Supporting Information

for

Synthesis of biotin-labeled core glycans of GPI anchors and their application to the study of GPI interaction with pore-forming bacterial toxin

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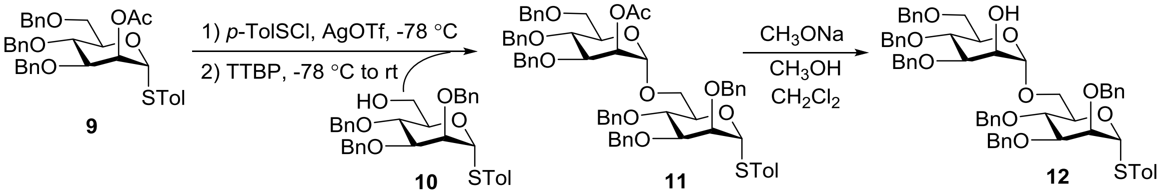
I. Experimental procedures for the synthesis

General Methods: Chemicals and materials were purchased from commercial sources and were used as received without further purification unless otherwise noted. Molecular sieve 4Å (MS 4Å) was flame-dried under high vacuum and cooled to rt under a N_2 atmosphere immediately before use. Analytical TLC was carried out on Silica Gel 60Å F254 plates with detection by UV and/or by charring with 15% H_2SO_4 in EtOH (w/v). Mass spectrometry (MS) was performed using a high resolution ESI-TOF MS machine. NMR spectra were recorded on a 500 or 600 MHz machine with chemical shifts reported in ppm (δ) downfield from internal tetramethylsilane (TMS) reference. NMR signals are described as s (singlet), d (doublet), t (triplet) or m (multiplet), and the coupling constants are reported in Hz.

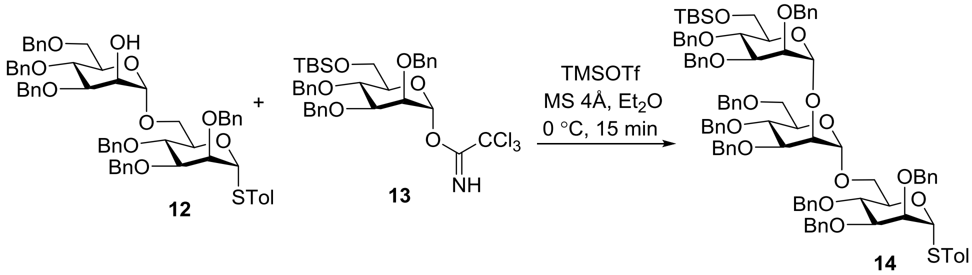
p-Tolyl (3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→6)-2,3,4-tri-*O*-benzyl-1-thio-α-D-mannopyranoside 12:



A mixture of glycosyl donor **9** (700 mg, 1.17 mmol) and freshly activated MS 4Å in anhydrous CH_2Cl_2 (6 mL) was stirred at rt for 1 h and then cooled to -78 °C. A solution of AgOTf (903 mg, 3.51 mmol) in acetonitrile (1 mL) was added. After 15 min of stirring, *p*-TolSCl (169 μL, 1.17 mmol) was added quickly. Fifteen minutes later, a solution of glycosyl acceptor **10** (591 mg, 1.06 mmol) and TTBP (263 mg, 1.06 mmol) in anhydrous CH_2Cl_2 (2.0 mL) was added. The reaction mixture was then warmed to rt in 1.5 h, stirred for another 20 min, and then quenched with Et_3N, diluted with CH_2Cl_2, and filtered. The filtrate was concentrated under vacuum, and the residue that contained compound **11** was dissolved in CH_2Cl_2 and CH_3OH (1:2, 12 mL). To this solution was added CH_3ONa (0.5 M in CH_3OH) until the pH reached 10. After 1 h of stirring, Amberlyst 15-hydrogen resin was added to neutralize the reaction mixture, which was filtered. The filtrate was concentrated under vacuum, and the residue was purified by silica column chromatography with EtOAc and hexane (1:2) as the eluents to afford **12** (818 mg, 78% for two steps) as a foamy solid. ^1H NMR (600 MHz, $CDCl_3$) δ: 7.40 – 7.23 (m, 30H), 7.20 – 7.16 (m, 2H), 7.09 (d, J = 8.4 Hz, 2H), 5.52 (d, J = 1.8 Hz, 1H), 5.04 (d, J = 1.2 Hz, 1H), 4.95 (d, J = 11.4 Hz, 1H), 4.84 (d, J =

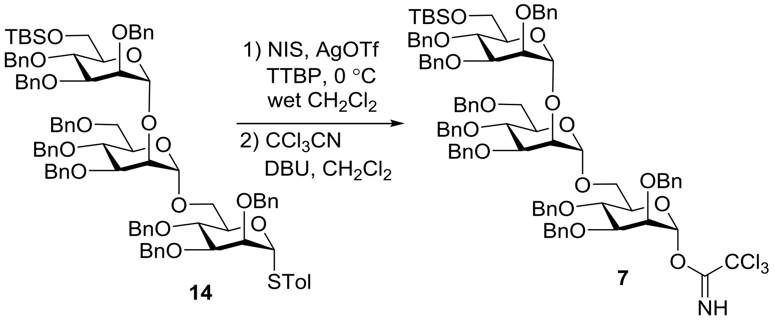
10.8 Hz, 1H), 4.71 (d, *J* = 12.6 Hz, 1H), 4.67 – 4.58 (m, 5H), 4.56 – 4.46 (m, 4H), 4.28 – 4.24 (m, 1H), 4.09 (s, 1H), 4.04 – 4.02 (m, 1H), 4.00 – 3.94 (m, 2H), 3.88 (m, 3H), 3.83 – 3.78 (m, 1H), 3.77 – 3.73 (m, 1H), 3.72 (dd, *J* = 10.8, 4.2 Hz, 1H), 3.63 (dd, *J* = 10.8, 1.8 Hz, 1H), 2.43 (d, *J* = 2.4 Hz, 1H), 2.22 (s, 3H). ^{13}C NMR (150 MHz, CDCl$_3$) δ: 138.52, 138.36, 138.17, 138.09, 137.88, 137.82, 137.52, 131.54, 130.85, 129.89, 128.50, 128.45, 128.41, 128.38, 128.32, 128.27, 127.97, 127.93, 127.91, 127.88, 127.85, 127.80, 127.78, 127.65, 127.57, 127.54, 99.53, 85.92, 80.20, 79.70, 76.36, 75.15, 75.09, 74.73, 74.21, 73.35, 72.35, 72.02, 71.98, 71.62, 70.99, 68.71, 68.09, 66.47, 21.00. HR ESI-TOF MS (m/z): calcd for $C_{61}H_{65}O_{10}S$ [M + H]$^+$, 989.4298; found, 989.4297.

***p*-Tolyl [2,3,4-tri-*O*-benzyl-6-*O*-(*tert*-butyldimethylsilyl)-α-D-mannopyranosyl]-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→6)-2,3,4-tri-*O*-benzyl-1-thio-α-D- mannopyranoside 14:**



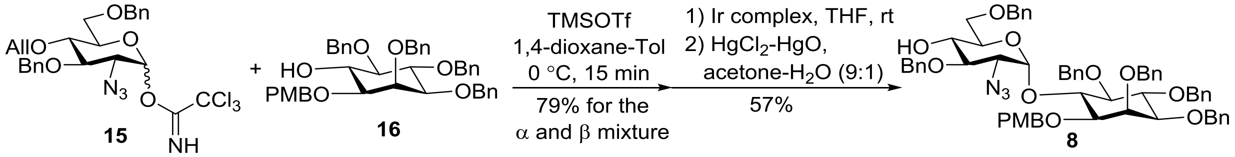
To a stirred mixture of **12** (700 mg, 0.708 mmol), **13** (602 mg, 0.849 mmol) and freshly activated MS 4Å in anhydrous diethyl ether (6 mL) was added TMSOTf (15 µL, 0.085 mmol) at 0 °C under N_2 protection. After the mixture was stirred for another 15 min, it was neutralized with Et_3N, filtered, and concentrated. The residue was subjected to silica gel column chromatography with EtOAc and hexane (1:5) as the eluents to give **14** (946 mg, 87%) as a foamy solid. ^{1}H NMR (600 MHz, CDCl$_3$) δ: 7.40 – 7.12 (m, 47H), 7.09 (d, *J* = 8.0 Hz, 2H), 5.54 (s, 1H), 5.21 (s, 1H), 4.95 (d, *J* = 11.4, 1H), 4.92 (d, *J* = 11.4, 1H), 4.88 (s, 1H), 4.85 (d, *J* = 10.8 Hz, 1H), 4.74 (d, *J* = 12.0 Hz, 1H), 4.66 (d, *J* = 12.0 Hz, 2H), 4.60 (m, 3H), 4.54 (dd, *J* = 10.8, 5.4 Hz, 2H), 4.51 – 4.44 (m, 7H), 4.28 (m, 1H), 4.13 (m, 1H), 4.05 – 4.02 (m, 2H), 3.99 (dd, *J* = 10.8, 5.4 Hz, 1H), 3.95 – 3.86 (m, 5H), 3.79 (m, 4H), 3.73 – 3.65 (m, 3H), 3.60 (d, *J* = 11.4 Hz, 1H), 2.16 (s, 3H), 0.89 (s, 9H), 0.05 (s, 3H), 0.04 (s, 3H). ^{13}C NMR (150 MHz, CDCl$_3$) δ: 139.03, 138.79, 138.73, 138.59, 138.55, 138.40, 138.15, 138.08, 137.92, 137.42, 131.45, 131.14, 129.89, 128.41, 128.40, 128.38, 128.31, 128.27, 128.25, 128.24, 128.19, 128.07, 127.99, 127.86, 127.75, 127.72, 127.70, 127.63, 127.49, 127.41, 127.34, 127.31, 127.24, 99.30, 98.82, 86.10, 80.39, 79.94, 79.72, 76.37, 75.12, 75.07, 74.99, 74.78, 74.76, 74.63, 73.55, 73.23, 73.21, 72.18, 72.13, 72.00, 71.94, 71.89, 71.85, 69.19, 66.73, 62.61, 25.96, 20.94, 18.33, -5.02, -5.27. HR ESI-TOF MS (m/z): calcd for $C_{94}H_{107}O_{15}SSi$ $[M + H]^+$, 1535.7100; found, 1535.7158.

6-*O*-{[2,3,4-tri-*O*-Benzyl-6-*O*-(*tert*-butyldimethylsilyl)-α-D-mannopyranosyl]-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)}-2,3,4-tri-*O*-benzyl-α-D-mannopyranosyl trichloroacetimidate 7:



To a solution of **14** (800 mg, 0.521 mmol) and TTBP (388 mg, 1.563 mmol) in wet CH_2Cl_2 (8 mL) were added *N*-iodosuccinimide (234 mg, 1.042 mmol) and AgOTf (268 mg, 1.042 mmol) at 0 °C. The mixture was stirred at rt for 2 h, quenched with saturated aq. $Na_2S_2O_3$ solution, and diluted with CH_2Cl_2 (200 mL). The organic phase, after being washed with saturated aq. NaCl solution, was dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by silica gel column chromatography with EtOAc and hexane (1:4) as the eluents to afford a white solid. To a solution of this product in anhydrous CH_2Cl_2 (8 mL) was added trichloroacetonitrile (261 µL, 2.605 mmol) and DBU (15 µL, 0.104 mmol) at 0 °C under N_2 protection. After 1 h of stirring, the mixture was concentrated in vacuum. The residue was purified on an Et_3N-neutralized silica gel column with EtOAc and hexane (1:10) as the eluents to afford **7** (582 mg, 71%) as a white foamy solid. ^{1}H NMR (600 MHz, $CDCl_3$) δ: 8.57 (s, 1H), 7.42 (d, *J* = 7.5 Hz, 2H), 7.38 – 7.12 (m, 43H), 6.34 (d, *J* = 1.8 Hz, 1H), 5.20 (s, 1H), 4.92 (d, *J* = 11.4 Hz, 1H), 4.91 (d, *J* = 11.4 Hz, 1H), 4.88 (s, 1H), 4.81 (d, *J* = 10.8 Hz, 1H), 4.79 (d, *J* = 12.6 Hz, 1H), 4.73 (d, *J* = 12.6 Hz, 1H), 4.65 (d, *J* = 12.0 Hz, 2H), 4.61 (m, 2H), 4.54 – 4.44 (m, 9H), 4.12 (d, *J* = 1.8 Hz, 1H), 4.03 (t, *J* = 9.6 Hz, 1H), 3.99 – 3.84 (m, 9H), 3.74 (m, 6H), 3.58 (d, *J* = 11.4 Hz, 1H), 0.91 (s, 9H), 0.09 (s, 3H), 0.07 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ: 160.05, 139.02, 138.79, 138.75, 138.69, 138.58, 138.20, 138.17, 138.00, 137.88, 128.40, 128.39, 128.35, 128.25, 128.23, 128.21, 128.18, 128.07, 127.99, 127.96, 127.88, 127.86, 127.83, 127.78, 127.76, 127.74, 127.68, 127.64, 127.61, 127.46, 127.39, 127.32, 127.25, 127.23, 99.13, 98.89, 95.76, 90.99, 79.89, 79.75, 79.18, 75.17, 75.06, 74.97, 74.93, 74.66, 74.07, 74.05, 73.83, 73.55, 73.26, 73.17, 72.68, 72.26, 72.18, 71.92, 71.89, 71.83, 69.21, 66.25, 62.65, 25.99, 18.35, -5.00, -5.21.

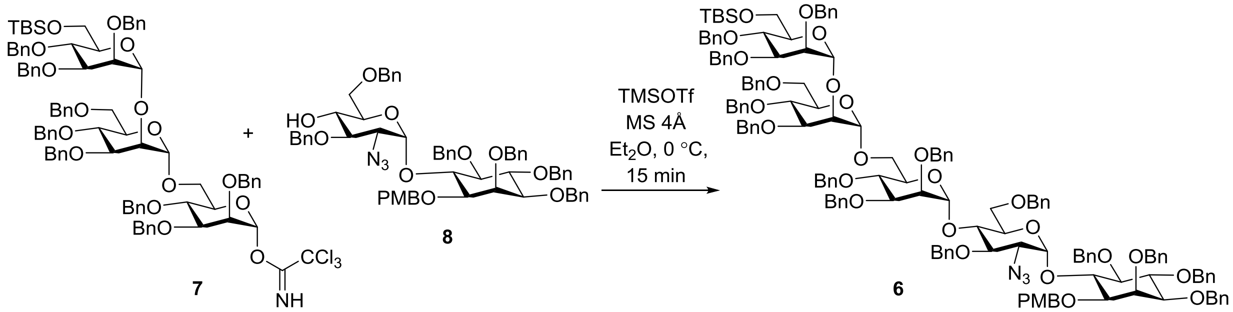
6-*O*-(2-Azido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-2,3,4,5-tetra-*O*-benzyl-1-*O*-(4-methoxybenzyl)-*myo*-inositol 8:



To a stirred mixture of **15** (569 mg, 1.00 mmol), **16** (600 mg, 0.909 mmol), and freshly activated MS 4Å in a mixture of 1,4-dioane and toluene (1:1, v/v, 6 mL) was added TMSOTf (18 μL, 0.10 mmol) at 0 °C under N_2 protection. After the mixture was stirred for another 15 min, it was neutralized with Et_3N, filtered, and concentrated. The residue was subjected to silica gel column chromatography with EtOAc and hexane (1:10) as the eluents to afford the α,β-mixture (767 mg, 79%) as colorless syrup. After the solution of $[Ir(COD)(PMePh_2)_2]PF_6$ (59 mg, 0.070 mmol) in THF (3 mL) was stirred under H_2 atmosphere at rt until the red color turned to pale yellow (in ca. 10 min) and H_2 was exchanged with argon for three times, to this solution was added slowly the above α,β-mixture (750 mg, 0.702 mmol) in anhydrous THF (4 mL). The mixture was stirred at rt for 30 min, at which point TLC showed the complete reaction. The mixture was concentrated in vacuum. The residue was dissolved in acetone and water (10 mL, 9:1, v/v), and the solution was treated with $HgCl_2$ (954 mg, 3.51 mmol) and HgO (23 mg, 0.105 mmol). Ten minutes later, the solution was concentrated, and the residue was purified by silica gel column chromatography with EtOAc and hexane (1:7) as the eluents to afford **8** (411 mg, 57%) as a white foamy solid. ^{1}H NMR (600 MHz, $CDCl_3$) δ: 7.46 – 7.17 (m, 32H), 6.87 (d, *J* = 8.4 Hz, 2H), 5.74 (d, *J* = 3.0 Hz, 1H), 5.04 (d, *J* = 11.4 Hz, 1H), 4.97 (d, *J* = 10.2 Hz, 1H), 4.91 – 4.86 (m, 2H), 4.85 – 4.78 (m, 3H), 4.69 – 4.61 (m, 3H), 4.51 (s, 2H), 4.42 (d, *J* = 12.0 Hz, 1H), 4.27 (m, 2H), 4.14 (t, *J* = 9.6 Hz, 1H), 4.04 (s, 1H), 4.00 – 3.96 (m, 1H), 3.82 – 3.76 (m, 4H), 3.71 (dt, *J* = 9.6, 3.6 Hz, 1H), 3.50 – 3.39 (m, 3H), 3.31 (dd, *J* = 10.2, 3.6 Hz, 1H), 3.21 (dt, *J* = 10.2, 3.6 Hz, 2H), 1.96 (d, *J* = 3.6 Hz, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) δ: 159.30, 138.81, 138.58, 138.35, 138.17, 138.15, 137.86, 129.62, 129.58, 128.55, 128.44, 128.32, 128.25, 128.19, 127.92, 127.79, 127.77, 127.74, 127.72, 127.64, 127.57, 127.52, 127.47, 127.44, 113.86, 97.38, 81.96, 81.88, 81.59, 80.82, 79.30, 75.71, 75.60, 74.80, 74.72, 74.16, 73.46, 73.36, 72.76, 71.99, 71.86, 69.43, 68.83, 62.57, 55.29.

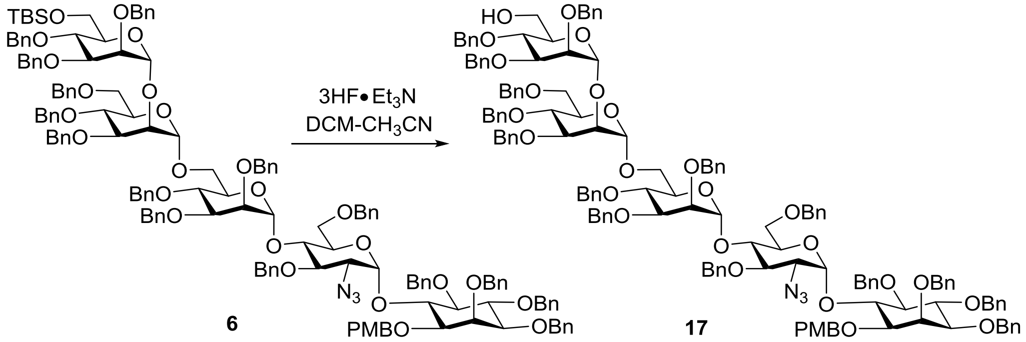
The data were consistant with those reported in the literature (Z. Wu, X. Guo, J. Gao, and Z. Guo, *Chem. Commun*. **2013**, *49*, 11689-11691).

6-*O*-{[2,3,4-tri-*O*-Benzyl-6-*O*-(*tert*-butyldimethylsilyl)-α-D-mannopyranosyl]-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→6)-(2,3,4-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→4)-(2-azido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)}-2,3,4,5-tetra-*O*-benzyl-1-*O*-(4-methoxybenzyl)-*myo*-inositol 6:



To a stirred mixture of **7** (505 mg, 0.321 mmol), **8** (300 mg, 0.292 mmol), and freshly activated MS 4Å in 8 mL of anhydrous diethyl ether was added TMSOTf (5.8 μL, 0.032 mmol) at 0 °C under N_2 protection. After 15 min of stirring, the mixture was neutralized with Et_3N, filtered, and concentrated. The residue was subjected to silica gel column chromatography with EtOAc and hexane (1:6) as the eluents to afford **6** (620 mg, 87%) as a white foamy solid. ^{1}H NMR (600 MHz, $CDCl_3$) δ: 7.39 (d, J = 7.2 Hz, 2H), 7.36 – 7.07 (m, 75H), 6.86 (d, J = 9.0 Hz, 2H), 5.80 (d, J = 3.6 Hz, 1H), 5.24 (s, 1H), 5.16 (s, 1H), 5.00 – 4.88 (m, 5H), 4.86 – 4.70 (m, 6H), 4.64 (m, 4H), 4.57 – 4.30 (m, 17H), 4.22 (d, J = 12.0 Hz, 1H), 4.13 – 4.03 (m, 6H), 3.98 – 3.76 (m, 11H), 3.74 – 3.64 (m, 4H), 3.51 (m, 9H), 3.32 (d, J = 10.2 Hz, 1H), 3.28 (dd, J = 11.4, 3.6 Hz, 1H), 3.20 (dd, J = 10.2, 3.6 Hz, 1H), 0.88 (s, 9H), 0.05 (s, 3H), 0.03 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ: 159.29, 139.11, 138.96, 138.82, 138.71, 138.61, 138.58, 138.55, 138.41, 138.27, 138.22, 138.03, 137.83, 129.63, 129.48, 128.51, 128.42, 128.32, 128.27, 128.22, 128.18, 128.15, 128.12, 128.03, 127.97, 127.85, 127.76, 127.75, 127.72, 127.67, 127.64, 127.60, 127.49, 127.43, 127.39, 127.34, 127.28, 127.23, 127.17, 127.14, 127.12, 127.06, 126.82, 113.85, 100.52, 99.29, 98.72, 97.32, 81.94, 81.86, 81.55, 80.87, 80.02, 79.89, 79.65, 77.42, 76.26, 75.77, 75.50, 75.10, 75.08, 74.98, 74.88, 74.55, 74.43, 74.29, 74.20, 73.76, 73.44, 73.27, 73.18, 73.16, 72.72, 72.10, 71.97, 71.90, 71.76, 71.73, 71.66, 69.57, 69.06, 68.59, 66.43, 63.07, 62.40, 55.28, 25.98, 18.32, -4.95, -5.23. HR ESI-TOF MS (m/z): calcd for $C_{149}H_{164}O_{26}N_3Si$ $[M + H]^+$, 2439.1372; found, 2439.1265.

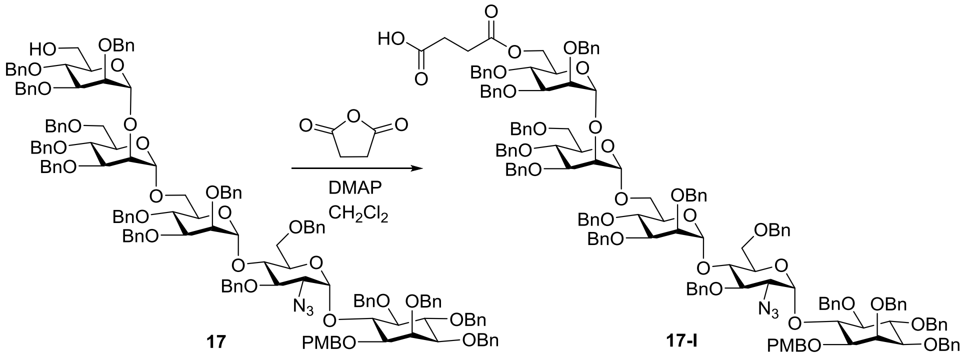
6-*O*-[(2,3,4-tri-*O*-Benzyl-α-D-mannopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→6)-(2,3,4-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→4)-(2-azido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)]-2,3,4,5-tetra-*O*-benzyl-1-*O*-(4-methoxybenzyl)-*myo*-inositol 17:



A solution of **6** (115 mg, 0.047 mmol) and triethylamine trihydrofluoride (1.0 mL) in CH_2Cl_2 and CH_3CN (1:1, 3 mL) was stirred at rt for one day under argon. The solution was quenched with dropwise addition of saturated aq. $NaHCO_3$ solution. The aq. phase was extracted with CH_2Cl_2 (3 × 30 mL), and the organic layer, after being dried over Na_2SO_4, was concentrated. The residue was purified by silica gel column chromatography with EtOAc and hexane (1:4) as the eluents to give **17** (96 mg, 88%) as colorless syrup. ^{1}H NMR (600 MHz, $CDCl_3$) δ: 7.40 (d, J = 6.6 Hz, 2H), 7.38 – 7.03 (m, 75H), 6.87 (d, J = 8.4 Hz, 2H), 5.79 (d, J = 3.6 Hz, 1H), 5.26 (s, 1H), 5.05 (s, 1H), 4.99 (d, J = 11.4 Hz, 1H), 4.95 – 4.35 (m, 30H), 4.32 (t, J = 9.6 Hz, 1H), 4.24 (d, J = 12.0 Hz, 1H), 4.17 (d, J = 12.0 Hz, 1H), 4.12 (t, J = 9.6 Hz, 1H), 4.07 – 4.04 (m, 2H), 3.97 (t, J = 9.6 Hz, 1H), 3.94 – 3.55 (m, 19H), 3.52 – 3.36 (m, 7H), 3.26 (dd, J = 11.4, 3.0 Hz, 1H), 3.21 (dd, J = 10.2, 3.6 Hz, 1H), 2.01 (m, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) δ: 159.30, 138.92, 138.84, 138.65, 138.61, 138.59, 138.55, 138.46, 138.41, 138.30, 138.27, 138.23, 138.10, 137.83, 129.63, 129.47, 128.49, 128.47, 128.42, 128.36, 128.31, 128.27, 128.20, 128.05, 127.98, 127.90, 127.81, 127.71, 127.67, 127.64, 127.62, 127.57, 127.49, 127.43, 127.36, 127.35, 127.30, 127.25, 127.17, 127.07, 127.00, 126.83, 100.44, 99.54, 99.36, 97.37, 81.94, 81.87, 81.55, 80.88, 80.03, 79.79, 79.72, 79.33, 77.13, 76.27, 75.78, 75.40, 75.16, 75.00, 74.92, 74.88, 74.73, 74.58, 74.27, 74.21, 74.17, 73.70, 73.27, 73.23, 72.80, 72.72, 72.34, 72.29, 72.16, 72.04, 71.95, 71.74, 71.72, 69.71, 69.09, 68.54, 66.05, 62.95, 62.18, 55.28. The data were consistant with those reported in the literature (Z. Wu, X. Guo, J. Gao, and Z. Guo, *Chem Commun*. **2013**, *49*, 11689-11691).

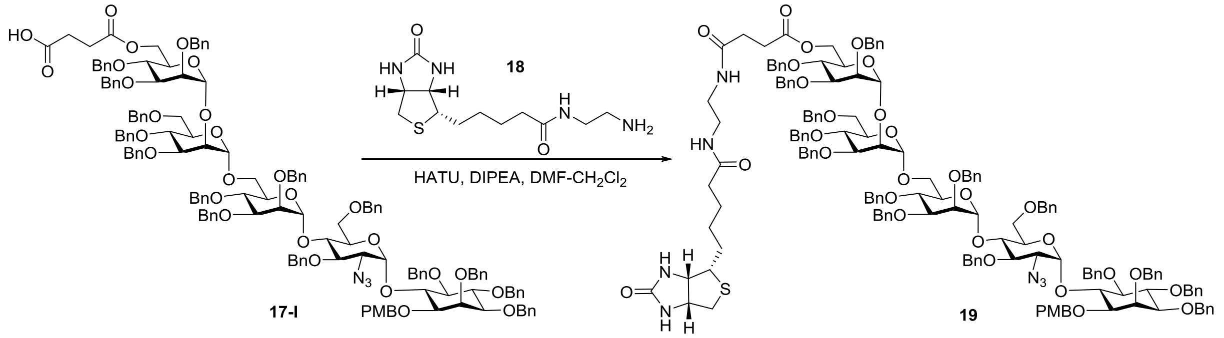
6-*O*-[(2,3,4-tri-*O*-Benzyl-6-*O*-succinyl-α-D-mannopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→6)-(2,3,4-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→4)-(2-azido-3,6-di-

***O*-benzyl-2-deoxy-α-D-glucopyranosyl)]-2,3,4,5-tetra-*O*-benzyl-1-*O*-(4-methoxybenzyl)-*myo*-inositol 17-I:**



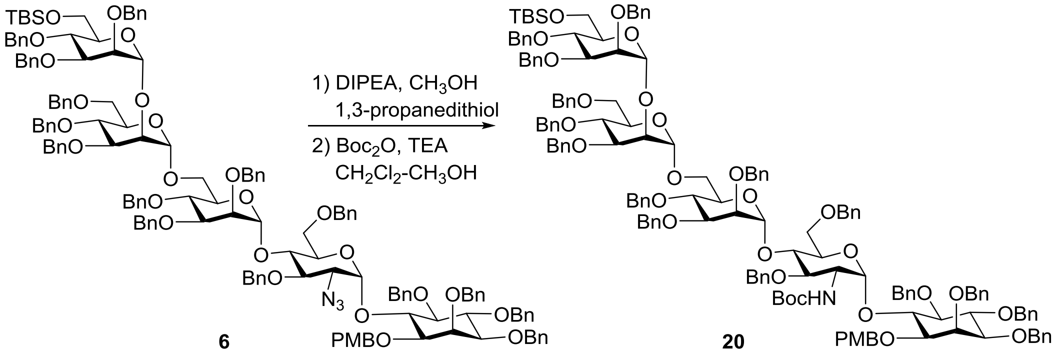
To a solution of **17** (85 mg, 0.037 mmol) in anhydrous CH_2Cl_2 (3.0 mL) were added succinic anhydride (14.8 mg, 0.148 mmol) and DMAP (18.1 mg, 0.148 mmol) at rt under N_2 protection. The mixture was stirred at rt overnight and then treated with AcOH, concentrated in vacuum, and purified by silica gel column chromatography with EtOAc and hexane (1:3) as the eluents to give **17-I** (72.7 mg, 82%) as a white solid. ^{1}H NMR (600 MHz, $CDCl_3$) δ: 7.57 – 6.93 (m, 77H), 6.87 (d, J = 8.4 Hz, 2H), 5.78 (d, J = 3.6 Hz, 1H), 5.31 (s, 1H), 5.21 (s, 1H), 5.01 – 4.89 (m, 5H), 4.85 – 4.71 (m, 6H), 4.63 (dt, J = 11.7, 8.2 Hz, 3H), 4.57 – 4.31 (m, 18H), 4.21 (m, 2H), 4.17 – 4.07 (m, 4H), 4.04 (s, 1H), 3.99 (t, J = 9.6 Hz, 1H), 3.91 – 3.74 (m, 12H), 3.72 – 3.66 (m, 2H), 3.58 – 3.32 (m, 10H), 3.29 – 3.25 (m, 2H), 2.53 – 2.47 (m, 1H), 2.46 – 2.37 (m, 2H), 2.29 – 2.23 (m, 1H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 173.16, 171.64, 159.31, 138.93, 138.82, 138.65, 138.55, 138.47, 138.42, 138.39, 138.31, 138.20, 138.02, 137.93, 137.68, 129.59, 129.51, 128.49, 128.46, 128.41, 128.38, 128.31, 128.26, 128.21, 128.16, 128.10, 127.99, 127.97, 127.93, 127.75, 127.71, 127.67, 127.64, 127.61, 127.56, 127.52, 127.42, 127.39, 127.29, 127.18, 127.13, 127.09, 126.97, 113.88, 100.05, 99.10, 98.34, 97.35, 81.89, 81.85, 81.52, 80.87, 80.20, 79.81, 79.65, 79.64, 76.33, 75.75, 75.45, 75.14, 75.06, 75.04, 74.90, 74.63, 74.51, 74.38, 74.19, 73.96, 73.66, 73.26, 73.20, 73.16, 72.71, 72.49, 72.19, 72.13, 71.97, 71.91, 71.85, 71.75, 71.60, 70.36, 69.76, 68.94, 68.54, 65.78, 64.33, 62.82, 55.28, 29.48, 28.60. HR ESI-TOF MS (m/z): calcd for $C_{147}H_{152}O_{29}N_3$ $[M - H]^+$, 2423.0512; found, 2423.0503.

6-*O*-{[2,3,4-tri-*O*-Benzyl-6-*O*-(*N*-biotinylethylenediaminyl-succinamide)-α-D-mannopyranosyl]-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→6)-(2,3,4-tri-*O*-benzyl-α-D-manno pyranosyl)-(1→4)-(2-azido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)}-2,3,4,5-tetra-*O*-benzyl-1-*O*-(4-methoxybenzyl)-*myo*-inositol 19:



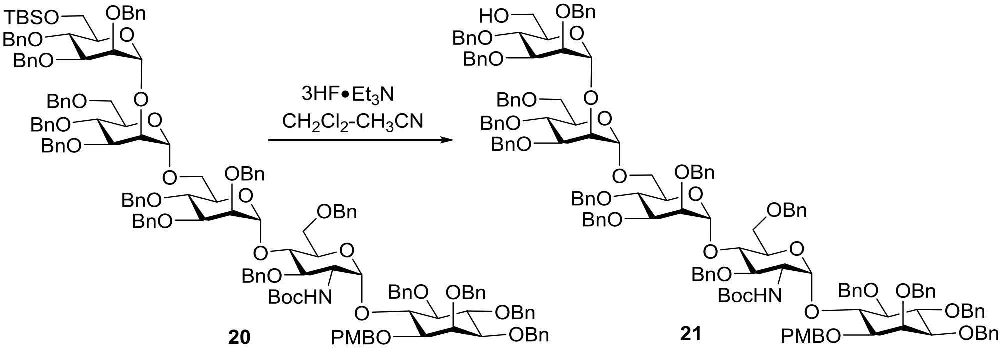
To a solution of HATU (12.2 mg, 0.032 mmol) in anhydrous DMF and DCM (3 mL, 1:1) was added **17-I** (65.0 mg, 0.027 mmol) at 0 °C under N_2 protection, followed by addition of DIPEA (11μL, 0.064 mmol). The mixture was stirred at 0 °C for 20 min and then biotin ethylenediamine **18** (14.8 mg, 0.040 mmol) was added. The reaction was allowed to continue for 10 h at rt. The mixture was poured into saturated aq. NH_4Cl, and the mixture was extracted with EtOAc. The organic phase, after being washed with saturated aq. NaCl, was dried over Na_2SO_4 and concentrated in vacuum. The residue was purified by silica gel column chromatography with EtOAc and hexane (1:2) as the eluents to give **19** (56.7 mg, 78%) as a white solid. ^{1}H NMR (600 MHz, $CDCl_3$) δ: 7.55 – 6.93 (m, 77H), 6.88 – 6.84 (d, J = 9.0 Hz, 2H), 6.80 (s, 1H), 6.37 (s, 1H), 6.28 (s, 1H), 5.77 (d, J = 3.0 Hz, 1H), 5.52 (s, 1H), 5.27 (s, 1H), 5.09 (s, 1H), 5.01 – 4.91 (m, 4H), 4.88 – 4.78 (m, 5H), 4.75 – 4.71 (m, 2H), 4.66 – 4.60 (m, 3H), 4.51 (m, 8H), 4.43 – 4.29 (m, 11H), 4.24 (d, J = 12.6 Hz, 1H), 4.19 (d, J = 12.0 Hz, 1H), 4.14 – 4.03 (m, 6H), 3.97 (t, J = 9.6 Hz, 1H), 3.92 – 3.75 (m, 12H), 3.73 (t, J = 9.6 Hz, 1H), 3.66 (s, 1H), 3.58 (d, J = 10.8 Hz, 1H), 3.55 – 3.39 (m, 6H), 3.36 – 3.30 (m, 2H), 3.27 – 3.23 (m, 2H), 3.20 – 3.13 (m, 3H), 3.00 – 2.97 (m, 1H), 2.74 (dd, J = 12.6, 4.2 Hz, 1H), 2.60 (d, J = 12.6 Hz, 1H), 2.58 – 2.49 (m, 2H), 2.28 – 2.21 (m, 2H), 2.15 – 2.08 (m, 2H), 1.66 – 1.54 (m, 4H), 1.35 – 1.33 (m, 2H). ^{13}C NMR (150 MHz, $CDCl_3$) δ: 173.69, 172.80, 172.45, 163.76, 159.31, 138.91, 138.82, 138.58, 138.54, 138.47, 138.39, 138.37, 138.29, 138.25, 138.20, 138.09, 137.97, 137.79, 129.62, 129.51, 128.49, 128.42, 128.37, 128.33, 128.32, 128.27, 128.21, 128.19, 128.15, 128.06, 128.00, 127.94, 127.75, 127.73, 127.71, 127.64, 127.57, 127.51, 127.44, 127.38, 127.31, 127.24, 127.18, 127.13, 127.04, 126.83, 113.88, 100.33, 99.17, 99.10, 97.36, 81.87, 81.51, 80.88, 80.21, 79.65, 79.49, 76.37, 75.75, 75.48, 75.20, 74.97, 74.88, 74.56, 74.36, 74.21, 74.17, 74.13, 73.75, 73.30, 73.23, 73.19, 72.72, 72.26, 72.24, 72.05, 72.03, 71.97, 71.91, 71.80, 71.75, 70.34, 69.61, 69.00, 68.60, 66.26, 63.39, 62.96, 61.47, 60.10, 55.40, 55.30, 40.40, 40.05, 39.22, 38.60, 35.62, 30.80, 29.72, 27.84, 27.81, 25.21. ESI-TOF MS (m/z): calcd for $C_{159}H_{174}O_{30}N_7S$ $[M + H]^+$, 2693.2026; found, 2693.2097.

6-*O*-{[2,3,4-tri-*O*-Benzyl-6-*O*-(*tert*-butyldimethylsilyl)-α-D-mannopyranosyl]-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→6)-(2,3,4-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→4)-[2-(*tert*-butyl-carbamate)-2-deoxy-3,6-di-*O*-benzyl-α-D-glucopyranosyl]}-2,3,4,5-tetra-*O*-benzyl-1-*O*-(4-methoxybenzyl)-*myo*-inositol 20:



To a solution of **6** (155 mg, 0.064 mmol) in CH_3OH were added 1,3-propanedithiol (127 µL, 1.27 mmol) and DIPEA (40 µL). After being stirred at rt overnight, the mixture was concentrated *in vacuo*, and the residue was purified by flash silica gel column chromatography with CH_3OH and CH_2Cl_2 (1:40) as the eluents to afford a free amine as colorless syrup. This product was dissolved in CH_3OH and CH_2Cl_2 (1:1, 3 mL), and to it were added di-*tert*-butyl dicarbonate (29 µL, 0.128 mmol) and Et_3N (60 µL). After being stirred at rt overnight, the mixture was concentrated under reduced pressure, and the residue was purified by silica gel column chromatography with EtOAc and hexane (1:3) as the eluent to give **20** (126 mg, 79%) as colorless syrup. ^1H NMR (600 MHz, $CDCl_3$) δ: 7.39 – 6.98 (m, 77H), 6.78 (d, *J* = 8.4 Hz, 2H), 5.62 (d, *J* = 10.2 Hz, 1H), 5.44 (d, *J* = 3.0 Hz, 1H), 5.18 (s, 1H), 5.14 (s, 1H), 4.92 – 4.72 (m, 10H), 4.65 – 4.31 (m, 20H), 4.23 (t, *J* = 9.6 Hz, 1H), 4.14 (br d, 2H), 4.10 – 3.60 (m, 22H), 3.60 – 3.26 (m, 11H), 1.25 (s, 9H), 0.86 (s, 9H), 0.03 (s, 3H), 0.02 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ: 159.35, 155.11, 139.08, 138.88, 138.81, 138.70, 138.66, 138.56, 138.54, 138.32, 138.26, 138.17, 137.96, 129.37, 129.21, 128.37, 128.30, 128.27, 128.26, 128.21, 128.15, 128.11, 128.06, 127.98, 127.84, 127.70, 127.62, 127.58, 127.46, 127.36, 127.32, 127.21, 127.13, 127.11, 126.46, 113.88, 100.27, 99.40, 99.28, 98.69, 82.86, 81.90, 81.06, 80.60, 80.07, 79.85, 79.63, 79.13, 77.74, 77.67, 75.84, 75.70, 75.55, 75.04, 74.96, 74.82, 74.62, 74.53, 74.45, 74.26, 73.78, 73.74, 73.44, 73.16, 73.12, 72.75, 72.59, 72.14, 72.06, 72.03, 71.87, 71.83, 71.73, 71.63, 71.11, 69.43, 69.04, 66.49, 62.42, 55.08, 54.74, 28.30, 25.97, 18.31, -4.97, -5.24. HR ESI-TOF MS (m/z): calcd for $C_{154}H_{174}O_{28}NSi$ $[M + H]^+$, 2513.1992; found, 2513.1960.

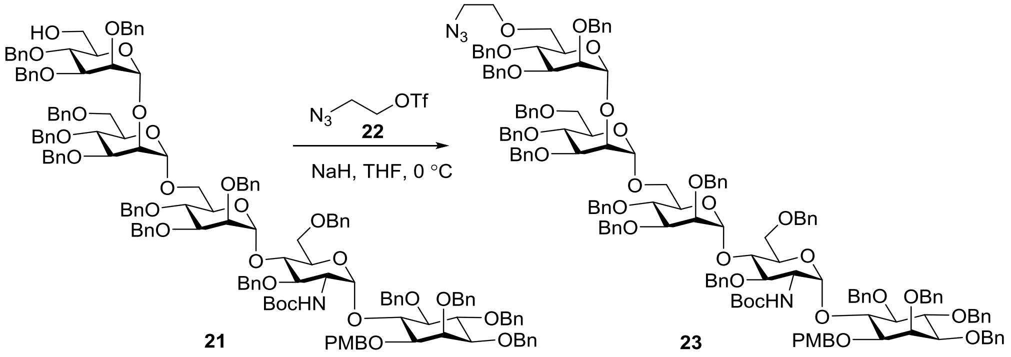
6-*O*-{(2,3,4-tri-*O*-Benzyl-α-D-mannopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→6)-(2,3,4-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→4)-[3,6-di-*O*-benzyl-2-(*tert*-butylcarbamate)-2-deoxy-α-D-glucopyranosyl]}-2,3,4,5-tetra-*O*-benzyl-1-*O*-(4-methoxybenzyl)-*myo*-inositol 21:



A solution of **20** (115 mg, 0.046 mmol) and triethylamine trihydrofluoride (1.0 mL) in CH_2Cl_2 and CH_3CN (1:1, 3.0 mL) was stirred at rt overnight under a N_2 atmosphere. The mixture was quenched with dropwise addition of saturated aq. $NaHCO_3$, and the water phase was extracted with CH_2Cl_2 (3 × 30 mL). The organic layer was dried over Na_2SO_4 and then concentrated under reduced pressure. The residue was purified by silica gel column chromatography with EtOAc and hexane (1:3) as the eluents to give **21** (95 mg, 87%) as colorless syrup. ^{1}H NMR (600 MHz, $CDCl_3$) δ: 7.37 – 7.08 (m, 77H), 6.80 (d, *J* = 8.4 Hz, 2H), 5.62 (d, *J* = 10.2 Hz, 1H), 5.46 (d, *J* = 3.0 Hz, 1H), 5.21 (s, 1H), 5.06 (s, 1H), 4.94 – 4.73 (m, 10H), 4.61– 4.55 (m, 4H), 4.53 – 4.45 (m, 8H), 4.43 – 4.33 (m, 8H), 4.28 – 4.14 (m, 3H), 4.11 – 3.97 (m, 5H), 3.96 – 3.86 (m, 2H), 3.88 – 3.58 (m, 16H), 3.56 – 3.51 (m, 4H), 3.40 – 3.32 (m, 6H), 2.03 (br d, 1H), 1.26 (s, 9H). ^{13}C NMR (150 MHz, $CDCl_3$) δ: 159.36, 155.13, 138.81, 138.67, 138.65, 138.63, 138.58, 138.52, 138.38, 138.35, 138.29, 138.19, 138.05, 129.41, 129.22, 128.39, 128.35, 128.30, 128.28, 128.18, 128.02, 127.94, 127.89, 127.77, 127.75, 127.70, 127.64, 127.62, 127.52, 127.50, 127.46, 127.44, 127.38, 127.33, 127.24, 127.13, 113.89, 100.13, 99.40, 99.30, 82.83, 81.92, 81.07, 81.02, 80.61, 79.97, 79.73, 79.38, 79.16, 77.67, 77.38, 75.86, 75.75, 75.50, 75.01, 74.89, 74.88, 74.80, 74.55, 74.18, 74.11, 73.80, 73.55, 73.22, 73.20, 72.79, 72.60, 72.18, 72.13, 72.11, 72.07, 71.93, 71.80, 71.70, 71.18, 69.34, 69.04, 66.22, 62.20, 55.10, 54.62, 28.31. HR ESI-TOF MS (m/z): calcd for $C_{148}H_{160}O_{28}N$ $[M + H]^+$, 2399.1127; found, 2399.1033.

