Chemical Synthesis and Immunological Evaluation of Entirely Carbohydrate Conjugate Globo H-PS A1

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Supporting Information

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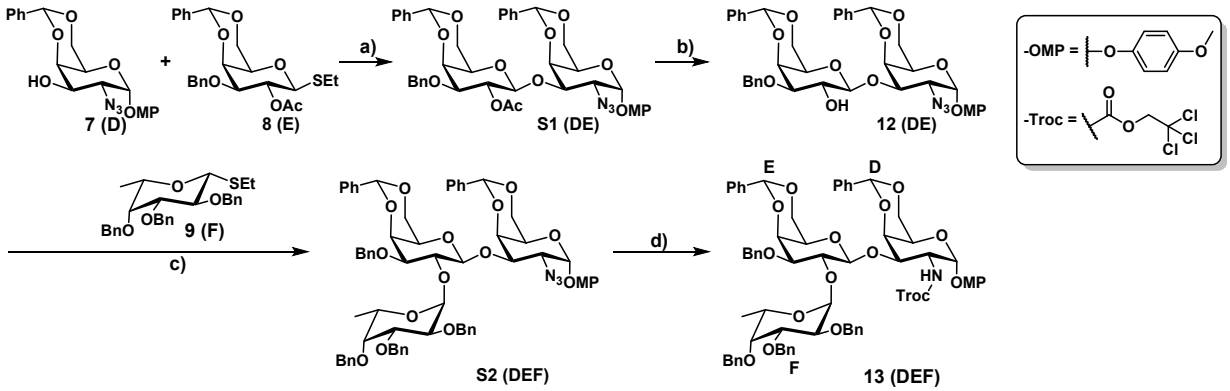
General Experimental Methods

Galactosamine hydrochloride were purchased form Ningbo Hongxiang Biotechonology Co. ltd. Relevant reagents and solvents were purchased from commercial sources and used without further purification. MS 4Å were activated by heating at 140 °C overnight under vacuum. Compounds were purified using flash chromatography with SiliCycle® Inc. 60 Å 230-400 mesh silica gel, or size exclusion chromatography with Bio-Rad Biogel P-2 or Sephadex® G-10. Thin layer chromatography (TLC) was performed with SiliCycle® Inc. silica gel TLC 250 μm w/h F-254. $CDCl_3$, MeOD, and D_2O used as standard NMR solvent. ^1H, ^{13}C, DEPT 135, ^1H-^1H COSY and HMQC NMR spectra were recorded using Bruker Avance III 600 Ultrafield Cryoprobe spectrometers. The residual $CDCl_3$ was referenced to δ 77.26 and δ 7.26 ppm in carbon and proton spectra respectively. The residual HDO was referenced at δ 4.79 with spectra taken in D_2O. Chemical shifts are reported in δ ppm. Data for ^1H NMR are reported as follows: chemical shift, multiplicity (s = singlet, br s = broad singlet, d = doublet, dd = doublet of doublet, t = triplet, m = multiplet), integration and coupling constants in Hertz (Hz). Low resolution mass spectrometry data were taken on an LCQ Deca ESI-MS machine. High resolution mass spectrometry data were collected using a SCIEX Orbitrap Fusion™ Tribrid™ Mass Spectrometer. MALDI spectrometry data were collected on a Bruker UltrafleXtreme MALDI-TOF/TOF Mass Spectrometer. Yields refer to chromatographically and spectroscopically pure material unless otherwise noted. Zwitterionic polysaccharide PS A1 was isolated and purified as described previously.[1-3]

Synthesis of DEF trisaccharide 13



Scheme S1. Synthesis of **DEF** trisaccharide **13**. Reagents and conditions: a) NIS/TMSOTf, CH_2Cl_2, 4 Å molecular sieves, -30 °C, 30 min, 73%; b) NaOMe/MeOH (0.1 M), 40 °C, 12 h, 81%; c) compound **9**, NIS/TMSOTf, 4 Å molecular sieves, CH_2Cl_2/Et_2O (1:1), -30 °C, 30 min, 70%; d) (i) 1,3-propanedithiol, Et_3N, CH_2Cl_2/MeOH (1:1), reflux, 12 h; (ii) TrocCl, $NaHCO_3$, THF, 3 h, 92%.

4-Methoxyphenyl (2-*O*-acetyl-3-*O*-benzyl-4,6-*O*-benzylidene-β-D-galactopyranosyl)-(1→3)-2-azido-4,6-*O*-benzylidene-2-deoxy-α-D-galactopyranoside (S1):

Under an argon atmosphere, compounds **7** (1.00 g, 2.50 mmol) and **8** (1.33 g, 2.99 mmol) were dissolved in anhydrous DCM (25 mL). MS 4Å (0.7 g) were then added and the mixture was allowed to stir at room temperature for 45 minutes. N-iodosuccinimide (0.81 g, 3.60 mmol) was added and the reaction mixture cooled to -30 °C. After 10 minutes at the same temperature, trifluoromethanesulfonate (25 μL, 0.25 mmol) was added and the reaction mixture was stirred for another 30 min until it completion. Progress was noted by TLC. The reaction mixture was quenched with saturated sodium bicarbonate. The aqueous layer was extracted with 2x DCM. Combined organic layers were washed with sodium thiosulfate solution and brine, then dried over anhydrous Na_2SO_4, filtered through a pad of Celite® and concentrated under reduced pressure to give the crude product. This crude material was then purified on a silica gel column using 1:2 hexane/EtOAc to afford compound **S1** as a white solid (1.43 g, 1.83 mmol, 73%).

^{1}H NMR (600 MHz, $CDCl_3$): δ 7.58-6.85 (m, 19H, Ar-H), 5.63 (d, J = 3.2 Hz, 1H, 1_D), 5.62 (s, 1H, PhC*H*), 5.52 (s, 1H, PhC*H*), 5.48 (dd, J = 8.0, 9.9 Hz, 1H, 2_E), 4.92 (d, J = 7.9 Hz, 1H, 1_E), 4.74 (d, J = 12.6 Hz, 1H, PhCH_2), 4.68 (d, J = 12.6 Hz, 1H, PhCH_2), 4.60 (d, J = 12.6 Hz, 1H, 4_D), 4.52 (dd, J = 3.3, 10.7 Hz, 1H, 3_D), 4.38 (dd, J = 1.4, 12.3 Hz, 1H, 6_{E1}), 4.26 (dd, J = 1.4, 12.6 Hz, 1H, 6_{E1}), 4.22 (d, J = 3.1 Hz, 1H, 4_E), 4.10-4.05 (m, 2H, 6_{D2}, 6_{E2}), 3.95 (dd, J = 3.3, 10.7 Hz, 1H, 2_D), 3.85 (br, 1H, 5_D), 3.80 (s, 3H, OCH_3), 3.66 (dd, J = 3.4, 10.0 Hz, 1H, 3_E), 3.51 (br, 1H, 5_E), 2.07 (s, 3H, 6_F)

^{13}C NMR (150 MHz, $CDCl_3$): δ 169.8 (*C*OCH_3) , 155.4-114.9 (Ar-C), 101.6 (1_E), 101.5 (PhC*H*), 100.8 (PhC*H*), 98.6 (1_D), 77.7 (3_E), 75.9 (4_D), 73.3 (3_D), 73.2 (4_E), 71.4 (OBn), 70.4 (2_E), 69.3 (6_D), 69.2 (6_E), 66.9 (5_E), 64.0 (5_D), 59.0 (2_D), 55.9 (O*C*H_3), 21.2 (CO*C*H_3). ESI-MS [(M + Na)] calculated for C_{42} $H_{43}N_3NaO_{12}$ is 804.2744, found 804.2756

4-Methoxyphenyl (3-*O*-benzyl-4,6-*O*-benzylidene-β-D-galactopyranosyl)-(1→3)-2-azido-4,6-*O*-benzylidene-2-deoxy-α-D-galactopyranoside (12):

A solution of compound **S1** (1.43 g, 1.83 mmol) in 0.1 M MeONa in MeOH (25 mL) was stirred at 40 °C for 12 h. The reaction mixture was neutralized with DOWEX® 50X8-100 ion exchange resin (H^+ form), filtered and then evaporated to dryness. Residual water in the crude mixture was removed by azeotrope with anhydrous toluene under vacuum. Purified **12** was obtained from crystallization with 1:1 DCM/*n*-hexane (1.09 g, 1.47 mmol, 81%).

^{1}H NMR (600 MHz, $CDCl_3$): δ 7.58-6.86 (m, 19H, Ar-H), 5.66 (d, *J* = 3.3 Hz, 1H, 1_D), 5.64 (s, 1H, PhC*H*), 5.50 (s, 1H, PhC*H*), 4.87-4.80 (m, 2H, 2 PhCH_2), 4.73 (d, *J* = 7.6 Hz, 1H, 1_E), 4.61 (d, *J* = 3.1 Hz, 1H, 4_D), 4.48-4.46 (m, 1H, 3_D), 4.33 (d, *J* = 12.3 Hz, 1H, 6_{E1}), 4.27 (d, *J* = 12.6 Hz, 1H, 6_{D1}), 4.16-4.14 (m, 2H, 2_E, 4_E), 4.11-4.04 (m, 3H, 2_D, 6_D, 6_E), 3.88 (br, 1H, 5_D), 3.80 (s, 3H, OCH_3), 3.59 (dd, *J* = 3.4, 9.8 Hz, 1H, 3_E), 3.48 (br, 1H, 5_E).

^{13}C NMR (150 MHz, $CDCl_3$): δ 155.5-114.9 (Ar-C), 104.5 (1_E), 101.3 (PhC*H*), 100.1 (PhC*H*), 98.4 (1_D), 78.8 (3_E), 76.3 (4_D), 74.7 (3_D), 73.6 (2_E), 72.1 (OBn), 70.5 (4_E), 69.4 (6_D), 69.3 (6_E), 67.0 (5_E), 64.0 (5_D), 59.1 (2_D), 55.9 (O*C*H_3). ESI-LRMS [(M+Na)] calcd for $C_{40}H_{41}N_3NaO_{11}$ 762.3, found 762.4.

4-Methoxyphenyl (2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-(1→2)-(3-*O*-benzyl-4,6-*O*-benzylidene-β-D-galactopyranosyl)-(1→3)-2-azido-4,6-*O*-benzylidene-2-deoxy-α-D-galactopyranoside (S2):

To a solution of compound **12** (1.09 g, 1.47 mmol) and compound **9** (0.85 g, 1.47 mmol) in anhydrous DCM/diethyl ether (1:1; 30 mL), 4 Å molecular sieves (1.0 g) were added and the reaction mixture was stirred under argon at room temperature for 45 minutes. NIS (0.48 g, 2.13 mmol) was added and the reaction mixture cooled to -30 °C. After 10 minutes at -30 °C, trifluoromethanesulfonate (20 μL, 0.2 mmol) was added and the reaction mixture was stirred until completion of the reaction (30 minutes). Reaction progress was monitored by TLC. After 30 min, the reaction mixture was quenched with saturated aqueous $NaHCO_3$ and aqueous 5% $Na_2S_2O_3$ and the aqueous layer was extracted with 2x DCM. Combined organic layers were washed 3x with 5% $Na_2S_2O_3$ and brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to give crude product. This crude material was purified over silica gel chromatography using hexane–EtOAc (3:1) as the eluent to furnish compound **S2** as an off-white solid (1.10 g, 0.95 mmol, 70%).

^{1}H NMR (600 MHz, $CDCl_3$): δ 7.59-6.92 (m, 34H, Ar-H), 5.70 (d, *J* = 3.3 Hz, 1H, 1_D), 5.69 (s, 1H, PhC*H*), 5.68 (d, *J* = 3.8 Hz, 1H, 1_F), 5.47 (s, 1H, PhC*H*), 5.00 (d, *J* = 7.6 Hz, 1H, 1_E), 4.91-4.85 (m, 2H, 2 PhCH_2), 4.78-4.70 (m, 3H, 3 PhCH_2), 4.66 (d, J = 2.9 Hz, 1H, 4_D), 4.64-4.61 (m, 2H, 2 PhCH_2), 4.57-4.55 (m, 2H, 3_D, PhCH_2), 4.53-4.50 (m, 1H, 5_F), 4.40 (d, *J* = 11.9 Hz, 1H, 6_{E1}), 4.36-4.33 (m, 1H, 2_E), 4.29 (d, *J* = 12.6 Hz, 1H, 6_{D1}), 4.20 (d, *J* = 3.4 Hz, 1H, 4_E), 4.13-4.09 (m, 3H, 6_D, 6_E, 3_F), 4.06-4.04 (m, 1H, 2_F), 3.94 (br, 1H, 5_D), 3.91 (dd, *J* = 3.6, 9.4 Hz, 1H, 3_E), 3.85 (s, 3H, OCH_3), 3.78 (dd, *J* = 3.2, 10.9 Hz, 1H, 2_D), 3.54-3.53 (m, 2H, 5_E, 4_F), 0.97 (d, *J* = 6.5 Hz, 3H, 6_F).

^{13}C NMR (150 MHz, $CDCl_3$): δ 155.6-114.9 (Ar-C), 102.4 (1_E), 101.2 (PhC*H*), 101.0 (PhC*H*), 99.2 (1_D), 97.4 (1_F), 81.7 (3_E), 79.5 (3_F), 78.6 (4_F), 76.6 (4_D), 76.0 (2_F), 74.9 (OBn), 72.9 (OBn), 72.8 (4_E), 72.6 (OBn), 72.2 (3_D), 72.0 (2_E), 71.0 (OBn), 69.4 (6_D), 69.2 (6_E), 66.6 (5_E), 66.5 (5_F), 64.1 (5_D), 59.0 (2_D), 55.9 (O*C*H_3), 16.5 (6_F). ESI-HRMS [(M+Na)] calcd for $C_{67}H_{69}N_3NaO_{15}$ 1178.4626, found 1178.4623

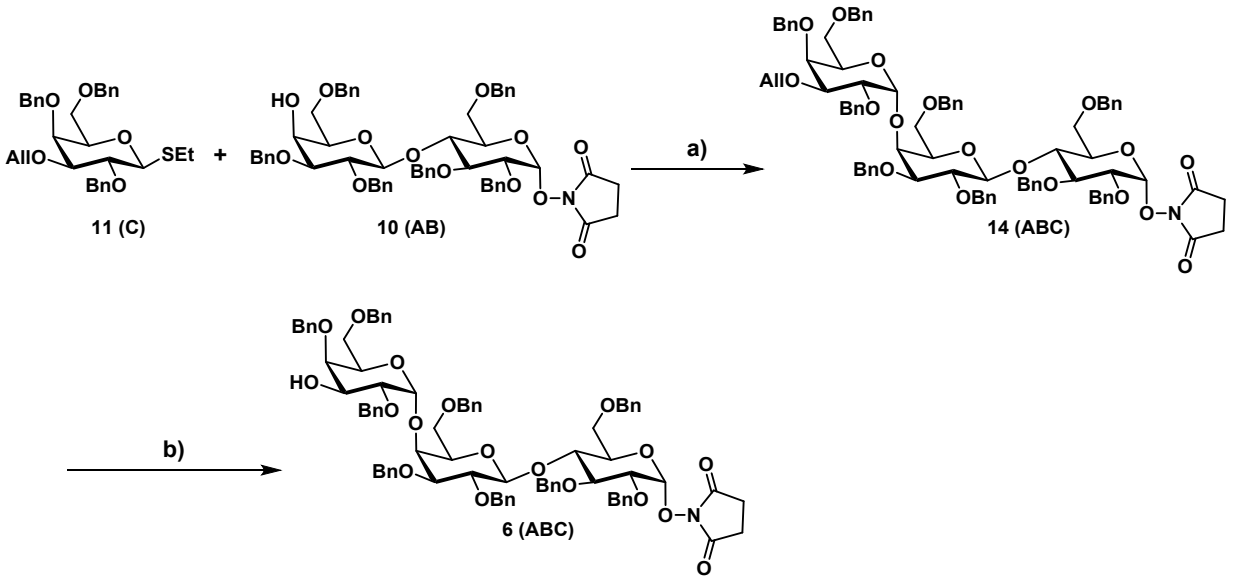
4-Methoxyphenyl (2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-(1→2)-(3-*O*-benzyl-4,6-*O*-benzylidene-β-D-galactopyranosyl)-(1→3)-4,6-*O*-benzylidene-2-deoxy-(2′,2′,2′-trichloroethoxycarbonylamino)-α-D-galactopyranoside (13):

To a solution of compound **S2** (1.10 g, 0.95 mmol) in a mixture of DCM:MeOH (1:1; 30 mL), 1,3-propanedithiol (1.03 mL, 8.8 mmol) and Et_3N (0.96 mL, 13.1 mmol) were added and the reaction was allowed to stir under reflux overnight with an argon atmosphere. Upon complete conversion of **S2**, the reaction mixture was diluted by adding DCM (100 mL), then the organic layer was washed 3x with saturated $NaHCO_3$ and water, dried over Na_2SO_4, filtered, and concentrated under vacuum. The resulting residue was dissolved in anhydrous THF (16 mL) and then $NaHCO_3$ (0.20 g, 2.38 mmol) was added and the mixture stirred for another 10 min. After 10 min, TrocCl (0.17 mL, 1.24 mmol) was added dropwise while the reaction was stirred at room temperature under an atmosphere of argon for 3 h to reach completion. The reaction mixture was filtered, and the filtrate was concentrated and then diluted with DCM (50 mL). The DCM layer was washed with aqueous $NaHCO_3$ and brine, dried over anhydrous Na_2SO_4 and the solvent removed under reduced pressure to give a crude product. This crude material was purified over silica gel chromatography using hexane/EtOAc (3:1) as the eluent to afford compound **13** (1.14 g 0.87 mmol, 92%).

^{1}H NMR (600 MHz, $CDCl_3$): δ 7.59-6.85 (m, 34H, Ar-H), 5.72 (d, *J* = 9.0 Hz, 1H, N*H*), 5.67 (d, *J* = 9.0 Hz, 1H, 1_D), 5.62 (br, 2H, 1_F, PhC*H*), 5.48 (s, 1H, PhC*H*), 4.90-4.85 (m, 2H, 2 PhCH_2), 4.82 (d, *J* = 7.6 Hz, H, 1_E), 4.79 (d, *J* = 12.1 Hz, 1 H, PhCH_2), 4.74-4.70 (m, 2H, PhCH_2, CH_{2a} of NHTroc), 4.65-4.48 (m, 7H, 2_D, 4_D, 4 PhCH_2, CH_{2b} of NHTroc), 4.37-4.24 (m, 5H, 3_D, 6_D, 2_E, 6_E, 4_F), 4.18 (d, *J* = 2.8 Hz, 1H, 4_E), 4.10-4.02 (m, 4H, 6_D, 6_E, 2_F, 5_F), 3.86 (br, 1H, 5_D), 3.80 (s, 3H, OCH_3), 3.76-3.74 (m, 3H, 3_E), 3.52 (br, 1H, 3_F), 3.47 (br, 1H, 5_E), 0.89 (d, *J* = 6.24 Hz, 3H, 6_F).

^{13}C NMR (150 MHz, $CDCl_3$): δ 155.5-114.9 (Ar-C), 102.5 (1_E), 101.5 (PhC*H*), 101.1 (PhC*H*), 98.3 (1_D), 97.5 (1_F), 95.7 (*C*Cl_3), 80.9 (3_E), 79.4 (3_F), 78.4 (4_F), 76.4 (3_D), 76.0 (2_F), 74.9 (OBn), 74.7 (OBn), 73.5 (4_E), 72.2 (4_D), 72.9 (OBn), 72.5 (OBn),72.4 (2_E), 71.2 (CH_2 of NHTroc), 69.4 (6_D), 69.3 (6_E), 66.9 (5_F), 66.7 (5_E), 64.0 (5_D), 55.9 (O*C*H_3), 51.3 (2_D), 16.5 (6_F). ESI-HRMS $[M+Na]^+$ calcd for $C_{70}H_{72}Cl_3NNaO_{17}$ 1326.3764, found 1326.3759.

Synthesis of ABC trisaccharide 6



Scheme S2. Synthesis of **ABC** trisaccharide **6**. a) NIS/TMSOTf, CH_2Cl_2, 4 Å molecular sieves, -30 °C, 30 min, 66%; b). $PdCl_2$, CH_2Cl_2:MeOH (4:1), 3 h, rt, 86%;

***O*-succinimidyl-(3-allyl-2,4,6-tri-*O*-benzyl-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 (14):**

To a solution of compound **10** (1.00 g, 1.02 mmol) and compound **11** (0.65 g, 1.21 mmol) in anhydrous DCM/Ether (4:1, 20 mL) preactivated 4 Å molecular sieves (0.5 g) were added and the reaction mixture was stirred under argon at room temperature for 45 minutes. NIS (0.33 g, 1.47 mmol) was added and the reaction mixture and then it was cooled to 0 °C. After 10 minutes at the same temperature, trifluoromethanesulfonate (TMSOTf; 20 μL) was added and the reaction mixture was stirred until completion of the reaction (1 h) was noted by TLC. The reaction mixture was quenched with saturated aqueous $NaHCO_3$ and aqueous 5% $Na_2S_2O_3$ and was filtered through a bed of Celite®-545 and washed with DCM (100 mL). The organic layer was washed 3x with aqueous $NaHCO_3$ and water, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to give a crude product. This crude material was purified over SiO_2 using hexane–EtOAc (2:1) as the eluent to furnish pure α compound **14** (0.97 g, 0.66 mmol, 66%).[4]

^{1}H NMR (600 MHz, $CDCl_3$): δ 7.49-7.16 (m, 45H, Ar-H), 5.80-5.74 (m, 1H, C*H*=CH_2), 5.52 (d, *J* = 4.1 Hz, 1H, 1_A), 5.20-5.17 (m, 1H, CH=CH_2), 5.12 (d, *J* = 11.0 Hz, 1H, PhCH_2), 5.06-5.04 (m, 1H, CH=CH_2), 5.04 (d, *J* = 3.2 Hz, 1H, 1_C), 4.95-4.69 (m, 10H, 5_A, 9 PhCH_2), 4.52-4.48 (m, 3H, 3 PhCH_2), 4.37-4.35 (m, 1H, 5_C), 4.33-4.32 (d, *J* = 7.7 Hz, 1H, 1_B), 4.31-4.26 (m, 3H, PhCH_2), 4.20-4.13 (m, 3H, 6_{B1}, 2 PhCH_2), 4.06-3.96 (m, 7H, 3_A, 4_A, 6_{A1}, 4_B, 2_C, 4_C, OCH_2=CH), 3.91-3.87 (m, 2H, 3_C, OCH_2=CH), 3.66-3.62 (m, 2H, 2_A, 2_B), 3.57-3.48 (m, 3H, 6_{A2}, 6_{B2}, 6_{C1}), 3.29 (dd, $J_{1/2,1/3}$ = 5.6, 7.9 Hz, 1H, 5_B), 3.22 (dd, $J_{1/2,1/3}$ = 2.6, 10.0 Hz, 1H, 3_B), 3.30-3.17 (m, 1H, 6_{C2}), 2.75 (br, 4H, succinimide).

^{13}C NMR (150 MHz, $CDCl_3$): 171.0 (2 C, -*C*(O)CH_2-CH_2-*C*(O)), 139.5-115.8 (Ar-C), 103.1 (1_B), 101.6 (1_A), 101.1 (1_C), 81.9 (3_B), 79.7 (3_A), 79.3 (3_C), 79.1 (2_B), 77.7 (2_A), 76.5 (2_C), 76.3 (4_C), 75.9 (OBn), 75.7 (CH=*C*H_2), 75.4 (4_B), 75.0 (OBn), 74.6 (4_A), 74.0 (2 C, 2 OBn), 73.7 (5_B), 73.4 (2 C, 2 OBn), 73.2 (2 C, 2 OBn), 72.5 (5_A), 72.3 (OBn), 71.1 (O*C*H_2=CH), 69.6 (5_C), 68.1 (6_A),

68.0 (6_C), 67.7 (6_B), 25.7 (succinimide). ESI-HRMS $[M+Na]^+$ calcd for $C_{88}H_{93}NNaO_{18}$ 1474.6290, found 1474.6287.

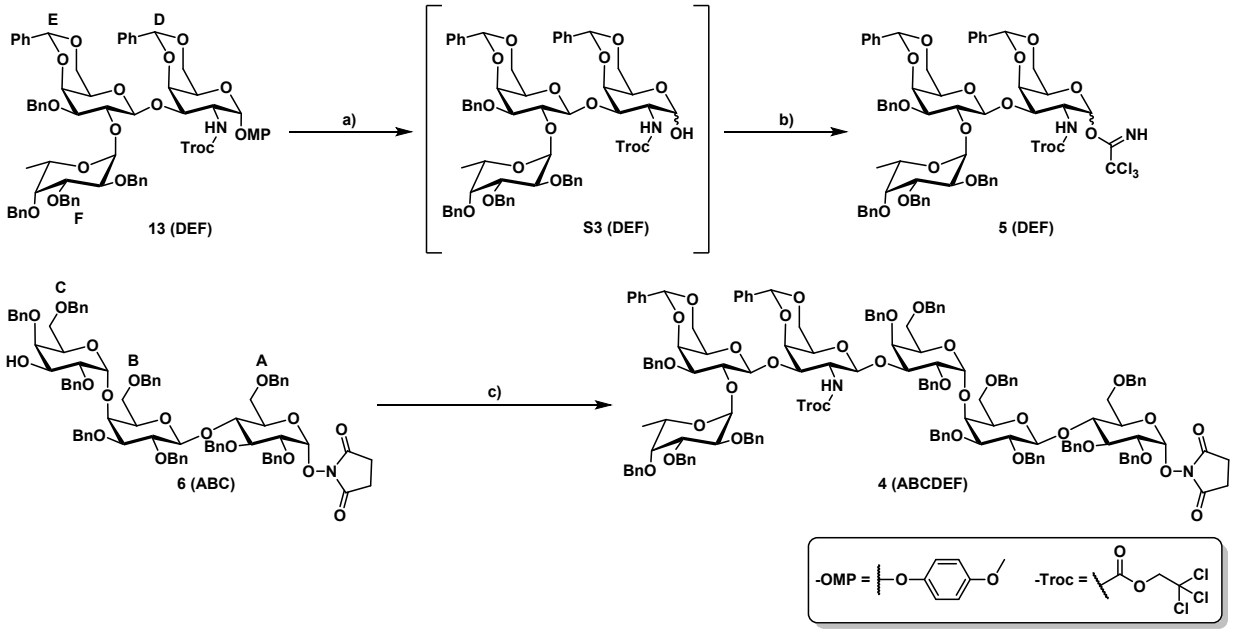
_O_-succinimidyl-(2,4,6-tri-_O_-benzyl-α-D-galactopyranosyl)-(1→4)-(2,3,6-tri-_O_-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-_O_-benzyl-β-D-glucopyranoside (6):

To a solution of compound **14** (0.97 g, 0.66 mmol) in a mixture of DCM:MeOH (4:1; 20 mL) was added $PdCl_2$ (200 mg, 1.13 mmol) and the reaction mixture was allowed to stir at room temperature for 3 h. After the time elapsed, the solvent was removed under vacuum and the reaction mixture was diluted with DCM (50 mL). The organic layer was washed 3x with aqueous $NaHCO_3$ and water in succession, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to give a crude product. The crude material was purified over SiO_2 using hexane–EtOAc (1.5:1) as the eluent to furnish pure α compound **6f** (0.81 g, 0.66 mmol, 86%).

^{1}H NMR (600 MHz, $CDCl_3$): δ 7.49-7.19 (m, 45H, Ar-H), 5.54 (d, *J* = 4.1 Hz, 1H, 1_A), 5.15 (d, *J* = 3.2 Hz, 1H, 1_C), 5.13 (d, *J* = 11.2 Hz, 1H, PhC*H*$_2$), 4.97 (d, *J* = 11.2 Hz, 1H, PhC*H*$_2$), 4.85-4.71 (m, 8H, 5_A, 7 PhC*H*$_2$), 4.60-4.51 (m, 4H, 4 PhC*H*$_2$), 4.44-4.42 (m, 1H, 5_C), 4.39-4.36 (m, 2H, 2 PhC*H*$_2$), 4.35 (d, J = 7.7 Hz, 1H, 1_B), 4.30 (d, J = 11.9 Hz, 1H, PhC*H*$_2$), 4.21 (d, J = 11.7 Hz, 1H, PhC*H*$_2$), 4.16 (d, J = 11.7 Hz, 1H, PhC*H*$_2$), 4.13-4.08 (m, 2H, 6_{A1}, 3_C), 4.06-4.02 (m, 3H, 4_A, 4_B, 6_{B1}), 3.99-3.97 (m, 2H, 3_A, 4_C), 3.85-3.82 (m, 1H, 2_C), 3.68-3.61 (m, 2H, 2_A, 2_B), 3.56-3.52 (m, 3H, 6_{A2}, 6_{B2}, 6_{C1}), 3.31-3.22 (m, 3H, 3_B, 5_B, 6_{C2}), 2.75 (br, 4H, succinimide).

^{13}C NMR (150 MHz, $CDCl_3$):): δ 171.1 (2 C, -*C*(O)C*H*$_2$-C*H*$_2$-*C*(O)), 139.7-127.5 (Ar-C), 102.9 (1_B), 101.7 (1_A), 100.0 (1_C), 81.9 (3_B), 79.9 (3_A), 79.2 (2_B), 78.0 (2_C), 77.8 (2_A), 77.3 (4_C), 76.2 (4_B), 75.9 (OBn), 75.8 (OBn), 75.5 (OBn), 75.4 (4_A), 73.5 (2 C, 5_B, OBn), 73.4 (2 C, 2 OBn), 73.3 (2 C, 2 OBn), 72.6 (5_A), 72.4 (OBn), 70.3 (3_C), 69.5 (5_C), 68.1 (6_A), 68.0 (6_C), 67.9 (6_B), 25.8 (succinimide). ESI-HRMS $[M+Na]^+$ calcd for $C_{85}H_{89}NNaO_{18}$ 1434.5977, found 1434.5983.

Synthesis of ABCDEF hexasaccharide 4



Scheme S3. Synthesis of **ABCDEF** hexasaccharide **4**. a) Ceric Ammonium Nitrate (CAN), CH_3CN/H_2O (4:1), rt, 3 h; b) Trichloroacetonitrile, DBU, CH_2Cl_2, 0 °C, 1 h, 92%; c) **5**, TMSOTf, CH_2Cl_2, 4 Å molecular sieves, -30 °C, 45 min, 39%

Trichloroacetimidate-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-(1→2)-(3-*O*-benzyl-4,6-*O*-benzylidene-β-D-galactopyranosyl)-(1→3)-4,6-*O*-benzylidene-2-deoxy-(2′,2′,2′-trichloroethoxycarbonylamino)-D-galactopyranoside (5)

To a solution of compound **13** (1.14 g 0.87 mmol) in CH_3CN/H_2O (4:1, 25 mL), ceric ammonium nitrate (CAN; 1.87 g, 3.42 mmol) was added and the reaction mixture was stirred at room temperature for 3 h. The reaction mixture was diluted with DCM (50 mL) and the organic layer was washed 3x with aqueous $NaHCO_3$ and water. The organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to give crude intermediate **DEF** trisaccharide hemiacetal **S3**. Trichloroacetonitrile (0.21 mL, 1.48 mmol) was added to the solution of **S3** in anhydrous DCM (20 mL) at 0 °C. DBU (0.1 mL, 0.65 mmol) was added to the cooled reaction mixture and allowed to stir for another 1 h. Upon complete consumption of the starting material, as noted by TLC, the reaction mixture was evaporated to dryness and the crude product was passed through a short silica gel flash column (hexane/EtOAc=1:1) to furnish compound **5** (0.76 g, 0.56 mmol, two steps 65%), which was immediately used for the next step reaction. **Note:** *Silica gel was neutralized with 1% triethylamine in hexane before packing the column; **5** is not bench stable and extended hours at room temperature will lead to decomposition. Furthermore, rotary evaporator water bath temperature was set at 25°C. **5** should be immediately subjected to the next glycosylation procedure.*

***O*-succinimidyl-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-(1→2)-(3-*O*-benzyl-4,6-*O*-benzylidene-β-D-galactopyranosyl)-(1→3)-(4,6-*O*-benzylidene-2-deoxy-(2′,2′,2′-trichloroethoxycarbonylamino)-α-D-galactopyranosyl)-(1→3)-(2,4,6-tri-*O*-benzyl-α-D-**

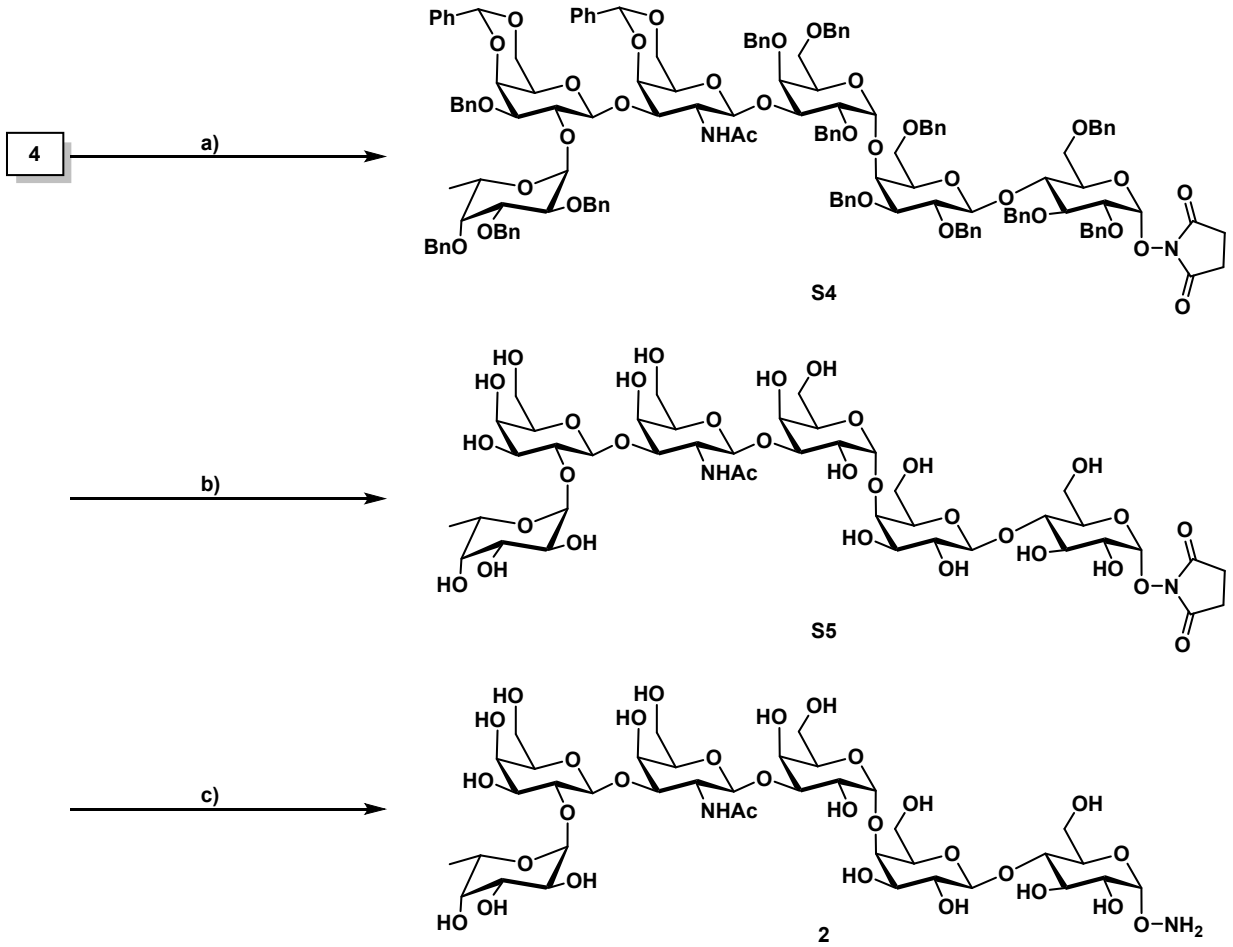
galactopyranosyl)-(1→4)-(2,3,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (4):

5 (**DEF**, 0.76 g, 0.56 mmol) and **6** (**ABC**, 0.61 g, 0.43 mmol) were dissolved in anhydrous DCM (20 mL). 4 Å Molecular sieves (1.0 g) were added and the reaction mixture was stirred at 0 °C for 30 minutes under an atmosphere of argon. Then the reaction mixture was cooled to -30 °C and TMSOTf (25 μL) was added and the reaction was allowed to stir at the same temperature for 45 minutes to reach completion. The reaction was quenched with excess triethylamine and was diluted with DCM (75 mL). The organic layer was washed with saturated $NaHCO_3$ and brine, dried over Na_2SO_4 and evaporated to dryness. The crude product was purified over a silica gel column using hexane-EtOAc (1:1) as eluent to give **4** (0.43 g, 0.16 mmol, 39%).

