Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Supporting Information

Chemoenzymatic synthesis of the oligosaccharide moiety of the tumor-associated antigen disialosyl globopentaosylceramide

Ingrid M.E. 't Hart,[a] Tiehai Li,[b] Margreet A. Wolfert,[a,b]
Shuo Wang,[b] Kelley W. Moremen,[b,c] Geert-Jan Boons[a,b,d] *

[a]Department of Chemical Biology and Drug Discovery, Utrecht Institute for Pharmaceutical Sciences, and Bijvoet Center for Biomolecular Research, Utrecht University, Universiteitsweg 99, 3584 CG Utrecht, The Netherlands, [b]Complex Carbohydrate Research Center, University of Georgia, 315 Riverbend Road, Athens, GA 30602, USA; [c]Department of Biochemistry and Molecular Biology, University of Georgia, Athens, GA 30602, USA, [d]Department of Chemistry, University of Georgia, Athens, GA 30602, USA.

Table of Contents

1. Chemical synthesis

1.1 General procedures

All chemicals were purchased from commercial sources. NMR spectra (^{1}H, ^{13}C, COSY, HSQC) were obtained on an Agilent 400-MR DD2 or Bruker 750 MHz. Chemical shifts are reported in part per million (ppm) relative to $CDCl_3$ (7.26 ppm), TMS (0.00 ppm) or D_2O (4.79 ppm). NMR data is presented as: chemical shift, multiplicity (where s = singlet, d = doublet, t = triplet, dd = doublet of doublets, m = multiplet) and the coupling constant in Hertz (Hz). Mass spectra were obtained on a Shimadzu ESI LC-MS QP8000 or Kratos Analytical Maxima-CFR MALDI-TOF system (using 2,5-dihydroxybenzoic acid matrix). Reported HRMS data was obtained on an Agilent technologies 6560 Ion mobility Q-TOF. Semi-preparative HPLC was performed on an Applied Biosystems 400 solvent delivery system and 757 Absorbance Detector (UV absorbance set on 214 nm) using HILIC column (XBridge® Amide 5 µm, 4.6 mm x 250 mm column, Waters). The mobile phase for analytical and semi-preparative HPLC runs consisted of buffers A and B. For C_{18} columns buffer A is 0.1 % TFA in H_2O and buffer B is 10 % A + 90 % CH_3CN and a gradient was used. For HILIC column chromatography buffer A is 10 mM NH_4COOH in H_2O (pH = 4) and B is 10 % A + 90 % CH_3CN at isocratic conditions. Size exclusion chromatography was performed on Bio-Gel P-2 (45-90 µm) with water as the eluent. Column chromatography was performed on silica gel G60 (Silicycle 60 – 200 µm, 60 Å). TLC analysis was conducted on silica gel 60 F254 (EMD Chemicals Inc.) with detection by UV light (254 nm) and staining by 10 % H_2SO_4 in EtOH or *p*-anisaldehyde solution, followed by heating for visualization. Molecular sieves (4 Å) were flame-dried prior to use.

1.2 NMR nomenclature

The monosaccharides of glycan DSGb5 have been labeled as shown in **Figure S1.** Starting from the reducing end of the pentasaccharide core Gb5, these were labeled as Glc-I, Gal-II, Gal-III, GalNAc-IV, Gal-V. The sialosides were named Neu5Ac-VI for the α2,3-linked sialic acid and Neu5Ac-VII for the α2,6-linked sialic acid.

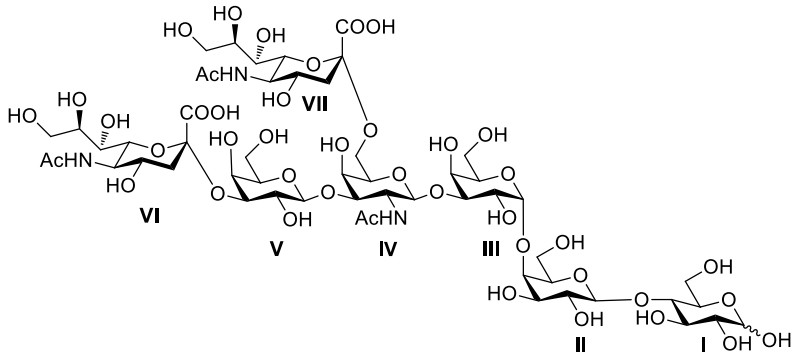
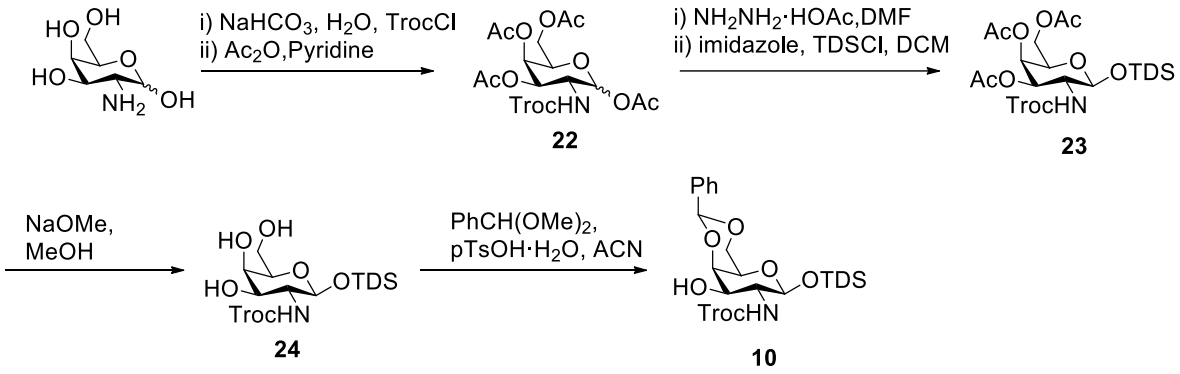


Figure S1. Monosaccharide labeling system for DSGb5

1.3 Experimental procedures

2,2,2-Trichloroacetimidate 2,3,4,6-*O*-acetyl-α-D-galactopyranoside (9). Compound **9** was synthesized according to previous synthesis.[1] NMRs of the α-anomer of the title compound are described below. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.66 (1H, s, N*H*), 6.58 (1H, d, *J* = 3.1 Hz, H-1), 5.55 (1H, d, *J* = 2.6 Hz, H-4), 5.45 – 5.31 (2H, m, H-3, H-2), 4.43 (1H, t, *J* = 6.6 Hz, H-5), 4.20 – 4.01 (2H, m, H-6), 2.15 (3H, s, OAc), 2.03 – 1.98 (9H, m, 3x OAc). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.2 (C, OAc), 170.0 (C, OAc), 170.0 (C, OAc), 169.9 (C, OAc), 160.8 (C=NH), 93.4 (C-1), 68.9 (C-5), 67.40, 67.27, 66.80, 61.2 (C-6), 20.6 (CH_3, OAc), 20.5 (CH_3, OAc), 20.5 (CH_3, OAc), 20.4 (CH_3, OAc).



Scheme S1. Synthesis of GalNHTroc acceptor **10** (similar to the procedure as described for $GlcNH_2$).[2]

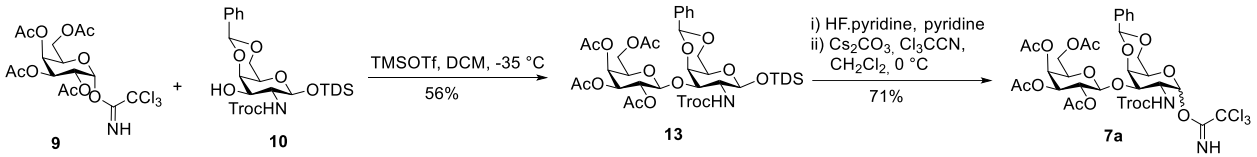
1,3,4,6-tetra-*O*-acetyl-2-[(2,2,2-trichloromethoxy)carbonylamino]-β-D-galactopyranoside (22). $NaHCO_3$ (23 g, 278 mmol) was added to Galactosamine·HCl (20 g, 93 mmol) in H_2O (180 mL) and after 30 min 2,2,2-trichloroethyl chloroformate (15.3 mL, 111 mmol) was added. After overnight stirring the white solids were filtered off, washed with H_2O and dried under high vacuum overnight. The solids were dissolved in pyridine (100 mL) and acetic anhydride (80 mL) was added. The reaction mixture was stirred for 3 h and concentrated *in vacuo*. The obtained oil was dissolved in DCM, washed with 1M HCl (2x), H_2O, sat. aq. $NaHCO_3$ and dried (Na_2SO_4), filtered and concentrated *in vacuo* to afford **22** (19 g, 40%, over two steps). ESI HRMS (*m/z*): $[M + Na]^+$ calcd for $C_{17}H_{22}Cl_3NO_{11}$, 544.0156; found 544.0155. $[\alpha]_{589}^{25}$ = 326.1° (C = 0.1; $CHCl_3$).

Dimethylthexylsilyl 3,4,6-tri-*O*-acetyl-2-[(2,2,2-trichloromethoxy)carbonylamino]-β-D-galactopyranoside (23). Hydrazine acetate (3.7 g, 40 mmol) was added to a solution of compound **22** (19 g, 36 mmol) in DMF (60 mL). The mixture was stirred overnight, concentrated *in vacuo*, dissolved in EtOAc, washed with sat. aq. $NaHCO_3$, H_2O and dried (Na_2SO_4), filtered and concentrated *in vacuo*. The resulting crude was dissolved in DCM (80 mL) and imidazole (7.4 g, 109 mmol) was added. When all imidazole was dissolved, *tert*-hexyldimethylsilyl chloride (8.6 mL, 44 mmol) was added. The mixture was stirred overnight, washed with 1 M HCl, sat. aq. $NaHCO_3$, dried (Na_2SO_4), filtered and concentrated *in vacuo*. The obtained residue was purified by silica column chromatography using Toluene:EtOAc (1:0 to 6:4 v/v) as the eluent to afford **23** (17.2 g, 76%, over two steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.35 (1H, d, *J* = 3.2 Hz, H-4), 5.16 (1H, d, *J* = 9.9 Hz, H-3), 4.95 (1H, d, *J* = 8.3 Hz, N*H*), 4.84 – 4.68 (1H, m, H-1; CH*H*, Troc), 4.62 (1H, d, *J* = 11.7 Hz, CH*H*, Troc), 4.20 – 4.05 (2H, m, H-6), 3.89 (1H, t, *J* = 6.6 Hz, H-5), 3.78 (1H, dd, *J* = 18.4, 9.2 Hz, H-2), 2.16 (2H, s,

OAc), 2.04 (3H, s, OAc), 1.99 (3H, s, OAc), 1.66 – 1.58 (1H, m, CH, TDS), 0.95 – 0.76 (12H, m, 4x CH_3, TDS), 0.17 (3H, s, CH_3-Si), 0.14 (3H, s, CH_3-Si). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.5 (C, OAc), 170.4 (C, OAc), 170.3 (C, OAc), 154.0 (C=O, Troc), 96.4 (C-1), 95.3 (CCl_3), 74.5 (CH_2, Troc), 70.7 (C-5), 69.9 (C-3), 66.9 (C-4), 61.8 (C-6), 54.7 (C-2), 33.9 (CH, TDS), 24.8 (C, TDS), 20.7 (CH_3, OAc), 20.6 (CH_3, OAc), 20.6 (CH_3, OAc), 19.9 (2x CH_3, TDS), 18.5 (2x CH_3, TDS), -1.9 (CH_3-Si), -3.4 (CH_3-Si). ESI HRMS (*m/z*): $[M + NH_4]^+$ calcd for $C_{23}H_{38}Cl_3NO_{10}Si$, 639.1669; found 639.1675. $[\alpha]_{589}^{25}$ = -32.7° (C = 0.1; $CHCl_3$).

Dimethylthexylsilyl 2-[(2,2,2-trichloromethoxy)carbonylamino]-β-D-galactopyranoside (24). Freshly prepared NaOMe was added to compound **23** (17.3 g, 28 mmol) in MeOH (50 mL). After 2 h the reaction was quenched by addition of Amberlite H^+ resin, filtered and concentrated *in vacuo* to afford **24** (13 g, 95%). This product was then used in the next step without additional purification. ESI HRMS (*m/z*): $[M + Na]^+$ calcd for $C_{17}H_{32}Cl_3NO_7Si$, 518.0911; found 518.0913. $[\alpha]_{589}^{25}$ = -77.0° (C = 0.1; $CHCl_3$).

Dimethylthexylsilyl 4,6-*O*-benzylidene-2-[(2,2,2-trichloromethoxy)carbonylamino]-β-D-galacto-pyranoside (10). Benzaldehyde dimethyl acetal (4.57 mL, 30.4 mmol) and pTsOH·H_2O (1.05 g, 5.5 mmol) were added to a solution of compound **24** (13 g, 26 mmol) in CH_3CN (90 mL). After 1 h the mixture was quenched with Et_3N, concentrated *in vacuo* and the obtained residue was purified by silica column chromatography using Hexane:EtOAc (1:0 to 3:1 v/v) as the eluent to obtain compound **10** (4.6 g, 30%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.55 – 7.48 (2H, m, H-Ar), 7.43 – 7.35 (3H, m, H-Ar), 5.57 (1H, s, C*H*-C_6H_5), 5.05 (1H, s, N*H*), 4.80 (1H, d, *J* = 7.8 Hz, H-1), 4.69 (2H, s, CH_2, Troc), 4.28 (1H, d, *J* = 12.3, 1.3 Hz, H-6a), 4.20 (1H, d, *J* = 3.6 Hz, H-4), 4.07 (1H, d, *J* = 12.4, 1.8 Hz, H-6b), 3.92 (1H, d, *J* = 8.9 Hz, H-3), 3.63 (1H, d, *J* = 9.4 Hz, H-2), 3.47 (1H, s, H-5), 2.73 (1H, d, *J* = 9.0 Hz, O*H*), 1.66 – 1.58 (1H, m, C*H*, TDS), 0.94 – 0.78 (12H, m, 4x CH_3, TDS), 0.22 (3H, s, CH_3-Si), 0.17 (3H, s, CH_3-Si). ^{13}C NMR (101 MHz, $CDCl_3$) δ 154.7 (C=O, Troc), 137.5 (C, Ar), 129.3, 128.5, 128.3, 126.4, 101.4 (C*H*-C_6H_5), 95.7 (C-1), 75.0 (C-4), 74.7 (CH_2, Troc), 70.4 (C-3), 69.3 (C-6), 66.5 (C-5), 57.8 (C-2), 34.0 (*C*H, TDS), 20.1 (CH_3, TDS), 20.1 (CH_3, TDS), 18.5 (CH_3, TDS), 18.5 (CH_3, TDS), -1.7 (CH_3-Si), -2.9 (CH_3-Si). ESI HRMS (*m/z*): $[M + Na]^+$ calcd for $C_{24}H_{36}Cl_3NO_7Si$, 606.1224; found 606.1227. $[\alpha]_{589}^{25}$ = -22.5° (C = 1; $CHCl_3$).

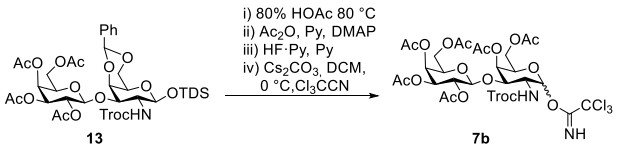


Scheme S2. Chemical glycosylation of donor **9** and acceptor **10** and formation of disaccharide donor **7a**.

Dimethylthexylsilyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→3)-4,6-*O*-benzylidene-2-[(2,2,2-trichloromethoxy)carbonylamino]-β-D-galactopyranoside (13). A mixture of acceptor **10** (1.0 g, 1.7 mmol), donor **9** (1.3 g, 2.6 mmol) and 4 Å molecular sieves was stirred in DCM (5 mL) for 2 h. The reaction mixture was cooled to -35°C and TMSOTf (62 µL, 0.3 mmol) was added. After 30 min the reaction was quenched with Et_3N, filtered over a pad of Celite and concentrated *in* vacuo. The obtained residue was purified by silica column chromatography using Toluene:EtOAc (1:0 to 8.5:1.5 v/v) as the eluent to afford compound **13** (878 mg, 56 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.58 – 7.52 (2H, m,

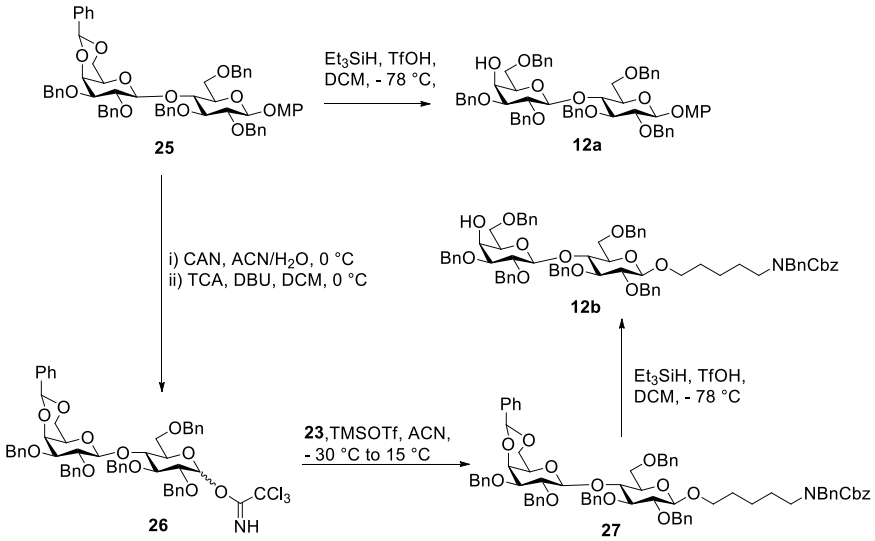
H-Ar), 7.42 – 7.31 (3H, m, H-Ar), 5.55 (1H, s, C*H*-C_6H_5), 5.36 (1H, dd, *J* = 3.4, 0.8 Hz, H-4, Gal-V), 5.32 – 5.26 (1H, m, N*H*), 5.21 (1H, d, *J* = 7.9 Hz, H-1, GalNAc-IV), 5.13 (1H, d, *J* = 7.7 Hz, H-2, Gal-V), 4.96 (1H, dd, *J* = 10.4, 3.5 Hz, H-3, Gal-V), 4.78 (1H, d, *J* = 7.9 Hz, H-1, Gal-V), 4.75 – 4.60 (2H, m, CH_2, Troc), 4.46 (1H, dd, *J* = 11.1, 2.7 Hz, H-3, GalNAc-IV), 4.29 (1H, d, *J* = 3.3 Hz, H-4, GalNAc-IV), 4.25 (1H, d, *J* = 12.2, 1.1 Hz, H-6a, GalNAc-IV), 4.22 – 4.07 (2H, m, H-6, Gal-V), 4.03 (1H, d, H-6b, GalNAc-IV), 3.88 (1H, t, *J* = 6.3 Hz, H-5, Gal-V), 3.54 – 3.36 (2H, m, H-2, GalNAc-IV; H-5, GalNAc-IV), 2.15 (3H, s, OAc), 2.06 (3H, s, OAc), 2.04 (3H, s, OAc), 1.97 (3H, s, OAc), 1.63 (1H, p, *J* = 13.7, 6.9 Hz, C*H*, TDS), 0.92 – 0.78 (12H, m, 4x CH_3, TDS), 0.19 (3H, s, CH_3-Si), 0.13 (3H, s, CH_3-Si). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.2 (C, OAc), 170.0 (C, OAc), 169.3 (C, OAc), 153.8 (C=O, Troc), 138.0 (C, C_6H_5), 129.0, 128.2, 126.3, 101.6 (C-1, Gal-V), 100.7 (*C*H-C_6H_5), 95.20 (CCl_3), 94.5 (C-1, GalNAc-IV), 76.1 (C-4, GalNAc-IV), 75.7 (C-3, GalNAc-IV), 74.5 (CH_2, Troc), 70.8 (C-5, Gal-V), 70.8 (C-3, Gal-V), 69.3 (C-6, GalNAc-IV), 68.8 (C-2, Gal-V), 67.0 (C-4, Gal-V), 66.4 (C-5, GalNAc-IV), 61.6 (C-6, Gal-V), 55.9 (C-2, GalNAc-IV), 34.0 (C*H*, TDS), 24.8 (C, TDS), 20.7 (CH_3, OAc), 20.7 (CH_3, OAc), 20.7 (CH_3, OAc), 20.5 (CH_3, OAc), 20.1 (CH_3, TDS), 20.0 (CH_3, TDS), 18.6 (CH_3, TDS), 18.5 (CH_3, TDS), -1.8 (CH_3-Si), -3.1 (CH_3-Si). ESI HRMS (*m/z*): $[M + NH_4]^+$ calcd for $C_{38}H_{54}Cl_3NO_{16}Si$, 931.2616; found 931.2634. $[\alpha]\frac{25}{589}$ = 223.8° (C = 0.1; $CHCl_3$).

2,2,2-Trichloroacetimidate 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→3)-4,6-*O*-benzylidene-2-[(2,2,2-trichloromethoxy)carbonylamino]-α-D-galactopyranoside (7a). HF·Pyridine (70% HF, 1.2 mL) was added to a stirring solution of disaccharide **13** (1.2 g, 1.3 mmol) in pyridine (12 mL) in a plastic round bottom flask. After 2.5 h, the mixture was diluted with DCM and quenched by addition of sat. aq. $NaHCO_3$. The organic phase was washed with sat. aq. $NaHCO_3$ (2 x), dried (Na_2SO_4), filtered, concentrated *in vacuo* and 2 x co-evaporated with toluene. The obtained intermediate was dissolved in DCM, stirred with 4 Å molecular sieves for 30 min and 2,2,2-Trichloroethyl chloroformate (615 µL, 6.14 mmol) and Cs_2CO_3 (400 mg, 1.23 mmol) were added. After 3 h the reaction mixture was concentrated *in vacuo* and the obtained residue was purified by silica column chromatography using Hexane:EtOAc (1:0 to 1:1 v/v) as the eluent to isolate the α-anomer of the title compound. (859 mg, 71 %, over 2 steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.72 (1H, s, C=N*H*), 7.59 – 7.47 (2H, m, Ar-H), 7.44 – 7.29 (3H, m, Ar-H), 6.67 (1H, d, *J* = 3.2 Hz, H-1, GalNAc-IV), 5.54 (1H, s, C*H*-C_6H_5), 5.41 (1H, d, *J* = 2.8 Hz, H-4, Gal-V), 5.32 – 5.20 (2H, m, H-2, Gal-V; N*H*Troc), 5.02 (1H, dd, *J* = 10.3, 3.3 Hz, H-3, Gal-V), 4.88 (1H, d, *J* = 8.1 Hz, H-1, Gal-V), 4.81 (1H, d, *J* = 12.1 Hz, CH*H*, Troc), 4.63 (1H, d, *J* = 12.1 Hz, CH*H*, Troc), 4.56 (1H, dd, *J* = 10.9, 7.4, 3.1 Hz, H-2, GalNAc-IV), 4.46 (1H, d, *J* = 2.8 Hz, H-4, GalNAc-IV), 4.34 (1H, d, *J* = 11.9 Hz, H-6a, GalNAc-IV), 4.27 (1H, dd, *J* = 11.2, 3.1 Hz, H-3, GalNAc-IV), 4.22 – 3.98 (4H, m, H-6, Gal-V; H-6b, GalNAc-IV; H-5, Gal-V), 3.88 (1H, s, H-5, GalNAc-IV), 2.18 (3H, s, OAc), 2.05 (3H, s, OAc), 2.04 (3H, s, OAc), 1.99 (3H, s, OAc). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.2 (C, OAc), 170.0 (C, OAc), 169.9 (C, OAc), 169.6 (C, OAc), 160.2 (C=NH), 154.0 (C=O, Troc), 137.4 (C, C_6H_5), 129.1, 128.2, 126.2, 101.0 (*C*H-C_6H_5), 100.0 (C-1, Gal-V), 96.3 (C-1, GalNAc-IV), 95.4 (CCl_3), 74.6 (C-4, GalNAc-IV), 74.5 (CH_2, Troc), 71.8 (C-3, GalNAc-IV), 71.3 (C-5, Gal-V), 70.8 (C-3, Gal-V), 68.9 (C-6, GalNAc-IV), 68.4 (C-2, Gal-V), 66.4 (C-4, Gal-V), 65.3 (C-5, GalNAc-IV), 60.9 (C-6, Gal-V), 49.9 (C-2, GalNAc-IV), 20.7 (CH_3, OAc), 20.7 (CH_3, OAc), 20.6 (CH_3, OAc), 20.5 (CH_3, OAc).



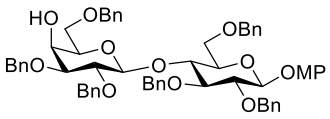
Scheme S3. Formation of disaccharide donor **7b** from disaccharide **13**.

2,2,2-Trichloroacetimidate 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→3)-4,6-di-*O*-acetyl-2-[(2,2,2-trichloromethoxy)carbonylamino]-β-D-galactopyranoside (7b). A solution of disaccharide **13** (350 mg, 0.38 mmol) in 80% aq AcOH (4 mL) was heated to 80°C for 4 h. The mixture was allowed to cool to room temperature (RT) and was diluted with EtOAc, washed with H_2O, sat aq. $NaHCO_3$ (3x), dried (Na_2SO_4), filtered and concentrated *in vacuo.* The obtained crude was dissolved in pyridine (3 mL) and Ac_2O (2 mL) was slowly added, followed by DMAP (cat.). After 1 h, the reaction showed complete conversion by TLC and the mixture was concentrated *in vacuo*. The crude was dissolved in pyridine (3.5 mL) and transferred to a plastic round bottom flask. HF·Pyridine (70% HF, 350 µL) was added and the mixture was stirred overnight. The reaction mixture was diluted with EtOAc, washed with sat. aq. $NaHCO_3$ (3x), dried (Na_2SO_4), filtered, concentrated *in vacuo* and co-evaporated with toluene. Quick silica column purification Hexane:EtOAc (1:0 to 1:3 v/v) provided the intermediate in 81% yield. The obtained intermediate (237 mg, 0.31 mmol) was dissolved in DCM and stirred with 4 Å molecular sieves at 0°C. 2,2,2-trichloroethyl chloroformate (297 µL, 2.96 mmol) and Cs_2CO_3 (301 mg, 0.92 mmol) were added after 30 min. After 22 h the reaction mixture was concentrated *in vacuo* and the obtained residue was purified by silica column chromatography using Hexane:EtOAc (1:0 to 1:1 v/v) as the eluent to isolate the α-anomer of the title compound (143 mg, 53 %). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.75 (1H s, C=N*H*), 6.57 (1H, d, *J* = 3.3 Hz, H-1, GalNAc-IV), 5.50 – 5.34 (3H, m, H-4, GalNAc-IV; N*H*Troc; H-4, Gal-V), 5.28 – 5.20 (1H, m, H-2, Gal-V), 4.99 (1H, dd, *J* = 10.3, 3.2 Hz, H-3, Gal-V), 4.84 (1H, , *J* = 12.1 Hz, CH*H*, Troc), 4.76 (1H, d, *J* = 8.2 Hz, H-1, Gal-V), 4.62 (1H, d, *J* = 12.0 Hz, CH*H*, Troc), 4.41 – 4.34 (1H, m, H-2, GalNAc-IV), 4.31 (1H, t, *J* = 6.4 Hz, H-5, GalNAc-IV), 4.25 – 4.04 (4H, m, H-6a, GalNAc-IV; H-3, GalNAc-IV; H-6, Gal-V), 4.04 – 3.92 (2H, m, H-5, Gal-V; H-6b, GalNAc-IV), 2.20 (3H, s, OAc), 2.15 (3H, s, OAc), 2.10 (3H, s, OAc), 2.04 (6H, s, 2x OAc), 1.98 (3H, s, OAc).



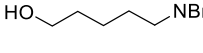
Scheme S4. Synthesis of disaccharide acceptors **12a** and **12b** from protected lactose **25**.

***Para*-methoxyphenyl 2,3,6-tri-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-gluco-pyranoside (12a).** Compound **25**[3] (8.1 g, 8.2 mmol) was stirred in DCM (50 mL) with 4 Å molecular sieves for 3 h. The mixture was cooled to -78°C and after adding Et_3SiH (6.5 mL, 41 mmol) stirring was continued for another 30 min. TfOH (1.45 mL, 16.4 mmol) was introduced and after 2.5 h, the reaction mixture was quenched with Et_3N. The resulting mixture was filtered over Celite and the filtrate

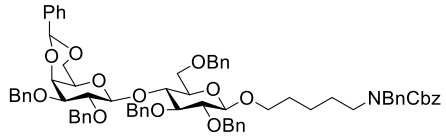


was washed with H_2O, sat. aq. $NaHCO_3$, dried (Na_2SO_4), filtered and concentrated *in vacuo*. The obtained residue was purified by silica gel chromatography using Toluene:EtOAc (1:0 to 20:1 v/v) as the eluent to afford compound **12a** (2.28g, 64 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.54 – 7.14 (30H, m, Ar-H), 7.02 (2H, d, *J* = 9.1 Hz, OMP), 6.79 (2H, d, *J* = 9.1 Hz, OMP), 5.00 (2H, t, *J* = 10.7 Hz, CH_2, Bn), 4.85 (1H, d, *J* = 7.4 Hz, H-1, Glc-I), 4.83 – 4.64 (6H, m, 3x, CH_2, Bn), 4.53 – 4.35 (5H, m, 2x CH_2, Bn; H-1, Gal-II), 4.06 – 3.96 (2H, m, H-4, Gal-II; H-5 Gal-II), 3.82 – 3.74 (4H, m, CH_3, OMP; H-6 Glc-I), 3.72 – 3.57 (4H, m, H-4, Glc-I; H-6a, Gal-II; H-2, Glc-I; H-2, Gal-II), 3.52 – 3.45 (2H, m, H-5, Glc-I; H6-b, Gal-II), 3.40 (1H, dd, *J* = 9.3, 3.4 Hz, H-3, Gal-II), 3.35 (1H, t, H-3, Glc-I), 2.39 (1H, d, *J* = 2.0 Hz, O*H*). ^{13}C NMR (101 MHz, $CDCl_3$) δ 155.2 (C, OMP), 151.6 (C, OMP), 139.0 (C, OBn), 138.5 (C, OBn), 138.4 (C, OBn), 138.3 (C, OBn, 138.2 (C, OBn), 137.9 (C, OBn), 128.43, 128.34, 128.28, 128.25, 128.20, 128.08, 128.04, 127.83, 127.78, 127.73, 127.62, 127.59, 127.54, 127.40, 127.25, 118.4 (2x CH, OMP), 114.5 (2x CH, OMP), 102.8 (C-1, Glc-I), 102.6 (C-1, Gal-II), 82.9 (C-4, Glc-I), 81.6 (C-2, Glc-I), 81.1 (C-3, Gal-II), 79.4 (C-2, Gal-II), 77.2 (C-5, Gal-II), 75.4 (CH_2), 75.3 (2x CH_2), 75.1 (C-5, Glc-I), 73.5 (CH_2), 73.1 (CH_2), 72.8 (C-3, Glc-I), 72.0 (CH_2), 68.4 (C-6, Glc-I), 68.3 (C-6, Gal-II), 66.1 (C-4, Gal-II), 55.6 (CH_3, OMP). ESI HRMS (*m/z*): $[M + NH_4]^+$ calcd for $C_{61}H_{64}O_{12}$, 1006.4736; found 1006.4750.

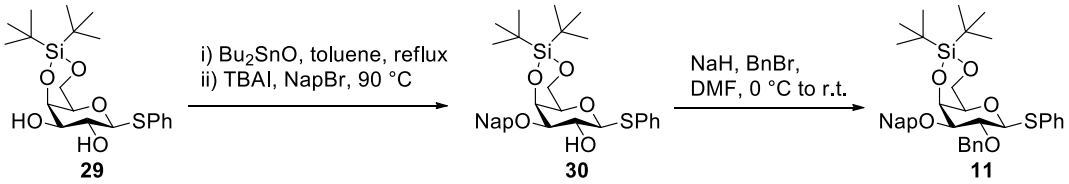
***N*-(Benzyl)-benzyloxycarbonyl-5-aminopentan-1-ol (28).** The protected aminopentanol linker was synthesized as described before.[4]



***N*-(Benzyl)-benzyloxycarbonyl-5-aminopentyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (27).** Oven-dried (90°C, 1.5 h) ceric ammonium nitrate (1.67 g, 3.0 mmol) was added to a stirring solution of compound **25**[3] (2.0 g, 2.0 mmol) in CH_3CN/H_2O (40/10 mL) at 0°C. After 30 min, the mixture was diluted with DCM, washed with sat. aq. $NaHCO_3$ (2 x), brine, dried (Na_2SO_4), filtered and concentrated *in vacuo*. Purification by silica column chromatography using Hexane:EtOAc (1:0 to 1:1 v/v) as the eluent provided the product, which was directly used in the next step. 2,2,2-Trichloroacetonitrile (848 µL, 8.5 mmol) and DBU (51 µL, 0.3 mmol) were added to the intermediate (1.49 g, 1.7 mmol) in DCM (3 mL) with 4 Å molecular sieves at 0°C. After 15 min the reaction mixture was concentrated *in vacuo* and the obtained crude was directly purified by silica column chromatography using Toluene:EtOAc (1:0 to 8:2 v/v) as the eluent. The obtained compound **26** was directly used in the next step. A mixture of acceptor **28** (100 mg, 0.3 mmol), donor **26** (468 mg, 0.46 mmol) and 4 Å molecular sieves was stirred in CH_3CN (5 mL) for 1 h. The mixture was cooled to -30°C and TMSOTf (11 µL, 0.06 mmol) was added. The reaction mixture was allowed to warm to 15°C over 2 h. The reaction mixture was with Et_3N, filtered over a pad of Celite and concentrated *in vacuo*. The obtained residue was diluted with EtOAc, washed with $NaHCO_3$. The organic layer was dried (Na_2SO_4), filtered and concentrated *in vacuo.* The obtained crude was purified by silica column chromatography using Toluene:EtOAc (1:0 to 7:3 v/v) as the eluent to afford compound **27** (305 mg, 84 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.56 – 7.07 (40H, m, Ar-H), 5.45 (1H, s, C*H*-C_6H_5), 5.22 – 5.09 (3H, m), 4.93 – 4.67 (6H, m), 4.54 (1H, d), 4.50 – 4.41 (3H, m, H-1, Glc-I; CH_2, pentyl), 4.39 – 4.14 (4H, m, H-1, Gal-II), 4.01 (1H d, *J* = 3.5 Hz, H-4, Gal-II), 3.97 (1H, dd, *J* = 11.6, 7.1 Hz, H-5, Glc), 3.93 – 3.66 (5H, m, H-2, Glc-I), 3.66 – 3.58 (1H, m, H-3, Gal-II), 3.57 – 3.31 (4H, m, H-2, Gal-II; H-3, Glc-I; H-4, Glc-I), 3.28 – 3.10 (2H, m, CH_2, pentyl), 2.92 (1H, s, H-5, Gal-II), 1.74 – 1.42 (4H, m, 2x CH_2, pentyl), 1.41 – 1.17 (2H, m, CH_2, pentyl). ^{13}C NMR (101 MHz, $CDCl_3$) δ 163.5 (C=O, Cbz), 138.9, 138.8, 138.7, 138.5, 138.3, 138.1, 129.0, 128.8, 128.6, 128.6, 128.5, 128.4, 128.4, 128.3, 128.3, 128.3, 128.2, 128.2, 128.2, 128.1, 128.0, 127.8, 127.8, 127.7, 127.6, 127.5, 127.5, 127.4, 126.6, 103.6 (C-1, Glc-I), 102.9 (C-1, Gal-II), 101.4 (*C*H-C_6H_5), 92.1, 83.0 (C-3, Gal-II), 81.8 (C-2, Gal-II), 79.6, 78.9 (C-2, Glc-I), 77.6, 7578, 75.3, 75.0, 75.0, 73.7 (C-4, Gal-II), 73.0, 71.7, 69.9, 69.0, 68.3, 67.2, 66.3 (C-5, Gal-II), 50.6, 50.3, 47.2, 46.3, 29.5, 28.0, 23.4.



