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Synthesis of a dimer of the repeating unit of type Ia Group B *Streptococcus* extracellular capsular polysaccharide and immunological evaluations of related protein conjugates

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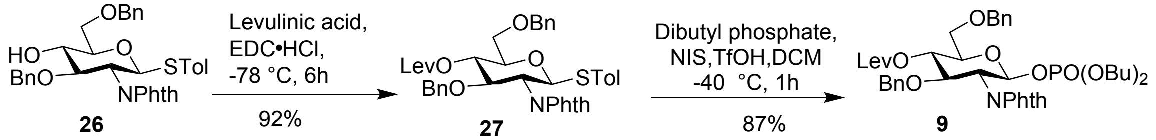
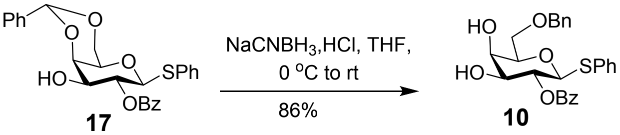
I. Experimental Section

General Methods. Materials were obtained from commercial sources without further purification unless otherwise noted. Anhydrous solvents were obtained either commercially or from a solvent purification system. Molecular sieves were flame-dried under high vacuum and used immediately after cooling. Analytical thin-layer chromatography (TLC) was performed on silica gel 60 Å plates (thickness: 0.25 mm) and detected by UV, charring with phosphomolybdic acid in EtOH, or charring with 5% H_2SO_4 in EtOH. Column chromatography was performed using flash silica gel (32-63 μm). ^{1}H NMR spectra were recorded at 500 or 600 MHz with chemical shifts reported in ppm (δ) relative to $CHCl_3$ (7.26 ppm) or tetramethylsilane (0.00 ppm). ^{13}C NMR spectra were recorded at 125 or 150 MHz relative to the ^{13}C signal of $CDCl_3$ (77.23 ppm). Coupling constants (*J*) are reported in hertz (Hz). Mass spectrometry was performed using either a MALDI TOF MS or high resolution (HR) ESI TOF MS machine.

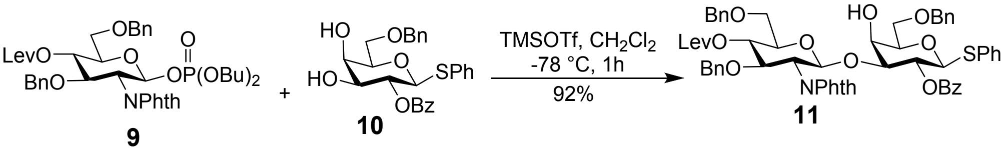
Note: Compounds are listed in order of their appearance in the manuscript. Intermediates that were not numbered in the manuscript receive numbers beginning with 26.



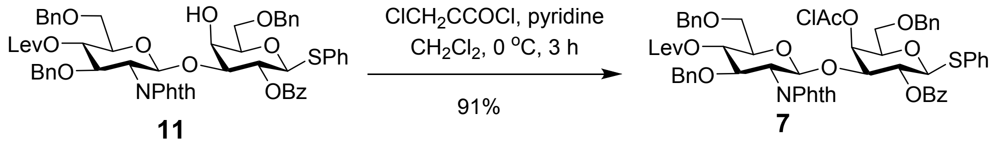
Synthesis of compound 9: A solution of **26** (0.98 g, 1.65 mmol), which was prepared according to a reported procedure (Huang, L.; Wang, Z.; Li, X.; Ye, X.-H.; Huang, X. *Carbohydr. Res.* **2006**, 341, 1669-1697), levlinic acid (0.30 g, 2.47 mmol), and EDC·HCl (0.38 g, 1.98 mmol) in DCM (30 mL) was stirred at room temperature for 6 h, and then it was washed with water and brine, dried with anhydrous Na_2SO_4, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (ethyl acetate/hexanes, 1:6) to give **27** (1.05 g, 92%) as a foamy solid. ^1H NMR (600 MHz, $CDCl_3$) δ: 7.79 (d, *J* = 7.2 Hz, 1 H, Ph), 7.66 (m, 3 H, Ph), 7.36 – 7.25 (m, 7 H, Ph), 6.99 – 6.83 (m, 7 H, Ph), 5.48 (d, *J* = 10.6, 1 H, H-1), 5.12 (dd, *J* = 10.1, 8.9 Hz, 1 H), 4.64 (d, *J* = 12.1 Hz, 1 H, H-3), 4.52 (s, 2 H, O-CH_2-Ph), 4.43 (dd, *J* = 10.2, 8.8 Hz, 1 H), 4.33 – 4.25 (m, 2 H, H-2), 3.80 (ddd, *J* = 9.5, 5.7, 3.5 Hz, 1 H), 3.64 (t, *J* = 4.6 Hz, 2 H), 2.63 (td, *J* = 6.4, 3.6 Hz, 2 H, -$COCH_2$-), 2.44 (t, *J* = 6.5 Hz, 2 H, -$COCH_2$-), 2.25 (s, 3 H, Ph-CH_3), 2.12 (d, *J* = 1.4 Hz, 3 H, -$COCH_3$). ^{13}C NMR (150 MHz, $CDCl_3$) δ: 206.2, 171.5, 168.2, 167.0, 138.2, 138.1, 137.6, 134.0, 133.8, 133.0, 131.6, 131.5, 129.6, 128.3, 128.1, 128.0, 127.9, 127.8, 127.5, 127.4, 123.5, 123.3, 83.7 (C-1), 78.0, 77.7, 74.2, 73.5, 72.5, 69.7, 54.6, 37.7, 29.8, 27.9, 21.1. HR ESI-TOF MS (*m/z*): calcd for $C_{40}H_{39}NO_8S$ $[M + Na]^+$, 716.2289; found, 716.2294. After a mixture of **27** (562 mg, 0.81 mmol), dibutyl phosphate (340 mg, 1.62 mmol) and 4Å molecular sieves (3 g) in 15 mL of dry DCM was stirred under an argon atmosphere for 2 h, it was cooled to -40 ˚C and NIS (275 mg, 1.22 mmol) and 20 µL of triflic acid were added sequentially. After 1 h, the mixture was warmed to 0 ˚C over a period of 2 h, and diluted with DCM. Saturated aq. $NaHCO_3$ solution was added to quench the reaction. The molecular sieves were filtered off, and the organic layer was washed with $Na_2S_2O_3$ solution and brine, dried, and concentrated under vacuum. The residue was purified by silica gel column chromatography (ethyl acetate/hexanes, 1:4) to afford **9** (551 mg, 87%) as a foamy solid. ^1H NMR (400 MHz, $CDCl_3$) δ 7.69 (m, 4 H, Ph), 7.33 (m, 4 H, Ph), 7.31 – 7.27 (m, 1 H, Ph), 7.00 (m, 2 H, Ph), 6.95 – 6.81 (m, 3 H, Ph), 5.83 (m, 1 H, H-1), 5.26 – 5.19 (m, 1 H, H-5), 4.67 (d, *J* = 12.3 Hz, 1 H, H-3), 4.59 – 4.53 (m, 1 H, H-4), 4.52 (s, 2 H, PhCH_2-), 4.35 (s, 1 H), 4.33 – 4.29 (m, 1 H, H-2), 4.01 – 3.90 (m, 2 H, -OCH_2-), 3.90 – 3.84 (m, 1 H, H-6), 3.77 – 3.67 (m, 2 H, -OCH_2-), 3.67 – 3.58 (m, 2 H, -OCH_2-), 2.64 (td, *J* = 6.4, 2.0 Hz, 2 H, -$COCH_2$-), 2.44 (t, *J* = 6.5 Hz, 2 H, -$COCH_2$-), 2.13 (d, *J* = 0.8 Hz, 3 H, -$COCH_3$), 1.54 – 1.44 (m, 2 H, -CH_2-), 1.31 – 1.21 (m, 4 H, 2 -CH_2-), 1.09 – 0.98 (m, 2 H , -CH_2-), 0.82 (td, *J* = 7.4, 0.8 Hz, 3 H, -CH_3), 0.73 – 0.66 (m, 3 H, -CH_3) ppm. ^{13}C NMR (150 MHz, $CDCl_3$) δ 206.1, 171.4, 137.9, 137.7, 133.9, 131.5, 128.3, 128.0, 127.8, 127.7, 127.6, 127.3, 123.3, 94.0 (C-1), 77.3, 77.1, 76.8, 76.5, 74.1, 73.8, 73.5, 72.0, 69.0, 68.0, 68.0, 67.8, 67.8, 55.8, 55.8, 37.7, 31.9, 31. 9, 31.8, 31.7, 29.7, 27.9, 18.5, 18.3, 13.5, 13.4. HR ESI-TOF MS (*m/z*): calcd for $C_{41}H_{50}NNaO_{12}P$ [M+Na]$^+$ 802.2968; found, 802. 2974.



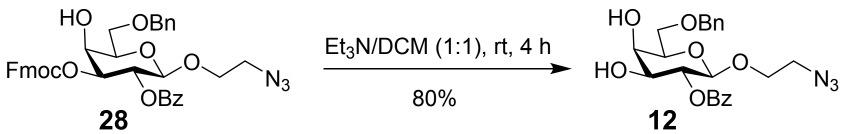
Synthesis of compound 10: A mixture of **17** (6.0 g, 8.73 mmol), which was prepared according to a reported procedure (Mondal, P. K.; Liao, G.; Mondal, M. A.; Guo Z. *Org Lett*, **2015**, 17, 1102-1105), 4Å molecular sieves (8.0 g), $NaCNBH_3$ (5.48 g, 87.30 mmol) and a pinch of methyl orange indicator in 10 mL of dry THF was stirred at room temperature for 2 h, and then HCl•OEt_2 was added dropwise addition at 0 °C until an intense pink color persisted. The mixture was allowed to slowly warm to rt, and then quenched with saturated $NaHCO_3$ solution, extracted with DCM. The combined organic layer was dried over anhydrous Na_2SO_4, filtered, and condensed under vacuum. The residue was purified by silica gel chromatography (ethyl acetate/hexanes, 1:5) to give **10** (5.18 g, 86%) as a white solid. ^{1}H NMR (600 MHz, $CDCl_3$) δ: 8.00 – 7.92 (m, 2 H, Ph), 7.51 – 7.44 (m, 1 H, Ph), 7.39 (m, 2 H, Ph), 7.34 (m, 2 H, Ph), 7.25 (m, 4 H, Ph), 7.20 (m, 1 H, Ph), 7.18 – 7.10 (m, 3 H, Ph), 5.18 (t, J = 9.6 Hz, 1 H, 2-H), 4.71 (d, J = 10.0 Hz, 1 H, H-1), 4.49 (s, 2 H, $PhCH_2O$), 3.99 (d, J = 3.3 Hz, 1 H, H-4), 3.74 (dd, J = 5.4, 2.6 Hz, 2 H, H-5, H-6), 3.70 (dd, J = 9.3, 3.3 Hz, 1 H, H-3), 3.63 (t, J = 5.4 Hz, 1 H, H-6), 3.24 – 3.09 (m, 2 H).^{13}C NMR (150 MHz, $CDCl_3$) δ: 166.7, 137.8, 133.4, 133.0, 132.3, 130.0, 129.6, 128.9, 128.8, 128.5, 128.4, 127.8, 127.8, 86.2 (C-1), 77.5, 73.8, 73.7, 72.0, 69.8, 69.7. HR ESI-TOF MS (m/z): calcd for $C_{26}H_{27}O_6S$ [M+H]$^+$, 467.1528; found, 467.1530.



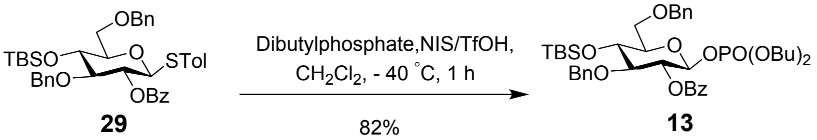
Synthesis of compound 11: After a mixture of **9** (140.8 mg, 0.2 mmol), **10** (93.3 mg, 0.2 mmol) and 4Å molecular sieves in 5 mL of dry DCM was stirred at rt for 2 h, TMSOTf (43 μL, 0.24 mmol) was added dropwise at −78 °C. The mixture was stirred under argon for 1 h, quenched with saturated $NaHCO_3$ and filtered off, and the organic layer was dried and removed under vacuum. The residue was purified by silica gel column chromatography (ethyl acetate/hexanes, 1:4) to give **11** (190.6 mg, 82%) as a white solid. ^{1}H NMR (600 MHz, $CDCl_3$) δ: 7.68 – 7.48 (m, 2 H, Ph), 7.39 (m, 2 H, Ph), 7.36 – 7.20 (m, 12 H, Ph), 7.20 – 7.06 (m, 6 H, Ph), 6.91 – 6.84 (m, 2 H, Ph), 6.81 (m, 3 H, Ph), 5.36 (t, *J* = 9.7 Hz, 1 H, H-2^{Gal}), 5.33 (d, *J* = 8.2 Hz, 1 H, H-1^{GlcN}), 5.04 (t, *J* = 9.4 Hz, 1 H, H-4^{GlcN}), 4.66 (d, *J* = 1 0.1 Hz, 1 H, H-1^{Gal}), 4.57 – 4.44 (m, 5 H, PhCH), 4.32 (dd, *J* = 10.6, 8.8 Hz, 1 H, , H-3^{GlcN}), 4.28 – 4.24 (m, 1 H, H-2^{GlcN}), 4.24 – 4.19 (m, 2 H, , PhCH), 3.85 – 3.75 (m, 3 H, H-3^{Gal}, H-5^{Gal} H-5^{GlcN}), 3.73 – 3.63 (m, 2 H), 3.60 – 3.52 (m, 2 H), 2.68 – 2.60 (m, 2 H, -COCH$_{2-}$), 2.49 – 2.36 (m, 2 H,- COCH$_2$-), 2.13 (s, 3 H, -COCH$_3$). ^{13}C NMR (150 MH z, CDCl$_3$) δ: 206.2, 171.6, 164.5, 138.2, 137.8, 137.5, 133.6, 132.6, 131.8, 129.5, 129.3, 128.7, 128.4, 128.3, 128.1, 127.9, 127.7, 127.7, 127.6, 127.6, 127.4, 127.3, 98.7 (C-1^{GlcN}), 86.7 (C-1^{Gal}), 82.2, 77.6, 76.6, 73.9, 73.5, 73.5, 72.3, 69.9, 69.6, 69.0, 68.5, 55.1, 37.7, 29.7, 29.7, 27.8. HR ESI-TOF MS (*m*/*z*): calcd for $C_{59}H_{57}NO_{14}S$ [M + Na] $^+$, 1058.3392; found, 1058.3359.



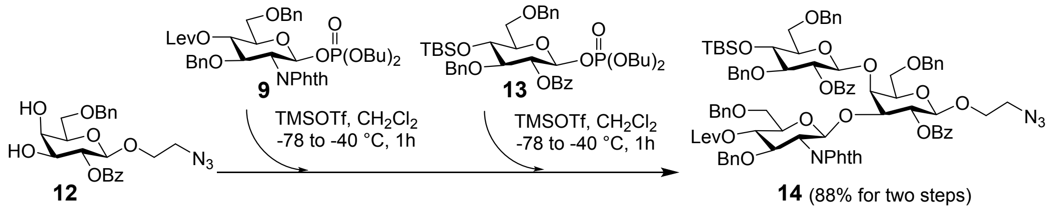
Synthesis of compound 7: To a stirred solution of **11** (200 mg, 0.19 mmol) in DCM (5 mL) and pyridine (200 μL) was added dropwise chloroacetyl chloride (120 μL, 1.50 mmol) at 0 °C. After the mixture was stirred for 3 h, it was allowed to slowly warm to rt. Solvents were removed by co-evaporation with toluene, and the residue was purified by silica gel column chromatography (ethyl acetate/toluene, 1:8) to give **7** (195.4 mg, 91%) as a white solid. ^{1}H NMR (600 MHz, CDCl$_3$) δ: 7.61 (m, 3 H, Ph), 7.53 (m, 2 H, Ph), 7.47 – 7.40 (m, 1 H, Ph), 7.32 (m, 13 H, Ph), 7.24 – 7.21 (m, 1 H, Ph), 7.17 (m, 1 H, Ph), 7.12 (m, 2 H, Ph), 6.99 (m, 1 H, Ph), 6.87 (m, 2 H, Ph), 6.86 – 6.80 (m, 3 H, Ph), 5.65 (d, *J* = 3.4 Hz, 1 H, H-4^{Gal}), 5.24 – 5.19 (m, 1 H, H-2^{Gal}), 5.19 (d, *J* = 8.5 Hz, 1 H, H-1^{GlcN}), 5.05 (t, *J* = 9.4 Hz, 1 H, H-4^{GlcN}), 4.66 (d, *J* = 10.1 Hz, 1 H, H-1^{Gal}), 4.55 (s, 2 H, CH$_2$Cl), 4.48 (m, 3 H, PhCH$_2$), 4.35 (t, *J* = 10.8, 8.8 Hz, 1 H, H-3^{GlcN}), 4.26 – 4.18 (m, 2 H, PhCH$_2$), 4.11 – 4.07 (m, 2 H, H-2^{GlcN}, PhCH), 3.99 (dd, *J* = 9.5, 3.5 Hz, 1 H, H-3^{Gal}), 3.78 (m, 1 H, H-5^{Gal}), 3.72 (m, 1 H, H-5^{GlcN}), 3.64 – 3.60 (m, 2 H, H-6^{GlcN}), 3.56 (m, 2 H, H-5^{Gal}), 2.60 (t, *J* = 6.5 Hz, 2 H, -COCH$_2$-), 2.45 – 2.33 (m, 2 H, -COCH$_2$-), 2.11 (s, 3 H, -COCH$_3$). ^{13}C NMR (150 MHz, CDCl$_3$) δ: 206.1, 171.4, 166.8, 164.5, 138.2, 137.7, 133.2, 133.0, 131.9, 129.7, 129.1, 128.8, 128.4, 128.4, 128.2, 127.9, 127.9, 127.8, 127.6, 127.6, 127.4, 127.2, 98.7 (C-1^{GlcN}), 86.9 (C-1^{Gal}), 77.5, 77.3, 77.0, 76.8, 76.6, 76.4, 73.8, 73.6, 73.5, 72.4, 72.1, 69.8, 69.3, 68.70 55.4, 40.9, 37.7, 29.7, 27.8. HR ESI-TOF MS (*m*/*z*): calcd for $C_{61}H_{58}ClNO_{15}S$ [M +Na]$^+$, 1134.3108; found, 1134.2891.



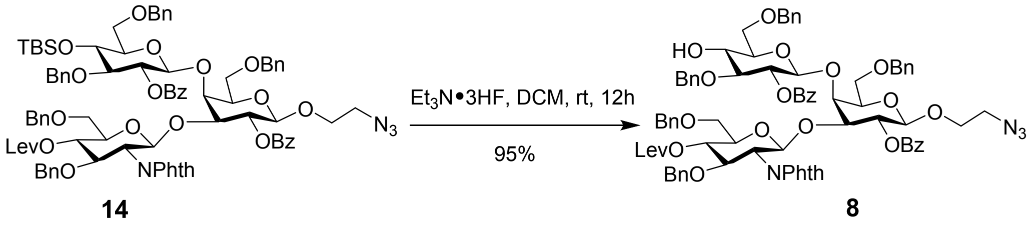
Synthesis of compound 12: A solution of **28** (3.6 g, 5.41 mmol), which was prepared from D-galactose according to reported procedures (Mondal,P. K.; Liao, G.; Mondal, M. A.; Guo Z. *Org Lett*, **2015**, 17, 1102-1105), in triethylamine (25 mL) and DCM (25 mL) was stirred at rt for 4 h, and the solvents were removed under vacuum. The crude product was purified by silica gel column chromatography (ethyl acetate/hexanes 1:4) to give **12** (1.91 g, 80%) as a white solid. ^{1}H NMR (600 MHz, CDCl$_3$) δ: 8.07 (m, 2 H, Ph), 7.57 (s, 1 H, Ph), 7.44 (m, 2 H, Ph), 7.41 – 7.33 (m, 4H, Ph), 7.31 (m, 1 H, Ph), 5.23 (dd, *J* = 9.6, 7.9 Hz, 1 H, H-2), 4.64 (d, *J* = 7.9 Hz, 1 H, H-2), 4.61 (d, *J* = 1.7 Hz, 2 H, PhCH$_2$O), 4.06 (d, *J* = 3.6 Hz, 1 H, H-4), 4.05 – 4.02 (m, 1 H), 3.86 – 3.80 (m, 2 H), 3.79 (dd, *J* = 9.8, 3.2 Hz, 1 H, H-3), 3.74 – 3.69 (m, 2 H), 3.44 (ddd, *J* = 12.5, 8.1, 3.9 Hz, 1 H, -CHN$_3$), 3.33 – 3.28 (m, 1 H, , -CHN$_3$).^{13}C NMR (150 MHz, CDCl$_3$) δ: 167.0, 137.7, 133.3, 129.9, 129.7, 128.5, 128.4, 128.3, 127.9, 127.8, 101.0 (C-1), 73.8, 73.7, 73.5, 72.9, 69.5, 69.4, 67.9, 50.7. HR ESI-TOF MS (*m*/*z*): calcd for $C_{22}H_{25}N_3O_7$ [M + Na]$^+$, 466.1585; found, 466.1590.



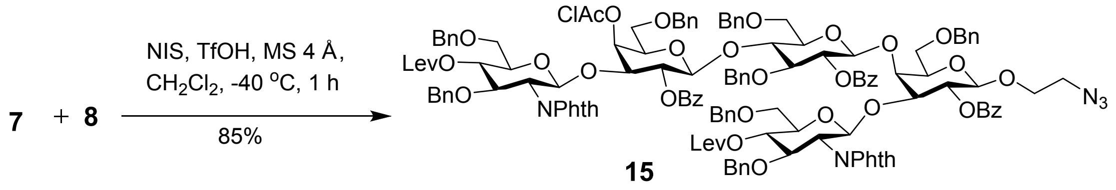
Synthesis of compound 13: After a mixture of **29** (500 mg, 0.77 mmol), which was prepared according to reported procedures (Mondal,P. K.; Liao, G.; Mondal, M. A.; Guo Z. *Org Lett*, **2015**, 17, 1102-1105), dibutyl phosphate (323.4 mg, 1.54 mmol) and 4Å molecular sieves (2 g) in 10 mL of dry DCM was stirred under an argon atmosphere for 2 h, it was cooled to -40 ˚C, and then NIS (260.3 mg, 1.16 mmol) and 10 µL of triflic acid were added sequentially. After 1 h, the mixture was warmed to 0 ˚C, diluted with DCM, quenched with saturated aq. NaHCO$_3$, washed with Na$_2$S$_2$O$_3$ solution and brine, and filtered off. The organic layer was dried with anhydrous Na$_2$SO$_4$ and condensed under vacuum. The residue was purified by silica gel column chromatography (ethyl acetate/hexanes, 1:10) to afford **13** (487.1 mg, 82%) as a white solid. ^{1}H NMR (600 MHz, CDCl$_3$) δ: 7.99 (m, 2 H, Ph), 7.57 – 7.52 (m, 1 H, Ph), 7.39 (m, 2 H, Ph), 7.36 (m, 4 H, Ph), 7.32 – 7.27 (m, 1 H, Ph), 7.18 – 7.11 (m, 5 H, Ph), 5.93 – 5.87 (m, 1 H, H-1), 5.21 (dt, *J* = 9.5, 2.7 Hz, 1 H, H-2), 4.86 – 4.74 (m, 2 H, -OCH$_2$ Ph), 4.58 (ddd, *J* = 58.9, 12.1, 1.5 Hz, 2 H, -OCH$_2$ Ph), 4.10 – 4.05 (m, 2 H, H-3,), 4.03 – 3.98 (m, 2 H), 3.98 – 3.80 (m, 3 H), 3.76 – 3.67 (m, 2 H, CH$_2$), 1.54 (dt, *J* = 12.9, 6.8 Hz, 2 H, CH$_2$), 1.40 – 1.34 (m, 2 H, CH$_2$), 1.34 – 1.26 (m, 2 H, 2 -CH$_{2-}$), 1.20 – 1.13 (m, 2 H, 2 -CH$_{2-}$), 0.89 (d, *J* = 1.8 Hz, 9 H, 3 -CH$_3$), 0.86 (dd, *J* = 7.4, 1.6 Hz, 3 H, -CH$_3$), 0.77 (td, *J* = 7.4, 1.6 Hz, 3 H, -CH$_3$), 0.05 (s, 6 H, 2 -SiCH$_3$).^{13}C NMR (150MHz, CDCl$_3$) δ: 165.5, 138.1, 138.0, 133.8, 133.3, 129.7, 129.3, 128.3, 128.3, 128.1, 127.5, 127.5, 127.3, 127.2, 94.5 (C-1), 94.4 (C-1), 79.6, 75.2, 73.8, 73.5, 73.5, 73.3, 70.2, 68.4, 67.7, 67.7, 67.6, 32.3, 32.1, 32.1, 32.0, 31.9, 25.9, 18.5, 18.4, 18.0, 13.6, 13.6, 13.5, -3.8, -4.9. HR ESI-TOF MS (*m*/*z*): calcd for $C_{42}H_{59}NO_{10}$Psi [M+NH$_4$] $^+$, 796.3646; found, 796.3641.



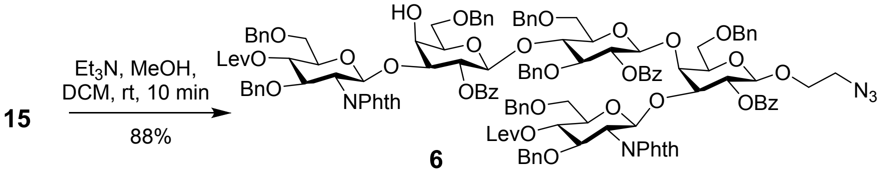
Synthesis of compound 14: After a mixture of **12** (88.7 mg, 0.2 mmol), **9** (156.0 mg, 0.2 mmol) and 4Å molecular sieves in 10 mL of dry DCM was stirred at rt for 2 h, TMSOTf (43 μL, 0.24 mmol) was added dropwise at − 78 °C. The mixture was stirred under argon for 1 h, and allowed to slowly warm to – 40 °C for 30 min. The mixture was cooled to – 78 °C again and **13** (185.0 mg, 0.24 mmol) was added to allow for glycosylation reaction proceeding under the same conditions described. The mixture was quenched with saturated aq. NaHCO$_3$ and filtered off, and the organic layer was dried with Na$_2$SO$_4$ and condensed under vacuum. The residue was purified by silica gel column chromatography (ethyl acetate/hexanes, 1: 4) to give **14** (277.0 mg, 88%) as a white solid. ^{1}H NMR (600 MHz,, CDCl$_3$) δ: 7.88 – 7.82 (m, 2 H, Ph), 7.60 – 7.57 (m, 2 H, Ph), 7.57 – 7.50 (m, 2 H, Ph), 7.47 (s, 1 H, Ph), 7.37 – 7.14 (m, 24 H, Ph), 7.11 (2 H, Ph), 7.01 (s, 1 H, Ph), 6.94 – 6.90 (m, 2 H, Ph), 6.90 – 6.83 (m, 3 H, Ph), 5.37 (d, *J* = 7.9 Hz, 1 H, H-1^{Glc}), 5.29 – 5.25 (m, 1 H, H-2^{Glc}), 5.23 (d, *J* = 8.4 Hz, 1 H, H-1^{GlcN}), 5.19 – 5.12 (m, 1 H, H-2^{Gal}), 4.97 (dd, *J* = 10.0, 8.0 Hz, 1 H), 4.76 (q, *J* = 11.1 Hz, 2 H), 4.59 (d, *J* = 12.1 Hz, 1 H, H-3^{GlcN}), 4.53 – 4.49 (m, 2 H), 4.47 (s, 2 H), 4.47 – 4.45 (d, d, *J* = 8.0 Hz 1 H, H-1^{Gal}), 4.44 (m, 2 H), 4.43 – 4.39 (m, 2 H), 4.26 (dd, *J* = 11.2, 8.8 Hz, 2 H), 3.98 (dd, *J* = 10.0, 2.8 Hz, 1 H), 3.89 (dt, *J* = 29.8, 8.7 Hz, 2 H), 3.76 (ddd, *J* = 13.9, 8.2, 3.6 Hz, 2 H), 3.72 – 3.56 (m, 7 H), 3.33 (ddd, *J* = 11.1, 6.7, 4.5 Hz, 1 H), 2.89 (dq, *J* = 6.7, 4.0, 3.1 Hz, 2 H), 2.61 (q, *J* = 6.3 Hz, 2 H, -COCH$_2$-), 2.40 (t, *J* = 6.5 Hz, 2 H, -COCH$_2$-), 2.12 (s, 3 H, -COCH$_3$), 0.90 (s, 9 H, 3 CH$_3$), 0.00 (d, *J* = 11.3 Hz, 6 H, 2 -SiCH$_3$). ^{13}C NMR (150 MHz, CDCl$_3$) δ: 206.1, 171.6, 167.9, 166.8, 165.9, 164.1, 138.5, 138.5, 138.0, 137.6, 133.4, 132.6, 132.2, 130.0, 129.9, 129.6, 129.6, 128.4, 128.3, 128.3, 128.2, 127.99, 127.97, 127.94, 127.7, 127.64, 127.61, 127. 6, 127.5, 127.4, 127.35, 127.32, 127.2, 127.0, 123.1, 99.7 (C-1^{Gal}), 99.2 (C-1^{GlcN}), 99.04 (C-1^{Glc}), 72.1, 71.2, 70.3, 70.0, 69.9, 69.1, 64.0, 56.0, 50.6, 37.7, 29.8, 27.9, 26.1, 18.1, -3.7, -4.9. HR ESI-TOF MS (*m/z*): calcd for $C_{88}H_{96}N_4O_{21}Si$ [M + NH$_4$]$^+$, 1590.6675; found, 1590.6644.



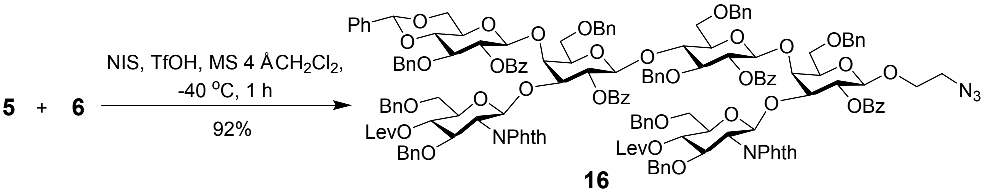
Synthesis of compound 8: To a solution of **14** (213 mg, 0.14 mmol) in THF (4 mL) was added 4 mL of triethylamine-trihydrofluoride. The mixture was stirred at rt for 12 h and concentrated under vacuum. The residue was purified by silica gel column chromatography (ethyl acetate/hexanes, 1:3) to give **8** (187.7 mg, 95%) as a white solid. ^{1}H NMR (600 MHz, CDCl$_3$) δ: 7.94 – 7.89 (m, 2 H, Ph), 7.64 – 7.60 (m, 2 H, Ph), 7.60 – 7.47 (m, 4 H, Ph), 7.46 – 7.38 (m, 2 H, Ph), 7.38 – 7.23 (m, 21 H, 21xPh), 7.18 (t, J = 7.7 Hz, 2 H, Ph), 7.15 – 7.06 (m, 1 H, Ph), 6.95 – 6.91 (m, 2 H, Ph), 6.91 – 6.82 (m, 3 H, Ph), 5.43 (d, J = 8.1 Hz, 1 H, H-1$^{\text{Glc}}$), 5.27 – 5.17 (m, 2 H, H-2$^{\text{Glc}}$, H-1$^{\text{GlcN}}$), 5.12 (dd, J = 10.1, 8.8 Hz, 1 H, H-2$^{\text{Gal}}$), 5.03 (dd, J = 10.1, 8.0 Hz, 1 H), 4.81 (d, J = 1.6 Hz, 2 H), 4.58 (d, J = 12.2 Hz, 1 H), 4.54 – 4.46 (m, 5 H), 4.46 (dd, J = 8.2 Hz, J = 2.5 Hz, 1 H, H-1$^{\text{Gal}}$), 4.44 (s, 2 H), 4.30 – 4.19 (m, 2 H), 4.05 – 3.97 (m, 2 H), 3.86 (t, J = 8.9 Hz, 1 H), 3.80 – 3.62 (m, 8 H), 3.56 (dd, J = 10.7, 6.2 Hz, 1 H), 3.30 (ddd, J = 11.0, 6.6, 4.5 Hz, 1 H), 3.22 (dt, J = 10.4, 4.7 Hz, 1 H), 2.92 – 2.78 (m, 2 H), 2.72 (d, J = 8.9 Hz, 1 H), 2.62 (t, J = 6.5 Hz, 2 H, -COCH$_2$-), 2.45 – 2.34 (m, 2 H, -COCH$_2$-), 2.12 (s, 3 H, -COCH$_3$). ^{13}C NMR (151 MHz, CDCl$_3$) δ: 206.2, 171.5, 165.9, 164.1, 138.6, 138.4, 138.0, 137.7, 133.5, 132.7, 132.4, 130.0, 129.9, 129.8, 129.6, 129.5, 128.53, 128.45, 128.4, 128.36, 128.34, 128.32, 128.3, 128.2, 128.04, 128.0, 127.98, 127.9, 127.8, 127.74, 127.65, 127.6, 127.57, 127.5, 127.4, 127.3, 123.1, 99.8 (C-1$^{\text{Gal}}$), 99.0 (C-1$^{\text{GlcN}}$), 98.9 (C-1$^{\text{Glc}}$), 82.9, 79.5, 77.3, 77.1, 76.9, 76.7, 74.7, 74.37, 74.3, 74.1, 73.6, 73.55, 73.52, 73.4, 73.4, 72.8, 72.4, 71.7, 70.4, 69.9, 69.8, 64.2, 56.1, 50.5, 37.7, 29.8, 27.9, 27.8. HR ESI-TOF MS (m/z): calcd for $C_{82}H_{82}N_4O_{21}$ [M + Na]$^+$, 1481.5364; found, 1481.5690.



Synthesis of compound 15: After a mixture of **7** (126.1 mg, 0.11 mmol), **8** (165 mg, 0.11 mmol) and 4Å molecular sieves (1 g) in 5 mL of dry DCM was stirred under an argon atmosphere for 2 h, it was cooled to -40 ˚C and NIS (52.2 mg, 0.23 mmol) and 5 µL of triflic acid were added sequentially. After 1 h, the mixture was warmed to 0 ˚C, diluted with DCM, quenched with saturated aq. NaHCO$_3$ solution and filtered off. The organic layer was washed with Na$_2$S$_2$O$_3$ solution and brine and dried over anhydrous Na$_2$SO$_4$ and condensed under vacuum. The residue was purified by silica gel column chromatography (ethyl acetate/toluene, 1:8) to afford **15** (237.2 mg, 85%) as a white solid. ^{1}H NMR (600 MHz, CDCl$_3$) δ: 7.69 (m, 4 H, Ph), 7.54 (m, 3 H, Ph), 7.47 (m, 4 H, Ph), 7.36 – 7.22 (m, 30 H, Ph), 7.16 – 7.07 (m, 8 H, Ph), 7.03 (m, 2 H, Ph), 6.90 (m, 7 H, Ph), 6.83 (m, 5 H, Ph), 5.64 (d, J = 2.7 Hz, 1 H), 5.14 – 5.06 (m, 5 H, 3 H-1), 5.02 – 4.95 (m, 2 H), 4.91 (d, J = 11.2 Hz, 1 H), 4.59 – 4.21 (m, 21 H, 2 H-1), 4.14 – 3.54 (m, 17 H), 3.53 – 3.06 (m, 8 H), 2.88 – 2.72 (m, 2 H, -CH$_2$N$_3$), 2.68 – 2.61 (m, 4 H, 2 -COCH$_2$-), 2.49 – 2.34 (m, 4 H, 2 -COCH$_2$-), 2.18 (s, 3 H, 2 –COCH$_3$), 2.13 (s, 3 H, 2 –COCH$_3$). ^{13}C NMR (150 MHz, CDCl$_3$) δ: 206.2, 206.1, 171.5, 171.4, 168.0, 168.0, 167.1, 166.8, 166.5, 165.6, 164.4, 164.1, 139.2, 138.4, 138.1, 138.0, 138.0, 137.93, 137.9, 137.8, 133.5, 133.0, 132.7, 132.1, 131.3, 130.0, 129.8, 129.7, 129.65, 129.63, 129.6, 129.5, 129.2, 129.1, 128.5, 128.4, 128.38, 128.35, 128.3, 128.28, 128.2, 128.17, 128.02, 127.99, 127.96, 127.9, 127.8, 127.73, 127.69, 127.65, 127.6, 127.57, 127.5, 127.45, 127.43, 127.4, 127.34, 127.2, 127.1, 126.9, 113.7, 100.1 (C-1), 99.7 (C-1), 98.6 (2 C-1), 98.6 (C-1), 80.6, 78.5, 76. 8, 76.5, 76.5, 76.2, 74.7, 74.2, 74.1, 74.0, 73.8, 73.7, 73.67, 73.6, 73.4, 73.3, 73.27, 73.2, 72.5, 72.4, 72.35, 71.9, 71.8, 71.5, 70.1, 69.9, 69.6, 67.6, 67.3, 63.8, 55.7, 55.5, 55.3, 55.2, 50.5, 40.9, 37.7, 29.8, 29.6, 27.9, 27.9. HR ESI-TOF MS (*m*/*z*): calcd for $C_{137}H_{134}ClN_5O_{36}$ $[M + H]^+$, 2460.8570; found, 2460.7856.

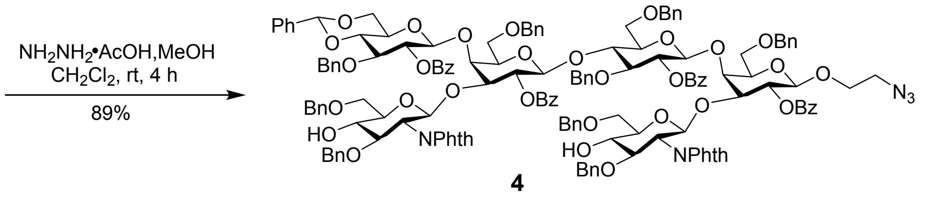


Synthesis of compound 6: After **15** (100 mg, 40.6 µmol) was dissolved in MeOH (10 mL) and DCM (1 mL) at rt, Et_3N (300 µL) was added. The resulting mixture was stirred for 10 min. Solvents were removed under vacuum, and the residue was purified by silica gel column chromatography (ethyl acetate/toluene, 1:8) to give **6** (85.3 mg, 88%) as a white solid. ^{1}H NMR (600 MHz, $CDCl_3$) δ: 7.74 (m, 2 H, Ph), 7.65 (m, 2 H, Ph), 7.54 – 7.35 (m, 10 H, Ph), 7.35 – 7.27 (m, 18 H, Ph), 7.24 – 7.20 (m, 8 H, Ph), 7.18 – 7.04 (m, 12 H, Ph), 6.93 – 6.80 (m, 11 H, Ph), 5.32 (t, *J* = 8.9 Hz, 1 H), 5.28 (d, *J* = 7.5 Hz, 1 H, H-1), 5.02 (dt, *J* = 60.0, 9.8 Hz, 6 H, 2 H-1), 4.57 – 4.48 (m, 6 H, H-1), 4.45 – 4.35 (m, 8 H, H-1), 4.32 – 4.16 (m, 8 H), 4.03 – 3.91 (m, 4 H), 3.85 – 3.75 (m, 3 H), 3.58 (d, *J* = 13.0 Hz, 7 H), 3.53 – 3.44 (m, 3 H), 3.42 – 3.26 (m, 3 H), 3.17 (ddd, *J* = 24.0, 10.9, 4.9 Hz, 3 H),3.23 – 3.12 (m, 3 H), 2.87 – 2.77 (m, 2 H, $-CH_2N_3$), 2.64 (q, *J* = 6.8 Hz, 4 H, 2 $-COCH_2-$), 2.48 – 2.34 (m, 4 H, 2 $-COCH_2-$), 2.15 (d, *J* = 18.5 Hz, 6 H, 2 $-COCH_3$). ^{13}C NMR (150 MHz, $CDCl_3$) δ: 206.1, 171.6, 171.3, 165.6, 164.3, 164.1, 139.2, 138.5, 138.4, 138.2, 138.0, 137.84, 137.8, 137.6, 132.7, 132.6, 132.1, 131.3, 130.0, 129.8, 129.7, 129.6, 129.4, 129.3, 128.4, 128.4, 128.3, 128.2, 128.15, 128.1, 128.03, 128.0, 127.98, 127.95, 127.93, 127.8, 127.79, 127.7, 127.68, 127.64, 127.6, 127.56, 127.5, 127.46, 127.4, 127.37, 127.3, 127.26, 127.2, 126.8, 113.8, 100.1 (C-1), 99.6 (C-1), 98.9 (C-1), 98.7 (C-1), 98.5 (C-1), 81.1, 80.8, 76.7, 76.7, 76.3, 74.8, 74.3, 74.2, 73.9, 73.8, 73.6, 73.5, 73.5, 73.4, 73.4, 73.2, 73.16, 72.4, 72.35, 71.8, 71.3, 70.1, 69.7, 69.6, 69.59, 68.9, 68.2, 67.7, 63.7, 55.7, 55.2, 50.5, 37.7, 37.7, 29.8, 29.75, 27.9, 27.9. ESI-TOF MS (*m*/*z*): calcd for $C_{135}H_{133}N_5NaO_{35}$ $[M + Na]^+$, 2406.8679; found, 2406.8685.



Synthesis of compound 16: After a mixture of **5** (41.5 mg, 71.3 µmol), which was prepared according to reported procedures (Liao, G.; Burgula, S.; Zhou, Z.; Guo, Z. *Eur. J. Org. Chem.*, **2015**, 13, 2942-2951), **6** (85.2 mg, 35.6 µmol) and 4Å molecular sieves (1 g) in anhydrous DCM (4 mL) was stirred under an argon atmosphere for 2 h, it was cooled to -40 ˚C and NIS (26 mg, 106.8 µmol) and 3 µL of triflic acid were added sequentially. After 1 h, the mixture was warmed to 0 ˚C, diluted with DCM, quenched with saturated $NaHCO_3$ solution and filtered off. The organic layer was washed with $Na_2S_2O_3$ solution and brine, dried over anhydrous Na_2SO_4 and condensed under vacuum. The residue was purified by silica gel column chromatography (ethyl acetate/toluene, 1:8) to afford **16** (93.4 mg, 92%) as a white solid. ^{1}H NMR (600 MHz, $CDCl_3$) δ: 8.08 (m, 2 H, Ph), 7.81 – 7.44 (m, 12 H, Ph), 7.39 – 7.29 (m, 28 H, Ph), 7.20 (m, 8 H, Ph), 7.16 – 6.97 (m, 8 H, Ph), 6.91 (m, 10 H, Ph), 6.10 (t, *J* = 8.2 Hz, 1 H), 5.71 (d, *J* = 7.1 Hz, 1 H, H-1), 5.52 (t, *J* = 7.6 Hz, 1 H), 5.44 (s, 1 H, Ph-CH-), 5.23 (t, *J* = 9.2 Hz, 1 H), 5.17 – 5.11 (m, 2 H, H-1), 5.10 (d, *J* = 8.6 Hz, 1 H, H-1), 5.06 – 5.01 (m, 1 H), 4.97 (dt, *J* = 14.7, 8.5 Hz, 3 H), 4.72 (d, *J* = 11.7 Hz, 1 H), 4.53-4.47 (q, *J* = 12.2, 11.7 Hz, 7 H, H-1), 4.46 (s, 1 H), 4.45 – 4.14 (m, 17 H, H-1), 4.15 – 3.75 (m, 7 H), 3.76 – 3.44 (m, 12 H), 3.44 – 3.00 (m, 5 H), 2.82 (q, *J* = 7.9, 6.5 Hz, 1 H), 2.71 – 2.64 (m, 4 H, -$COCH_2$-), 2.64 – 2.55 (m, 2 H, -$COCH_2$-), 2.47 – 2.33 (m, 4 H, 2 -$COCH_2$-), 2.18 (s, 3 H,–$COCH_3$), 2.13 (s, 3 H, –$COCH_3$). ^{13}C NMR (150 MHz, $CDCl_3$) δ: 206.2, 206.1, 177.3, 175.5, 171.5, 171.4, 167.9, 166.7, 165.6, 165.59, 164.3, 164.1, 163.8, 139.3, 138.7, 138.4, 138.3, 137.95, 137.91, 137.85, 137.83, 137.1, 132.8, 132.7, 132.6, 132.4, 132.1, 131.3, 131.2, 130.13, 130.1, 130.05, 129.99, 129.81, 129.67, 129.64, 129.58, 129.4, 128.8, 128.44, 128.0, 128.37, 128.3, 128.25, 128.1, 128.04, 128.0, 127.96, 127.9, 127.8, 127.77, 127.74, 127.7, 127.68, 127.64, 127.55, 127.5, 127.46, 127.4, 127.4, 127.3, 127.2, 127.1, 126.5, 126.4, 101.5 (Ph-C), 100.5 (C-1), 99.6 (C-1), 99.5 (C-1), 99.1 (C-1), 98.6 (C-1), 98.5 (C-1), 80.7, 79.6, 78.9, 78.5, 76.5, 74.7, 74.3, 74.2, 74.0, 73.9, 73.86, 73.7, 73.5, 73.47, 73.4, 73.38, 73.35, 73.24, 73.20, 73.17, 72.6, 72.4, 72.3, 72.1, 71.7, 70.1, 69.9, 69.8, 69.7, 69.0, 67.5, 65.9, 63.7, 55.6, 55.5, 50.5, 37.8, 37.7, 29.8, 29.76, 29.5, 27.9, 27.9. HR ESI-TOF MS (*m/z*): calcd for $C_{162}H_{155}N_5O_{42}$ [M + H] $^+$, 2843.0219; found, 2843.0085.

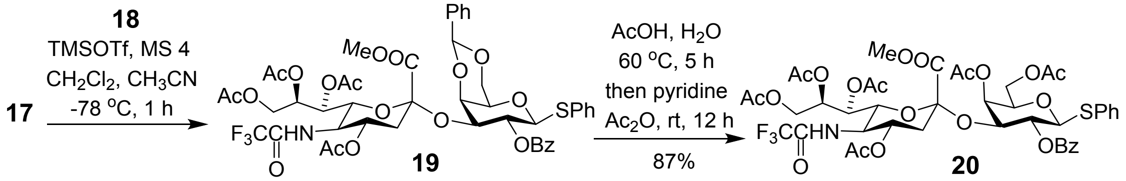
16



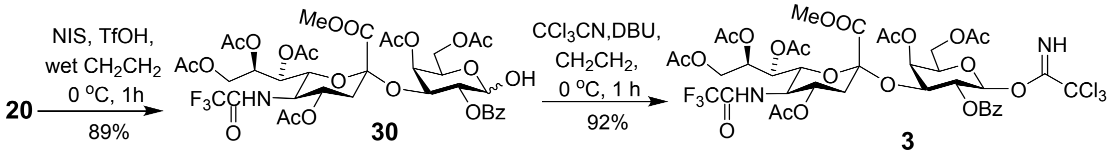
4

Synthesis of compound 4: To a solution of **16** (46 mg, 16.1 µmol) in DCM (2.0 mL) were added MeOH (2 drops) and hydrazine acetate at rt. The reaction was stirred for 4 h and quenched with saturated aq. $NaHCO_3$ and extracted with DCM. The combined organic layers were dried over anhydrous Na_2SO_4 and condensed under vacuum. The residue was purified by silica gel column chromatography (ethyl acetate/toluene, 1:8) to afford **4** (37.7 mg, 92%) as a foamy solid.

