METHODS

Glycan microarray analysis

Synthetic oligosaccharides (0.2 mM solutions in printing buffer (50 mM sodium phosphate (NaPi) pH 8.5)), polysaccharides (0.02 or 0.04 µg/ml in printing buffer) and proteins (0.5 µM in printing buffer) were spotted onto "CodeLink" glass slides (SurModics Inc.) using an automated piezoelectric arraying robot (Scienion) at 0.4 nl per spot, incubated for 24 h at room temperature in a humified chamber, quenched using 100 mM ethanolamine in 0.1 M NaPi pH 9 and dried. Slides were blocked with 1% (w/v) BSA in phosphate buffered saline (PBS) and antibody dilutions were applied. Sera were diluted 1:50 or 1:100 unless stated otherwise. Slides were incubated for 16 h at 4 °C, washed with 0.1 % (v/v) Tween-20 in PBS (PBS-T) and incubated with secondary antibodies. Slides were washed, and fluorescence read-out was performed using an Axon GenePix 4300A microarray scanner and GenePix Pro 7 software (MDS). Brightness and contrast of related images (e.g. all sera at different time points of the same animal) were adjusted equally using Photoshop CS5 (Adobe Systems).

Conjugation of tetrasaccharide 12 to CRM197 using *N*-hydroxysuccinimide chemistry

To a stirred solution of di-*N*-succinimidyl adipate (DSAP, 10 mg, 29 µmol) and triethylamine (10 µl, 72 µmol) in anhydrous DMSO (150 µl) was added at room temperature dropwise a solution of tetrasaccharide **12** (approx. 2 mg, 2.8 µmol) in anhydrous DMSO (150 µl). The reaction was stirred for 2 h at that temperature under argon atmosphere and treated with conjugation buffer (100 mM NaPi pH 7.4, 200 µl). The mixture was extracted with chloroform (10 ml) and the phases separated by centrifugation (2 min, 1800 g). The organic phase was discarded and the extraction step was repeated twice. The aqueous layer was separated by centrifugation in a 1.5 ml reaction tube (1 min, 14500 g) and added to a stirring solution of CRM197 (1 mg, 17.3 nmol) in conjugation buffer (1 ml). The mixture was stirred for 16 h at room temperature and dialyzed against water four times to remove excess oligosaccharide using a centrifugal filter (10 kDa MWCO, Millipore). An aliquot was taken for characterization and the mixture was dialyzed three times against PBS. The glycoconjugate was characterized by MALDI/TOF-MS and SDS-PAGE.

Conjugation of synthetic ST8 oligosaccharides to CRM197 using 4-nitrophenyl ester chemistry

To a stirred solution of the oligosaccharide (2 µmol) in a 1:3 (v/v) mixture of anhydrous DMSO and anhydrous pyridine (100-200 µl) were added bis(4-nitrophenyl)adipate (DNAP, 9.3 mg, 24 µmol) and triethylamine (10 µl). The reaction was stirred for 2 h at room temperature under argon atmosphere. The mixture was shock-frozen and lyophilized. The residue was triturated with chloroform (4x0.5 ml) and dichloromethane (4x0.5 ml), transferred to a new reaction tube using DMSO as a solvent and lyophilized again. CRM197 (3 mg, 52 nmol) was dialyzed twice against 0.1 M sodium phosphate buffer pH 8.0 using a centrifugal filter (10 kDa MWCO), concentrated to 300 µl and added to the activated oligosaccharide. The mixture was stirred at room temperature for 16 h and dialyzed against water four times to remove excess oligosaccharide. An aliquot was taken for characterization and the mixture was dialyzed three times against PBS. The glycoconjugates were characterized by MALDI-TOF MS, SDS-PAGE and immunoblot. Conjugates were stable for at least two months at 4 °C (if handled under sterile conditions) or at -20 °C.

Generation of monoclonal antibodies against ST8 frameshift C

Monoclonal antibodies were prepared using BM-Condimed H1 (Roche) according to the manufacturer's instructions. After fusion of plasma cells with P3X63Ag8.653 (ATCC CRL-1580) cells, single clones were generated using limited dilution and two subsequent rounds of subcloning. Antibody production was monitored by glycan array and enzyme-linked immunosorbent assay (ELISA). Thirty-three clones were eventually isolated that produced mAbs recognizing both ST8 frameshift C(**3**) and ST8 CPS (Fig. S6). Clones 1H8C6H4 (termed "1H8") and 1F1F7H2 (termed "1F1") were expanded in ISF-1 serum-free medium (Biochrom) supplemented with Penicillin and Streptomycin (Life Technologies). mAb 1H8 was purified using a Protein G Antibody Purification kit (Pro-Chem). mAb 1F1 was purified by gel filtration chromatography using a Superdex HiLoad 16/600 200 prep grade column mounted on an ÄKTA 900 system (GE Healthcare). mAb isotypes were determined using a Mouse Isotyping Test Kit (AbD Serotec) and purity was confirmed by SDS-PAGE (fig. S6, B and CB). Antibodies were stored in PBS supplemented with 0.02% (w/v) sodium azide at 4 °C or in 50% (v/v)

glycerol/PBS at -20 °C. mAbs were dialyzed against azide-free PBS before application in animal or cell-based experiments.

Isotype-matched control mAbs employed were an anti-*Y. pestis* lipopolysaccharide core trisaccharide mAb IgG1 (clone 1E12) and a purified mouse myeloma IgM (cat. no. 02-6800, Invitrogen). Fluorescent antibodies were used from commercial sources: Goat anti-Rabbit IgG H+L FITC conjugate (abcam), goat anti-human IgG H+L Alexa Fluor 647 conjugate (life Technologies), goat anti-mouse IgG H+L FITC conjugate (Sigma-Aldrich), Goat anti-Mouse IgG H+L Alexa Fluor 635 conjugate (life Technologies), Goat anti-mouse IgM H chain Alexa Fluor 680 conjugate (life Technologies), donkey anti-mouse IgM H chain Alexa Fluor 594 conjugate (dianova).

Secondary antibodies used for ELISA and immunoblot were horseradish peroxidase (HRP)-labeled: goat anti-mouse IgG HRP conjugate (dianova), goat anti-mouse IgM H chain HRP conjugate (life Technologies) or goat anti-rabbit IgG (whole molecule)-peroxidase conjugate (Sigma-Aldrich) and used according to the manufacturers' specifications.

Immunofluorescence of UV-inactivated *S. pneumoniae*

S. pneumoniae serotype 8 (ATCC 6308), serotype 1 (ATCC 6301) or serotype 3 (PN36, NCTC7978) bacteria (a gift from S. Hammerschmidt, Universität Greifswald) were plated from frozen stocks on Columbia Agar plates with 5% (v/v) sheep blood, grown for approx. 9 h at 37 °C/5% CO_2 and inoculated as single colonies in Todd Hewitt Broth with 0.5% (w/v) yeast extract (growth medium). Bacteria were cultured at 37 °C/5% CO_2 to log phase (OD_{600} approx. 0.3) and harvested by centrifugation. For UV-inactivation, bacteria were washed with PBS once, harvested, suspended in PBS to approx. $4x10^8$ colony-forming units (cfu)/ml and inactivated by irradiation at λ = 254 nm for 10 min at room temperature. Cells were harvested, washed once with PBS and frozen at approx. $8x10^8$ cfu/ml in growth medium supplemented with with 20% (v/v) glycerol (freezing medium) at -20 °C.

Bacteria were thawed, harvested by centrifugation (16800 g, 15 min, r.t.) and washed once in 50 mM $NaHCO_3$, 100 mM NaCl, pH 7.5 (Buffer A). Cells were resuspended in Buffer A (1 ml) and treated with fluorescein isothiocyanate (FITC, Sigma-Aldrich) solution (10 mg/ml in DMSO) to a final FITC concentration of 0.1 mg/ml. Bacteria were labeled in the dark for 1 h at 37 °C, harvested by centrifugation and washed twice with 0.25% (w/v) BSA in PBS (1 ml).

Labeling was monitored by fluorescence microscopy using an Axio Imager.M2 system equipped with a LSM 700 confocal laser scanning microscope (Carl Zeiss Microscopy). Cells were suspended in 1% (w/v) BSA in PBS (1 ml for ST8, 0.5 ml for ST1) and the suspension was distributed into two aliquots. The suspensions were treated with mAb 1H8 or mAb 1E12 as an isotype control mAb to a final antibody concentration of 10 µg/ml. Bacteria were incubated in the dark for 16 h at 4 °C under agitation and washed with 1% (w/v) BSA in PBS (0.5 ml). The cells were suspended in a solution of goat anti-mouse IgG-Alexa Fluor 635 conjugate (1:100 dilution in 200 µl 1% (w/v) BSA in PBS, Invitrogen), incubated in the dark for 1.5 h at room temperature and washed with 1% (w/v) BSA in PBS and PBS (0.5 ml, respectively). Fluorescently labeled bacteria were visualized by fluorescence microscopy and images were processed with using Zen 2011 software (Carl Zeiss Microscopy).

Flow cytometry of fluorescently labeled bacteria

S. pneumoniae ST8, ST1, and ST3 were UV-inactivated, FITC-labeled and treated with a fluorescent secondary antibody (anti-mouse IgG-Alexa Fluor 635 conjugate or anti-mouse IgM-Alexa Fluor 680 conjugate) as described in "Immunofluorescence of UV-inactivated *S. pneumoniae*". Flow cytometry was performed by counting 10,000 bacteria using a FACSCanto II flow cytometer (BD Pharmingen) and analyzed using FlowJo software (Tree Star Inc).

Surface plasmon resonance

Surface plasmon resonance was performed on a Biacore T100 instrument (GE Healthcare). Murine antibodies were immobilized using the Mouse Antibody Capture Kit and Amine Coupling Kit (GE Healthcare). Approx. 10,000 response units (RU) of capture antibody were immobilized. A commercial mouse IgG (Invitrogen) was immobilized as a dummy in the reference cell (approx. 10,000 RU). Approx. 1000 RU of mAb 1H8 or 500 RU of mAb 1F1 were captured prior to every run. Runs were performed using PBS (for ST8 CPS analyte) or PBS supplemented with 0.001% (v/v) Tween 20 (for synthetic oligosaccharide analytes) as running buffers at a flow rate of 30-50 µl/min with 120 s association and 280-600 s dissociation periods. Flow cells were regenerated using 10 mM glycine-HCl pH 1.7 and 100 mM glycine-NaOH pH 12 with 0.3% (v/v) Triton-X100. To calculate the concentration of ST8

polysaccharide, a molecular weight of 150 kDa was assumed (*14*). Affinities and standard errors were calculated using Biacore T100 Evaluation Software (GE Healthcare).

To generate ST8 CPS fragments, a literature protocol was applied (*16*). Briefly, ST8 CPS (200 µg) in 0.5 M aqueous trifluoroacetic acid (200 µl) was warmed to 100 °C in a sealed tube. The solution was directly shock-frozen and lyophilized to remove excess acid.

Immobilization of oligosaccharides was performed using the Amine coupling Kit (GE Healthcare) according to the manufacturer's recommendations with 0.66 mM glycan solutions in printing buffer. Approx. 200 RU of each glycan were immobilized.

Enzyme-linked immunosorbent assay (ELISA)

ELISA was performed using Costar high-binding polystyrene 96-well plates (Corning). Plates were coated using native pneumococcal polysaccharides (SSI Diagnostica) at a concentration of 10 µg/ml in PBS for 20 h at 4 °C. Plates were blocked with 10% (v/v) fetal calf serum in PBS for 2 h at 37 °C and washed once with PBS-T. Human pooled pneumococcal antiserum (WHO 1st International Standard for Human Anti-pneumococcal capsule Reference Serum, prod. no. 007sp, NIBSC) and pneumococcal ST8 typing serum (Type 8 Neufeld antiserum, SSI Diagnostica) were pre-incubated with pneumococcal cell wall polysaccharide (CWPS, SSI Diagnostica) at a concentration of 10 µg/ml in PBS. Sera from ST8 glycoconjugate-immunized rabbits were pre-adsorbed with CWPS and *S. pneumoniae* serotype 22F CPS (SSI Diagnostica) at concentrations of 5 µg/ml each to abrogate non-specific binding. After applying antibody samples (30-50 µl), plates were incubated for 1 h at 37 °C, washed with PBS-T three times and treated with a horseradish peroxidase (HRP)-labeled secondary antibody (see below). Plates were washed with PBS-T three times and HRP activity was measured with TMB substrate (BD Biosciences) according to the manufacturer's instructions. Endpoint titers were determined as the reciprocal of the highest dilution resulting in an absorbance above a value of 0.1 arbitrary units.

Characterization of serotype 8 glycoconjugate adsorption to Prevnar 13

A flow cytometry-based assay was used to assess adsorption of ST8 glycoconjugates to Prevnar 13. Aliquots of 10% (50 µl) of a dose of Prevnar 13 were mixed with different amounts of ST8 glycoconjugates, equivalent to 25%, 100% or 400% of the normal glycan content (2.2 µg glycan/dose) of CPSs of other serotypes in the vaccine. Suspensions were

incubated overnight at 4 °C, particles were harvested (3000 g, 5 min, room temperature) and blocked with 10% (w/v) BSA in PBS (50 µl) for 30 min at room temperature. Particles were harvested and incubated with primary antibody samples (1:100 dilutions of ST1 typing serum and 10 µg/ml mAb 1H8 in 1% (w/v) BSA in PBS) for 30 min at room temperature. Particles were harvested, washed once with PBS (100 µl) and incubated with fluorescently labeled secondary antibodies (1:100 dilutions in 1% (w/v) BSA in PBS, 100 µl) for 20 min at room temperature. Particles were washed, suspended in PBS and analyzed by flow cytometry. Control samples included non-treated or CRM197-treated (9.2 µg CRM197/aliquot, corresponding to the CRM197 dose of the highest ST8 glycoconjugate concentration) Prevnar 13 particles. Gates were set by omitting primary antibodies. Pooled murine sera against Prevnar 13 were used as a positive control for mouse antibody binding. Co-adsorbed glycoconjugates were stable for at least one week after adsorption.

Synthetic chemistry

Commercial grade solvents and reagents were used for chemical reactions unless stated otherwise. Anhydrous solvents were obtained from a Dry Solvent System (Waters). Solvents for chromatography were of technical grade and distilled under reduced pressure prior to use. Sensitive reactions were carried out in oven-dried glassware and under an argon atmosphere. Molecular sieves were activated by heating under high vacuum prior to use. Analytical thin layer chromatography (t.l.c.) was performed on Kieselgel 60 F254 glass plates pre-coated with silica gel of 0.25 mm thickness (Macherey-Nagel). Spots were visualized with sugar stain (0.1% (v/v) 3-methoxyphenol, 2.5% (v/v) sulfuric acid in EtOH) or CAM stain (5% (w/v) ammonium molybdate, 1% (w/v) cerium(II) sulfate, and 10% (v/v) sulfuric acid in water) dipping solutions. Flash chromatography was performed on Kieselgel 60 with 230-400 mesh (Sigma-Aldrich). Solvents were removed under reduced pressure using a rotary evaporator and high vacuum (<1 mbar). Freeze-drying of aqueous solutions was performed using an Alpha 2-4 LD Lyophilizer (Christ).

High performance liquid chromatography (HPLC)

HPLC chromatograms were recorded on a 1100 Series chromatography system using a 1200 evaporating light scattering detector (ELSD) (Agilent). Preparative HPLC was performed on a 1200 Series chromatography system with ELSD detection (Agilent). Deprotected

oligosaccharides were analyzed by reverse-phase HPLC (column: Hypercarb, 150 X 4.6 mm (Thermo Scientific); flow rate: 0.7 ml/min; eluents: 0.1% (v/v) formic acid in water (A) and 0.1% (v/v) formic acid in MeCN (B); gradient: 0% B (10 min), 0-30% (in 30 min), 30-100% (in 5 min), 100% (varying time); detection: ELSD).

For preparative HPLC purification of tetrasaccharides **SI-1**, **SI-2**, **SI-3** and **SI-4**, the crude residue after photocleavage (see below) was carefully dissolved in a minimum volume of CH_2Cl_2 and 0.9 ml of 20% (v/v) hexane in EtOAc. The crude solution was injected for purification (column: Luna® 5µ Silica, 260 X 10 mm (Phenomenex); flow rate: 5 ml/min; eluents: 5% (v/v) CH_2Cl_2 in hexanes (A) and 5% (v/v) CH_2Cl_2 in EtOAc (B); gradient: 20% B (5 min), 20-60% B (in 40 min) 60-100% (in 5 min); detection: UV absorption at λ = 280 nm and ELSD) to afford protected tetrasaccharides **SI-1**, **SI-2**, **SI-3** and **SI-4**.

For preparative HPLC of tetrasaccharides **1**, **2**, **3** and **4**, the residue after global deprotection was injected for purification (column: Hypercarb, 150 X 10 mm (Thermo Scientific); flow rate: 3.6 ml/min; eluents: 0.1% (v/v) formic acid in water (A) and 0.1% (v/v) formic acid in MeCN (B); gradient: 0% B (10 min), 0-30% (in 30 min), 30-100% (in 5 min); detection: ELSD) to afford the corresponding unprotected oligosaccharide.

Nuclear magnetic resonance (NMR) spectroscopy

^1H, ^{13}C, and two-dimensional NMR spectra were measured with a Varian 400-MR spectrometer or a Varian 600 spectrometer at 296 K (Agilent). Chemical shifts (δ) are reported in parts per million (ppm) relative to the respective residual solvent peaks ($CDCl_3$: δ 7.26 in ^1H and 77.16 in ^{13}C NMR; acetone-D_6: δ 2.05 in ^1H and 29.84 in ^{13}C NMR; D_2O: δ 4.79 in ^1H NMR). Two-dimensional NMR experiments (HH-COSY, CH-HSQC, CH-HMBC) were performed to confirm newly-formed linkages. The following abbreviations are used to indicate peak multiplicities: *s* singlet; *d* doublet; *dd* doublet of doublets; *t* triplet; *dt* doublet of triplets; *m* multiplet. Coupling constants (*J*) are reported in Hertz (Hz). Optical rotation (OR) measurements were carried out with a UniPol L1000 polarimeter (Schmidt+Haensch) at λ = 589 nm and a concentration (c) expressed in g/100 ml in the solvent noted in parentheses. High resolution mass spectrometry by electrospray ionization (ESI-HRMS) was performed at Freie Universität Berlin, Mass Spectrometry Core Facility, with an Agilent 6210 ESI-TOF mass spectrometer. Matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) high resolution mass spectra were recorded on a Daltonics Autoflex Speed

spectrometer (Bruker, Billerica, USA). Infrared (IR) spectra were measured with a Spectrum 100 FTIR spectrometer (Perkin-Elmer, Waltham, USA).

The syntheses of deprotected saccharides **14** and **16**-**19** as well as precursors **27** and **41** are reported elsewhere (*17*).

Automated assembly of ST8 tetrasaccharides 1-4

All automated glycosylations were performed on a prototype automated oligosaccharide synthesizer using anhydrous solvents. ST8 CPS-related tetrasaccharides **1**-**4** were synthesized by automated glycan assembly using a polystyrene resin equipped with photocleavable linker **5** (*10*) and orthogonally protected building blocks **6** (*39*), **7** (*10*), **8**, **9** (*31*), **10** (*31*) and **11** (*40*) (Fig. S1). Permanent protecting groups at the C2-positions were chosen to confer either 1,2-*trans*-selectivities (benzoate esters at C2-positions in building blocks **6** and **7**) or 1,2-*cis*-selectivities (benzyl ethers at C2-positions in building blocks **8**-**11**) during glycosylations. Further enhancement of 1,2-*cis*-selectivities was achieved by employing ester or carbonate protecting groups at the C4-positions in galactose building blocks **8** and **11**, or at the C6-positions in glucose building blocks **9** and **10**. Following photocleavage from the resin, protected tetrasaccharides **SI-1**, **SI-2** and **SI-4** were obtained as the major products with minor amounts of impurities. Tetrasaccharide **SI-3** was isolated along with the respective reducing end β-anomer as the major product (not shown). All protected tetrasaccharides were purified by preparative HPLC, subjected to a two-step global deprotection sequence and HPLC purification to yield ST8 CPS frameshifts A-D(**1**-**4**). Analytical HPLC traces of the purified, deprotected ST8 tetrasaccharides are provided along with the spectral characterization.

Solutions for automated glycan assembly

Building block solutions and equivalents

Glycosylating agents were co-evaporated with anhydrous toluene three times before use.

All glycosylating agents (**6**-**11**) were delivered in two cycles with five equivalents each, using solutions of 0.25 mmol glycosylating agent in 2 ml CH_2Cl_2 (**6**, **7**, **9** and **10**) or a 1:3 (v/v) mixture of CH_2Cl_2 and Et_2O (**8** and **11**).

Acidic TMSOTf wash

For acidic TMSOTf washes, a solution of TMSOTf (480 µl, 2.71 mmol) in 20 ml CH_2Cl_2 was used.

Thioglycoside activator solution

To activate thioglycosides, *N*-iodosuccinimide (1.35 g, 6.03 mmol) was dissolved in a 1:1 (v/v) mixture of CH_2Cl_2 and 1,4-dioxane (40 ml) and treated with TfOH (60 µL, 0.71 mmol) at 0 °C. This solution was kept at 0 °C.

Glycosyl phosphate activator solution

To activate glycosyl phosphates, a solution of TMSOTf (480 µl, 2.71 mmol) in 20 ml CH_2Cl_2 was used.

Fmoc deprotection solution

A solution of 20% (v/v) triethylamine in DMF was used for Fmoc deprotection. Loading of functionalized resins was determined using a UV-MINI-1240 UV spectrometer (Shimadzu).

Glycosylation temperatures

Glycosylations using glycosylating agents **6**, **7**, **9** and **10** were performed for 5 min at -30 °C, then for 50 min at -10 °C. Glycosylations using glycosylating agents **8** and **11** were performed for 5 min at -40 °C, then for 50 min at -20 °C.

Synthesizer modules

Module 1: acidic TMSOTf wash

The resin was washed with DMF, THF, CH_2Cl_2 (3x2 ml each for 25 s) and TMSOTf wash solution (0.35 ml) for 1 min at -20 °C. The resin was then swollen in CH_2Cl_2 (2 ml) and the temperature of the reaction vessel was adjusted to the glycosylation temperature (*see* above).

Module 2: glycosylation using thioglycosides

Building block solution (1 ml) was delivered to the reaction vessel. After the glycosylation temperature was reached, thioglycoside activator solution (1 ml) was added. Following glycosylation, the reactor was drained and the resin was washed with CH_2Cl_2 (6x2 ml for 15 s). This procedure was repeated.

Module 3: glycosylation using glycosyl phosphates

Building block solution (1 ml) was delivered to the reaction vessel. After the glycosylation temperature was reached, glycosyl phosphate activator solution (1 ml) was added. Following glycosylation, the reactor was drained and the resin was washed with CH_2Cl_2 (6x2 ml for 15 s). This procedure was repeated.

Module 4: Fmoc deprotection

The resin was washed with DMF (6x2 ml for 15 s), swollen in DMF (2 ml) and the temperature of the reaction vessel was adjusted to 25 °C. The resin was treated with Fmoc deprotection solution (2 ml), incubated for 5 min at that temperature, and the reactor was drained. This procedure was repeated two times.

Automated tetrasaccharide assembly

The synthesizer reaction vessel was charged with functionalized polystyrene resin and swollen in CH_2Cl_2 (2 ml). The resin was first washed using Module 1. Reaction and washing steps were performed using Modules 1-4 and the appropriate building block solutions (Table S1).

Photocleavage

Photocleavage of oligosaccharides from the resin was performed as described previously (*10*). Prior to photocleavage, the fluorinated ethylene propylene tubing wrapped around the light source was washed with CH_2Cl_2 (20 ml, flow rate 5 ml/min). The resin was slowly injected from a disposable syringe (20 ml) into the reactor and transported through the tubing using CH_2Cl_2 (18 ml, flow rate 0.6ml/min) to carry out photocleavage. The tubing was washed with CH_2Cl_2 (20 ml, flow rate 2 ml/min). The combined suspension was filtered, the tubing was re-equilibrated with CH_2Cl_2 (20 ml, flow rate 5 ml/min) and the resin was re-subjected to the photocleavage conditions. The combined filtrate was evaporated and the residue was characterized by NMR and mass spectrometry (see below).

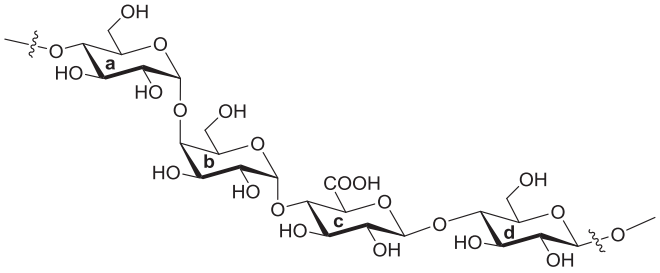
Purification of protected tetrasaccharides SI-1, SI-2, SI-3 and SI-4

Following cleavage from the solid support, protected intermediates were purified by preparative HPLC to give tetrasaccharides **SI-1**, **SI-2**, **SI-3** and **SI-4**.

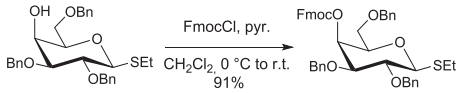
Global deprotection of tetrasaccharides SI-1, SI-2, SI-3 and SI-4

To a stirred solution of the protected tetrasaccharide in MeOH (5 ml) was added at 40 °C NaOMe (0.5 M in MeOH, 58 µL). Upon completion, the reaction was neutralized with Amberlite IR120 (H^+ form), filtered and concentrated. The residue was dissolved in EtOAc/MeOH/AcOH (0.5:5:0.2, 5 ml) and treated with Pd/C (5% (w/w) loading). The suspension was purged with argon and hydrogen, and stirred under hydrogen atmosphere for 16 h at room temperature. The reaction mixture was filtered and concentrated to give the crude deprotected tetrasaccharides **1** (2.6 mg, 3.40 µmol, 45%), **2** (0.8 mg, 1.05 µmol, 38%), **3** (0.9 mg, 1.18 µmol, 22%) and **4** (1.9 mg, 2.48 µmol, 47%)

NMR peaks are assigned as follows:



Ethyl 2,3,6-tri-*O*-benzyl-4-*O*-fluorenylmethoxycarbonyl-1-thio-β-D-galactopyranoside (8)

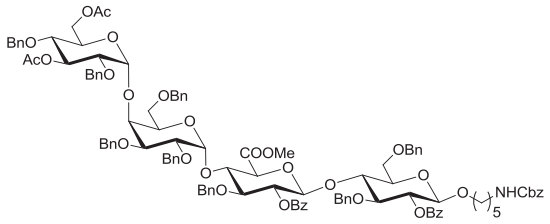


SI-5 **8**

To a stirred solution of alcohol **SI-5** (*40*) (0.61 g, 1.21 mmol) in CH_2Cl_2 (6.1 ml) were added at 0 °C FmocCl (0.63 g, 2.43 mmol) and pyridine (0.29 ml, 3.64 mmol). The reaction mixture was warmed to room temperature and stirred for 16 h. The mixture was diluted with CH_2Cl_2 (12 ml) and HCl (1 M aq. solution, 6 ml). After separation, the aqueous fraction was extracted with CH_2Cl_2 (12 ml), the combined organic fractions were dried over $MgSO_4$, filtered and concentrated. The residue was purified by flash chromatography (EtOAc/CH_2Cl_2/hexanes 1:1:18 to 4:1:16) to give carbonate **8** (0.79 g, 1.11 mmol, 91%) as a white foam. $[\alpha]_D^{20}$ = +5.59 (c = 3.60, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.78 (d, *J* = 7.6 Hz, 2H), 7.65 (d, *J* = 7.5 Hz, 1H), 7.60 (d, *J* = 7.5 Hz, 1H), 7.44 – 7.37 (m, 4H), 7.34 – 7.27 (m, 11H), 7.25 – 7.17 (m, 4H),

5.49 (d, *J* = 2.9 Hz, 1H), 4.87 (d, *J* = 10.2 Hz, 1H), 4.82 (m, 2H), 4.57 (d, *J* = 11.3 Hz, 1H), 4.50 (m, 3H), 4.44 (dd, *J* = 10.4, 7.2 Hz, 1H), 4.32 – 4.27 (m, 1H), 4.21 (t, *J* = 7.5 Hz, 1H), 3.76 (t, *J* = 6.4 Hz, 1H), 3.68 (m, 3H), 3.63 – 3.57 (m, 1H), 2.86 – 2.70 (m, 2H), 1.33 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) *δ* 155.2, 143.8, 143.4, 141.4, 138.3, 137.9, 137.8, 128.6, 128.52, 128.47, 128.4, 128.1, 128.0, 127.9, 127.8, 127.3, 125.6, 125.3, 120.15, 120.12, 85.5, 81.2, 77.9, 76.1, 75.8, 73.9, 72.1, 71.4, 70.2, 68.3, 46.8, 25.1, 15.3; IR (thin film) 3031, 2869, 1748, 1452, 1255, 1101 cm^{-1}; HRMS (ESI) calculated for $C_{44}H_{44}O_7S$ $(M+Na)^+$ 739.2700 found 739.2703 *m/z*.

_N_-Benzyloxycarbonyl-5-amino-pentanyl-3,6-di-_O_-acetyl-2,4-di-_O_-benzyl-α-D-glucopyranosyl-(1→4)-2,3,6-tri-_O_-benzyl-α-D-galactopyranosyl-(1→4)-methyl[2-_O_-benzoyl-3-_O_-benzyl-β-D-glucopyranosyl]uronate-(1→4)-2-_O_-benzoyl-3,6-di-_O_-benzyl-β-D-glucopyranoside (SI-1)

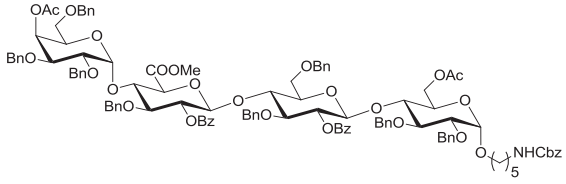


SI-1

Tetrasaccharide **SI-1** (12.9 mg, 6.70 µmol, 23%) was obtained after eleven on-resin steps following the sequence I-II-III-V. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.88 (d, *J* = 8.1 Hz, 2H), 7.83 (d, *J* = 8.0 Hz, 2H), 7.57 (t, *J* = 7.4 Hz, 1H), 7.51 (t, *J* = 7.0 Hz, 1H), 7.45 – 7.18 (m, 37H), 7.15 (m, 2H), 7.10 (m, 2H), 7.06 – 6.97 (m, 6H), 6.97 – 6.89 (m, 2H), 5.58 (t, *J* = 9.7 Hz, 1H), 5.28 (d, *J* = 3.4 Hz, 1H), 5.24 (t, *J* = 8.8 Hz, 1H), 5.17 – 5.09 (m, 2H), 5.04 (s, 2H), 4.86 – 4.69 (m, 6H), 4.64 – 4.38 (m, 10H), 4.32 – 4.27 (m, 3H), 4.23 – 4.16 (m, 2H), 4.09 – 4.00 (m, 2H), 3.97 (dd, *J* = 10.6, 3.4 Hz, 1H), 3.88 – 3.42 (m, 13H), 3.35 (s, 3H), 3.32 – 3.23 (m, 2H), 3.15 (d, *J* = 9.5 Hz, 1H), 2.85 (dd, *J* = 13.0, 6.6 Hz, 2H), 2.01 (s, 3H), 1.94 (s, 3H), 1.47 – 1.33 (m, 2H), 1.31 – 1.17 (m, 2H), 1.17 – 1.01 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.6, 169.9, 168.0, 165.2, 164.7, 156.3, 138.5, 138.4, 138.12, 138.09, 138.03, 137.9, 137.7, 136.8, 133.4, 133.1, 130.1, 129.8, 129.5, 128.8, 128.62, 128.58, 128.53, 128.51, 128.4, 128.3, 128.2, 128.1, 127.84, 127.79, 127.7, 127.4, 127.2, 101.2 ($J_{1,2}$ = 160.8 Hz, C-1d), 100.6 ($J_{1,2}$ = 167.6 Hz, C-1c), 98.8 ($J_{1,2}$ = 173.6 Hz, C-1b), 98.4 ($J_{1,2}$ = 174.0 Hz, C-1a), 81.2, 80.2, 78.3, 77.4, 76.1, 76.0, 75.8 75.2, 74.7, 74.6, 74.4, 74.1, 73.8, 73.7, 73.6, 73.3, 73.2, 72.9, 69.5, 68.5, 67.4, 66.6, 66.2, 62.7, 52.5,

40.9, 29.4, 28.9, 23.2, 21.3, 21.1; HRMS (ESI) calculated for $C_{112}H_{119}NO_{28}$ $(M+Na)^+$ 1948.7811 found 1948.7806 *m/z*.

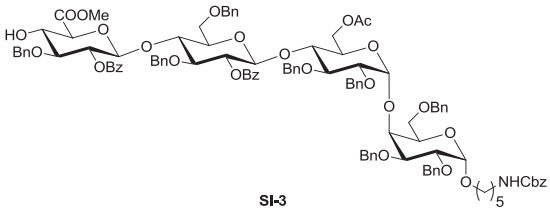
_N_-Benzyloxycarbonyl-5-amino-pentanyl-4-_O_-acetyl-2,3,6-tri-_O_-benzyl-α-D-galactopyranosyl-(1→4)-methyl[2-_O_-benzoyl-3-_O_-benzyl-β-D-glucopyranosyl]uronate-(1→4)-2-_O_-benzoyl-3,6-di-_O_-benzyl-β-D-glucopyranosyl-(1→4)-6-_O_-acetyl-2,3-di-_O_-benzyl-α-D-glucopyranoside (SI-2)



SI-2

Tetrasaccharide **SI-2** (12.1 mg, 6.29 μmol, 21%) was obtained after ten on-resin steps following the sequence IV-I-II-VI. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.90 (d, *J* = 7.3 Hz, 2H), 7.79 (d, *J* = 7.3 Hz, 2H), 7.60 (t, *J* = 7.4 Hz, 1H), 7.56 – 7.51 (m, 1H), 7.44 – 7.17 (m, 36H), 7.14 (dd, *J* = 9.0, 5.5 Hz, 1H), 7.09 – 7.05 (m, 3H), 7.04 – 6.98 (m, 7H), 6.89 (d, *J* = 7.3 Hz, 2H), 5.69 (d, *J* = 1.9 Hz, 1H), 5.33 (d, *J* = 3.7 Hz, 1H), 5.19 (dd, *J* = 9.3, 8.4 Hz, 1H), 5.15 (dd, *J* = 9.3, 8.2 Hz, 1H), 5.08 (s, 2H), 4.98 – 4.90 (m, 1H), 4.86 – 4.80 (m, 4H), 4.77 (d, *J* = 11.5 Hz, 1H), 4.71 (d, *J* = 11.3 Hz, 1H), 4.61 (d, *J* = 12.1 Hz, 1H), 4.58 – 4.53 (m, 6H), 4.52 (d, *J* = 1.8 Hz, 1H), 4.48 (dd, *J* = 11.9, 5.3 Hz, 2H), 4.43 (d, *J* = 11.8 Hz, 1H), 4.36 (d, *J* = 11.3 Hz, 1H), 4.20 (t, *J* = 9.0 Hz, 1H), 4.11 (d, *J* = 10.4 Hz, 1H), 4.06 (t, *J* = 9.2 Hz, 1H), 4.03 (d, *J* = 12.2 Hz, 1H), 3.99 – 3.92 (m, 2H), 3.88 – 3.84 (m, 2H), 3.77 – 3.72 (m, 2H), 3.62 – 3.56 (m, 2H), 3.52 (m, 2H), 3.48 (s, 3H), 3.45 (m, 1H), 3.44 – 3.42 (m, 1H), 3.38 (dd, *J* = 10.9, 2.8 Hz, 1H), 3.36 – 3.31 (m, 2H), 3.24 (dd, *J* = 16.2, 6.7 Hz, 1H), 3.20 (d, *J* = 10.1 Hz, 1H), 3.12 (dd, *J* = 12.9, 6.5 Hz, 2H), 2.93 (d, *J* = 9.6 Hz, 1H), 2.01 (s, 3H), 1.69 (s, 3H), 1.62 – 1.49 (m, 2H), 1.32 – 1.24 (m, 4H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 173.0, 172.7, 170.6, 167.6 , 167.2, 159.0, 142.2, 140.9, 140.8, 140.7, 140.6, 140.4, 140.2, 139.3, 135.9, 135.7, 132.5, 132.34, 132.27, 132.0, 131.3, 131.1, 131.06, 131.96, 130.9, 130.84, 130.81, 130.7, 130.7, 130.62, 130.57, 130.5, 130.5, 130.4, 130.22, 130.19, 130.1, 129.9, 129.7, 129.5, 129.2, 126.7, 126.1, 104.0 ($J_{1,2}$ = 165.0 Hz, C-1d), 102.8 ($J_{1,2}$ = 166.2 Hz, C-1c), 101.0 ($J_{1,2}$ = 174.6 Hz, C-1b), 99.1 ($J_{1,2}$ = 169.8 Hz, C-1a), 84.0, 83.0, 82.8, 82.2, 80.9, 79.9, 79.7, 79.4, 79.2, 78.4, 77.7, 77.5, 77.35, 77.30, 76.8, 76.5, 76.41, 76.37, 76.5, 75.7, 74.2, 70.8, 70.6, 70.5, 67.0, 69.7, 69.5, 69.2, 64.9, 55.11, 43.5, 32.32, 32.26, 31.4, 25.9, 23.5, 23.1; HRMS (ESI) calculated for $C_{112}H_{119}NO_{28}$ $(M+Na)^+$ 1948.7811 found 1948.7796 *m/z*.

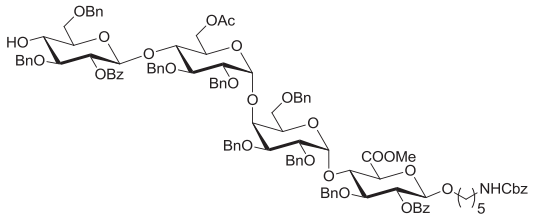
***N*-Benzyloxycarbonyl-5-amino-pentanyl methyl[2-*O*-benzoyl-3-*O*-benzyl-β-D-glucopyranosyl]uronate-(1→4)-2-*O*-benzoyl-3,6-di-*O*-benzyl-β-D-glucopyranosyl-(1→4)-6-*O*-acetyl-2,3-di-*O*-benzyl-α-D-glucopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-α-D-galactopyranoside (SI-3)**



SI-3

Tetrasaccharide **SI-3** (5.3 mg, 2.81 μmol, 9%) was obtained after eleven on-resin steps following the sequence III-IV-I-II. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.87 (m, 4H), 7.64 (t, *J* = 7.4 Hz, 1H), 7.50 – 6.95 (m, 50H), 5.31 – 5.25 (m, 1H), 5.19 – 5.13 (m, 1H), 5.11 (d, *J* = 11.8 Hz, 1H), 5.06 (s, 2H), 4.90 (d, *J* = 11.5 Hz, 1H), 4.84 – 4.45 (m, 13H), 4.44 – 4.30 (m, 3H), 4.22 – 4.09 (m, 4H), 4.05 – 3.83 (m, 6H), 3.78 – 3.62 (m, 6H), 3.58 (d, *J* = 9.9 Hz, 1H), 3.54 – 3.47 (m, 4H), 3.45 – 3.30 (m, 5H), 3.26 (d, *J* = 10.5 Hz, 1H), 3.08 (m, 3H), 1.71 (s, 3H), 1.58 – 1.47 (m, 2H), 1.45 – 1.36 (m, 2H), 1.32 – 1.22 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.5, 169.9, 164.9, 156.5, 139.8, 138.7, 138.6, 138.5, 138.2, 138.0, 133.5, 133.1, 129.9, 129.6, 128.7, 128.64, 128.58, 128.4, 128.3, 128.1, 128.0, 127.9, 127.8, 127.7, 127.5, 127.1, 126.9, 126.6, 101.7 ($J_{1,2}$ = 163.6 Hz, C-1d), 100.4 ($J_{1,2}$ = 165.2 Hz, C-1c), 99.1 ($J_{1,2}$ = 175.6 Hz, C-1a), 97.9 ($J_{1,2}$ = 165.0 Hz, C-1b), 81.1, 80.7, 80.3, 79.8, 78.3, 76.6, 75.2, 74.87, 74.77, 74.7, 74.5, 73.94, 73.86, 73.7, 73.3, 73.0, 72.6, 72.3, 69.4, 69.0, 68.2, 67.9, 67.4, 66.7, 62.0, 52.8, 40.9, 29.8, 29.5, 28.9, 23.3, 20.8; HRMS (ESI) calculated for $C_{110}H_{117}NO_{27}$ $(M+Na)^+$ 1906.7705 found 1906.7590 *m/z*.

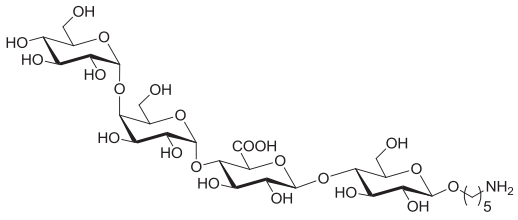
***N*-Benzyloxycarbonyl-5-amino-pentanyl 2-*O*-benzoyl-3,6-di-*O*-benzyl-β-D-glucopyranosyl-(1→4)-6-*O*-acetyl-3,4-di-*O*-benzyl-α-D-glucopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-α-D-galactopyranosyl-(1→4)-methyl[2-*O*-benzoyl-3-*O*-benzyl-β-D-glucopyranosyl]uronate (SI-4)**



SI-4

Tetrasaccharide **SI-4** (7.1 mg, 3.78 μmol, 13%) was obtained after eleven on-resin steps following the sequence II-III-IV-I. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.94 (d, *J* = 7.7 Hz, 2H), 7.88 (d, *J* = 7.7 Hz, 2H), 7.52 (t, *J* = 7.3 Hz, 1H), 7.39 – 7.18 (m, 39H), 7.15 (d, *J* = 7.0 Hz, 2H), 7.12 (d, *J* = 7.1 Hz, 4H), 7.08 – 7.01 (m, 3H), 6.98 (d, *J* = 7.2 Hz, 2H), 5.30 – 5.26 (m, 1H), 5.21 – 5.16 (m, 1H), 5.11 (d, *J* = 3.5 Hz, 1H), 5.06 (m, 3H), 5.00 (d, *J* = 3.3 Hz, 1H), 4.83 (d, *J* = 11.1 Hz, 1H), 4.74 (m, 2H), 4.71 – 4.68 (m, 1H), 4.66 – 4.55 (m, 4H), 4.51 – 4.44 (m, 3H), 4.43 – 4.34 (m, 3H), 4.31 – 4.17 (m, 6H), 4.00 (s, 1H), 3.93 – 3.83 (m, 4H), 3.77 (m, 5H), 3.66 (dd, *J* = 10.5, 2.3 Hz, 1H), 3.62 (t, *J* = 9.1 Hz, 1H), 3.56 (dd, *J* = 9.4, 5.1 Hz, 1H), 3.54 – 3.47 (m, 2H), 3.46 – 3.41 (m, 4H), 3.41 – 3.31 (m, 2H), 3.23 (dd, *J* = 9.0, 5.0 Hz, 1H), 2.99 (brs, 1H), 2.90 (m, 2H), 1.90 (s, 3H), 1.49 – 1.36 (m, 2H), 1.36 – 1.23 (m, 2H), 1.21 – 1.09 (m, 2H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.7, 168.6, 165.1, 164.9, 156.4 , 139.6, 138.42, 138.40, 138.3, 138.14, 138.12, 137.7, 136.9, 133.2, 130.0, 129.9, 129.8, 129.6, 128.63, 128.61, 128.55, 128.52, 128.47, 128.44, 128.39, 128.37, 128.21, 128.18, 128.0, 127.9, 127.80, 127.77, 127.75, 127.74, 127.73, 127.71, 127.6, 127.5, 127.4, 127.3, 127.2, 101.5 ($J_{1,2}$ = 162.6 Hz, C-1c), 101.3 ($J_{1,2}$ = 162.0 Hz, C-1d), 99.0 ($J_{1,2}$ = 172.8 Hz, C-1b), 98.8 ($J_{1,2}$ = 174.0 Hz, C-1a), 82.4, 81.0, 79.89, 79.85, 78.0, 77.3, 75.8, 75.7, 75.4, 75.1, 75.0, 74.7, 74.3, 74.1, 73.8, 73.7, 73.1, 71.8, 71.2, 69.7, 69.0, 66.6, 66.3, 62.3, 52.4, 40.9, 29.9, 29.5, 28.9, 23.2, 21.0; HRMS (ESI) calculated for $C_{110}H_{117}NO_{27}$ $(M+Na)^+$ 1906.7705 found 1906.7624 *m/z*.

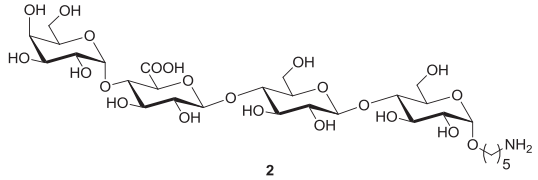
5-Amino-pentanyl α-D-glucopyranosyl-(1→4)-α-D-galactopyranosyl-(1→4)-β-D-glucopyranosyluronate-(1→4)-β-D-glucopyranoside (1)



1

^{1}H NMR (600 MHz, D_2O) δ 5.42 (d, *J* = 3.9 Hz, 1H, H-1b), 4.83 (d, *J* = 3.9 Hz, 1H, H-1a), 4.40 (d, *J* = 8.0 Hz, 1H, H-1c or H-1d), 4.35 (d, *J* = 8.0 Hz, 1H, H-1c or H-1d), 4.00 – 3.96 (m, 1H), 3.95 (d, *J* = 2.6 Hz, 1H), 3.90 – 3.78 (m, 4H), 3.76 – 3.71 (m, 2H), 3.70 – 3.63 (m, 7H), 3.62 – 3.44 (m, 5H), 3.42 (dd, *J* = 10.0, 3.8 Hz, 1H), 3.35 – 3.31 (m, 1H), 3.26 (t, *J* = 8.5 Hz, 1H), 3.19 – 3.15 (m, 1H), 2.88 (t, *J* = 7.5 Hz, 2H), 1.56 (m, 4H), 1.38 – 1.28 (m, 2H). ^{13}C NMR (150 MHz, D_2O) δ 177.7, 104.9 (C-1c or C-1d), 104.8 (C-1c or C-1d), 100.8 (C-1b), 100.4 (C-1a), 81.3, 81.0, 78.9, 78.7, 78.6, 77.4, 76.6, 75.7, 75.5, 74.3, 73.6, 73.2, 73.1, 71.8, 71.5, 71.0, 70.5, 63.3, 62.5, 42.0, 30.7, 29.1, 25.1; HRMS (ESI) calculated for $C_{29}H_{51}NO_{22}$ $(M+H)^+$ 766.2975 found 766.3030 *m/z*.

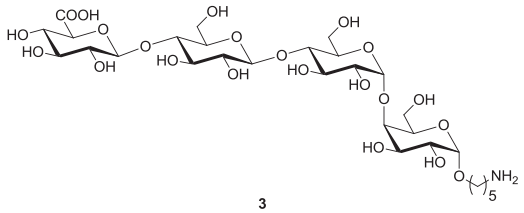
5-Amino-pentanyl α-D-galactopyranosyl-(1→4)-β-D-glucopyranosyluronate-(1→4)-β-D-glucopyranosyl-(1→4)-α-D-glucopyranoside (2)



2

^{1}H NMR (600 MHz, D_2O) δ 5.53 (d, *J* = 3.7 Hz, 1H, H-1b), 4.93 (d, *J* = 3.5 Hz, 1H, H-1a), 4.55 (2xd, *J* = 8.1 and 8.2 Hz, 2H, H-1c, H-1d), 4.00 – 3.96 (m, 3H), 3.93 (d, *J* = 11.3 Hz, 1H), 3.88 – 3.61 (m, 17H), 3.59 – 3.54 (m, 1H), 3.41 (t, *J* = 8.2 Hz, 1H), 3.38 – 3.34 (m, 1H), 3.03 (t, *J* = 7.5 Hz, 2H), 1.75 – 1.66 (m, 4H), 1.49 (m, 2H); ^{13}C NMR (150 MHz, D_2O) δ 177.7, 104.9 (C-1c or C-1d), 104.8 (C-1c or C-1d), 100.8 (C-1b), 100.4 (C-1a), 81.3, 81.0, 78.9, 78.7, 78.6, 77.4, 76.6, 75.7, 75.5, 74.3, 73.6, 73.2, 73.1, 71.8, 71.5, 71.0, 70.5, 63.3, 62.5, 42.0, 30.7, 29.1, 25.1; HRMS (ESI) calculated for $C_{29}H_{51}NO_{22}$ $(M+H)^+$ 766.2975 found 766.3066 *m/z*.

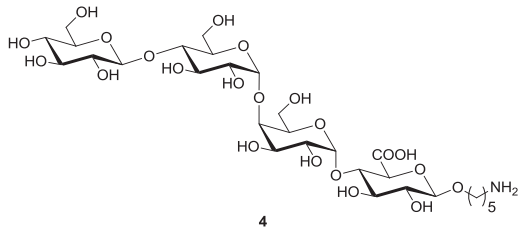
5-Amino-pentanyl β-D-glucopyranosyluronate-(1→4)-β-D-glucopyranosyl-(1→4)-α-D-glucopyranosyl-(1→4)-α-D-galactopyranoside (3)



3

^{1}H NMR (600 MHz, D_2O) δ 5.01 (d, *J* = 4.0 Hz, 1H, H-1a or H-1b), 4.94 (d, *J* = 3.7 Hz, 1H, H-1a or H-1b), 4.58 (d, *J* = 8.0 Hz, 1H, H-1c or H-1d), 4.54 (d, *J* = 7.9 Hz, 1H, H-1c or H-1d), 4.25 (d, *J* = 9.8 Hz, 1H), 4.08 (s, 1H), 4.03 – 3.82 (m, 10H), 3.78 (dd, *J* = 9.9, 5.2 Hz, 2H), 3.73 – 3.53 (m, 8H), 3.41 – 3.36 (m, 2H), 3.05 – 3.01 (m, 2H), 1.76 – 1.65 (m, 4H), 1.53 – 1.44 (m, 2H); HRMS (ESI) calculated for $C_{29}H_{51}NO_{22}$ $(M+H)^+$ 766.2975 found 766.3047 *m/z*.

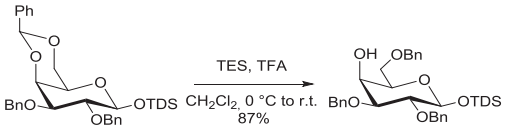
5-Amino-pentanyl β-D-glucopyranosyl-(1→4)-α-D-glucopyranosyl-(1→4)-α-D-galactopyranosyl-(1→4)-β-D-glucopyranosyluronate (4)



4

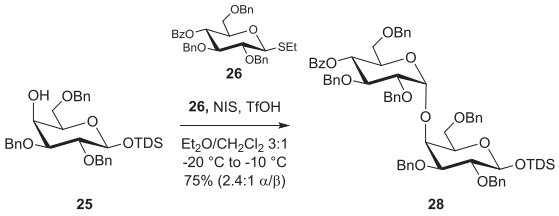
^{1}H NMR (600 MHz, D_2O) δ 5.54 (d, *J* = 3.7 Hz, 1H, H-1b), 4.96 (d, *J* = 3.7 Hz, 1H, H-1a), 4.53 (d, *J* = 7.9 Hz, 1H, H-1c or H-1d), 4.48 (d, *J* = 8.0 Hz, 1H, H-1c or H-1d), 4.24 (d, *J* = 10.1 Hz, 1H), 4.08 (d, *J* = 2.2 Hz, 1H), 4.01 (t, *J* = 6.5 Hz, 1H), 3.94 – 3.67 (m, 15H), 3.60 (dd, *J* = 10.0, 3.7 Hz, 1H), 3.51 (dt, *J* = 9.6, 7.4 Hz, 2H), 3.43 (t, *J* = 9.4 Hz, 1H), 3.33 (dd, *J* = 16.2, 8.1 Hz, 2H), 3.01 (t, *J* = 7.5 Hz, 2H), 1.73 – 1.64 (m, 4H), 1.52 – 1.42 (m, 2H); ^{13}C NMR (150 MHz, D_2O) δ 177.9, 105.1 (C-1c or C-1d), 104.7 (C-1c or C-1d), 102.4 (C-1a), 100.9 (C-1b), 80.9, 80.8, 79.2, 79.1, 78.7, 78.6, 78.1, 75.8, 75.7, 74.1, 73.9, 73.4, 73.2, 72.7, 72.0, 71.3, 71.1, 63.2, 62.3, 62.1, 42.0, 30.7, 28.8, 24.5; HRMS (ESI) calculated for $C_{29}H_{51}NO_{22}$ $(M+H)^+$ 766.2975 found 766.3052 *m/z*.

***T*hexyl 2,3,6-tri-*O*-benzyl-β-D-galactopyranoside (25)**



To a stirred solution of benzylidene acetal **SI-6** (*41*) (1.68 g, 2.84 mmol) in CH_2Cl_2 (60 ml) over activated MS (3 Å-AW) were added at 0 °C triethylsilane (2.72 ml, 17.06 mmol) and trifluoroacetic acid (1.81 ml, 17.06 mmol). The mixture was slowly warmed to room temperature and stirred for 16 h at that temperature. The reaction was quenched with Et_3N (2 ml), filtered through celite and concentrated. The residue was purified by flash chromatography (EtOAc/hexanes 1:20 to 1:7) to give alcohol **25** (1.46 g, 2.46 mmol, 87%) as a clear oil. R_f (EtOAc/toluene/hexanes 1:1:3) = 0.68; $[\alpha]_D^{20}$ = +12.5° (c = 2.20, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42 – 7.27 (m, 15H), 4.93 (d, *J* = 11.0 Hz, 1H), 4.75 (d, *J* = 11.0 Hz, 1H), 4.70 (s, 2H), 4.62 – 4.56 (m, 3H), 4.01 (s, 1H), 3.80 (dd, *J* = 9.8, 5.9 Hz, 1H), 3.70 (dd, *J* = 9.8, 6.0 Hz, 1H), 3.57 (m, 2H), 3.49 (dd, *J* = 9.4, 3.4 Hz, 1H), 2.51 (d, *J* = 1.6 Hz, 1H), 1.69 (dt, *J* = 13.7, 6.8 Hz, 1H), 0.92 – 0.85 (m, 12H), 0.20 (d, *J* = 10.5 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.8, 138.2, 138.1, 128.6, 128.5, 128.4, 128.1, 127.99, 127.98, 127.8, 127.7, 98.4, 81.0, 80.9, 75.4, 73.8, 73.3, 72.6, 69.5, 67.1, 33.9, 25.0, 20.3, 20.1, 18.8, 18.6, -1.6, -3.0; IR (thin film) 2866, 1497, 1454, 1365, 1252, 1181, 1072, 1029, 876, 832, 781, 735, 696 cm^{-1}; HRMS (ESI) calculated for $C_{35}H_{48}O_6Si$ $(M+Na)^+$ 615.3117 found 615.3104 *m/z*.

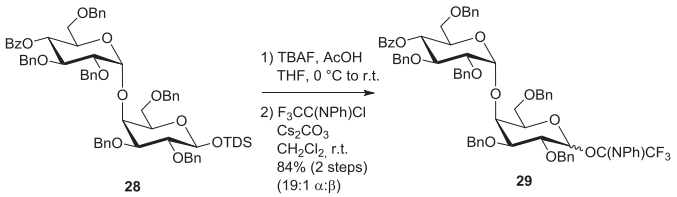
***T*hexyl 4-*O*-benzoyl-2,3,6-tri-*O*-benzyl-α-D-glucopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-galactopyranoside (28)**



Alcohol **25** (550 mg, 0.93 mmol) and thioglycoside **26** (*42*) (667 mg, 1.11 mmol) were co-evaporated with anhydrous toluene (3x10 ml) and kept under high vacuum for 30 min. The mixture was dissolved in Et_2O (14 ml) and CH_2Cl_2 (2.8 ml) and stirred over activated molecular sieves (3 Å-AW) for 30 min at room temperature. The solution was cooled to -20 °C and treated with NIS (250 mg, 1.11 mmol) and TfOH (16 µl, 0.19 mmol). The mixture was

stirred for 1 h and slowly warmed to -10 °C. The reaction was quenched with Et_3N (0.05 ml), diluted with CH_2Cl_2 (20 ml), filtered through celite and concentrated. The residue was purified by flash chromatography (EtOAc/hexanes 0:1 to 1:8 to 1:6) to give disaccharide **28** (553 mg, 0,490 mmol, 53%) along with the corresponding β-anomer (231 mg, 0.205 mmol, 22%). Analytical data for **28**: Clear oil. R_f (EtOAc/hexanes 1:1) = 0.28; $[\alpha]_D^{20}$ = +70.6° (c = 1.46, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.95 (dd, *J* = 8.3, 1.3 Hz, 2H), 7.64 – 7.52 (m, 1H), 7.49 – 7.02 (m, 32H), 5.52 (dd, *J* = 10.1, 9.5 Hz, 1H), 5.12 (d, *J* = 3.4 Hz, 1H), 4.99 (d, *J* = 11.1 Hz, 1H), 4.88 (t, *J* = 12.0 Hz, 2H), 4.84 – 4.59 (m, 6H), 4.49 – 4.33 (m, 2H), 4.33 – 4.20 (m, 4H), 4.14 (d, *J* = 2.9 Hz, 1H), 4.01 (m, 1H), 3.83 – 3.63 (m, 2H), 3.62 – 3.48 (m, 2H), 3.44 (dd, *J* = 10.0, 2.9 Hz, 1H), 3.05 (m, 2H), 1.80 – 1.64 (m, 1H), 1.04 – 0.84 (m, 12H), 0.29 (s, 3H), 0.23 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.2, 138.9, 138.61, 138.58, 138.2, 138.1, 137.9, 133.0, 130.3, 129.8, 128.5, 128.43, 128.39, 128.37, 128.3, 128.2, 128.14, 128.12, 128.0, 127.8, 127.7, 127.6, 127.5, 127.50, 127.45, 127.4, 99.5 (C-1a), 98.7 (C-1b), 81.3, 80.8, 80.3, 79.0, 75.1, 75.0, 74.9, 73.7, 73.6, 73.4, 73.3, 72.7, 70.8, 69.5, 68.1, 67.6, 34.2, 25.0, 20.4, 18.8, 18.7, -1.6, -2.3; IR (thin film) 2866, 1727, 1497, 1454, 1364, 1267, 1096, 834, 782, 734, 697 cm^{-1}; HRMS (ESI) calculated for $C_{69}H_{80}O_{12}Si$ $(M+Na)^+$ 1151.5316 found 1151.5293 *m/z*.

4-*O*-Benzoyl-2,3,6-tri-*O*-benzyl-α-D-glucopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-αβ-D-galactopyranosyl trifluoro-(*N*-phenyl)acetimidate (29)

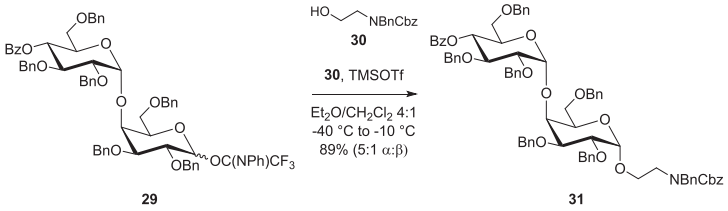


To a stirred solution of silyl ether **28** (470 mg, 0.416 mmol) in THF (8.3 ml) were added at 0 °C acetic acid (0.24 ml, 4.19 mmol) and TBAF (1.0 M solution in THF, 4.2 ml, 4.20 mmol). The reaction was slowly warmed to room temperature and stirred for 2 h at that temperature. Acetic acid (0.24 ml, 4.19 mmol) and TBAF (1.0 M solution in THF, 4.2 ml, 4.20 mmol) were added to drive the reaction to completion and the mixture was stirred for 16 h at room temperature. The reaction was diluted with Et_2O (50 ml), washed with water (3x30 ml) and the aqueous phase was re-extracted with Et_2O (2x20 ml). The combined organic fractions were dried over Na_2SO_4, filtered and concentrated. The residue was filtered through a short

plug of silica gel (EtOAc/hexanes 1:3 to 1:1) to give the intermediate lactol mixture as a clear oil.

To a stirred solution of the lactol mixture in CH_2Cl_2 (7.8 ml) were added at room temperature cesium carbonate (318 mg, 0.975 mmol) and $F_3CC(NPh)Cl$ (202 mg, 0.975 mmol). The mixture was stirred for 2.5 h at that temperature, diluted with hexanes containing 0.5% (v/v) Et_3N (10 ml) and filtered through celite. The residue was purified by flash chromatography (EtOAc/hexanes 0:1 + 0.5% (v/v) Et_3N to 1:3 + 0.5% (v/v) Et_3N) to give imidate mixture **29** (404 mg, 0.349 mmol, 84% over two steps, predominantly α-isomer) as a clear oil. R_f (EtOAc/hexanes 1:3) = 0.63 – 0.74; $[\alpha]_D^{20}$ = +65.4° (c = 1.00, acetone); ^{1}H NMR (400 MHz, acetone-D_6) δ 8.03 – 7.95 (m, 2H), 7.76 – 7.61 (m, 1H), 7.56 – 7.00 (m, 35H), 6.93 – 6.72 (m, 2H), 5.44 (t, *J* = 9.8 Hz, 1H), 5.25 (d, *J* = 3.3 Hz, 1H), 4.96 – 4.58 (m, 9H), 4.55 – 4.46 (m, 1H), 4.45 – 3.96 (m, 10H), 3.77 (m, 1H), 3.64 (m, 1H), 3.30 – 3.13 (m, 2H); ^{13}C NMR (100 MHz, acetone-D_6) δ 165.7, 139.6, 139.4, 133.9, 131.3, 130.4, 129.9, 129.7, 129.4, 129.2, 129.1, 128.8, 128.8, 128.6, 128.5, 128.41, 128.37, 128.3, 128.2, 128.1, 128.0, 126.6, 125.1, 121.6, 120.1, 99.8, 81.6, 81.3, 79.7, 78.5, 75.5, 75.4, 74.1, 73.8, 73.5, 73.1, 72.2, 70.3, 69.5; IR (thin film) 2868, 1724, 1454, 1315, 1268, 1209, 1102, 1028, 737, 697 cm^{-1}; HRMS (ESI) calculated for $C_{69}H_{66}F_3NO_{12}$ $(M+Na)^+$ 1180.4434 found 1180.4458 *m/z*.

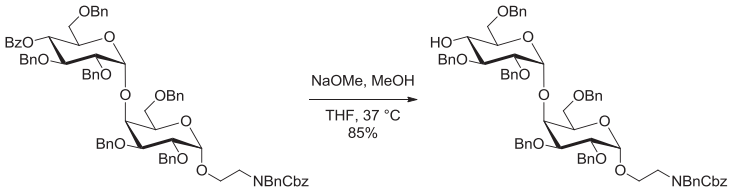
_N_-Benzyl-_N_-benzyloxycarbonyl-2-amino-ethyl 4-_O_-benzoyl-2,3,6-tri-_O_-benzyl-α-D-glucopyranosyl-(1→4)-2,3,6-tri-_O_-benzyl-α-D-galactopyranoside (31)



Imidate **29** (200 mg, 0.173 mmol) and alcohol **30** (*43*) (74 mg, 0.259 mmol) were co-evaporated with anhydrous toluene (2x5 ml) and kept under high vacuum for 30 min. The mixture was dissolved in Et_2O (2.8 ml) and CH_2Cl_2 (0.7 ml) and stirred over activated molecular sieves (3 Å-AW) for 30 min at room temperature. The solution was cooled to -40 °C and treated with TMSOTf (6.2 µl, 35 µmol). The mixture was stirred for 10 min at that temperature and then slowly warmed to -10 °C. The reaction was quenched with sat. aq. $NaHCO_3$ (5 ml), extracted with CH_2Cl_2 (3x20 ml) and the combined organic fractions were dried over Na_2SO_4, filtered and concentrated. The residue was purified by flash

chromatography (EtOAc/hexanes 0:1 to 1:6 to 1:4) to give disaccharide **31** (160 mg, 0.128 mmol, 74%) along with the corresponding β-anomer (32 mg, 0.026 mmol, 15%). Analytical data for **31**: Clear oil. R_f (EtOAc/hexanes 1:3) = 0.58; $[\alpha]_D^{20}$ = +68.2° (c = 1.48, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.03 – 7.86 (m, 2H), 7.58 (t, *J* = 7.4 Hz, 1H), 7.48 – 7.04 (m, 42H), 5.52 (t, *J* = 9.8 Hz, 1H), 5.22 – 5.12 (m, 2H), 5.08 (s, 1H), 4.94 – 4.65 (m, 8H), 4.65 – 4.52 (m, 3H), 4.45 (d, *J* = 8.8 Hz, 1H), 4.38 (d, *J* = 12.1 Hz, 1H), 4.17 (m 5H), 4.06 – 3.96 (m, 1H), 3.96 – 3.79 (m, 3H), 3.76 – 3.60 (m, 2H), 3.58 – 3.34 (m, 4H), 3.12 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.2, 156.6, 156.3, 138.7, 138.33, 138.30, 138.2, 138.0, 137.9, 136.8, 133.1, 130.3, 129.8, 128.6, 128.53, 128.51, 128.4, 128.3, 128.2, 128.1, 128.01, 127.96, 127.9, 127.82, 127.77, 127.61, 127.57, 127.5, 127.4, 127.3, 99.6 (C-1a), 98.1, 98.0 (C-1b), 80.1, 79.7, 77.4, 76.2, 76.1, 75.7, 75.6, 75.3, 74.1, 73.6, 73.3, 73.2, 73.1, 72.7, 71.1, 69.7, 69.6, 69.4, 68.1, 68.1, 67.7, 67.43, 67.37, 66.9, 51.4, 46.5, 45.6; IR (thin film) 3031, 2922, 1727, 1699, 1497, 1453, 1417, 1363, 1267, 1097, 1041, 1028, 736, 697 cm^{-1}; HRMS (ESI) calculated for $C_{78}H_{79}NO_{14}$ $(M+Na)^+$ 1276.5398 found 1276.5405 *m/z*.

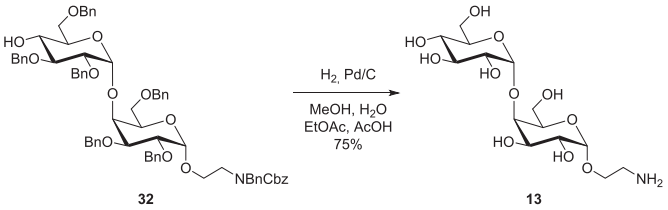
_N_-Benzyl-_N_-benzyloxycarbonyl-2-amino-ethyl 2,3,6-tri-_O_-benzyl-α-D-glucopyranosyl-(1→4)-2,3,6-tri-_O_-benzyl-α-D-galactopyranoside (32)



To a stirred solution of ester **31** (126 mg, 0.100 mmol) in THF (5 ml) and MeOH (5 ml) was added at 0 °C NaOMe (0.5 M in MeOH, 1 ml, 0.500 mmol). The reaction was slowly warmed to room temperature and stirred for 24 h. NaOMe (0.5 M in MeOH, 1 ml, 0.500 mmol) was added and the reaction was warmed to 37 °C. The mixture was stirred for 7 h at that temperature, neutralized with Amberlite IR120 (H^+ form), filtered and concentrated. The residue was purified by flash chromatography (EtOAc/hexanes 0:1 to 1:6 to 1:4) to give alcohol **32** (98 mg, 85 μmol, 85%) as a clear oil. R_f (EtOAc/hexanes 1:3) = 0.51; $[\alpha]_D^{20}$ = +65.3° (c = 0.5, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44 – 7.07 (m, 40H), 5.19 – 5.10 (m, 2H), 5.01 (s, 1H), 4.94 (d, *J* = 11.3 Hz, 1H), 4.89 – 4.72 (m, 5H), 4.72 – 4.61 (m, 3H), 4.55 (q, *J* = 15.5 Hz, 2H), 4.40 (d, *J* = 12.2 Hz, 1H), 4.22 (dd, *J* = 15.8, 11.1 Hz, 4H), 4.09 (d, *J* = 6.0 Hz, 1H), 4.01 – 3.67 (m, 7H), 3.65 – 3.27 (m, 6H), 3.19 (dd, *J* = 10.2, 3.9 Hz, 1H), 2.44 (s, 1H); ^{13}C NMR (100

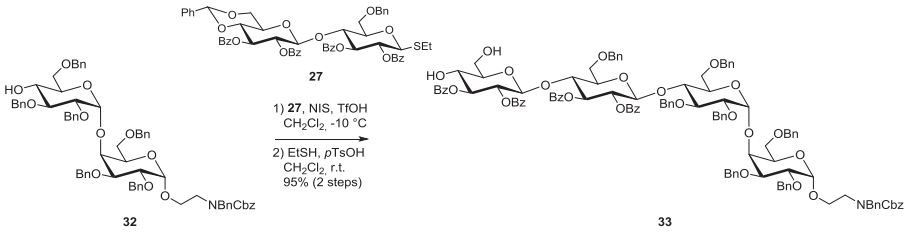
MHz, $CDCl_3$) δ 156.6, 156.3, 139.1, 138.82, 138.77, 138.63, 138.55, 138.4, 138.2, 138.14, 138.05, 137.9, 137.7, 136.8, 136.7, 128.6, 128.52, 128.50, 128.45, 128.4, 128.3, 128.1, 128.0, 127.78, 127.75, 127.7, 127.6, 99.7 (C-1a), 98.2 (C-1b), 81.8, 80.1, 77.4, 76.4, 76.3, 75.5, 75.4, 73.9, 73.5, 73.4, 73.2, 72.7, 71.8, 70.4, 69.7, 69.6, 69.2, 68.1, 68.0, 67.4, 66.9, 66.8, 51.4, 46.5, 45.5; IR (thin film) 3031, 2925, 1699, 1497, 1454, 1364, 1234, 1096, 1059, 736, 698 cm^{-1}; HRMS (ESI) calculated for $C_{71}H_{75}NO_{13}$ $(M+Na)^+$ 1172.5136 found 1172.5103 *m/z*.

2-Amino-ethyl α-D-glucopyranosyl-(1→4)-α-D-galactopyranoside (13)



Benzyl ether **32** (10 mg, 8.69 µmol) in EtOAc (1 ml) was added at room temperature to a suspension of Pd/C (30 mg) in MeOH (3 ml), water (0.5 ml) and AcOH (3 drops). The reaction was purged with hydrogen, stirred under hydrogen atmosphere for 24 h, filtered and concentrated to give disaccharide **13** (acetate salt, 2.9 mg, 6.55 µmol, 75%) as a white solid. ^{1}H NMR (600 MHz, D_2O) δ 5.04 (d, *J* = 3.7 Hz, 1H, H-1a or H-1b), 4.91 (d, *J* = 3.8 Hz, 1H, H-1a or H-1b), 4.16 – 4.05 (m, 2H), 3.99 (m, 3H), 3.91 (m, 2H), 3.86 – 3.69 (m, 5H), 3.54 (dd, *J* = 10.1, 3.8 Hz, 1H), 3.45 (t, *J* = 9.7 Hz, 1H), 3.32 – 3.21 (m, 2H); ^{13}C NMR (150 MHz, D_2O) δ 102.8 (C-1a or C-1b), 101.2 (C-1a or C-1b), 81.2, 75.3, 74.6, 74.3, 74.2, 71.8, 71.4, 70.9, 66.7, 63.1, 62.7, 41.9, 25.8. HRMS (ESI) calculated for $C_{14}H_{27}NO_{11}$ $(M+Na)^+$ 408.1481 found 408.1499 *m/z*.