***N*-(Benzyl)-benzyloxycarbonyl-5-aminopentyl 2,3,6-tri-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (12b).** Compound **27** (300 mg, 0.25 mmol) was stirred in DCM with 4 Å molecular sieves for 2 h. The mixture was cooled to -78°C and after adding Et_3SiH (201 µL, 1.26 mmol) stirring was continued for another 30 min. TfOH (45 µL, 0.50 mmol) was introduced. More Et_3SiH (200 µL) and TfOH (70 µL, 0.8 mmol) were added over time and after 2.5 h the reaction mixture was quenched with Et_3N. The quenched mixture was filtered over Celite, washed with H_2O, sat. aq. $NaHCO_3$, dried (Na_2SO_4), filtered and concentrated *in vacuo*. The obtained residue was purified by silica gel chromatography using Toluene:EtOAc (1:0 to 5:1 v/v) as the eluent to afford compound **12b** (191 mg, 64 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.59 – 6.95 (40H, m, Ar-H), 5.15 (2H, d, *J* = 6.7 Hz), 4.97 (1H, d, *J* = 10.7 Hz), 4.89 – 4.61 (6H, m), 4.61 – 4.27 (8H, m, H-1 Glc-I; H-1, Gal-II), 4.01 (1H, s, H-4, Gal-II), 3.99 – 3.91 (1H, m), 3.91 – 3.28 (13H, m, H-2, Glc-I; H-2, Gal-II), 3.28 – 3.10 (2H, m, CH_2, pentyl), 2.40 (1H, s, O*H*), 1.71 – 1.42 (4H, m, 2x CH_2, pentyl), 1.42 – 1.17 (2H, m, CH_2, pentyl). ^{13}C NMR (101 MHz, $CDCl_3$) δ 139.1, 138.7, 138.6, 138.3, 138.2, 137.9, 128.5, 128.5, 128.4, 128.3, 128.0, 127.9, 127.8, 127.8, 127.8, 127.7, 127.6, 127.5, 127.5, 127.2, 103.6 (C-1, Glc-I), 102.5 (C-1, Gal-II), 82.9, 81.8 (C-2, Glc-I), 81.1, 79.4 (C-2, Gal-II), 76.6 (C-3, Gal-II), 75.3, 75.2, 75.1, 74.9, 73.5, 73.1, 72.7, 72.0, 68.4, 68.3, 67.1, 66.1 (C-4, Gal-II), 50.5, 50.2, 47.2, 46.2, 29.4, 28.0, 27.5, 23.4.



Scheme S5. Synthesis of galactosyl donor **11** from compound **29**.[5]

Phenyl 3-*O*-(2-naphthyl)methyl-4,6-*O*-di-*tert*-butylsilanediyl-1-thio-β-D-galactopyranoside (30). Bu2SnO (5.78 g, 23 mmol) was added to compound **29**[5] (7.98 g, 19 mmol) in toluene (100 mL) and the suspension was heated under reflux for 3 h. The resulting clear solution was cooled to 90 °C and after the addition of 2-(bromomethyl)naphthalene (4.70 g, 21 mmol), tetrabutylammonium iodide (7.86 g, 21 mmol) was added portionwise over 1 h. After overnight stirring at 90 °C, the reaction mixture was concentrated *in vacuo*. The obtained crude was dissolved in DCM, washed with sat. aq. $NaHCO_3$, extracted with DCM (2x), washed with brine, dried (Na_2SO_4) and filtered over a pad of Celite. The concentrated crude was purified by silica column chromatography using Toluene:EtOAc (1:0 to 10:1 v/v) as the eluent to afford compound **30** (5.88 g, 55 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.88 – 7.75 (4H, m, Ar-H), 7.58 – 7.52 (3H, m, Ar-H), 7.51 – 7.43 (2H, m, Ar-H), 7.34 – 7.22 (3H, m, Ar-H), 4.96 (1H, d, *J* = 11.9 Hz, CH*H*, Nap), 4.81 (1H, d, *J* = 11.9 Hz, CH*H*, Nap), 4.61 – 4.54 (2H, m, H-1, H-4), 4.23 (2H, dd, *J* = 12.5, 1.9 Hz, H-6), 4.06 (1H, dd, *J* = 9.5, 1.9 Hz, H-2), 3.41 (1H, dd, *J* = 9.1, 3.0 Hz, H-3), 3.35 (1H, s, H-5), 2.60 (1H, d, *J* = 1.9 Hz, O*H,* H-2), 1.08 (9H, s, *t*-Bu), 1.07 (9H, s, *t*-Bu). ^{13}C NMR (101 MHz, $CDCl_3$) δ 135.6, 133.60, 133.25, 133.09, 132.53, 128.85, 128.37, 127.87, 127.72, 127.67, 126.61, 126.19, 125.99, 125.85, 89.2 (C-1), 81.8 (C-3), 75.1 (C-5), 70.5 (CH_2, Nap), 69.5 (C-4), 68.6 (C-2), 67.4 (C-6), 27.7 (3x CH_3, *t*-Bu), 27.6 (3x CH_3, *t*-Bu), 23.4 (C, *t*-Bu), 20.7 (C, *t*-Bu). ESI HRMS (*m/z*): [M + Na]$^+$ calcd for $C_{31}H_{40}O_5SSi$, 575,2263; found 575.2260. [α] $\frac{25}{589}$ = -10.0° (C = 0.01; $CHCl_3$).

Phenyl 2-*O*-benzyl-3-*O*-(2-naphthyl)methyl-4,6-*O*-di-*tert*-butylsilanediyl-1-thio-β-D-galactopyranoside (11). A mixture of compound **30** (1.9 g, 3.4 mmol) and NaH (60 % dispersion in oil; 275 mg, 6.9 mmol) in DMF (10 mL) was stirred at 0°C for 10 min. Benzyl bromide (612 µL, 5.2 mmol) was added dropwise and the mixture was stirred at RT for another 30 min before quenching with AcOH in MeOH. The mixture was concentrated *in vacuo*, diluted with DCM and washed with 1M HCl and water. The organic phase was dried (Na_2SO_4), filtered and concentrated *in vacuo*. Crystallization from MeOH provided compound **11** (1.39 g, 63 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.86 – 7.77 (3H, m, Ar-H), 7.76 – 7.69 (1H, m, Ar-H), 7.57 – 7.50 (3H, m, Ar-H), 7.50 – 7.40 (3H, m, Ar-H), 7.38 – 7.18 (7H, m, Ar-H), 4.98 – 4.82 (4H, m, 2x CH_2, Bn, Nap), 4.66 (1H, d, *J* = 9.8 Hz, H-1), 4.52 (1H, d, *J* = 2.7 Hz, H-4), 4.18 (2H, dd, *J* = 12.4, 1.9 Hz, H-6), 3.88 (3H, t, *J* = 9.4 Hz, H-2), 3.52 (1H, dd, *J* = 9.1, 3.0 Hz, H-3), 3.26 (1H, s, H-5), 1.15 (9H, s, *t*-Bu), 1.09 (9H, s, *t*-Bu). ^{13}C NMR (101 MHz, $CDCl_3$) δ 138.37, 135.83, 134.82, 133.21, 133.01, 132.02, 128.70, 128.41, 128.29, 128.19, 127.84, 127.69, 127.66, 127.22, 126.44, 126.07, 125.86, 88.7 (C-1), 82.6 (C-3) 77.3 (C-2), 75.9, 74.7 (C-5), 71.1, 70.1 (C-4), 67.3 (C-6), 27.7 (CH_3, *t*-Bu), 27.6 (CH_3, *t*-Bu), 23.4 (C, *t*-Bu), 20.7 (C, *t*-Bu). ESI HRMS (*m/z*): $[M + Na]^+$ calcd for $C_{38}H_{46}O_5SSi$, 665.2733; found 665.2731. $[\alpha]\frac{25}{589}$ = 4.5° (C = 0.1; $CHCl_3$).

***Para*-methoxyphenyl 2-*O*-benzyl-3-*O*-(2-naphthyl)methyl-4,6-*O*-di-*tert*-butylsilanediyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (15a).** A mixture of acceptor **12a** (1.54 g, 1.56 mmol), donor **11** (1.20 g, 1.87 mmol) and 4 Å molecular sieves was stirred in DCM (15 mL) for 1 h. The reaction mixture was cooled to -30°C and *N*-iodosuccinimide (700 mg, 3.1 mmol) and triflic acid (14 µL, 0.16 mmol) were added. After 35 min the reaction was quenched with Et_3N, filtered over a pad of Celite and concentrated *in vacuo*. The obtained residue was purified by silica gel chromatography using Toluene:EtOAc (1:0 to 20:1 v/v) as the eluent to afford compound **15a** (2.13 g, 90 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.84 – 7.64 (4H, m, Ar-H), 7.49 – 7.39 (4H, m, Ar-H), 7.36 – 7.13 (34H, m, Ar-H), 7.01 (2H, d, *J* = 9.0 Hz, OMP), 6.78 (2H, d, *J* = 9.0 Hz, OMP), 5.12 – 5.07 (1H, d, *J* = 11.3 Hz, CH*H*), 5.01 – 4.92 (2H, m, H-1, Gal-III; CH*H*), 4.85 (1H, d, *J* = 7.3 Hz, H-1, Glc-I), 4.83– 4.64 (8H, m, 4x CH_2), 4.62 – 4.52 (2H, m, 2x CH*H*), 4.52 – 4.41 (3H, m, CH*H*; H-1, Gal-II; H-4, Gal-III), 4.40 – 4.24 (3H, m, CH_2; CH*H*), 4.17 – 3.87 (6H, m, H-6a, Gal-II; H-4, Glc-I; H-2, Gal-III, H-5, Gal-II; H-4, Gal-II; H-3, Gal-III) 3.83 – 3.78 (4H, m, H-6, Glc-I; H-6, Gal-III), 3.76 (3H, s, CH_3, OMP), 3.70 – 3.60 (2H, m, H-2, Glc-I; H-5, Gal-III), 3.59 – 3.43 (3H, m, H-2, Gal-II; H-6b, Gal-II; H-5, Glc-I), 3.35 (1H, dd, *J* = 8.1, 5.5 Hz, H-3, Glc-I), 3.29 (1H, dd, *J* = 10.0, 2.7 Hz, H-3, Gal-II), 1.01 (9H, s, *t*-Bu), 0.99 (9H, s, *t*-Bu). ^{13}C NMR (101 MHz, $CDCl_3$) δ 155.2 (C, OMP), 151.6 (C, OMP), 139.2 (C, Ar), 138.7 (C, Ar), 138.5 (C, Ar), 138.4 (C, Ar), 138.3 (C, Ar), 138.2 (C, Ar), 136.7 (C, Ar), 133.2, 132.9, 129.0, 128.5, 128.4, 128.4, 128.3, 128.2, 128.2, 128.2, 128.1, 127.9, 127.8, 127.7, 127.7, 127.6, 127.6, 127.6, 127.5, 127.4, 127.4, 127.4, 127.4, 126.0, 125.9, 125.9, 125.7, 118.5 (2x CH, OMP), 114.5 (2x CH, OMP), 103.1 (C-1, Glc-I), 102.7 (C-1, Gal-II), 100.2 (C-1, Gal-III), 82.5 (C-5, Gal-III), 81.5 (C-2, Glc-I), 81.1 (C-3, Gal-II), 79.1 (C-2, Gal-II), 78.0 (C-3, Gal-III), 77.2 (C-5, Gal-II), 75.3 (C-5, Glc-I), 75.2, 75.1, 74.9, 74.3 (C-2, Gal-III), 73.7, 73.5 (C-4, Glc-I), 73.4 (C-3, Glc-I), 73.2, 73.1, 72.2, 71.2 (C-4, Gal-III), 70.6, 68.4 (C-6, Glc-I), 67.7 (C-6, Gal-II), 67.5 (C-4, Gal-II), 67.1 (C-6, Gal-III), 55.6 (CH_3, OMP), 27.7 (CH_3, *t*-Bu), 27.3 (CH_3, *t*-Bu), 23.3 (C, *t*-Bu), 20.7 (C, *t*-Bu). ESI HRMS (*m/z*): $[M + NH_4]^+$ calcd for $C_{93}H_{104}O_{17}Si$, 1538.7381; found 1538.7403. $[\alpha]\frac{25}{589}$ = 357.2° (C = 0.1; $CHCl_3$).

***Para*-methoxyphenyl 2-*O*-benzyl-4,6-*O*-di-*tert*-butylsilanediyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (8a).** DDQ (377 mg, 1.7 mmol) was added to a stirring solution of compound **15a** (2.1 g, 1.4 mmol) in DCM (120 mL) and PBS buffer (pH 7.4, 5 mL) and the reaction mixture was kept in darkness. After 3 h, the mixture was diluted with DCM, washed with sat. aq. $NaHCO_3$ (2 x), H_2O, dried (Na_2SO_4), filtered and concentrated *in vacuo*. Purification by silica column chromatography using Toluene:EtOAc (1:0 to 4:1 v/v) as the eluent provided compound **8a** (987 mg, 52 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42 (2H, d, *J* = 7.0 Hz, Ar-H), 7.36 – 7.17 (33H, m, Ar-H), 7.01 (2H, d, *J* = 9.0 Hz, OMP), 6.78 (2H, d, *J* = 9.1 Hz, OMP), 5.09 (1H, d, *J* = 11.6 Hz, CH*H*), 5.01 – 4.94 (2H, m, H-1, Gal-III; CH*H*), 4.86 (1H, d, *J* = 7.5 Hz, H-1, Glc-I), 4.81 – 4.56 (8H, m, 4x CH_2), 4.51 – 4.42 (2H, m, H-1, Gal-II; CH*H*), 4.40 – 4.27 (3H, m, CH_2; CH*H*), 4.24 (1H, d, *J* = 3.2 Hz, H-4, Gal-III), 4.09 – 3.95 (5H, m, H-4, Gal-II; H-4, Glc-I; H-6a, Gal-II; H-3, Gal-III; H-5, Gal-II), 3.79 (2H, d, *J* = 3.1 Hz, H-6, Glc-I), 3.76 (3H, s, CH_3, OMP), 3.72 (2H, s, H-6, Gal-III), 3.70 – 3.61 (3H, m, H-2, Glc-I; H-5, Gal-III; H-2, Gal-III), 3.56 (1H, dd, *J* = 9.9, 7.7 Hz, H-2, Gal-II), 3.51 – 3.43 (2H, m, H-6b, Gal-II; H-5, Glc-I), 3.34 (1H, dd, *J* = 8.3, 5.5 Hz, H-3, Glc-I), 3.29 (1H, dd, *J* = 10.0, 2.7 Hz, H-3, Gal-II), 2.40 – 2.31 (1H, m, O*H*), 0.97 (9H, s, *t*-Bu), 0.90 (9H, s, *t*-Bu). ^{13}C NMR (101 MHz, $CDCl_3$) δ 155.2 (C, OMP), 151.6 (C, OMP), 139.3 (C, Ar), 138.4 (C, Ar), 138.4 (C, Ar), 138.4 (2 C, Ar), 138.2 (C, Ar), 138.1 (C, Ar), 128.4, 128.3, 128.3, 128.3, 128.2, 128.2, 128.1, 128.0, 127.9, 127.7, 127.7, 127.6, 127.6, 127.6, 127.5, 127.4, 127.3, 127.1, 118.4 (2x CH, OMP), 114.5 (2x CH, OMP), 103.1 (C-1, Gal-II), 102.7 (C-1, Glc-I), 99.4 (C-1, Gal-III), 82.7 (C-5, Gal-III), 81.6 (C-2, Glc-I), 81.1 (C-3, Gal-II), 79.0 (C-2, Gal-II), 77.3 (C-5, Gal-II), 75.5 (C-2, Gal-III), 75.3 (C-5, Glc-I), 75.1, 75.0, 75.0, 73.9 (C-4, Gal-III), 73.2 (C-4, Glc-I), 73.1 (C-3, Glc-I), 73.0, 72.3, 70.1 (C-3, Gal-III), 68.4 (C-6, Glc-I), 67.5 (C-6, Gal, II), 67.1 (C-4, Gal-II), 66.7 (C-6, Gal-III), 55.6 (CH_3, OMP), 27.5 (CH_3, *t*-Bu), 27.2 (CH_3, *t*-Bu), 23.2 (C, *t*-Bu), 20.6 (C, *t*-Bu). ESI HRMS (*m/z*): $[M + NH_4]^+$ calcd for $C_{82}H_{96}O_{17}Si$, 1398.6755; found 1398.6760. $[\alpha]\frac{25}{589}$ = 54.2° (C = 0.05; $CHCl_3$).

***N*-(Benzyl)-benzyloxycarbonyl-5-aminopentyl 2-*O*-benzyl-3-*O*-(2-naphthyl)methyl-4,6-*O*-di-*tert*-butylsilanediyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (15b).** A mixture of acceptor **12b** (189 mg, 0.19 mmol), donor **11** (147 mg, 0.23 mmol) and 4 Å molecular sieves was stirred in DCM (1.5 mL) for 1 h. The reaction mixture was cooled to -30°C and *N*-iodosuccinimide (86 mg, 0.38 mmol) and triflic acid (1.7 µL, 0.02 mmol) were added. After 20 min the reaction was quenched with Et_3N, filtered over a pad of Celite and concentrated *in vacuo*. The obtained residue was purified by silica gel chromatography using Toluene:EtOAc (1:0 to 5:1 v/v) as the eluent to afford compound **15b** (197 mg, 60%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.95 – 7.64 (m, 4H, Ar-H), 7.54 – 6.79 (48H, m, Ar-H), 5.15 (2H, d, *J* = 6.3 Hz), 5.06 (1H, d, *J* = 11.1 Hz), 4.97 (1H, d, *J* = 3.4 Hz, H-1, Gal-III), 4.87 – 4.62 (7H, m), 4.62 – 4.38 (7H, m, H-1, Gal-II), 4.37 – 4.17 (4H, m, H-1, Glc-I), 4.16 – 4.07 (1H, m), 4.06 – 3.98 (2H, m, H-2, Gal-III), 3.98 – 3.63 (10H, m), 3.62 – 3.07 (10H, m, H-2, Gal-II, H-2, Glc-I), 1.68 – 1.40 (4H, m, 2x, CH_2, pentyl), 1.40 – 1.16 (2H, m, CH_2, pentyl), 1.00 (9H, s, *t*-Bu), 0.97 (9H, s, *t*-Bu). ^{13}C NMR (101 MHz, $CDCl_3$) δ 139.3, 138.7, 138.7, 138.5, 138.3, 138.2, 136.7, 133.2, 132.9, 129.0, 128.5, 128.4, 128.4, 128.3, 128.2, 128.2, 128.1, 127.9, 127.8, 127.7, 127.6, 127.6, 127.4, 127.4, 127.3, 126.0, 125.9, 125.9, 125.6, 125.3, 103.5 (C-1, Glc-I), 102.9 (C-1, Gal-II), 100.1 (C-1, Gal-III), 82.5, 81.7 (C-2, Glc-I), 81.1, 79.0 (C-2, Gal-II), 78.0, 77.2, 75.1, 75.0, 74.9, 74.9, 74.2, 73.6 (C-2, Gal-III), 73.1, 73.1, 72.1, 71.2, 70.6, 70.1, 69.7, 68.3, 67.6, 67.5, 67.1, 67.1, 50.2, 47.2, 46.2, 29.4, 27.6 (CH_3, *t*-Bu), 27.6, 27.3 (CH_3, *t*-Bu), 23.3 (C, *t*-Bu), 20.7 (C, *t*-Bu). $[\alpha]\frac{25}{589}$ = -23.5° (C = 0.02; $CHCl_3$).

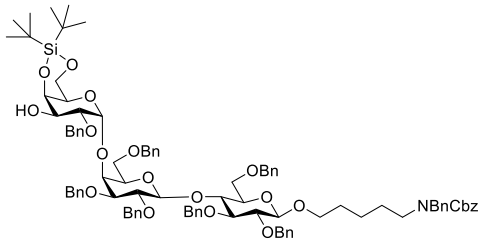
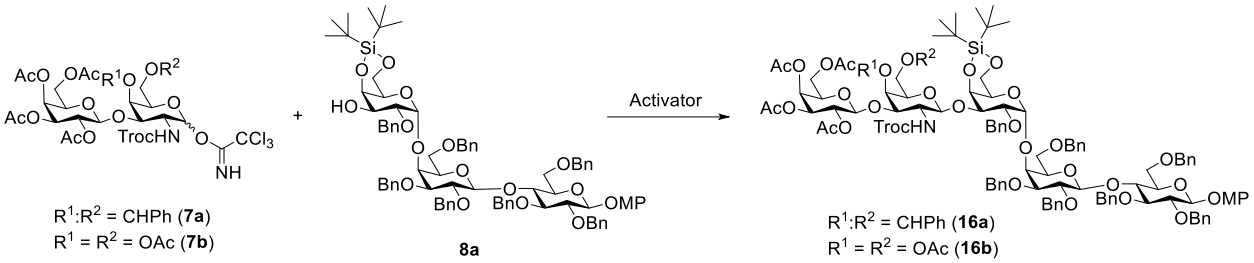
***N*-(Benzyl)-benzyloxycarbonyl-5-aminopentyl 2-*O*-benzyl-4,6-*O*-di-*tert*-butylsilanediyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (8b).**  β-Pinene (71 µL, 0.45 mmol) and DDQ (51 mg, 0.22 mmol) were added to a stirring solution of compound **15b** (2.1 g, 1.4 mmol) in DCM/H_2O (9/1 mL). The reaction mixture was kept in darkness and stirred overnight. The mixture was diluted with DCM, washed with sat. aq. $NaHCO_3$ (2 x), dried (Na_2SO_4), filtered and concentrated *in vacuo*. Purification by silica column chromatography using Toluene:EtOAc (1:0 to 10:1.2 v/v) as the eluent provided compound **8b** (105 mg, 59 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42 – 7.09 (45H, m, Ar-H), 5.15 (2H, d, *J* = 7.0 Hz, CH_2), 5.05 (1H, d, *J* = 11.6 Hz, C*H*H), 4.98 (1H, d, *J* = 3.2 Hz, H-1, Gal-III), 4.89 – 4.80 (1H, m, C*H*H), 4.80 – 4.26 (16H, m, H-1, Gal-II; H-1, Glc-I), 4.23 (1H, d, *J* = 2.8 Hz, H-4, Gal-III), 4.09 – 3.68 (10H, m, H-3, Gal-III), 3.65 (1H, dd, *J* = 9.9, 3.2 Hz, H-2, Gal-III), 3.61 – 3.09 (10H, m, H-2, Gal-II; H-2, Glc-I), 2.39 – 2.31 (1H, m, O*H*), 1.78 – 1.42 (4H, m, 2x CH_2, pentyl), 1.38 – 1.18 (2H, m, CH_2, pentyl), 0.97 (9H, s, *t*-Bu), 0.90 (9H, s, *t*-Bu). ^{13}C NMR (101 MHz, $CDCl_3$) δ 139.4, 138.6, 138.4, 138.4, 138.2, 128.5, 128.4, 128.3, 128.3, 128.2, 128.2, 128.1, 128.0, 127.9, 127.8, 127.7, 127.7, 127.6, 127.6, 127.5, 127.4, 127.3, 127.1, 103.5 (C-1, Glc-I), 103.0 (C-1, Gal-II), 99.4 (C-1, Gal-III), 82.6, 81.7 (C-2, Glc-I), 81.0, 78.9 (C-2, Gal-II), 77.2, 75.5 (C-2, Gal-III), 75.1, 75.0, 74.9, 73.9 (C-4, Gal-III), 73.2, 73.1, 73.1, 73.0, 72.2, 70.1 (C-3, Gal-III), 68.4, 67.5, 67.1, 66.7, 29.4, 27.5 (CH_3, *t*-Bu), 27.2 (CH_3, *t*-Bu), 23.4, 23.2 (C, *t*-Bu), 20.6 (C, *t*-Bu). [α] $\frac{25}{589}$ = -53° (C = 0.01; $CHCl_3$).

Table S1. Glycosylation conditions of trisaccharide acceptor **8a** and disaccharide donor **7a** or **7b** to afford protected Gb5 (**16a, 16b**).



Donor	Activator	Temperature	Total product	Isolated β-product*
8a	TMSOTf (0.2eq)	-30°C	56-59%	37-42%
8a	TMSOTf (0.2-0.3eq)	-60°C	72-76%	42-45%
8a	TfOH (0.1eq)	-10°C	32%	32%
8a	TfOH (0.1eq)	-50°C	43%	24-28%
8b	TfOH (0.1eq)	-10 °C	19-44%	19-44%
8b	TfOH (0.1eq)	-50 °C	52%	52%

The highest overall yield of the glycosylation was obtained at the coldest activation temperature. However, changes in temperature or activator did not improve β/α selectivities when donor **8a** was used. The

glycosylation proved most successful with donor **7b** and acceptor **8a** in DCM with molecular sieves at -50 °C, since no α-product was formed.

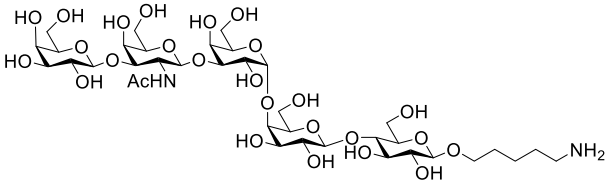
***Para*-methoxyphenyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→3)-4,6-*O*-benzylidene-2-[(2,2,2-trichloromethoxy)carbonylamino]-β-D-galactopyranosyl-(1→3)-2-*O*-benzyl-4,6-*O*-di-*tert*-butyl-silanediyl-α-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (16a).** A mixture of acceptor **8a** (987 mg, 0.71 mmol), donor **7a** (845 mg, 0.92 mmol) and 4 Å molecular sieves was stirred in DCM (9 mL) for 1 h. The mixture was cooled to -35°C and TMSOTf (26 µL, 0.14 mmol) was added. After 5 min the reaction was quenched by addition of Et_3N. The mixture was filtered over Celite and concentrated *in vacuo*. The obtained residue was purified by silica gel chromatography using Toluene:EtOAc (1:0 to 8:2 v/v) as the eluent to give compound **16a** as an oil. The β-anomer of the title pentasaccharide (562 mg, 37 %). (total isolated α/β yield: 59%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.58 – 7.09 (40H, m, H-Ar), 7.00 (2H, d, *J* = 8.8 Hz, OMP), 6.78 (2H, d, *J* = 8.8 Hz, OMP), 5.47 (1H, s, C*H*-C_6H_5), 5.36 (1H, s), 5.16 (1H, dd), 5.09 – 4.94 (3H, m), 4.93 – 3.95 (32H, m, 5x H-1), 3.95 – 3.70 (10H, m), 3.70 – 3.55 (3H, m), 3.53 – 3.42 (2H, m), 3.37 – 3.27 (2H, m), 2.90 (1H, s), 2.14 (3H, s, OAc), 2.06 (3H, s, OAc), 2.01 (3H, s, OAc), 1.95 (3H, s, OAc), 1.03 – 0.85 (18H, m, 2x *t*-Bu). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.3 (C, OAc), 170.1 (C, OAc), 169.4 (C, OAc), 155.2 (C, OMP), 153.7 (C=O, Troc), 151.5 (C, OMP), 139.4 (C, Ar), 138.5 (C, Ar), 138.4 (C, Ar), 138.3 (C, Ar), 138.1 (C, Ar), 138.0 (C, Ar), 137.9 (C, Ar), 129.0, 129.0, 128.5, 128.4, 128.3, 128.2, 128.1, 128.0, 127.8, 127.7, 127.7, 127.6, 127.5, 127.5, 127.4, 126.5, 125.3, 118.4 (2x CH, OMP), 114.5 (2x CH, OMP), 103.1 (C-1, Gal-II), 102.7 (C-1, Glc-I), 101.5 (C-1, Gal-V), 101.4 (C-1, GalNAc-IV), 100.8 (C<u>H</u>-C_6H_5), 99.9 (C-1, Gal-III), 95.3 (CCl_3), 81.6, 81.2, 79.3, 78.9, 77.2, 75.9, 75.5, 75.3, 75.1, 74.9, 74.3, 74.2, 73.8, 73.5, 73.3, 73.1, 73.0, 72.1, 70.8, 68.9, 68.7, 68.4, 67.8, 67.5, 67.0, 66.0, 61.4, 55.6 (CH_3, OMP), 53.7, 27.5 (CH_3, *t*-Bu), 27.4 (CH_3, *t*-Bu), 23.30, 20.8 (CH_3, OAc), 20.7 (CH_3, OAc), 20.7 (CH_3, OAc), 20.5 (CH_3, OAc). ESI HRMS (*m/z*): $[M + Na]^+$ calcd for $C_{112}H_{130}Cl_3NO_{32}Si$ 2156.7309; 2156.7454 found. $[\alpha]\frac{25}{589} = 55.0°$ (C = 0.05; $CHCl_3$).

***N*-(Benzyl)-benzyloxycarbonyl-5-aminopentyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→3)-4,6-*O*-benzylidene-2-[(2,2,2-trichloromethoxy)carbonylamino]-β-D-galactopyranosyl-(1→3)-2-*O*-benzyl-4,6-*O*-di-*tert*-butylsilanediyl-α-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (16c).** A mixture of acceptor **8b** (100 mg, 0.063 mmol), donor **7a** (75 mg, 0.082 mmol) and 4 Å molecular sieves was stirred in DCM (9 mL) for 1 h. The mixture was cooled to -30°C and TMSOTf (2 µL, 0.013 mmol) was added. After 20 min the reaction was quenched by addition of Et_3N. The mixture was filtered over Celite and concentrated *in vacuo*. The obtained residue was purified by silica gel chromatography using Toluene:EtOAc (1:0 to 8:2 v/v) as the eluent to give compound **16c** as an oil. The β-anomer of the title pentasaccharide (62 mg, 42%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.61 – 6.95 (50H, m, Ar-H), 5.47 (1H, s, C*H*-C_6H_5), 5.37 (1H, d, *J* = 3.1 Hz), 5.20 – 5.11 (3H, m), 5.00 (1H, d, *J* = 11.2 Hz), 4.94 – 4.41 (19H, m, H-1, Gal-

III; H-1, Gal-V; H-1, GalNAc-IV; H-1, Gal-II), 4.40 – 3.63 (24H, m, H-1, Glc-I), 3.62 – 3.08 (10H, m), 2.91 (1H, s), 2.15 (3H, s, OAc), 2.06 (3H, s, OAc), 2.01 (3H, s, OAc), 1.96 (3H, s, OAc), 1.67 – 1.43 (4H, m, 2x CH_2, pentyl), 1.39 – 1.19 (2H, m, CH_2, pentyl), 0.97 – 0.92 (18H, m, 2x *t*-Bu). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.3, 170.1, 169.3, 153.6, 139.5, 139.5, 138.6, 138.5, 138.4, 138.3, 138.1, 138.1, 138.0, 137.9, 137.8, 133.8, 129.1, 129.0, 128.5, 128.5, 128.4, 128.3, 128.2, 128.2, 128.0, 127.9, 127.8, 127.7, 127.6, 127.5, 127.4, 126.5, 125.5, 125.3, 111.9, 103.5 (C-1, Glc-I), 102.9 (C-1, Gal-II), 101.5 (C-1, Gal-V), 101.3 (C-1, GalNAc-IV), 100.7 (*C*H-C_6H_5), 99.5 (C-1, Gal-III), 95.3, 88.0, 81.7, 81.6, 81.2, 79.3, 78.7, 77.2, 76.8, 76.2, 75.9, 75.5, 75.0, 74.9, 74.2, 74.2, 73.9, 73.4, 73.2, 73.1, 72.1, 70.8, 69.7, 68.9, 68.6, 68.4, 67.7, 67.5, 67.1, 67.0, 67.0, 66.0, 61.4, 57.4, 53.8, 53.4, 50.5, 50.2, 47.1, 46.2, 29.7, 29.4, 27.5 (CH_3, *t*-Bu), 27.4 (CH_3, *t*-Bu), 23.4, 23.3 (C, *t*-Bu), 20.8 (C, *t*-Bu), 20.8 (CH_3, OAc), 20.7 (CH_3, OAc), 20.6 (CH_3, OAc), 20.5 (CH_3, OAc). $[\alpha]\frac{25}{589}$ = -84° (C = 0.01; $CHCl_3$).

β-D-Galactopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-galactopyranosyl-(1→3)-α-D-galactopyranosyl-(1→4)-β-D-galactopyranosyl-(1→4)-α/β-D-glucopyranose (4a). Pentasaccharide **16a** was deprotected in a total of six steps, all steps were monitored by TLC and MALDI-TOF-MS. HF·Pyridine (300 µL of 70%) was added to a mixture of compound **16a** (590 mg, 0.28 mmol) in pyridine (6 mL). The mixture was stirred at RT overnight, diluted with EtOAc, washed with sat. aq. $NaHCO_3$ (3 x), dried (Na_2SO_4), filtered and concentrated *in vacuo*. The obtained crude was co-evaporated with toluene (3 x) and dissolved in THF (10 mL). To this mixture 1 M NaOH (10 mL) was added and after heating to refluxed (80 °C) overnight, the mixture was concentrated *in vacuo* and co-evaporated with toluene (2 x). The resulting intermediate was dissolved in pyridine (10 mL) and Ac_2O (8 mL) was added. The mixture was stirred at RT for 6 h, diluted with EA, washed with 1 M HCl, H_2O, sat. aq. $NaHCO_3$ (4 x), dried (Na_2SO_4), filtered and concentrated *in vacuo*. The obtained residue was purified by silica column chromatography using Hexane:EtOAc (1:0 to 1:4 v/v) as the eluent to obtain the intermediate product (417 mg, 76 % over 3 steps). Ammonium cerium(IV) nitrate (587 mg, 1.07 mmol) was added to a solution of this intermediate in CH_3CN (10 mL)/H_2O (2.5 mL) at 0 °C. After 7 min the mixture was quenched by addition of sat. aq. $NaHCO_3$. The layers were separated and the organic layer was washed with sat. aq. $NaHCO_3$ (2x), H_2O, dried (Na_2SO_4), filtered and concentrated *in vacuo*. The obtained crude was dissolved in MeOH (3 mL), freshly prepared NaOMe was added and the mixture was stirred at r.t for 2 h. The mixture was neutralized with Dowex H^+ resin, filtered, concentrated *in vacuo*. The crude intermediate was dissolved in a mixture of MeOH/H_2O/HOAc (3/3/1 mL), followed by the addition of $Pd(OH)_2$/C (560 mg, 20 %, Degussa type) and the reaction mixture was left stirring overnight under the atmosphere of hydrogen. The mixture was filtered over a pad of Celite, concentrated *in vacuo* and purified by Bio-Gel P-2 size exclusion chromatography to give the title compound as a white amorphous solid (131 mg, 70 %, over three steps) Additional purification by HPLC with a semi-preparative HILIC column (XBridge® Amide 5 µm, 4.6 mm x 250 mm column, Waters) under isocratic conditions (74% B) with UV detection (210 nm) affords analytically pure glycan. ^{1}H NMR (400 MHz, D_2O) δ 5.21 (0.5H, d, *J* = 3.7 Hz, H-1α, Glc-I), 4.90 (1H, d, *J* = 3.8 Hz, H-1, Gal-III), 4.70 – 4.61 (1.5H, m, H-1, GalNAc-IV; H-1β Glc-I), 4.50 (1H, d, *J* = 7.7 Hz, H-1, Gal-II), 4.45 (1H, d, *J* = 7.7 Hz, H-1, Gal-V), 4.38 (1H, t, *J* = 6.3 Hz, H-5, Gal-III), 4.24 (1H, d, *J* = 2.3 Hz, H-4, Gal-III), 4.17 (1H, d, *J* = 2.9 Hz, H-4, GalNAc-IV), 4.10 – 4.01 (2H, m, H-2, GalNAc-IV; H-4, Gal-II), 3.99 – 3.54 (23H, m, H-2, Gal-III; H-2, Gal-II), 3.50 (1H, dd, *J* = 9.8, 7.8 Hz, H-2, Gal-V), 3.27 (0.5H, t, *J* = 8.4 Hz, H-2, Glc-Iβ), 2.02 (3H, s, NHAc). ^{13}C NMR (101 MHz, D_2O) δ 175.0 (C=O NHAc), 104.7 (C-1, Gal-V), 103.2 (C-1, Gal-II), 102.9 (C-1, GalNAc-IV), 100.3 (C-1, Gal-III), 95.6 (C-1β, Glc-I), 92.1 (C-1α, Glc-I), 79.5, 78.6, 78.6, 77.1, 75.4, 74.8, 74.5, 74.4, 73.8 (C-2, Glc-I), 72.4, 72.0, 71.4, 71.1 (C-2, Gal-III), 70.81 (C-2, Gal-II), 70.51 (C-2, Gal-V), 70.2, 70.1, 68.9, 68.5, 67.9, 67.5, 60.9, 60.9, 60.3, 60.3, 60.3, 51.4 (C-2, GalNAc-IV), 22.2 (CH_3, NHAc). ESI HRMS (*m/z*): $[M + Na]^+$ calcd for $C_{32}H_{55}NO_{26}$, 892.2910; found 892.2912.

5-aminopentyl β-D-Galactopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-galactopyranosyl-(1→3)-α-D-galactopyranosyl-(1→4)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (4b). Pentasaccharide **16c**



was deprotected in a total of 5 steps, all steps were the same as for Gb5-OMP, without the CAN reaction. (4.57 mg, 19 %, over 5 steps). ^{1}H NMR (400 MHz, D_2O) δ 4.89 (1H, d, *J* = 3.5 Hz, H-1, Gal-III), 4.67 (1H, d, *J* = 8.5 Hz, H-1, GalNAc-IV), 4.54 – 4.40 (3H, m, H-1, Gal-II; H-1, Glc-I; H-1, Gal-V), 4.37 (1H, t, *J* = 6.3 Hz, H-5, Gal-III), 4.23 (1H, s, H-4, Gal-III) 4.16 (1H, d, *J* = 2.4 Hz, H-4, GalNAc-IV), 4.10 – 3.46 (28H, m), 3.28 (1H, t, *J* = 8.2 Hz, H-2, Glc-I), 3.03 – 2.94 (2H, m, CH_2, pentyl), 2.01 (3H, s, NHAc), 1.76 – 1.58 (4H, m, 2x CH_2, pentyl), 1.50 – 1.38 (2H, m, CH_2 pentyl). ^{13}C NMR (101 MHz, D_2O) δ 175.0 (C=O, NHAc), 104.7 (C-1, Gal-V), 103.2 (C-1, Gal-II), 102.8 (C-1, GalNAc-IV), 101.9 (C-1, Glc-I), 100.3 (C-1, Gal-III), 79.5, 78.7, 78.6, 77.1, 75.3, 74.9, 74.7, 74.5, 74.4, 72.8 (C-2, Glc-I), 72.3, 72.0, 70.8 (C-2, Gal-II), 70.5 (C-2, Gal-V), 70.2, 70.0, 68.8 (C-2, Gal-III), 68.5, 67.9, 67.5, 60.9, 60.8, 60.2, 60.2, 59.9, 51.4 (C-2, GalNAc-IV), 39.2 (CH_2), 28.1 (CH_2), 26.3 (CH_2), 22.2 (CH_3, NHAc), 22.0 (CH_2). ESI HRMS (*m/z*): $[M + H]^+$ calcd for $C_{37}H_{66}N_2O_{26}$, 955.3977; found 955.3979.

