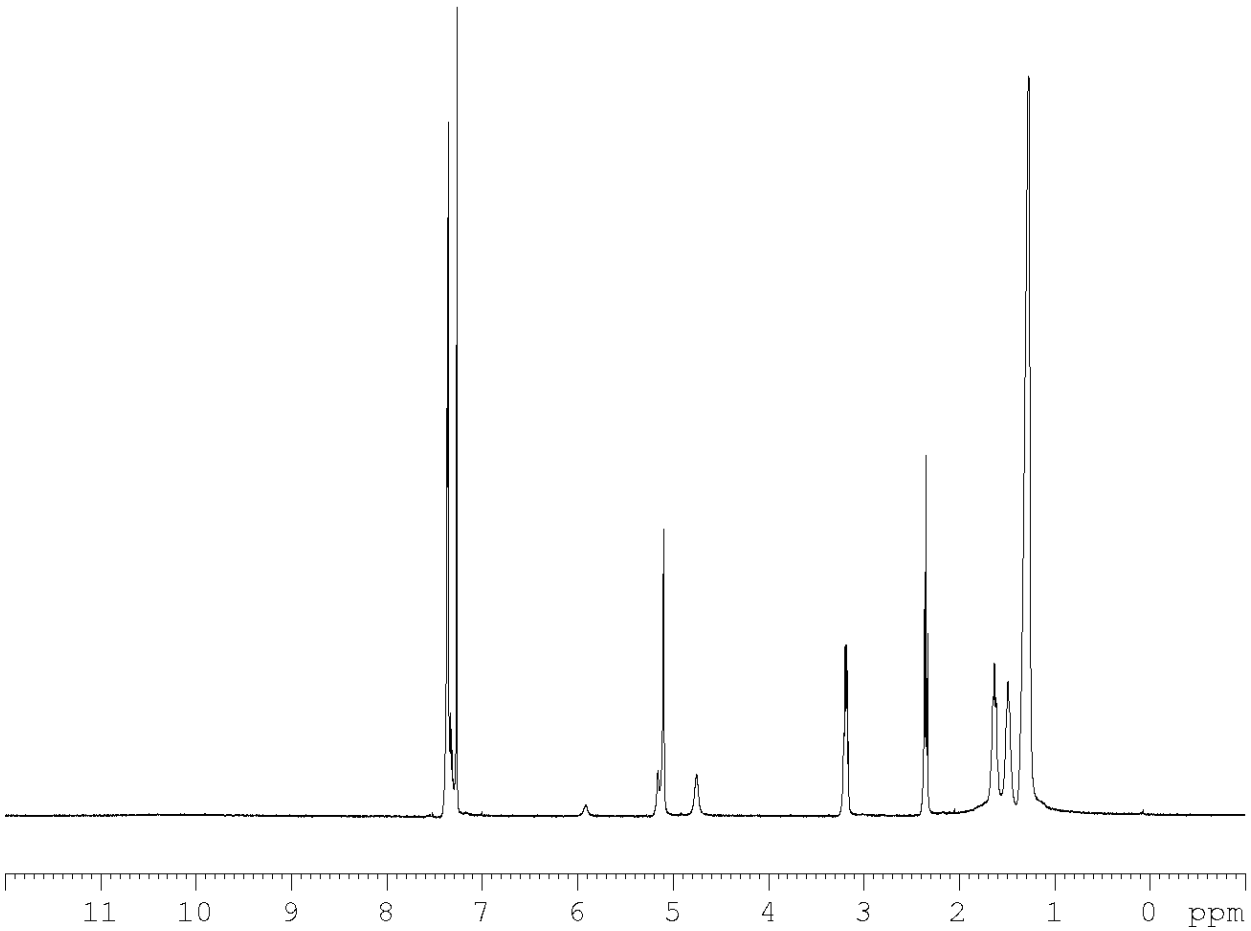
======== CHANNEL f1 ========
SFO1         100.6530073 MHz
NUC1                13C
P1                 9.00 usec
PLW1         58.70000076 W

======== CHANNEL f2 ========
SFO2         400.2516010 MHz
NUC2                1H
CPDPRG[2         waltz16
PCPD2              90.00 usec
PLW2         16.70000076 W
PLW12         0.32991999 W
PLW13         0.26723999 W

F2 - Processing parameters
SI                 32768
SF           100.6429354 MHz
WDW                  EM
SSB       0
LB                 1.00 Hz
GB        0
PC                 1.40
```

12-(*N*-Benzyloxycarbonyl)aminododecanoic acid **96**






```
Instrument DQX400
Chemist ds
Group SJC
IDS229 - recryst
h1acq.au CDCl3 {E:\NMR2013} sjcgrp 29

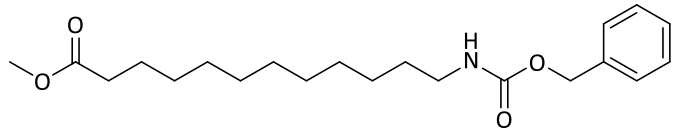
Current Data Parameters
NAME      IDS229 - Jan22-2013-29
EXPNO                  1
PROCNO                 1

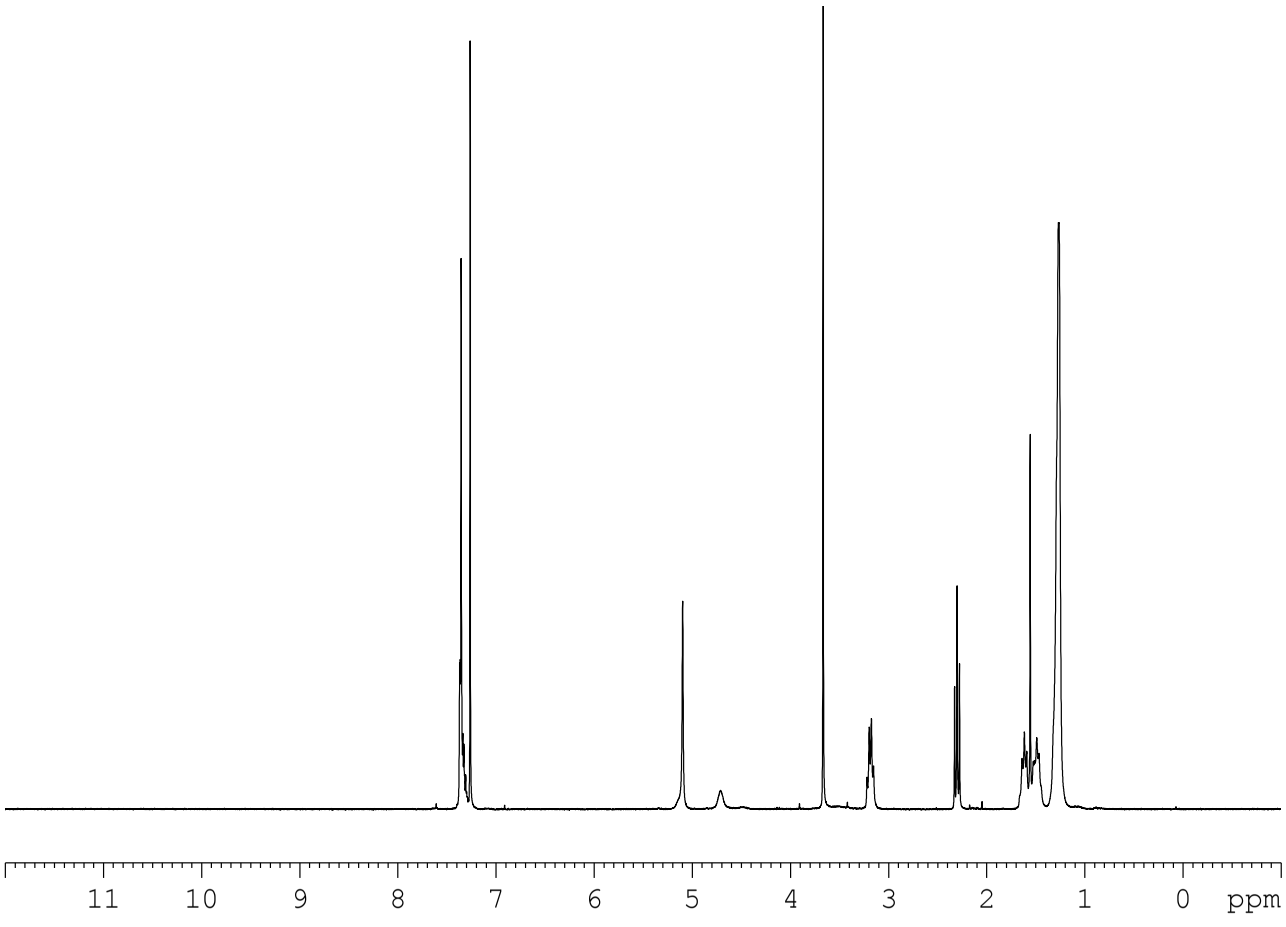
F2 - Acquisition Parameters
Date_           20130122
Time               12.55
INSTRUM            av400
PROBHD   5 mm QNP 1H/13
PULPROG             zg60
TD                 65536
SOLVENT            CDCl3
NS                    16
DS                     2
SWH             8278.146 Hz
FIDRES          0.126314 Hz
AQ             3.9583745 sec
RG                   256
DW                60.400 usec
DE                 7.50 usec
TE                294.4 K
D1           1.00000000 sec

======== CHANNEL f1 ========
NUC1                  1H
P1                10.10 usec
PL1       0 dB
SFO1         400.2024714 MHz

F2 - Processing parameters
SI                 32768
SF          400.2000063 MHz
WDW                   EM
SSB       0
LB                 0.30 Hz
GB        0
PC                 1.00
```

Methyl 12-(*N*-benzyloxycarbonyl)aminododecanoate **97**






```
                Instrument DPX300
                 Chemist Name ds
               Research Group SJC
                 IDS264 - crude

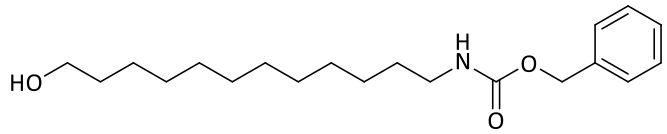
Current Data Parameters
NAME      IDS264 crude - Jan22-2013-49
EXPNO                  1
PROCNO                 1

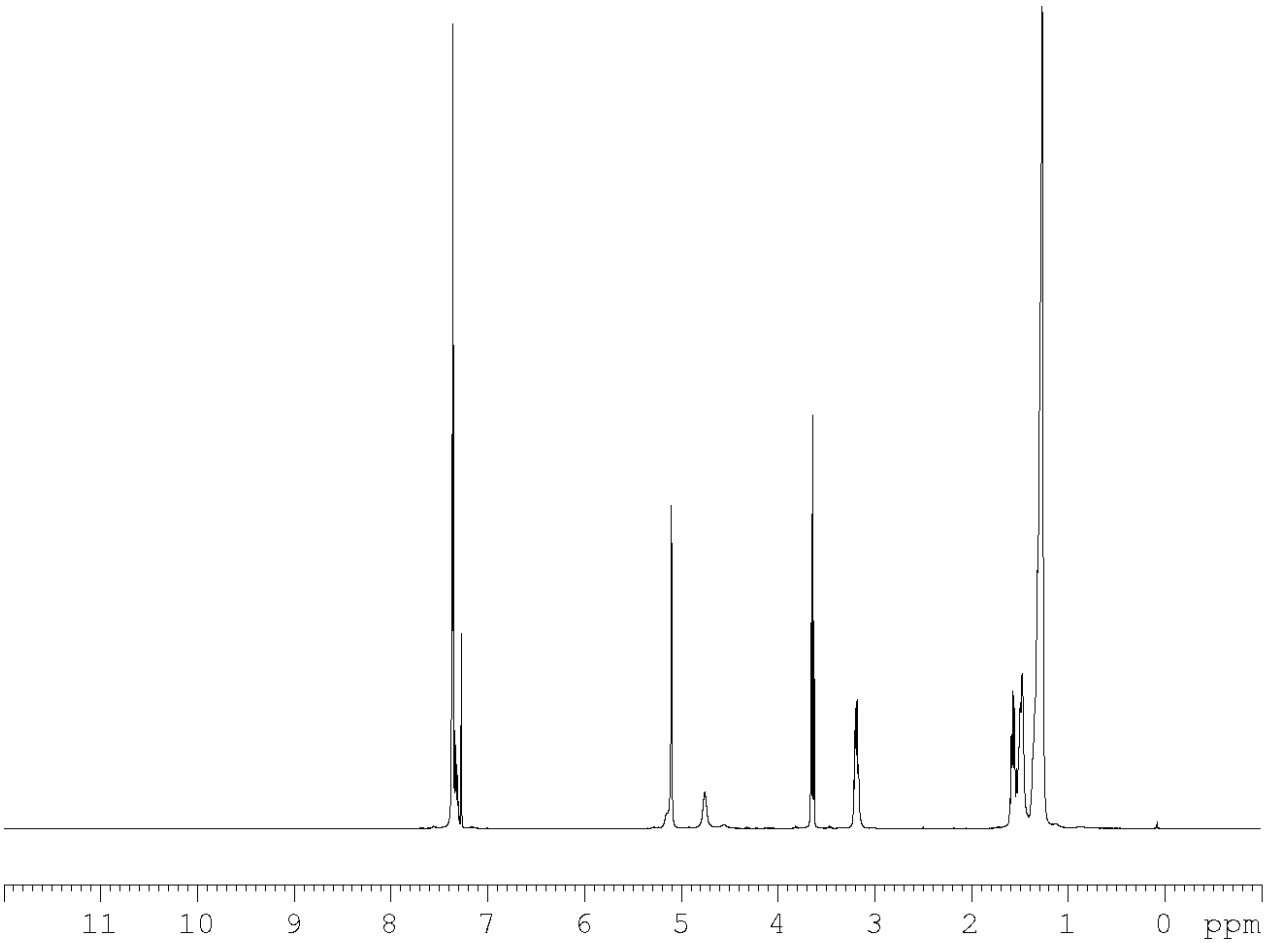
F2 - Acquisition Parameters
Date_           20130122
Time                19.39
INSTRUM           DPX300
PROBHD   5 mm DUL 13C-1
PULPROG              zg60
TD                  32768
SOLVENT             CDCl3
NS                     16
DS                      2
SWH              4789.272 Hz
FIDRES           0.146157 Hz
AQ               3.4209793 sec
RG                    512
DW                104.400 usec
DE                  6.00 usec
TE                 300.0 K
D1            1.00000000 sec
TD0                    1

======== CHANNEL f1 ========
NUC1                  1H
P1                 16.50 usec
PL1                -6.00 dB
SFO1         300.1315007 MHz

F2 - Processing parameters
SI                  32768
SF           300.1300064 MHz
WDW                    EM
SSB       0
LB                  0.30 Hz
GB        0
PC                  1.00
```

12-(*N*-Benzyloxycarbonyl)aminododecanol **58**






```
                Instrument AVN400
                   Chemist ds
                   Group SJC
                     IDS265
 h1acq.crl CDCl3 {C:\NMR} sjcgrp 23

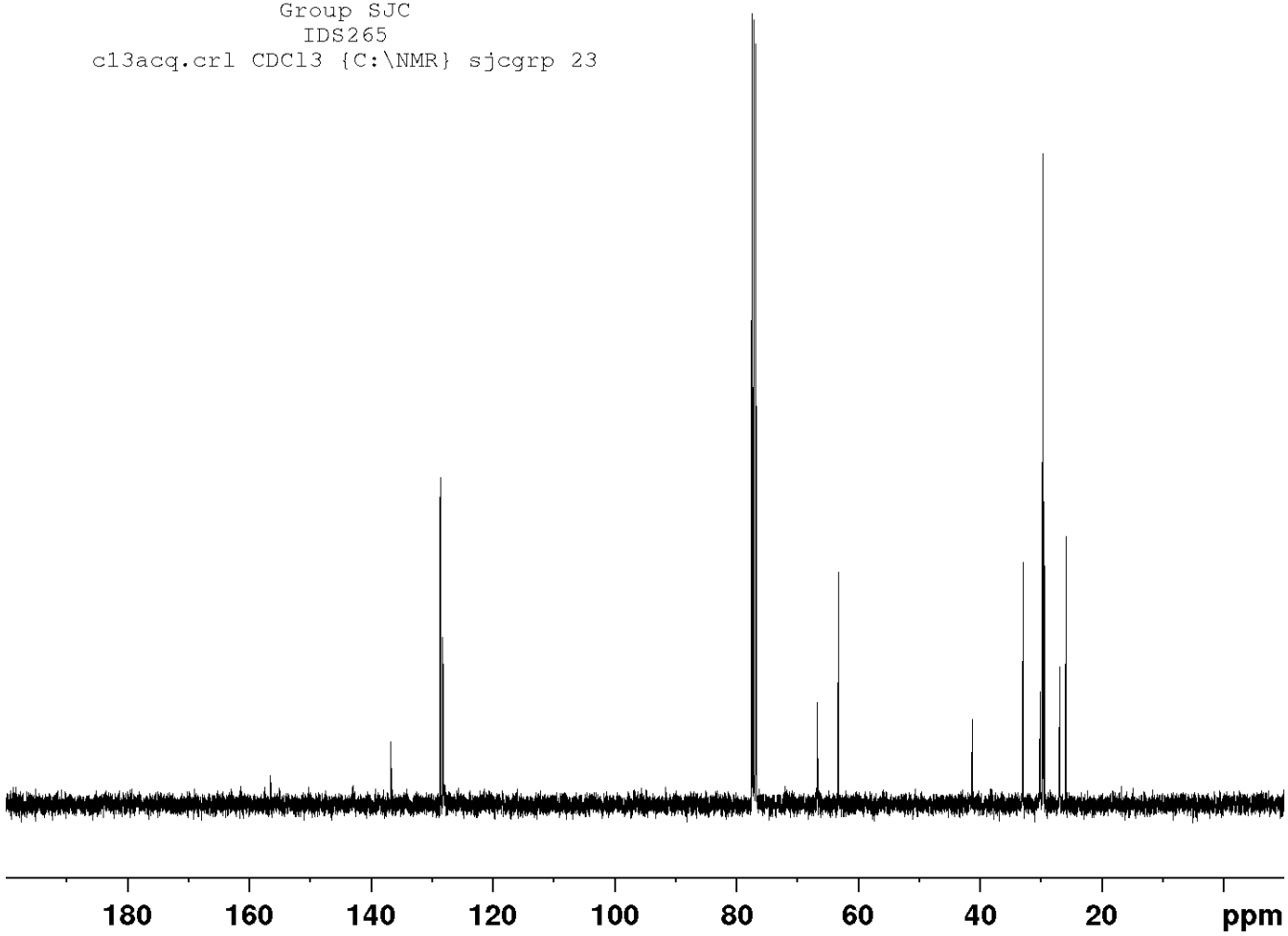
Current Data Parameters
NAME      IDS265 - Jan28-2013-23
EXPNO                      1
PROCNO                     1

F2 - Acquisition Parameters
Date_            20130129
Time                11.40
INSTRUM           avn400
PROBHD    5 mm PABBO BB/
PULPROG              zg60
TD                  65536
SOLVENT             CDCl3
NS                     16
DS                      2
SWH              8012.820 Hz
FIDRES           0.122266 Hz
AQ              4.0894465 sec
RG                  57.58
DW                62.400 usec
DE                 6.50 usec
TE                296.4 K
D1             1.00000000 sec
TD0                     1

======== CHANNEL f1 ========
SFO1         400.2524015 MHz
NUC1                   1H
P1                  12.65 usec
PLW1         16.70000076 W

F2 - Processing parameters
SI                  32768
SF           400.2500112 MHz
WDW                    EM
SSB       0
LB                  0.30 Hz
GB        0
PC                  1.00
```

12-(*N*-Benzyloxycarbonyl)aminododecanol **58**




```
Current Data Parameters
NAME      IDS265 - Jan28-2013-23
EXPNO                          3
PROCNO                         1

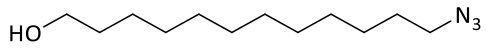
F2 - Acquisition Parameters
Date_                   20130129
Time                       11.54
INSTRUM                  avn400
PROBHD    5 mm PABBO BB/
PULPROG                  zgpg30
TD                        32768
SOLVENT                   CDCl3
NS                          256
DS                            4
SWH                   26041.666 Hz
FIDRES                 0.794729 Hz
AQ                     0.6291456 sec
RG                        205.43
DW                        19.200 usec
DE                         6.50 usec
TE                        297.4 K
D1                   1.00000000 sec
D11                  0.03000000 sec
TD0                           1

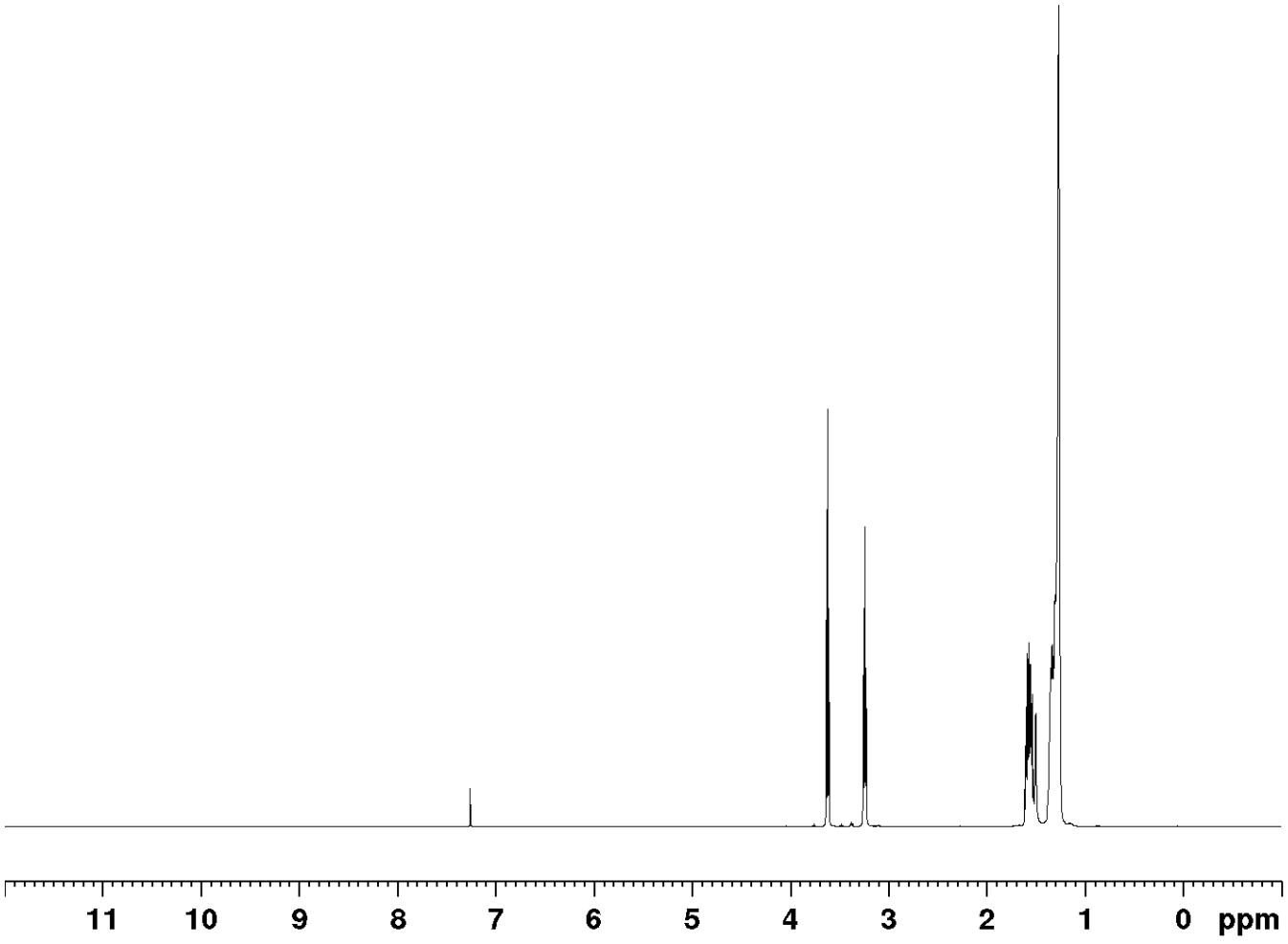
======== CHANNEL f1 ========
SFO1                100.6530073 MHz
NUC1                        13C
P1                        9.00 usec
PLW1                58.70000076 W

======== CHANNEL f2 ========
SFO2                400.2516010 MHz
NUC2                        1H
CPDPRG[2                waltz16
PCPD2                     90.00 usec
PLW2                16.70000076 W
PLW12                0.32991999 W
PLW13                0.26723999 W

F2 - Processing parameters
SI                        32768
SF                  100.6429315 MHz
WDW                         EM
SSB       0
LB                        1.00 Hz
GB        0
PC                        1.40
```

12-Azidododecanol **62**






```
DRX500 BBO Probe IDS368
h1acqbbo CDCl3 {C:\NMR} sjcogroup 1

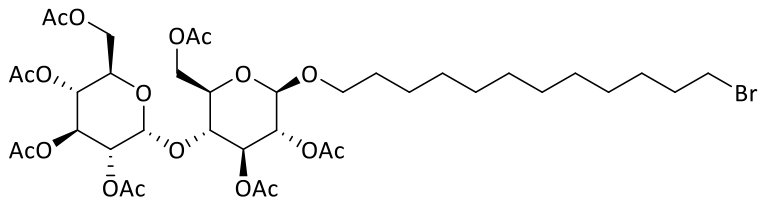
Current Data Parameters
NAME                 IDS368
EXPNO                     1
PROCNO                    1

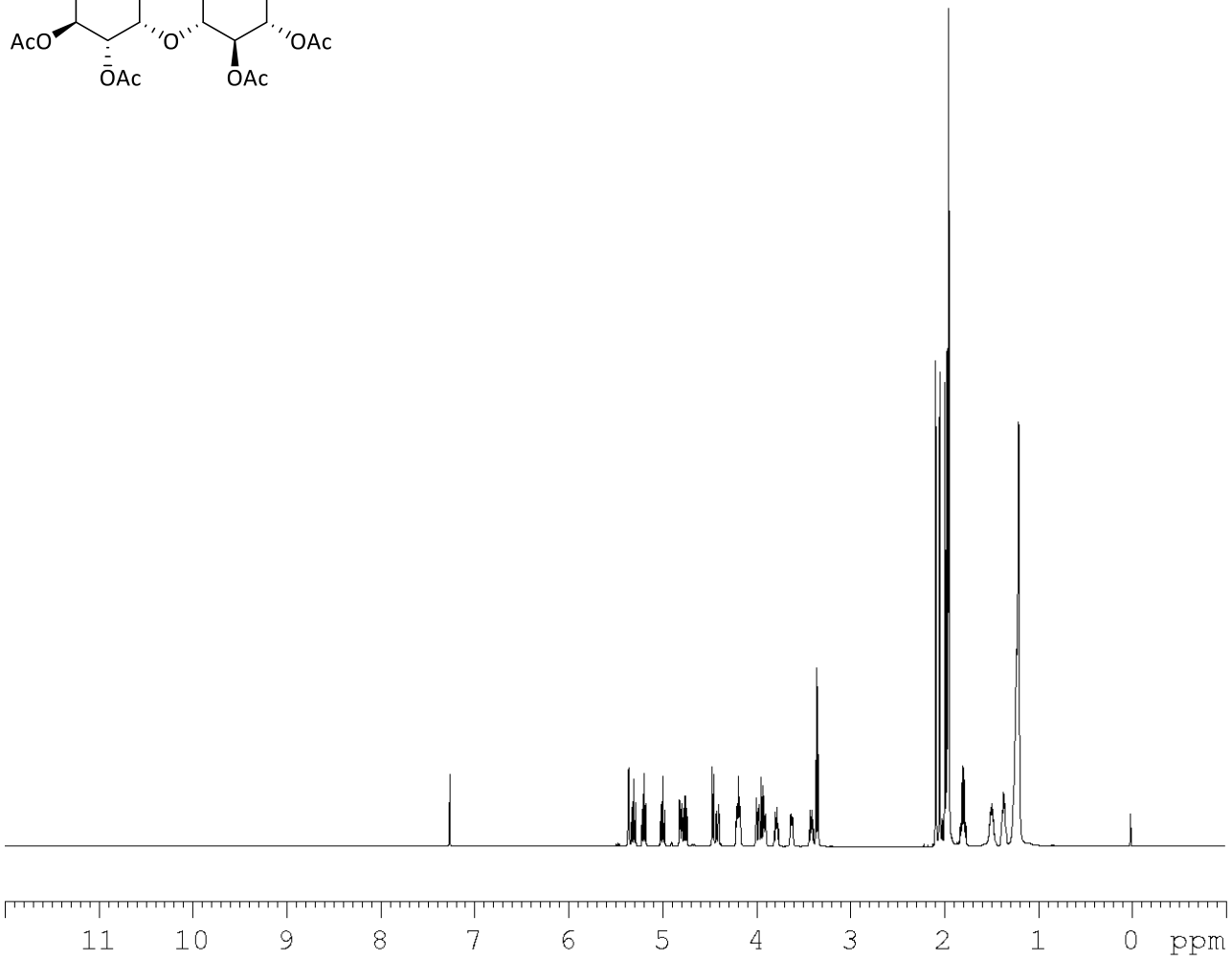
F2 - Acquisition Parameters
Date_              20131205
Time                  19.57
INSTRUM              drx500
PROBHD   5 mm PABBO BB/
PULPROG                zg60
TD                    65536
SOLVENT               CDCl3
NS                       16
DS                        2
SWH              10330.578 Hz
FIDRES            0.157632 Hz
AQ                3.1719425 sec
RG                       32
DW                  48.400 usec
DE                  17.00 usec
TE                   297.7 K
D1              1.00000000 sec

======== CHANNEL f1 ========
NUC1                     1H
P1                   11.00 usec
PL1       0 dB
SFO1          500.1325007 MHz

F2 - Processing parameters
SI                       32768
SF            500.1300284 MHz
WDW                         EM
SSB       0
LB                    0.30 Hz
GB        0
PC                    1.00
```

n-12-Bromododecyl-β-D-maltose heptaacetate **63**






```
DRX500 BBO Probe IDS279 - R3F21-R4F4 product
h1acqbbo CDCl3 {C:\NMR} sjcogroup 3

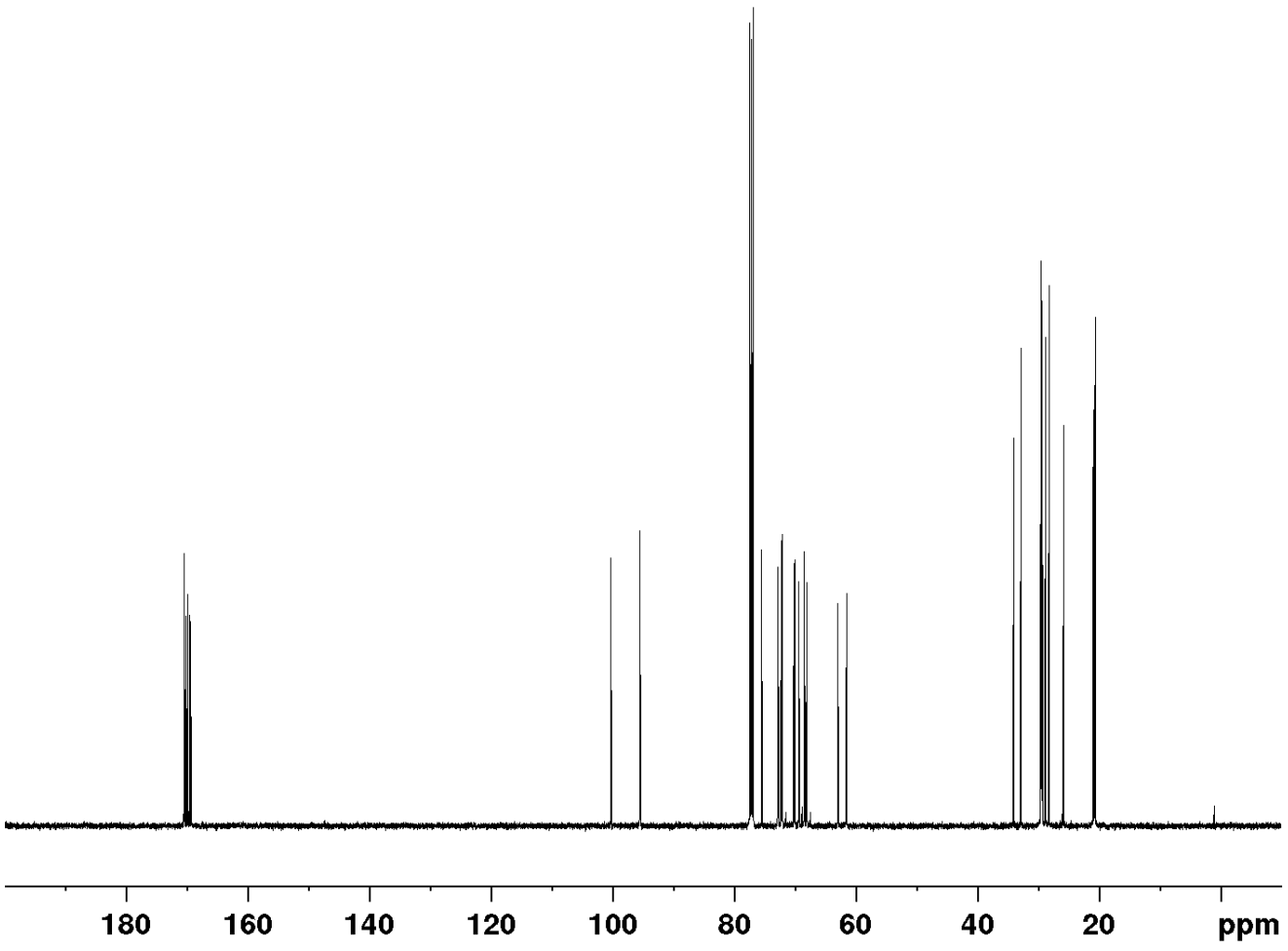
Current Data Parameters
NAME      IDS279
          R3F21-R4F4 product
EXPNO                  1
PROCNO                 1

F2 - Acquisition Parameters
Date_           20130311
Time               19.45
INSTRUM           drx500
PROBHD   5 mm PABBO BB/
PULPROG             zg60
TD                 65536
SOLVENT            CDCl3
NS                    16
DS                     2
SWH           10330.578 Hz
FIDRES         0.157632 Hz
AQ            3.1719425 sec
RG                 14.3
DW                48.400 usec
DE                17.00 usec
TE                298.0 K
D1           1.00000000 sec

======== CHANNEL f1 ========
NUC1                   1H
P1                 11.00 usec
PL1       0 dB
SFO1         500.1325007 MHz

F2 - Processing parameters
SI                 32768
SF           500.1300282 MHz
WDW                   EM
SSB       0
LB                 0.30 Hz
GB        0
PC                 1.00
```

n-12-Bromododecyl-β-D-maltose heptaacetate **63**




```
Current Data Parameters
NAME      IDS279
          R3F21-R4F4 product
EXPNO                   2
PROCNO                  1

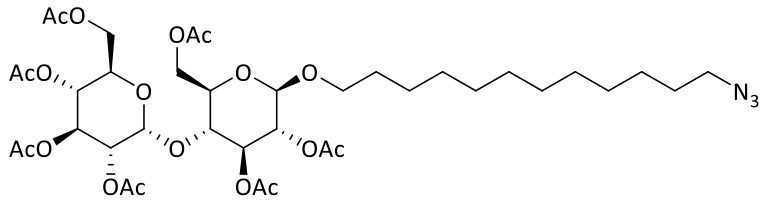
F2 - Acquisition Parameters
Date_           20130311
Time               20.39
INSTRUM           drx500
PROBHD   5 mm PABBO BB/
PULPROG           zgpg30
TD                65536
SOLVENT           CDCl3
NS                 1000
DS                    4
SWH           30303.031 Hz
FIDRES         0.462388 Hz
AQ            1.0813440 sec
RG               1824.6
DW                16.500 usec
DE                11.00 usec
TE                298.0 K
D1           2.00000000 sec
d11          0.03000000 sec
DELTA        1.89999998 sec
TD0                   1

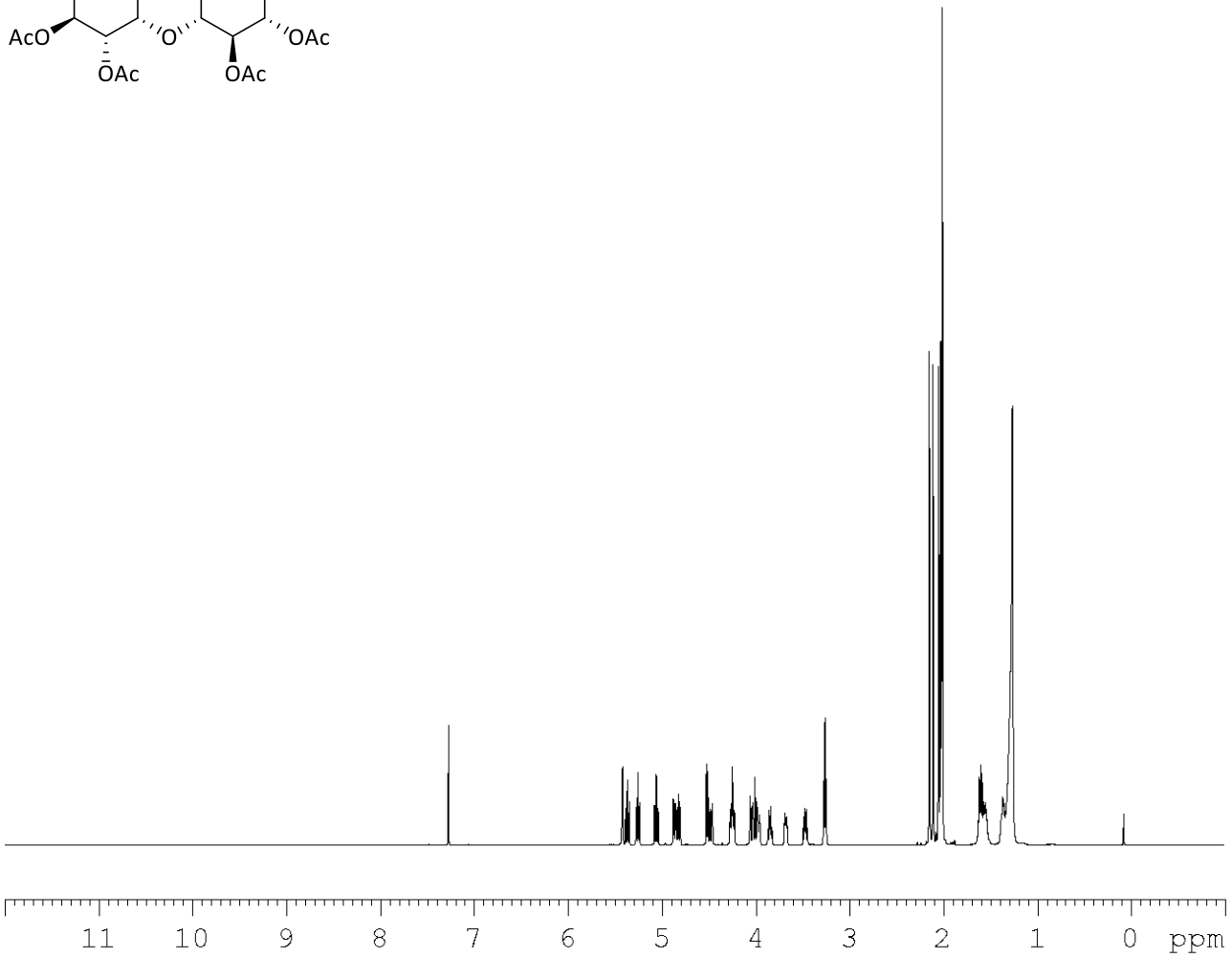
======== CHANNEL f1 ========
NUC1                 13C
P1                  6.80 usec
PL1                 5.00 dB
SFO1        125.7703148 MHz

======== CHANNEL f2 ========
CPDPRG[2         waltz16
NUC2                 1H
PCPD2             100.00 usec
PL2      0 dB
PL12                19.00 dB
PL13                23.00 dB
SFO2        500.1320005 MHz

F2 - Processing parameters
SI                  32768
SF          125.7577863 MHz
WDW                  EM
SSB      0
LB                  1.00 Hz
GB       0
PC                  1.40
```

n-12-Azidododecyl-β-D-maltose heptaacetate **59**






```
DRX500 BBO Probe IDS278
h1acqbbo CDCl3 {C:\NMR} sjcogroup 1

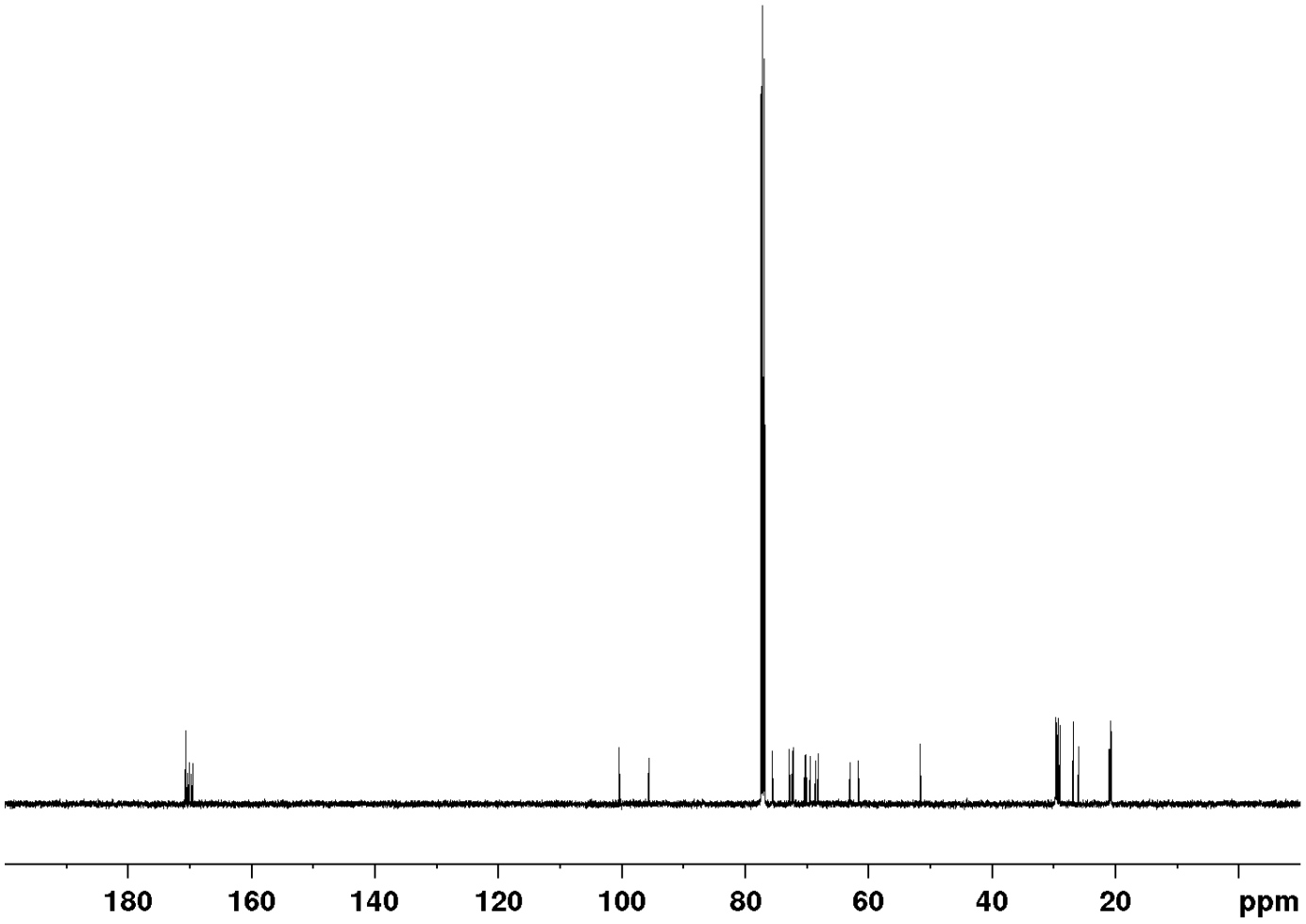
Current Data Parameters
NAME                 IDS278
EXPNO                     1
PROCNO                    1

F2 - Acquisition Parameters
Date_              20130310
Time                  15.57
INSTRUM              drx500
PROBHD    5 mm PABBO BB/
PULPROG                zg60
TD                    65536
SOLVENT               CDCl3
NS                       16
DS                        2
SWH              10330.578 Hz
FIDRES            0.157632 Hz
AQ                3.1719425 sec
RG                    101.6
DW                   48.400 usec
DE                   17.00 usec
TE                   298.0 K
D1              1.00000000 sec

======== CHANNEL f1 ========
NUC1                      1H
P1                   11.00 usec
PL1       0 dB
SFO1          500.1325007 MHz

F2 - Processing parameters
SI                    32768
SF            500.1300237 MHz
WDW                      EM
SSB       0
LB                    0.30 Hz
GB        0
PC                    1.00
```

n-12-Azidododecyl-β-D-maltose heptaacetate **59**




```
Current Data Parameters
NAME            IDS278_2
EXPNO                  2
PROCNO                 1

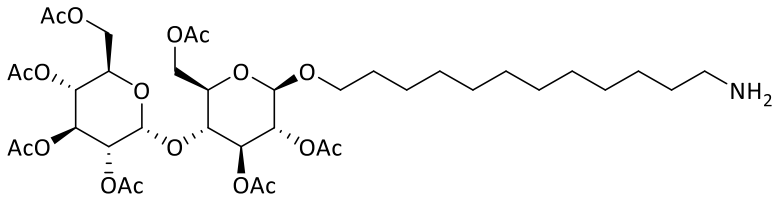
F2 - Acquisition Parameters
Date_           20130314
Time               19.17
INSTRUM           drx500
PROBHD   5 mm PABBO BB/
PULPROG          zgpg30
TD                65536
SOLVENT            CDCl3
NS                  1000
DS                     4
SWH           30303.031 Hz
FIDRES         0.462388 Hz
AQ             1.0813440 sec
RG                2580.3
DW                16.500 usec
DE                11.00 usec
TE                298.0 K
D1            2.00000000 sec
d11           0.03000000 sec
DELTA         1.89999998 sec
TD0                    1

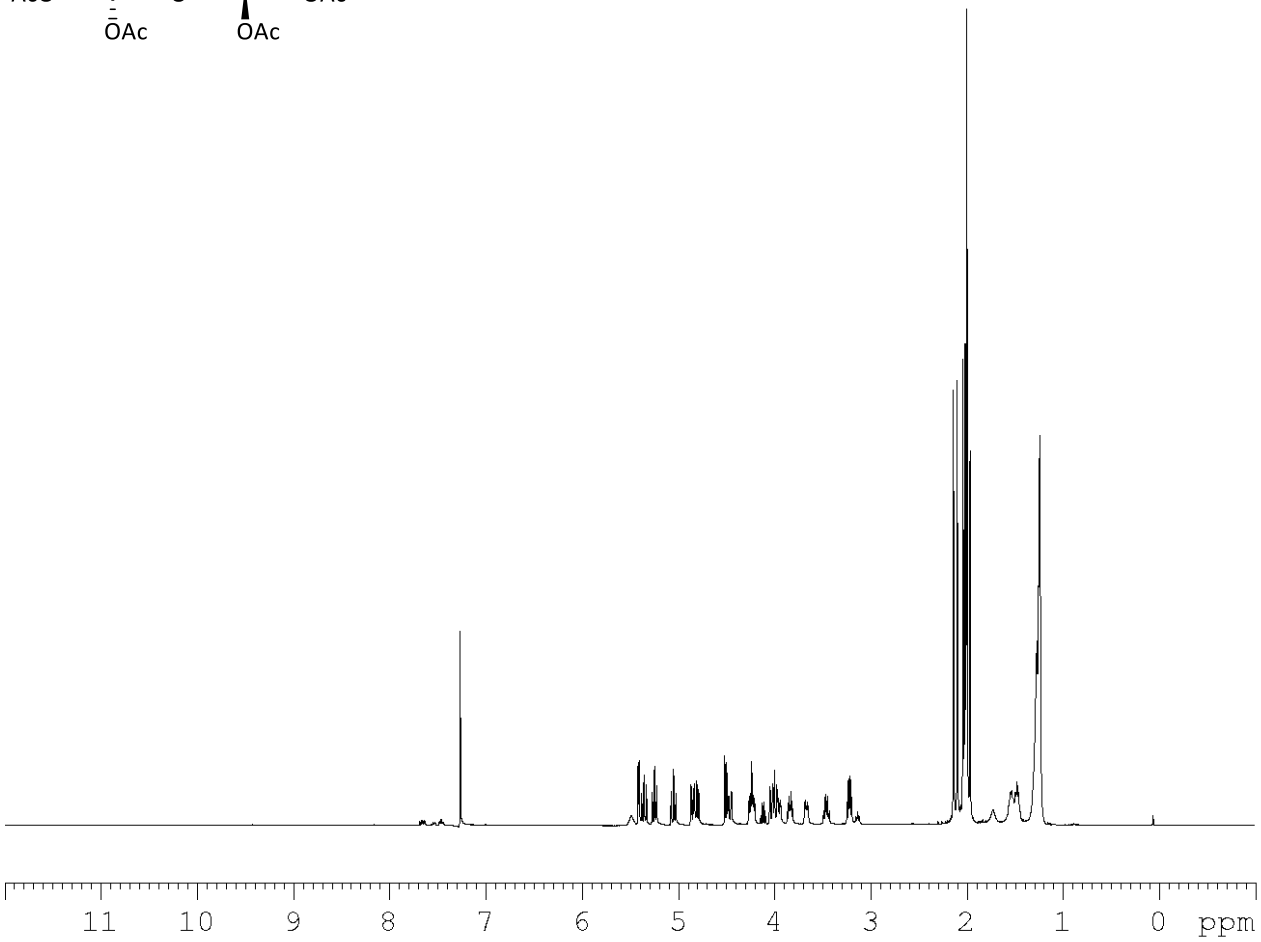
======== CHANNEL f1 ========
NUC1                  13C
P1                   6.80 usec
PL1                  5.00 dB
SFO1         125.7703148 MHz

