Automated Glycan Assembly Using the Glyconeer 2.1® Synthesizer

Heung Sik Hahm,[1,2] Mark K. Schlegel,[1,5,6] Mattan Hurevich,[1,7] Steffen Eller,[1,8] Frank Schuhmacher,[1,2] Johanna Hofmann,[3,4] Kevin Pagel[3,4] and Peter H. Seeberger[1,2] *

1 Max Planck Institute of Colloids and Interfaces, Department of Biomolecular System, Am Mühlenberg 1, 14476 Potsdam, Germany.

2 Institute of Chemistry and Biochemistry, Free University Berlin, Arnimallee 22, 14195 Berlin, Germany.

3 Fritz Haber Institute of the Max Planck Society, Faradayweg 4-6, 14195 Berlin, Germany.

4 Institute of Chemistry and Biochemistry, Free University Berlin, Takustrasse 3, 14195 Berlin, Germany.

5 GlycoUniverse GmbH&CoKG, Arnimallee 22, 14195 Berlin, Germany.

6 Current address: Alnylam Pharmaceuticals, 300 Third Street, Cambridge, 02142, MA, USA.

7 Current address: Institute of Chemistry, The Hebrew University of Jerusalem, Safra Campus, Givat Ram, 91904, Jerusalem, Israel.

8 Current address: Chemspeed Technologies AG, Rheinstrasse 32, 4302, Augst, Switzerland.

* Fax: (+49) 30 838 459301, E-mail: peter.seeberger@mpikg.mpg.de

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General Information

All chemicals used were reagent grade and used as supplied, except where noted. Prior to use, molecular sieves were activated by heating under high vacuum. All reactions were performed in oven-dried glassware under an argon atmosphere, unless noted otherwise. *N*,*N*-Dimethylformamide, (DMF) dichloromethane (DCM), toluene and tetrahydrofuran (THF) were purified in a Cycle-Tainer Solvent Delivery System, unless noted otherwise. Analytical thin layer chromatography (TLC) was performed on Merck silica gel 60 F254 plates (0.25 mm). Compounds were visualized by UV-irradiation, or by dipping the plate either in a cerium sulfate ammonium molybdate (CAM) solution or a 1:1 mixture of H_2SO_4 (2 N) and resorcine monomethylether (0.2%) in ethanol. Flash column chromatography was carried out using forced flow of the indicated solvent on Fluka silica gel 60 (230-400 mesh). All automated glycosylations were performed on a GlycoUniverse Glyconeer 2.1® automated oligosaccharide synthesizer using anhydrous solvents of the Cycle-Tainer Solvent Delivery System. LCMS chromatograms were recorded on an Agilent 1100 Series HPLC coupled to an ESI-MS spectrometer. Preparative HPLC purifications were performed on an Agilent 1200 Series. Loading determination of functionalized resins was obtained using a Shimadzu UV-MINI-1240 UV spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Mercury 400 (400 MHz), 600 (600 MHz) or Bruker DRX700 (700 MHz) spectrometer in $CDCl_3$ or D_2O with chemical shifts referenced to internal standards ($CDCl_3$: 7.26 ppm ^{1}H, 77.16 ppm ^{13}C; CD_3OD: 4.87 or 3.13 ppm ^{1}H, 49.0 ppm ^{13}C) unless stated otherwise. Splitting patterns are indicated as s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad singlet for ^{1}H-NMR data. NMR chemical shifts (δ) are reported in ppm and coupling constants (*J*) are reported in Hz. High resolution mass spectral (HRMS) analyses were performed by the MS-service in the Department of Biology, Chemistry, Pharmacy at Free University Berlin using an Agilent 6210 ESI-TOF (Agilent Technologies, Santa Clara, CA, USA). IR spectra were recorded on a Perkin-Elmer 1600 FTIR spectrometer. Optical rotations were measured with a UniPol L 1000 polarimeter (Schmidt & Haensch, Berlin, Germany), with concentrations expressed in g per 100 mL. MALDI-TOF spectra were recorded on a Bruker Daltonics Autoflex Speed, using a 2,4,6-trihydroxyacetophenone (THAP) matrix.

Synthesis

Preparation of photolabile linker bound resin

The synthesis of solid support **1** and the loading (0.392 mmol/g) determination method have been reported previously.(1-3)

Preparation and characterization of thioglycoside building blocks

The list of building blocks used in this study are presented in **Fig. S2**. Protocol for the synthesis of thioglucose **2** from a reported starting material (4), is presented in details. Building blocks **3-8** were synthesized according to known procedures.(5-8)

Ethyl-2,3-di-O-benzoyl-4-O-benzyl-6-O-fluorenylmethoxycarbonyl-1-thio-α-D-mannosylpyranose **2**

Compound **S2** (4) (**Fig. S2**, 4.45 g, 6.94 mmol, 1.0 eq.) was co-evaporated with toluene and dissolved under an argon atmosphere in DCM (50 mL, 0.17 M). To the solution a 1 M solution of BH_3 in THF (38.5 mL, 38.5 mmol, 4.5 eq.) and trimethylsilyl trifluoromethanesulfonate (TMSOTf, 0.77 mL, 4.27 mmol, 0.5 eq.) were added successively at 0 °C. The reaction mixture was stirred for 5 h at 0 °C, quenched with saturated aqueous $NaHCO_3$, and diluted with DCM. The combined organic layer was dried over $MgSO_4$ and evaporated *in vacuo*. To a solution of the crude product were added 9-fluorenylmethyl chloroformate (Fmoc-Cl, 3.59 g, 13.88 mmol, 2.0 eq.) and pyridine (1.68 mL, 20.82 mmol, 3.0 eq.) at 0 °C, and the reaction mixture was stirred overnight at room temperature. After the reaction mixture was quenched with 1 M aqueous HCl, diluted with DCM, the phases were separated. The organic phase was dried over $MgSO_4$ and evaporated *in vacuo*. The crude product was purified by column chromatography on silica gel (hexane/ethyl acetate/DCM = 9:0.5:0.5 → 9:1:0.5) to afford **2** (5.74 g, 6.65 mmol, 93%). R_f (hexane/ethyl acetate:DCM = 9:1:0.5) = 0.21; $[\alpha]_D^{20}$= -4.13 (c = 2.53, $CHCl_3$). IR (thin film): υ = 3067, 2964, 1727, 1603, 1452, 1256 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 8.14 (dd, *J* = 7.8, 1.2 Hz, 2H), 7.98 – 7.95 (m, 2H), 7.83 (dd, *J* = 7.4, 3.2 Hz, 2H), 7.71 (d, *J* = 7.5 Hz, 1H), 7.67 (d, *J* = 7.5 Hz, 1H), 7.64–7.55 (m, 2H), 7.52–7.44 (m, 4H), 7.41 (t, *J* = 7.6 Hz, 2H), 7.36–7.31 (m, 4H), 7.26 (s, 3H), 5.79–5.75 (m, 2H, H-2, H-3), 5.53 (s, 1H, *J* = 1.1 Hz, H-1), 4.79 (d, *J* = 10.9 Hz, 1H, CHHPh), 4.68 (d, *J* = 11.0 Hz, 1H, CHHPh), 4.61–4.53 (m, 3H, CH, CH_2 of Fmoc), 4.48 (d, J = 7.8 Hz, 2H, H-6), 4.37–4.29 (m, 2H, H-4, H-5), 2.91–2.54 (m, 2H), 1.39 (dt, *J* = 8.0, 3.9 Hz, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 165.47 (Bz), 165.39 (Bz), 155.26 (Fmoc), 143.57, 143.41, 141.46, 141.44, 137.33, 133.59, 133.39, 129.98, 129.81, 129.66, 128.72, 128.59, 128.57, 128.30, 128.15, 128.09, 127.34, 127.31, 125.35, 125.31, 120.26, 120.24 (Ar), 82.45 (C-1), 75.14 (CH_2Ph), 73.26 (C-4), 73.09 (C-2), 72.57 (C-3), 70.32 (CH of Fmoc), 70.26 (C-6), 66.71(CH_2 of Fmoc), 46.94 (C-5), 25.74 (Et), 15.05 (Me). MS ESI+-HRMS *m/z* $[M+Na]^+$ calcd for $C_{44}H_{40}O_9SNa$ 767.2291, found 767.2272.

Ethyl-2,3-di-O-benzoyl-5-O-fluorenylmethoxycarbonyl-1-thio-α-D-arabinofuranoside **3**

This compound had been synthesized previously please refer to the reference for details.[5]

Ethyl-2-O-benzoyl-3,5-di-O-fluorenylmethoxycarbonyl-1-thio-α-D-arabinofuranoside **4**

This compound had been synthesized previously please refer to the reference for details.(6)

Ethyl-2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-1-thio-α-D-mannosylpyranose **5**

This compound had been synthesized previously please refer to the reference for details.(8)

2-Methyl-5-*tert*-butylphenyl 2,3-di-*O*-benzoyl-6-*O*-benzyl-4-*O*-fluorenylmethoxycarbonyl-1-thio-β-D-glucopyranoside **6**

This compound had been synthesized previously please refer to the reference for details.(5)

Ethyl-2-*O*-benzoyl-4,6-di-*O*-benzyl-3-*O*-fluorenylmethoxycarbonyl-1-thio-β-D-galactopyranoside **7**

This compound had been synthesized previously please refer to the reference for details.(5)

Ethyl 2,3,4,6-tetra-O-benzyl-1-thio-β-D-galactopyranoside **8**

This compound had been synthesized previously please refer to the reference for details.(7)

Synthesizer Set-up

Solvent preparation and assembly: All solvents are purified in a Cycle-Tainer Solvent Delivery System or, alternatively, purchased in a safe/sure-seal bottles. Each solvent is transferred to the appropriate narrow-mouth glass bottle on the synthesizer's solvent drawer as fast as possible, fitted with two-way sealed cap and immediately pressurized with argon. The solvents are kept under a constant inert atmosphere.

Building Block Preparation (125 µmol/vial): Option 1) building blocks are co-evaporated three times with toluene in a RB flask and kept under vacuum overnight. The dried BB powder is transferred into the designated synthesizer's vials and are further dried by placing the vials under vacuum overnight after which the vials are purged with argon stream and closed immediately with a designated Septum sealed cap. Option 2) building blocks are placed directly into the designated synthesizer's vials and co-evaporated three times with toluene before being placed under vacuum for overnight drying. The vials are purged with argon stream and closed immediately with a designated Septum sealed cap. In both cases the vials were placed in the BB carousel according to the specific oligosaccharide sequence.

Reagent Preparation:

Reagent Bottle Preparation: In all cases, 250 mL amber glass narrow-mouth graduated laboratory bottles are used. The bottles are washed thoroughly and dried overnight in an oven before use. Shortly before filling the reagent solutions, the bottles are taken out of the oven and flushed with argon while cooling down to room temperature.

Acidic Washing Solution: A pre-dried reagent bottle is filled with anhydrous DCM (40 mL) under argon atmosphere and added trimethylsilyl trifluoromethanesulfonate (TMSOTf; 1.0 mL). The prepared reagent bottle is placed in the predetermined acid-wash position in the reagent drawer, fitted with a two-way sealed cap, pressurized and stored under argon atmosphere.

NIS Activation Solution: Into a pre-dried reagent bottle recrystallized *N*-iodosuccinimide (NIS, 1.24 g) is added and filled with a mixture of anhydrous DCM and anhydrous dioxane (v/v 9/1, 40.0 mL) under an argon atmosphere. Triflic acid (TfOH) (50 µL) is added to the NIS solution at 0 °C. The bottle is placed in the cooling block on the reagent drawer, fitted with a two-way seal cap, pressurized and stored under argon atmosphere.

Triethylamine Solution: A pre-dried reagent bottle is filled with DMF (80 mL) and triethylamine (TEA, 20 mL) is added. This bottle is placed on the Fmoc deprotection bottle position, fitted with a two-way seal cap, pressurized and stored under argon atmosphere.

Automated synthesis working modules

The timing and quantity of solvents/reagents transferred to the reaction vessel in each step is controlled by the software. The delivery system is based on valve-pressured control in which the entire platform is constantly pressurized so that the specific solvent/reagent is transferred by timing the opening and closing of the appropriate valves.

Module 1: Acidic Washing: The resin is washed with DMF, THF, DCM (six times each with 2 mL for 25 s). The resin is swollen in 2 mL DCM, and the temperature of the reaction vessel was adjusted to –20 °C. For acidic washing 0.5 mL of the solution of TMSOTf is delivered to the reaction vessel. After one minute, the solution is drained. Finally, 2 mL DCM is added to the reaction vessel.

Module 2: Glycosylation: Thioglycoside building block is dissolved in the proper solvent mixture (2 mL for two glycosylation cycles) in the designated building block vial on the carousel. The reaction vessel is set to reach the initial glycosylation temperature. During the adjustment of the temperature, the DCM in the reaction vessel is drained and half the solution of thioglycoside building block (5.0 eq. in 1.0 mL DCM) is delivered (slowly) from the building block vial to the reaction vessel. After the set temperature of -40 °C is reached, 1.0 mL NIS (5.5 eq. in 1.0 mL) and TfOH (0.2 eq. in 1.0 mL) solution in DCM and dioxane (v/v, 9:1) is delivered (slowly) to the reaction vessel. The glycosylation mixture is incubated for 5 min at -40 °C, linearly ramped to -10 °C, and after reaching -10 °C the reaction mixture as incubated for additional 25 min. Once incubation time is finished, the reaction mixture is drained and the resin is washed with DCM (six times with 2 mL for 15 s). This procedure is repeated twice.

Module 3: Fmoc Deprotection: The resin is washed with DMF (six times with 2 mL for 25 s), swollen in 2 mL DMF and the temperature of the reaction vessel is adjusted to 25 °C. For Fmoc deprotection the DMF is drained and 2 mL of a solution of 20% triethylamine in DMF was delivered to the reaction vessel. After 5 min the reaction solution is collected in the fraction collector of the oligosaccharide synthesizer and 2 mL of a solution of 20% triethylamine in DMF is delivered to the resin. This procedure is repeated three times.

Post-automation protocols, purification and analysis

Cleavage from resin

This procedure was describes in details by Eller *et al*. and Hurevich *et al*.(1, 3)

Photoreactor set-up: A medium pressure mercury lamp (450 W) was placed in double jacked photoreactor equipped with circulation water. A Pyrex filter rapped with or 1/8'' (inner diameter 0.06 inch) fluorinated-ethylene-propylene (FEP) tubing was placed around the double jacked apparatus. The entire setting was placed in a sealed aluminum box equipped with entry and exit points for the FEP tubing.

Oligosaccharide cleavage from solid support: The mercury lamp is turned on 30 min prior to the first cleavage event. The FEP tubing is washed with 20 mL DCM at a flow rate of 5 mL/min before cleavage. The solid support after AGA is pre-swelled in the dark in DCM for 30 min before being taken up with a 20 mL disposable syringe. The suspension of solid support in DCM is slowly injected from the disposable syringe (20 mL) into the FEP tubing using a syringe pump. The suspension is pushed through the FEP tubing into the photoreactor with additional 18 mL DCM (flow rate: 600 µL/min). The photocleavage takes place inside the reactor while solid support travels toward the exit point of the reactor. The suspension leaving the reactor is directed into a syringe equipped with polyethylene filter frit where the resin is filtered off and the solution containing the cleaved oligosaccharide is collected in a separate glass vial. The tubing is washed with 20 mL DCM (flow rate: 2 mL/min) until any remaining resin exits the reactor and the remaining oligosaccharide solution is collected. The tubing is re-equilibrated with 20 mL DCM using a flow rate of 5 mL/min and the entire cleavage procedure is repeated. The combined solution that was collected in the photocleavage process is evaporated *in vacuo* and the crude material was analyzed by MALDI-TOF, NMR and HPLC.

Purification and characterization of fully protected oligosaccharides 9, 10 and 11

Analytical HPLC: The crude material was analyzed by normal-phase (NP) HPLC: Column: Luna 5µm Silica 100Å, (260 x 4.60 mm); flow rate: 1 mL/min; eluents: 5% DCM in hexane/5% DCM in ethyl acetate; gradient: 20% (5 min), 60% (in 40 min), 100% (in 5 min); detection: 280 nm, and ELSD.

