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Supplemental Information

Automated Glycan Assembly of Oligo-*N*-Acetyllactosamine and Keratan Sulfate Probes to Study Virus-Glycan Interactions

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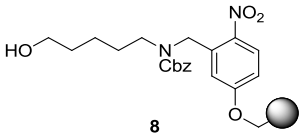
1. General Information

All chemicals used were reagent grade and used as supplied unless noted otherwise. Molecular sieves were activated by heating under high vacuum prior to use. All reactions were performed in oven-dried glassware under 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 (v/v) 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 Kieselgel 60 (230-400 mesh). Merrifield resin LL (loading 0.50 mmol/g, 100-200 mesh) was purchased from EMD Millipore. All automated glycosylations were performed on a prototype automated oligosaccharide synthesizer described before[S1] that was used here in a version modified for the synthesis of sulfated glucosaminoglycans[S2]. Anhydrous solvents of the Cycle-Tainer Solvent Delivery System were employed. LC-MS chromatograms were recorded on an Agilent 1100 Series spectrometer. Preparative HPLC purifications were performed on an Agilent 1200 Series system. Loading determination of functionalized resins was obtained using a Shimadzu UV-MINI-1240 UV spectrometer. ^{1}H and ^{13}C NMR spectra were recorded using a Varian Mercury 400 (400 MHz), 600 (600MHz) 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; brs, 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 Organic Chemistry 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.

2. Pre-Automation

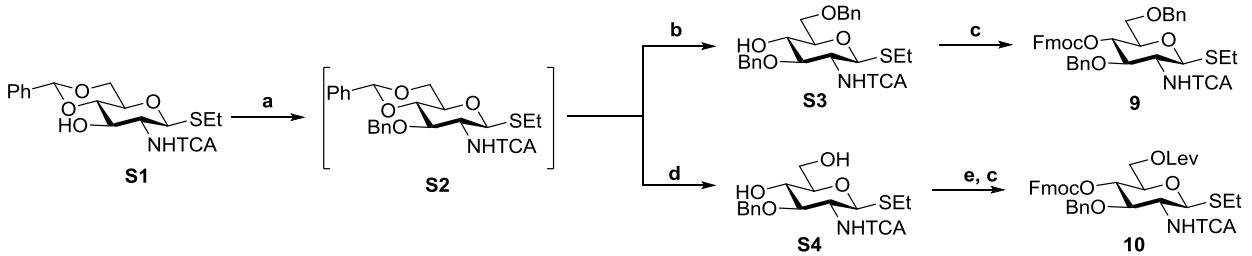
2.1 Polystyrene Resin Functionalized with Photolabile Linker 8.



8

The synthesis of solid support **1**[S2] and the loading (0.392 mmol/g)[S3] determination method has been reported previously.

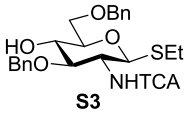
2.2 Building Blocks



S1 S2 S3 S4 9 10

Scheme S1. Synthesis of glucosamine building blocks 9 and 10. a) BnBr, NaH, DMF, 0 °C; b) $HSiEt_3$, TFAA, TFA, DCM, 0 °C to r.t. 91% over two steps; c) FmocCl, pyridine, DCM, 0 °C to r.t. 92% for **9**, 79% for **10**; d) DCM, TFA, r.t. 86% over two steps; e) 2-chloro-1-methylpyridinium iodide, LevOH, DABCO, DCM, -15 °C; BnBr = benzyl bromide, DABCO = 1,4-diazabicyclo[2.2.2]octane, FmocCl = fluorenylmethyloxycarbonyl chloride, $HSiEt_3$ = triethylsilane, LevOH = levulinic acid, TFAA = trifluoroacetic anhydride, TFA = trifluoroacetic acid.

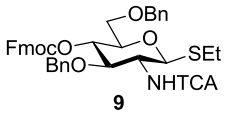
Ethyl 3,6-di-*O*-benzyl-2-deoxy-2-trichloroacetamino-1-thio-β-D-glucopyranoside (S3)



S3

To a solution of **S1**[S4] (4.13 g, 9.04 mmol) in DMF (45 mL, 0.2 M) were added BnBr (1.77 mL, 14.9 mmol) and NaH (1.19 g 60% in mineral oil, 29.7 mmol) at 0 °C, and the reaction mixture was stirred at 0 °C for 2 h. The reaction was quenched by addition of MeOH (10 mL) at 0 °C, followed by the addition of H_2O (25 mL) at 0 °C to form a precipitate. The precipitate was filtered off and washed with MeOH–H_2O (2:1, v/v, 5 mL, five times) and cold MeOH (5 mL, three times) to give the crude **S2** as a colorless powder. The precipitate was dissolved in DCM, dried over $MgSO_4$, and evaporated *in vacuo*. The crude **S2** was co-evaporated with toluene three times, and dissolved in DCM (41 mL, 0.18 M) under an Ar atmosphere. To a solution of the crude mixture **S2** were added triethylsilane (12.0 mL, 75.0 mmol) and trifluoroacetic anhydride (0.64 mL, 4.52 mmol) at 0 °C. After 30 min at 0 °C trifluoroacetic acid (3.90 mL, 50.6 mmol) was added dropwise, and the mixture was allowed to warm to room temperature overnight. The mixture was quenched with saturated aqueous $NaHCO_3$, diluted with DCM, and the organic layer was separated. The aqueous layer was extracted with DCM. The organic layers were combined, dried over $MgSO_4$ and concentrated *in vacuo*. The crude product was purified by column chromatography on silica gel (hexane–ethyl acetate = 9:1 to 7:3) to afford **S3** (4.37 g, 7.96 mmol, 88% over two steps). The analytical data (^1H, ^{13}C NMR and HRMS) was in agreement with literature data.[S4]

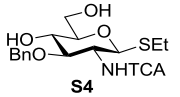
Ethyl 3,6-di-*O*-benzyl-4-*O*-fluorenylmethoxycarbonyl-2-deoxy-2-trichloroacetamino-1-thio-β-D-glucopyranoside (9)



9

Compound **S3** (8.9 g, 16.2 mmol) and FmocCl (8.39 g, 32.4 mmol) were dissolved in 4 mL (49.6 mmol) of pyridine at 0 °C, and the reaction mixture was stirred at room temperature for 15 h. The reaction was quenched with saturated aqueous $NaHCO_3$, diluted with DCM, and the organic layer was separated. The aqueous layer was extracted with DCM. The organic layers were combined, washed with 1 M aqueous HCl, dried over $MgSO_4$ and concentrated *in vacuo*. The crude product was purified by silica gel column chromatography (hexane–ethyl acetate–DCM = 7:2:1 to 7:3:1, v/v/v) to give **9** (3.50 g, 99%). The analytical data (^{1}H, ^{13}C NMR and HRMS) was in agreement with literature data.[S4]

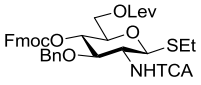
Ethyl 3-*O*-benzyl-2-deoxy-2-trichloroacetamino-1-thio-β-D-glucopyranoside (S4)



S4

To a solution of the compound **S1**[S3] (2.1 g, 4.60 mmol) in DMF (10 mL) was added NaH (0.603 g, in oil 60 w%) at 0 °C. After the reaction mixture was stirred for 5 min at 0 °C, 0.9 mL (2.5 mmol) of benzyl bromide was added. The mixture was stirred 0 °C for 0.5 h and quenched with the addition of MeOH (10 mL) at 0 °C followed by addition of H_2O (25 mL) at 0 °C to form a precipitate. The precipitate was filtered off and washed with MeOH–H_2O (2:1, v/v, 5 mL five times) and cold MeOH (5 mL, three times) to give the crude **S2**. To a solution of **S2** in DCM (100 mL) was added trifluoroacetic acid (5 mL) at 0 °C and the reaction mixture was stirred for 3 h at 0 °C. The reaction was quenched with saturated aqueous $NaHCO_3$, and extracted twice with DCM (150 mL). The organic layers were combined, washed with saturated aqueous $NaHCO_3$, and dried over Na_2SO_4. The crude mixture was concentrated *in vacuo* and re-dissolved in DCM–hexane (3:5, v/v, 80 mL) and stored at -20 °C overnight to form a precipitate. The precipitate was filtered, washed with DCM-hexane mixture (1:2, v/v), and hexane. The precipitation process was repeated twice to afford compound **S4** (4.37 g, 3.96 mmol, 86% over two steps). R_f =0.1 (cyclohexane:ethyl acetate = 1:1) ^{1}H-NMR (400 MHz, **CD_3OD**) : δ 7.39 – 7.00 (m, 5H), 4.87 (d, *J* = 10.9 Hz, 1H, C*H*HPh), 4.71 (d, *J* = 10.6 Hz, 2H, H-1, C*H*HPh), 3.92 – 3.82 (m, 2H, H-2, H-6), 3.73 – 3.65 (m, 2H, H-3, H-6), 3.56 – 3.47 (m, 1H, H-4), 3.35 (ddd, *J* = 9.7, 5.9, 2.2 Hz, 1H, H-5), 2.82 – 2.64 (m, 2H), 1.25 (t, *J* = 7.4 Hz, 3H). ^{13}C-NMR (100 MHz, **CD_3OD**) : δ 163.85 ($NHCOCCl_3$), 139.84 (CCl_3), 129.15, 128.79, 128.49 (Ar), 84.71 (C-1), 84.57 (C-3), 82.36 (C-5), 76.10 (CH_2Ph), 72.07 (C-4), 62.80 (C-6), 57.35 (C-2), 24.89 (CH_2, SEt), 15.28 (CH_3, SEt); HR-ESI MS: *m/z* $[M+Na]^+$ calcd for $C_{17}H_{22}NO_9Cl_3SNa^+$ 480.0176, found 480.0184.

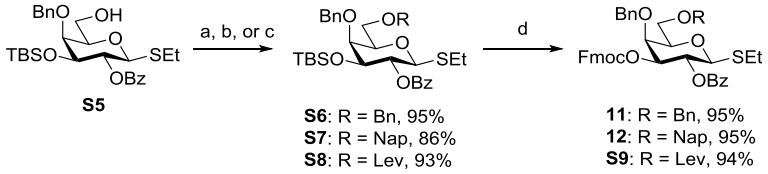
Ethyl 3-*O*-benzyl-4-*O*-fluorenylmethoxycarbonyl-6-*O*-levulinyl-2-deoxy-2-trichloro-acetamino-1-thio-β-D-glucopyranoside (10)



10

To a solution of **S4** (2.4 g, 5.23 mmol) in DCM (26 mL) were added levulinic acid (1.22 g, 10.5 mmol), 2-chloro-1-methylpyridinium iodide (2.94 g, 11.5 mmol), and 1,4-diazabicyclo[2.2.2]octane (DABCO) (2.35 g, 30.9 mmol) at –15 °C, and the mixture was stirred at –15 °C for 2 h. The mixture was quenched with saturated aqueous $NaHCO_3$, diluted with DCM, and the organic layer was separated. The aqueous layer was extracted with DCM. The organic layers were combined, washed with 1 M aqueous HCl, dried over $MgSO_4$ and concentrated *in vacuo*. The crude product was dissolved in pyridine (0.85 mL). To the solution, FmocCl (2.91 g, 1.62 mmol) was added at 0 °C. The reaction mixture was stirred at room temperature for 15 h. The reaction was quenched with saturated aqueous $NaHCO_3$, diluted with DCM, and the organic layer was separated. The aqueous layer was extracted with DCM. The organic layers were combined, washed with 1 M aqueous HCl, dried over $MgSO_4$ and concentrated. The crude product was purified by silica gel column chromatography (hexane–ethyl acetate–DCM = 7:2:1 to 7:3:1, v/v/v) to give **10** (3.2 g, 4.11 mmol, 79%). R_f = 0.1 (cyclohexane:EtOAc = 9:1) ^{1}H-NMR (400 MHz, $CDCl_3$) : δ 7.75 (dd, *J* = 7.6, 3.6 Hz, 2H), 7.57 (ddd, *J* = 15.9, 7.5, 0.7 Hz, 2H), 7.38 (t, *J* = 7.5 Hz, 2H), 7.30 – 7.24 (m, 2H), 7.23 – 7.12

(m, 5H), 6.93 (d, *J* = 7.8 Hz, 1H, NH), 5.05 (d, *J* = 10.3 Hz, 1H, H-1), 4.91 (dd, *J* = 9.9, 9.0 Hz, 1H, H-4), 4.62 (s, 2H, CH_2Ph), 4.49 (dd, *J* = 10.5, 6z.8 Hz, 1H, C*H*H, Fmoc), 4.34 (dd, *J* = 10.5, 7.3 Hz, 1H, C*H*H, Fmoc), 4.31 – 4.16 (m, 4H, H-4, H-6, CH of Fmoc), 3.76 (ddd, *J* = 10.0, 5.2, 2.8 Hz, 1H, H-5), 3.63 (td, *J* = 10.1, 7.8 Hz, 1H, H-2), 2.80 – 2.65 (m, 4H, CH_2 of SEt, CH_2 of Lev), 2.60 (ddd, *J* = 10.3, 6.4, 3.1 Hz, 2H, CH_2 of Lev), 2.16 (s, 3H, CH_3, Lev), 1.29 (t, *J* = 7.4 Hz, 3H, CH_3, SEt). ^{13}C-NMR (100 MHz, $CDCl_3$) : δ 206.50 (CO, Lev), 172.46 (CO, Lev), 161.84 (Fmoc), 154.34 ($NHCOCl_3$), 143.41, 143.16, 141.46, 141.42, 137.29, 128.57, 128.09, 128.07, 127.97, 127.37, 125.25, 125.08, 120.23, 120.21 (Ar), 92.39 (CCl_3), 82.65 (C-1), 78.71 (CH, Fmoc), 75.94 (C-5), 75.32 (C-4), 74.97 (CH_2Ph), 70.48 (CH_2, Fmoc), 62.86 (C-6), 57.81 (C-2), 46.85 (C-3), 38.02 (CH_2, Lev), 29.97 (CH_3, Lev), 28.01 (CH_2, Lev), 24.97 (CH_2, SEt), 15.29 (CH_3, SEt).; HR-ESI MS: *m/z* $[M+Na]^+$ calcd for $C_{37}H_{38}NO_9Cl_3SNa^+$ 800.1225, found 800.1194.



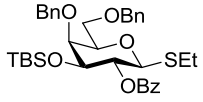
S5

S6: R = Bn, 95%
S7: R = Nap, 86%
S8: R = Lev, 93%

11: R = Bn, 95%
12: R = Nap, 95%
S9: R = Lev, 94%

Scheme S2. Synthesis of glucosamine building blocks 11, 12, and S9. a) BnBr, THF, DMF, 0 °C, 95%; b) NapBr, THF, DMF, 0 °C, 86%; c) LevOH, DIC, DMAP, DCM, -15 °C; d) (i) BF_3OEt_2, MeCN, 0 °C; (ii) FmocCl, pyridine, DCM, 0 °C, 95% for **11,** 95% for **12,** 94% for **S9**. DIC, *N*,*N*'-diisopropylcarbodiimide, NapBr = 2-naphthylmethyl bromide.

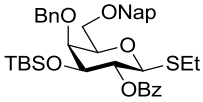
Ethyl 2-*O*-benzoyl-4,6-di-*O*-benzyl-3-*O*-*tert*-butyldimethylsilyl-1-thio-β-D-galactopyranoside (S6)



S6

To a solution of compound **S5**[S4] (4.01 g, 7.51 mmol) in anhydrous DMF–THF (1:9, v/v, 58.7 mL), were added BnBr (2.68 mL, 22.5 mmol) and NaH (0.721 mg, 18.0 mmol) at 0 °C. The reaction mixture was stirred at 0 °C for 2 h. The reaction was quenched with saturated aqueous NH_4Cl, diluted with DCM, and the organic layer was separated. The aqueous layer was extracted with DCM. The organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. The crude product was purified by column chromatography on silica gel (hexane–ethyl acetate-DCM = 9:0.5:0.5 to 9:1:1, v/v/v) to afford **S9** (4.58 g, 7.36 mmol, 98%). The analytical (^{1}H, ^{13}C NMR and HRMS) data was in agreement with literature data.[S3]

Ethyl 2-*O*-benzoyl-4-*O*-benzyl-3-*O*-*tert*-butyldimethylsilyl-6-*O*-(2-naphthylmethyl)-1-thio-β-D-galactopyranoside (S7)

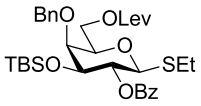


S7

To a solution of **S5** [S3] (3.92 g, 7.32 mmol) and NapBr (3.24 g, 14.64 mmol) in anhydrous DMF–THF (1:9, v/v, 53 mL) was added NaH (0.703 mg, 17.6 mmol) at 0 °C, and the reaction mixture was stirred at 0 °C for 1 h. The reaction was quenched with saturated aqueous NH_4Cl, extracted with Et_2O, and dried with Mg_2SO_4. The organic layer was concentrated *in vacuo*. The crude product was purified by silica gel column chromatography (hexane–ethylacetate–DCM = 9:0.5:1 to 9:1:1, v/v/v) to give **S7** as a colorless solid (4.23 g, 86%). R_f (hexane:ethyl acetate:DCM = 8:2:1) = 0.79; $[\alpha]_D^{20}$= +10.64 (c = 1.0, $CHCl_3$). IR (thin film): υ = 3060, 2954, 2856, 1727, 1602, 1452, 1349, 1265, 1152, 1105, 1070, 1027, 910, 838, 708cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.05 – 8.00 (m, 2H), 7.84 – 7.75 (m, 3H), 7.71 (s, 1H), 7.59 – 7.49 (m, 1H), 7.49 – 7.36 (m, 5H), 7.31 – 7.17 (m, 5H), 5.61 (t, *J* = 9.4 Hz, 1H, H-2), 5.06 (d, *J* = 11.5 Hz, 1H, C*H*HPh), 4.59 (q, *J* = 12.0 Hz, 2H, CH_2NAP), 4.49 (d, *J* = 11.5 Hz, 1H, C*H*HPh), 4.48 (d, *J* = 9.7 Hz, 1H, H-1), 3.99 – 3.90 (m, 1H, H-3), 3.85 (d, *J* = 2.3 Hz, 1H, H-4), 3.74 (dd, *J* = 6.9, 5.9 Hz, 1H, H-5), 3.67 – 3.61 (m, 1H, H-6), 2.79 – 2.54 (m, 2H. CH_2 thio), 1.17 (t, *J* = 7.5 Hz, 3H, Me thio), 0.75 (s, 9H, *tert*-Bu Si), 0.09 (s, 3H, Me Si), -0.11 (s, 3H, Me Si). ^{13}C NMR (101 MHz, $cdcl_3$) δ 165.48(C=O), 138.95, 135.50, 133.37, 133.16, 133.01, 130.49, 129.93, 128.40, 128.36, 128.29, 128.02, 127.82, 127.76, 127.48, 126.91,

126.29, 126.09, 125.99(Ar), 83.79(C-1), 77.69(C-5), 77.43(C-4), 75.80(C-3), 75.27(CH_2Ph), 73.77(CH_2 of NAP), 71.21(C-2), 68.81(C-6), 25.67(*tert*-Bu Si), 23.67(CH_2 thio), 17.93(Cq Si), 14.94(Me thio), -3.89(Me Si), -4.91(Me Si). MS ESI+-HRMS *m/z* $[M+Na]^+$ calcd for $C_{39}H_{48}O_6SSiNa$ 695.2839, found 695.2759.

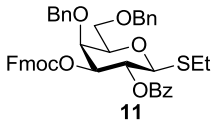
Ethyl 2-*O*-benzoyl-4-*O*-benzyl-3-*O*-*tert*-butyldimethylsilyl-6-*O*-levulinyl-1-thio-β-D-galactopyranoside (S8)



S8

To a solution of compound **S5**[S3] (4.28 g, 8.03 mmol) and DMAP (0.294 g, 2.41 mmol) in anhydrous DCM (40 mL) were added levulinic acid (2.80 g, 24.1 mmol) and DIC (3.75 mL, 24.10 mmol) at 0 °C, and the mixture was stirred for 1 h at 0 °C. The mixture was quenched with saturated aqueous $NaHCO_3$, diluted with DCM, and the organic layer was separated. The aqueous layer was extracted with DCM. The organic layers were combined, dried over $MgSO_4$, and concentrated *in vacuo*. The crude product was purified by column chromatography on silica gel (hexane–ethyl acetate-DCM = 9:1:1 to 7:3:1, v/v/v) to afford **S8** (4.7 g, 7.45 mmol, 93%). R_f (hexane:ethyl acetate:DCM = 7:3:1) = 0.51; $[\alpha]_D^{20}$= +48.68 (c = 2.0, $CHCl_3$). IR (thin film): υ = 2886, 1719, 1452, 1352, 1259, 1069cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.07 – 7.99 (m, 2H), 7.62 – 7.50 (m, 1H), 7.48 – 7.41 (m, 2H), 7.41 – 7.31 (m, 4H), 7.31 – 7.25 (m, 1H), 5.63 (t, *J* = 9.5 Hz, 1H, H-2), 5.12 (d, *J* = 11.5 Hz, 1H, CH*H*Ph), 4.59 (d, *J* = 11.5 Hz, 1H, CH*H*Ph), 4.51 (d, *J* = 9.7 Hz, 1H, H-1), 4.27 (dd, *J* = 11.2, 6.6 Hz, 1H, H-6a), 4.19 (dd, *J* = 11.2, 5.8 Hz, 1H, H-6b), 3.98 (d, *J* = 9.2 Hz, 1H, H-3), 3.82 – 3.70 (m, 2H, H-4,5), 2.82 – 2.61 (m, 4H, CH_2 thio, CH_2 Lev), 2.57 – 2.46 (m, 2H, CH_2 of Lev), 2.18 (s, 3H, CH_3 of Lev), 1.21 (t, *J* = 7.5 Hz, 3H, CH_3 thio), 0.78 (s, 9H, *tert*-Bu of Si), 0.13 (s, 3H, Me of Si), -0.07 (s, 3H, Me of Si). ^{13}C NMR (100 MHz, $CDCl_3$) δ 206.60(C=O, Lev), 172.61(C=O, Lev), 165.46(C=O, Bz), 138.66, 133.07, 130.41, 129.93, 128.42, 128.41, 127.94, 127.67(Ar), 83.85(C-1), 77.20(C-4), 76.23(C-15, 75.71(C-3), 75.16(CH_2Ph), 70.99(C-2), 63.62(C-6), 38.07(CH_2 Lev), 29.95(Me, Lev), 28.02(CH_2, Lev), 25.66(*tert*-Bu, Si), 23.86(CH_2, thio), 17.92(Cq, Si), 14.96(Me, thio), -3.89(Me, Si), -4.94(Me, Si). MS ESI+-HRMS *m/z* $[M+Na]^+$ calcd for $C_{33}H_{46}O8SSiNa$ 653.2580, found 653.2530.

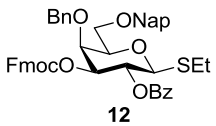
Ethyl 2-*O*-benzoyl-4,6-di-*O*-benzyl-3-*O*-fluorenylmethoxycarbonyl-1-thio-β-D-galacto-pyranoside (11)



11

To a solution of **S6** (3.77 g, 6.05 mmol) in anhydrous acetonitrile (76 mL, 0.08 M) was added boron trifluoride diethyl etherate ($BF_3{\cdot}OEt_2$) (0.92 mL, 7.26 mmol) at 0 °C. The reaction mixture was stirred at 0 °C for 20 min. The reaction was quenched with saturated aqueous $NaHCO_3$ (30 mL), diluted with DCM and the organic layer was separated. The aqueous layer was extracted with DCM. The organic layers were combined, dried over $MgSO_4$, and concentrated *in vacuo*. The crude product was dissolved to anhydrous DCM (30 mL). To the solution was added 9-FmocCl (3.91 g, 15.1 mmol) and pyridine (1.47 mL, 18.1 mmol) successively at 0 °C, and the mixture was stirred overnight at room temperature. The mixture was quenched with saturated aqueous $NaHCO_3$, diluted with DCM, and the organic layer was separated. The aqueous layer was extracted with DCM. The organic layers were combined, washed with 1 M aqueous HCl, dried over $MgSO_4$ and concentrated *in vacuo*. The crude product was purified by column chromatography on silica gel (hexane–ethylacetate–DCM = 8:1:1 to 8:2:1, v/v/v) to afford **11** (4.21 g, 5.76 mmol, 95%). The analytical data (^{1}H, ^{13}C NMR and HRMS) was in agreement with literature data.[S3]

Ethyl 2-*O*-benzoyl-4-*O*-benzyl-3-*O*-fluorenylmethoxycarbonyl-6-*O*-(2-naphthylmethyl)-1-thio-β-D-galactopyranoside (12)

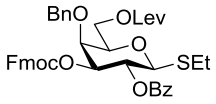


12

To a solution of **S7** (4.23 g, 5.42 mmol) in anhydrous acetonitrile (68 mL, 0.08 M) was added $BF_3{\cdot}OEt_2$ (0.76 mL, 5.96 mmol) at 0 °C. The reaction mixture was stirred at 0 °C for 20 min. The reaction was

quenched with saturated aqueous $NaHCO_3$ (30 mL), diluted with DCM and the organic layer was separated. The aqueous layer was extracted with DCM. The organic layers were combined, dried over $MgSO_4$, and concentrated *in vacuo*. The crude product was dissolved in anhydrous DCM (30 mL). To the solution was added 9-FmocCl (2.80 g, 10.8 mmol) and pyridine (1.10 mL, 13.6 mmol) successively at 0 °C, and the mixture was stirred overnight at room temperature. The mixture was quenched with saturated aqueous $NaHCO_3$, diluted with DCM, and the organic layer was separated. The aqueous layer was extracted with DCM. The organic layers were combined, washed with 1 M aqueous HCl, dried over $MgSO_4$ and concentrated *in vacuo*. The crude product was purified by column chromatography on silica gel (hexane–ethylacetate–DCM = 8:1:1 to 8:2:1, v/v/v) to afford **11** (3.97 g, 5.09 mmol, 94%). R_f: 0.31 (hexane: ethyl acetate:DCM = 9:1:1); $[\alpha]_D^{20}$= +32.12 (c = 2.67, $CHCl_3$); IR (thin film): υ = 2870, 1745, 1450, 1272, 1154, 1095, 1027, 819, 740, 709cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.20 – 8.01 (m, 2H), 7.94 – 7.82 (m, 3H), 7.78 (s, 1H), 7.75 – 7.65 (m, 2H), 7.63 – 7.23 (m, 15H), 7.14 (dtd, *J* = 8.6, 7.5, 1.1 Hz, 2H), 5.78 (t, *J* = 9.9 Hz, 1H, H-2), 5.11 (dd, *J* = 10.0, 3.0 Hz, 1H, H-3), 4.82 (d, *J* = 11.4 Hz, 1H, CH*H*Ph), 4.67 (dd, *J* = 26.7, 11.0 Hz, 3H, H-1, CH_2Nap), 4.52 (d, *J* = 11.5 Hz, 1H, CH*H*Ph), 4.33 (dd, *J* = 10.4, 7.2 Hz, 1H, H-6a), 4.24 (dd, *J* = 10.4, 7.8 Hz, 1H, H-6b), 4.18 (dd, *J* = 2.9, 0.6 Hz, 1H, H-4), 4.08 (t, *J* = 7.4 Hz, 1H, H-5), 3.88 (dd, *J* = 7.4, 6.3 Hz, 1H, CH, Fmoc), 3.79 – 3.68 (m, 2H, CH_2 Fmoc), 2.84 – 2.69 (m, 2H, CH_2 thio), 1.26 (t, *J* = 7.5 Hz, 3H, Me thio). ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.36(C=O, Bz), 154.65(C=O, Fmoc), 143.38, 142.96, 141.32, 141.22, 137.98, 135.29, 133.36, 133.32, 133.18, 130.06, 129.71, 128.50, 128.40, 128.23, 128.03, 127.92, 127.84, 127.82, 127.23, 127.20, 126.89, 126.32, 126.13, 125.95, 125.29, 125.07, 120.07(Ar), 83.86(C-1), 79.17(C-3), 77.42(*C*H, Fmoc), 75.20(CH_2, Bn), 74.13(C-4), 73.79(CH_2, Nap), 70.24(C-6), 68.72(C-2), 68.21(CH_2, Fmoc), 46.59(C-5), 24.01(CH_2 thio), 14.93(CH_3 thio). MS ESI+-HRMS *m/z* $[M+Na]^+$ calcd for $C_{48}H_{44}O_8SNa$ 803.2655, found 803.2601.

Ethyl 2-*O*-benzoyl-4-*O*-benzyl-3-*O*-fluorenylmethoxycarbonyl-6-*O*-levulinyl-1-thio-β-D-galactopyranoside (S9)



S9

To a solution of compound **S8** (4.7 g, 7.45 mmol) in anhydrous acetonitrile (93 mL) was added $BF_3{\cdot}OEt_2$ (1.13 mL, 8.94 mmol) and the mixture was stirred at 0 °C for 20 min. The mixture was quenched with saturated aqueous $NaHCO_3$, diluted with DCM, and the organic layer was separated. The aqueous layer was extracted with DCM. The organic layers were combined, dried over $MgSO_4$, and concentrated *in vacuo*. The crude product was dissolved in anhydrous DCM (36 mL). To the solution was added 9-fluorenylmethylchloroformate (4.63 g, 17.91 mmol) and pyridine (1.74 mL, 21.49 mmol) successively at 0 °C, and the mixture was stirred overnight at room temperature. The mixture was quenched with saturated aqueous $NaHCO_3$, diluted with DCM, and the organic layer was separated. The aqueous layer was extracted with DCM. The organic layers were combined, washed with 1 M aqueous HCl, dried over $MgSO_4$ and concentrated *in vacuo*. The crude product was purified by column chromatography on silica gel (hexane–ethyl acetate–DCM = 8:1:1 to 8:2:1, v/v/v) to afford **S9** (5.0 g, 6.77 mmol, 94%). R_f (hexane:ethyl acetate:DCM = 7:3:1) = 0.29; $[\alpha]_D^{20}$= +33.58 (c = 2.0, $CHCl_3$). IR (thin film): υ = 3060, 1732, 1602, 1451, 1359, 1272, 1156 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.14 – 7.92 (m, 2H), 7.71 – 7.65 (m, 2H), 7.56 – 7.50 (m, 1H), 7.49 – 7.27 (m, 11H), 7.18 – 7.06 (m, 2H), 5.75 (t, *J* = 10.0 Hz, 1H, H-2), 5.08 (dd, *J* = 10.0, 2.9 Hz, 1H, H-3), 4.85 (d, *J* = 11.4 Hz, 1H, CH*H*Ph), 4.62 (d, *J* = 9.9 Hz, 1H, H-1), 4.55 (d, *J* = 11.4 Hz, 1H, CH*H*Ph), 4.37 – 4.20 (m, 3H, CH_2 Fmoc, H-6a), 4.16 (dd, *J* = 11.1, 6.4 Hz, 1H, H-6b), 4.07 (dd, *J* = 13.9, 5.2 Hz, 2H, C*H* Fmoc, H-4), 3.82 (dd, *J* = 6.8, 6.0 Hz, 1H, H-5), 2.84 – 2.66 (m, 4H, CH_2 Lev, CH_2 thio), 2.53 (t, *J* = 6.4 Hz, 2H, CH_2 Lev), 2.19 (s, 3H, Me Lev), 1.24 (t, *J* = 7.5 Hz, 3H, Me Lev). ^{13}C NMR ($CDCl_3$) ^{13}C NMR (100 MHz, $CDCl_3$) δ 206.41(C=O, Lev), 172.30(C=O, Lev), 165.20(C=O, Bz), 154.49(C=O, Fmoc), 143.21, 142.75, 141.20, 141.08, 137.42, 133.25, 129.93, 129.47, 128.41, 128.39, 128.36, 127.94, 127.83, 127.11, 127.07, 125.15, 124.91, 119.98, 119.97(Ar), 83.82(C-1), 78.96(C-2), 75.90(C-5), 75.01(CH_2Ph), 73.52(C-4), 70.21(C-6), 68.36(C-2), 62.66(CH_2, Fmoc), 46.43(CH, Fmoc), 37.86(CH_2, Lev), 29.85(Me, Lev), 27.75(CH_2, Lev), 24.06(CH_2, thio), 14.82(Me, thio), MS ESI+-HRMS *m/z* $[M+Na]^+$ calcd for $C_{42}H_{42}O_{10}SNa$ 761.2396, found 761.2333.

3. Automated Synthesis and Post-Automation Steps

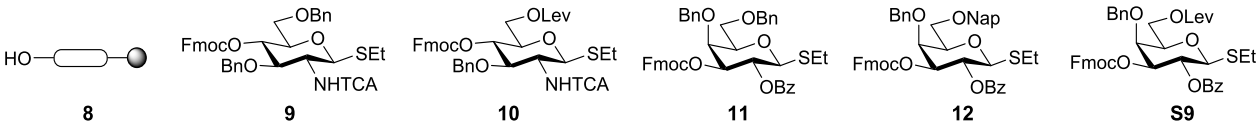


Figure S1. Functionalized resin and complete set of monosaccharide building blocks.

The fully automated, computer-controlled solid-phase oligosaccharide synthesizer was reported in 2012.[S1]. To synthesize chondroitin sulfates, a family of glycosaminoglycans, a modified synthesizer was developed in 2013.[S2] The latter was employed here without any further modifications. Automated solid phase synthesis was carried out in a temperature-controlled double jacketed cylinder shape vessel with glass filter (50 mL, see the detailed pictures in references S1 and S2). Mixing of resin and reagents was achieved by Argon bubbling. Reaction temperature and solution transfer were controlled with the J-KEM program as described.[S1]

3.1 Preparation of Reagent Solutions and Modules

Building Block Solutions
For the glycosylation using twice five equivalents, 0.25 mmol of building block was dissolved in 2 mL of DCM.
For the glycosylation using twice 7.5 equivalents, 0.375 mmol of building block was dissolved in 2 mL of DCM.

Acidic TMSOTf Wash Solution
For Acidic TMSOTf wash, 480 µL TMSOTf were dissolved in 20 mL DCM.

Activator Solution
For thioglycosides, *N*-Iodosuccinimide (1.35 g) was dissolved in a 9:1 (v/v) mixture of anhydrous DCM and dioxane (40 mL) and then TfOH (60 µL) was added at 0 °C.

Fmoc Deprotection Solution
The solution was 20% (v/v) triethylamine in DMF.

Acetylation Capping Solution
Ac_2O was directly used.

Nap Deprotection Solution: 0.1 M of 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) solution
For Nap deprotection, DDQ (454 mg, 2 mmol) was dissolved in 16 mL DCE, 4 mL MeOH and 0.25 mL phosphate buffer (pH 7.4).