======== CHANNEL f2 ========
CPDPRG[2          waltz16
NUC2                  1H
PCPD2              100.00 usec
PL2       0 dB
PL12                19.00 dB
PL13                23.00 dB
SFO2         500.1320005 MHz

F2 - Processing parameters
SI                 32768
SF           125.7577764 MHz
WDW                   EM
SSB       0
LB                   1.00 Hz
GB        0
PC                   1.40
```

n-12-Aminododecyl-β-D-maltose heptaacetate **60**






```
Instrument DQX400
Chemist ds
Group SJC
IDS314
h1acq.au CDCl3 {E:\NMR2013} sjcgrp 6

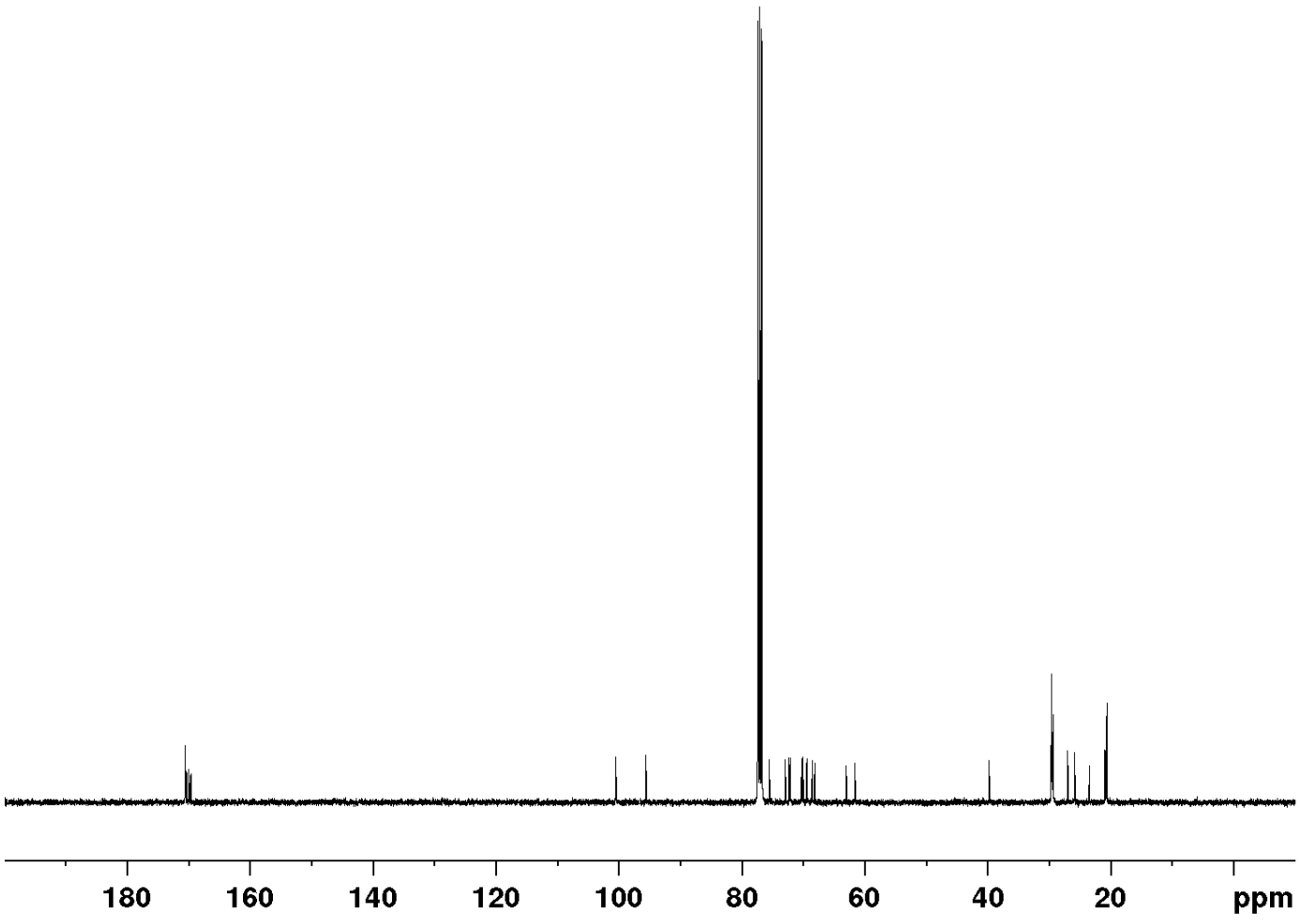
Current Data Parameters
NAME      IDS314 - Jul02-2013-6
EXPNO                         1
PROCNO                        1

F2 - Acquisition Parameters
Date_               20130702
Time                    9.31
INSTRUM                av400
PROBHD    5 mm QNP 1H/13
PULPROG                 zg60
TD                     65536
SOLVENT                CDCl3
NS                        16
DS                         2
SWH                 8278.146 Hz
FIDRES              0.126314 Hz
AQ                  3.9583745 sec
RG                       90.5
DW                     60.400 usec
DE                      7.50 usec
TE                     300.0 K
D1                1.00000000 sec

======== CHANNEL f1 ========
NUC1                       1H
P1                     10.10 usec
PL1       0 dB
SFO1           400.2024714 MHz

F2 - Processing parameters
SI                      32768
SF            400.2000066 MHz
WDW                        EM
SSB       0
LB                      0.30 Hz
GB        0
PC                      1.00
```

n-12-Aminododecyl-β-D-maltose heptaacetate **60**




```
Current Data Parameters
NAME         IDS376 29mg
EXPNO                  2
PROCNO                 1

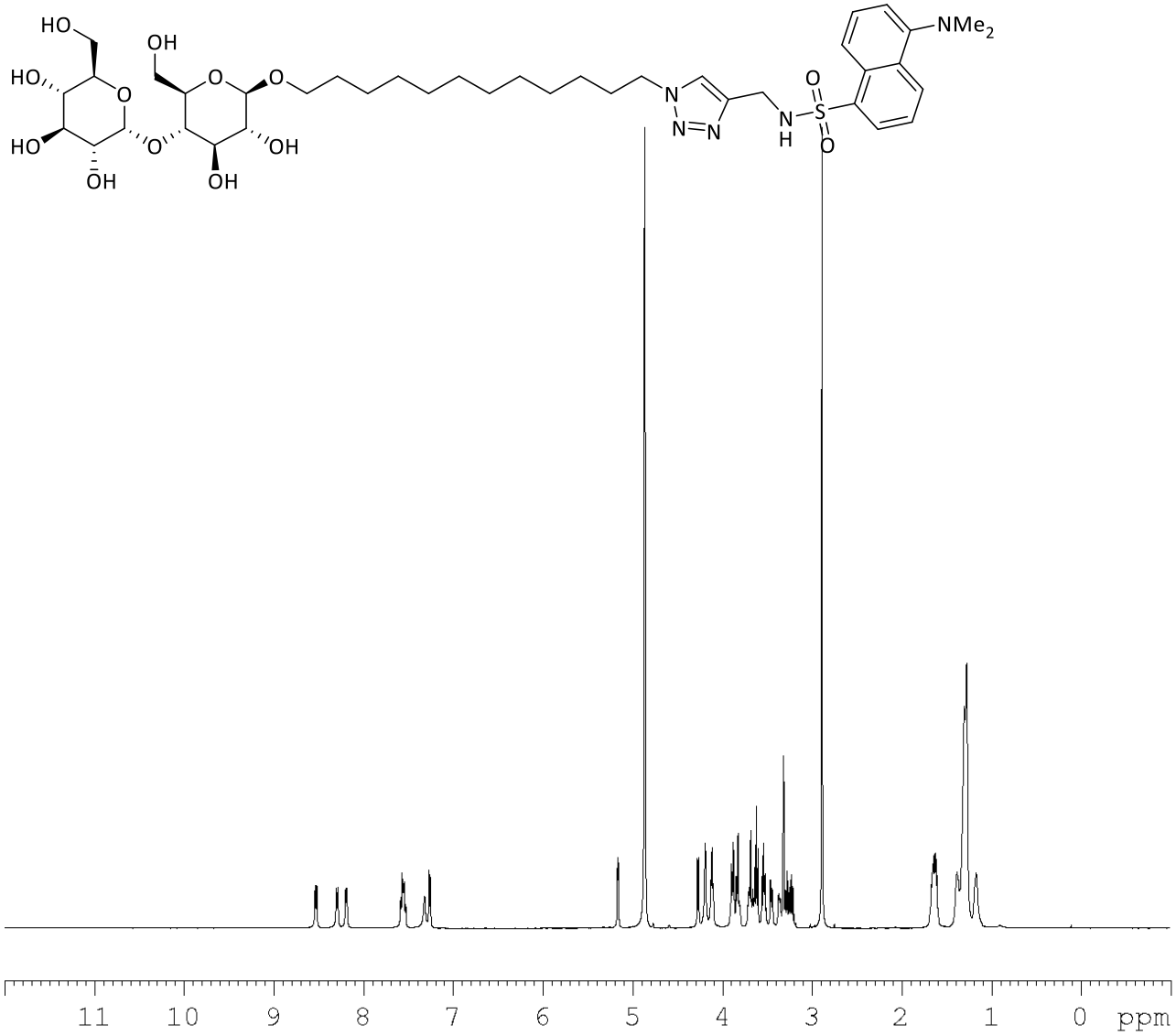
F2 - Acquisition Parameters
Date_           20140209
Time               17.13
INSTRUM          avb400
PROBHD   5 mm PABBO BB/
PULPROG          zgpg30
TD                65536
SOLVENT           CDCl3
NS                 2048
DS                    4
SWH           24038.461 Hz
FIDRES         0.366798 Hz
AQ             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           100.6127543 MHz
WDW                  EM
SSB       0
LB                1.00 Hz
GB        0
PC                1.40
```

n-12-(4-[*N*-Dansyl]-aminomethyl-1*H*-1,2,3-triazol-1-yl)-dodecyl-β-D-maltoside **44**




```
DRX500 BBO Probe IDS299+305
h1acqbbo MeOD {C:\NMR} sjcogroup 13

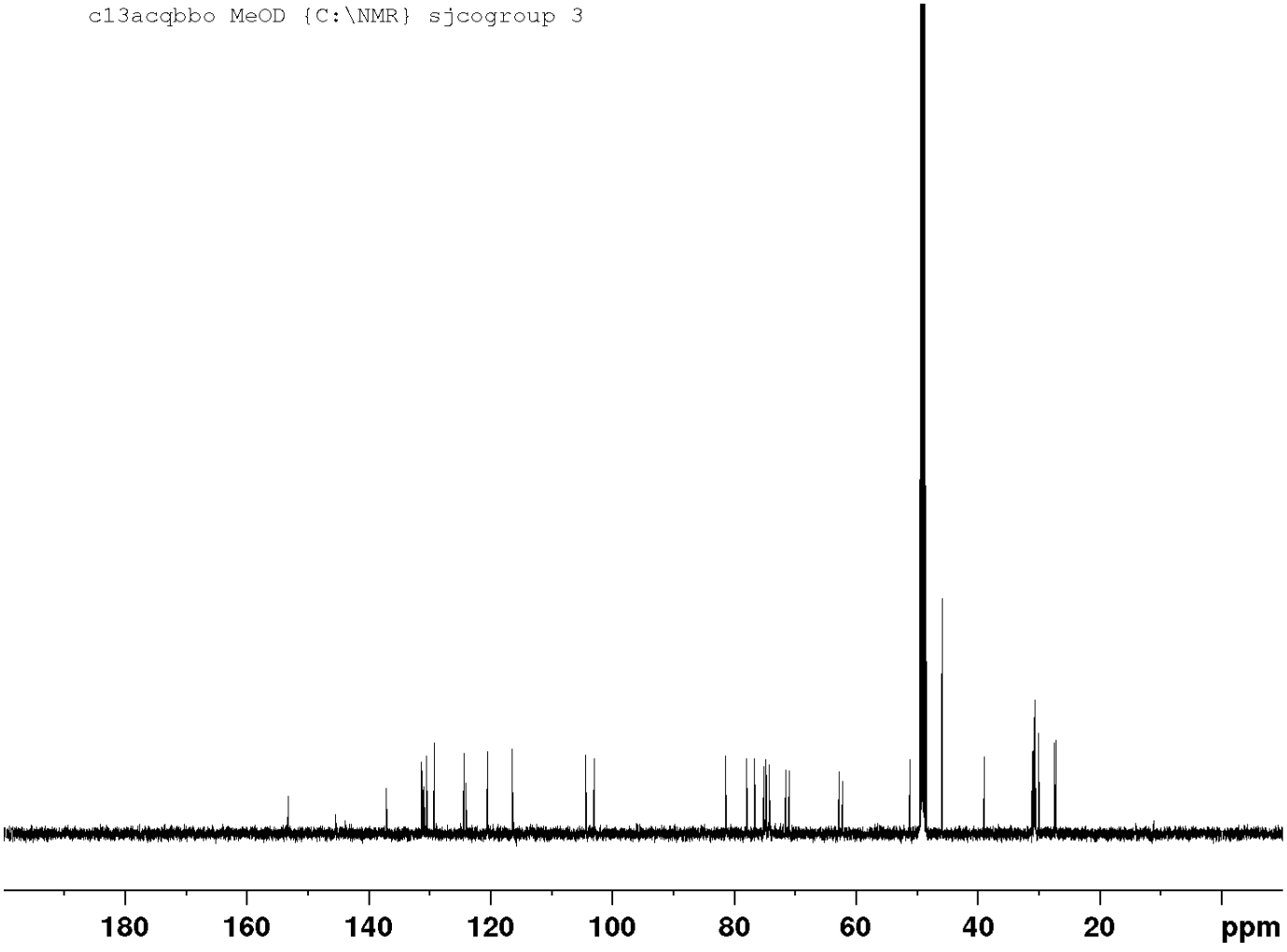

Current Data Parameters
NAME          IDS299+305
EXPNO                  1
PROCNO                 1

F2 - Acquisition Parameters
Date_           20131029
Time                4.06
INSTRUM           drx500
PROBHD   5 mm PABBO BB/
PULPROG             zg60
TD                 65536
SOLVENT             MeOD
NS                    16
DS                     2
SWH           10330.578 Hz
FIDRES         0.157632 Hz
AQ             3.1719425 sec
RG                  80.6
DW                48.400 usec
DE                17.00 usec
TE                298.7 K
D1           1.00000000 sec

======== CHANNEL f1 ========
NUC1                  1H
P1                11.00 usec
PL1       0 dB
SFO1        500.1325007 MHz

F2 - Processing parameters
SI                 32768
SF          500.1300110 MHz
WDW                   EM
SSB       0
LB                 0.30 Hz
GB        0
PC                 1.00
```

n-12-(4-[*N*-Dansyl]-aminomethyl-1*H*-1,2,3-triazol-1-yl)-dodecyl-β-D-maltoside **44**




```
Current Data Parameters
NAME              IDS331
EXPNO                  3
PROCNO                 1

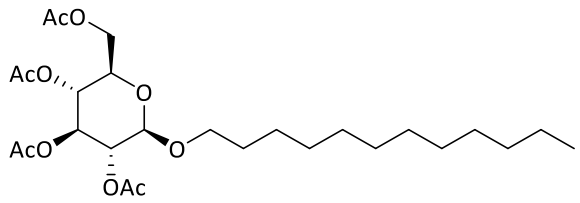
F2 - Acquisition Parameters
Date_           20130826
Time               23.43
INSTRUM           drx500
PROBHD   5 mm PABBO BB/
PULPROG           zgpg30
TD                 65536
SOLVENT              MeOD
NS                   1024
DS                      4
SWH           30303.031 Hz
FIDRES         0.462388 Hz
AQ            1.0813440 sec
RG               4597.6
DW                16.500 usec
DE                11.00 usec
TE                298.7 K
D1           2.00000000 sec
d11          0.03000000 sec
DELTA        1.89999998 sec
TD0                    1

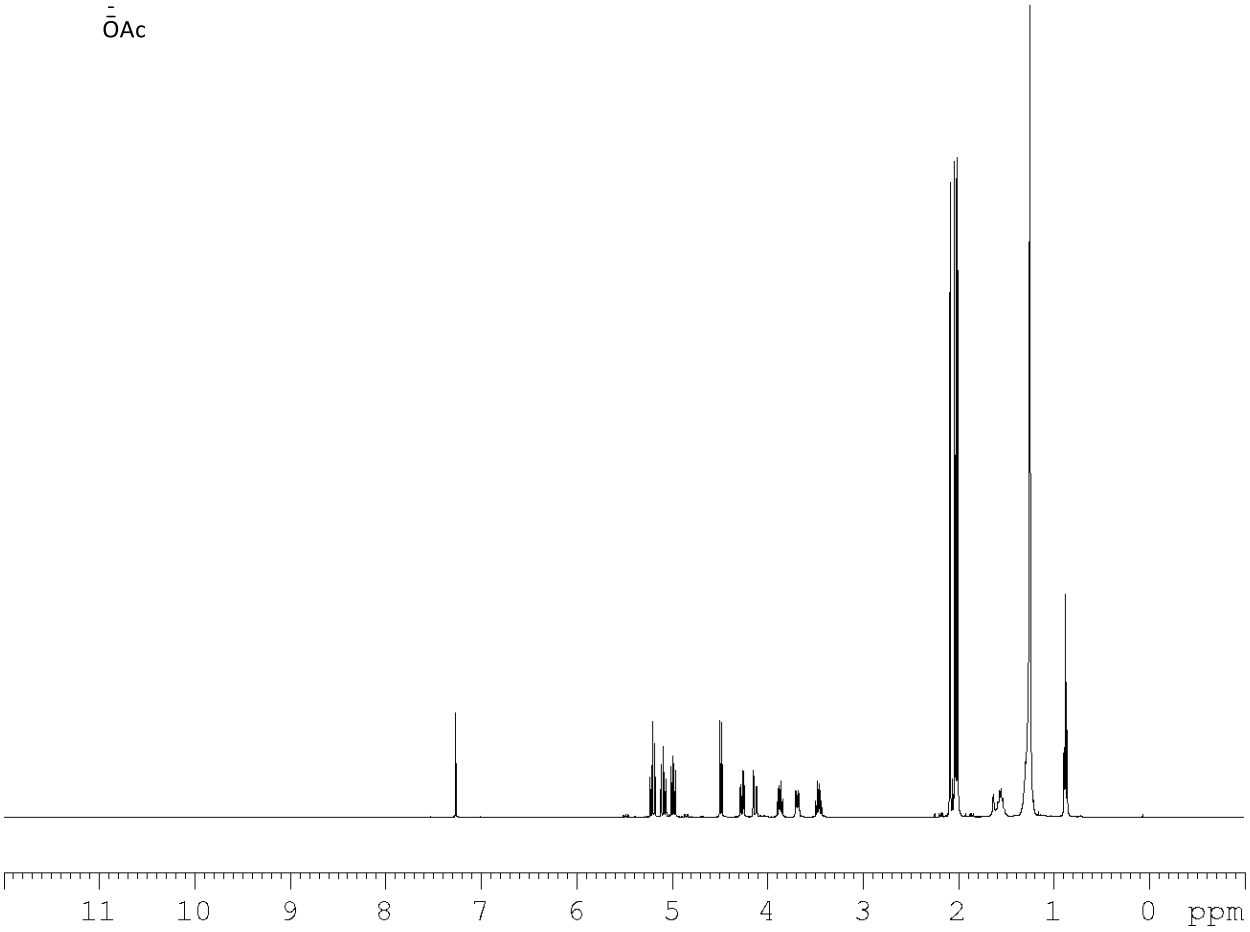
======== CHANNEL f1 ========
NUC1                  13C
P1                   6.80 usec
PL1                  5.00 dB
SFO1        125.7703148 MHz

======== CHANNEL f2 ========
CPDPRG[2         waltz16
NUC2                  1H
PCPD2              100.00 usec
PL2      0 dB
PL12                19.00 dB
PL13                23.00 dB
SFO2        500.1320005 MHz

F2 - Processing parameters
SI                 32768
SF          125.7576139 MHz
WDW                   EM
SSB      0
LB                   1.00 Hz
GB       0
PC                   1.40
```

n-Dodecyl-(2,3,4,6-*O*-tetraacetyl)-β-D-glucopyranoside **65**






```
          Instrument DQX400
             Chemist ds
             Group SJC
          IDS332 - columned
hlacq.au CDCl3 {E:\NMR2013} sjcgrp 42

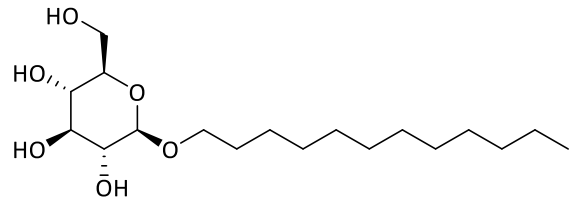

Current Data Parameters
NAME      IDS332 columned
           Aug08-2013-42
EXPNO                   1
PROCNO                  1

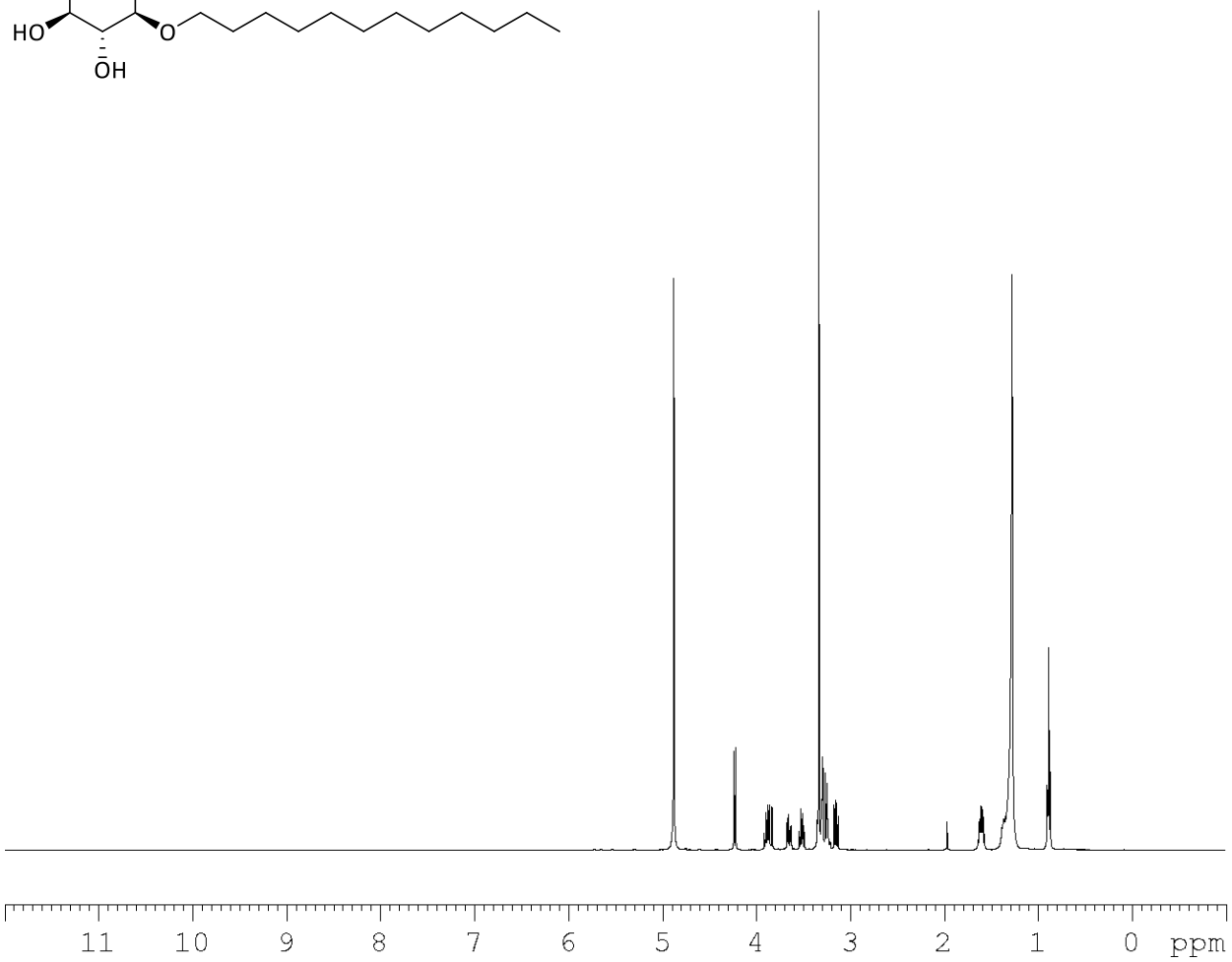
F2 - Acquisition Parameters
Date_           20130808
Time               10.09
INSTRUM            av400
PROBHD   5 mm QNP 1H/13
PULPROG             zg60
TD                 65536
SOLVENT            CDCl3
NS                    16
DS                     2
SWH             8278.146 Hz
FIDRES          0.126314 Hz
AQ             3.9583745 sec
RG                  90.5
DW                60.400 usec
DE                 7.50 usec
TE                300.0 K
D1           1.00000000 sec

======== CHANNEL f1 ========
NUC1                   1H
P1                 10.10 usec
PL1       0 dB
SFO1         400.2024714 MHz

F2 - Processing parameters
SI                 32768
SF           400.2000067 MHz
WDW                   EM
SSB       0
LB                 0.30 Hz
GB        0
PC                 1.00
```

n-Dodecyl-β-D-glucopyranoside **34**






```
          Instrument DQX400
             Chemist ds
             Group SJC
          IDS338 - columned
h1acq.au MeOD {E:\NMR2013} sjcgrp 11

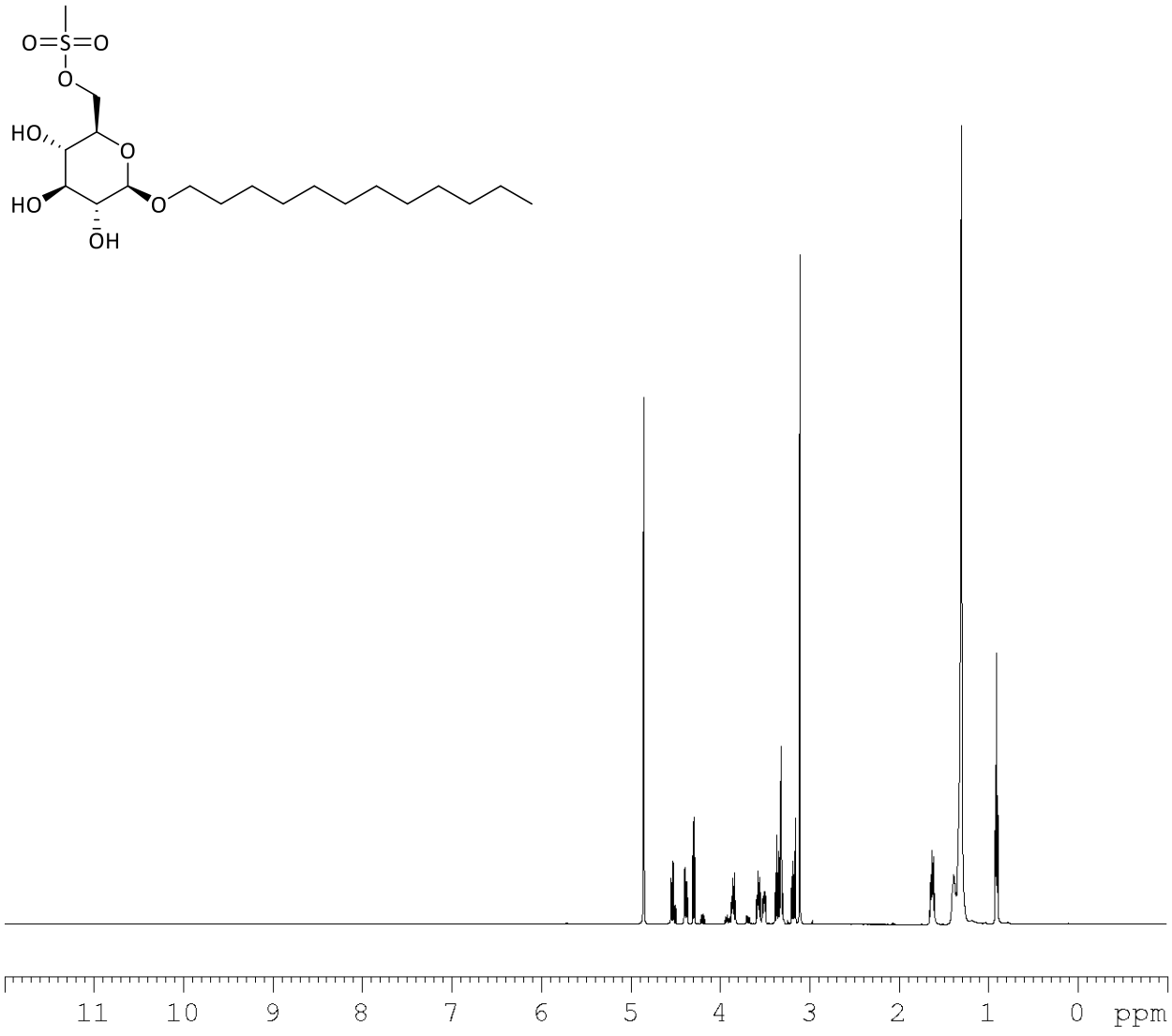
 Current Data Parameters
 NAME      IDS338 COSY
          Aug16-2013-11
 EXPNO                  1
 PROCNO                 1

 F2 - Acquisition Parameters
 Date_           20130816
 Time               17.55
 INSTRUM            av400
 PROBHD   5 mm QNP 1H/13
 PULPROG             zg60
 TD                 65536
 SOLVENT             MeOD
 NS                    16
 DS                     2
 SWH             8278.146 Hz
 FIDRES          0.126314 Hz
 AQ             3.9583745 sec
 RG                  90.5
 DW                60.400 usec
 DE                 7.50 usec
 TE                300.0 K
 D1           1.00000000 sec

 ======== CHANNEL f1 ========
 NUC1                  1H
 P1                10.10 usec
 PL1       0 dB
 SFO1         400.2024714 MHz

 F2 - Processing parameters
 SI                 32768
 SF           400.2000135 MHz
 WDW                  EM
 SSB       0
 LB                 0.30 Hz
 GB        0
 PC                 1.00
```

n-Dodecyl-(6-*O*-mesyl)-β-D-glucopyranoside **102**




```
DRX500 BBO Probe IDS346 prod spot
h1acqbbo MeOD {C:\NMR} sjcogroup 3

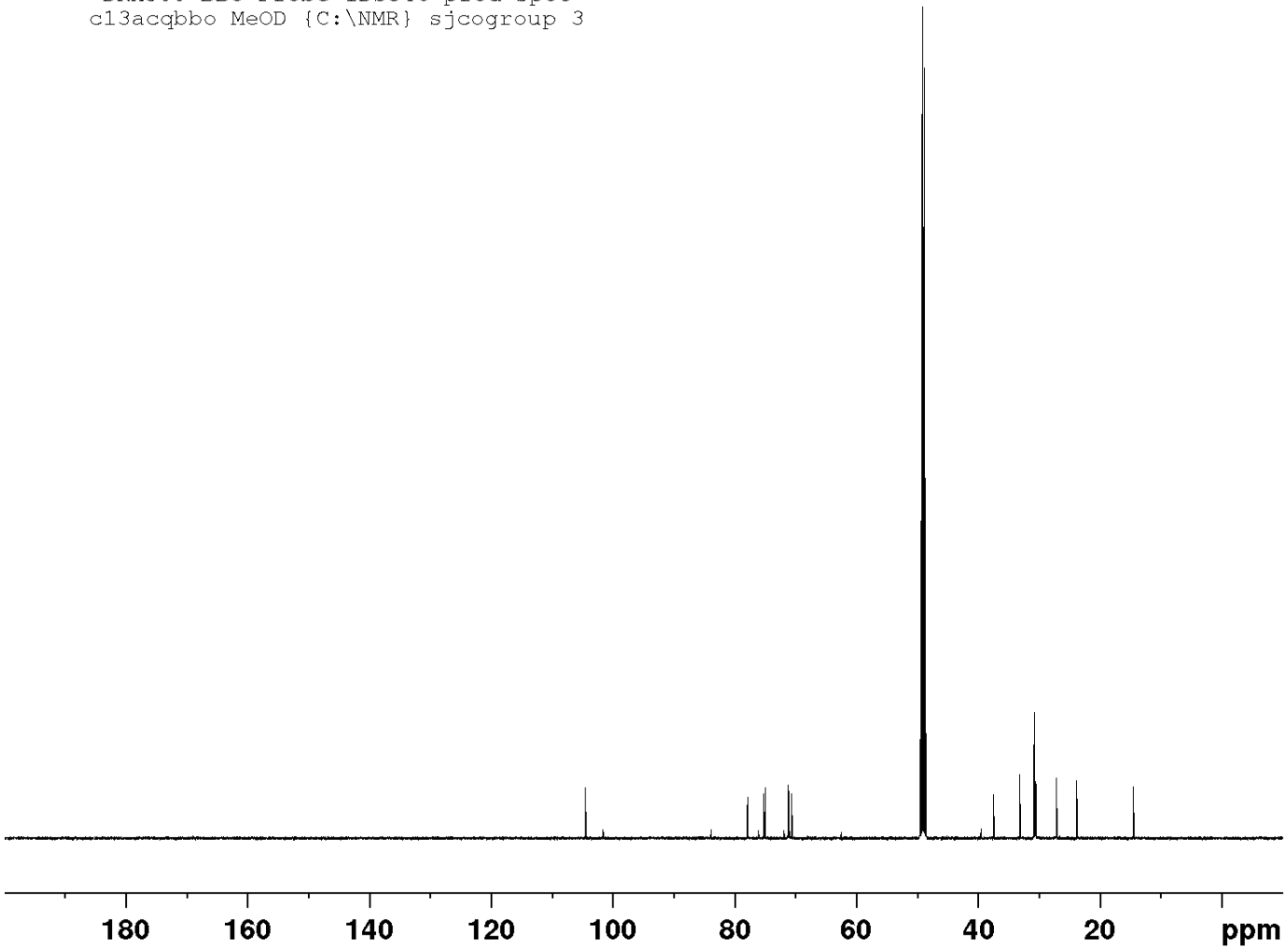
Current Data Parameters
NAME      IDS346 prod spot
EXPNO                    1
PROCNO                   1

F2 - Acquisition Parameters
Date_           20130904
Time               23.14
INSTRUM          drx500
PROBHD   5 mm PABBO BB/
PULPROG             zg60
TD                 65536
SOLVENT             MeOD
NS                    16
DS                     2
SWH           10330.578 Hz
FIDRES         0.157632 Hz
AQ            3.1719425 sec
RG                  90.5
DW                48.400 usec
DE                17.00 usec
TE                297.7 K
D1           1.00000000 sec

======== CHANNEL f1 ========
NUC1                   1H
P1                 11.00 usec
PL1       0 dB
SFO1        500.1325007 MHz

F2 - Processing parameters
SI                  32768
SF          500.1300107 MHz
WDW                    EM
SSB       0
LB                  0.30 Hz
GB        0
PC                  1.00
```

n-Dodecyl-(6-*O*-mesyl)-β-D-glucopyranoside **102**




```
Current Data Parameters
NAME      IDS346 prod spot
EXPNO                    3
PROCNO                   1

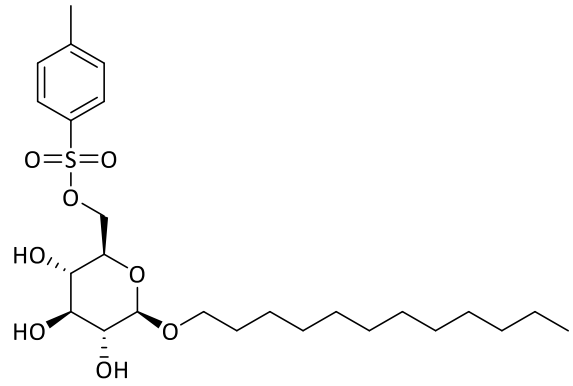
F2 - Acquisition Parameters
Date_           20130905
Time                1.16
INSTRUM           drx500
PROBHD   5 mm PABBO BB/
PULPROG           zgpg30
TD                 65536
SOLVENT              MeOD
NS                   2048
DS                      4
SWH           30303.031 Hz
FIDRES         0.462388 Hz
AQ            1.0813440 sec
RG                  8192
DW                16.500 usec
DE                11.00 usec
TE                297.7 K
D1           2.00000000 sec
d11          0.03000000 sec
DELTA        1.89999998 sec
TD0                     1

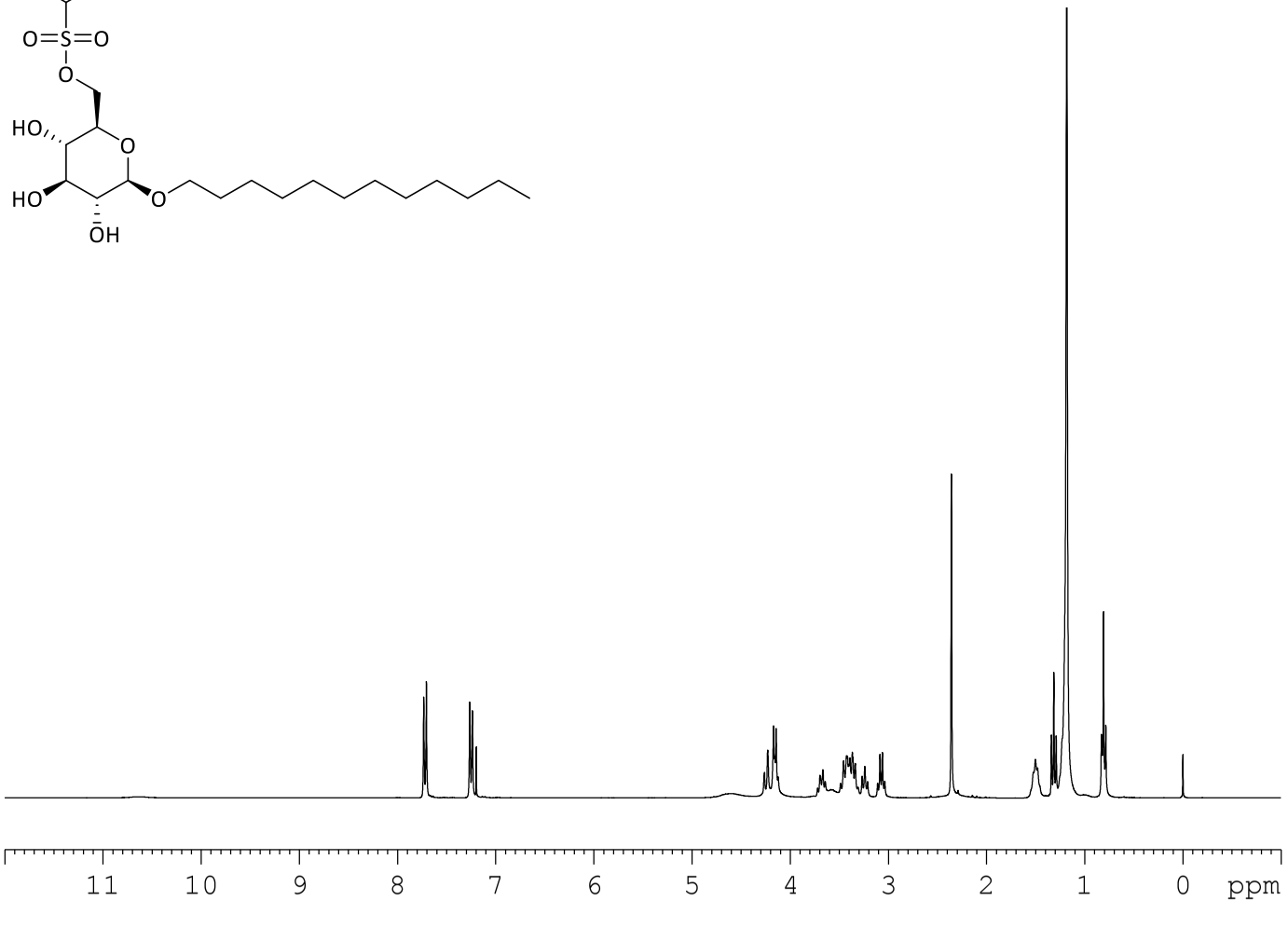
======== CHANNEL f1 ========
NUC1                   13C
P1                  6.80 usec
PL1                 5.00 dB
SFO1         125.7703148 MHz

======== CHANNEL f2 ========
CPDPRG[2          waltz16
NUC2                   1H
PCPD2             100.00 usec
PL2       0 dB
PL12                19.00 dB
PL13                23.00 dB
SFO2         500.1320005 MHz

F2 - Processing parameters
SI                  32768
SF           125.7576130 MHz
WDW                    EM
SSB       0
LB                  1.00 Hz
GB        0
PC                  1.40
```

n-Dodecyl-(6-*O*-tosyl)-β-D-glucopyranoside **103**






```
Instrument DPX300
Chemist Name ds
Research Group SJC
IDS351ii

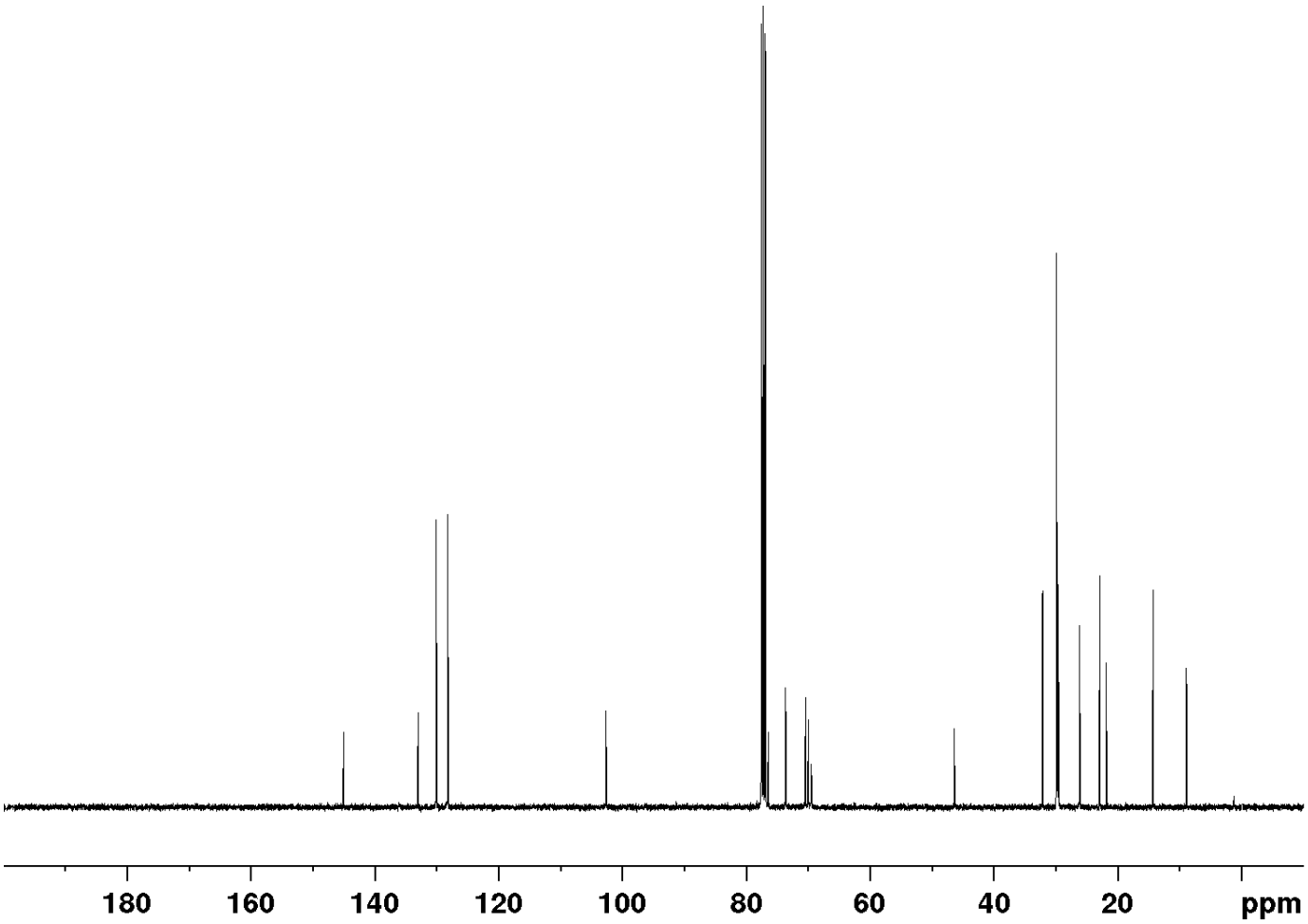
Current Data Parameters
NAME      IDS351iiDec17-2013-3
EXPNO                        2
PROCNO                       1

F2 - Acquisition Parameters
Date_              20131217
Time                 17.24
INSTRUM              DPX300
PROBHD   5 mm DUL 13C-1
PULPROG               zg60
TD                   32768
SOLVENT              CDCl3
NS                      16
DS                       2
SWH               4789.272 Hz
FIDRES           0.146157 Hz
AQ               3.4209793 sec
RG                     114
DW                 104.400 usec
DE                   6.00 usec
TE                  300.0 K
D1             1.00000000 sec
TD0                      1

======== CHANNEL f1 ========
NUC1                    1H
P1                   16.50 usec
PL1                  -6.00 dB
SFO1          300.1315007 MHz

F2 - Processing parameters
SI                   32768
SF            300.1300248 MHz
WDW                     EM
SSB       0
LB                   0.30 Hz
GB        0
PC                   1.00
```

n-Dodecyl-(6-*O*-tosyl)-β-D-glucopyranoside **103**




```
Current Data Parameters
NAME             IDS351i
EXPNO                  3
PROCNO                 1

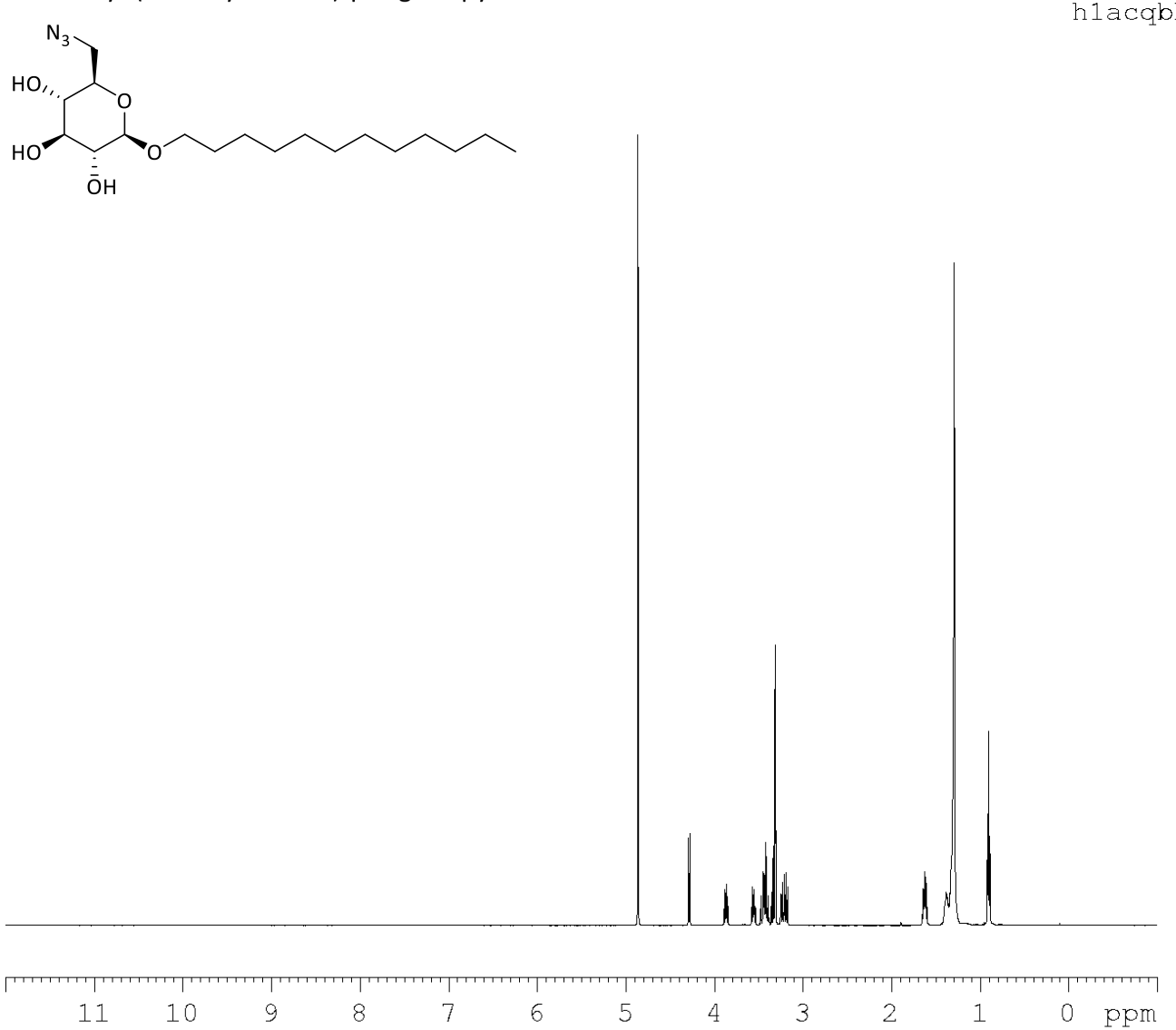
F2 - Acquisition Parameters
Date_           20131217
Time               16.19
INSTRUM           avb400
PROBHD   5 mm PABBO BB/
PULPROG           zgpg30
TD                 65536
SOLVENT            CDCl3
NS                  1536
DS                     4
SWH           24038.461 Hz
FIDRES         0.366798 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           100.6127557 MHz
WDW                  EM
SSB       0
LB                 1.00 Hz
GB        0
PC                 1.40
```

n-Dodecyl-(6-deoxy-6-azido)-β-D-glucopyranoside **66**




