

Electronic Supporting Information

Design and synthesis of a “click” high-mannose oligosaccharide mimic emulating Man₈ binding affinity towards Con A

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1 : General Information

General methods and materials :

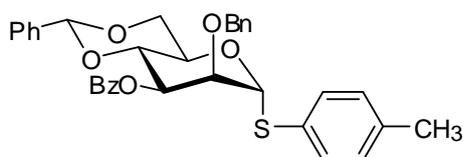
All reactions were monitored by TLC on Kieselgel 60 F₂₅₄ (E. Merck). Detection was achieved by charring with vanillin-H₂SO₄ reagent. Preparative column chromatography was performed using 230-240 mesh Merck silica gel (purchased from Aldrich). Optical rotations were measured with a JASCO DIP-370 digital polarimeter, using a sodium lamp ($\lambda = 589$ nm) at 20°C. Micro-waves irradiation was performed in a CEM Discover[®] System. All NMR experiments were recorded at 300.13 and 600.13 MHz using Bruker DPX300 and IP600 spectrometers respectively equipped with a Z-gradient unit for pulsed-field gradient spectroscopy. Assignments were performed by stepwise identification using COSY, HSQC and HMBC experiments using standard pulse programs from the Bruker library. Chemical shifts are given relative to external TMS with calibration involving the residual solvent signals. When D₂O was used, TMS was used as internal standard reference in a previous ¹³C NMR experiment performed in the same experimental conditions. The length of the 90° pulse was approximately 7 μs (¹H NMR) and 10 μs (¹³C NMR). 1D NMR data spectra were collected using 16 K data points. 2D experiments were run using 1K data points and 512 time increments. The phase-sensitive (TPI) sequence was used and processing resulted in a 1K*1K (real-real) matrix. A 45° flip angle (3.5 μs) and a total recovery time of 5 s were used to ensure complete relaxation of the protons and quantitative measurements. The digital integration of the transformed spectra was performed after polynomial baseline correction. High-resolution mass spectra were recorded in positive mode on a ZabSpec TOF (Micromass, UK) tandem hybrid mass spectrometer with EBETOF geometry. The compounds were individually dissolved in 1:1 water-MeCN at a concentration of 10 μg cm⁻³ and then infused into the electrospray ion source at a flow rate of 10 mm³ min⁻¹ at 60°C. The mass spectrometer was operated at 4 kV whilst scanning the magnet at a typical range of 4000-100 Da. The mass spectra were collected as continuum profile data. Accurate mass measurement was achieved using polyethylene glycol as internal reference with a resolving power set to a minimum of 10 000 (10% valley). Preparative HPLC was carried out with a Waters Prep LC 4000 System chromatograph fitted with evaporative light scattering detector PL-ELS 1000 (Polymer Laboratories) and a Prevail Carbohydrates

ES column (5 μm , 10 x 250 mm). Commercial reagents were used without purification. Air and/or moisture-sensitive reactions were carried out under an atmosphere of argon using oven/flamed-dried glassware and standard syringe/septa techniques.

The high-mannose oligosaccharides **Man**₅₋₉ used in the comparative ELLA experiments were purchased from Ludger (Abingdon, UK; Refs: CN-MAN5, CN-MAN6, CN-MAN7, CN-MAN8 and CN-MAN9) and stored at -20 °C. Purity was controlled by ¹H NMR.

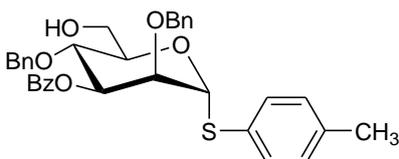
2 : Experimental Procedures

2.1 : *p*-methylphenyl 3-*O*-benzoyl-2-*O*-benzyl-4,6-*O*-benzylidene-1-thio- α -D-mannopyranoside (**7**)



p-methylphenyl 2-*O*-benzyl-4,6-*O*-benzylidene-1-thio- α -D-mannopyranoside^[1] **6** (2.69 g, 5.79 mmol) was dissolved in pyridine (70 mL) and cooled to 0°C. Benzoyl chloride (1.34 mL, 11.60 mmol) was added and the reaction mixture was stirred for 30 min at room temperature then quenched by the addition of MeOH (5 mL) at 0°C. The solvent was evaporated, and the residue was diluted with CH₂Cl₂ (150 mL) and washed with saturated KHSO₄ (2 x 70 mL), NaHCO₃ (2 x 70 mL), and water (1 x 70 mL), then dried over sodium sulfate, filtered, and concentrated. The crude product was purified by column chromatography (Cyclohexane/EtOAc, 9:1) to afford **7** (3.27 g, 5.75 mmol, 99%): white solid; $[\alpha]_D^{20}$: + 56° (c = 0.21, CHCl₃); ¹H NMR (CDCl₃, 300 MHz) 8.11-8.05 (2H, m, C₆H₅COO); 7.62-7.14 (17H, m, C₆H₅COO, C₆H₅CH₂, SC₆H₄CH₃); 5.66 (1H, s, H-7); 5.59 (1H, dd, *J*₂₋₃ = 3.4 Hz, *J*₃₋₄ = 9.9 Hz, H-3); 5.53 (1H, d, *J*₁₋₂ = 1.3 Hz, H-1); 4.67 (1H, d, *J* = 11.9 Hz, C₆H₅CH₂); 4.55 (1H, d, C₆H₅CH₂); 4.52-4.42 (2H m, H-4, H-5); 4.38 (1H, dd, H-2); 4.29 (1H, dd, *J*₅₋₆ = 4.3 Hz, *J*_{6-6'} = 10.2 Hz, H-6); 3.94 (1H, t, *J*_{6-6'} = *J*₆₋₅ = 10.2 Hz, H-6'); 2.37 (3H, s, CH₃); ¹³C NMR (CDCl₃, 75 MHz) 165.9 (C₆H₅COO); 138.2-126.3 (C₆H₅COO, C₆H₅, SC₆H₄CH₃, C₆H₅CH₂); 101.9 (C-7); 87.0 (C-1); 77.8 (C-2); 76.5 (C-4); 73.2 (C₆H₅CH₂); 71.3 (C-3); 68.7 (C-6); 65.4 (C-5); 21.3 (SC₆H₄CH₃); HRMS (ESI) calcd for [C₃₄H₃₂O₆S + Na]⁺: 591.1817, Found: 591.1799.