Preparative HPLC: The crude mixture was dissolved in a minimum volume of DCM and 0.9 mL of 20% hexane in ethyl acetate. The crude solution was purified using preparative NP-HPLC. Column: Luna 5µm Silica (260 x 10 mm); flow rate: 5 mL/min; eluents: 5% DCM in hexane/5%

DCM in ethyl acetate; gradient: 20% (5 min), 60% (in 40 min), 100% (in 5 min); detection: 280 nm, and ELSD) to afford the fully protected target oligosaccharide. The HPLC Chromatograms of oligosaccharides **4**, **5** and **6** are presented in **Supplementary Figures 4-6**

NMR Characterization

N-Benzyloxycarbonyl-5-amino-pentyl (2,3-di-*O*-benzoyl-α-D-arabinofuranosyl)-(1→5)-(2,3-di-*O*-benzoyl-α-D-arabinofuranosyl)-(1→5)-(2,3-di-*O*-benzoyl-α-D-arabinofuranosyl)-(1→6)-(2,3-di-*O*-benzoyl-α-D-mannopyranosyl)-(1→6)-(2,3-di-*O*-benzoyl-α-D-mannopyranosyl)-(1→6)-2,3-di-*O*-benzoyl-α-D-mannopyranoside **9**

Hexasaccharide **9** was synthesized using a synthesizer sequence: I-I-I-II-II-II as described in **Table S1**, isolated (25.3 mg; 9.47 μmol; 38%) and characterized by NMR

^{1}H NMR (600 MHz, $CDCl_3$) δ 8.15–8.09 (m, 2H), 8.08–8.03 (m, 8H), 8.01 (dd, *J* = 8.3, 1.2 Hz, 2H), 7.95–7.90 (m, 6H), 7.90–7.88 (m, 2H), 7.85 (dd, *J* = 8.3, 1.2 Hz, 2H), 7.81 (dd, *J* = 8.3, 1.2 Hz, 2H), 7.60–7.28 (m, 35H), 7.27–7.20 (m, 4H), 7.21–7.07 (m, 15H), 6.99–6.95 (m, 2H), 5.88 (dd, *J* = 3.1, 1.9 Hz, 1H), 5.86 – 5.80 (m, 4H), 5.77 (dd, *J* = 9.7, 3.4 Hz, 1H), 5.64 – 5.62 (m, 2H), 5.60 (d, *J* = 4.0 Hz, 2H), 5.58 (d, *J* = 4.3 Hz, 1H), 5.50 (s, 1H, **H$_{Araf}$-1**), 5.42 (dd, *J* = 5.8, 2.0 Hz, 2H, **H$_{Araf}$-1**), 5.38 (s, 1H, **H$_{Araf}$-1**), 5.22 (d, *J* = 1.6 Hz, 1H, **H$_{Man}$-1**), 5.16 (s, 1H, **H$_{Man}$-1**), 5.07 (d, *J* = 12.4 Hz, 2H), 4.94 (s, 1H, **H$_{Man}$-1**), 4.79 (dd, *J* = 11.2, 8.9 Hz, 2H), 4.67 (dd, *J* = 11.3, 1.3 Hz, 2H), 4.60 – 4.51 (m, 3H), 4.50 – 4.42 (m, 2H), 4.40 – 4.30 (m, 2H), 4.26 (dd, *J* = 6.7, 4.0 Hz, 1H), 4.19 – 4.12 (m, 3H), 4.07 (dd, *J* = 15.5, 6.4 Hz, 2H), 4.02 – 3.89 (m, 7H), 3.86 (d, *J* = 9.8 Hz, 1H), 3.82 (dd, *J* = 11.2, 2.4 Hz, 1H), 3.77 (t, *J* = 9.5 Hz, 2H), 3.73 (d, *J* = 10.1 Hz, 1H), 3.47 (dd, *J* = 15.5, 6.3 Hz, 1H), 3.19 (dd, *J* = 13.0, 6.6 Hz, 2H), 2.29 (s, 1H), 1.67 – 1.59 (m, 2H), 1.59 – 1.50 (m, 2H), 1.47 – 1.38 (m, 2H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 166.19 (Bz), 165.85 (Bz), 165.69 (Bz), 165.67 (Bz), 165.60 (Bz), 165.50 (Bz), 165.30 (Bz), 165.24 (Bz), 165.18 (Bz), 156.60 (Cbz), 137.81, 137.00, 133.66, 133.39, 133.31, 133.27, 133.20, 133.15, 130.00, 129.96, 129.87, 129.76, 129.72, 129.66, 129.62, 129.30, 129.25, 129.15, 129.11, 129.08, 128.84, 128.79, 128.71, 128.67, 128.55, 128.52, 128.48, 128.43, 128.37, 128.15, 128.01, 127.92, 127.89, 127.86, 127.80, 127.25 (Ar), 106.24 (**C$_{Araf}$-1**), 106.01 (2 x **C$_{Araf}$-1**), 98.54 (**C$_{Man}$-1**), 98.31 (**C$_{Man}$-1**), 97.74 (**C$_{Man}$-1**), 83.77, 83.41, 82.14, 81.83, 81.68, 81.61, 77.96, 77.84, 77.45, 75.30, 75.28, 75.16, 73.49, 73.43, 73.26, 73.15, 73.09, 73.05, 71.45, 71.33, 71.04, 70.55, 70.49, 68.29, 66.53, 66.18, 65.98, 65.57, 65.46, 62.46, 41.16, 29.85, 29.10, 23.70.; MS ESI+-HRMS *m/z* $[M+Na]^+$ calcd for $C_{151}H_{139}NO_{42}Na$ 2660.8664, found 2660.8608.

N-Benzyloxycarbonyl-5-amino-pentanyl (2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-α-D-manno-pyranosyl)-(1→5)-(2-*O*-benzoyl-3,5-di-*O*-benzyl-α-D-arabinofuranosyl)-(1→5)-(2-*O*-benzoyl-3-O-[(2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→5)-(2-*O*-benzoyl-3,5-di-*O*-benzyl-α-D-arabinofuranosyl-(1→3)]-(α-D-arabinofuranosyl)-(1→5)-2,3-di-*O*-benzoyl-α-D-arabinofuranoside **10**

Pentasaccharide **10** was synthesized using a synthesizer sequence II-III-II-IV as described in **Table S2**, isolated (19.1 mg; 7.40 μmol; 31%) and characterized by NMR

^{1}H NMR (600 MHz, $CDCl_3$) δ 8.04 (d, *J* = 8.0 Hz, 6H), 8.01 (dd, *J* = 7.2, 1.1 Hz, 4H), 7.99–7.96 (m, 4H), 7.95–7.89 (m, 4H), 7.51 (dt, *J* = 16.5, 7.1 Hz, 3H), 7.45–7.23 (m, 39H), 7.23–7.07 (m, 20H), 5.66 (d, *J* = 1.9 Hz, 1H), 5.63 (d, *J* = 1.8 Hz, 1H), 5.59 (d, *J* = 7.8 Hz, 2H, **H$_{Araf}$-1**), 5.52 (d, *J* = 0.9 Hz, 2H), 5.50 (s, 1H), 5.46 (d, *J* = 5.0 Hz, 1H), 5.43 (s, 2H), 5.39 (s, 1H, **H$_{Araf}$-1**), 5.37 (s, 1H, **H$_{Araf}$-1**), 5.18 (s, 1H, **H$_{Araf}$-1**), 5.07 (s, 2H), 5.05 (s, 1H, **H$_{Man}$-1**), 4.99 (s, 1H, **H$_{Man}$-1**), 4.85 (s, 1H), 4.75 (dd, *J* = 16.6, 11.0 Hz, 2H), 4.69 (dd, *J* = 19.5, 12.0 Hz, 2H), 4.54 – 4.36 (m, 11H), 4.15 (dd, *J* = 11.2, 4.7 Hz, 1H), 4.12 – 4.03 (m, 7H), 3.97 – 3.86 (m, 7H), 3.83 (td, *J* = 11.1, 3.3 Hz, 2H), 3.78 – 3.69 (m, 4H), 3.51 – 3.42 (m, 1H), 3.15 (dd, *J* = 12.7, 6.3 Hz, 2H), 1.66 – 1.55 (m, 2H), 1.55 – 1.46 (m, 2H), 1.45 – 1.36 (m, 2H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 165.73 (Bz), 165.68 (Bz), 165.62 (Bz), 165.59 (Bz), 165.57 (Bz), 165.53 (Bz), 165.24 (Bz), 165.16 (Bz), 156.51 (Cbz), 138.68, 138.66, 138.18, 136.85, 133.62, 133.57, 133.42, 133.37, 133.16, 133.10, 130.08, 129.95, 129.46, 129.37, 129.29, 129.24, 128.74, 128.67, 128.62, 128.53, 128.49, 128.44, 128.43, 128.40, 128.33, 128.30, 128.24, 128.23, 128.16, 128.08, 128.05, 128.01, 127.57, 127.55, 127.53, 127.48 (Ar), 106.27 (**C$_{Araf}$-1**), 105.98 (**C$_{Araf}$-1**), 105.71 (**C$_{Araf}$-1**), 105.35 (**C$_{Araf}$-1**), 98.52 (**C$_{Man}$-1**), 98.47 (**C$_{Man}$-1**), 82.72, 82.40, 82.34, 82.21, 82.11, 81.89, 81.79, 80.96, 78.81, 78.75, 77.64, 77.37, 75.26, 75.19, 74.27, 74.20, 73.50, 73.45, 71.99, 71.57, 69.05, 68.99, 68.94, 67.42, 66.84, 66.66, 66.13, 65.97, 41.14, 29.80, 29.20, 23.52.; MS ESI+-HRMS *m/z* $[M+Na]^+$ calcd for $C_{150}H_{143}NO_{38}Na$ 2588.9180, found 2588.9158.

N-Benzyloxycarbonyl-5-amino-pentanyl (2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)-(1→3)-(2-*O*-benzoyl-3,6-di-*O*-benzyl-α-D-galactopyranosyl)-(1→4)-(2,3-di-*O*-benzoyl-6-*O*-benzyl-α-D-glucopyranoside **11**

Trisaccharide **11** was synthesized using a synthesizer sequence V-VI-VII as described in **Table S3**, isolated (10.4 mg; 6.00 μmol; 24%)and characterized by NMR

^{1}H NMR ($CDCl_3$) δ 7.97 – 7.88 (m, 4H), 7.83 (d, *J* = 7.3 Hz, 2H), 7.47 (dd, *J* = 15.3, 7.5 Hz, 2H), 7.40 – 7.06 (m, 47H), 5.55 (m, 2H, H'-2, H-3), 5.31 (br, 1H, H-2), 5.07 (s, 2H, CH_2, Cbz), 4.96 (d, *J* = 11.8 Hz, 1H, CHHPh), 4.90 (d, *J* = 3.3 Hz, 1H, H''-1), 4.78 (dd, *J* = 11.4, 6.0 Hz, 2H, 2 x CHHPh), 4.62–4.42 (m, 6H, H-1, H'-1), 4.37 (d, *J* = 11.3 Hz, 1H, CHHPh), 4.31–4.14 (m, 4H), 4.10-4.00 (m, 3H, H-4, CH_2Ph), 3.97 (dd, *J* = 10.1, 3.3 Hz, 1H, H''-2), 3.90–3.83 (m, 2H, H'-4, H''-5), 3.81 (br, 1H, -OCHH$(CH_2)_4$), 3.73 (dd, *J* = 10.1, 2.5 Hz, 1H, H''-3), 3.67–3.46 (m, 5H, H'-3, H''-4, H-5, H-6), 3.39 (s, 1H, -OCHH$(CH_2)_4$-NHCbz), 3.21 (dd, *J* = 9.9, 7.1 Hz, 2H, H'-5, H''-6), 2.99 (dd, *J* = 8.7, 5.5 Hz, 1H, H''-6), 2.91 (s, 2H, CH_2NHCbz), 2.86 (d, *J* = 6.6 Hz, 2H, H'-6), 1.54–1.37 (m, 2H, CH_2, pentane), 1.34 – 1.24 (m, 2H, CH_2, pentane), 1.18 (s, 2H, CH_2, pentane). ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.36 (Bz), 165.29 (Bz), 164.84 (Bz), 156.39 (Cbz), 139.39, 138.85, 138.74, 138.55, 138.41, 138.26, 136.88, 133.15, 133.05, 132.58, 130.50, 130.19, 130.03, 129.92, 129.87, 129.76, 128.62, 128.48, 128.41, 128.23, 128.15, 128.12, 128.07, 127.85, 127.80, 127.75, 127.66, 127.61, 127.54, 127.49, 127.43, 127.05 (Ar), 101.27 (**C-1 or C'-1**), 101.14 (**C-1 or C'-1**), 99.11 (**C''-1**), 80.51, 79.04, 77.36, 76.53, 75.63, 74.95, 74.92, 74.85, 74.63, 74.25, 73.80, 73.60, 73.37, 73.28, 73.12, 72.72, 72.26, 69.82, 69.75, 68.11, 68.06, 67.30, 66.62, 40.95, 29.52, 29.03, 23.19.; MS ESI+-HRMS *m/z* $[M+Na]^+$ calcd for $C_{101}H_{103}NO_{21}Na$ 1688.6920, found 1688.6920.

Final deprotection and purification protocols, compounds characterization, analysis and quality control.

Deprotection protocol

To the solution of the purified fully protected oligosaccharide (**9**, **10** or **11**) in methanol (0.2 mL/µmol of oligosaccharide) was added 58 µL of 0.5 M NaOMe solution (0.25 eq. per acetyl of benzoyl group) in methanol at 40 °C. The mixture was stirred until complete conversion, neutralized by the addition of 200 mg of Amberite (400 mg per 100 µL of NaOMe solution). After filtration, the filtrate was dissolved in methanol, ethyl acetate, and acetic acid (v/v/v =5:0.5:0.2) followed by adding 5% palladium on carbon (Pd/C) (50% w/w = Pd/oligosaccharide), purged first with argon and then with hydrogen, and stirred overnight at room temperature under balloon pressure. The reaction mixture was filtered through modified cellulose filter, washed with 20 mL water/methanol (9:1) and the combined solution was evaporated *in vacuo* to provide the crude product.

Purification and characterization of oligosaccharides 12, 13 and 14

Analytical HPLC: The crude material was analyzed by HPLC: column: Hypercarb®, (150 x 4.60 mm); flow rate: 0.8 mL/min; eluents: 0.1% formic acid (FA) in acetonitrile/0.1% FA in water; gradient: 0% (10 min), 30% (in 30 min), 100% (in 5 min); detection: ELSD.

Preparative HPLC: The crude solution is purified to afford the unprotected oligosaccharide by preparative HPLC: column: Hypercarb®, (150 X 10.00 mm); flow rate: 3.6 mL/min; eluents: gradient: 0.1% FA in acetonitrile/0.1% FA in water; gradient: 0% (10 min), 30% (in 30 min), 100% (in 5 min); detection: ELSD. HPLC Chromatograms of oligosaccharides **12**, **13** and **14** are presented in **Figures S7-S9**.

5-Amino-pentanyl α-D-arabinofuranosyl-(1→5)-α-D-arabinofuranosyl-(1→5)-α-D-arabinofuranosyl-(1→6)-α-D-mannopyranosyl-(1→6)-α-D-mannopyranosyl-(1→6)-α-D-mannopyranoside **12** (5.3 mg; 5.37 µmol; 56% over two steps)

^{1}H NMR (600 MHz, D_2O) δ 8.50 (s, 1H, HCO_2H), 5.14 (s, 2H, 2 x **H-1**), 5.13 (d, *J* = 1.5 Hz, 1H, **H-1**), 4.95 (d, *J* = 1.6 Hz, 1H, **H-1**), 4.93 (d, *J* = 1.5 Hz, 1H, **H-1**), 4.90 (d, *J* = 1.6 Hz, 1H, **H-1**), 4.26 (dq, *J* = 8.7, 2.9 Hz, 2H), 4.18 (tt, *J* = 15.2, 6.8 Hz, 3H), 4.14 (td, *J* = 5.9, 3.3 Hz, 1H), 4.06 (dt, *J* = 6.0, 2.9 Hz, 2H), 4.03 (dt, *J* = 3.4, 1.8 Hz, 2H), 4.02–3.97 (m, 5H), 3.95–3.74 (m, 19H), 3.61 (dt, *J* = 10.0, 6.2 Hz, 1H), 3.07–3.03 (m, 2H), 1.78–1.65 (m, 4H), 1.58–1.40 (m, 2H). ^{13}C NMR (600 MHz, D_2O) δ 173.62 (HCO_2H), 110.11 (**C-1**), 110.03 (**C-1**), 109.88 (**C-1**), 102.52 (**C-1**), 102.04 (**C-1**), 101.97 (**C-1**), 86.59, 84.96, 84.72, 83.54, 83.47, 83.44, 79.35, 79.30, 79.15, 73.61, 73.57, 73.52, 73.45, 73.35, 73.20, 72.70, 72.62, 72.57, 70.25, 69.49, 69.40, 69.28, 69.22, 69.21, 68.96, 68.32, 68.23, 63.81, 42.02, 30.66, 29.20, 25.16.; MS ESI+-HRMS *m/z* $[M+Na]^+$ calcd for $C_{38}H_{67}NO_{28}Na$ 1008.3742, found 1008.3778.

5-Amino-pentanyl α-D-mannopyranosyl-(1→5)-α-D-arabinofuranosyl-(1→5)-[α-D-mannopyranosyl-(1→5)-α-D-arabinofuranosyl-(1→3)]-(α-D-arabinofuranosyl)-(1→5)-α-D-arabinofuranoside **13** (3.1 mg; 3.24 µmol; 55%)

^{1}H NMR (600 MHz, D_2O) δ 8.32 (s, 1H, HCO_2H), 5.02 (d, *J* = 1.3 Hz, 1H**, H-1**), 4.98 (s, 1H**, H-1**), 4.96 (d, *J* = 1.3 Hz, 1H**, H-1**), 4.88 (d, *J* = 2.0 Hz, 1H**, H-1**), 4.79 (d, *J* = 1.7 Hz, 2H**, 2 x H-1**), 4.19–4.13 (m, 2H), 4.08 (td, *J* = 5.5, 3.0 Hz, 1H), 4.02 (ddd, *J* = 9.5, 4.5, 2.3 Hz, 4H), 3.95 (dd, *J* = 5.4, 2.1 Hz, 1H), 3.94–3.88 (m, 4H), 3.87–3.85 (m, 2H), 3.82 (dd, *J* = 11.4, 5.6 Hz, 1H), 3.73 (dddd, *J* = 15.5, 12.5, 9.0, 5.5 Hz, 8H), 3.66 (t, *J* = 4.2 Hz, 1H), 3.63 (ddd, *J* = 13.2, 6.8, 4.1 Hz, 5H), 3.57–3.50 (m, 4H), 3.45 (dt, *J* = 10.0, 6.4 Hz, 1H), 2.90–2.86 (m, 2H), 1.60–1.48 (m, 4H), 1.36–1.27 (m, 2H). ^{13}C NMR (600 MHz, D_2O) δ 173.62 (HCO_2H), 109.98 (**2 x C-1**), 109.83 (**C-1**), 109.72 (**C-1**), 102.42 (**C-1**), 102.39 (**C-1**), 85.07, 84.68, 84.55, 84.29, 84.26, 83.89, 83.61, 83.43, 81.70, 79.14, 79.09, 78.85, 75.53, 75.51, 73.15, 73.10, 72.50, 70.70, 69.30, 69.16, 68.88, 68.64, 68.47, 63.53, 41.97, 30.67, 29.04, 24.86.; MS ESI+-HRMS *m/z* $[M+H]^+$ calcd for $C_{38}H_{68}NO_{28}$ 956.3817, found 956.3834.