2. Enzymatic synthesis

2.1 Human glycosyl transferase expression

The catalytic domains of human glycosyl transferases (see Table S2 below) were expressed as soluble, secreted fusion proteins by transient transfection of HEK293 suspension cultures.[6] The coding regions were amplified from Mammalian Gene Collection clones using primers that appended a tobacco etch virus (TEV) protease cleavage site[6b, 7] to the NH_2-terminal end of the coding region and attL1 and attL2 Gateway adaptor sites to the 5′ and 3′ terminal ends of the amplimer products. The amplimers were recombined via BP clonase reaction into the pDONR221 vector and the DNA sequences were confirmed. The pDONR221 clone was then recombined via LR clonase reaction into a custom Gateway adapted version of the pGEn2 mammalian expression vector[6, 8] to assemble a recombinant coding region comprised of a 25 amino acid NH_2-terminal signal sequence from the *T. cruzi* lysosomal α-mannosidase[9] followed by an 8xHis tag, 17 amino acid AviTag,[10] "superfolder" GFP,[11] the nine amino acid sequence encoded by attB1 recombination site, followed by the TEV protease cleavage site and the respective glycosyltransferase catalytic domain coding region.

Suspension culture HEK293 cells (Freestyle 293-F cells, Life Technologies, Grand Island, NY) were transfected as previously described[6] and the culture supernatant was subjected to Ni-NTA superflow chromatography (Qiagen, Valencia, CA). Enzyme preparations eluted with 300 mM imidazole were concentrated to ~1 mg mL^{-1} using an ultrafiltration pressure cell membrane (Millipore, Billerica, MA) with a 10 kDa molecular weight cutoff.

Table S2. Enzyme expression details.[6b]

Enzyme	Amino Acid Residues	Uniprot ID
ST3GAL1	52 - 340	Q11201
ST6GALNAC5	50 - 336	Q9BVH7
ST6GALNAC6	31 - 333	Q969X2

2.2 Experimental procedures for enzymatic synthesis

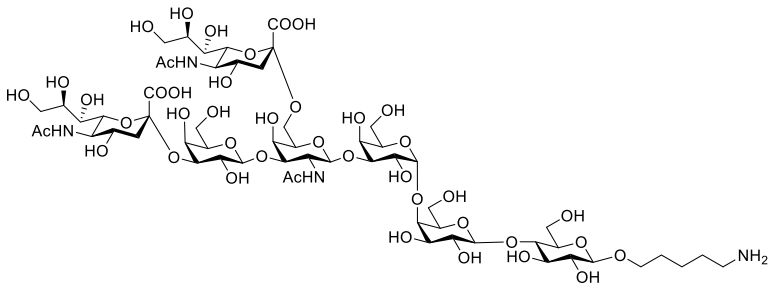
αNeu5Ac-(2→3)-β-D-Galactopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-galactopyranosyl-(1→3)-α-D-galactopyranosyl-(1→4)-β-D-galactopyranosyl-(1→4)-α/β-D-glucopyranose (5a). ST3Gal1 and CIAP were added to compound **4a** (6.5 mg, 10 mM final concentration) in H_2O with CMP-Neu5Ac (15 mM), $MgCl_2$ (20 mM), sodium cacodylate buffer (50 mM, pH 7.5). The mixture was shaken at 37°C for 94 h and monitored by TLC (EA:MeOH:H_2O:HOAc 4:3:2:1). More enzymes were added until no more starting material could be observed. Purification by Bio-Gel P-2 size exclusion chromatography and semi-preprarative HILIC column (XBridge® Amide 5 µm, 4.6 mm x 250 mm column, Waters, 70% B isocratic) provided compound **5a** (4.57 mg, 53 %). ^{1}H NMR (750 MHz, D_2O) δ 5.24 (0.5H, d, *J* = 3.7 Hz, H-1α, Glc-I), 4.93 (1H, d, *J* = 3.9 Hz, H-1, Gal-III), 4.70 (1H, d, *J* = 8.5 Hz, H-1, GalNAc-IV), 4.68 (0.5H, d, *J* = 8.0 Hz, H-1β, Glc-I), 4.55 – 4.51 (2H, m, H-1, Gal-V; H-1, Gal-II), 4.41 – 4.37 (1H, m, H-5, Gal-III), 4.26 (1H, s, H-4, Gal-III), 4.19 (1H, d, *J* = 3.0 Hz, H-4, GalNAc-IV), 4.08 (2H, dd, *J* = 9.9, 3.2 Hz, H-2, GalNAc-IV; H-3, Gal-V), 4.05 (1H, d, *J* = 2.9 Hz, H-4, Gal-II), 4.01 – 3.57 (29.5H, m), 3.55 (1H, dd, H-2, Gal-V), 3.29 (0.5H, t, *J* = 8.6 Hz, H-2, Glc-I), 2.76 (1H, dd, *J* = 12.4, 4.6 Hz, H-3eq, Neu5Ac-VI), 2.04 (6H, s, 2x NHAc), 1.79 (1H, t, *J* = 12.1 Hz, H-3ax, Neu5Ac-VI). ESI HRMS (*m/z*): $[M + Na]^+$ calcd for $C_{43}H_{72}N_2O_{34}$, 1183.3864; found 1183.3861.

5-aminopentyl αNeu5Ac-(2→3)-β-D-Galactopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-galacto-pyranosyl-(1→3)-α-D-galactopyranosyl-(1→4)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranose (5b). ST3Gal1 and CIAP were added to compound **4b** (2.0 mg, 10 mM final concentration) in H_2O with CMP-Neu5Ac (15 mM), $MgCl_2$ (20 mM), sodium cacodylate buffer (50 mM, pH 7.5). The mixture was shaken at 37°C for 24 h and monitored by TLC (EA:MeOH:H_2O:HOAc 4:4:3.3:2). Purification by Bio-Gel P-2 size exclusion chromatography provided compound **5b** (1.8 mg, 69%). ^{1}H NMR (600 MHz, D_2O) δ 4.92 (1H, d, *J* = 3.9 Hz, H-1, Gal-III), 4.71 (1H, d, *J* = 8.5 Hz, H-1, GalNAc-IV), 4.56 – 4.49 (3H, m, H-1 Gal-V; H-1, Gal-II; H-1, Glc-I), 4.39 (1H, t, *J* = 6.4 Hz, H-5, Gal-III), 4.26 (1H, d, *J* = 2.4 Hz, H-4, Gal-III), 4.19 (1H, d, *J* = 2.8 Hz, H-4, GalNAc-IV), 4.13 – 3.48 (34H, m), 3.36 – 3.27 (1H, m, H-2, Glc-I), 3.02 (2H, t, *J* = 7.5 Hz, CH_2), 2.76 (2H, dd, *J* = 12.4, 4.6 Hz, H-3eq, Neu5Ac-VI), 2.04 (6H, s, 2x NHAc), 1.79 (1H, t, *J* = 12.1 Hz, H-3ax, Neu5Ac-VI), 1.75 – 1.65 (4H, m, 2x CH_2, pentyl), 1.52 – 1.43 (2H, m, CH_2, pentyl). ESI HRMS (*m/z*): $[M + H]^+$ calcd for $C_{48}H_{83}N_3O_{34}$, 1246.4858; found 1246.4970.

αNeu5Ac-(2→3)-β-D-Galactopyranosyl-(1→3)-[αNeu5Ac-(2→6)]-2-acetamido-2-deoxy-β-D-galacto-pyranosyl-(1→3)-α-D-galactopyranosyl-(1→4)-β-D-galactopyranosyl-(1→4)-α/β-D-glucopyranose (6a). ST6GalNAc5 and CIAP were added to a mixture of compound **5a** (4.2 mg, 10 mM final concentration) in H_2O with CMP-Neu5Ac (15 mM), $MgCl_2$ (20 mM), sodium cacodylate buffer (50 mM, pH 7.5). The mixture was shaken at 37°C overnight and monitored by TLC (EA:MeOH:H_2O:HOAc 3:3:3:2). More enzymes were added until no more starting material could be observed. Purification by Bio-Gel

P-2 size exclusion chromatography and semi-preprarative HILIC column (XBridge® Amide 5 µm, 4.6 mm x 250 mm column, Waters, 70% B isocratic) provided compound **6a** (2.64 mg, 50 %). ^{1}H NMR (750 MHz, D_2O) δ 5.24 (0.5H, d, *J* = 3.7 Hz, H-1α, Glc-I, 4.93 (1H, d, *J* = 3.8 Hz, H-1, Gal-III), 4.70 – 4.65 (1.5H, m, H-1β, Glc-I; H-1, GalNAc-IV), 4.54 – 4.50 (2H, m, H-1, Gal-II, H-1, Gal-V), 4.43 – 4.39 (1H, m, H-5, Gal-III), 4.28 (1H, s, H-4, Gal-III), 4.20 (1H, d, *J* = 3.0 Hz, H-4, GalNAc-IV), 4.10 – 4.04 (3H, m, H-2, GalNAc-IV; H-3, Gal-V), 4.02 – 3.57 (36.5H, m), 3.55 (1H, dd, *J* = 9.6, 8.1 Hz, H-2, Gal-V), 3.29 (0.5H, dd, *J* = 9.0, 8.2 Hz, H-2, Glc-I), 2.79 – 2.70 (2H, m, H-3eq, Neu5Ac-VI; H-3eq, Neu5Ac-VII), 2.07 – 2.01 (9H, m, 3x NHAc), 1.80 (1H, t, *J* = 12.2 Hz, H-3ax, Neu5Ac-VI), 1.66 (1H, t, *J* = 12.2 Hz, H-3ax, Neu5Ac-VII). ESI HRMS (*m/z*): $[M + Na]^+$ calcd for $C_{54}H_{89}N_3O_{42}$, 1474.4818; found 1474.4807.

5-aminopentyl αNeu5Ac-(2→3)-β-D-Galactopyranosyl-(1→3)-[αNeu5Ac-(2→6)]-2-acetamido-2-deoxy-β-D-galactopyranosyl-(1→3)-α-D-galactopyranosyl-(1→4)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (6b). ST6GalNAc5 and CIAP were added to a mixture of compound **5b** (1.0 mg, 10 mM final concentration) in H_2O with CMP-Neu5Ac (15 mM), $MgCl_2$ (20 mM), sodium cacodylate buffer (50 mM, pH 7.5). The mixture was shaken at 37°C for 4 h and monitored by TLC (EA:MeOH:H_2O:HOAc 3:3:3:2). Purification by Bio-Gel P-2 size exclusion chromatography provided compound **6b** (0.7 mg, 57%). ^{1}H NMR (600 MHz, D_2O) δ 4.92 (1H, d, *J* = 4.0 Hz, H-1, Gal-III), 4.69 (1H, d, *J* = 8.5 Hz, H-1, GalNAc-IV), 4.55 – 4.49 (3H, m, H-1, Gal-II; H-1, Gal-V; H-1, Glc-I), 4.40 (1H, t, *J* = 6.5 Hz, H-5, Gal-III), 4.28 (1H, d, *J* = 2.7 Hz, H-4, Gal-III), 4.20 (1H, d, *J* = 3.0 Hz, H-4, GalNAc-IV), 4.11 – 4.04 (3H, m, H-2, GalNAc-IV; H-3, Gal-V, H-4, Gal-II), 4.03 – 3.53 (39H, m), 3.31 (1H, t, *J* = 8.5 Hz, H-2, Glc-I), 3.06 – 2.99 (2H, m, CH_2, pentyl), 2.80 – 2.70 (2H, m, H-3eq, Neu5Ac-VI; H-3eq, Neu5Ac-VII), 2.07 – 2.00 (9H, m, 3x NHAc), 1.79 (1H, t, *J* = 12.1 Hz, H-3ax, Neu5Ac-VI), 1.75 – 1.63 (5H, m, 2x CH_2, pentyl; H-3ax, Neu5Ac-VII), 1.51 – 1.43 (2H, m, CH_2, pentyl). ESI HRMS (*m/z*): $[M + H]^+$ calcd for $C_{59}H_{100}N_4O_{42}$, 1537.5812; found 1537.5874.



3. Microarray

3.1 Experimental procedures

The synthetic glycans (100 µM in sodium phosphate (250 mM), pH 8.5 buffer) were printed on activated glass slides (Nexterion Slide H, Schott Inc) by piezoelectric non-contact printing (sciFLEXARRAYER S3, Scienion Inc) with a drop volume of ~400 pL and 1 drop per spot at 50 % relative humidity. The compounds were printed as replicates of 6 with on each slide 24 subarrays (3x8). The slides were incubation overnight in a saturated NaCl chamber (providing a 75% relative humidity environment), after which the remaining activated esters were quenched with ethanolamine (50 mM) in TRIS (100 mM), pH 9.0. Slides were rinsed with DI water, dried by centrifugation, and stored in a desiccator at RT.

Sub-arrays were incubated with biotinylated lectins (*Maackia amurensis* leukagglutinin (MAL-II), Soybean agglutinin (SBA) and Wheat Germ agglutinin (WGA); from Vector Labs) at 10 µg/mL premixed with Streptavidin-AlexaFluor635 (5 µg/mL; ThermoFisher Scientific, S32364) in TSM binding buffer (20 mM Tris Cl, pH 7.4, 150 mM NaCl, 2 mM $CaCl_2$, 2 mM $MgCl_2$, 0.05% Tween, 1% BSA) for 1 h followed by washing. Wash steps involved 4 successive washes with each 5 min soak time with 1) TSM wash buffer (20 mM Tris

Cl, pH 7.4, 150 mM NaCl, 2 mM $CaCl_2$, 2 mM $MgCl_2$, 0.05% Tween-20); 2) TSM buffer (20 mM Tris Cl, pH 7.4, 150 mM NaCl, 2 mM $CaCl_2$, 2 mM $MgCl_2$); 3) deionized H_2O; and 4) deionized H_2O.

Biotin-conjugated ganglioside GM1 polyclonal antibody (2 µg/mL; Bioss, bs-2367R-Biotin) in TSM binding buffer was incubated for 1 h followed by washing as described above. Next the subarray was incubated with Streptavidin-AlexaFluor635 (5 µg/mL) for 1 h followed by washing.

Using the same buffers as above, recombinant human Siglec-7 comp (a gift from Dr. R.L. Schnaar, Johns Hopkins University School of Medicine, Baltimore, MD, USA) was assayed at 50 µg/mL premixed with 6x-His Tag monoclonal antibody-AlexaFluor647 (5 µg/mL; ThermoFisher Scientific MA1-135-A647) with an incubation for 2 h.

All incubation and wash steps were performed at RT. Washed arrays were dried by centrifugation and immediately scanned for fluorescence on a GenePix 4000 B microarray scanner (Molecular Devices) using a detection gain adjusted to avoid saturation of the signal. The data were processed with GenePix Pro 7 software and further analyzed using our home written Microsoft Excel macro. The lowest and highest value of the 6 replicates were excluded, after which the mean fluorescence intensities (corrected for mean background) and standard deviations (SD) were calculated (n=4). Data were fitted using Prism software (GraphPad Software, Inc). The lowest concentration required for good responsiveness in the optimum dynamic range was selected for all proteins examined.

3.2 Results and discussion printing controls

The printing of the synthetic compounds was validated by the plant lectins MAL II, SBA and WGA and a GM1 antibody.

MAL-II binds the terminal trisaccharide sequence Neu5Ac(α2-3)Gal(β1–4)GlcNAc/Glc.[12] Compounds **17** and **20** (Neu5Ac(α2-8)-Neu5Ac(α2-3)Gal(β1–4)Glc) have three terminal intact sugars, and as expected binds to MAL II. Compounds **5b** and **6b**, with the Neu5Ac(α2-3)-Gal-β1,3-GalNAc epitope at the terminal end, are not recognized by MAL II. Similarly, GT1b (**21**) with the same terminal epitope as **5b** and **6b** also did not show binding to MAL II.

SBA preferentially binds GalNAc, and also recognizes Gal residues although at much lower affinity.[13] Binding to SBA was observed for compounds with either a GalNAc or Gal at the terminal residue: **18** (GM2; with GalNAc at the terminal residue) and **4b** (Gb5; with Gal at terminal residue). As expected sialylated compounds **5b** and **6b** (sialylated Gb5; no Gal at the terminal residue) didn't show any binding. Also compound **19** (GM1a; with Gal at the terminal residue) didn't show any binding, due to the inhibition effect of Neu5Ac.

WGA preferentially binds GlcNAc moieties, and also interacts with some glycoproteins via terminal sialic acid residues. Indeed the terminal sialylated compound **21** (GT1b) and sialylated Gb5 (**5b** and **6b**) showed binding, while the non-terminal sialylated compounds **18** (GM2), **19** (GM1a) and **4b** (Gb5) did not bind. Compound **20** (GD3; with terminal α2,8-Neu5Ac-a2,3-Neu5Ac) also did not bind to WGA, apparently WGA does not recognize this sialylated epitope.

As expected only GM1a (**19**) showed binding to the GM1 antibody.

4. References

1 S. Vudhgiri, S. R. Routhu, C. G. Kumar, R. B. N. Prasad and R. C. R. Jala, *Med. Chem. Res.*, 2018, **27**, 285.

2 G.J. Boons and Z. Wang, US Utility Patent Number 9,938,312, titled "*Compounds and Methods for Chemical and Chemo-Enzymatic Synthesis of Complex Glycans*" issued April 10, 2018. Assignee: University of Georgia Research Foundation, Inc.

3 H. Kuyama, T. Nukada, Y. Nakahara and T. Ogawa, *Tetrahedron Lett.*, 1993, **34**, 2171.

4 C. Noti, J. L. de Paz, L. Polito and P. H. Seeberger, *Chem. Eur. J.*, 2006, **12**, 8664.

5 A. Imamura, H. Ando, S. Korogi, G. Tanabe, O. Muraoka, H. Ishida and M. Kiso, *Tetrahedron Lett.*, 2003, **44**, 6725.

6 (a) L. Meng, F. Forouhar, D. Thieker, Z. Gao, A. Ramiah, H. Moniz, Y. Xiang, J. Seetharaman, S. Milaninia, M. Su, R. Bridger, L. Veillon, P. Azadi, G. Kornhaber, L. Wells, G. T. Montelione, R. J. Woods, L. Tong and K. W. Moremen, *J. Biol. Chem.*, 2013, **288**, 34680; (b) K. W. Moremen, A. Ramiah, M. Stuart, J. Steel, L. Meng, F. Forouhar, H. A. Moniz, G. Gahlay, Z. Gao, D. Chapla, S. Wang, J. Y. Yang, P. K. Prabhakar, R. Johnson, M. D. Rosa, C. Geisler, A. V. Nairn, J. Seetharaman, S. C. Wu, L. Tong, H. J. Gilbert, J. LaBaer and D. L. Jarvis, *Nat. Chem. Biol.*, 2018, **14**, 156.

7 J. Phan, A. Zdanov, A. G. Evdokimov, J. E. Tropea, H. K. Peters, R. B. Kapust, M. Li, A. Wlodawer and D. S. Waugh, *J. Biol. Chem.*, 2002, **277**, 50564.

8 A. W. Barb, L. Meng, Z. Gao, R. W. Johnson, K. W. Moremen and J. H. Prestegard, *Biochemistry*, 2012, **51**, 4618.

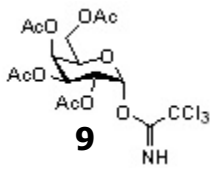
9 A. S. Vandersall-Nairn, R. K. Merkle, K. O'Brien, T. N. Oeltmann and K. W. Moremen, *Glycobiology*, 1998, **8**, 1183.

10 D. Beckett, E. Kovaleva and P. J. Schatz, *Protein Sci.*, 1999, **8**, 921.

11 J. D. Pedelacq, S. Cabantous, T. Tran, T. C. Terwilliger and G. S. Waldo, *Nat. Biotechnol.*, 2006, **24**, 79.

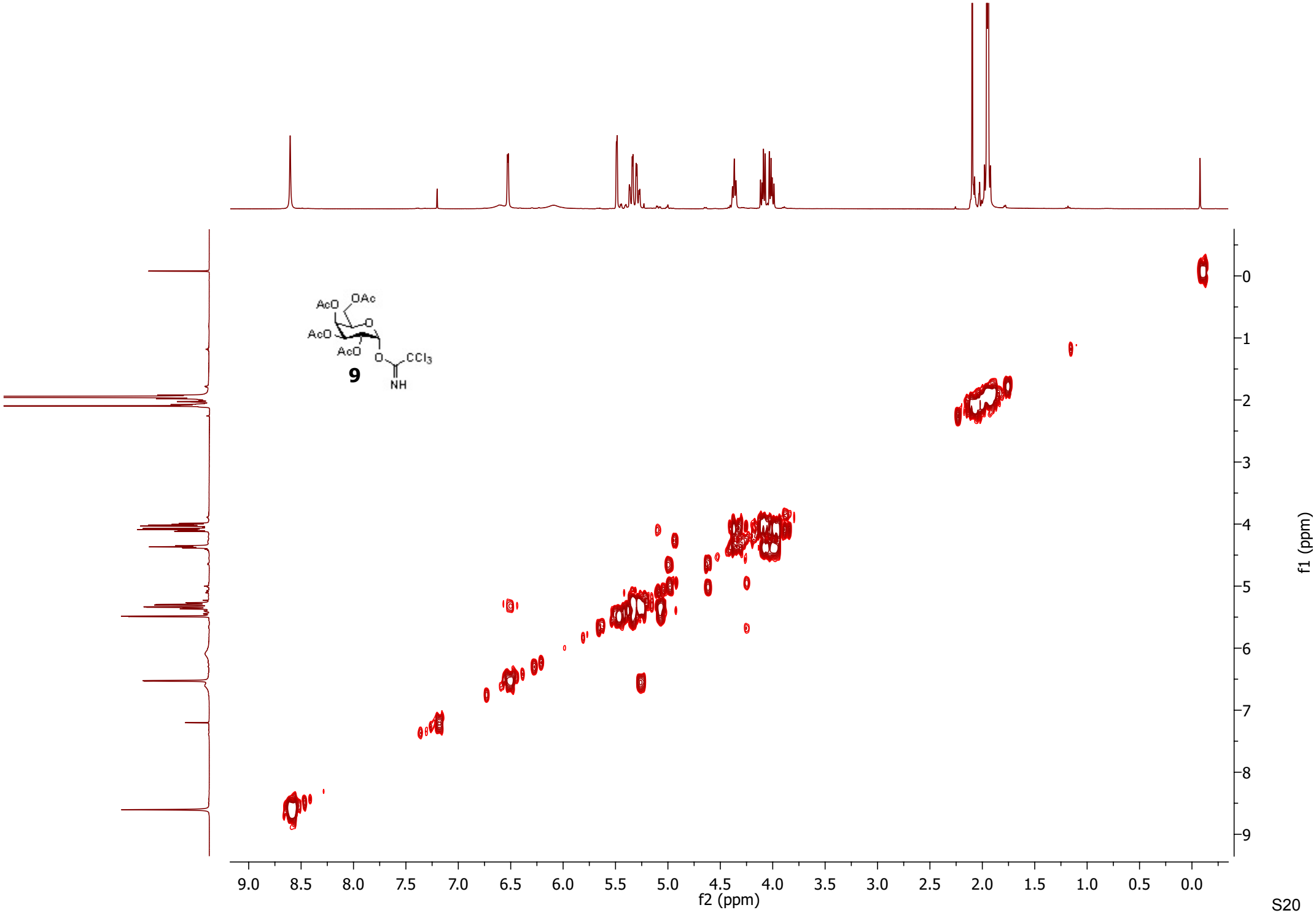
12 I. J. Goldstein, H. C. Winter and R. D. Poretz, in *New Comprehensive Biochemistry*, Elsevier, 1997, vol. 29, Part B, pp. 403.

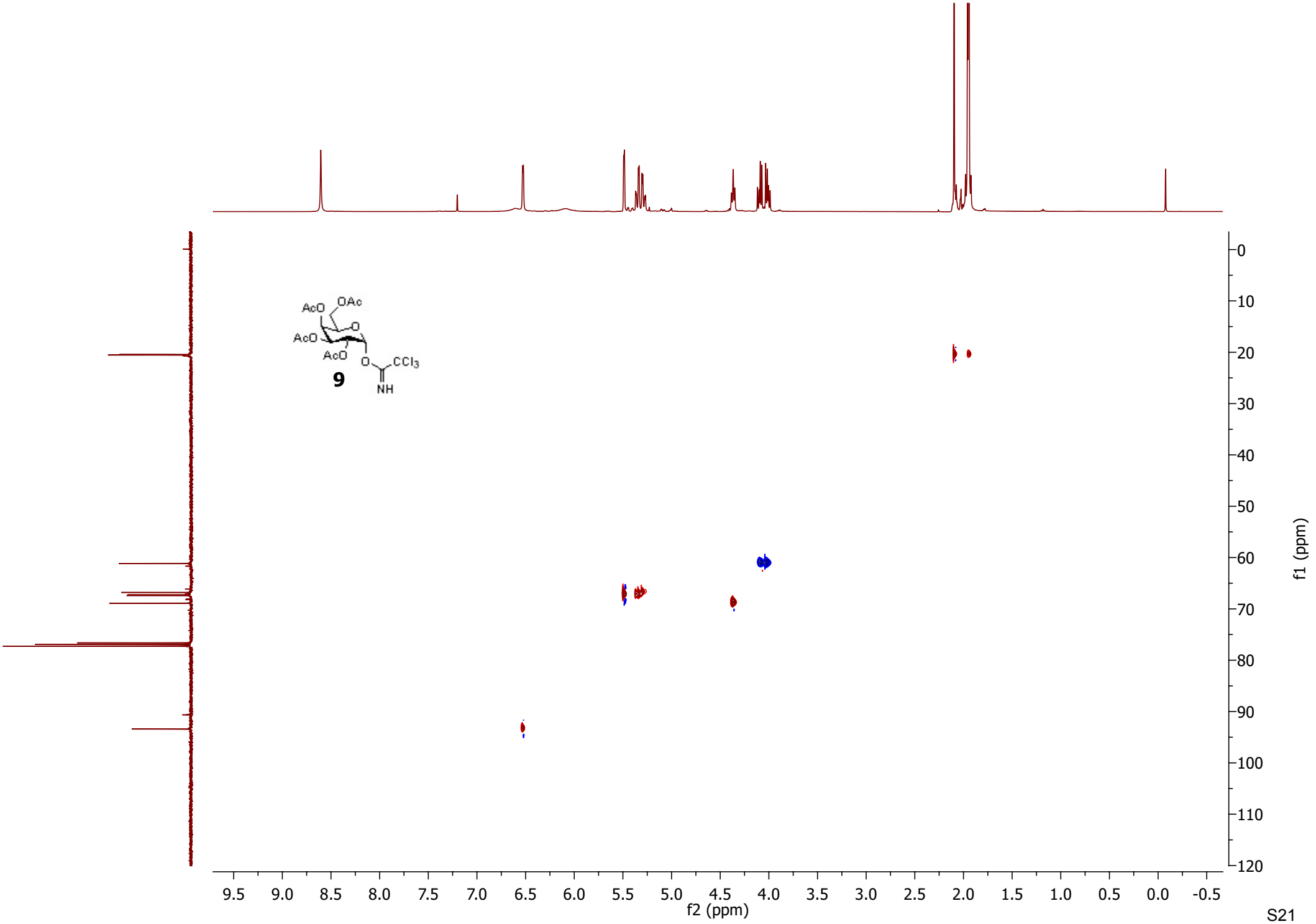
13 V. S. R. Rao, K. Lam and P. K. Qasba, *J. Biomol. Struct. Dyn.*, 1998, **15**, 853.

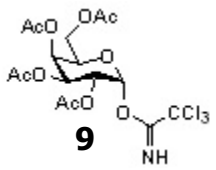

AcO
OAc
O
AcO
AcO
O
CCl3
NH

9

2300
2200
2100
2000
1900
1800
1700
1600
1500
1400
1300
1200
1100
1000
900
800
700
600
500
400
300
200
100
0
-100
-200
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

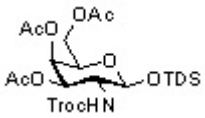

AcO
OAc
AcO
AcO
O
O
CCl3
NH
9
f2 (ppm)
f1 (ppm)


AcO
OAc
AcO
AcO
O
O
CCl3
NH
9
f2 (ppm)
f1 (ppm)
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
0 10 20 30 40 50 60 70 80 90 100 110 120


AcO
OAc
O
AcO
AcO
O
CCl3
NH

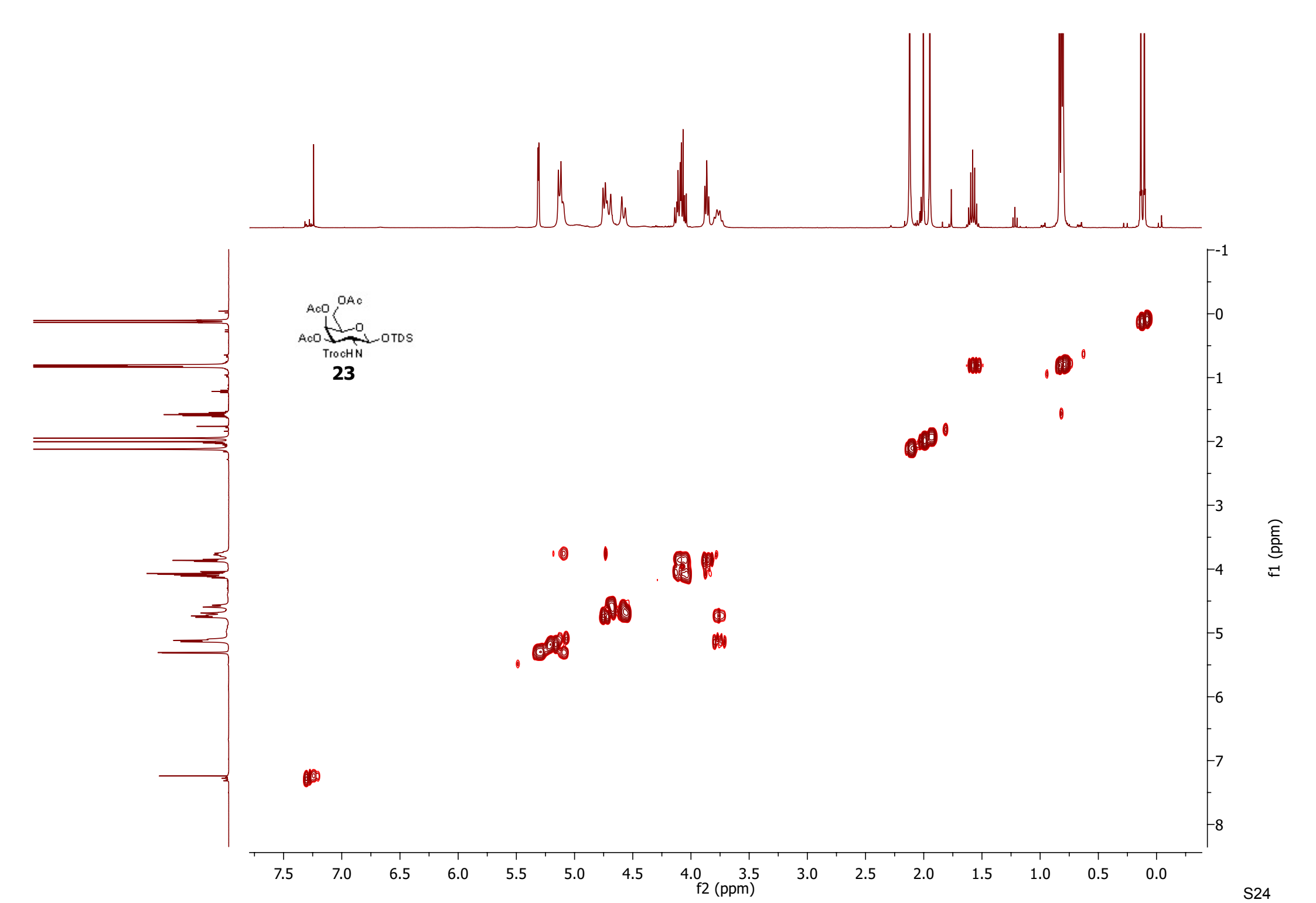
9

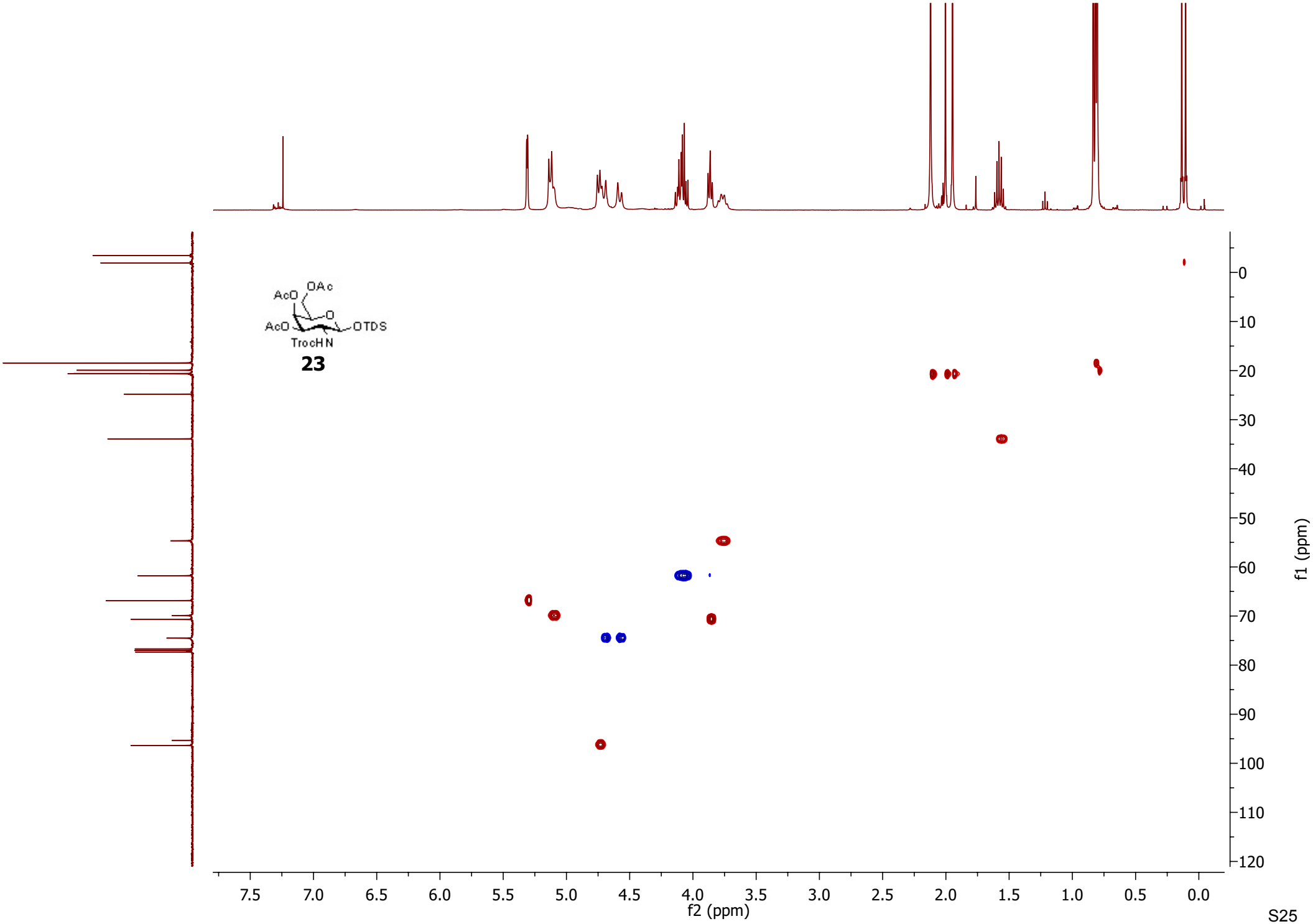
100
90
80
70
60
50
40
30
20
10
0
-10
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

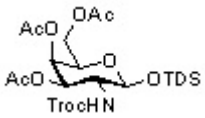

AcO
OAc
O
AcO
OTDS
TrocHN

23

1800
1700
1600
1500
1400
1300
1200
1100
1000
900
800
700
600
500
400
300
200
100
0
-100
11.0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)

