Supporting Information

for

Synthesis and Immunogenicity Evaluation of Oligosaccharide Epitopes for the Development of Glycoconjugate Vaccine against *Streptococcus pneumoniae* Serotype 3

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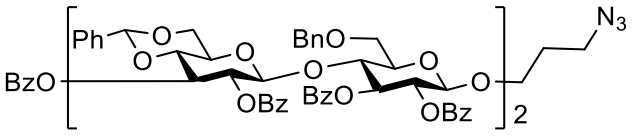
I. Experimental Section

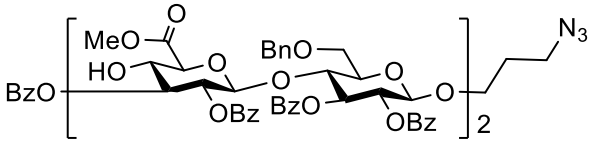
General Information. Chemical reagents and solvents were obtained from commercial sources and used as received without additional purification unless otherwise noted. Molecular sieves (MS) 4Å were activated by the muffle furnace at 350℃ for 3 h and cooled to room temperature (rt) under an argon atmosphere before use. Analytical thin-layer chromatography (TLC) was performed with silica gel HF_{254} plates detected by charring with 30% (v/v) H_2SO_4 in MeOH or by a UV-light (λ = 254 nm) detector. Flash column chromatography was performed with silica gel (100−200 mesh) and employed a solvent polarity correlated with TLC mobility, or size-exclusion gel chromatography (Sephadex G-10). Nuclear Magnetic Resonance (NMR) spectra were recorded on a 600 MHz NMR spectrometer and chemical shifts (δ) were given in ppm downfield from internal TMS or with DHO signal as a reference when $CDCl_3$ or D_2O was used as the solvent. Chemical shifts and coupling constants were obtained from a first-order analysis of one-dimensional spectra and assignments of proton and carbon resonances were based on ^{1}H-^{1}H COSY and ^{1}H-^{13}C HSQC experiments. High-resolution mass spectra (HRMS) were measured on an IT-TOF spectrometer using the electrospray ionization (ESI) technique to introduce the sample. MALDI-TOF mass spectra were recorded with sinapic acid (SA) as the matrix. The ST3 capsular polysaccharide (CPS) and ST3 CPS-TT conjugate samples were supplied from Yuxi Walvax Biotechnology Co., Ltd.



9

3-Azidopropyl 2-*O*-benzoyl-4,6-*O*-benzylidene-3-*O*-benzoyl-β-D-glucopyranosyl-(1→4)-2,3-di-*O*-benzoyl-6-*O*-benzyl-β-D-glucopyranosyl-(1→3)-2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-glucopyranosyl-(1→4)-2,3-di-*O*-benzoyl-6-*O*-benzyl-β-D-glucopyranoside (9). To a stirred solution of disaccharide **4** (500 mg, 0.48 mmol), disaccharide **6** (395 mg, 0.43 mmol), and activated MS 4Å (2.0 g) in anhydrous DCM (10 mL) were added 2,4,6-tri-*tert*-butylpyrimidine (TTBP) (131 mg, 0.53 mmol), silver trifluoromethane sulfonate (AgOTf) (308 mg, 1.20 mmol) at rt under an argon atmosphere. Then, the reaction mixture was cooled to -78℃ with a stir for 30 min, and *p*-toluenethiol chloride (*p*-TolSCl, 82 μL, 0.57 mmol) was added dropwise. After the reaction was stirred for another 2 h with the temperature slowly warming up to rt, it was neutralized with trimethylamine (Et_3N), diluted with DCM, filtered, and concentrated. The resulting residue was

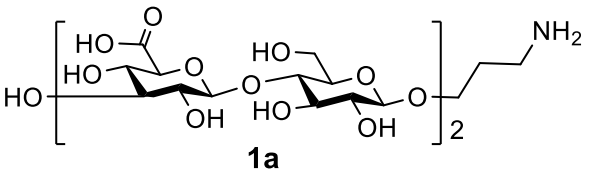
purified by flash column chromatography (toluene/ethyl acetate, 10:1) to give tetrasaccharide **9** (591 mg, 75%) as white foamy solid. $[\alpha]_D^{25}$ +19.6 (*c* 0.8, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.01–7.96 (m, 2H, Ar*H*), 7.92–7.81 (m, 8H, Ar*H*), 7.64–7.58 (m, 2H, Ar*H*), 7.56–7.51 (m, 2H, Ar*H*), 7.50–7.25 (m, 25H, Ar*H*), 7.25–7.19 (m, 10H, Ar*H*), 7.17–7.13 (m, 4H, Ar*H*), 7.08–7.02 (m, 3H, Ar*H*), 5.54–5.47 (m, 2H, H-3^A, H-3^D), 5.36 (t, *J* = 9.0 Hz, 1H, H-3^C), 5.28 (dd, *J* = 9.6, 7.8 Hz, 1H, H-2^A), 5.24 (dd, *J* = 9.6, 7.8 Hz, 1H, H-2^D), 5.19 (dd, *J* = 9.6, 7.8 Hz, 1H, H-2^C), 5.13 (s, 1H, PhC*H*), 5.10 (t, *J* = 9.0 Hz, 1H, H-2^B), 4.92 (s, 1H, PhC*H*), 4.66 (d, *J* = 7.8 Hz, 1H, H-1^D), 4.60 (d, *J* = 7.8 Hz, 1H, H-1^C), 4.54 (d, *J* = 12.0 Hz, 1H, PhCH_2), 4.45 (d, *J* = 7.8 Hz, 2H, H-1^A, H-1^B), 4.26 (d, *J* = 12.0 Hz, 1H, PhCH_2), 4.23 (d, *J* = 12.0 Hz, 1H, PhCH_2), 4.18 (d, *J* = 12.0 Hz, 1H, PhCH_2), 4.06 (t, *J* = 9.6 Hz, 1H, H-4^A), 3.98 (t, *J* = 9.6 Hz, 1H, H-4^C), 3.86–3.81 (m, 1H, -OCH_2CH$_2$-), 3.79 (t, *J* = 9.0 Hz, 1H, H-3^B), 3.60 (dd, *J* = 10.8, 4.8 Hz, 1H, H-6a^B), 3.53–3.48 (m, 2H, H-4^D, H-6a^D), 3.46–3.41 (m, 3H, H-4^B, H-6a^C, -OCH_2CH$_2$-), 3.32–3.26 (m, 3H, H-6b^C, H-6a^A, H-5^A), 3.21–3.08 (m, 6H, H-5^D, H-6b^A, H-5^C, H-5^B, -CH_2N$_3$), 2.66–2.57 (m, 2H, H-6b^B, H-6b^D), 1.76–1.62 (m, 2H, -OCH$_2$CH_2CH$_2$N$_3$); ^{13}C NMR (150 MHz, $CDCl_3$): δ 165.5, 165.2, 165.0, 164.9, 164.6, 163.8, 138.0, 137.9, 136.8, 136.6, 133.3, 133.1, 133.04, 133.00, 132.98, 132.95, 132.5, 130.1, 129.9, 129.81, 129.78, 129.69, 129.67, 129.6, 129.6, 129.3, 129.1, 129.1, 128.99, 128.96, 128.5, 128.5, 128.43, 128.35, 128.3, 128.23, 128.21, 128.19, 128.1, 128.02, 128.00, 127.9, 126.0, 125.9, 101.3 (Ph*C*H), 101.0 (2C, C-1^C, Ph*C*H), 100.9 (C-1^B), 100.8 (C-1^A), 100.0 (C-1^C), 79.3 (C-4^B), 78.24 (C-3^B), 78.18 (C-4^D), 76.1 (C-4^C), 75.5 (C-4^A), 74.4 (C-5^A), 74.0 (C-5^C), 73.5 (C-3^C), 73.3 (Ph*C*H$_2$), 73.2 (2C, C-3^A, Ph*C*H$_2$), 72.9 (C-2^B), 72.4 (C-2^D), 72.09 (C-2^C), 72.06 (C-3^D), 71.7 (C-2^A), 67.8 (C-6^B), 67.6 (C-6^D), 66.92 (C-6^C), 66.86 (C-6^A), 66.3 (-O*C*H$_2$CH$_2$-), 66.1 (C-5^D), 66.0 (C-5^B), 47.8 (-*C*H$_2$N$_3$), 28.9 (-OCH$_2$*C*H$_2$CH$_2$N$_3$); ESI HRMS: calcd for ($C_{104}H_{95}N_3O_{28}$ + NH_4^+) *m/z*, 1851.6440; found, 1851.6460.



10

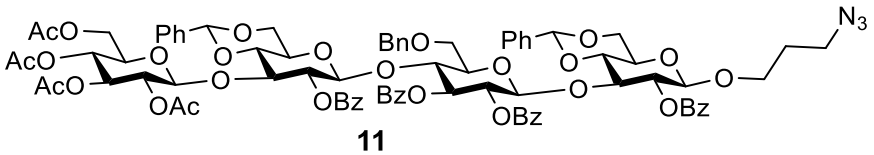
3-Azidopropyl (methyl 2,3-di-*O*-benzoyl-β-D-glucopyranosyluronate)-(1→4)-2,3-di-*O*-benzoyl-6-*O*-benzyl-β-D-glucopyranosyl-(1→3)-(methyl 2-*O*-benzoyl-β-D-glucopyranosyluronate)-(1→4)-2,3-di-*O*-benzoyl-6-*O*-benzyl-β-D-glucopyranoside (10). A solution of **9** (300 mg, 0.16 mmol) in 80% AcOH (25 mL) was stirred at 80℃ for 2 h, and then it

was co-evaporated with toluene to dryness. To a solution of the above product in DCM/H_2O (21 mL, 2:1, v/v) was added TEMPO (15 mg, 98 μmol) at 0℃, followed by the addition of BAIB (386 mg, 1.23 mmol) after being stirred for 10 min. The reaction mixture was warmed up to rt and stirred for another 5 h, then diluted with ethyl acetate and quenched by saturated aq. $Na_2S_2O_3$. The organic layer was washed with brine twice, dried over anhydrous Na_2SO_4, filtered, and concentrated. The crude product obtained above was dissolved in MeOH/Et_2O (15 mL, 1:4, v/v) at rt under an Ar atmosphere, and $TMSCHN_2$ (2 M in hexanes, 0.49 mL, 0.98 mmol) was added. After being stirred for 15 min, the reaction mixture was quenched with AcOH and concentrated. The residue was purified by flash column chromatography (petroleum ether/ethyl acetate, 3:2) to give tetrasaccharide **10** (185 mg, 66% over three steps) as white foamy solid. $[\alpha]_D^{25}$ +19.2 (*c* 0.5, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.90–7.84 (m, 8H, Ar*H*), 7.75 (d, *J* = 8.4 Hz, 2H, Ar*H*), 7.53–7.38 (m, 11H, Ar*H*), 7.38-7.31 (m, 14H, Ar*H*), 7.29–7.25 (m, 6H, Ar*H*), 7.17 (t, *J* = 7.8 Hz, 2H, Ar*H*), 7.06 (t, *J* = 7.8 Hz, 2H, Ar*H*), 5.50 (t, *J* = 9.0 Hz, 1H, H-3^C), 5.45 (t, *J* = 9.6 Hz, 1H, H-3^A), 5.29 (t, *J* = 9.6 Hz, 1H, H-3^D), 5.26 (t, *J* = 9.6 Hz, 1H, H-2^D), 5.24–5.20 (m, 2H, H-2^A, H-2^C), 5.02 (t, *J* = 9.0 Hz, 1H, H-2^B), 4.64 (d, *J* = 7.2 Hz, 1H, H-1^D), 4.59 (d, *J* = 12.0 Hz, 1H, PhC*H*$_2$), 4.52 (d, *J* = 7.8 Hz, 1H, H-1^C), 4.44 (d, *J* = 7.8 Hz, 1H, H-1^B), 4.43 (d, *J* = 7.8 Hz, 1H, H-1^A), 4.40 (d, *J* = 12.0 Hz, 1H, PhC*H*$_2$), 4.22 (d, *J* = 12.0 Hz, 1H, PhC*H*$_2$), 4.16 (d, *J* = 12.0 Hz, 1H, PhC*H*$_2$), 4.12 (t, *J* = 9.0 Hz, 1H, H-4^C), 4.09 (t, *J* = 9.0 Hz, 1H, H-4^A), 4.03 (s, 1H, -O*H*), 3.89 (td, *J* = 9.0, 3.0 Hz, 1H, H-4^D), 3.83–3.76 (m, 2H, H-4^B, -OC*H*$_2$CH$_2$-), 3.62–3.58 (m, 3H, H-5^A, H-5^B, H-5^D), 3.53–3.50 (m, 2H, H-6^A), 3.46 (dd, *J* = 10.8, 3.0 Hz, 1H, H-6a^C), 3.43–3.39 (m, 2H, H-3^B, -OC*H*$_2$CH$_2$-), 3.38 (s, 3H, COOC*H*$_3$), 3.34 (s, 4H, H-6b^C, COOC*H*$_3$), 3.26–3.23 (m, 1H, H-5^C), 3.20–3.09 (m, 2H, -C*H*$_2$N$_3$), 3.07 (d, *J* = 3.6 Hz, 1H, -O*H*), 1.73–1.59 (m, 2H, -OCH$_2$C*H*$_2$CH$_2$N$_3$); ^{13}C NMR (150 MHz, $CDCl_3$): δ 168.1, 167.3, 166.4, 165.3, 165.2, 165.1, 164.8, 164.7, 163.6, 137.9, 137.1, 133.6, 133.4, 133.1, 132.90, 132.87, 132.6, 132.5, 129.9, 129.8, 129.7, 129.6, 129.5, 129.5, 129.4, 129.32, 129.28, 128.81, 128.78, 128.7, 128.6, 128.6, 128.4, 128.3, 128.3, 128.2, 128.2, 128.1, 127.93, 127.91, 100.9 (C-1^A), 100.8 (C-1^C), 100.7 (C-1^D), 100.5 (C-1^B), 84.3 (C-3^B), 75.6 (C-4^A), 75.2 (C-5^A), 74.69 (C-4^C), 74.65 (C-3^D), 74.4 (C-5^C), 74.2 (C-5^D), 74.1 (C-5^B), 73.6 (PhCH$_2$), 73.4 (PhCH$_2$), 73.0 (C-3^C), 72.9 (C-3^A), 72.0 (C-2^A), 71.7 (C-2^C), 71.4 (C-2^B), 71.24 (C-2^D), 70.15 (C-4^D), 69.9 (C-4^B), 67.5 (C-6^A), 67.0 (C-6^C), 66.2 (-OCH$_2$CH$_2$-), 52.5 (COOCH$_3$), 52.2 (COOCH$_3$), 47.8 (-CH$_2$N$_3$), 28.9 (-OCH$_2$CH$_2$CH$_2$N$_3$); ESI HRMS: calcd for ($C_{92}H_{87}N_3O_{30}$+Na^+) *m/z*, 1736.5267; found, 1736.5294.



1a

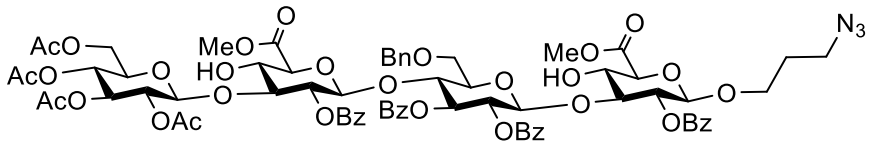
3-Aminopropyl β-D-glucopyranosyluronic acid-(1→4)-β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyluronic acid-(1→4)-β-D-glucopyranoside (1a). To a stirred solution of tetrasaccharide **10** (80 mg, 47 μmol) in THF (8 mL) was added 1 M LiOH at rt until the pH value reached 11. The reaction was stirred overnight and neutralized with 1 M HCl until the pH value reached 6. Then, the reaction mixture was concentrated under reduced pressure, and the crude product was simply purified by Sephadex G-10 column chromatography with distilled H_2O as the eluent. After lyophilization of the desired fractions, the resulting residue was dissolved in H_2O (5 mL), followed by the addition of 10% Pd/C (15 mg). The reaction mixture was stirred for 18 h under H_2 atmosphere, then filtered to remove the catalyst, and concentrated. The residue was purified by size-exclusion chromatography on a Sephadex G-10 column (H_2O eluent) to give target product **1a** (27 mg, 77% over two steps) as white powder after lyophilization. $[\alpha]_D^{25}$ -6.7 (*c* 0.1, H_2O); ^{1}H NMR (600 MHz, D_2O): δ 4.69 (d, J = 7.8 Hz, 1H, H-1), 4.43 (d, J = 7.8 Hz, 1H, H-1), 4.41 (d, J = 7.8 Hz, 1H, H-1), 4.37 (d, J = 7.8 Hz, 1H, H-1), 3.94–3.89 (m, 1H, -OC*H*$_2$CH$_2$-), 3.87–3.77 (m, 5H), 3.70–3.65 (m, 4H, H-6b×2, -OC*H*$_2$CH$_2$-), 3.59–3.39 (m, 12H), 3.27–3.23 (m, 2H), 3.20–3.17 (m, 1H), 3.02 (t, J = 7.2 Hz, 2H, -C*H*$_2$NH$_2$), 1.90–1.85 (m, 2H, -OCH$_2$C*H*$_2$CH$_2$NH$_2$); ^{13}C NMR (150 MHz, D_2O): δ 173.5, 173.3, 102.3 (2C, C-1×2), 102.1 (C-1), 102.0 (C-1), 82.7, 78.8 (2C), 75.1, 74.8 (2C), 74.7 (2C), 74.2, 74.0, 73.1, 72.8, 72.7 (2C), 71.3, 69.84, 67.80 (-O*C*H$_2$CH$_2$-), 59.9 (2C, C-6×2), 37.5 (-*C*H$_2$NH$_2$), 26.6 (-OCH$_2$*C*H$_2$CH$_2$NH$_2$); ESI HRMS: calcd for ($C_{27}H_{45}NO_{23}$+H^+) *m/z*, 752.2455; found, 752.2458.



11

3-Azidopropyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl-(1→3)-2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-glucopyranosyl-(1→4)-2,3-di-*O*-benzoyl-6-*O*-benzyl-β-D-glucopyranosyl-(1→3)-2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-glucopyranoside (11). To a stirred solution of trisaccharide **7** (320 mg, 0. 25mmol), monosaccharide **8** (138 mg, 0.30 mmol) and activated MS 4Å (1.3 g) in anhydrous DCM (15 mL) were added TTBP (69 mg, 0.28 mmol) and AgOTf (162

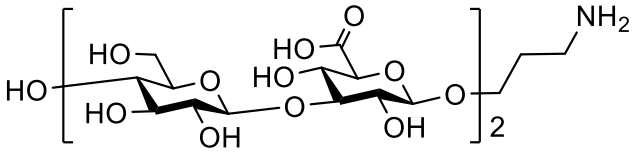
mg, 0.63 mmol) at rt under an argon atmosphere. The reaction mixture was then cooled to -78℃, and *p*-TolSCl (40 μL, 0.28 mmol) was dropwise added. The reaction was stirred for another 2 h with the temperature slowly warming up to rt, then neutralized with Et_3N, diluted with DCM, and filtered. The filtrate was concentrated, and the resulting residue was purified by flash column chromatography (petroleum ether/ethyl acetate, 5:2) to give tetrasaccharide **11** (266 mg, 66%) as white foamy solid. $[\alpha]_D^{25}$ +8.4 (*c* 0.75, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.92 (d, *J* = 6.6 Hz, 2H, Ar*H*), 7.87 (d, *J* = 7.2 Hz, 2H, Ar*H*), 7.73–7.65 (m, 3H, Ar*H*), 7.52 (t, *J* = 7.2 Hz, 2H, Ar*H*), 7.50–7.41 (m, 6H, Ar*H*), 7.39–7.26 (m, 15H, Ar*H*), 7.13 (t, *J* = 8.4 Hz, 2H, Ar*H*), 7.10–7.05 (m, 3H, Ar*H*), 5.38 (t, *J* = 9.6 Hz, 1H, H-3^B), 5.29 (s, 1H, PhC*H*), 5.28–5.25 (m, 1H, H-2^B), 5.22 (t, *J* = 8.4 Hz, 1H, H-2^A), 5.18 (s, 1H, PhC*H*), 5.08 (t, *J* = 9.0 Hz, 1H, H-2^C), 4.95 (t, *J* = 9.6 Hz, 1H, H-4^D), 4.86–4.83 (m, 2H, H-2^D, H-3^D), 4.73 (d, *J* = 7.8 Hz, 1H, H-1^B), 4.53 (d, *J* = 7.8 Hz, 1H, H-1^C), 4.49 (d, *J* = 7.8 Hz, 1H, H-1^A), 4.45 (d, *J* = 7.8 Hz, 1H, H-1^D), 4.41 (d, *J* = 12.0 Hz, 1H, PhCH_2), 4.30 (dd, *J* = 10.8, 4.8 Hz, 1H, H-6a^A), 4.18 (d, *J* = 12.0 Hz, 1H, PhCH_2), 4.06 (t, *J* = 9.0 Hz, 1H, H-3^A), 4.05–4.02 (m, 1H, H-6a^D), 4.01 (t, *J* = 9.6 Hz, 1H, H-4^B), 3.90–3.82 (m, 3H, H-3^C, H-6b^D, -OCH_2CH$_2$-), 3.79 (t, *J* = 9.0 Hz, 1H, H-4^A), 3.73 (t, *J* = 10.2 Hz, 1H, H-6b^A), 3.54 (dd, *J* = 10.8, 4.2 Hz, 1H, H-6a^C), 3.46 (td, *J* = 9.6, 4.8 Hz, 1H, H-5^A), 3.41–3.34 (m, 3H, H-4^C, H-5^D, -OCH_2CH$_2$-), 3.30 (dd, *J* = 10.8, 3.0 Hz, 1H, H-6a^B), 3.18–3.08 (m, 4H, H-5^C, H-5^B, H-6b^B, -CH_2N$_3$), 3.06–3.00 (m, 1H, -CH_2N$_3$), 2.55 (t, *J* = 10.8 Hz, 1H, H-6b^C), 1.94 (s, 3H, COCH_3), 1.93 (s, 3H, COCH_3), 1.87 (s, 3H, COCH_3), 1.67–1.62 (m, 2H, -OCH$_2$CH_2CH$_2$N$_3$), 1.59 (s, 3H, COCH_3); ^{13}C NMR (150 MHz, $CDCl_3$): δ 170.7, 170.3, 169.2, 169.1, 164.9, 164.8, 164.5, 163.9, 138.3, 137.0, 136.9, 133.5, 133.0, 132.9, 132.7, 130.0, 129.8, 129.7, 129.5, 129.5, 129.3, 129.2, 129.1, 129.1, 129.1, 128.6, 128.5, 128.4, 128.3, 128.2, 128.2, 128.1, 128.0, 127.9, 126.0, 125.9, 101.6 (Ph*C*H), 101.4 (C-1^A), 100.9 (Ph*C*H), 100.8 (C-1^C), 100.3 (2C, C-1^B, C-1^D), 79.7 (C-4^A), 78.9 (C-3^C), 78.5 (C-4^C), 78.4 (C-3^A), 75.6 (C-4^B), 74.2 (C-5^B), 73.3 (C-3^B), 73.3 (Ph*C*H$_2$), 73.2 (C-2^C), 72.9 (C-2^A), 72.8 (C-2^D), 72.1 (C-2^B), 71.4 (C-5^D), 70.7 (C-3^D), 68.7 (C-6^A), 68.1 (C-4^D), 67.6 (C-6^C), 66.8 (C-6^B), 66.3 (C-5^A), 66.3 (-O*C*H$_2$CH$_2$-), 66.1 (C-5^C), 61.8 (C-6^D), 47.6 (-*C*H$_2$N$_3$), 28.8 (-OCH$_2$*C*H$_2$CH$_2$N$_3$), 20.7 (CO*C*H$_3$), 20.54 (CO*C*H$_3$), 20.51 (CO*C*H$_3$), 19.9 (CO*C*H$_3$); ESI HRMS: calcd for ($C_{84}H_{85}N_3O_{29}$+Na^+) *m/z*, 1622.5161; found, 1622.5168.



12

3-Azidopropyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl-(1→3)-(methyl 2-*O*-benzoyl-β-D-glucopyranosyluronate)-(1→4)-2,3-di-*O*-benzoyl-6-*O*-benzyl-β-D-glucopyranosyl-(1→3)-(methyl 2-*O*-benzoyl-β-D-glucopyranosyluronate) (12). After a solution of **11** (230 mg, 0.14 mmol) in 80% AcOH (16 mL) was stirred at 80℃ for 2 h, it was then co-evaporated with toluene to dryness. To a solution of the above residue in DCM/H_2O (18 mL, 2:1, v/v) was added TEMPO (14 mg, 86 μmol) at 0℃, followed by the addition of BAIB (347 mg, 1.08mmol) after being stirred for 10 min. The resulting mixture was stirred with the temperature warming up to rt within 4 h, then diluted with ethyl acetate and quenched by saturated aq. $Na_2S_2O_3$. The organic layer was washed with brine twice, dried over anhydrous Na_2SO_4, filtered, and concentrated. The crude product was dissolved in MeOH/Et_2O (15 mL, 1:4, v/v) under an argon atmosphere, and then $TMSCHN_2$ (2 M in hexanes, 0.44 mL, 0.87 mmol) was added at rt. The reaction mixture was stirred for 30 min, then quenched with AcOH and concentrated. The residue was purified by flash column chromatography (petroleum ether/ethyl acetate, 1:1) to give tetrasaccharide **12** (134 mg, 63% over three steps) as white foamy solid. $[\alpha]_D^{25}$ +5.4 (*c* 0.35, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.88 (d, *J* = 9.0 Hz, 2H, Ar*H*), 7.73 (d, *J* = 9.0 Hz, 2H, Ar*H*), 7.65–7.60 (m, 1H, Ar*H*), 7.48–7.38 (m, 10H, Ar*H*), 7.37–7.33 (m, 1H, Ar*H*), 7.32–7.25 (m, 5H, Ar*H*), 7.18 (t, *J* = 9.0, 2H, Ar*H*), 7.01 (t, *J* = 9.0 Hz, 2H, Ar*H*), 5.50 (t, *J* = 9.6 Hz, 1H, H-3^B), 5.25 (dd, *J* = 9.6, 7.8 Hz, 1H, H-2^B), 5.13 (t, *J* = 7.8 Hz, 1H, H-2^A), 5.10 (t, *J* = 8.4 Hz, 1H, H-2^C), 5.00 (t, *J* = 9.0 Hz, 1H, H-3^D), 4.94 (t, *J* = 9.6 Hz, 1H, H-4^D), 4.87 (dd, *J* = 9.6, 8.4 Hz, 1H, H-2^D), 4.63 (d, *J* = 7.8 Hz, 1H, H-1^B), 4.51 (d, *J* = 8.4 Hz, 1H, H-1^C), 4.48 (d, *J* = 12.0 Hz, 1H, PhCH_2), 4.44 (d, *J* = 7.8 Hz, 1H, H-1^D), 4.40 (d, *J* = 7.8 Hz, 1H, H-1^A), 4.20–4.16 (m, 2H, -OH, PhCH_2), 4.15–4.10 (m, 2H, H-6^D), 4.06 (t, *J* = 9.6 Hz, 1H, H-4^B), 3.96 (td, *J* = 8.4, 1.8 Hz, 1H, H-4^A), 3.85–3.81 (m, 2H, H-5^A, -OCH_2CH$_2$-), 3.78 (s, 3H, COOCH_3), 3.76–3.72 (m, 2H, H-4^C, H-5^D), 3.69 (t, *J* = 9.0 Hz, 1H, H-3^A), 3.65 (d, *J* = 1.8 Hz, 1H, -OH), 3.59 (d, *J* = 9.6 Hz, 1H, H-5^C), 3.57–3.54 (m, 1H, H-5^B), 3.52 (t, *J* = 9.0 Hz, 1H, H-3^C), 3.50–3.43 (m, 2H, H-6^B), 3.40–3.35 (m, 1H, -OCH_2CH$_2$-), 3.35 (s, 3H, COOCH_3), 3.08–3.03 (m, 1H, -CH_2N$_3$), 3.01–2.96 (m, 1H, -CH_2N$_3$), 2.02 (s, 3H, COCH_3), 1.98 (s, 3H, COCH_3), 1.89 (s, 3H, COCH_3), 1.66–1.60 (m, 1H, -OCH$_2$CH_2CH$_2$N$_3$), 1.56–1.51 (m, 1H,

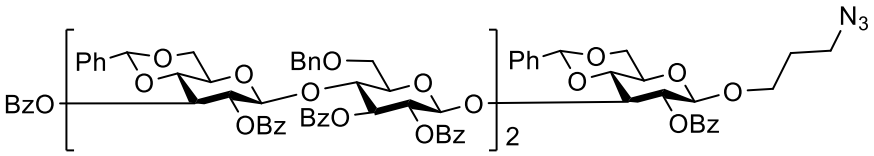
-OCH$_2$C*H*$_2$CH$_2$N$_3$), 1.50 (s, 3H, COC*H*$_3$); ^{13}C NMR (150 MHz, CDCl$_3$): δ 170.4, 170.1, 169.1, 169.0, 168.3, 167.0, 165.1, 164.8, 164.2, 164.0, 137.5, 133.8, 132.9, 132.8, 132.6, 129.6, 129.6, 129.5, 129.28, 129.26, 129.0, 128.80, 128.75, 128.7, 128.3, 128.2, 128.2, 128.1, 127.9, 101.5 (C-1^A), 101.2 (C-1^B), 101.0 (C-1^D), 100.6 (C-1^C), 84.8 (C-3^A), 84.5 (C-3^C), 75.4 (C-4^B), 75.3 (C-5^A), 75.0 (C-5^C), 74.1 (C-5^B), 73.5 (Ph*C*H$_2$), 72.7 (C-3^B), 72.4 (C-3^D), 71.9 (C-5^D), 71.7 (C-2^B), 71.7 (C-2^C), 71.5 (C-2^A), 70.6 (C-2^D), 70.2 (C-4^A), 69.9 (C-4^C), 68.2 (C-4^D), 67.5 (C-6^B), 66.3 (-O*C*H$_2$CH$_2$-), 61.9 (C-6^D), 52.7 (COO*C*H$_3$), 52.3 (COO*C*H$_3$), 47.6 (-*C*H$_2$N$_3$), 28.7 (-OCH$_2$*C*H$_2$CH$_2$N$_3$), 20.5 (2C, CO*C*H$_3$), 20.4 (CO*C*H$_3$), 19.7 (CO*C*H$_3$); ESI HRMS: calcd for (C$_{72}$H$_{77}$N$_3$O$_{31}$+Na$^+$) *m/z*, 1502.4433; found, 1502.4489.



1b

3-Aminopropyl β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyluronic acid-(1→4)-β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyluronic acid (1b). To a stirred solution of tetrasaccharide **12** (60 mg, 41 μmol) in THF (12 mL) was added 1 M LiOH at rt until the pH value reached 11. The reaction solution was stirred for 20 h and neutralized with 1 M HCl until the pH value reached 6. Then, after the reaction mixture was concentrated, the crude product was simply purified by Sephadex G-10 column chromatography with distilled H_2O as the eluent. The desired fractions were collected and concentrated. The resulting residue was dissolved in H_2O (8 mL), and 10% Pd/C (20 mg) was added at rt. The resulting mixture was stirred for 15 h under H_2 atmosphere, then filtered to remove the catalyst, and concentrated. The resulting residue was purified via size-exclusion chromatography on a Sephadex G-10 column with distilled H_2O as the eluent, which was followed by lyophilization of the desired fractions to give target product **1b** as white powder (23 mg, 75% over two steps). $[\alpha]_D^{25}$ -30.0 (*c* 0.1, H_2O); ^{1}H NMR (600 MHz, D_2O): δ 4.62–4.60 (m, 2H, H-1×2), 4.35 (d, *J* = 7.8 Hz, 1H, H-1), 4.33 (d, *J* = 7.8 Hz, 1H, H-1), 3.87–3.83 (m, 1H, -OC*H*$_2$CH$_2$-), 3.80 (dd, *J* = 12.0, 1.8 Hz, 1H, H-6a), 3.75–3.72 (m, 1H, H-6a), 3.68–3.31 (m, 19H, H-6b×2, -OC*H*$_2$CH$_2$-), 3.30–3.26 (m, 1H), 3.24–3.19 (m, 2H), 3.16 (dd, *J* = 9.6, 8.4 Hz, 1H), 3.00–2.95 (m, 2H, -C*H*$_2$NH$_2$), 1.85–1.80 (m, 2H, -OCH$_2$C*H*$_2$CH$_2$NH$_2$); ^{13}C NMR (150 MHz, D_2O): δ 173.5, 173.3, 102.4 (C-1), 102.2 (C-1), 102.0 (C-1), 101.8 (C-1), 83.1, 82.6, 78.8, 75.9, 75.7, 75.6, 75.4, 74.8, 74.0, 73.3, 73.0, 72.9, 72.7, 70.1 (2C), 69.4, 67.7 (-O*C*H$_2$CH$_2$-), 60.6 (C-6), 59.9 (C-6),

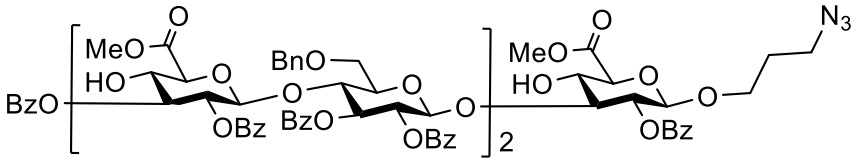
37.4 (-*C*H_2NH_2), 26.5 (-OCH$_2$*C*$H_2CH_2NH_2$); ESI HRMS: calcd for($C_{27}H_{45}NO_{23}$+H^+) *m/z*, 752.2455; found, 752.2474.



13

3-Azidopropyl 2,3-di-*O*-benzoyl-4,6-*O*-benzylidene-β-D-glucopyranosyl-(1→4)-2,3-di-*O*-benzoyl-6-*O*-benzyl-β-D-glucopyranosyl-(1→3)-2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-glucopyranosyl-(1→4)-2,3-di-*O*-benzoyl-6-*O*-benzyl-β-D-glucopyranosyl-(1→3)-2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-glucopyranoside (13). To a stirred solution of disaccharide **4** (400 mg, 0.38 mmol) and activated MS 4Å (1.6 g) in anhydrous DCM (6 mL) were successively added TTBP (236 mg, 0.95 mmol) and AgOTf (781 mg, 3.1 mmol) at rt under an argon atmosphere. The reaction mixture was cooled to -78°C with a stir for 20 min, and then *p*-TolSCl (58 μL, 0.40 mmol) was added dropwise by micro-syringe. After **4** was completely activated as monitored by TLC (toluene/ethyl acetate, 8:1), a solution of disaccharide **5** (321 mg, 0.34 mmol) in DCM (2 mL) was added dropwise to the above mixture. The resulting mixture was stirred with the temperature warming up to rt, and concomitantly monitored by MALDI-TOF MS to confirm the complete formation of tetrasaccharide product. Then, after the reaction was cooled to -78°C again, a solution of monosaccharide **8** (138 mg, 0.30 mmol) in DCM (1 mL) and *p*-TolSCl (49 μL, 0.34 mmol) was added subsequently. After that, the reaction mixture was naturally warmed to rt in 3 h with a stir, then neutralized with Et_3N, diluted with DCM, filtered, and concentrated. The residue was purified by flash column chromatography (petroleum ether/ethyl acetate/toluene, 3:1:1) to give pentasaccharide **13** (348 mg, 53%) as white foamy solid. $[\alpha]_D^{25}$ +18.0 (*c* 0.5, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.87–7.81 (m, 8H, Ar*H*), 7.69 (d, *J* = 7.8 Hz, 2H, Ar*H*), 7.61 (d, *J* = 7.8 Hz, 2H, Ar*H*), 7.55 (t, *J* = 7.2, 1H, Ar*H*), 7.52–7.26 (m, 31H, Ar*H*), 7.25–7.19 (m, 7H, Ar*H*), 7.18–7.14 (m, 4H, Ar*H*), 7.14–7.10 (m, 4H, Ar*H*), 7.08–6.99 (m, 6H, Ar*H*), 5.49 (t, *J* = 9.6 Hz, 1H, H-3^E), 5.32 (t, *J* = 9.6 Hz, 2H, H-3^B, H-3^D), 5.26 (s, 1H, PhC*H*), 5.23 (t, *J* = 7.8 Hz, 1H, H-2^E), 5.21 (t, *J* = 7.8 Hz, 1H, H-2^D), 5.18 (t, *J* = 9.0 Hz, 1H, H-2^A), 5.15 (t, *J* = 8.4 Hz, 1H, H-2^B), 5.12 (s, 1H, PhC*H*), 5.05 (t, *J* = 8.4 Hz, 1H, H-2^C), 4.87 (s, 1H, PhC*H*), 4.69 (d, *J* = 7.8 Hz, 1H, H-1^E), 4.65 (d, *J* = 7.8 Hz, 1H, H-1^D), 4.57 (d, *J* = 7.8 Hz, 1H, H-1^B), 4.47 (d, *J* = 7.8 Hz, 1H, H-1^A), 4.43 (d, *J* = 7.8 Hz, 1H, H-1^C), 4.29–4.24 (m, 3H, H-6b^A, PhCH_2, PhCH_2), 4.18 (d, *J* = 12.0 Hz, 1H,

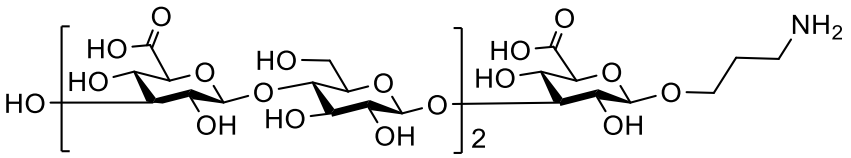
PhCH_2), 4.08 (d, J = 12.0 Hz, 1H, PhCH_2), 4.03 (t, J = 9.0 Hz, 1H, H-3^A), 3.96 (t, J = 9.0 Hz, 1H, H-4^D), 3.92 (t, J = 9.0 Hz, 1H, H-4^B), 3.83–3.78 (m, 2H, -OCH_2CH$_2$-, H-3^C), 3.76 (t, J = 9.6 Hz, 1H, H-4^A), 3.69 (t, J = 10.2 Hz, 1H, H-6b^A), 3.54–3.47 (m, 3H, H-6a^E, H-6a^C, H-4^E), 3.44 (td, J = 9.6, 4.8 Hz, 1H, H-5^A), 3.40–3.36 (m, 2H, H-4^C, -OCH_2CH$_2$-), 3.27–3.23 (m, 1H, H-6a^D), 3.22–3.15 (m, 2H, H-6a^B, H-5^E), 3.15–3.11 (m, 1H, H-6b^D), 3.12–3.00 (m, 6H, H-5^D, H-5^B, H-6b^B, H-5^C, -CH_2N$_3$), 2.59 (t, J = 10.2 Hz, 1H, H-6b^E), 2.52 (t, J = 10.2 Hz, 1H, H-6b^C), 1.67–1.60 (m, 2H, -OCH$_2$CH_2CH$_2$N$_3$); ^{13}C NMR (150 MHz, CDCl$_3$): δ 165.5, 165.0, 164.82, 164.79, 164.6, 164.5, 163.7, 138.2, 137.9, 136.9, 136.7, 136.6, 133.3, 133.0, 132.92, 132.86, 132.50, 132.46, 130.01, 129.96, 129.8, 129.68, 129.67, 129.62, 129.56, 129.52, 129.48, 129.3, 129.2, 129.14, 129.09, 129.06, 129.02, 128.95, 128.5, 128.42, 128.36, 128.3, 128.22, 128.20, 128.17, 128.15, 128.1, 128.02, 128.01, 127.99, 127.96, 127.8, 126.03, 125.94, 125.9, 101.5 (Ph*C*H), 101.4 (C-1^A), 101.3 (Ph*C*H), 101.10 (Ph*C*H), 101.07 (C-1^D), 100.8 (C-1^C), 100.2 (C-1^E), 99.9 (C-1^B), 79.6 (C-4^A), 79.3 (C-4^C), 78.33 (C-3^C), 78.29 (C-3^A), 78.2 (C-4^E), 76.1 (C-4^D), 75.7 (C-4^B), 74.2 (C-5^D), 74.0 (C-5^B), 73.5 (C-3^D), 73.4 (C-3^B), 73.14 (Ph*C*H$_2$), 73.11 (Ph*C*H$_2$), 73.0 (C-2^A), 72.9 (C-2^C), 72.4 (C-2^E), 72.14 (2C, C-2^D, C-2^B), 72.08 (C-3^E), 68.6 (C-6^A), 67.7 (C-6^C), 67.6 (C-6^E), 66.9 (C-6^D), 66.8 (C-6^B), 66.4 (C-5^A), 66.2 (-O*C*H$_2$CH$_2$-), 66.0 (C-5^E), 65.9 (C-5^C), 47.7 (-*C*H$_2$N$_3$), 28.8 (-OCH$_2$*C*H$_2$CH$_2$N$_3$); ESI HRMS: calcd for (C$_{124}$H$_{113}$N$_3$O$_{34}$+Na$^+$) *m/z*, 2210.7098; found, 2210.7082.