```
IDS375i - columned
h1acqbbo MeOD {C:\NMR} sjcogroup 3

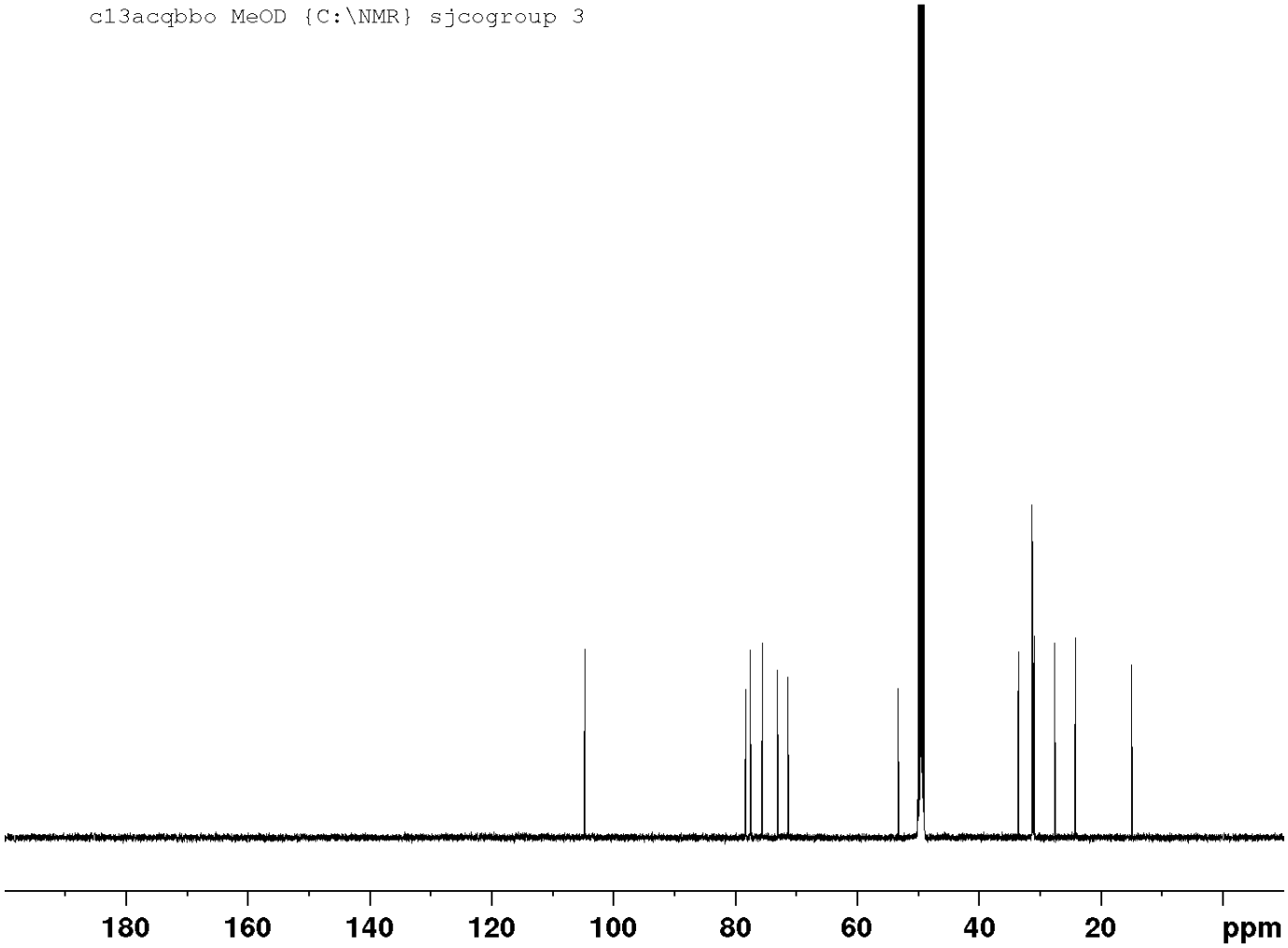
Current Data Parameters
NAME              IDS375i
EXPNO                   1
PROCNO                  1

F2 - Acquisition Parameters
Date_            20131220
Time                21.28
INSTRUM           drx500
PROBHD   5 mm PABBO BB/
PULPROG             zg60
TD                 65536
SOLVENT             MeOD
NS                    16
DS                     2
SWH           10330.578 Hz
FIDRES         0.157632 Hz
AQ            3.1719425 sec
RG                143.7
DW               48.400 usec
DE                17.00 usec
TE                297.7 K
D1           1.00000000 sec

======== CHANNEL f1 ========
NUC1                  1H
P1                11.00 usec
PL1       0 dB
SFO1        500.1325007 MHz

F2 - Processing parameters
SI                 32768
SF          500.1300109 MHz
WDW                   EM
SSB       0
LB                 0.30 Hz
GB        0
PC                 1.00
```

n-Dodecyl-(6-deoxy-6-azido)-β-D-glucopyranoside **66**




```
Current Data Parameters
NAME            IDS375i
EXPNO                 3
PROCNO                1

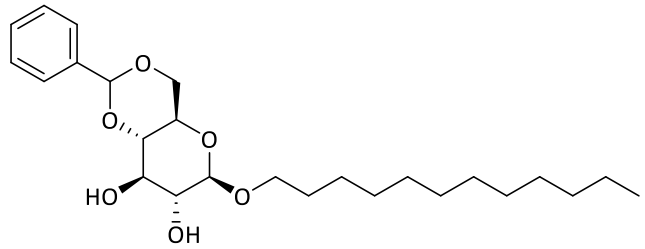
F2 - Acquisition Parameters
Date_          20131221
Time               2.27
INSTRUM          drx500
PROBHD   5 mm PABBO BB/
PULPROG          zgpg30
TD                65536
SOLVENT             MeOD
NS                 5120
DS                    4
SWH           30303.031 Hz
FIDRES         0.462388 Hz
AQ            1.0813440 sec
RG               9195.2
DW                16.500 usec
DE                11.00 usec
TE                297.7 K
D1           2.00000000 sec
d11          0.03000000 sec
DELTA        1.89999998 sec
TD0                   1

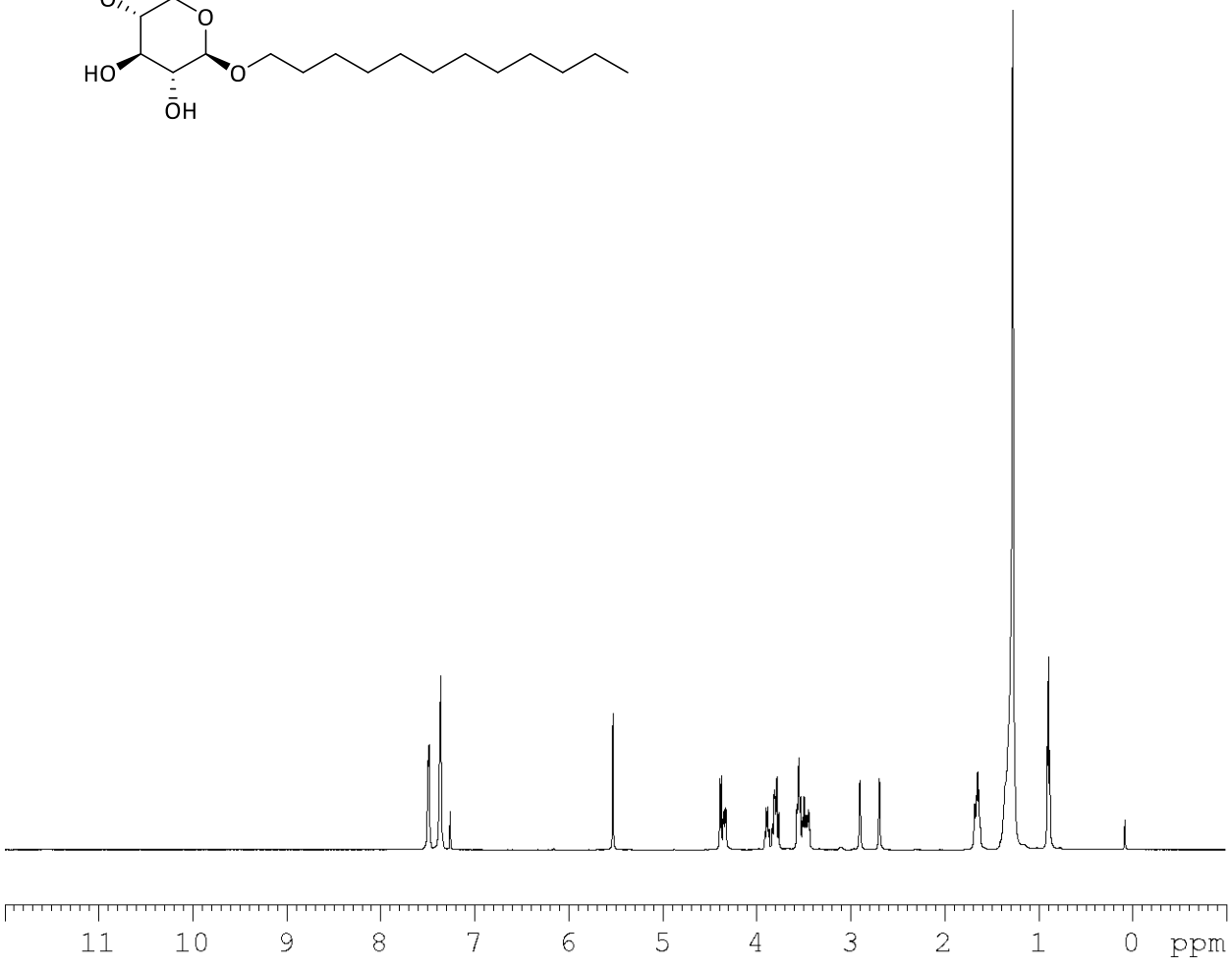
======== CHANNEL f1 ========
NUC1                 13C
P1                  6.80 usec
PL1                 5.00 dB
SFO1        125.7703148 MHz

======== CHANNEL f2 ========
CPDPRG[2         waltz16
NUC2                 1H
PCPD2             100.00 usec
PL2      0 dB
PL12                19.00 dB
PL13                23.00 dB
SFO2        500.1320005 MHz

F2 - Processing parameters
SI                  32768
SF          125.7575604 MHz
WDW                  EM
SSB       0
LB                  1.00 Hz
GB        0
PC                  1.40
```

n--Dodecyl-(4,6-*O*-bezylidene)-β-D-glucopyranoside **99**






```
                DRX500 BBO Probe IDS354
h1acqbbo CDCl3 {C:\NMR} sjcogroup 11

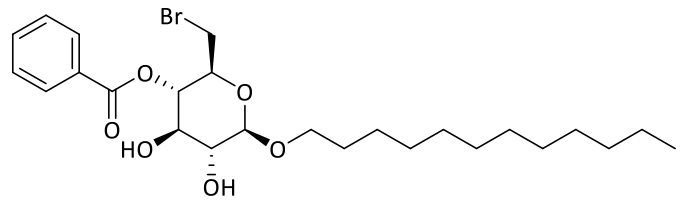
Current Data Parameters
NAME                 IDS354
EXPNO                     1
PROCNO                    1

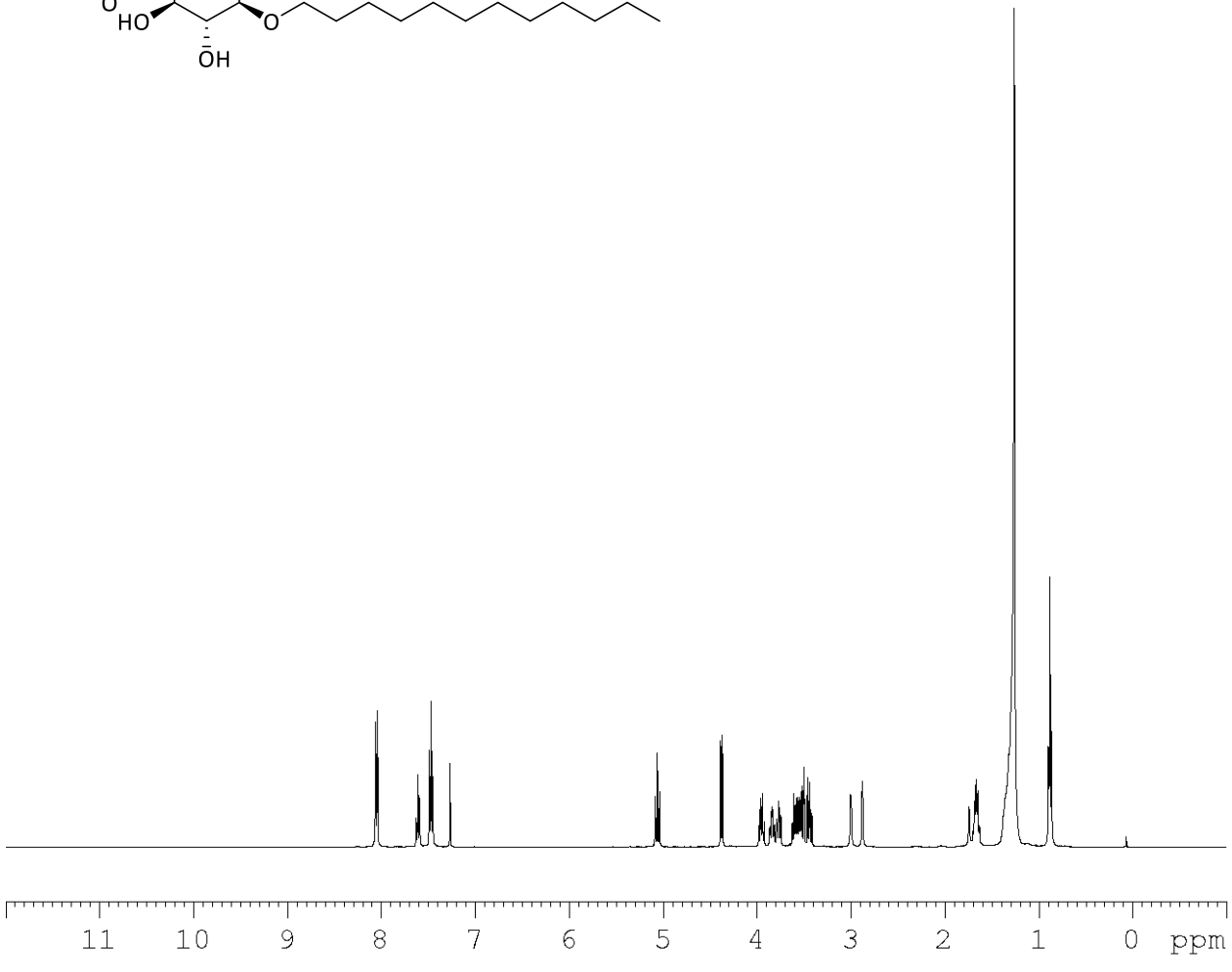
F2 - Acquisition Parameters
Date_              20131028
Time                  19.27
INSTRUM              drx500
PROBHD    5 mm PABBO BB/
PULPROG                zg60
TD                    65536
SOLVENT               CDCl3
NS                       16
DS                        2
SWH              10330.578 Hz
FIDRES            0.157632 Hz
AQ               3.1719425 sec
RG                       64
DW                  48.400 usec
DE                  17.00 usec
TE                   298.7 K
D1              1.00000000 sec

======== CHANNEL f1 ========
NUC1                     1H
P1                   11.00 usec
PL1       0 dB
SFO1          500.1325007 MHz

F2 - Processing parameters
SI                       32768
SF             500.1300283 MHz
WDW                      EM
SSB       0
LB                    0.30 Hz
GB        0
PC                    1.00
```

n-Dodecyl-(4-*O*-benzoyl-6-deoxy-6-bromo)-β-D-glucopyranoside **100**






```
          Instrument AVF400
             Chemist ds
             Group SJC
          IDS357 - columned
  h1acq.crl CDCl3 {C:\NMR} sjcgrp 6

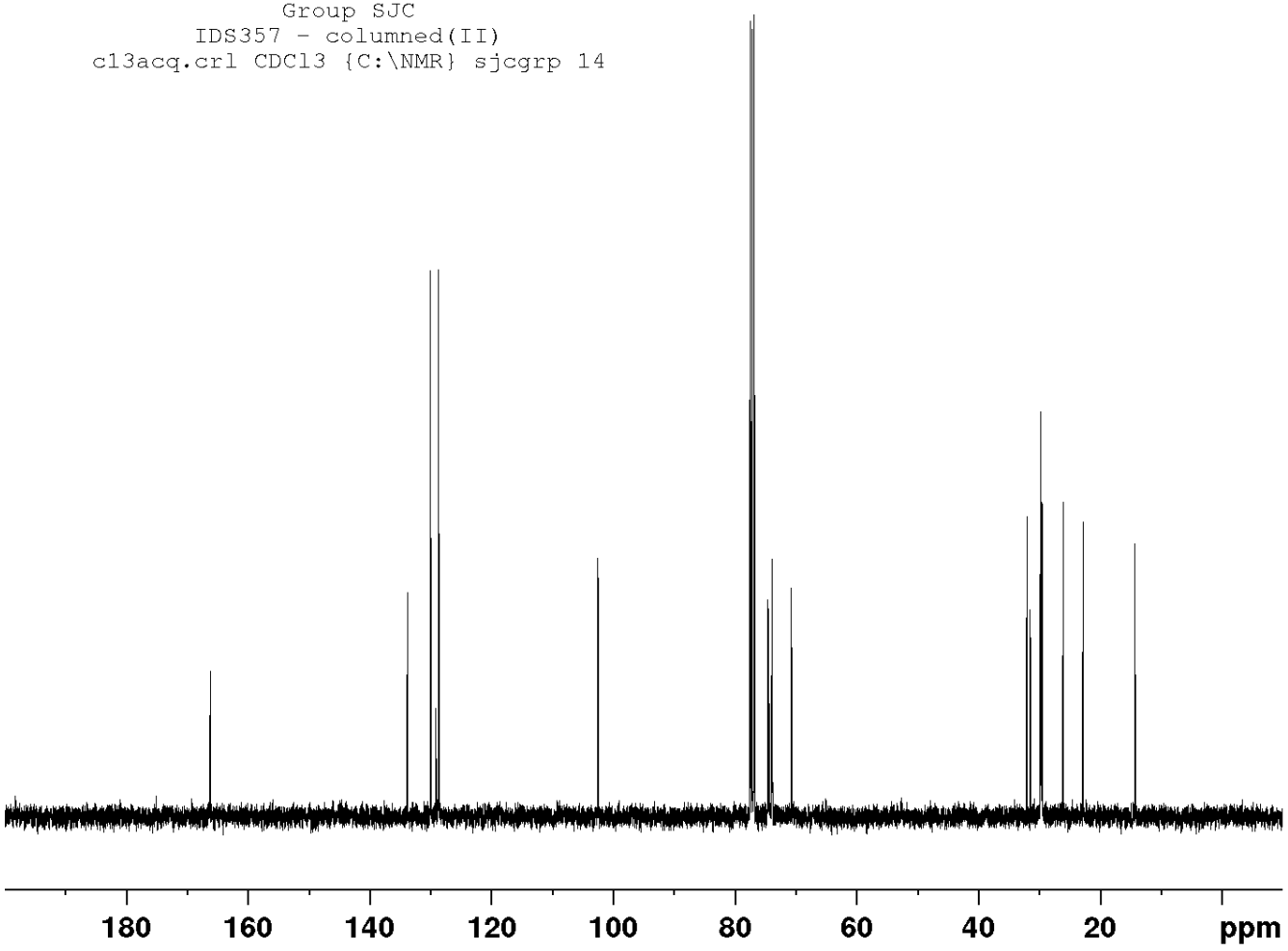
Current Data Parameters
NAME      IDS358(I) - Oct21-2013-6
EXPNO                  1
PROCNO                 1

F2 - Acquisition Parameters
Date_           20131021
Time               10.00
INSTRUM          avf400
PROBHD   5 mm PABBO BB/
PULPROG             zg60
TD                 65536
SOLVENT            CDCl3
NS                    16
DS                     2
SWH             8012.820 Hz
FIDRES          0.122266 Hz
AQ              4.0894465 sec
RG                 39.49
DW                62.400 usec
DE                 6.50 usec
TE                294.5 K
D1           1.00000000 sec
TD0                    1

======== CHANNEL f1 ========
SFO1         400.2524015 MHz
NUC1                   1H
P1                 12.65 usec
PLW1         16.70000076 W

F2 - Processing parameters
SI                 32768
SF           400.2500123 MHz
WDW                   EM
SSB       0
LB                 0.30 Hz
GB        0
PC                 1.00
```

n-Dodecyl-(4-*O*-benzoyl-6-deoxy-6-bromo)-β-D-glucopyranoside **100**




```
Current Data Parameters
NAME      IDS358(II)
          Oct21-2013-14
EXPNO                2
PROCNO               1

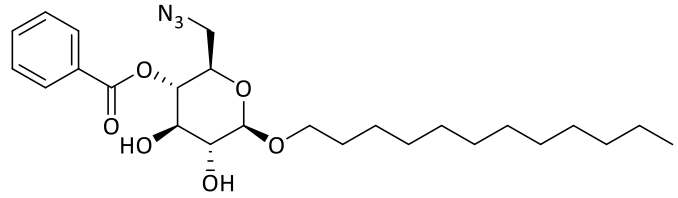
F2 - Acquisition Parameters
Date_          20131021
Time              12.26
INSTRUM          avf400
PROBHD   5 mm PABBO BB/
PULPROG          zgpg30
TD                32768
SOLVENT           CDCl3
NS                  256
DS                    4
SWH           26041.666 Hz
FIDRES         0.794729 Hz
AQ            0.6291456 sec
RG                205.43
DW                19.200 usec
DE                 6.50 usec
TE                295.3 K
D1           1.00000000 sec
D11          0.03000000 sec
TD0                  1

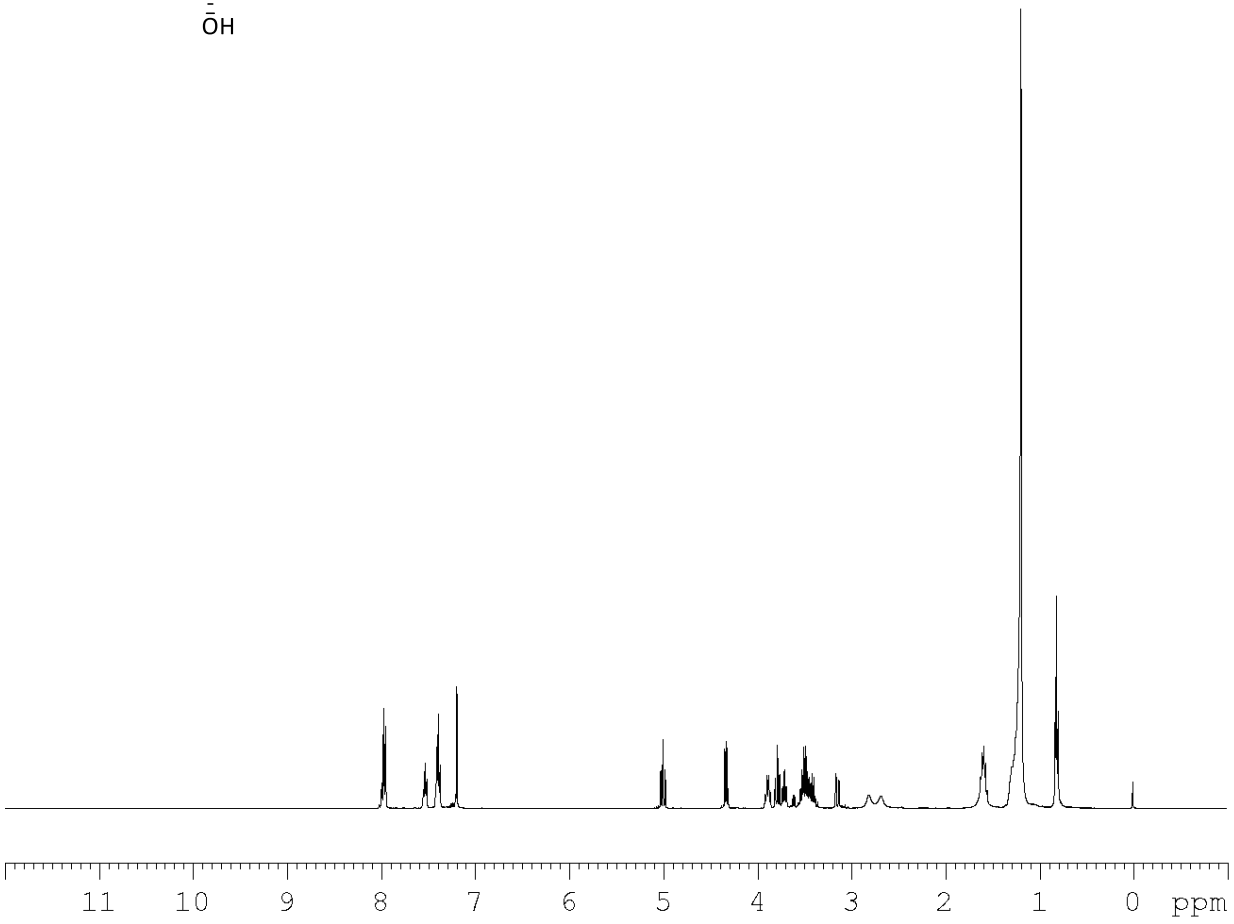
======== CHANNEL f1 ========
SFO1        100.6530073 MHz
NUC1                13C
P1                 9.00 usec
PLW1        58.70000076 W

======== CHANNEL f2 ========
SFO2        400.2516010 MHz
NUC2                1H
CPDPRG[2         waltz16
PCPD2             90.00 usec
PLW2        16.70000076 W
PLW12        0.32991999 W
PLW13        0.26723999 W

F2 - Processing parameters
SI                32768
SF          100.6429315 MHz
WDW                 EM
SSB       0
LB                 1.00 Hz
GB        0
PC                 1.40
```

n-Dodecyl-(4-*O*-benzoyl-6-deoxy-6-azido)-β-D- glucopyranoside **101**






```
        Instrument AVG400
            Group SJC
            Chemist ds
     IDS365 columned R1 F4-11
hlacq.crl CDCl3 {C:\NMR} sjcgrp 46

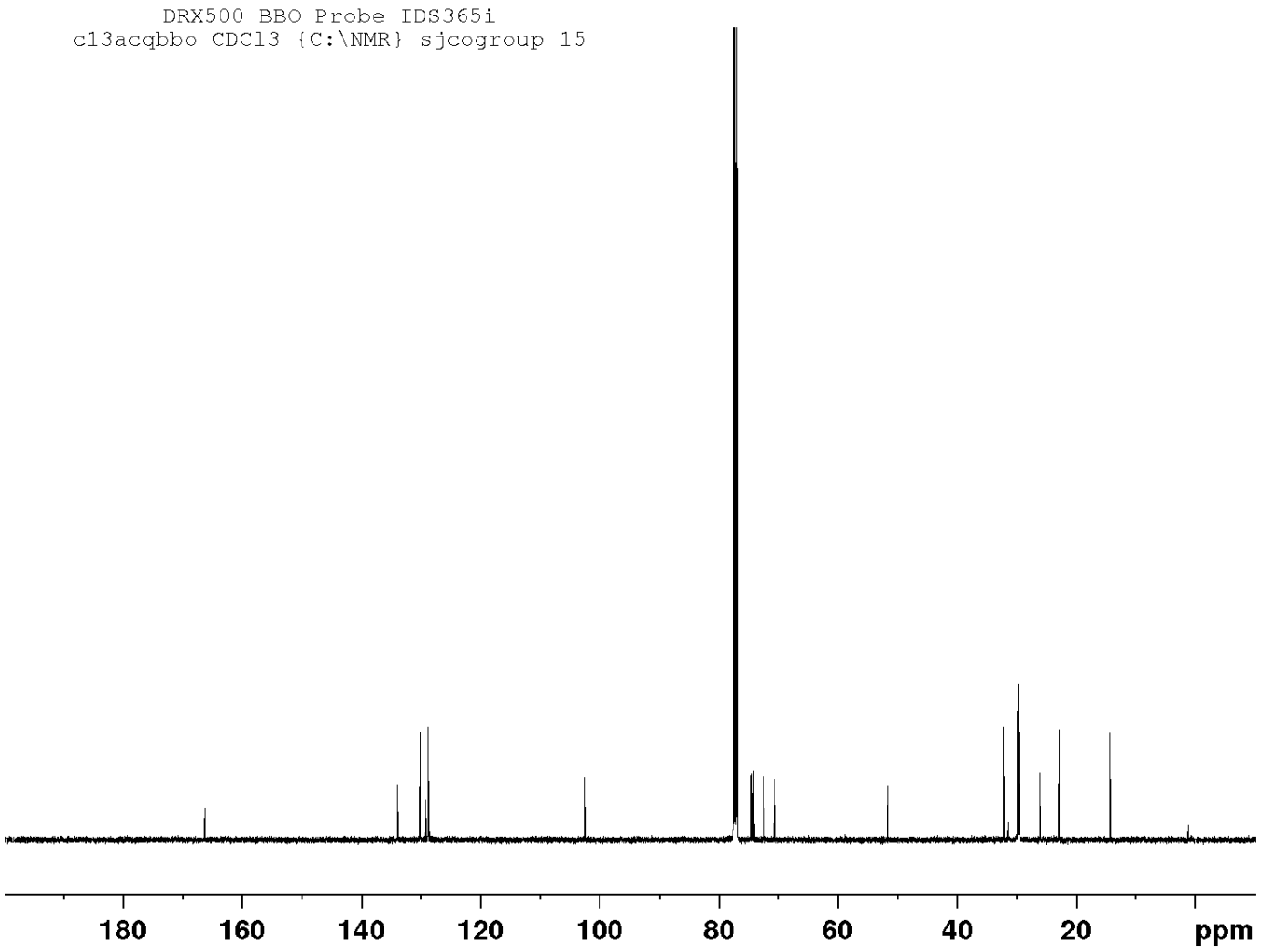
   Current Data Parameters
   NAME      IDS365 columned
             Nov24-2013-46
   EXPNO                   1
   PROCNO                  1

   F2 - Acquisition Parameters
   Date_            20131124
   Time                14.09
   INSTRUM           avg400
   PROBHD   5 mm QNP 1H/13
   PULPROG              zg30
   TD                  65536
   SOLVENT             CDCl3
   NS                     16
   DS                      2
   SWH            10000.000 Hz
   FIDRES          0.152588 Hz
   AQ             3.2767999 sec
   RG                 221.01
   DW                 50.000 usec
   DE                   6.50 usec
   TE                 294.8 K
   D1            1.00000000 sec
   TD0                     1

   ======== CHANNEL f1 ========
   SFO1         400.2024714 MHz
   NUC1                   1H
   P1                 12.23 usec
   PLW1         11.30000019 W

   F2 - Processing parameters
   SI                  65536
   SF           400.2000404 MHz
   WDW                    EM
   SSB       0
   LB                  0.30 Hz
   GB        0
   PC                  1.00
```

n-Dodecyl-(4-*O*-benzoyl-6-deoxy-6-azido)-β-D- glucopyranoside **101**




```
Current Data Parameters
NAME              IDS365i
EXPNO                   3
PROCNO                  1

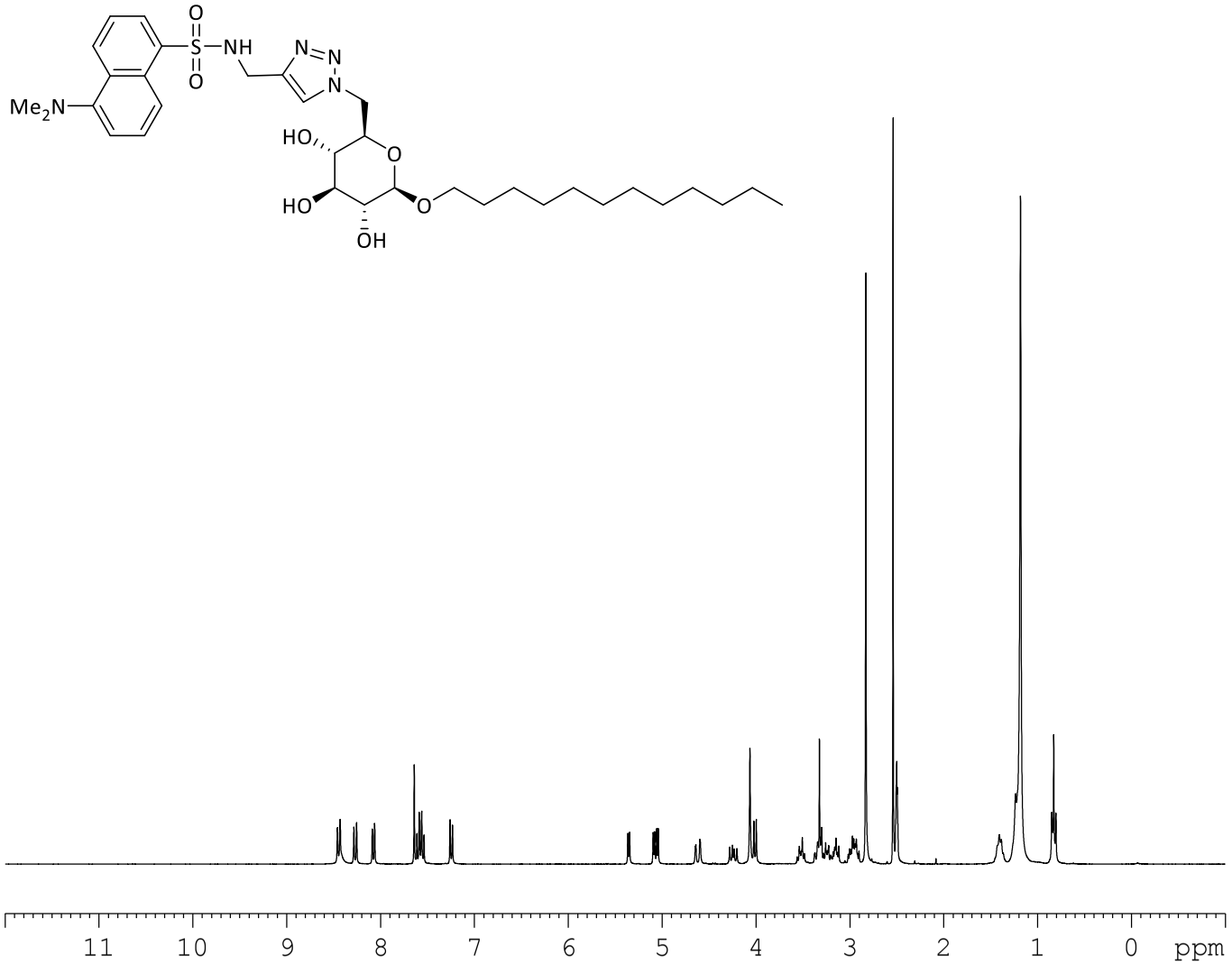
F2 - Acquisition Parameters
Date_           20131125
Time               22.40
INSTRUM          drx500
PROBHD   5 mm PABBO BB/
PULPROG          zgpg30
TD                65536
SOLVENT           CDCl3
NS                 3072
DS                    4
SWH           30303.031 Hz
FIDRES         0.462388 Hz
AQ            1.0813440 sec
RG               4597.6
DW               16.500 usec
DE               11.00 usec
TE                297.7 K
D1           2.00000000 sec
d11          0.03000000 sec
DELTA        1.89999998 sec
TD0                   1

======== CHANNEL f1 ========
NUC1                 13C
P1                  6.80 usec
PL1                 5.00 dB
SFO1        125.7703148 MHz

======== CHANNEL f2 ========
CPDPRG[2         waltz16
NUC2                 1H
PCPD2             100.00 usec
PL2       0 dB
PL12                19.00 dB
PL13                23.00 dB
SFO2        500.1320005 MHz

F2 - Processing parameters
SI                  32768
SF          125.7577761 MHz
WDW                    EM
SSB       0
LB                  1.00 Hz
GB        0
PC                  1.40
```

n-Dodecyl-(6-deoxy-6-[4-{*N*-dansyl}aminomethyl-1*H*-1,2,3-triazol-1-yl]-β-D-glucopyranoside **45**




```
Instrument DPX300
Chemist Name ds
Research Group SJC
IDS373i

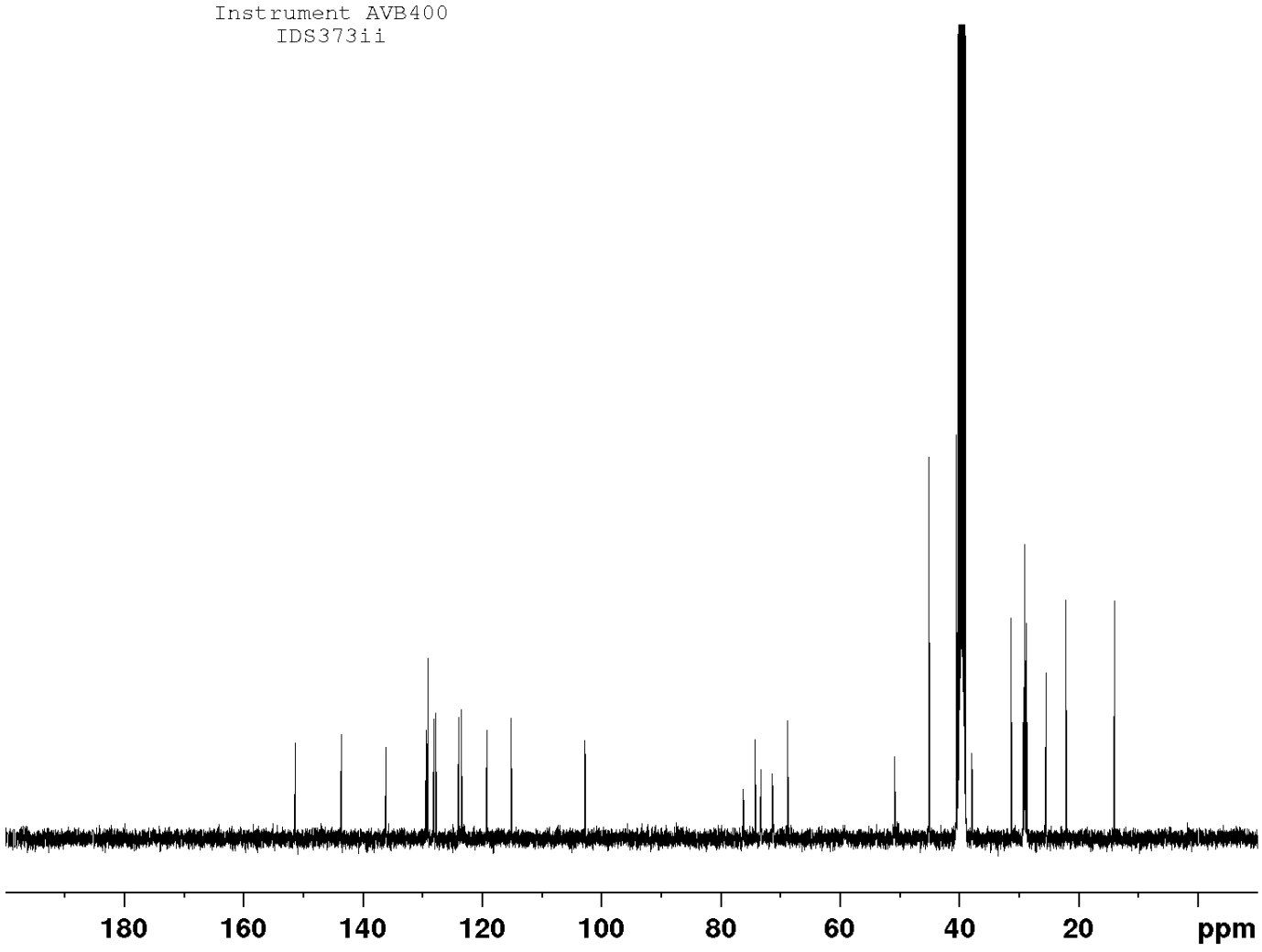
Current Data Parameters
NAME      IDS373i - Dec17-2013-2
EXPNO                  1
PROCNO                 1

F2 - Acquisition Parameters
Date_           20131217
Time                16.14
INSTRUM           DPX300
PROBHD   5 mm DUL 13C-1
PULPROG              zg60
TD                  32768
SOLVENT              DMSO
NS                     16
DS                      2
SWH              4789.272 Hz
FIDRES           0.146157 Hz
AQ              3.4209793 sec
RG                    256
DW                104.400 usec
DE                  6.00 usec
TE                 300.0 K
D1           1.00000000 sec
TD0                    1

======== CHANNEL f1 ========
NUC1                  1H
P1                 16.50 usec
PL1                -6.00 dB
SFO1         300.1315007 MHz

F2 - Processing parameters
SI                  32768
SF           300.1300011 MHz
WDW                    EM
SSB       0
LB                  0.30 Hz
GB        0
PC                  1.00
```

n-Dodecyl-(6-deoxy-6-[4-{*N*-dansyl}aminomethyl-1*H*-1,2,3-triazol-1-yl]-β-D-glucopyranoside **45**




```
Current Data Parameters
NAME            IDS373ii
EXPNO                  2
PROCNO                 1

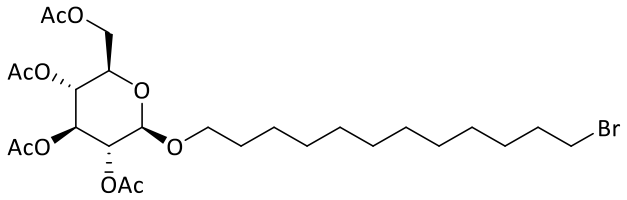
F2 - Acquisition Parameters
Date_           20131217
Time               18.29
INSTRUM          avb400
PROBHD   5 mm PABBO BB/
PULPROG          zgpg30
TD                65536
SOLVENT            DMSO
NS                 1900
DS                    4
SWH           24038.461 Hz
FIDRES         0.366798 Hz
AQ            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

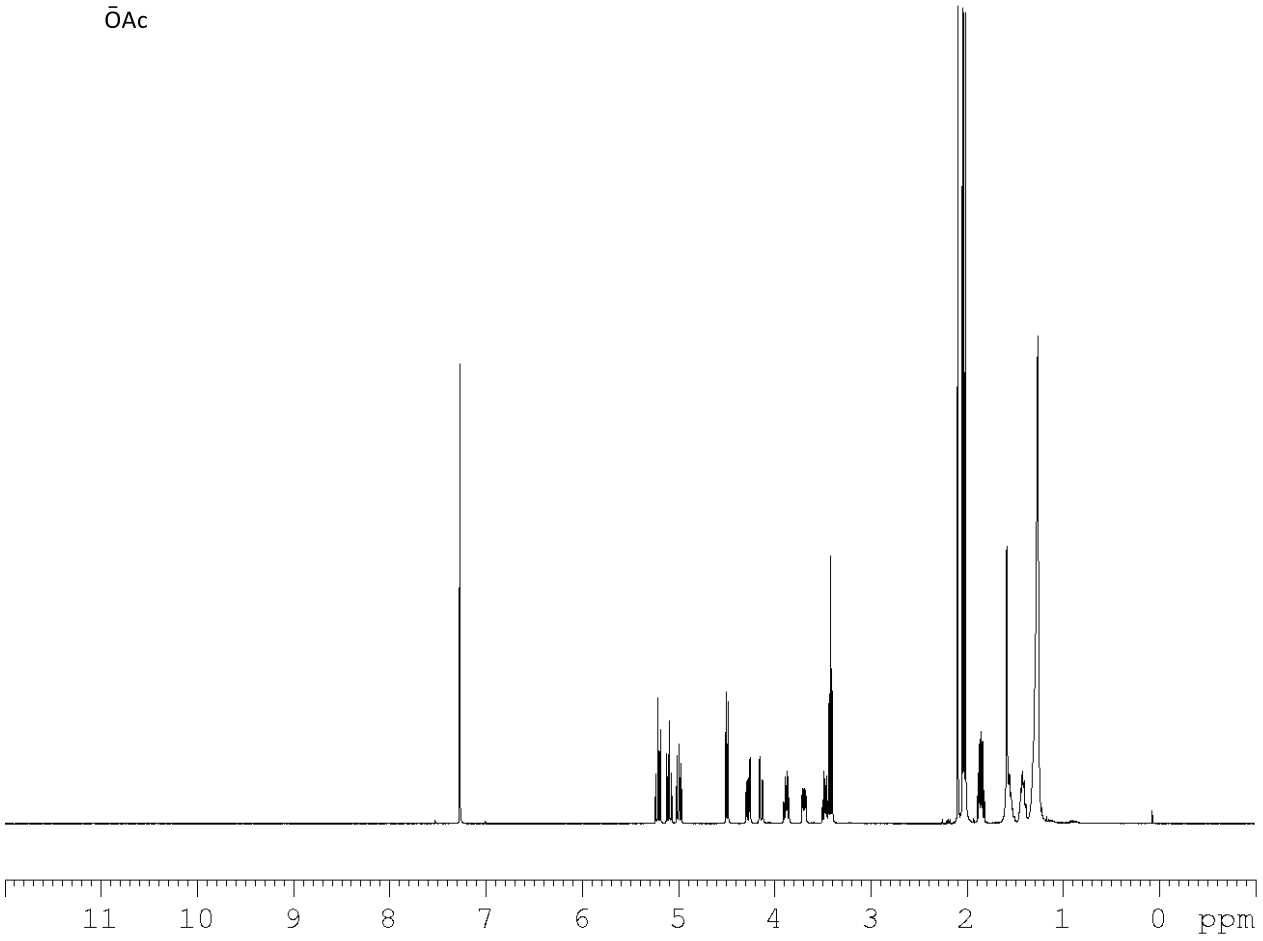
======== CHANNEL f1 ========
SFO1        100.6228303 MHz
NUC1                13C
P1                10.00 usec
PLW1        60.95399857 W

======== 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          100.6128197 MHz
WDW                  EM
SSB       0
LB                1.00 Hz
GB        0
PC                1.40
```

n-12-Bromododecyl-(2,3,4,6-*O*-tetraacetyl)-β-D-glucopyranoside **104**






```
        Instrument DQX400
            Chemist ds
            Group SJC
        IDS333 - columned
h1acq.au CDCl3 {E:\NMR2013} sjcgrp 16

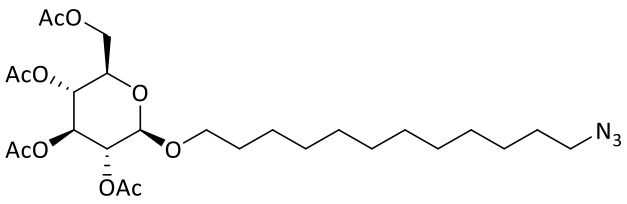
    Current Data Parameters
    NAME      IDS333 columned
              Aug09-2013-16
    EXPNO                    1
    PROCNO                   1