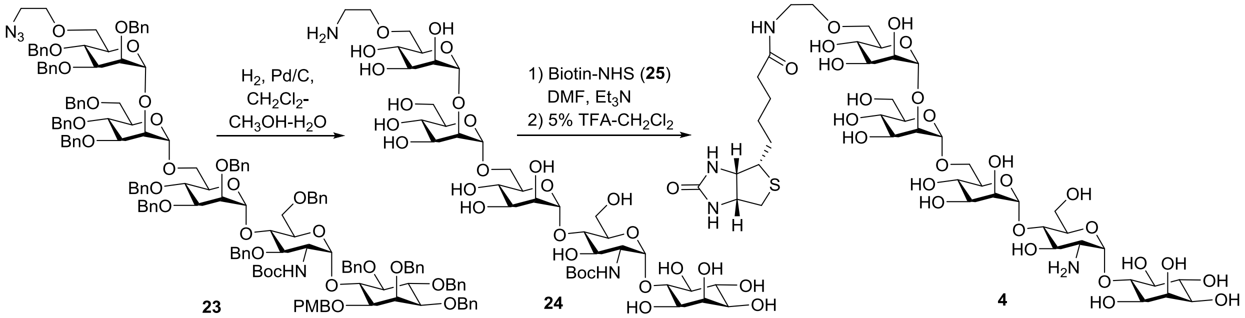
6-*O*-{(6-*O*-Azidoethyl-2,3,4-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→6)-(2,3,4-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→4)-[3,6-di-*O*-

benzyl-2-(*tert*-butylcarbamate)-2-deoxy-α-D-glucopyranosyl]}-2,3,4,5-tetra-*O*-benzyl-1-*O*-(4-methoxybenzyl)-*myo*-inositol 23:



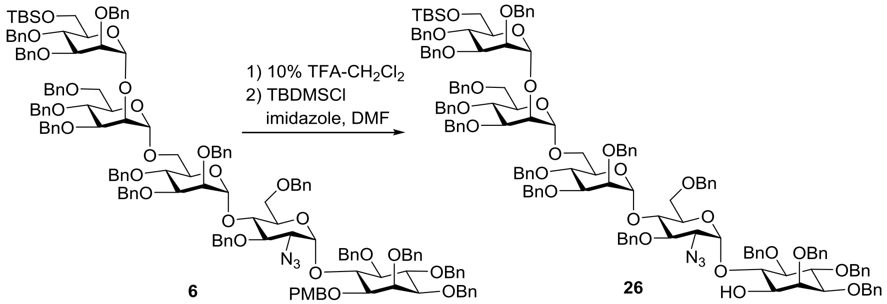
To a solution of **21** (90 mg, 0.038 mmol) in anhydrous THF (4 mL) was added NaH (5.5 mg, 0.228 mmol) at 0 °C under N_2 atmosphere. After stirring for 15 min, a THF solution containing **22** (33 mg, 0.152 mmol) was added, and the reaction was monitored by TLC. After 1 h of stirring at 0 °C, excessive NaH was decomposed with saturated aq. NaCl solution, and the mixture was diluted with ethyl acetate. The organic layer, after being washed with saturated aq. NaCl solution, was dried with Na_2SO_4 and concentrated *in vacuo*. The residue was purified by silica gel column chromatography with EtOAc and hexane (1:4) as the eluents to afford **23** (64 mg, 69%) as colorless syrup. ^{1}H NMR (600 MHz, $CDCl_3$) δ: 7.38 – 7.06 (m, 77H), 6.79 (d, J = 8.4 Hz, 2H), 5.63 (d, J = 10.2 Hz, 1H), 5.44 (d, J = 2.4 Hz, 1H), 5.21 (s, 1H), 5.11 (s, 1H), 4.94 – 4.73 (m, 9H), 4.65 – 4.31 (m, 19H), 4.23 (t, J = 9.4 Hz, 1H), 4.17 (m, 2H), 4.04 (m, 6H), 3.93 (t, J = 9.4 Hz, 1H), 3.82 (m, 7H), 3.74 – 3.25 (m, 24H), 3.15 m, 1H), 1.25 (s, 9H). ^{13}C NMR (150 MHz, $CDCl_3$) δ: 159.35, 155.12, 138.87, 138.70, 138.65, 138.59, 138.55, 138.45, 138.31, 138.28, 138.17, 138.13, 137.96, 129.56, 129.37, 129.21, 129.07, 128.38, 128.32, 128.29, 128.28, 128.26, 128.18, 128.15, 128.06, 128.00, 127.98, 127.85, 127.81, 127.75, 127.71, 127.66, 127.63, 127.61, 127.56, 127.49, 127.41, 127.38, 127.29, 127.24, 127.08, 127.05, 126.94, 126.50, 113.88, 100.21, 99.46, 99.39, 99.19, 82.82, 81.91, 81.12, 81.06, 80.60, 80.05, 79.73, 79.53, 79.17, 77.73, 77.48, 75.96, 75.73, 75.53, 74.93, 74.81, 74.80, 74.73, 74.60, 74.51, 74.20, 73.79, 73.72, 73.18, 72.73, 72.60, 72.20, 72.13, 72.04, 72.01, 71.97, 71.90, 71.84, 71.68, 71.16, 71.13, 70.60, 70.47, 70.32, 70.15, 69.49, 69.32, 69.04, 66.49, 55.09, 54.76, 50.74, 28.31. HR ESI-TOF MS (m/z): calcd for $C_{150}H_{163}O_{28}N_4$ $[M + H]^+$, 2468.1454; found, 2468.1511.

6-*O*-{[6-*O*-(*N*-Biotinylsuccinamide-ethyl)-α-D-mannopyranosyl]-(1→2)-(α-D-mannopyranosyl)-(1→6)-(α-D-mannopyranosyl)-(1→4)-[2-amino-2-deoxy-α-D-glucopyranosyl]}-*myo*- inositol 4:



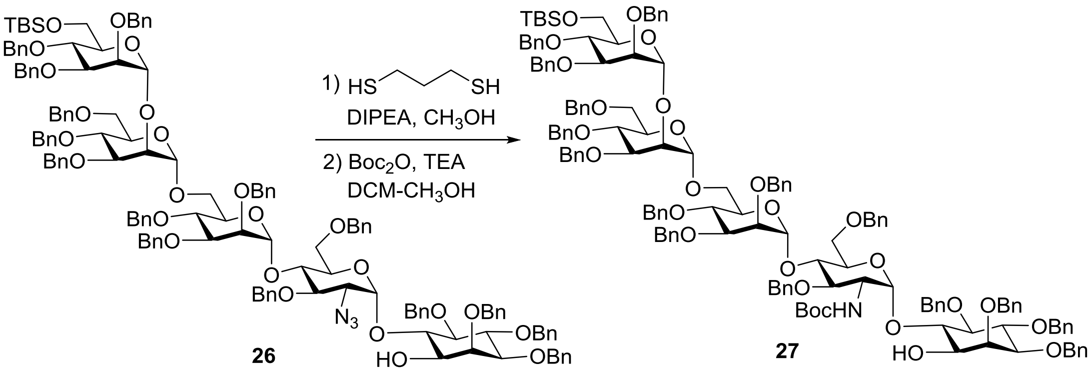
After a mixture of **23** (50 mg, 0.020 mmol) and 10% Pd/C (10 mg) in CH_2Cl_2, CH_3OH, and H_2O (1:4:0.5, 3.3 mL) was stirred at rt under H_2 atmosphere (50 psi) for 1 day, it was filtered off, and the filtrate was concentrated *in vacuo* to afford **24** (17.7 mg, 90%) as an off-white solid: MALDI TOF MS (positive mode): calcd for $C_{37}H_{67}O_{27}N_2$ $[M + H]^+$, 971.3; found, 971.8. To a solution of **24** (16.0 mg, 0.016 mmol) in anhydrous DMF (1 mL) were added **25** (6.7 mg, 0.020 mmol) and one drop Et_3N. The reaction mixture was stirred at rt for 1 h before showing a complete reaction by MALDI-TOF MS. The solution was concentrated under reduced pressure, and the residue was dissolved in 5% TFA in CH_2Cl_2 (v/v). After 20 min of stirring, the solvent was removed under reduced pressure by co-evaporation with toluene three times. The residue was purified by size-exclusion chromatography on a G15 column with water as the eluent to give **4** (12.7 mg, 70% for the last two steps) as a foamy solid. ^{1}H NMR (600 MHz, D_2O) δ: 5.26 (d, J = 3.0 Hz, 1H), 5.05 (s, 1H), 4.93 (s, 1H), 4.84 (s, 1H), 4.47 – 4.40 (m, 1H), 4.29 – 4.22 (m, 1H), 3.98 (d, J = 10.2 Hz, 1H), 3.93 – 3.87 (m, 3H), 3.86 – 3.78 (m, 4H), 3.74 – 3.44 (m, 22H), 3.34 (dd, J = 10.2, 1.8 Hz, 1H), 3.26 – 3.16 (m, 5H), 3.06 (d, J = 4.8 Hz, 1H), 2.83 (dd, J = 13.2, 4.8 Hz, 1H), 2.61 (d, J = 12.6 Hz, 1H), 2.11 (t, J = 7.2 Hz, 2H), 1.60 – 1.39 (m, 4H), 1.28 – 1.22 (m, 2H). ^{13}C NMR (150 MHz, D_2O) δ: 176.85, 165.27, 102.28, 101.93, 98.21, 96.11, 80.24, 78.79, 76.54, 72.61, 72.54, 72.44, 72.26, 72.12, 71.74, 71.48, 70.91, 70.78, 70.36, 70.12, 70.05, 69.75, 69.72, 69.67, 69.19, 66.70, 66.29, 66.19, 61.98, 60.75, 60.17, 59.98, 55.22, 54.31, 39.60, 38.85, 35.36, 27.79, 27.58, 25.07. HR ESI-TOF MS (m/z): calcd for $C_{42}H_{73}O_{27}N_4S$ $[M + H]^+$, 1097.4183; found, 1097.4235.

6-*O*-{[2,3,4-tri-*O*-Benzyl-6-*O*-(*tert*-butyldimethylsilyl)-α-D-mannopyranosyl]-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→6)-(2,3,4-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→4)-(2- azido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)}-2,3,4,5-tetra-*O*-benzyl-*myo*-inositol 26:



To a solution of **6** (290 mg, 0.119 mmol) in CH_2Cl_2 (3 mL) was added 20% TFA in CH_2Cl_2 (3 mL) to give a final concentration of about 10% TFA. The mixture was stirred for 1 h, when TLC showed the completion of reaction. The solution was concentrated and then co-evaporated with toluene three times to remove TFA completely. The residue was dissolved in DMF (4 mL), and then to the solution were added TBSCl (27 mg, 0.178 mmol) and imidazole (16 mg, 0.238 mmol) at 0 °C. The mixture was stirred at rt for 2 h and diluted with EtOAc (100 mL). The organic layer, after washed with saturated aq. NaCl solution, was dried with Na_2SO_4 and concentrated *in vacuo*. The residue was purified by silica gel column chromatography with EtOAc and hexane (1:4) as the eluents to give **26** (198 mg, 72% for two steps) as colorless syrup. ^{1}H NMR (600 MHz, $CDCl_3$) δ: 7.39 – 6.92 (m, 75H), 5.50 (d, J = 3.6 Hz, 1H), 5.26 (s, 1H), 5.15 (s, 1H), 5.01 (d, J = 11.4 Hz, 1H), 4.97 – 4.87 (m, 5H), 4.78 – 4.67 (m, 7H), 4.65 – 4.60 (m, 2H), 4.56 – 4.24 (m, 16H), 4.18 (d, J = 12.0 Hz, 1H), 4.09 – 3.60 (m, 22H), 3.50 – 3.46 (m, 2H), 3.43 – 3.31 (m, 6H), 2.96 (d, J = 7.2 Hz, 1H), 0.87 (s, 9H), 0.04 (s, 3H), 0.02 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ: 139.06, 138.79, 138.66, 138.57, 138.55, 138.48, 138.43, 138.35, 138.33, 138.14, 138.05, 138.01, 137.64, 128.45, 128.42, 128.32, 128.31, 128.29, 128.22, 128.21, 128.15, 128.14, 128.06, 128.01, 127.92, 127.85, 127.77, 127.70, 127.68, 127.65, 127.63, 127.58, 127.53, 127.48, 127.41, 127.38, 127.28, 127.20, 127.16, 127.10, 126.87, 100.14, 99.26, 98.73, 97.57, 81.75, 81.32, 80.91, 80.52, 79.97, 79.66, 77.29, 76.03, 75.77, 75.33, 75.04, 74.97, 74.93, 74.84, 74.55, 74.48, 74.31, 74.25, 73.48, 73.30, 73.19, 73.17, 73.11, 73.03, 72.20, 72.08, 71.97, 71.90, 71.82, 71.68, 70.40, 69.04, 68.71, 66.39, 64.03, 62.47, 60.03, 25.97, 18.32, -4.96, -5.23. HR ESI-TOF MS (m/z): calcd for $C_{141}H_{156}O_{25}N_3Si$ $[M + H]^+$, 2319.0797; found, 2319.0837.

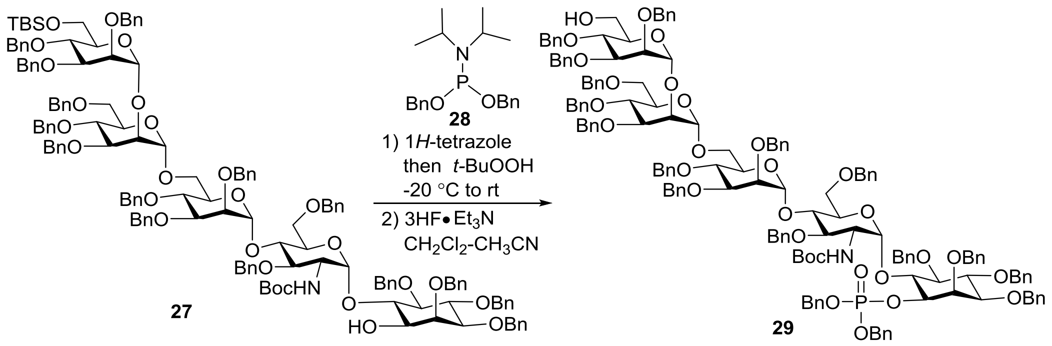
6-*O*-{[2,3,4-tri-*O*-Benzyl-6-*O*-(*tert*-butyldimethylsilyl)-α-D-mannopyranosyl]-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→6)-(2,3,4-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→4)-[3,6-di-*O*-benzyl-2-(*tert*-butylcarbamate)-2-deoxy-α-D-glucopyranosyl]}-2,3,4,5-tetra-*O*-benzyl-*myo*-inositol 27:



26 **27**

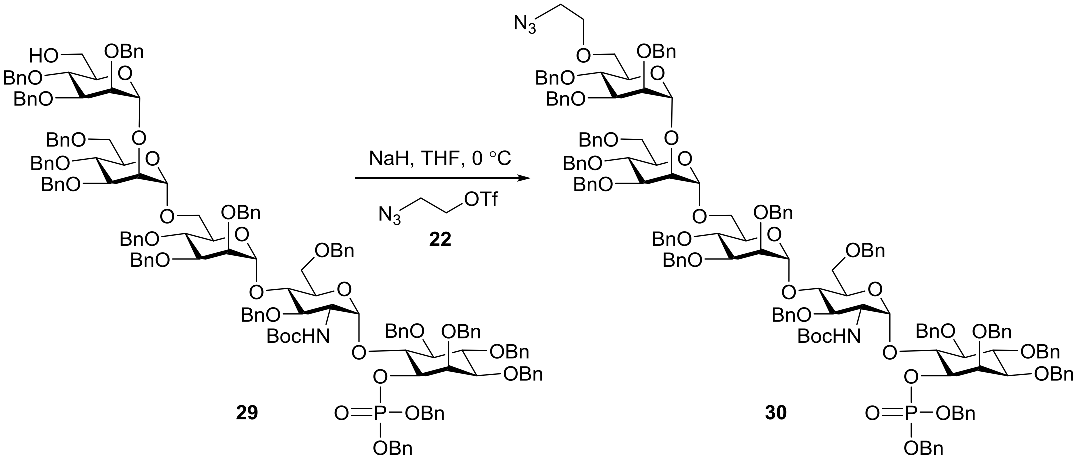
A mixture of **26** (180 mg, 0.078 mmol), 1,3-propanedithiol (155 µL, 1.55 mmol), and DIPEA (50 µL) in CH_3OH (2 mL) was stirred at rt overnight. The mixture was concentrated *in vacuo* and the residue was purified by flash silica gel column chromatography with CH_3OH and CH_2Cl_2 (1:40) as the eluents to afford the free amine as colorless oil. This product was dissolved in CH_3OH and CH_2Cl_2 (1:1, 3 mL), and to the solution were added di-*tert*-butyl dicarbonate (36 µL, 0.155 mmol) and Et_3N (80 µL). After stirred at rt overnight, the solvent was removed under reduced pressure, and the residue was purified by silica gel column chromatography with EtOAc and hexane (1:2) as the eluents to afford **27** (150 mg, 81% for two steps) as colorless syrup. ^{1}H NMR (600 MHz, $CDCl_3$) δ: 7.34 – 7.06 (m, 75H), 5.42 (d, J = 8.4 Hz, 1H), 5.34 (s, 1H), 5.19 (s, 1H), 5.15 (s, 1H), 5.06 (d, J = 11.4 Hz, 1H), 4.94 – 4.63 (m, 14H), 4.56 – 4.32 (m, 14H), 4.19 – 3.76 (m, 16H), 3.74 – 3.29 (m, 16H), 2.60 (d, J = 9.6 Hz, 1H), 1.27 (s, 9H), 0.87 (s, 9H), 0.05 (s, 3H), 0.03 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ: 155.30, 139.06, 138.88, 138.80, 138.70, 138.56, 138.49, 138.35, 138.09, 137.98, 128.55, 128.48, 128.41, 128.30, 128.27, 128.25, 128.22, 128.16, 128.12, 128.00, 127.93, 127.87, 127.83, 127.72, 127.71, 127.67, 127.62, 127.59, 127.54, 127.50, 127.46, 127.37, 127.30, 127.26, 127.19, 127.15, 127.11, 100.27, 99.27, 98.70, 98.30, 81.46, 81.36, 80.98, 80.74, 80.18, 80.02, 79.88, 79.63, 79.31, 77.54, 77.45, 75.88, 75.68, 75.02, 74.98, 74.85, 74.77, 74.60, 74.53, 74.48, 74.22, 73.82, 73.47, 73.29, 73.16, 72.25, 72.07, 71.88, 71.85, 71.75, 71.64, 71.02, 69.43, 69.04, 66.43, 62.45, 54.52, 28.25, 25.98, 18.33, -4.96, -5.22. HR ESI-TOF MS (m/z): calcd for $C_{146}H_{166}O_{27}NSi$ $[M + H]^+$, 2393.1417; found, 2393.1431.

6-*O*-{(2,3,4-tri-*O*-Benzyl-α-D-mannopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→6)-(2,3,4-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→4)-[3,6-di-*O*-benzyl -2-(*tert*-butylcarbamate)-2-deoxy-α-D-glucopyranosyl]}-2,3,4,5-tetra-*O*-benzyl-1-*O*-(di-*O*-benzylphosphoryl)-*myo*-inositol 29:



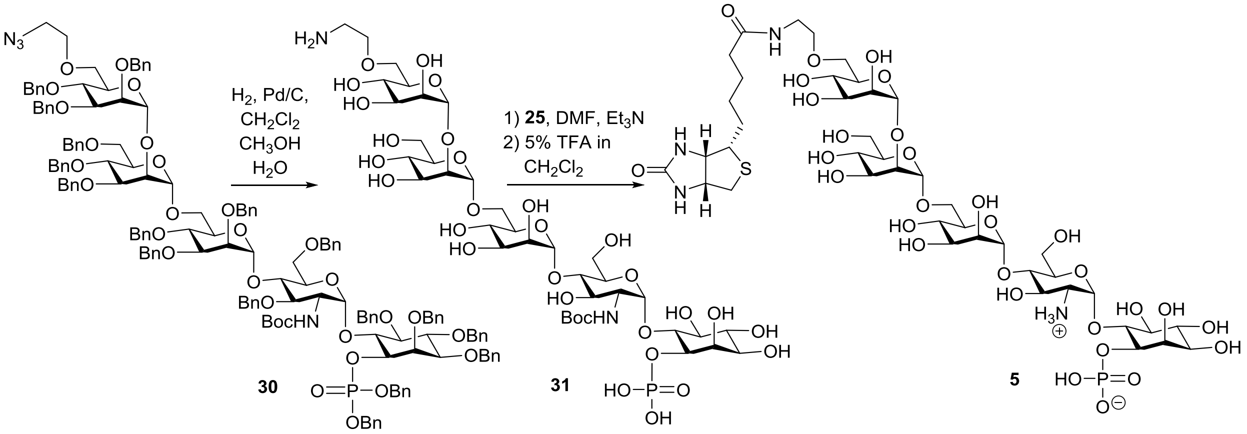
To a mixture of **27** (135 mg, 0.056 mmol), dibenzyl diisopropylphosphoramidate (76 μL, 0.226 mmol) and MS 4 Å in anhydrous CH_2Cl_2 (3 mL) was added 1*H*-tetrazole (0.45 M in CH_3CN, 0.5 mL, 0.226 mmol). After stirred at rt under N_2 atmosphere for 1 h, the mixture was cooled to −20 °C, and *t*-BuOOH (5.5 M in decane; 61 μL, 0.338 mmol) was added. After 2 h of stirring at 0 °C, excessive *t*-BuOOH was quenched wirh saturated aq. $NaHCO_3$ solution. The mixture was extracted with CH_2Cl_2 (3 × 50 mL). The organic layer was dried with Na_2SO_4 and concentrated *in vacuo*. The residue was dissolved in CH_2Cl_2 and CH_3CN (1:1, 2 mL), and to the solution was added triethylamine trihydrofluoride (1.0 mL). The mixture was stirred at rt overnight under N_2 atmosphere. The reaction was quenched with dropwise addition of saturated aq. $NaHCO_3$ solution. The aq. phase was extracted with CH_2Cl_2 (3 × 50 mL); the organic layer was dried over Na_2SO_4 and concentrated in vacuum. The residue was purified by silica gel column chromatography with EtOAc and hexane (1:2) as the eluents to give **29** (95 mg, 66% for three steps) as colorless syrup. ^{1}H NMR (600 MHz, $CDCl_3$) δ: 7.53 – 6.77 (m, 85H), 5.37 (d, J = 10.2 Hz, 1H), 5.25 (s 2H), 5.07 – 4.82 (m, 12H), 4.78 (d, J = 11.4 Hz, 1H), 4.68 (m, 2H), 4.63 – 4.34 (m, 18H), 4.26 – 4.20 (m, 1H), 4.17 – 4.00 (m, 7H), 3.96 – 3.88 (m, 2H), 3.88 – 3.68 (m, 12H), 3.62 – 3.50 (m, 5H), 3.48 – 3.34 (m, 4H), 3.31 – 3.25 (m, 2H), 2.05 (s, 1H), 1.24 (s, 9H). ^{13}C NMR (150 MHz, $CDCl_3$) δ: 155.34, 138.91, 138.64, 138.59, 138.57, 138.52, 138.48, 138.38, 138.24, 138.21, 138.11, 138.03, 137.88, 135.49, 135.43, 135.39, 128.79, 128.74, 128.70, 128.66, 128.61, 128.48, 128.40, 128.35, 128.31, 128.28, 128.26, 128.19, 128.15, 128.07, 127.97, 127.93, 127.77, 127.71, 127.62, 127.52, 127.44, 127.38, 127.33, 127.24, 127.06, 126.57, 100.23, 99.38, 99.32, 98.89, 81.67, 81.63, 80.71, 80.17, 79.92, 79.72, 79.33, 78.57, 78.54, 77.34, 76.27, 76.22, 76.01, 75.80, 75.71, 75.48, 75.01, 74.89, 74.87, 74.79, 74.73, 74.53, 74.09, 73.77, 73.22, 73.15, 72.77, 72.49, 72.18, 72.11, 72.05, 71.90, 71.75, 71.73, 71.17, 69.96, 69.92, 69.52, 69.48, 69.22, 69.02, 66.17, 62.19, 54.51, 28.27. ^{31}P NMR (162 MHz, $CDCl_3$) δ: -1.82. HR ESI-TOF MS (m/z): calcd for $C_{154}H_{165}O_{30}NP$ [M + H]$^+$, 2539.1154; found, 2539.1279.

6-*O*-{(2,3,4-tri-*O*-Benzyl-6-*O*-azidoethyl-α-D-mannopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→6)-(2,3,4-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→4)-[3,6-di-*O*-benzyl-2-(*tert*-butylcarbamate)-2-deoxy-α-D-glucopyranosyl]}-2,3,4,5-tetra-*O*-benzyl-1-*O*-(di-*O*-benzylphosphoryl)-*myo*-inositol 30:



To a solution of **29** (85.0 mg, 0.033 mmol) in anhydrous THF (3 mL) was added NaH (4.8 mg, 0.2 mmol) at 0 °C under N_2 atmosphere. After stirring for 15 min, a THF solution of **22** (29.0 mg, 0.132 mmol) was added, and the reaction was monitored by TLC. After 1 h of stirring at 0 °C, excessive NaH was quenched with saturated aq. NaCl solution, and the mixture was diluted with EtOAc. The organic layer, after washed with saturated aq. NaCl solution, was dried with Na_2SO_4 and concentrated *in vacuo*. The residue was purified by silica gel column chromatography with EtOAc and hexane (1:3) as the eluents to give **30** (58.5mg, 67%) as colorless syrup. ^1H NMR (600 MHz, $CDCl_3$) δ: 7.34 – 7.04 (m, 85H), 5.38 (d, J = 10.2 Hz, 1H), 5.24 (s, 2H), 5.12 (s, 1H), 5.05 – 4.97 (m, 3H), 4.93 – 4.75 (m, 10H), 4.70 – 4.62 (m, 3H), 4.55 (d, J = 12.0 Hz, 2H), 4.49 – 4.32 (m, 14H), 4.24 – 4.20 (m, 2H), 4.08 (m, 8H), 3.94 (t, J = 9.6 Hz, 1H), 3.88 – 3.75 (m, 8H), 3.68 (m, 4H), 3.58 (m, 3H), 3.54 – 3.40 (m, 6H), 3.29 (m, 4H), 3.19 – 3.14 (m, 1H), 1.23 (s, 9H). ^{13}C NMR (151 MHz, $CDCl_3$) δ: 155.34, 138.95, 138.89, 138.71, 138.67, 138.55, 138.51, 138.46, 138.38, 138.23, 138.18, 138.09, 137.95, 137.87, 135.43, 135.38, 128.79, 128.75, 128.69, 128.67, 128.48, 128.39, 128.33, 128.31, 128.26, 128.20, 128.18, 128.15, 128.11, 128.07, 128.01, 127.97, 127.91, 127.86, 127.76, 127.71, 127.61, 127.58, 127.52, 127.48, 127.43, 127.41, 127.38, 127.30, 127.24, 127.19, 127.07, 127.04, 126.99, 100.32, 99.37, 99.18, 98.95, 81.65, 81.63, 80.81, 80.17, 80.01, 79.74, 79.50, 79.33, 78.56, 78.52, 77.49, 76.33, 76.29, 76.11, 75.78, 75.68, 75.50, 74.93, 74.82, 74.79, 74.65, 74.59, 74.51, 74.11, 74.00, 73.68, 73.18, 73.13, 72.49, 72.23, 72.05, 72.00, 71.97, 71.90, 71.83, 71.80, 71.72, 71.11, 70.34, 70.13, 69.96, 69.92, 69.53, 69.49, 69.22, 69.03, 66.40, 54.63, 50.76, 28.27. ^{31}P NMR (162 MHz, $CDCl_3$) δ: -1.65. HR ESI-TOF MS (m/z): calcd for $C_{156}H_{168}O_{30}N_4P$ [M + H]$^+$, 2608.1481; found, 2608.1472.

6-*O*-{[6-*O*-(*N*-Biotinylsuccinamide-ethyl)-α-D-mannopyranosyl]-(1→2)-(α-D-mannopyranosyl)-(1→6)-(α-D-mannopyranosyl)-(1→4)-[2-amino-2-deoxy-α-D-glucopyranosyl]}-1-*O*-phosphoryl-*myo*-inositol 5:

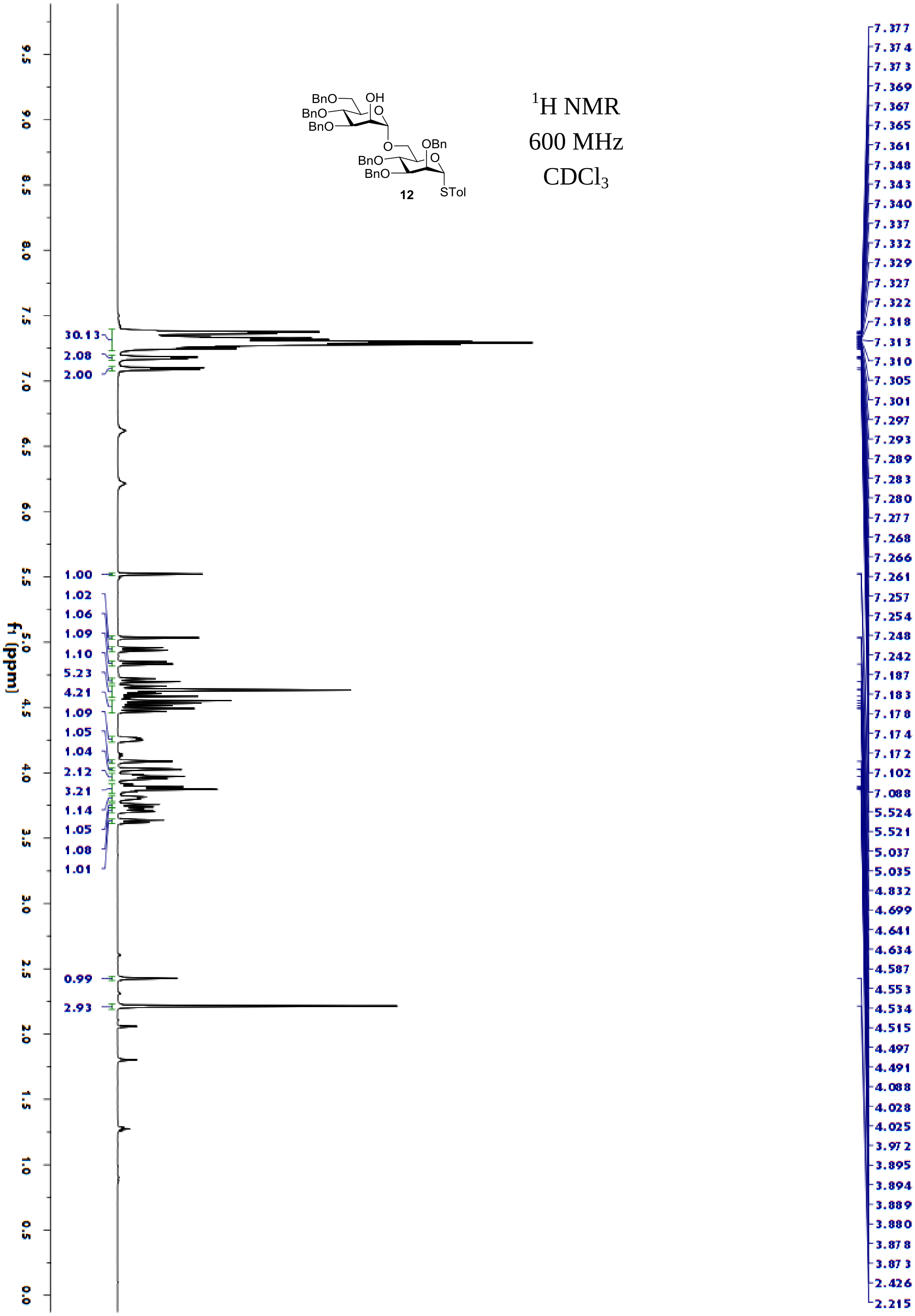


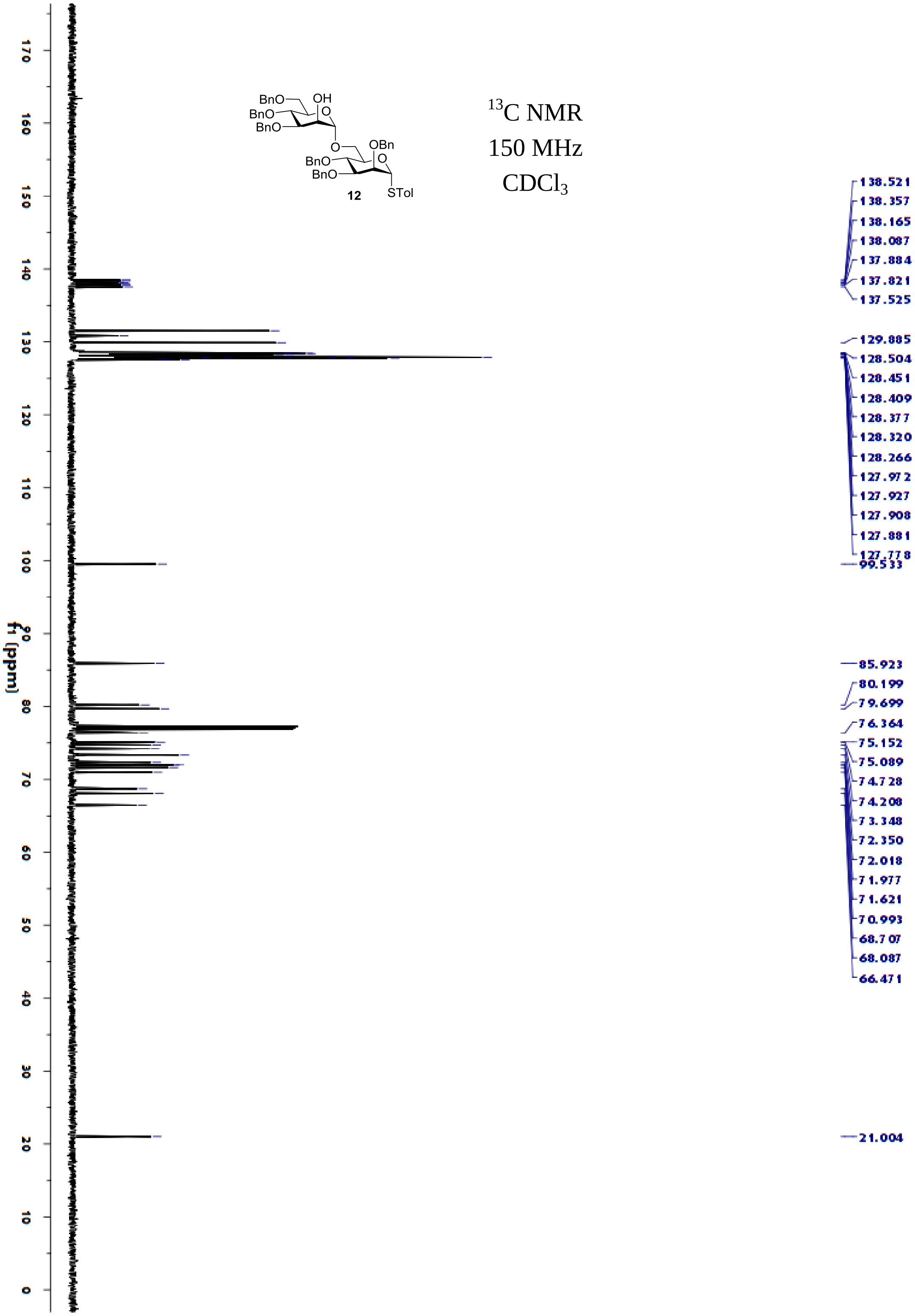
A mixture of **30** (50.0 mg, 0.019 mmol) and 10% Pd/C (10 mg) in CH_2Cl_2, CH_3OH, and H_2O (1:4:0.5, 3.3 mL) was stirred at rt under H_2 atmosphere (50 psi) for 1 day. The solution was filtered, and the filtrate was concentrated *in vacuo* to give deprotected intermediate **31** (17.5 mg, 87%) as an off-white solid, which was directly used in the next step. MALDI-TOF MS (negtive mode): calcd for $C_{37}H_{66}O_{30}N_2P$ [M–H]$^-$, 1049.3; found, 1049.4. To the solution of **31** (14.0 mg, 0.013 mmol) in anhydrous DMF (1 mL) were added **25** (5.5 mg, 0.016 mmol) and one drop of Et_3N. The mixture was stirred at rt for 1 h before MALDI-TOF MS showed a complete reaction. The solvent was removed under reduced pressure, and the residue was dissolved in 5% TFA in CH_2Cl_2. After 20 min of stirring, the solvent was removed, and the residue was co-evaporated with toluene three times and then purified by size exclusion column chromatography on a G15 column with water as the eluent to give **5** (10.6 mg, 68% for the last two steps) as a foamy solid. ^{1}H NMR (600 MHz, D_2O) δ: 5.44 (s, 1H), 5.05 (s, 1H), 4.92 (s, 1H), 4.84 (s, 1H), 4.46 – 4.41 (m, 1H), 4.27 – 4.24 (m, 1H), 4.03 – 3.95 (m, 3H), 3.92 – 3.86 (m, 3H), 3.83 – 3.78 (m, 3H), 3.77 – 3.43 (m, 22H), 3.39 (d, *J* = 9.6 Hz, 1H), 3.27 – 3.15 (m, 5H), 2.83 (dd, *J* = 13.2, 4.8 Hz, 1H), 2.61 (d, *J* = 13.2 Hz, 1H), 2.11 (t, *J* = 7.2 Hz, 2H), 1.58 –1.40 (m, 4H), 1.28 – 1.22 (m, 2H). ^{13}C NMR (150 MHz, D_2O) δ: 176.87, 165.26, 102.26, 101.86, 98.22, 95.16, 78.79, 77.35, 76.41, 75.66, 72.66, 72.61, 72.18, 72.12, 71.75, 71.47, 70.79, 70.36, 70.34, 70.21, 70.12, 70.04, 69.75, 69.64, 69.20, 66.73, 66.70, 66.33, 66.22, 61.98, 60.76, 60.17, 60.07, 55.21, 53.75, 39.60, 38.83, 35.36, 27.78, 27.57, 25.05. ^{31}P NMR (162 MHz, D_2O) δ: -1.30. HR ESI-TOF MS (m/z): calcd for $C_{42}H_{72}O_{30}N_4P$ $[M–H]^-$, 1175.3690; found, 1175.3744.

II. Experimental procedures for ELISA

The 96-well ELISA plates were incubated with a solution of CAMP factor (2 μg/mL, 100 μL/well) dissolved in coating buffer (0.1 M bicarbonate, pH 9.6) at 37 °C for 1 h, which was followed by treatment with a blocking buffer (1% BSA in PBST) and washing 3 times with PBST (containing 0.05% Tween-20). Then, the solution of compounds **4** and **5** with serial half-log dilutions from 200 μg/mL to 0.0244 μg/mL in PBS was added to the plates (100 μL/well). The plates were incubated at 37 °C for 2 h and washed with PBST. Then, to the plates was added a 1:1000 diluted solution of horseradish peroxidase (HRP)-linked streptavidin (100 μL/well), and the plates were incubated at rt for 1 h and washed with PBST. Thereafter, the Pierce™ TMB Substrate Kit solution was added to the plates (100 μL/well), and the plates were incubated at rt for 15 min followed by the addition of 1 M H_2SO_4 solution (100 μL) according to the instructions of the manufacturer. Finally, the light absorptions of the plates were measured with a microplate reader for the colorimetric readout at 450 nm wavelength.