5-Amino-pentanyl α-D-galactopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside **14**[7] (1.7 mg; 2.88 µmol; 46%)

^{1}H NMR (600 MHz, D_2O) δ 8.48 (s, 1H, HCO_2H), 5.17 (d, *J* = 4.0 Hz, 1H, H, H''-1), 4.55 (d, *J* = 8.0 Hz, 1H, H-1), 4.52 (d, *J* = 8.1 Hz, 1H, H-1), 4.25 – 4.19 (m, 2H), 4.05 (d, *J* = 3.1 Hz, 1H), 4.02 (dd, *J* = 12.3, 1.8 Hz, 1H), 3.99–3.94 (m, 2H), 3.89 (dd, *J* = 10.4, 3.9 Hz, 1H), 3.86–3.65 (m, 12H), 3.64–3.61 (m, 1H), 3.36–3.32 (m, 1H), 2.98 (t, *J* = 7.3 Hz, 2H, $OCH_2(CH_2)_4NH_2$), 1.73–1.66 (m, 4H, $OCH_2CH_2CH_2CH_2CH_2NH_2$), 1.52–1.44 (m, 2H $OCH_2CH_2CH_2CH_2CH_2NH_2$). ^{13}C NMR (600 MHz, D_2O) δ 173.63 (CO_2H), 105.45 (C-1), 104.62(C-1), 98.03(C''-1), 81.28, 79.81, 77.66, 77.35, 77.13, 75.41, 73.44, 72.75, 72.18, 71.89, 71.73, 70.79, 67.41, 63.59, 63.53, 62.77, 42.09 (CH_2NH_2), 30.80, 29.63, 24.73.; MS ESI+-HRMS *m/z* $[M+Na]^+$ calcd for $C_{23}H_{44}NO_{16}Na$ 590.2655, found 590.2644.

Quality control and sequence analysis of oligosaccharides 12-14

Ionmobility-mass spectrometry (IM-MS) was used to analyze the stereo-purity of the resulting oligosaccharides. In addition, MS/MS prior to the ion mobility separation was used to sequence the oligosaccharides. All analyses were performed on a Synapt G2-S HDMS (Waters Corporation, Manchester).(9, 10) Samples were dissolved in water/methanol (1:1, v/v) and ionized using a nano-electrospray source (nESI) from platinum-palladium-coated borosilicate capillaries prepared in-house.

For each measurement, high and low intensity scans were performed. The high intensity scan (an average signal intensity of at least 10^4 counts per second) was used to identify components and impurities of low concentration. Low intensity scans (approximately 10^3 counts per second) are performed to avoid peak broadening caused by detector saturation, which results in narrower peaks for molecules of high concentration. As a result, a better separation, especially of poorly resolved species, can generally be obtained. Molecule **14** was studied previously (2); IM-MS data of **12** and **13** are shown in **Figures S9-S12**.

Typical settings for positive ion mode: Source temperature, 25 °C; needle voltage, 1.0 kV; sample cone voltage, 25 V; source offset, 25 V. Ion mobility parameters were: trap gas flow, 2 mL/min; helium cell gas flow, 180 mL/min; IM gas flow, 90 mL/min; drift time trimming, 5 bins; mobility delay after trap release, 0 µs; trap DC entrance, 3 V; trap DC bias, 35 V; trap DC exit, 0 V; IM wave velocity, 800 m/s; IM wave height, 40 V; for MS/MS: trap collision energy, 42 V.

Typical settings for negative ion mode: Source temperature, 25 °C; needle voltage, 0.8 kV; sample cone voltage, 25 V, source offset, 25 V. Ion mobility parameters were: trap gas flow, 2 mL/min; helium cell gas flow, 180 mL/min; IM gas flow, 90 mL/min; mobility delay after trap release, 1000 µs; trap DC entrance, 3 V; trap DC bias, 45 V; trap DC exit 0 V; IM wave velocity, 600 m/s; IM wave height, 40 V; for MS/MS: trap collision energy, 55-70 V.

Collision cross sections (CCSs) can be determined from the drift times of the ions by either using a calibration for travelling wave IM-MS instruments or using the Mason-Schamp equations when drift tube IM-MS instruments are used.(11-13) Here, a Synapt instrument that was modified with a linear drift cell, using a design reported previously (14), was used to measure absolute CCSs in the drift gas nitrogen. Drift times were measured at an IMS gas pressure of 2.2 Torr nitrogen at eight different drift voltages. Details of the experimental procedure can be found elsewhere.(13, 15)

An MS/MS study is performed to further confirm composition of the oligosaccharides **12** and **13** **(Figures S12** and **S13)**. The MS/MS study provides the monosaccharide fingerprint of the oligosaccharides. This analysis is very useful for the sequencing of **12** and **13** as it allows to clearly differentiate between the arabinose and mannose monosaccharides. For MS/MS experiments the

species of interest were selected in the quadrupole and fragmented in the trap cell of the instrument by collision-induced dissociation using argon as a collision gas. The resulting fragments are subsequently separated in the ion mobility cell and their *m/z* and drift times are measured.

SI Tables

Table S1 | The set of sequences used for the automated oligosaccharide synthesis of 9

Glycosylation sequence	**Module**	**Details**	**Condition**	**Repeating cycle**
I	1	0.5 mL TMSOTf solution	- 20 °C for 1 min	*once*
	2	5 eq. BB **2**, 5 eq. NIS solution	T_1 = - 40 °C T_2 = - 10 °C	*twice*
	3	20% TEA in DMF	room temperature for 5 min	*twice*
I	1	0.5 mL TMSOTf solution	- 20 °C for 1 min	*once*
	2	5 eq. BB **2**, 5 eq. NIS solution	T_1 = - 40 °C T_2 = - 10 °C	*twice*
	3	20% TEA in DMF	room temperature for 5 min	*twice*
I	1	0.5 mL TMSOTf solution	- 20 °C for 1 min	*once*
	2	5 eq. BB **2**, 5 eq. NIS solution	T_1 = - 40 °C T_2 = - 10 °C	*twice*
	3	20% TEA in DMF	room temperature for 5 min	*twice*
II	1	0.5 mL TMSOTf solution	- 20 °C for 1 min	*once*
	2	5 eq. BB **3**, 5 eq. NIS solution	T_1 = - 40 °C T_2 = - 10 °C	*twice*
	3	20% TEA in DMF	room temperature for 5 min	*twice*
II	1	0.5 mL TMSOTf solution	- 20 °C for 1 min	*once*
	2	5 eq. BB **3**, 5 eq. NIS solution	T_1 = - 40 °C T_2 = - 10 °C	*twice*
	3	20% TEA in DMF	room temperature for 5 min	*twice*
II	1	0.5 mL TMSOTf solution	- 20 °C for 1 min	*once*
	2	5 eq. BB **3**, 5 eq. NIS solution	T_1 = - 40 °C T_2 = - 10 °C	*twice*
	3	20% TEA in DMF	room temperature for 5 min	*twice*

Table S2 | Glycosylation sequences used for the automated oligosaccharide synthesis of **10**

Glycosylation sequence	Module	Details	Condition	Repeating cycle
II	1	0.5 mL TMSOTf solution	- 20 °C for 1 min	*once*
	2	5 eq. BB **3**, 5 eq. NIS solution	T_1 = - 40 °C T_2 = - 10 °C	*twice*
	3	20% TEA in DMF	room temperature for 5 min	*twice*
III	1	0.5 mL TMSOTf solution	- 20 °C for 1 min	*once*
	2	5 eq. BB **4**, 5 eq. NIS solution	T_1 = - 40 °C T_2 = - 10 °C	*twice*
	3	20% TEA in DMF	room temperature for 5 min	*twice*
II	1	0.5 mL TMSOTf solution	- 20 °C for 1 min	*once*
	2	5 eq. BB **3**, 5 eq. NIS solution	T_1 = - 40 °C T_2 = - 10 °C	*four times*
	3	20% TEA in DMF	room temperature for 5 min	*twice*
IV	1	0.5 mL TMSOTf solution	- 20 °C for 1 min	*once*
	2	5 eq. BB **5**, 5 eq. NIS solution	T_1 = - 40 °C T_2 = - 10 °C	*four times*
	3	20% TEA in DMF	room temperature for 5 min	*twice*

Table S3 | Glycosylation sequences used for the automated oligosaccharide synthesis of **11**

Glycosylation sequence	Module	Details	Condition	Repeating cycle
V	1	0.5 mL TMSOTf solution	- 20 °C for 1 min	*once*
	2	5 eq. BB **6**, 5 eq. NIS solution	T_1 = - 40 °C T_2 = - 10 °C	*twice*
	3	20% TEA in DMF	room temperature for 5 min	*twice*
VI	1	0.5 mL TMSOTf solution	- 20 °C for 1 min	*once*
	2	5 eq. BB **7**, 5 eq. NIS solution	T_1 = - 40 °C T_2 = - 10 °C	*twice*
	3	20% TEA in DMF	room temperature for 5 min	*twice*
VII	1	0.5 mL TMSOTf solution	- 20 °C for 1 min	*once*
	2	5 eq. BB **8**, 5 eq. NIS solution	T_1 = - 40 °C T_2 = - 10 °C	*twice*
	3	20% TEA in DMF	room temperature for 5 min	*twice*

SI Figures

Figure S1. The Glyconeer 2.1® is a versatile automated platform for the synthesis of glycans on solid support. It features a collection of argon-pressurized bottles for the storage of up to eight solvents (**d**) and 16 reagent bottles of various sizes, two of which can be cooled to -4 °C (**e**). The solvents and reagents are delivered via argon pressure to a triple-jacketed reaction vessel (**b**). The reaction vessel is covered with a jacket that is connected to a computer controlled cryostat that allows to control the temperature in a range that varies from -40 °C to +80 °C. Building blocks can be stored as solids or solutions in the building block carousel for 64 individual vials (**a**). This carousel features a two-way needle to deliver both solvents (from any of the solvents currently attached to the synthesizer) to dissolve solid building blocks as well as deliver the building block solution to the reaction vessel. The contents of the reaction vessel may be mixed, and maintained under an inert argon atmosphere, via bubbling of argon gas through the bottom frit of the vessel. Coupling efficiency can be monitored via a programmable on-line UV detector for the detection of UV transmittance of specific protecting groups release from the resin-bound oligosaccharide. An additional fraction collector allows for the separation of specific reaction mixtures from the main waste stream (**c**). Finally, the Glyconeer 2.1® features user-friendly software allowing the user to program synthetic cycles, add additional "approved" building blocks and their reaction conditions, add and/or adjust reaction modules, and analyze previous runs and results.

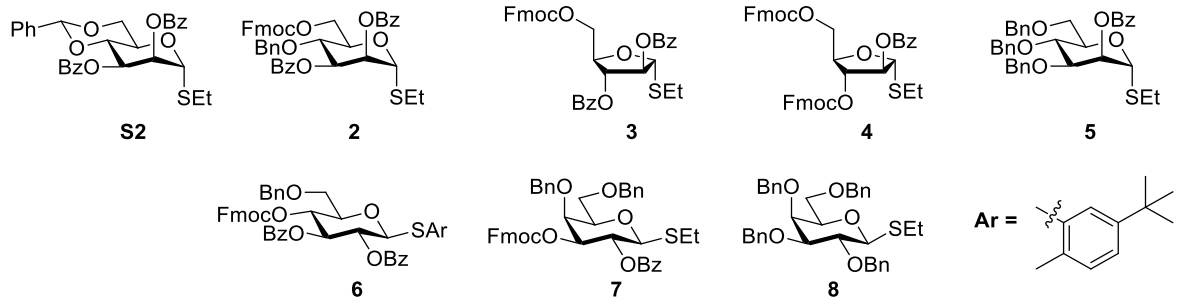


Figure S2. Set of building blocks used for automated synthesis.

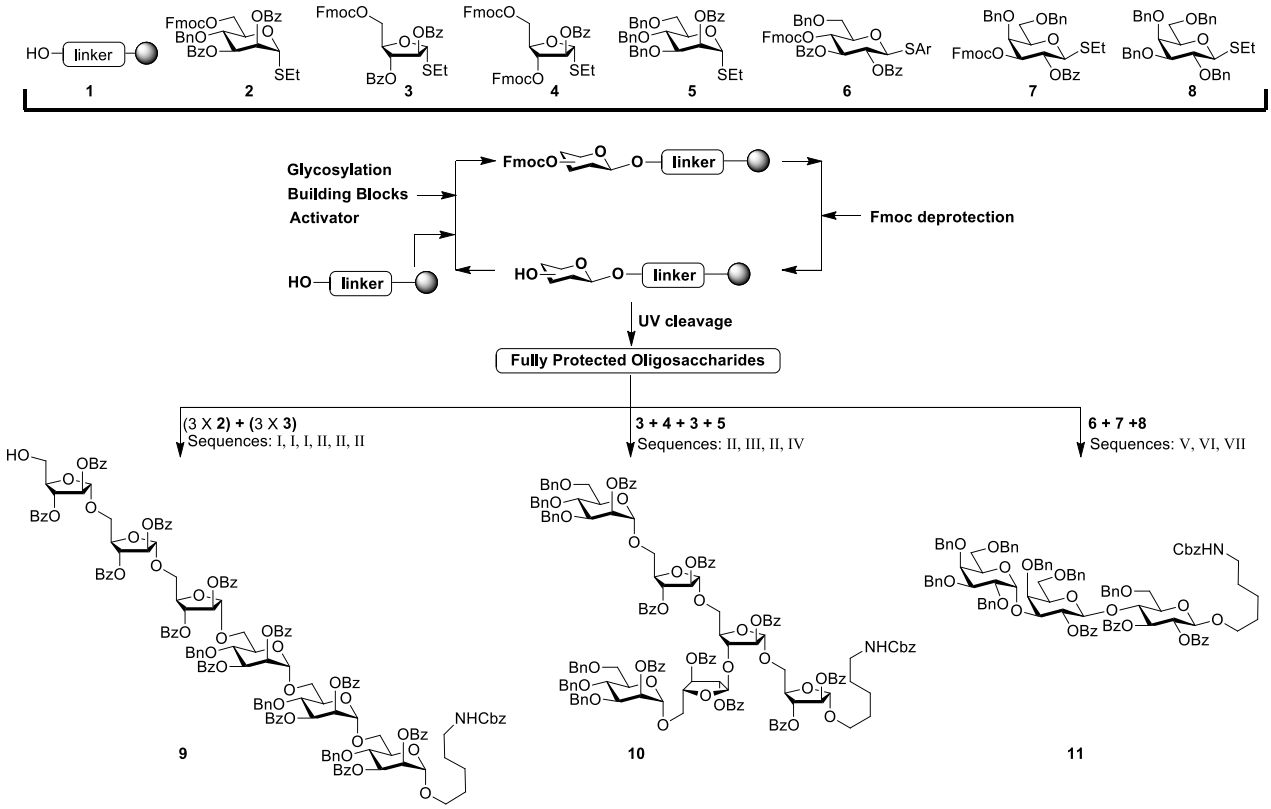


Figure S3 Automated synthesis of oligosaccharides **9**–**11.** The corresponding building block used for each oligosaccharide is presented. The exact automated synthesis protocol is shown in Latin numerals for each of the three oligosaccharides. Functionalized resin **1** (64 mg; loading 0.392 mmol/g; 25.1 μmol) was loaded into the reaction vessel of the synthesizer and swollen in 2 mL DCM. The sequences described in **Supplementary Tables 1-3** were executed to assemble the target protected oligosaccharides **9**, **10** and **11**, respectively.

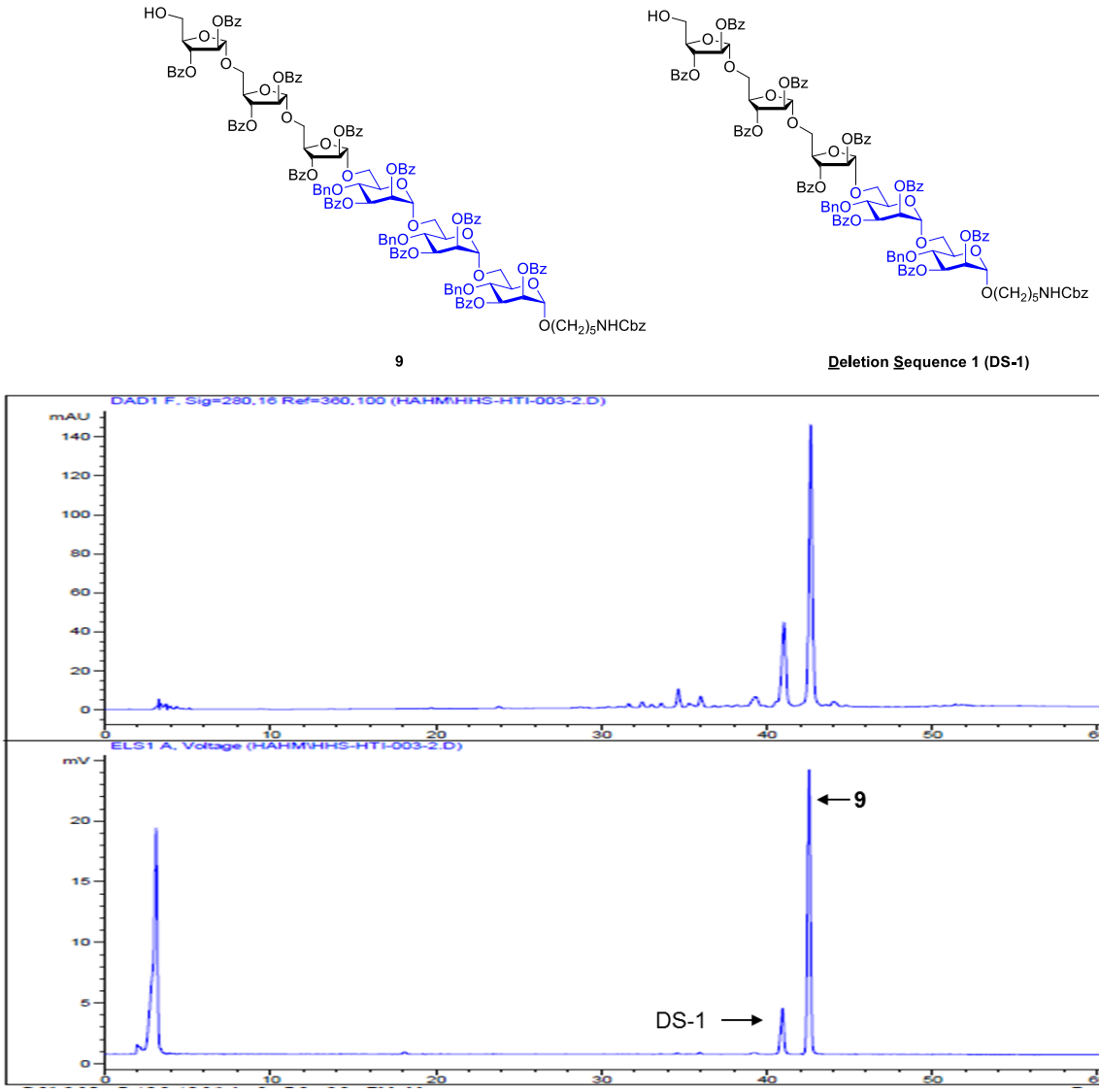
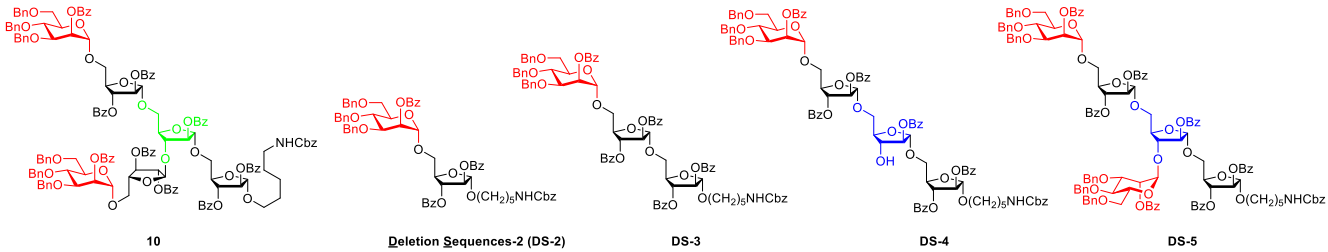


Figure S4 | Purification of hexasaccharide **9** by NP-HPLC. Column: Luna 5µm Silica (260 x 10 mm); flow rate: 5 mL/min; eluents: 5% DCM in hexane/5% DCM in ethyl acetate; gradient: 20% (5 min), 60% (in 40 min), 100% (in 5 min); detection: 280 nm (upper), and ELSD (lower).