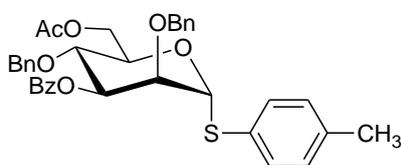
2.2 : *p*-methylphenyl 3-*O*-benzoyl-2,4-di-*O*-benzyl-1-thio- α -D-mannopyranoside (**5**)



The compound **7** (2.27 g, 3.99 mmol) was treated with 1M BH₃-THF solution (12.78 mL, 12.78 mmol) and 1M Bu₂BOTf-CH₂Cl₂ solution (4.23 mL, 4.23 mmol) at 0°C. The reaction mixture was stirred for 4 h at 0°C then the reaction was quenched by dropwise addition of MeOH up to the end of the gas release. The

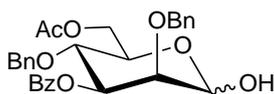
solvent was evaporated and the crude product was purified by column chromatography (Cyclohexane/EtOAc, 85:15) to afford **5** (1.67 g, 2.92 mmol, 74%): white solid; $[\alpha]_D^{20}$: + 53.3° (c = 0.32, CHCl₃); ¹H NMR (CDCl₃, 300 MHz) 7.94-6.96 (19H; m; C₆H₅CH₂, SC₆H₄CH₃, C₆H₅COO); 5.42 (1H; dd; J₂₋₃ = 3.0 Hz, J₃₋₄ = 9.2 Hz; H-3); 5.38 (1H, d, J₁₋₂ = 1.6 Hz, H-1); 4.82 (1H, d, J = 11 Hz, C₆H₅CH₂); 4.71 (1H, d, J = 11 Hz, C₆H₅CH₂); 4.67 (1H, d, J = 12 Hz, C₆H₅CH₂); 4.55 (1H, d, J = 12 Hz, C₆H₅CH₂); 4.22-4.14 (3H, m, H-2, H-4, H-5); 3.74 (2H, m, H-6, H-6'); 2.18 (3H, s, SC₆H₄CH₃); 2.16 (1H, bs, OH); ¹³C NMR (CDCl₃, 75 MHz) 165.7 (C₆H₅COO); 138.1-127.9 (C₆H₅CH₂, C₆H₅COO, SC₆H₄CH₃); 86.2 (C-1); 77.3 (C-2); 75.2 (C₆H₅CH₂); 74.6 (C-3); 73.3, 73.3 (C-4, C-5); 72.7 (C₆H₅CH₂); 62.0 (C-6); 21.3 (SC₆H₄CH₃); HRMS (ESI) calcd for [C₃₄H₃₄O₆S + Na]⁺: 593.1974, Found: 593.1971.

2.3 : *p*-methylphenyl 6-O-acetyl-3-O-benzoyl-2,4-di-O-benzyl-1-thio- α -D-mannopyranoside (**8**)



The compound **5** (1.45 g, 2.54 mmol) was dissolved in pyridine (40 mL) and cooled to 0°C. Acetic anhydride (0.94 mL, 10.17 mmol) was added and the reaction mixture was stirred for 4 h at room temperature then quenched by the addition of MeOH (5 mL) at 0°C. The solvent was evaporated, and the residue was diluted with CH₂Cl₂ (40 mL) and successively washed with saturated aq.KHSO₄ (2 x 20 mL), NaHCO₃ (2 x 20 mL), and water (1 x 10 mL), dried over sodium sulfate, filtered, and concentrated. The crude product was purified by column chromatography (Cyclohexane/EtOAc, 8:2) to afford **8** (1.35 g, 2.20 mmol, 87%): white solid; $[\alpha]_D^{20}$: + 57° (c = 0.23, CHCl₃); ¹H NMR (CDCl₃, 300 MHz) 8.05-7.02 (19 H, m, C₆H₅CH₂, SC₆H₄CH₃, C₆H₅COO); 5.45 (1H, d, J₁₋₂ = 1.7 Hz, H-1); 5.42 (1H, dd, J₂₋₃ = 3.2 Hz, J₃₋₄ = 9.3 Hz, H-3); 4.69 (1H, d, J = 10.8 Hz, C₆H₅CH₂); 4.58 (1H, d, J = 12.1 Hz, C₆H₅CH₂); 4.51 (1H, d, J = 10.8 Hz, C₆H₅CH₂); 4.40 (1H, d, J = 12.1 Hz, C₆H₅CH₂); 4.39 (1H, m, H-5); 4.28 (2H, m, H-6, H-6'); 4.16 (1H, dd, H-2); 4.08 (1H, t, J₄₋₅ = 9.5 Hz, H-4); 2.24 (3H, s; SC₆H₄CH₃); 1.97 (3H, s, CH₃COO); ¹³C NMR (CDCl₃, 75 MHz) 170.9 (CH₃COO); 165.6 (C₆H₅COO); 138.1-127.9 (C₆H₅CH₂, C₆H₅COO, SC₆H₄CH₃); 85.8 (C-1); 77.1 (C-2); 75.1 (C₆H₅CH₂); 74.6 (C-3); 73.7 (C-4); 72.4 (C₆H₅CH₂); 70.7 (C-5); 63.5 (C-6); 21.3 (SC₆H₄CH₃); 21.0 (CH₃COO); HRMS (ESI) calcd for [C₃₆H₃₆O₇S + Na]⁺: 635.2079, Found: 635.2073.

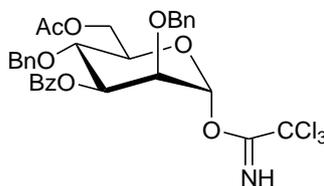
2.4 : 6-O-acetyl-3-O-benzoyl-2,4-di-O-benzyl-D-mannopyranose (**9**)



A solution of **8** (1.17 g, 1.92 mmol) in acetone (70 mL) was cooled to 0°C then NBS (513 mg, 2.88 mmol) was added in one portion. The mixture was warmed up to room temperature and after 35 min the reaction was quenched by the addition of solid ammonium chloride (166.5 mg) followed by stirring for 10 min. The reaction mixture was then diluted with EtOAc (50 mL) and washed with water (1 x 30 mL). The aqueous phase was extracted with EtOAc (2 x 40 mL). The combined organic phases were dried over sodium