^{1}H NMR (600 MHz, $CDCl_3$): δ 7.57-7.07 (m, 75H, Ar-H), 5.54 (d, *J* = 3.9 Hz, 1H, 1_A), 5.15 (d, *J* = 3.5 Hz, 1H, 1_F), 5.49 (s, 1H, PhC*H*), 5.47 (s, 1H, PhC*H*), 5.26 (d, *J* = 11.4 Hz, 1H, PhCH_2), 5.12 (d, *J* = 10.9 Hz, 1H, PhCH_2), 4.97 (d, *J* = 2.9 Hz, 1H, 1_C), 4.95 (d, *J* = 11.5 Hz, 1H, PhCH_2), 4.88-4.85 (m, 3H, 2 PhCH_2, CH_{2a} of NHTroc), 4.81-4.69 (m, 8H, 7 PhCH_2, CH_{2b} of NHTroc), 4.67-4.63 (m, 3H, 5_A, 2 PhCH_2), 4.58- 4.46 (m, 6H, 1_E, 5 PhCH_2), 4.43-4.38 (m, 4H, 5_C, 1_D, , 2 PhCH_2), 4.32- 4.24 (m, 5H, 1_B, 6_{D1}, 3 PhCH_2), 4.17-4.07 (m, 12H, 6_{B1}, 2_C, 3_C, 4_C, 4_D, 6_{D2}, 2_E, 4_E, 6_{E1}, 4_F, 2 PhCH_2), 4.03-3.98 (m, 4H, 4_A, 6_{A2}, 4_B, 2_D), 3.96-3.86 (m, 3H, 3_A, 6_{E2}, 2_F), 3.69-3.65 (m, 2H, 2_A, 2_B), 3.60-3.55 (m, 2H, 3_E, 5_F), 3.54-3.45 (m, 4H, 6_{A2}, 6_{B2}, 6_{C1}, 3_F), 3.32 (br, 1H, 5_D), 3.28-3.23 (m, 2H, 3_B, 5_B), 3.21-3.19 (m, 1H, 6_{C2}), 2.94 (br, 1H, 5E), 2.74 (br, 4H, succinimide), 0.45 (d, *J* = 6.2 Hz, 3H, 6_F).

^{13}C NMR (150 MHz, $CDCl_3$): δ 171.0 (2 C, -*C*(O)CH_2-CH_2-*C*(O)), 139.7-126.4 (Ar-C), 103.0 (1_D), 102.9 (1_B), 102.1 (1_E), 101.7 (PhC*H*), 101.3 (1_A), 100.9 (2 C, 1_C, PhC*H*), 97.4 (1_F), 95.8 (*C*Cl_3), 81.6 (3_B), 81.2 (3_E), 80.8 (4_E), 79.3 (2_B), 79.2 (4_F), 79.0 (3_A), 78,8 (3_F), 77.8 (2_A), 77.7 (4_C), 77.4 (3_D), 76.4 (2_F), 76.0 (2_C), 75.9 (4_D), 75.6 (OBn), 75.5 (4_B), 75.4 (OBn), 75.1 (OBn), 75.0 (OBn), 74.6 (4_A), 74.5 (OBn), 73.6 (2 C, 2_E ,OBn), 73.3 (5_B), 73.2 (2 C, 2 OBn),73.1 (OBn), 72.8 (OBn), 72.5 (OBn), 72.4 (5_A), 72.4 (5_F), 71.9 (OBn), 71.2 (OBn), 69.5 (5_C), 69.4 (6_D), 69.1 (6_E), 68.0 (6_C), 67.8 (6_B), 67.7 (6_A), 66.9 (5_D), 66.7 (3_C), 66.4 (5_E), 54.3 (2_D), 25.7 (succinimide), 16.1 (6_F). ESI-HRMS $[M+Na]^+$ calcd for $C_{148}H_{153}Cl_3N_2NaO_{33}$ 2613.9320, found 2613.9315.

Synthesis of aminooxy Globo H (2)



Scheme S4. Synthesis of aminooxy Globo H (**2**)**.** a) activated Zn, THF/AcOH/Ac_2O (3:2:1), 0 °C to rt, 72%; b) 10% Pd-C, H_2, MeOH, rt, 3 h; c) NH_2-NH_2·H_2O, MeOH/H_2O (1:1), rt, 12 h, 57%.

***O*-succinimidyl-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-(1→2)-(3-*O*-benzyl-4,6-*O*-benzylidene-β-D-galactopyranosyl)-(1→3)-(4,6-*O*-benzylidene-2-deoxy-(2-acetamido)-α-D-galactopyranosyl)-(1→3)-(2,4,6-tri-*O*-benzyl-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 (S4)**

To a solution of compound **15** (0.43 g, 0.16 mmol, 1 equiv.), in anhydrous THF (12 mL) at 0 °C, acetic acid (8 mL/g), acetic anhydride (4 mL/g), and zinc dust (600 mg), were added. After addition of all the reagents, the reaction was slowly allowed to run at room temperature and complete consumption of the starting material (5 h) was noted by TLC. The reaction mixture was filtered through a pad of Celite®-545. The acid was quenched with saturated $NaHCO_3$. The reaction mixture was extracted 3x with DCM. The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude product was purified over a silica gel column using hexane-EtOAc (1:1) as the eluent to give compound **S4** (0.29 g, 0.12 mmol, 72%).

^{1}H NMR (600 MHz, $CDCl_3$): δ 7.57-7.10 (m, 75H, Ar-H), 5.56 (d, *J* = 3.4 Hz, 1H, 1_F), 5.55-5.53 (m, 2H, 1_A, PhC*H*), 5.47 (s, 1H, PhC*H*), 5.27 (d, *J* = 11.6 Hz, 1H, PhCH_2), 5.14 (d, *J* = 11.3 Hz, 1H, PhCH_2), 5.07 (d, *J* = 8.2 Hz, 1H, N*H*), 4.98 (d, *J* = 2.9 Hz, 1H, 1_C), 4.96 (d, *J* = 11.4 Hz, 1H,

PhCH_2), 4.88-4.61 (m, 14H, 5_A, 13 PhCH_2), 4.55-4.46 (m, 7H, 1_D, 1_E, 5 PhCH_2), 4.45-4.42 (m, 2H, 4_C, 5_C), 4.34-430 (m, 3H, 1_B, 6_{D1}, PhCH_2), 4.27-4.24 (m, 2H, 2 PhCH_2), 4.22-4.18 (m, 11H, 6_{B1}, 3_C, 2_D, 4_D, 6_{D2}, 2_E, 4_E, 6_{E1}, 5_F, 2 PhCH_2), 4.14-3.87 (m, 8H, 3_A, 4_A, 6_{A1},4_B, 2_C, 2_F, 3_F, 6_{E2}), 3.71-3.66 (m, 5H, 2_A, 2_B, 3_D, 3_E, 4_F), 3.54-3.47 (m, 3H, 6_{A2}, 6_{B2}, 6_{C1}), 3.39 (br, 1H, 5_D), 3.29-3.22 (m, 3H, 3_B, 5_B, 6_{C2}), 3.07 (br, 1H, 5_E), 2.74 (br, 4H, succinimide), 1.54 (s, 3H, COCH_3), 0.64 (d, J = 6.4 Hz, 3H, 6_F).

^{13}C NMR (150 MHz, $CDCl_3$): δ 171.0 (2 C, -*C*(O)CH_2-CH_2-*C*(O)), 169.9 (*C*OCH$_3$), 139.7-126.4 (Ar-C), 103.3 (1_E), 102.9 (1_B), 102.6 (1_D), 101.4 (PhC*H*), 101.3 (1_A), 100.9 (PhC*H*), 100.8 (1_C), 97.2 (1_F), 81.5 (3_B), 81.4 (2 C, 4_E, 4_F), 79.3 (3_F), 79.2 (2_B), 78.8 (2_A), 78.5 (3_F), 77.7 (2 C, 3_A, 4_C), 76.0 (3_D), 75.9 (2_F), 75.8 (2_C), 75.6 (4_D), 75.5 (OBn), 75.3 (OBn), 75.0 (OBn), 74.7 (OBn), 74.5 (4_B), 74.3 (4_A), 73.6 (OBn), 73.3 (2_E), 73.2 (2 C, 2 OBn), 73.1 (2 C, 2 OBn), 72.7 (5_B), 72.4 (5A), 72.3 (2 C, 2 OBn), 72.1 (OBn), 72.0 (5_F), 71.8 (OBn), 70.7 (OBn), 69.5 (5_C), 69.4 (6_D), 69.2 (6_E), 68.2 (6_C), 67.7 (6_B), 67.6 (6_A), 66.8 (3_C), 66.7 ($_{5D}$), 66.4 (5_E), 52.3 (2_D), 25.7 (succinimide), 23.7 (NHCOCH_3), 16.0 (C*C*H$_3$). ESI-MS [(M + Na)] calculated for $C_{147}H_{154}N_2NaO_{32}$ is 2482.0382, found 2482.0452.

_O_-succinimidyl-(α-L-fucopyranosyl)-(1→2)-(β-D-galactopyranosyl)-(1→3)-(2-deoxy-2-acetamido-α-D-galactopyranosyl)-(1→3)-(α-D-galactopyranosyl)-(1→4)-(β-D-galactopyranosyl)-(1→4)-β-D-glucopyranoside (S5)

To a solution of compound **S4** (0.29 g, 0.12 mmol) in methanol (30 mL) was added 10% Pd/C (0.10 g) and the mixture was stirred at room temperature under a 1 atm of hydrogen gas. Reaction progress was monitored by ESI mass spectra (LRMS). Upon the completion (3-4 hours), the reaction mixture was filtered through a pad of Celite®-545 and then washed with MeOH (20 mL). The combined filtrate was evaporated under reduced pressure to obtain the crude product, which was purified using a Bio-Rad Biogel P-2 size exclusion column using 100% H_2O as the eluent to give **S5** (49 mg, 0.12 mmol, 38%). **Note:** *Minor decomposition was detected after 3 hours. We found that recharging the reaction mixture with fresh Pd/C can alleviate this problem. LR-ESI mass spec indicated the loss of the O-succinimidyl group at the reducing end.*

^{1}H NMR (600 MHz, D_2O): δ 5.34 (d, J = 3.7 Hz, 1H), 5.11 (d, J = 4.0 Hz, 1H), 4.78 (d, J = 3.8 Hz, 1H), 4.50 (d, J = 7.7 Hz, 1H), 4.43 (d, J = 7.6 Hz, 2H), 4.35-4.33 (m, 1H), 4.30 (t, J = 6.4 Hz, 1H), 4.13-4.11 (m, 2H), 3.99 (d, J = 2.2 Hz, 1H), 3.92 (d, J = 2.2 Hz, 1H), 3.88-3.51 (m, 28H), 2.70 (br, 4H), 1.92 (s, 3H), 1.10 (d, J = 6.5 Hz, 1H).

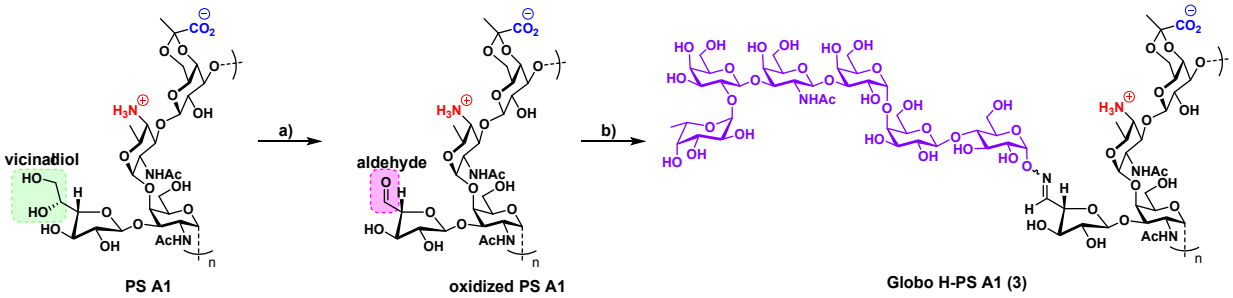
^{13}C NMR (150 MHz, D_2O): δ 174.9 (2 Carbonyl), 174.2, 104.0, 103.1, 103.0, 102.0, 100.4, 97.2, 78.3, 77.5, 77.0, 76.3, 76.1, 75.5, 75.0, 74.6, 73.5, 72.5, 72.0, 71.8, 70.9, 70.8, 70.3, 70.0, 69.4, 69.2, 69.0, 68.4, 68.0, 67.8, 66.7, 60.9, 60.3, 59.3, 52.3, 25.3, 22.2, 15.3. LRMS $[M+Na]^+$ calcd for $C_{42}H_{68}N_2NaO_{32}$ 1135.4, found 1135.5.

Aminooxy-(α-L-fucopyranosyl)-(1→2)-(β-D-galactopyranosyl)-(1→3)-(2-deoxy-2-acetamido-α-D-galactopyranosyl)-(1→3)-(α-D-galactopyranosyl)-(1→4)-(β-D-galactopyranosyl)-(1→4)-α-D-glucopyranoside (2):

S5 (49 mg, 0.12 mmol) was treated with hydrazine hydrate (0.2 mL, 10 equiv.) in 2 mL MeOH/H_2O (1:1) and the mixture was stirred at room temperature overnight. The reaction was monitored by TLC (product R_f 0.3, H_2O/n-Butanol/AcOH = 4:1:1) and ESI mass spec (LRMS). After completion of the reaction was noted, the mixture was concentrated to 1 mL under reduced pressure, which was then loaded onto a Bio-Rad Biogel P-2 size exclusion column using H_2O as the eluent to give pure compound **2** (25 mg, 0.02 mmol, 57%).

^{1}H NMR (600 MHz, D_2O): δ 5.11 (d, *J* = 3.8 Hz, 1H), 4.89 (d, *J* = 3.8 Hz, 1H), 4.77 (d, *J* = 3.5 Hz, 1H), 4.50 (d, *J* = 7.7 Hz, 1H), 4.43 (d, *J* = 7.6 Hz, 1H), 4.41 (d, *J* = 7.9 Hz, 1H), 4.29 (t, *J* = 6.3 Hz, 1H), 4.13-4.11 (m, 2H), 3.99 (br, 1H), 3.92 (br, 1H), 3.88-3.46 (m, 29H), 1.93 (s, 3H), 1.10 (d, *J* = 6.5 Hz, 3H).
^{13}C NMR (151MHz, D_2O): δ 174.3, 104.0, 103.2, 102.0, 101.2, 100.4, 99.2, 78.4, 78.3, 77.0, 76.3, 76.1, 75.5, 75.0, 74.6, 73.5, 72.0, 71.8, 71.6, 70.8, 70.5, 70.4, 70.1, 69.5, 69.2, 69.1, 68.4, 68.0, 67.8, 66.8, 60.3, 59.6, 51.6, 22.2, 15.3. ESI-HRMS [(M + Na)] calculated for $C_{38}H_{66}N_2NaO_{30}$ is 1053.3598, found 1053.3605.

Preparation of Globo H-PS A1 (3)

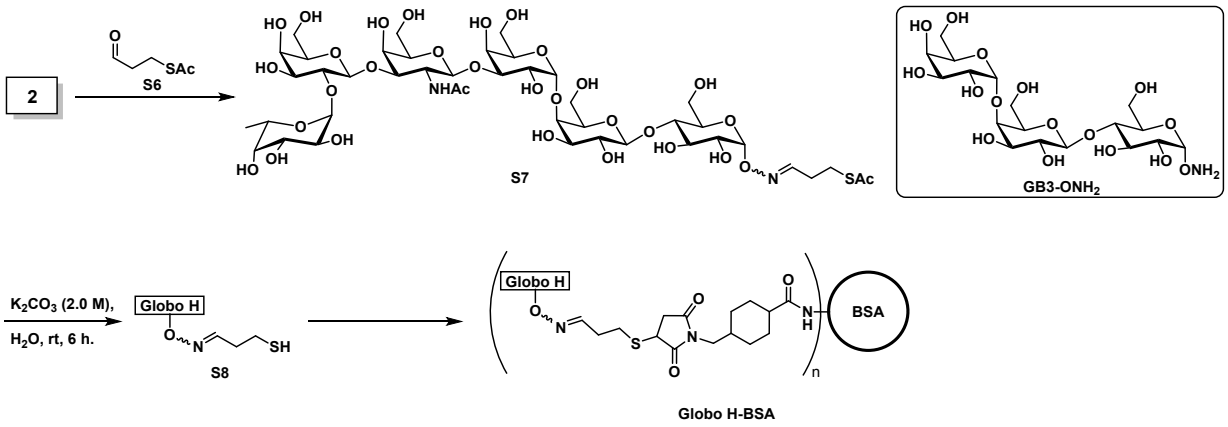


Scheme S5. Preparation of Globo H-PS A1 **(3)**. a) $NaIO_4$, NaOAc buffer (0.1 M, pH 5.0), rt, in the dark, 1.5 h.; b) Globo H-PS A1 (**3**), NaOAc buffer (0.1 M, pH 5.0), rt, 18 h.

Globo H-PS A1 (3):

PS A1 (1 mg, 9.1 x 10^{-9} mol) was dissolved in NaOAc buffer (1.0 mL, 0.1 M, pH 5.0). To this solution was added 55 µL of 0.01 M $NaIO_4$ solution (5.5 x 10^{-7} mol). The reaction was stirred for 90 min, exclusive from light, at room temperature. The excess $NaIO_4$ was quenched by adding ethylene glycol and the mixture was allowed to stir for another 20 min in the dark. The oxidized PS A1 was purified with a centrifugal filter (Vivaspin®, molecular cut-off 10 kDa) and the residue was then dissolved in NaOAc buffer (1.0 mL, 0.1 M, pH = 5.0). Subsequently, 2.9 mg (4.4 x 10^{-6} mol) of aminooxy Globo H (**2**) was added and the reaction mixture was shaken gently for another 16 hours at room temperature. The resulting conjugate was purified by centrifugal filter (Vivaspin®, molecular cut-off 10 kDa), washed 2x with 300 µL deionized water. Globo H-PS A1 (**3**) was obtained as porous solid (1.1 mg) after lyophilization.[5-7]

Preparation of Globo H-BSA conjugates



Scheme S6. Preparation of Globo H-BSA conjugates.

Aminooxy Globo H (**2**) was treated with aldehyde linker **S6** (3.0 equiv.) in sodium acetate buffer (0.1 M, pH 5.0) for 18 hours at room temperature. The reaction mixture was passed through a Sephadex® G-10 size exclusion column and eluted with deionized water. The fractions containing **S7** were combined and lyophilized to obtain purified **S6** as an off-white solid.[8]

^{1}H NMR (D_2O,600MHz): δ 7.56 (t, *J*=6.2 Hz, 1H), 5.34 (d, *J*=3.7 Hz, 1H), 5.12 (d, *J*=4.4 Hz, 1H), 4.75 - 4.83 (m, 8H), 4.74 (brs, 7H), 4.64 - 4.70 (m, 11H), 4.49 - 4.53 (m, 1H), 4.40 - 4.46 (m,

3H), 4.27 - 4.31 (m, 1H), 4.10 - 4.15 (m, 3H), 3.98 - 4.01 (m, 1H), 3.90 - 3.93 (m, 2H), 3.77 - 3.89 (m, 9H), 3.71 - 3.76 (m, 7H), 3.51 - 3.70 (m, 26H), 3.49 - 3.50 (m, 1H), 3.48 (t, *J*=2.0 Hz, 1H), 2.95 - 3.03 (m, 2H), 2.40 - 2.50 (m, 1H), 2.24 - 2.29 (m, 3H), 1.91 - 1.95 (m, 3H), 1.07 - 1.13 ppm (m, 3H).
LRMS $[M+Na]^+$ calcd for $C_{43}H_{72}N_2NaO_{31}S$ 1167.4, found 1167.6.

Compound **S7** (2 mg, 1.75 μM) was deacetylated in a K_2CO_3 solution (2.0 M, 2mL). The reaction mixture was shaken gently for 6 hours at room temperature. The resulting mixture was allowed to pass through a short column of AmberLite™ IRC-120H ion exchange resin (H^+ form, 1×4 cm) and the column was then flashed with additional deionized water. All the eluent was collected and lyophilized to give **S8** (1.6 mg, 1.45 μM). Intermediate **S8** was then dissolved in 250 μL 20 mM PBS buffer (230 mM NaCl, 2 mM EDTA, pH 7.2). 1 mg of maleimide activated BSA (15-20 maleimide per protein, Thermo Scientific, catalog number: 77116) was dissolved in 1000 μL 0.1 M PBS buffer (0.15 M NaCl, 0.1 M EDTA, pH 7.2). The solution of **S8** was then added to a maleimide activated BSA solution and mixed thoroughly. The resulting mixture was shaken gently for 18 hours at room temperature to afford Globo H-BSA. Globo H-BSA was then purified by centrifugal ultrafiltration (Vivaspin®, molecular weight cut-off 10 kDa) and washed 3x with 200 μL of PBS buffer (20 mM, pH 7.2). Globo H-BSA conjugate was subjected to MALDI-TOF analysis and the average molecule weight was determined to be 92.25 kDa. The percent loading by mass was calculated to be 23.2%. The antigen/protein molar ratio was determined to be 18.8:1. Therefore, on average, 18-19 Globo H antigens were covalently conjugated to one BSA molecule. A Gb3-BSA conjugate was prepared in a similar manner.[8, 9] The antigen/protein molar ratio was determined to be 19.1:1.

Percent loading was calculated using the following equation:

$$\%\ Loading = \frac{M2 - M1}{M2} \times 100$$

*M*1= average MW of maleimide activated BSA (70.83 kDa)
*M*2= average MW of Globo H-BSA conjugate (92.25 kDa)

Antigen/protein molar ratio was determine using following equation

$$moles\ antigen\ per\ protein = \frac{M2 - M1}{M3}$$

*M*1= average MW of maleimide activated BSA (70.83 kDa)
*M*2= average MW of Globo H-BSA conjugate (92.25 kDa)
*M*3= MW of **S8** (1103 Da)

Animal Immunizations

Jax C57BL/6 male mice (6 weeks) were obtained from The Jackson Laboratories and maintained by the Division of Laboratory Animal Resources (DLAR) at the University of Toledo. Animal study was performed in strict compliance with protocols approved by the by the Institutional Animal Care and Use Committee of the University of Toledo (Toledo, Ohio, United States), NIH guidelines for the care and use of laboratory animals (NIH publication No. 85-23, rev. 1985) were observed. Two groups of mice (C57BL/6J, male, n=5) were vaccinated using interperitoneal injections (i.p.) with the Globo H-PS A1 construct formulating 20 μg of Globo H-PS A1 conjugate with 20 μg of Sigma Adjuvant System® (SAS) or 20 μg of TiterMAX® Gold (TMG) in 200 μL PBS buffer. The SAS groups were immunized on day 0, 21, 42 as per manufactures instructions. Blood sera were obtained using a cardiac puncture technique on day 52. The TMG groups were immunized on day 0, 14, 28, 42. Blood sera were obtained using a cardiac puncture technique on day 52. A group of mice (C57BL/6J, male, n=5) was immunized with 20 μg of Globo H-PS A1 conjugate in 200 μL PBS buffer on day 0, 14, 28, 42 and blood sera collected on day 52. Lastly, a group of mice (C57BL/6J, male, n=5) was used as a negative control and only injected 200 μL PBS buffer on day 0, 14, 28, 42. Blood sera was collected on day 52.

Enzyme Linked Immunosorbent Assay (ELISA)

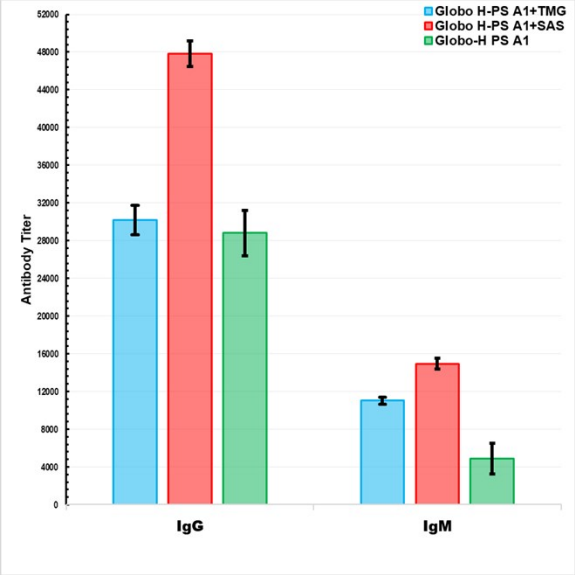


Figure S1. Cross reactivity test of anti-Globo H-PS A1 IgG/IgM antibodies to Gb3 glycan.

ELISA plates (Immulon® Microtiter™ 4 HBX) were coated with 0.1 μg/well of target glycoprotein (Globo H-BSA or Gb3-BSA). The plates were incubated overnight at 4 °C. Nonspecific sites were blocked with 3% bovine serum albumin (BSA) for 2 hours and washed 3x with PBS buffer (pH 7.2). Serially diluted antiserum was added to each well. After 1 hour of incubation, the plates were washed and alkaline phosphatase labelled goat anti-mouse IgM or IgG (Jackson ImmunoResearch) was added at 1:200 dilution. After 30min of incubation in the dark, a stop solution was added. Antibody titers were illustrated as the serum serial dilution resulting in an absorbance of OD405 equal to twice the mean background of the assay.

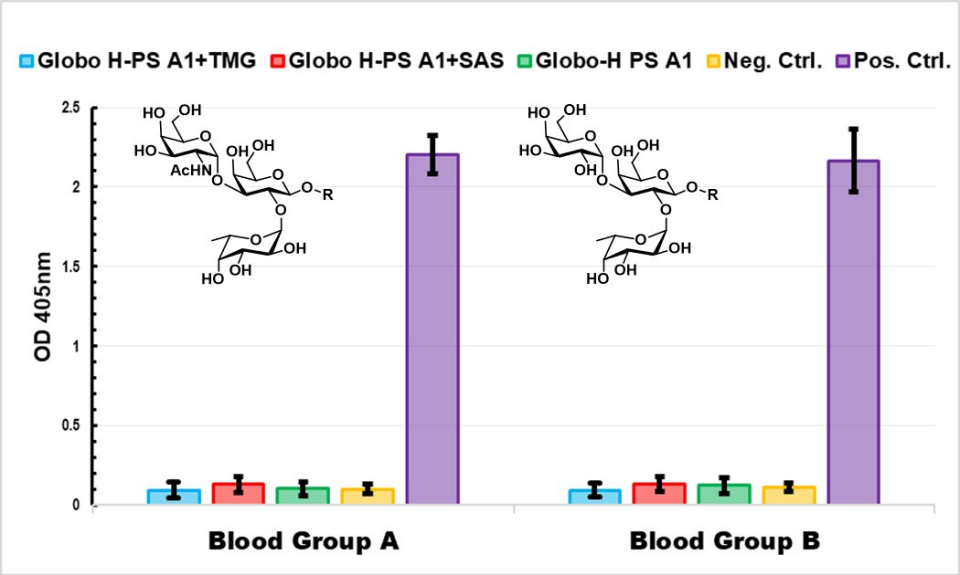


Figure S2. No cross reactivity of anti-Globo H-PS A1 antibodies to Blood Group A and Blood Group B antigens.

ELISA plates (Immulon® Microtiter™ 4 HBX) were coated with 0.1 µg/well of target glycolipids (Blood Group A and Blood Group B antigens). The plates were then incubated overnight at 4 °C. Nonspecific sites were blocked with 3% bovine serum albumin (BSA) and washed 3x with PBS buffer (pH 7.2). 1:200 dilution of antiserum was added to the designated well. After 1 hour of incubation, the plates were washed then alkaline phosphatase labelled goat anti-mouse light chain antibody (IgG, Chemicon®, catalog number: AP200A) was added at 1:200 dilution. After 30min of incubation, exclusive from light, a stop solution was added and the OD value was read at 405 nm. For the negative control (Neg. Ctrl.), antiserum was replaced by the PBS buffer. For the positive control (Pos. Ctrl.), antiserum was replaced with 1:1000 dilution of monoclonal anti-Blood Group A (IgM, Sigma-Aldrich, catalog number: SAB4700674) or anti-Blood Group B (IgM, Sigma-Aldrich, catalog number: SAB4700676) antibody.

Fluorescence-activated cell sorting (FACS)

Ovarian cancer cell line OVCAR-5 and breast cancer cell lines MCF-7 were provided by Dr. Frederic Valeriote at 21st Century Therapeutics. A suspension of 1.0 x 10^6 target cell was incubated with 1:50 dilution of selected anti-serums at 4 ºC for 1 h. The cells were washed 3x with FACS buffer (2 % FBS in PBS, 0.1 % sodium azide). 100 µL 1:50 dilution goat antimouse-IgG labelled with Alexa Fluor® 488 (Jackson ImmunoResearch) was added to the cell suspension and incubated at 4 ºC for 1 h. The resulting cells were collected and washed 3x with FACS buffer. The cells were fixed with freshly made 1% paraformaldehyde solution. Percent positive cells and Mean Fluorescent Intensity (MFI) of stained cells were recorded using a BD FACSCalibur™ flow cytometer. Data were processed and analysed with BD CellQuest™ Pro software (BD Biosciences) and FlowJo™ software (FlowJo LLC).

Complement Dependent Cytotoxicity (CDC)

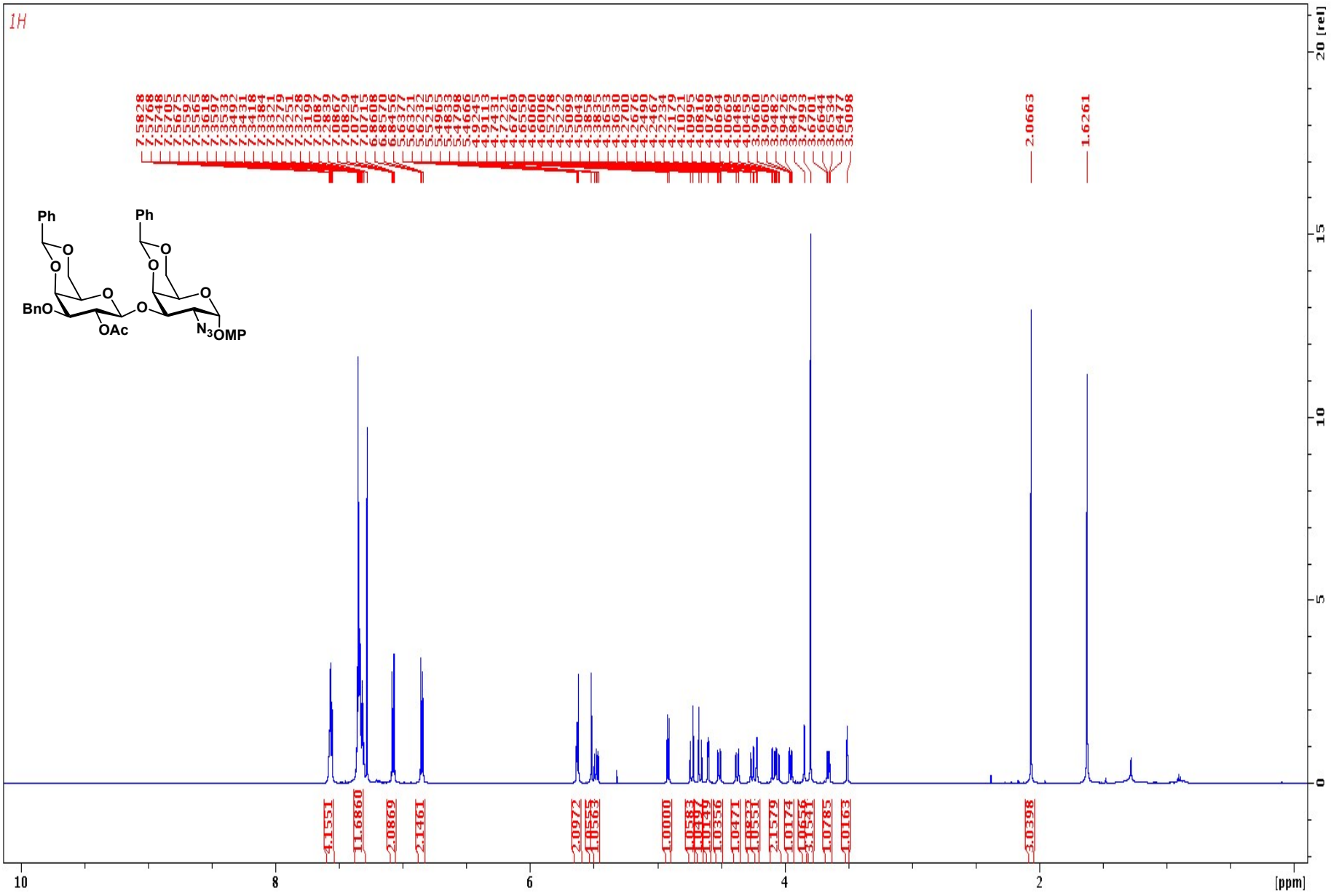
Complement dependent cytotoxicity was determined using commercially available LDH assay kit (Roche, catalog number: 11644793001) following manufacturer's instructions without further optimization. Human mammary cell lines MCF-10A were provided by Dr. Kadance Williams at the University of Toledo College of Medicine and Life Sciences. Targeted cells (1.0×10^4) were seeded in a 96 well plates and incubated overnight at 37 ºC in a 5% CO_2 incubator. The plates were washed with 2% BSA in DPBS and incubated with 100 µL of 1:20 dilution experimental anti-sera for 1 h. Afterwards, the wells were washed and incubated with 1:10 dilution rabbit complement (Pel-Freez) for 1 h at 37 ºC. No antisera was added to the low control (spontaneous LDH release). For the high control (maximum LDH release), both the antisera and complement were replaced with 100 µL of 2% Triton X-100. After incubation, 50 µL of the supernatant from each well was transferred to another 96 well-plate, then diluted with 50 µL of DPBS buffer. 100 µL of the LDH detection reagent was added to each well and the plate was incubated for 1 h at room temperature. The optical absorption (A) of each well was read at 490 nm using a plate reader. The percentage of cytotoxicity was calculated according to the following equation:

$$cytotoxicity\ \% = \frac{experimental\ A - low\ control\ A}{high\ control\ A - low\ control\ A} \times 100$$

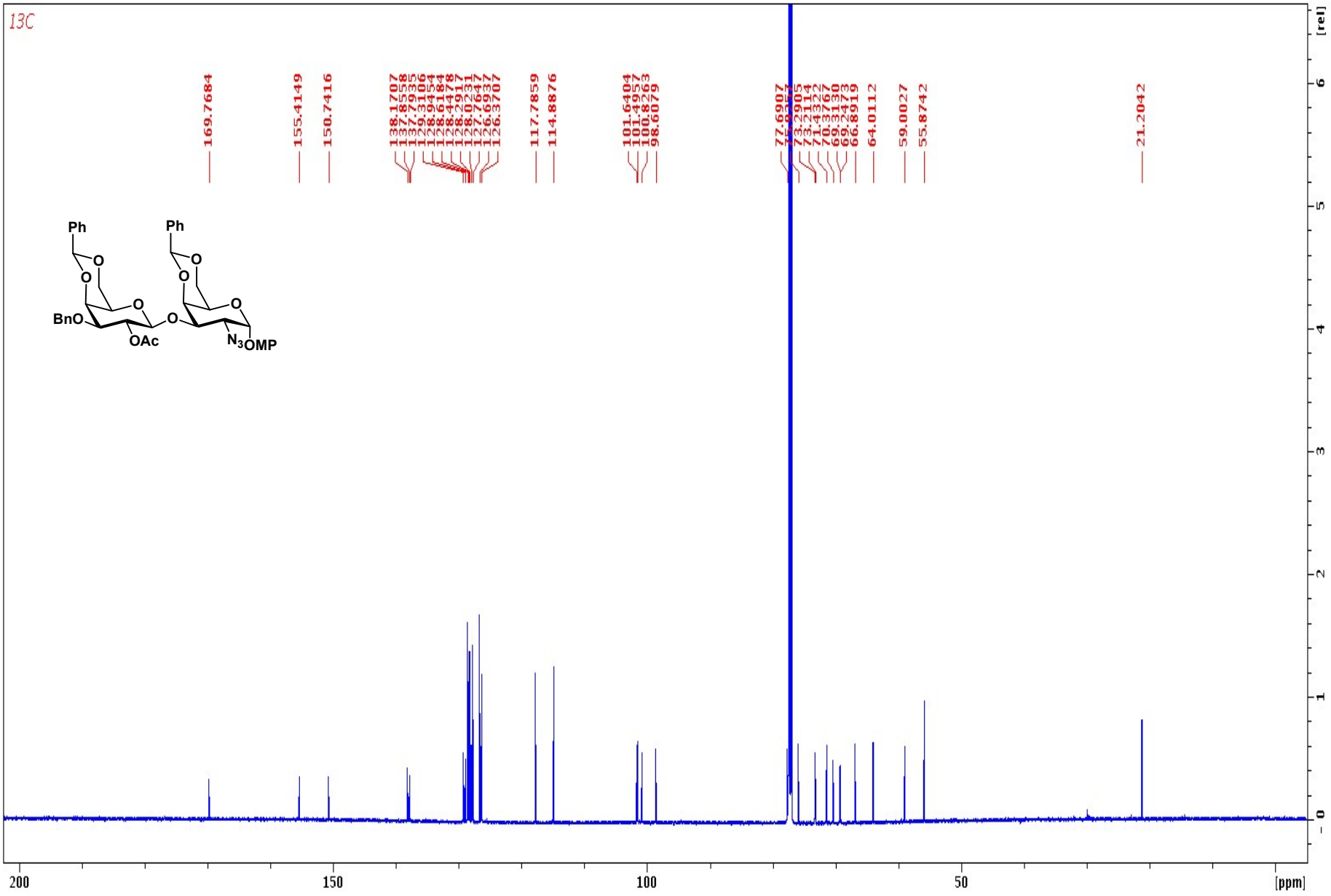
experimental A = the optical absorption at 490 nm of cells lysed by treatment of antisera and complement;
low control A = the optical absorption of cells lysed without antisera and complement;
high control A = the optical absorption of cells lysed with 2% Triton X-100 solution.