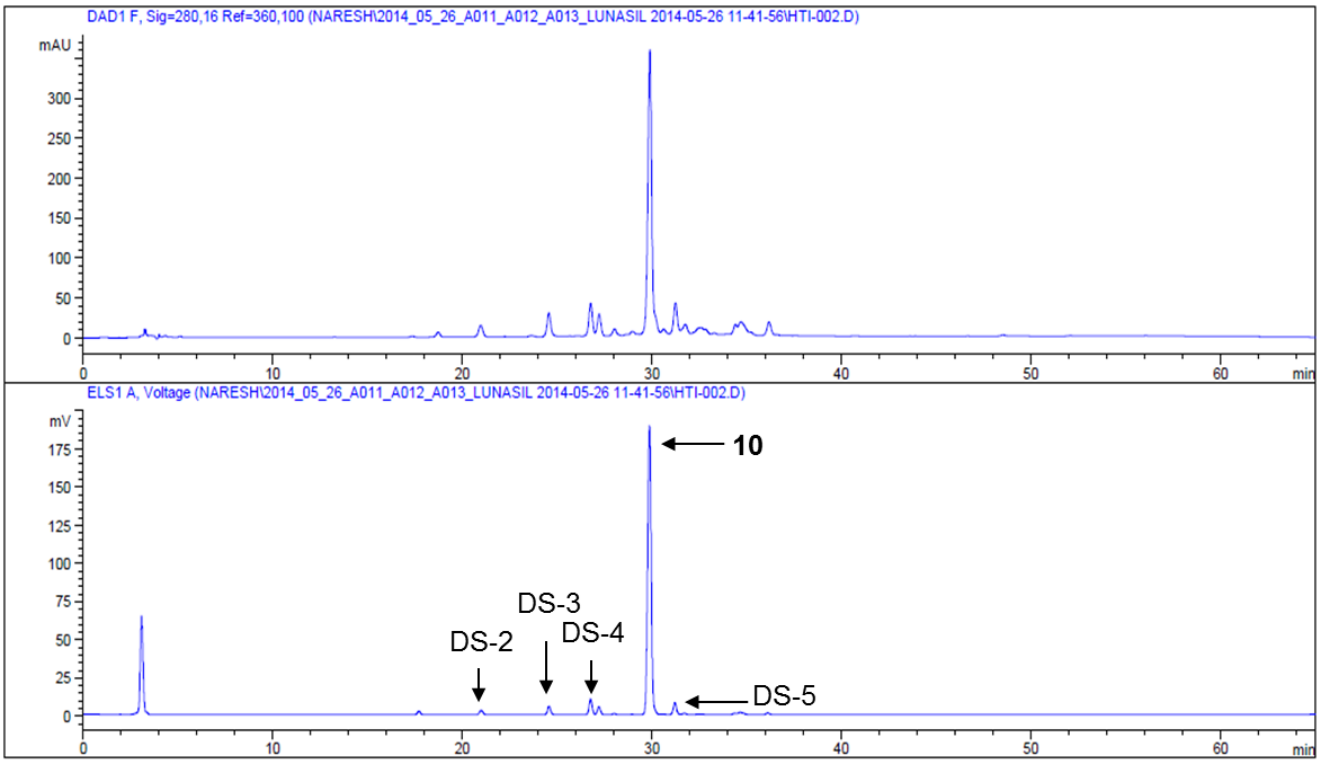
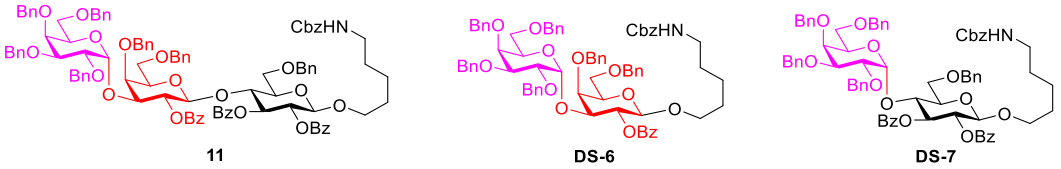


Figure S5. Purification of hexasaccharide **10** by NP-HPLC. Column: Luna 5µm Silica (260 x 10 mm); flow rate: 5 mL/min; eluents: 5% DCM in hexane/5% DCM in ethyl acetate; gradient: 20% (5 min), 60% (in 40 min), 100% (in 5 min); detection: 280 nm (upper), and ELSD (lower).



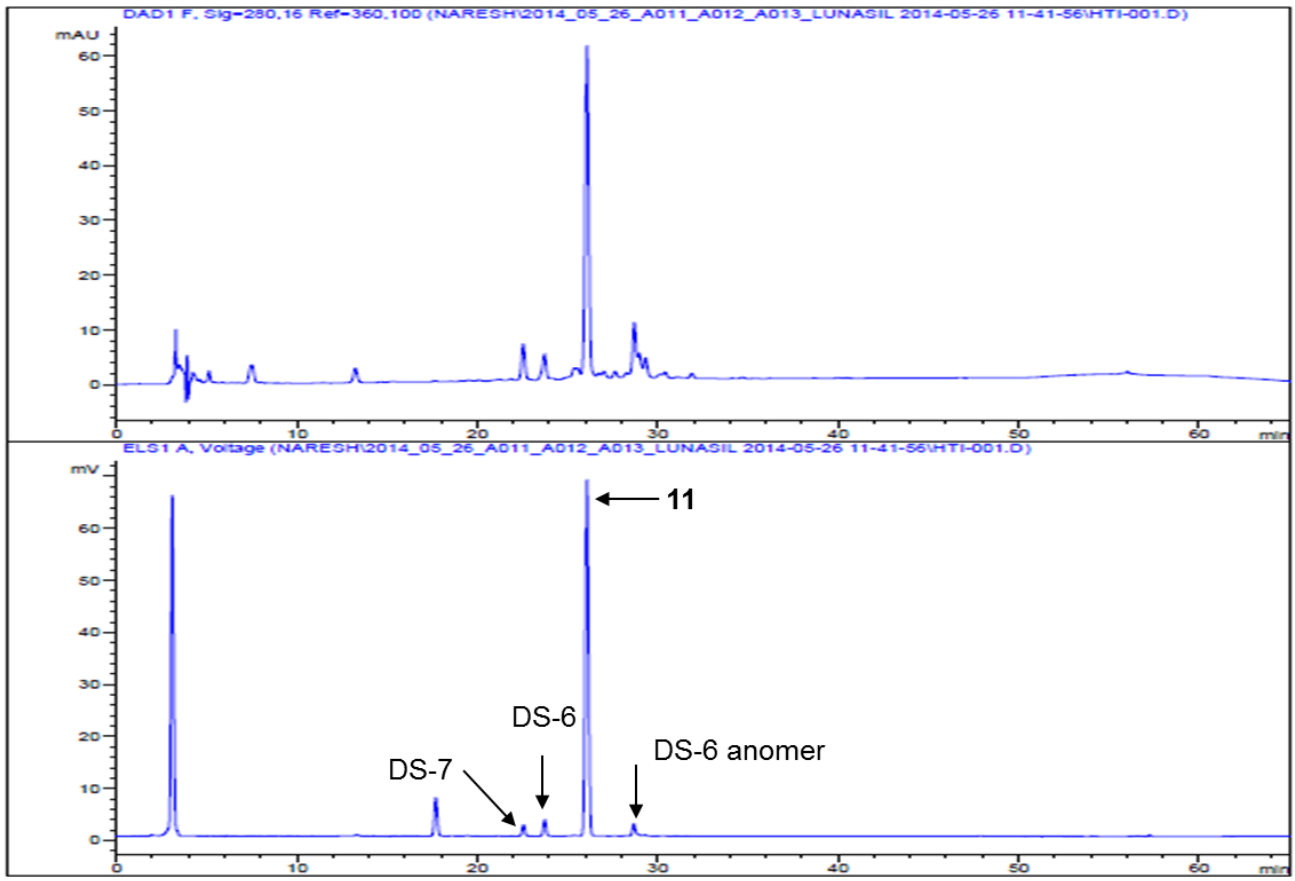


Figure S6. Purification of trisaccharide **11** by NP-HPLC column: Luna 5µm Silica (260 x 10 mm); flow rate: 5 mL/min; eluents: 5% DCM in hexane/5% DCM in ethyl acetate; gradient: 20% (5 min), 60% (in 40 min), 100% (in 5 min); detection: 280 nm (upper), and ELSD (lower).

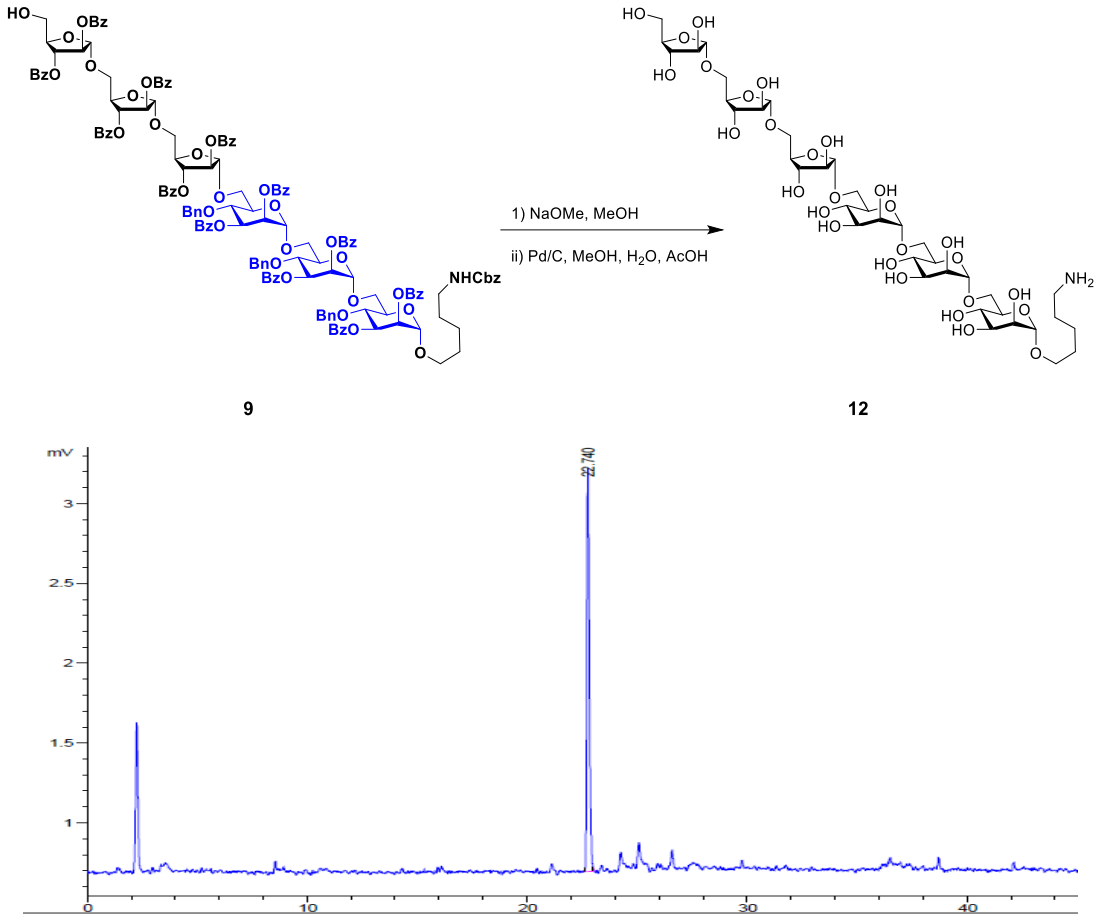


Figure S7. Purification of linear hexasaccharide **12** by Hypercarb HPLC. Column: Hypercarb®, (150 X 10.00 mm); flow rate: 3.6 mL/min; eluents: gradient: 0.1% FA in acetonitrile/0.1% FA in water; gradient: 0% (10 min), 30% (in 30 min), 100% (in 5 min); detection: ELSD.

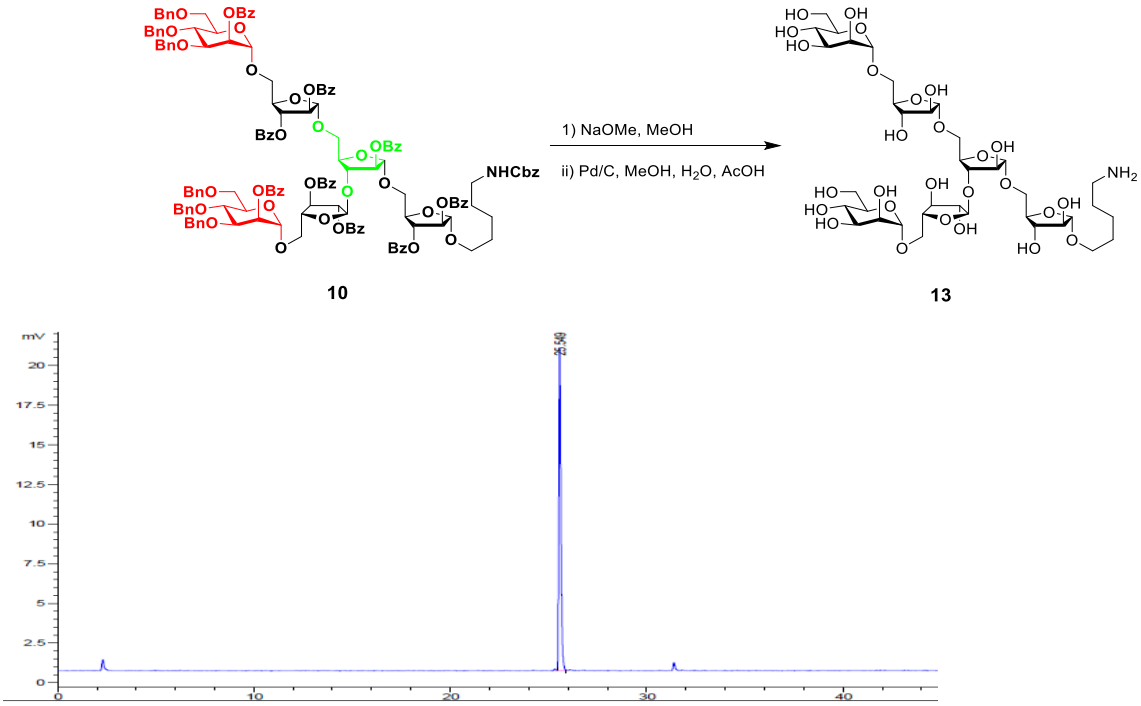


Figure S8. Purification of branched hexasaccharide **13** by Hypercarb HPLC. Column: Hypercarb®, (150 X 10.00 mm); flow rate: 3.6 mL/min; eluents: gradient: 0.1% FA in acetonitrile/0.1% FA in water; gradient: 0% (10 min), 30% (in 30 min), 100% (in 5 min); detection: ELSD.

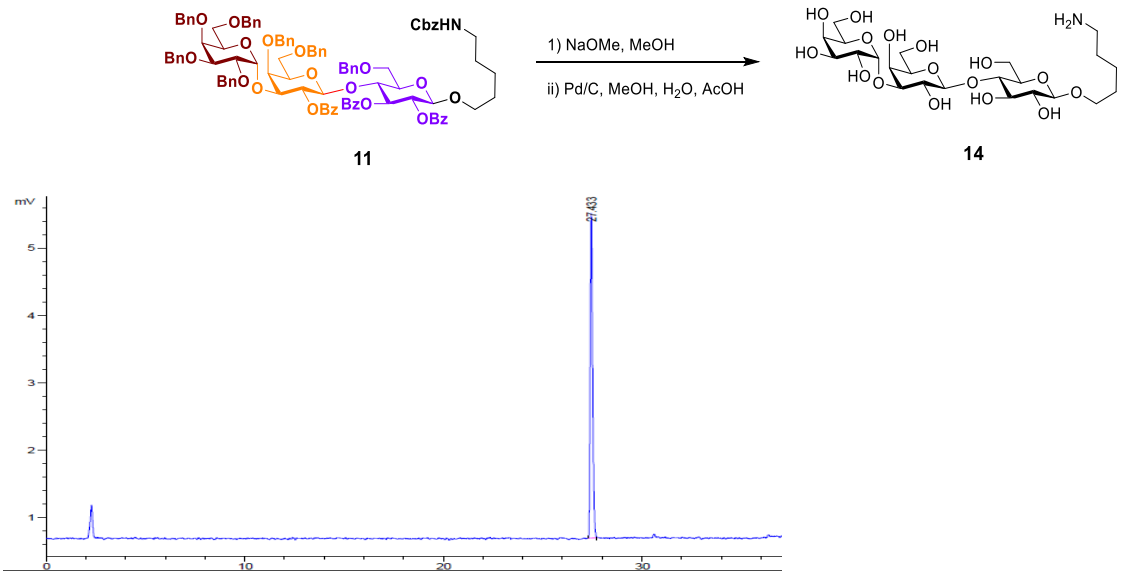


Figure S9. Purification of trisaccharide **14** by Hypercarb HPLC. Column: Hypercarb®, (150 X 10.00 mm); flow rate: 3.6 mL/min; eluents: gradient: 0.1% FA in acetonitrile/0.1% FA in water; gradient: 0% (10 min), 30% (in 30 min), 100% (in 5 min); detection: ELSD.