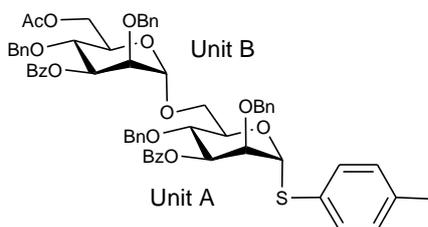
sulfate, filtered, and concentrated. The crude product was purified by column chromatography (Cyclohexane/EtOAc, 6:4) to afford **9** (967 mg, 1.90 mmol, 99%): white solid; $^1\text{H NMR}$ (CDCl_3 , 300 MHz) 8.09-7.16 (30H, m, $\text{C}_6\text{H}_5\text{CH}_2$, $\text{C}_6\text{H}_5\text{COO}$ anomer α and β); 5.62 (1H, dd, $J_{3-2} = 3.2$ Hz, $J_{3-4} = 8.7$ Hz, H-3 α); 5.34-5.30 (2H, m, H-1 α , H-3 β); 4.90-4.86 (3H, m, H-1 β , $\text{C}_6\text{H}_5\text{CH}_2\beta$); 4.77-4.57 (3H, m, $\text{C}_6\text{H}_5\text{CH}_2\alpha$); 4.41 (1H, dd, $J_{5-6} = 1.8$ Hz, $J_{6-6'} = 11.8$ Hz, H-6 α); 4.30 (1H, dd, $J_{5-6'} = 4.4$ Hz, H-6' α); 4.21-4.06 (3H, m, H-4 α , H-5 α , $\text{C}_6\text{H}_5\text{CH}_2\alpha$); 4.03 (1H, m, H-2 α); 2.08 (3H, s, $\text{CH}_3\text{COO}\alpha$); 2.04 (3H, s, $\text{CH}_3\text{COO}\beta$); $^{13}\text{C NMR}$ (CDCl_3 , 75 MHz) 171.1 (CH_3COO); 165.7 ($\text{C}_6\text{H}_5\text{COO}$); 137.9-127.8 ($\text{C}_6\text{H}_5\text{CH}_2$, $\text{C}_6\text{H}_5\text{COO}$); 93.7 (C-1 β); 92.8 (C-1 α); 77.2, 77.0 (2C- β); 76.3 (C-2 α); 75.6, 75.3 (2 x $\text{C}_6\text{H}_5\text{CH}_2\beta$); 75.0 ($\text{C}_6\text{H}_5\text{CH}_2\alpha$); 74.2 (C-3 α); 73.5 (C-4 α or C-5 α); 73.4 (C- β); 73.1 ($\text{C}_6\text{H}_5\text{CH}_2\alpha$); 73.0 (C- β); 70.1 (C-4 α or C-5 α); 63.6 (C-6 α); 63.5 (C-6 β); 21.1 (CH_3COO); HRMS (ESI) calcd for $[\text{C}_{29}\text{H}_{30}\text{O}_8 + \text{Na}]^+$: 529.1837, Found: 529.1852.

2.5 : 6-O-acetyl-3-O-benzoyl-2,4-di-O-benzyl-D-mannopyranosyl trichloroacetimidate (**4**)



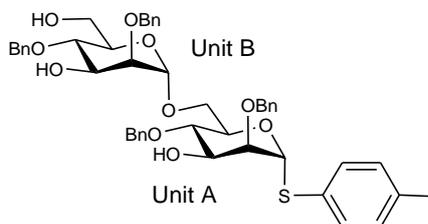
To a solution of compound **9** (857.2 mg, 1.69 mmol) in dry CH_2Cl_2 (60 mL) were added trichloroacetonitrile (2.71 mL, 27.09 mmol) at room temperature and 1.8-diazabicyclo-[5,4,0]-undec-7-ene (15.31 μL , 0.101 mmol) at 0°C . The reaction mixture was stirred for 3 h at 0°C then the solvent was evaporated. The crude product was purified by column chromatography (Cyclohexane/EtOAc, 6:4, 2% of Et_3N) to afford **4** (1.03 g, 1.58 mmol, 95%): white solid; $[\alpha]_D^{20}$: $+28^\circ$ ($c = 0.20$, CHCl_3); $^1\text{H NMR}$ (CDCl_3 , 300 MHz) 8.60 (1H, s, OCNHCCl_3); 8.07-7.16 (15H, m, $\text{C}_6\text{H}_5\text{CH}_2$, $\text{C}_6\text{H}_5\text{COO}$); 6.40 (1H, d; $J_{1-2} = 2.2$ Hz, H-1); 5.57 (1H, dd, $J_{2-3} = 3.3$ Hz, $J_{3-4} = 8.6$ Hz, H-3); 4.82 (1H, d, $J = 10.8$ Hz, $\text{C}_6\text{H}_5\text{CH}_2$); 4.75 (1H, d, $J = 12$ Hz, $\text{C}_6\text{H}_5\text{CH}_2$); 4.62 (1H, d, $J = 10.8$ Hz, $\text{C}_6\text{H}_5\text{CH}_2$); 4.61 (1H, d, $J = 12$ Hz, $\text{C}_6\text{H}_5\text{CH}_2$); 4.42 (1H, dd, $J_{5-6} = 2.0$ Hz, $J_{6-6'} = 12.1$ Hz, H-6); 4.32 (1H, dd; $J_{5-6'} = 4.3$ Hz, H-6'); 4.24 (1H, dd; $J_{2-3} = 3.4$ Hz, H-2); 4.19-4.13 (2H, m, H-4, H-5); 2.07 (3H, s, CH_3COO); $^{13}\text{C NMR}$ (CDCl_3 , 75 MHz) 170.9 (CH_3COO); 165.7 ($\text{C}_6\text{H}_5\text{COO}$); 160.6 (OCNHCCl_3); 137.4-127.9 ($\text{C}_6\text{H}_5\text{CH}_2$, $\text{C}_6\text{H}_5\text{COO}$); 95.7 (C-1); 75.2 ($\text{C}_6\text{H}_5\text{CH}_2$); 74.1 (C-2); 73.9 (C-3); 73.0 ($\text{C}_6\text{H}_5\text{CH}_2$); 72.8, 72.6 (C-4, C-5); 63.0 (C-6); 21.0 (CH_3COO); MS (ESI) calcd for $[\text{C}_{31}\text{H}_{30}\text{Cl}_3\text{NO}_8 + \text{Na}]^+$: 673.9, Found: 673.9.

2.6 : *p*-methylphenyl 6-(6-*O*-acetyl-3-*O*-benzoyl-2,4-*O*-benzyl- α -D-mannopyranosyl)-3-*O*-benzoyl-2,4-di-*O*-benzyl-1-thio- α -D-mannopyranoside (10)