Lev Deprotection Solution: 0.56 M of hydrazine monohydrate solution
For Lev deprotection a 0.56 M solution of hydrazine monohydrate (0.68 mL) was dissolved in pyridine–acetic acid (3:2, v/v, 25 mL).

Sulfation Solution: 0.5 M of sulfur trioxide pyridine complex in DMF/pyridine
For sulfation, sulfur trioxide pyridine complex (1.6 g) was dissolved in DMF–pyridine (1:1, v/v, 20 mL).

Preparation of the resin and the synthesizer for automated synthesis: The functionalized resin was loaded into the reaction vessel of the synthesizer and swollen in 2 mL DCM. The building blocks were co-evaporated with toluene three times, dissolved in DCM under an argon atmosphere and transferred into the vials that were placed on the corresponding port in the synthesizer. Reagents were dissolved in the

corresponding solvents under an Ar atmosphere in bottles that were placed on the corresponding port in the synthesizer.

Module I – Acidic TMSOTf Wash: The resin is washed with DMF, THF, DCM (three times each, with 2 mL for 10 s), and 0.350 mL of solution of TMSOTf in DCM for one minute at -20 °C. The resin is swollen in 2 mL DCM and the temperature of the reaction vessel is adjusted to $\mathbf{T_1}$.

Module II – Glycosylation using thioglycoside: For the glycosylation the DCM is drained and a solution of the thioglycoside building block (BB) (5 equiv. in 1.0 mL DCM) is delivered to the reaction vessel. After the set temperature is reached ($\mathbf{T_a}$), the reaction starts with the addition of 1 mL of activator solution. The glycosylation is performed at $\mathbf{T_a}$ for 5 min and at $\mathbf{T_i}$ for 25 min. After the reaction the solution is drained and the resin is washed with DCM (six times 2 mL for 15 s). This procedure is repeated twice.

Module III- Glycosylation (2 x 7.5 equivalents of the donor): For glycosylation the DCM is drained and a solution of the thioglycoside **11** (**7.5 equiv.** in 1.0 mL DCM) is delivered to the reaction vessel. After the set temperature is reached ($\mathbf{T_a}$), the reaction starts with the addition of **1.5 mL** of activator solution. The glycosylation is performed $\mathbf{T_a}$ for 5 min and at $\mathbf{T_i}$ for 25 min. After the reaction the solution is drained and the resin is washed with DCM (six times 2 mL for 15 s). This procedure is repeated twice.

Module IV - Fmoc deprotection: The resin is washed with DMF (six times with 2 mL for 15 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 3 mL of a solution of 20% Et_3N in DMF is 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% Et_3N in DMF is delivered to the resin. This procedure is repeated twice.

Module V – Lev deprotection: The resin is washed with DCM (six times with 2 mL for 25 s), swollen in 1.3 mL DCM and the temperature of the reaction vessel is adjusted to 25 °C. For Lev deprotection 0.8 mL of the hydrazine hydrate solution is delivered into the reaction vessel. After 30 min the reaction solution is drained and the resin is washed with 0.2 M acetic acid in DCM and DCM (six times each with 2 mL for 25 s). The entire procedure is performed three times.

Module VI – Nap deprotection: The resin is washed with DCM (six times with 2 mL for 15 s), swollen in 1.0 mL DCM and the temperature of the reaction vessel is adjusted to 25 °C. For Nap deprotection 2.0 mL of the DDQ solution is delivered. After 25 min the reaction solution is drained and the resin is washed with THF, DMF, and DCM (six times each with 2 mL for 15 s). The entire procedure is performed three times.

Module VII – Acetylation: The resin is washed with pyridine (six times each with 2 mL for 15 s), and swollen in 2 mL pyridine. The temperature of the reaction vessel is adjusted to 25 °C. The reaction is started by addition of 1 mL of acetic anhydride to the reaction vessel. After 30 min, the reaction solution is drained and the resin is washed with CH_2Cl_2 and pyridine (six times with 2 mL for 15 s). This acetylation procedure is performed three times.

Module VIII – Sulfation: The resin is washed with DMF and pyridine (three times each with 2 mL for 15 s), swollen in 2 mL pyridine and the temperature of the reaction vessel is adjusted to 50 °C. For sulfation, 2 mL of a 0.5 M solution of sulfur trioxide pyridine complex in DMF/pyridine, 1:1 is added. After 3 h, the reaction solution is drained and the resin is washed with DMF and pyridine (three times each with 2 mL for 15 s). The entire procedure is performed three times.

Table S1. Glycosylation conditions for building blocks 9-13.

Building block	Glycosylation conditions	
	Activation	Incubation
9	5 min at -30 °C	25 min at -10 °C
10		

11	5 min at -40 °C	25 min at -20 °C
12		
S9		

3.2 HPLC Conditions for Linear and Branched LacNAc Structures

For protected oligosaccharides 13-15

Analytical NP-HPLC: The crude material was analyzed by HPLC (column: Luna 5µ Silica 100A, (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: ELSD).

Deprotection conditions: To a solution of the fully protected oligosaccharide in MeOH (5 mL) was added 0.5 M NaOMe solution (0.25 equiv. per acetyl or benzoyl group) in MeOH at 40 °C. The mixture was stirred until the reaction completed, then neutralized by 200 mg Amberite-120 (400 mg per 100 µL NaOMe solution). This crude mixture was dissolved in MeOH–EtOAc–AcOH (5:0.5:0.2, v/v/v). To the mixture was added 5% Pd/C (W/V), purged first with argon and then with hydrogen, and the reaction mixture was stirred at room temperature for overnight under balloon pressure. The reaction mixture was filtered through a modified cellulose filter, washed with 20 mL of water/MeOH (9:1, v/v) and the combined filtrate was concentrated to provide the crude reaction product.

For conjugation-ready oligosaccharides 1-3

Analytical HPLC: The crude material was analyzed by HPLC with Hypercarb® (150 X 4.60 mm) column at the; flow rate of 0.8 mL/min (solvent A: 0.1% formic acid in acetonitrile; solvent B: 0.1% formic acid in water; gradient (solvent A): 0% (10 min) 30% (in 30 min) 100% (in 5 min); detection: ELSD).

Preparative HPLC: The crude solution was purified by preparative HPLC with Hypercarb® (150 X 10.00 mm) column at the flow rate of 3.6 mL/min (solvent A: 0.1% formic acid in acetonitrile; solvent B: 0.1% formic acid in water; gradient (solvent A): 0% (10 min) 30% (in 30 min) 100% (in 5 min); detection: ELSD) to afford the unprotected oligosaccharide.

3.3 HPLC Conditions for Sulfated LacNAc Structures

For fully protected sulfated tetrasaccharides 17-20

Analytical RP-HPLC: The crude material was analyzed by HPLC with C18-Nucleodur (21x250 mm; 5 µm) column at the flow rate of 1.0 mL/min (solvent A: 3% isopropanol in acetonitrile; solvent B: 3% isopropanol in 0.01 M NH_4HCO_3 in water; gradient (solvent A): 0% (10 min) 30% (in 30 min) 100% (in 5 min); detection: ELSD).

Note that analytical HPLC chromatograms of the crude protected oligosaccharides 18 and 20 indicated the presence of impurities that were removed by RP-HPLC prior to global deprotection to obtain the corresponding conjugation-ready sulfated glycans.

Preparative RP-HPLC: The crude material was analyzed by HPLC with C18-Nucleodur (21x250 mm; 5 µm) column at the flow rate of 10.0 mL/min (solvent A: 3% isopropanol in acetonitrile; solvent B: 3% isopropanol in 0.01 M NH_4HCO_3 in water; gradient (solvent A): 0% (10 min) 30% (in 30 min) 100% (in 5 min); detection: ELSD).

For the conjugation-ready sulfated tetrasaccharides 4-7

Analytical RP-HPLC: The crude material was analyzed by HPLC with Hypercarb® (150 X 4.60 mm) column at the flow rate of 0.8 mL/min (solvent A: acetonitrile; solvent B: 0.01 M NH_4HCO_3 in water; gradient (solvent A): 0% (10 min) 30% (in 30 min) 100% (in 5 min); detection: ELSD).

Preparative RP-HPLC: The crude solution is purified by preparative HPLC with Hypercarb®(150 X 10.00 mm) column at the flow rate of 3.6 mL/min (solvent A: acetonitrile; solvent B: 0.01 M NH_4HCO_3 in water; gradient (solvent A): 0% (10 min) 30% (in 30 min) 100% (in 5 min); detection: ELSD) to afford the unprotected sulfated tetrasaccharides. The collected solution was passed through Dowex-50WX8 resin (Na^+ form), and lyophilized. Non-carbohydrate impurities derived from the ion-exchange step and resin were observed by NMR spectroscopy. These impurities could be removed using G-25 (or G-10 P-2) columns.

3.4 Automated Synthesis of Linear LacNAcs

***N*-Benzyloxycarbonyl-5-amino-pentyl (2-*O*-benzoyl-4,6-di-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-(3,6-di-*O*-benzyl-2-deoxy-2-trichloracetamido-β-D-glucopyranosyl)-(1→3)-(2-*O*-benzoyl-4,6-di-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-trichloracetamido-β-D-glucopyranoside (13)**

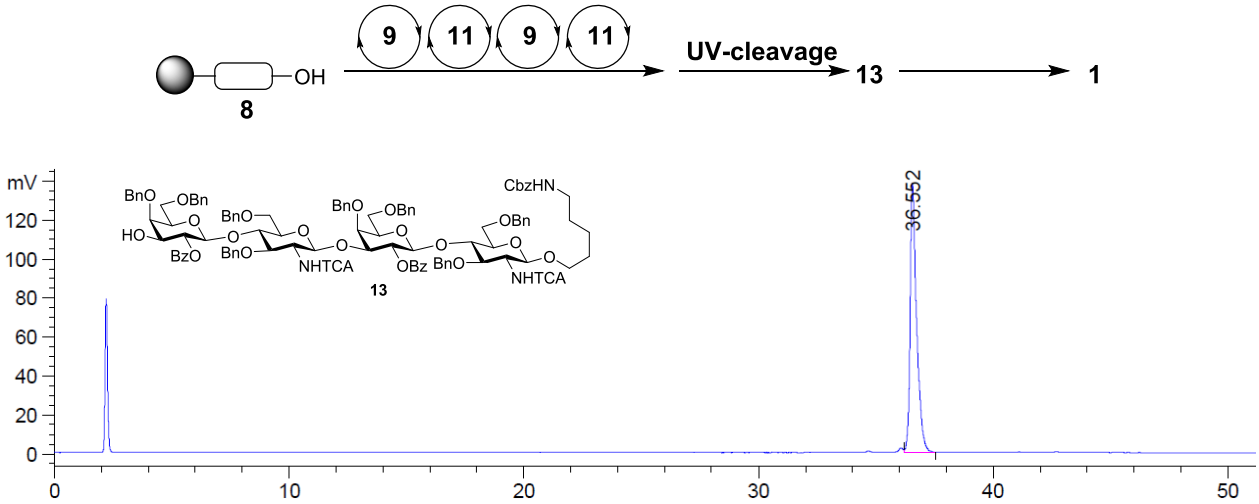


Figure S2. Analytical HPLC chromatogram of the crude protected tetrasaccharide **13**.

5-Amino-pentyl β-D-galactopyranosyl-(1→4)-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-acetamido-2-deoxy-β-D-glucopyranoside (1) (5.1 mg, 6.1 μmol, 14% over fourteen steps)

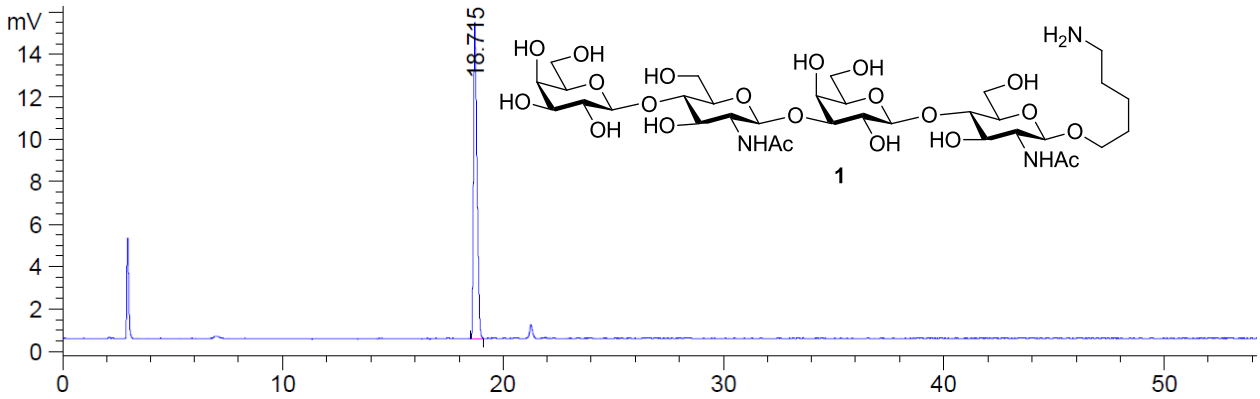
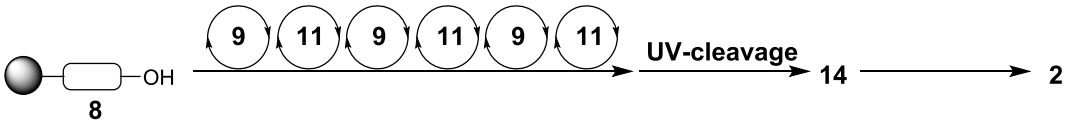


Figure S3. Analytical HPLC chromatogram of the crude tetrasaccharide **1**.

^{1}H NMR (600 MHz, D_2O) δ 4.72 (d, *J* = 8.4 Hz, 1H, **H-1**), 4.54 (d, *J* = 7.5 Hz, 1H, **H-1**), 4.50 (d, *J* = 7.9 Hz, 1H, **H-1**), 4.48 (d, *J* = 8.0 Hz, 1H, **H-1**), 4.17 (d, *J* = 2.8 Hz, 1H), 4.02 – 3.90 (m, 4H), 3.78 (dtdd, *J* = 31.4, 13.7, 11.1, 3.9 Hz, 17H), 3.64 – 3.54 (m, 5H), 3.02 – 2.98 (m, 2H), 2.05 (s, 6H), 1.72 – 1.66 (m, 2H), 1.61 (dt, *J* = 13.5, 6.6 Hz, 2H), 1.45 – 1.37 (m, 2H ^{13}C NMR (150 MHz, D_2O) δ 177.49 (NHAc), 177.01 (NHAc), 173.62 (formic acid), 105.51 (**C-1**), 105.47 (**C-1**), 105.34 (**C-1**), 103.70 (**C-1**), 84.69, 81.13, 80.79, 77.96, 77.49, 77.36, 77.16, 75.12, 75.01, 74.79, 73.57, 72.70, 72.55, 71.15, 70.90, 63.63, 63.55, 62.68, 62.48, 57.80, 57.66, 41.94, 30.68, 28.98, 24.79, 24.76, 24.72.; MS ESI+-HRMS *m/z* $[M+H]^+$ calcd for $C_{38}H_{60}N_3O_{21}$ 834.3714, found 834.3722.

***N*-Benzyloxycarbonyl-5-amino-pentyl (2-*O*-benzoyl-4,6-di-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-(3,6-di-*O*-benzyl-2-deoxy-2-trichloracetamido-β-D-glucopyranosyl)-(1→3)-(2-*O*-benzoyl-4,6-di-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-(3,6-di-*O*-benzyl-2-deoxy-2-trichloracetamido-β-D-glucopyranosyl)-(1→3)-(2-*O*-benzoyl-4,6-di-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-trichloracetamido-β-D-glucopyranoside (14)**



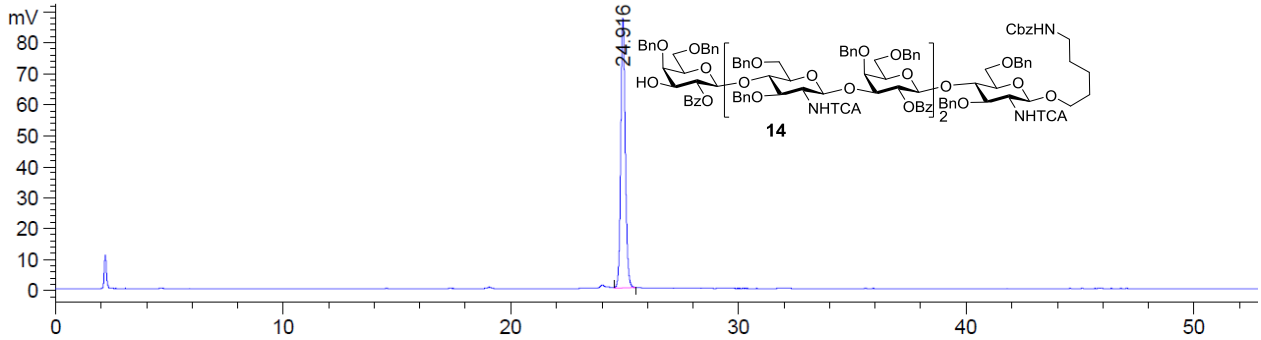


Figure S4. Analytical HPLC chromatogram of the crude protected hexasaccharide **14.**

5-Amino-pentyl β-D-galactopyranosyl-(1→4)-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-acetamido-2-deoxy-β-D-glucopyranoside (2) (4.8 mg, 4.0 umol, 14% over fourteen steps)

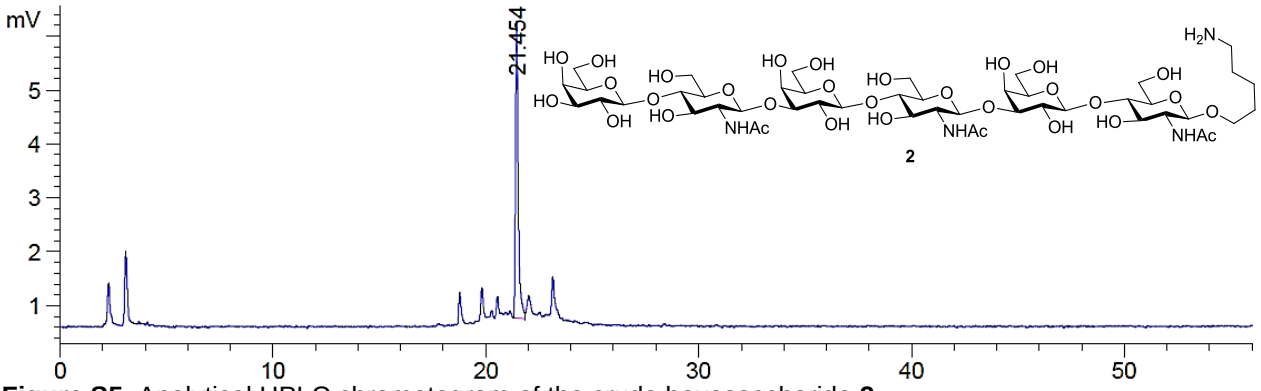


Figure S5. Analytical HPLC chromatogram of the crude hexasaccharide **2**.

^{1}H NMR (600 MHz, D_2O) δ 4.57 (d, *J* = 8.1 Hz, 2H, 2 x **H-1**), 4.39 (d, *J* = 7.3 Hz, 1H, **H-1**), 4.34 (dd, *J* = 13.3, 6.8 Hz, 3H, 3 x **H-1**), 4.03 (s, 2H), 3.89 – 3.38 (m, 36H), 2.86 (t, *J* = 7.4 Hz, 2H), 1.91 (s, 9H), 1.58 – 1.51 (m, 2H), 1.50 – 1.42 (m, 2H), 1.31 – 1.22 (m, 2H). ^{13}C NMR (150 MHz, D_2O) δ 177.50 (2 x NHAc), 177.01 (NHAc), 173.63 (formic acid), 105.50 (3 x **C-1**), 105.47 (**C-1)**, 105.35 (**C-1)**, 103.71 (**C-1)**, 84.68, 81.12, 80.80, 77.96, 77.48, 77.36, 77.16, 75.12, 75.01, 74.79, 73.57, 72.71, 72.56, 71.15, 70.91, 63.63, 63.55, 62.67, 62.47, 57.80, 57.67, 41.94, 30.68, 28.99, 24.78.; MS ESI+-HRMS *m/z* [M+H]+ calcd for $C_{47}H_{84}N_4O_{31}$ 1199.5036, found 1199.4991.

3.5 Automated Synthesis of Branched LacNAc 3

Three strategies can be used to assemble branched hexasaccharide **3** that differ in the number of glycosylation reactions to be performed (Table S2 and Figure S6).

Approach A: The first approach relies on the complete assembly of the linear tetrasaccharide, followed by the removal of a specific Nap (or Lev) protective group and linear chain extension to place the disaccharide branch. This approach requires six glycosylation cycles and **14 steps on resin in total**.

Approach B: Alternatively, simultaneous growth of both branches by placement of monosaccharides to a disaccharide can be pursued.[S4] For that purpose two hydroxyls are liberated by Fmoc cleavage followed and Nap (or Lev) removal to set the stage for extension. This strategy utilizes four glycosylation cycles to complete bis-glycosylation at C-4 in glucosamine **9**.

Approach C: Finally, a new approach is devised using a modified glycosylation conditions that perform glycosylation reaction with 7.5 equivalents of a donor twice to save glycosylating agents and reduce assembly time.

Table S2. Comparison of three different approaches to assemble 16a-16c.

Entry	Total amount of building blocks		Glycosylation reactions	Total reaction time (min)
	9 (equiv.)	**11 (equiv.)**		
Approach A	30	20	12	360
Approach B	30	20	12	360
Approach C	30	**15**	**10**	**300**

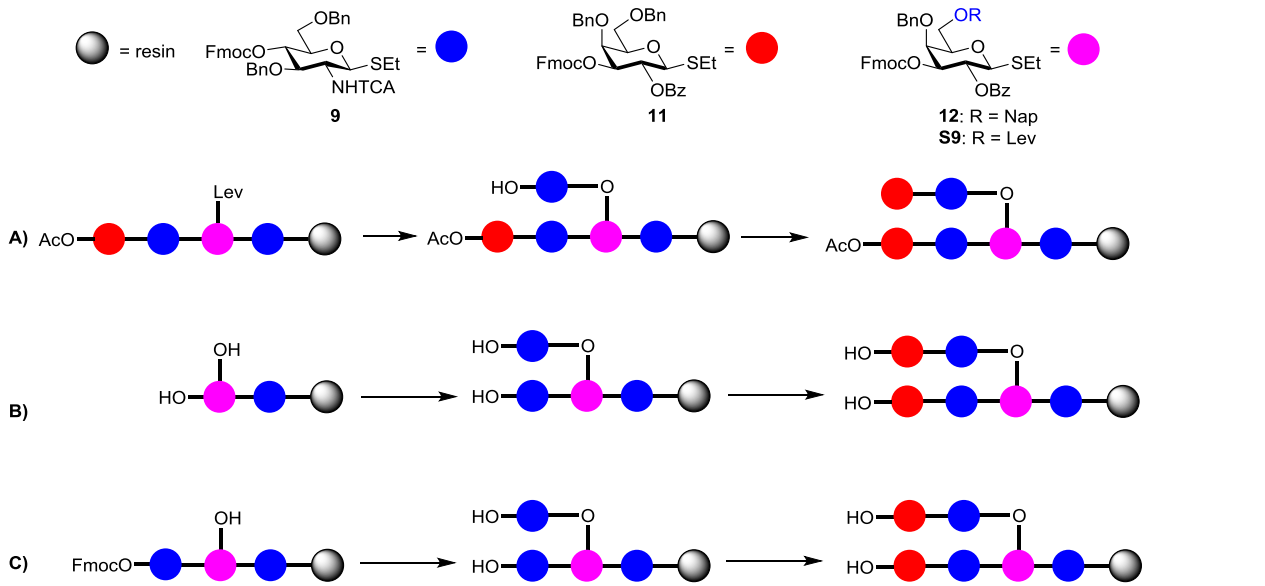
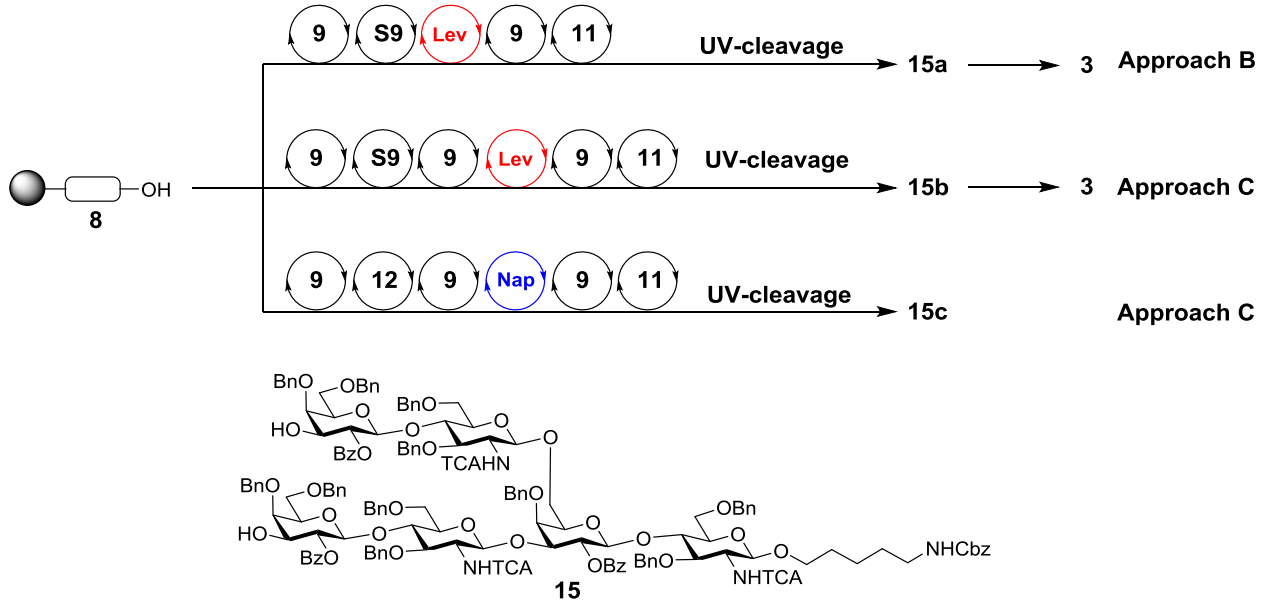


Figure S6. Three approaches to synthesize a branched LacNAc hexasaccharide. A) Step-wise approach. Following assembly of a tetrasaccharide backbone, branched disaccharide was elongated. B) Double glycosylation approach using five equiv. of building block four times. C) Double glycosylation approach using 7.5 equiv. of building block twice.

Two approaches (B and C, Figure S6) and two building blocks (**12** and **S9**) were tested to assemble branched hexasaccharide **3.** For this assembly, Lev and Nap deprotection modules were introduced to automated synthesis. Lev deprotection removes a levulinoyl ester (Lev) using a solution of hydrazine in acetic acid and pyridine (v/v, 3:2) to allow for the other hydroxyl groups (Module IV). Nap deprotection was performed to liberate the hydroxyl group to undergo glycosylation using a homogeneous solution of DDQ in DCE, MeOH, and phosphate buffer (Module V). Following AGA, the resulting oligosaccharide was released by UV irradiation.



Scheme S3. Automated glycan assembly of 15 using three different approaches.

To assemble the branched hexasaccharide following Approach B, the disaccharide LacNAc consisting of **9** and **S9** was initially assembled (Figure S6B). For the bis-glycosylation, following Lev deprotection module, the glycosylation reactions, firstly using five equiv. of **9** four times (4 x 5 eq. of **9**) and five equiv. of **11** four times (4 x 5 eq. of **11**) were executed to afford the protected branched hexasaccharide **15a** (Scheme S3). To evaluate Approach C, a trisaccharide backbone consisting of **9** and **S9** was substituted with glucosamine **9** at C-6 on galactose **S9** to give a branched tetrasaccharide, and then a newly devised glycosylation module (2 x 7.5 equiv. of **11**) afforded the desired compound **15b** (Scheme S3). Lastly, the branched hexasaccharide **16** (Figure S6C) was assembled using the building blocks **12** instead of **13** by Approach C in order to investigate compatibility of Nap deprotection in this synthetic context (Scheme S3). Analysis of the crude hexasaccharide **15a-15c** assembled by three different approaches using two respective building blocks (**12** and **S9**) was indicated to be almost identical by analytical NP-HPLC (Figure S7). Removal of protecting groups provided the branched hexasaccharide **3** in 18% yield over 13 steps through **15a** and 5.3 mg, 4.4 μmol, 18% yield over 12 steps through **15c**.

***N*-Benzyloxycarbonyl-5-amino-pentyl (2-*O*-benzoyl-4,6-di-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-(3,6-di-*O*-benzyl-2-deoxy-2-trichloracetamido-β-D-glucopyranosyl)-(1→3)-[(2-*O*-benzoyl-4,6-di-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-(3,6-di-*O*-benzyl-2-deoxy-2-trichloracetamido-β-D-glucopyranosyl)-(1→6)]-(2-*O*-benzoyl-4,6-di-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-trichloracetamido-β-D-glucopyranoside (15)**

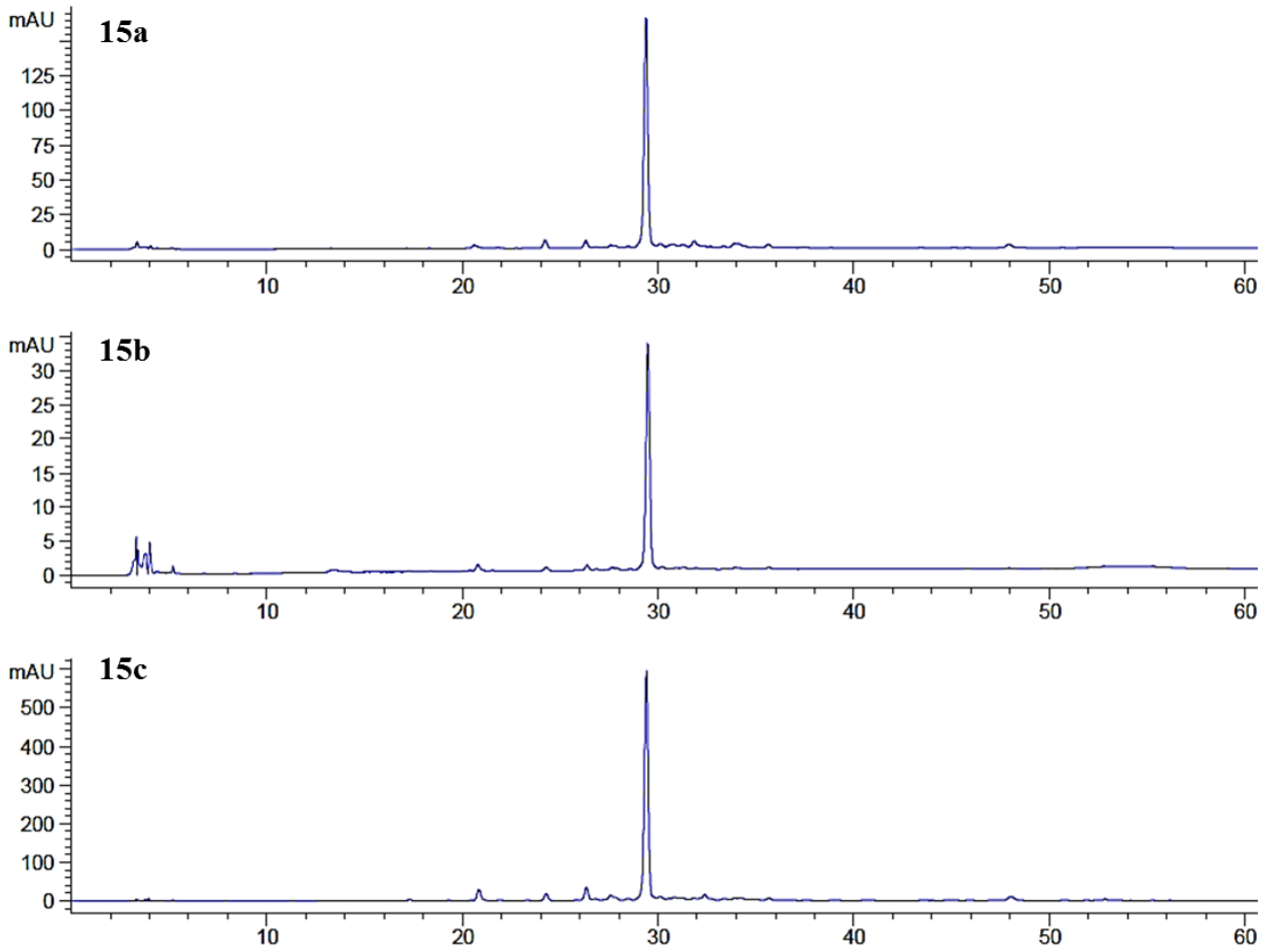


Figure S7. Analytical HPLC chromatogram of the crude protected hexasaccharides **15a-15c.**

5-Amino-pentyl β-D-galactopyranosyl-(1→4)-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-[β-D-galactopyranosyl-(1→4)-acetamido-2-deoxy-β-D-glucopyranosyl-(1→6)]-β-D-galactopyranosyl-(1→4)-acetamido-2-deoxy-β-D-glucopyranoside (3) (5.3 mg, 4.4 µmol, 14% over thirteen steps)

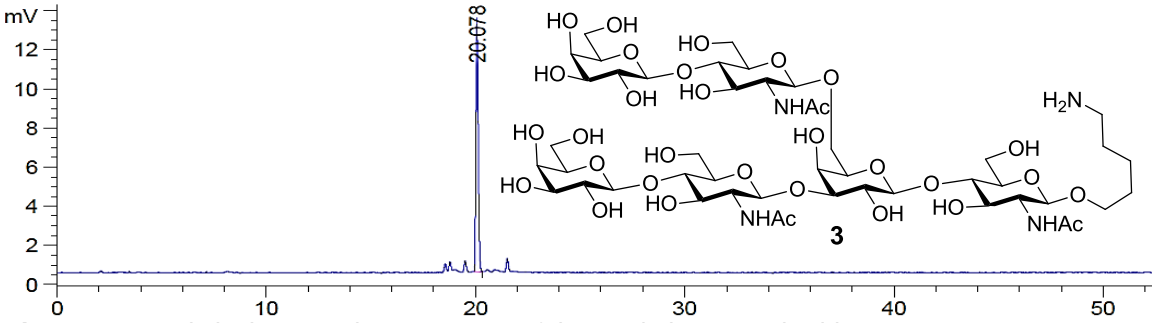
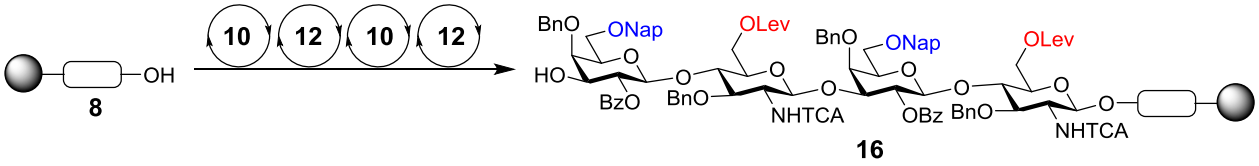


Figure S8. Analytical HPLC chromatogram of the crude hexasaccharide **3.**

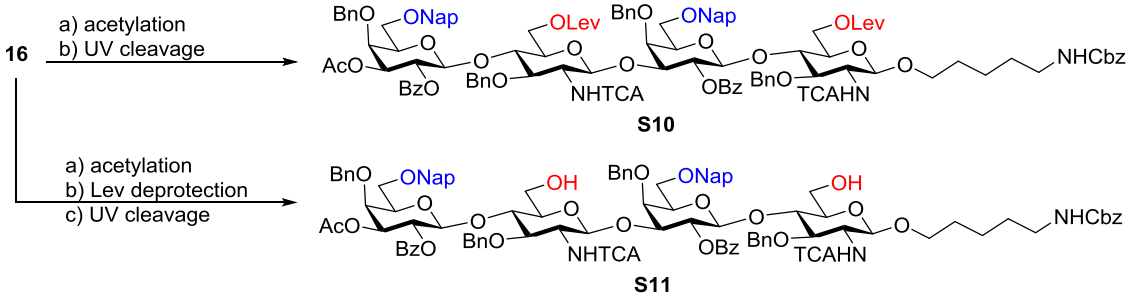
^{1}H NMR (600 MHz, D_2O) δ 4.72 (d, *J* = 8.4 Hz, 1H, **H-1**), 4.63 (d, *J* = 7.8 Hz, 1H, **H-1**), 4.54 (d, *J* = 8.2 Hz, 1H, **H-1**), 4.51 – 4.47 (m, 3H, 3 x **H-1**), 4.17 (d, *J* = 3.0 Hz, 1H), 4.02 – 3.90 (m, 7H), 3.89 – 3.54 (m, 30H), 3.01 (t, *J* = 7.6 Hz, 2H), 2.08 (s, 3H), 2.05 (d, *J* = 4.0 Hz, 6H), 1.69 (dt, *J* = 15.4, 7.7 Hz, 2H), 1.65 – 1.59 (m, 2H), 1.46 – 1.38 (m, 2H). ^{13}C NMR (150 MHz, D_2O) δ 177.47 (NHAc), 177.07 (NHAc), 176.90 (NHAc), 171.67 (formic acid), 105.53 (**C-1**), 105.47 (2 x **C-1**), 105.32 (**C-1**), 103.71 (**C-1**), 103.50 (**C-1**), 84.42, 81.60, 80.97, 80.77, 77.95, 77.28, 77.16, 76.32, 75.07, 74.95, 74.78, 73.57, 72.71, 72.41, 71.26, 71.15, 63.62, 62.59, 57.81, 57.64, 41.94, 30.68, 28.98, 25.03, 24.78, 24.72.; MS ESI+-HRMS *m/z* [M+Na]+ calcd for $C_{47}H_{83}N_4O_{31}Na$ 1221.4855, found 1221.4836.