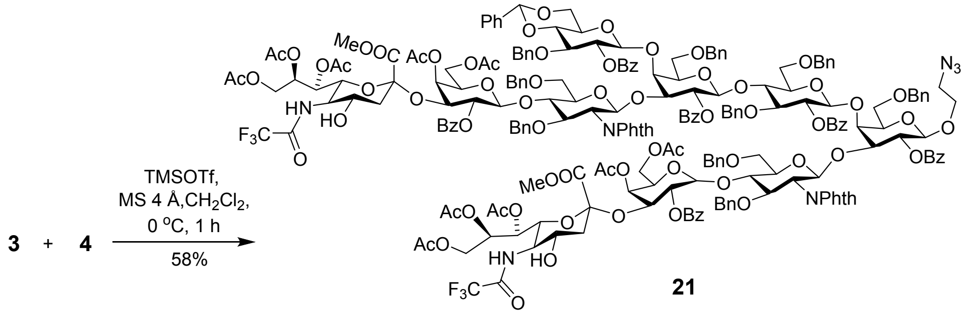
^{1}H NMR (600 MHz, $CDCl_3$) δ: 7.98 (s, 2 H, Ph), 7.72 (m, 4 H, Ph), 7.47 (m, 4 H, Ph), 7.41 – 7.27 (m, 14 H, Ph), 7.25 – 7.13 (m, 24 H, Ph), 7.12 – 7.03 (m, 7 H, Ph), 7.01 – 6.85 (m, 14 H, Ph), 6.01 (t, *J* = 9.1 Hz, 1 H), 5.65 (d, *J* = 7.6 Hz, 1 H, H-1), 5.45 – 5.39 (m, 2 H, Ph-CH-), 5.06 – 4.96 (m, 4 H, 2 H-1), 4.94 (d, *J* = 8.1 Hz, 1 H, H-1), 4.85 – 4.79 (m, 1 H), 4.62 (d, *J* = 11.4 Hz, 1 H), 4.58 – 4.53 (m, 3 H, H-1), 4.50 – 4.43 (m, 3 H), 4.37 – 4.30 (m, 6 H, H-1), 4.29 – 4.14 (m, 8 H), 4.11 – 4.03 (m, 2 H), 3.94 – 3.83 (m, 4 H), 3.81 – 3.66 (m, 6 H), 3.59 – 3.42 (m, 7 H), 3.39 – 3.21 (m, 6 H), 3.12 (td, *J* = 10.1, 9.2, 4.0 Hz, 2 H), 2.98 (d, *J* = 9.8 Hz, 1 H), 2.84 – 2.72 (m, 2 H), 2.58 (d, *J* = 11.7 Hz, 2 H). ^{13}C NMR (150 MHz, $CDCl_3$) δ: 168.1, 167.1, 167.07, 166.2, 165.76, 165.68, 165.6, 163.9, 163.7, 139.2, 138.7, 138.39, 138.37, 138.18, 138.16, 137.54, 137.5, 137.0, 133.5, 132.7, 132.63, 132.60, 132.4, 132.16, 131.26, 131.17, 130.1, 130.08, 130.05, 129.97, 129.95, 129.6, 129.55, 129.39, 128.9, 128.6, 128.6, 128.4, 128.3, 128.27, 128.25, 128.22, 128.19, 128.14, 128.12, 128.02, 127.98, 127.82, 127.80, 127.73, 127.56, 127.51, 127.44, 127.38, 127.37, 127.34, 126.6, 126.3, 123.1, 122.6, 101.5 (Ph-C), 100.5 (C-1), 99.7 (C-1), 99.5 (C-1), 99.3 (C-1), 99.0 (C-1), 98.8 (C-1), 80.8, 79.7, 79.2, 79.1, 78.42, 78.38, 74.7, 74.6, 74.3, 74.2, 74.1, 74.0, 73.7, 73.5, 73.44, 73.4, 73.33, 73.25, 73.22, 73.00, 72.7, 72.0, 71.8, 70.7, 70.4, 69.9, 69.8, 69.0, 67.9, 66.1, 63.9, 55.6, 55.5, 50.6. HR ESI-TOF MS (*m/z*): calcd for $C_{152}H_{143}N_5O_{38}$ $[M + H]^+$, 2646.9484; found, 2646.9456.



Synthesis of compound 20: After a mixture of **17** (1.64 g, 2.22 mmol), **18** (1.03 g, 2.22 mmol), which was prepared according to reported procedures (Mondal, P. K.; Liao, G.; Mondal, M. A.; Guo Z. *Org Lett*, **2015**, 17, 1102-1105), and 4Å molecular sieves (5 g) in dry DCM (18 mL) and CH_3CN (9 mL) was stirred under argon for 2 h, it was cooled to -78 ˚C and then 482 µL of TMSOTf was added dropwise. After 1 h, the mixture was warmed to -40 ˚C, quenched with saturated aq. $NaHCO_3$ solution, diluted with DCM, and filtered off. The organic layer was dried over anhydrous Na_2SO_4 and concentrated to give **19** (2.21 g) as a white solid, which was directly applied to the next reaction. HR ESI-TOF MS (*m/z*): calcd for $C_{46}H_{48}F_3NO_{17}S$ $[M + K]^+$, 1014.2227; found, 1014.2440. After **19** (660 mg, 0.67 mmol) was dissolved in AcOH (6 mL) and H_2O (4 mL), the solution was heated at 60 ˚C for 5 h. TLC indicated that **19** was completely consumed. The solvents were removed under vacuum, and the residue was co-evaporated with toluene (5 x). The resulting product was dissolved in Ac_2O and pyridine (4:1; 10 mL), and the mixture was stirred at rt for 12 h. The solvents were removed under vacuum. The crude product was purified by silica gel column chromatography (ethyl acetate/toluene, 1:3) to give **20** (566.9 mg, 87%) as a white solid. ^{1}H NMR (600 MHz, $CDCl_3$) δ: 8.14 (m, 2 H, Ph), 7.56 (m, 1 H, Ph), 7.47 (m, 2 H, Ph), 7.43 (m, 2 H, Ph), 7.25 – 7.24 (m, 3 H, Ph), 6.59 (s, 1 H, -NHCO-), 5.47 (ddd, J = 8.4, 6.0, 2.2 Hz, 1 H), 5.27 – 5.21 (m, 1 H), 5.13 (d, J = 8.9 Hz, 1 H), 5.02 (d, J = 9.9 Hz, 1 H, H-1), 4.98 (d, J = 3.1 Hz, 1 H), 4.91 (ddd, J = 12.1, 9.9, 4.8 Hz, 1 H), 4.74 (dd, J = 9.6, 3.1 Hz, 1 H), 4.30 (dd, J = 12.5, 2.0 Hz, 1 H), 4.06 (d, J = 5.8 Hz, 2 H), 3.95 (t, J = 6.4 Hz, 1 H), 3.88 (dd, J = 12.5, 6.0 Hz, 1 H), 3.80 (s, 3 H), 3.77 – 3.70 (m, 2 H), 2.54 (dd, J = 12.8, 4.7 Hz, 1 H), 2.14 (d, J = 4.2 Hz, 3 H), 2.05 (s, 3 H), 2.03 (s, 3 H), 1.97 (s, 3 H), 1.92 (s, 3 H), 1.67 (t, J = 12.5 Hz, 1 H), 1.38 (s, 3 H). ^{13}C NMR (151 MHz, $CDCl_3$) δ: 170.7, 170.6, 170.4, 170.2, 169.7, 167.8, 165.2, 157.6, 157.4, 133.2, 132.7, 132.4, 130.2, 130.1, 128.7, 128.5, 127.9, 116.2, 114.3, 96.8 (C-1), 86.1 (C-1), 74.3, 72.3, 71.1, 69.1, 68.6, 68.0, 67.8, 66.3, 62.3, 62.2, 53.2, 49.5, 37.3, 21.4, 20.7, 20.6, 20.4, 19.9. HR ESI-TOF MS (*m/z*): calcd for $C_{39}H_{44}C_{13}F_3N_2O_{21}$ $[M + H]^+$, 1039.1527; found, 1039.3655.

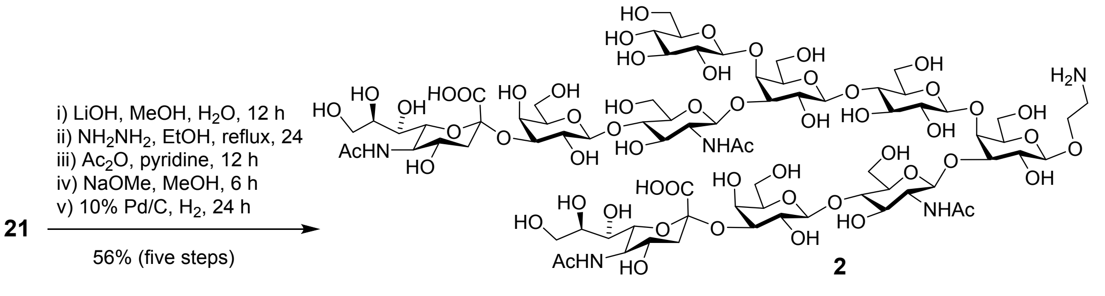


Synthesis of compound 3: To a solution of **20** (200 mg, 0.20 mmol) and NIS (100 mg, 0.40 mmol) in wet DCM (12 mL) was added dropwise 3.2 µL of triflic acid at 0 ˚C. The mixture was stirred for 1 h, and diluted with ethyl acetate. The organic layer was washed sequentially with saturated aq. $NaHCO_3$, NaS_2O_3 solutions, and brine, dried and concentrated under vacuum. The residue was purified by silica gel chromatography (ethyl acetate/hexanes, 1:4) to give the mixture of anomeric hemiacetals **30** (161.5 mg, 89%). To a solution of **30** (150.3 mg, 0.17 mmol) in dry DCM (5 mL) were added CCl_3CN (96.4 mg, 0.67 mmol) and DBU (5 µL) at 0 ˚C sequentially. The solution was stirred for 1 h and then concentrated. The residue was purified on a triethylamine-neutralized silica gel column (ethyl acetate/hexanes, 1:3) to afford **3** (160.6 mg, 92%) as a white solid. This relatively unstable product was directly applied to the subsequent glycosylation reaction.

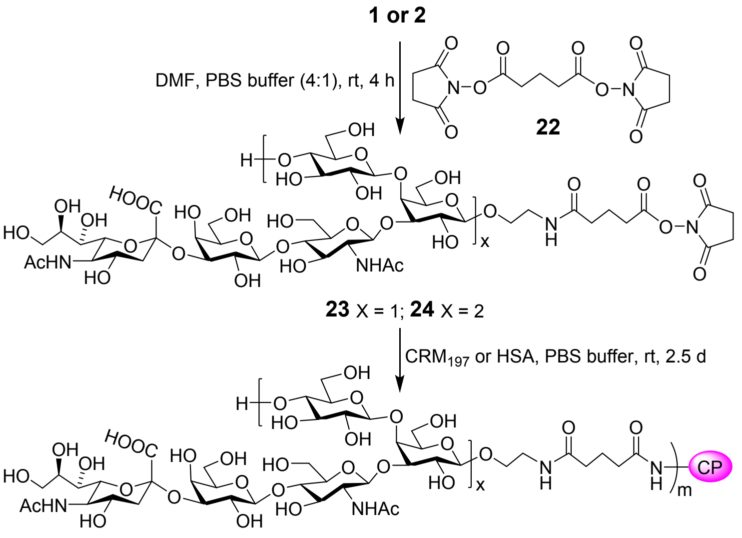


Synthesis of compound 21: After a mixture of **3** (130.4 mg, 0.13 mmol), **4** (79.9 mg, 31.3 µmol) and 4Å molecular sieves (1 g) in dry DCM (5 mL) was stirred under argon for 2 h, it was cooled to -78 ˚C and then 10 µL of TMSOTf was added dropwise. After 1 h, the mixture was warmed to

-0 ˚C, quenched with saturated $NaHCO_3$ solution, diluted with DCM, and filtered off. The organic layer was dried over anhydrous Na_2SO_4 and concentrated. The residue was purified by silica gel column chromatography (ethyl acetate/toluene, 1:7) to give **21** (79.9 mg, 58%) as a white solid. ^1H NMR (600 MHz, $CDCl_3$) δ: 8.32 (m, 2 H), 8.20 (m, 2 H), 8.05 (m, 2 H), 7.70 (m, 2 H), 7.64 – 7.28 (m, 44 H), 7.22 – 6.86 (m, 44 H), 6.11 – 6.02 (m, 3 H), 5.72 – 5.63 (m, 3 H, 2 H-1), 5.48 (s, 1 H), 5.45 – 5.41 (m, 1 H), 5.32 – 5.10 (m, 6 H，H-1), 5.03 – 4.85 (m, 12 H，6 H-1), 4.73 – 4.66 (m, 2 H), 4.48 – 4.17 (m, 20 H，H-1), 3.96 – 3.72 (m, 27 H), 3.60 (d, J = 10.7 Hz, 1 H), 3.57 – 3.46 (m, 7 H), 3.44 – 3.39 (m, 1 H), 3.31 (dd, J = 25.4, 8.6 Hz, 2 H), 3.22 (q, J = 10.6, 10.0 Hz, 2 H), 3.11 (d, J = 12.0 Hz, 1 H), 3.06 – 2.97 (m, 3 H), 2.78 – 2.70 (m, 2 H), 2.57 (d, J = 8.0 Hz, 2 H), 2.21 (s, 3 H), 2.15 (s, 3 H), 2.03 (s, 3 H), 2.01 (s, 6 H), 1.99 (s, 3 H), 1.95 (d, J = 5.7 Hz, 6 H), 1.93 (s, 3 H), 1.82 (s, 3 H), 1.46 (s, 3 H), 1.43 (s, 3 H). ^{13}C NMR (151 MHz, $CDCl_3$) δ: 170.8, 170.7, 170.5, 170.5, 170.4, 170.1, 169.8, 169.7, 167.9, 167.7, 167.6, 167.1, 167.0, 165.8, 165.5, 165.4, 165.1, 164.8, 164.1, 163.9, 157.7, 157.68, 157.44, 157.40, 139.2, 138.9, 138.8, 138.6, 138.4, 138.3, 138.2, 138.1, 137.1, 133.8, 133.5, 133.4, 133.1, 132.8, 132.7, 132.2, 132.0, 131.44, 131.27, 131.23, 130.4, 130.2, 130.1, 129.95, 129.92, 129.9, 129.6, 129.59, 129.51, 128.8, 128.6, 128.5, 128.4, 128.34, 128.31, 128.23, 128.20, 128.04, 127.94, 127.91, 127.75, 127.73, 127.68, 127.59, 127.55, 127.42, 127.39, 127.21, 127.19, 127.18, 127.04, 126.96, 126.8, 126.5, 126.1, 123.8, 123.6, 122.8, 122.7, 116.2, 114.3, 101.3, 100.6 (C-1), 100.4 (C-1), 99.9 (C-1), 99.5 (C-1), 99.4 (C-1), 98.8 (C-1), 98.4 (C-1), 97.1 (C-1), 96.9 (C-1), 96.9 (C-1), 80.6, 79.9, 78.7, 78.0, 77.4, 76.6, 76.6, 76.0, 74.6, 74.5, 74.3, 74.1, 73.9, 73.9, 73.4, 73.4, 73.2, 73.0, 72.74, 72.7, 72.6, 71.8, 71.6, 71.58, 71.4, 71.0, 70.5, 70.4, 70.3, 69.3, 69.2, 69.0, 68.8, 68.6, 68.5, 67.7, 67.6, 67.3, 67.2, 67.0, 66.5, 66.3, 66.0, 63.3, 62.2, 62.0, 61.5, 61.2, 56.1, 56.0, 53.3, 53.28, 51.0, 50.5, 49.7, 49.6, 37.3, 37.2, 21.4, 21.3, 20.8, 20.7, 20.6, 20.6, 20.57, 20.5, 20.42, 20.41, 20.1, 20.08. HR ESI-TOF MS (*m/z*): calcd for $C_{226}H_{227}F_6N_7O_{78}$ $[M + H]^+$, 4400.3916; found, 4401.19.



Synthesis of compound 2: To a solution of **25** (50 mg, 11.4 μmol) in MeOH (9 mL) and H_2O (6 ML) was added LiOH-H_2O (25 mg) at rt. After the mixture was stirred at 80 ˚C for 12 h, solvents were removed under vacuum, and the residue was dissolved in a mixture of EtOH (8 ML) and hydrazine monohydrate (2 mL). The solution was refluxed for 24 hand concentrated. The residue was dissolved in pyridine (6 mL) and acetic anhydrate and stirred at rt for 12 h. It was concentrated under vacuum, and the residue was dissolved in MeOH (10 mL) followed by addition of NaOMe (40 mg) at 0 ˚C. The reaction was stirred at rt for 6 h and neutralized with 0.1 N HCl at 0 ˚C. To the resulting solution was added 10% Pd/C (30 mg), and the mixture was shaken under hydrogen at 50 psi for 24 h. The catalyst was removed by filtration, and the filtrate was concentrated under vacuum. The residue was purified on a Sephadex G-25 gel filtration column using water as the eluent, and the fractions were combined and lyophilized to give **2** (13.3 mg) as a fluffy solid. ^{1}H NMR (600 MHz, D_2O) δ: 4.90 (s, 1 H), 4.74 (s, 1 H，H-1), 4.54 (s, 4 H, H-1), 4.44 – 4.35 (m, 3 H, 2 H-1), 4.24 (t, J = 28.5 Hz, 6 H, 5 H-1), 3.95 (d, J = 9.6 Hz, 3 H, H-1), 3.82 – 3.40 (m, 53 H), 3.30 – 3.17 (m, 4 H), 3.09 (s, 3 H), 2.01 (d, J = 3.4 Hz, 2 H), 1.92 – 1.77 (m, 12 H), 1.63 (s, 2 H). ^{13}C NMR (151 MHz, D_2O) δ: 181.0, 177.0, 174.9, 174.7, 174.1, 173.8, 128.7, 128.6, 128.52, 128.5, 126.2, 126.2, 103.1 (C-1), 102.84 (C-1), 102.81 (C-1), 102.8 (C-1), 102.5 (C-1), 102.48 (C-1), 102.0 (C-1), 99.9 (C-1), 99.8 (C-1), 99.7 (C-1), 81.8, 79.5, 78.2, 76.3, 76.2, 75.8, 75.6, 75.4, 75.1, 74.6, 74.4, 74.4, 74.3, 73.6, 72.8, 72.1, 71.7, 71.66, 70.2, 69.7, 69.3, 68.3, 68.26, 68.0, 67.64, 67.6, 67.4, 62.53, 62.52, 62.49, 61.6, 61.0, 60.9, 60.8, 60.77, 60.75, 60.7, 60.6, 60.1, 55.2, 55.2, 51.6, 39.7, 39.6, 39.4, 32.4, 32.1, 23.2, 22.2, 22.0, 20.7, 20.4, 20.0, 13.26. HR ESI-TOF MS (*m*/*z*): calcd for $C_{226}H_{227}F_6N_7O_{78}$ [M + 2H]$^{2+}$, 1067.8296; found, 1067.7161.



1a X = 1; CP = CRM_{197}
1b X = 1; CP = HSA
2a X = 2; CP = CRM_{197}
2b X = 2; CP = HSA

Synthesis of glycoconjugates 1a,b and 2a,b. To a stirred solution of oligosaccharide **1** or **2** (4 mg) in DMF (0.4 ml) and PBS (0.1 M, 0.1 ml) was added disuccinimidal glutarate (15 equiv). After the mixture was stirred at rt for 4 h, solvents were removed under vacuum. The residue was washed with EtOAc 10 times, and then dried under vacuum for 1 h to get **23** and **24**, respectively, which were directly used to conjugate with proteins. To a stirred solution of CRM$_{197}$ or HSA (5 mg) in PBS (0.1 M, 0.4 mL) was added **23** or **24** obtained above. The mixture was stirred at rt for 2.5 days in dark and was then directly applied to a Biogel A 0.5 column for purification using 0.1 M PBS as the eluent. The fractions containing glycoproteins, as indicated by the bicinchoninic acid (BCA) assay, were combined and dialyzed against distilled water for 1 day. Lyophilization of the solution produced the desired glycoconjugates **1a,b** and **2a,b**, respectively, as white fluffy solids.

Analysis of the carbohydrate loadings of glycoconjugates 1a,b and 2a,b. To a solution of the resorcinol reagent (2.0 mL) in distilled water (1 mL) was added of an exactly weighted conjugate sample (0.3-0.6 mg) at rt. The mixture was heated in a boiling water bath for 30 min, cooled to rt, and then 1-butanol acetate and 1-butanol (v/v = 85/15, 3 mL) were addded. After vigorous shaking and standing still for 10 min, the organic layer was separated and transferred to a 1.0-cm cuvette. The absorbance was determined at 580 nm with an UV-Vis spectrometer, using a blank extraction solution as the negative control. The *N*-acetyl sialic acid content in each sample was determined by comparison with a calibration curve created with standard solutions of *N*-acetyl sialic acid under the same conditions. The *N*-acetyl sialic acid content in each glycoconjugate sample was used to calculated the carbohydrate loading according to the following equations.

For conjugates **1a,b:** carbohydrate loading (%)= $\frac{sialic\ acid\ content(mg)\ in\ the\ sample\ \times\ 999}{weight\ of\ the\ glycocojugate\ sample(mg)\ \times\ 309}$ ×100%

For conjugates **2a,b:** carbohydrate loading (%)= $\frac{sialic\ acid\ content(mg)\ in\ the\ sample\ \times\ 1980}{weight\ of\ the\ glycocojugate\ sample(mg)\ \times\ 618}$ ×100%

Procedure for the analysis of glycoconjugates 1a and 2a by SDS-PAGE: After the solution of a glycoconjugate **1a** or **2a** (1 μg) with 0.1 M PSB (1 μl), NuPAGE® LDS NuPAGE® sample buffer (4×, 2.5 μl), reducing agent (10×, 1 μl), and deionized water (5.5 μl) was heated at 70 °C for 10 min, it was loaded onto the sodium dodecyl sulfate polyacrylamide gel (SDS-PAGE). The upper buffer chamber was filled with 200 ml of 1× SDS running buffer containing 500 µl of NuPAGE® antioxidant and the lower buffer chamber was filled with 600 ml of 1× SDS running buffer. The gel was run at 200 V for 2 h, and stained with silver. The results are shown in Figure S1.

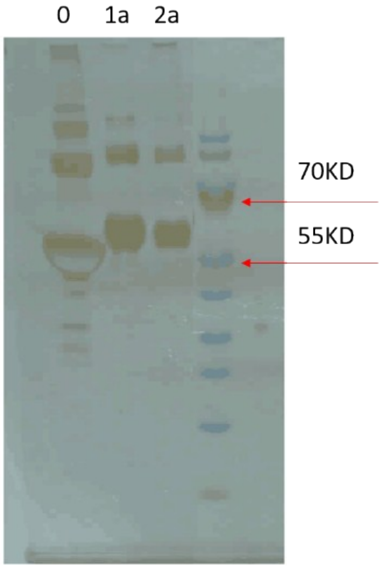


Figure S1. SDS-PAGE results of oligosaccharide-CRM$_{197}$ conjugates **1a** and **2a**. Lane 0: CRM$_{197}$ protein used for conjugation reaction; Lane 2: glycoconjugate **1a**; Lane 3: glycoconjugate **2a**.

The mouse immunization protocol: Twelve female C57BL/6 J mice (6-8 weeks) were randomly separated into 2 groups. On day 1, to each mouse was intramuscularly (i.m.) injected an emulsion of a glycoconjugate **1a** or **2a** (0.1 mL/mouse, containing 3.0 µg of the carbohydrate antigen), which was prepared by dissolving **1a** or **2a** in 1× PBS and then thorough mixing with CFA using a three-way syringe. On day 14, an IFC emulsion of the same glycoconjugate (0.1 mL/mouse, containing 3.0 µg of the carbohydrate antigen) was subcutaneously (s.c.) injected to each mouse for the second immunization. Each mouse was bled by its saphenous vein on day 0 and day 21 and blood samples were centrifuged at 5000 dr/min for 7 min at -4℃ to prepare sera according to standard protocols. The sera were stored at -80 ℃. The animal use protocol was approved by the Institutional Animal Care and Use Committee (IACUC) of Wayne State University.

The ELISA protocol: After ELISA plates were incubated with a solution of HSA conjugate **1b** or **2b** dissolved in a coating buffer (2 µg/mL, 100 µL) at 4 ℃ overnight and then at 37 ℃ for 1 h, they were washed with PBST 3 times and then treated with the blocking buffer 37 ℃ for 1 h. After being washed with PBST 3 times, the plates were loaded with the day 0 and day 21 mouse sera with serial half-log dilutions from 1:300 to 1:218700 in PBS (100 µL/well). Thereafter, the plates were incubated at 37 ℃ for 2 h, followed by washing with PBST 3 times. A secondary antibody, AP-linked goat anti-mouse kappa, IgG or IgM antibody (1:1000 dilution with 1× PBS), was then added into each well of the plates (100 µL/well) and the plates were incubated at rt for 1 h, followed by washing with PBST 3 times. The plates were finally developed with a *p*-nitrophenylphosphate (PNPP) solution (1.67 mg/mL, 100 µL/well) for 30 min at rt. The OD values at 405 nm wavelength were measured with a microplate reader and used to plot against dilution numbers to obtain a best-fit line by linear regression analysis for each sample. The equation of this line was used to calculate the dilution value at which an OD of 0.1 was achieved. ELISA experiments to measure the cross-reactivity between groups were performed by the same protocol using each HSA conjugate as the capture antigen to detect antibodies in both antisera.

Statistical analysis. ELISA data were assessed by student's t-test analysis. Statistical significance was defined as P value < 0.05.

ELISA results to test cross-reactive IgM antibodies in both antisera (Figure S2): Experimental protocol was presented above.

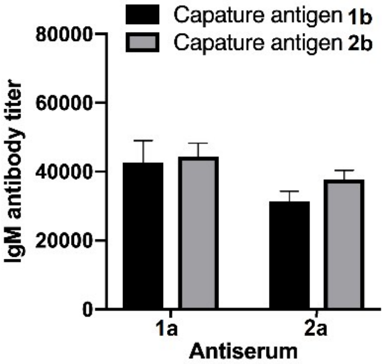
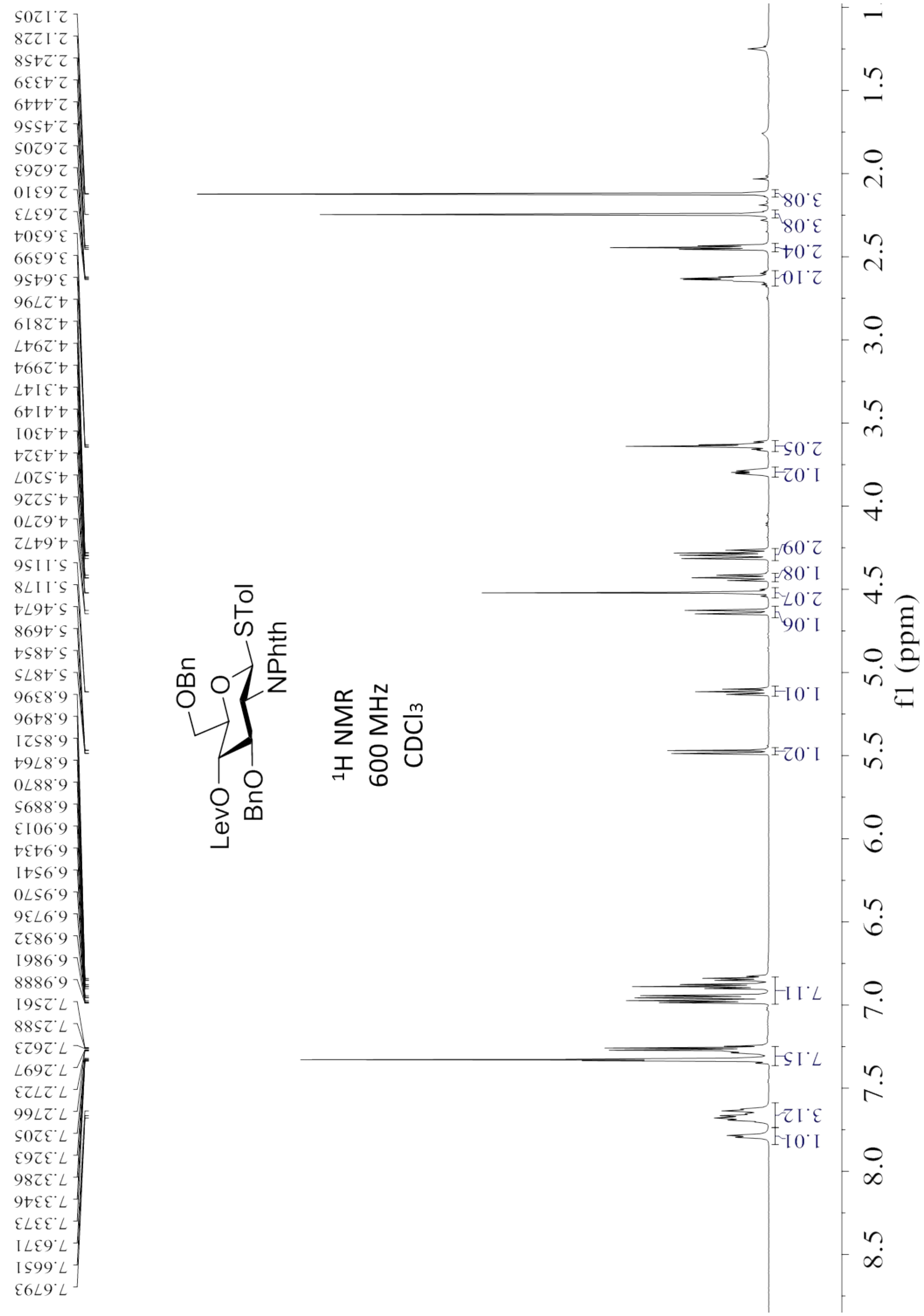
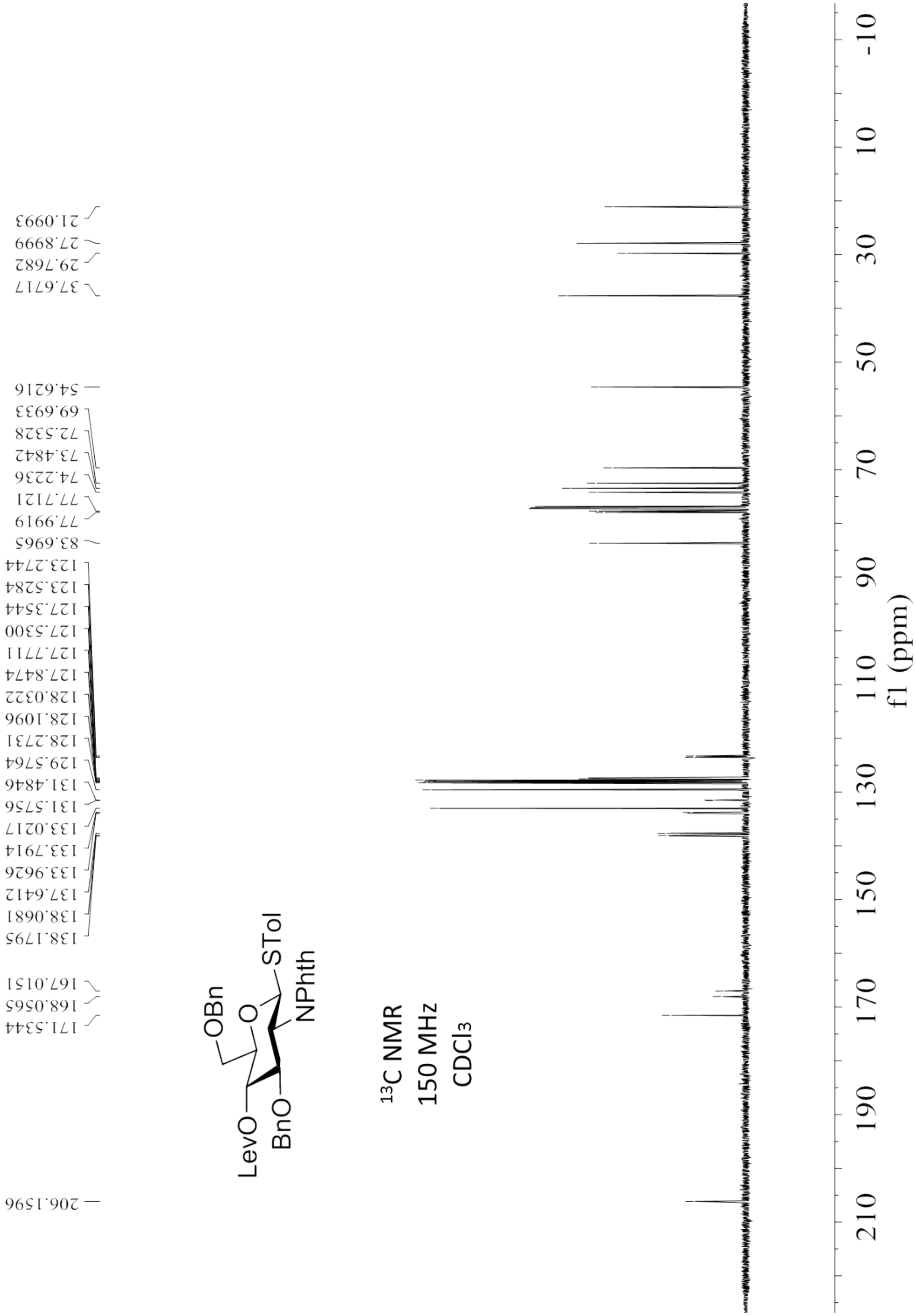


Figure S2. ELISA results to disclose the reactivity of each antiserum with both oligosaccharides with HSA conjugates **1b** and **2b** as coating antigens to detect IgM antibody in each pooled antiserum. The mean antibody titer of three parallel experiments was presented for each sample, and the error bar showed the SEM.

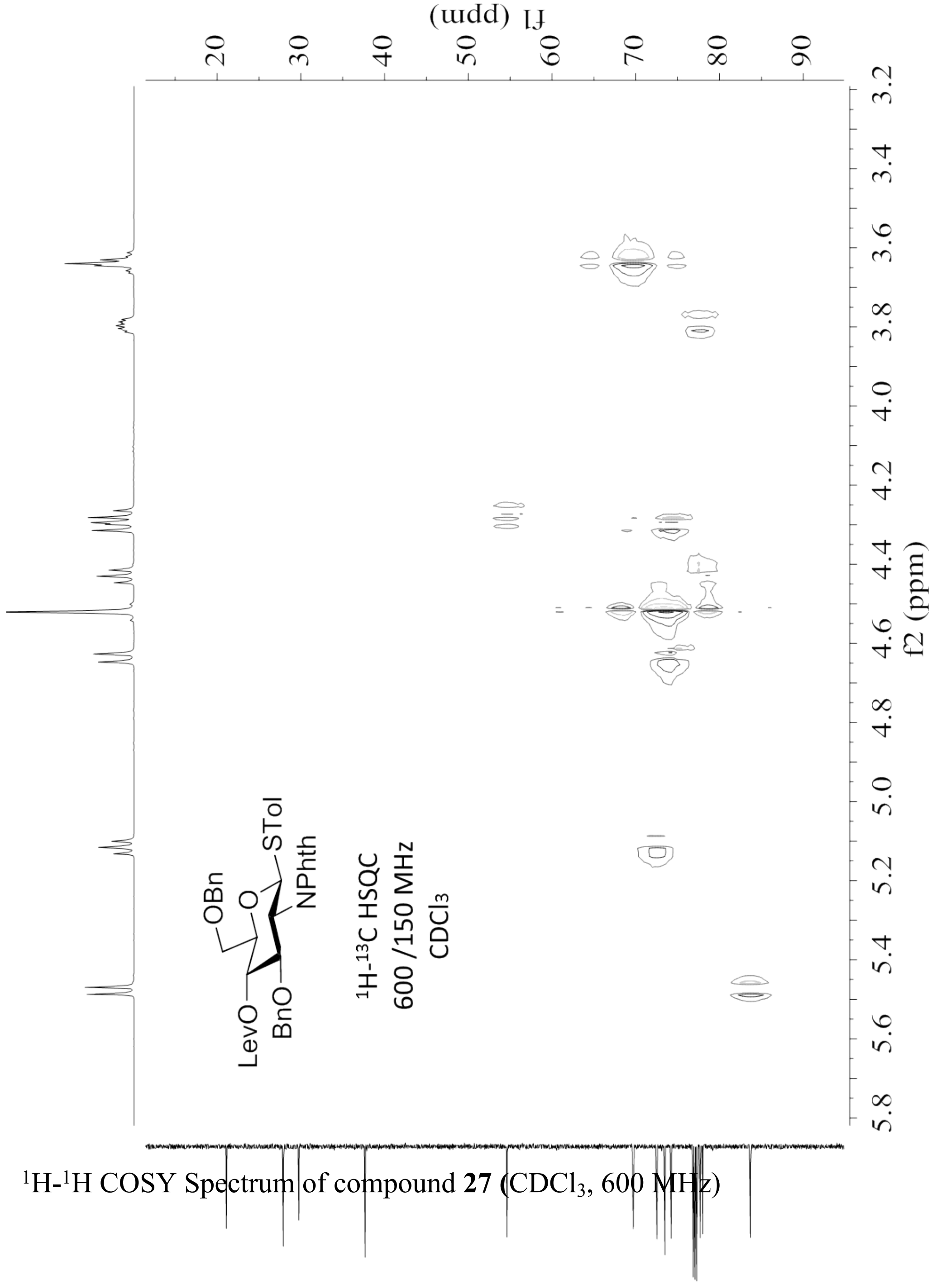
II. NMR and MS Spectra of Synthesized Compounds



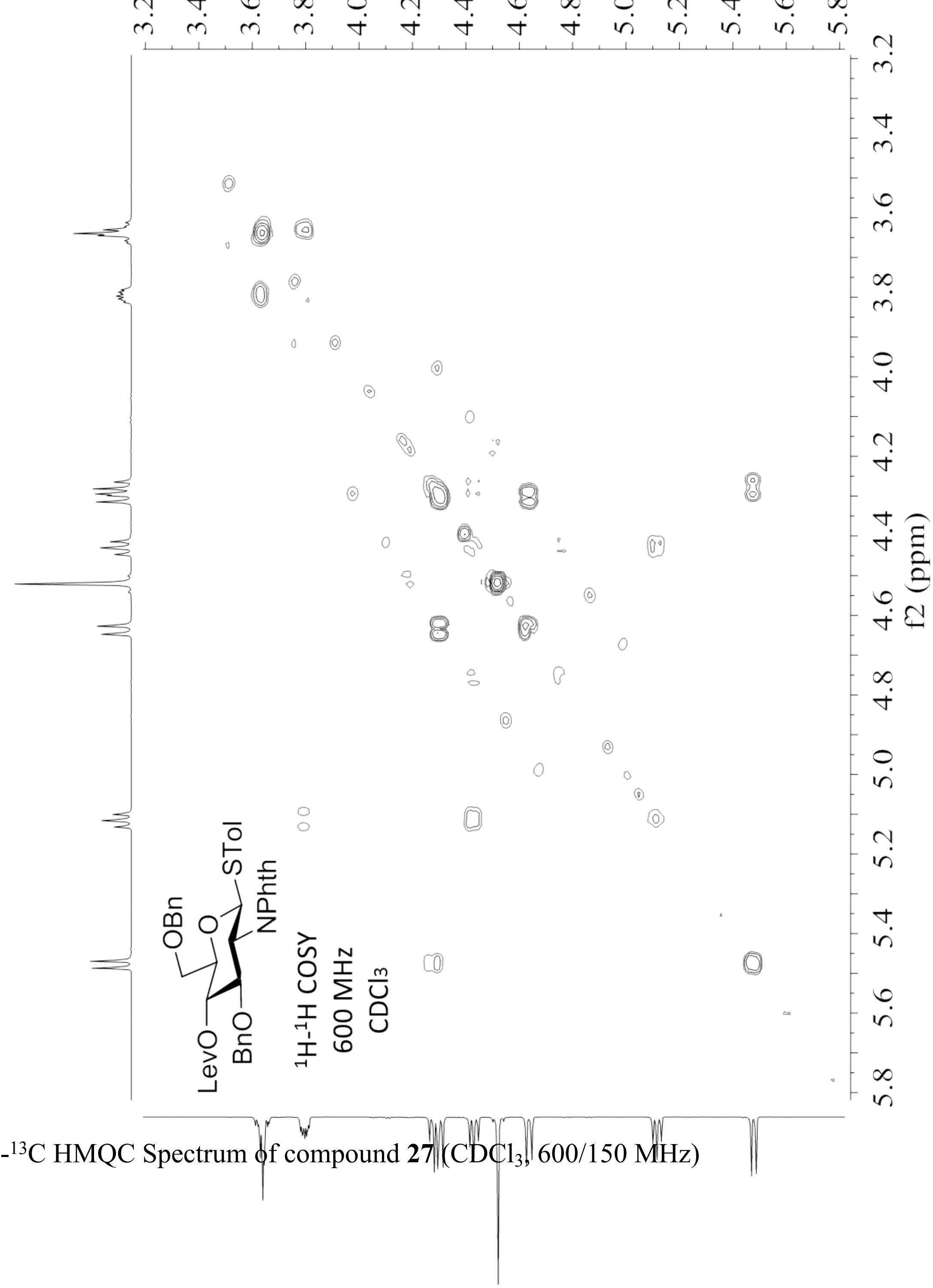
^{1}H NMR Spectrum of compound **27** (CDCl$_3$, 600 MHz)



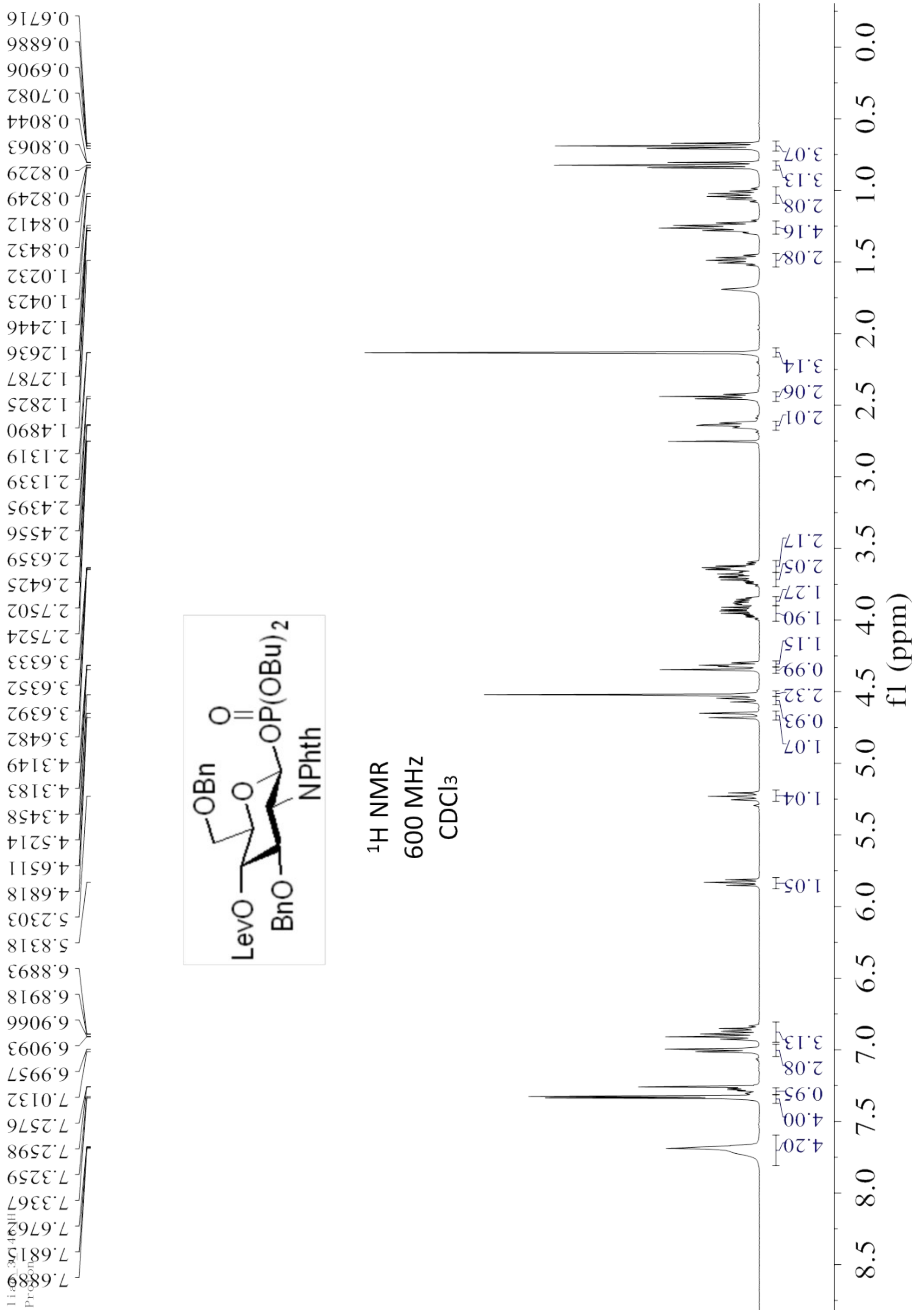
^{13}C NMR Spectrum of compound **27** ($CDCl_3$, 150 MHz)



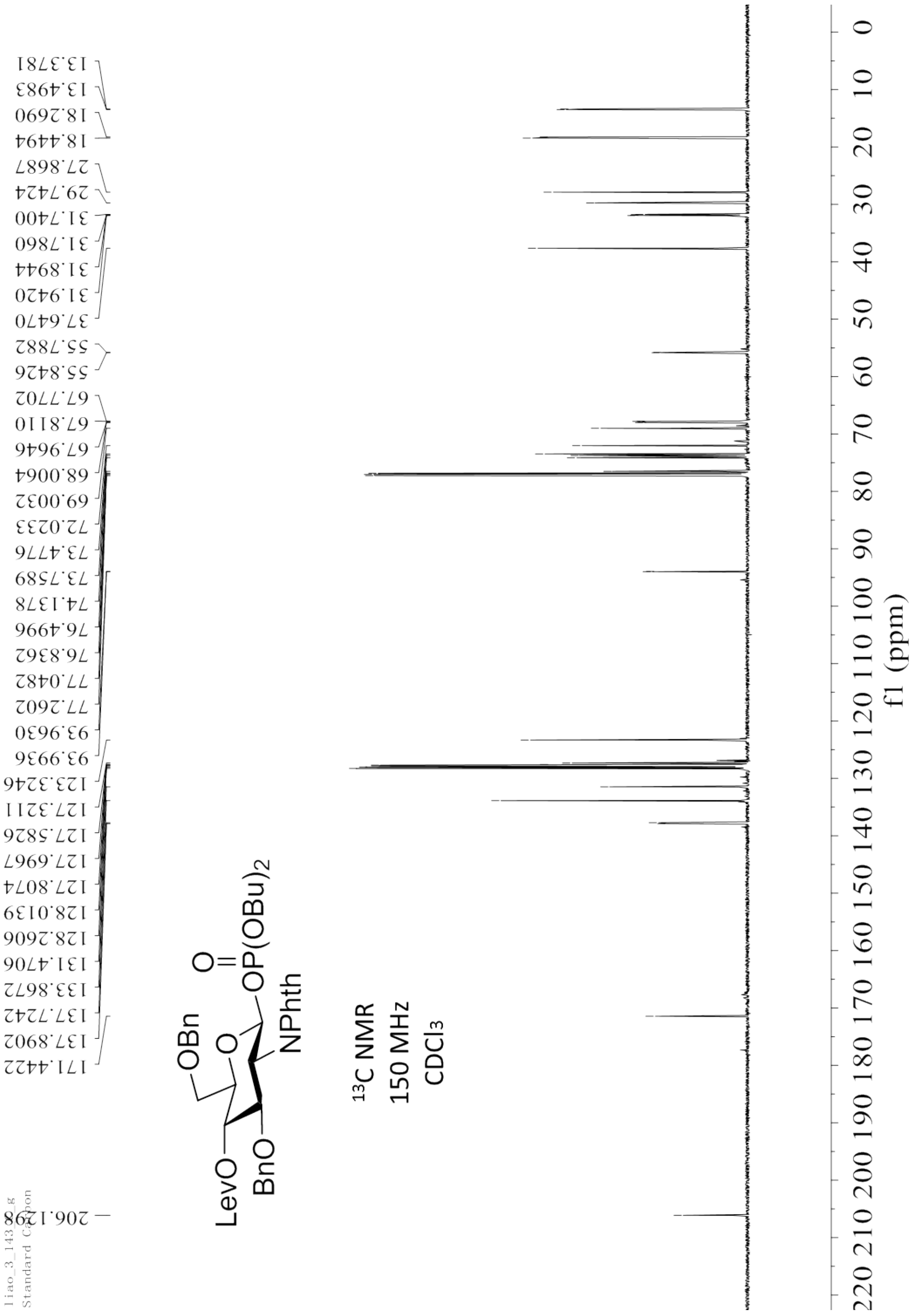
^{1}H-^{1}H COSY Spectrum of compound **27** (CDCl$_3$, 600 MHz)