14

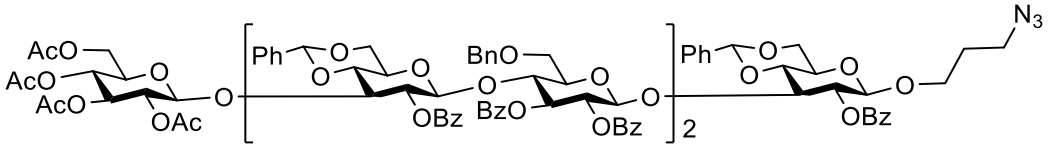
3-Azidopropyl (methyl 2,3-di-*O*-benzoyl-β-D-glucopyranosyluronate)-(1→4)-2,3-di-*O*-benzoyl-6-*O*-benzyl-β-D-glucopyranosyl-(1→3)-(methyl 2-*O*-benzoyl-β-D-glucopyranosyluronate)-(1→4)-2,3-di-*O*-benzoyl-6-*O*-benzyl-β-D-glucopyranosyl-(1→3)-(methyl 2-*O*-benzoyl-β-D-glucopyranosyluronate) (14). A solution of **13** (166 mg, 76 μmol) in 80% AcOH (10 mL) was stirred at 80°C for 4 h, and it was co-evaporated with toluene to dryness. To a solution of the product generated above in DCM/H_2O (9 mL, 2:1, v/v) was added TEMPO (9.5 mg, 61 μmol) at 0°C. After the mixture was stirred for 10 min, BAIB (244 mg, 0.76 mmol) was added to the mixture. The resulting reaction mixture was stirred at rt for 7 h, then diluted with ethyl acetate and quenched by saturated aq. $Na_2S_2O_3$. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated. The above crude product was then

dissolved in MeOH/Et_2O (10 mL, 1:4, v/v) under an argon atmosphere, and $TMSCHN_2$ (2 M in hexanes, 0.34 mL, 0.68 mmol) was added at rt. After being stirred for 40 min, the reaction mixture was quenched with AcOH and concentrated. The resultant residue was purified by flash column chromatography (petroleum ether/ethyl acetate, 1:1) to give pentasaccharide **14** (91 mg, 60% over three steps) as white foamy solid. $[\alpha]_D^{25}$ +14.2 (*c* 0.6, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.88 (d, *J* = 7.8 Hz, 2H, Ar*H*), 7.84 (d, *J* = 7.8 Hz, 2H, Ar*H*), 7.75 (d, *J* = 7.8 Hz, 2H, Ar*H*), 7.71 (d, *J* = 8.4 Hz, 2H, Ar*H*), 7.53–7.29 (m, 27H, Ar*H*), 7.29–7.25 (m, *J* = 6.4 Hz, 5H, Ar*H*), 7.21 (d, *J* = 7.8 Hz, 2H, Ar*H*), 7.17 (t, *J* = 7.8 Hz, 4H, Ar*H*), 7.05 (t, *J* = 7.8 Hz, 2H, Ar*H*), 6.99 (t, *J* = 7.8 Hz, 2H, Ar*H*), 5.49 (t, *J* = 9.0 Hz, 1H, H-3^D), 5.41 (t, *J* = 9.0 Hz, 1H, H-3^B), 5.30–5.24 (m, 2H, H-3^E, H-2^E), 5.22 (t, *J* = 9.6 Hz, 1H, H-2^D), 5.19 (t, *J* = 9.6 Hz, 1H, H-2^B), 5.09 (t, *J* = 9.0 Hz, 1H, H-2^C), 5.02 (t, *J* = 9.0 Hz, 1H, H-2^A), 4.63 (d, *J* = 7.8 Hz, 1H, H-1^E), 4.56 (d, *J* = 7.8 Hz, 1H, H-1^D), 4.53 (d, *J* = 7.8 Hz, 1H, H-1^B), 4.40–4.35 (m, 3H, H-1^A, H-1^C, PhCH_2), 4.34 (d, *J* = 12.0 Hz, 1H, PhCH_2), 4.16–4.12 (m, 2H, -OH, PhCH_2), 4.10 (t, *J* = 9.6 Hz, 1H, H-4^D), 4.07–4.03 (m, 2H, -OH, PhCH_2), 3.97 (t, *J* = 9.6 Hz, 1H, H-4^B), 3.92 (t, *J* = 9.0 Hz, 1H, H-4^C), 3.87 (t, *J* = 9.6 Hz, 1H, H-4^E), 3.83–3.79 (m, 2H, H-5^C, -OCH_2CH$_2$-), 3.76 (s, 4H, H-4^A, COOCH_3), 3.64 (t, *J* = 9.6 Hz, 1H, H-3^C), 3.62–3.57 (m, 2H, H-5^A, H-5^D), 3.55–3.48 (m, 3H, H-5^E, H-6^D), 3.45–3.40 (m, 2H, H-3^A, H-5^B), 3.38 (s, 3H, COOCH_3), 3.36–3.33 (m, 3H, -OCH_2CH$_2$-, H-6^B), 3.29 (s, 3H, COOCH_3), 3.07–3.01 (m, 2H, -OH, -CH_2N$_3$), 3.00–2.94 (m, 1H, -CH_2N$_3$), 1.60–1.48 (m, 2H, -OCH$_2$CH_2CH$_2$N$_3$); ^{13}C NMR (150 MHz, $CDCl_3$): δ 168.3, 168.1, 167.1, 166.4, 165.2, 165.0, 164.82, 164.75, 164.64, 164.2, 163.6, 137.3, 137.0, 133.6, 133.4, 133.1, 132.90, 132.85, 132.7, 132.53, 132.48, 129.8, 129.7, 129.6, 129.52, 129.50, 129.47, 129.4, 129.32, 129.29, 129.26, 128.80, 128.75, 128.7, 128.63, 128.56, 128.5, 128.4, 128.3, 128.20, 128.18, 128.10, 128.0, 127.9, 127.8, 101.4 (C-1^A), 101.1 (C-1^D), 100.9 (C-1^B), 100.8 (C-1^C), 100.7 (C-1^E), 84.7 (C-3^C), 84.3 (C-3^A), 75.6 (C-4^D), 75.4 (C-4^B), 75.3 (C-5^C), 75.2 (C-5^E), 74.7 (C-3^E), 74.2 (C-5^A), 74.1 (2C, C-5^D, C-5^B), 73.5 (Ph*C*H$_2$), 73.4 (Ph*C*H$_2$), 73.0 (C-3^D), 72.7 (C-3^B), 71.7 (C-2^D), 71.6 (C-2^B), 71.5 (C-2^C), 71.4 (C-2^A), 71.2 (C-2^E), 70.18 (C-4^C), 70.15 (C-4^E), 69.9 (C-4^A), 67.5 (C-6^D), 67.3 (C-6^B), 66.3 (-O*C*H$_2$CH$_2$-), 52.6 (COO*C*H$_3$), 52.5 (COO*C*H$_3$), 52.1 (COO*C*H$_3$), 47.6 (-*C*H$_2$N$_3$), 28.7 (-OCH$_2$*C*H$_2$CH$_2$N$_3$); ESI HRMS: calcd for ($C_{106}H_{101}N_3O_{37}$+Na$^+$) *m/z*, 2030.6006; found, 2030.6084.



2b

3-Aminopropyl β-D-glucopyranosyluronic acid-(1→4)-β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyluronic acid-(1→4)-β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyluronic acid (2b). To a stirred solution of pentasaccharide **14** (66 mg, 33 μmol) in THF (7 mL) was added 1 M LiOH at rt until the pH value reached 11. The reaction mixture was stirred for 48 h and neutralized with 1 M HCl until the pH value reached 6. Then, the mixture was concentrated and the resultant product was purified by Sephadex G-10 column chromatography (H_2O eluent) to give a deacylated product. The residue was then dissolved in H_2O (4 mL), and 10% Pd/C (10 mg) was added at rt. The resulting reaction mixture was stirred for 20 h under H_2 atmosphere, filtered to remove the catalyst, and concentrated. Purification of the residue via size-exclusion chromatography on a Sephadex G-10 column with distilled H_2O as the eluent, afforded target product **2b** (22 mg, 75% over two steps) as white powder after lyophilization. $[\alpha]_D^{25}$ -23.3 (*c* 0.1, H_2O); ^{1}H NMR (600 MHz, D_2O): δ 4.71–4.60 (m, 2H, H-1×2), 4.38–4.33 (m, 3H, H-1×3), 3.89–3.84 (m, 1H, -OC*H*$_2$CH$_2$-), 3.82 (d, *J* = 12.0, 2H, H-6a×2), 3.70–3.66 (m, 1H, -OC*H*$_2$CH$_2$-), 3.66–3.58 (m, 7H, H-6b×2), 3.57–3.33 (m, 14H), 3.24–3.17 (m, 3H), 3.02-2.97 (m, 2H, -C*H*$_2$NH$_2$), 1.87–1.81 (m , 2H, -OCH$_2$C*H*$_2$CH$_2$NH$_2$); ^{13}C NMR (150 MHz, D_2O): δ 175.4, 175.3, 175.2, 102.3 (C-1), 102.21 (C-1), 102.19 (C-1), 102.1 (C-1), 101.8 (C-1), 83.1, 82.6, 78.9 (2C), 75.7 (2C), 75.2, 74.8, 74.7, 74.1 (2C), 73.1 (2C), 73.0, 72.8, 72.7, 71.6, 70.08 (2C), 70.05, 67.8 (-O*C*H$_2$CH$_2$-), 59.9 (2C, C-6×2), 37.4 (-*C*H$_2$NH$_2$), 26.6 (-OCH$_2$*C*H$_2$CH$_2$NH$_2$); ESI HRMS: calcd for ($C_{33}H_{53}NO_{29}$+H^+) *m/z*, 928.2772; found, 928.2800.

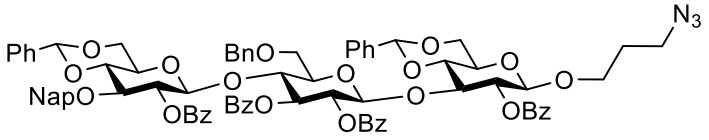


15

3-Azidopropyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl-(1→3)-2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-glucopyranosyl-(1→4)-2,3-di-*O*-benzoyl-6-*O*-benzyl-β-D-glucopyranosyl-(1→3)-2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-glucopyranosyl-(1→4)-2,3-di-*O*-benzoyl-6-*O*-benzyl-β-D-glucopyranosyl-(1→3)-2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-glucopyranoside

(15). ***Method A:*** To a stirred solution of trisaccharide **7** (62 mg, 49 μmol) and activated MS 4Å (240 mg) in anhydrous DCM (3 mL) were successively added TTBP (30 mg, 12 mmol) and AgOTf (100 mg, 0.39 mmol) at rt under an argon atmosphere. The reaction mixture was then cooled to -78°C with a stir for 30 min, and *p*-TolSCl (7 μL, 51 μmol) was added dropwise by micro-syringe. After the reaction mixture was stirred for 30 min at which time TLC (petroleum ether/ethyl acetate, 3:2) indicated that donor **7** was completely activated, a solution of acceptor **5** (42 mg, 44 μmol) in DCM (0.3 mL) was added. The resultant mixture was stirred with temperature warming up to rt and monitored by MALDI-TOF MS to confirm the complete formation of pentasaccharide intermediates. After that, the reaction was cooled to -78°C again, and a solution of monosaccharide **8** (18 mg, 39 μmol) in DCM (0.1 mL) was then added, followed by the dropwise addition of *p*-TolSCl (6 μL, 44 μmol). The resulting reaction was stirred for another 3 h with the temperature slowly warming up to rt, neutralized with Et_3N, diluted with DCM, and filtered. The filtrate was concentrated, and the resulting residue was purified by flash column chromatography (petroleum ether/ethyl acetate/toluene, 2:1:1) to give hexasaccharide **15** (17 mg, 18%). ***Method B:*** After a reaction mixture of activated MS 4Å (560 mg), trisaccharide **7** (140 mg, 0.11 mmol), and **16** (140 mg, 0.11 mmol) in DCM (5 mL) were stirred at rt for 20 min under an argon atmosphere, it was cooled down to -20°C, and then NIS (27 mg, 0.12 mmol) and AgOTf (3 mg, 11 μmol) was added subsequently. The resulting reaction was stirred at this condition for another 30 min, then neutralized with Et_3N, filtered, and concentrated. The residue was purified by flash column chromatography (petroleum ether/ethyl acetate, 3:2) to give hexasaccharide **15** (186 mg, 70%) as white foamy solid. $[\alpha]_D^{25}$ +24.5 (*c* 0.6, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.92–7.90 (m, 2H, Ar*H*), 7.86–7.82 (m, 4H, Ar*H*), 7.70–7.68 (m, 2H, Ar*H*), 7.59–7.57 (m, 2H, Ar*H*), 7.51–7.40 (m, 12H, Ar*H*), 7.38–7.30 (m, 15H, Ar*H*), 7.24–7.19 (m, 6H, Ar*H*), 7.18–7.10 (m, 10H, Ar*H*), 7.04 (m, 7H, Ar*H*), 5.32 (t, *J* = 9.6 Hz, 1H, H-3^D), 5.29–5.25 (m, 2H, H-3^B, PhC*H*), 5.22 (t, *J* = 8.4 Hz, 1H, H-2^D), 5.18 (t, *J* = 9.0 Hz, 1H, H-2^A), 5.17 (s, 1H, PhC*H*), 5.13 (t, *J* = 9.0 Hz, 1H, H-2^B), 5.06–5.01 (m, 2H, H-2^C, H-2^E), 4.96–4.92 (m, 1H, H-4^F), 4.88 (s, 1H, PhC*H*), 4.85–4.82 (m, 2H, H-2^F, H-3^F), 4.69 (d, *J* = 8.4 Hz, 1H, H-1^D), 4.55 (d, *J* = 8.4 Hz, 1H, H-1^B), 4.49 (d, *J* = 7.8 Hz, 1H, H-1^E), 4.47 (d, *J* = 7.8 Hz, 1H, H-1^A), 4.44 (d, *J* = 7.8 Hz, 1H, H-1^F), 4.42 (d, *J* = 7.8 Hz, 1H, H-1^C), 4.30 (d, *J* = 12.0 Hz, 1H, PhCH_2), 4.28–4.24 (m, 2H, H-6a^A, PhCH_2), 4.11 (d, *J* = 12.0 Hz, 1H, PhCH_2), 4.07 (d, *J* = 12.0 Hz, 1H, PhCH_2), 4.05–4.00 (m, 2H, H-3^A, H-6a^F), 3.95-3.90 (m, 2H, H-4^B, H-4^D), 3.88–3.79 (m, 3H, H-4^A, H-6b^F, -OCH_2CH$_2$-), 3.80–3.74 (m, 2H, H-3^C, H-3^E), 3.69 (t,

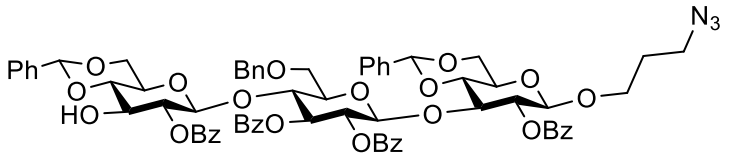
J = 9.0 Hz, 1H, H-6b^{A}), 3.54–3.47 (m, 2H, H-6a^{C}, H-6a^{E}), 3.46–3.41 (m, 1H, H-5^{A}), 3.38–3.33 (m, 4H, H-4^{C}, H-4^{E}, H-5^{F}, -OCH_2CH$_2$-), 3.25–3.16 (m, 2H, H-6a^{B}, H-6a^{D}), 3.11–3.01 (m, 8H, H-5^{B}, H-5^{C}, H-5^{D}, H-5^{E}, H-6b^{B}, H-6b^{D}, -CH_2N$_3$), 2.55–2.48 (m, 2H, H-6b^{C}, H-6b^{E}), 1.93 (s, 3H, COCH_3), 1.92 (s, 3H, COCH_3), 1.86 (s, 3H, COCH_3), 1.66–1.59 (m, 2H, -OCH$_2$CH_2CH$_2$N$_3$), 1.57 (s, 3H, COCH_3); ^{13}C NMR (150 MHz, CDCl$_3$): δ 170.7, 170.3, 169.2, 169.1, 165.0, 164.8, 164.7, 164.5, 163.9, 163.8, 138.18, 138.16, 136.9, 136.84, 136.76, 133.5, 133.0, 132.94, 132.88, 132.5, 132.5, 128.99, 129.97, 129.73, 129.67, 129.62, 129.56, 129.52, 129.48, 129.3, 129.2, 129.13, 129.10, 129.06, 129.03, 128.97, 128.6, 128.41, 128.37, 128.35, 128.3, 128.18, 128.16, 128.14, 128.08, 128.02, 127.97, 127.83, 127.78, 125.94, 125.92, 101.5 (Ph*C*H), 101.4 (C-1^{A}), 101.3 (Ph*C*H), 100.9 (Ph*C*H), 100.8 (2C, C-1^{C}, C-1^{E}), 100.3 (C-1^{F}), 100.2 (C-1^{D}), 100.0 (C-1^{B}), 79.6 (C-3^{E}), 79.2 (C-4^{C}), 78.9 (C-4^{A}), 78.5 (C-4^{E}), 78.4 (C-3^{C}), 78.3 (C-3^{A}), 75.7 (C-4^{D}), 75.6 (C-4^{B}), 74.1 (C-5^{D}), 74.0 (C-5^{B}), 73.37 (C-3^{D}), 73.35 (C-3^{B}), 73.3 (C-2^{E}), 73.14 (Ph*C*H$_2$), 73.11 (Ph*C*H$_2$), 73.0 (C-2^{A}), 72.9 (C-2^{C}), 72.8 (C-2^{F}), 72.1 (C-2^{D}), 72.0 (C-2^{B}), 71.4 (C-5^{F}), 70.8 (C-3^{F}), 68.6 (C-6^{A}), 68.1 (C-4^{F}), 67.7 (C-6^{E}), 67.6 (C-6^{C}), 66.84 (C-6^{D}), 66.77 (C-6^{B}), 66.4 (C-5^{A}), 66.2 (-O*C*H$_2$CH$_2$-), 66.1 (C-5^{E}), 65.9 (C-5^{C}), 61.8 (C-6^{F}), 47.7 (-*C*H$_2$N$_3$), 28.8 (-OCH$_2$*C*H$_2$CH$_2$N$_3$), 20.7 (CO*C*H$_3$), 20.52 (CO*C*H$_3$), 20.49 (CO*C*H$_3$), 19.9 (CO*C*H$_3$); ESI HRMS: calcd for (C$_{131}$H$_{127}$N$_3$O$_{42}$+Na$^+$) *m/z*, 2436.7786; found, 2436.7765.



19

3-Azidopropyl 2-*O*-benzoyl-3-*O*-(2-Naphthylmethyl)-4,6-*O*-benzylidene-β-D-glucopyranosyl-(1→4)-2,3-di-*O*-benzoyl-6-*O*-benzyl-β-D-glucopyranosyl-(1→3)-2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-glucopyranoside (19). To a stirred solution of monosaccharide **17** (445 mg, 0.72 mmol) and activated MS 4Å (1.8 g) in anhydrous DCM (10 mL) were added TTBP (447 mg, 1.8 mmol) and AgOTf (1480 mg, 5.8 mmol) at rt under an argon atmosphere. The reaction mixture was then cooled to -78°C with a stir, and *p*-TolSCl (109 μL, 0.76 mmol) was added dropwise by micro-syringe. After the resulting mixture was stirred for 30 min at which time TLC (toluene/ethyl acetate, 10:1) indicated that **17** was completely activated, a solution of **18** (379 mg, 0.65 mmol) in DCM (2 mL) was added dropwise. The reaction was stirred with the temperature slowly warming to rt and concomitantly monitored by MALDI-TOF MS to confirm

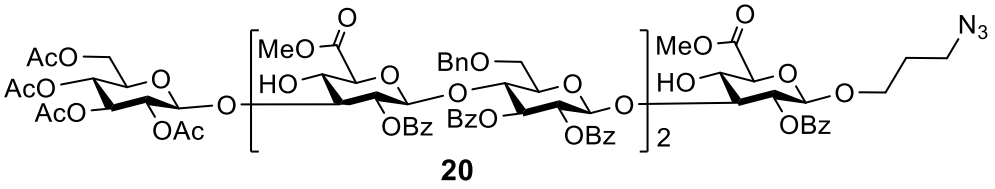
the complete formation of disaccharide intermediates. After the reaction mixture was cooled to -78°C again, and a solution of monosaccharide **8** (262 mg, 0.58 mmol) in DCM (2 mL) was added, together with the subsequent addition of *p*-TolSCl (93 μL, 0.65 mmol). After that, the reaction mixture was stirred and naturally warmed to rt within 3 h, neutralized with Et_3N, filtered, and concentrated. The residue was purified by flash column chromatography (petroleum ether/ethyl acetate/toluene, 4:1:1) to give trisaccharide **19** (547 mg, 67%) as white foamy solid. $[\alpha]_D^{25}$ +21.5 (*c* 0.4, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.86 (d, *J* = 8.4 Hz, 2H, Ar*H*), 7.81 (d, *J* = 8.4 Hz, 2H, Ar*H*), 7.71 (d, *J* = 8.4 Hz, 2H, Ar*H*), 7.67 (d, *J* = 7.8 Hz, 1H, Ar*H*), 7.62 (t, *J* = 7.8 Hz, 1H, Ar*H*), 7.50–7.31 (m, 22H, Ar*H*), 7.26–7.22 (m, 4H, Ar*H*), 7.17–7.08 (m, 6H, Ar*H*), 7.07-7.04 (m, 2H, Ar*H*), 5.38 (t, *J* = 9.6 Hz, 1H, H-3^B), 5.27 (s, 1H, PhC*H*), 5.26–5.23 (m, 2H, H-2^B, PhC*H*), 5.21 (t, *J* = 7.8 Hz, 1H, H-2^A), 5.08 (t, *J* = 8.4 Hz, 1H, H-2^C), 4.85 (d, *J* = 12.0 Hz, 1H, PhCH_2), 4.76–4.69 (m, 2H, H-1^B, PhCH_2), 4.49 (d, *J* = 7.8 Hz, 1H, H-1^A), 4.47 (d, *J* = 7.8 Hz, 1H, H-1^C), 4.32–4.26 (m, 2H, PhCH_2, H-6a^A), 4.11 (d, *J* = 12.0 Hz, 1H, PhCH_2), 4.06 (t, *J* = 9.0 Hz, 1H, H-3^A), 3.98 (t, *J* = 9.6 Hz, 1H, H-4^B), 3.85–3.81 (m, 1H, -OCH_2CH$_2$-), 3.78 (t, *J* = 9.0 Hz, 1H, H-4^A), 3.71 (t, *J* = 10.2 Hz, 1H, H-6b^A), 3.61 (t, *J* = 9.6 Hz, 1H, H-3^C), 3.53 (dd, *J* = 10.8, 4.8 Hz, 1H, H-6a^C), 3.49 (t, *J* = 9.6 Hz, 1H, H-4^C), 3.47–3.43 (m, 1H, H-5^A), 3.41–3.37 (m, 1H, -OCH_2CH$_2$-), 3.26 (dd, *J* = 11.4, 4.2 Hz, 1H, H-6a^B), 3.16–3.12 (m, 2H, H-5^B, H-6b^B), 3.12–3.09 (m, 1H, -CH_2N$_3$), 3.07–3.01 (m, 2H, -CH_2N$_3$, H-5^C), 2.58 (t, *J* = 10.2 Hz, 1H, H-6b^C), 1.69–1.62 (m, 2H, -OCH$_2$CH_2CH$_2$N$_3$); ^{13}C NMR (150 MHz, $CDCl_3$): δ 165.0, 164.8, 164.6, 164.5, 138.1, 137.1, 136.8, 135.2, 133.2, 133.00, 132.97, 132.95, 132.8, 132.5, 130.1, 129.9, 129.62, 129.55, 129.52, 129.48, 129.2, 129.1, 129.0, 128.4, 128.3, 128.2, 128.2, 128.10, 128.05, 128.02, 127.98, 127.9, 127.82, 127.79, 127.6, 126.9, 126.1, 126.00, 125.97, 125.9, 125.7, 101.6 (Ph*C*H), 101.4 (C-1^A), 101.1 (C-1^C), 101.0 (Ph*C*H), 100.2 (C-1^B), 81.4 (C-4^C), 79.7 (C-4^A), 78.4 (C-3^A), 77.4 (C-3^C), 75.7 (C-4^B), 74.2 (C-5^B), 73.7 (Ph*C*H$_2$), 73.6 (C-3^B), 73.3 (C-2^C), 73.2 (Ph*C*H$_2$), 72.9 (C-2^A), 72.1 (C-2^B), 68.7 (C-6^A), 67.7 (C-6^C), 66.9 (C-6^B), 66.4 (C-5^A), 66.3 (-O*C*H$_2$CH$_2$-), 65.7 (C-5^C), 47.7 (-*C*H$_2$N$_3$), 28.8 (-OCH$_2$*C*H$_2$CH$_2$N$_3$); ESI HRMS: calcd for ($C_{81}H_{75}N_3O_{20}$+Na$^+$) *m/z*, 1432.4836; found, 1432.4891.



16

3-Azidopropyl 2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-glucopyranosyl-(1→4)-2,3-di-*O*-benzoyl-6-*O*-benzyl-β-D-glucopyranosyl-(1→3)-2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-glucopyranoside (16). To a stirred solution of **19** (430 mg, 0.31 mmol) in DCM/H_2O (33 mL, 10:1, v/v) was added *β*-pinene (166 μL, 1.05 mmol) and DDQ (141 mg, 0.62 mmol) at 0℃. After the mixture was stirred at rt for 3 h, it was diluted with DCM and successively washed by saturated aq. $NaHCO_3$ and brine. The organic layer was dried over anhydrous Na_2SO_4, filtered, and concentrated. The residue was purified by flash column chromatography (petroleum ether/ethyl acetate, 5:2) to give **16** (314 mg, 80%) as white foamy solid. $[\alpha]_D^{25}$ +22.2 (*c* 1.0, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): *δ* 7.99 (d, *J* = 6.6 Hz, 2H, Ar*H*), 7.88 (d, *J* = 7.2 Hz, 2H, Ar*H*), 7.73 (d, *J* = 6.6 Hz, 2H, Ar*H*), 7.65 (t, *J* = 7.8 Hz, 1H, Ar*H*), 7.47–7.44 (m, 6H, Ar*H*), 7.42–7.31 (m, 12H, Ar*H*), 7.31–7.26 (m, 6H, Ar*H*), 7.15–7.12 (m, 2H, Ar*H*), 7.09–7.07 (m, 2H, Ar*H*), 5.40 (t, *J* = 9.6 Hz, 1H, H-3^B), 5.30–5.26 (m, 2H, PhC*H*, H-2^B), 5.23 (t, *J* = 8.4 Hz, 1H, H-2^A), 5.17 (s, 1H, PhC*H*), 5.00 (dd, *J* = 9.0, 7.8 Hz, 1H, H-2^C), 4.76 (d, *J* = 7.8 Hz, 1H, H-1^B), 4.62 (d, *J* = 7.8 Hz, 1H, H-1^C), 4.51 (d, *J* = 7.8 Hz, 1H, H-1^A), 4.40 (d, *J* = 12.0 Hz, 1H, PhCH_2), 4.30 (dd, *J* = 10.2, 4.8 Hz, 1H, H-6a^A), 4.26 (d, *J* = 12.0 Hz, 1H, PhCH_2), 4.08 (t, *J* = 9.0 Hz, 1H, H-3^A), 4.04 (t, *J* = 9.6 Hz, 1H, H-4^B), 3.86–3.83 (m, 1H, -OCH_2CH$_2$-), 3.81 (t, *J* = 9.0 Hz, 1H, H-4^A), 3.79–3.74 (m, 1H, H-3^C), 3.72 (t, *J* = 10.8 Hz, 1H, H-6b^A), 3.52 (dd, *J* = 10.8, 5.4 Hz, 1H, H-6a^C), 3.47 (td, *J* = 9.6, 4.8 Hz, 1H, H-5^A), 3.43–3.38 (m, 2H, H-6a^B, -OCH_2CH$_2$-), 3.29–3.24 (m, 2H, H-4^C, H-6b^B), 3.20–3.16 (m, 1H, H-5^B), 3.14–3.11 (m, 1H, -CH_2N$_3$), 3.08–3.03 (m, 2H, H-5^C, -CH_2N$_3$), 2.55 (t, *J* = 10.2 Hz, 1H, H-6b^C), 2.46 (d, *J* = 3.6 Hz, 1H, -OH), 1.69–1.63 (m, 1H, -OCH$_2$CH_2CH$_2$N$_3$), 1.61–1.55 (m, 1H, -OCH$_2$CH_2CH$_2$N$_3$); ^{13}C NMR (150 MHz, $CDCl_3$): *δ* 165.3, 165.0, 164.8, 164.6, 138.2, 136.8, 136.7, 133.4, 133.0, 132.6, 130.0, 129.9, 129.6, 129.52, 129.49, 129.4, 129.3, 129.2, 129.10, 129.06, 128.49, 128.47, 128.4, 128.3, 128.2, 128.0, 127.9, 126.2, 126.00, 101.7 (Ph*C*H), 101.6 (Ph*C*H), 101.4 (C-1^A), 100.9 (C-1^C), 100.2 (C-1^B), 80.4 (C-4^C), 79.7 (C-4^A), 78.4 (C-3^A), 75.9 (C-4^B), 74.6 (C-2^C), 74.3 (C-5^B), 73.5 (C-3^B), 73.3 (Ph*C*H$_2$), 73.0 (C-2^A), 72.5 (C-3^C), 72.1 (C-2^B), 68.7 (C-6^A), 67.6 (C-6^C), 67.0 (C-6^B), 66.4 (C-5^A), 66.3 (-O*C*H$_2$CH$_2$-), 65.7 (C-5^C), 47.7 (-*C*H$_2$N$_3$),

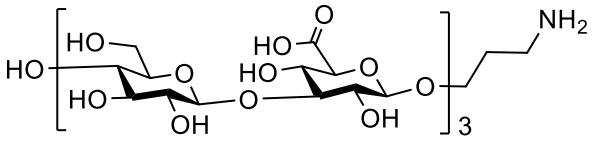
28.8 (-$OCH_2CH_2CH_2N_3$); ESI HRMS: calcd for ($C_{70}H_{67}N_3O_{20}$+Na^+) *m/z*, 1292.4210; found, 1292.4292.



20

3-Azidopropyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl-(1→3)-(methyl 2-*O*-benzoyl-β-D-glucopyranosyluronate)-(1→4)-2,3-di-*O*-benzoyl-6-*O*-benzyl-β-D-glucopyranosyl-(1→3)-(methyl 2-*O*-benzoyl-β-D-glucopyranosyluronate)-(1→4)-2,3-di-*O*-benzoyl-6-*O*-benzyl-β-D-glucopyranosyl-(1→3)-(methyl 2-*O*-benzoyl-β-D-glucopyranosyluronate) (20). A solution of **15** (120 mg, 50 μmol) in 80% AcOH (10 mL) was stirred at 80℃ for 5 h and then co-evaporated with toluene to dryness. The hexasaccharide generated above was then dissolved in DCM/H_2O (9 mL, 2:1, v/v), and TEMPO (8 mg, 50 μmol) was added at 0℃. After the mixture was stirred at this condition for 15 min, BAIB (192 mg, 0.60 mmol) was added to the reaction solution. The reaction mixture was stirred at rt for 5 h, then diluted with ethyl acetate and quenched by saturated aq. $Na_2S_2O_3$. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated. After the crude product was dissolved in MeOH/Et_2O (5 mL, 1:4, v/v) under an argon atmosphere, $TMSCHN_2$ (2 M in hexanes, 0.23 mL, 0.45 mmol) was added dropwise at rt. The resulting mixture was stirred for 45 min, then quenched with AcOH and concentrated. The residue was purified by flash column chromatography (petroleum ether/ethyl acetate, 2:3) to give hexasaccharide **20** (58 mg, 53% over three steps) as white foamy solid. $[\alpha]_D^{25}$ +7.7 (*c* 0.35, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.89 (d, *J* = 7.8 Hz, 2H, Ar*H*), 7.73–7.68 (m, 4H, Ar*H*), 7.61 (t, *J* = 7.8 Hz, 1H, Ar*H*), 7.47–7.37 (m, 15H, Ar*H*), 7.35–7.31 (m, 6H, Ar*H*), 7.29-7.26 (m, 2H, Ar*H*), 7.26–7.23 (m, 5H, Ar*H*), 7.22–7.15 (m, 6H, Ar*H*), 7.04 (t, *J* = 9.0 Hz, 2H, Ar*H*), 7.01–6.97 (t, *J* = 9.0 Hz, 2H, Ar*H*), 5.46 (t, *J* = 9.0 Hz, 1H, H-3^B), 5.41 (t, *J* = 9.0 Hz, 1H, H-3^D), 5.20 (t, *J* = 9.6, 1H, H-2^D), 5.16 (t, *J* = 9.6, 1H, H-2^B), 5.12–5.07 (m, 2H, H-2^A, H-2^E), 5.00 (t, *J* = 9.0 Hz, 2H, H-2^C, H-3^F), 4.94 (t, *J* = 9.6 Hz, 1H, H-4^F), 4.88 (t, *J* = 8.4 Hz, 1H, H-2^F), 4.56 (d, *J* = 7.8 Hz, 1H, H-1^D), 4.50 (d, *J* = 7.8 Hz, 1H, H-1^B), 4.48 (d, *J* = 7.8 Hz, 1H, H-1^E), 4.45 (d, *J* = 8.4 Hz, 1H, H-1^F), 4.39 (d, *J* = 12.0 Hz, 1H, PhCH_2), 4.37 (d, *J* = 7.8 Hz, 1H, H-1^A), 4.36 (d, *J* = 7.2 Hz, 1H, H-1^C), 4.35 (d, *J* = 12.0 Hz, 1H, PhCH_2), 4.17 (d, *J* = 1.8 Hz, 1H, -OH), 4.14–4.10 (m, 3H, H-6^F, PhCH_2), 4.06 (d, *J* = 12.0 Hz, 1H, PhCH_2), 4.03 (t, *J* = 9.0 Hz, 1H, H-4^E), 4.00 (t, *J* = 9.0 Hz, 1H,

H-4^{B}), 3.97 (t, J = 9.6 Hz, 1H, H-4^{D}), 3.92 (t, J = 8.4 Hz, 1H, H-4^{A}), 3.84–3.80 (m, 1H, -OCH_2CH$_2$-), 3.82 (d, J = 10.2 Hz, 1H, H-5^{A}), 3.77 (s, 3H, COOCH_3), 3.75–3.71 (m, 3H, -OH, H-4^{C}, H-5^{F}), 3.67–3.63 (m, 2H, -OH, H-3^{A}), 3.57 (d, J = 9.6 Hz, 1H, H-5^{E}), 3.55–3.50 (m, 3H, H-5^{C}, H-3^{E}, H-5^{B}), 3.45–3.39 (m, 4H, H-3^{C}, H-5^{D}, H-6^{B}), 3.39–3.34 (m, 2H, H-6a^{D}, -OCH_2CH$_2$-), 3.34 (s, 3H, COOCH_3), 3.32–3.30 (m, 1H, H-6b^{D}), 3.28 (s, 3H, COOCH_3), 3.08–3.03 (m, 1H, -CH_2N$_3$), 3.01–2.95 (m, 1H, -CH_2N$_3$), 2.02 (s, 3H, COCH_3), 1.98 (s, 3H, COCH_3), 1.90 (s, 3H, COCH_3), 1.55 (m, 2H, -OCH$_2$CH_2CH$_2$N$_3$), 1.50 (s, 3H, COCH_3); ^{13}C NMR (150 MHz, CDCl$_3$): δ 170.4, 170.1, 169.2, 169.0, 168.3, 167.1, 167.0, 165.1, 165.0, 164.9, 164.8, 164.2, 164.1, 163.7, 137.3, 137.3, 133.9, 133.1, 132.9, 132.7, 132.5, 129.6, 129.6, 129.51, 129.47, 129.31, 129.27, 129.2, 128.9, 128.8, 128.7, 128.7, 128.6, 128.59, 128.57, 128.4, 128.3, 128.20, 128.15, 128.11, 128.05, 128.0, 127.89, 127.85, 101.4 (C-1^{A}), 101.1 (C-1^{D}), 100.9 (C-1^{F}), 100.84 (C-1^{B}), 100.76 (C-1^{C}), 100.6 (C-1^{E}), 84.7 (C-3^{A}), 84.4 (C-5^{B}), 84.3 (C-5^{D}), 75.6 (C-4^{B}), 75.4 (C-4^{D}), 75.3 (C-5^{A}), 75.2 (C-5^{C}), 74.9 (C-5^{E}), 74.0 (2C, C-6^{B}, C-3^{E}), 73.5 (PhCH$_2$), 73.4 (PhCH$_2$), 72.8 (C-3^{B}), 72.7 (C-3^{D}), 72.4 (C-3^{F}), 71.9 (C-5^{F}), 71.8 (C-2^{E}), 71.7 (C-2^{B}), 71.6 (C-2^{D}), 71.5 (C-2^{A}), 71.4 (C-2^{C}), 70.6 (C-2^{F}), 70.2 (C-4^{A}), 69.9 (C-4^{C}), 68.2 (C-4^{F}), 67.5 (C-6^{B}), 67.2 (C-6^{D}), 66.3 (-OCH$_2$CH$_2$-), 61.9 (C-6^{F}), 53.4 (C-6^{D}), 52.6 (COOCH$_3$), 52.3 (COOCH$_3$), 52.1 (COOCH$_3$), 47.6 (-CH$_2$N$_3$), 28.7 (-OCH$_2$$CH_2CH_2N_3$), 20.5 (2C, CO$CH_3$), 20.4 (CO$CH_3$), 19.7 (CO$CH_3$); ESI HRMS: calcd for (C$_{131}$H$_{115}$N$_3$O$_{45}$+Na$^+$) *m/z*, 2256.6695; found, 2256.6698.