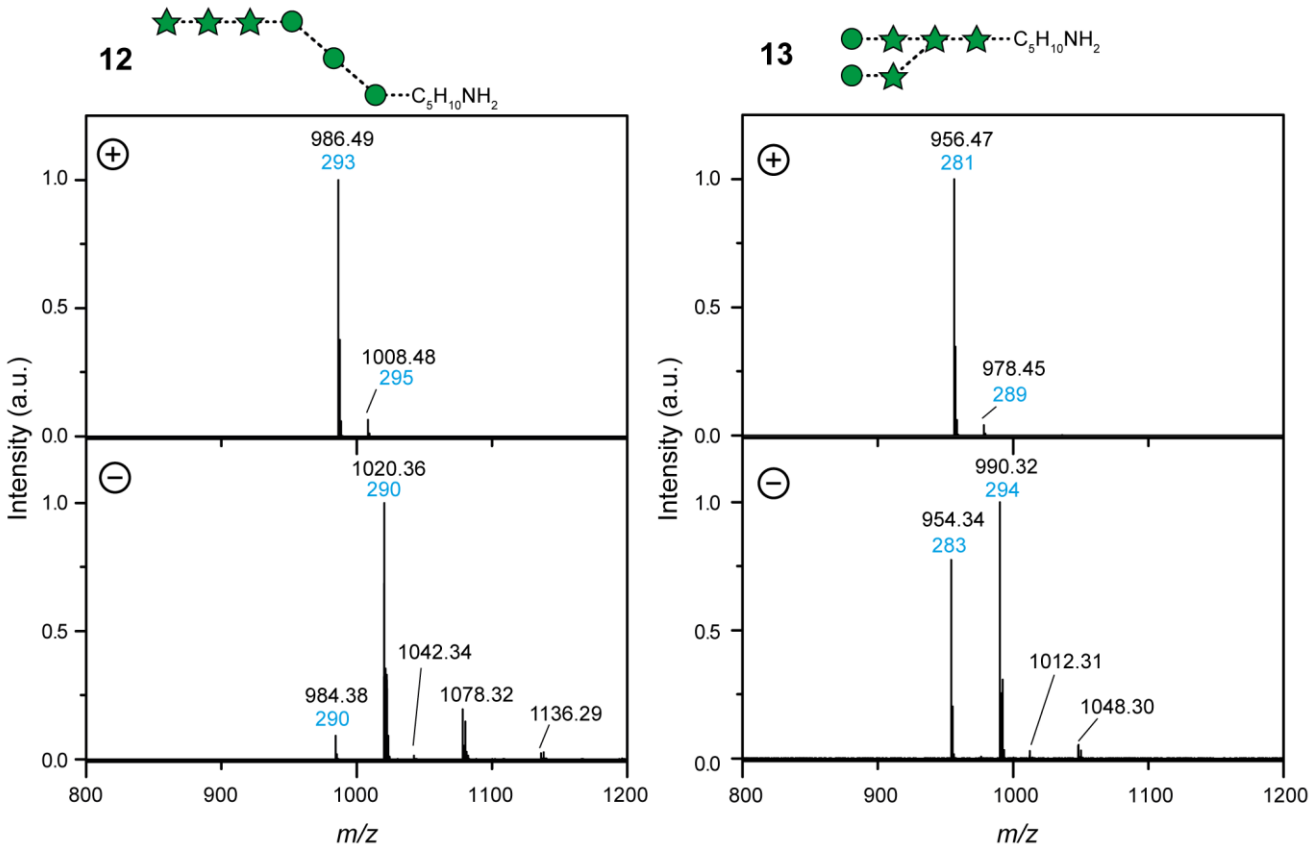


Figure S10. Mass spectra of **12** (Left) and **13** (right) in the positive (top) and negative (bottom) ion mode. *m/z* values are given in black and corresponding absolute nitrogen CCSs in blue. Chemical structures are depicted as cartoons according to the SNFG nomenclature: green circles, mannose; green star, arabinofuranose; horizontal lines, α1,4 bonds; upwards diagonals, α1→6 bonds; downwards diagonals, α1→3 bonds.

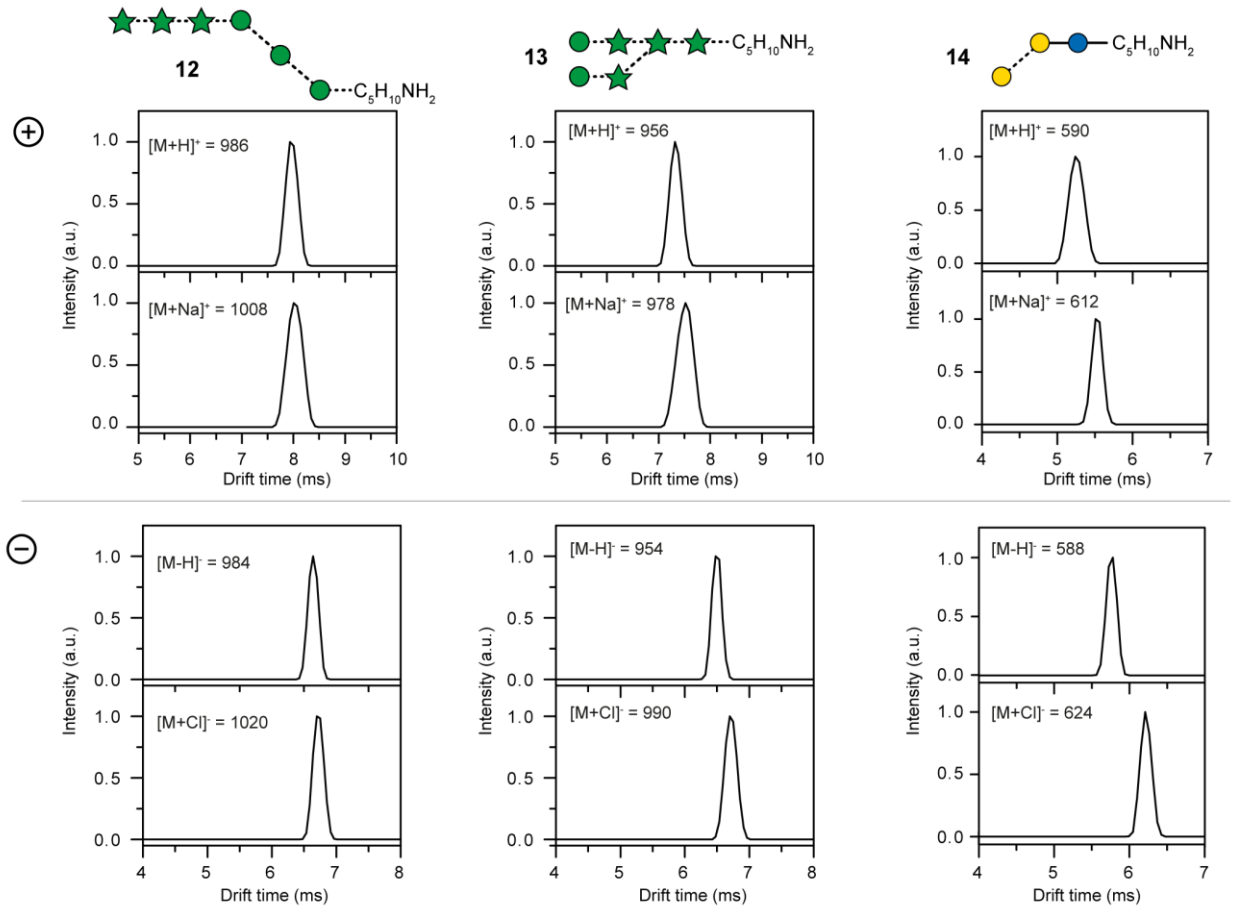


Figure S11. IM-MS arrival time distributions (ATDs) of the intact glycan ions **12-14**. Chemical structures are depicted as cartoons according to the SNFG nomenclature: green circles, mannose; green star, arabinofuranose; yellow circles, galactose; blue circles, glucose; horizontal lines, 1→4 bonds; upwards diagonals, 1→6 bonds; downwards diagonals, 1→3 bonds; dashed lines, α linkages; solid lines, β linkages.

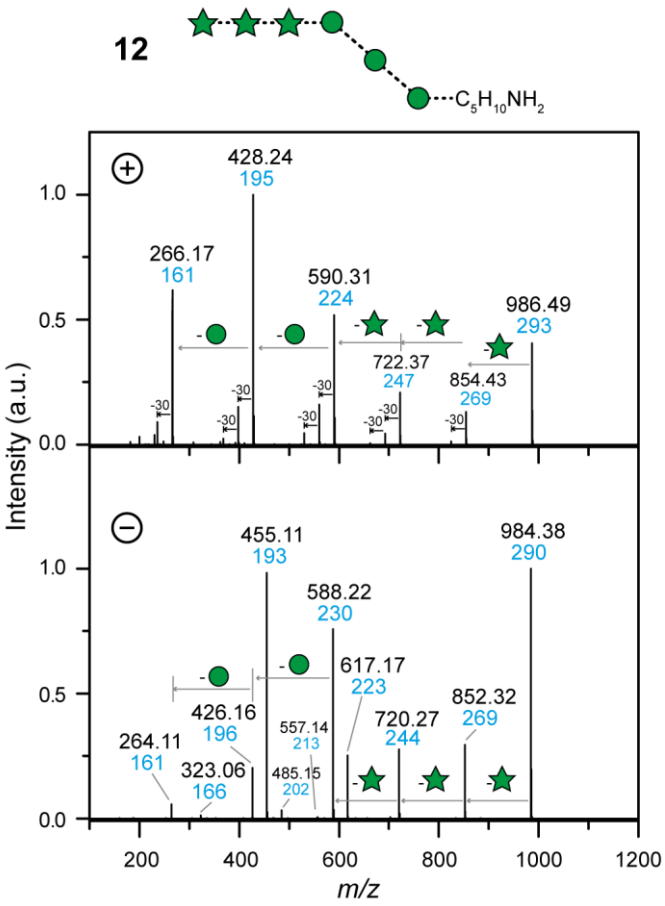


Figure S12. MS/MS spectra of **12** in posivite and negative ion mode. A sequential loss of monocaccharides is obverved. *m/z* values are given in black and corresponding absolute nitrogen CCSs in blue.

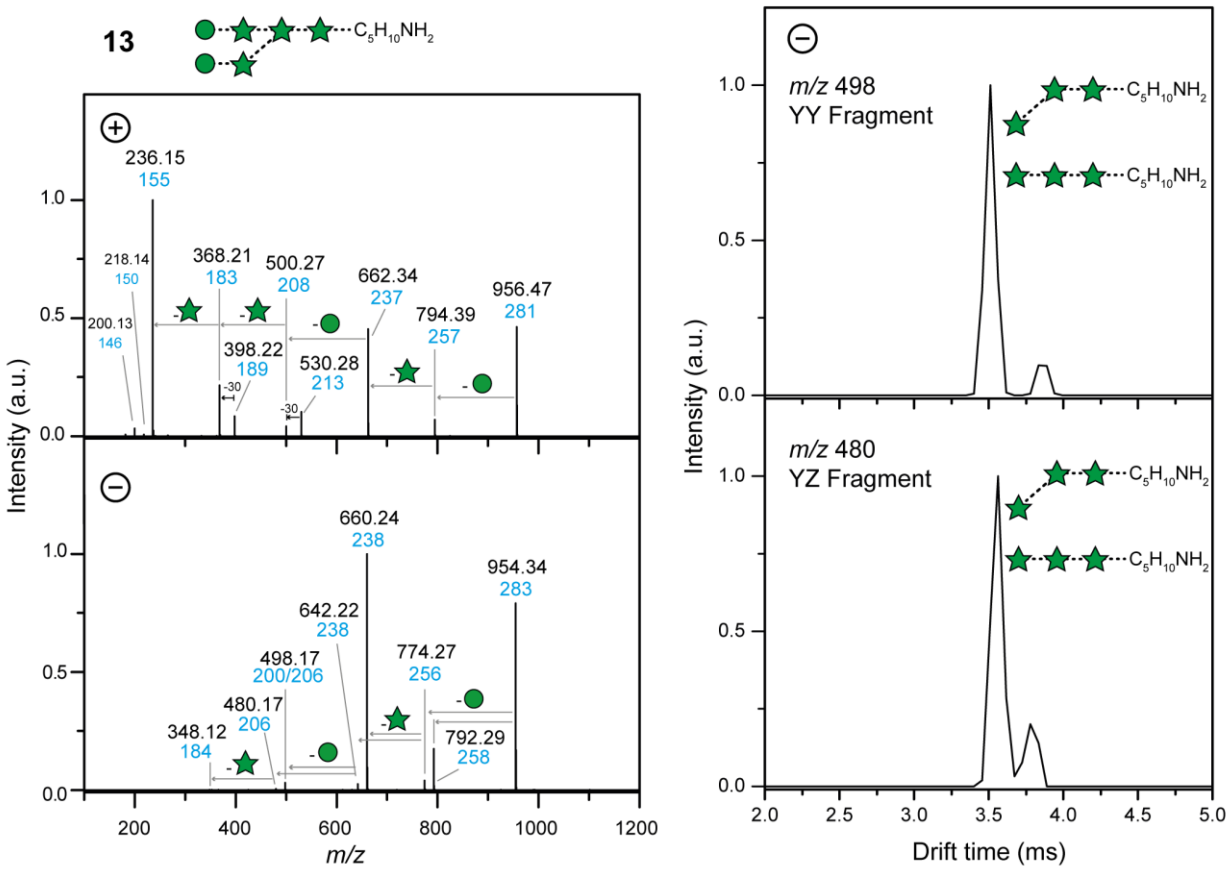


Figure S13. MS/MS spectra of **13** in posivite and negative ion mode. Due to the branced structure of the molecule, two possible trisaccharide fragments can occour during collision-induced dissociation. As a result, two drift peaks are observed for the deprotonated ions *m/z* 498 and 480 (right panel). *m/z* values are given in black and corresponding absolute nitrogen CCSs in blue.

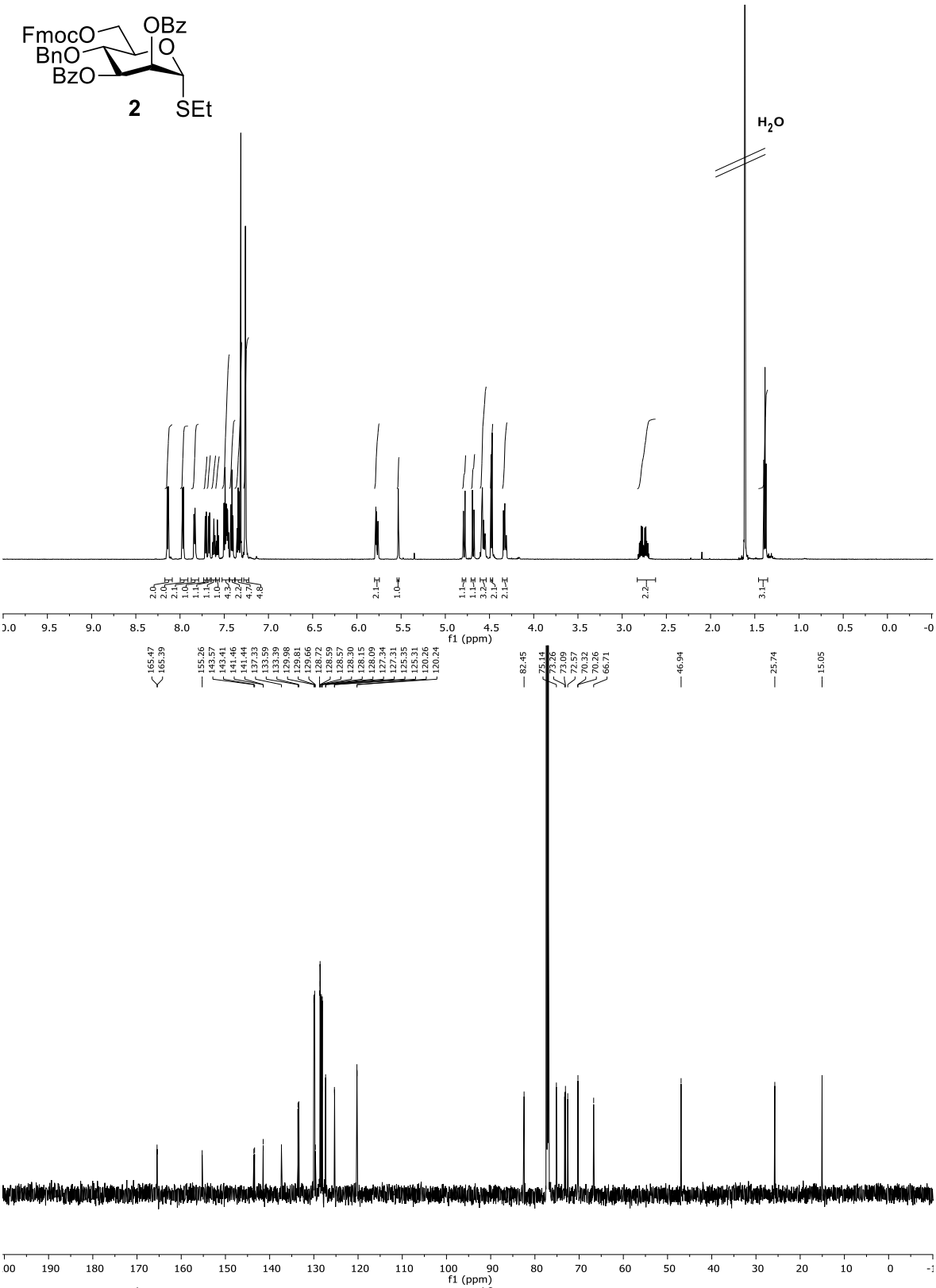


Figure S14. ^{1}H NMR (400 MHz, CDCl3) and ^{13}C NMR (125 MHz, $CDCl_3$) of **2**