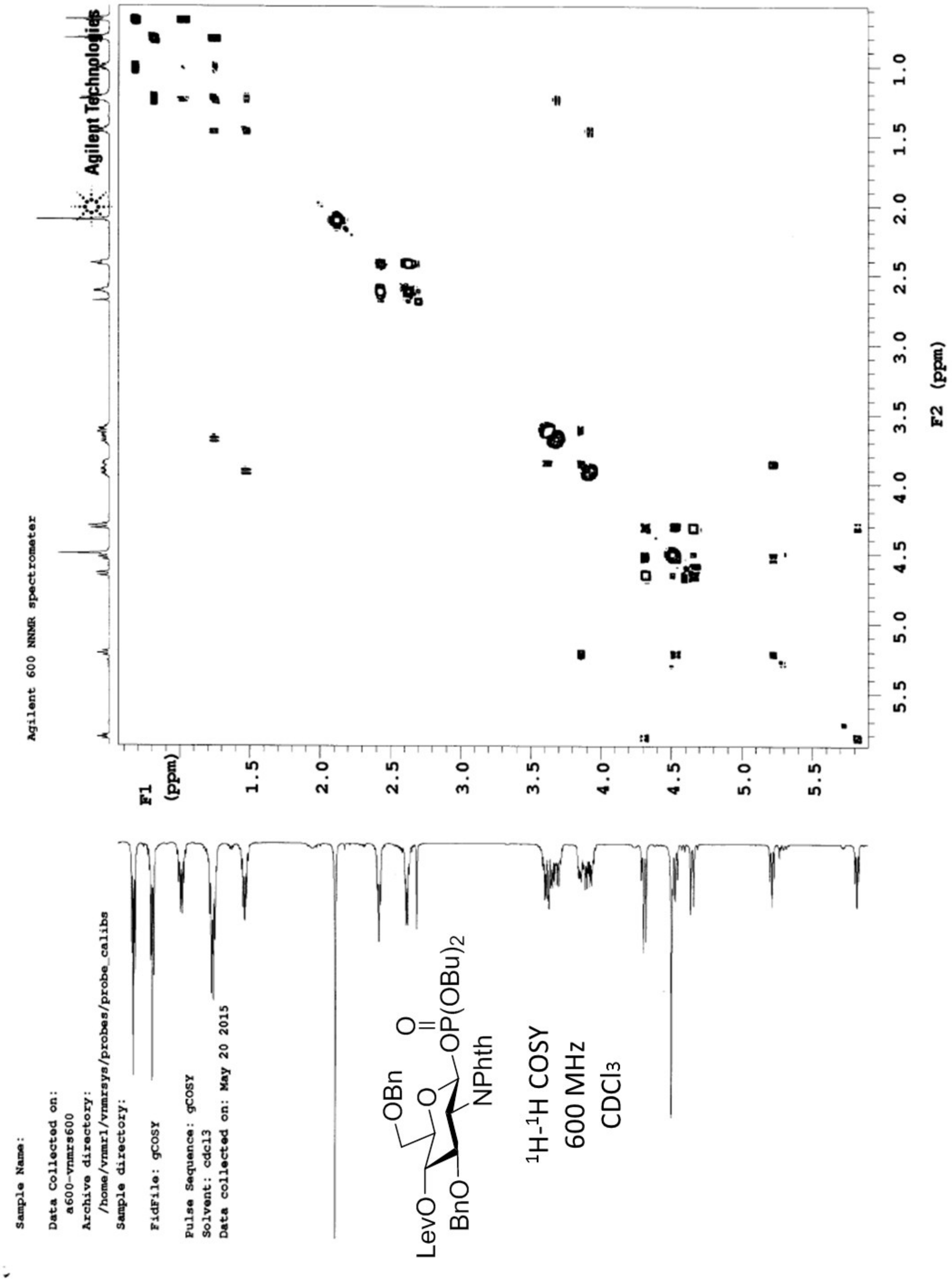
^{1}H-^{13}C HMQC Spectrum of compound **27** (CDCl$_3$, 600/150 MHz)



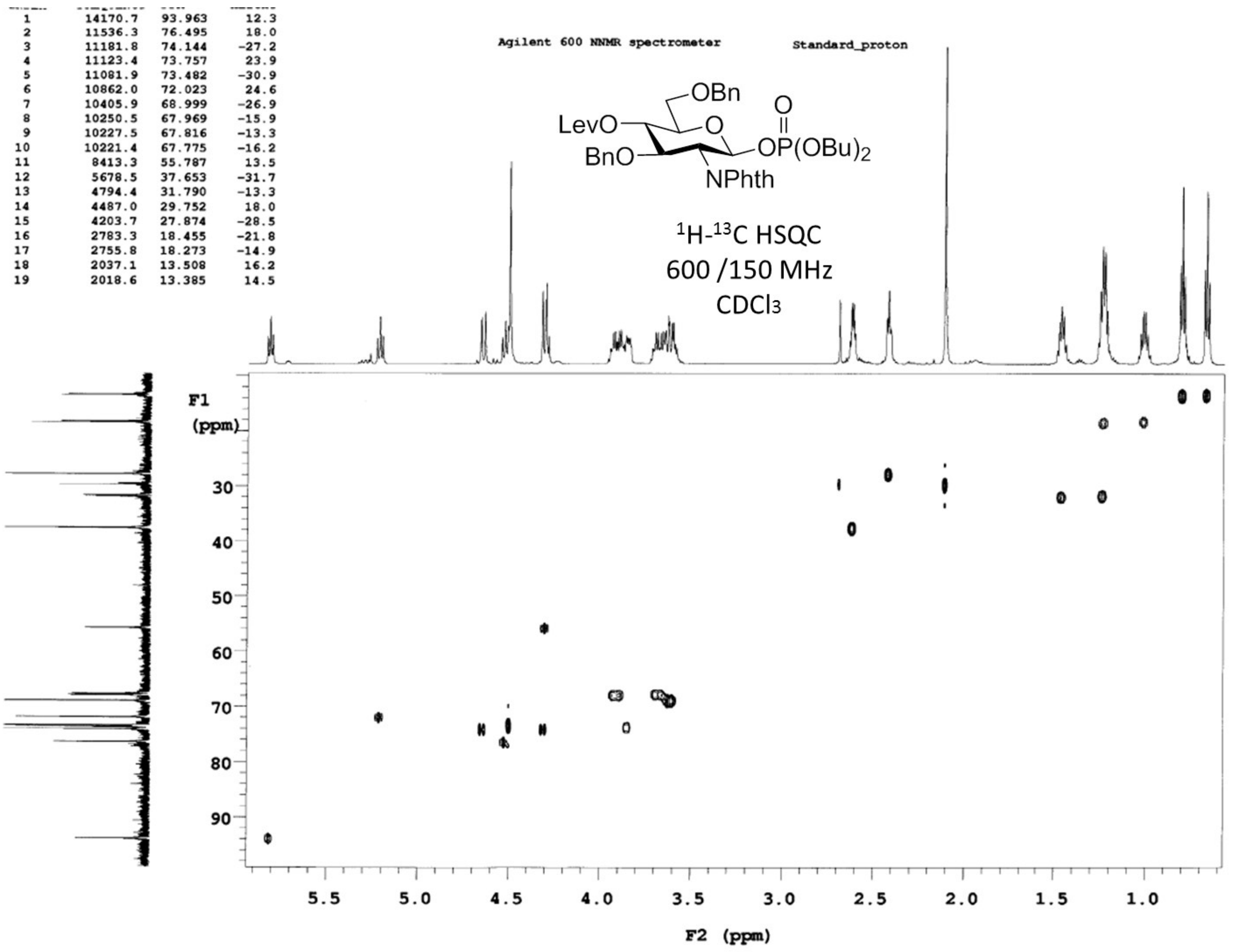
^{1}H NMR Spectrum of compound **9** ($CDCl_3$, 600 MHz)



^{13}C NMR Spectrum of compound **9** (CDCl$_3$, 150 MHz)

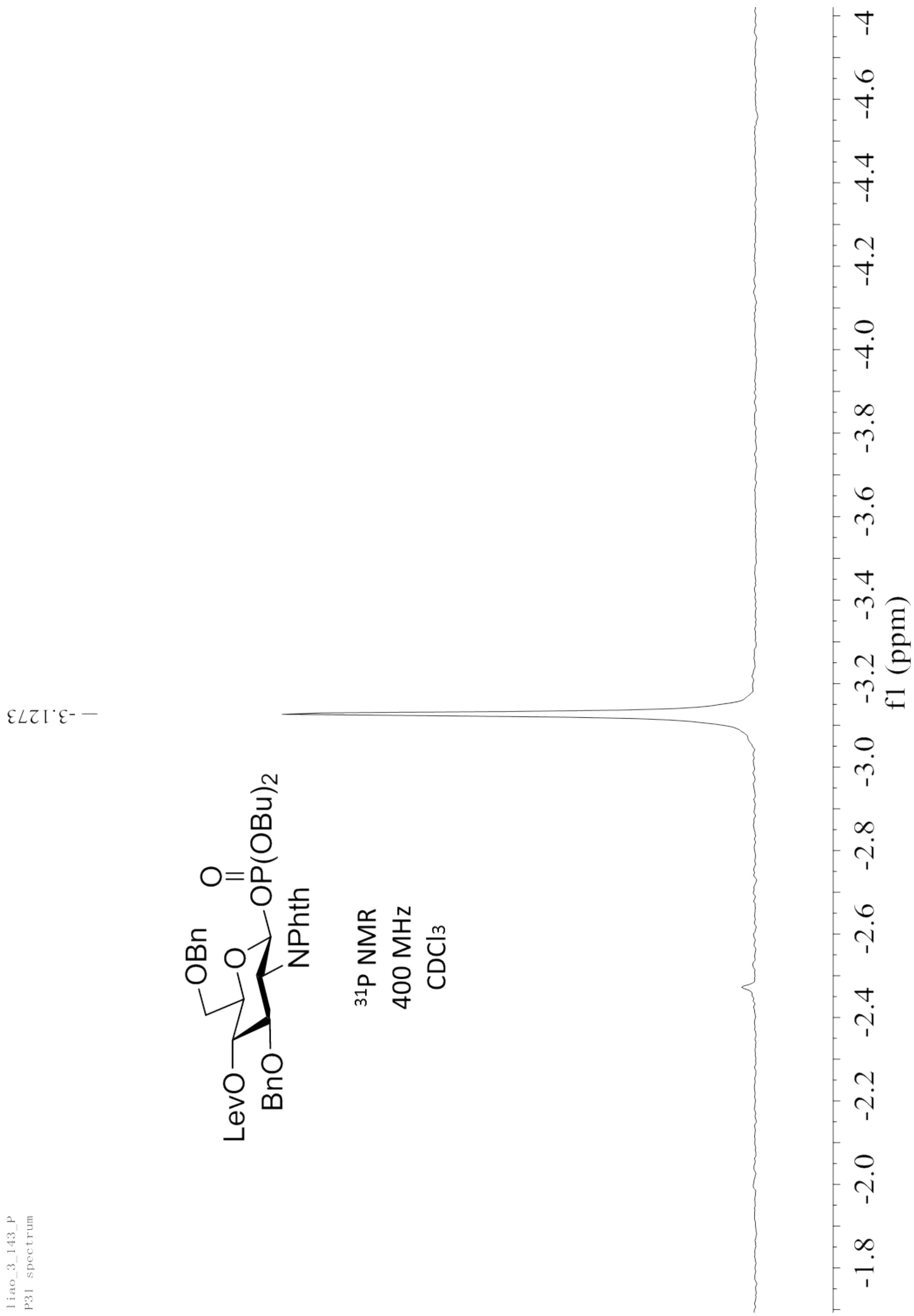


^{1}H-^{1}H COSY Spectrum of compound **9** (CDCl$_3$, 600 MHz)

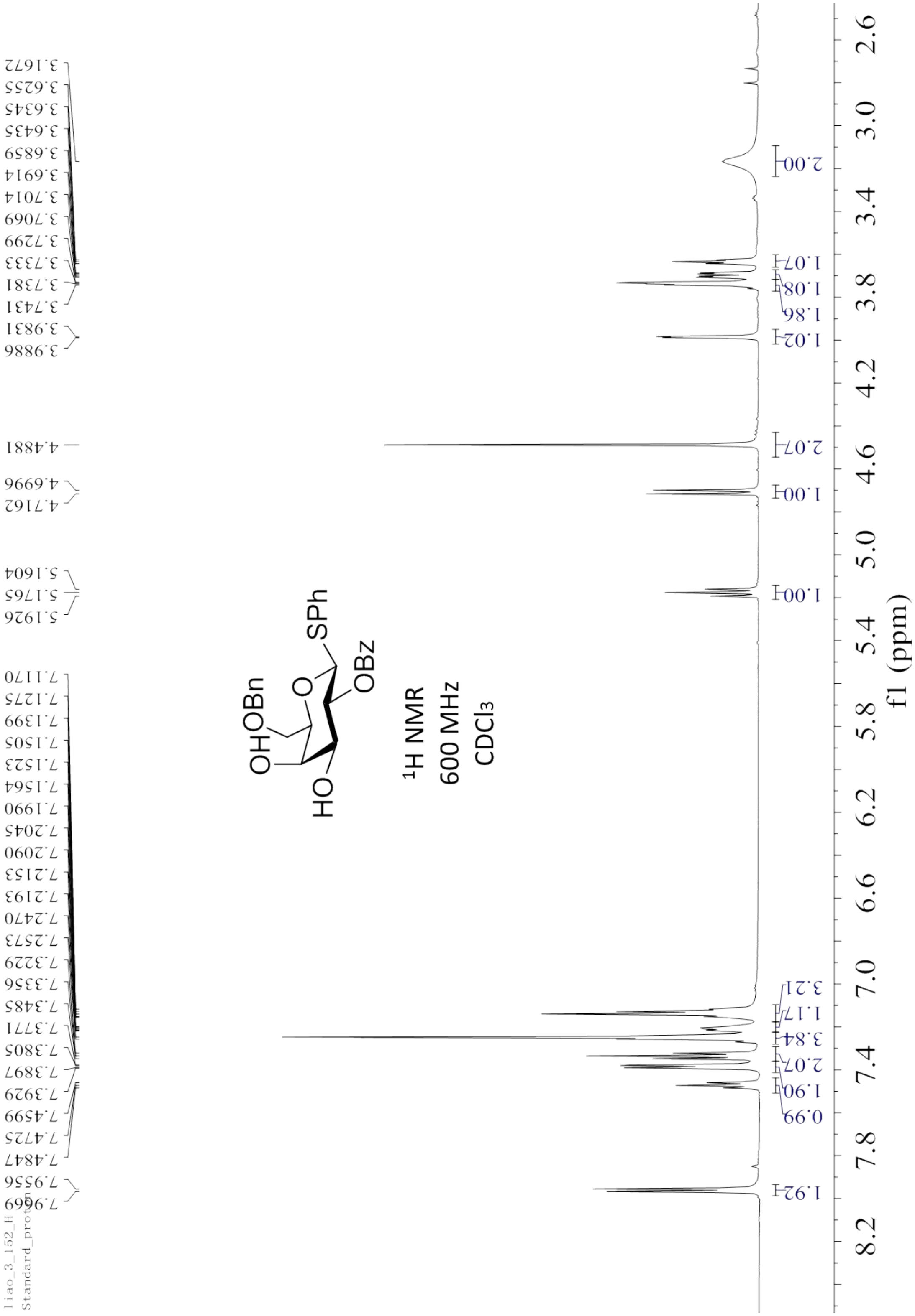


1	14170.7	93.963	12.3
2	11536.3	76.495	18.0
3	11181.8	74.144	-27.2
4	11123.4	73.757	23.9
5	11081.9	73.482	-30.9
6	10862.0	72.023	24.6
7	10405.9	68.999	-26.9
8	10250.5	67.969	-15.9
9	10227.5	67.816	-13.3
10	10221.4	67.775	-16.2
11	8413.3	55.787	13.5
12	5678.5	37.653	-31.7
13	4794.4	31.790	-13.3
14	4487.0	29.752	18.0
15	4203.7	27.874	-28.5
16	2783.3	18.455	-21.8
17	2755.8	18.273	-14.9
18	2037.1	13.508	16.2
19	2018.6	13.385	14.5

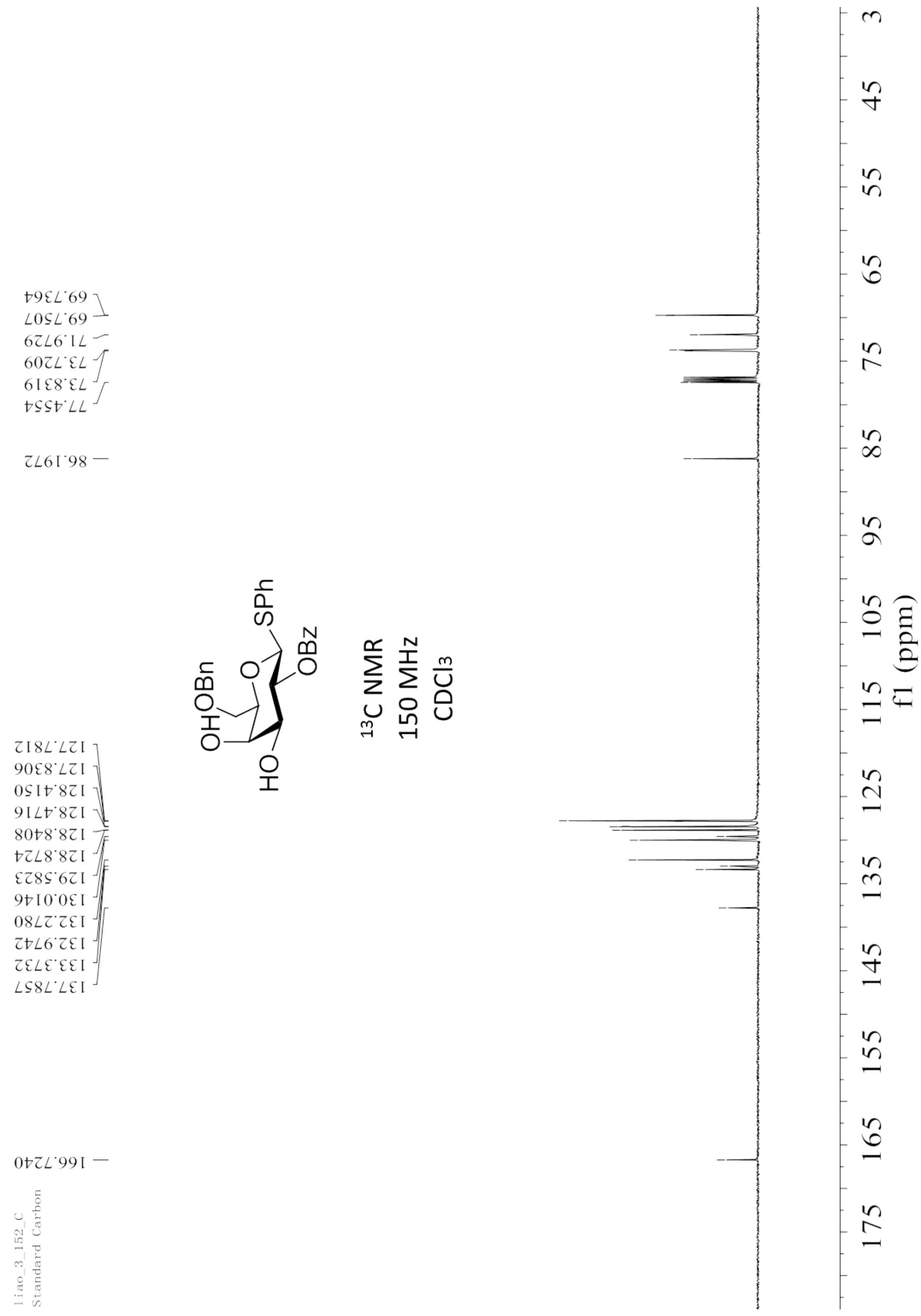
^{1}H-^{13}C HMQC Spectrum of compound **9** (CDCl$_3$, 600/150 MHz)



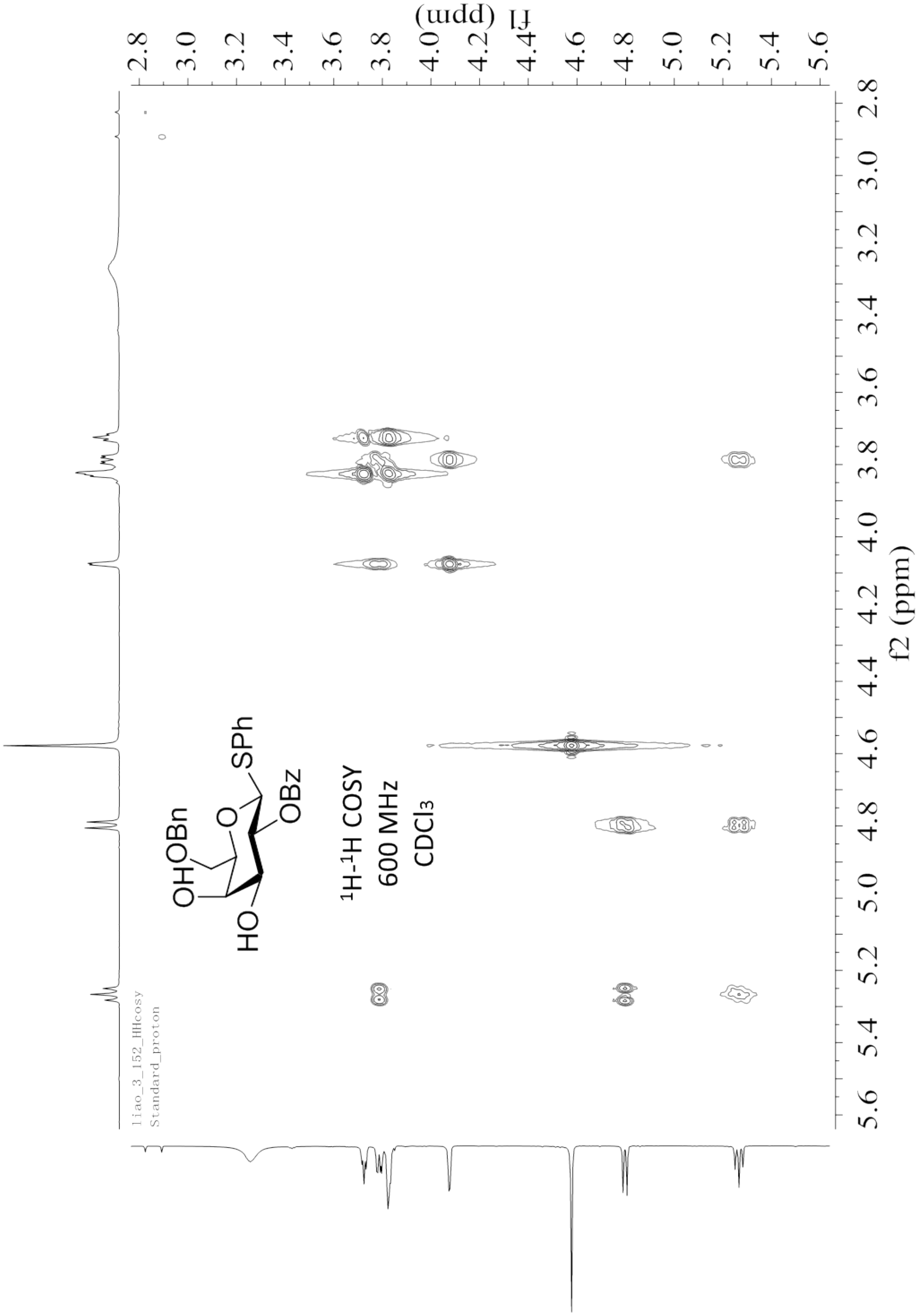
^{31}P NMR Spectrum of compound **9** ($CDCl_3$, 400 MHz)



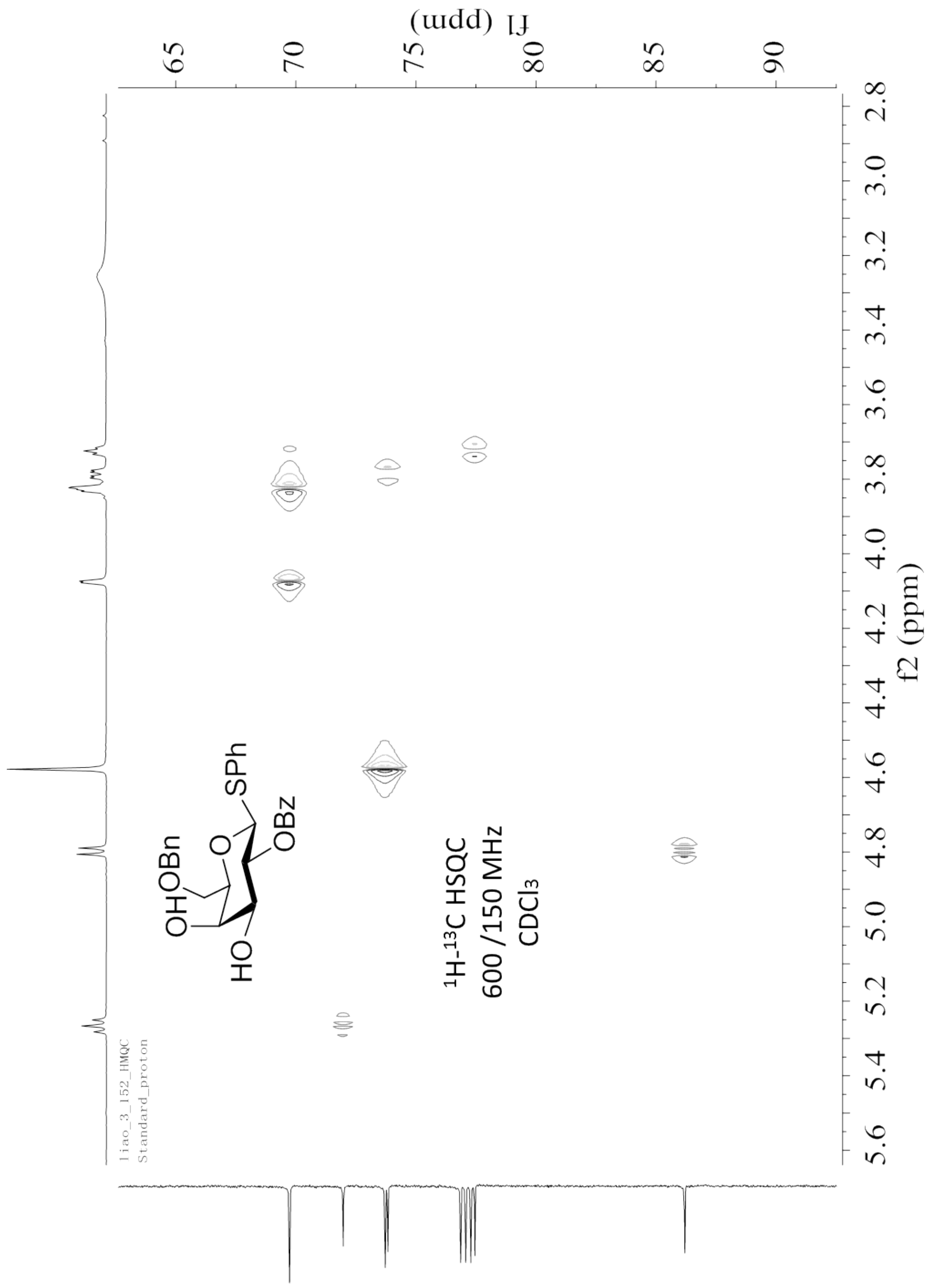
^{1}H NMR Spectrum of compound **10** (CDCl$_3$, 600 MHz)



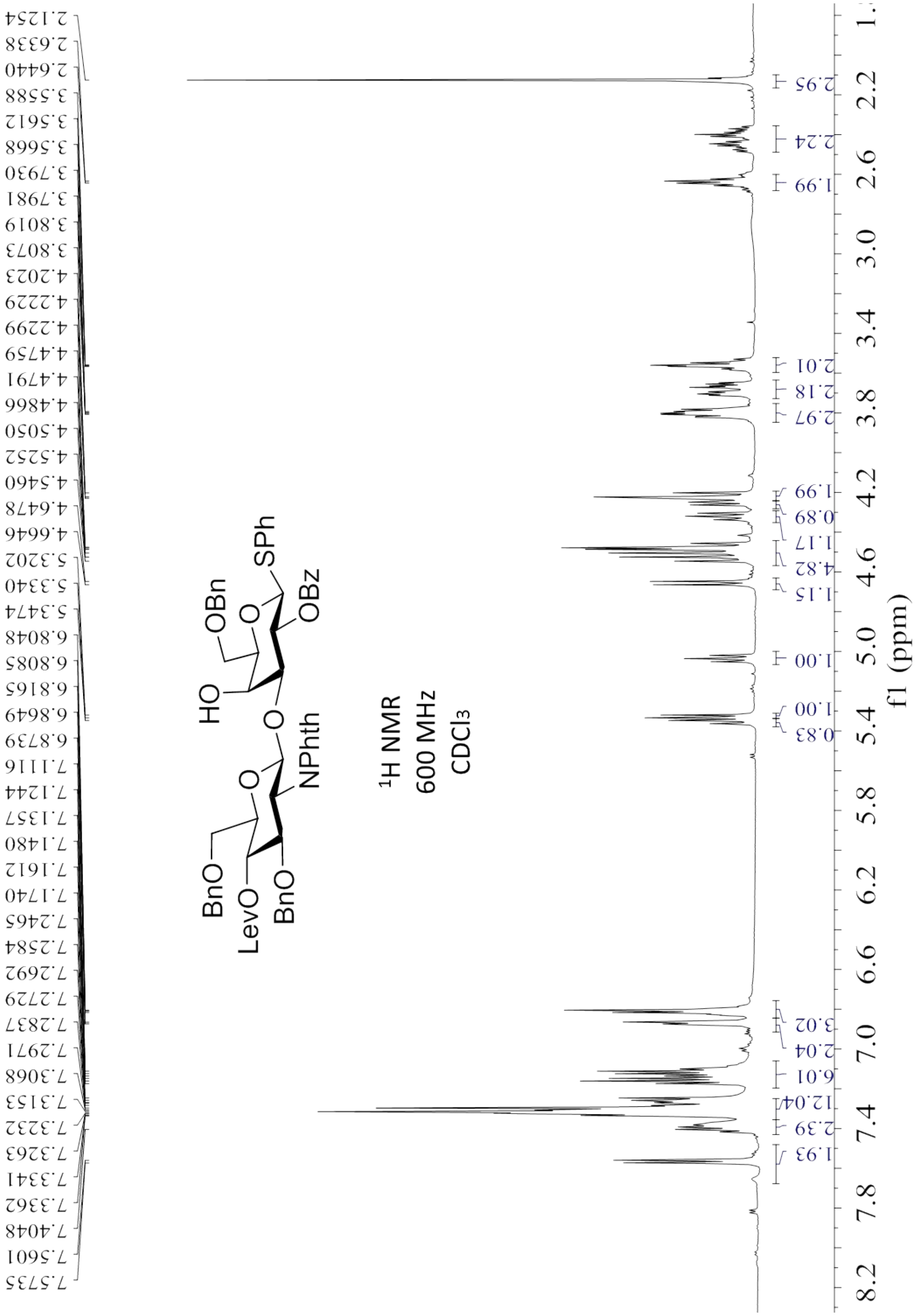
^{13}C NMR Spectrum of compound **10** (CDCl$_3$, 150 MHz)



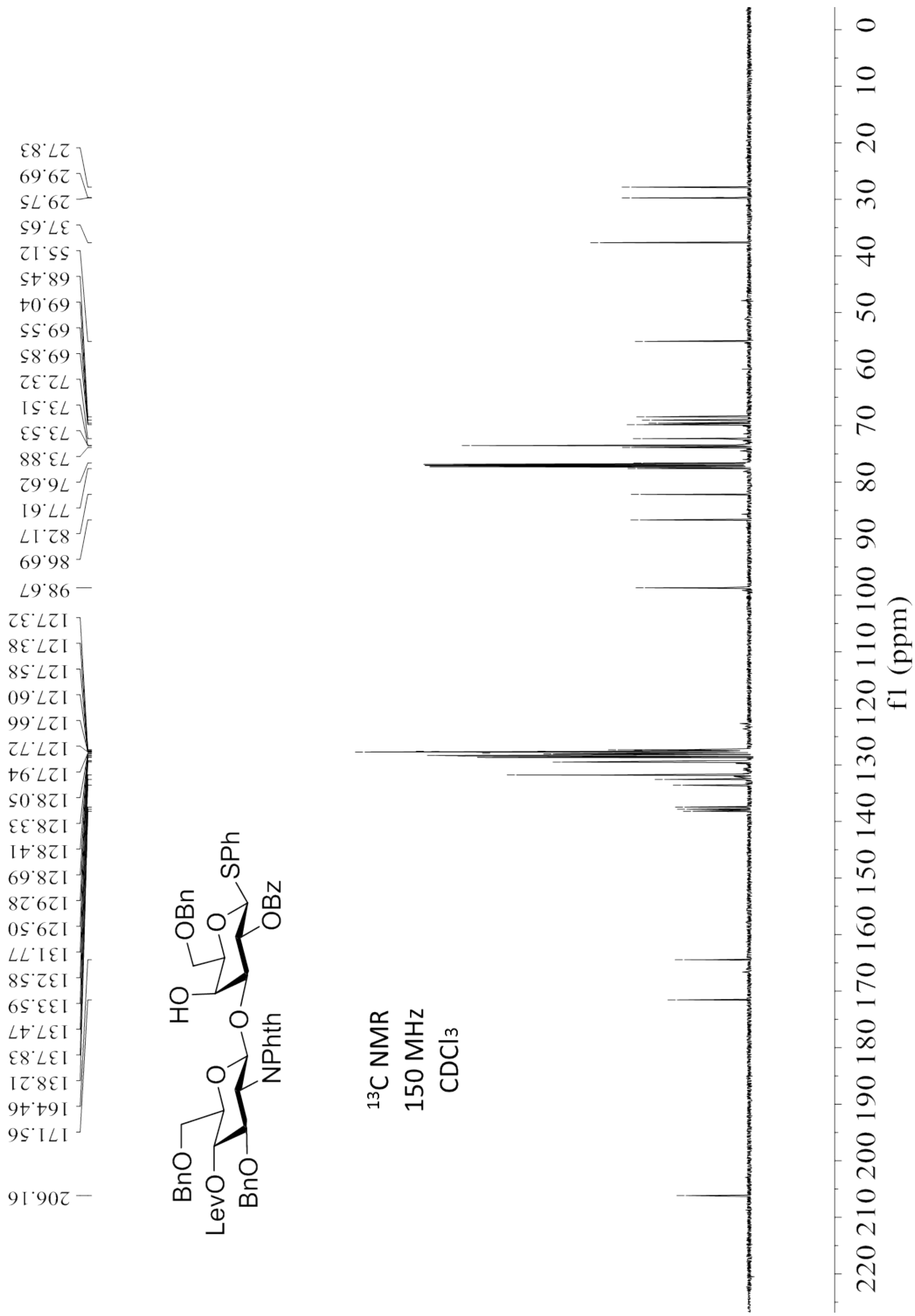
^{1}H-^{1}H COSY Spectrum of compound **10** **(**CDCl$_3$, 600 MHz)



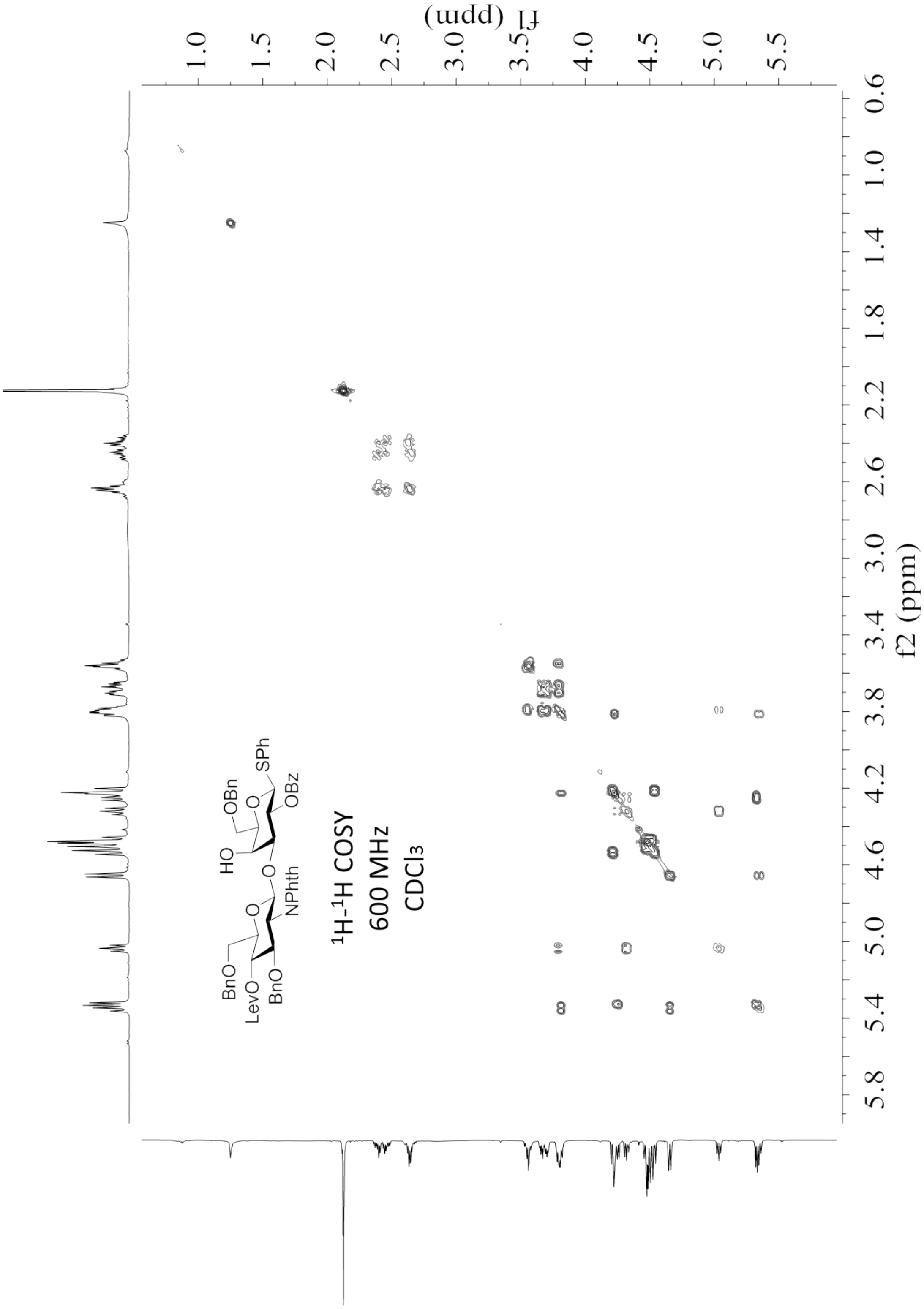
^{1}H-^{13}C HMQC Spectrum of compound **10** (CDCl$_3$, 600/150 MHz)



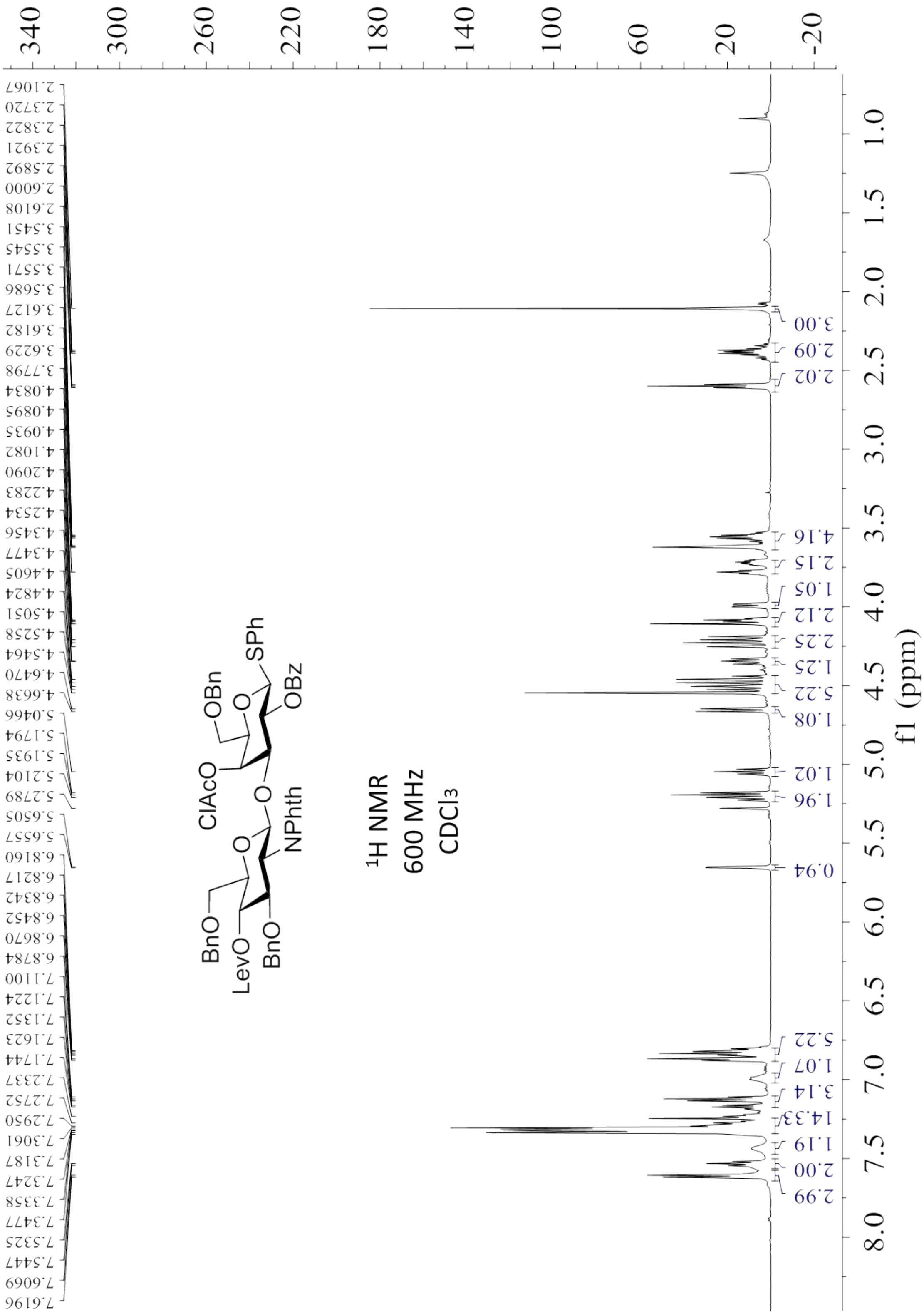
^{1}H NMR Spectrum of compound **11** ($CDCl_3$, 600 MHz)



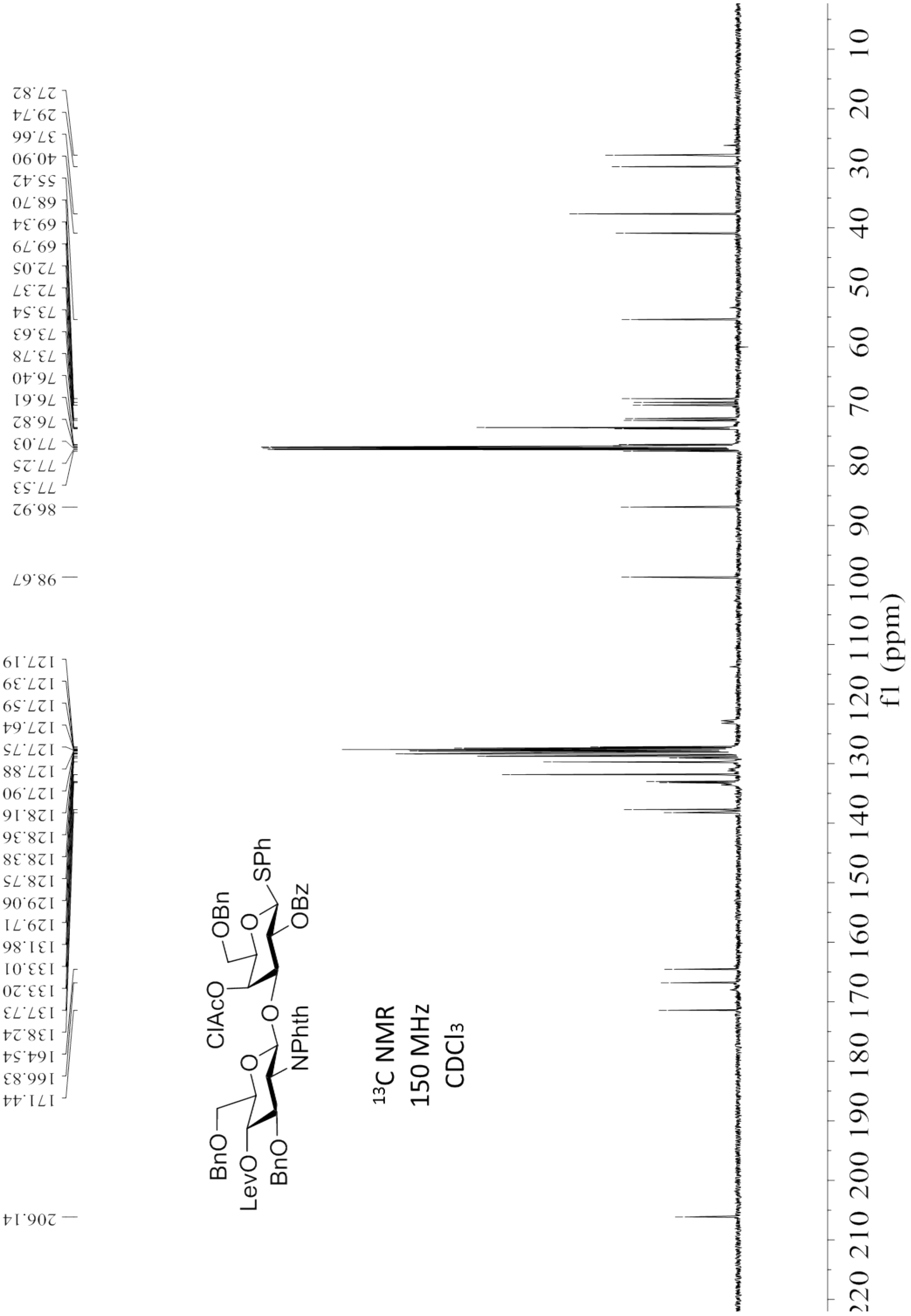
^{13}C NMR Spectrum of compound **11** ($CDCl_3$, 150 MHz)