The compound **4** (230.4 mg, 0.355 mmol) and compound **5** (202.4 mg, 0.355 mmol) were both dissolved in dry CH_2Cl_2 under argon atmosphere and cooled to -80°C for the addition of trimethylsilyl trifluoromethanesulfonate (32.2 μL , 0.177 mmol). The reaction mixture was stirred for 45 min at -80°C then neutralized by the addition of Et_3N . The solvent was then evaporated and the crude product was purified by column chromatography (Cyclohexane/ EtOAc , 9:1) to afford the compound **10** (328 mg, 87%): white solid; $[\alpha]_D^{20}$: $+29^\circ$ ($c = 0.21$, CHCl_3); $^1\text{H NMR}$ (CDCl_3 , 300 MHz) 8.04-6.94: (34H, m, $\text{C}_6\text{H}_5\text{CH}_2$, $\text{C}_6\text{H}_5\text{COO}$, $\text{SC}_6\text{H}_4\text{CH}_3$); 5.56 (1H, dd, $J_{2-3} = 3.3$ Hz, $J_{3-4} = 9.4$ Hz, H-3B); 5.42 (1H, dd, $J_{2-3} = 3.1$ Hz, $J_{3-4} = 12.1$ Hz, H-3A); 5.38 (1H, d, $J_{1-2} = 1.7$ Hz, H-1A); 5.08 (1H, d, $J_{1-2} = 1.7$ Hz, H-1B); 4.81-4.37 (7H, m, 4 x $\text{C}_6\text{H}_5\text{CH}_2$); 4.30-4.21 (5H, m, H-4A, H-5A, H-6B, H-6'B, $\text{C}_6\text{H}_5\text{CH}_2$); 4.15 (1H, dd, H-2A); 4.08-4.02 (2H, m, H-4B, H-2B); 3.91 (2H, m, H-6A, H-5B); 3.74 (1H, dd; $J_{5-6} = 1.1$ Hz, $J_{6-6'} = 11.9$ Hz, H-6'A); 2.19, 1.97 (6H, 2s, CH_3COO , $\text{SC}_6\text{H}_4\text{CH}_3$); $^{13}\text{C NMR}$ (CDCl_3 , 75 MHz) 171.0 (CH_3COO); 165.8, 165.6 ($\text{C}_6\text{H}_5\text{COO}$); 138.2-129.9 ($\text{C}_6\text{H}_5\text{CH}_2$, $\text{C}_6\text{H}_5\text{COO}$, $\text{SC}_6\text{H}_4\text{CH}_3$); 98.4 (C-1B); 86.3 (C-1A); 77.4 (C-2A); 76.5 (C-2B); 75.2, 75.1 (2 x $\text{C}_6\text{H}_5\text{CH}_2$); 74.8 (C-3A); 74.5 (C-3B); 73.5 (C-4A); 73.4 (C-4B); 72.9 ($\text{C}_6\text{H}_5\text{CH}_2$); 72.8 (C-5A); 72.6 ($\text{C}_6\text{H}_5\text{CH}_2$); 70.0 (C-5B); 66.3 (C-6A); 63.4 (C-6B); 21.2, 21.0 (CH_3COO , $\text{SC}_6\text{H}_4\text{CH}_3$); HRMS (ESI) calcd for $[\text{C}_{63}\text{H}_{62}\text{O}_{13}\text{S} + \text{NH}_4]^+$: 1076.4255, Found: 1076.4252.

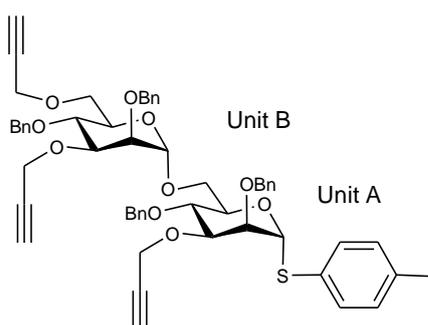
2.7 : *p*-methylphenyl 6-(2,4-*O*-benzyl- α -D-mannopyranosyl)-2,4-di-*O*-benzyl-1-thio- α -D-mannopyranoside (11)



To a solution of compound **10** (328 mg, 0.31 mmol) in MeOH (9 mL) was added sodium methoxide (1.85 mL, 1M in MeOH) The reaction mixture was stirred for 15 h at room temperature then neutralized with Amberlite® IR120 H^+ resin, filtered, and concentrated. The crude product was purified by column chromatography (Cyclohexane/ EtOAc , 75:25) to afford **11** (207 mg, 82%): white solid; $[\alpha]_D^{20}$: $+86^\circ$ ($c = 0.07$, CHCl_3); $^1\text{H NMR}$ (CDCl_3 , 300 MHz) 7.39-7.09 (24H, m, $\text{C}_6\text{H}_5\text{CH}_2$, $\text{SC}_6\text{H}_4\text{CH}_3$); 5.52 (1H, s, H-1A); 5.00-4.42 (9H, m, 4 x $\text{C}_6\text{H}_5\text{CH}_2$, H-1B); 4.29-4.25 (1H, m, H-5A or H-5B); 4.05-3.96 (2H, m, H-2A, H-5A or H-5B); 3.91 (1H; dd; $J_{5-6} = 5.4$ Hz; $J_{6-6'} = 11.4$ Hz, H-6A); 3.76 (1H, dd, $J_{1-2} = 1.6$ Hz; $J_{2-3} = 3.7$ Hz; H-2B);

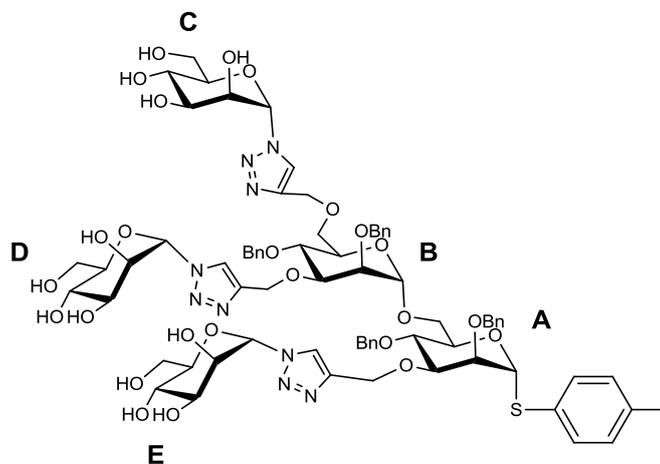
3.74-3.62 (7H, H-3A, H-3B, H-4A, H-4B, H-6'A, H-6B, H-6'B); 2.40 (1H, d, $J = 9.2$ Hz, OH); 2.28 (3H, s; $\text{SC}_6\text{H}_4\text{CH}_3$); 2.17 (1H, d, $J = 9.3$ Hz, OH); 1.84 (1H, bs, OH); ^{13}C NMR (CDCl_3 , 75 MHz) 138.2-127.8 ($\text{C}_6\text{H}_5\text{CH}_2$, $\text{SC}_6\text{H}_4\text{CH}_3$); 97.2 (C-1B); 85.4 (C-1A); 79.8 (C-2A); 78.7 (C-2B); 77.3, 76.8, 76.6, 72.5, 71.8, 71.5 (C-3A, C-3B, C-4A, C-4B, C-5A, C-5B); 75.1 ($\text{C}_6\text{H}_5\text{CH}_2$); 74.9 ($\text{C}_6\text{H}_5\text{CH}_2$); 73.0 ($\text{C}_6\text{H}_5\text{CH}_2$); 72.4 ($\text{C}_6\text{H}_5\text{CH}_2$); 66.4 (C-6A); 62.3 (C-6B); 21.2 ($\text{SC}_6\text{H}_4\text{CH}_3$); HRMS (ESI) calcd for $[\text{C}_{47}\text{H}_{52}\text{O}_{10}\text{S} + \text{NH}_4]^+$: 826.3625, Found: 826.3639.