3b

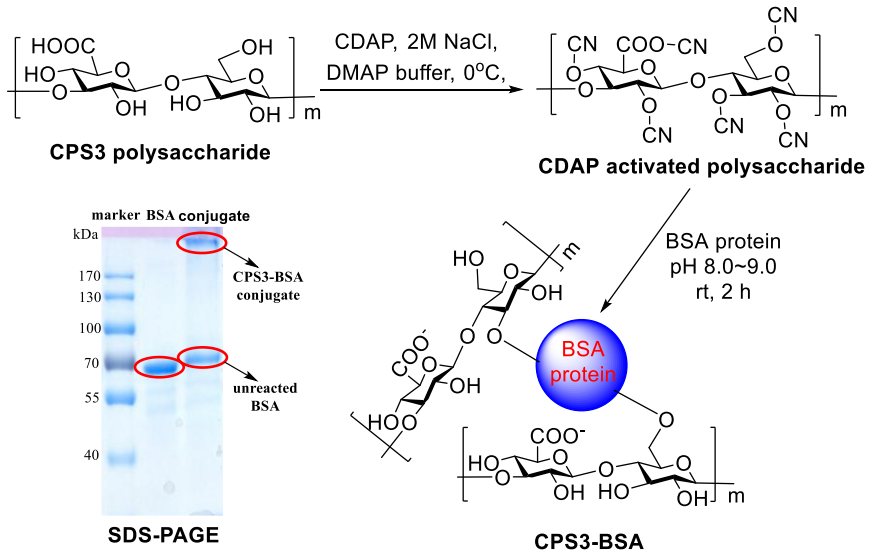
3-Aminopropyl β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyluronic acid-(1→4)-β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyluronic acid-(1→4)-β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyluronic acid (3b). To a stirred solution of hexasaccharide **20** (40 mg, 18 μmol) in THF (4 mL) was added 1 M LiOH at rt until the pH value reached 11. The reaction solution was stirred overnight and neutralized with 1 M HCl until the pH value reached 6. Then, the reaction mixture was concentrated, and the crude product was simply purified on size-exclusion chromatography (H$_2$O eluent). To a solution of the desired product in H$_2$O (4 mL) was added 10% Pd/C (12 mg) at rt. The resulting mixture was stirred for 18 h under H$_2$ atmosphere, then filtered to remove the catalyst, and concentrated. The residue was purified via size-exclusion

chromatography on a Sephadex G-10 column with distilled H_2O as the eluent to give target product **3b** (14 mg, 75% over two steps) as white powder after lyophilization. $[\alpha]_D^{25}$ -37.5 (*c* 0.1, H_2O); ^1H NMR (600 MHz, D_2O): δ 4.67–4.63 (m, 3H, H-1×3), 4.38–4.35 (m, 3H, H-1×3), 3.89–3.84 (m, 1H, -OC*H*$_2$CH$_2$-), 3.82 (d, *J* = 12.0 Hz, 3H, H-6a×2), 3.75 (d, *J* = 12.0 Hz, 1H, H-6a), 3.71–3.66 (m, 1H, -OC*H*$_2$CH$_2$-), 3.65–3.28 (m, 39H, H-6b×3), 3.24–3.16 (m, 6H), 3.02-2.97 (m, 2H, -C*H*$_2$NH$_2$), 1.87–1.81 (m, 2H, -OCH$_2$C*H*$_2$CH$_2$NH$_2$) ; ^{13}C NMR (150 MHz, D_2O): δ 175.3, 175.2, 175.0, 102.4 (C-1), 102.21 (C-1), 102.19 (C-1), 102.08 (C-1), 102.05 (C-1), 101.8 (C-1), 83.1, 82.6 (2C), 78.8 (2C), 75.9, 75.4, 74.8, 74.7, 74.1 (2C), 73.3 (2C), 73.1 (2C), 72.8 (2C), 72.8, 72.7, 70.09 (2C), 70.05, 69.5 (2C), 67.8, 60.6 (C-6), 59.9 (2C, C-6×2), 37.4 (-*C*H$_2$NH$_2$), 26.6 (-OCH$_2$*C*H$_2$CH$_2$NH$_2$); ESI HRMS: calcd for ($C_{39}H_{63}NO_{34}$+H^+) *m/z*, 1090.3304; found, 1090.3322.

Preparation of glycoproteins 25-30a-b and CPS3-BSA. For preparation of oligosaccharide conjugates **25-30a-b**: A mixture of oligosaccharides **1-3a-b** (3 mg) and di-(*N*-succinimidyl) glutarate **21** (DSG, 15.0 equiv) was dissolved in DMF/H_2O (0.5 mL, 4:1, v/v) at rt. The reaction mixture was gently stirred for 2 h and concomitantly monitored by MALDI-TOF MS analysis. After MALDI-TOF MS indicated the complete consumption of oligosaccharide haptens, the reaction solvents were then concentrated under reduced pressure. The resulting residue was washed successively with ethyl acetate (×10 times) to remove excessive DSG linker. The activated monoesters **22-24a-b** were finally obtained as white solids and directly used for protein conjugation without further purification**.** Thereby, a mixture of each activated ester and TT or BSA protein in 1:2 mass ratio was dissolved in PBS buffer (0.1 M, 0.3 mL, pH 8.0) and gently stirred at 37℃ for 1 day. After MALDI-TOF MS analysis indicated no further increase on molecular weight of the formed glycocnjugates, the reaction mixture was treated with ultrafiltration using 30 kDa or 10 kDa ultrafiltrer tube (3000 rpm; 4℃; 4×washes) to remove PBS and unreacted oligosaccharide monoester, yielding the target glycoproteins **25-30a-b** as white fluffy powders after lyophilization. The average molecular weight of each glycoprotein was measured by MALDI-TOF MS, and their carbohydrate loading on carrier proteins were calculated according to the following Equation：

$$Carbohydrate\ \ loading\% = \frac{MS_{conjugate} - MS_{protein}}{MS_{conjugate}} \times 100\%$$

For preparation of polysaccharide conjugate **CPS3-BSA**: To a stirred solution of ST3 CPS polysaccharide (10 mg) in aq. NaCl (2 M, 1 mL) with pH value adjusted at 8.5 using DMAP buffer was quickly added CDAP (150 μL, 100 mg/mL in acetonitrile) at 0°C. After being stirred 15 min at this condition, the above reaction solution was added with BSA (10 mg) and then diluted to final volume of 2 mL. The resulting mixtures were stirred for 2 h at rt with reaction pH maintained at 8.0~9.0 through the intermittent addition of aq. NaOH (0.2 M), and then quenched by glycine (~7.5 equiv of CDAP.). The resulting solution was gently agitated overnight at rt and then subjected to ultrafiltration purification using 30 kDa ultrafilter tube (3000 rpm; 4°C; 4 × washes) to generate the conjugate **CPS3-BSA** as white fluffy powder after lyophilization. The conjugation condition of **CPS3-BSA** was generally determined by SDS-PAGE analysis as shown in Scheme S1.



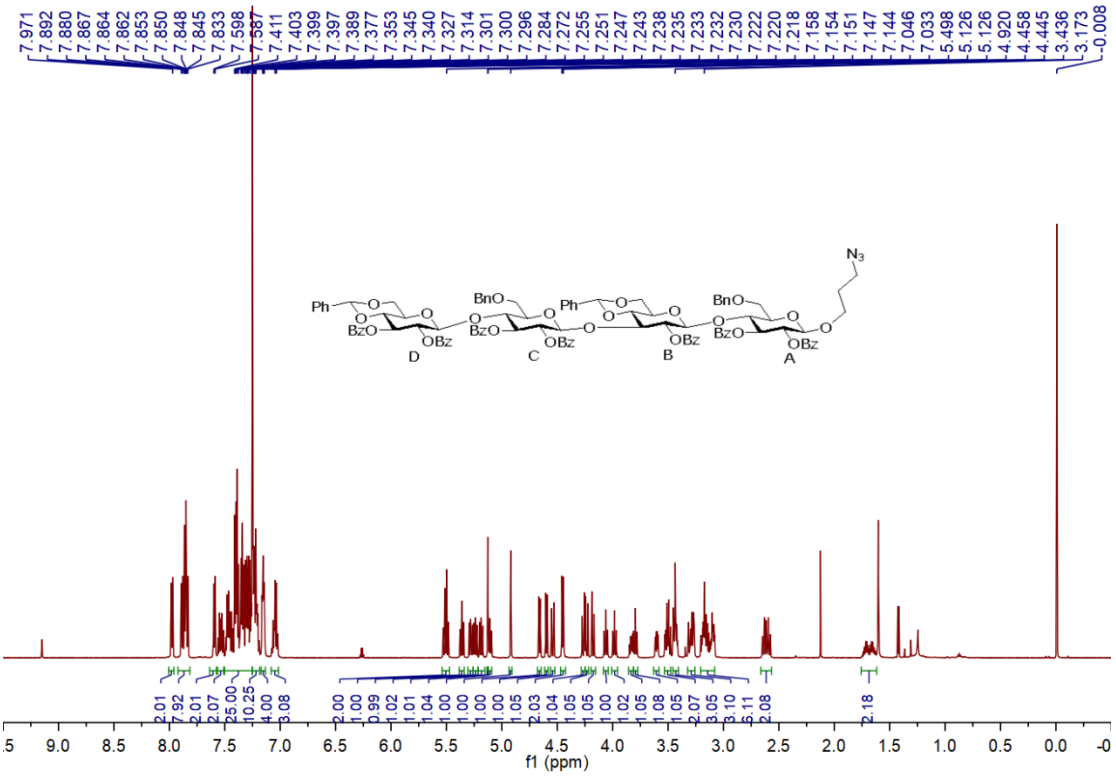
Scheme S1. Conjugation of ST3 polysaccharide with BSA protein via CDAP chemistry.

Immunization of Mice. Immunization study was carried out with female Balb/c mice aged 6–8 weeks. Each TT conjugate **25-27a-b** (containing 30 μg of the corresponding oligosaccharide) was dissolved in 0.5 mL of 2 × PBS buffer., and then were well-mixed with 0.5 mL of Freund's complete adjuvant (FCA, F5881, Sigma)/Freund's incomplete adjuvant (FIA, F5506, Sigma) to generate an emulsion according to the manufacturer's instructions. Each group of six mice was primary immunization with 0.1 mL of the FCA emulsion on day 1 by subcutaneous injection and boosted three times with 0.1 mL of the FIA emulsion on days 15, 22, and 29. Similar immunization

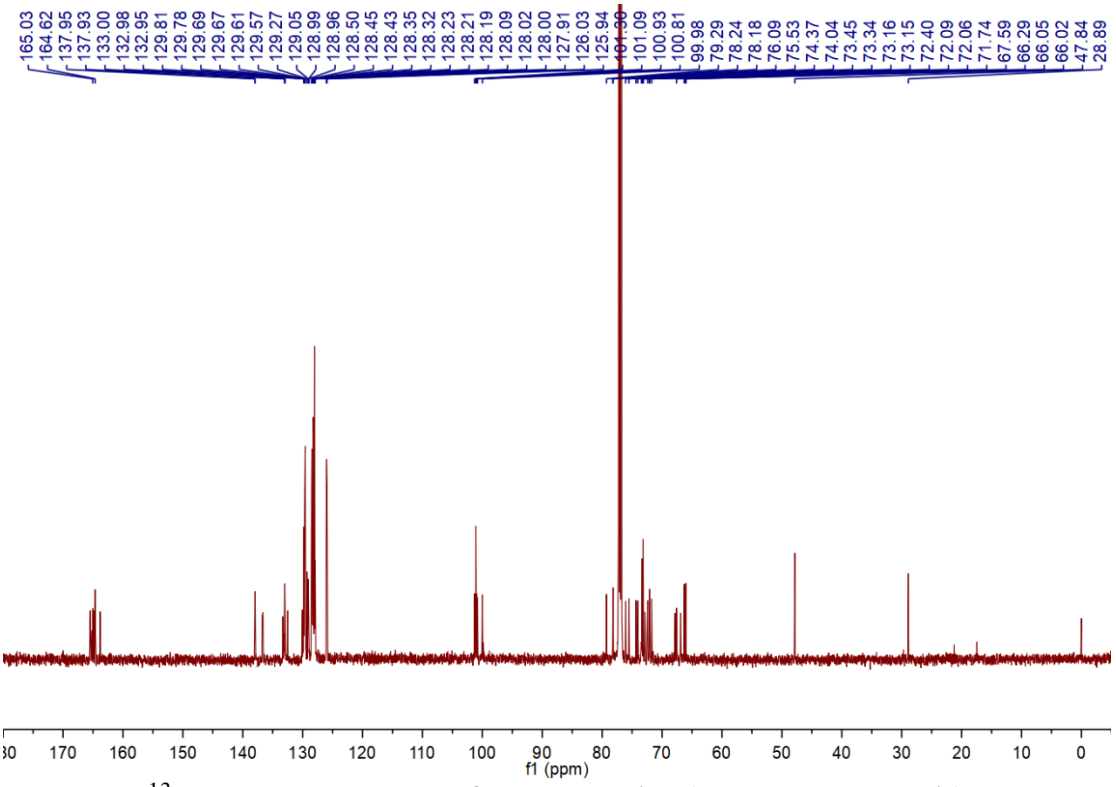
schedule was employed for the positive group that immunized with ST3 CPS-TT conjugate **CPS3-TT** (~1 μg of ST3 polysaccharide per mouse per inoculation). Accordingly, 150 μL of blood samples were collected via tail vein of each mouse on day 35. The antisera samples were obtained by standard protocols and stored at -80 ℃ for immunological analysis. The animal protocols were approved by the Institutional Animal Care and Use Committee at Shandong University.

ELISA Assay. Antibody titers were measured by ELISA from antisera of six mice's blood as previously described. ELISA plates were treated with a solution of BSA conjugate **28-30a-b** (100 μL/well, 2 μg/mL) and **CPS3-BSA** (100 μL/well, 3 μg/mL) in coating buffer (0.1 M bicarbonate, pH 9.6) at 4℃ overnight and at 37℃ for 1 h, and then each well was washed three times with PBST (150 μL/well, PBS buffer supplemented with 0.05% Tween-20). Subsequently, plates were then incubated with the blocking buffer (100 μL/well, 1% BSA in PBS) at rt for 1 h and washed with PBST three times. Each mouse serum with serial dilution from 1:300 to 1:218700 in PBS (100 μL/well) was added to the coated plates and then incubated at 37℃ for 2 h. In the meanwhile, the negative control (100 μL/well, 0.1% BSA in PBS) was carried out according to the same protocol. After being washed by PBST three times, the plates were added a 1:2000 diluted solution of Horseradish Peroxidase (HRP)-conjugate goat anti-mouse IgG, IgM, IgG1, IgG2a, and IgG2b antibody (100 μL/well, 0.1 μL/mL in buffer) and incubated at 37℃ for 1 h. Again, the plates were washed by PBST three times and developed with a 3,3',5,5'-tetramethylbenzidine (TMB) solution (200 μL/well, 0.25 mg/mL in buffer) to stain the bound products at rt for 15 min. The reactions were quenched by 0.5 M H_2SO_4 (50 μL/well), which were immediately followed by colorimetric readout using a microplate reader at 450 nm wavelength. The OD values were plotted against serum dilution values, and a best-fit curve was obtained. The positive cut-off value was based on 2.1 times OD of negative control, and the antibody titers were obtained using calibration curves.

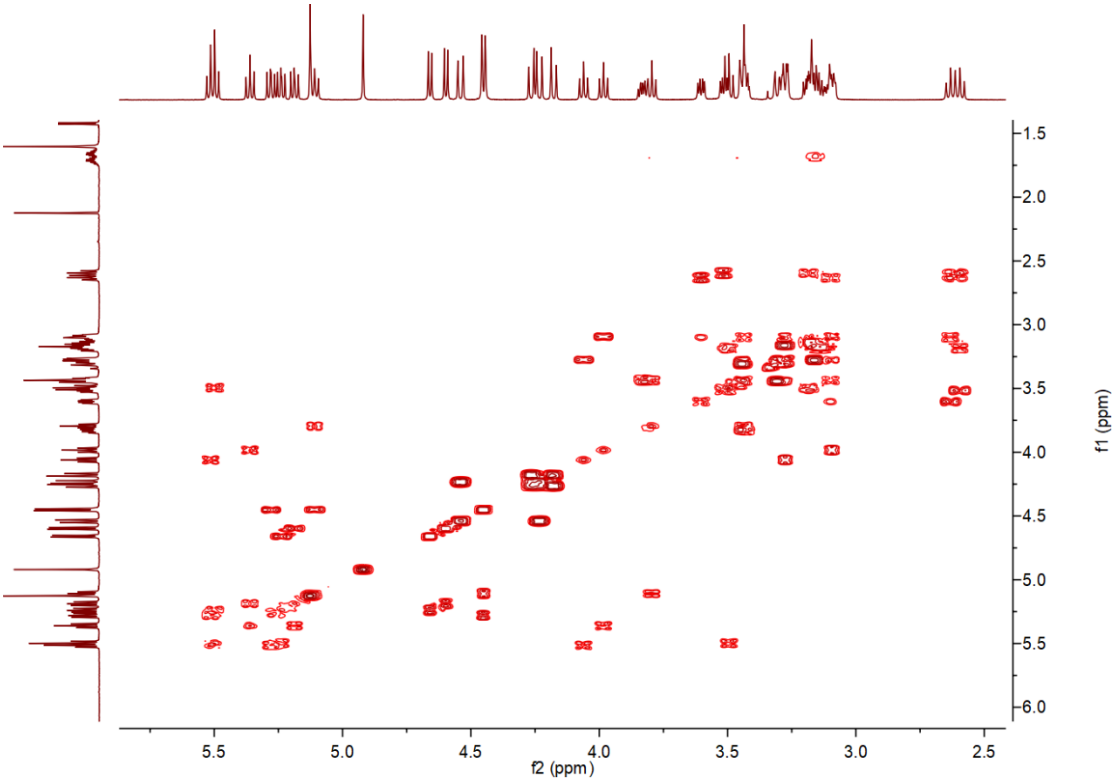
II. NMR spectra of synthetic compounds



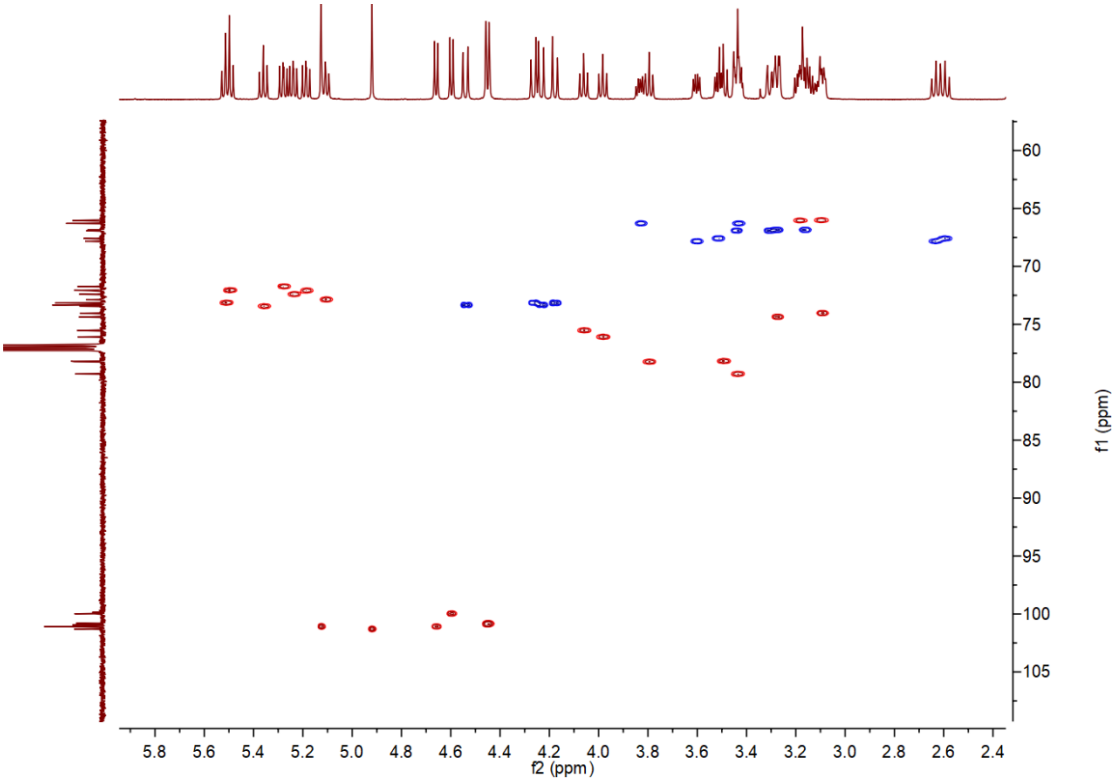
^{1}H NMR spectrum of compound **9** (600 MHz, $CDCl_3$)



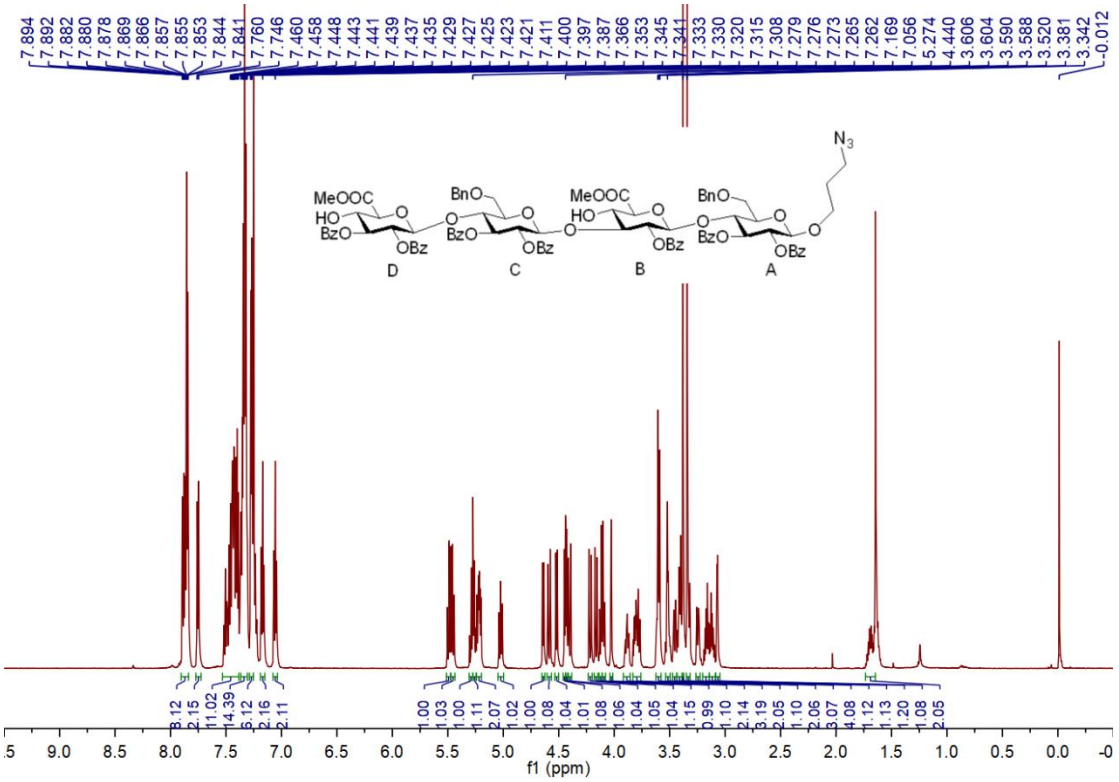
^{13}C NMR spectrum of compound **9** (150 MHz, $CDCl_3$)



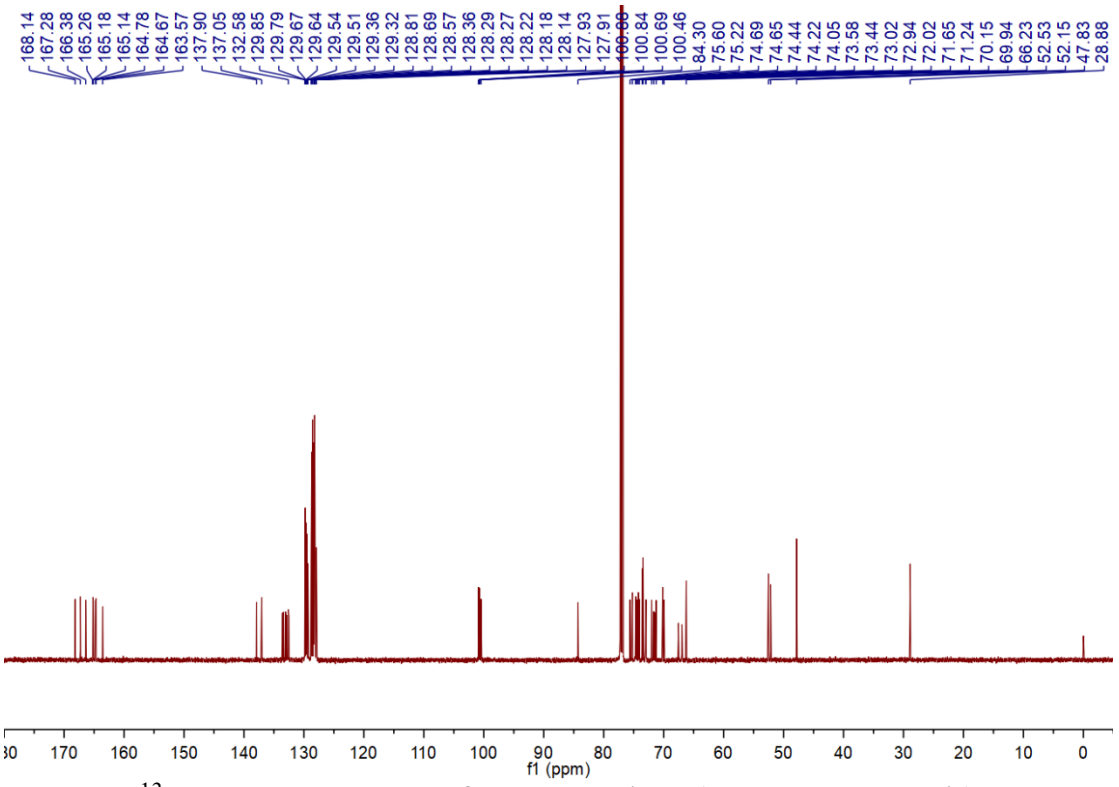
^{1}H-^{1}H COSY spectrum of compound **9** (600/600 MHz, $CDCl_3$)



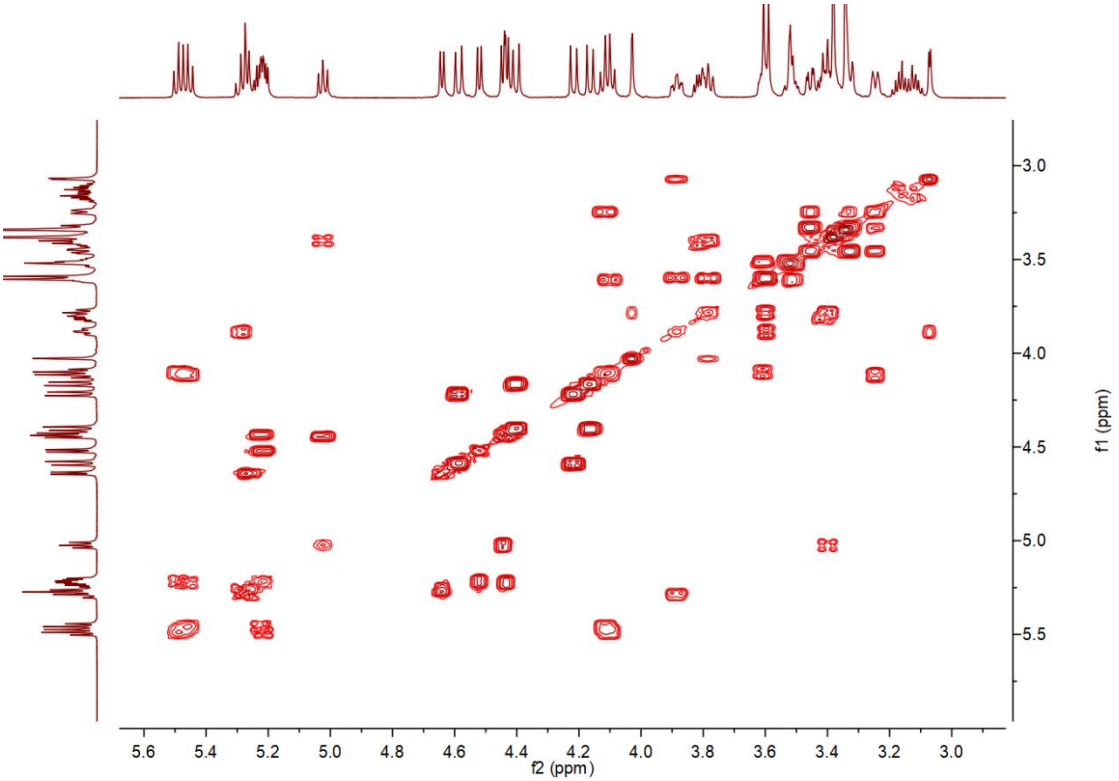
^{1}H-^{13}C HSQC spectrum of compound **9** (600/150 MHz, $CDCl_3$)



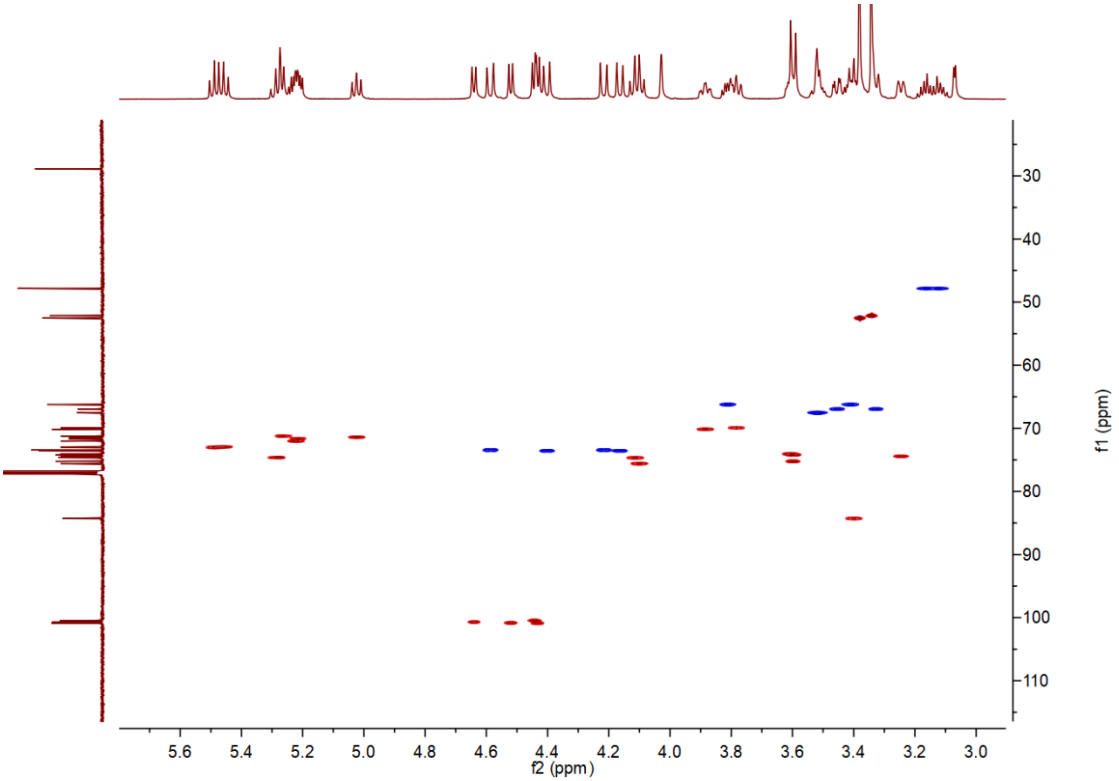
^{1}H NMR spectrum of compound **10** (600 MHz, $CDCl_3$)



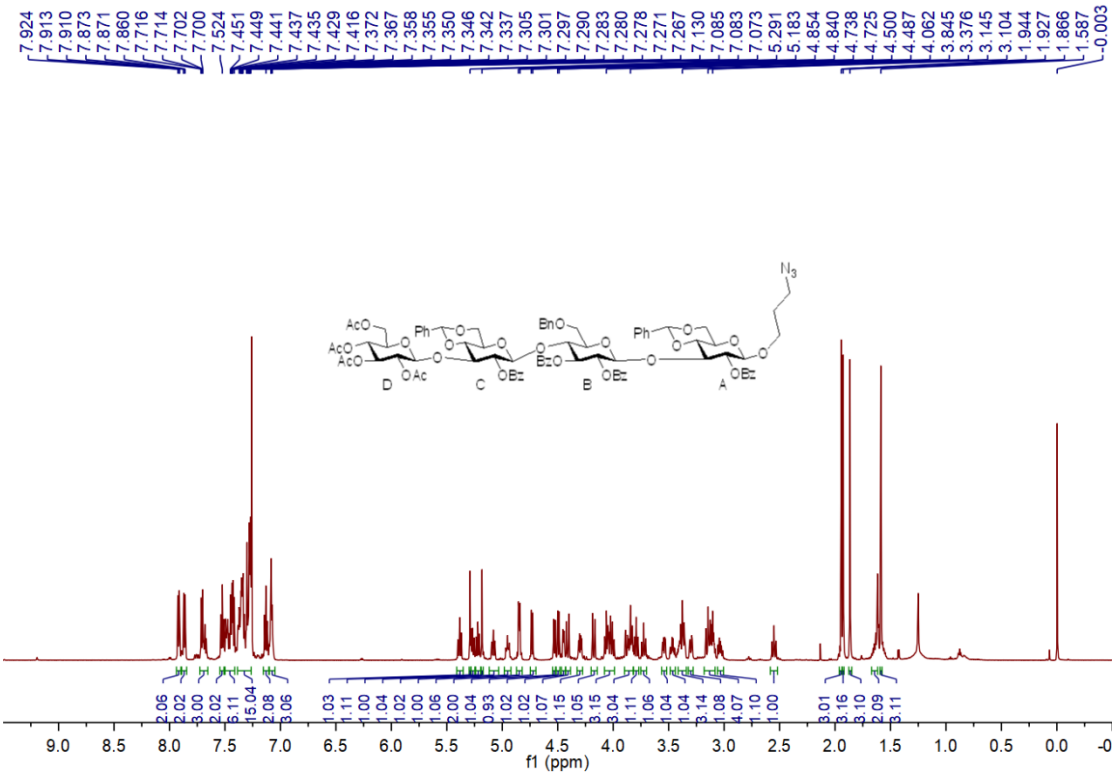
^{13}C NMR spectrum of compound **10** (150 MHz, $CDCl_3$)



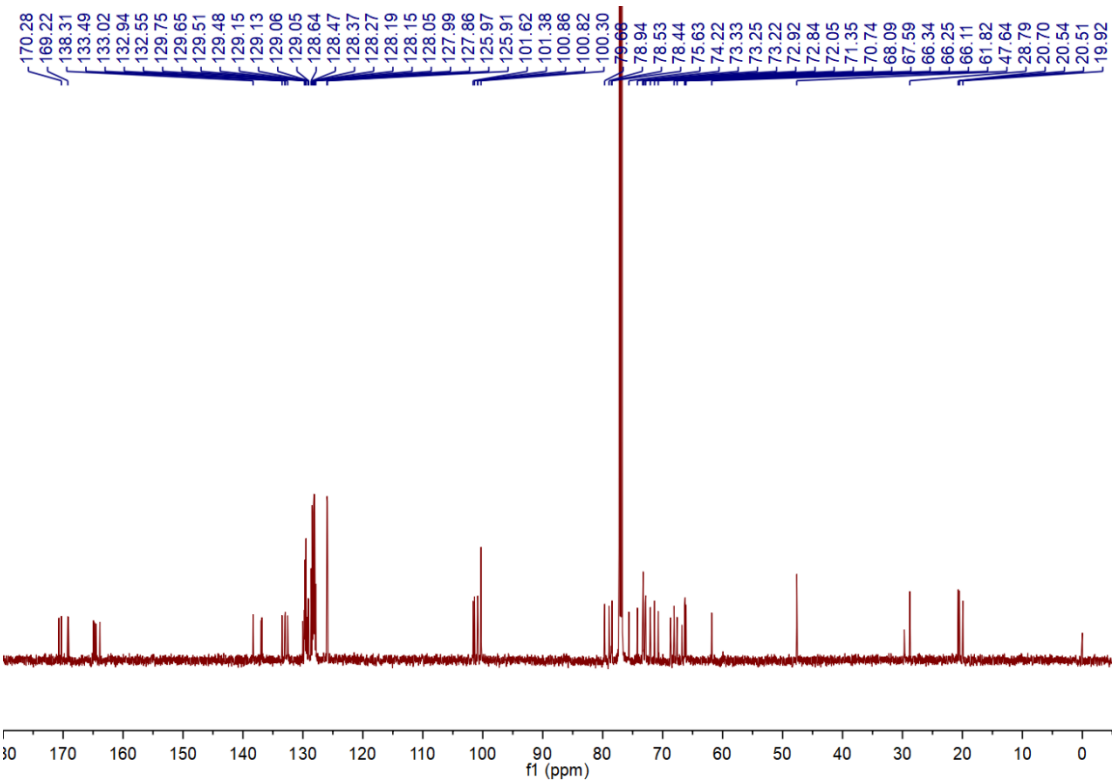
^{1}H-^{1}H COSY spectrum of compound **10** (600/600 MHz, $CDCl_3$)



