

Rewriting the Bacterial Glycocalyx via Suzuki-Miyaura Cross-Coupling

Journal:	<i>ChemComm</i>
Manuscript ID:	CC-COM-12-2012-038824
Article Type:	Communication
Date Submitted by the Author:	09-Dec-2012
Complete List of Authors:	Spicer, Chris; University of Oxford, Department of Chemistry Davis, Benjamin; University of Oxford, Department of Chemistry

Cite this: DOI: 10.1039/c0xx00000x

www.rsc.org/xxxxxx

ARTICLE TYPE

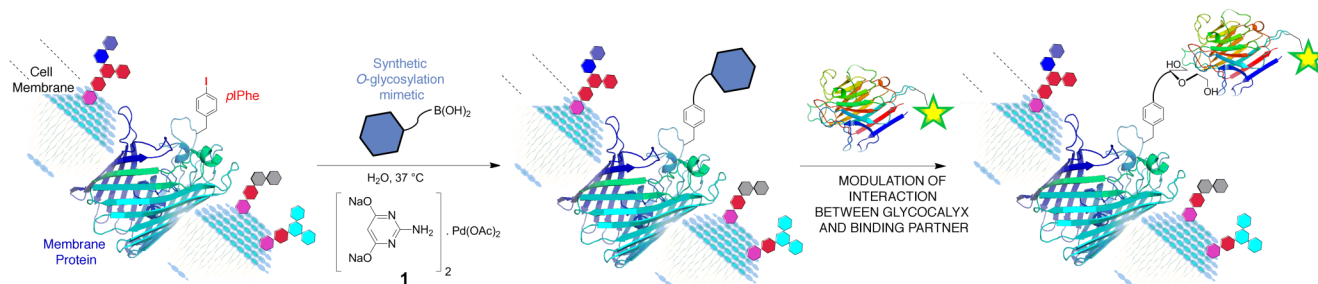
Rewriting the Bacterial Glycocalyx via Suzuki-Miyaura Cross-Coupling

Christopher D. Spicer^a and Benjamin G. Davis^{*a}

Received (in XXX, XXX) Xth XXXXXXXXXX 20XX, Accepted Xth XXXXXXXXXX 20XX

DOI: 10.1039/b000000x

5

Fig. 1 Proposed procedure for cell surface carbohydrate modification of *E. coli*.

Suzuki-Miyaura cross-coupling has been used to couple novel carbohydrate-based boronic acids, site-selectively, to the surface of *E. coli* at an unnatural amino acid. In this way, benign metal-catalyzed cellular switching allowed modulation of interactions with biomolecular partners via prokaryotic *O*-glycosylation mimics.

Until recently protein glycosylation was thought to be a primarily eukaryotic phenomena.¹ However, since the discovery of the first prokaryotic glycoprotein in the archaeobacteria *Halobacterium salinarum*,² and the first description of prokaryotic *N*-glycosylation in *Campylobacter jejuni*,³ several cases have been described.⁴ Indeed, whilst the full extent can only now be estimated, it is clear that prokaryotic protein glycosylation may be common, particularly on bacterial cell surfaces.⁵

Over the past decade much research has been undertaken to uncover the mechanisms and effects of glycosylation, and the selectivities towards interacting partners. Yet new tools are still required to allow further study and analysis.⁶ Of particular appeal are new techniques for installing natural analogues or mimics of such glycosyl modifications in a site-specific manner, into relevant biomolecules and complex biological contexts.⁷⁻⁹

We have recently developed a water soluble, phosphine-free palladium catalyst (Pd(OAc)₂(ADHP)₂, **1**, see Fig. 1)¹⁰ for protein modification via Suzuki-Miyaura cross-couplings¹¹ at genetically incorporated unnatural amino acids.¹² This system and related variants have subsequently shown utility in a variety of protein systems.^{13, 14} One such amino acid, the aryl halide *p*-iodophenylalanine (*p*IPhe), can be incorporated into both bacterial¹⁵ and eukaryotic¹⁶ systems site-selectively via amber-stop codon suppression.¹⁷ We recently reported the use of this method to incorporate a *p*IPhe 'tag' onto cell surfaces and

demonstrated the applicability of our catalyst for the cell surface Suzuki-Miyaura fluorescent labelling of 'tagged' cells.¹⁸ In the course of this work, we demonstrated biological compatibility, as well as low associated toxicity. In addition, a critical Pd threshold effect was observed, leading to a 'switch-like' dose response.

Herein, we describe the synthesis of novel carbohydrate boronic acids and their cross-coupling to the surface of *E. coli*. This demonstrates the covalent conjugation of biomolecules to cell surfaces and the potential applicability of this system in the elucidation of prokaryotic glycobiology, through the selective modulation of cell surface binding partner interactions.

We envisaged the synthesis of both aryl **2** (as mimics of Tyr *O*-glycosylation)¹⁹ and vinyl **10** (as mimics of Thr/Ser *O*-glycosylation)²⁰ boronic acids. D-mannose (Man) and D-galactose (Gal) were chosen as suitable glycans as the most common hexose motifs found in natural glycoproteins,²¹ whilst D-glucose (Glc) was also chosen due to its high natural abundance and widespread occurrence in biopolymers.²²

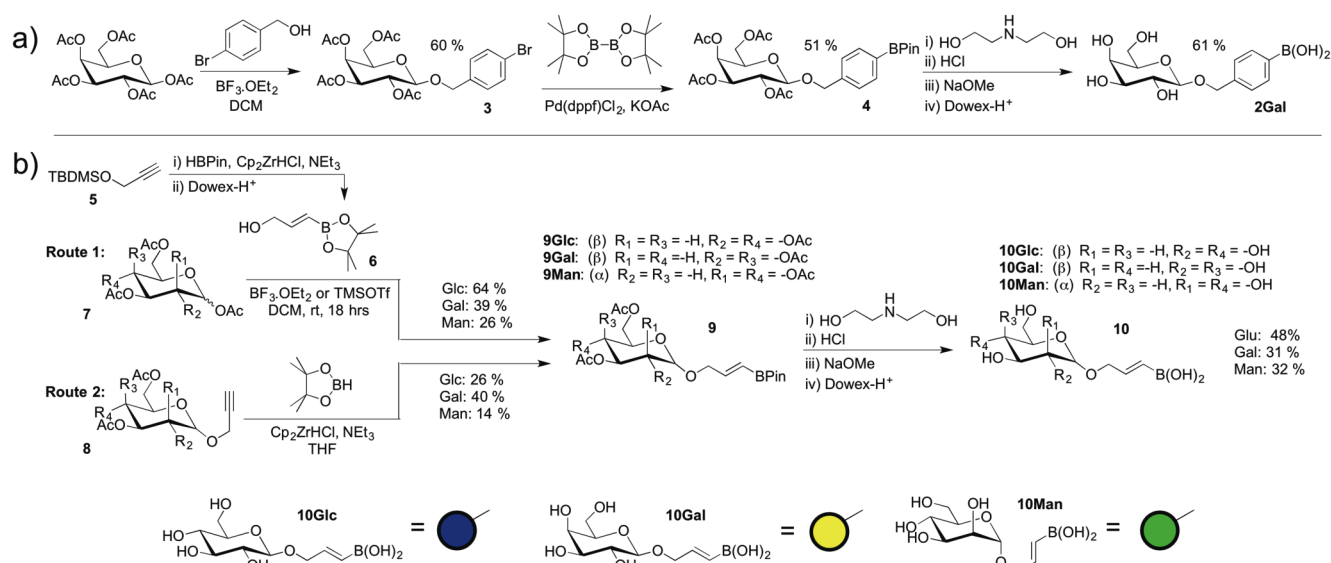
To synthesise the D-galactoside aryl boronic acid, **2Gal**, the corresponding β-benzyl bromide, **3**, was first synthesized via BF₃-promoted glycosylation (Scheme 1a). Subsequent, Miyaura borylation²³ with bis(pinacolato)diboron gave the corresponding boronic pinacol ester, **4**. Removal of the ester was achieved through treatment with diethanolamine followed by acidic hydrolysis. Global Zemplén deprotection with NaOMe yielded free aryl sugar boronic acid **2Gal**.

The vinyl boronic acids were synthesised via two convergent methods (Scheme 1b). In our initial approach (Route 1), hydroboration of protected propargyl alcohol **5** and subsequent acidic deprotection gave the corresponding vinyl boronic ester alcohol, **6**, in good yields. Glycosylation of this acceptor, **6**, using the appropriate per-acetylated sugars as glycosyl donors was

Cite this: DOI: 10.1039/c0xx00000x

www.rsc.org/xxxxxx

ARTICLE TYPE

Scheme 1 Synthesis of sugar functionalised boronic acids **2Gal**, **10Glc**, **10Gal** and **10Man**.

subsequently undertaken in the presence of a Lewis acidic promoter.¹⁰ Whilst the use of $\text{BF}_3 \cdot \text{etherate}$ proved sufficient for glycosylation using D-glucose and D-galactose donors in moderate yields (**9Glc** and **9Gal**), the use of TMSOTf was required to access the corresponding D-mannoside, **9Man**. Whilst complete consumption of the glycosyl donors was observed in all cases, overall reduced yield was attributed to hydrodeboration of the product under the acidic reaction conditions, giving corresponding deborylated vinyl sugars as unwanted side products.²⁴ As a result, we investigated alternative access (Route 2) to **9** via hydroboration of the corresponding propargyl derivatives, **8**, themselves synthesised via previously reported boron trifluoride promoted glycosylations.²⁵ A number of hydroboration conditions were investigated with little success: use of an excess of catecholborane²⁶ under solvent-less conditions failed to yield desired product, nor use of dibromoborane,²⁷ dicyclohexylborane²⁸ or diisopinocampheylborane²⁹. However, the use of pinacolborane in the presence of catalytic amounts of Schwartz's Reagent (Cp_2ZrHCl) and triethylamine³⁰ provided the protected boronic esters, **9**, albeit in modest yields. Deprotection of the boronic esters was again achieved with diethanolamine, followed by deprotection with sodium methoxide to yield the desired sugars, **10**, as crystalline solids (Scheme 1).

With these boronic acids (**2Gal**, **10Glc**, **10Gal** and **10Man**) in hand, Suzuki-Miyaura cross-couplings were undertaken on *pIPhe* 'tagged' *E. coli*.¹⁸ Briefly, *E. coli* cell line JW2203-1 (an OmpC knockout strain from the Keio collection³¹) was co-transformed with plasmids pEVOL(IPhe)¹⁷ and pOmpC-(Y232-), to allow amber-suppressed incorporation of *pIPhe* into a mutant OmpC membrane protein at position Y232 (see SI). After overnight induction of protein expression with 1 mM IPTG, 0.02 % L-arabinose and 2 mM *pIPhe*, cells were collected by centrifugation

and washed extensively with pH 8.0 phosphate buffer. The cells were then labelled at an $\text{OD}_{600} = 0.2$ using a palladium concentration of 0.5 mM and a boronic acid concentration of 3.5 mM. After labelling at 37 °C for 1 hr, cells were collected by centrifugation and washed extensively to ensure the complete removal of unreacted boronic acid. Disappointingly, the Gal-tyrosinyl mimic reagent **2Gal** proved to be poorly soluble in water under conditions amenable to biological systems and so could not be used for cellular labelling. The glycosyl-serinyl mimic reagents **10Glc**, **10Gal** and **10Man**, however, were all highly soluble under relevant conditions and proved to be highly effective coupling partners.

Having thus synthetically altered the composition of the cell surface glycocalyx through C-C coupling, we investigated the modulation of its interactions with glycan-selective binding protein partners. *Lens culinaris* agglutinin (LCA)³² and *Griffonia simplicifolia* lectin I (GSL)³³ were chosen as α -Man- and β -Gal-selective lectins, respectively, whilst Concanavalin A (ConA) was used as a positive control due to its known affinity for the existing glycan lipo-polysaccharide (LPS) of *E. coli* outer membranes.³⁴ Gratifyingly, when cell surface glycans were 'switched' to alternatives via Suzuki-Miyaura cell-surface modification, a high specificity of recognition was observed. Modulation of the cell surface with Gal (from **10Gal**) resulted in strong interaction with GSL (Fig. 2 middle), whilst synthetic 'switching' to Man (from **10Man**) resulted in a high interaction specificity for LCA (Fig. 2 top). Glc modification of the cell surfaces resulted in no interaction with either of these proteins. Experiments run in the absence of the key coupling partners (palladium, boronic acid or in cells grown in the absence of *pIPhe*) also failed to result in any binding, strongly supporting a Suzuki-Miyaura-induced switching mechanism (see SI). Notably,

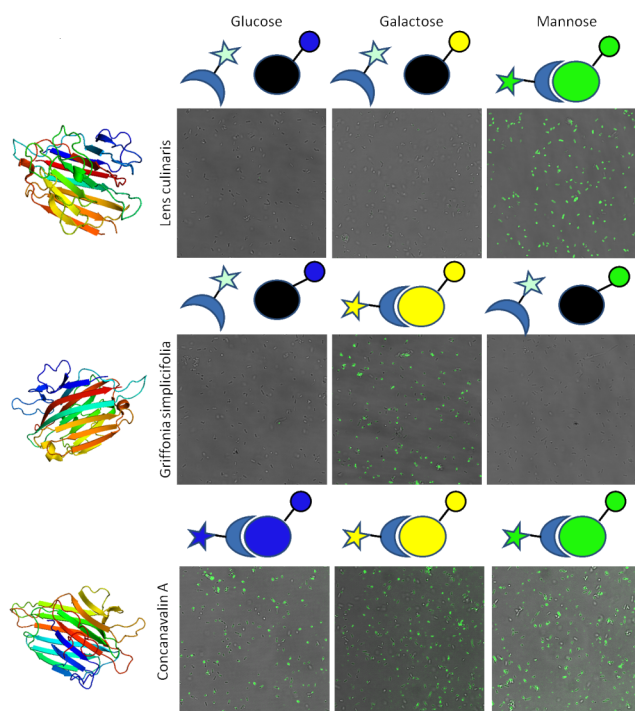


Fig. 2 Interaction of fluorescein-lectin conjugates with *E. coli* labelled with monosaccharide boronic acids via Suzuki-Miyaura coupling.

the existing, natural *E. coli* glycocalyx was not disrupted during the course of the reaction, allowing the inherent interaction with ConA to be maintained in all cases (Fig. 2 bottom). In addition to confirming Suzuki-Miyaura glycoconjugations at bacterial cell surfaces, these results also demonstrated the accessibility and potential applicability of these biologically relevant ligands for future applications.

In conclusion, we have synthesised a series of novel carbohydrate based boronic acids, and applied these as cross-coupling partners for the Suzuki-Miyaura labelling of pIPhe 'tagged' cells. The cognate interactions of these biologically relevant ligands could be visualised on the cell surface via the selective binding of fluorescein-lectin conjugates, thus demonstrating the functionality of such ligands in a complex and relevant biological context. The use of such couplings further demonstrates the power of Pd-mediated control of Biology,³⁵ here in cellular interactions. We are currently working towards utilising such methods in eukaryotic systems, particularly in the potential blood typing ('blood groups') of mammalian samples.

We would like to thank UCB and BBSRC for funding and Drs R. Alexander and J. Porter for helpful discussions. BGD is a Royal Society Wolfson Research Merit Award recipient.

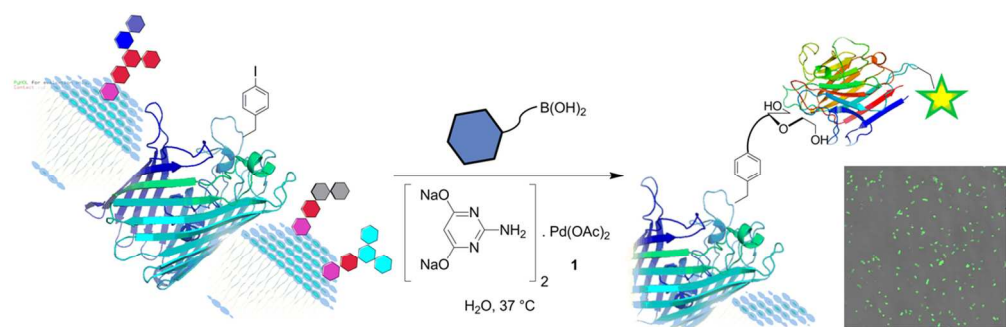
Notes and references

^a Chemistry Research Laboratory, University of Oxford, 12 Mansfield Road, Oxford, OX1 3TA. Fax: +44 (0) 1865 275674; Tel: +44 (0) 1865 275652; E-mail: Ben.Davis@chem.ox.ac.uk

[†] Electronic Supplementary Information (ESI) available: [Full experimental details, compound characterisation and fluorescence microscopy images]. See DOI: 10.1039/b000000x/

- R. K. Upreti, M. Kumar and V. Shankar, *Proteomics*, 2003, **3**, 363-379.
- M. F. Mescher, J. L. Strominger and S. W. Watson, *J. Bacteriol.*, 1974, **120**, 945-954.

- M. Wacker, D. Linton, P. G. Hitchen, M. Nita-Lazar, S. M. Haslam, S. J. North, M. Panico, H. R. Morris, A. Dell, B. W. Wren and M. Aebi, *Science*, 2002, **298**, 1790-1793.
- N. M. Young, J.-R. Brisson, J. Kelly, D. C. Watson, L. Tessier, P. H. Lanthier, H. C. Jarrell, N. Cadotte, F. St. Michael, E. Aberg and C. M. Szymanski, *J. Biol. Chem.*, 2002, **277**, 42530-42539.
- C. M. Szymanski and B. W. Wren, *Nat. Rev. Microbiol.*, 2005, **3**, 225-237.
- E. Weerapana and B. Imperiali, *Glycobiology*, 2006, **16**, 91R-101R.
- B. G. Davis, *Chem. Rev.*, 2002, **102**, 579-602.
- D. P. Gamblin, E. M. Scanlan and B. G. Davis, *Chem. Rev.*, 2009, **109**, 131-163.
- J. M. Chalker, G. J. L. Bernardes and B. G. Davis, *Acc. Chem. Res.*, 2011, **44**, 730-741.
- J. M. Chalker, C. S. C. Wood and B. G. Davis, *J. Am. Chem. Soc.*, 2009, **131**, 16346-16347.
- N. Miyaura and A. Suzuki, *Chem. Rev.*, 1995, **95**, 2457-2483.
- C. D. Spicer and B. G. Davis, *Chem. Commun.*, 2011, **47**, 1698-1700.
- N. Li, R. K. V. Lim, S. Edwardraja and Q. Lin, *J. Am. Chem. Soc.*, 2011, **133**, 15316-15319.
- Y.-S. Wang, W. K. Russell, Z. Wang, W. Wan, L. E. Dodd, P.-J. Pai, D. H. Russell and W. R. Liu, *Mol. Biosyst.*, 2011, **7**, 714-717.
- J. Xie, L. Wang, N. Wu, A. Brock, G. Spraggon and P. G. Schultz, *Nat. Biotechnol.*, 2004, **22**, 1297-1301.
- J. W. Chin, T. A. Cropp, J. C. Anderson, M. Mukherji, Z. Zhang and P. G. Schultz, *Science*, 2003, **301**, 964-967.
- T. S. Young, I. Ahmad, J. A. Yin and P. G. Schultz, *J. Mol. Biol.*, 2010, **395**, 361-374.
- C. D. Spicer, T. Triemer and B. G. Davis, *J. Am. Chem. Soc.*, 2012, **134**, 800-803.
- I. R. Rodriguez and W. J. Whelan, *Biochem. Biophys. Res. Commun.*, 1985, **132**, 829-836.
- B. C. O'Connell and L. A. Tabak, *J. Dent. Res.*, 1993, **72**, 1554-1558.
- A. Varki, R. D. Cummings, J. D. Esko, H. H. Freeze, P. Stanley, C. R. Bertozzi, G. W. Hart and M. E. Etzler, *Essentials of Glycobiology*, 2nd edn., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, 2009.
- W. S. Hu and A.-P. Zeng, *Genomics and Systems Biology of Mammalian Cell Culture*, Springer, 2012.
- T. Ishiyama, M. Murata and N. Miyaura, *J. Org. Chem.*, 1995, **60**, 7508-7510.
- H. C. Brown, D. Basavaiah, S. U. Kulkarni, H. D. Lee, E. Negishi and J. J. Katz, *J. Org. Chem.*, 1986, **51**, 5270-5276.
- H. B. Mereyala and S. R. Gurralla, *Carbohydr. Res.*, 1998, **307**, 351-354.
- H. C. Brown and S. K. Gupta, *J. Am. Chem. Soc.*, 1972, **94**, 4370-4371.
- H. C. Brown and J. B. Campbell, *J. Org. Chem.*, 1980, **45**, 389-395.
- H. C. Brown, A. K. Mandal and S. U. Kulkarni, *J. Org. Chem.*, 1977, **42**, 1392-1398.
- P. Martinez-Fresneda and M. Vaultier, *Tetrahedron Lett.*, 1989, **30**, 2929-2932.
- Y. D. Wang, G. Kimball, A. S. Prashad and Y. Wang, *Tetrahedron Lett.*, 2005, **46**, 8777-8780.
- T. Baba, T. Ara, M. Hasegawa, Y. Takai, Y. Okumura, M. Baba, K. A. Datsenko, M. Tomita, B. L. Wanner and H. Mori, *Mol. Syst. Biol.*, 2006, **2**.
- A. Foriers, E. Lebrun, R. Van Rapenbusch, R. de Neve and A. D. Strosberg, *J. Biol. Chem.*, 1981, **256**, 5550-5560.
- L. A. Murphy and I. J. Goldstein, *J. Biol. Chem.*, 1977, **252**, 4739-4742.
- T. G. Pistole, *Annu. Rev. Microbiol.*, 1981 **35** 85-112.
- J. Li and P. R. Chen, *ChemBioChem*, 2012, **13**, 1728-1731.