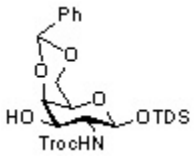

AcO
OAc
AcO
O
OTDS
TrocHN
23
f2 (ppm)
f1 (ppm)


AcO
OAc
O
AcO
OTDS
TrocHN
23
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f2 (ppm)
0
10
20
30
40
50
60
70
80
90
100
110
120
f1 (ppm)


AcO
OAc
O
AcO
OTDS
TrocHN

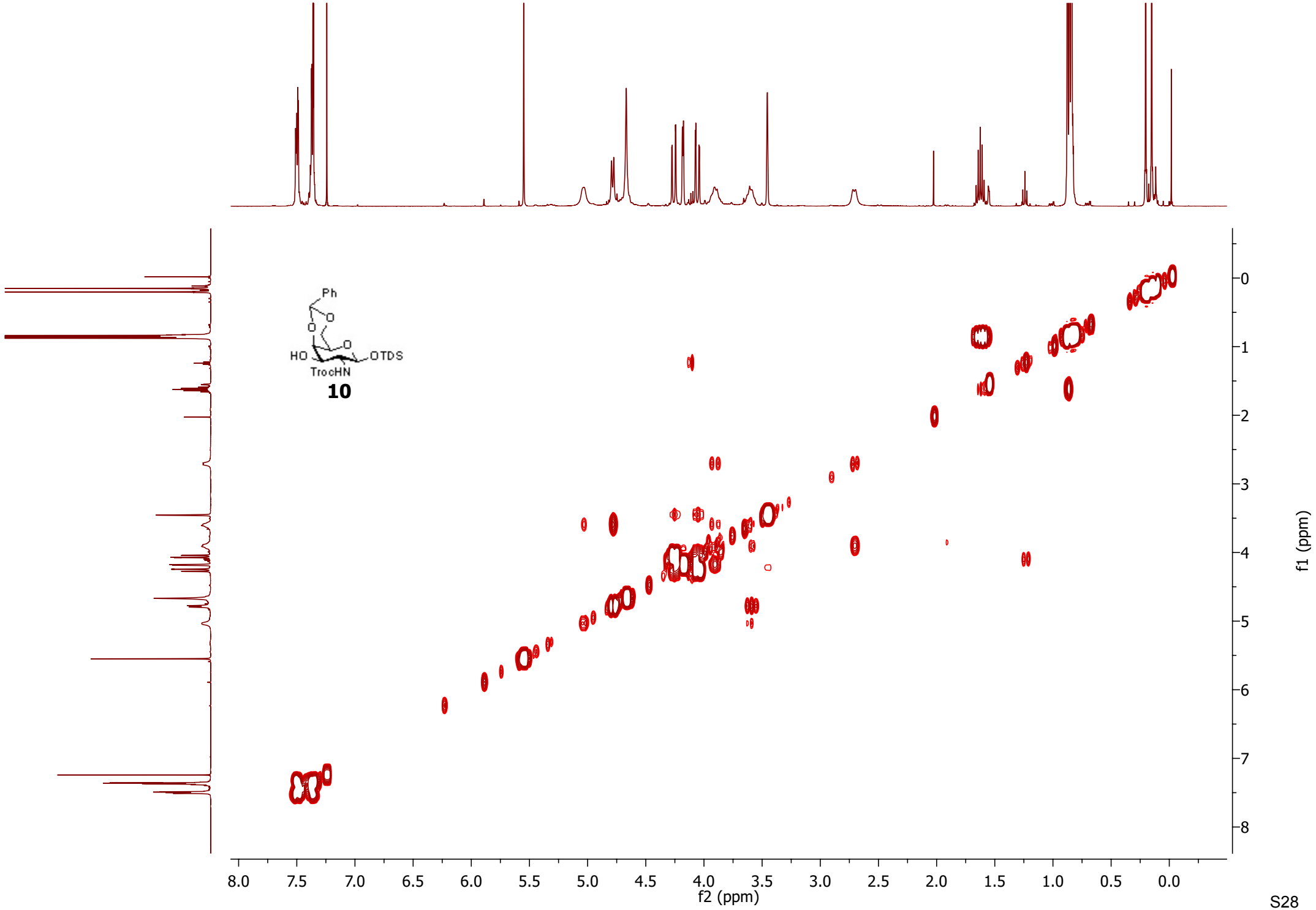
23

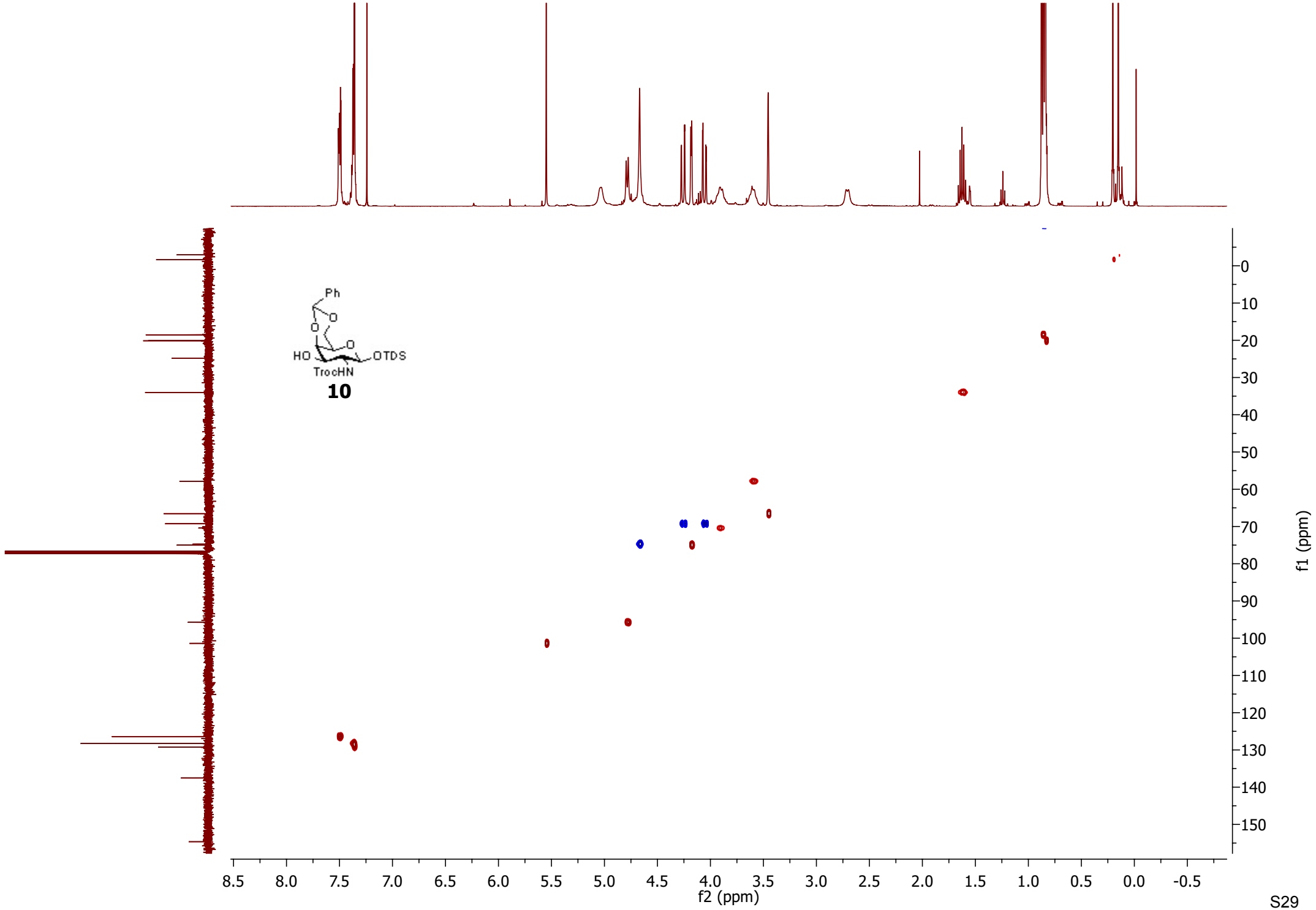
80
75
70
65
60
55
50
45
40
35
30
25
20
15
10
5
0
-5
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)

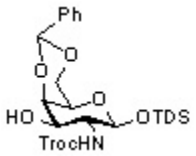

Ph
O
O
O
HO
OTDS
TrocHN

10

1700
1600
1500
1400
1300
1200
1100
1000
900
800
700
600
500
400
300
200
100
0
-100
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

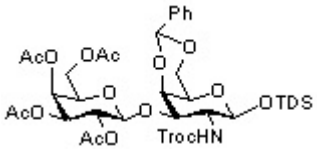

Ph
O
O
O
HO
OTDS
TrocHN
10
f2 (ppm)
f1 (ppm)


Ph
O
O
O
HO
OTDS
TrocHN
10
f2 (ppm)
f1 (ppm)
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150


Ph
O
O
O
HO
OTDS
TrocHN

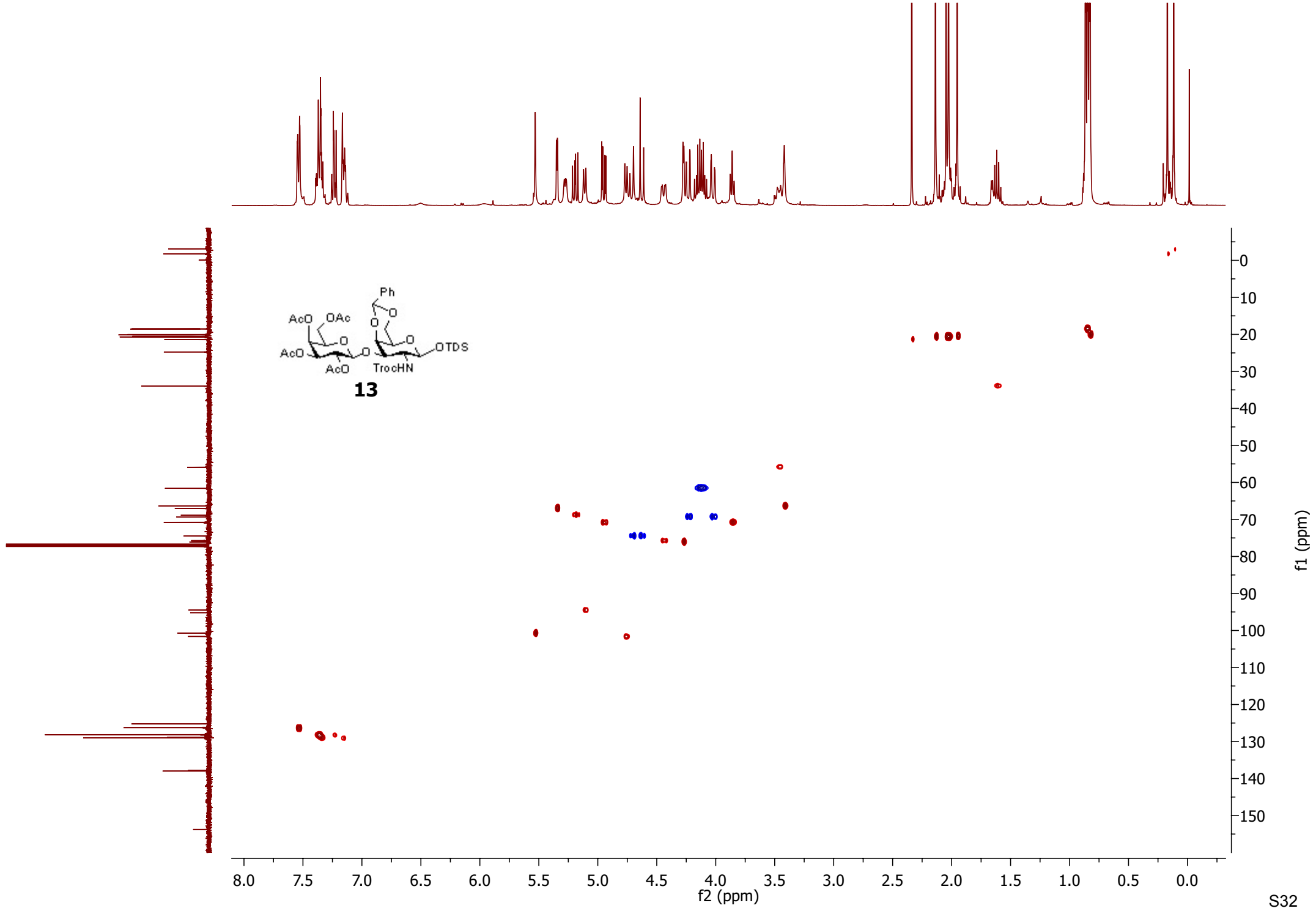
10

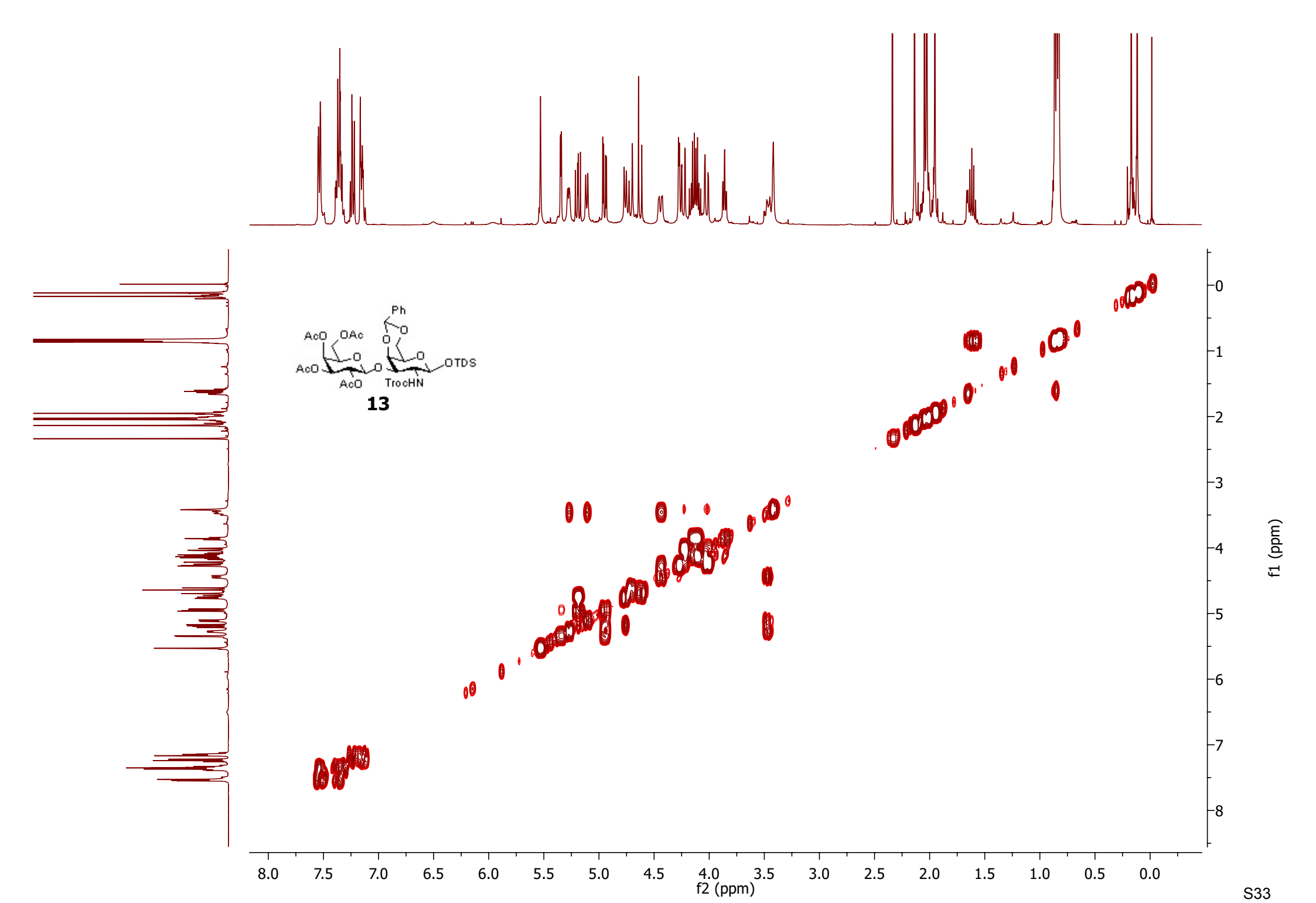
30
28
26
24
22
20
18
16
14
12
10
8
6
4
2
0
-2
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)

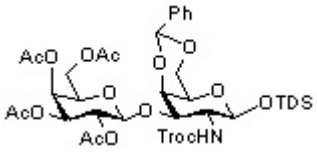

Ph
AcO
OAc
AcO
AcO
TrocHN
OTDS

13

1200
1100
1000
900
800
700
600
500
400
300
200
100
0
-100
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)

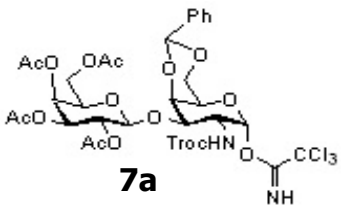

Ph
AcO
OAc
AcO
AcO
TrocHN
OTDS
13
f2 (ppm)
f1 (ppm)


Ph
AcO
OAc
AcO
AcO
TrocHN
OTDS
13
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f2 (ppm)
0
1
2
3
4
5
6
7
8
f1 (ppm)


Ph
AcO
OAc
AcO
AcO
TrocHN
OTDS

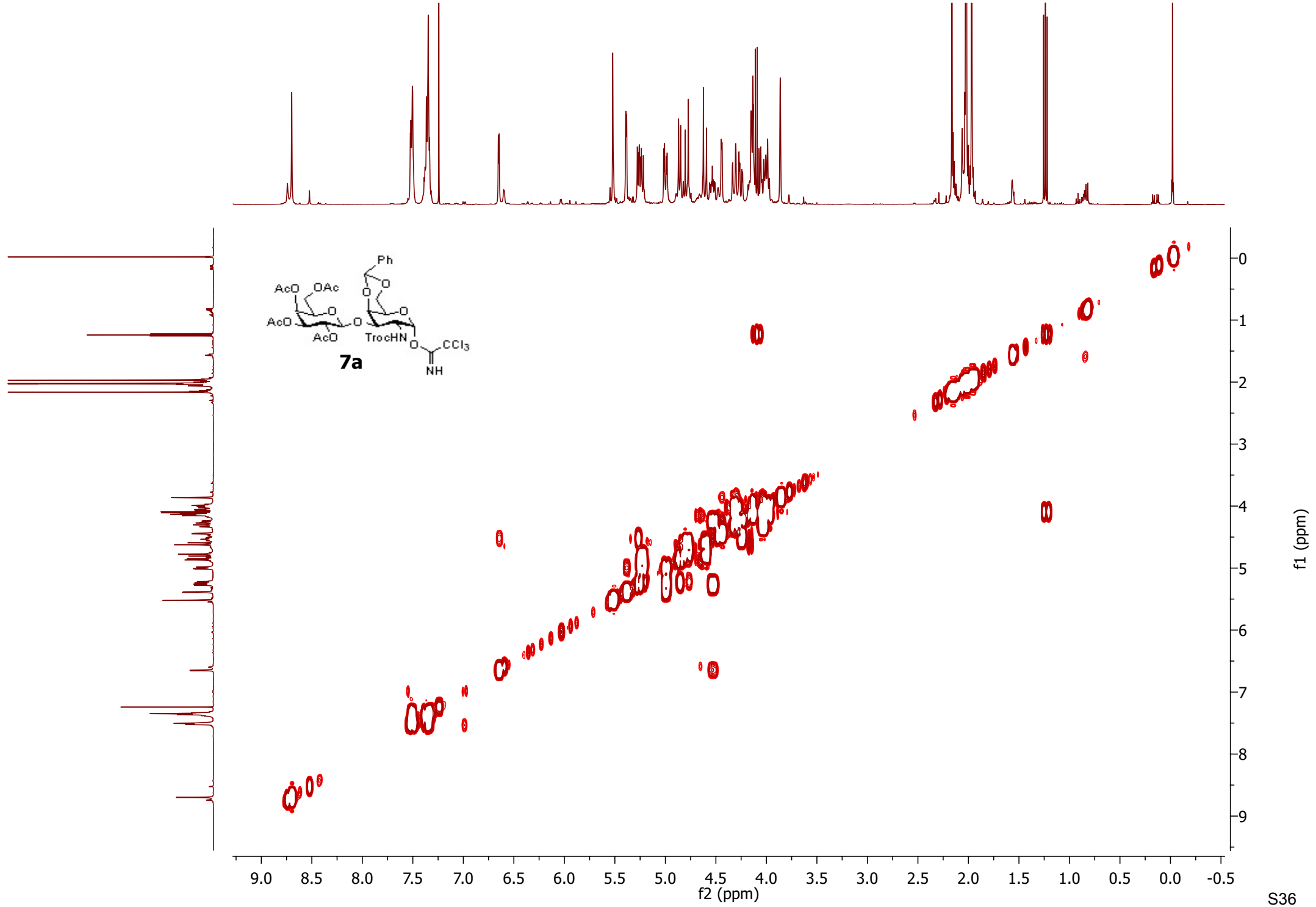
13

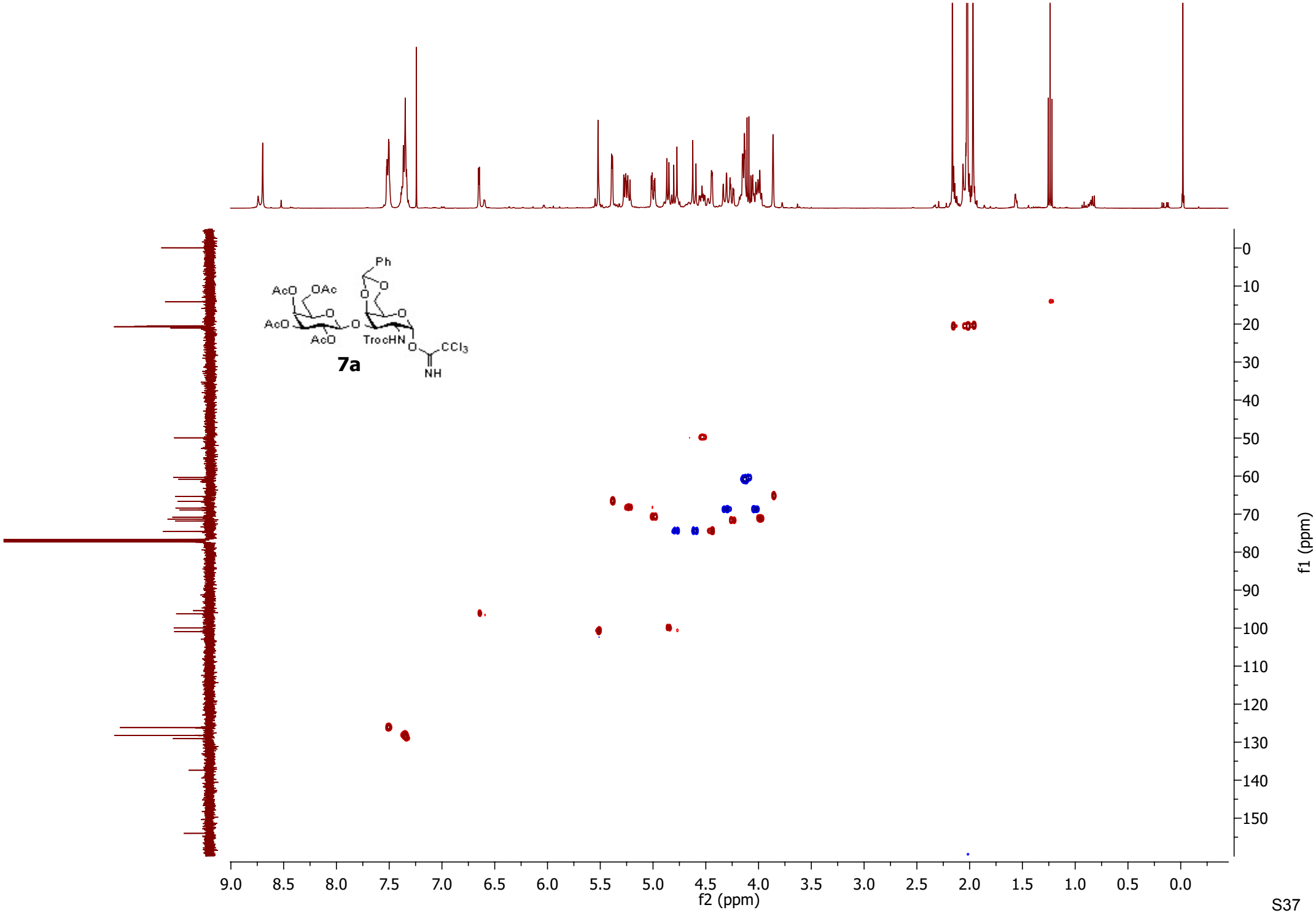
35
30
25
20
15
10
5
0
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)

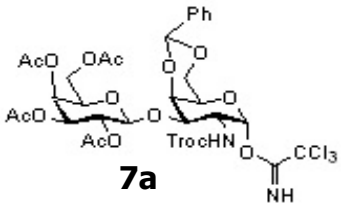

Ph
AcO
OAc
AcO
AcO
TrocHN
CCl3
NH

7a

3200
3000
2800
2600
2400
2200
2000
1800
1600
1400
1200
1000
800
600
400
200
0
-200
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

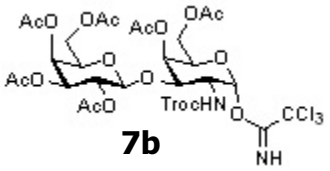

Ph
AcO
OAc
AcO
AcO
TrocHN
CCl3
NH
7a
f2 (ppm)
f1 (ppm)


Ph
AcO
OAc
AcO
AcO
TrocHN
CCl3
NH
7a
f2 (ppm)
f1 (ppm)


Ph
AcO
OAc
AcO
AcO
TrocHN
CCl3
NH

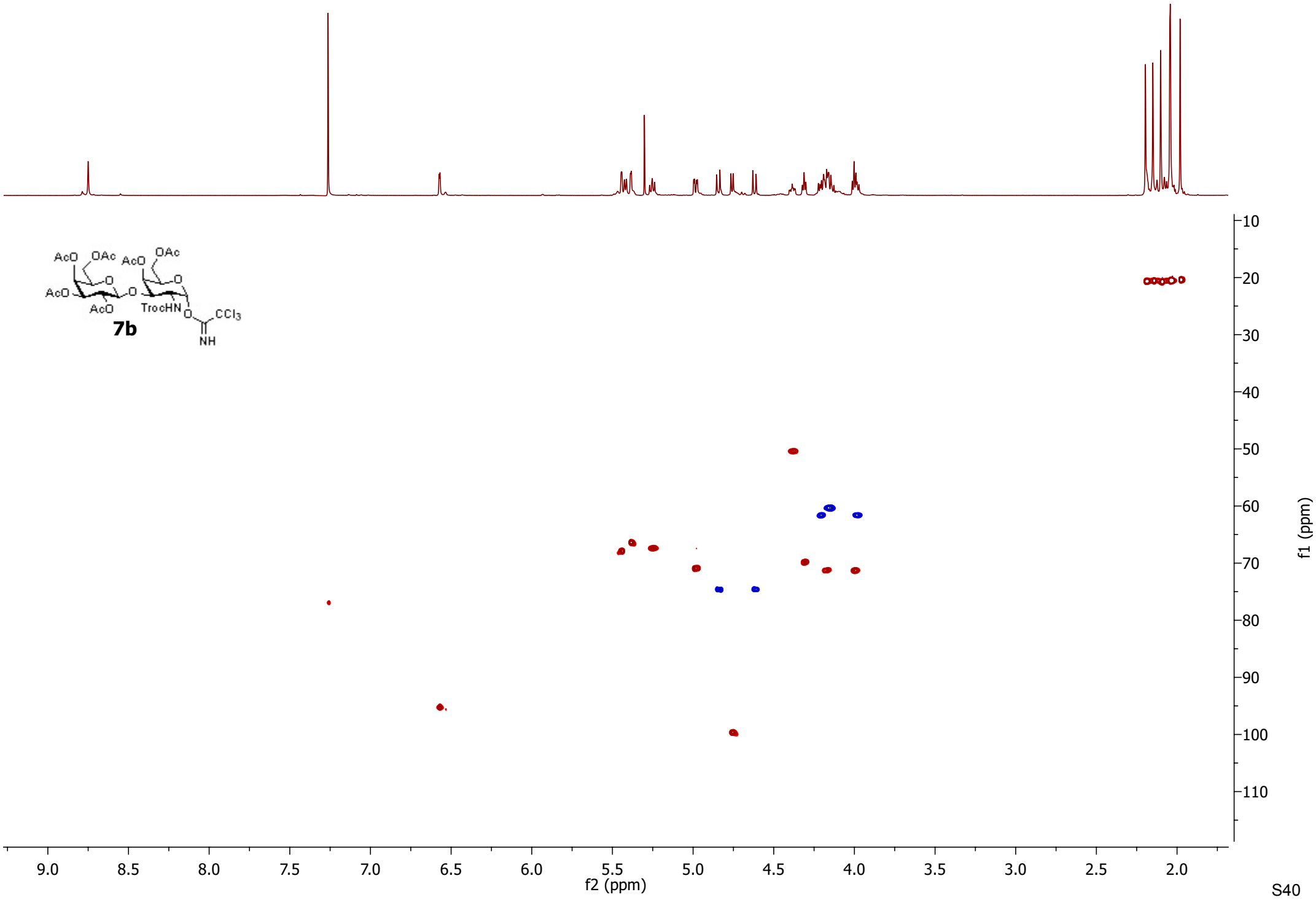
7a

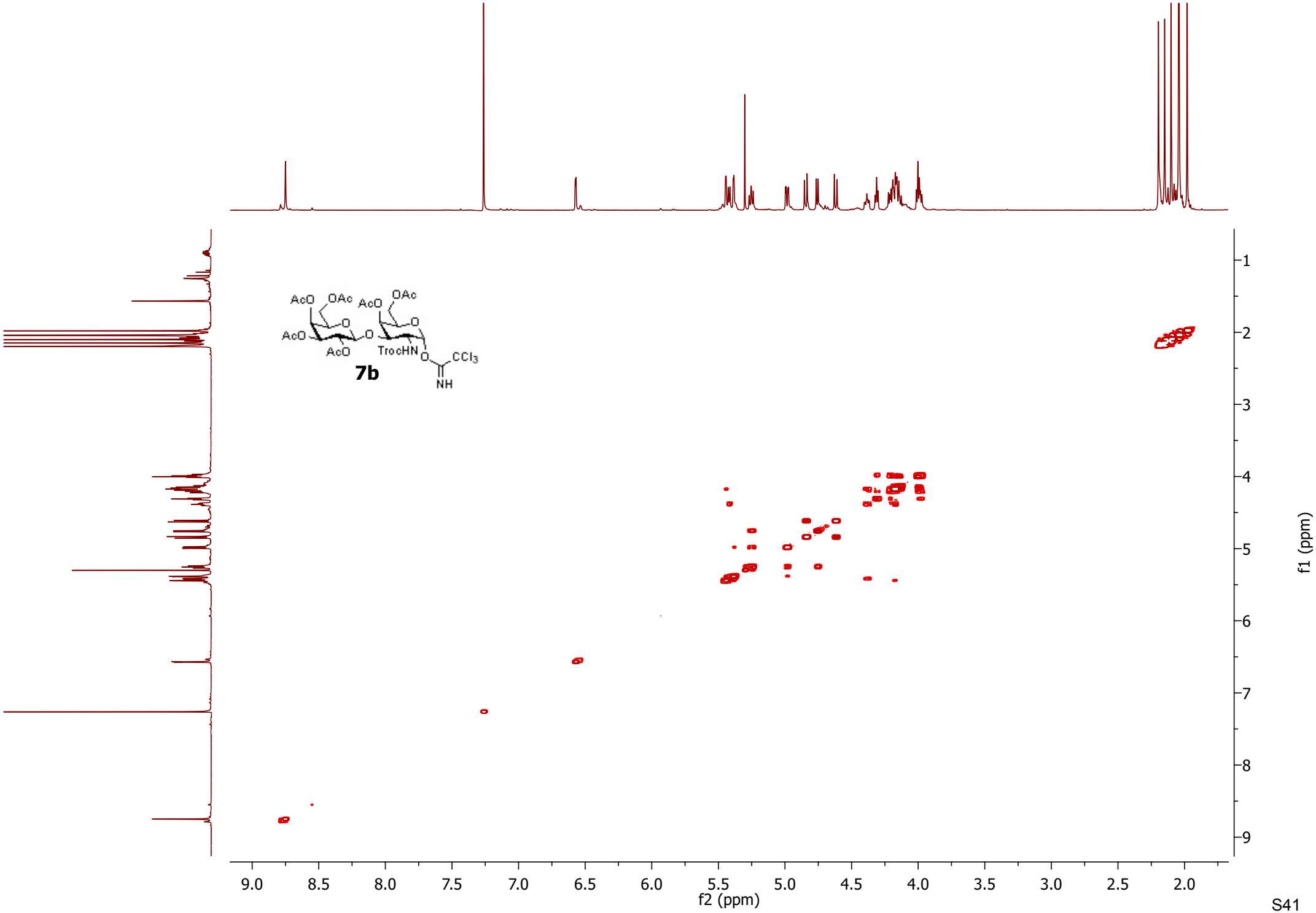
35
30
25
20
15
10
5
0
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)

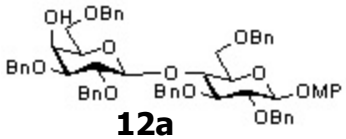

AcO
OAc
AcO
OAc
AcO
AcO
TrocHN
O
CCl3
NH

7b

4E+08
4E+08
3E+08
2E+08
2E+08
2E+08
1E+08
5E+07
0
8.8
8.6
8.4
8.2
8.0
7.8
7.6
7.4
7.2
7.0
6.8
6.6
6.4
6.2
6.0
5.8
5.6
5.4
5.2
5.0
4.8
4.6
4.4
4.2
4.0
3.8
3.6
3.4
f1 (ppm)

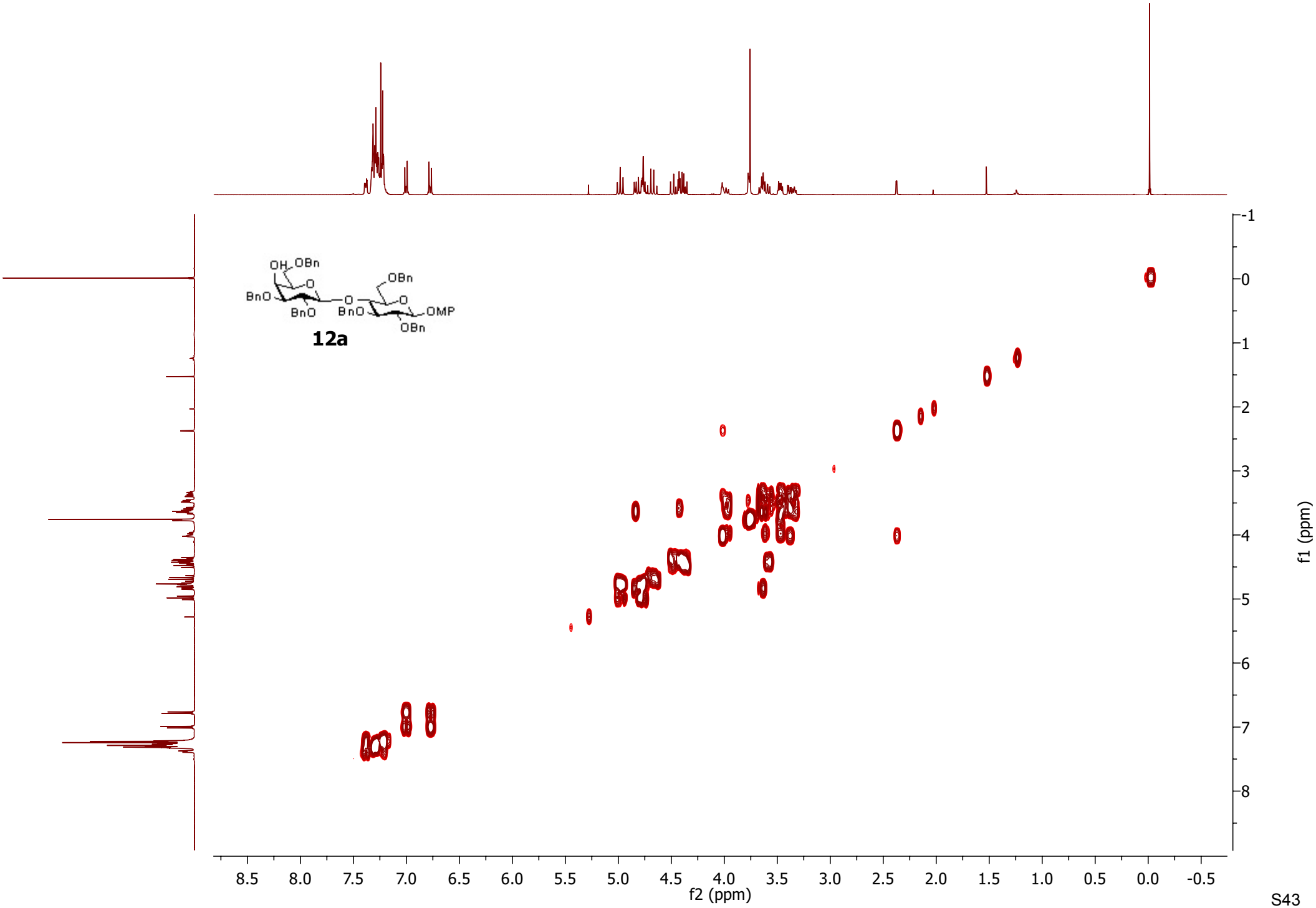

AcO
OAc
AcO
OAc
AcO
AcO
TrocHN
O
CCl3
NH
7b
10
20
30
40
50
60
70
80
90
100
110
f1 (ppm)
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
f2 (ppm)