2.8 : *p*-methylphenyl 6-(2,4-O-benzyl-3,6-di-O-propargyl- α -D-mannopyranosyl)-2,4-di-O-benzyl-3-O-propargyl-1-thio- α -D-mannopyranoside (3)



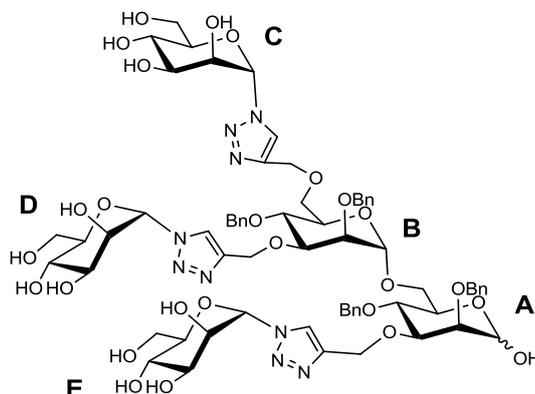
The compound **11** (202 mg, 0.250 mmol) was dissolved in dry DMF (5.5 mL) and cooled to 0°C then NaH (70 mg, 1.74 mmol) and propargyl bromide (267 μL , 2.24 mmol) were added. The reaction mixture was stirred for 30 min at -0°C and then slowly warmed up to room temperature for 2h. The reaction mixture was then diluted with H_2O (5 mL) and water phase was extracted with Et_2O (3 x 20 mL). The combined organic phase was dried with sodium sulfate, filtered, and concentrated. The crude product was purified by column chromatography (Cyclohexane/ EtOAc , 8:2) to afford **3** (126 mg, 96%): yellowish oil; $[\alpha]_D^{20}$: +71° ($c = 0.22$, CHCl_3); ^1H NMR (CDCl_3 , 300 MHz) 7.35-7.24 (24H, m, $\text{C}_6\text{H}_5\text{CH}_2$, $\text{SC}_6\text{H}_4\text{CH}_3$); 5.44 (1H, d, $J_{1-2} = 1.6$ Hz, H-1A); 5.01 (1H, d, $J_{1-2} = 1.8$ Hz, H-1B); 4.95-4.88 (2H, m, $\text{C}_6\text{H}_5\text{CH}_2$); 4.71-4.60 (6H, m, 3 x $\text{C}_6\text{H}_5\text{CH}_2$); 4.29-4.10 (7H, m, 3 x OCH_2CCH , + one "unidentified" H); 4.07 (1H, dd, $J_{2-3} = 2.7$ Hz; H-2A); 4.00-3.90 (5H, m, H-6B, H-5A + 3 "unidentified" H); 3.87 (1H, m, H-2B); 3.86-3.78 (2H; H-6A + 1 "unidentified" H); 3.71-3.66 (2H, m, H-6'A, H-6'B); 2.46 (1H, t, $J = 2.4$ Hz, OCH_2CCH), 2.34 (2H, m, 2 x OCH_2CCH); 2.24 (3H, s, $\text{SC}_6\text{H}_4\text{CH}_3$); ^{13}C NMR (CDCl_3 , 75 MHz) 138.9-127.5 ($\text{C}_6\text{H}_5\text{CH}_2$, $\text{SC}_6\text{H}_4\text{CH}_3$); 98.5 (C-1B); 86.2 (C-1A); 80.2, 80.0 (2 x OCH_2CCH); 79.9 (1 "unidentified" C); 79.8 (OCH_2CCH); 79.6 (1 "unidentified" C); 76.7 (C-2A); 75.6 (C-2B); 75.3, 75.2 (2 x $\text{C}_6\text{H}_5\text{CH}_2$); 74.9, 74.7 (2 x OCH_2CCH); 74.7 (2 "unidentified" C); 74.7 (OCH_2CCH); 72.6 ($\text{C}_6\text{H}_5\text{CH}_2$); 72.6 (1 "unidentified" C); 72.3 ($\text{C}_6\text{H}_5\text{CH}_2$); 71.6 (1 "unidentified" C); 68.7 (C-6A); 66.6 (C-6B); 58.6, 57.7, 57.6 (3 x OCH_2CCH); 21.1 ($\text{SC}_6\text{H}_4\text{CH}_3$); HRMS (ESI) calcd for $[\text{C}_{56}\text{H}_{58}\text{O}_{10}\text{S} + \text{Na}]^+$: 945.3648, Found: 945.3632.

2.9 : (Tris) *N*-(α -D-mannopyranosyl)-triazole of *p*-methylphenyl 6-(2,4-*O*-benzyl-3,6-di-*O*-propargyl- α -D-mannopyranosyl)-2,4-di-*O*-benzyl-3-*O*-propargyl-1-thio- α -D-mannopyranoside (12**)**



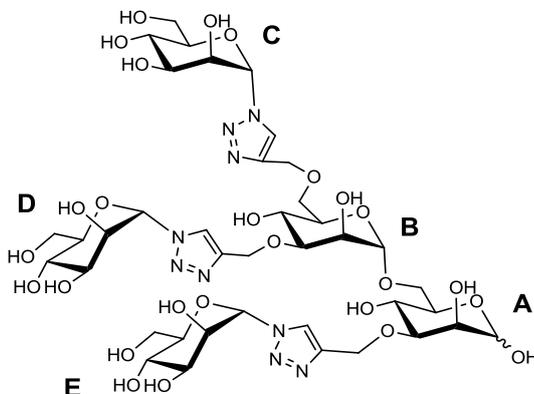
To a vigorous stirred solution of compound **3** (257 mg, 0.28 mmol) and α -D-mannopyranosyl azide^[2] **2** (200 mg, 0.97 mmol) in dry DMF (5.8 mL) was added dropwise a freshly prepared solution of copper sulfate (42 mg, 0.17 mmol) and sodium ascorbate (110mg, 0.33 mmol) in water (1 mL). The reaction mixture was placed under micro-waves irradiation at 100°C for 30 min then concentrated. The crude product was purified by reverse phase chromatography (Water/Acetonitrile, 30% of water to 15%) to afford the compound **12** (307 mg, 72%); white solid; $[\alpha]_D^{20}$: + 90.1° (*c* = 0.5, MeOH); ¹H NMR (MeOD, 600 MHz) selected data 8.13 (1H, s, H-5 triazole); 7.98 (1H, s, H-5 triazole); 7.84 (1H, s, H-5 triazole); 7.34-7.09 (24H, m, C₆H₅CH₂, SC₆H₄CH₃); 5.96 (1H, d, *J*₁₋₂ = 2.52 Hz; H-1C or D or E); 5.90 (1H, d, *J*₁₋₂ = 2.22 Hz, H-1C or D or E); 5.88 (1H, d, *J*₁₋₂ = 2.52 Hz, H-1C or D or E); 5.46 (1H, d, *J*₁₋₂ = 1.44 Hz, H-1A); 4.88 (1H, d, *J*₁₋₂ = 1.74 Hz, H-1B); 2.19 (3H, s, SC₆H₄CH₃); ¹³C NMR (MeOD, 75 MHz) 146.3, 146.1, 145.9 (C-4 triazole); 140.0, 139.8, 139.4, 139.1, 133.2-128.7 (C₆H₅CH₂, SC₆H₄CH₃); 125.24, 125.19, 124.99 (C-5 triazole); 99.4 (C-1B); 88.43, 88.38, 88.27 (C-1C-E); 87.7 (C-1A); 81.7, 80.8, 78.4, 78.1 76.2 75.8, 75.7, 73.5, 73.1, 72.6, 70.1, 68.5, 68.4 (C-2A-E, C-3A-E, C-4A-E, C-5A-E); 76, 73.8, 73.7, 70.5, 67.6, 65.1, 63.9, 63.5, 62.4 (C-6A-E, 3 x OCH₂ triazole, 4 x C₆H₅CH₂); 21.1 (SC₆H₄CH₃); HRMS (ESI) calc for [C₇₄H₉₁N₉O₂₅S + Na]⁺: 1560.5745, Found: 1560.5673.