3.6 Automated Synthesis of Sulfated LacNAc/KS Oligosaccharides



Scheme S4. Automated glycan assembly of a common tetrasaccharide backbone.

***N*-Benzyloxycarbonyl-5-amino-pentyl 3-*O*-acetyl-2-*O*-benzoyl-4-*O*-benzyl-6-*O*-(2-methylnaphthyl)-β-D-galactopyranosyl-(1→4)-3-*O*-benzyl-6-*O*-levulinyl-2-deoxy-2-trichloracetamido-β-D-glucopyranosyl-(1→3)-2-*O*-benzoyl-4-*O*-benzyl-6-*O*-(2-methylnaphthyl)-β-D-galactopyranosyl-(1→4)-3-*O*-benzyl-6-*O*-levulinyl-2-deoxy-2-trichloracetamido-β-D-glucopyranoside S10**



Scheme S5. Automated glycan assembly of tetrasaccharides S10 and S11.

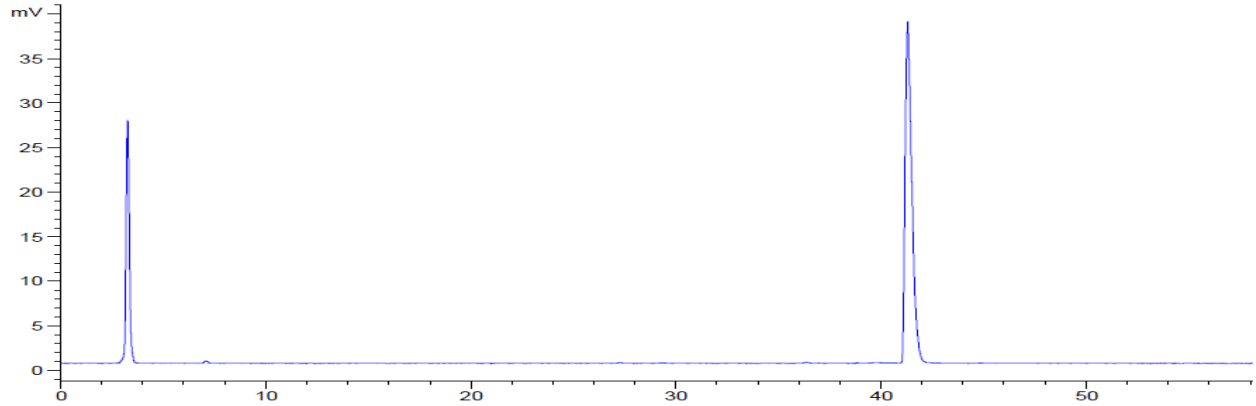


Figure S9. Analytical HPLC chromatogram of the crude tetrasaccharide **S10**. Conditions: column: Luna 5µ Silica 100A, (260 X 4.60 mm); flow rate: 1 mL/min; eluents: 5% DCM in hexane / 5% DCM in EtOAc; gradient: 20% (5 min) 60% (in 40 min) 100% (in 5 min); detection: ELSD.

^{1}H NMR (600 MHz, $CDCl_3$) δ 8.05 (dd, *J* = 23.0, 7.5 Hz, 4H), 7.78 (ddd, *J* = 12.0, 9.3, 3.5 Hz, 6H), 7.63 – 7.42 (m, 13H), 7.35 – 7.00 (m, 26H), 6.58 (d, *J* = 8.1 Hz, 1H), 5.62 (dd, *J* = 10.3, 7.9 Hz, 1H), 5.55 (dd, *J* = 9.9, 8.1 Hz, 1H), 5.29 (dd, *J* = 10.4, 3.0 Hz, 1H), 5.06 (s, 2H), 4.95 (dd, *J* = 11.0, 4.8 Hz, 2H), 4.85 (t, *J* = 8.8 Hz, 3H), 4.77 – 4.68 (m, 2H), 4.61 – 4.42 (m, 9H), 4.32 (dd, *J* = 12.0, 3.5 Hz, 2H), 4.25 (dd, *J* = 11.9, 3.8 Hz, 1H), 4.19 – 3.99 (m, 6H), 3.91 – 3.75 (m, 5H), 3.71 (t, *J* = 6.1 Hz, 1H), 3.69 – 3.55 (m, 3H), 3.52 – 3.45 (m, 1H), 3.44 – 3.32 (m, 4H), 3.29 (dd, *J* = 9.1, 5.3 Hz, 1H), 3.18 (dd, *J* = 15.7, 6.5 Hz, 1H), 3.11 (dd, *J* = 12.8, 6.3 Hz, 2H), 2.75 – 2.56 (m, 2H), 2.52 – 2.28 (m, 5H), 2.26 – 2.16 (m, 1H), 2.06 (s, 3H), 1.98 (s, 3H), 1.87 (s, 3H), 1.50 – 1.37 (m, 4H), 1.33 – 1.20 (m, 2H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 206.62, 206.56, 172.36, 170.40, 165.28, 165.14, 161.90, 161.81, 156.50, 138.96, 138.21, 136.76, 135.68, 135.39, 133.53, 133.29, 133.08, 130.17, 130.09, 129.52, 129.35, 128.80, 128.75, 128.61, 128.40, 128.25, 128.21, 128.09, 127.99, 127.80, 127.50, 127.33, 126.73, 126.64, 126.26, 126.06, 125.95, 100.91, 100.49, 99.60, 92.56, 92.09, 79.18, 77.85, 77.68, 75.86, 75.20, 75.11, 74.70, 74.49, 74.09, 73.68, 73.56, 73.45, 73.37, 73.24, 72.40, 70.99, 69.71, 68.73, 67.62, 66.65, 63.04, 62.32, 57.76, 56.76, 41.02, 37.82, 37.75, 29.83, 29.78, 29.64, 29.00, 27.82, 27.76, 23.28, 20.90.; MS ESI+-HRMS *m/z* [M+Na]+ calcd for $C_{117}H_{121}N_3O_{30}Na$ 2280.6058, found 2280.6069.

_N_-Benzyloxycarbonyl-5-amino-pentyl 3-_O_-acetyl-2-_O_-benzoyl-4-_O_-benzyl-6-_O_-(2-methylnaphthyl)-β-D-galactopyranosyl-(1→4)-3-_O_-benzyl-2-deoxy-2-trichloracetamido-β-D-glucopyranosyl-(1→3)-2-_O_-benzoyl-4-_O_-benzyl-6-_O_-(2-methylnaphthyl)-β-D-galactopyranosyl-(1→4)-3-_O_-benzyl-2-deoxy-2-trichloracetamido-β-D-glucopyranoside (S11)

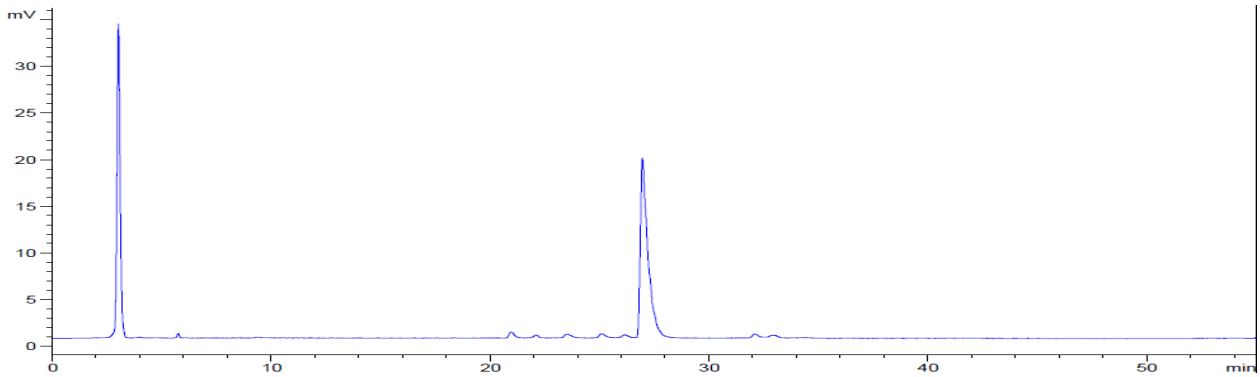
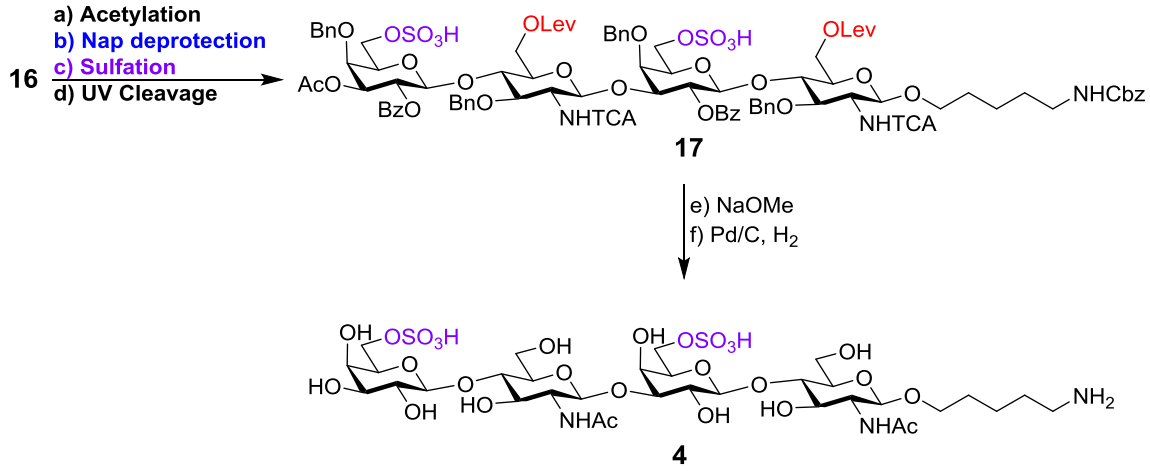


Figure S10. Analytical HPLC chromatogram of the crude tetrasaccharide **S11**. Conditions: column: Luna 5µ Silica 100A, (260 X 4.60 mm); flow rate: 1 mL/min; eluents: 5% DCM in hexane / 5% DCM in EtOAc; gradient: 50% (5 min) 80% (in 30 min) 100% (in 5 min); detection: ELSD.

^{1}H NMR (600 MHz, $CDCl_3$) δ 8.07 (d, *J* = 7.5 Hz, 2H), 8.02 (d, *J* = 7.5 Hz, 2H), 7.80 (dt, *J* = 18.4, 7.3 Hz, 6H), 7.63 – 7.55 (m, 4H), 7.52 – 7.43 (m, 9H), 7.26 (ddt, *J* = 37.6, 15.8, 6.0 Hz, 19H), 7.10 (dt, *J* = 21.9, 6.9 Hz, 4H), 7.03 (t, *J* = 7.3 Hz, 2H), 6.97 (d, *J* = 7.8 Hz, 1H), 6.55 (d, *J* = 8.1 Hz, 1H), 5.59 (dd, *J* = 18.2, 8.0 Hz, 2H), 5.18 (dd, *J* = 10.5, 3.0 Hz, 1H), 5.06 (s, 2H), 4.96 – 4.86 (m, 3H), 4.80 (d, *J* = 7.7 Hz, 1H), 4.76 (s, 1H), 4.73 (dd, *J* = 9.6, 5.4 Hz, 2H), 4.66 (d, *J* = 7.9 Hz, 1H), 4.57 (d, *J* = 7.6 Hz, 1H), 4.54 (d, *J* = 10.6 Hz, 1H), 4.49 (dt, *J* = 15.1, 8.0 Hz, 5H), 4.35 (dd, *J* = 11.9, 2.8 Hz, 2H), 4.03 (d, *J* = 2.6 Hz, 1H), 3.87 (ddd, *J* = 22.5, 14.7, 8.9 Hz, 5H), 3.76 – 3.72 (m, 1H), 3.66 (tt, *J* = 16.4, 8.3 Hz, 6H), 3.58 (s, 3H), 3.52 – 3.48 (m, 1H), 3.46 (t, *J* = 8.5 Hz, 2H), 3.35 (ddd, *J* = 18.8, 9.0, 5.3 Hz, 2H), 3.28 (d, *J* = 8.9 Hz, 1H), 3.18 (d, *J* = 8.1 Hz, 1H), 3.12 (d, *J* = 5.8 Hz, 3H), 1.89 (s, 3H), 1.50 – 1.41 (m, 4H), 1.29 (dd, *J* = 15.5, 7.1 Hz, 2H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.40 (Ac), 165.38 (Bz), 164.99 (Bz), 161.88 (2 x NHTCA), 156.56 (Cbz), 138.73, 138.30, 138.16, 138.11, 136.79, 135.52, 135.41, 133.71, 133.64, 133.39, 133.20, 129.95, 129.86, 129.68, 129.54, 128.88, 128.65, 128.45, 128.39, 128.37, 128.30, 128.22, 128.04, 128.00, 127.86, 127.62, 127.54, 127.48, 126.77, 126.72, 126.31, 126.10, 125.96, 125.92, 101.07, 101.00, 100.57, 99.84, 92.68, 92.24, 78.94, 78.01, 77.89, 77.37, 77.16, 76.95, 76.21, 76.12, 76.00, 75.87, 75.27, 74.95, 74.70, 74.52, 74.38, 73.93, 73.78, 73.74, 73.69, 72.70, 71.11, 69.83, 68.28, 67.72, 66.75, 61.02, 57.72, 57.55, 41.04, 29.63, 29.06, 23.26, 20.85.; MS ESI+-HRMS *m/z* [M+Na]+ calcd for $C_{107}H_{119}O_{26}Na$ 2084.5323, found 2084.5340.

_N_-Benzyloxycarbonyl-5-amino-pentyl 3-_O_-acetyl-2-_O_-benzoyl-4-_O_-benzyl-6-_O_-sulfato-β-D-galactopyranosyl-(1→4)-3-_O_-benzyl-6-_O_-levulinyl-2-deoxy-2-trichloracetamido-β-D-glucopyranosyl-(1→3)-2-_O_-benzoyl-4-_O_-benzyl-6-_O_-6-_O_-sulfato-β-D-galactopyranosyl-(1→4)-3-_O_-benzyl-6-_O_-levulinyl-2-deoxy-2-trichloracetamido-β-D-glucopyranoside (18)



Scheme S6. Automated glycan assembly of 4.

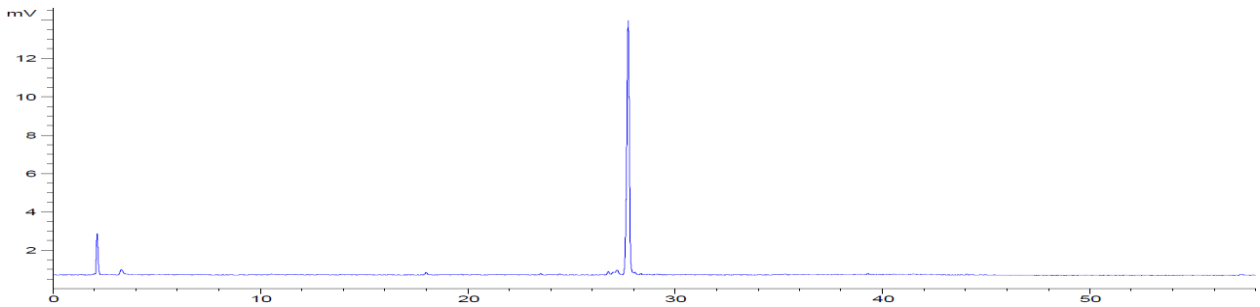


Figure S11. Analytical HPLC chromatogram of the crude tetrasaccharide **17.**

5-Amino-pentyl 6-*O*-sulfato-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-6-*O*-sulfato-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranoside (4) (3.5 mg, 3.5 µmol, 14% over fourteen steps)

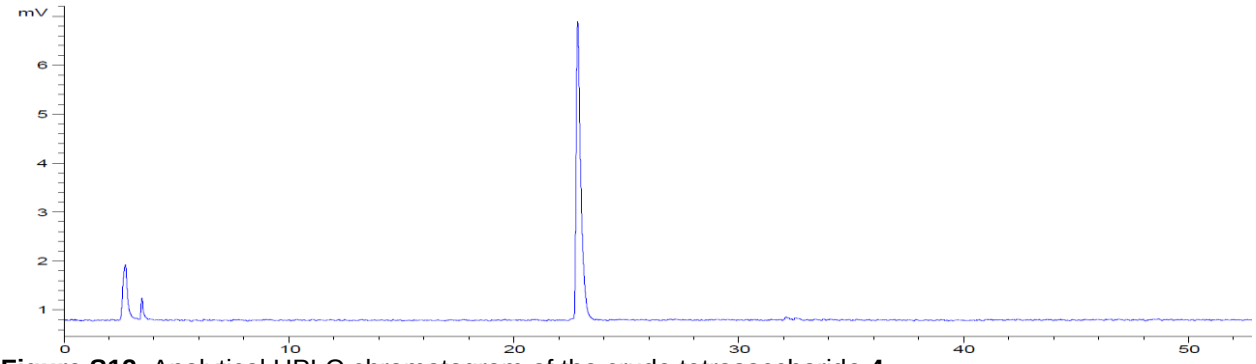
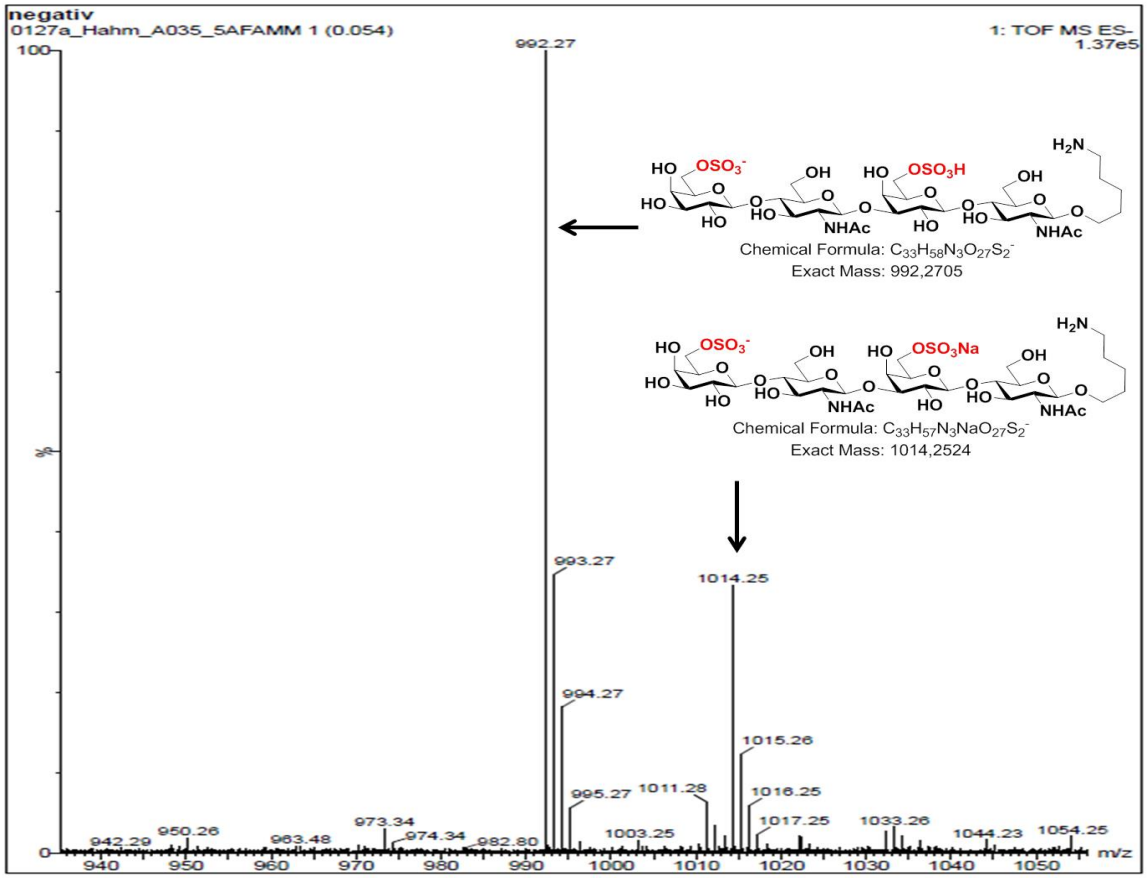
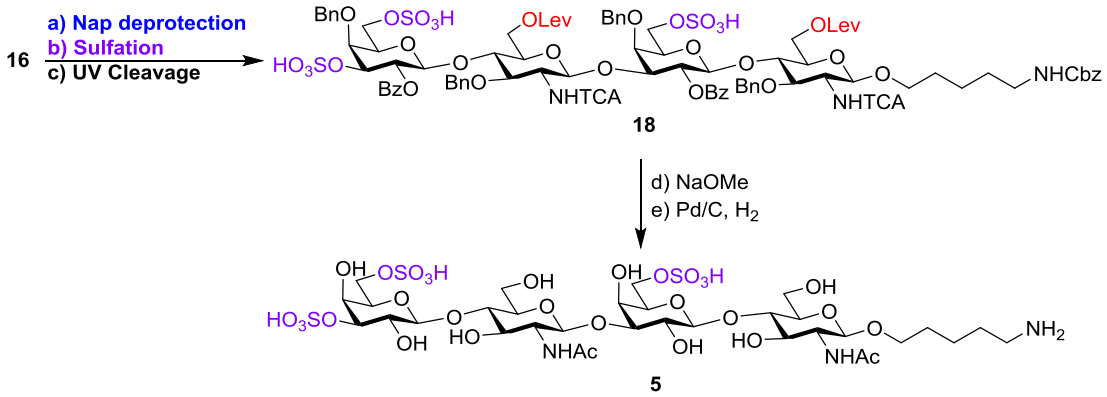


Figure S12. Analytical HPLC chromatogram of the crude tetrasaccharide **4.**

^{1}H NMR (700 MHz, D_2O) δ 4.64 (d, *J* = 8.0 Hz, 1H, **H-1**), 4.44 (t, *J* = 8.5 Hz, 3H, 3 x **H-1**), 4.12 (dt, *J* = 21.2, 12.1 Hz, 4H), 3.94 – 3.86 (m, 4H), 3.85 – 3.81 (m, 1H), 3.80 – 3.59 (m, 12H), 3.57 – 3.50 (m, 4H), 3.50 – 3.46 (m, 1H), 2.92 (t, *J* = 7.5 Hz, 2H), 1.96 (s, 6H), 1.63 – 1.57 (m, 2H), 1.53 (dt, *J* = 12.9, 6.6 Hz, 2H), 1.36 – 1.30 (m, 2H). ^{13}C NMR (176 MHz, D_2O) δ 174.90, 174.43, 102.91 (**C-1**), 102.77 (**C-1**), 102.62 (**C-1**), 101.08 (**C-1**), 82.09, 79.18, 79.00, 74.74, 74.50, 72.80, 72.48, 72.31, 72.07, 70.81, 70.02, 69.73, 68.24, 67.97, 67.35, 67.17, 60.28, 60.00, 55.23, 55.17, 39.34, 28.06, 28.03, 26.36, 26.34, 26.32, 22.20, 22.17, 22.15, 22.09.; MS ESI+-HRMS *m/z* $[M-H]^-$ calcd for $C_{33}H_{58}N_3O_{27}S_2^-$ 992.2705, found 992.2708.



***N*-Benzyloxycarbonyl-5-amino-pentyl 2-*O*-benzoyl-4-*O*-benzyl-3,6-di-*O*-sulfato-β-D-galactopyranosyl-(1→4)-3-*O*-benzyl-6-*O*-levulinyl-2-deoxy-2-trichloracetamido-β-D-glucopyranosyl-(1→3)-2-*O*-benzoyl-4-*O*-benzyl-2,6-di-*O*-sulfato-β-D-galactopyranosyl-(1→4)- 3-*O*-benzyl-6-*O*-levulinyl-2-deoxy-2-trichloracetamido-β-D-glucopyranoside (18)** (21.1 mg, 9.7 μmol, 14% over eleven steps)



Scheme S7. Automated glycan assembly of 5.

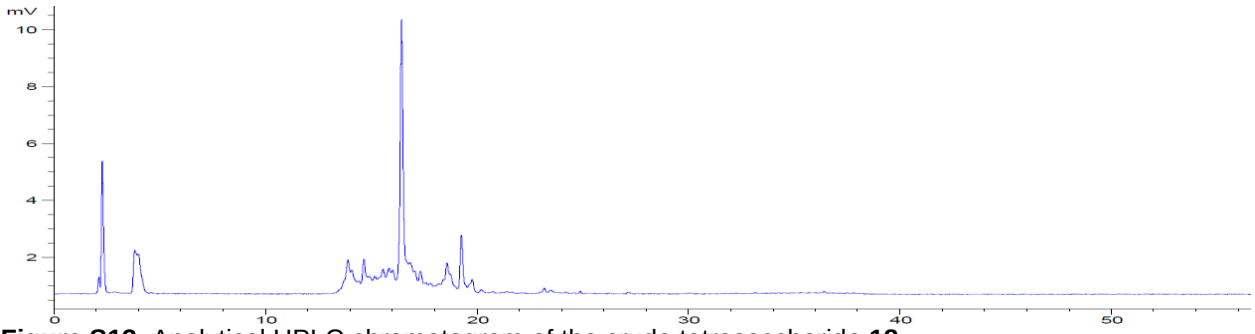
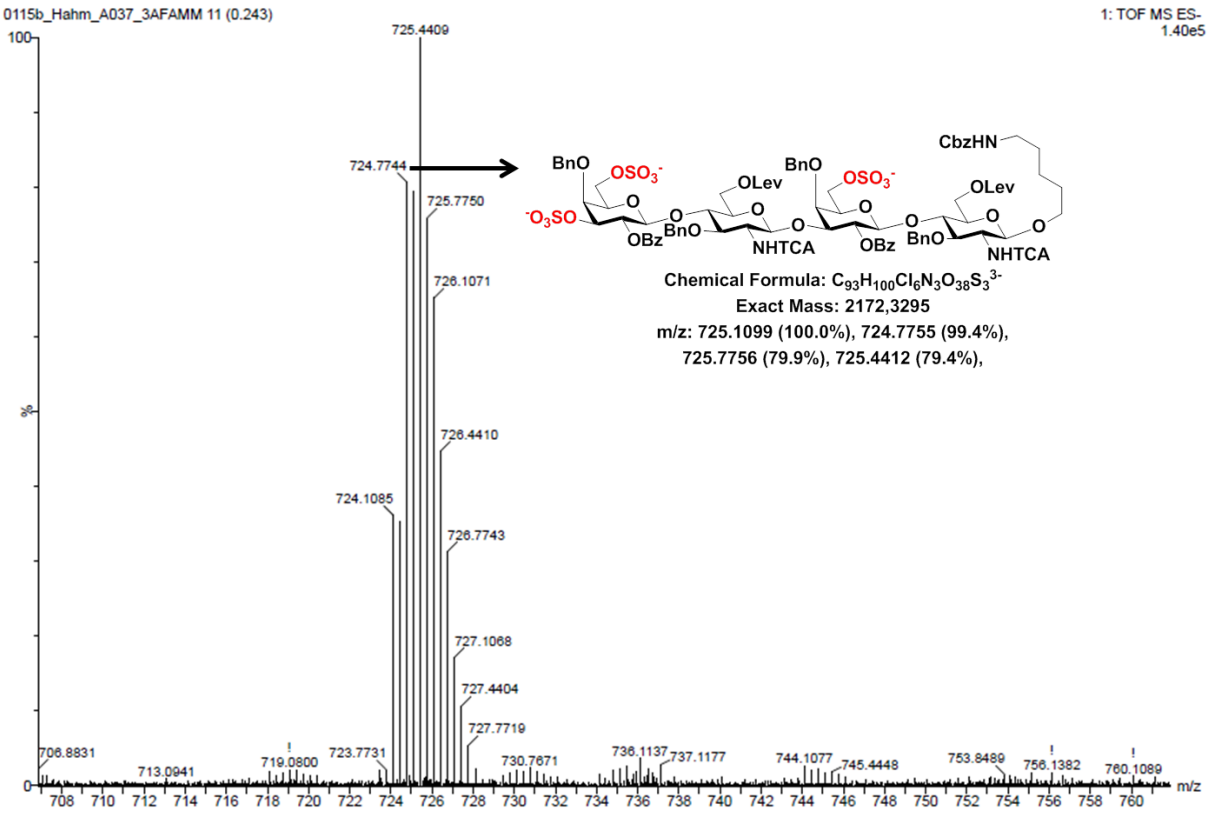


Figure S13. Analytical HPLC chromatogram of the crude tetrasaccharide **18.**

^{1}H NMR (600 MHz, CD_3OD) δ 8.13 (d, *J* = 7.4 Hz, 2H), 8.04 (d, *J* = 7.4 Hz, 2H), 7.74 – 7.69 (m, 1H), 7.62 (dt, *J* = 7.9, 3.0 Hz, 2H), 7.59 (t, *J* = 7.5 Hz, 1H), 7.50 (t, *J* = 7.7 Hz, 2H), 7.45 (dd, *J* = 16.3, 8.0 Hz, 6H), 7.32 (d, *J* = 4.3 Hz, 3H), 7.28 (dd, *J* = 15.1, 7.6 Hz, 5H), 7.22 (ddd, *J* = 7.6, 6.8, 4.4 Hz, 6H), 7.11 (dd, *J* = 14.5, 7.1 Hz, 5H), 5.51 (dd, *J* = 10.2, 7.9 Hz, 1H), 5.47 (dd, *J* = 10.2, 7.9 Hz, 1H), 5.13 (d, *J* = 10.8 Hz, 1H), 5.04 (d, *J* = 13.3 Hz, 3H), 4.93 (s, 2H), 4.76 – 4.69 (m, 3H), 4.64 (d, *J* = 11.0 Hz, 1H), 4.57 (s, 1H), 4.51 – 4.43 (m, 4H), 4.31 – 4.25 (m, 3H), 4.22 (dt, *J* = 9.9, 5.0 Hz, 3H), 4.17 – 4.11 (m, 2H), 4.04 (dt, *J* = 13.4, 6.5 Hz, 4H), 3.99 (dd, *J* = 8.9, 5.2 Hz, 2H), 3.92 – 3.83 (m, 3H), 3.79 – 3.72 (m, 3H), 3.69 (dd, *J* = 15.8, 6.2 Hz, 1H), 3.50 – 3.44 (m, 1H), 3.35 (dt, *J* = 11.6, 5.5 Hz, 1H), 3.27 (d, *J* = 8.4 Hz, 1H), 3.11 – 3.06 (m, 1H), 3.03 (t, *J* = 7.0 Hz, 2H), 2.84 – 2.66 (m, 4H), 2.56 – 2.37 (m, 4H), 2.15 (s, 3H), 2.07 (s, 3H), 1.48 – 1.39 (m, 6H).; ^{13}C NMR (150 MHz, CD_3OD) δ 209.73, 209.38, 174.28, 174.20, 169.34, 167.28, 166.79, 164.10, 164.02, 158.83, 140.44, 139.61, 139.50, 138.46, 134.48, 134.32, 133.58, 132.40, 131.35, 131.21, 131.07, 129.85, 129.77, 129.68, 129.60, 129.44, 129.20, 129.13, 129.09, 129.01, 128.90, 128.73, 128.40, 128.34, 128.28, 102.81, 101.94, 101.90, 101.57, 94.10, 93.57, 80.66, 80.23, 80.12, 79.23, 78.42, 78.19, 76.93, 76.52, 76.41, 75.94, 75.53, 75.39, 74.61, 74.43, 74.12, 73.98, 73.63, 72.67, 70.59, 69.12, 67.35, 67.26, 66.48, 63.85, 58.44, 58.06, 49.43, 49.28, 49.14, 49.00, 48.86, 48.72, 48.57, 47.93, 41.69, 40.18, 38.81, 38.62, 31.63, 30.47, 30.23, 30.14, 29.88, 28.97, 28.83, 25.55, 24.96, 24.24, 24.02.; MS ESI+-HRMS *m/z* $[M-3H]^{3-}$ calcd for $C_{93}H_{101}N_3O_{38}Cl_6S_3$ 725.1099, found 725.4409.