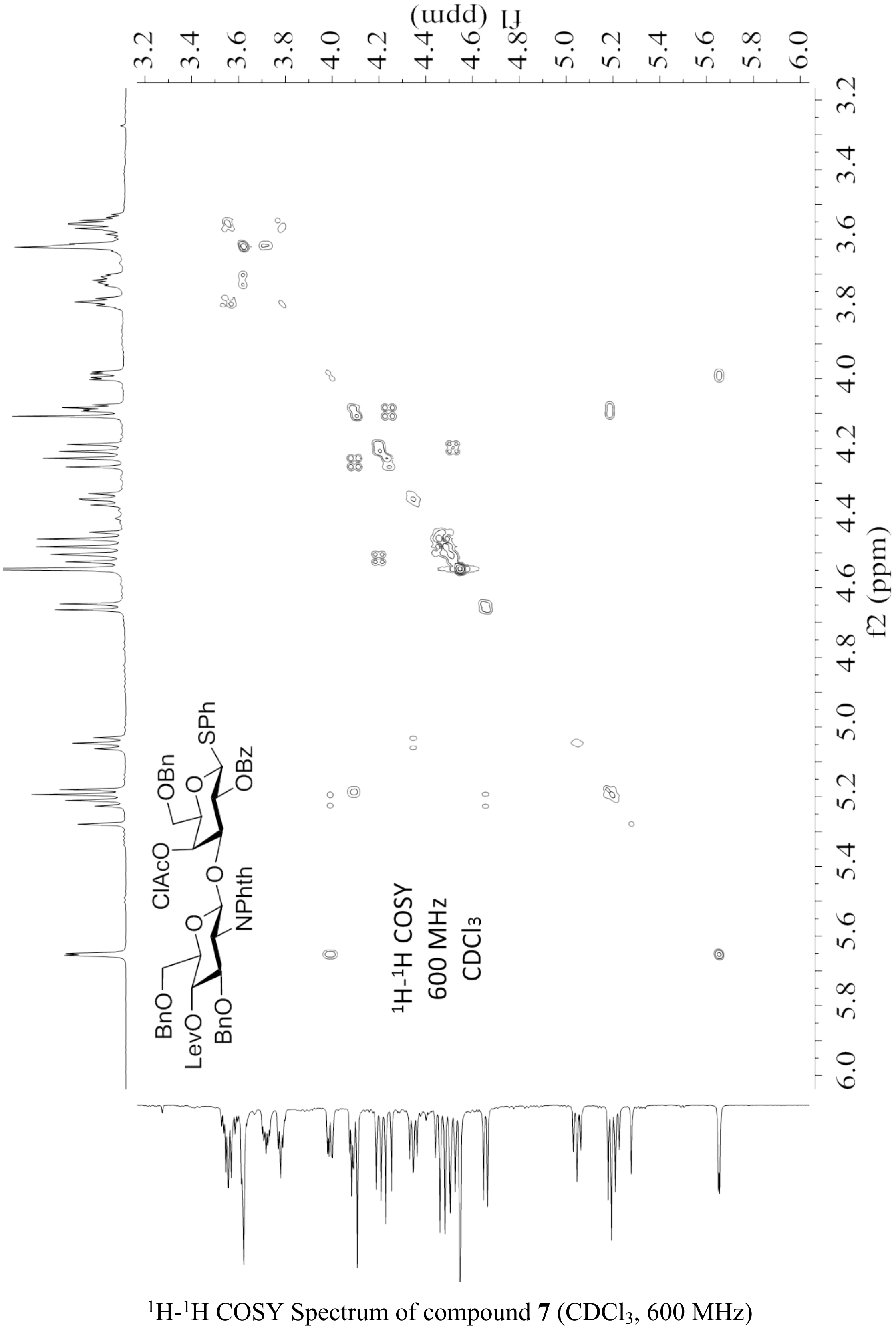
^{1}H-^{1}H COSY Spectrum of compound **11** ($CDCl_3$, 600 MHz)



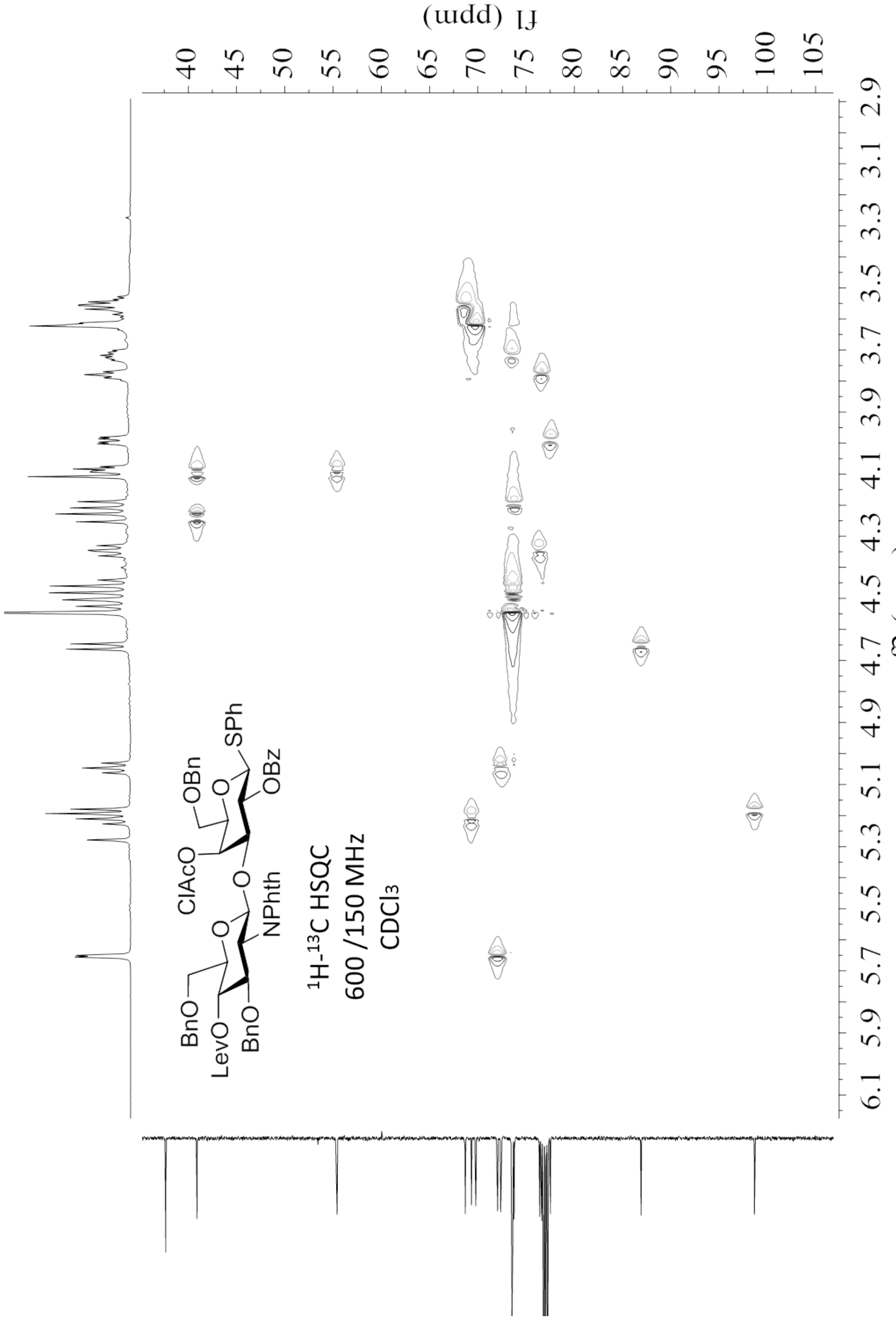
^{1}H NMR Spectrum of compound **7** (CDCl$_3$, 600 MHz)



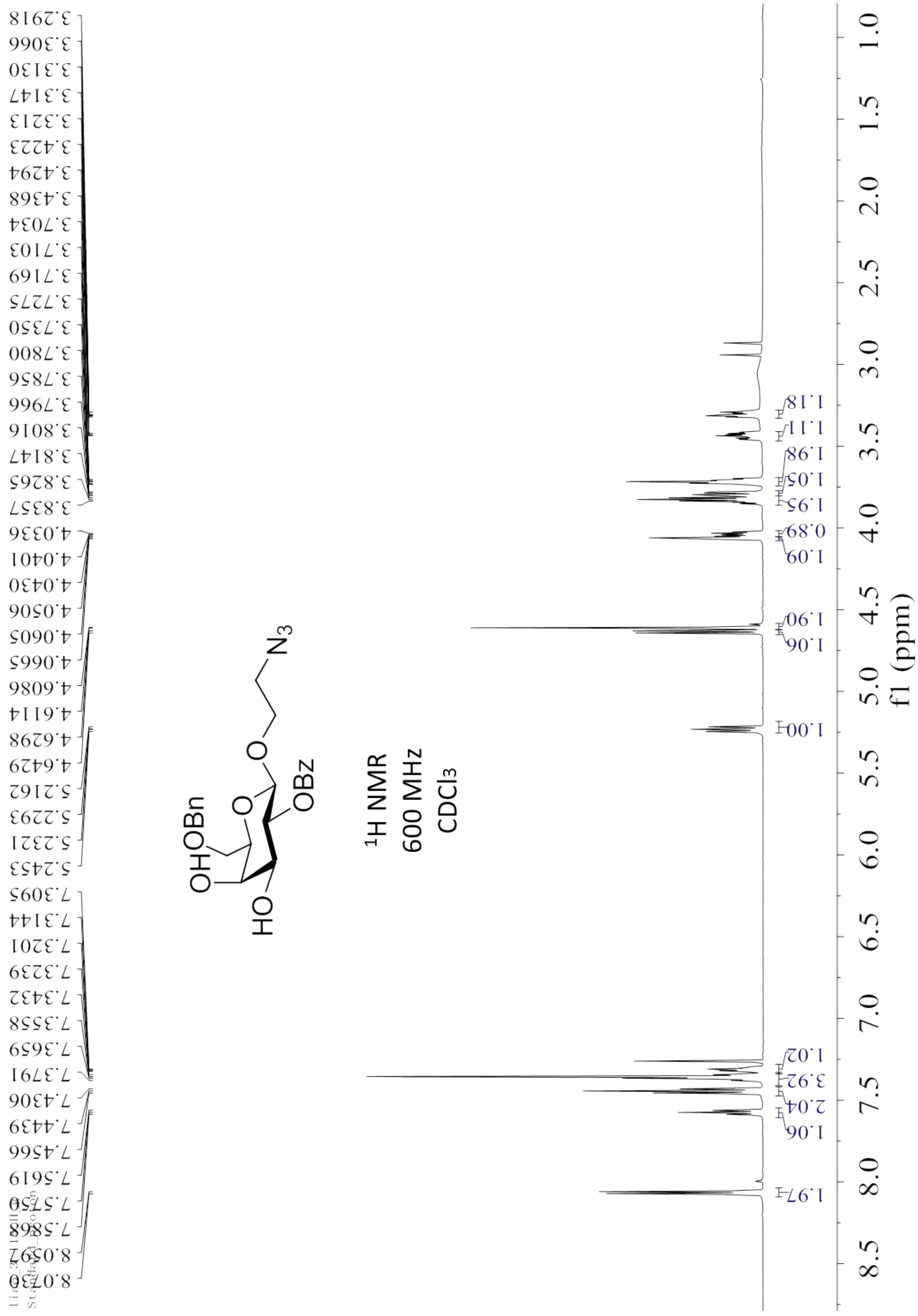
^{13}C NMR Spectrum of compound **7** ($CDCl_3$, 150 MHz)



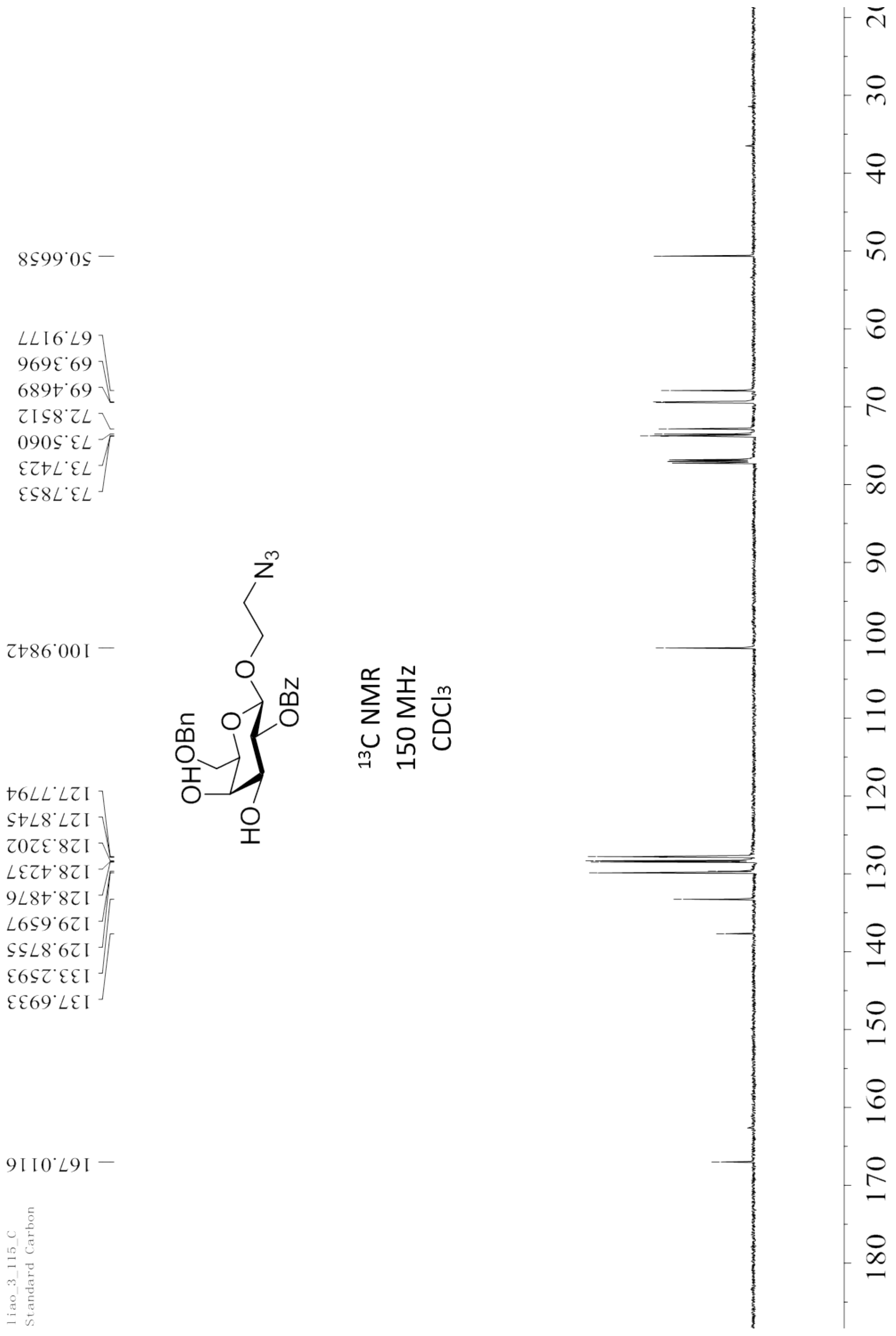
^{1}H-^{1}H COSY Spectrum of compound **7** (CDCl$_3$, 600 MHz)



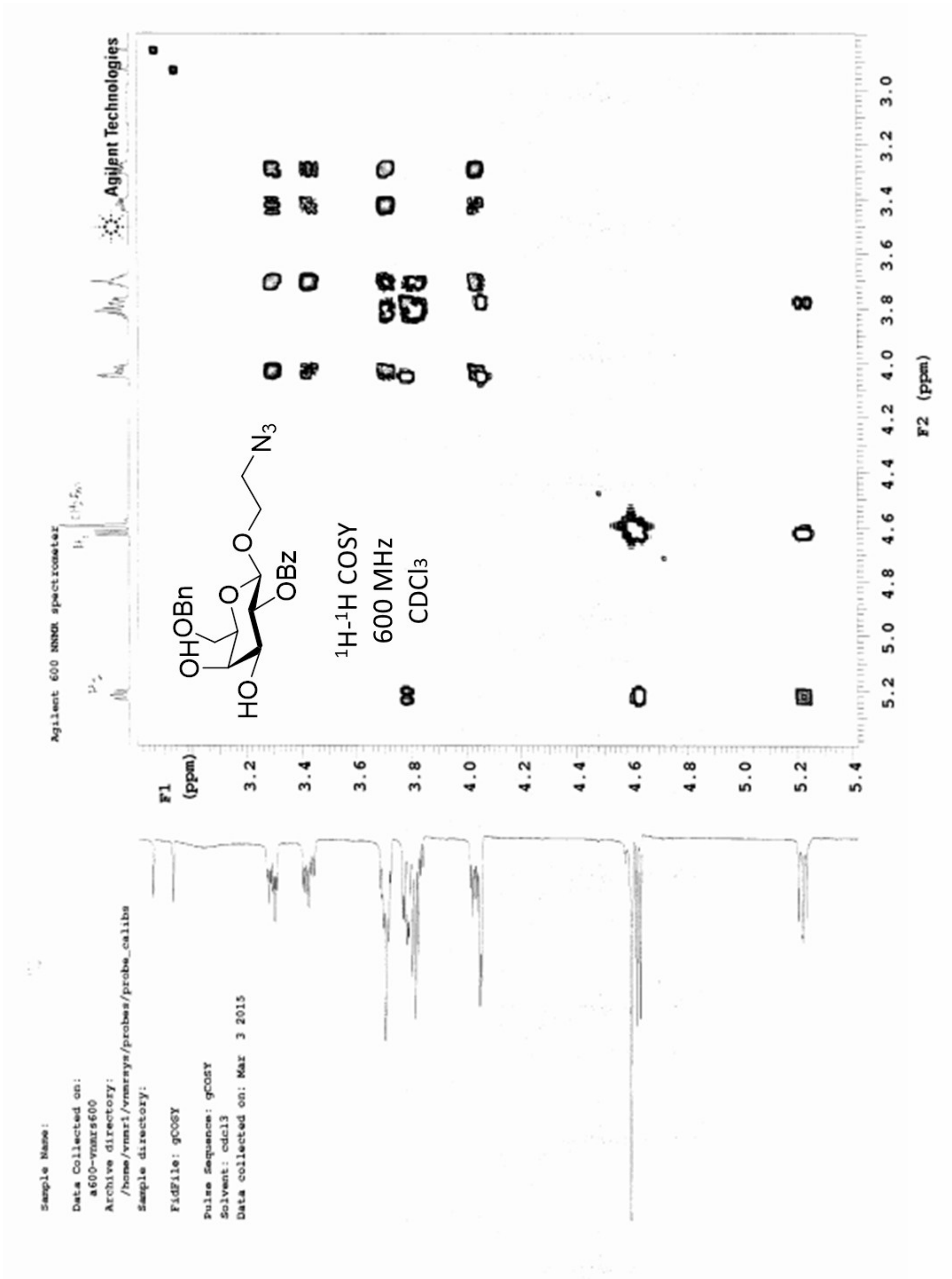
^{1}H-^{13}C HMQC Spectrum of compound **7** (CDCl$_3$, 600/150 MHz)



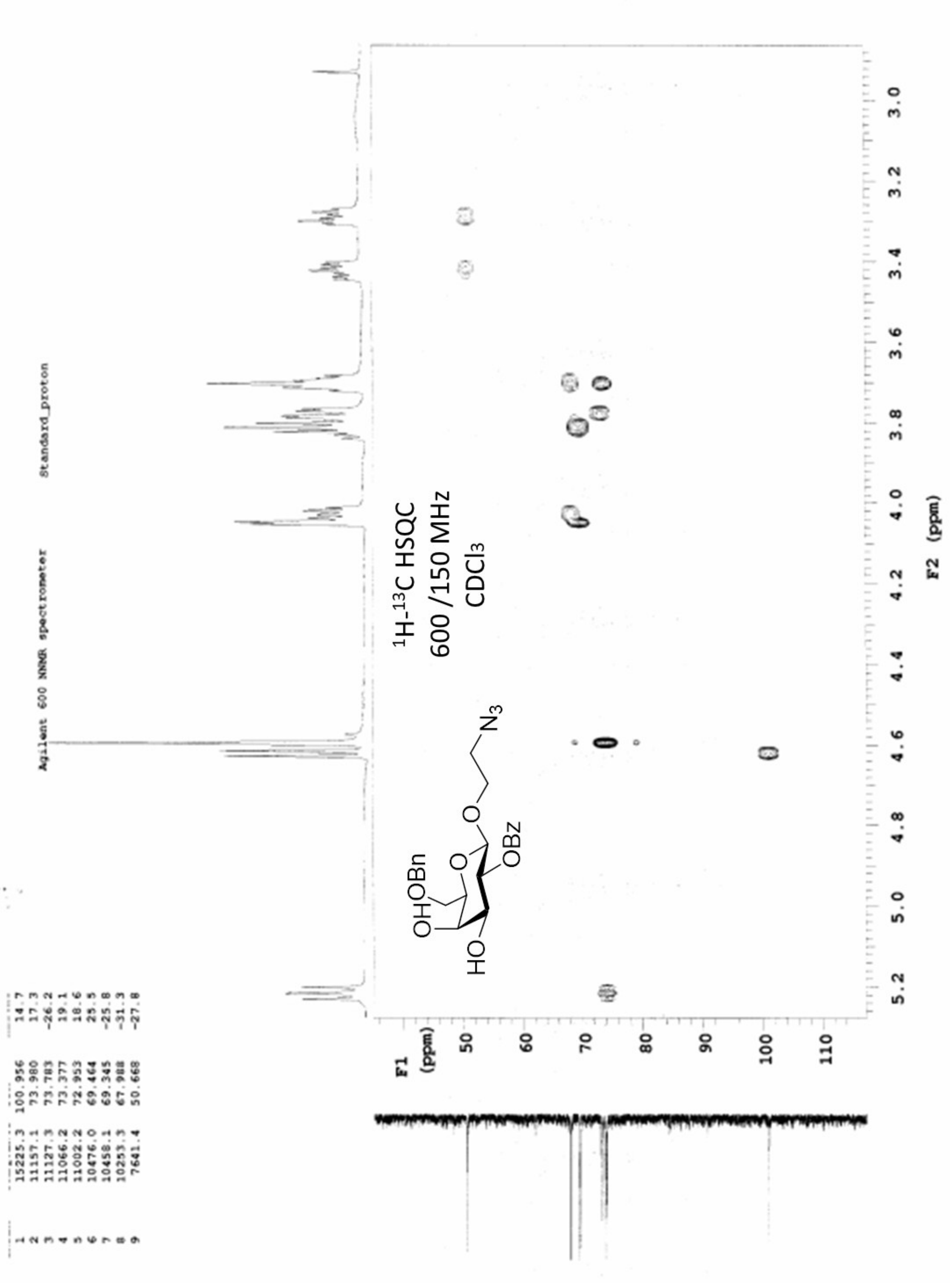
^{1}H NMR Spectrum of compound **12** (CDCl$_3$, 600 MHz)



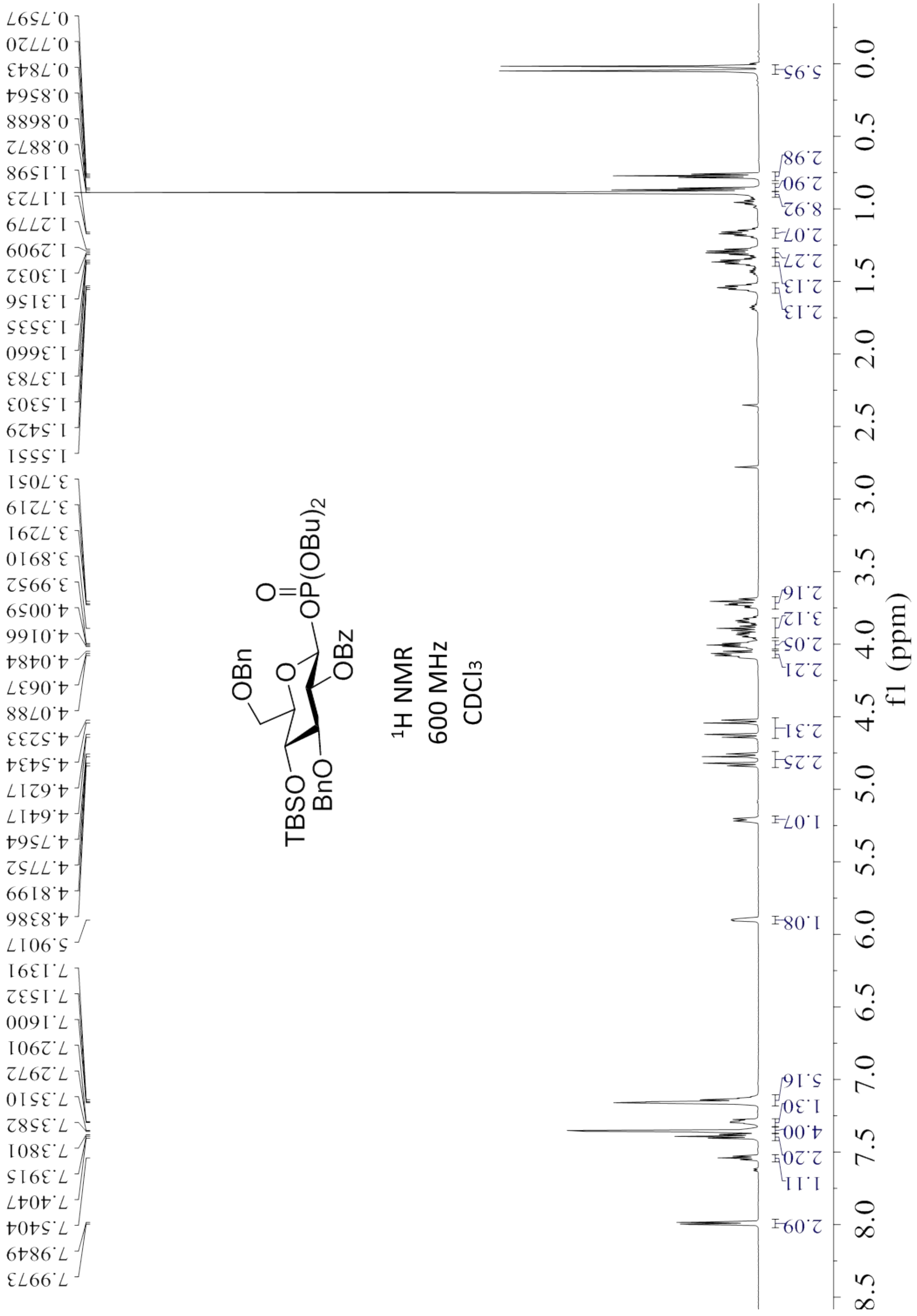
^{13}C NMR Spectrum of compound **12** ($CDCl_3$, 150 MHz)



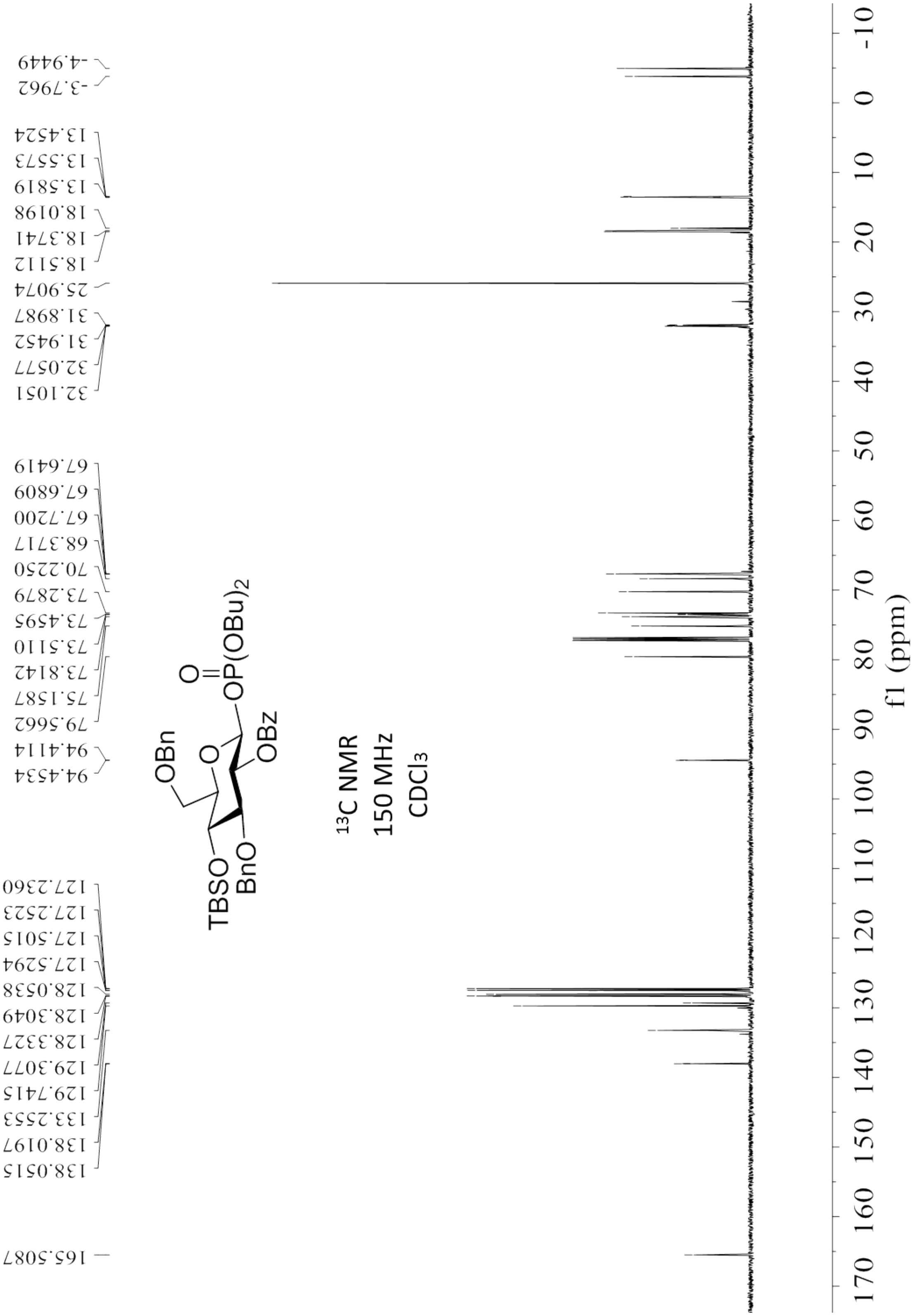
^{1}H-^{1}H COSY Spectrum of compound **12** ($CDCl_3$, 600 MHz)



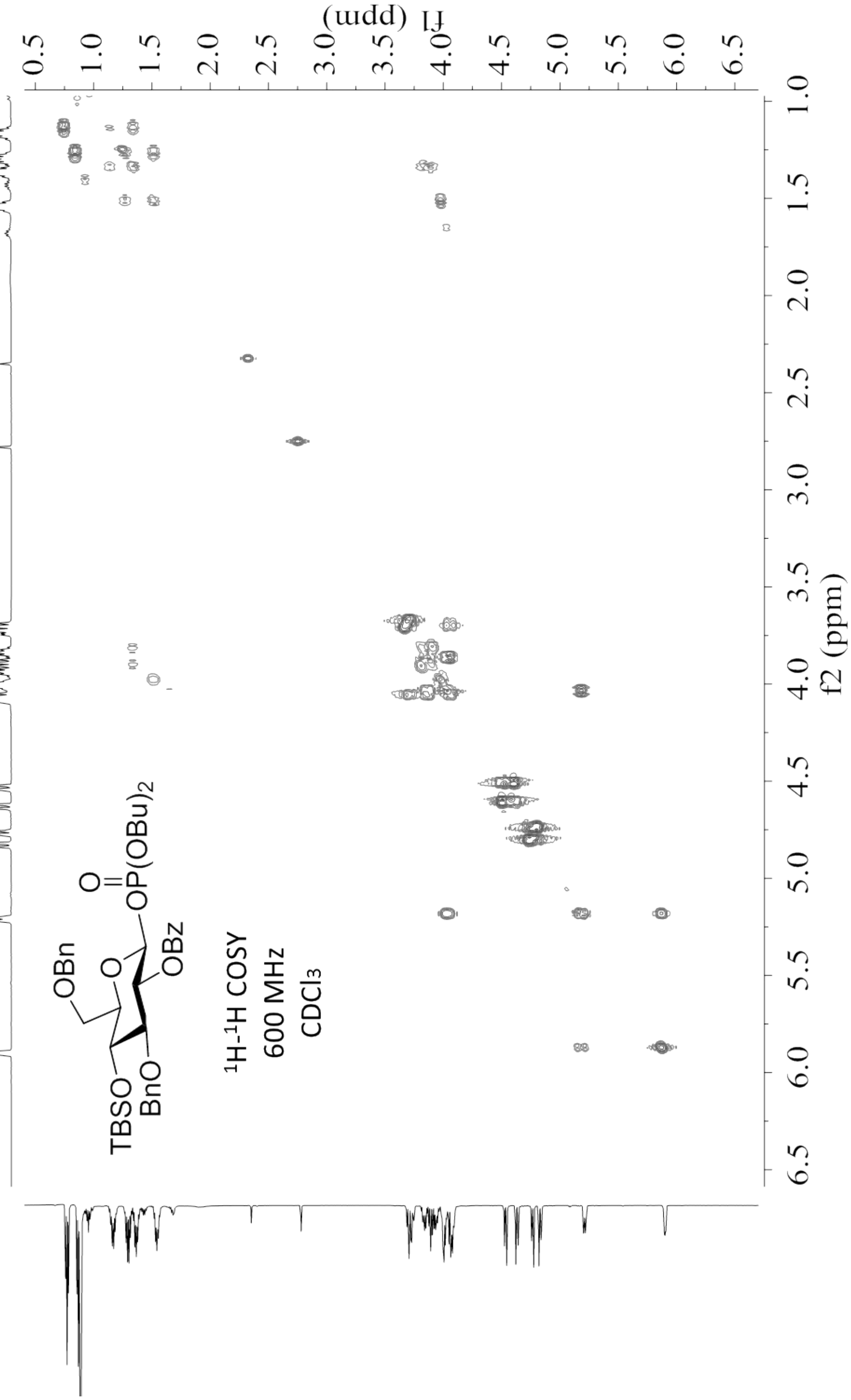
^{1}H-^{13}C HMQC Spectrum of compound **12** (CDCl$_3$, 600/150 MHz)



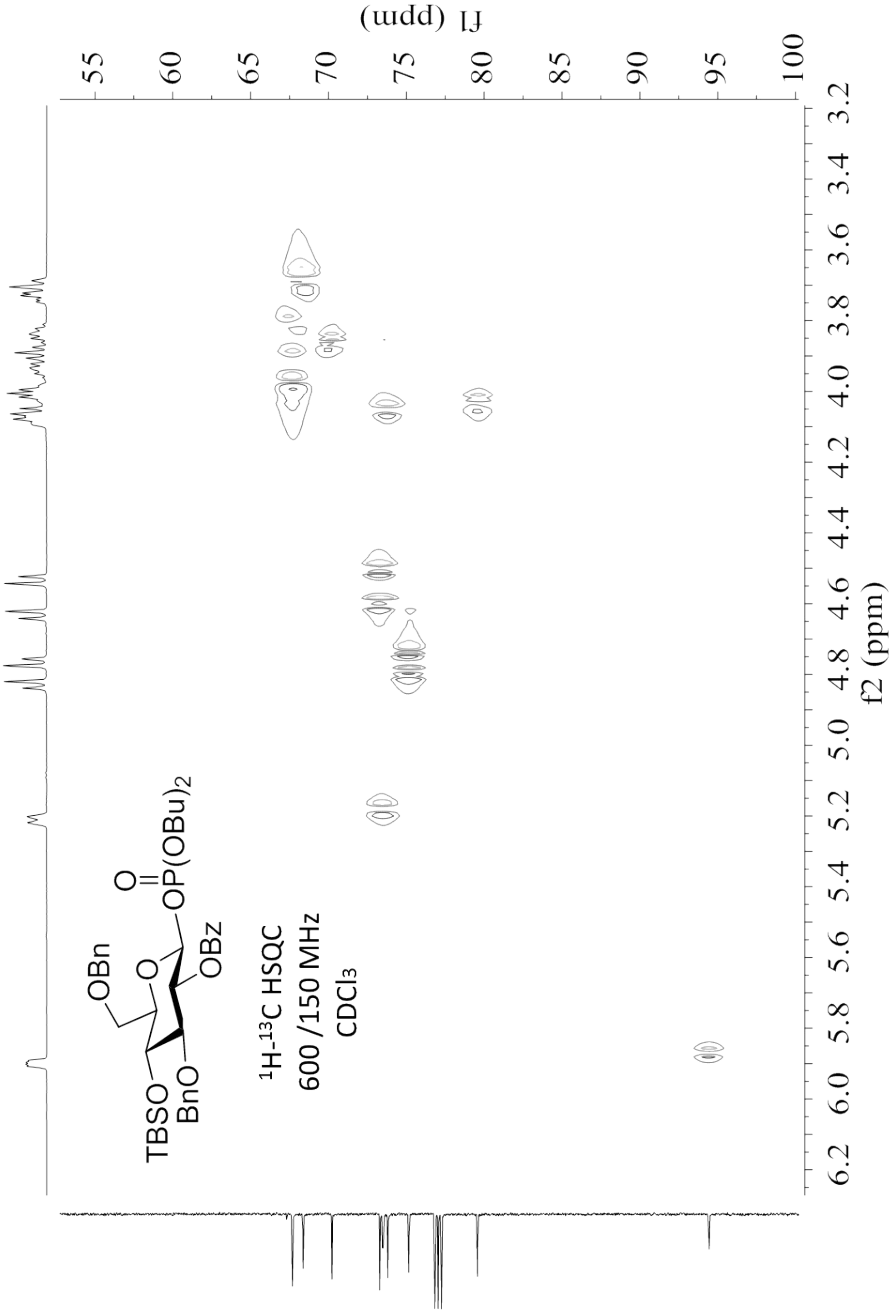
^{1}H NMR Spectrum of compound **13** ($CDCl_3$, 600 MHz)



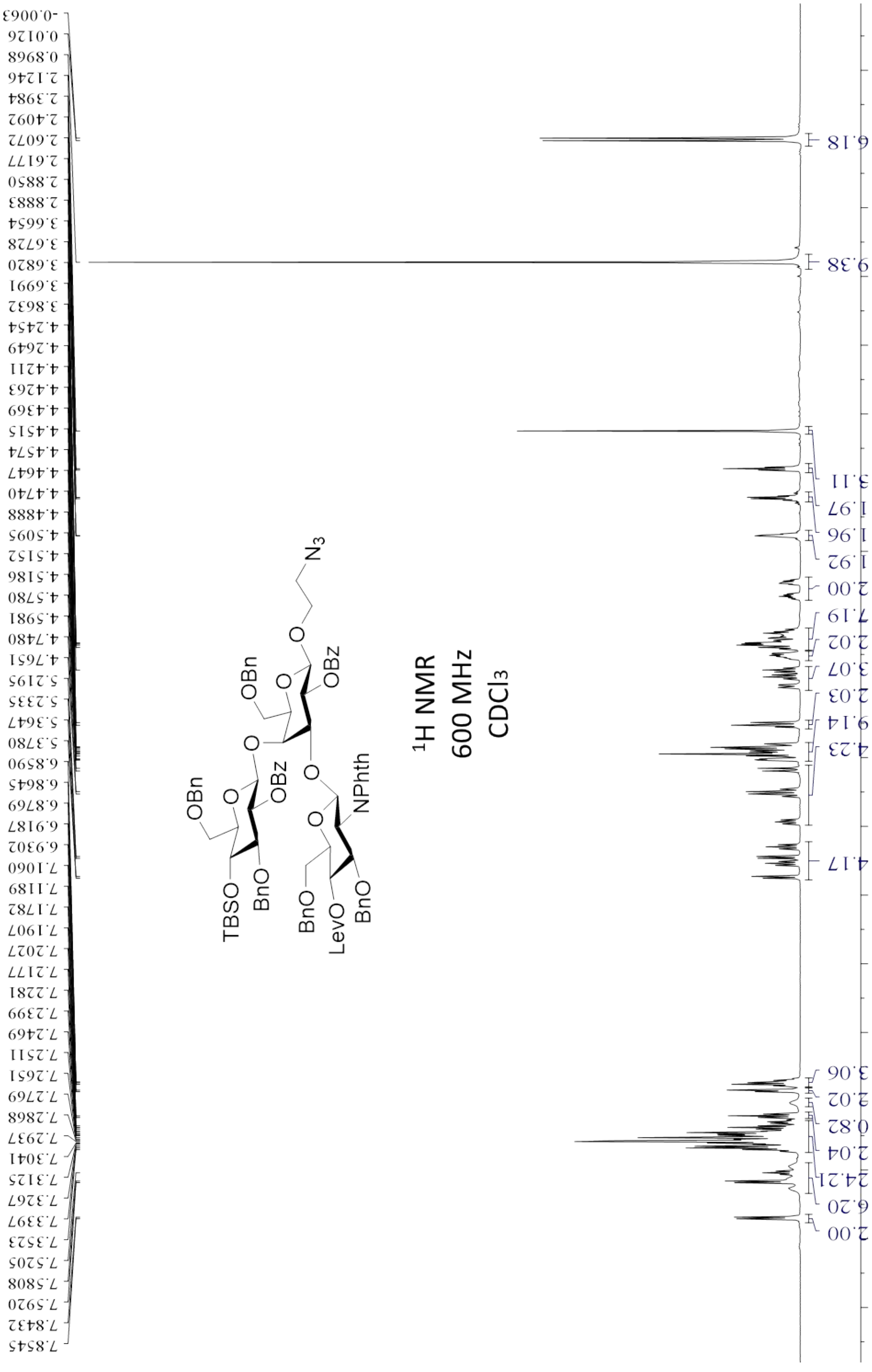
^{13}C NMR Spectrum of compound **13** ($CDCl_3$, 150 MHz)



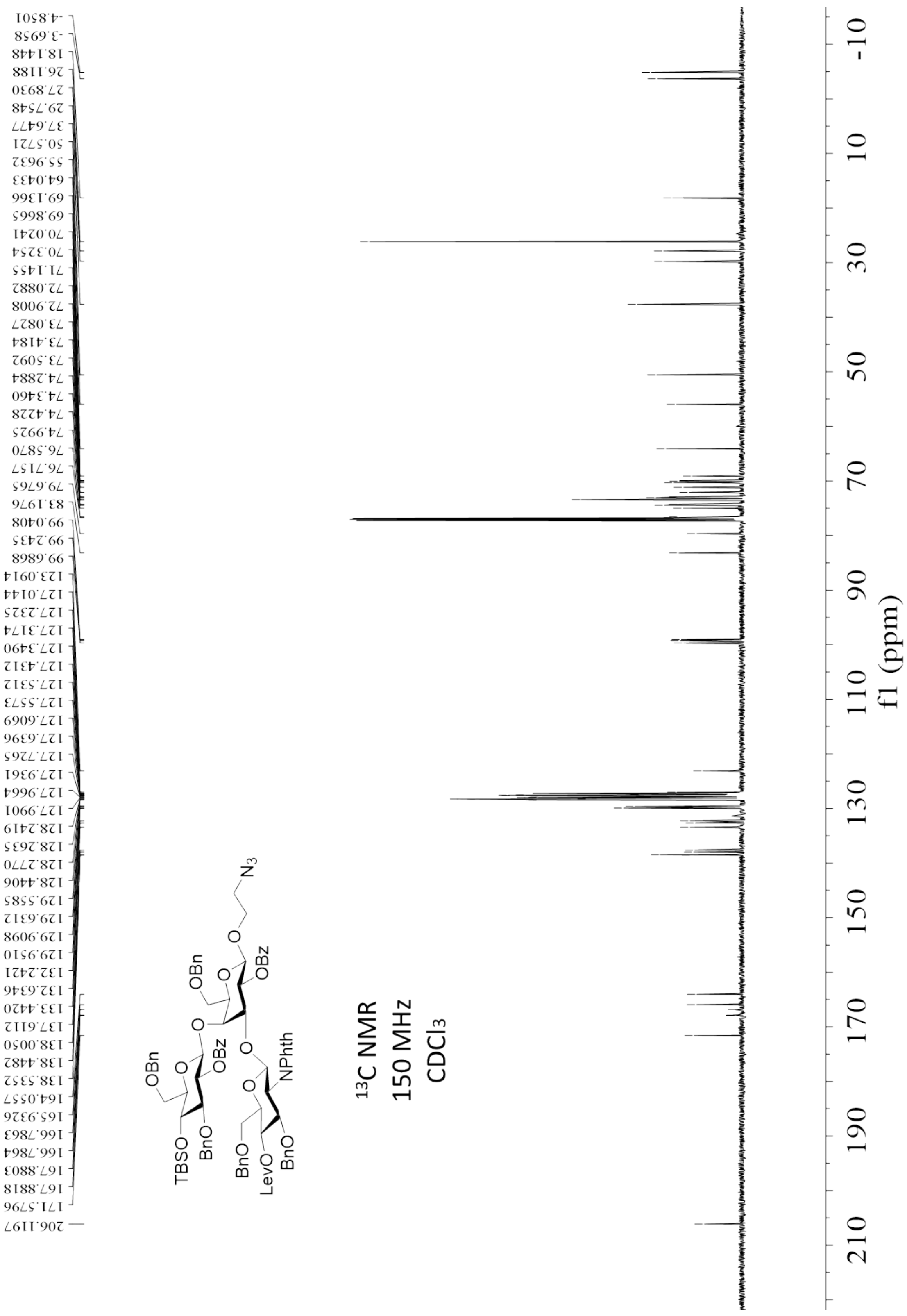
^{1}H-^{1}H COSY Spectrum of compound **13** ($CDCl_3$, 600 MHz)



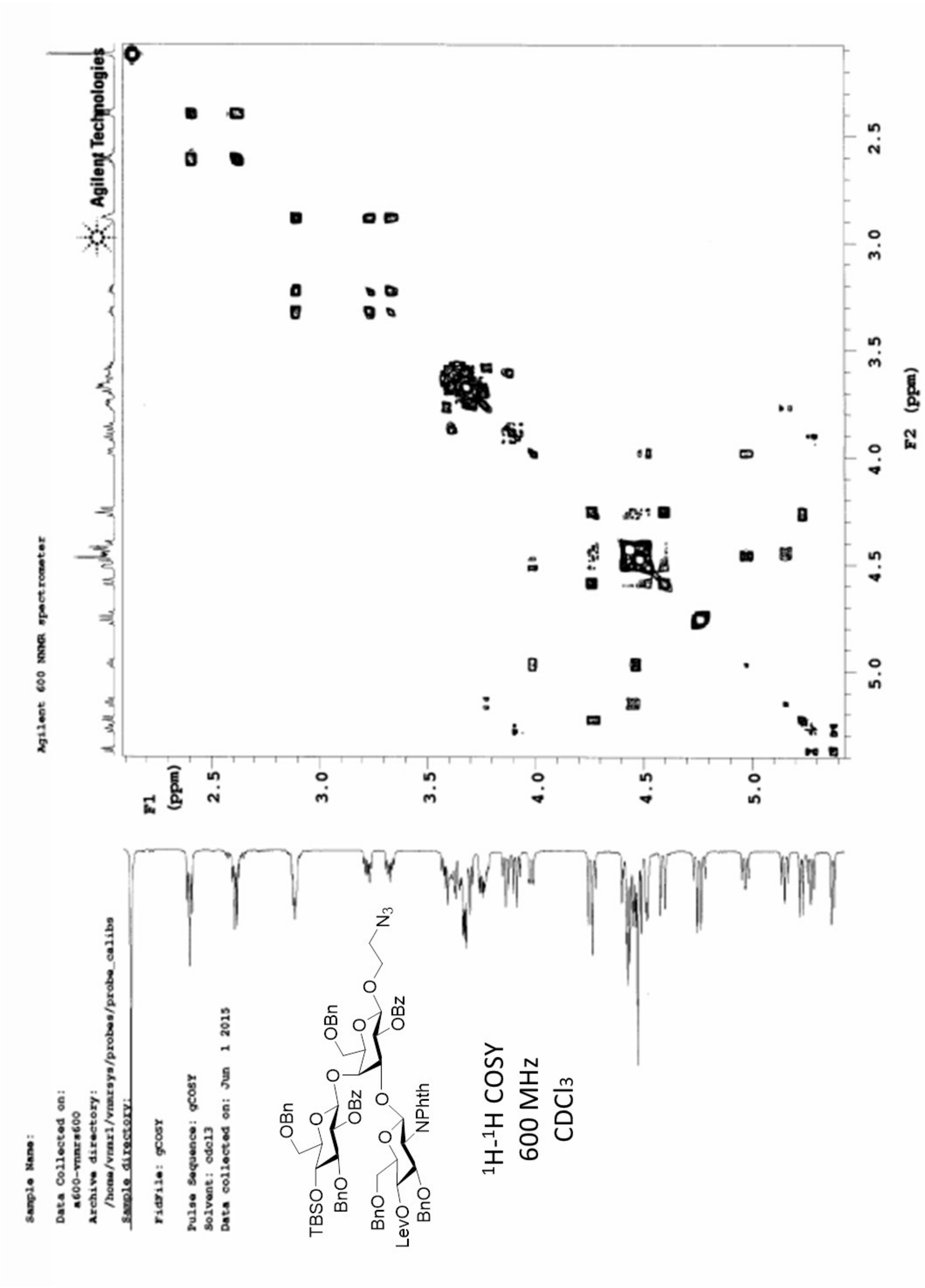
^{1}H-^{13}C HMQCSpectrum of compound **13** (CDCl$_3$, 600/150 MHz)