III. NMR spectra of synthetic intermediates and final products





13C NMR
150 MHz
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BnO
OH
BnO
O
BnO
O
OBn
BnO
O
BnO
12
STol
138.521
138.357
138.165
138.087
137.884
137.821
137.525
129.885
128.504
128.451
128.409
128.377
128.320
128.266
127.972
127.927
127.908
127.881
127.778
99.533
85.923
80.199
79.699
76.364
75.152
75.089
74.728
74.208
73.348
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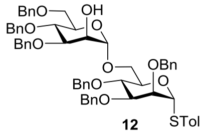
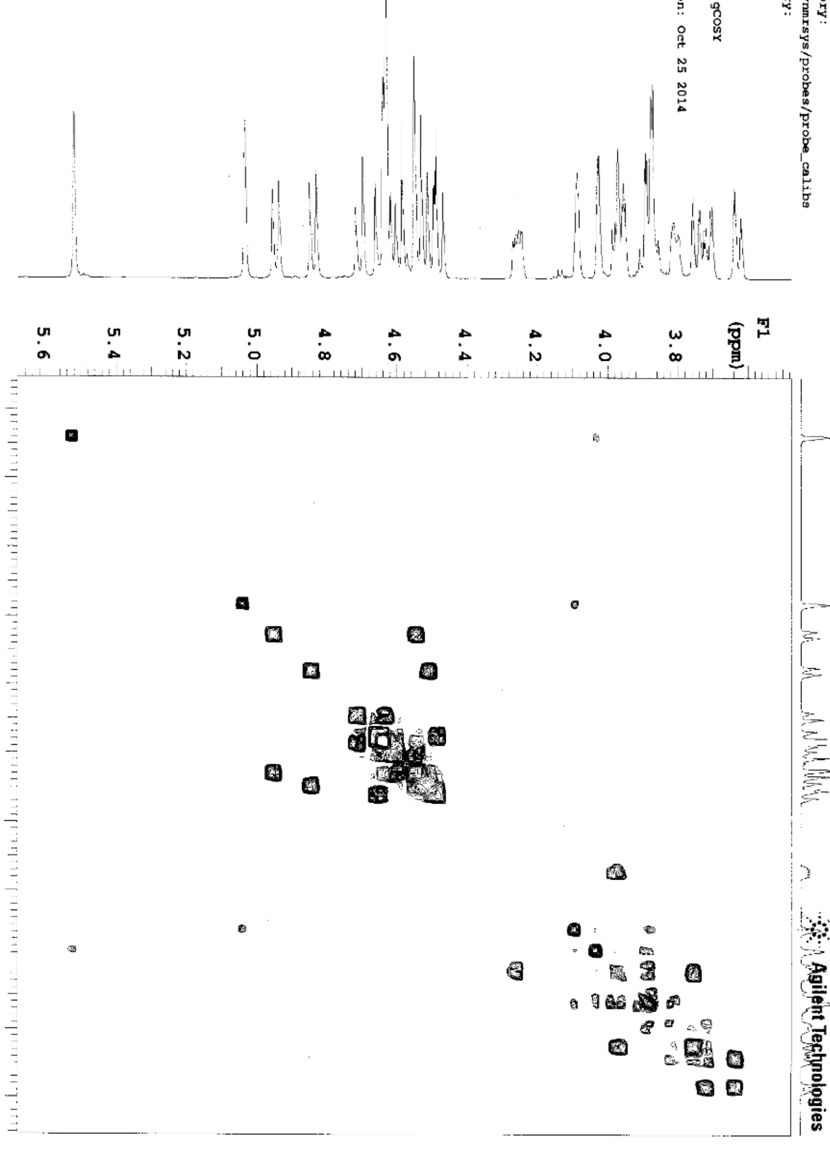
66.471
21.004
f1 (ppm)
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0

^1H-^1H COSY
600 MHz
$CDCl_3$



12

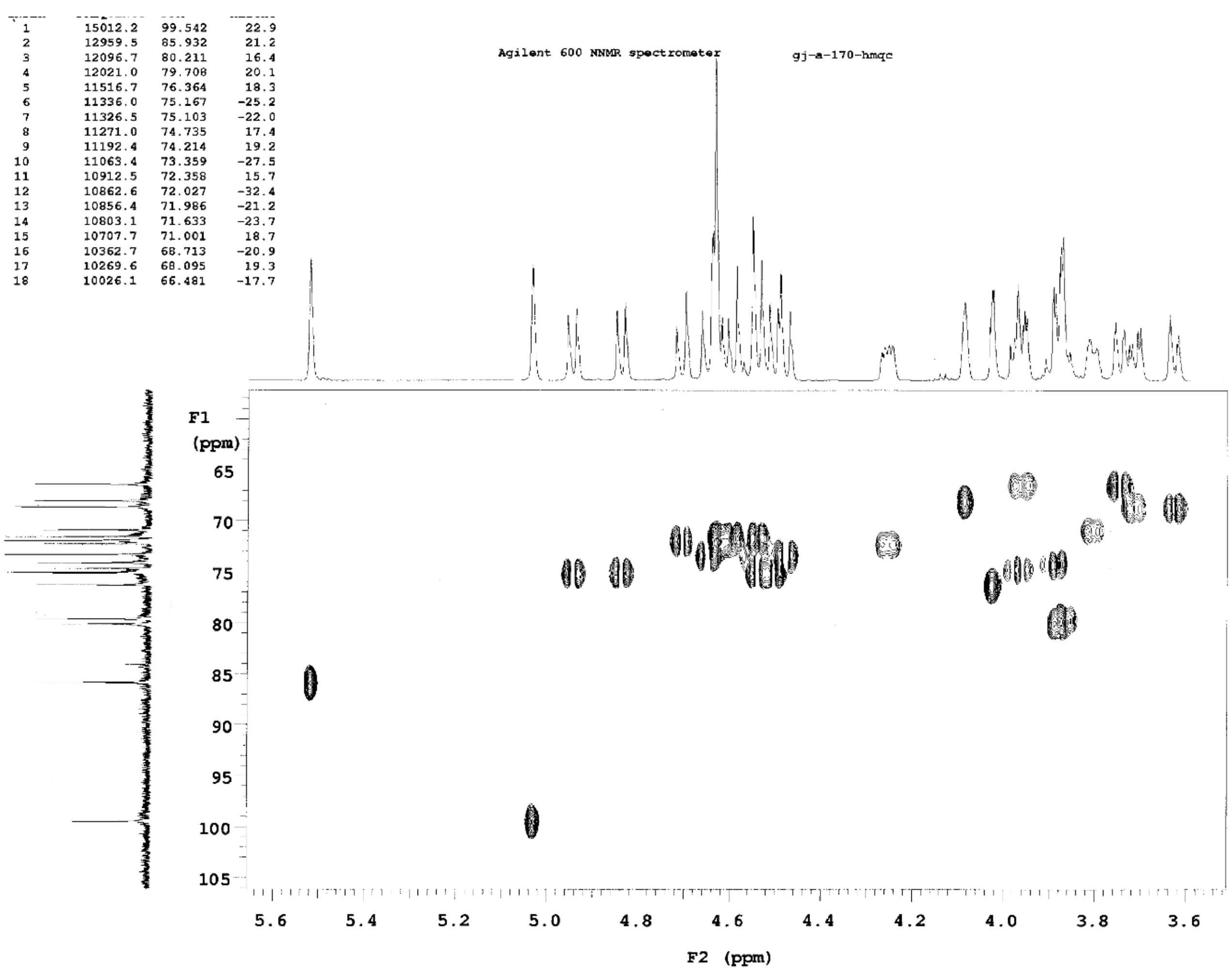


Sample Name:

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: Oct 25 2014



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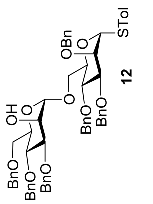
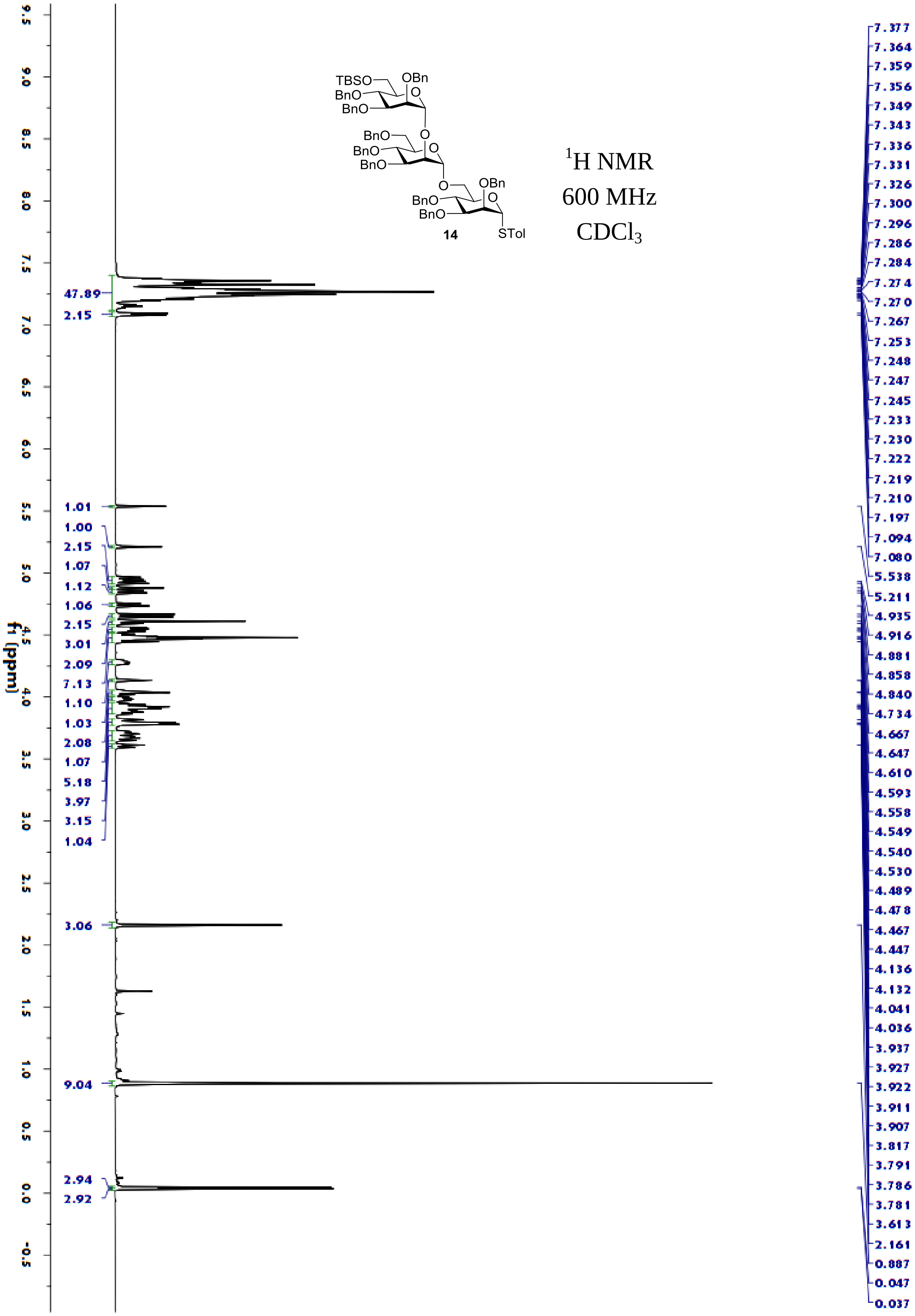
5	11516.7	76.364	18.3
6	11336.0	75.167	-25.2
7	11326.5	75.103	-22.0
8	11271.0	74.735	17.4
9	11192.4	74.214	19.2
10	11063.4	73.359	-27.5
11	10912.5	72.358	15.7
12	10862.6	72.027	-32.4
13	10856.4	71.986	-21.2
14	10803.1	71.633	-23.7
15	10707.7	71.001	18.7
16	10362.7	68.713	-20.9
17	10269.6	68.095	19.3
18	10026.1	66.481	-17.7

^{1}H-^{13}C HMQC
600/150 MHz
CDCl$_3$



12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O
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OBn
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BnO
14
STol
^{1}H NMR
600 MHz
$CDCl_3$
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)
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1.00
2.15
1.07
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1.06
2.15
3.01
2.09
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1.03
2.08
1.07
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4.935
4.916
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4.840
4.734
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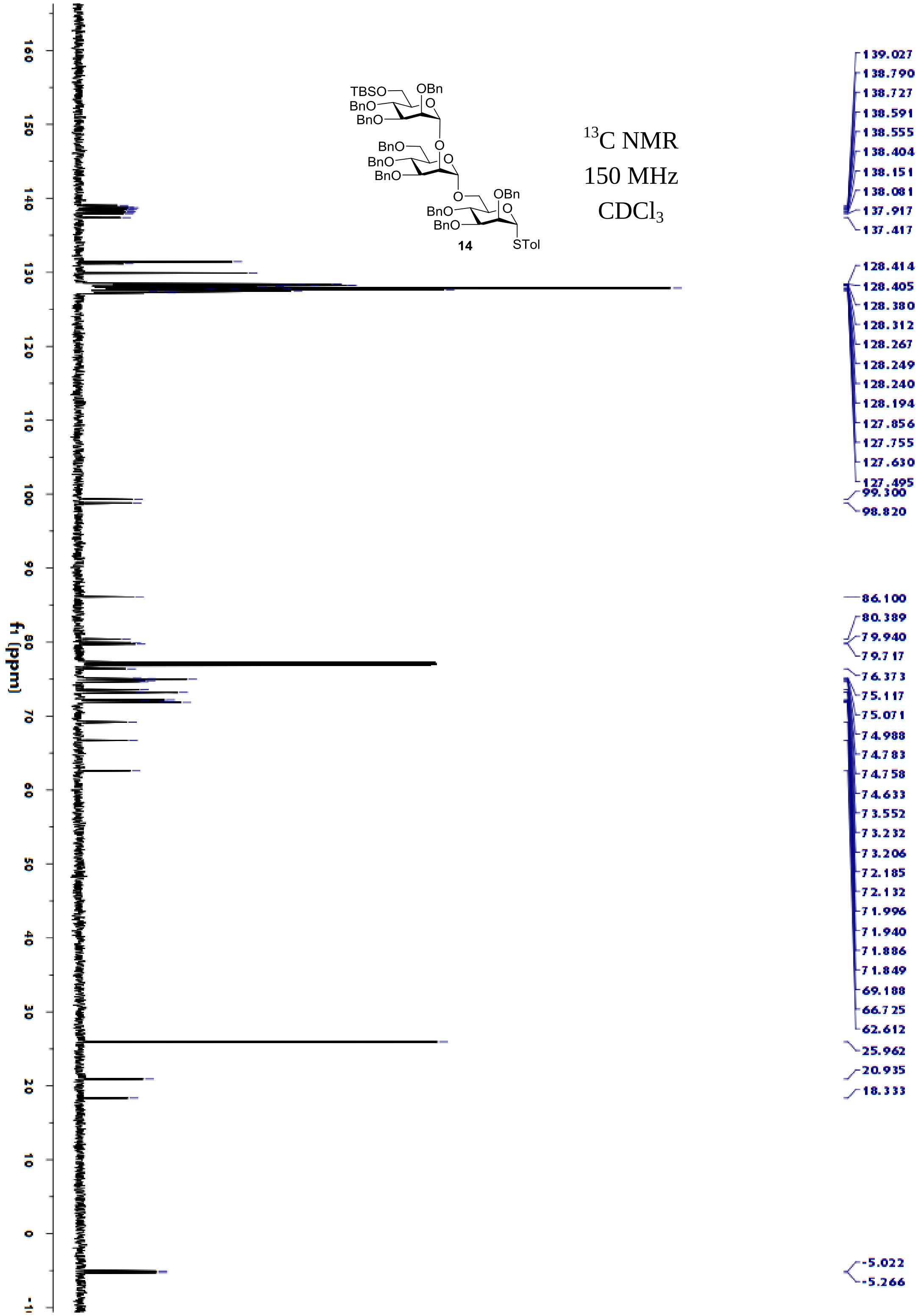
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O
14
STol
^{13}C NMR
150 MHz
CDCl$_3$
f1 (ppm)
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
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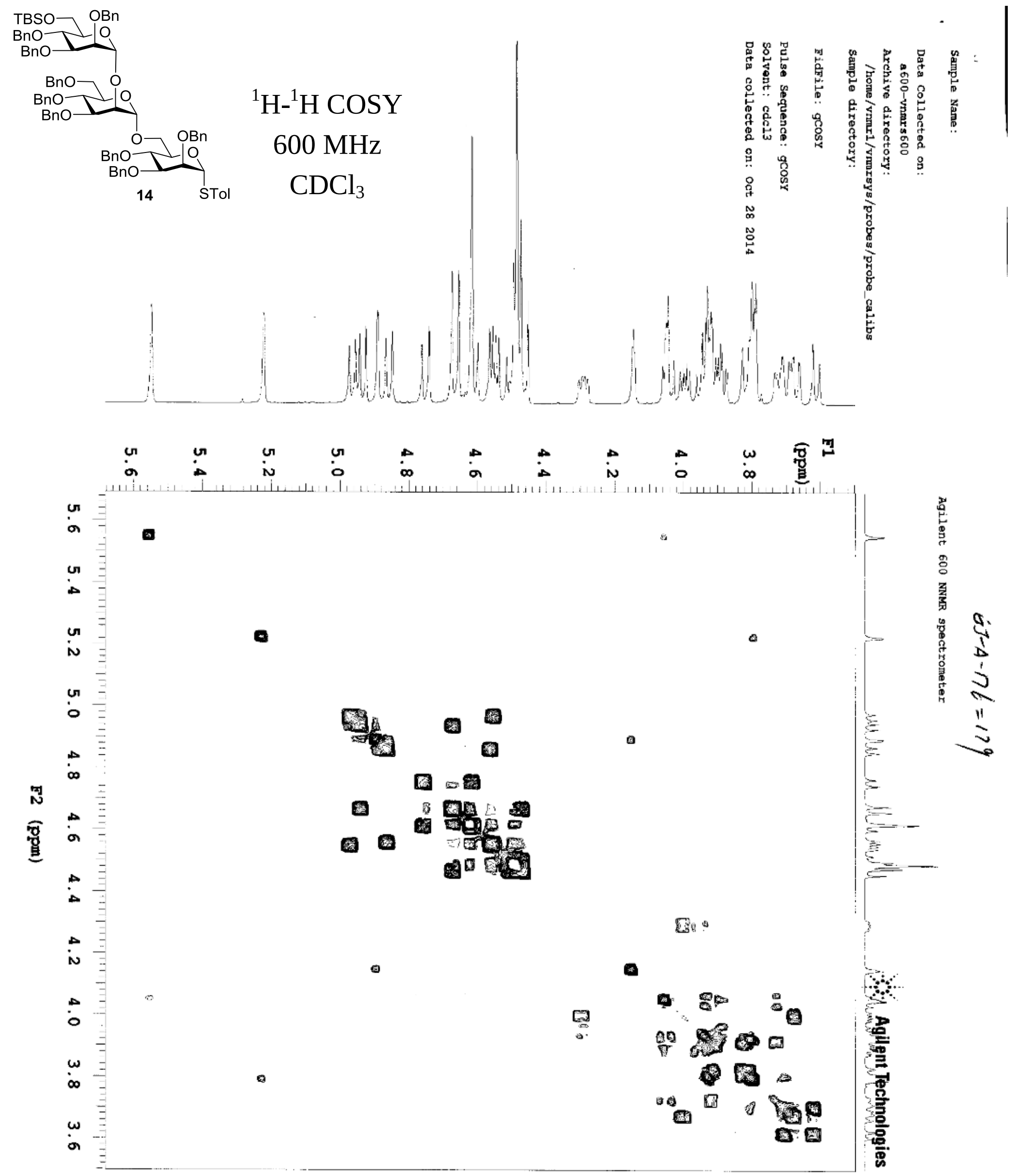
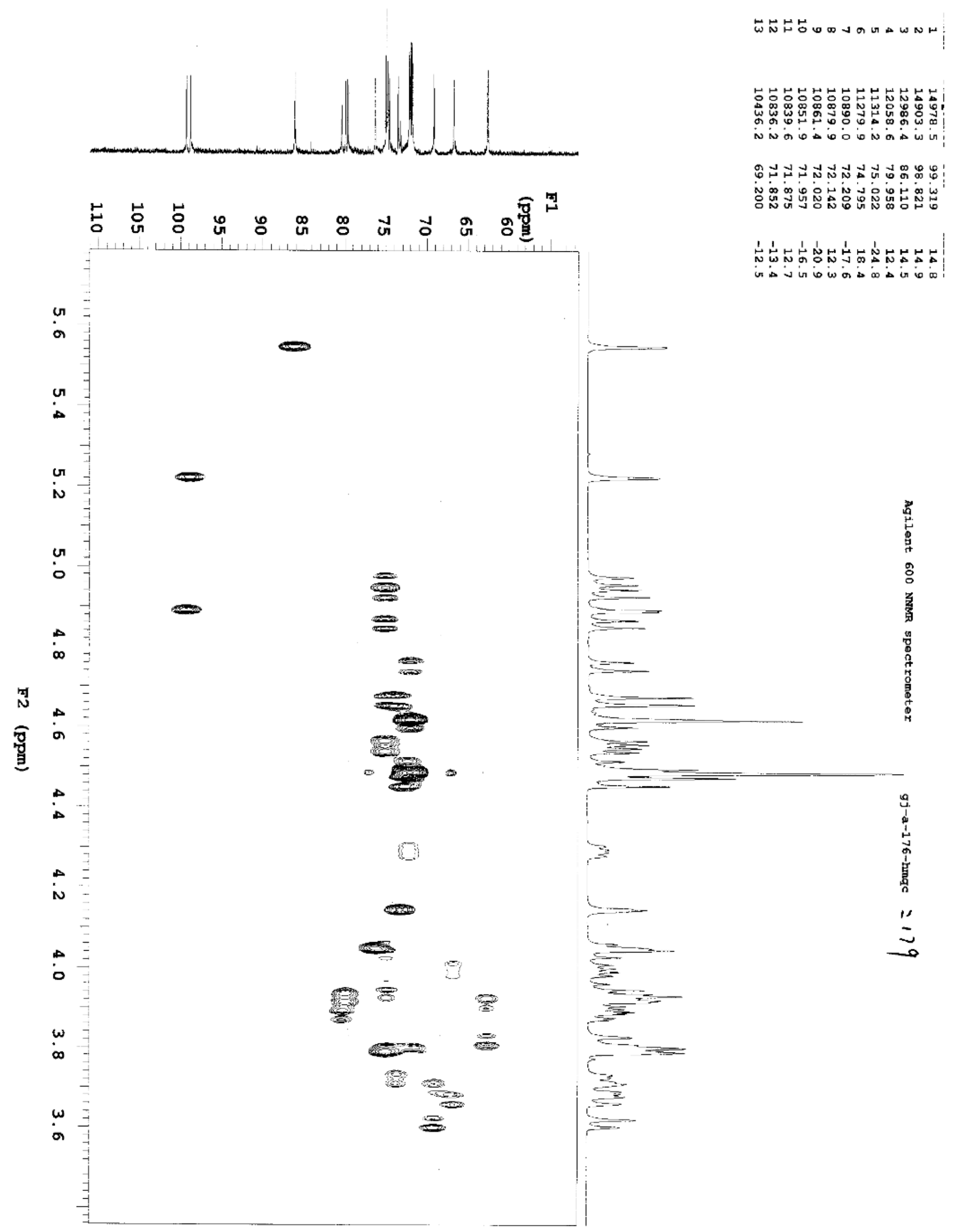


GJ-A-176=179

14

^{1}H-^{1}H COSY
600 MHz
$CDCl_3$



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4	12058.6	79.958	12.4
5	11314.2	75.022	-24.8
6	11279.9	74.795	18.4
7	10890.0	72.209	-17.6
8	10879.9	72.142	12.3
9	10861.4	72.020	-20.9
10	10851.9	71.957	-16.5
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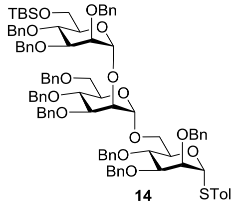
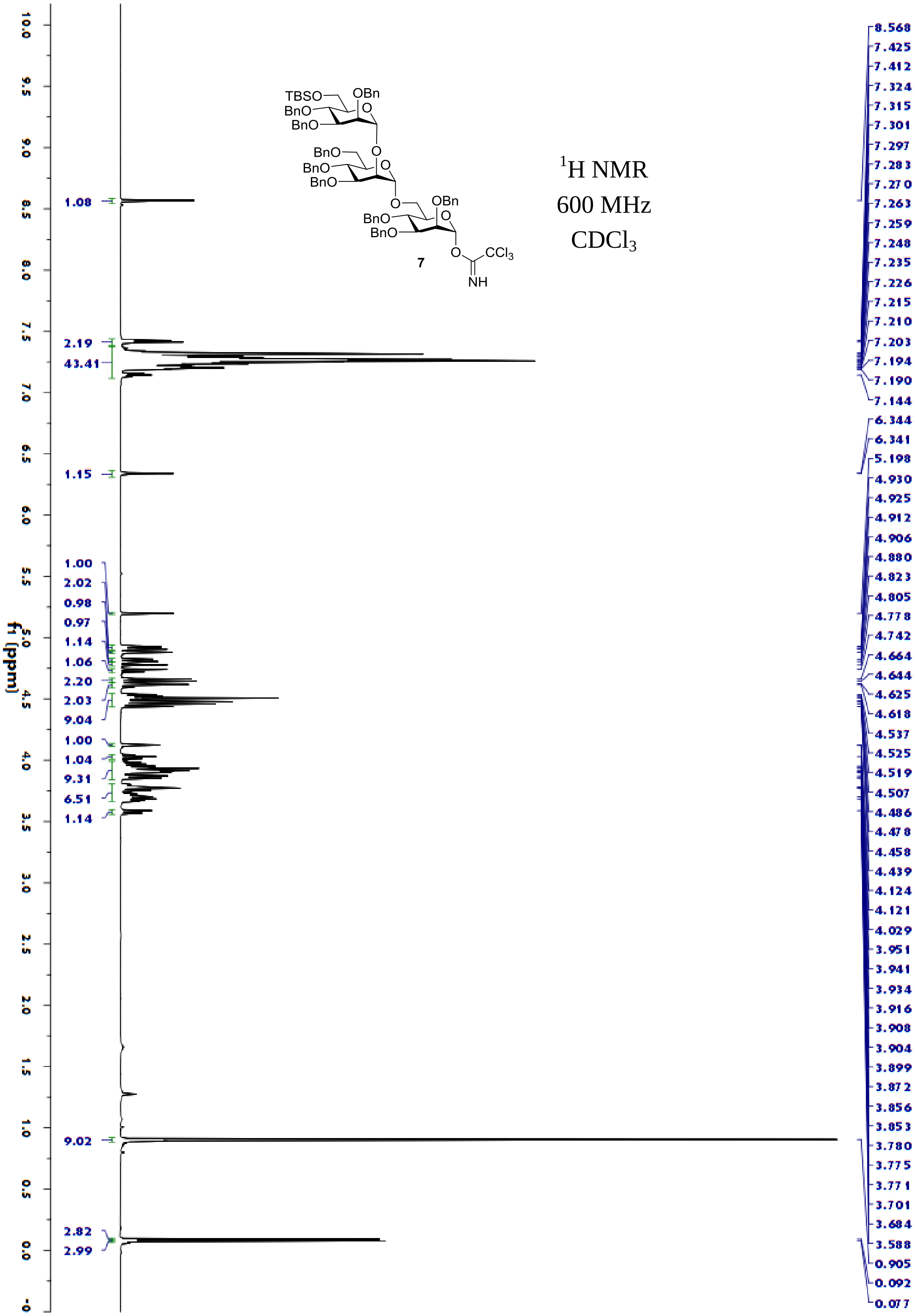


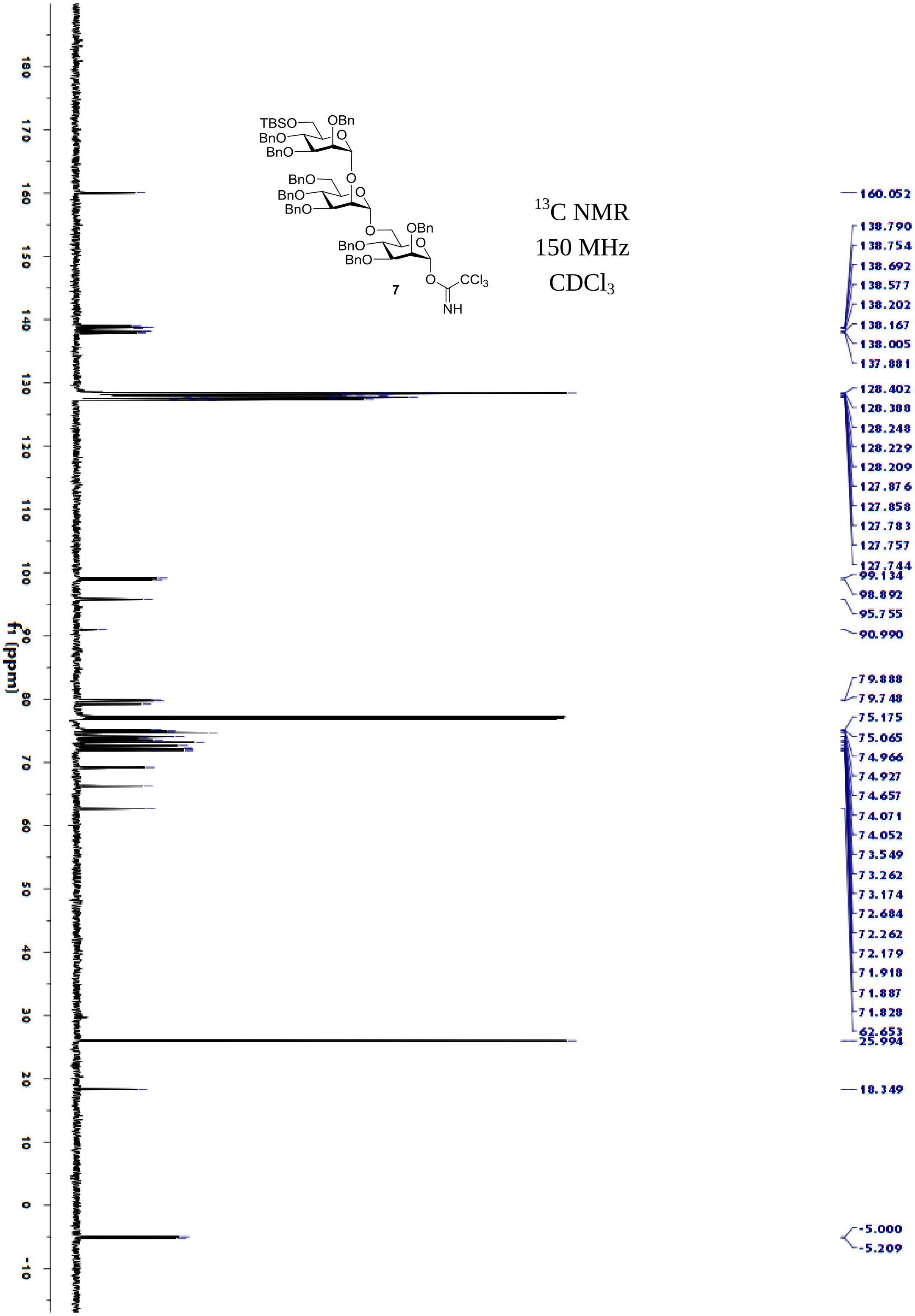
14

^{1}H-^{13}C HMQC
600/150 MHz
CDCl$_3$

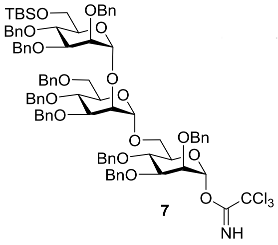


7

^{1}H NMR
600 MHz
$CDCl_3$

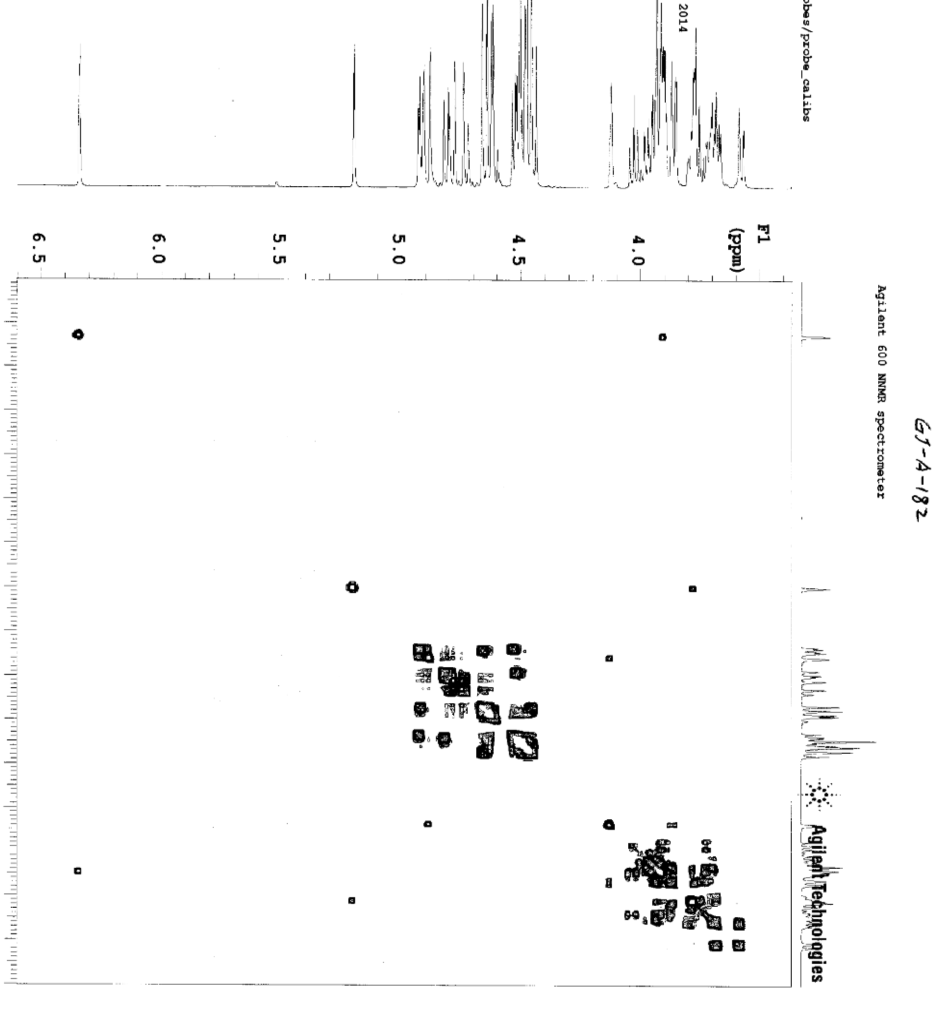


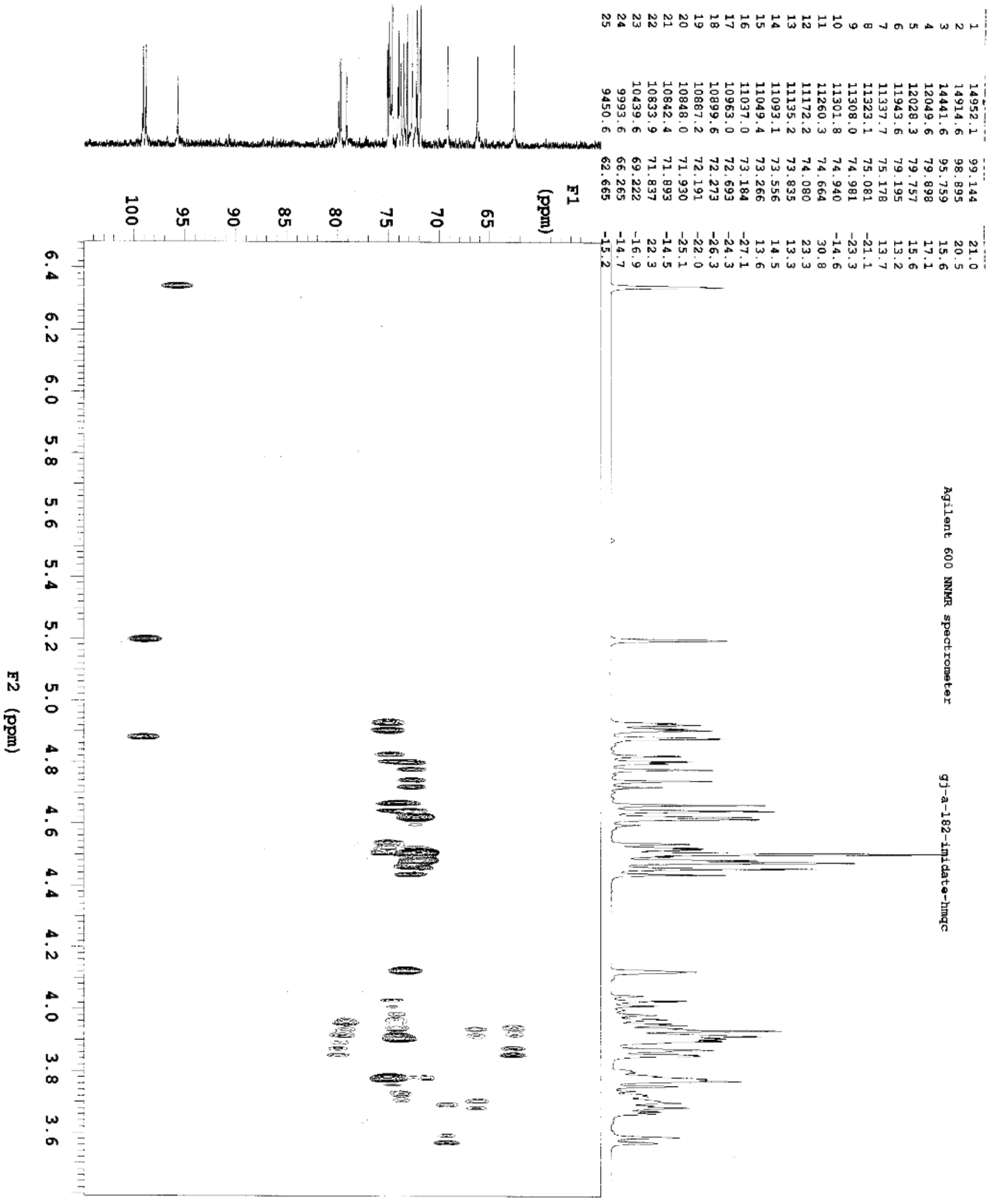
TBSO
OBn
O
BnO
BnO
O
BnO
BnO
BnO
O
O
OBn
BnO
BnO
O
O
CCl3
NH
7
13C NMR
150 MHz
CDCl3
f1 (ppm)
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
160.052
138.790
138.754
138.692
138.577
138.202
138.167
138.005
137.881
128.402
128.388
128.248
128.229
128.209
127.876
127.858
127.783
127.757
127.744
99.134
98.892
95.755
90.990
79.888
79.748
75.175
75.065
74.966
74.927
74.657
74.071
74.052
73.549
73.262
73.174
72.684
72.262
72.179
71.918
71.887
71.828
62.653
25.994
18.349
-5.000
-5.209



7

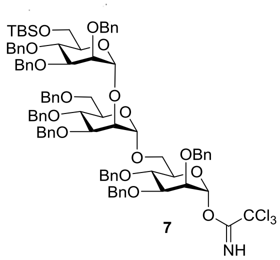
^{1}H-^{1}H COSY
600 MHz
CDCl$_3$

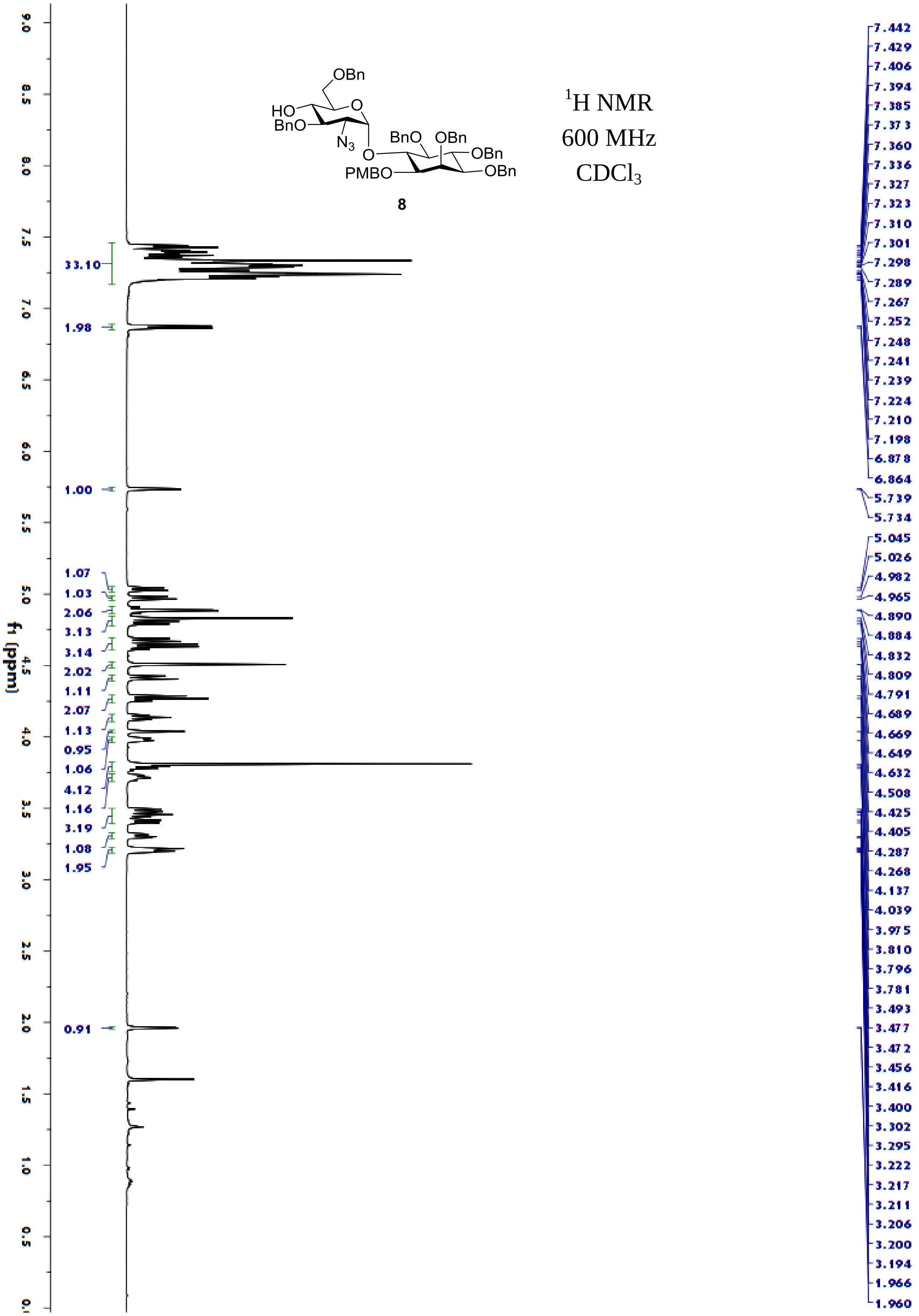




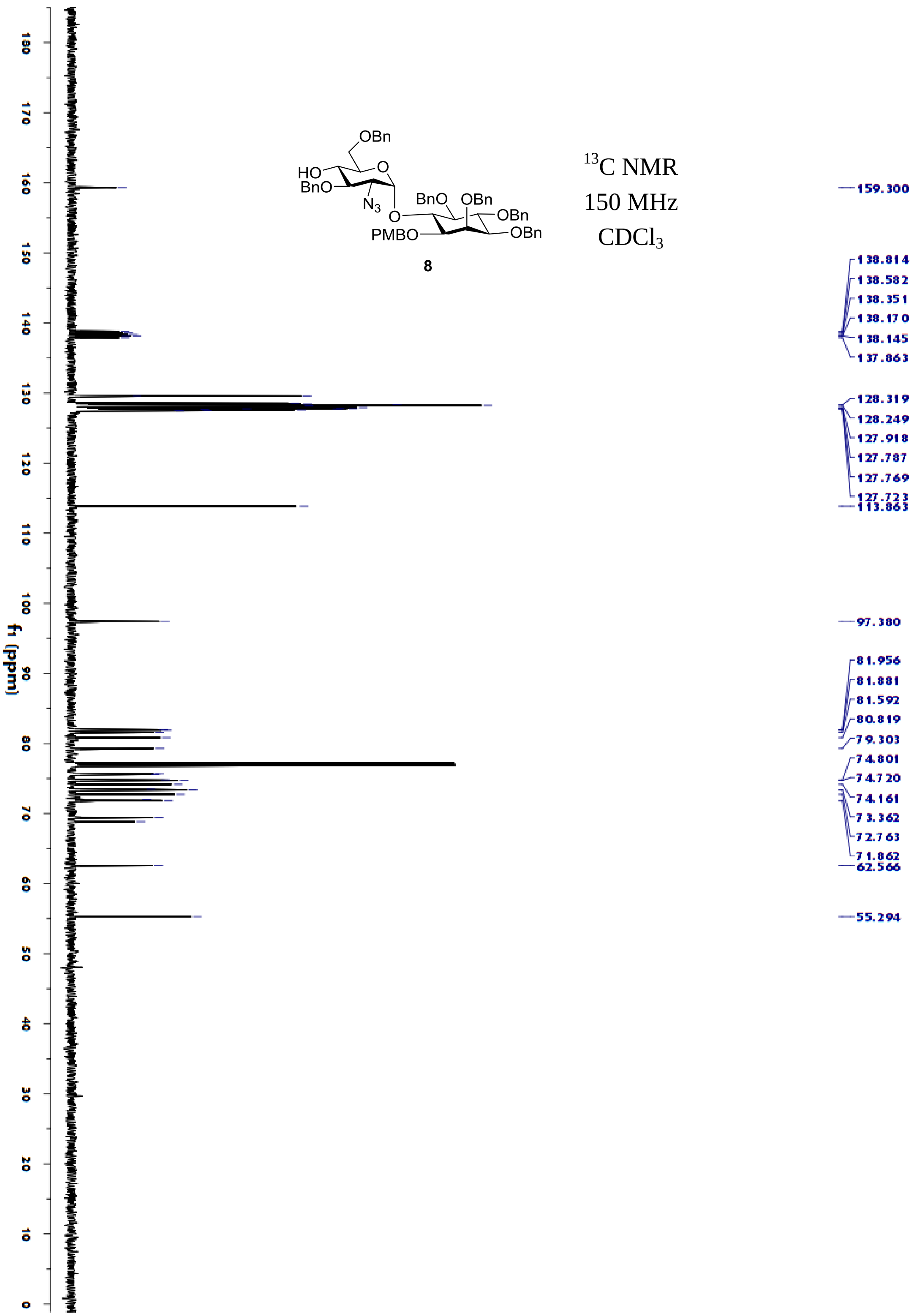
1	14952.1	99.144	21.0
2	14914.6	98.895	20.5
3	14441.6	95.759	15.6
4	12049.6	79.898	17.1
5	12028.3	79.757	15.6
6	11943.6	79.195	13.2
7	11337.7	75.178	13.7
8	11323.1	75.081	-21.1
9	11308.0	74.981	-23.3
10	11301.8	74.940	-14.6
11	11260.3	74.664	30.8
12	11172.2	74.080	23.3
13	11135.2	73.835	13.3
14	11093.1	73.556	14.5
15	11049.4	73.266	13.6
16	11037.0	73.184	-27.1
17	10963.0	72.693	-24.3
18	10899.6	72.273	-26.3
19	10887.2	72.191	-22.0
20	10848.0	71.930	-25.1
21	10842.4	71.893	-14.5
22	10833.9	71.837	22.3
23	10439.6	69.222	-16.9
24	9993.6	66.265	-14.7
25	9450.6	62.665	-15.2