110x36mm (300 x 300 DPI)

S1**Supplementary Information****Re-writing the Bacterial Glycocalyx via Suzuki-Miyaura Cross-Coupling**

Christopher D. Spicer and Benjamin G. Davis

Chemistry Research Laboratory, University of Oxford, 12 Mansfield Road,
Oxford, UK OX1 3TA. E-mail: ben.davis@chem.ox.ac.uk;

Fax: +44 (0)1865 275674; Tel: +44 (0)1865 275652

Table of contents

S1	General Considerations
S4	Chemical Syntheses
S20	Induction of Protein Expression
S21	General Procedure for Cell Labelling
S22	Labelling in the Absence of Palladium
S23	Labelling in the Absence of Boronic Acid
S24	Labelling in the Absence of Aryl Halide
S25	Cells in the Absence of Fluorescein-Labelled Lectins
S26	References
S27	NMRs of Novel Compounds

General Considerations

Proton nuclear magnetic resonance (^1H NMR) spectra were recorded on a Bruker AV400 (400 MHz) spectrometer. Carbon nuclear magnetic resonance (^{13}C NMR) spectra were recorded on a Bruker AV400 (100 MHz) or a Bruker AVII500 (125 MHz) spectrometer as indicated. NMR shifts were assigned using COSY, HSQC and HMBC spectra. All chemical shifts are quoted on the δ scale in ppm using residual solvent as the internal standard (^1H NMR: CDCl_3 = 7.26; D_2O = 4.79; DMSO-d_6 = 2.50 and ^{13}C NMR: CDCl_3 = 77.16, DMSO-d_6

S2

= 39.52). Coupling constants (J) are reported in Hz with the following splitting abbreviations: s = singlet, d = doublet, t = triplet, q = quartet, quin = quintet, app = apparent, m = multiplet. Melting points (m.p.) were recorded on a Leica Galen III hot stage microscope equipped with a Testo 720 thermocouple probe and are uncorrected. Infrared (IR) spectra were recorded on a Bruker Tensor 27 Fourier Transform spectrophotometer with a diamond ATR module. Absorption maxima (ν_{\max}) are reported in wavenumbers (cm^{-1}). Low resolution mass spectra (LRMS) were recorded on a Waters Micromass LCT Premier TOF spectrometer using electrospray ionization (ESI) and high resolution mass spectra (HRMS) were recorded on a Bruker MicroTOF ESI mass spectrometer. Nominal and exact m/z values are reported in Daltons. Optical rotations were measured on a Perkin-Elmer 241 polarimeter with a path length of 1.0 dm and are reported with implied units of $10^{-1} \text{ deg cm}^2 \text{ g}^{-1}$. Concentrations (c) are given in g/100 mL. Thin layer chromatography (TLC) was carried out using aluminium backed sheets coated with 60F254 silica gel (Merck). Visualization of the silica plates was achieved using a UV lamp ($\lambda_{\max} = 254 \text{ nm}$ or 318 nm), and/or ammonium molybdate (5 % in 2M H_2SO_4), and/or potassium permanganate (5 % KMnO_4 in 1M NaOH with 5 % potassium carbonate). Flash column chromatography was carried out using Geduran Si 60 (40-63 μm) silica (Merck). Mobile phases are reported as % volume of more polar solvent in less polar solvent. Anhydrous solvents were purchased from Acros and used as supplied, with the exception of DCM and THF which were dried through an activated alumina column prior to use. All other solvents were used as supplied (Analytical or HPLC grade), without prior purification. Deionized water was used for chemical reactions and Milli-Q purified water for protein and cellular manipulations. Reagents were purchased from Aldrich and used as supplied, unless otherwise indicated. Bis(pinacolato) diborane was purchased from Frontier Scientific. 'Petrol' refers to the fraction of light petroleum ether boiling in the range 40-60 $^{\circ}\text{C}$. All reactions using anhydrous conditions were performed using flame-dried apparatus under

S3

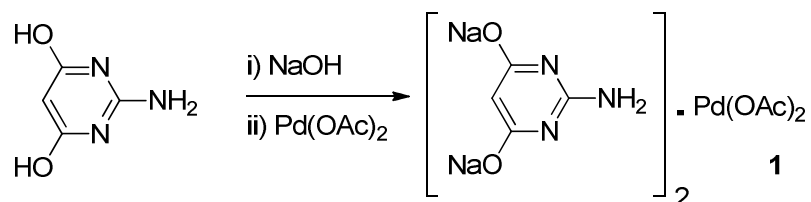
an atmosphere of nitrogen. Brine refers to a saturated solution of sodium chloride. Anhydrous magnesium sulfate (MgSO_4) or sodium sulphate (Na_2SO_4) were used as the drying agent after reaction workup as specified.

Plasmid pEVOL (*pIPhe*) was generously donated by the group of Prof. P. G. Schultz at The Scripps Institute. *E. coli* cell line JW2203-1, deficient for host OmpC production, was purchased from the Yale *E. coli* Genetic Stock Centre. Plasmid pOmpC was synthesised by Genscript. SDS-PAGE gels were run using pre-cast gels purchased from Invitrogen (NuPAGE 4-12 % Bis-Tris gel), and stained using InstantBlue™ (Expedeon). Chloramphenicol and kanamycin were both used at a concentration of 50 mg L^{-1} and ampicillin was used at a concentration of 100 mg L^{-1} . All biological manipulations were undertaken under sterile conditions in a HERAsafe KSP12 laminar flow hood (Thermo Scientific). Fluorescein labelled Lens Culinaris Agglutinin, Griffonia simplicifolia Lectin and Concanavalin A were purchased from Vector Labs

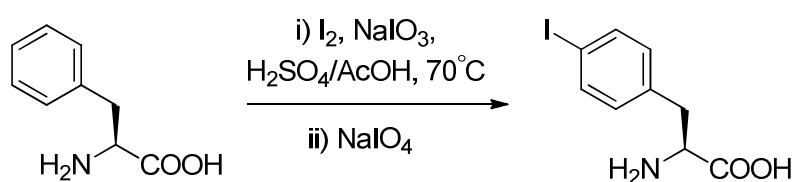
Microscopy experiments were performed on a Leica Microsystems SP5 Inverted Confocal Microscope. All experiments were performed with the pinhole set at 1 Airy diameter. All images were captured at 512×512 pixels at 400 Hz or 1400 Hz capture rates. All experiments were performed with the 62 x oil objective with further magnification being achieved with the optical zoom. Fluorescein was excited at 488 nm (Ar laser) and emission collected between 500-640 nm. Images were processed using the Leica LAS software. Gains were kept constant throughout measurements, at a point where saturation was not reached in the most fluorescent sample.

S4

Chemical Syntheses



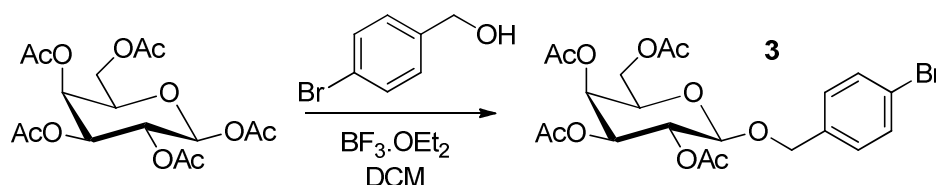
2-amino-4,6-dihydroxypyrimidine (13 mg, 0.1 mmol) was dissolved in NaOH (0.1 M, 2 mL) at 65 °C. Palladium acetate (11 mg, 0.05 mmol) was then added and the solution stirred at 65 °C for 30 min. The orange solution was then allowed to cool to rt and diluted to 5 mL with distilled water to give a stock 0.01 M catalyst solution, **1**.²



To a solution of L-phenylalanine (41.3 g, 250 mmol) in acetic acid (250 mL) and concentrated sulphuric acid (31 mL) was added Iodine (25.2 g, 100 mmol) and sodium iodate (9.9 g, 50 mmol). The mixture was heated to 70 °C for 18 hrs. Two portions of sodium periodate (2 × 1.05 g) were then added and stirring continued at 70 °C for ~30 min until the mixture turned orange. The acetic acid was then removed *in vacuo*, and the crude mixture was diluted with water (400 mL) then washed with ether (2 x 200 mL) and DCM (2 x 300 mL). To the aqueous layer was added 2 M NaOH until a white precipitate was formed. The solid was collected by filtration and re-crystallized from boiling water:ethanol (160 mL:100 mL). The resulting crystals were collected and dried *in vacuo*, to yield L-p-iodophenylalanine as white crystals. A yield of 33.01 g, 113 mmol (57 %) was obtained. Spectroscopic data was consistent with that previously reported.¹ ¹H NMR (400 MHz, NaOD/D₂O): δ = 7.60 (2H, d, *J* = 8.7 Hz, *ortho*-H), 6.93 (2H, d, *J* = 8.7 Hz, *meta*-H), 3.37 (1H, t, *J* = 6.1 Hz, H_α), 2.82 (1H,

S5

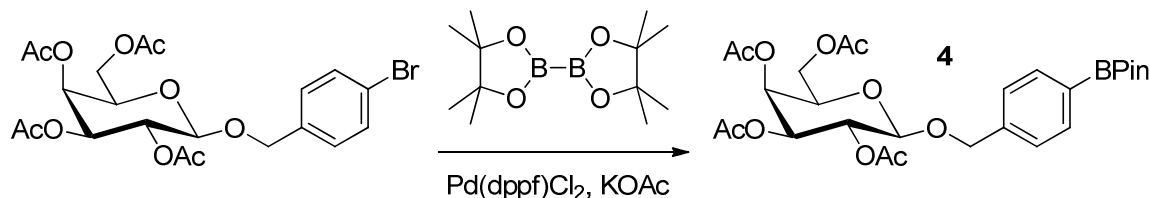
dd, $J = 14.5, 6.1$ Hz, $-\underline{\text{CH}}_2\text{Ar}$ ABX system), 2.68 (1H, dd, $J = 14.5, 6.1$ Hz, $-\underline{\text{CH}}_2\text{Ar}$ ABX system); m.p: 260-263 °C; $[\alpha]_{\text{D}} = +20$ ($c = 1.0, 0.5$ M HCl) (Lit²: $+20, c = 1.0, 0.5$ M HCl);



β -D-galactose penta-acetate (2.8 g, 7.2 mmol) was dissolved in dry DCM (30 mL) under nitrogen and cooled to 0 °C. Boron trifluoride diethyl etherate (1.35 mL, 10.8 mmol) was added drop-wise, followed by 4-bromobenzyl alcohol (2.01 g, 10.8 mmol). After warming to rt, the reaction was stirred for 18 hrs. Sat. NaHCO_3 (50 mL) was then added to quench the reaction and the mixture stirred for a further 30 min. The mixture was extracted with DCM (2 x 50 mL) and the organics dried with MgSO_4 , filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography, eluting with 20-28 % EtOAc:Petrol. Pure fractions were concentrated to give the DP as a colourless oil. A yield of 2.2 g, 4.3 mmol (60 %) was obtained. ^1H NMR (400 MHz, CDCl_3): $\delta = 7.47$ (2H, d, $J = 8.5$ Hz, $o\text{-H}$), 7.16 (2H, d, $J = 8.5$ Hz, $m\text{-H}$), 5.38 (1H, dd, $J = 3.4, 0.9$ Hz, $\underline{\text{H}}_4$), 5.27 (1H, dd, $J = 10.5, 8.0$ Hz, $\underline{\text{H}}_2$), 4.99 (1H, dd, $J = 10.5, 3.4$ Hz, $\underline{\text{H}}_3$), 4.85 (1H, d, $J = 12.5$ Hz, $-\underline{\text{CH}}_2\text{Ar}$), 4.57 (1H, d, $J = 12.5$ Hz, $-\underline{\text{CH}}_2\text{Ar}$), 4.51 (1H, d, $J = 8.0$ Hz, $\underline{\text{H}}_1$), 4.06-4.10 (2H, m, $\underline{\text{H}}_6$), 3.89 (1H, td, $J = 6.7, 1.0$ Hz, $\underline{\text{H}}_5$), 2.15 (3H, s, $-\text{OAc}$), 2.03 (3H, s, $-\text{OAc}$), 2.02 (3H, s, $-\text{OAc}$), 1.97 (3H, s, $-\text{OAc}$) ppm; ^{13}C NMR (100 MHz, CDCl_3): $\delta = 171.34$ ($-\text{OAc}$), 170.20 ($-\text{OAc}$), 170.07 ($-\text{OAc}$), 169.33 ($-\text{OAc}$), 135.81 ($p\text{-H}$), 131.56 ($o\text{-H}$), 129.22 ($m\text{-H}$), 121.86 ($i\text{-H}$), 99.99 ($\underline{\text{C}}_1$), 70.83 ($\underline{\text{C}}_5$), 70.76 ($\underline{\text{C}}_3$), 69.95 ($-\underline{\text{CH}}_2\text{Ar}$), 68.77 ($\underline{\text{C}}_2$), 66.99 ($\underline{\text{C}}_4$), 61.25 ($\underline{\text{C}}_6$), 20.71 ($-\text{OAc}$), 20.65 ($-\text{OAc}$), 20.63 ($-\text{OAc}$), 20.53 ($-\text{OAc}$) ppm; HRMS m/z (ESI⁺): Found: 539.0523/541.0504 ($\text{M}+\text{Na}$)

S6

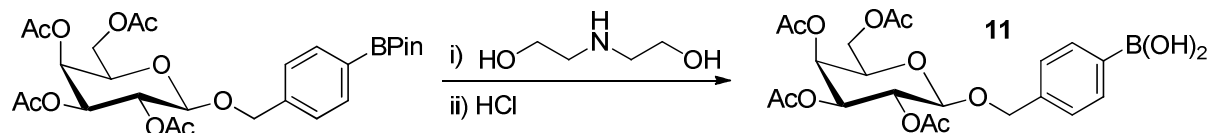
Calc.: 539.0516/541.0503; IR (ν_{\max} , oil): 1743, 1367, 1214, 1045 cm^{-1} ; $[\alpha]_{\text{D}} = -29$ ($c = 1.0$, CHCl_3);



Benzyl bromide **3** (2 g, 3.9 mmol), bis(pinacolato) diborane (1.5 g, 5.8 mmol) and potassium acetate (1.53 g, 15.6 mmol) were charged under nitrogen and dissolved in dry dioxane (30 mL). Pd(dppf)Cl₂ (142 mg, 0.2 mmol) was then added and the mixture refluxed for 18 hrs. The mixture was cooled and concentrated *in vacuo*. The residue was re-dissolved in DCM (250 mL) and washed with H₂O (100 mL). The aqueous was extracted with DCM (100 mL) and the combined organics washed with brine (100 mL). The organics were then dried with MgSO₄, filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography eluting with 20-25 % EtOAc:Petrol. Pure fractions were concentrated *in vacuo* to give the DP as a colourless oil. A yield of 1.37 g, 1.9 mmol (51 %) was obtained. ¹H NMR (400 MHz, CDCl₃): δ = 7.78 (2H, d, J = 8.0 Hz, *o*-H), 7.28 (2H, d, J = 8.0 Hz, *m*-H), 5.37 (1H, d, J = 3.2 Hz, H₄), 5.27 (1H, dd, J = 10.4, 8.0 Hz, H₂), 4.94 (1H, dd, J = 10.4, 3.2 Hz, H₃), 4.92 (1H, d, J = 12.8 Hz, -CH₂Ar), 4.64 (1H, d, J = 12.8 Hz, -CH₂Ar), 4.47 (1H, d, J = 8.0 Hz, H₁), 4.19 (1H, dd, J = 11.3, 6.7 Hz, H₆), 4.12 (1H, dd, J = 11.3, 7.3 Hz, H₆), 3.86 (1H, dd, J = 7.3, 6.7 Hz, H₅), 2.15 (3H, s, -OAc), 2.06 (3H, s, -OAc), 2.02 (3H, s, -OAc), 1.96 (3H, s, -OAc), 1.34 (12H, s, Pin) ppm; ¹³C NMR (100 MHz, CDCl₃): δ = 170.38 (-OAc), 170.27(-OAc), 170.10(-OAc), 169.40 (-OAc), 139.72 (*p*-C), 134.89 (*o*-C), 126.89 (*m*-C), 99.61 (C₁), 83.84 (-BOCR₃), 70.88 (C₃), 70.68 (C₅), 70.42 (-CH₂Ar), 68.79 (C₂), 67.05 (C₄), 61.32 (C₆), 24.83 (-BOC(CH₃)₂), 20.74 (-OAc), 20.68 (-OAc), 20.66 (-OAc), 20.55 (-

S7

OAc) ppm; HRMS m/z (ESI⁺): Found: 587.2269 (M+Na) Calc.: 587.2275; IR (ν_{\max} , oil): 2979, 1746, 1359, 1214, 1143, 1046 cm^{-1} ; $[\alpha]_{\text{D}} = -23$ ($c = 0.5$, CHCl_3);

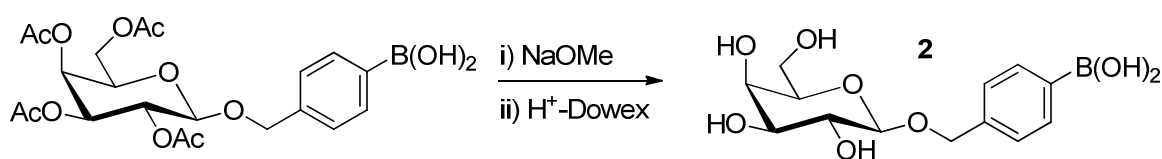


Pinacol ester **4** (700 mg, 1.2 mmol) was dissolved in diethyl ether (30 mL) and diethanolamine (195 mg, 1.9 mmol) was added. After stirring for 18 hrs the resultant white precipitate was collected by filtration and washed with diethyl ether (30 mL). NMR confirmed the presence of the intermediate DEA ester: ¹H NMR (400 MHz, DMSO): $\delta = 7.43$ (2H, d, $J = 7.9$ Hz, *o*-H), 7.11 (2H, d, $J = 7.9$ Hz, *m*-H), 6.84 - 6.91 (1H, m, -NH), 5.26 (1H, d, $J = 3.6$ Hz, H₄), 5.16 (1H, dd, $J = 10.4$, 3.6 Hz, H₃), 4.98 (1H, dd, $J = 10.4$, 7.9 Hz, H₂), 4.75 (1H, d, $J = 7.9$ Hz, H₁), 4.73 (1H, d, $J = 12.0$ Hz, -CH₂Ar), 4.51 (1H, d, $J = 12.0$ Hz, -CH₂Ar), 4.21 (1H, dd, $J = 6.6$, 6.3 Hz, H₅), 4.11 (1H, dd, $J = 11.3$, 6.6 Hz, H₆), 4.07 (1H, dd, $J = 11.3$, 6.3 Hz, H₆), 3.83 - 3.91 (2H, m, -CH₂NH-), 3.75 - 3.81 (2H, m, -CH₂NH-), 3.03 - 3.14 (2H, m, -BOCH₂-), 2.81 - 2.88 (2H, m, -BOCH₂-), 2.13 (3H, s, -OAc), 2.04 (3H, s, -OAc), 2.00 (3H, s, -OAc), 1.92 (3H, s, -OAc) ppm;

The residue was re-suspended in diethyl ether (30 mL) and hydrochloric acid (0.1 M, 30 mL) was added. After stirring for 2 hrs, the aqueous was washed with diethyl ether (100 mL) and the combined organics dried with MgSO₄, filtered and concentrated *in vacuo*. The DP was obtained as white crystals. A yield of 350 mg, 0.72 mmol (61 %) was obtained. ¹H NMR (400 MHz, DMSO): $\delta = 8.03$ (2H, s, -B(OH)₂), 7.78 (2H, d, $J = 7.9$ Hz, *o*-H), 7.25 (2H, d, $J = 7.9$ Hz, *m*-H), 5.27 (1H, dd, $J = 3.5$, 1.0 Hz, H₄), 5.18 (1H, dd, $J = 10.4$, 3.5 Hz, H₃), 5.00 (1H, dd, $J = 10.4$, 7.9 Hz, H₂), 4.79 - 4.82 (2H, m, -CH₂Ar), 4.60 (1H, d, $J = 12.6$ Hz, H₁), 4.22 (1H, td, $J = 6.3$, 1.0 Hz, H₅), 4.05 - 4.12 (2H, m, H₆), 2.13 (3H, s, -OAc), 2.03 (3H, s, -

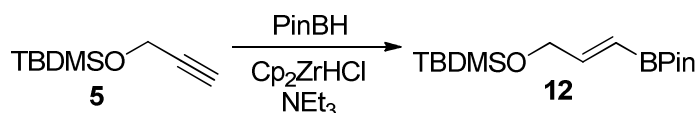
S8

OAc), 2.00 (3H, s, -OAc), 1.92 (3H, s, -OAc) ppm; ^{13}C NMR (100 MHz, DMSO): δ = 169.97 (-OAc), 169.91 (-OAc), 169.49 (-OAc), 169.15 (-OAc), 139.06 (*p*-C), 134.12 (*o*-C), 126.39 (*m*-C), 99.24 (C1), 70.16 (C3), 70.10 (-CH₂Ar), 69.90 (C5), 68.75 (C2), 67.32 (C4), 61.26 (C6), 20.52 (-OAc), 20.48 (-OAc), 20.40 (-OAc), 20.33 (-OAc) ppm; HRMS m/z (ESI+): Found: 505.1481 (M+Na) Calc.: 505.1492; IR (ν_{max} , solid): 1743, 1367, 1217, 1045, 819, 648 cm^{-1} ; M.p: >300 °C;

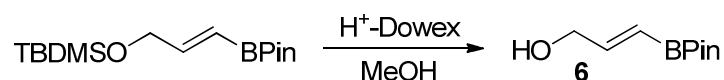


Boronic acid **11** (400 mg, 0.83 mmol) was dissolved in methanol (10 mL) and sodium methoxide was added (179 mg, 3.32 mmol). After stirring for 2 hrs, a white precipitate had formed. The reaction was neutralised with pre-activated Dowex-50WX8 and the mixture stirred for 20 min. The residue was collected by filtration and washed with methanol (2 x 20 mL). Water (2 x 20 mL) was then added and the suspension stirred for 1 hr. The water was then collected by filtration and concentrated *in vacuo* to afford the DP as white crystals. A yield of 94 mg, 0.3 mmol (36 %) was obtained. ^1H NMR (400 MHz, NaOD/D₂O): δ = 6.97 (2H, d, J = 7.9 Hz, *o*-H), 6.76 (2H, d, J = 7.9 Hz, *p*-H), 4.28 (1H, d, J = 11.3 Hz, -CH₂Ar), 4.07 (1H, d, J = 11.3 Hz, -CH₂Ar), 3.76 (1H, d, J = 7.2 Hz, H1), 3.26 (1H, d, J = 2.8 Hz, H4), 3.07-3.12 (2H, m, H2 and H3), 2.90 (1H, dd, $J_1 = J_2 = 6.0$ Hz, H5), 2.77-2.87 (2H, m, H6) ppm; ^{13}C NMR (125 MHz, NaOD/D₂O): δ = 133.52 (*p*-C), 131.03 (*o*-C), 127.45 (*m*-C), 102.96 (C1), 76.49 (C5), 74.52 (C6), 71.14 (C7), 69.43 (C4), 60.96 (C3), 60.96 (C2) ppm; HRMS m/z (ESI-): Found: 341.1400 (M-H) Calc.: 341.1416; IR (ν_{max} , solid): 1611, 1311, 1261, 1174, 1076, 1041 cm^{-1} ; M.p: >300 °C; $[\alpha]_{\text{D}} = -29$ (c = 0.7, 2 M NaOH);

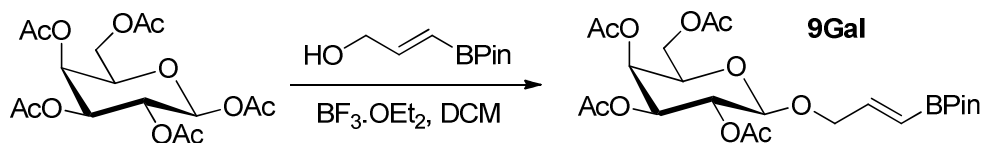
S9



Tert-butyl-dimethyl(2-propynyloxy) silane (12.16 mL, 60 mmol) was charged under nitrogen and pinacolborane (9.5 mL, 66 mmol) was added, followed by Schwartz's reagent (1.59 g, 6 mmol) and triethylamine (842 μ L, 6 mmol). After heating to 60 $^{\circ}$ C for 18 hrs, petrol (40 mL) was added and the mixture filtered through silica. Petrol and remaining starting material were removed *in vacuo* to give the DP, **12**, as a colourless oil. A yield of 15.8 g, 53 mmol (88 %) was obtained. Spectroscopic data was consistent with that previously reported.³ ^1H NMR (400 MHz, CDCl_3): δ = 6.68 (1H, dt, J = 17.9, 3.4 Hz, $-\text{CHCHBPin}$), 5.76 (1H, dt, J = 17.9, 2.0 Hz, $-\text{CHBPin}$), 4.25 (2H, dd, J = 3.4, 2.0 Hz, $-\text{CH}_2\text{OR}$), 1.27 (12H, s, Pin), 0.91 (9H, s, *t*-Bu), 0.06 (6H, s, Me) ppm;