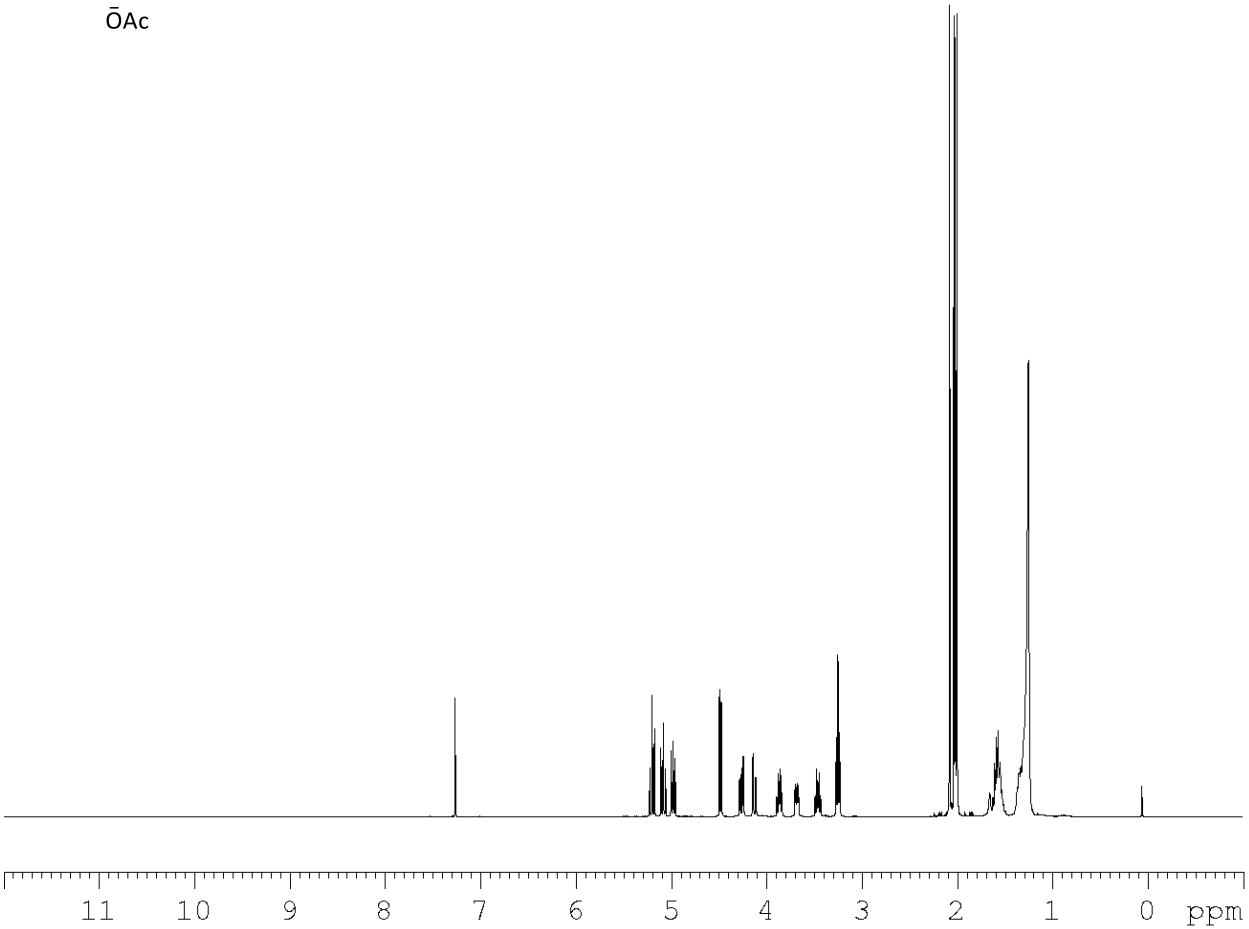
    F2 - Acquisition Parameters
    Date_             20130809
    Time                 10.35
    INSTRUM              av400
    PROBHD   5 mm QNP 1H/13
    PULPROG               zg60
    TD                   65536
    SOLVENT              CDCl3
    NS                      16
    DS                       2
    SWH               8278.146 Hz
    FIDRES           0.126314 Hz
    AQ               3.9583745 sec
    RG                     256
    DW                  60.400 usec
    DE                   7.50 usec
    TE                  300.0 K
    D1             1.00000000 sec

    ======== CHANNEL f1 ========
    NUC1                    1H
    P1                   10.10 usec
    PL1       0 dB
    SFO1          400.2024714 MHz

    F2 - Processing parameters
    SI                   32768
    SF            400.2000068 MHz
    WDW                     EM
    SSB       0
    LB                    0.30 Hz
    GB        0
    PC                    1.00
```

n-12-Azidododecyl-(2,3,4,6-*O*-tetraacetyl)-β-D-glucopyranoside **67**






```
        Instrument AVN400
            Chemist ds
            Group SJC
        IDS339 - columned
h1acq.crl CDCl3 {C:\NMR} sjcgrp 17

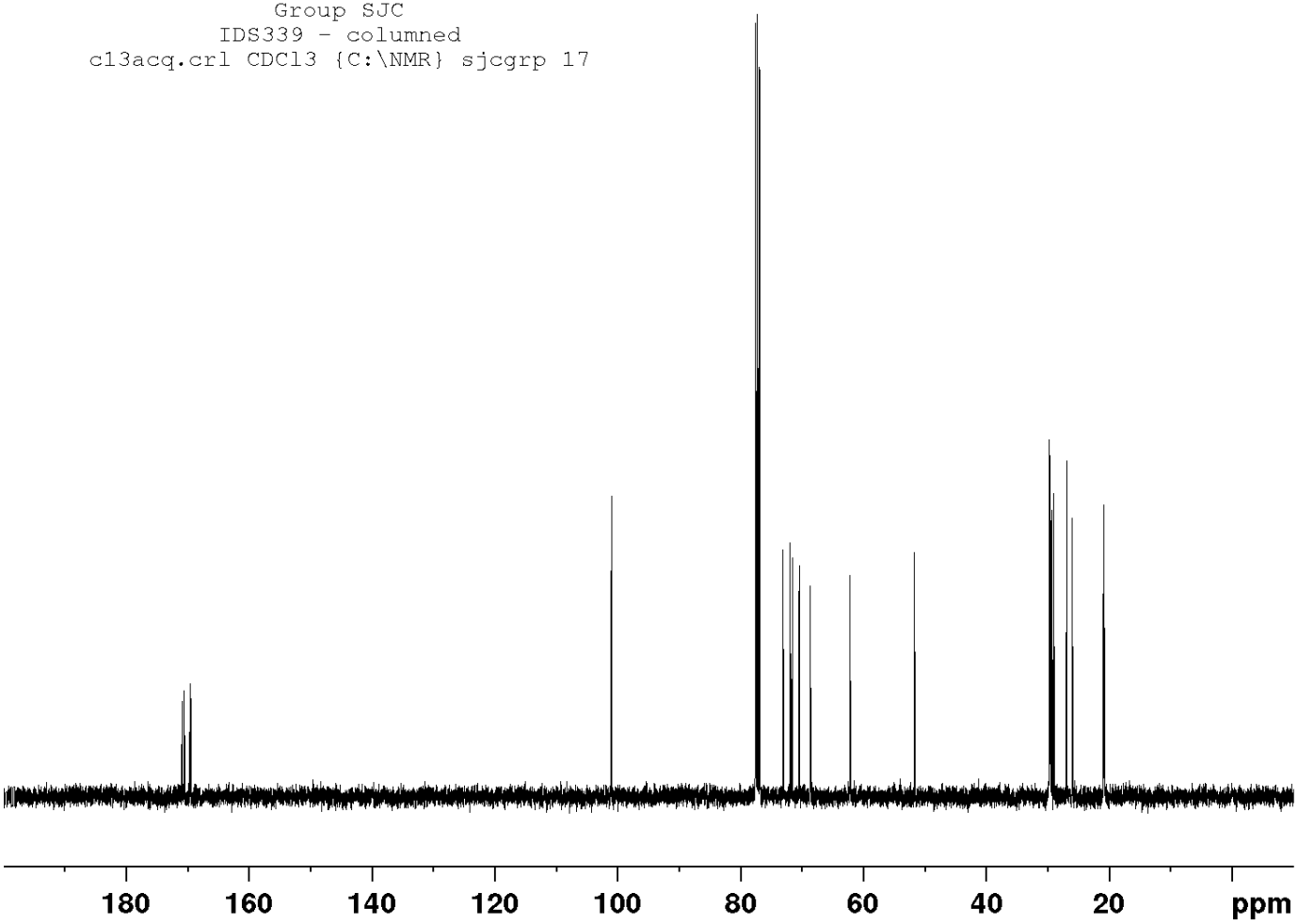
   Current Data Parameters
   NAME      IDS339
             Aug19-2013-17
   EXPNO                   1
   PROCNO                  1

   F2 - Acquisition Parameters
   Date_           20130819
   Time               17.41
   INSTRUM           avn400
   PROBHD   5 mm PABBO BB/
   PULPROG             zg60
   TD                 65536
   SOLVENT            CDCl3
   NS                    16
   DS                     2
   SWH             8012.820 Hz
   FIDRES          0.122266 Hz
   AQ              4.0894465 sec
   RG                 39.49
   DW                62.400 usec
   DE                 6.50 usec
   TE                294.4 K
   D1             1.00000000 sec
   TD0                    1

   ======== CHANNEL f1 ========
   SFO1         400.2524015 MHz
   NUC1                   1H
   P1                 12.65 usec
   PLW1         16.70000076 W

   F2 - Processing parameters
   SI                 32768
   SF           400.2500117 MHz
   WDW                   EM
   SSB       0
   LB                 0.30 Hz
   GB        0
   PC                 1.00
```

n-12-Azidododecyl-(2,3,4,6-*O*-tetraacetyl)-β-D-glucopyranoside **67**




```
Current Data Parameters
NAME      IDS339 - Aug19-2013-17
EXPNO                          3
PROCNO                         1

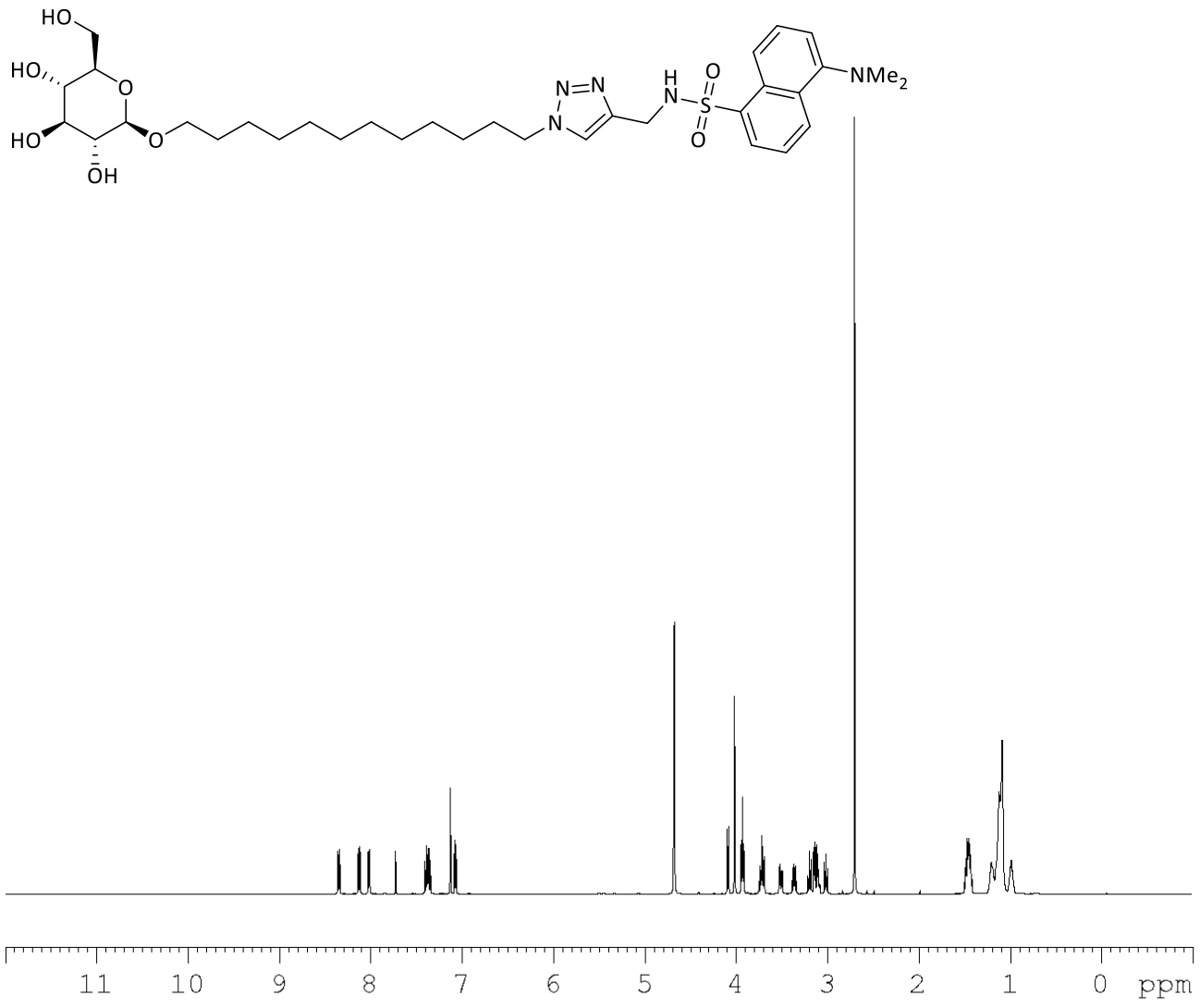
F2 - Acquisition Parameters
Date_                   20130819
Time                       21.53
INSTRUM                  avn400
PROBHD   5 mm PABBO BB/
PULPROG                  zgpg30
TD                        32768
SOLVENT                   CDCl3
NS                          256
DS                            4
SWH                   26041.666 Hz
FIDRES                 0.794729 Hz
AQ                     0.6291456 sec
RG                        205.43
DW                        19.200 usec
DE                         6.50 usec
TE                        295.9 K
D1                    1.00000000 sec
D11                   0.03000000 sec
TD0                           1

======== CHANNEL f1 ========
SFO1                100.6530073 MHz
NUC1                        13C
P1                         9.00 usec
PLW1                58.70000076 W

======== CHANNEL f2 ========
SFO2                400.2516010 MHz
NUC2                        1H
CPDPRG[2                waltz16
PCPD2                     90.00 usec
PLW2                16.70000076 W
PLW12                0.32991999 W
PLW13                0.26723999 W

F2 - Processing parameters
SI                        32768
SF                  100.6429317 MHz
WDW                         EM
SSB       0
LB                         1.00 Hz
GB        0
PC                         1.40
```

n-12-(4-[*N*-Dansyl]-aminomethyl-1*H*-1,2,3-triazol-1-yl)-dodecyl-β-D-glucopyranoside **46**




```
DRX500 BBO Probe IDS340
h1acqbbo MeOD {C:\NMR} sjcogroup 20

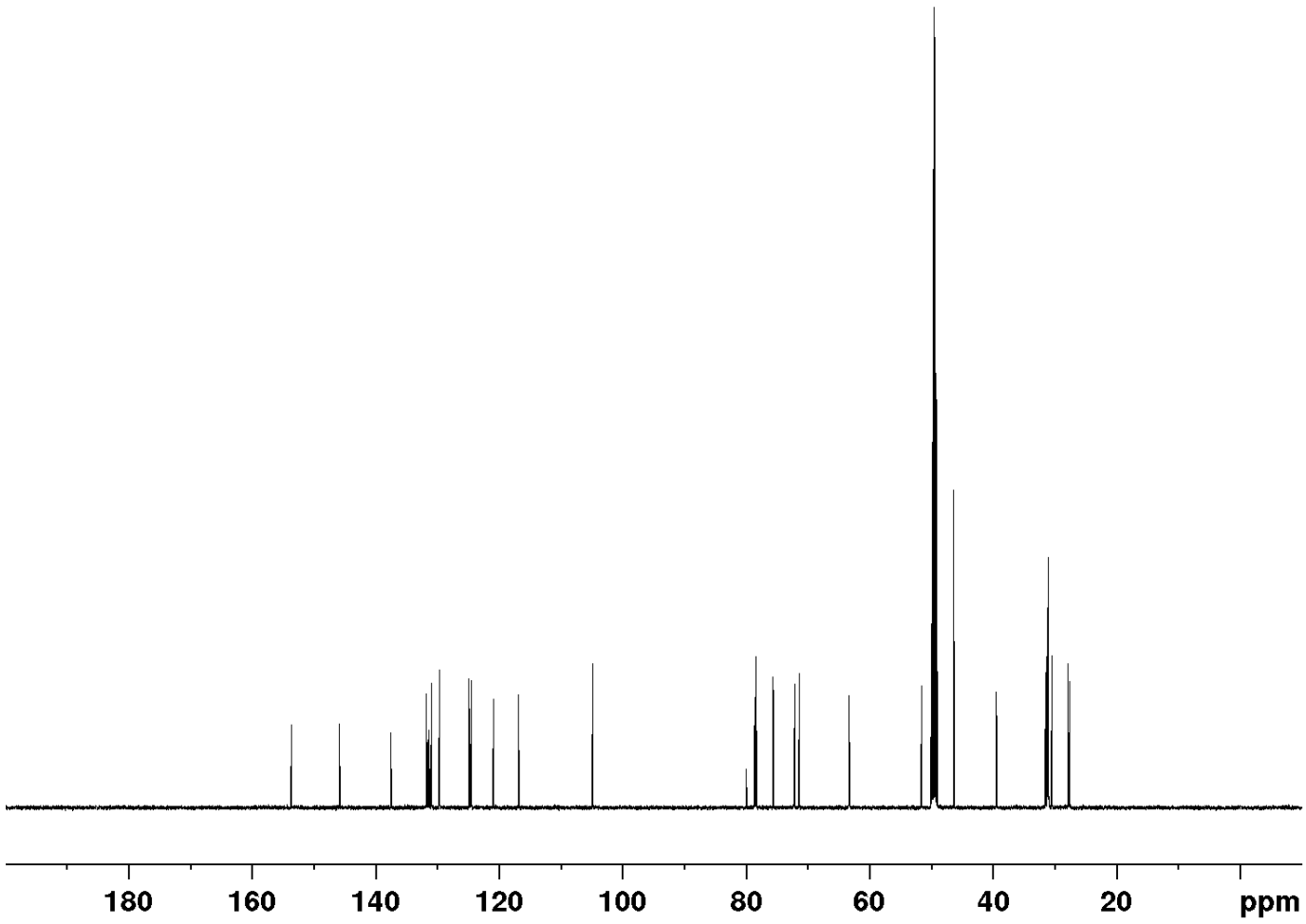
Current Data Parameters
NAME              IDS340d
EXPNO                   1
PROCNO                  1

F2 - Acquisition Parameters
Date_            20131030
Time                 5.27
INSTRUM            drx500
PROBHD   5 mm PABBO BB/
PULPROG              zg60
TD                  65536
SOLVENT              MeOD
NS                     16
DS                      2
SWH            10330.578 Hz
FIDRES          0.157632 Hz
AQ             3.1719425 sec
RG                     32
DW                48.400 usec
DE                17.00 usec
TE                298.7 K
D1           1.00000000 sec

======== CHANNEL f1 ========
NUC1                   1H
P1                 11.00 usec
PL1       0 dB
SFO1         500.1325007 MHz

F2 - Processing parameters
SI                  32768
SF           500.1300970 MHz
WDW                    EM
SSB       0
LB                  0.30 Hz
GB        0
PC                  1.00
```

n-12-(4-[*N*-Dansyl]-aminomethyl-1*H*-1,2,3-triazol-1-yl)-dodecyl-β-D-glucopyranoside **46**




```
Current Data Parameters
NAME            IDS340d
EXPNO                 3
PROCNO                1

F2 - Acquisition Parameters
Date_          20131030
Time               7.01
INSTRUM          drx500
PROBHD   5 mm PABBO BB/
PULPROG          zgpg30
TD                65536
SOLVENT             MeOD
NS                  1536
DS                     4
SWH           30303.031 Hz
FIDRES         0.462388 Hz
AQ            1.0813440 sec
RG              10321.3
DW               16.500 usec
DE               11.00 usec
TE               298.7 K
D1           2.00000000 sec
d11          0.03000000 sec
DELTA        1.89999998 sec
TD0                   1

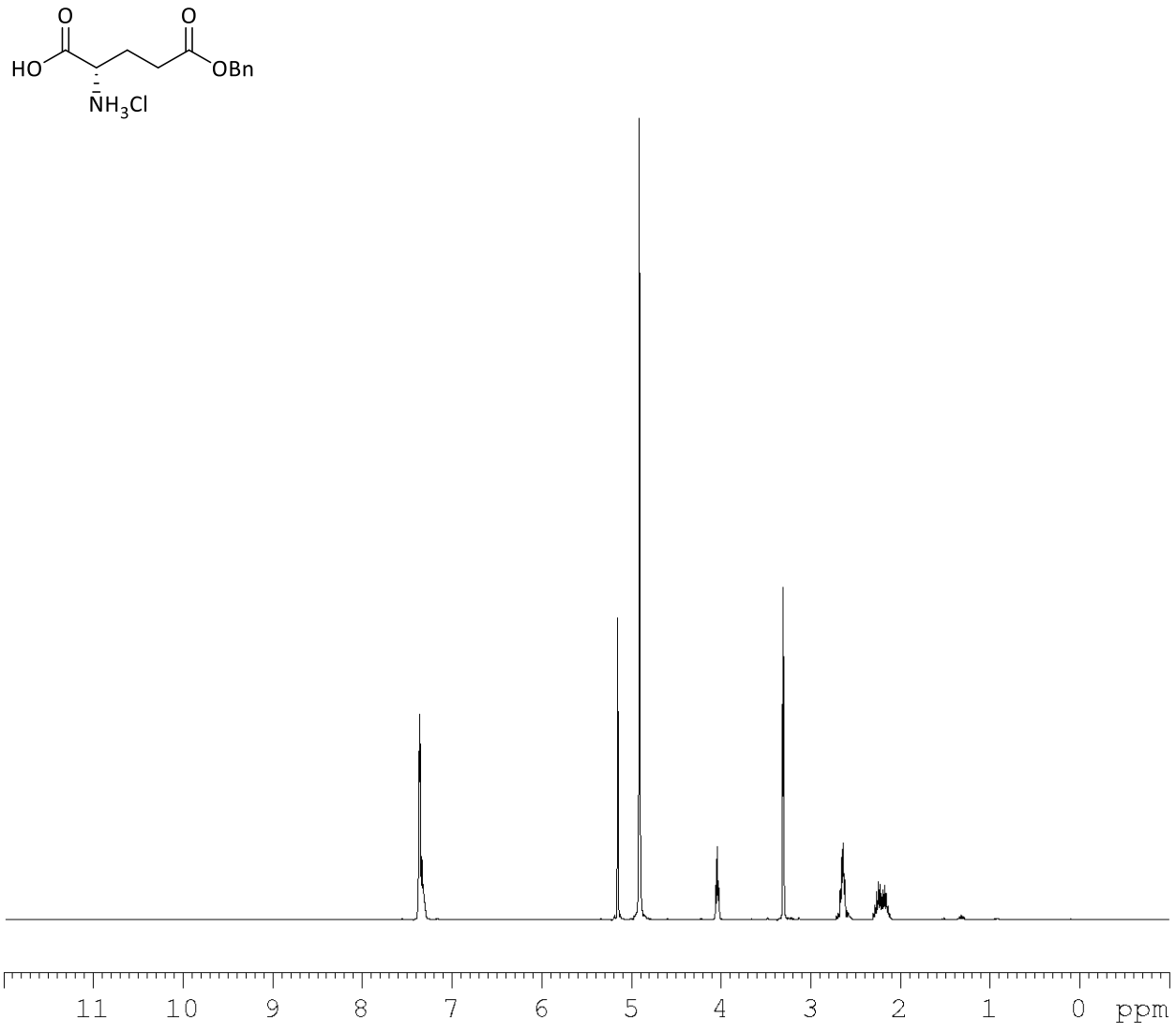
======== CHANNEL f1 ========
NUC1                 13C
P1                6.80 usec
PL1               5.00 dB
SFO1        125.7703148 MHz

======== CHANNEL f2 ========
CPDPRG[2         waltz16
NUC2                 1H
PCPD2            100.00 usec
PL2      0 dB
PL12              19.00 dB
PL13              23.00 dB
SFO2        500.1320005 MHz

F2 - Processing parameters
SI                32768
SF          125.7575604 MHz
WDW                  EM
SSB      0
LB                1.00 Hz
GB       0
PC                1.40
```

NMR data for the synthesis of small molecule probes for Kef

Glutamic acid 5-benzyl ester hydrochloride salt **143**




```
        Instrument DPX400
            Chemist ds
            Group sjc
              IDS65
Qh1acq.au MeOD {C:\NMR} sjcgrp 4

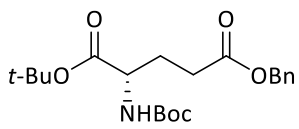
Current Data Parameters
NAME      IDS065 - Mar25-2011-4
EXPNO                         1
PROCNO                        1

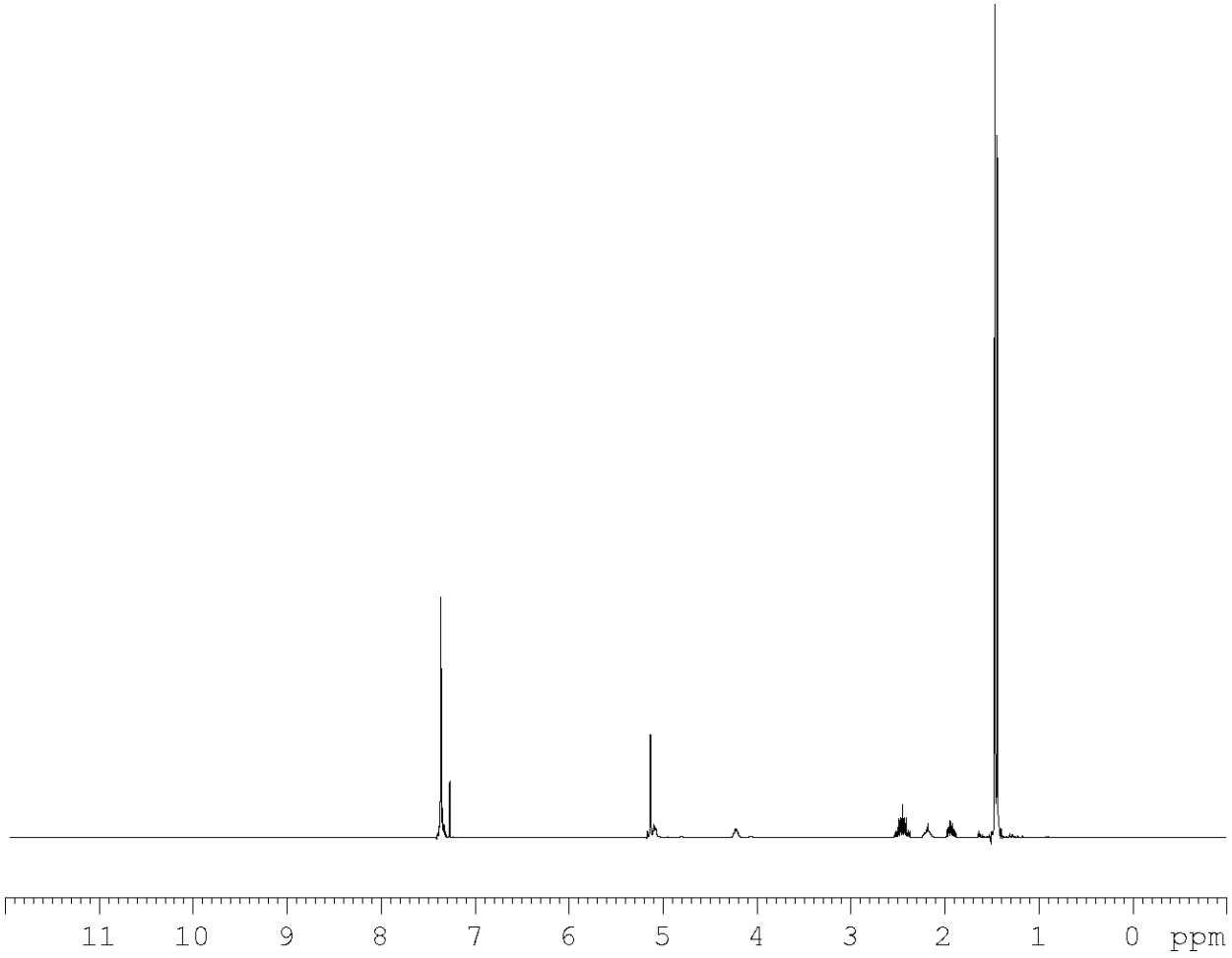
F2 - Acquisition Parameters
Date_                  20110325
Time                      10.09
INSTRUM                 dpx400
PROBHD    5 mm Dual 1H/1
PULPROG                    zg60
TD                        32768
SOLVENT                    MeOD
NS                           16
DS                            2
SWH                    5592.841 Hz
FIDRES                 0.170680 Hz
AQ                     2.9294591 sec
RG                         287.4
DW                        89.400 usec
DE                        17.00 usec
TE                        300.0 K
D1                   1.00000000 sec

======== CHANNEL f1 ========
NUC1                          1H
P1                         7.30 usec
PL1       0 dB
SFO1             400.1320007 MHz

F2 - Processing parameters
SI                        32768
SF                 400.1300075 MHz
WDW                          EM
SSB       0
LB                         0.30 Hz
GB        0
PC                         0.60
```

1-(*tert*-Butyl) 5-benzyl Boc-L-glutamate **138**






```
        Instrument DPX400
            Chemist ds
            Group sjc
          IDS123 - clean
Qh1acq.au CDCl3 {C:\NMR} sjcgrp 38

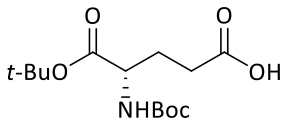
  Current Data Parameters
  NAME      IDS123 clean
             Oct19-2011-38
  EXPNO                  1
  PROCNO                 1

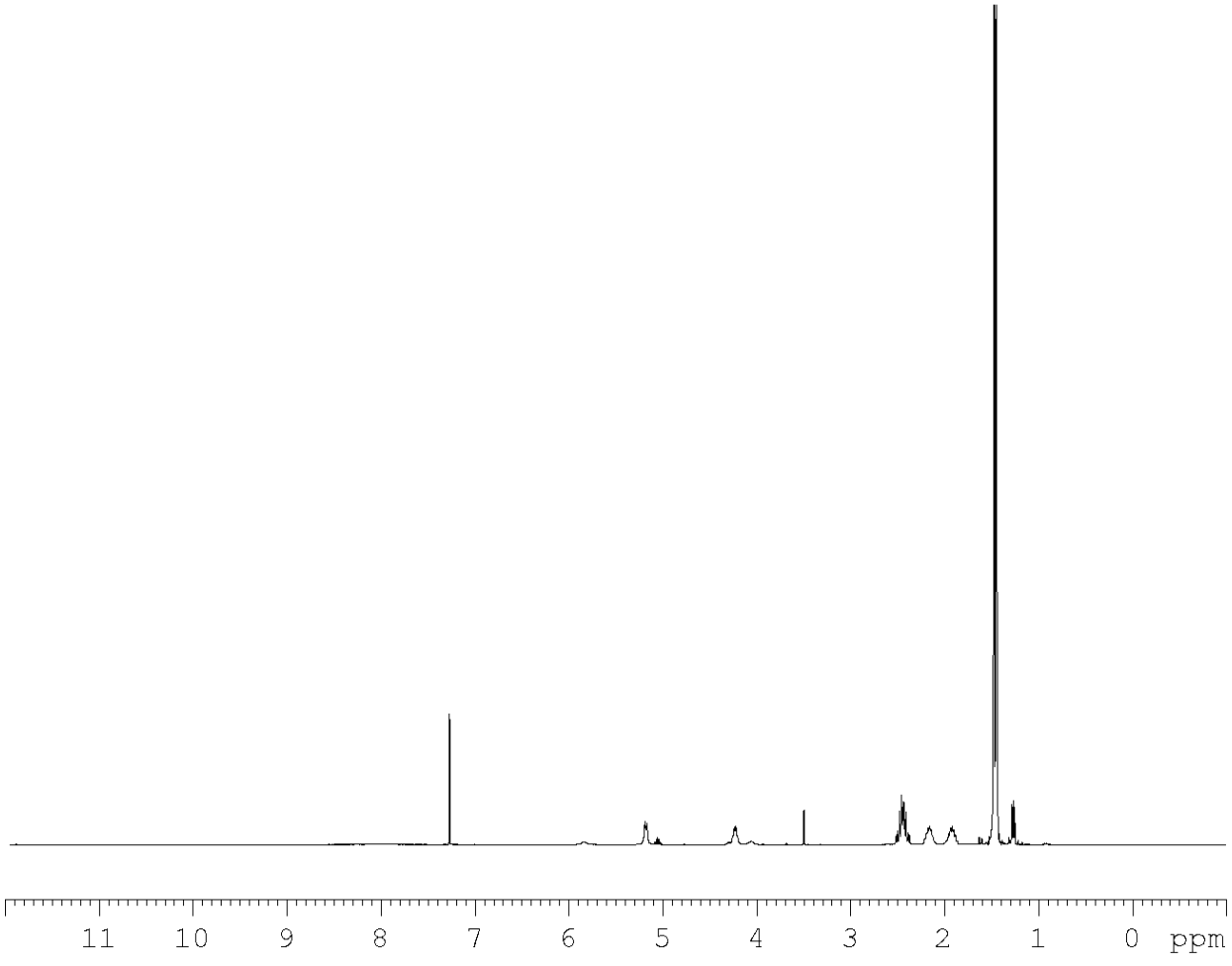
  F2 - Acquisition Parameters
  Date_           20111019
  Time               10.52
  INSTRUM          dpx400
  PROBHD   5 mm Dual 1H/1
  PULPROG            zg60
  TD                32768
  SOLVENT           CDCl3
  NS                   16
  DS                    2
  SWH            5592.841 Hz
  FIDRES         0.170680 Hz
  AQ             2.9294591 sec
  RG                45.3
  DW               89.400 usec
  DE               17.00 usec
  TE               300.0 K
  D1           1.00000000 sec

  ======== CHANNEL f1 ========
  NUC1                 1H
  P1                7.30 usec
  PL1       0 dB
  SFO1         400.1320007 MHz

  F2 - Processing parameters
  SI                32768
  SF          400.1300182 MHz
  WDW                  EM
  SSB       0
  LB                0.30 Hz
  GB        0
  PC                0.60
```

Boc-L-glutamic acid 1-*tert*-butyl ester **131**






```
Instrument DPX400
Chemist ds
Group sjc
IDS125 - pure
Qh1acq.au CDCl3 {C:\NMR} sjcgrp 18

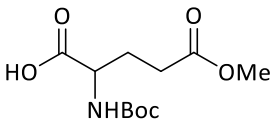
Current Data Parameters
NAME      IDS125 - Oct24-2011-18
EXPNO                  1
PROCNO                 1

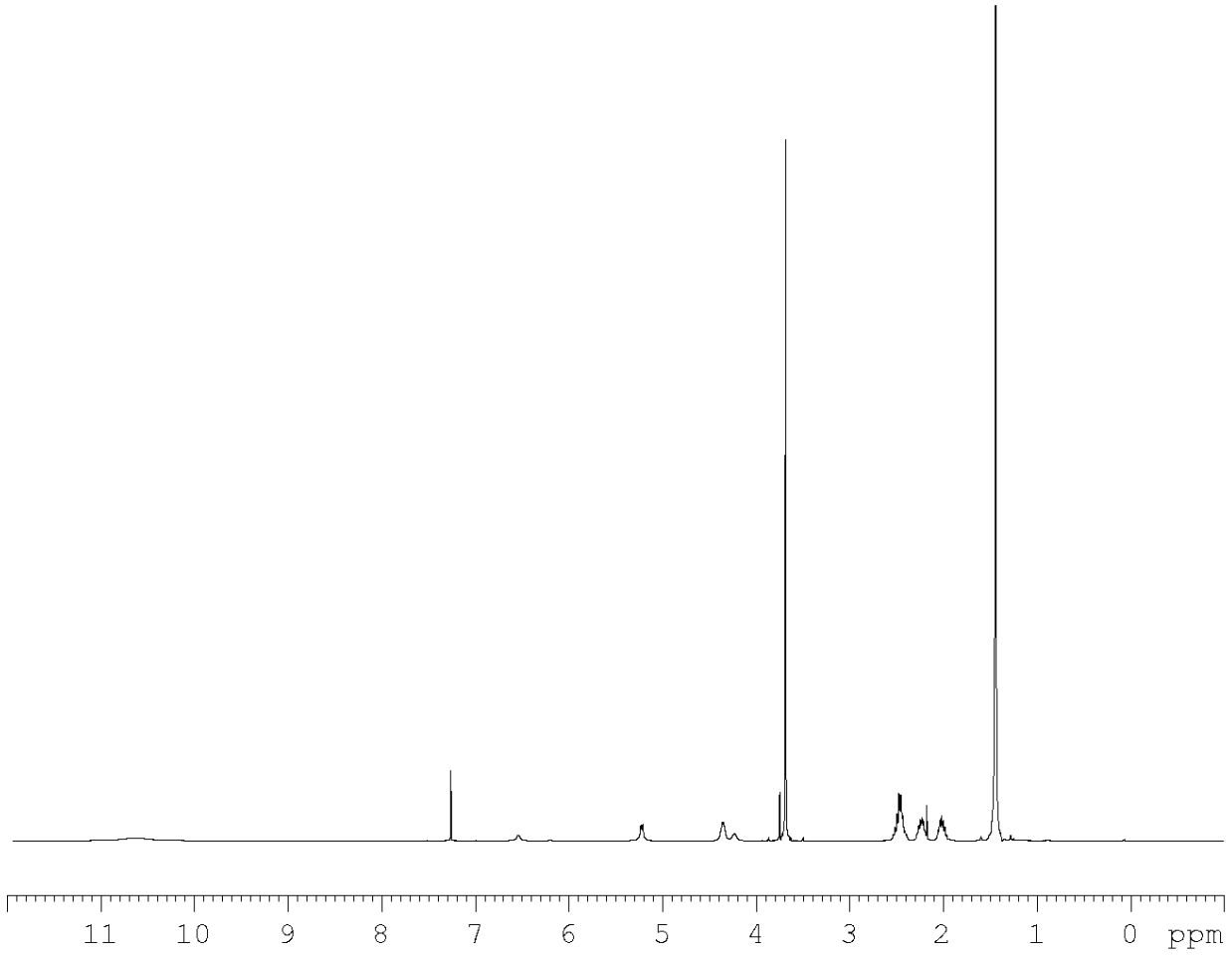
F2 - Acquisition Parameters
Date_           20111024
Time              14.21
INSTRUM           dpx400
PROBHD   5 mm Dual 1H/1
PULPROG             zg60
TD                 32768
SOLVENT            CDCl3
NS                    16
DS                     2
SWH             5592.841 Hz
FIDRES          0.170680 Hz
AQ              2.9294591 sec
RG                 71.8
DW                89.400 usec
DE                17.00 usec
TE                300.0 K
D1           1.00000000 sec

======== CHANNEL f1 ========
NUC1                   1H
P1                  7.30 usec
PL1       0 dB
SFO1         400.1320007 MHz

F2 - Processing parameters
SI                 32768
SF           400.1300182 MHz
WDW                   EM
SSB       0
LB                  0.30 Hz
GB        0
PC                  0.60
```

Boc-(DL)-glutamic acid 5-methyl ester **146**






```
        Instrument DPX400
            Chemist ds
            Group sjc
      IDS60 - redried in CDCl3
Qh1acq.au CDCl3 {C:\NMR} sjcgrp 60

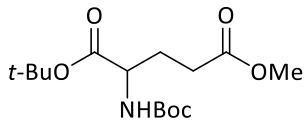
   Current Data Parameters
   NAME      IDS060 CDCl3
             Mar02-2011-60
   EXPNO                  1
   PROCNO                 1

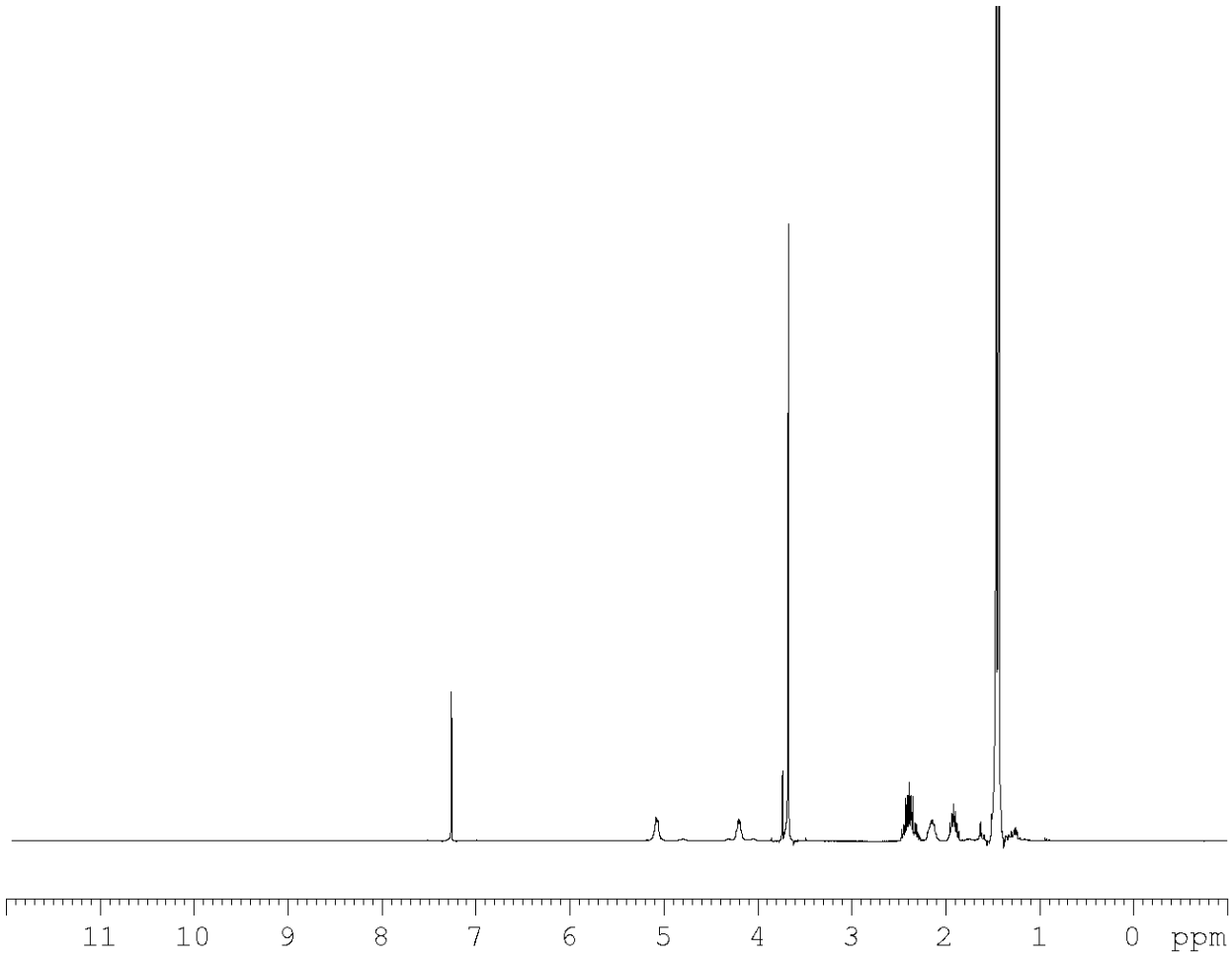
   F2 - Acquisition Parameters
   Date_           20110302
   Time               14.10
   INSTRUM          dpx400
   PROBHD   5 mm Dual 1H/1
   PULPROG             zg60
   TD                 32768
   SOLVENT            CDCl3
   NS                    16
   DS                     2
   SWH             5592.841 Hz
   FIDRES          0.170680 Hz
   AQ             2.9294591 sec
   RG                  71.8
   DW                89.400 usec
   DE                17.00 usec
   TE                300.0 K
   D1           1.00000000 sec

   ======== CHANNEL f1 ========
   NUC1                  1H
   P1                 7.30 usec
   PL1       0 dB
   SFO1        400.1320007 MHz

   F2 - Processing parameters
   SI                 32768
   SF          400.1300214 MHz
   WDW                   EM
   SSB       0
   LB                 0.30 Hz
   GB        0
   PC                 0.60
```

1-(*tert*-Butyl) 5-methyl Boc-(DL)-glutamate **147**






```
          Instrument DPX400
              Chemist ds
              Group sjc
IDS61 - tBu protection (2 x tBu peaks?)
   Qh1acq.au CDCl3 {C:\NMR} sjcgrp 6

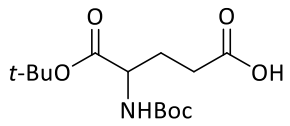
 Current Data Parameters
 NAME      IDS061 - Mar21-2011-6
 EXPNO                         1
 PROCNO                        1

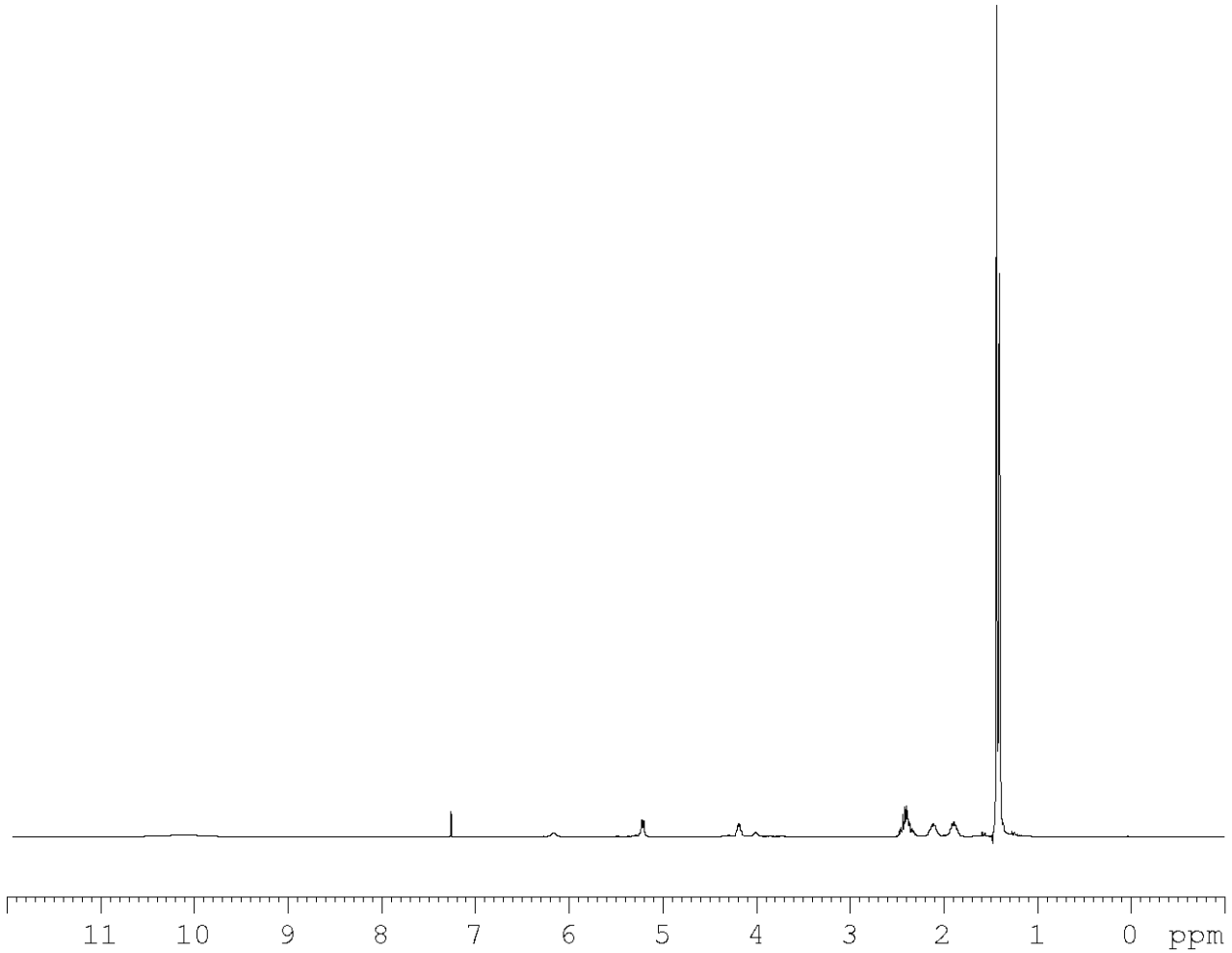
 F2 - Acquisition Parameters
 Date_                  20110321
 Time                      17.33
 INSTRUM                 dpx400
 PROBHD   5 mm Dual 1H/1
 PULPROG                   zg60
 TD                       32768
 SOLVENT                  CDCl3
 NS                          16
 DS                           2
 SWH                   5592.841 Hz
 FIDRES                0.170680 Hz
 AQ                    2.9294591 sec
 RG                         80.6
 DW                       89.400 usec
 DE                        17.00 usec
 TE                        300.0 K
 D1                  1.00000000 sec

 ======== CHANNEL f1 ========
 NUC1                        1H
 P1                        7.30 usec
 PL1       0 dB
 SFO1           400.1320007 MHz

 F2 - Processing parameters
 SI                       32768
 SF                400.1300218 MHz
 WDW                         EM
 SSB       0
 LB                        0.30 Hz
 GB        0
 PC                        0.60
```

Boc-(DL)-glutamic acid 1-tert-butyl ester **148**






```
Instrument DPX400
    Chemist ds
    Group sjc
IDS68 - demethylation
Qh1acq.au CDCl3 {C:\NMR} sjcgrp 6

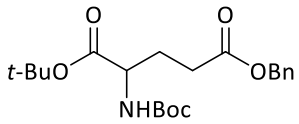

Current Data Parameters
NAME      IDS068
          demethylation Mar28-2011-6
EXPNO                 1
PROCNO                1