Spectroscopy data of compounds

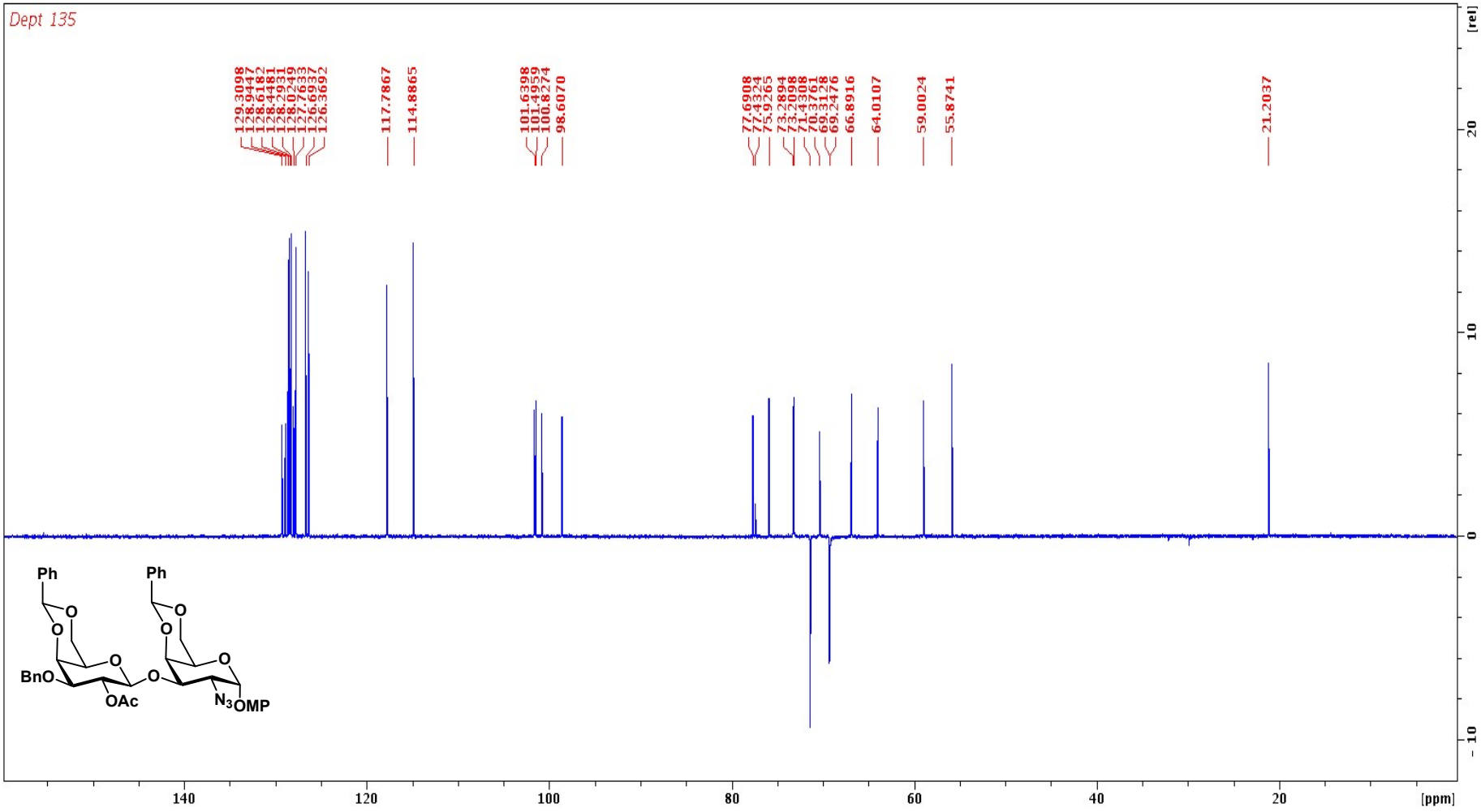
^{1}H NMR Spectrum of Compound **S1**



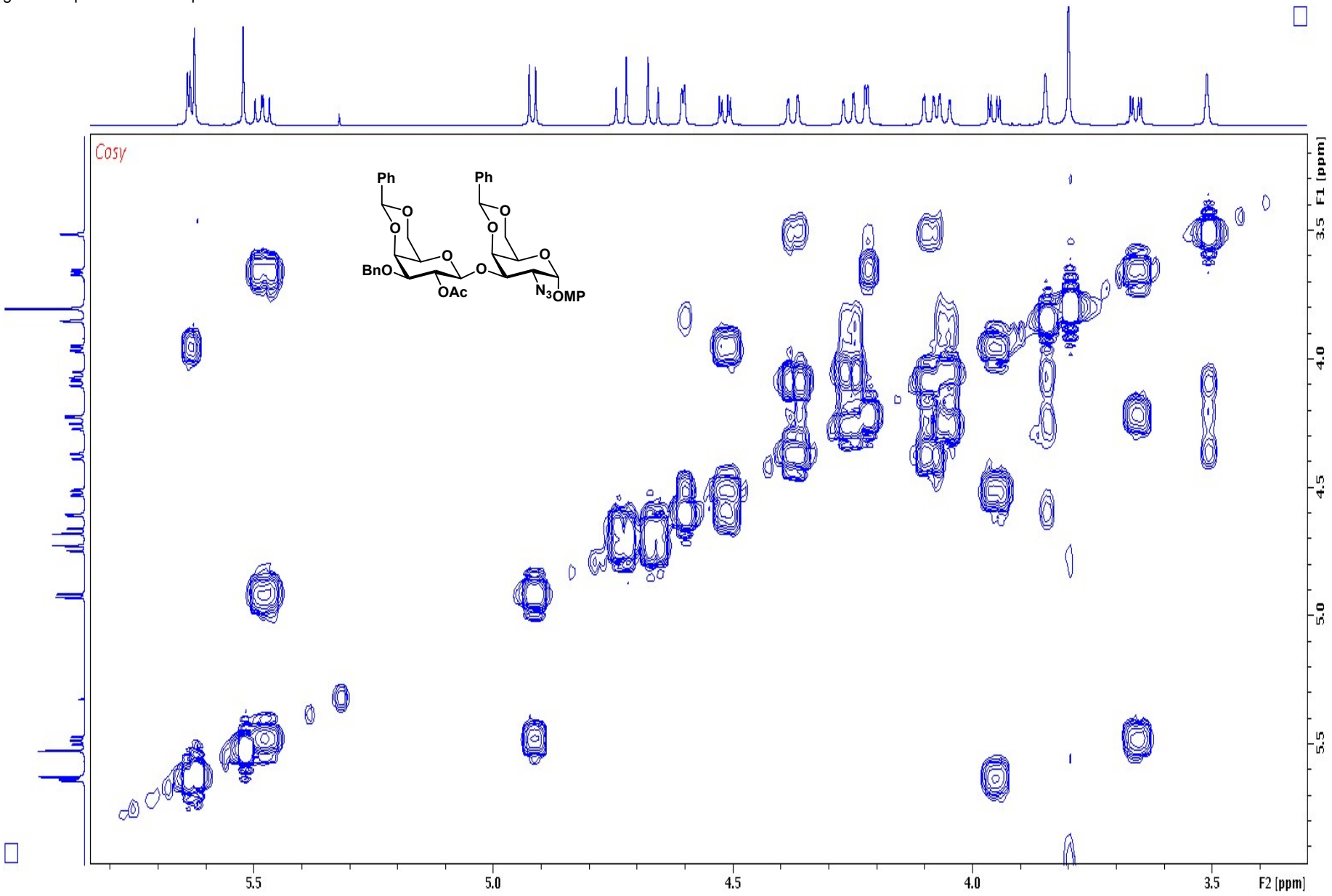
13C NMR Spectrum of Compound **S1**



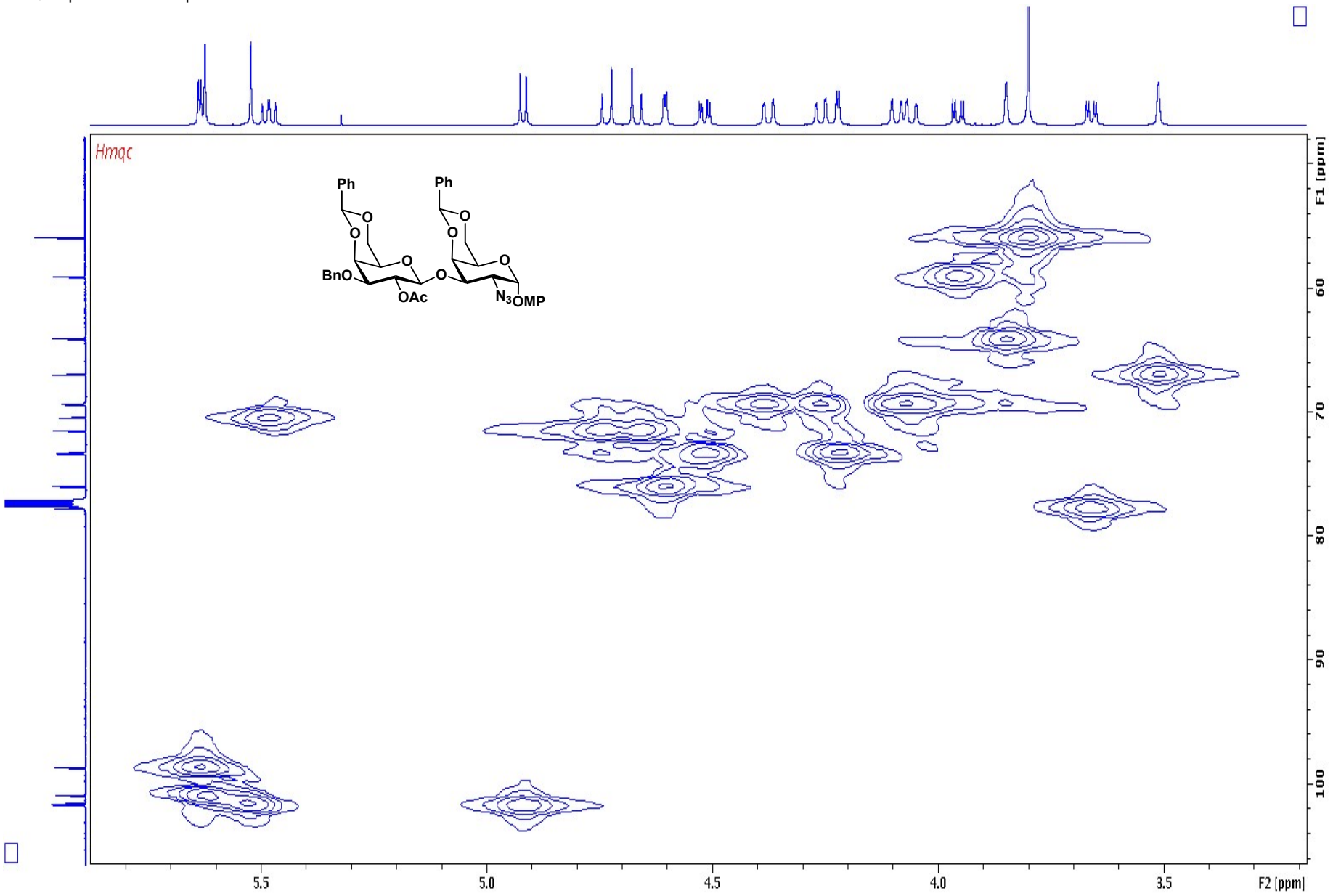
DEPT 135 Spectrum of Compound **S1**



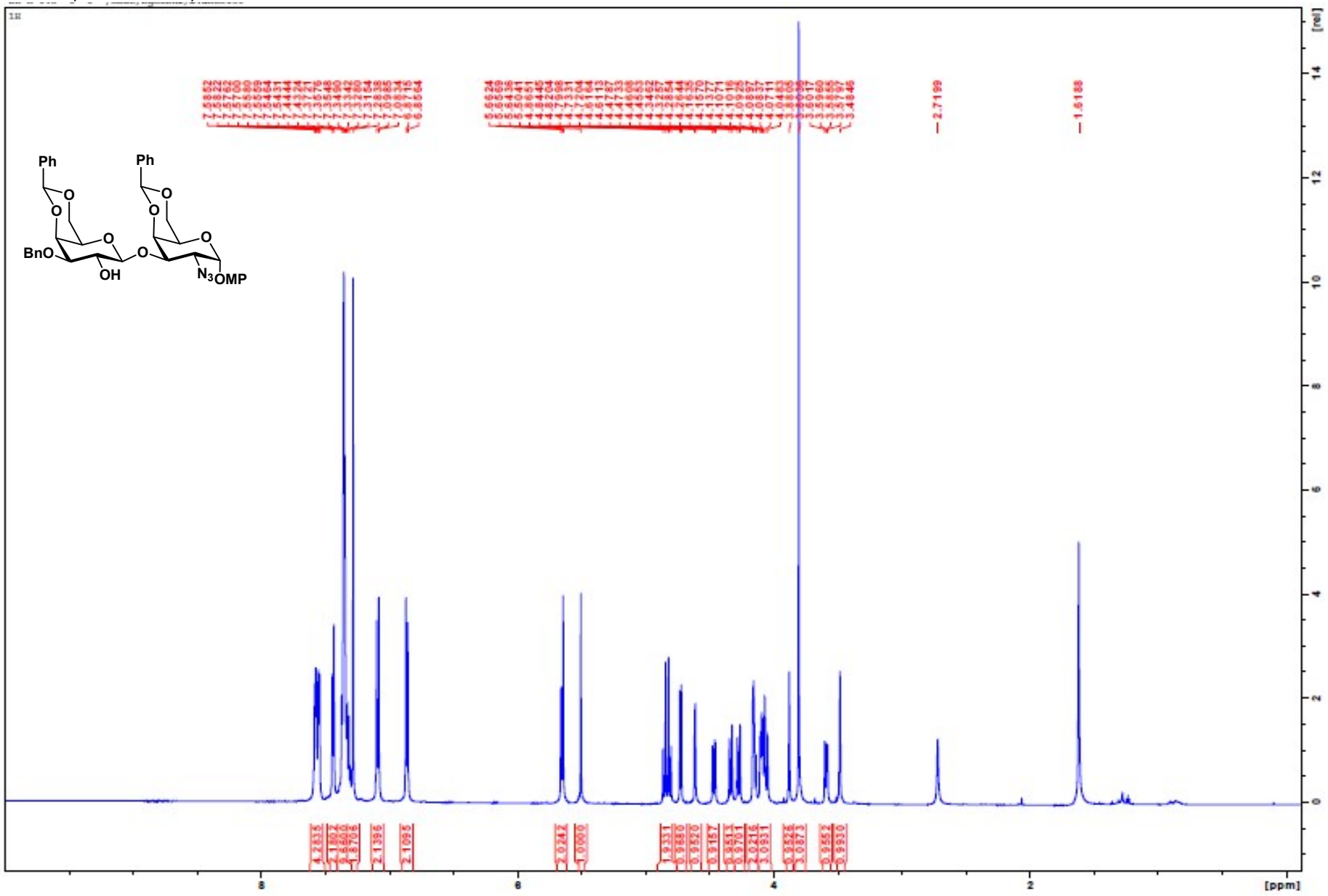
gCOSY Spectrum of Compound **S1**



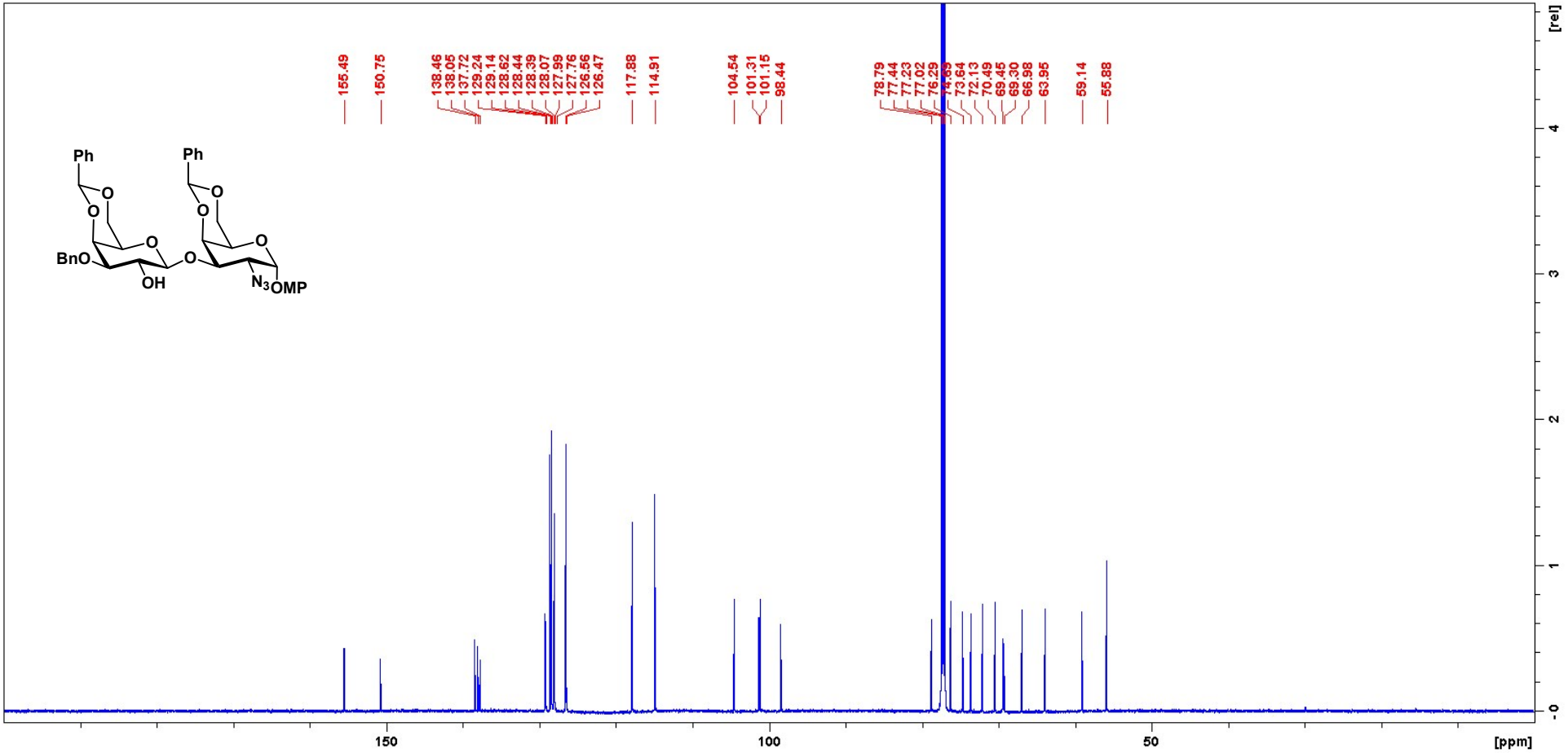
HMQC Spectrum of Compound **S1**



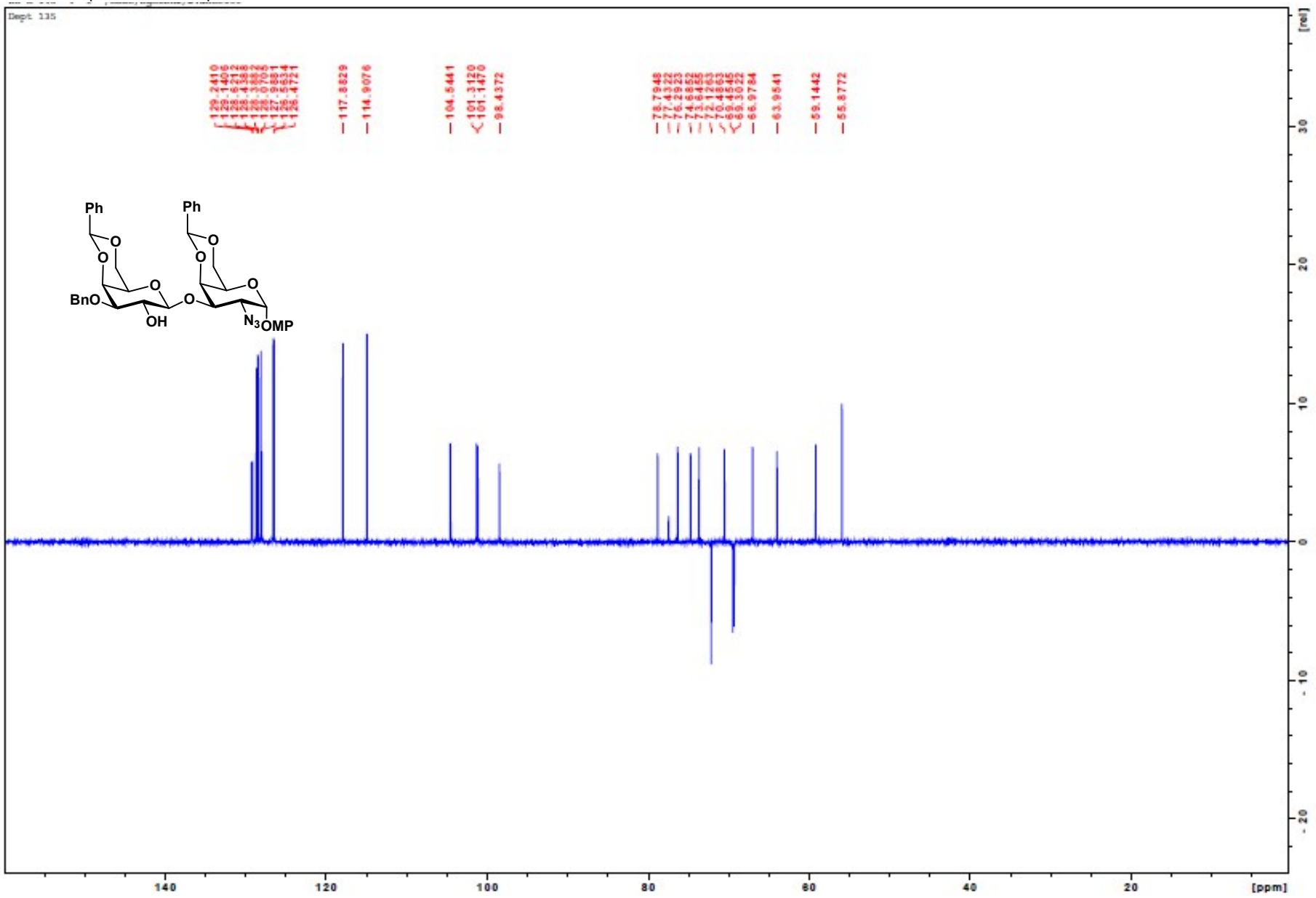
^{1}H NMR Spectrum of Compound **12**



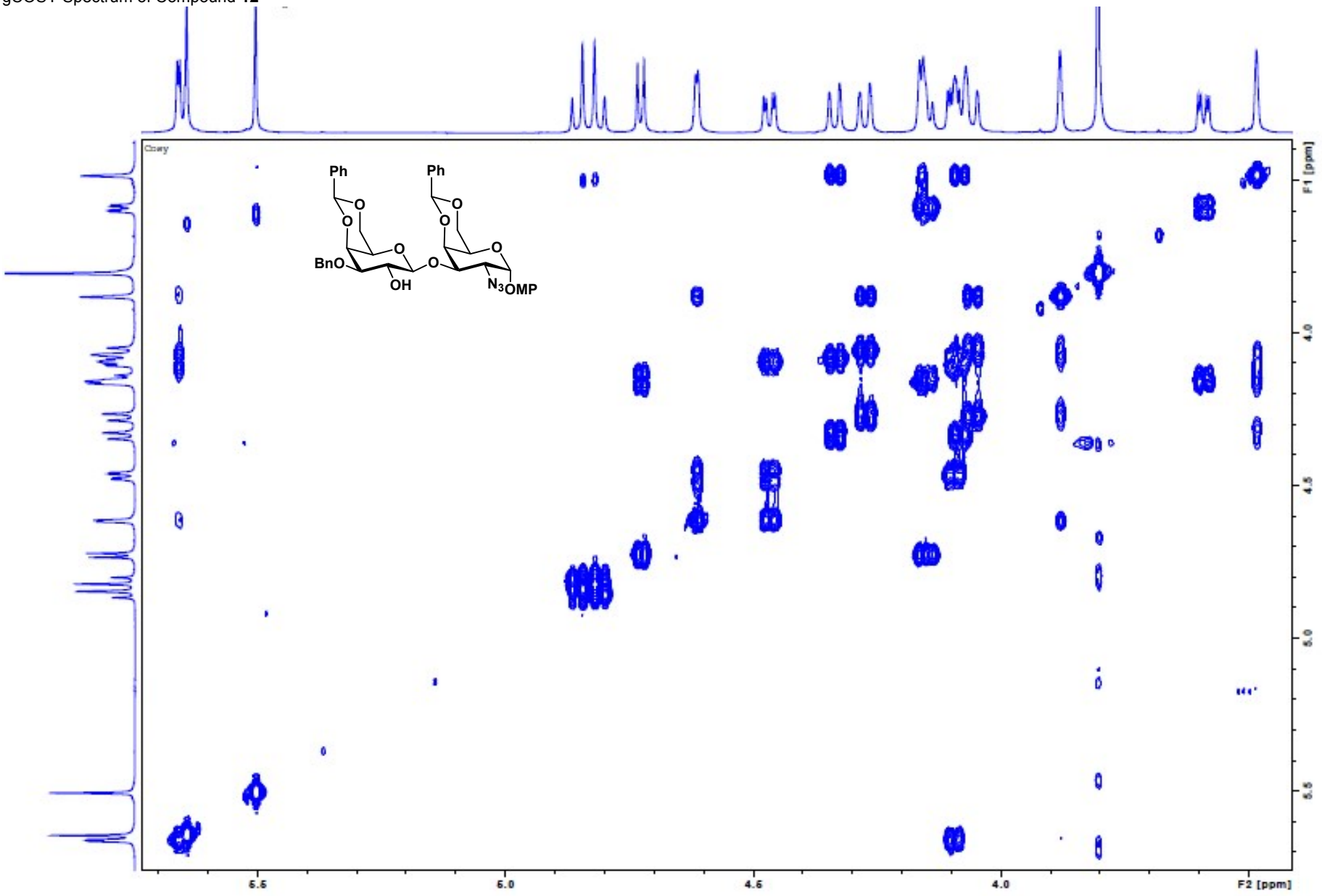
^{13}C NMR Spectrum of Compound **12**



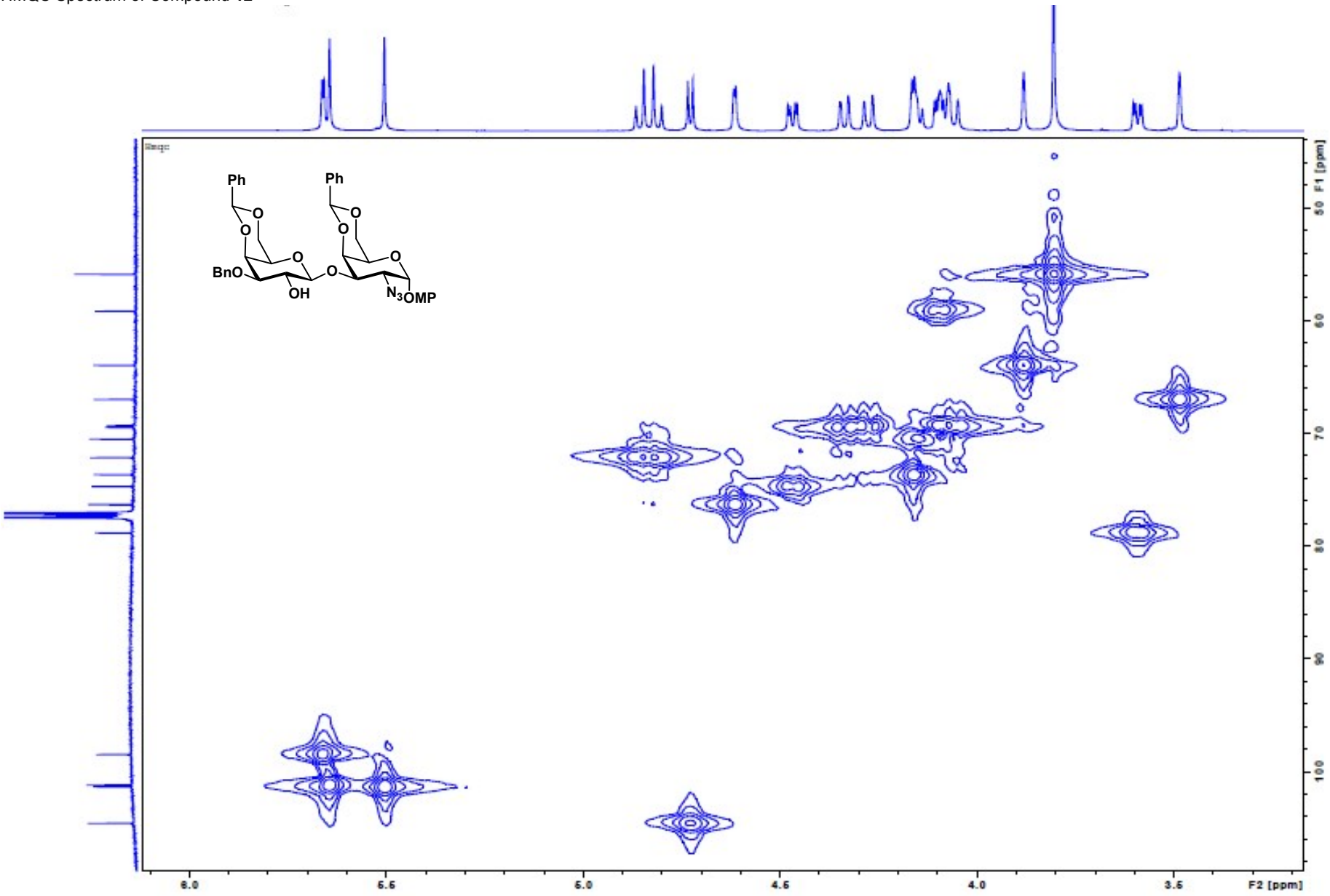
DEPT 135 Spectrum of Compound **12**



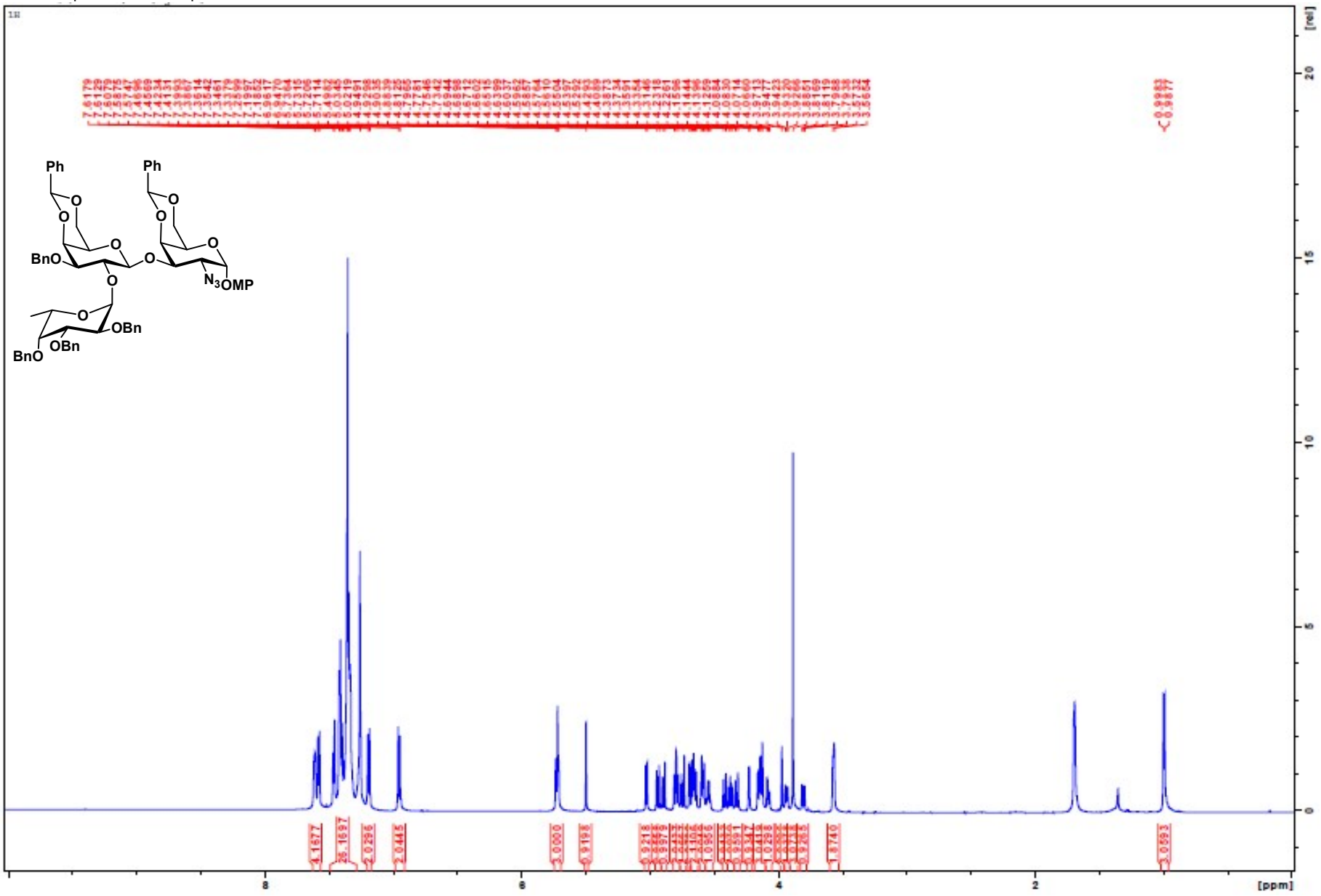
gCOSY Spectrum of Compound **12**



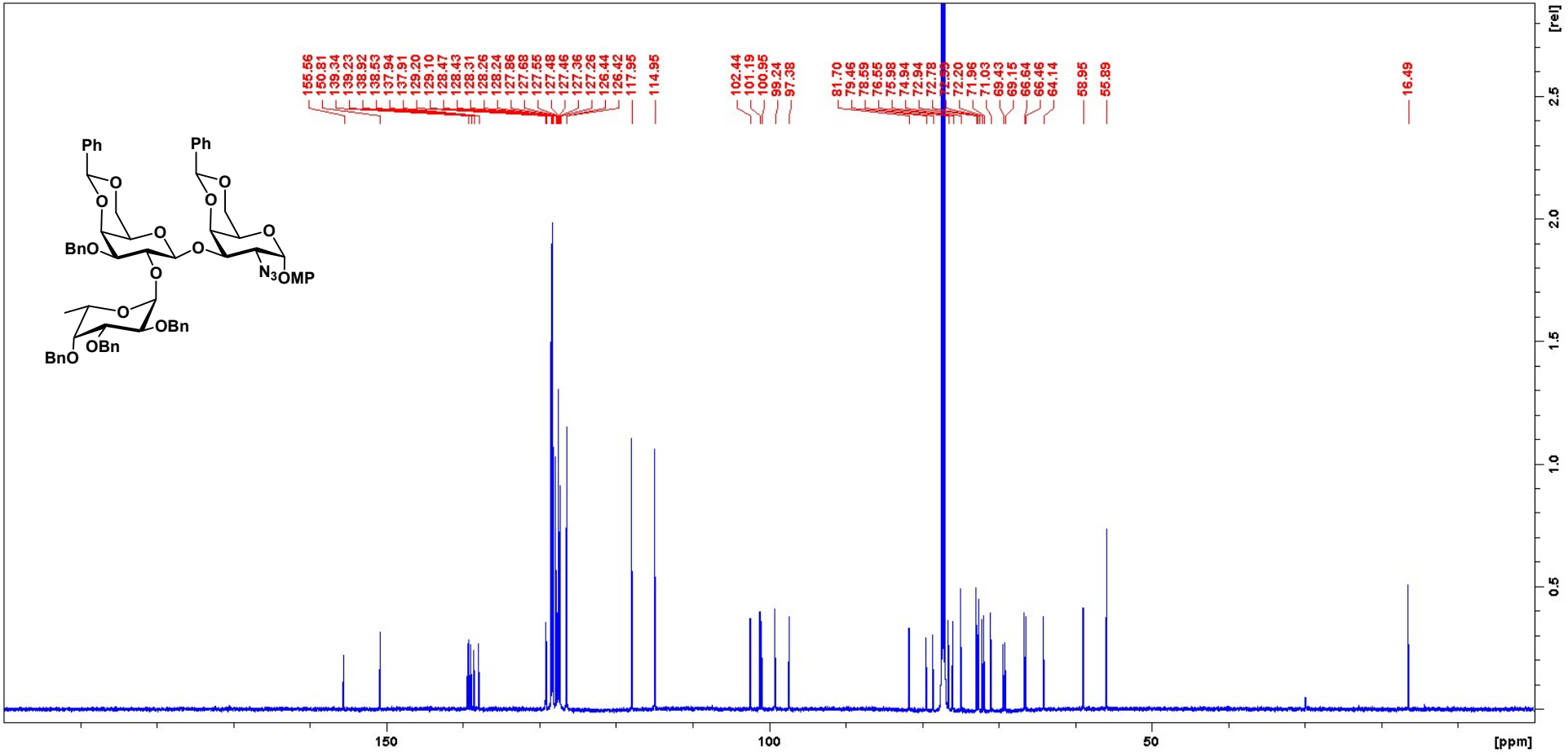
HMQC Spectrum of Compound **12**