***N*-Benzyl-*N*-benzyloxycarbonyl-2-amino-ethyl 2,3-di-*O*-benzoyl-β-D-glucopyranosyl-(1→4)-2,3-di-*O*-benzoyl-6-*O*-benzyl-β-D-glucoyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-α-D-glucopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-α-D-galactopyranoside (33)**

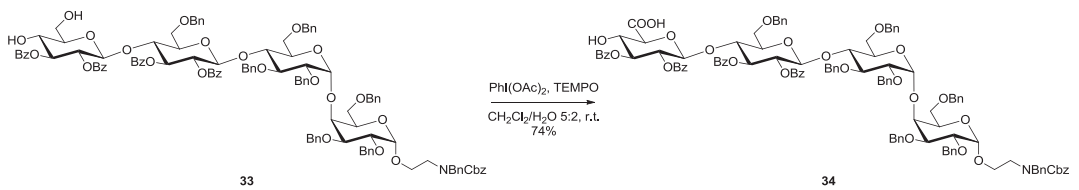


Alcohol **32** (47 mg, 41 µmol) and thioglycoside **27** (*17*) (60 mg, 61 µmol) were co-evaporated with anhydrous toluene (2x5 ml) and kept under high vacuum for 30 min. The mixture was

dissolved in CH_2Cl_2 (2 ml) and stirred over activated molecular sieves (3 Å-AW) for 30 min at room temperature. The solution was cooled to -10 °C and treated with NIS (13.8 mg, 61 µmol) and TfOH (1 µl, 11 µmol). The mixture was kept for 1 h at that temperature and slowly warmed to 0 °C. The reaction was quenched with Et_3N (50 µl), filtered and concentrated to give the intermediate benzylidene acetal as a yellow oil.

To a stirred solution of the intermediate benzylidene acetal in CH_2Cl_2 (2 ml) were added at room temperature ethanethiol (0.3 ml, 4.06 mmol) and *p*-toluenesulfonic acid (10 mg, 0.053 mmol). The mixture was stirred for 1 h at that temperature, quenched with Et_3N (20 µl) and concentrated. The residue was purified by flash chromatography (EtOAc/hexanes 0:1 to 1:3 to 1:2) to give diol **33** (78 mg, 39 µmol, 95% over two steps) as a clear oil. R_f (EtOAc/hexanes 1:1) = 0.67; $[\alpha]_D^{20}$ = +46.1° (c = 0.5, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.00 (d, *J* = 7.8 Hz, 2H), 7.95 (d, *J* = 7.9 Hz, 2H), 7.89 (d, *J* = 7.8 Hz, 2H), 7.70 (d, *J* = 7.8 Hz, 2H), 7.59 (t, *J* = 7.4 Hz, 1H), 7.55 – 7.10 (m, 56H), 5.44 – 5.26 (m, 3H), 5.26 – 5.05 (m, 4H), 4.96 (s, 1H), 4.75 (t, *J* = 13.1 Hz, 2H), 4.69 – 4.56 (m, 4H), 4.55 – 4.42 (m, 4H), 4.35 – 4.05 (m, 8H), 4.03 – 3.84 (m, 5H), 3.79 – 3.60 (m, 5H), 3.59 – 3.25 (m, 10H), 3.23 – 3.08 (m, 2H), 3.02 (d, *J* = 10.0 Hz, 2H), 2.00 (s, 1H), 0.98 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.5, 165.0, 164.9, 164.7, 156.5, 156.2, 140.0, 138.8, 138.7, 138.5, 138.3, 138.2, 138.0, 137.9, 137.5, 136.8, 133.7, 133.6, 133.4, 133.2, 130.1, 130.0, 129.8, 129.7, 129.4, 129.2, 129.1, 129.0, 128.9, 128.84, 128.80, 128.73, 128.65, 128.6, 128.43, 128.37, 128.3, 128.13, 128.10, 128.0, 127.9, 127.6, 127.5, 127.4, 127.2, 127.0, 100.2 (C-1d), 99.9 (C-1c), 99.8 (C-1a), 98.5 (C-1b), 98.4, 80.7, 79.0, 77.4, 76.6, 76.5, 75.7, 75.2, 74.9, 74.7, 74.5, 74.3, 74.1, 73.8, 73.7, 73.6, 73.4, 73.0, 72.1, 72.0, 71.5, 70.4, 69.5, 67.74, 67.68, 67.3, 67.2, 66.9, 66.7, 61.7, 51.3, 46.4, 45.5, 29.8; IR (thin film) 2926, 1733, 1602, 1453, 1364, 1315, 1272, 1094, 1070, 1028, 737, 710 cm^{-1}; HRMS (ESI) calculated for $C_{118}H_{117}NO_{27}$ $(M+Na)^+$ 2002.7710 found 2002.7731 *m/z*.

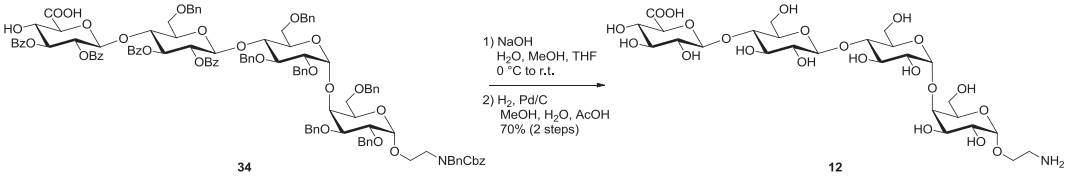
_N_-Benzyl-_N_-benzyloxycarbonyl-2-amino-ethyl 2,3-di-_O_-benzoyl-β-D-glucopyranosyluronate-(1→4)-2,3-di-_O_-benzoyl-6-_O_-benzyl-β-D-glucoyranosyl-(1→4)-2,3,6-tri-_O_-benzyl-α-D-glucopyranosyl-(1→4)-2,3,6-tri-_O_-benzyl-α-D-galactopyranoside (34)



33 34

To a vigorously stirred solution of alcohol **33** (45 mg, 23 µmol) in CH_2Cl_2 (2 ml) and water (0.8 ml) were added at 0 °C TEMPO (three crystals) and $PhI(OAc)_2$ (15.4 mg, 48 µmol). The reaction was stirred for 20 min at that temperature and slowly warmed to room temperature. After 1 h, TEMPO (two crystals) and $PhI(OAc)_2$ (10 mg, 31 µmol) were added and the mixture was stirred for 2 h at room temperature. The reaction was diluted with CH_2Cl_2 (5 ml) and quenched with 10% (w/v) aq. $Na_2S_2O_3$ (5 ml). The aqueous phase was extracted with EtOAc (2x10 ml), the combined organic fractions were dried over Na_2SO_4, filtered and concentrated. The residue was purified by flash chromatography twice (EtOAc/hexanes 0:1 to 1:2 to 8:1, then EtOAc/hexanes 1:1 + 1% (v/v) AcOH) and co-evaporated with heptane repeatedly to give acid **34** (33 mg, 17 µmol, 74%) as a clear oil. R_f (EtOAc/hexanes 1:1 + 0.5% (v/v) AcOH) = 0.63; $[\alpha]_D^{20}$ = +33.7° (c = 0.5, CH_2Cl_2); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.88 (dd, *J* = 22.7, 7.7 Hz, 6H), 7.65 (d, *J* = 7.5 Hz, 2H), 7.56 (t, *J* = 7.4 Hz, 1H), 7.52 – 7.27 (m, 26H), 7.24 – 7.06 (m, 30H), 5.36 – 5.26 (m, 3H), 5.23 (d, *J* = 9.3 Hz, 2H), 5.17 – 5.03 (m, 2H), 4.92 (s, 1H), 4.75 – 4.66 (m, 3H), 4.62 – 4.52 (m, 4H), 4.51 – 4.40 (m, 4H), 4.35 (t, *J* = 10.1 Hz, 2H), 4.22 (m, 3H), 4.13 (d, *J* = 9.9 Hz, 2H), 4.04 (d, *J* = 11.9 Hz, 2H), 3.96 (d, *J* = 11.9 Hz, 2H), 3.91 – 3.79 (m, 4H), 3.76 – 3.56 (m, 5H), 3.46 (m, 6H), 3.30 (m, 3H), 3.05 (d, *J* = 9.3 Hz, 1H), 2.97 (d, *J* = 9.6 Hz, 1H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 166.2, 166.1, 164.9, 156.6, 156.3, 140.0, 138.8, 138.5, 138.4, 138.3, 138.0, 137.9, 137.6, 136.8, 136.7, 133.6, 133.4, 133.2, 130.0, 129.9, 129.7, 129.3, 129.2, 129.0, 128.9, 128.72, 128.69, 128.64, 128.56, 128.5, 128.4, 128.3, 128.1, 128.0, 127.9, 127.7, 127.6, 127.5, 127.4, 127.2, 127.0, 100.1 ($J_{1,2}$ = 162.2 Hz, C-1c), 100.0 ($J_{1,2}$ = 166.6 Hz, C-1d), 99.8 ($J_{1,2}$ = 170.6 Hz, C-1a), 98.4, 98.3 ($J_{1,2}$ = 170.3 Hz, C-1b), 80.6, 79.3, 76.7, 75.2, 75.0, 74.4, 74.2, 74.1, 73.6, 73.4, 73.1, 72.4, 72.2, 71.3, 70.5, 70.2, 69.6, 69.5, 67.9, 67.8, 67.5, 67.4, 67.0, 66.84, 66.78, 51.4, 46.5, 45.5; IR (thin film) 3031, 2924, 1735, 1602, 1496, 1453, 1363, 1316, 1272, 1094, 1070, 1028, 737, 710 cm^{-1}; HRMS (ESI) calculated for $C_{118}H_{115}NO_{28}$ $(M+Na)^+$ 2016.7503 found 2016.7558 *m/z*.

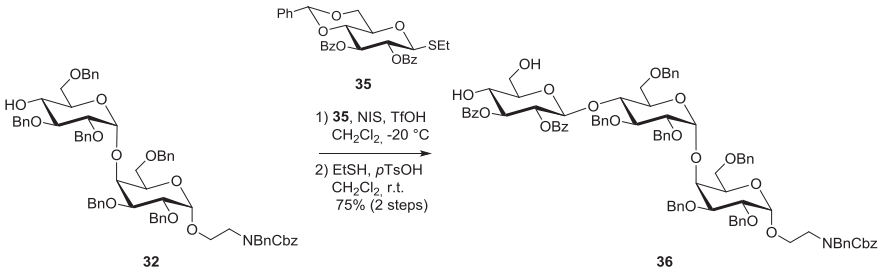
_N_-Benzyl-_N_-benzyloxycarbonyl-2-amino-ethyl β-D-glucopyranosyluronate-(1→4)-β-D-glucopyranosyl-(1→4)-α-D-glucopyranosyl-(1→4)-α-D-galactopyranoside (12)



To a stirred solution of ester **34** (45 mg, 23 µmol) in THF (4 ml) and MeOH (0.5 ml) was added at 0 °C NaOH (1 M aq. solution, 1 ml). The reaction was slowly warmed to room temperature and stirred for 16 h at that temperature. The solution was neutralized at 0 °C with 0.5 M aq. $NaHSO_4$ and extracted with EtOAc (5x5 ml). The combined organic fractions were dried over Na_2SO_4, filtered and concentrated to give the intermediate alcohol as a white foam.

The intermediate alcohol in MeOH (3 ml) was added at room temperature to a suspension of Pd/C (20 mg) in MeOH (6 ml), water (six drops) and AcOH (three drops). The suspension was purged with hydrogen, stirred under hydrogen atmosphere for 96 h, filtered and concentrated. Since the reaction had not proceeded to completion, the residue was subjected to the same conditions again and stirred for 72 h at room temperature. The mixture was filtered and concentrated, the residue was purified by solid phase extraction (Chromabond C18, Macherey-Nagel) and lyophilized to give tetrasaccharide **12** (11.3 mg, 16 µmol, 70% over two steps) as a white solid. ^{1}H NMR (600 MHz, D_2O) δ 5.07 (d, *J* = 3.7 Hz, 1H, H-1b), 4.93 (d, *J* = 3.9 Hz, 1H, H-1a), 4.58 (d, *J* = 8.0 Hz, 1H, H-1c or H-1d), 4.53 (d, *J* = 7.9 Hz, 1H, H -1c or H-1d), 4.28 – 4.21 (m, 1H), 4.10 (d, *J* = 2.7 Hz, 1H), 4.06 – 3.97 (m, 4H), 3.97 – 3.90 (m, 3H), 3.90 – 3.82 (m, 4H), 3.80 – 3.72 (m, 2H), 3.72 – 3.64 (m, 4H), 3.62 (dd, *J* = 10.1, 3.9 Hz, 1H), 3.56 – 3.51 (m, 2H), 3.40 – 3.36 (m, 2H), 3.35 – 3.26 (m, 2H); ^{13}C NMR (150 MHz, D_2O) δ 178.1, 104.9 (C-1c or C-1d), 104.8 (C-1c or C-1d), 102.5 (C-1a), 101.2 (C-1b), 81.23, 81.17, 80.9, 78.4, 77.9, 77.4, 76.7, 75.53, 75.52, 74.3, 74.2, 74.1, 73.8, 73.3, 71.4, 70.8, 66.6, 63.1, 62.6, 62.1, 41.9; HRMS (MALDI) calculated for $C_{26}H_{45}NO_{22}$ $(M+Na)^+$ 746.2330 found 746.2416 *m/z*.

_N_-Benzyl-_N_-benzyloxycarbonyl-2-amino-ethyl 2,3-di-_O_-benzoyl-β-D-glucopyranosyl-(1→4)-2,3,6-tri-_O_-benzyl-α-D-glucopyranosyl-(1→4)-2,3,6-tri-_O_-benzyl-α-D-galactopyranoside (36)

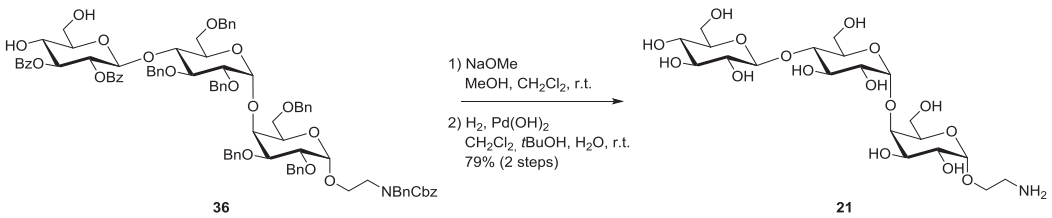


Alcohol **32** (15 mg, 13 µmol) and thioglycoside **35** (*17*) (20.4 mg, 39 µmol) were co-evaporated with anhydrous toluene (2x5 ml) and kept under high vacuum for 10 min. The

mixture was dissolved in CH_2Cl_2 (1.3 ml) and stirred over activated molecular sieves (3 Å-AW) for 30 min at room temperature. The solution was cooled to -20 °C and treated with NIS (8.8 mg, 39 µmol) and TfOH (1 µl, 11 µmol). The mixture was stirred for 1 h at that temperature and slowly warmed to 0 °C. The reaction was quenched with a 1:1 (v/v) mixture of sat. aq. $NaHCO_3$ (10 ml) and 10% (w/v) Na_2SO_3 (5 ml) and extracted with CH_2Cl_2 (4x10 ml). The combined organic fractions were dried over Na_2SO_4, filtered and concentrated. The residue was purified by flash chromatography (EtOAc/hexanes 1:5 to 1:4 to 1:3) to give the intermediate benzylidene acetal as a yellow oil.

To a stirred solution of the intermediate benzylidene acetal in CH_2Cl_2 (2 ml) were added at room temperature ethanethiol (0.2 ml, 2.8 mmol) and *p*-toluenesulfonic acid (6 mg, 32 µmol). The mixture was stirred for 1 h at that temperature, quenched with Et_3N (100 µl) and concentrated. The residue was purified by flash chromatography (EtOAc/hexanes 0:1 to 1:3 to 2:3) to give diol **36** (14.7 mg, 9.7 µmol, 75% over two steps) as a clear oil. R_f (EtOAc/hexanes 1:1) = 0.37; $[\alpha]_D^{20}$ = +26.4° (c = 0.1, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.91 (d, *J* = 7.3 Hz, 2H), 7.61 (d, *J* = 7.4 Hz, 2H), 7.52 – 7.26 (m, 27H), 7.24 – 6.97 (m, 19H), 5.38 – 5.26 (m, 1H), 5.14 – 5.07 (m, 2H), 5.05 (d, *J* = 11.0 Hz, 1H), 5.01 – 4.94 (m, 2H), 4.84 – 4.80 (m, 2H), 4.70 (dd, *J* = 14.1, 12.4 Hz, 2H), 4.62 – 4.39 (m, 6H), 4.33 – 4.25 (m, 1H), 4.18 (m, 3H), 4.10 – 3.91 (m, 4H), 3.86 (m, 2H), 3.69 (m, 6H), 3.55 – 3.19 (m, 8H), 3.02 (s, 1H), 2.95 (d, *J* = 9.5 Hz, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 167.8, 164.9, 139.54, 138.47, 138.3, 137.8, 133.7, 133.3, 130.1, 129.7, 129.1, 129.0, 128.9, 128.8, 128.6, 128.5, 128.4, 128.1, 128.0, 127.8, 127.7, 127.6, 127.4, 127.2, 99.89 (C-1d), 99.87 (C-1a), 98.5 (C-1b), 80.1, 79.3, 77.8, 76.0, 75.2, 74.2, 73.8, 73.1, 72.3, 71.8, 70.51, 70.48, 69.6, 67.9, 67.4, 66.9, 66.8, 62.1; IR (thin film) 3453, 2928, 1733, 1701, 1454, 1273, 1094, 1029, 739, 699 cm^{-1}; HRMS (MALDI) calculated for $C_{91}H_{93}NO_{20}$ $(M+Na)^+$ 1542.6188 found 1542.6145 *m/z*.

2-Amino-ethyl β-D-glucoyranosyl-(1→4)-α-D-glucopyranosyl-(1→4)-α-D-galactopyranoside (21)

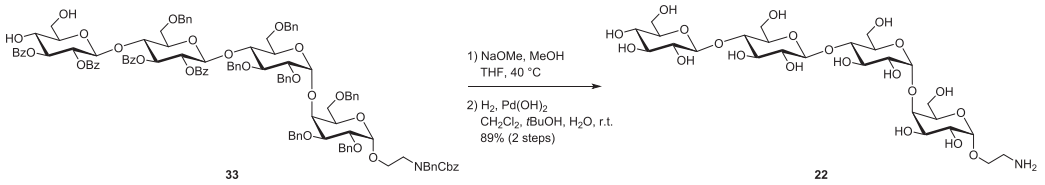


36 21

To a stirred solution of ester **36** (26 mg, 17 μmol) in CH_2Cl_2 (1 ml) and MeOH (1 ml) was added at room temperature NaOMe (0.5 M solution in MeOH, 0.5 ml). The reaction was stirred for 2 h at that temperature, neutralized at 0 °C with Amberlite IR-120 (H^+ form), filtered and concentrated. The residue was purified by flash chromatography (EtOAc/hexanes 1:3 to 2:1) to give the intermediate tetraol as a white foam.

The intermediate tetraol in CH_2Cl_2/*t*BuOH/water (1:6:2, 5 ml) was purged with argon and treated at 0 °C with a suspension of $Pd(OH)_2$ on carbon (20% (w/w) loading, 30 mg) in the same solvent mixture (1 ml). The suspension was purged with hydrogen, stirred under a hydrogen atmosphere for 24 h, filtered and concentrated. Since the reaction had not proceeded to completion, the residue was subjected to the same conditions again and stirred for 48 h at room temperature. The mixture was filtered and concentrated, the residue was purified by solid phase extraction (Chromabond C18, Macherey-Nagel) and lyophilized to give trisaccharide **21** (7.3 mg, 13 μmol, 79% over two steps) as a white solid. ^{1}H NMR (400 MHz, D_2O) δ 5.01 (d, *J* = 3.3 Hz, 1H, H-1b), 4.87 (d, *J* = 3.4 Hz, 1H, H-1a), 4.49 (d, *J* = 7.9 Hz, 1H, H-1d), 4.19 (d, *J* = 10.1 Hz, 1H), 4.03 (s, 1H), 4.00 – 3.74 (m, 10H), 3.67 (m, 3H), 3.55 (dd, *J* = 10.0, 3.5 Hz, 1H), 3.52 – 3.34 (m, 3H), 3.24 (m, 3H); ^{13}C NMR (100 MHz, D_2O) δ 102.4 (C-1d), 99.8 (C-1b), 98.4 (C-1a), 78.4, 78.2, 75.8, 75.4, 73.0, 71.5, 71.3, 71.1, 70.5, 69.3, 68.7, 68.1, 63.8, 60.4, 60.3, 59.3, 39.1; HRMS (ESI) calculated for $C_{20}H_{37}NO_{16}$ $(M+Na)^+$ 570.2010 found 570.2000 *m/z*.

2-Amino-ethyl β-D-glucopyranosyl-(1→4)-β-D-glucopyranosyl-(1→4)-α-D-glucopyranosyl-(1→4)-α-D-galactopyranoside (22)

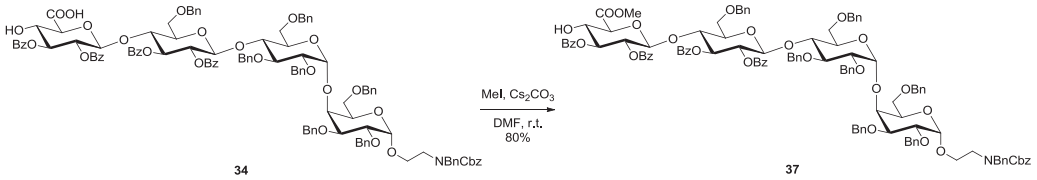


To a stirred solution of ester **33** (20 mg, 10.1 μmol) in THF (1 ml) and MeOH (0.33 ml) was added at room temperature NaOMe (0.5 M solution in MeOH, 0.5 ml). The reaction was warmed to 40 °C and stirred for 5 h at that temperature. The mixture was cooled to room temperature and stirred for 16 h at that temperature. The reaction was neutralized with Amberlite IR-120 (H^+ form), filtered and concentrated. The residue was purified by size

exclusion chromatography (Sephadex LH-20, CH_2Cl_2/MeOH 2:1) to give the intermediate hexaol as a white foam.

The intermediate hexaol in CH_2Cl_2/*t*BuOH/water (1:16:8, 1 ml) was purged with argon and treated at 0 °C with a suspension of $Pd(OH)_2$ on carbon (20% (w/w) loading, 20 mg) in the same solvent mixture (1 ml). The suspension was purged with hydrogen, stirred under a hydrogen atmosphere for 18 h, filtered and concentrated. The residue was purified by solid phase extraction (Chromabond C18, Macherey-Nagel) and lyophilized to give tetrasaccharide **22** (6.8 mg, 9.0 µmol, 89% over two steps) as a white solid. ^{1}H NMR (600 MHz, D_2O) δ 5.15 (d, *J* = 3.5 Hz, 1H, H-1b), 5.02 (d, *J* = 3.7 Hz, 1H, H-1a), 4.66 (d, *J* = 7.9 Hz, 1H, H-1c or H-1d), 4.61 (d, *J* = 7.9 Hz, 1H, H-1c or H-1d), 4.33 (d, *J* = 10.1 Hz, 1H), 4.18 (d, *J* = 2.2 Hz, 1H), 4.14 – 4.06 (m, 4H), 4.05 – 3.97 (m, 4H), 3.96 – 3.89 (m, 4H), 3.87 – 3.67 (m, 7H), 3.64 – 3.56 (m, 2H), 3.55 – 3.49 (m, 1H), 3.46 (t, *J* = 8.4 Hz, 1H), 3.45 – 3.34 (m, 3H); ^{13}C NMR (150 MHz, D_2O) δ 105.2 (C-1c or C-1d), 104.9 (C-1c or C-1d), 102.5 (C-1a), 101.2 (C-1b), 81.2, 81.0, 80.9, 78.6, 78.1, 77.4, 76.7, 75.7, 75.6, 74.2, 74.1, 73.8, 73.3, 72.1, 71.4, 70.9, 66.6, 63.2, 63.1, 62.5, 62.1, 41.9; HRMS (ESI) calculated for $C_{26}H_{47}NO_{21}$ $(M+Na)^+$ 732.2538 found 732.2504 *m/z*.

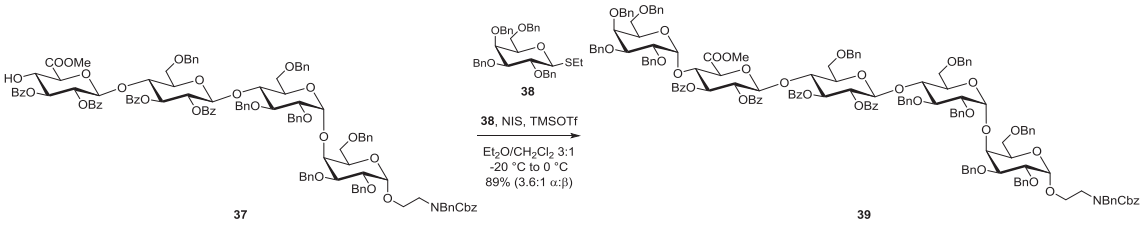
_N_-Benzyl-_N_-benzyloxycarbonyl-2-amino-ethyl methyl[2,3-di-_O_-benzoyl-β-D-glucopyranosyl]uronate-(1→4)-2,3-di-_O_-benzoyl-6-_O_-benzyl-β-D-glucopyranosyl-(1→4)-2,3,6-tri-_O_-benzyl-α-D-glucopyranosyl-(1→4)-2,3,6-tri-_O_-benzyl-α-D-galactopyranoside (37)



To a stirred solution of carboxylic acid **34** (100 mg, 50 µmol) in DMF (2.5 ml) were added at room temperature Cs_2CO_3 (24.5 mg, 75 µmol) and methyl iodide (10.7 mg, 75 µmol) and the reaction was stirred at that temperature. After 2 h, methyl iodide (10.7 mg, 75 µmol) was added and the mixture was stirred for another 2 h at room temperature. The reaction was quenched with sat. aq. NH_4Cl (5 ml), extracted with EtOAc (4x10 ml), the combined organic fractions were dried over Na_2SO_4, filtered and concentrated. The residue was purified by flash chromatography (EtOAc/hexanes 0:1 to 2:3) to give methyl ester **37** (81 mg, 40 µmol, 80%) as a white foam. R_f (EtOAc/hexanes 1:1) = 0.83; $[\alpha]_D^{20}$ = +33.1° (c = 0.25, CH_2Cl_2); ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.07 – 7.91 (m, 6H), 7.76 (d, *J* = 7.4 Hz, 2H), 7.66 (t, *J* = 7.4 Hz, 1H),

7.62 – 7.03 (m, 56H), 5.39 (m, 3H), 5.30 (t, *J* = 9.5 Hz, 1H), 5.25 (t, *J* = 9.5 Hz, 1H), 5.21 – 5.14 (m, 2H), 5.00 (s, 1H), 4.81 (d, *J* = 11.9 Hz, 1H), 4.77 (d, *J* = 11.4 Hz, 1H), 4.71 (d, *J* = 8.0 Hz, 1H), 4.68 – 4.62 (m, 3H), 4.60 – 4.47 (m, 5H), 4.32 (m, 4H), 4.22 (d, *J* = 9.2 Hz, 2H), 4.12 (d, *J* = 12.1 Hz, 2H), 4.08 – 3.87 (m, 6H), 3.83 – 3.64 (m, 5H), 3.62 – 3.47 (m, 6H), 3.45 (s, 3H), 3.42 – 3.29 (m, 2H), 3.17 (d, *J* = 2.6 Hz, 1H), 3.05 (d, *J* = 9.4 Hz, 2H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 168.4, 166.7, 165.6, 164.83, 164.79, 156.6, 156.3, 140.1, 138.80, 138.76, 138.6, 138.4, 138.34, 138.29, 138.1, 138.0, 137.9, 137.6, 136.83, 136.76, 133.6, 133.5, 133.1, 132.6, 130.4, 130.0, 129.84, 129.80, 129.7, 129.3, 129.18, 129.15, 129.1, 129.0, 128.9, 128.8, 128.7, 128.6, 128.51, 128.46, 128.44, 128.35, 128.3, 128.2, 128.1, 128.0, 127.94, 127.87, 127.7, 127.6, 127.5, 127.43, 127.40, 127.0, 100.3 (C-1c), 100.2 (C-1d), 99.8 (C-1a), 98.5 (C-1b), 80.7, 79.2, 76.7, 76.6, 75.2, 75.12, 75.07, 75.0, 74.8, 74.6, 74.40, 74.36, 74.1, 73.7, 73.62, 73.59, 73.4, 73.1, 72.5, 72.1, 71.4, 70.5, 69.6, 69.5, 67.8, 67.3, 67.0, 66.8, 52.6, 51.4, 46.5, 45.5; IR (thin film) 2928, 1734, 1602, 1453, 1364, 1272, 1094, 1070, 740, 710 cm^{-1}; HRMS (MALDI) calculated for $C_{119}H_{117}NO_{28}$ $(M+Na)^+$ 2030.7659 found 2030.7660 *m/z*.

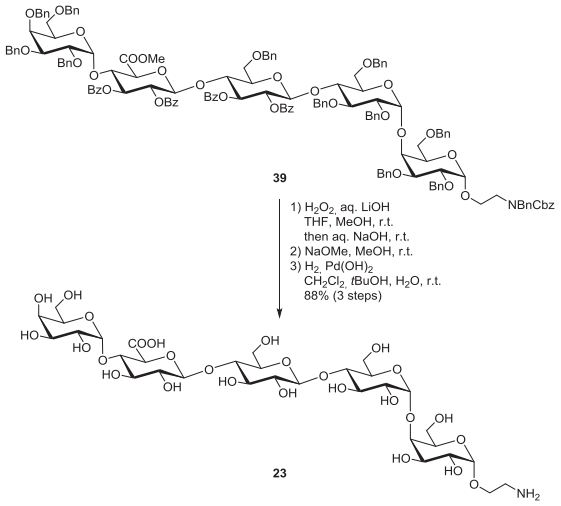
_N_-Benzyl-_N_-benzyloxycarbonyl-2-amino-ethyl 2,3,4,6-tetra-_O_-benzyl-α-D-galactopyranosyl-(1→4)-methyl[2,3-di-_O_-benzoyl-β-D-glucopyranosyl]uronate-(1→4)-2,3-di-_O_-benzoyl-6-_O_-benzyl-β-D-glucoyranosyl-(1→4)-2,3,6-tri-_O_-benzyl-α-D-glucopyranosyl-(1→4)-2,3,6-tri-_O_-benzyl-α-D-galactopyranoside (39)



Alcohol **37** (14 mg, 7 µmol) and thioglycoside **38** (*39*) (16 mg, 28 µmol) were co-evaporated with anhydrous toluene (3x10 ml) and kept under high vacuum for 30 min. The mixture was dissolved in Et_2O (1.05 ml) and CH_2Cl_2 (0.35 ml) and stirred over activated molecular sieves (3 Å-AW) for 30 min at room temperature. The solution was cooled to -20 °C and treated with NIS (6.3 mg, 28 µmol) and TMSOTf (1 µl, 5.5 µmol). The mixture was stirred for 1 h at that temperature and slowly warmed to 0 °C. The reaction was quenched with a 1:1 (v/v) mixture of sat. aq. $NaHCO_3$ (10 ml) and 10% (w/v) Na_2SO_3 (5 ml) and extracted with CH_2Cl_2 (4x10 ml). The combined organic fractions were dried over Na_2SO_4, filtered and concentrated. The residue was purified by flash chromatography (EtOAc/hexanes 0:1 to 1:4 to 1:3) to give

pentasaccharide **39** (12.5 mg, 4.9 µmol, 70%) along with the corresponding β-anomer (3.4 mg, 1.3 µmol, 19%). Analytical data for **39**: Clear oil. R_f (EtOAc/hexanes 2:3) = 0.63; $[\alpha]_D^{20}$ = +20.4° (c = 0.33, CH_2Cl_2); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.97 – 7.82 (m, 5H), 7.67 (d, *J* = 7.3 Hz, 2H), 7.57 (t, *J* = 7.4 Hz, 1H), 7.48 – 6.98 (m, 77H), 5.52 (t, *J* = 9.6 Hz, 1H), 5.37 (dd, *J* = 9.9, 8.2 Hz, 1H), 5.27 (t, *J* = 9.1 Hz, 2H), 5.17 (t, *J* = 9.5 Hz, 1H), 5.11 (t, *J* = 9.9 Hz, 2H), 4.92 (s, 1H), 4.82 (d, *J* = 11.3 Hz, 1H), 4.77 – 4.53 (m, 9H), 4.52 – 4.37 (m, 7H), 4.32 – 4.07 (m, 9H), 4.03 (d, *J* = 6.9 Hz, 1H), 3.96 (d, *J* = 12.0 Hz, 2H), 3.91 – 3.78 (m, 6H), 3.76 – 3.56 (m, 7H), 3.54 – 3.39 (m, 7H), 3.38 – 3.21 (m, 4H), 3.07 (s, 3H), 3.05 – 2.91 (m, 2H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 166.8, 165.49, 165.46, 164.84, 164.79, 156.6, 156.3, 140.0, 139.1, 138.9, 138.8, 138.6, 138.5, 138.4, 138.3, 138.2, 138.1, 138.0, 137.5, 136.8, 133.5, 133.0, 132.5, 130.2, 129.9, 129.8, 129.7, 129.4, 129.2, 129.1, 129.03, 128.95, 128.8, 128.7, 128.6, 128.5, 128.43, 128.41, 128.35, 128.28, 128.26, 128.22, 128.15, 128.03, 127.98, 127.93, 127.91, 127.8, 127.6, 127.52, 127.49, 127.45, 127.2, 127.0, 100.5 (C-1c), 100.2 (C-1d), 99.8 (C-1b), 99.47 (C-1a), 98.46 (C-1b), 80.7, 79.2, 78.4, 76.8, 76.61, 76.55, 75.34, 75.30, 75.26, 75.1, 75.0, 74.9, 74.8, 74.5, 74.2, 73.7, 73.6, 73.5, 73.4, 73.3, 73.1, 72.6, 72.1, 71.6, 70.5, 70.0, 69.6, 67.9, 67.5, 67.3, 67.0, 66.9, 52.2, 51.4, 46.5, 45.5; IR (thin film) 2928, 1737, 1498, 1454, 1271, 1094, 1047, 1028, 738, 699 cm^{-1}; HRMS (MALDI) calculated for $C_{153}H_{151}NO_{33}$ $(M+Na)^+$ 2553.0066 found 2553.0066 *m/z*.

2-Amino-ethyl α-D-galactopyranosyl-(1→4)-β-D-glucopyranosyluronate-(1→4)-β-D-glucoyranosyl-α-D-glucopyranosyl-(1→4)-α-D-galactopyranoside (23)

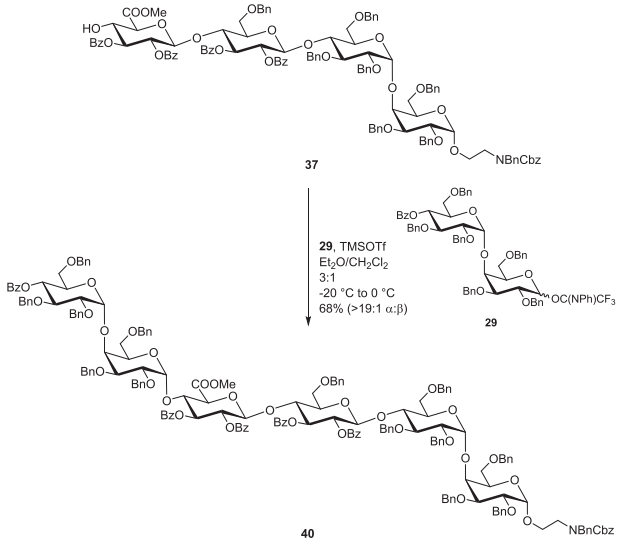


To a stirred solution of ester **39** (26 mg, 10.3 µmol) in THF (1 ml) and MeOH (1 ml) was added at 0 °C a 1:1 (v/v) mixture (450 µl) of hydrogen peroxide (6% (v/v) aq. solution, 397

μmol) and LiOH (0.5 M aq. solution, 113 μmol). The reaction was warmed to room temperature and stirred for 1 h at that temperature. The reaction was treated with NaOH (0.5 M aq. solution, 1 ml) and stirred for 16 h at room temperature. The solvents were evaporated under reduced pressure, the residue was co-evaporated with toluene (2x5 ml) and dissolved in MeOH (1 ml). The solution was treated at room temperature with NaOMe (0.5 M in MeOH, 1 ml) and stirred for 16 h at that temperature. The reaction was diluted with water (0.5 ml) and CH_2Cl_2 (0.5 ml), neutralized at 0 °C with Amberlite IR-120 (H^+ form), filtered and concentrated. The residue was purified by flash chromatography (EtOAc/hexanes 0:1 to 1:4 to 1:2 to 1:2 + 1% (v/v) AcOH to 1:1 + 1% (v/v) AcOH) to give the intermediate carboxylic acid as a clear oil.

The intermediate carboxylic acid in CH_2Cl_2/*t*BuOH/water (1:16:8, 1 ml) was purged with argon and treated at 0 °C with a suspension of $Pd(OH)_2$ on carbon (20% (w/w) loading, 20 mg) in the same solvent mixture (0.5 ml). The suspension was purged with hydrogen, stirred under a hydrogen atmosphere for 16 h, filtered and concentrated. Since the reaction had not proceeded to completion, the residue was subjected to the same conditions again and stirred for 24 h at room temperature. The mixture was filtered and concentrated, the residue was purified by solid phase extraction (Chromabond C18, Macherey-Nagel) and lyophilized to give pentasaccharide **23** (8.1 mg, 9.1 μmol, 88% over three steps) as a white solid. ^{1}H NMR (600 MHz, D_2O) δ 5.52 (d, *J* = 3.5 Hz, 1H, H-1b), 5.07 (d, *J* = 3.7 Hz, 1H, H-1b), 4.93 (d, *J* = 3.8 Hz, 1H, H-1a), 4.56 (2xd, *J* = 8.4 and 8.4 Hz, 2H, H-1c and H-1d), 4.24 (d, *J* = 10.0 Hz, 1H), 4.09 (d, *J* = 2.6 Hz, 1H), 4.07 – 3.78 (m, 19H), 3.77 – 3.58 (m, 8H), 3.39 (dt, *J* = 24.4, 8.5 Hz, 2H), 3.34 – 3.26 (m, 2H); ^{13}C NMR (150 MHz, D_2O) δ 104.86 (C-1c and C-1d), 104.85 (C-1a), 102.5 (C-1b), 101.2 (C-1b), 101.0, 81.2, 81.0, 80.9, 78.7, 78.6, 77.4, 76.6, 75.7, 75.5, 74.2, 74.1, 73.8, 73.31, 73.29, 71.8, 71.6, 71.5, 71.0, 70.9, 66.5, 63.3, 63.1, 62.5, 62.1, 41.9; HRMS (MALDI) calculated for $C_{32}H_{55}NO_{27}$ $(M+Na)^+$ 884.2883 found 884.2942 *m/z*.

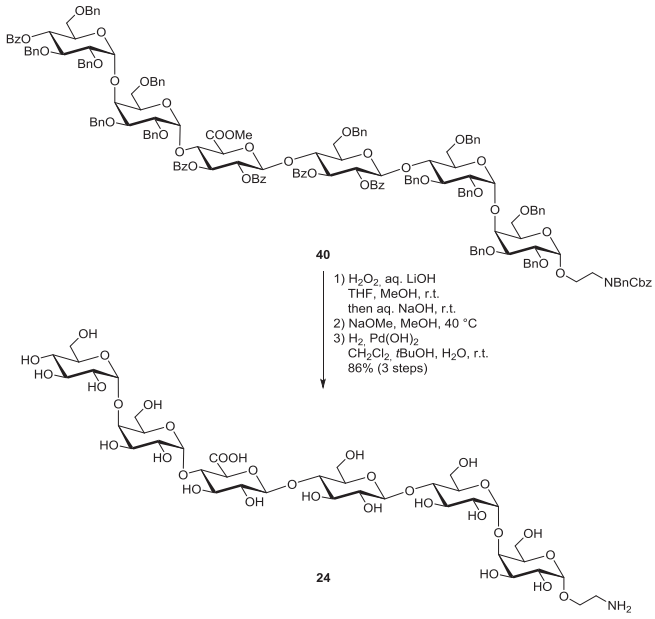
***N*-Benzyl-*N*-benzyloxycarbonyl-2-amino-ethyl 4-*O*-benzoyl-2,3,6-tri-*O*-benzyl-α-D-glucopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-α-D-galactopyranosyl-(1→4)-methyl[2,3-di-*O*-benzoyl-β-D-glucopyranosyl]uronate-(1→4)-2,3-di-*O*-benzoyl-6-*O*-benzyl-β-D-glucopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-α-D-glucopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-α-D-galactopyranoside (40)**



Alcohol **37** (50 mg, 25 µmol) and imidate **29** (72.1 mg, 62 µmol) were co-evaporated with anhydrous toluene (3x10 ml) and kept under high vacuum for 30 min. The mixture was dissolved in Et_2O (2 ml) and CH_2Cl_2 (0.67 ml) and stirred over activated molecular sieves (3 Å-AW) for 30 min at room temperature. The solution was cooled to -20 °C and treated with TMSOTf (2 µl, 11 µmol). The mixture was stirred for 1 h at that temperature and slowly warmed to 0 °C. The reaction was quenched with sat. aq. $NaHCO_3$ (10 ml) and extracted with CH_2Cl_2 (4x10 ml). The combined organic fractions were dried over Na_2SO_4, filtered and concentrated. The residue was purified by flash chromatography (EtOAc/hexanes 0:1 to 1:3 to 3:7 to 1:2) to give hexasaccharide **40** (51 mg, 17 µmol, 68%) as a clear oil. R_f (EtOAc/hexanes 2:3) = 0.63; $[\alpha]_D^{20}$ = +36.6° (c = 0.21, CH_2Cl_2); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.95 (d, *J* = 7.3 Hz, 2H), 7.89 (d, *J* = 7.7 Hz, 4H), 7.84 (d, *J* = 7.3 Hz, 2H), 7.67 (d, *J* = 7.4 Hz, 2H), 7.55 (dt, *J* = 26.6, 7.4 Hz, 2H), 7.48 – 6.98 (m, 88H), 5.49 (dt, *J* = 19.5, 9.8 Hz, 2H), 5.39 (dd, *J* = 9.9, 8.2 Hz, 1H), 5.28 (m, 2H), 5.16 (t, *J* = 9.5 Hz, 1H), 5.09 (m, 3H), 4.92 (s, 1H), 4.74 (dd, *J* = 11.7, 9.1 Hz, 2H), 4.71 – 4.66 (m, 3H), 4.60 (m, 7H), 4.53 – 4.41 (m, 6H), 4.35 – 4.28 (m, 3H), 4.28 – 4.20 (m, 5H), 4.18 – 4.01 (m, 8H), 4.00 – 3.78 (m, 9H), 3.77 – 3.59 (m, 8H), 3.55 (d, *J* = 9.6 Hz, 1H), 3.53 – 3.20 (m, 8H), 3.17 (dd, *J* = 8.8, 4.9 Hz, 1H), 3.06 (s, 3H), 2.97 (m, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 167.0, 165.54, 165.50, 165.2, 164.8, 156.6, 156.3, 140.1, 138.9,

138.79, 138.76, 138.6, 138.41, 138.36, 138.3, 138.2, 138.1, 138.0, 137.6, 136.84, 136.77, 133.5, 133.1, 133.0, 132.5, 130.4, 130.2, 130.1, 129.9, 129.8, 129.7, 129.3, 129.2, 129.1, 129.04, 128.95, 128.8, 128.7, 128.6, 128.44, 128.41, 128.37, 128.35, 128.33, 128.30, 128.25, 128.18, 128.15, 128.1, 128.03, 127.99, 127.96, 127.94, 127.85, 127.8, 127.7, 127.64, 127.61, 127.55, 127.4, 127.3, 127.2, 127.0, 100.4 ($J_{1,2}$ = 165.6 Hz, C-1c), 100.2 ($J_{1,2}$ = 165.6 Hz, C-1d), 100.0 ($J_{1,2}$ = 171.3 Hz, C-1a), 99.8 ($J_{1,2}$ = 171.0 Hz, C-1b), 99.3 ($J_{1,2}$ = 172.8 Hz, C-1a), 98.5 ($J_{1,2}$ = 174.0 Hz, C-1b), 80.7, 80.1, 79.7, 79.2, 77.6, 76.64, 76.56, 75.9, 75.3, 75.2, 74.9, 74.43, 74.41, 74.2, 73.9, 73.62, 73.57, 73.4, 73.3, 73.2, 73.1, 73.0, 72.6, 72.4, 72.1, 71.6, 71.1, 70.5, 70.1, 69.6, 69.5, 69.2, 67.9, 67.8, 67.6, 67.3, 67.0, 66.9, 66.8, 66.3, 52.2, 51.40, 51.37, 46.5, 45.5; IR (thin film) 2926, 1736, 1454, 1271, 1095, 1045, 737, 699 cm^{-1}; HRMS (MALDI) calculated for $C_{180}H_{177}NO_{39}$ $(M+2Na)^{2+}$ 1511.0847 found 1511.0576 *m/z*.

2-Amino-ethyl α-D-glucopyranosyl-(1→4)-α-D-galactopyranosyl-(1→4)-β-D-glucopyranosyluronate-(1→4)-β-D-glucopyranosyl-(1→4)-α-D-glucopyranosyl-(1→4)-α-D-galactopyranoside (24)

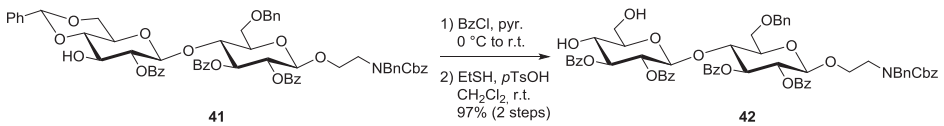


To a stirred solution of ester **40** (22 mg, 7.4 µmol) in THF (1 ml) and MeOH (1 ml) was added at 0 °C a 1:1 (v/v) mixture (296 µl) of hydrogen peroxide (6% (v/v) aq. solution, 295 µmol) and LiOH (0.5 M aq. solution, 74 µmol). The reaction was warmed to room temperature and treated after 2 h and 4 h with another 294 µl of the same lithium peroxide solution, respectively. The mixture was stirred for 16 h at room temperature and treated with NaOH (1 M aq. solution, 0.5 ml) and MeOH (0.5 ml). The reaction was stirred for 20 h at that

temperature, quenched with 10% aq. Na_2SO_3 (0.8 ml) and concentrated under reduced pressure. The residue was dissolved in water (4 ml), acidified at 0 °C with 0.5 M aq. $NaHSO_4$ to approx. pH 4 and extracted with EtOAc (4x10 ml). The combined organic fractions were dried over Na_2SO_4, filtered and concentrated. The residue was treated with NaOMe (0.5 M solution in MeOH, 1 ml), warmed to 40 °C and stirred for 5 h at that temperature. The reaction was cooled to room temperature, stirred for another 16 h at that temperature and treated with water (0.5 ml). The mixture was neutralized with Amberlite IR-120 (H^+ form), filtered and concentrated. The residue was purified by flash chromatography (EtOAc/hexanes 0:1:0 to 1:4 + 2% (v/v) AcOH to 1:1 + 2% (v/v) AcOH) to give the intermediate carboxylic acid as a clear oil.

The intermediate carboxylic acid in CH_2Cl_2/*t*BuOH/water (1.5:16:8, 3 ml) was purged with argon and treated at 0 °C with a suspension of $Pd(OH)_2$ on carbon (20% (w/w) loading, 30 mg) in the same solvent mixture (1 ml). The suspension was purged with hydrogen, warmed to room temperature, stirred under a hydrogen atmosphere for 18 h, filtered and concentrated. The residue was purified by solid phase extraction (Chromabond C18, Macherey-Nagel) and lyophilized to give hexasaccharide **24** (7 mg, 6.7 µmol, 86% over three steps) as a white solid. ^{1}H NMR (600 MHz, D_2O) δ 5.57 (d, *J* = 3.2 Hz, 1H, H-1b), 5.07 (d, *J* = 2.9 Hz, 1H, H-1b), 4.97 (d, *J* = 3.0 Hz, 1H, H-1a), 4.94 (d, *J* = 3.0 Hz, 1H, H-1a), 4.56 (2xd, *J* = 8.0 and 7.9 Hz, 2H, H-1c and H-1d), 4.24 (d, *J* = 9.8 Hz, 1H), 4.15 – 4.07 (m, 3H), 4.01 (m, 4H), 3.89 (m, 16H), 3.77 – 3.60 (m, 8H), 3.56 (dd, *J* = 9.9, 3.1 Hz, 1H), 3.47 (t, *J* = 9.6 Hz, 1H), 3.43 – 3.28 (m, 4H); ^{13}C NMR (150 MHz, D_2O) δ 104.9 (C-1c or C-1d), 104.82 (C-1c or C-1d), 102.80 (C-1a), 102.5 (C-1a), 101.2 (C-1b), 101.0 (C-1b), 81.2, 81.01, 80.98, 80.9, 78.68, 78.67, 77.4, 76.6, 75.7, 75.5, 75.4, 74.5, 74.4, 74.2, 74.1, 73.8, 73.6, 73.3, 71.9, 71.5, 71.3, 71.1, 70.9, 66.5, 63.1, 62.7, 62.5, 62.4, 62.1, 41.9; HRMS (ESI) calculated for $C_{38}H_{65}NO_{32}$ $(M+Na)^+$ 1070.3387 found 1070.3391 *m/z*.

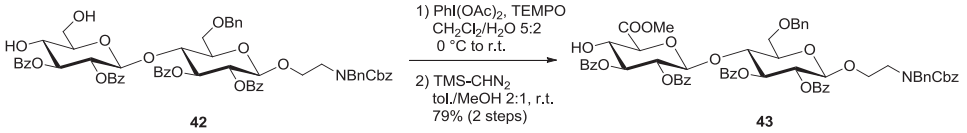
_N_-Benzyl-_N_-benzyloxycarbonyl-2-amino-ethyl 2,3-di-_O_-benzoyl-β-D-glucopyranosyl-(1→4)-2,3-Di-_O_-benzoyl-6-_O_-benzyl-β-D-glucopyranoside (42)



To a stirred solution of alcohol **41** (*17*) (400 mg, 0.36 mmol) in pyridine (5.0 ml) was added at 0 °C benzoyl chloride (63 µl, 0.55 mmol). The reaction was slowly warmed to room temperature and stirred for 16 h at that temperature. An additional 0.5 equiv. BzCl was added to drive the reaction to completion. The mixture was stirred for 2 h at room temperature, quenched with water (30 ml) and diluted with EtOAc (50 ml). After separation, the organic fraction was washed with 0.1 M HCl (20 ml) and the aqueous fraction was re-extracted with EtOAc (30 ml). The combined organic fractions were washed with sat. aq. $NaHCO_3$ (20 ml) and brine (10 ml), dried over Na_2SO_4, filtered and concentrated to give the intermediate tetrabenzoate as a yellow oil.

To a stirred solution of the intermediate tetrabenzoate in CH_2Cl_2 (6.5 ml) were added at room temperature ethanethiol (0.36 ml, 4.9 mmol) and *p*-toluenesulfonic acid (12 mg, 0.06 mmol). The mixture was stirred for 2 h at that temperature, quenched with Et_3N (50 µl) and concentrated. The residue was purified by flash chromatography (EtOAc/hexanes 0:1 to 1:10 to 1:5) to give diol **42** (389 mg, 0.349 mmol, 97% over two steps) as a white foam. R_f (EtOAc/hexanes 1:1) = 0.34; $[\alpha]_D^{20}$ = +9.1° (c = 1.0, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.02 (d, *J* = 8.0 Hz, 2H), 7.98 – 7.79 (m, 5H), 7.62 – 7.08 (m, 27H), 7.04 – 6.86 (m, 1H), 5.68 – 5.52 (m, 1H), 5.51 – 5.34 (m, 1H), 5.30 – 5.24 (m, 1H), 5.19 (m, 1H), 5.15 – 4.95 (m, 2H), 4.69 (t, *J* = 10.8 Hz, 2H), 4.53 (d, *J* = 7.9 Hz, 1H), 4.48 – 4.29 (m, 3H), 4.28 – 4.14 (m, 1H), 4.04 – 3.93 (m, 1H), 3.84 (dd, *J* = 10.3, 5.3 Hz, 1H), 3.64 (m, 2H), 3.52 (d, *J* = 10.4 Hz, 1H), 3.48 – 3.13 (m, 7H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.3, 165.4, 165.2, 164.9, 156.3, 156.2, 137.92, 137.85, 137.79, 136.7, 136.6, 133.60, 133.55, 133.47, 133.3, 130.0, 129.8, 129.5, 129.4, 129.2, 129.0, 128.9, 128.8, 128.6, 128.53, 128.48, 128.4, 128.3, 128.2, 128.1, 128.0, 127.8, 127.3, 127.2, 101.2, 100.3, 77.4, 76.9, 75.8, 75.3, 74.6, 73.8, 73.5, 71.9, 71.8, 71.6, 69.3, 68.9, 67.4, 67.2, 67.1, 61.6, 51.7, 46.8, 45.8; IR (thin film) 3448, 2945, 1729, 1602, 1452, 1418, 1365, 1315, 1264, 1093, 1069, 1027, 989, 854, 709 cm^{-1}; HRMS (ESI) calculated for $C_{64}H_{61}NO_{17}$ $(M+Na)^+$ 1138.3837 found 1138.3850 *m/z*.

_N_-Benzyl-_N_-benzyloxycarbonyl-2-amino-ethyl methyl(2,3-di-_O_-benzoyl-β-D-glucopyranosyl)uronate-(1→4)-2,3-di-_O_-benzoyl-6-_O_-benzyl-β-D-glucopyranoside (43)

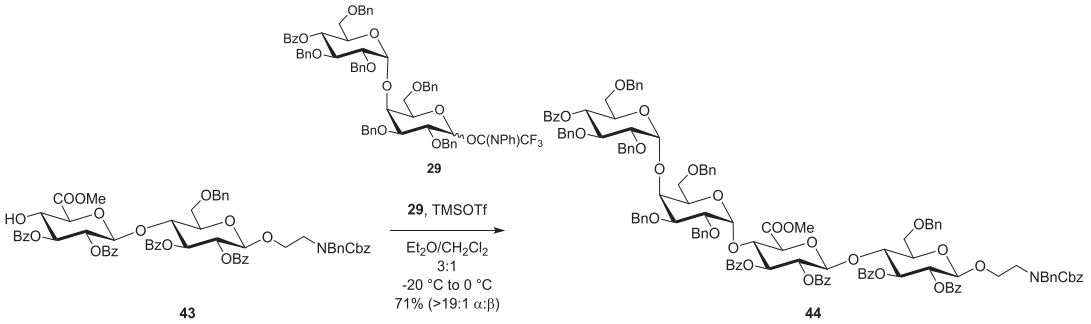


42 **43**

To a stirred solution of alcohol **42** (90 mg, 0.081 mmol) in CH_2Cl_2 (2.0 ml) and water (0.8 ml) were added at 0 °C TEMPO (2.5 mg, 0.016 mmol) and $PhI(OAc)_2$ (55 mg, 0.170 mmol). The reaction was stirred for 20 min at that temperature and warmed to room temperature. The mixture was stirred for 2 h at that temperature and diluted with EtOAc (20 ml) and water (10 ml). After separation, the aqueous fraction was extracted with EtOAc (2x10 ml), the combined organic fractions were dried over Na_2SO_4, filtered and concentrated. The residue was purified by flash chromatography (EtOAc/hexanes 1:2 to 1:1, then 1:1 + 5% AcOH) to give the intermediate carboxylic acid as a white foam.

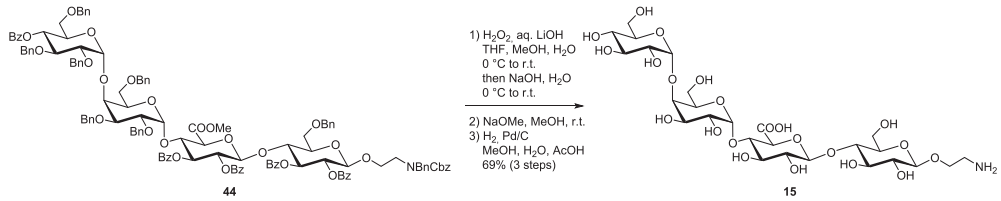
To a stirred solution of the intermediate carboxylic acid in toluene (1.6 ml) and MeOH (0.8 ml) was added at room temperature TMS-diazomethane (40 µl, 0.081 mmol). The reaction was stirred for 2 h at that temperature. An additional 0.25 equiv. TMS-diazomethane was added to drive the reaction to completion. The mixture was stirred for 1 h, quenched with AcOH (0.1 ml) and concentrated. The residue was purified by flash chromatography (EtOAc/hexanes 0:1 to 1:1) to give methyl ester **43** (73 mg, 0.064 mmol, 79% over two steps) as a clear oil. R_f (EtOAc/hexanes 1:1) = 0.47; $[\alpha]_D^{20}$ = +16.7° (c = 1.47, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.90 (m, 8H), 7.58 – 7.29 (m, 20H), 7.24 – 6.93 (m, 7H), 5.71 – 5.49 (m, 1H), 5.50 – 5.22 (m, 3H), 5.14 – 4.94 (m, 2H), 4.77 – 4.62 (m, 2H), 4.55 – 4.17 (m, 5H), 4.09 – 3.78 (m, 2H), 3.65 (m, 3H), 3.59 – 3.38 (m, 5H), 3.34 – 3.19 (m, 2H), 3.12 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.4, 166.6, 165.6, 165.4, 164.8, 156.4, 156.2, 138.0, 137.9, 137.8, 136.6, 133.5, 133.31, 133.26, 133.0, 132.9, 130.06, 130.05, 130.0, 129.9, 129.7, 129.42, 129.38, 129.37, 129.11, 129.10, 129.05, 128.9, 128.6, 128.53, 128.49, 128.3, 128.2, 128.10, 128.06, 128.0, 127.8, 127.3, 127.2, 101.3, 100.4, 77.4, 75.1, 74.8, 74.6, 74.4, 73.8, 73.7, 73.4, 72.2, 72.1, 71.4, 70.4, 68.9, 67.4, 67.2, 52.7, 51.7, 46.8, 45.8. ; IR (thin film) 3442, 2951, 1731, 1602, 1452, 1366, 1270, 1094, 1069, 1028, 992, 752, 709 cm^{-1}; HRMS (ESI) calculated for $C_{65}H_{61}NO_{18}$ $(M+Na)^+$ 1166.3786 found 1166.3762 *m/z*.

***N*-Benzyl-*N*-benzyloxycarbonyl-2-amino-ethyl 4-*O*-benzoyl-2,3,6-tri-*O*-benzyl-α-D-glucopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-α-D-galactopyranosyl-(1→4)-methyl [2,3-Di-*O*-benzoyl-β-D-glucopyranosyl]uronate-(1→4)-2,3-di-*O*-benzoyl-6-*O*-benzyl-β-D-glucoyranoside (44)**



Alcohol **43** (100 mg, 87 µmol) and imidate **29** (121 mg, 105 µmol) were co-evaporated with anhydrous toluene (3x10 ml) and kept under high vacuum for 30 min. The mixture was dissolved in Et_2O (3.3 ml) and CH_2Cl_2 (1.1 ml) and stirred over activated molecular sieves (3 Å-AW) for 30 min at room temperature. The solution was cooled to -20 °C and treated with TMSOTf (3.2 µl, 17 µmol). The mixture was stirred for 1 h at that temperature and slowly warmed to 0 °C. The reaction was quenched with sat. aq. $NaHCO_3$ (10 ml), extracted with CH_2Cl_2 (3x20 ml) and the combined organic fractions were dried over Na_2SO_4, filtered and concentrated. The residue was purified by flash chromatography (EtOAc/hexanes/toluene 1:3:3 to 1:2:2) to give tetrasaccharide **44** (130 mg, 62 µmol, 71%) as a clear oil. R_f (EtOAc/hexanes/toluene 1:1:2) = 0.66; $[\alpha]_D^{20}$ = +35.8° (c = 1.03, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.92 (m, 10H), 7.64 – 6.97 (m, 60H), 5.54 (m, 3H), 5.37 (m, 2H), 5.16 – 4.97 (m, 3H), 4.86 – 4.58 (m, 8H), 4.56 – 4.45 (m, 1H), 4.46 – 4.17 (m, 10H), 4.19 – 4.07 (m, 3H), 4.03 – 3.60 (m, 10H), 3.57 – 3.37 (m, 3H), 3.32 – 3.18 (m, 3H), 3.13 (s, 3H), 3.07 – 2.92 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.0, 165.6, 165.5, 165.4, 165.1, 164.8, 156.3, 156.2, 138.8, 138.5, 138.4, 138.3, 138.1, 138.0, 137.9, 137.8, 136.74, 136.66, 133.5, 133.3, 133.0, 132.7, 130.3, 129.94, 129.85, 129.7, 129.42, 129.38, 129.2, 129.0, 128.6, 128.54, 128.49, 128.3, 128.2, 128.1, 127.9, 127.84, 127.79, 127.7, 127.63, 127.58, 127.5, 127.4, 127.3, 127.2, 101.2 (C-1c), 100.6 (C-1d), 100.0 (C-1b), 99.3 (C-1a), 80.1, 79.7, 77.4, 77.0, 75.8, 75.24, 75.17, 75.1, 74.7, 74.2, 73.9, 73.8, 73.5, 73.4, 73.2, 73.0, 72.4, 72.3, 71.6, 71.0, 70.0, 69.1, 68.9, 67.4, 67.2, 67.1, 66.3, 52.3, 51.7; IR (thin film) 2870, 1732, 1602, 1496, 1453, 1363, 1269, 1094, 1070, 1051, 1027, 738, 709 cm^{-1}; HRMS (ESI) calculated for $C_{126}H_{121}NO_{29}$ $(M+Na)^+$ 2134.7921 found 2134.7879 *m/z*.

2-Amino-ethyl α-D-glucopyranosyl-(1→4)-α-D-galactopyranosyl-(1→4)-β-D-glucopyranosyluronate-(1→4)-β-D-glucoyranoside (15)

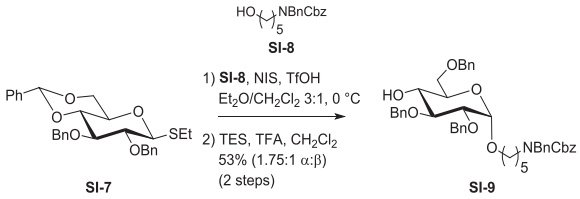


44 15

To a stirred solution of ester **44** (56 mg, 26 µmol) in THF (5 ml) and MeOH (1 ml) were added at 0 °C hydrogen peroxide (6% aq. solution, 265 µl, 530 µmol) and LiOH (1 M aq. solution, 265 µl, 132 mol). The reaction was stirred for 1 h and warmed to room temperature. The reaction was kept at that temperature and treated after 2 h with hydrogen peroxide (6% aq. solution, 265 µl, 530 µmol) and LiOH (1 M aq. solution, 265 µl, 132 µmol). After 2 h, NaOH (15% (w/v) aq. solution, 1 ml) was added and the mixture was stirred for 72 h at room temperature. The solvents were evaporated under reduced pressure, the residue was co-evaporated with toluene (2x5 ml) and dissolved in MeOH (5 ml). The solution was treated at room temperature with NaOMe (143 mg, 2.65 mmol) and stirred for 96 h at that temperature. The solvent was evaporated and the residue was dissolved in water (5 ml). The solution was acidified at 0 °C with 0.5 M aq. $NaHSO_4$ to approx. pH 4 and extracted with EtOAc (5x5 ml). The combined organic fractions were dried over Na_2SO_4, filtered and concentrated to give the intermediate acid as a white foam.

The intermediate acid in MeOH (2 ml) was added at room temperature to a suspension of Pd/C (50 mg) in MeOH (1 ml), water (0.1 ml) and AcOH (five drops). The mixture was purged with hydrogen, stirred under hydrogen atmosphere for 48 h at room temperature, filtered and concentrated. The residue was purified by solid phase extraction (Chromabond C18, Macherey-Nagel) and lyophilized to give tetrasaccharide **15** (acetate salt, 13.6 mg, 18 µmol, 69% over three steps) as a white solid. ^{1}H NMR (400 MHz, D_2O) δ 5.49 (d, *J* = 3.8 Hz, 1H, H-1b), 4.89 (d, *J* = 3.8 Hz, 1H, H-1a), 4.51 – 4.43 (2xd, *J* = 7.9 and 7.9 Hz, 2H, H-1c and H-1d), 4.11 – 4.01 (m, 3H), 3.97 – 3.83 (m, 4H), 3.82 – 3.53 (m, 13H), 3.48 (dd, *J* = 10.0, 3.8 Hz, 1H), 3.44 – 3.27 (m, 3H), 3.20 (t, *J* = 5.0 Hz, 2H); ^{13}C NMR (150 MHz, D_2O) δ 177.7, 104.7 (C-1c), 104.5 (C-1d), 102.8 (C-1a), 100.9 (C-1b), 81.01, 80.97, 78.8, 78.7, 78.6, 77.4, 76.6, 75.7, 75.4, 75.3, 74.5, 74.4, 73.5, 71.9, 71.3, 71.1, 68.4, 62.7, 62.5, 62.4, 42.0; HRMS (MALDI) calculated for $C_{26}H_{45}NO_{22}$ $(M+Na)^+$ 746.2330 found 746.2323 *m/z*.

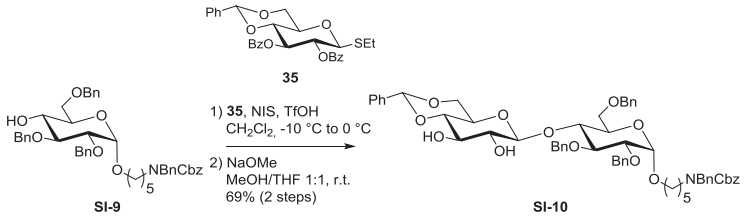
***N*-Benzyloxycarbonyl-5-amino-pentanyl 2,3,6-tri-*O*-benzyl-α-D-glucopyranoside (SI-8)**



Thioglycoside **SI-7** (*44*) (5.47 g, 11.10 mmol) and alcohol **SI-8** (*45*) (4.72 g, 14.43 mmol) were dissolved in Et_2O (75 ml) and CH_2Cl_2 (25 ml) and stirred over activated molecular sieves (4 Å) for 15 min at room temperature. The solution was cooled to 0 °C and treated with NIS (2.30 g, 13.32 mmol) and TfOH (0.148 ml, 1.67 mmol). The mixture was stirred for 1 h at that temperature, quenched with 10% aq. $Na_2S_2O_3$ (25 ml) and filtered through celite. The solution was diluted with CH_2Cl_2 (150 ml) and washed with brine (10 ml). The organic layer was dried over Na_2SO_4, filtered and concentrated. The residue was purified by flash chromatography (3:7 EtOAc/hexanes) to give the intermediate glycoside as a 1.75:1 α/β mixture (6.2 g) as a yellow oil.

The intermediate glycoside mixturewas dissolved in CH_2Cl_2 (100 ml) and stirred in presence over activated molecular sieves (4 Å) for 10 min at room temperature. The mixture was cooled to 0 °C and treated with triethylsilane (10.45 ml, 65.4 mmol) and dropwise with trifluoroacetic acid (3.78 ml, 49.1 mmol). The mixture was warmed to room temperature, stirred for 16 h at that temperature and quenched with water (30 ml). The aqueous layer was extracted with CH_2Cl_2 (3x30 ml), the combined organic fractions werewashed with water (3x20 ml) and brine (20 ml), dried over Na_2SO_4, filtered and concentrated.The residue was purified by flash chromatography (1:5 to 1:4 EtOAc/hexanes)to give alcohol **SI-9** (2.86 g, 3.77 mmol, 34% over two steps) along with the corresponding β-anomer (1.64 g, 2.15 mmol 19% over two steps) as a colorless oil. Analytical data for **SI-9**: Clear oil. $[\alpha]_D^{20}$ = +39.17° (c = 0.5, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.51 – 7.04 (m, 25H), 5.17 (d, *J* = 16.9 Hz, 2H), 5.00 (d, *J* = 11.4 Hz, 1H), 4.80 – 4.42 (m, 8H), 3.93 – 3.04 (m, 11H), 1.70 – 1.43 (m, 4H), 1.39 – 1.13 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.8, 156.3, 139.0, 138.3, 138.2, 138.0, 137.0, 136.8, 128.7, 128.6, 128.5, 128.4, 128.1, 128.0, 128.0, 127.7, 127.4, 127.3, 97.0, 81.7, 79.9, 75.5, 73.7, 73.0, 71.0, 70.2, 69.6, 68.1, 67.3, 50.6, 50.4, 47.4, 46.3, 29.2, 28.1, 27.7, 23.8, 23.6; HRMS (ESI) calculated for $C_{47}H_{53}NO_8$ $(M + Na)^+$ 782.3669 found 782.3664 *m*/*z*.

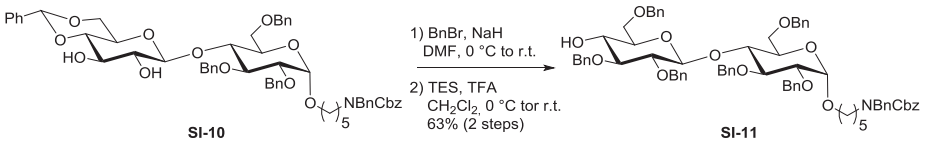
***N*-Benzyloxycarbonyl-5-amino-pentanyl 4,6-*O*-benzylidene-β-D-glucopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-α-D-glucopyranoside (SI-10)**



Alcohol **SI-9** (3.0 g, 3.95 mmol) and thioglycoside **35** (2.57 g, 4.94 mmol) were dissolved in CH_2Cl_2 (50 ml) and stirred over activated molecular sieves (4 Å) for 15 min at room temperature. The solution was cooled to -10 °C and treated with NIS (1.07 g, 4.74 mmol) and TfOH (53 µl, 0.59 mmol). The mixture was stirred for 3 h at that temperature, quenched with 10% aq. $Na_2S_2O_3$ (25 ml) and filtered through celite. The solution was diluted with CH_2Cl_2 (150 ml) and washed with sat. aq. $NaHCO_3$ (50 ml) and brine (25 ml). The organic layer was dried over Na_2SO_4, filtered and concentrated. The residue was purified by flash chromatography (1:4 to 1:0 EtOAc/hexanes) to give the intermediate glycoside as (4.0 g) as a clear oil.

To a stirred solution of the intermediate glycoside in MeOH (40 ml) and THF (40 ml) was added at room temperature NaOMe (0.5 M in MeOH, 16.42 ml, 8.21 mmol). The solution was and stirred for 18 h and concentrated. The residue was dissolved in CH_2Cl_2 (100 ml) and water (100 ml) and the layers were separated. The aqueous layer was extracted with CH_2Cl_2 (3x50 ml) and the combined organic fractions were dried over Na_2SO_4, filtered and concentrated. The residue was purified by flash chromatography (2:5 to 3:5 EtOAc/hexanes) to give diol **SI-10** (2.75 g, 2.72 mmol, 69% over two steps) as a clear oil. $[\alpha]_D^{20}$ = +48.05° (c = 1.00, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.58 – 7.04 (m, 30H), 5.44 (s, 1H), 5.17 (d, *J* = 14.6 Hz, 2H), 4.97 – 4.81 (m, 2H), 4.79 – 4.64 (m, 3H), 4.63 – 4.41 (m, 5H), 4.07 – 3.87 (m, 5H), 3.78 (dd, *J* = 18.1, 9.6 Hz, 1H), 3.69 – 3.18 (m, 10H), 3.10 (td, *J* = 9.8, 5.0 Hz, 1H), 2.64 (d, *J* = 16.9 Hz, 1H), 1.66 – 1.46 (m, 4H), 1.43 – 1.24 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 157.0, 156.4, 139.3, 138.4, 137.9, 137.6, 137.2, 129.4, 128.7, 128.6, 128.5, 128.5, 128.3, 128.2, 128.1, 128.0, 127.5, 127.3, 126.5, 103.6, 101.9, 97.0, 80.8, 80.5, 79.7, 77.6, 75.6, 75.4, 73.8, 73.5, 73.2, 69.8, 68.8, 68.5, 68.2, 67.7, 67.4, 66.4, 50.5, 50.2, 47.2, 46.0, 29.2, 28.0, 27.5, 23.8; HRMS (ESI) calculated for $C_{60}H_{67}NO_{13}$ $(M + Na)^+$ 1032.4510 found 1032.4505 *m*/*z*.