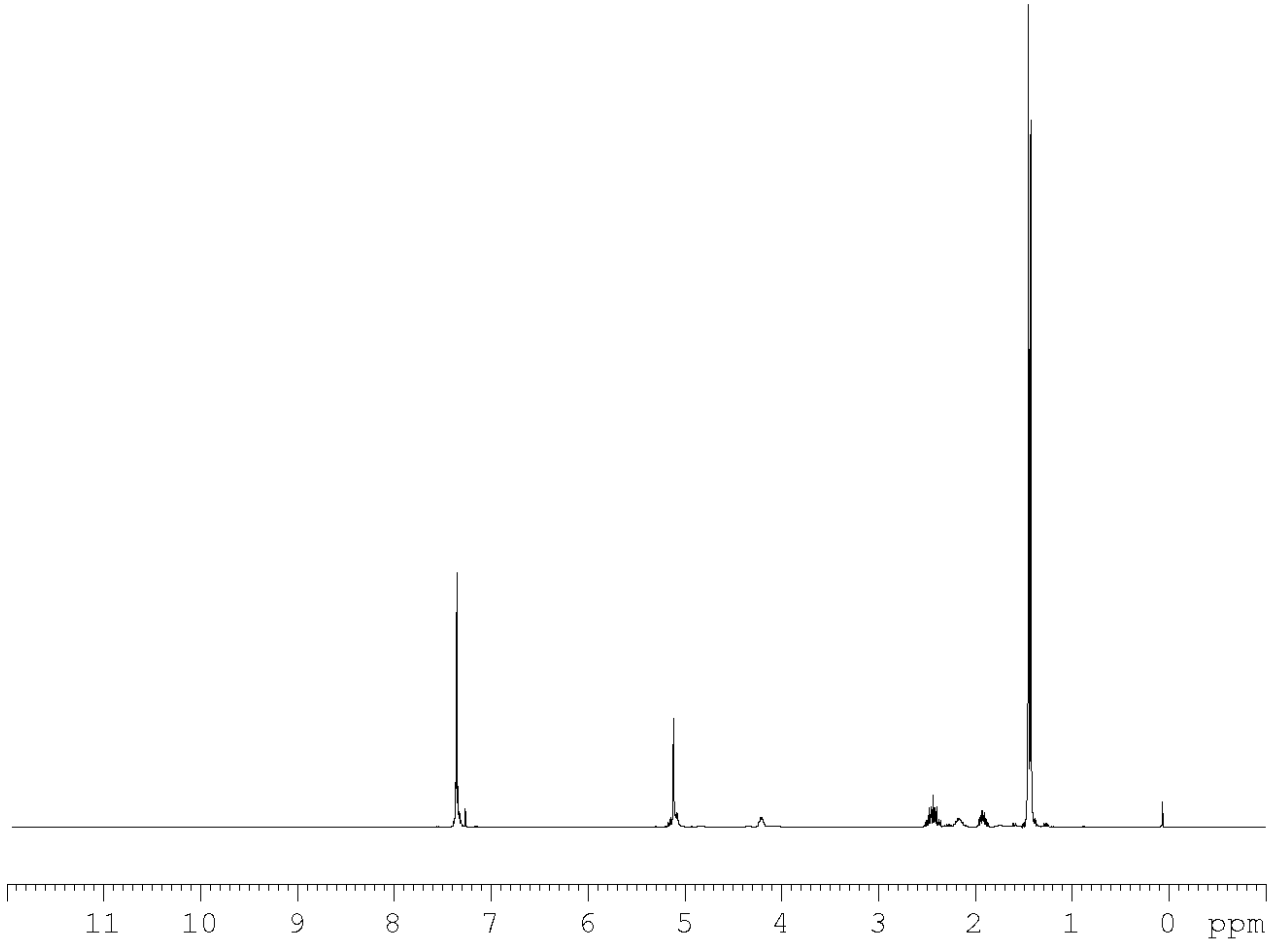
F2 - Acquisition Parameters
Date_           20110328
Time              11.09
INSTRUM           dpx400
PROBHD   5 mm Dual 1H/1
PULPROG            zg60
TD                32768
SOLVENT           CDCl3
NS                   16
DS                    2
SWH            5592.841 Hz
FIDRES         0.170680 Hz
AQ            2.9294591 sec
RG                   16
DW               89.400 usec
DE               17.00 usec
TE               300.0 K
D1           1.00000000 sec

======== CHANNEL f1 ========
NUC1                 1H
P1                7.30 usec
PL1       0 dB
SFO1        400.1320007 MHz

F2 - Processing parameters
SI                32768
SF          400.1300212 MHz
WDW                  EM
SSB       0
LB                0.30 Hz
GB        0
PC                0.60
```

1-(*tert*-Butyl) 5-benzyl Boc-(DL)-glutamate **142**






```
Instrument DPX400
Chemist ds
Group sjc
IDS80 - post MeCN/Heaxane wash (MeCN LAYER)
Qh1acq.au CDCl3 {C:\NMR} sjcgrp 49

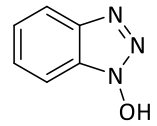
Current Data Parameters
NAME      IDS080
          post MeCN-Hexane wash
          May18-2011-49
EXPNO                   1
PROCNO                  1

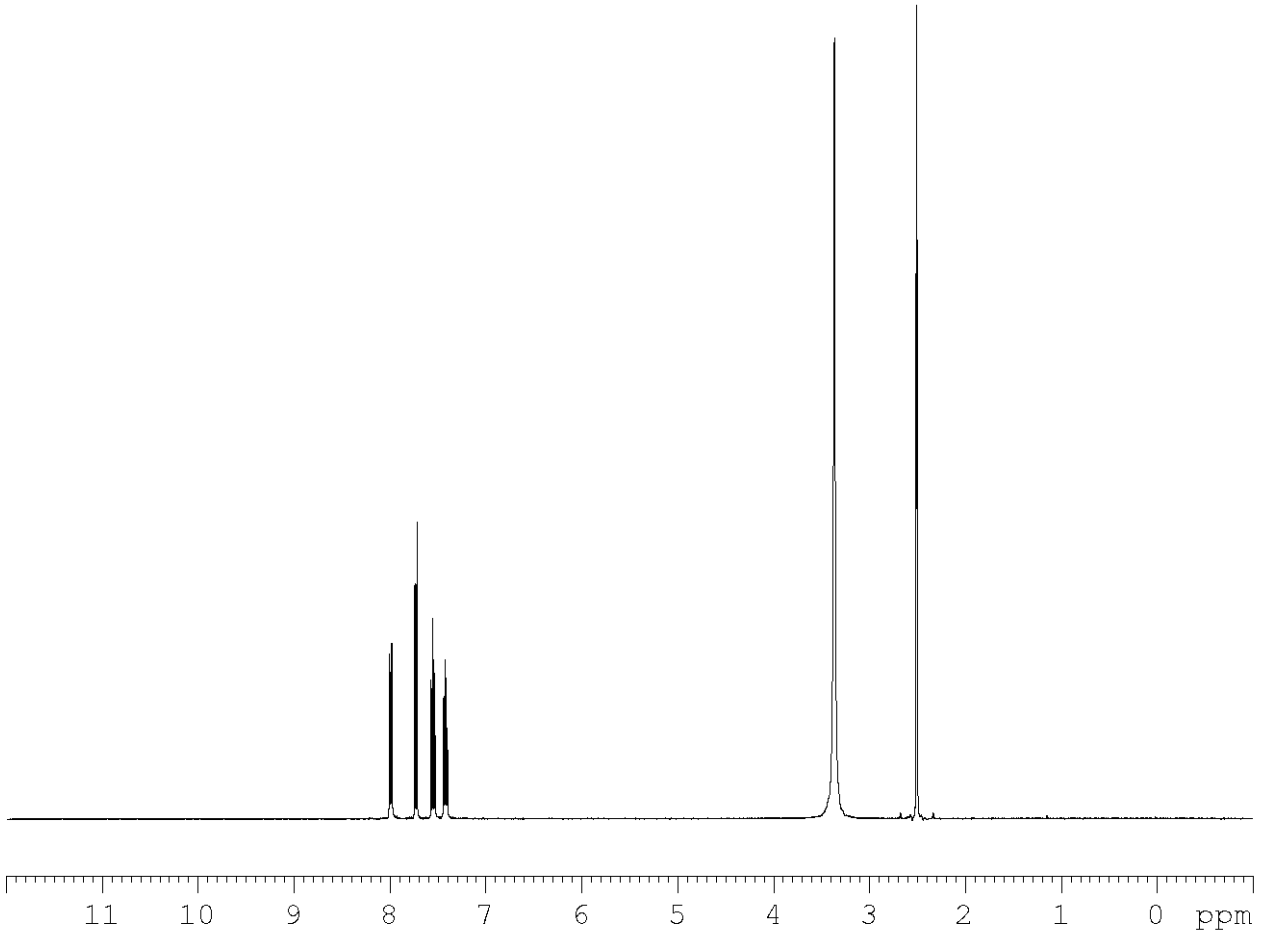
F2 - Acquisition Parameters
Date_           20110518
Time               11.13
INSTRUM           dpx400
PROBHD   5 mm Dual 1H/1
PULPROG             zg60
TD                 32768
SOLVENT            CDCl3
NS                    16
DS                     2
SWH             5592.841 Hz
FIDRES          0.170680 Hz
AQ              2.9294591 sec
RG                  45.3
DW                89.400 usec
DE                17.00 usec
TE                300.0 K
D1           1.00000000 sec

======== CHANNEL f1 ========
NUC1                   1H
P1                  7.30 usec
PL1       0 dB
SFO1         400.1320007 MHz

F2 - Processing parameters
SI                 32768
SF          400.1300212 MHz
WDW                   EM
SSB       0
LB                 0.30 Hz
GB        0
PC                 0.60
```

1*H*-Benzotriazol-1-ol **125**






```
Instrument DPX400
    Chemist ds
     Group sjc
    IDS22 - HOBT
Qh1acq.au DMSO {C:\NMR} sjcgrp 59

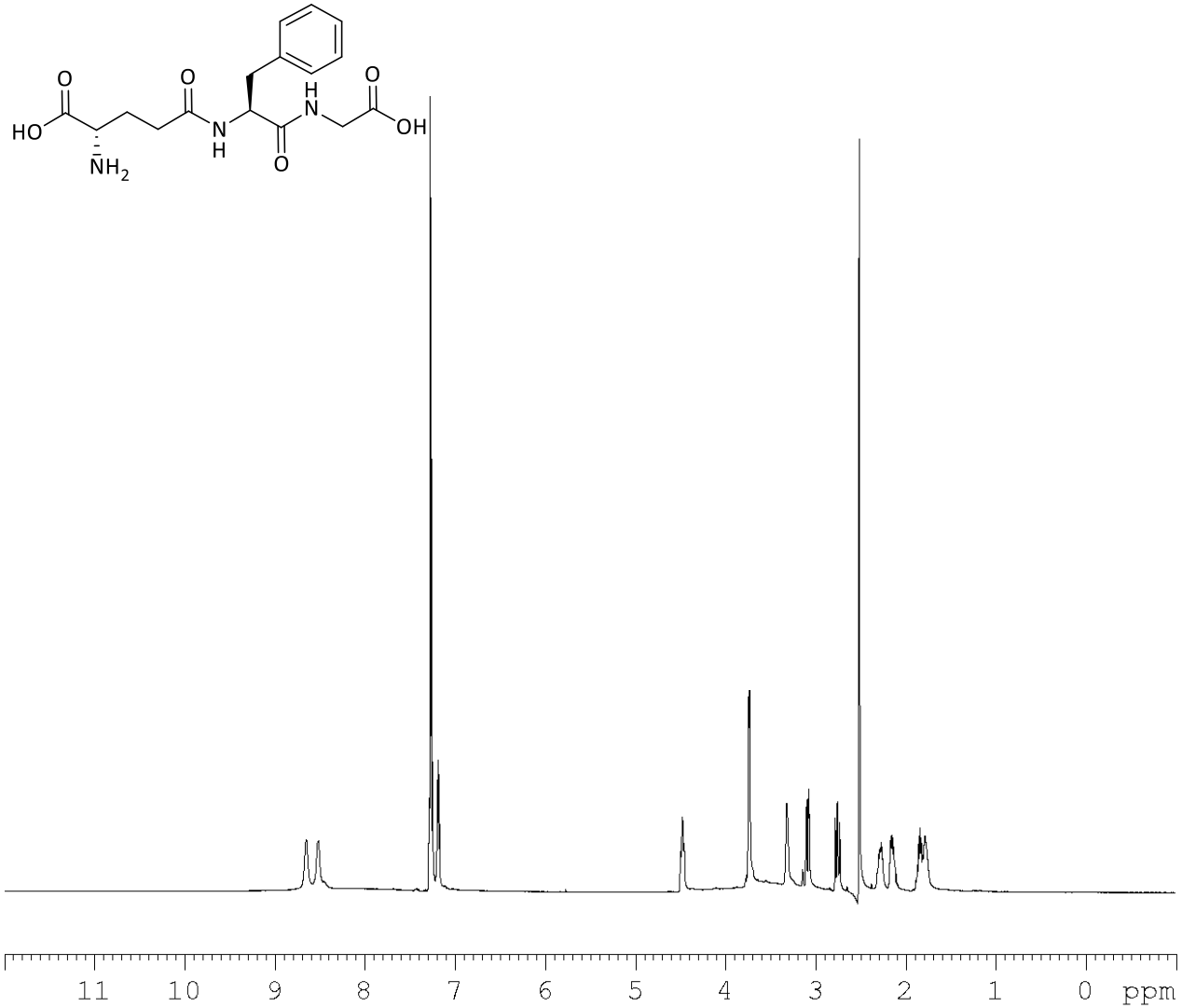
    Current Data Parameters
    NAME      IDS022 - Nov11-2010-59
    EXPNO                   1
    PROCNO                  1

    F2 - Acquisition Parameters
    Date_            20101111
    Time                13.36
    INSTRUM            dpx400
    PROBHD   5 mm Dual 1H/1
    PULPROG              zg60
    TD                  32768
    SOLVENT              DMSO
    NS                     16
    DS                      2
    SWH              5592.841 Hz
    FIDRES           0.170680 Hz
    AQ               2.9294591 sec
    RG                 406.4
    DW                89.400 usec
    DE                17.00 usec
    TE                300.0 K
    D1           1.00000000 sec

    ======== CHANNEL f1 ========
    NUC1                   1H
    P1                  7.30 usec
    PL1       0 dB
    SFO1         400.1320007 MHz

    F2 - Processing parameters
    SI                  32768
    SF           400.1300000 MHz
    WDW                    no
    SSB       0
    LB        0 Hz
    GB        0
    PC                   0.60
```

γ-Glu-Phe-Gly **127**




```
Instrument AVC500
Group SJC
7608 Diane Swallow 20/1/12

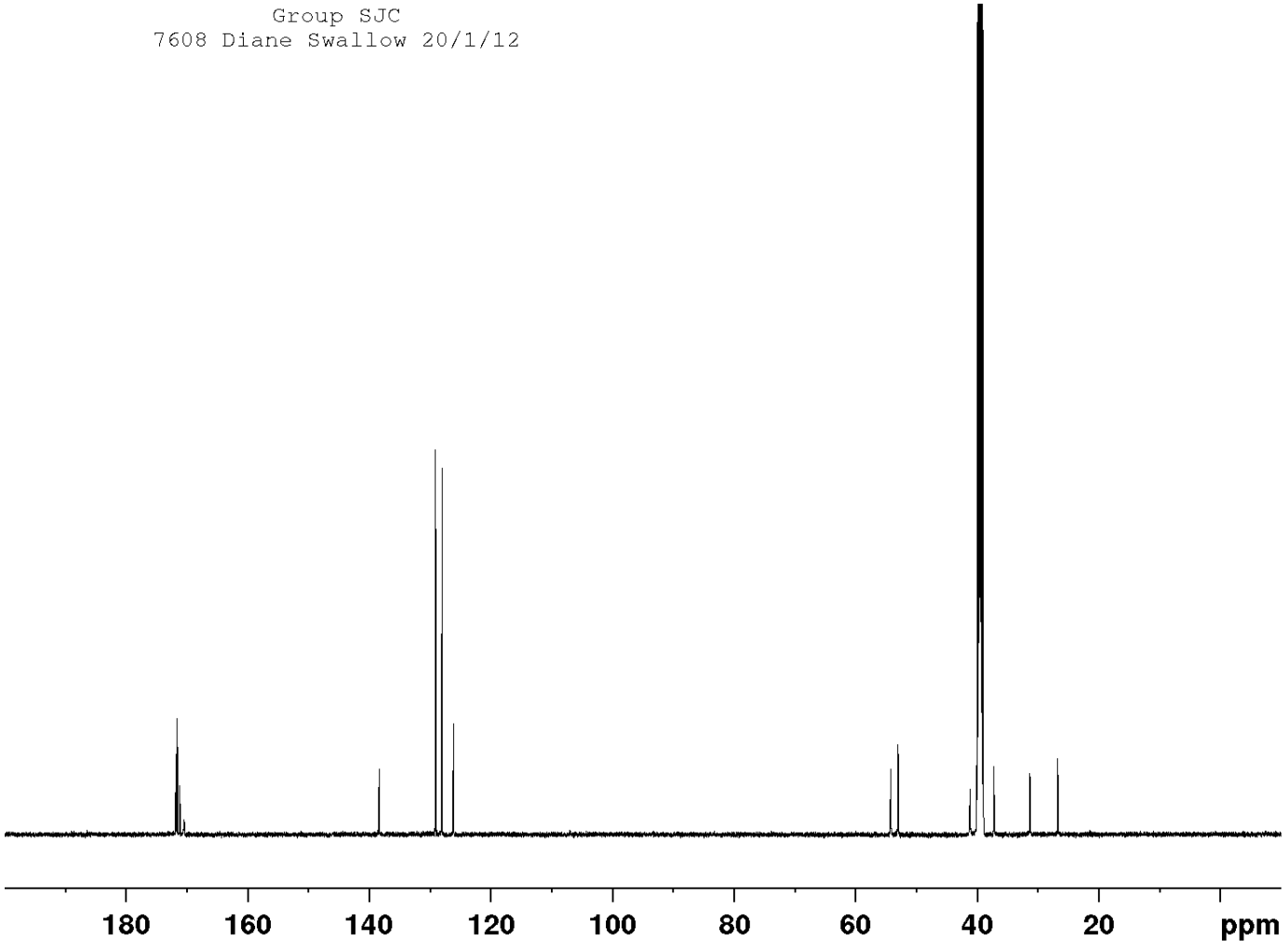
Current Data Parameters
NAME      IDS144 - ds76082001
EXPNO                    1
PROCNO                   1

F2 - Acquisition Parameters
Date_             20120123
Time                  0.18
INSTRUM             avc500
PROBHD   5 mm CPDUL 13C
PULPROG               zg30
TD                   65536
SOLVENT               DMSO
NS                      16
DS                       2
SWH              10330.578 Hz
FIDRES            0.157632 Hz
AQ                3.1719425 sec
RG                       4
DW                  48.400 usec
DE                   6.00 usec
TE                  298.0 K
D1              1.00000000 sec
TD0                      1

======== CHANNEL f1 ========
NUC1                    1H
P1                   9.60 usec
PL1                 -6.00 dB
PL1W         15.19999981 W
SFO1         500.3030896 MHz

F2 - Processing parameters
SI                   32768
SF            500.3000000 MHz
WDW                     EM
SSB       0
LB                   0.30 Hz
GB        0
PC                   1.00
```

γ-Glu-Phe-Gly **127**




```
Current Data Parameters
NAME      IDS144 - ds76082001
EXPNO                      4
PROCNO                     1

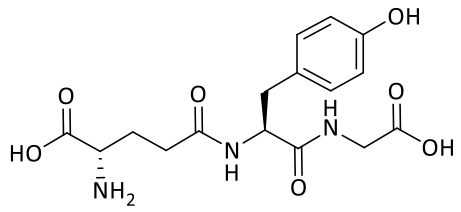
F2 - Acquisition Parameters
Date_            20120123
Time                 1.33
INSTRUM            avc500
PROBHD    5 mm CPDUL 13C
PULPROG            zgpg30
TD                  65536
SOLVENT               DMSO
NS                    1024
DS                       2
SWH             31250.000 Hz
FIDRES           0.476837 Hz
AQ              1.0485760 sec
RG                    1820
DW                16.000 usec
DE                20.00 usec
TE                298.0 K
D1             2.00000000 sec
D11            0.03000000 sec
TD0                      1

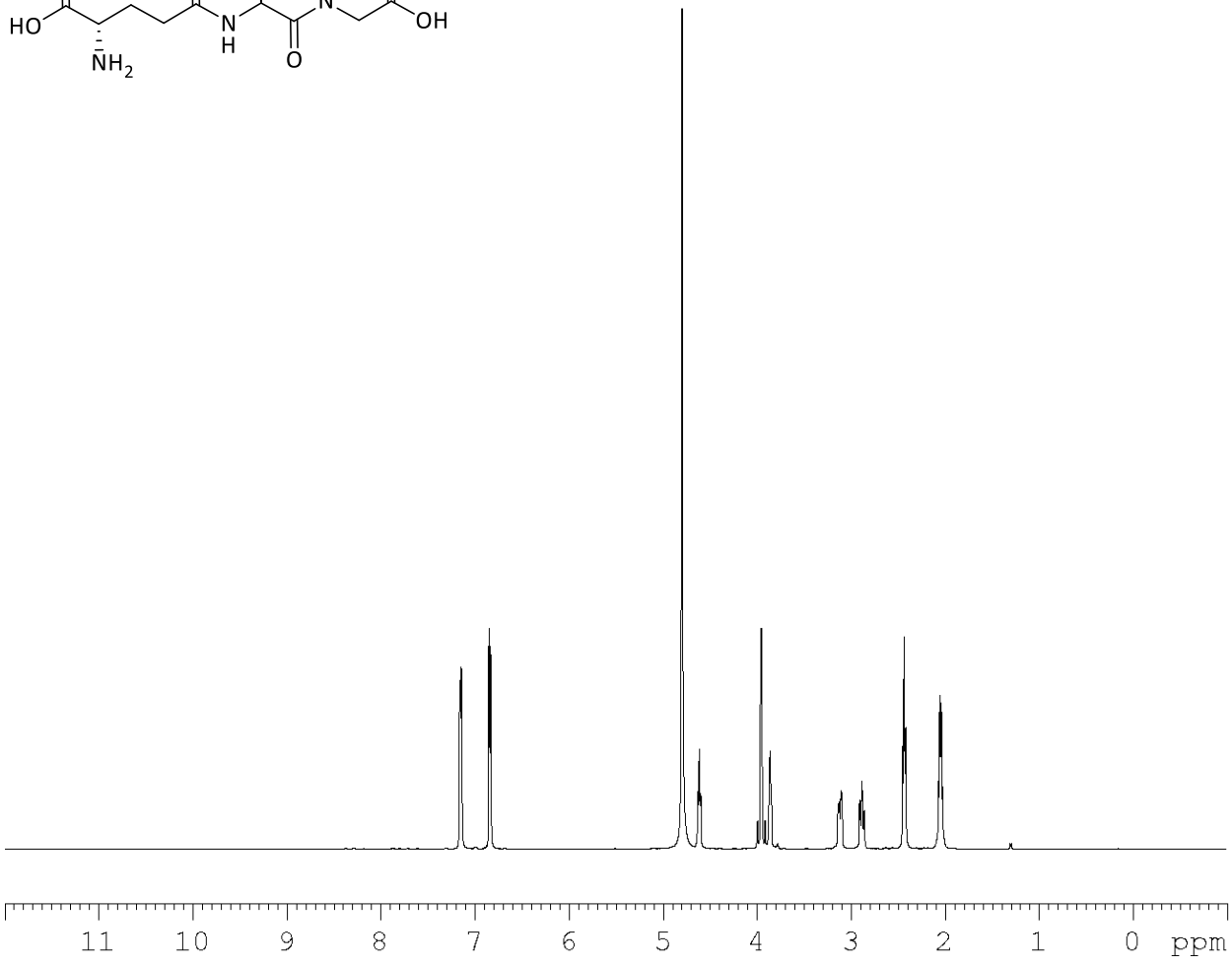
======== CHANNEL f1 ========
NUC1                    13C
P1                  10.00 usec
PL1                 -4.40 dB
PL1W          28.15752029 W
SFO1          125.8131151 MHz

======== CHANNEL f2 ========
CPDPRG[2           waltz16
NUC2                    1H
PCPD2                80.00 usec
PL2                  -6.00 dB
PL12                 12.42 dB
PL13                 18.42 dB
PL2W          15.19999981 W
PL12W          0.21869738 W
PL13W          0.05493430 W
SFO2          500.3020012 MHz

F2 - Processing parameters
SI                    32768
SF            125.8005918 MHz
WDW                      EM
SSB       0
LB                   1.00 Hz
GB        0
PC                   1.40
```

γ-Glu-Tyr-Gly **128**






```
Instrument AVC500
Group SJC
7099 Diane Swallow 6/12/11

Current Data Parameters
NAME      IDS131 - ds70990612
EXPNO                    1
PROCNO                   1

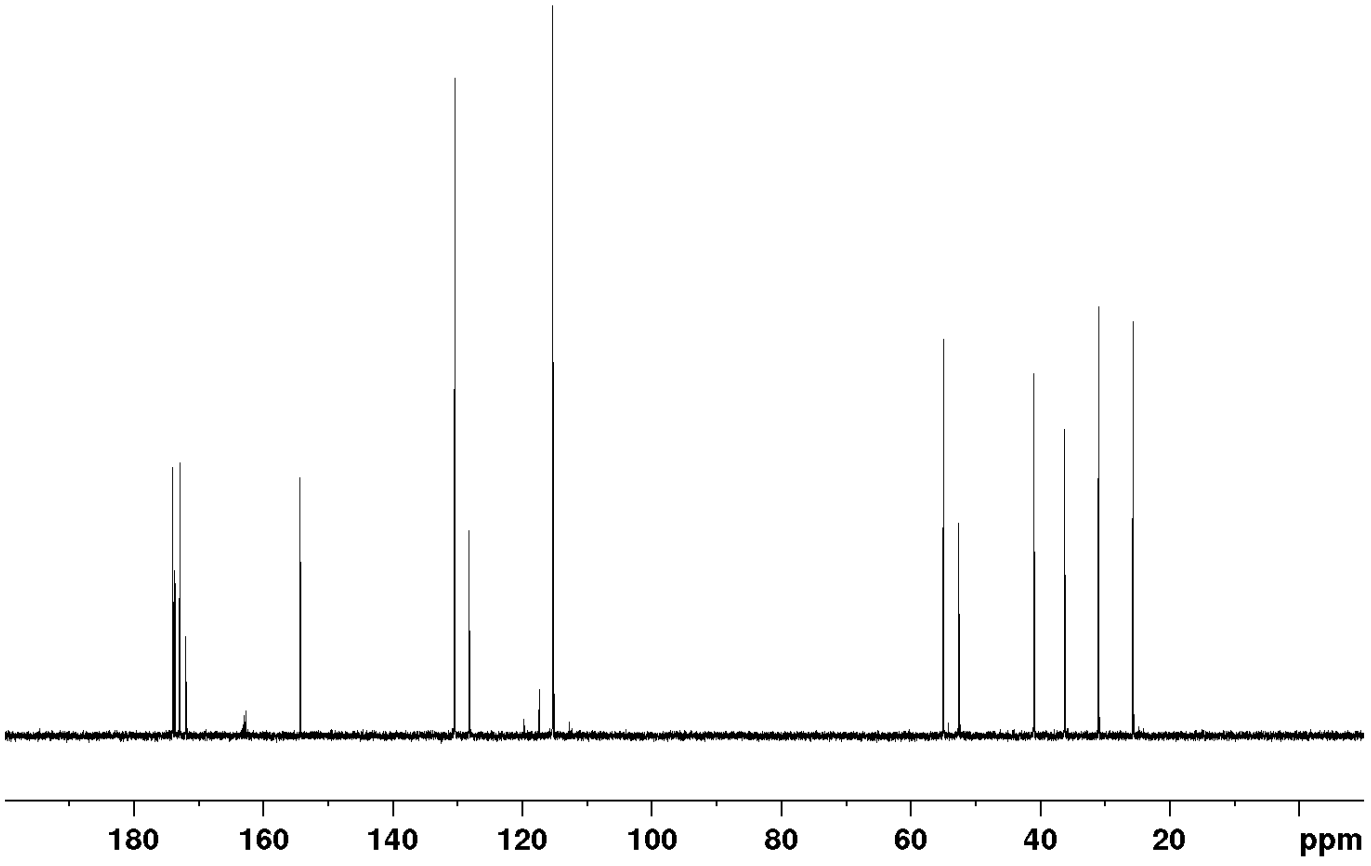
F2 - Acquisition Parameters
Date_           20111208
Time                5.21
INSTRUM          avc500
PROBHD   5 mm CPDUL 13C
PULPROG             zg30
TD                 65536
SOLVENT              D2O
NS                    16
DS                     2
SWH           10330.578 Hz
FIDRES         0.157632 Hz
AQ             3.1719425 sec
RG                     4
DW                48.400 usec
DE                 6.00 usec
TE                298.0 K
D1           1.00000000 sec
TD0                    1

======== CHANNEL f1 ========
NUC1                  1H
P1                 9.60 usec
PL1               -6.00 dB
PL1W        15.19999981 W
SFO1        500.3030896 MHz

F2 - Processing parameters
SI                 32768
SF          500.2999578 MHz
WDW                   EM
SSB       0
LB                 0.30 Hz
GB        0
PC                 1.00
```

γ-Glu-Tyr-Gly **128**

Instrument AVC500
Group SJC
7099 Diane Swallow 6/12/11




```
Current Data Parameters
NAME      IDS131 - ds70990612
EXPNO                       4
PROCNO                      1

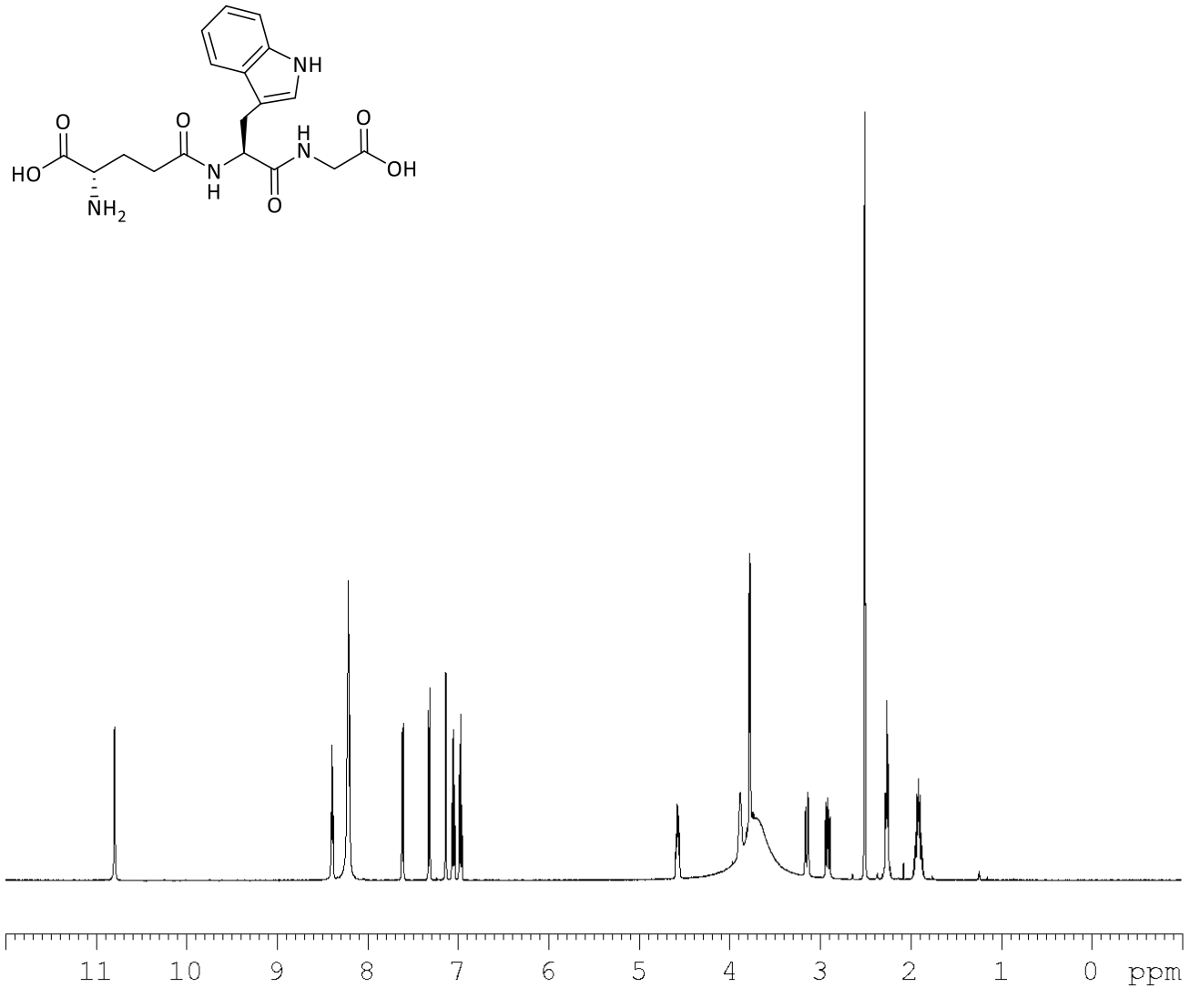
F2 - Acquisition Parameters
Date_            20111208
Time                 6.09
INSTRUM           avc500
PROBHD   5 mm CPDUL 13C
PULPROG           zgpg30
TD                65536
SOLVENT             D2O
NS                  512
DS                    2
SWH           31250.000 Hz
FIDRES         0.476837 Hz
AQ            1.0485760 sec
RG                 1820
DW               16.000 usec
DE               20.00 usec
TE               298.0 K
D1           2.00000000 sec
D11          0.03000000 sec
TD0                   1

======== CHANNEL f1 ========
NUC1                 13C
P1                10.00 usec
PL1               -4.40 dB
PL1W        28.15752029 W
SFO1        125.8131151 MHz

======== CHANNEL f2 ========
CPDPRG[2         waltz16
NUC2                 1H
PCPD2             80.00 usec
PL2               -6.00 dB
PL12              12.42 dB
PL13              18.42 dB
PL2W        15.19999981 W
PL12W        0.21869738 W
PL13W        0.05493430 W
SFO2        500.3020012 MHz

F2 - Processing parameters
SI                32768
SF          125.8005350 MHz
WDW                 EM
SSB       0
LB                1.00 Hz
GB        0
PC                1.40
```

γ-Glu-Trp-Gly **129**




```
                Instrument DRX500
h1acqbbo DMSO {C:\NMR} sjcogroup 49

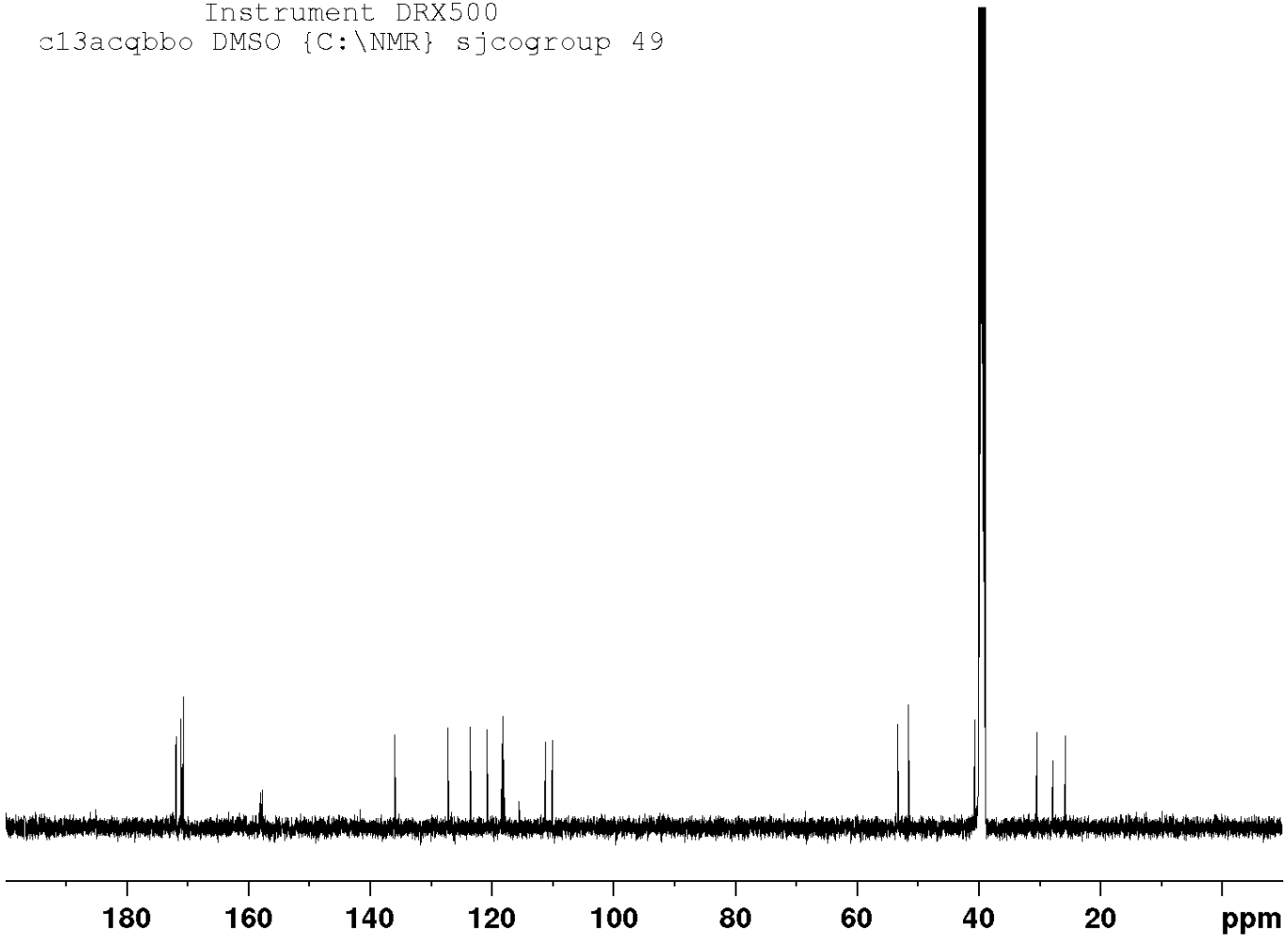

Current Data Parameters
NAME      IDS Glu-Trp-Gly II
EXPNO                    2
PROCNO                   1

F2 - Acquisition Parameters
Date_           20140426
Time                5.05
INSTRUM           drx500
PROBHD   5 mm PABBO BB/
PULPROG             zg60
TD                 65536
SOLVENT             DMSO
NS                    16
DS                     2
SWH           10330.578 Hz
FIDRES         0.157632 Hz
AQ            3.1719425 sec
RG                 228.1
DW                48.400 usec
DE                17.00 usec
TE                297.7 K
D1           1.00000000 sec

======== CHANNEL f1 ========
NUC1                   1H
P1                 11.00 usec
PL1       0 dB
SFO1        500.1325007 MHz

F2 - Processing parameters
SI                 32768
SF          500.1300053 MHz
WDW                   EM
SSB       0
LB                  0.30 Hz
GB        0
PC                  1.00
```

γ-Glu-Trp-Gly **129**




```
Current Data Parameters
NAME      IDS Glu-Trp-Gly II
EXPNO                    1
PROCNO                   1

F2 - Acquisition Parameters
Date_           20140426
Time                5.03
INSTRUM           drx500
PROBHD   5 mm PABBO BB/
PULPROG           zgpg30
TD                65536
SOLVENT             DMSO
NS                  6144
DS                     4
SWH           30303.031 Hz
FIDRES         0.462388 Hz
AQ            1.0813440 sec
RG               9195.2
DW               16.500 usec
DE                11.00 usec
TE                297.7 K
D1           2.00000000 sec
d11          0.03000000 sec
DELTA        1.89999998 sec
TD0                    1

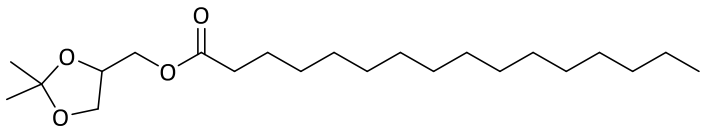
======== CHANNEL f1 ========
NUC1                  13C
P1                 6.80 usec
PL1                5.00 dB
SFO1        125.7703148 MHz

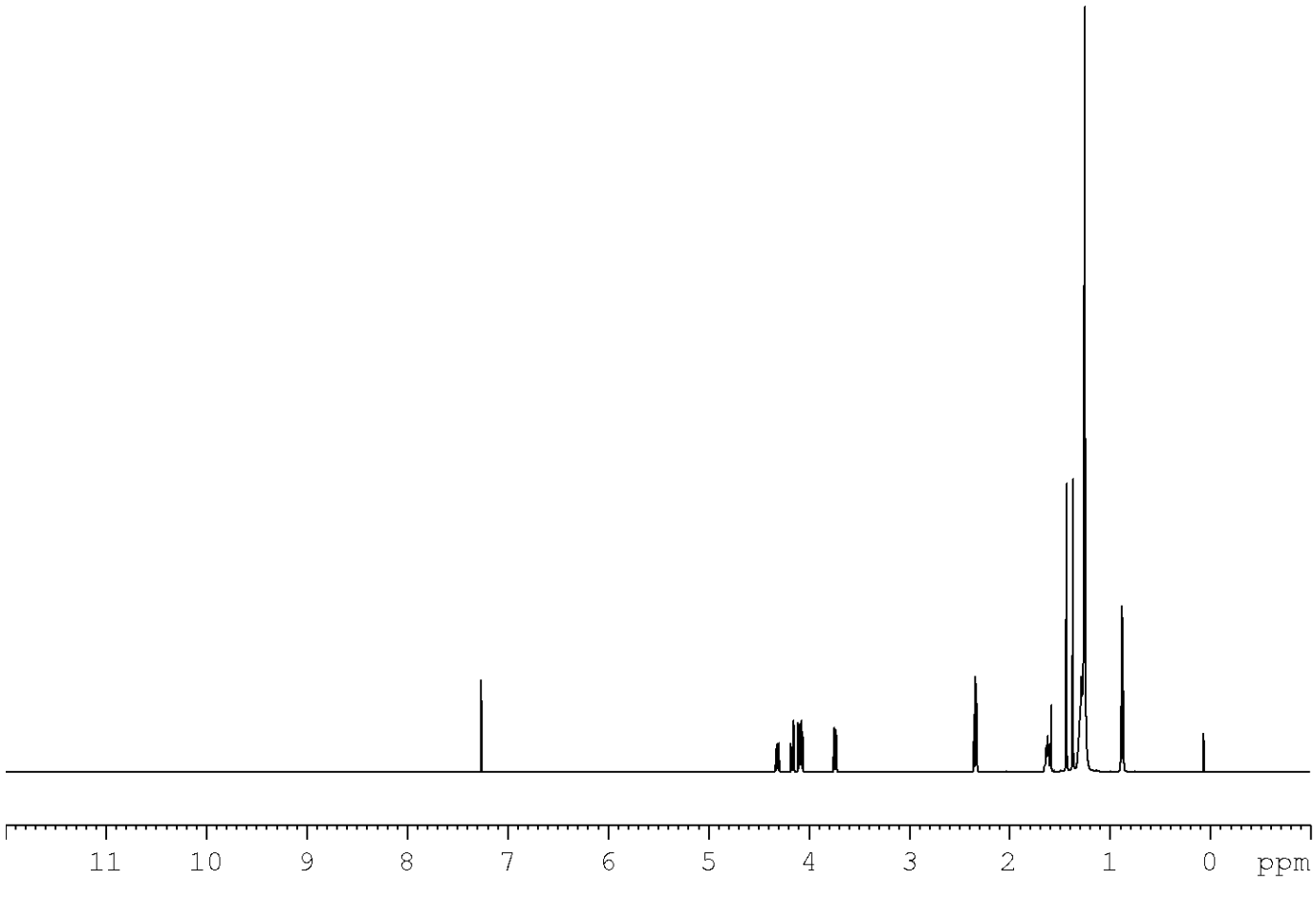
======== CHANNEL f2 ========
CPDPRG[2         waltz16
NUC2                  1H
PCPD2             100.00 usec
PL2       0 dB
PL12               19.00 dB
PL13               23.00 dB
SFO2        500.1320005 MHz

F2 - Processing parameters
SI                 32768
SF          125.7578472 MHz
WDW                   EM
SSB       0
LB                 1.00 Hz
GB        0
PC                 1.40
```

NMR data for the synthesis of a small molecule probe for MscS

(2,2-Dimethyl-1,3-dioxolan-4-yl)methyl palmitate **165**






```
Instrument AVC500
Group SJC
5730 Diane Swallow 1/9/11

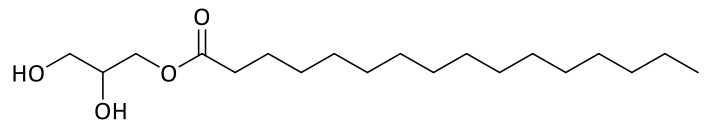
Current Data Parameters
NAME      IDS064 - ds57300109
EXPNO                    1
PROCNO                   1

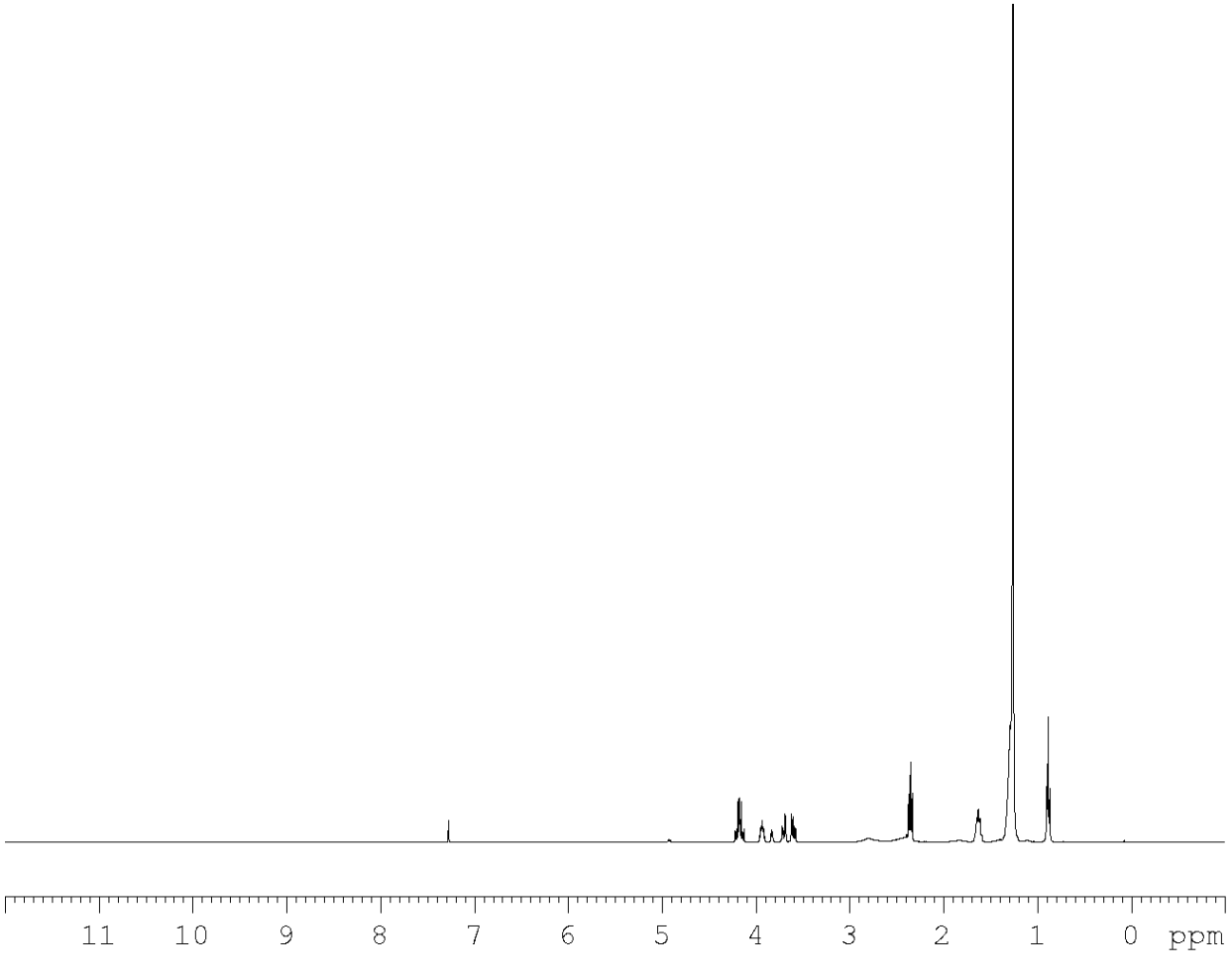
F2 - Acquisition Parameters
Date_           20110903
Time                2.31
INSTRUM          avc500
PROBHD   5 mm CPDUL 13C
PULPROG             zg30
TD                 65536
SOLVENT            CDCl3
NS                    16
DS                     2
SWH           10330.578 Hz
FIDRES         0.157632 Hz
AQ            3.1719425 sec
RG                     4
DW               48.400 usec
DE                6.00 usec
TE               298.0 K
D1           1.00000000 sec
TD0                    1