^{1}H NMR Spectrum of compound **14** ($CDCl_3$, 600 MHz)



^{13}C NMR Spectrum of compound **14** (CDCl$_3$, 150 MHz)



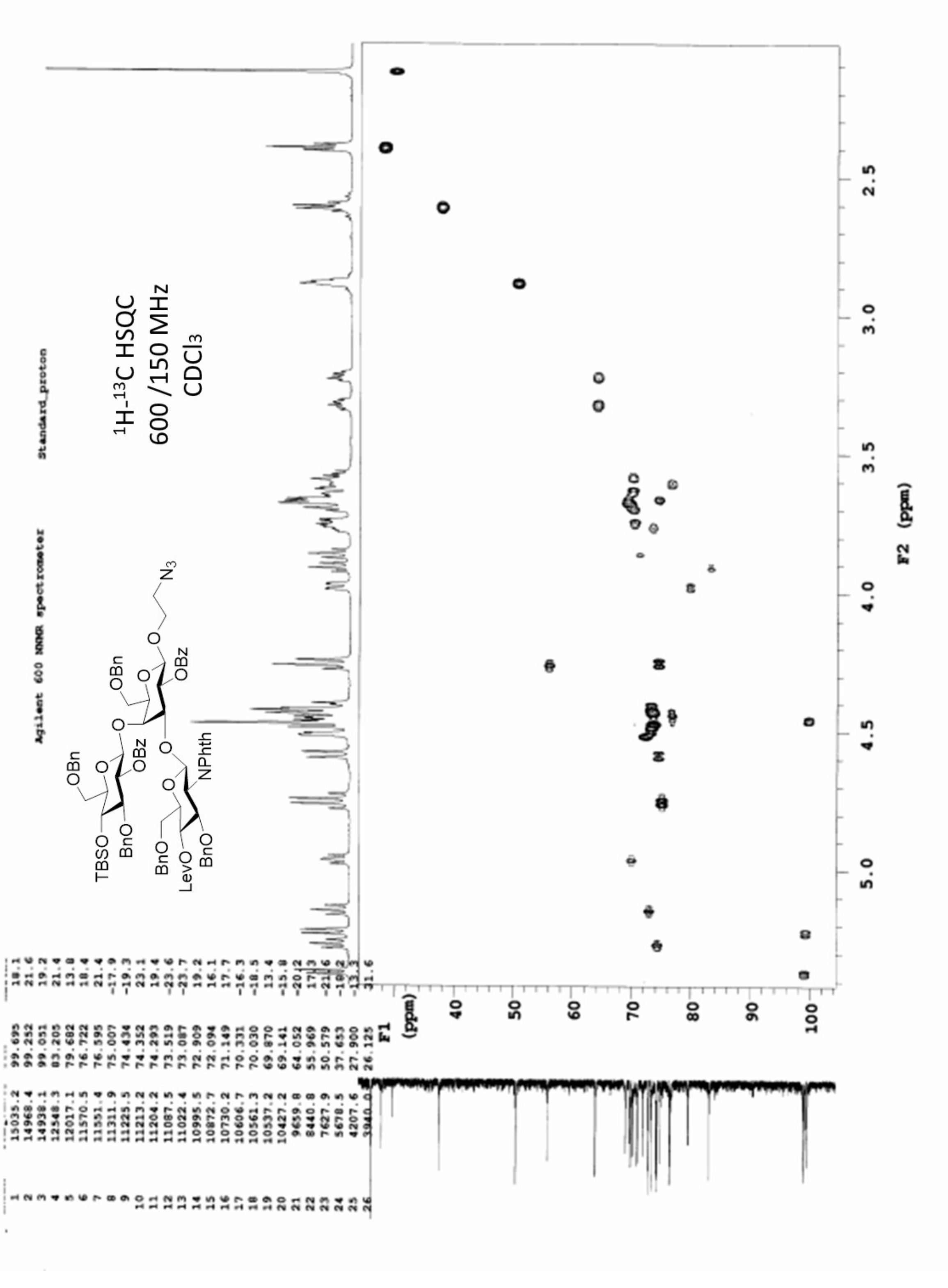
Sample Name:

Data Collected on:
a600-vnmrs600
Archive directory:
/home/vnmr1/vnmrsys/probes/probe_calibs
Sample directory:

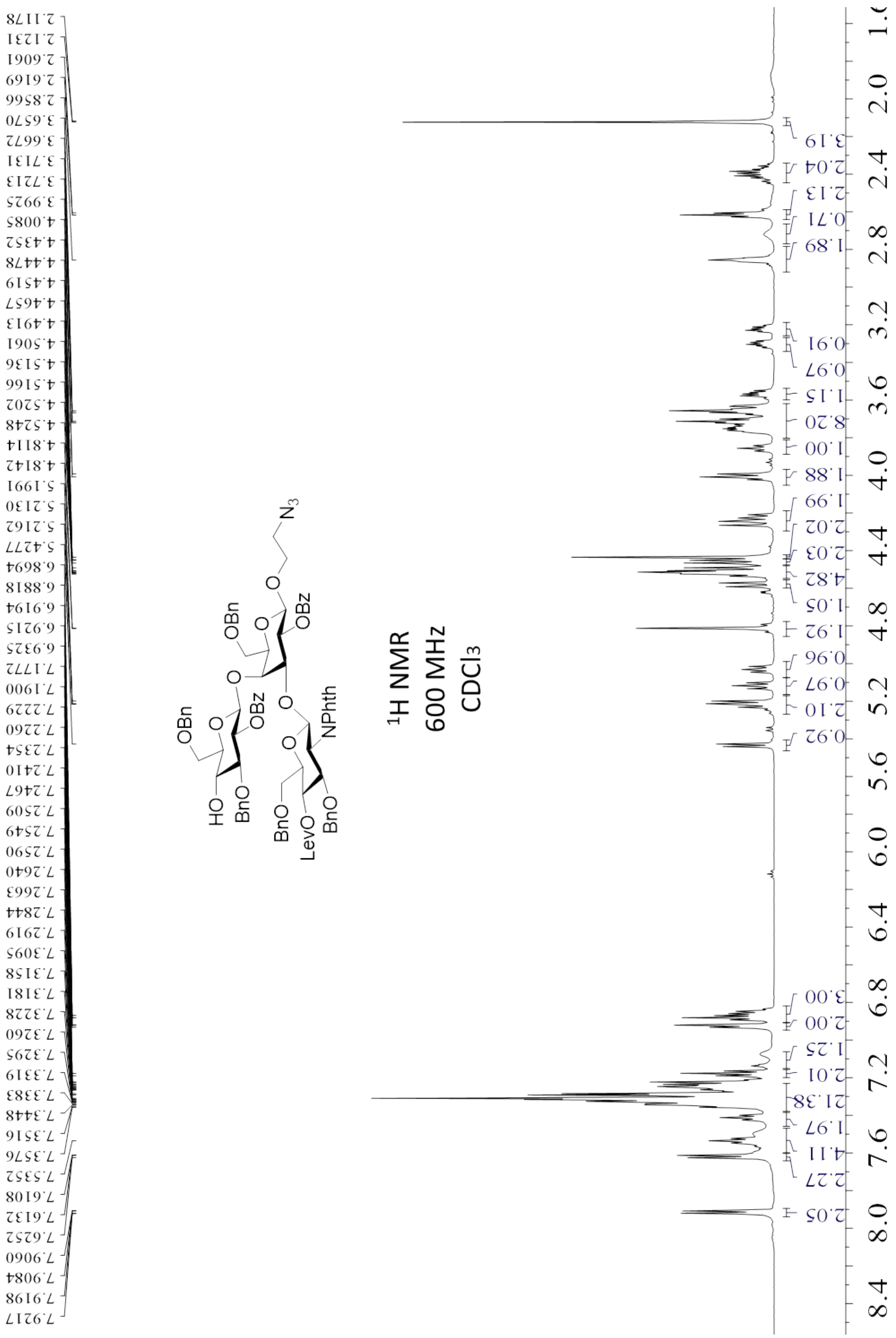
FidFile: gCOSY

Pulse Sequence: gCOSY
Solvent: cdcl3
Data collected on: Jun 1 2015

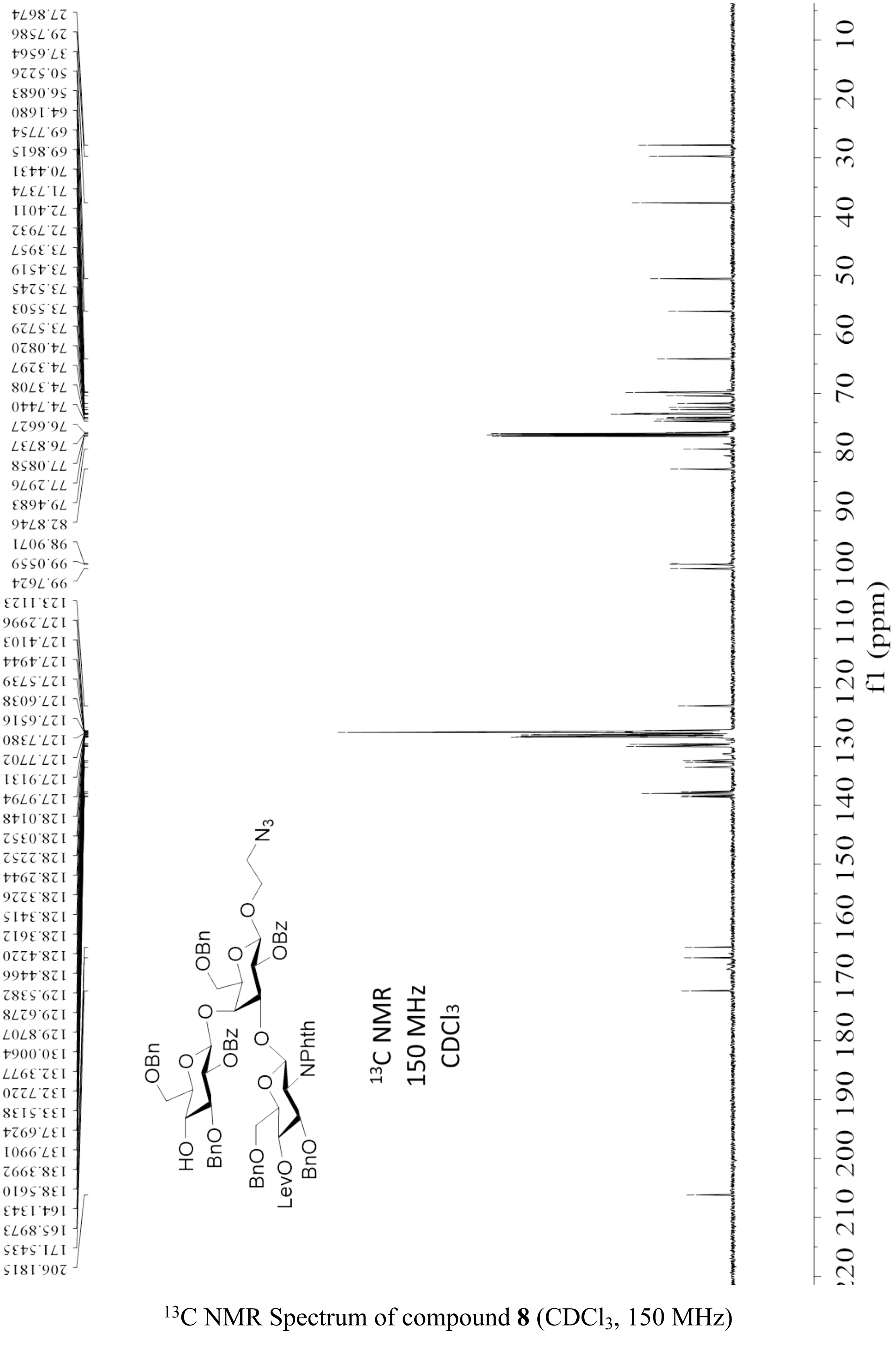
^1H-^1H COSY Spectrum of compound **14** ($CDCl_3$, 600 MHz)



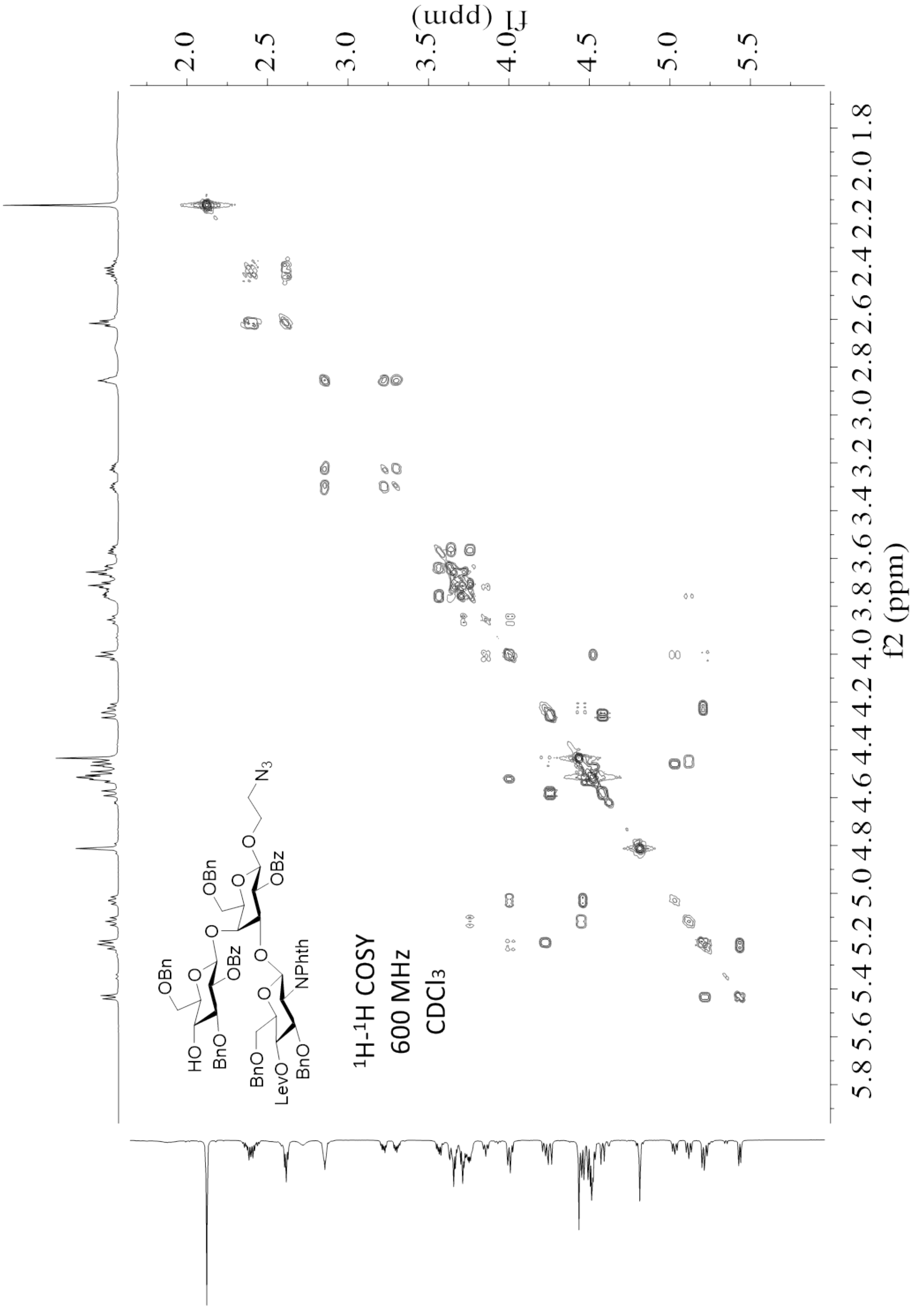
^{1}H-^{13}C HMQC Spectrum of compound **14** ($CDCl_3$, 600/150 MHz)



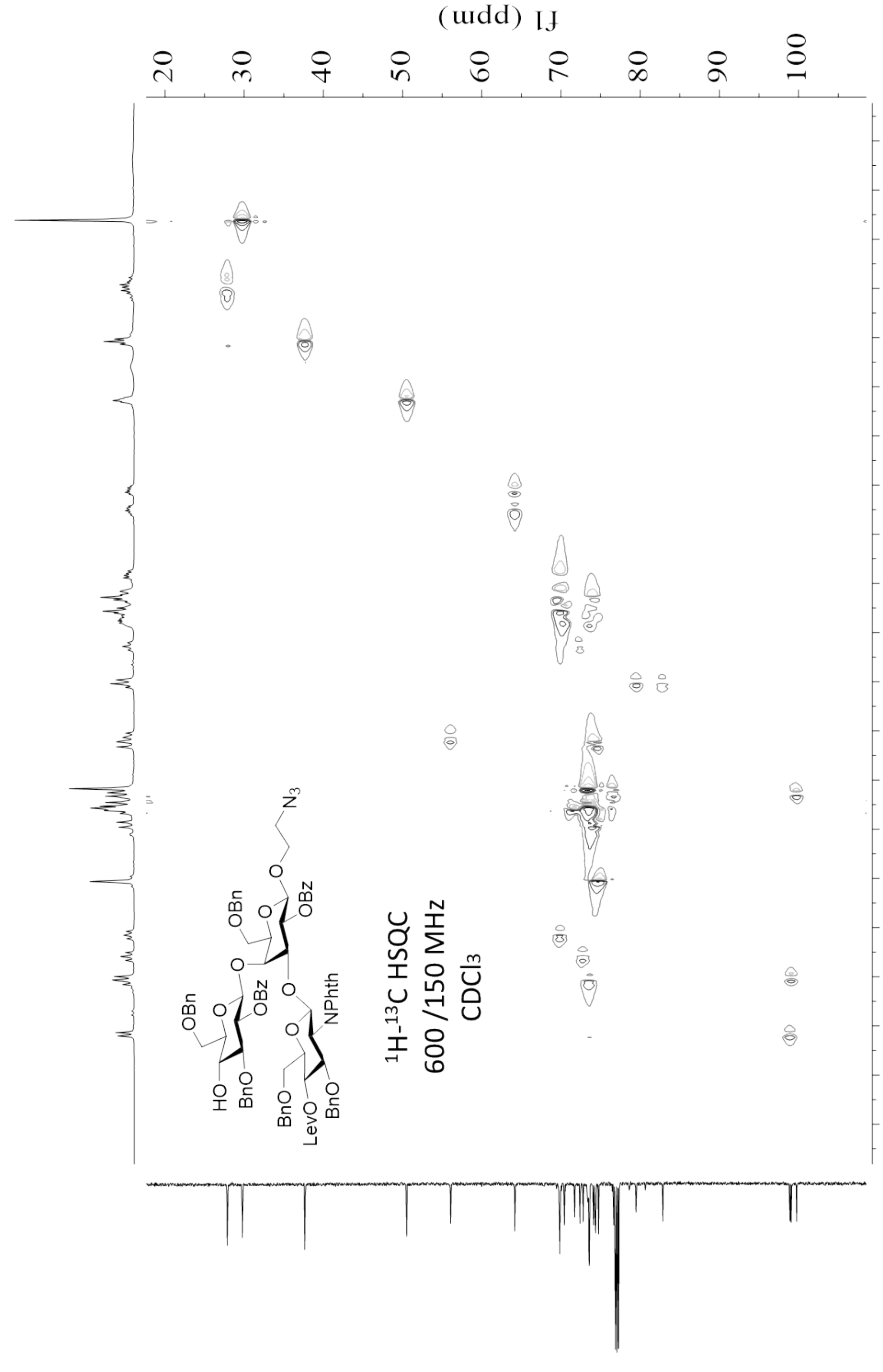
^{1}H NMR Spectrum of compound **8** ($CDCl_3$, 600 MHz)



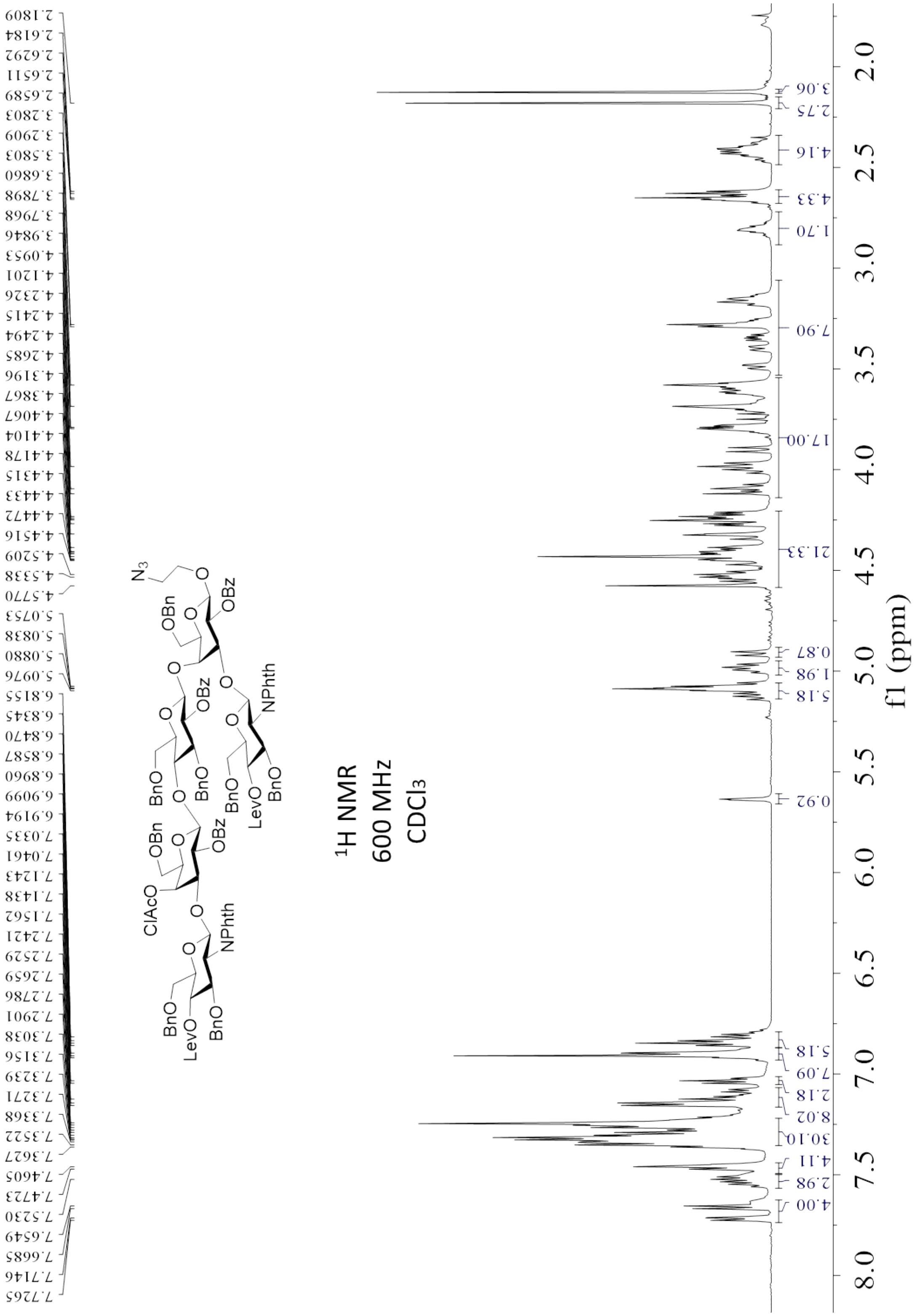
^{13}C NMR Spectrum of compound **8** (CDCl$_3$, 150 MHz)



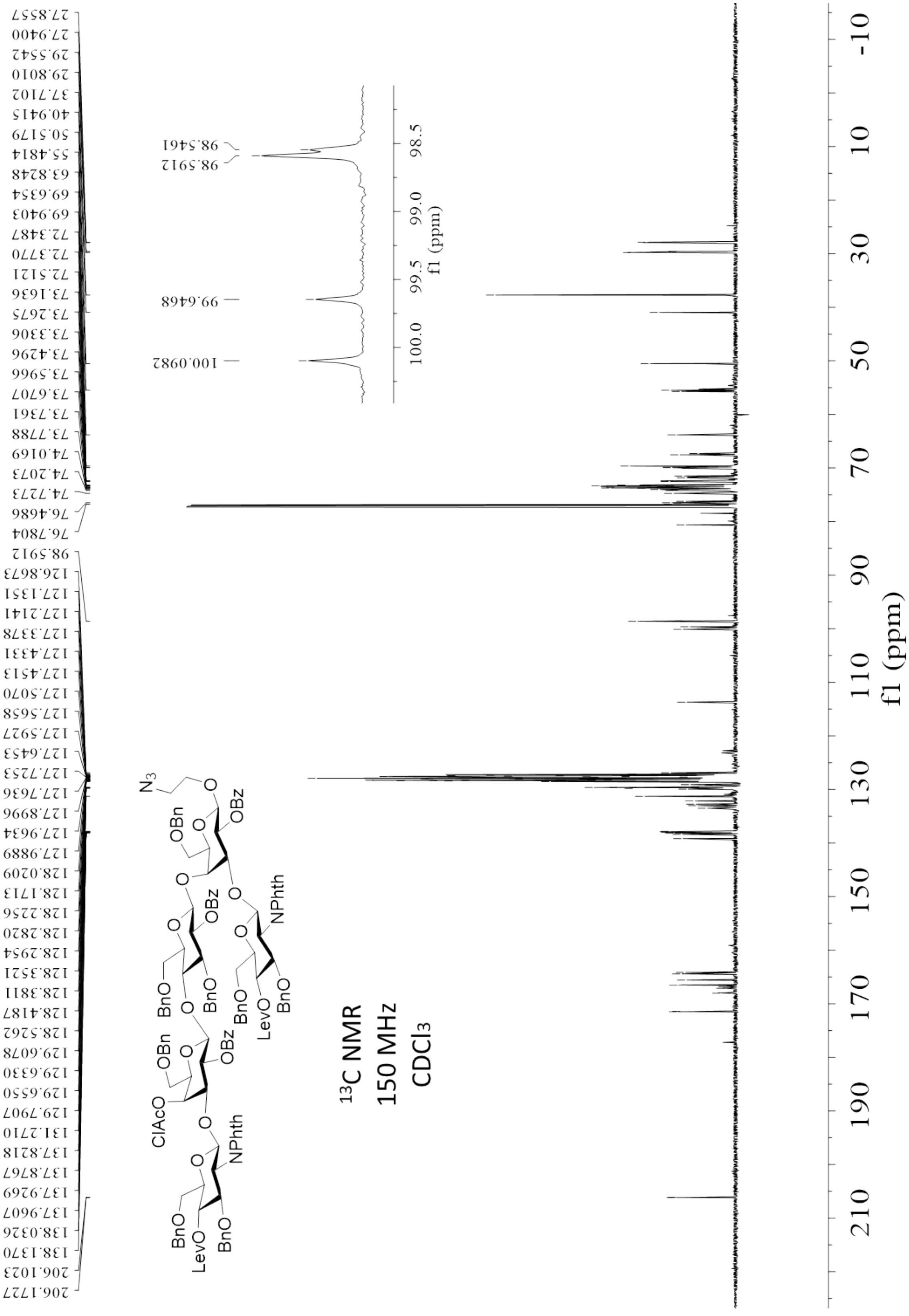
^{1}H-^{1}H COSY Spectrum of compound **8** (CDCl$_3$, 600 MHz)



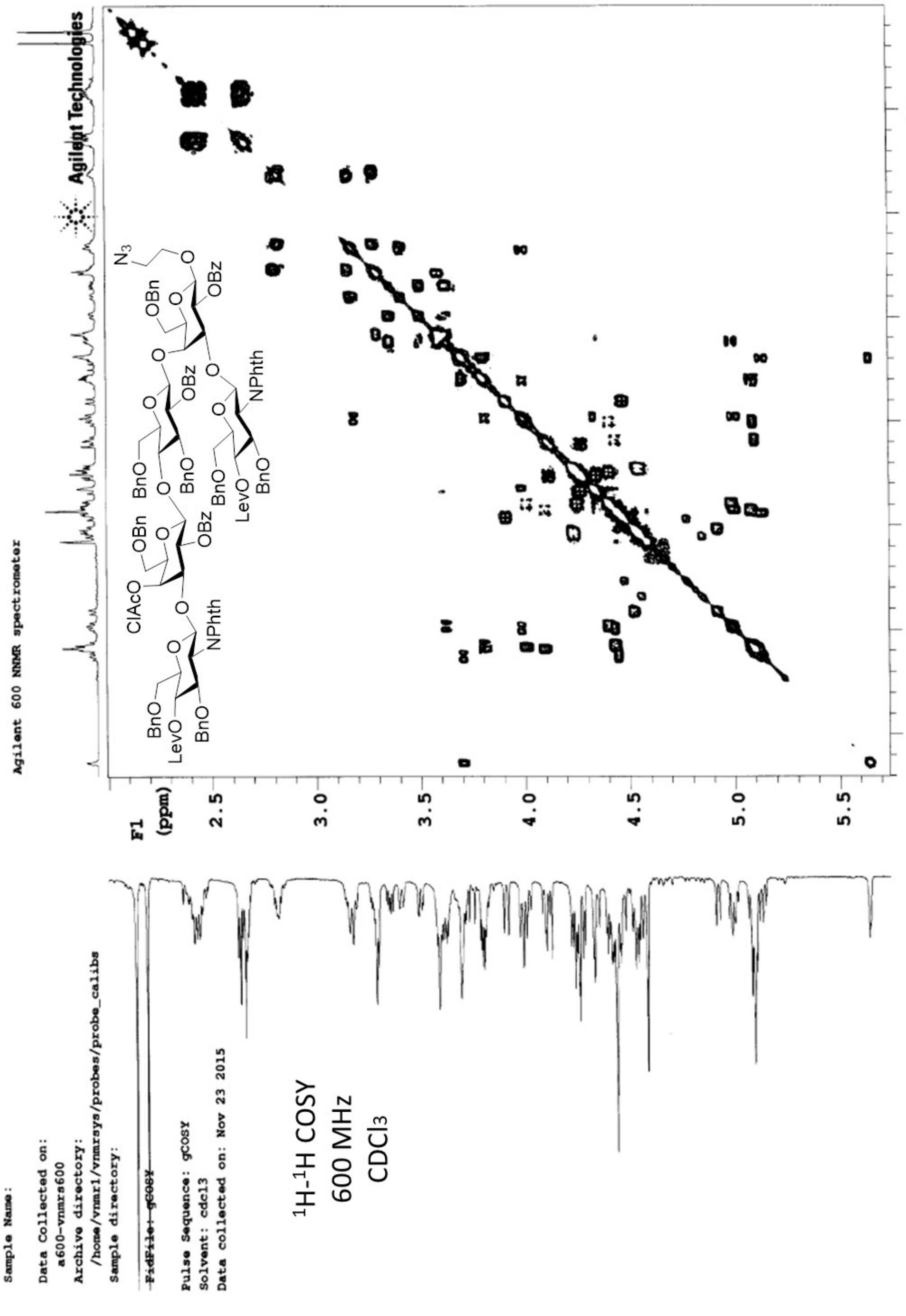
^{1}H-^{13}C HMQC Spectrum of compound **8** (CDCl$_3$, 600/150 MHz)



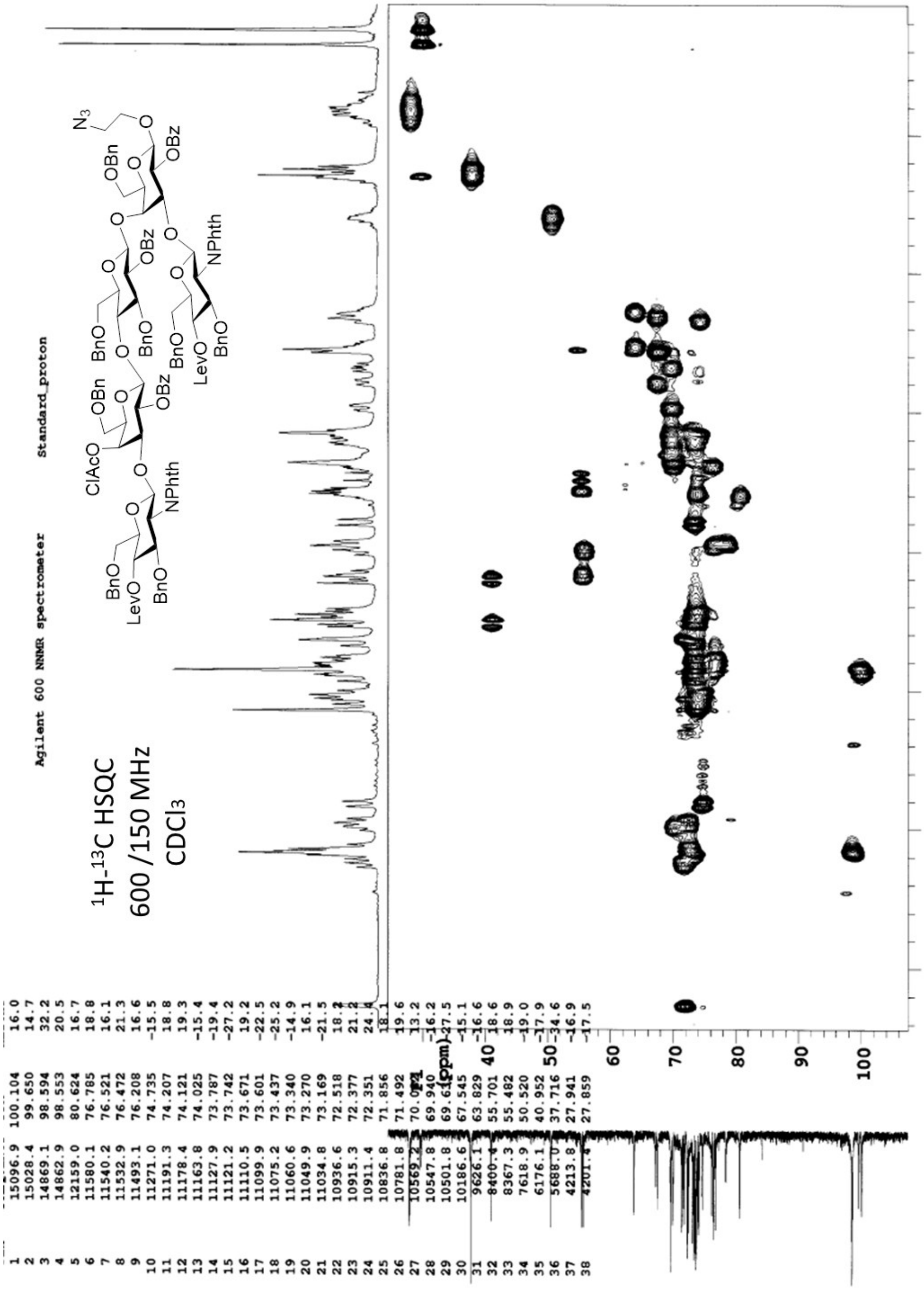
^{1}H NMR Spectrum of compound **15** ($CDCl_3$, 600 MHz)



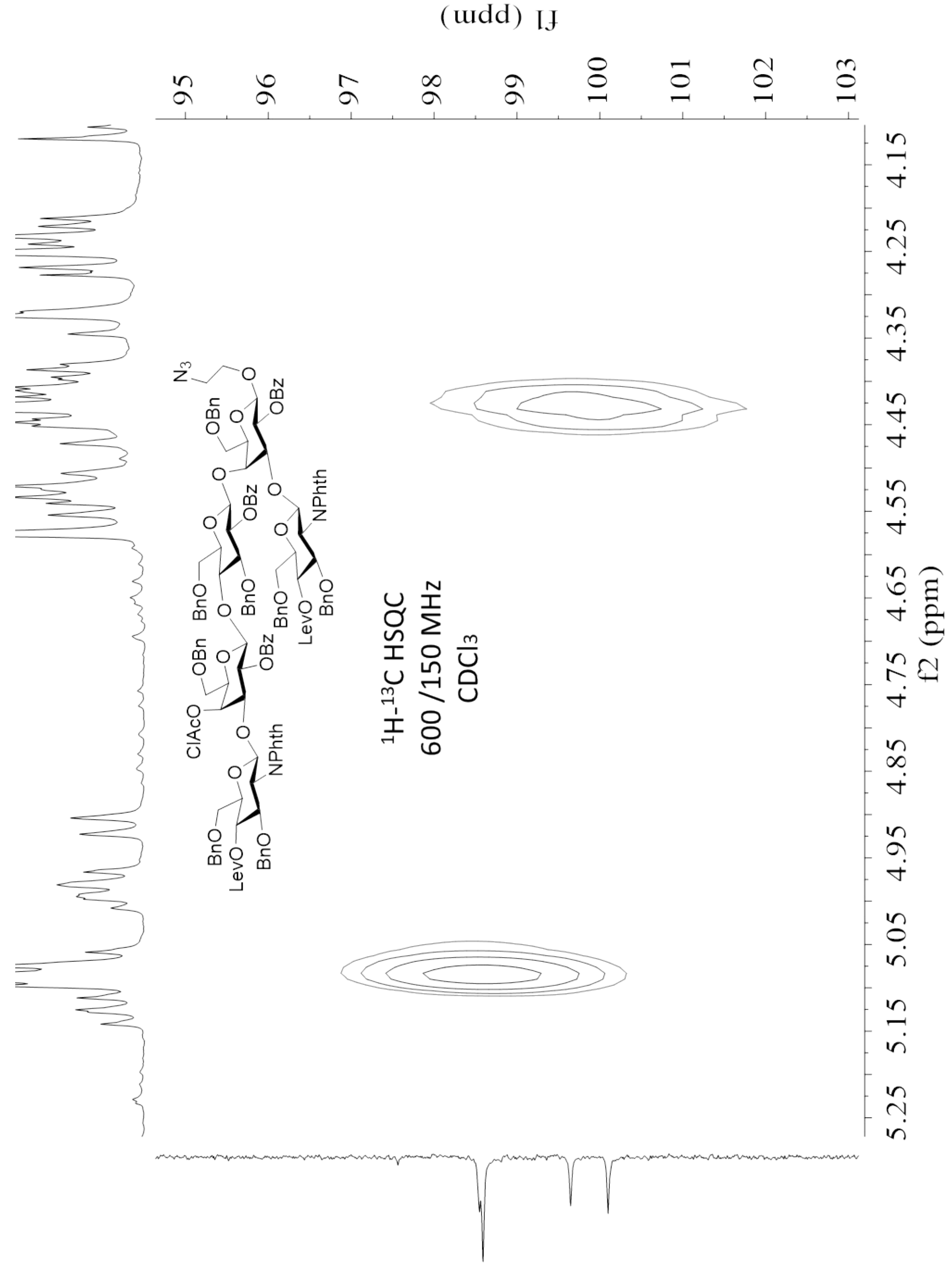
^{13}C NMR Spectrum of compound **15** (CDCl$_3$, 150 MHz)



^{1}H-^{1}H COSY Spectrum of compound **15** (CDCl$_3$, 600 MHz)



^{1}H-^{13}C HMQC Spectrum of compound **15** ($CDCl_3$, 600/150 MHz)



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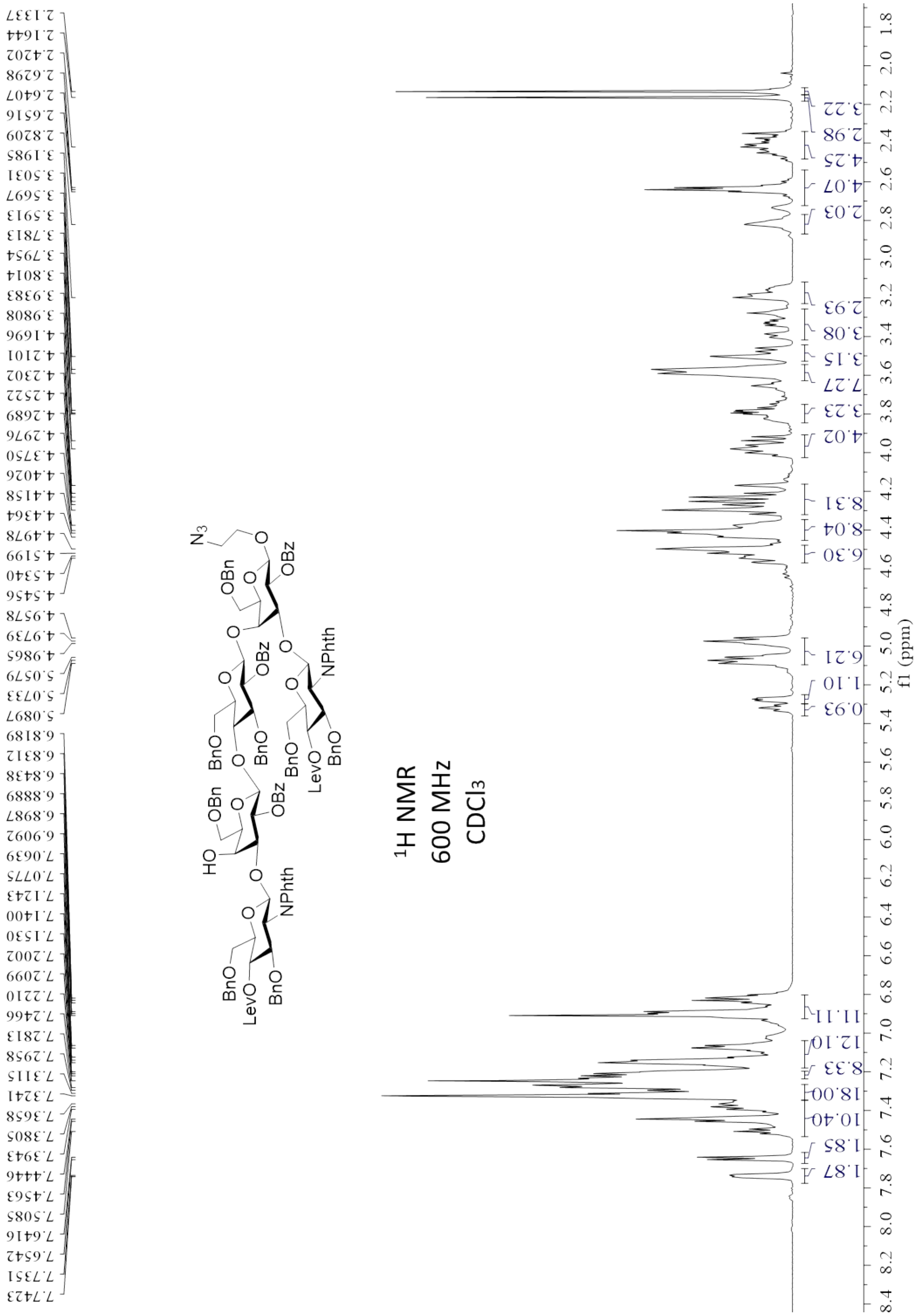
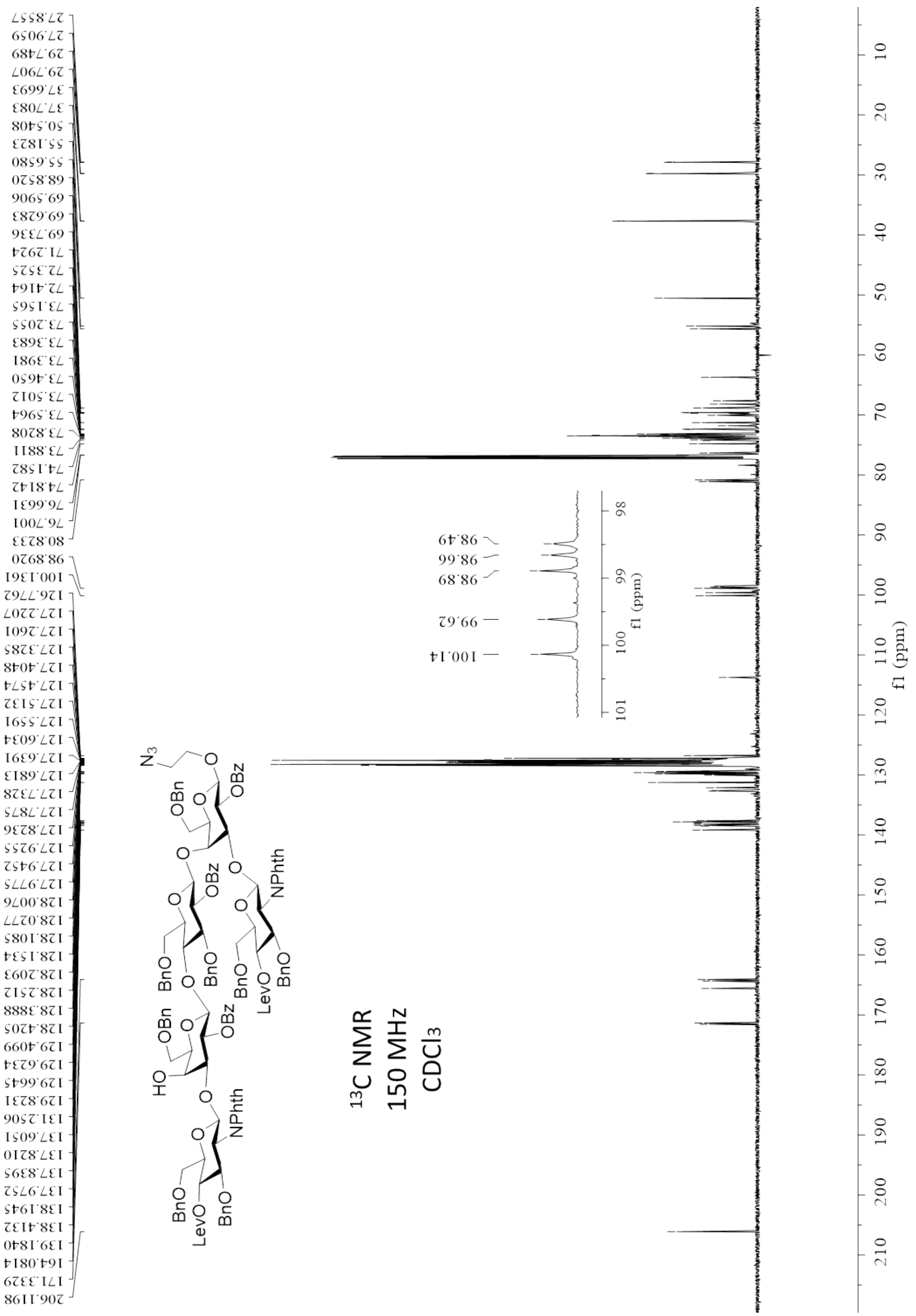
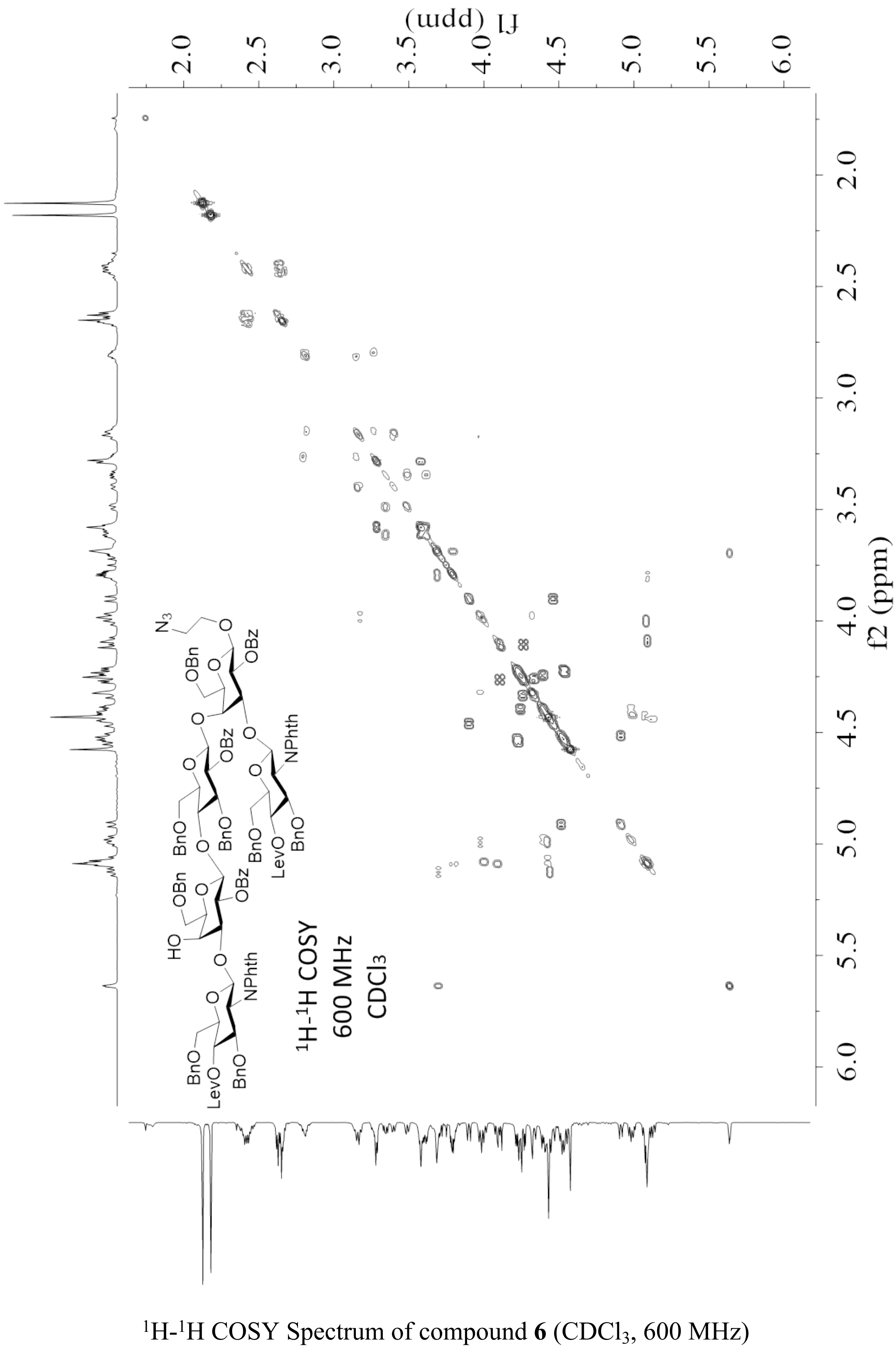