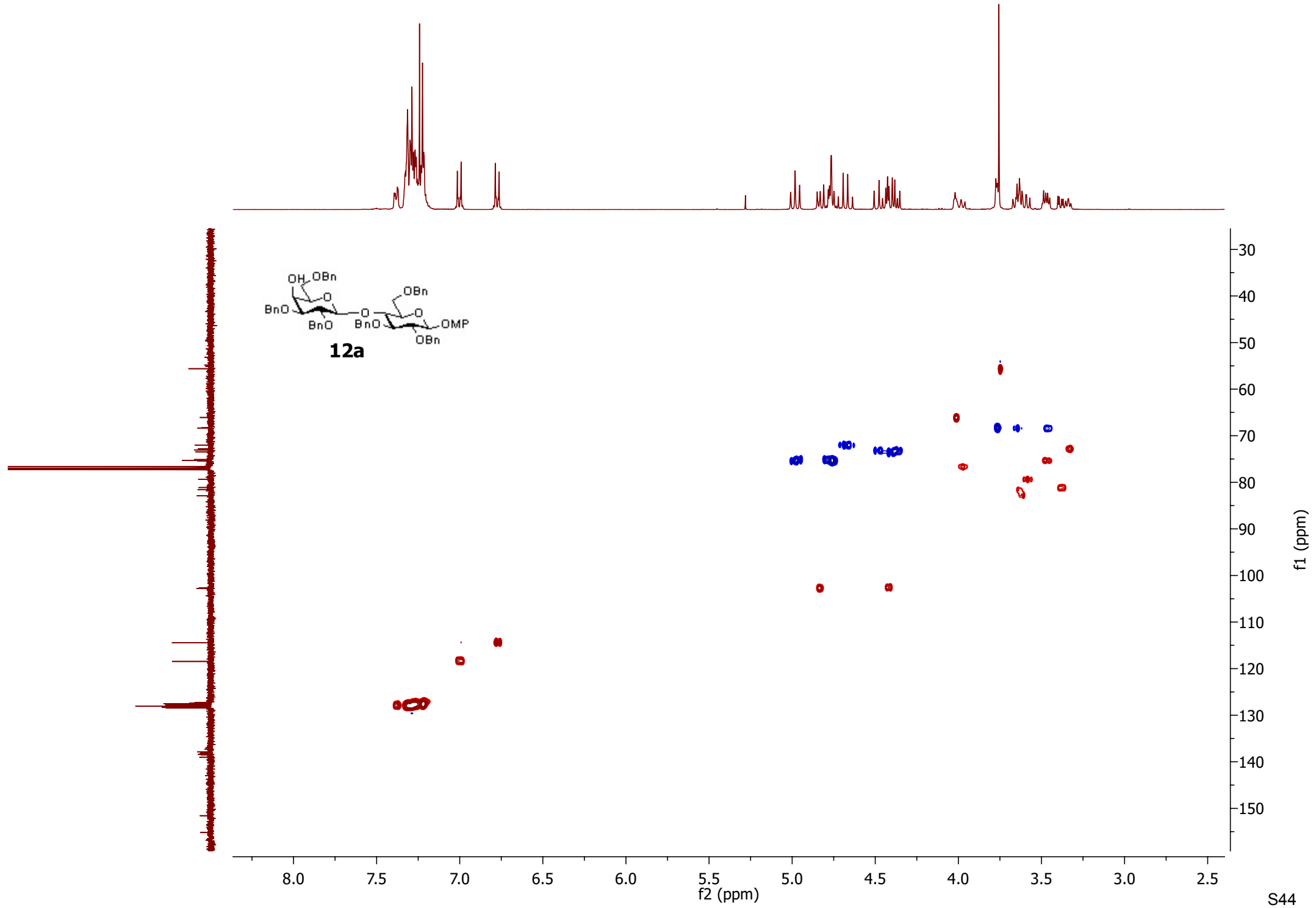

AcO
OAc
AcO
OAc
AcO
O
O
AcO
TrocHN
O
CCl3
NH
7b
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
f2 (ppm)
1
2
3
4
5
6
7
8
9
f1 (ppm)

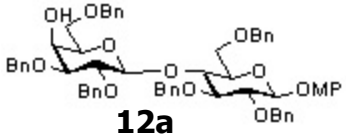

OH
OBn
O
BnO
BnO
O
OBn
O
BnO
OMP
OBn

12a

2200
2100
2000
1900
1800
1700
1600
1500
1400
1300
1200
1100
1000
900
800
700
600
500
400
300
200
100
0
-100
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)

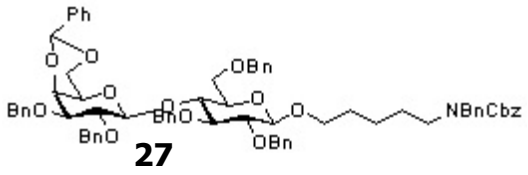

OH
OBn
BnO
O
BnO
OBn
O
BnO
OMP
OBn
12a
f2 (ppm)
f1 (ppm)
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
-1
0
1
2
3
4
5
6
7
8


OH
OBn
BnO
O
BnO
OBn
O
BnO
OMP
OBn
12a
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
f2 (ppm)
30
40
50
60
70
80
90
100
110
120
130
140
150
f1 (ppm)


OH
OBn
O
BnO
BnO
O
OBn
O
BnO
OMP
OBn

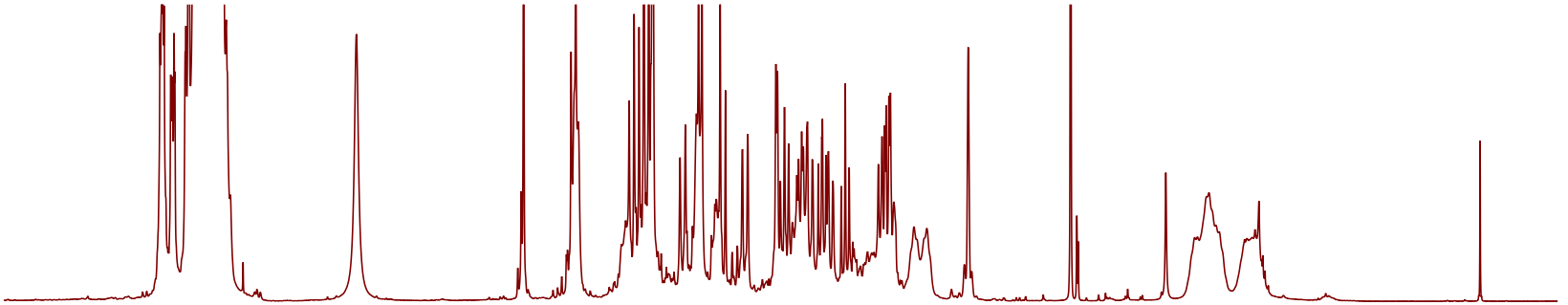
12a

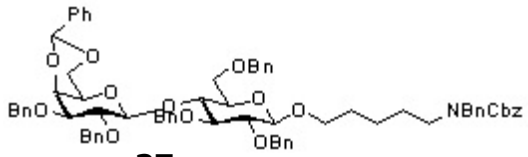
18
17
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
-1
-2
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)


Ph
OBn
BnO
BnO
BnO
OBn
NBnCbz

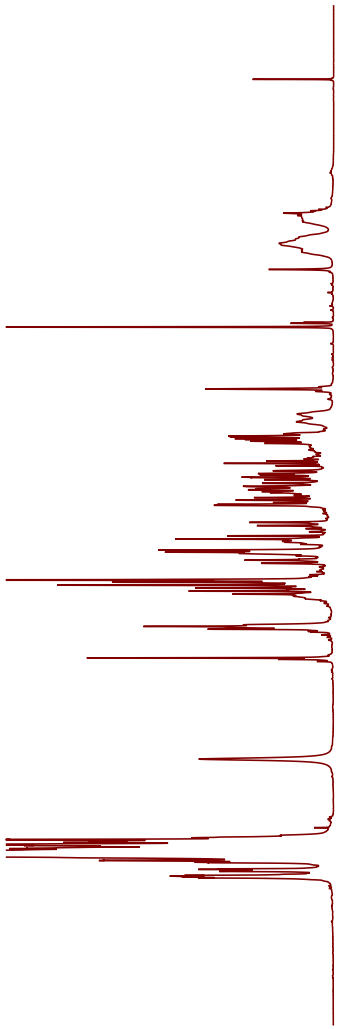
27

1500
1400
1300
1200
1100
1000
900
800
700
600
500
400
300
200
100
0
-100
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

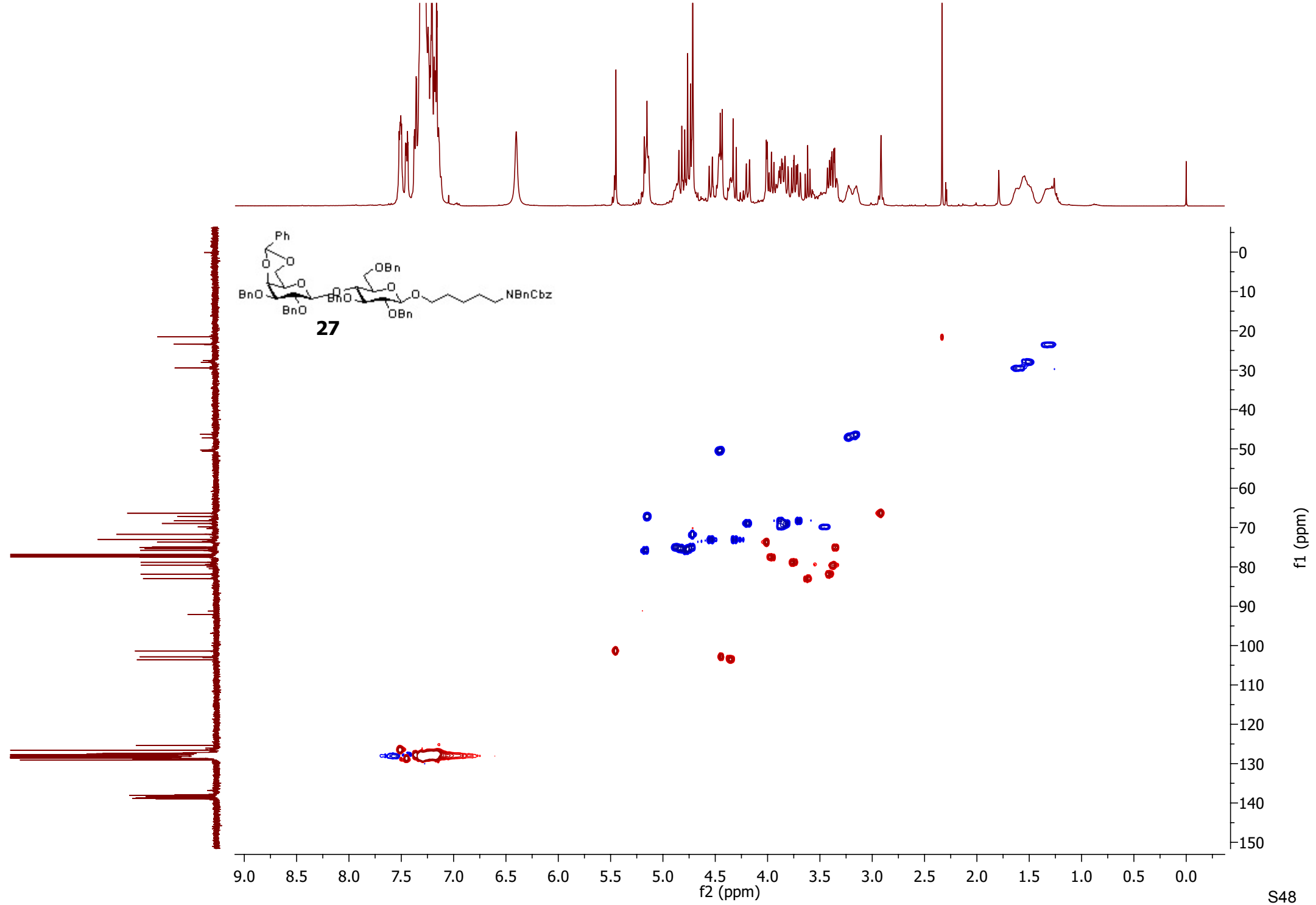


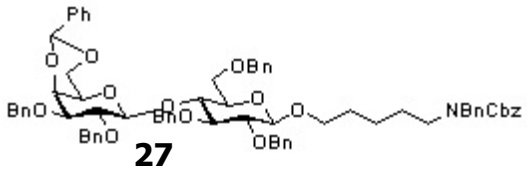

Ph
O
O
O
BnO
BnO
O
BnO
OBn
O
O
NBnCbz
OBn

27

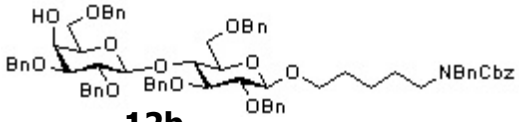


0
1
2
3
4
5
6
7
8
f1 (ppm)
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f2 (ppm)


Ph
OBn
BnO
BnO
BnO
OBn
NBnCbz
27
f2 (ppm)
f1 (ppm)

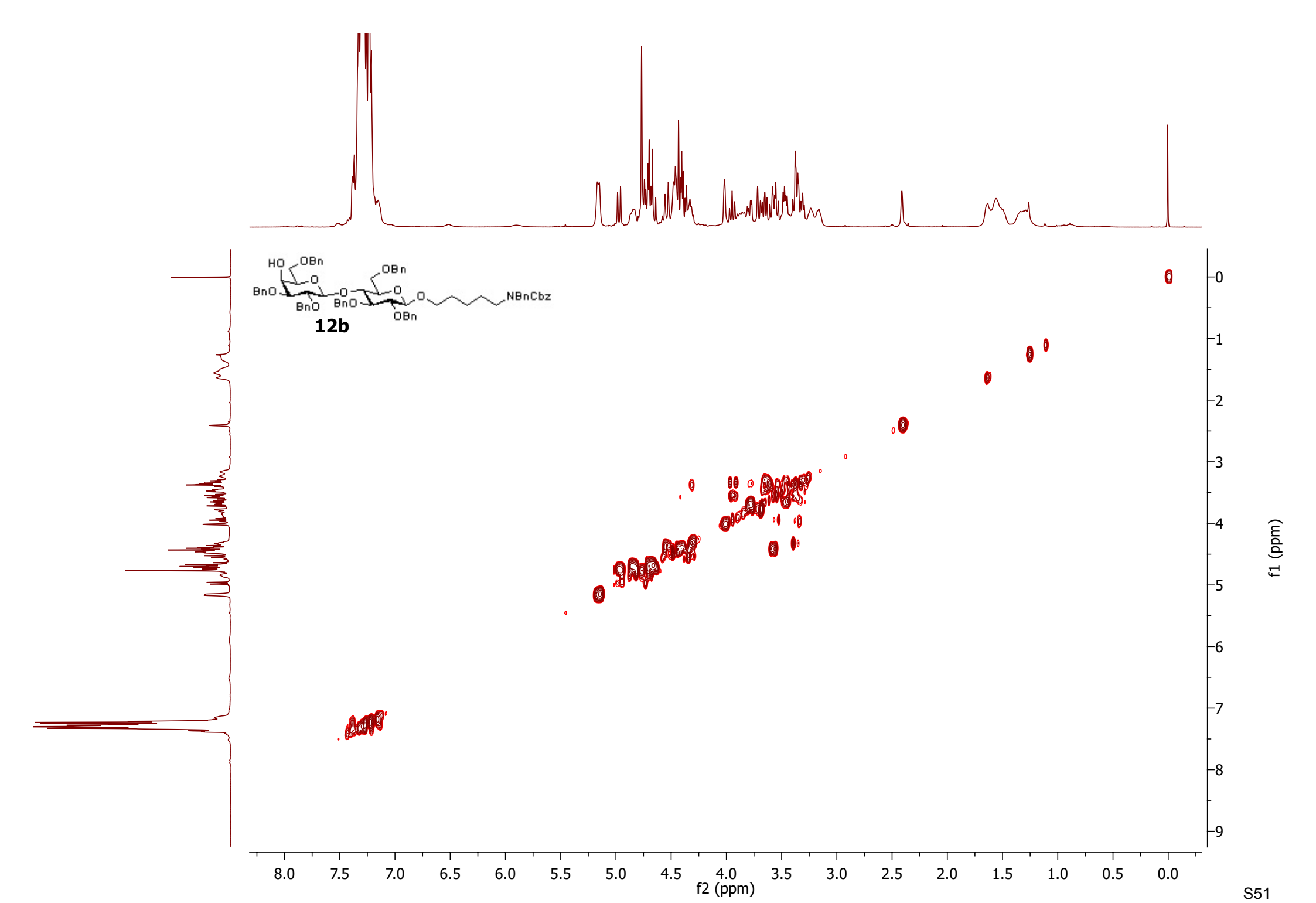


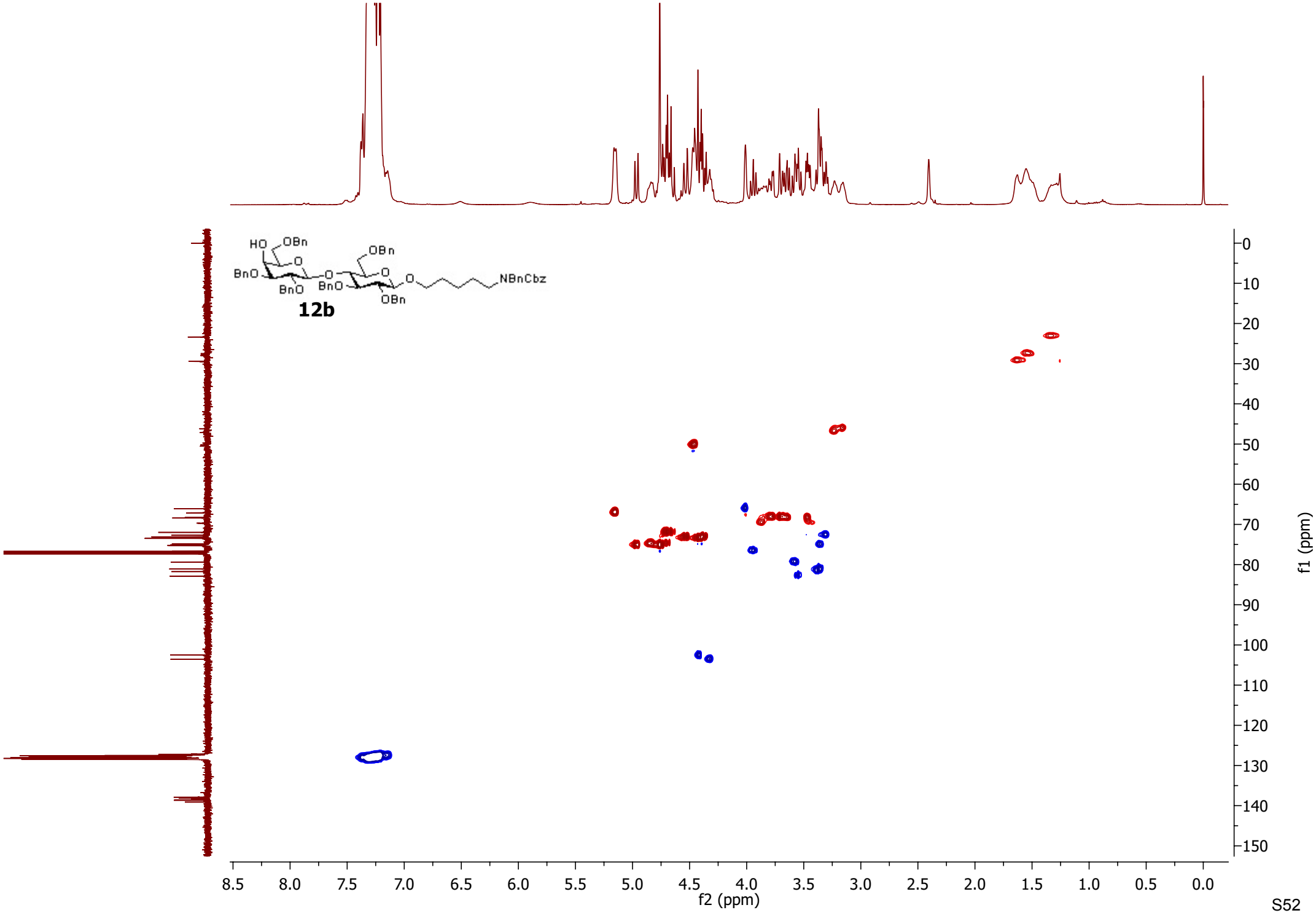
27

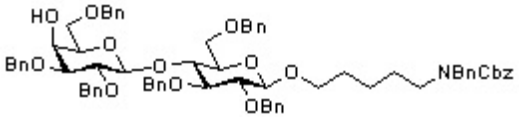

HO
OBn
O
BnO
O
OBn
O
O
NBnCbz
BnO
BnO
OBn
OBn

12b

1500
1400
1300
1200
1100
1000
900
800
700
600
500
400
300
200
100
0
-100
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

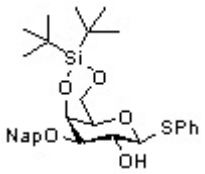

HO
OBn
BnO
BnO
BnO
OBn
OBn
NBnCbz
12b
f2 (ppm)
f1 (ppm)


HO
OBn
BnO
BnO
BnO
OBn
OBn
NBnCbz
12b
f2 (ppm)
f1 (ppm)


HO
OBn
O
BnO
BnO
O
OBn
O
BnO
O
NBnCbz
OBn

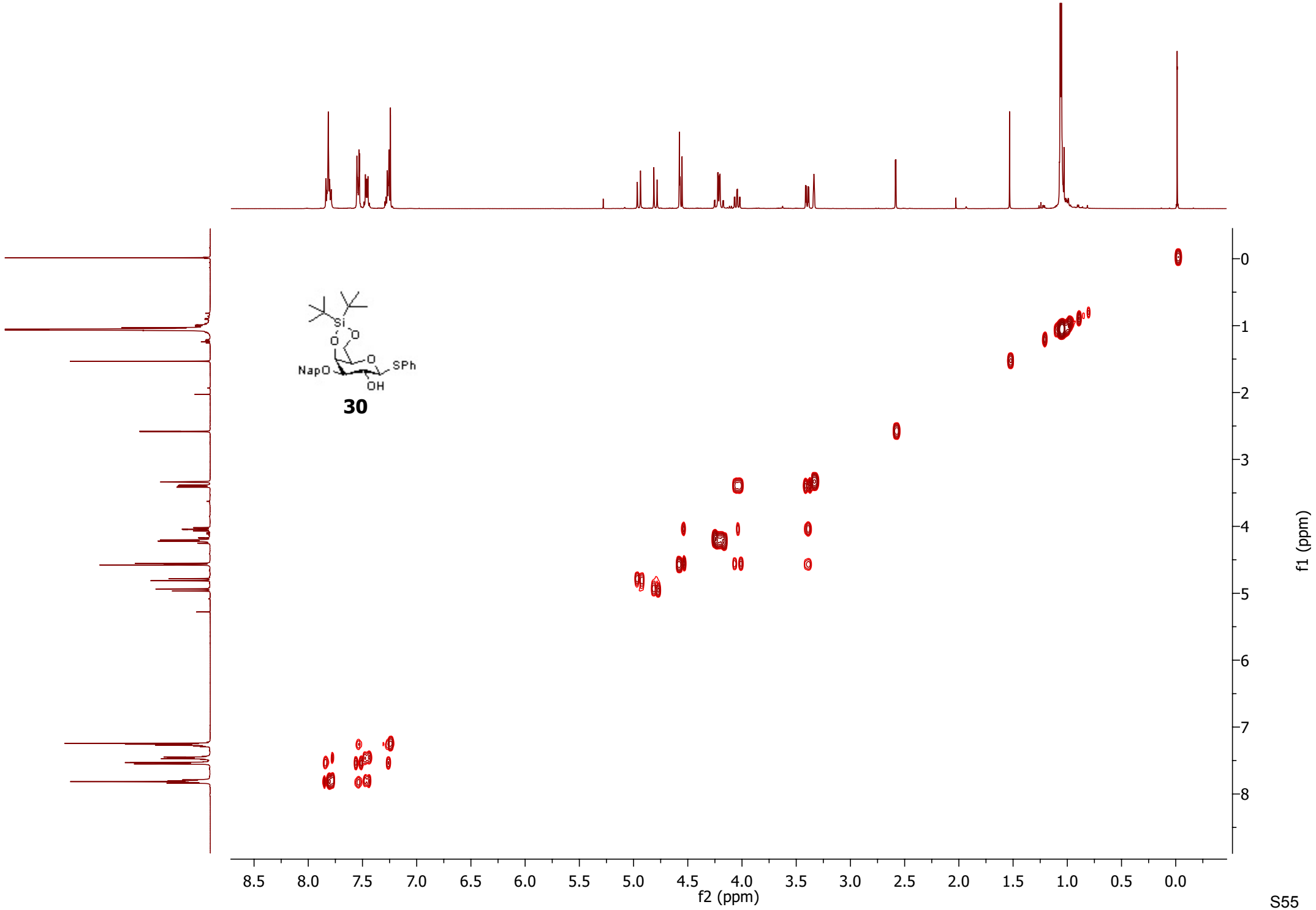
12b

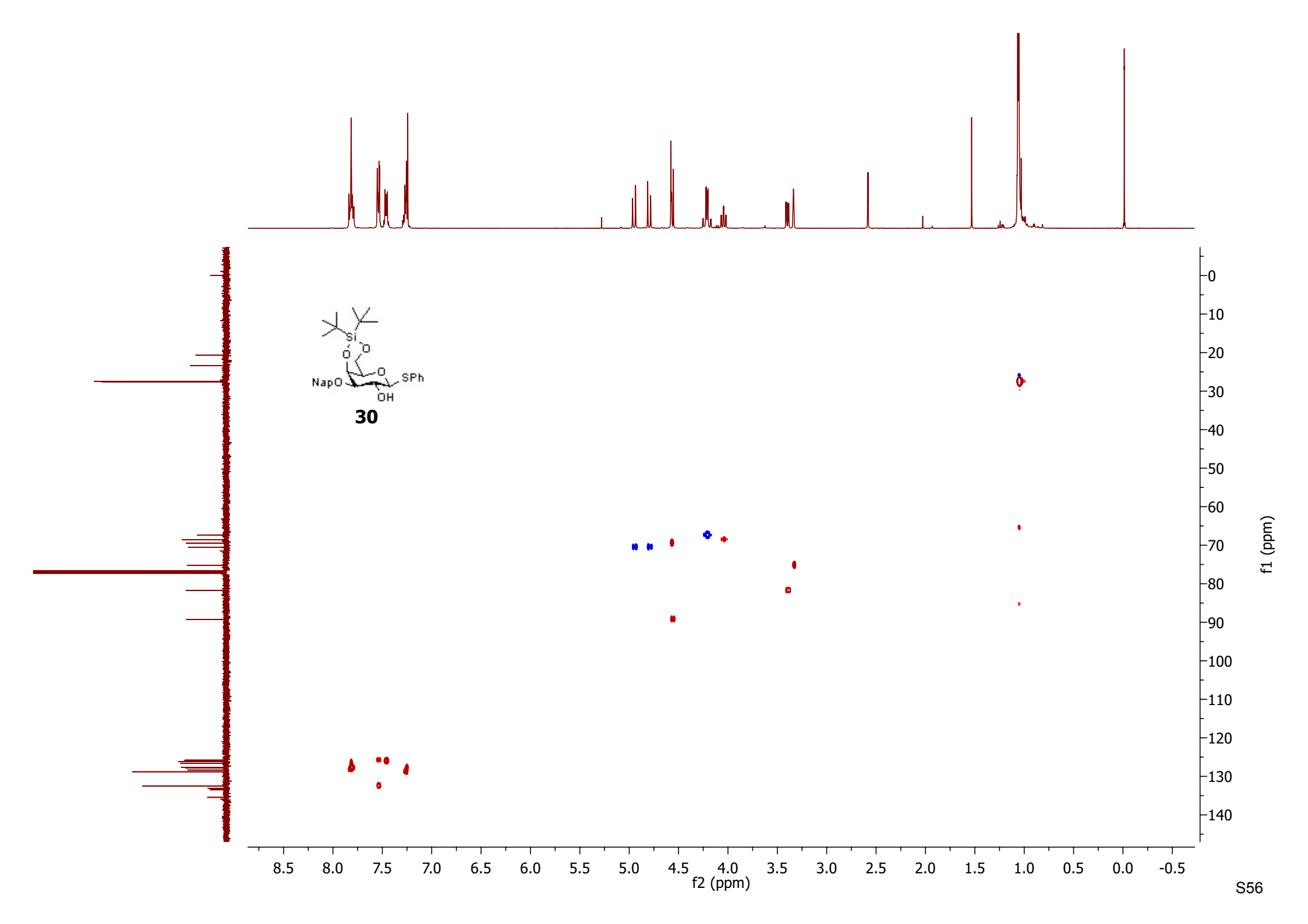
45
40
35
30
25
20
15
10
5
0
-5
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

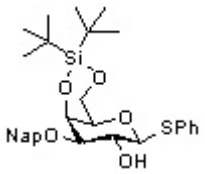

Si
O
O
O
NapO
SPh
OH

30

10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

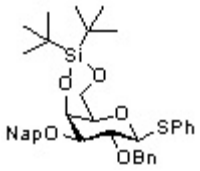

Si
O
O
O
O
NapO
SPh
OH
30
f2 (ppm)
f1 (ppm)


Si
O
O
O
NapO
SPh
OH
30
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f2 (ppm)
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
f1 (ppm)


Si
O
O
O
NapO
SPh
OH

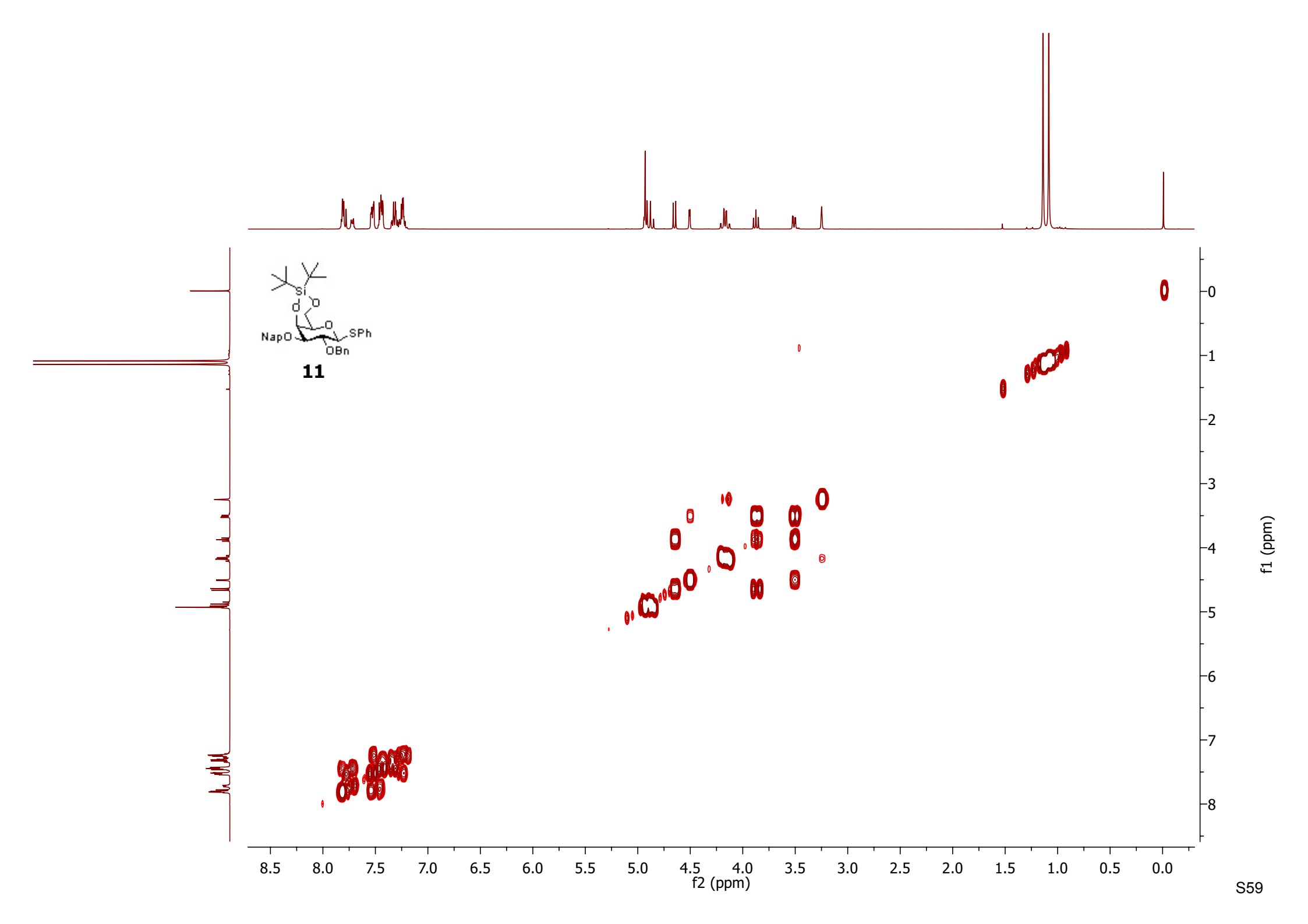
30

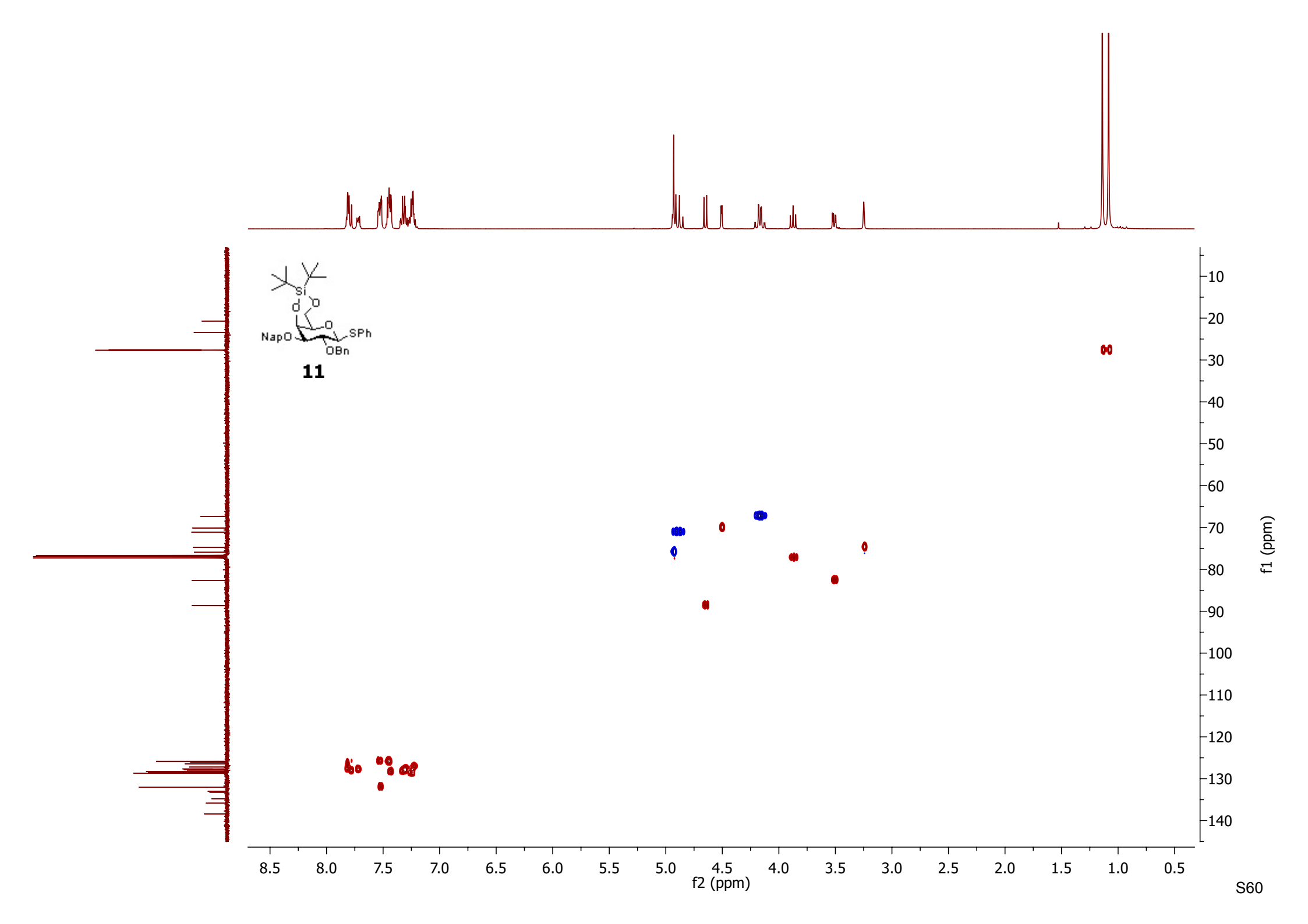
32
30
28
26
24
22
20
18
16
14
12
10
8
6
4
2
0
-2
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