======== CHANNEL f1 ========
NUC1                  1H
P1                 9.60 usec
PL1               -6.00 dB
PL1W        15.19999981 W
SFO1        500.3030896 MHz

F2 - Processing parameters
SI                 32768
SF          500.3000285 MHz
WDW                   EM
SSB      0
LB                 0.30 Hz
GB       0
PC                 1.00
```

2,3-Dihydroxypropyl palmitate **166**






```
                Instrument DQX400
                  Chemist ds
                  Group sjc
                    IDS75
h1acq.au CDCl3 {E:\NMR2011} sjcgrp 28

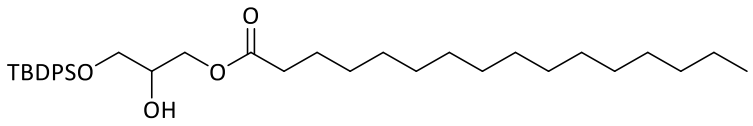
   Current Data Parameters
   NAME      IDS075 - May25-2011-28
   EXPNO                   1
   PROCNO                  1

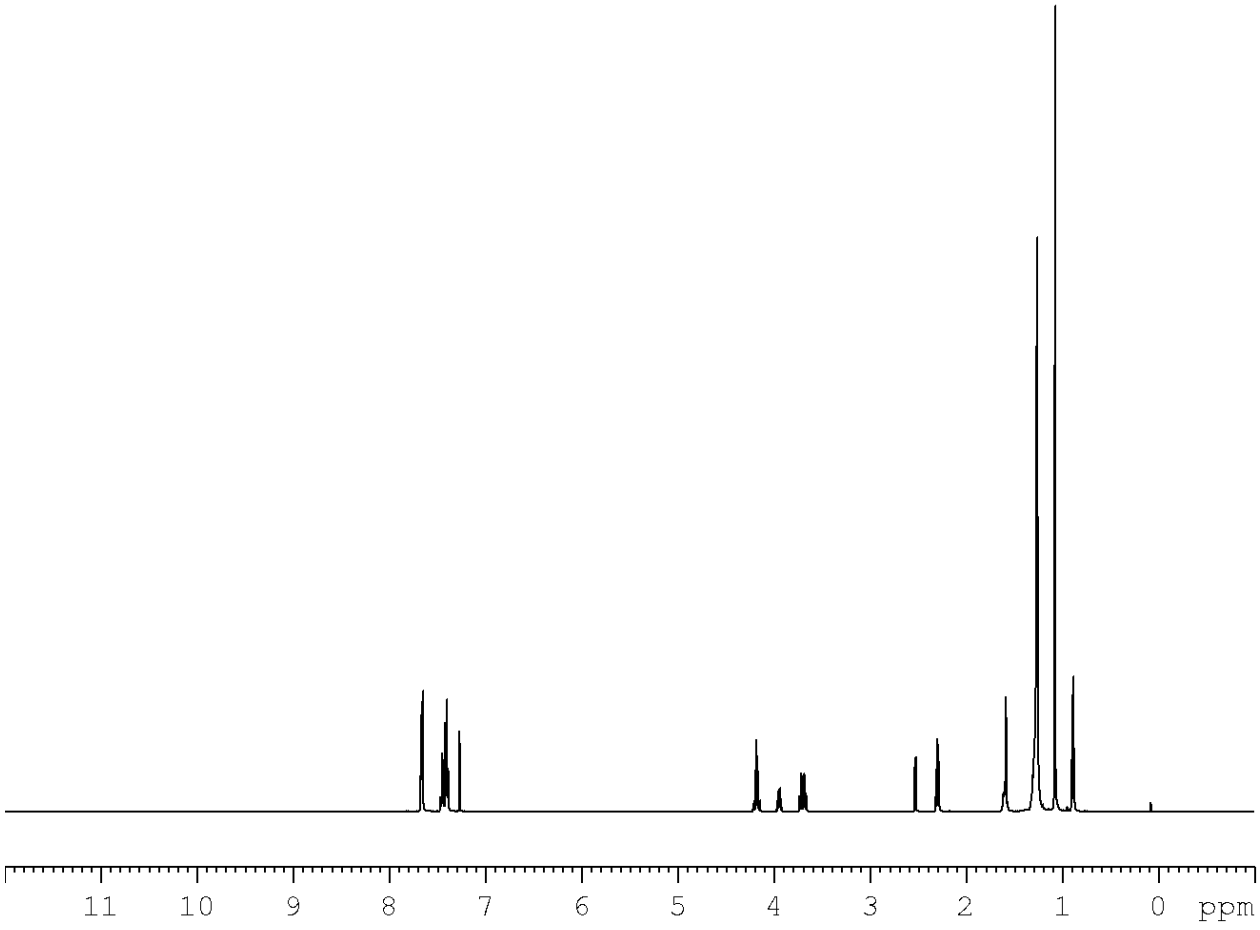
   F2 - Acquisition Parameters
   Date_           20110527
   Time                3.22
   INSTRUM            av400
   PROBHD   5 mm QNP 1H/13
   PULPROG             zg60
   TD                 65536
   SOLVENT            CDCl3
   NS                    16
   DS                     2
   SWH             8278.146 Hz
   FIDRES          0.126314 Hz
   AQ              3.9583745 sec
   RG                  40.3
   DW                60.400 usec
   DE                 7.50 usec
   TE                300.0 K
   D1           1.00000000 sec

   ======== CHANNEL f1 ========
   NUC1                  1H
   P1                 9.00 usec
   PL1       0 dB
   SFO1         400.2024714 MHz

   F2 - Processing parameters
   SI                 32768
   SF           400.2000028 MHz
   WDW                   EM
   SSB       0
   LB                 0.30 Hz
   GB        0
   PC                 1.00
```

2-Hydroxy-3-(*tert*-butyldiphenylsilyl)oxypropyl palmitate **201**






```
        Instrument AVC500
            Group SJC
    5739 Diane Swallow 1/9/11

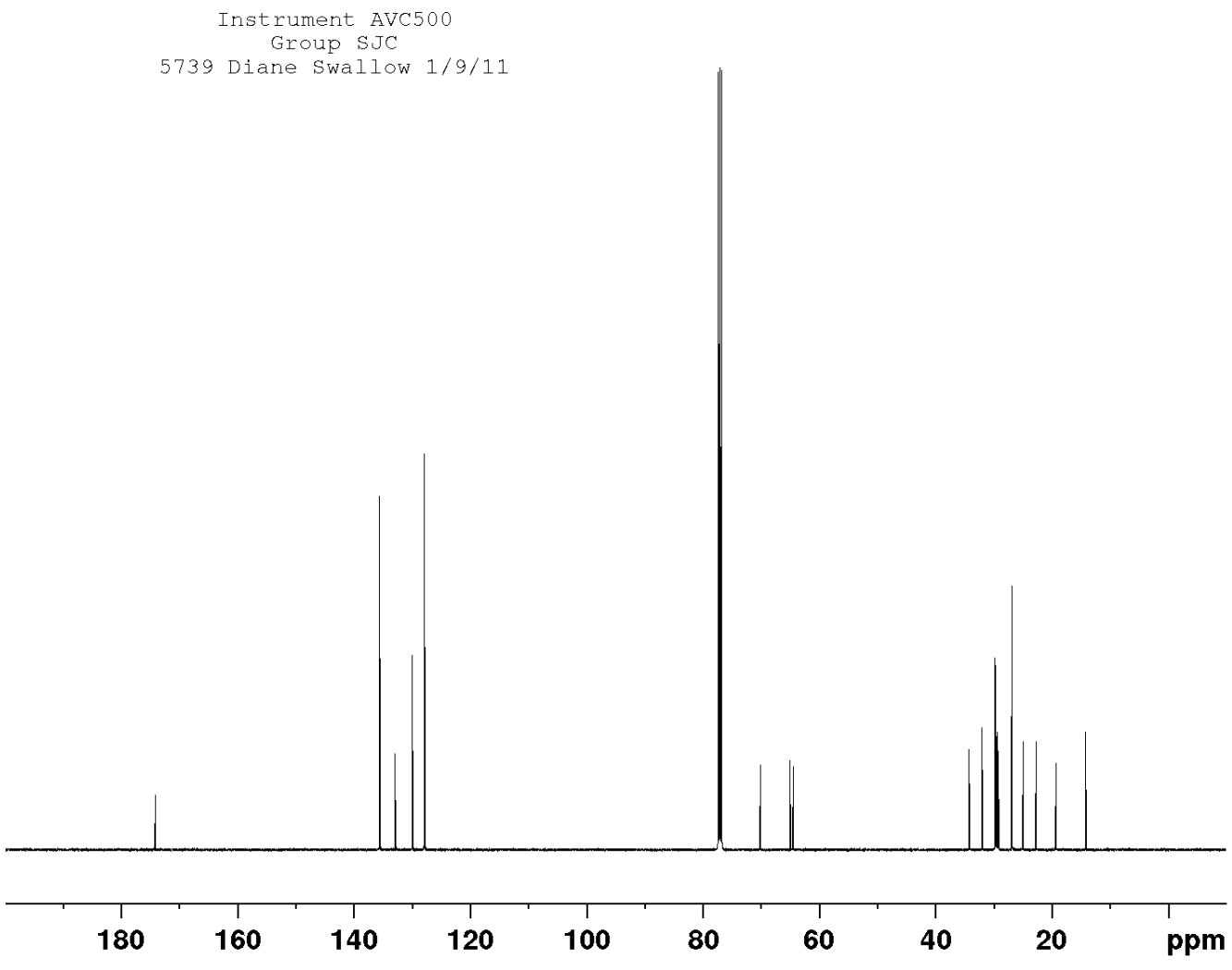

Current Data Parameters
NAME      IDS088 product - ds57390109
EXPNO                   1
PROCNO                  1

F2 - Acquisition Parameters
Date_           20110903
Time               16.39
INSTRUM          avc500
PROBHD   5 mm CPDUL 13C
PULPROG             zg30
TD                 65536
SOLVENT            CDCl3
NS                    16
DS                     2
SWH           10330.578 Hz
FIDRES         0.157632 Hz
AQ            3.1719425 sec
RG                     4
DW                48.400 usec
DE                 6.00 usec
TE                298.0 K
D1           1.00000000 sec
TD0                    1

======== CHANNEL f1 ========
NUC1                  1H
P1                  9.60 usec
PL1                -6.00 dB
PL1W        15.19999981 W
SFO1        500.3030896 MHz

F2 - Processing parameters
SI                 32768
SF          500.3000240 MHz
WDW                   EM
SSB      0
LB                 0.30 Hz
GB       0
PC                 1.00
```

2-Hydroxy-3-(*tert*-butyldiphenylsilyl)oxypropyl palmitate **201**




```
Current Data Parameters
NAME      IDS088 product - ds57390109
EXPNO                   4
PROCNO                  1

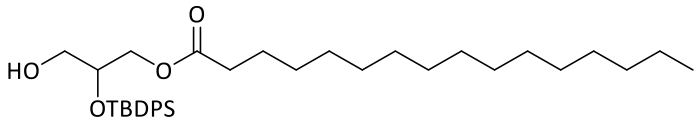
F2 - Acquisition Parameters
Date_            20110903
Time                17.09
INSTRUM           avc500
PROBHD   5 mm CPDUL 13C
PULPROG           zgpg30
TD                 65536
SOLVENT            CDCl3
NS                   256
DS                     2
SWH           31250.000 Hz
FIDRES         0.476837 Hz
AQ            1.0485760 sec
RG                  1820
DW               16.000 usec
DE               20.00 usec
TE               298.0 K
D1           2.00000000 sec
D11          0.03000000 sec
TD0                    1

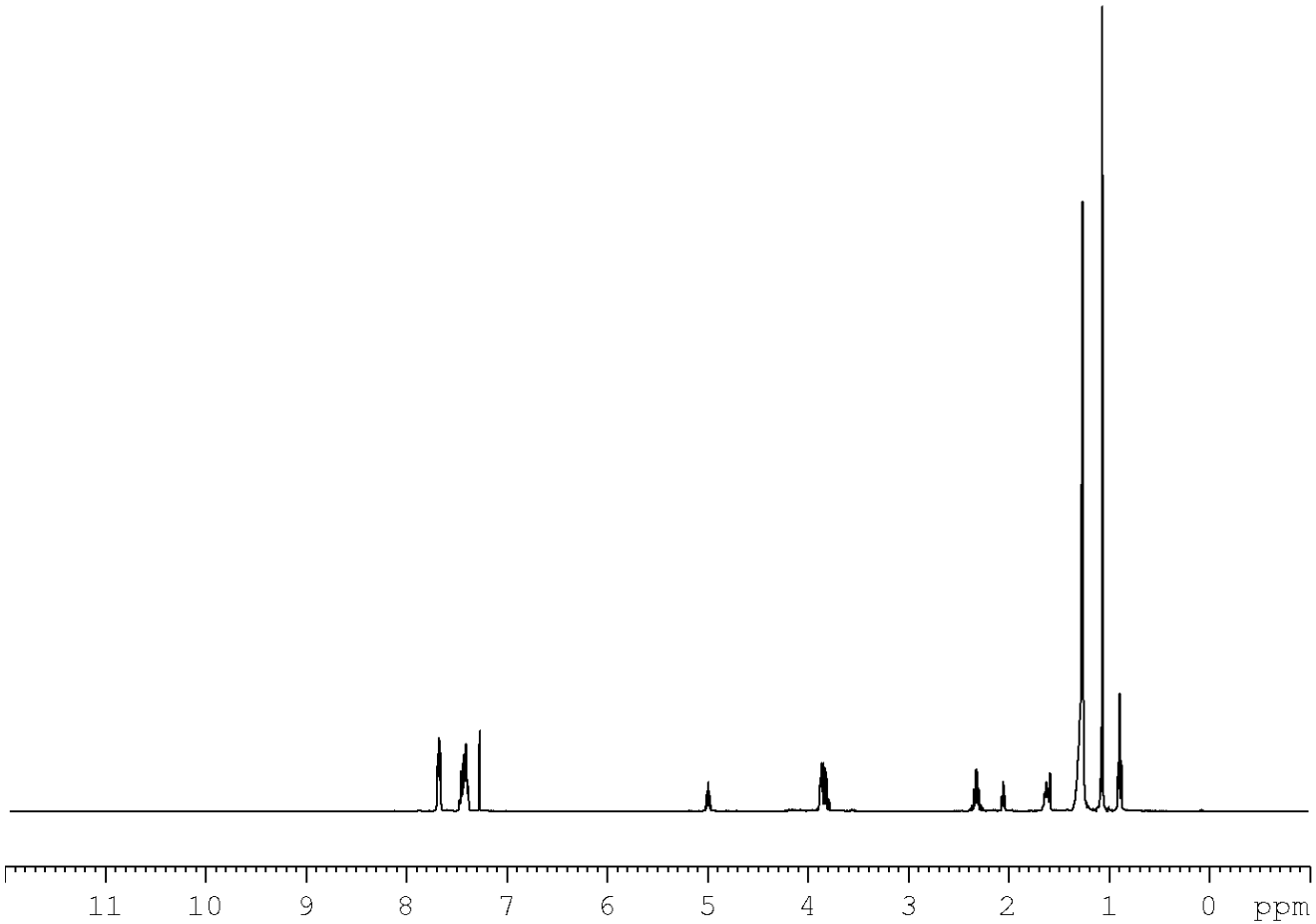
======== CHANNEL f1 ========
NUC1                  13C
P1                 10.00 usec
PL1                -4.40 dB
PL1W        28.15752029 W
SFO1        125.8131151 MHz

======== CHANNEL f2 ========
CPDPRG[2          waltz16
NUC2                  1H
PCPD2              80.00 usec
PL2                -6.00 dB
PL12               12.42 dB
PL13               18.42 dB
PL2W        15.19999981 W
PL12W        0.21869738 W
PL13W        0.05493430 W
SFO2        500.3020012 MHz

F2 - Processing parameters
SI                 32768
SF          125.8005242 MHz
WDW                   EM
SSB       0
LB                 1.00 Hz
GB        0
PC                 1.40
```

2-(*tert*-Butyldiphenylsilyl)oxy-3-hydroxypropyl palmitate **202**






```
Instrument DPX400
Chemist ds
Group sjc
IDS88 by-product
Qh1acq.au CDCl3 {C:\NMR} sjcgrp 25

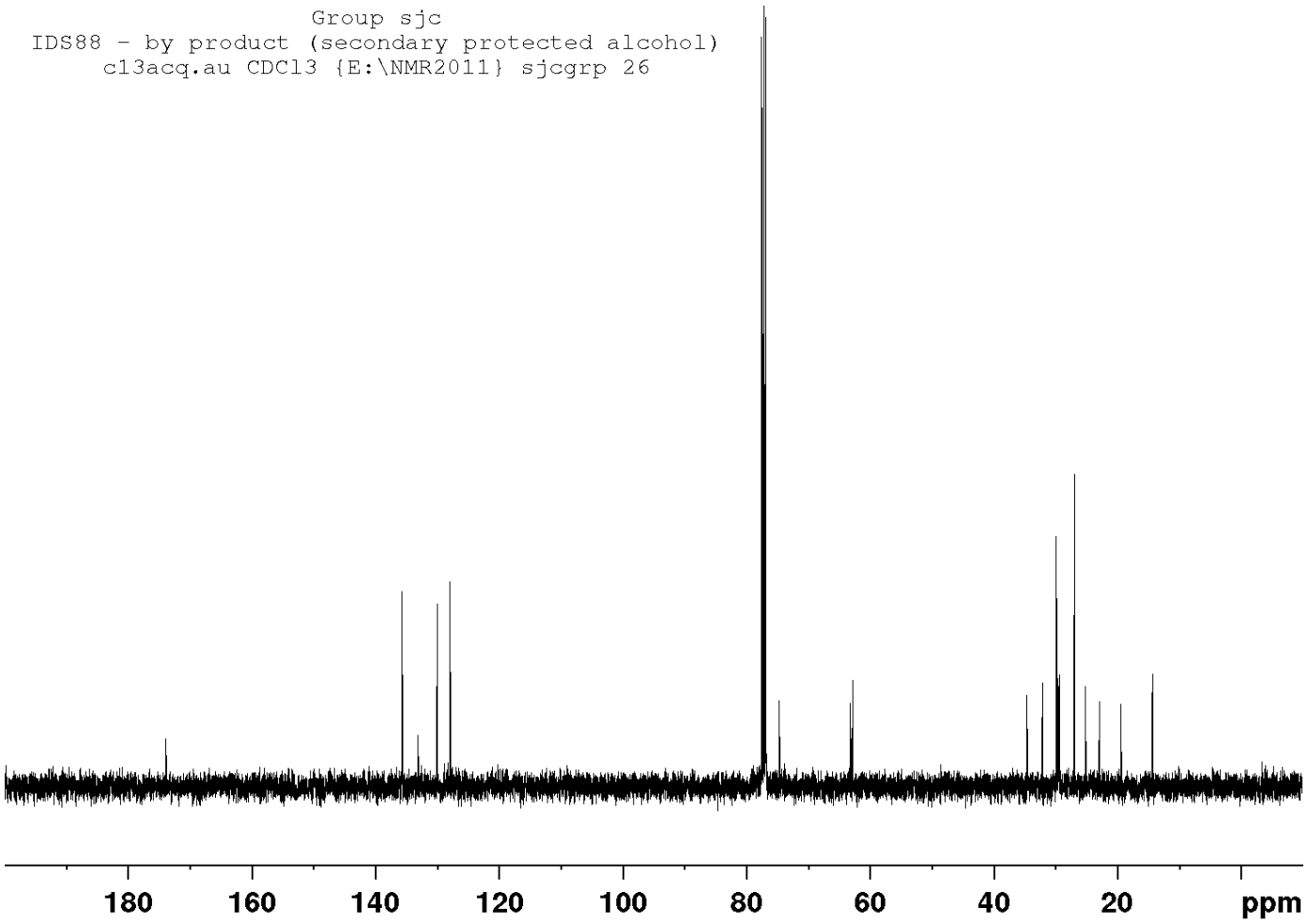
Current Data Parameters
NAME      IDS088
          by-product Aug31-2011-25
EXPNO                  1
PROCNO                 1

F2 - Acquisition Parameters
Date_           20110831
Time               19.14
INSTRUM          dpx400
PROBHD   5 mm Dual 1H/1
PULPROG             zg60
TD                 32768
SOLVENT            CDCl3
NS                    16
DS                     2
SWH             5592.841 Hz
FIDRES          0.170680 Hz
AQ              2.9294591 sec
RG                  71.8
DW                89.400 usec
DE                17.00 usec
TE                300.0 K
D1           1.00000000 sec

======== CHANNEL f1 ========
NUC1                  1H
P1                 7.30 usec
PL1       0 dB
SFO1         400.1320007 MHz

F2 - Processing parameters
SI                 32768
SF           400.1300182 MHz
WDW                   EM
SSB       0
LB                 0.30 Hz
GB        0
PC                 0.60
```

2-(*tert*-Butyldiphenylsilyl)oxy-3-hydroxypropyl palmitate **202**




```
Current Data Parameters
NAME      IDS088
          by-product Jul04-2011-26
EXPNO                 3
PROCNO                1

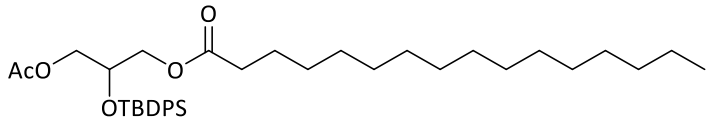
F2 - Acquisition Parameters
Date_          20110704
Time              17.42
INSTRUM           av400
PROBHD   5 mm QNP 1H/13
PULPROG          zgpg30
TD                32768
SOLVENT           CDCl3
NS                  256
DS                    4
SWH           26178.010 Hz
FIDRES         0.798889 Hz
AQ            0.6258688 sec
RG                32768
DW               19.100 usec
DE                7.50 usec
TE                300.0 K
D1           1.00000000 sec
D11          0.03000000 sec
TD0                   1

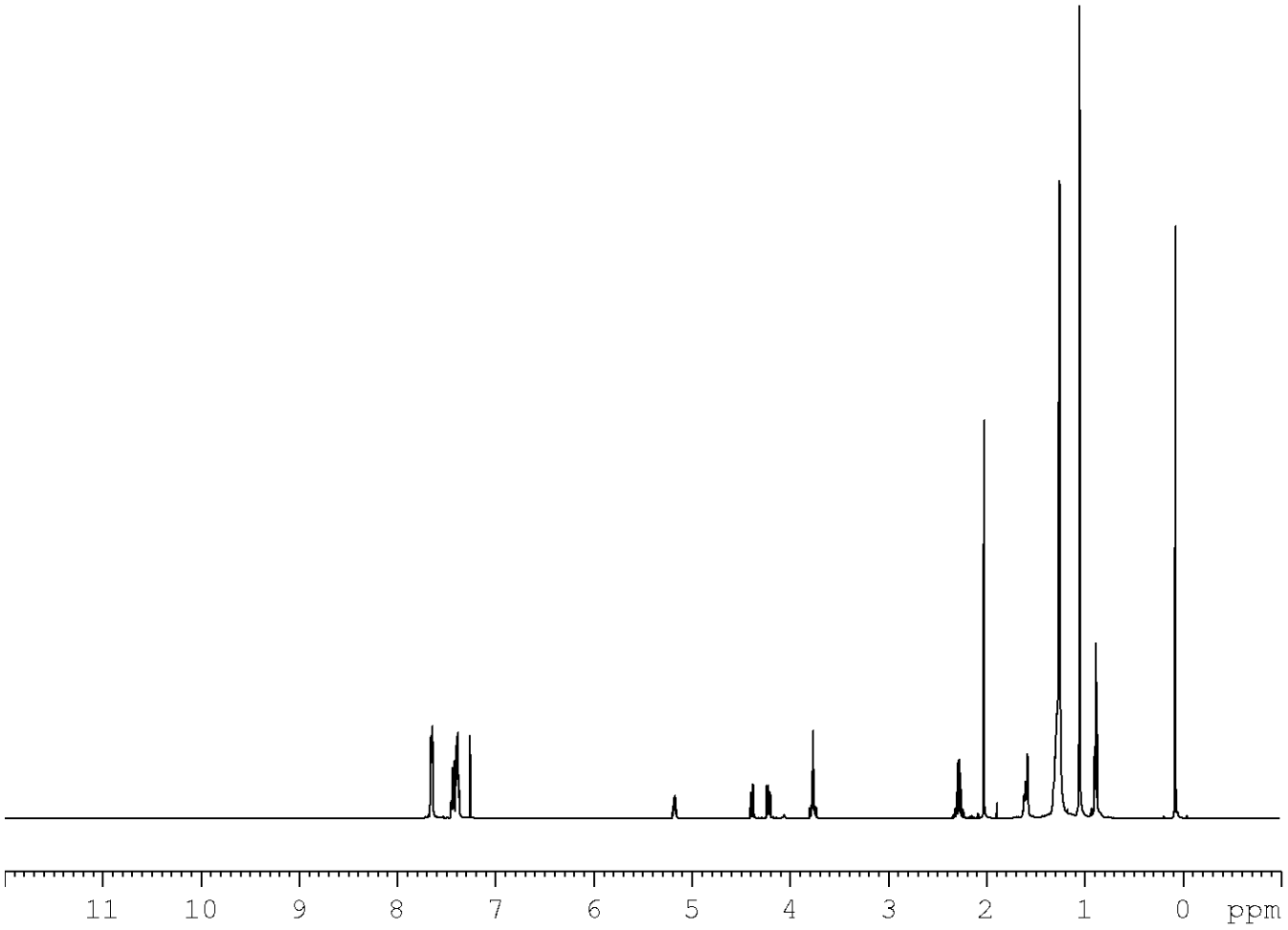
======== CHANNEL f1 ========
NUC1                 13C
P1                 9.50 usec
PL1       0 dB
SFO1        100.6403931 MHz

======== CHANNEL f2 ========
CPDPRG[2         waltz16
NUC2                 1H
PCPD2             80.00 usec
PL2       0 dB
PL12              19.00 dB
PL13              25.00 dB
SFO2         400.2016008 MHz

F2 - Processing parameters
SI                32768
SF           100.6303561 MHz
WDW                  EM
SSB       0
LB                1.00 Hz
GB        0
PC                1.40
```

2-(*tert*-Butyldiphenylsilyl)oxy-3-acetoxypropyl palmitate **203**






```
      Instrument AVC500
         Group SJC
 5230 Diane Swallow 25/7/11

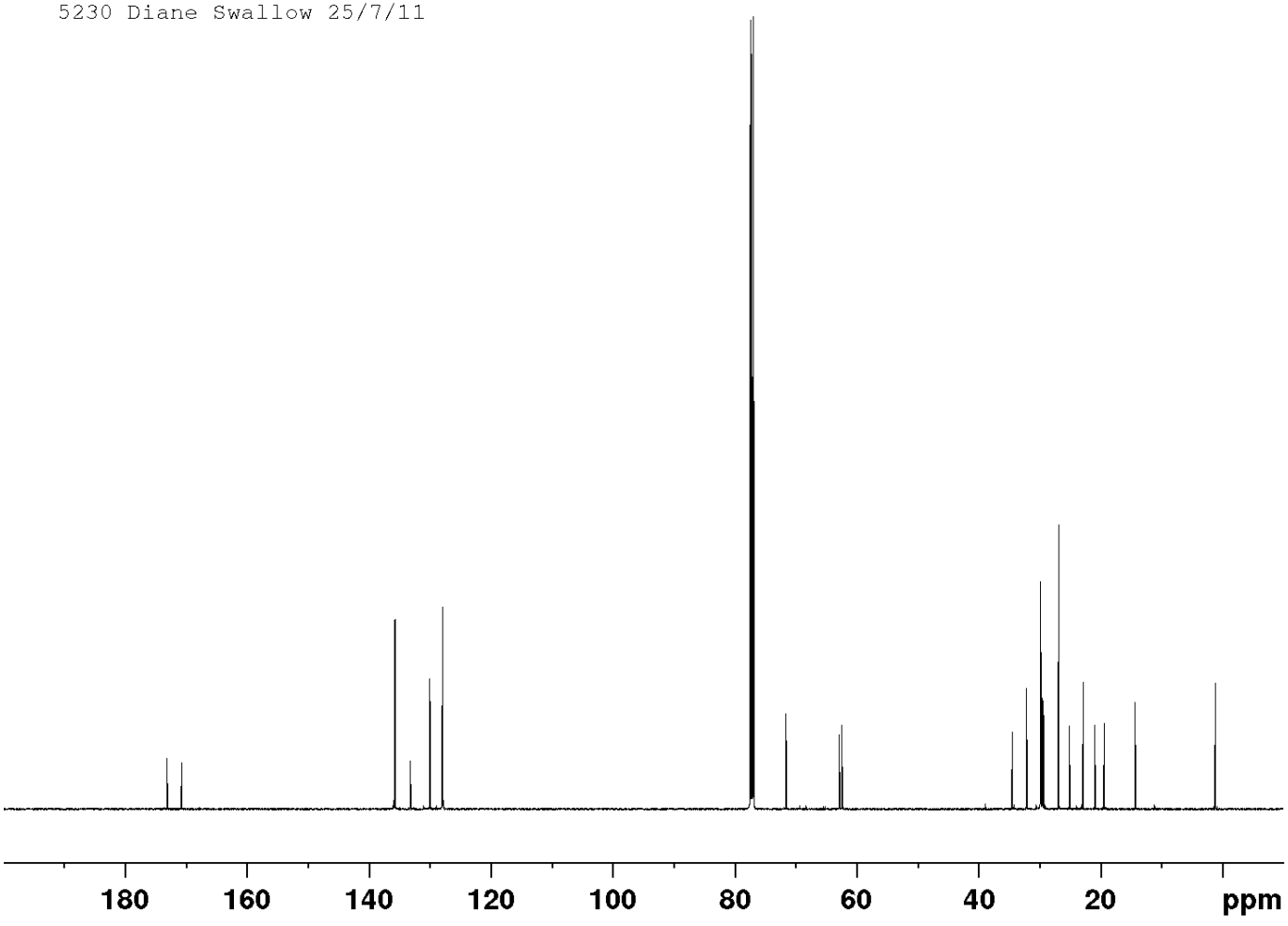
Current Data Parameters
NAME      IDS094 service - ds52
EXPNO                    1
PROCNO                   1

F2 - Acquisition Parameters
Date_           20110726
Time                7.32
INSTRUM           avc500
PROBHD   5 mm CPDUL 13C
PULPROG             zg30
TD                 65536
SOLVENT            CDCl3
NS                    16
DS                     2
SWH           10330.578 Hz
FIDRES         0.157632 Hz
AQ            3.1719425 sec
RG                     4
DW               48.400 usec
DE                6.00 usec
TE                298.0 K
D1           1.00000000 sec
TD0                    1

======== CHANNEL f1 ========
NUC1                  1H
P1                 9.60 usec
PL1               -6.00 dB
PL1W        15.19999981 W
SFO1        500.3030896 MHz

F2 - Processing parameters
SI                 32768
SF          500.3000298 MHz
WDW                   EM
SSB      0
LB                0.30 Hz
GB       0
PC                1.00
```

2-(*tert*-Butyldiphenylsilyl)oxy-3-acetoxypropyl palmitate **203**




```
Current Data Parameters
NAME      IDS094 service
          ds52302507
EXPNO                   4
PROCNO                  1

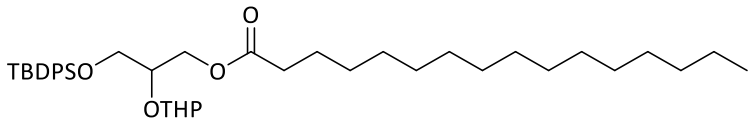
F2 - Acquisition Parameters
Date_           20110726
Time                8.15
INSTRUM           avc500
PROBHD   5 mm CPDUL 13C
PULPROG           zgpg30
TD                65536
SOLVENT           CDCl3
NS                  512
DS                    2
SWH           31250.000 Hz
FIDRES         0.476837 Hz
AQ            1.0485760 sec
RG                 1820
DW               16.000 usec
DE               20.00 usec
TE               298.0 K
D1           2.00000000 sec
D11          0.03000000 sec
TD0                   1

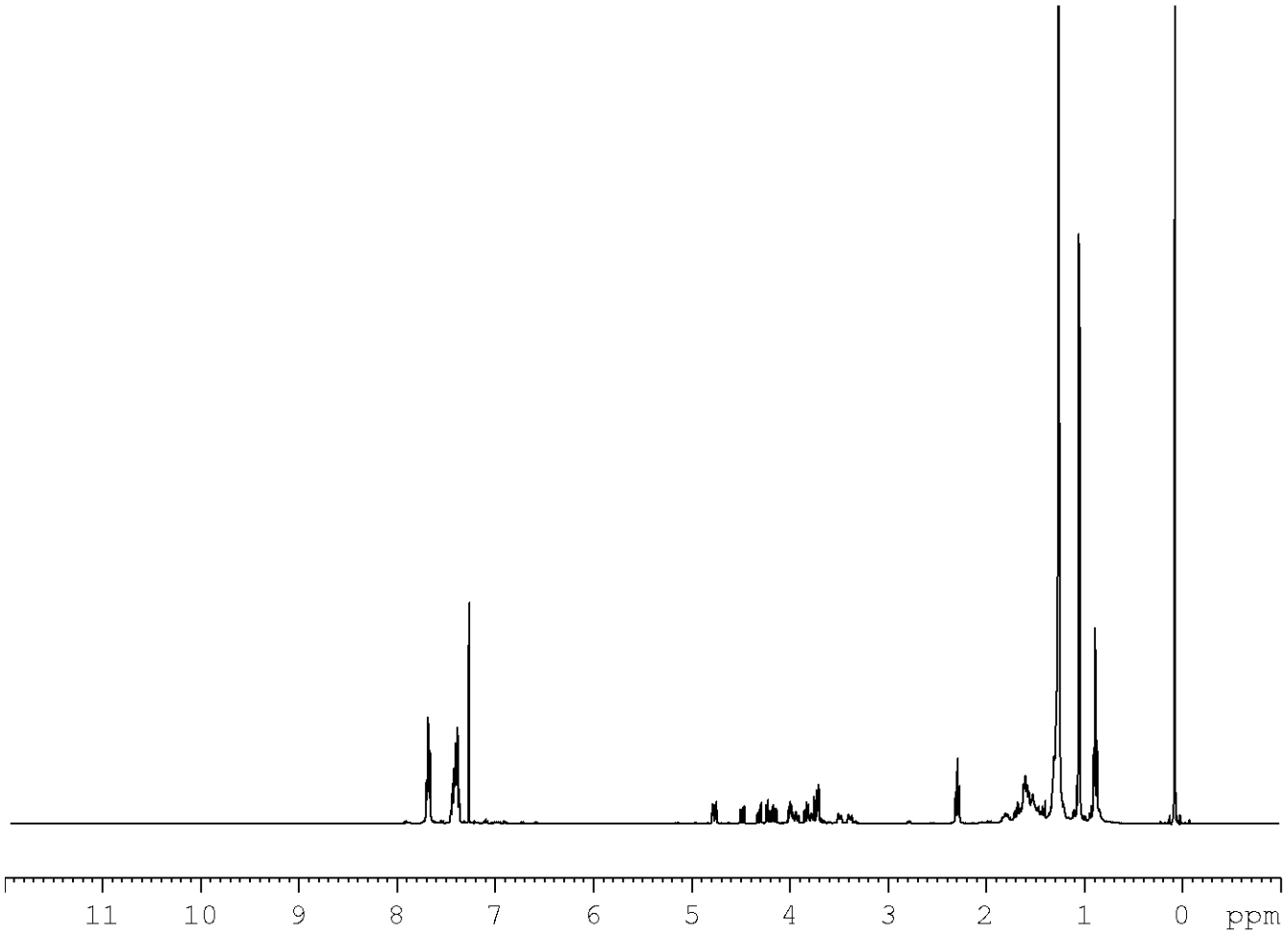
======== CHANNEL f1 ========
NUC1                 13C
P1                 9.10 usec
PL1               -4.40 dB
PL1W        28.15752029 W
SFO1       125.8131151 MHz

======== CHANNEL f2 ========
CPDPRG[2         waltz16
NUC2                 1H
PCPD2             80.00 usec
PL2               -6.00 dB
PL12              12.42 dB
PL13              18.42 dB
PL2W        15.19999981 W
PL12W        0.21869738 W
PL13W        0.05493430 W
SFO2       500.3020012 MHz

F2 - Processing parameters
SI                32768
SF         125.8005230 MHz
WDW                  EM
SSB      0
LB                1.00 Hz
GB       0
PC                1.40
```

2-(Tetrahydro-2*H*-pyran-2-yl)oxy-3-(*tert*-butyldiphenylsilyl)oxypropyl palmitate **204**






```
Instrument DPX400
Chemist ds
Group sjc
IDS99 - product
Qh1acq.au CDCl3 {C:\NMR} sjcgrp 43

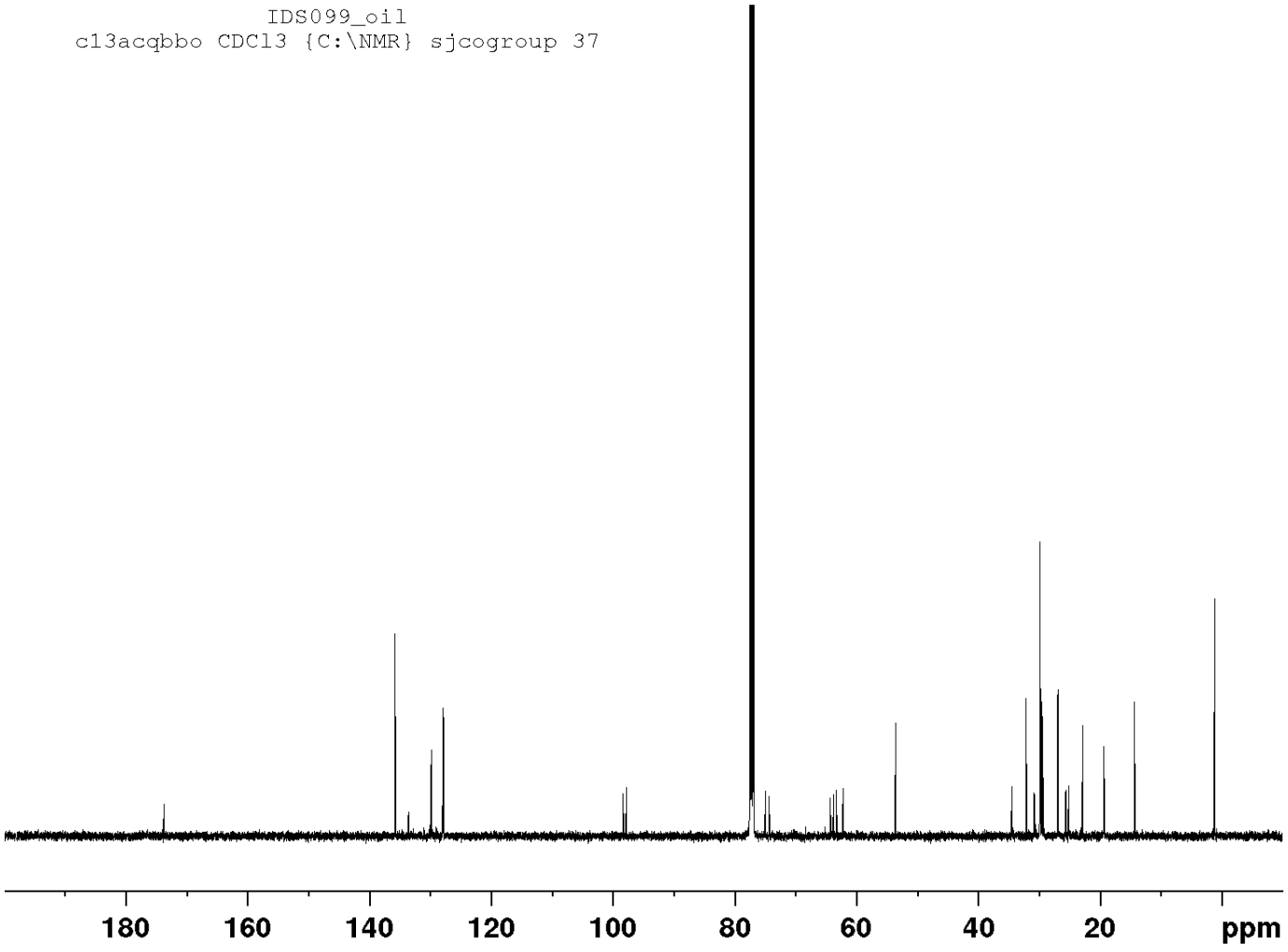
Current Data Parameters
NAME      IDS099 - Aug07-2011-43
EXPNO                  1
PROCNO                 1

F2 - Acquisition Parameters
Date_           20110807
Time              15.58
INSTRUM           dpx400
PROBHD   5 mm Dual 1H/1
PULPROG             zg60
TD                32768
SOLVENT           CDCl3
NS                   16
DS                    2
SWH            5592.841 Hz
FIDRES         0.170680 Hz
AQ            2.9294591 sec
RG                 80.6
DW                89.400 usec
DE                17.00 usec
TE                300.0 K
D1           1.00000000 sec

======== CHANNEL f1 ========
NUC1                1H
P1                7.30 usec
PL1       0 dB
SFO1        400.1320007 MHz

F2 - Processing parameters
SI                32768
SF          400.1300215 MHz
WDW                 EM
SSB       0
LB                0.30 Hz
GB        0
PC                0.60
```

2-(Tetrahydro-2*H*-pyran-2-yl)oxy-3-(*tert*-butyldiphenylsilyl)oxypropyl palmitate **204**




```
Current Data Parameters
NAME          IDS099_oil
EXPNO                  2
PROCNO                 1

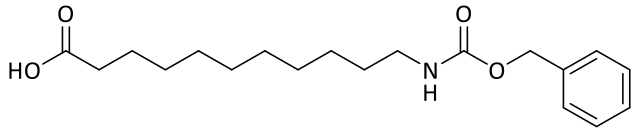
F2 - Acquisition Parameters
Date_           20140115
Time               4.39
INSTRUM           drx500
PROBHD   5 mm PABBO BB/
PULPROG          zgpg30
TD                65536
SOLVENT           CDCl3
NS                 4096
DS                    4
SWH           30303.031 Hz
FIDRES         0.462388 Hz
AQ            1.0813440 sec
RG               3649.1
DW               16.500 usec
DE               11.00 usec
TE               297.7 K
D1           2.00000000 sec
d11          0.03000000 sec
DELTA        1.89999998 sec
TD0                   1

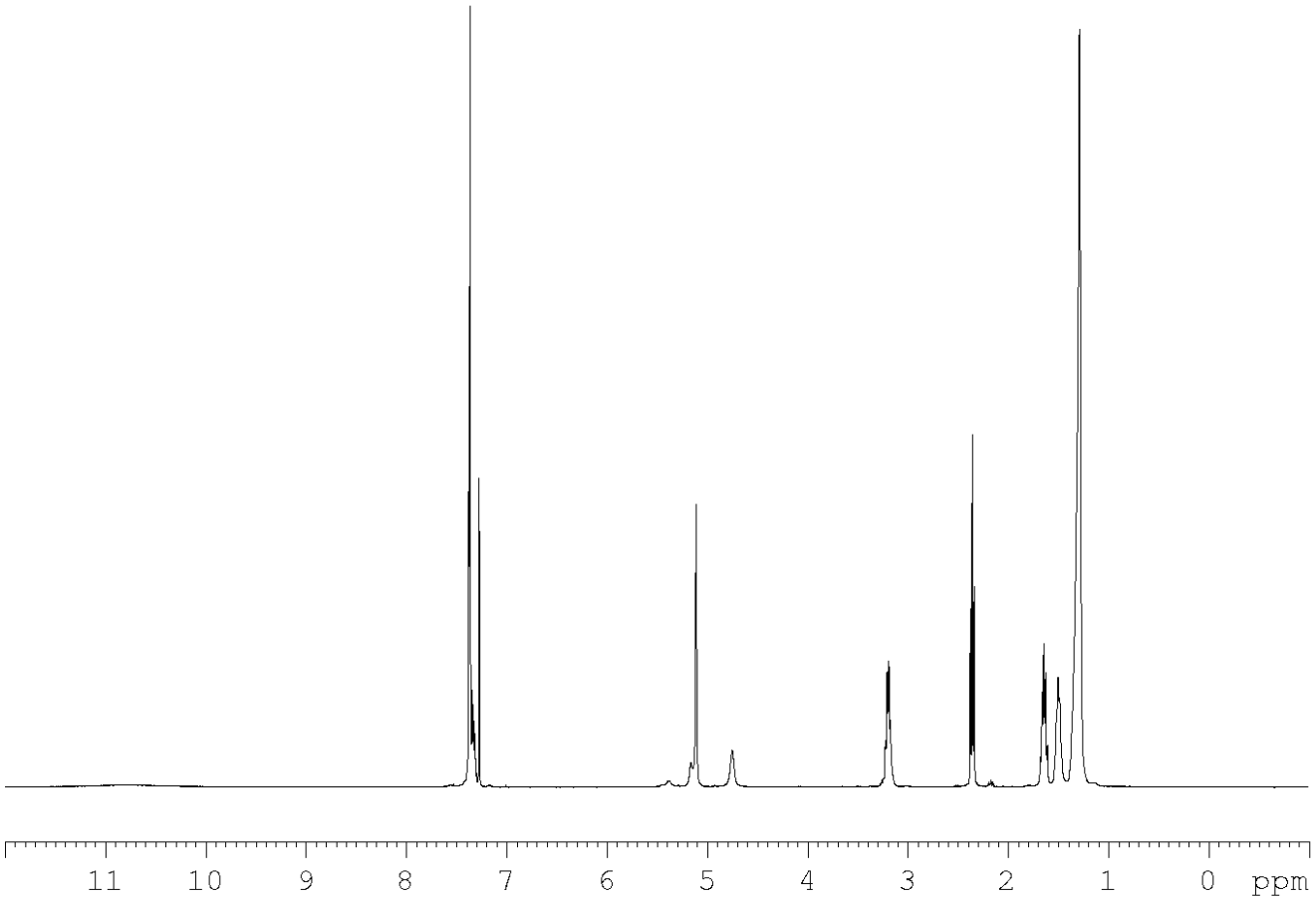
======== CHANNEL f1 ========
NUC1                 13C
P1                6.80 usec
PL1               5.00 dB
SFO1        125.7703148 MHz

======== CHANNEL f2 ========
CPDPRG[2         waltz16
NUC2                 1H
PCPD2            100.00 usec
PL2      0 dB
PL12              19.00 dB
PL13              23.00 dB
SFO2        500.1320005 MHz

F2 - Processing parameters
SI                32768
SF          125.7577756 MHz
WDW                  EM
SSB      0
LB                1.00 Hz
GB       0
PC                1.40
```

11-(*N*-Benzyloxycarbonyl)aminoundecanoic acid **188**






```
Instrument DQX400
    Chemist ds
     Group sjc
IDS45 - CBZ protection
h1acq.au CDCl3 {C:\NMR} sjcgrp 32

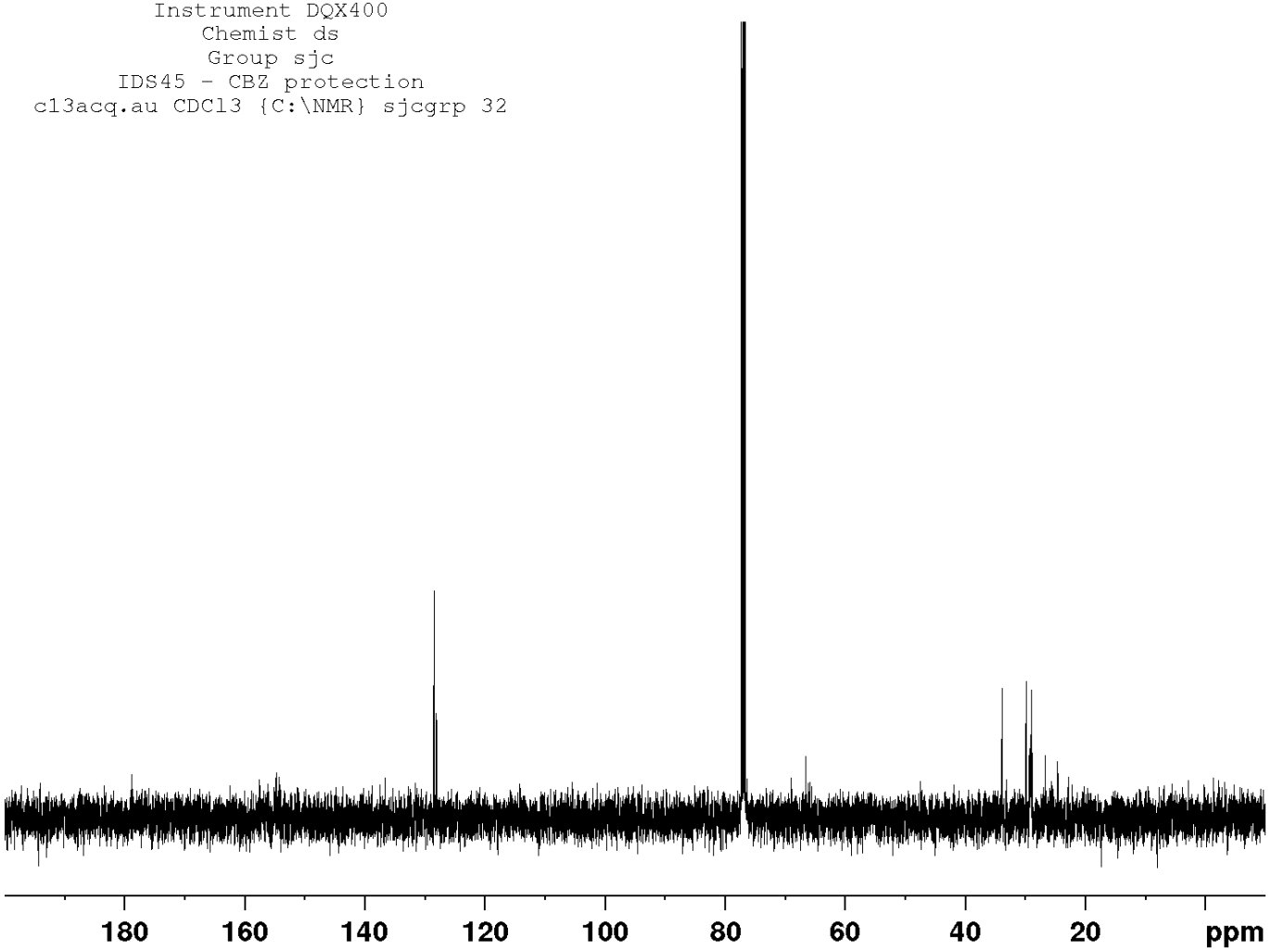
Current Data Parameters
NAME      IDS045 - Jan24-2011-32
EXPNO                      5
PROCNO                     1