2.10 : (Tris) *N*-(α -D-mannopyranosyl)-triazole of 6-(2,4-*O*-benzyl-3,6-di-*O*-propargyl- α -D-mannopyranosyl)-2,4-di-*O*-benzyl-3-*O*-propargyl- α -D-mannopyranoside (13**)**



To a solution of compound **12** (182 mg, 0.12 mmol) in acetone/H₂O (11 mL, 90:10 v/v) was cooled to 0°C then NBS (43 mg, 0.24 mmol) was added in one portion. The mixture was warmed up to room temperature and after 2h the reaction was quenched by the addition of solid ammonium chloride (387 mg) followed by stirring for 10 min. Then the reaction was filtered and the residue was concentrated. The crude product was purified by reverse phase chromatography (Water/Acetonitrile, 85% of water down to 0%) to afford the compound **13** as a α/β mixture (92:8) (101 mg, 60%); white solid; $[\alpha]_D^{20}$: + 62.7° (c = 1, MeOH); ¹H NMR (MeOD, 600 MHz) selected data : 8.12, 7.97, 7.91 (3H, s, H-5 triazole anomer α); 8.09, 7.98, 7.89 (3H, s, H-5 triazole anomer β); 7.36-7.10 (20H, m, C₆H₅CH₂); 5.98 (1H, d, J_{1-2} = 2,22 Hz, H-1C or D or E); 5.93 (1H, d, J_{1-2} = 2,52 Hz, H-1C or D or E); 5.89 (1H, d, J_{1-2} = 2,52 Hz, H-1C or D or E); 5.22 (1H, d; J_{1-2} = 1,56 Hz, H-1A); 4.98 (1H, d, J_{1-2} = 1,02 Hz, H-1B); ¹³C NMR (MeOD, 150 MHz) 146.4, 146.3, 145.9 (C-4 triazole); 139.95, 139.91, 139.82, 139.81, 129.4-128.5 (C₆H₅CH₂); 125.3, 125.1, 125.0 (C-5 triazole); 99.6 (C-1B); 93.6 (C-1A); 88.4, 88.3, 88.2 (C-1C-E); 81.4, 80.9, 78.4, 78.3, 78.2, 77.6, 76.3, 75.8, 75.6, 73.1, 72.6, 72.5, 72.3, 70.2, 70.11, 70.1, 68.5, 68.4, 68.40 (C-2A-E, C-3A-E, C-4A-E, C-5A-E); 76.0, 75.9, 74.3, 73.6, 70.5, 67.7, 65.2, 63.8, 63.5, 62.5, 62.4, 62.3 (C-6A-E, 3 x OCH₂ triazole, 4 x C₆H₅CH₂); HRMS (ESI) calc for [C₆₇H₈₅N₉O₂₆ + Na]⁺: 1454.5503, Found: 1454.5533.

2.11 : (Tris) *N*-(α -D-mannopyranosyl)-triazole of 6-(3,6-di-O-propargyl- α -D-mannopyranosyl)-3-O-propargyl-1-thio- α -D-mannopyranose (1**)**



To a stirred solution **13** (50 mg; 35 μ mol) in a mixture of THF (4 mL), MeOH (4 mL) and H₂O (1 mL) were added Pd/C (10% ww, 38 mg; 0.35 mmol) and ammonium formate (192 mg; 3.02 mmol). The reaction mixture was stirred and heated to 50°C for 14 h in a sealed flask. The mixture was then cooled to room temperature prior to venting the pressurized gas in the reaction vessel and the crude mixture was filtered through celite. The obtained residue is then purified by HPLC to afford compound **1** as a α/β mixture (70:30) (12.4 mg; 40%): white solid; $[\alpha]_D^{20}$: +22.1° (c = 0.5, H₂O); ¹H NMR (MeOD, 600 MHz) selected data 8.3, 8.24, 8.23 (3H, s, H-5 triazole α or β); 8.26, 8.25, 8.23 (3H, s, H-5 triazole α or β); 6.16 (1H, d, J_{1-2} = 2,4 Hz, H-1C or D or E); 6.15 (1H, d, J_{1-2} = 2,4 Hz, H-1C or D or E); 6.14 (1H, d, J_{1-2} = 2,22 Hz, H-1C or D or E); 5.21 (1H, d, J_{1-2} = 1,74 Hz, H-1A α); 4.91 (1H, s, H-1B); 4.90 (1H, s; H-1A β); ¹³C NMR (MeOD, 150 MHz) 145.2, 145.1, 144.9 (C-4 triazole); 125.4 (3C, C-5 triazole); 100.1 (C-1 B); 94.8 (C-1 A α or β); 94.5 (C-1 A α or β); 87.4, 87.3 (3C, C-1 C, D and E); 79.1, 79.0, 76.7, 71.8, 71.3, 71.1, 68.9, 68.1, 67.1, 66.2, 66.1 (C-2 A, C-3 A, C-4 A, C-5 A, C-2 B, C-3 B, C-4 B, C-5 B, C-2 C, C-3 C, C-4 C, C-5 C, C-2 D, C-3 D, C-4 D, C-5 D, C-2 E, C-3 E, C-4 E, C-5 E); 69.6, 66.4, 63.8, 62.3, 62.2, 61.1 (C-6A, C-6B, C-6E, C-6C, C-6D, 3 x OCH₂ triazole); HRMS (ESI) calcd for [C₃₉H₆₁N₉O₂₆ + Na]⁺: 1094.3625, Found: 1094.3636.