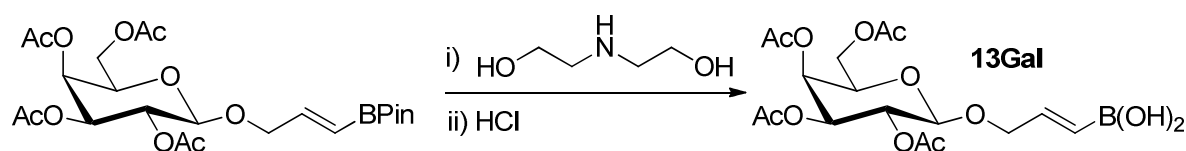


Silane **12** (15.8 g, 53 mmol) was dissolved in methanol (60 mL) and activated Dowex-50WX8 (~5 g) was added. After stirring for 72 hrs, the methanol was removed *in vacuo* and the residue purified by flash column chromatography, eluting with 30 % EtOAc: Petrol. Pure fractions were concentrated *in vacuo* to give the DP as a colourless oil. A yield of 9.7 g, 53 mmol (100 %) was obtained. Spectroscopic data matched that previously obtained.² ^1H NMR (400 MHz, CDCl_3): δ = 6.73 (1H, dt, J = 18.2, 4.2 Hz, $-\text{CHCHBPin}$), 5.68 (1H, dt, J = 18.2, 1.9 Hz, $-\text{CHCHBPin}$), 4.21 (2H, app s, $-\text{CH}_2\text{OH}$), 1.25 (12H, s, Pin) ppm;



S10

β -D-Galactose pentaacetate (3.90 g, 10 mmol) was dissolved in dry DCM (50 mL) under nitrogen in a flame dried flask and cooled to 0 °C. Boron trifluoride diethyl etherate (1.88 mL, 15 mmol) was added followed by vinyl alcohol **6** (2.20 g, 12 mmol). The reaction was allowed to warm to rt and stirred for 18 hrs, The reaction was then quenched with water (50 mL) and the aqueous extracted with DCM (50 mL). The combined organics were dried with MgSO_4 , filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography eluting with 30 % EtOAc:Petrol. Pure fractions were concentrated *in vacuo* to give a light yellow oil. A yield of 2.1 g, 3.9 mmol (39 %) was obtained. ^1H NMR (400 MHz, CDCl_3): δ = 6.56 (1H, dt, J = 18.3, 4.8 Hz, $-\text{CHCHBPin}$), 5.68 (1H, dt, J = 18.3, 1.7 Hz, $-\text{CHBPin}$), 5.38 (1H, dd, J = 3.4, 0.85 Hz, $\underline{\text{H}}_4$), 5.25 (1H, dd, J = 10.6, 8.0 Hz, $\underline{\text{H}}_2$), 5.02 (1H, dd, J = 10.6, 3.4 Hz, $\underline{\text{H}}_3$), 4.51 (1H, d, J = 8.0 Hz, $\underline{\text{H}}_1$), 4.42 (1H, ddd, J = 14.7, 4.3, 0.85 Hz, $\underline{\text{H}}_5$), 4.39 (1H, d, J = 4.8 Hz, $-\text{CH}_2\text{CHCHBPin}$), 4.11-4.22 (2H, m, $\underline{\text{H}}_6$), 3.89 (1H, dd, J = 6.7, 1.0 Hz, $-\text{CH}_2\text{CHCHBPin}$), 2.16 (3H, s, $-\text{OAc}$), 2.09 (3H, s, $-\text{OAc}$), 2.06 (3H, s, $-\text{OAc}$), 1.99 (3H, s, $-\text{OAc}$), 1.27 (12H, s, Pin) ppm; ^{13}C NMR (125 MHz, CDCl_3): δ = 170.30 ($-\text{OAc}$), 170.21 ($-\text{OAc}$), 170.06 ($-\text{OAc}$), 169.37 ($-\text{OAc}$), 147.24 ($-\text{CHCHBPin}$), 119.96 ($-\text{CHBPin}$), 100.15 ($\underline{\text{C}}_1$), 83.23 ($-\text{BOCR}_3$), 70.85 ($\underline{\text{C}}_3$), 70.55 ($\underline{\text{C}}_5$), 70.35 ($-\text{CH}_2\text{CHCHBPin}$), 68.75 ($\underline{\text{C}}_2$), 67.00 ($\underline{\text{C}}_4$), 61.22 ($\underline{\text{C}}_6$), 24.71 ($-\text{BOC}(\underline{\text{C}}\text{H}_3)_2$), 20.68 ($-\text{OAc}$), 20.55 ($-\text{OAc}$), 20.46 ($-\text{OAc}$) ppm; HRMS m/z (ESI $^+$): Found: 537.2118 ($\text{M}+\text{Na}$) Calc: 537.2099; IR (ν_{max} , oil): 2979, 1746, 1645, 1368, 1219, 1143, 1062 cm^{-1} ; $[\alpha]_{\text{D}} = +6.2$ (c = 0.5, CHCl_3);



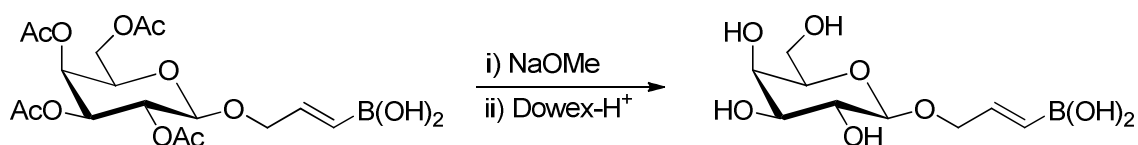
Vinyl galactose **9Gal** (2 g, 3.9 mmol) was dissolved in diethyl ether (80 mL) and diethanolamine (613 mg, 5.8 mmol) was added. After stirring for 18 hrs the resultant white

S11

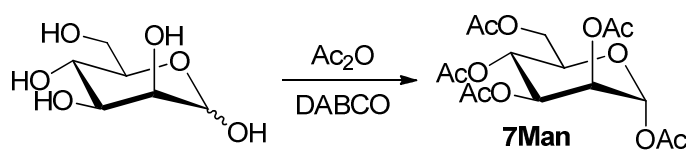
precipitate was collected by filtration, washed with diethyl ether (50 mL) and dried *in vacuo*. The presence of the corresponding DEA ester was confirmed by ^1H NMR (400 MHz, DMSO): δ = 6.75 (1H, br s, -NH), 5.72 (1H, dt, J = 17.6, 5.3 Hz, -CHCHBOR₂), 5.56 (1H, d, J = 17.6, -CHBOR₂), 5.26 (1H, dd, J = 3.4, 0.5 Hz, H₄), 5.15 (1H, dd, J = 10.4, 3.4 Hz, H₃), 4.94 (1H, dd, J = 10.4, 8.0 Hz, H₂), 4.68 (1H, d, J = 8.0 Hz, H₁), 4.16 (2H, dd, J = 12.6, 5.3 Hz, -CH₂CHCHBOR₂), 4.02-4.70 (2H, m, H₆), 3.98 (1H, ddd, J = 13.0, 5.6, 0.5 Hz, H₅), 3.69-3.75 (2H, m, -OCH₂CH₂NH-), 3.58-3.64 (2H, m, -OCH₂CH₂NH-), 2.93-3.03 (2H, m, -CH₂NH-), 2.69-2.76 (2H, m, -CH₂NH-), 2.12 (3H, s, -OAc), 2.03 (3H, s, -OAc), 2.01 (3H, s, -OAc), 1.92 (3H, s, -OAc) ppm;

The white solid was resuspended in ether (30 mL) and stirred with hydrochloric acid (0.1 M, 30 mL) for 2 hrs. The aqueous was washed with ether (100 mL) and the combined organics dried with MgSO₄, filtered and dried *in vacuo* to give the DP as white crystals. A yield of 520 mg, 1.2 mmol (31 %) was obtained. ^1H NMR (400 MHz, DMSO): δ = 7.71 (2H, br s, -B(OH)₂), 6.41 (1H, dt, J = 18.0, 4.5 Hz, -CHCHB(OH)₂), 5.45 (1H, d, J = 18.0 Hz, -CHB(OH)₂), 5.27 (1H, d, J = 3.4 Hz, H₄), 5.20 (1H, dd, J = 10.2, 3.4 Hz, H₃), 4.98 (1H, dd, J = 10.2, 7.9 Hz, H₂), 4.73 (1H, d, J = 7.9 Hz, H₁), 4.05-4.34 (4H, m, H₅, H₆ and -CH₂CHCHB(OH)₂), 2.14 (3H, s, -OAc), 2.06 (3H, s, -OAc), 2.03 (3H, s, -OAc), 1.94 (3H, s, -OAc) ppm; ^{13}C NMR (125 MHz, DMSO): δ = 169.95 (-OAc), 169.90 (-OAc), 169.50 (-OAc), 169.19 (-OAc), 144.31 (-CHCHBPin), 125.56 (-CHBPin), 99.25 (C₁), 70.23 (-CH₂CHCHBPin), 70.19 (C₃), 69.83 (C₅), 68.89 (C₂), 67.33 (C₄), 61.24 (C₆), 20.52 (-OAc), 20.49 (-OAc), 20.39 (-OAc), 20.33 (-OAc) ppm; M.p: >300 °C; IR (ν_{max} , solid): 2916, 2849, 1742, 1645, 1368, 1220, 1045 cm⁻¹;

S12



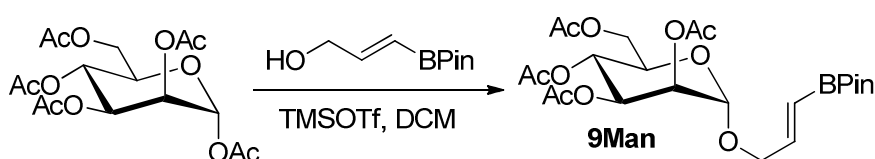
Protected galactose boronic acid **13Gal** (432 mg, 1 mmol) was dissolved in methanol (10 mL) and sodium methoxide (216 mg, 4 mmol) was added. After stirring for 90 min, pre-activated Dowex-50WX8 was added until the pH was neutral, and the mixture stirred for a further 90 min. The reaction was then filtered and the solvent removed *in vacuo* to give 230 mg of DP as a white solid. A further 120 mg was obtained by washing the Dowex with water, and concentrating in *vacuo*. ^1H NMR (400 MHz, D_2O): δ = 6.42 (1H, dt, J = 18.3, 5.1 Hz, - $\text{CHCHB}(\text{OH})_2$), 5.64 (1H, d, J = 18.3 Hz, - $\text{CHB}(\text{OH})_2$), 4.35 (1H, ddd, J = 14.2, 4.8, 1.7 Hz, - $\text{CH}_2\text{CH}=\text{CH}$), 4.30 (1H, d, J = 7.9 Hz, H1), 4.20 (1H, ddd, J = 14.2, 5.3, 1.4 Hz, - $\text{CH}_2\text{CH}=\text{CH}$), 3.79 (1H, d, J = 3.4 Hz, H4), 3.55-3.69 (3H, m, H5 and H6), 3.52 (1H, dd, J = 9.9, 3.4 Hz, H3), 3.42 (1H, dd, J = 9.9, 7.8 Hz, H2) ppm; ^{13}C NMR (125 MHz, D_2O): δ = 145.47 (- $\text{CHCHB}(\text{OH})_2$), 124.11 (- $\text{CHB}(\text{OH})_2$), 102.01 (C1), 75.14 (C5), 72.74 (C3), 71.05 (- $\text{CH}_2\text{CH}=\text{CH}$), 70.75 (C2), 68.61 (C4), 60.93 (C6) ppm; HRMS m/z (ESI+): Found: 287.0909 (M+Na) Calc: 287.0910; M.p: >300 °C; IR (ν_{max} , solid): 3364, 1641, 1419, 1362, 1323, 1266, 1204, 1034 cm^{-1} ; $[\alpha]_{\text{D}} = -12$ (c = 1.0, H_2O);



The synthesis was adapted from Ch *et al.*⁴ D-Mannose (1.8 g, 10 mmol) was suspended in acetic anhydride (5.7 mL, 60 mmol) and DABCO (1.12 g, 10 mmol) was added, causing a large exotherm. After stirring for 18 hrs, the resultant light brown solution was poured into ice/water (100 mL). The product was extracted with DCM (2 x 100 mL) and the combined organics washed with sat. NaHCO_3 (50 mL), dried with Na_2SO_4 , filtered and concentrated *in*

S13

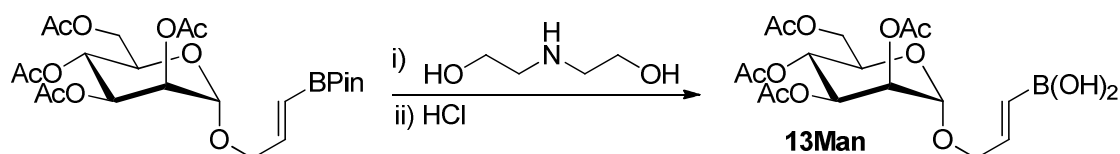
vacuo. The DP was obtained as a colourless oil. A yield of 3.62 g, 9.3 mmol (93 %) was obtained. Spectroscopic data matched that previously obtained.⁵ ^1H NMR (400 MHz, CDCl_3): δ = 6.08 (1H, d, J = 1.9 Hz, H1), 5.33-5.37 (2H, m, H3 and H4), 5.26 (1H, t, J = 2.2 Hz, H2), 4.28 (1H, dd, J = 12.3, 5.0 Hz, H6), 4.10 (1H, dd, J = 12.5, 2.4 Hz, H6), 4.03-4.06 (1H, m, H5), 2.22 (3H, s, -OAc), 2.18 (3H, s, -OAc), 2.17 (3H, s, -OAc), 2.10 (3H, s, -OAc), 2.05 (3H, s, -OAc), 2.01 (3H, s, -OAc) ppm;



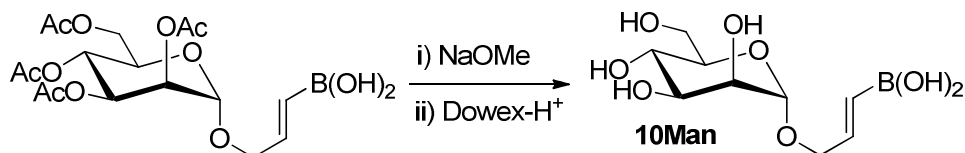
D-Mannose pentaacetate (3.2 g, 8.2 mmol) and vinyl alcohol **6** (2.02 g, 11 mmol) were dissolved in dry DCM (50 mL) under nitrogen and cooled to 0 °C. Trimethylsilyl trifluoromethanesulfonate (1.99 mL, 11 mmol) was then added drop-wise and the reaction allowed to warm to rt. After stirring for 18 hrs the reaction was washed with water (20 mL) and the organics dried with MgSO_4 , filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography eluting with 25 % EtOAc:Petrol. Pure fractions were concentrated to give the DP as a light yellow oil. A yield of 1.1 g, 2.1 mmol (26 %) was obtained. ^1H NMR (400 MHz, CDCl_3): δ = 6.60 (1H, dt, J = 18.0, 4.7 Hz, -CHCHBPin), 5.73 (1H, dt, J = 18.0, 1.6 Hz, -CHBPin), 5.37 (1H, dd, J = 10.1, 3.5 Hz, H3), 5.26-5.30 (H2 and H4), 4.85 (1H, d, J = 1.6 Hz, H1), 4.25-4.30 (2H, m, H6 and -CH2CHCHBPin), 4.07-4.14 (2H, m, H6 and -CH2CHCHBPin), 3.97-4.02 (1H, m, H5), 2.15 (3H, s, -OAc), 2.10 (3H, s, -OAc), 2.04 (3H, s, -OAc), 1.99 (3H, s, -OAc), 1.28 (12H, s, -BPin) ppm; ^{13}C NMR (125 MHz, CDCl_3): δ = 170.63 (-OAc), 169.97 (-OAc), 169.74 (-OAc), 146.72 (-CHCHBPin), 120.69 (-CHBPin), 96.92 (C1), 83.44 (-BOCR₃), 69.47 (C2), 69.18 (-CH2CHCHBPin), 69.05 (C3), 68.55 (C5), 66.09 (C4), 62.38 (C6), 24.75 (-BOCR(CH₃)₂), 20.87 (-OAc), 20.72 (-

S14

OAc), 20.68 (-OAc), 20.65 (-OAc) ppm; HRMS m/z (ESI⁺): Found: 537.2111 (M+Na) Calc.: 537.2118; IR (ν_{\max} , solid): 1743, 1367, 1359, 1217, 1045 cm^{-1} ; $[\alpha]_{\text{D}} = +26$ ($c = 1.0$, CHCl_3);



Pinacol ester **9Man** (900 mg, 1.75 mmol) was dissolved in ether (30 mL) and diethanolamine (273 mg, 2.6 mmol) was added. After stirring for 7 hrs, the intermediate DEA ester was collected by filtration and dried under suction. NMR confirmed the presence of the intermediate DEA ester. ^1H NMR (400 MHz, DMSO): δ = 6.59 (1H, br s, -NH), 5.79 (1H, dt, $J = 17.6$, 5.8 Hz, -CHCHBR₃), 5.64 (1H, d, $J = 17.6$ Hz, -CHBR₃), 5.07-5.15 (2H, m, H₃ and H₄), 4.88 (1H, d, $J = 1.2$ Hz, H₁), 4.02-4.20 (3H, m, H₅ and H₆), 3.89-3.97 (2H, m, -CH₂CHCHBR₃), 3.69-3.77 (2H, m, -BOCH₂-), 3.58-3.65 (2H, m, -BOCH₂-), 2.95-3.05 (2H, m, -NHCH₂-), 2.69-2.77 (2H, m, -NHCH₂-), 2.11 (3H, s, -OAc), 2.04 (3H, s, -OAc), 2.03 (3H, s, -OAc), 1.95 (3H, s, -OAc) ppm; The DEA ester was suspended in ether and hydrochloric acid (10 %, 50 mL) was added. After stirring for 1 hr the aqueous was washed with ether (100 mL) and the combined organics dried with MgSO_4 , filtered and concentrated *in vacuo* to give the DP as white crystals. A yield of 329 mg, 0.76 mmol (44 %) was obtained. The product was used in the subsequent de-protection step without further analysis.



Boronic acid **13Man** (300 mg, 0.69 mmol) was dissolved in methanol (10 mL) and sodium methoxide (150 mg, 2.76 mmol) was added. After stirring for 1 hr, pre-activated Dowex-

S15

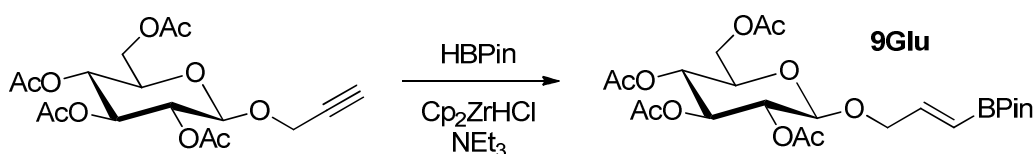
50WX8 was added in small portions until the pH was neutral. Stirring was continued for 30 min and the reaction mixture was then filtered and concentrated *in vacuo*. The resultant brown oil was azeotroped with DCM (4 x 20 mL) to give the DP as a light pink solid. A yield of 132 mg, 0.5 mmol (72 %) was obtained. ^1H NMR (400 MHz, D_2O): δ = 6.42 (1H, dt, J = 18.3, 4.8 Hz, $-\text{CHCHB}(\text{OH})_2$), 5.59 (1H, dt, J = 18.3, 1.5 Hz, $-\text{CHB}(\text{OH})_2$), 4.78 (1H, d, J = 1.7 Hz, $\underline{\text{H1}}$), 4.19 (1H, ddd, J = 14.3, 4.6, 1.7 Hz, $-\text{CH}_2\text{CHCHB}(\text{OH})_2$), 4.04 (1H, ddd, J = 14.3, 5.1, 1.5 Hz, $-\text{CH}_2\text{CHCHB}(\text{OH})_2$), 3.89 (1H, dd, J = 3.5, 1.6 Hz, $\underline{\text{H2}}$), 3.79 (1H, dd, J = 12.3, 1.3 Hz, $\underline{\text{H2}}$), 3.73-3.76 (1H, m, $\underline{\text{H3}}$), 3.64-3.69 (1H, m, $\underline{\text{H6}}$), 3.55-3.60 (2H, m, $\underline{\text{H4}}$ and $\underline{\text{H5}}$) ppm; ^{13}C NMR (125 MHz, D_2O): δ = 148.38 ($-\text{CHCHB}(\text{OH})_2$), 123.68 ($-\text{CHB}(\text{OH})_2$), 99.11 ($\underline{\text{C1}}$), 72.83 ($\underline{\text{C3}}$), 70.54 ($\underline{\text{C5}}$), 69.95 ($\underline{\text{C2}}$), 68.48 ($-\text{CH}_2\text{CHCHB}(\text{OH})_2$), 66.70 ($\underline{\text{C4}}$), 60.85 ($\underline{\text{C6}}$) ppm; HRMS m/z (ESI $^{+}$): Found: 287.0908 ($\text{M}+\text{Na}$) Calc: 287.0910; M.p: >300 °C; IR (ν_{max} , solid): 3334, 2917, 1643, 1328, 1029 cm^{-1} ; $[\alpha]_{\text{D}} = +20$ ($c = 0.5$, H_2O);



D-Glucose penta-acetate (5 g, 12.8 mmol) was dissolved in dry DCM (100 mL) under nitrogen and cooled to 0 °C. Propargyl alcohol (897 μL , 15.4 mmol) was added, followed by drop-wise addition of boron trifluoride diethyl etherate (2.41 mL, 19.2 mmol). After warming to rt, the reaction was stirred for 18 hrs. Potassium carbonate (2 g) was added to quench the reaction and stirred for 30 min. After filtering, the reaction was washed with water (2 x 50 mL) and the aqueous extracted with DCM (100 mL). The combined organics were dried with Na_2SO_4 , filtered and concentrated *in vacuo*. The residue was re-crystallised from 10 mL petrol: 10 mL DCM. The DP was obtained as white crystals. A yield of 2.4 g, 6.2 mmol (49

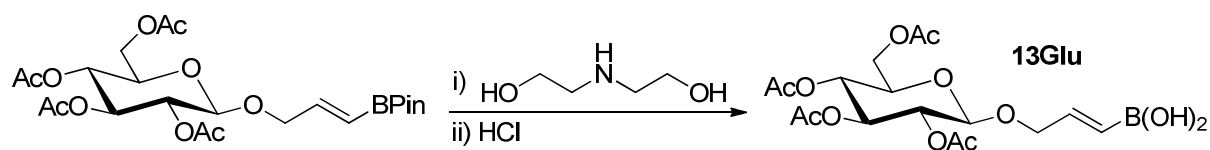
S16

%) was obtained. Spectroscopic data matched that previously reported.⁶ ¹H NMR (400 MHz, CDCl₃): δ = 5.25 (1H, dd, J = 9.6, 9.4 Hz, H3), 5.11 (1H, dd, J = 9.7, 9.6 Hz, H4), 5.02 (1H, dd, J = 9.4, 8.0 Hz, H2), 4.78 (1H, d, J = 8.0 Hz, H1), 4.37 (2H, d, J = 2.4 Hz, -CH₂CCH), 4.28 (1H, dd, J = 12.3, 4.6 Hz, H6), 4.15 (1H, dd, J = 12.3, 2.4 Hz, H6), 3.74 (1H, ddd, J = 9.7, 4.6, 2.4 Hz, H5), 2.48 (1H, t, J = 2.4 Hz, -CCH), 2.09 (3H, s, -OAc), 2.06 (3H, s, -OAc), 2.03 (3H, s, -OAc), 2.01 (3H, s, -OAc) ppm;

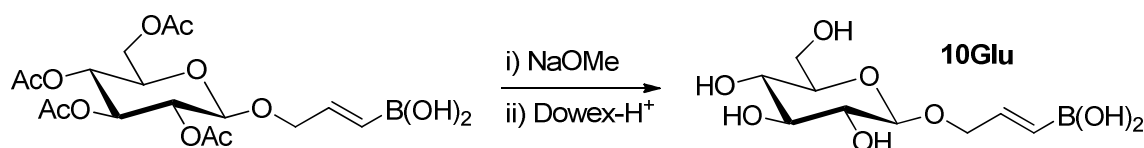


Pinacolborane (838 μ L, 5.83 mmol) was added to glucose derivative **8Glu** (1.5 g, 3.89 mmol) and Schwartz's reagent (100 mg, 0.39 mmol) under nitrogen. Triethylamine (55 μ L, 0.39 mmol) was then added and the reaction heated to 65 °C for 18 hrs. After cooling to rt, the reaction was diluted with DCM (10 mL), filtered and purified by flash column chromatography eluting with 30-50 % EtOAc:Petrol. Pure fractions were concentrated *in vacuo* to give the DP as a colourless oil. A yield of 520 mg, 1.0 mmol (26 %) was obtained. Spectroscopic data matched that previously reported.² ¹H NMR (400 MHz, CDCl₃): δ = 6.58 (1H, dt, J = 18.1, 4.8 Hz, -CHCHBPin), 5.66 (1H, dt, J = 18.1, 1.7 Hz, -CHBPin), 5.25 (1H, dd, $J_1 = J_2 = 9.7$ Hz,), 4.98-5.06 (2H, m,), 4.57 (1H, d, J = 8.0 Hz, H1), 4.38 (1H, ddd, J = 14.5, 4.6, 1.9 Hz, -CH₂CHCHBPin), 4.22 (1H, ddd J = 14.5, 5.0, 1.5 Hz, -CH₂CHCHBPin), 3.72 (1H, dd, J = 12.5, 2.4 Hz, H6), 3.60 (1H, dd, J = 12.5, 5.0 Hz, H6), 3.50 (1H, ddd, J = 9.9, 5.0, 2.4 Hz, H5), 2.07 (3H, s, -OAc), 2.05 (3H, s, -OAc), 2.01 (3H, s, -OAc), 1.27 (12H, s, Pin) ppm;

S17



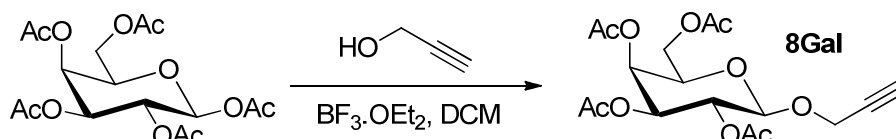
Pinacol ester **9Glu** (500 mg, 0.97 mmol) was dissolved in diethyl ether (20 mL) and diethanolamine (153 mg, 1.45 mmol) was added. After stirring for 18 hrs the resultant precipitate was collected by filtration and dried *in vacuo*. The residue was then resuspended in ether (20 mL) and hydrochloric acid (10 %, 10 mL) was added. After stirring for 1 hr the aqueous was extracted with ether (20 mL) and the combined organics dried with MgSO_4 , filtered and concentrated *in vacuo*. The resultant white solid was used in the subsequent deprotection without further purification or analysis.