^{1}H-^{13}C HMQC
600/150 MHz
$CDCl_3$



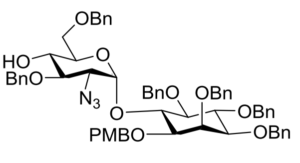


OBn
HO
BnO
N3
O
BnO
OBn
OBn
PMBO
OBn
8
1H NMR
600 MHz
CDCl3
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)
33.10
1.98
1.00
1.07
1.03
2.06
3.13
3.14
2.02
1.11
2.07
1.13
0.95
1.06
4.12
1.16
3.19
1.08
1.95
0.91
7.442
7.429
7.406
7.394
7.385
7.373
7.360
7.336
7.327
7.323
7.310
7.301
7.298
7.289
7.267
7.252
7.248
7.241
7.239
7.224
7.210
7.198
6.878
6.864
5.739
5.734
5.045
5.026
4.982
4.965
4.890
4.884
4.832
4.809
4.791
4.689
4.669
4.649
4.632
4.508
4.425
4.405
4.287
4.268
4.137
4.039
3.975
3.810
3.796
3.781
3.493
3.477
3.472
3.456
3.416
3.400
3.302
3.295
3.222
3.217
3.211
3.206
3.200
3.194
1.966
1.960

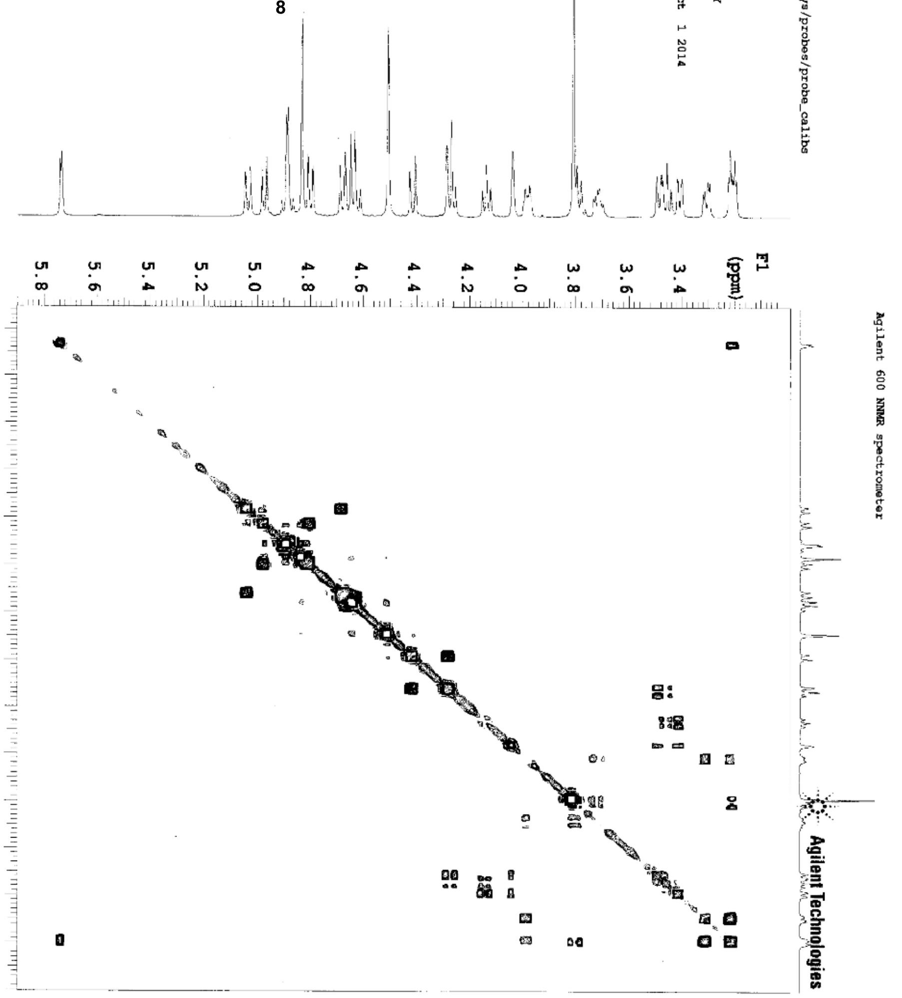


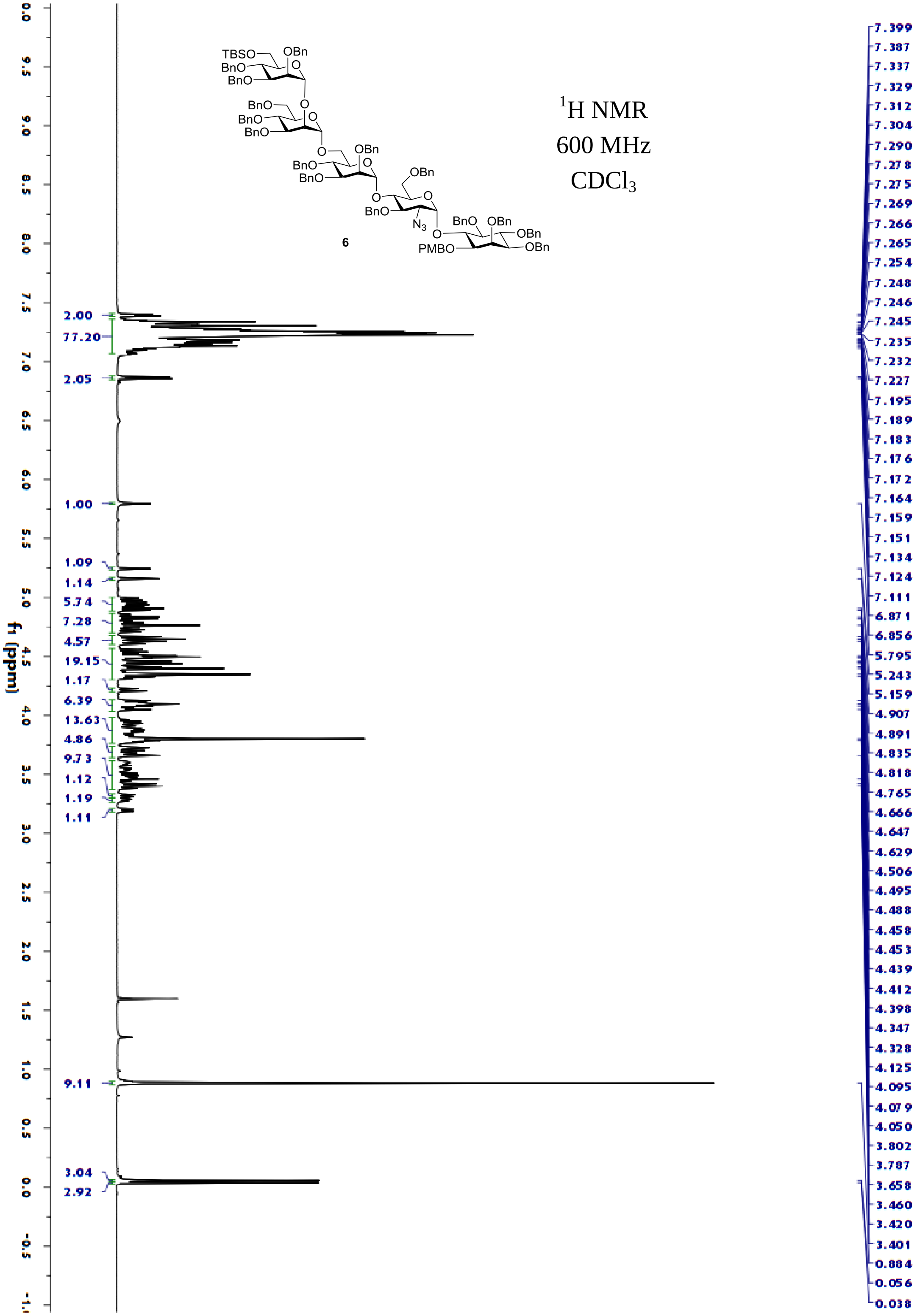
OBn
HO
O
BnO
N3
O
BnO
OBn
OBn
PMBO
OBn
8
13C NMR
150 MHz
CDCl3
159.300
138.814
138.582
138.351
138.170
138.145
137.863
128.319
128.249
127.918
127.787
127.769
127.723
113.863
97.380
81.956
81.881
81.592
80.819
79.303
74.801
74.720
74.161
73.362
72.763
71.862
62.566
55.294
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

^{1}H-^{1}H COSY
600 MHz
CDCl$_3$

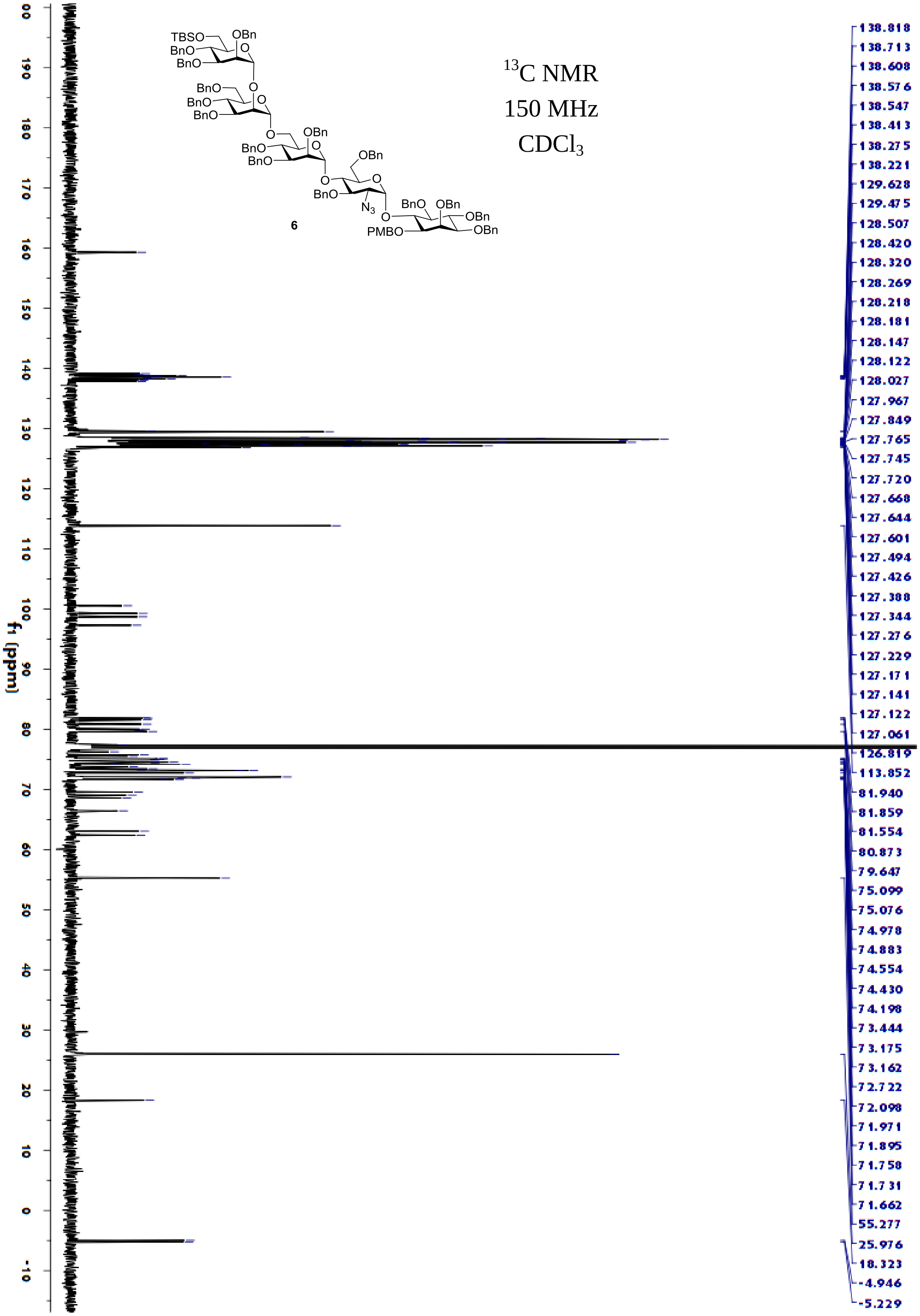


8



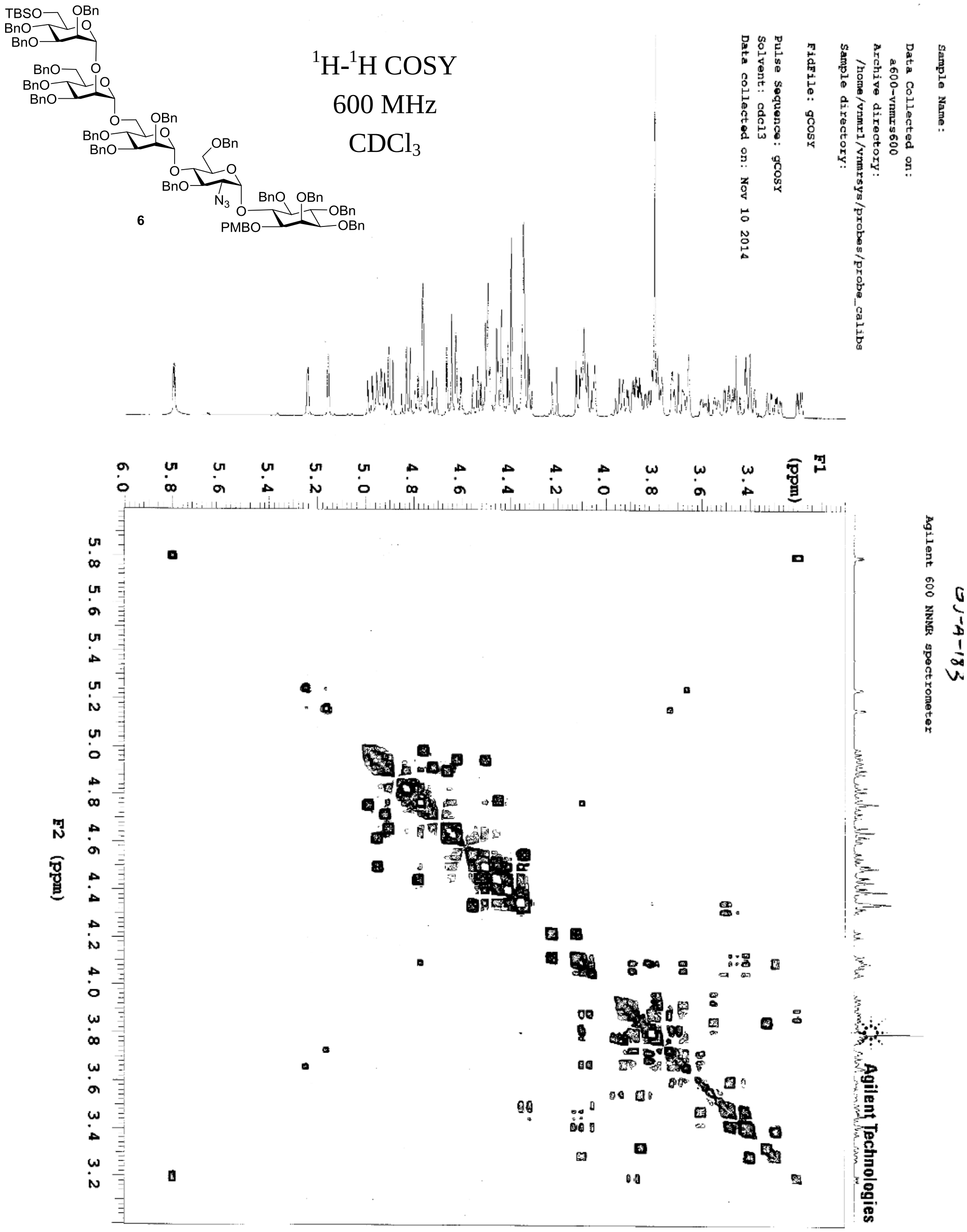


^{1}H NMR
600 MHz
$CDCl_3$



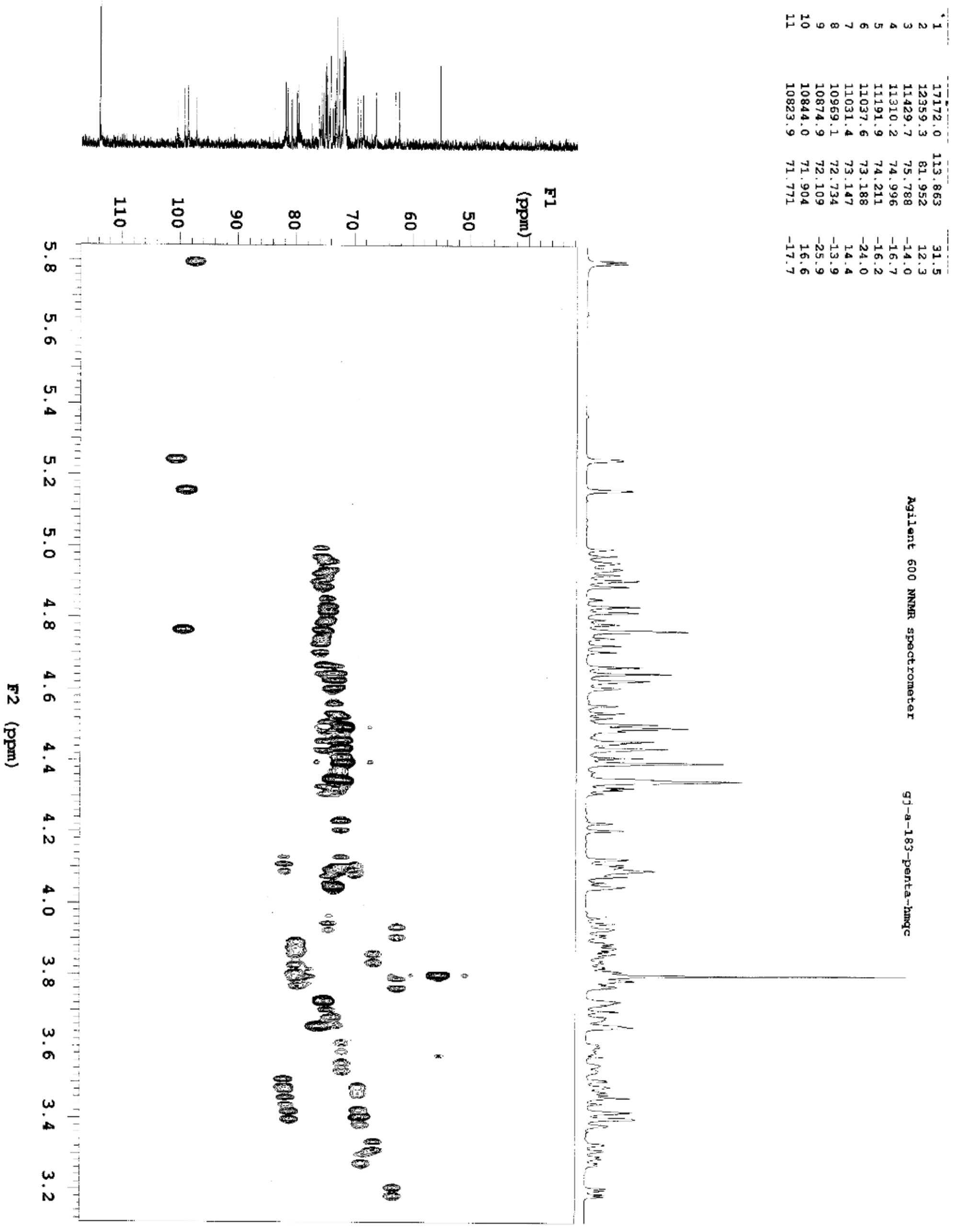
6

^{13}C NMR
150 MHz
$CDCl_3$

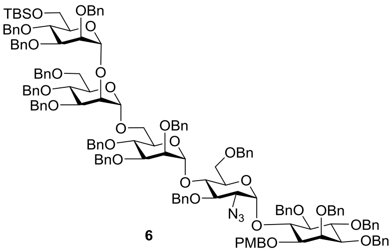


6

^{1}H-^{1}H COSY
600 MHz
CDCl$_3$

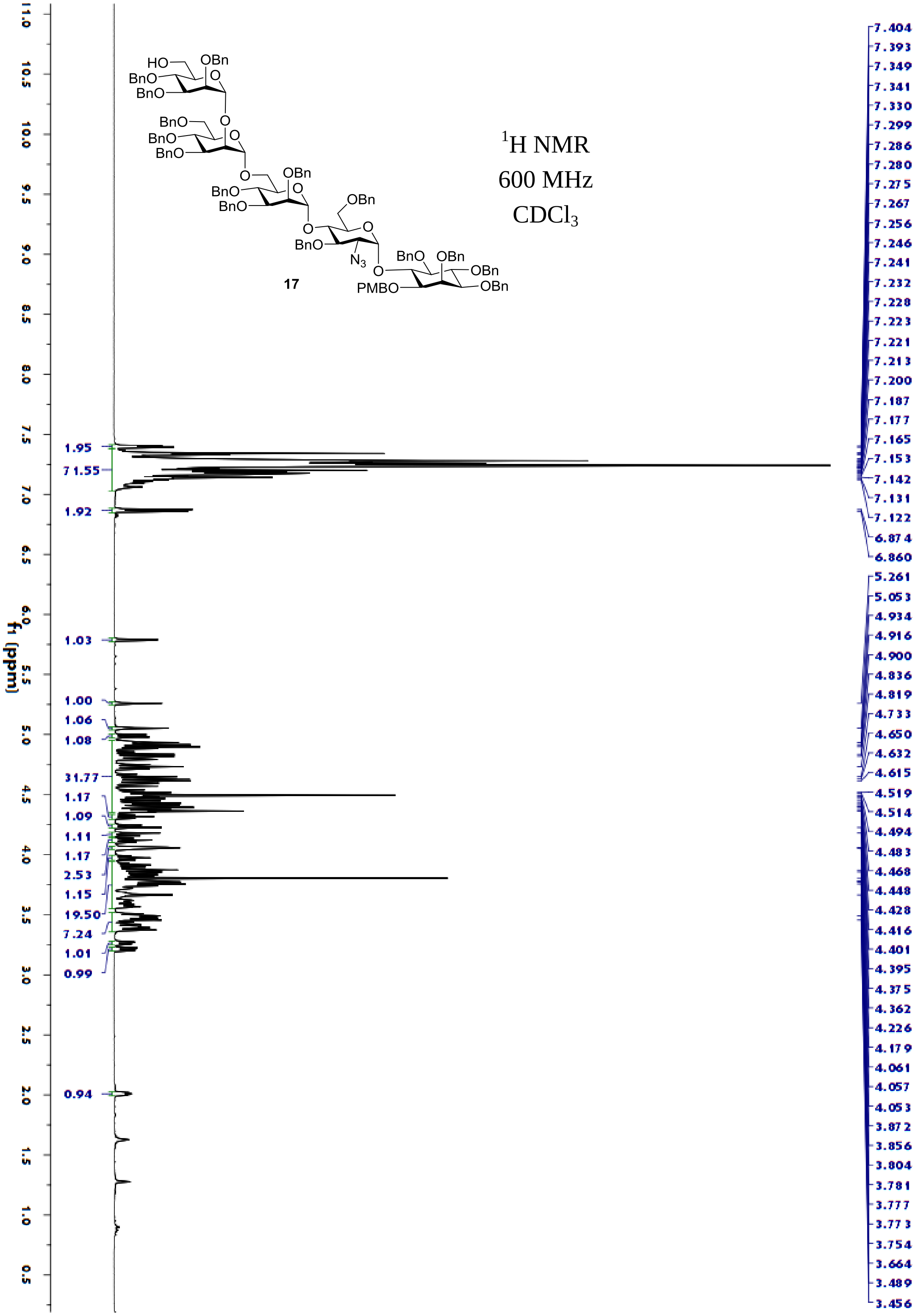


1	17172.0	113.863	31.5
2	12359.3	81.952	12.3
3	11429.7	75.788	-14.0
4	11310.2	74.996	-16.7
5	11191.9	74.211	-16.2
6	11037.6	73.188	-24.0
7	11031.4	73.147	14.4
8	10969.1	72.734	-13.9
9	10874.9	72.109	-25.9
10	10844.0	71.904	16.6
11	10823.9	71.771	-17.7

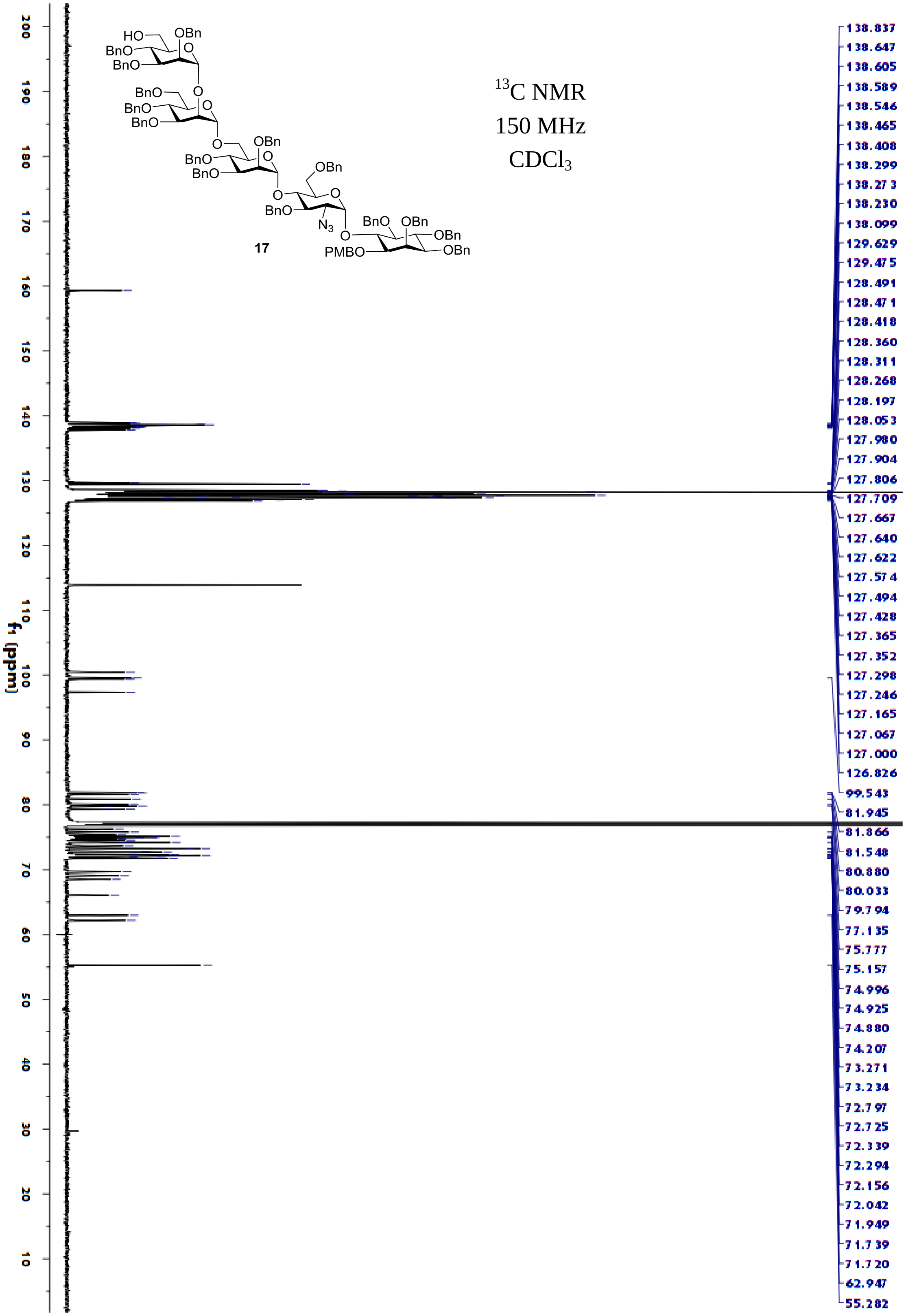


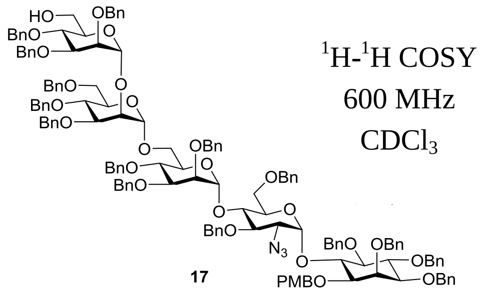
6

^{1}H-^{13}C HMQC
600/150 MHz
$CDCl_3$



17
HO
OBn
BnO
BnO
O
BnO
BnO
BnO
O
O
O
OBn
BnO
BnO
O
OBn
O
BnO
N3
O
BnO
OBn
OBn
OBn
PMBO
¹H NMR
600 MHz
CDCl3
f1 (ppm)
11.0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
1.95
71.55
1.92
1.03
1.00
1.06
1.08
31.77
1.17
1.09
1.11
1.17
2.53
1.15
19.50
7.24
1.01
0.99
0.94
7.404
7.393
7.349
7.341
7.330
7.299
7.286
7.280
7.275
7.267
7.256
7.246
7.241
7.232
7.228
7.223
7.221
7.213
7.200
7.187
7.177
7.165
7.153
7.142
7.131
7.122
6.874
6.860
5.261
5.053
4.934
4.916
4.900
4.836
4.819
4.733
4.650
4.632
4.615
4.519
4.514
4.494
4.483
4.468
4.448
4.428
4.416
4.401
4.395
4.375
4.362
4.226
4.179
4.061
4.057
4.053
3.872
3.856
3.804
3.781
3.777
3.773
3.754
3.664
3.489
3.456


13C NMR
150 MHz
CDCl3
HO
OBn
BnO
BnO
O
BnO
BnO
BnO
O
O
OBn
BnO
BnO
O
O
OBn
BnO
N3
O
BnO
OBn
OBn
OBn
PMBO
17
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)
138.837
138.647
138.605
138.589
138.546
138.465
138.408
138.299
138.273
138.230
138.099
129.629
129.475
128.491
128.471
128.418
128.360
128.311
128.268
128.197
128.053
127.980
127.904
127.806
127.709
127.667
127.640
127.622
127.574
127.494
127.428
127.365
127.352
127.298
127.246
127.165
127.067
127.000
126.826
99.543
81.945
81.866
81.548
80.880
80.033
79.794
77.135
75.777
75.157
74.996
74.925
74.880
74.207
73.271
73.234
72.797
72.725
72.339
72.294
72.156
72.042
71.949
71.739
71.720
62.947
55.282

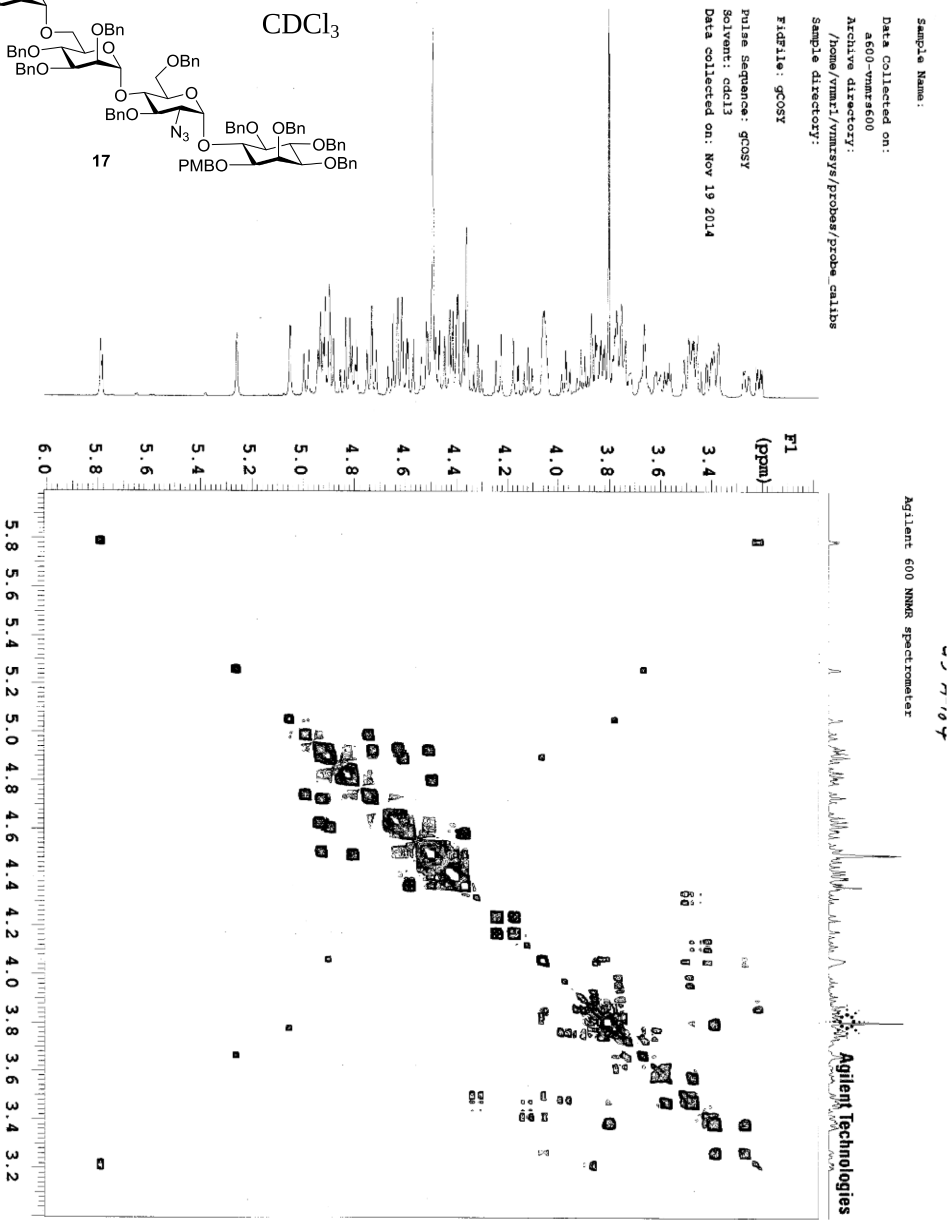


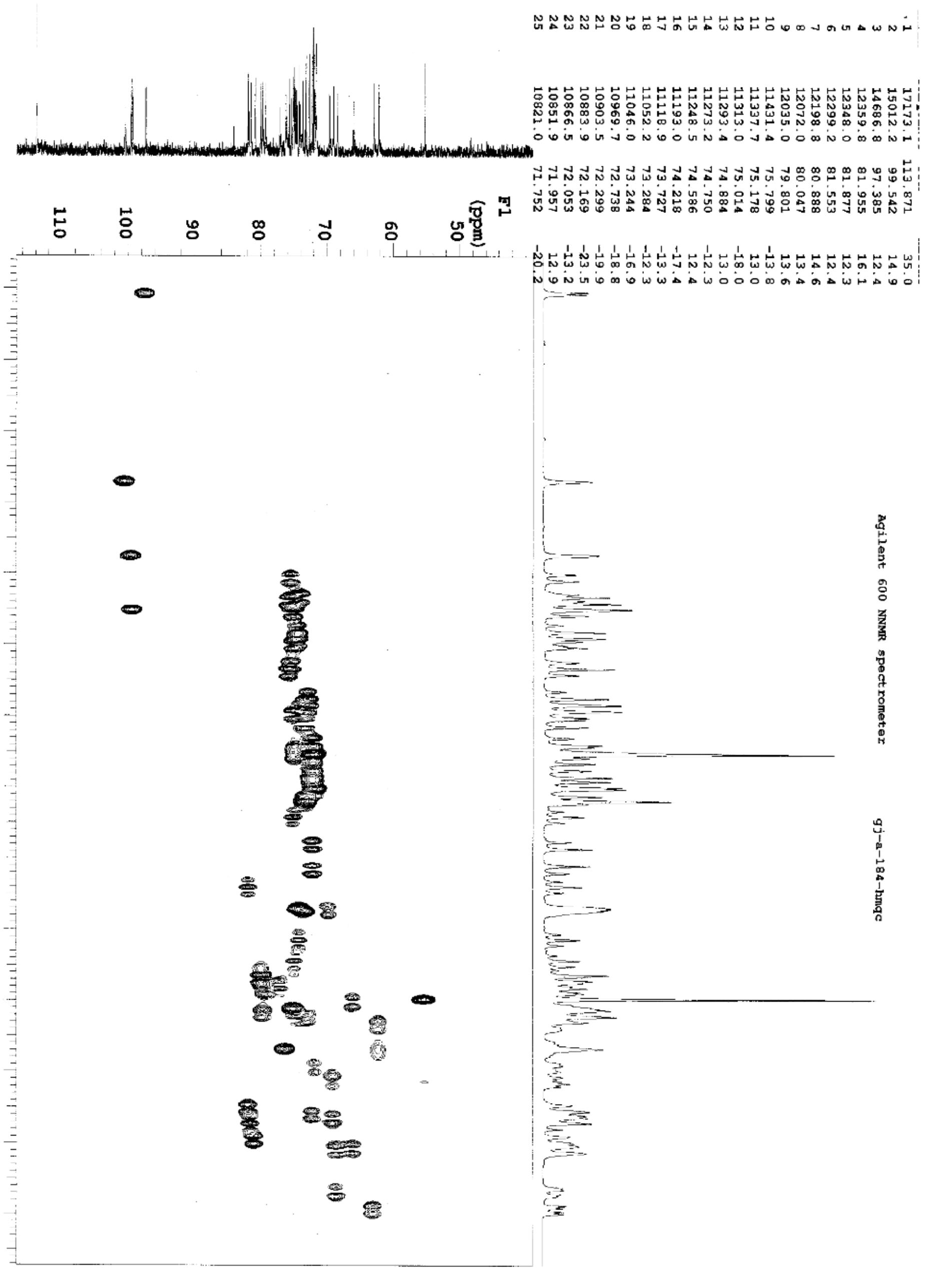
17

^{1}H-^{1}H COSY

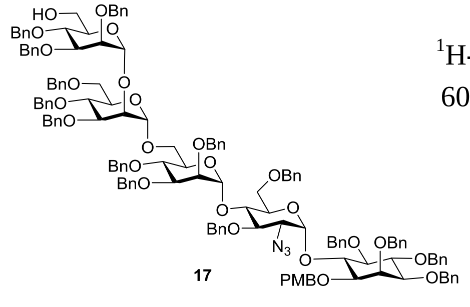
600 MHz

$CDCl_3$



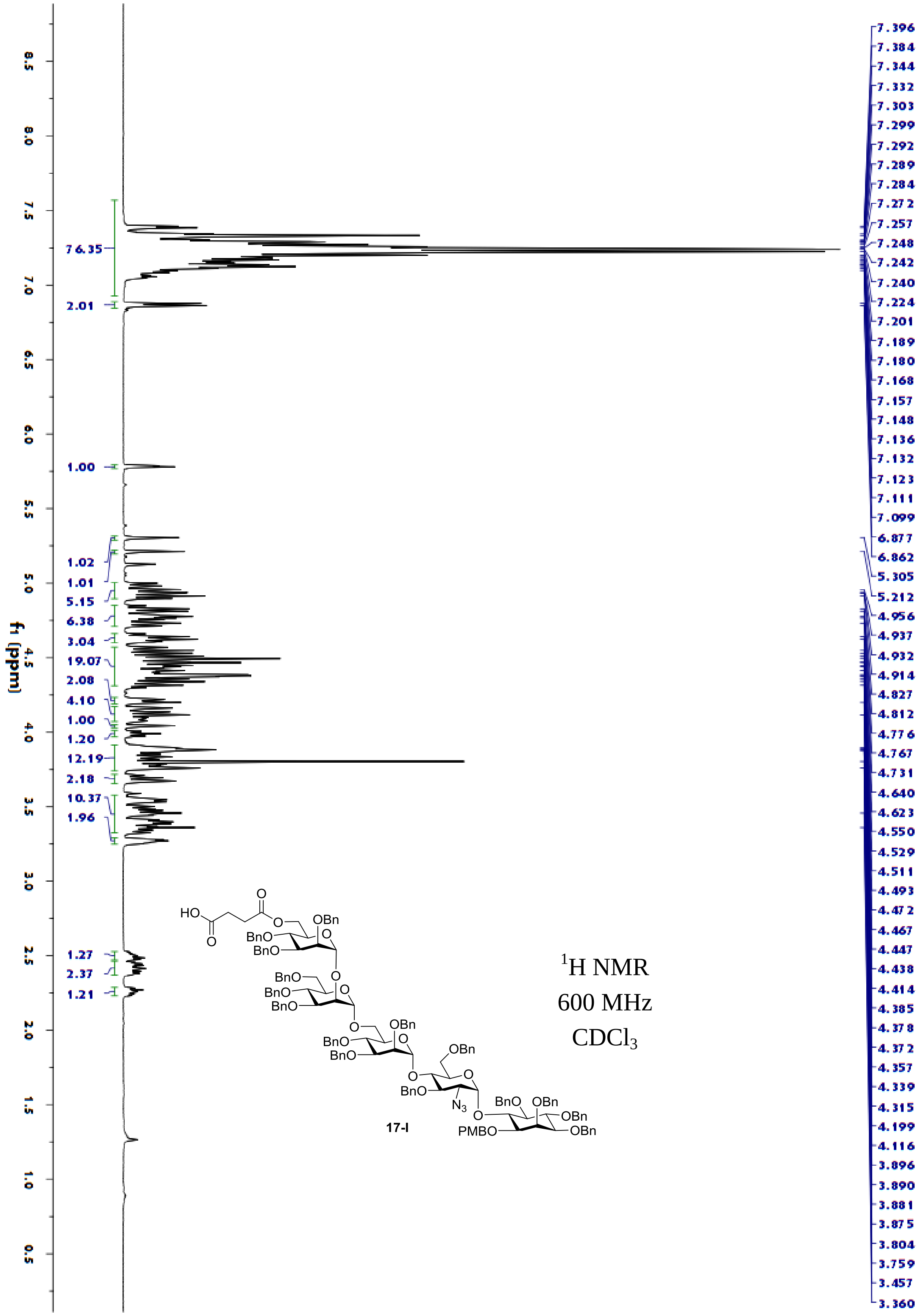


1	17173.1	113.871	35.0
2	15012.2	99.542	14.9
3	14686.8	97.385	12.4
4	12359.8	81.955	16.1
5	12348.0	81.877	12.3
6	12299.2	81.553	12.4
7	12198.8	80.888	14.6
8	12072.0	80.047	13.4
9	12035.0	79.801	13.6
10	11431.4	75.799	-13.8
11	11337.7	75.178	13.0
12	11313.0	75.014	-18.0
13	11293.4	74.884	13.0
14	11273.2	74.750	-12.3
15	11248.5	74.586	12.4
16	11193.0	74.218	-17.4
17	11118.9	73.727	-13.3
18	11052.2	73.284	-12.3
19	11046.0	73.244	-16.9
20	10969.7	72.738	-18.8
21	10903.5	72.299	-19.9
22	10883.9	72.169	-23.5
23	10866.5	72.053	-13.2
24	10851.9	71.957	12.9
25	10821.0	71.752	-20.2