F2 - Acquisition Parameters
Date_              20110124
Time                 16.24
INSTRUM              av400
PROBHD    5 mm QNP 1H/13
PULPROG               zg60
TD                   65536
SOLVENT              CDCl3
NS                      16
DS                       2
SWH               8278.146 Hz
FIDRES             0.126314 Hz
AQ                3.9583745 sec
RG                      256
DW                   60.400 usec
DE                    7.50 usec
TE                   300.0 K
D1             1.00000000 sec

======== CHANNEL f1 ========
NUC1                     1H
P1                    9.00 usec
PL1       0 dB
SFO1          400.2024714 MHz

F2 - Processing parameters
SI                    32768
SF            400.2000028 MHz
WDW                      EM
SSB       0
LB                    0.30 Hz
GB        0
PC                    1.00
```

11-(*N*-Benzyloxycarbonyl)aminoundecanoic acid **188**




```
Current Data Parameters
NAME      IDS045 - Jan24-2011-32
EXPNO                      7
PROCNO                     1

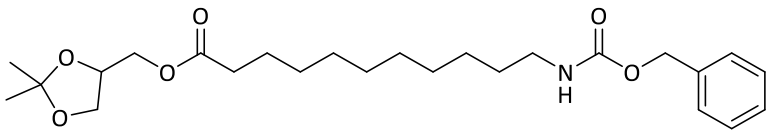
F2 - Acquisition Parameters
Date_            20110124
Time                16.36
INSTRUM             av400
PROBHD   5 mm QNP 1H/13
PULPROG            zgpg30
TD                  32768
SOLVENT             CDCl3
NS                    256
DS                      4
SWH             26178.010 Hz
FIDRES           0.798889 Hz
AQ               0.6258688 sec
RG                  32768
DW                 19.100 usec
DE                  7.50 usec
TE                 300.0 K
D1              1.00000000 sec
D11             0.03000000 sec
TD0                        1

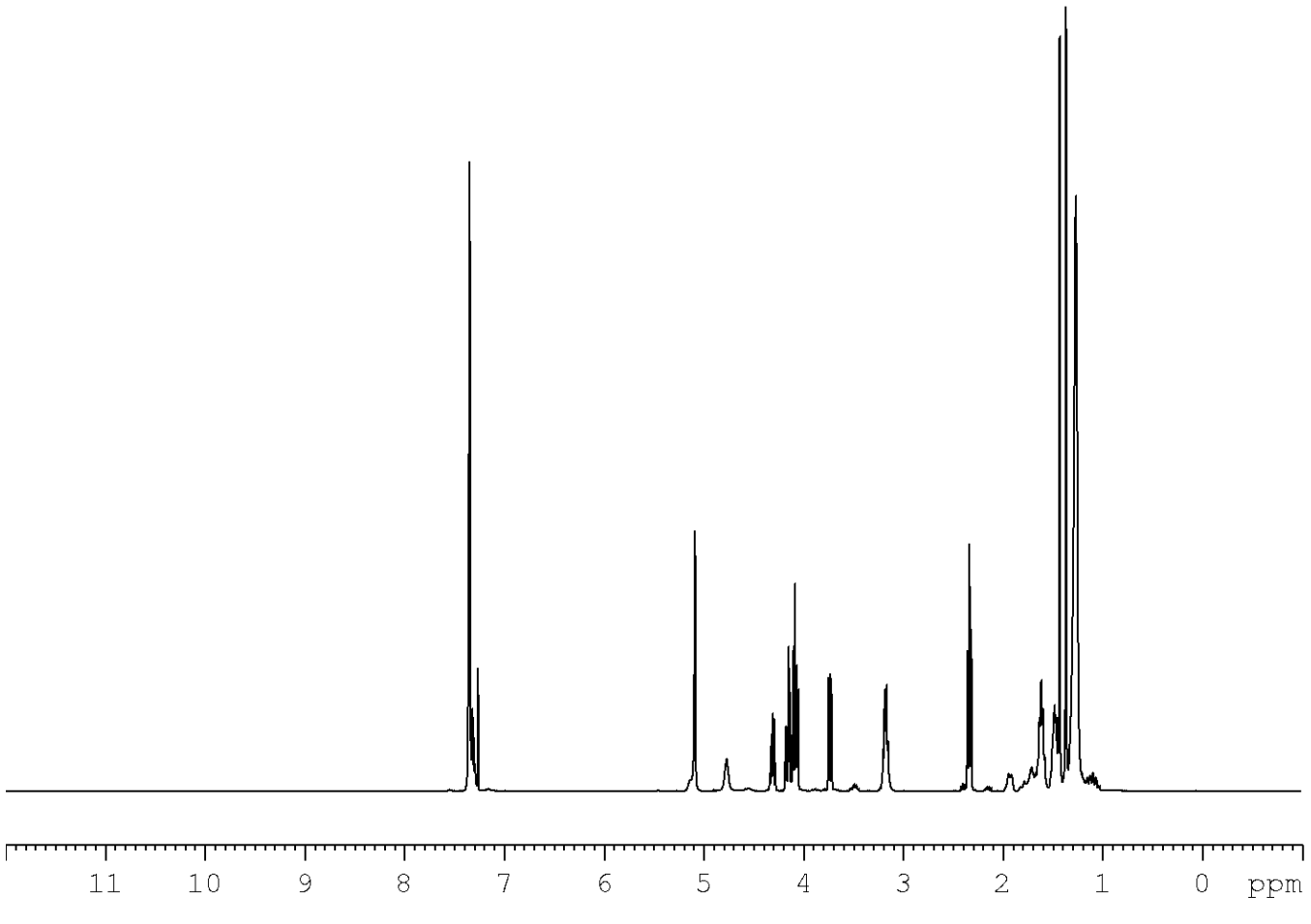
======== CHANNEL f1 ========
NUC1                   13C
P1                    9.50 usec
PL1       0 dB
SFO1          100.6403931 MHz

======== CHANNEL f2 ========
CPDPRG[2           waltz16
NUC2                   1H
PCPD2                80.00 usec
PL2       0 dB
PL12                 19.00 dB
PL13                 25.00 dB
SFO2          400.2016008 MHz

F2 - Processing parameters
SI                  32768
SF             100.6303718 MHz
WDW                    EM
SSB       0
LB                    1.00 Hz
GB        0
PC                    1.40
```

(2,2-Dimethyl-1,3-dioxolan-4-yl)methyl 11-(*N*-benzyloxycarbonyl)aminoundecanoate **192**






```
Instrument DQX400
    Chemist ds
    Group sjc
    IDS63(II)
h1acq.au CDCl3 {E:\NMR2011} sjcgrp 6

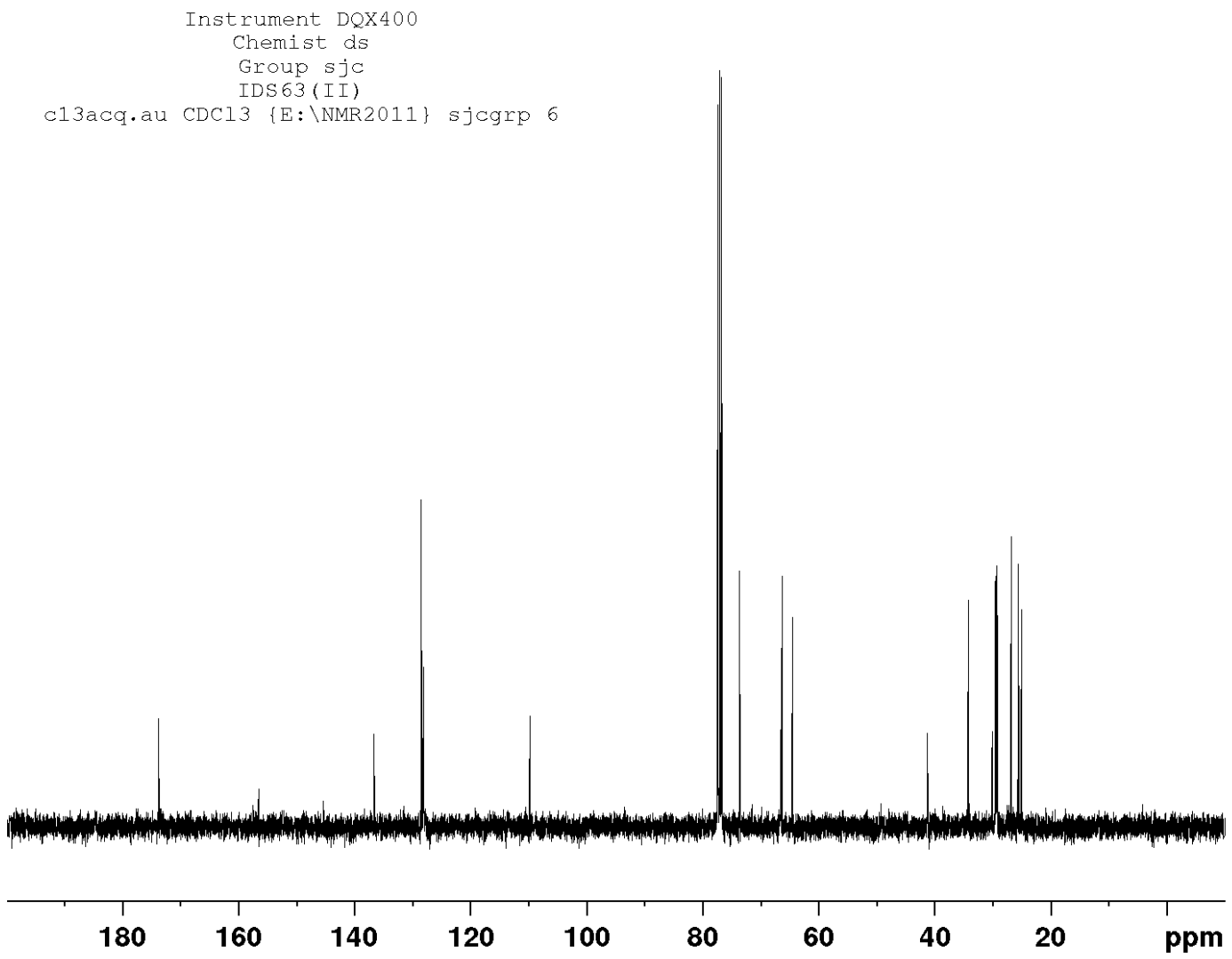
Current Data Parameters
NAME      IDS063(II) - May31-2011-6
EXPNO                  1
PROCNO                 1

F2 - Acquisition Parameters
Date_           20110601
Time                6.10
INSTRUM           av400
PROBHD   5 mm QNP 1H/13
PULPROG             zg60
TD                 65536
SOLVENT            CDCl3
NS                    16
DS                     2
SWH             8278.146 Hz
FIDRES          0.126314 Hz
AQ              3.9583745 sec
RG                  90.5
DW                60.400 usec
DE                 7.50 usec
TE                300.0 K
D1           1.00000000 sec

======== CHANNEL f1 ========
NUC1                  1H
P1                 9.00 usec
PL1       0 dB
SFO1         400.2024714 MHz

F2 - Processing parameters
SI                 32768
SF           400.2000063 MHz
WDW                   EM
SSB       0
LB                 0.30 Hz
GB        0
PC                 1.00
```

(2,2-Dimethyl-1,3-dioxolan-4-yl)methyl 11-(*N*-benzyloxycarbonyl)aminoundecanoate **192**




```
Current Data Parameters
NAME      IDS063(II) - May31-2011-6
EXPNO                  2
PROCNO                 1

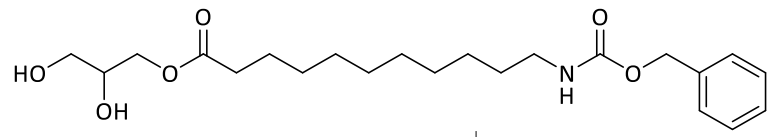
F2 - Acquisition Parameters
Date_           20110601
Time               6.18
INSTRUM           av400
PROBHD   5 mm QNP 1H/13
PULPROG          zgpg30
TD                32768
SOLVENT           CDCl3
NS                  256
DS                    4
SWH           26178.010 Hz
FIDRES         0.798889 Hz
AQ            0.6258688 sec
RG                32768
DW               19.100 usec
DE                7.50 usec
TE                300.0 K
D1           1.00000000 sec
D11          0.03000000 sec
TD0                   1

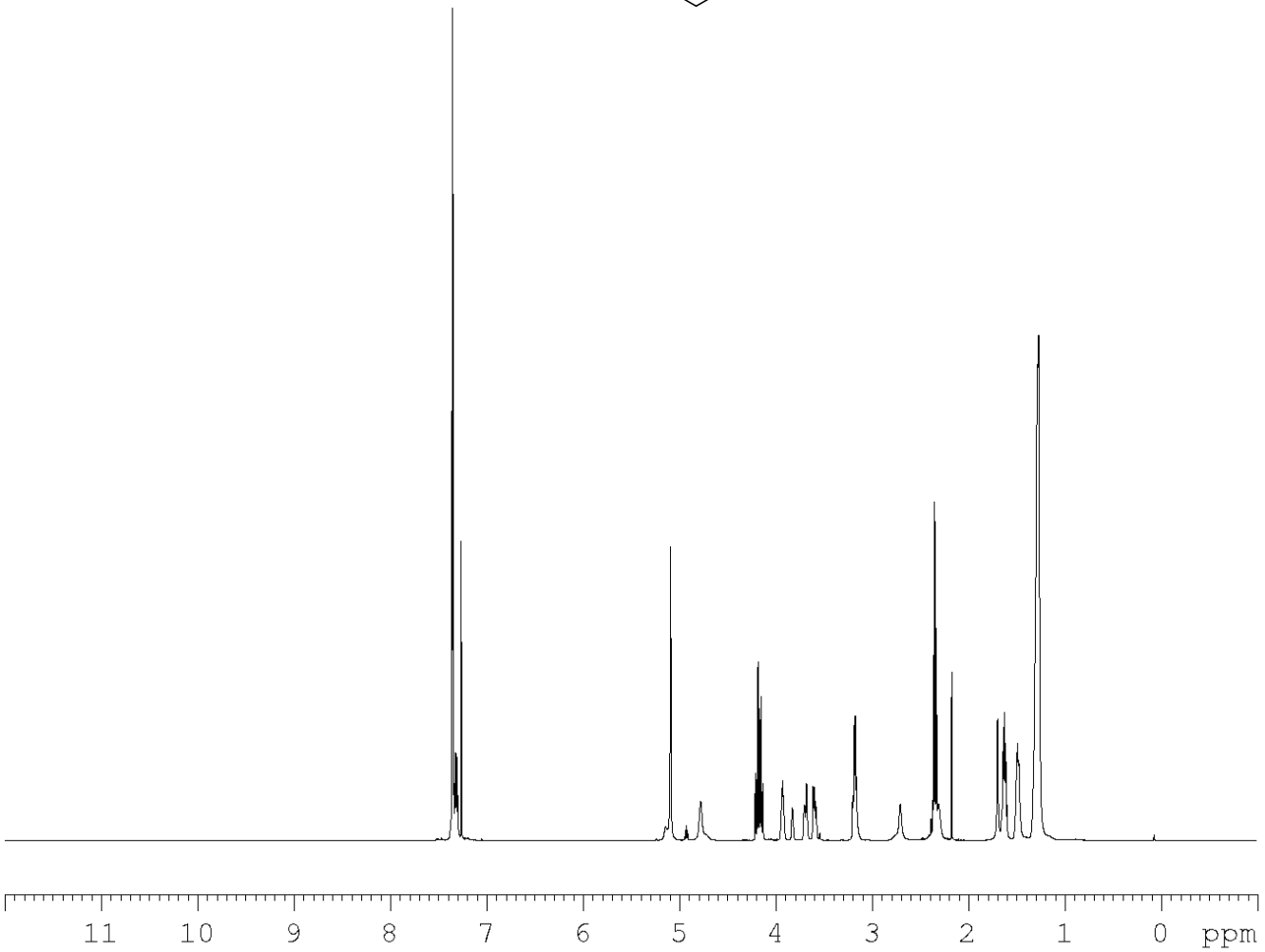
======== CHANNEL f1 ========
NUC1                 13C
P1                  9.50 usec
PL1       0 dB
SFO1        100.6403931 MHz

======== CHANNEL f2 ========
CPDPRG[2         waltz16
NUC2                 1H
PCPD2              80.00 usec
PL2       0 dB
PL12              19.00 dB
PL13              25.00 dB
SFO2         400.2016008 MHz

F2 - Processing parameters
SI                32768
SF          100.6303587 MHz
WDW                  EM
SSB       0
LB                1.00 Hz
GB        0
PC                1.40
```

2,3-Dihydroxypropyl 11-(*N*-benzyloxycarbonyl)aminoundecanoate **186**






```
Instrument AVC500
Group SJC
6244 Diane Swallow 4/10/11

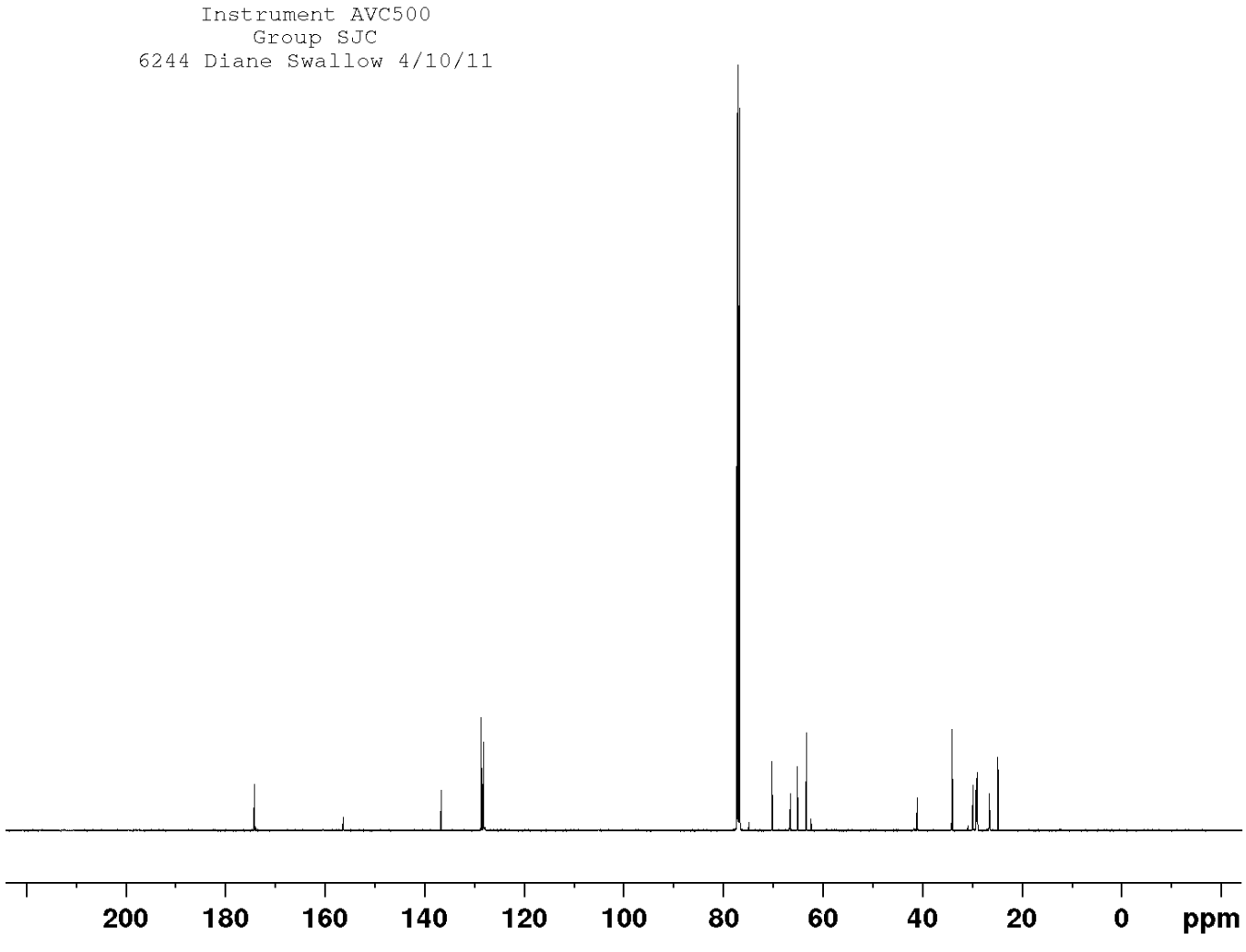
Current Data Parameters
NAME      IDS115- ds62440410
EXPNO                      1
PROCNO                     1

F2 - Acquisition Parameters
Date_              20111004
Time                  17.25
INSTRUM              avc500
PROBHD   5 mm CPDUL 13C
PULPROG                zg30
TD                    65536
SOLVENT               CDCl3
NS                       16
DS                        2
SWH              10330.578 Hz
FIDRES            0.157632 Hz
AQ                3.1719425 sec
RG                        4
DW                   48.400 usec
DE                    6.00 usec
TE                   298.1 K
D1              1.00000000 sec
TD0                       1

======== CHANNEL f1 ========
NUC1                     1H
P1                    9.60 usec
PL1                  -6.00 dB
PL1W          15.19999981 W
SFO1         500.3030896 MHz

F2 - Processing parameters
SI                    32768
SF           500.3000284 MHz
WDW                      EM
SSB       0
LB                    0.30 Hz
GB        0
PC                    1.00
```

2,3-Dihydroxypropyl 11-(*N*-benzyloxycarbonyl)aminoundecanoate **186**




```
Current Data Parameters
NAME      IDS115- ds62440410
EXPNO                      4
PROCNO                     1

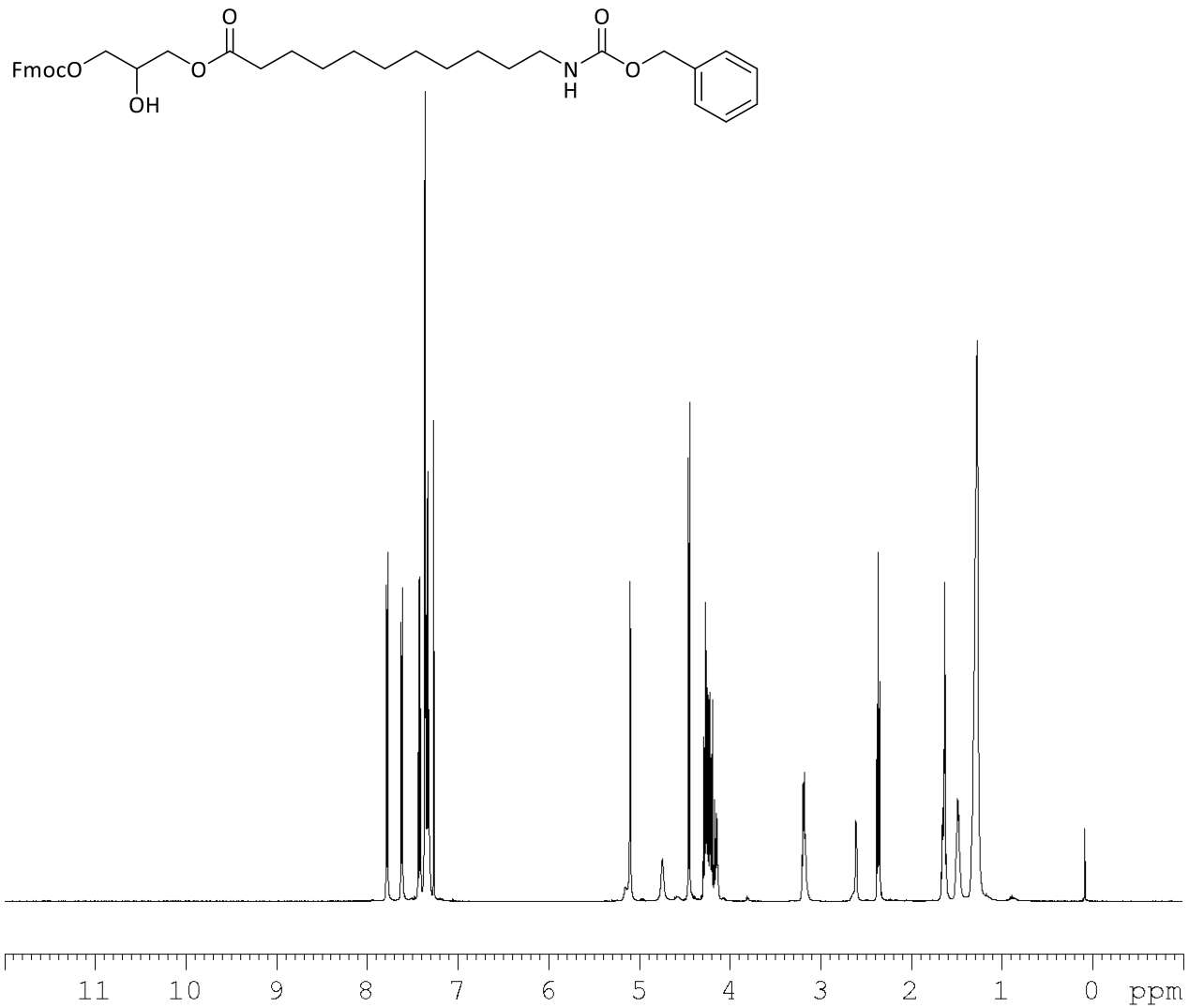
F2 - Acquisition Parameters
Date_            20111004
Time                 18.08
INSTRUM            avc500
PROBHD    5 mm CPDUL 13C
PULPROG            zgpg30
TD                  65536
SOLVENT             CDCl3
NS                    512
DS                      2
SWH             31250.000 Hz
FIDRES          0.476837 Hz
AQ              1.0485760 sec
RG                   1820
DW                 16.000 usec
DE                 20.00 usec
TE                 298.0 K
D1            2.00000000 sec
D11           0.03000000 sec
TD0                     1

======== CHANNEL f1 ========
NUC1                   13C
P1                  10.00 usec
PL1                 -4.40 dB
PL1W         28.15752029 W
SFO1         125.8131151 MHz

======== CHANNEL f2 ========
CPDPRG[2          waltz16
NUC2                   1H
PCPD2               80.00 usec
PL2                 -6.00 dB
PL12                12.42 dB
PL13                18.42 dB
PL2W         15.19999981 W
PL12W         0.21869738 W
PL13W         0.05493430 W
SFO2         500.3020012 MHz

F2 - Processing parameters
SI                  32768
SF           125.8005238 MHz
WDW                    EM
SSB       0
LB                  1.00 Hz
GB        0
PC                  1.40
```

2-Hydroxy-3-([9*H*-fluoren-9-yl]methoxycarbonyl)oxypropyl
11-(*N*-benzyloxycarbonyl)aminoundecanoate **209**




```
Instrument AVC500
Group SJC
8431 Diane Swallow 6/3/12

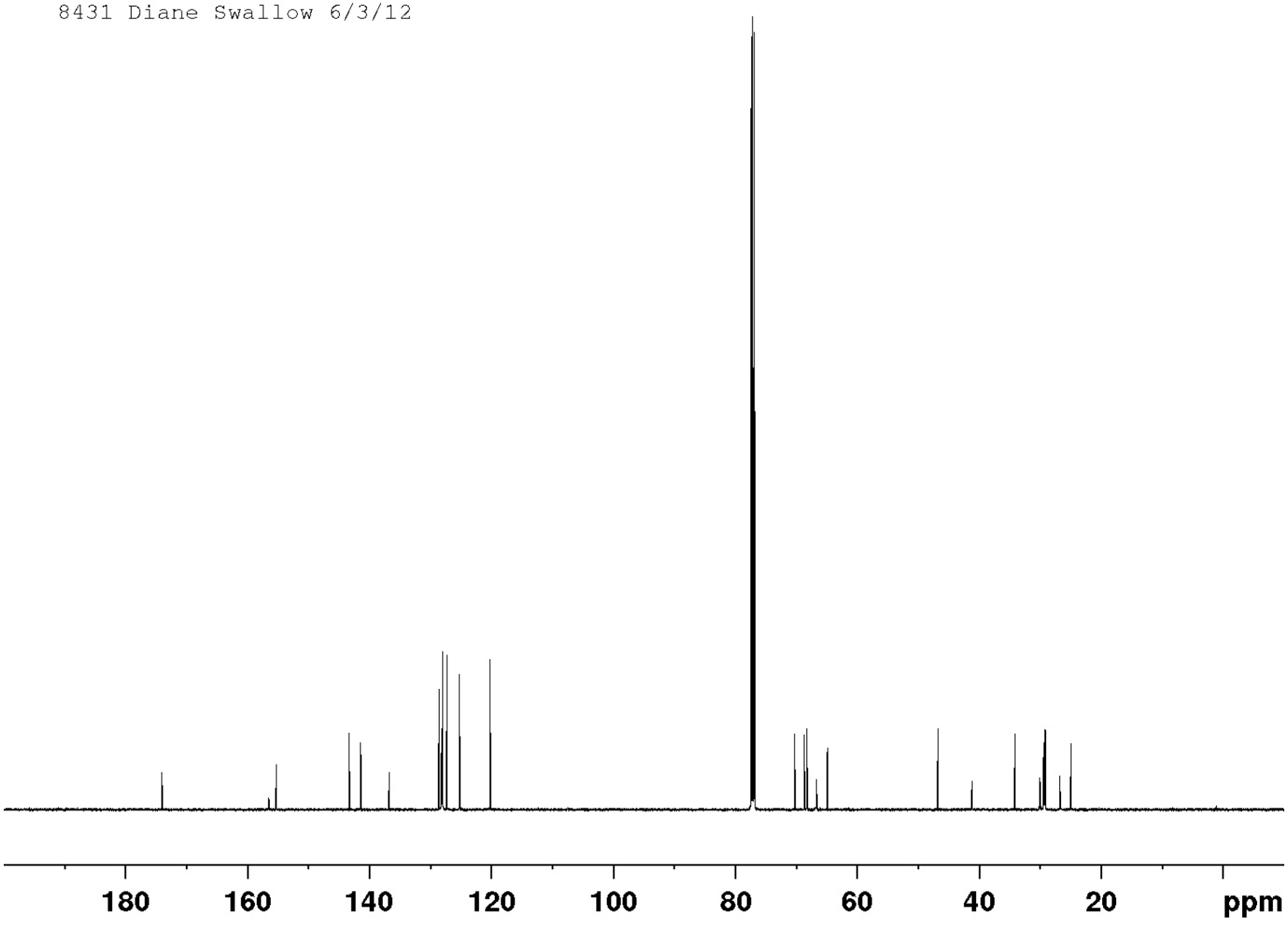

Current Data Parameters
NAME      IDS161 - ds84310603
EXPNO                    1
PROCNO                   1

F2 - Acquisition Parameters
Date_             20120307
Time                 19.44
INSTRUM             avc500
PROBHD    5 mm CPDUL 13C
PULPROG               zg30
TD                   65536
SOLVENT              CDCl3
NS                      16
DS                       2
SWH              10330.578 Hz
FIDRES            0.157632 Hz
AQ                3.1719425 sec
RG                       4
DW                  48.400 usec
DE                   6.00 usec
TE                  298.0 K
D1              1.00000000 sec
TD0                      1

======== CHANNEL f1 ========
NUC1                    1H
P1                   9.60 usec
PL1                 -6.00 dB
PL1W          15.19999981 W
SFO1          500.3030896 MHz

F2 - Processing parameters
SI                   32768
SF            500.3000282 MHz
WDW                     EM
SSB       0
LB                   0.30 Hz
GB        0
PC                   1.00
```

2-Hydroxy-3-([9*H*-fluoren-9-yl]methoxycarbonyl)oxypropyl 11-(*N*-benzyloxycarbonyl)aminoundecanoate **209**




```
Current Data Parameters
NAME      IDS161 - ds84310603
EXPNO                       4
PROCNO                      1

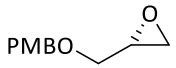
F2 - Acquisition Parameters
Date_            20120307
Time                20.19
INSTRUM           avc500
PROBHD   5 mm CPDUL 13C
PULPROG           zgpg30
TD                65536
SOLVENT           CDCl3
NS                  256
DS                    2
SWH           31250.000 Hz
FIDRES         0.476837 Hz
AQ            1.0485760 sec
RG                 1440
DW               16.000 usec
DE               20.00 usec
TE               298.0 K
D1           2.00000000 sec
D11          0.03000000 sec
TD0                   1

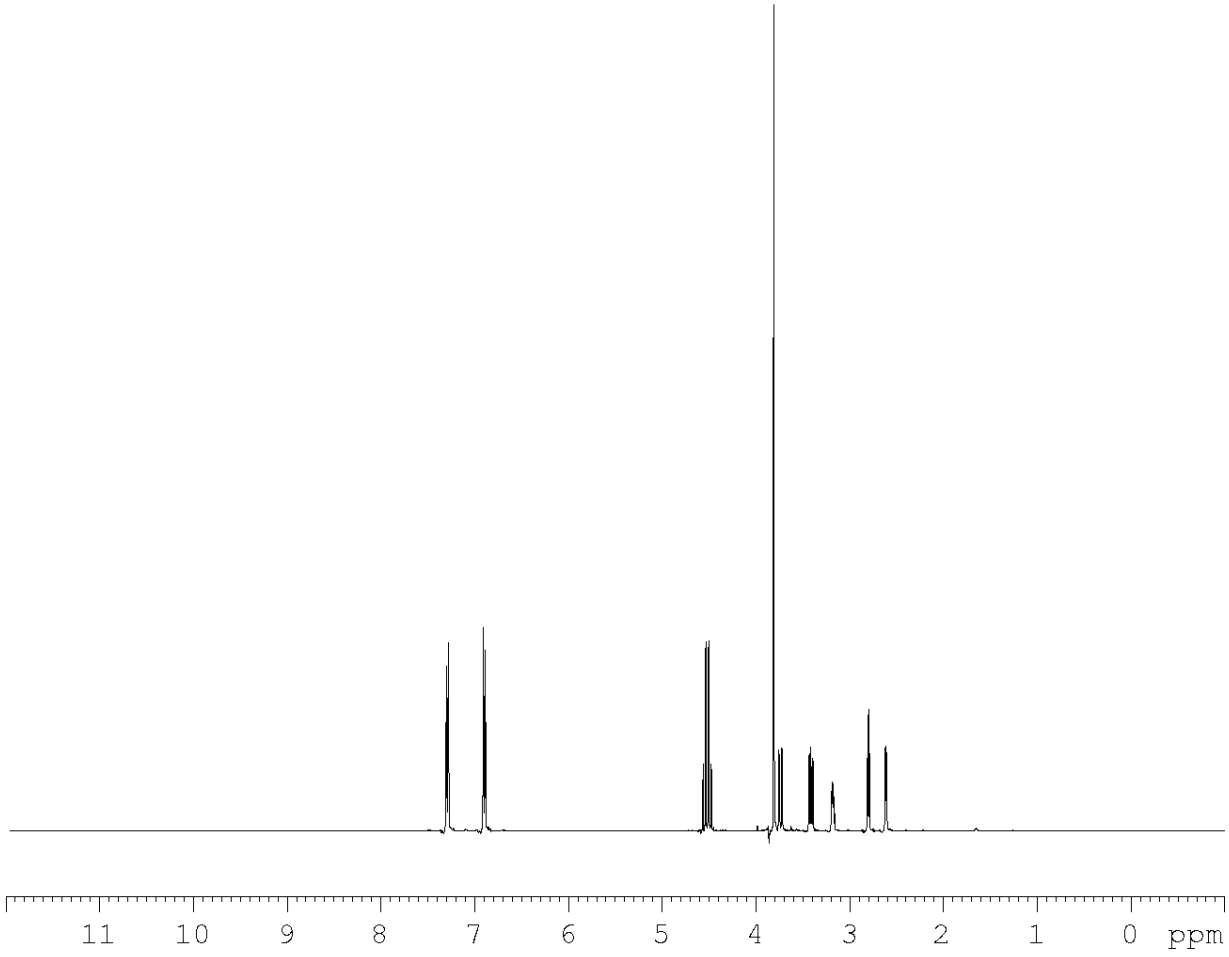
======== CHANNEL f1 ========
NUC1                 13C
P1                10.00 usec
PL1               -4.40 dB
PL1W        28.15752029 W
SFO1       125.8131151 MHz

======== CHANNEL f2 ========
CPDPRG[2         waltz16
NUC2                 1H
PCPD2             80.00 usec
PL2               -6.00 dB
PL12              12.42 dB
PL13              18.42 dB
PL2W        15.19999981 W
PL12W        0.21869738 W
PL13W        0.05493430 W
SFO2       500.3020012 MHz

F2 - Processing parameters
SI                32768
SF          125.8005249 MHz
WDW                  EM
SSB       0
LB                1.00 Hz
GB        0
PC                1.40
```

(*R*)-2-([{4-Methoxybenzyl}oxy]methyl)oxirane **199**






```
Instrument DPX400
    Chemist ds
     Group sjc
   IDS174 - clean
Qh1acq.au CDCl3 {C:\NMR} sjcgrp 20

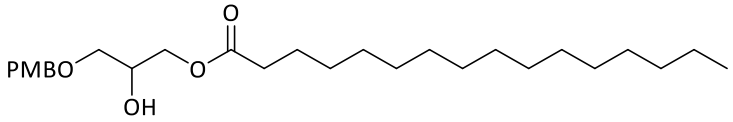
Current Data Parameters
NAME      IDS174 clean
          Apr11-2012-20
EXPNO                  1
PROCNO                 1

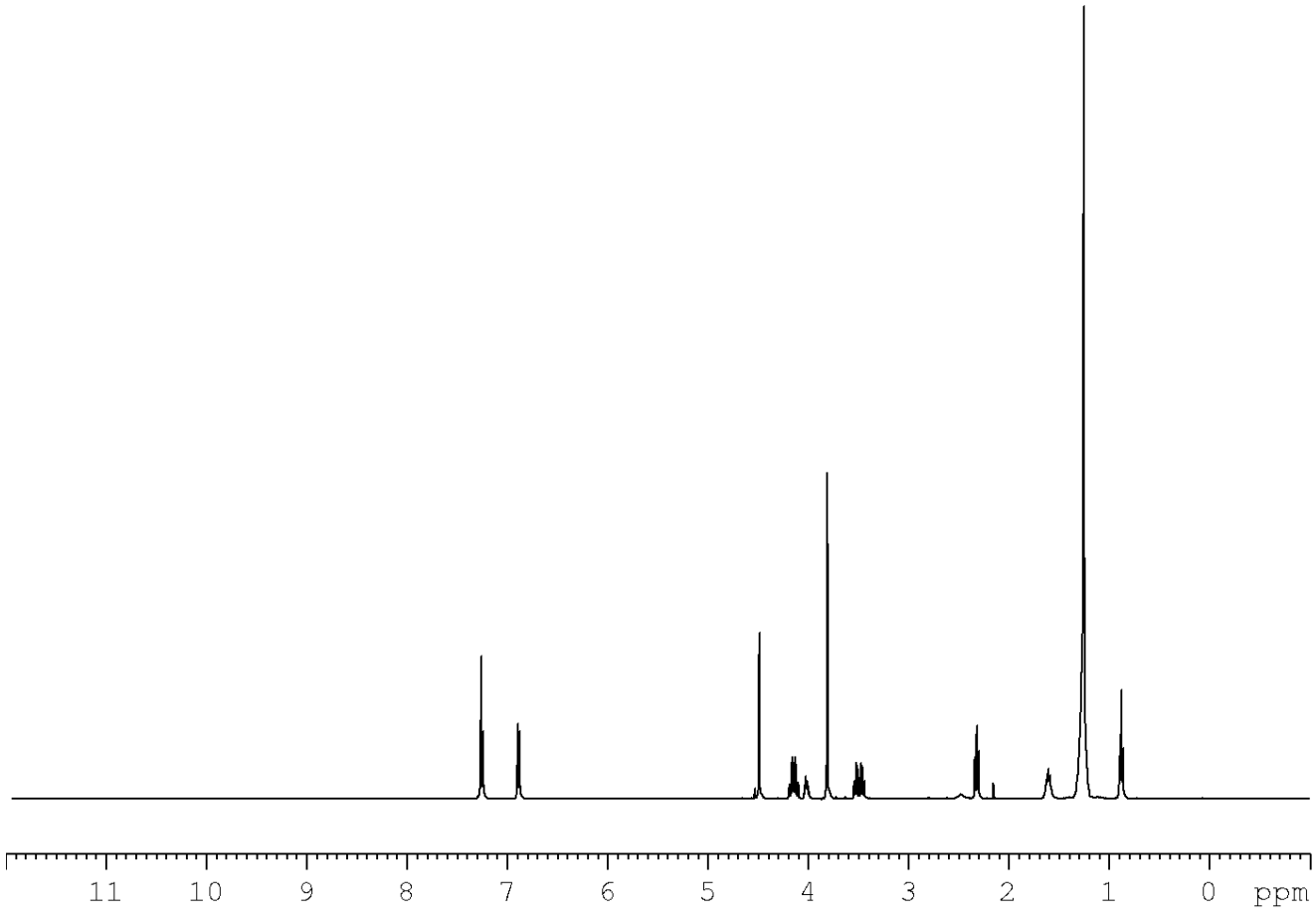
F2 - Acquisition Parameters
Date_           20120411
Time                9.47
INSTRUM           dpx400
PROBHD   5 mm Dual 1H/1
PULPROG             zg60
TD                 32768
SOLVENT            CDCl3
NS                    16
DS                     2
SWH             5592.841 Hz
FIDRES          0.170680 Hz
AQ             2.9294591 sec
RG                  45.3
DW                89.400 usec
DE                17.00 usec
TE                300.0 K
D1           1.00000000 sec

======== CHANNEL f1 ========
NUC1                  1H
P1                 7.30 usec
PL1       0 dB
SFO1         400.1320007 MHz

F2 - Processing parameters
SI                 32768
SF          400.1300182 MHz
WDW                   EM
SSB       0
LB                 0.30 Hz
GB        0
PC                 0.60
```

(S)-2-Hydroxy-3-(4-methoxybenzyl)oxypropyl palmitate **197**






```
Instrument DPX400
    Chemist ds
    Group sjc
 IDS175 - 'clean'
Qh1acq.au CDCl3 {C:\NMR} sjcgrp 56

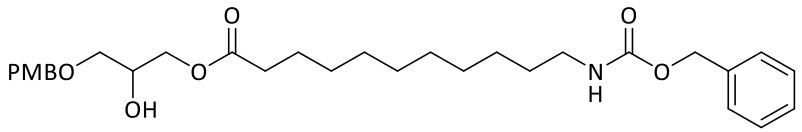
Current Data Parameters
NAME      IDS175 clean
           Apr16-2012-56
EXPNO                  1
PROCNO                 1

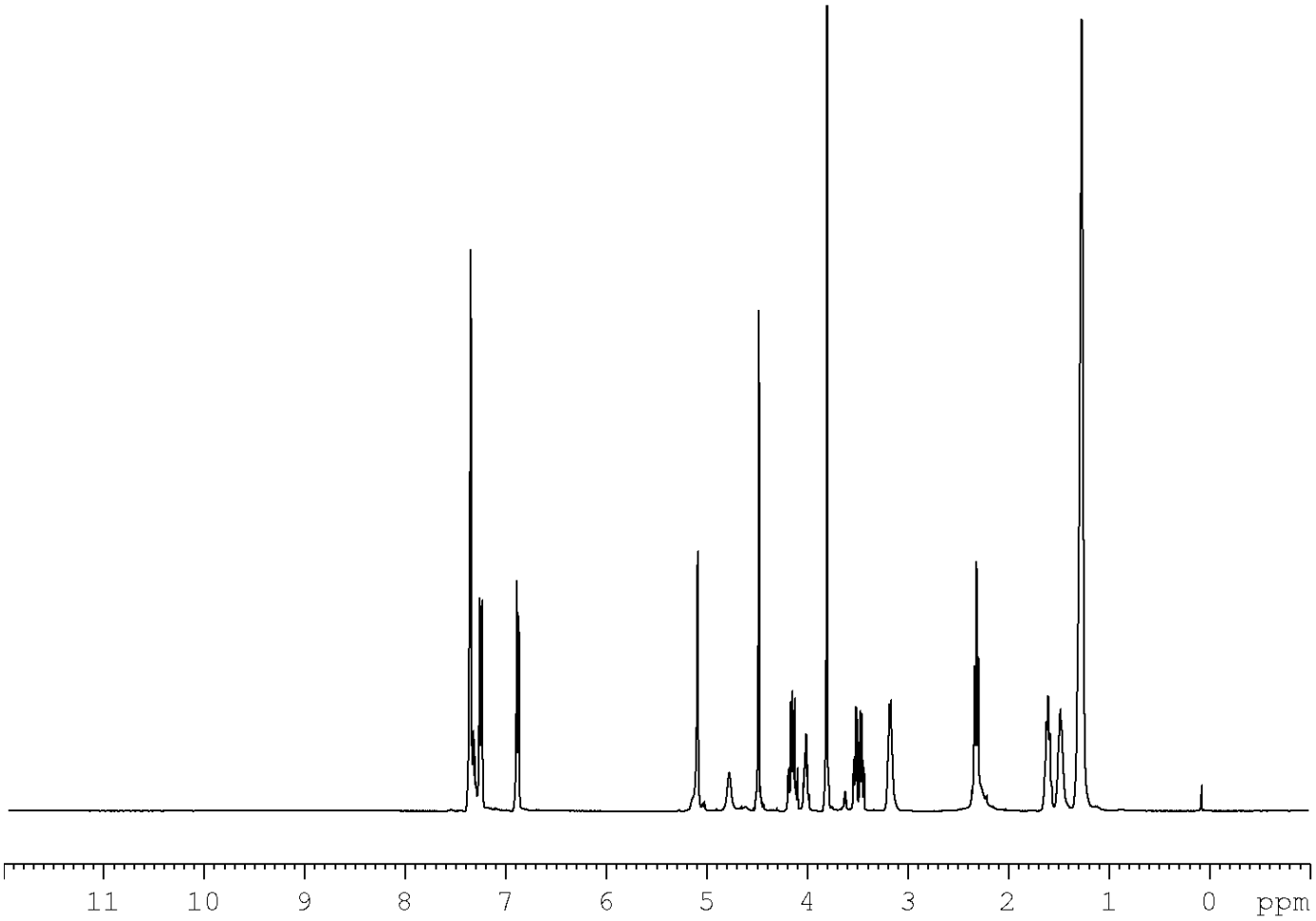
F2 - Acquisition Parameters
Date_           20120416
Time               21.08
INSTRUM          dpx400
PROBHD   5 mm Dual 1H/1
PULPROG            zg60
TD                32768
SOLVENT           CDCl3
NS                   16
DS                    2
SWH            5592.841 Hz
FIDRES         0.170680 Hz
AQ            2.9294591 sec
RG                101.6
DW               89.400 usec
DE               17.00 usec
TE               300.0 K
D1          1.00000000 sec

======== CHANNEL f1 ========
NUC1                  1H
P1                 7.30 usec
PL1       0 dB
SFO1        400.1320007 MHz

F2 - Processing parameters
SI                32768
SF          400.1300182 MHz
WDW                  EM
SSB       0
LB                 0.30 Hz
GB        0
PC                 0.60
```

(*S*)-2-Hydroxy-3-(4-methoxybenzyl)oxypropyl 11-(*N*-benzyloxycarbonyl)aminoundecanoate **198**






```
Instrument DPX400
    Chemist ds
     Group sjc
  IDS184 - F22-25
Qh1acq.au CDCl3 {C:\NMR} sjcgrp 21