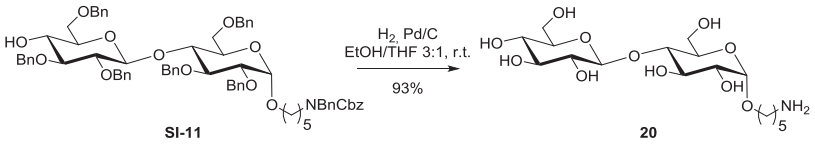
***N*-Benzyloxycarbonyl-5-amino-pentanyl 2,3,6-*O*-tri-*O*-benzyl-β-D-glucopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-α-D-glucopyranoside (SI-11)**



To a stirred solution of diol **SI-10** (5.8 g, 5.74 mmol) in anhydrous DMF (60 ml) was added at 0 °C sodium hydride (60% (w/w) emulsion in mineral oil, 0.92 g, 22.97 mmol) and the mixture was stirred at that temperature for 35 min. Benzyl bromide (2.05 ml, 17.22 mmol) was added dropwise at 0 °C, the reaction was warmed to room temperature and stirred for 24 h. The mixture was quenched with water (150 ml) and the aqueous fractions was extracted with EtOAc (3x100 ml). The combined organic fractions were washed with water (3x50 ml) and brine (20 ml), dried over Na_2SO_4, filtered and concentrated. The residue was purified by flash chromatography (1:4 to 2:5 EtOAc/hexanes) to give the intermediate ether as a white foam.

The intermediate ether was dissolved in CH_2Cl_2 (100 ml) and stirred over activated molecular sieves (4 Å) for 10 min at room temperature. The mixture was cooled to 0 °C and treated with triethylsilane (6.98 ml, 43.7 mmol) and dropwise with trifluoroacetic acid (2.52 ml, 32.8 mmol). The mixture was warmed to room temperature, stirred for 16 h at that temperature and quenched with water (30 ml). The aqueous layer was extracted with CH_2Cl_2 (3x30 ml), the combined organic fractions werewashed with water (3x20 ml) and brine (20 ml), dried over Na_2SO_4, filtered and concentrated. The residue was purified by flash chromatography (1:3 to 2:5 EtOAc/hexanes) to give alcohol **SI-11** (4.3 g, 3.61 mmol, 63%) as a clear oil. $[\alpha]_D^{20}$ = 29.71° (c = 1.00, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.66 – 6.89 (m, 40H), 5.19 (d, *J* = 7.9 Hz, 2H), 4.99 (d, *J* = 11.0 Hz, 1H), 4.92 – 4.66 (m, 7H), 4.61 (d, *J* = 12.1 Hz, 2H), 4.54 – 4.44 (m, 3H), 4.43 – 4.31 (m, 3H), 4.02 – 3.91 (m, 1H), 3.90 – 3.79 (m, 2H), 3.73 – 3.40 (m, 7H), 3.42 – 3.13 (m, 6H), 2.95 (d, *J* = 1.8 Hz, 1H), 1.76 – 1.50 (m, 4H), 1.41 – 1.17 (m, 2H); 13C NMR (100 MHz, $CDCl_3$) δ 156.8, 156.2, 139.7, 138.9, 138.6, 138.0, 136.8, 128.6, 128.6, 128.5, 128.4, 128.3, 128.2, 128.1, 128.03, 127.95, 127.93, 127.87, 127.79, 127.76, 127.7, 127.6, 127.4, 127.3, 127.2, 102.6, 97.1, 84.3, 82.1, 80.4, 79.2, 76.8, 75.3, 74.9, 73.8, 73.7, 73.4, 73.1, 71.4, 70.1, 67.9, 67.3, 50.6, 50.3, 47.2, 46.3, 29.2, 28.1, 27.7, 23.6; HRMS (ESI) calculated for $C_{74}H_{81}NO_{13}$ $(M + Na)^+$ 1214.5606 found 1214.5615 *m/z*.

5-Amino-pentanyl β-D-glucopyranosyl-(1→4)-α-D-glucopyranoside (20)



To a stirred solution of benzyl ether **SI-11** (45 mg, 38 µmol) in EtOH (2 ml) and THF (1 ml) was added at room temperature a suspension of Pd/C (50 mg) in EtOH (1 ml). The mixture was purged with hydrogen and stirred under hydrogen atmosphere for 16 h at room temperature. A suspension of Pd/C (10 mg) in EtOH (1 ml) was added, the mixture was purged with hydrogen and stirred under hydrogen atmosphere for 36 h. The suspension was filtered through PTFE, the filter was washed with MeOH (3x5 ml) and water (2x5 ml) and the combined solutions were concentrated. The residue was lyophilized to give disaccharide **20** (15.0 mg, 35 µmol, 92% over three steps) as a white solid. ^{1}H NMR (600 MHz, D_2O) δ 4.93 (d, *J* = 3.8 Hz, 1H), 4.53 (d, *J* = 7.9 Hz, 1H), 3.93 (dt, *J* = 12.1, 2.3 Hz, 2H), 3.89 – 3.72 (m, 5H), 3.69 – 3.48 (m, 5H), 3.46 – 3.41 (m, 1H), 3.33 (dd, *J* = 9.3, 8.1 Hz, 1H), 3.08 – 2.97 (m, 2H), 1.78 – 1.61 (m, 4H), 1.54 – 1.42 (m, 2H); ^{13}C NMR (150 MHz, D_2O) δ 105.1, 100.4, 81.4, 78.6, 78.2, 75.8, 74.3, 73.6, 73.1, 72.1, 70.5, 63.2, 62.6, 42.0, 30.7, 29.1, 25.1; HRMS (ESI) calculated for $C_{17}H_{33}NO_{11}$ $(M + H)^+$ 428.2132 found 428.2194 *m*/*z*.

SUPPLEMENTARY FIGURES

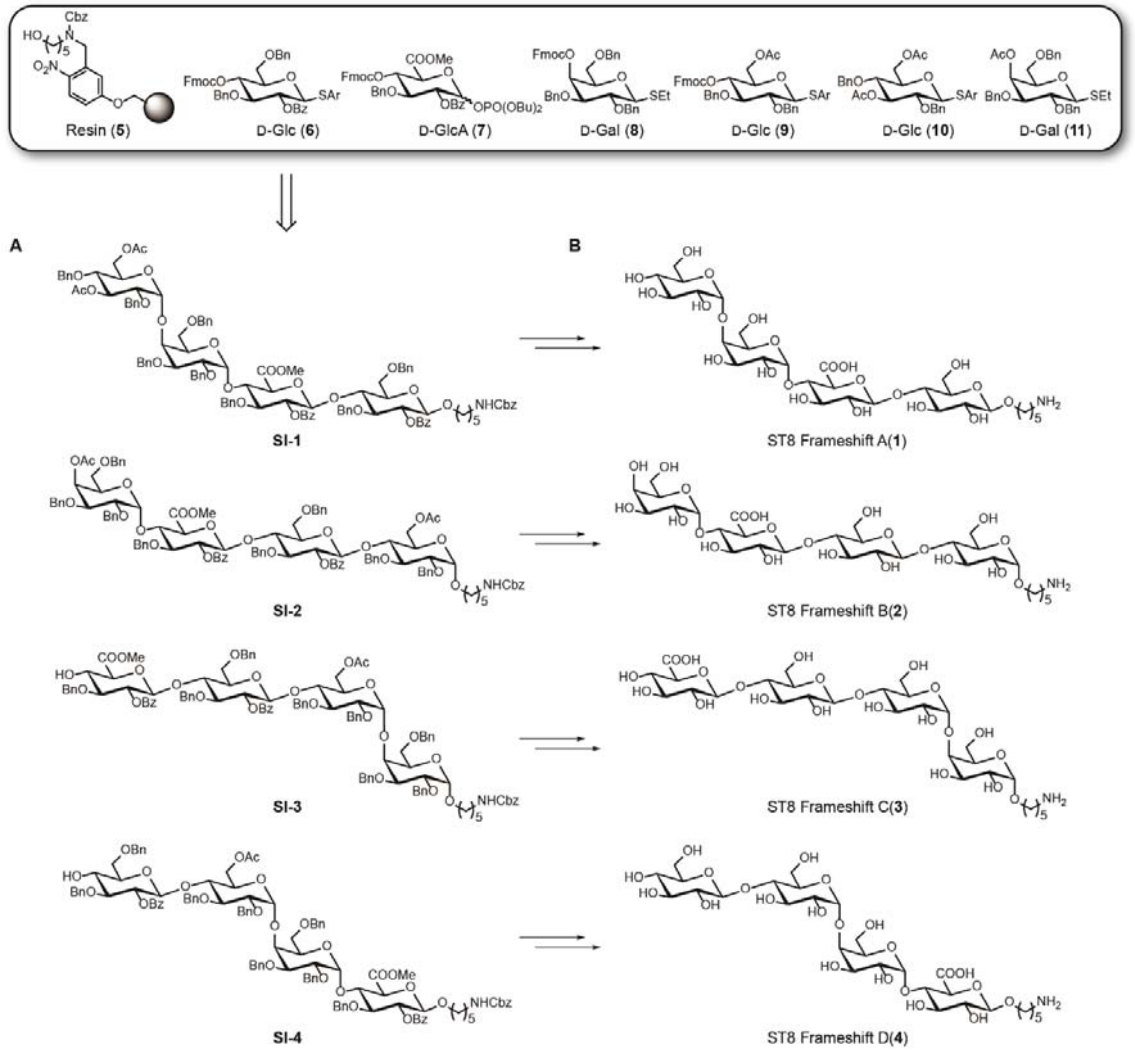


Fig. S1. AGA of tetrasaccharides 1 to 4. (**A**) Polystyrene resin **5** and building blocks **6**-**11** were used to assemble protected tetrasaccharide precursors. Intermediates **SI-1**-**SI-4** were obtained after cleavage from the resin. (**B**) Global deprotection afforded tetrasaccharides **1**-**4**.

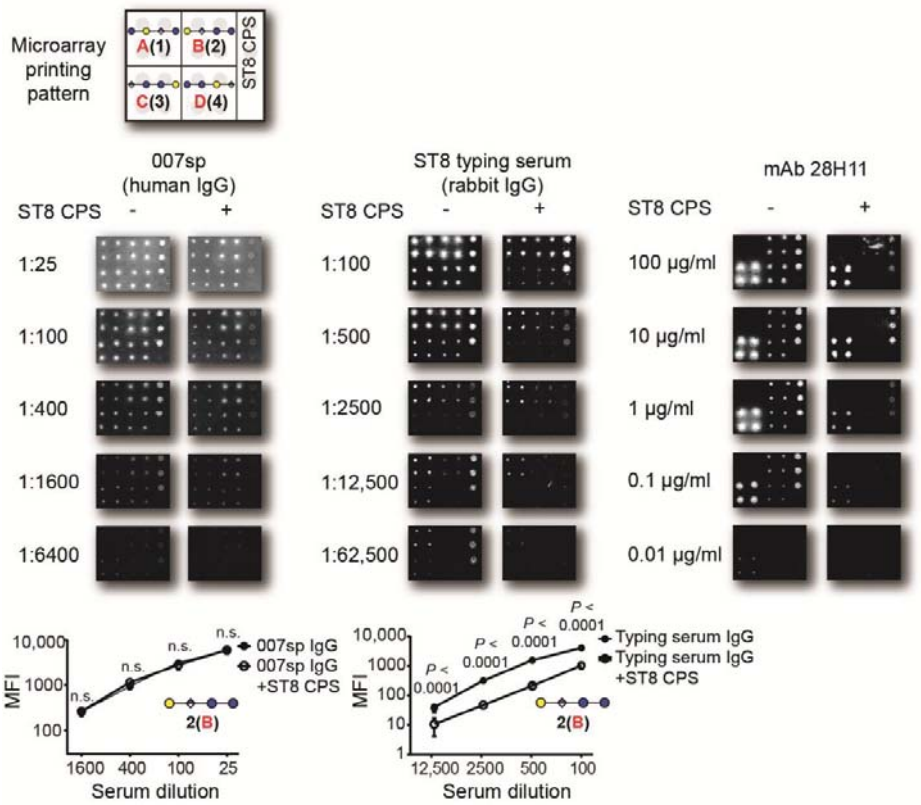


Fig. S2. Differential immune recognition of synthetic ST8 CPS frameshifts. Glycan microarray analysis of pooled human sera 007sp (top) with quantitative analysis of FS B(**2**) binding (bottom), rabbit ST8 typing serum (top) with quantitative analysis of FS B(**2**) binding (bottom), and protective murine mAb 28H11 at different concentrations with or without pre-incubation with native ST8 CPS. Data are means ± SD of 8 spots as technical replicates from one representative out of at least two independent experiments. *P* values are determined by one-tailed, unpaired t test with Welch's correction.

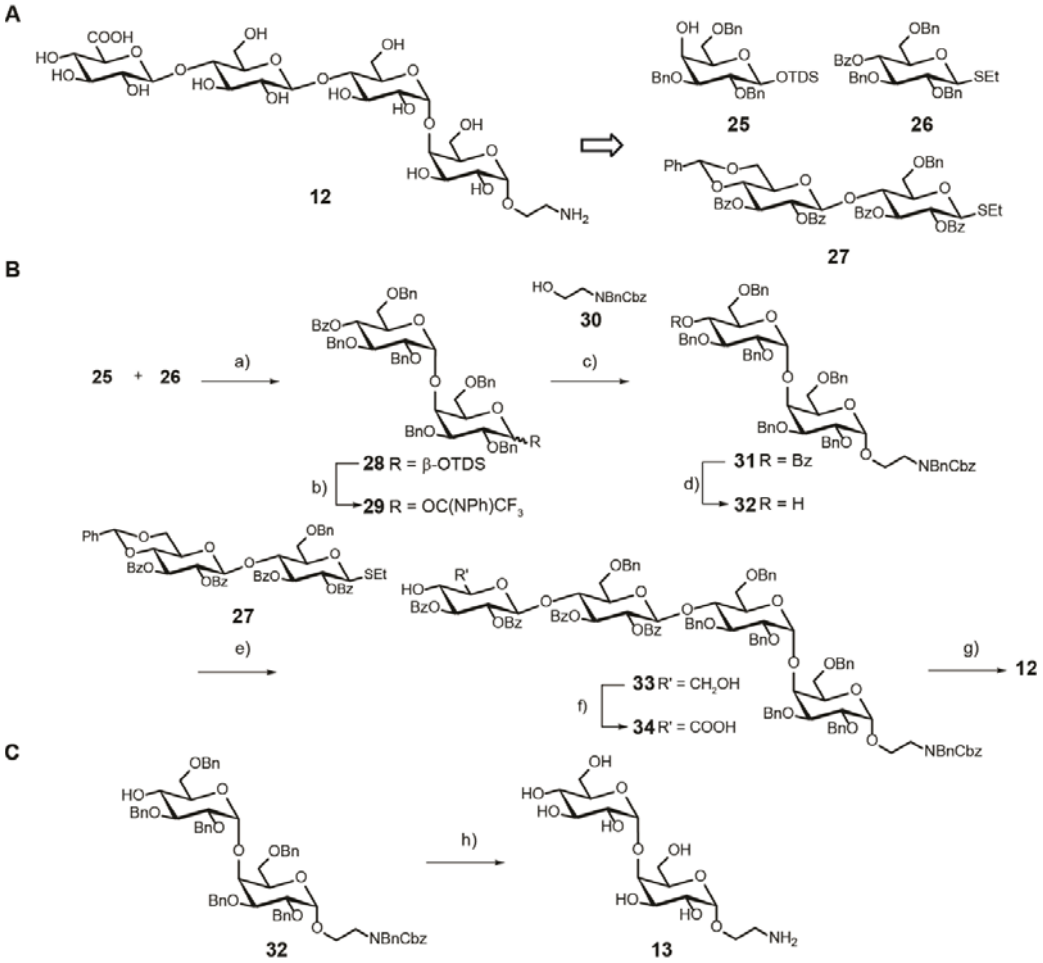


Fig. S3. Solution-phase syntheses of ST8 CPS frameshift C(12) and disaccharide 13. (**A**) Retrosynthesis of tetrasaccharide **12**. (**B**) Total synthesis of **12**. (**C**) Total synthesis of **13**. Reagents and conditions: a) NIS, TfOH, Et_2O/CH_2Cl_2 3:1, -20 °C to -10 °C, 75% (2.4:1 α:β); b) i. TBAF, AcOH, THF, 0 °C to r.t.; ii. $F_3CC(NPh)Cl$, Cs_2CO_3, CH_2Cl_2, r.t., 84% (2 steps); c) **30**, TMSOTf, Et_2O/CH_2Cl_2 4:1, -40 °C to -10 °C, 89% (5:1 α:β); d) NaOMe, MeOH, THF, 37 °C, 85%; e) i. **27**, NIS, TfOH, CH_2Cl_2, -10 °C, ii. EtSH, *p*TsOH, CH_2Cl_2, r.t., 95% (2 steps); f) $PhI(OAc)_2$, TEMPO, CH_2Cl_2/H_2O 5:2, r.t., 74%; g) i. NaOH, THF, MeOH, H_2O, 0 °C to r.t.; ii. H_2, Pd/C, MeOH, H_2O, AcOH, r.t., 70% (2 steps); h) H_2, Pd/C, EtOAc, MeOH, H_2O, AcOH, r.t., 75%. NIS, *N*-iodosuccinimide; TBAF, tetra-n-butylammonium fluoride; TEMPO, 2,2,6,6-tetramethylpiperidine 1-oxyl; *p*TsOH, *p*-toluenesulfonic acid; TfOH, trifluoromethanesulfonic acid; TMSOTf, trimethylsilyl trifluoromethanesulfonate.

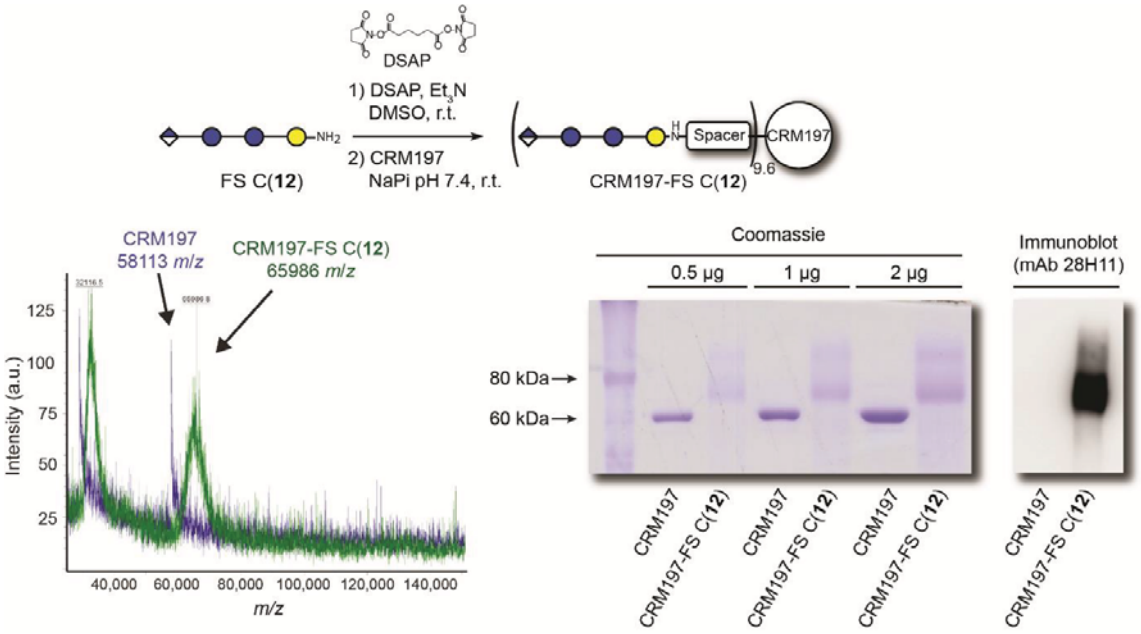


Fig. S4. Characterization of the CRM197–frameshift C(12) glycoconjugate. Conjugation reaction and characterization by MALDI-TOF MS, SDS-PAGE and immunoblot decorated with mAb 28H11.

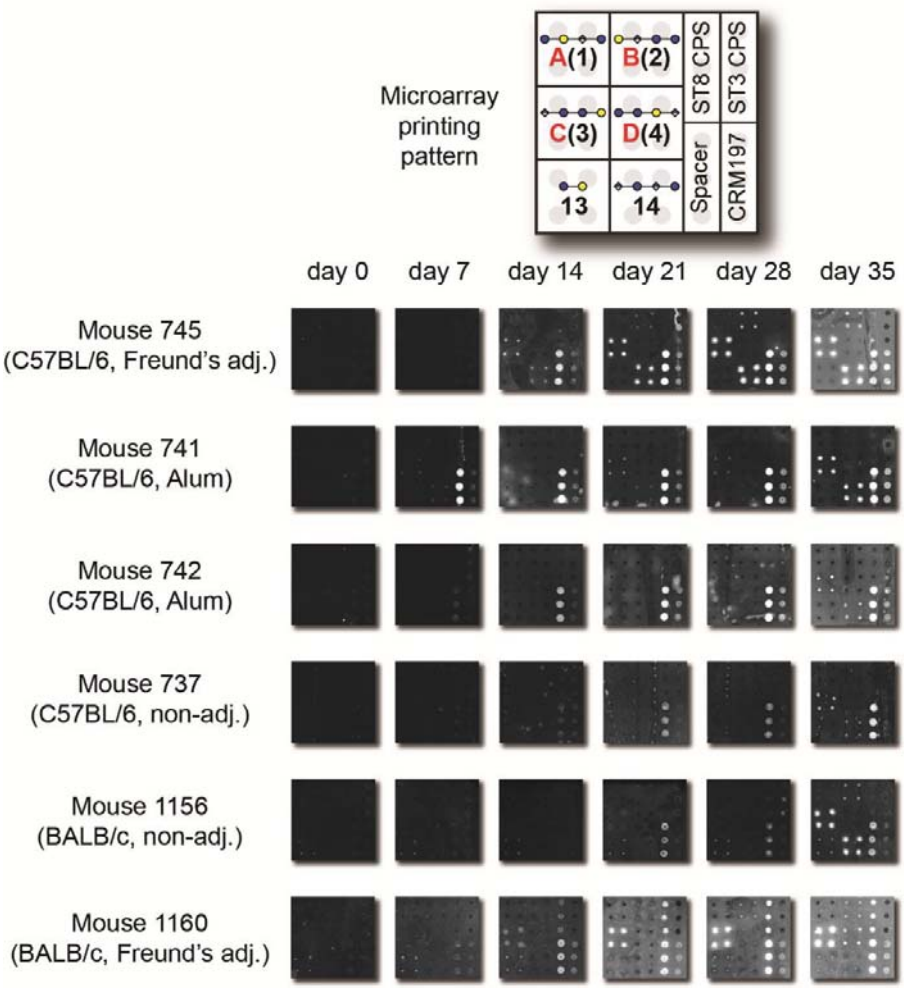


Fig. S5. **Time course of antiglycan immune responses after immunization with CRM197–frameshift C(12)**. Glycan microarray analysis of mice showing an anti-glycan immune response. Splenocytes from mouse 1160 were taken for mAb development.

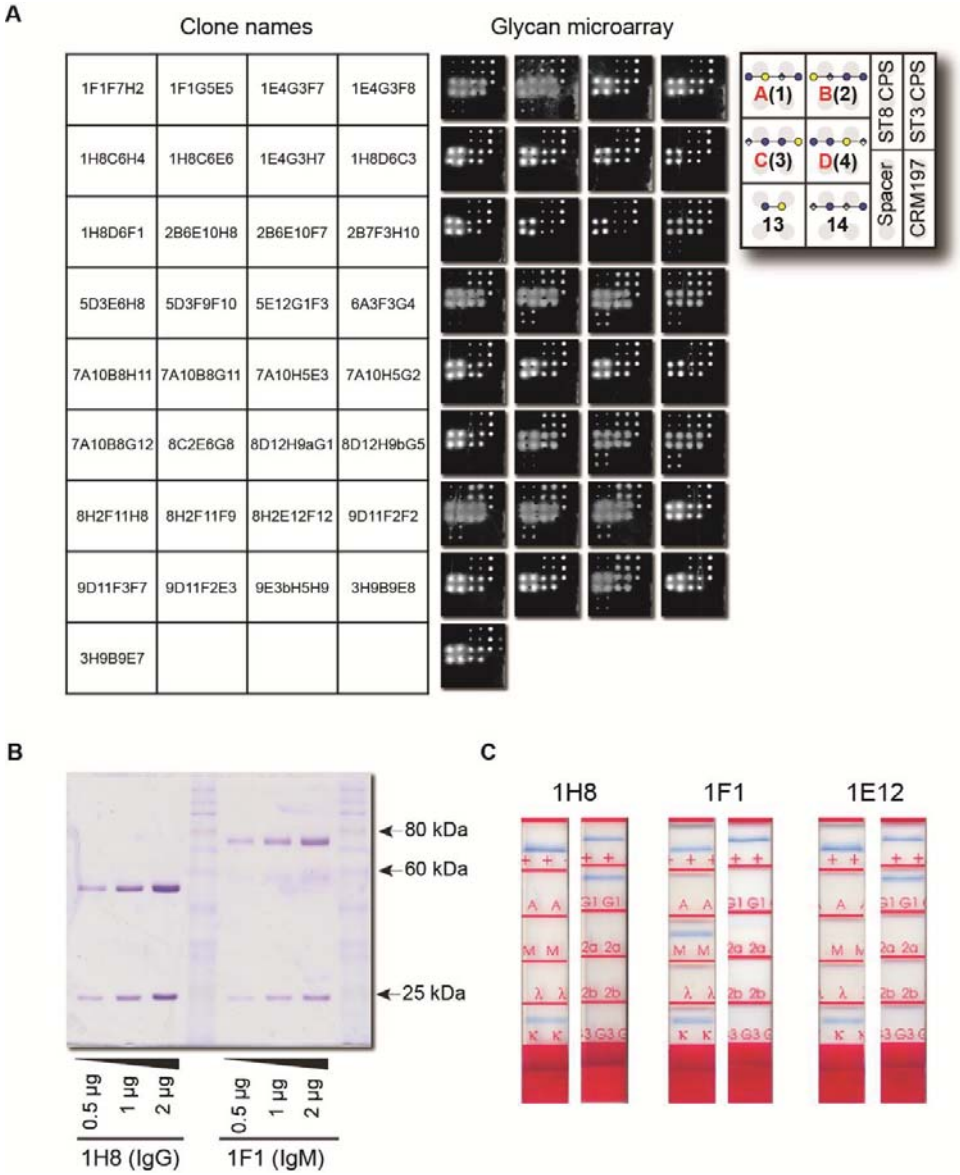


Fig. S6. Characterization of mAbs raised against ST8 frameshift C(12). (**A**) Clone names (left) and glycan binding of cell culture supernatants by glycan microarray. Anti-mouse IgG secondary antibodies that display cross-reactivity towards IgM were used for ELISA and glycan microarray analyses. (**B**) Analysis of purified mAbs 1H8 and 1F1 by SDS-PAGE. (**C**) Determination of mAb isotypes of 1H8 and 1F1 and anti-*Y. pestis* LPS core mAb 1E12 that was used as an IgG1 isotype-matched control in subsequent experiments.

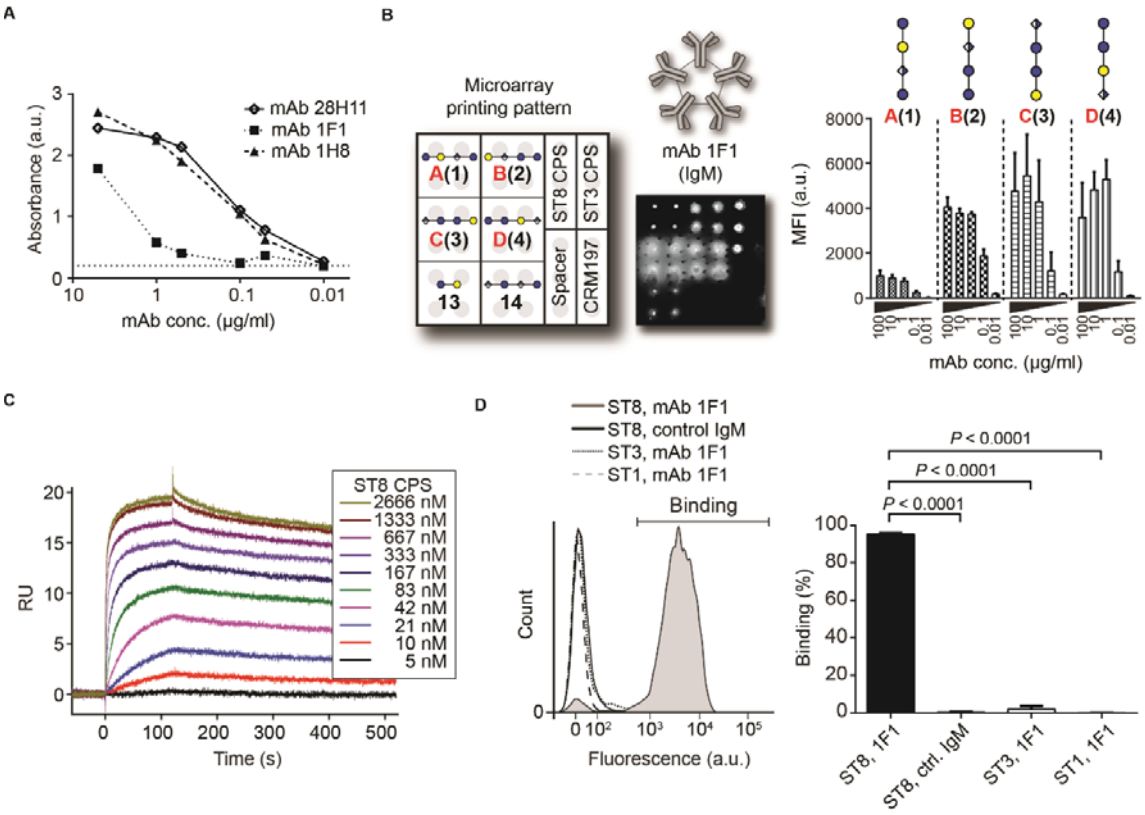


Fig. S7. Binding of ST8-related glycans by mAbs 1H8, 28H11 and 1F1. (**A**) Comparison of ST8 CPS binding of mAbs 1H8 and 1F1 with mAb 28H11 by polysaccharide ELISA. (**B**) Glycan microarray analysis of mAb 1F1 binding to synthetic ST8-related glycans. Data are means + SD of fluorescence intensities of 8 spots as technical replicates from one representative out of two independent experiments. (**C**) Surface plasmon resonance analysis of immobilized mAb 1F1 using ST8 CPS as analyte in the indicated concentrations. (**D**) Flow cytometry of fluorescently labeled, inactivated pneumococci. Data are a representative flow cytometry result with gating strategy for binding after incubation with mAb 1F1 or IgM control mAb and Alexa Fluor 680-labeled secondary antibody, and cumulated results of three labeling experiments. Data are means + SD of positive binding from *n* = 3 independent experiments. *P* values are determined by one-tailed, paired t test.

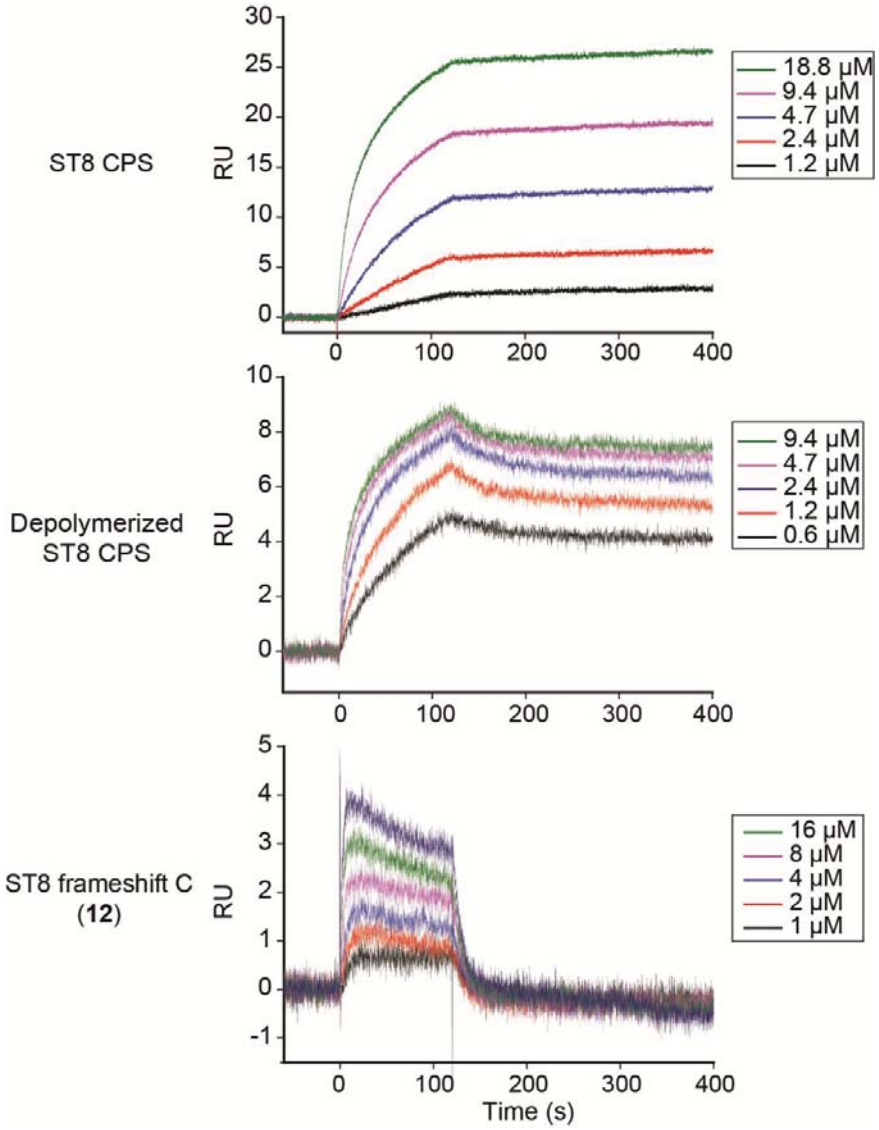


Fig. S8. Qualitative comparison of binding kinetics of mAb 1H8 toward ST8 CPS–derived glycans of different chain lengths. mAb 1H8 was immobilized and subjected to a flow of native ST8 CPS, depolymerized ST8 CPS containing one to eight repeating units or synthetic tetrasaccharide **12**. Analyte concentrations are calculated based on repeating units present (M_r = 664 for polysaccharide preparations and M_r = 723 for tetrasaccharide **12**).

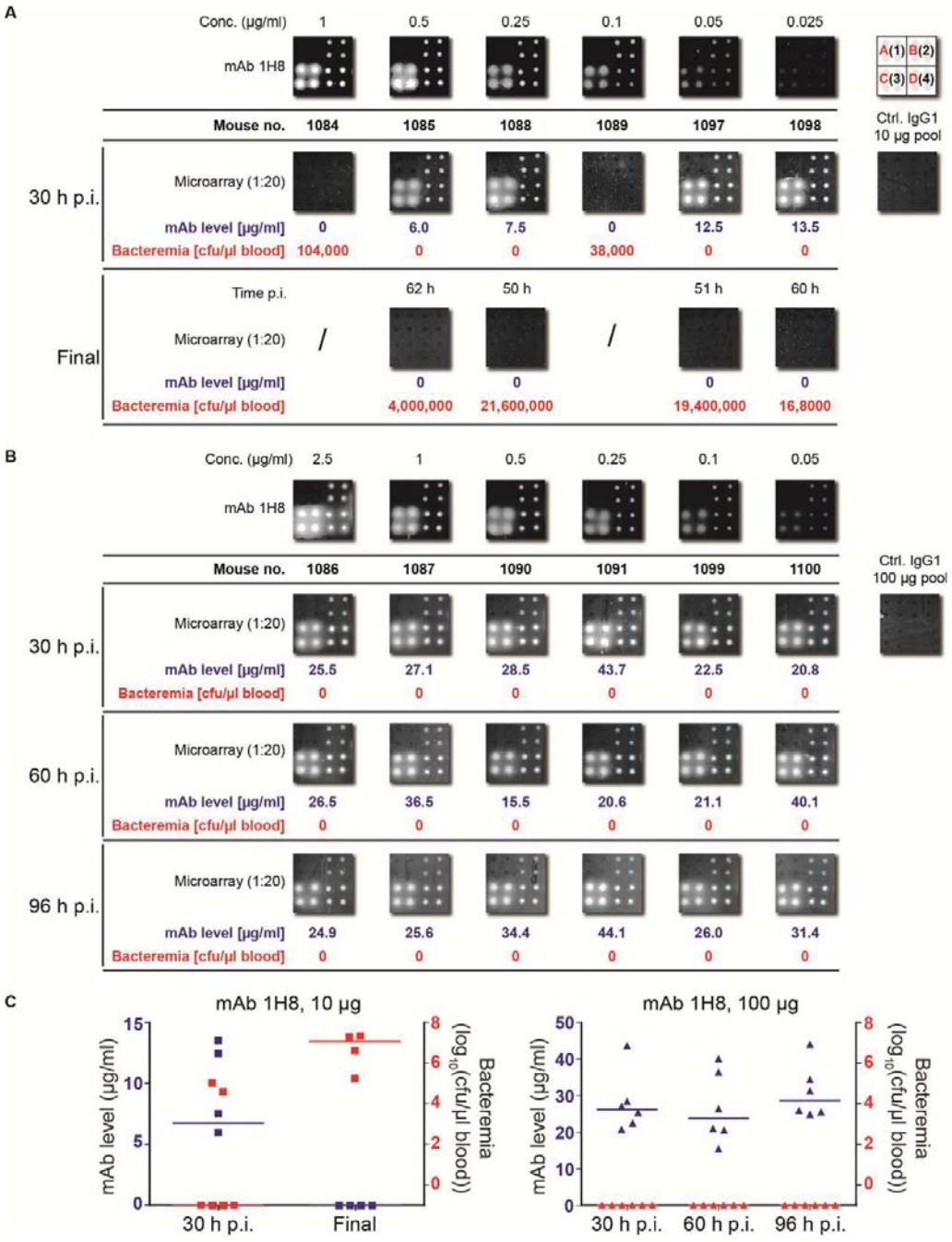


Fig. S9. Correlation between mAb 1H8 levels and bacterial burden in blood after passive immunization and lethal pneumococcal challenge. Assessment of mAb 1H8 levels (glycan microarray) and bacterial burden in blood of passively immunized and ST8 infected mice. mAb concentrations were assessed by comparing fluorescence to standards of mAb 1H8. Data are mean fluorescence values of at least 8 spots as technical replicates. Pooled sera of control mAb-treated mice 30 h p.i. are shown for comparison. (**A**) mAb 10 µg dose. Blood was analyzed 30 h p.i. and at indicated time points upon euthanizing ("final"). (**B**) mAb 100 µg doses. Blood was analyzed after 30 h, 60 h and 96 h. (**C**-**D**) Correlation of mAb levels and bacterial burden in blood of mice receiving a 10 µg (C) or a 100 µg mAb dose (D). Data are individual data from *n* = 5-6 mice pre group and median.

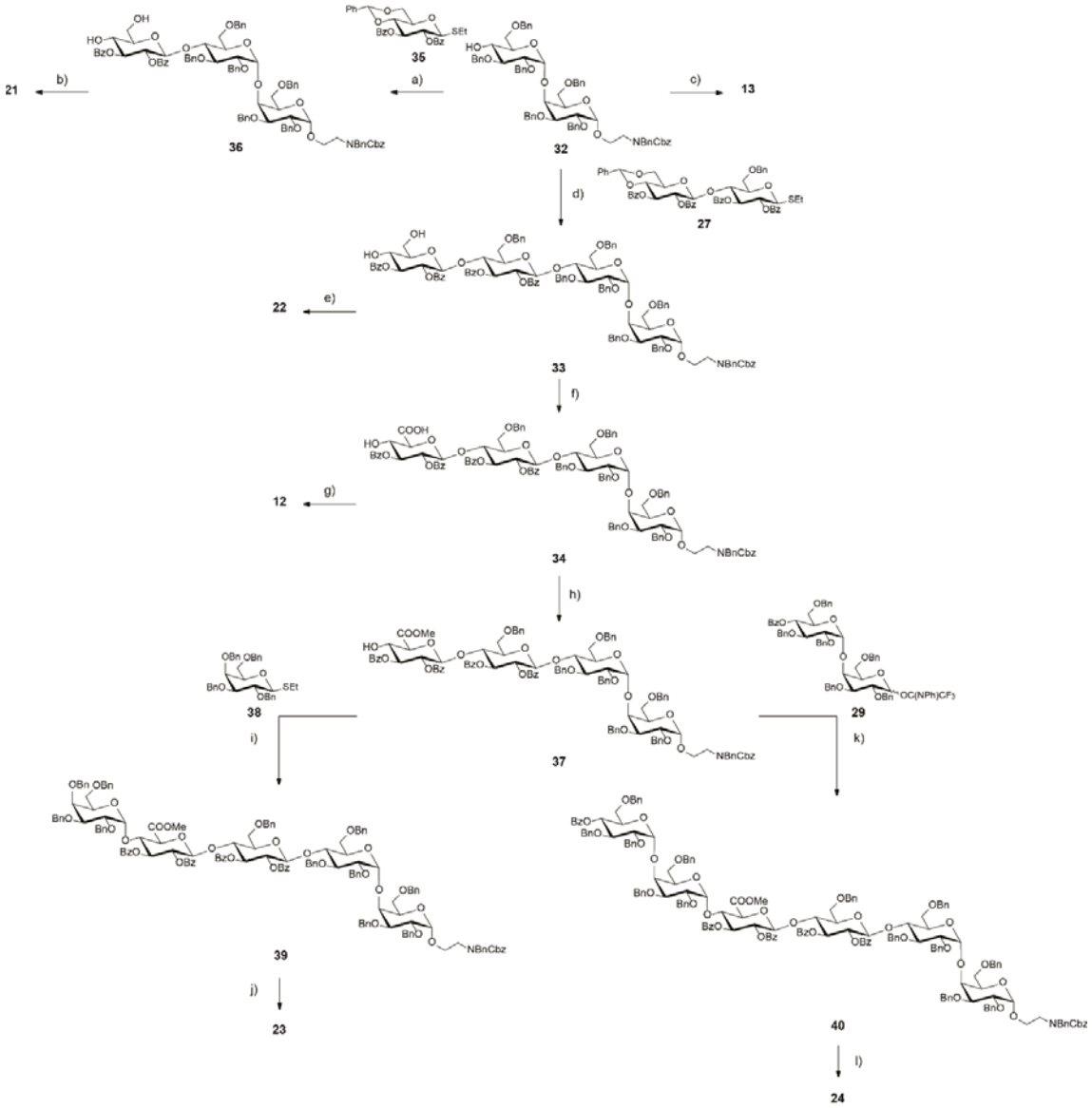


Fig. S10. Divergent total syntheses of ST8 frameshift C-related glycans 21 to 24 from versatile precursor 32. Reagents and conditions: a) i. **35**, NIS, TfOH, CH_2Cl_2, -20 °C, ii. EtSH, *p*TsOH, CH_2Cl_2, r.t., 75% (2 steps); b) i. NaOMe, MeOH, CH_2Cl_2, r.t.; ii. H_2, $Pd(OH)_2$, CH_2Cl_2, *t*BuOH, H_2O, r.t., 79% (2 steps); c) H_2, Pd/C, EtOAc, MeOH, H_2O, AcOH, r.t., 87%; d) **27**, NIS, TfOH, CH_2Cl_2, -10 °C, ii. EtSH, *p*TsOH, CH_2Cl_2, r.t., 95% (two steps); e) i. NaOMe, THF, MeOH, 40 °C; ii. H_2, $Pd(OH)_2$, CH_2Cl_2, *t*BuOH, H_2O, r.t., 89% (2 steps); f) $PhI(OAc)_2$, TEMPO, CH_2Cl_2/H_2O 5:2, r.t., 74%; g) i. NaOH, THF, MeOH, H_2O, 0 °C to r.t.; ii. H_2, Pd/C, MeOH, H_2O, AcOH, r.t., 70% (2 steps); h) MeI, Cs_2CO_3, DMF, r.t., 80%; i) **38**, NIS, TMSOTf, Et_2O/CH_2Cl_2 3:1, -20 °C to 0 °C, 89% (3.6:1 α:β); j) i. H_2O_2, aq. LiOH, THF, MeOH, H_2O, r.t., then aq. NaOH, r.t., ii. NaOMe, MeOH, r.t.; iii. H_2, $Pd(OH)_2$, CH_2Cl_2, *t*BuOH, H_2O, r.t., 88% (3 steps); k) **29**, TMSOTf, Et_2O/CH_2Cl_2 3:1, -20 °C to 0 °C, 68% (>19:1 α:β); l) i. H_2O_2, aq. LiOH, THF, MeOH, H_2O, r.t., then aq. NaOH, r.t., ii. NaOMe, MeOH, r.t.; iii. H_2, $Pd(OH)_2$, CH_2Cl_2, *t*BuOH, H_2O, r.t., 86% (3 steps).

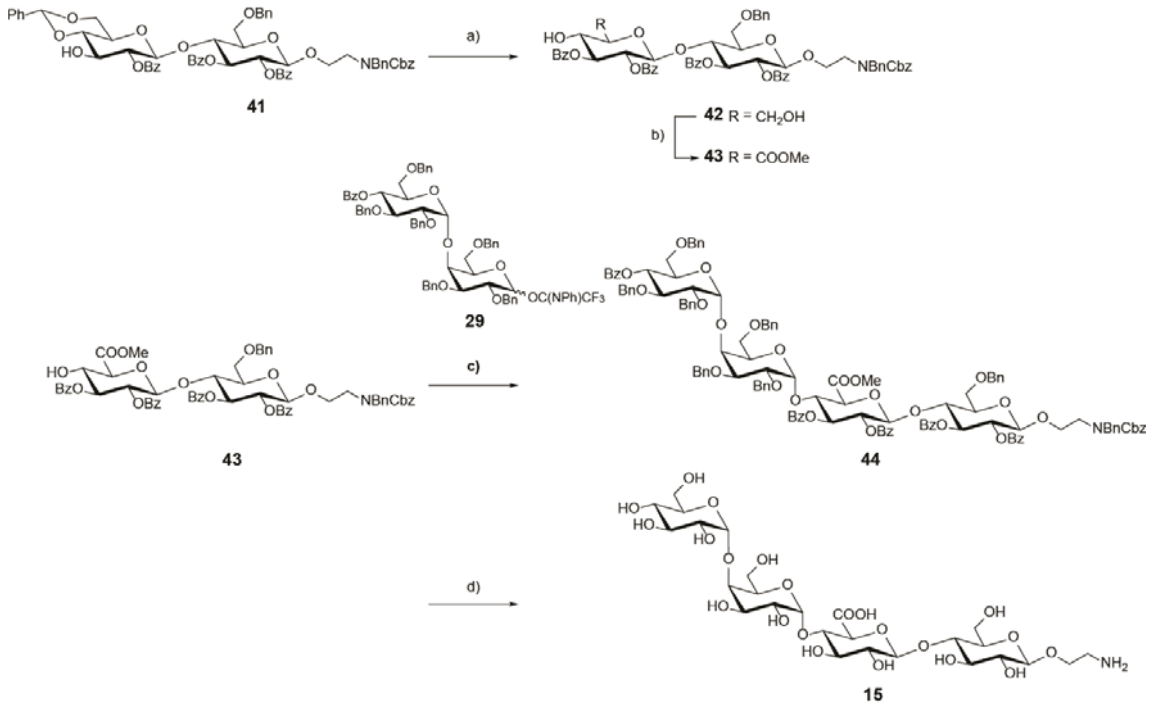


Fig. S11. Total synthesis of ST8 tetrasaccharide 15. Reagents and conditions: a) i. BzCl, pyr., 0 °C to r.t., ii. EtSH, *p*TsOH, CH_2Cl_2, r.t., 97% (2 steps); b) i. $PhI(OAc)_2$, TEMPO, CH_2Cl_2/H_2O 5:2, 0 °C to r.t., ii. TMS-CHN_2, tol./MeOH 2:1, r.t., 79% (2 steps) ; c) **29**, TMSOTf, Et_2O/CH_2Cl_2 3:1, -20 °C to 0 °C, 71% (>19:1 α:β); d) H_2O_2, aq. LiOH, THF, MeOH, H_2O, 0 °C to r.t., then aq. NaOH, 0 °C to r.t., ii. NaOMe, MeOH, r.t.; iii. H_2, Pd/C, MeOH, H_2O, AcOH, r.t., 69% (3 steps).

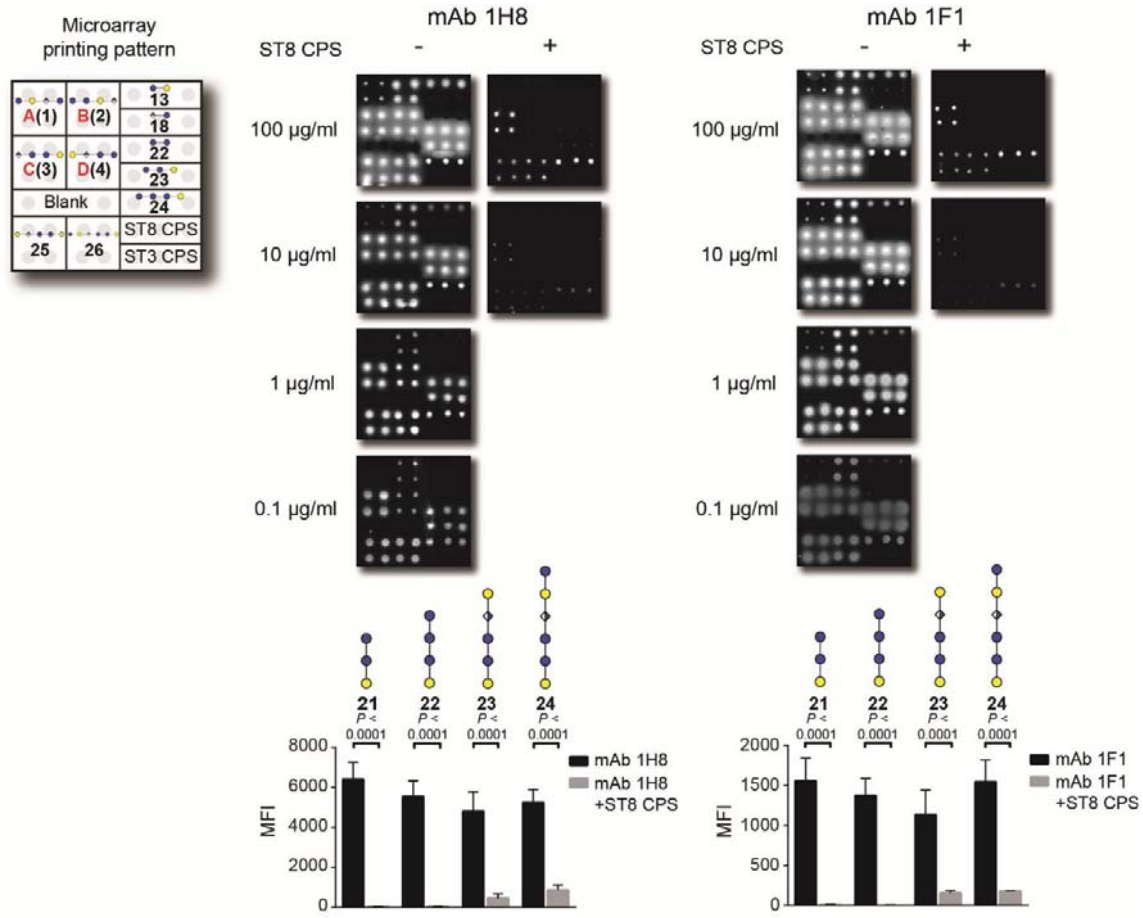


Fig. S12. Binding characterization of mAbs 1H8 and 1F1 to ST8 CPS–derived substructures by glycan microarray. Glycan microarray of mAbs 1H8 and 1F1 with or without pre-adsorption to ST8 CPS (top) and quantitation of binding inhibition to oligosaccharides **21**-**24** by native ST8 CPS (bottom). Data are means + SD of at least 6 spots as technical replicates from one representative out of at least two independent experiments. *P* values are determined by one-tailed, unpaired t test with Welch's correction.

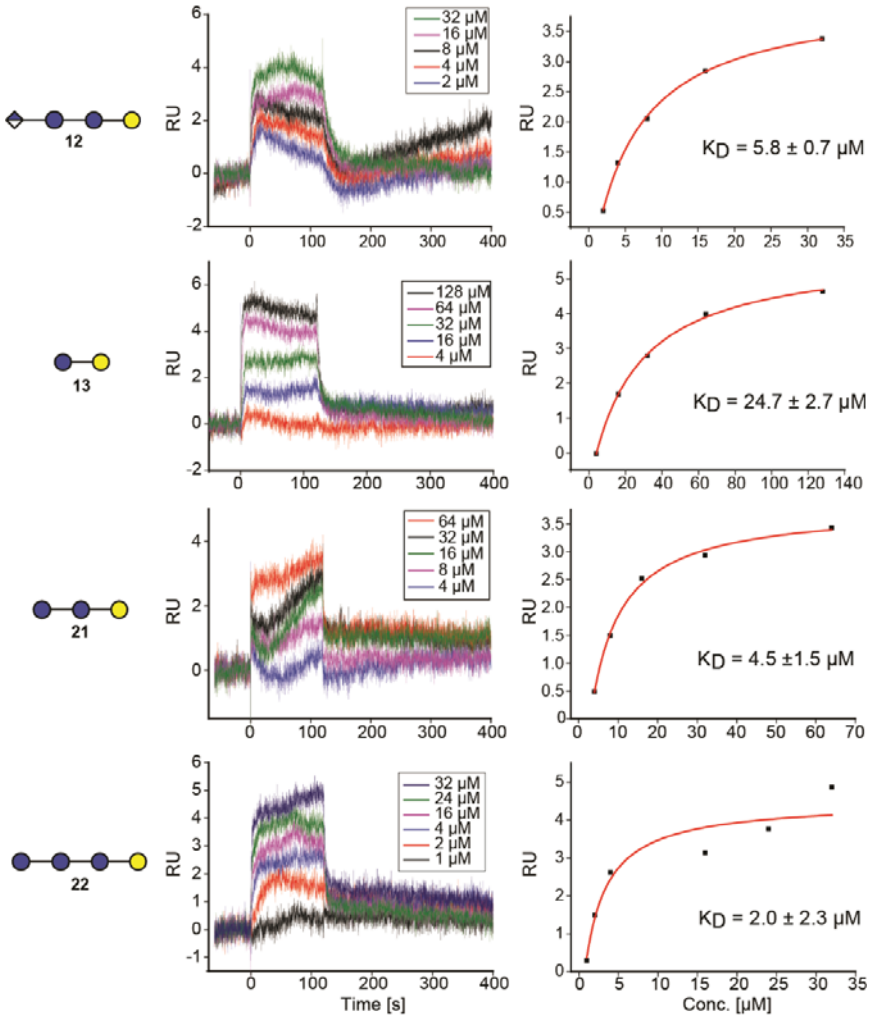


Fig. S13. Determination of affinities of mAb 1H8 toward synthetic ST8 CPS–related oligosaccharides. mAb 1H8 was immobilized and subjected to a flow of synthetic oligosaccharides. Data were fit on general 1:1 binding curves.

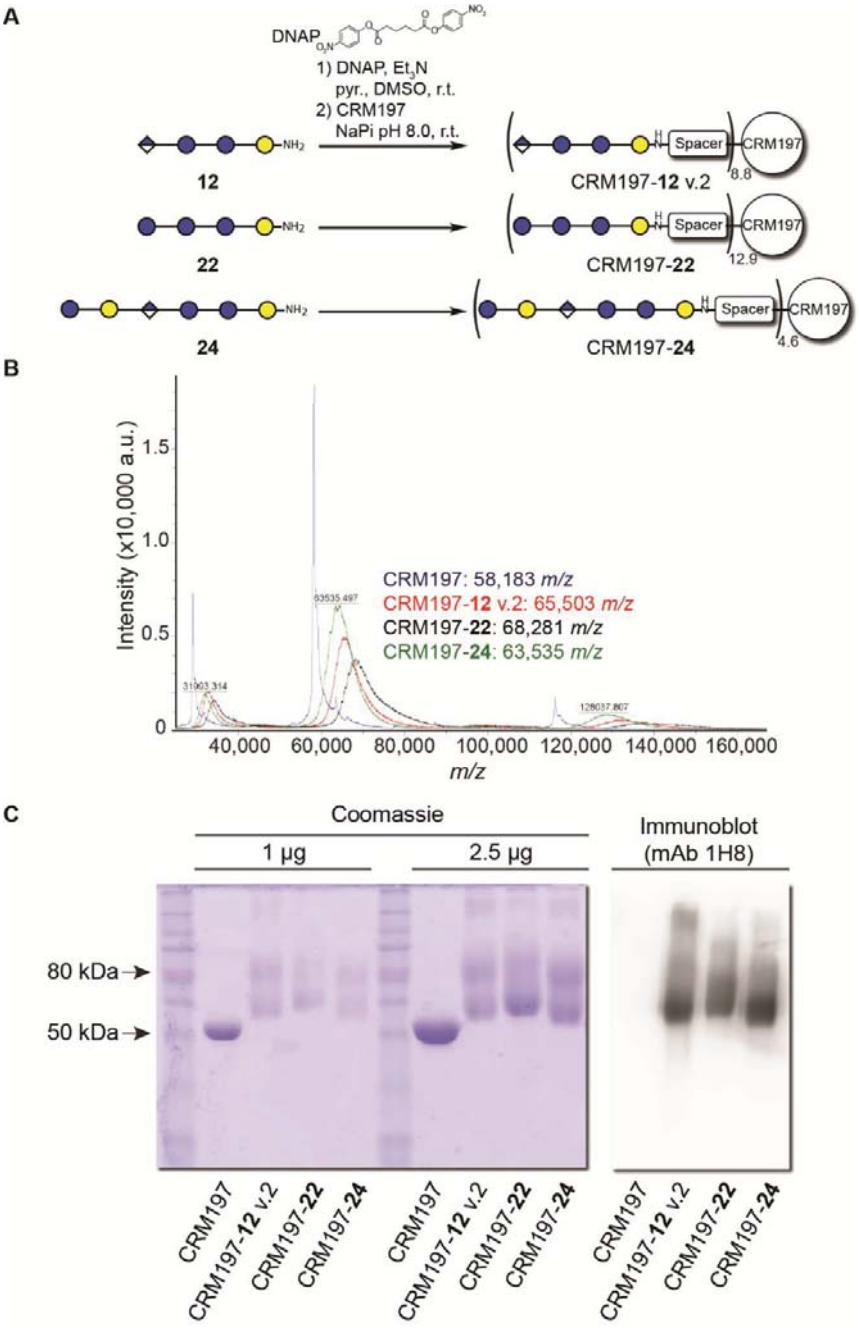


Fig. S14. **Conjugation of synthetic oligosaccharides to CRM197 using the bifunctional spacer bis(4-nitrophenyl)adipate (DNAP).** (**A**) Conjugation reactions of saccharides **12**, **22** and **24**. (**B**) Characterization of glycoconjugates by MALDI-TOF MS. (**C**) Characterization by SDS-PAGE and immunoblot decorated with mAb 1H8.

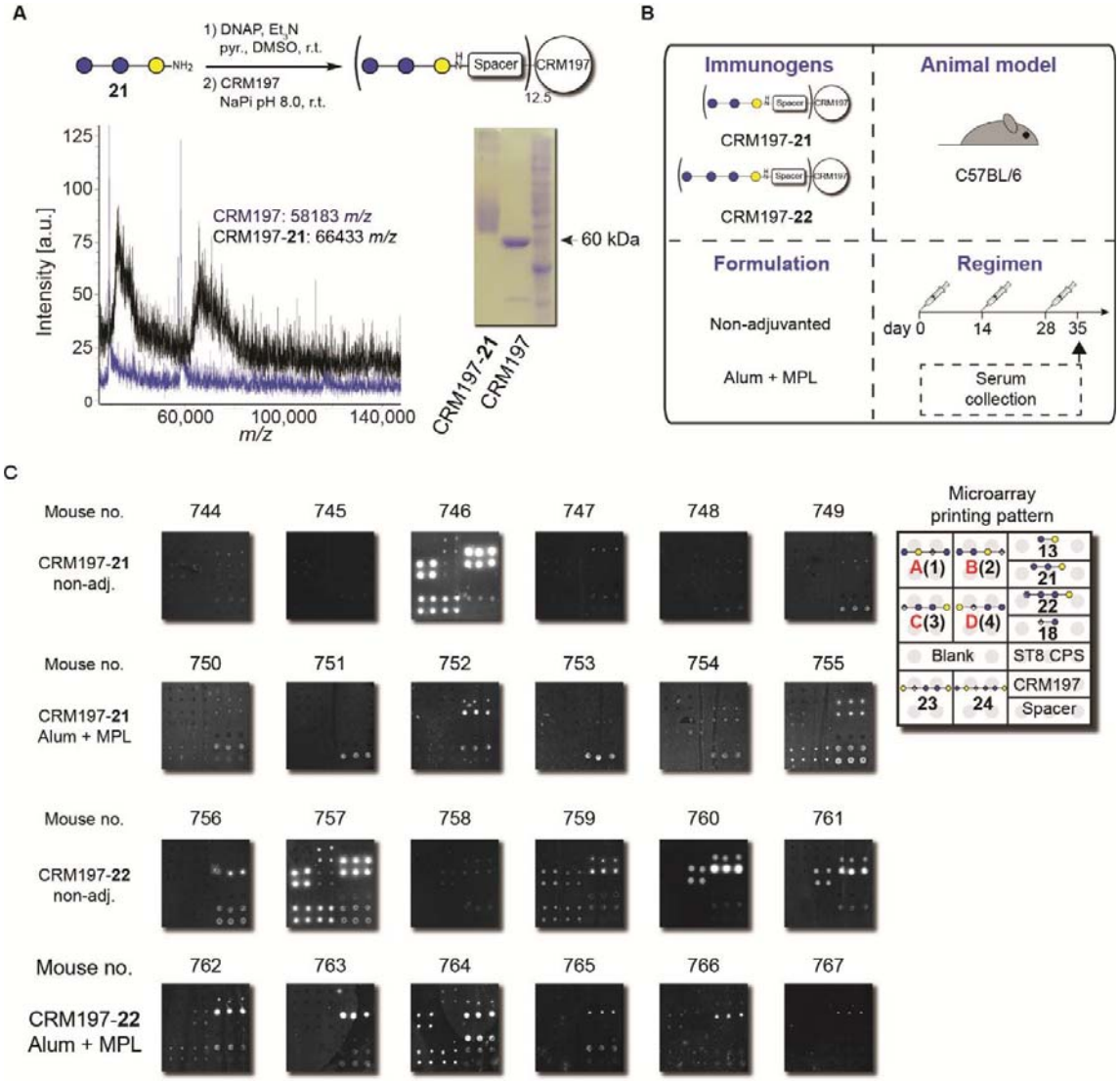


Fig. S15. Evaluation of CRM197 glycoconjugates of trisaccharide 21 and tetrasaccharide 22 in mice. (**A**) Conjugation of trisaccharide **21** to CRM197 and characterization of the glycoconjugate by MALDI-TOF MS and SDS-PAGE. (**B**) Immunization strategy. Mice (*n* = 6 per group) were s.c. immunized three times with CRM197-**21** or CRM197-**22** glycoconjugates either without adjuvant or formulated with Alum and monophosphoryl lipid A. Serum was collected one week after the third immunization. (**C**) Immune responses of individual mice at day 35 as assessed by glycan microarray.

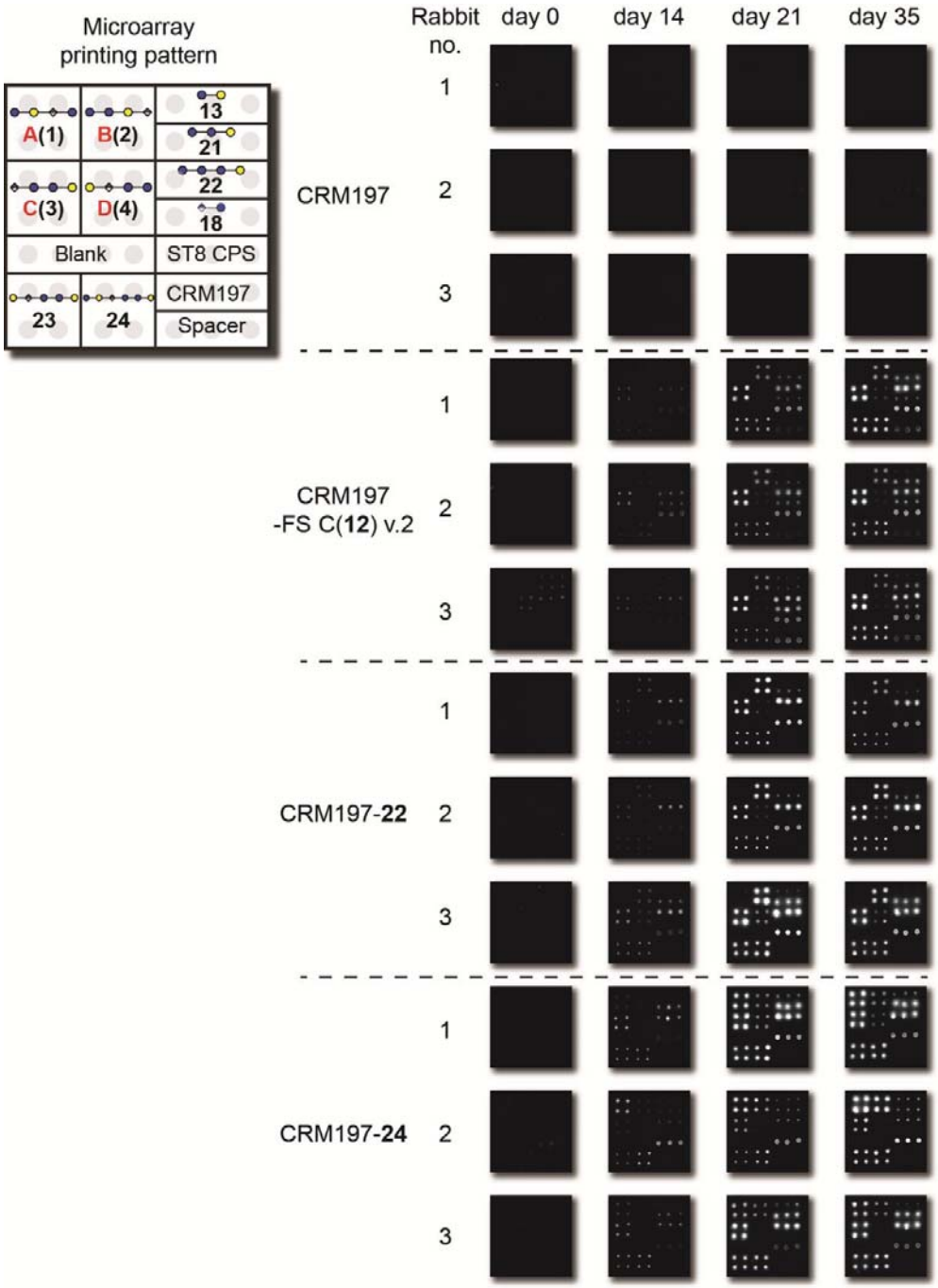


Fig.S16. Immune response against semisynthetic ST8 glycoconjugates in rabbits. Time course of immune responses of individual rabbits as assessed by glycan microarray.

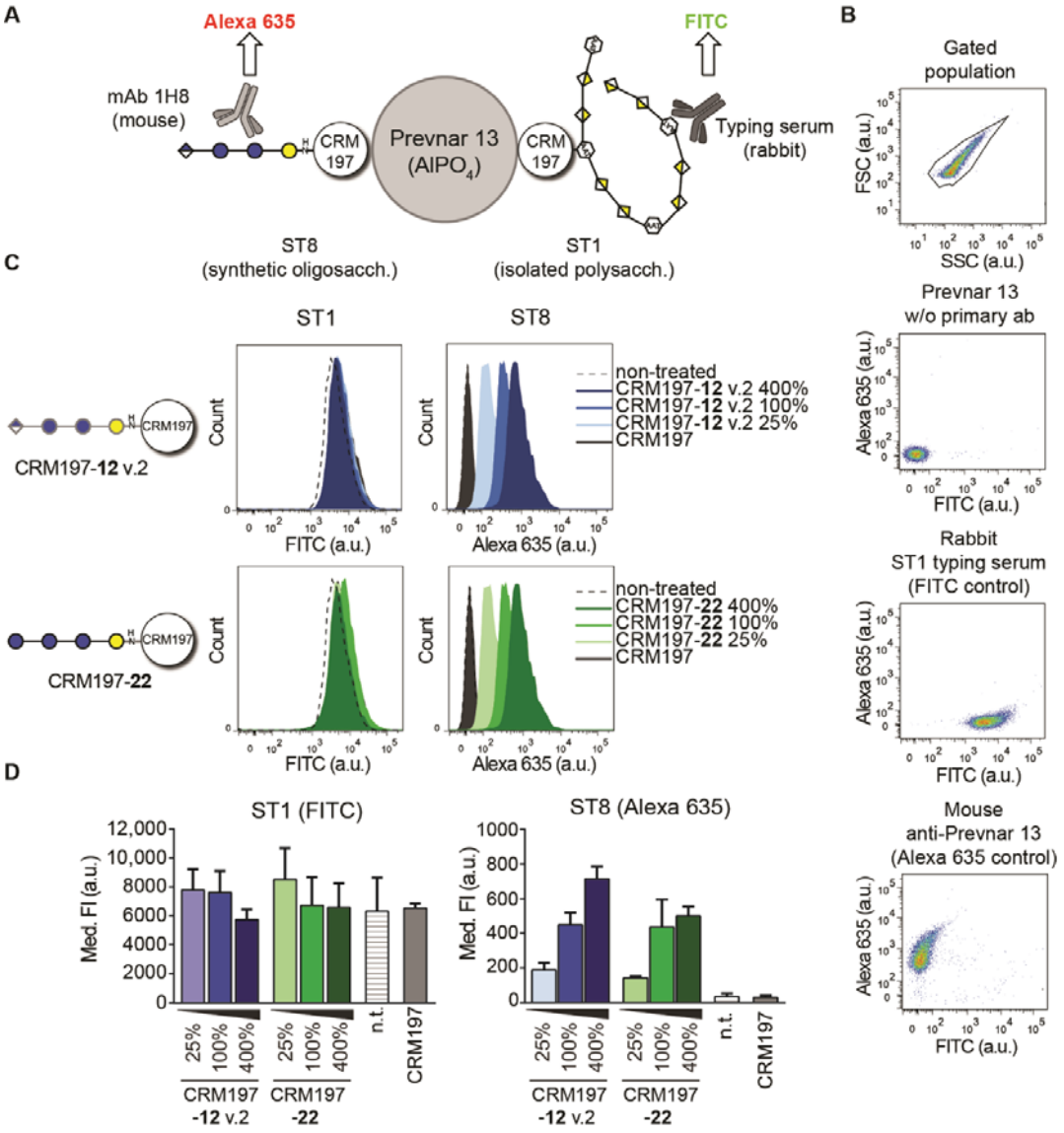


Fig. S17. **Adsorption of ST8 glycoconjugates to Prevnar 13, as assessed by flow cytometry.** (**A**) ST8 glycoconjugates were adsorbed to Prevnar 13 (aluminium phosphate) particles and detected by flow cytometry using mAb 1H8 and an Alexa Fluor 635-labeled detection antibody. Adsorbed ST1 glycoconjugates (contained in Prevnar 13) were detected using a rabbit-derived ST1 typing serum and a FITC-labeled anti-detection antibody. (**B**) Flow cytometry settings and controls. Graphs depict gated population, a control using only detection antibodies and positive binding controls. (**C**) Dose-dependent adsorption of ST8 glycoconjugates to Prevnar 13. 25%, 100% or 400% of a full equivalent (2.2 µg glycan per dose Prevnar 13) of ST8 glycoconjugates were adsorbed to Prevnar 13 particles and adsorption was monitored by flow cytometry. Controls are non-treated and CRM197-treated Prevnar 13 particles. Data are histograms of a representative labeling experiment. (**D**) Cumulated results of three replicate experiments. Data are means + SD of the median fluorescence intensities of *n* = 3 independent experiments.