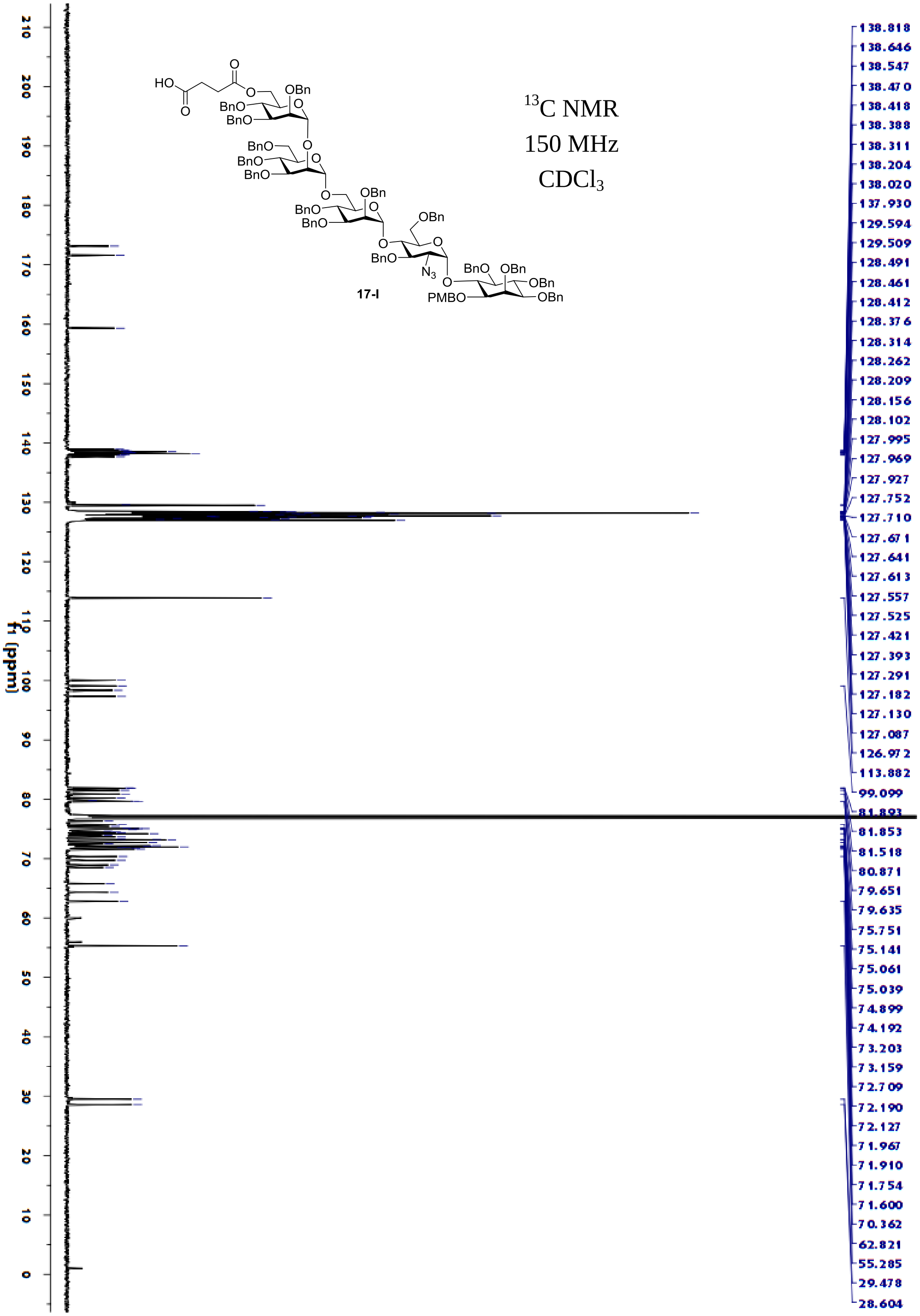
17

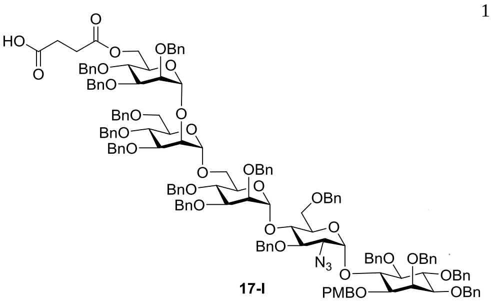
^{1}H-^{13}C HMQC
600/150 MHz
CDCl$_3$



17-I

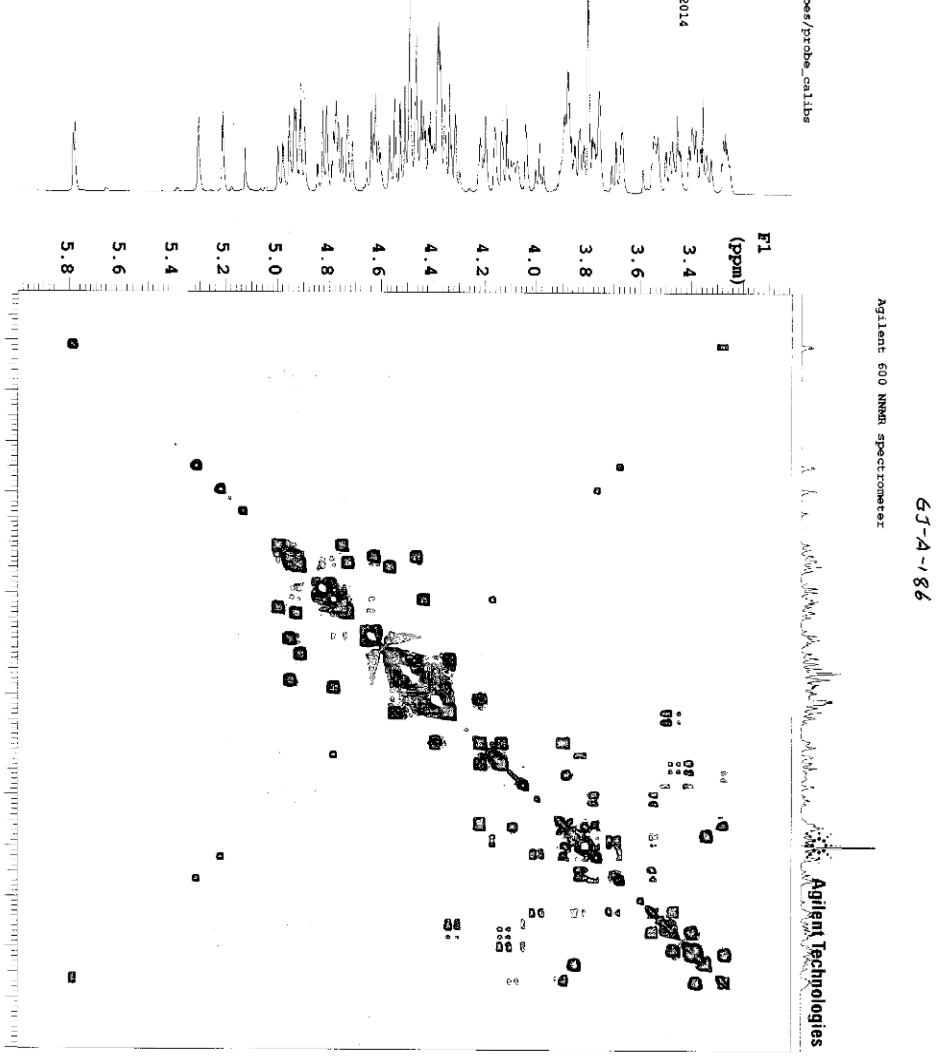
^{1}H NMR
600 MHz
CDCl$_3$

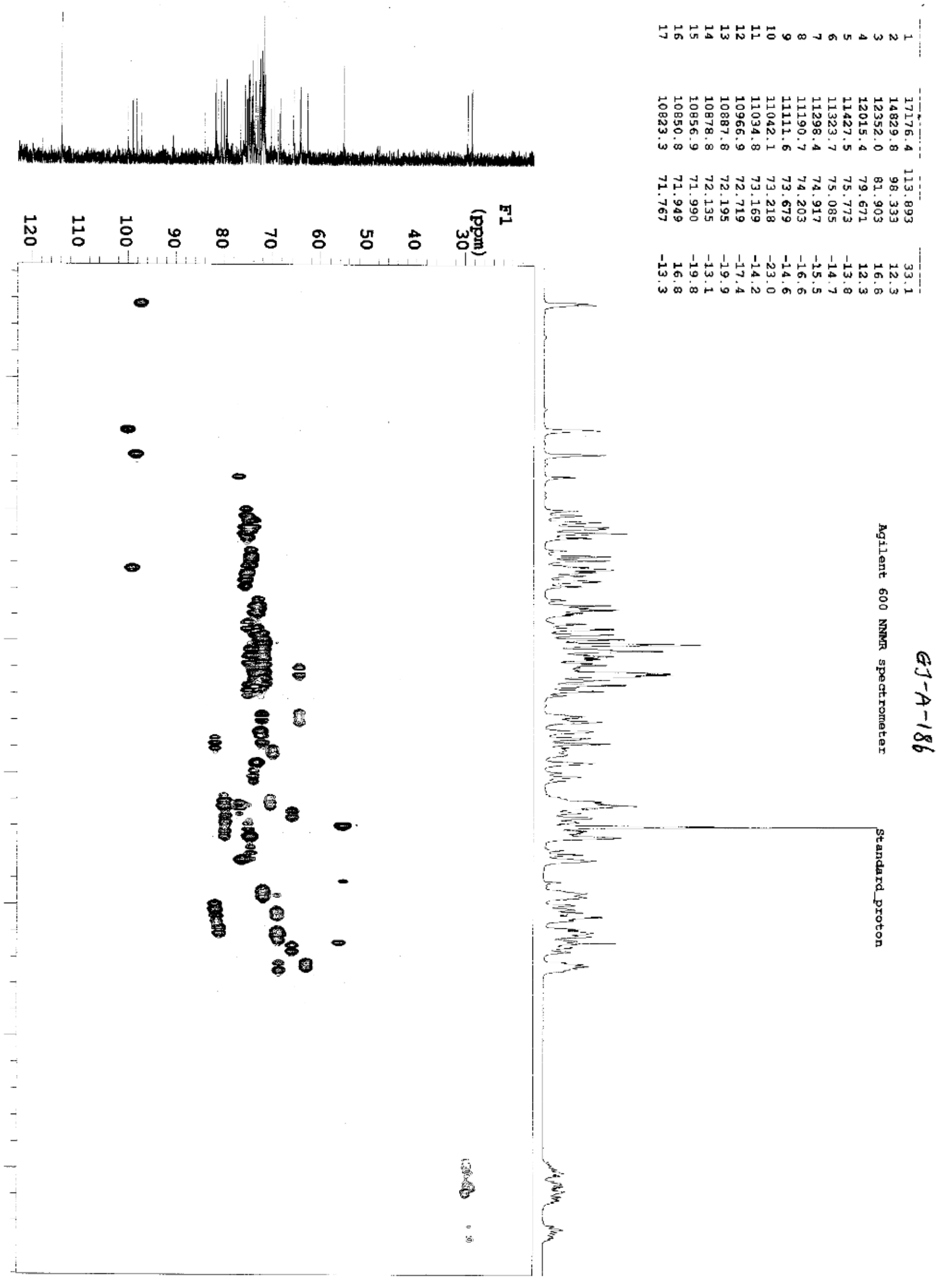

^{13}C NMR
150 MHz
CDCl$_3$
17-I
HO
O
O
OBn
BnO
BnO
BnO
BnO
BnO
O
OBn
BnO
BnO
OBn
BnO
N$_3$
BnO
OBn
OBn
OBn
PMBO
f1 (ppm)
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
138.818
138.646
138.547
138.470
138.418
138.388
138.311
138.204
138.020
137.930
129.594
129.509
128.491
128.461
128.412
128.376
128.314
128.262
128.209
128.156
128.102
127.995
127.969
127.927
127.752
127.710
127.671
127.641
127.613
127.557
127.525
127.421
127.393
127.291
127.182
127.130
127.087
126.972
113.882
99.099
81.893
81.853
81.518
80.871
79.651
79.635
75.751
75.141
75.061
75.039
74.899
74.192
73.203
73.159
72.709
72.190
72.127
71.967
71.910
71.754
71.600
70.362
62.821
55.285
29.478
28.604



17-I

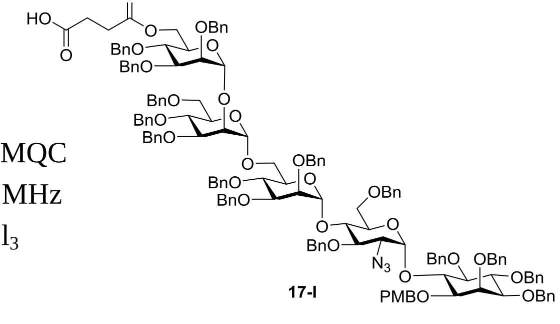
^{1}H-^{1}H COSY
600 MHz
$CDCl_3$



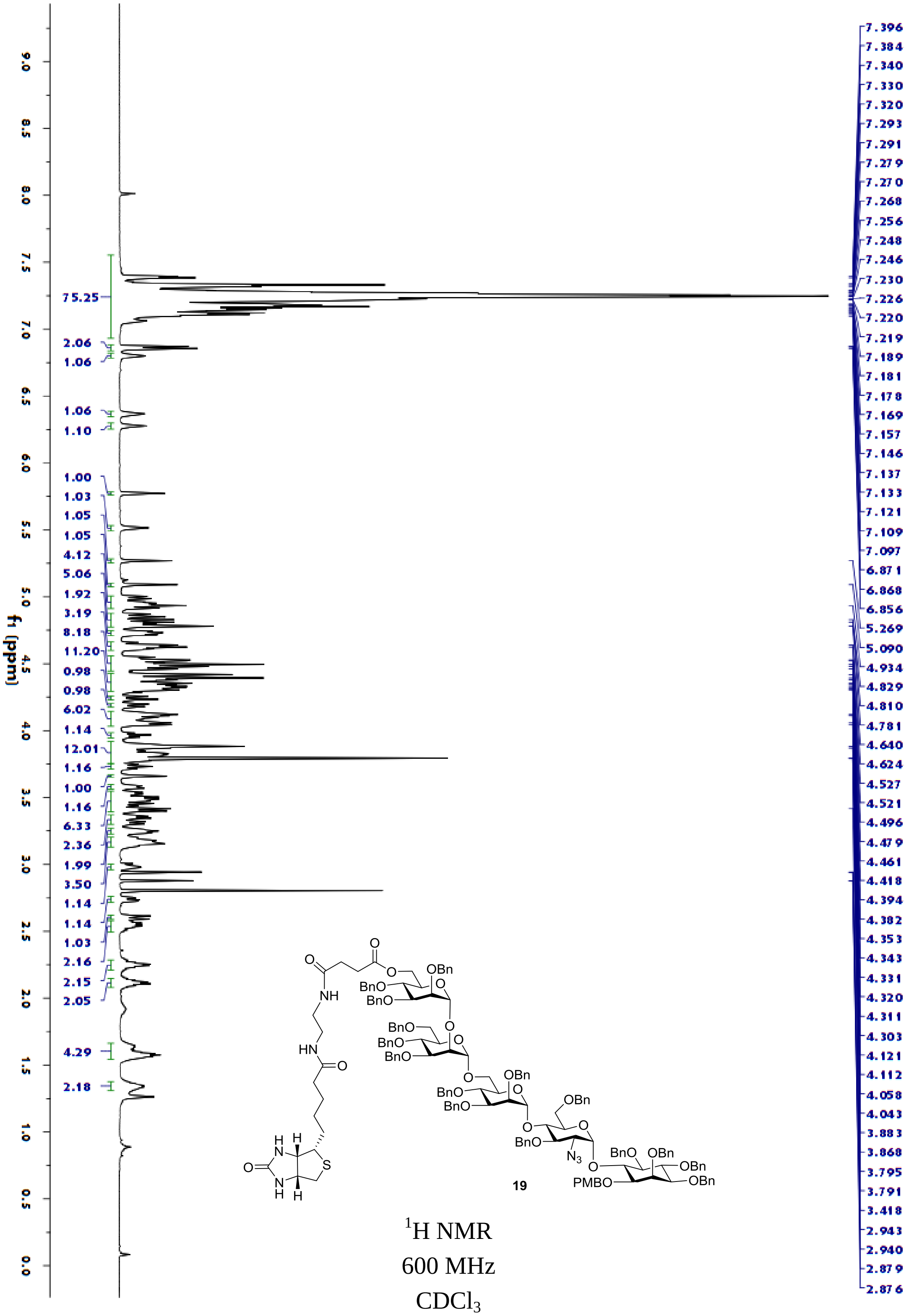


1	17176.4	113.893	33.1
2	14829.8	98.333	12.3
3	12352.0	81.903	16.6
4	12015.4	79.671	12.3
5	11427.5	75.773	-13.8
6	11323.7	75.085	-14.7
7	11298.4	74.917	-15.5
8	11190.7	74.203	-16.6
9	11111.6	73.679	-14.6
10	11042.1	73.218	-23.0
11	11034.8	73.169	-14.2
12	10966.9	72.719	-17.4
13	10887.8	72.195	-19.9
14	10878.8	72.135	-13.1
15	10856.9	71.990	-19.8
16	10850.8	71.949	16.8
17	10823.3	71.767	-13.3

^{1}H-^{13}C HMQC
600/150 MHz
CDCl$_3$



17-I

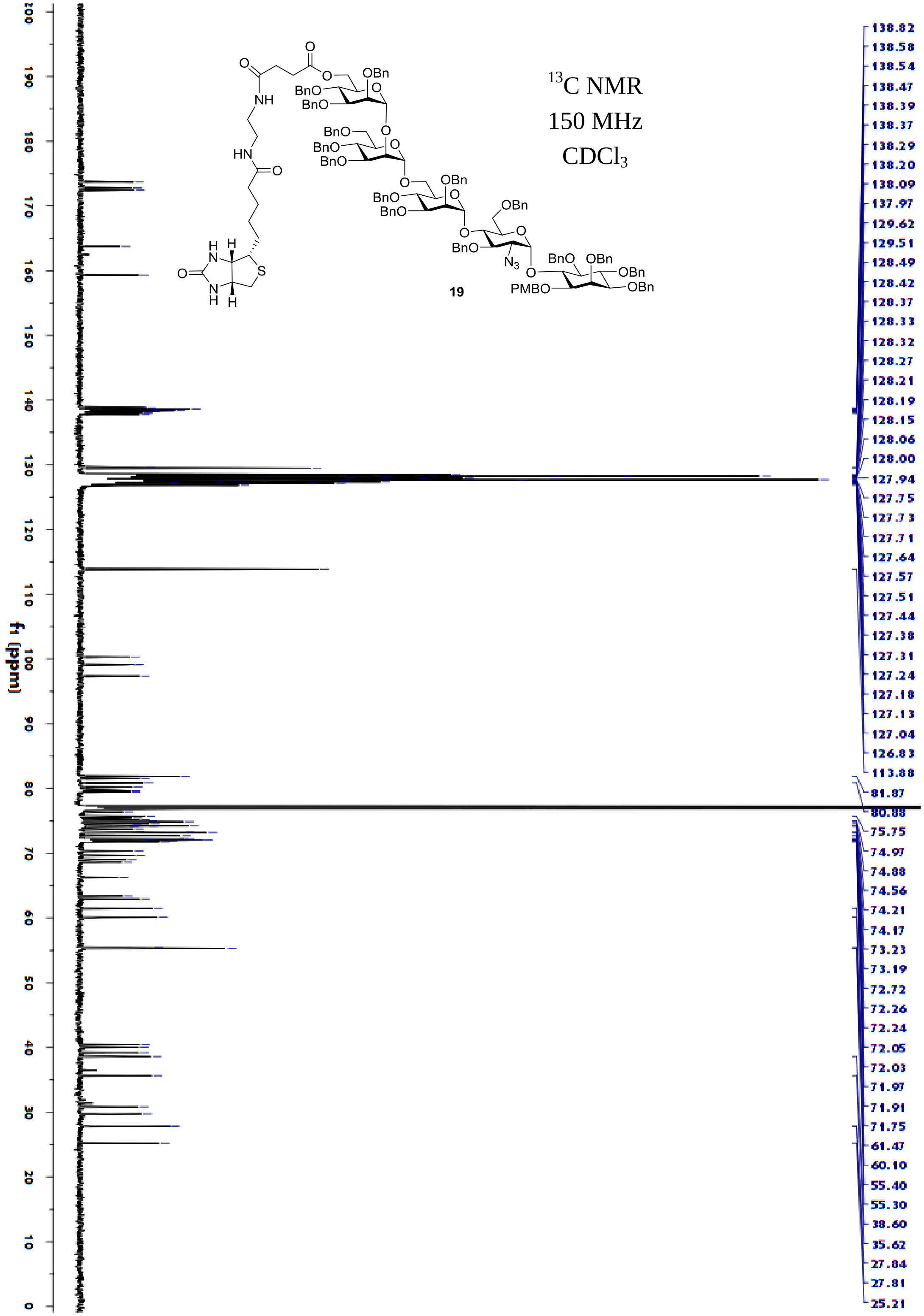


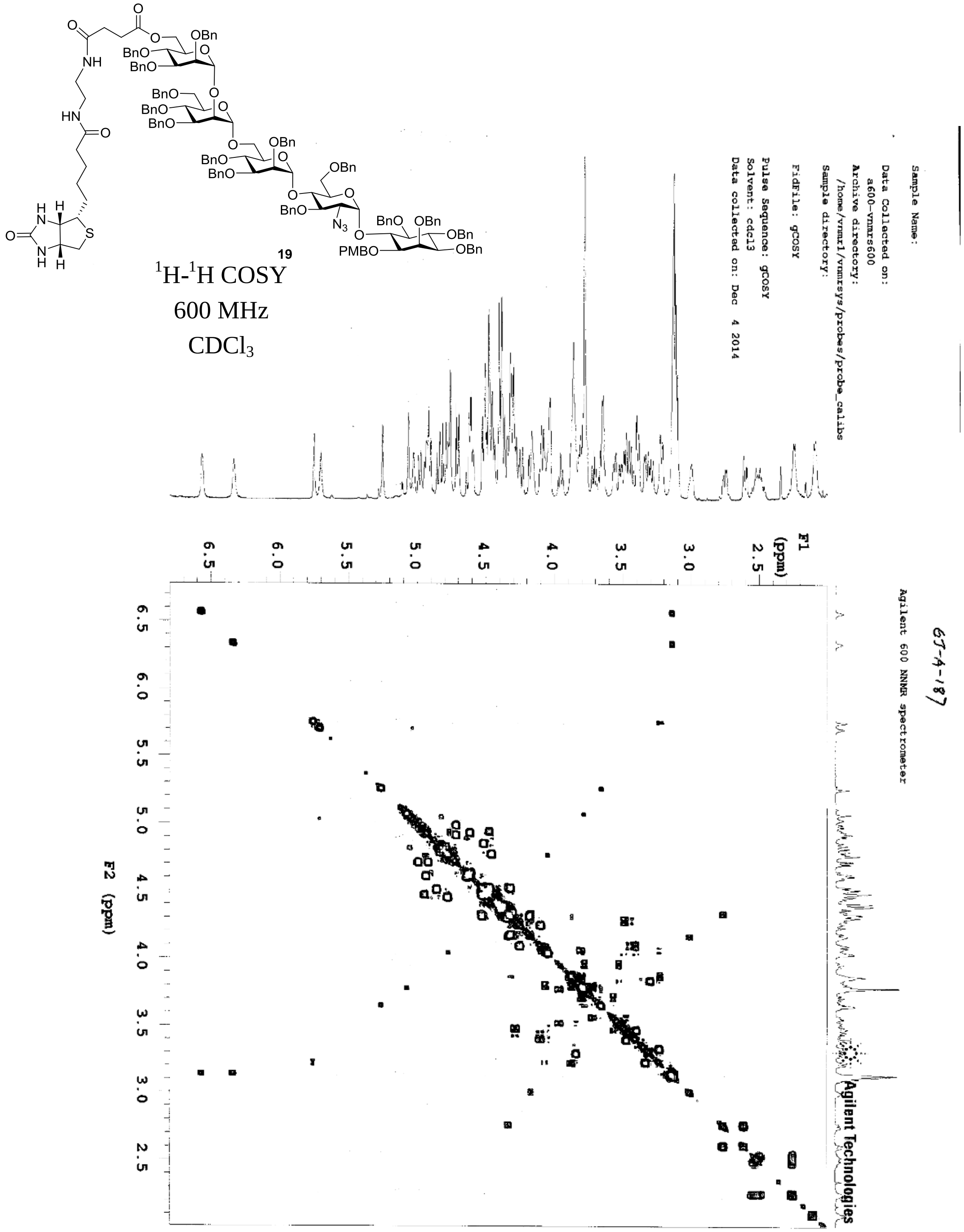
19

^{1}H NMR

600 MHz

$CDCl_3$

13C NMR
150 MHz
CDCl3
19
BnO
OBn
N3
PMBO
NH
HN
S
f1 (ppm)
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
138.82
138.58
138.54
138.47
138.39
138.37
138.29
138.20
138.09
137.97
129.62
129.51
128.49
128.42
128.37
128.33
128.32
128.27
128.21
128.19
128.15
128.06
128.00
127.94
127.75
127.73
127.71
127.64
127.57
127.51
127.44
127.38
127.31
127.24
127.18
127.13
127.04
126.83
113.88
81.87
80.88
75.75
74.97
74.88
74.56
74.21
74.17
73.23
73.19
72.72
72.26
72.24
72.05
72.03
71.97
71.91
71.75
61.47
60.10
55.40
55.30
38.60
35.62
27.84
27.81
25.21

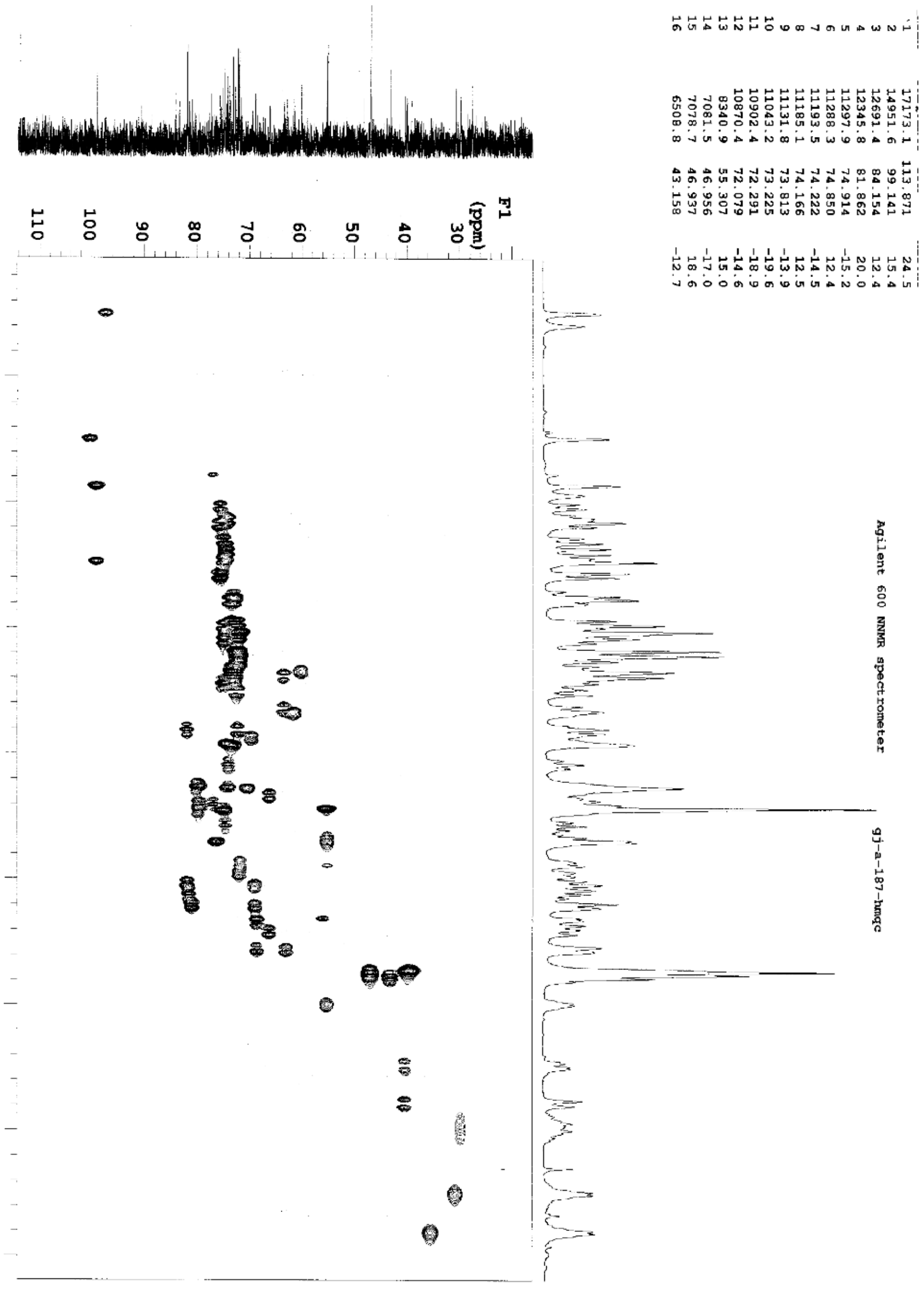


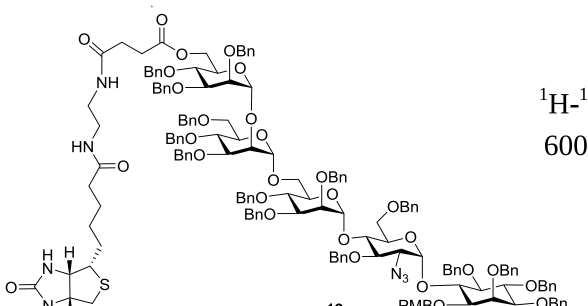
19

^{1}H-^{1}H COSY

600 MHz

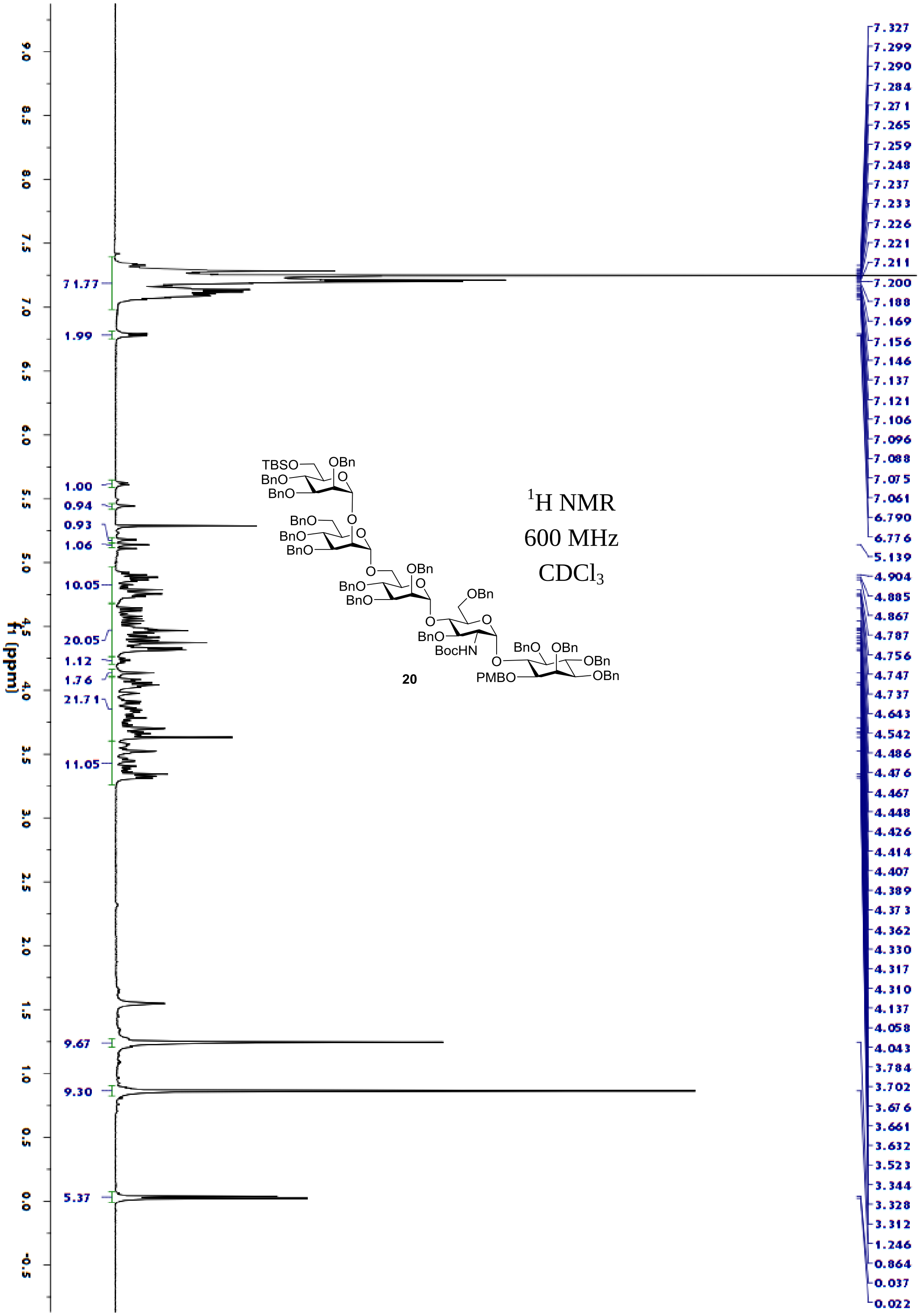
$CDCl_3$





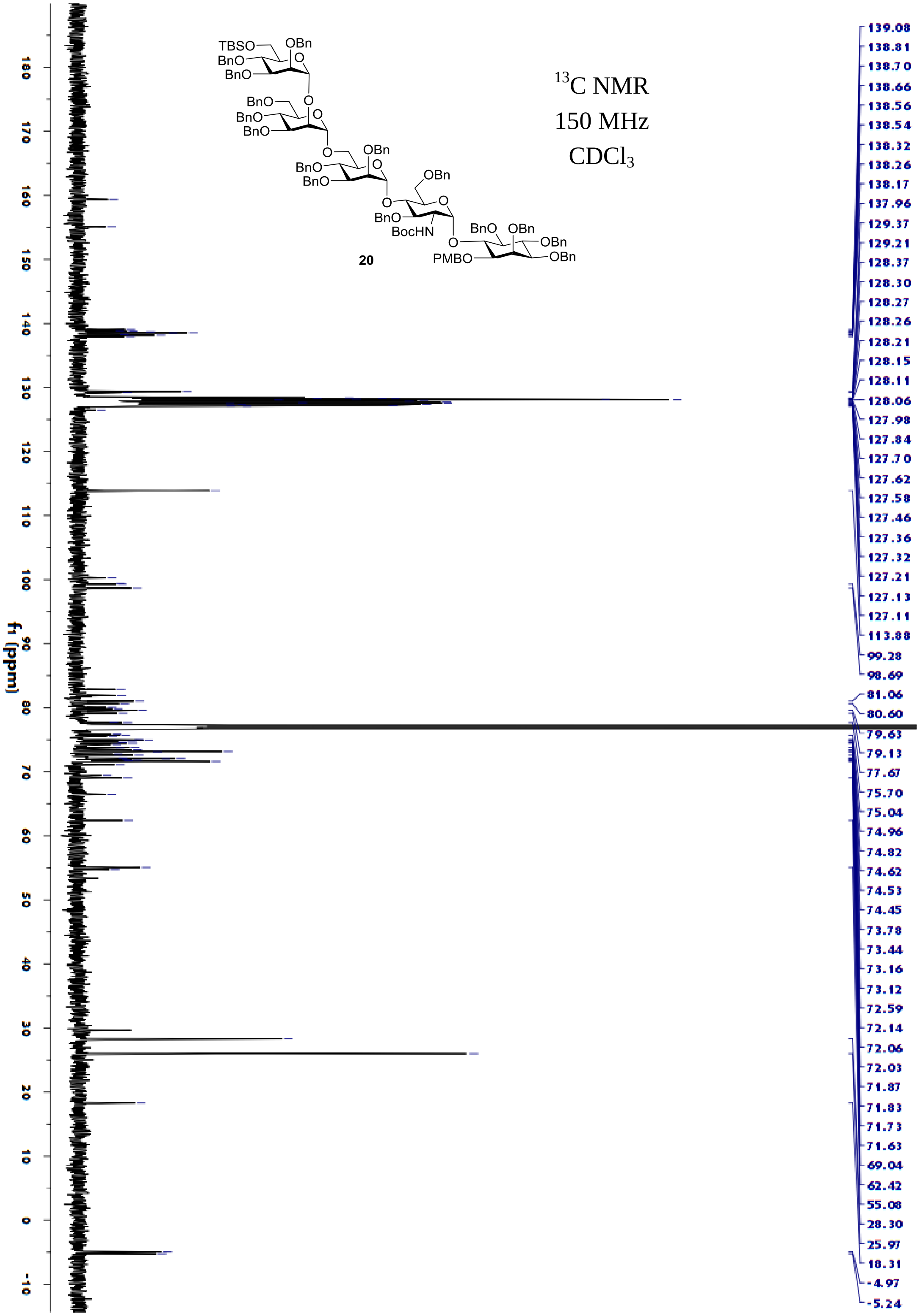
19

^{1}H-^{13}C HMQC
600/150 MHz
$CDCl_3$



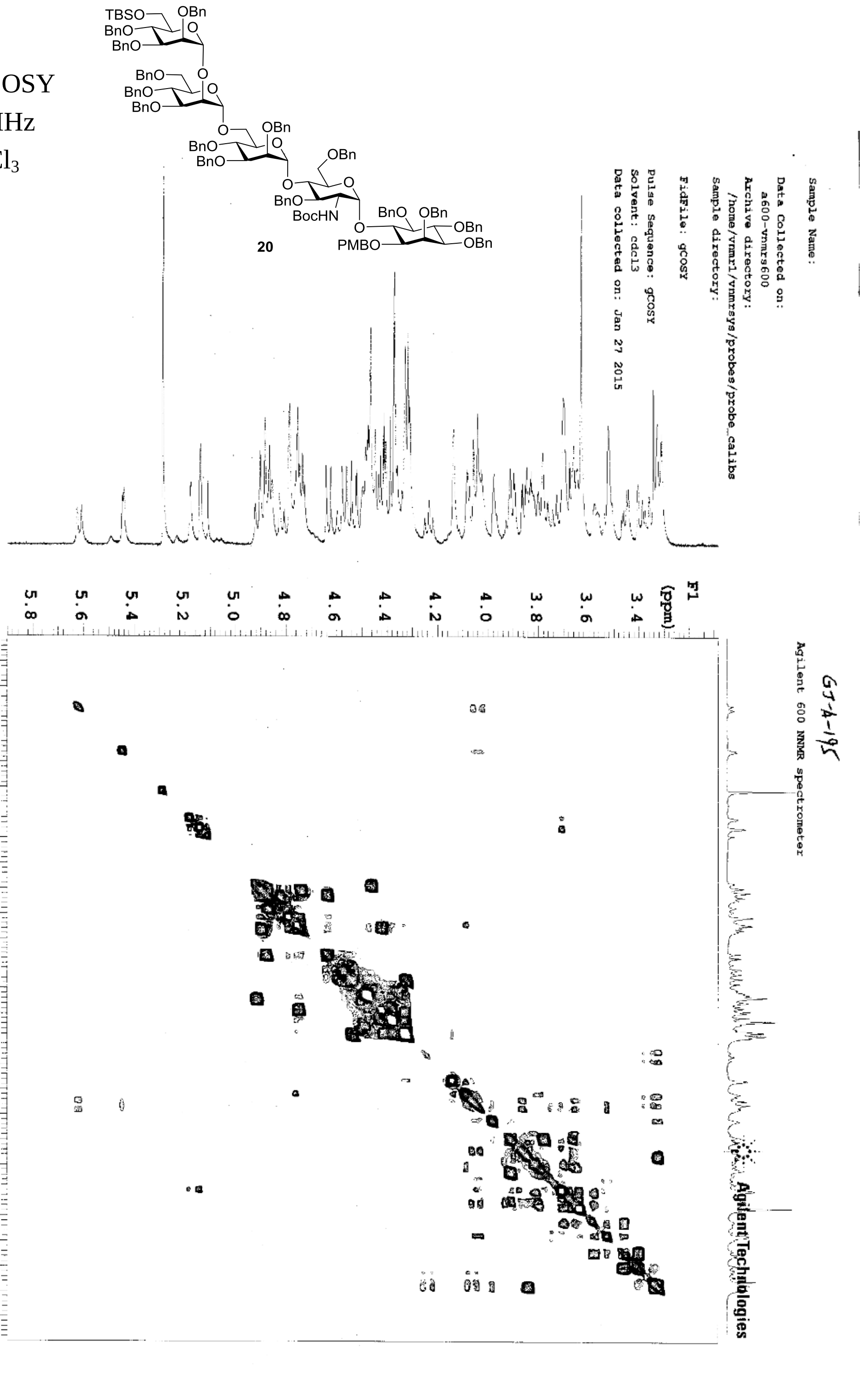
20

^{1}H NMR
600 MHz
CDCl$_3$

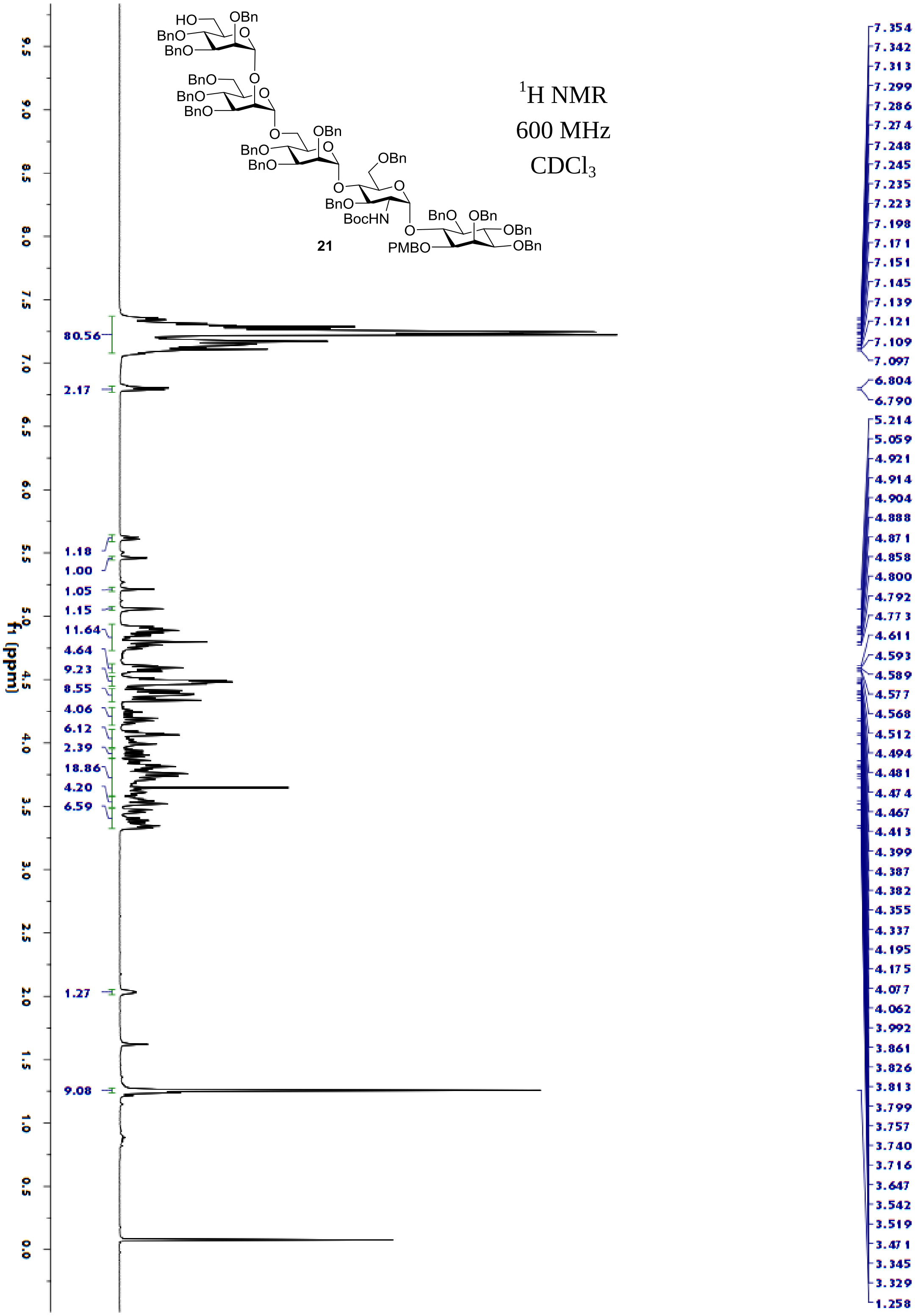

20

TBSO
OBn
BnO
BnO
O
BnO
BnO
BnO
O
O
OBn
BnO
BnO
O
OBn
O
BnO
BocHN
O
BnO
OBn
OBn
PMBO
OBn
^{13}C NMR
150 MHz
CDCl$_3$
f1 (ppm)
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
139.08
138.81
138.70
138.66
138.56
138.54
138.32
138.26
138.17
137.96
129.37
129.21
128.37
128.30
128.27
128.26
128.21
128.15
128.11
128.06
127.98
127.84
127.70
127.62
127.58
127.46
127.36
127.32
127.21
127.13
127.11
113.88
99.28
98.69
81.06
80.60
79.63
79.13
77.67
75.70
75.04
74.96
74.82
74.62
74.53
74.45
73.78
73.44
73.16
73.12
72.59
72.14
72.06
72.03
71.87
71.83
71.73
71.63
69.04
62.42
55.08
28.30
25.97
18.31
-4.97
-5.24