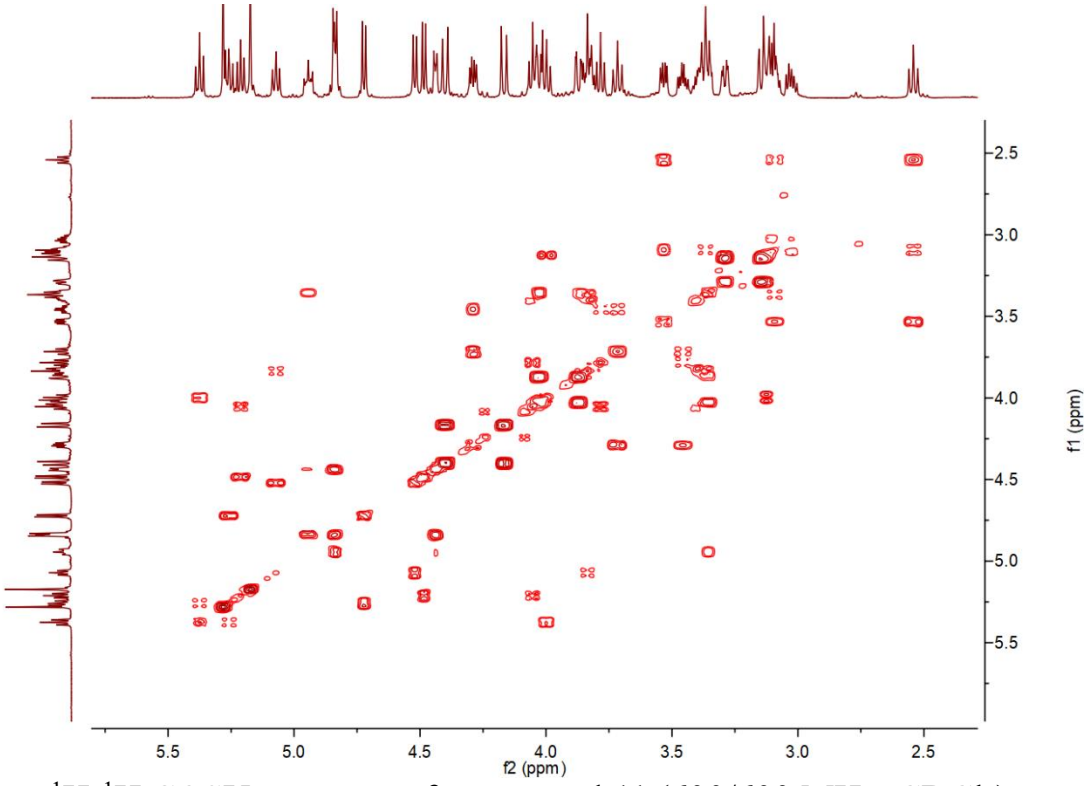
^{1}H-^{13}C HSQC spectrum of compound **10** (600/150 MHz, $CDCl_3$)



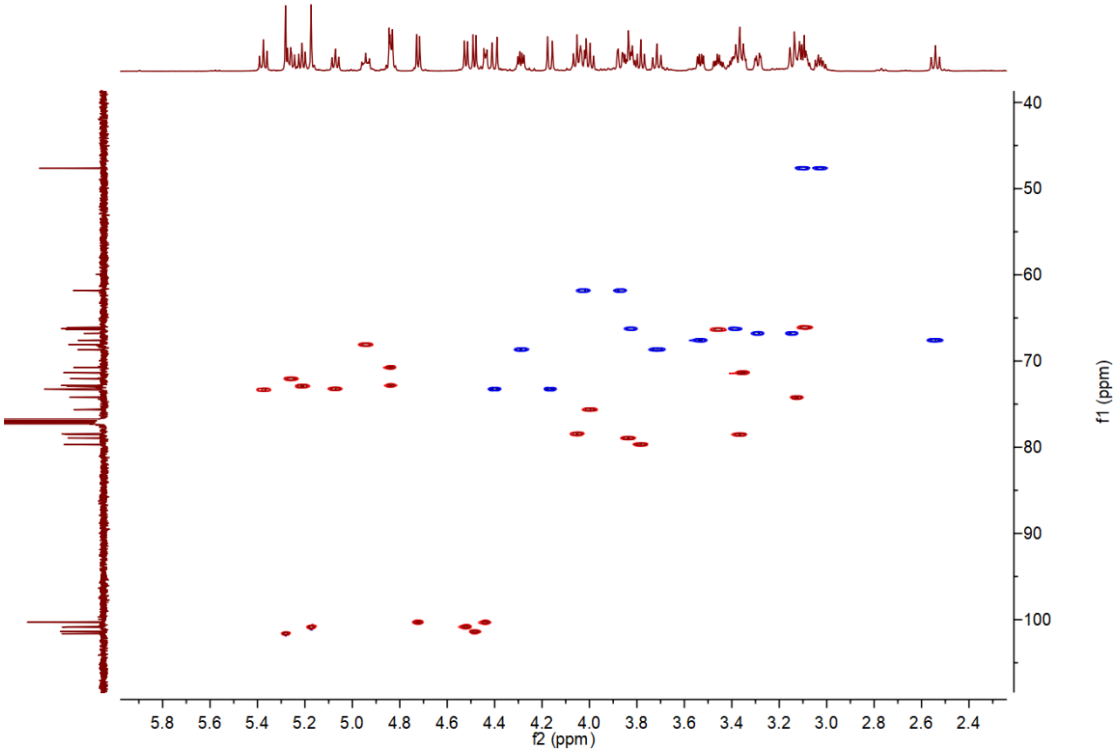
^{1}H NMR spectrum of compound **11** (600 MHz, $CDCl_3$)



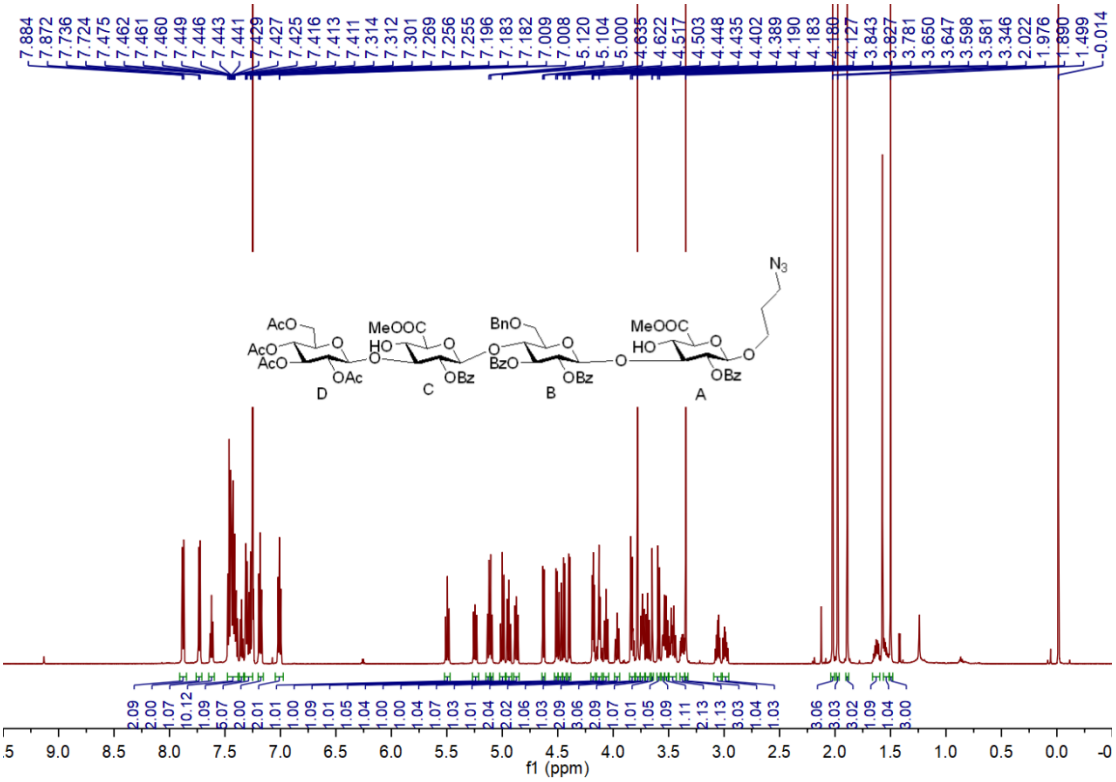
^{13}C NMR spectrum of compound **11** (150 MHz, $CDCl_3$)



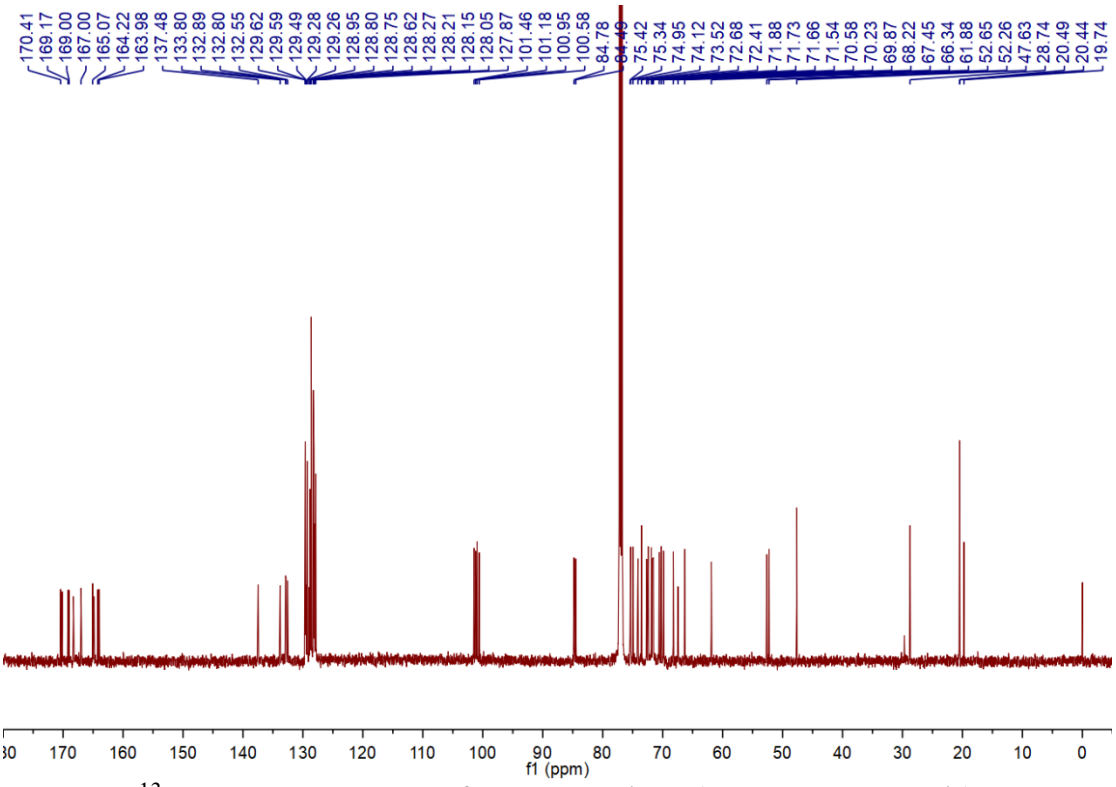
^{1}H-^{1}H COSY spectrum of compound **11** (600/600 MHz, $CDCl_3$)



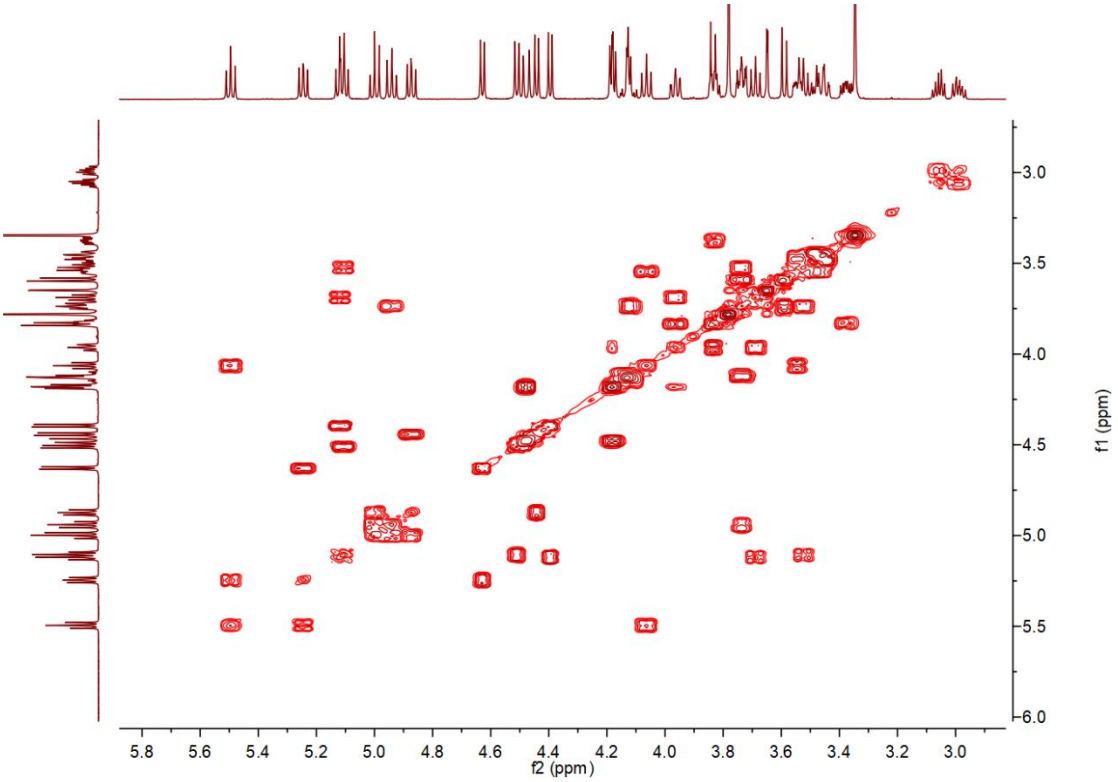
^{1}H-^{13}C HSQC spectrum of compound **11** (600/150 MHz, $CDCl_3$)



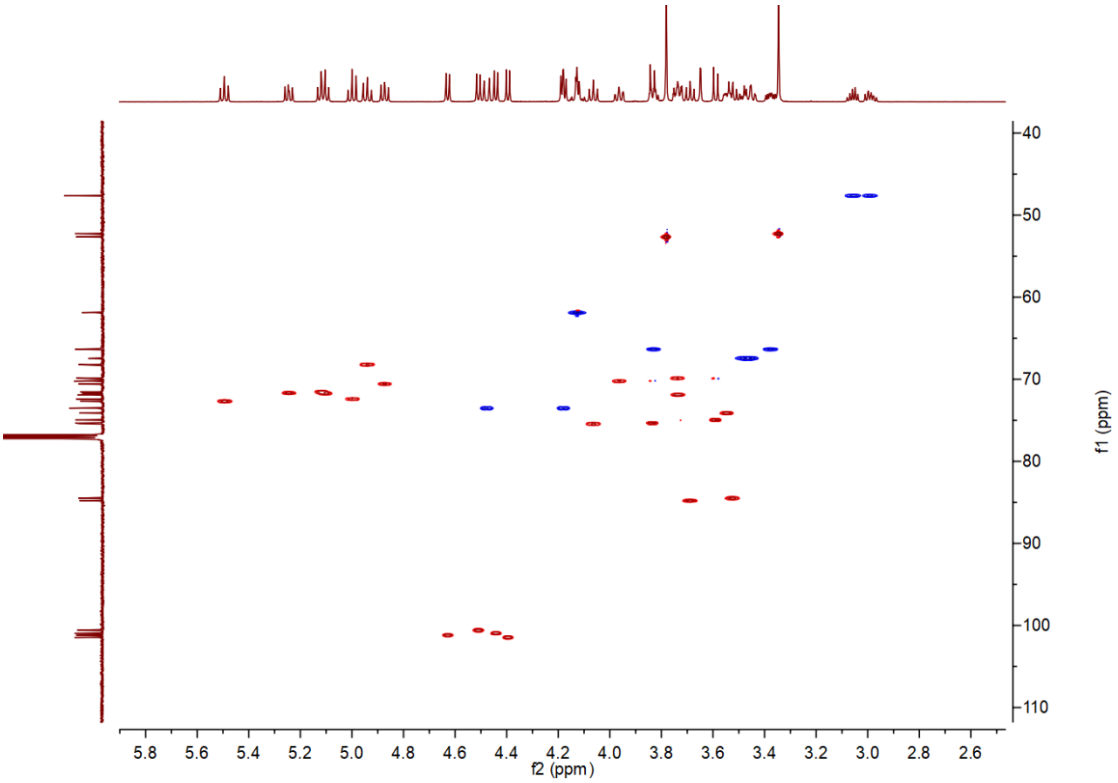
^{1}H NMR spectrum of compound **12** (600 MHz, $CDCl_3$)



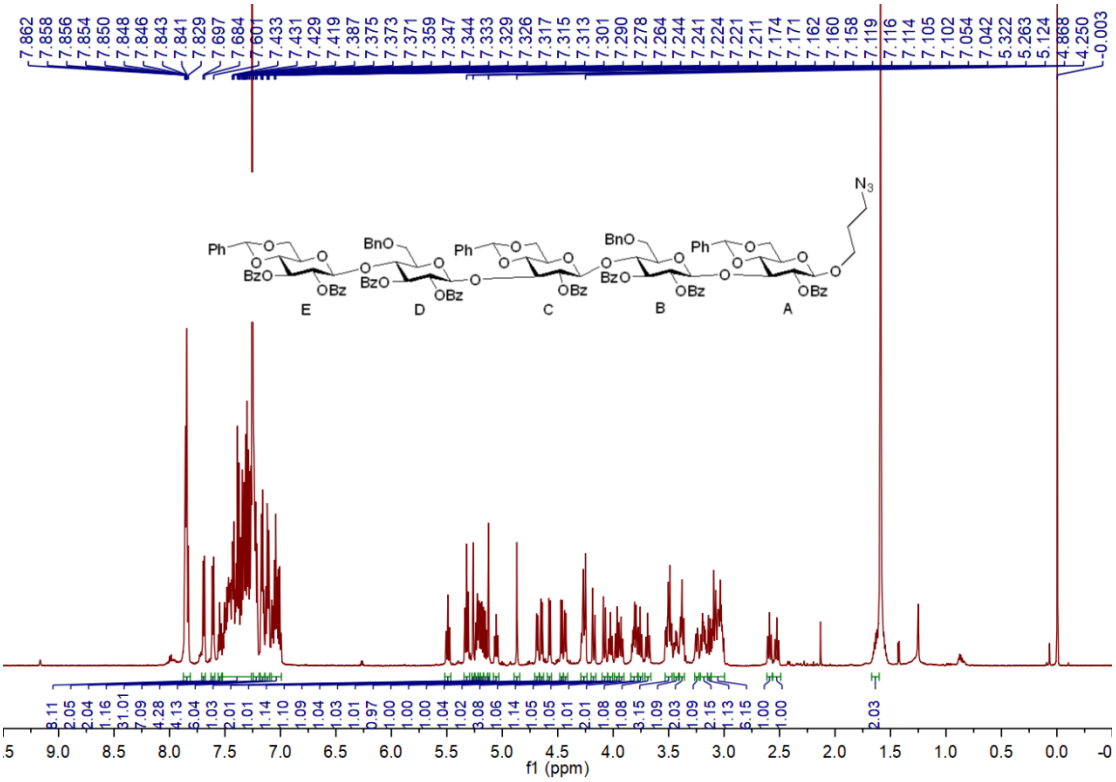
^{13}C NMR spectrum of compound **12** (150 MHz, $CDCl_3$)



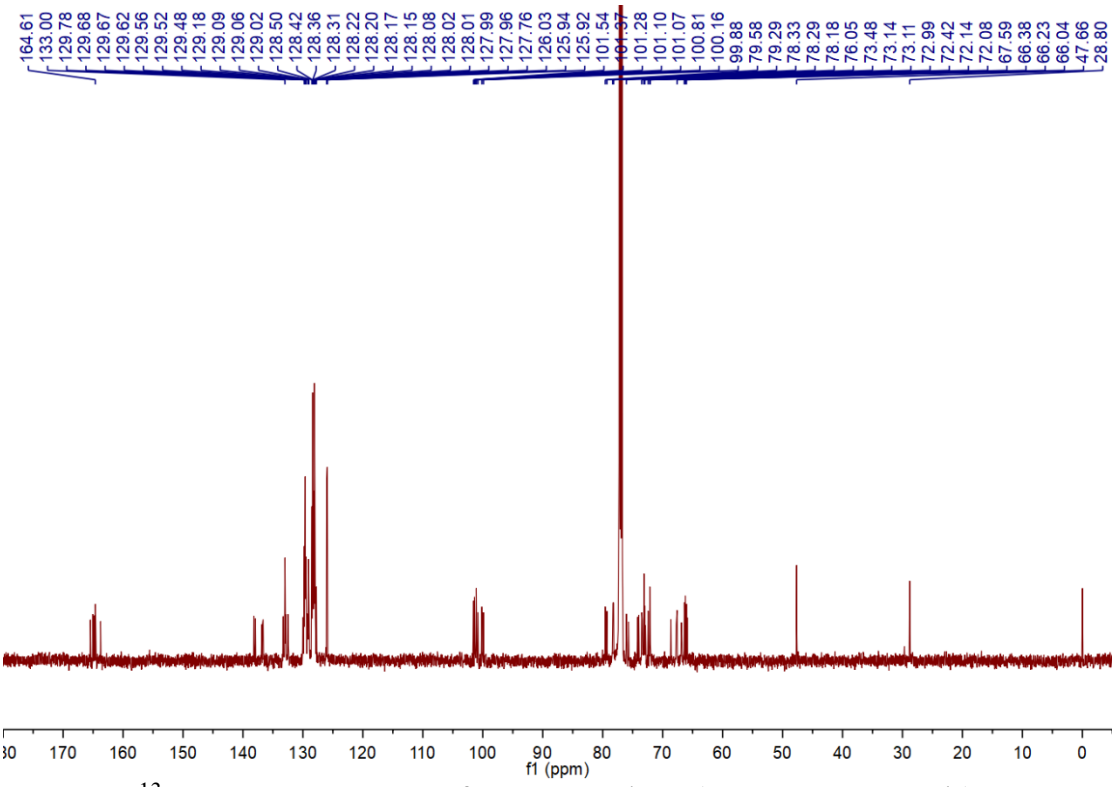
^{1}H-^{1}H COSY spectrum of compound **12** (600/600 MHz, $CDCl_3$)



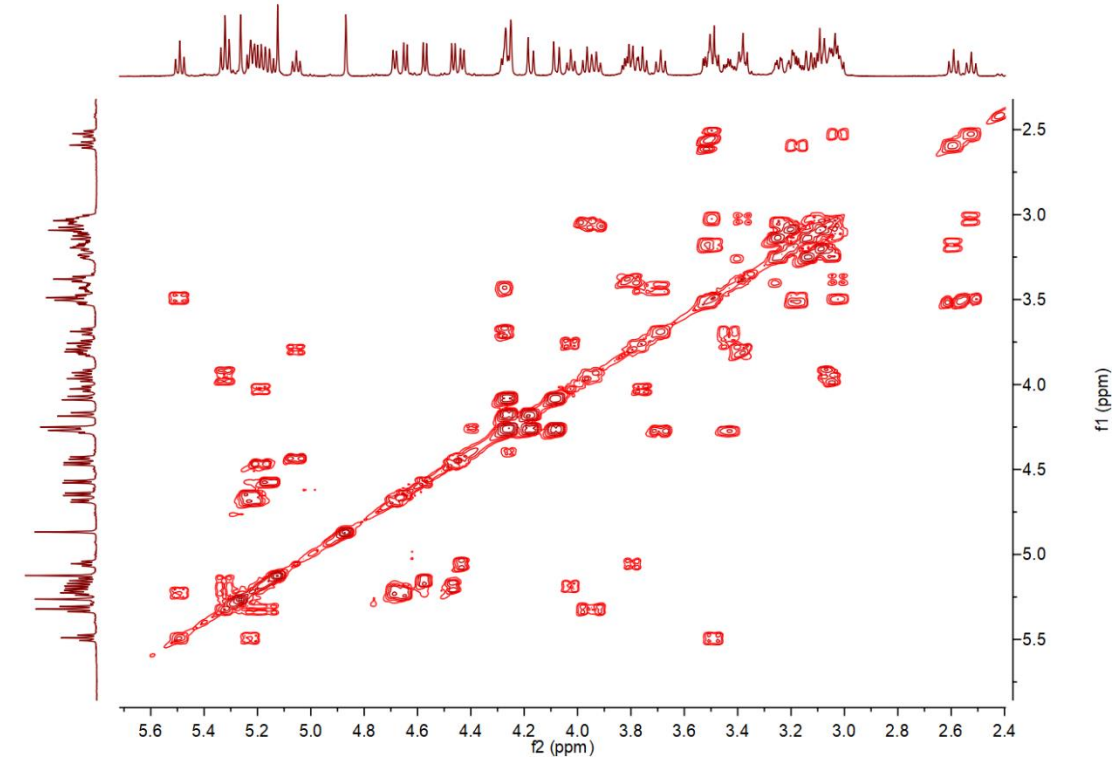
^{1}H-^{13}C HSQC spectrum of compound **12** (600/150 MHz, $CDCl_3$)



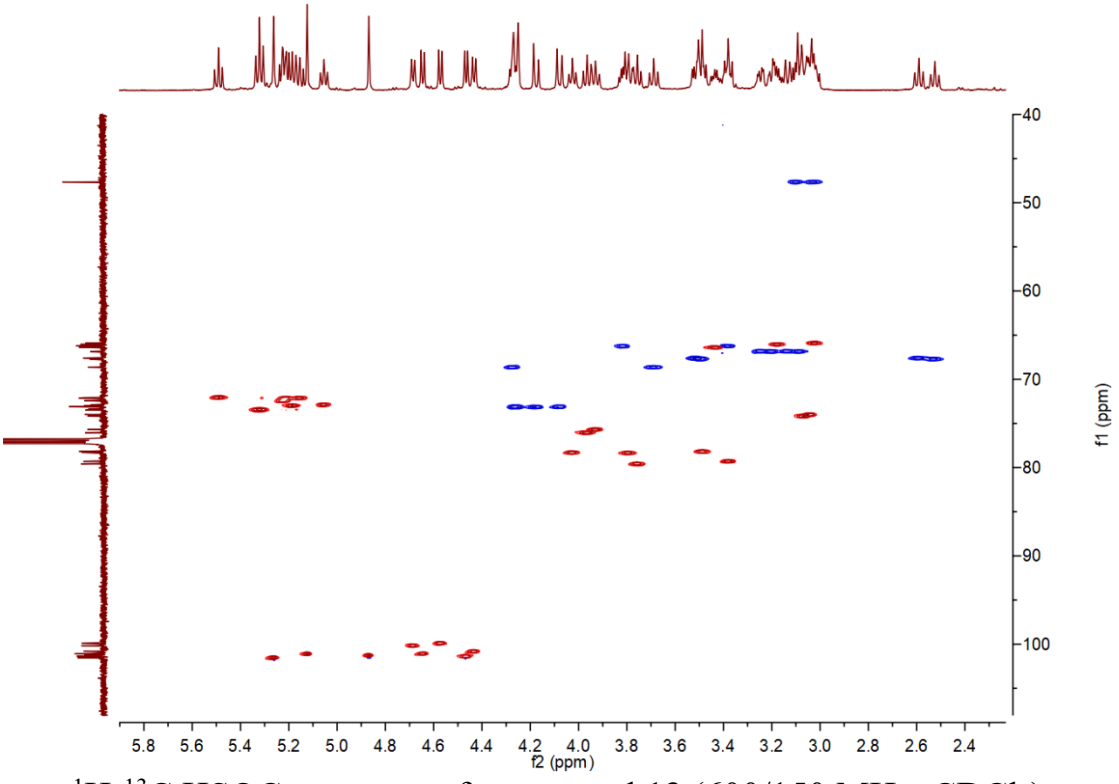
^{1}H NMR spectrum of compound **13** (600 MHz, $CDCl_3$)



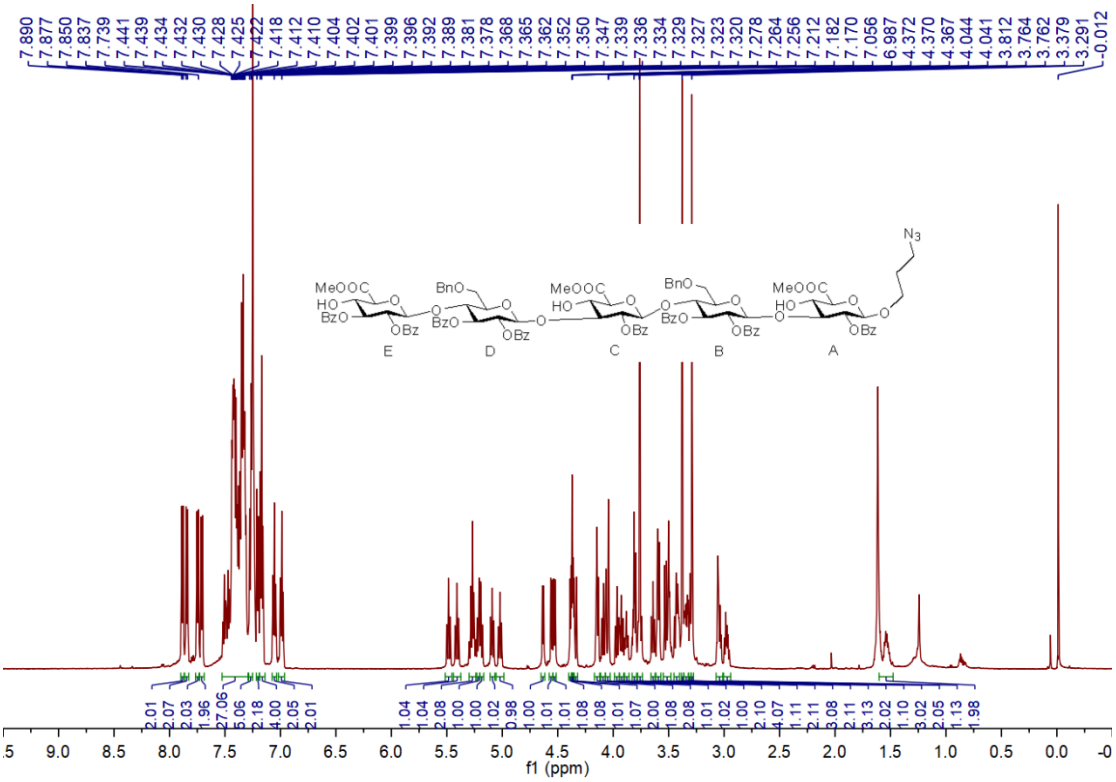
^{13}C NMR spectrum of compound **13** (150 MHz, $CDCl_3$)



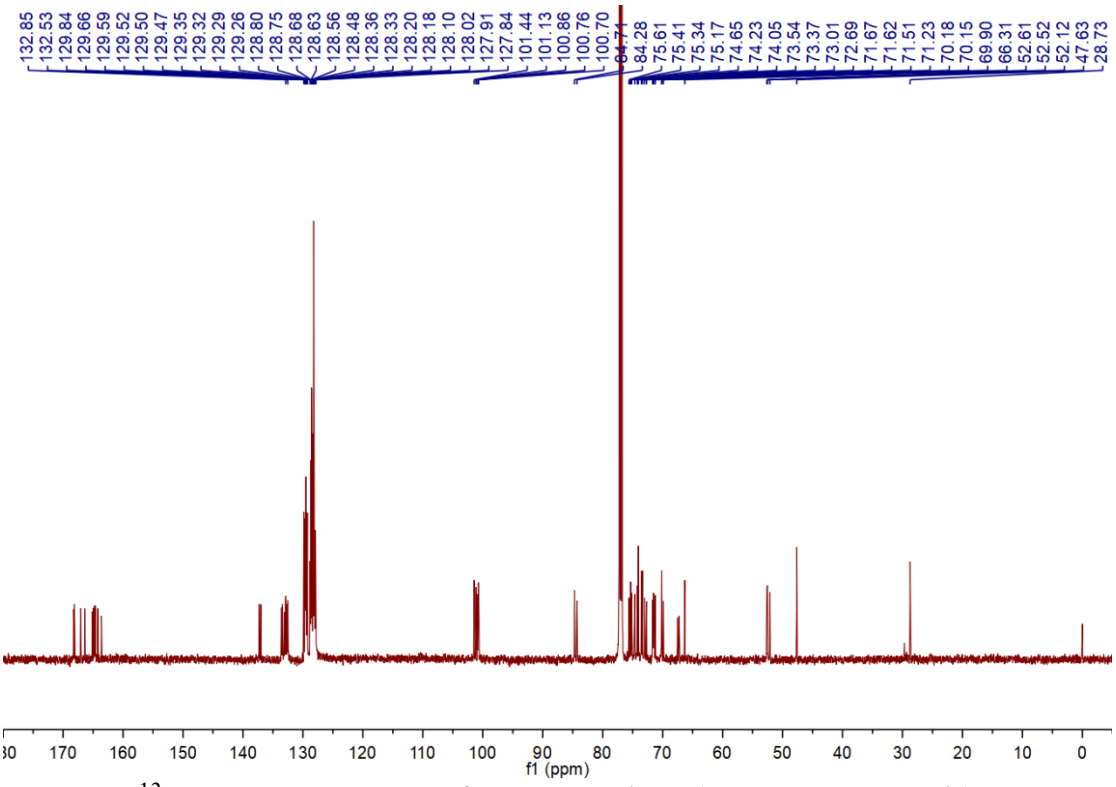
^{1}H-^{1}H COSY spectrum of compound **13** (600/600 MHz, $CDCl_3$)



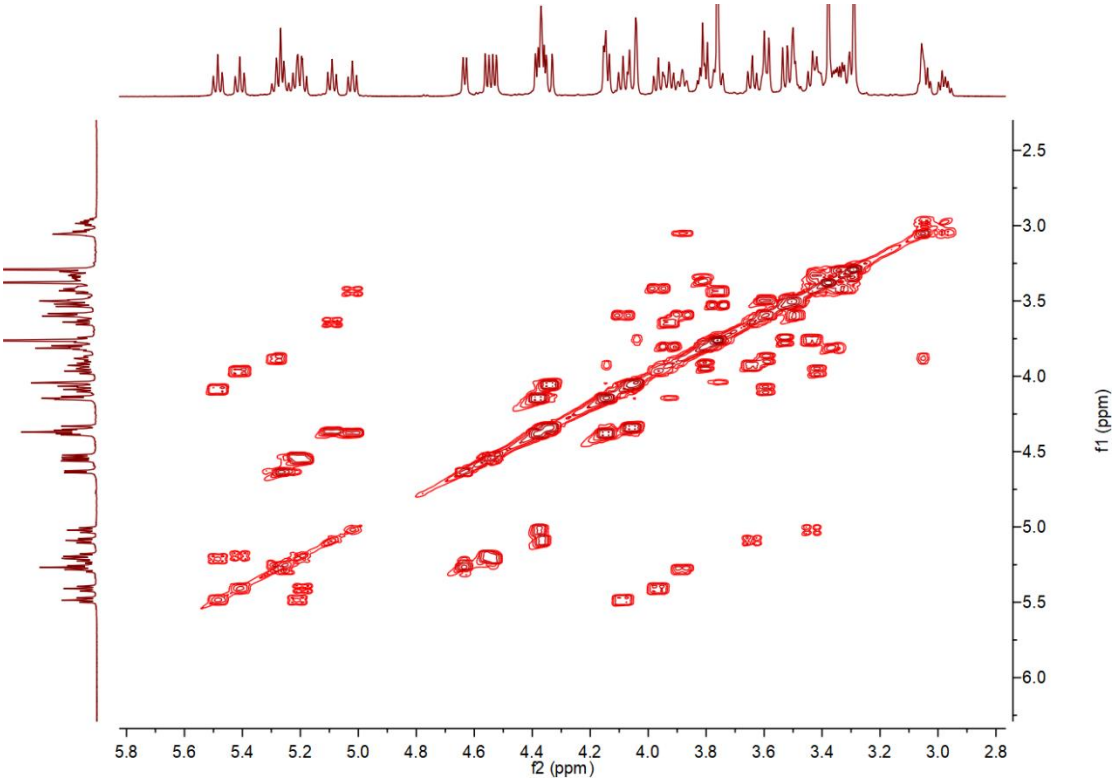
^{1}H-^{13}C HSQC spectrum of compound **13** (600/150 MHz, $CDCl_3$)



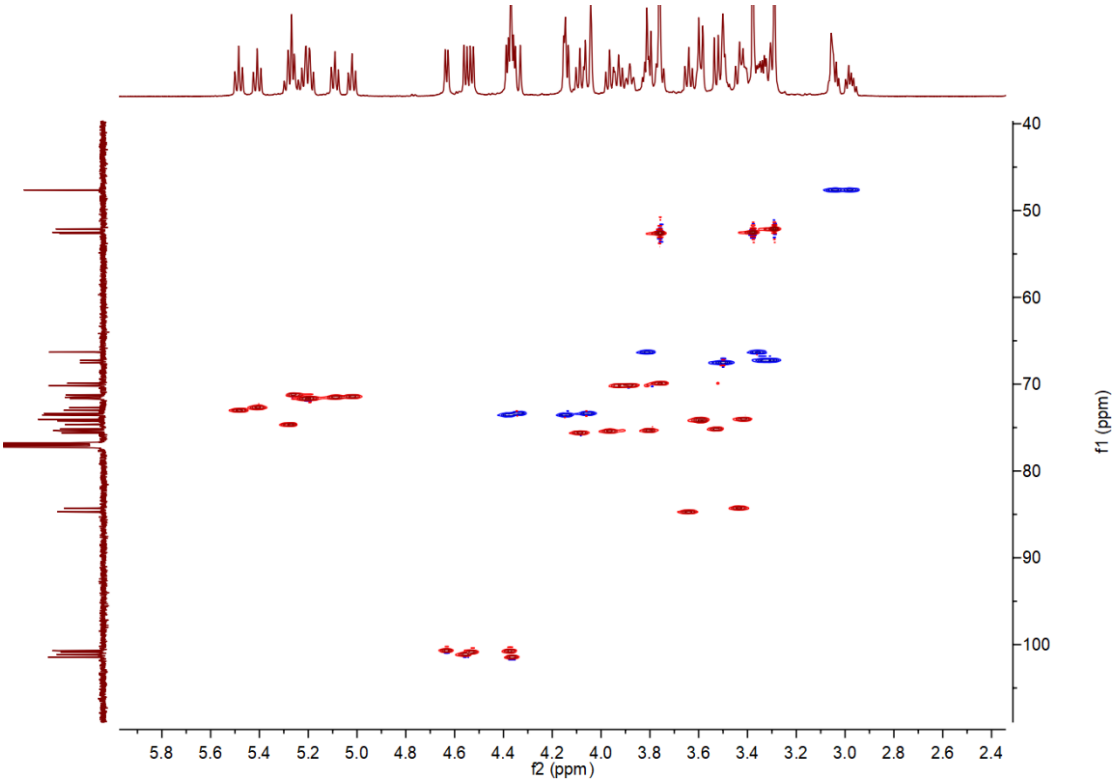
^{1}H NMR spectrum of compound **14** (600 MHz, $CDCl_3$)