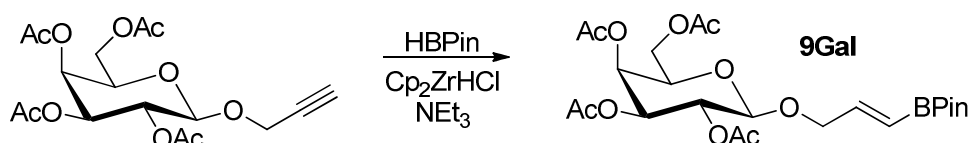
Boronic acid **13Glu** (240 mg, 0.55 mmol) was dissolved in methanol (10 mL) and sodium methoxide (120 mg, 2.22 mmol) was added. After stirring for 2 hrs the reaction was quenched through the addition of pre-activated Dowex-50WX8 until the pH was approximately neutral. After stirring for a further 30 min, the solid was removed by filtration and washed with methanol (20 mL). The filtrate was concentrated *in vacuo* to give the DP as an off white solid. A yield of 125 mg, 0.46 mmol (84 %) was obtained. ^1H NMR (400 MHz, D_2O): δ = 6.43 (1H, dt, J = 18.2, 5.1 Hz, $-\text{CHCHB}(\text{OH})_2$), 5.64 (1H, d, J = 18.2 Hz, $-\text{CHB}(\text{OH})_2$), 4.38 (1H, d, J = 8.1 Hz, $\underline{\text{H}}_1$), 4.36 (1H, dd, J = 14.2, 4.3 Hz, $-\text{CH}_2\text{CHCHB}(\text{OH})_2$), 4.21 (1H, dd, J = 14.1, 5.3 Hz, $-\text{CH}_2\text{CHCHB}(\text{OH})_2$), 3.80 (1H, app d, J = 11.6 Hz, $\underline{\text{H}}_6$), 3.60 (1H, dd, J = 11.6, 5.8 Hz, $\underline{\text{H}}_6$), 3.23-3.41 (3H, m, $\underline{\text{H}}_3$, $\underline{\text{H}}_4$ and $\underline{\text{H}}_5$), 3.19 (1H, dd, J = 8.8, 8.1 Hz, $\underline{\text{H}}_2$) ppm; ^{13}C NMR (125 MHz, D_2O): δ = 145.35 ($-\text{CHCHB}(\text{OH})_2$),

S18

124.26 (Weak, $-\underline{\text{CHB}}(\text{OH})_2$), 101.41 ($\underline{\text{C1}}$), 75.89 ($\underline{\text{C5}}$), 75.72 ($\underline{\text{C3}}$), 73.10 ($\underline{\text{C2}}$), 71.08 ($-\underline{\text{CH}_2\text{CHCHB}}(\text{OH})_2$), 69.62 ($\underline{\text{C4}}$), 60.71 ($\underline{\text{C6}}$) ppm; HRMS m/z (ESI⁺): Found: 287.0912 (M+Na) Calc: 287.0910; M.p: >300 °C; IR (ν_{max} , solid): 3351, 2910, 1643, 1319, 1262, 1070, 1000 cm^{-1} ; $[\alpha]_{\text{D}} = -18$ ($c = 1.0$, H_2O);

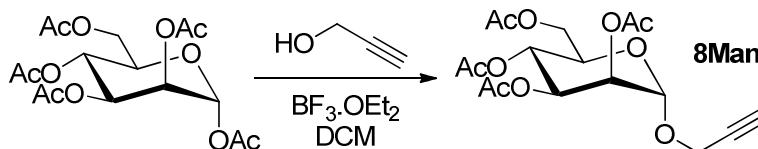


D-Galactose pentaacetate (3.91 g, 10 mmol) was dissolved in dry DCM (50 mL) under nitrogen and cooled to 0 °C. Propargyl alcohol (700 μL , 12 mmol) was added, followed by drop-wise addition of boron trifluoride diethyl etherate (1.88 mL, 15 mmol). After warming to rt, the reaction was stirred for 18 hrs. To quench the reaction, potassium carbonate (2 g) was added and stirred for 30 min. After filtration, the mixture was washed with water (2 x 75 mL) and the aqueous extracted with DCM (75 mL). The combined organics were dried with MgSO_4 , filtered and concentrated *in vacuo* to give the DP as a light yellow oil. A yield of 3.9 g, 10 mmol (100 %) was obtained. Spectroscopic data matched that previously reported.⁶ ^1H NMR (400 MHz, CDCl_3): $\delta = 5.40$ (1H, dd, $J = 3.4, 1.0$ Hz, $\underline{\text{H4}}$), 5.22 (1H, dd, $J = 10.6, 8.0$ Hz, $\underline{\text{H2}}$), 5.06 (1H, dd, $J = 10.6, 3.4$ Hz, $\underline{\text{H3}}$), 4.74 (1H, d, $J = 8.0$ Hz, $\underline{\text{H1}}$), 4.39 (2H, d, $J = 2.4$ Hz, $-\underline{\text{CH}_2\text{CCH}}$), 4.14-4.22 (2H, m, $\underline{\text{H6}}$), 3.94 (1H, td, $J = 6.8, 1.0$ Hz, $\underline{\text{H5}}$), 2.47 (1H, t, $J = 2.4$ Hz, $-\underline{\text{CCH}}$), 2.16 (3H, s, $-\underline{\text{OAc}}$), 2.08 (3H, s, $-\underline{\text{OAc}}$), 2.06 (3H, s, $-\underline{\text{OAc}}$), 1.99 (3H, s, $-\underline{\text{OAc}}$) ppm;



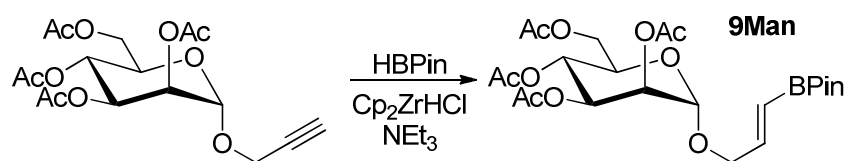
S19

β -Propargylgalactose tetra-acetate, **8Gal**, (0.5 g, 1.3 mmol) and Schwartz's reagent (84 mg, 0.33 mmol) were charged under nitrogen and pinacolborane (745 μ L, 5.2 mmol) was added. Triethylamine (46 μ L, 0.33 mmol) was then added and the mixture heated to 65 °C for 18 hrs. The mixture was then cooled to rt, diluted with ethyl acetate (10 mL) and filtered through a short plug of silica. The flow-through was concentrated *in vacuo* and purified by flash column chromatography, eluting with 25-30 % EtOAc:Petrol. Pure fractions were concentrated *in vacuo* to give the DP as a colourless oil. A yield of 270 mg, 0.53 mmol (40 %) was obtained. Spectroscopic data matched that previously obtained.



D-Mannose pentaacetate (1.5 g, 3.84 mmol) was dissolved in dry DCM (20 mL) under nitrogen and cooled to 0 °C. Propargyl alcohol (1.12 mL, 19.21 mmol) was added, followed by boron trifluoride diethyl etherate (4.86 mmol, 38.4 mmol) dropwise. After warming to rt, the reaction was stirred for 18 hrs. DCM (50 mL) was then added and the mixture washed sequentially with water (30 mL), sat. NaHCO₃ (3 x 30 mL) then water (50 mL). The organics were dried with Na₂SO₄, filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography eluting with 30 % EtOAc:Petrol. Pure fractions were concentrated *in vacuo* to give the DP as an off white solid. A yield of 1.2 g, 3.11 mmol (81 %) was obtained. Spectroscopic data matched that previously reported.⁷ ¹H NMR (400 MHz, CDCl₃): δ = 5.27-5.37 (3H, m, H2, H3 and H4), 5.04 (1H, d, J = 1.5 Hz, H1), 4.27-4.33 (3H, m, H6 and -CH₂CCH), 4.12 (1H, dd, J = 12.3 Hz, 2.4 Hz, H6), 4.03 (1H, ddd, J = 9.0, 5.1, 2.4 Hz, H5), 2.48 (1H, t, J = 2.4 Hz, -CCH), 2.17 (3H, s, -OAc), 2.11 (3H, s, -OAc), 2.05 (3H, s, -OAc), 2.00 (3H, s, -OAc) ppm;

S20



α -Propargylmannose tetra-acetate, **8Man**, (1 g, 2.6 mmol) and Schwartz's reagent (134 mg, 0.52 mmol) were charged under nitrogen and pinacolborane (518 μL , 3.9 mmol) was added. Triethylamine (73 μL , 0.52 mmol) was then added, and the reaction heated to 65 $^{\circ}\text{C}$ for 18 hrs. After cooling to rt, the mixture was diluted with DCM (30 mL) and washed with water (10 mL). The organics were dried with Na_2SO_4 , filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography eluting with 25-50 % EtOAc:Petrol. Pure fractions were concentrated *in vacuo* to give the DP as a yellow oil. A yield of 190 mg, 0.37 mmol (14 %) was obtained. Spectroscopic data was identical to that previously obtained.

Induction of Protein Expression

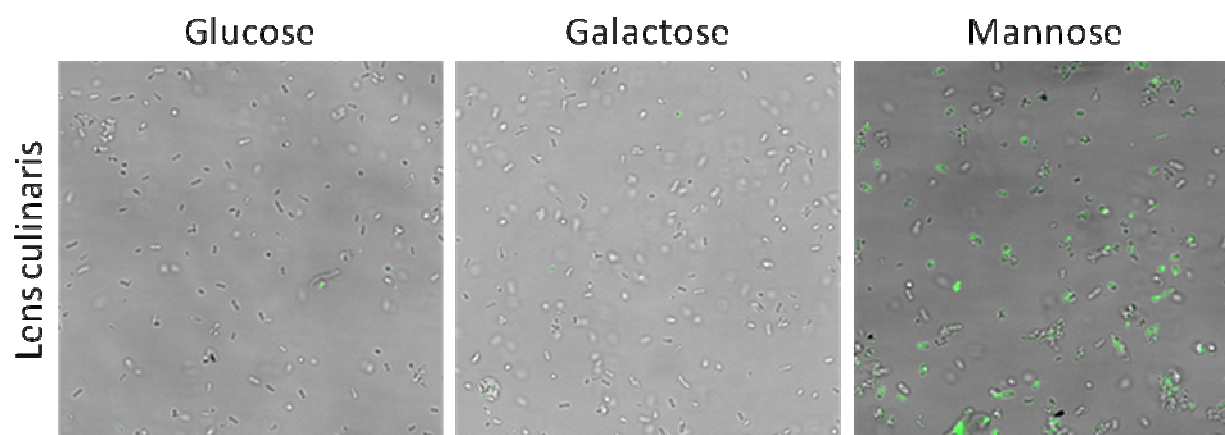
Cell line JW2203-1-pEvol(*pIPhe*)-OmpC(Y232-) was generated as a glycerol stock in a previous report.⁸ The cell line codes for the expression of OmpC with an amber stop codon at position 232, with a pEVOL plasmid for *pIPhe* incorporation in response to the amber codon. The plasmids are carried by an OmpC-knockout cell line.

Overnight cultures were grown from the above glycerol stock in LB media containing ampicillin and chloramphenicol. 0.2 mL of this culture was then diluted in fresh media (30 mL) and grown to an $\text{O.D}_{600} \sim 0.6$ at 37 $^{\circ}\text{C}$. Protein production was then induced through the addition of 1 mM IPTG, 0.02 % *L*-arabinose, and 2 mM *L-p*-iodophenylalanine. Cells were then incubated at 30 $^{\circ}\text{C}$ for 14 hrs.

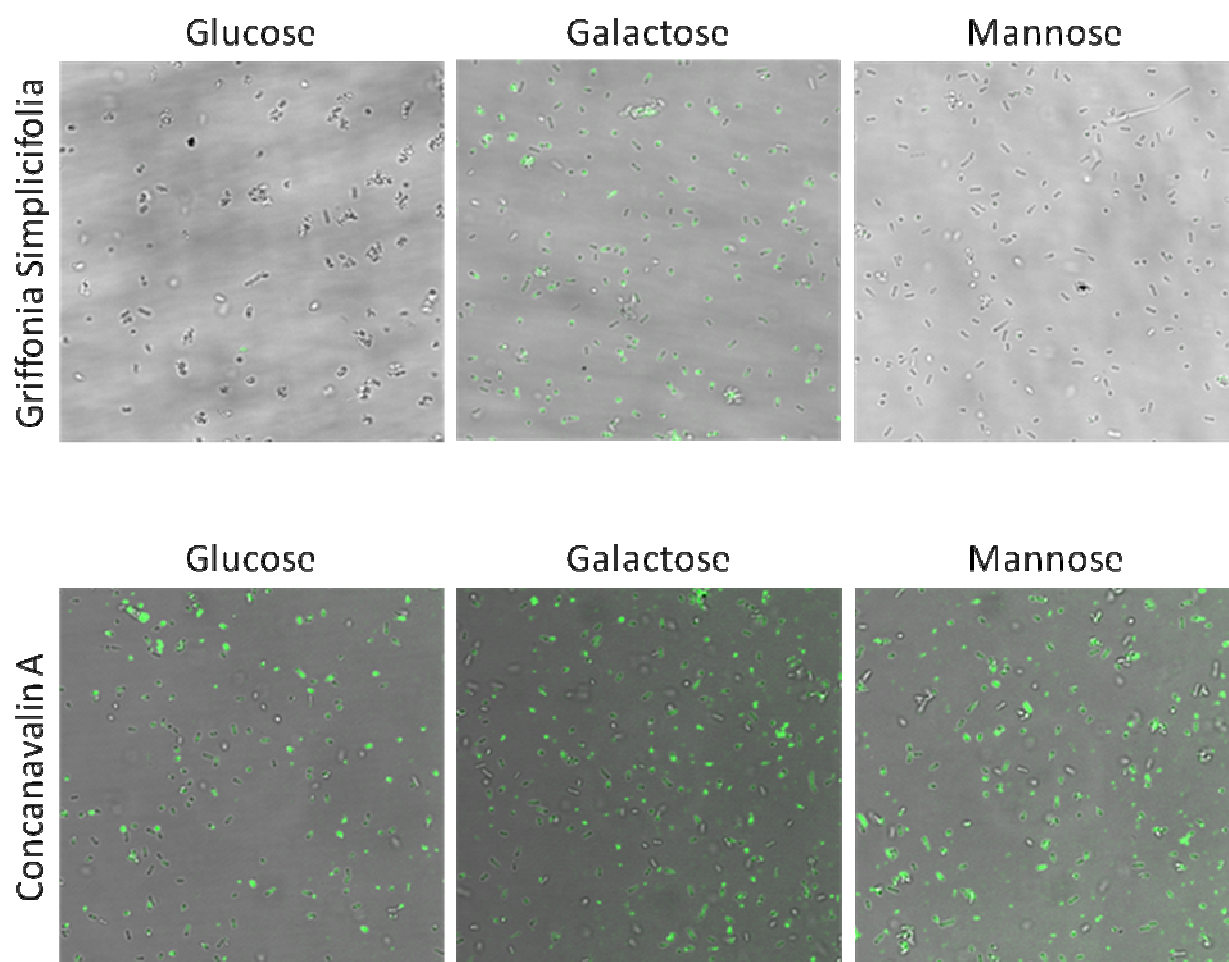
S21

General Procedure for Cell Labelling

Induced cells were collected by centrifugation (rt, 10 min, 3000 rpm) and re-suspended in phosphate buffer (20 mL, 50 mM NaCl, 20 mM NaH₂PO₄, pH 8.0). This process was repeated twice in order to remove residual LB media and any residual unincorporated unnatural amino acid. The O.D₆₀₀ was then measured, and the culture diluted with phosphate buffer to give an O.D₆₀₀ = 0.2. In a standard experiment, a 200 µL aliquot was then added to a 1.5 mL Eppendorf tube. A stock solution of sugar boronic acid was prepared by dissolving 5 mg of the boronic acid with 5 mg of Na₂HPO₄ in 300 µL of water at 37 °C (18.9 µmol boronic acid, 35.2 µmol Na₂HPO₄). A 12 µL aliquot was added to the cells (0.76 µmol), followed by a 10 µL aliquot of stock palladium catalyst **1** (0.1 µmol). After the cells were incubated with shaking at 37 °C for 1 hr they were collected by centrifugation (rt, 5 min, 10,000 rpm) and re-suspended in a 0.85 % saline solution (400 µL). This process was repeated 5 times, to ensure complete removal of un-reacted boronic acid and palladium. Finally, the cells were re-suspended in 200 µL saline solution and fluorescein-labelled lectins were added to a final concentration of 25 µg/mL. Cells were then visualised by fluorescence microscopy.



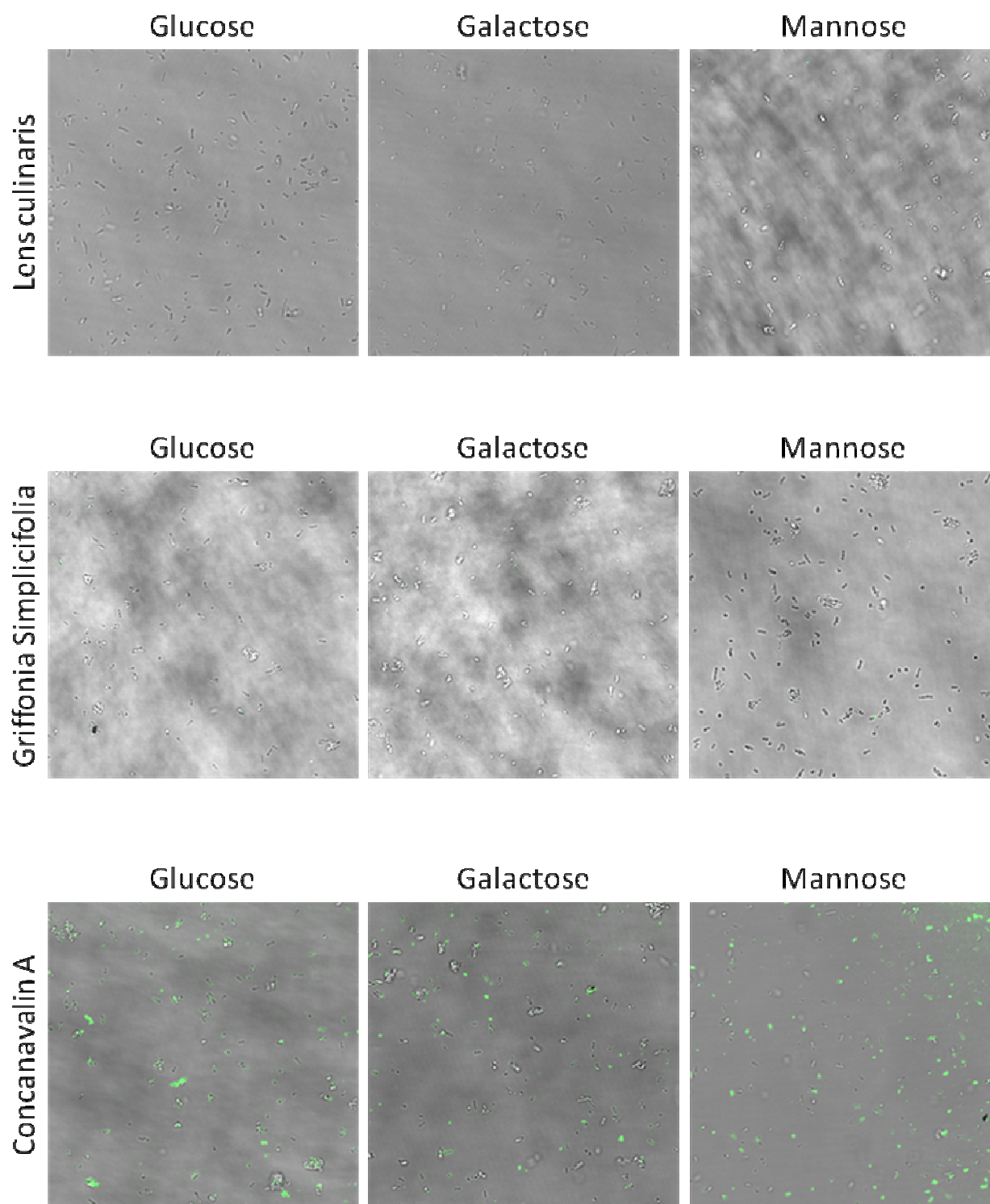
S22



Labelling in the Absence of Palladium

Experiments were run as described above, replacing palladium with the addition of phosphate buffer. Cells were then visualised by fluorescence microscopy.