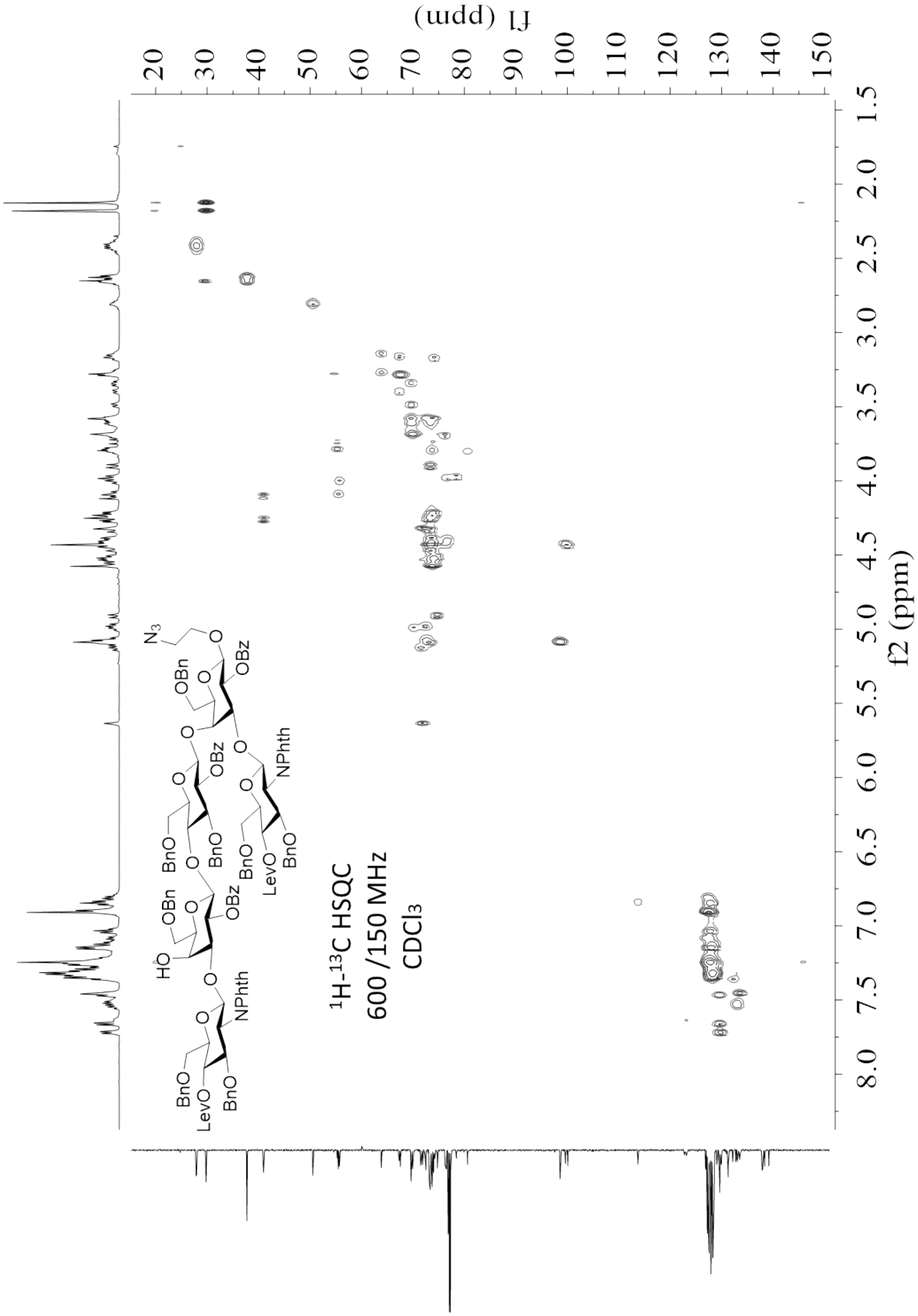
^{1}H NMR Spectrum of compound **6** ($CDCl_3$, 600 MHz)



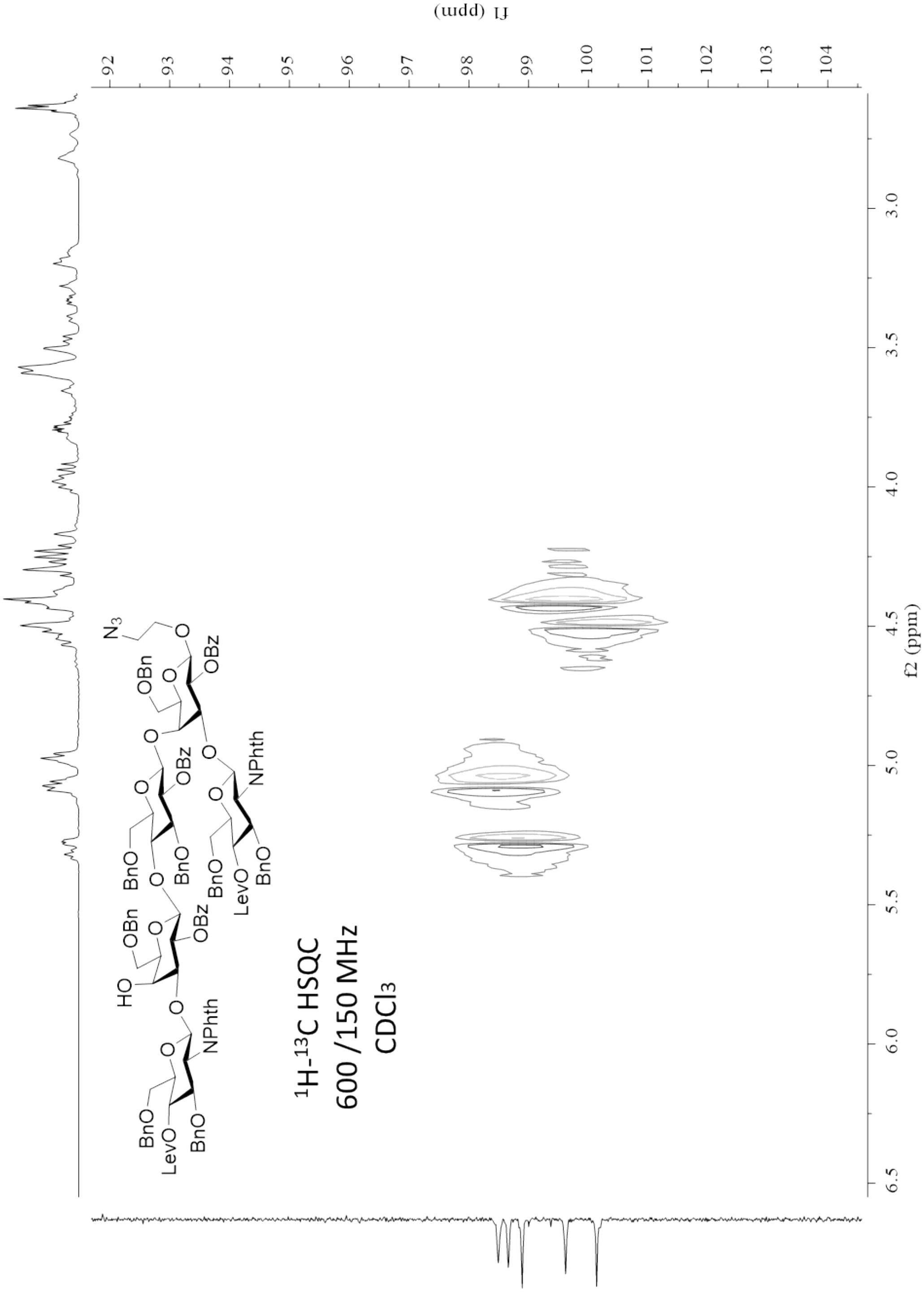
^{13}C NMR Spectrum of compound **6** ($CDCl_3$,150 MHz)



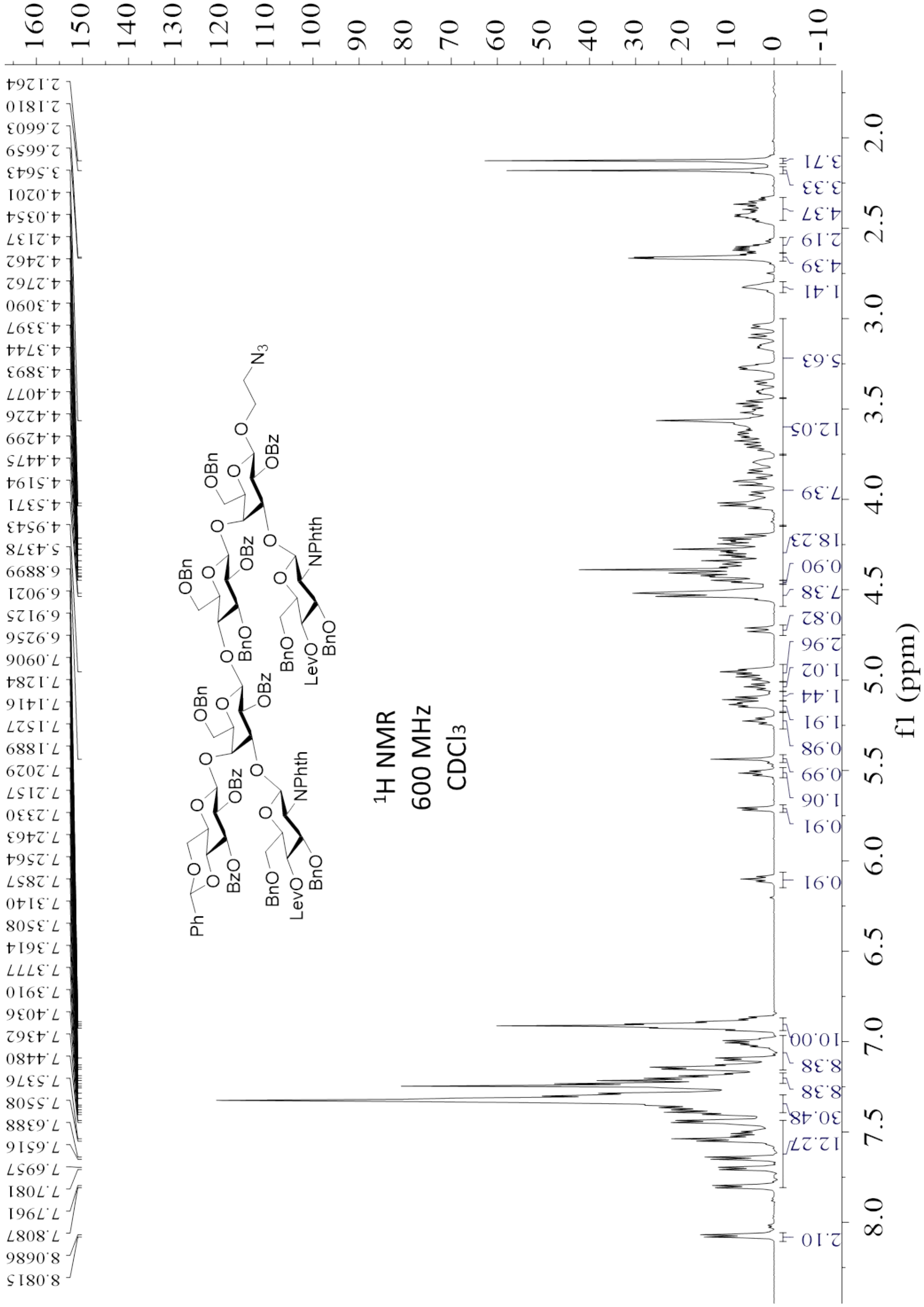
^{1}H-^{1}H COSY Spectrum of compound **6** (CDCl$_3$, 600 MHz)



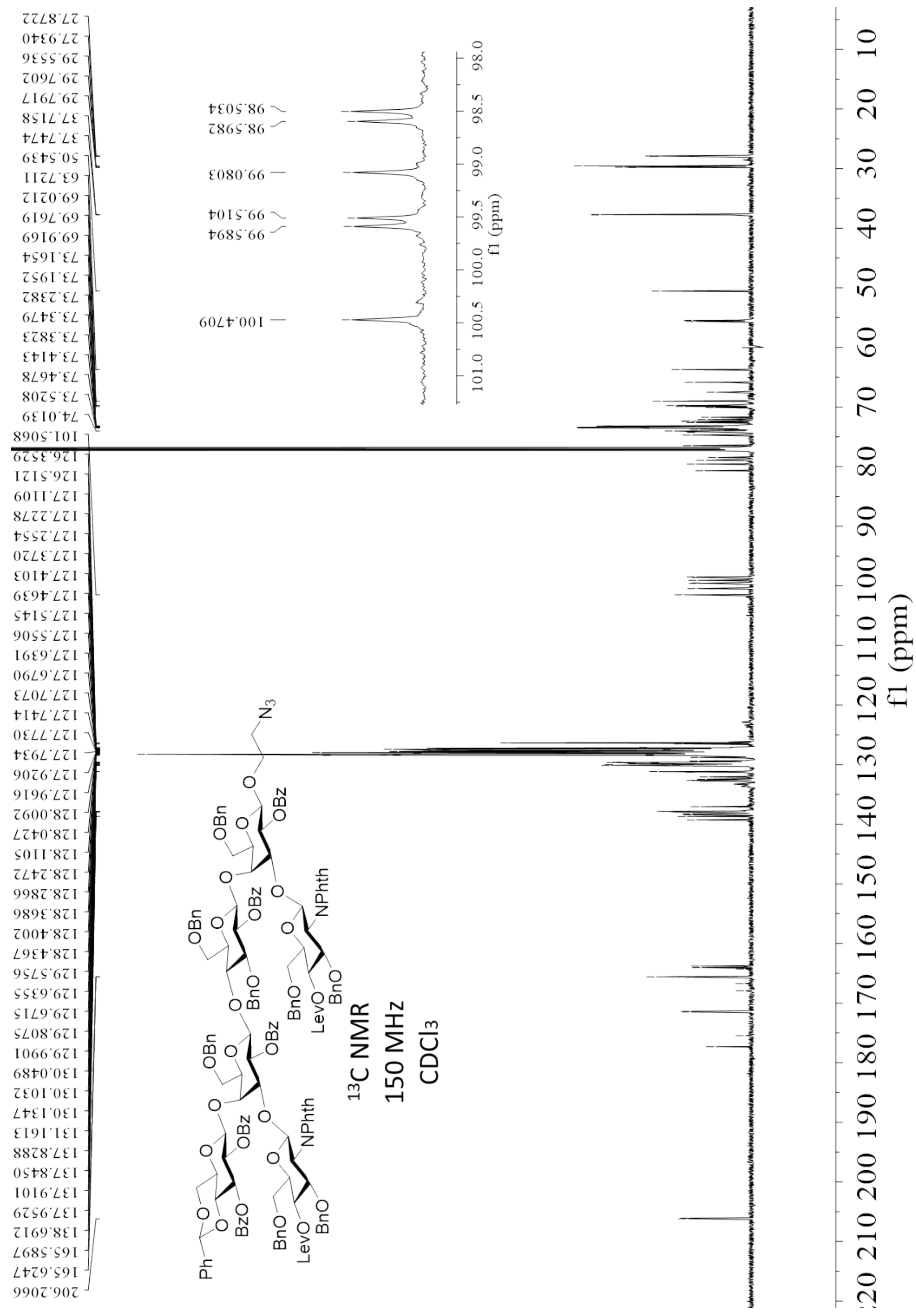
^{1}H-^{13}C HMQC Spectrum of compound **6** (CDCl$_3$, 600/150 MHz)



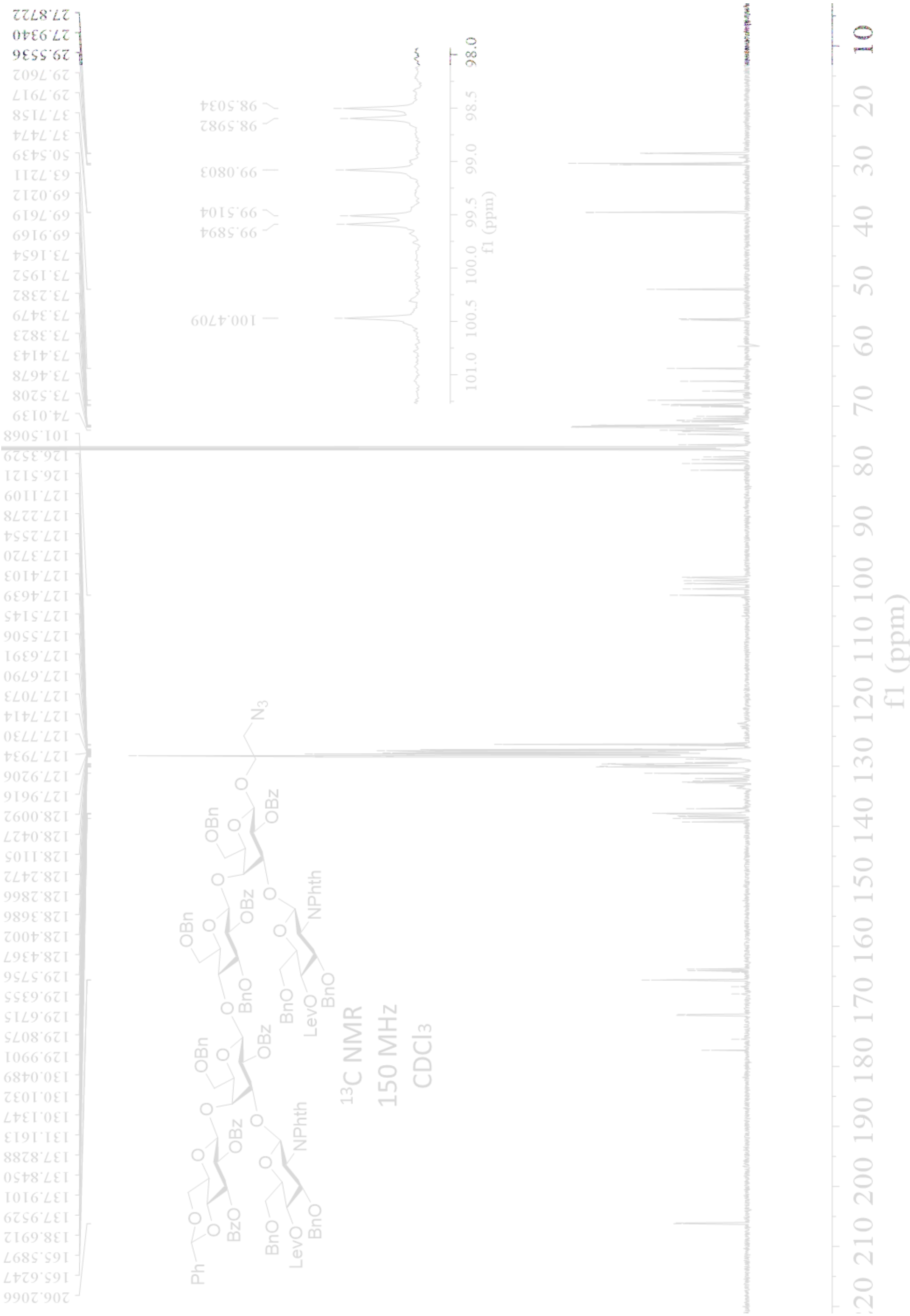
^{1}H-^{13}C HMQC Spectrum of compound **6** (CDCl$_3$, 600/150 MHz)



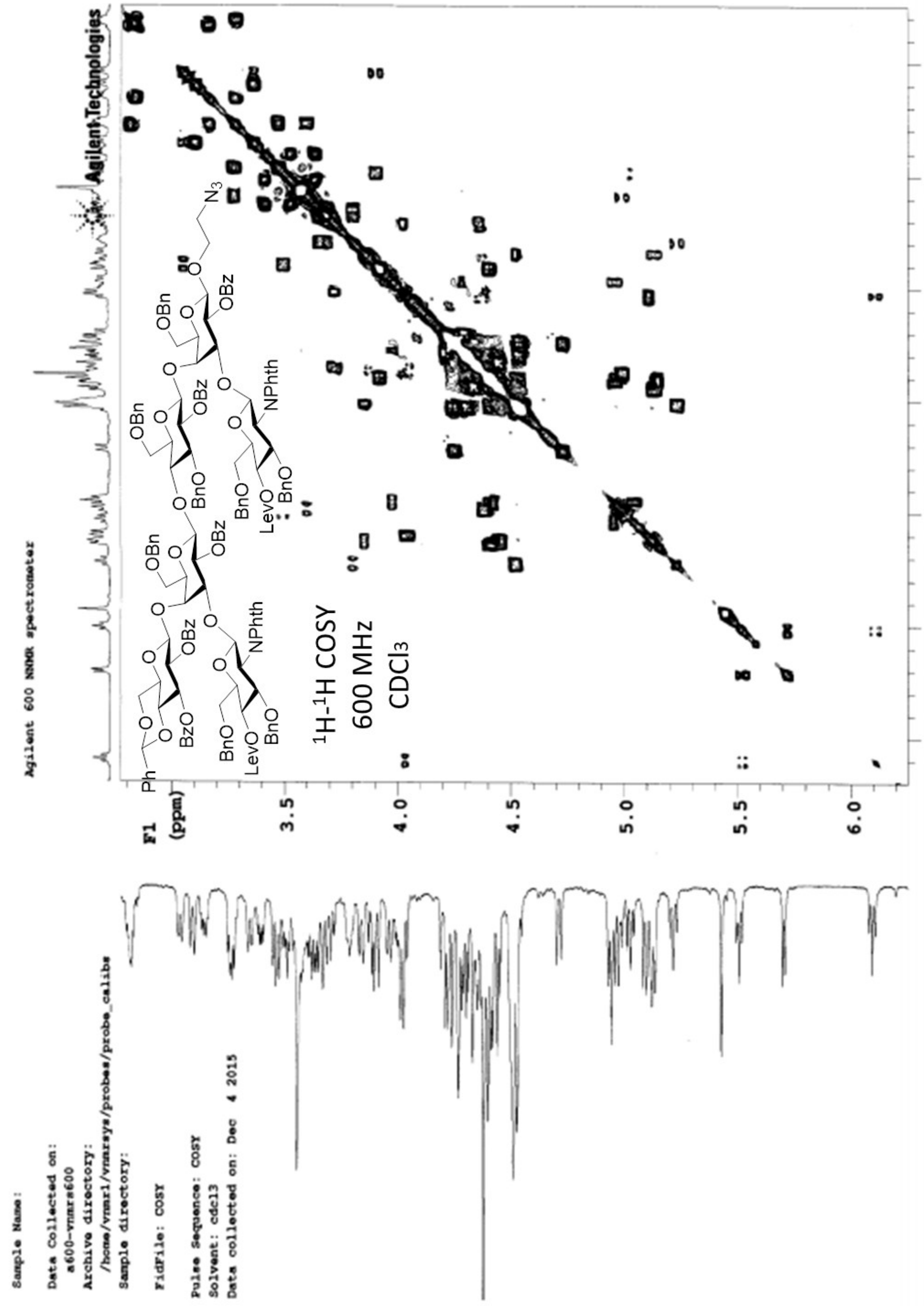
^{1}H NMR Spectrum of compound **16** ($CDCl_3$, 600 MHz)



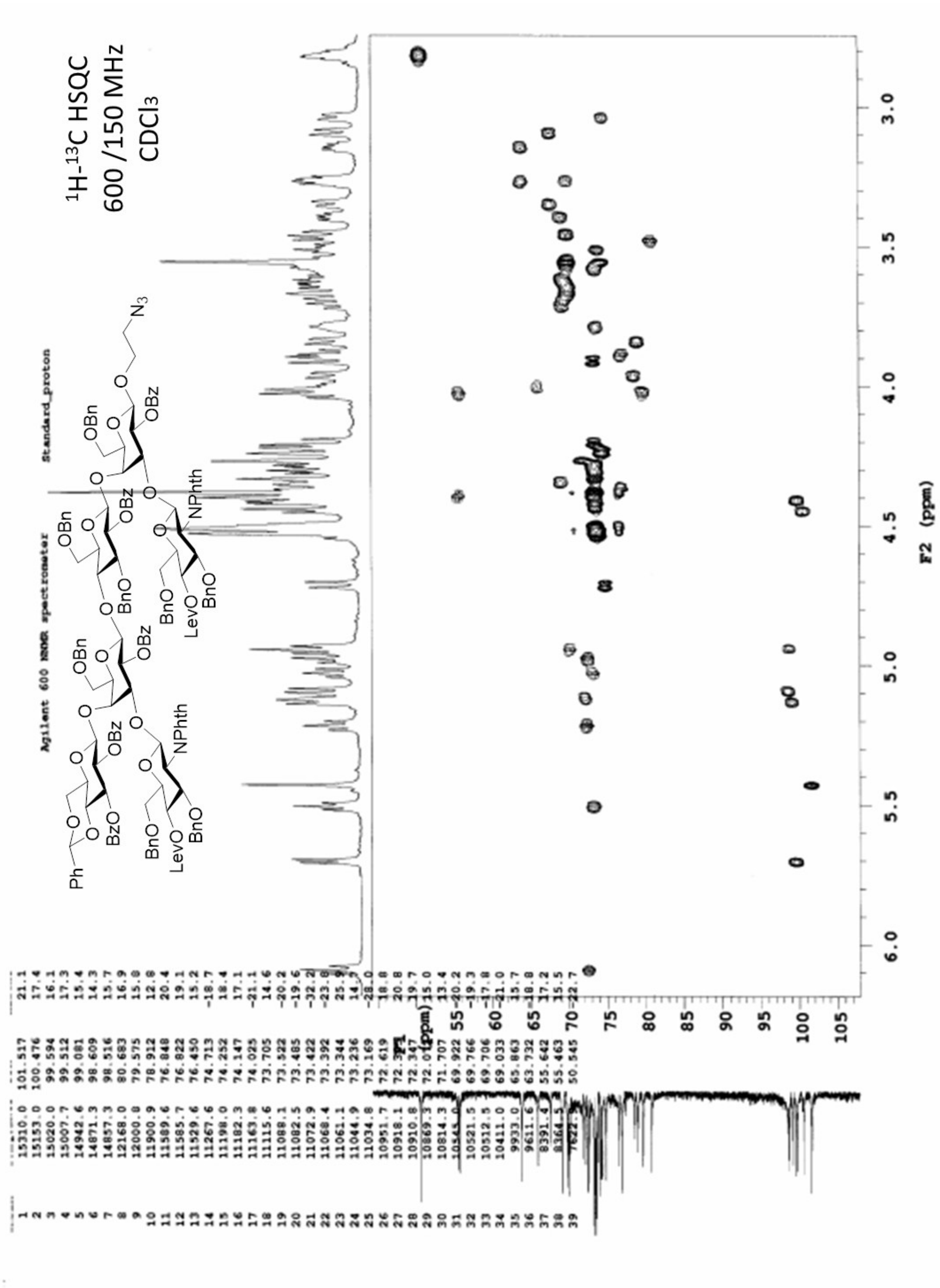
^{13}C NMR Spectrum of compound **16** (CDCl$_3$, 150 MHz)



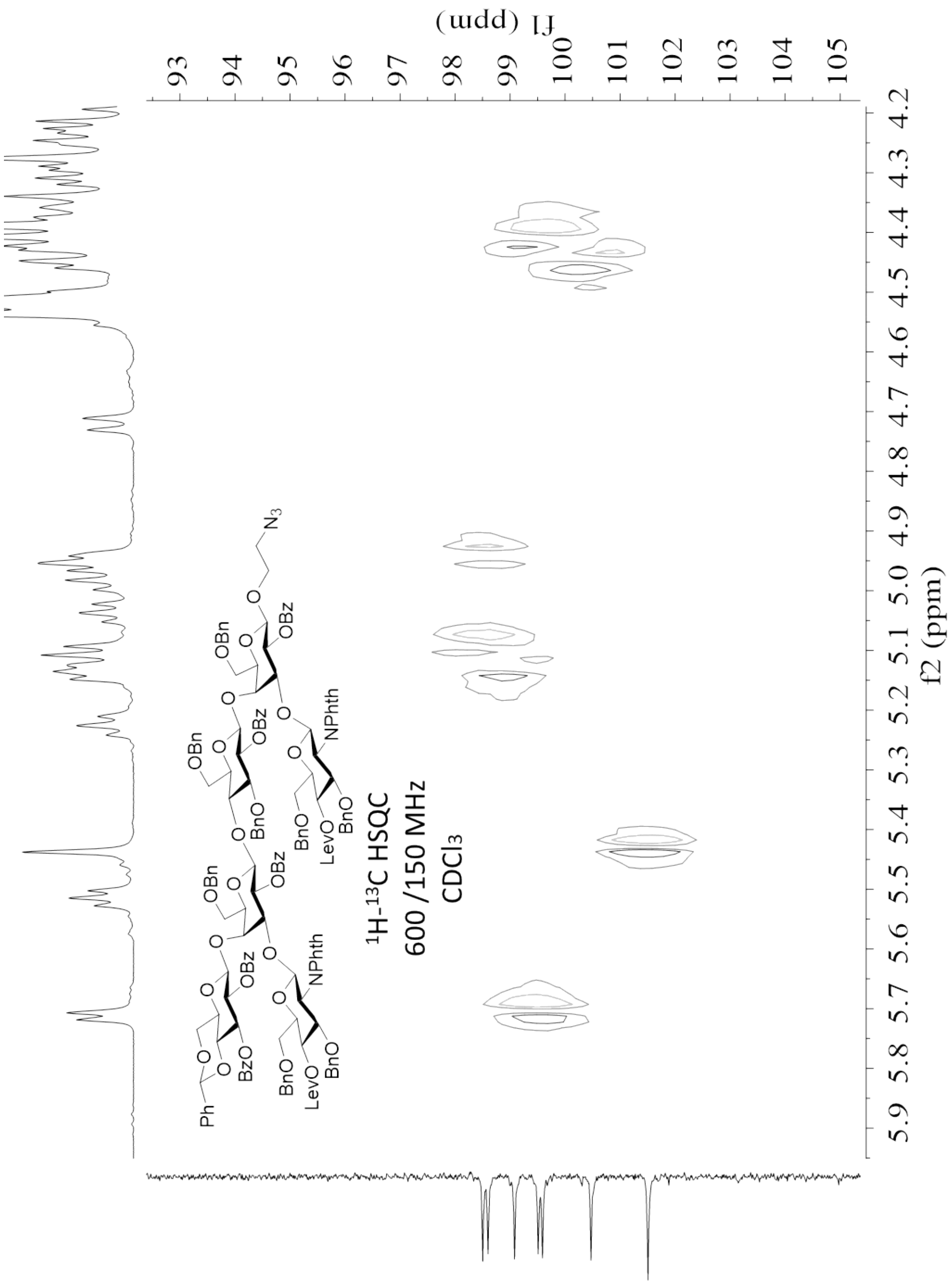
^{13}C NMR Spectrum of compound **16** ($CDCl_3$, 150 MHz)



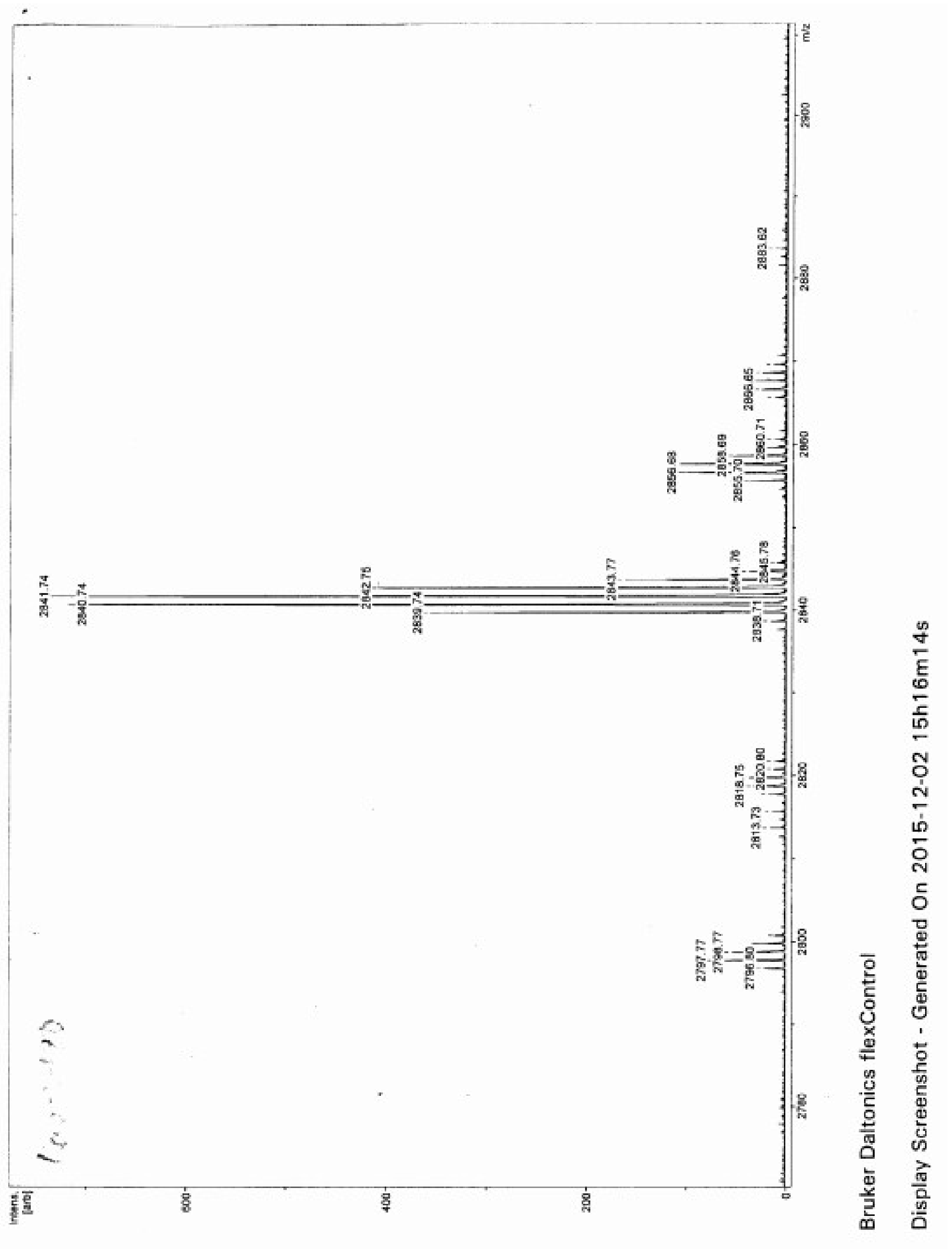
^{1}H-^{1}H COSY Spectrum of compound **16** (CDCl$_3$, 600 MHz)



^{1}H-^{13}C HMQC Spectrum of compound **16** (CDCl$_3$, 600/150 MHz)



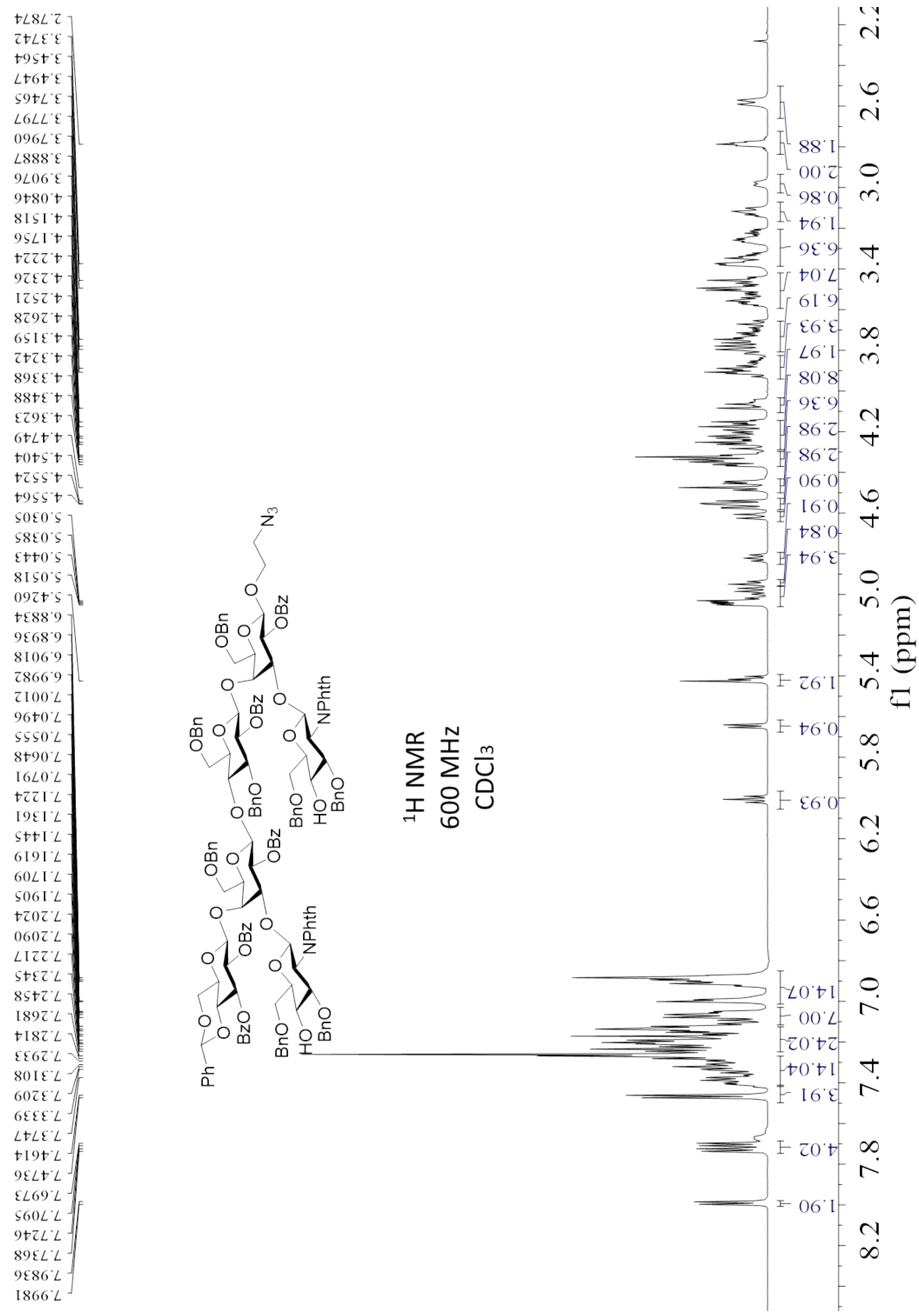
^{1}H-^{13}C HMQC Spectrum of compound **16** (CDCl$_3$, 600/150 MHz)



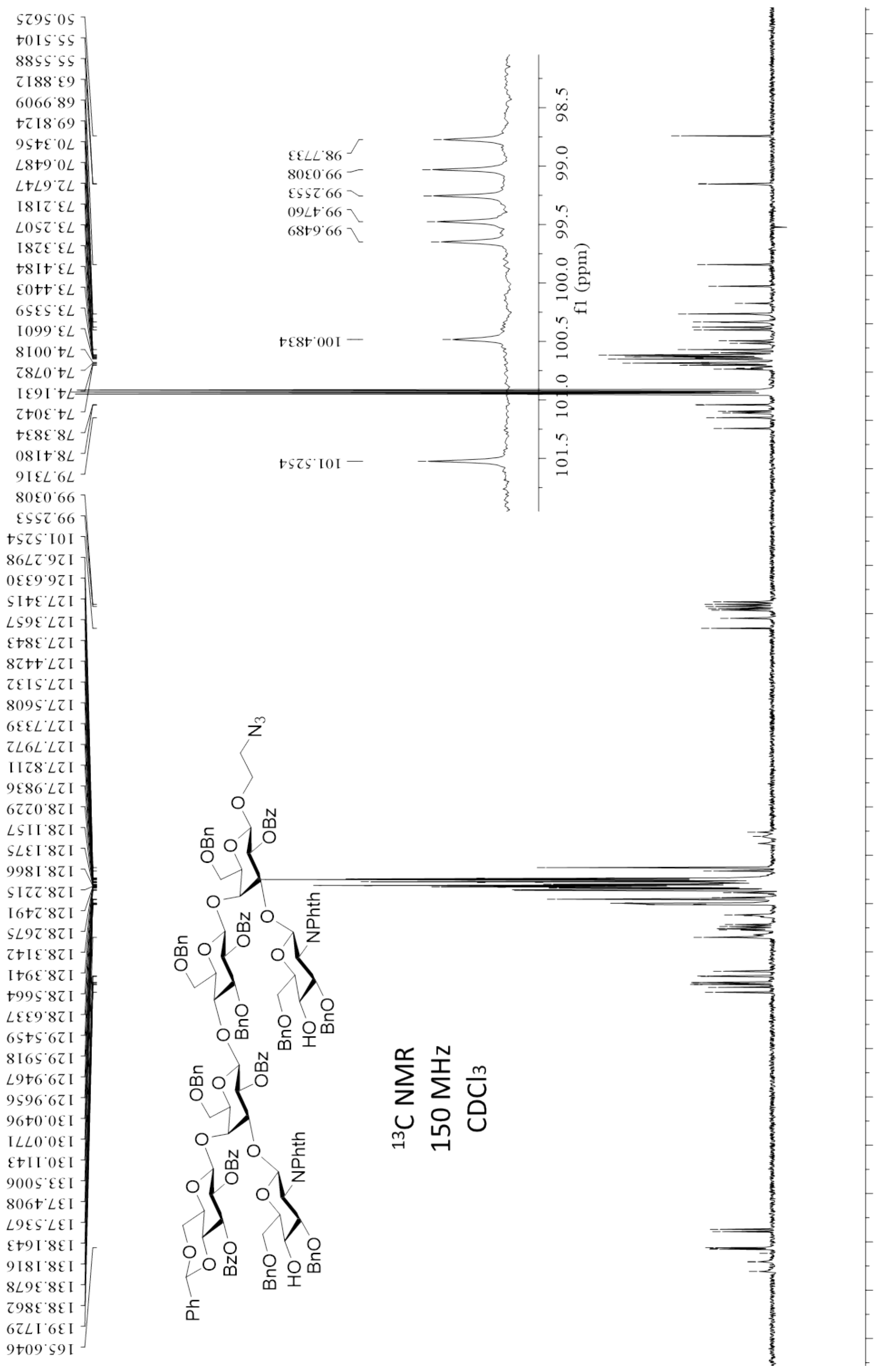
Bruker Daltonics flexControl

Display Screenshot - Generated On 2015-12-02 15h16m14s

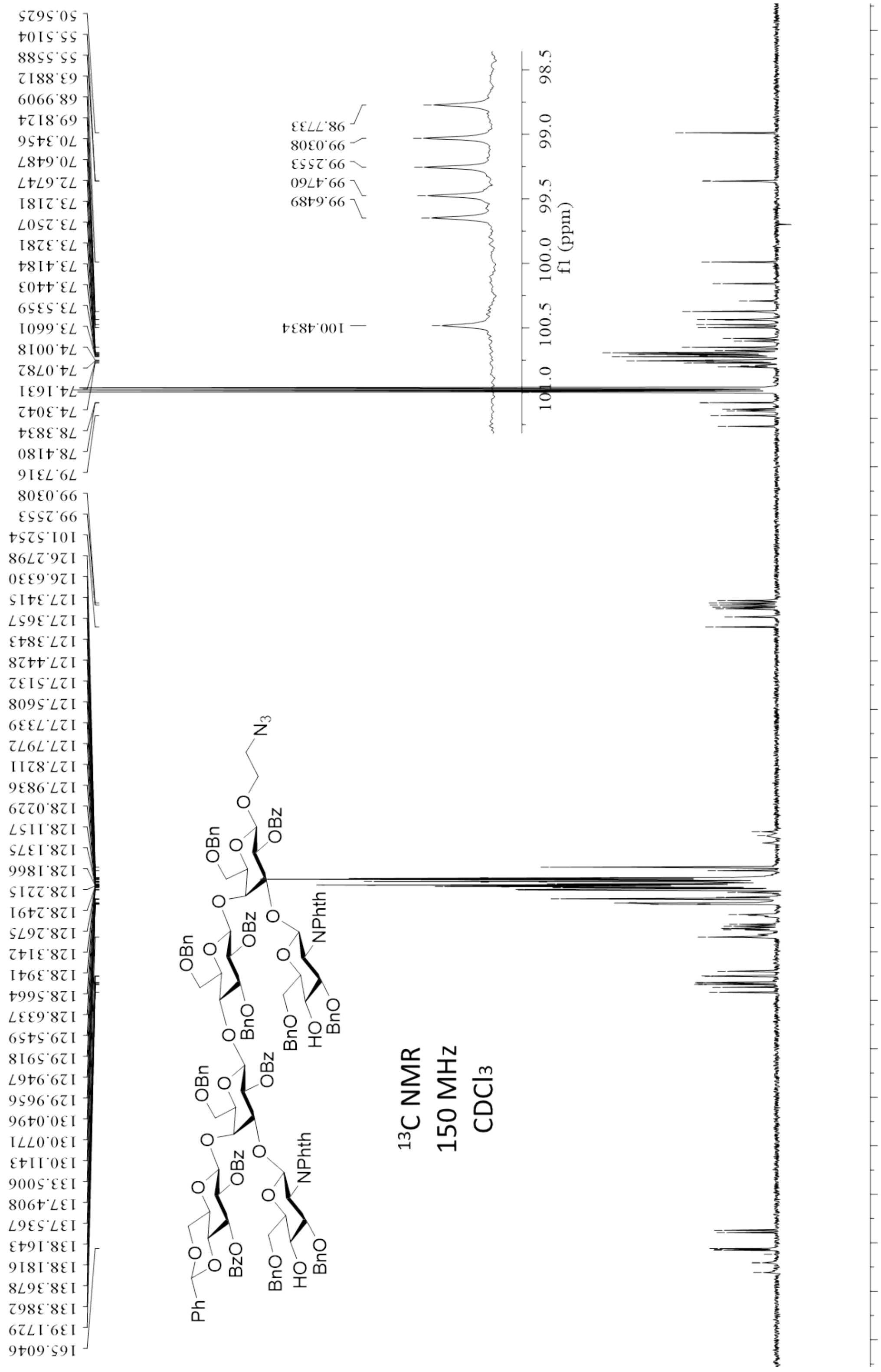
HR ESI-TOF MS Spectrum of compound **16**