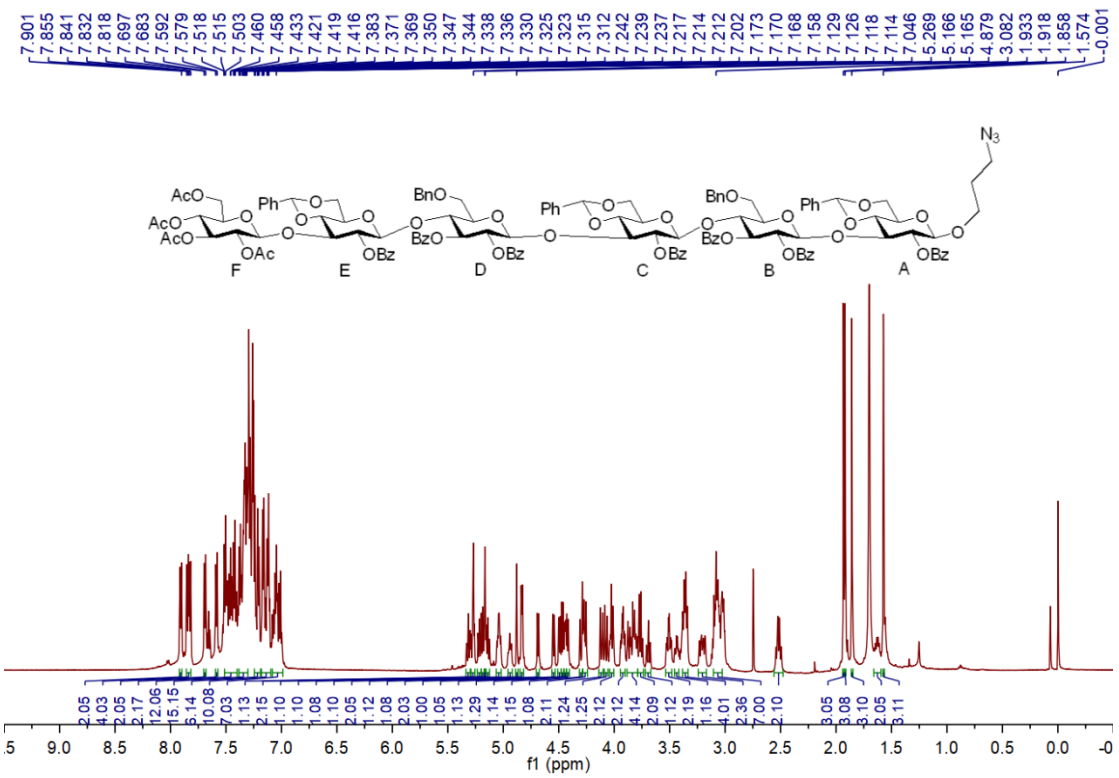
^{13}C NMR spectrum of compound **14** (150 MHz, $CDCl_3$)



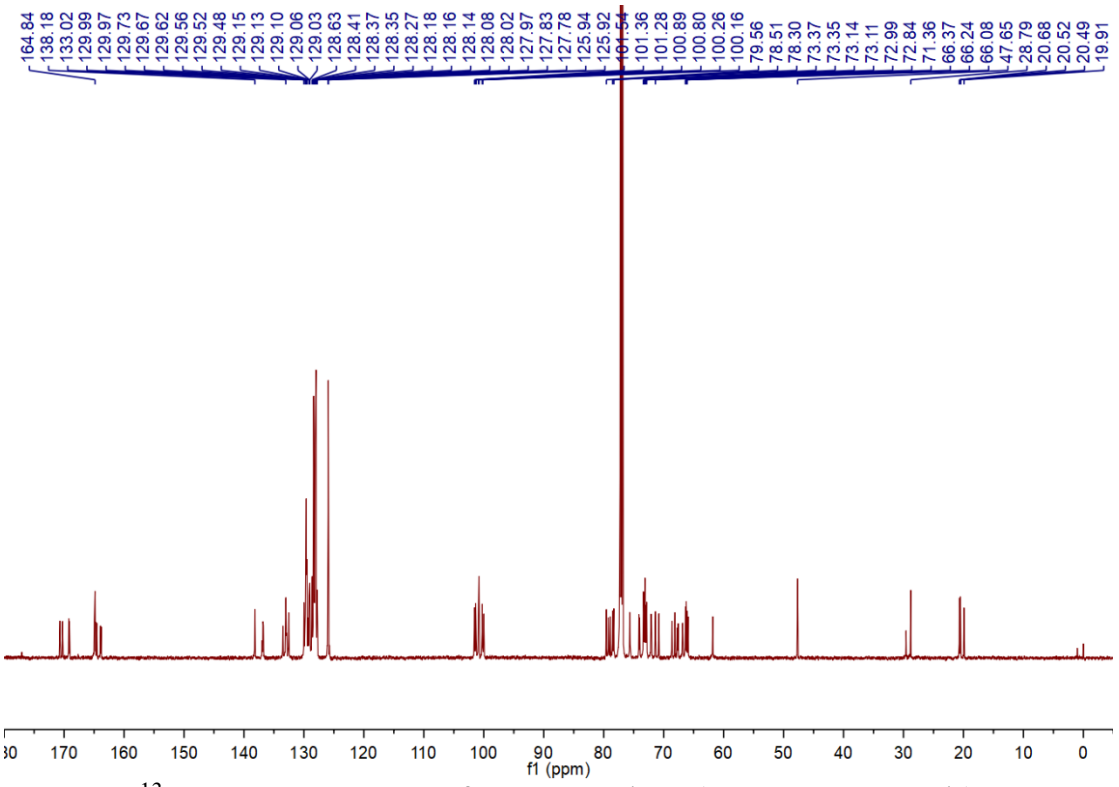
^{1}H-^{1}H COSY spectrum of compound **14** (600/600 MHz, $CDCl_3$)



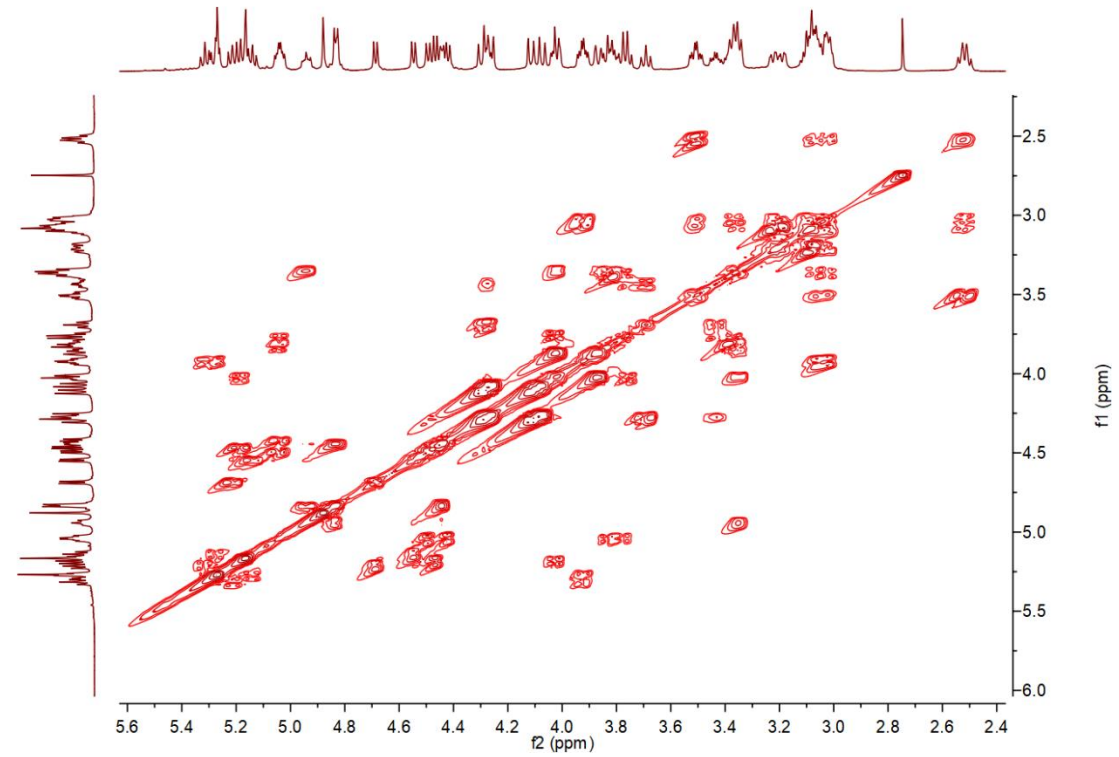
^{1}H-^{13}C HSQC spectrum of compound **14** (600/150 MHz, $CDCl_3$)



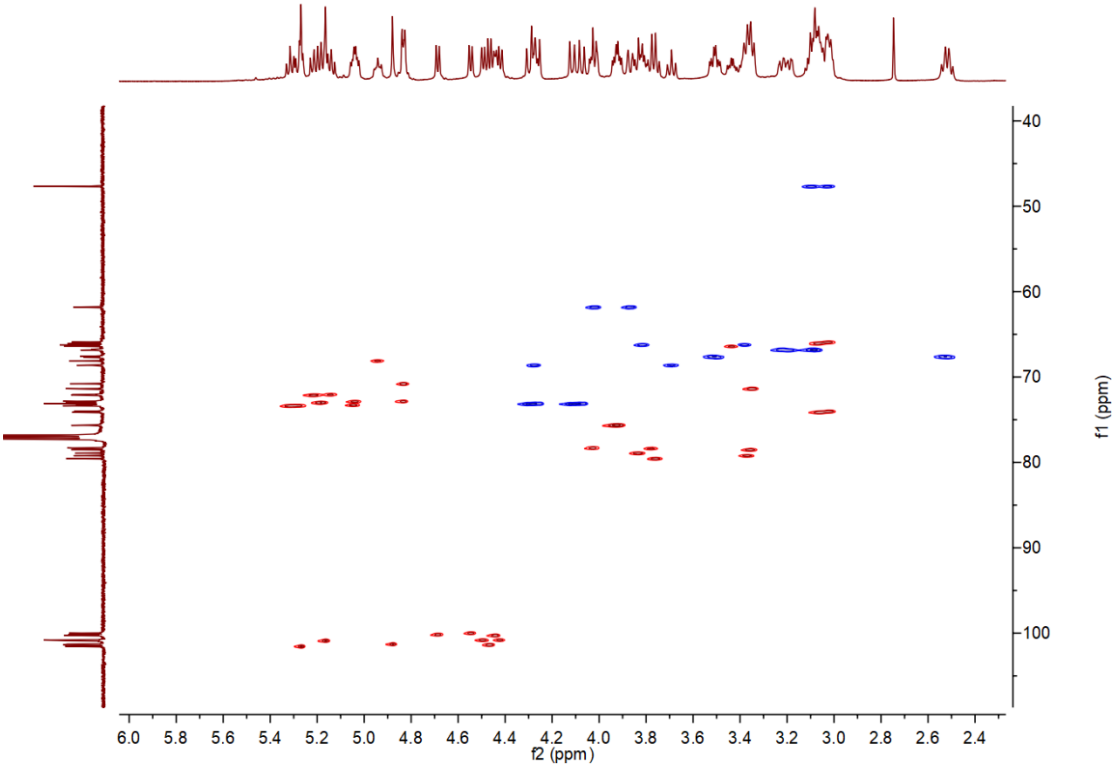
^{1}H NMR spectrum of compound **15** (600 MHz, $CDCl_3$)



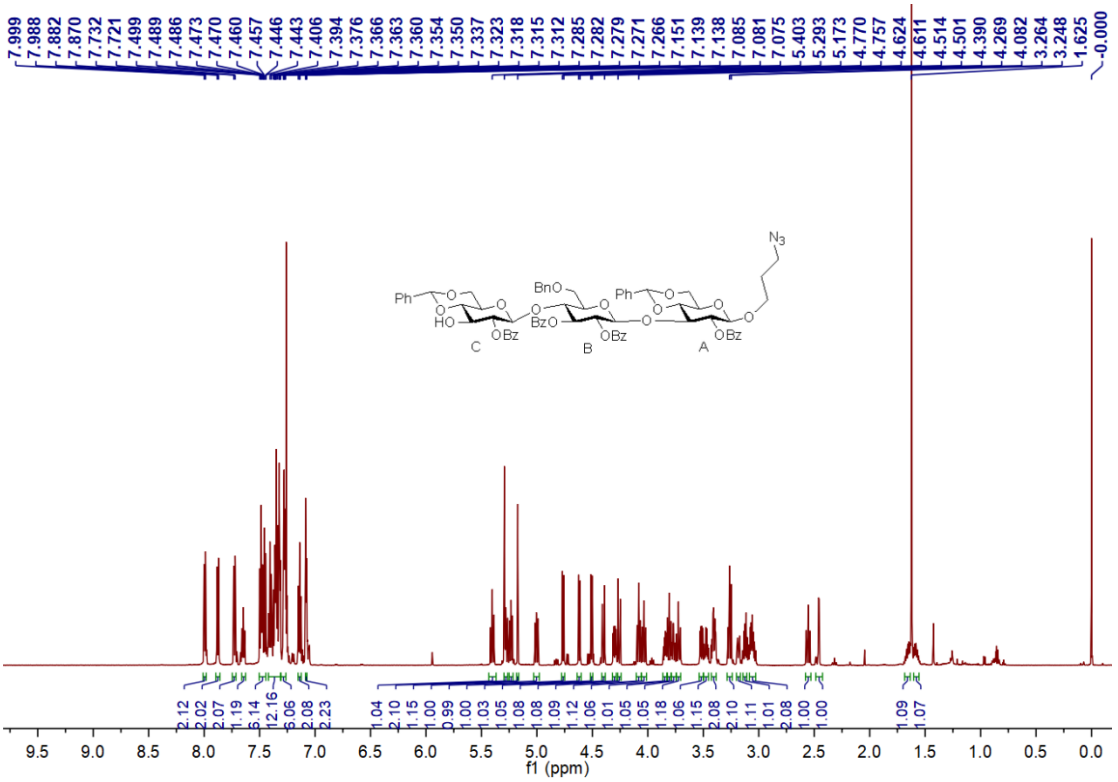
^{13}C NMR spectrum of compound **15** (150 MHz, $CDCl_3$)



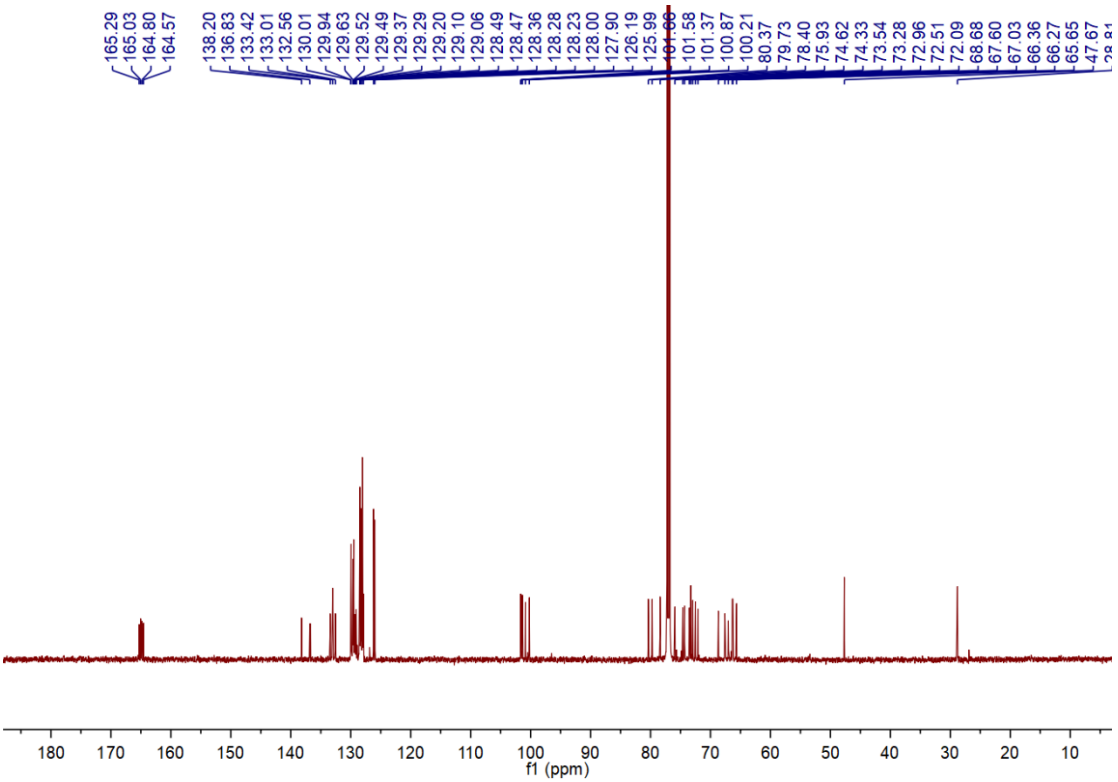
^{1}H-^{1}H COSY spectrum of compound **15** (600/600 MHz, $CDCl_3$)



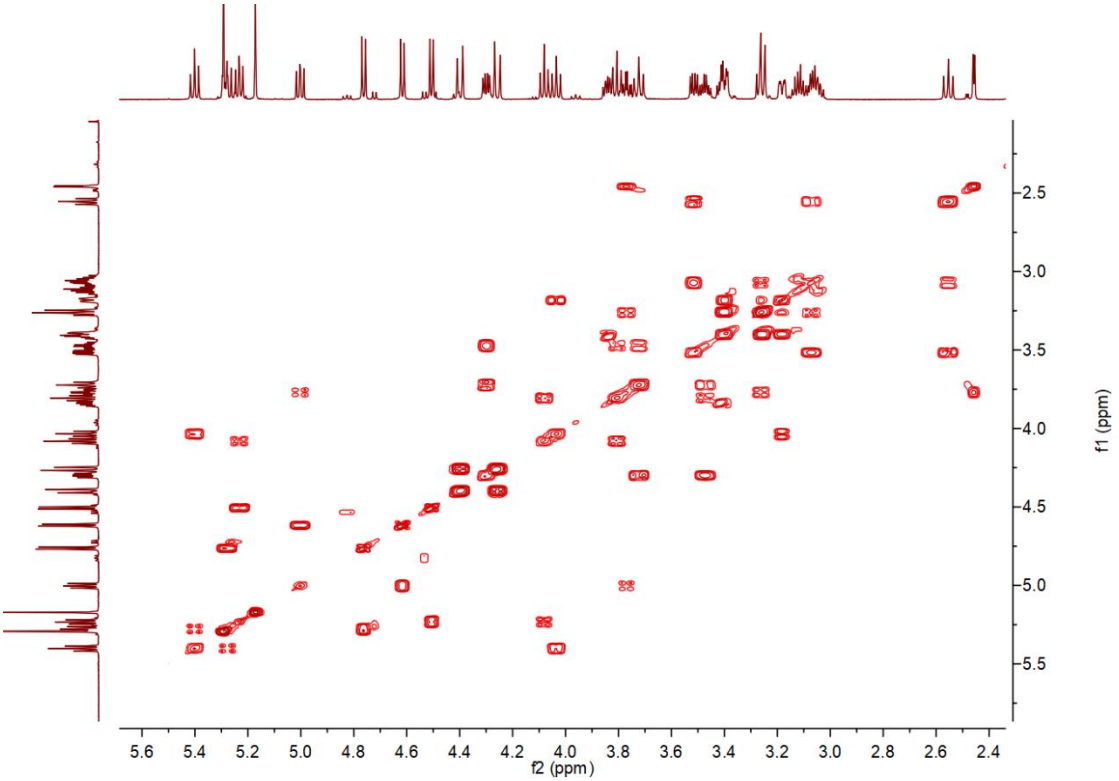
^{1}H-^{13}C HSQC spectrum of compound **15** (600/150 MHz, $CDCl_3$)



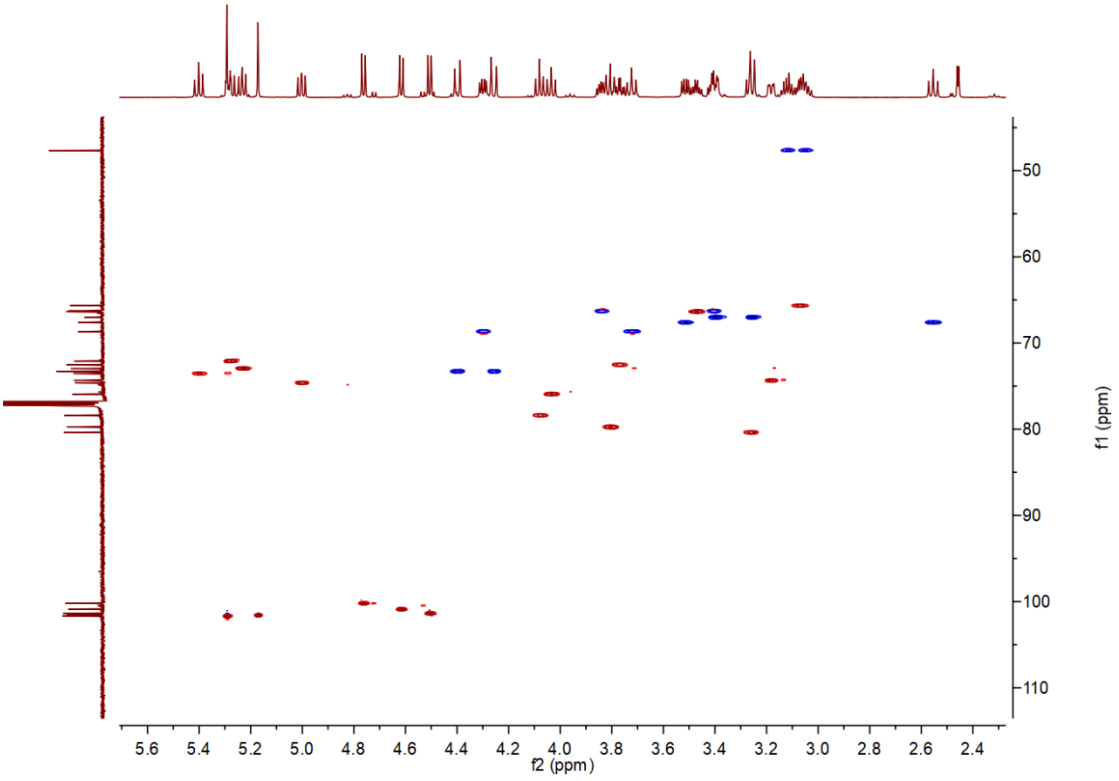
^{1}H NMR spectrum of compound **16** (600 MHz, $CDCl_3$)



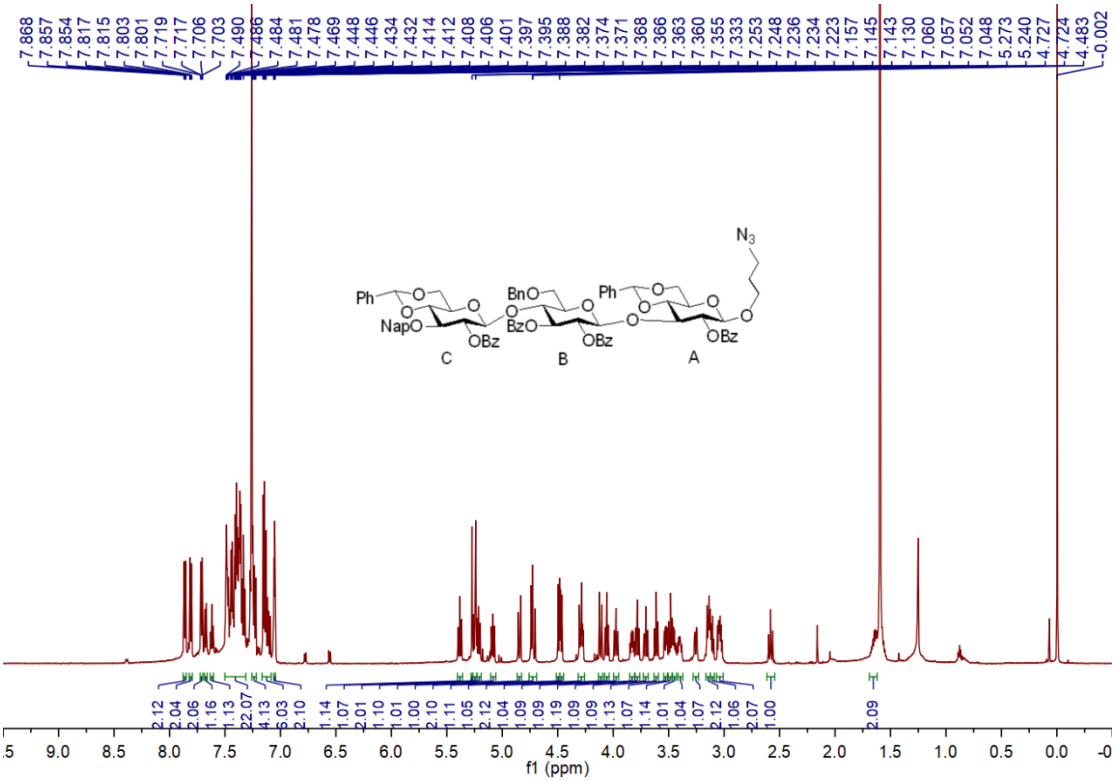
^{13}C NMR spectrum of compound **16** (150 MHz, $CDCl_3$)



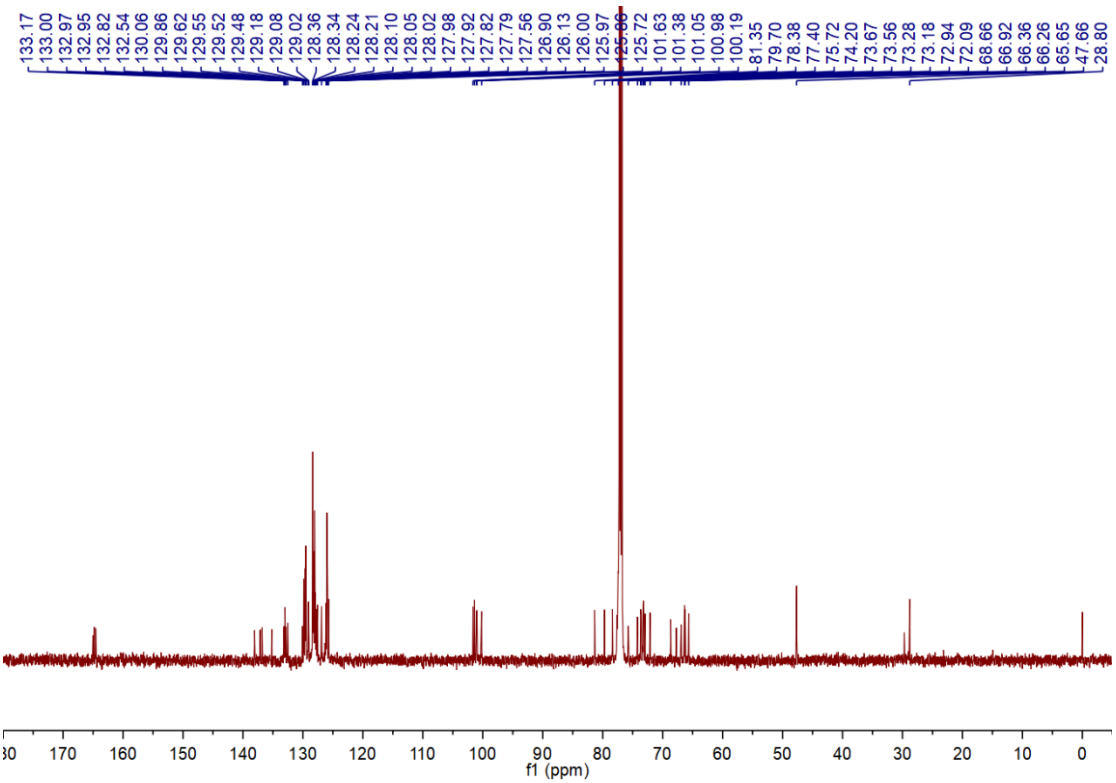
^{1}H-^{1}H COSY spectrum of compound **16** (600/600 MHz, $CDCl_3$)



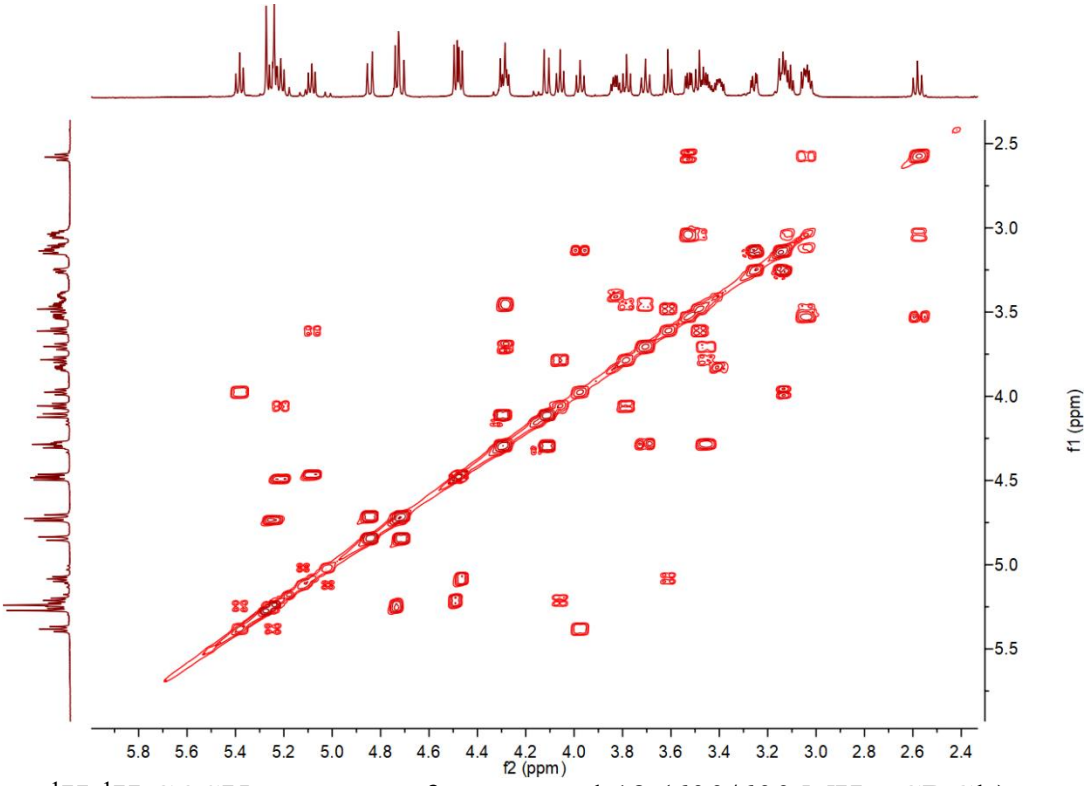
^{1}H-^{13}C HSQC spectrum of compound **16** (600/150 MHz, $CDCl_3$)



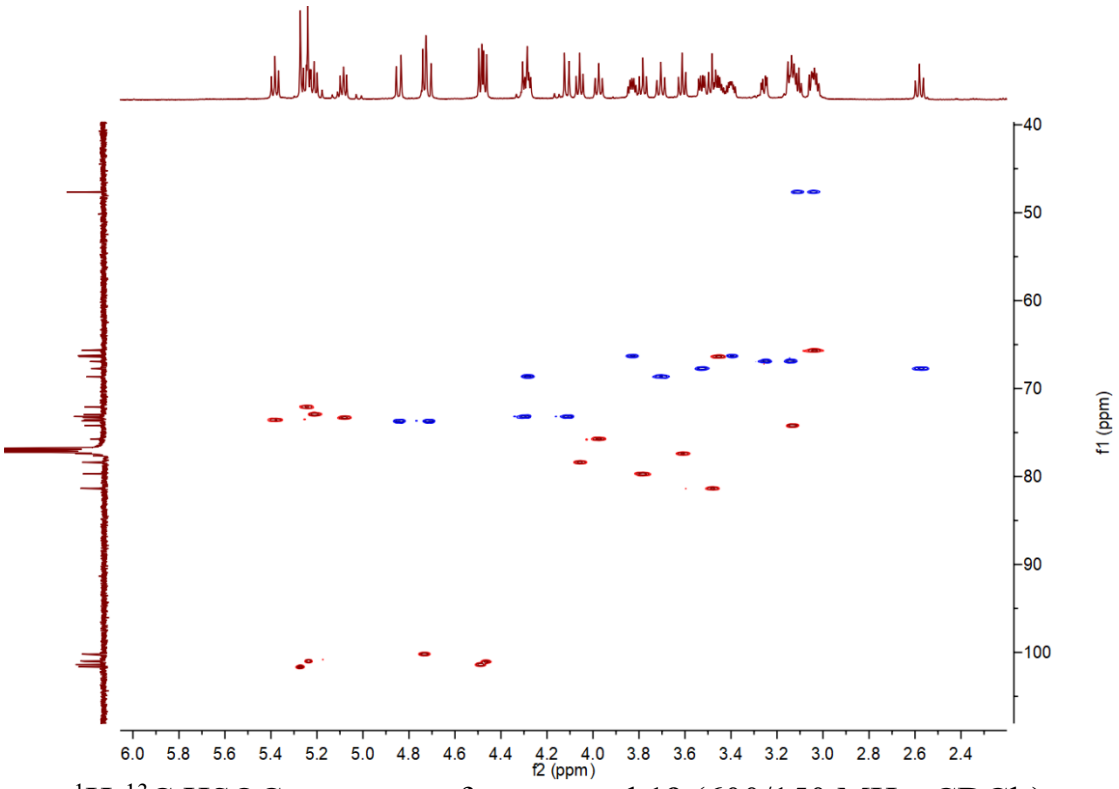
^{1}H NMR spectrum of compound **19** (600 MHz, $CDCl_3$)



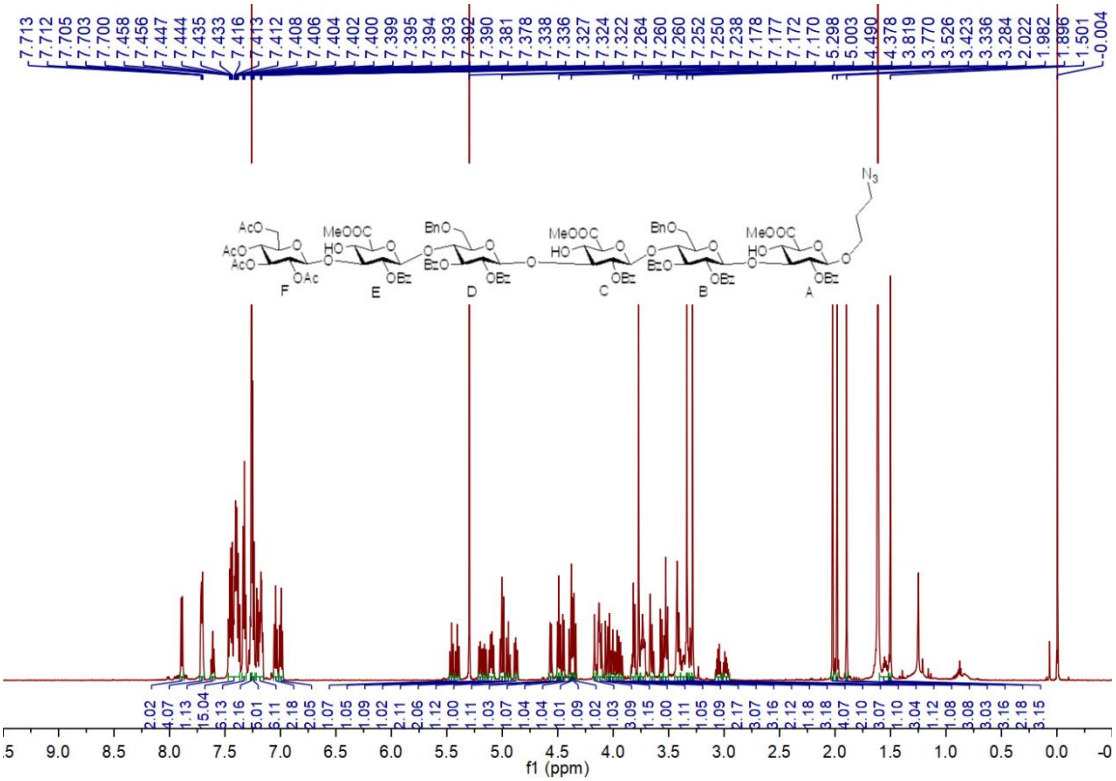
^{13}C NMR spectrum of compound **19** (150 MHz, $CDCl_3$)



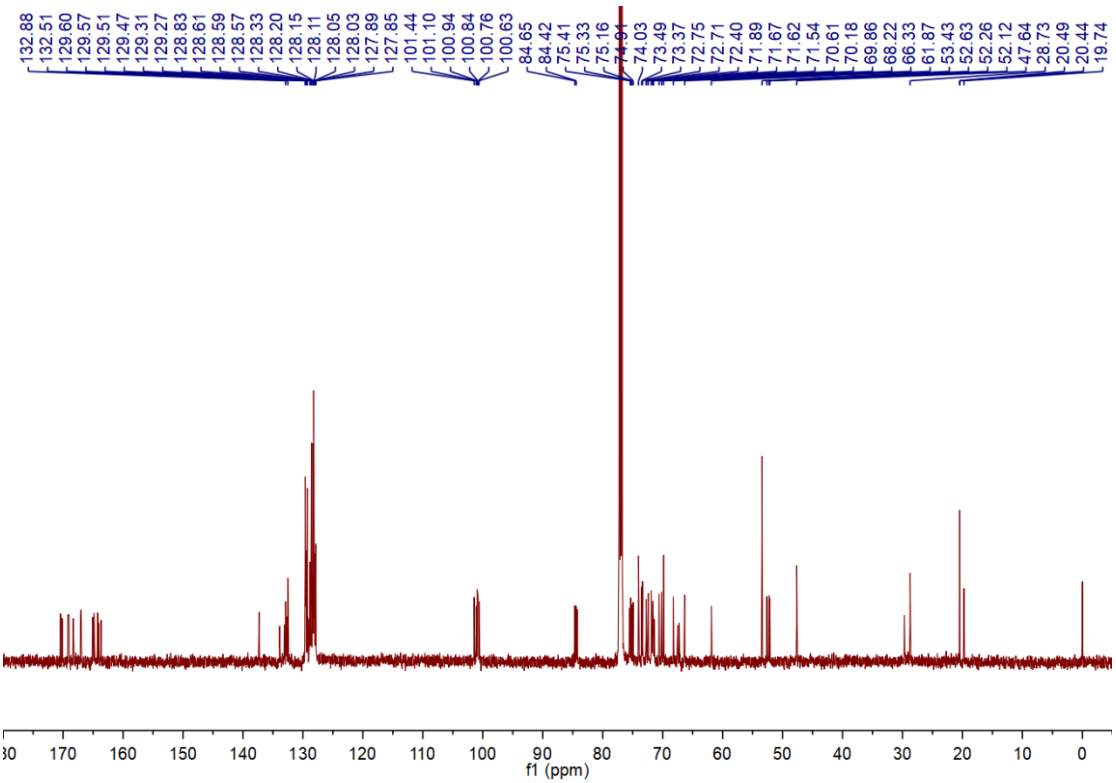
^{1}H-^{1}H COSY spectrum of compound **19** (600/600 MHz, $CDCl_3$)