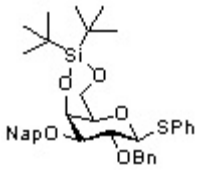

Si
O
O
O
NapO
SPh
OBn

11

4500
4000
3500
3000
2500
2000
1500
1000
500
0
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

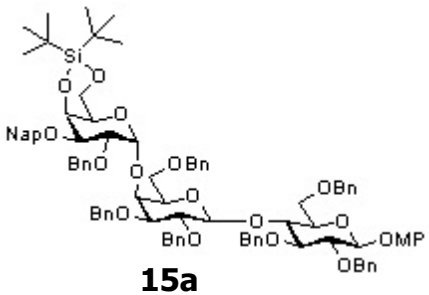

NapO
SPh
OBn
11
f2 (ppm)
f1 (ppm)


NapO
SPh
OBn
11
f2 (ppm)
f1 (ppm)


Si
O
O
O
NapO
SPh
OBn

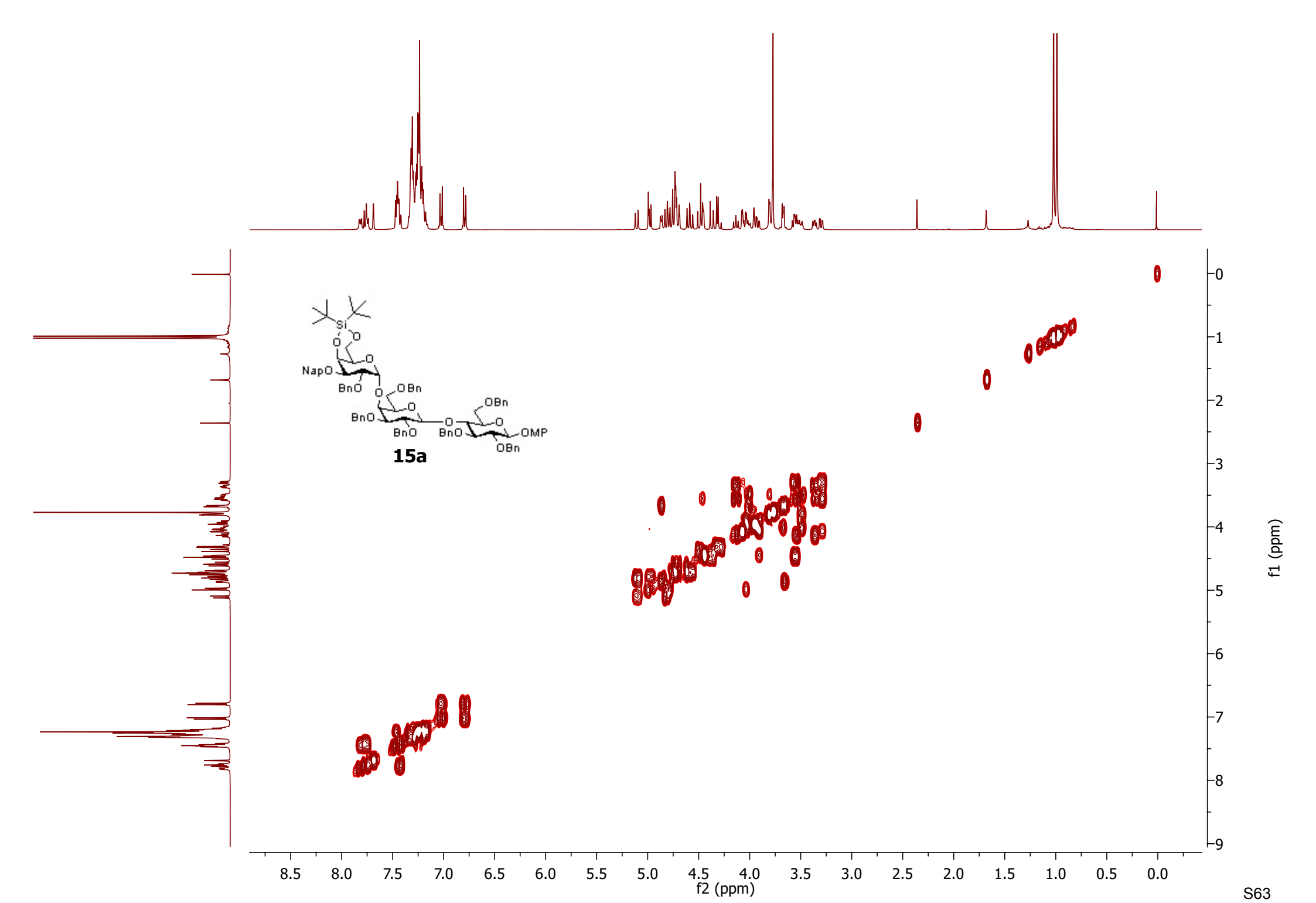
11

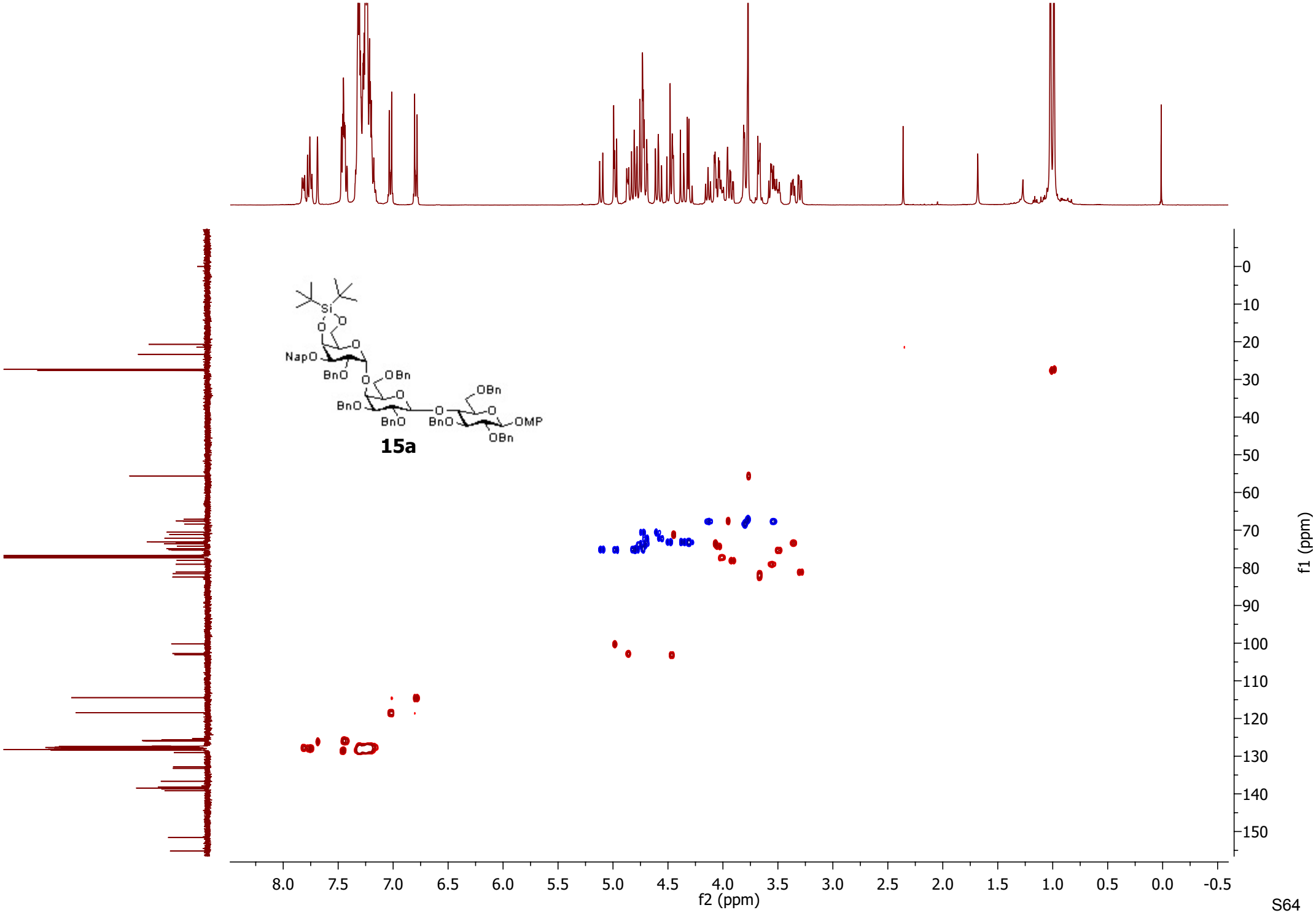
40
35
30
25
20
15
10
5
0
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)

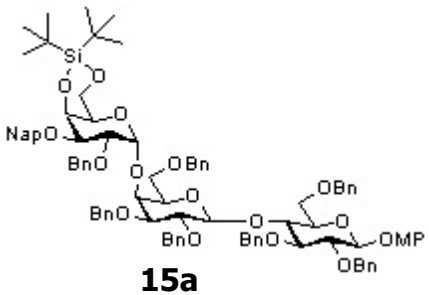

Si
O
O
O
O
NapO
BnO
O
OBn
O
BnO
BnO
O
OBn
BnO
O
OMP
OBn

15a

1300
1200
1100
1000
900
800
700
600
500
400
300
200
100
0
-100
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

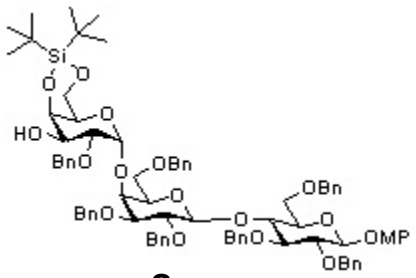

NapO
BnO
OBn
BnO
BnO
OBn
BnO
OMP
OBn
15a
f2 (ppm)
f1 (ppm)


15a
f2 (ppm)
f1 (ppm)


Si
O
O
O
O
NapO
BnO
O
OBn
O
BnO
BnO
O
OBn
BnO
OMP
OBn

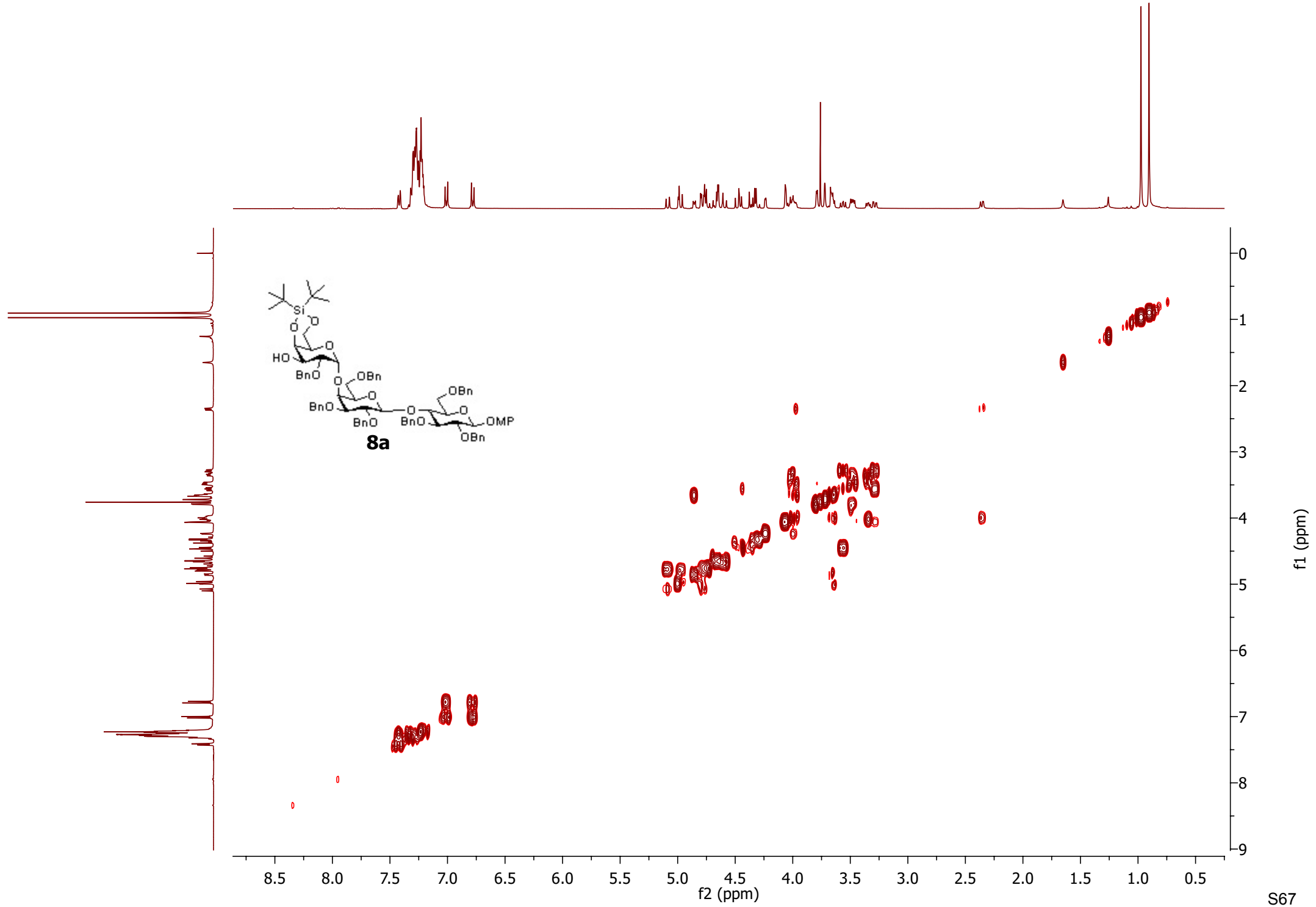
15a

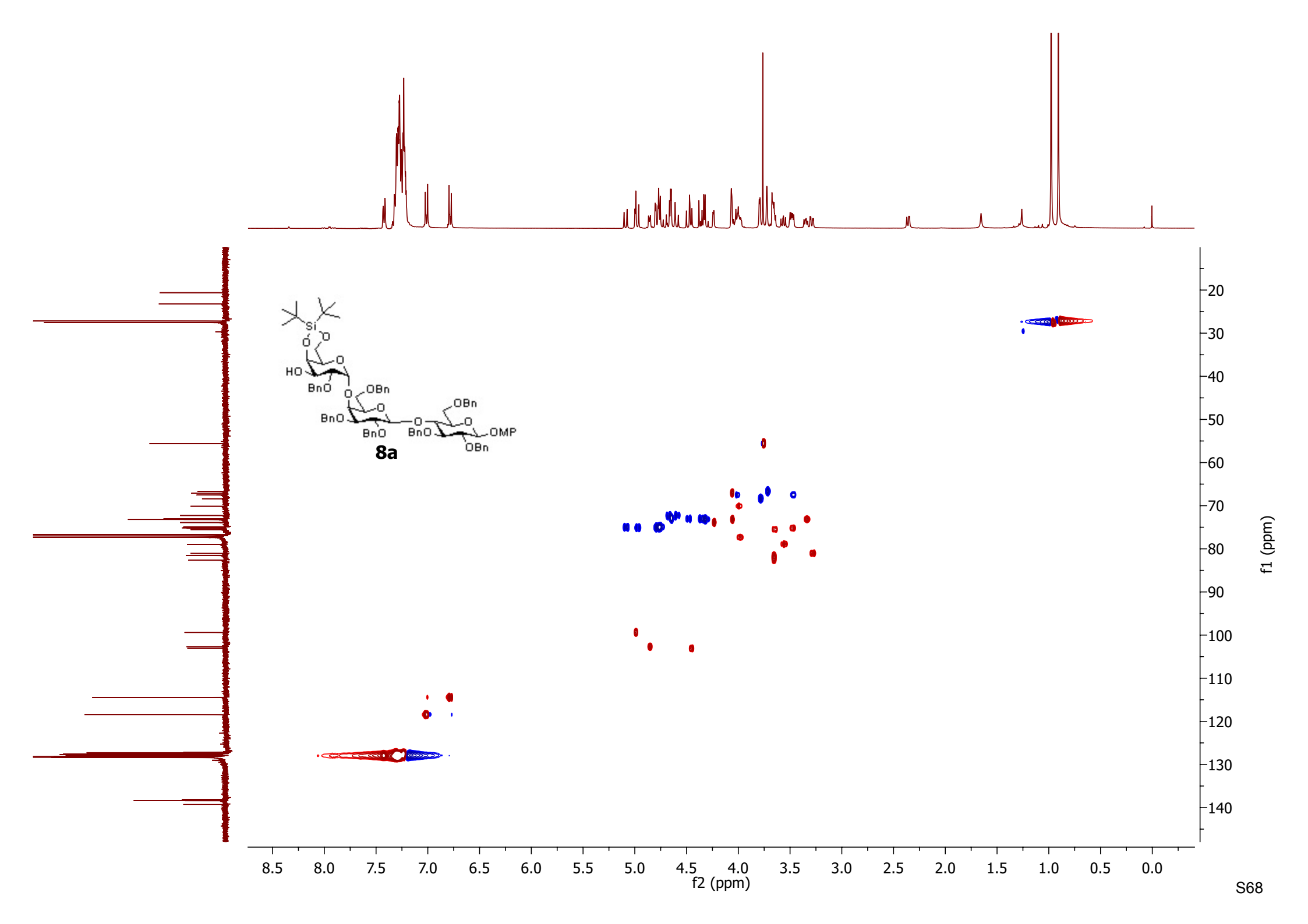
36
34
32
30
28
26
24
22
20
18
16
14
12
10
8
6
4
2
0
-2
-4
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

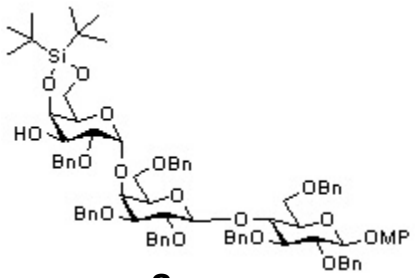

Si
O
O
O
HO
BnO
O
OBn
O
BnO
BnO
O
BnO
OBn
O
OMP
OBn

8a

1400
1300
1200
1100
1000
900
800
700
600
500
400
300
200
100
0
-100
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

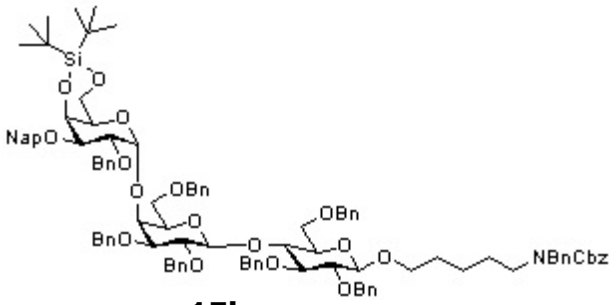

HO
BnO
OBn
BnO
BnO
OBn
BnO
OMP
OBn
8a
f2 (ppm)
f1 (ppm)


HO
BnO
OBn
BnO
BnO
OBn
BnO
OMP
OBn
8a
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f2 (ppm)
20
30
40
50
60
70
80
90
100
110
120
130
140
f1 (ppm)


Si
O
O
O
O
HO
BnO
O
OBn
O
BnO
BnO
O
BnO
OBn
O
OMP
OBn

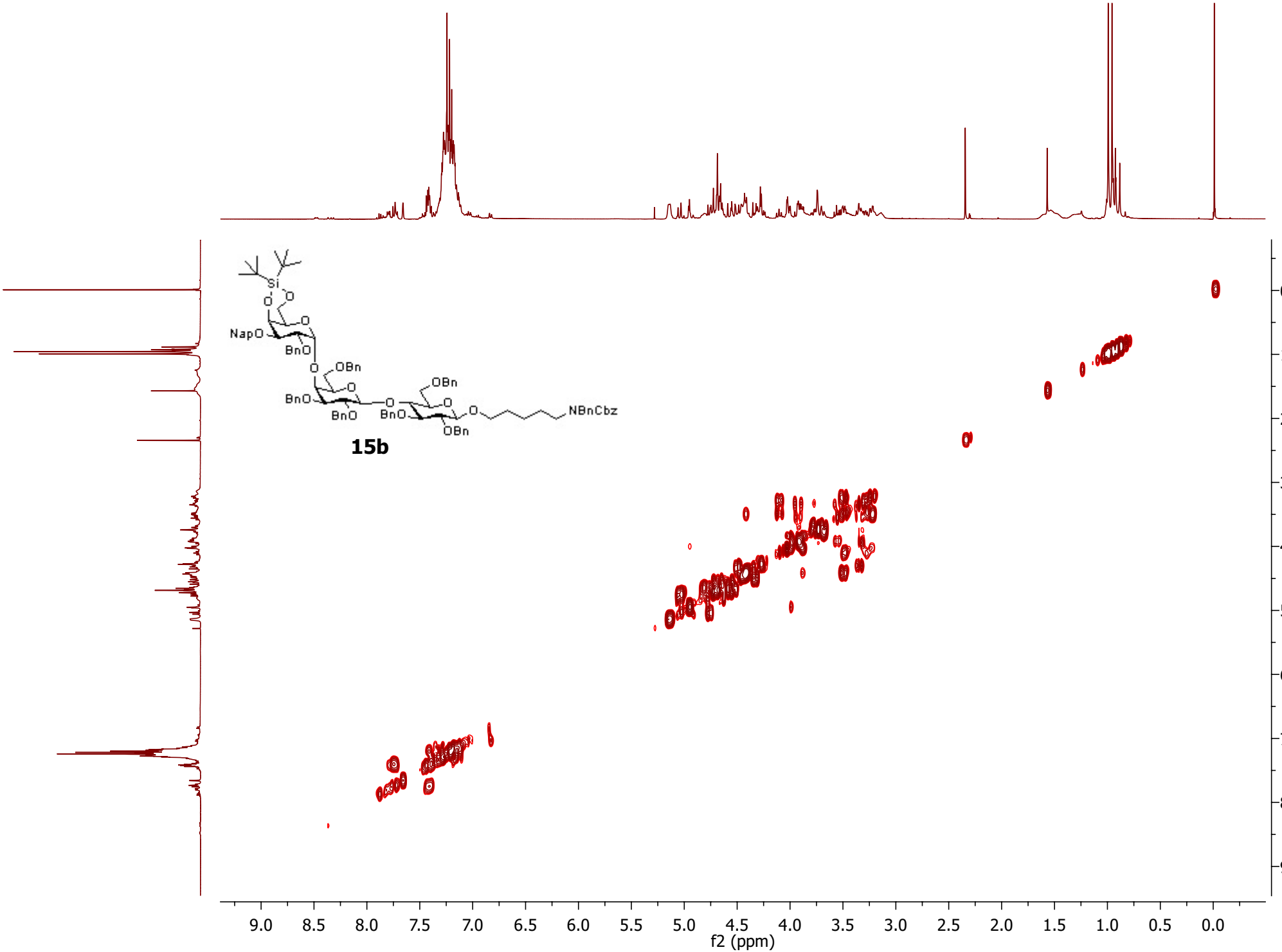
8a

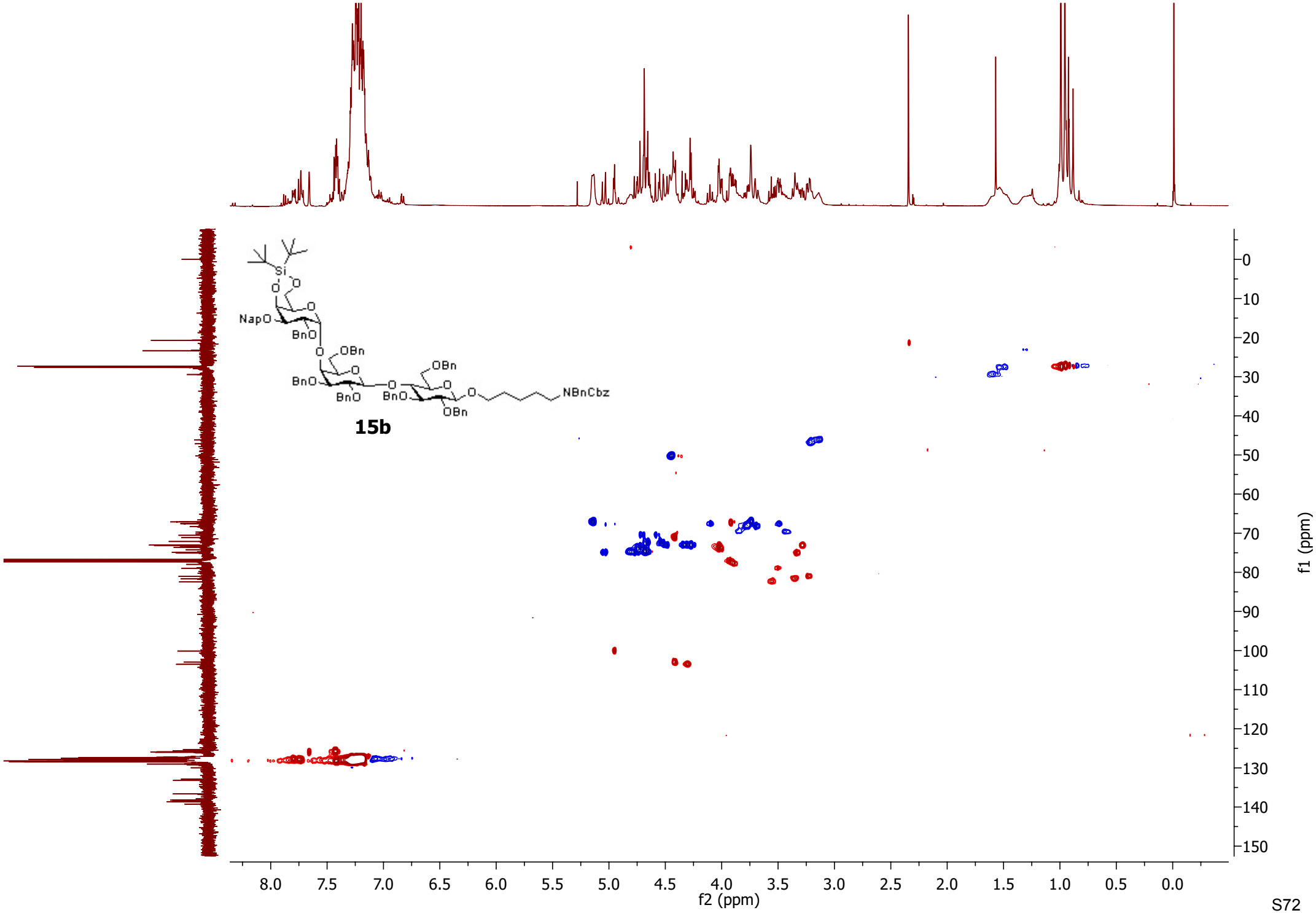
34
32
30
28
26
24
22
20
18
16
14
12
10
8
6
4
2
0
-2
-4
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

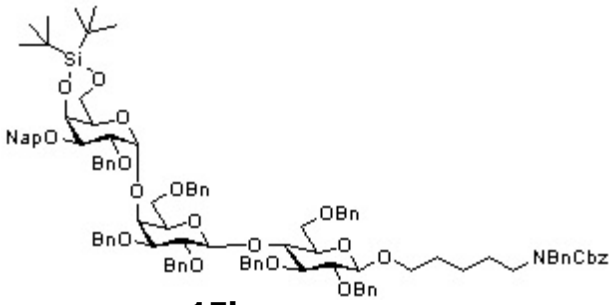

Si
O
O
O
NapO
BnO
O
OBn
O
BnO
BnO
O
BnO
OBn
O
OBn
O
NBnCbz

15b

1500
1400
1300
1200
1100
1000
900
800
700
600
500
400
300
200
100
0
-100
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)

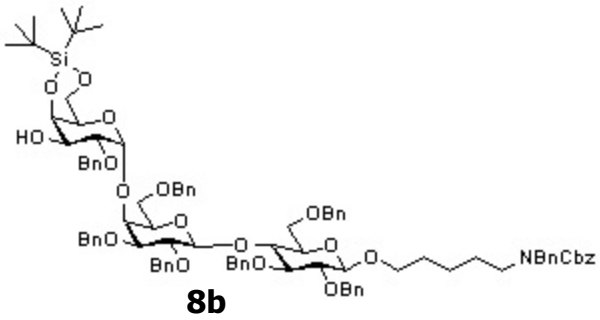

NapO
BnO
OBn
BnO
BnO
OBn
BnO
BnO
OBn
NBnCbz
15b
f2 (ppm)
f1 (ppm)


NapO
BnO
OBn
NBnCbz
15b
f2 (ppm)
f1 (ppm)


Si
O
O
O
NapO
BnO
O
OBn
O
BnO
O
BnO
BnO
OBn
O
BnO
O
NBnCbz
OBn

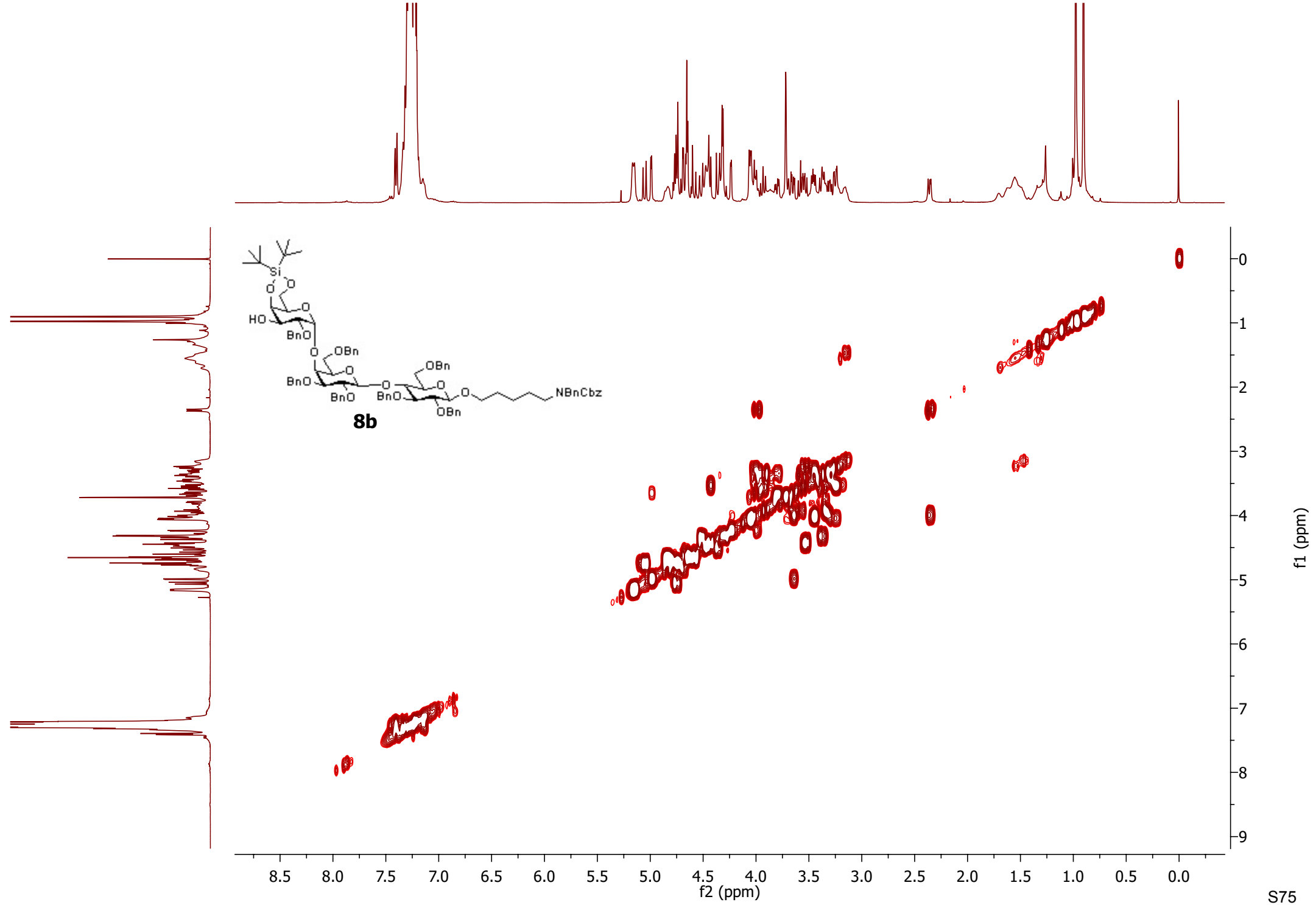
15b

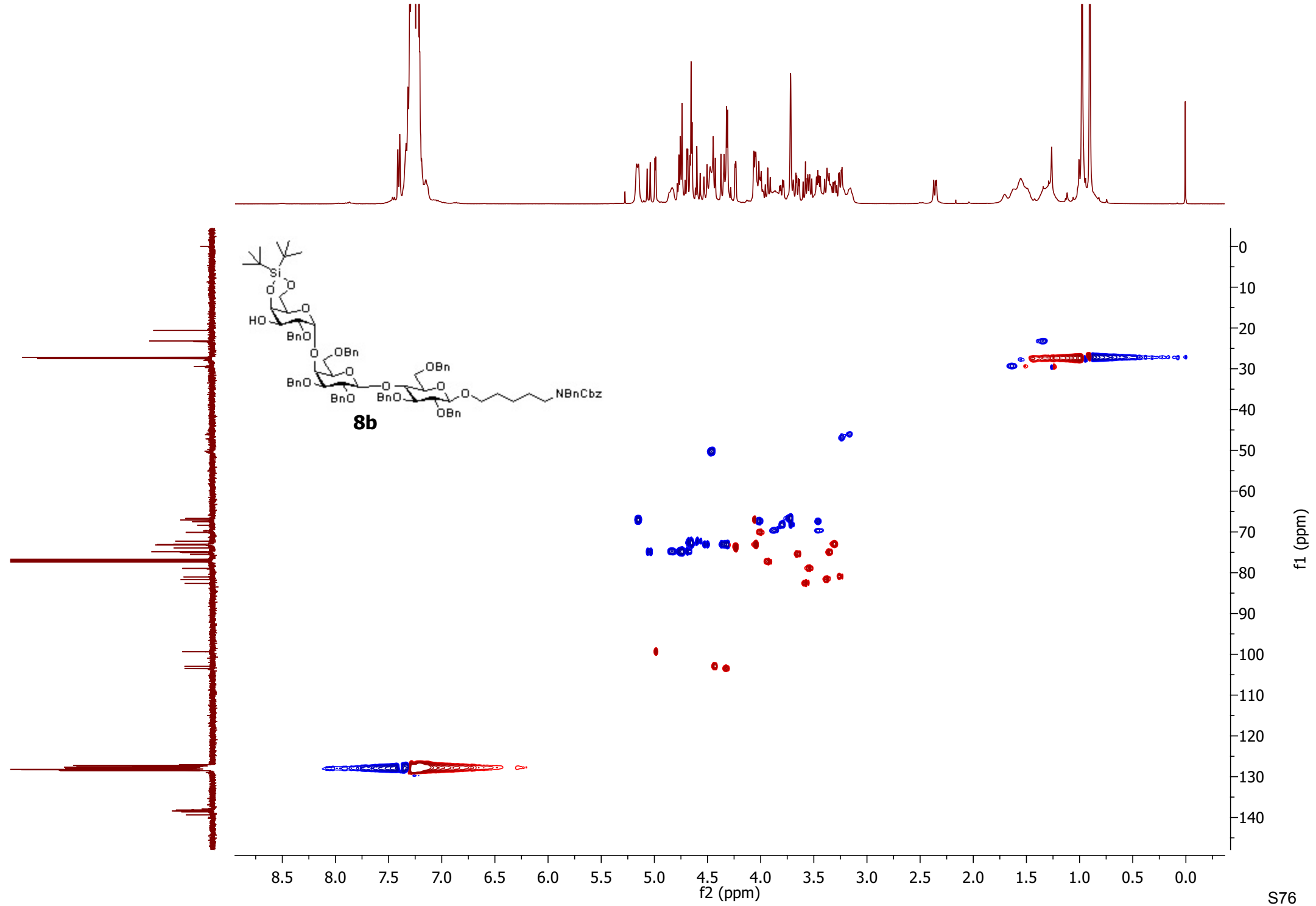
28
26
24
22
20
18
16
14
12
10
8
6
4
2
0
-2
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