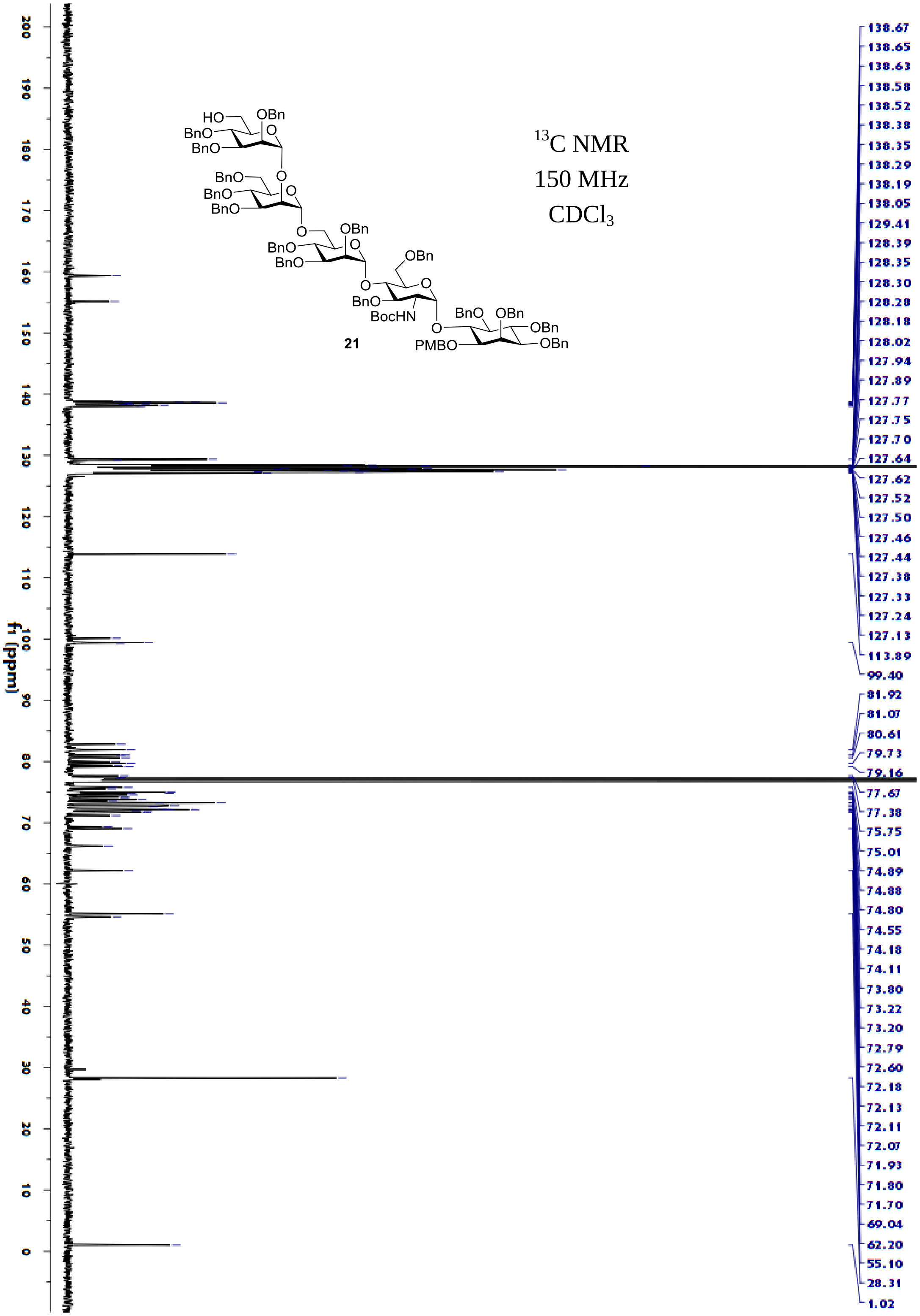
^{1}H-^{1}H COSY
600 MHz
CDCl$_3$



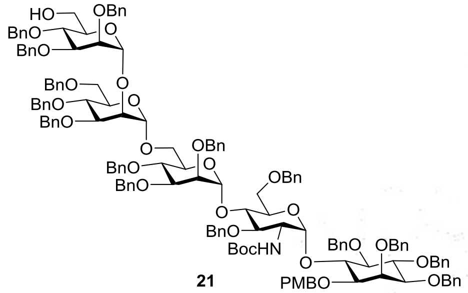
20



HO
OBn
BnO
O
BnO
BnO
O
O
BnO
BnO
O
OBn
BnO
O
BnO
OBn
O
O
BnO
BocHN
O
BnO
OBn
OBn
PMBO
OBn
21
^{1}H NMR
600 MHz
CDCl$_3$
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)
80.56
2.17
1.18
1.00
1.05
1.15
11.64
4.64
9.23
8.55
4.06
6.12
2.39
18.86
4.20
6.59
1.27
9.08
7.354
7.342
7.313
7.299
7.286
7.274
7.248
7.245
7.235
7.223
7.198
7.171
7.151
7.145
7.139
7.121
7.109
7.097
6.804
6.790
5.214
5.059
4.921
4.914
4.904
4.888
4.871
4.858
4.800
4.792
4.773
4.611
4.593
4.589
4.577
4.568
4.512
4.494
4.481
4.474
4.467
4.413
4.399
4.387
4.382
4.355
4.337
4.195
4.175
4.077
4.062
3.992
3.861
3.826
3.813
3.799
3.757
3.740
3.716
3.647
3.542
3.519
3.471
3.345
3.329
1.258



^{13}C NMR
150 MHz
CDCl$_3$
21
HO
OBn
BnO
BnO
O
BnO
BnO
BnO
O
OBn
BnO
BnO
OBn
BnO
BocHN
BnO
OBn
OBn
OBn
PMBO
f1 (ppm)
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
138.67
138.65
138.63
138.58
138.52
138.38
138.35
138.29
138.19
138.05
129.41
128.39
128.35
128.30
128.28
128.18
128.02
127.94
127.89
127.77
127.75
127.70
127.64
127.62
127.52
127.50
127.46
127.44
127.38
127.33
127.24
127.13
113.89
99.40
81.92
81.07
80.61
79.73
79.16
77.67
77.38
75.75
75.01
74.89
74.88
74.80
74.55
74.18
74.11
73.80
73.22
73.20
72.79
72.60
72.18
72.13
72.11
72.07
71.93
71.80
71.70
69.04
62.20
55.10
28.31
1.02

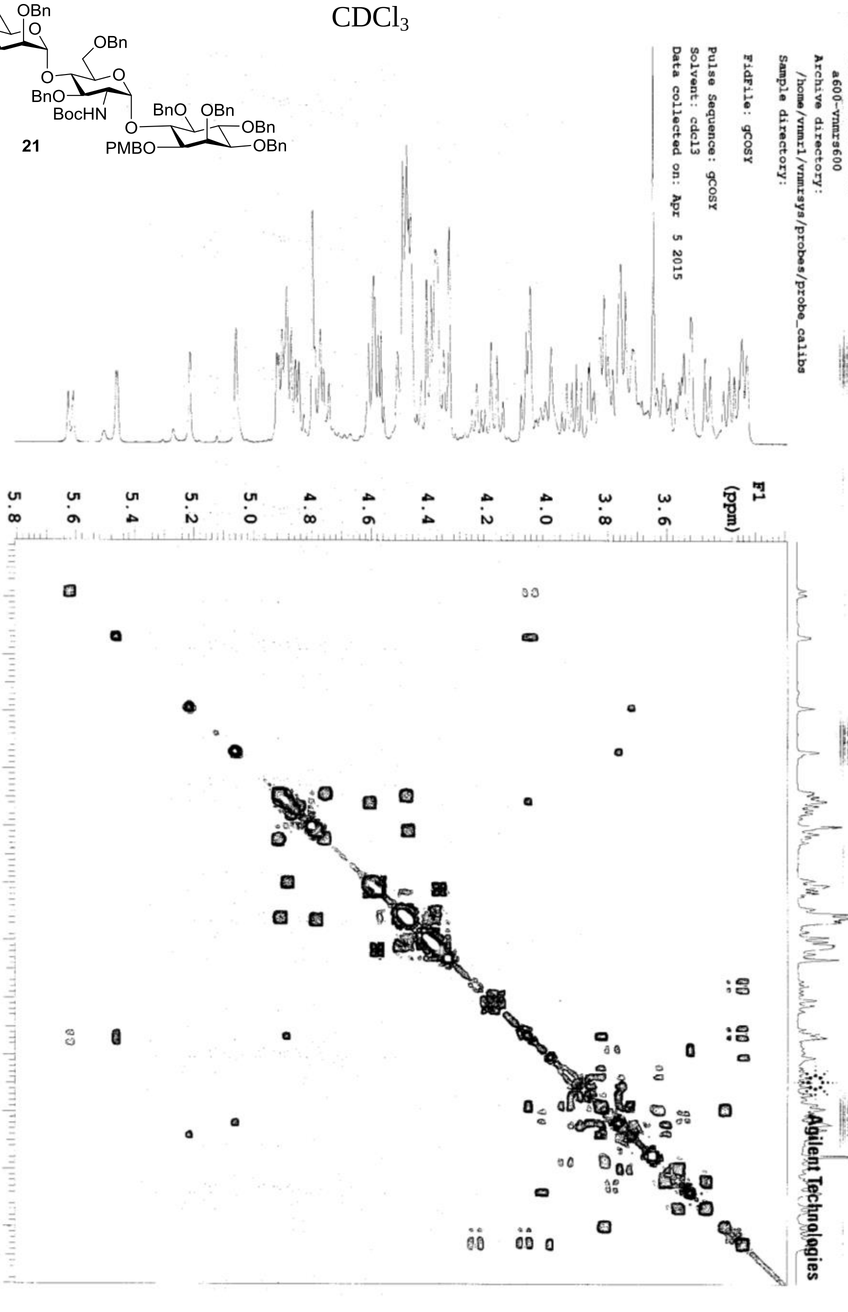


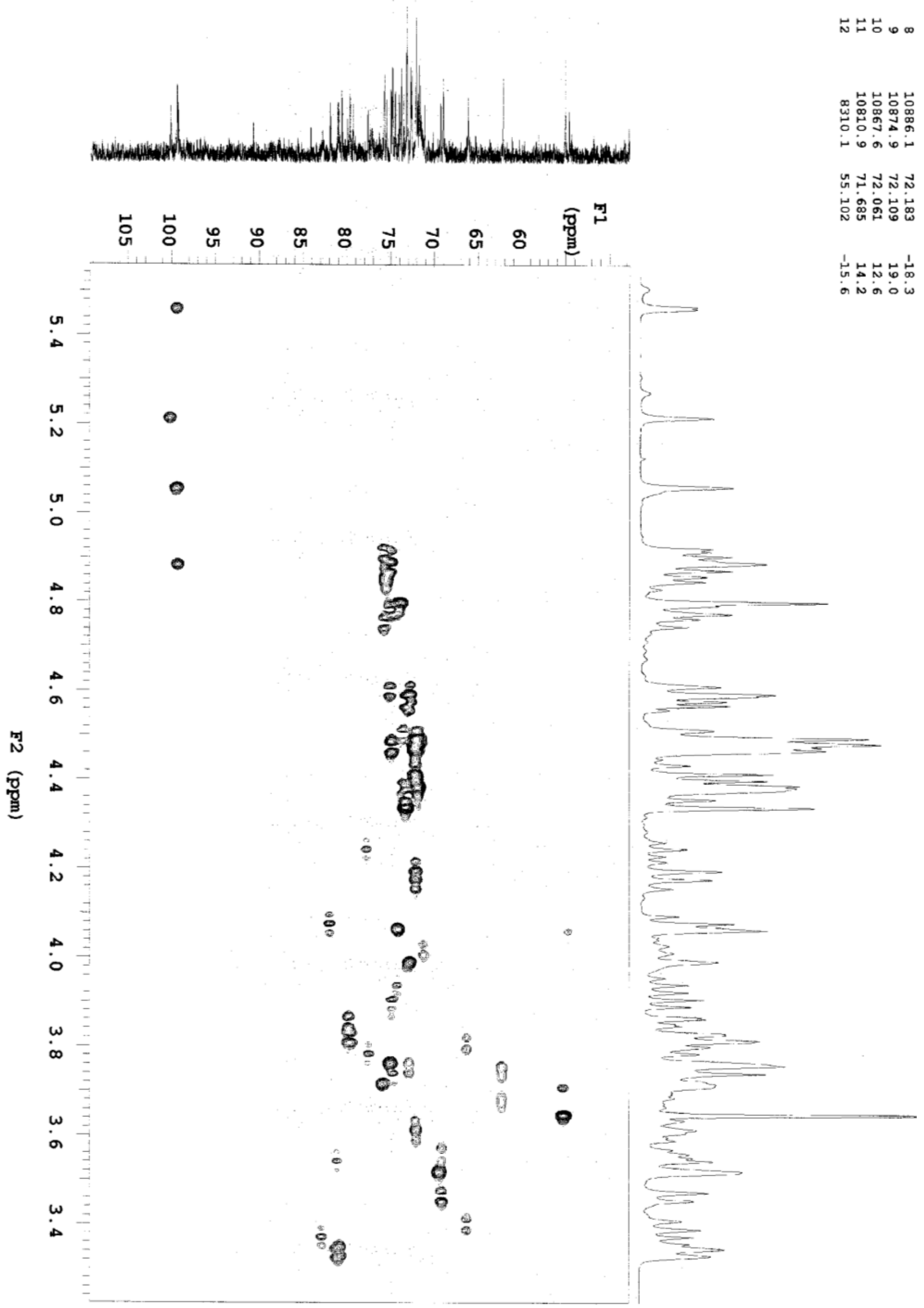
21

^{1}H-^{1}H COSY

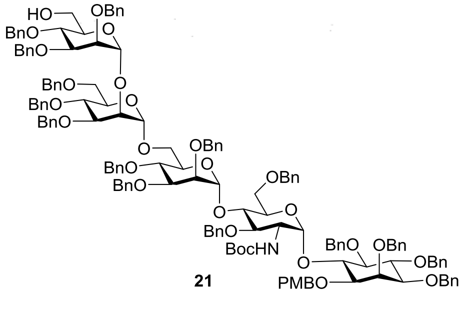
600 MHz

$CDCl_3$

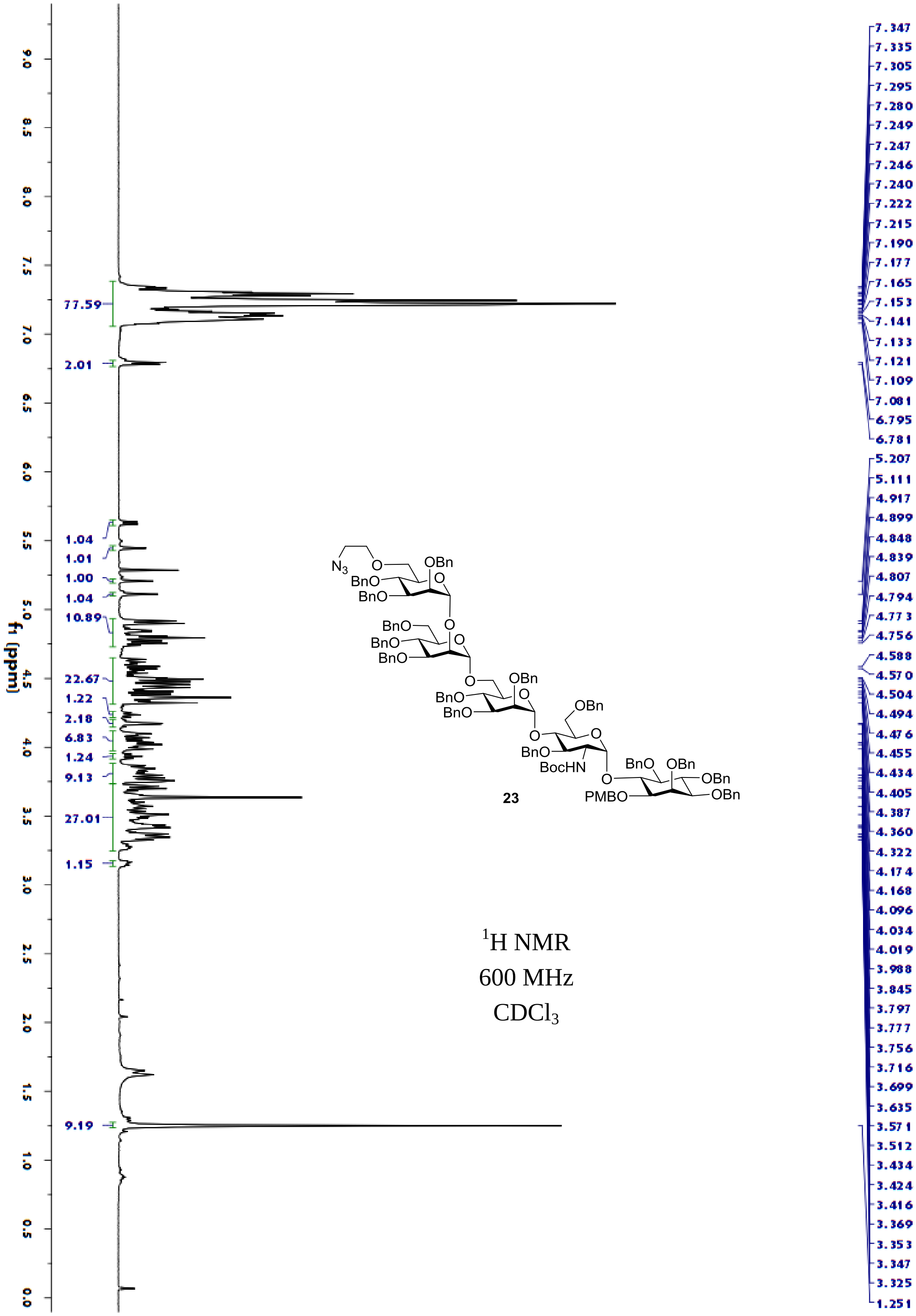




^{1}H-^{13}C HMQC
600/150 MHz
CDCl$_3$

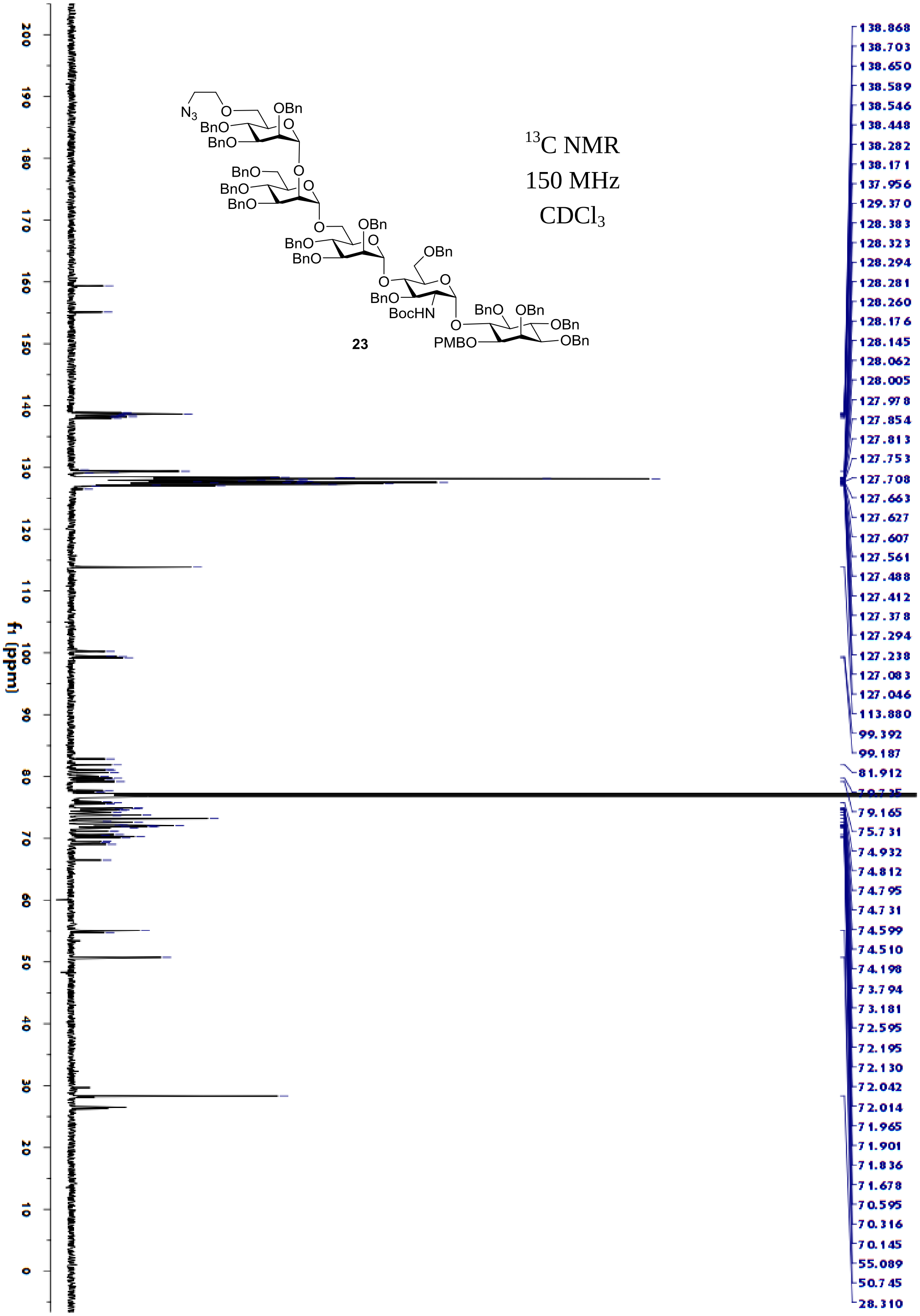


21

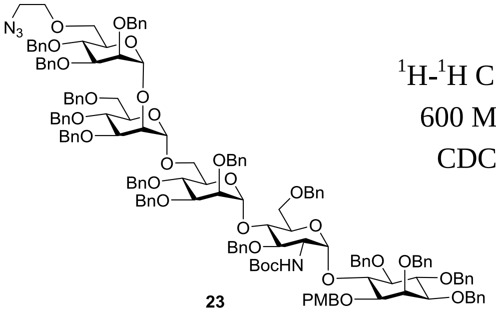


23

^{1}H NMR
600 MHz
CDCl$_3$

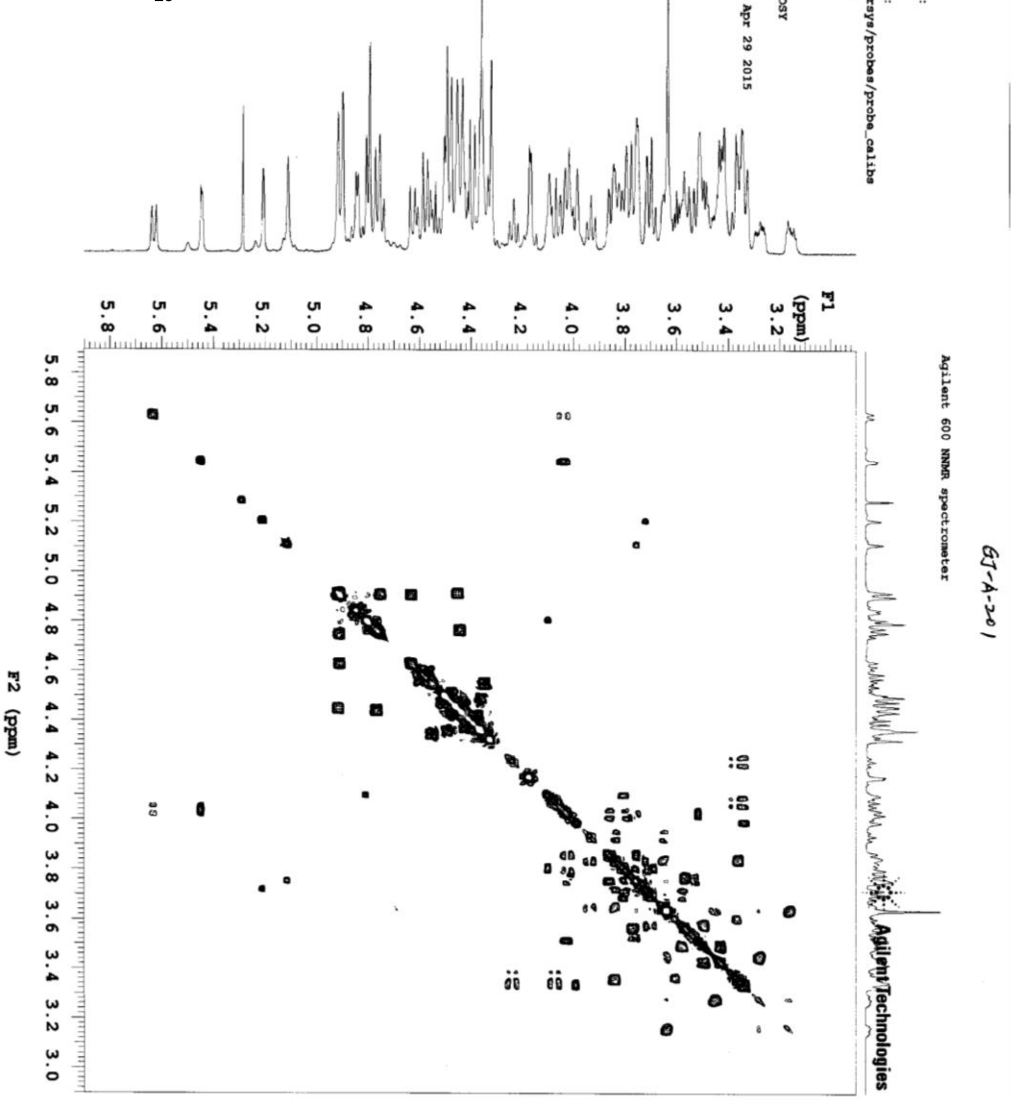


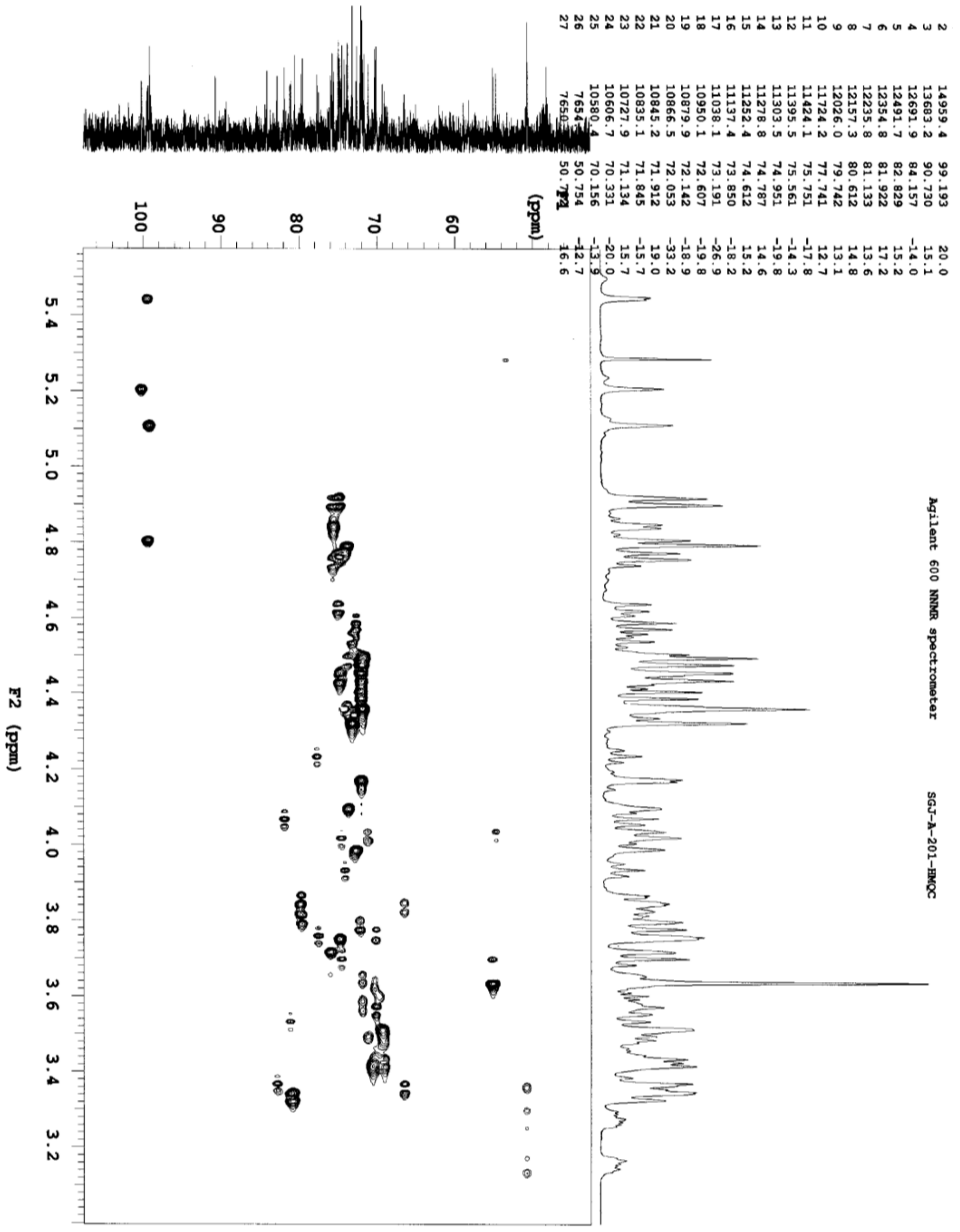
^{13}C NMR
150 MHz
CDCl$_3$
23
N$_3$
OBn
BnO
BnHN
BocHN
PMBO
f1 (ppm)
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
138.868
138.703
138.650
138.589
138.546
138.448
138.282
138.171
137.956
129.370
128.383
128.323
128.294
128.281
128.260
128.176
128.145
128.062
128.005
127.978
127.854
127.813
127.753
127.708
127.663
127.627
127.607
127.561
127.488
127.412
127.378
127.294
127.238
127.083
127.046
113.880
99.392
99.187
81.912
79.735
79.165
75.731
74.932
74.812
74.795
74.731
74.599
74.510
74.198
73.794
73.181
72.595
72.195
72.130
72.042
72.014
71.965
71.901
71.836
71.678
70.595
70.316
70.145
55.089
50.745
28.310



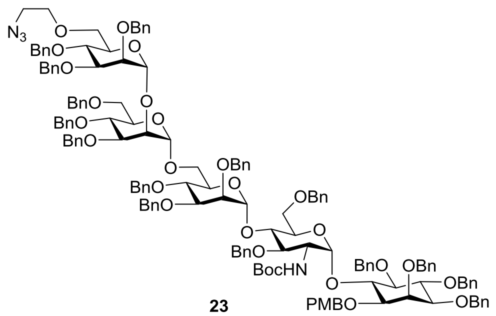
23

^{1}H-^{1}H COSY
600 MHz
$CDCl_3$



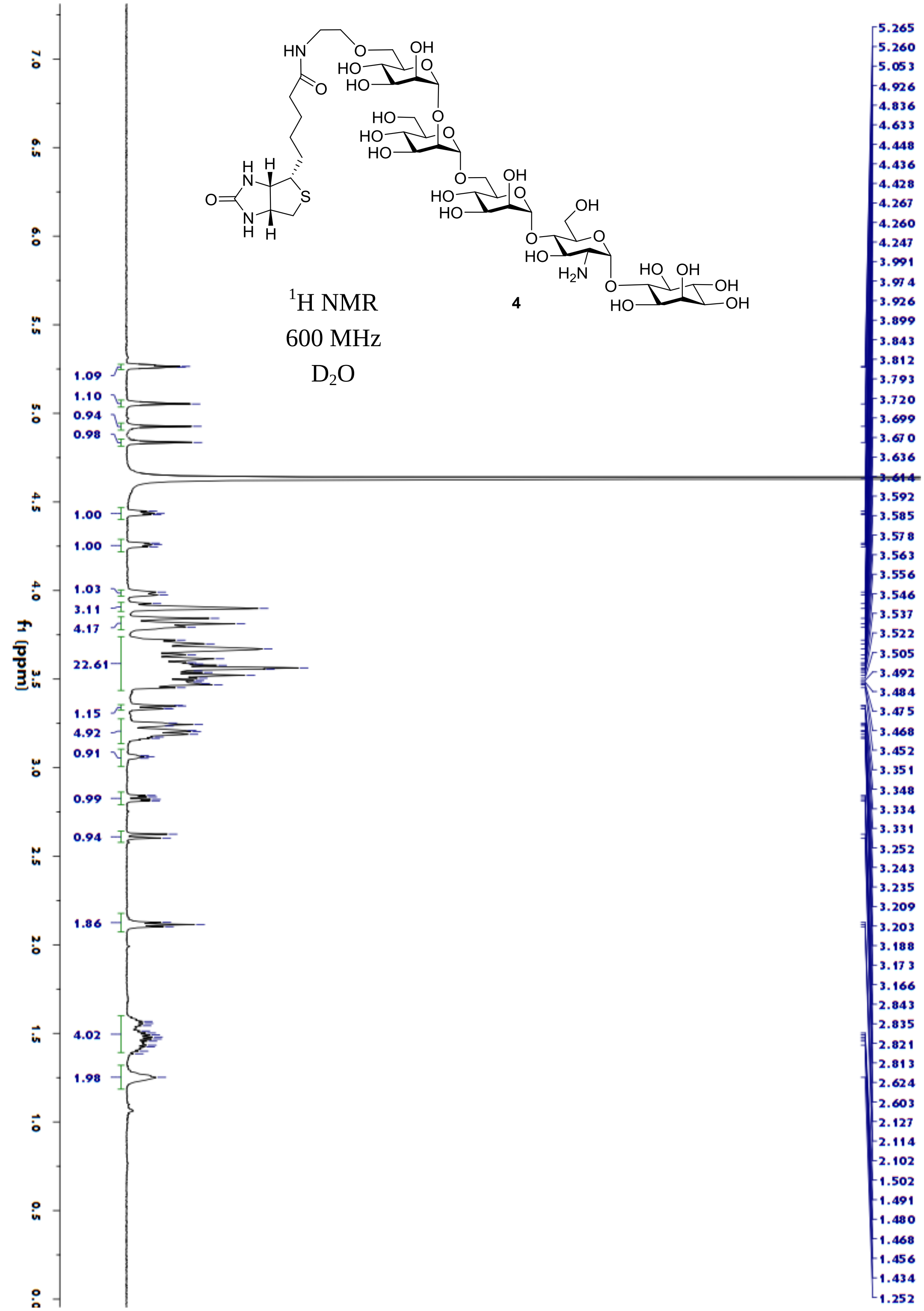


2	14959.4	99.193	20.0
3	13683.2	90.730	15.1
4	12691.9	84.157	-14.0
5	12491.7	82.829	15.2
6	12354.8	81.922	17.2
7	12235.8	81.133	13.6
8	12157.3	80.612	14.8
9	12026.0	79.742	13.1
10	11724.2	77.741	12.7
11	11424.1	75.751	-17.8
12	11395.5	75.561	-14.3
13	11303.5	74.951	-19.8
14	11278.8	74.787	14.6
15	11252.4	74.612	15.2
16	11137.4	73.850	-18.2
17	11038.1	73.191	-26.9
18	10950.1	72.607	-19.8
19	10879.9	72.142	-18.9
20	10866.5	72.053	-33.2
21	10845.2	71.912	19.0
22	10835.1	71.845	-15.7
23	10727.9	71.134	15.7
24	10606.7	70.331	-20.0
25	10580.4	70.156	-13.9
26	7654	50.754	-12.7
27	7650	50.7[illegible]	16.6