5-Amino-pentyl 3,6-di-*O*-sulfato-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-6-*O*-sulfato β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranoside (5) (3.2 mg, 3.0 μmol, 30% over two steps)

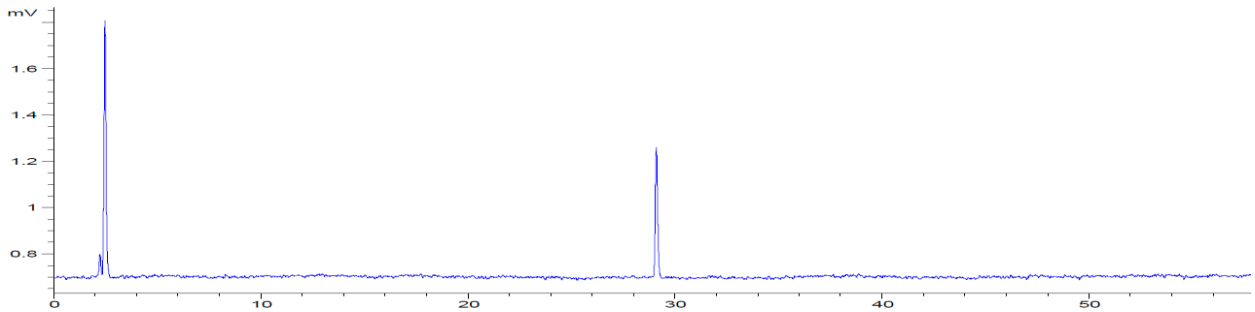
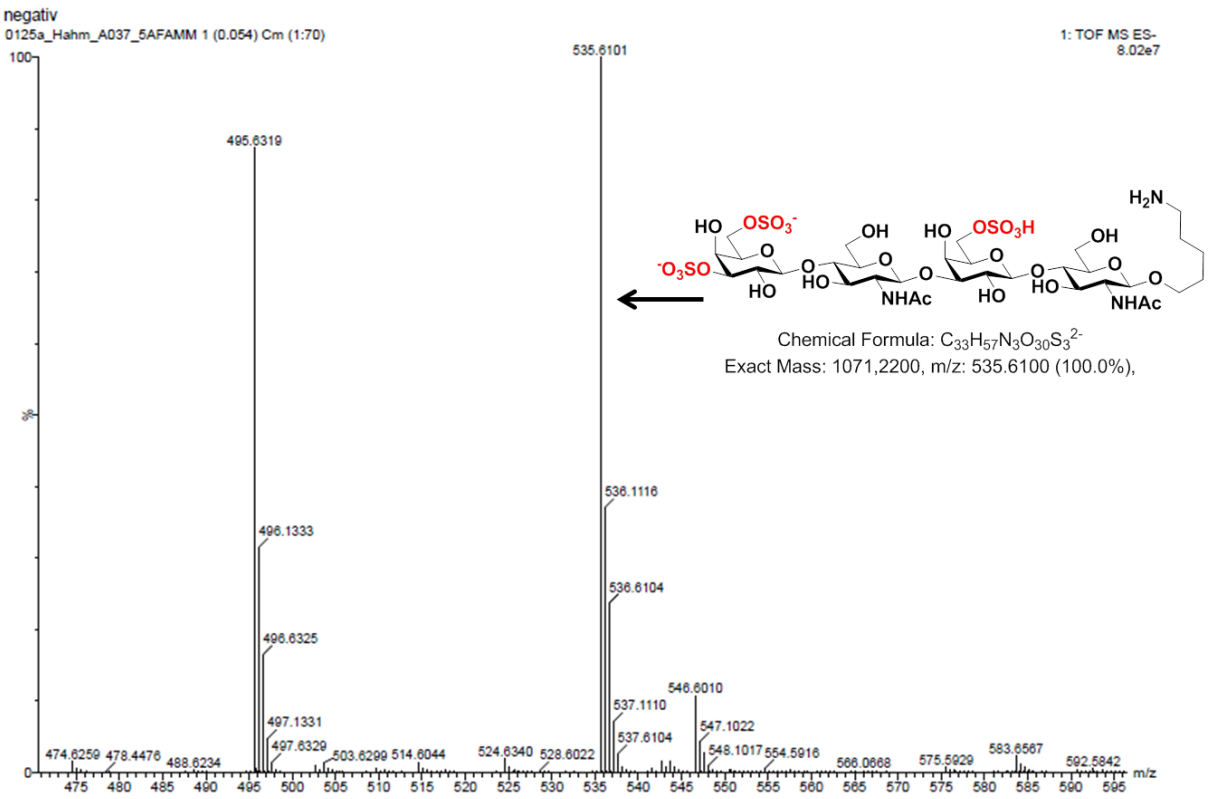
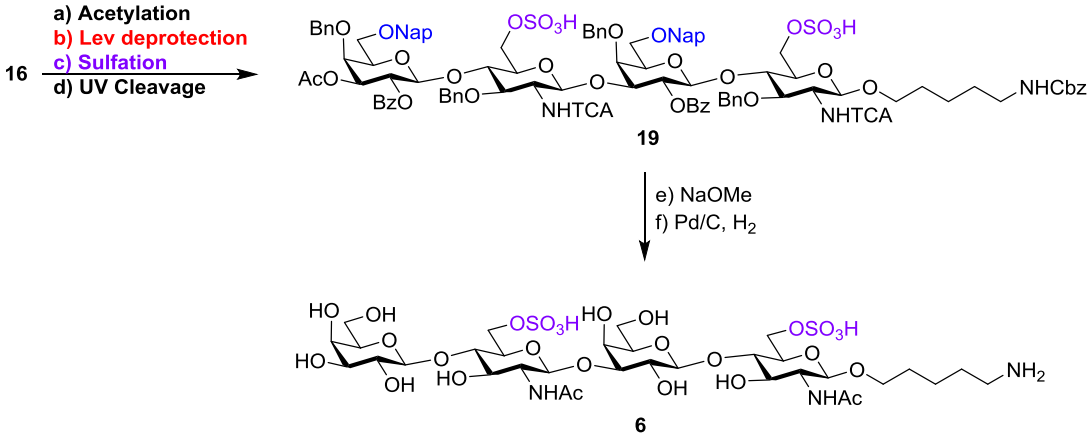


Figure S14. Analytical HPLC chromatogram of the crude tetrasaccharide **5.**

^{1}H NMR (600 MHz, D_2O) δ 4.72 (d, *J* = 8.1 Hz, 2H), 4.65 (d, *J* = 7.9 Hz, 1H), 4.53 (t, *J* = 8.0 Hz, 2H), 4.37 (dt, *J* = 6.1, 3.3 Hz, 2H), 4.26 – 4.16 (m, 5H), 4.05 (dd, *J* = 7.1, 5.3 Hz, 1H), 3.99 (ddd, *J* = 12.1, 10.8, 2.1 Hz, 3H), 3.94 – 3.66 (m, 11H), 3.66 – 3.57 (m, 4H), 3.02 – 2.98 (m, 2H), 2.05 (s, 2H), 2.04 (s, 3H), 1.72 – 1.65 (m, 2H), 1.64 – 1.58 (m, 2H), 1.45 – 1.37 (m, 2H). ^{13}C NMR (150 MHz, D_2O) δ 177.48, 177.02, 105.43, 105.36, 105.19, 105.01, 103.67, 84.67, 82.27, 81.72, 81.56, 77.35, 77.11, 75.06, 74.63, 72.62, 72.31, 71.63, 71.54, 70.63, 70.56, 69.79, 69.22, 62.88, 57.79, 41.95, 30.63, 28.97, 24.79, 24.69.; MS ESI+-HRMS *m/z* $[M-2H]^{2-}$ calcd for $C_{33}H_{57}N_3O_{30}S_3^{2-}$ 535.6100, found 535.6101.



***N*-Benzyloxycarbonyl-5-amino-pentyl (2-*O*-benzoyl-4,6-di-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-(3,6-di-*O*-benzyl-2-deoxy-2-trichloracetamido-β-D-glucopyranosyl)-(1→3)-(2-*O*-benzoyl-4,6-di-*O*-benzyl-β-D-galactopyranosyl)-(1→4)- 3,6-di-*O*-benzyl-2-deoxy-2-trichloracetamido-β-D-glucopyranoside (19)**



Scheme S8. Automated glycan assembly of 6.

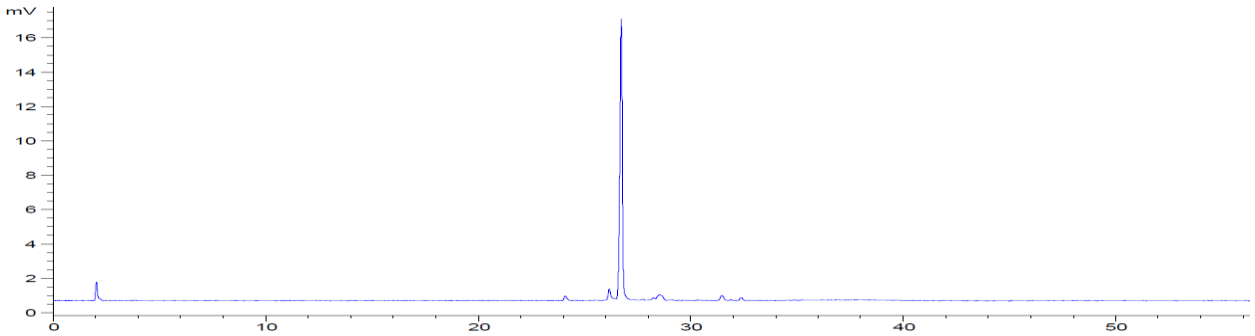
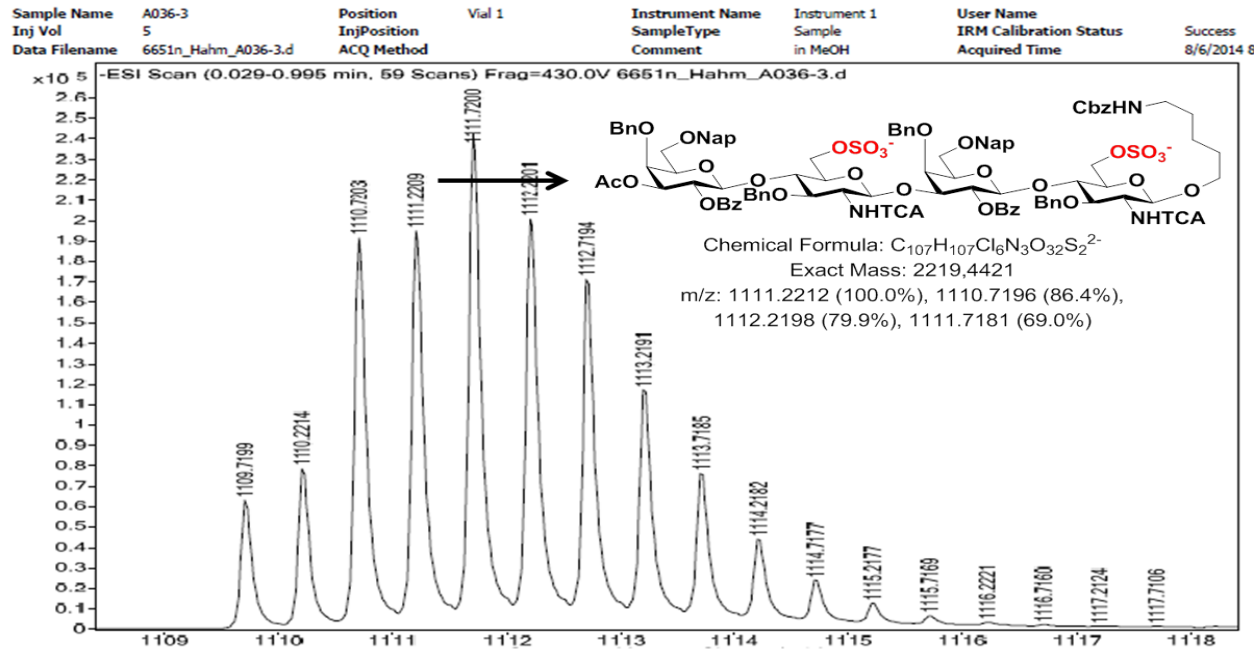


Figure S15. Analytical HPLC chromatogram of the crude tetrasaccharide **19.**

^{1}H NMR (600 MHz, CD_3OD) δ 8.22 – 8.18 (m, 2H), 8.12 (d, *J* = 8.1 Hz, 2H), 7.84 – 7.74 (m, 6H), 7.72 – 7.61 (m, 3H), 7.60 – 7.53 (m, 4H), 7.50 – 7.30 (m, 14H), 7.26 – 7.18 (m, 11H), 7.12 (t, *J* = 7.3 Hz, 1H), 7.01 (t, *J* = 7.4 Hz, 1H), 6.98 – 6.87 (m, 5H), 5.59 (dd, *J* = 10.3, 7.9 Hz, 1H), 5.49 (dd, *J* = 10.1, 8.1 Hz, 1H), 5.29 (dd, *J* = 10.4, 3.2 Hz, 1H), 5.24 (d, *J* = 7.9 Hz, 1H), 5.05 (d, *J* = 11.4 Hz, 1H), 5.03 – 4.97 (m, 4H), 4.91 (d, *J* = 8.0 Hz, 1H), 4.69 (dd, *J* = 9.8, 5.2 Hz, 2H), 4.61 (d, *J* = 12.0 Hz, 1H), 4.49 (ddd, *J* = 23.6, 17.8, 11.5 Hz, 7H), 4.38 (d, *J* = 8.2 Hz, 1H), 4.30 (d, *J* = 12.0 Hz, 1H), 4.20 (ddd, *J* = 13.2, 10.4, 4.2 Hz, 6H), 4.15 – 4.09 (m, 3H), 4.03 (t, *J* = 6.7 Hz, 1H), 3.94 – 3.89 (m, 2H), 3.84 (d, *J* = 9.1 Hz, 1H), 3.81 (t, *J* = 5.9 Hz, 1H), 3.68 (dd, *J* = 20.0, 10.6 Hz, 3H), 3.64 – 3.59 (m, 1H), 3.53 – 3.48 (m, 2H), 3.44 – 3.38 (m, 3H), 1.86 (d, *J* = 0.5 Hz, 3H), 1.47 – 1.37 (m, 6H).^{13}C NMR (150 MHz, CD_3OD) δ 171.67, 167.08, 164.12, 158.85, 140.61, 139.94, 139.84, 137.42, 137.03, 134.73, 134.50, 134.33, 132.39, 131.59, 131.12, 130.63, 129.93, 129.85, 129.67, 129.62, 129.41, 129.26, 129.20, 129.13, 129.05, 129.02, 128.85, 128.71, 128.57, 128.11, 127.97, 127.78, 127.53, 127.49, 127.24, 127.00, 126.76, 126.54, 103.27, 101.93, 101.84, 101.15, 80.69, 78.98, 76.91, 76.76, 76.39, 76.24, 76.12, 75.87, 75.79, 75.59, 74.70, 74.55, 74.49, 73.69, 70.46, 70.37, 69.12, 68.85, 67.24, 66.14, 58.31, 58.01, 49.43, 49.28, 49.14, 49.00, 48.86, 48.72, 48.57, 47.90, 41.71, 40.19, 31.64, 30.49, 30.22, 30.14, 24.96, 24.27, 24.03, 20.69.; MS ESI+-HRMS *m/z* $[M-3H]^{3-}$ calcd for $C_{107}H_{107}N_3O_{32}Cl_6S_2$ 1111.2212, found 1111.2209.



5-Amino-pentyl β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-6-*O*-sulfato-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy6-*O*-sulfato-β-D-glucopyranoside (6) (3.0 mg, 3.0 µmol, 12% over fourteen steps)

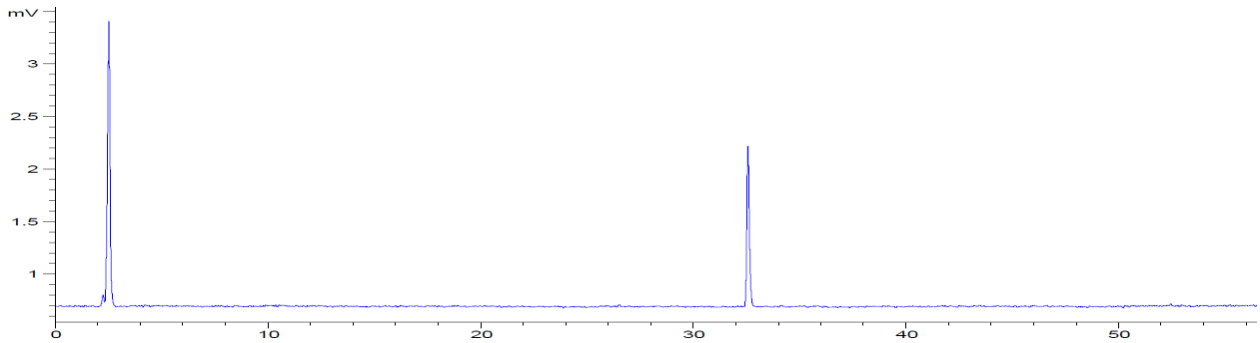
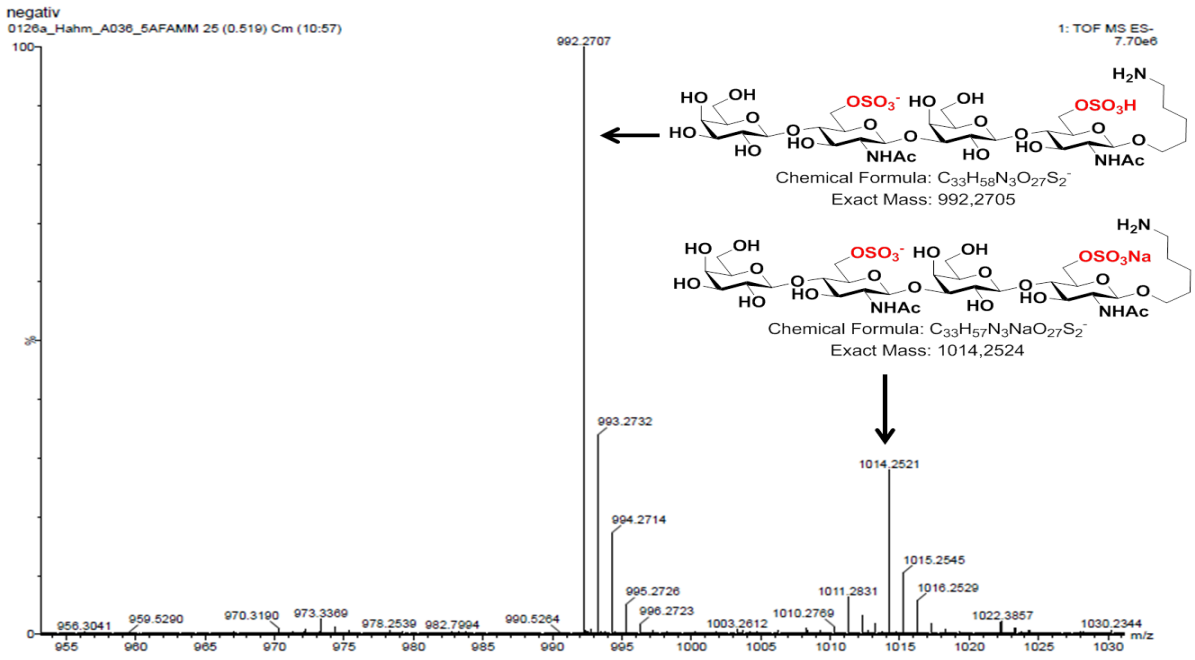
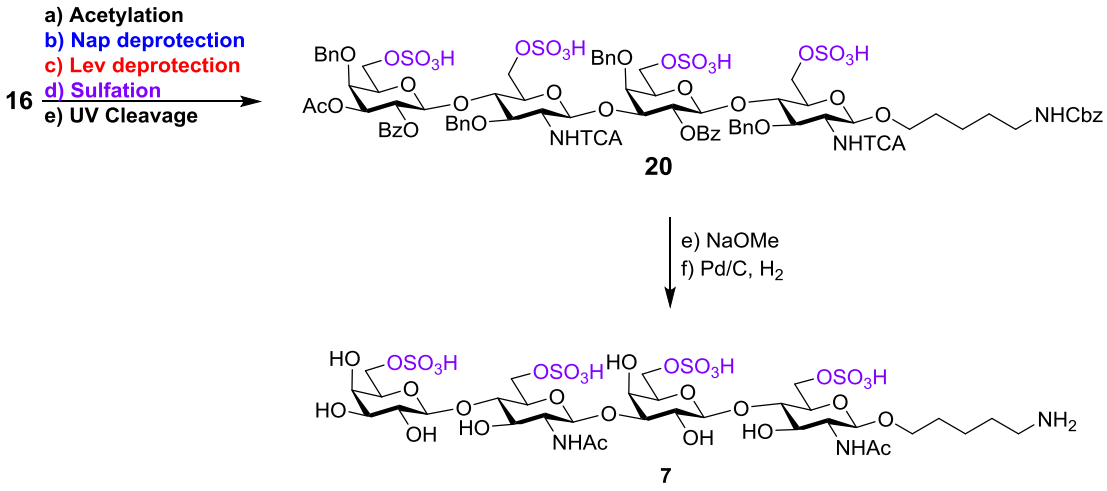


Figure S16. Analytical HPLC chromatogram of the crude tetrasaccharide **6.**

^{1}H NMR (600 MHz, D_2O) δ 4.72 (d, *J* = 8.7 Hz, 1H, **H-1**), 4.53 (dd, *J* = 18.2, 9.1 Hz, 3H, 3 x **H-1**), 4.45 – 4.28 (m, 4H), 4.19 (s, 1H), 3.93 (s, 1H), 3.90 – 3.62 (m, 18H), 3.56 (dt, *J* = 18.0, 8.8 Hz, 2H), 3.00 (t, *J* = 7.5 Hz, 2H), 2.04 (s, 3H), 2.03 (s, 3H), 1.71 – 1.65 (m, 2H), 1.63 – 1.56 (m, 2H), 1.49 – 1.35 (m, 2H).; ^{13}C NMR (150 MHz, D_2O) δ 177.53, 177.02, 105.46 (**C-1**), 105.26 (**C-1**), 105.11 (**C-1**), 103.76 (**C-1**), 85.02, 80.46, 79.91, 77.92, 77.63, 75.10, 75.05, 74.94, 74.72, 73.56, 72.83, 72.50, 71.20, 70.92, 68.98, 63.71, 63.63, 57.71, 57.60, 41.95, 30.67, 28.86, 24.78, 24.73, 24.61.; MS ESI+-HRMS *m/z* [M-H]$^-$ calcd for $C_{33}H_{58}N_3O_{27}S_2^-$ 992.2705, found 992.2707.



***N*-Benzyloxycarbonyl-5-amino-pentyl (2-*O*-benzoyl-4,6-di-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-(3,6-di-*O*-benzyl-2-deoxy-2-trichloracetamido-β-D-glucopyranosyl)-(1→3)-(2-*O*-benzoyl-4,6-di-*O*-benzyl-β-D-galactopyranosyl)-(1→4)- 3,6-di-*O*-benzyl-2-deoxy-2-trichloracetamido-β-D-glucopyranoside (20)** (19.9 mg, 3.8 μmol, 29% over twelve steps)



Scheme S9. Automated glycan assembly of 7.

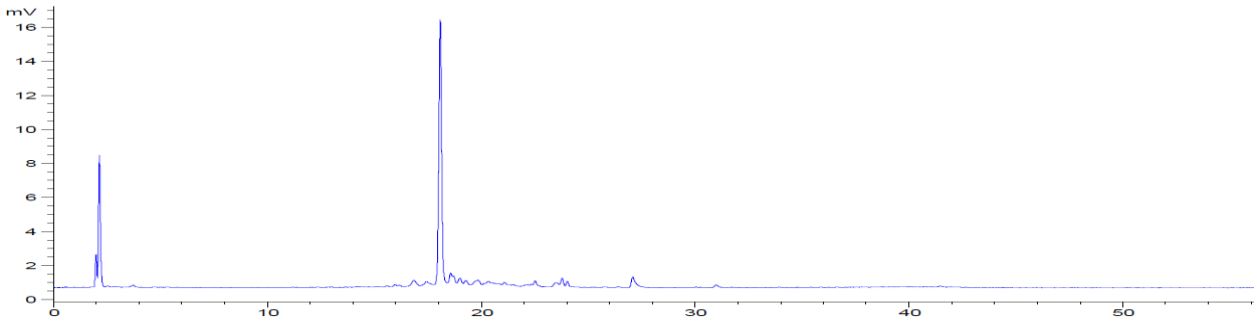
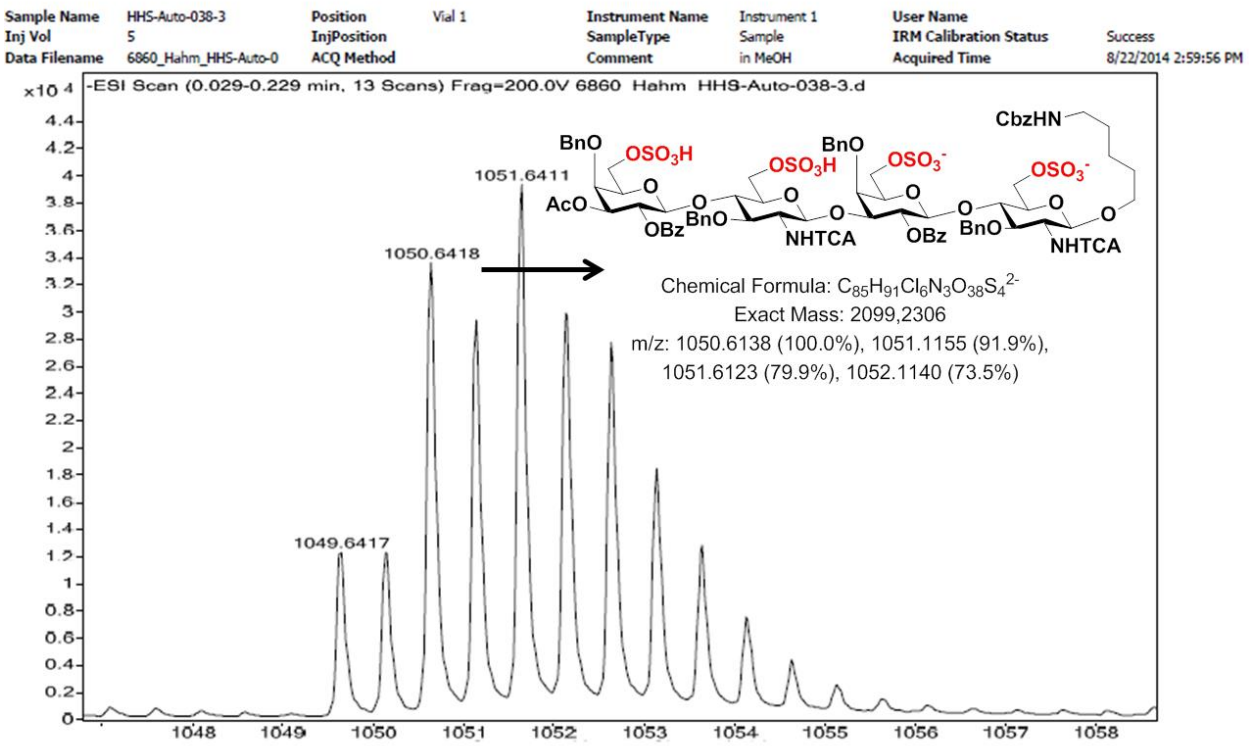


Figure S17. Analytical HPLC chromatogram of the crude tetrasaccharide **20.**

^{1}H NMR (600 MHz, CD_3OD) δ 8.18 (d, *J* = 8.0 Hz, 2H), 8.10 (d, *J* = 8.1 Hz, 2H), 7.73 – 7.60 (m, 2H), 7.60 – 7.52 (m, 3H), 7.49 – 7.42 (m, 6H), 7.33 – 7.21 (m, 13H), 7.18 (dd, *J* = 17.0, 9.9 Hz, 1H), 7.15 – 7.05 (m, 6H), 5.60 (dd, *J* = 10.0, 8.2 Hz, 1H), 5.50 (t, *J* = 8.8 Hz, 1H), 5.29 (dd, *J* = 10.3, 2.6 Hz, 1H), 5.24 (d, *J* = 7.9 Hz, 1H), 5.08 (d, *J* = 10.8 Hz, 1H), 5.02 (s, 2H), 4.95 (dt, *J* = 16.0, 8.9 Hz, 3H), 4.76 (dt, *J* = 28.5, 7.7 Hz, 2H), 4.68 (d, *J* = 8.2 Hz, 1H), 4.62 (d, *J* = 10.8 Hz, 1H), 4.47 (dd, *J* = 9.9, 6.3 Hz, 2H), 4.40 (dd, *J* = 21.6, 9.1 Hz, 2H), 4.18 (dt, *J* = 10.8, 9.1 Hz, 7H), 4.12 (d, *J* = 8.8 Hz, 1H), 4.11 – 4.00 (m, 4H), 3.94 – 3.85 (m, 4H), 3.68 (dd, *J* = 12.8, 6.0 Hz, 3H), 3.64 – 3.58 (m, 1H), 3.40 (d, *J* = 9.6 Hz, 1H), 3.34 – 3.31 (m, 1H), 3.06 – 2.99 (m, 2H), 1.85 (d, *J* = 2.9 Hz, 3H), 1.52 – 1.38 (m, 6H).^{13}C NMR (150 MHz, CD_3OD) δ 171.59, 169.33, 166.97, 164.14, 158.83, 140.45, 139.92, 139.43, 139.36, 138.44, 134.75, 134.42, 133.56, 132.40, 131.47, 131.08, 131.01, 130.60, 129.92, 129.85, 129.80, 129.71, 129.51, 129.39, 129.27, 129.14, 128.99, 128.88, 128.70, 128.59, 128.49, 128.40, 128.24, 102.80, 101.89, 101.50, 101.10, 94.08, 93.71, 80.62, 80.49, 79.96, 78.55, 76.79, 76.45, 76.42, 76.05, 75.52, 75.18, 74.72, 74.58, 73.81, 72.38, 70.54, 69.11, 67.67, 67.24, 66.16, 65.68, 58.31, 57.86, 41.67, 40.16, 31.61, 30.49, 30.20, 30.12, 24.94, 24.22, 24.01, 20.64, 14.39, 11.40, 9.41.; MS ESI+-HRMS *m/z* $[M-2H]^{2-}$ calcd for $C_{85}H_{91}N_3O_{38}Cl_6S_4$ 1050.6138, found 1050.6418.



5-Amino-pentyl 6-*O*-sulfato-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-6-*O*-sulfato-β-D-glucopyranosyl-(1→3)-6-*O*-sulfato-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-6-*O*-sulfato-β-D-glucopyranoside (7) (2.8 mg, 2.4 µmol, 25% over two steps)

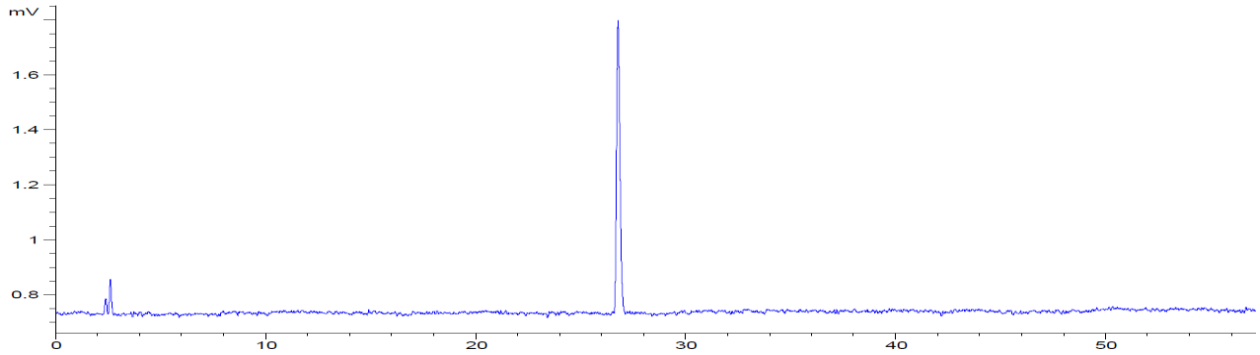


Figure S18. LC-MS chromatogram of 7.

^{1}H NMR (600 MHz, D_2O) δ 4.60 – 4.53 (m, 3H, 3 x **H-1**), 4.46 (d, *J* = 10.8 Hz, 1H), 4.42 (dd, *J* = 11.1, 2.1 Hz, 1H), 4.36 (dd, *J* = 11.1, 4.6 Hz, 1H), 4.31 (dd, *J* = 10.9, 5.5 Hz, 1H), 4.25 (d, *J* = 4.4 Hz, 1H), 4.22 (t, *J* = 8.7 Hz, 4H), 4.01 (d, *J* = 8.7 Hz, 3H), 4.92 – 3.88 (m, 4H), 3.81 – 3.71 (m, 8H), 3.70 – 3.65 (m, 1H), 3.64 – 3.60 (m, 1H), 3.59 – 3.54 (m, 1H), 3.02 (t, *J* = 7.6 Hz, 2H), 2.07 (s, 3H), 2.06 (s, 3H), 1.70 (dt, *J* = 14.5, 7.4 Hz, 2H), 1.66 – 1.60 (m, 2H), 1.44 (qd, *J* = 14.0, 7.3 Hz, 2H).; ^{13}C NMR (150 MHz, D_2O) δ 102.91 (**C-1**), 102.84 (**C-1**), 102.65 (**C-1**), 101.14 (**C-1**), 82.18, 79.18, 78.55, 74.47, 72.75, 72.67, 72.51, 72.37, 72.25, 72.15, 70.84, 70.19, 69.78, 68.21, 68.14, 67.86, 67.05, 66.89, 66.81, 66.49, 65.32, 55.09, 55.06, 39.37, 28.04, 26.23, 22.17, 21.99.; MS ESI+-HRMS *m/z* $[M-3H]^{3-}$ calcd for $C_{33}H_{56}N_3O_{33}S_4^{3-}$ 1150.1695, found 383.3905.