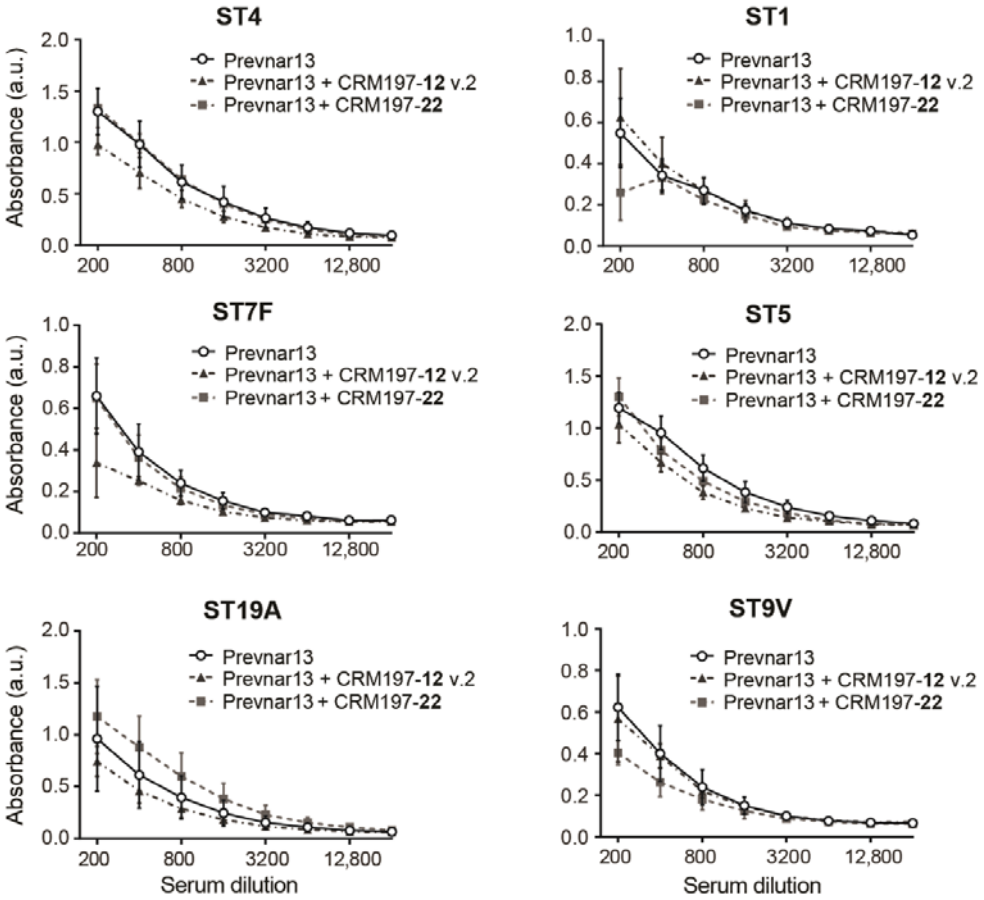


Fig. S18. Effect of coformulation of semisynthetic ST8 glycoconjugates with Prevnar 13 on the immune response against several pneumococcal CPSs. Antibody binding was assessed by polysaccharide ELISA. Data are means ± SD of polysaccharide binding of *n* = 3 rabbits per group.

Table S1. Sequences of automated assembly of protected ST8 CPS–related tetrasaccharide frameshifts. See Supporting Materials for experimental details on modules.

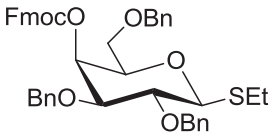
Sequence	Module	Details
I	1	TMSOTf wash
	2	Building block **6** (5 equiv.), thioglycoside activation
	4	Fmoc Removal
II	1	TMSOTf wash
	3	5 eq. building block **7** (5 equiv.), glycosyl phosphate activation
	4	Fmoc Removal
III	1	TMSOTf wash
	2	Building block **8** (5 equiv.), thioglycoside activation
	4	Fmoc Removal
IV	1	TMSOTf wash
	2	Building block **9** (5 equiv.), thioglycoside activation
	4	Fmoc Removal
V	1	TMSOTf wash
	2	Building block **10** (5 equiv.), thioglycoside activation
VI	1	TMSOTf wash
	2	Building block **11** (5 equiv.), thioglycoside activation

Table S2. Antipolysaccharide IgG end point titers of rabbits immunized with Prevnar 13 alone or coformulated with semisynthetic ST8 glycoconjugates. Titers were determined by polysaccharide ELISA.

	Prevnar 13			Prevnar 13 + CRM197-12 v.2			Prevnar 13 + CRM197-22		
Serotype	**Rabbit 1**	**Rabbit 2**	**Rabbit 3**	**Rabbit 1**	**Rabbit 2**	**Rabbit 3**	**Rabbit 1**	**Rabbit 2**	**Rabbit 3**
ST8	400	1600	1600	12800	12800	6400	6400	3200	3200
ST1	1600	1600	3200	6400	3200	1600	1600	1600	3200
ST4	6400	25600	12800	6400	6400	3200	12800	12800	12800
ST5	6400	12800	12800	6400	3200	3200	6400	3200	6400
ST7F	1600	6400	1600	800	1600	1600	1600	800	1600
ST9V	1600	3200	1600	3200	3200	1600	1600	800	3200
ST19A	1600	6400	6400	1600	6400	3200	12800	12800	3200

SPECTRA

^{1}H NMR, 600 MHz, $CDCl_3$



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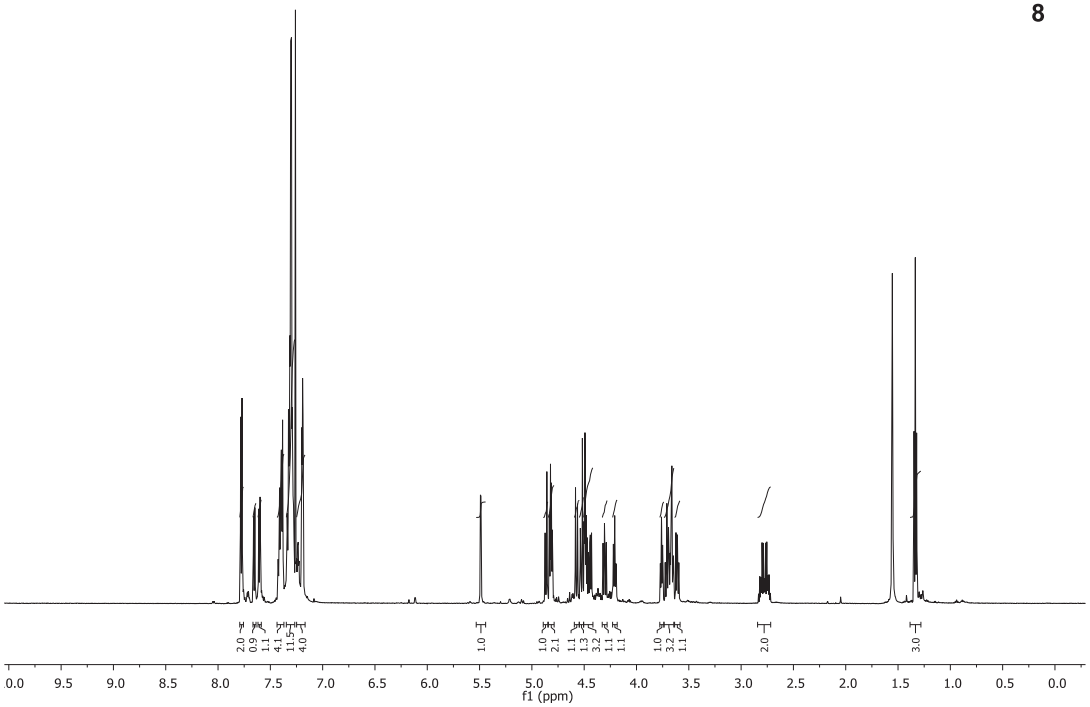

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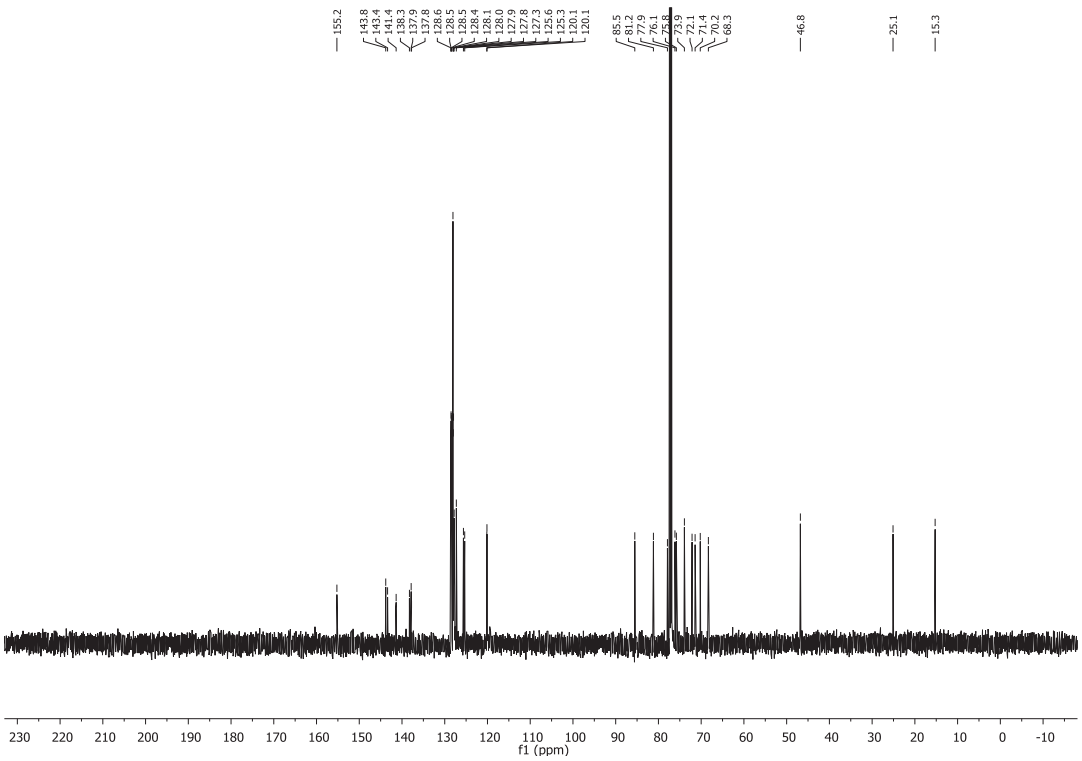

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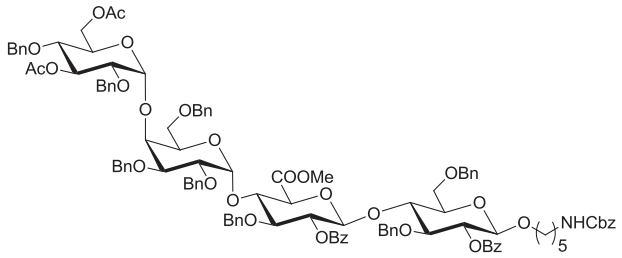

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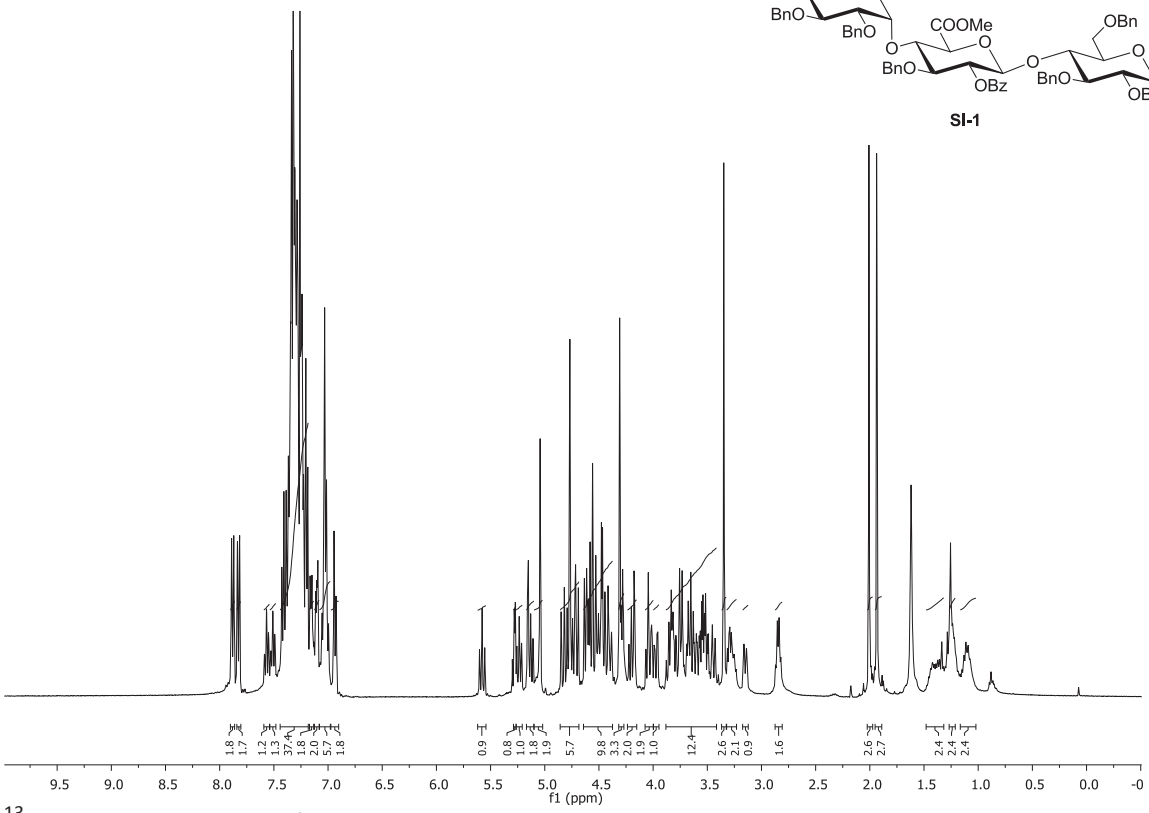

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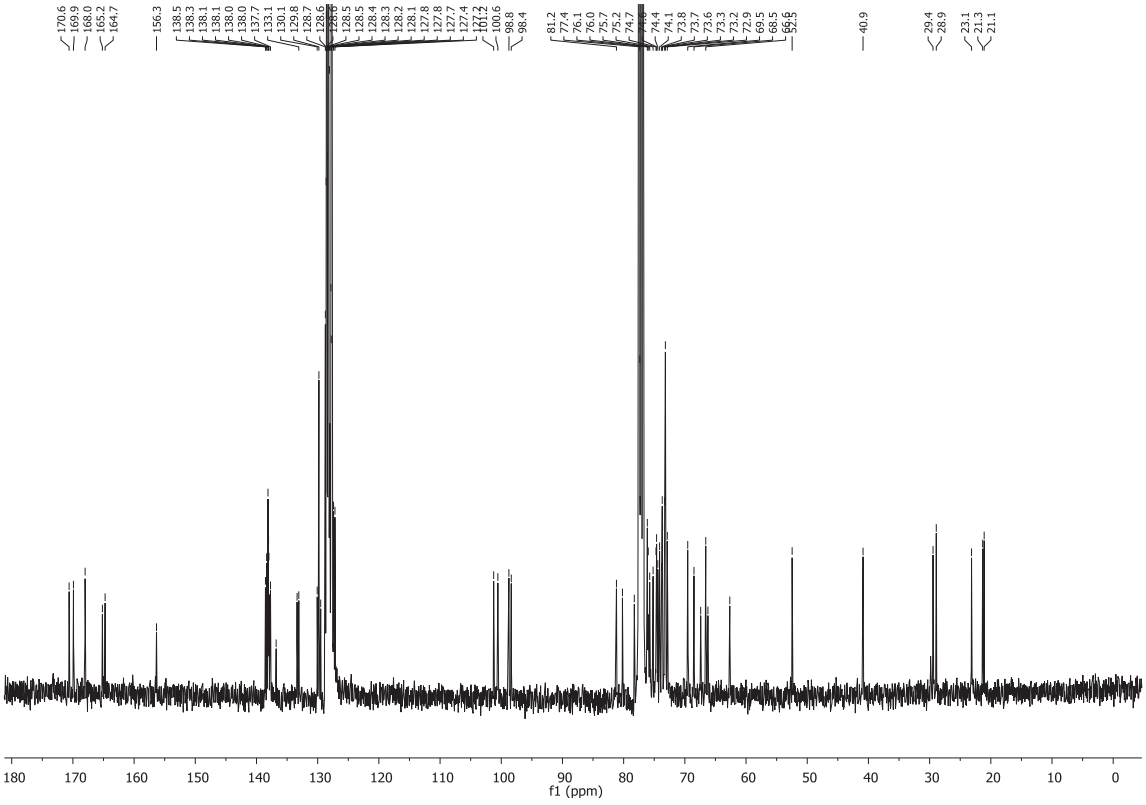

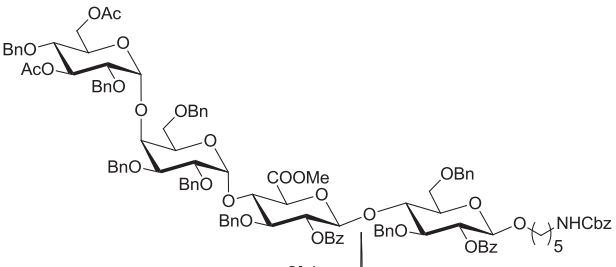

SI-1

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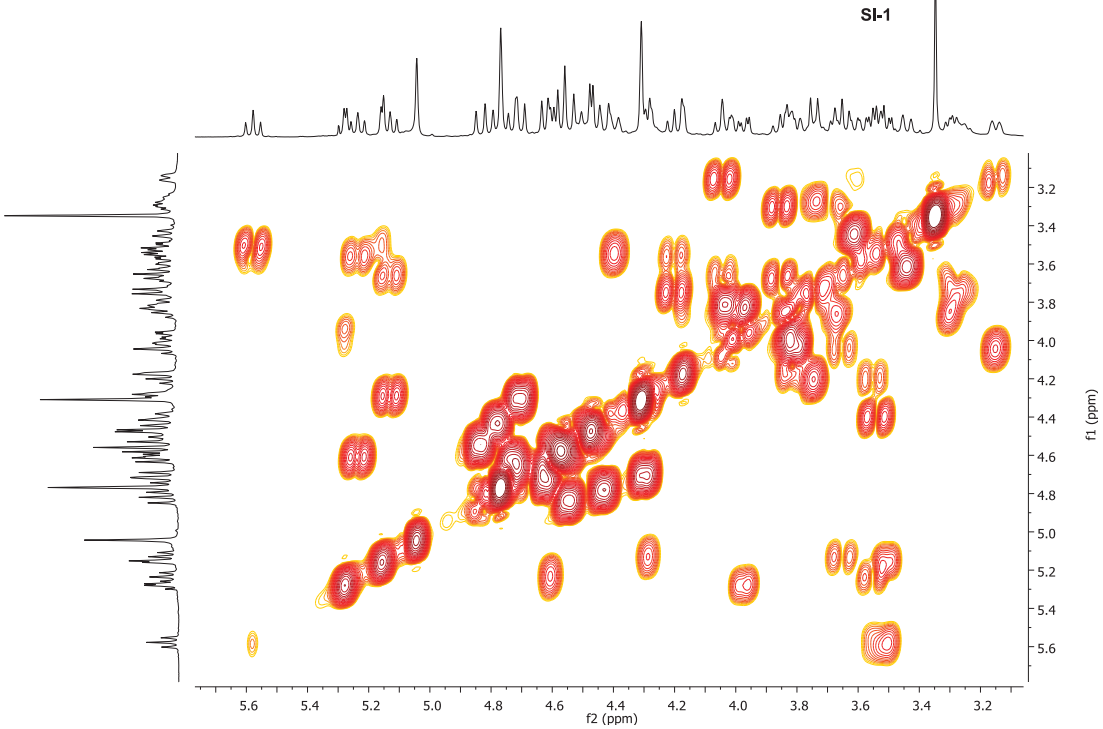

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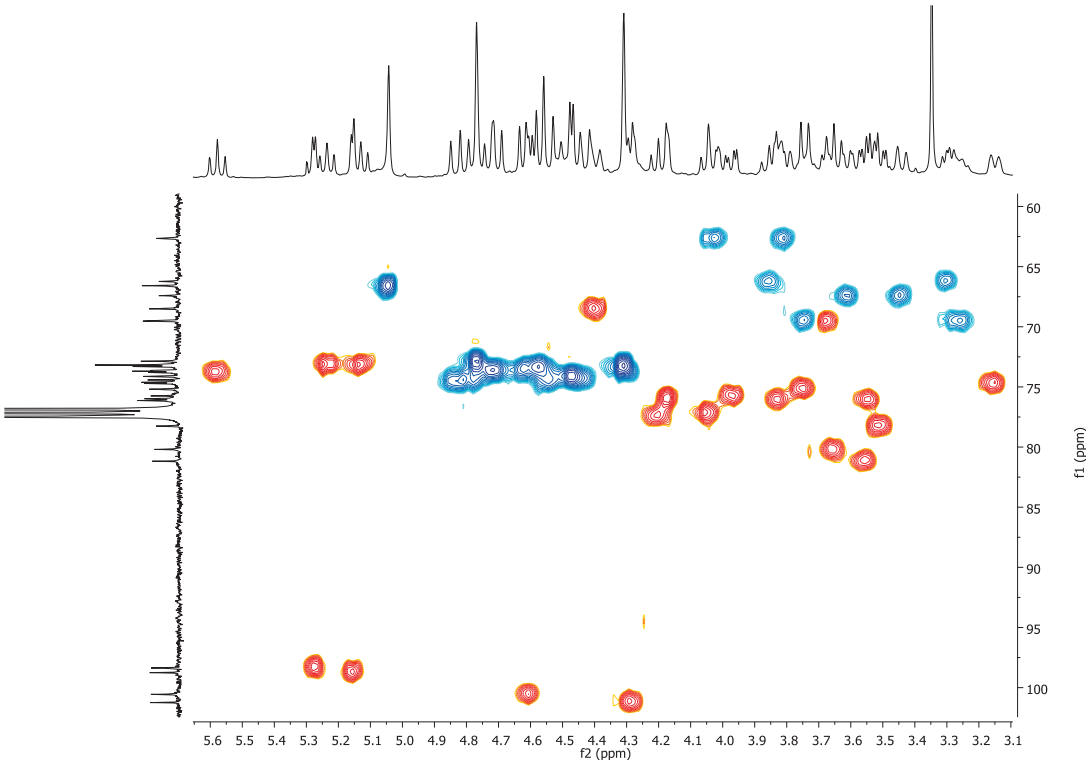

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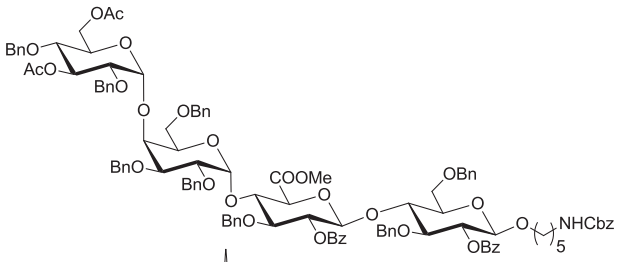

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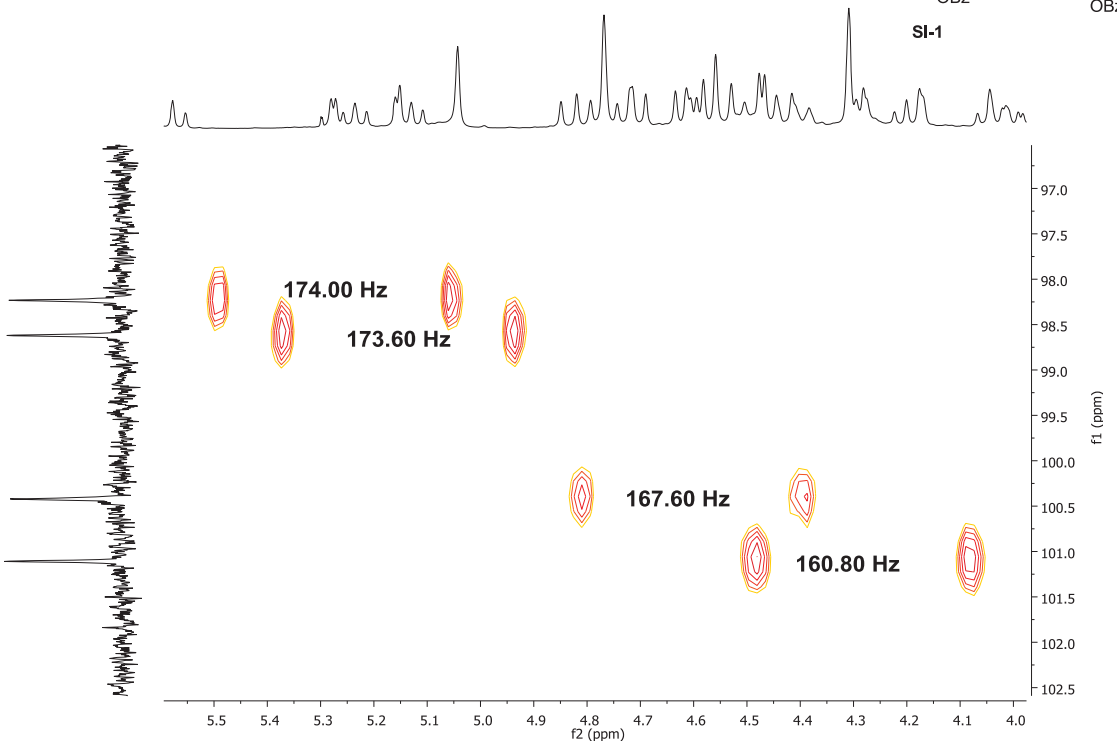

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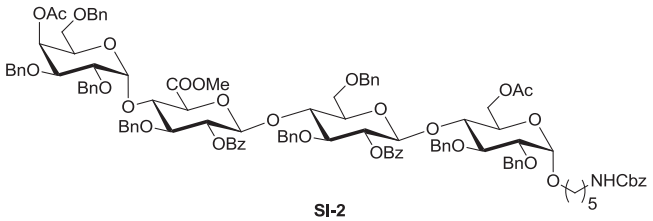

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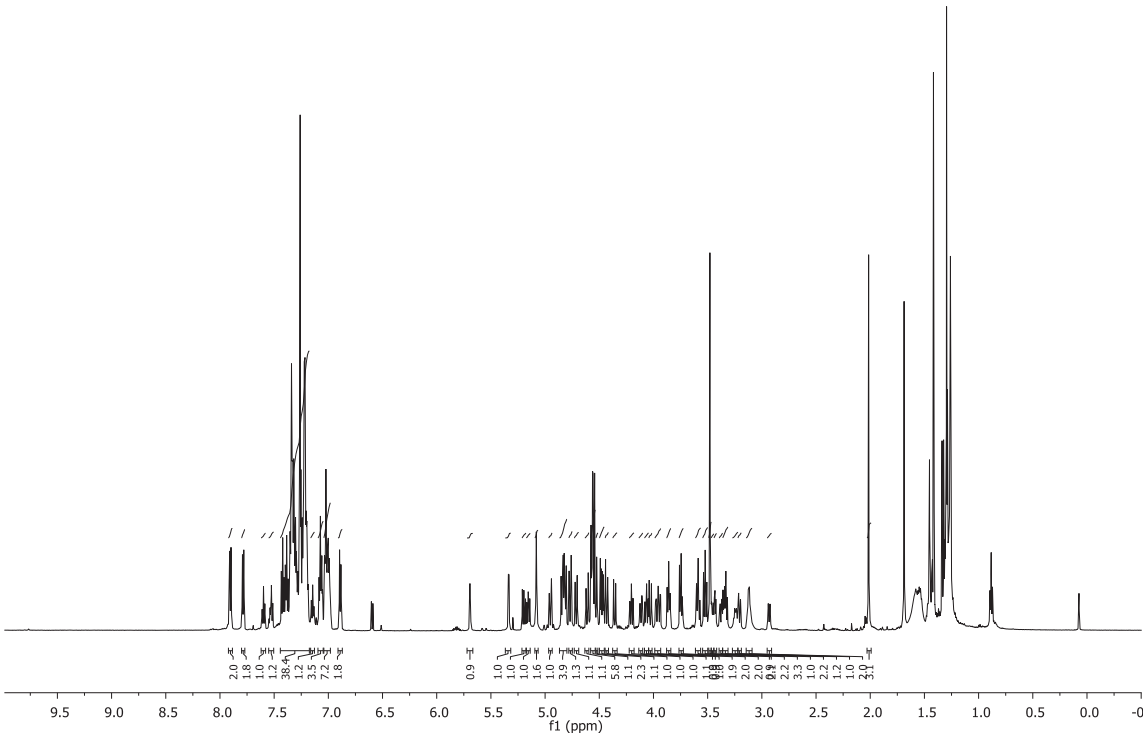

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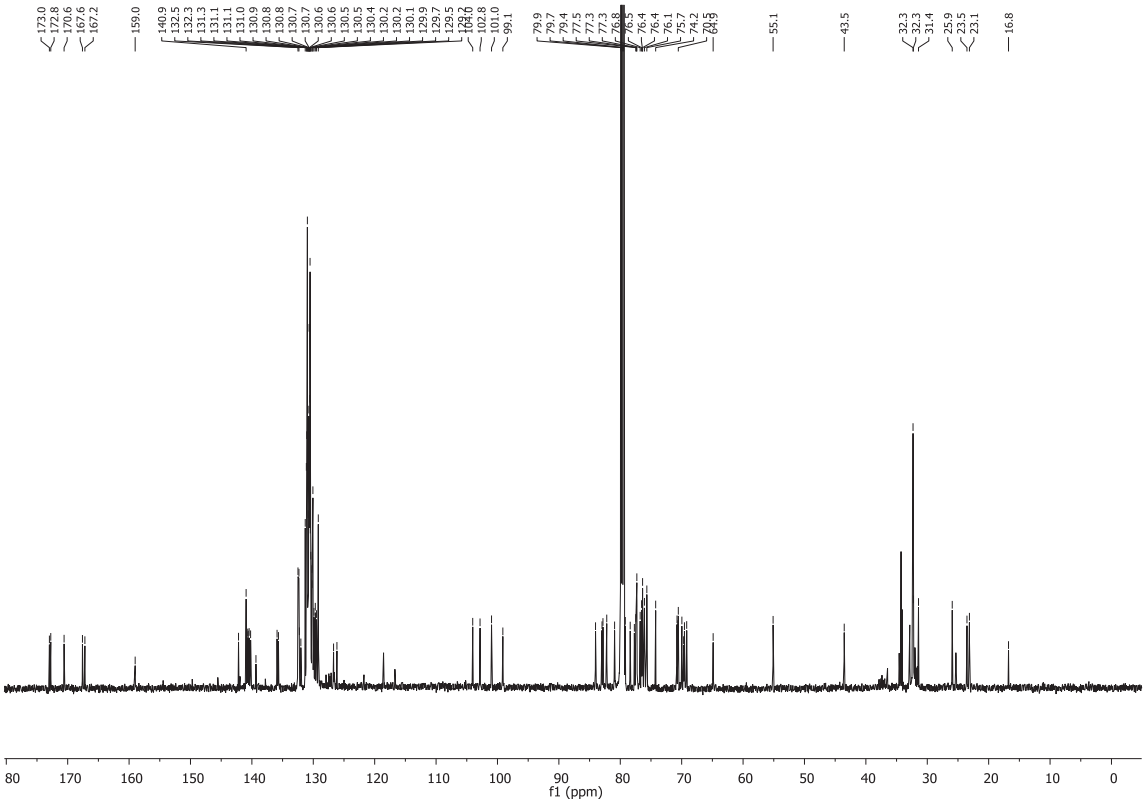
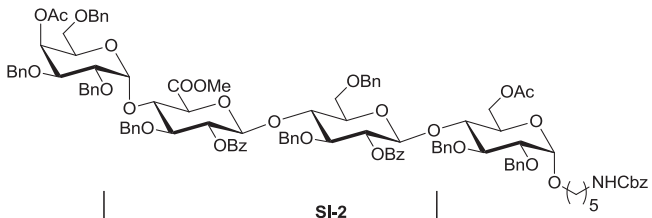
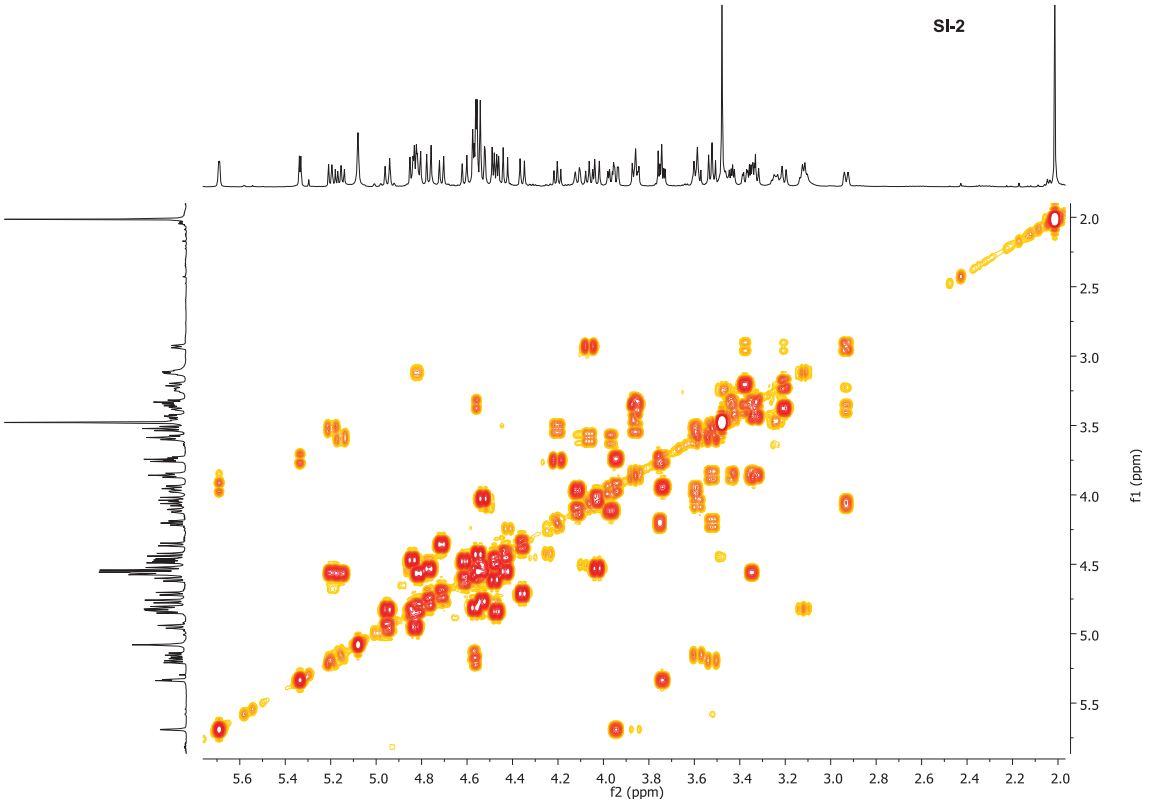
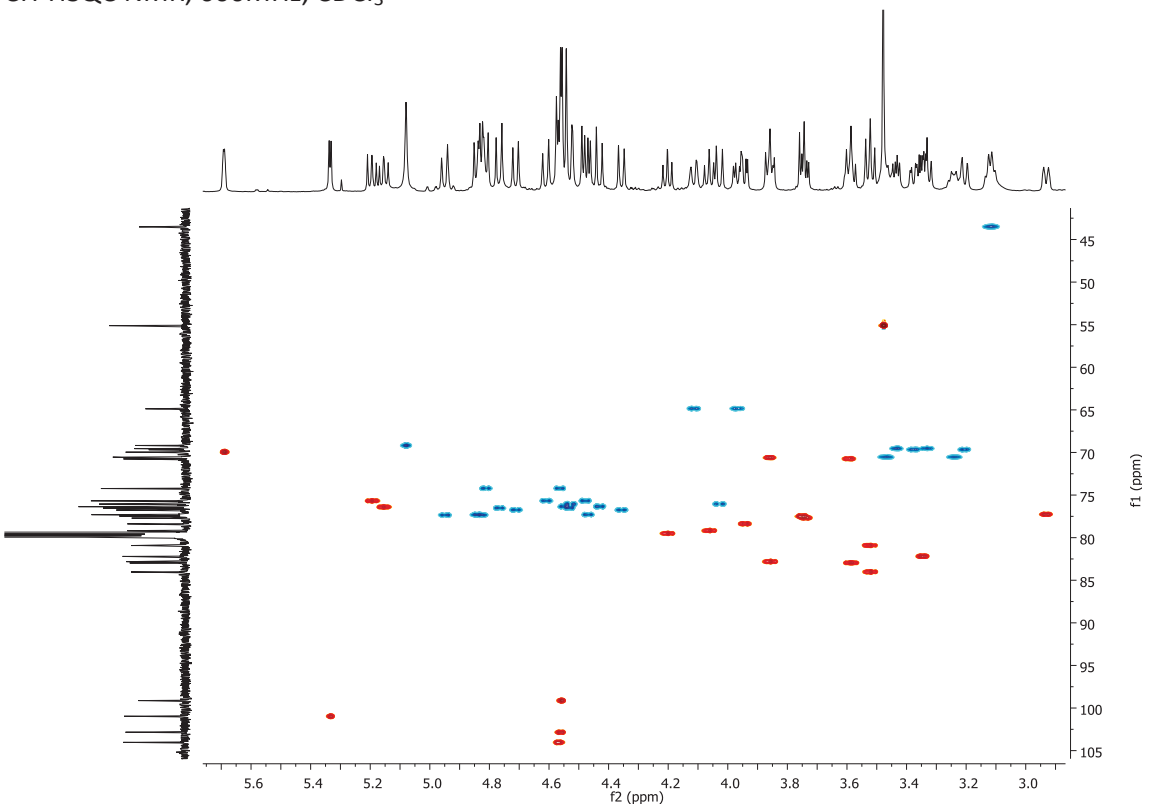


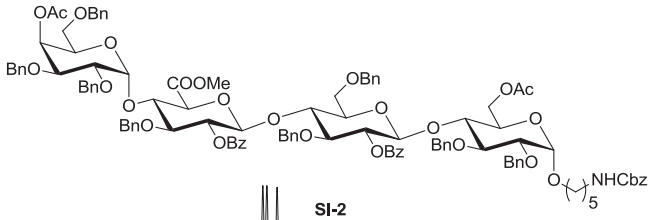
HH-COSY NMR, 600 MHz, $CDCl_3$





CH-HSQC NMR, 600MHz, $CDCl_3$





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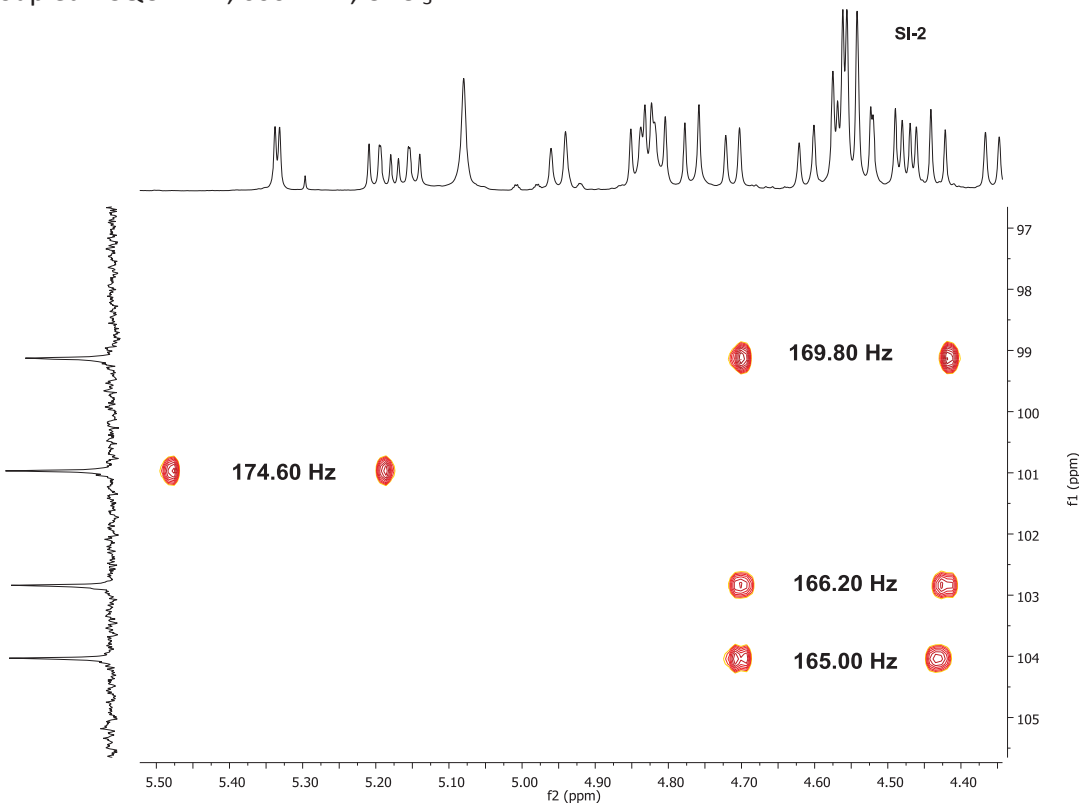

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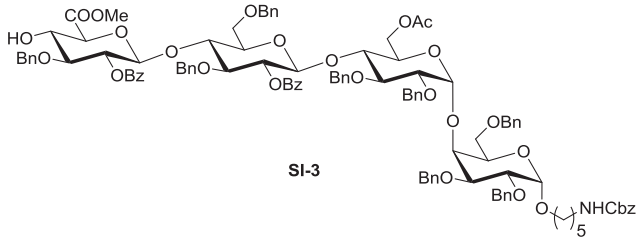

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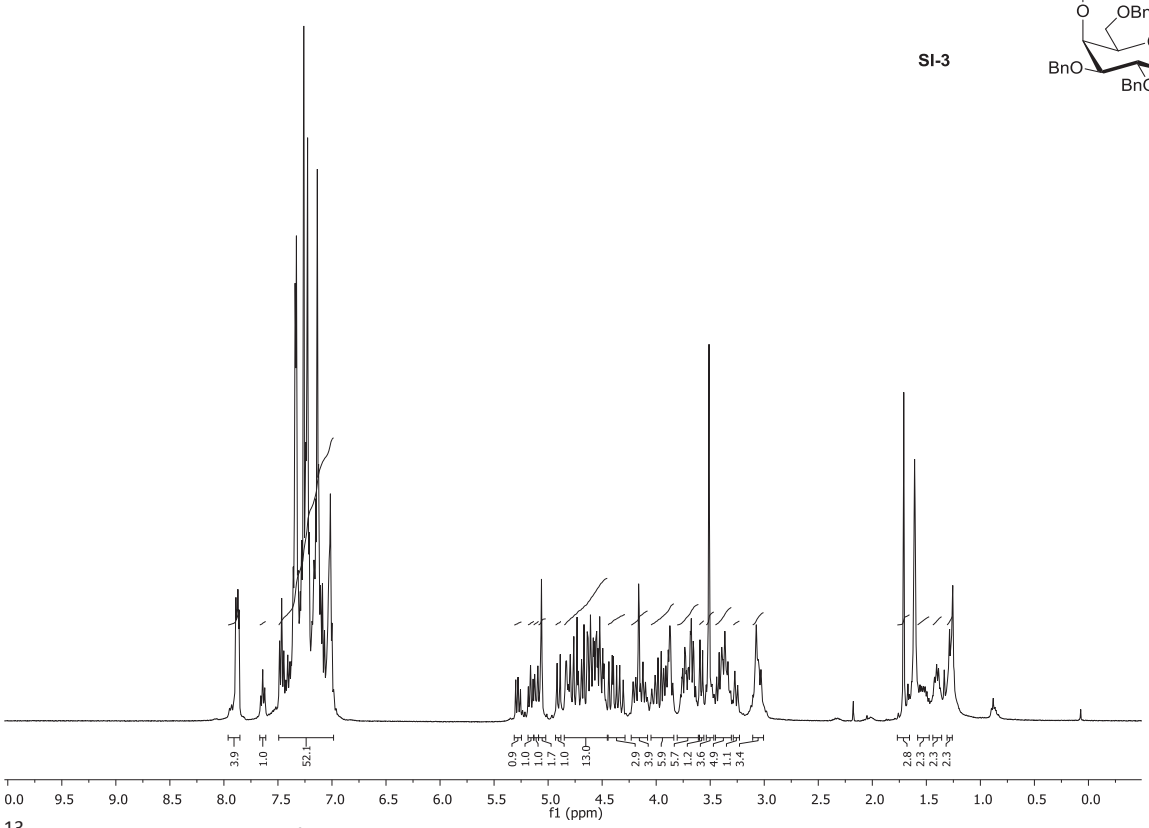

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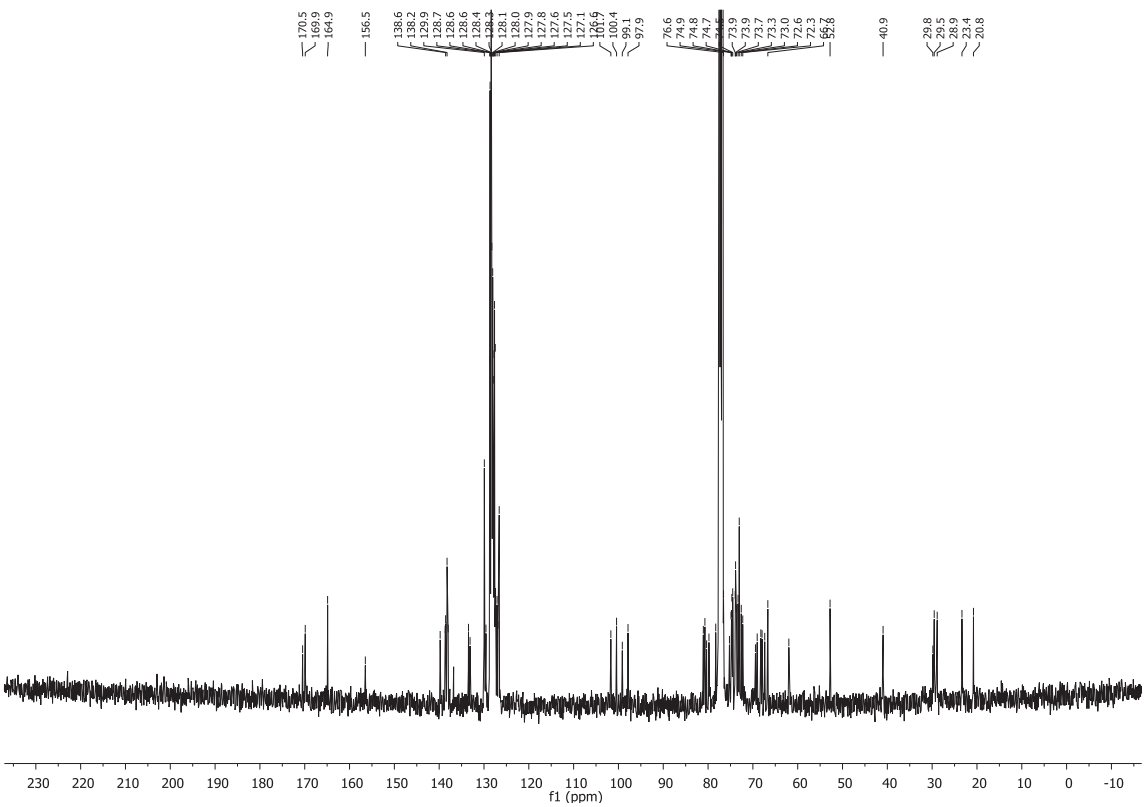

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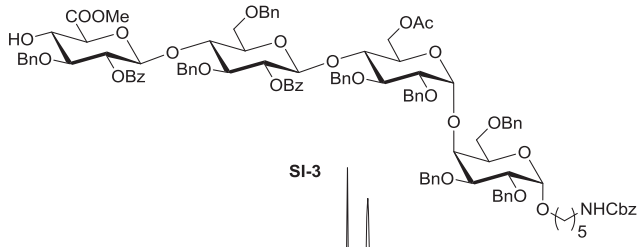

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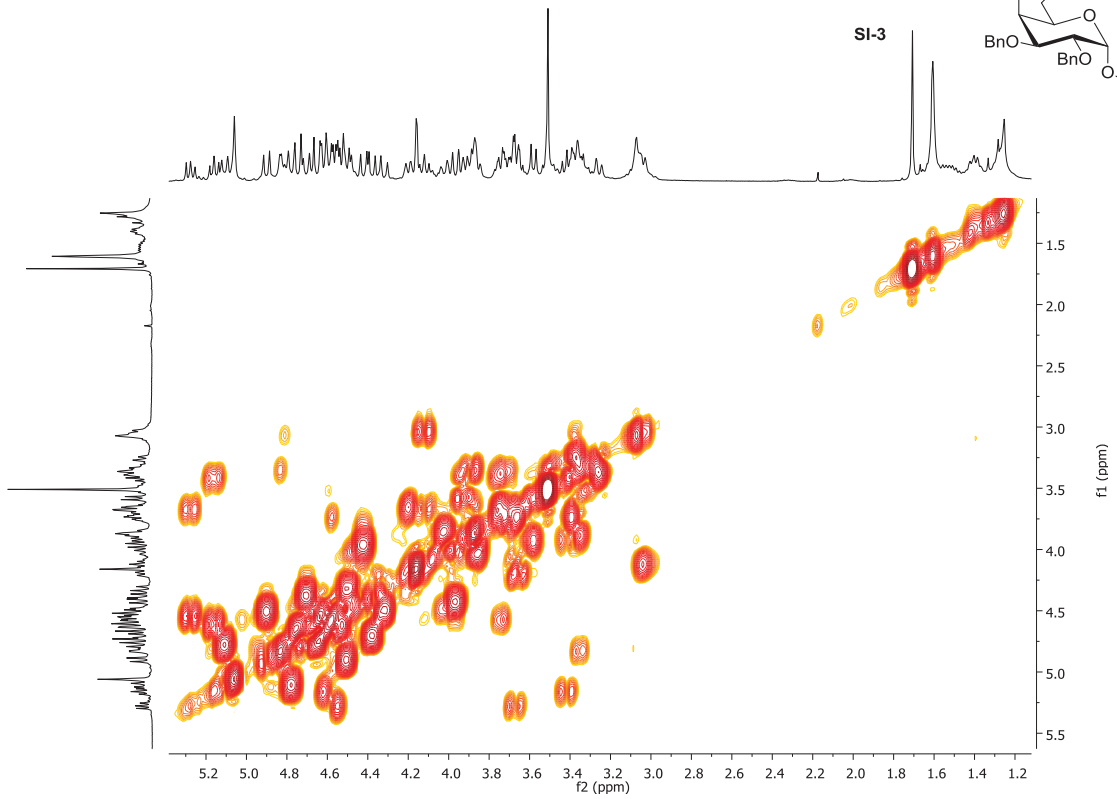
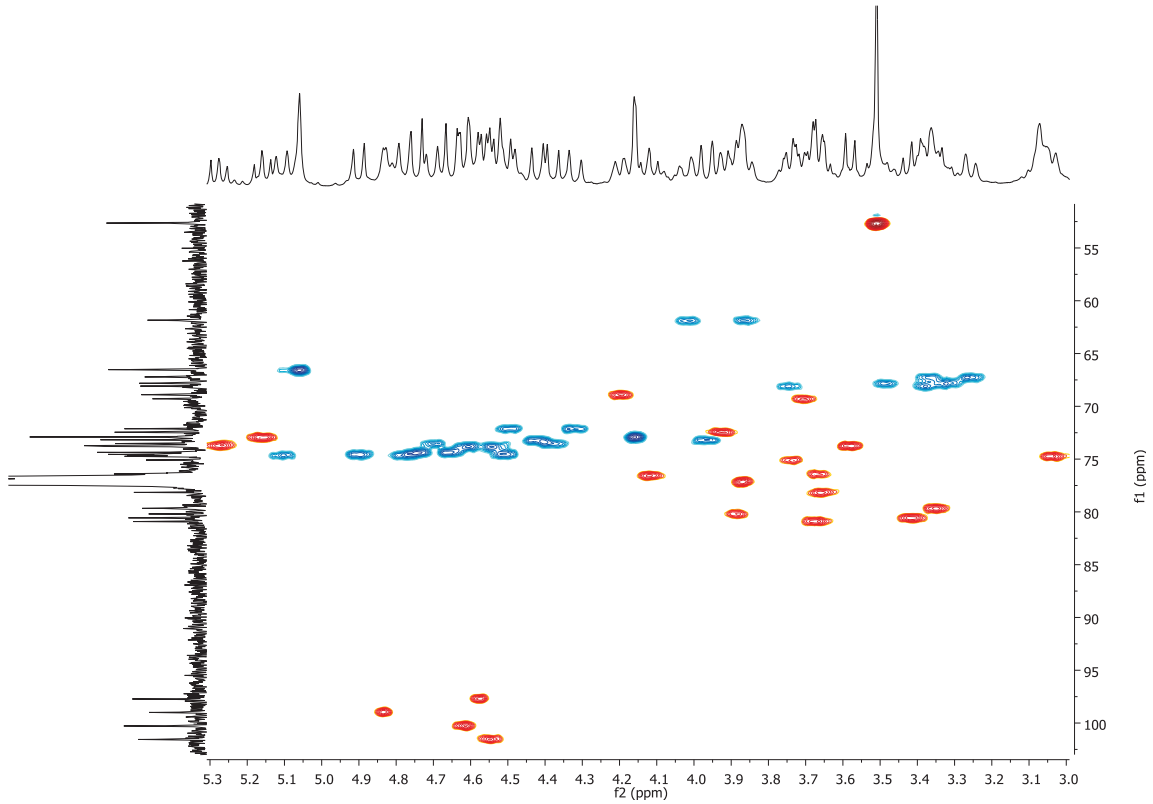
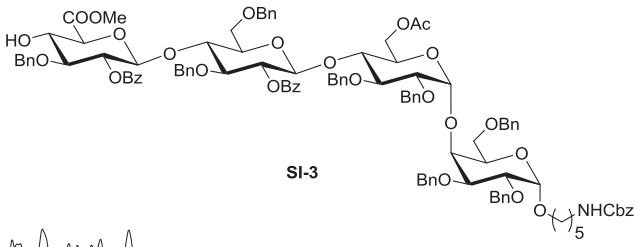