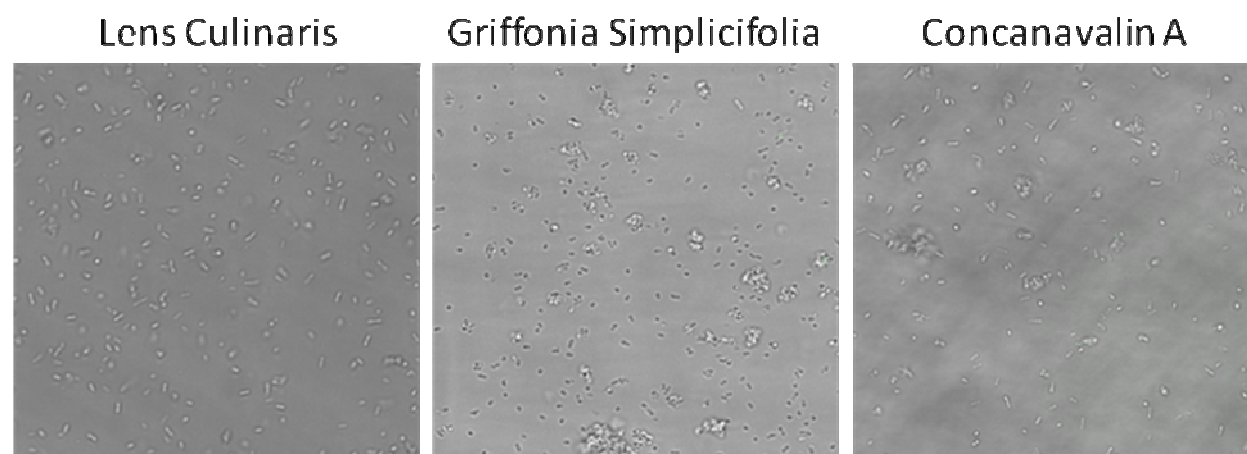
S23



Labelling in the Absence of Boronic Acid

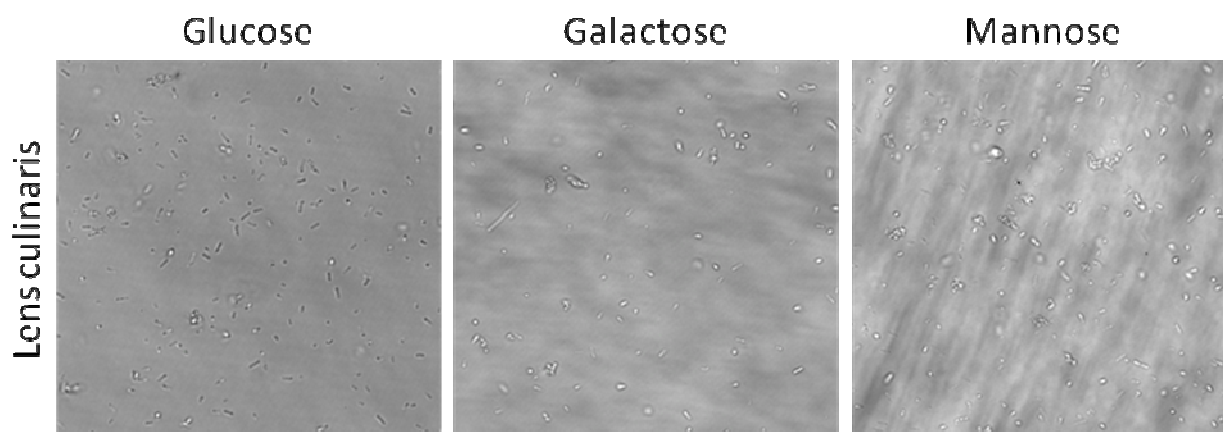
Experiments were run as described above, replacing the sugar boronic acid with the addition of phosphate buffer. Cells were then visualised by fluorescence microscopy.

S24

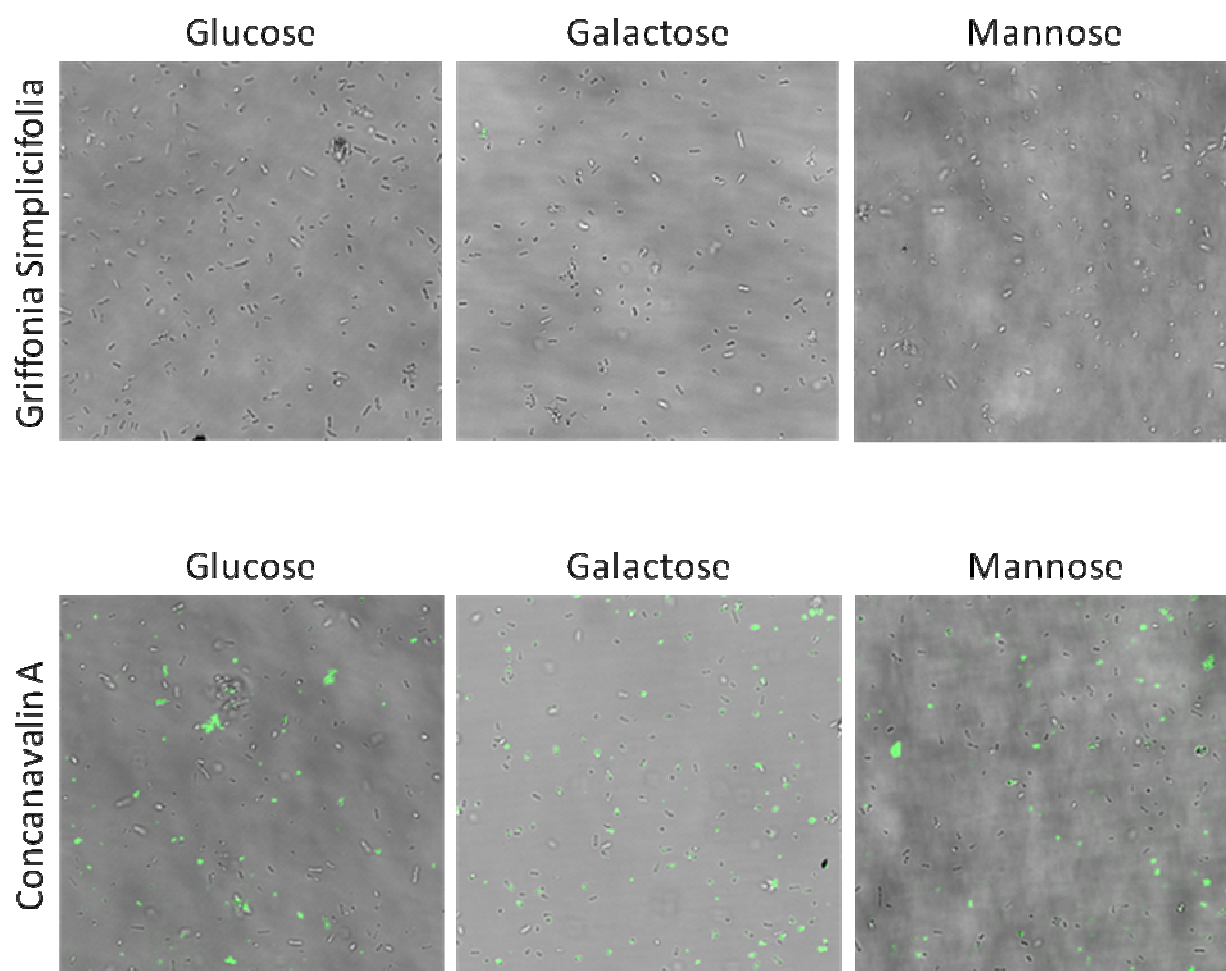


Labelling in the Absence of Amino Acid

Cells were grown as described above, omitting the addition of *p*-iodophenylalanine during the induction step, to generate cells deficient in aryl halide display on the cell surface. Coupling reactions were then run as described above and the cells visualised by fluorescence microscopy.

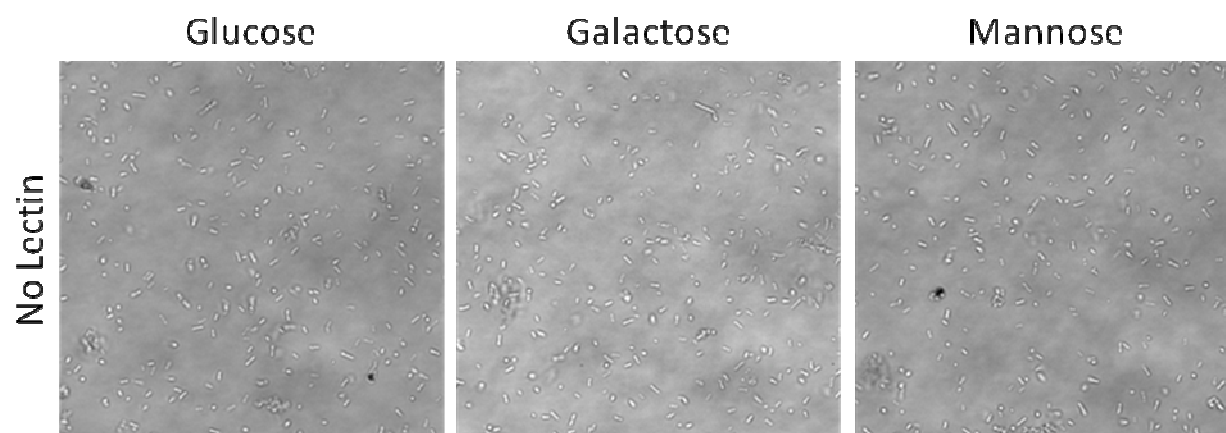


S25



Cells in the Absence of Fluorescein-Labelled Lectins

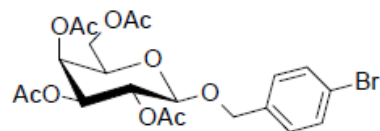
Experiments were run as described above, with phosphate buffer being added in place of fluorescent lectin conjugate. The cells were then visualised by fluorescence microscopy.



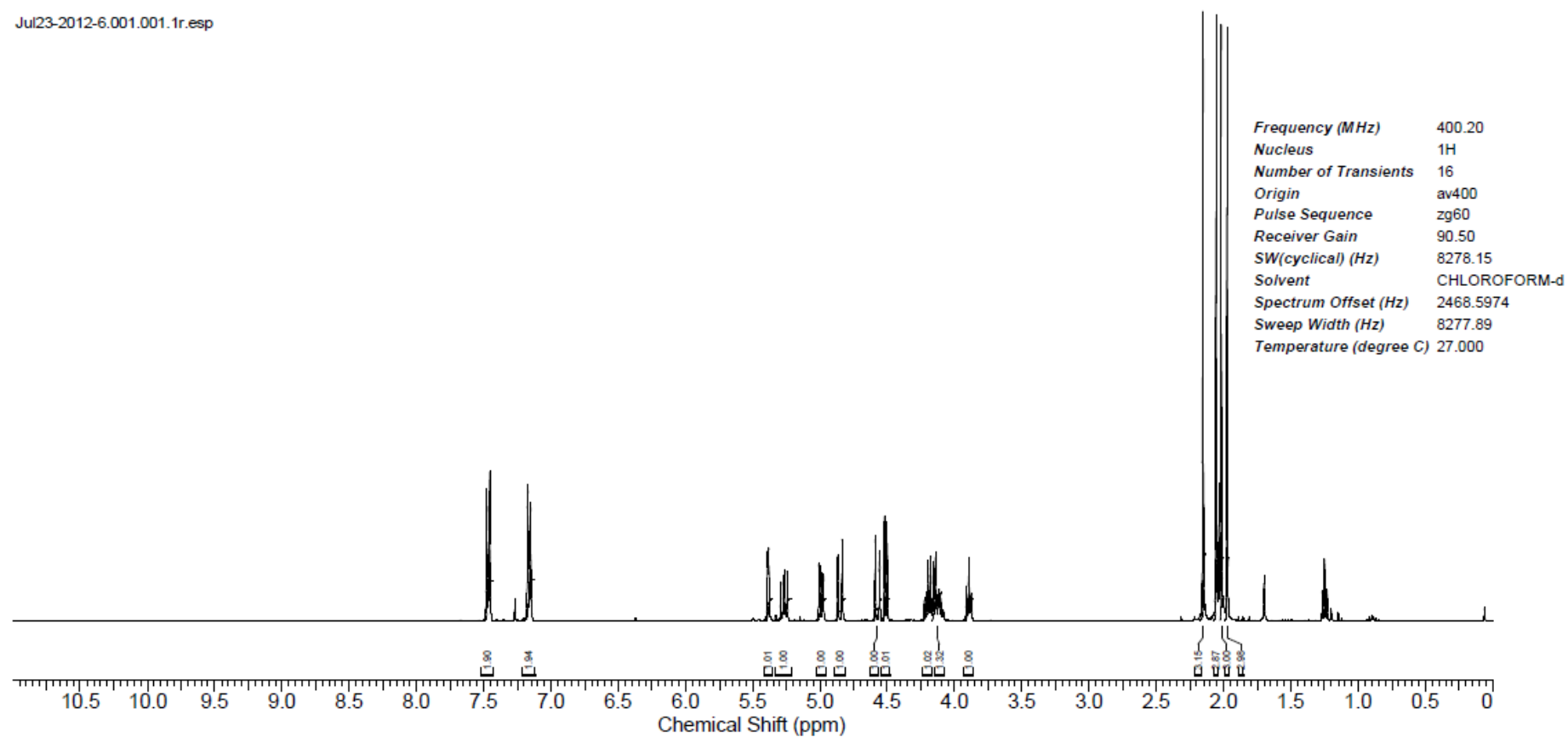
S26**References**

- (1) Lei, H.; Stoakes, M. S.; Schwabacher, A. W.; Herath, K. P. B.; Lee, J. *J. Org. Chem.* **1994**, *59*, 4206-4210.
- (2) Chalker, J. M.; Wood, C. S. C.; Davis, B. G. *J. Am. Chem. Soc.* **2009**, *131*, 16346-16347.
- (3) Fürstner, A.; Ackerstaff, J. *Chem. Comm.*, **2008**, *25*, 2870-2782.
- (4) Ch, R.; Tyagi, M.; Patil, P. R.; Ravindranathan Kartha K. P. *Tet. Lett.*, **2011**, *52*, 5841-5846.
- (5) Patel, M. K.; Vijayakrishnan. B.; Koeppe, J. R.; Chalker, J. M.; Doores, K. J.; Davis, B. G. *Chem. Comm.*, **2010**, *46*, 9119-9121.
- (6) Mereyala, H. B.; Gurralla, S. R. *Carb. Res.*, **1998**, *307*, 351-354.
- (7) Schmid, S.; Mena-Osteritz, E.; Kopyshev, A.; Bäuerle, P. *Org. Lett.*, **2009**, *11*, 5098-5101.
- (8) Spicer, C. D.; Triemer, T.; Davis. B. G. *J. Am. Chem. Soc.*, **2012**, *134*, 800-803.

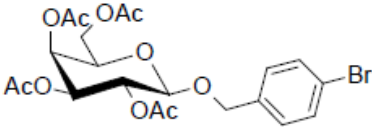
S27

¹H NMR (400 MHz, CDCl₃)

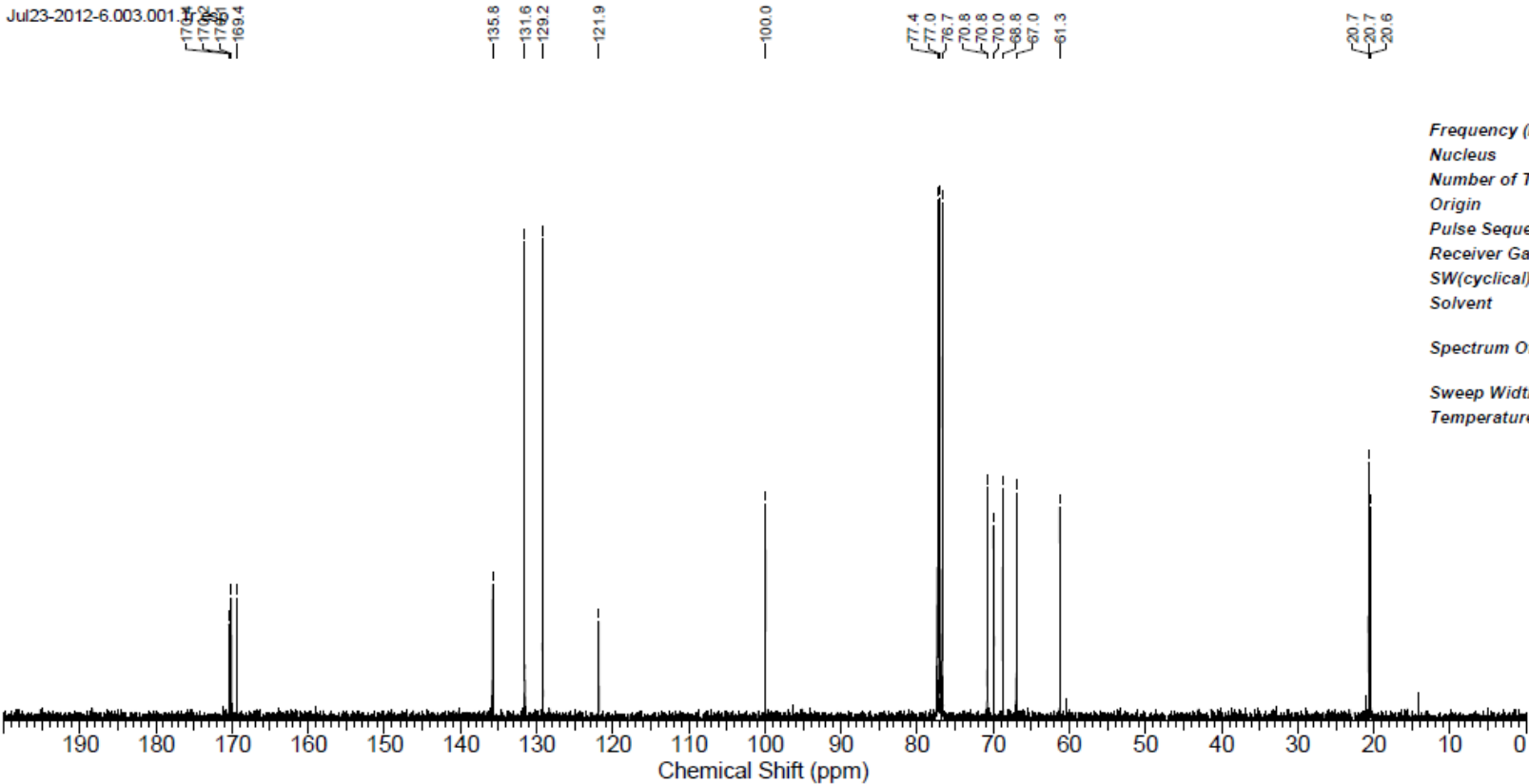
Jul23-2012-6.001.001.1r.esp



S28

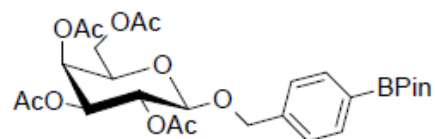


¹³C NMR (100 MHz, CDCl₃)

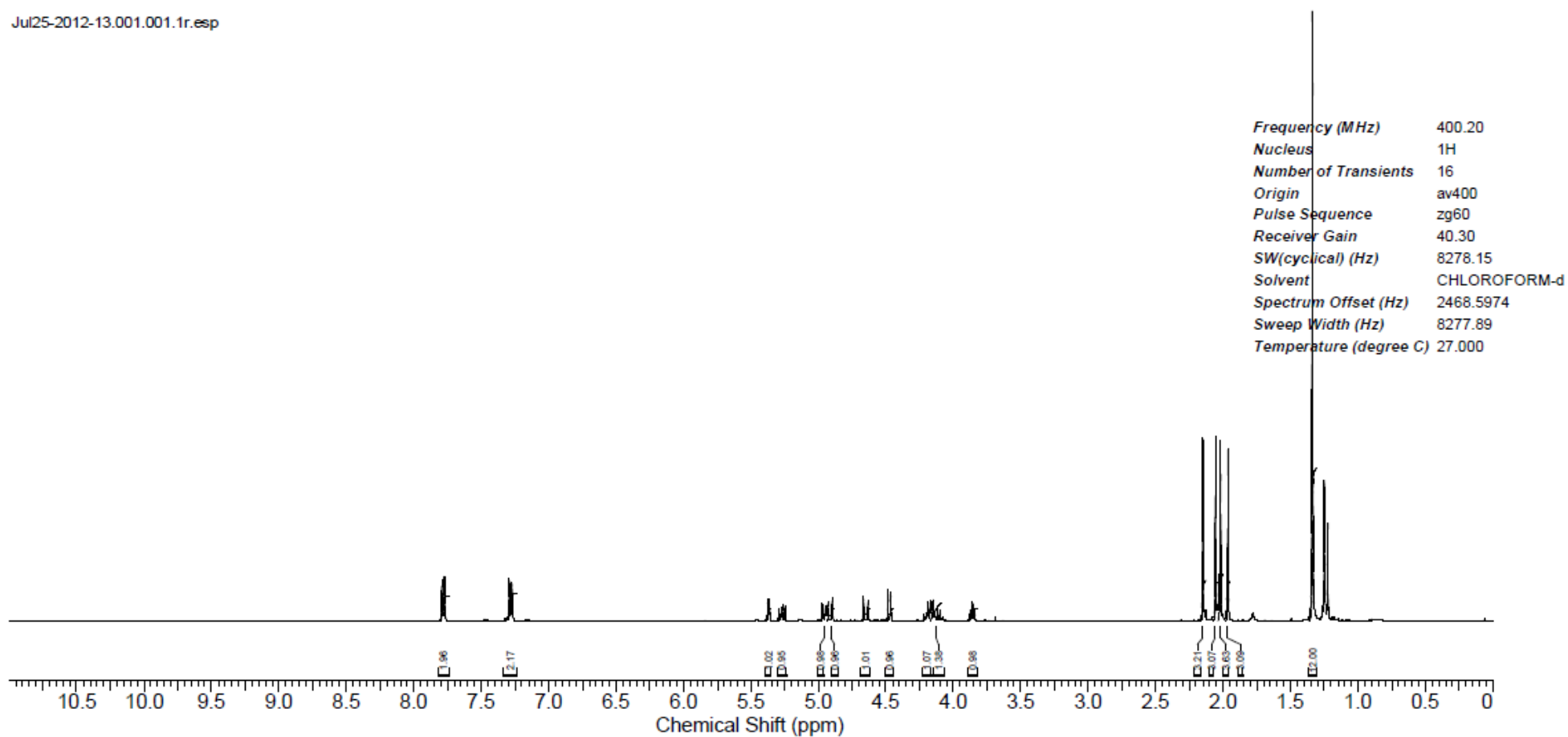


Frequency (MHz)	100.63
Nucleus	¹³ C
Number of Transients	256
Origin	av400
Pulse Sequence	zgpg30
Receiver Gain	32768.00
SW(cyclical) (Hz)	26178.01
Solvent	CHLORO FORM-d
Spectrum Offset (Hz)	10021.29 39
Sweep Width (Hz)	26177.21
Temperature (degree C)	27.000

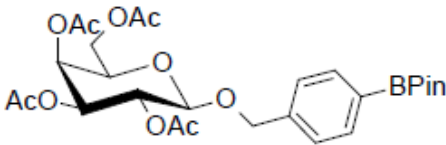
S29

 ^1H NMR (400 MHz, CDCl_3)