Sample Name	A038-5	**Position**	Vial 1	**Instrument Name**	Instrument 1	**User Name**	
Inj Vol	5	**InjPosition**		**SampleType**	Sample	**IRM Calibration Status**	Success
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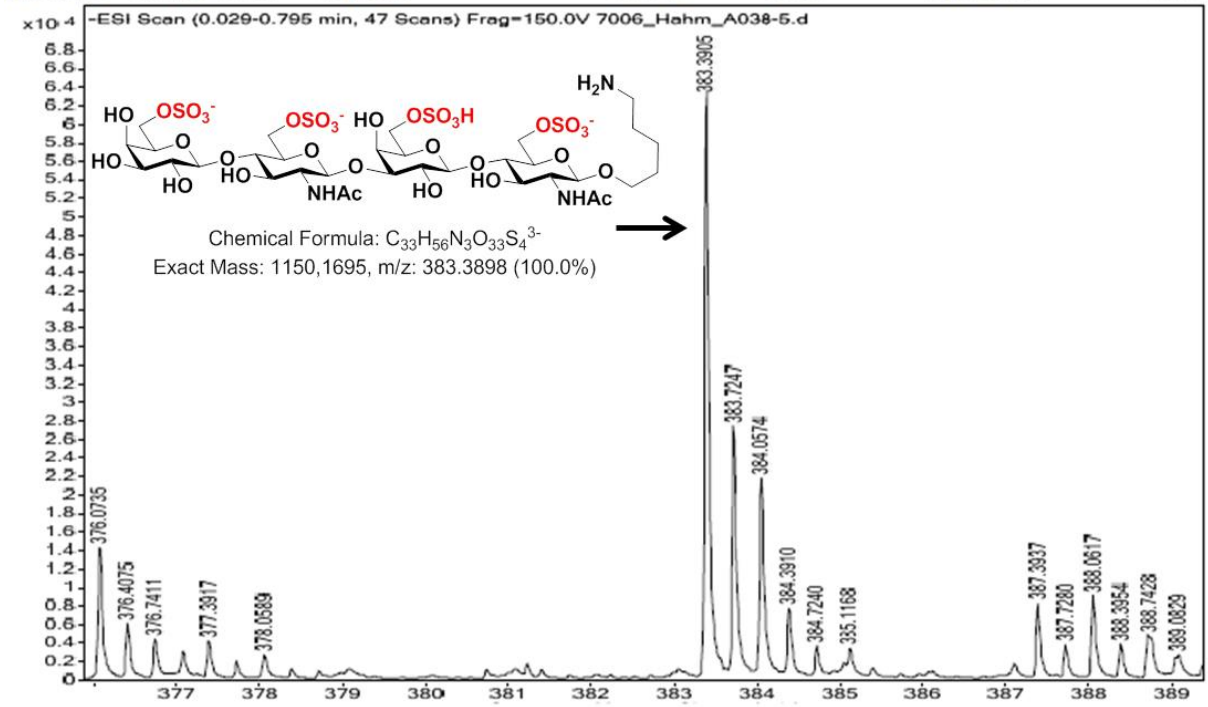

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/ol	5	**InjPosition**		**SampleType**	Sample	**IRM Calibration Status**	Success
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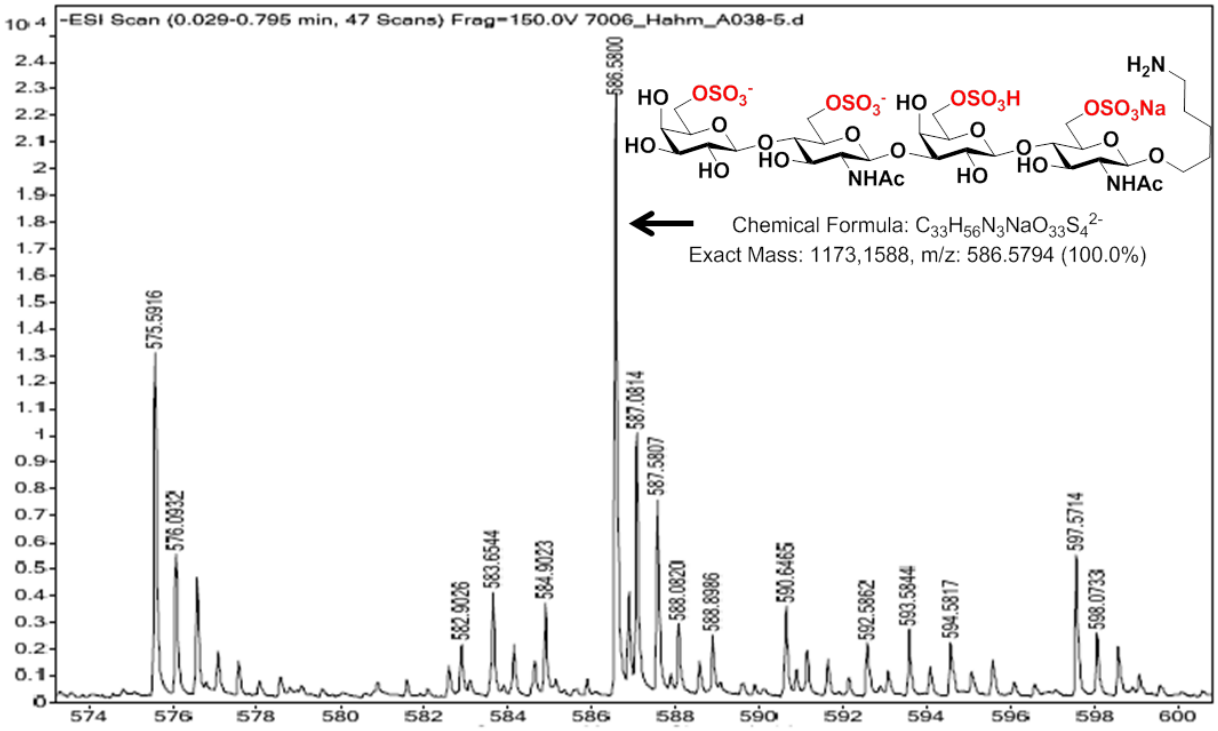

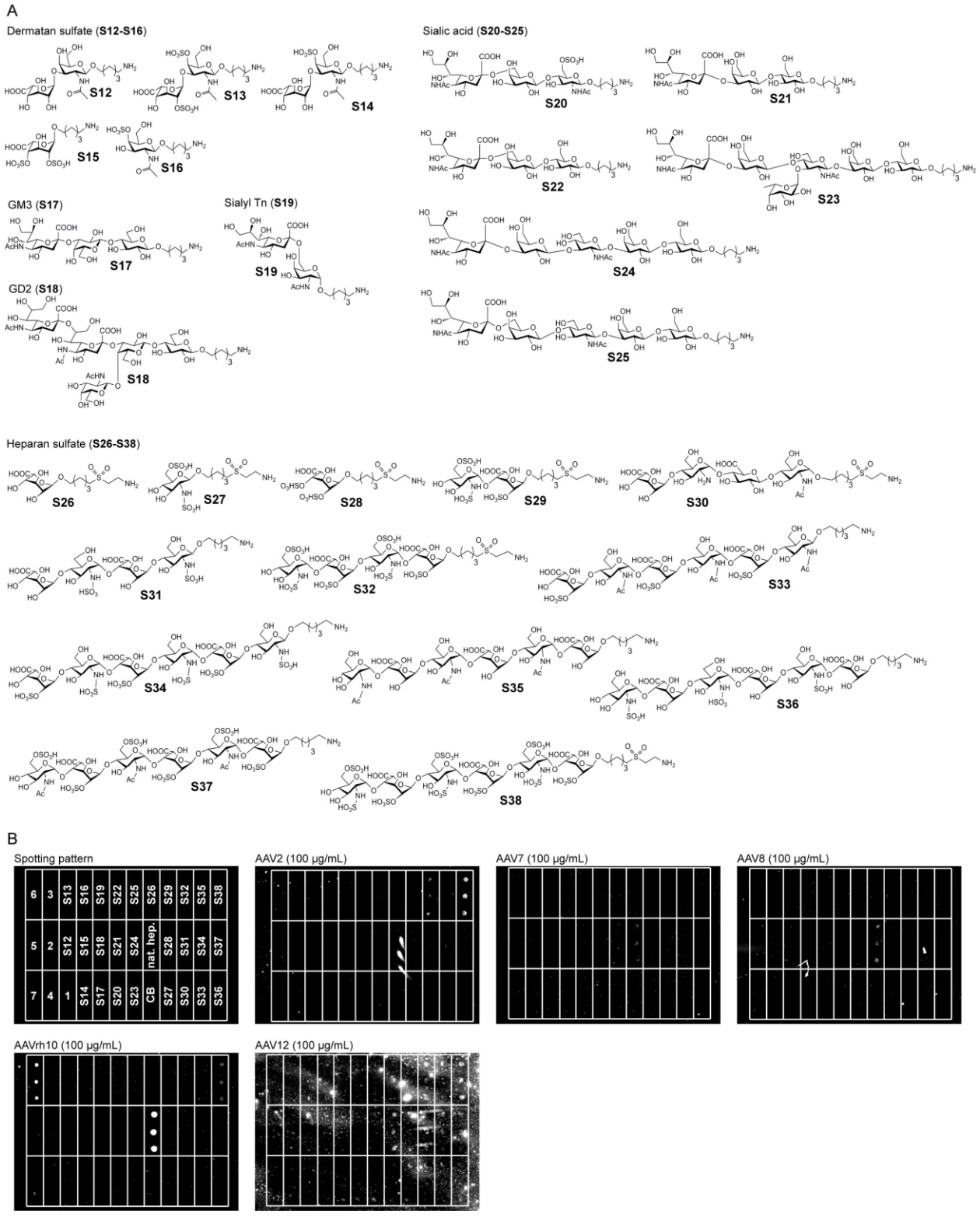

Figure S19. Glycan microarray binding studies of Adeno-associated virus particles. (A) Synthetic glycans used for microarray studies.[S5-S8] (B) Representative microarray scans of AAV particles. The spotting pattern is indicated, each compound was spotted in triplicate. nat. hep.; natural heparin (5 kDa); CB, coupling buffer.

4. Supplemental References

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5. NMR Spectra

Figure S20. ^{1}H (600 MHz, D_2O) and ^{13}C (150 MHz, D_2O) NMR of **1**.

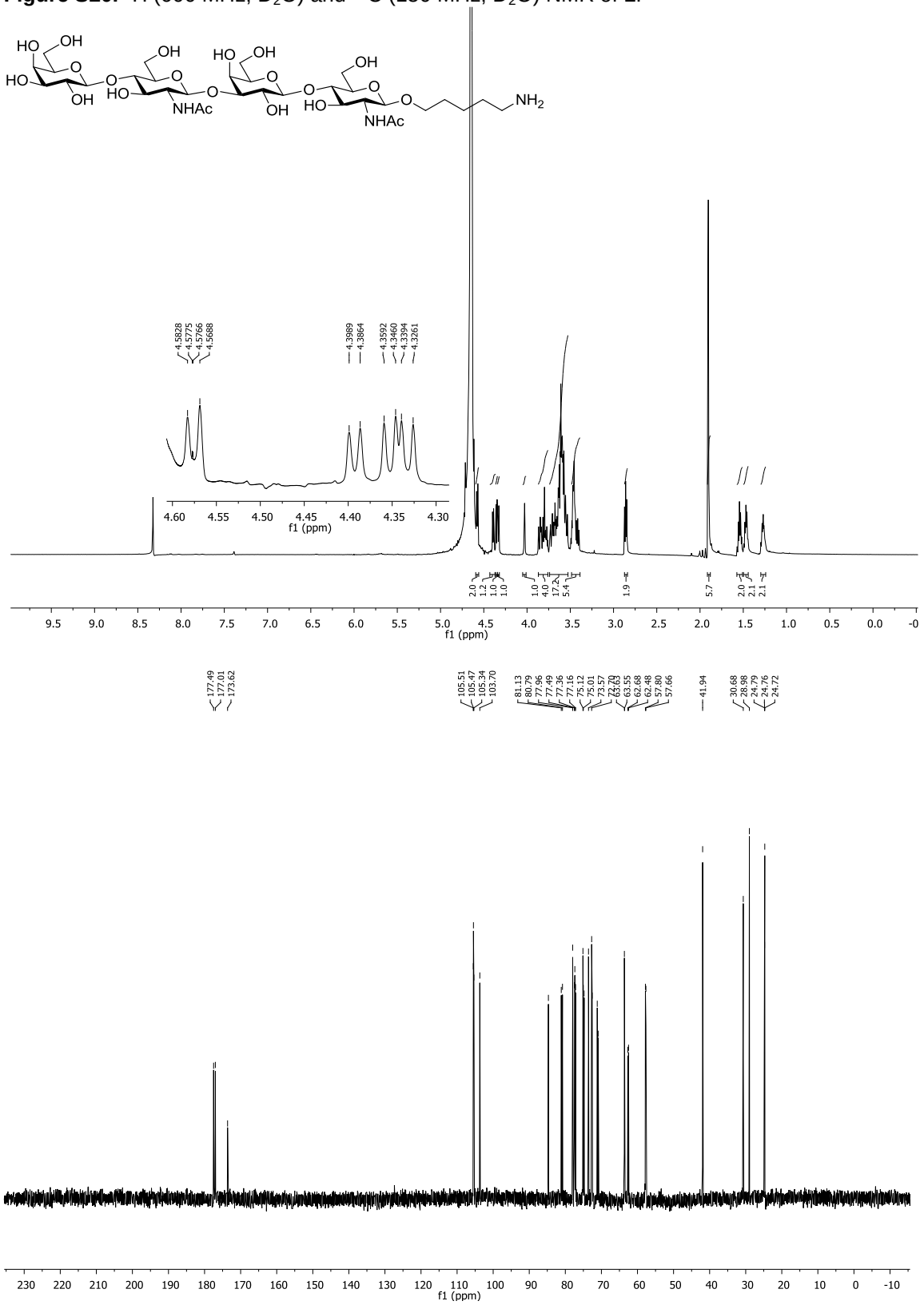

Figure S21. ^{1}H-COSY and ^{1}H-^{13}C-HSQC (600 MHz, D_2O) NMR of **1**.

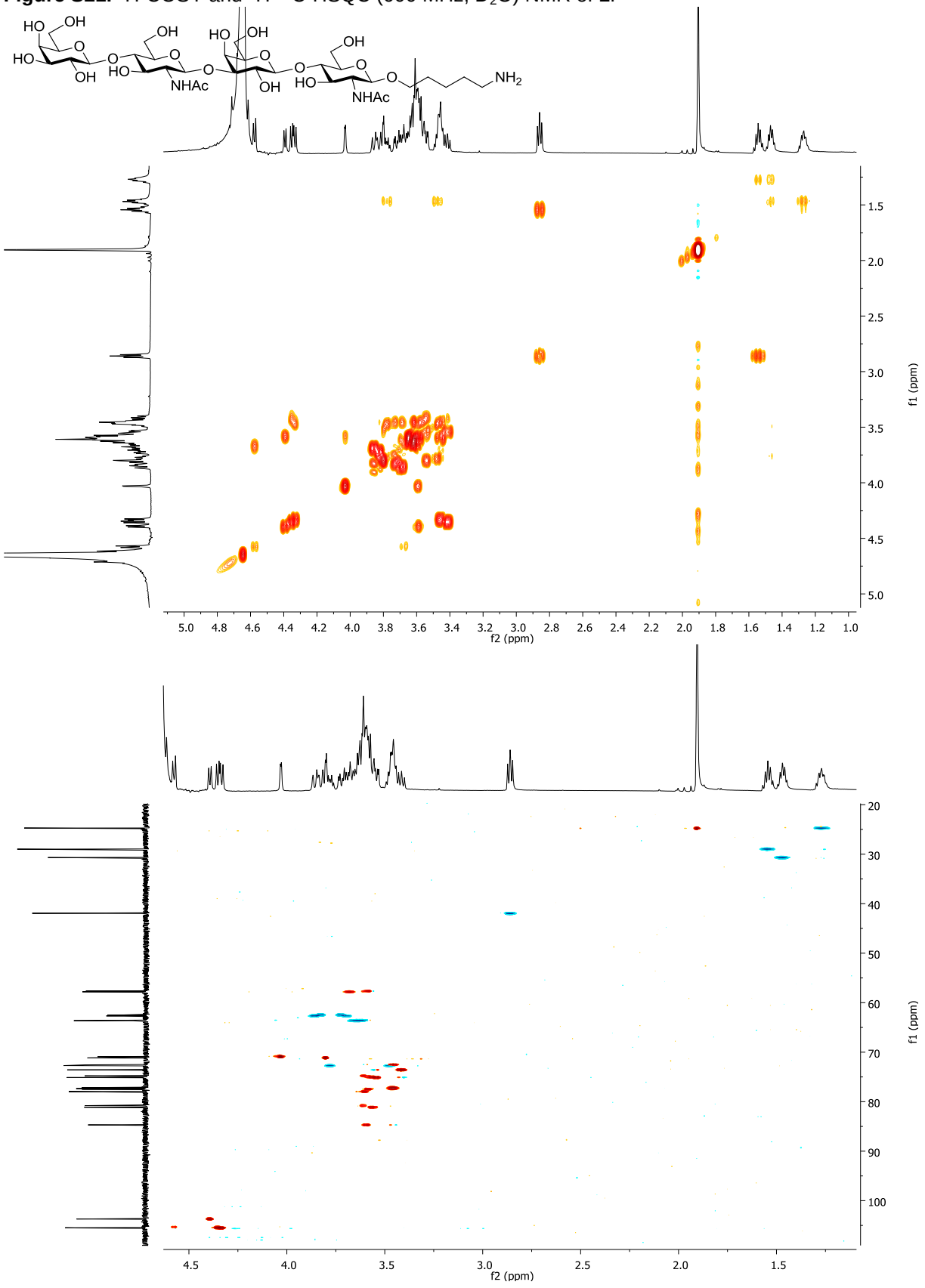

Figure S22. ^{1}H (600 MHz, D_2O) and ^{13}C (150 MHz, D_2O) NMR of **2**.

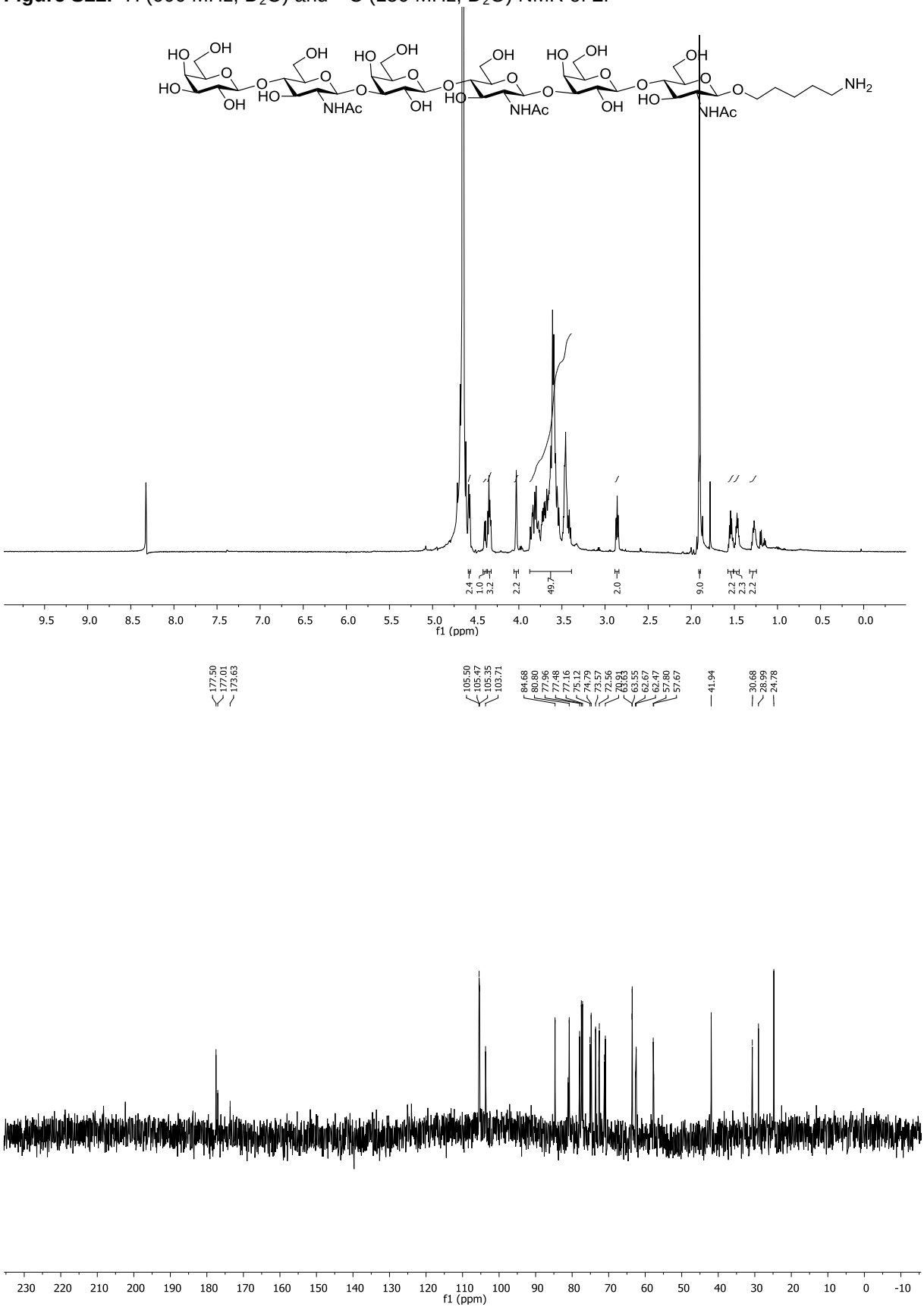

Figure S23. ^{1}H-COSY and ^{1}H-^{13}C-HSQC (600 MHz, D_2O) NMR of **2**.

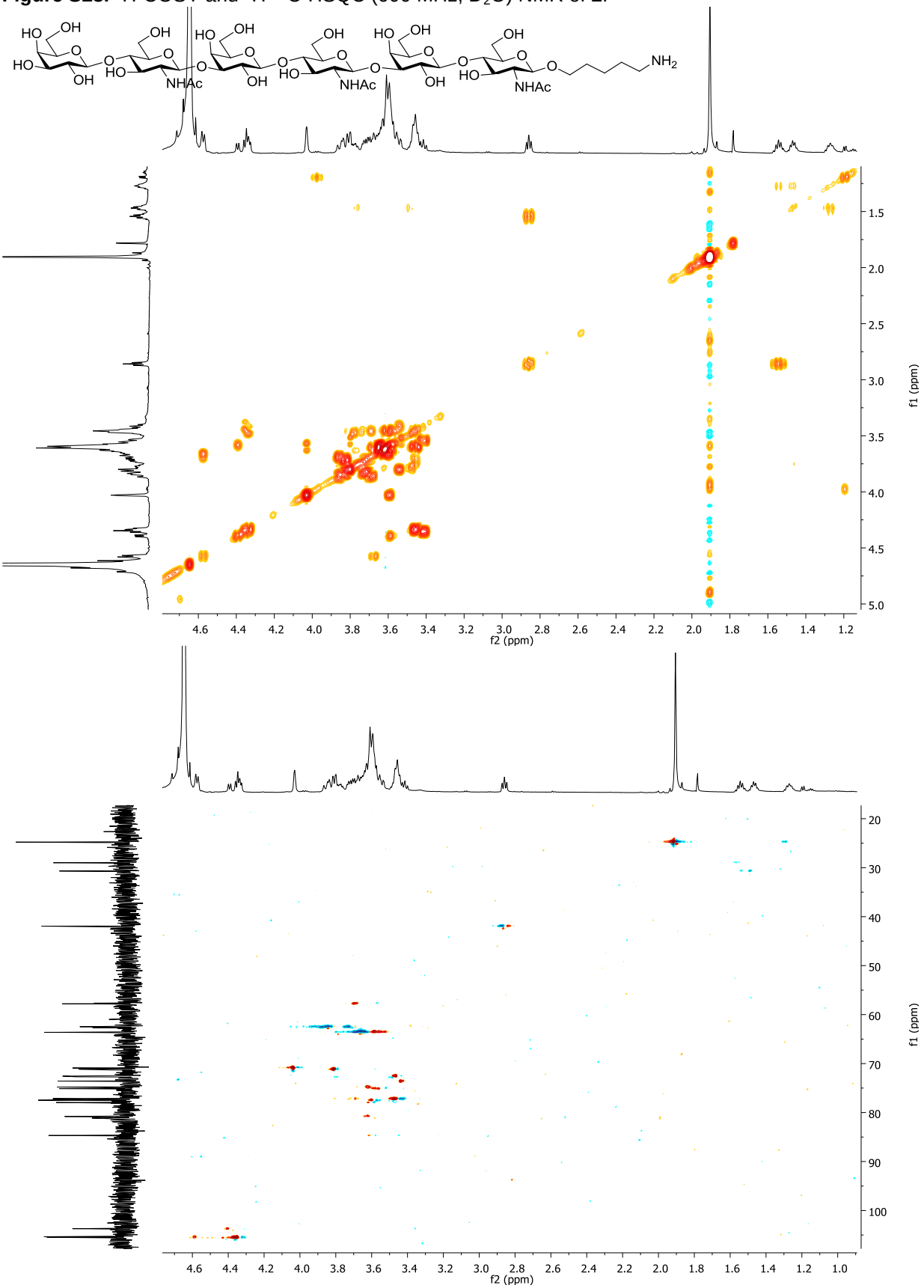

Figure S24. ^{1}H (600 MHz, D_2O) and ^{13}C (150 MHz, D_2O) NMR of **3**.

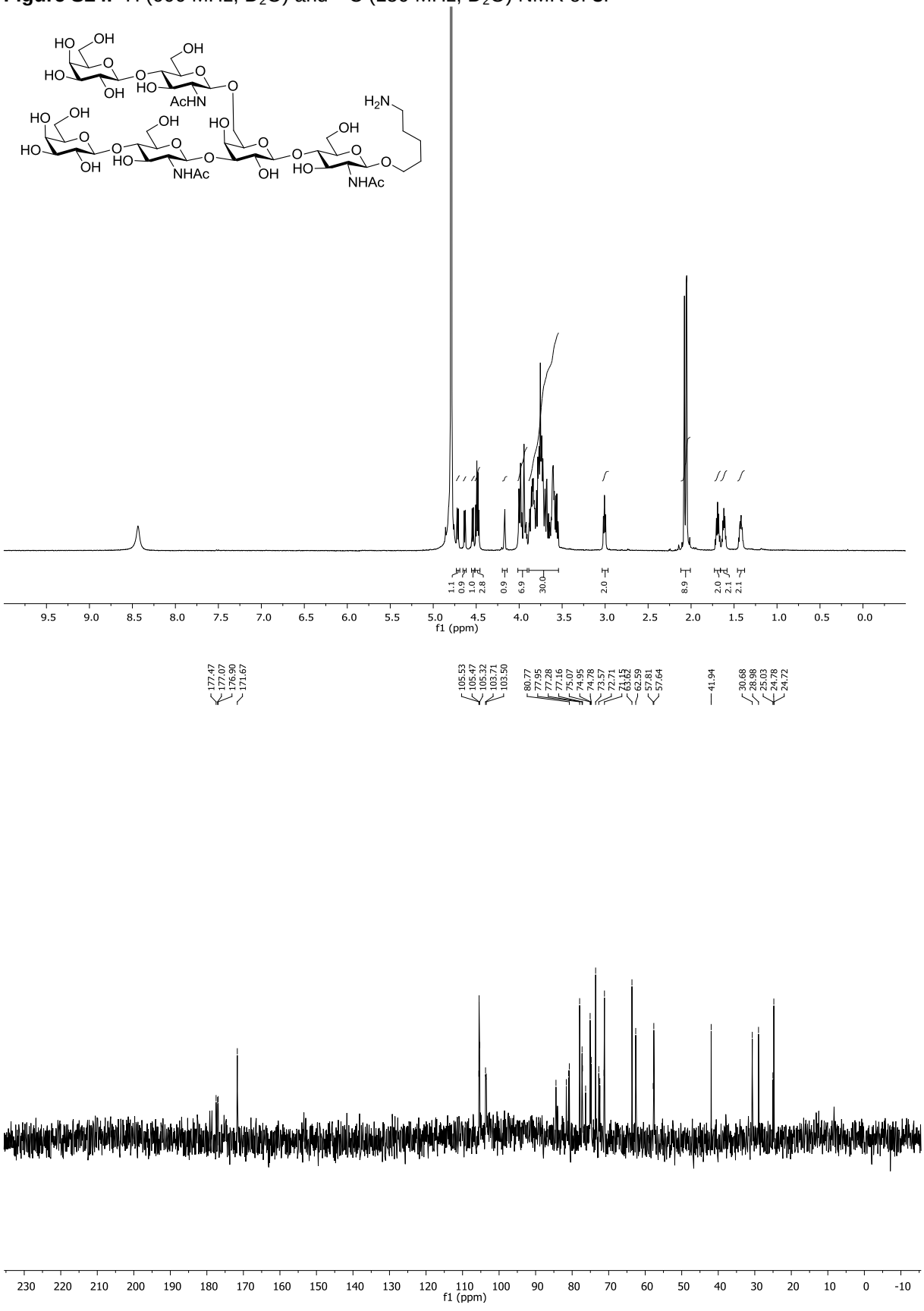

Figure S25. ^{1}H-COSY and ^{1}H-^{13}C-HSQC (600 MHz, D_2O) NMR of **3**.

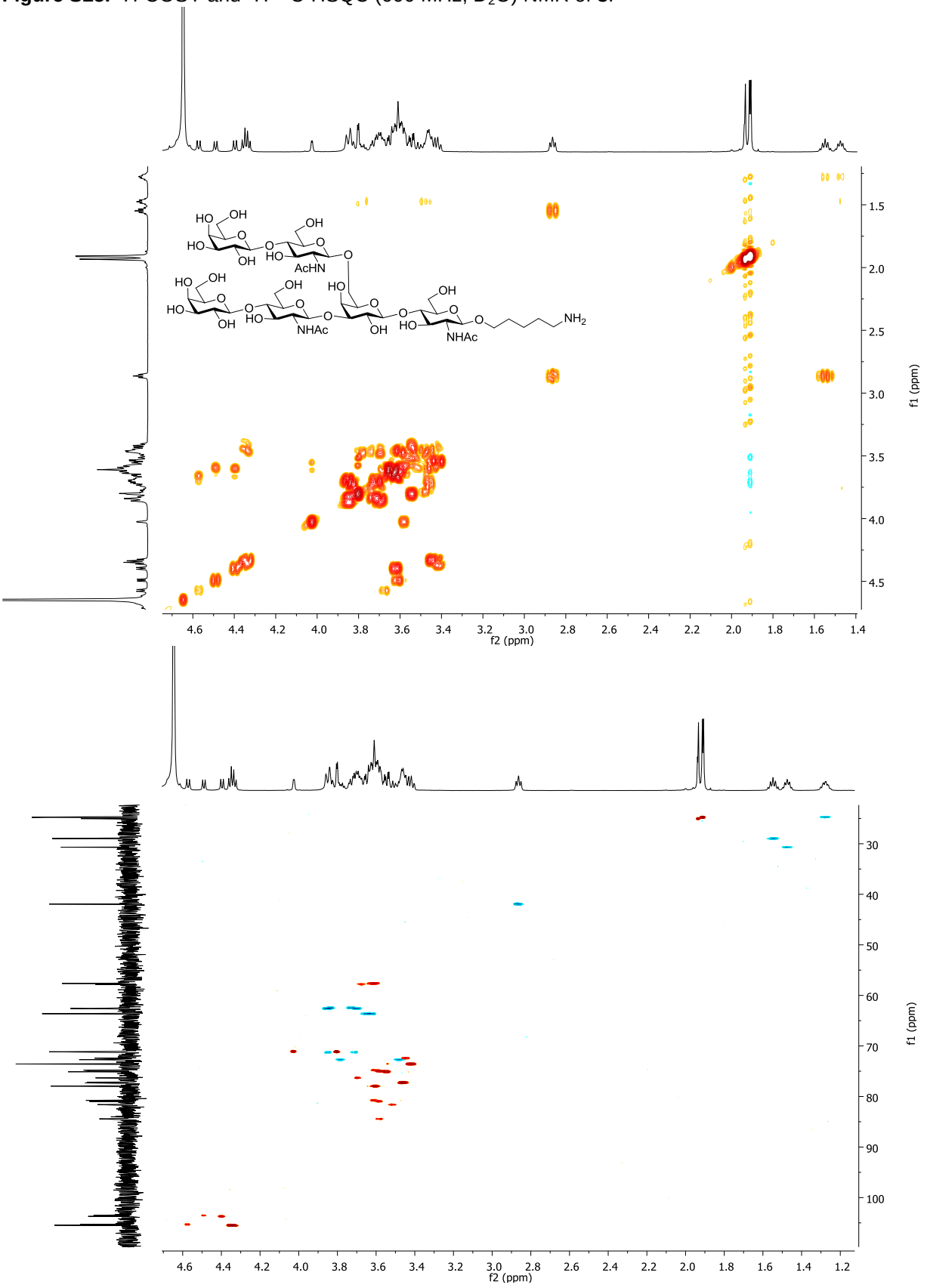

Figure S26. ^{1}H (600 MHz, D_2O) and ^{13}C (150 MHz, D_2O) NMR NMR of **4**.