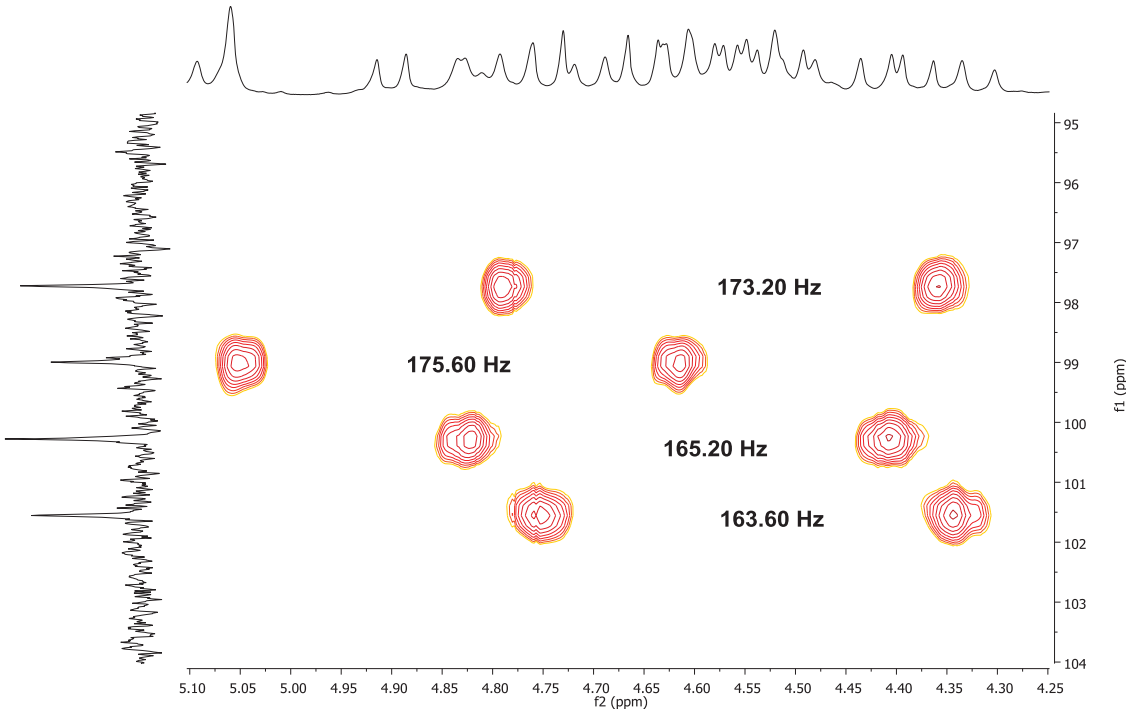
CH-HSQC NMR, 400 MHz, $CDCl_3$

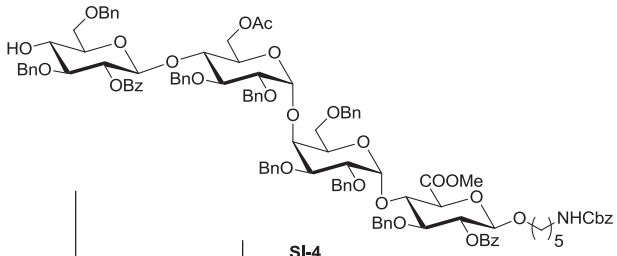


CH-coupled HSQC NMR, 400 MHz, $CDCl_3$



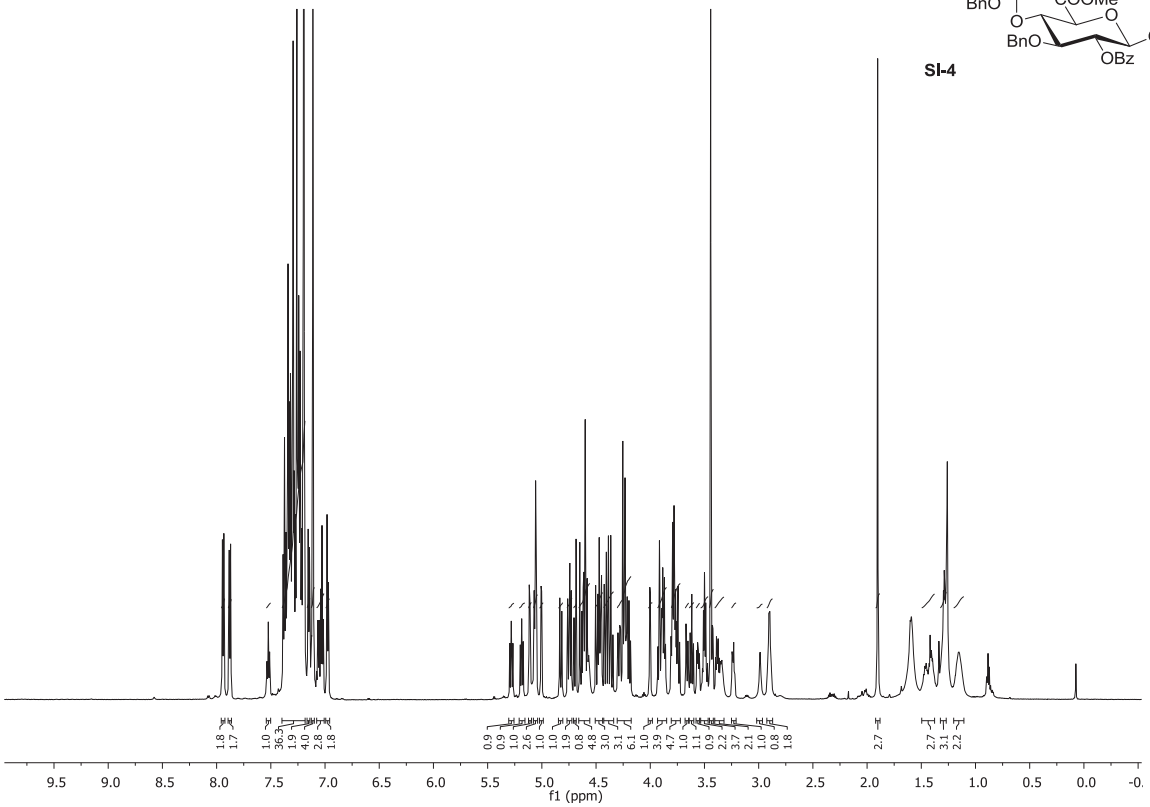
SI-3



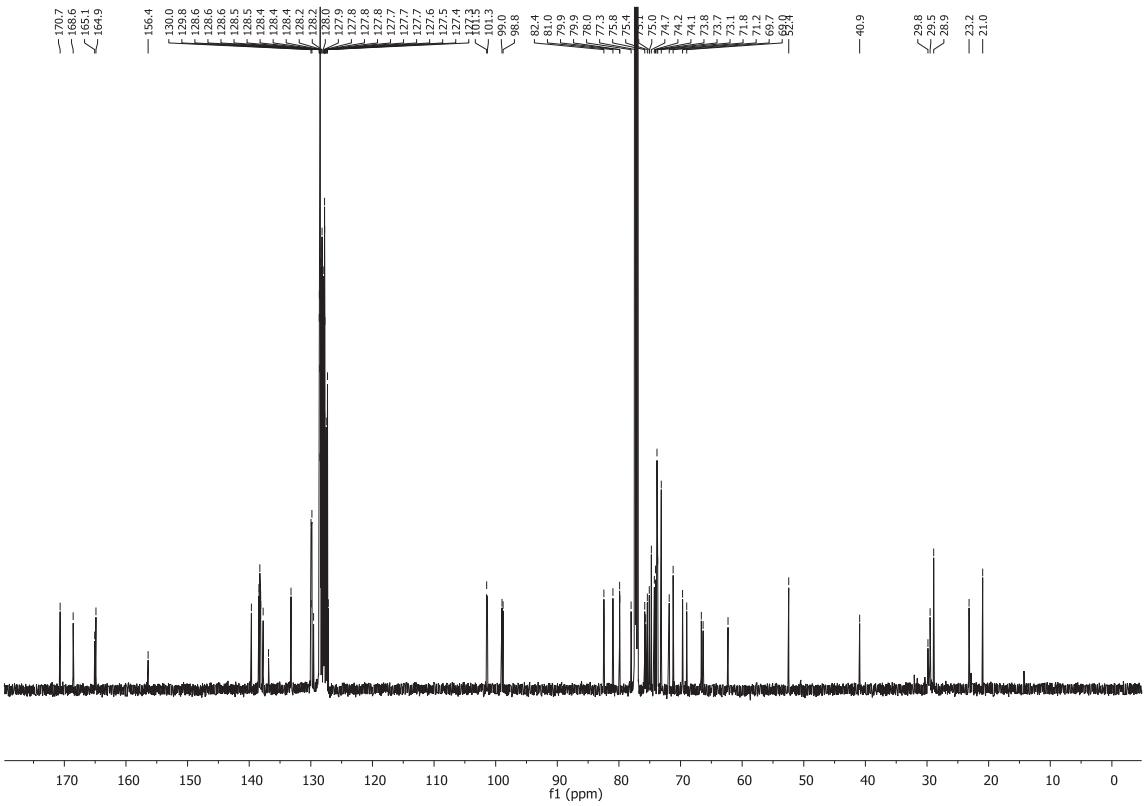


SI-4

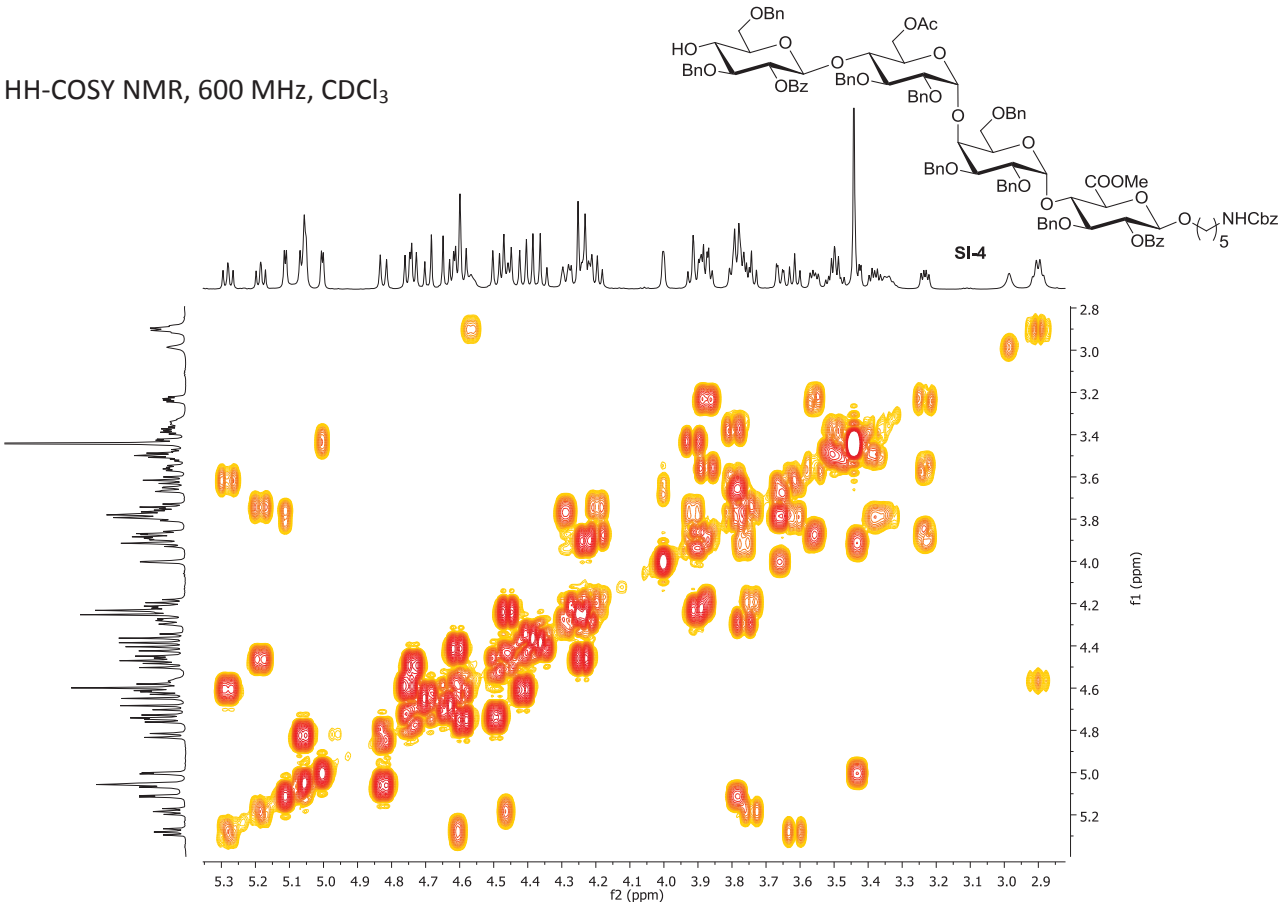
^{1}H NMR, 600 MHz, $CDCl_3$



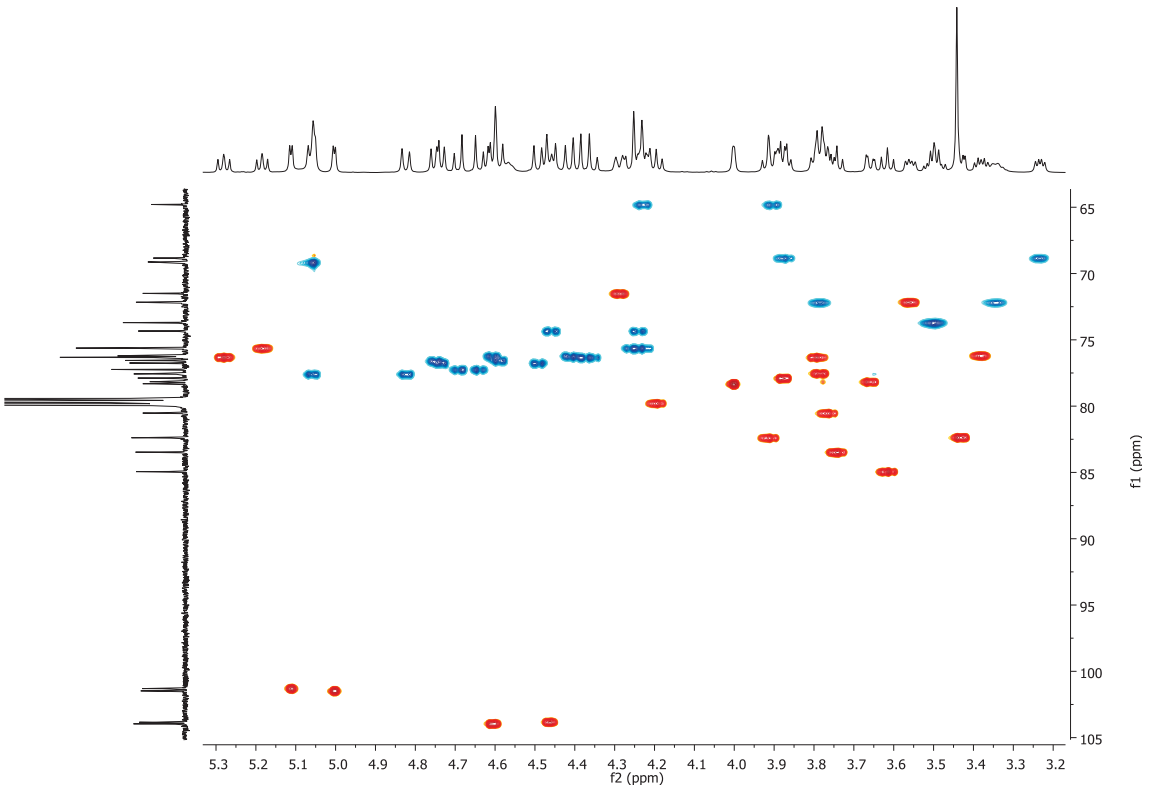
^{13}C NMR, 150 MHz, $CDCl_3$



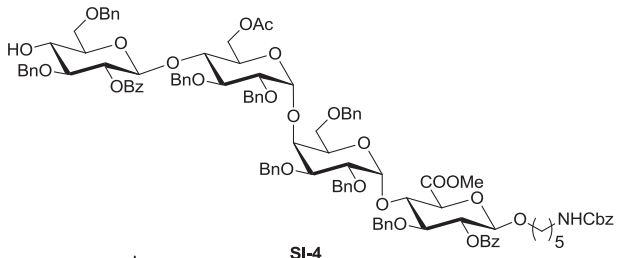
HH-COSY NMR, 600 MHz, $CDCl_3$



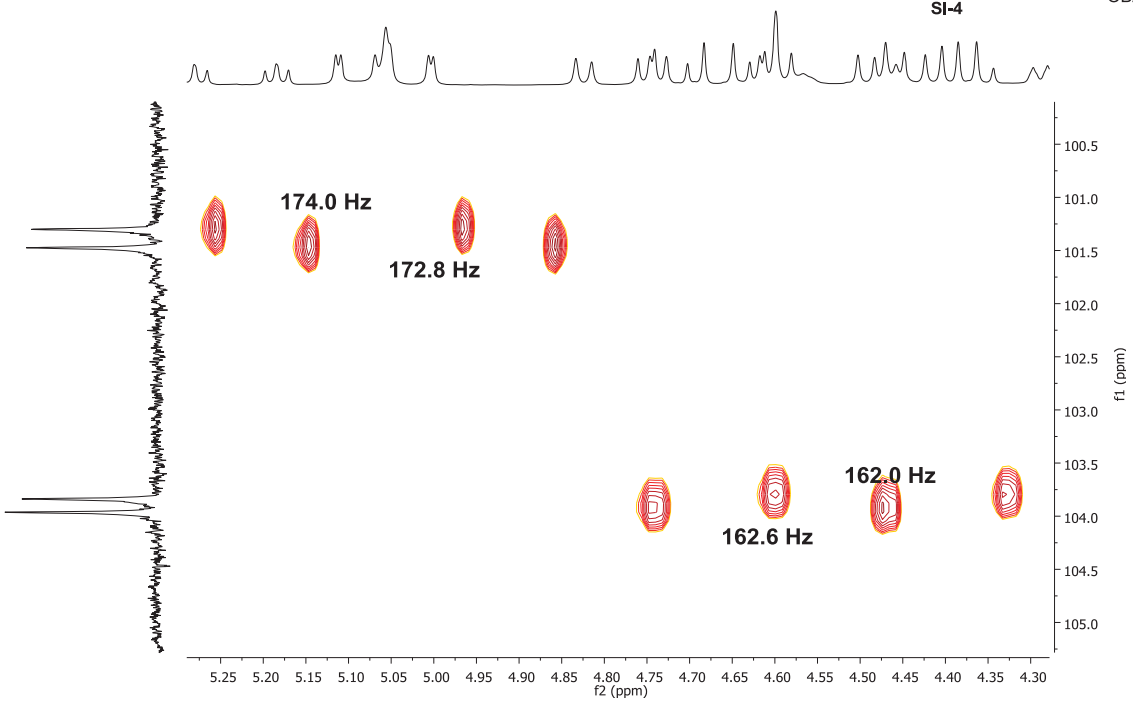
CH-HSQC NMR, 600 MHz, $CDCl_3$



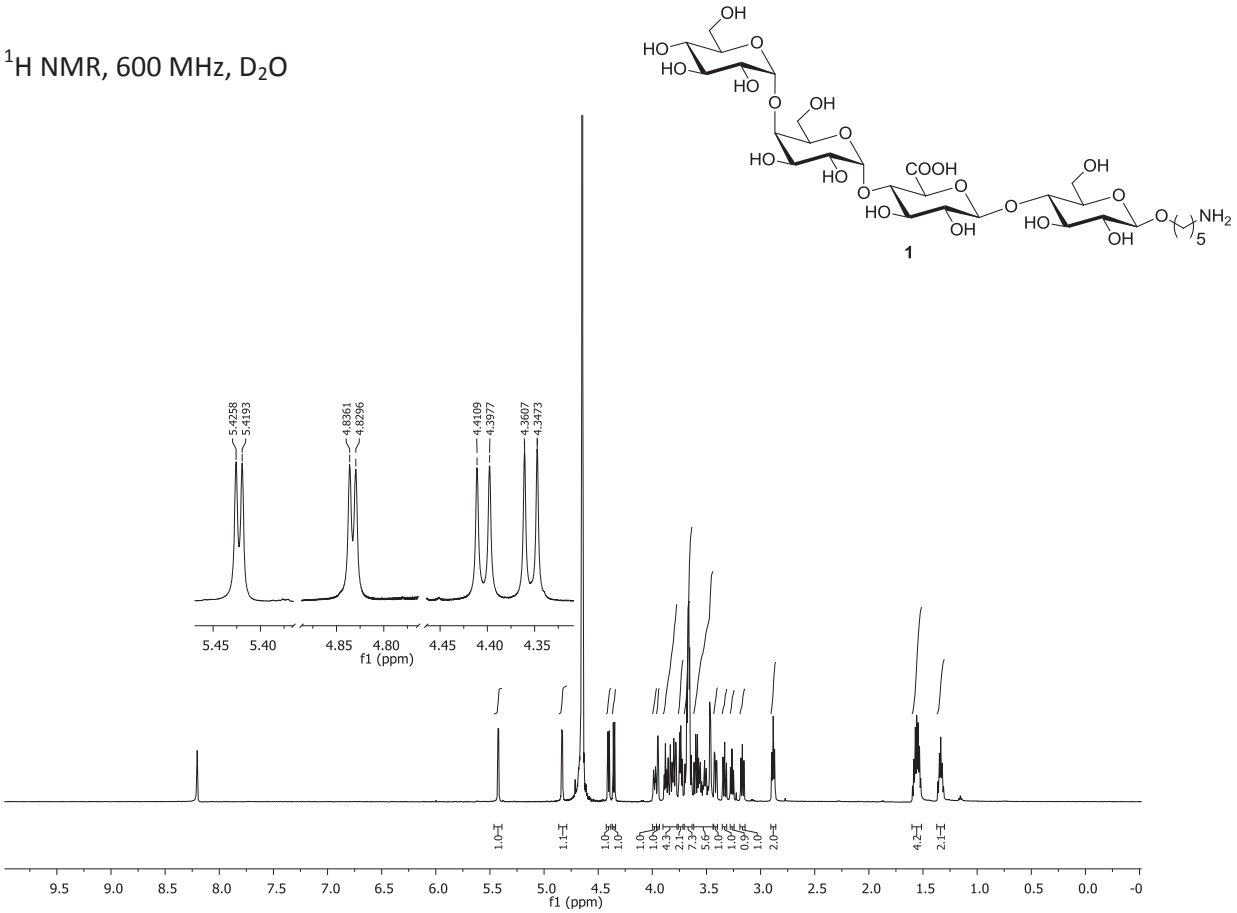
CH-coupled HSQC NMR, 600 MHz, $CDCl_3$



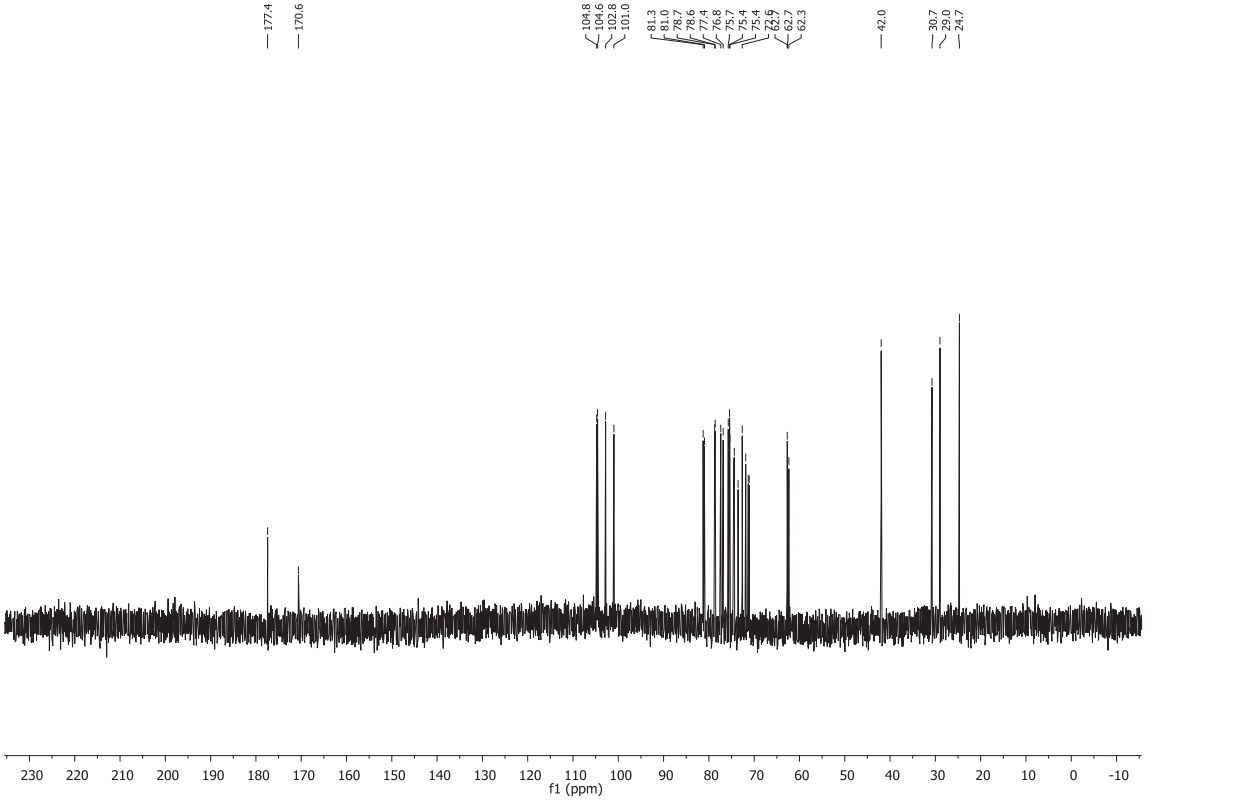
SI-4



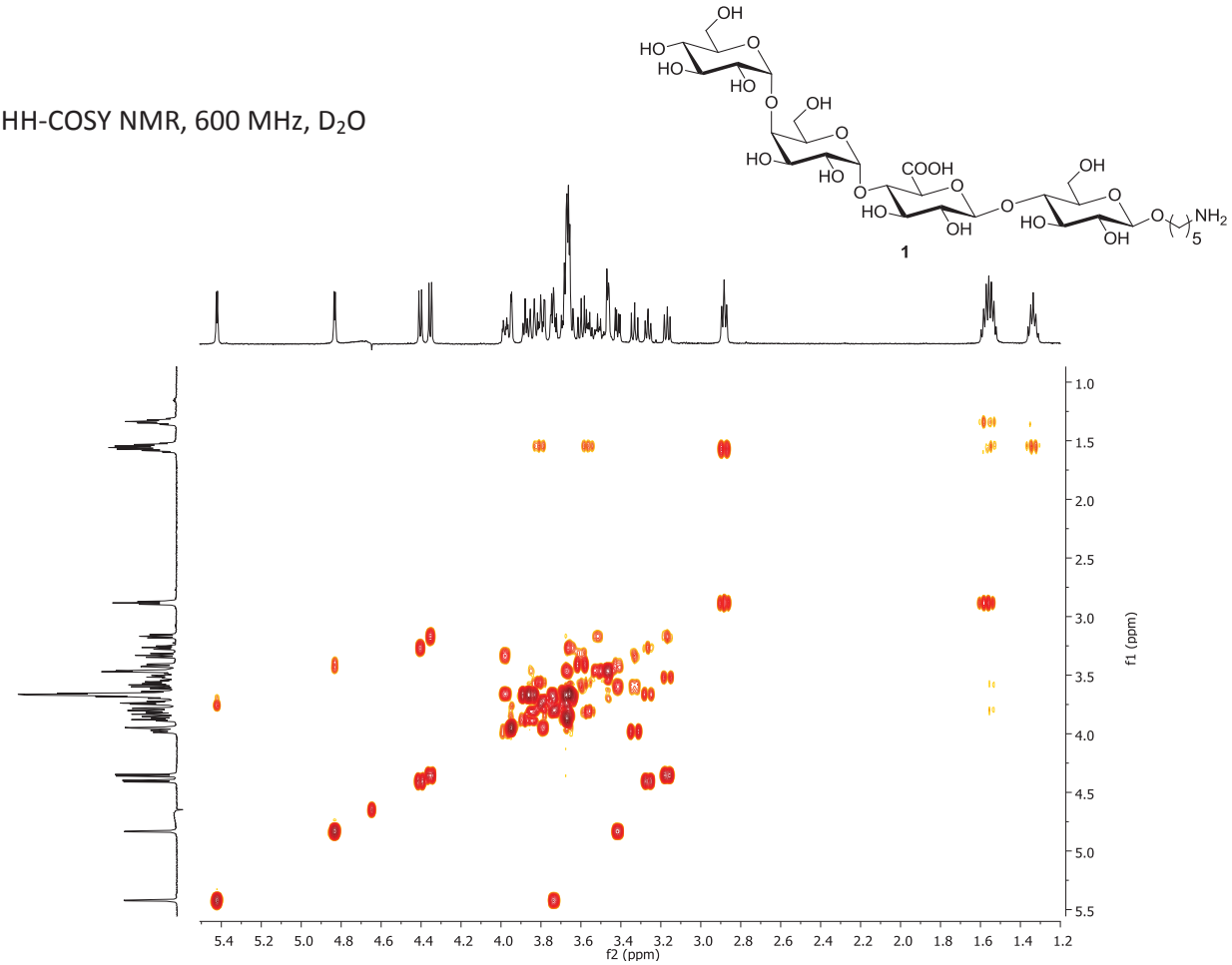
^{1}H NMR, 600 MHz, D_2O



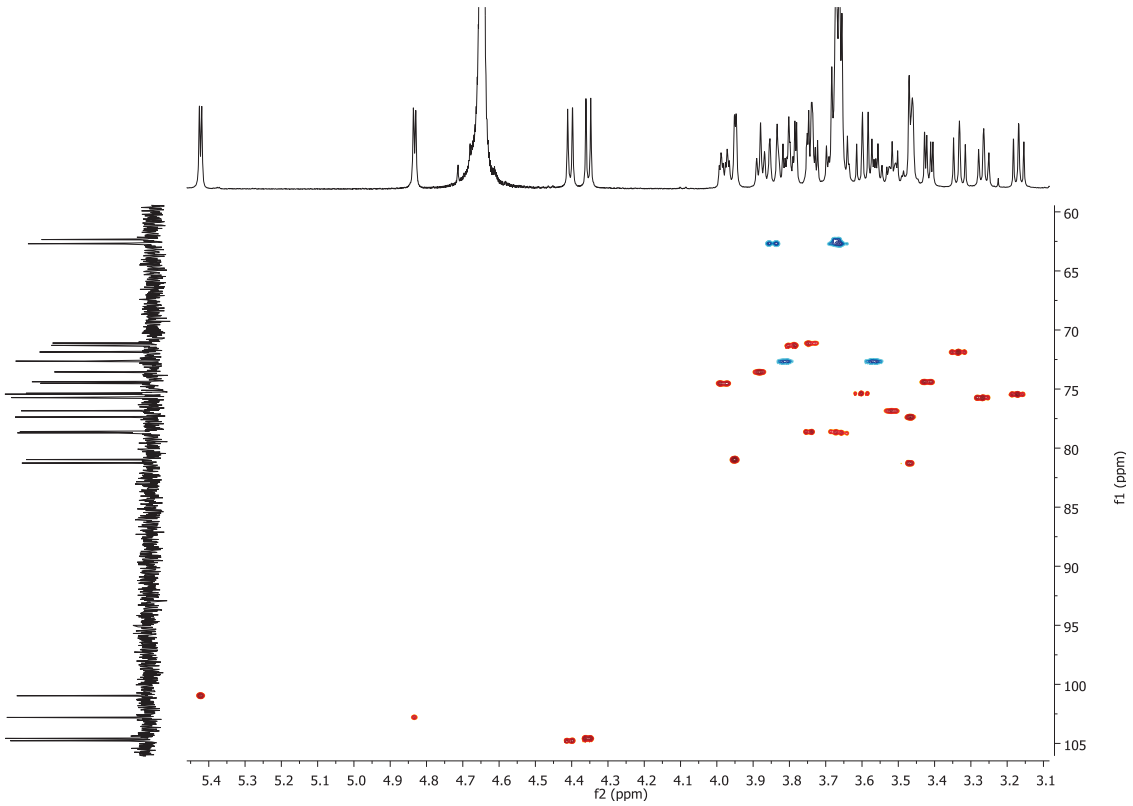
^{13}C NMR, 150 MHz, D_2O



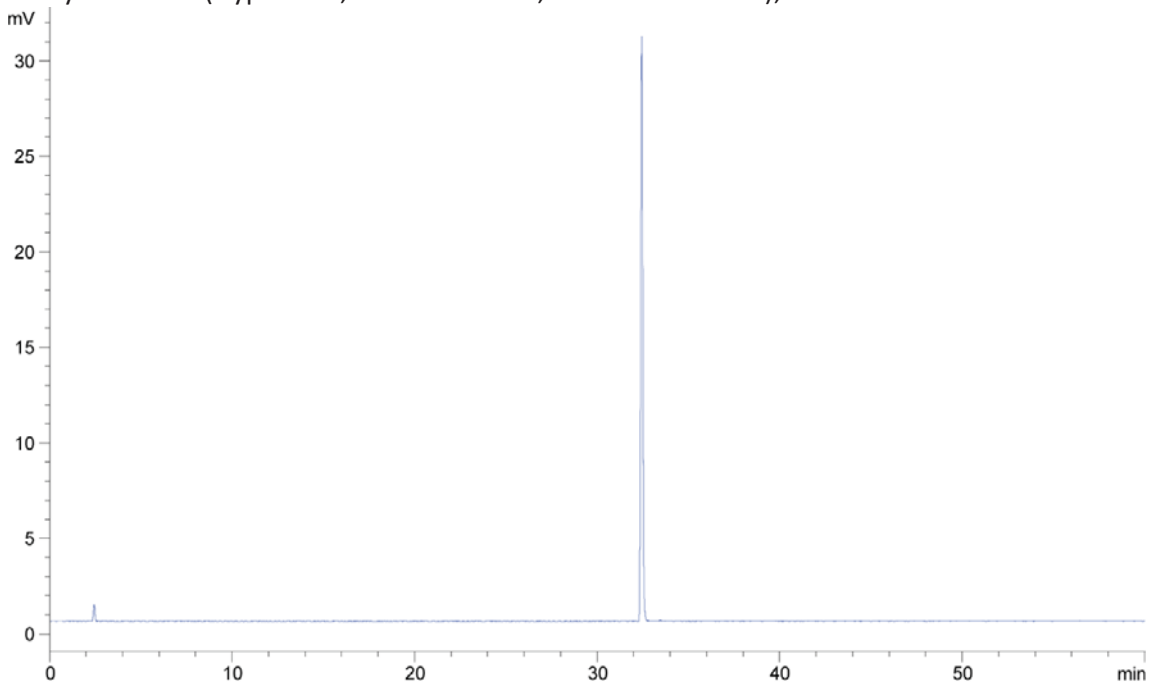
HH-COSY NMR, 600 MHz, D_2O



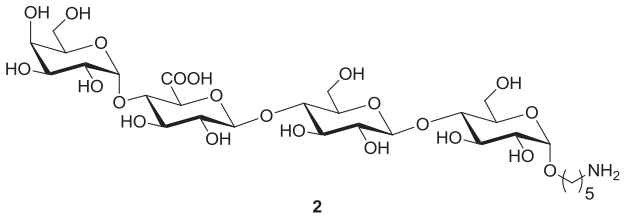
CH-HSQC NMR, 600 MHz, D_2O



Analytical HPLC (Hypercarb, 150 X 4.60 mm, Thermo Scientific); detector: ELSD



^{1}H NMR, 600 MHz, D_2O



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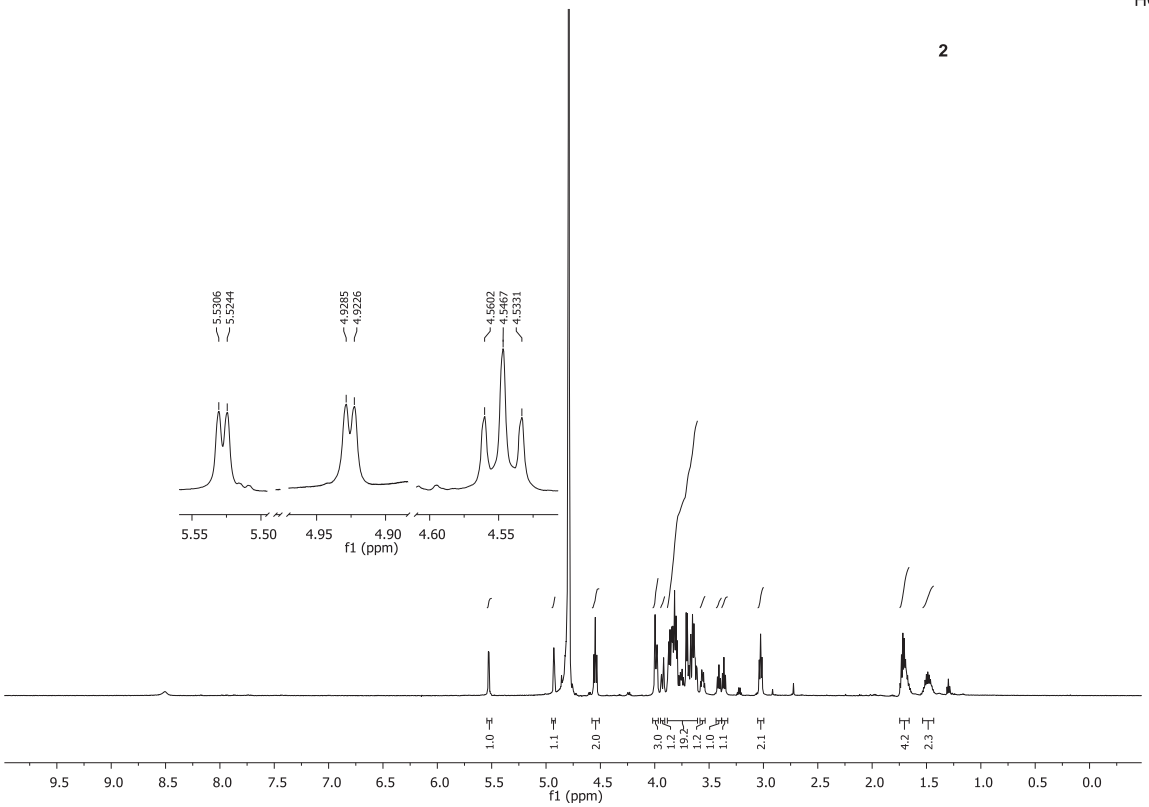
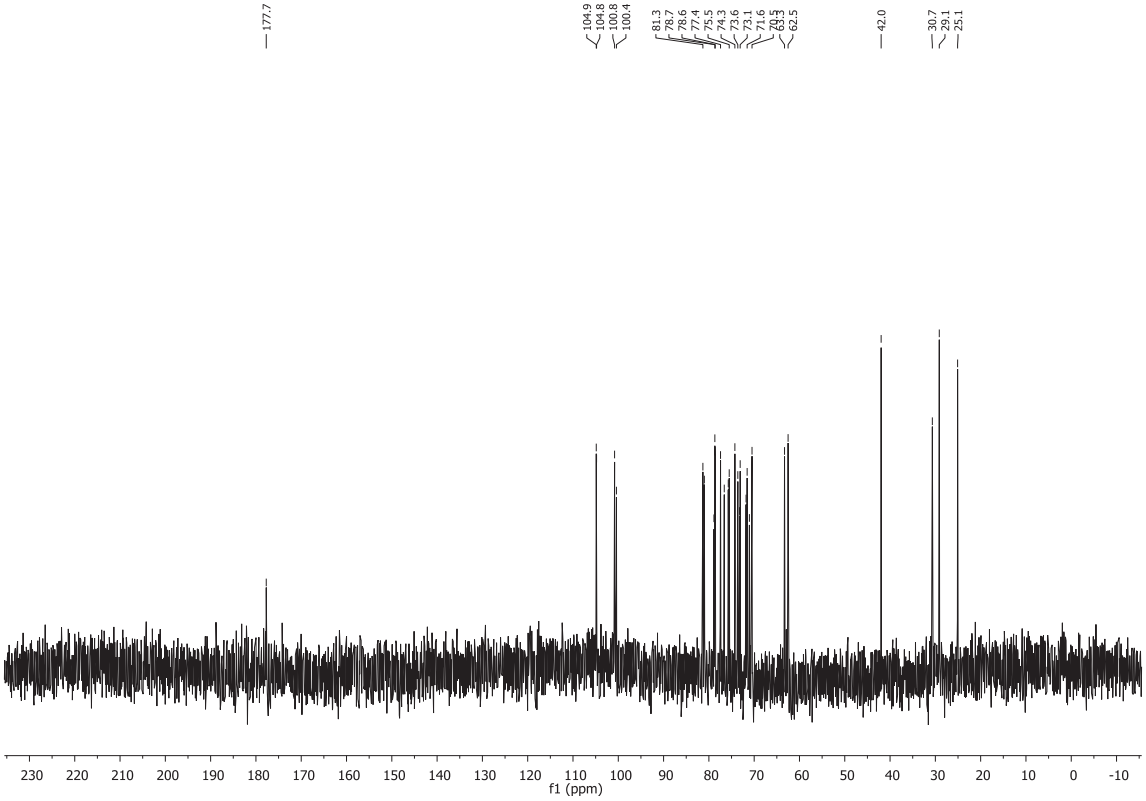
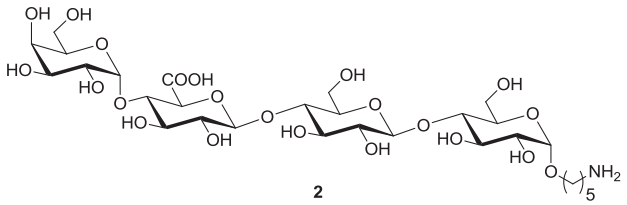


^{13}C NMR, 150 MHz, D_2O



HH-COSY NMR, 600 MHz, D_2O



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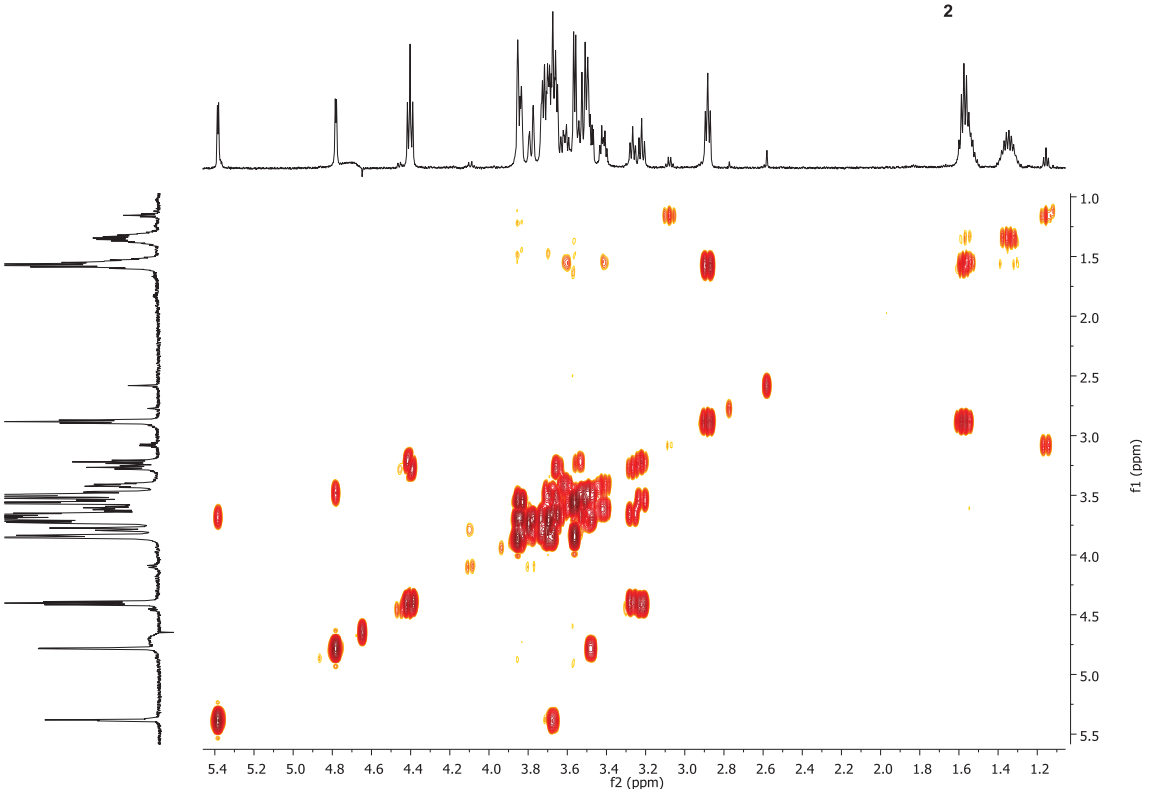
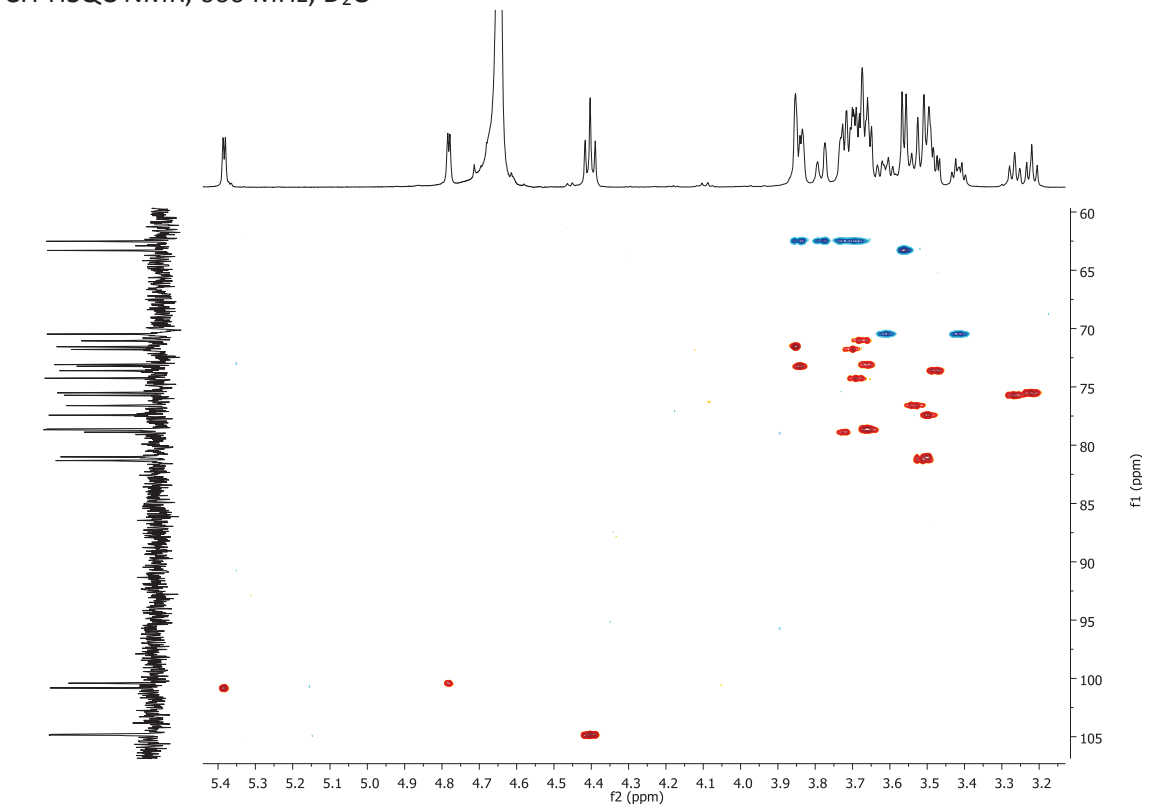
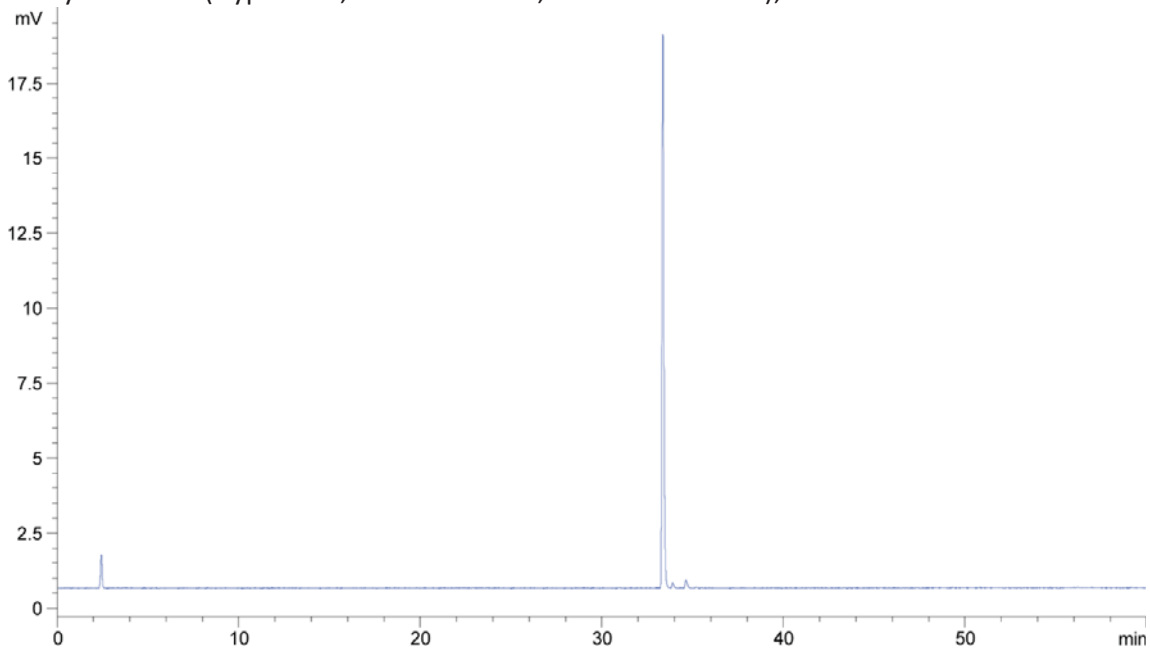


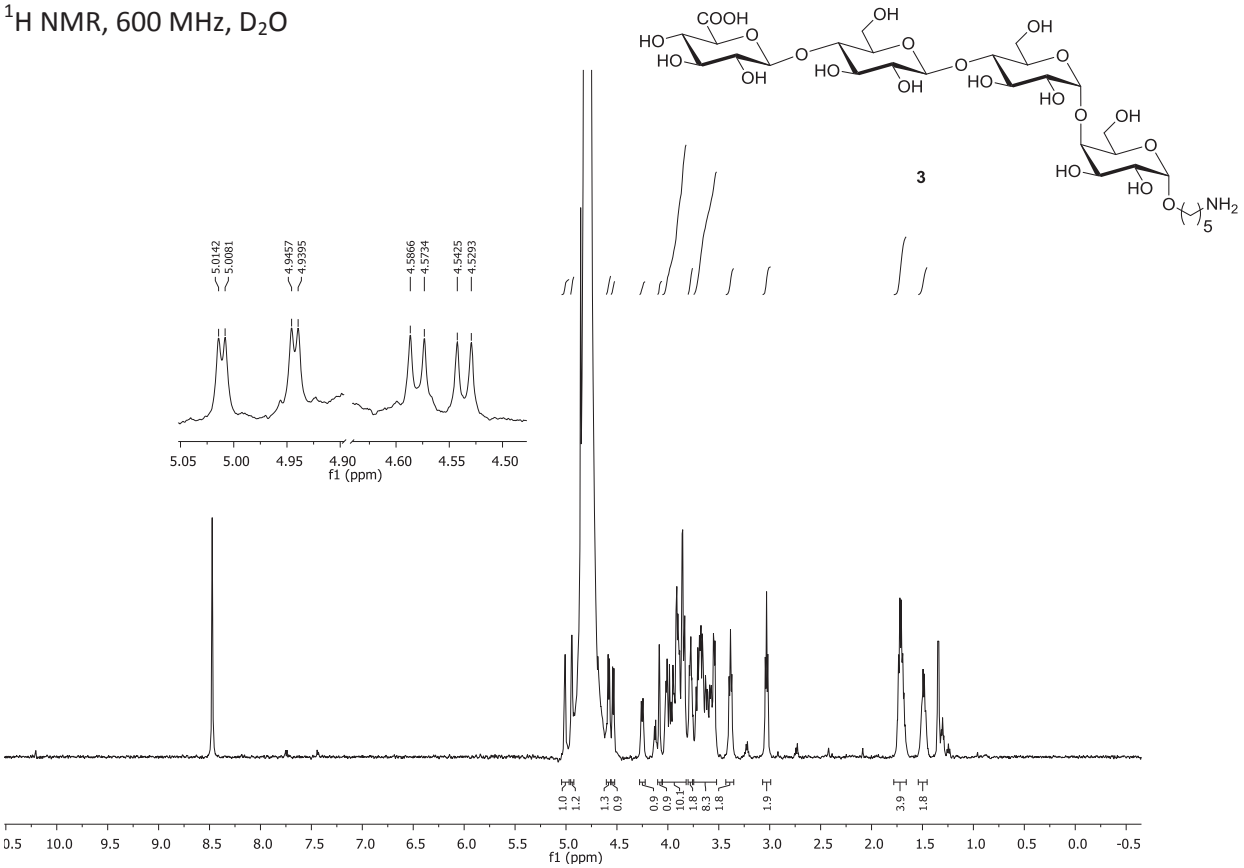
CH-HSQC NMR, 600 MHz, D_2O



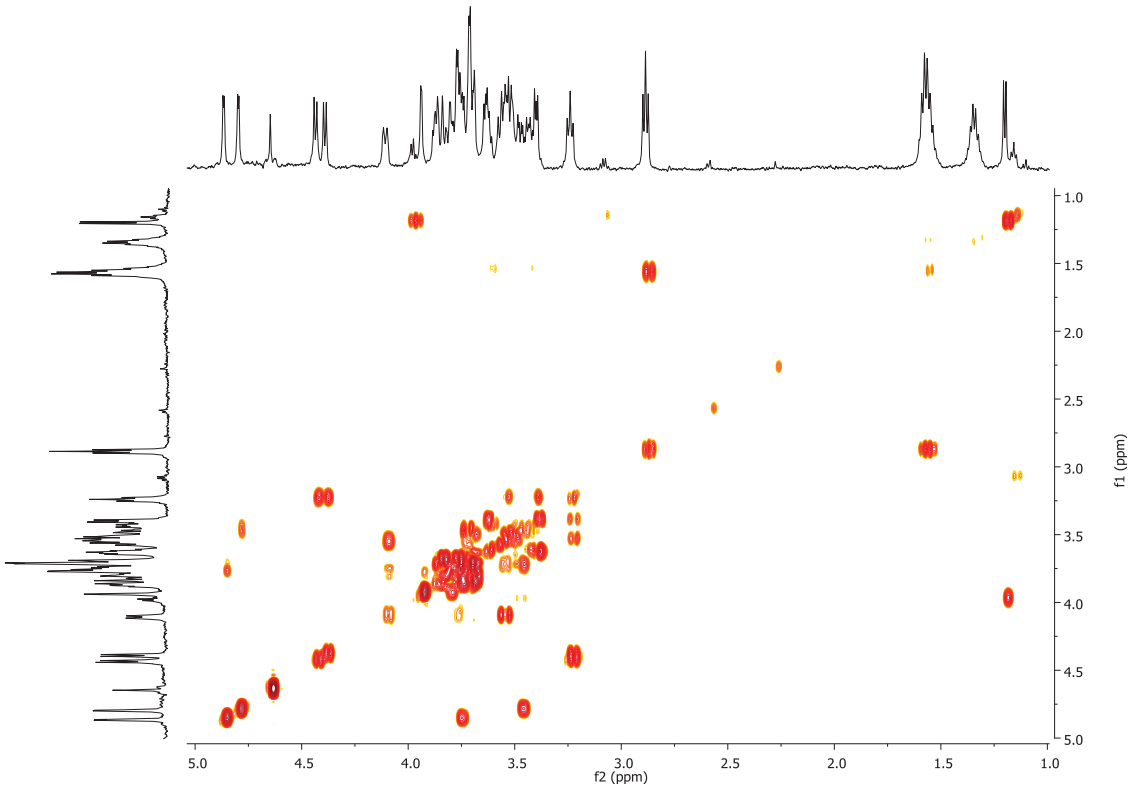
Analytical HPLC (Hypercarb, 150 X 4.60 mm, Thermo Scientific); detector: ELSD



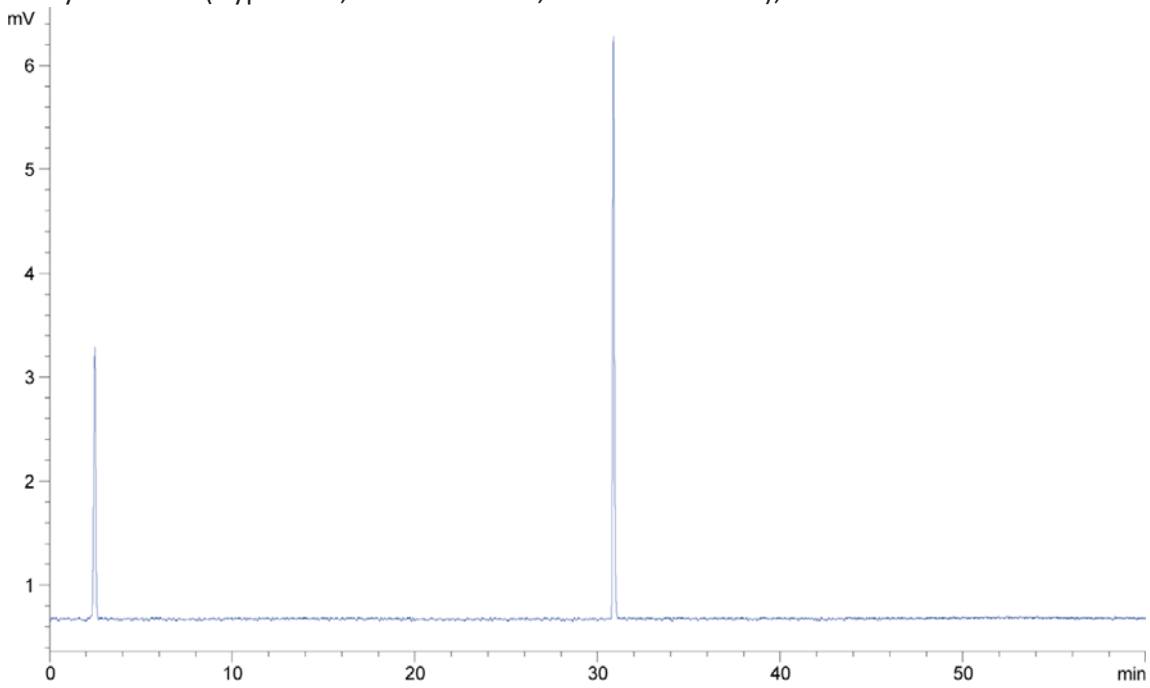
^{1}H NMR, 600 MHz, D_2O



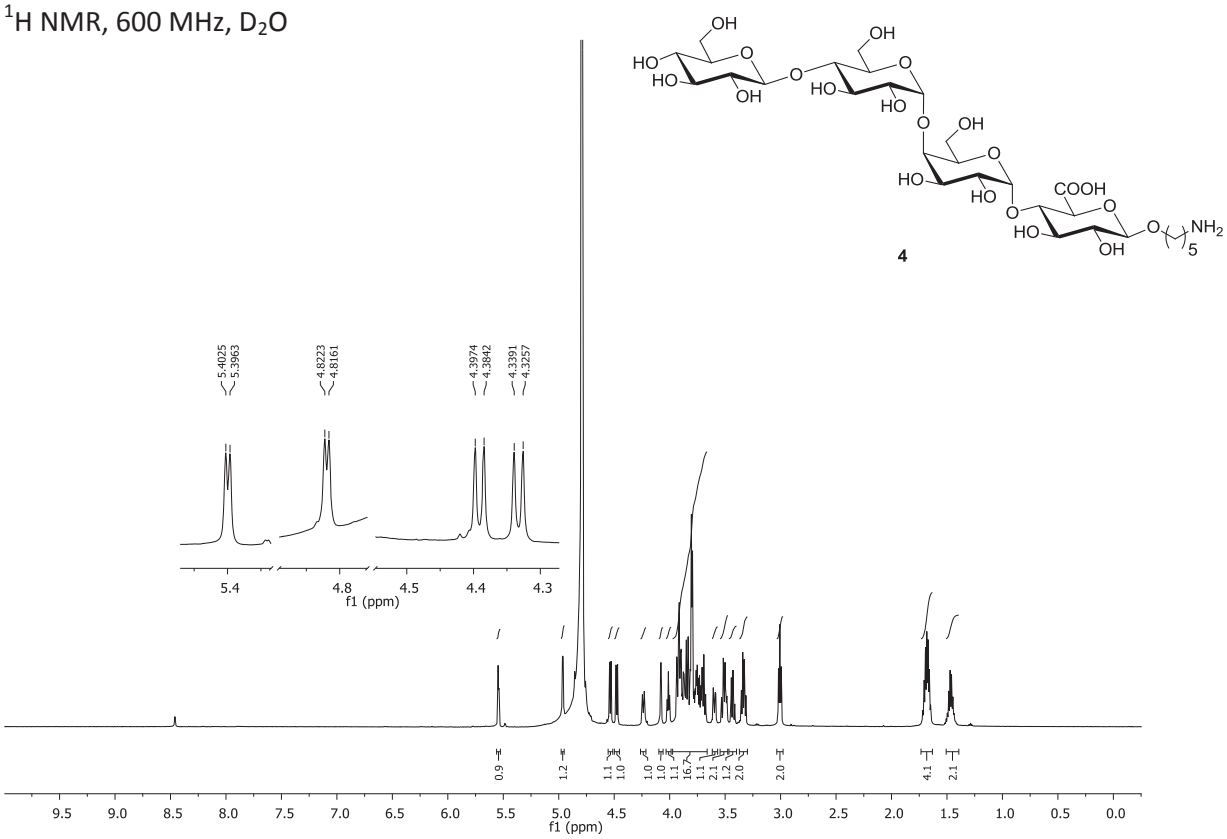
HH-COSY NMR, 600 MHz, D_2O



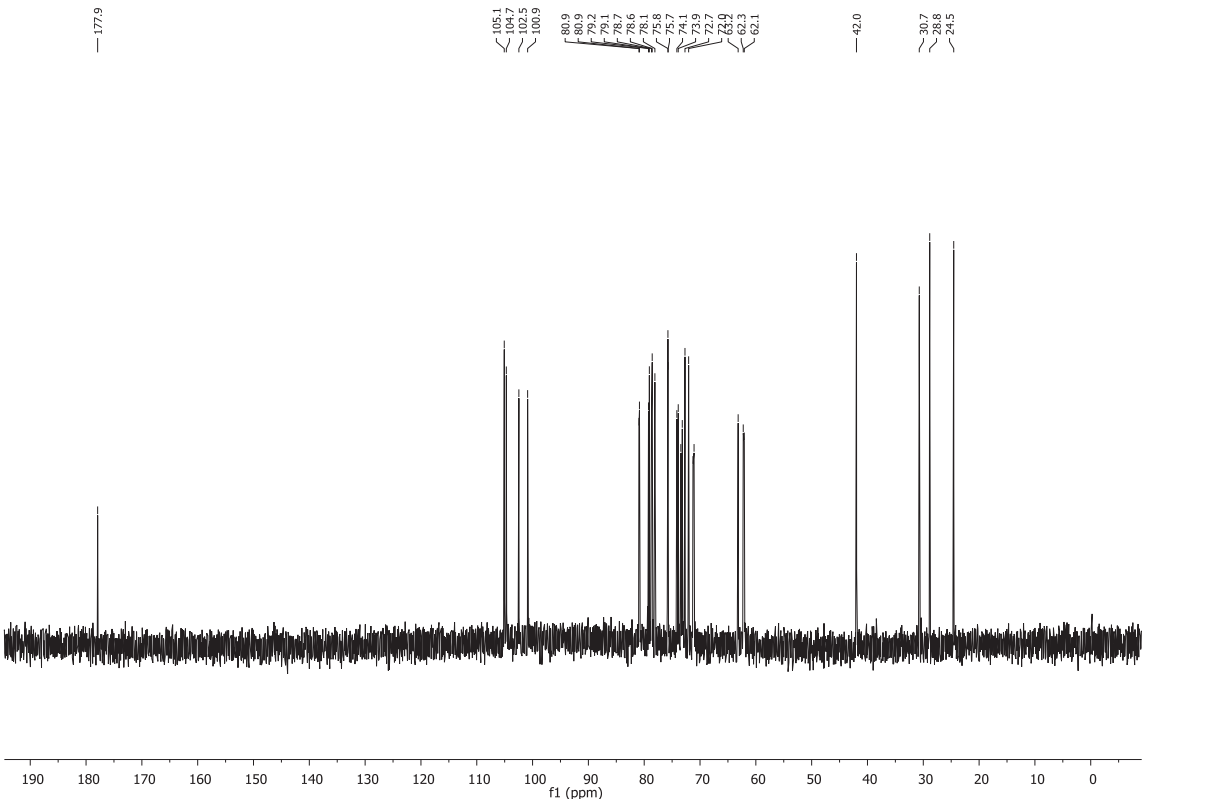
Analytical HPLC (Hypercarb, 150 X 4.60 mm, Thermo Scientific); detector: ELSD



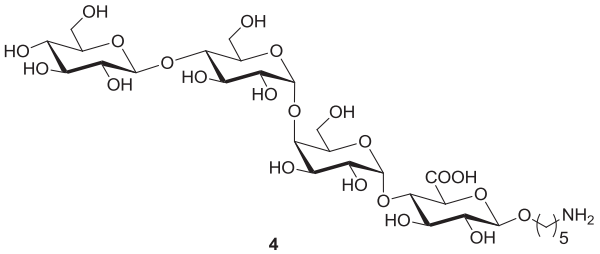
^{1}H NMR, 600 MHz, D_2O



^{13}C NMR, 600 MHz, D_2O



HH-COSY NMR, 400 MHz, D_2O



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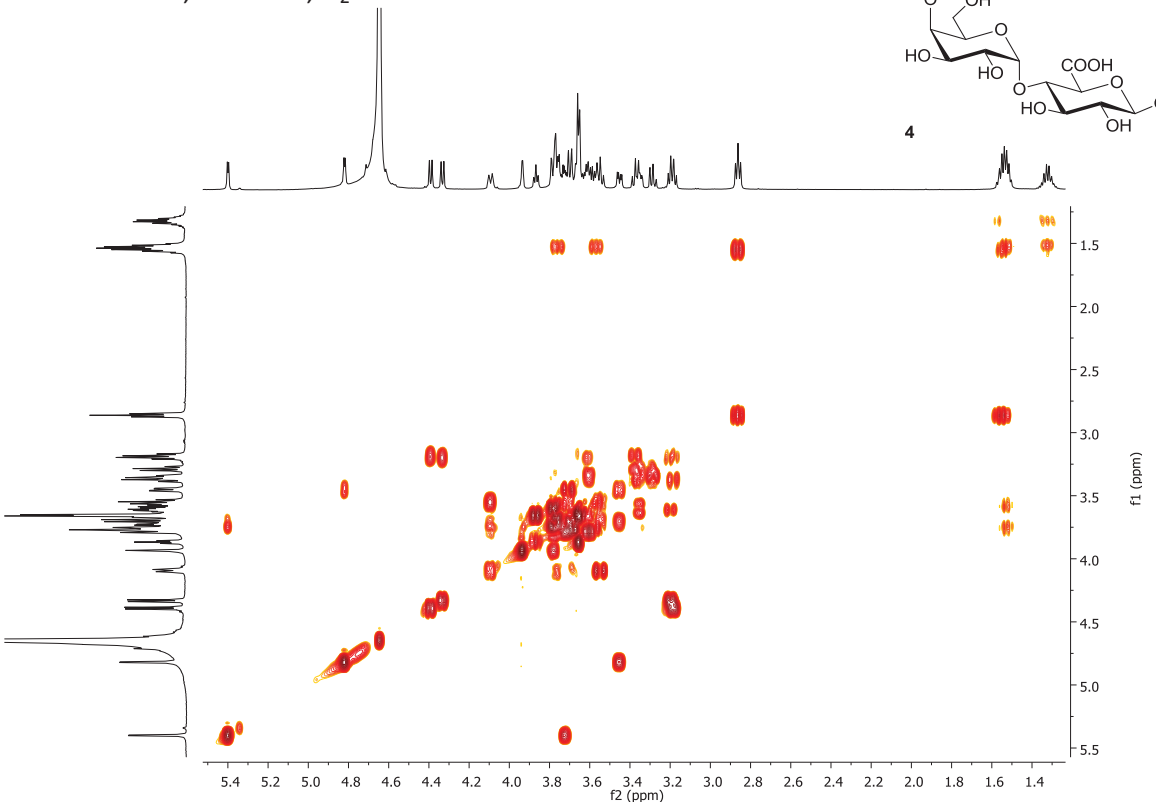
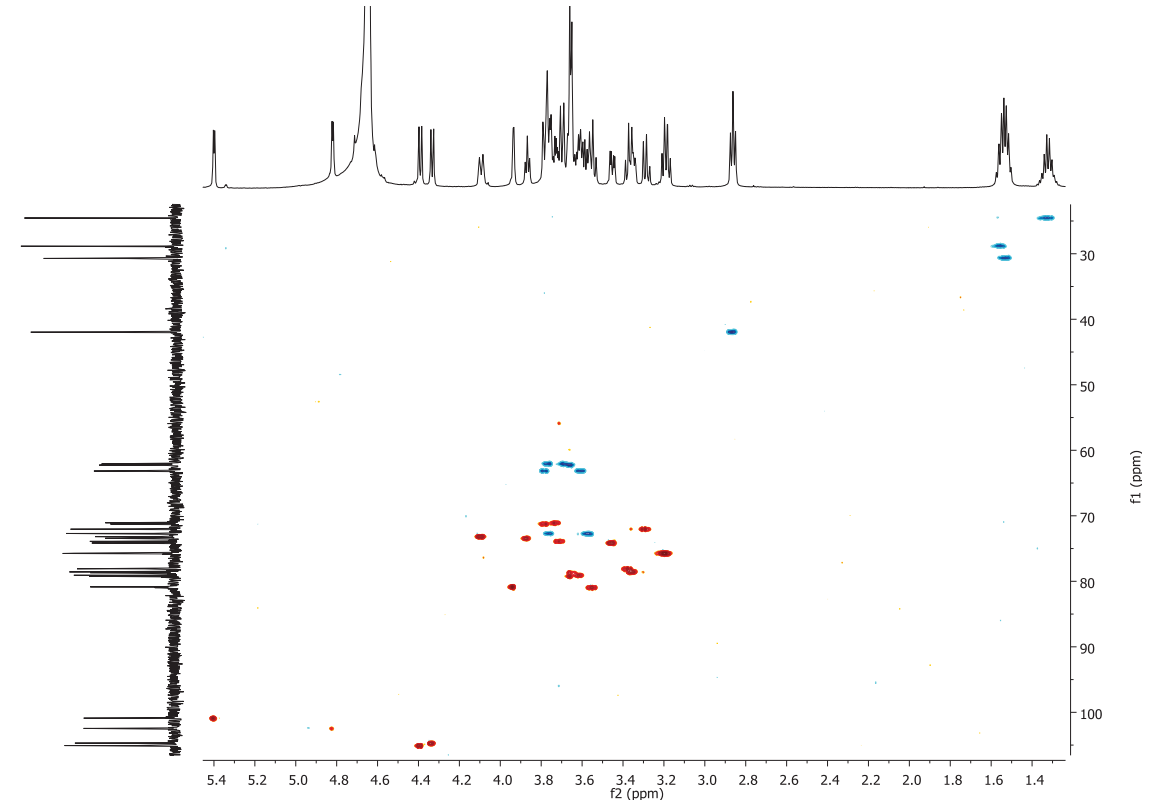
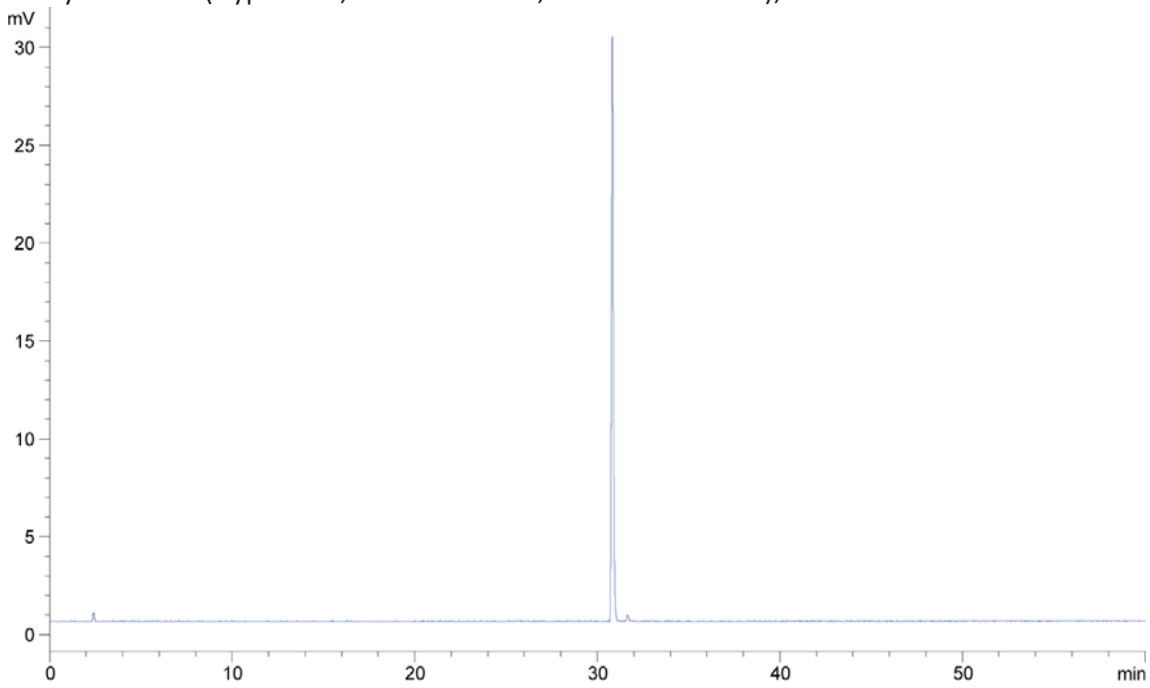


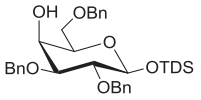
CH-HSQC NMR, 400 MHz, D_2O



Analytical HPLC (Hypercarb, 150 X 4.60 mm, Thermo Scientific); detector: ELSD



^{1}H NMR, 400 MHz, $CDCl_3$



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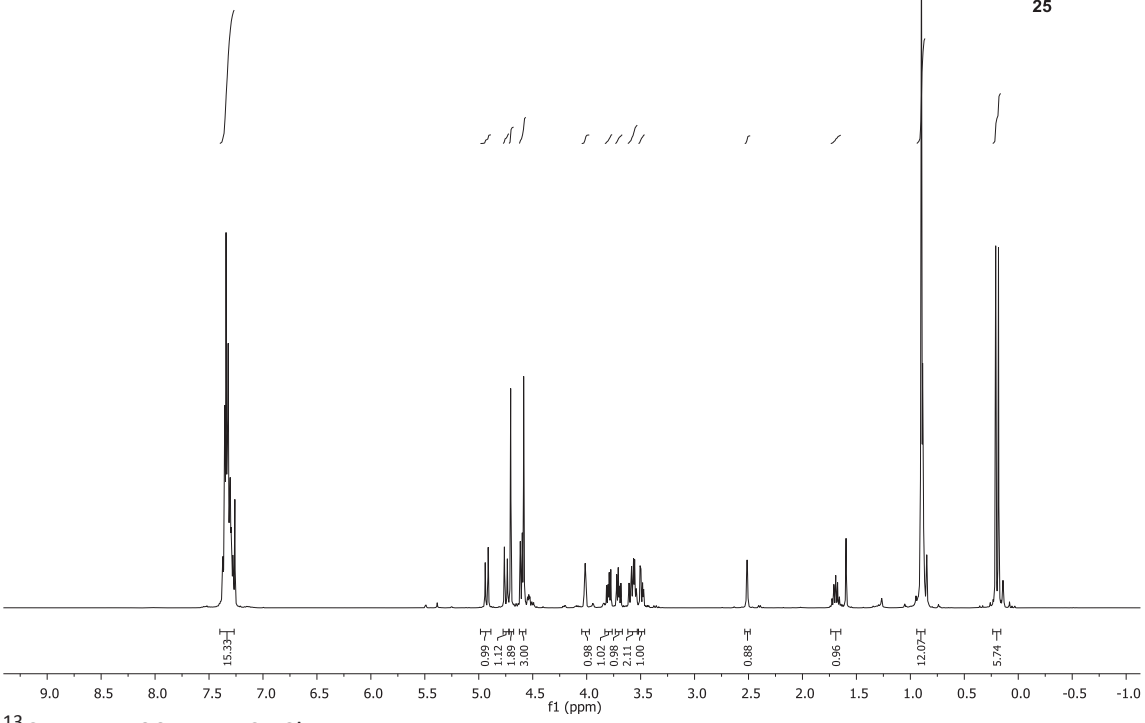
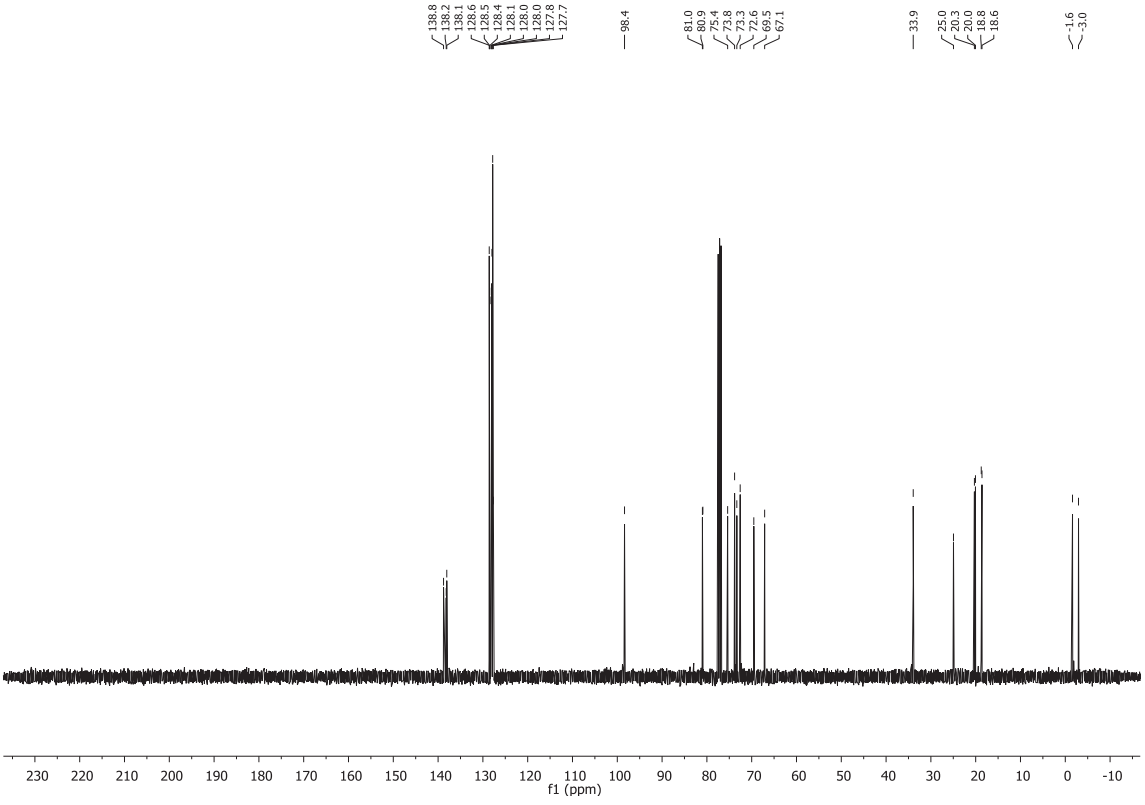
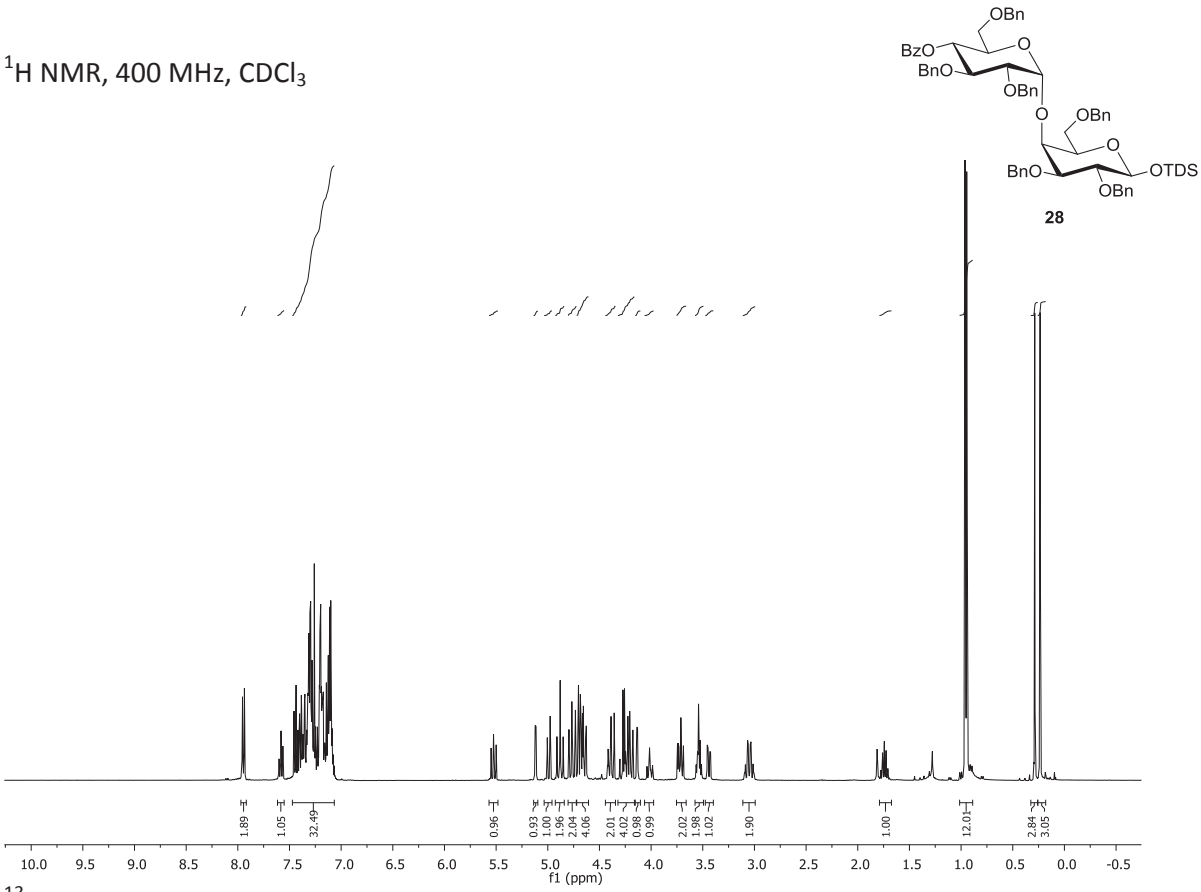


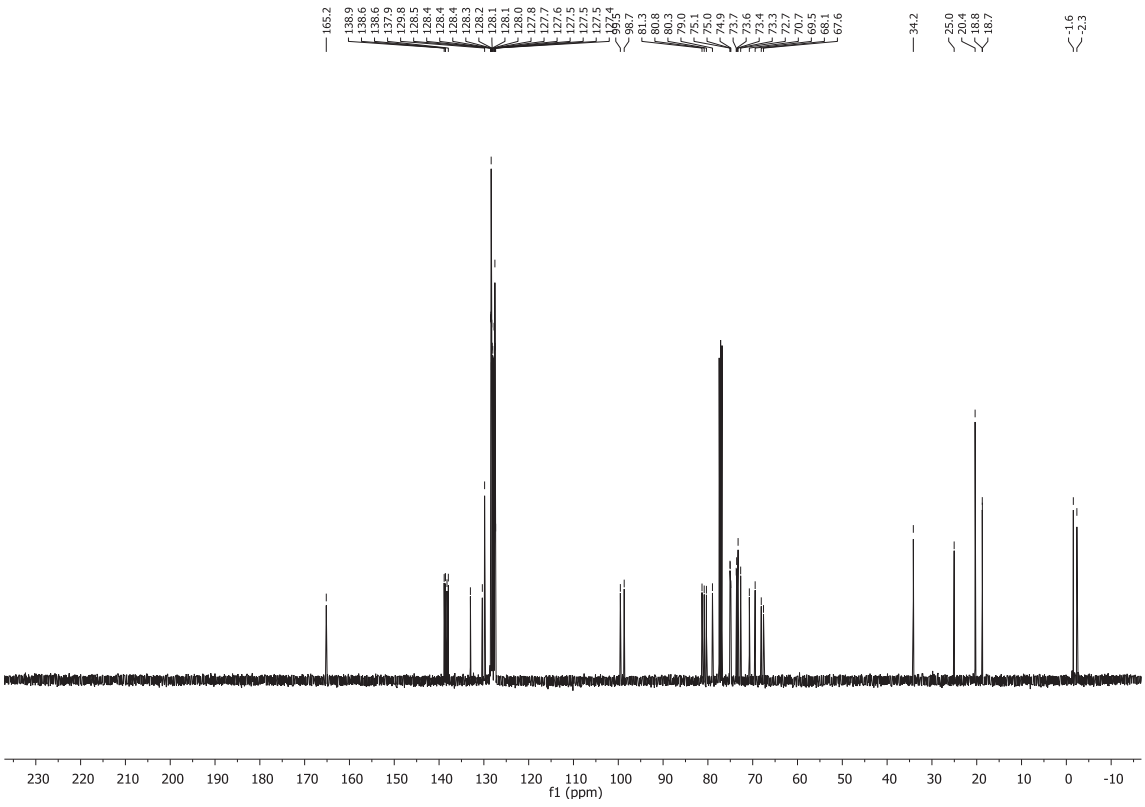
^{13}C NMR, 100 MHz, $CDCl_3$



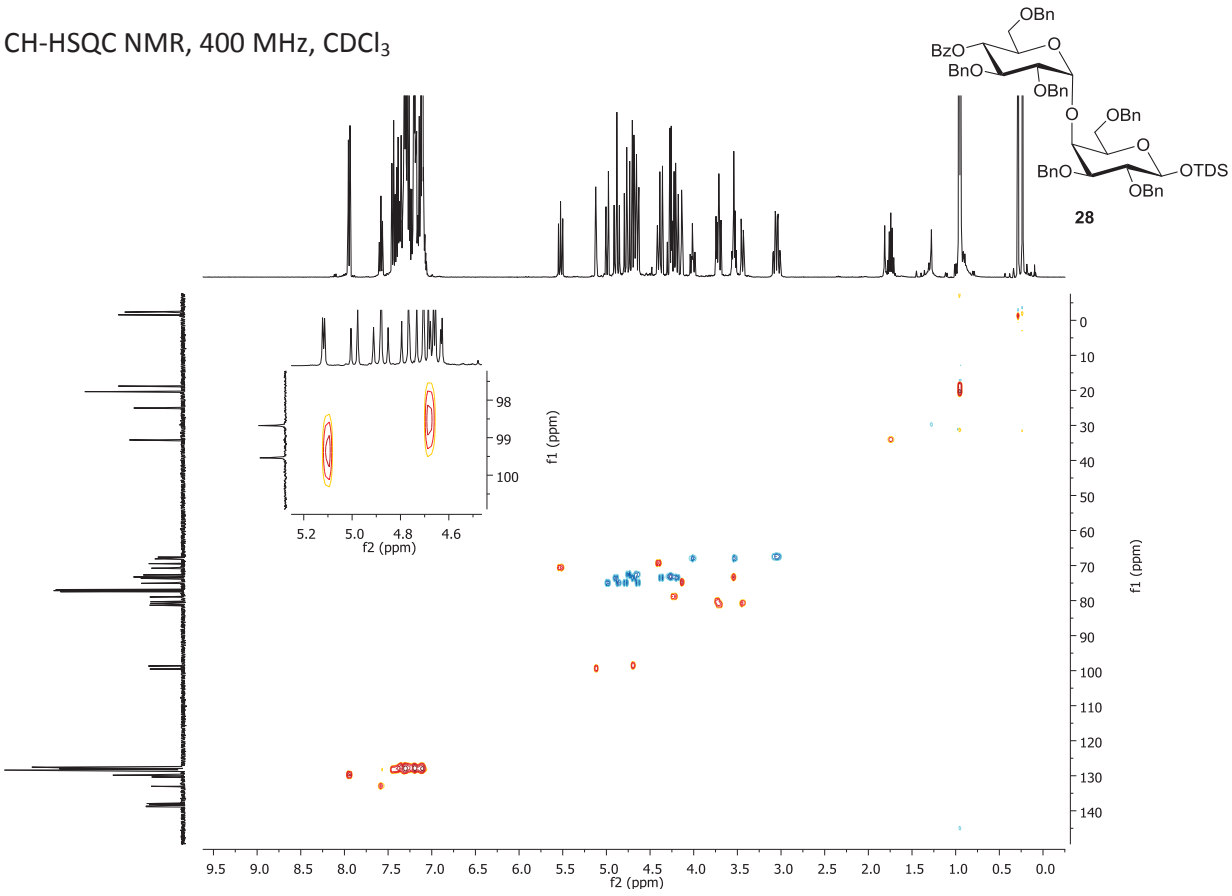
^{1}H NMR, 400 MHz, $CDCl_3$



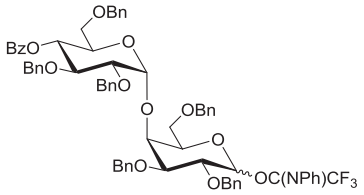
^{13}C NMR, 100 MHz, $CDCl_3$



CH-HSQC NMR, 400 MHz, $CDCl_3$



^{1}H NMR, 400 MHz, acetone-D_6



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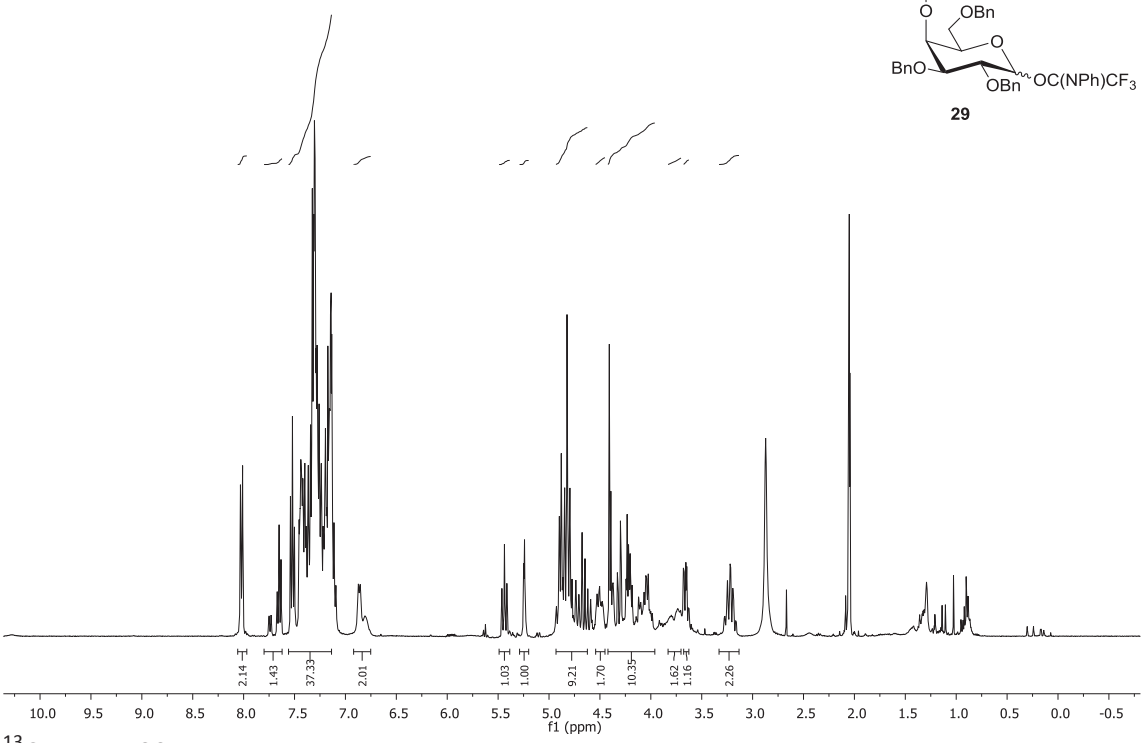
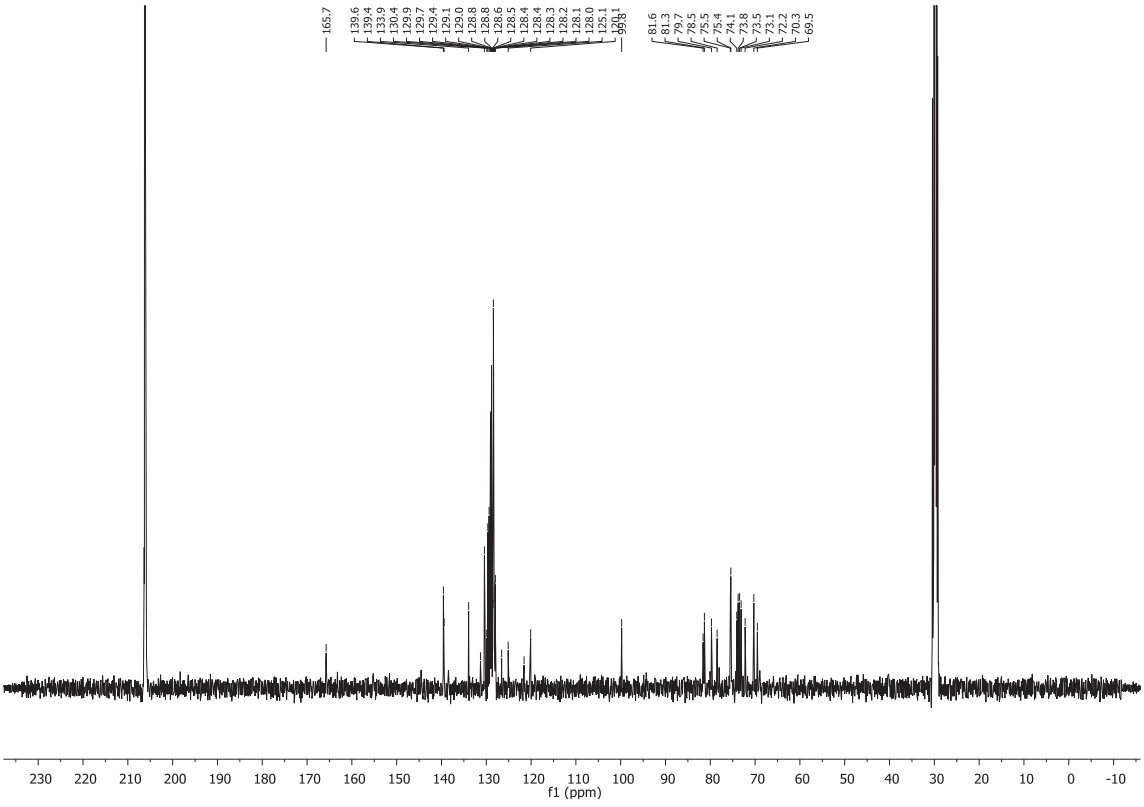
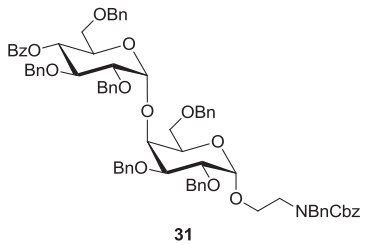