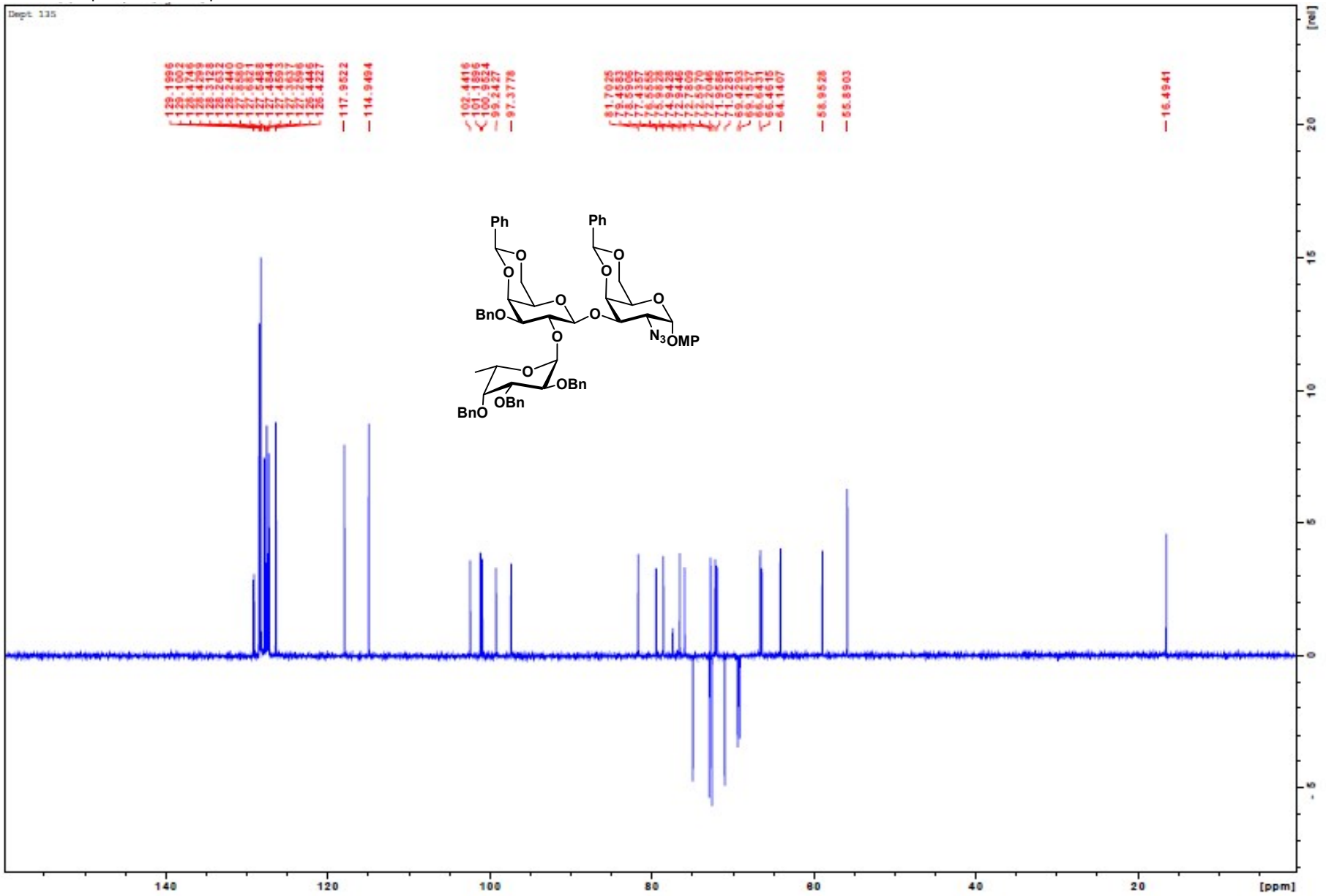
^{1}H NMR Spectrum of Compound **S2**



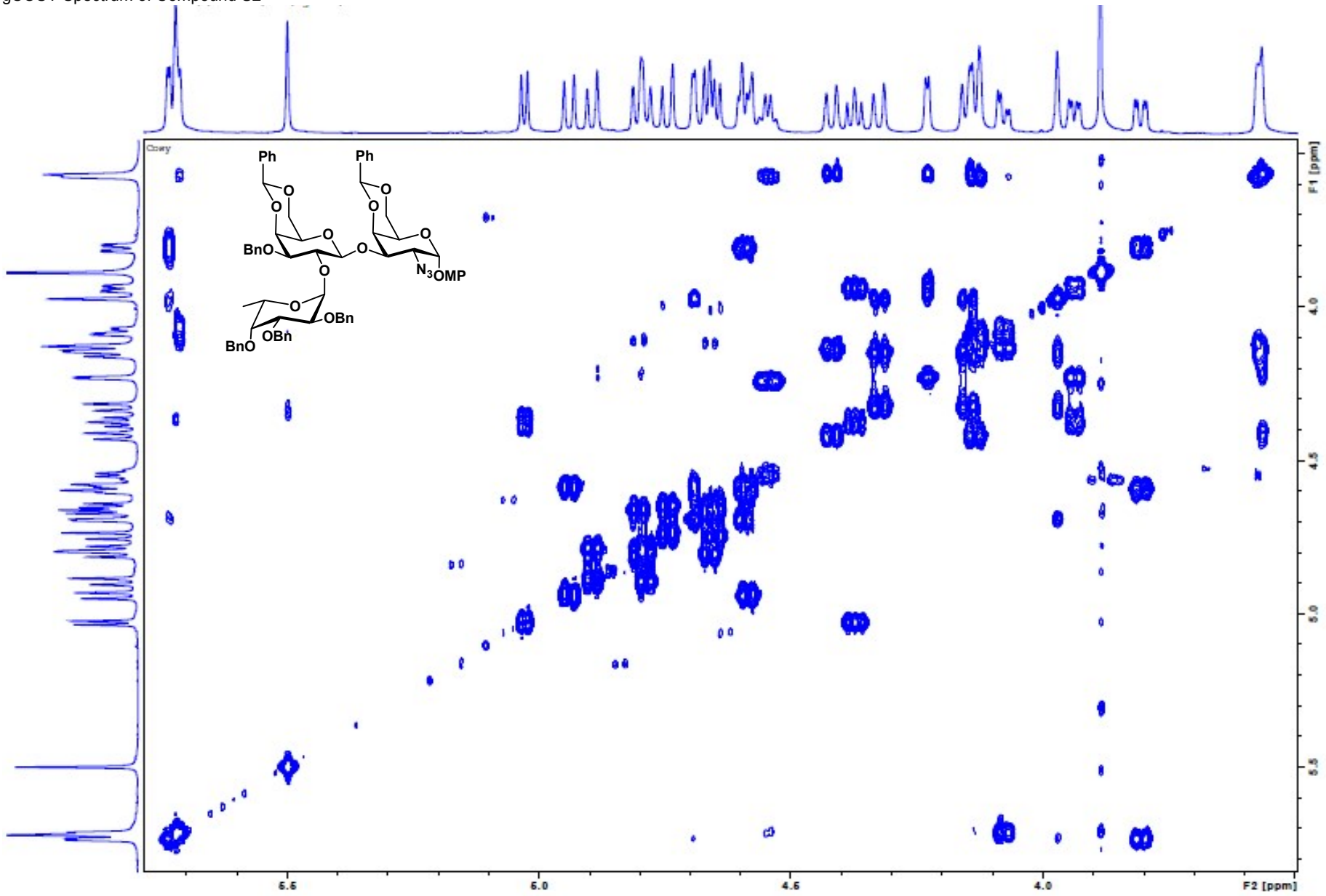
^{13}C NMR Spectrum of Compound **S2**



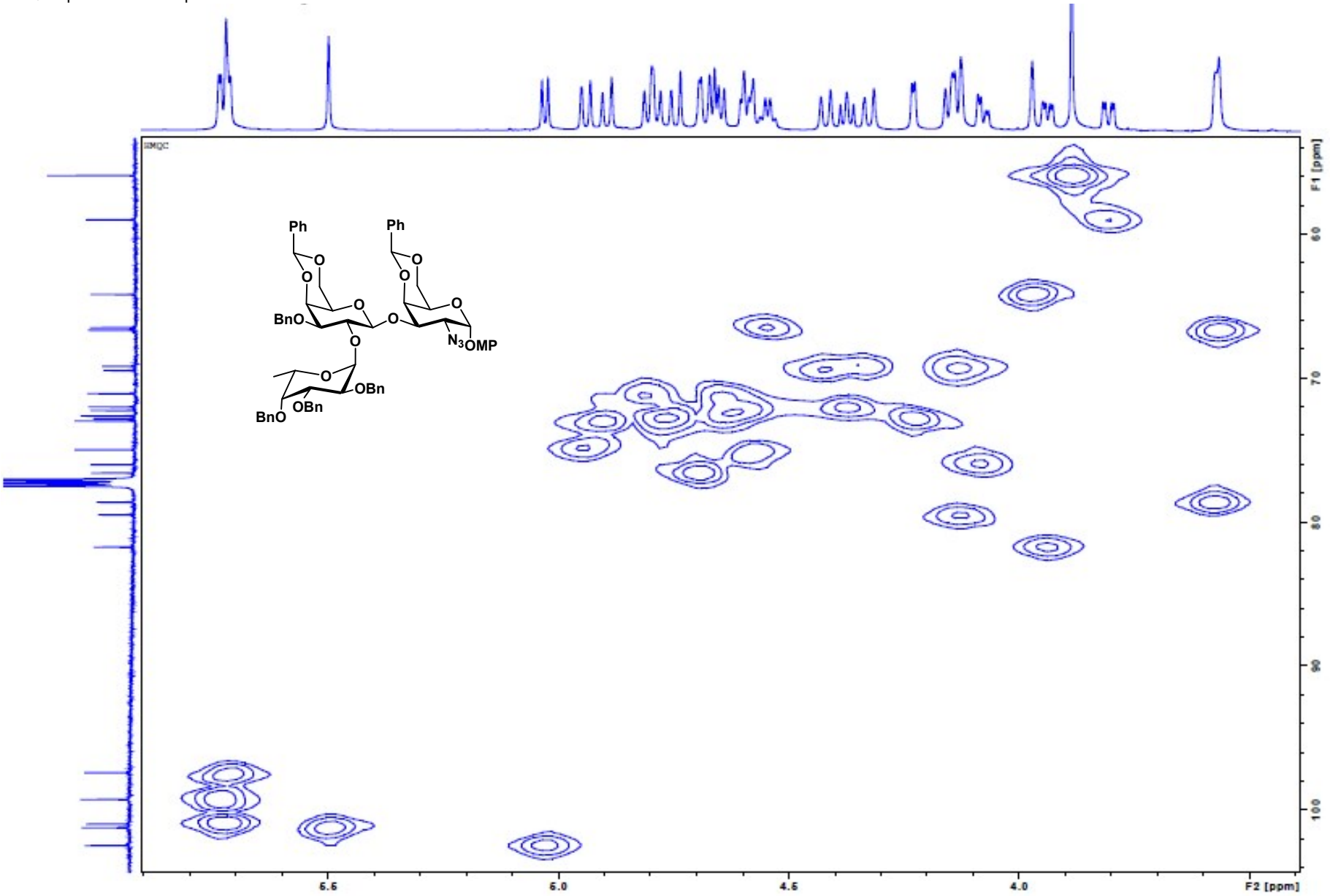
DEPT 135 Spectrum of Compound **S2**



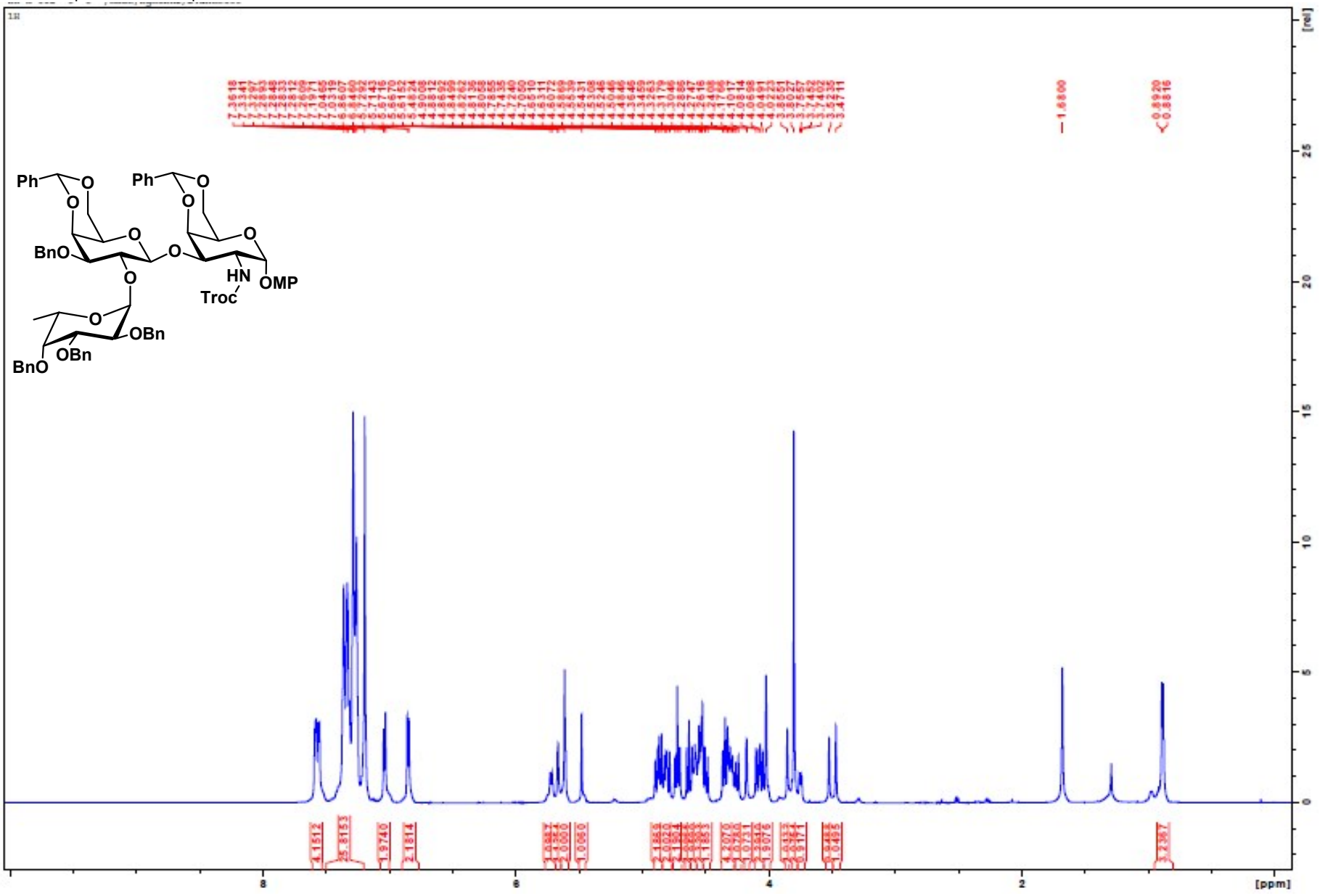
gCOSY Spectrum of Compound **S2**



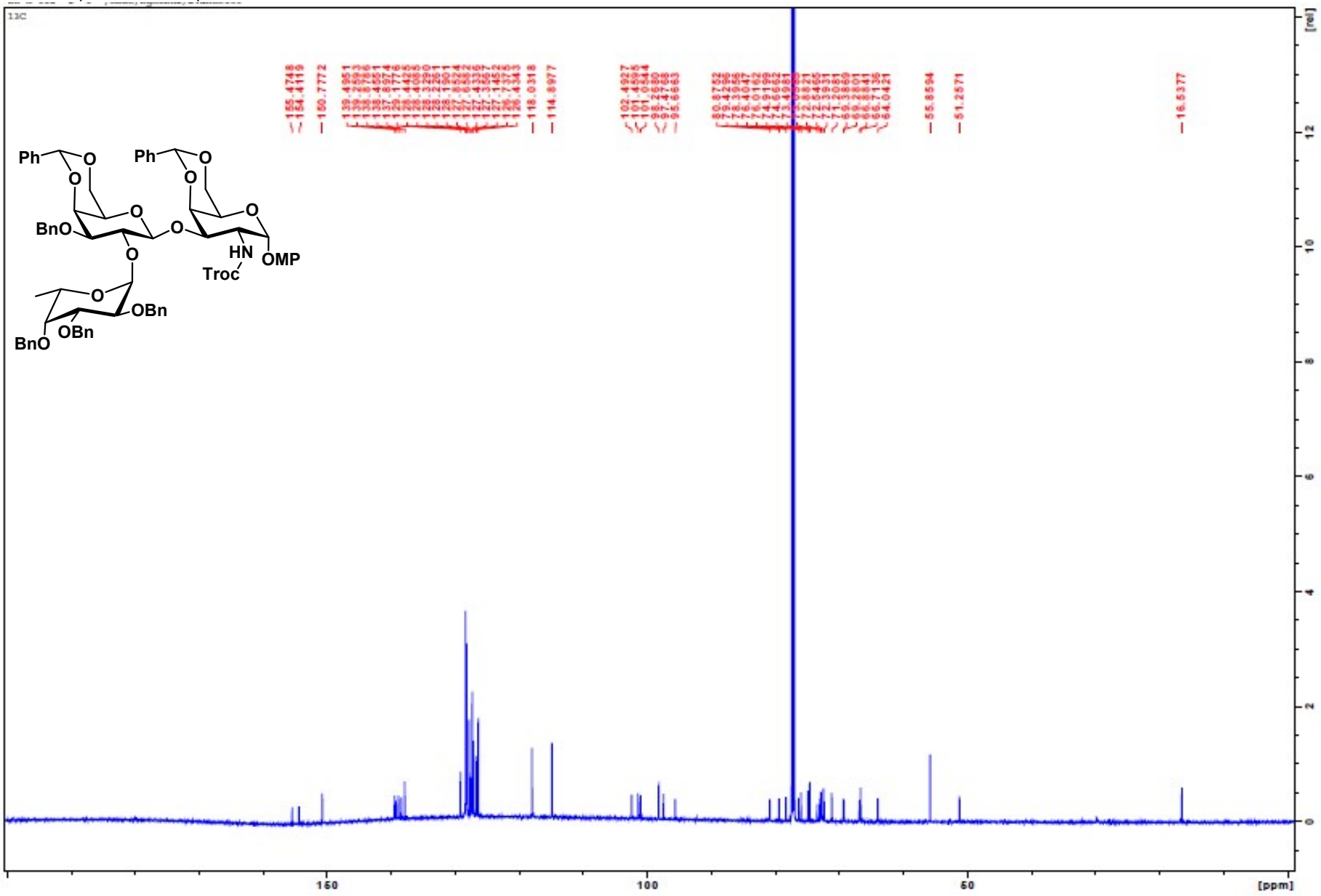
HMQC Spectrum of Compound **S2**



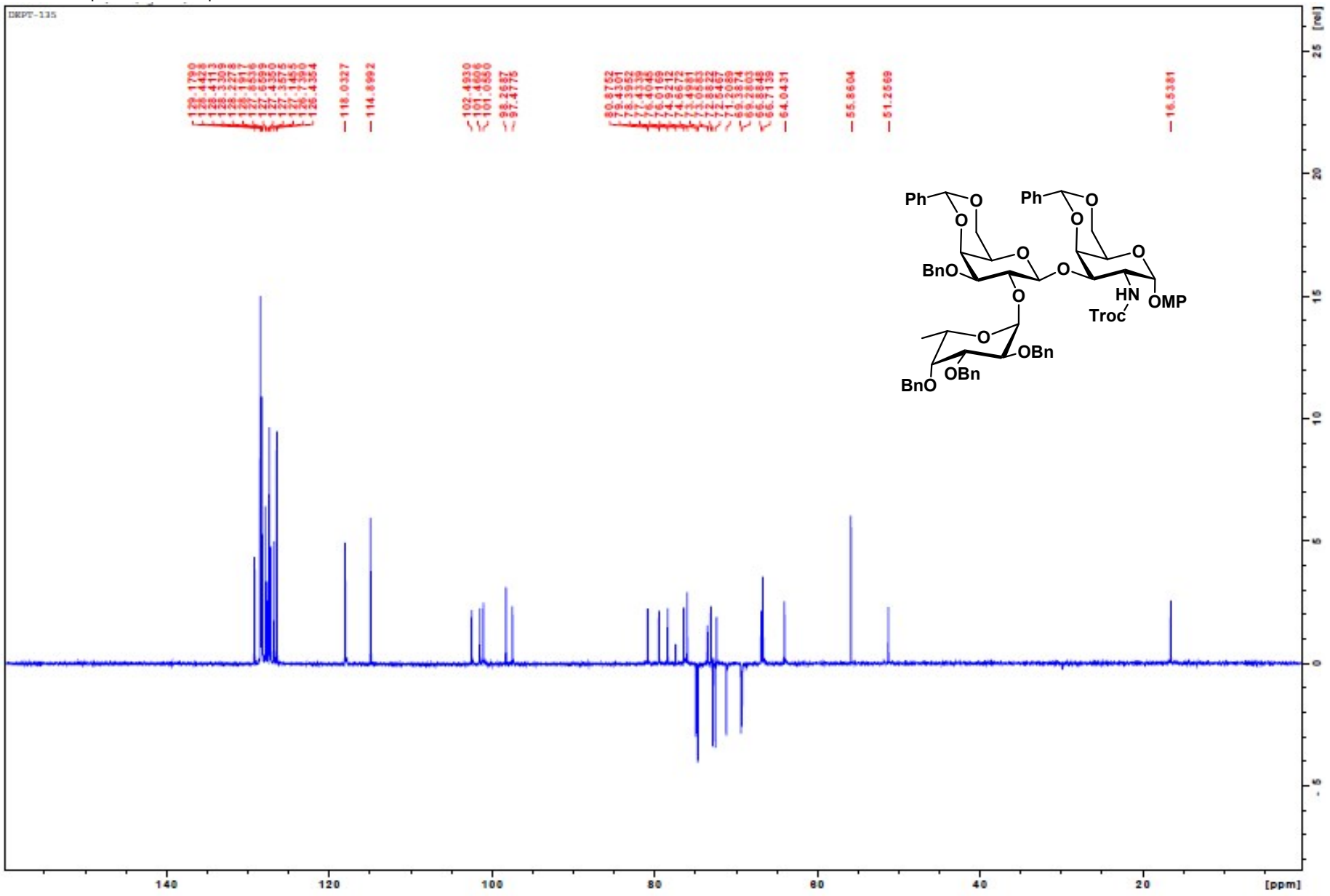
^{1}H NMR Spectrum of Compound **13**



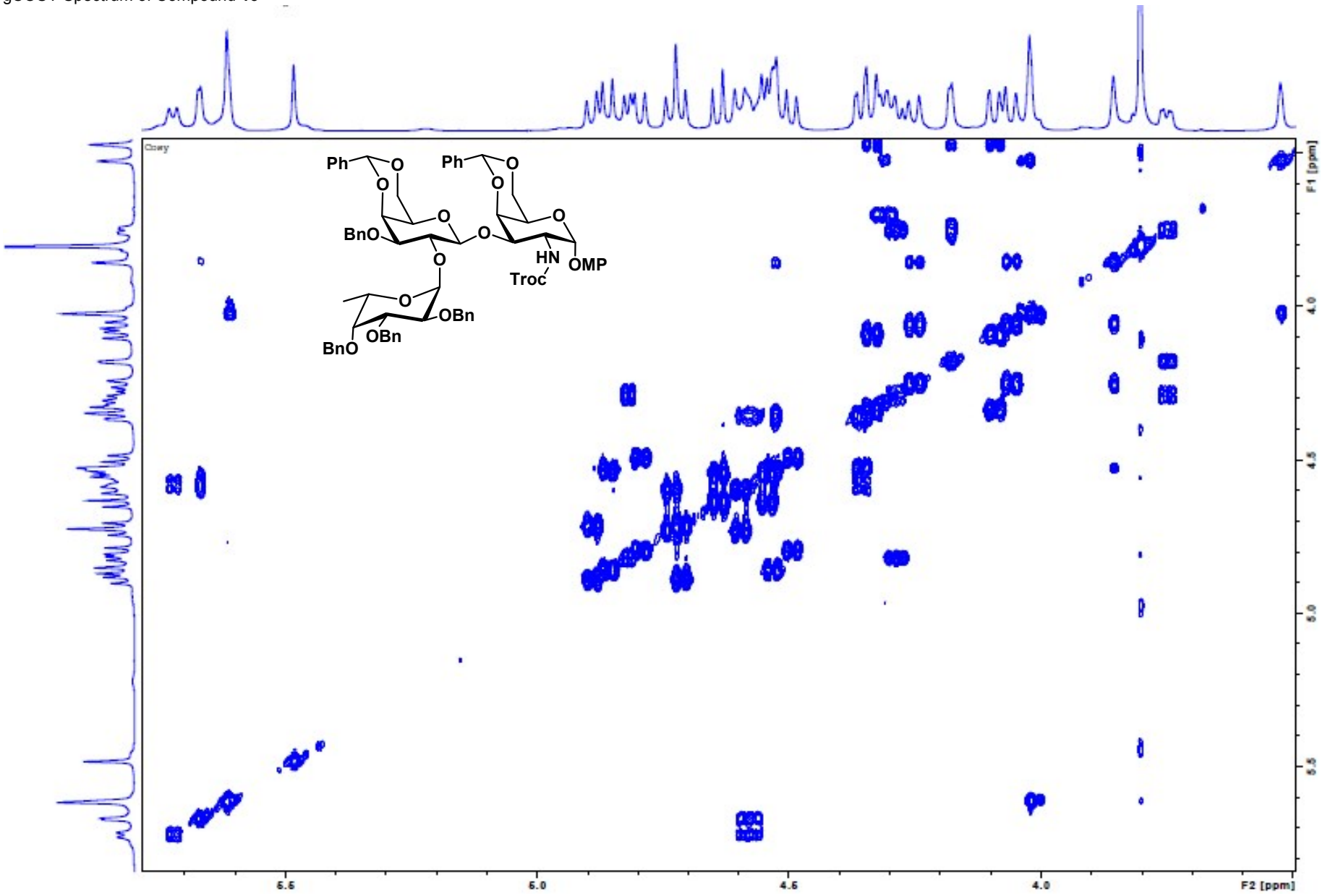
^{13}C NMR Spectrum of Compound **13**



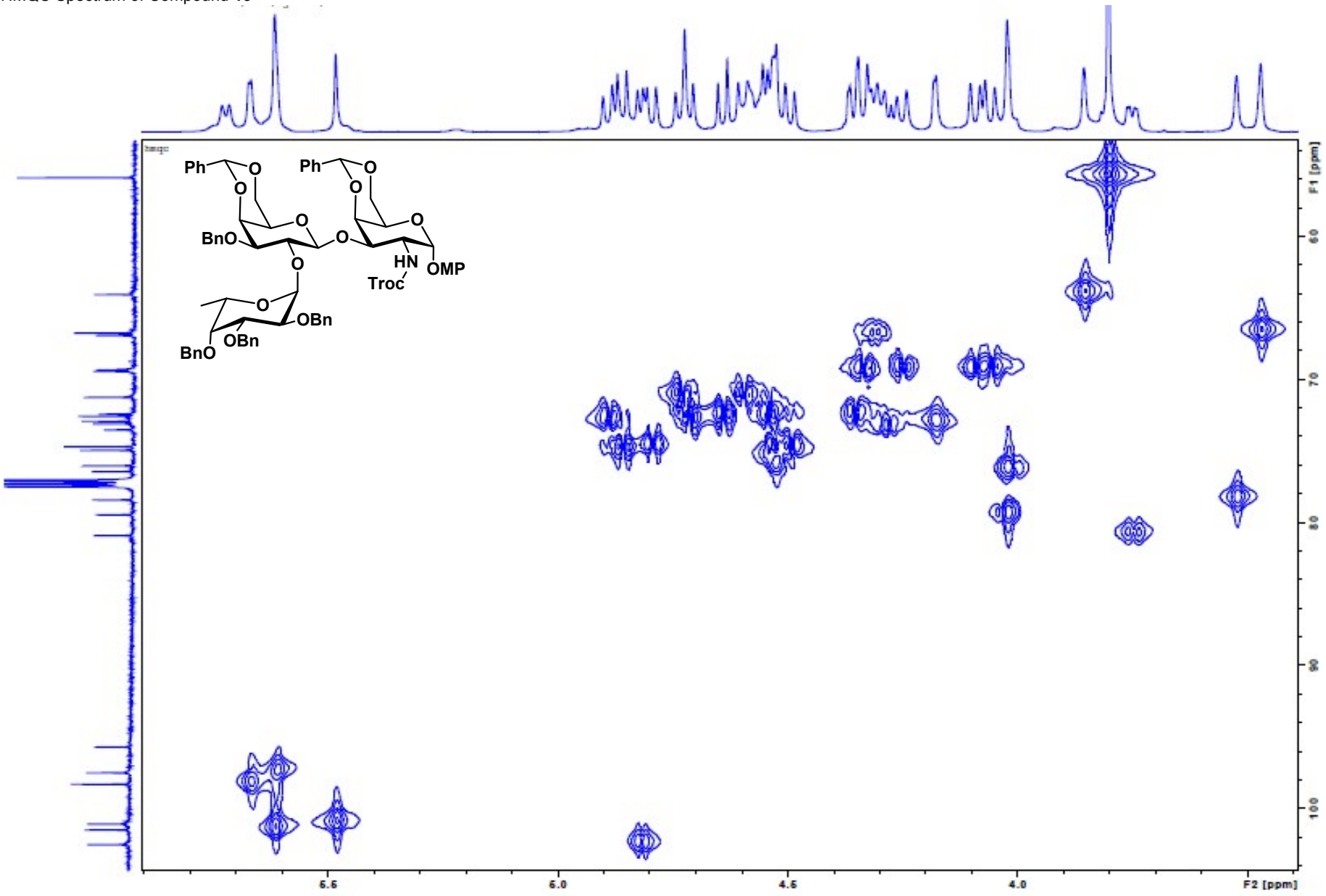
DEPT 135 Spectrum of Compound **13**



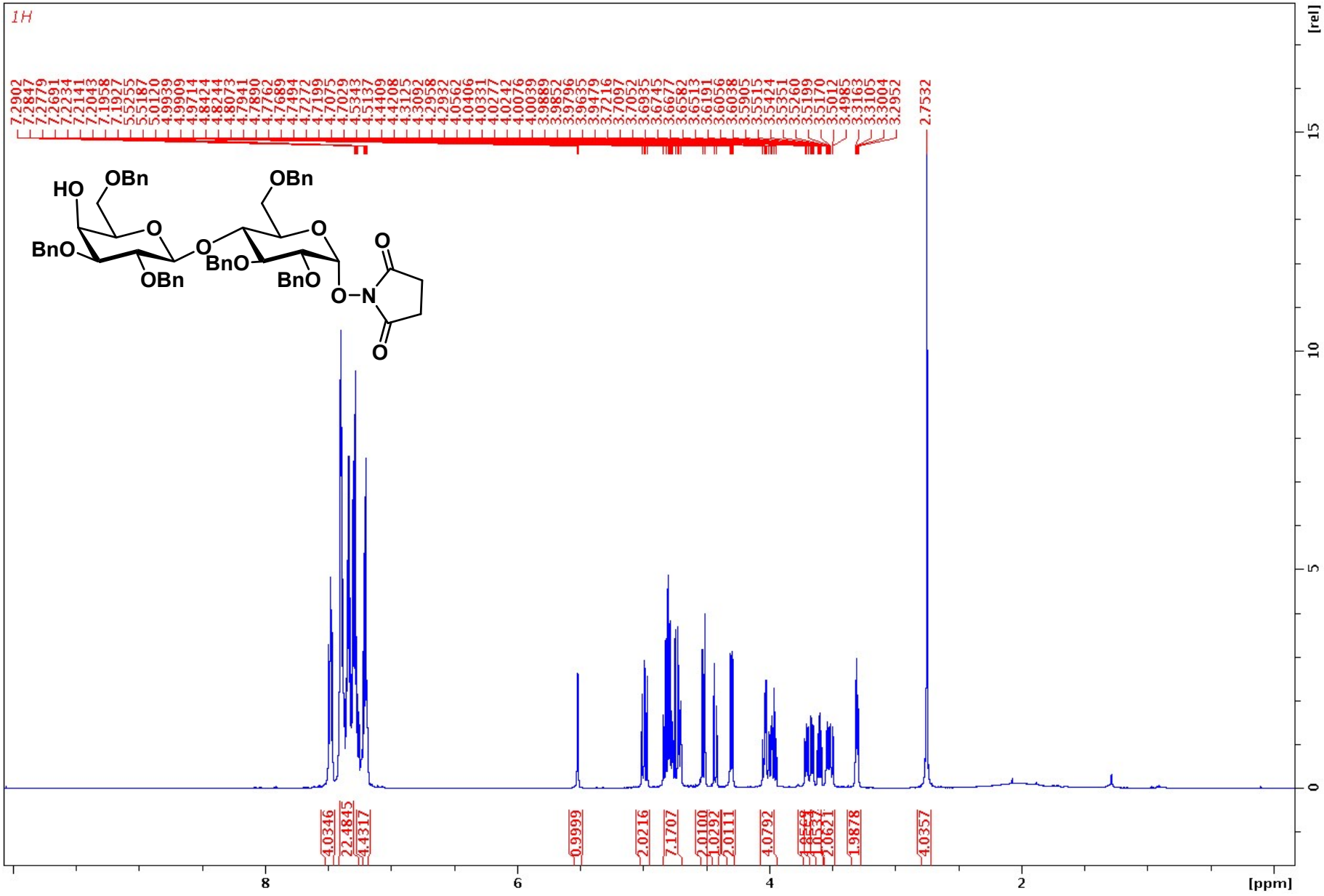
gCOSY Spectrum of Compound **13**



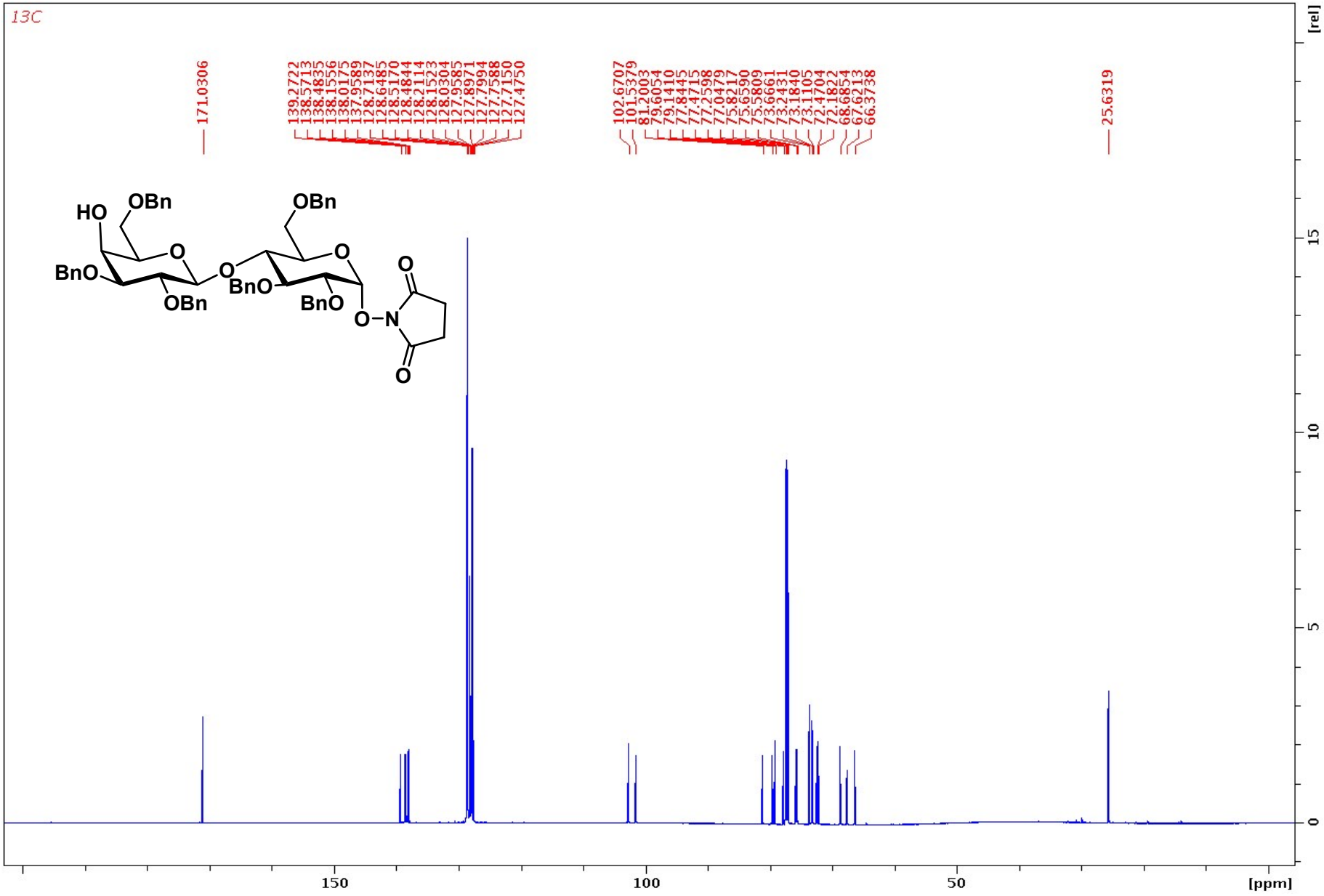
HMQC Spectrum of Compound **13**



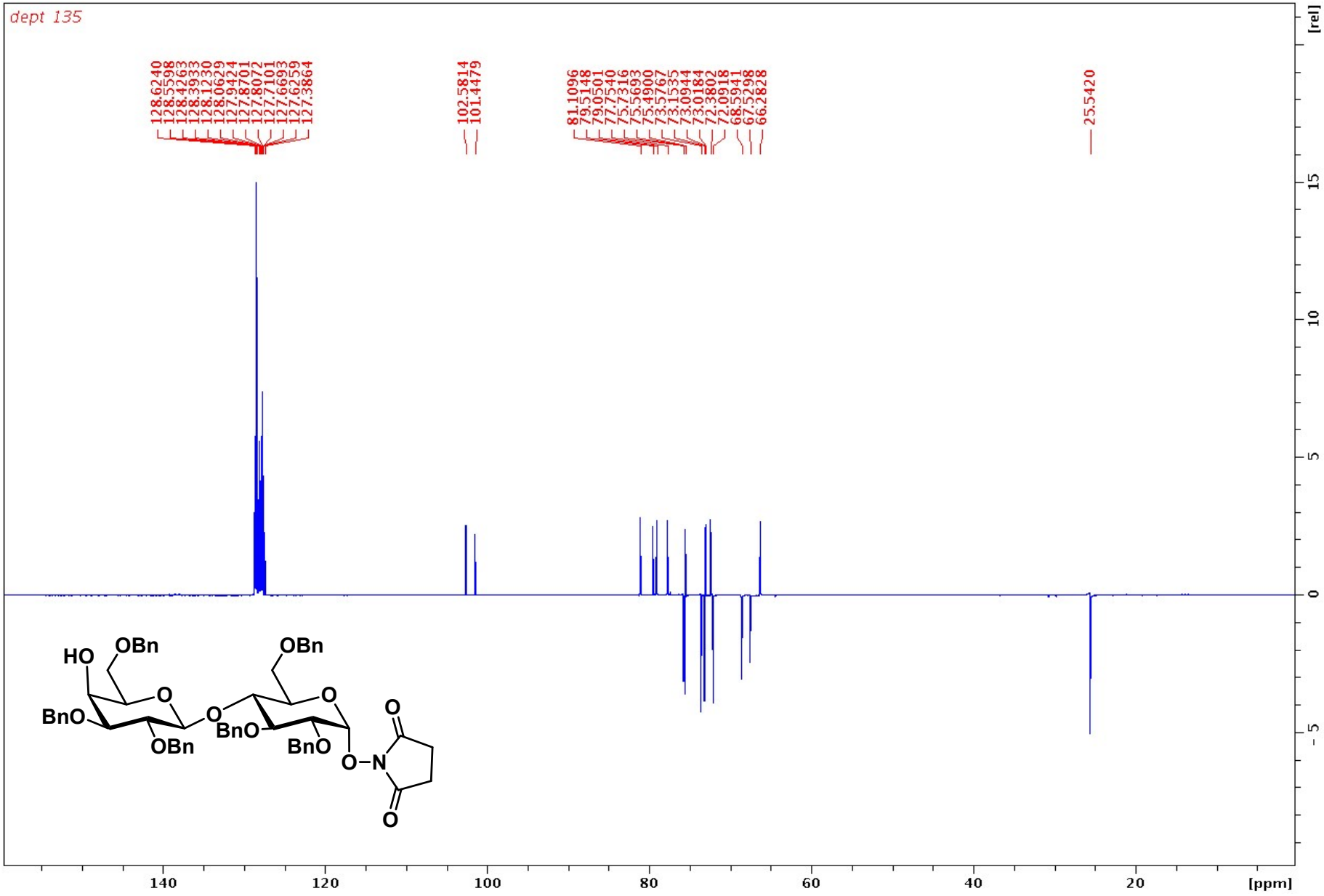