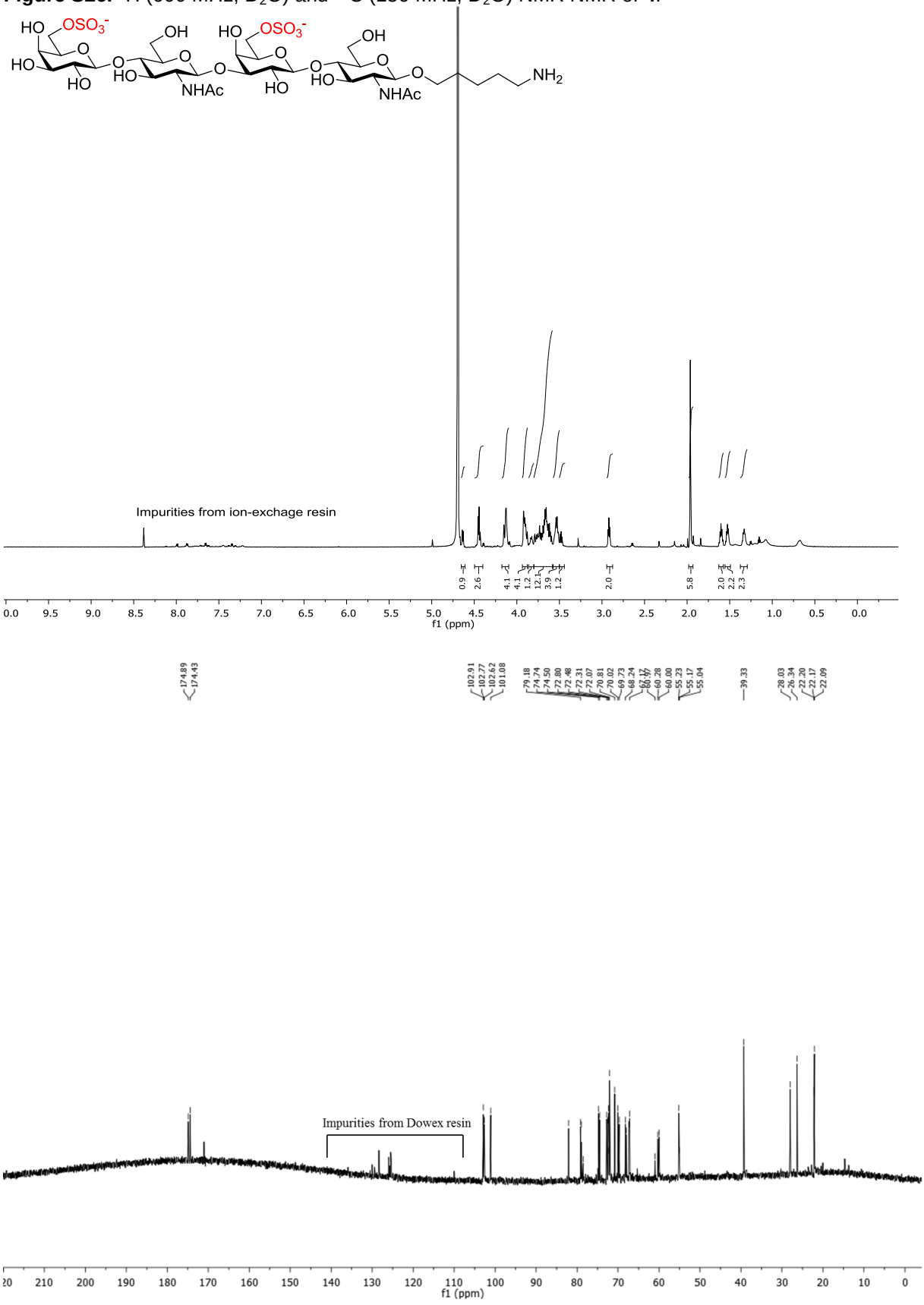

Figure S27. ^{1}H-COSY and ^{1}H-^{13}C-HSQC (600 MHz, D_2O) NMR of **4**.

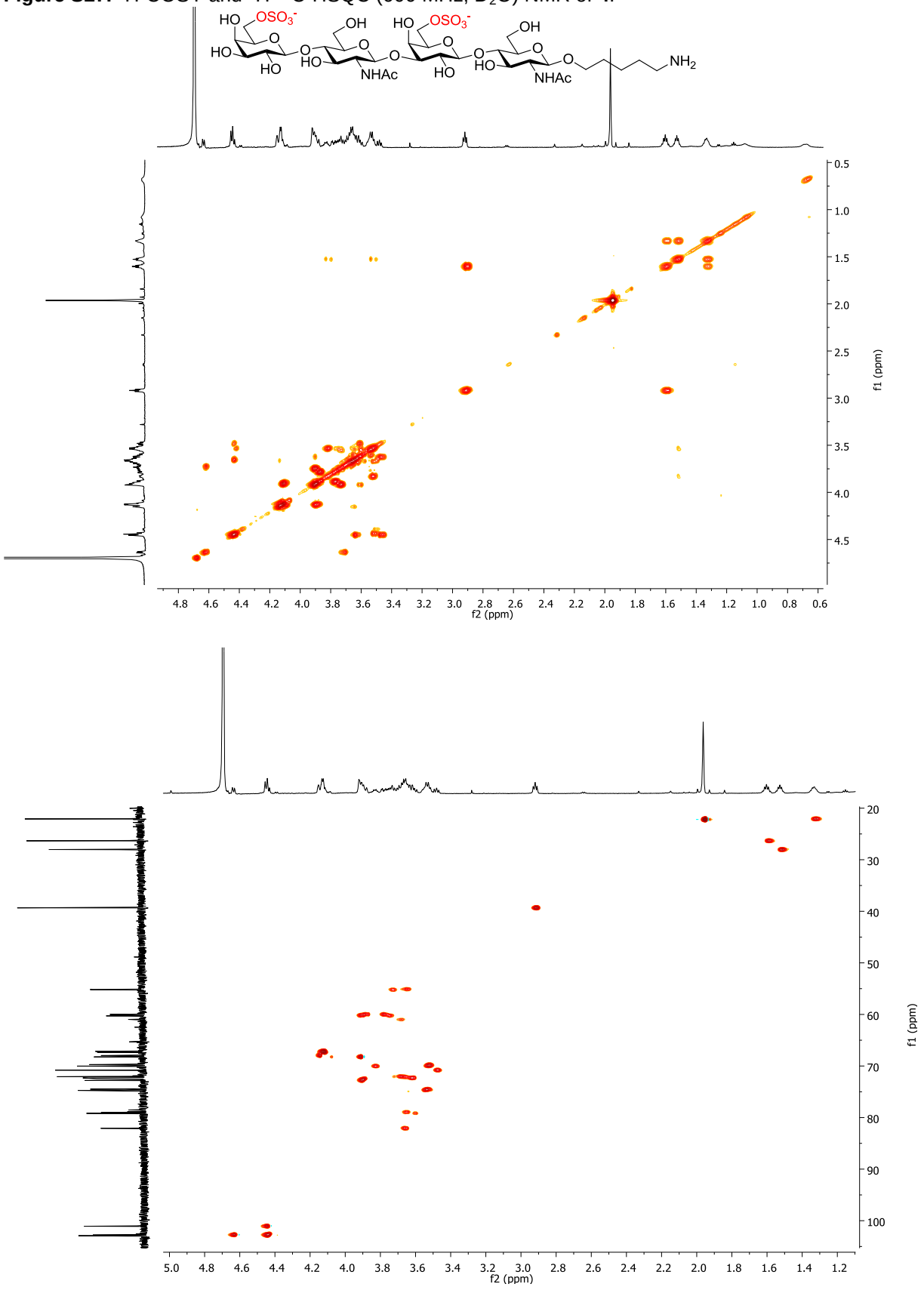

Figure S28. ^{1}H (600 MHz, D_2O) and ^{13}C (150 MHz, D_2O) NMR of **5**.

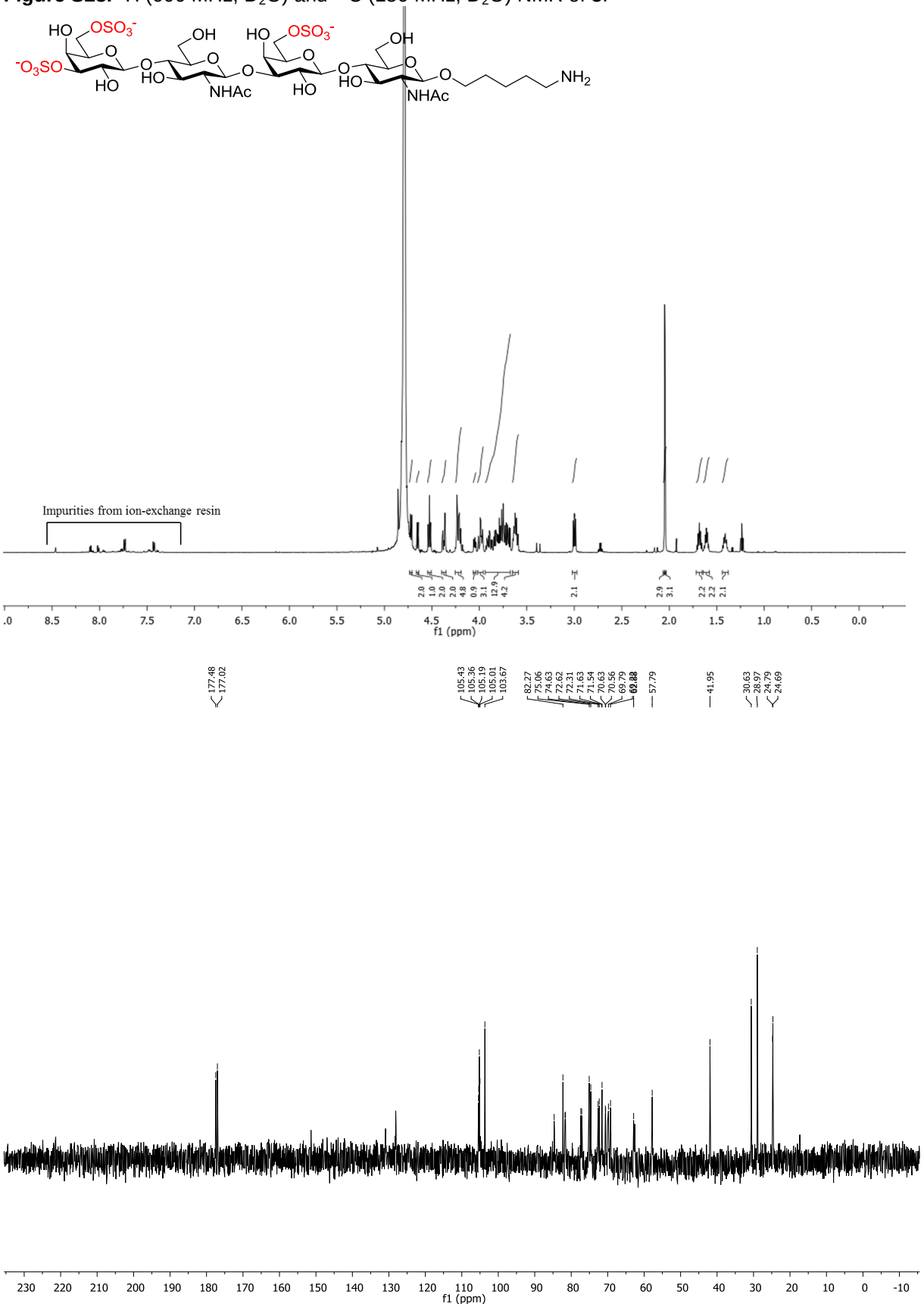

Figure S29. ^{1}H-COSY and ^{1}H-^{13}C-HSQC (600 MHz, D_2O) NMR of **5**.

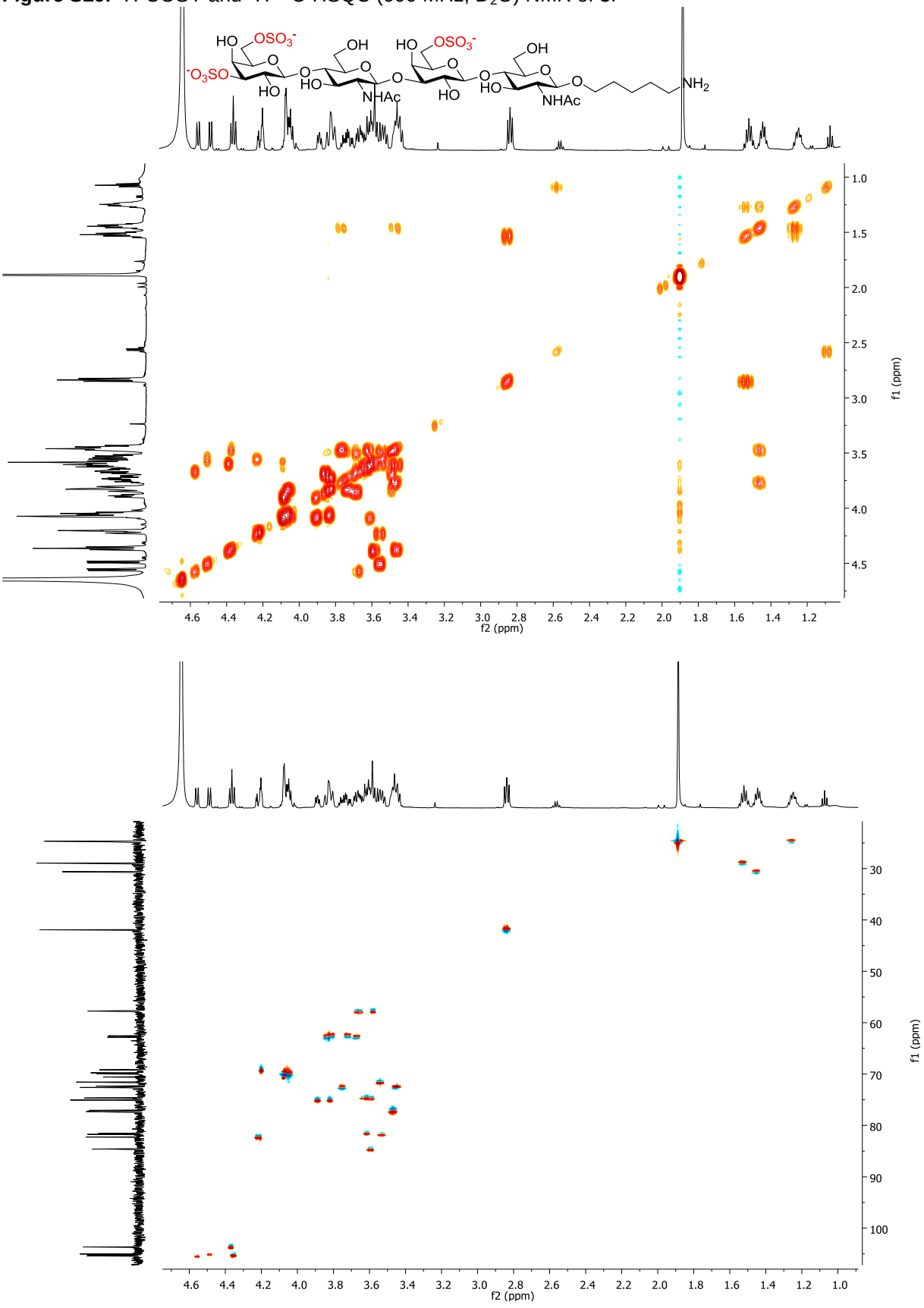

Figure S30. ^{1}H (600 MHz, D_2O) and ^{13}C (150 MHz, D_2O) NMR of **6**.

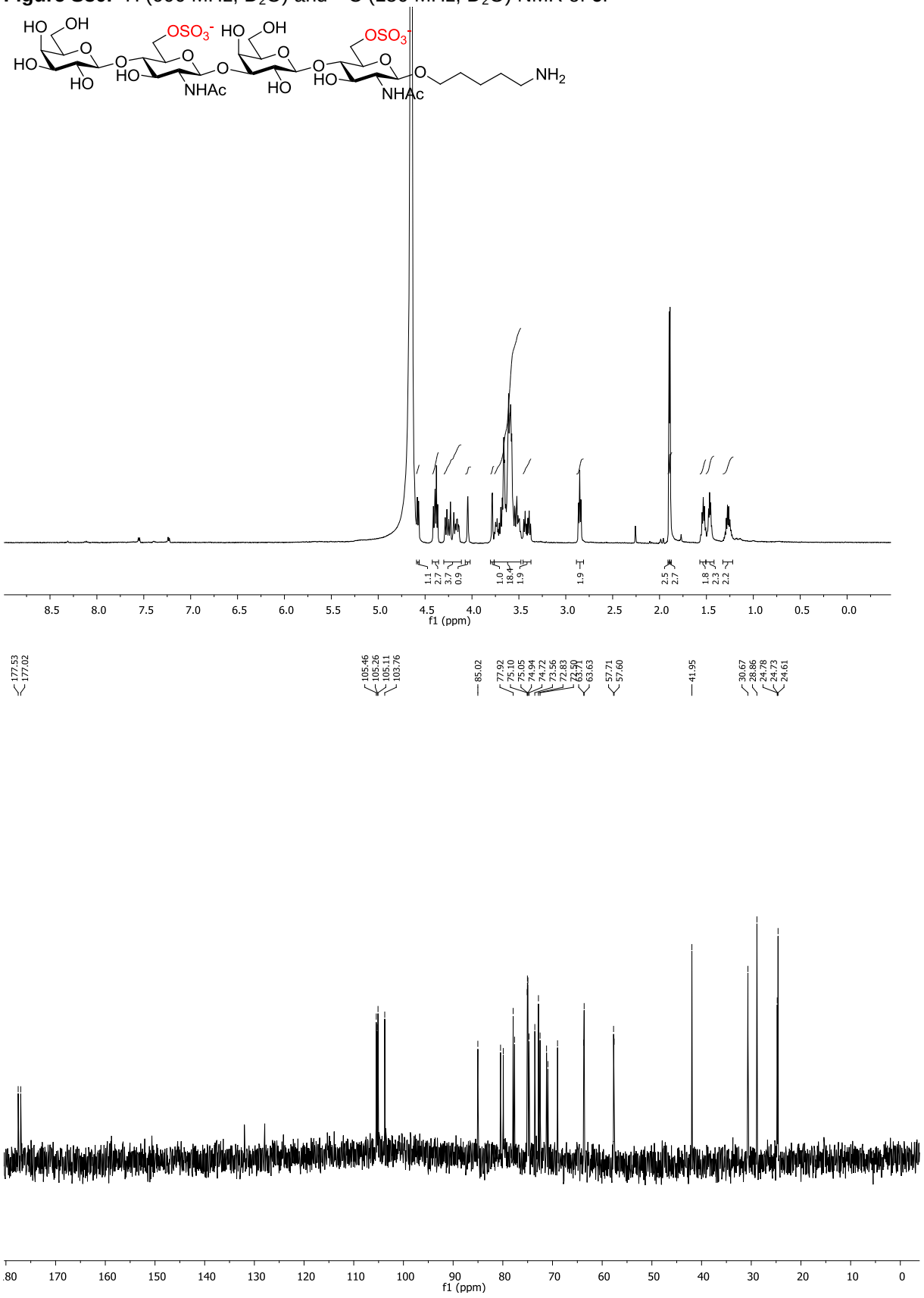

Figure S31. ^{1}H-COSY and ^{1}H-^{13}C-HSQC (600 MHz, D_2O) NMR of **6**.

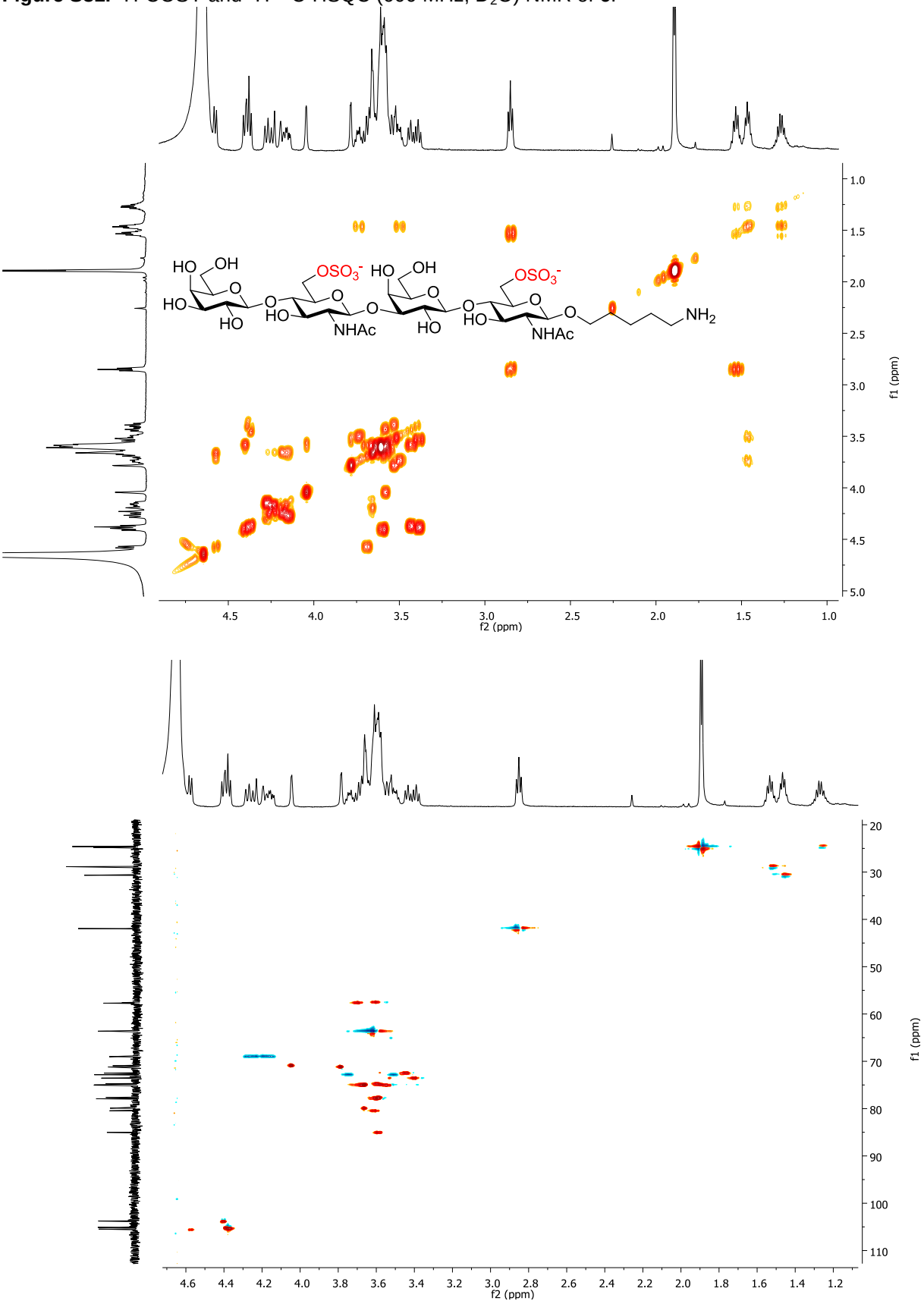

Figure S32. ^{1}H (600 MHz, D_2O) and ^{13}C (150 MHz, D_2O) NMR NMR of **7**.

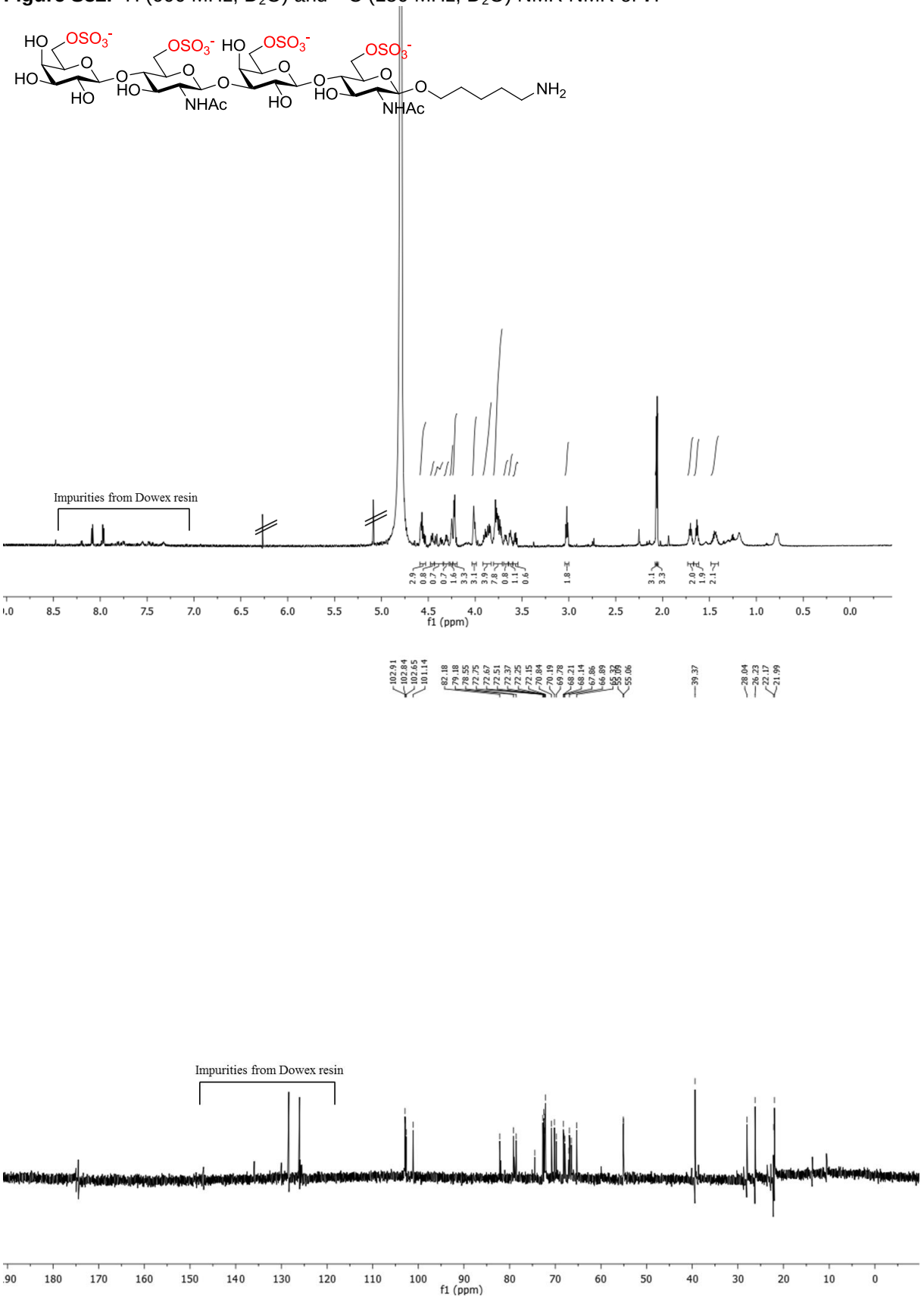

Figure S33. ^{1}H-COSY and ^{1}H-^{13}C-HSQC (600 MHz, D_2O) NMR of **7**.

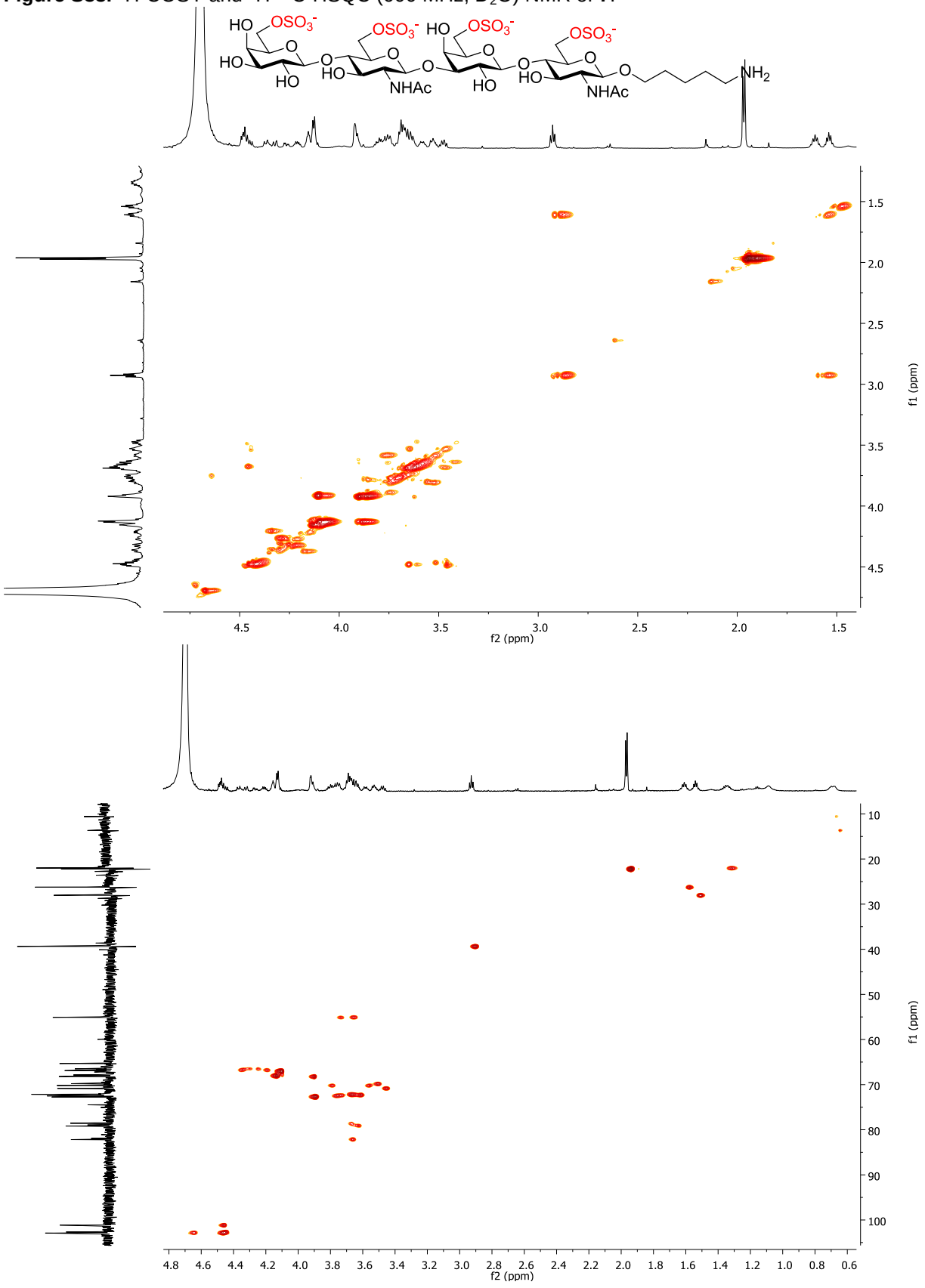

Figure S34. ^{1}H (400 MHz, $CDCl_3$) and ^{13}C (100 MHz, $CDCl_3$) NMR of **9**.

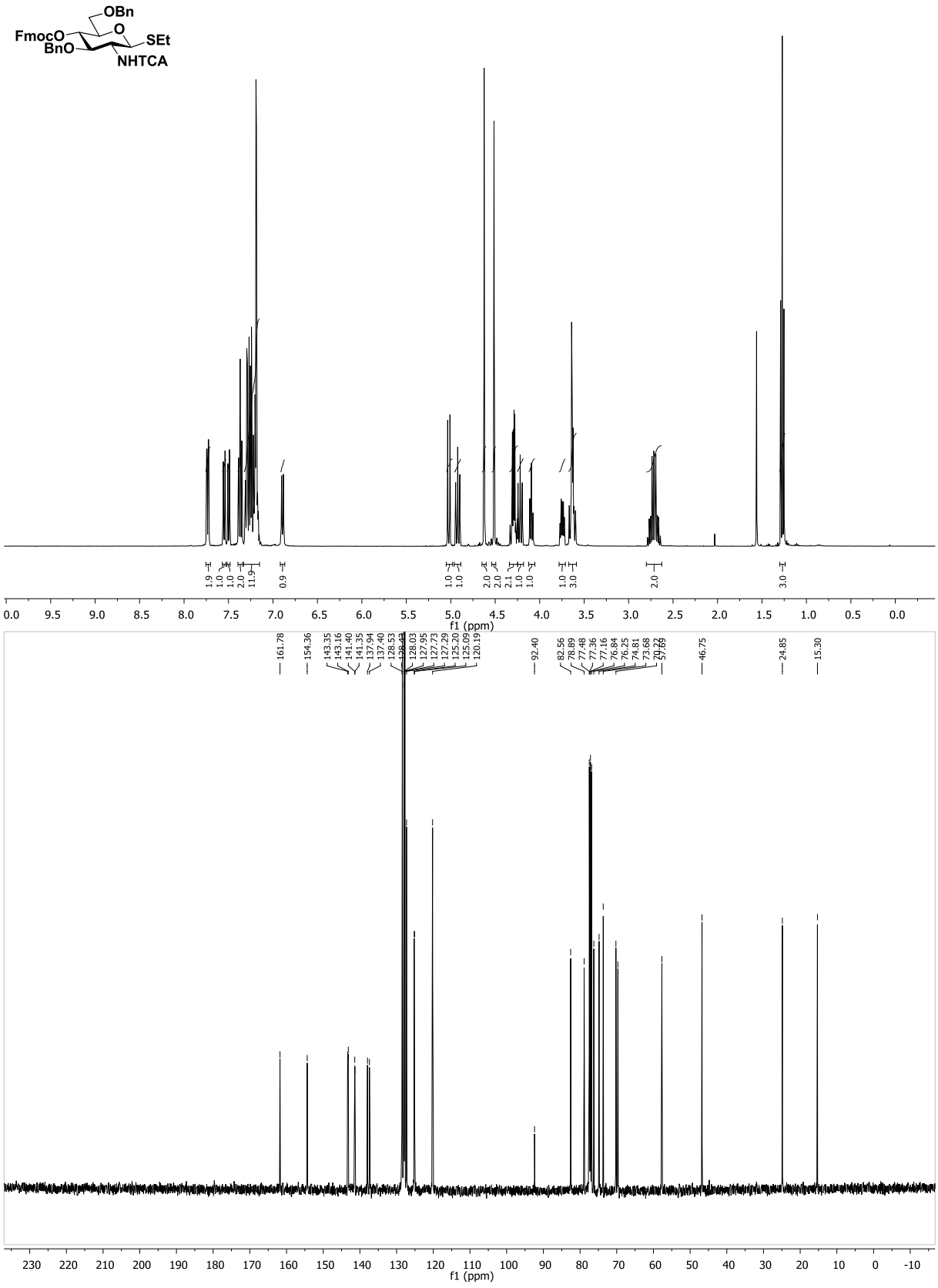

Figure S35. ^{1}H-COSY and ^{1}H-^{13}C-HSQC (400 MHz, $CDCl_3$) NMR of **9**.