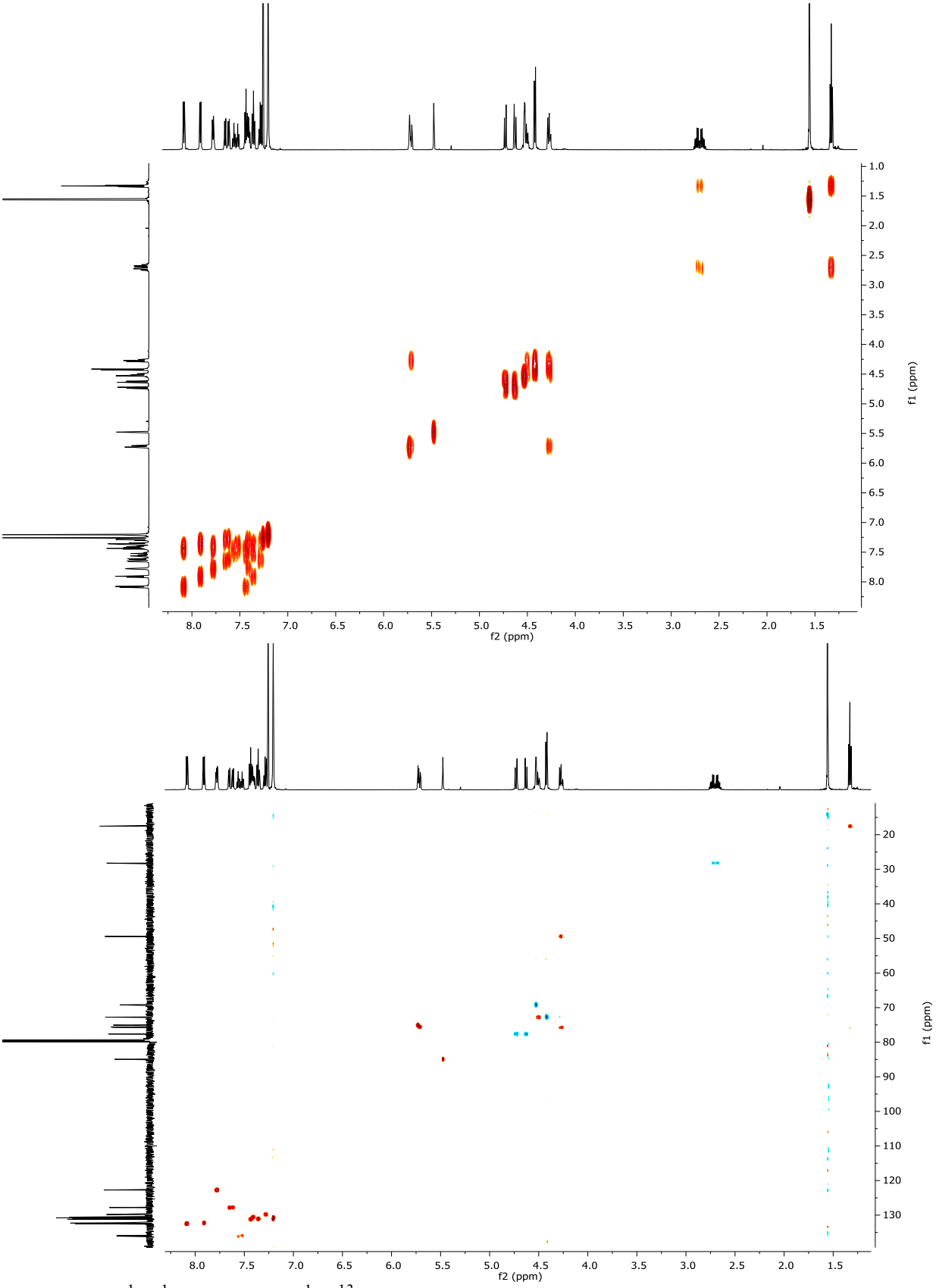


Figure S15. ^{1}H-^{1}H COSY and ^{1}H-^{13}C HSQC (400 MHz, $CDCl_3$) of **2**

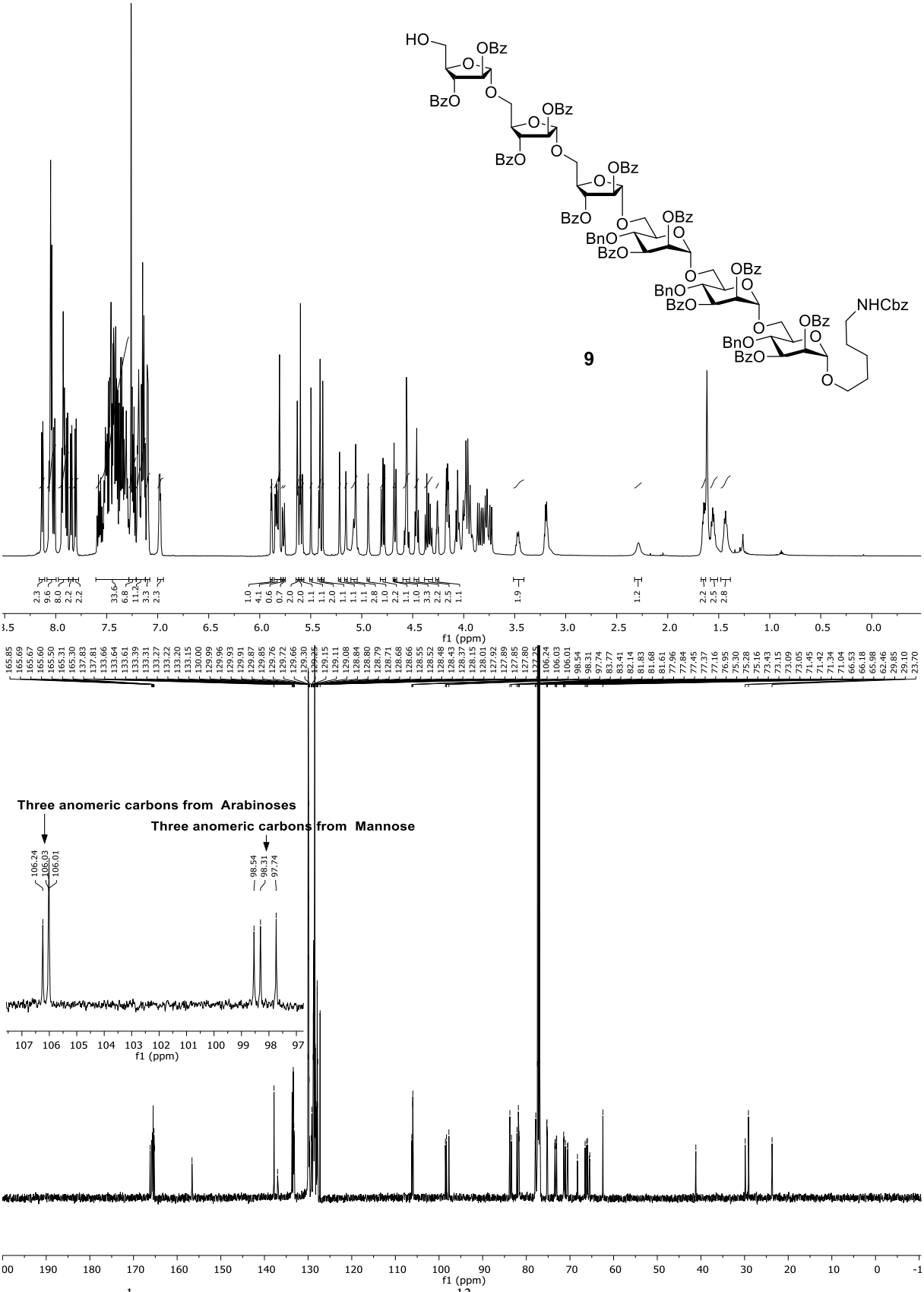


Figure S16. ^{1}H NMR (600 MHz, CDCl3) and ^{13}C NMR (150 MHz, $CDCl_3$) of **9**

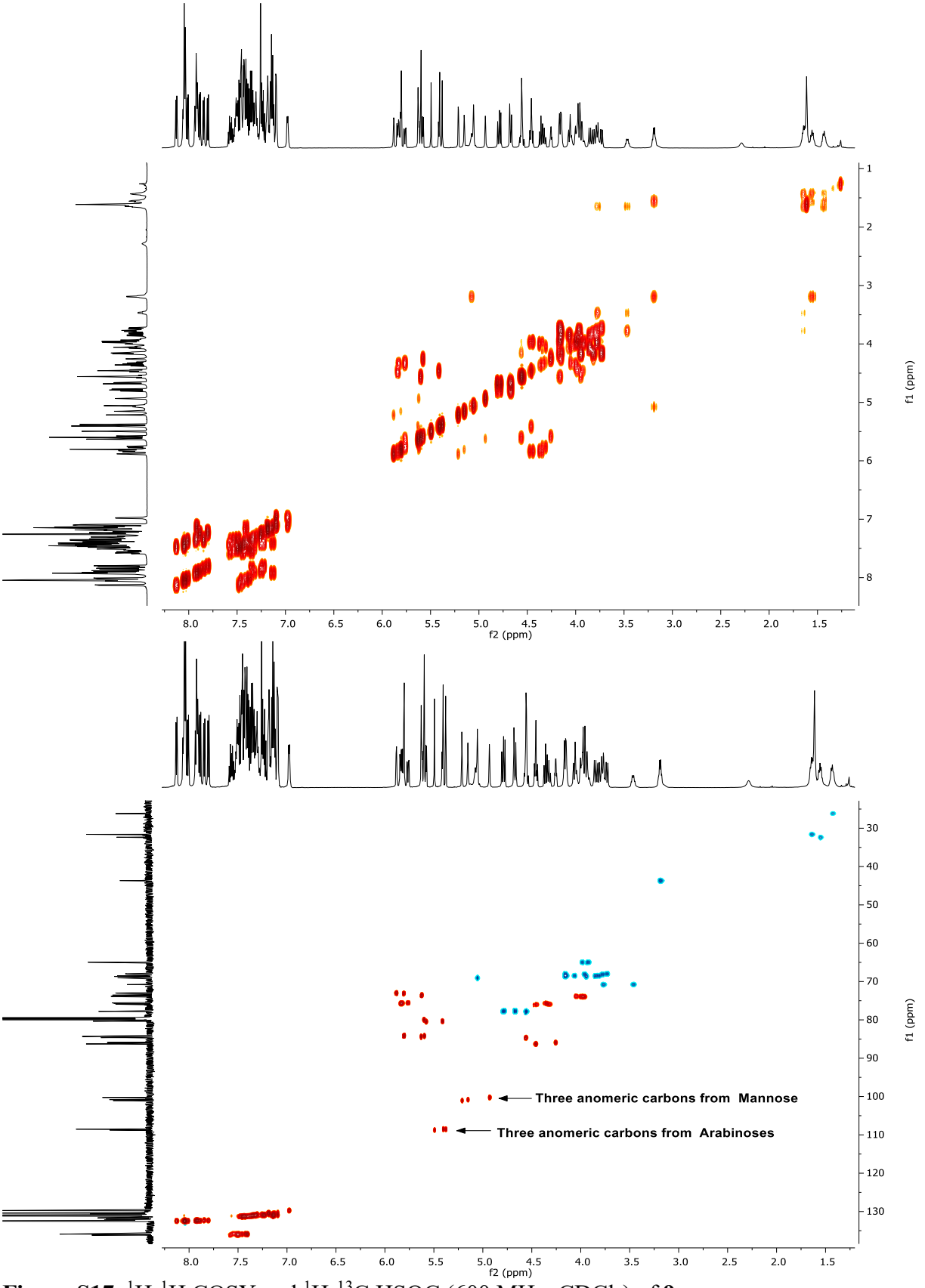


Figure S17. ^{1}H-^{1}H COSY and ^{1}H-^{13}C HSQC (600 MHz, $CDCl_3$) of **9**

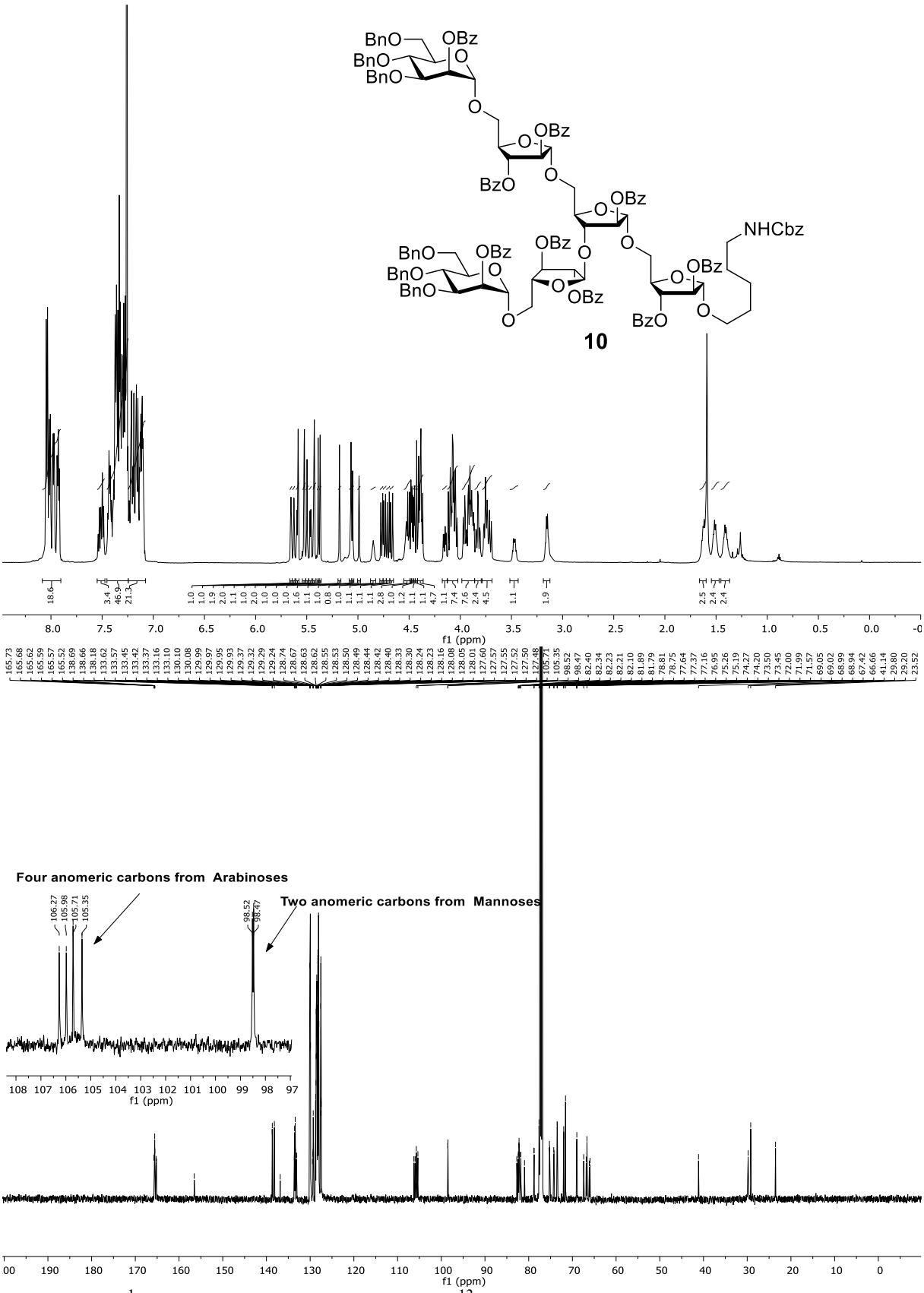


Figure S18. ^{1}H NMR (600 MHz, CDCl3) and ^{13}C NMR (150 MHz, $CDCl_3$) of **10**