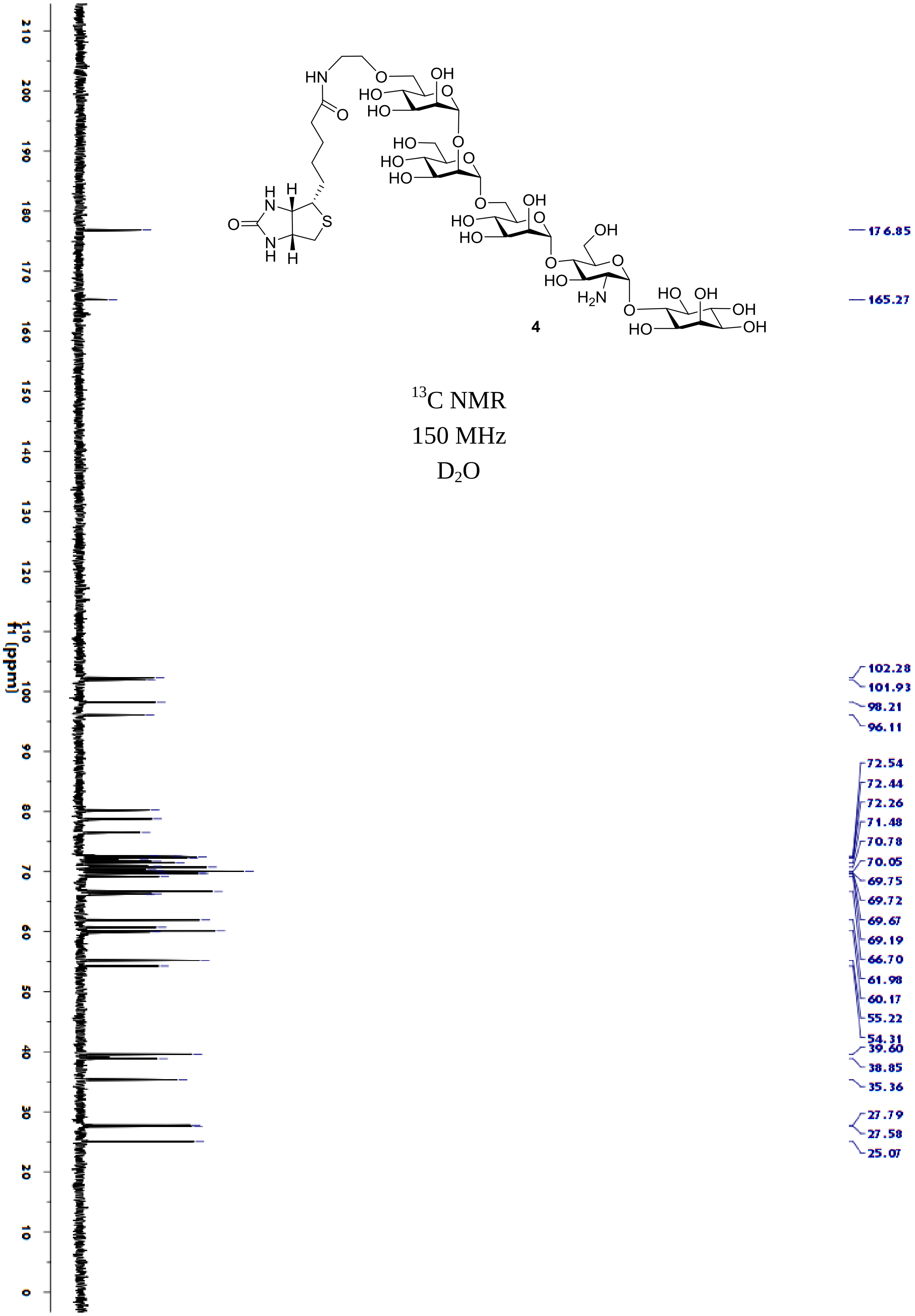
23

^{1}H-^{13}C HMQC
600/150 MHz
CDCl$_3$



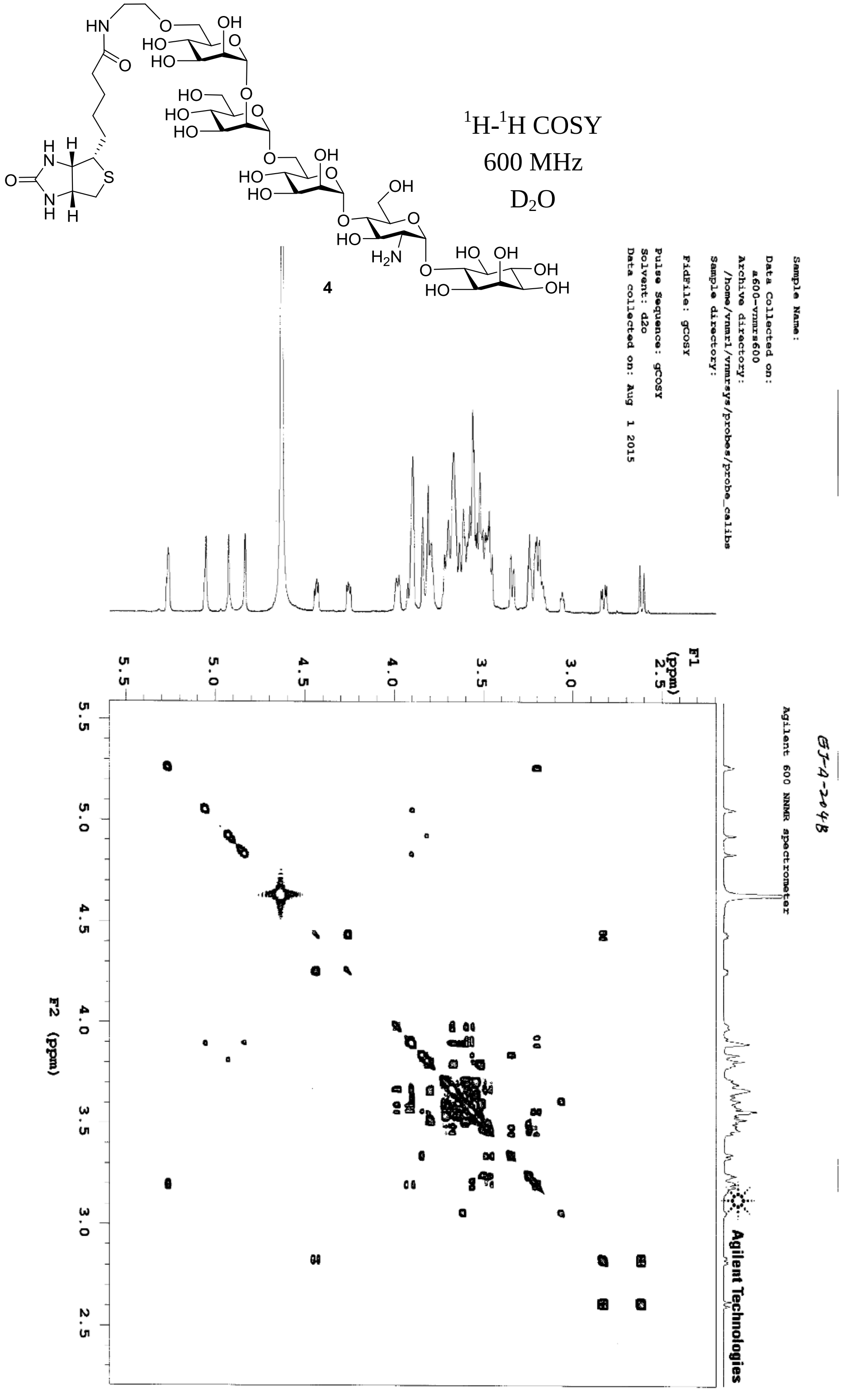
^{1}H NMR
600 MHz
D_2O

4



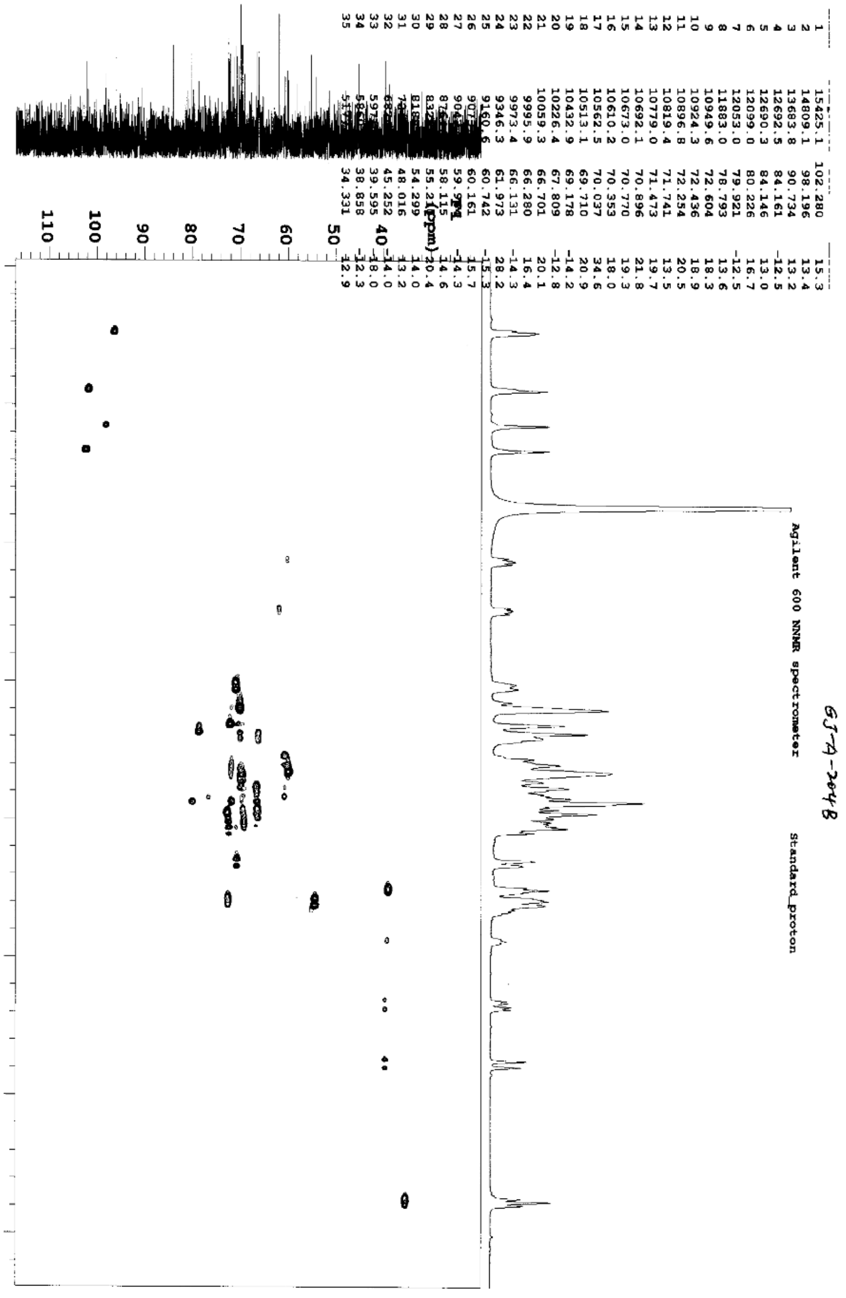
4

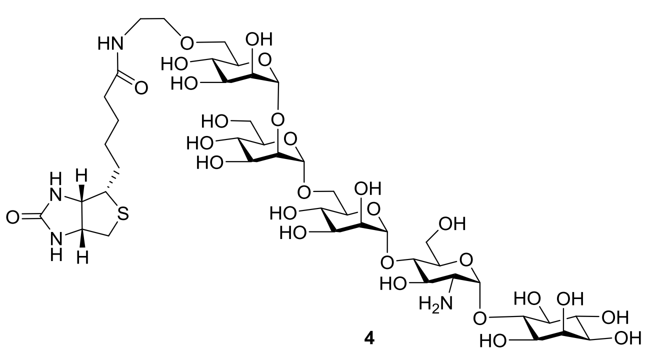
^{13}C NMR
150 MHz
D_2O



4

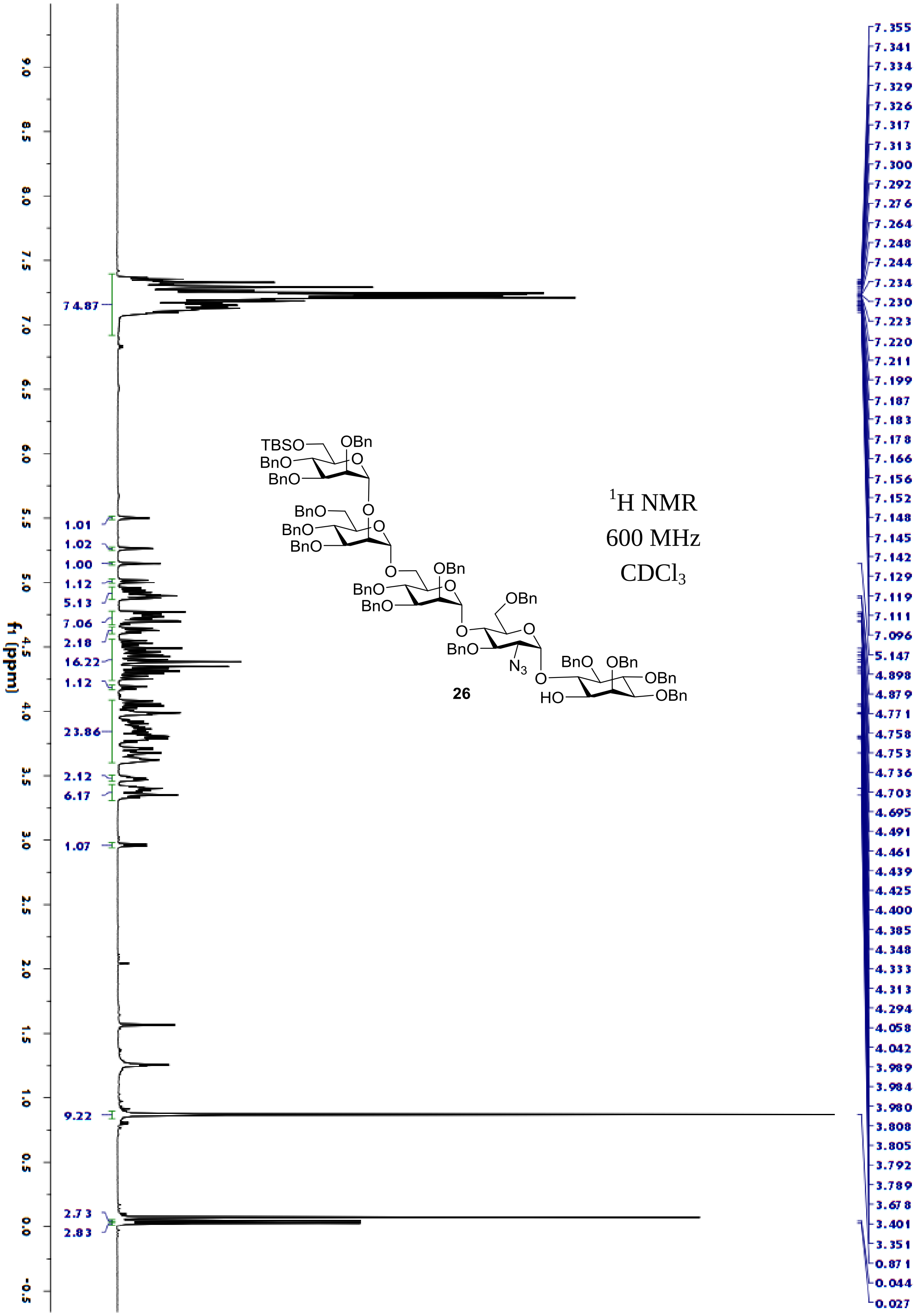
^{1}H-^{1}H COSY
600 MHz
D$_2$O





4

^{1}H-^{13}C HMQC
600/150 MHz
D_2O


1H NMR
600 MHz
CDCl3
26
TBSO
OBn
BnO
BnO
O
BnO
BnO
BnO
O
O
O
OBn
BnO
BnO
O
O
OBn
O
BnO
N3
O
BnO
OBn
OBn
HO
OBn
f1 (ppm)
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
74.87
1.01
1.02
1.00
1.12
5.13
7.06
2.18
16.22
1.12
23.86
2.12
6.17
1.07
9.22
2.73
2.83
7.355
7.341
7.334
7.329
7.326
7.317
7.313
7.300
7.292
7.276
7.264
7.248
7.244
7.234
7.230
7.223
7.220
7.211
7.199
7.187
7.183
7.178
7.166
7.156
7.152
7.148
7.145
7.142
7.129
7.119
7.111
7.096
5.147
4.898
4.879
4.771
4.758
4.753
4.736
4.703
4.695
4.491
4.461
4.439
4.425
4.400
4.385
4.348
4.333
4.313
4.294
4.058
4.042
3.989
3.984
3.980
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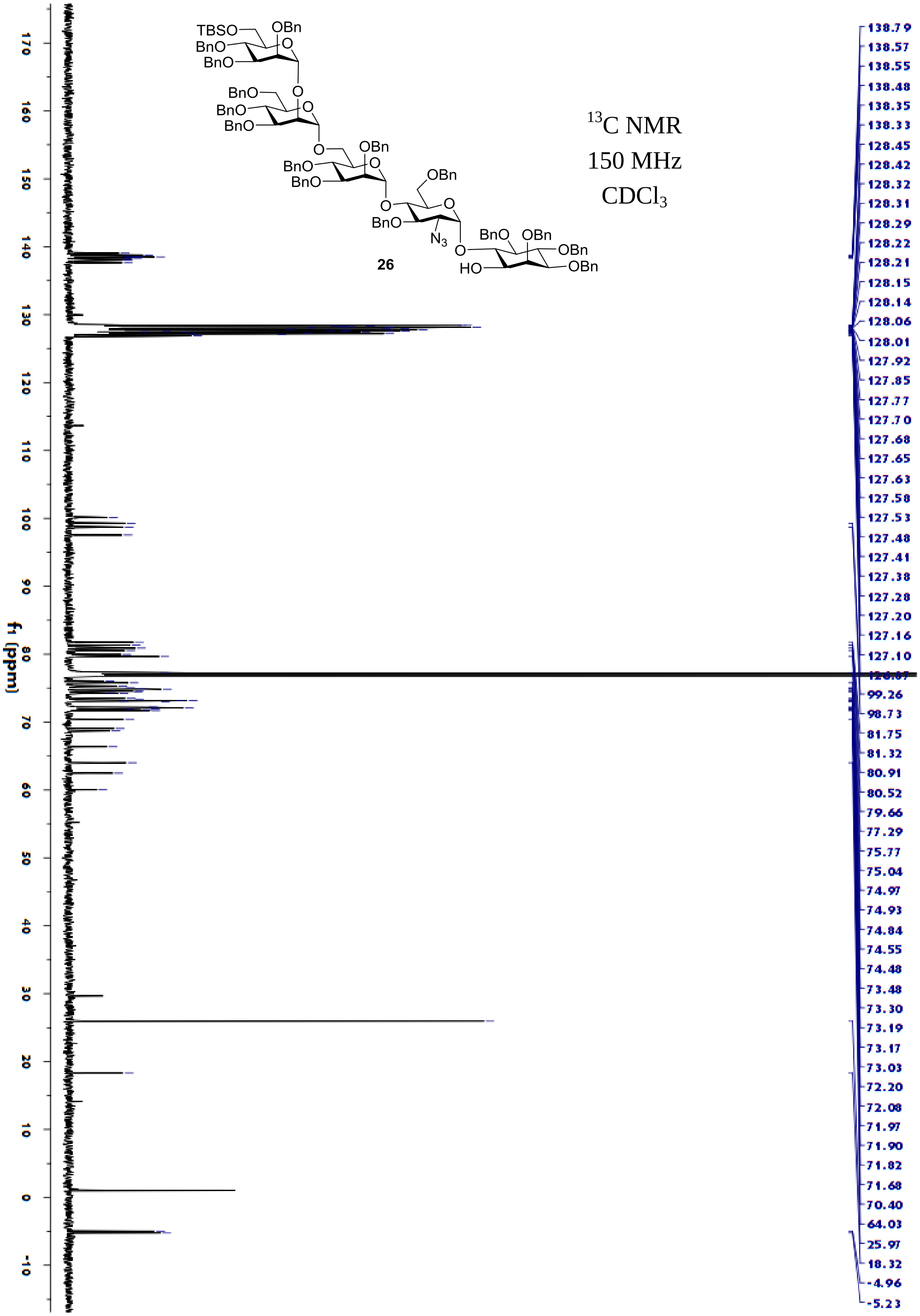
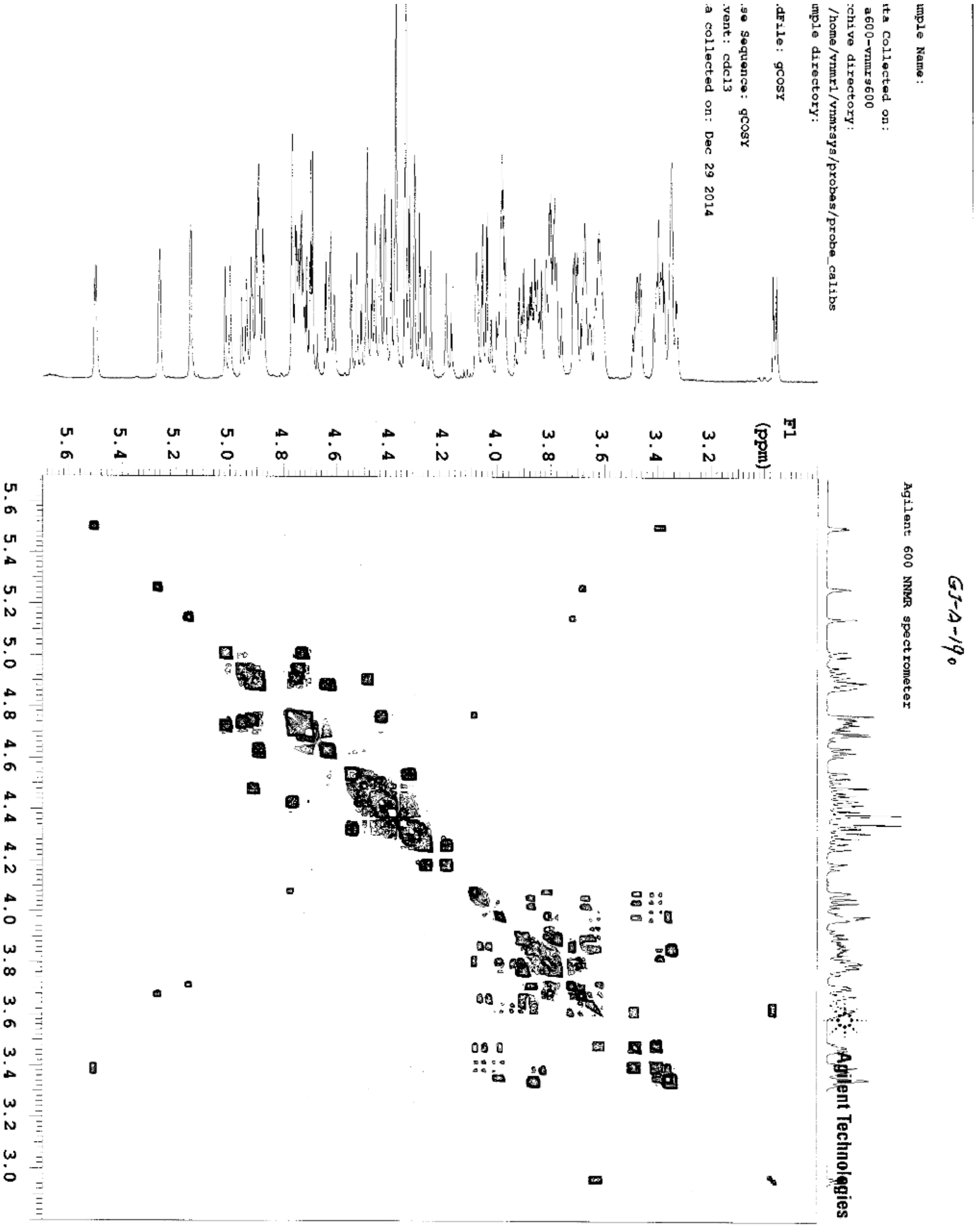
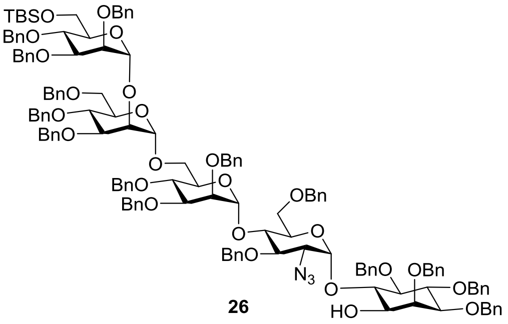
3.805
3.792
3.789
3.678
3.401
3.351
0.871
0.044
0.027



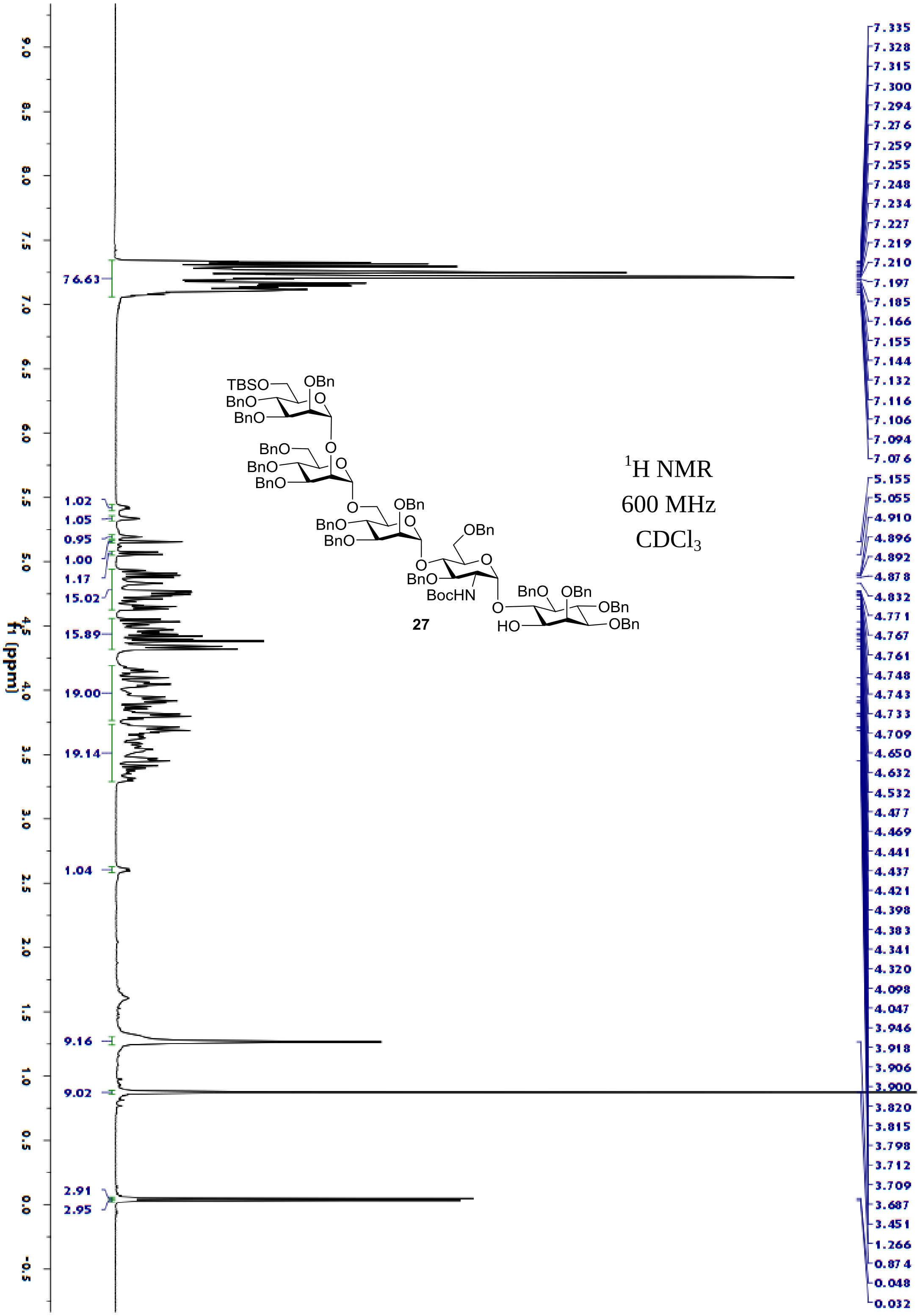
^{13}C NMR
150 MHz
CDCl$_3$
26
TBSO
OBn
BnO
BnO
O
BnO
BnO
BnO
O
O
OBn
BnO
BnO
O
O
OBn
BnO
N$_3$
O
BnO
OBn
OBn
HO
OBn
f1 (ppm)
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
138.79
138.57
138.55
138.48
138.35
138.33
128.45
128.42
128.32
128.31
128.29
128.22
128.21
128.15
128.14
128.06
128.01
127.92
127.85
127.77
127.70
127.68
127.65
127.63
127.58
127.53
127.48
127.41
127.38
127.28
127.20
127.16
127.10
126.87
99.26
98.73
81.75
81.32
80.91
80.52
79.66
77.29
75.77
75.04
74.97
74.93
74.84
74.55
74.48
73.48
73.30
73.19
73.17
73.03
72.20
72.08
71.97
71.90
71.82
71.68
70.40
64.03
25.97
18.32
-4.96
-5.23



^{1}H-^{1}H COSY
600 MHz
$CDCl_3$



26



1H NMR
600 MHz
CDCl3
27
TBSO
OBn
BnO
BnO
O
BnO
BnO
BnO
O
O
OBn
BnO
BnO
O
OBn
O
BnO
BocHN
O
BnO
OBn
OBn
OBn
HO
O
f1 (ppm)
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
76.63
1.02
1.05
0.95
1.00
1.17
15.02
15.89
19.00
19.14
1.04
9.16
9.02
2.91
2.95
7.335
7.328
7.315
7.300
7.294
7.276
7.259
7.255
7.248
7.234
7.227
7.219
7.210
7.197
7.185
7.166
7.155
7.144
7.132
7.116
7.106
7.094
7.076
5.155
5.055
4.910
4.896
4.892
4.878
4.832
4.771
4.767
4.761
4.748
4.743
4.733
4.709
4.650
4.632
4.532
4.477
4.469
4.441
4.437
4.421
4.398
4.383
4.341
4.320
4.098
4.047
3.946
3.918
3.906
3.900
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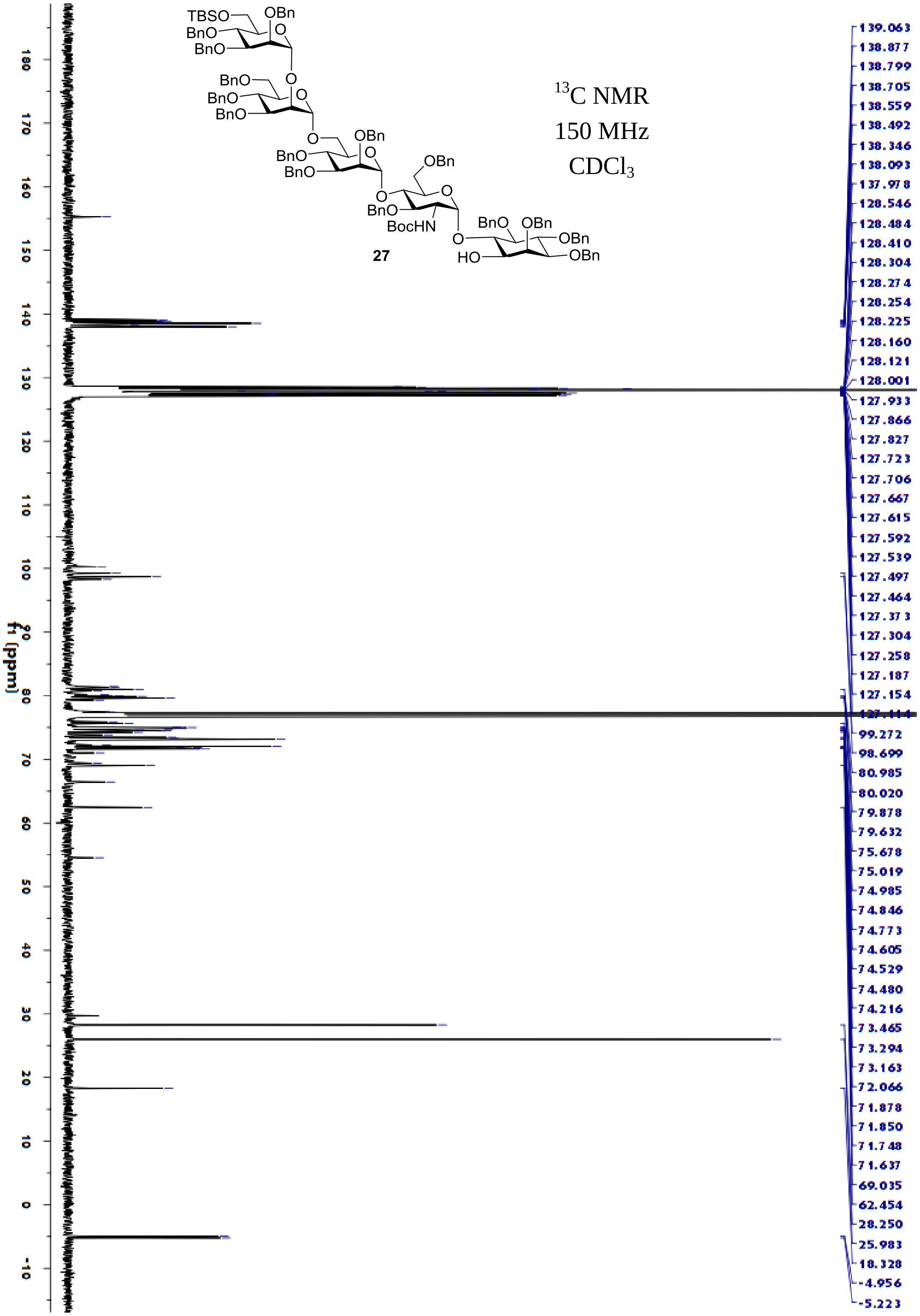
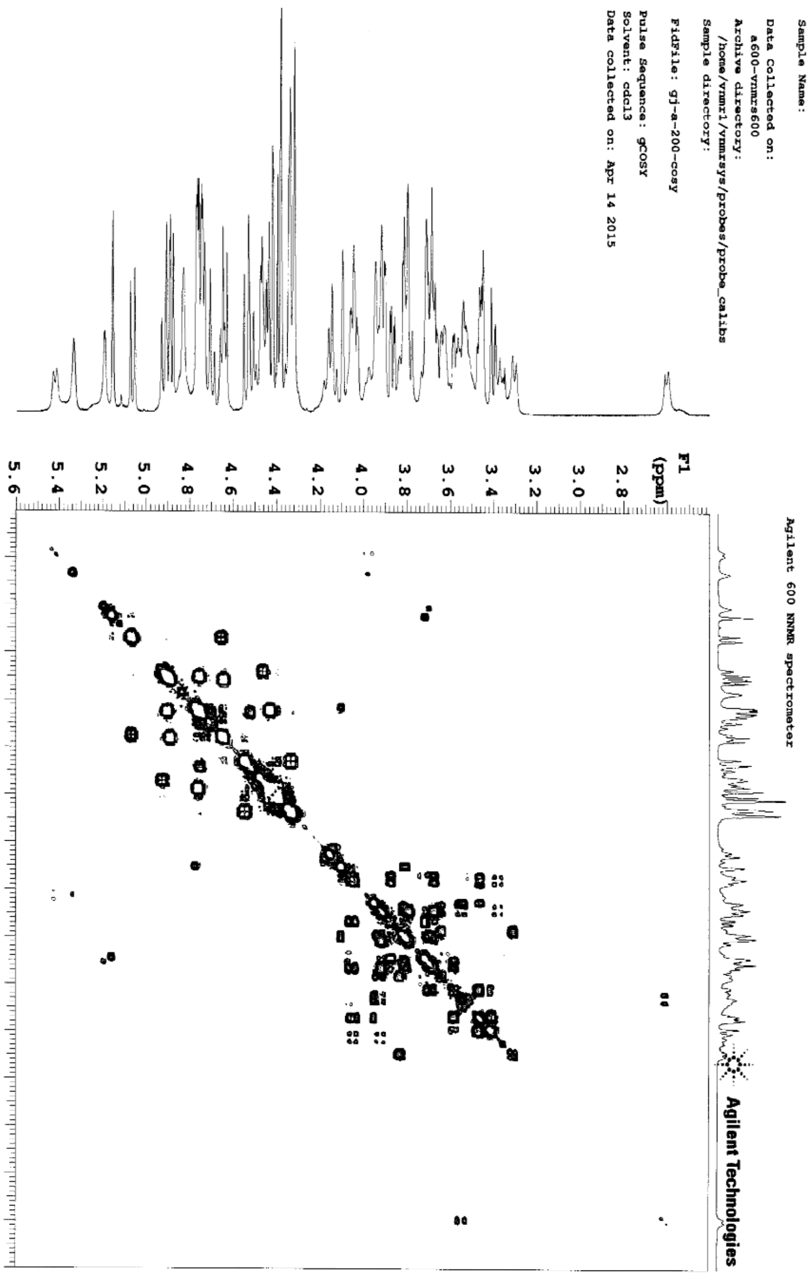
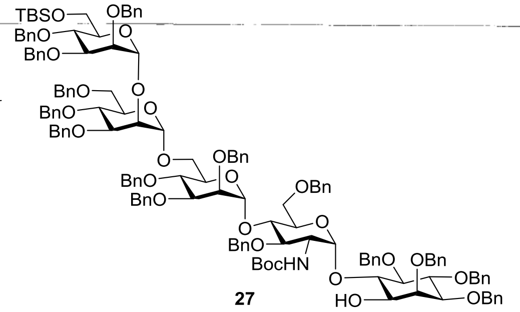
3.798
3.712
3.709
3.687
3.451
1.266
0.874
0.048
0.032

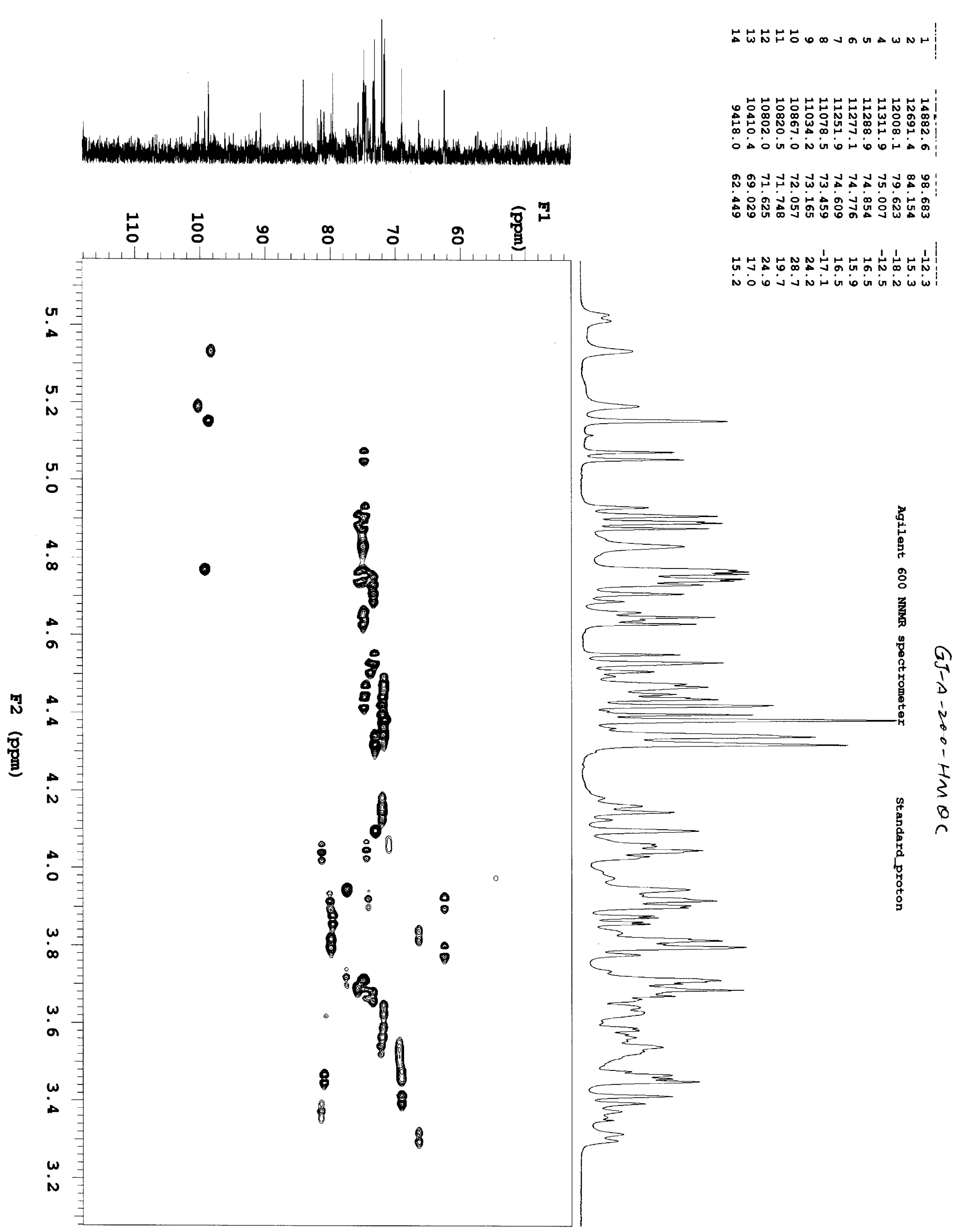




^{1}H-^{1}H COSY
600 MHz
$CDCl_3$

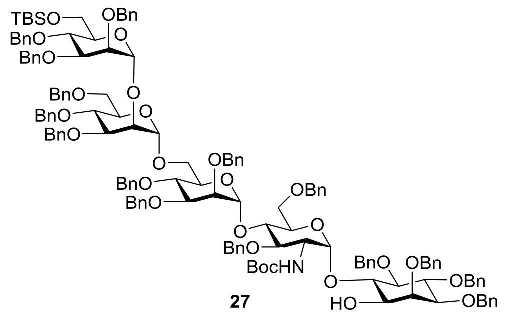


27



1	14882.6	98.683	-12.3
2	12691.4	84.154	15.3
3	12008.1	79.623	-18.2
4	11311.9	75.007	-12.5
5	11288.9	74.854	16.5
6	11277.1	74.776	15.9
7	11251.9	74.609	16.5
8	11078.5	73.459	-17.1
9	11034.2	73.165	24.2
10	10867.0	72.057	28.7
11	10820.5	71.748	19.7
12	10802.0	71.625	24.9
13	10410.4	69.029	17.0
14	9418.0	62.449	15.2

^{1}H-^{13}C HMQC
600/150 MHz
CDCl$_3$



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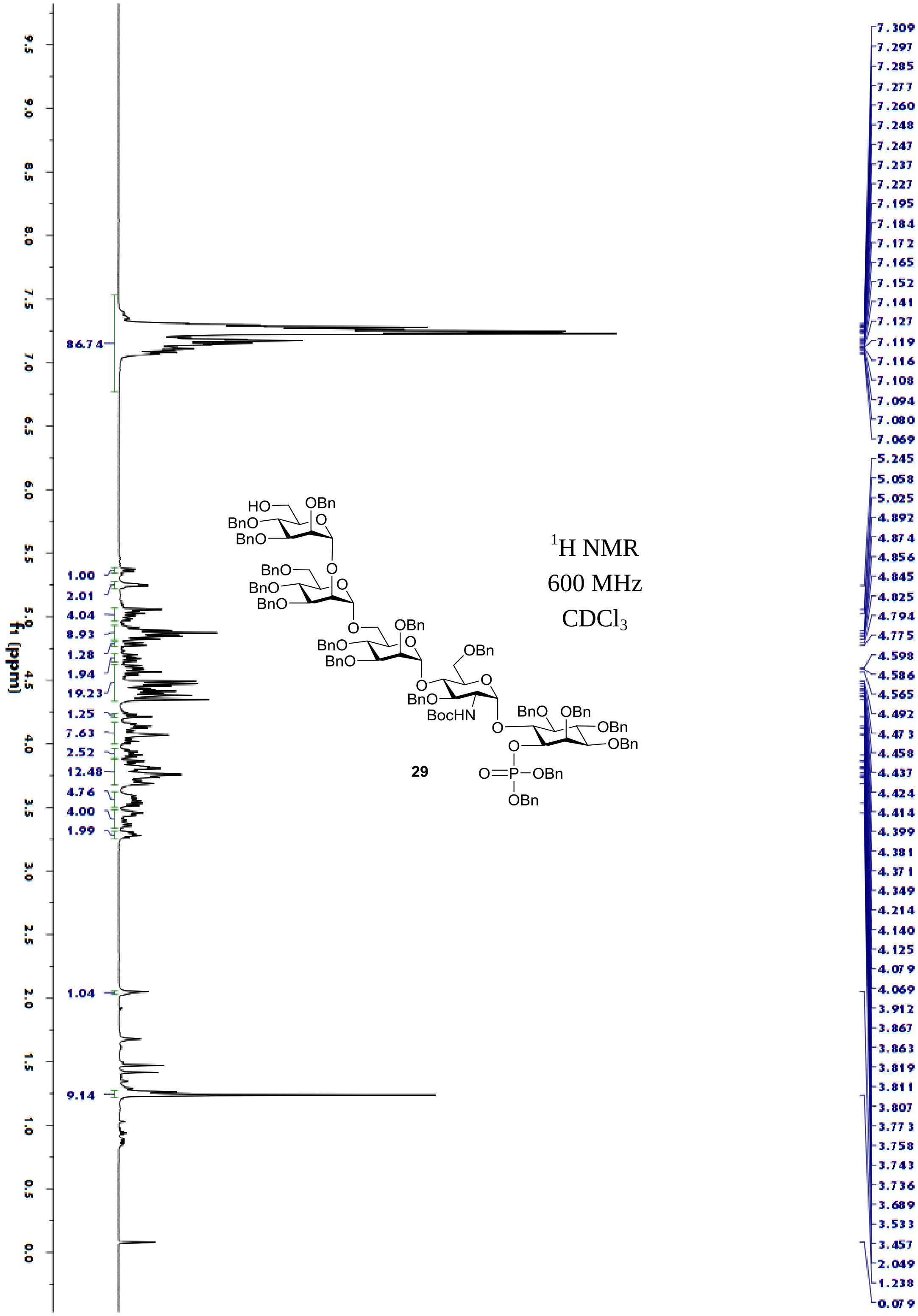
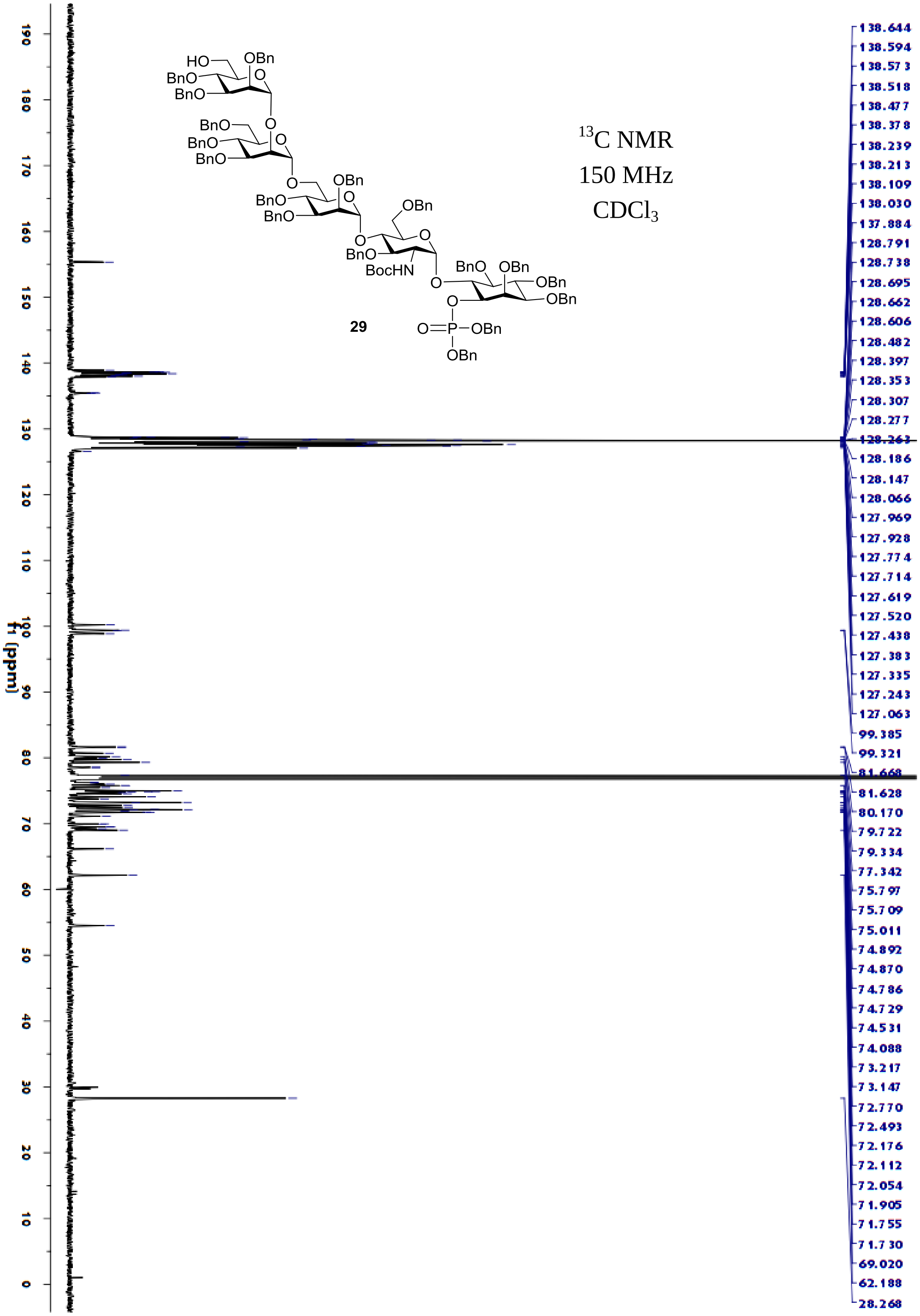
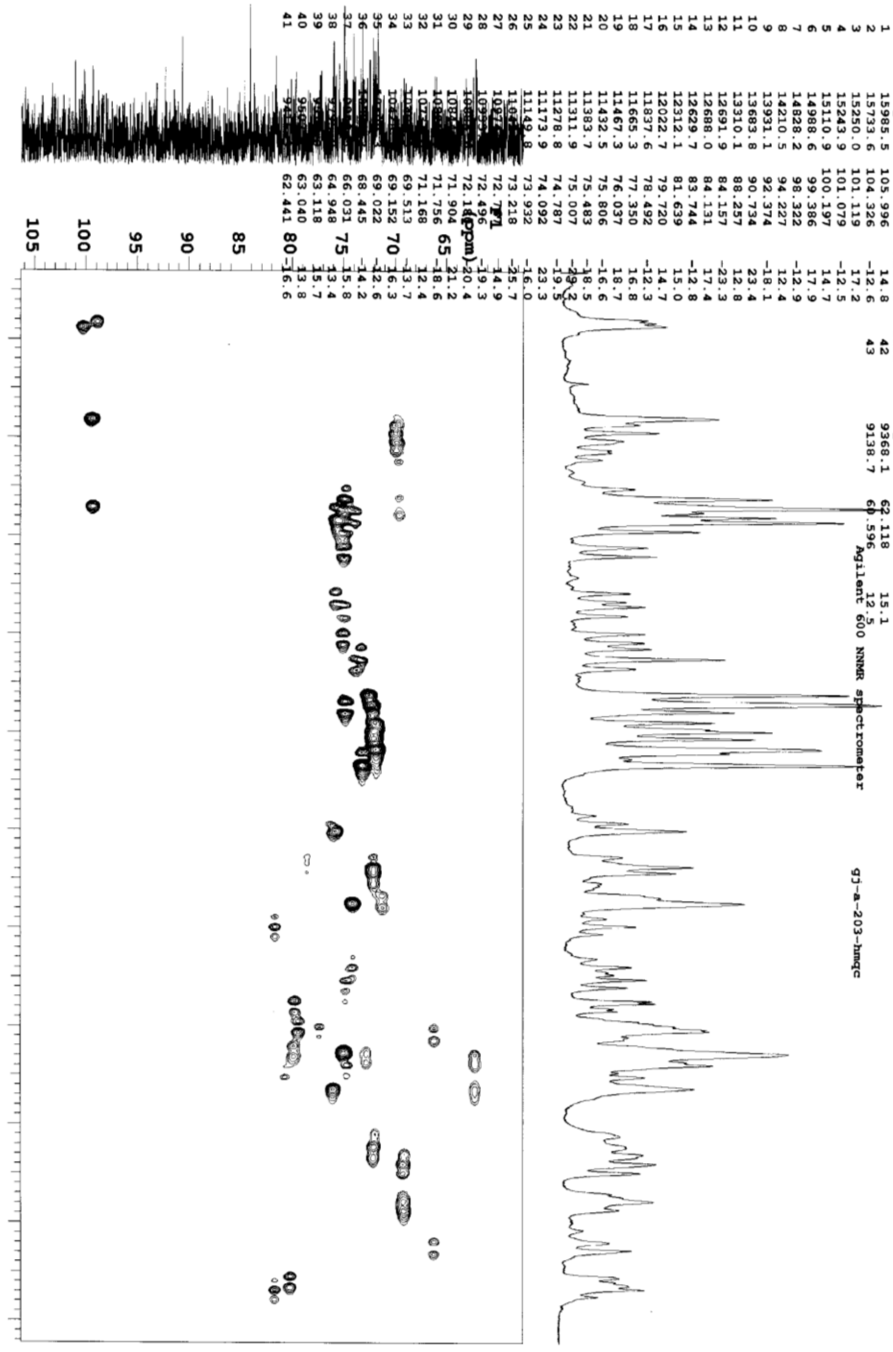