Jul25-2012-13.001.001.1r.esp

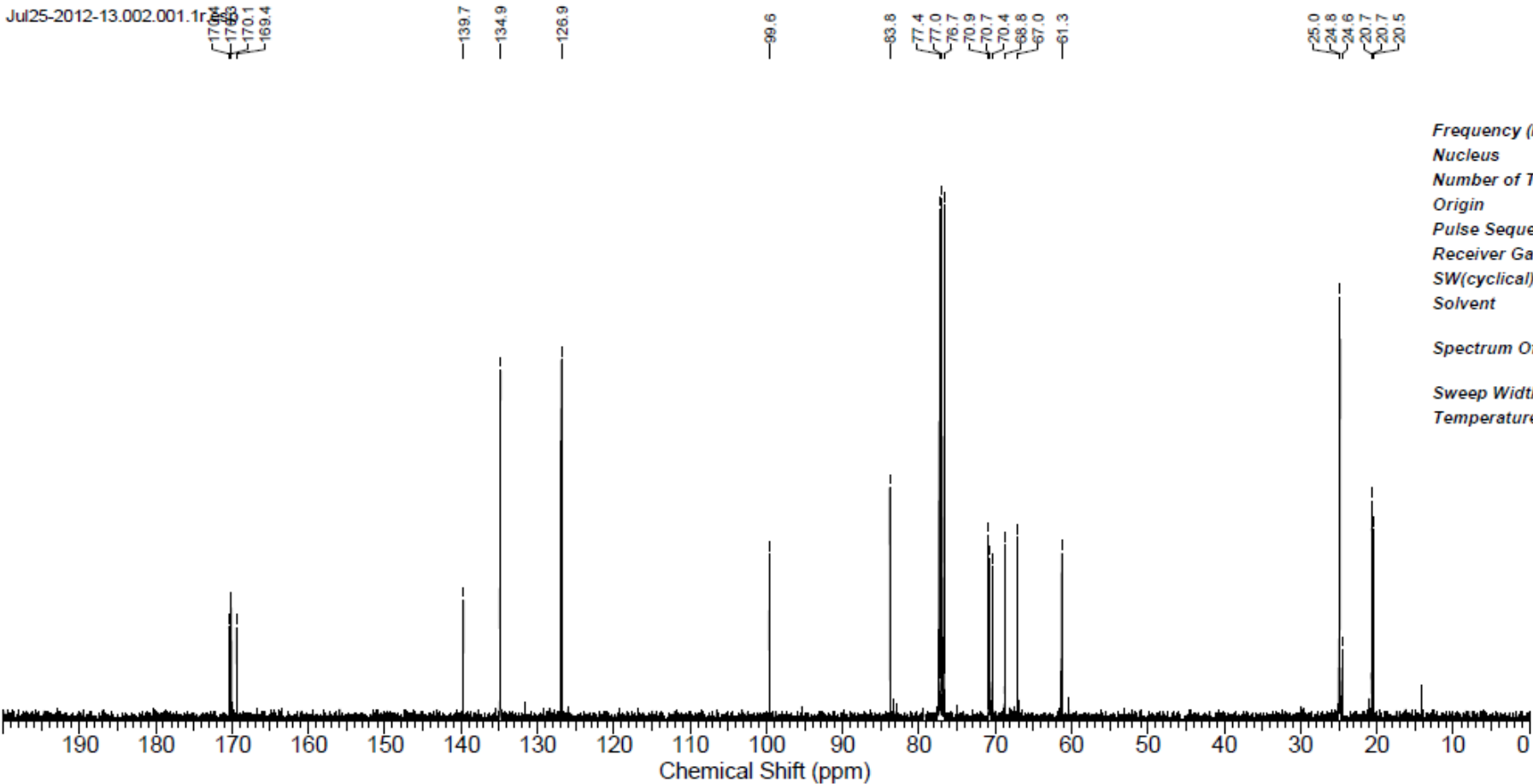


S30



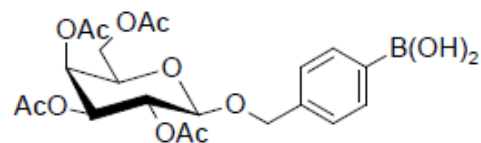
¹³C NMR (100 MHz, CDCl₃)

Jul25-2012-13.002.001.1r

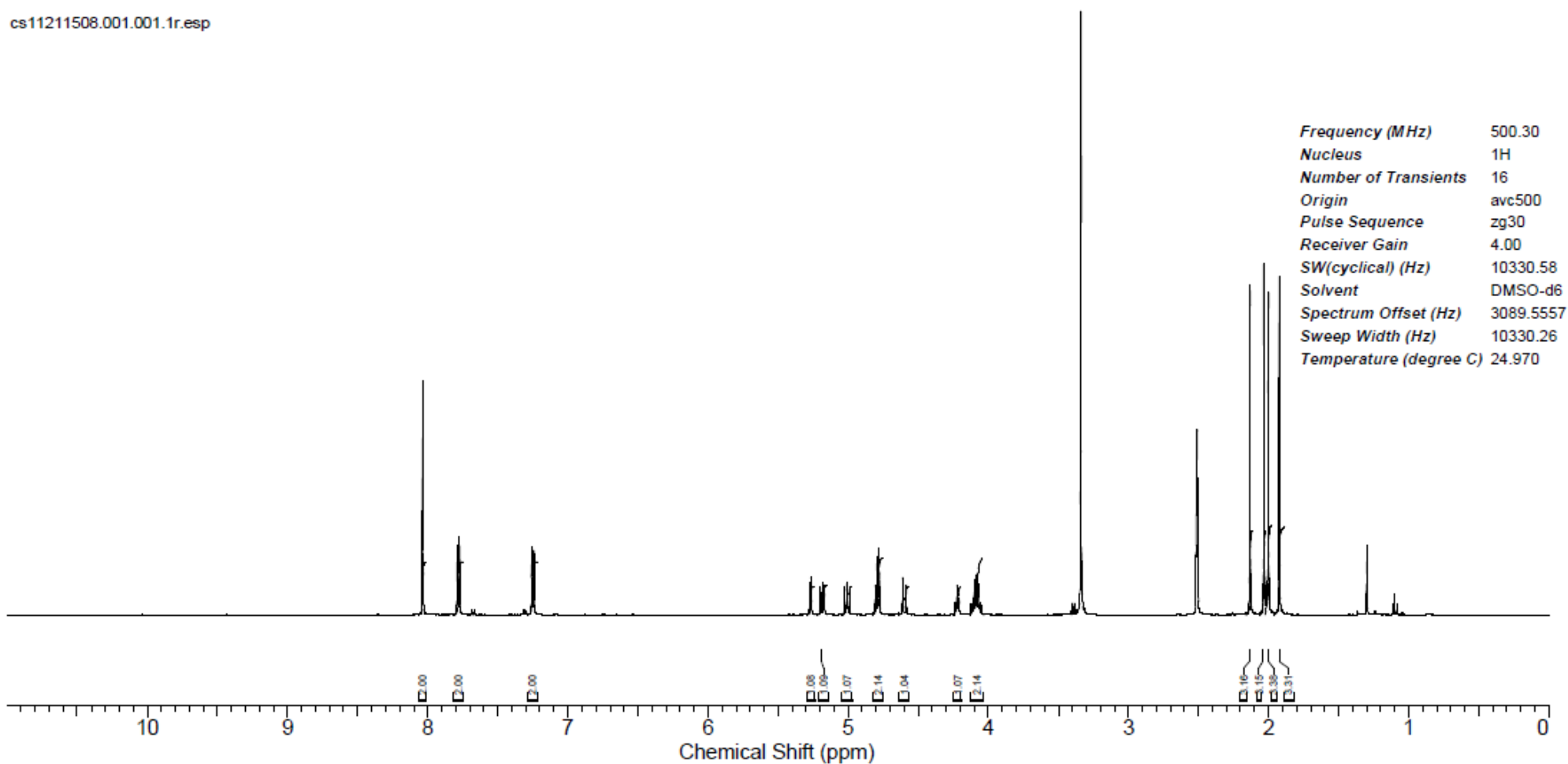


Frequency (MHz)	100.63
Nucleus	¹³ C
Number of Transients	256
Origin	av400
Pulse Sequence	zgpg30
Receiver Gain	32768.00
SW(cyclical) (Hz)	26178.01
Solvent	CHLORO FORM-d
Spectrum Offset (Hz)	10021.29 39
Sweep Width (Hz)	26177.21
Temperature (degree C)	27.000

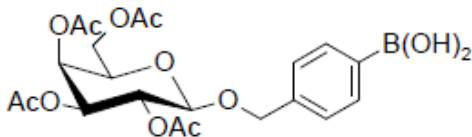
S31

¹H NMR (400 MHz, CDCl₃)

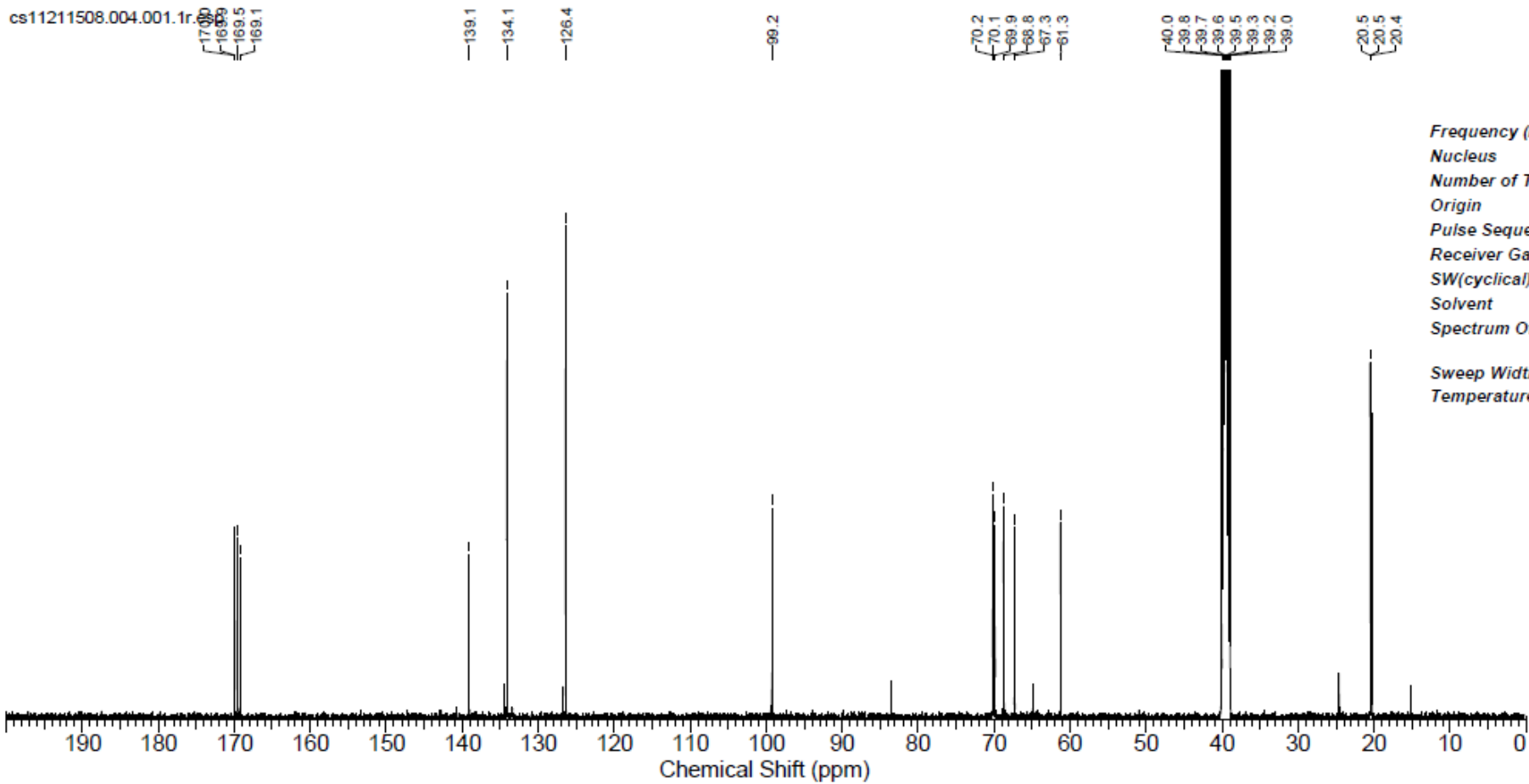
cs11211508.001.001.1r.esp



S32

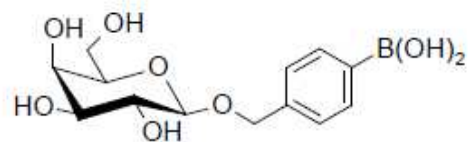


¹³C NMR (100 MHz, CDCl₃)

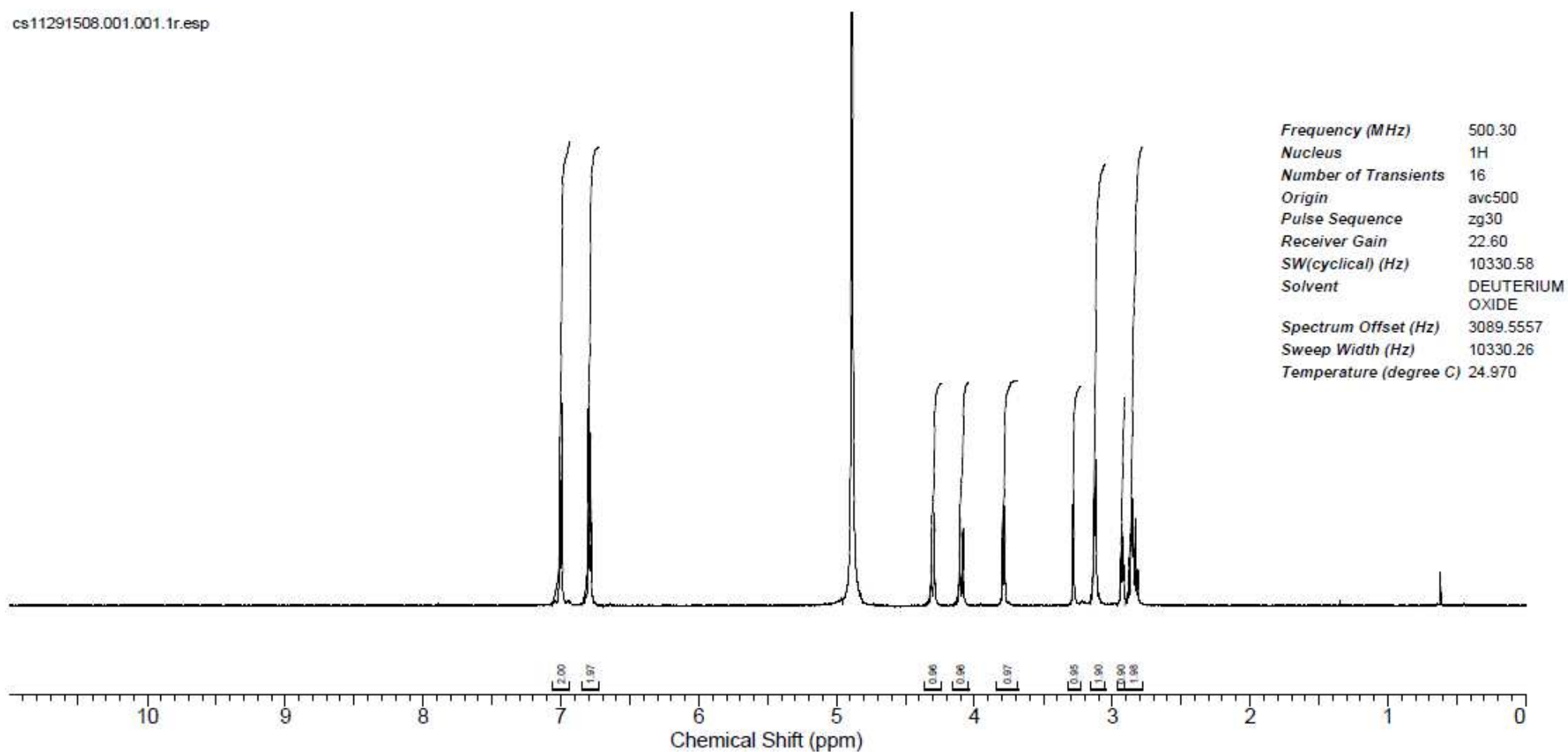


Frequency (MHz)	125.80
Nucleus	13C
Number of Transients	512
Origin	avc500
Pulse Sequence	zgpg30
Receiver Gain	1820.00
SW(cyclical) (Hz)	31250.00
Solvent	DMSO-d6
Spectrum Offset (Hz)	12519.72
	66
Sweep Width (Hz)	31249.05
Temperature (degree C)	24.970

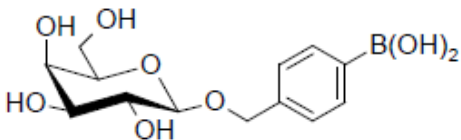
S33

 ^1H NMR (400 MHz, CDCl_3)

cs11291508.001.001.1r.esp

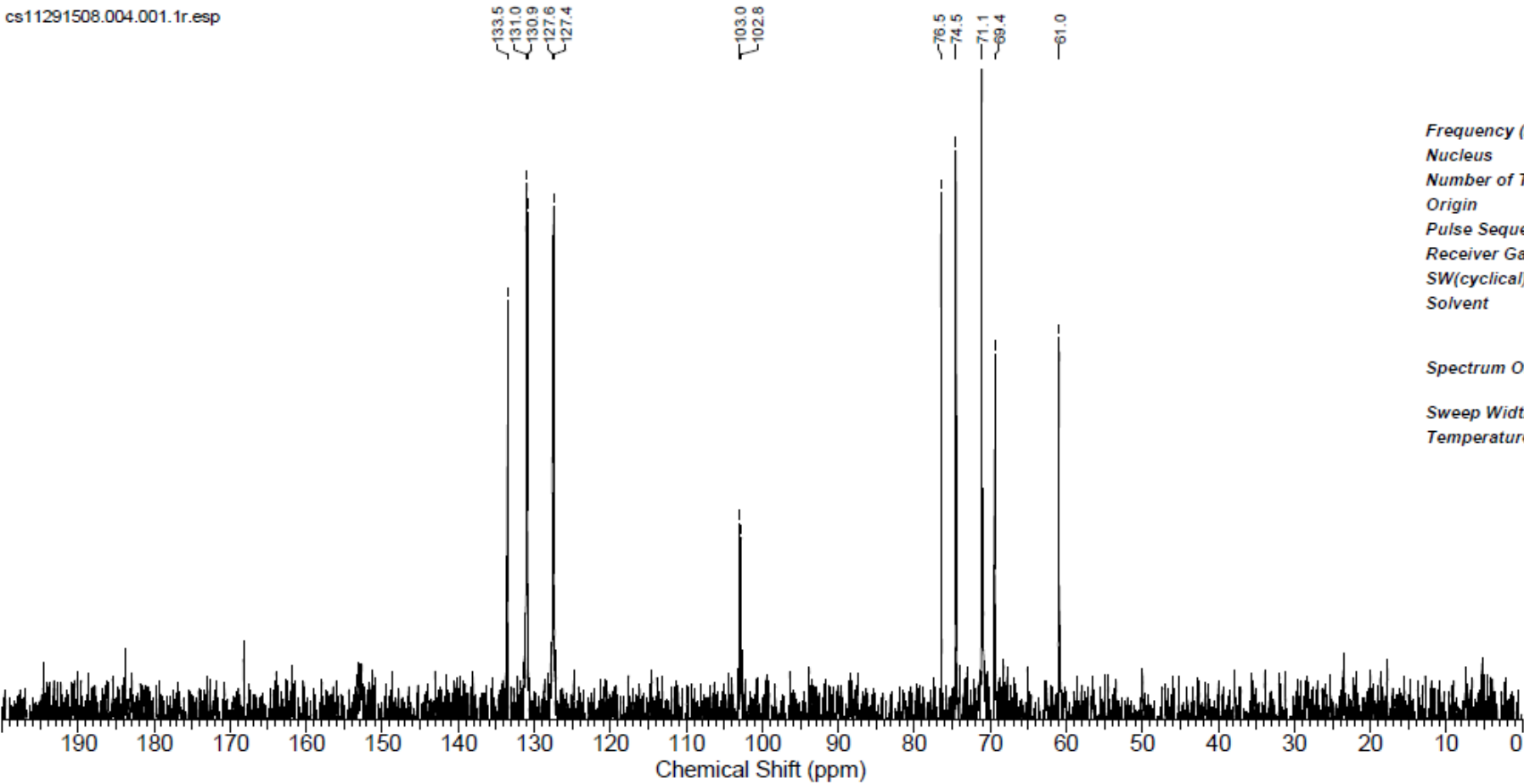


S34



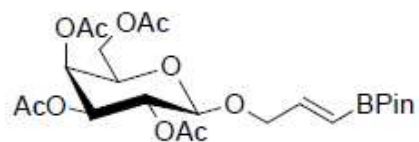
¹³C NMR (100 MHz, CDCl₃)

cs11291508.004.001.1r.esp

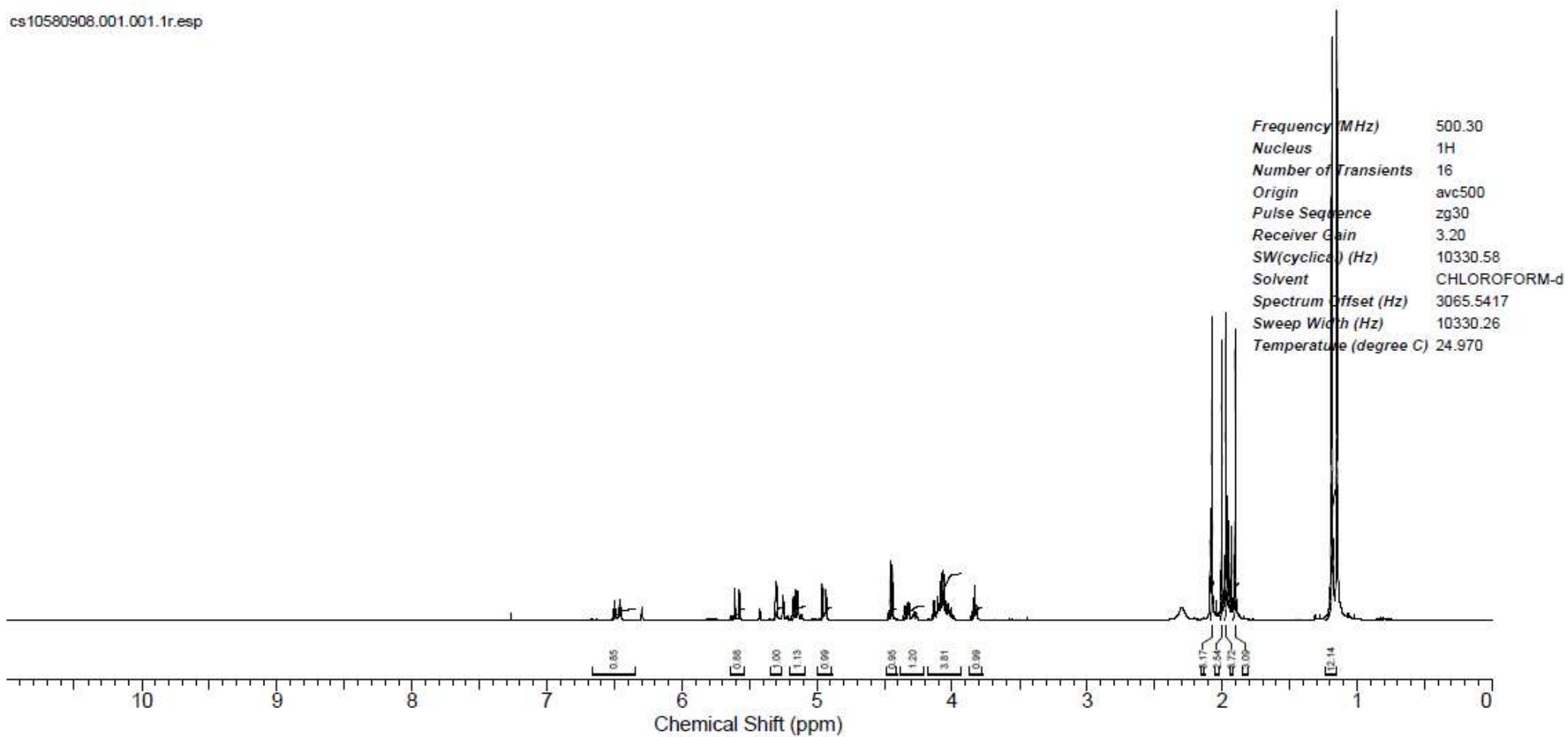


Frequency (MHz)	125.80
Nucleus	13C
Number of Transients	3072
Origin	avc500
Pulse Sequence	zgpg30
Receiver Gain	912.00
SW(cyclical) (Hz)	31250.00
Solvent	DEUTERIUM OXIDE
Spectrum Offset (Hz)	12580.10 94
Sweep Width (Hz)	31249.05
Temperature (degree C)	24.970