^{1}H NMR Spectrum of Compound **10**



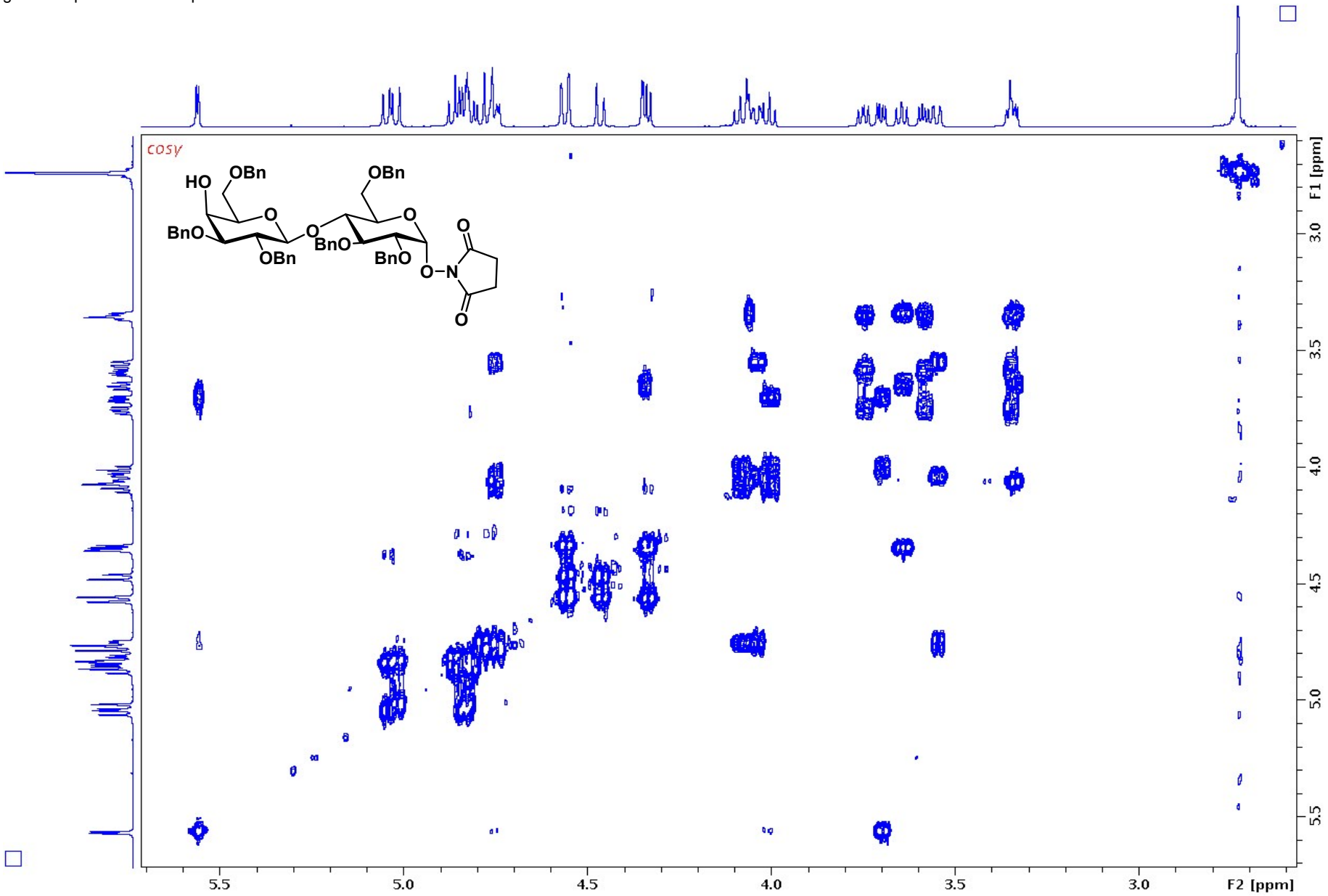
^{13}C NMR Spectrum of Compound **10**



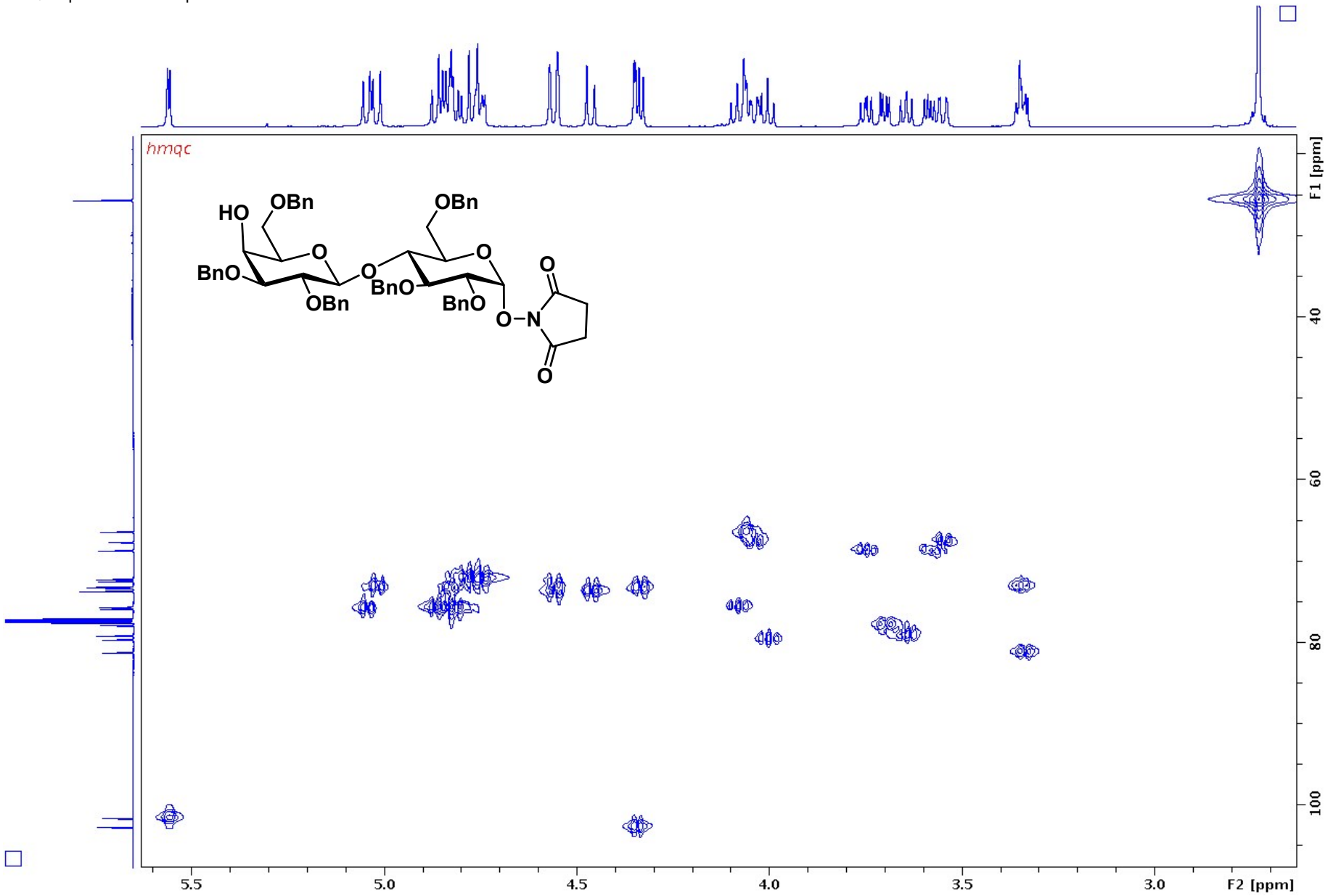
DEPT 135 Spectrum of Compound **10**



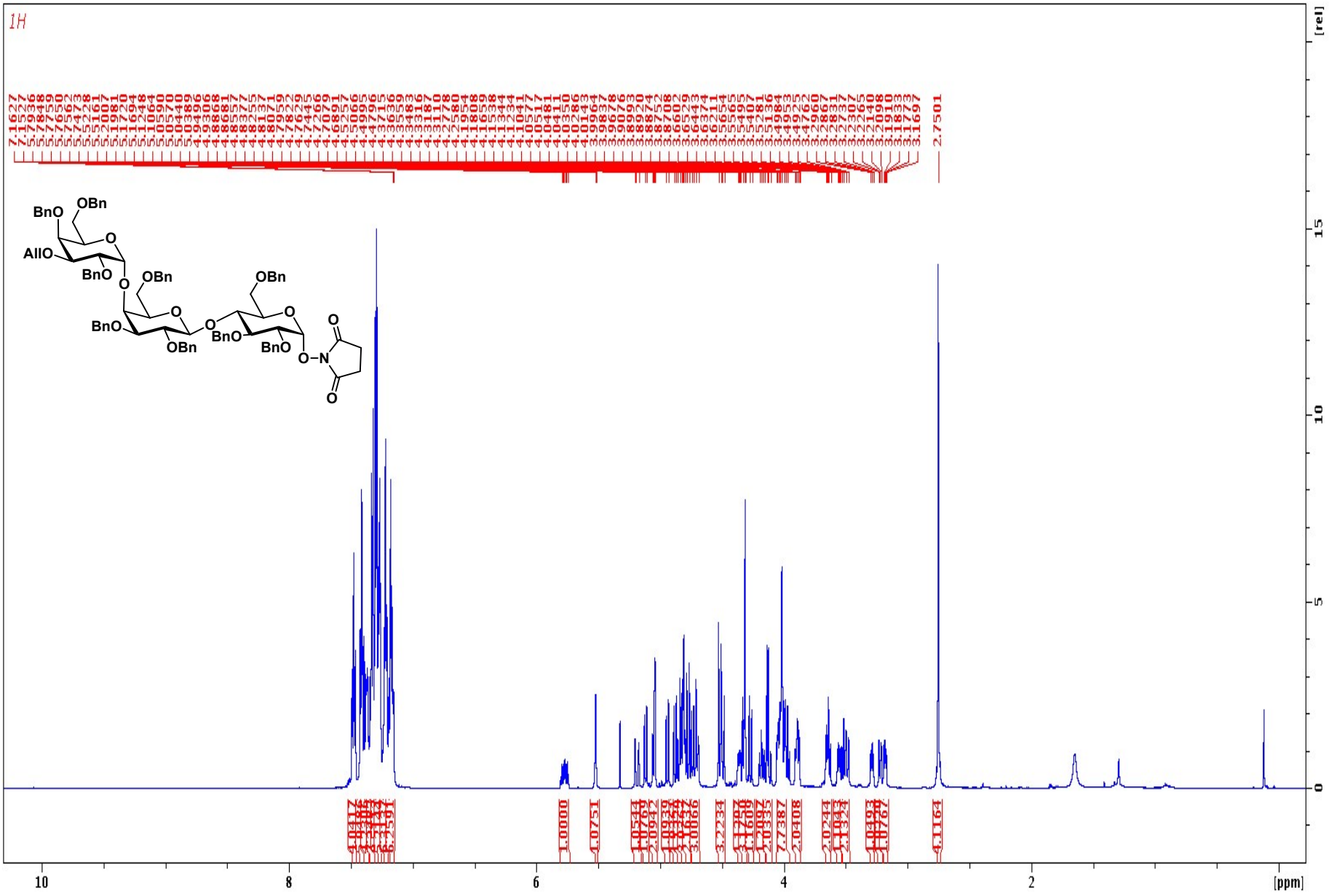
gCOSY Spectrum of Compound **10**



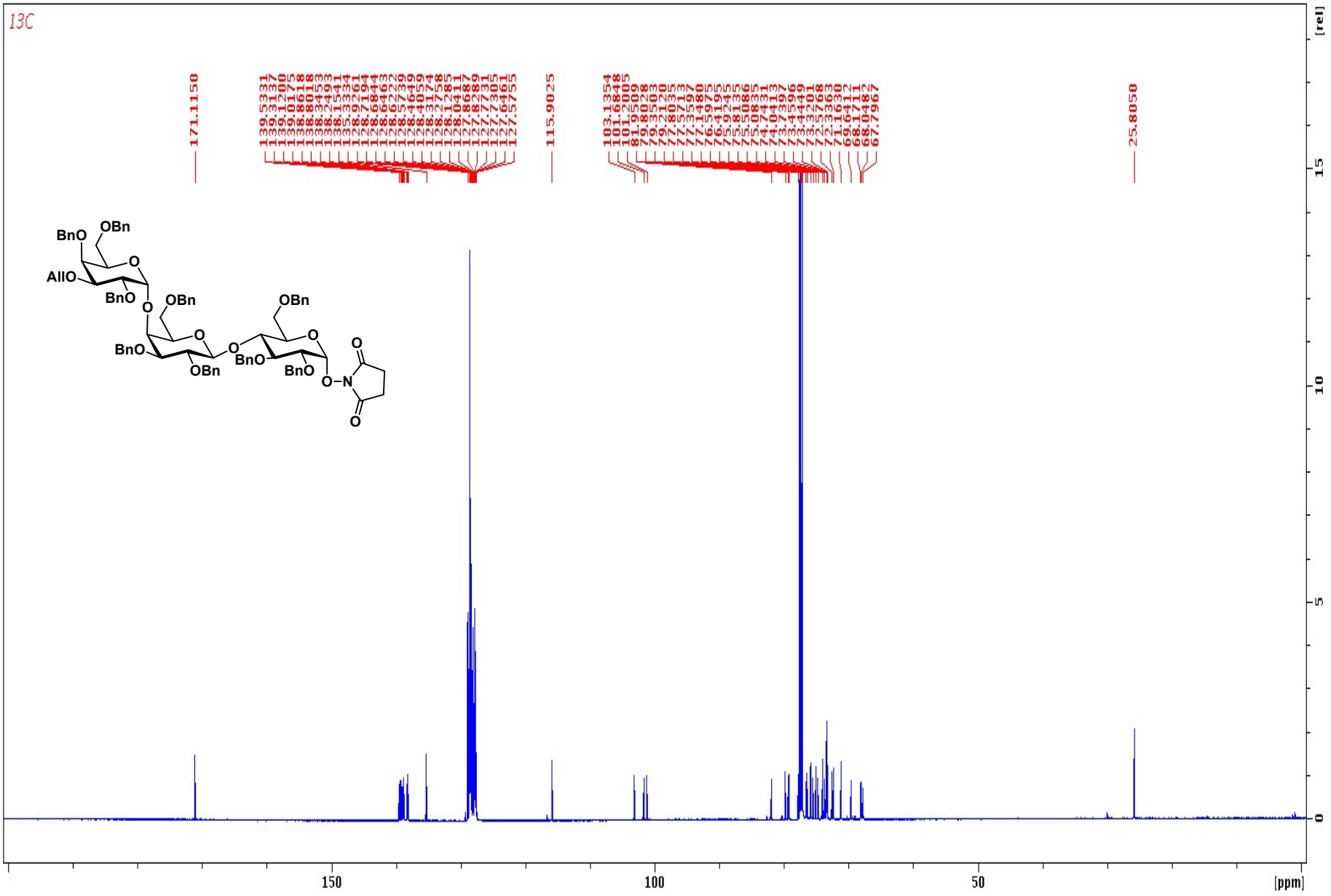
HMQC Spectrum of Compound **10**



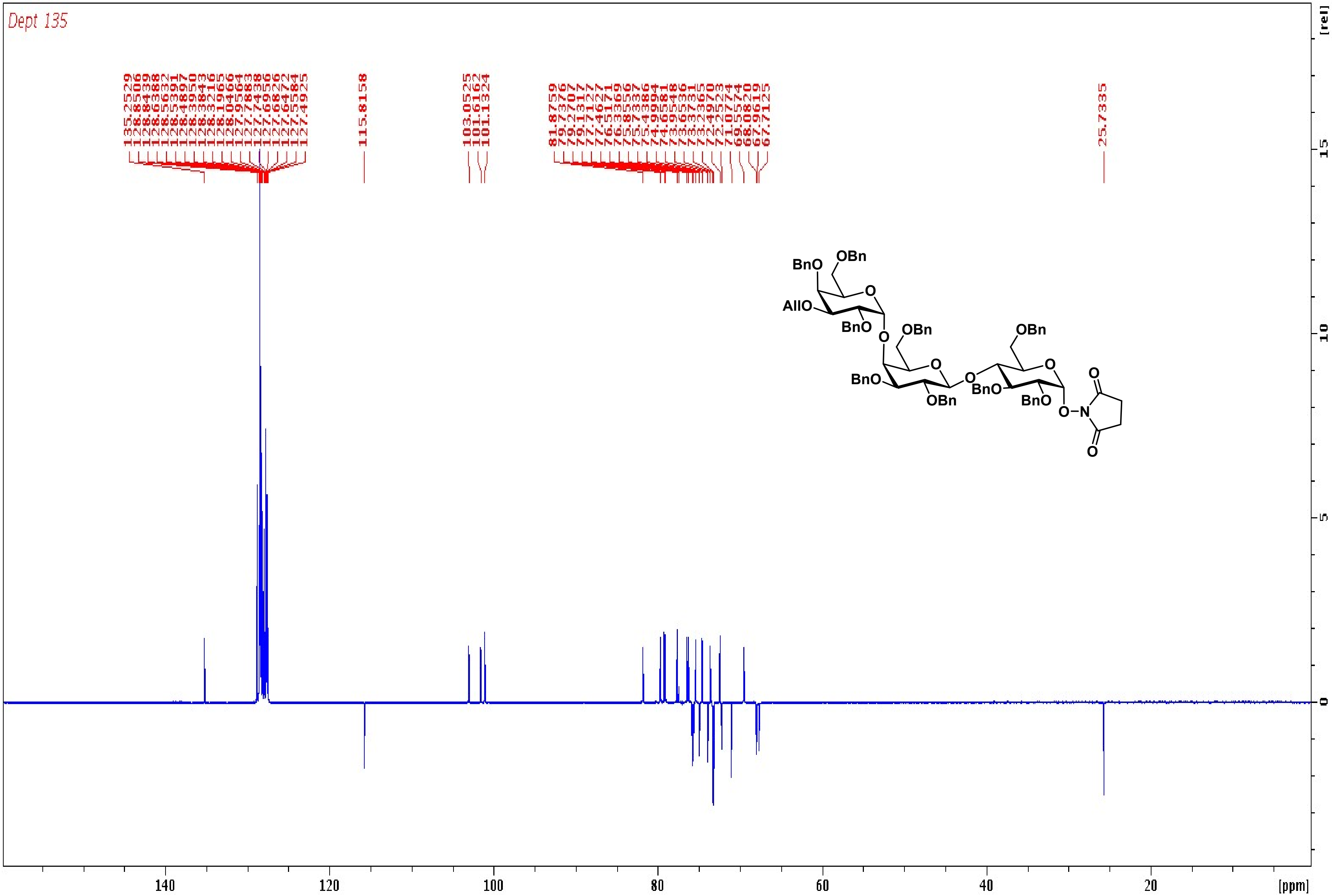
^{1}H NMR Spectrum of Compound **14**



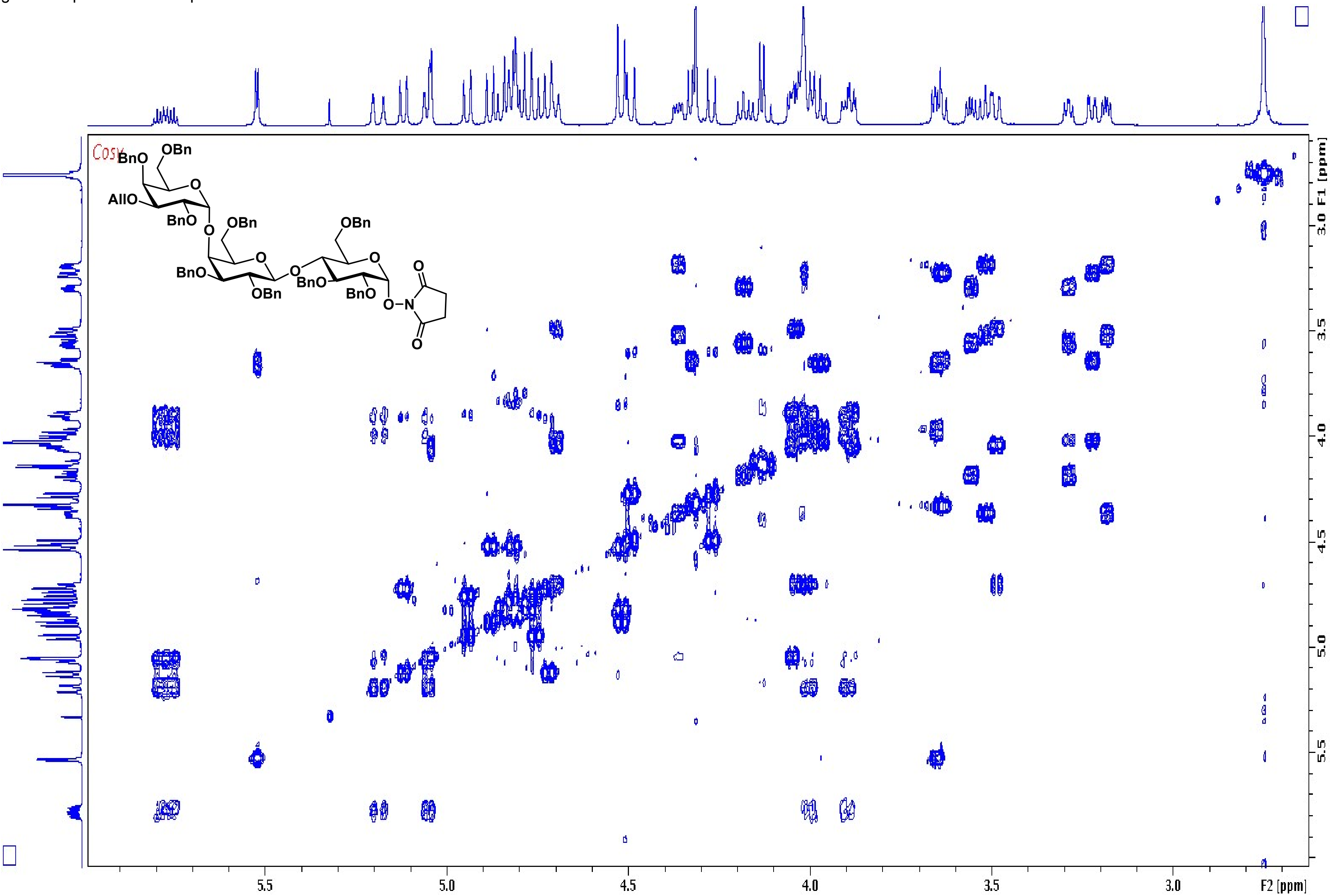
^{13}C NMR Spectrum of Compound **14**



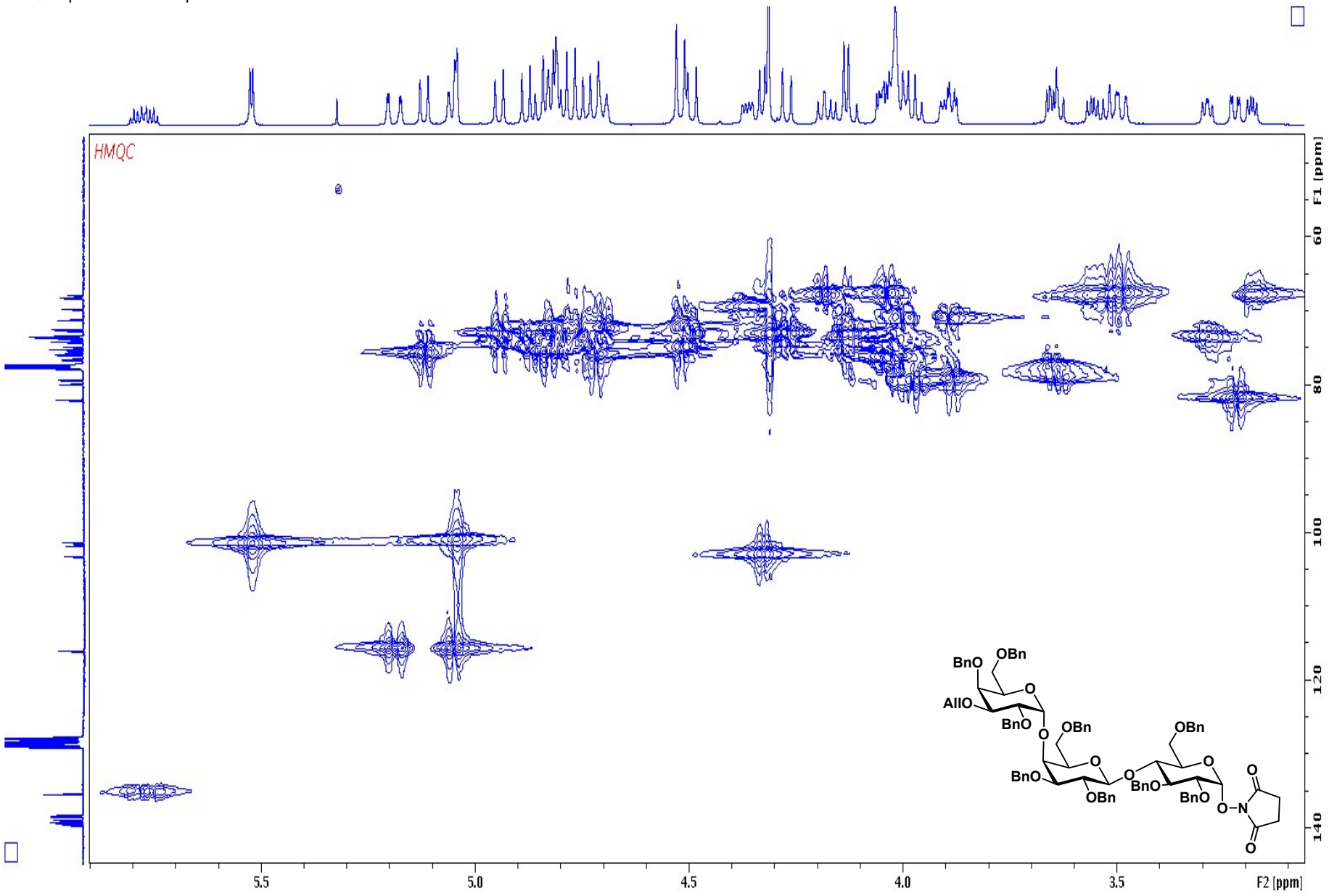
DEPT 135 Spectrum of Compound **14**



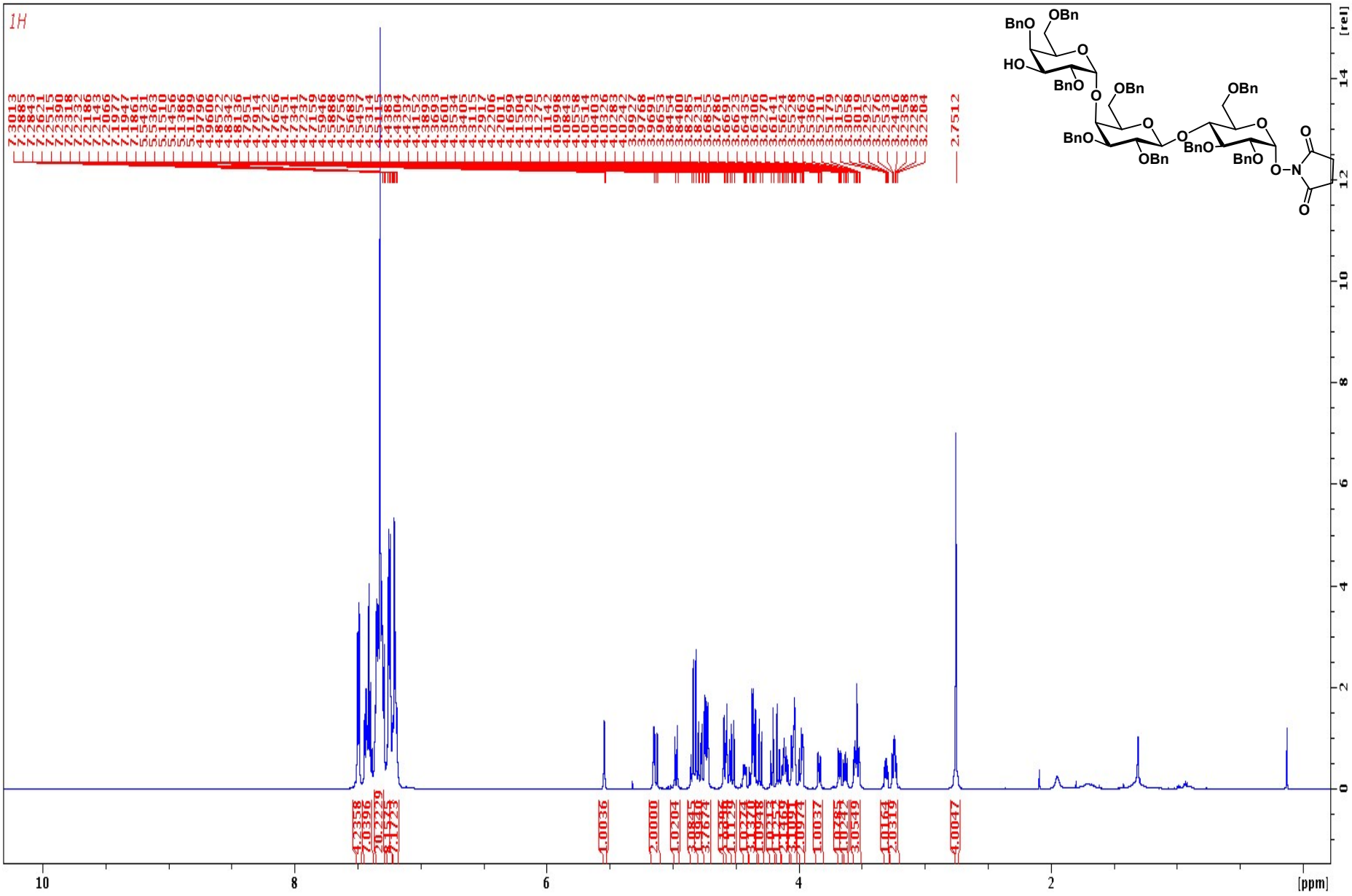
gCOSY Spectrum of Compound **14**



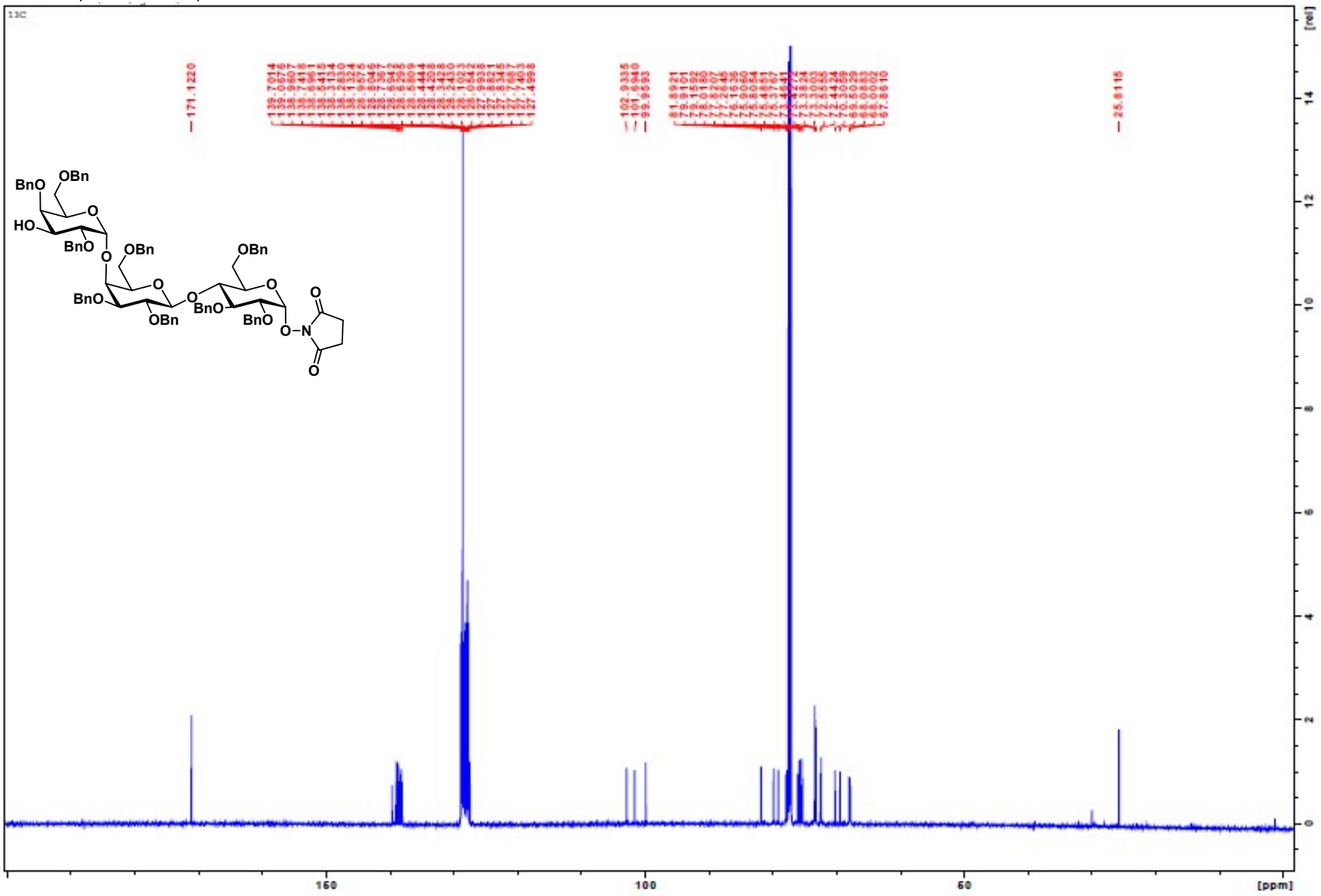
HMQC Spectrum of Compound **14**



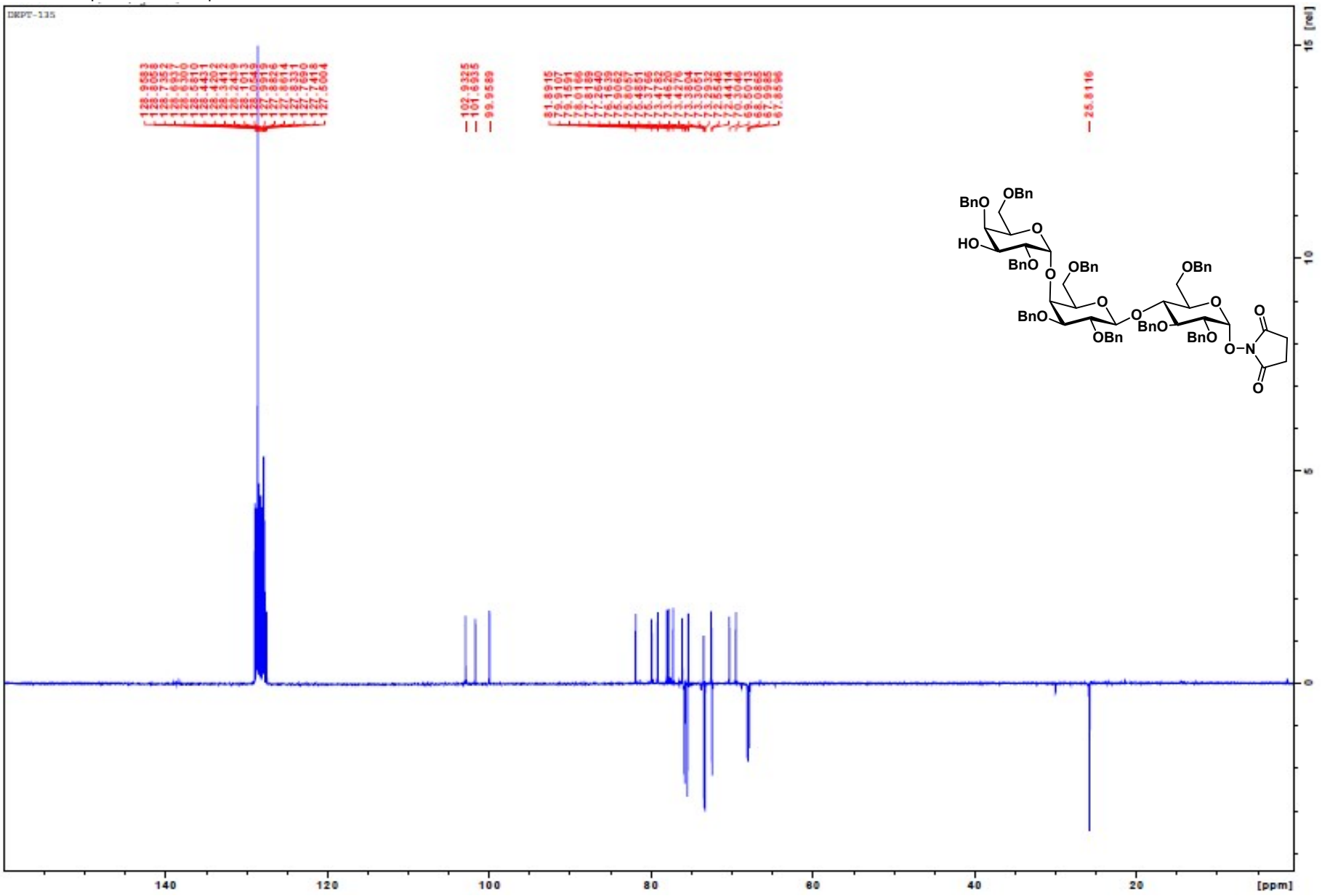
^{1}H NMR Spectrum of Compound **6**