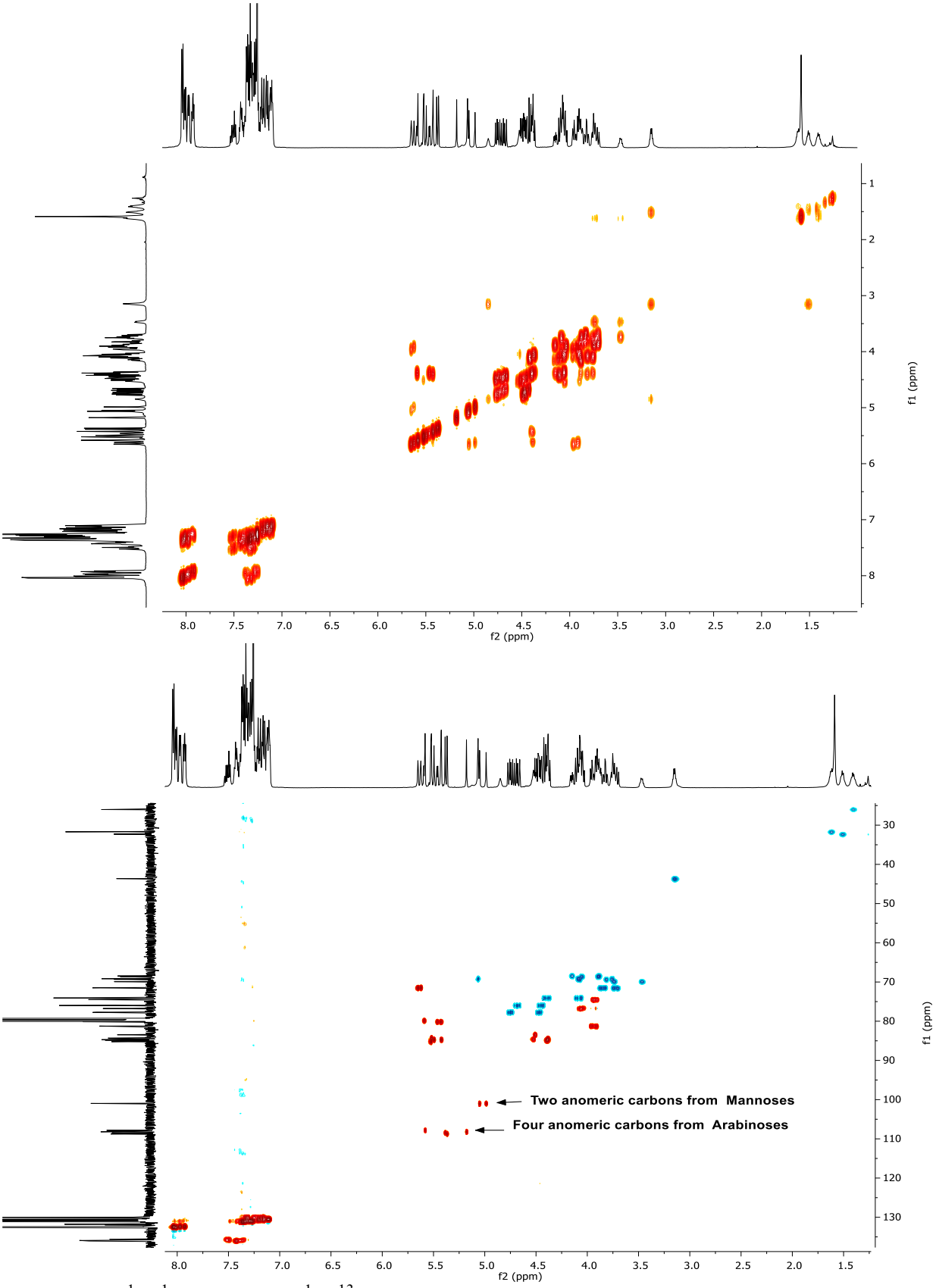


Figure S19. ^{1}H-^{1}H COSY and ^{1}H-^{13}C HSQC (600 MHz, $CDCl_3$) of **10**

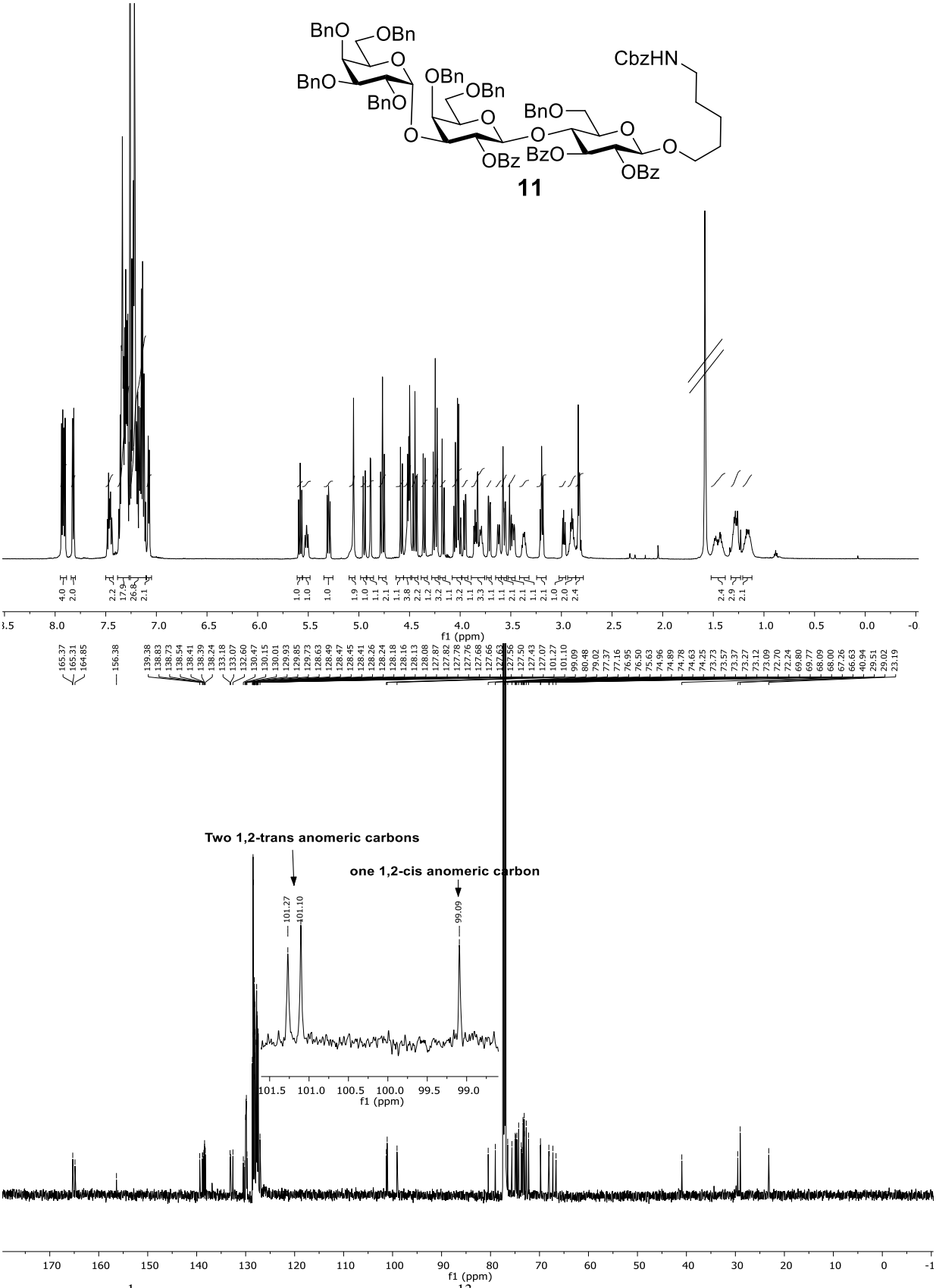


Figure S20. ^{1}H NMR (400 MHz, CDCl3) and ^{13}C NMR (125 MHz, $CDCl_3$) of **11**

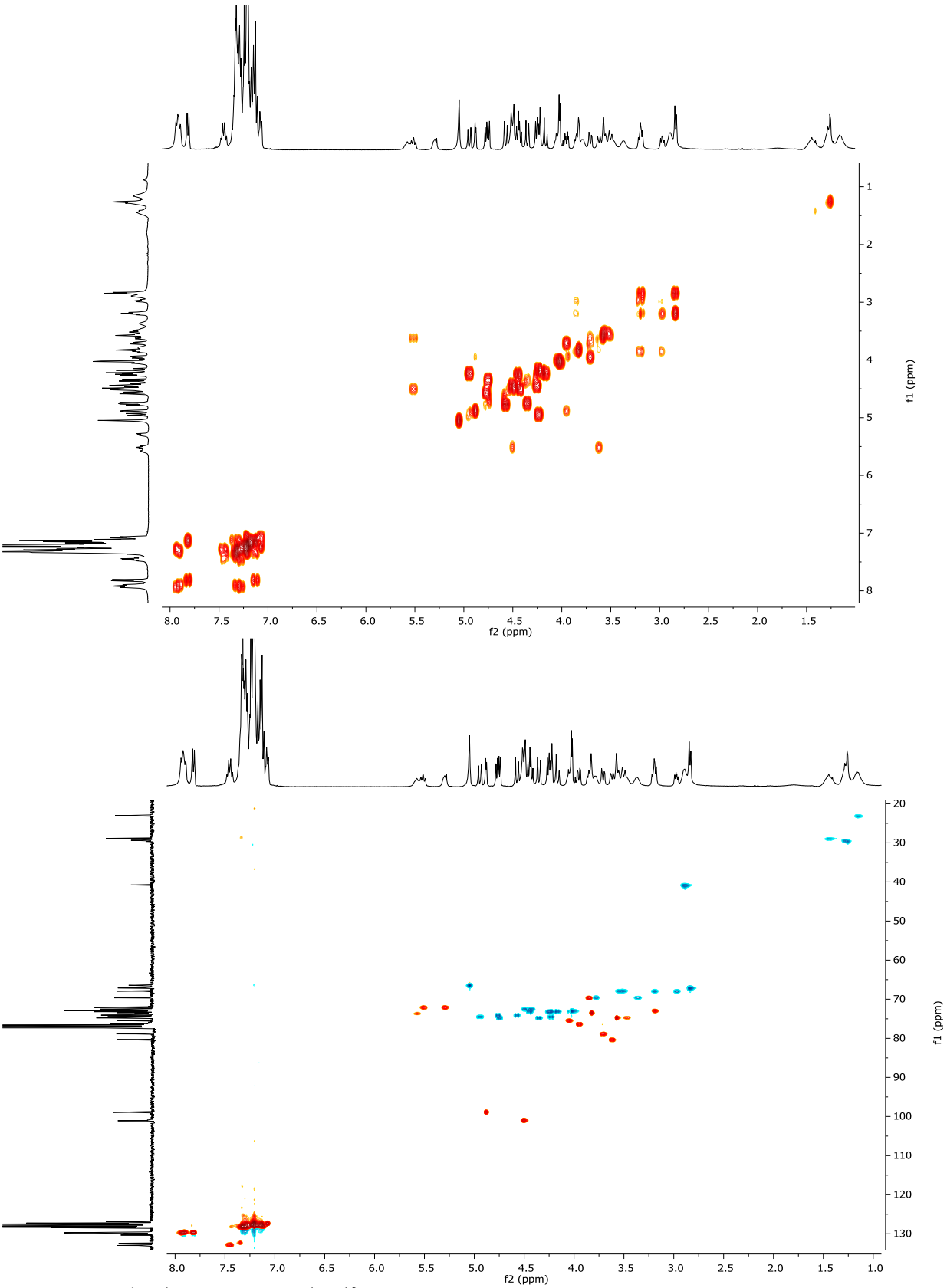


Figure S21. ^{1}H-^{1}H COSY and ^{1}H-^{13}C HSQC (400 MHz, $CDCl_3$) of **11**

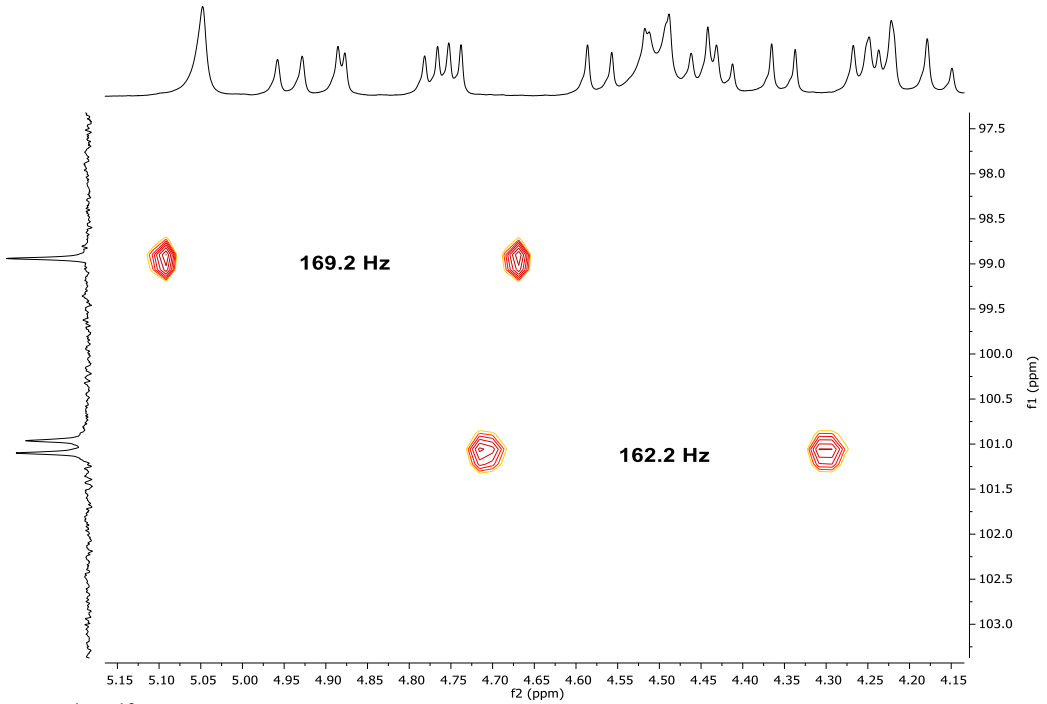


Figure S22. ^{1}H-^{13}C coupled-HSQC NMR (400 MHz, $CDCl_3$) of **11**

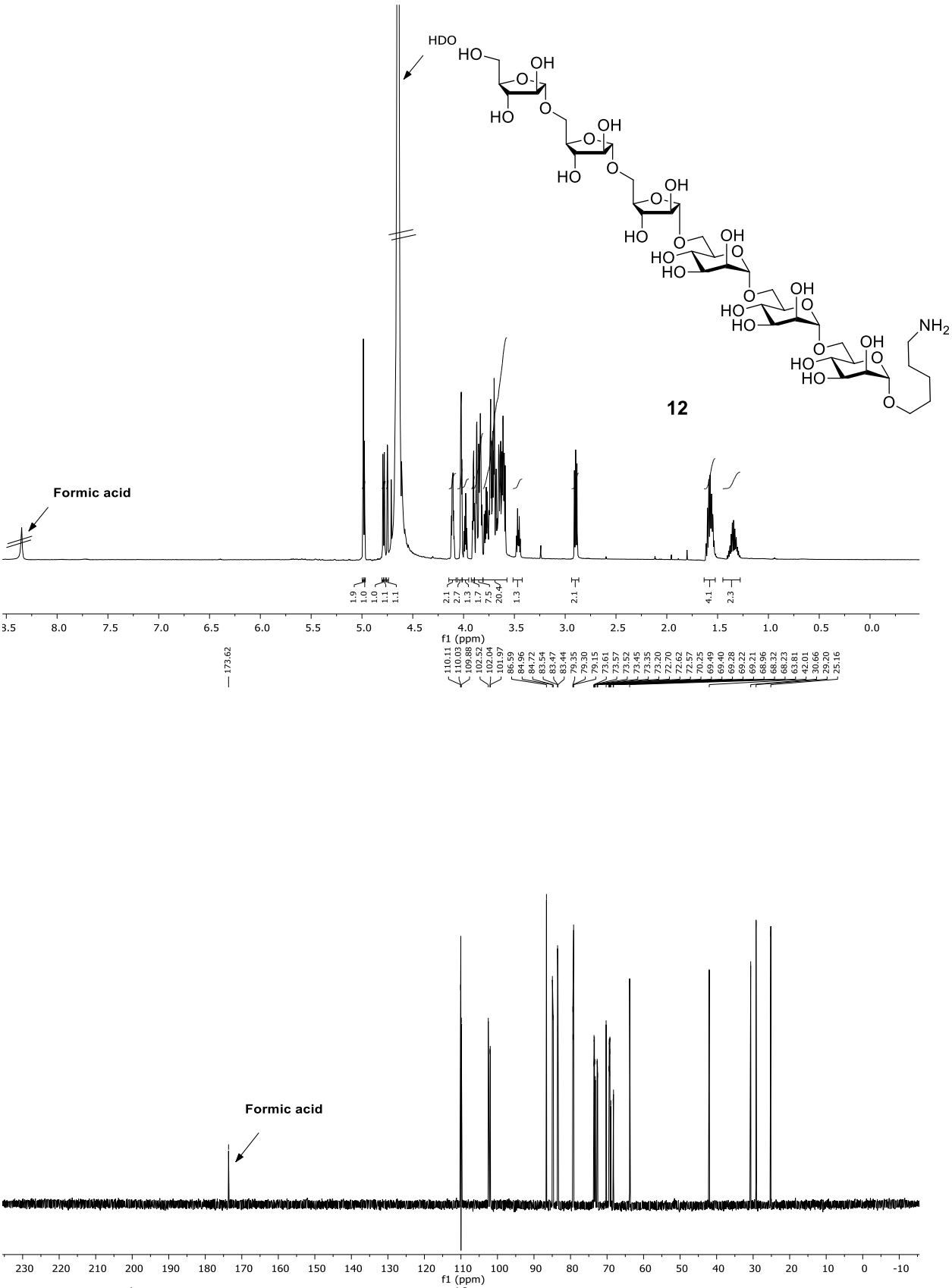


Figure S23. ^{1}H NMR (600 MHz, $CDCl_3$) and ^{13}C NMR (150 MHz, $CDCl_3$) of **12**

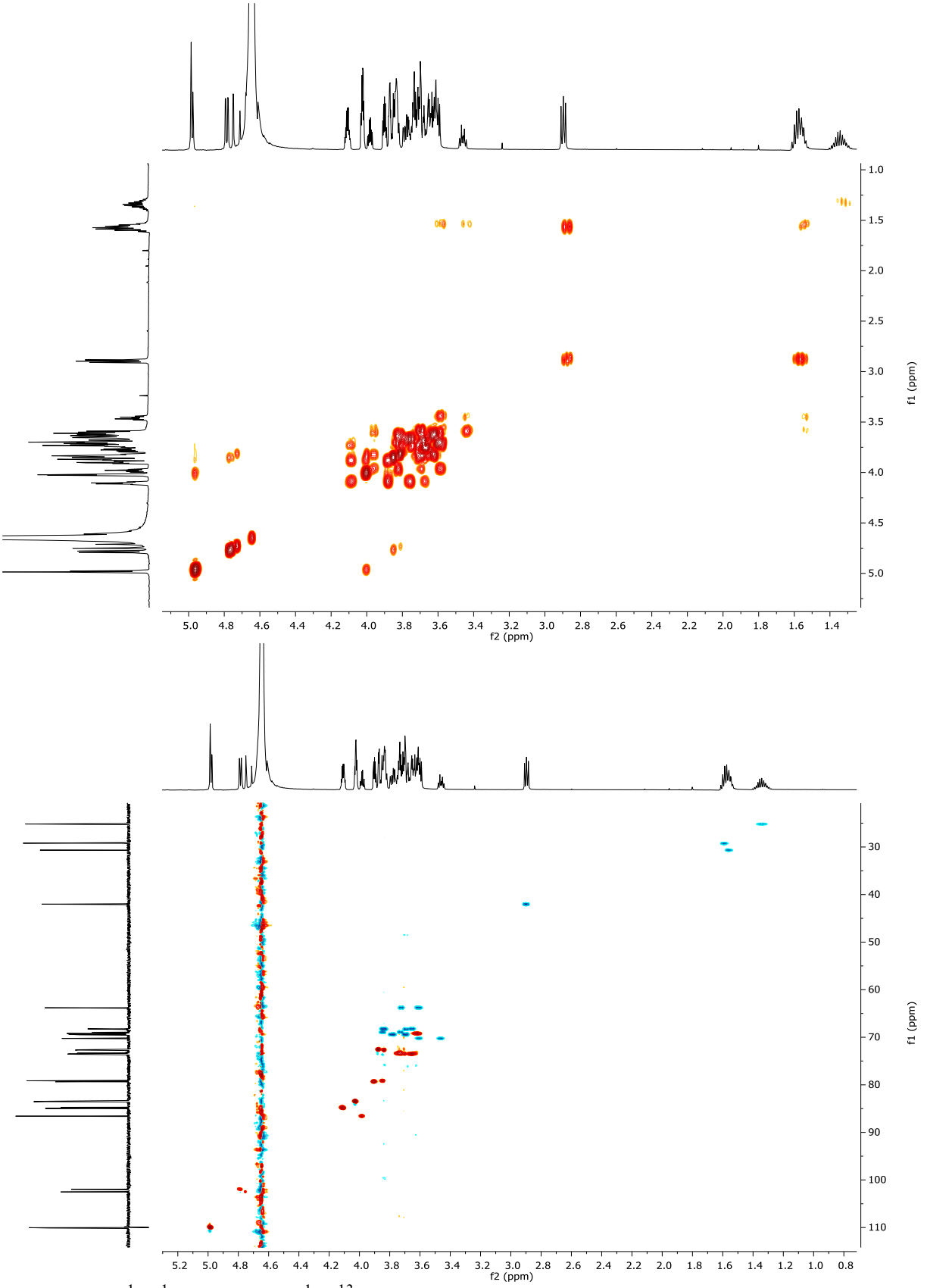


Figure S24. ^{1}H-^{1}H COSY and ^{1}H-^{13}C HSQC (600 MHz, $CDCl_3$) of **12**.