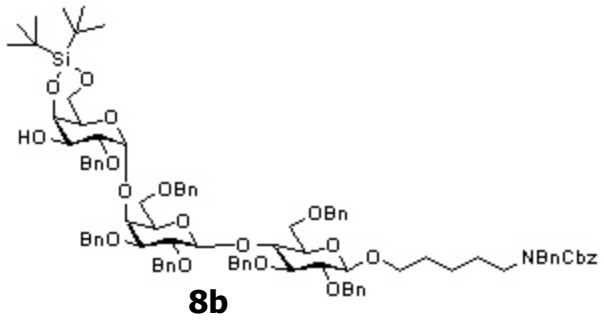

Si
O
O
O
HO
BnO
O
OBn
O
BnO
BnO
O
BnO
OBn
O
OBn
O
NBnCbz

8b

2100
2000
1900
1800
1700
1600
1500
1400
1300
1200
1100
1000
900
800
700
600
500
400
300
200
100
0
-100
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

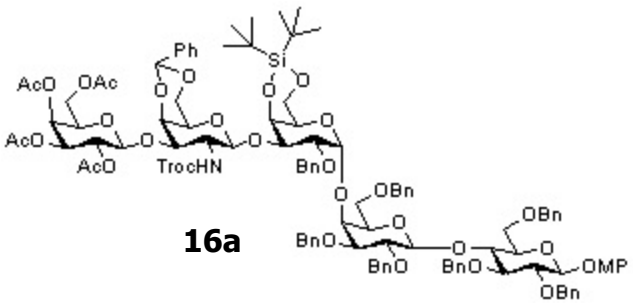

Si
HO
BnO
OBn
BnO
BnO
BnO
OBn
BnO
OBn
NBnCbz
8b
f2 (ppm)
f1 (ppm)


Si
HO
BnO
OBn
BnO
BnO
BnO
OBn
BnO
OBn
NBnCbz
8b
f2 (ppm)
f1 (ppm)


Si
O
O
O
HO
BnO
O
OBn
O
BnO
BnO
O
BnO
OBn
O
OBn
O
NBnCbz

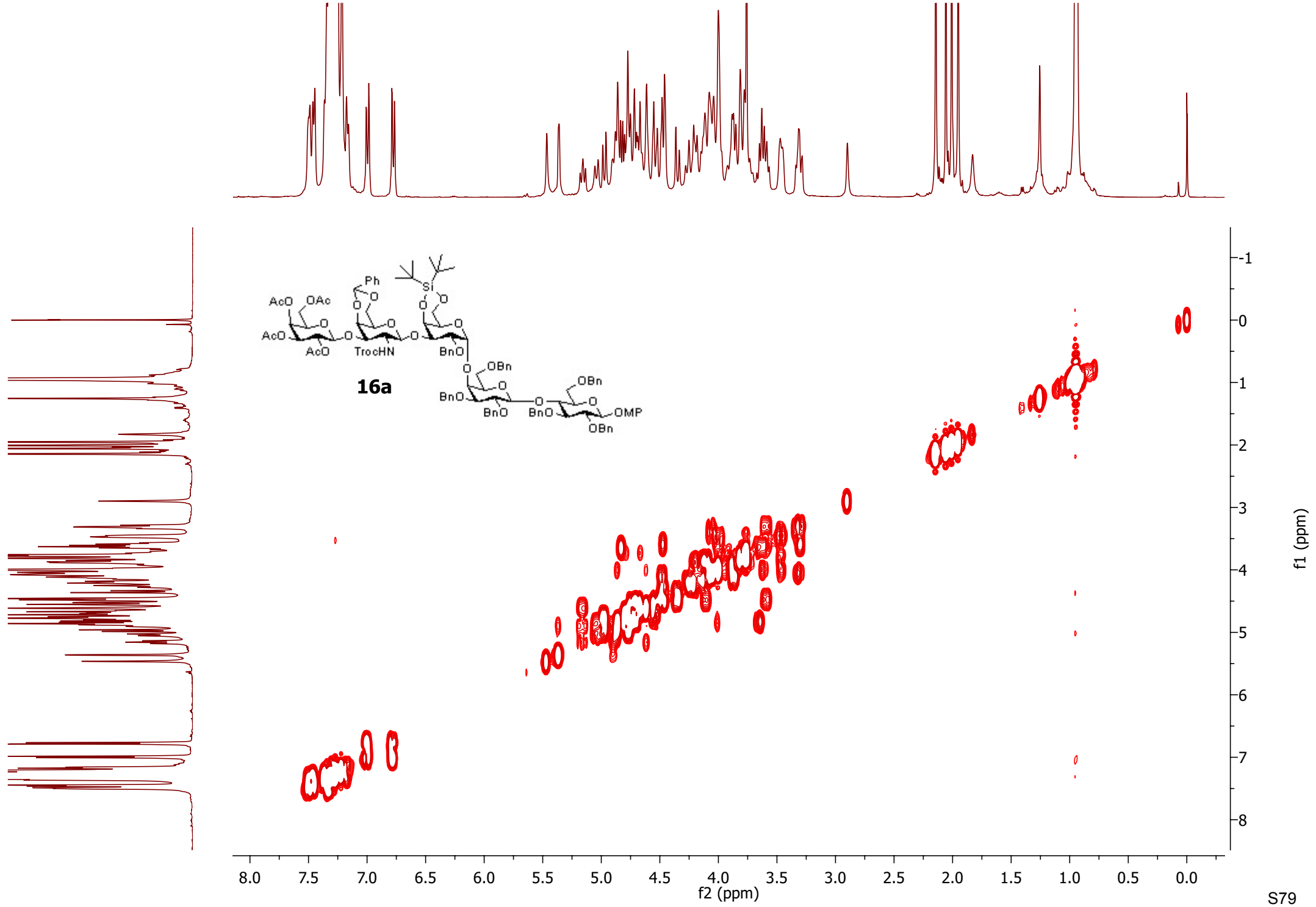
8b

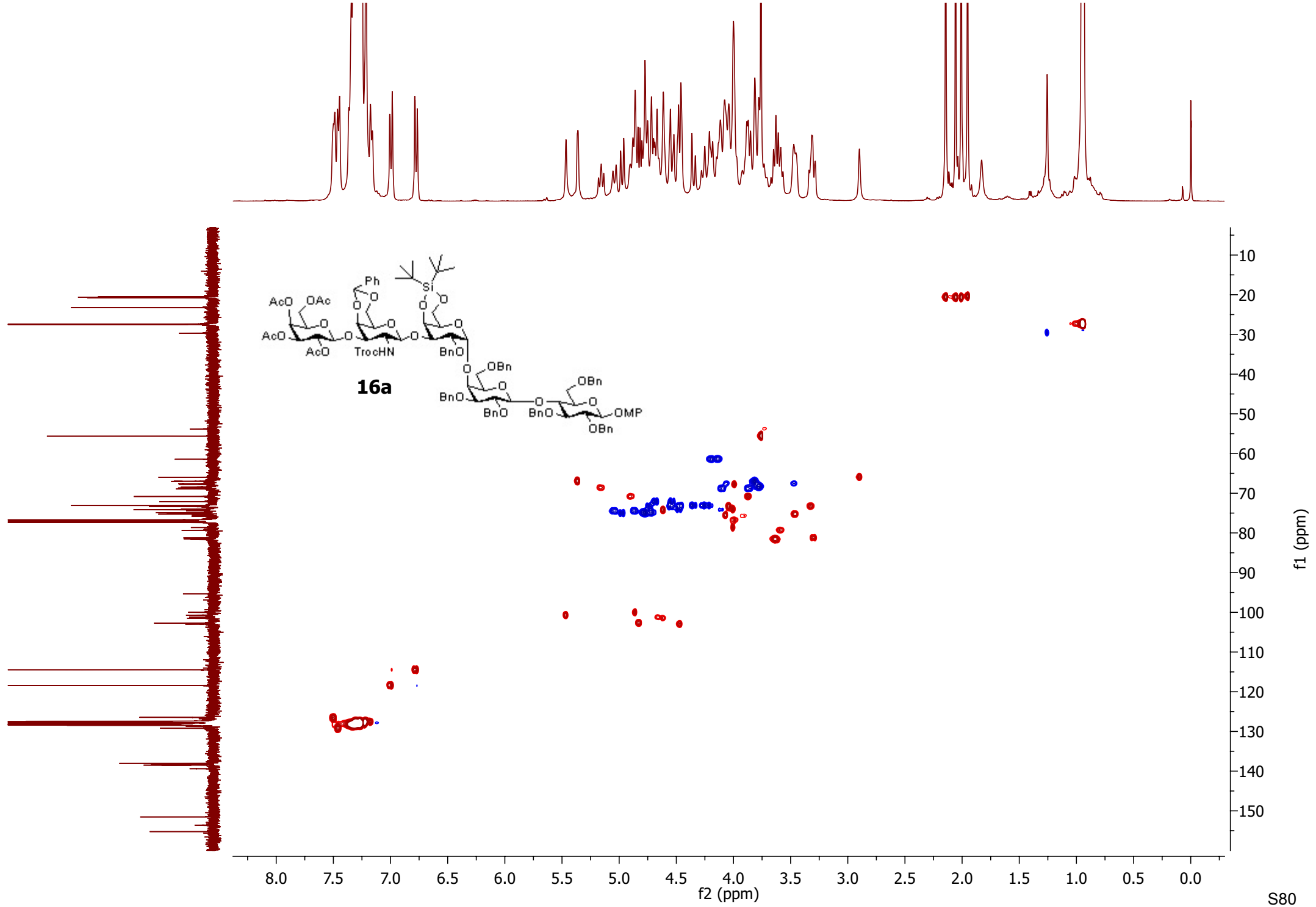
40
35
30
25
20
15
10
5
0
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

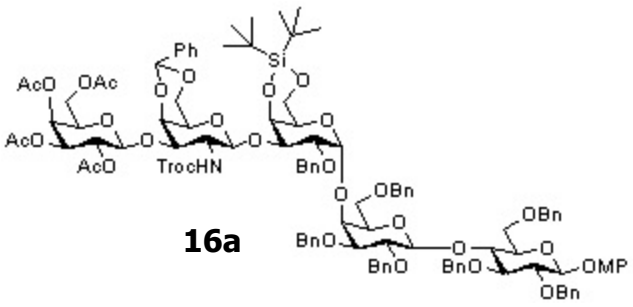

AcO
OAc
AcO
AcO
Ph
O
TrocHN
Si
BnO
OBn
BnO
BnO
BnO
OBn
OMP
OBn

16a

2000
1900
1800
1700
1600
1500
1400
1300
1200
1100
1000
900
800
700
600
500
400
300
200
100
0
-100
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)

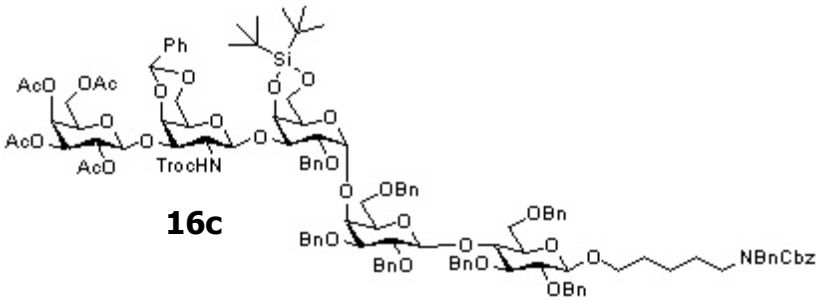

16a
AcO
OAc
AcO
AcO
TrocHN
Ph
Si
BnO
OBn
BnO
BnO
BnO
OBn
OMP
OBn
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f2 (ppm)
-1
0
1
2
3
4
5
6
7
8
f1 (ppm)


16a
f2 (ppm)
f1 (ppm)


AcO
OAc
AcO
AcO
Ph
O
TrocHN
Si
BnO
OBn
BnO
BnO
BnO
OBn
OMP
OBn

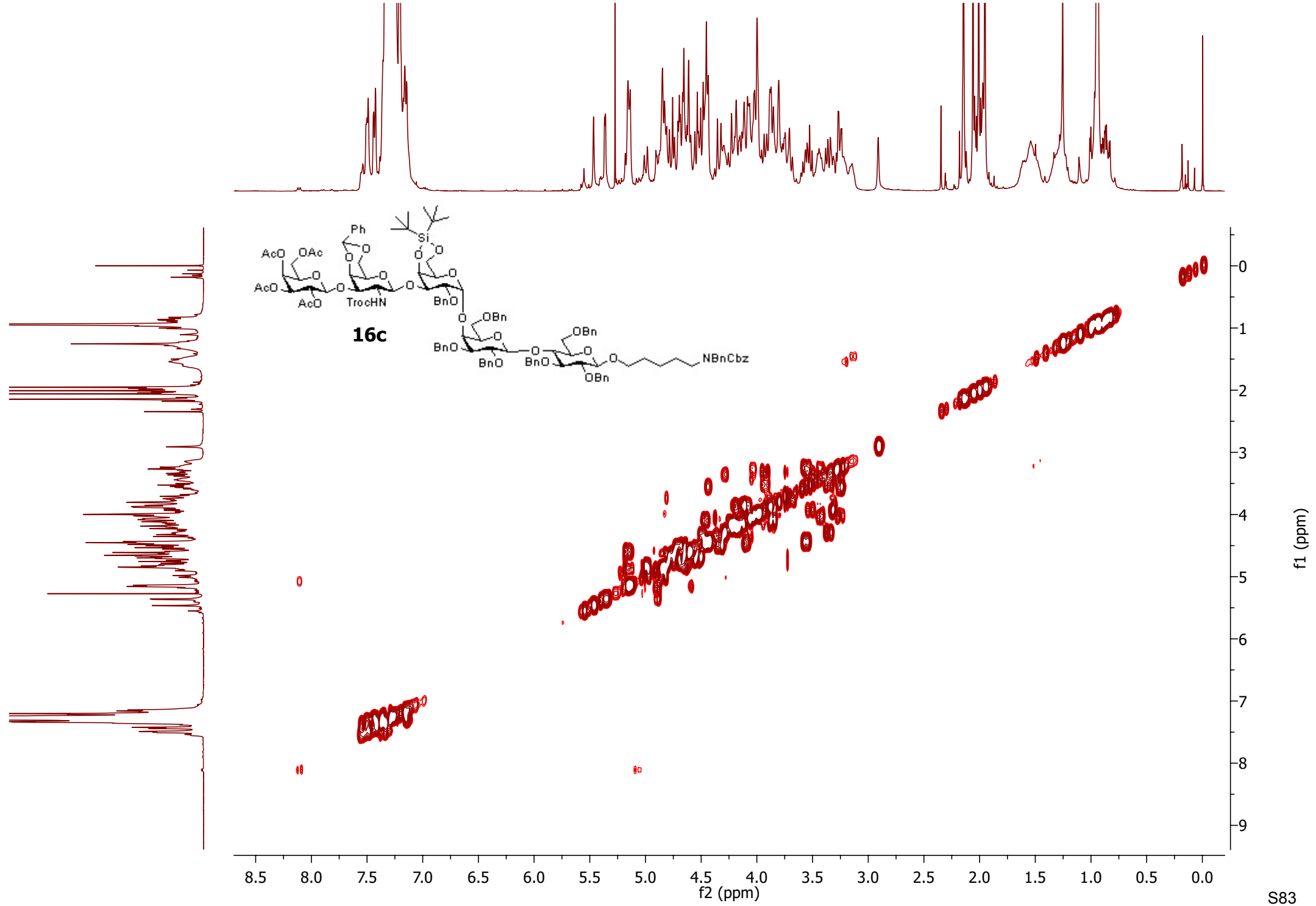
16a

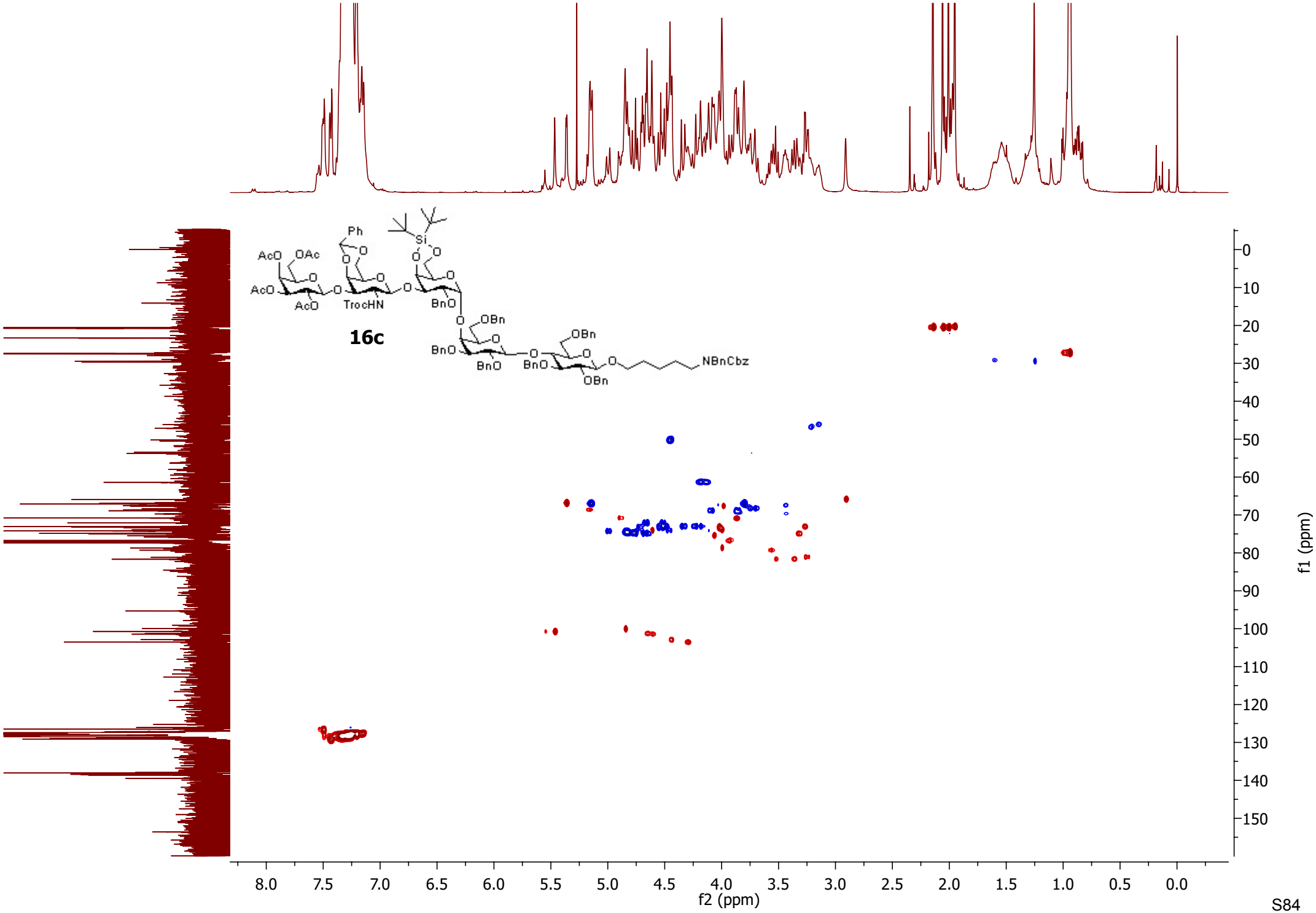
100
90
80
70
60
50
40
30
20
10
0
-10
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

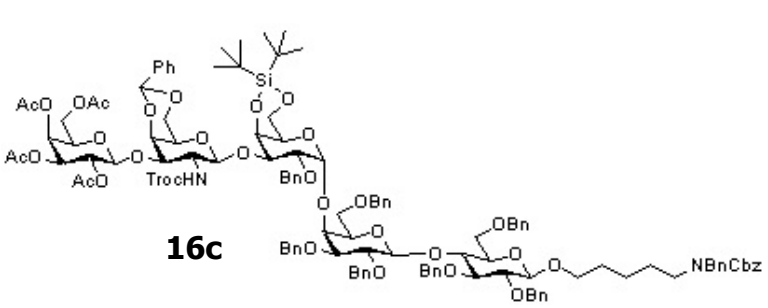


16c

f1 (ppm)

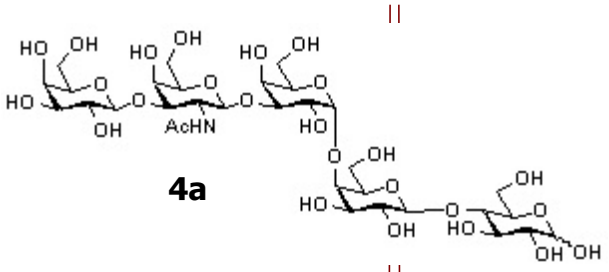

16c
AcO
OAc
AcO
AcO
TrocHN
Ph
Si
BnO
BnO
BnO
OBn
OBn
OBn
NBnCbz
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f2 (ppm)
0
1
2
3
4
5
6
7
8
9
f1 (ppm)


16c
AcO
OAc
AcO
AcO
TrocHN
Ph
Si
BnO
BnO
BnO
OBn
OBn
OBn
NBnCbz
f2 (ppm)
f1 (ppm)

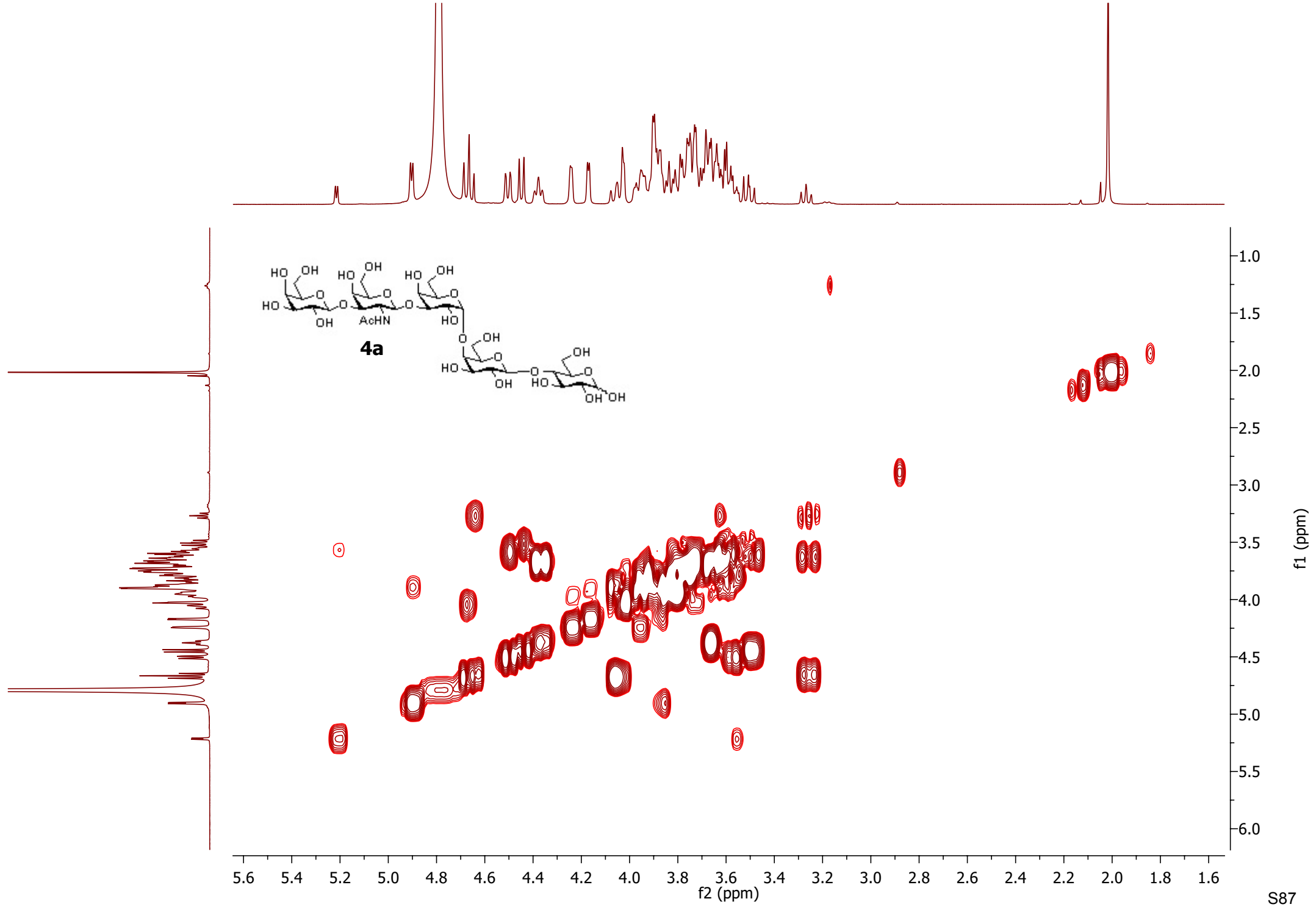

Ph
Si
AcO
OAc
AcO
AcO
TrocHN
BnO
OBn
BnO
BnO
BnO
OBn
OBn
NBnCbz

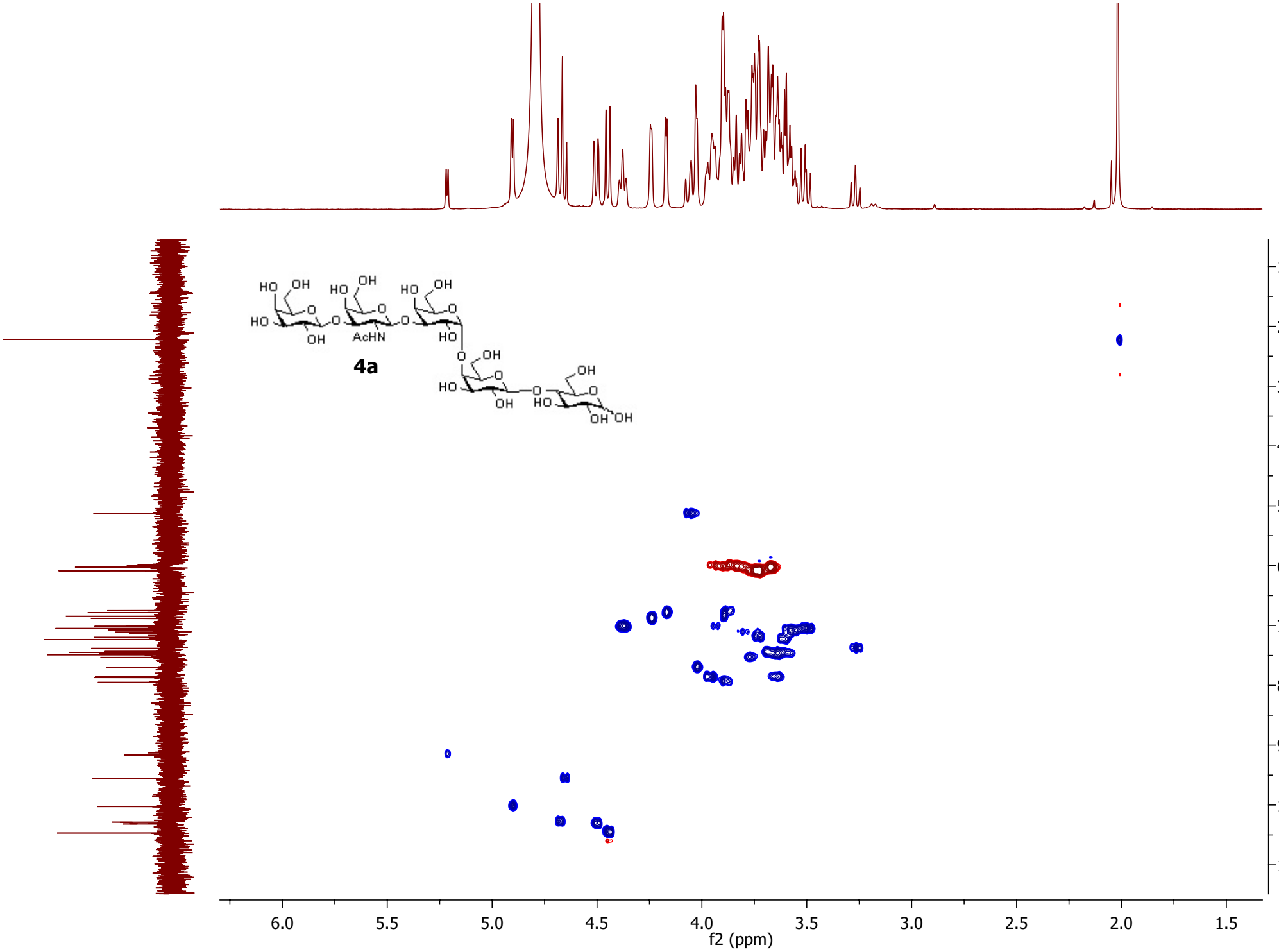
16c

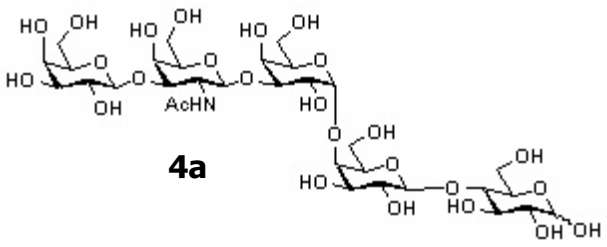
24
22
20
18
16
14
12
10
8
6
4
2
0
-2
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)


HO
OH
O
AcHN
4a

900
800
700
600
500
400
300
200
100
0
5.5
5.3
5.1
4.9
4.7
4.5
4.3
4.1
3.9
3.7
3.5
3.3
3.1
2.9
2.7
2.5
2.3
2.1
1.9
f1 (ppm)

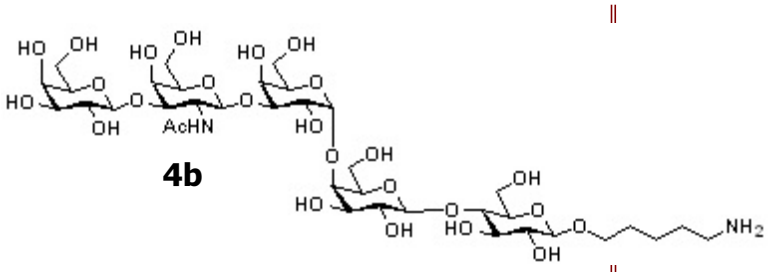

4a
AcHN
f2 (ppm)
f1 (ppm)


4a
AcHN
f2 (ppm)
f1 (ppm)
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
10
20
30
40
50
60
70
80
90
100
110


HO
OH
O
AcHN

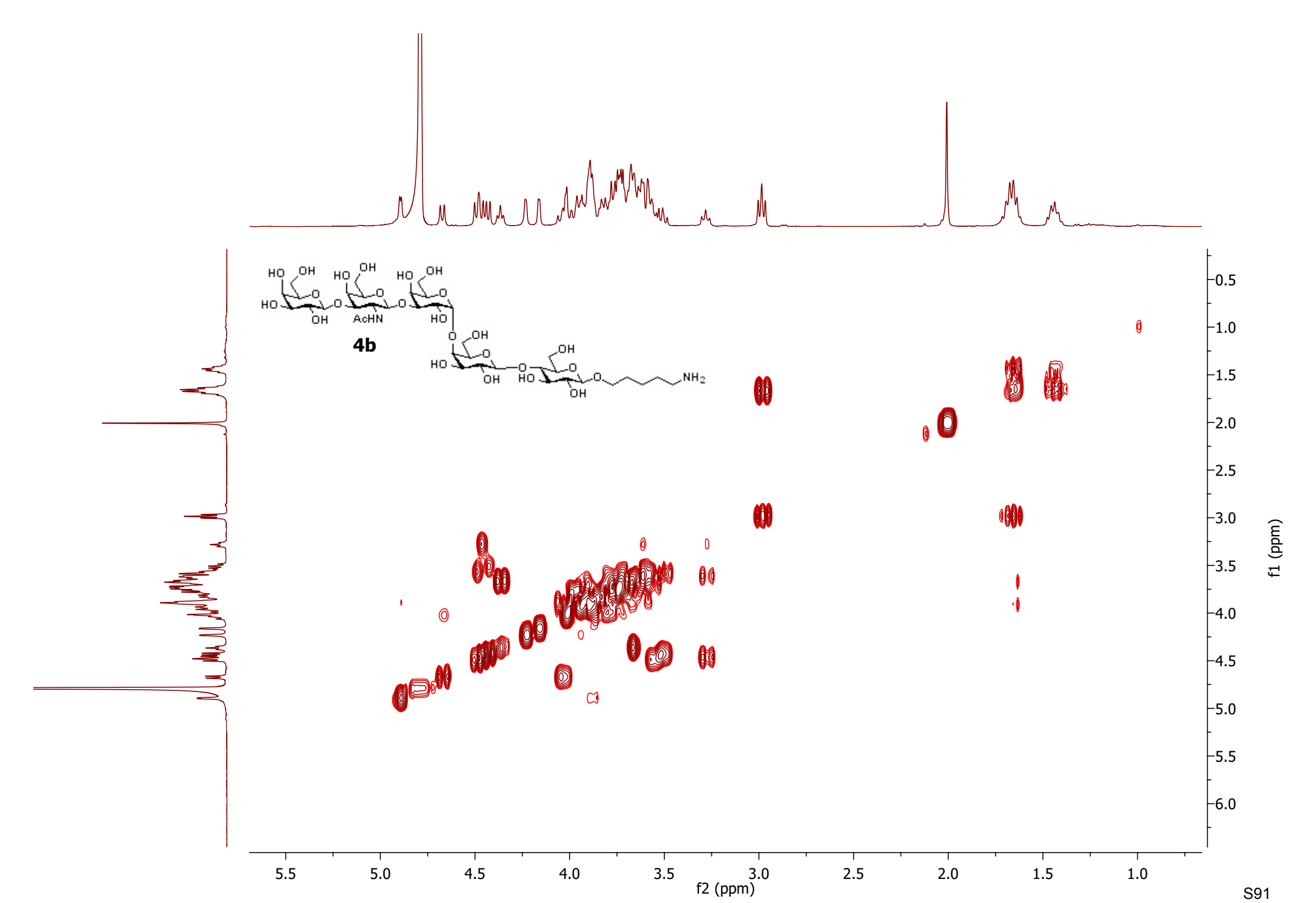
4a

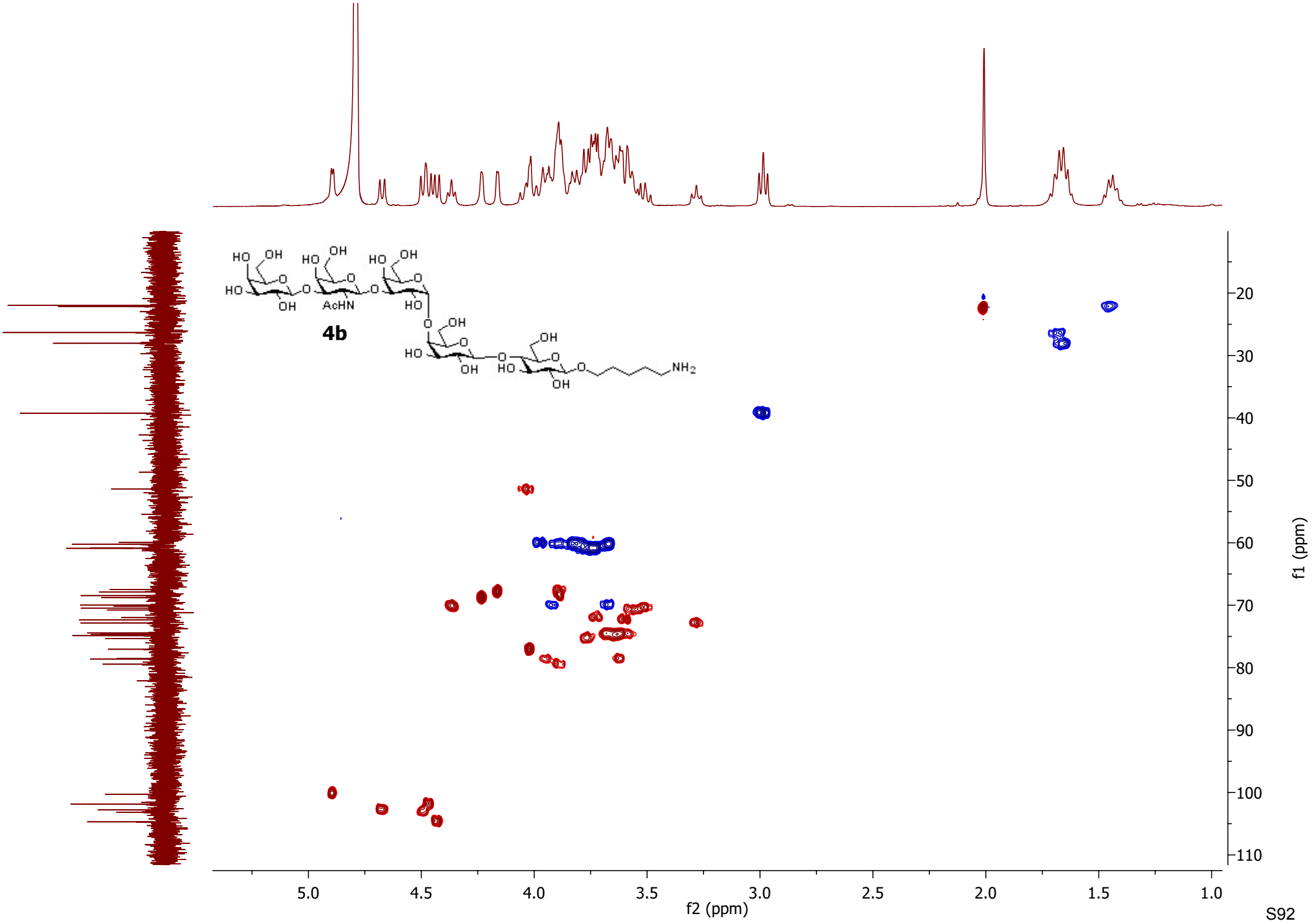
23
22
21
20
19
18
17
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
-1
-2
-3
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)