^{13}C NMR, 100 MHz, acetone-D_6



^{1}H NMR, 400 MHz, $CDCl_3$



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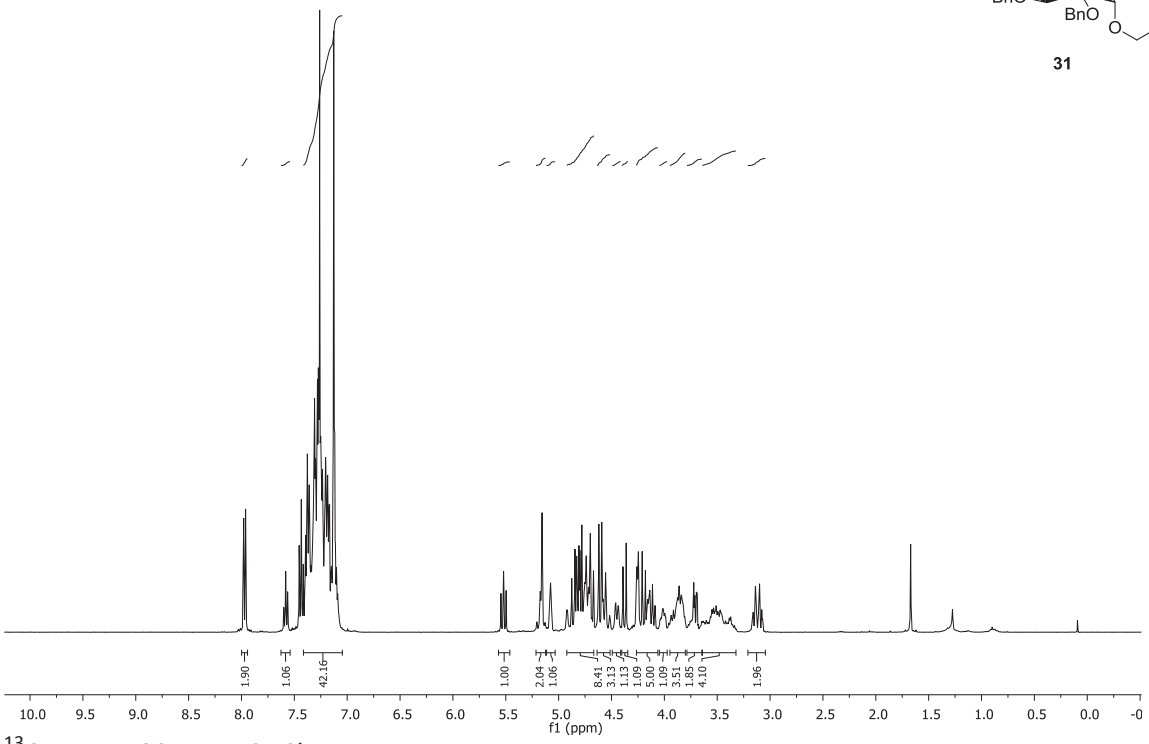
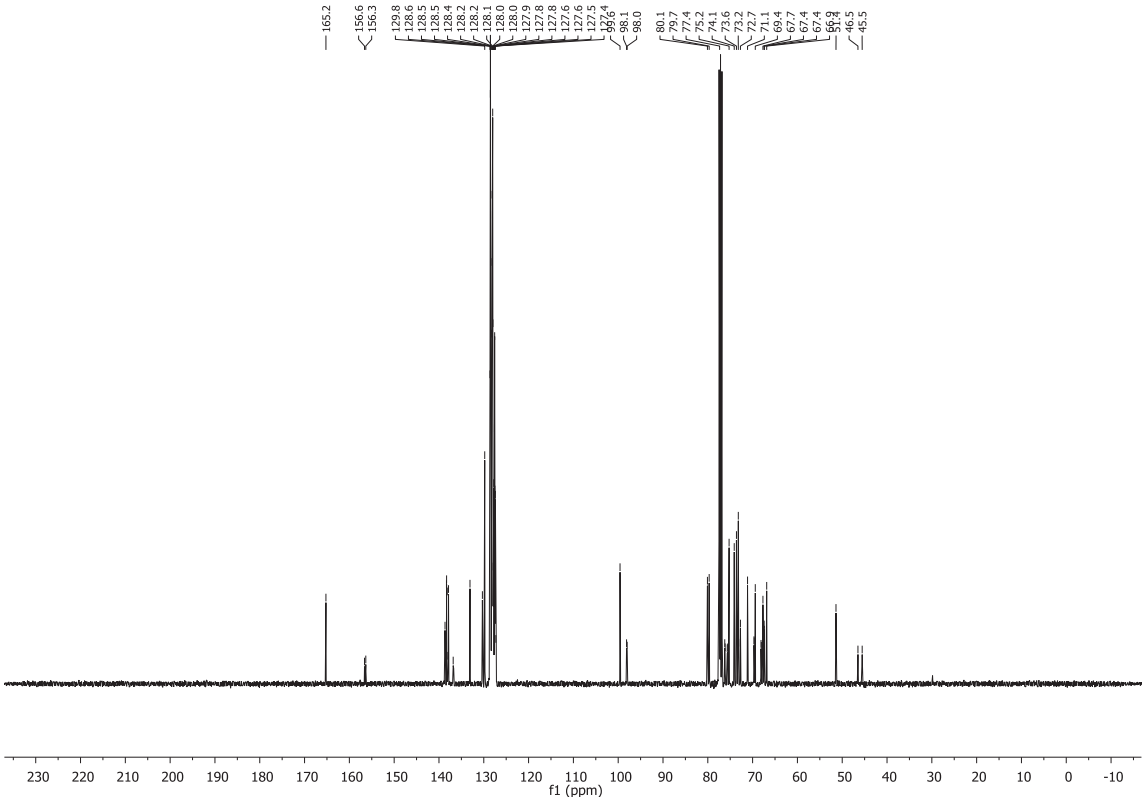
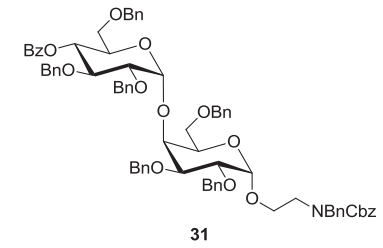


^{13}C NMR, 100 MHz, $CDCl_3$



CH-HSQC NMR, 400 MHz, $CDCl_3$



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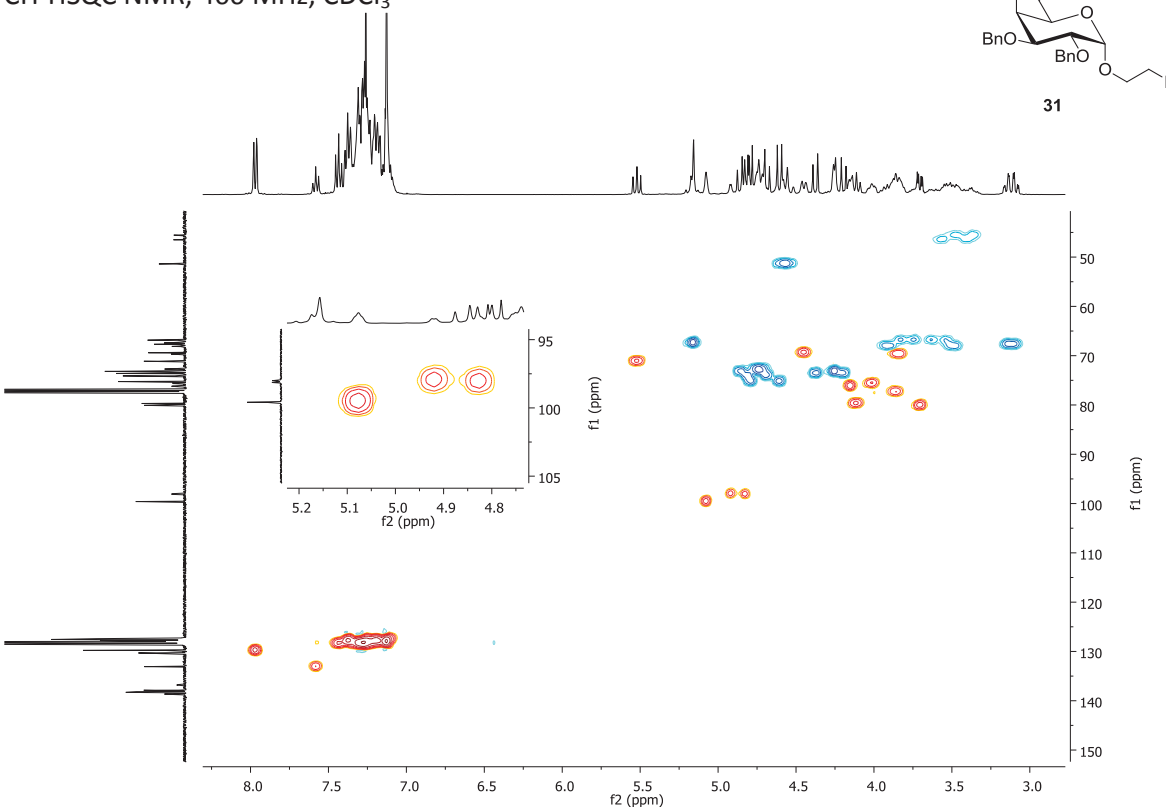

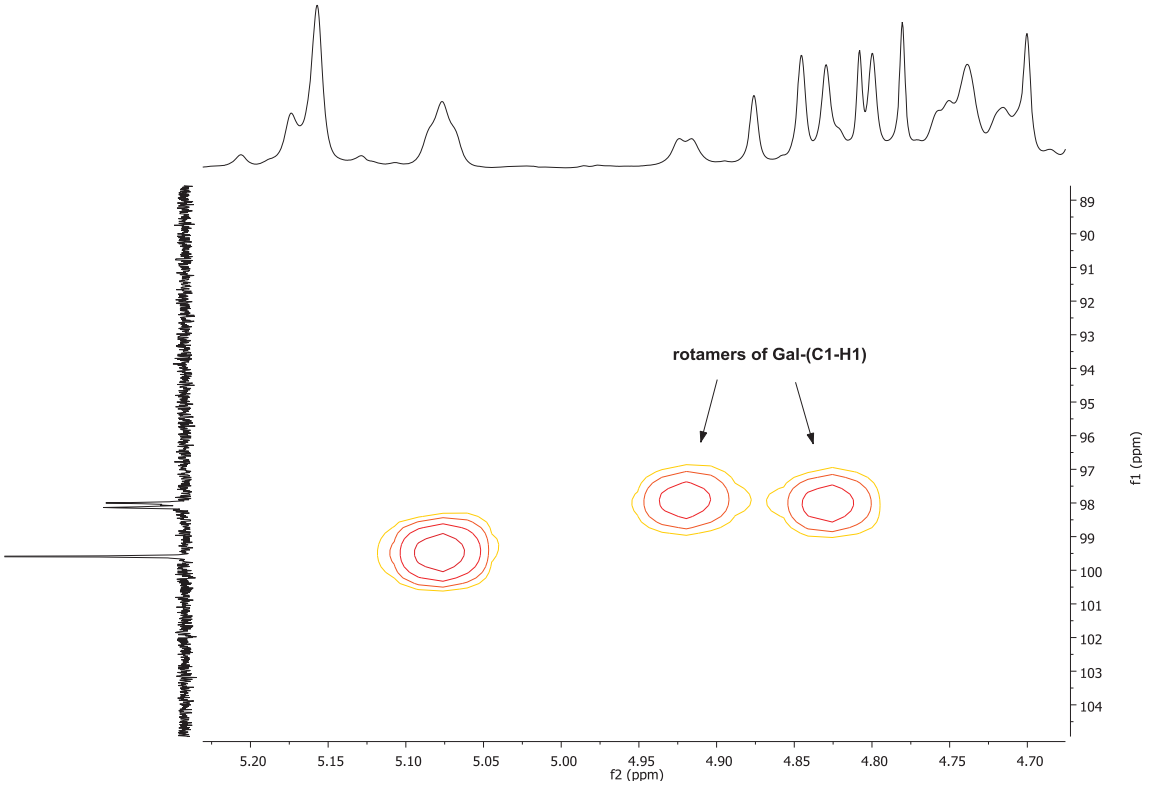

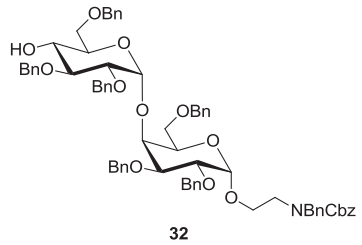

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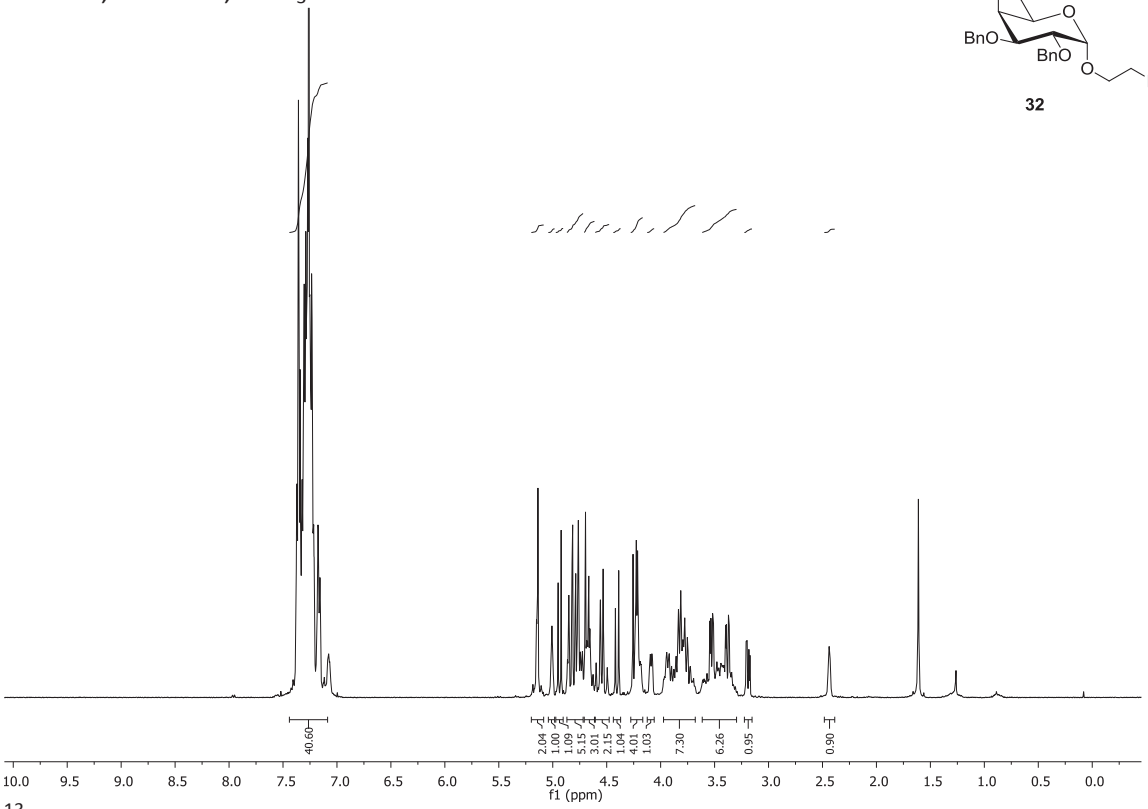
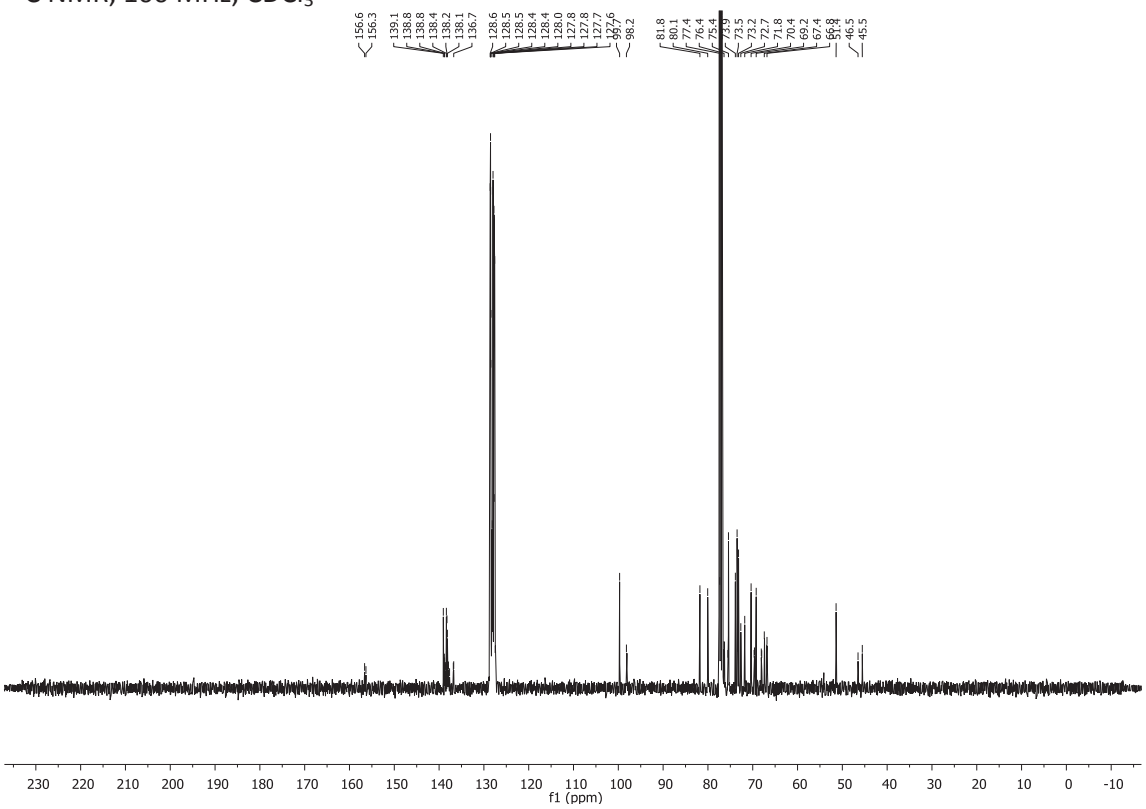
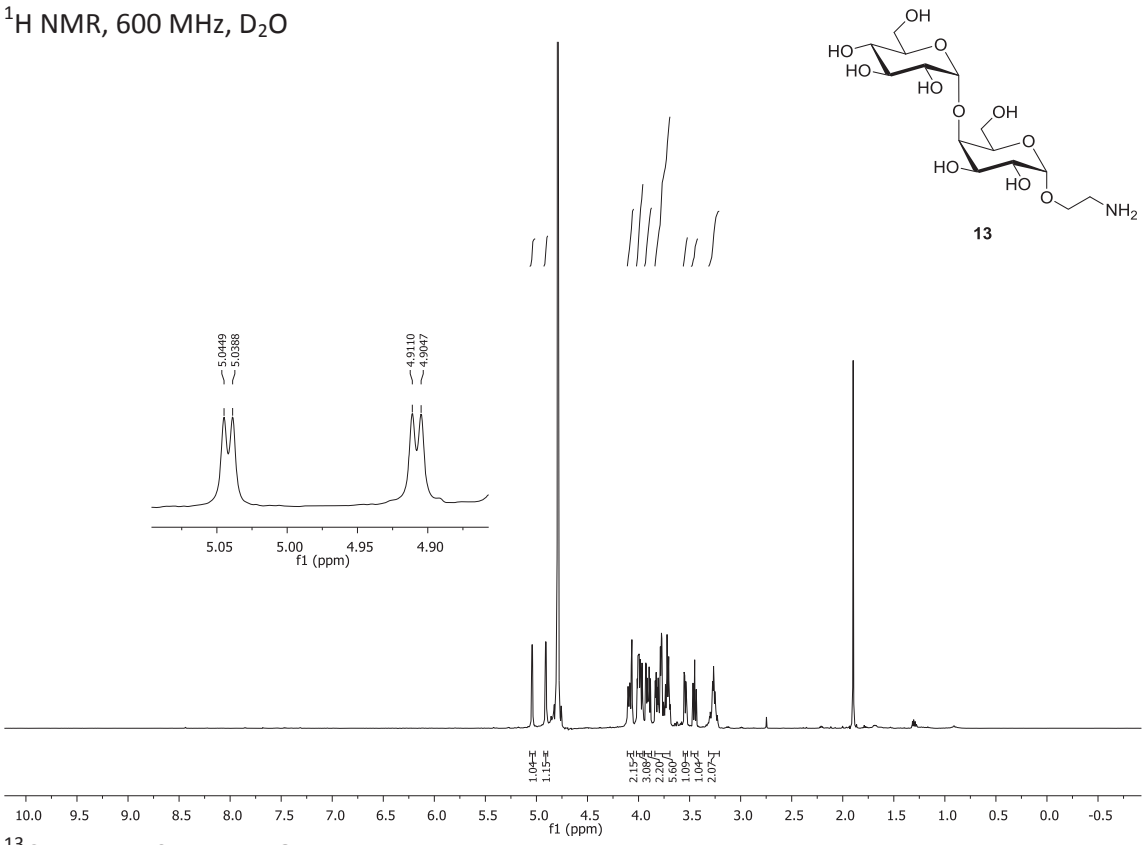
^{1}H NMR, 400 MHz, $CDCl_3$



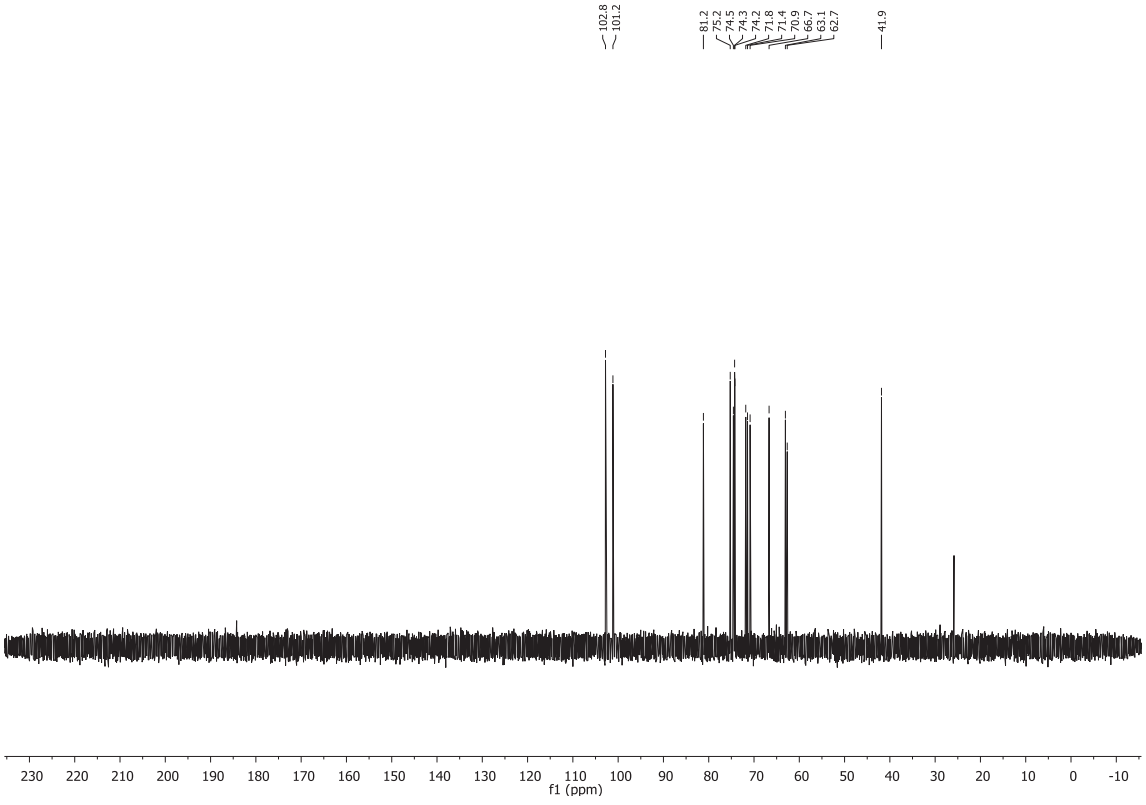
^{13}C NMR, 100 MHz, $CDCl_3$



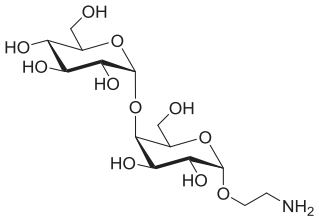
^{1}H NMR, 600 MHz, D_2O



^{13}C NMR, 150 MHz, D_2O



HH-COSY NMR, 600 MHz, D_2O



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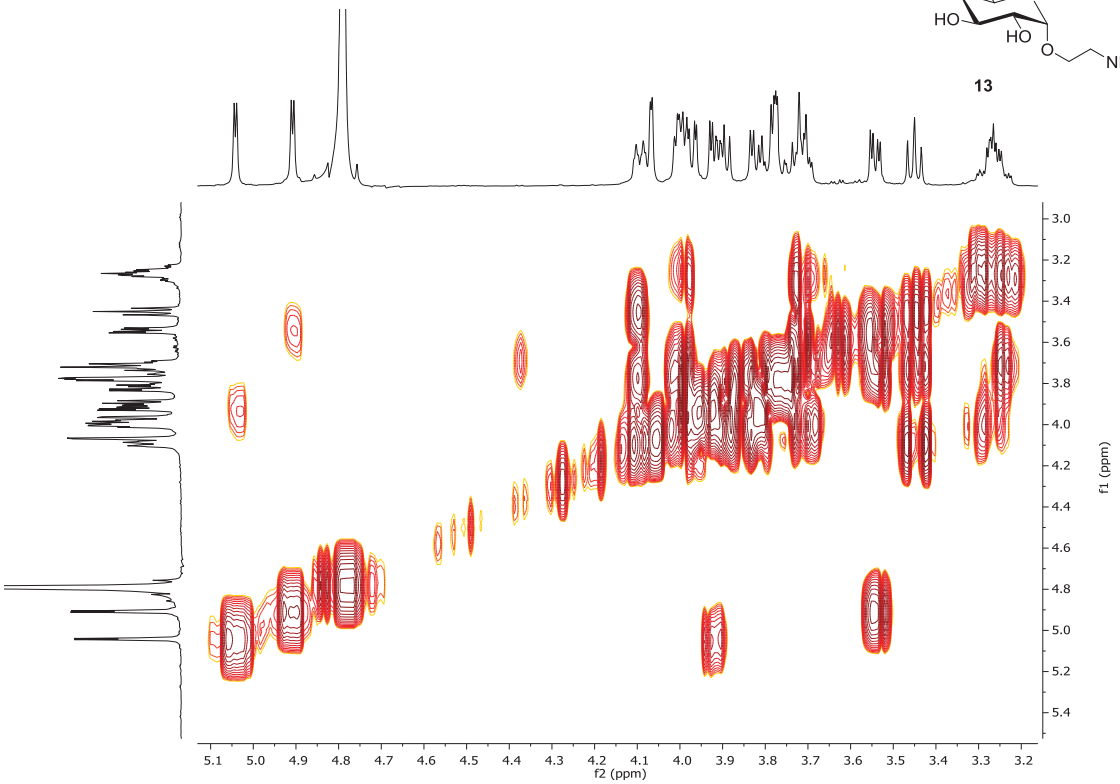
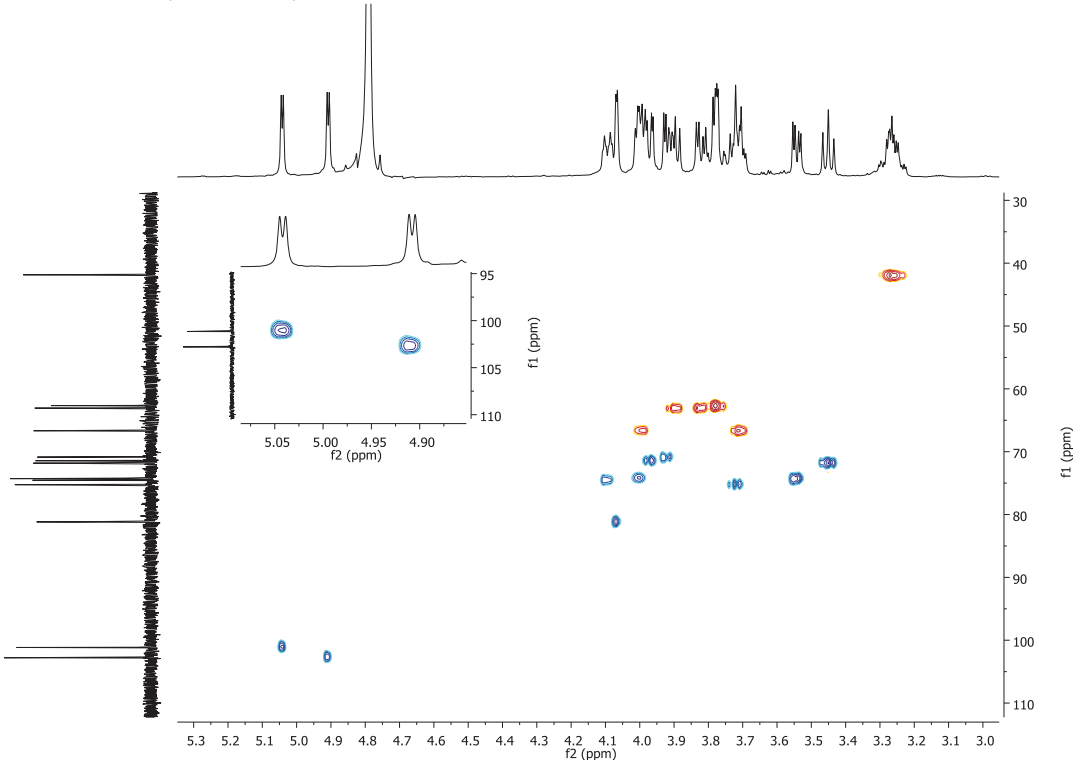
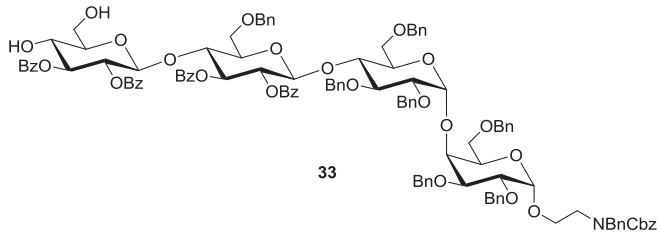


CH-HSQC NMR, 600 MHz, D_2O



^{1}H NMR, 400 MHz, $CDCl_3$



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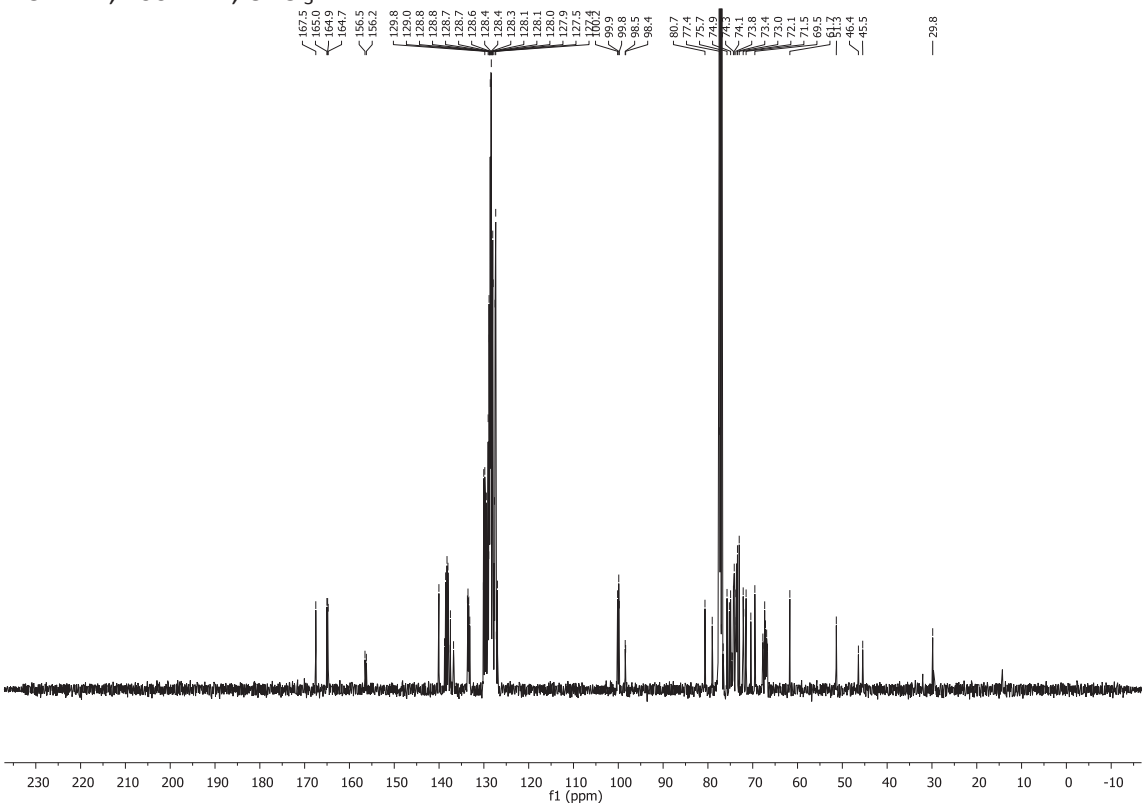
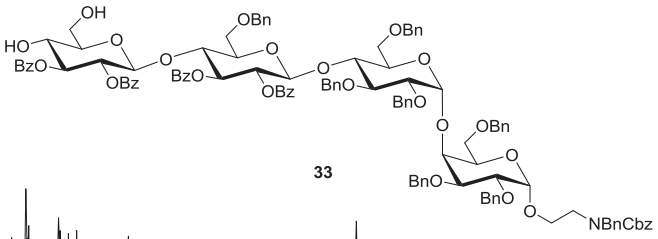
^{13}C NMR, 100 MHz, $CDCl_3$



HH-COSY NMR, 400 MHz, $CDCl_3$



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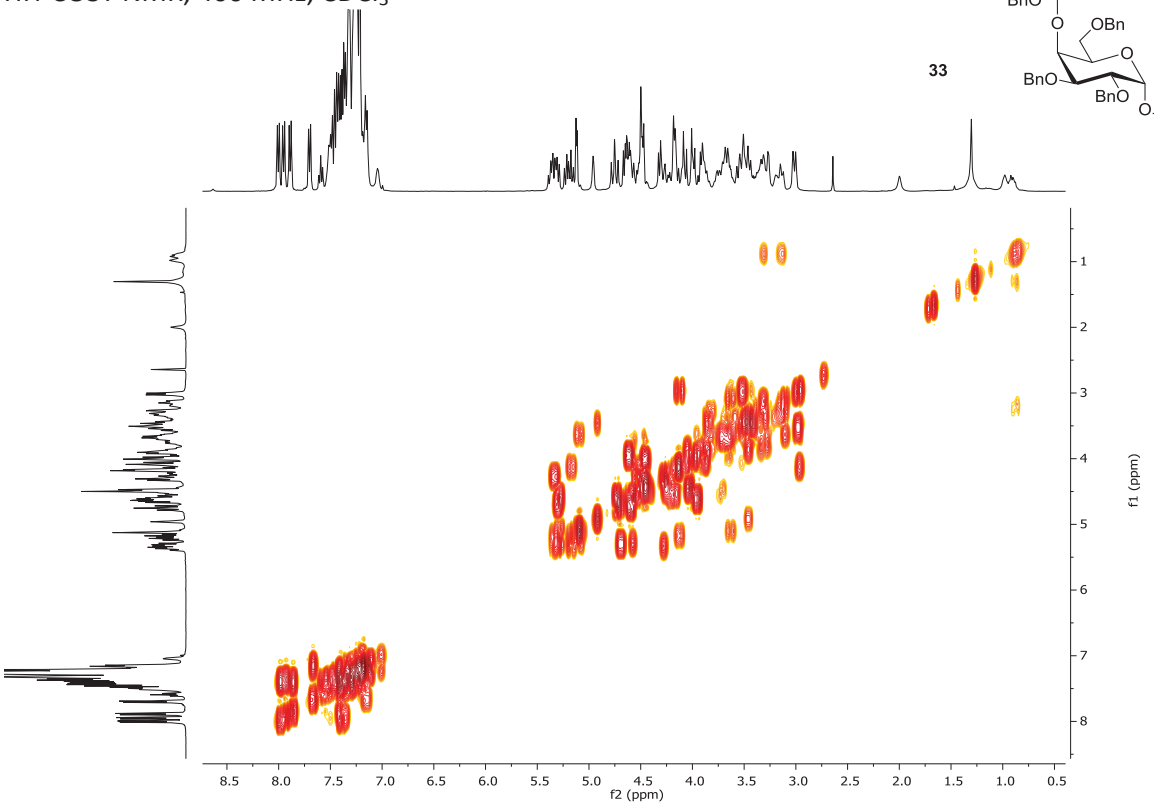
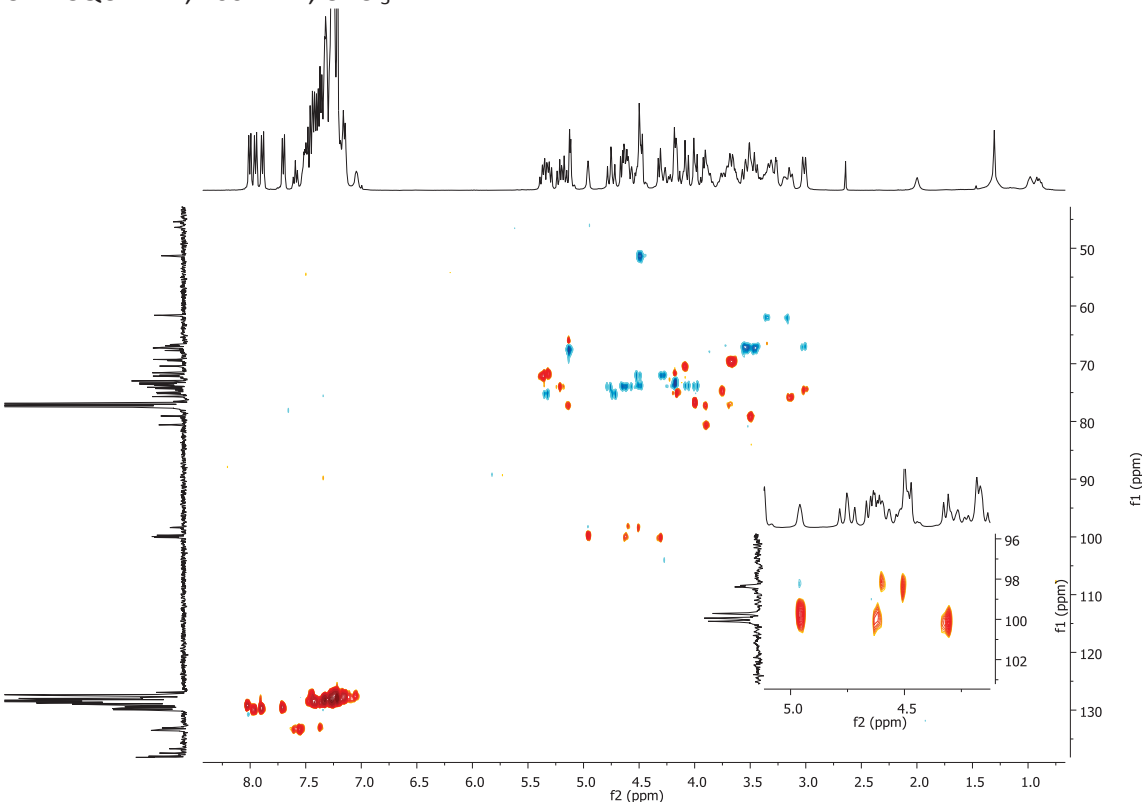
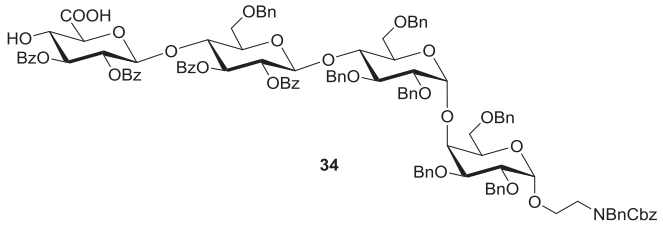


CH-HSQC NMR, 400 MHz, $CDCl_3$



^{1}H NMR, 600 MHz, $CDCl_3$



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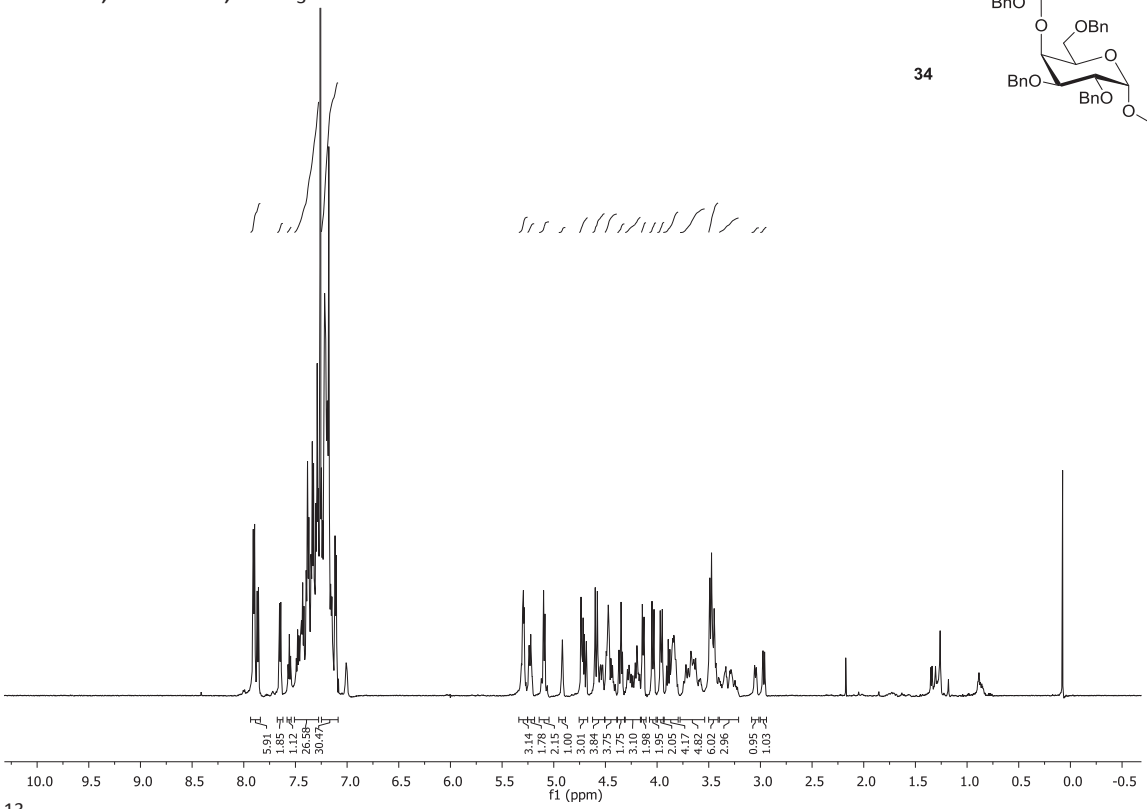
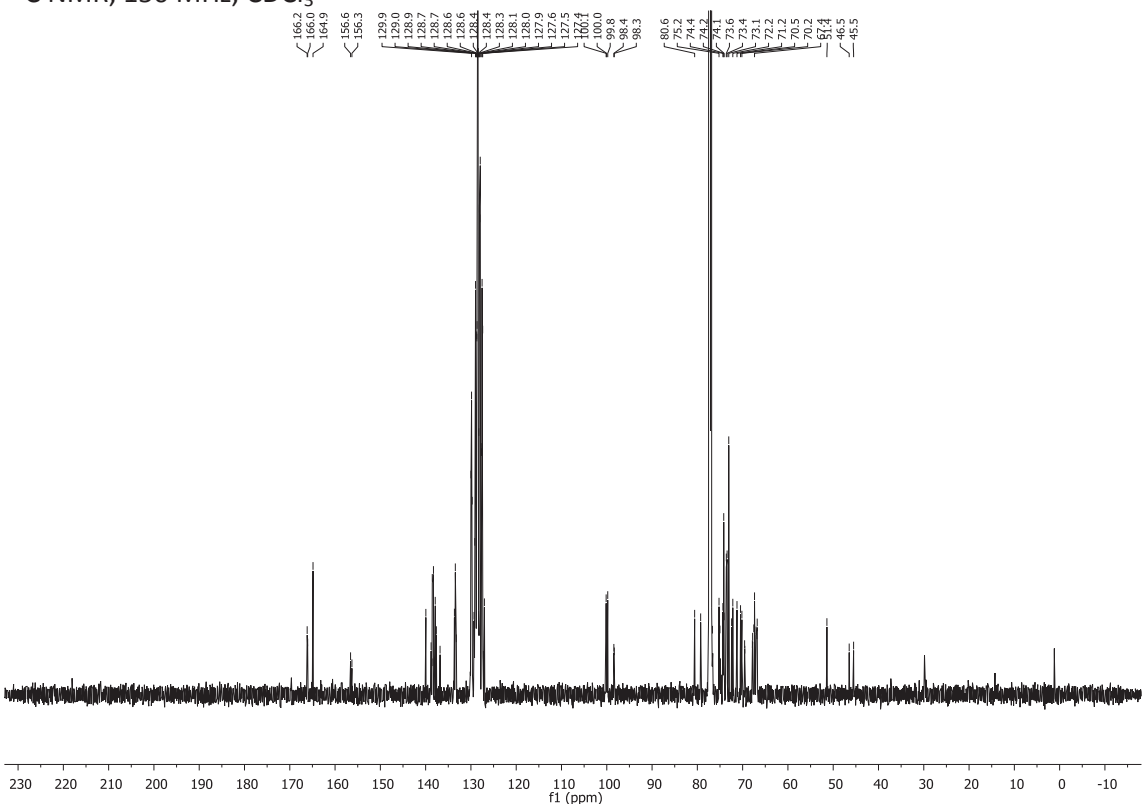
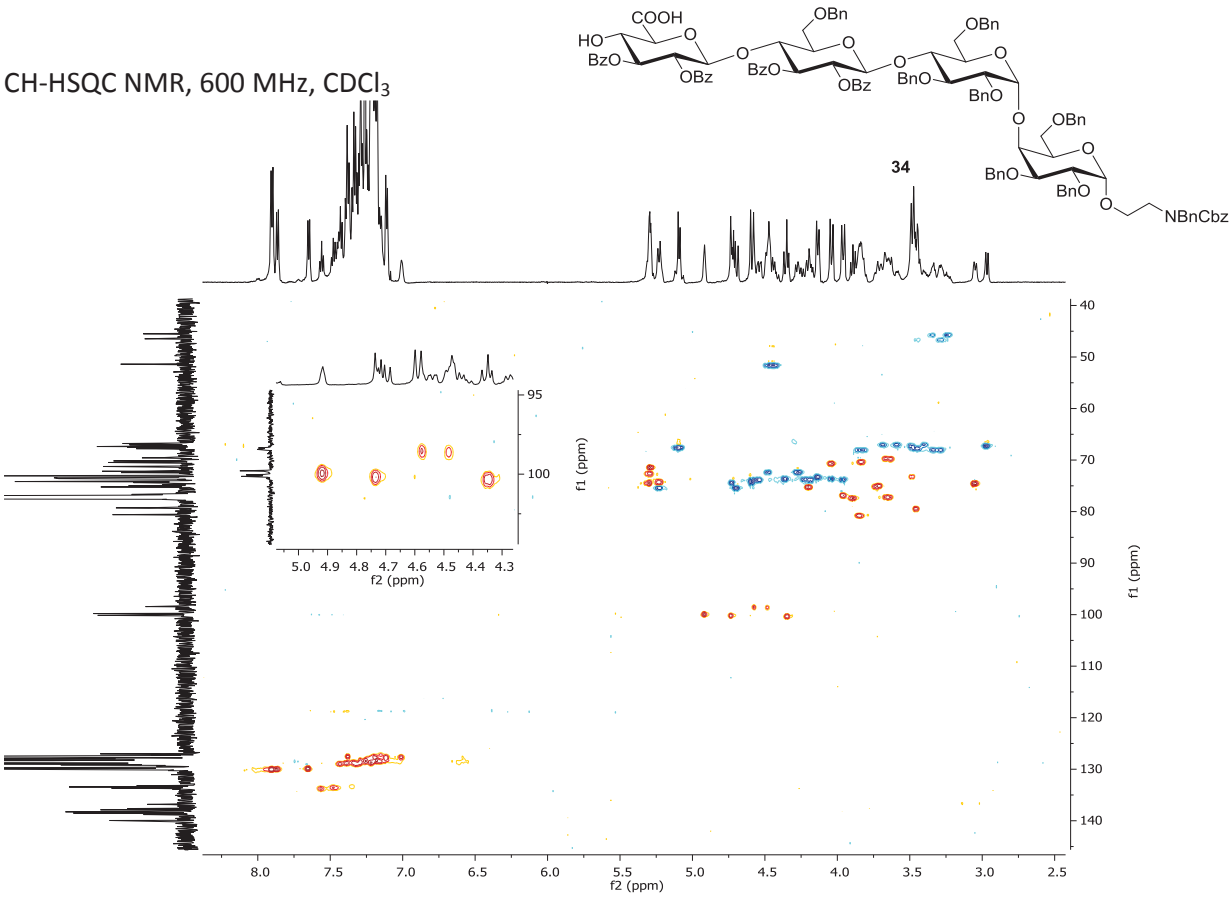


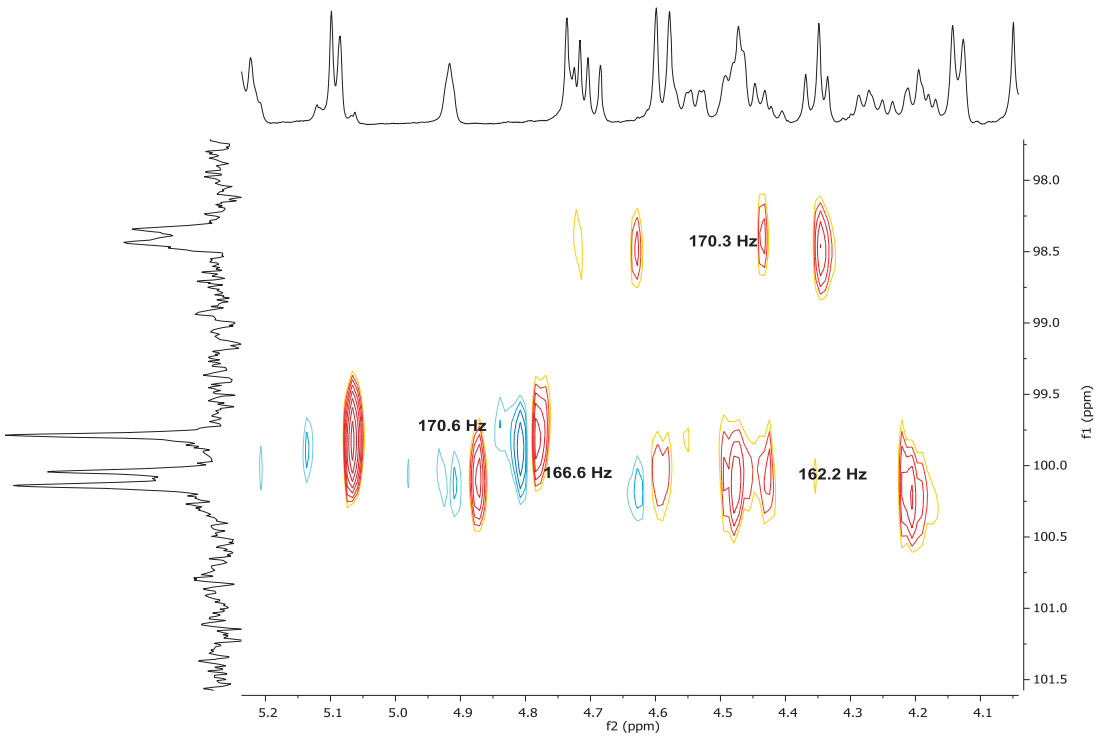
^{13}C NMR, 150 MHz, $CDCl_3$



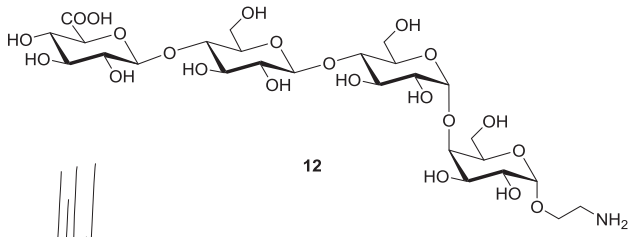
CH-HSQC NMR, 600 MHz, $CDCl_3$



CH-coupled HSQC NMR, 600 MHz



^{1}H NMR, 600 MHz, D_2O



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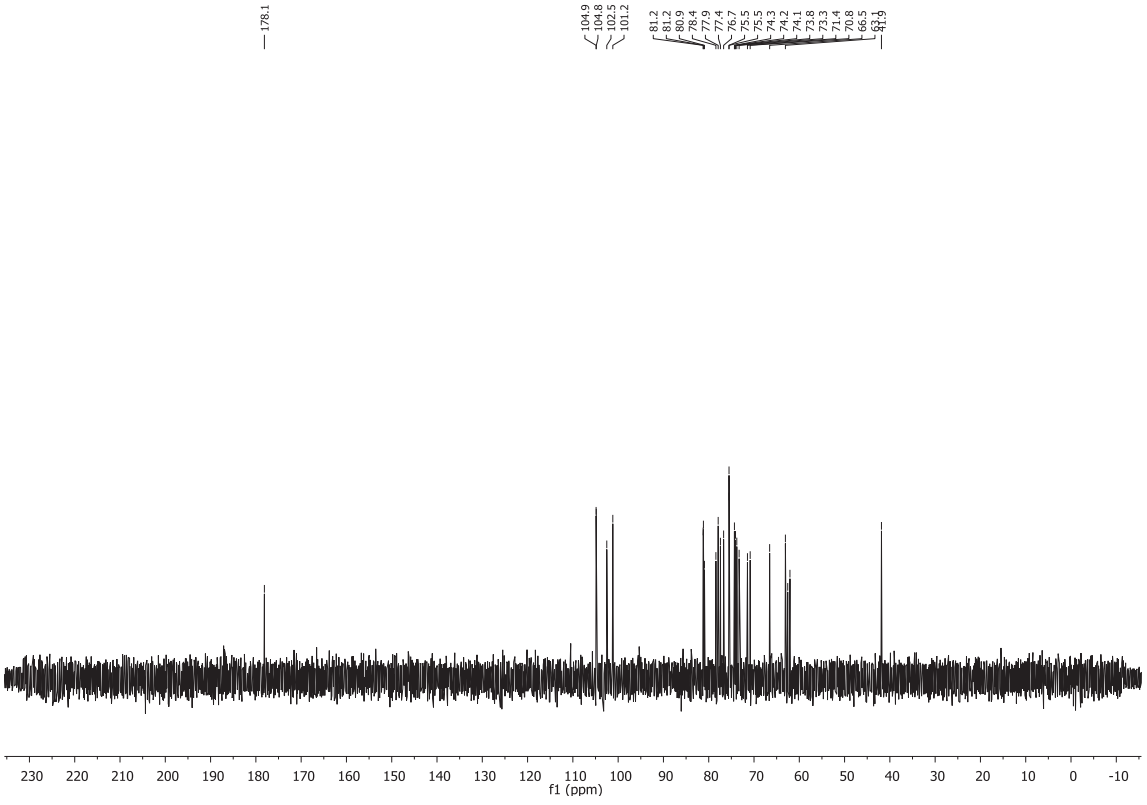
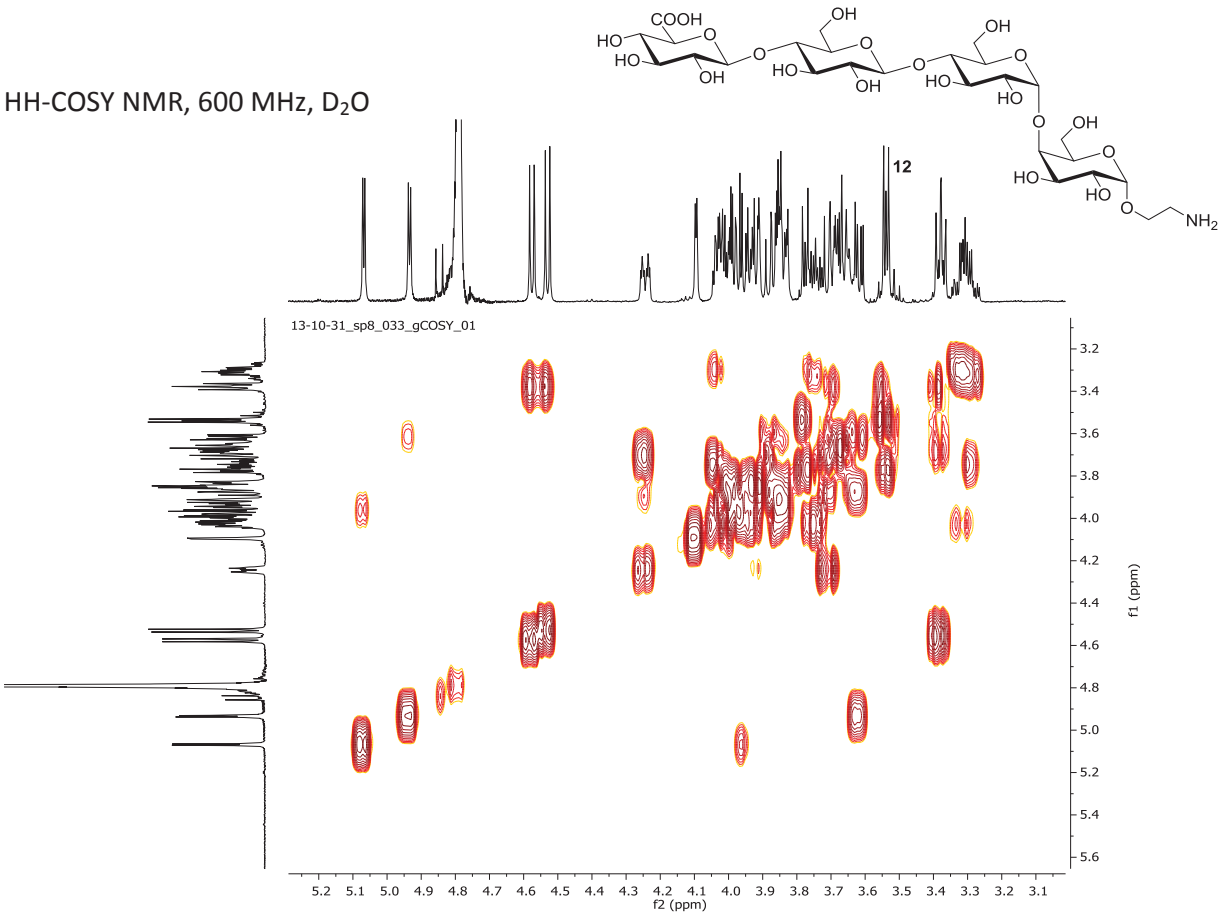
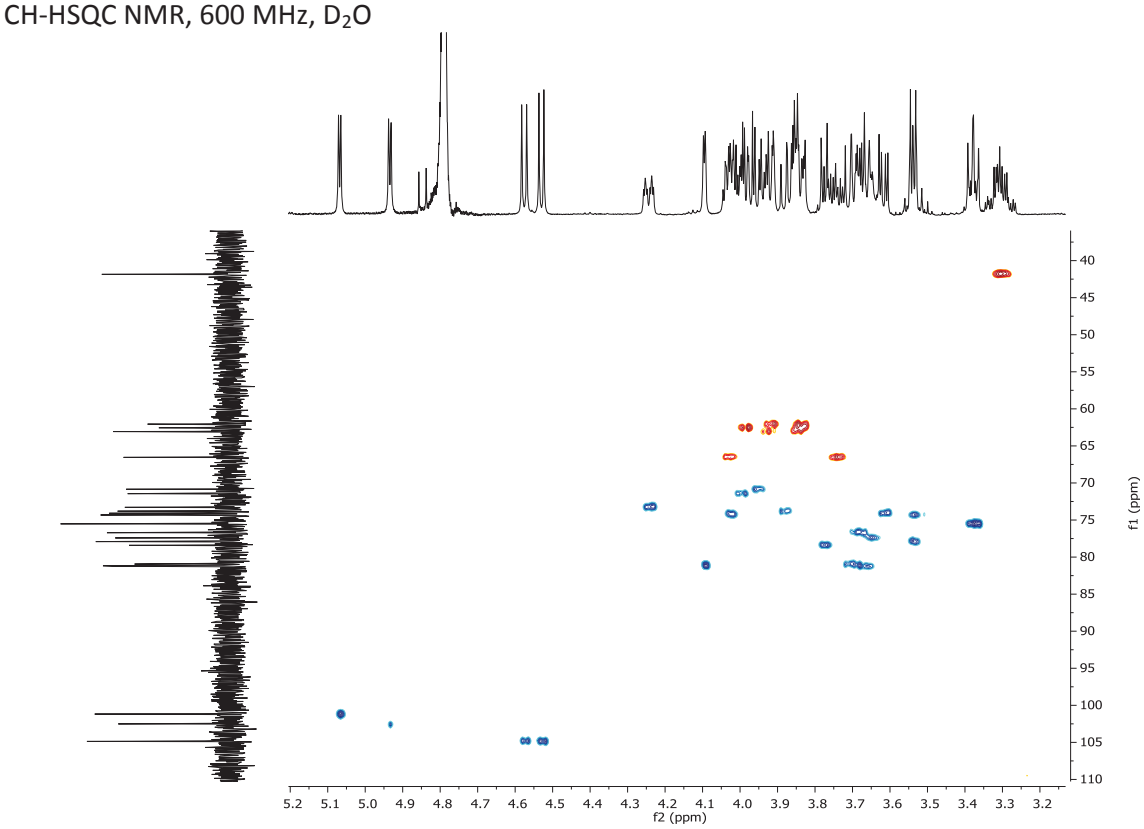
^{13}C NMR, 150 MHz, D_2O



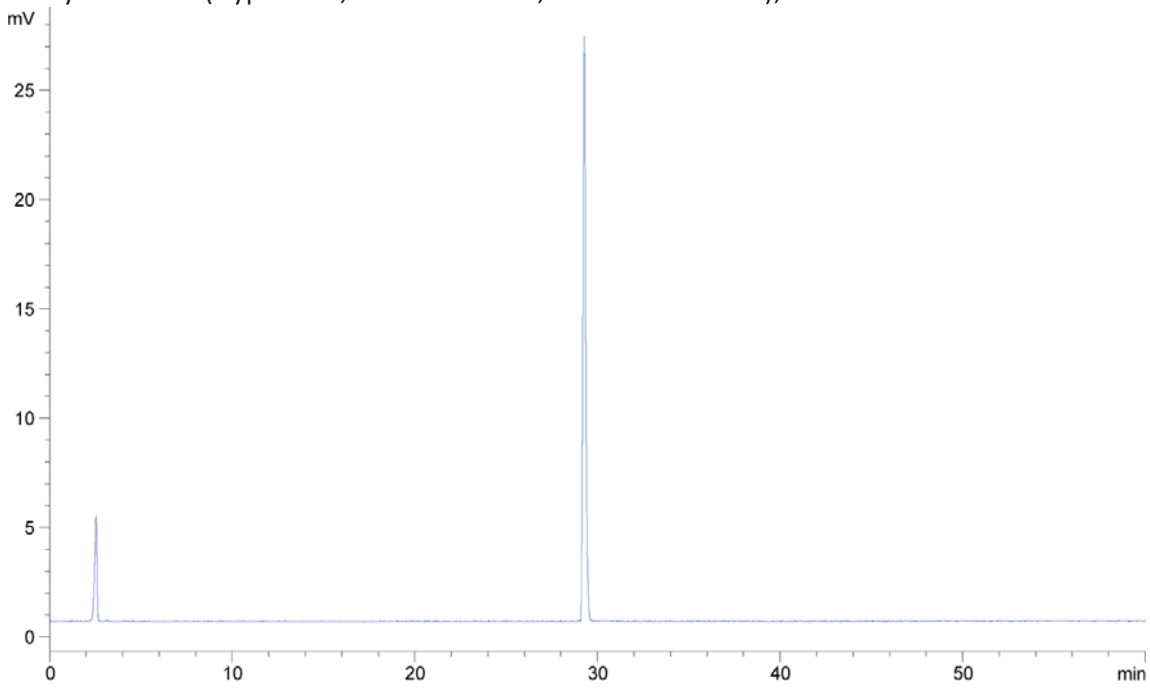
HH-COSY NMR, 600 MHz, D_2O

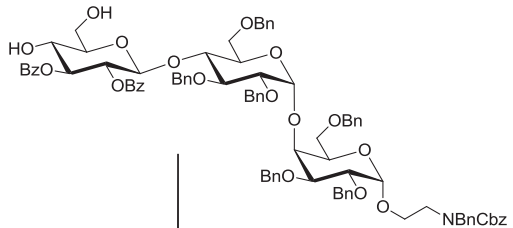


CH-HSQC NMR, 600 MHz, D_2O



Analytical HPLC (Hypercarb, 150 X 4.60 mm, Thermo Scientific); detector: ELSD





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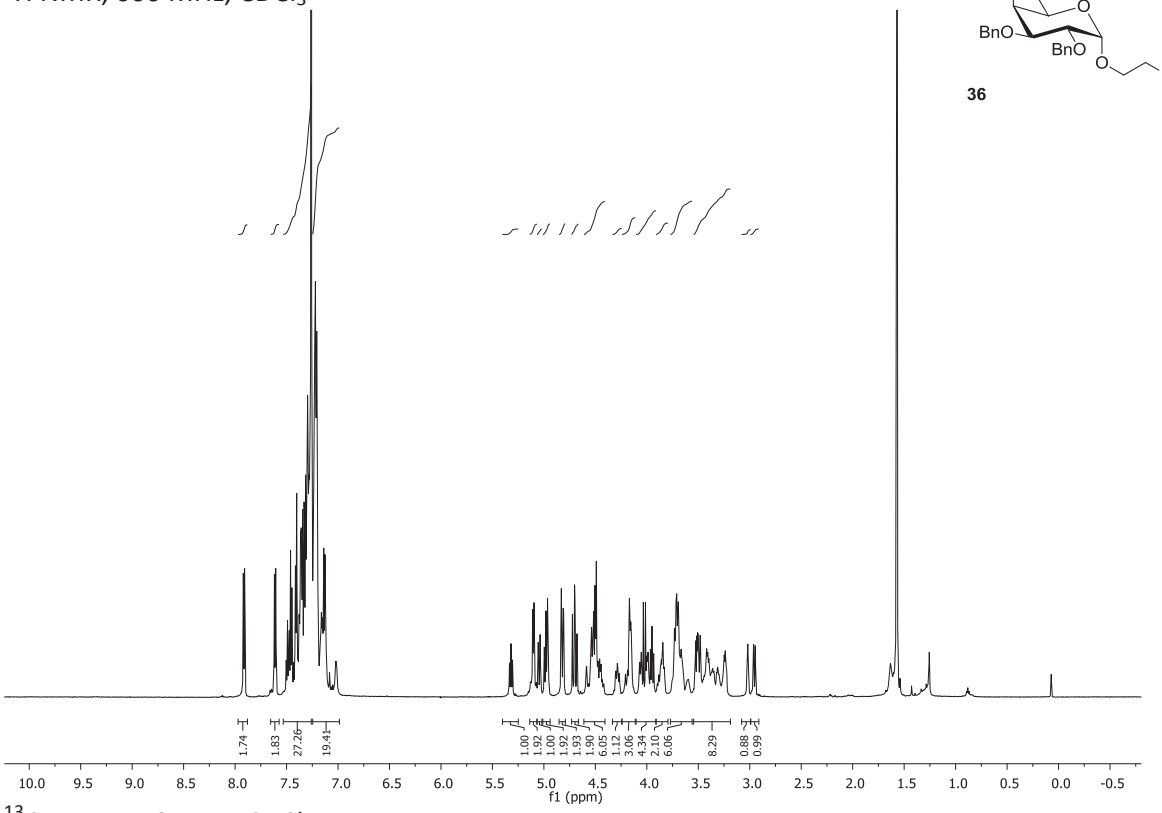
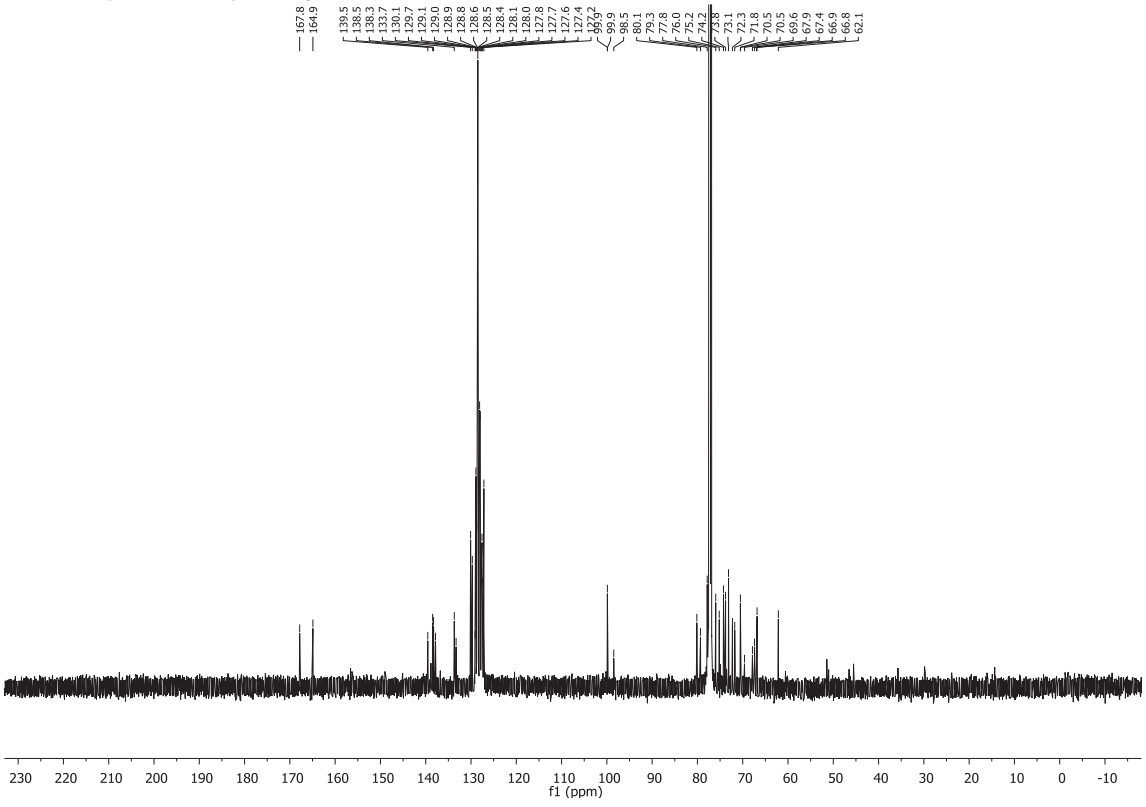
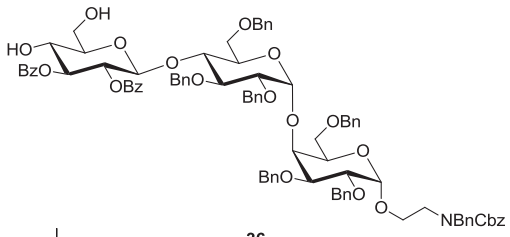
^{1}H NMR, 600 MHz, $CDCl_3$