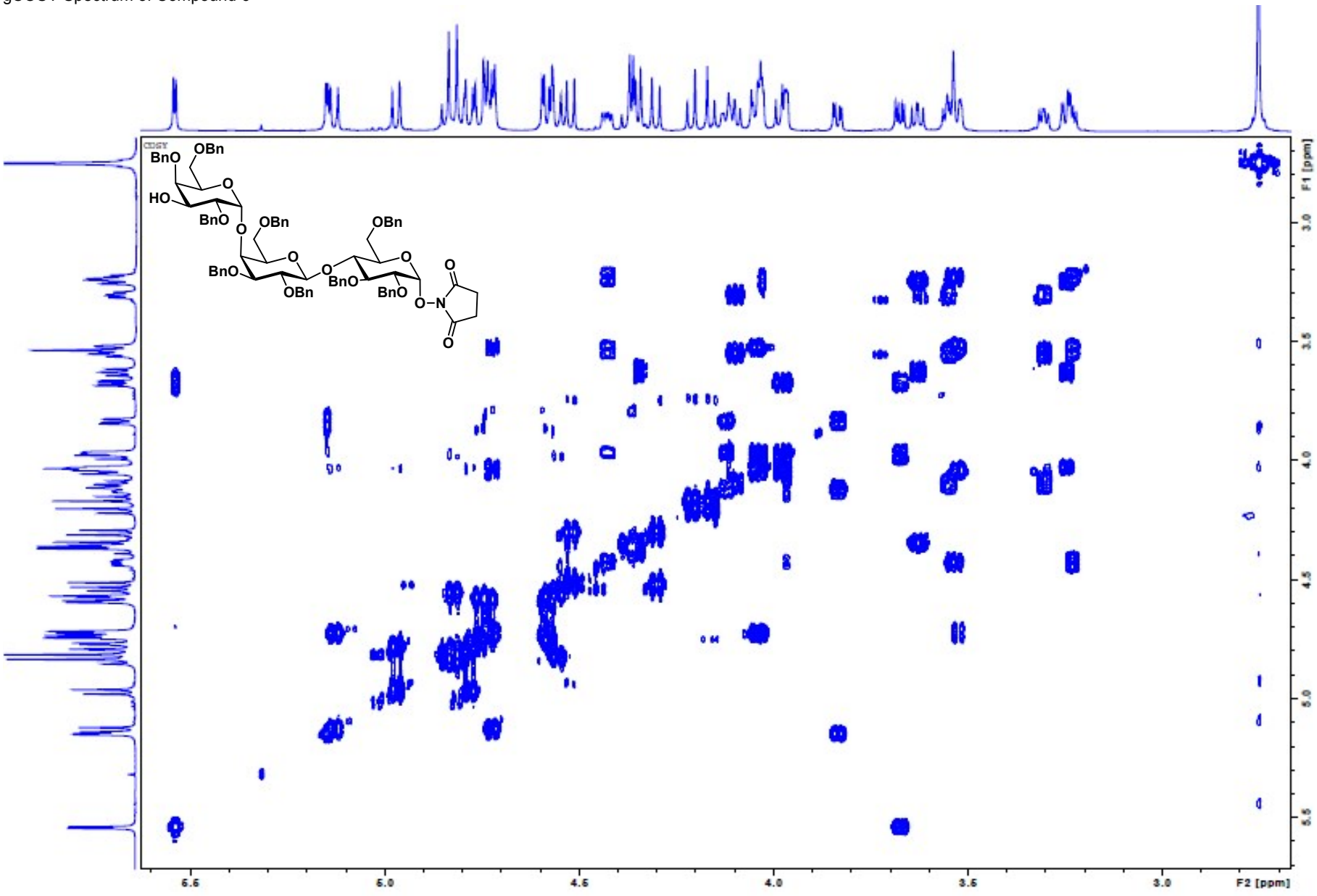
^{13}C NMR Spectrum of Compound **6**



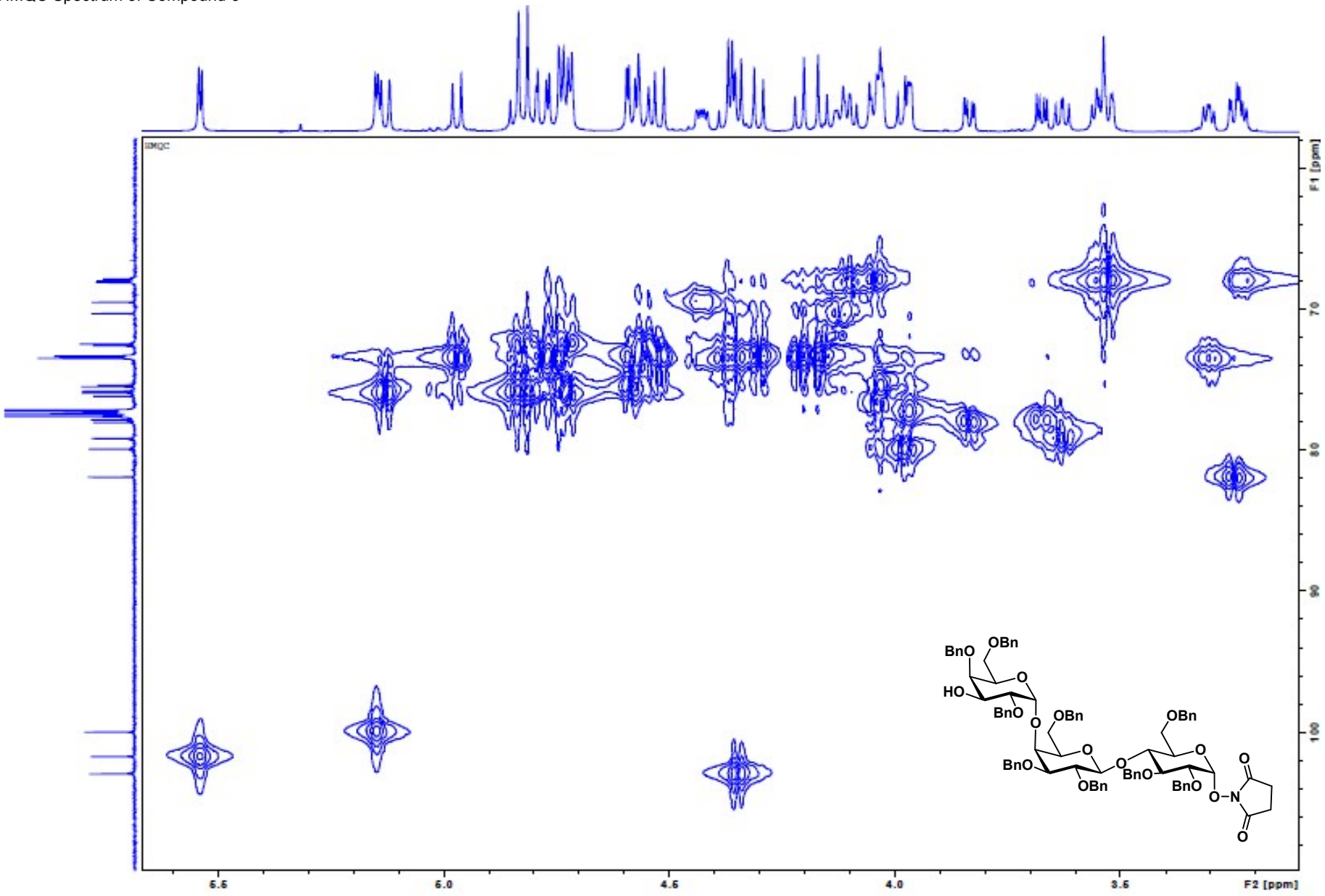
DEPT 135 Spectrum of Compound **6**



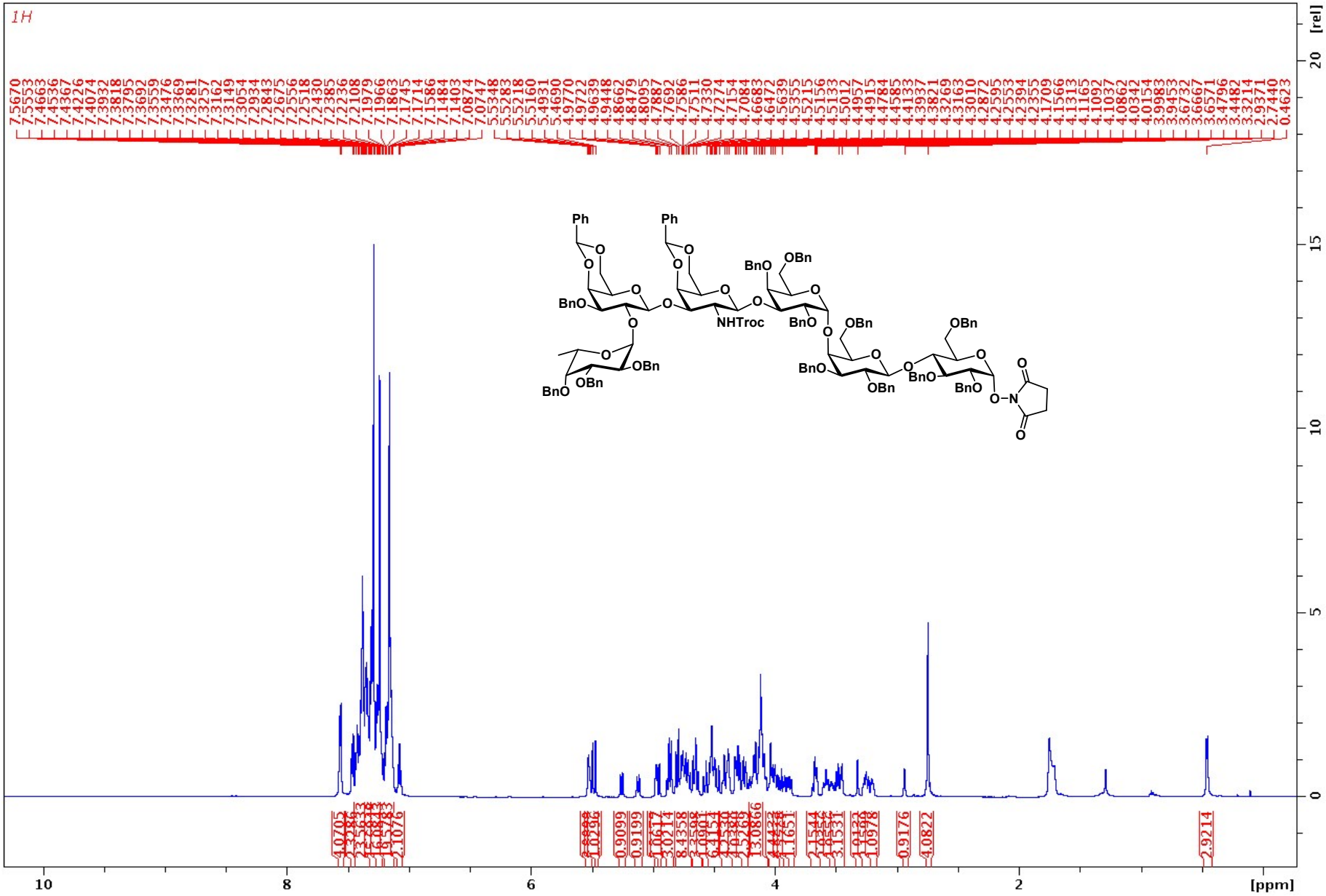
gCOSY Spectrum of Compound **6**



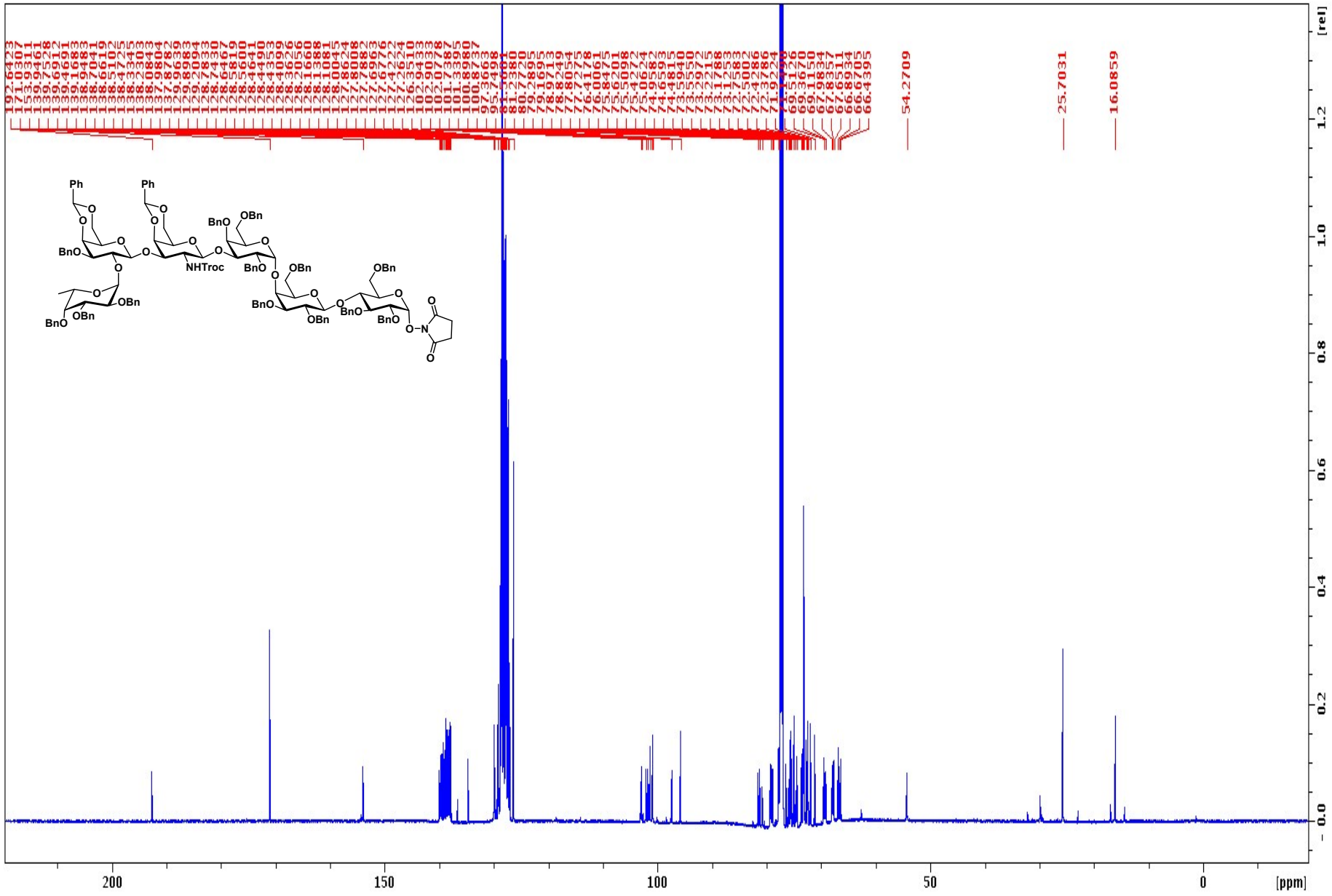
HMQC Spectrum of Compound **6**



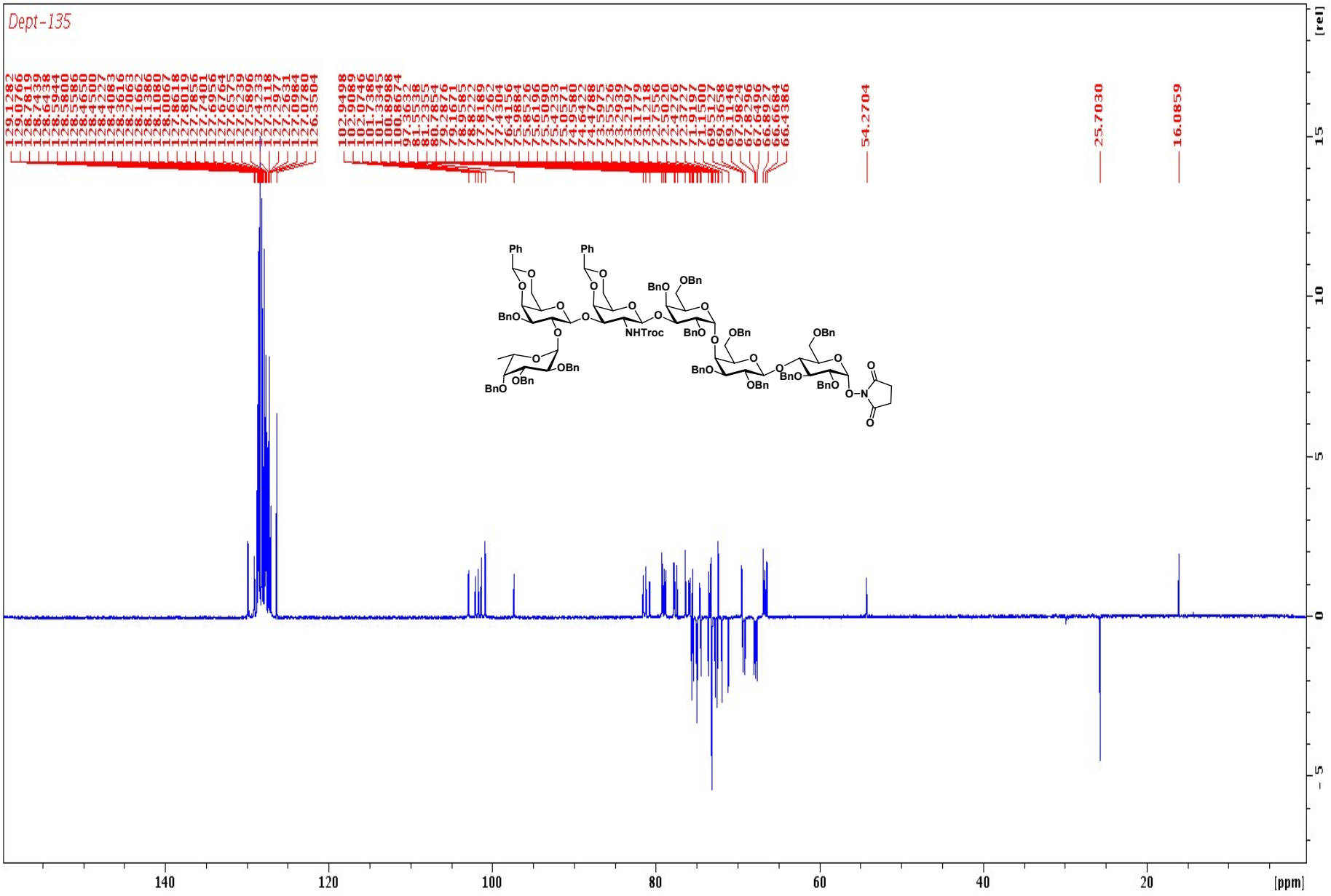
^{1}H NMR Spectrum of Compound **4**



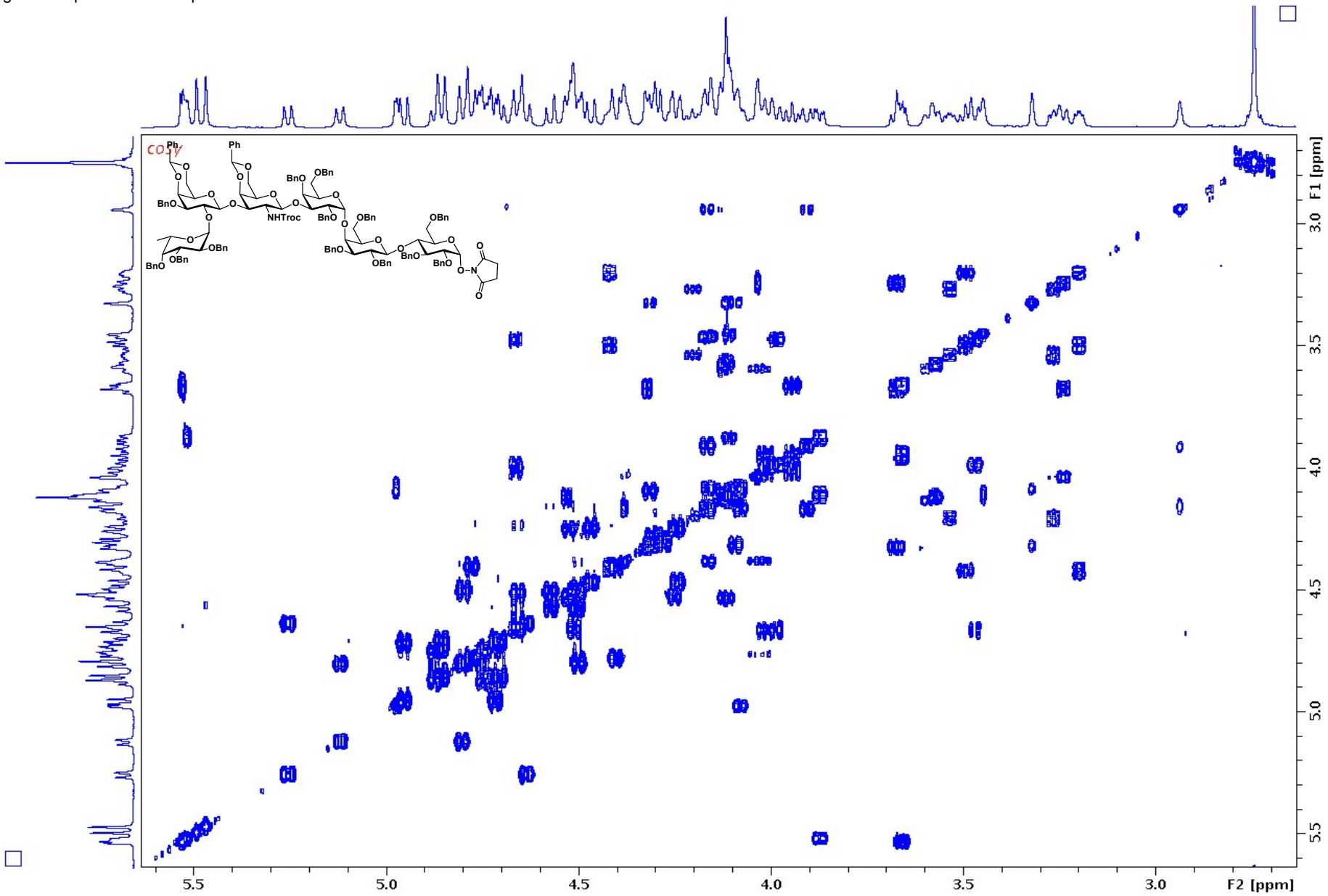
^{13}C NMR Spectrum of Compound **4**



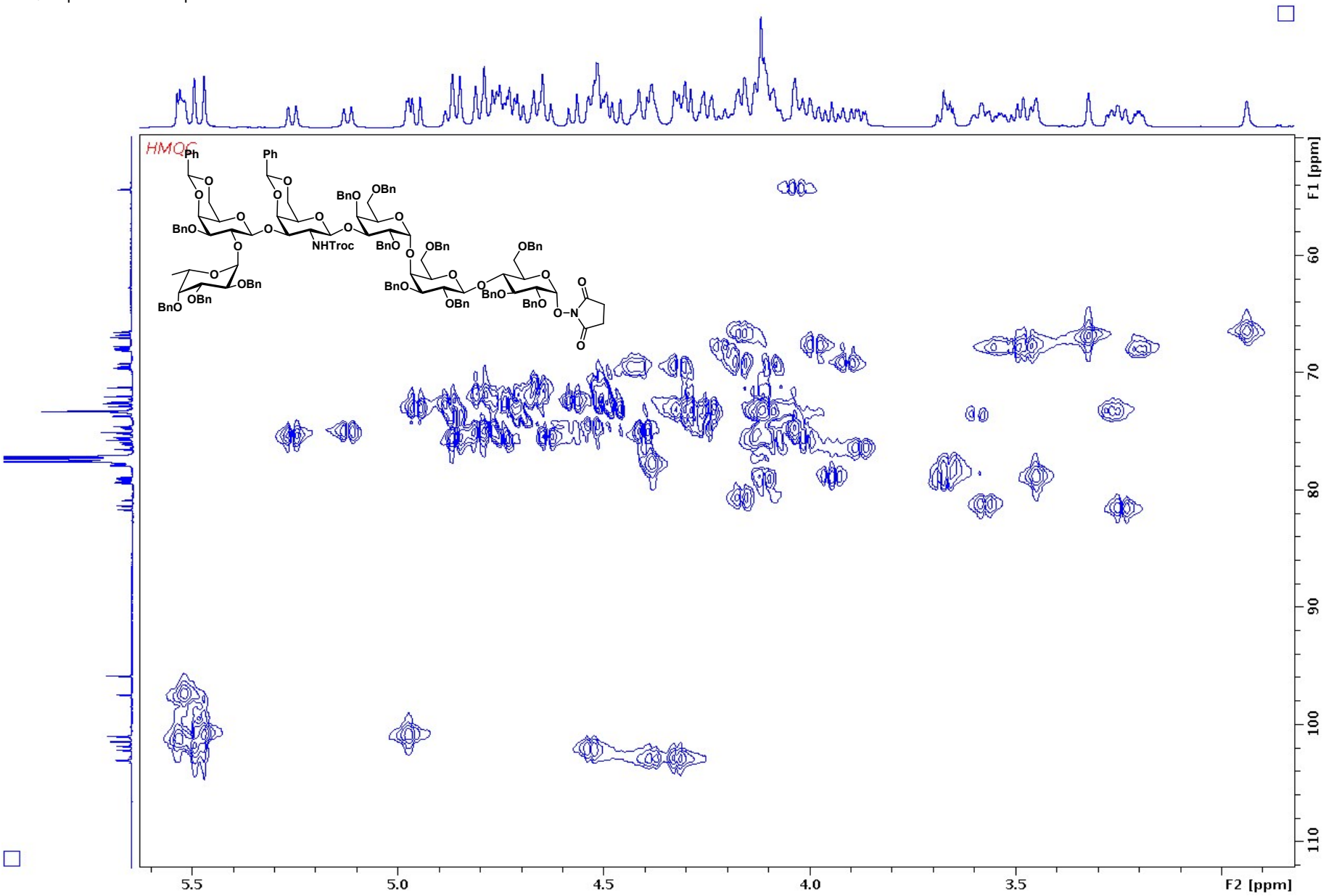
DEPT 135 Spectrum of Compound **4**



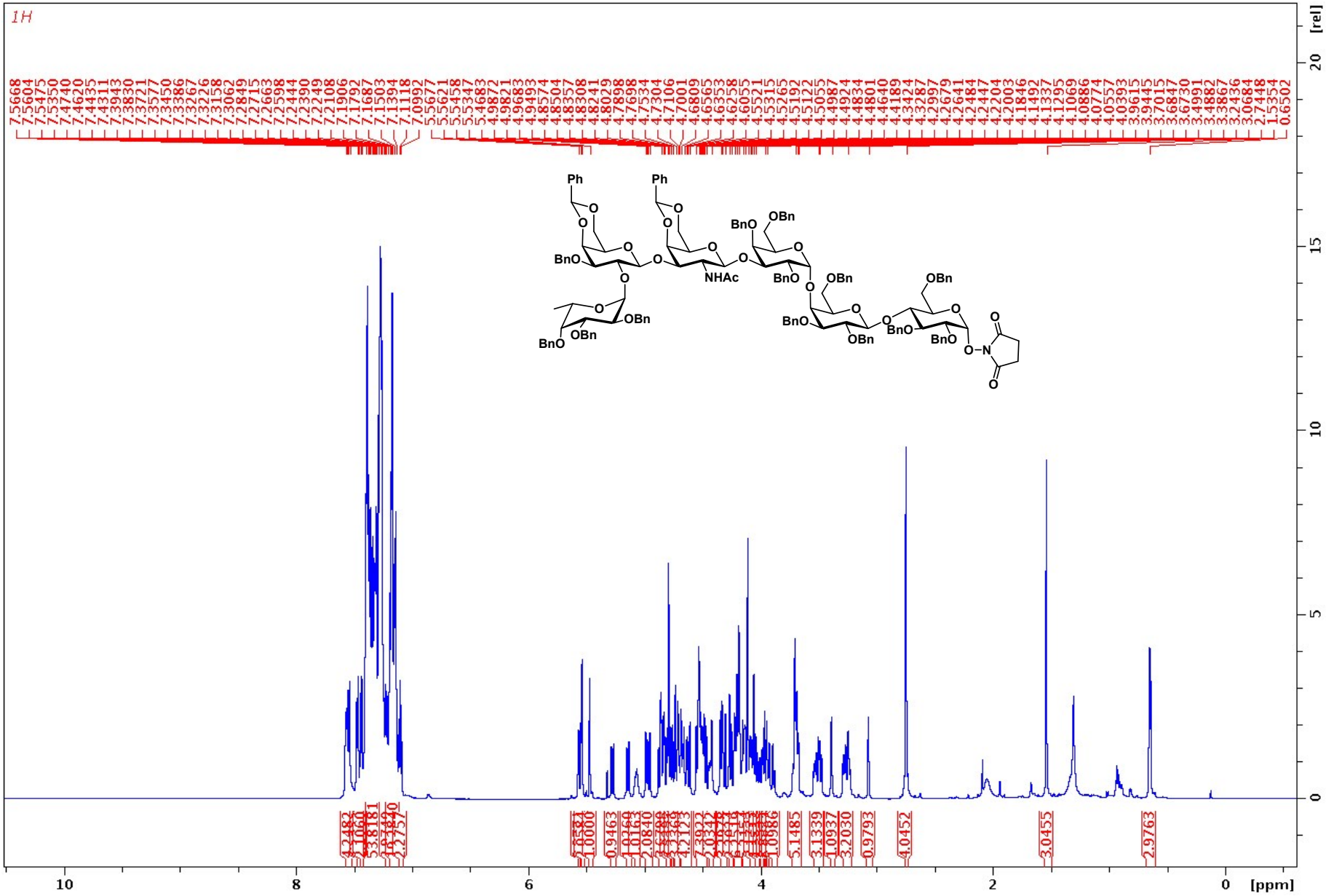
gCOSY Spectrum of Compound **4**



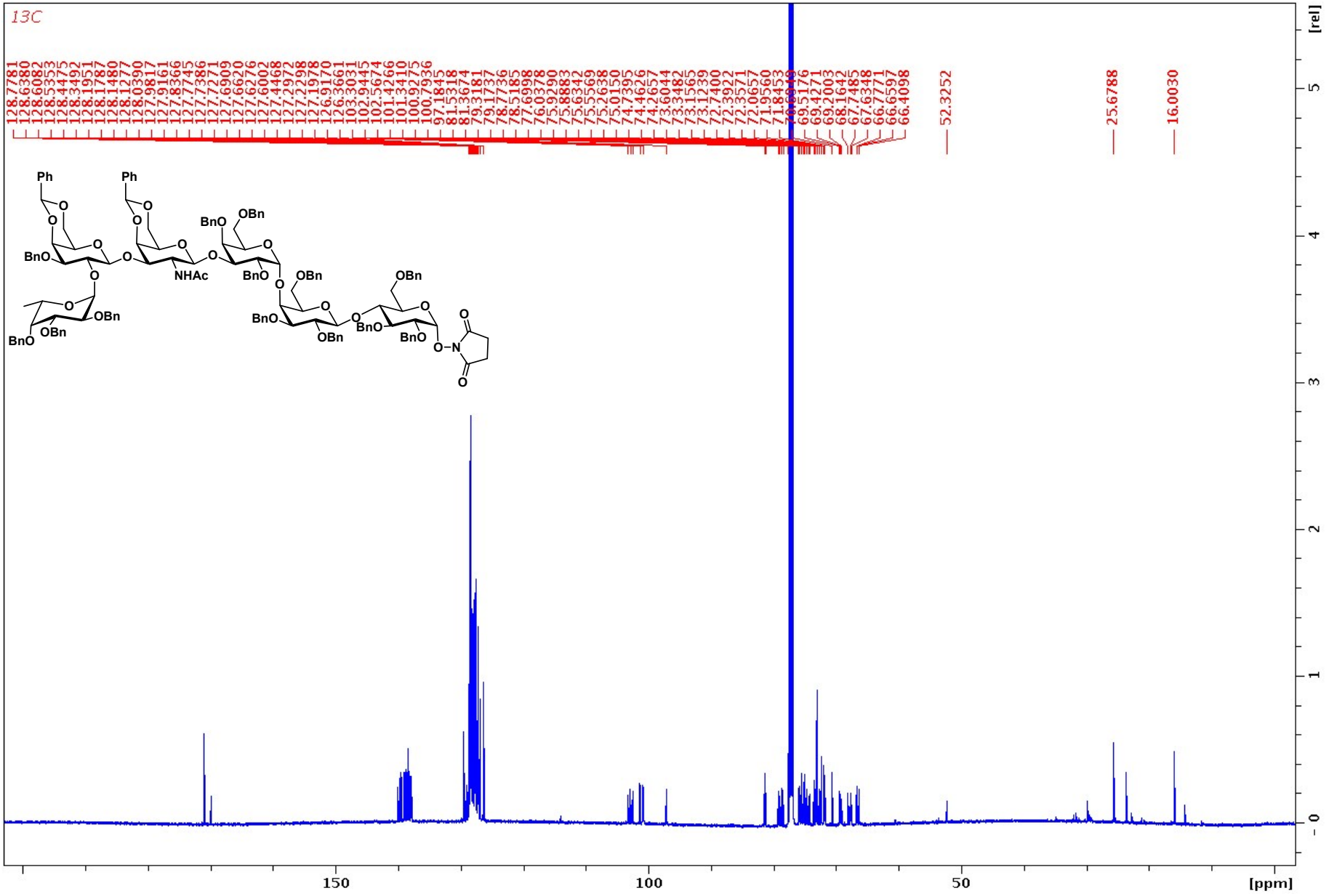
HMQC Spectrum of Compound **4**



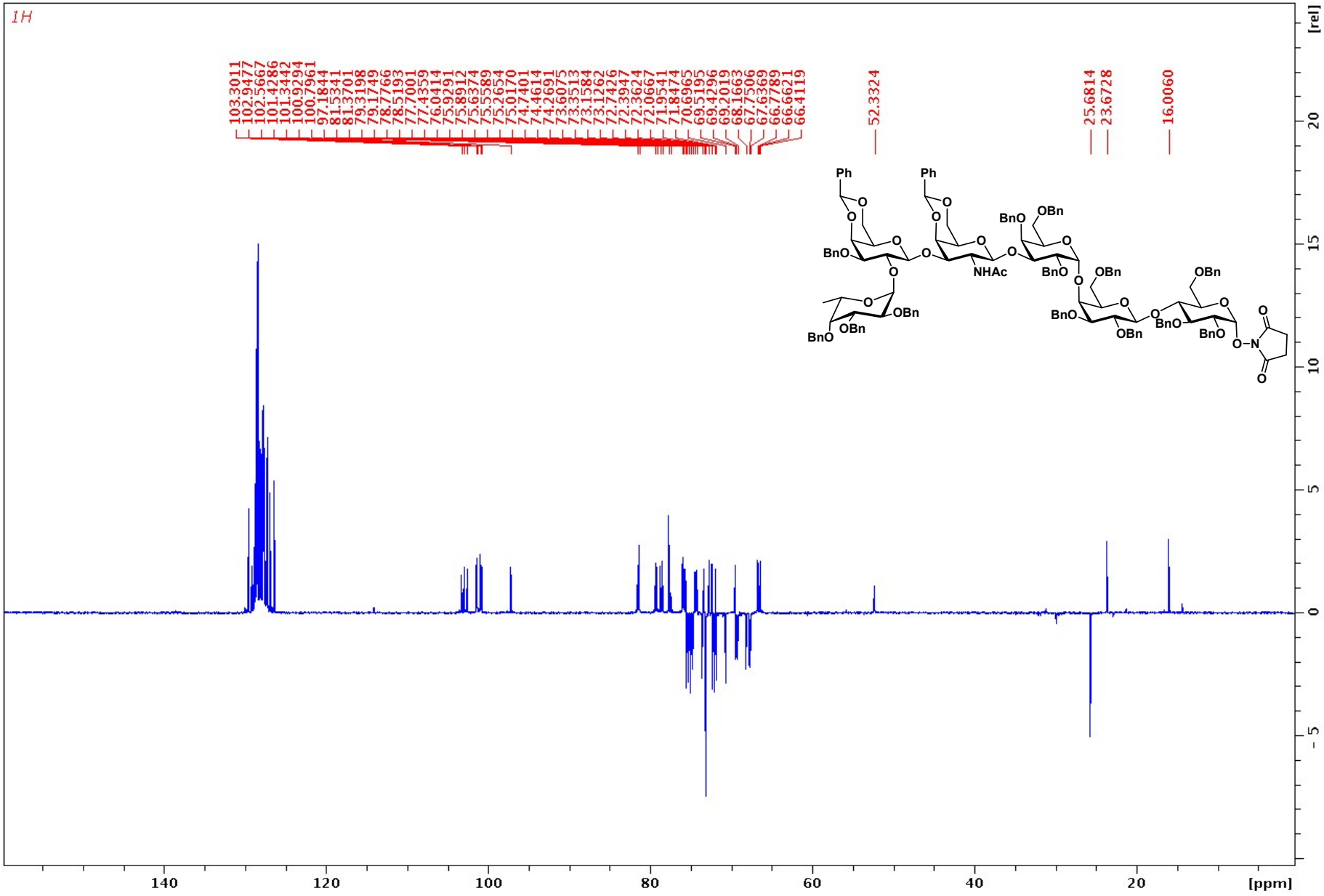
^{1}H NMR Spectrum of Compound **S4**



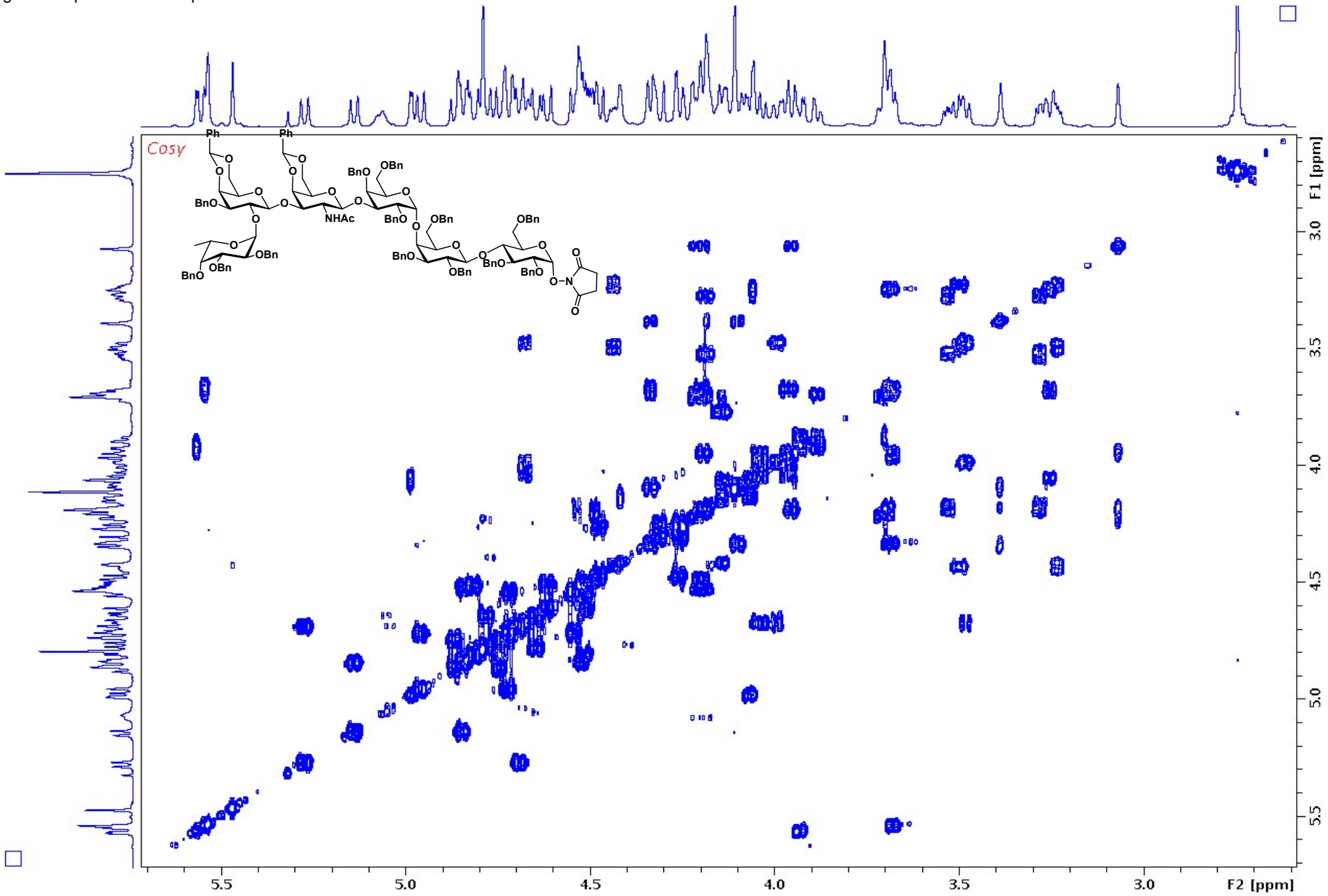