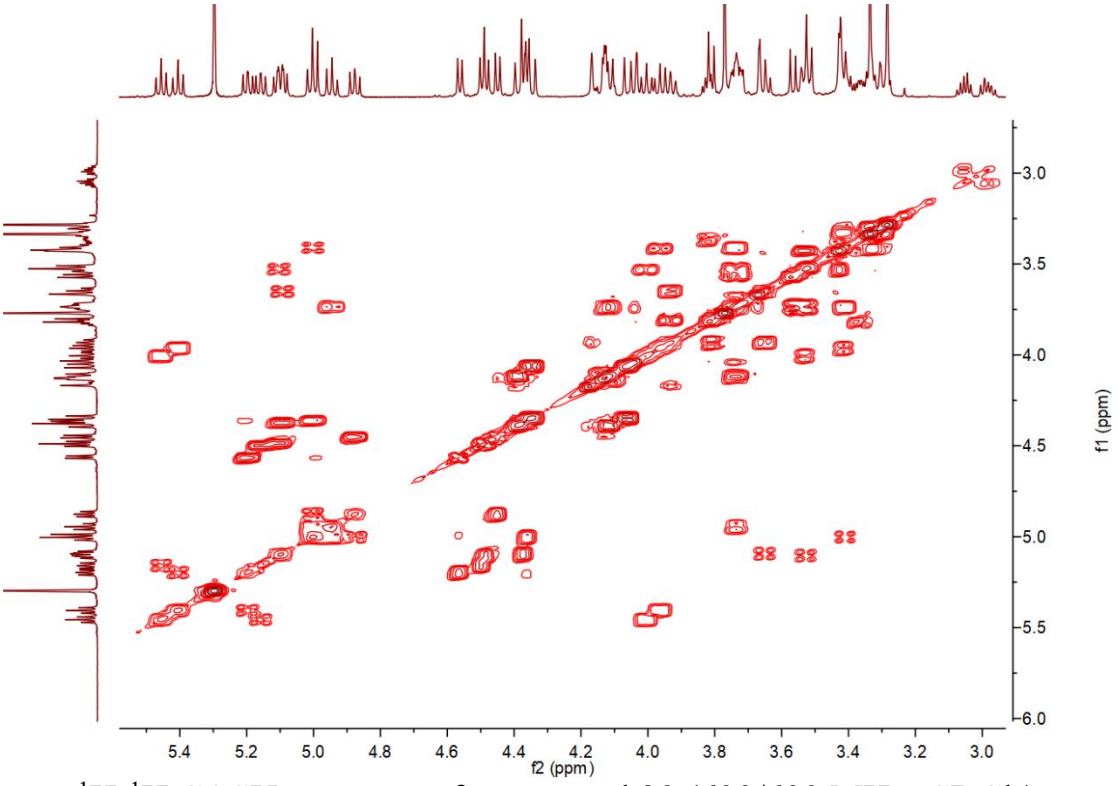
^{1}H-^{13}C HSQC spectrum of compound **19** (600/150 MHz, $CDCl_3$)



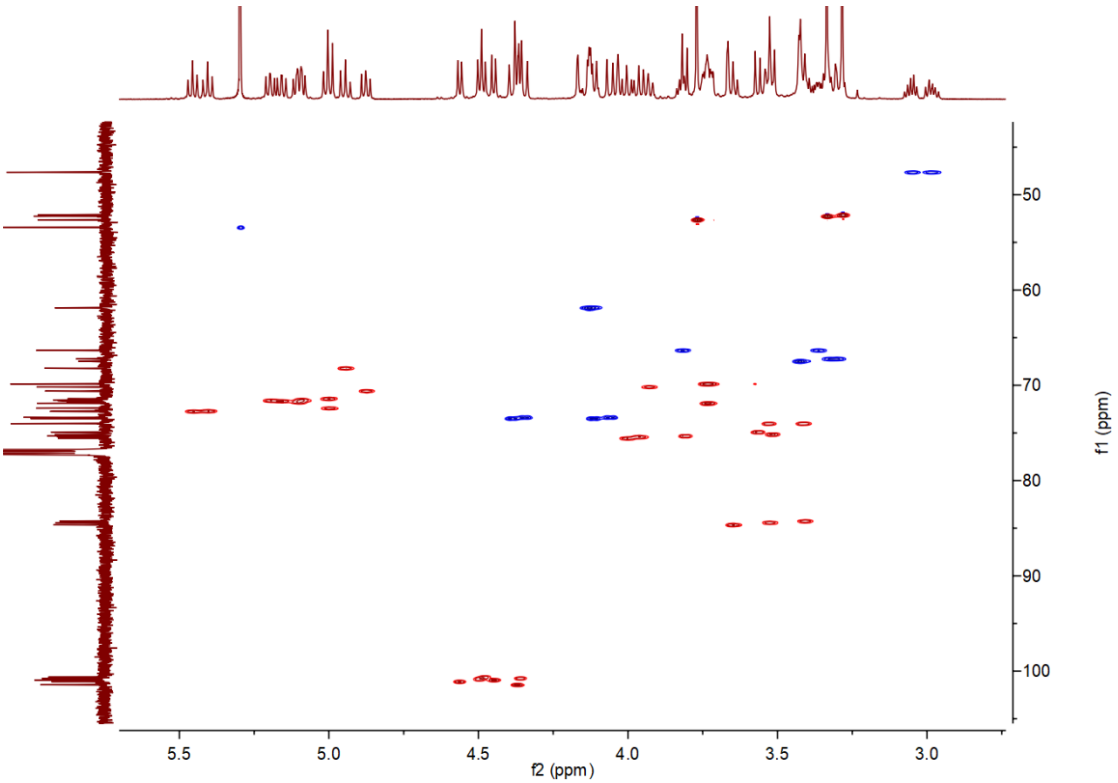
^{1}H NMR spectrum of compound **20** (600 MHz, $CDCl_3$)



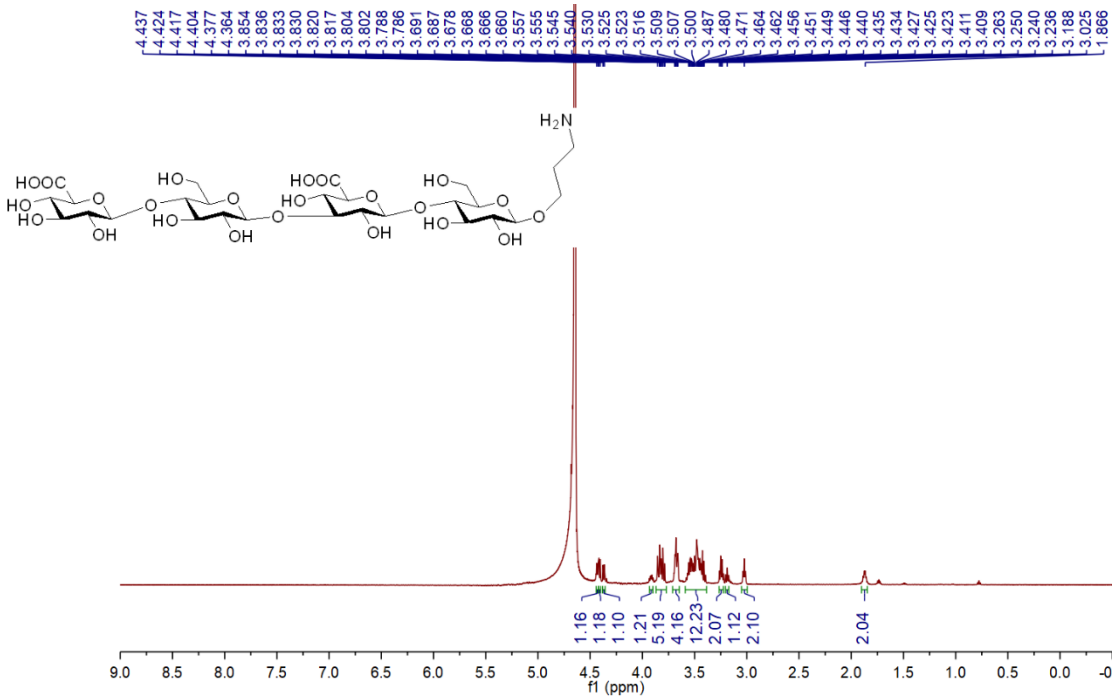
^{13}C NMR spectrum of compound **20** (150 MHz, $CDCl_3$)



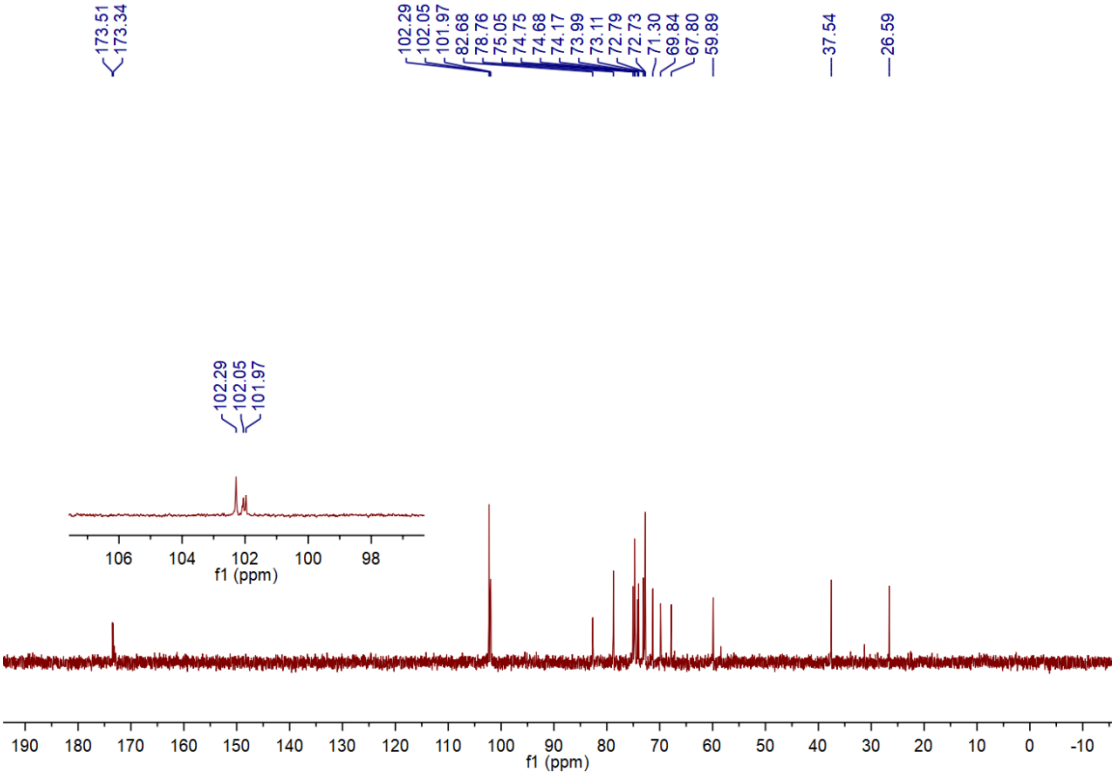
^{1}H-^{1}H COSY spectrum of compound **20** (600/600 MHz, $CDCl_3$)



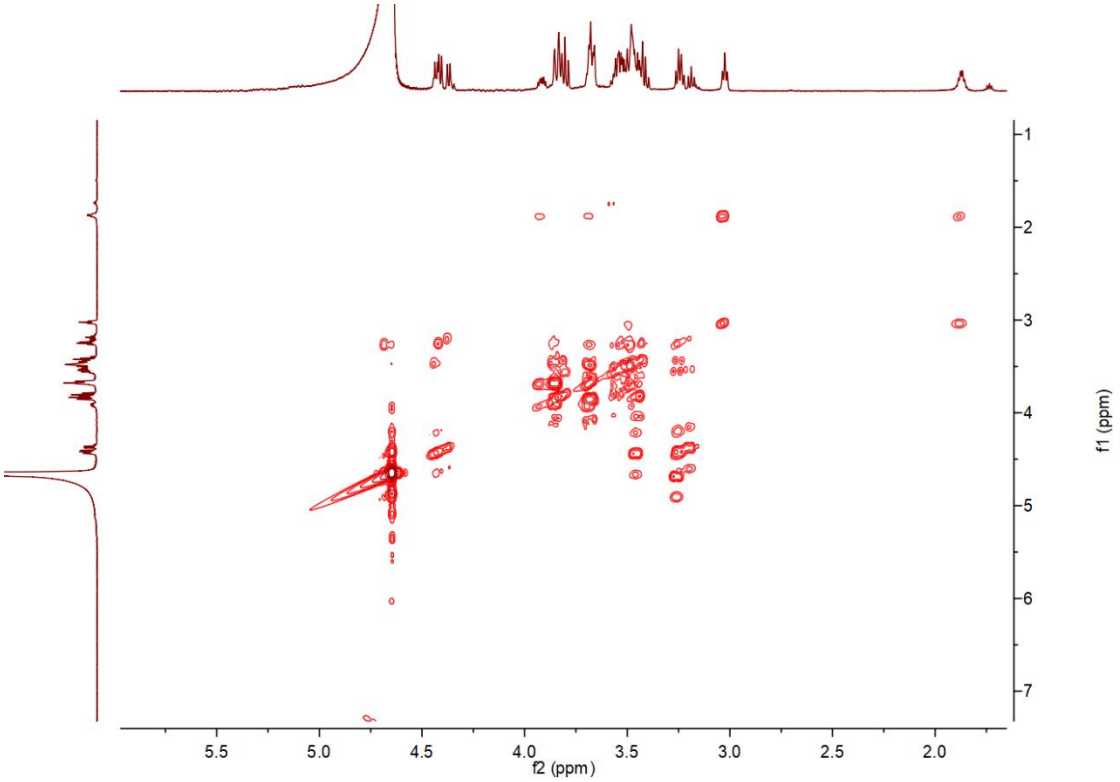
^{1}H-^{13}C HSQC spectrum of compound **20** (600/150 MHz, $CDCl_3$)



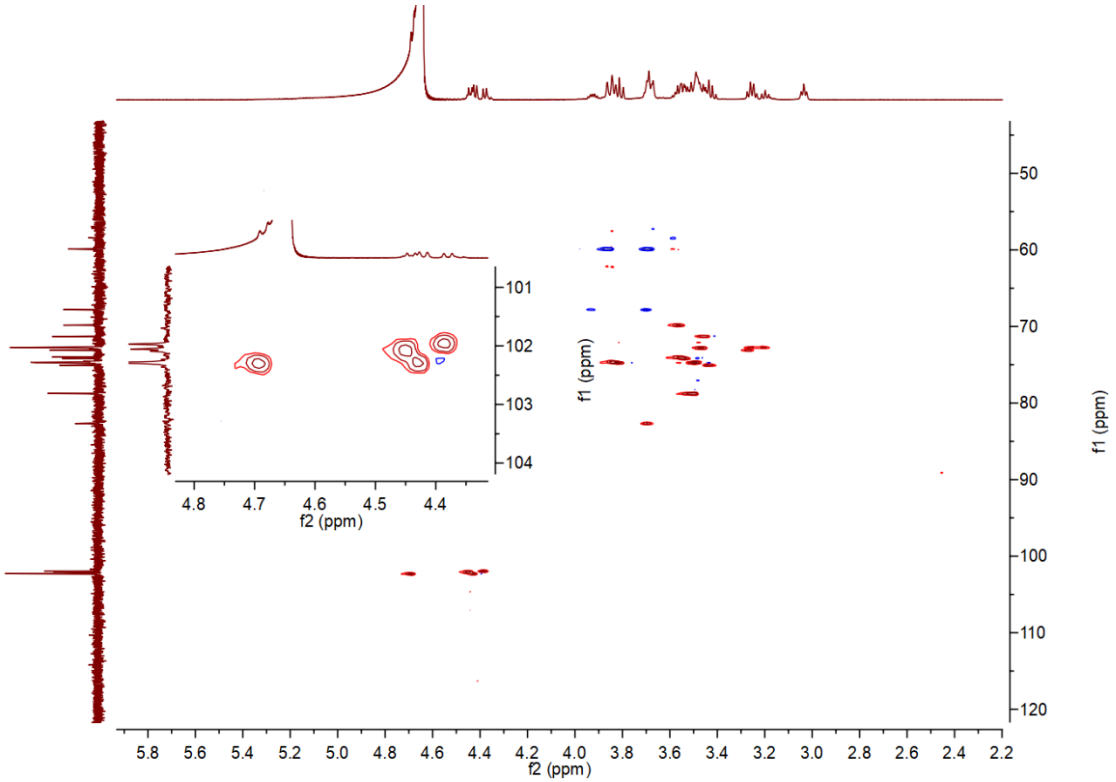
^{1}H NMR spectrum of compound **1a** (600 MHz, D_2O)



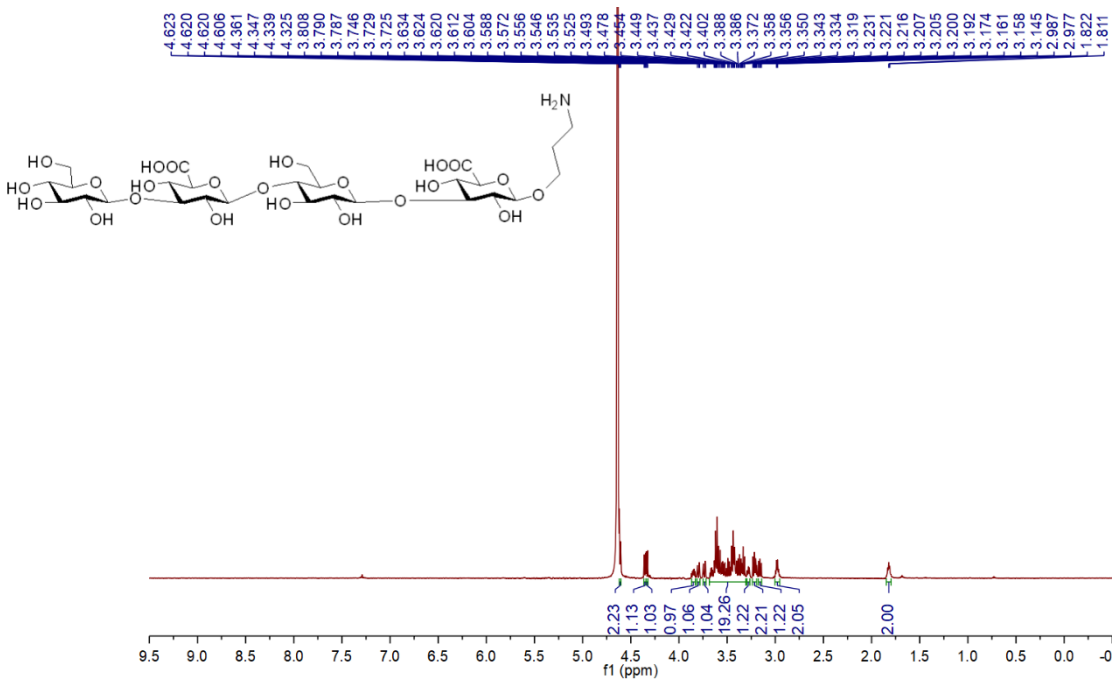
^{13}C NMR spectrum of compound **1a** (150 MHz, D_2O)



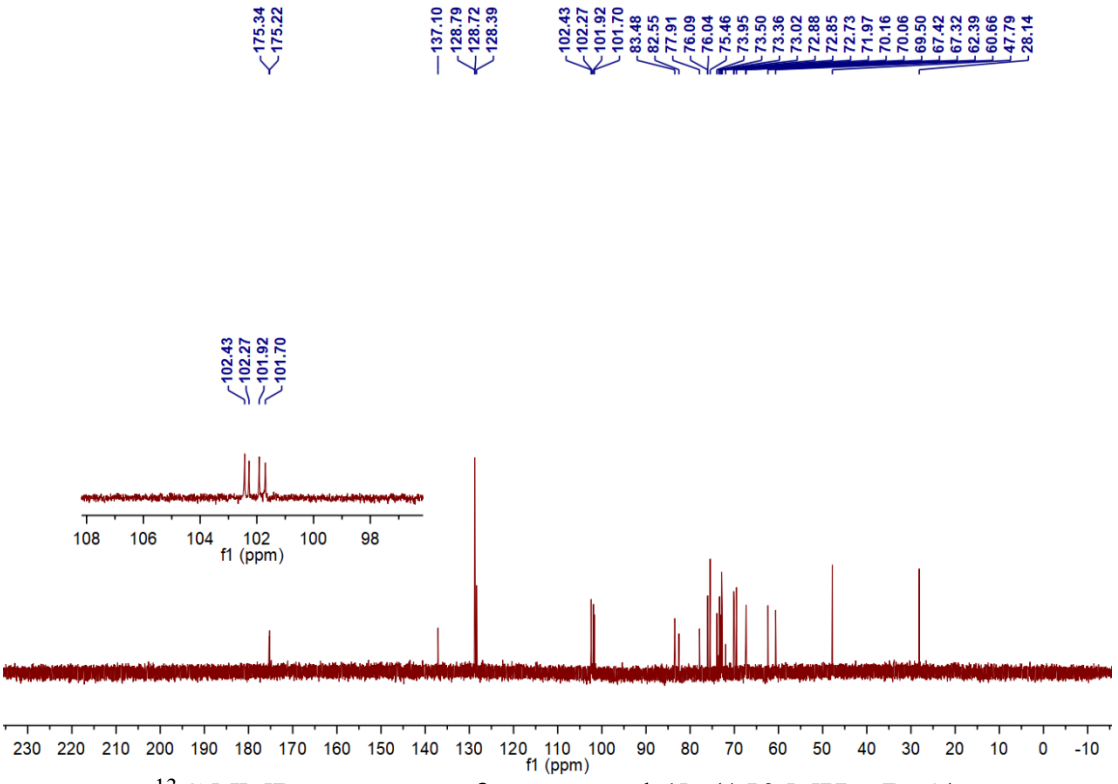
^{1}H-^{1}H COSY spectrum of compound **1a** (600/600 MHz, D_2O)



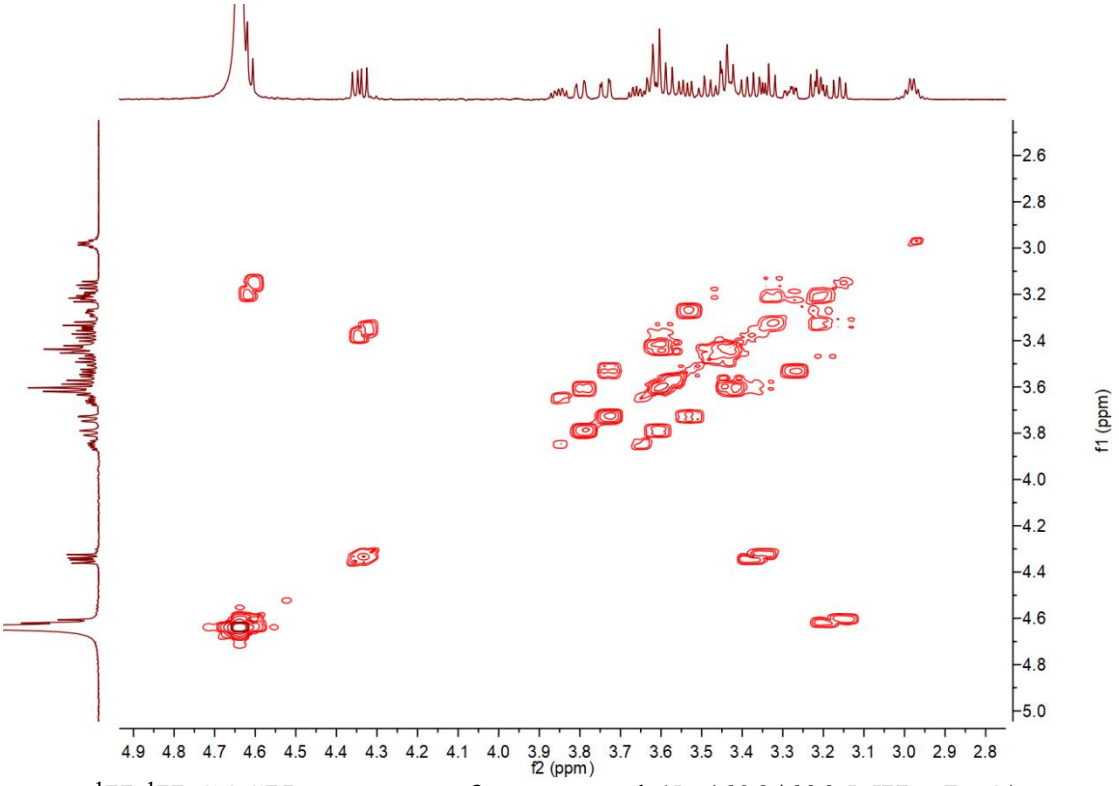
^{1}H-^{13}C HSQC spectrum of compound **1a** (600/150 MHz, D_2O)



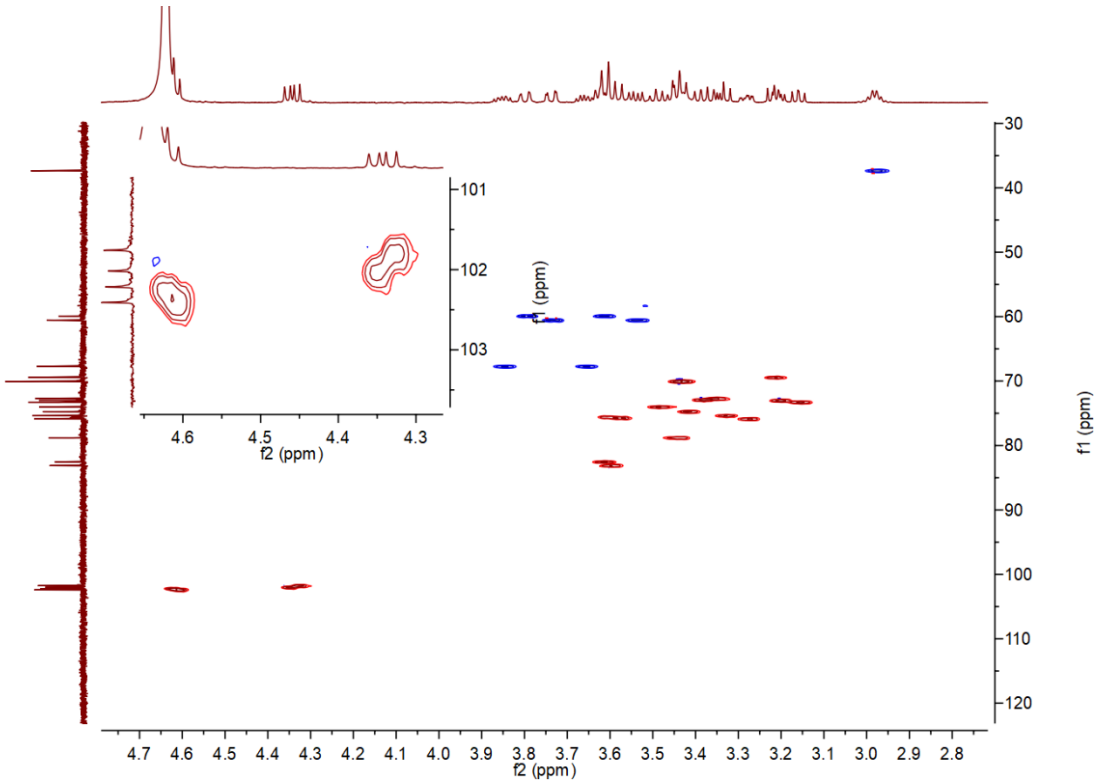
^{1}H NMR spectrum of compound **1b** (600 MHz, D_2O)



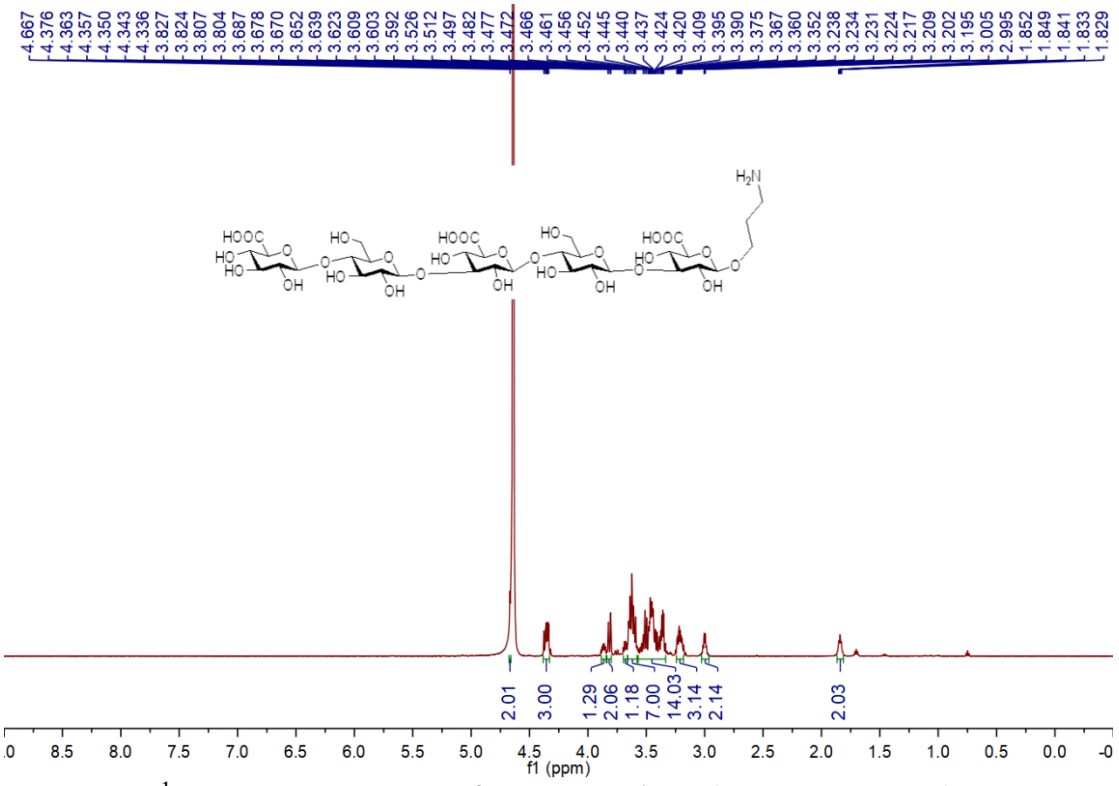
^{13}C NMR spectrum of compound **1b** (150 MHz, D_2O)



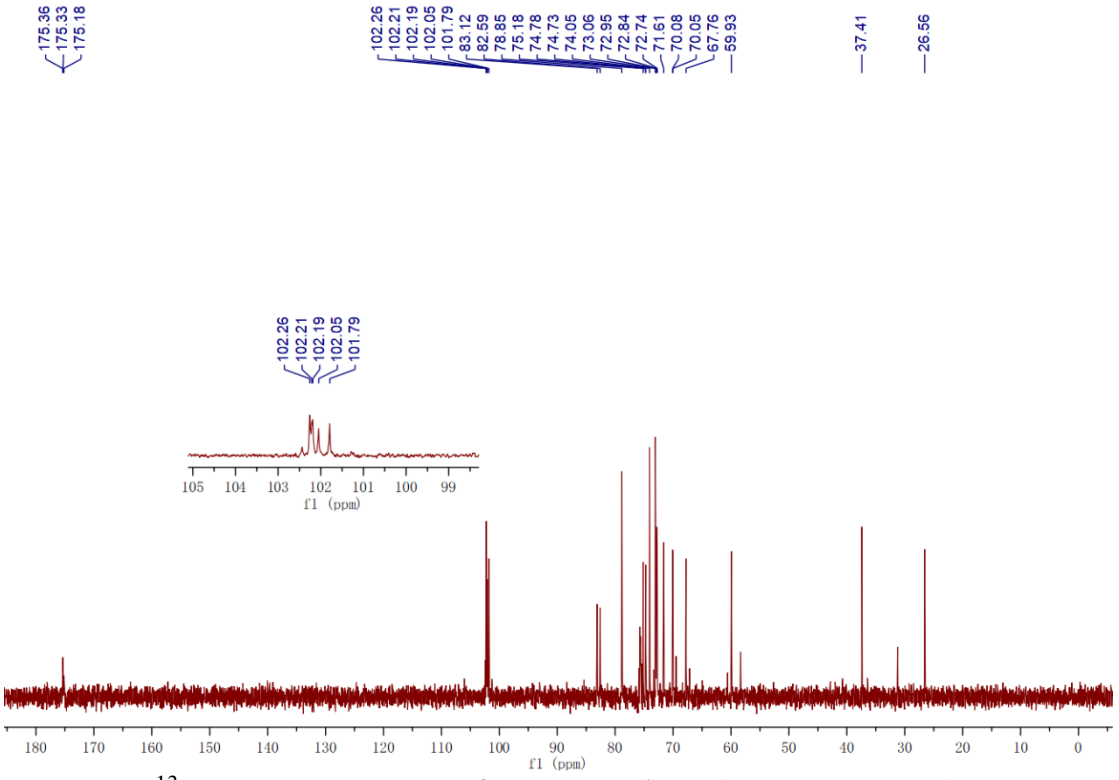
^{1}H-^{1}H COSY spectrum of compound **1b** (600/600 MHz, D_2O)



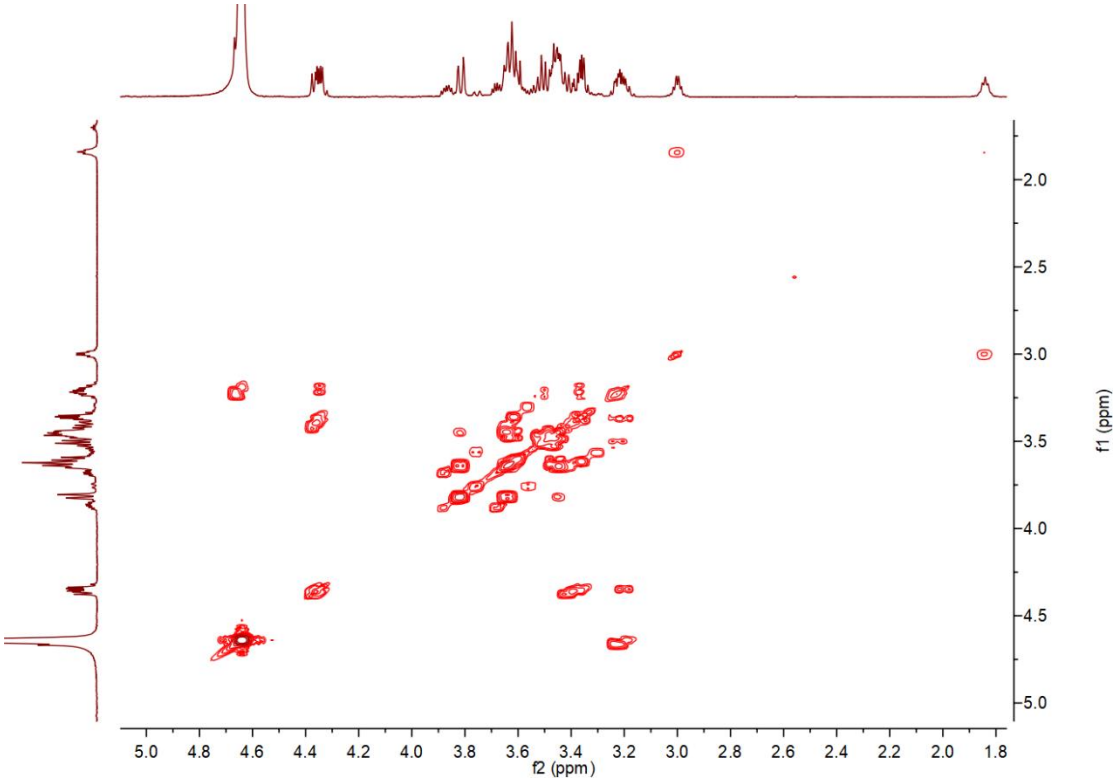
^{1}H-^{13}C HSQC spectrum of compound **1b** (600/150 MHz, D_2O)



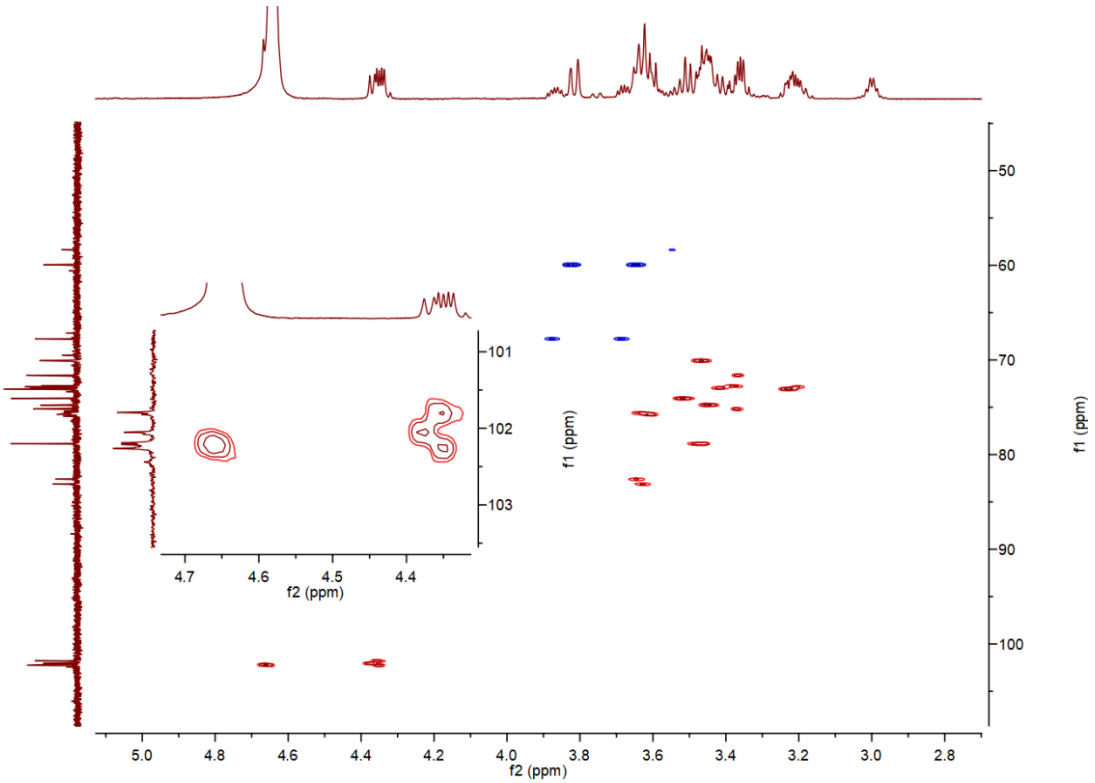
^{1}H NMR spectrum of compound **2b** (600 MHz, D_2O)