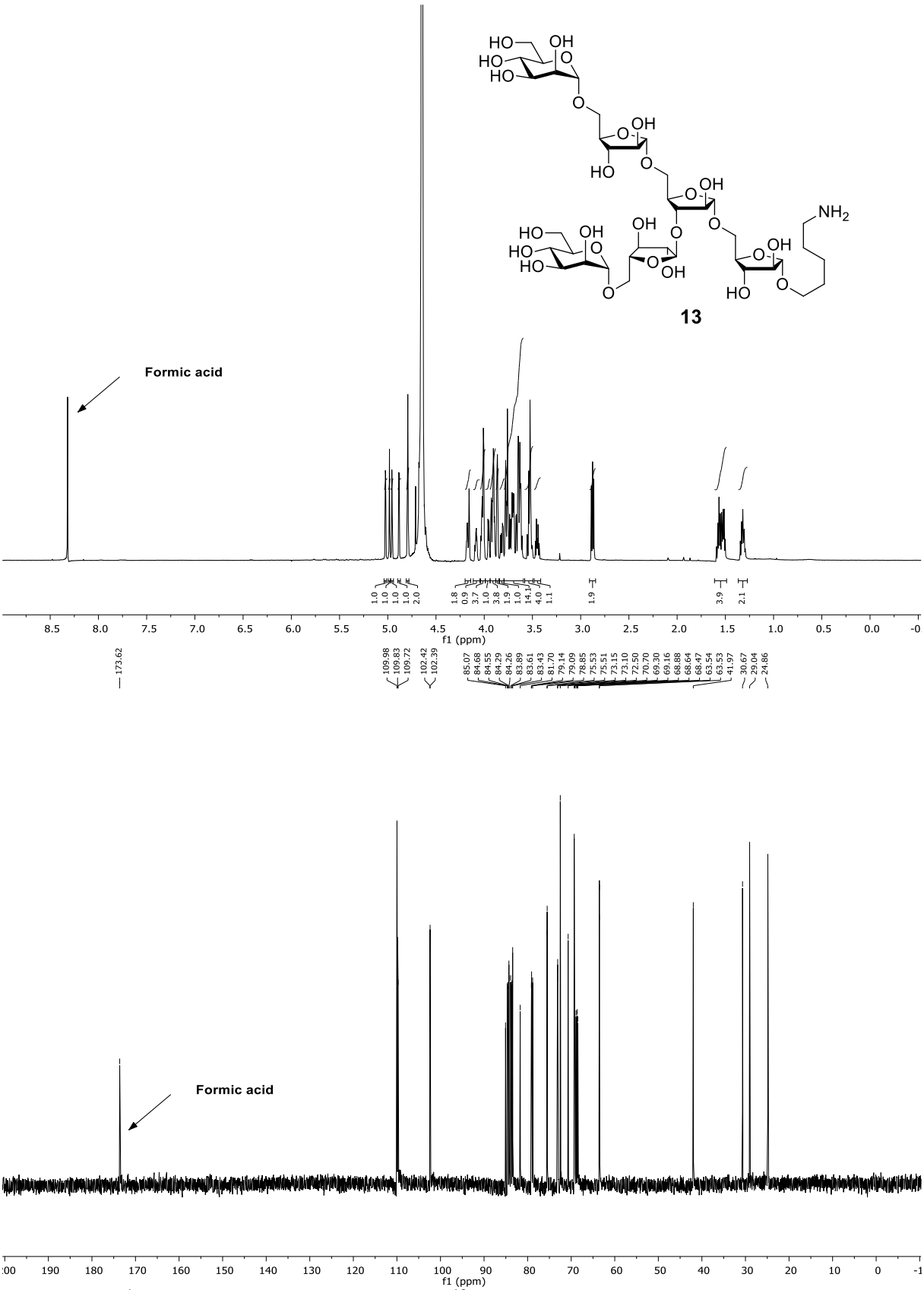


Figure S25. ^{1}H NMR (600 MHz, $CDCl_3$) and ^{13}C NMR (150 MHz, $CDCl_3$) of **13**

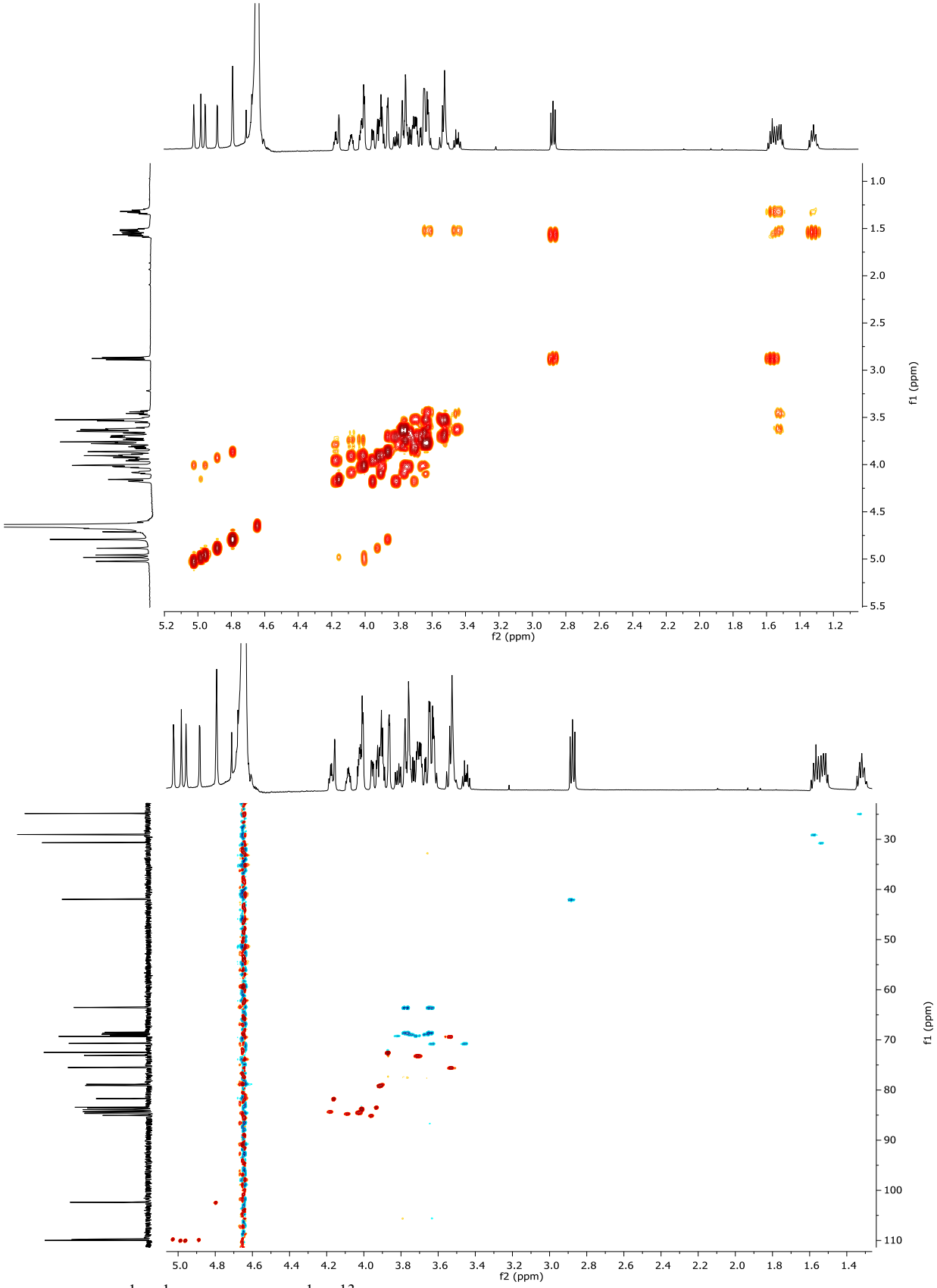
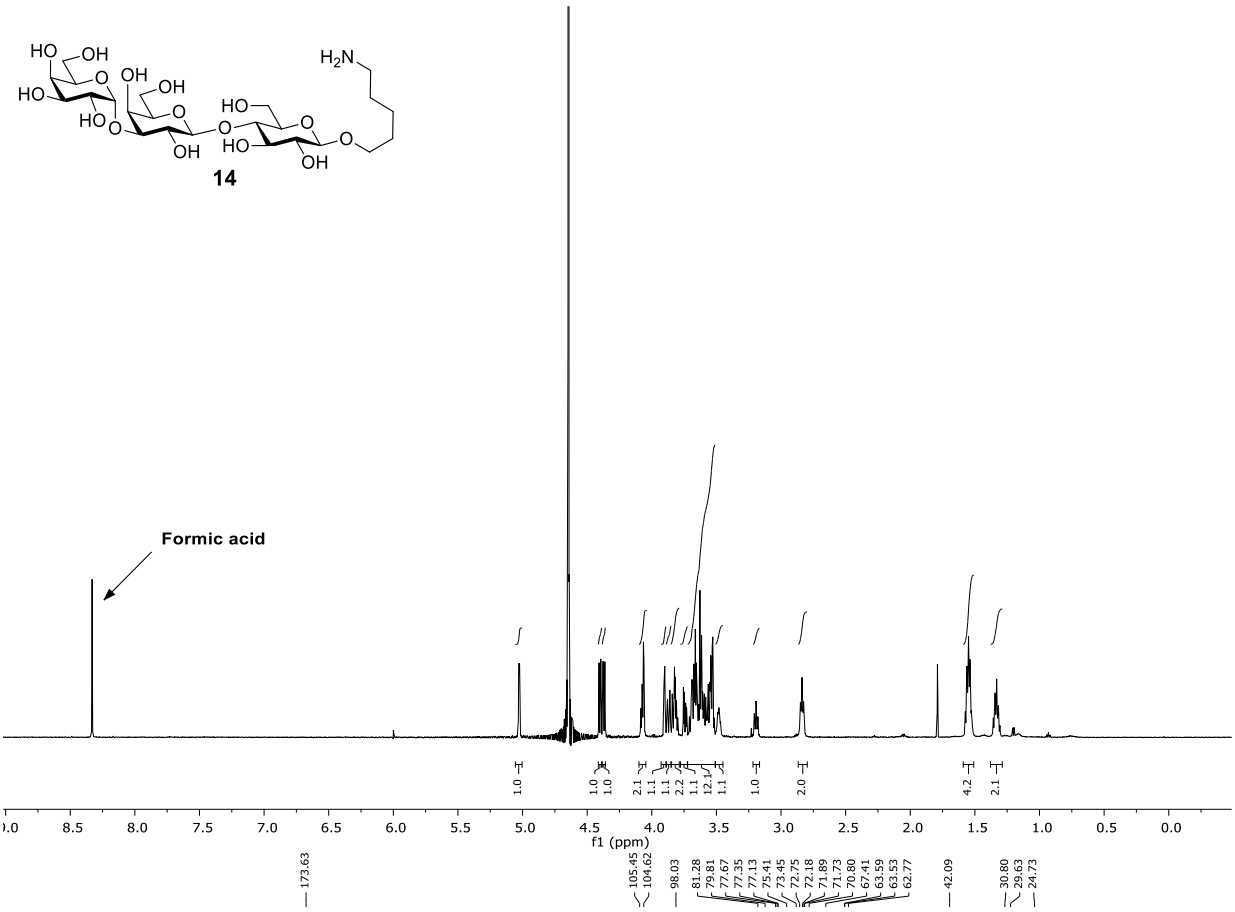


Figure S26. ^{1}H-^{1}H COSY and ^{1}H-^{13}C HSQC (600 MHz, $CDCl_3$) of **13**



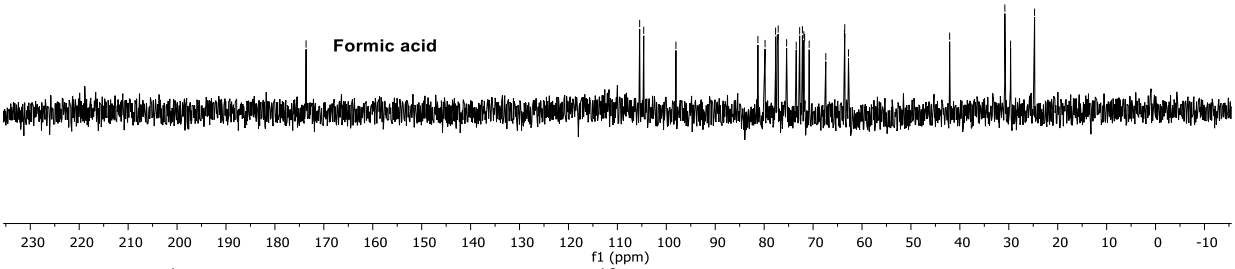


Figure S27. ^{1}H NMR (600 MHz, $CDCl_3$) and ^{13}C NMR (150 MHz, $CDCl_3$) of **14**

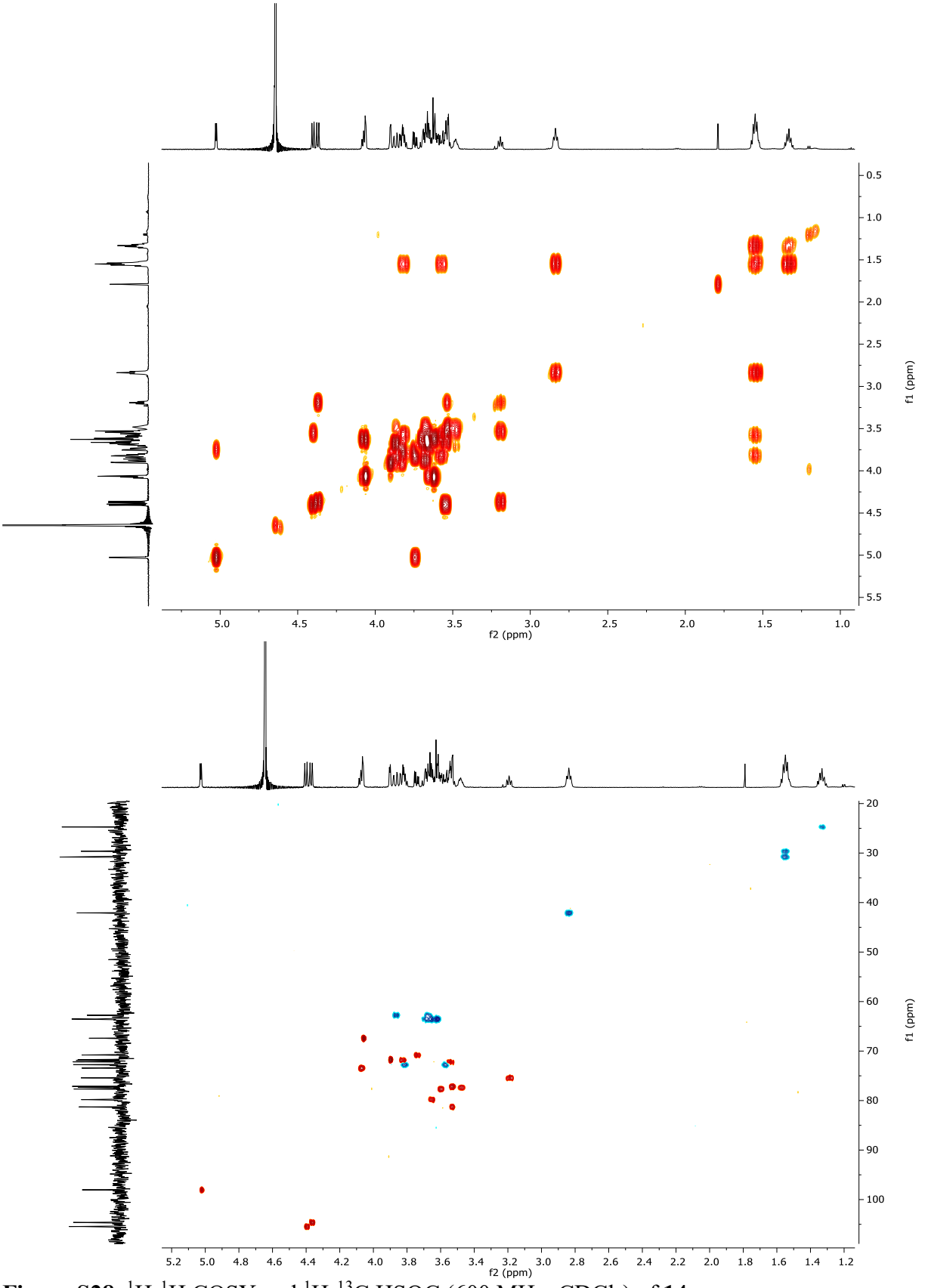


Figure S28. ^{1}H-^{1}H COSY and ^{1}H-^{13}C HSQC (600 MHz, $CDCl_3$) of **14**.

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