3 : Enzyme-Linked Lectin Assay (ELLA)

Nunc-Inmuno™ plates (MaxiSorp™) were coated overnight with yeast (*Saccharomyces cerevisiae*) mannan at 100 μ L/well diluted from a stock solution of 10 μ g·mL⁻¹ in 0.01 M phosphate buffer saline (PBS, pH 7.3 containing 0.1 mM Ca²⁺ and 0.1 mM Mn²⁺) at room temperature. The wells were then washed three times with 300 μ L of washing buffer (containing 0.05% (v/v) Tween 20) (PBST). The washing procedure was repeated after each of the incubations throughout the assay. The wells were then blocked with 150 μ L/well of 1% BSA/PBS for 1 h at 37 °C. After washing, the wells were filled with 100 μ L of serial dilutions of horseradish peroxidase labelled concanavalin A lectin (ConA-HRP) from 10⁻¹ to 10⁻⁵ mg mL⁻¹ in PBS,

and incubated at 37 °C for 1 h. The plates were washed and 50 µL/well of 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) (0.25 mg·mL⁻¹) in citrate buffer (0.2 M, pH 4.0 with 0.015% H₂O₂) was added. The reaction was stopped after 20 min by adding 50 µL/well of 1 M H₂SO₄ and the absorbances were measured at 405 nm. Blank wells contained citrate-phosphate buffer. The concentration of lectin-enzyme conjugate that displayed an absorbance between 0.8 and 1.0 was used for inhibition experiments.

In order to carry out the inhibition experiments, each oligosaccharide (**Man**₅₋₉ or **1**) was added in a serial of 2-fold dilutions (60 µL/well) in PBS with 60 µL of the desired Con A-HRP conjugate concentration on Nunclon™ (Delta) microtiter plates and incubated for 1 h at 37 °C. The above solutions (100 µL) were then transferred to the lactose polymer-coated microplates, which were incubated for 1 h at 37 °C. The plates were washed and the ABTS substrate was added (50 µL/well). Color development was stopped after 20 min and the absorbances were measured. The percent of inhibition was calculated as follows:

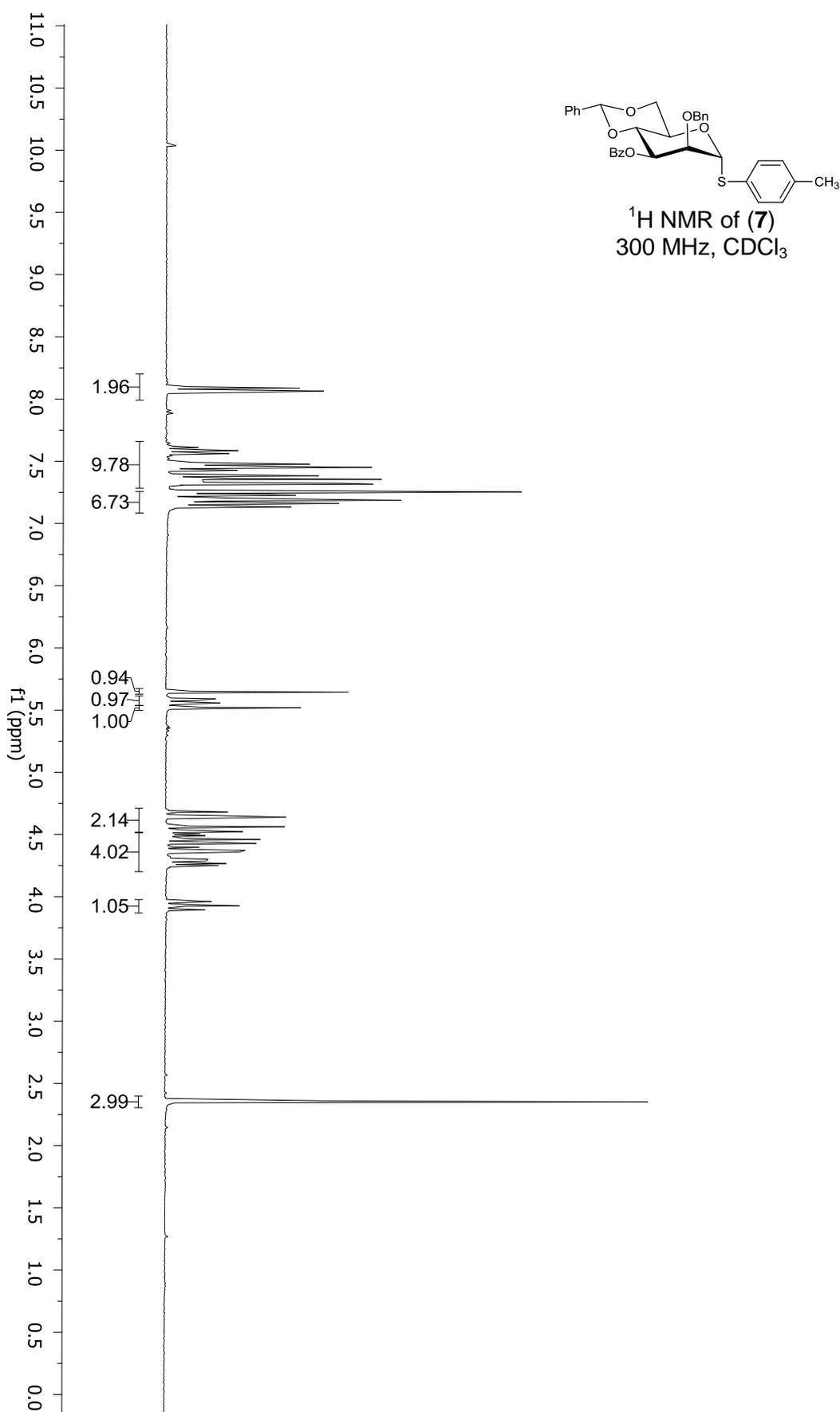
$$\% \text{ Inhibition} = (A_{(\text{no inhibitor})} - A_{(\text{with inhibitor})}) / A_{(\text{no inhibitor})} \times 100.$$

Results in triplicate were used for the plotting the inhibition curves for each individual ELLA experiment. Typically, the IC₅₀ values (concentration required for 50% inhibition of the Con A-yeast mannan association) obtained from several independently performed tests were in the range of ±12%. Nevertheless, the relative inhibition values calculated from independent series of data were highly reproducible.