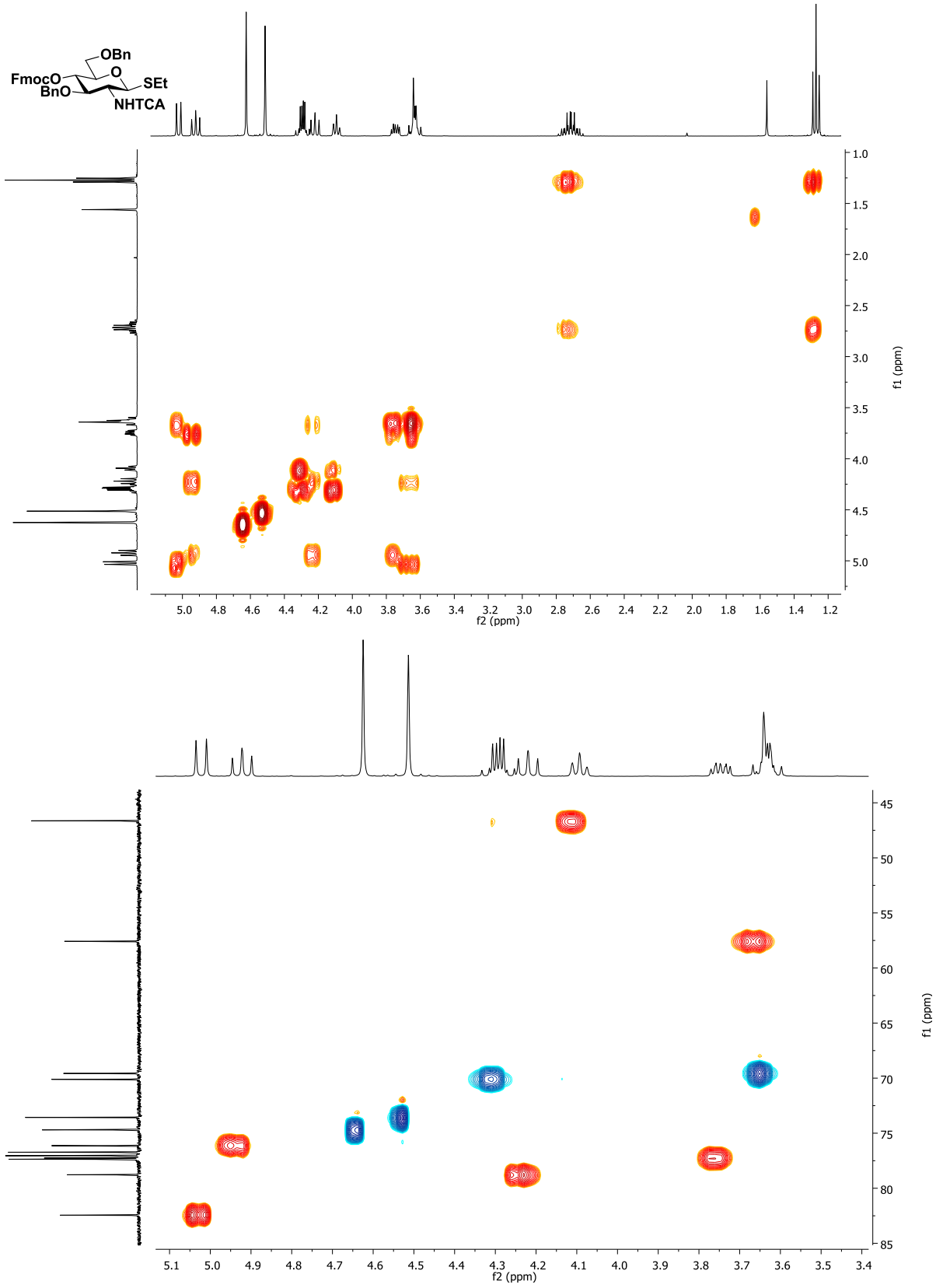

Figure S36. ^{1}H (400 MHz, $CDCl_3$) and ^{13}C (100 MHz, $CDCl_3$) NMR of **10**.

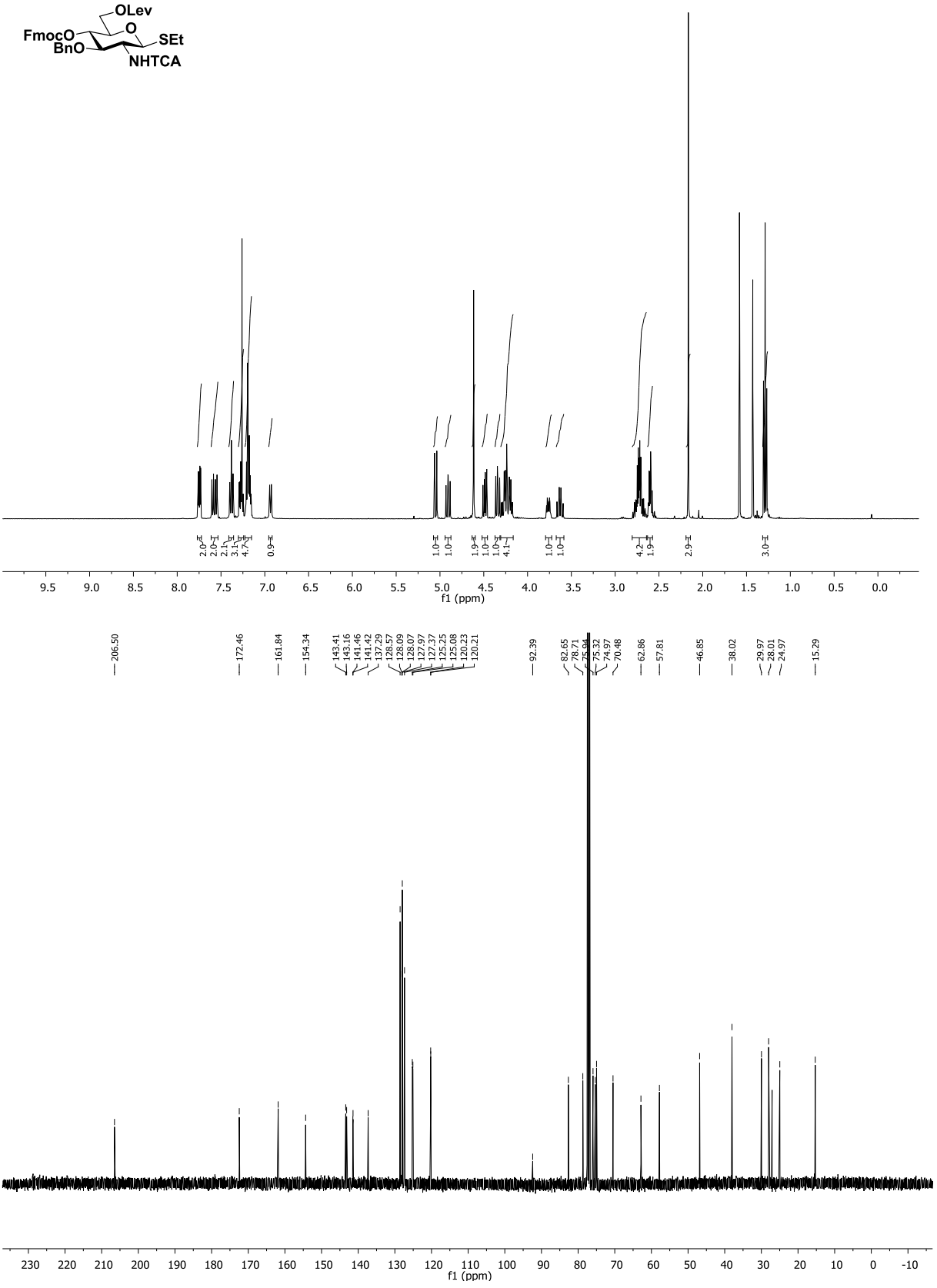

Figure S37. ^{1}H-COSY and ^{1}H-^{13}C-HSQC (400 MHz, $CDCl_3$) NMR of **10**.

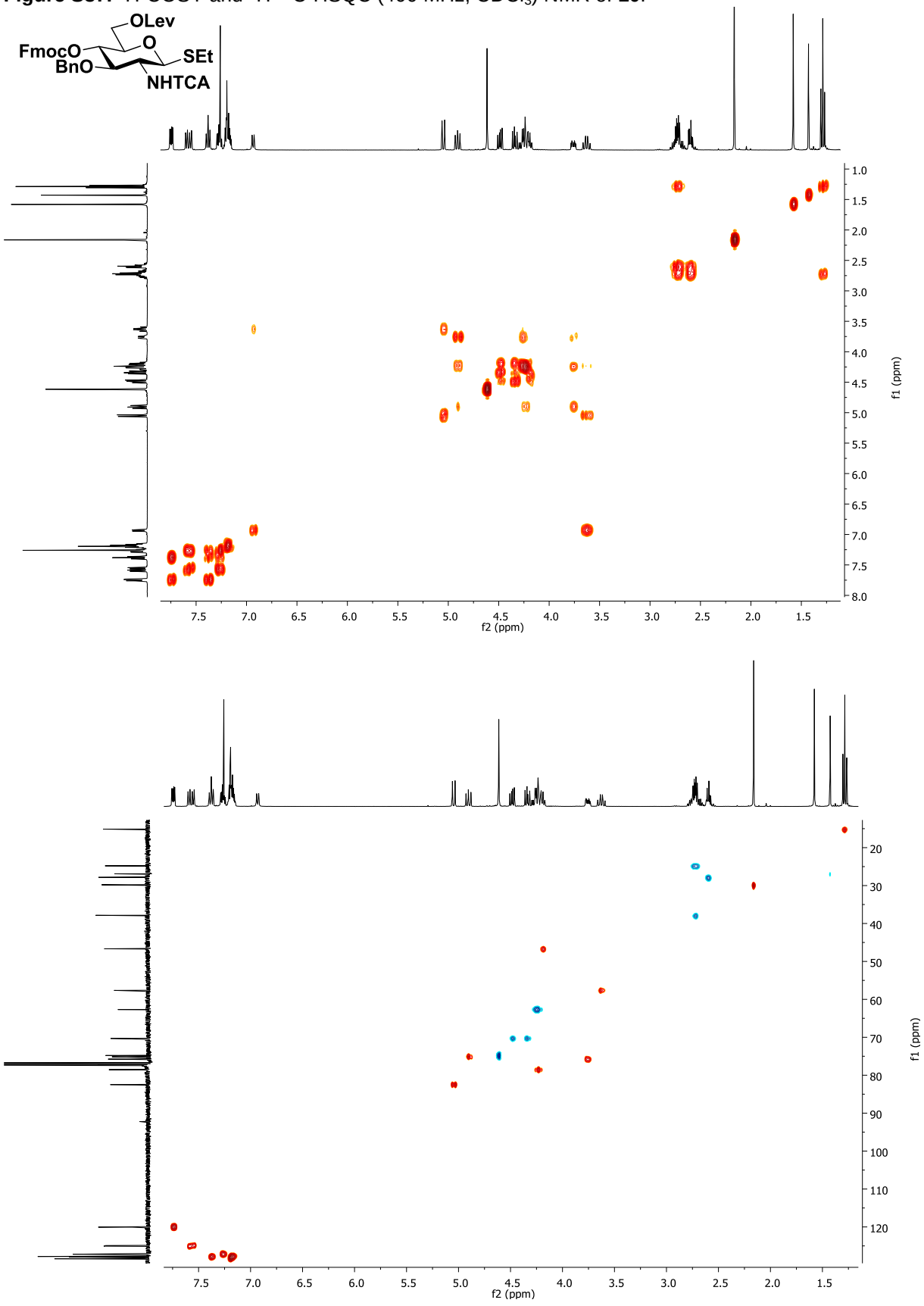

Figure S38. ^{1}H (400 MHz, $CDCl_3$) and ^{13}C (100 MHz, $CDCl_3$) NMR of **11**.

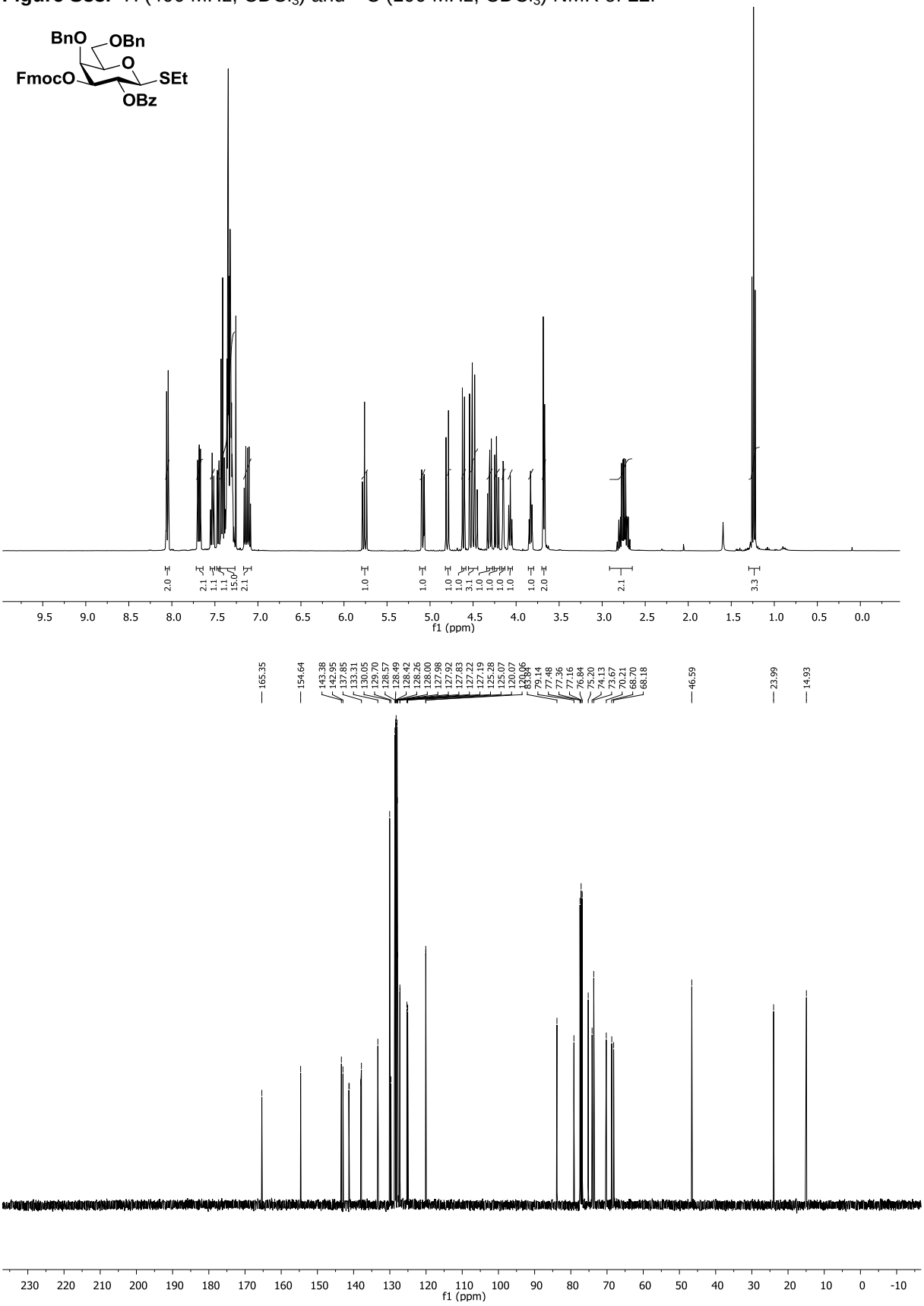

Figure S39. ^{1}H-COSY and ^{1}H-^{13}C-HSQC (400 MHz, $CDCl_3$) NMR of **11**.

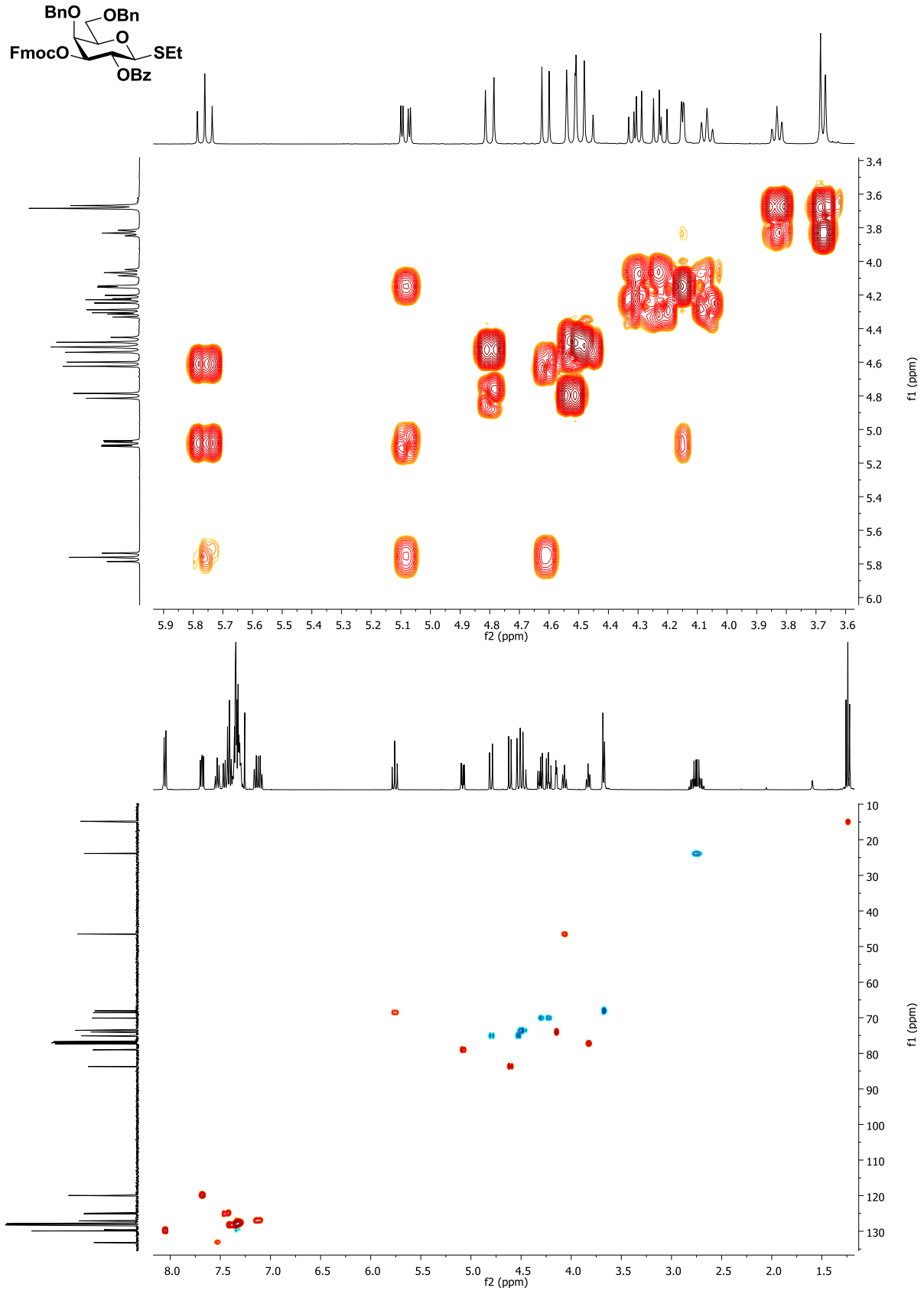

Figure S40. ^{1}H (400 MHz, $CDCl_3$) and ^{13}C (100 MHz, $CDCl_3$) NMR of **12**.

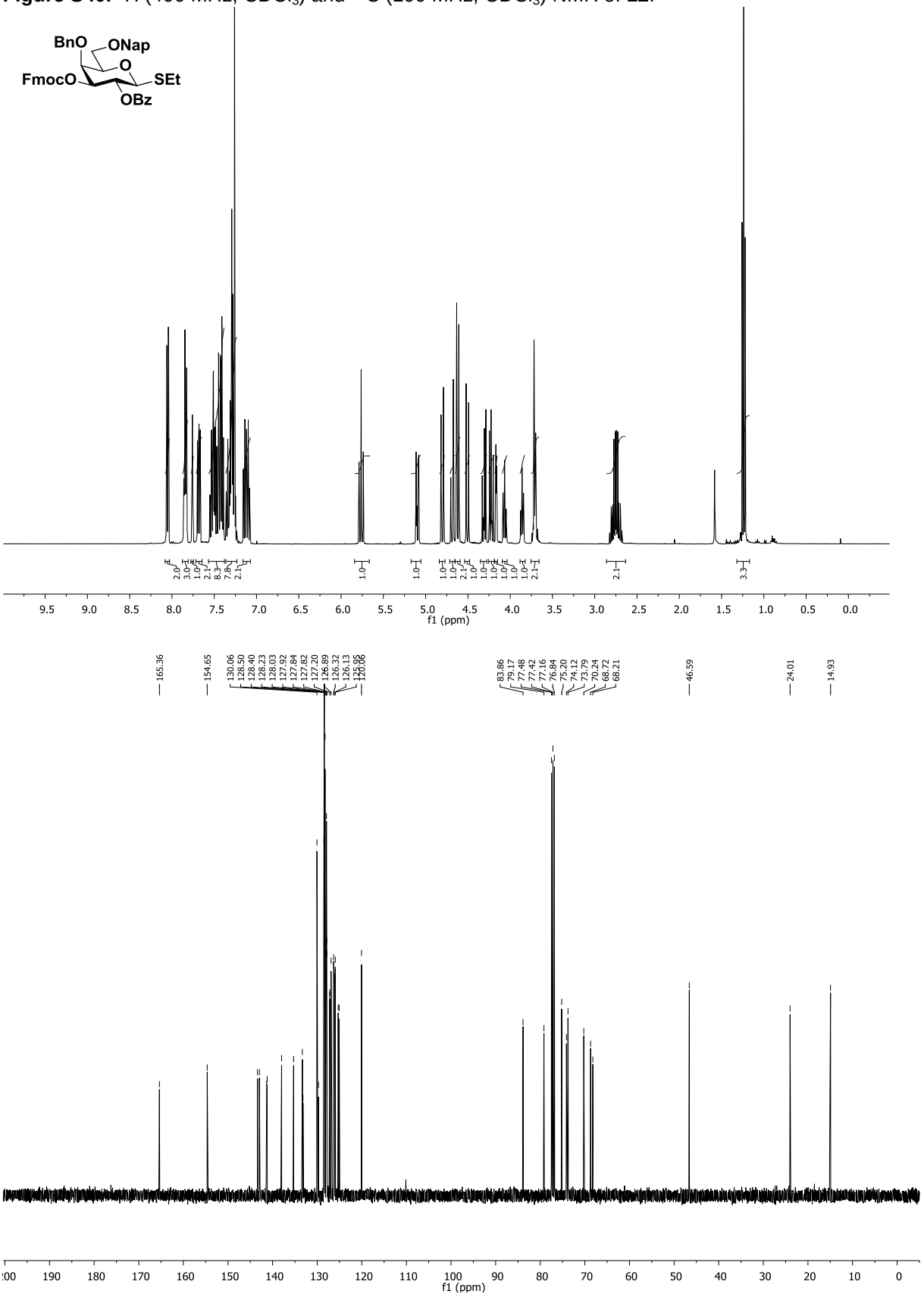

Figure S41. ^{1}H-COSY and ^{1}H-^{13}C-HSQC (400 MHz, $CDCl_3$) NMR of **12**.

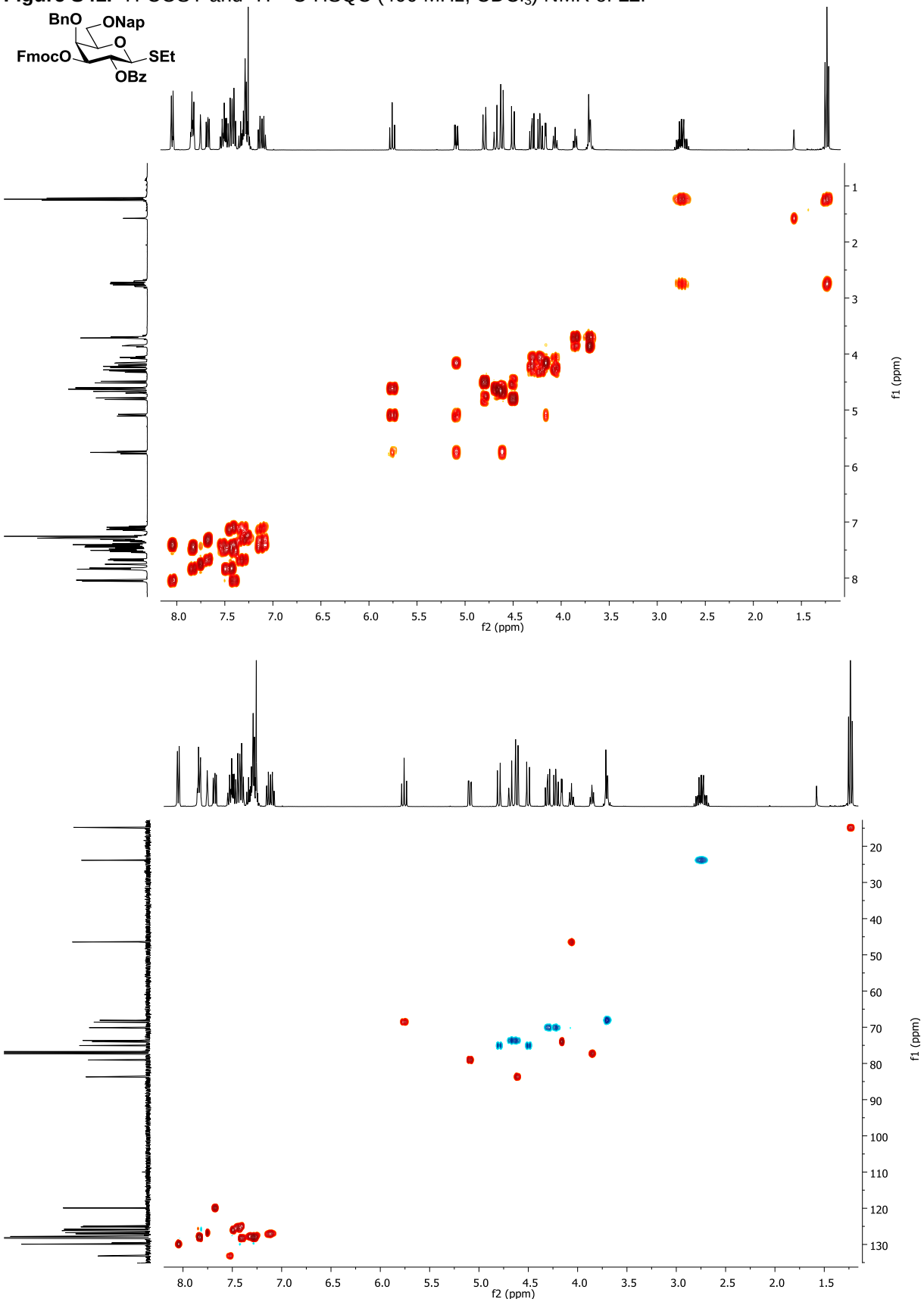

Figure S42. ^{1}H (400 MHz, $CDCl_3$) and ^{13}C (100 MHz, $CDCl_3$) NMR of **S9**.

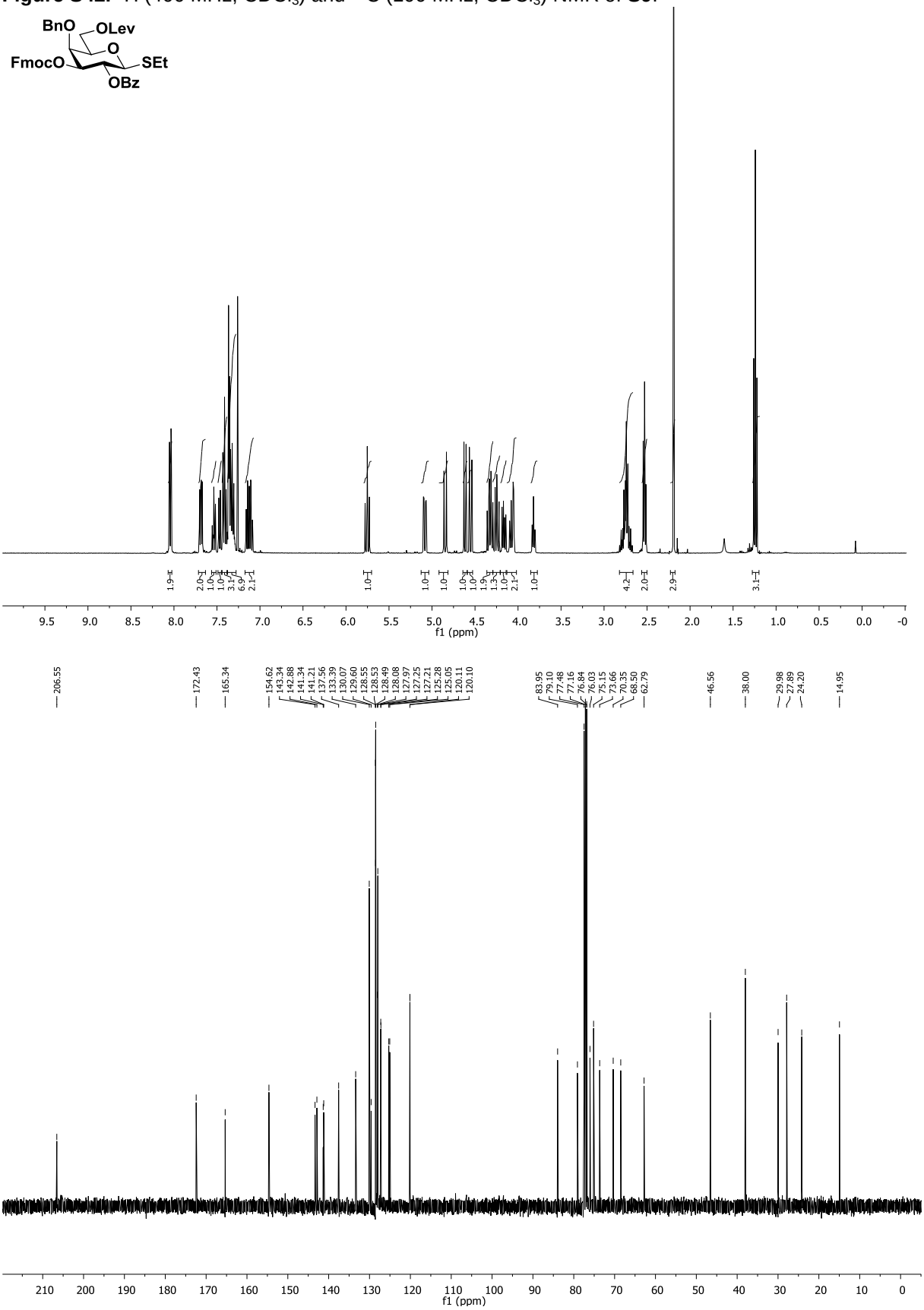

Figure S43. ^{1}H-COSY and ^{1}H-^{13}C-HSQC (400 MHz, $CDCl_3$) NMR of **S9**.

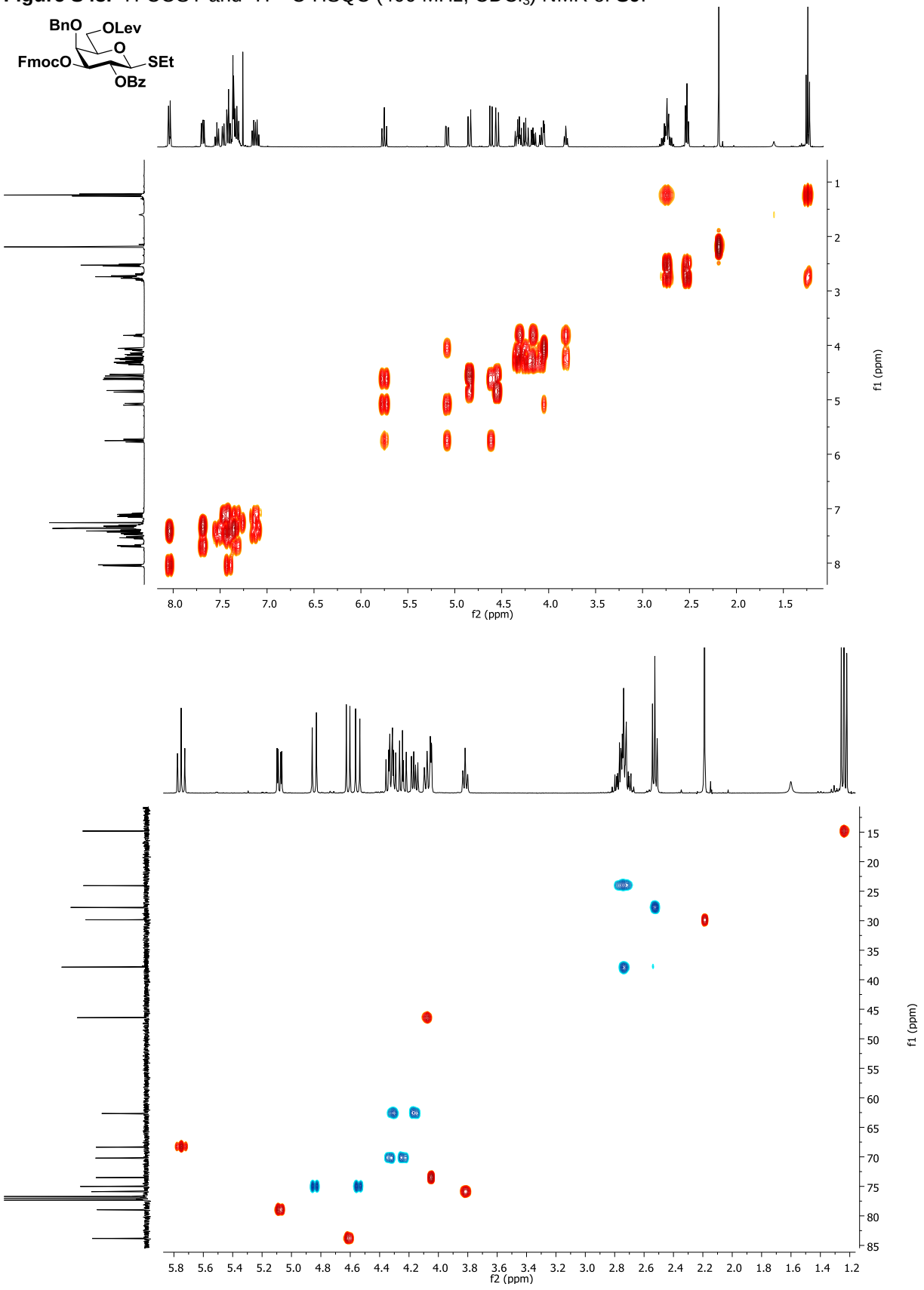

Figure S44. ^{1}H (400 MHz, $CDCl_3$) and ^{13}C (100 MHz, $CDCl_3$) NMR of **S10**.