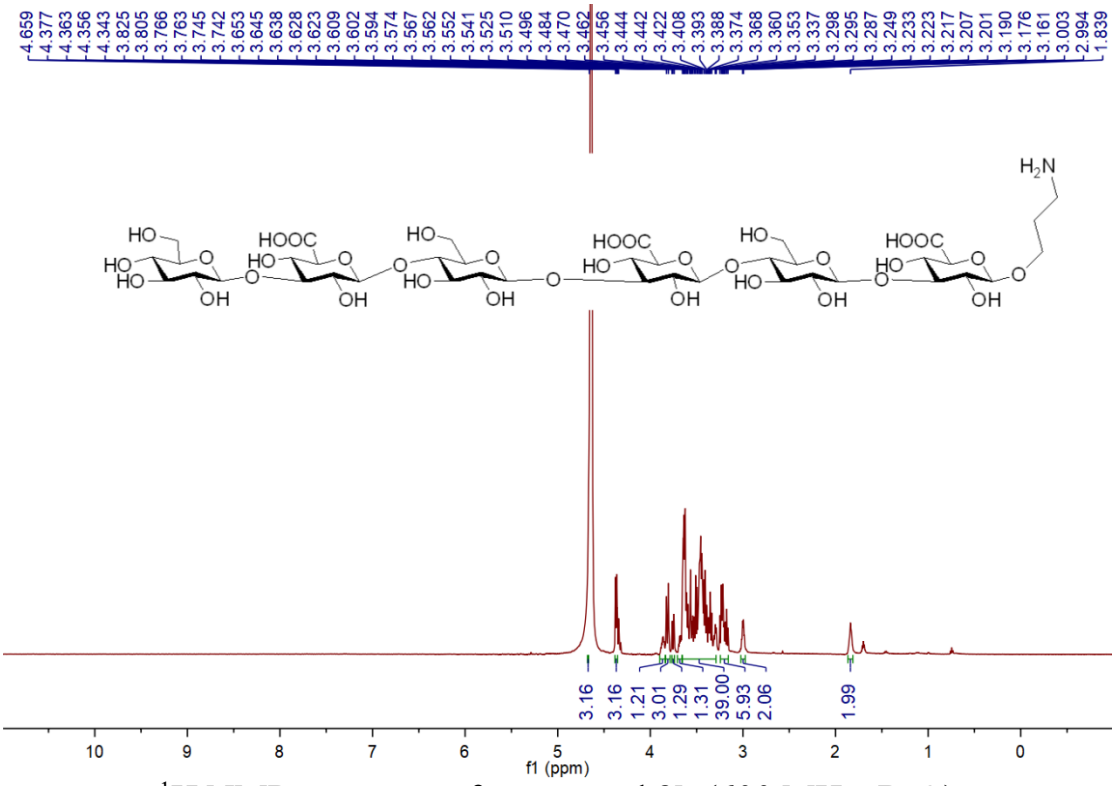
^{13}C NMR spectrum of compound **2b** (150 MHz, D_2O)



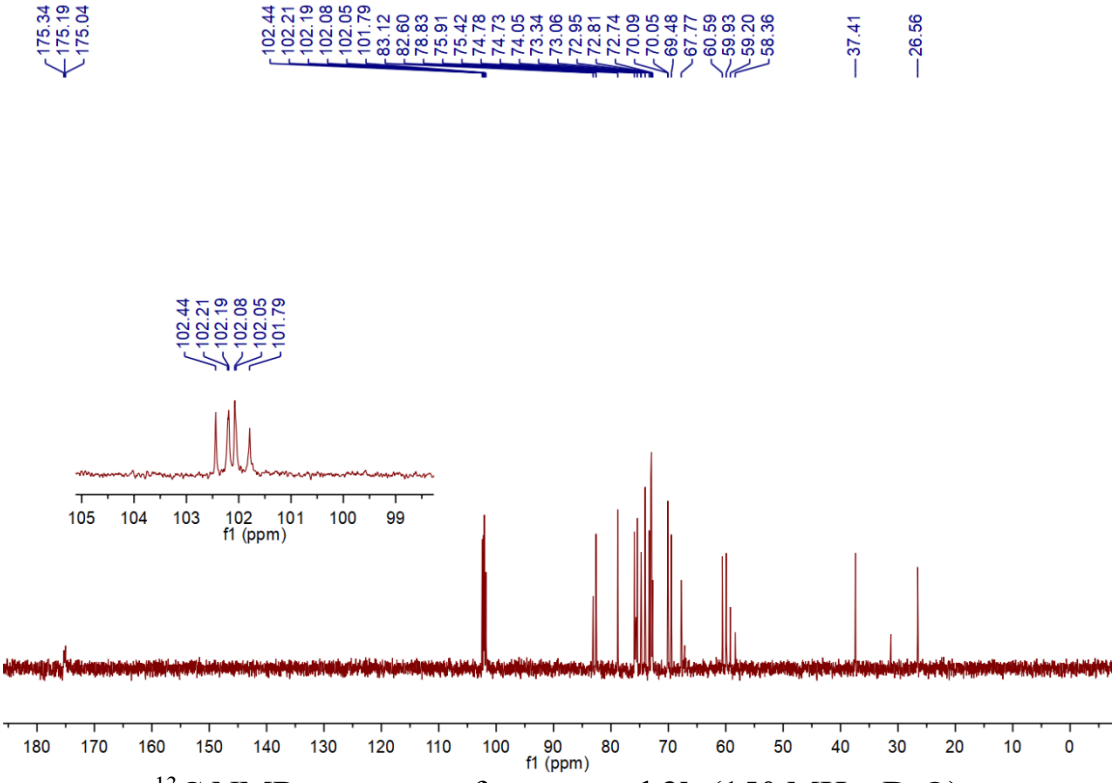
^{1}H-^{1}H COSY spectrum of compound **2b** (600/600 MHz, D_2O)



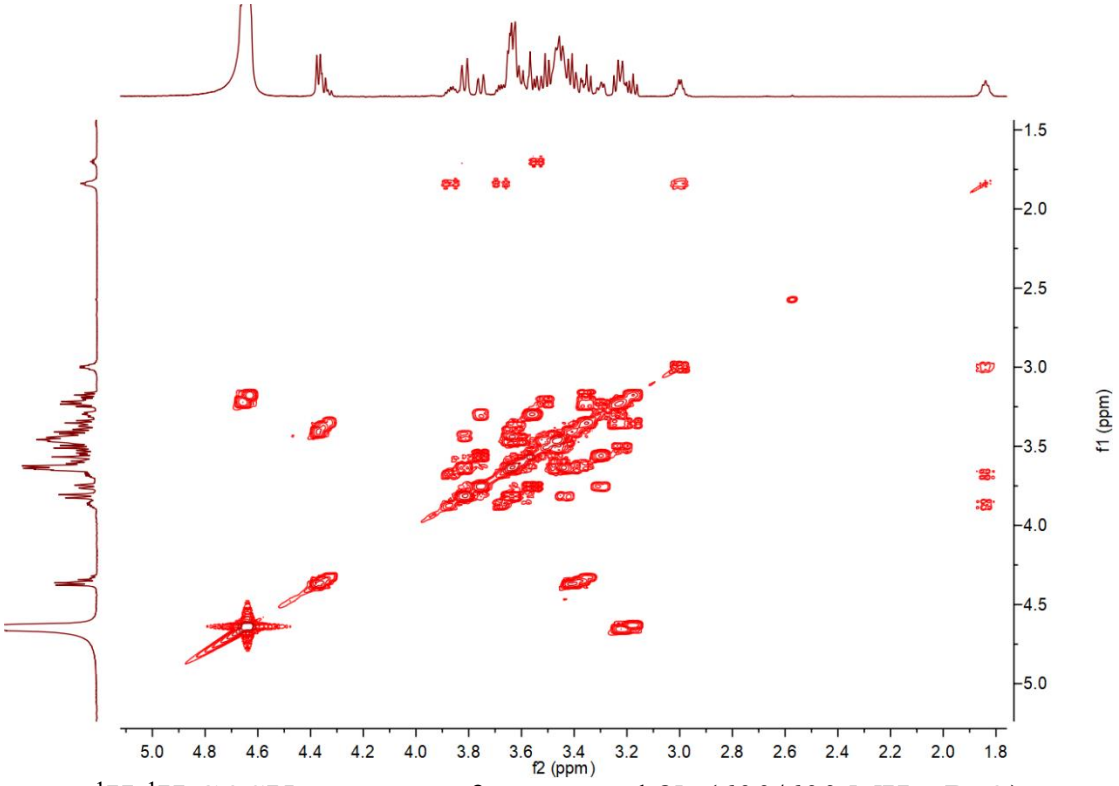
^{1}H-^{13}C HSQC spectrum of compound **2b** (600/150 MHz, D_2O)



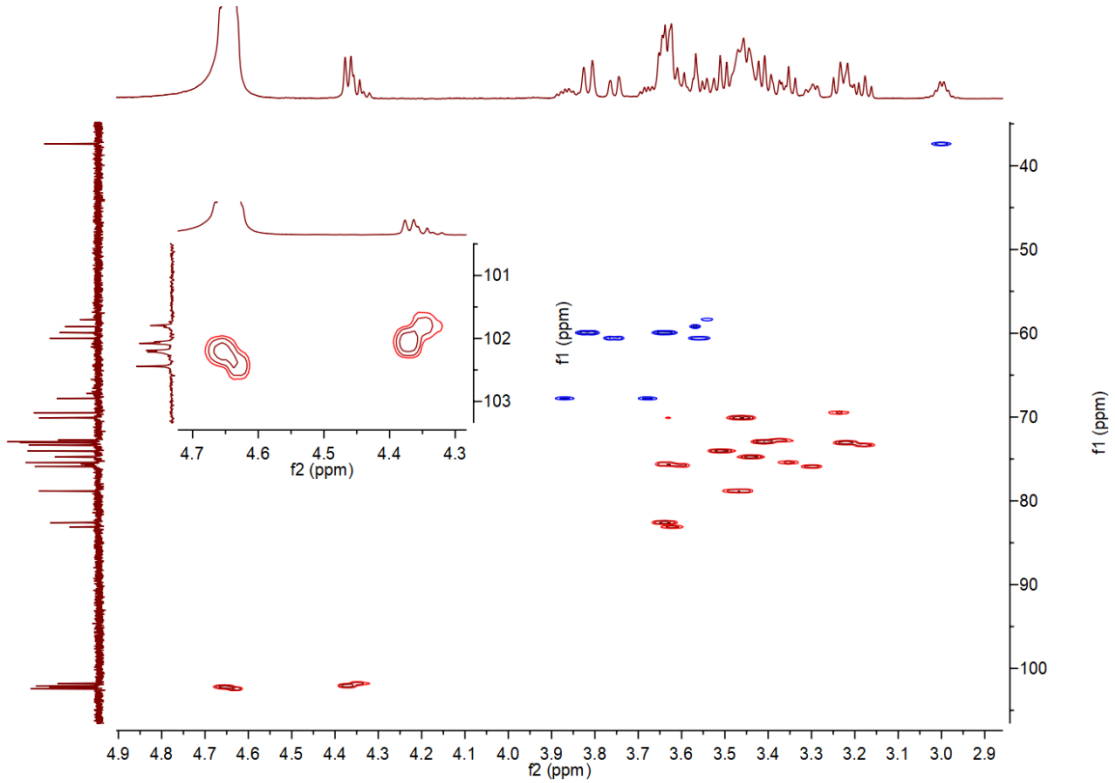
^{1}H NMR spectrum of compound **3b** (600 MHz, D_2O)



^{13}C NMR spectrum of compound **3b** (150 MHz, D_2O)

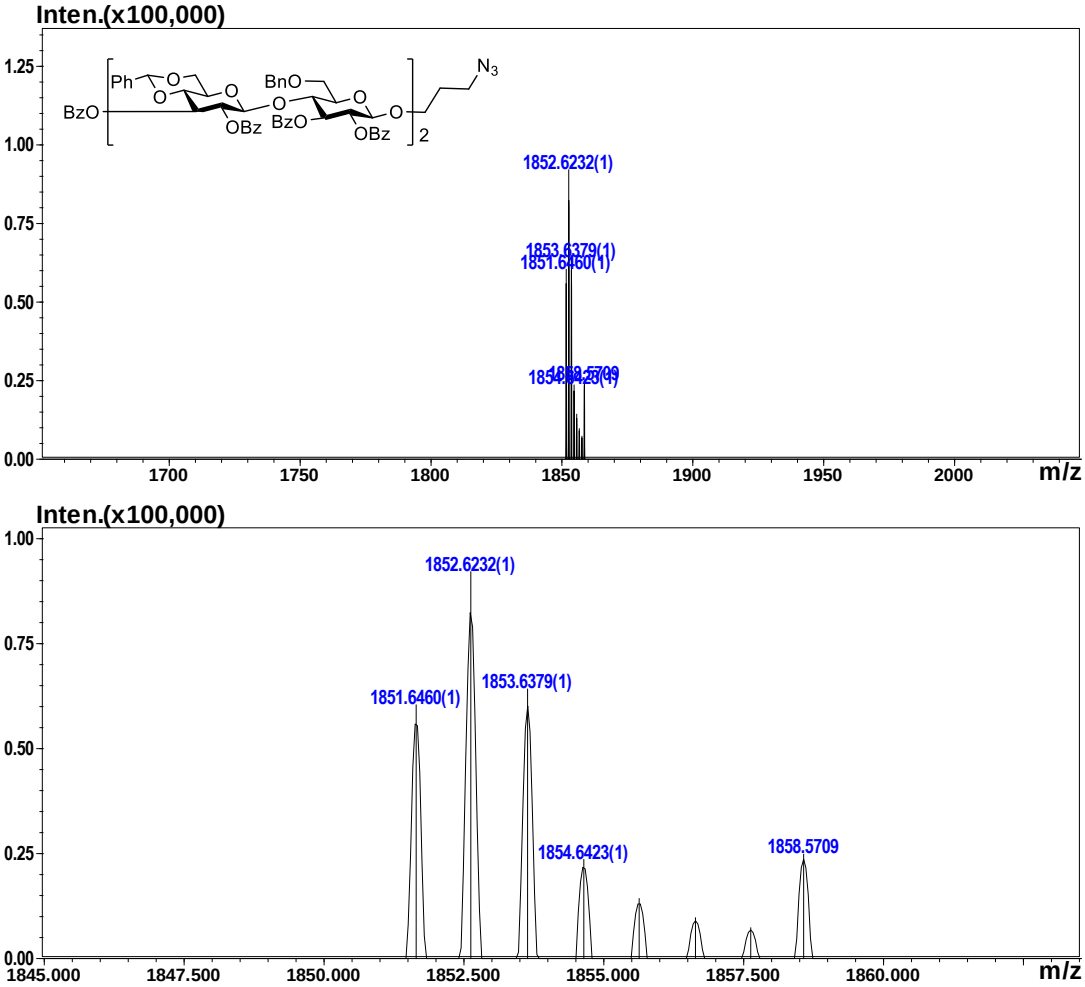


^{1}H-^{1}H COSY spectrum of compound **3b** (600/600 MHz, D_2O)

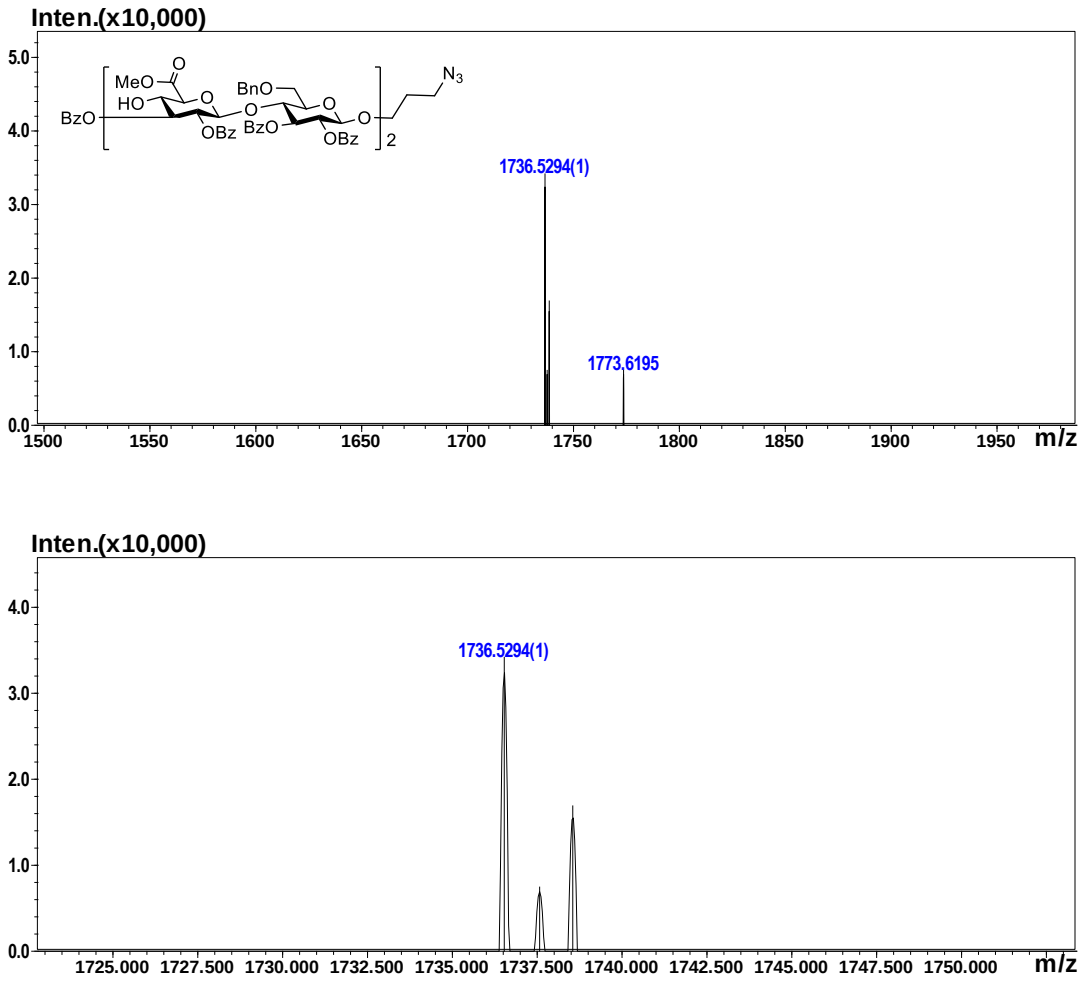


^{1}H-^{13}C HSQC spectrum of compound **3b** (600/150 MHz, D_2O)

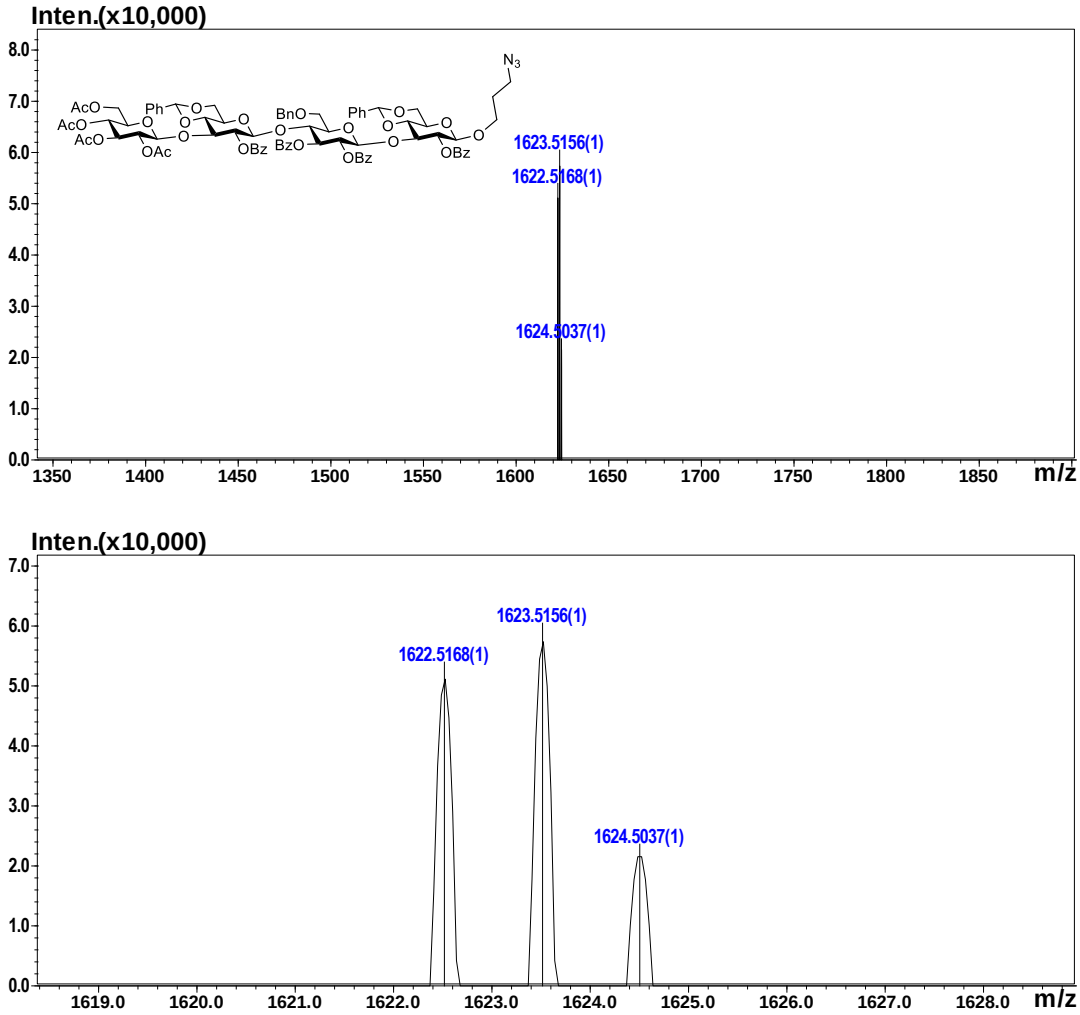
III. IT-TOF HRMS spectra of synthetic compounds