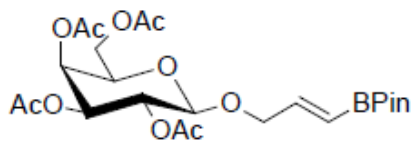
S35

 ^1H NMR (400 MHz, CDCl_3)

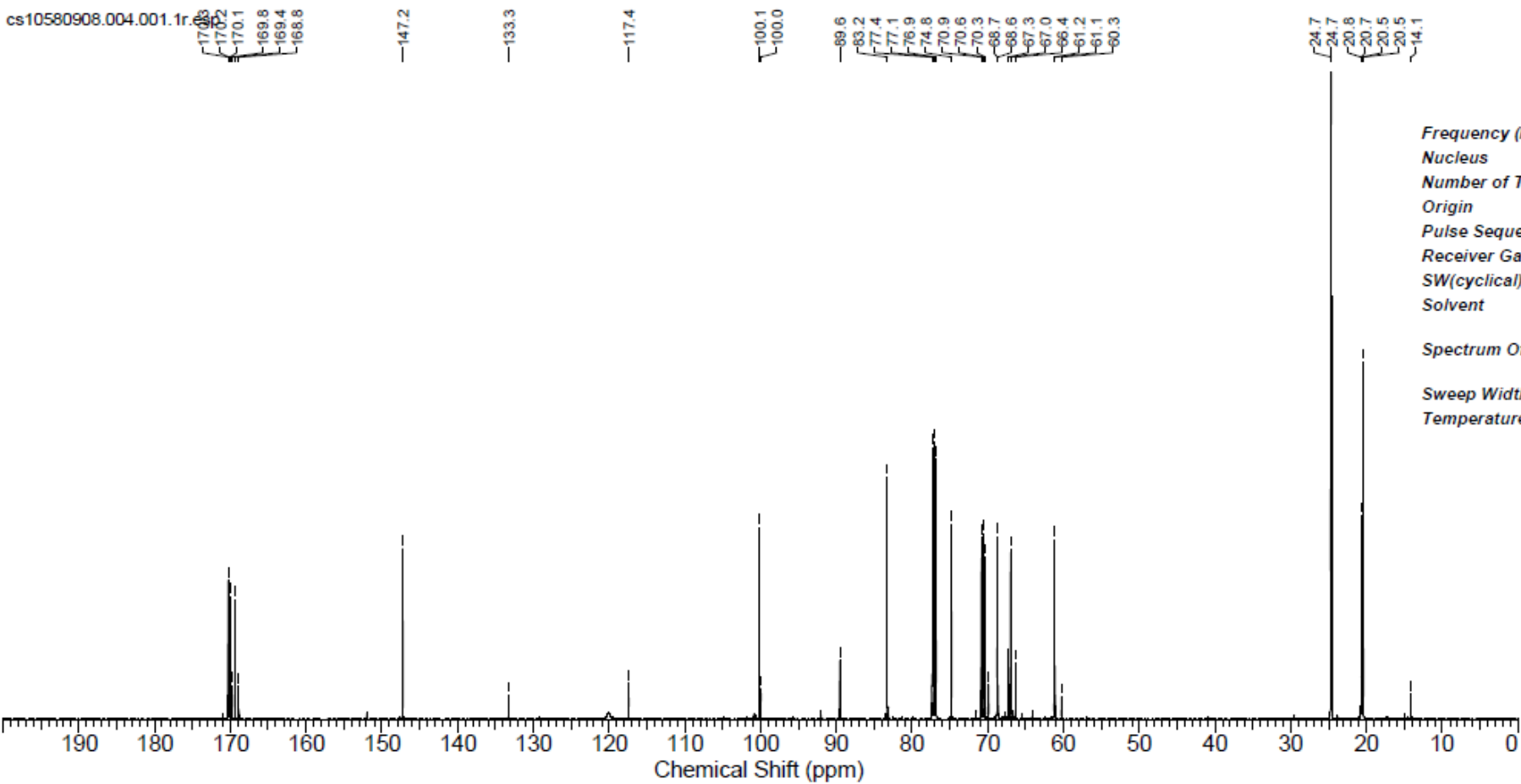
cs10580908.001.001.1r.esp



S36

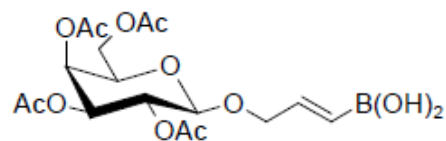


¹³C NMR (100 MHz, CDCl₃)

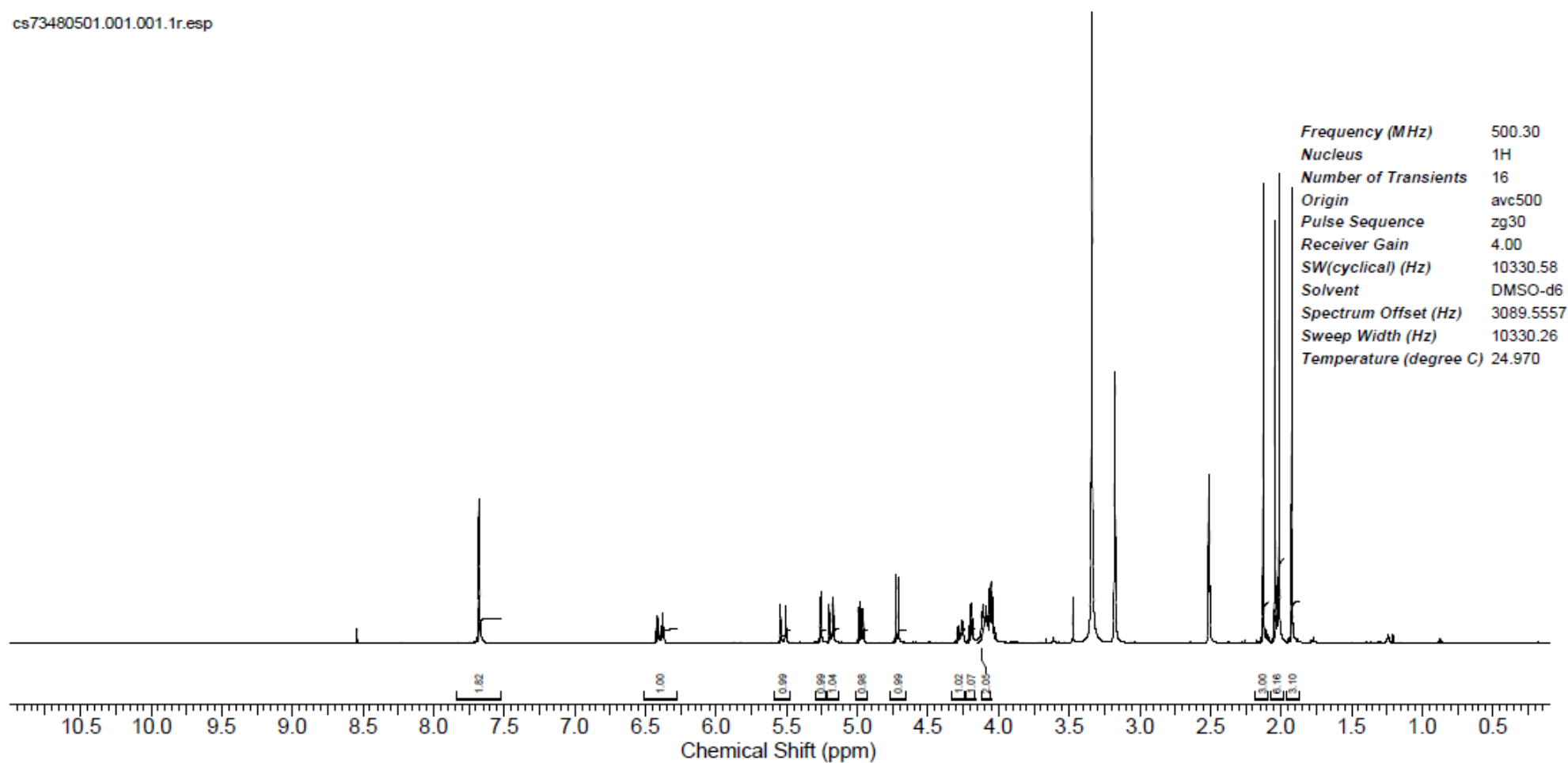


Frequency (MHz)	125.80
Nucleus	13C
Number of Transients	256
Origin	avc500
Pulse Sequence	zgpg30
Receiver Gain	1030.00
SW(cyclical) (Hz)	31250.00
Solvent	CHLORO FORM-d
Spectrum Offset (Hz)	12571.30
	47
Sweep Width (Hz)	31249.05
Temperature (degree C)	24.970

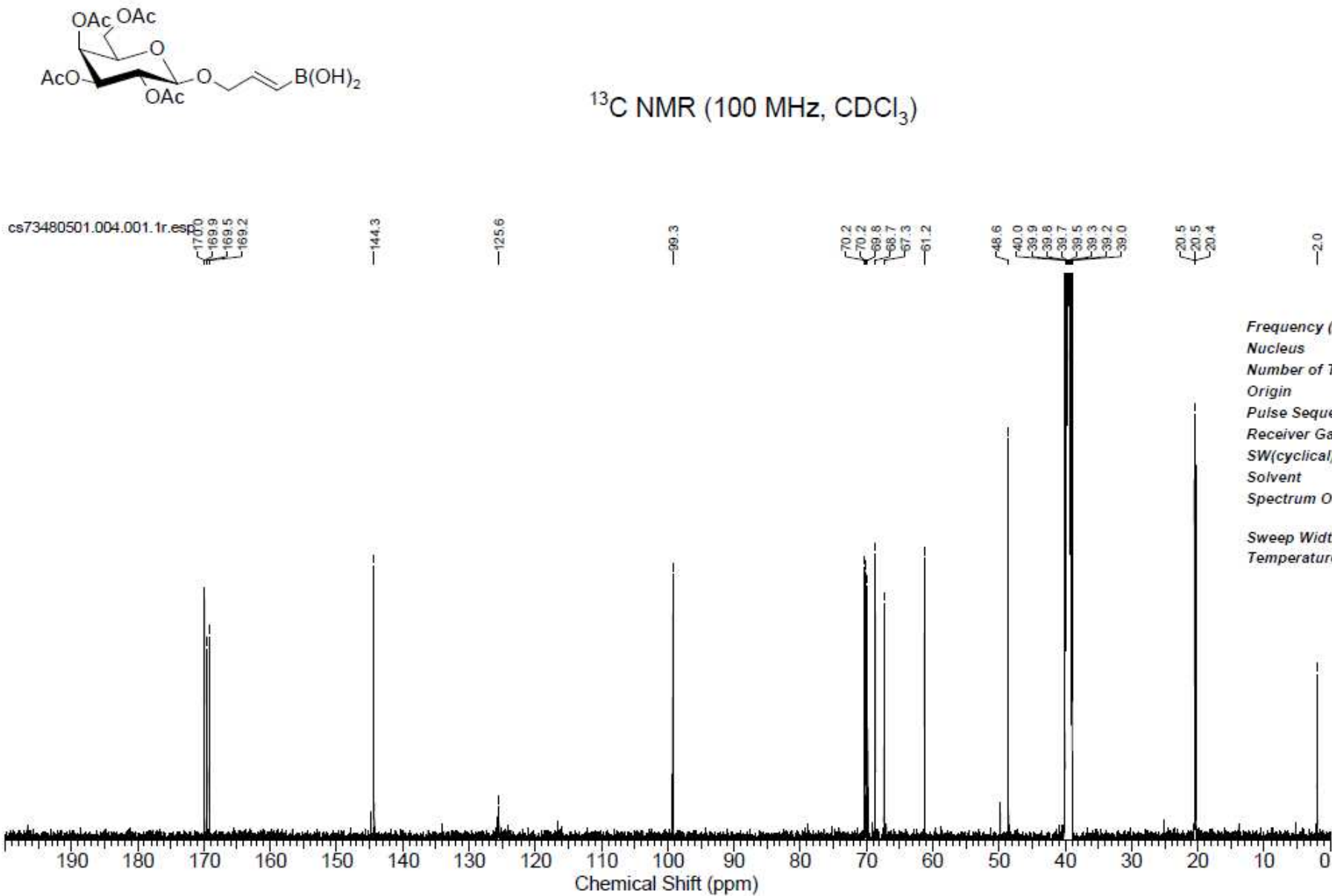
S37

 ^1H NMR (400 MHz, CDCl_3)

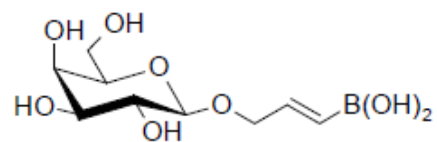
cs73480501.001.001.1r.esp



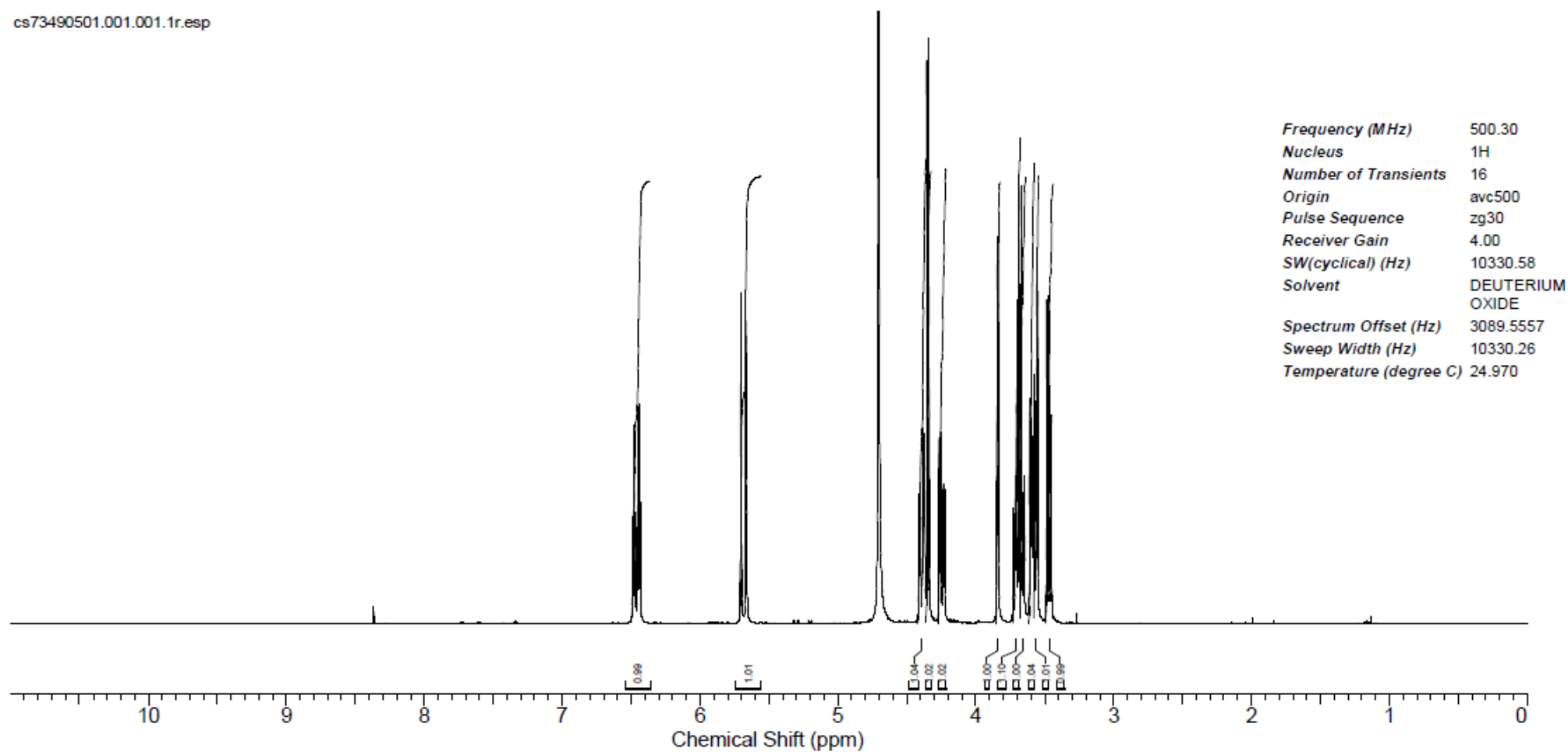
S38



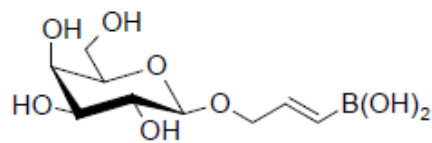
S39

¹H NMR (400 MHz, CDCl₃)

cs73490501.001.001.1r.esp

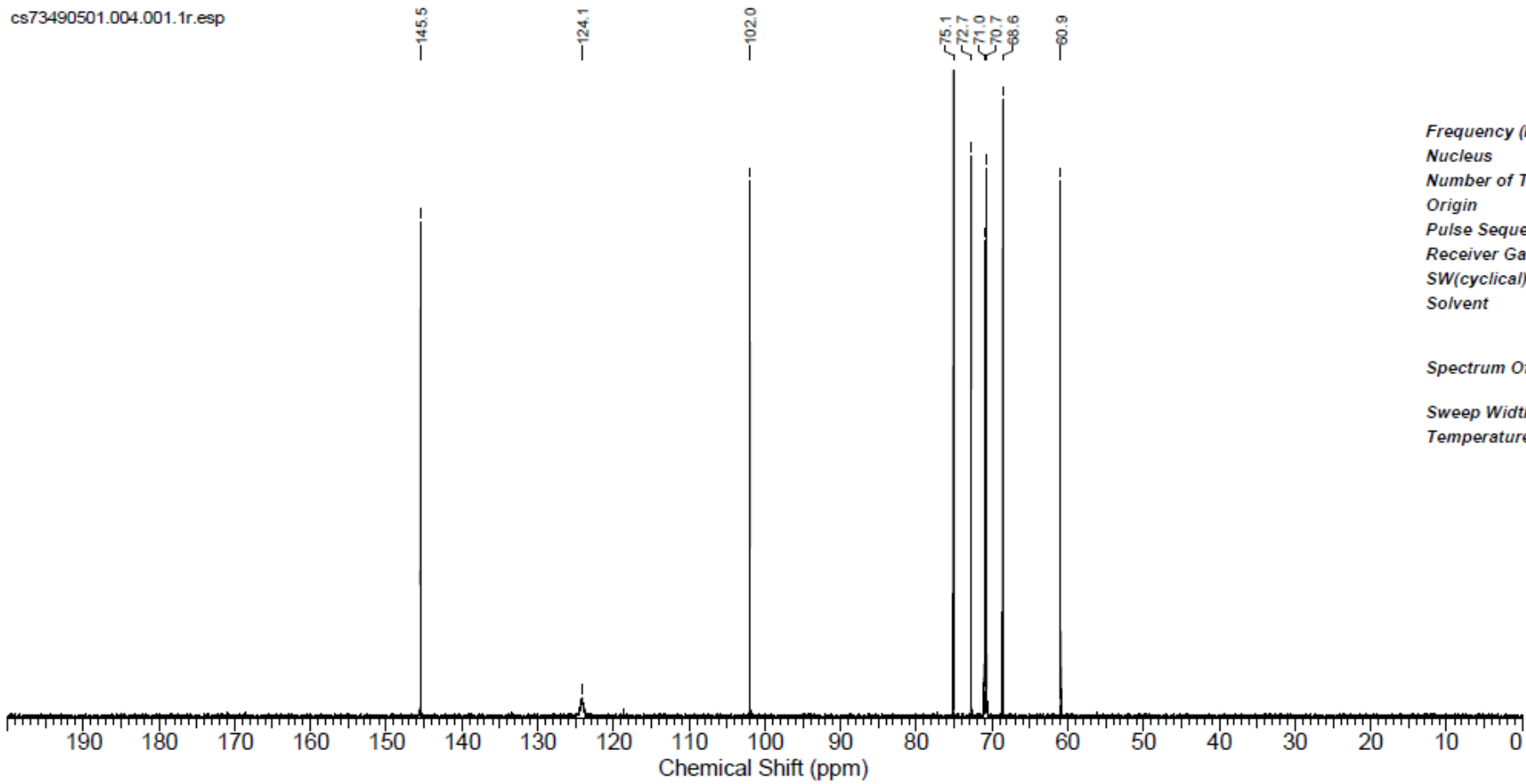


S40



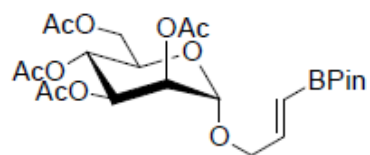
¹³C NMR (100 MHz, CDCl₃)

cs73490501.004.001.1r.esp

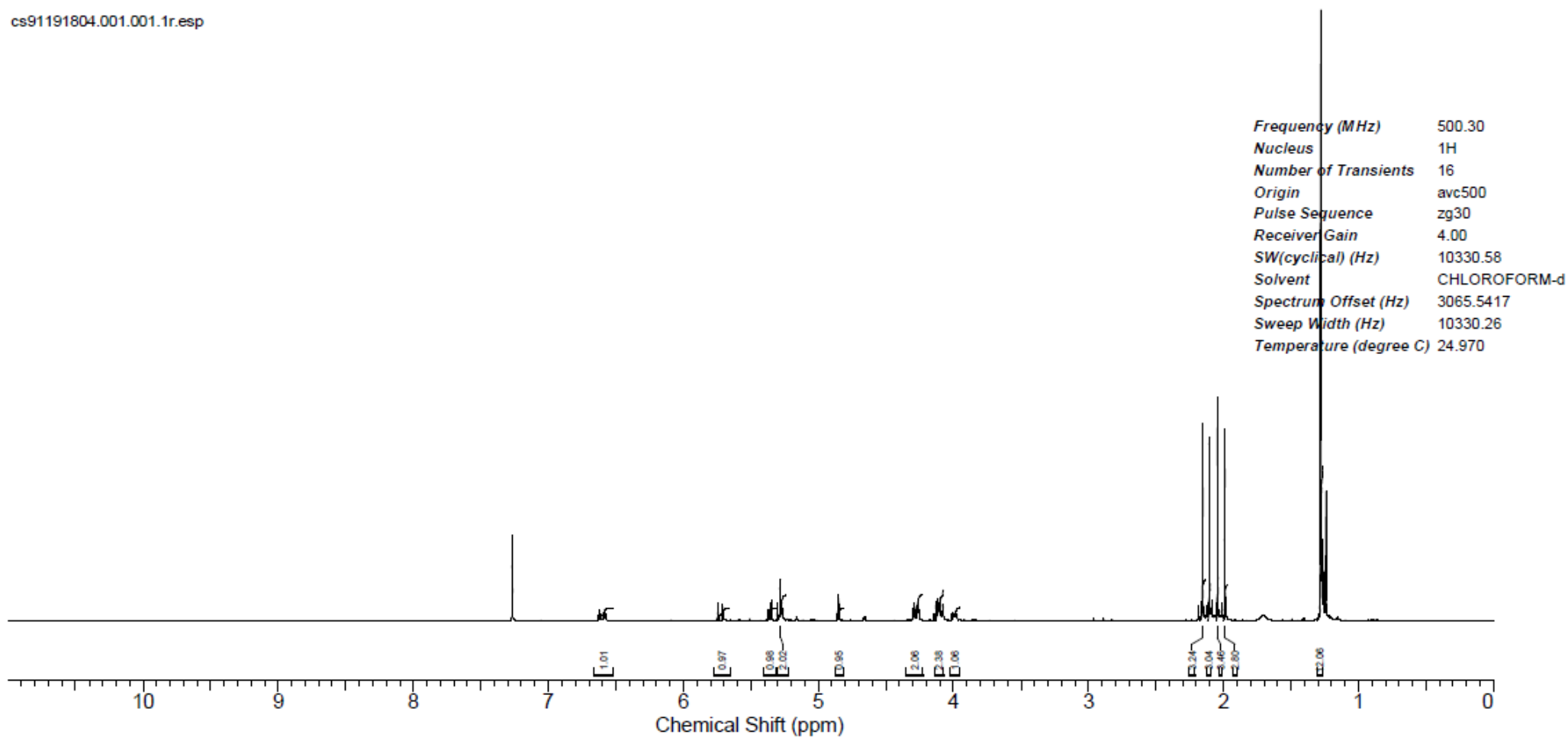


Frequency (MHz)	125.80
Nucleus	13C
Number of Transients	512
Origin	avc500
Pulse Sequence	zgpg30
Receiver Gain	1820.00
SW(cyclical) (Hz)	31250.00
Solvent	DEUTERI UM OXIDE
Spectrum Offset (Hz)	12580.10
	94
Sweep Width (Hz)	31249.05
Temperature (degree C)	24.970