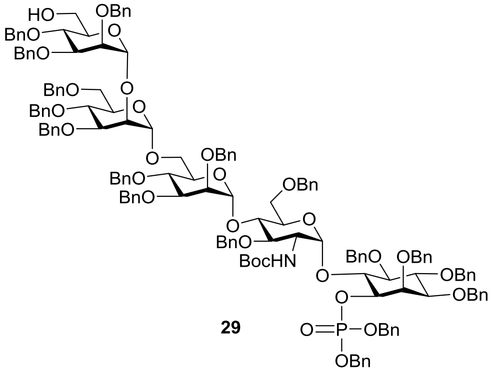
1H NMR
600 MHz
CDCl3
29
f1 (ppm)
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
86.74
1.00
2.01
4.04
8.93
1.28
1.94
19.23
1.25
7.63
2.52
12.48
4.76
4.00
1.99
1.04
9.14
7.309
7.297
7.285
7.277
7.260
7.248
7.247
7.237
7.227
7.195
7.184
7.172
7.165
7.152
7.141
7.127
7.119
7.116
7.108
7.094
7.080
7.069
5.245
5.058
5.025
4.892
4.874
4.856
4.845
4.825
4.794
4.775
4.598
4.586
4.565
4.492
4.473
4.458
4.437
4.424
4.414
4.399
4.381
4.371
4.349
4.214
4.140
4.125
4.079
4.069
3.912
3.867
3.863
3.819
3.811
3.807
3.773
3.758
3.743
3.736
3.689
3.533
3.457
2.049
1.238
0.079
HO
OBn
BnO
BnO
O
O
BnO
BnO
BnO
O
O
OBn
BnO
BnO
O
O
OBn
BnO
BocHN
O
O
BnO
OBn
OBn
OBn
O
O=P–OBn
OBn



29

^{13}C NMR
150 MHz
CDCl$_3$



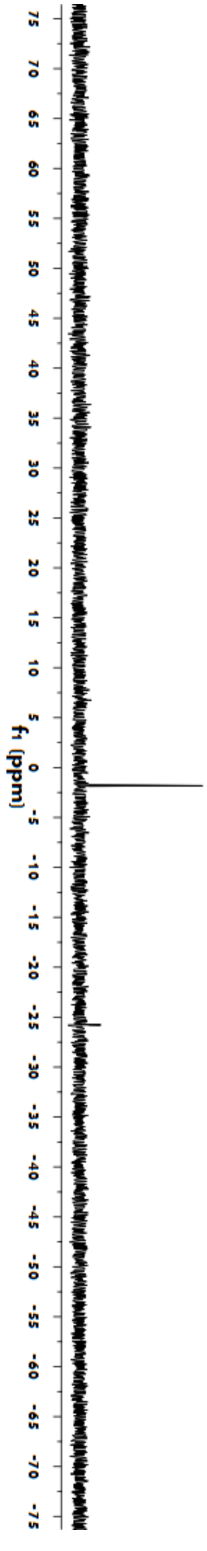


29

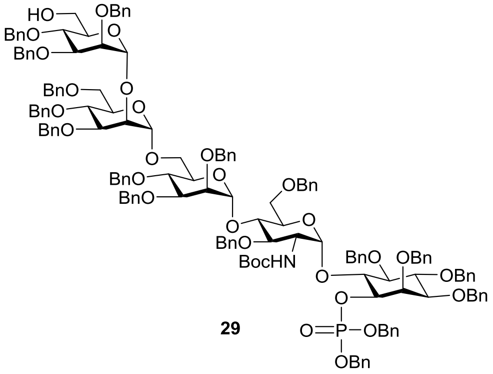
^{1}H-^{13}C HMQC

600/150 MHz

CDCl$_3$



^{31}P NMR
162 MHz
$CDCl_3$



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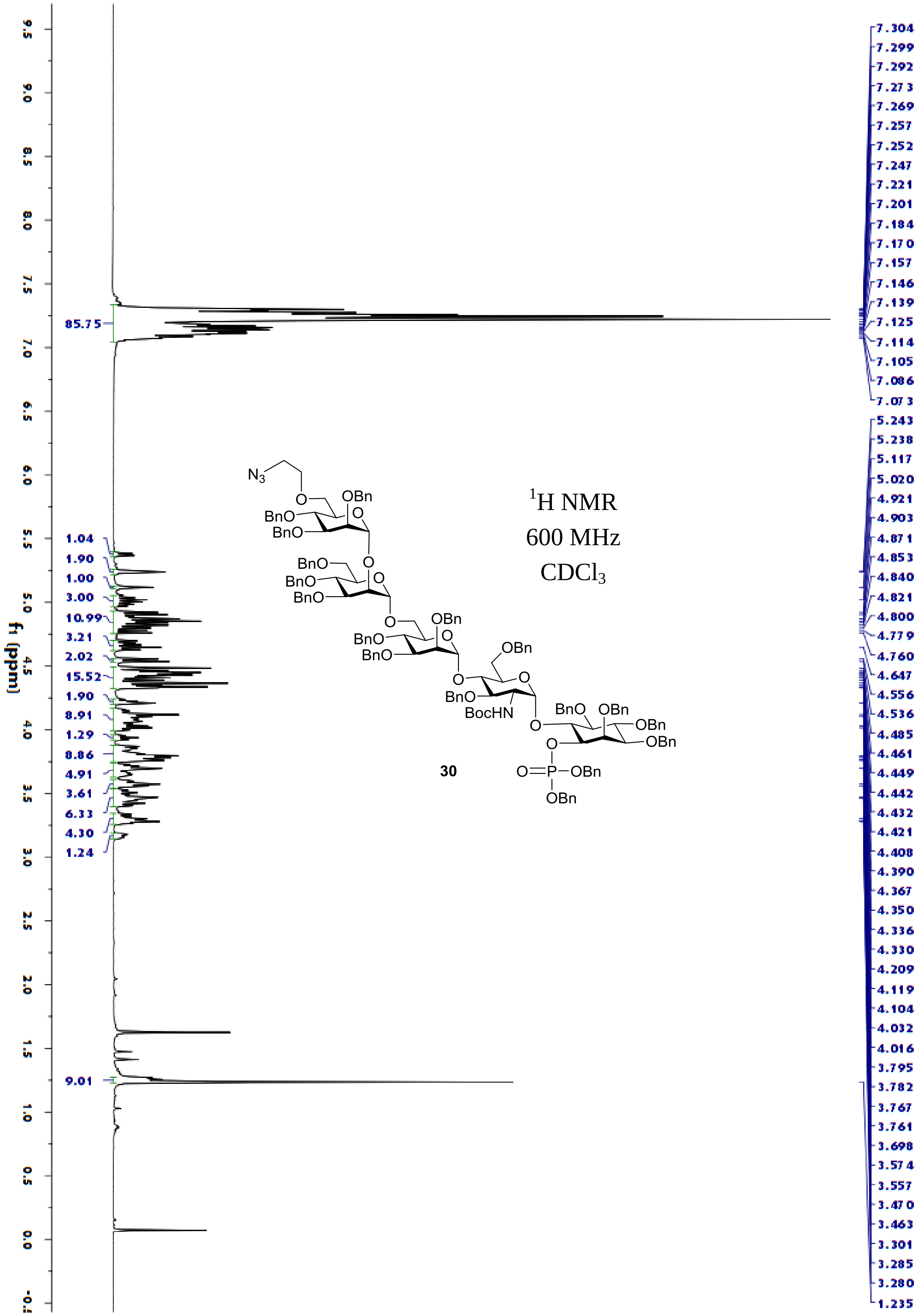
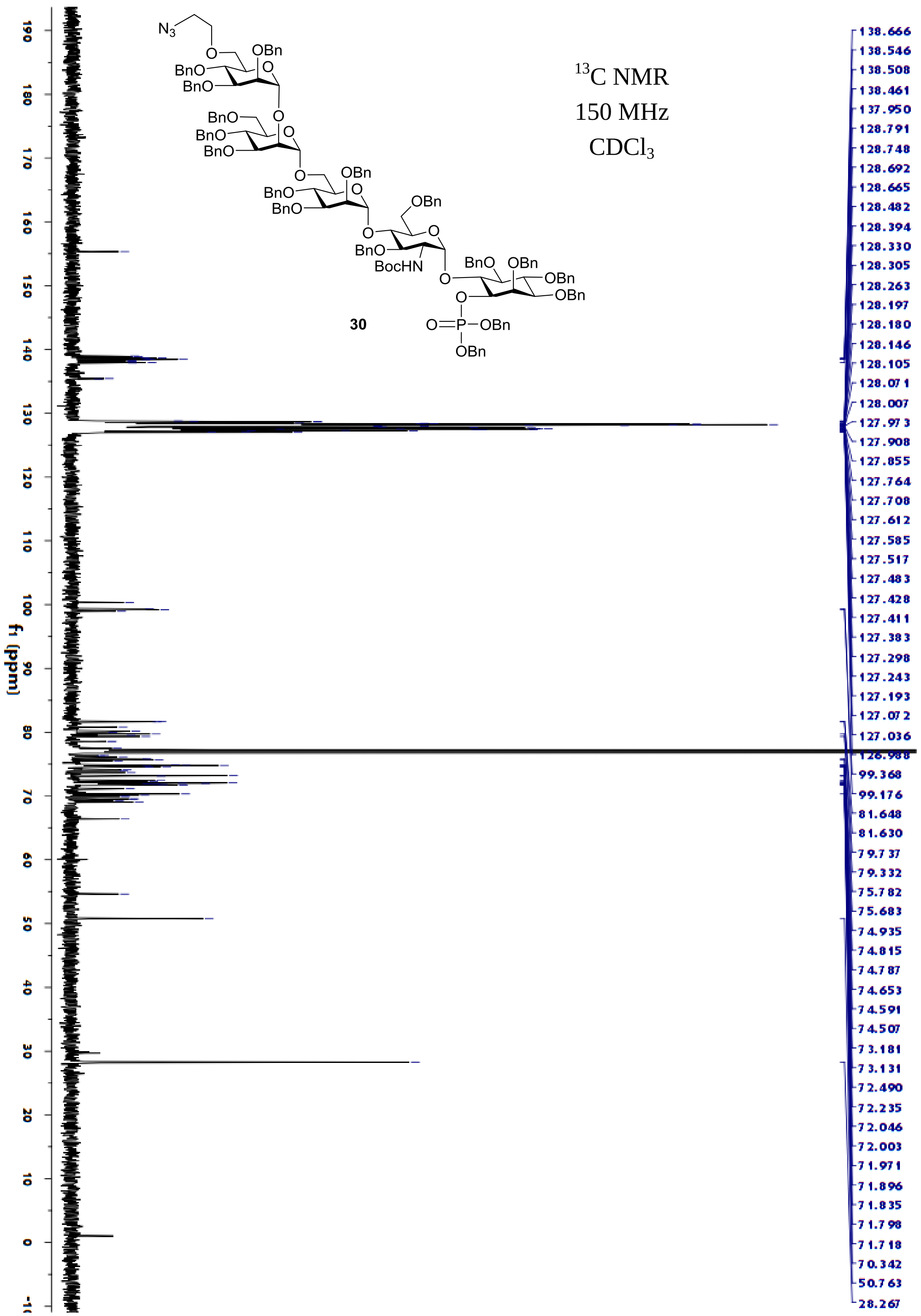
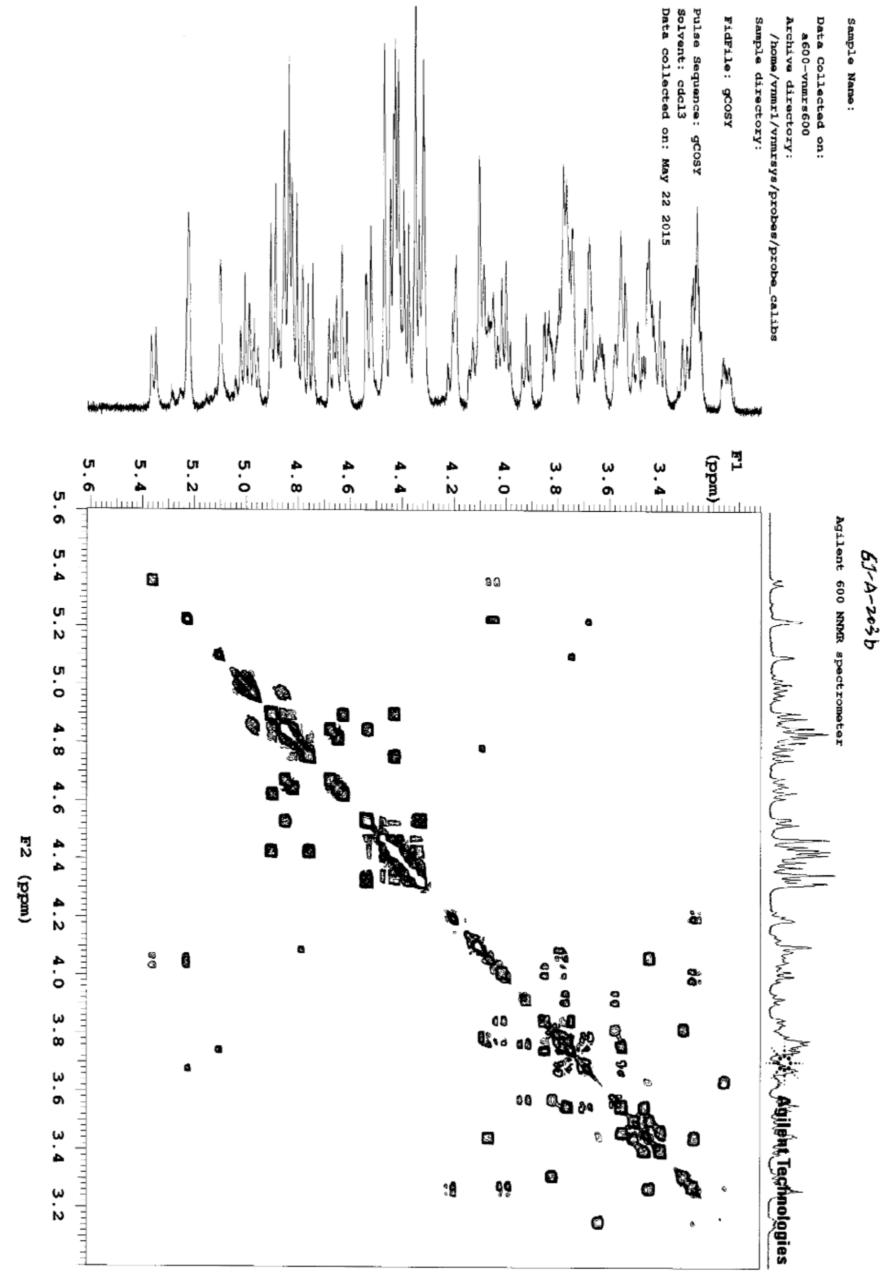


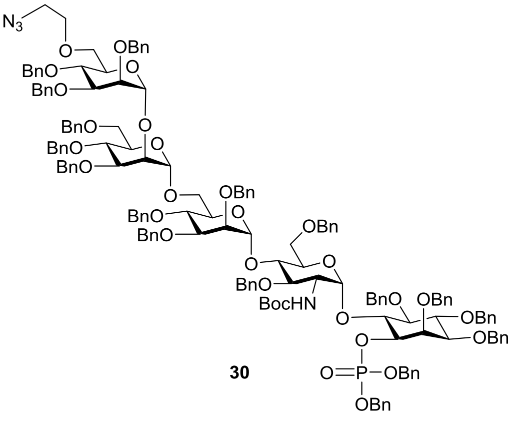
^{1}H NMR
600 MHz
$CDCl_3$

30


N3
OBn
BnO
BnO
O
O
BnO
BnO
BnO
OBn
BnO
BnO
OBn
BnO
BocHN
BnO
OBn
OBn
OBn
O=P–OBn
OBn
30
^{13}C NMR
150 MHz
CDCl$_3$
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-1
f1 (ppm)
138.666
138.546
138.508
138.461
137.950
128.791
128.748
128.692
128.665
128.482
128.394
128.330
128.305
128.263
128.197
128.180
128.146
128.105
128.071
128.007
127.973
127.908
127.855
127.764
127.708
127.612
127.585
127.517
127.483
127.428
127.411
127.383
127.298
127.243
127.193
127.072
127.036
126.988
99.368
99.176
81.648
81.630
79.737
79.332
75.782
75.683
74.935
74.815
74.787
74.653
74.591
74.507
73.181
73.131
72.490
72.235
72.046
72.003
71.971
71.896
71.835
71.798
71.718
70.342
50.763
28.267



^{1}H-^{1}H COSY
600 MHz
$CDCl_3$



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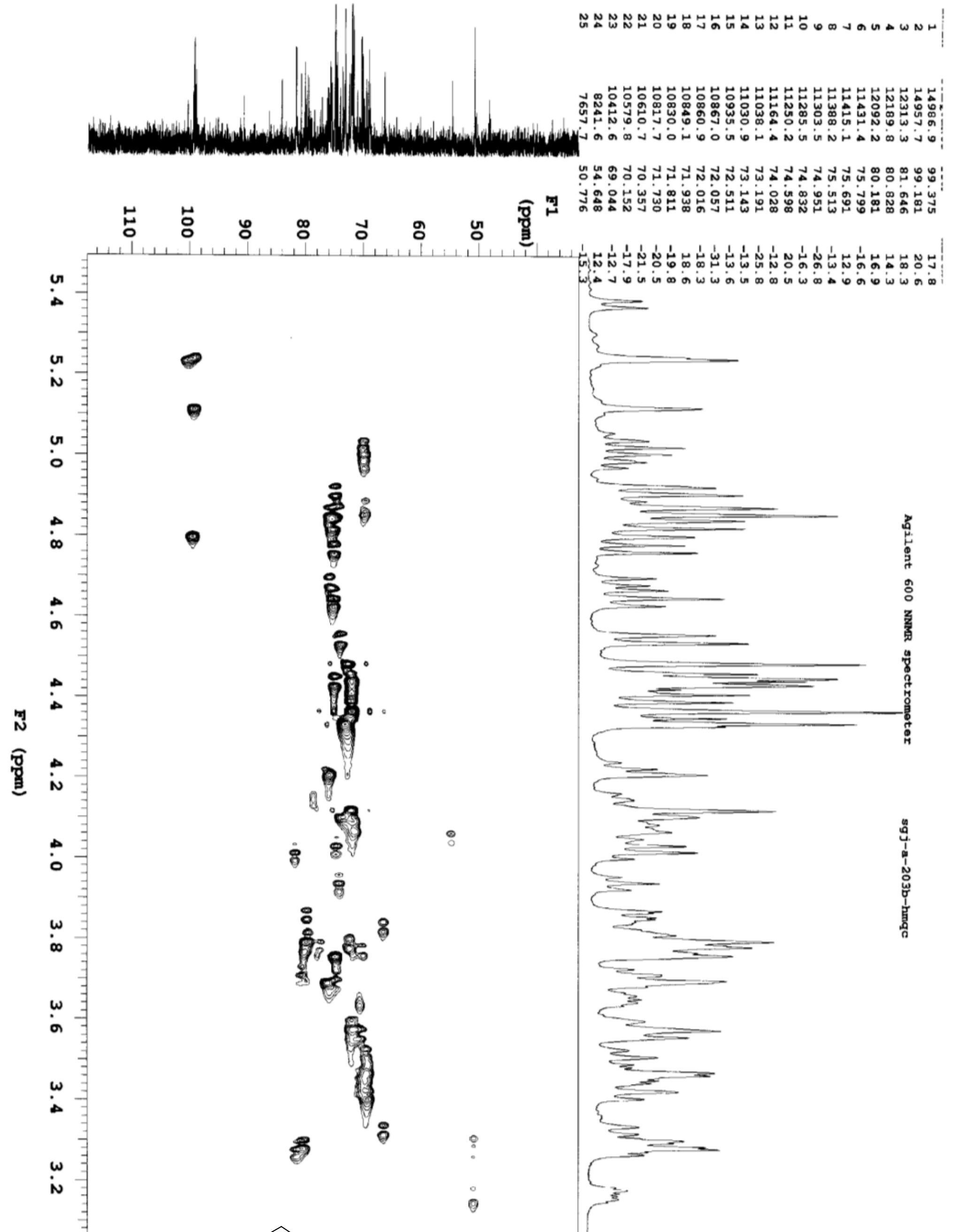

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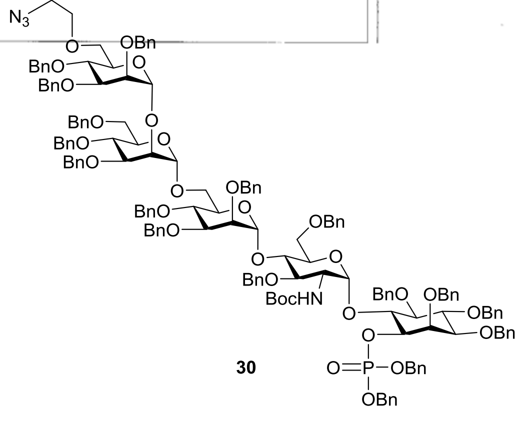
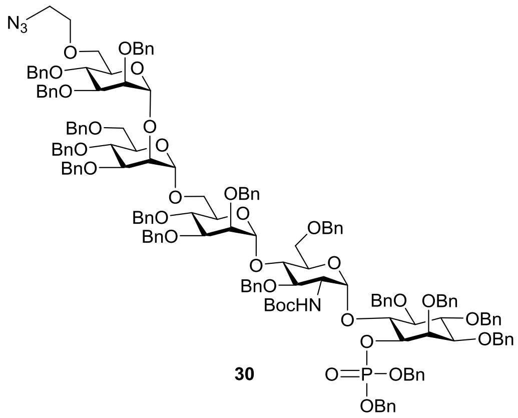
 2   14957.7   99.181   20.6
 3   12313.3   81.646   18.3
 4   12189.8   80.828   14.3
 5   12092.2   80.181   16.9
 6   11431.4   75.799  -16.6
 7   11415.1   75.691   12.9
 8   11388.2   75.513  -13.4
 9   11303.5   74.951  -26.8
10   11285.5   74.832  -16.3
11   11250.2   74.598   20.5
12   11164.4   74.028  -12.8
13   11038.1   73.191  -25.8
14   11030.9   73.143  -13.5
15   10935.5   72.511  -13.6
16   10867.0   72.057  -31.3
17   10860.9   72.016  -18.3
18   10849.1   71.938   18.6
19   10830.0   71.811  -19.8
20   10817.7   71.730  -20.5
21   10610.7   70.357  -21.5
22   10579.8   70.152  -17.9
23   10412.6   69.044  -12.7
24    8241.6   54.648   12.4
25    7657.7   50.776  -15.3
```

^{1}H-^{13}C HMQC
600/150 MHz
CDCl$_3$



30

^{31}P NMR
162 MHz
$CDCl_3$



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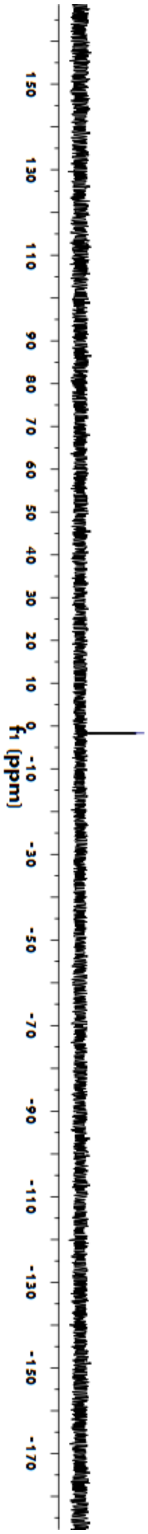
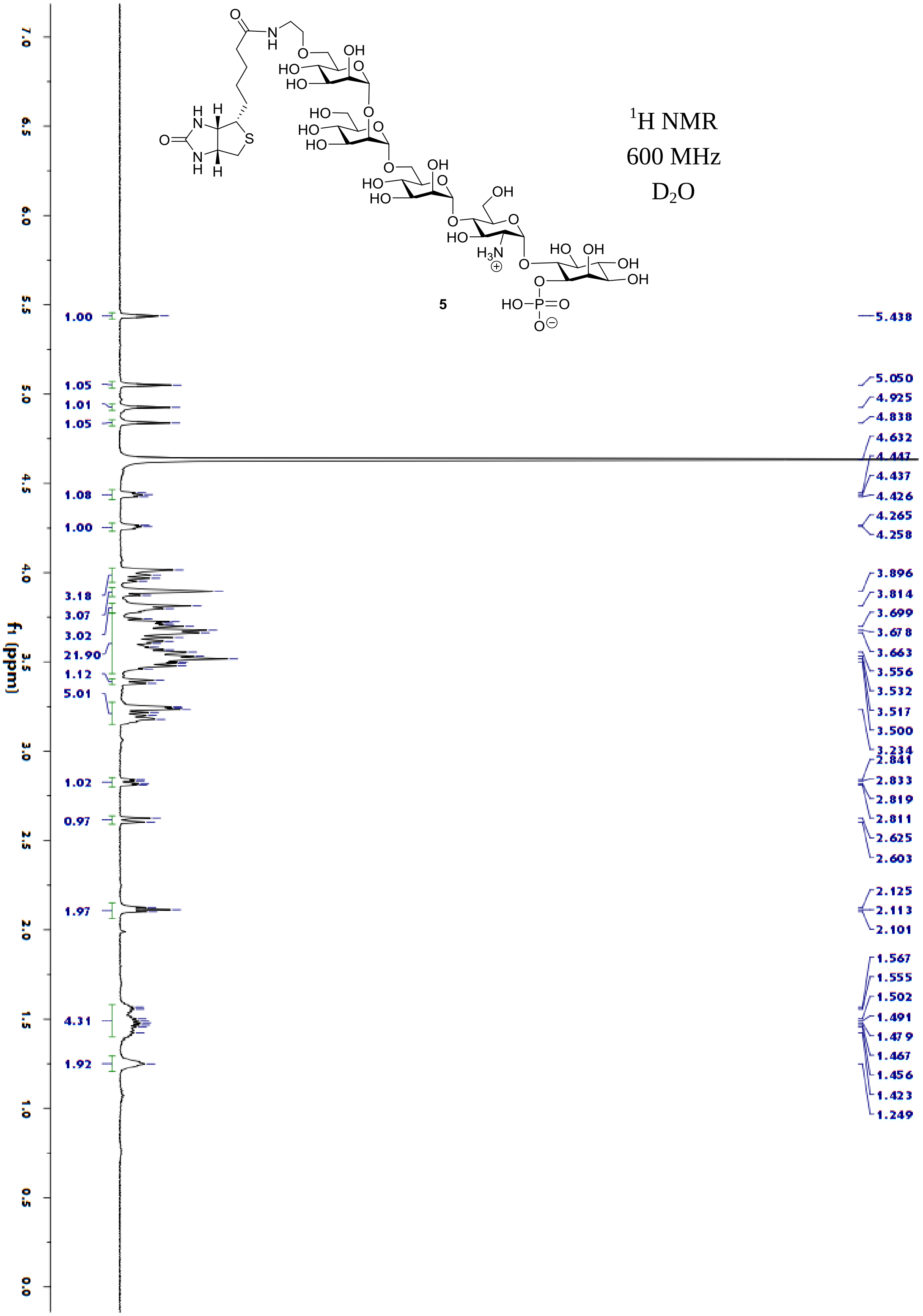
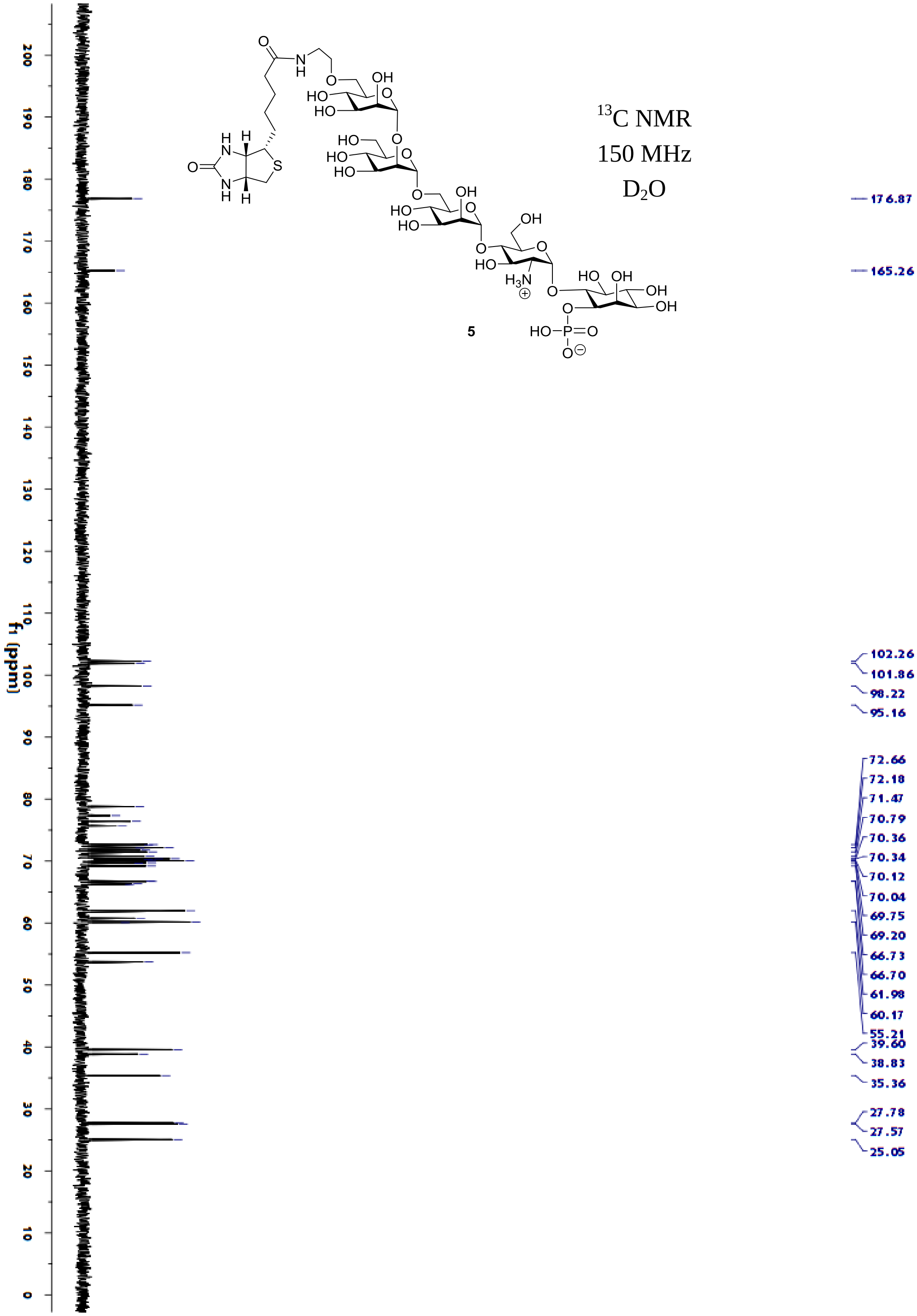


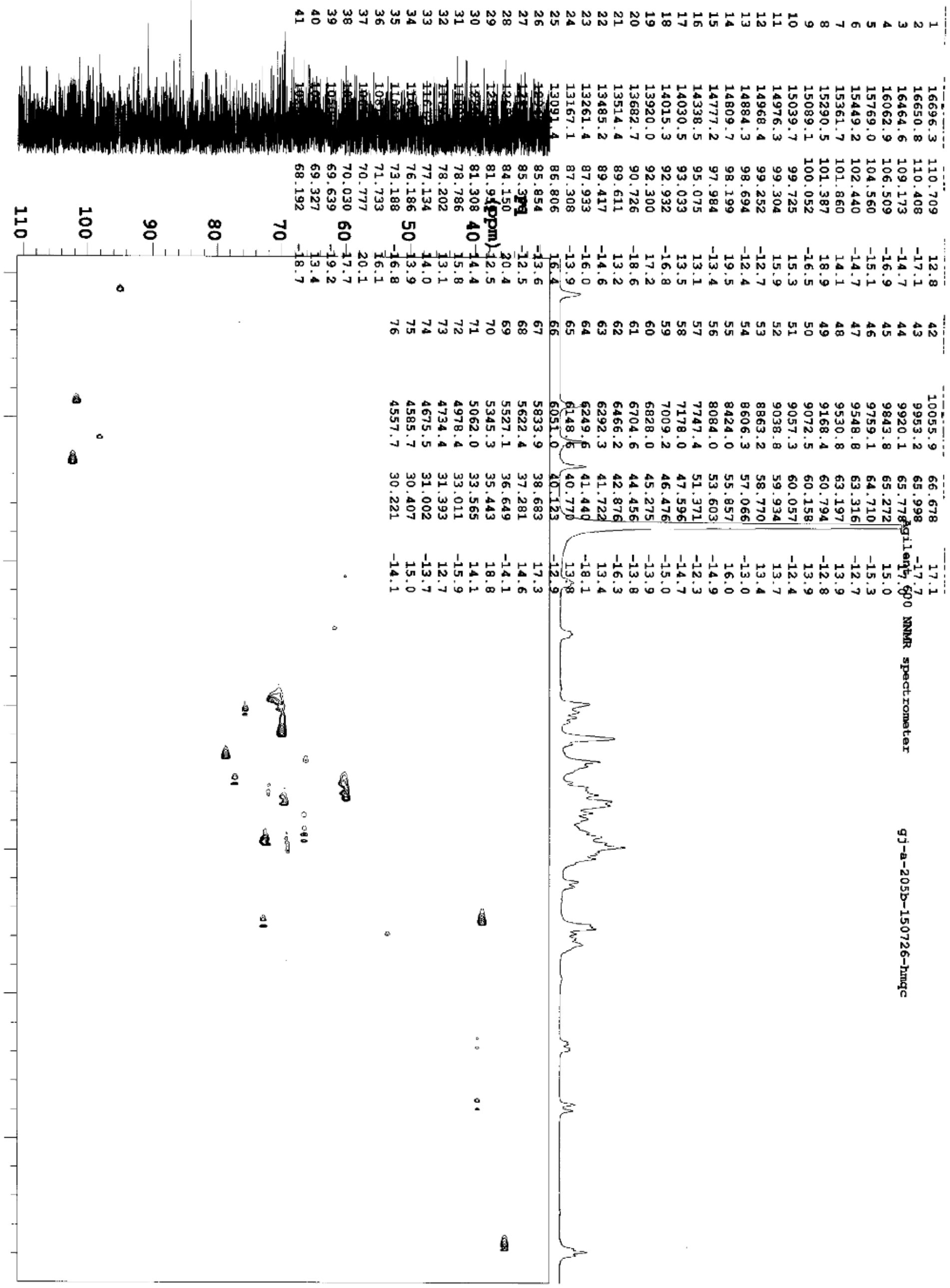


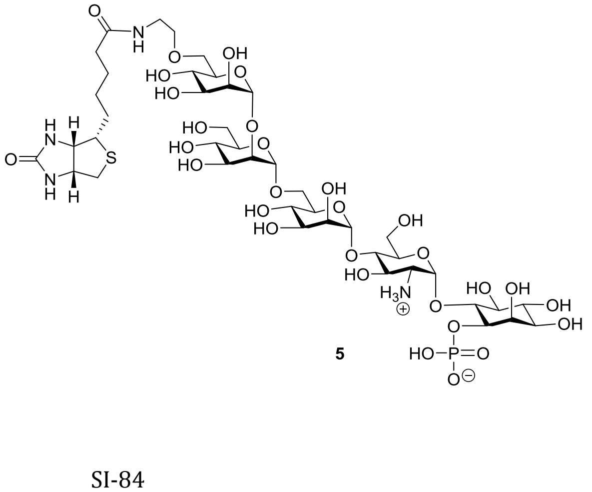
5

^{1}H NMR
600 MHz
D_2O


5

^{13}C NMR
150 MHz
D$_2$O
176.87
165.26
102.26
101.86
98.22
95.16
72.66
72.18
71.47
70.79
70.36
70.34
70.12
70.04
69.75
69.20
66.73
66.70
61.98
60.17
55.21
39.60
38.83
35.36
27.78
27.57
25.05
f1 (ppm)
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0



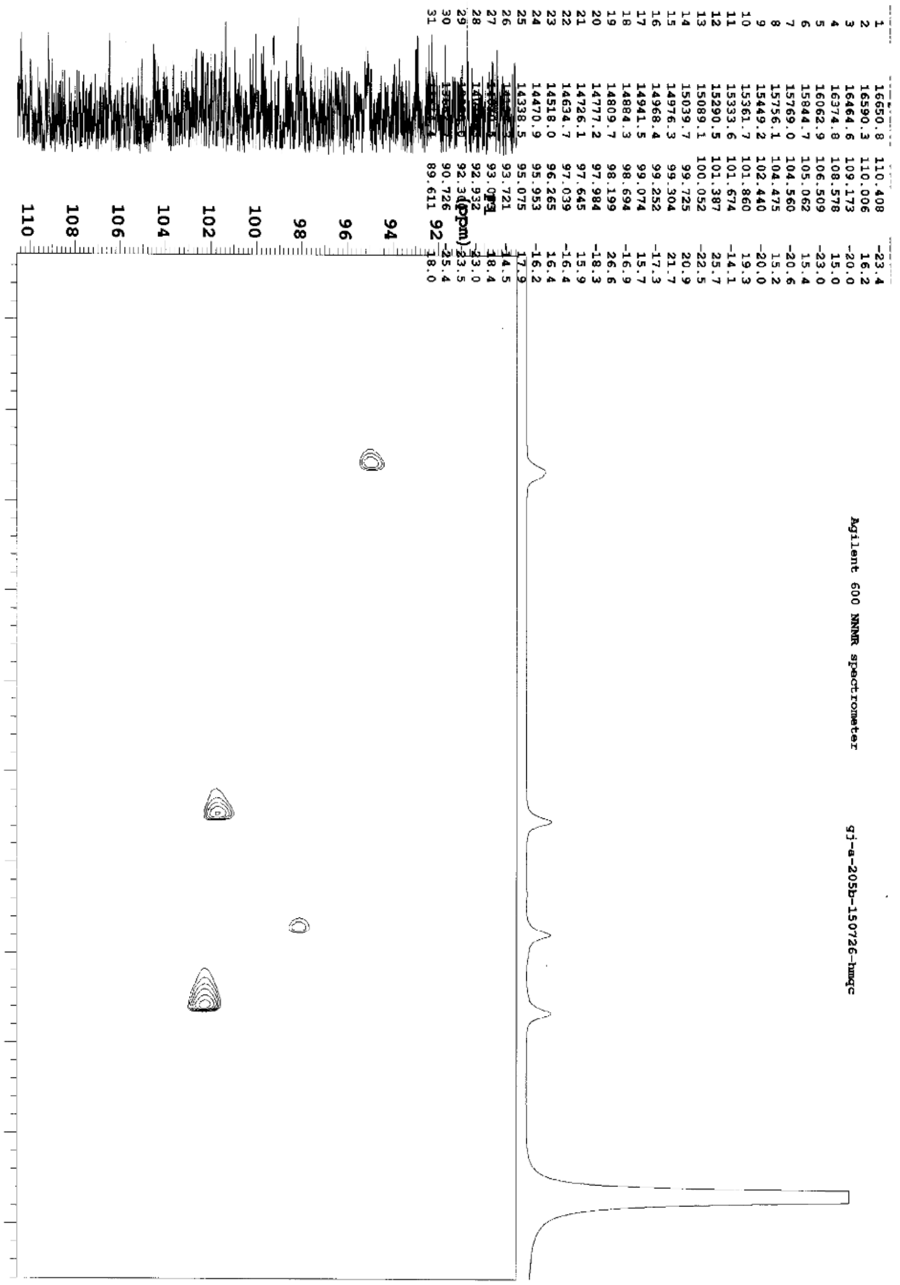


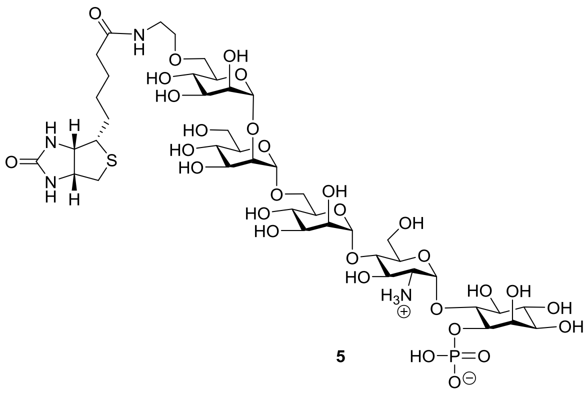
5

^{1}H-^{13}C COSY

600/150 MHz

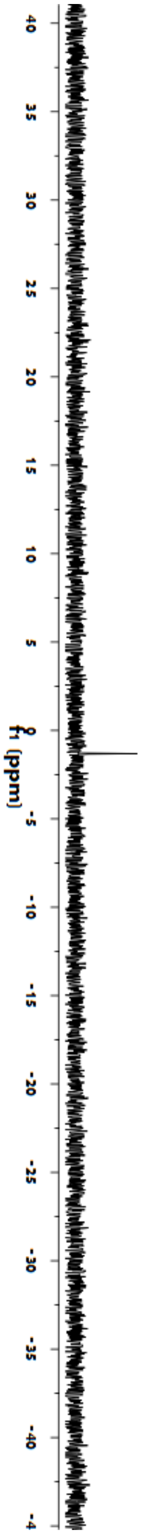
D_2O



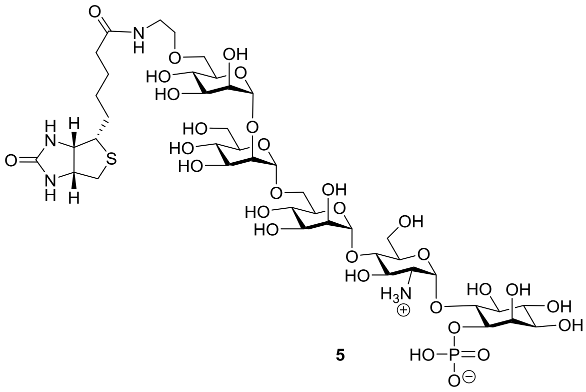


5

^{1}H-^{13}C HMQC
600/150 MHz
D$_2$O



^{31}P NMR
162 MHz
D_2O



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