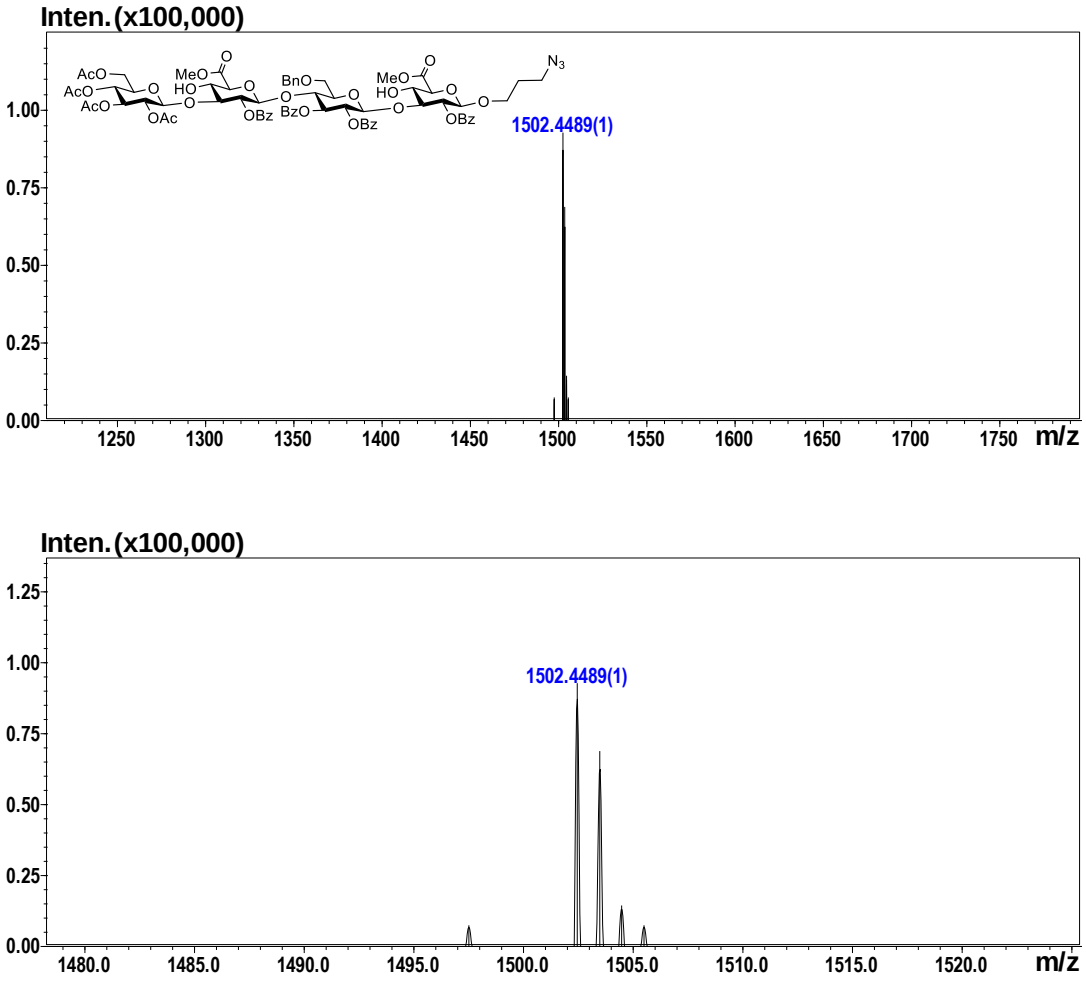
HR-ESI mass spectrum of compound **9**



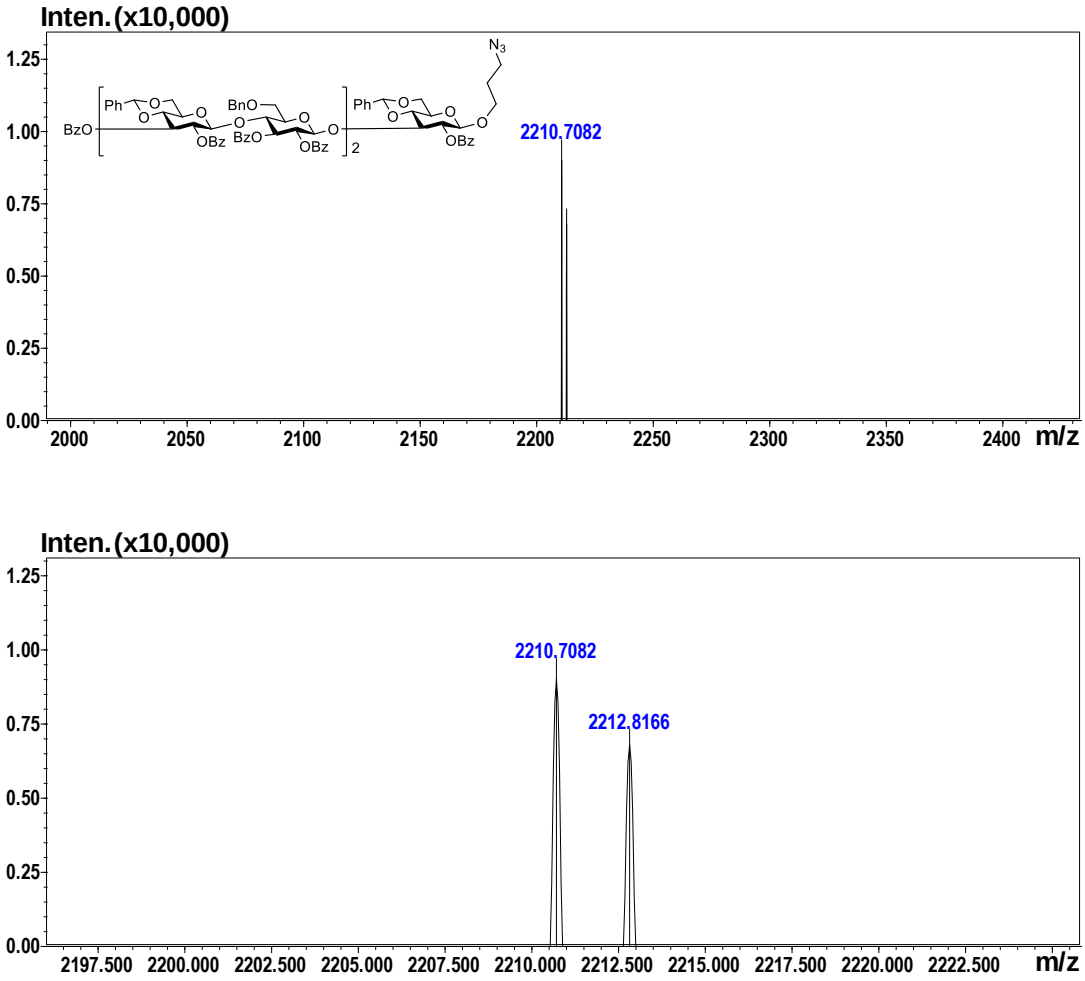
HR-ESI mass spectrum of compound **10**



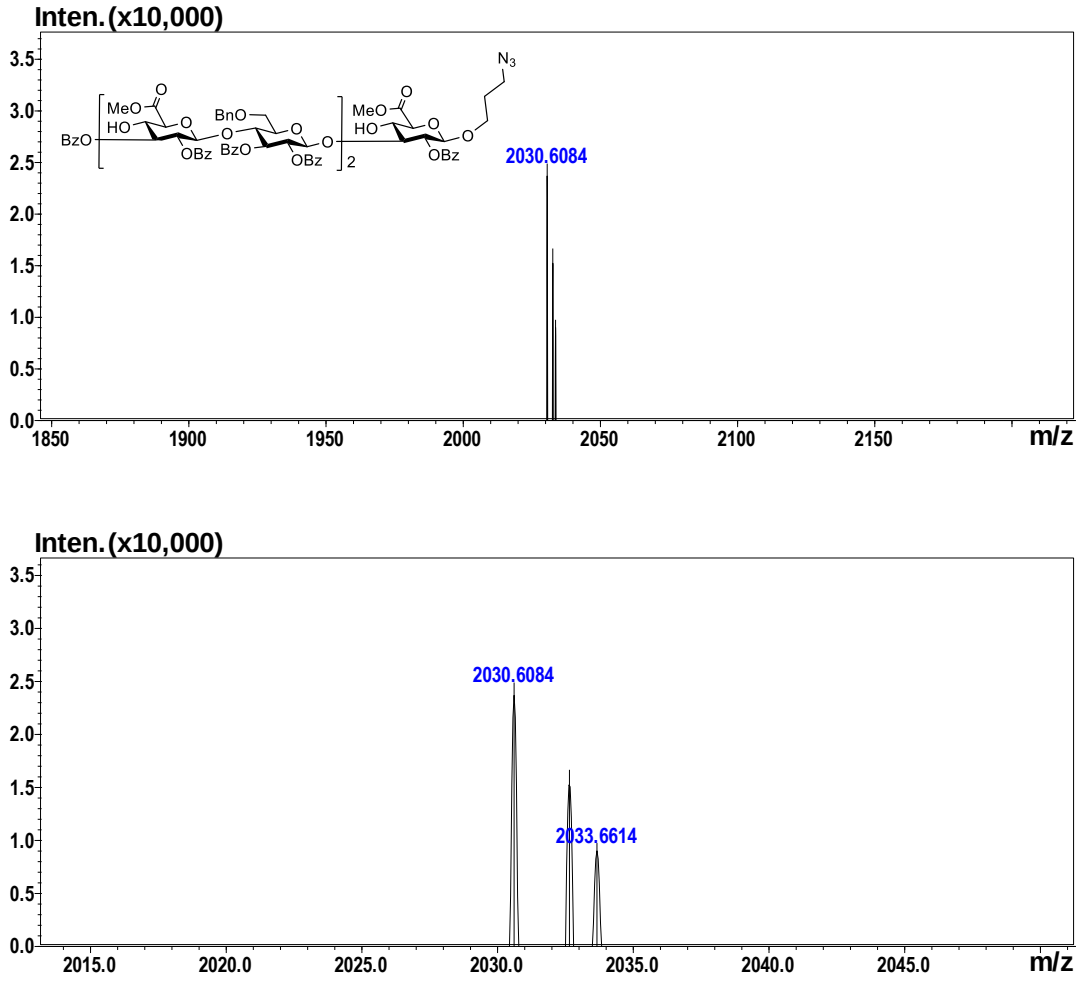
HR-ESI mass spectrum of compound **11**



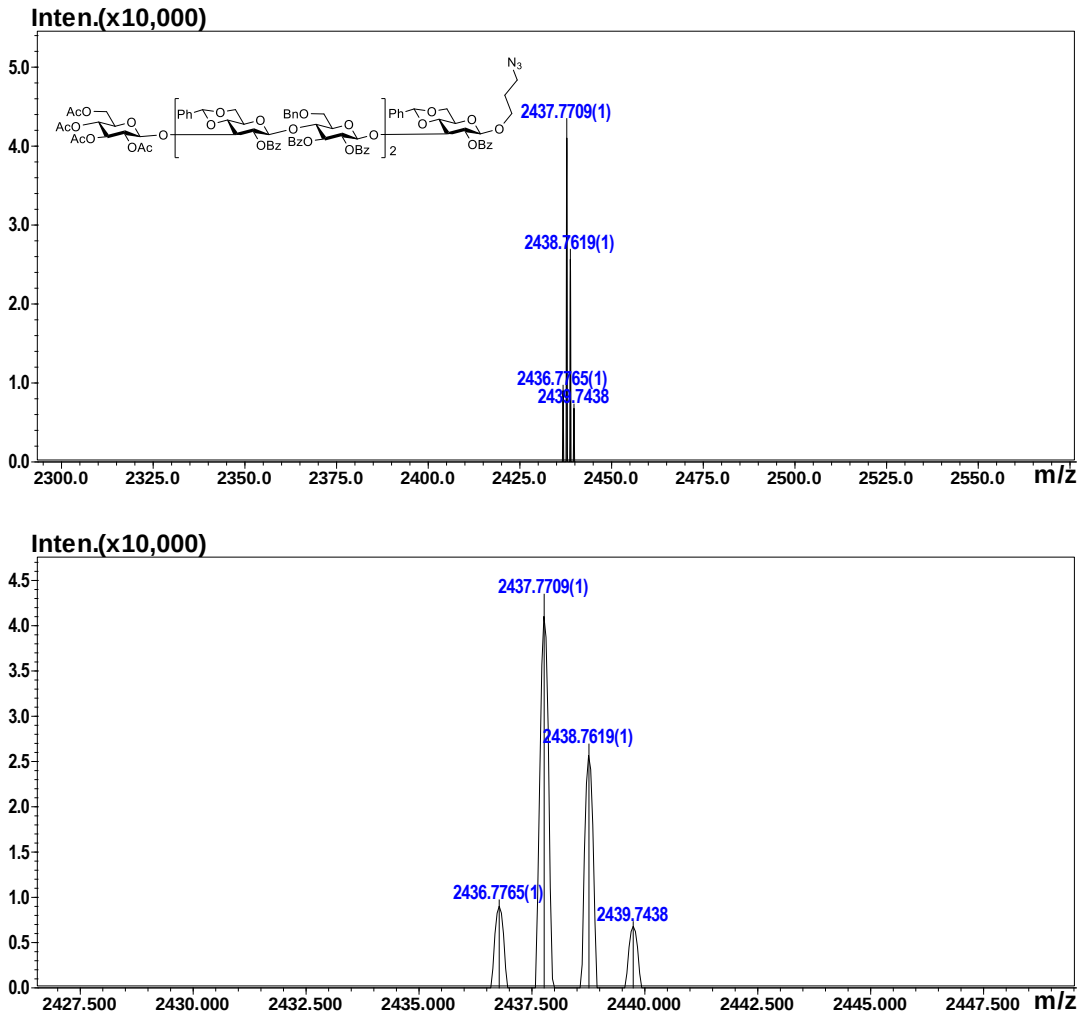
HR-ESI mass spectrum of compound **12**



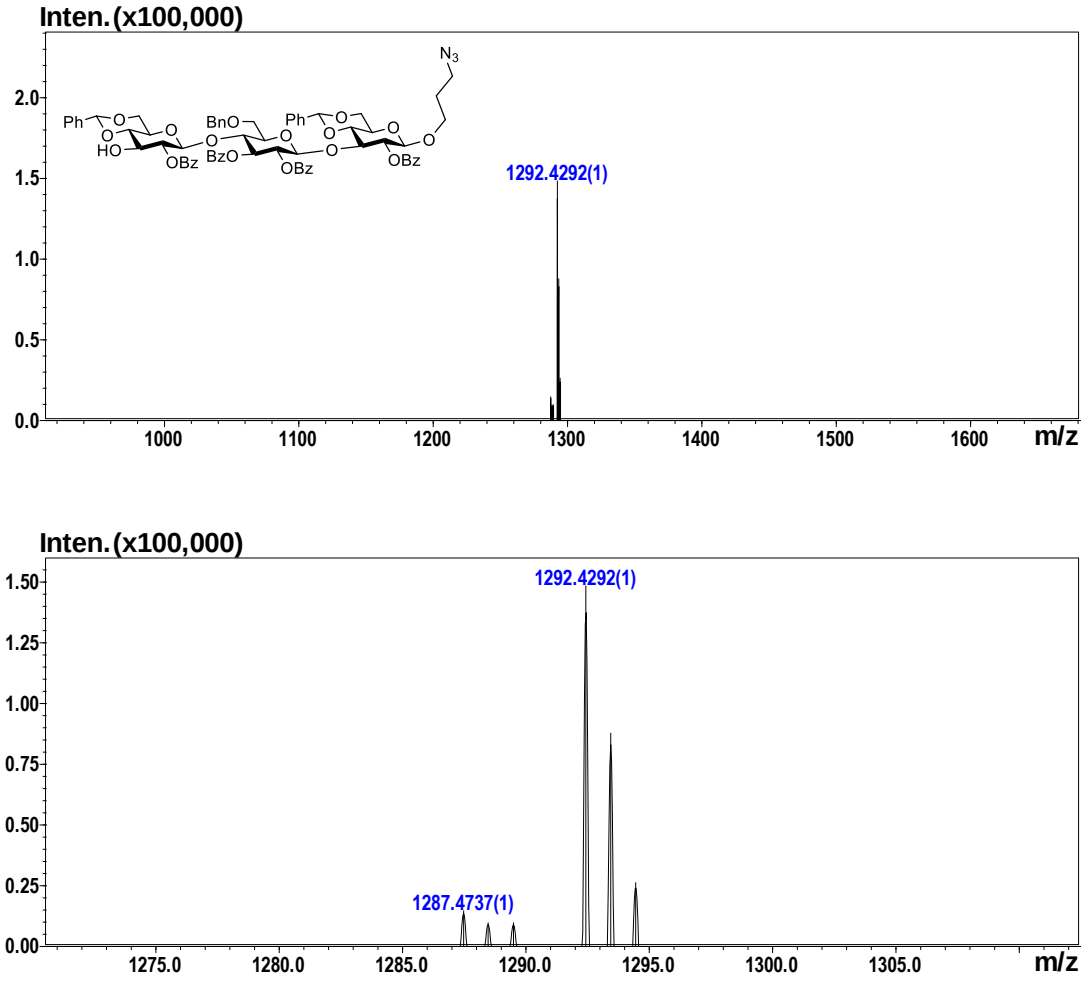
HR-ESI mass spectrum of compound **13**



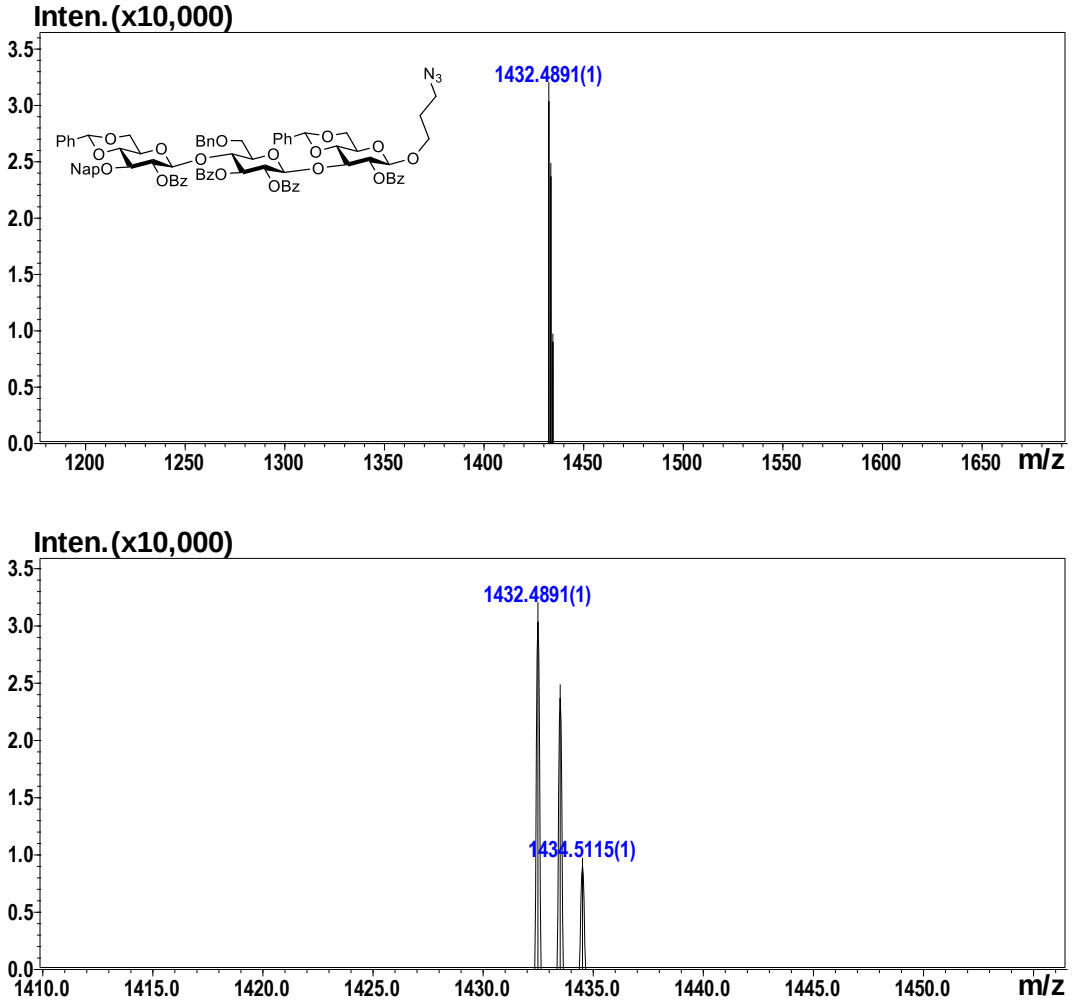
HR-ESI mass spectrum of compound **14**



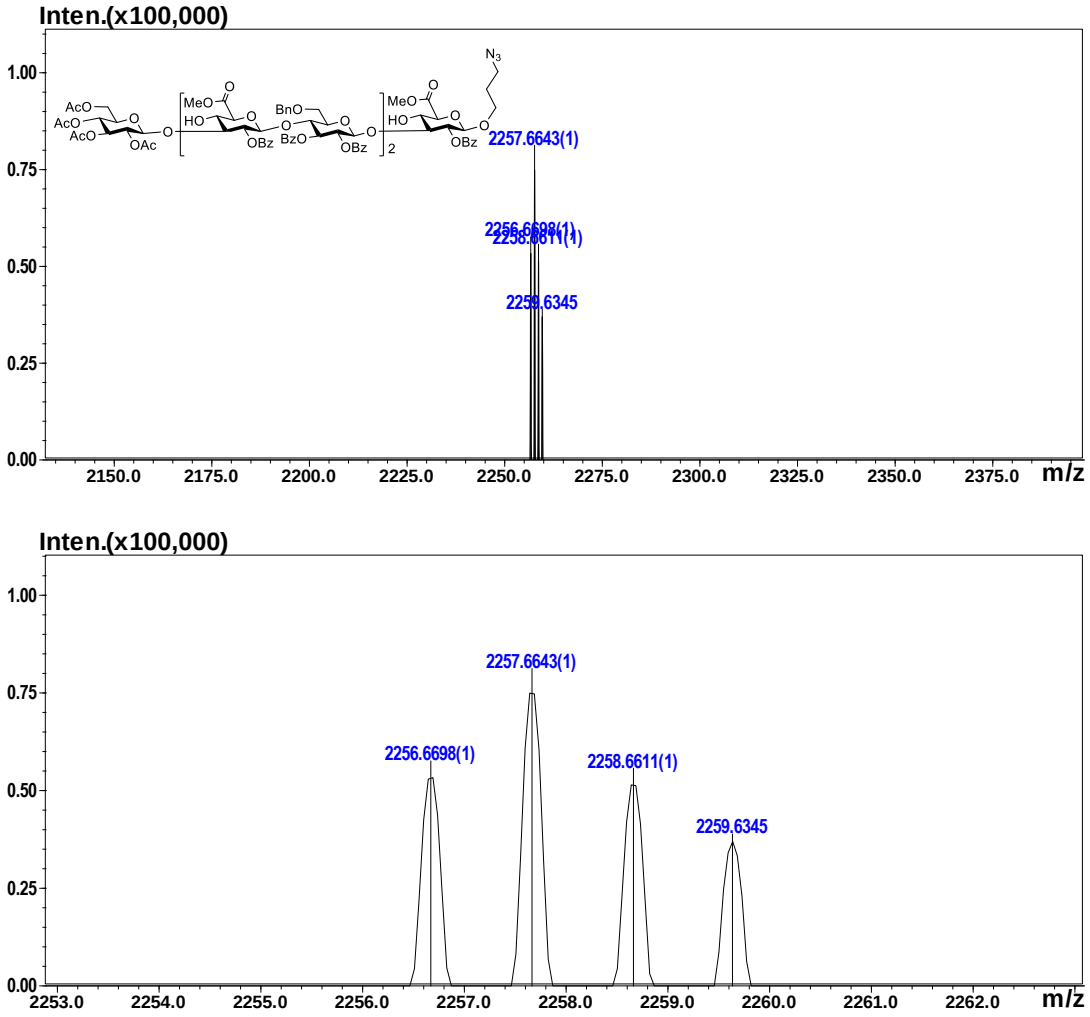
HR-ESI mass spectrum of compound **15**



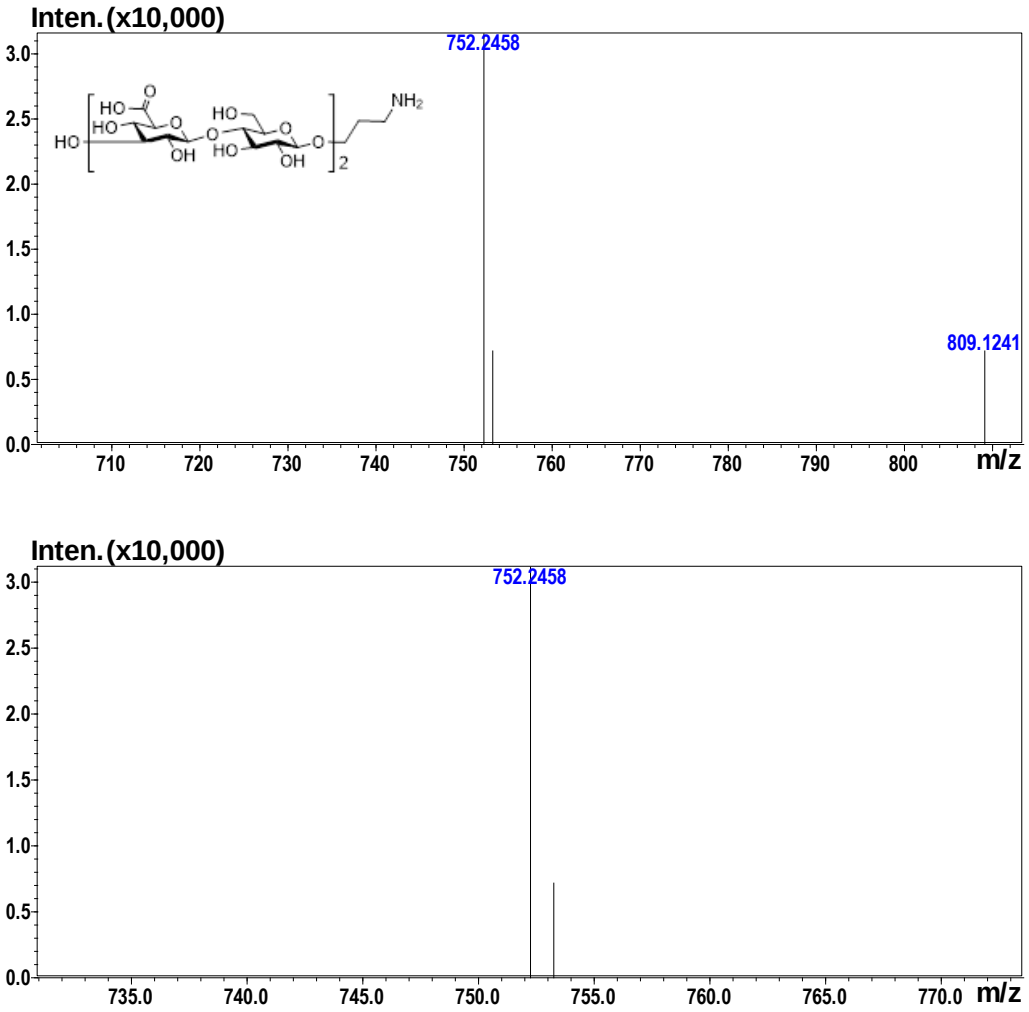
HR-ESI mass spectrum of compound **16**



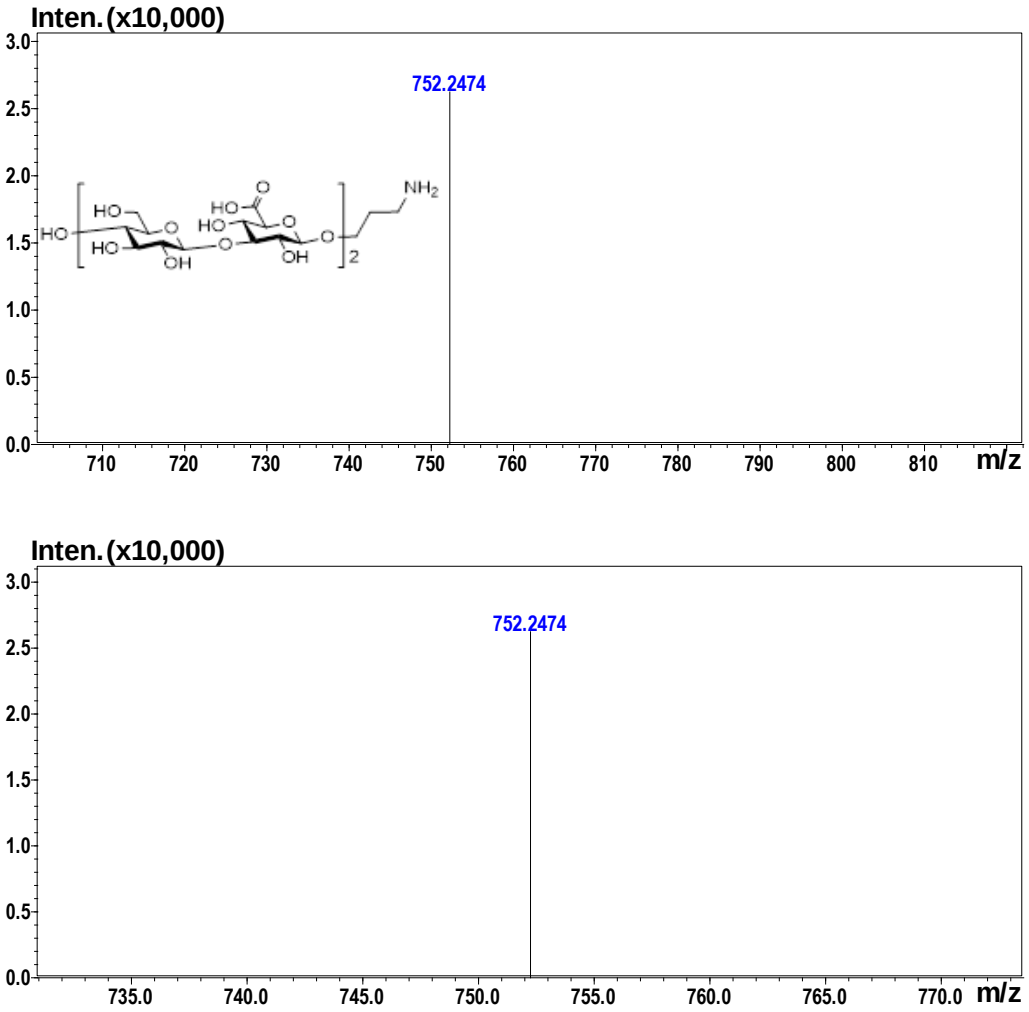
HR-ESI mass spectrum of compound **19**



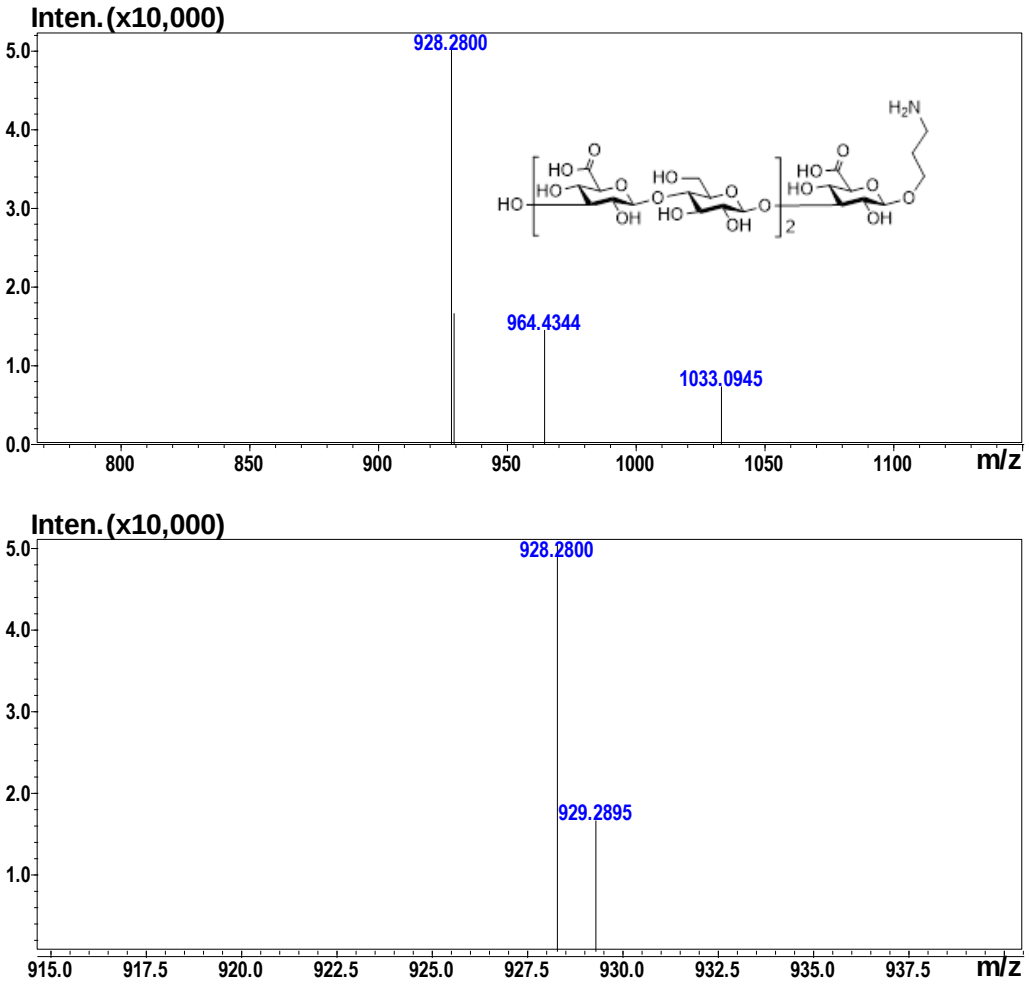
HR-ESI mass spectrum of compound **20**



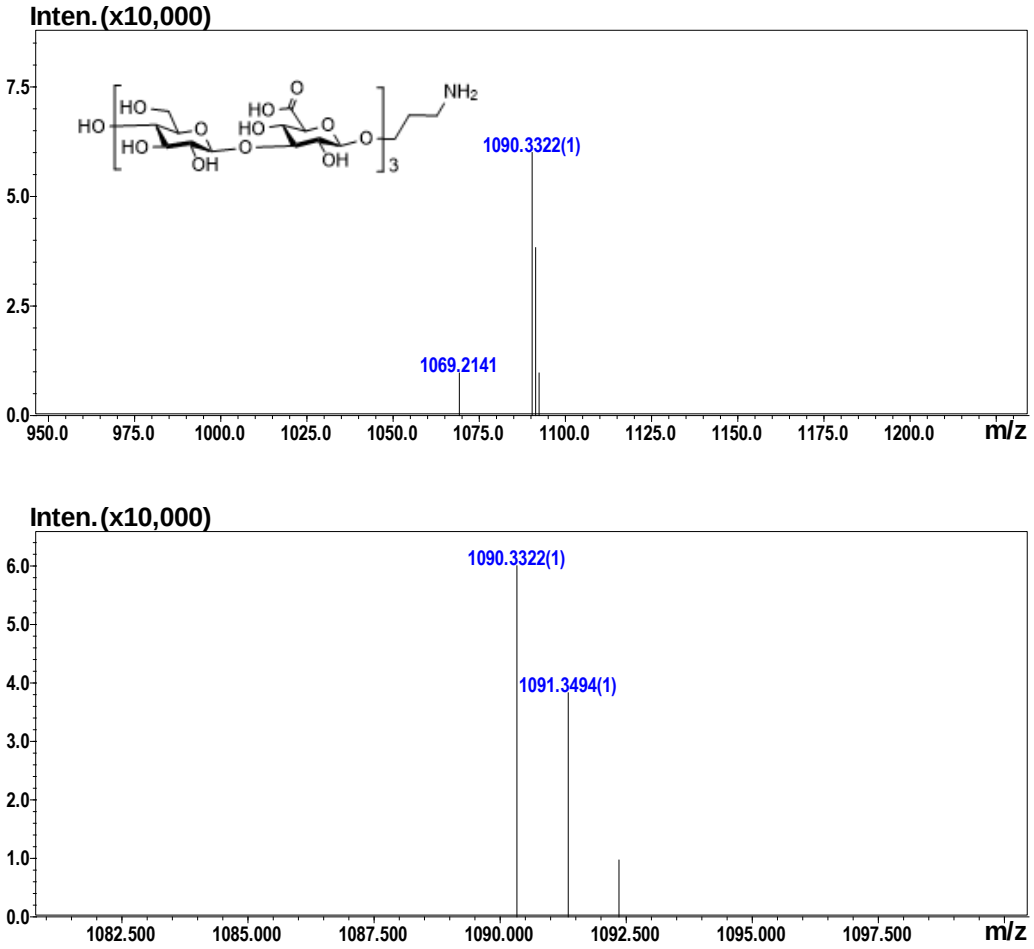
HR-ESI mass spectrum of compound **1a**



HR-ESI mass spectrum of compound **1b**

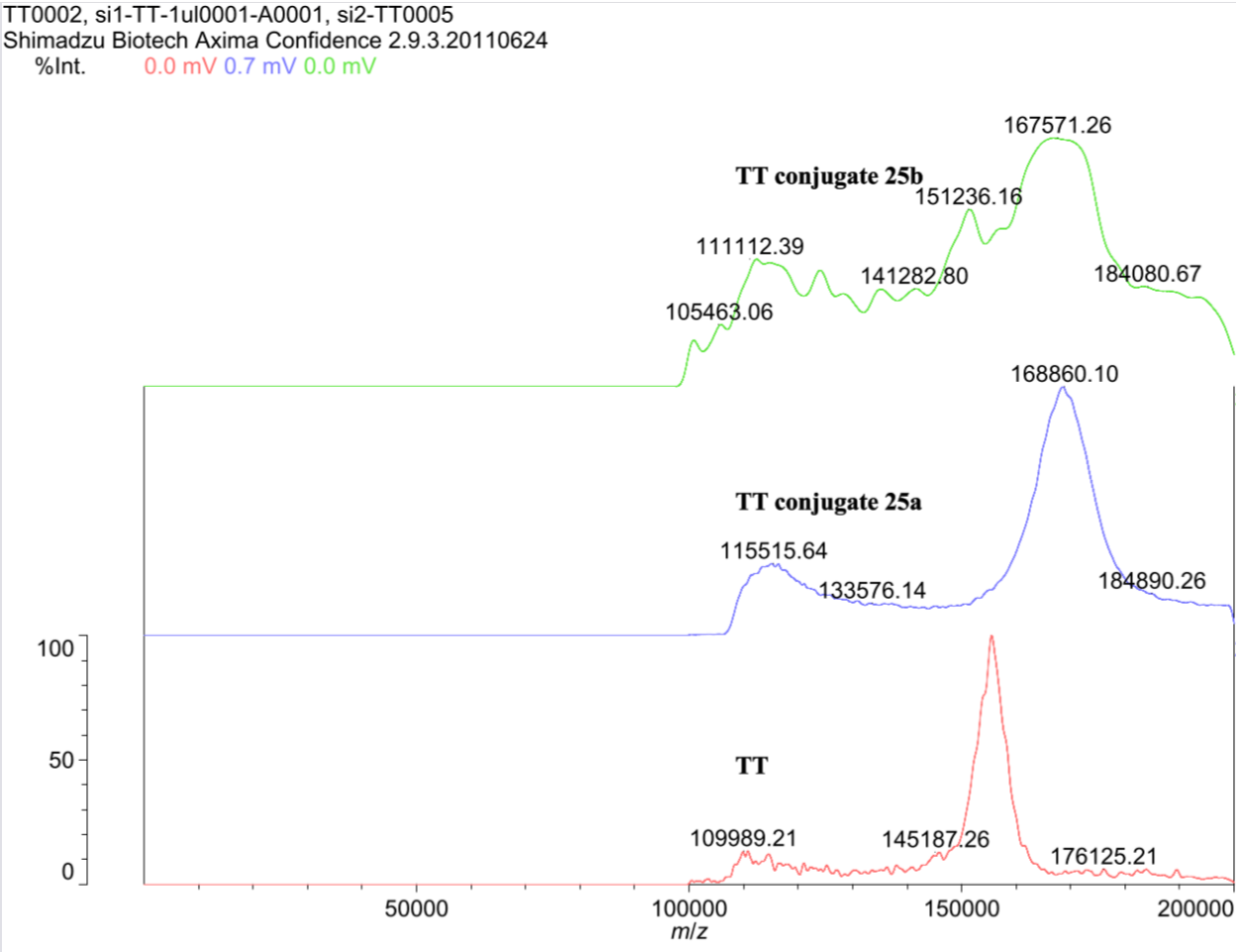


HR-ESI mass spectrum of compound **2b**

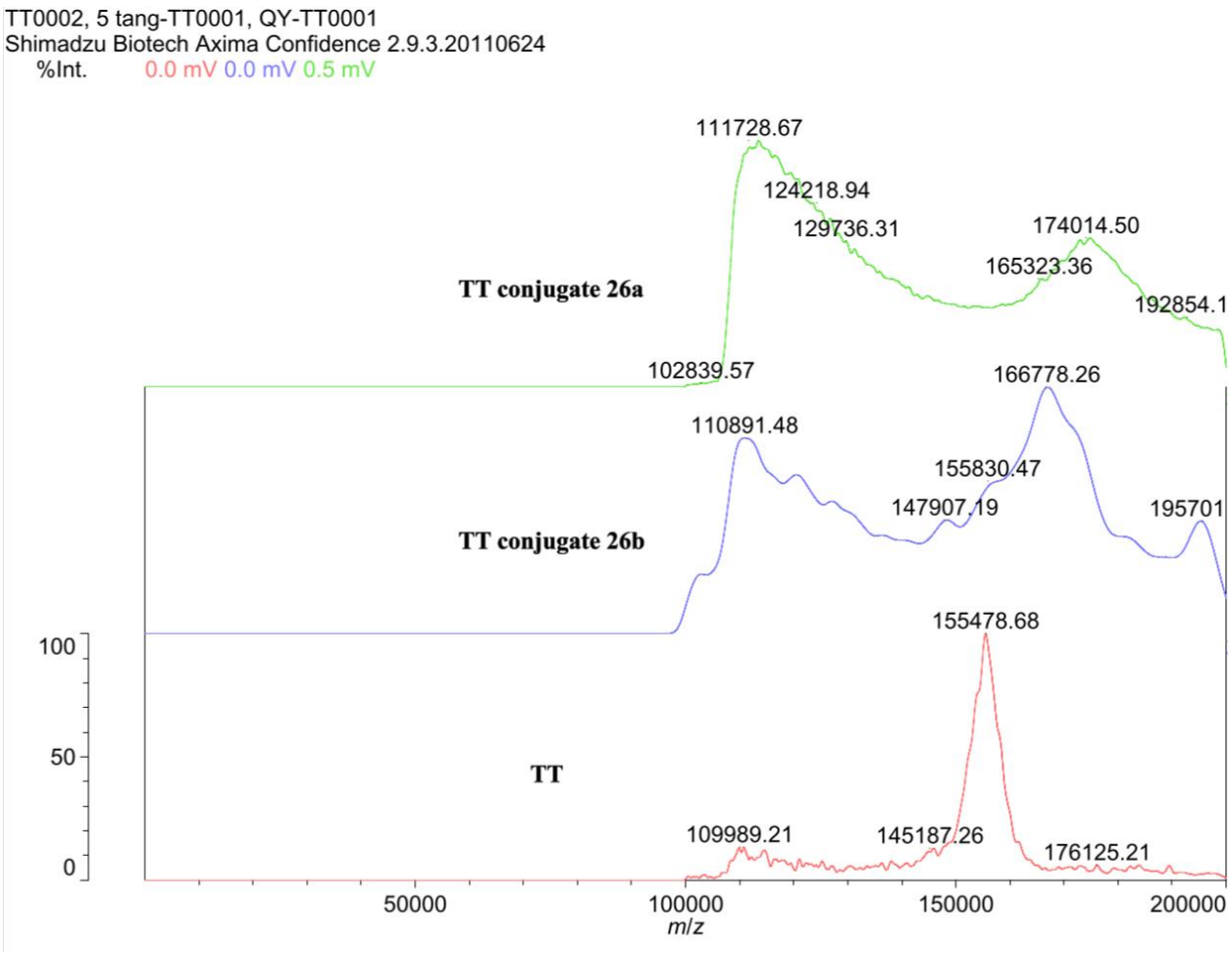


HR-ESI mass spectrum of compound **3b**

IV. MALDI-TOF MS analysis of ST3 oligosaccharide-protein conjugates

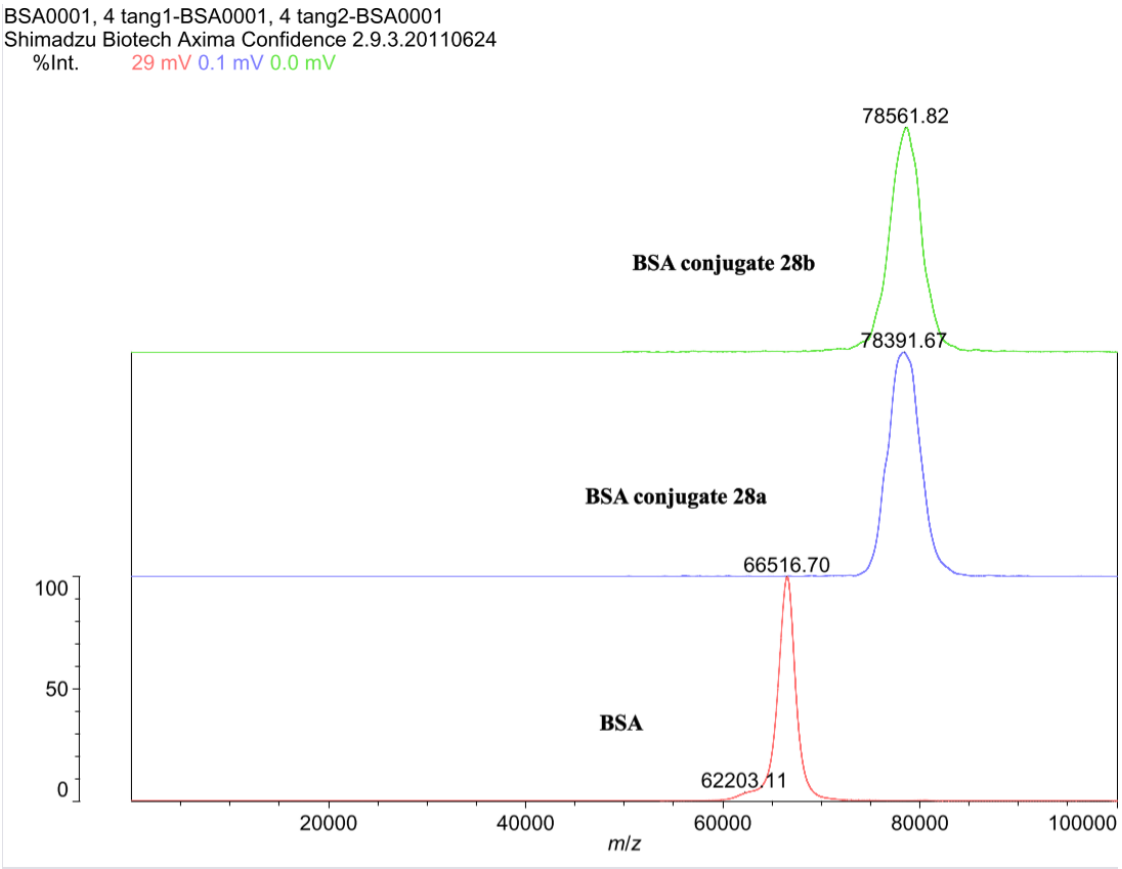


MALDI-TOF mass spectra of TT protein and tetrasaccharide-TT conjugates **25a-b**

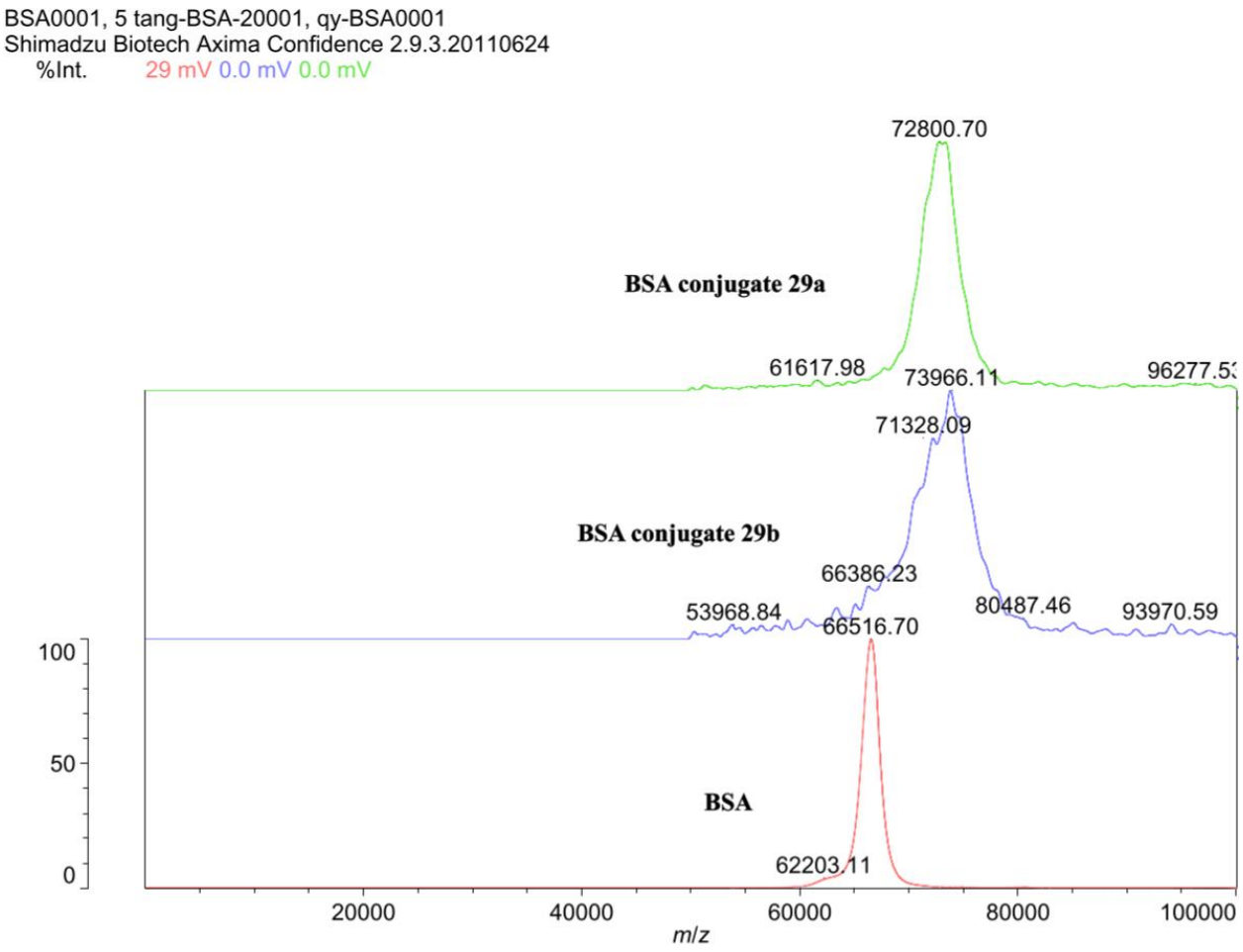


MALDI-TOF mass spectra of TT protein and pentasaccharide-TT conjugates **26a-b**

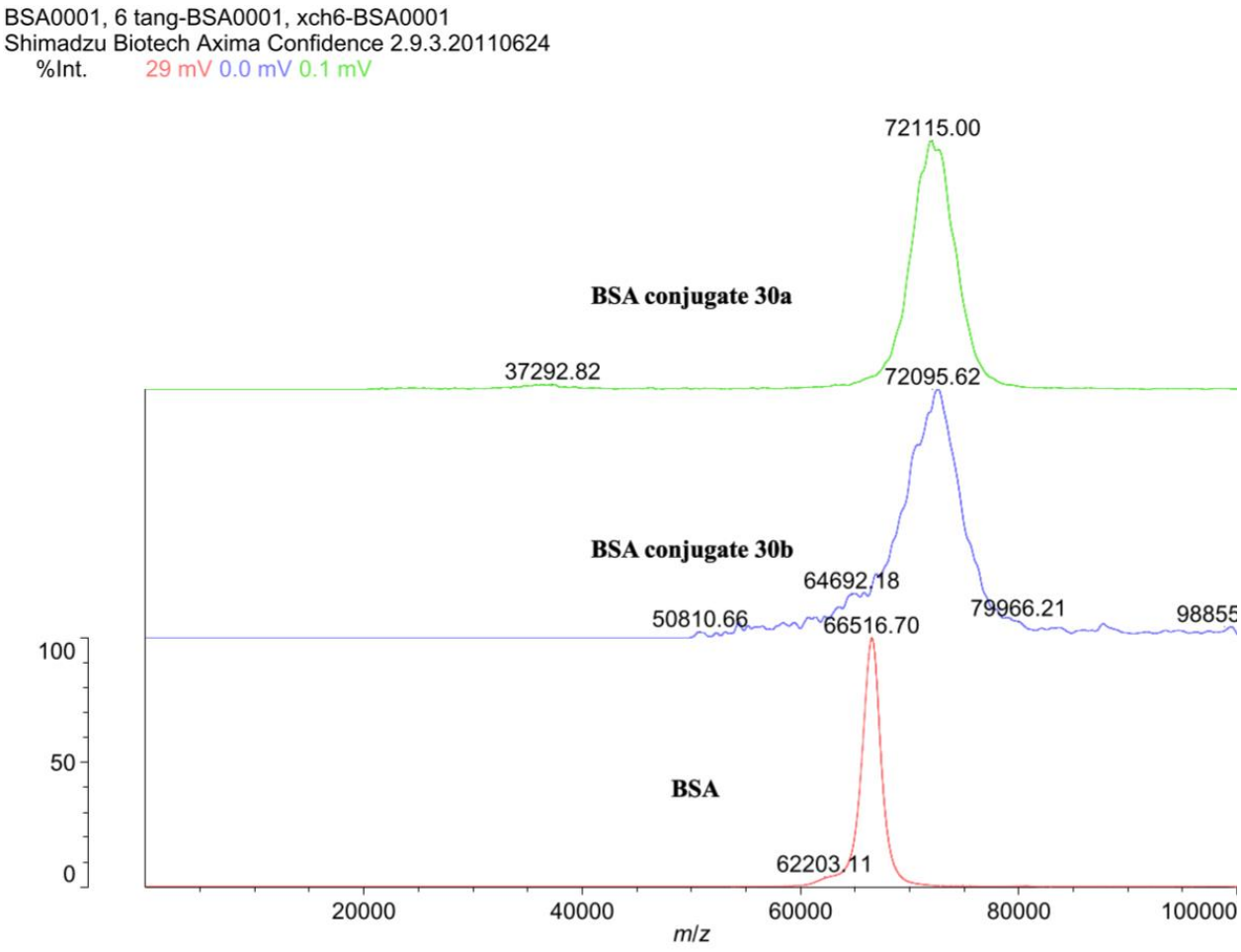
MALDI-TOF mass spectra of TT protein and hexasaccharide-TT conjugates **27a-b**



MALDI-TOF mass spectra of BSA protein and tetrasaccharide-BSA conjugates **28a-b**



MALDI-TOF mass spectra of BSA protein and pentasaccharide-BSA conjugates **29a-b**



MALDI-TOF mass spectra of BSA protein and hexasaccharide-BSA conjugates **30a-b**