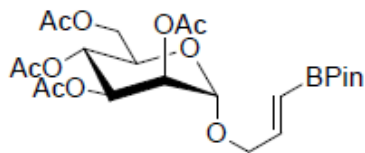
S41

 ^1H NMR (400 MHz, CDCl_3)

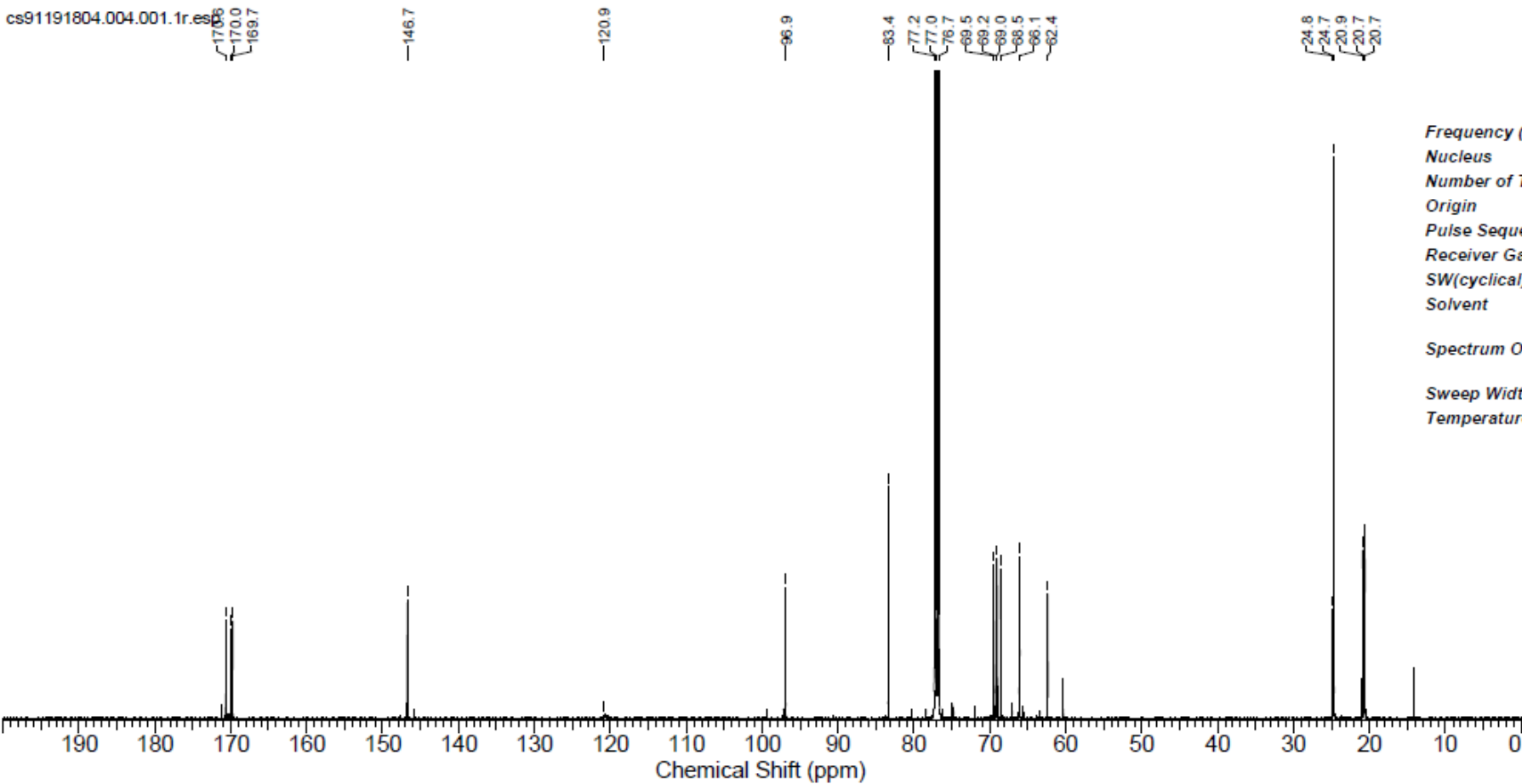
cs91191804.001.001.1r.esp



S42

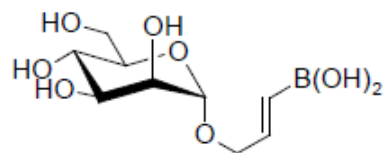


¹³C NMR (100 MHz, CDCl₃)

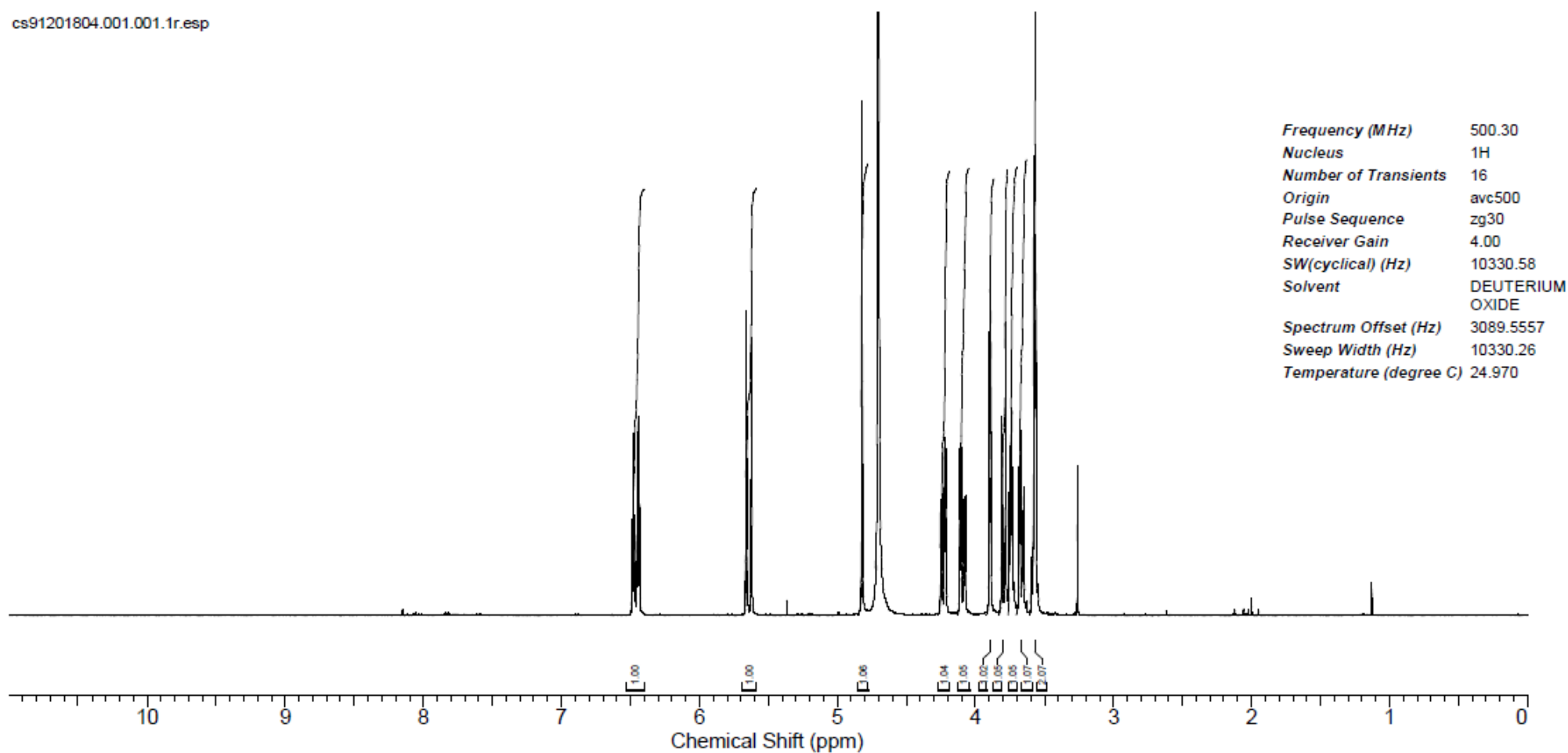


Frequency (MHz)	125.80
Nucleus	¹³ C
Number of Transients	1024
Origin	avc500
Pulse Sequence	zgpg30
Receiver Gain	1820.00
SW(cyclical) (Hz)	31250.00
Solvent	CHLORO FORM-d
Spectrum Offset (Hz)	12571.30 47
Sweep Width (Hz)	31249.05
Temperature (degree C)	24.970

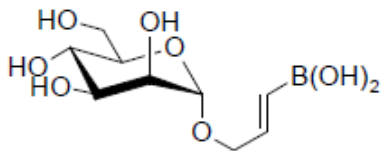
S43

¹H NMR (400 MHz, CDCl₃)

cs91201804.001.001.1r.esp

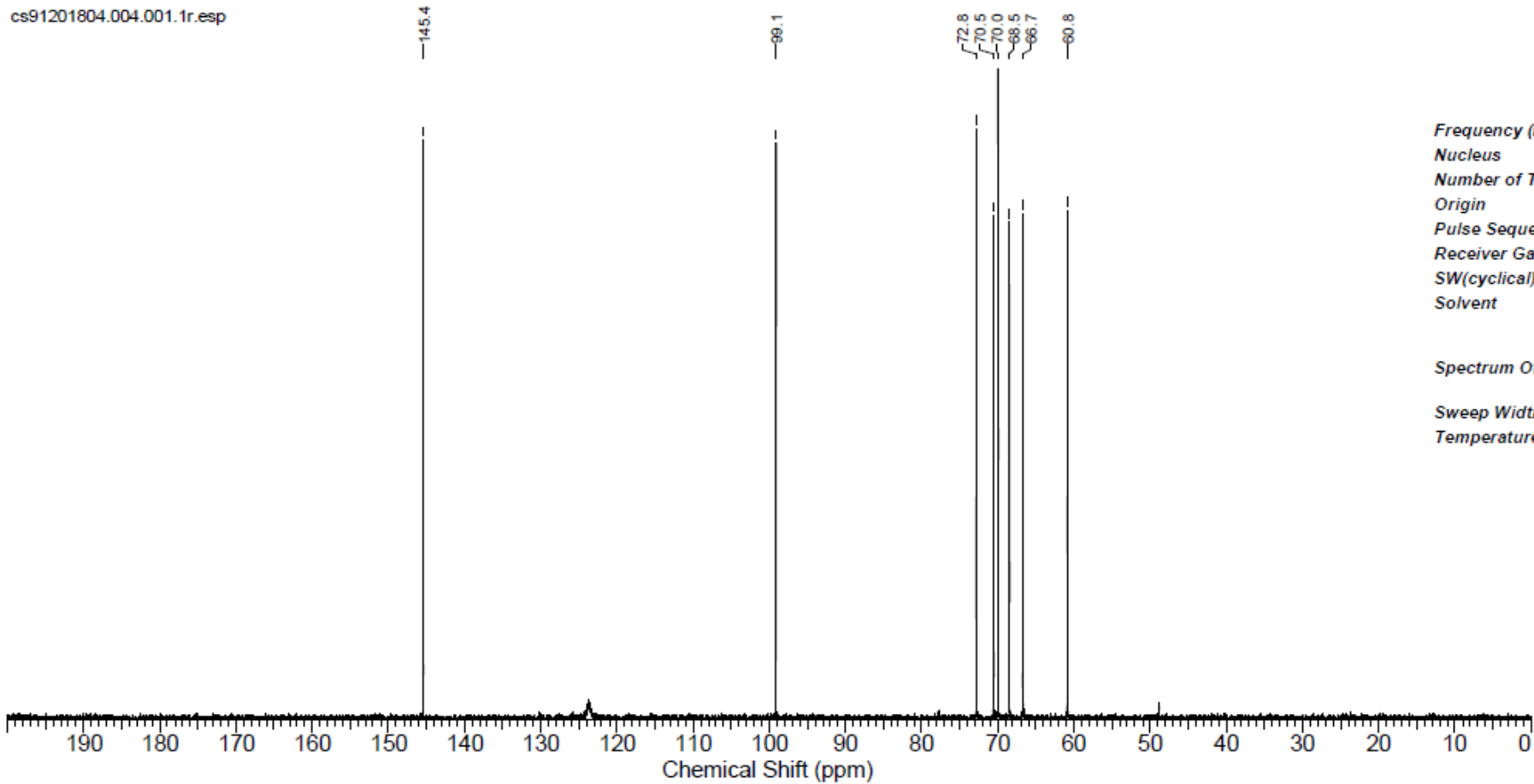


S44



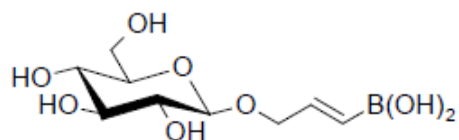
¹³C NMR (100 MHz, CDCl₃)

cs91201804.004.001.1r.esp

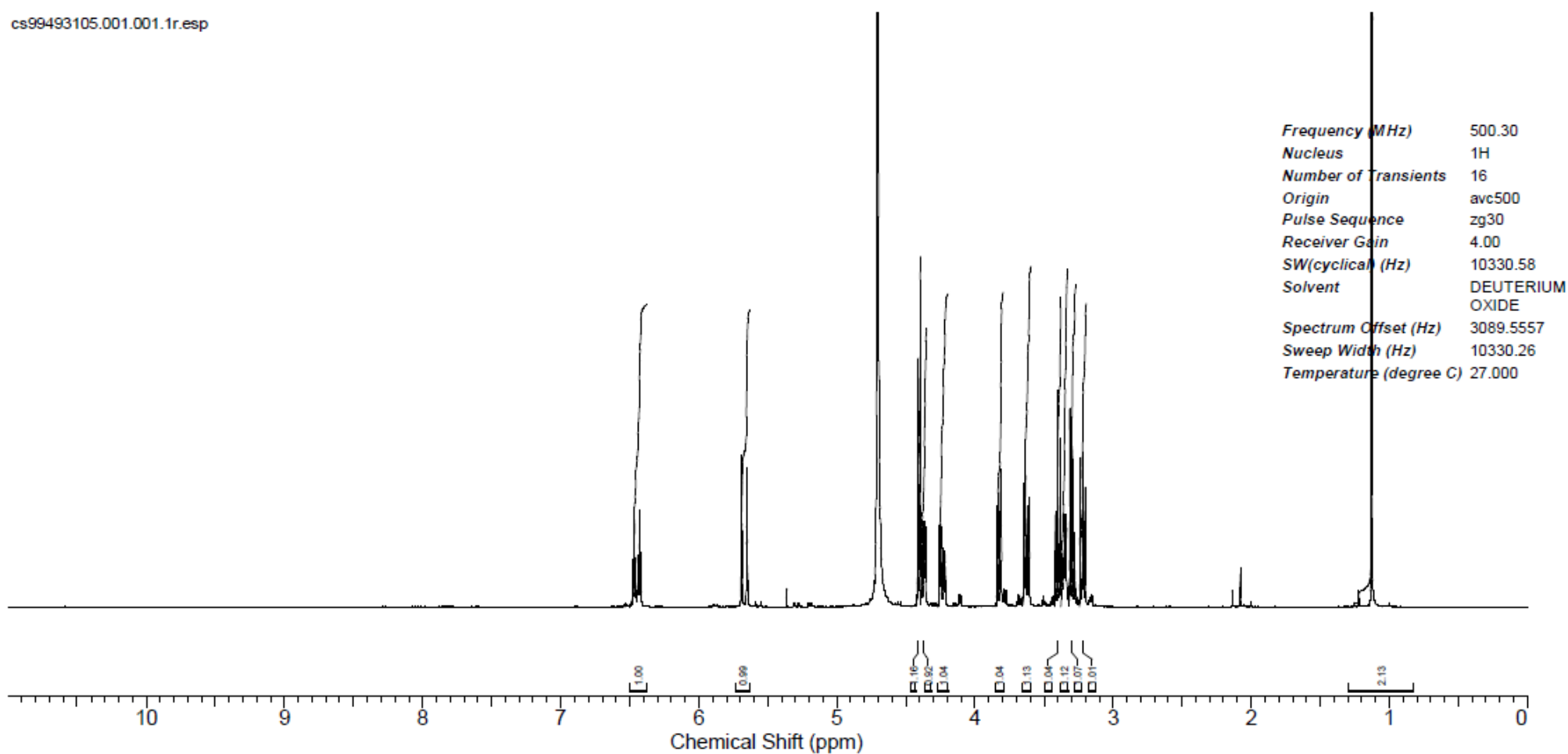


Frequency (MHz)	125.80
Nucleus	13C
Number of Transients	1024
Origin	avc500
Pulse Sequence	zgpg30
Receiver Gain	1820.00
SW(cyclical) (Hz)	31250.00
Solvent	DEUTERIUM OXIDE
Spectrum Offset (Hz)	12580.10 94
Sweep Width (Hz)	31249.05
Temperature (degree C)	24.970

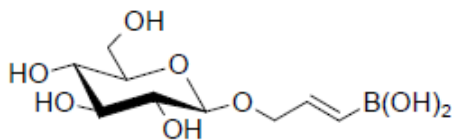
S45

 ^1H NMR (400 MHz, CDCl_3)

cs99493105.001.001.1r.esp



S46



¹³C NMR (100 MHz, CDCl₃)

cs99493105.004.001.1r.esp

145.3

101.4

75.9

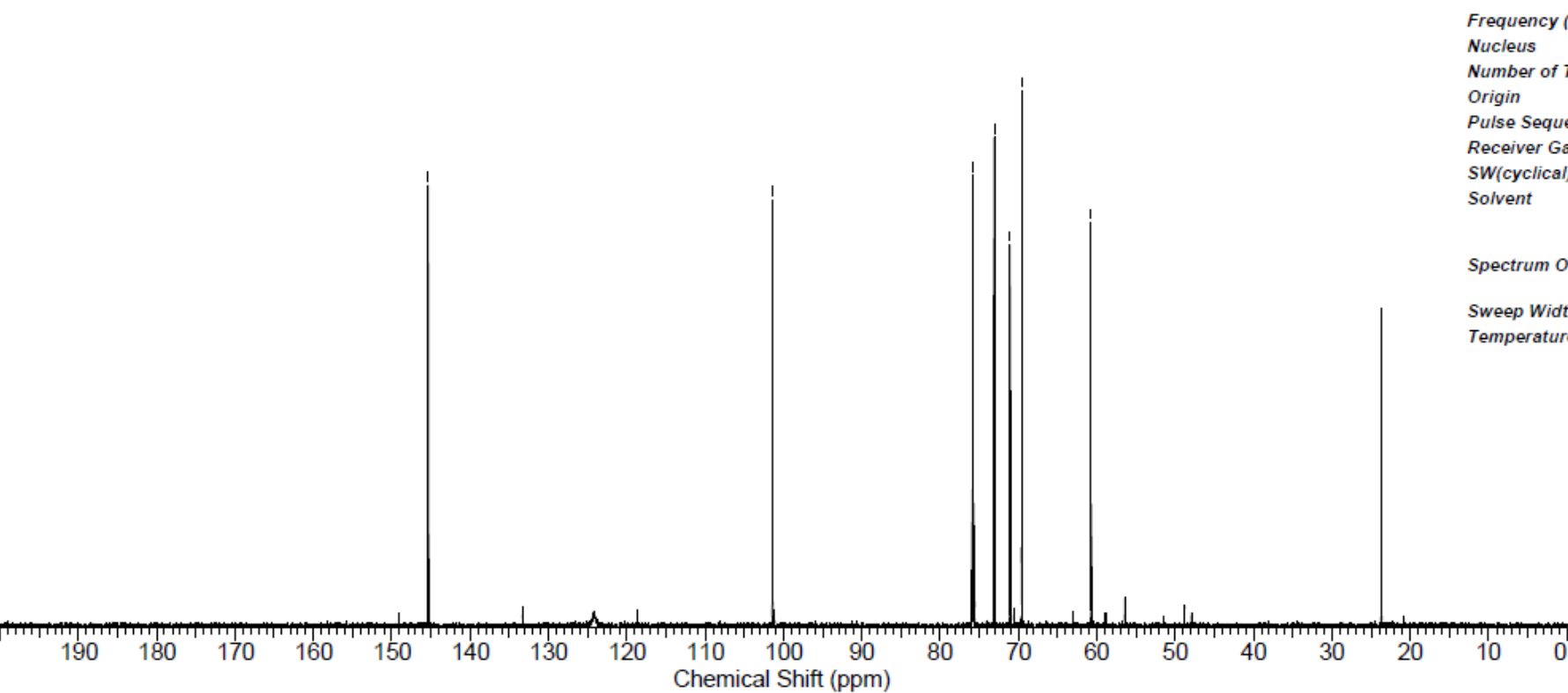
75.7

73.1

71.1

69.6

60.7



Frequency (MHz)	125.80
Nucleus	¹³ C
Number of Transients	1024
Origin	avc500
Pulse Sequence	zgpg30
Receiver Gain	1820.00
SW(cyclical) (Hz)	31250.00
Solvent	DEUTERIUM OXIDE
Spectrum Offset (Hz)	12580.10 94
Sweep Width (Hz)	31249.05
Temperature (degree C)	27.000