^{13}C NMR Spectrum of Compound **S4**



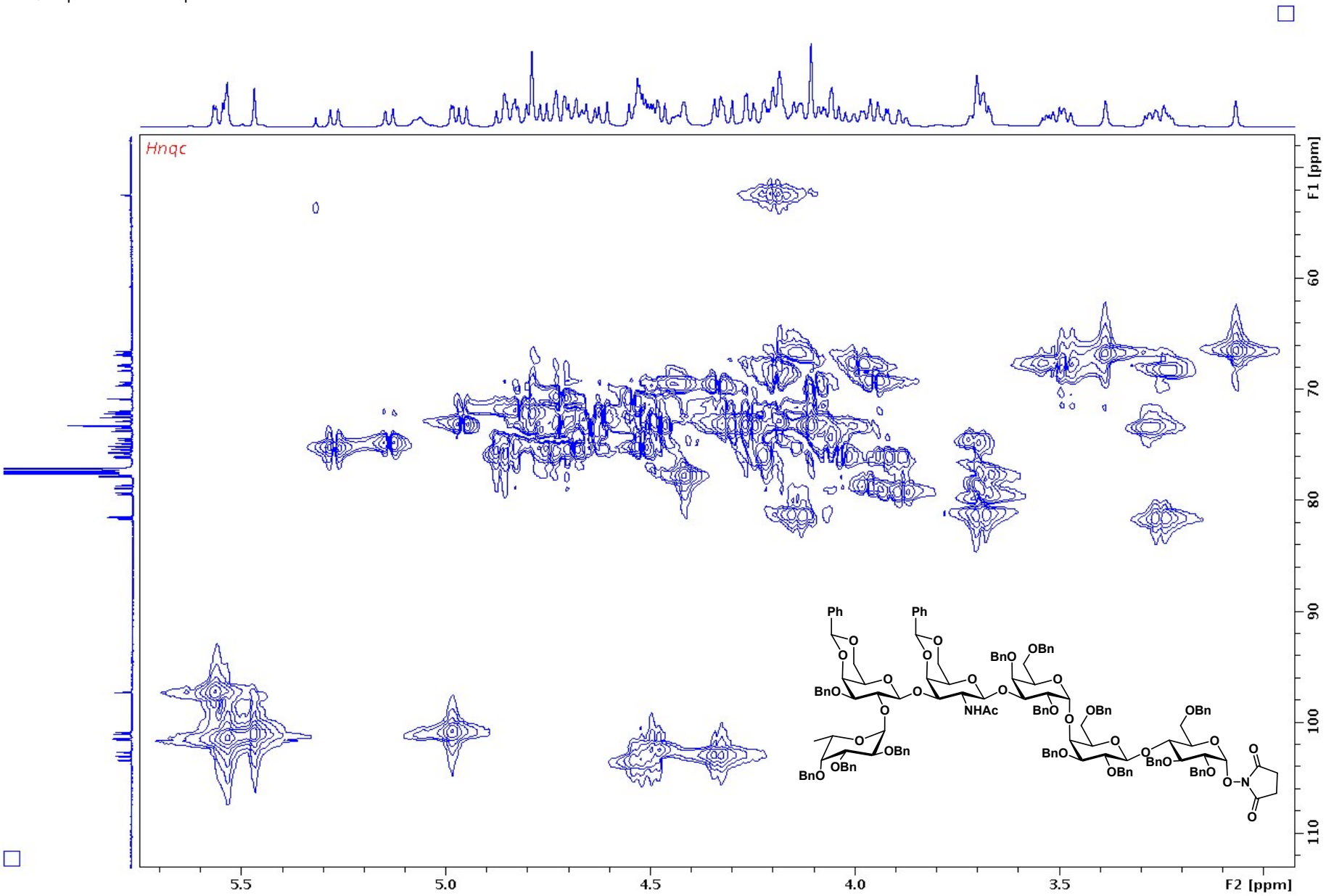
DEPT 135 Spectrum of Compound **S4**



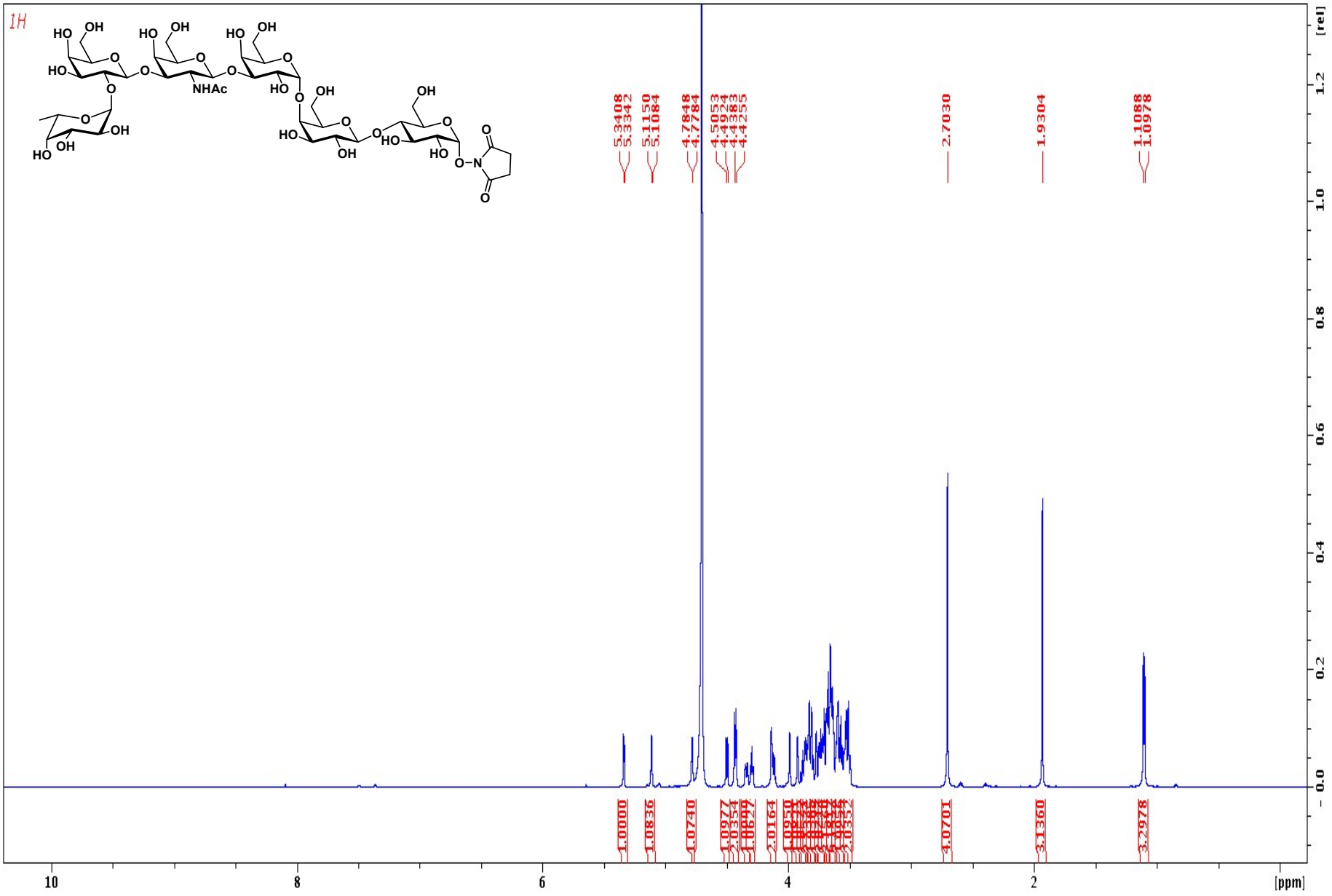
gCOSY Spectrum of Compound **S4**



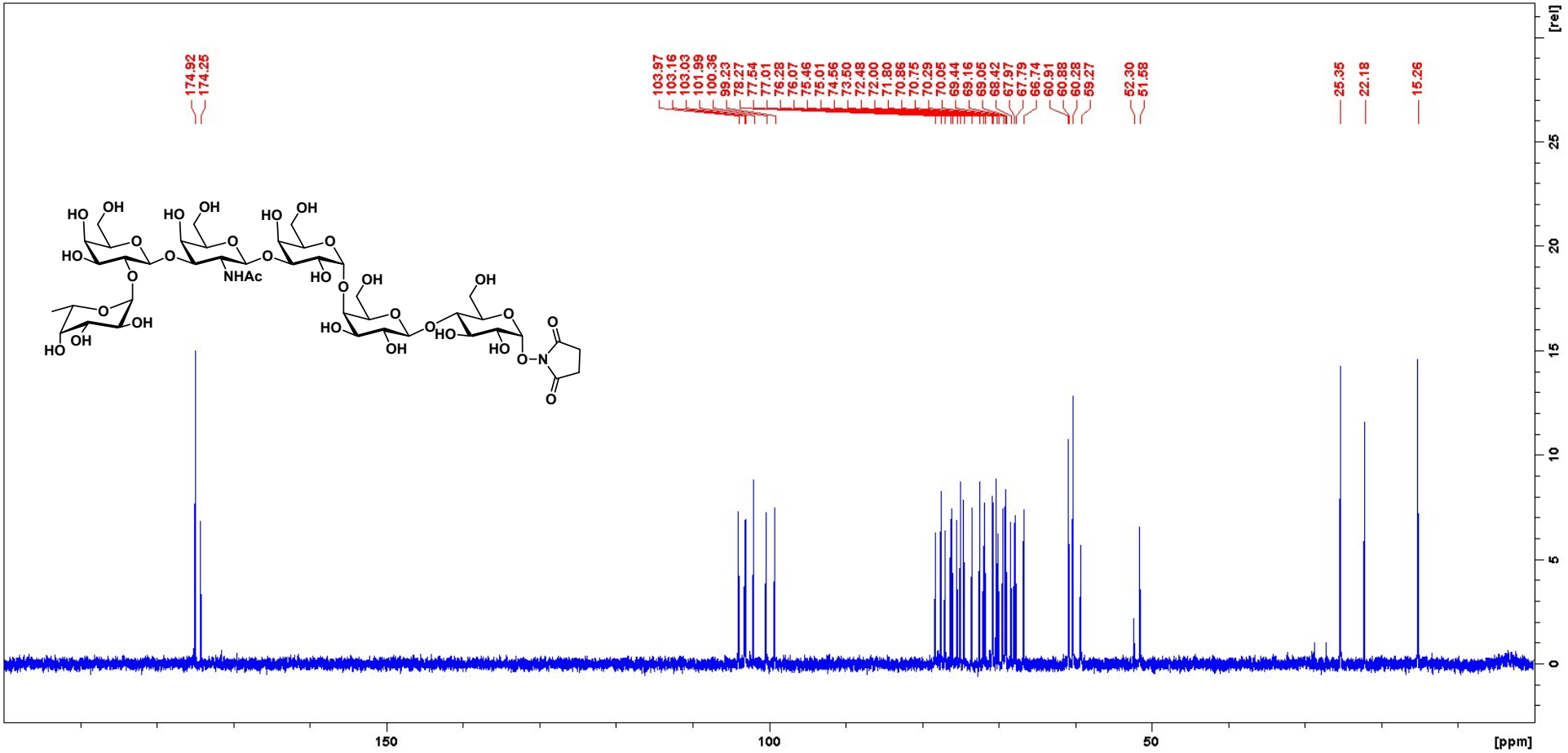
HMQC Spectrum of Compound **S4**



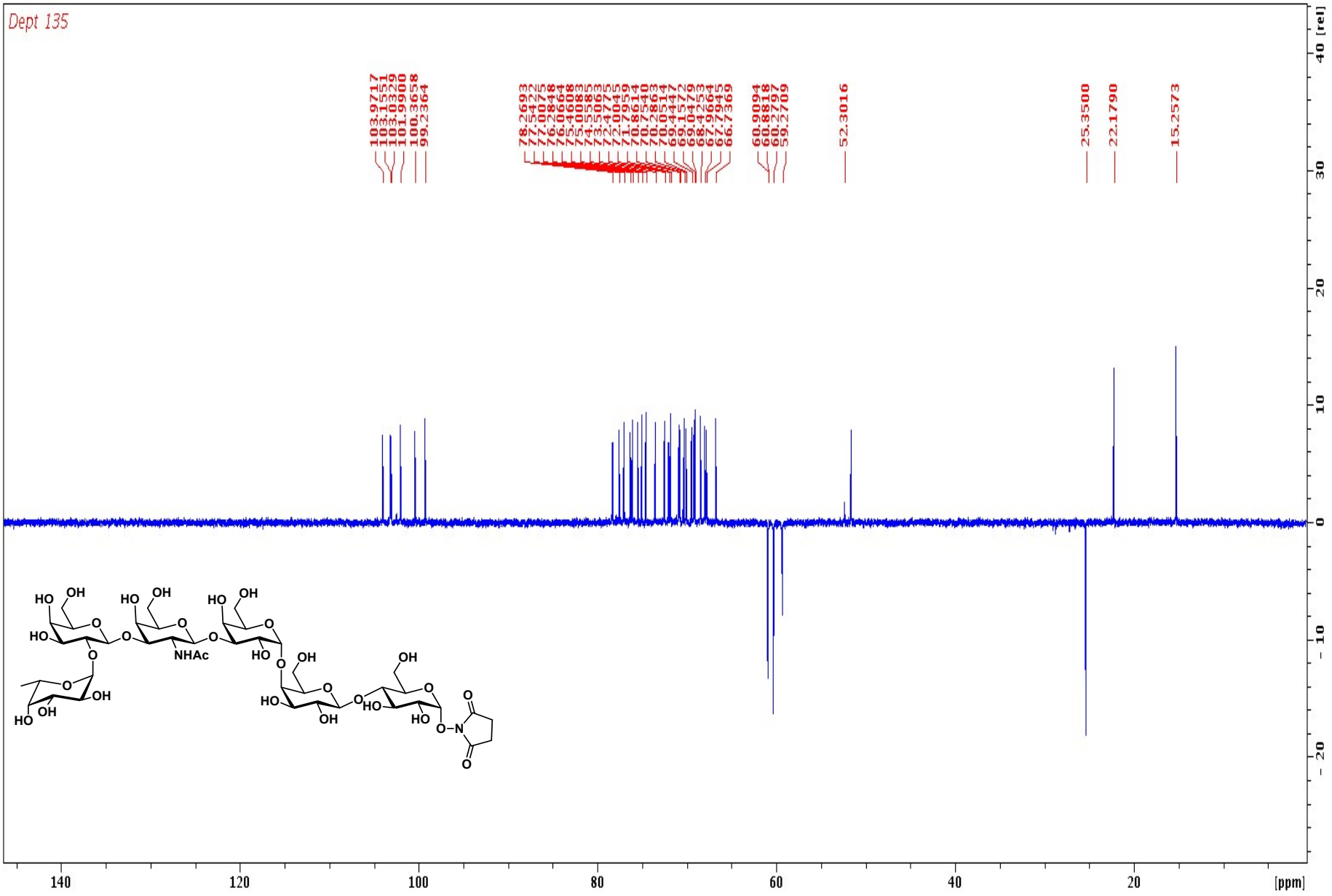
^{1}H NMR Spectrum of Compound **S5**



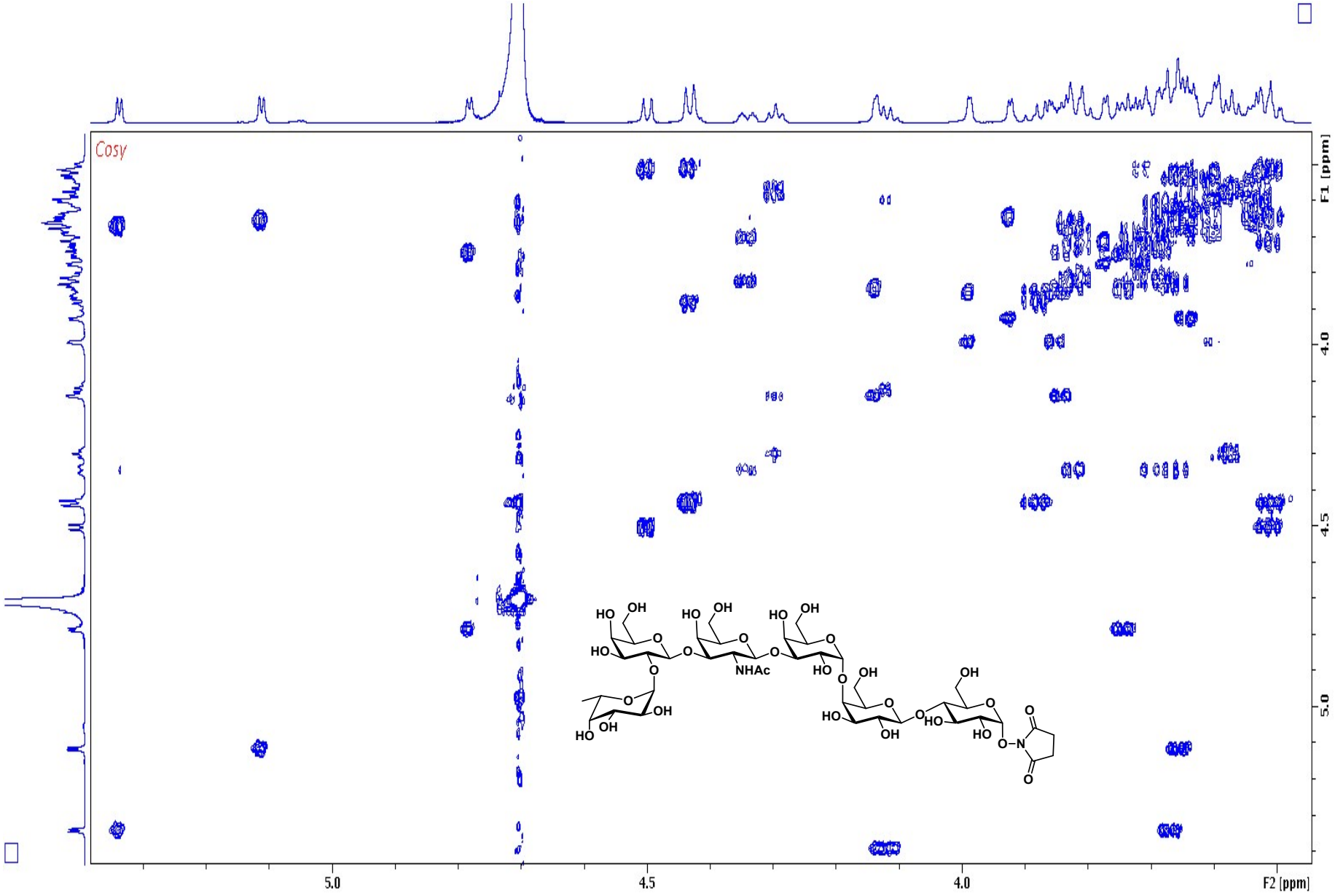
^{13}C NMR Spectrum of Compound **S5**



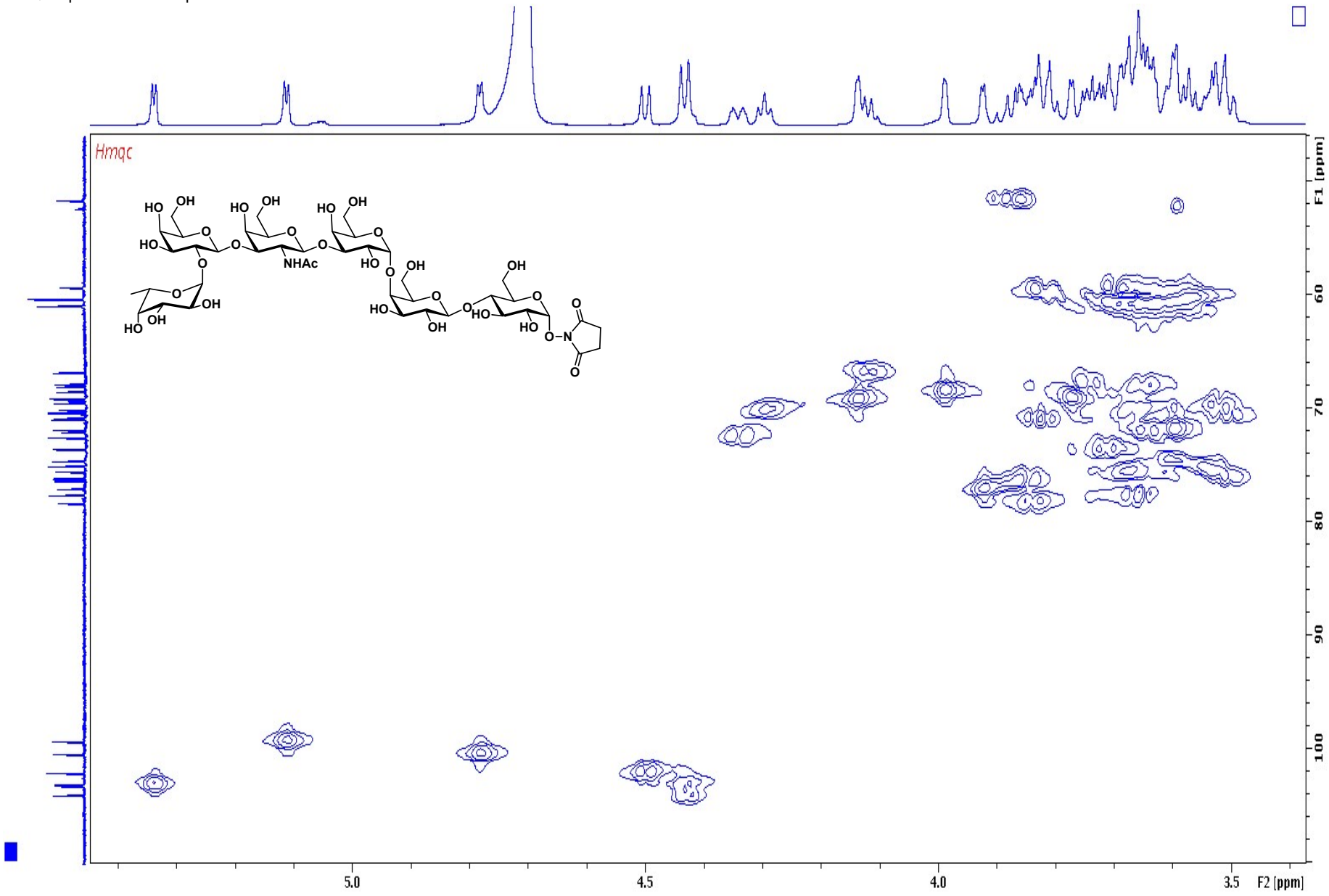
DEPT 135 Spectrum of Compound **S5**



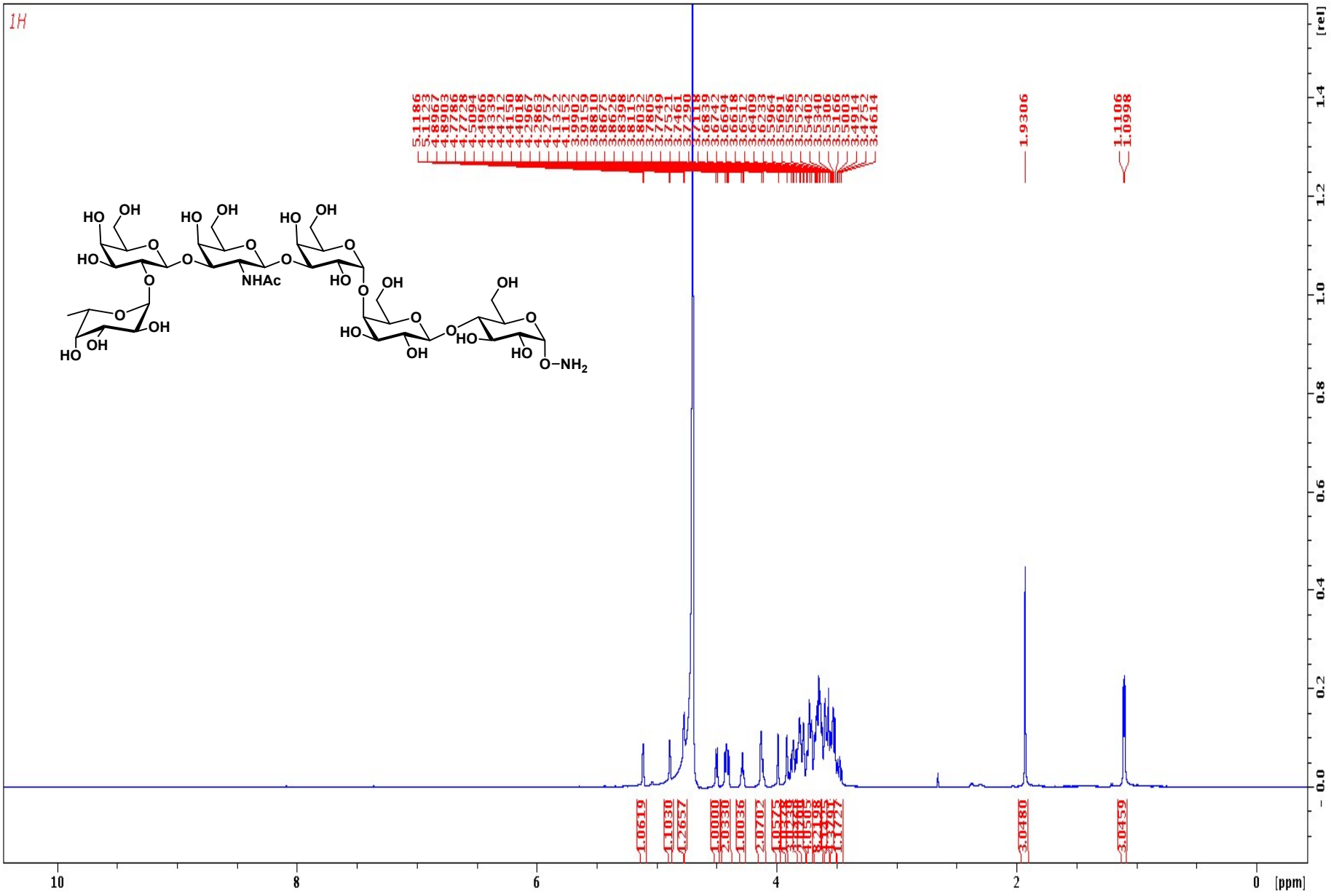
gCOSY Spectrum of Compound **S5**



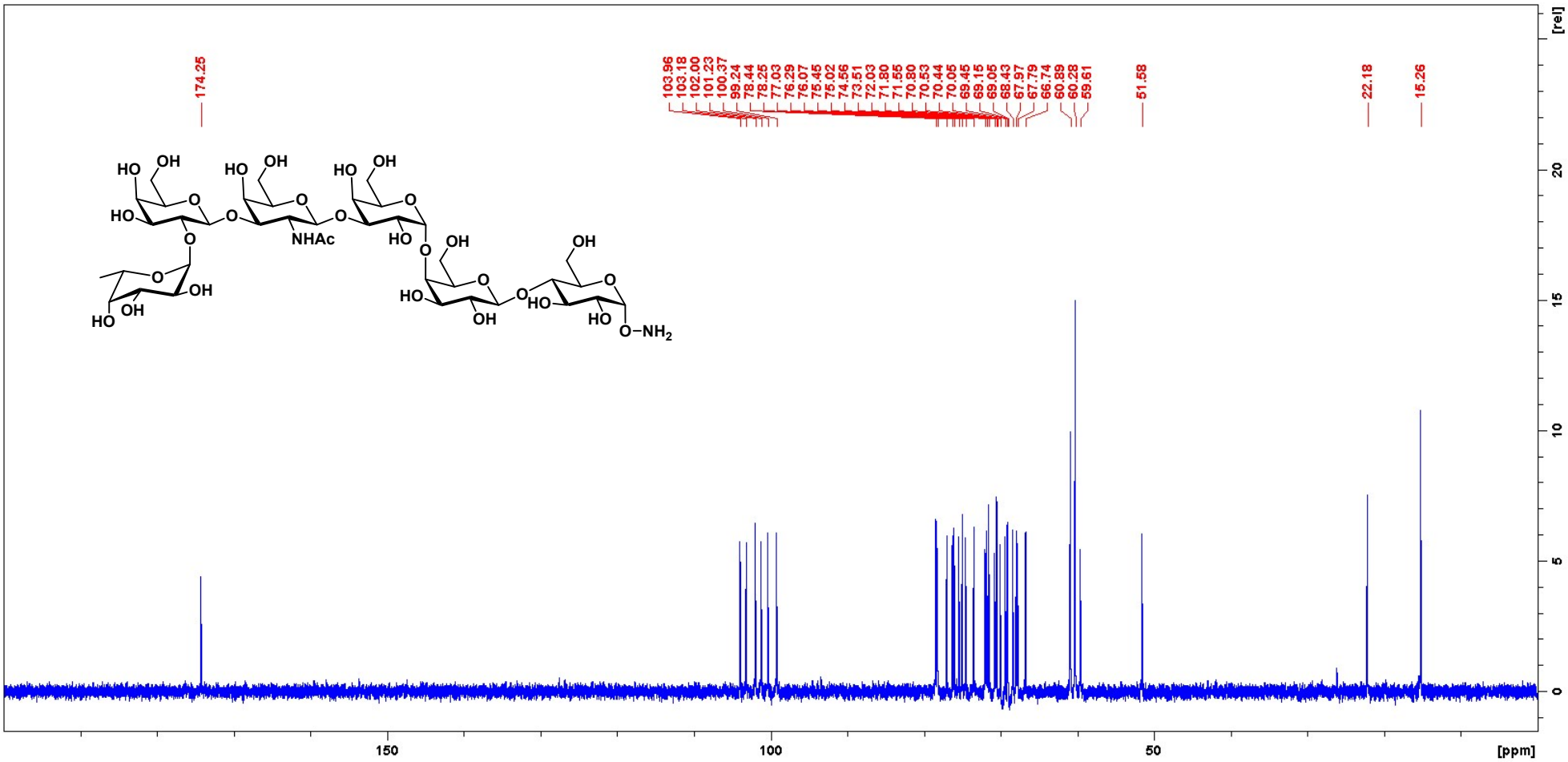
HMQC Spectrum of Compound **S5**



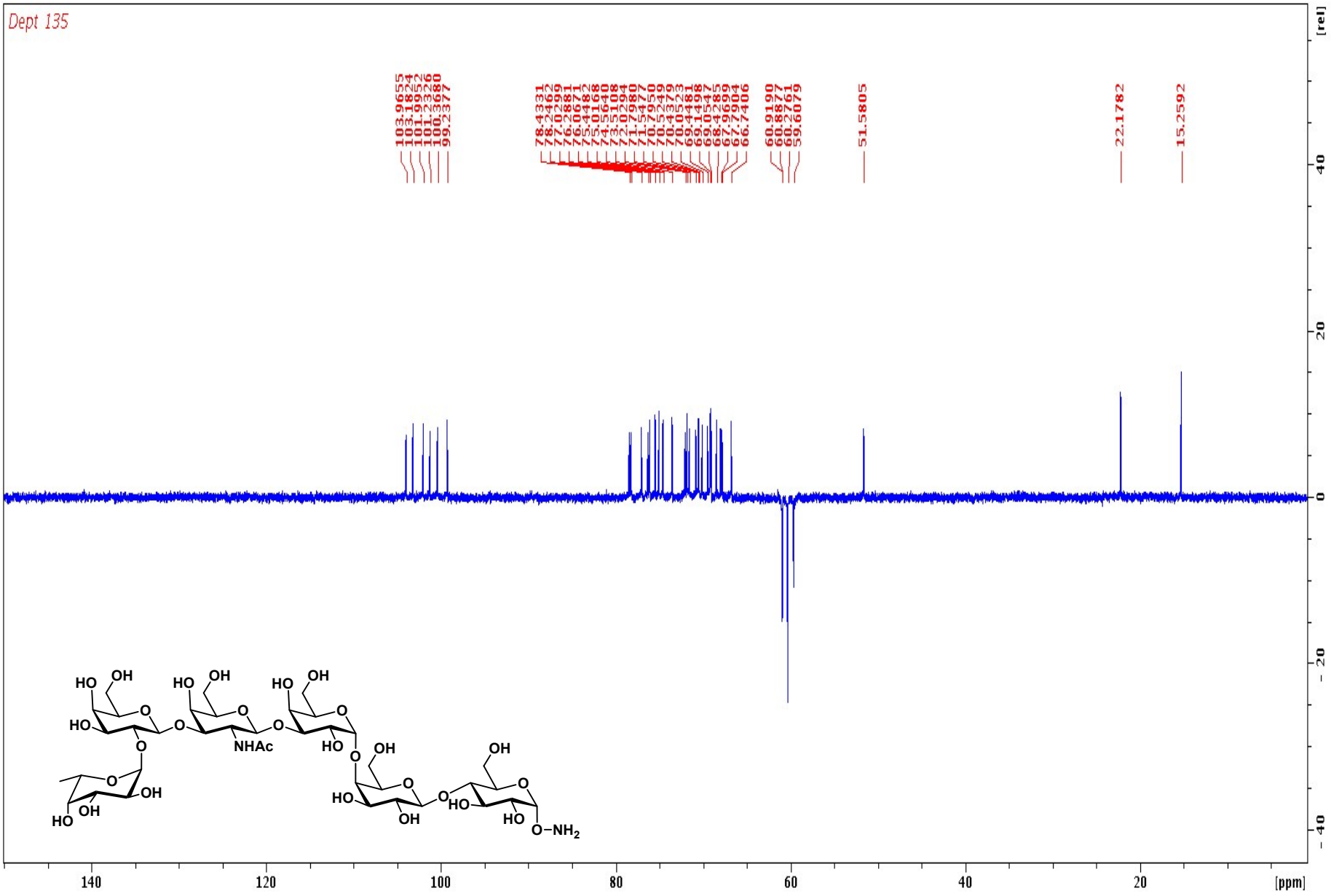
^{1}H NMR Spectrum of Compound **2**



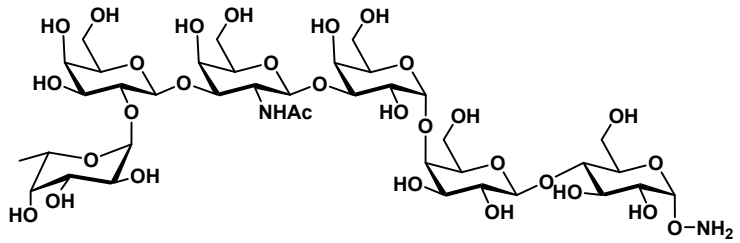
^{13}C NMR Spectrum of Compound **2**