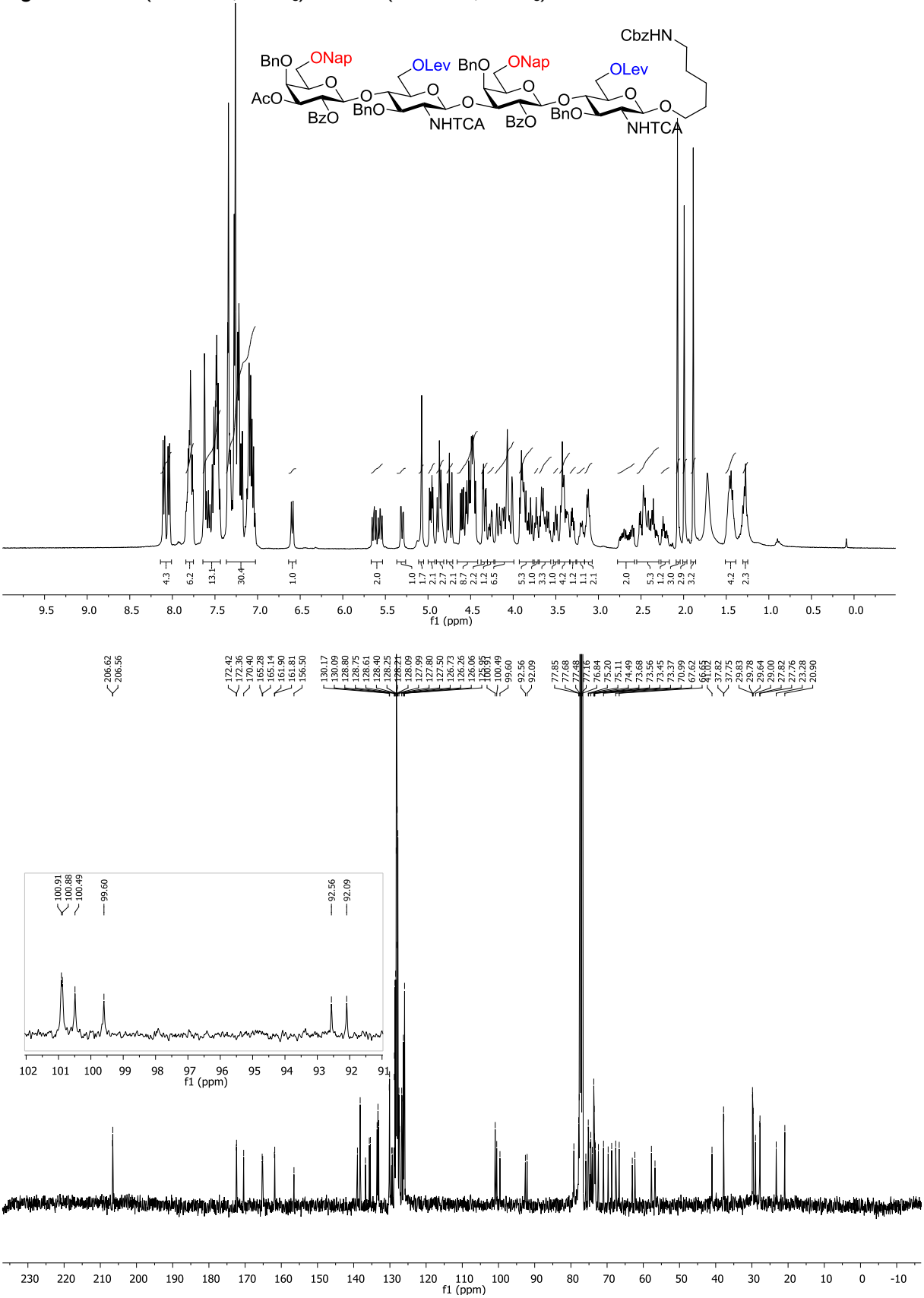

Figure S45. ^{1}H-COSY and ^{1}H-^{13}C-HSQC (400 MHz, $CDCl_3$) NMR of **S10**.

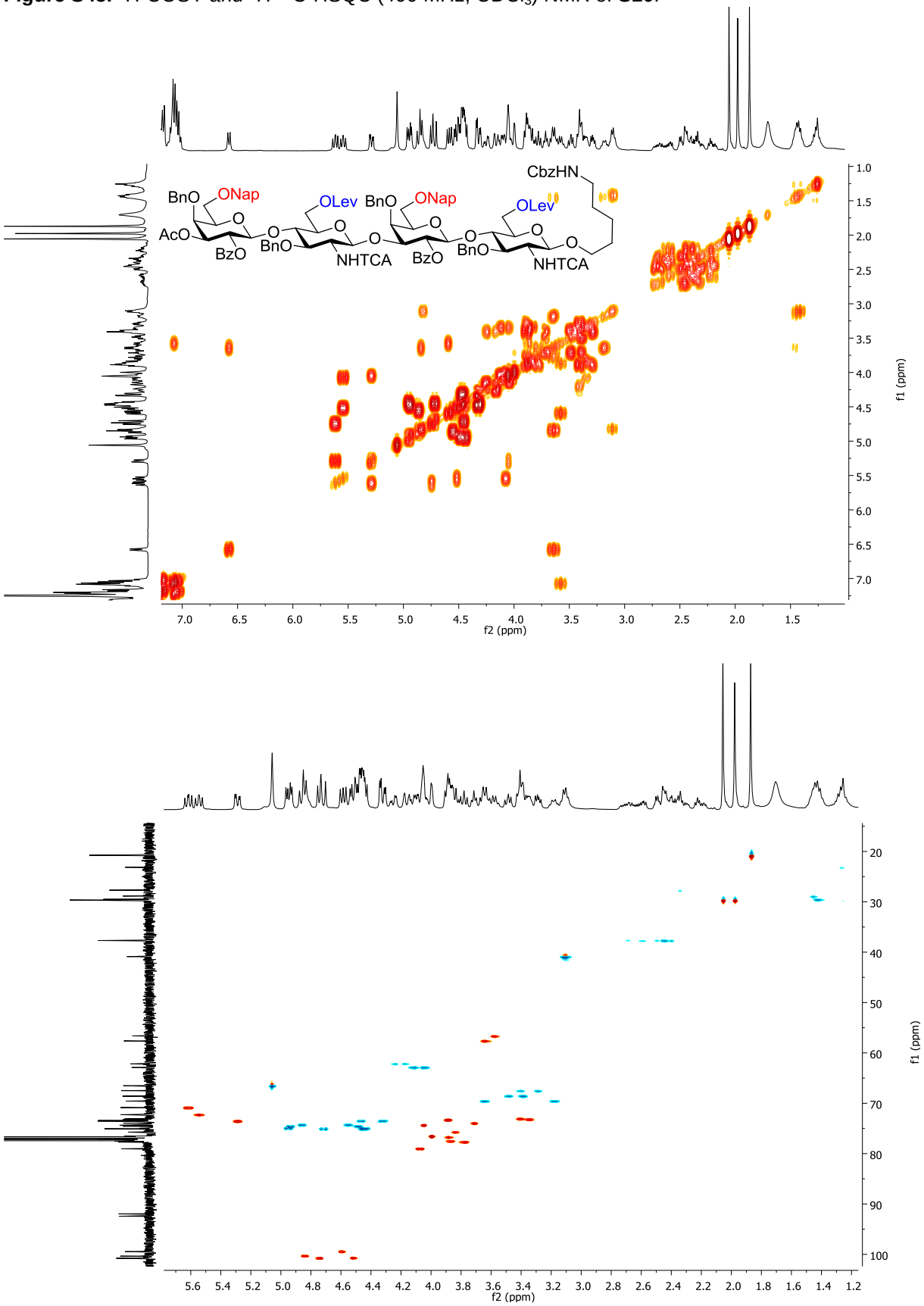

Figure S46. ^{1}H (400 MHz, $CDCl_3$) and ^{13}C (100 MHz, $CDCl_3$) NMR of **S11**.

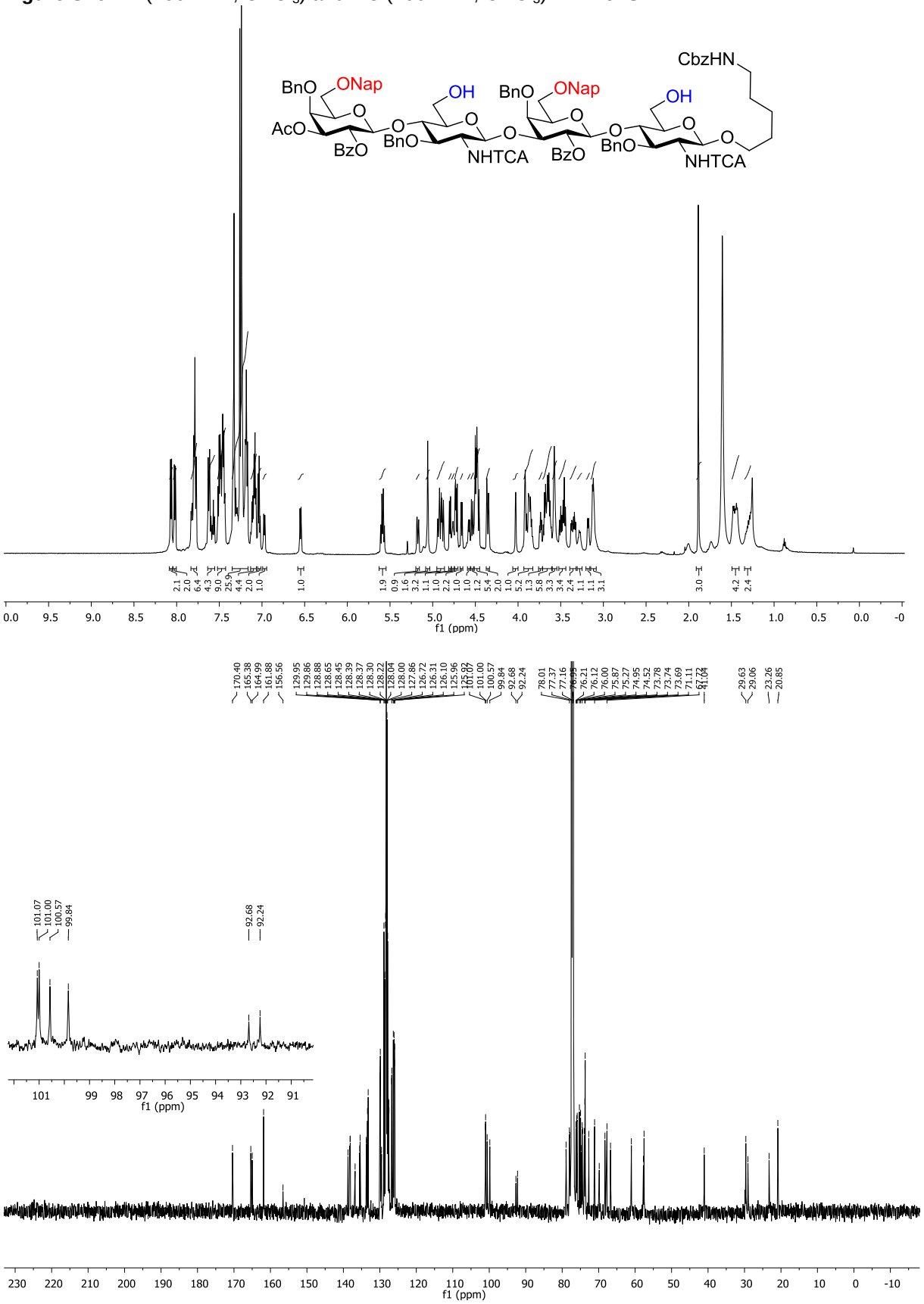

Figure S47. ^{1}H-COSY and ^{1}H-^{13}C-HSQC (400 MHz, $CDCl_3$) NMR of **S11**.

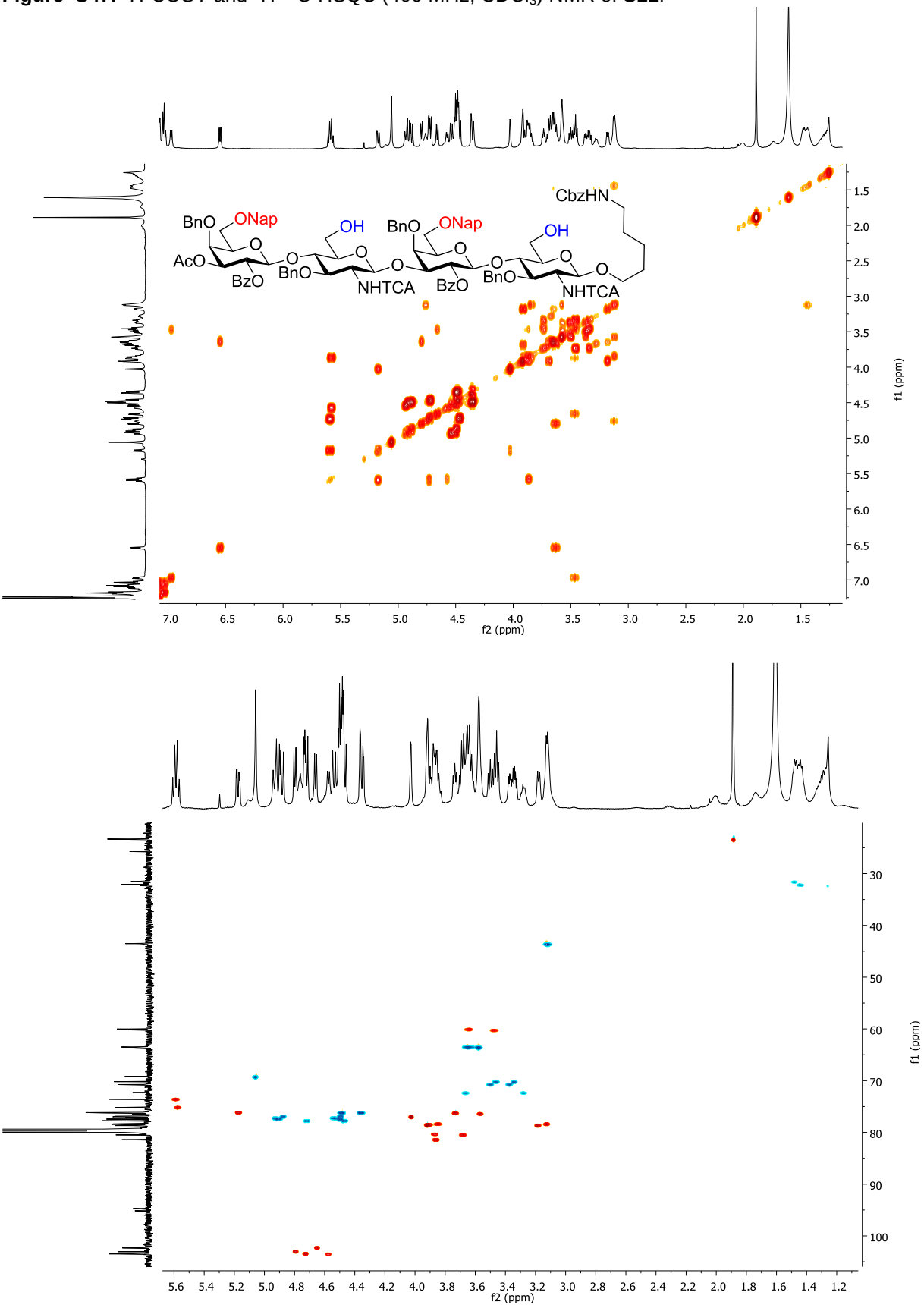

Figure S48. ^{1}H (600 MHz, CD_3OD) and ^{13}C (150 MHz, CD_3OD) NMR of **18**.

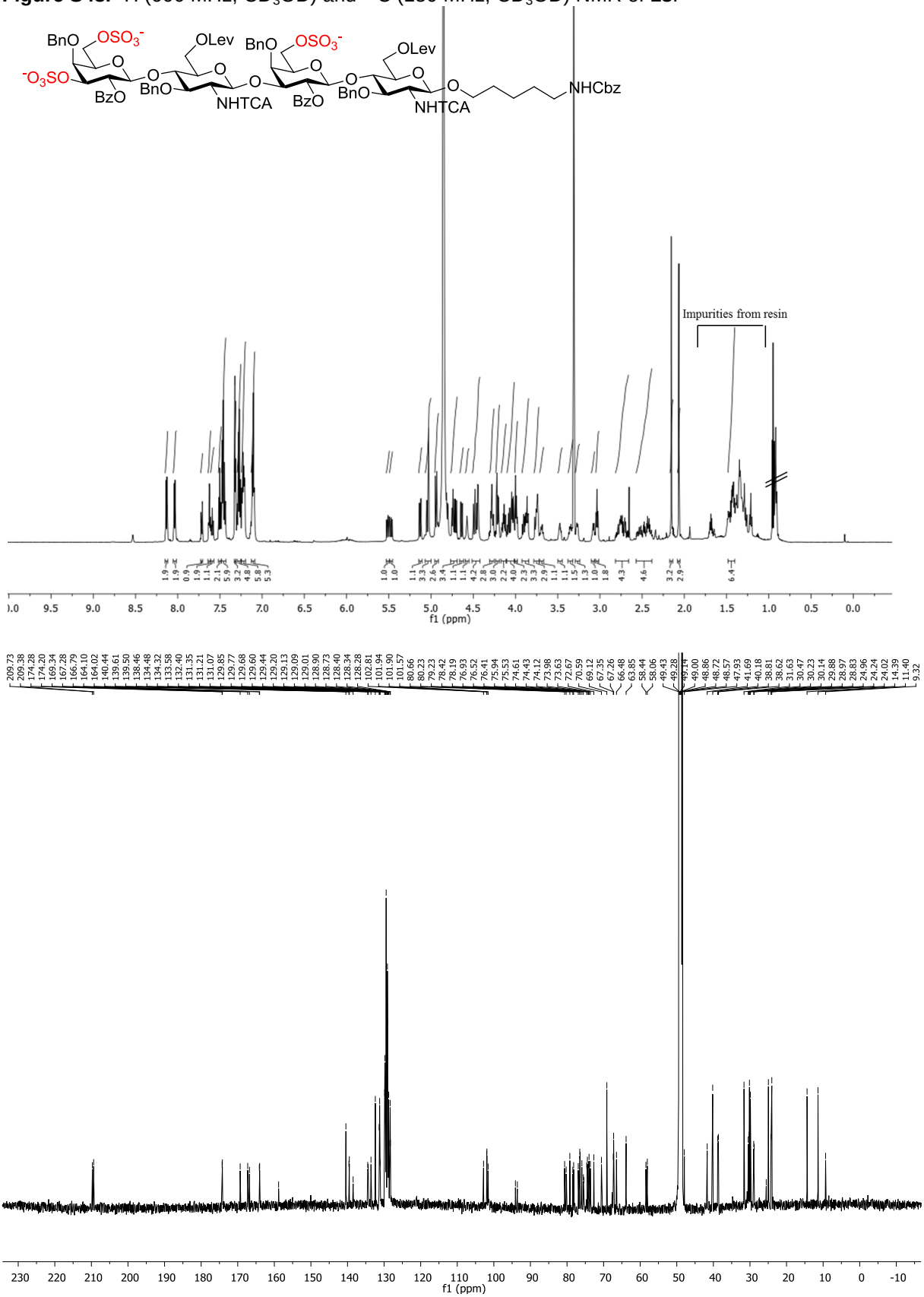

Figure S49. ^{1}H-COSY and ^{1}H-^{13}C-HSQC (600 MHz, CD_3OD) NMR of **18**.

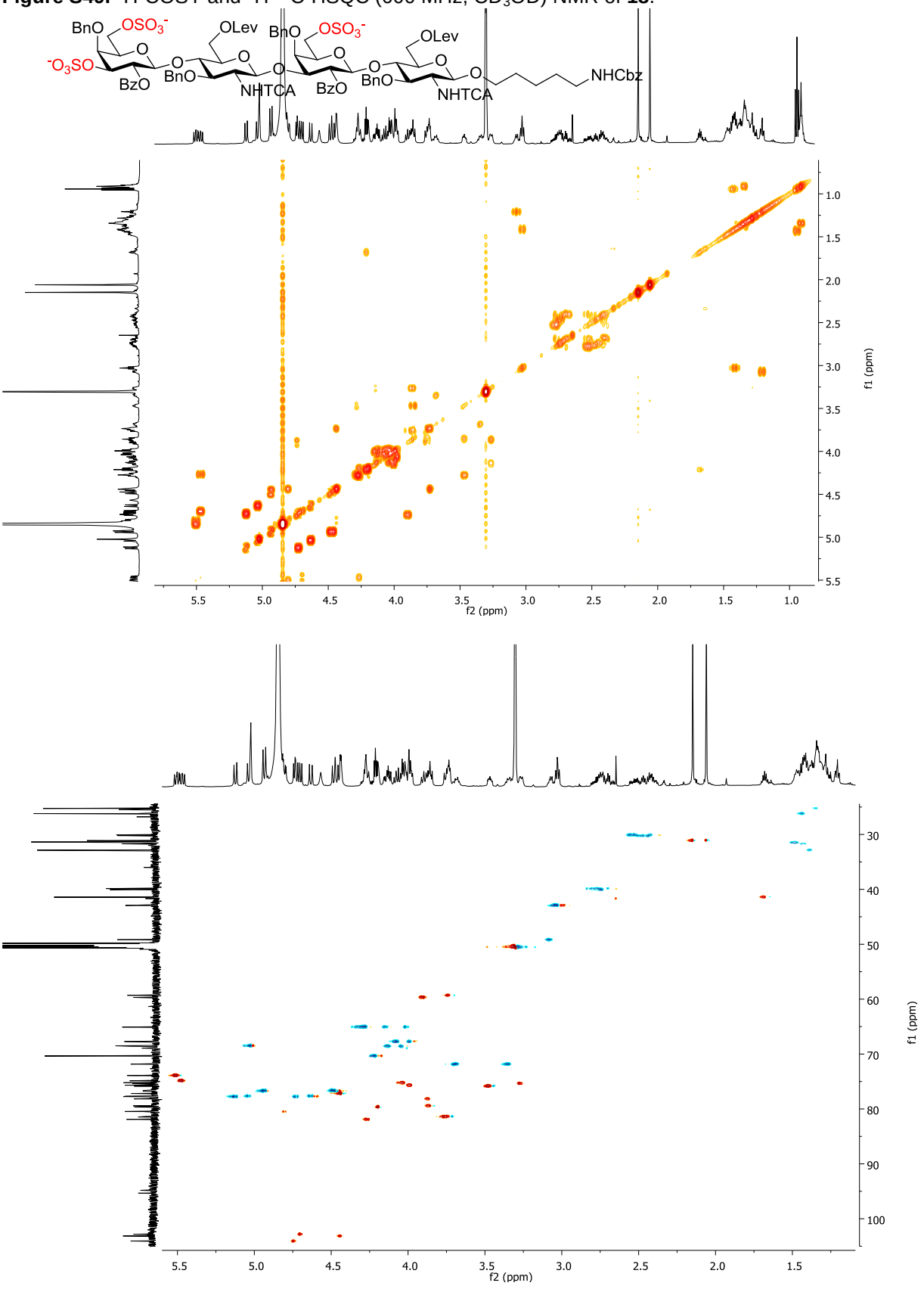

Figure S50. ^{1}H (600 MHz, CD_3OD) and ^{13}C (150 MHz, CD_3OD) NMR of **19**.

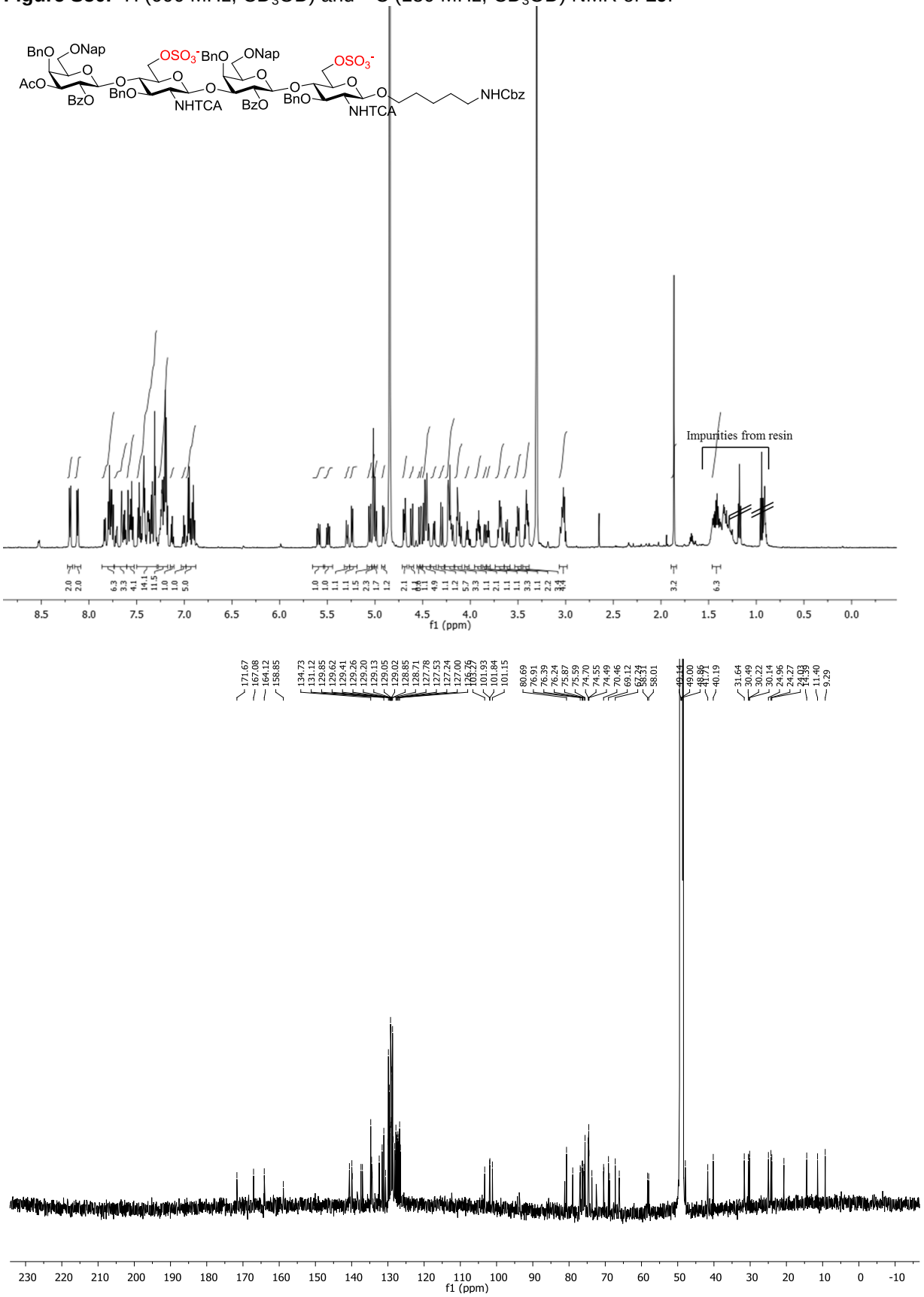

Figure S51. ^{1}H-COSY and ^{1}H-^{13}C-HSQC (600 MHz, CD_3OD) NMR of **19**.

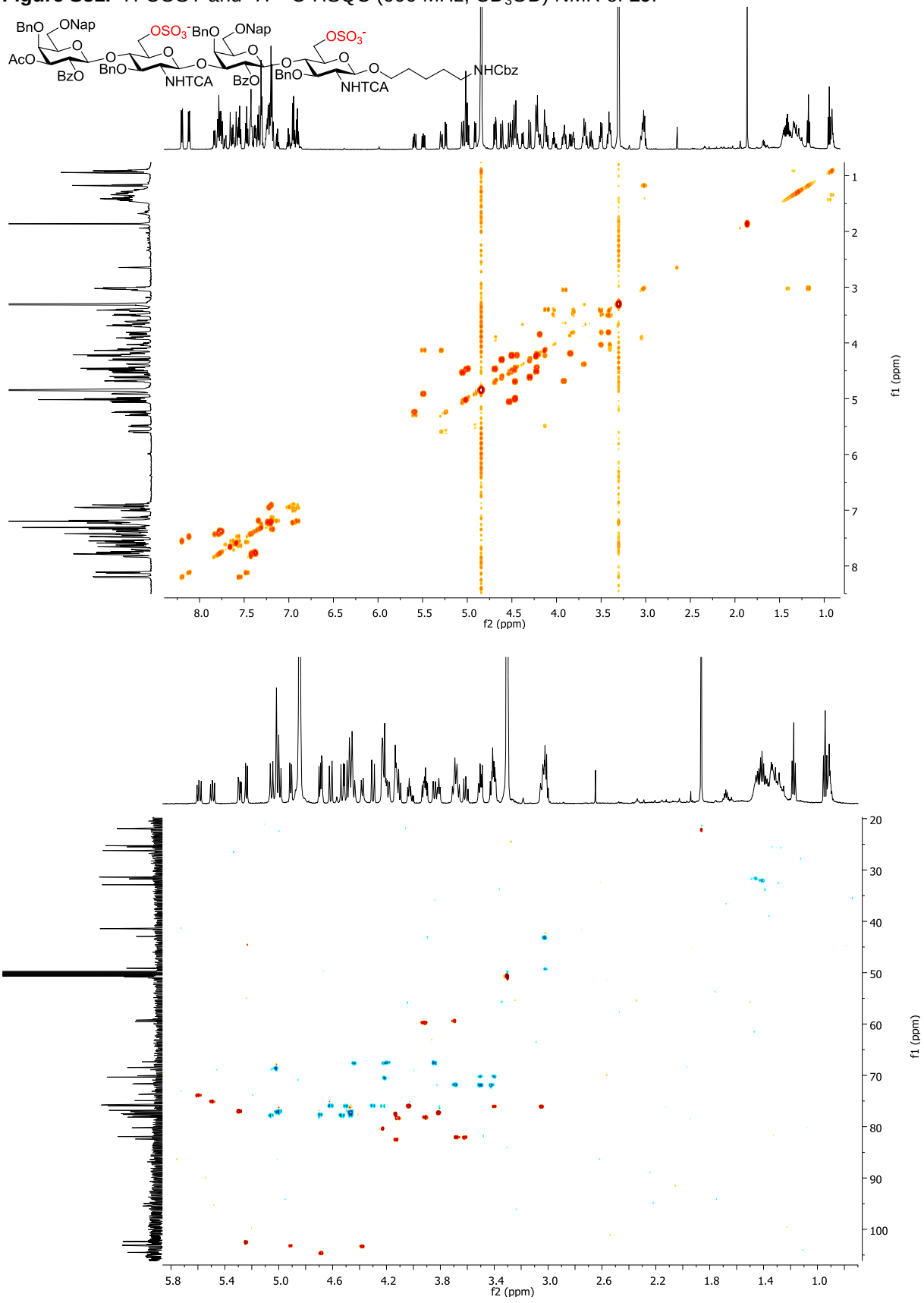

Figure S52. ^{1}H (600 MHz, CD_3OD) and ^{13}C (150 MHz, CD_3OD) NMR of **20**.

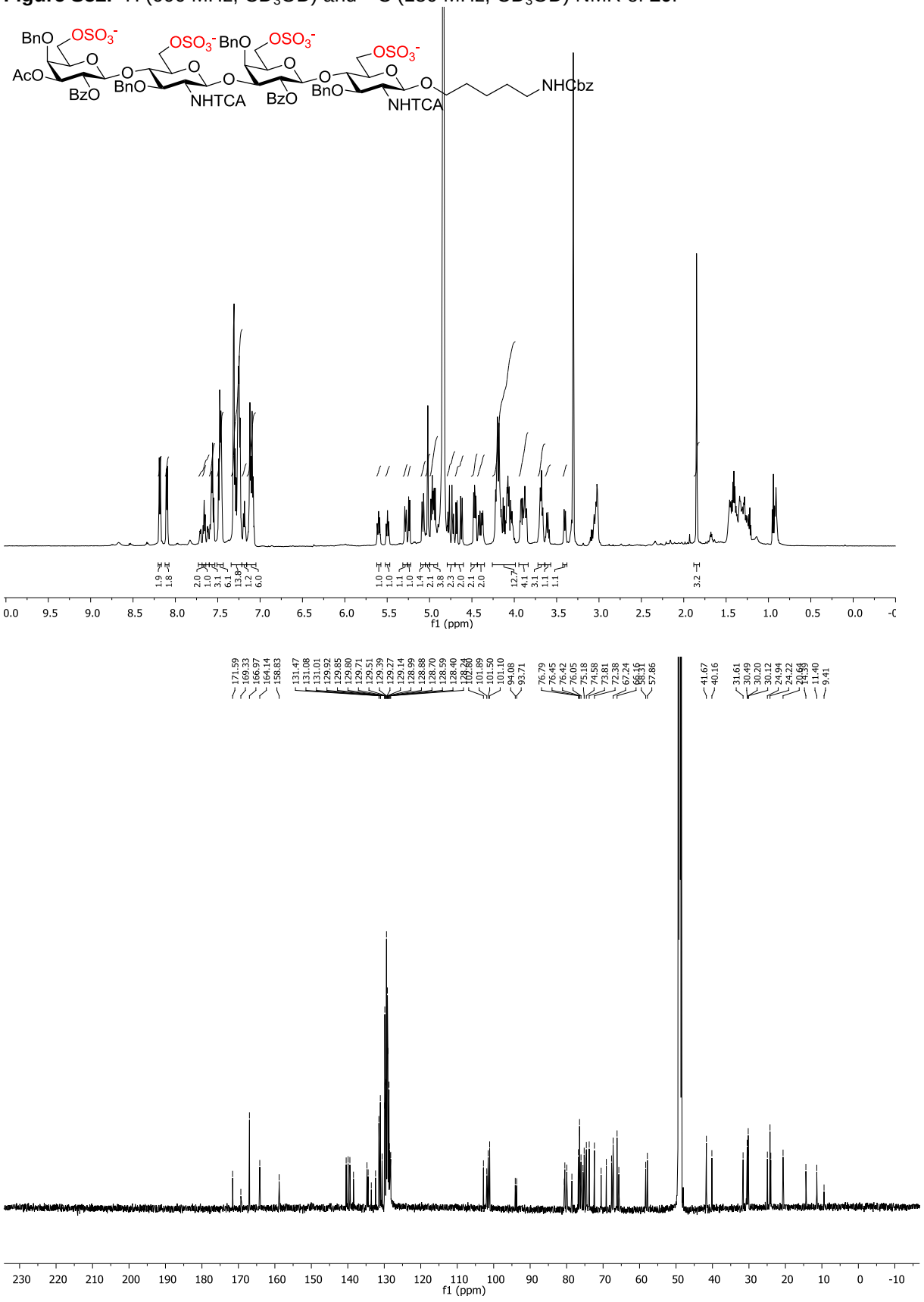

Figure S53. ^{1}H-COSY and ^{1}H-^{13}C-HSQC (600 MHz, CD_3OD) NMR of **20**.