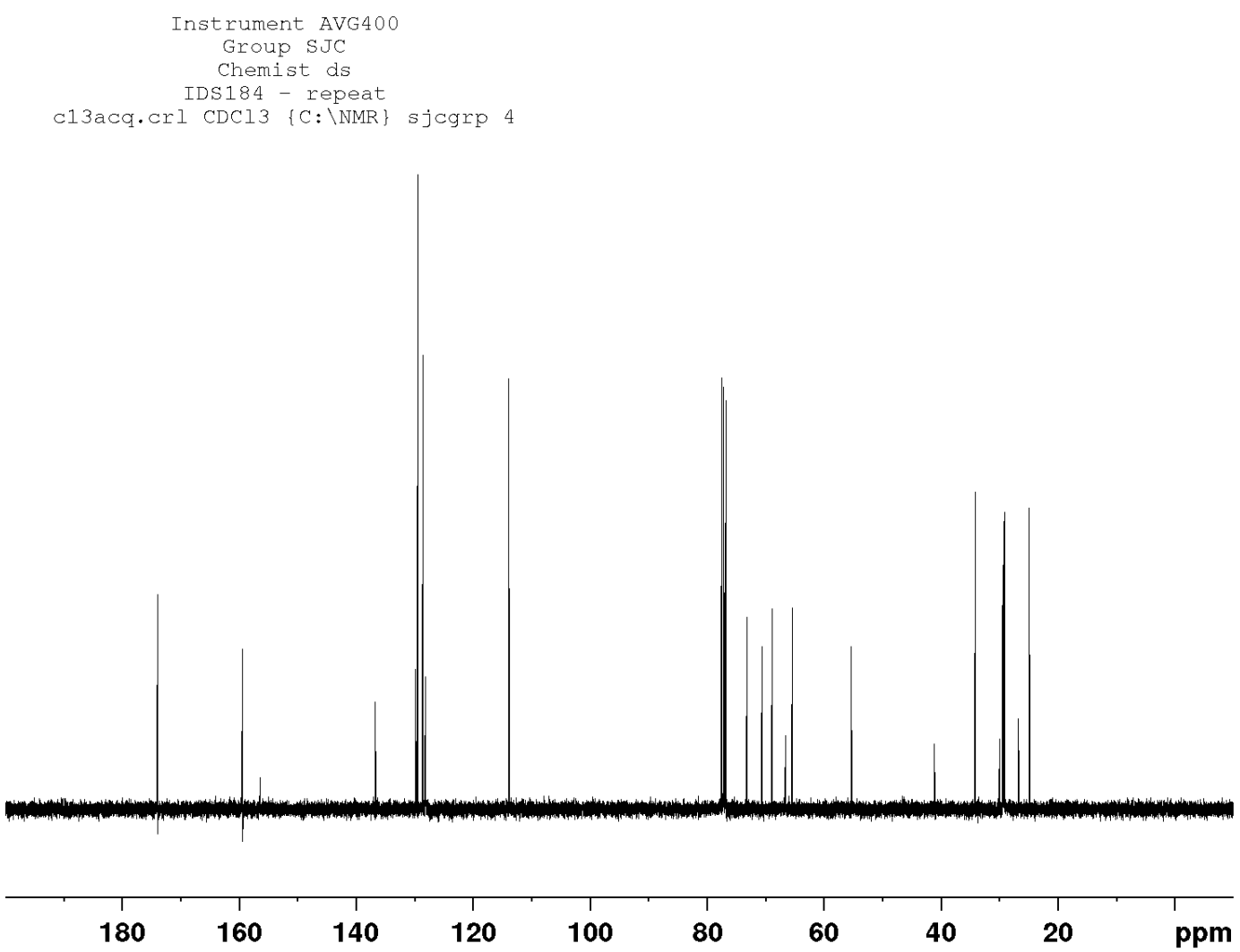
Current Data Parameters
NAME      IDS184 F22-25
          May30-2012-21
EXPNO                  1
PROCNO                 1

F2 - Acquisition Parameters
Date_           20120530
Time                9.53
INSTRUM           dpx400
PROBHD   5 mm Dual 1H/1
PULPROG             zg60
TD                 32768
SOLVENT            CDCl3
NS                    16
DS                     2
SWH             5592.841 Hz
FIDRES          0.170680 Hz
AQ              2.9294591 sec
RG                  45.3
DW                89.400 usec
DE                17.00 usec
TE                300.0 K
D1           1.00000000 sec

======== CHANNEL f1 ========
NUC1                  1H
P1                 7.30 usec
PL1       0 dB
SFO1         400.1320007 MHz

F2 - Processing parameters
SI                 32768
SF           400.1300182 MHz
WDW                   EM
SSB       0
LB                 0.30 Hz
GB        0
PC                 0.60
```

(*S*)-2-Hydroxy-3-(4-methoxybenzyl)oxypropyl 11-(*N*-benzyloxycarbonyl)aminoundecanoate **198**




```
Current Data Parameters
NAME        Oct19-2013-4
EXPNO                  2
PROCNO                 1

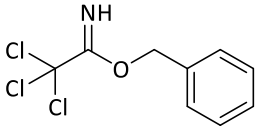
F2 - Acquisition Parameters
Date_           20131019
Time               15.53
INSTRUM          avg400
PROBHD   5 mm QNP 1H/13
PULPROG          zgpg30
TD                65536
SOLVENT           CDCl3
NS                  256
DS                    4
SWH           24038.461 Hz
FIDRES         0.366798 Hz
AQ             1.3631488 sec
RG                 2050
DW                20.800 usec
DE                 6.50 usec
TE                294.8 K
D1           2.00000000 sec
D11          0.03000000 sec
TD0                   1

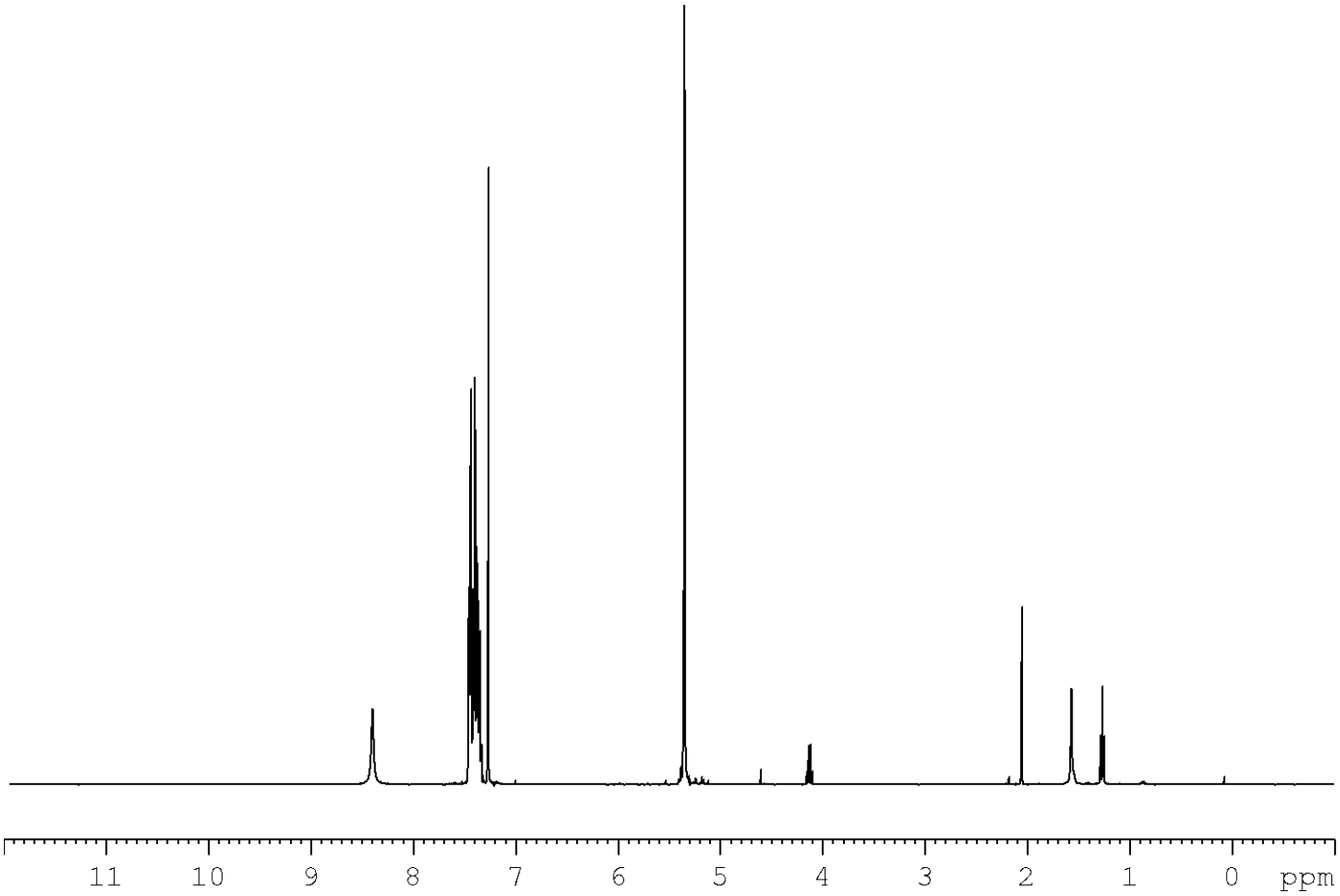
======== CHANNEL f1 ========
SFO1         100.6404326 MHz
NUC1                 13C
P1                10.00 usec
PLW1         40.00000000 W

======== CHANNEL f2 ========
SFO2         400.2016008 MHz
NUC2                 1H
CPDPRG[2         waltz16
PCPD2             90.00 usec
PLW2         11.29800034 W
PLW12         0.20863000 W
PLW13         0.16899000 W

F2 - Processing parameters
SI                32768
SF           100.6303661 MHz
WDW                  no
SSB       0
LB        0 Hz
GB        0
PC                 1.40
```

Benzyl-2,2,2-trichloroacetimidate **223**






```
Instrument DPX400
    Chemist ds
    Group sjc
      IDS181
Qh1acq.au CDCl3 {C:\NMR} sjcgrp 45

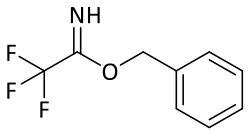
Current Data Parameters
NAME      IDS181
          May08-2012-45
EXPNO                   1
PROCNO                  1

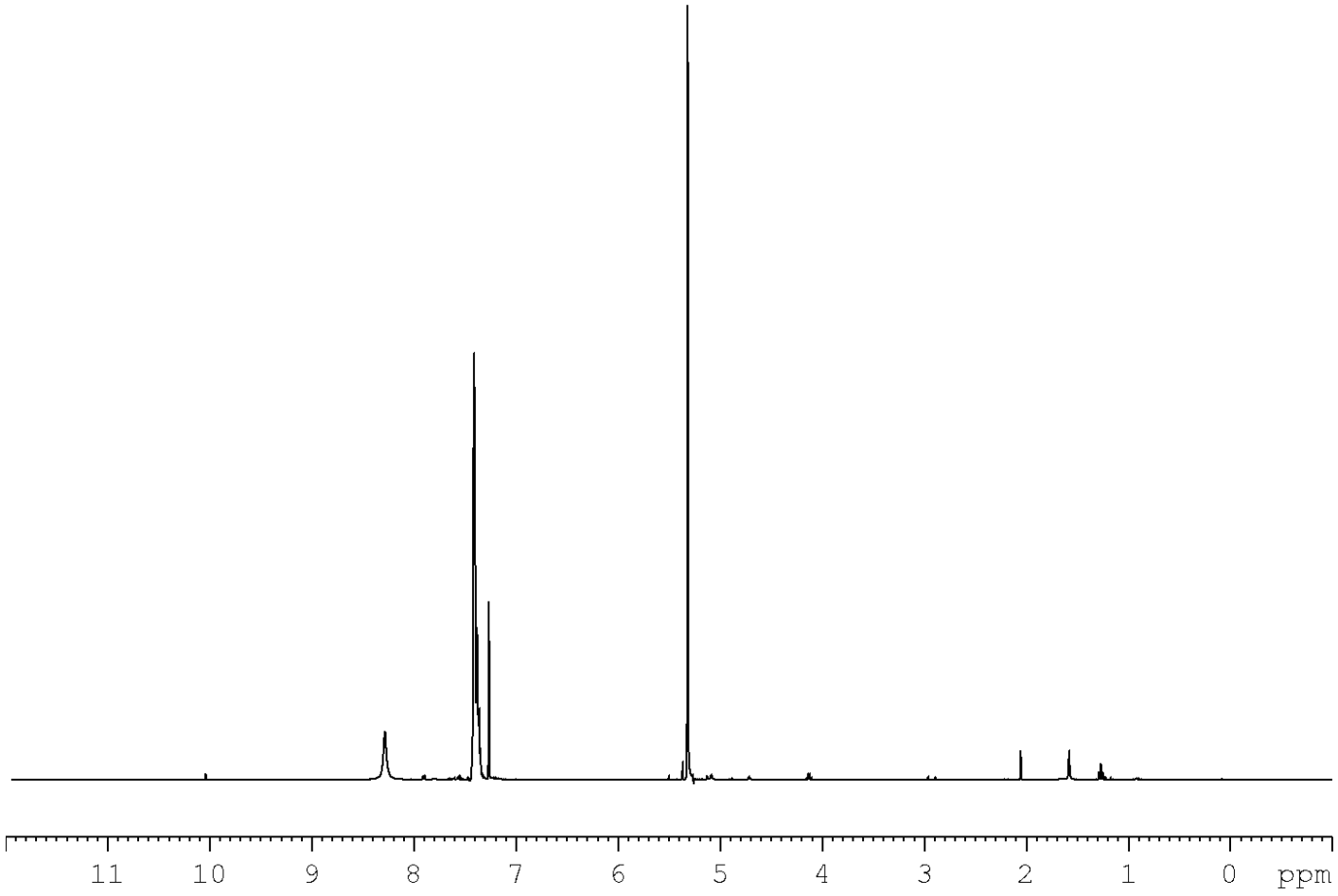
F2 - Acquisition Parameters
Date_           20120508
Time               14.44
INSTRUM          dpx400
PROBHD   5 mm Dual 1H/1
PULPROG             zg60
TD                 32768
SOLVENT            CDCl3
NS                    16
DS                     2
SWH             5592.841 Hz
FIDRES          0.170680 Hz
AQ             2.9294591 sec
RG                   256
DW                89.400 usec
DE                17.00 usec
TE                300.0 K
D1           1.00000000 sec

======== CHANNEL f1 ========
NUC1                  1H
P1                 7.30 usec
PL1       0 dB
SFO1        400.1320007 MHz

F2 - Processing parameters
SI                 32768
SF          400.1300182 MHz
WDW                   EM
SSB       0
LB                 0.30 Hz
GB        0
PC                 0.60
```

Benzyl-2,2,2-trifluoroacetimidate **225**






```
Instrument DPX400
    Chemist ds
     Group sjc
      IDS217
Qh1acq.au CDCl3 {C:\NMR} sjcgrp 54

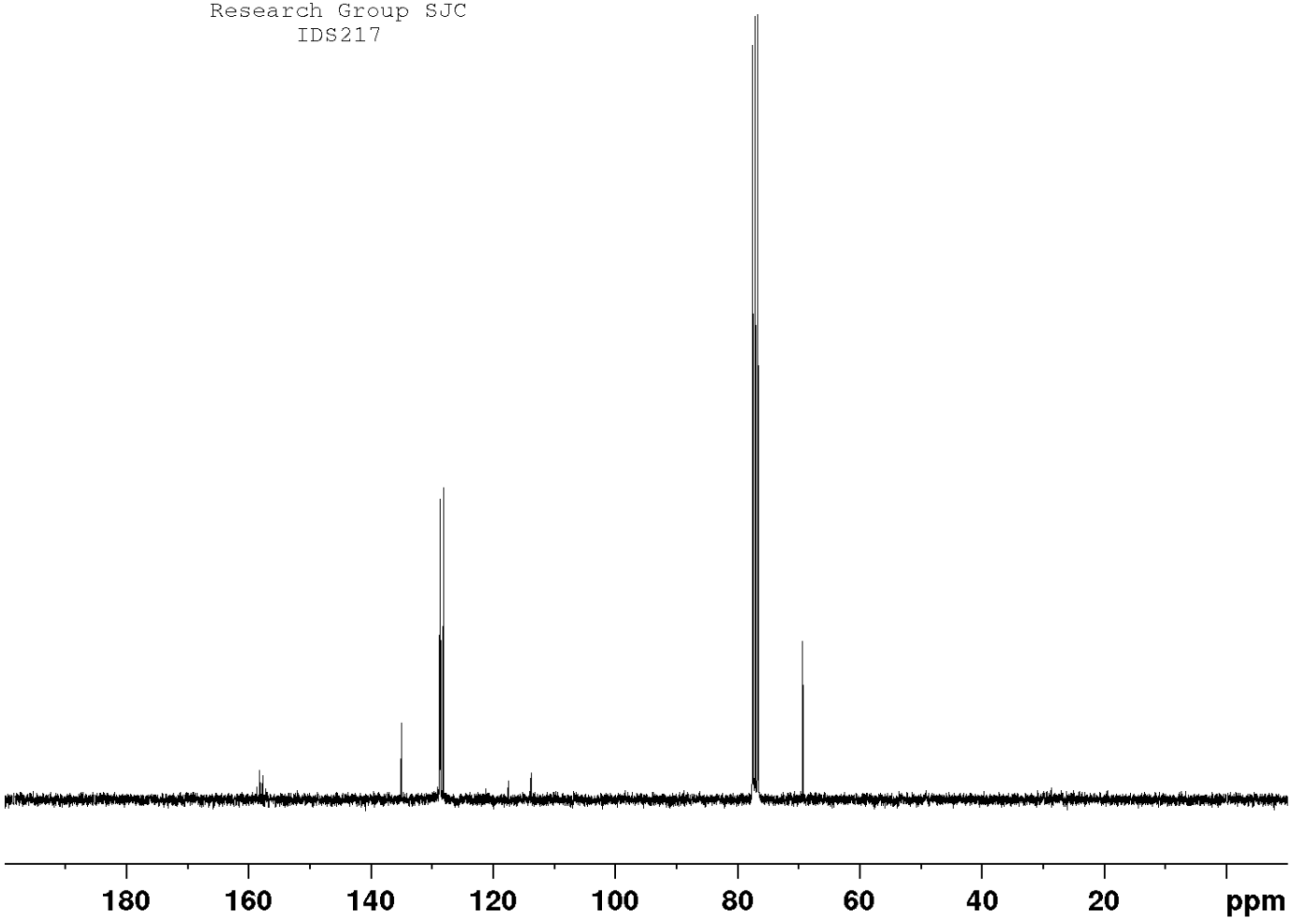

Current Data Parameters
NAME      IDS217
          Aug27-2012-54
EXPNO                 1
PROCNO                1

F2 - Acquisition Parameters
Date_          20120827
Time              11.16
INSTRUM           dpx400
PROBHD   5 mm Dual 1H/1
PULPROG             zg60
TD                 32768
SOLVENT            CDCl3
NS                    16
DS                     2
SWH             5592.841 Hz
FIDRES          0.170680 Hz
AQ              2.9294591 sec
RG                 143.7
DW                89.400 usec
DE                17.00 usec
TE                300.0 K
D1           1.00000000 sec

======== CHANNEL f1 ========
NUC1                  1H
P1                  7.30 usec
PL1       0 dB
SFO1         400.1320007 MHz

F2 - Processing parameters
SI                 32768
SF           400.1300182 MHz
WDW                   EM
SSB       0
LB                 0.30 Hz
GB        0
PC                 0.60
```

Benzyl-2,2,2-trifluoroacetimidate **225**




```
Current Data Parameters
NAME      IDS217 - Aug28-2012-23
EXPNO                  3
PROCNO                 1

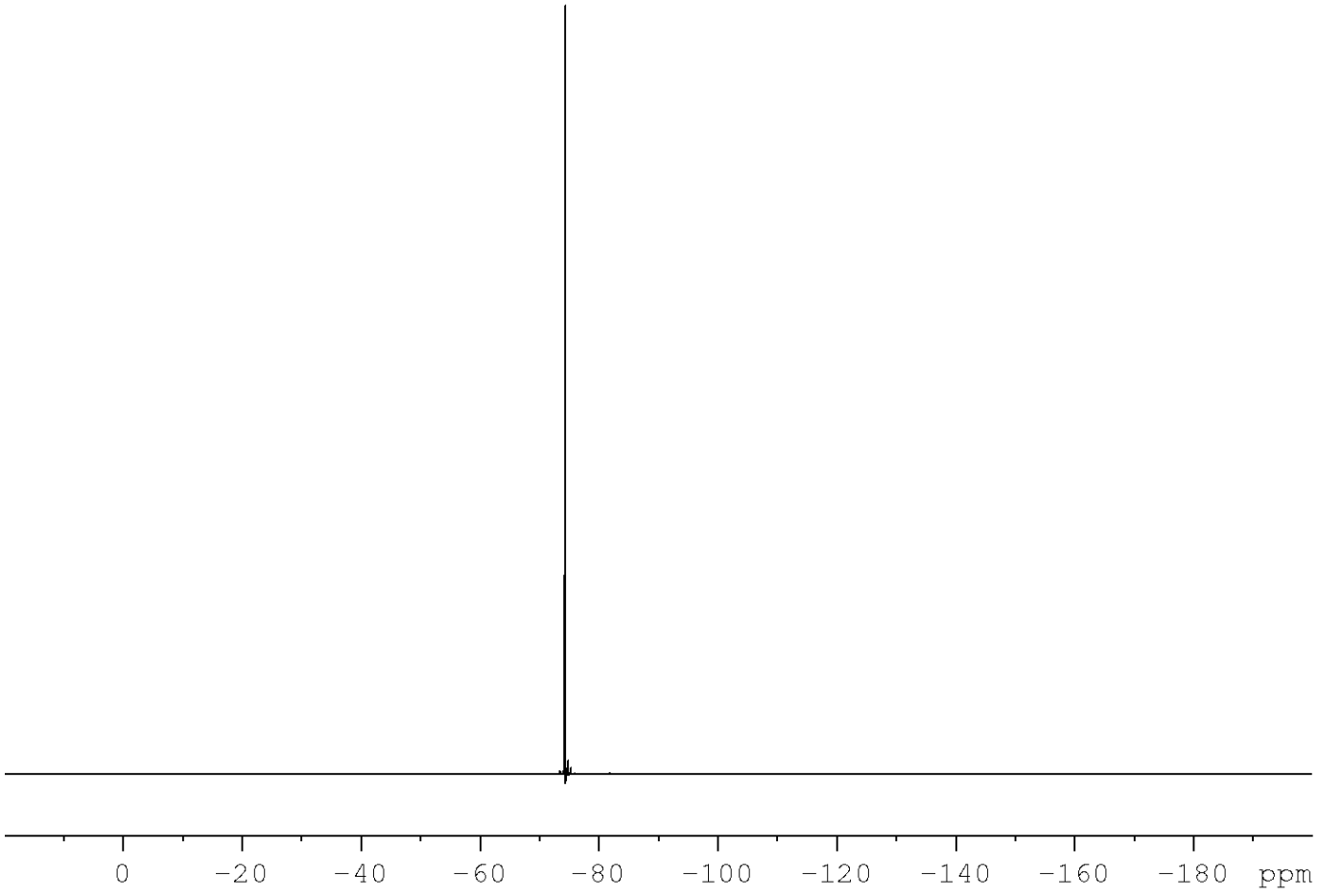
F2 - Acquisition Parameters
Date_           20120828
Time                9.51
INSTRUM           DPX300
PROBHD   5 mm DUL 13C-1
PULPROG           zgpg30
TD                65536
SOLVENT           CDCl3
NS                  400
DS                    4
SWH           17985.611 Hz
FIDRES          0.274439 Hz
AQ            1.8219008 sec
RG               5792.6
DW               27.800 usec
DE                6.00 usec
TE               300.0 K
D1           2.00000000 sec
d11          0.03000000 sec
DELTA        1.89999998 sec
TD0                   1

======== CHANNEL f1 ========
NUC1                 13C
P1                  8.25 usec
PL1                -2.40 dB
SFO1         75.4752953 MHz

======== CHANNEL f2 ========
CPDPRG[2         waltz16
NUC2                 1H
PCPD2              80.00 usec
PL2                -6.00 dB
PL12               11.00 dB
PL13               11.00 dB
SFO2        300.1312005 MHz

F2 - Processing parameters
SI                32768
SF           75.4677490 MHz
WDW                  EM
SSB      0
LB                1.00 Hz
GB       0
PC                1.40
```

Benzyl-2,2,2-trifluoroacetimidate **225**



Instrument AVB500
IDS217

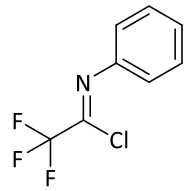
Current Data Parameters
NAME IDS217
EXPNO 1
PROCNO 1

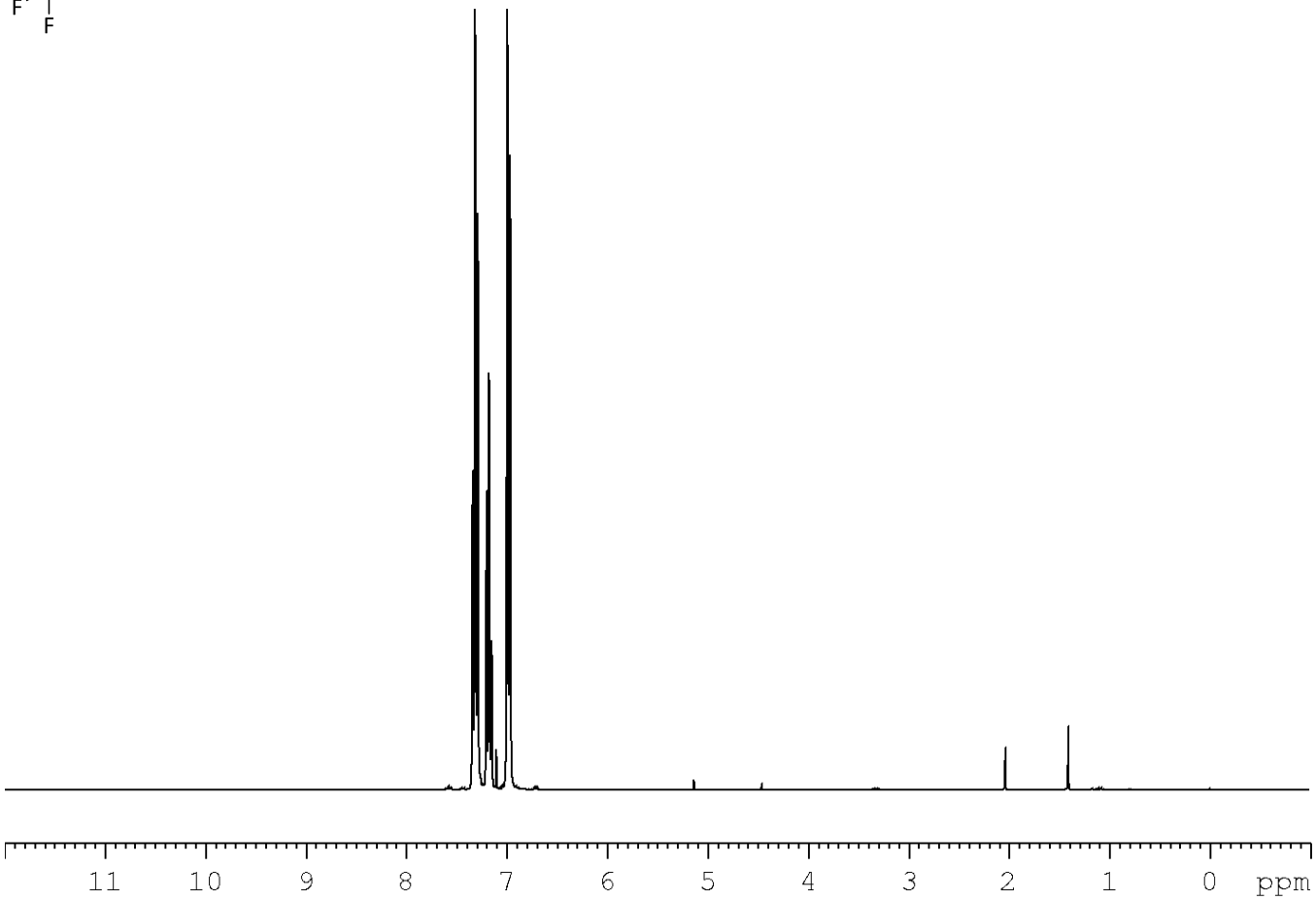
F2 - Acquisition Parameters
Date_ 20120827
Time 17.48
INSTRUM avb500
PROBHD 5 mm PATXI 1H/
PULPROG zgflqn
TD 131072
SOLVENT CDCl3
NS 16
DS 4
SWH 113636.367 Hz
FIDRES 0.866977 Hz
AQ 0.5767168 sec
RG 1150
DW 4.400 usec
DE 6.50 usec
TE 296.1 K
D1 1.00000000 sec
TD0 1

======== CHANNEL f1 ========
NUC1 19F
P1 10.00 usec
PL1 0.90 dB
SFO1 470.4041911 MHz

F2 - Processing parameters
SI 65536
SF 470.4512360 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

2,2,2-Trifluoro-*N*-phenylacetimidoyl chloride **227**






```
Instrument DPX300
Chemist Name ds
Research Group SJC
IDS218

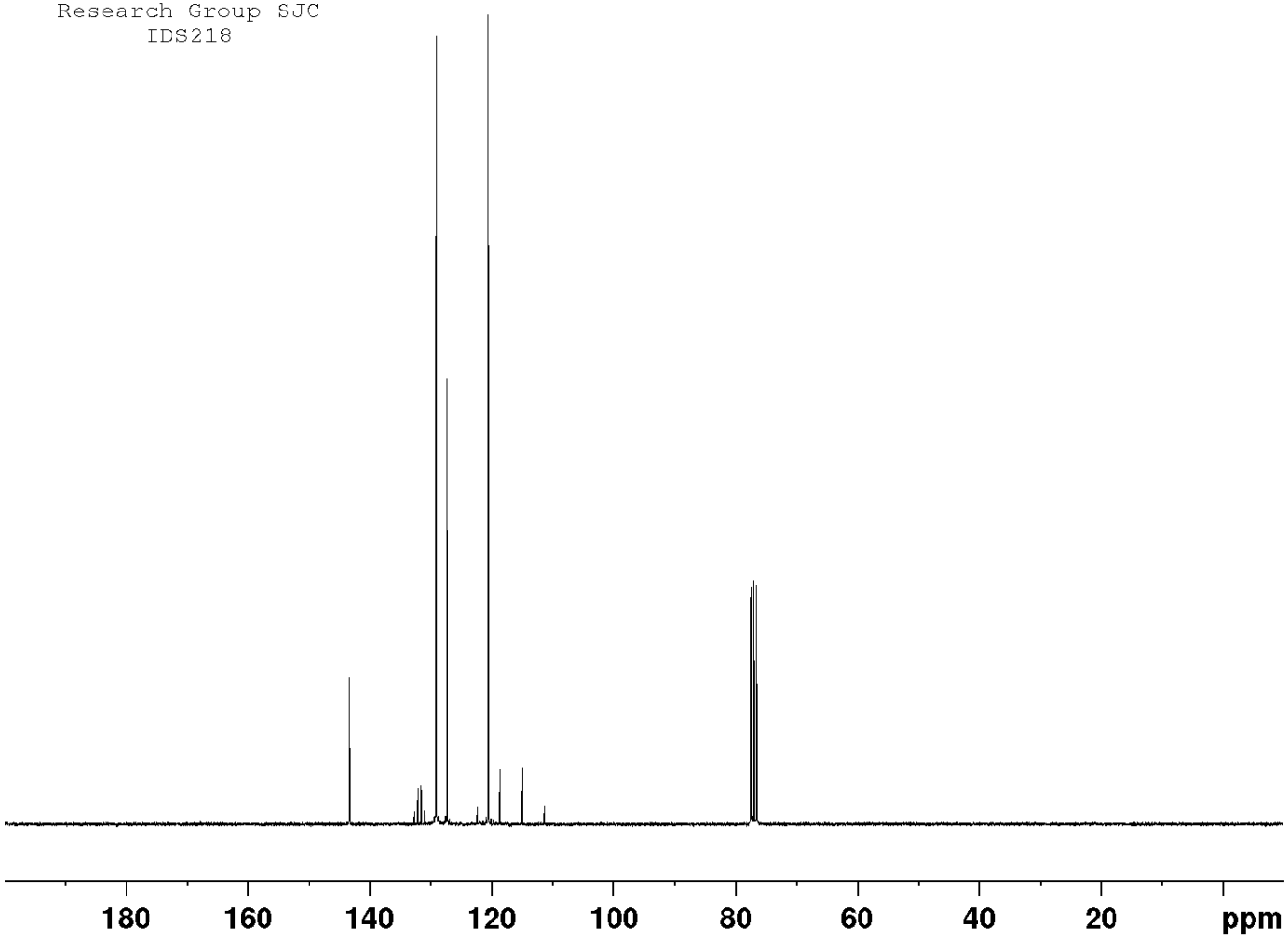
Current Data Parameters
NAME      IDS218 - Sep04-2012-46
EXPNO                  1
PROCNO                 1

F2 - Acquisition Parameters
Date_           20120904
Time              16.10
INSTRUM          DPX300
PROBHD   5 mm DUL 13C-1
PULPROG            zg60
TD                32768
SOLVENT           CDCl3
NS                   16
DS                    2
SWH            4789.272 Hz
FIDRES         0.146157 Hz
AQ             3.4209793 sec
RG                90.5
DW              104.400 usec
DE                6.00 usec
TE               300.0 K
D1          1.00000000 sec
TD0                   1

======== CHANNEL f1 ========
NUC1                  1H
P1                16.50 usec
PL1               -6.00 dB
SFO1        300.1315007 MHz

F2 - Processing parameters
SI                32768
SF          300.1300533 MHz
WDW                  EM
SSB       0
LB                0.30 Hz
GB        0
PC                1.00
```

2,2,2-Trifluoro-*N*-phenylacetimidoyl chloride **227**




```
Current Data Parameters
NAME      IDS218 - Sep04-2012-46
EXPNO                  2
PROCNO                 1

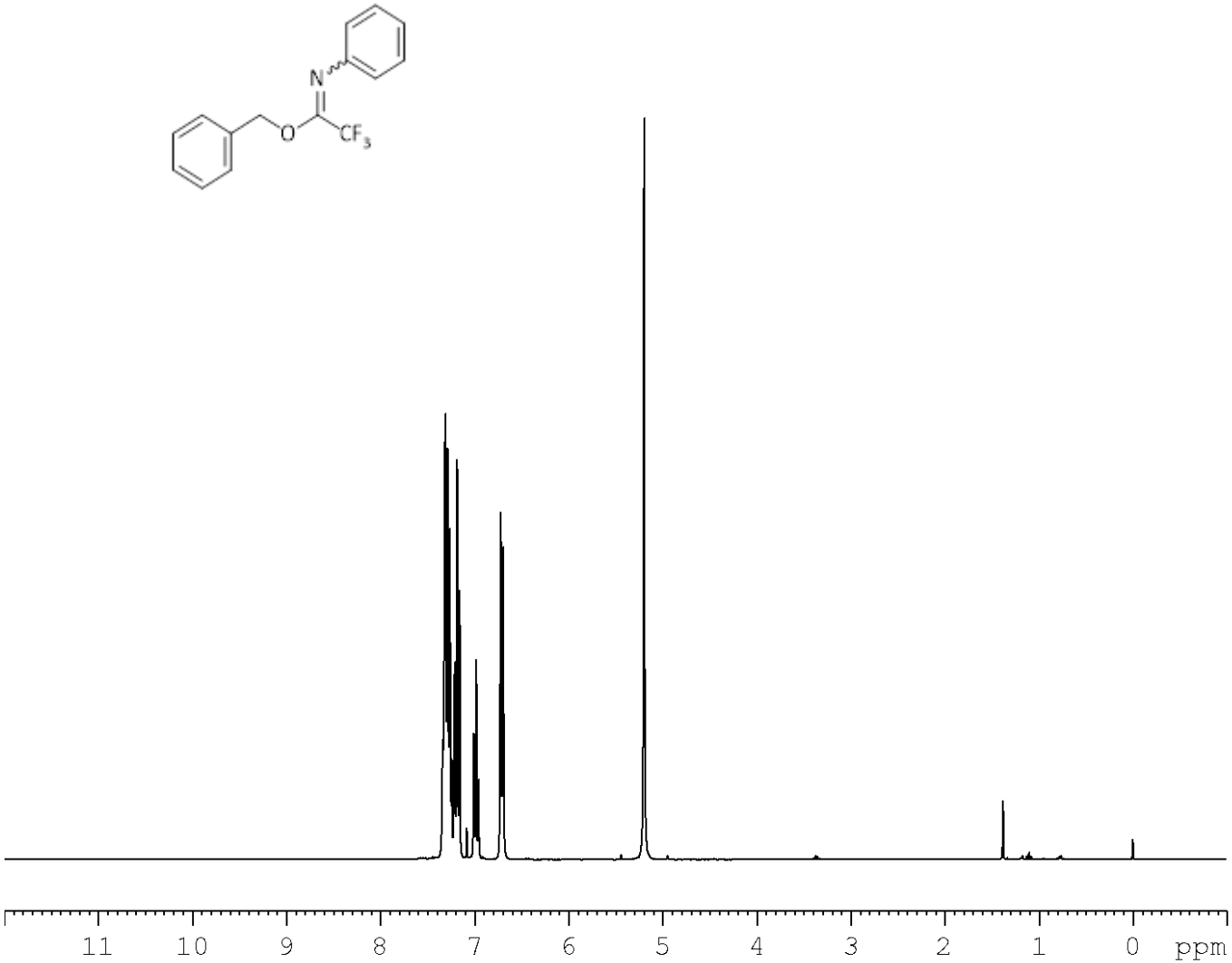
F2 - Acquisition Parameters
Date_           20120904
Time              17.00
INSTRUM           DPX300
PROBHD   5 mm DUL 13C-1
PULPROG           zgpg30
TD                65536
SOLVENT           CDCl3
NS                  750
DS                    4
SWH           17985.611 Hz
FIDRES         0.274439 Hz
AQ            1.8219008 sec
RG               5792.6
DW               27.800 usec
DE                6.00 usec
TE               300.0 K
D1           2.00000000 sec
d11          0.03000000 sec
DELTA        1.89999998 sec
TD0                   1

======== CHANNEL f1 ========
NUC1                 13C
P1                8.25 usec
PL1              -2.40 dB
SFO1         75.4752953 MHz

======== CHANNEL f2 ========
CPDPRG[2         waltz16
NUC2                 1H
PCPD2             80.00 usec
PL2               -6.00 dB
PL12              11.00 dB
PL13              11.00 dB
SFO2        300.1312005 MHz

F2 - Processing parameters
SI                32768
SF           75.4677490 MHz
WDW                  EM
SSB      0
LB                1.00 Hz
GB       0
PC                1.40
```

Benzyl-2,2,2-trifluoro-*N*-phenylacetimidate **228**




```
Instrument DPX300
Chemist Name ds
Research Group SJC
IDS219

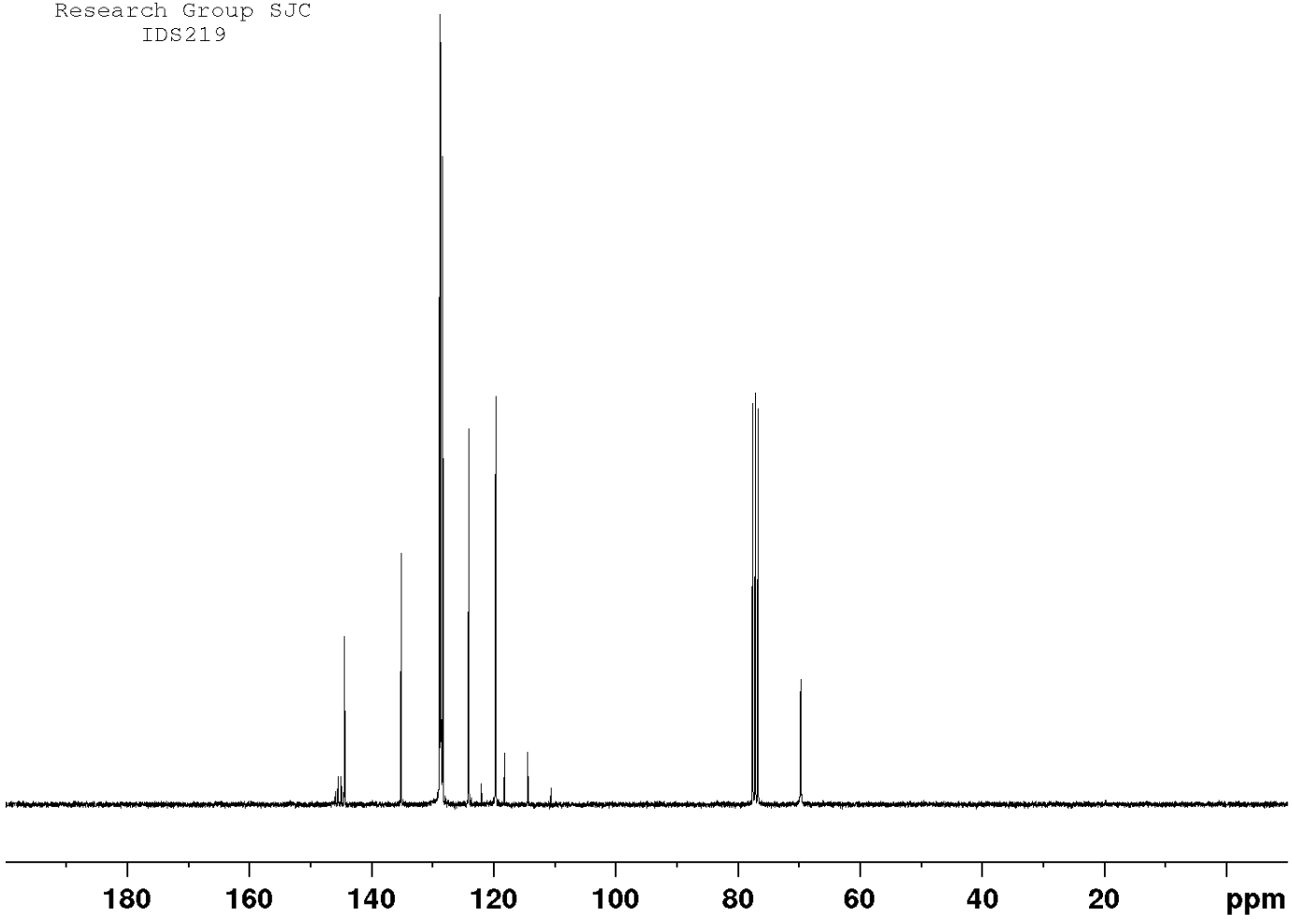

Current Data Parameters
NAME      IDS219 - Sep04-2012-47
EXPNO                  1
PROCNO                 1

F2 - Acquisition Parameters
Date_           20120904
Time              17.06
INSTRUM           DPX300
PROBHD   5 mm DUL 13C-1
PULPROG             zg60
TD                32768
SOLVENT           CDCl3
NS                   16
DS                    2
SWH            4789.272 Hz
FIDRES        0.146157 Hz
AQ            3.4209793 sec
RG                71.8
DW              104.400 usec
DE                6.00 usec
TE               300.0 K
D1           1.00000000 sec
TD0                   1

======== CHANNEL f1 ========
NUC1                  1H
P1                16.50 usec
PL1               -6.00 dB
SFO1        300.1315007 MHz

F2 - Processing parameters
SI                32768
SF          300.1300597 MHz
WDW                  EM
SSB       0
LB                0.30 Hz
GB        0
PC                1.00
```

Benzyl-2,2,2-trifluoro-*N*-phenylacetimidate **228**




```
Current Data Parameters
NAME      IDS219 - Sep04-2012-47
EXPNO                  2
PROCNO                 1

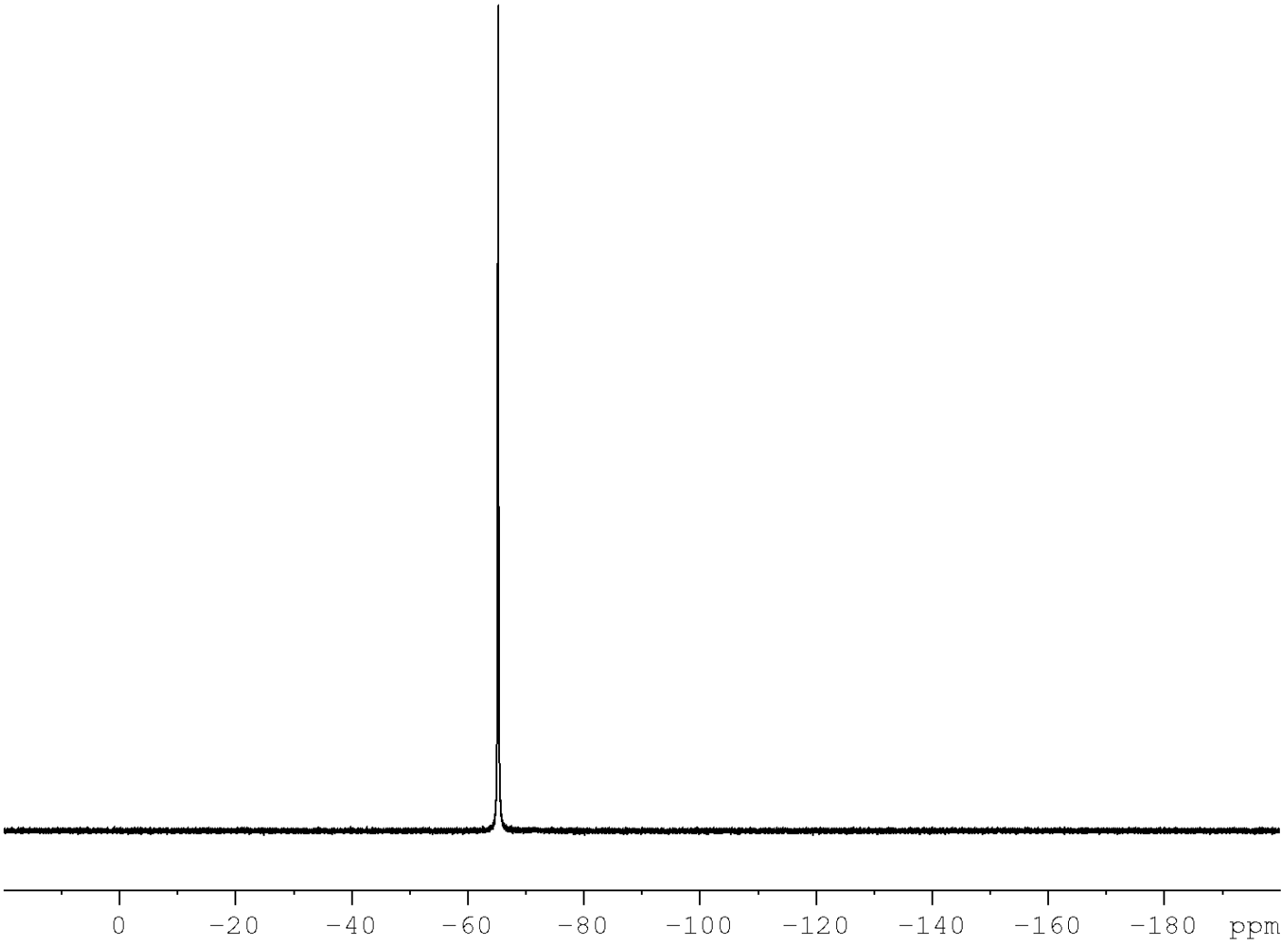
F2 - Acquisition Parameters
Date_           20120904
Time              17.55
INSTRUM           DPX300
PROBHD    5 mm DUL 13C-1
PULPROG           zgpg30
TD                65536
SOLVENT           CDCl3
NS                  750
DS                    4
SWH           17985.611 Hz
FIDRES         0.274439 Hz
AQ            1.8219008 sec
RG               5792.6
DW               27.800 usec
DE                6.00 usec
TE               300.0 K
D1           2.00000000 sec
d11          0.03000000 sec
DELTA        1.89999998 sec
TD0                   1

======== CHANNEL f1 ========
NUC1                 13C
P1                  8.25 usec
PL1                -2.40 dB
SFO1         75.4752953 MHz

======== CHANNEL f2 ========
CPDPRG[2         waltz16
NUC2                 1H
PCPD2              80.00 usec
PL2                -6.00 dB
PL12               11.00 dB
PL13               11.00 dB
SFO2        300.1312005 MHz

F2 - Processing parameters
SI                 32768
SF           75.4677490 MHz
WDW                  EM
SSB       0
LB                  1.00 Hz
GB        0
PC                  1.40
```

Benzyl-2,2,2-trifluoro-*N*-phenylacetimidate **228**



Instrument AVB500
IDS219

```
Current Data Parameters
NAME                 IDS219
EXPNO                     1
PROCNO                    1

F2 - Acquisition Parameters
Date_              20120827
Time                  17.55
INSTRUM               avb500
PROBHD    5 mm PATXI 1H/
PULPROG               zgflqn
TD                    131072
SOLVENT                CDCl3
NS                        16
DS                         4
SWH              113636.367 Hz
FIDRES             0.866977 Hz
AQ                 0.5767168 sec
RG                     1150
DW                    4.400 usec
DE                    6.50 usec
TE                    296.1 K
D1               1.00000000 sec
TD0                       1

======== CHANNEL f1 ========
NUC1                    19F
P1                    10.00 usec
PL1                    0.90 dB
SFO1           470.4041911 MHz

F2 - Processing parameters
SI                    65536
SF             470.4512360 MHz
WDW                      EM
SSB       0
LB                    0.30 Hz
GB        0
PC                    1.00
```