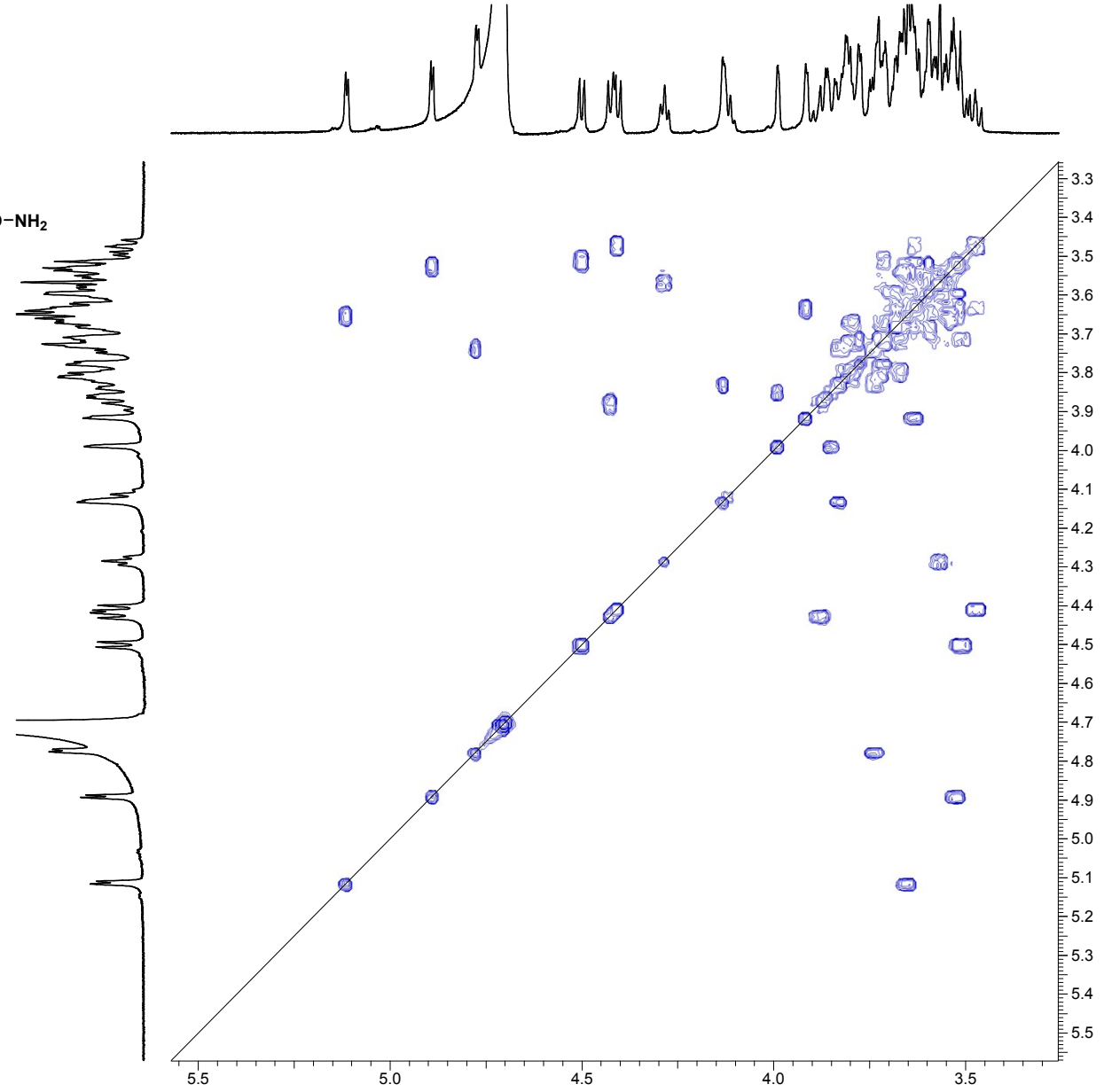


DEPT 135 Spectrum of Compound **2**

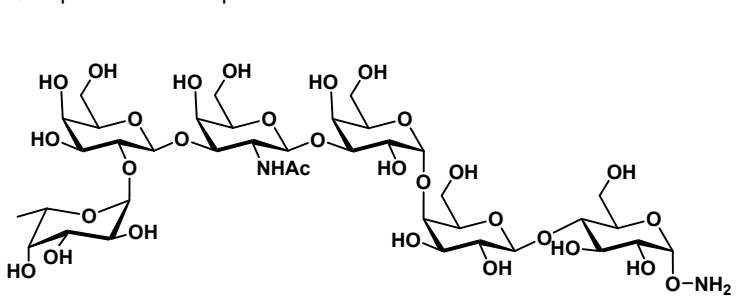


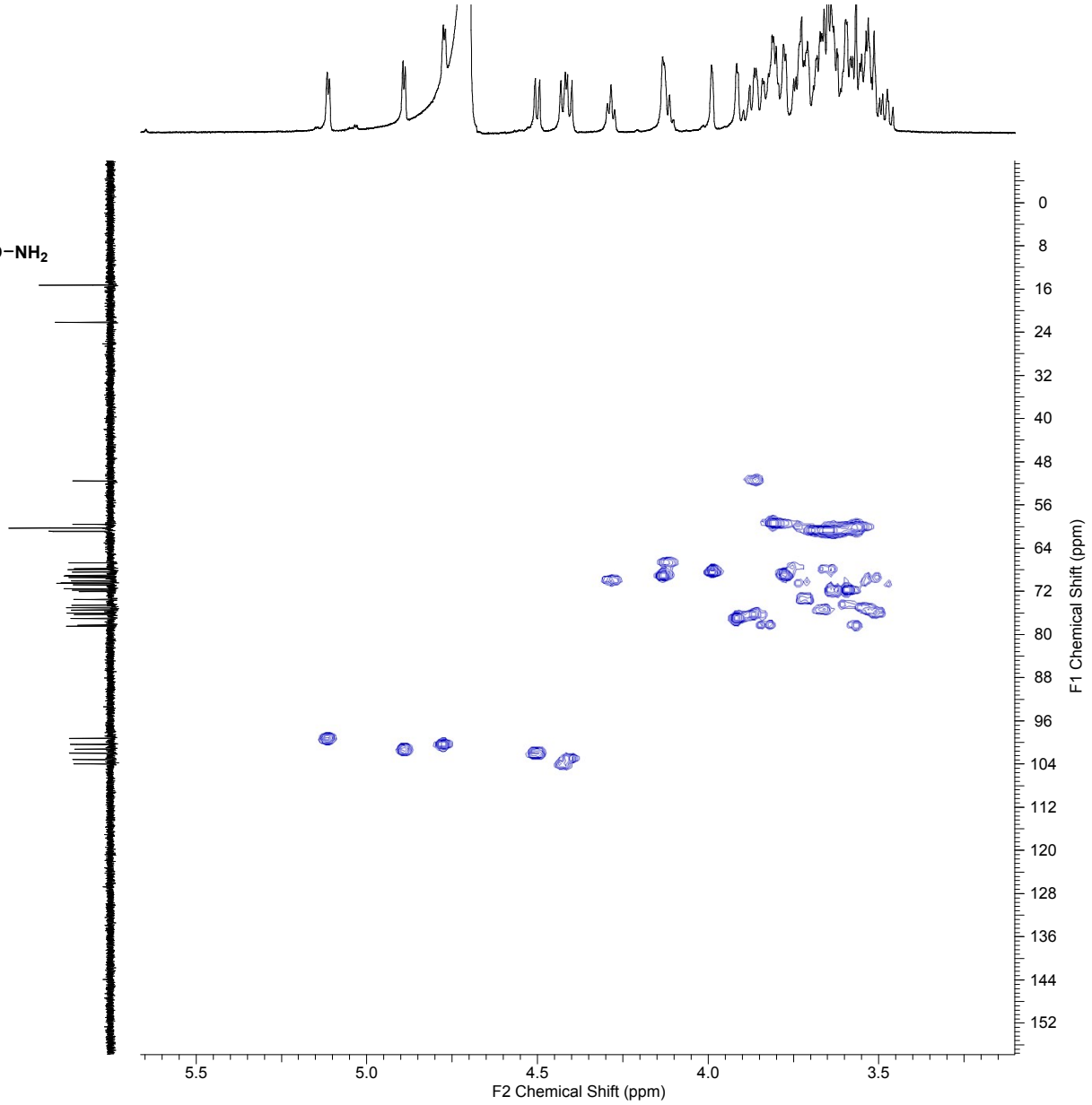
gCOSY Spectrum of Compound **2**



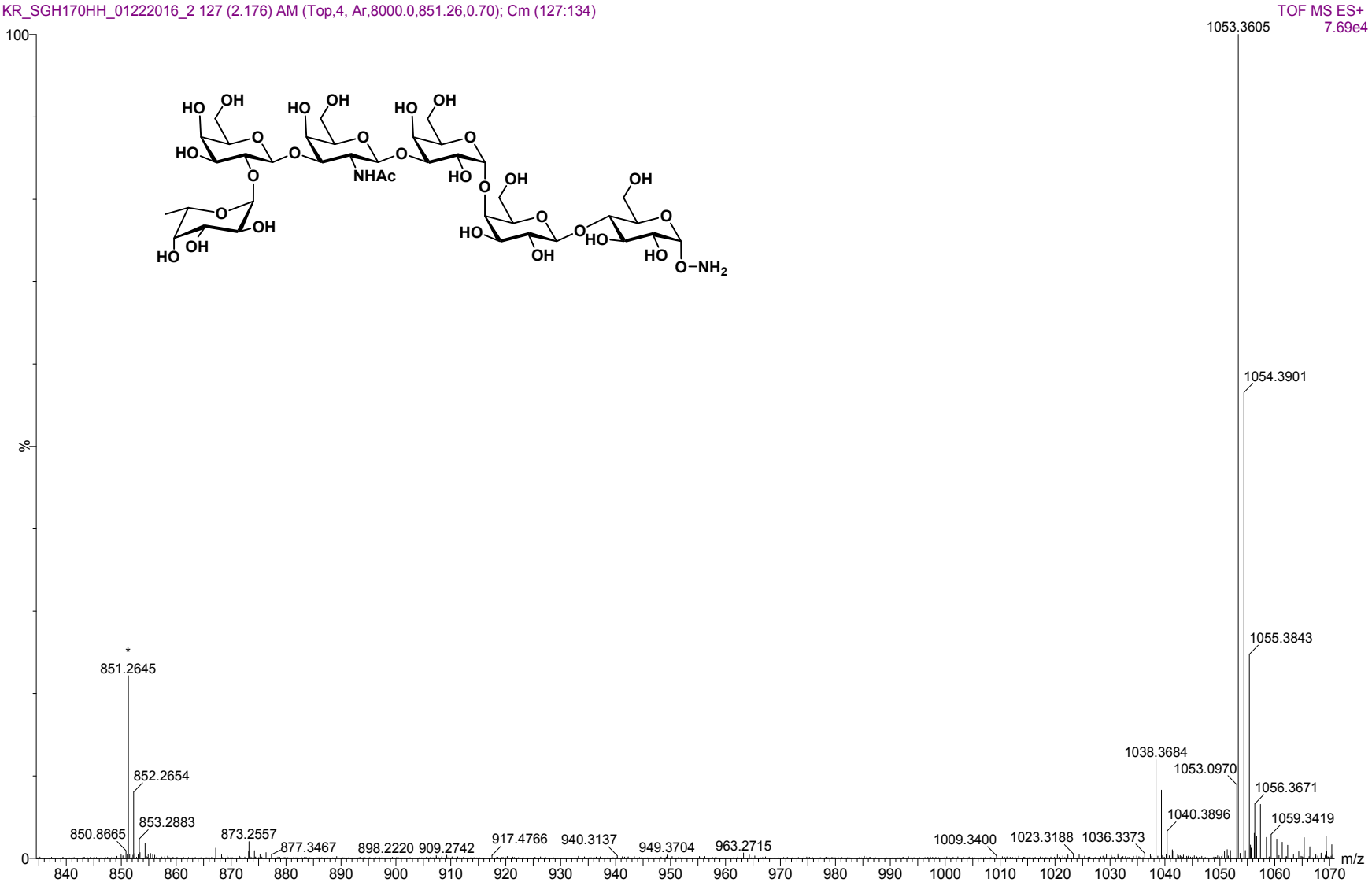


HMQC Spectrum of Compound **2**

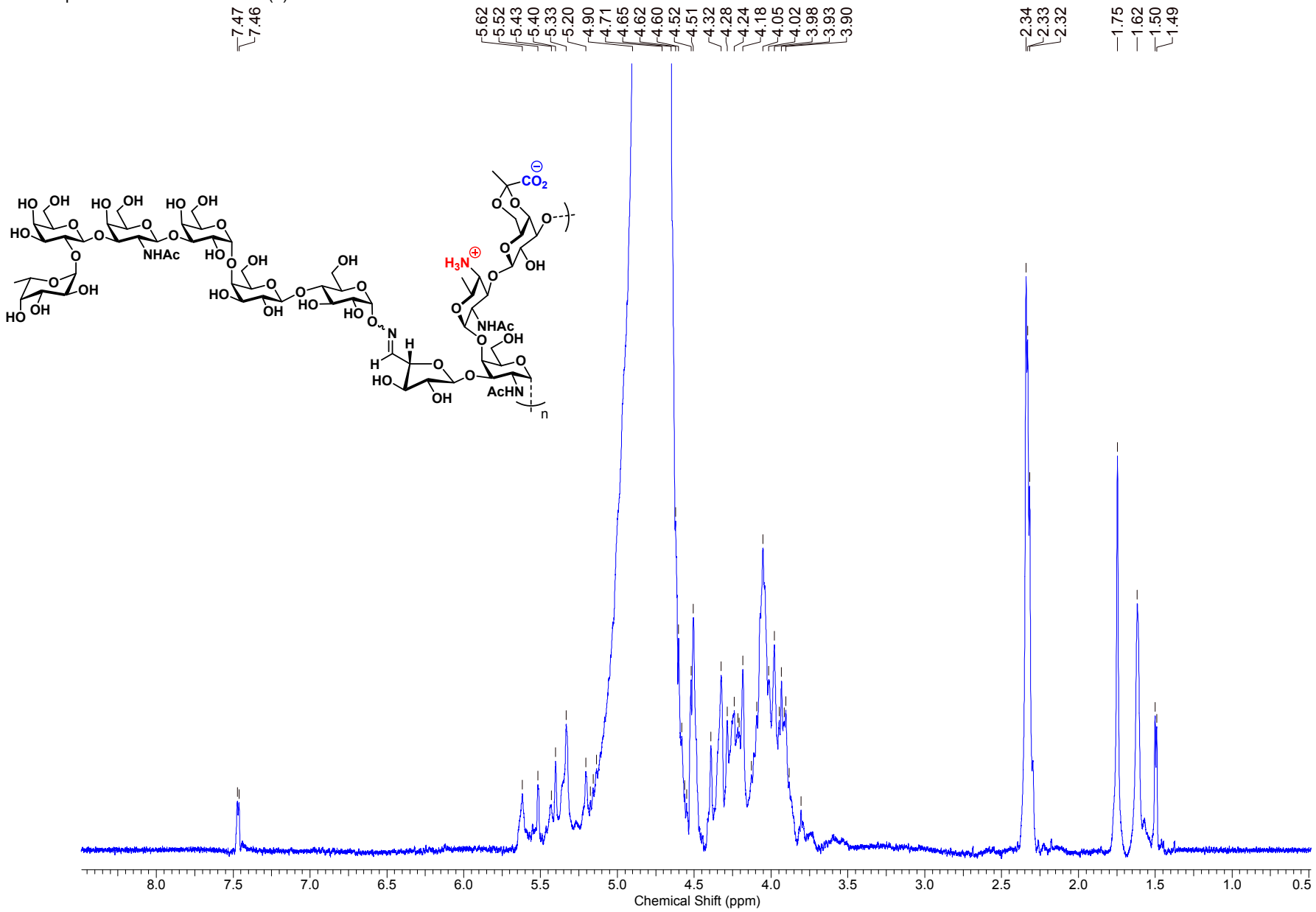




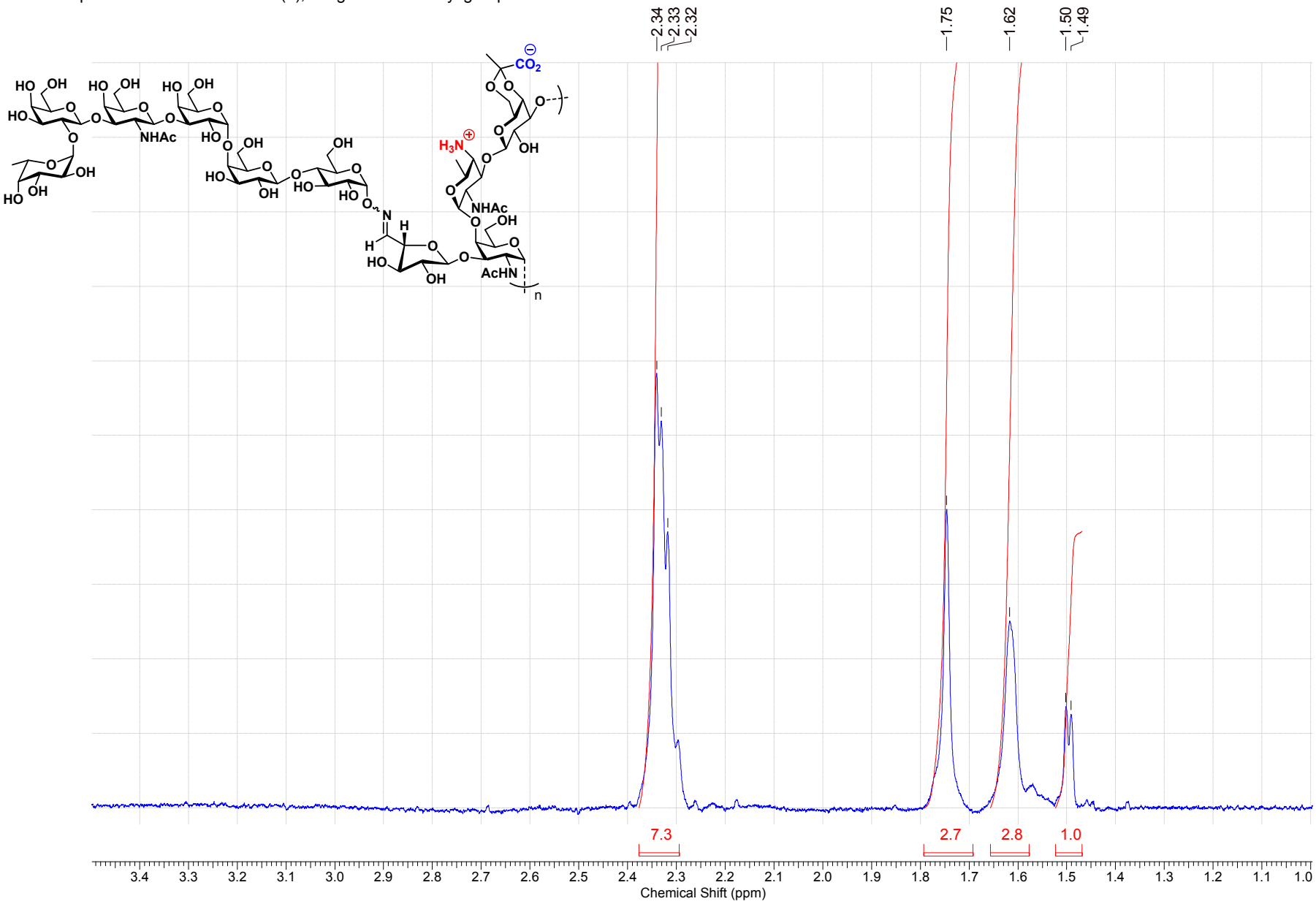
Hi-Res ESI-MS of Compound **2**



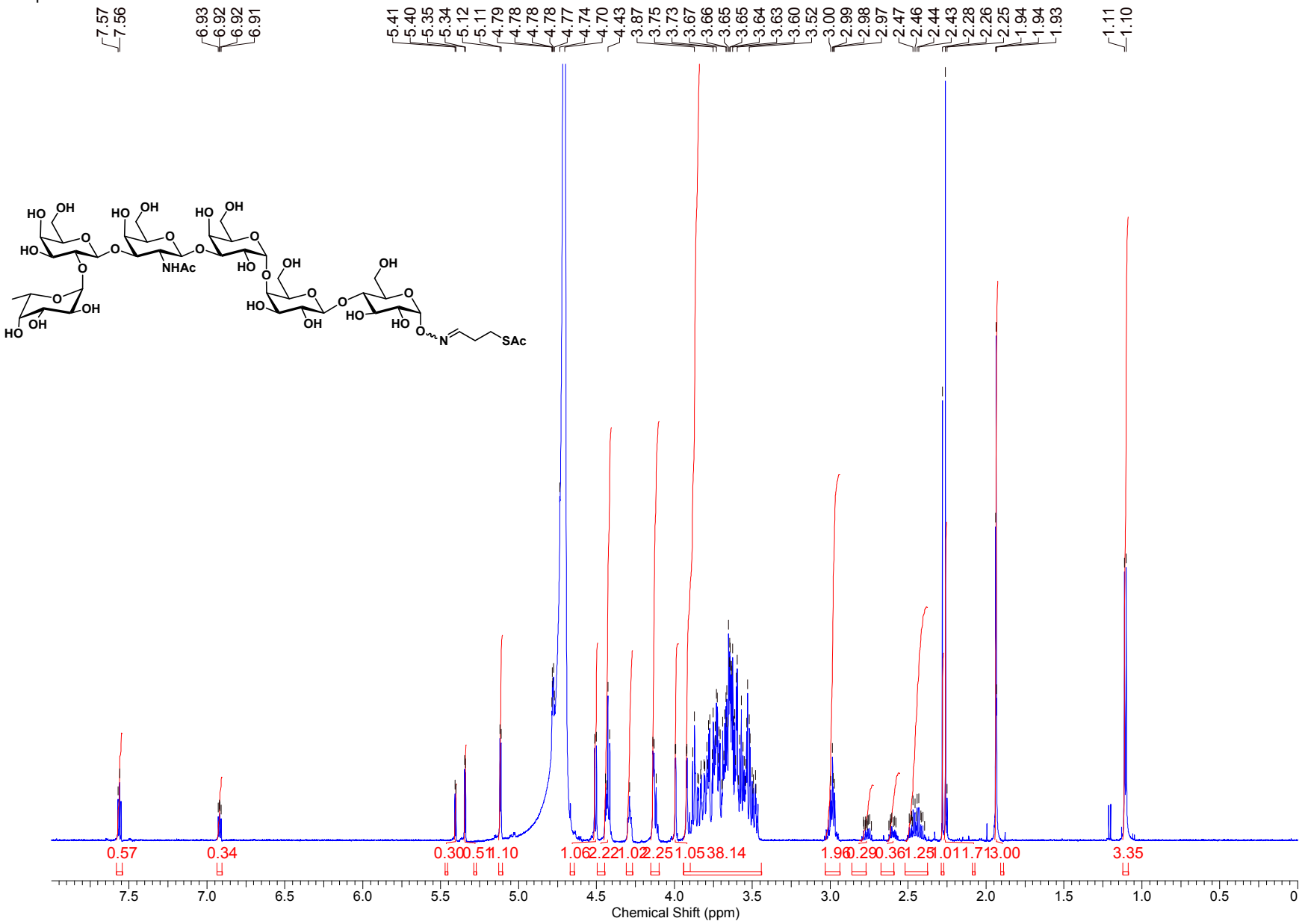
^{1}H NMR Spectrum of Globo H-PS A1 (**3**) at 60 °C



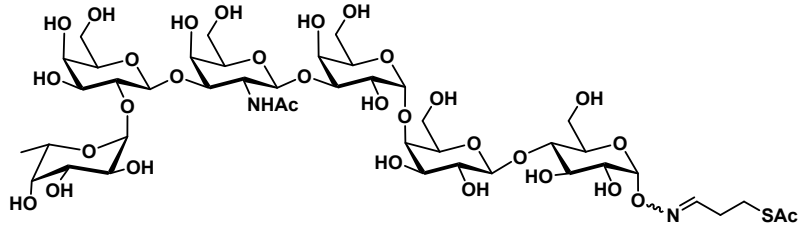
1H NMR Spectrum of Globo H-PS A1 (**3**), integration of methyl groups

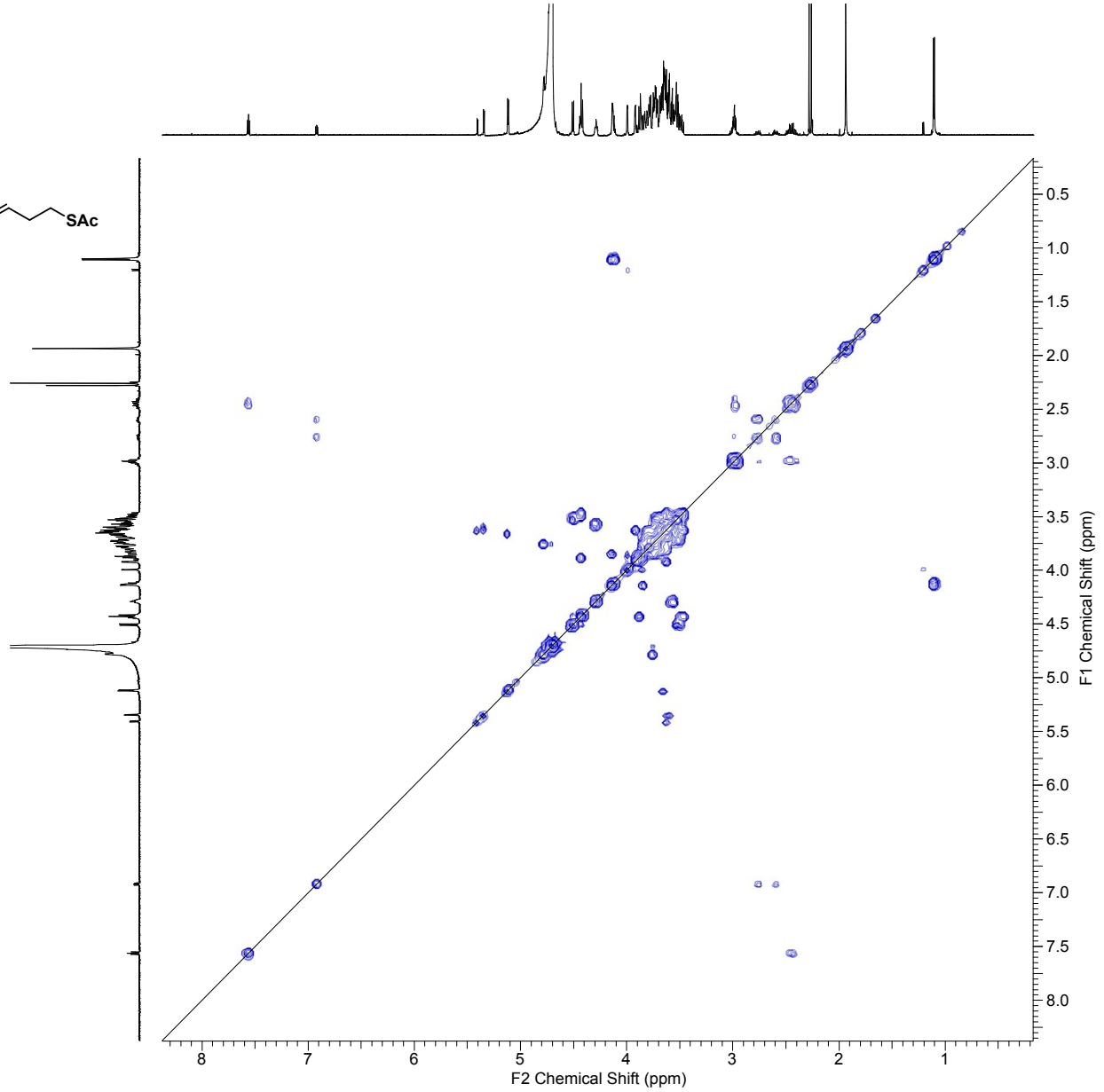


^{1}H NMR Spectrum of **S7**

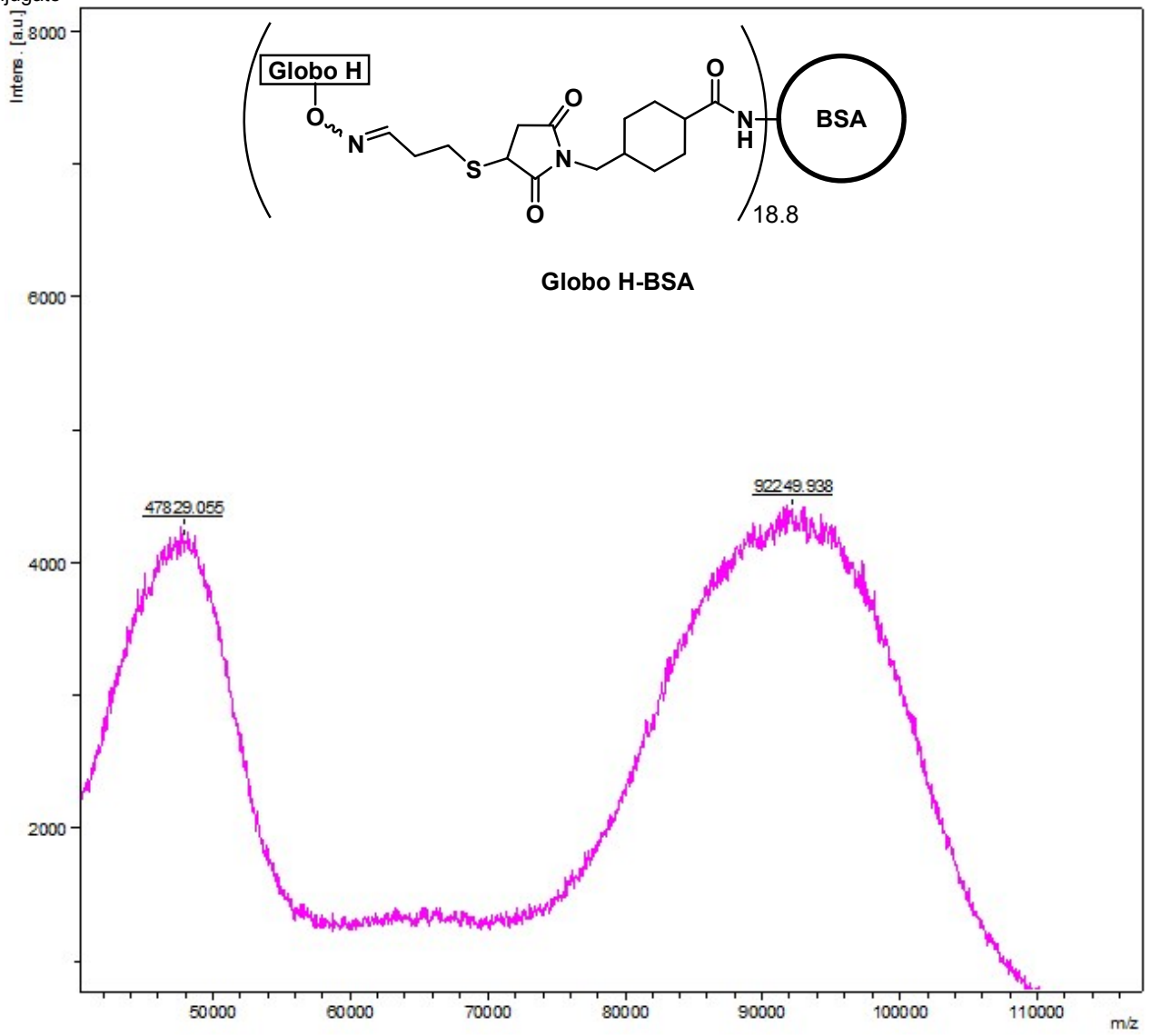


gCOSY Spectrum of **S7**





MALDI-TOF of Globo H-BSA conjugate



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