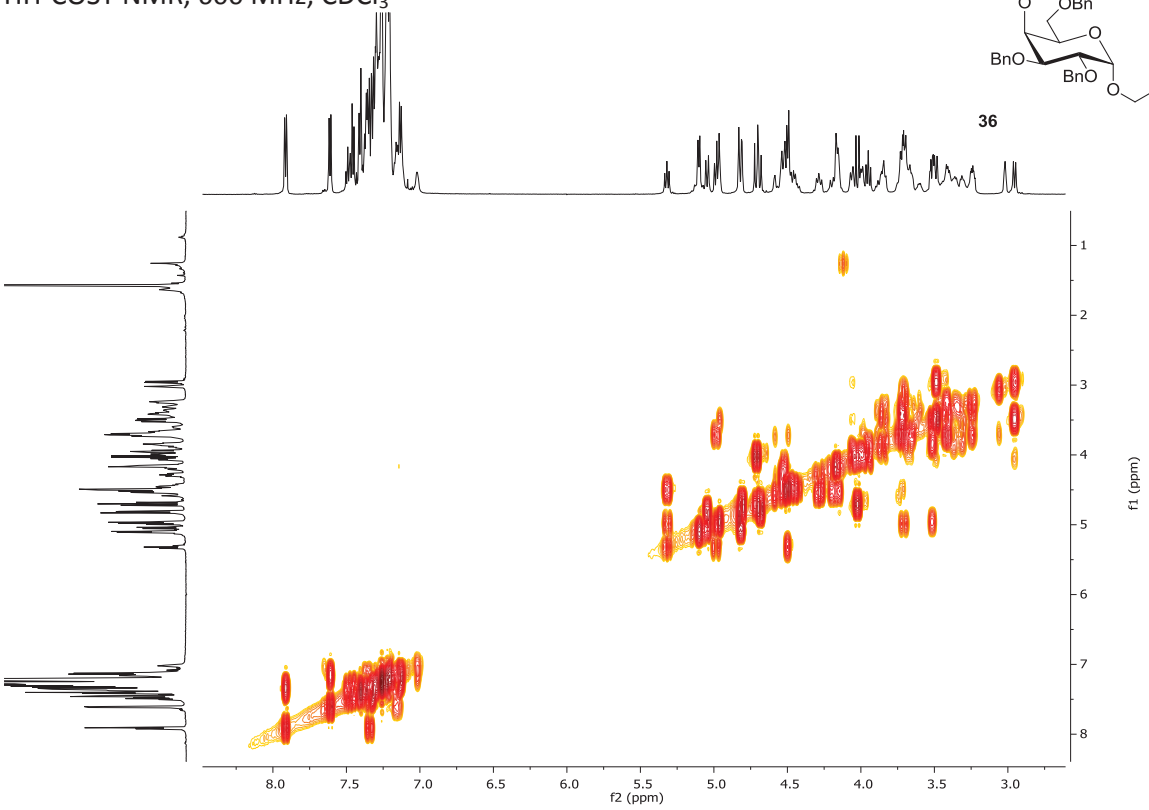
^{13}C NMR, 150 MHz, $CDCl_3$



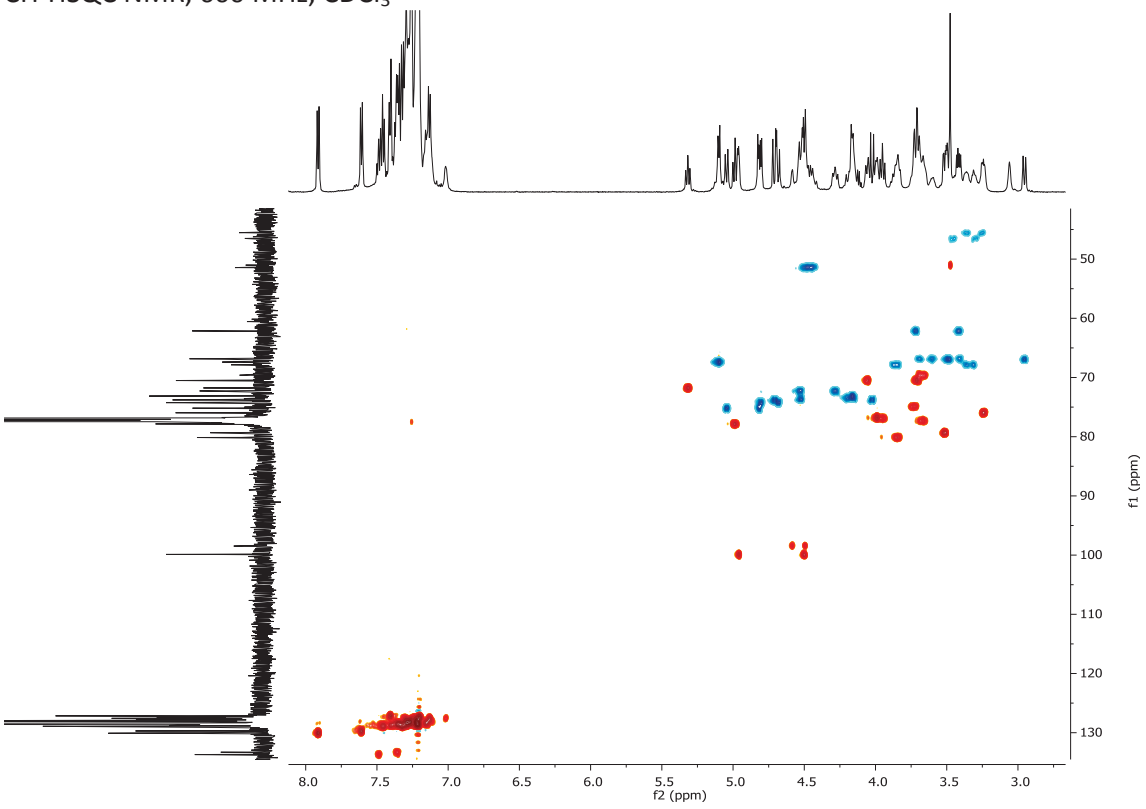


36

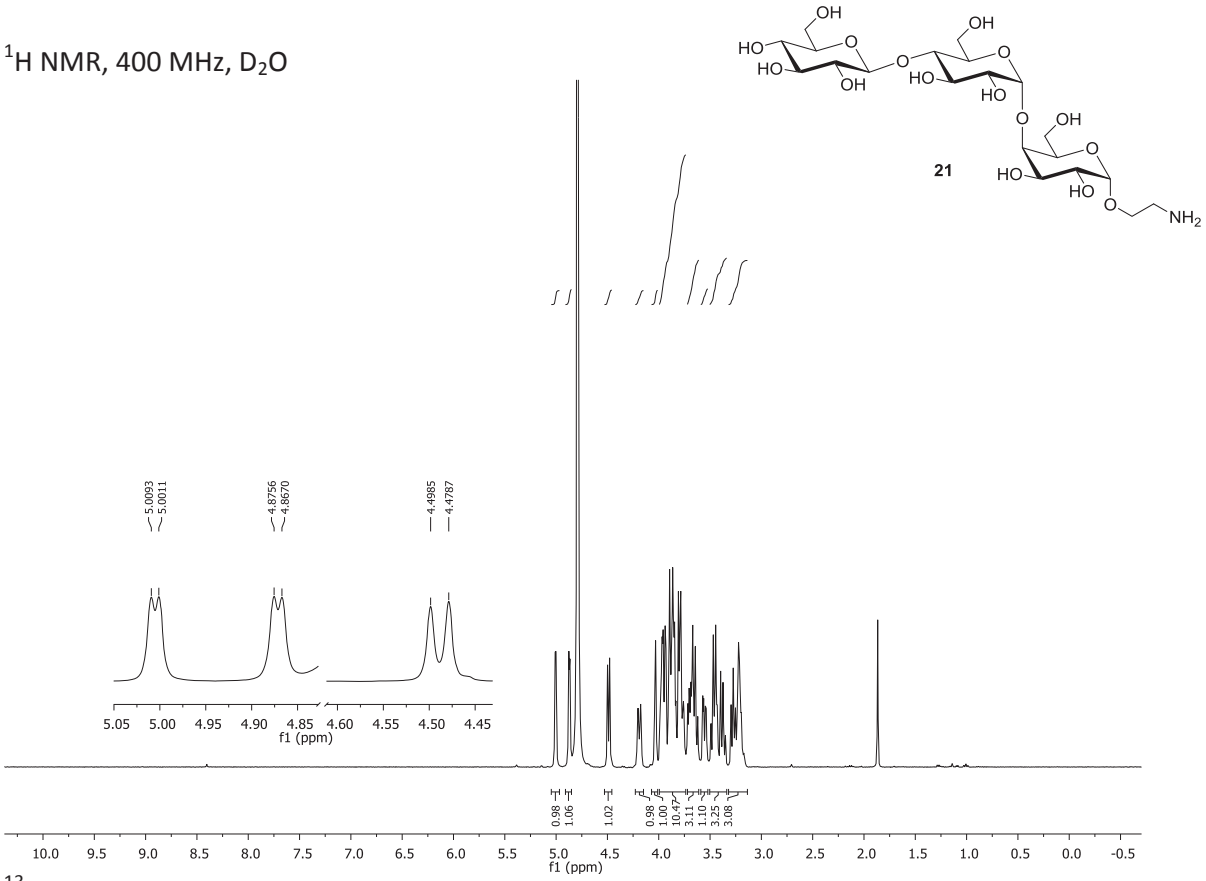
HH-COSY NMR, 600 MHz, $CDCl_3$



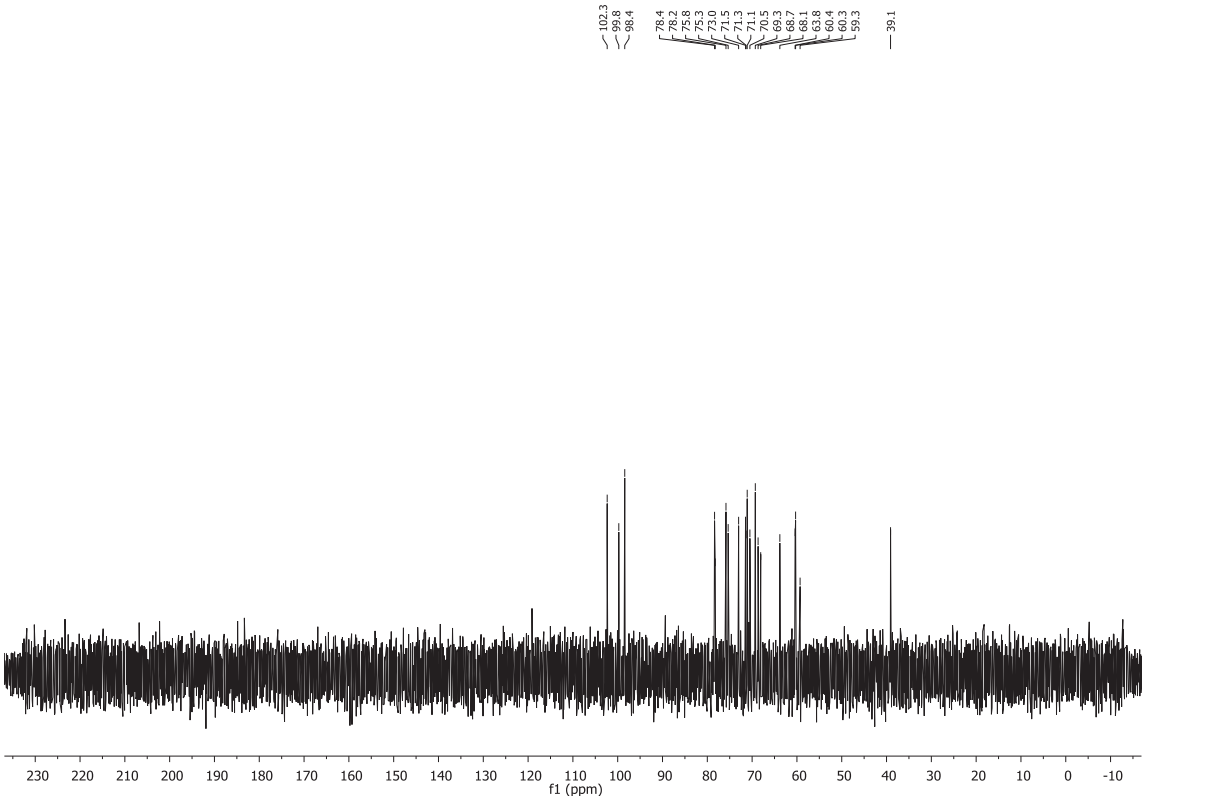
CH-HSQC NMR, 600 MHz, $CDCl_3$



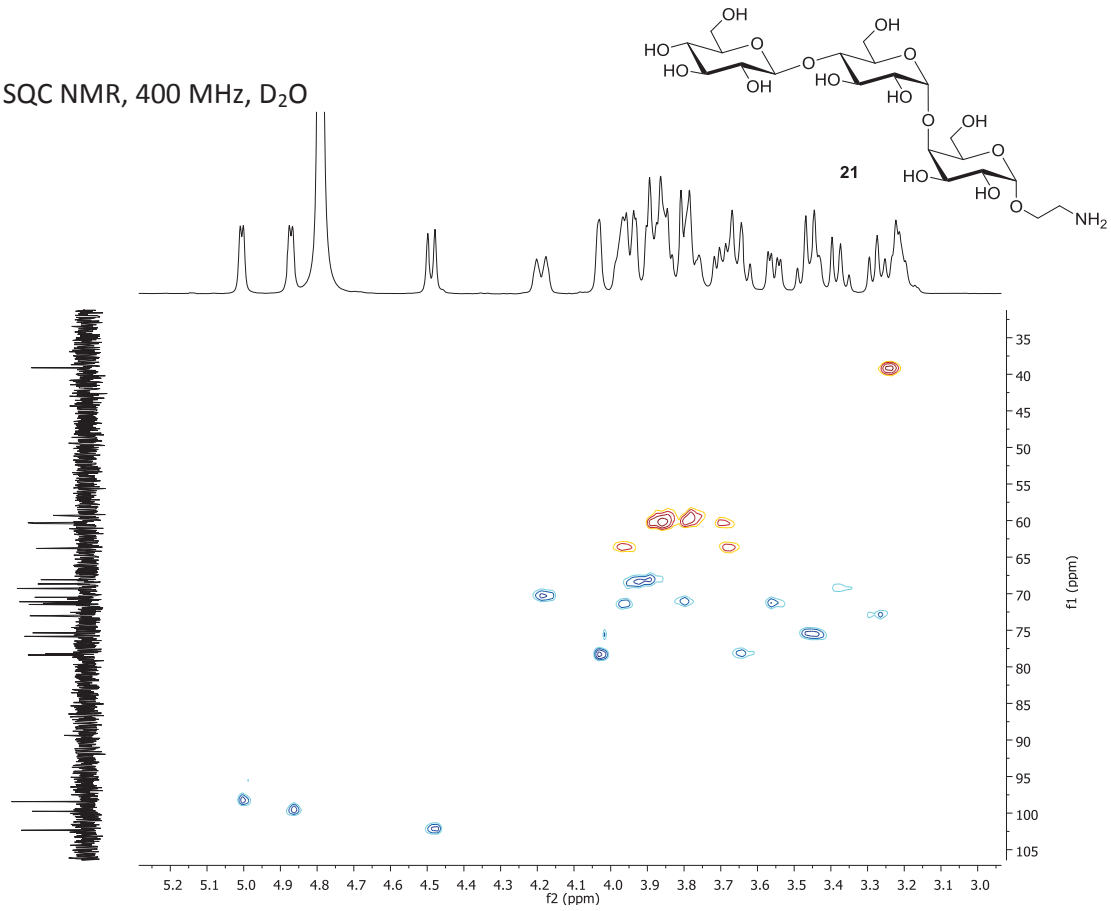
^{1}H NMR, 400 MHz, D_2O



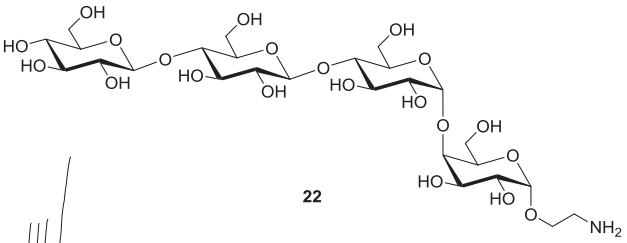
^{13}C NMR, 100 MHz, D_2O



CH-HSQC NMR, 400 MHz, D_2O



^{1}H NMR, 600 MHz, D_2O



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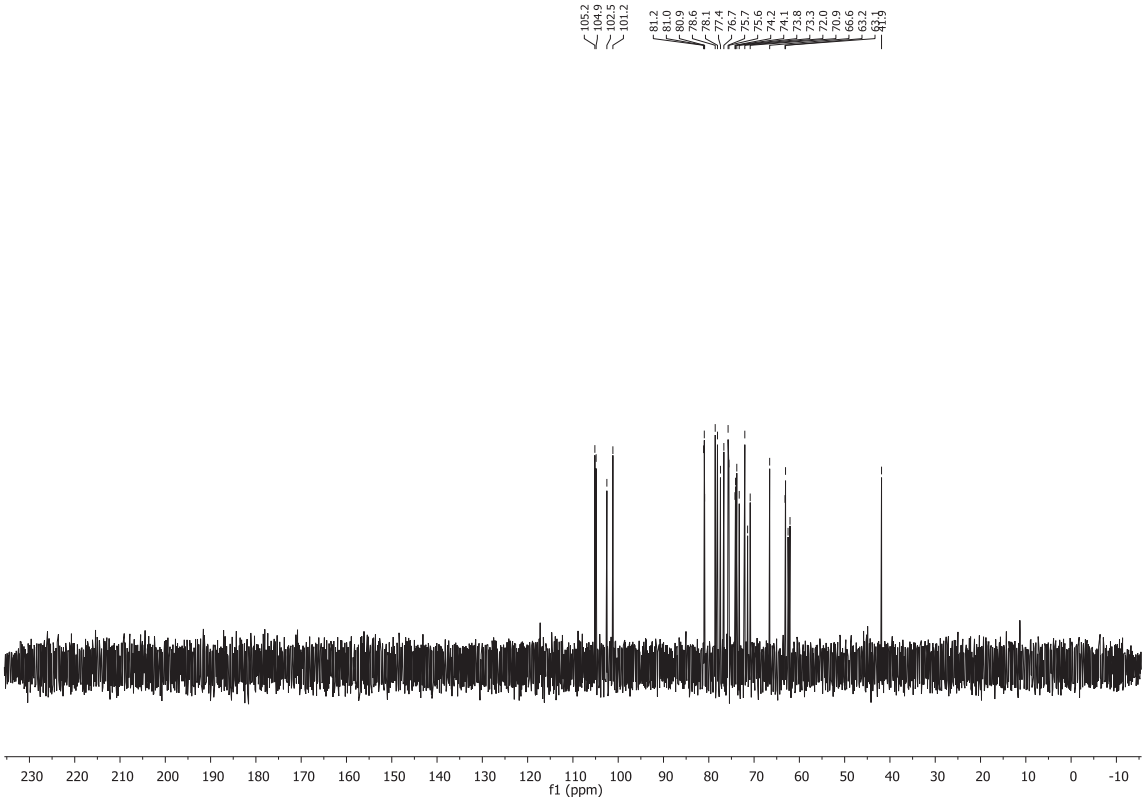
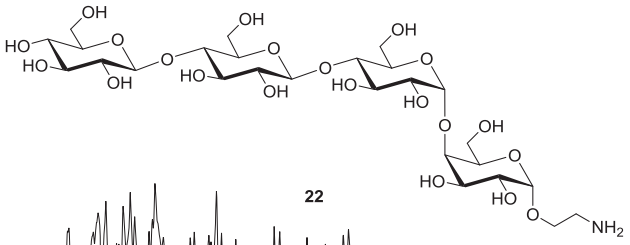
^{13}C NMR, 150 MHz, D_2O



CH-HSQC NMR, 600 MHz, D_2O



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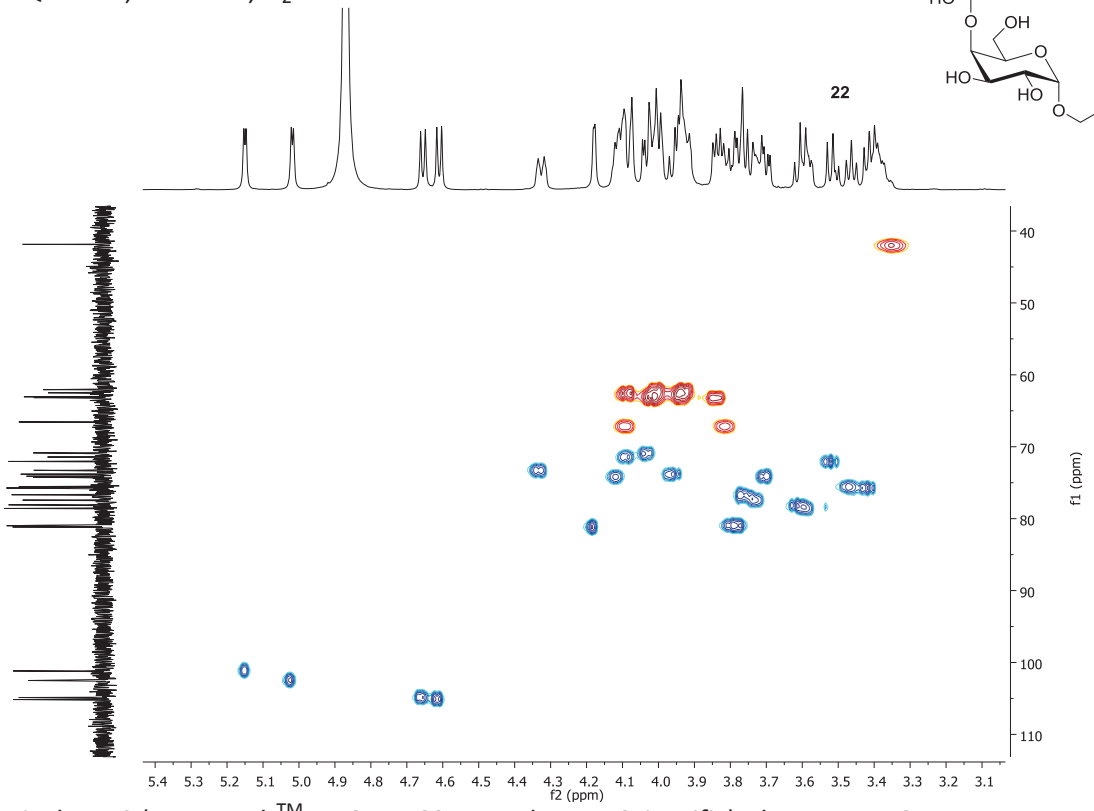
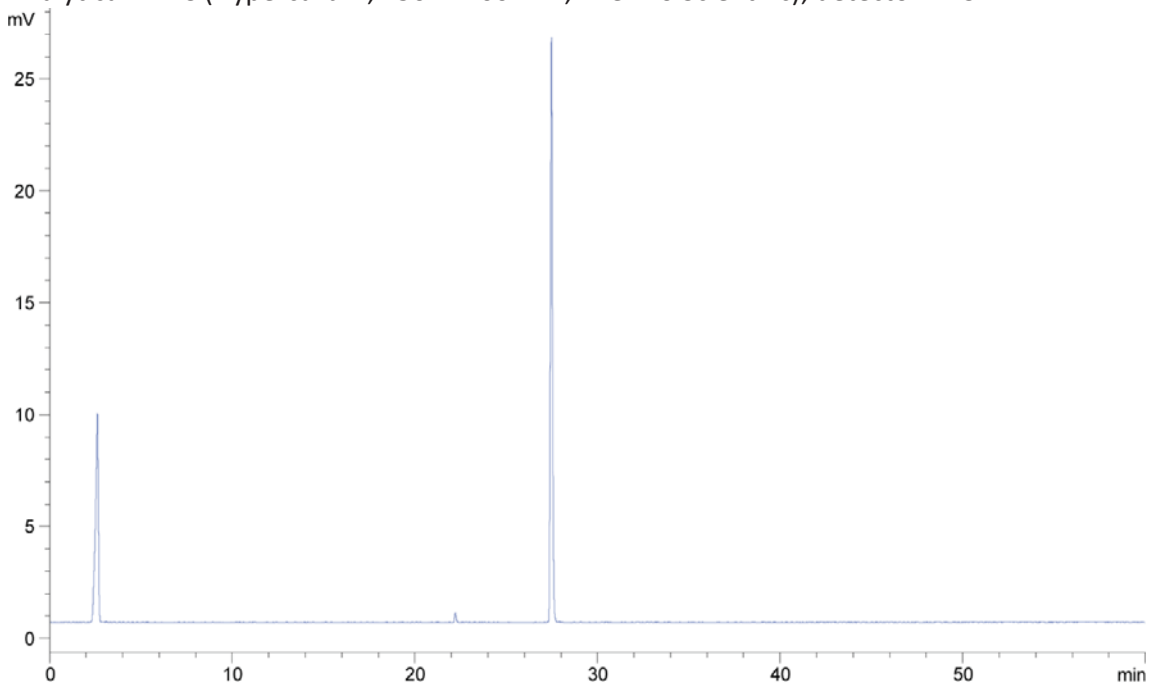
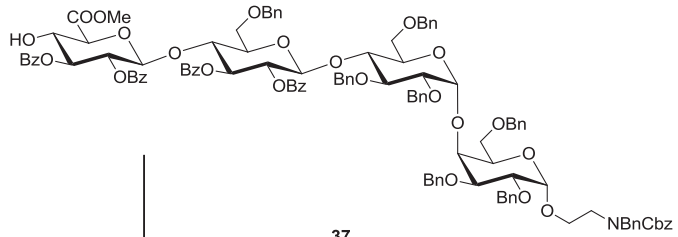


Analytical HPLC (Hypercarb^{TM}, 150 X 4.60 mm, Thermo Scientific); detector: ELSD



^{1}H NMR, 600 MHz, $CDCl_3$



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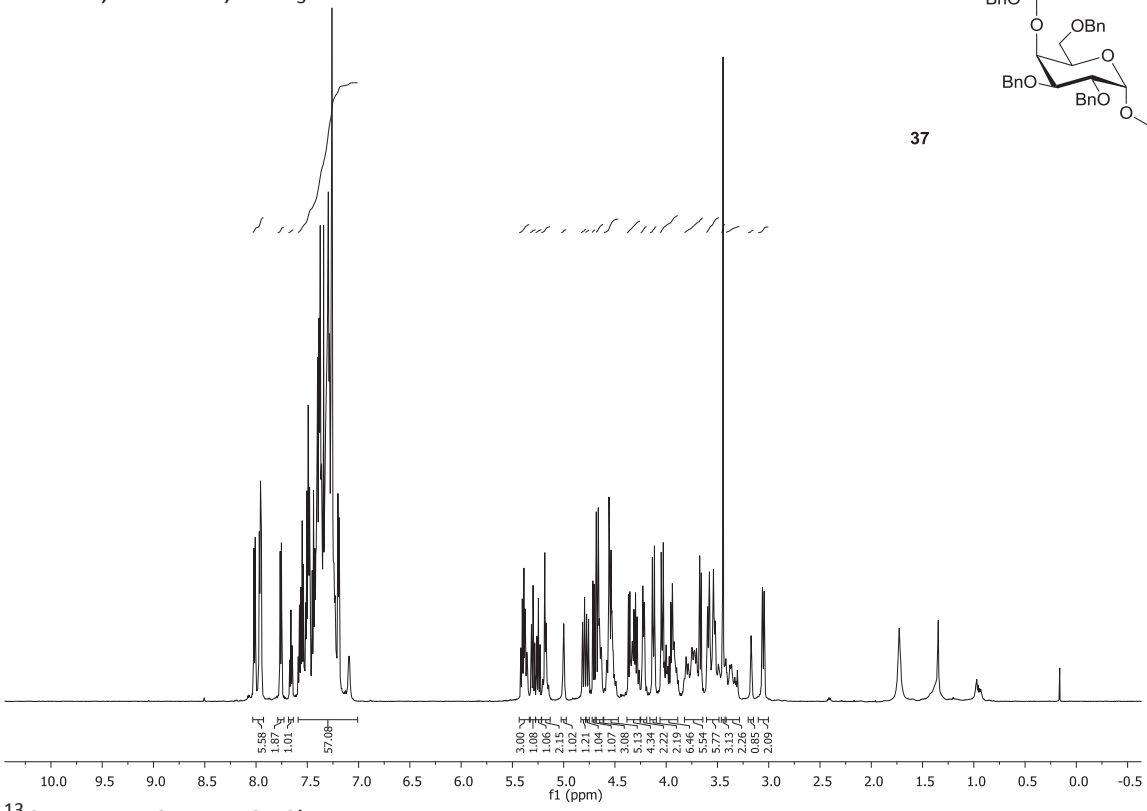
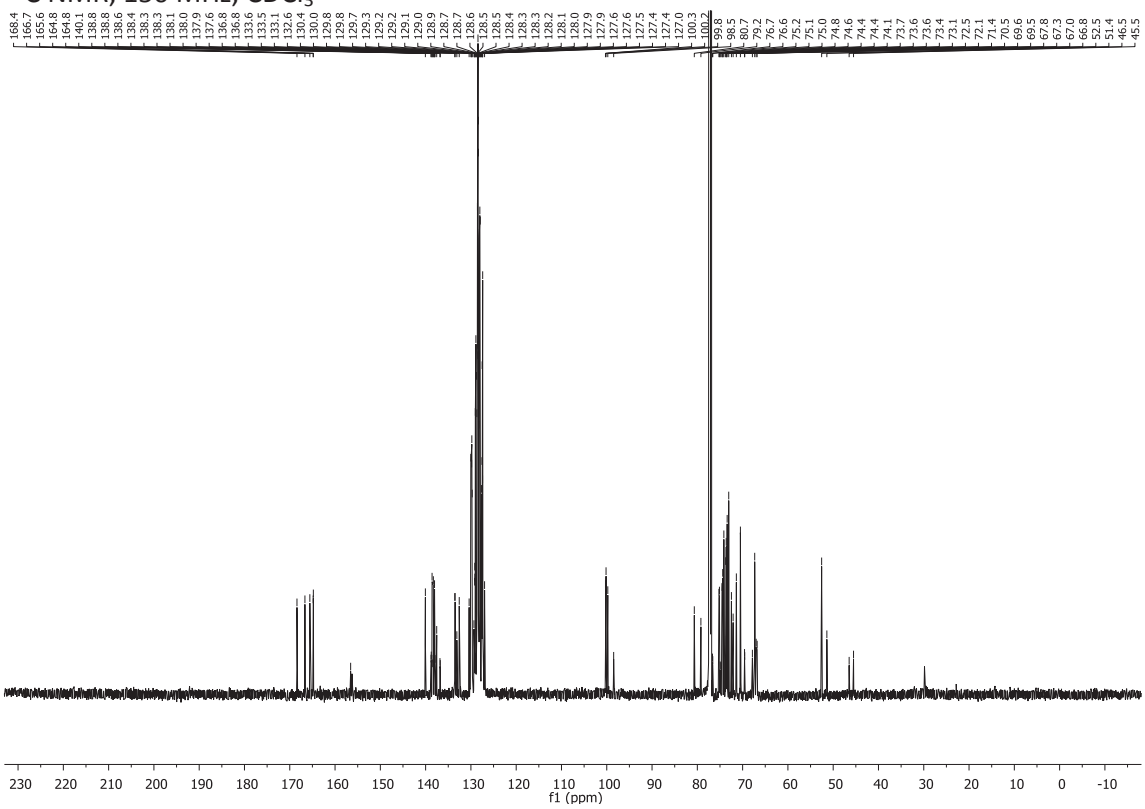
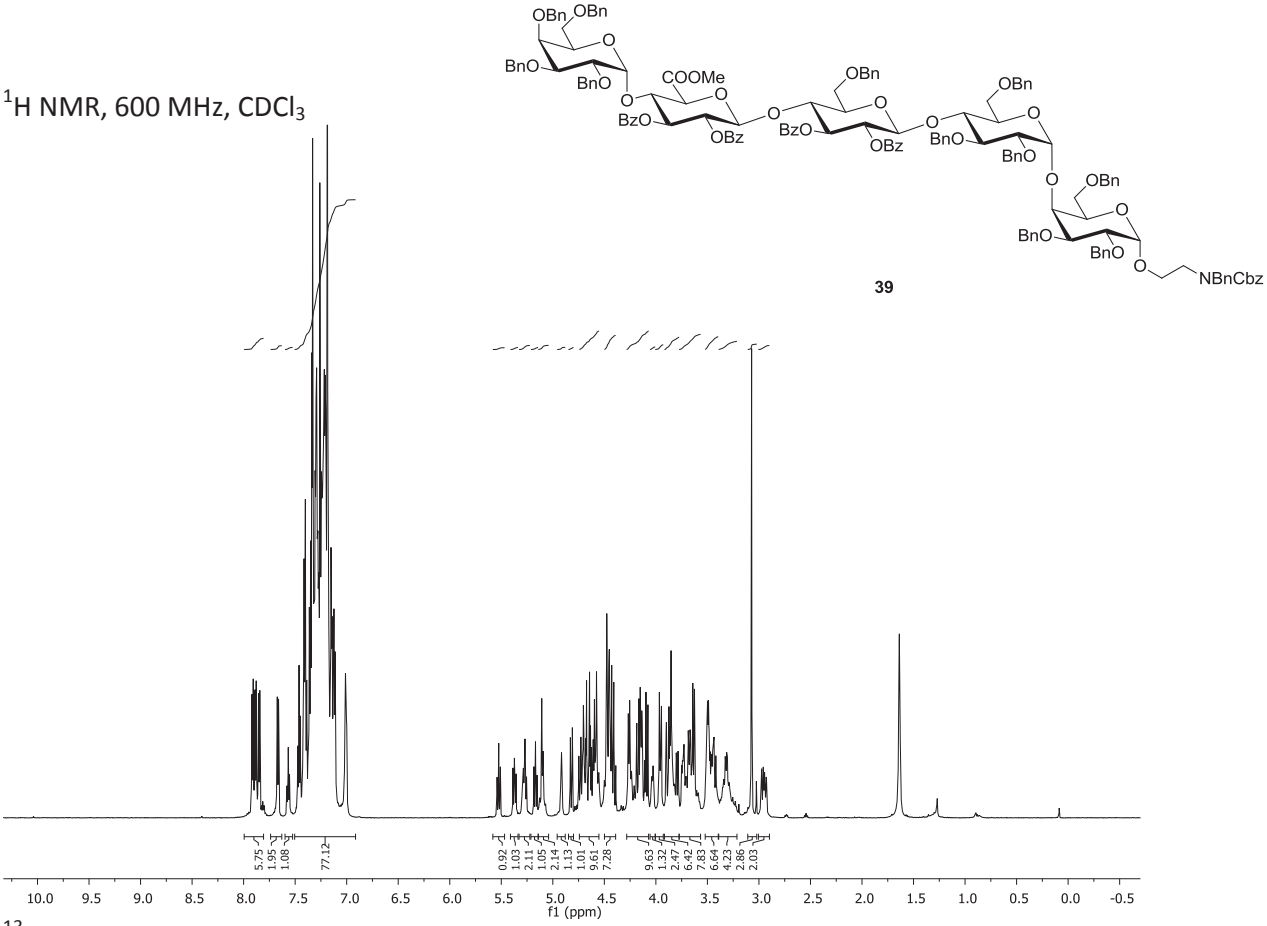


^{13}C NMR, 150 MHz, $CDCl_3$



^{1}H NMR, 600 MHz, $CDCl_3$



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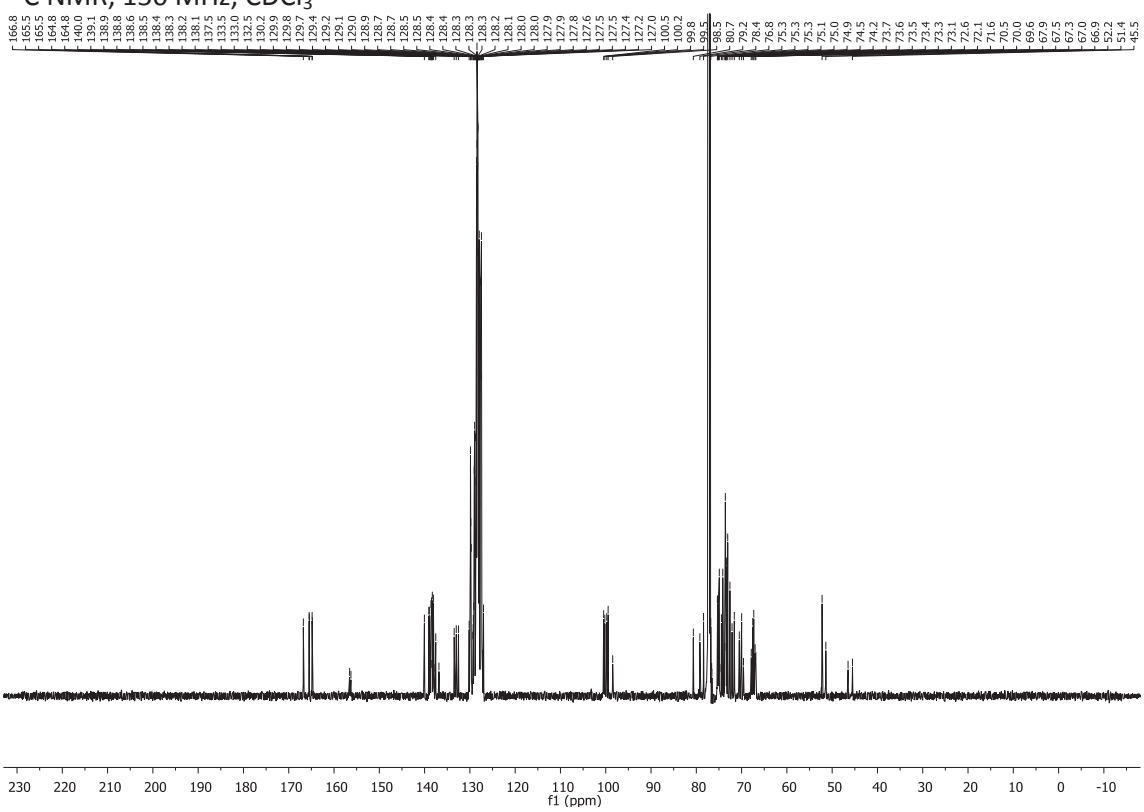
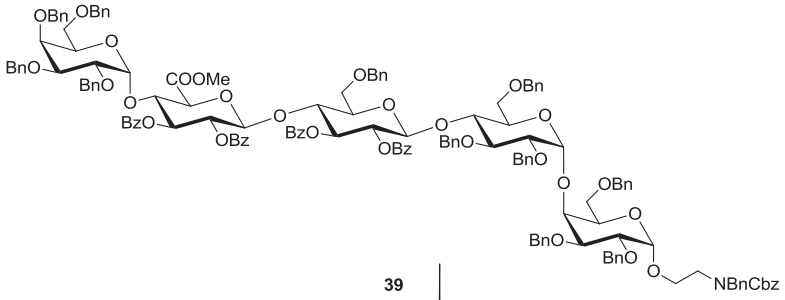
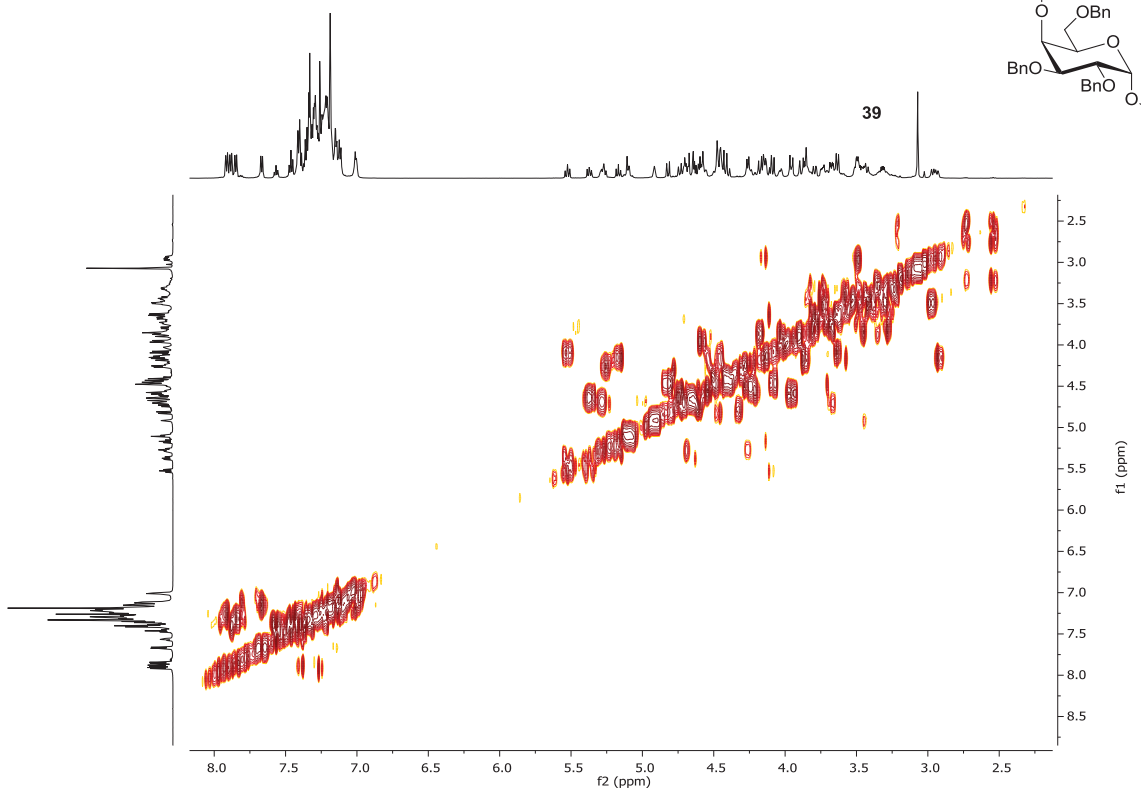
^{13}C NMR, 150 MHz, $CDCl_3$

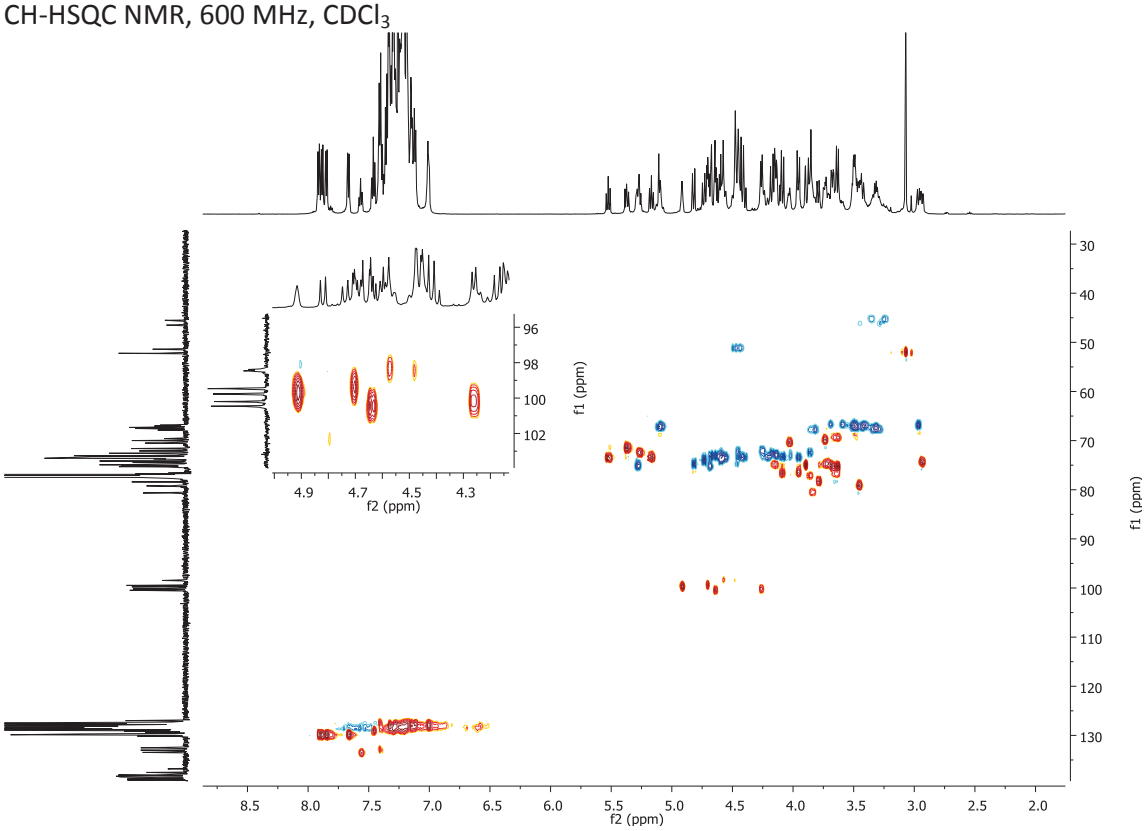




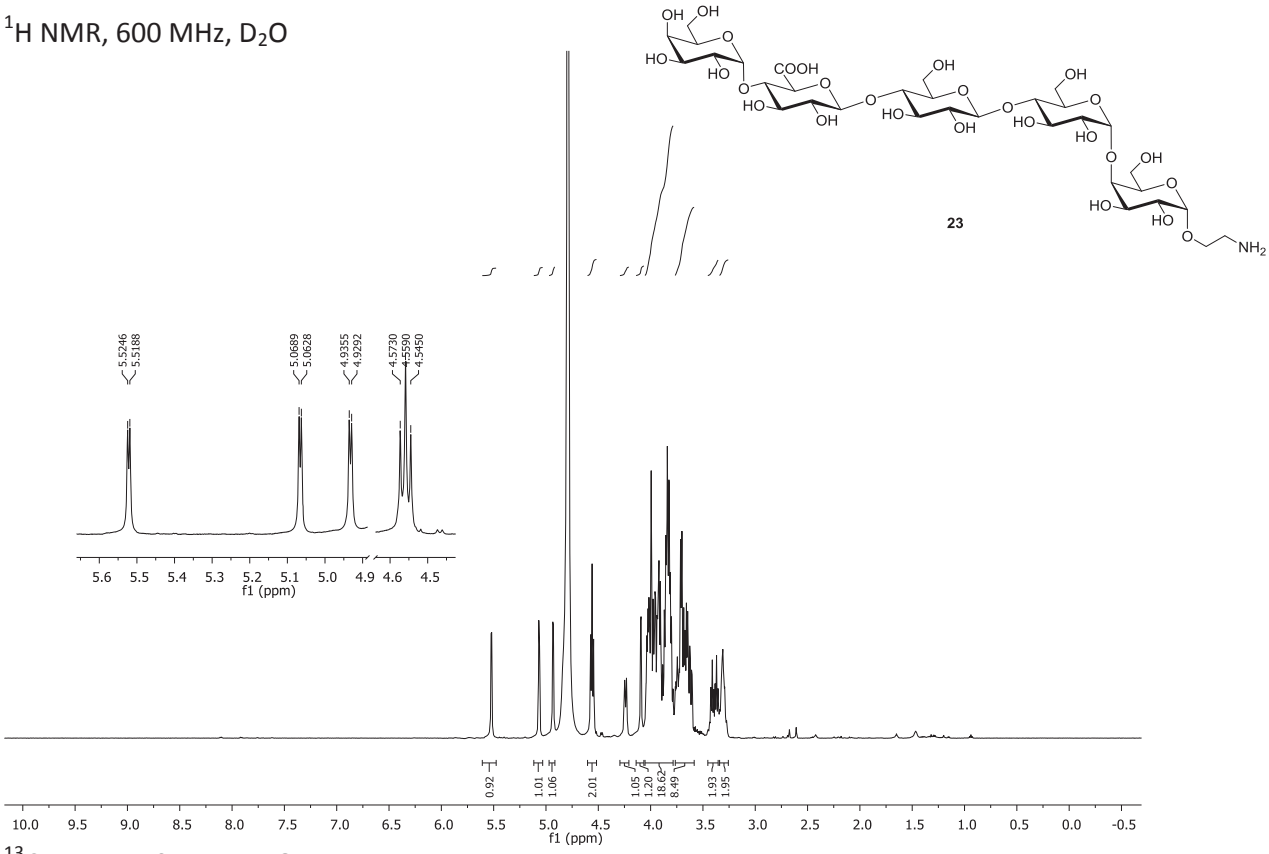
HH-COSY NMR, 600 MHz, $CDCl_3$



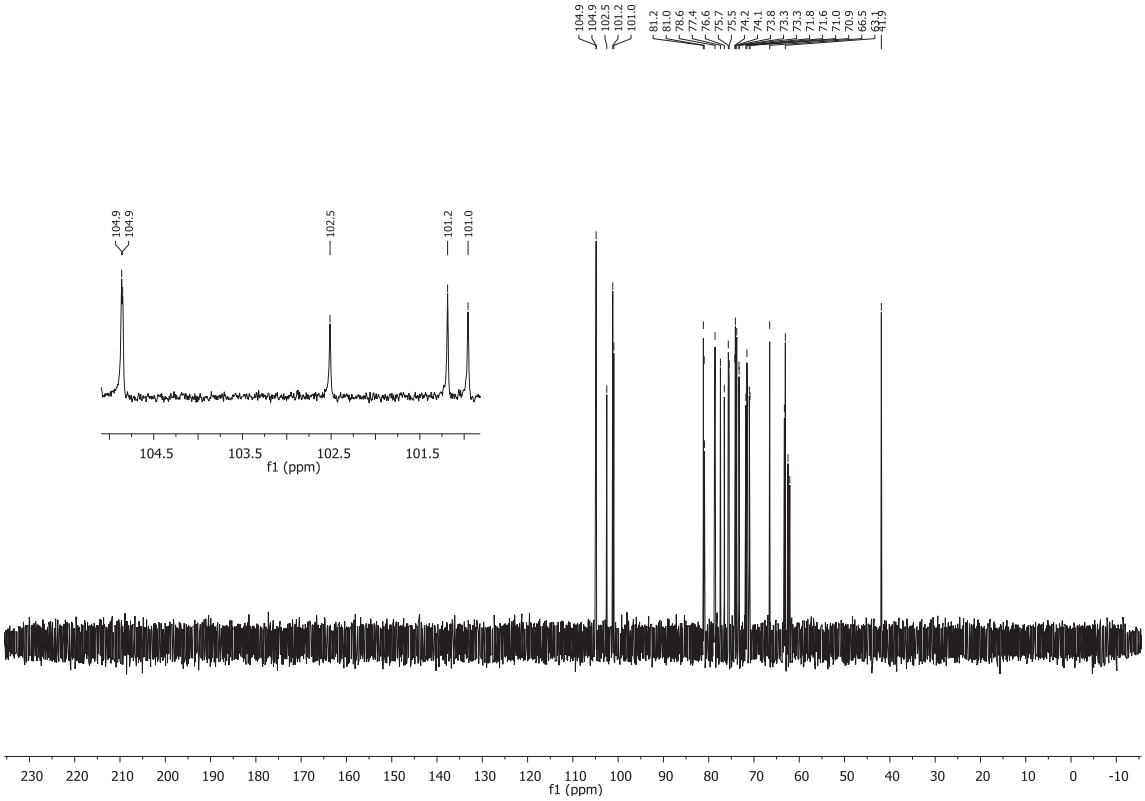
CH-HSQC NMR, 600 MHz, $CDCl_3$



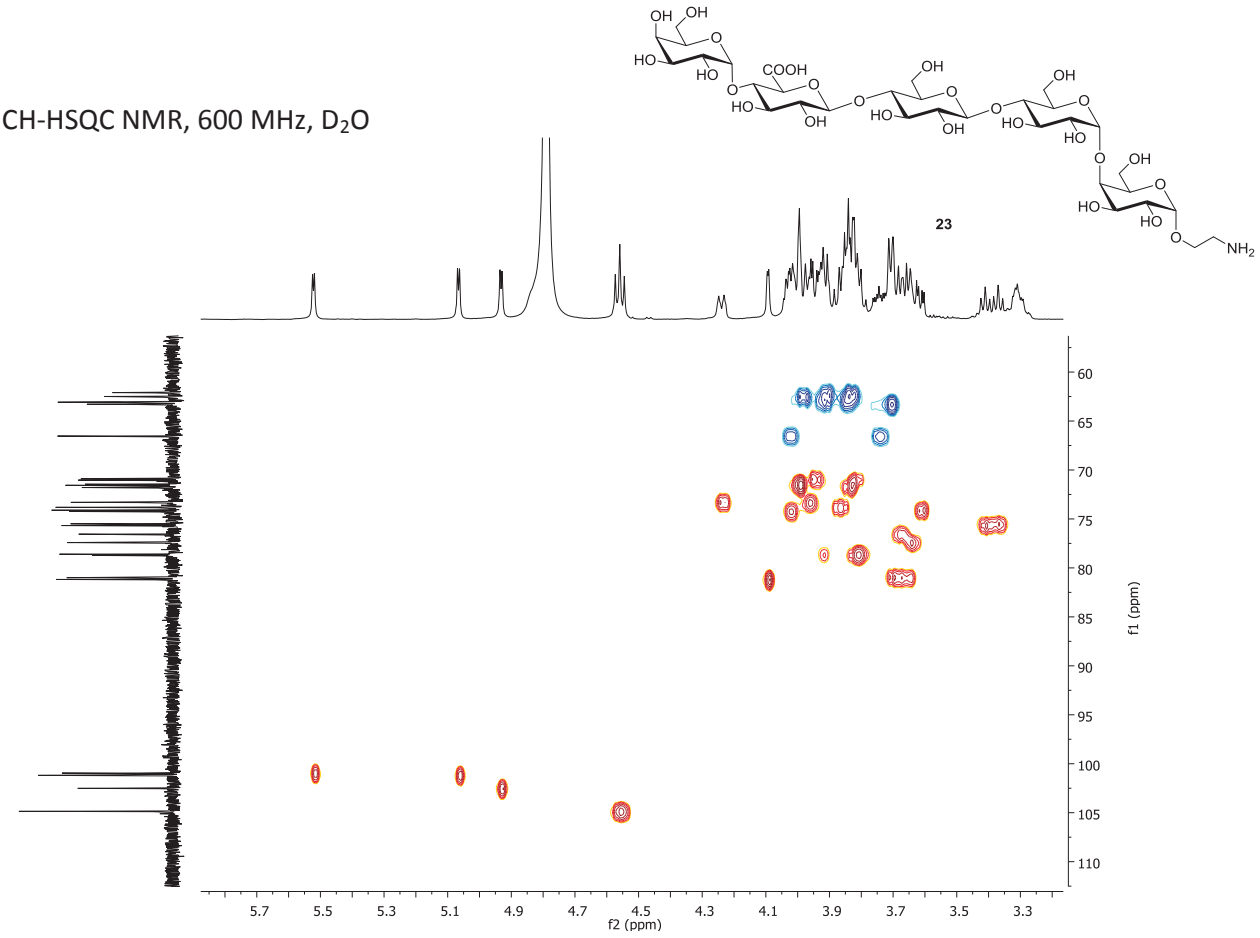
^{1}H NMR, 600 MHz, D_2O



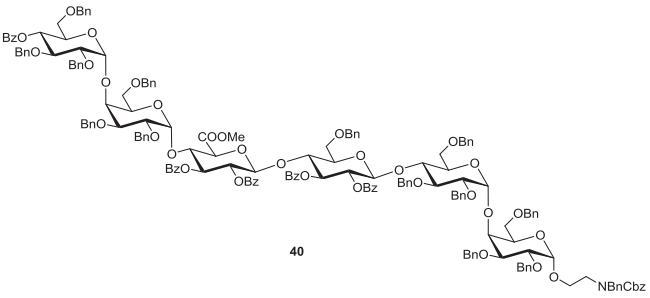
^{13}C NMR, 150 MHz, D_2O



CH-HSQC NMR, 600 MHz, D_2O



^{1}H NMR, 600 MHz, $CDCl_3$



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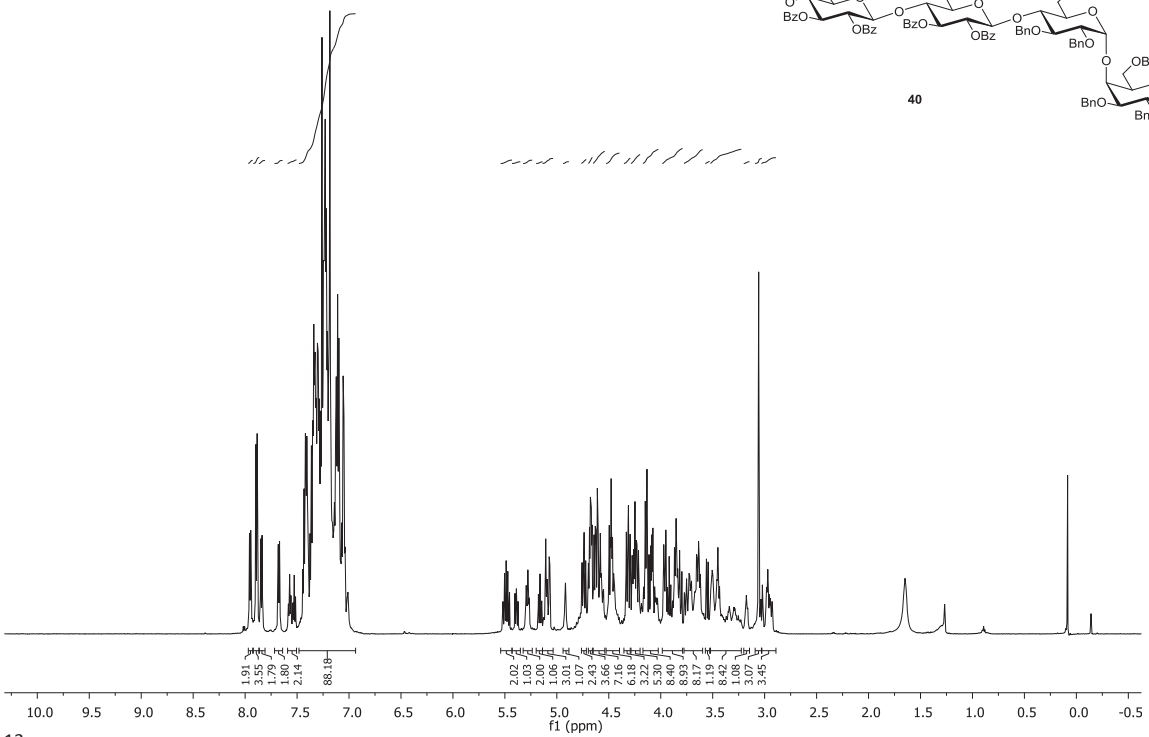
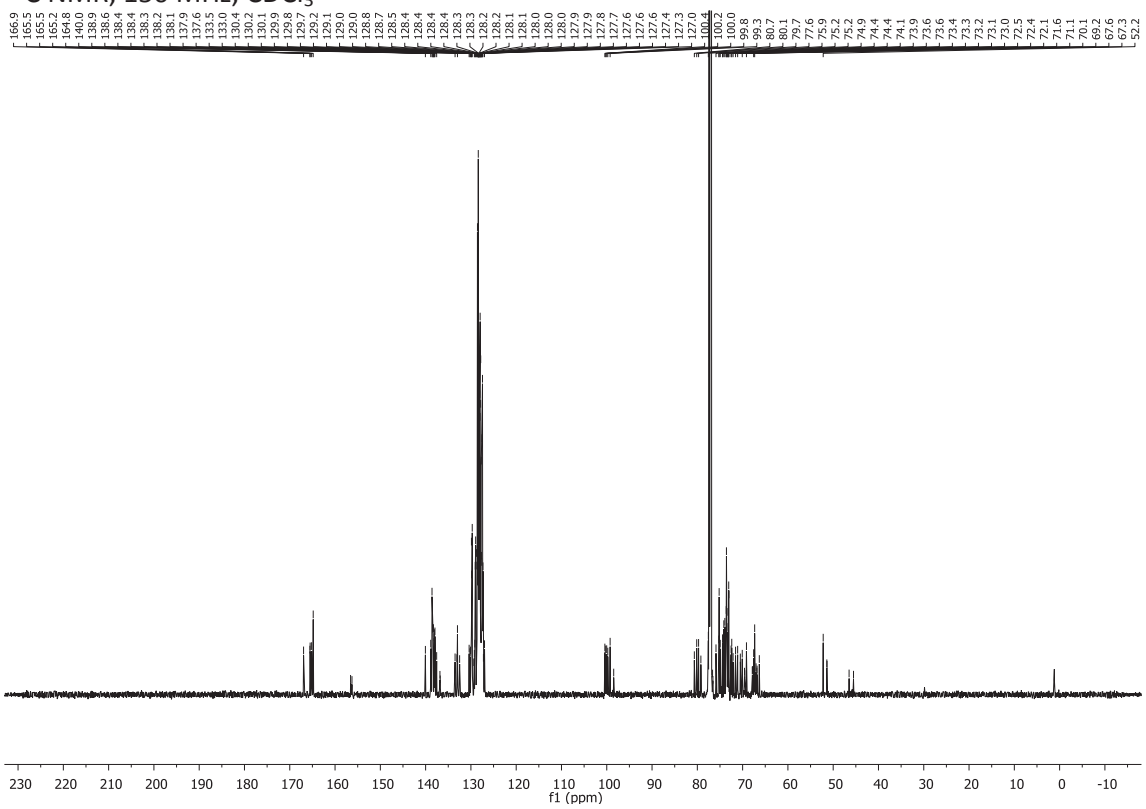
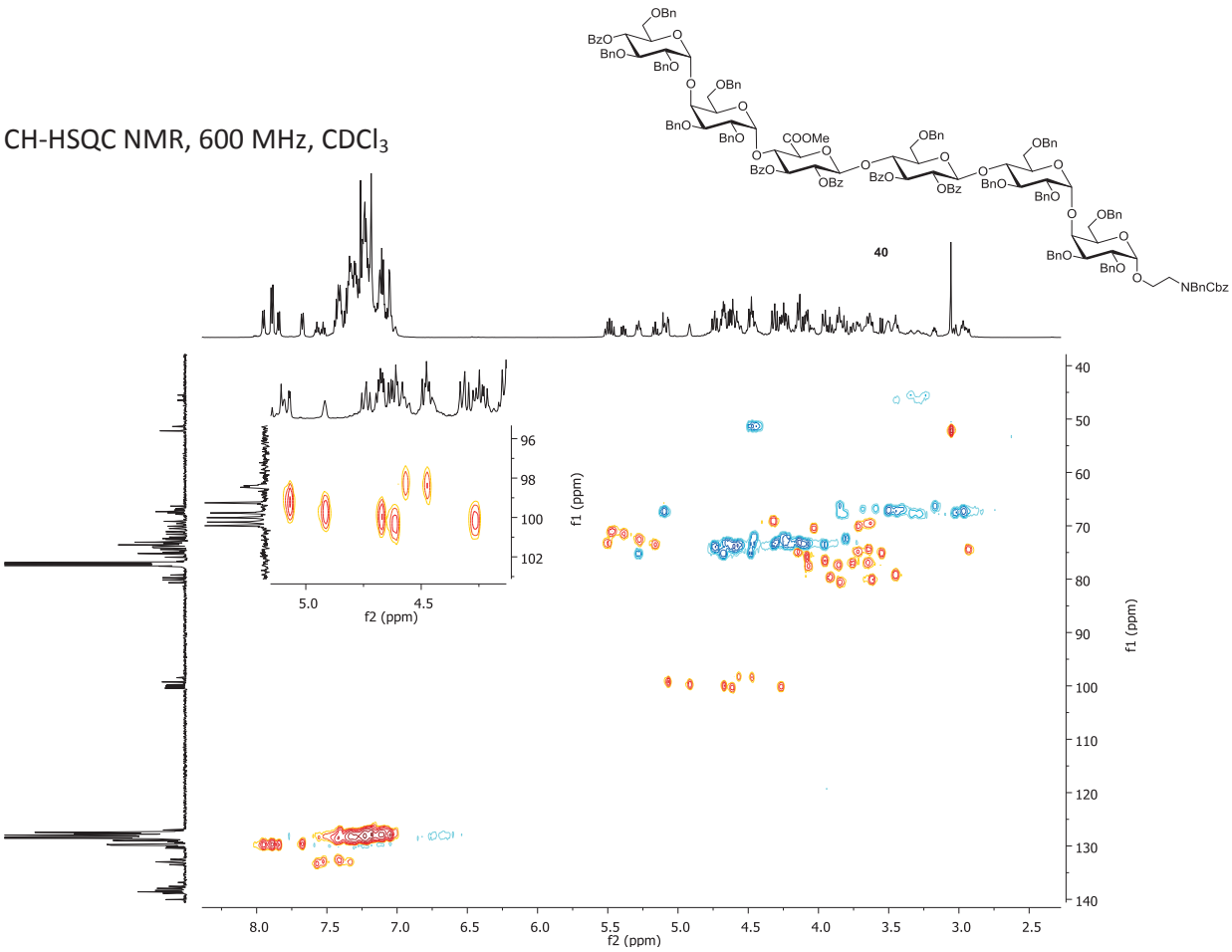