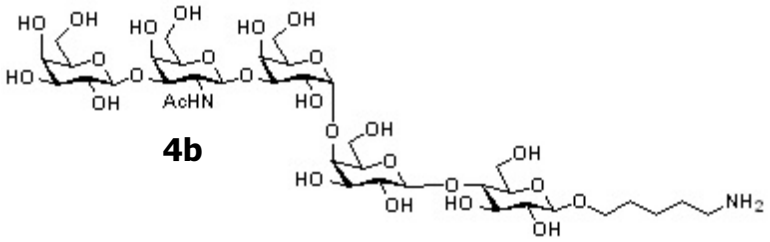

HO
OH
HO
OH
HO
OH
HO
O
HO
OH
AcHN
HO
OH
O
HO
OH
O
HO
OH
O
HO
OH
O
NH2

4b

1400
1300
1200
1100
1000
900
800
700
600
500
400
300
200
100
0
-100
1.32
1.00
2.49
1.01
1.02
0.98
20.92
0.92
1.64
2.09
3.78
1.87
6.6
6.4
6.2
6.0
5.8
5.6
5.4
5.2
5.0
4.8
4.6
4.4
4.2
4.0
3.8
3.6
3.4
3.2
3.0
2.8
2.6
2.4
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8
f1 (ppm)

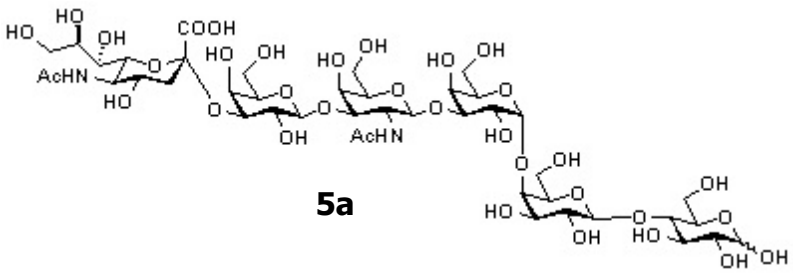

4b
AcHN
NH2
f2 (ppm)
f1 (ppm)
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
6.0


4b
AcHN
NH2
f2 (ppm)
f1 (ppm)
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
20
30
40
50
60
70
80
90
100
110


HO
OH
HO
O
OH
AcHN
HO
OH
HO
O
OH
HO
O
OH
HO
O
OH
HO
O
NH2
OH
OH
OH

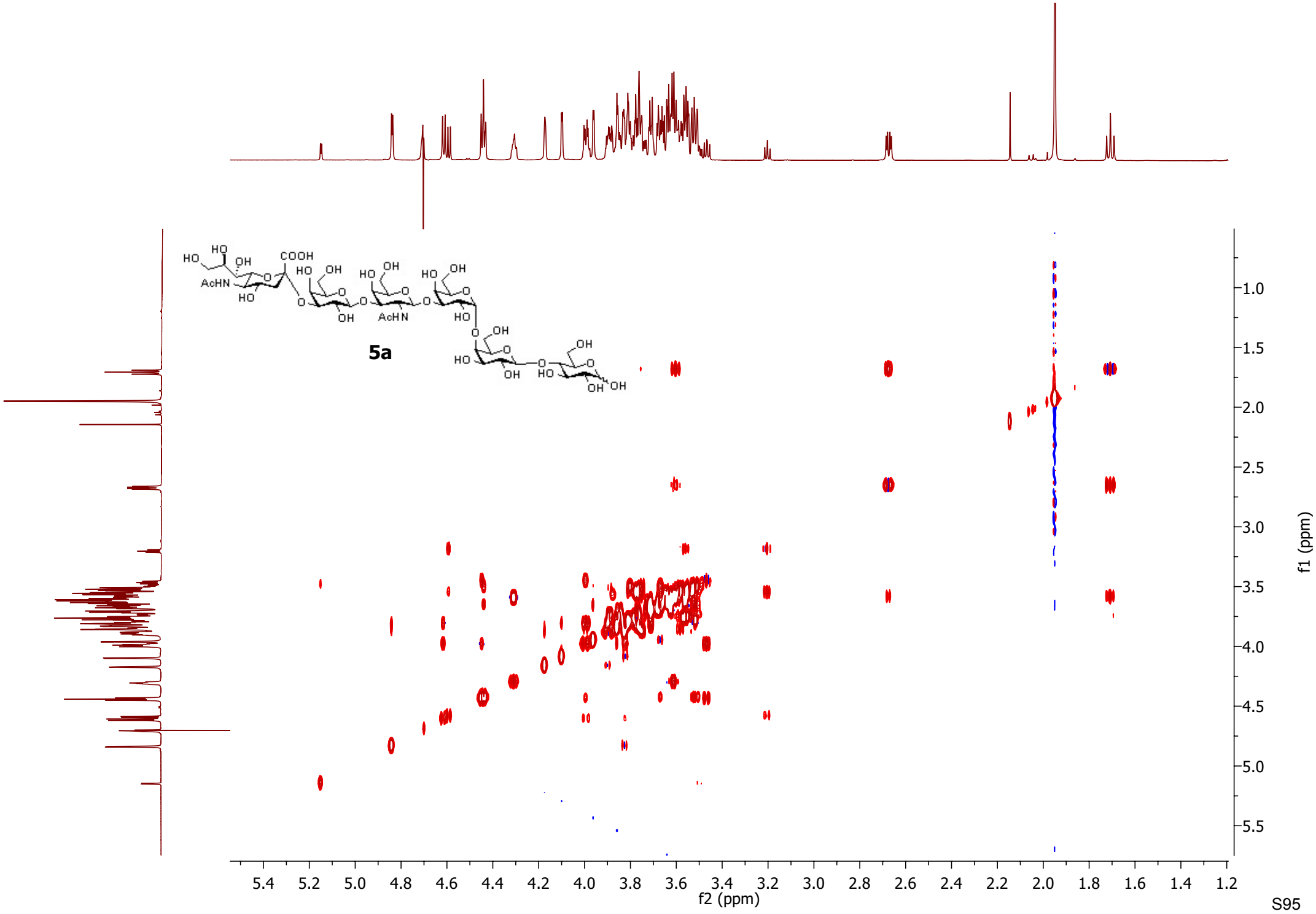
4b

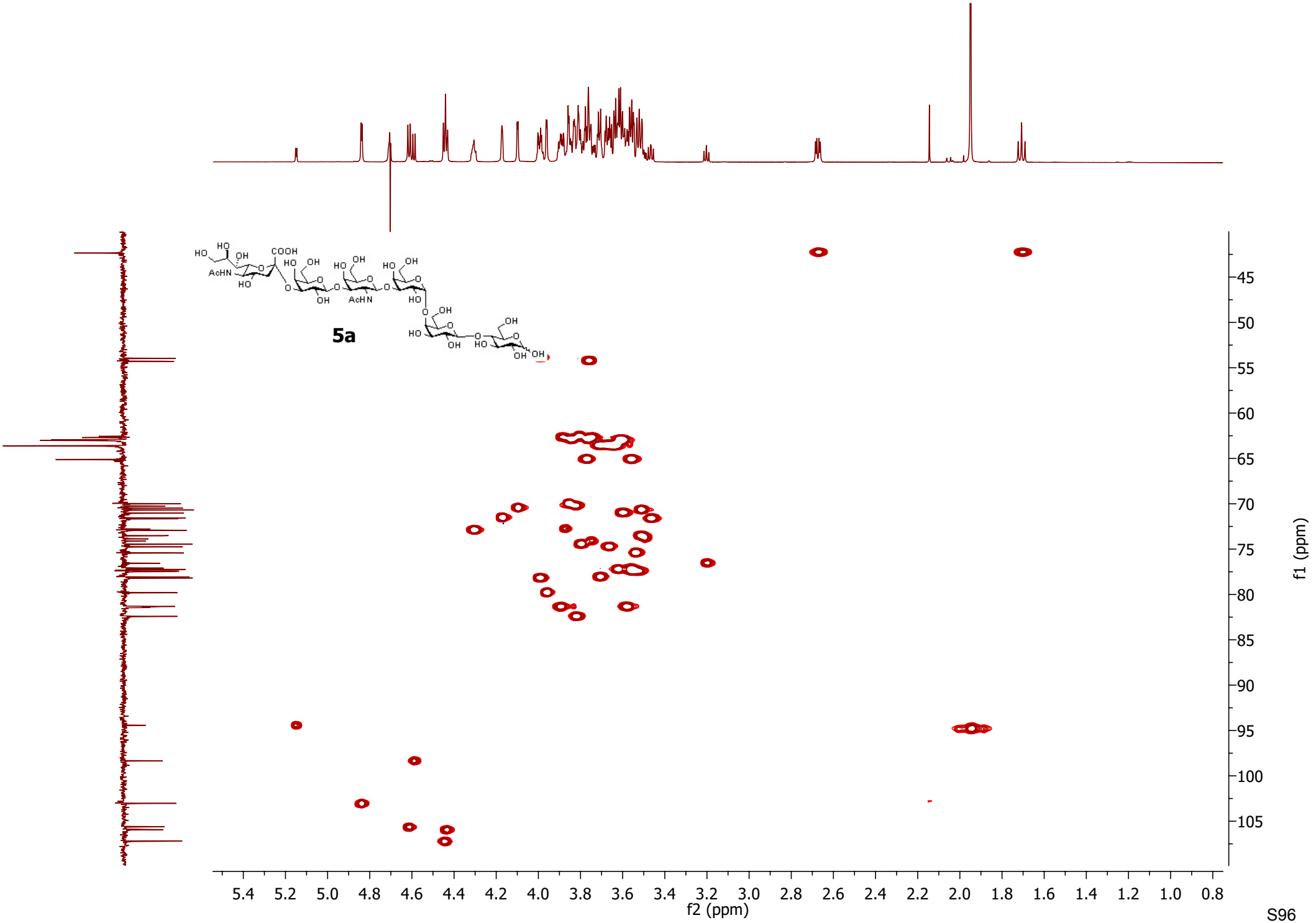
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
-1
-2
-3
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

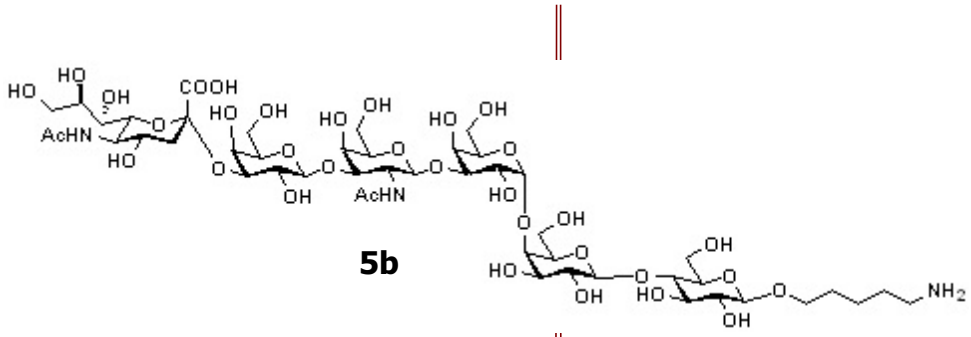

HO
OH
COOH
AcHN
HO
HO
OH
O
OH
AcHN
HO
OH
HO
OH
O
OH
HO
OH
HO
OH
OH
OH

5a

5500
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
5.6
5.4
5.2
5.0
4.8
4.6
4.4
4.2
4.0
3.8
3.6
3.4
3.2
3.0
2.8
2.6
2.4
2.2
2.0
1.8
1.6
1.4
f1 (ppm)

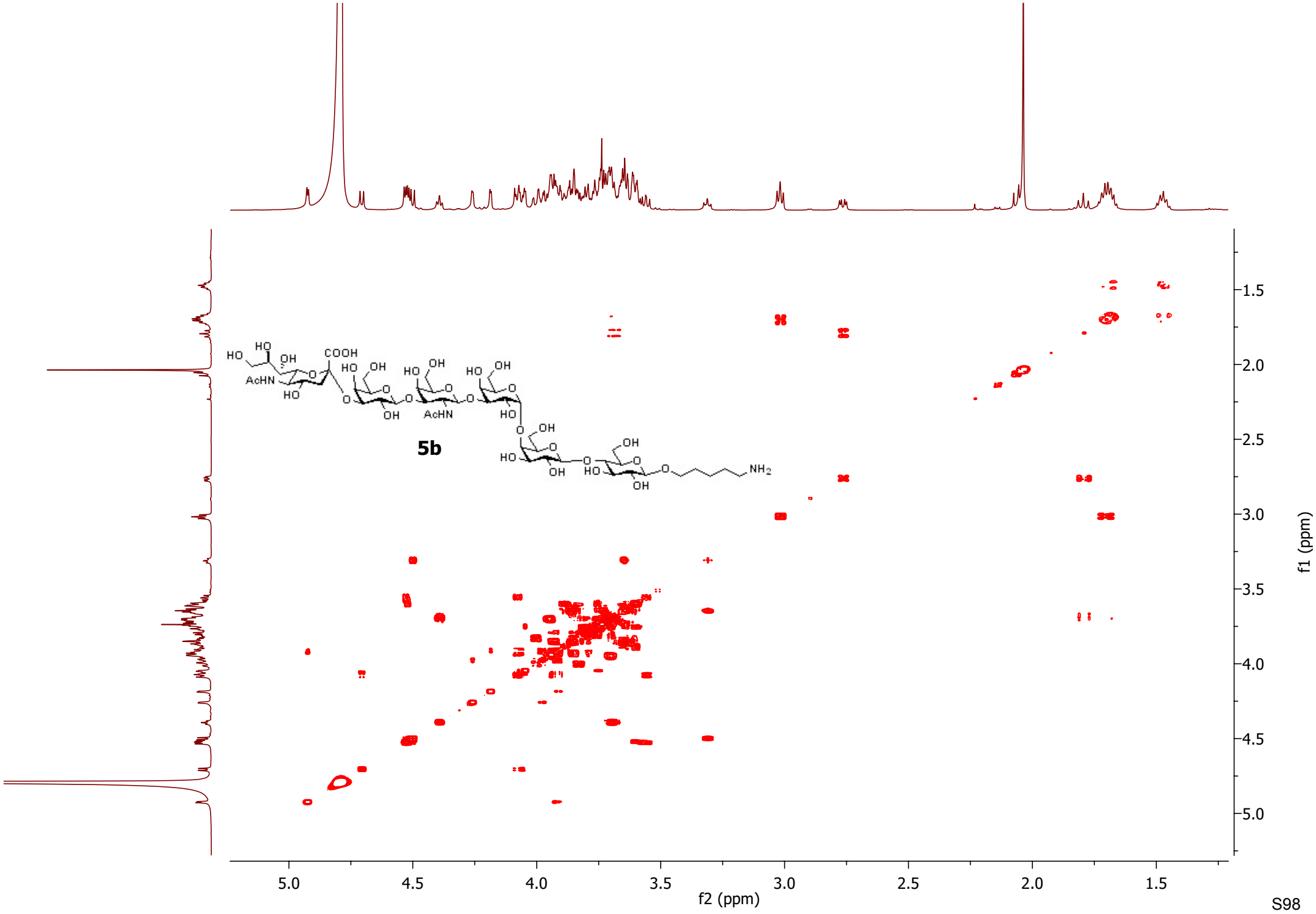

5a
f2 (ppm)
f1 (ppm)

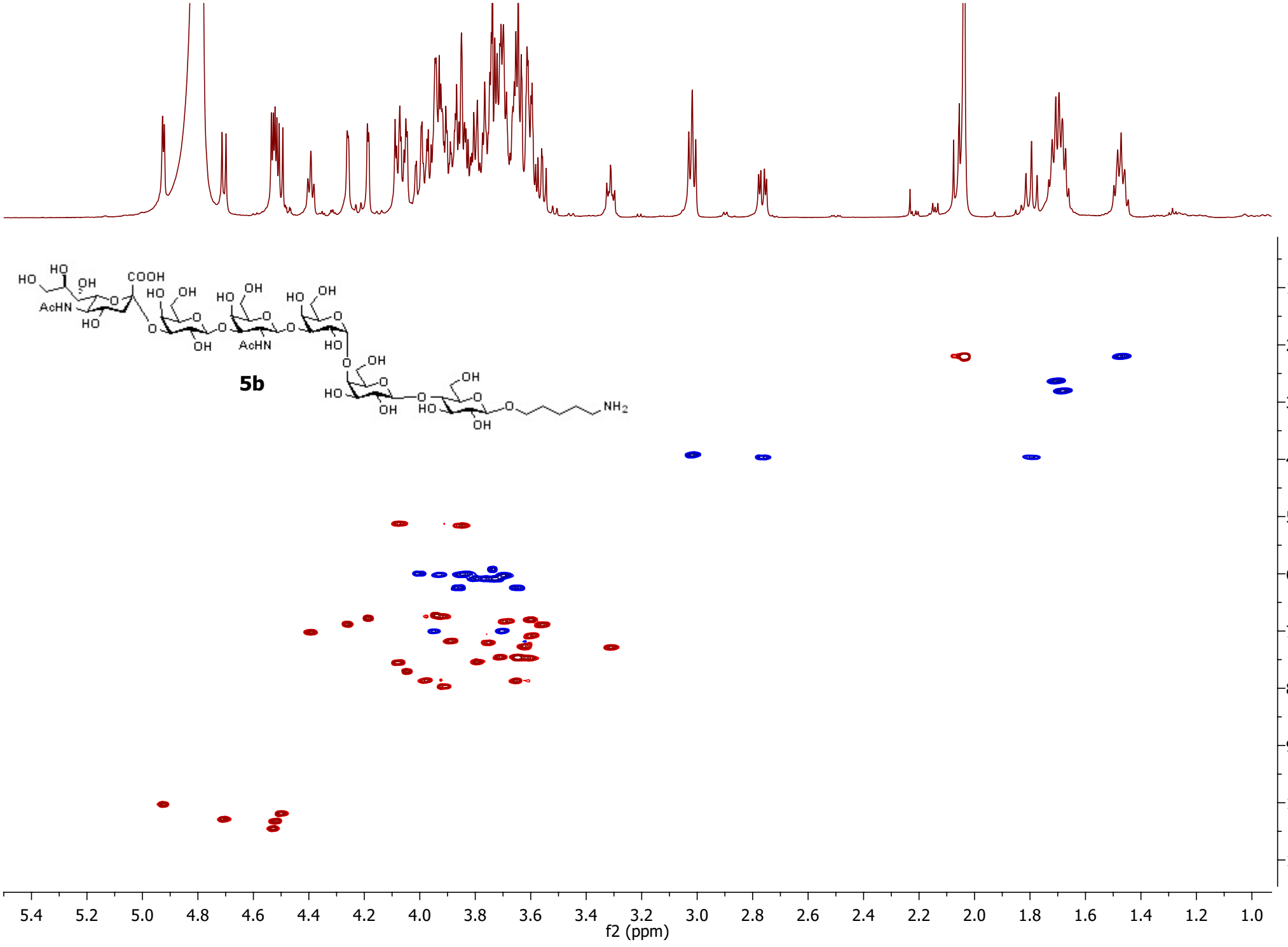

5a
f2 (ppm)
f1 (ppm)
5.4 5.2 5.0 4.8 4.6 4.4 4.2 4.0 3.8 3.6 3.4 3.2 3.0 2.8 2.6 2.4 2.2 2.0 1.8 1.6 1.4 1.2 1.0 0.8
45 50 55 60 65 70 75 80 85 90 95 100 105

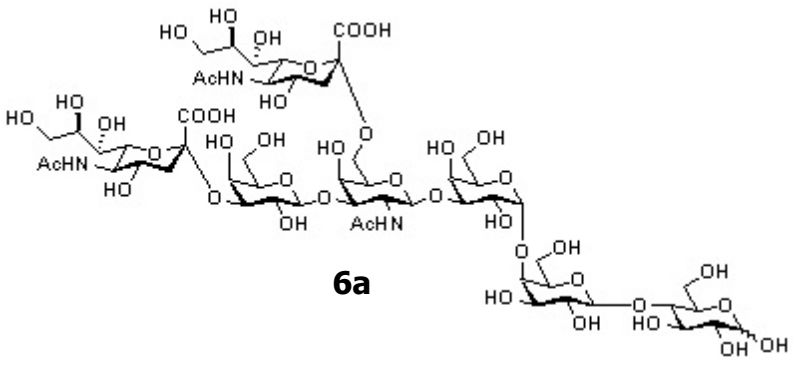

HO
HO
OH
AcHN
HO
COOH
HO
OH
O
OH
HO
OH
O
AcHN
HO
OH
O
HO
O
OH
HO
OH
O
OH
HO
O
OH
NH2

5b

3E+07
3E+07
2E+07
2E+07
2E+07
2E+07
2E+07
2E+07
1E+07
1E+07
1E+07
8E+06
6E+06
4E+06
2E+06
0
-2E+06
6.4 6.2 6.0 5.8 5.6 5.4 5.2 5.0 4.8 4.6 4.4 4.2 4.0 3.8 3.6 3.4 3.2 3.0 2.8 2.6 2.4 2.2 2.0 1.8 1.6 1.4 1.2 1.0 0.8 0.6 0.4
f1 (ppm)

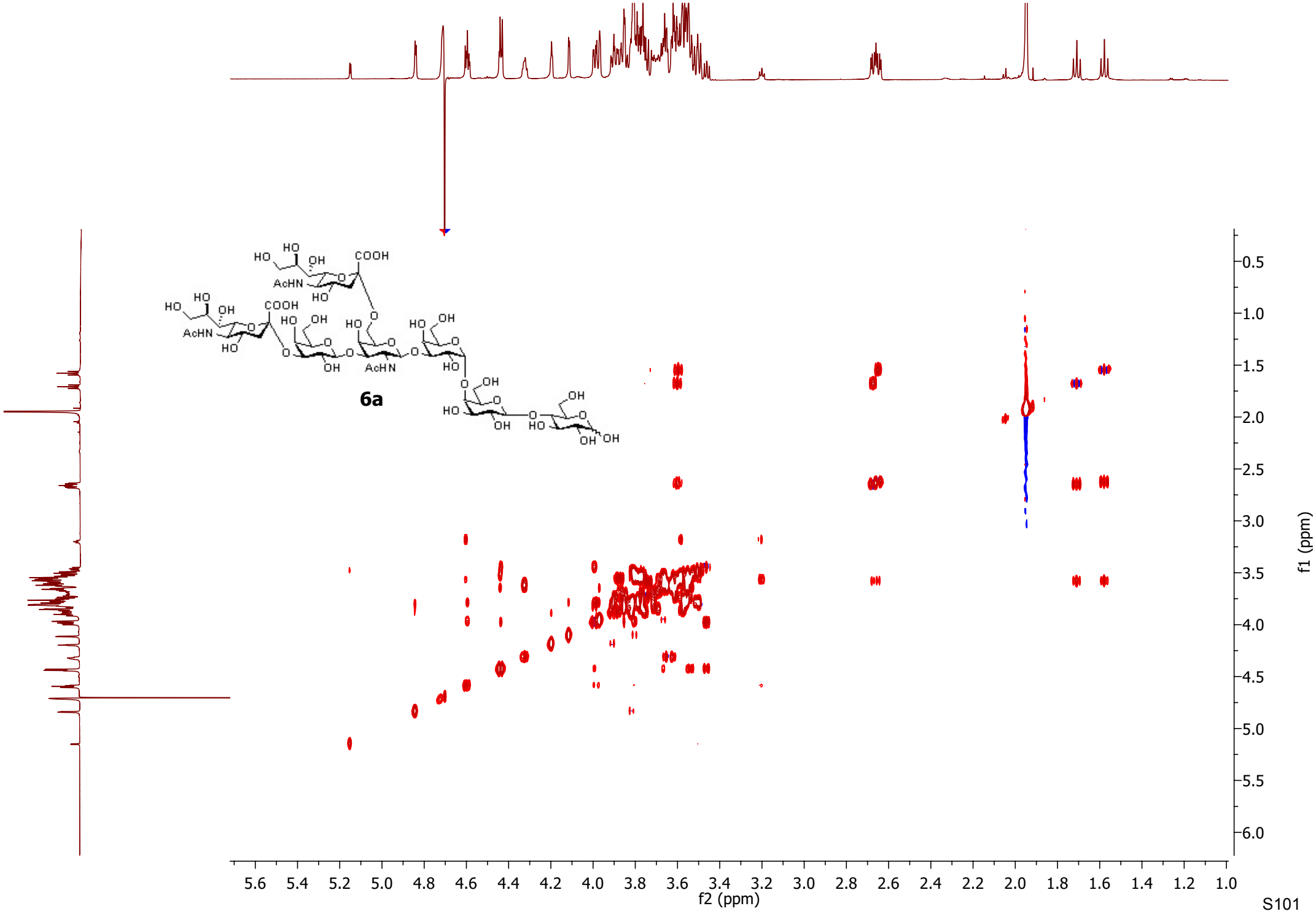

5b
f2 (ppm)
f1 (ppm)
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5

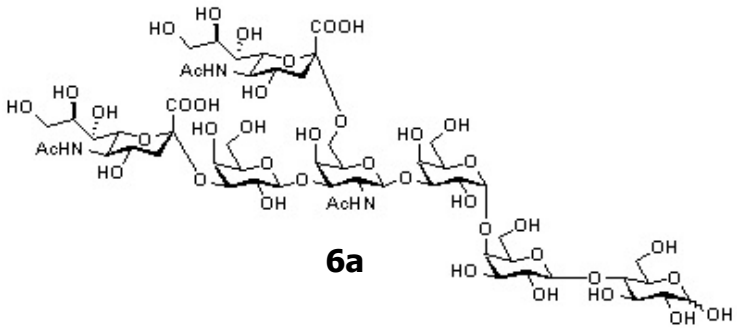

5b
f2 (ppm)
f1 (ppm)



6a

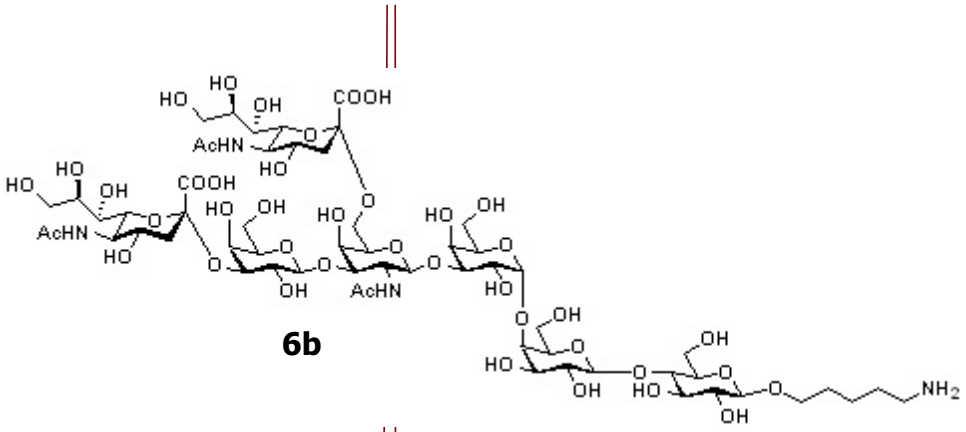
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
-500
-1000
5.4
5.2
5.0
4.8
4.6
4.4
4.2
4.0
3.8
3.6
3.4
3.2
3.0
2.8
2.6
2.4
2.2
2.0
1.8
1.6
1.4
1.2
f1 (ppm)
S100


6a
f2 (ppm)
f1 (ppm)
5.6 5.4 5.2 5.0 4.8 4.6 4.4 4.2 4.0 3.8 3.6 3.4 3.2 3.0 2.8 2.6 2.4 2.2 2.0 1.8 1.6 1.4 1.2 1.0
0.5 1.0 1.5 2.0 2.5 3.0 3.5 4.0 4.5 5.0 5.5 6.0

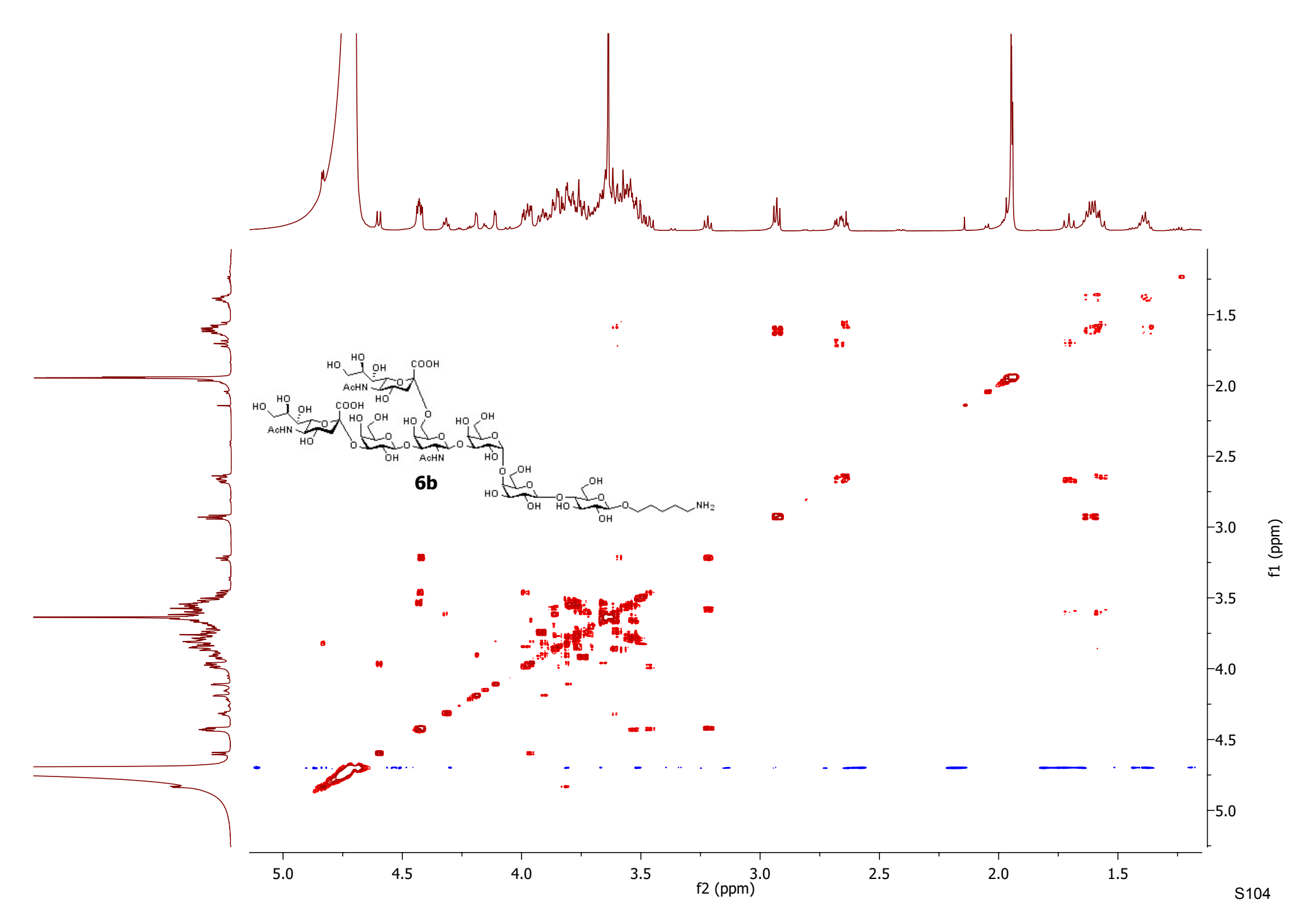

HO
HO
OH
COOH
AcHN
HO
HO
OH
COOH
AcHN
HO
HO
OH
AcHN
HO
OH
HO
OH
HO
OH
HO
OH
OH
OH
OH
OH

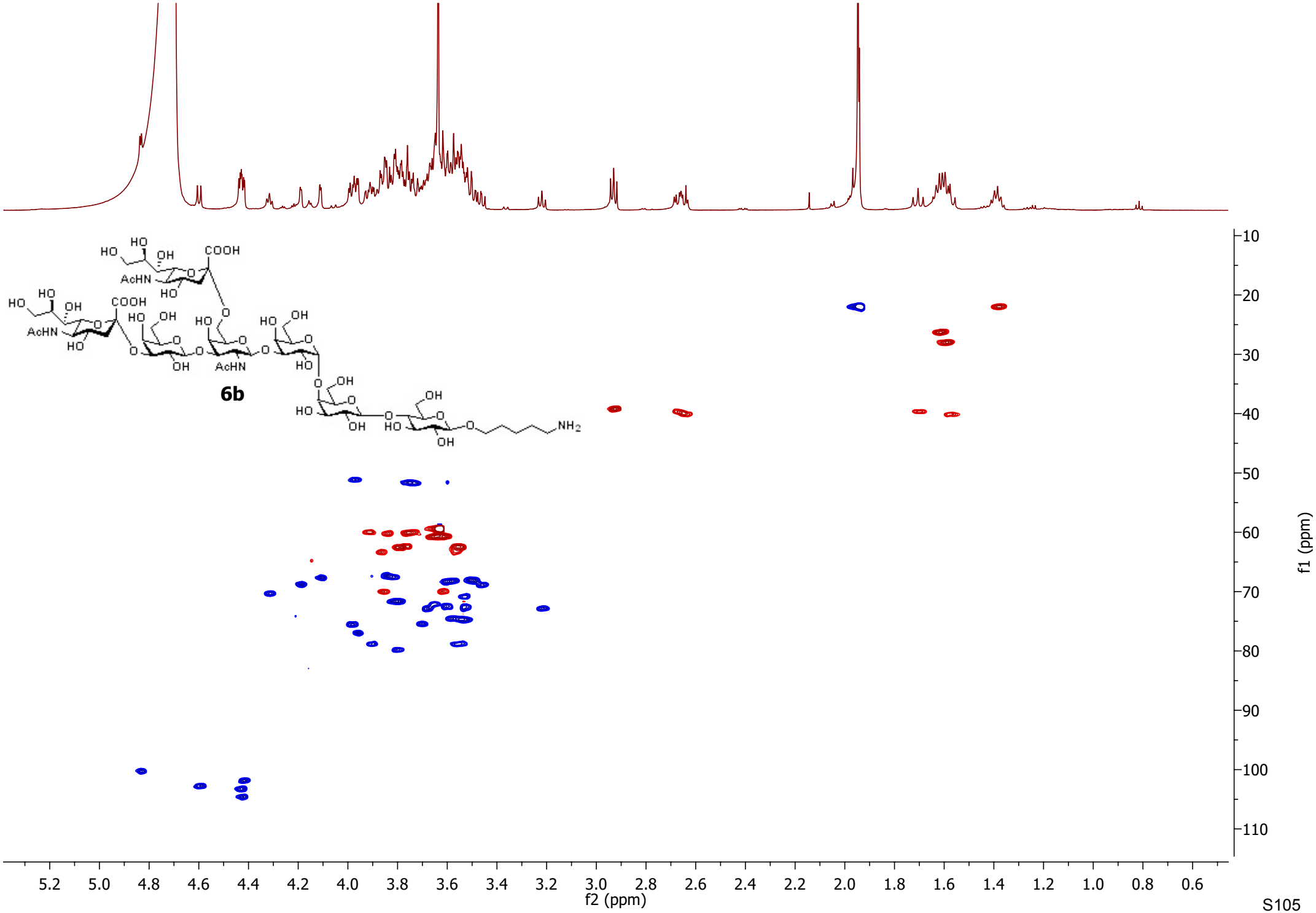
6a

5.6
5.4
5.2
5.0
4.8
4.6
4.4
4.2
4.0
3.8
3.6
3.4
3.2
3.0
2.8
2.6
2.4
2.2
2.0
1.8
1.6
1.4
1.2
f2 (ppm)
45
50
55
60
65
70
75
80
85
90
95
100
105
f1 (ppm)


6b

3E+07
2E+07
2E+07
2E+07
1E+07
5E+06
0
5.8
5.6
5.4
5.2
5.0
4.8
4.6
4.4
4.2
4.0
3.8
3.6
3.4
3.2
3.0
2.8
2.6
2.4
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8
f1 (ppm)


6b
f2 (ppm)
f1 (ppm)
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5


6b
f2 (ppm)
f1 (ppm)