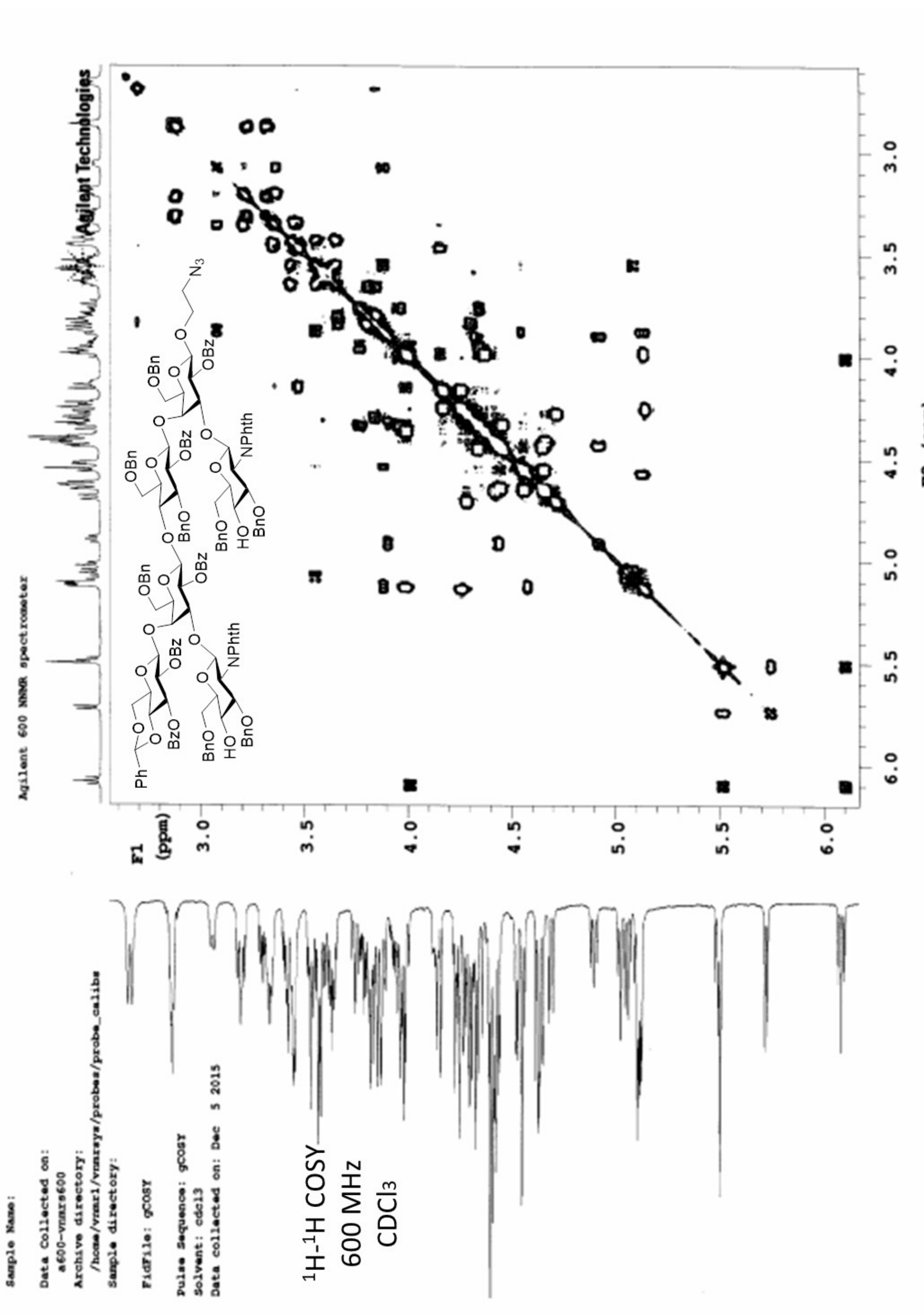
^{1}H NMR Spectrum of compound **4** ($CDCl_3$, 600 MHz)



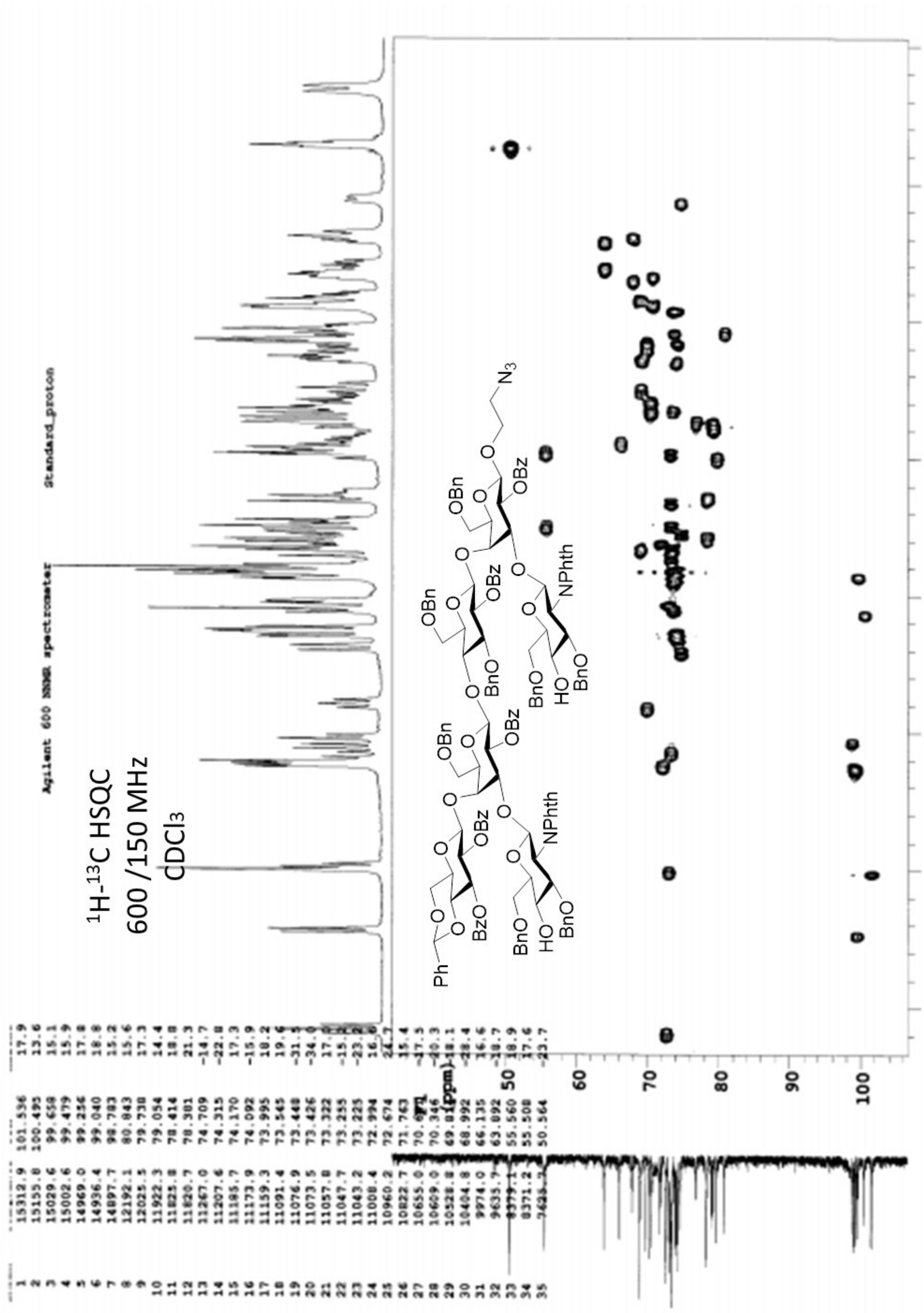
^{13}C NMR Spectrum of compound **4** (CDCl$_3$, 150 MHz)



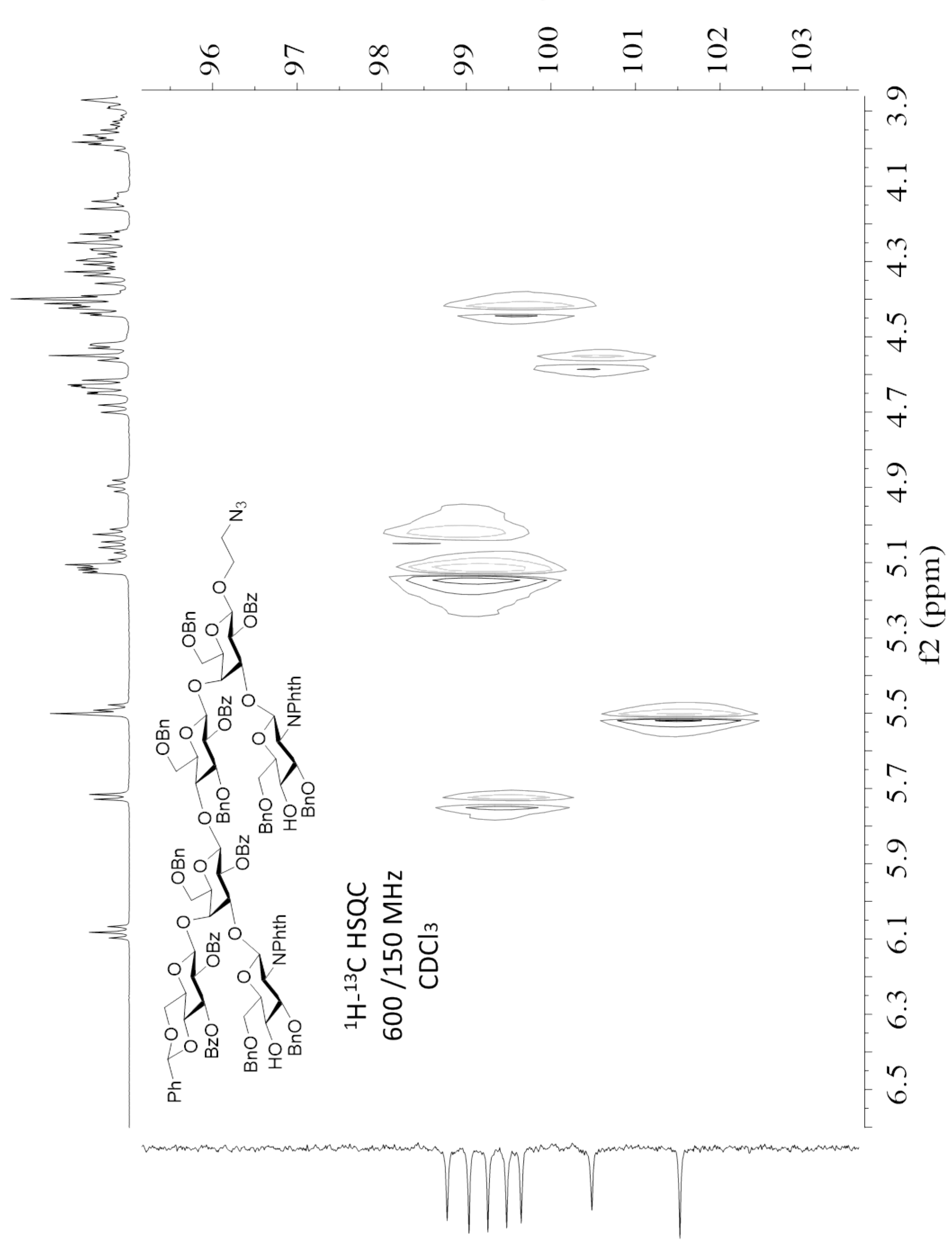
^{13}C NMR Spectrum of compound **4** (CDCl$_3$, 150 MHz)



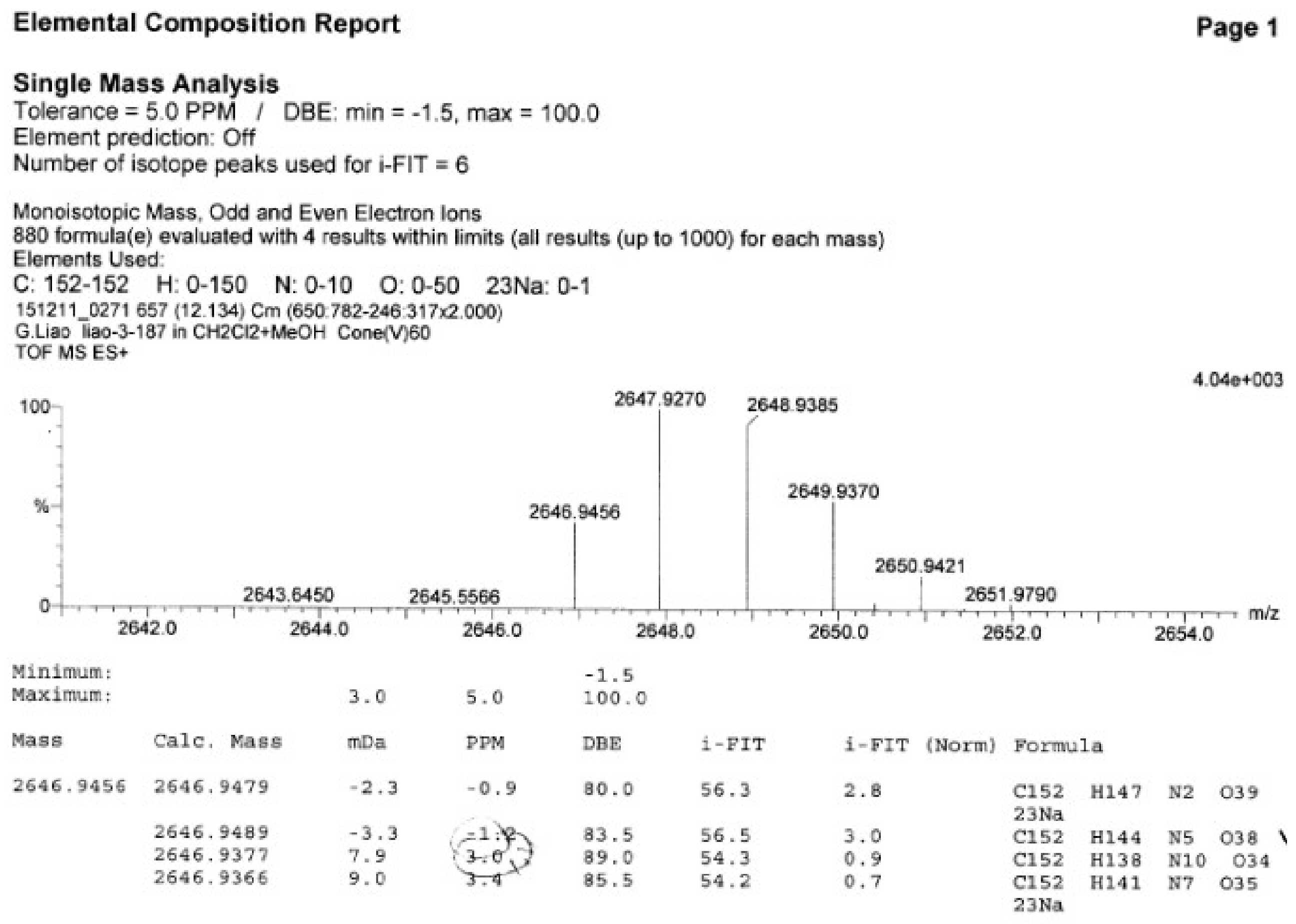
^{1}H-^{1}H COSY Spectrum of compound **4** ($CDCl_3$, 600 MHz)



^{1}H-^{13}C HMQC Spectrum of compound **4** (CDCl$_3$, 600/150 MHz)



^{1}H-^{13}C HMQC Spectrum of compound **4** (CDCl$_3$, 600/150 MHz)



Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 6

Monoisotopic Mass, Odd and Even Electron Ions
880 formula(e) evaluated with 4 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 152-152 H: 0-150 N: 0-10 O: 0-50 23Na: 0-1
151211_0271 657 (12.134) Cm (650:782-246:317x2.000)
G.Liao liao-3-187 in CH2Cl2+MeOH Cone(V)60
TOF MS ES+

```
Minimum:                                    -1.5
Maximum:                   3.0      5.0    100.0

Mass       Calc. Mass     mDa      PPM      DBE      i-FIT     i-FIT (Norm) Formula

2646.9456  2646.9479     -2.3     -0.9     80.0     56.3      2.8          C152  H147  N2  O39
                                                                           23Na
           2646.9489     -3.3     -1.2     83.5     56.5      3.0          C152  H144  N5  O38
           2646.9377      7.9      3.0     89.0     54.3      0.9          C152  H138  N10  O34
           2646.9366      9.0      3.4     85.5     54.2      0.7          C152  H141  N7  O35
                                                                           23Na
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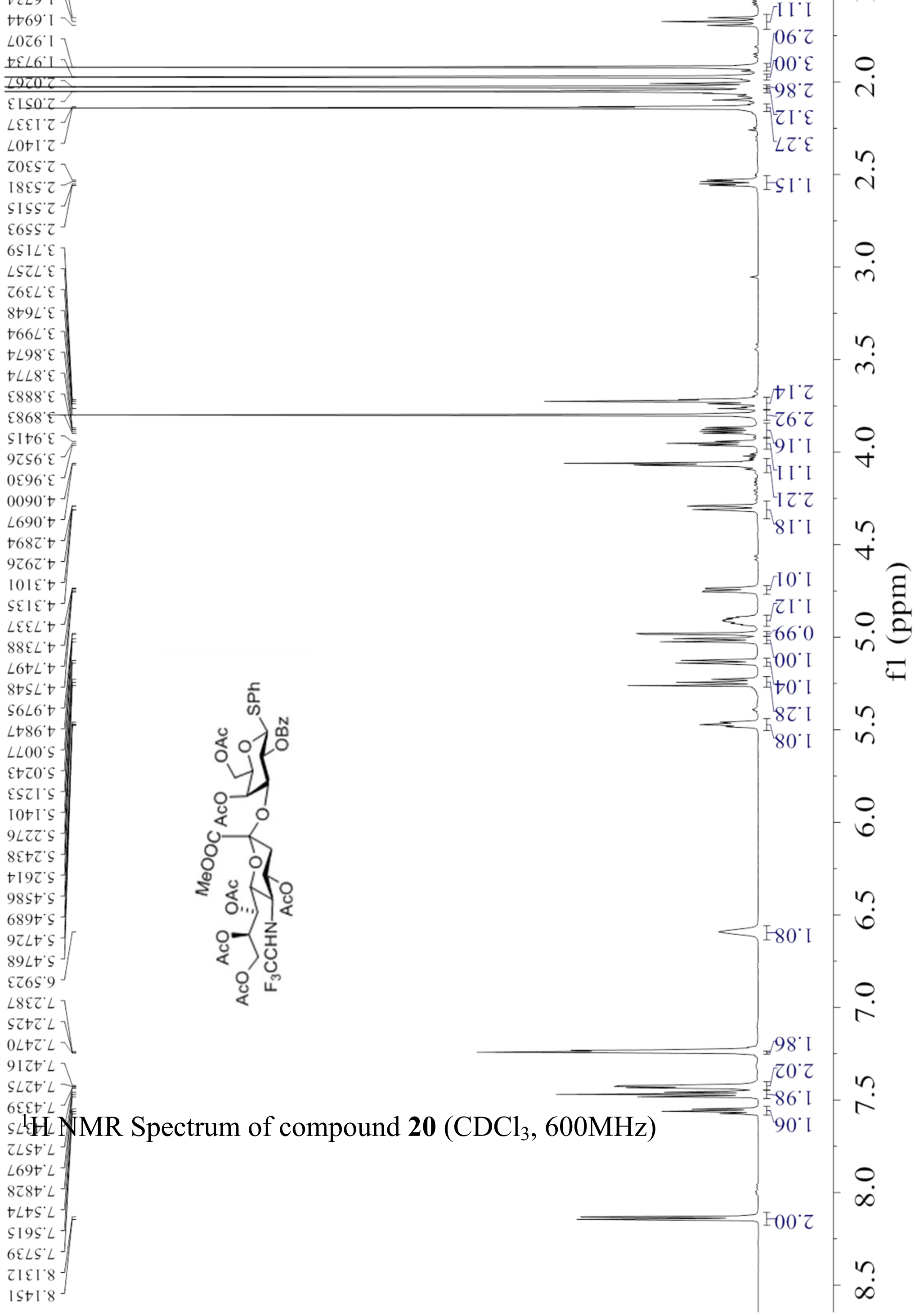
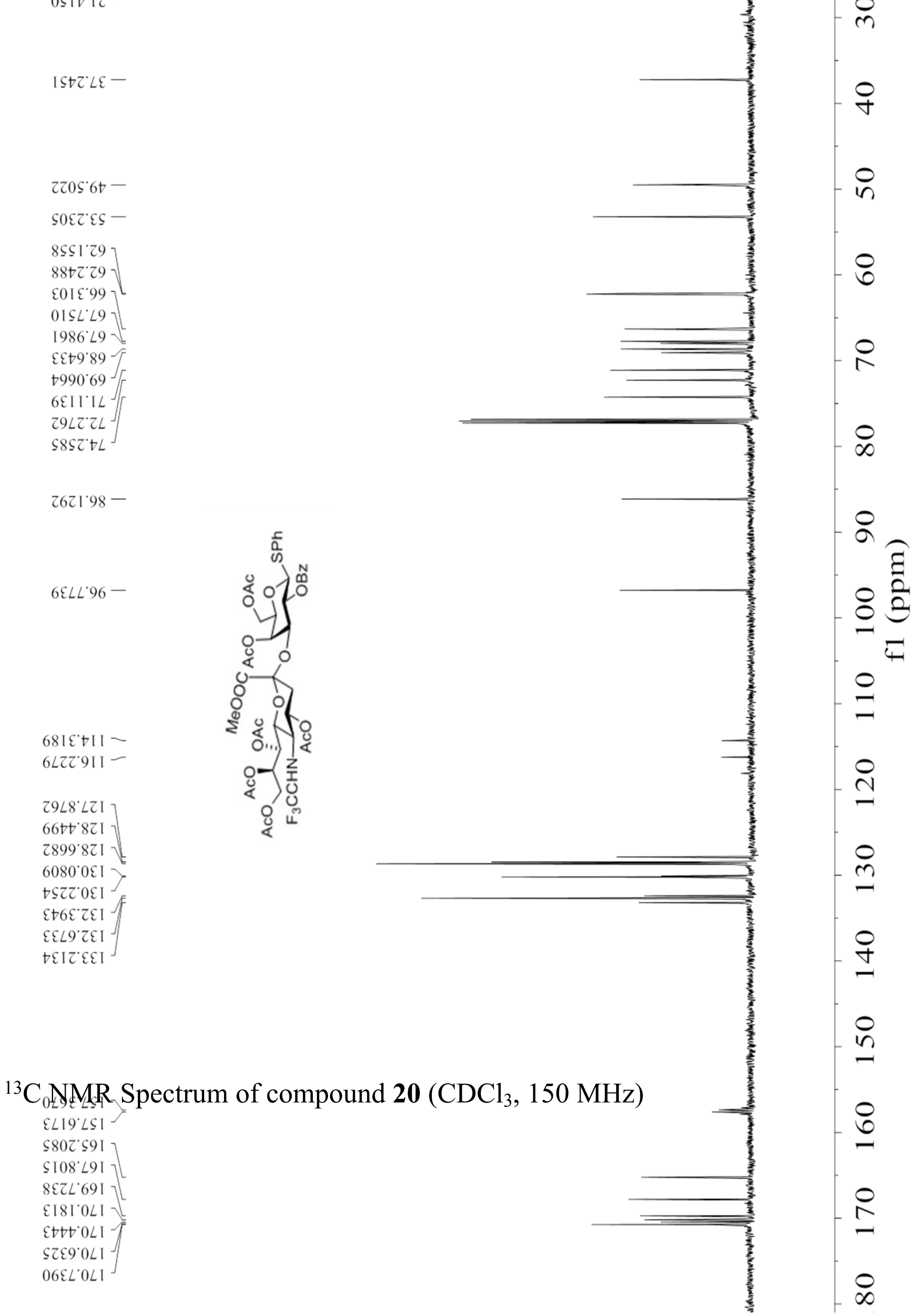
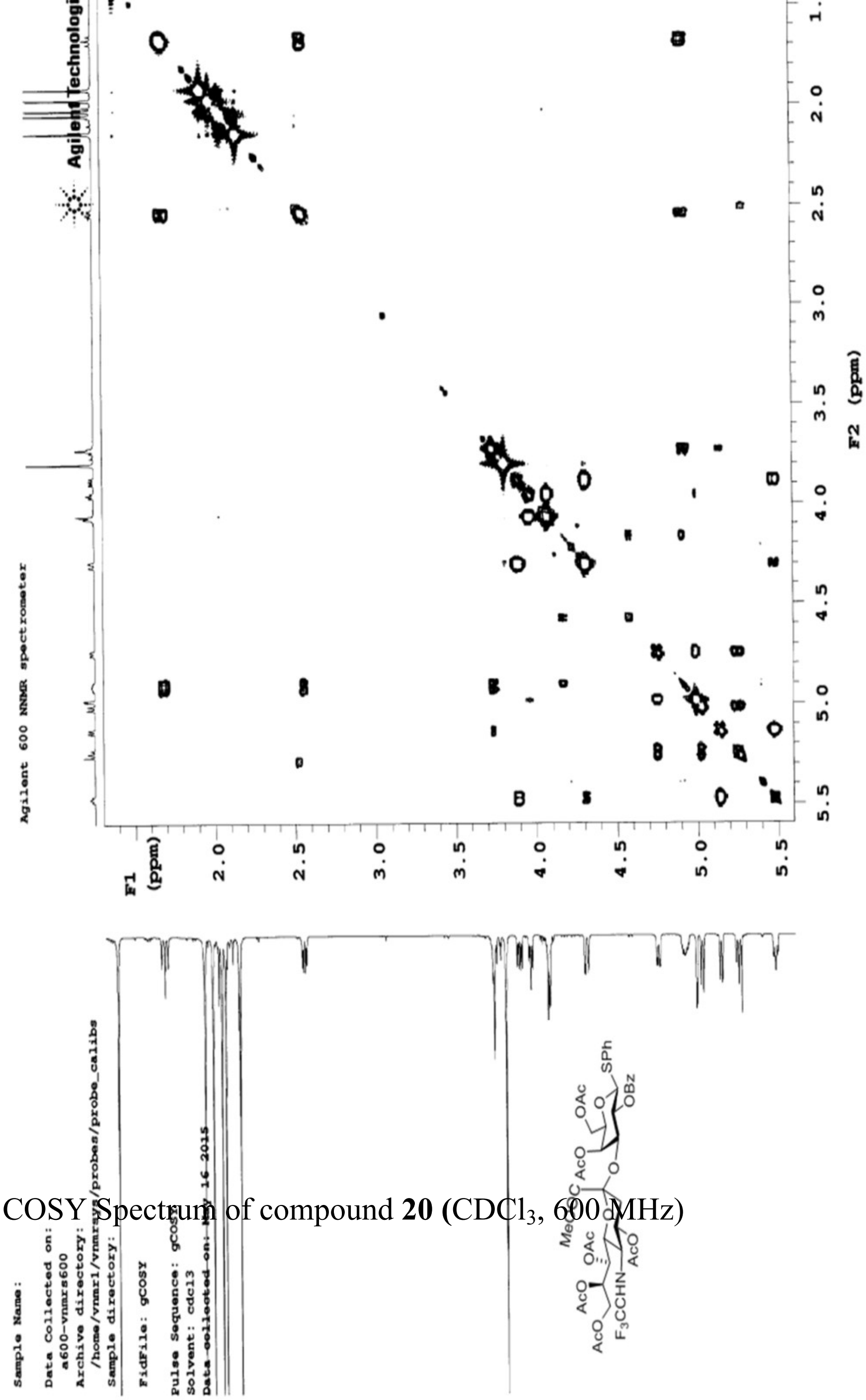
HR ESI-TOF MS Spectrum of compound **4**



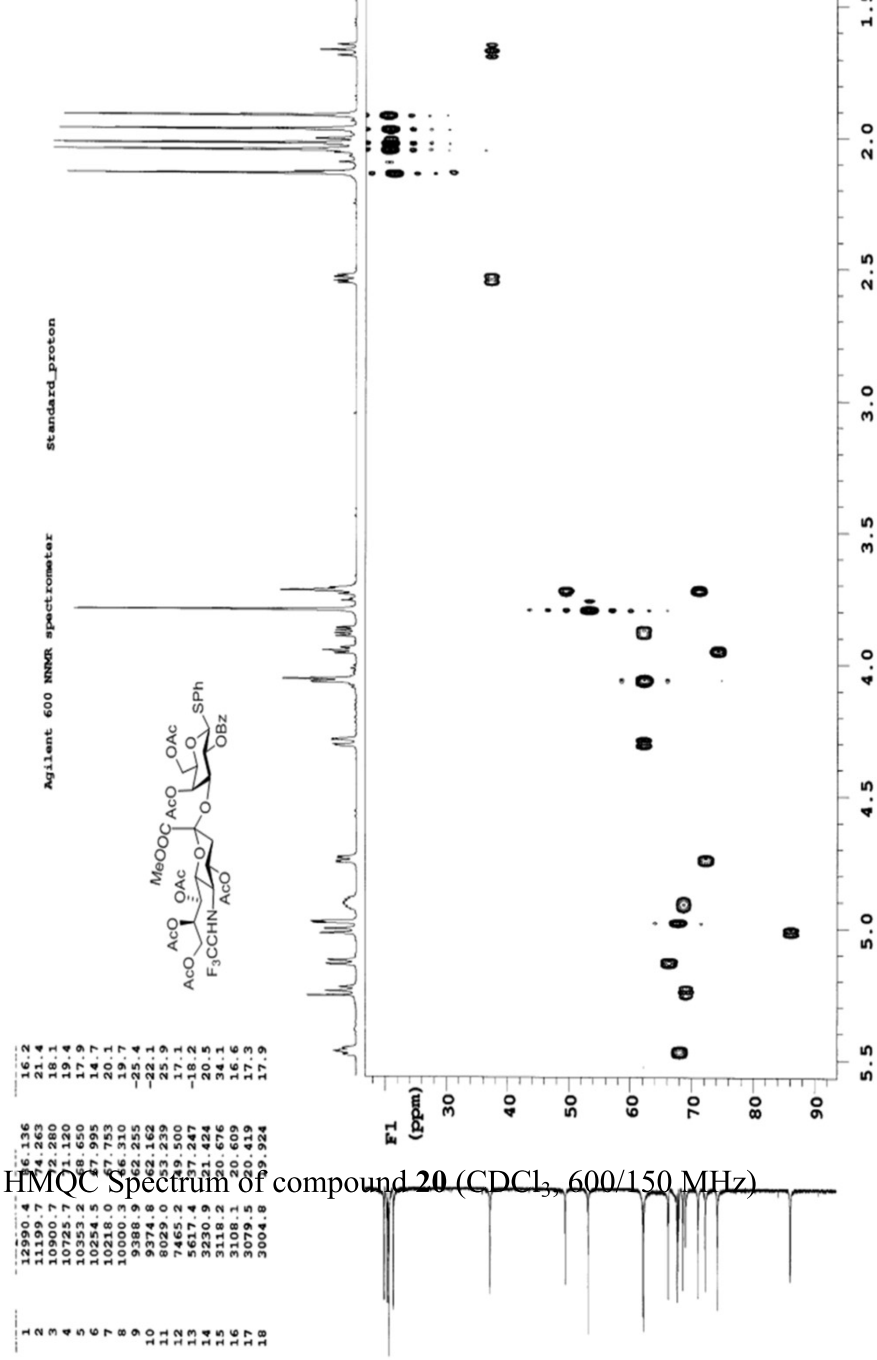
^{1}H NMR Spectrum of compound **20** ($CDCl_3$, 600MHz)



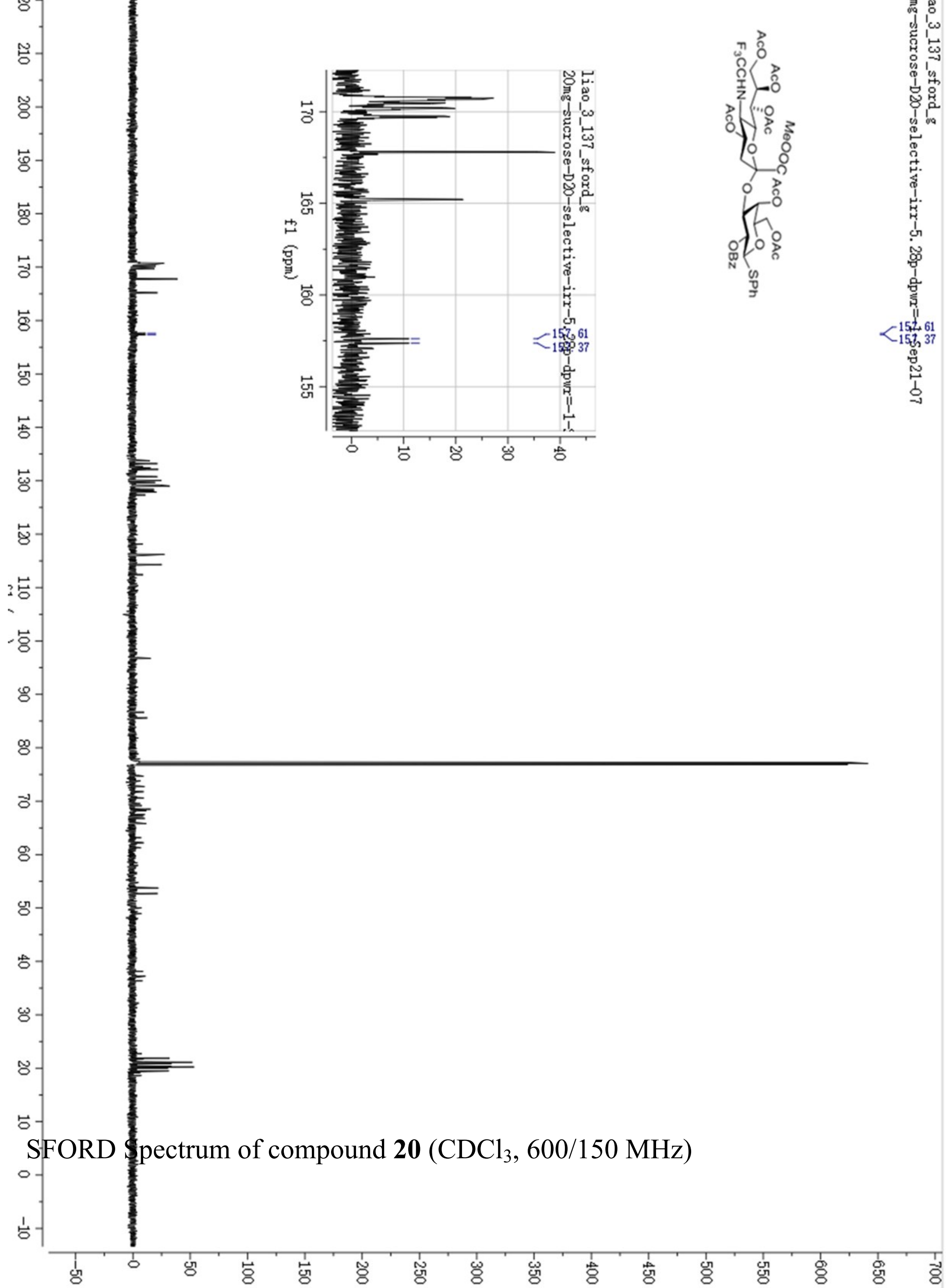
^{13}C NMR Spectrum of compound **20** ($CDCl_3$, 150 MHz)



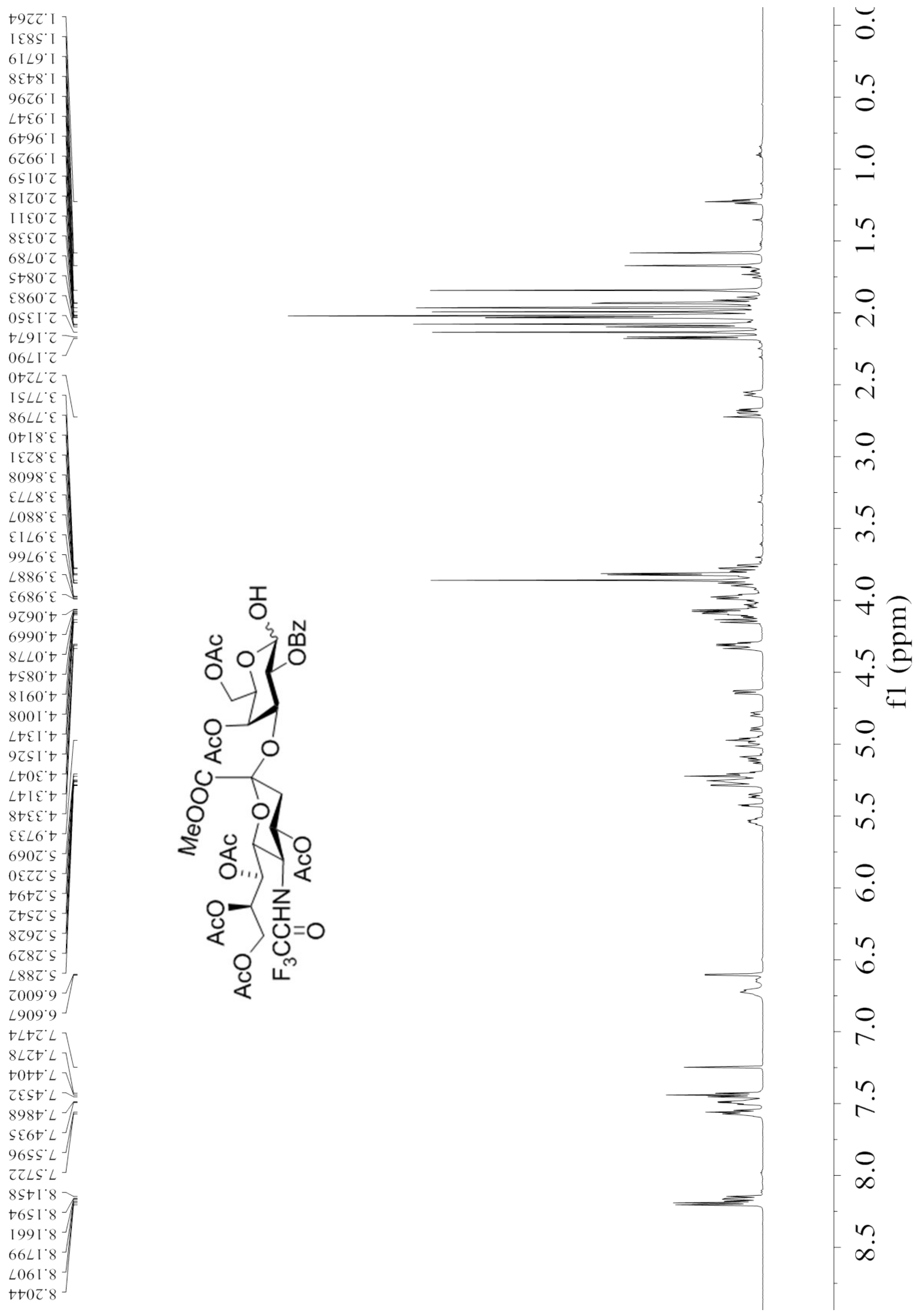
^1H-^1H COSY Spectrum of compound **20** ($CDCl_3$, 600 MHz)



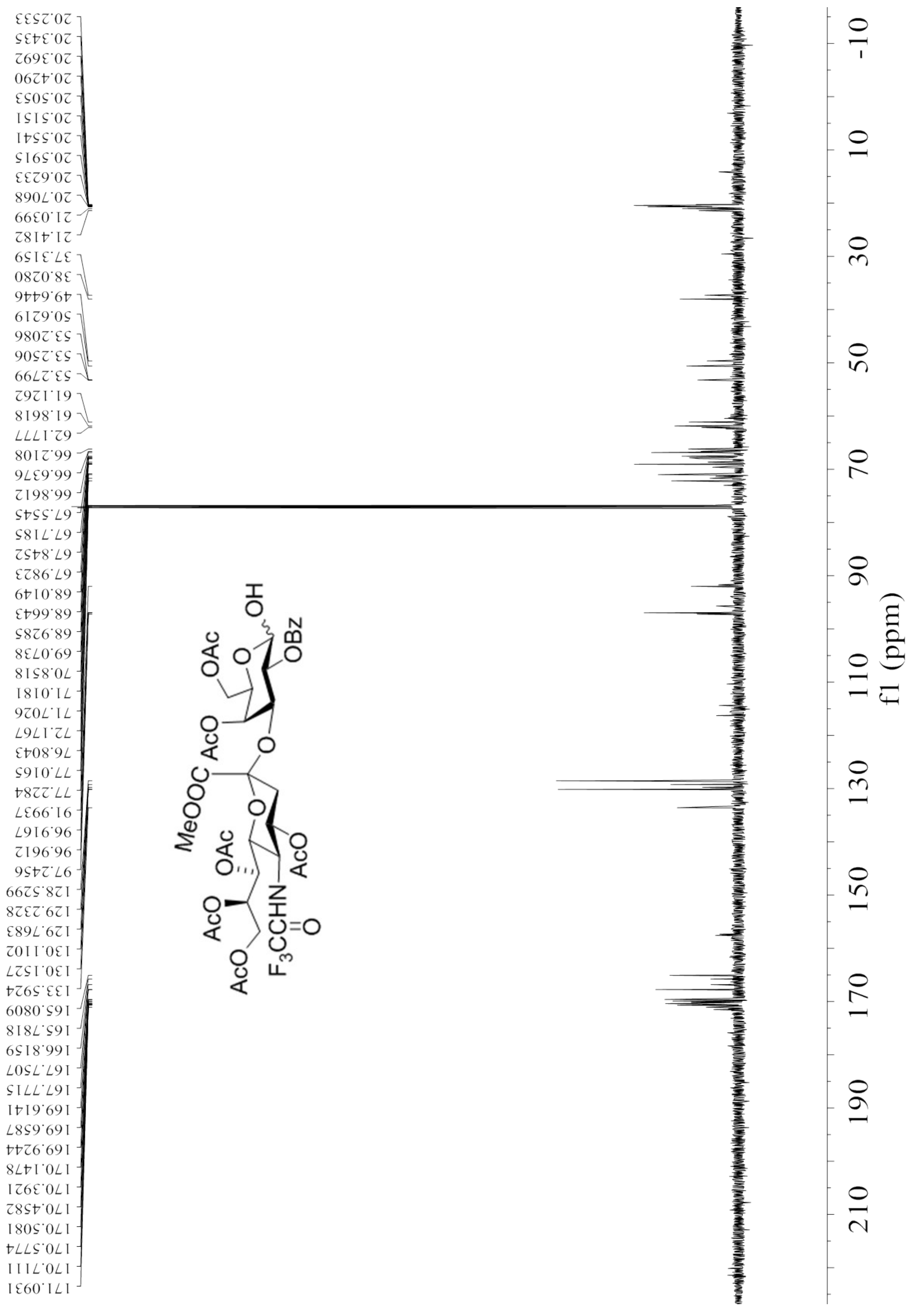
^{1}H-^{13}C HMQC Spectrum of compound **20** (CDCl$_3$, 600/150 MHz)



SFORD Spectrum of compound **20** ($CDCl_3$, 600/150 MHz)

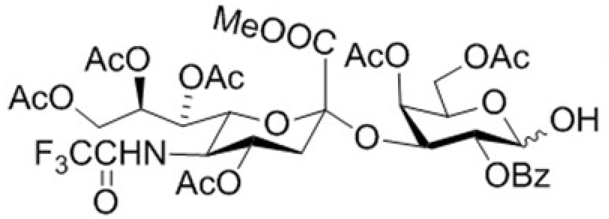


^{1}H NMR Spectrum of compound **23** ($CDCl_3$, 600 MHz)



^{13}C NMR Spectrum of compound **23**(CDCl$_3$, 150 MHz)

Elemental Composition Report



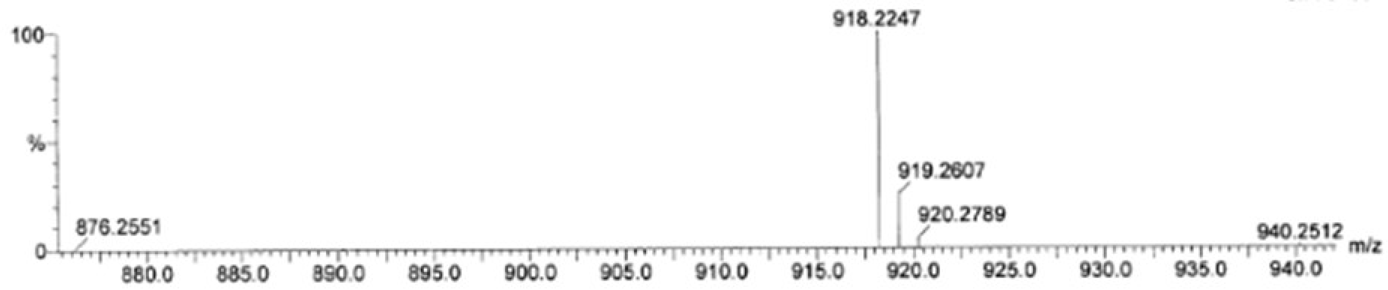
Single Mass Analysis

Tolerance = 5.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 6

Monoisotopic Mass, Odd and Even Electron Ions
567 formula(e) evaluated with 2 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 37-37 H: 0-50 N: 0-2 O: 0-25 F: 0-3 Na: 0-1

G. Liao liao-3-199 in CH2Cl2 Cone(V)60

LCT Premier KD128
TOF MS ES+

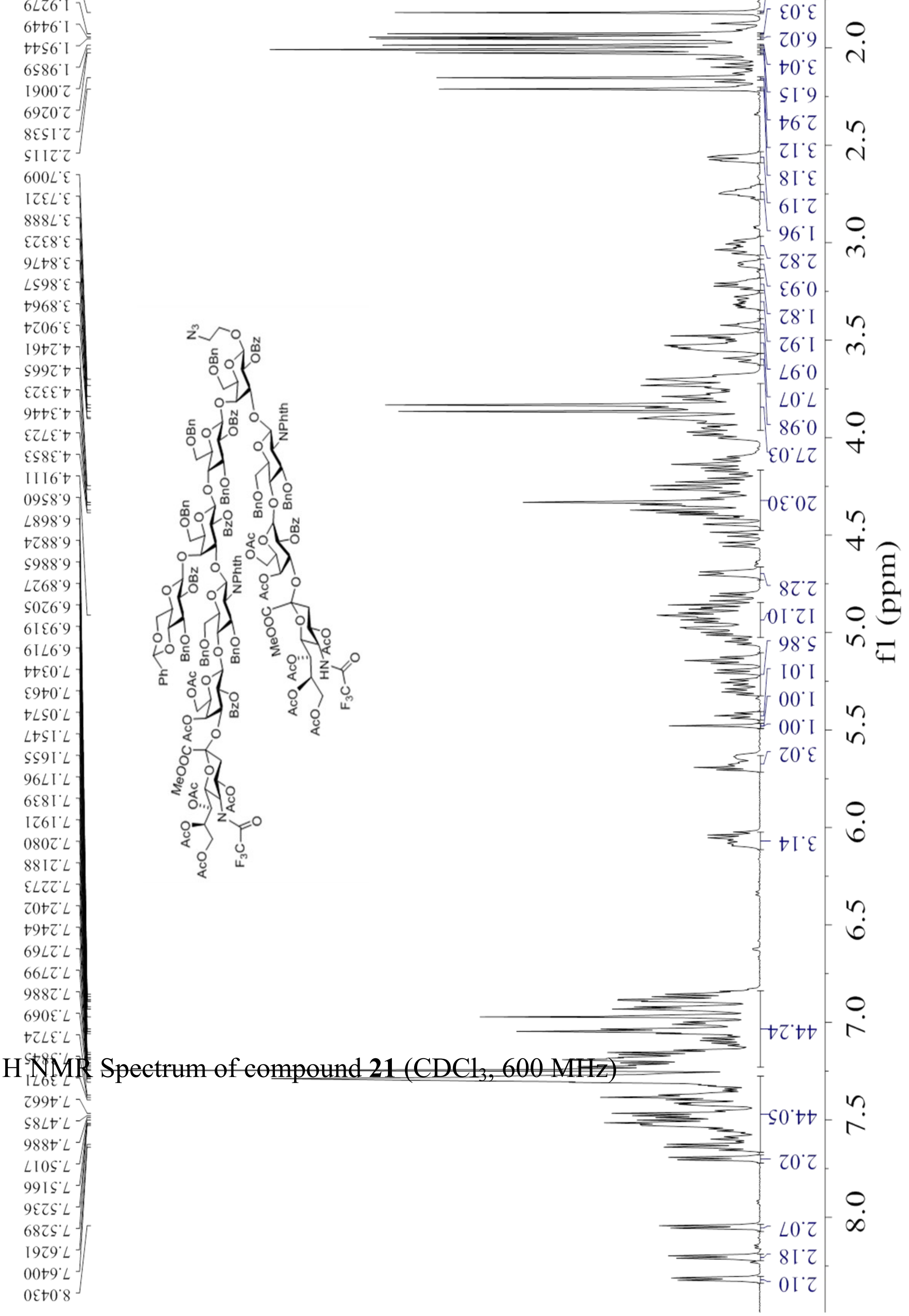