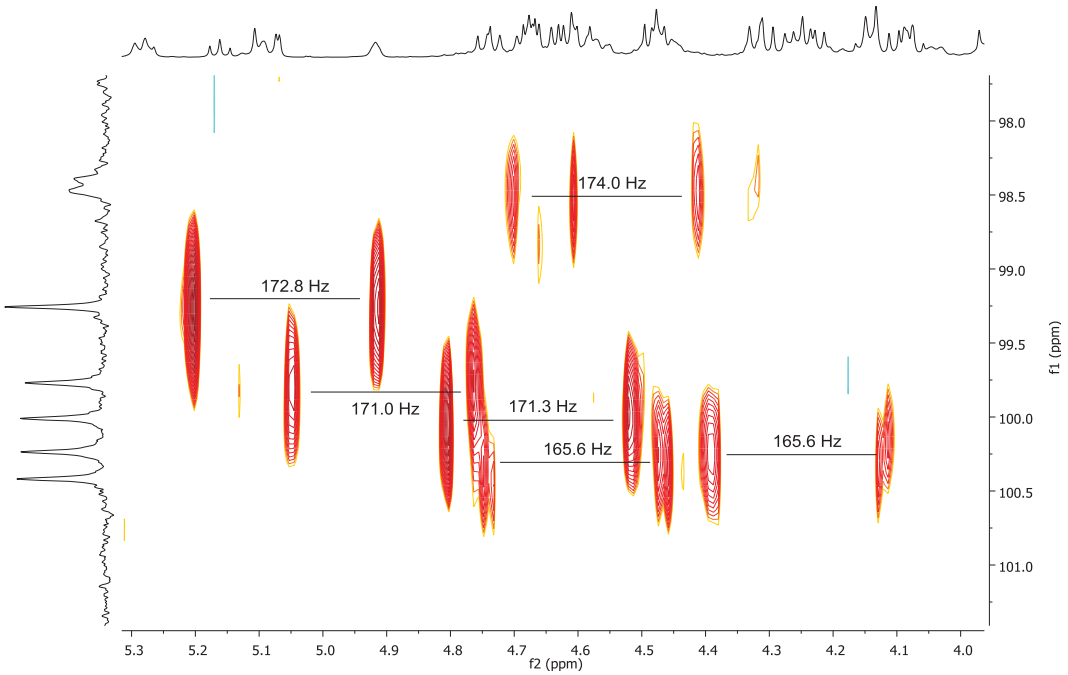
^{13}C NMR, 150 MHz, $CDCl_3$



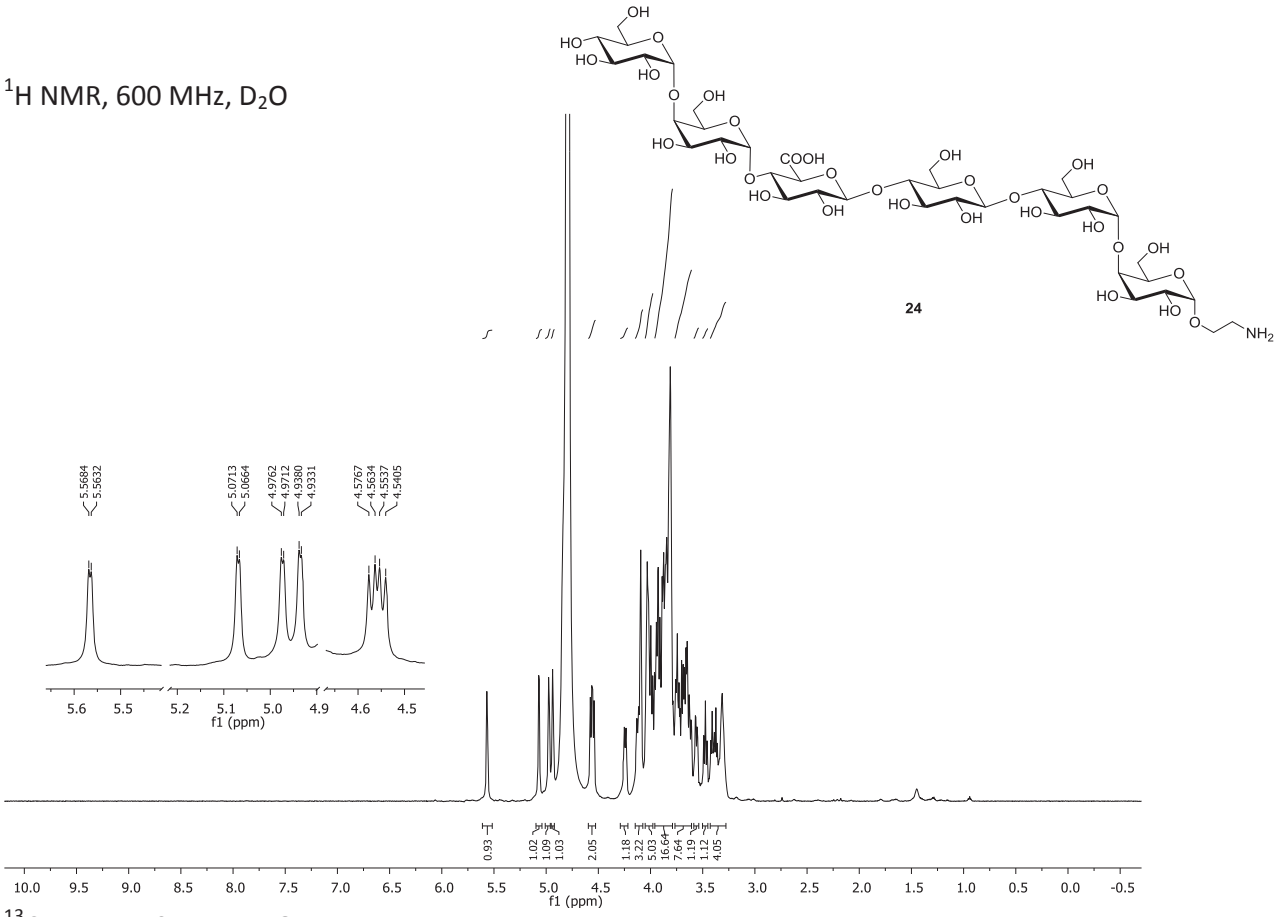
CH-HSQC NMR, 600 MHz, $CDCl_3$



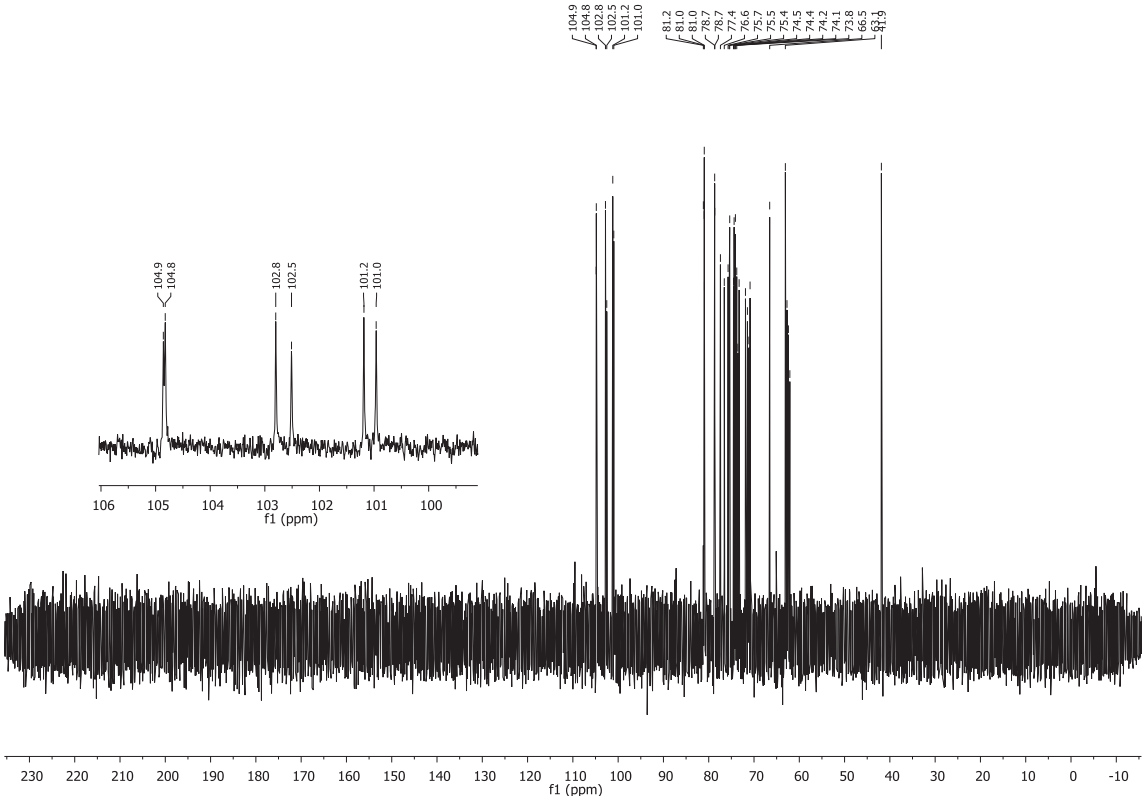
CH-coupled HSQC NMR, 600 MHz, $CDCl_3$



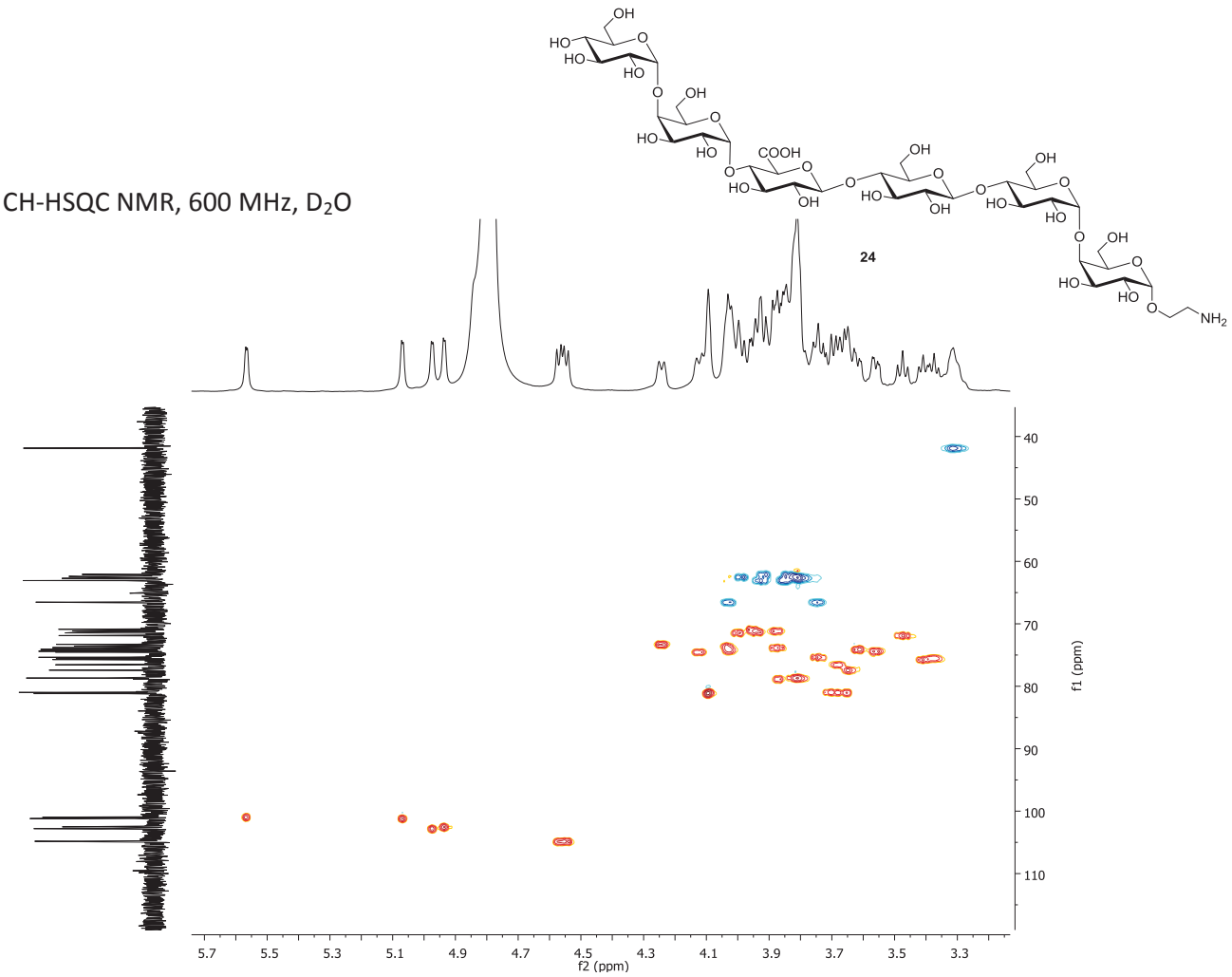
^{1}H NMR, 600 MHz, D_2O



^{13}C NMR, 150 MHz, D_2O



CH-HSQC NMR, 600 MHz, D_2O



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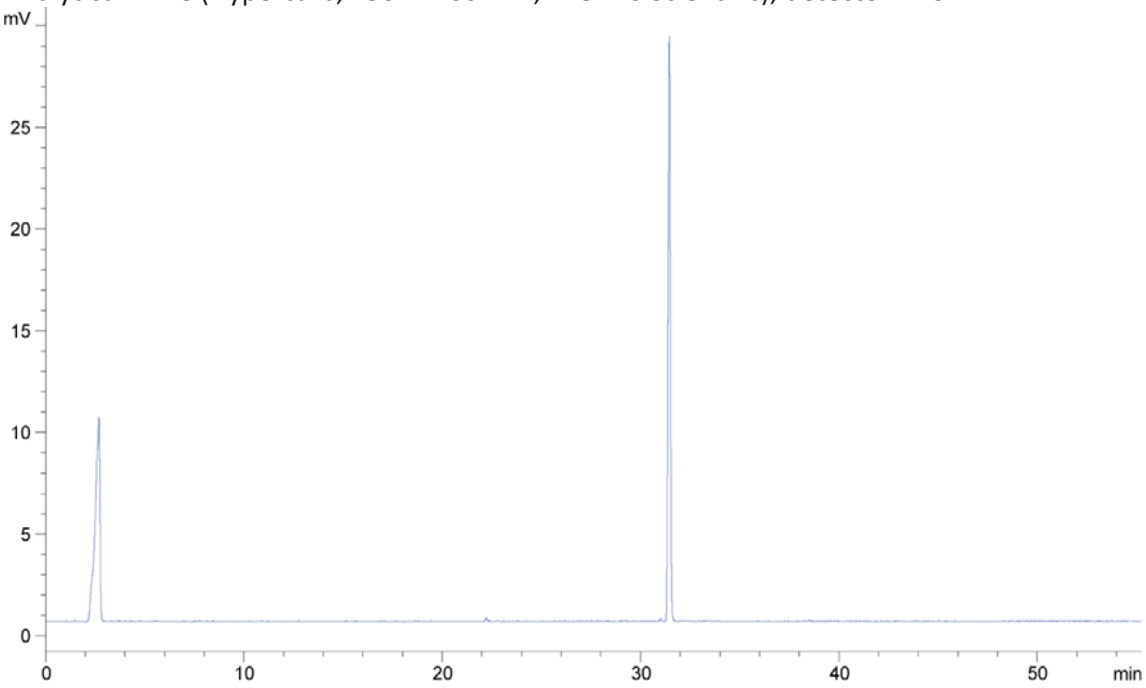
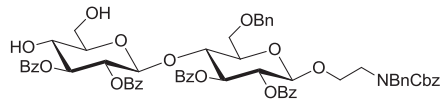


^{1}H NMR, 400 MHz, $CDCl_3$



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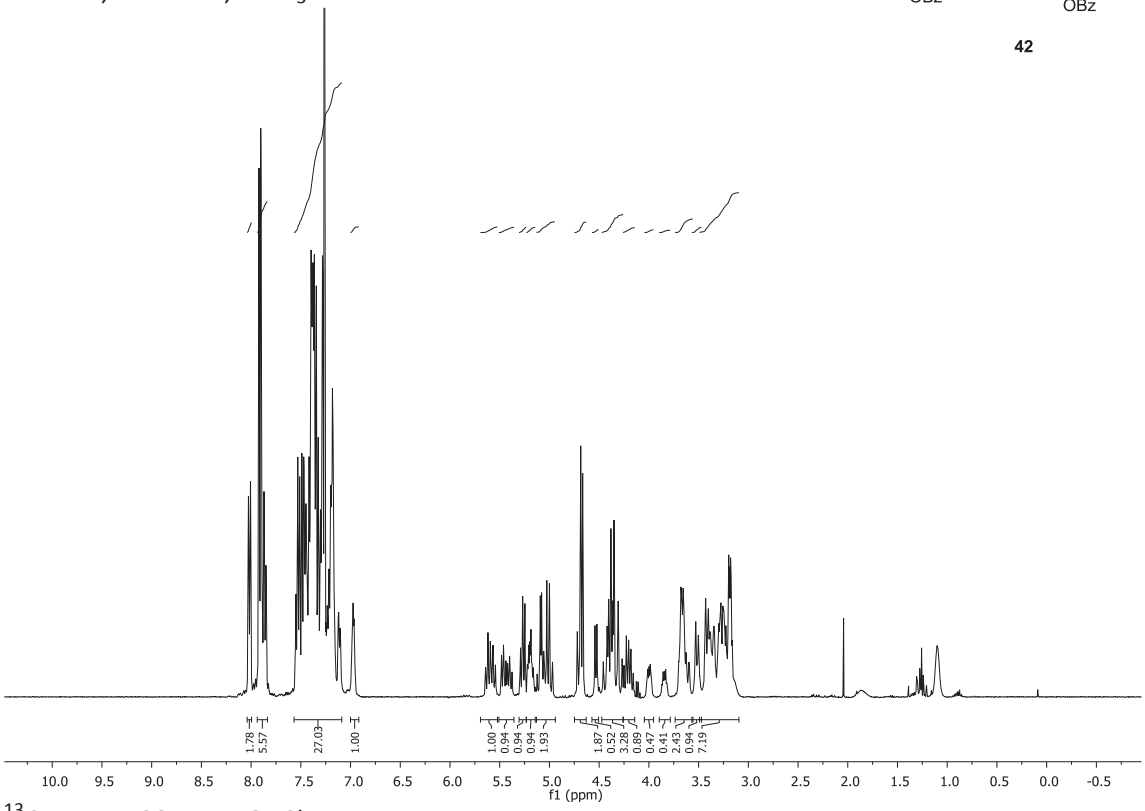
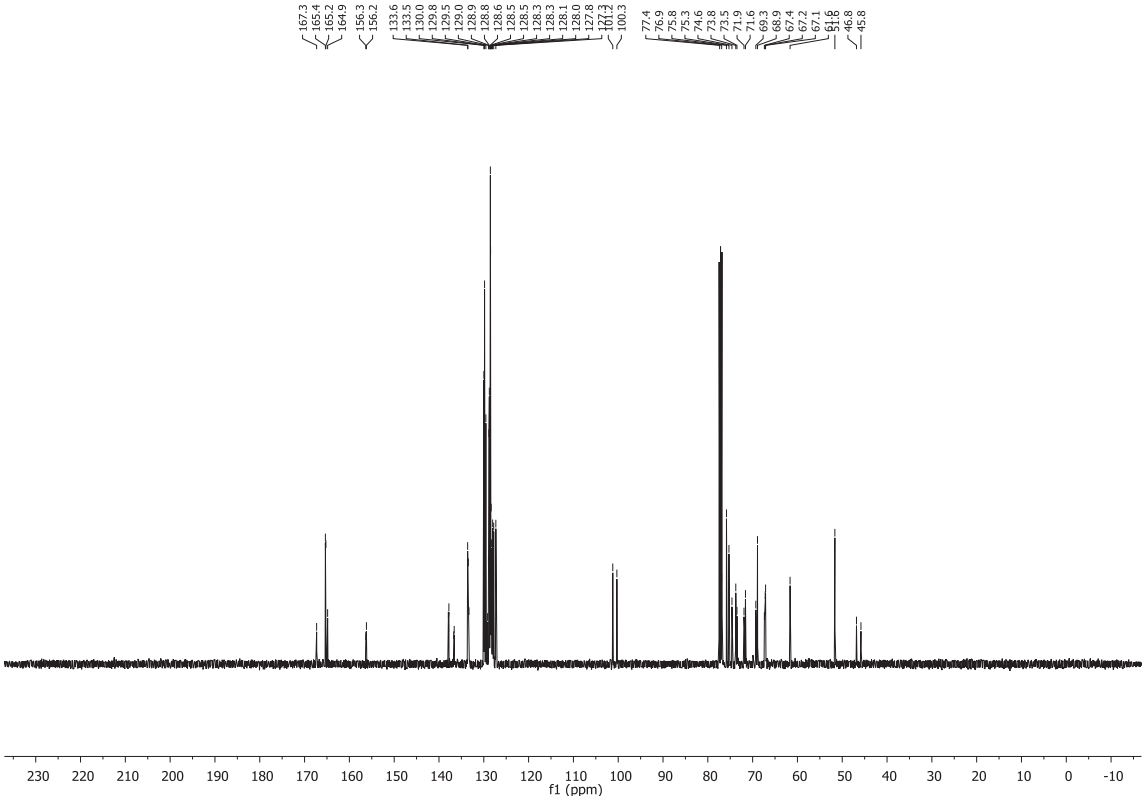
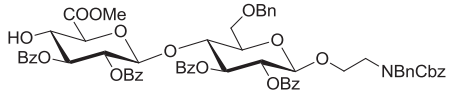


^{13}C NMR, 100 MHz, $CDCl_3$



^{1}H NMR, 400 MHz, $CDCl_3$



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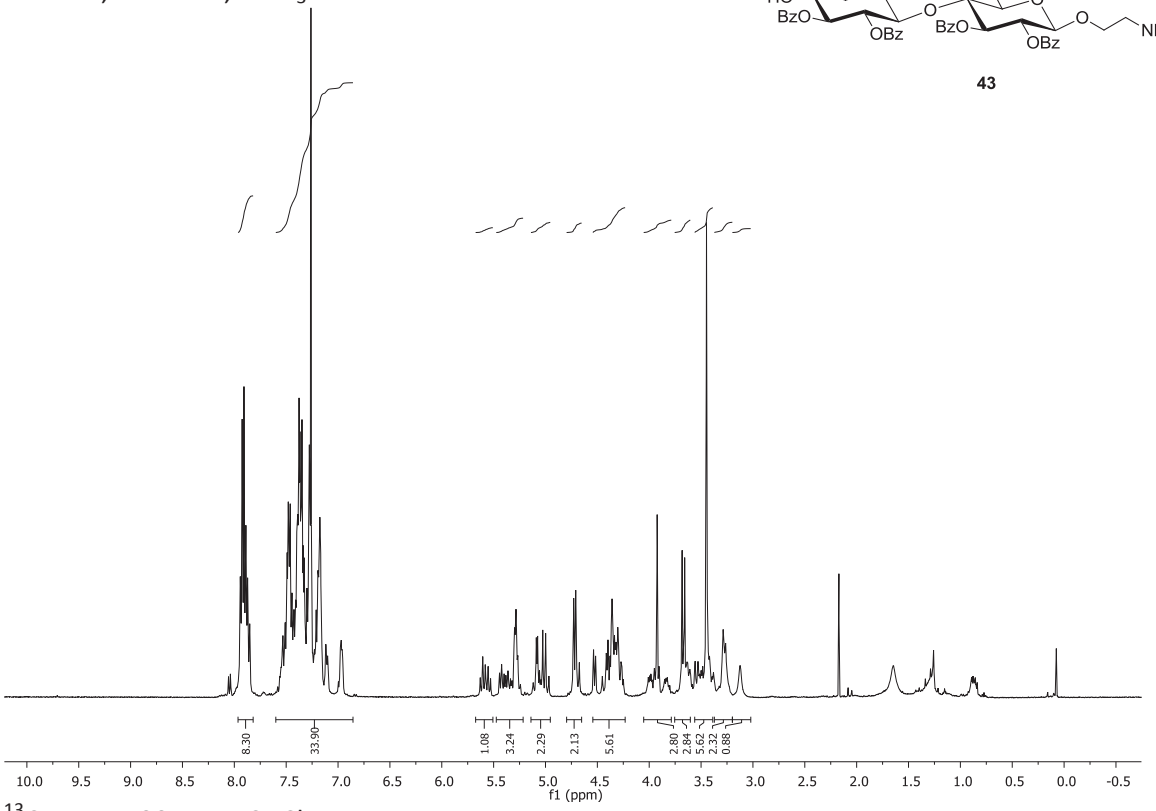
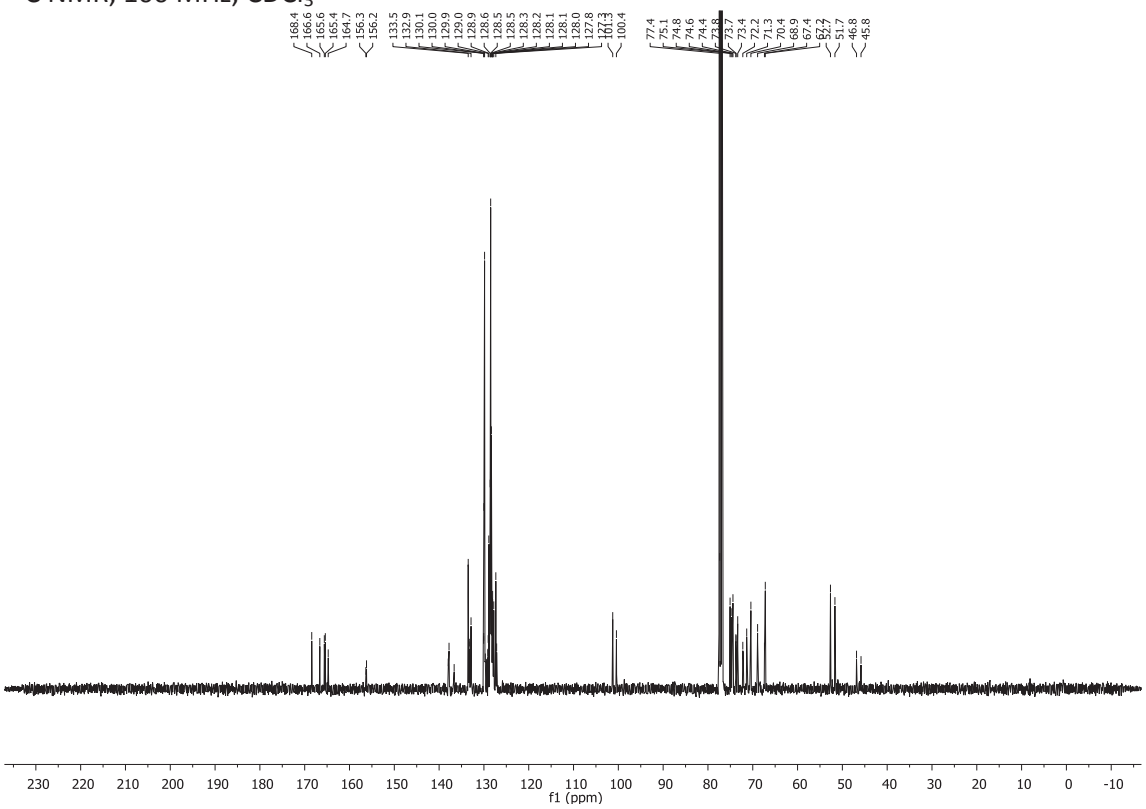
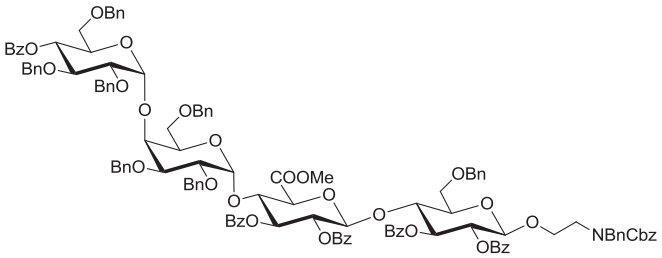


^{13}C NMR, 100 MHz, $CDCl_3$

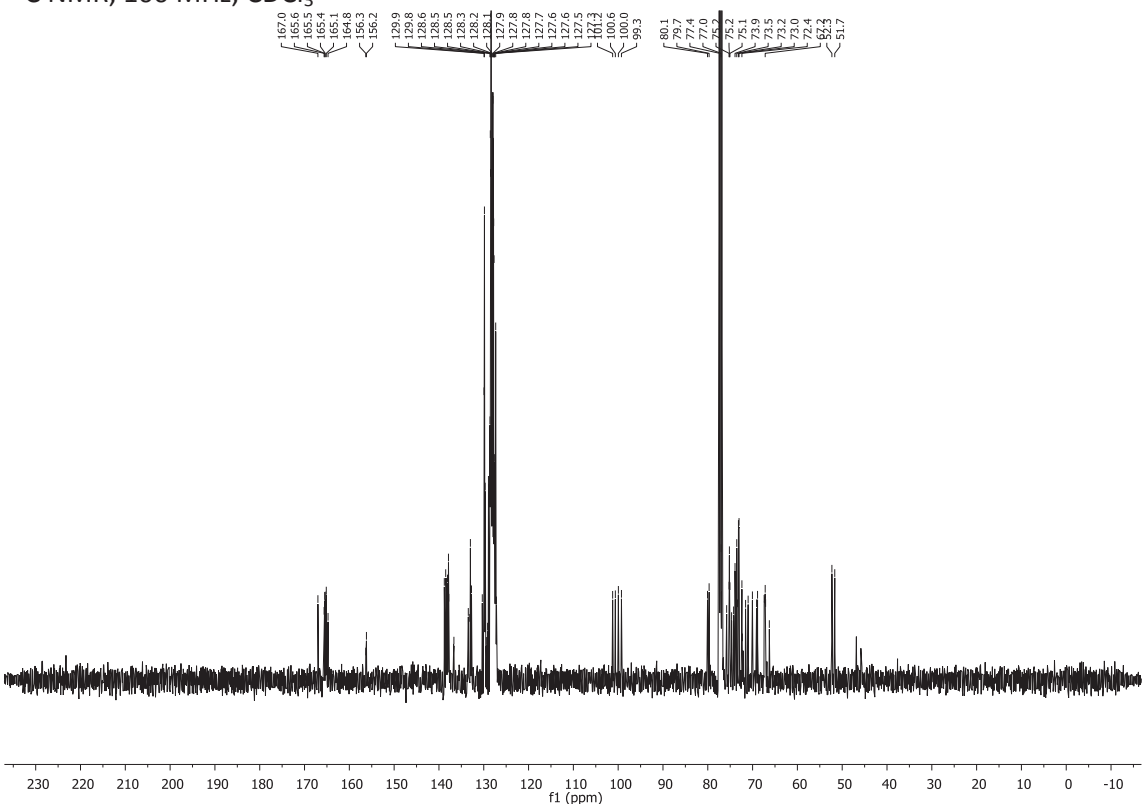


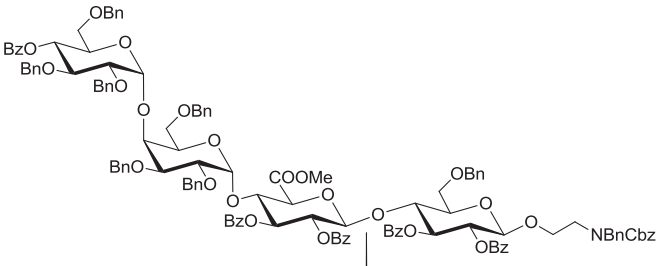
^{1}H NMR, 400 MHz, $CDCl_3$



44

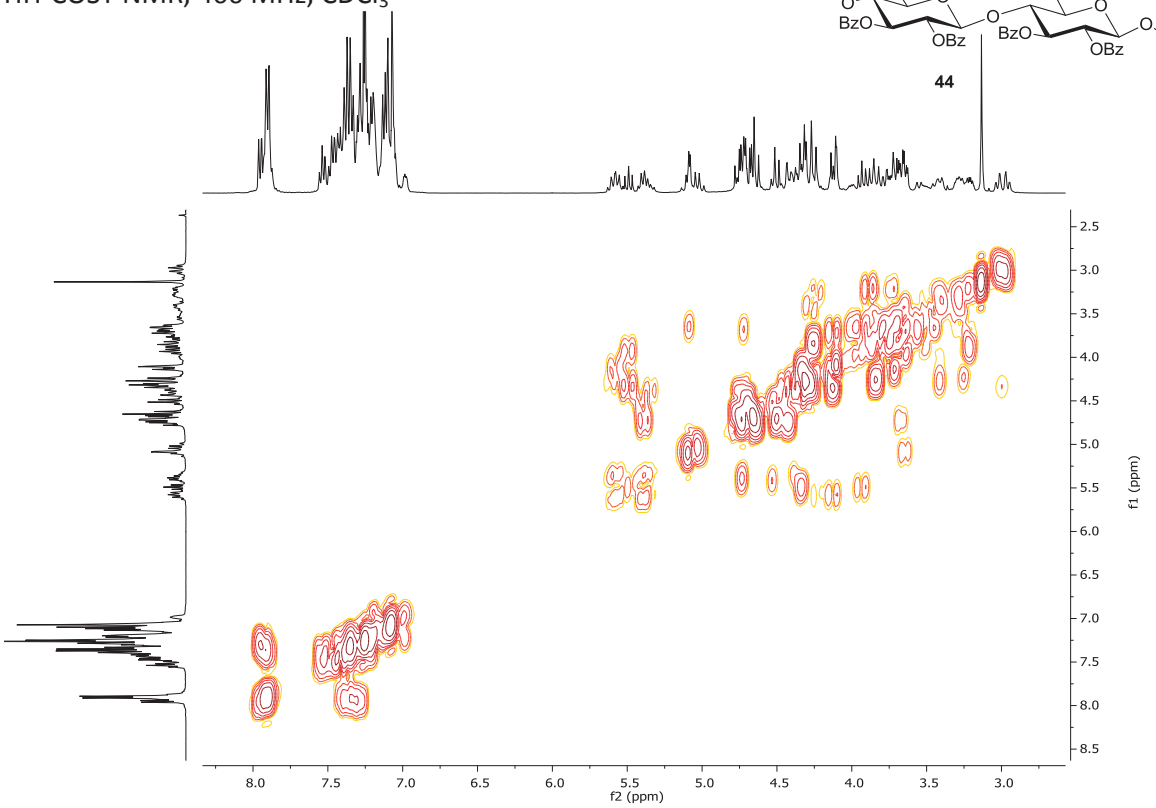
^{13}C NMR, 100 MHz, $CDCl_3$



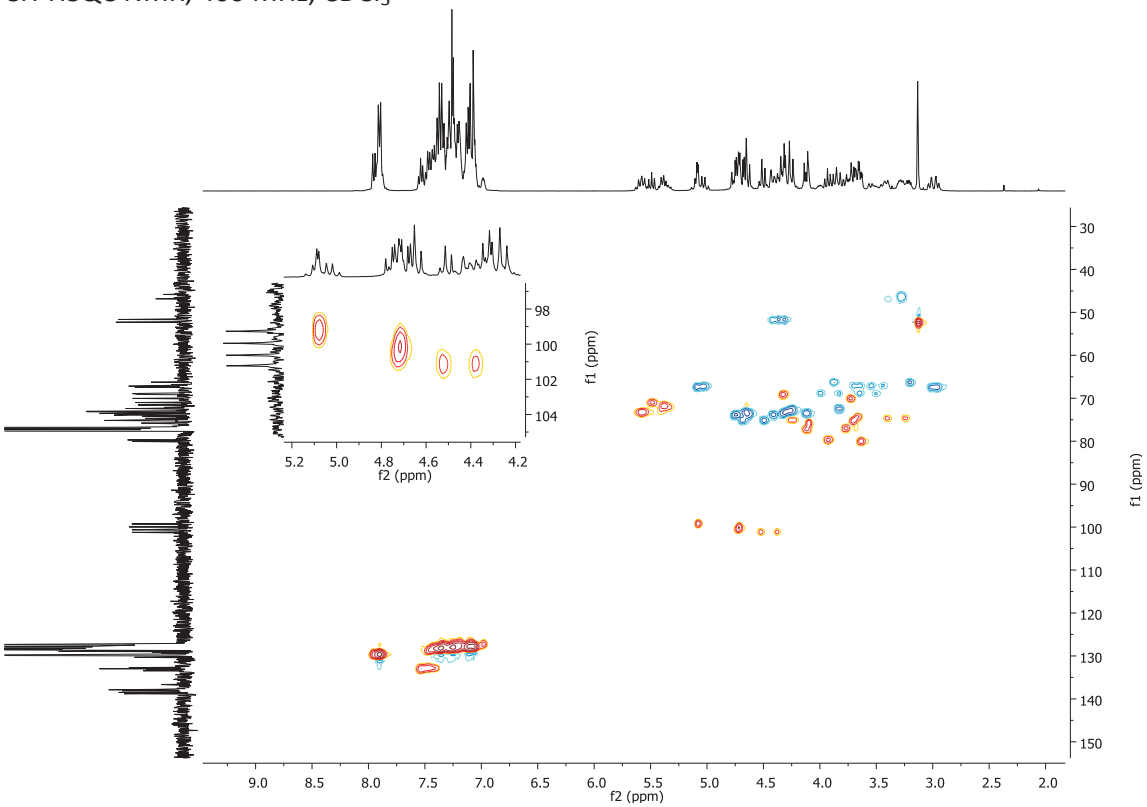


44

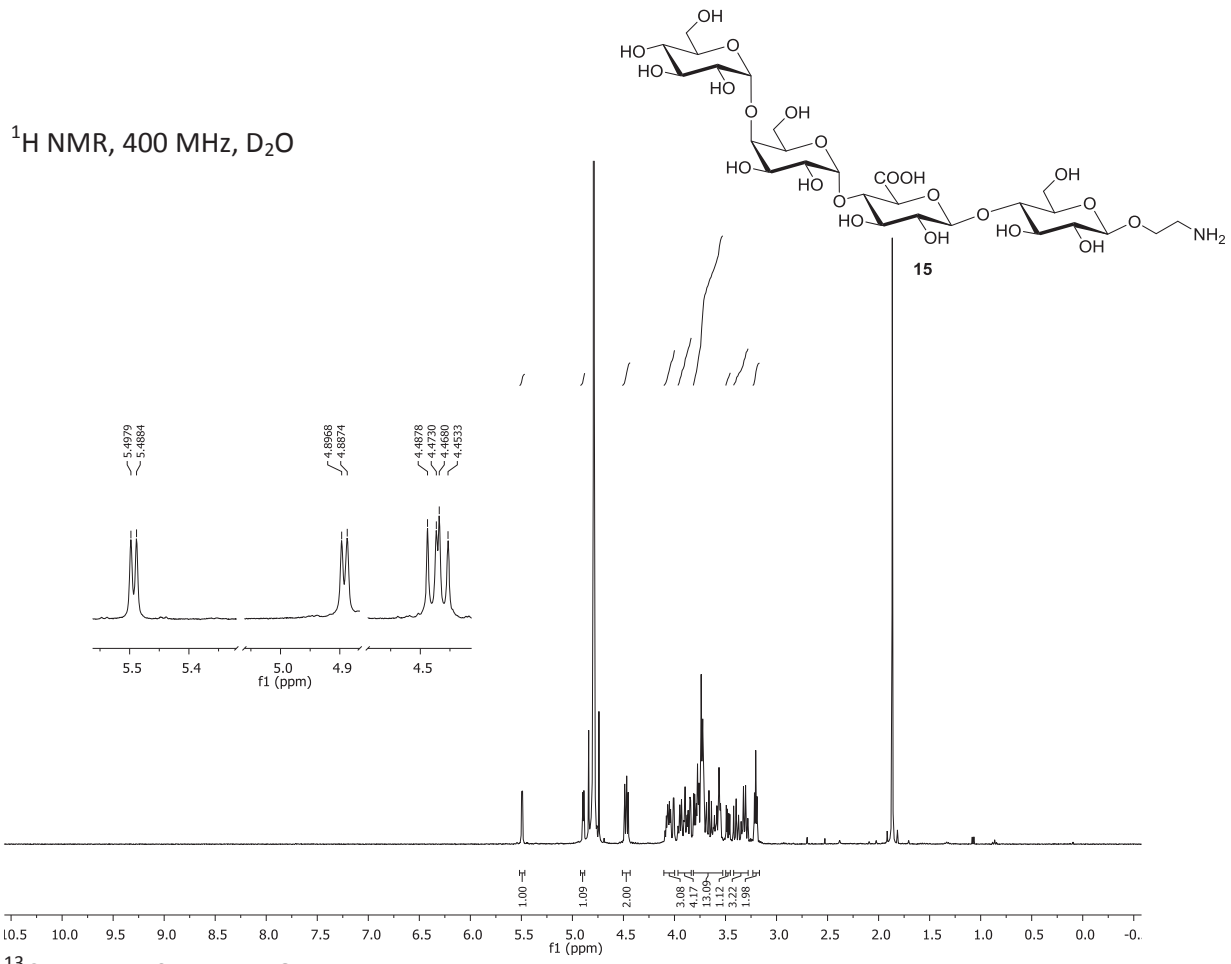
HH-COSY NMR, 400 MHz, $CDCl_3$



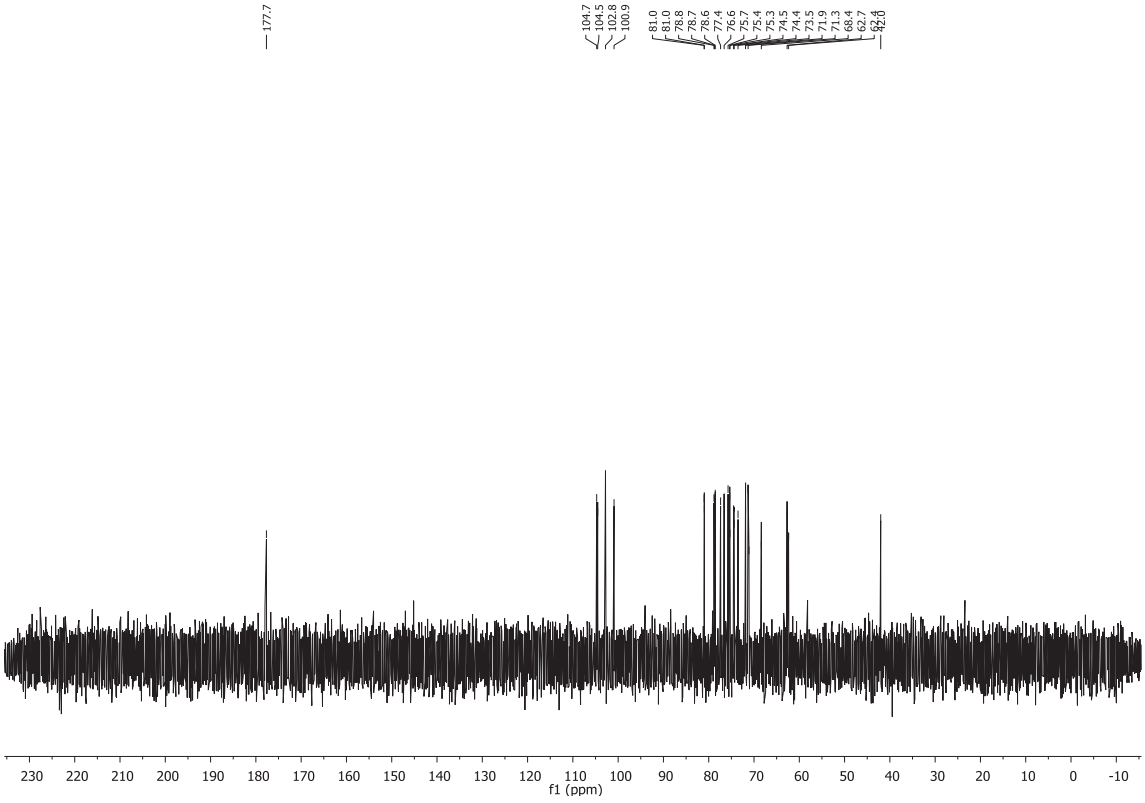
CH-HSQC NMR, 400 MHz, $CDCl_3$



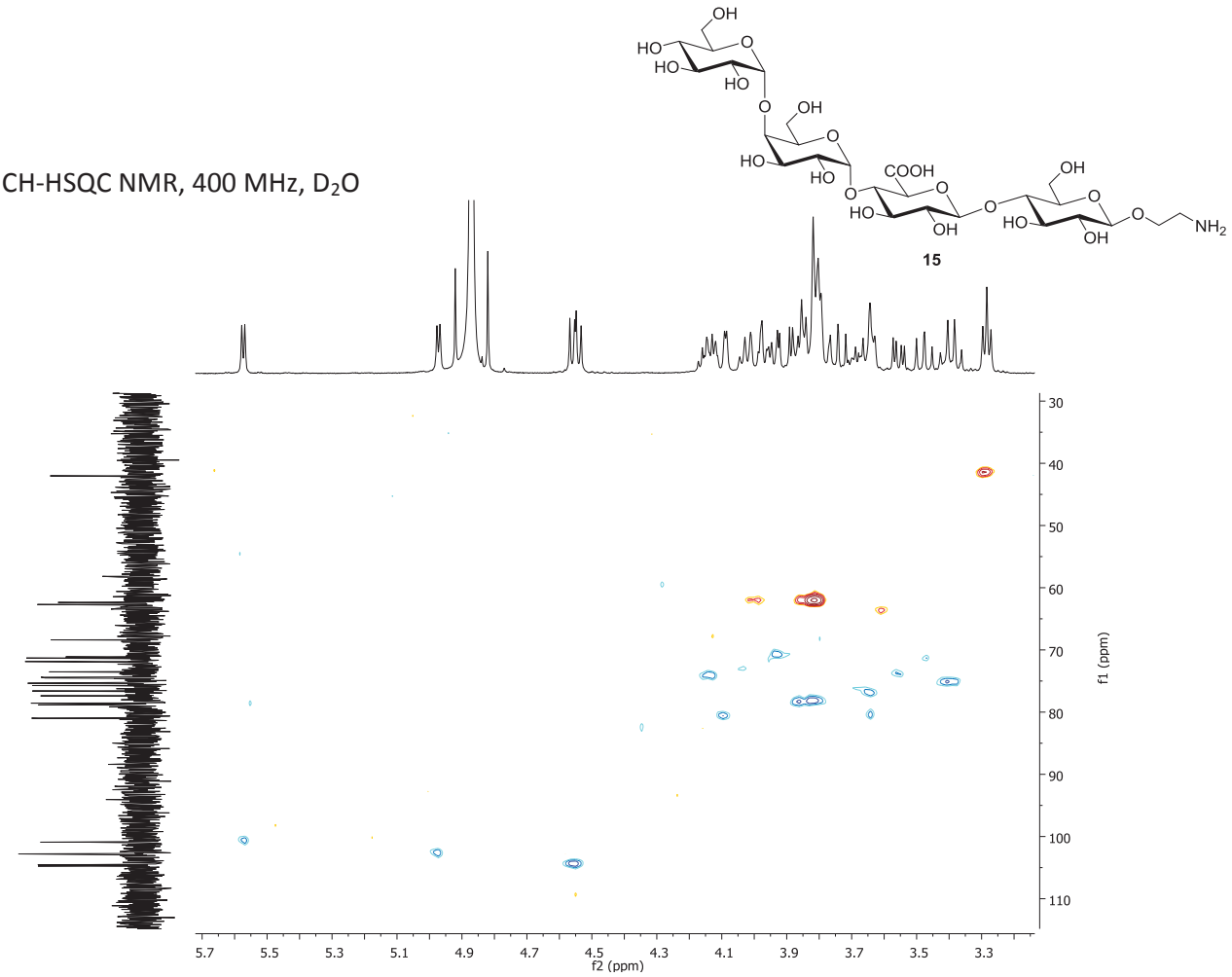
^{1}H NMR, 400 MHz, D_2O



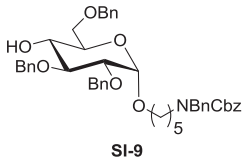
^{13}C NMR, 150 MHz, D_2O



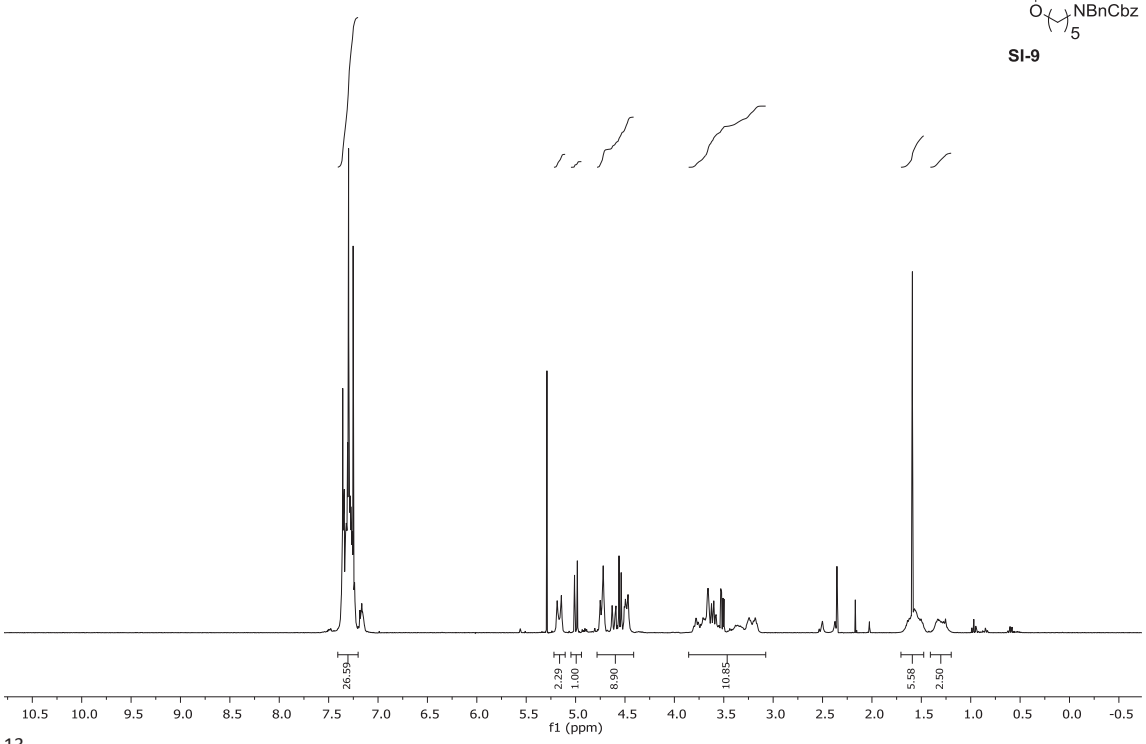
CH-HSQC NMR, 400 MHz, D_2O



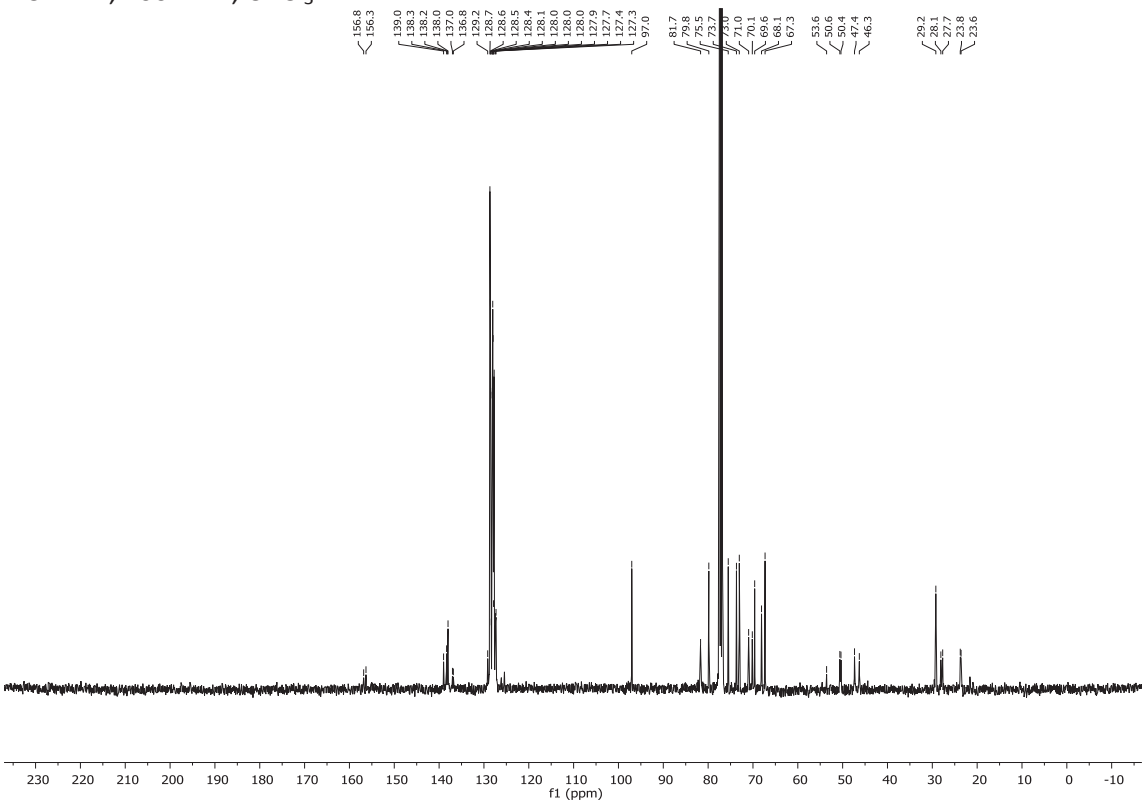
^{1}H NMR, 400 MHz, $CDCl_3$



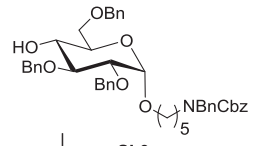
SI-9



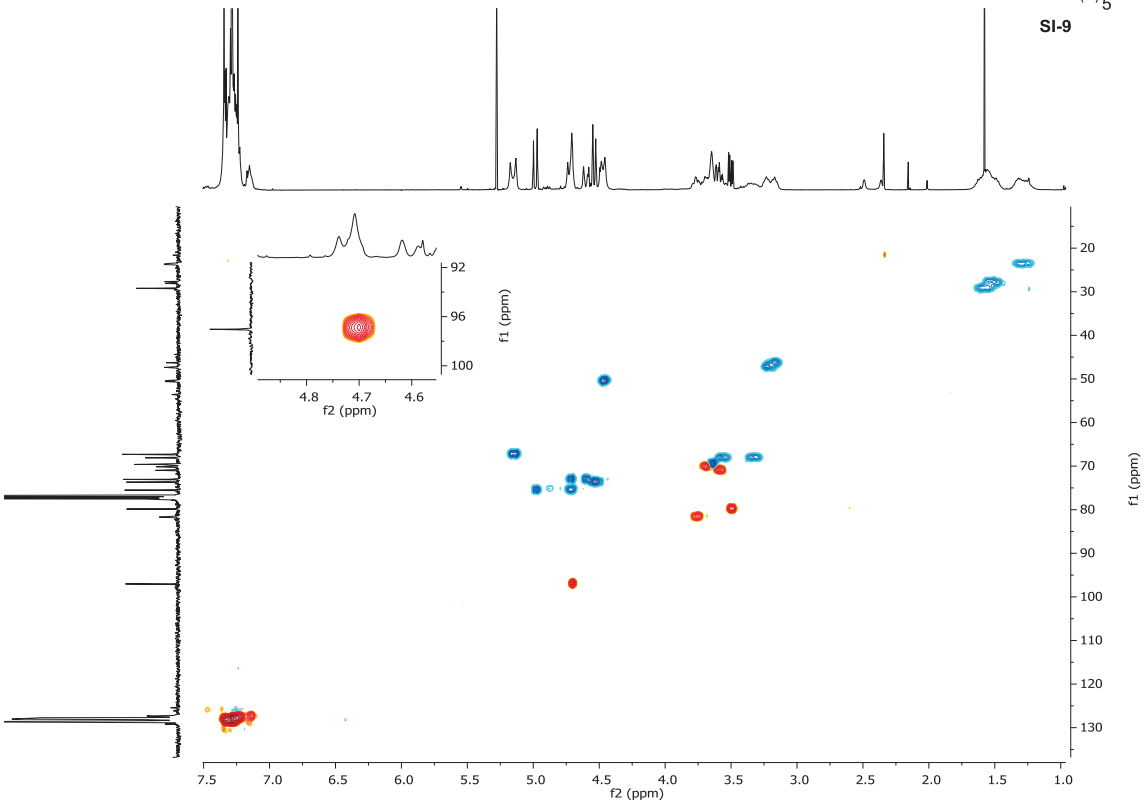
^{13}C NMR, 100 MHz, $CDCl_3$



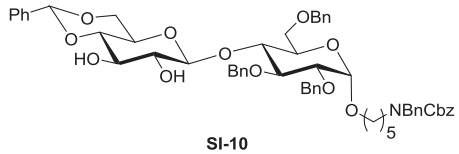
CH-HSQC NMR, 400 MHz, $CDCl_3$



SI-9

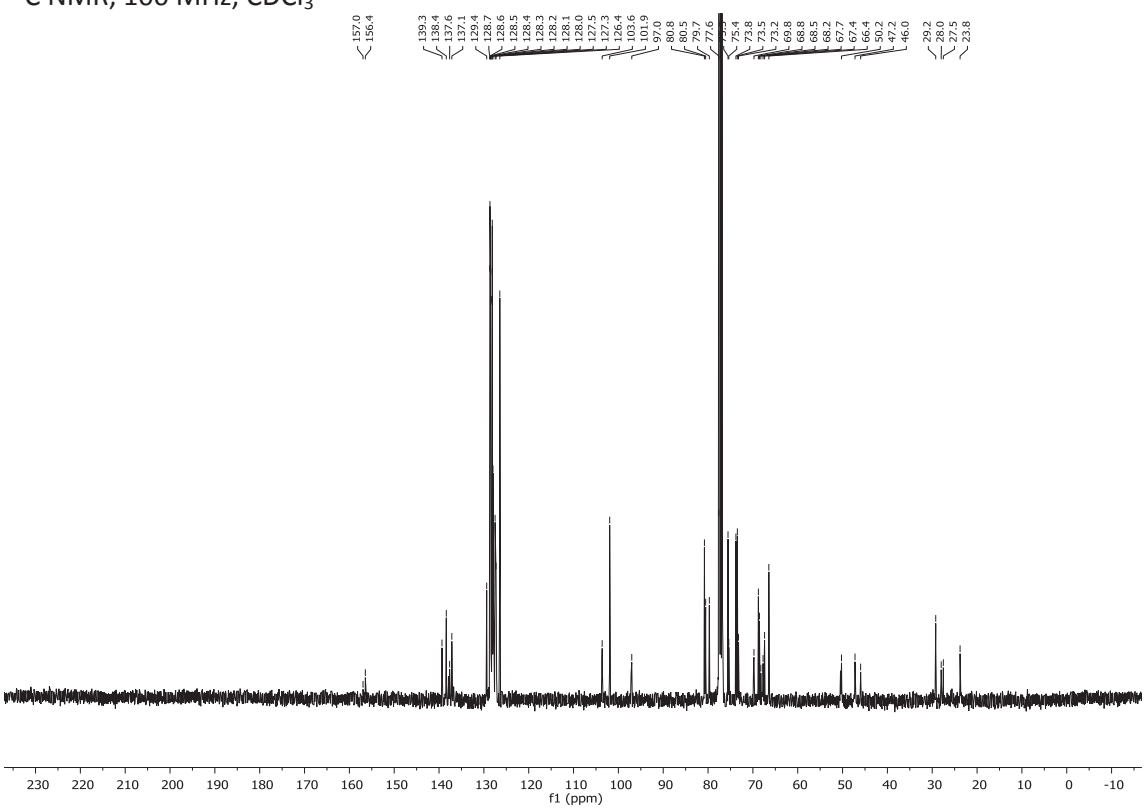


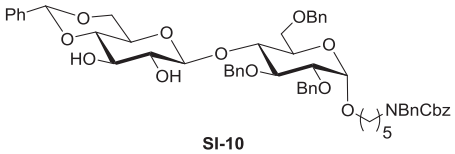
^{1}H NMR, 400 MHz, $CDCl_3$



SI-10

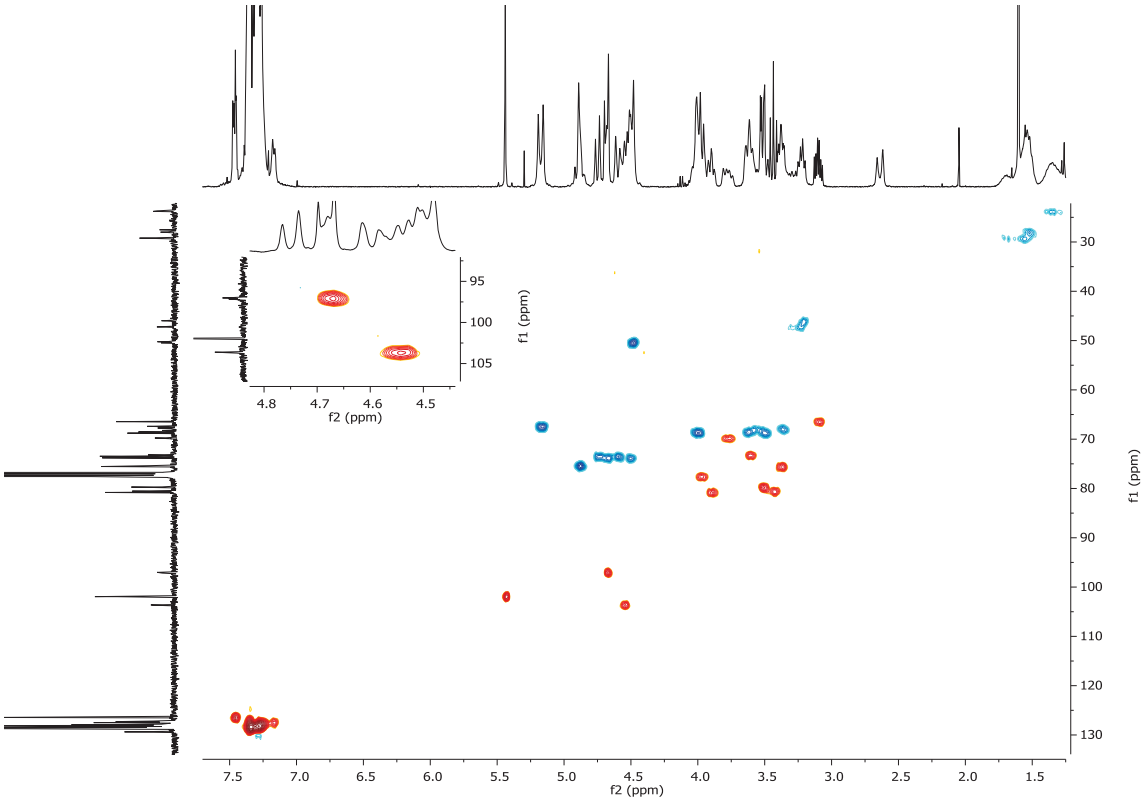
^{13}C NMR, 100 MHz, $CDCl_3$



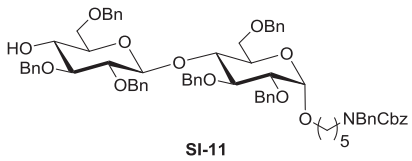


SI-10

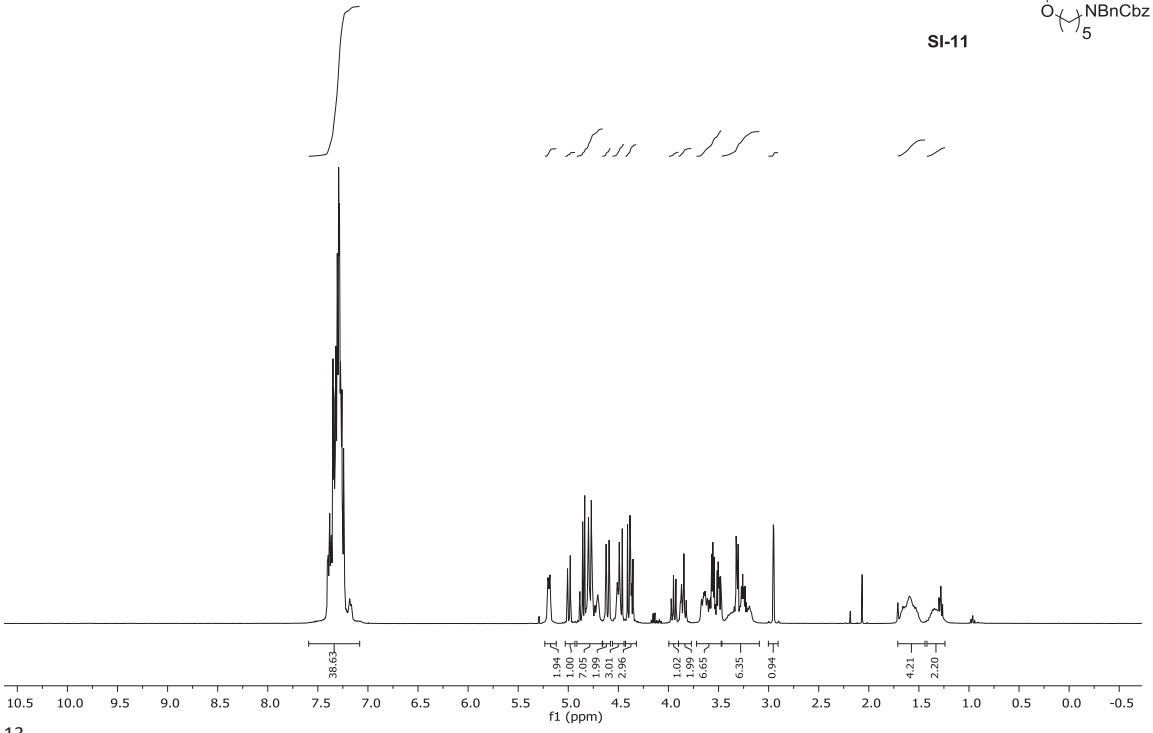
CH-HSQC, 400 MHz, $CDCl_3$



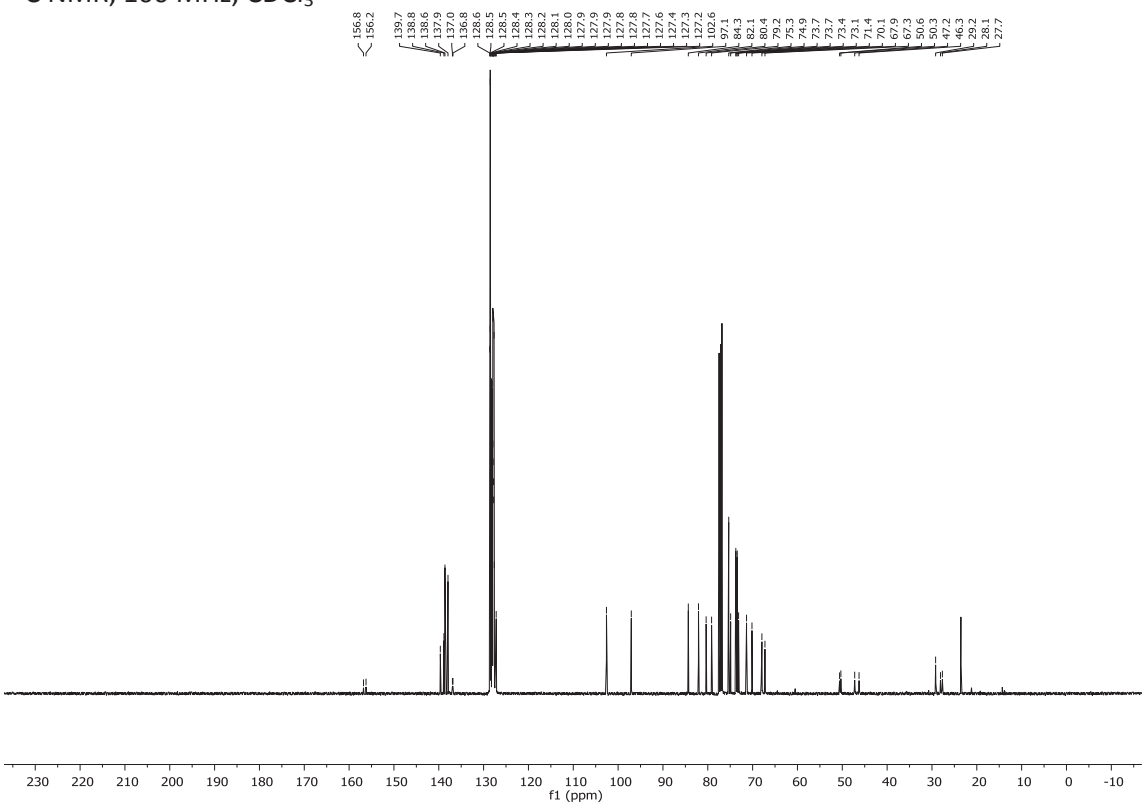
^{1}H NMR, 400 MHz, $CDCl_3$



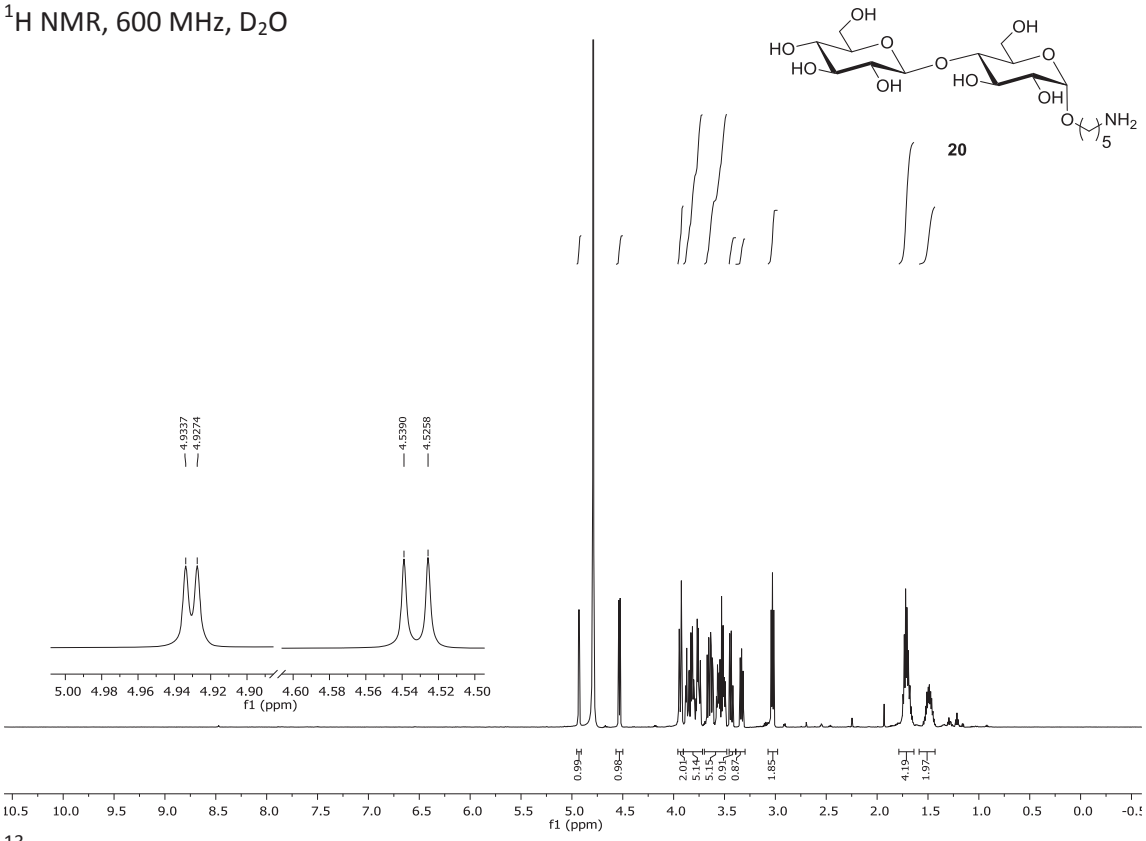
SI-11



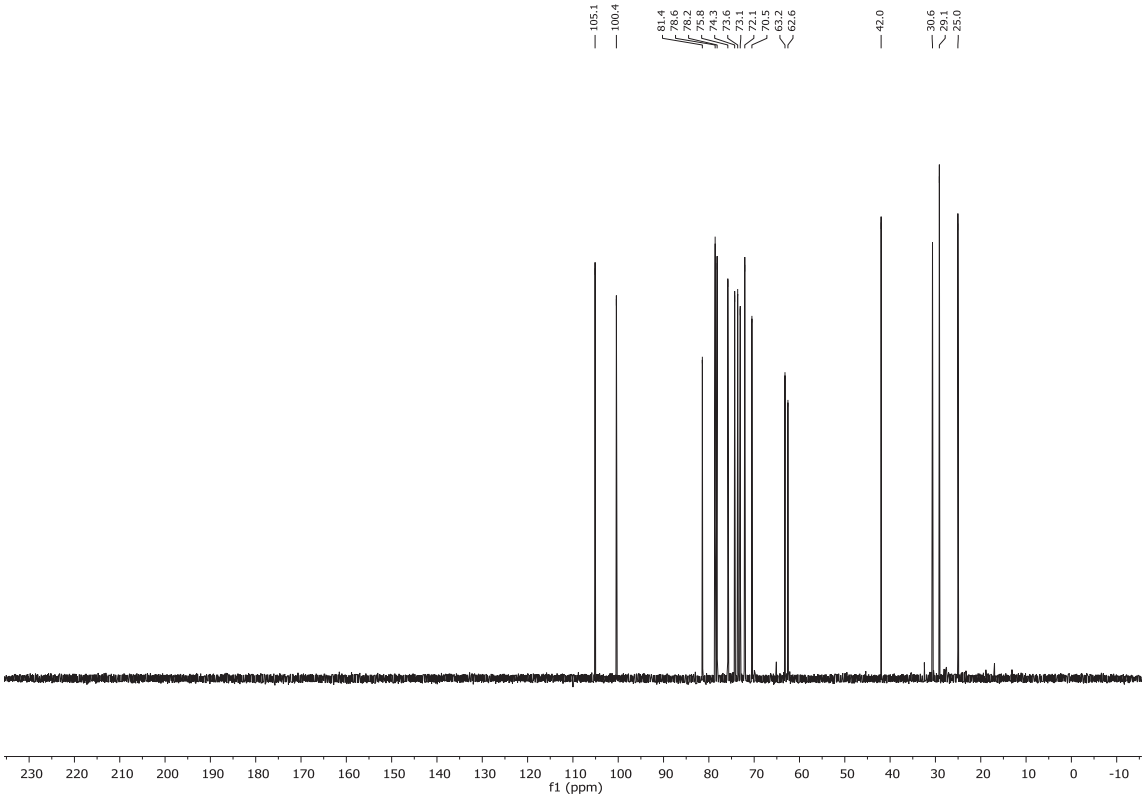
^{13}C NMR, 100 MHz, $CDCl_3$



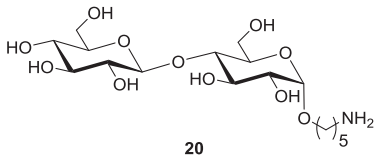
^{1}H NMR, 600 MHz, D_2O



^{13}C NMR, 150 MHz, $CDCl_3$



CH-HSQC, 600 MHz, D_2O



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