```
Minimum:                                  -1.5
Maximum:                  3.0       5.0   100.0

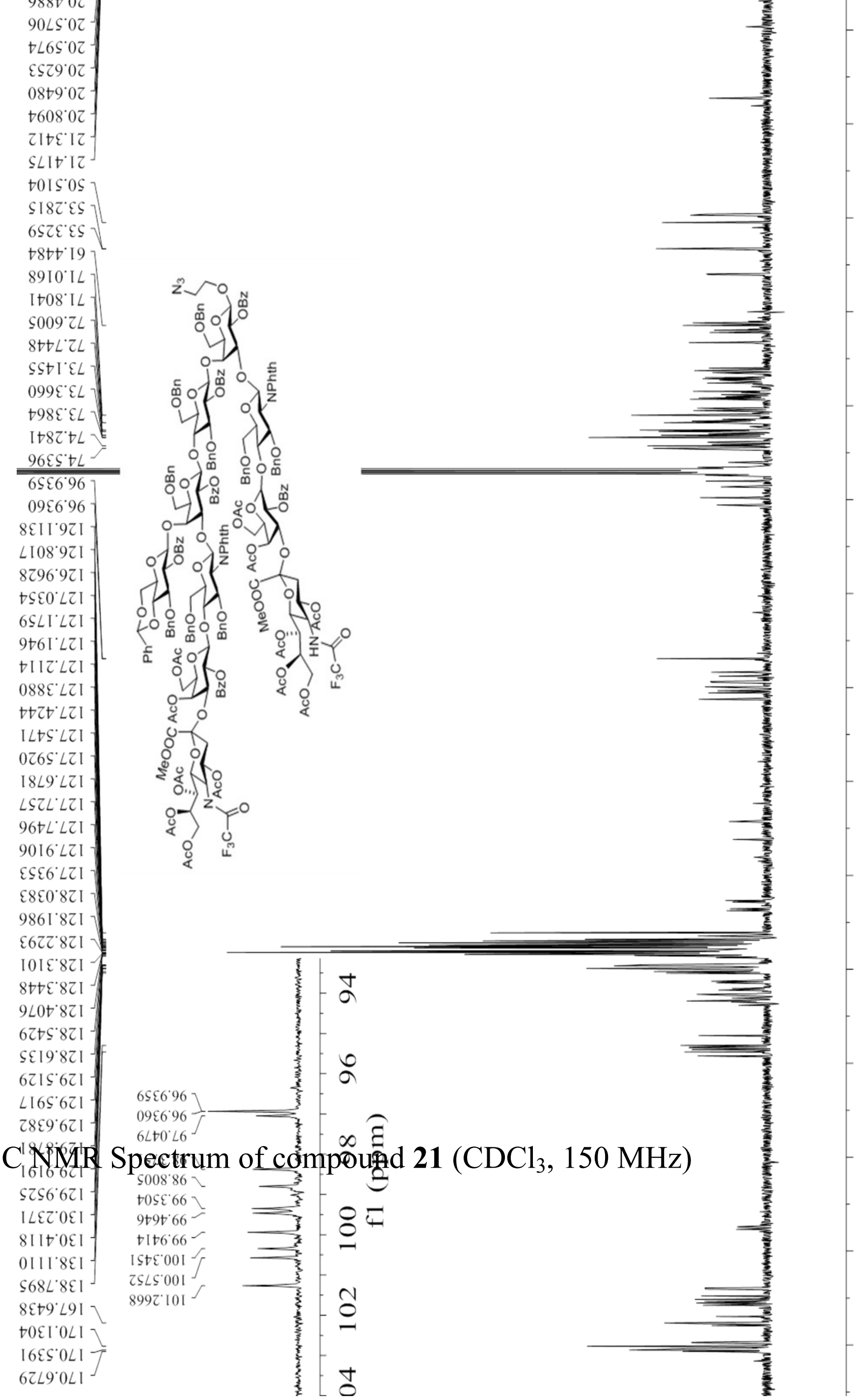
Mass       Calc. Mass     mDa       PPM       DBE       i-FIT     i-FIT (Norm)  Formula

918.2247   918.2256       -0.9      -1.0      14.5      56.3      0.5           C37  H44  N  O21  F3
                                                                                Na
           918.2217       3.0       3.3       14.0      56.7      0.9           C37  H45  O23  F2
                                                                                Na
```

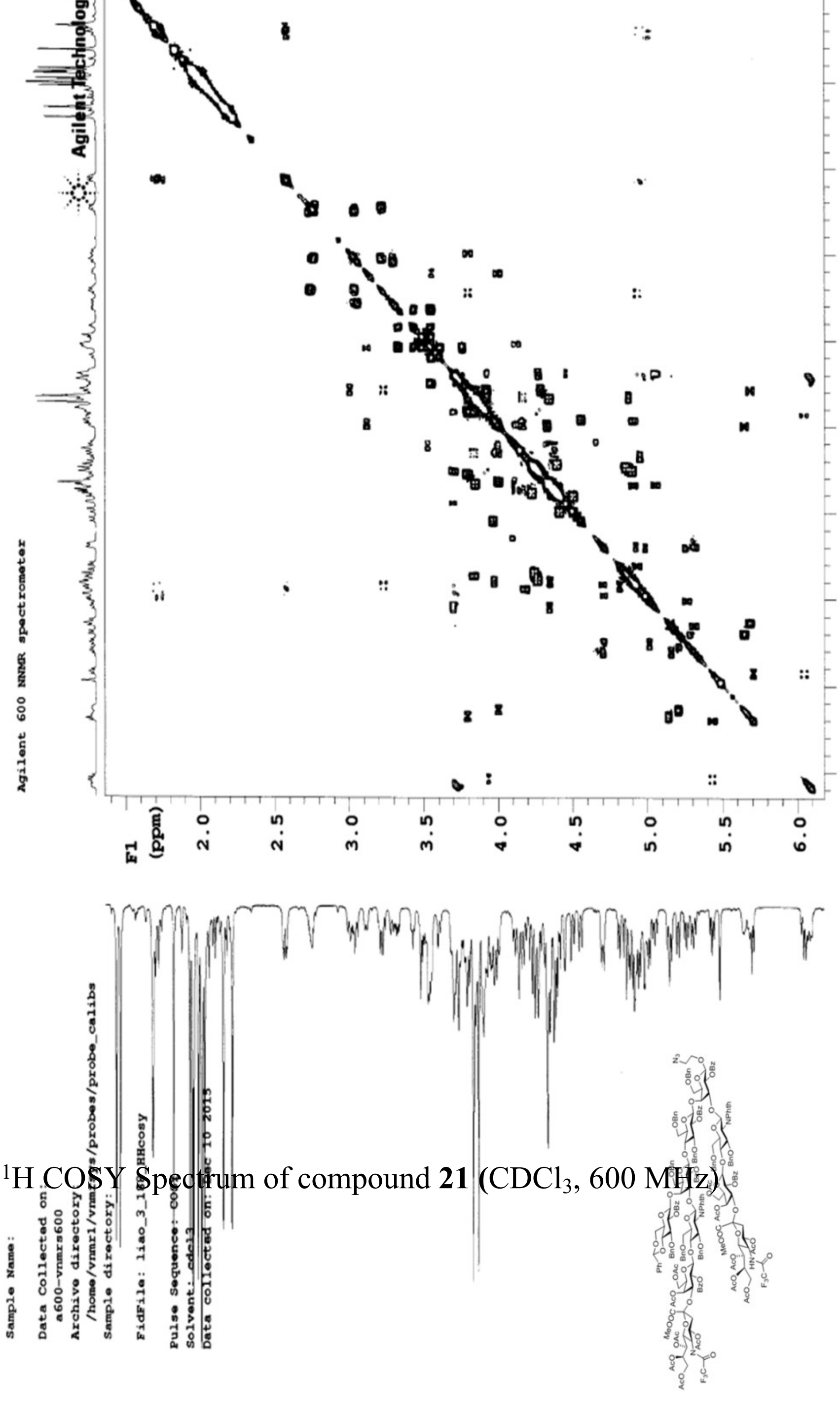
HR ESI-TOF MS Spectrum of compound **23**



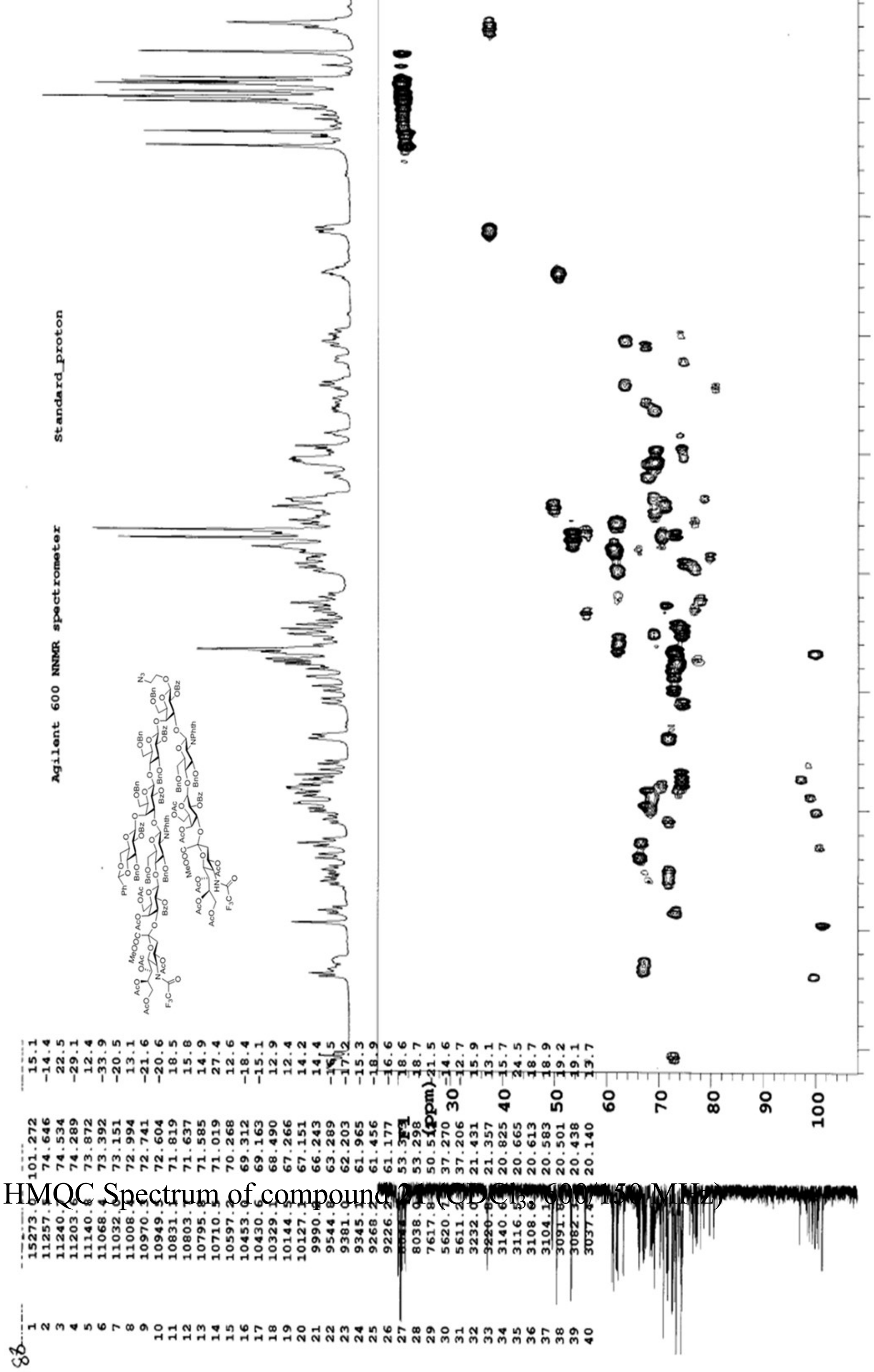
^{1}H NMR Spectrum of compound **21** (CDCl$_3$, 600 MHz)



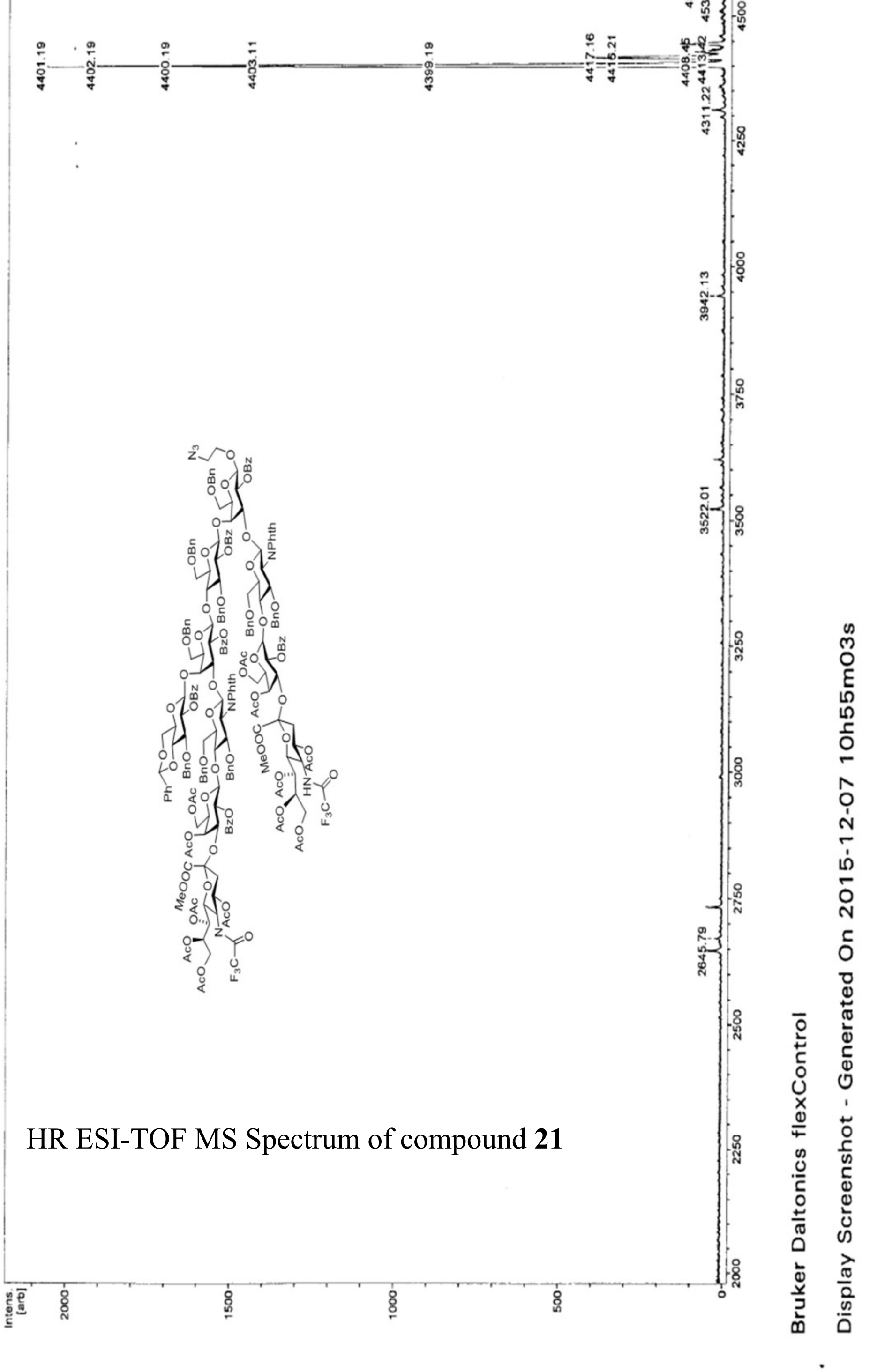
^{13}C NMR Spectrum of compound **21** (CDCl$_3$, 150 MHz)



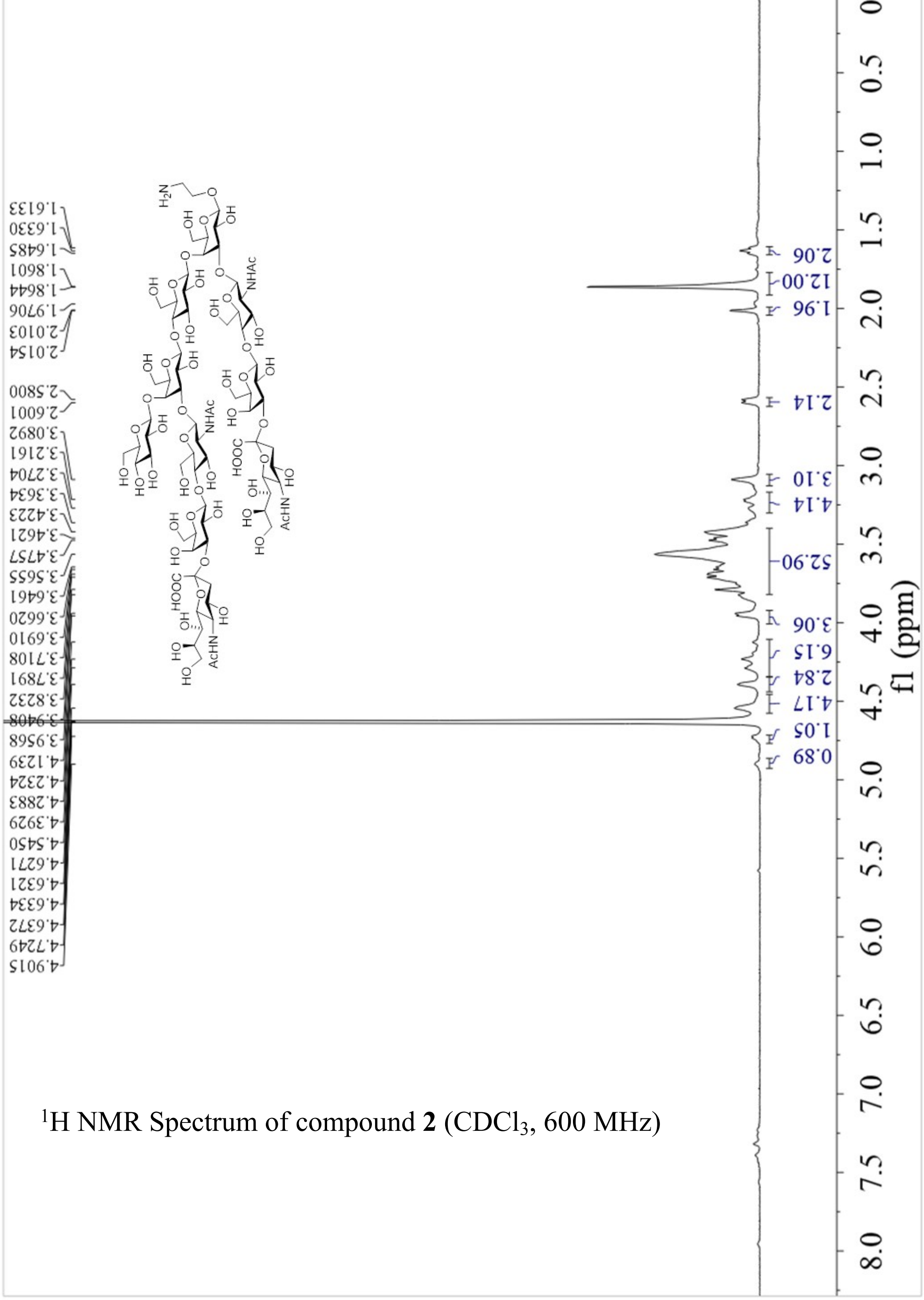
^{1}H-^{1}H COSY Spectrum of compound **21** (CDCl$_3$, 600 MHz)



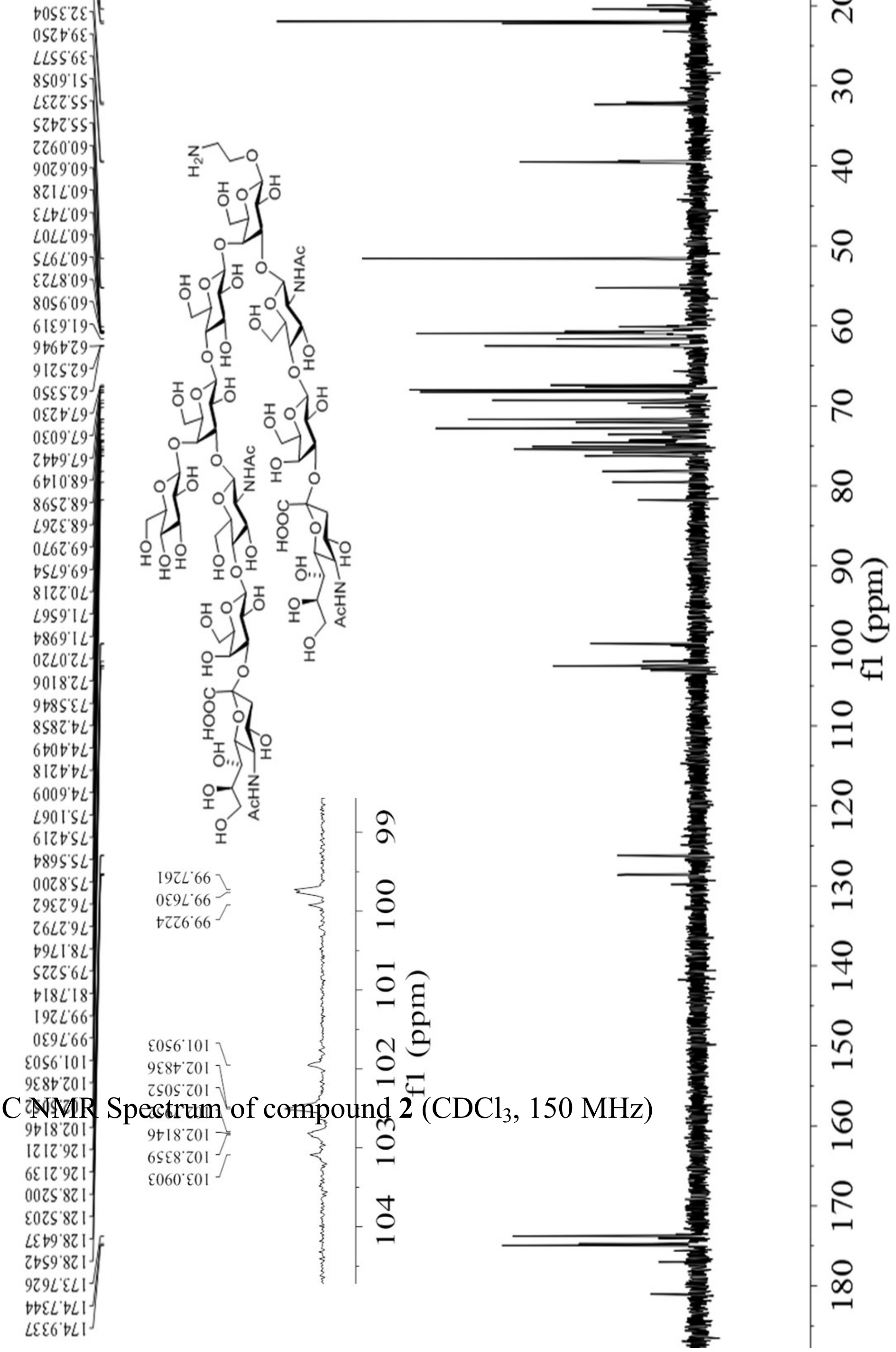
^1H-^{13}C HMQC Spectrum of compound 21 ($CDCl_3$, 600/150 MHz)



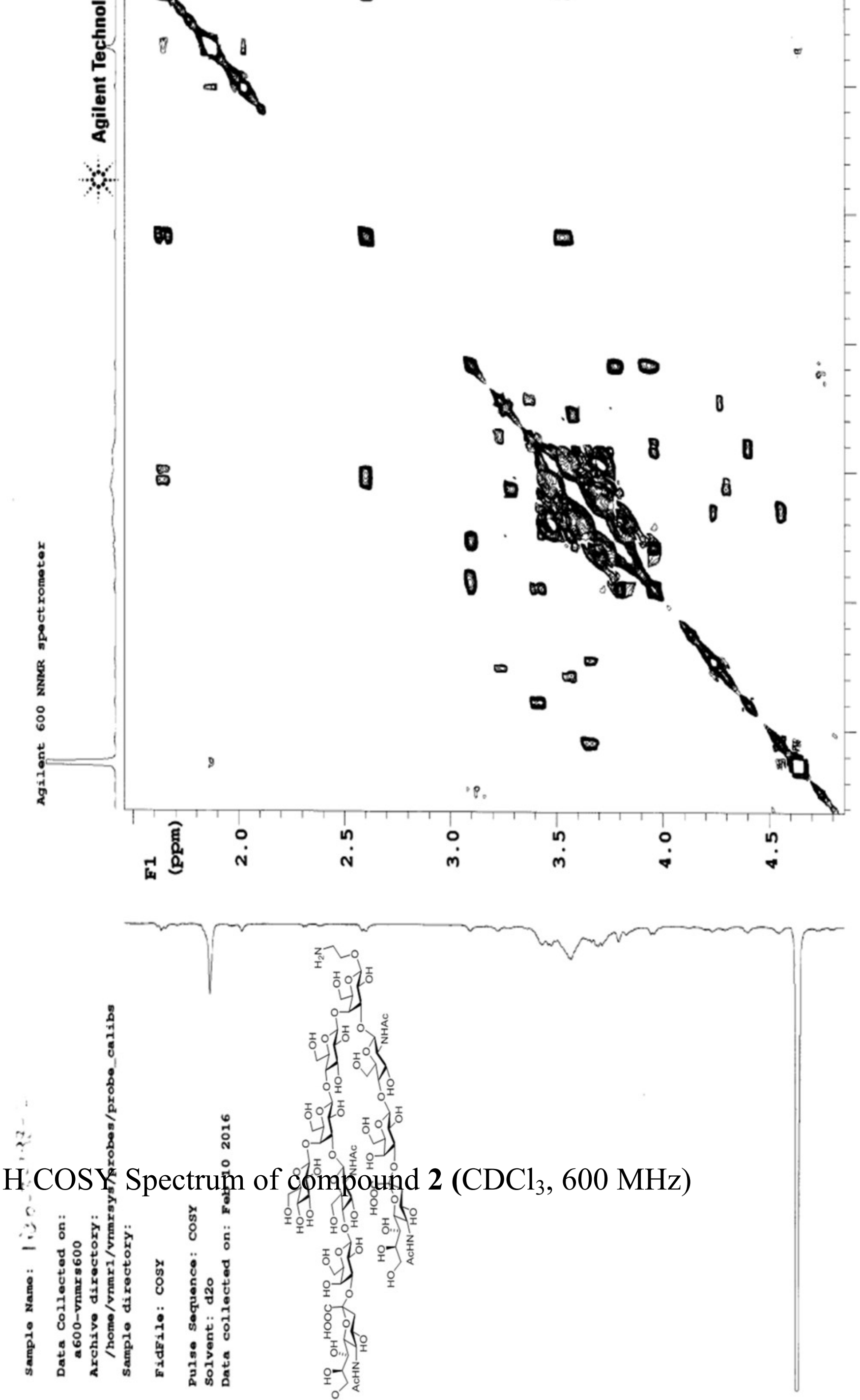
HR ESI-TOF MS Spectrum of compound **21**



^{1}H NMR Spectrum of compound **2** ($CDCl_3$, 600 MHz)



^{13}C NMR Spectrum of compound **2** (CDCl$_3$, 150 MHz)

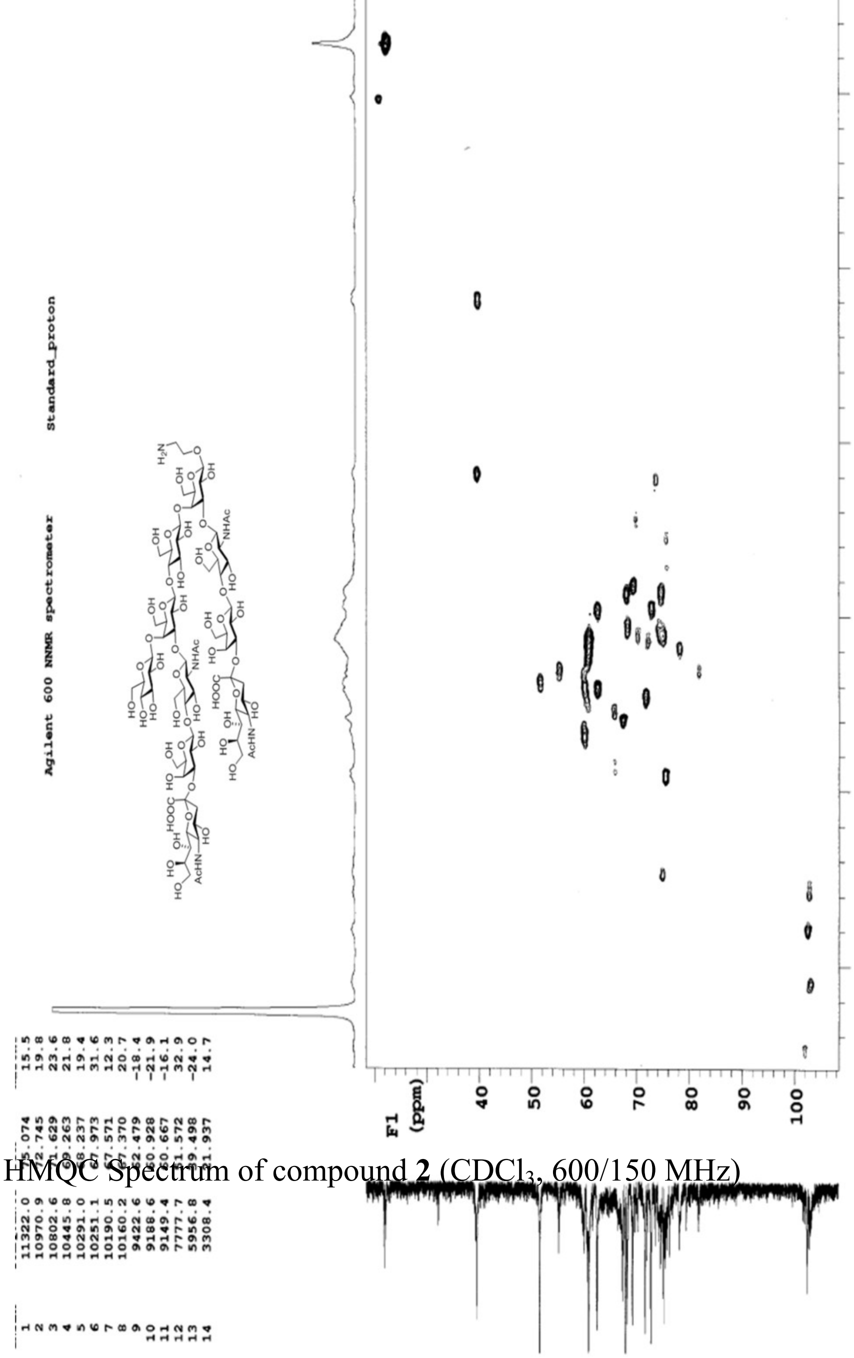


Sample Name:
Data Collected on:
a600-vnmrs600
Archive directory:
/home/vnmr1/vnmrsys/probes/probe_calibs
Sample directory:

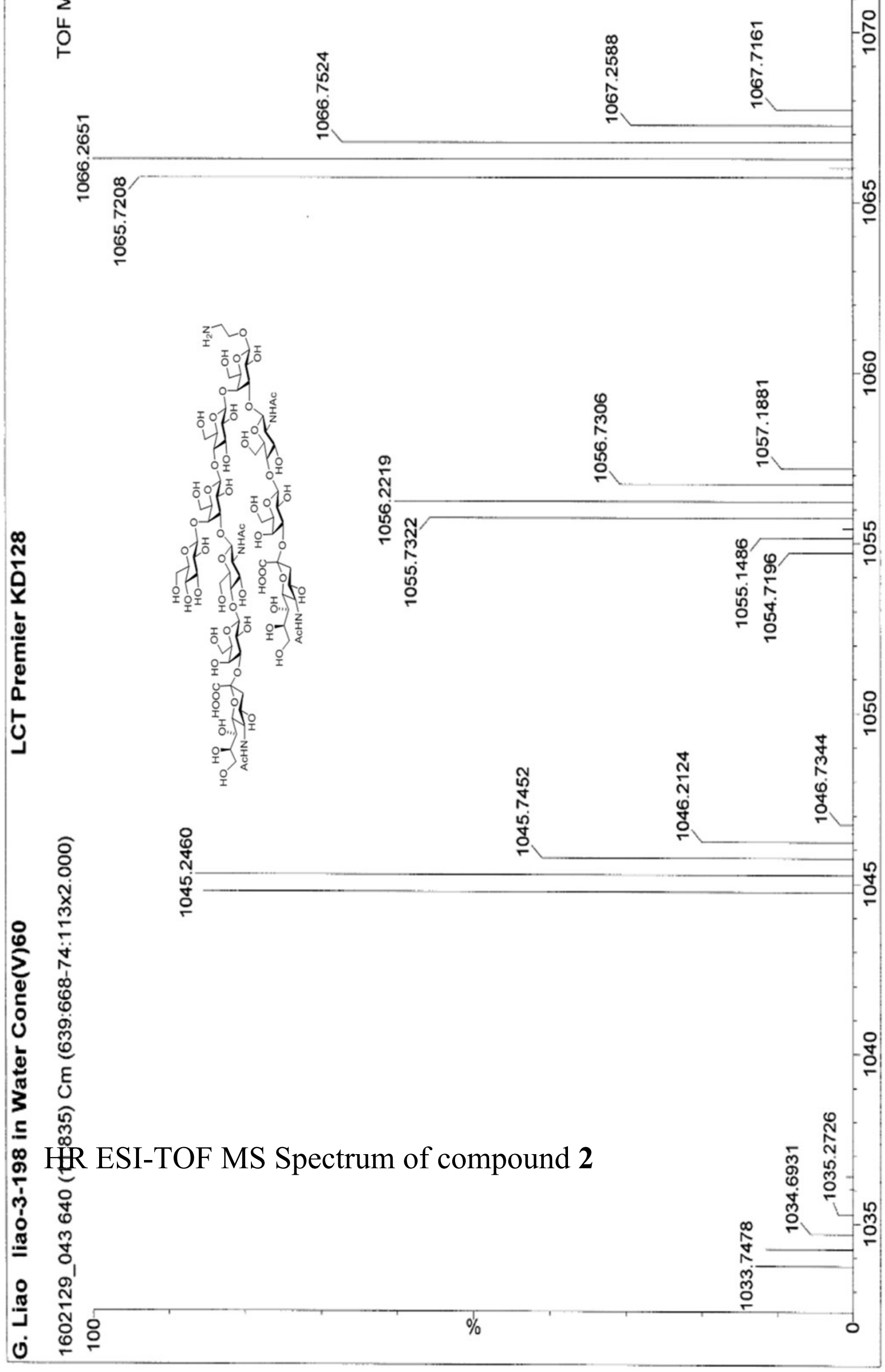
FidFile: COSY

Pulse Sequence: COSY
Solvent: d2o
Data collected on: Feb 10 2016

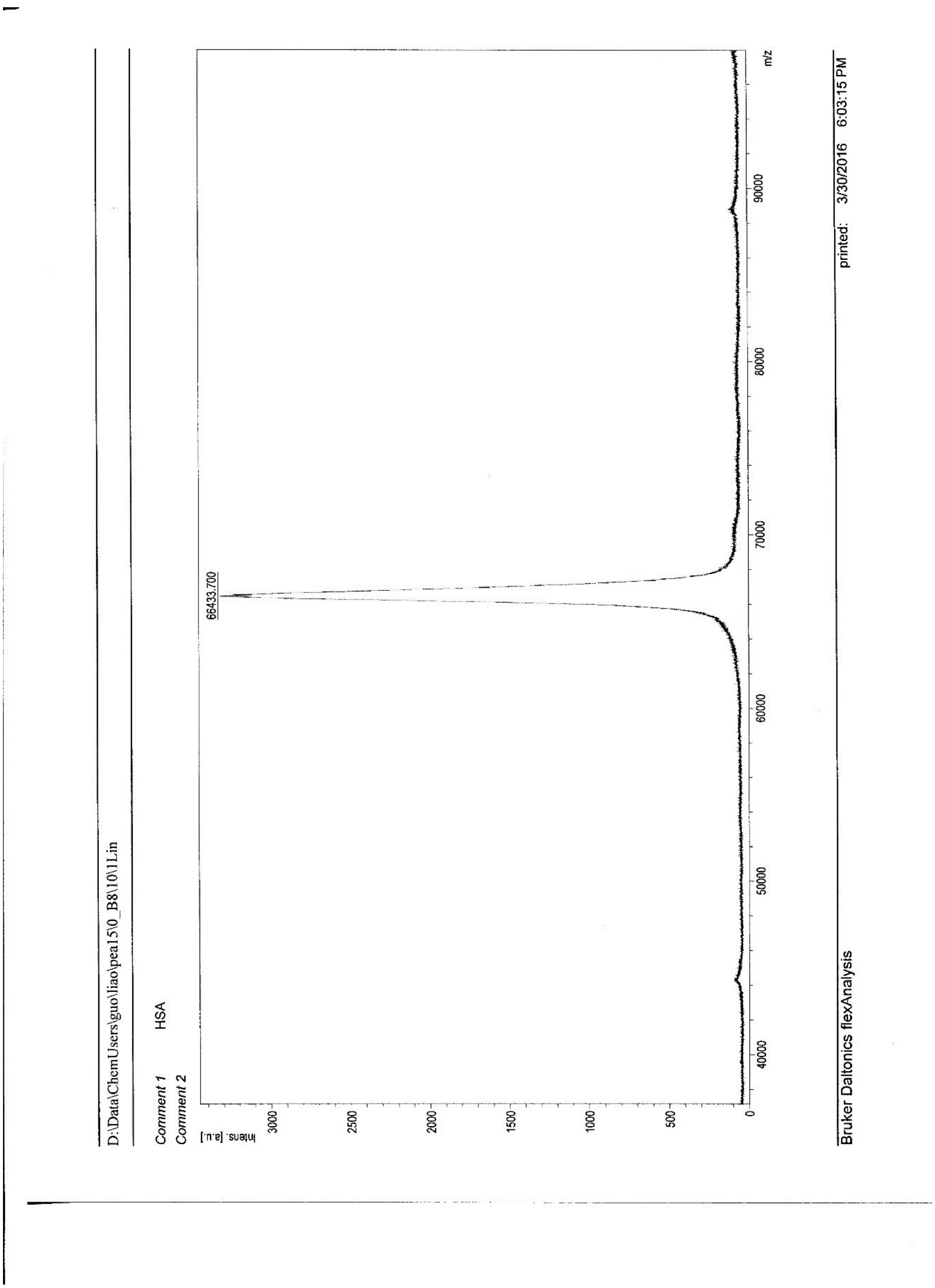
^{1}H-^{1}H COSY Spectrum of compound **2** (CDCl$_3$, 600 MHz)



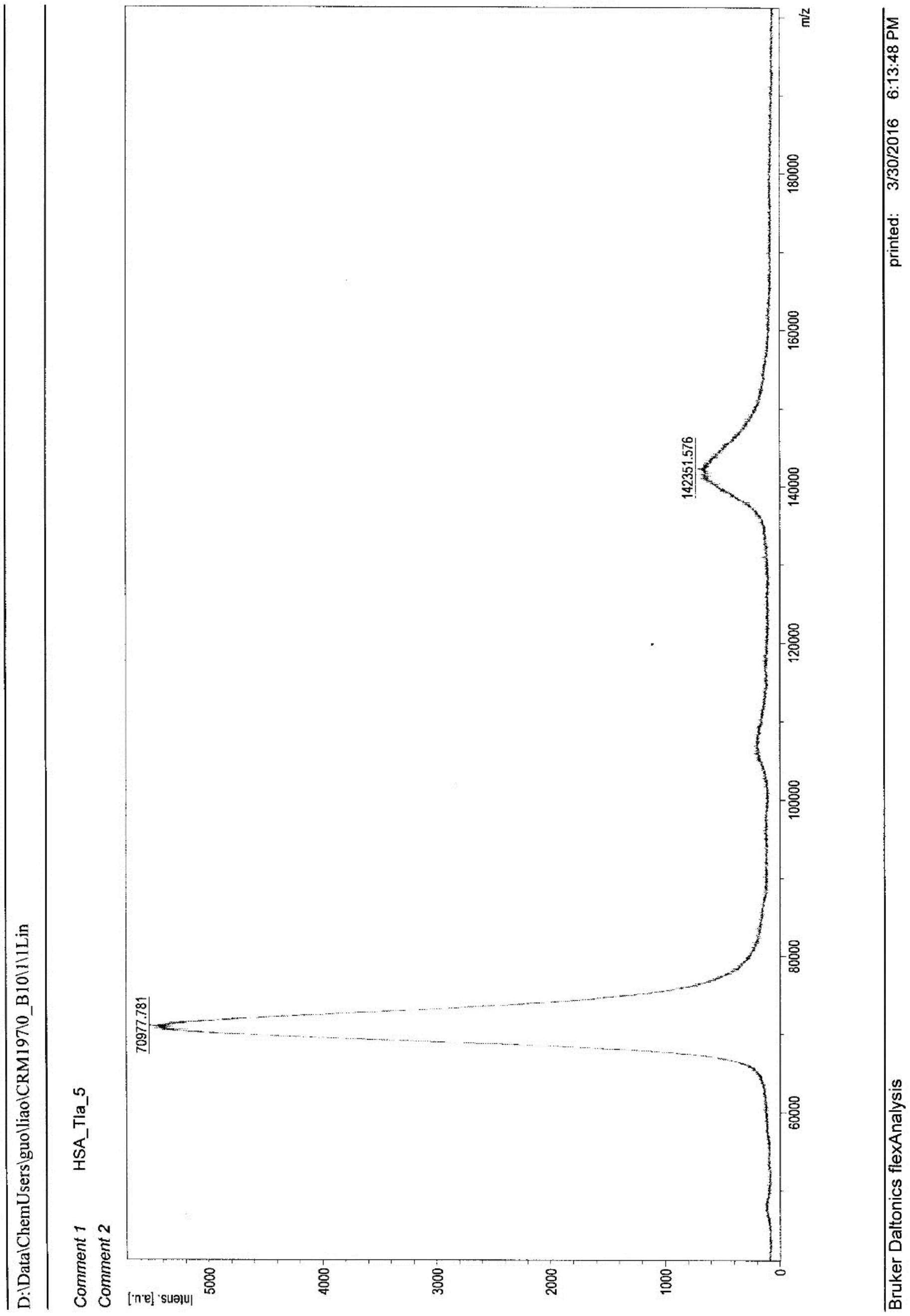
^{1}H-^{13}C HMQC Spectrum of compound **2** (CDCl$_3$, 600/150 MHz)



HR ESI-TOF MS Spectrum of compound **2**



MALDI TOF MS spectrum of HSA protein



D:\Data\ChemUsers\guo\liao\CRM197\0_B10\1\1Lin

Comment 1 HSA_Tla_5

Comment 2

Bruker Daltonics flexAnalysis

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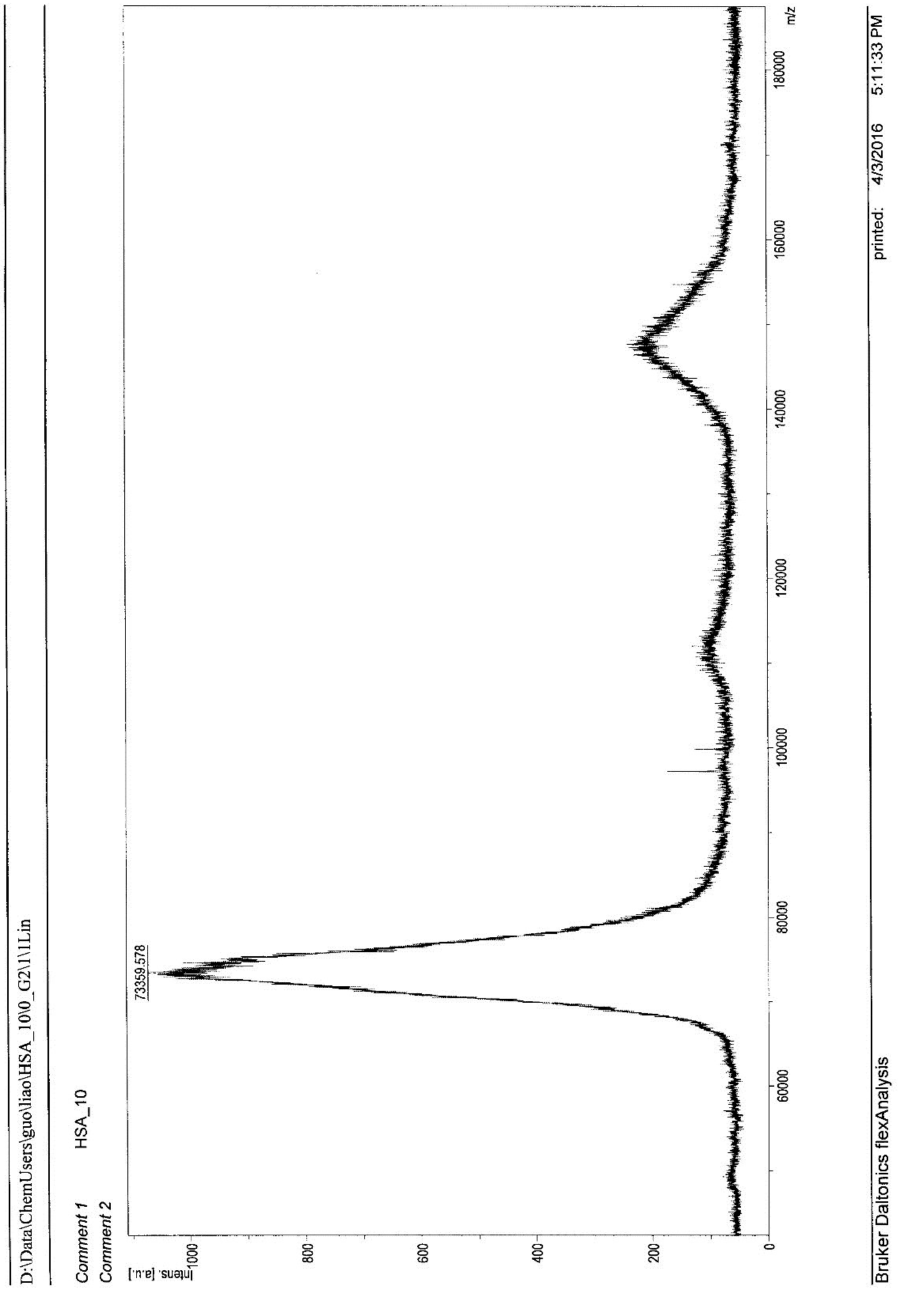
MALDI TOF MS spectrum of monomer - HSA conjugate **2a**



MALDI TOF MS spectrum of Dimer - HSA conjugate **2b**