4 : References

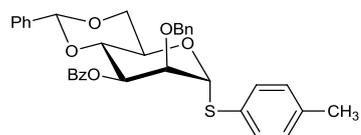
- [1] : D. Crich, W. Li, H. Li, *J. Am. Chem. Soc.* **2004**, *126*, 15081.
[2] : Z. Györgydea, H. Paulsen, *Liebigs Ann. Chem.* **1977**, 1987.

5 : ^1H and ^{13}C NMR Spectra of new compounds

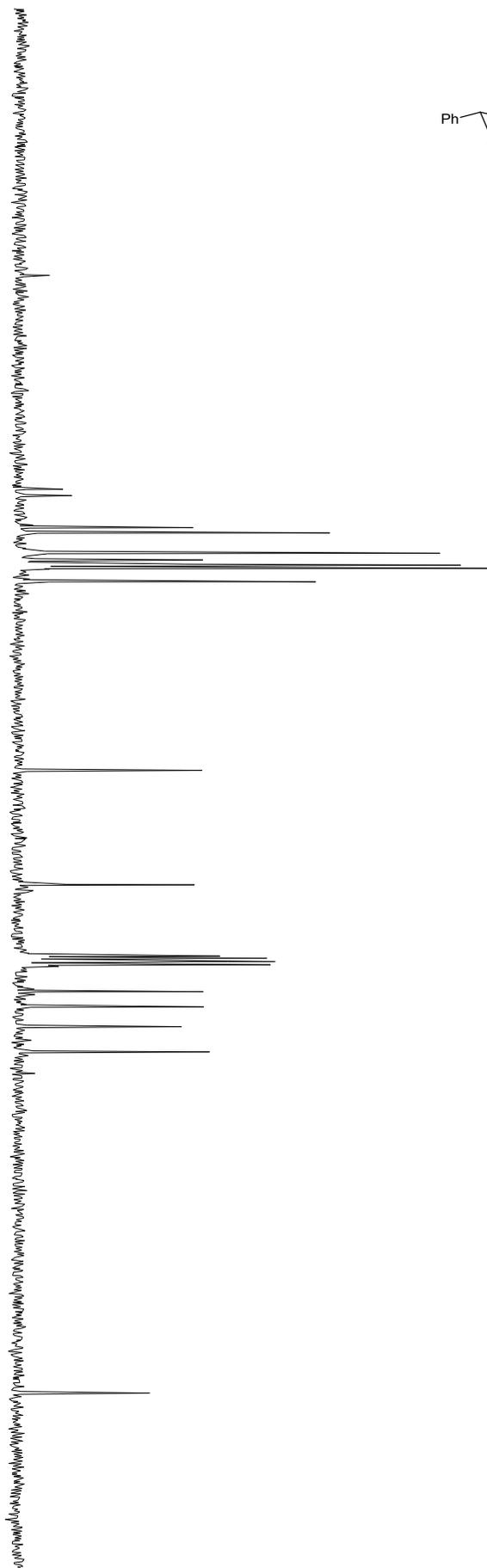


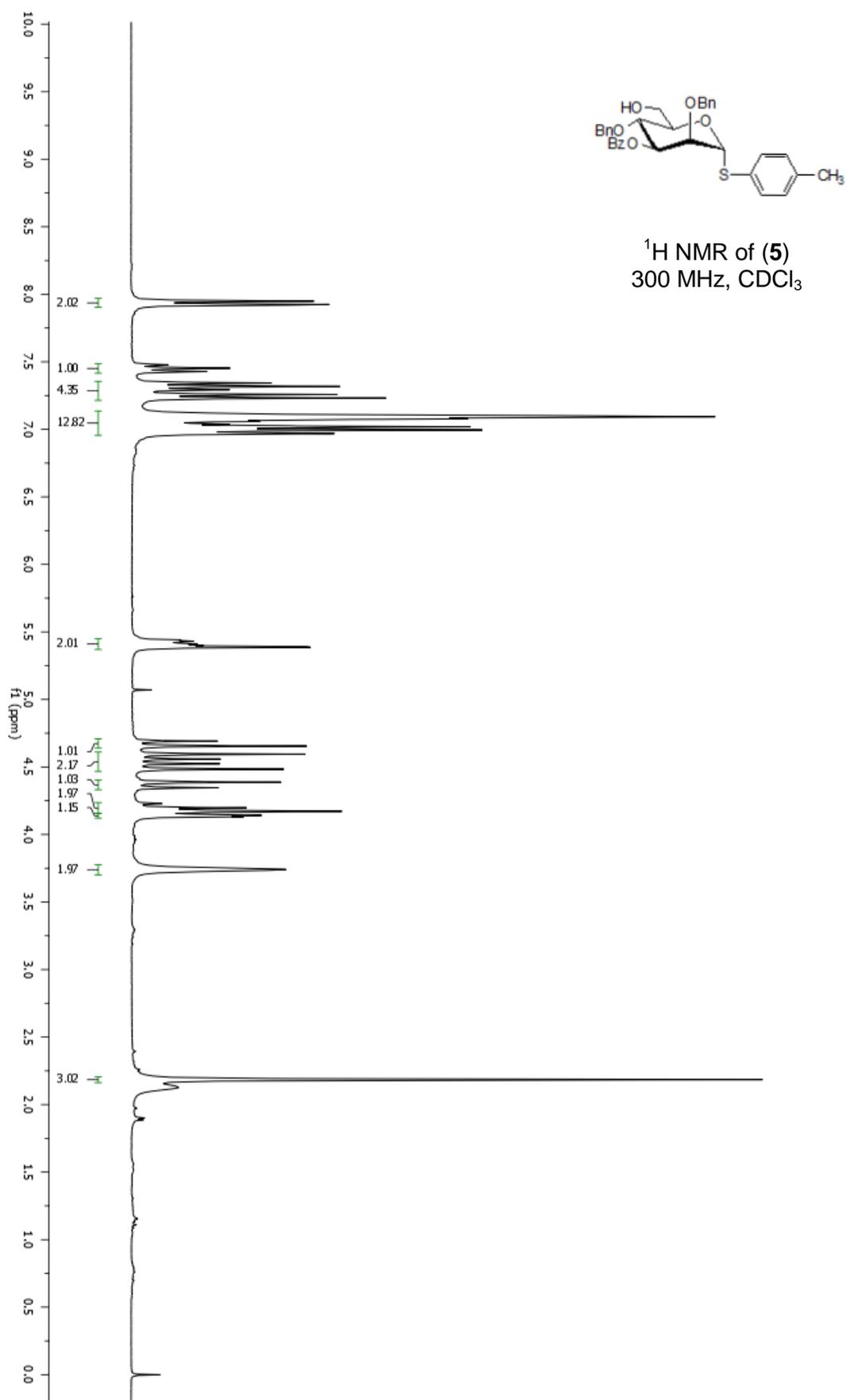
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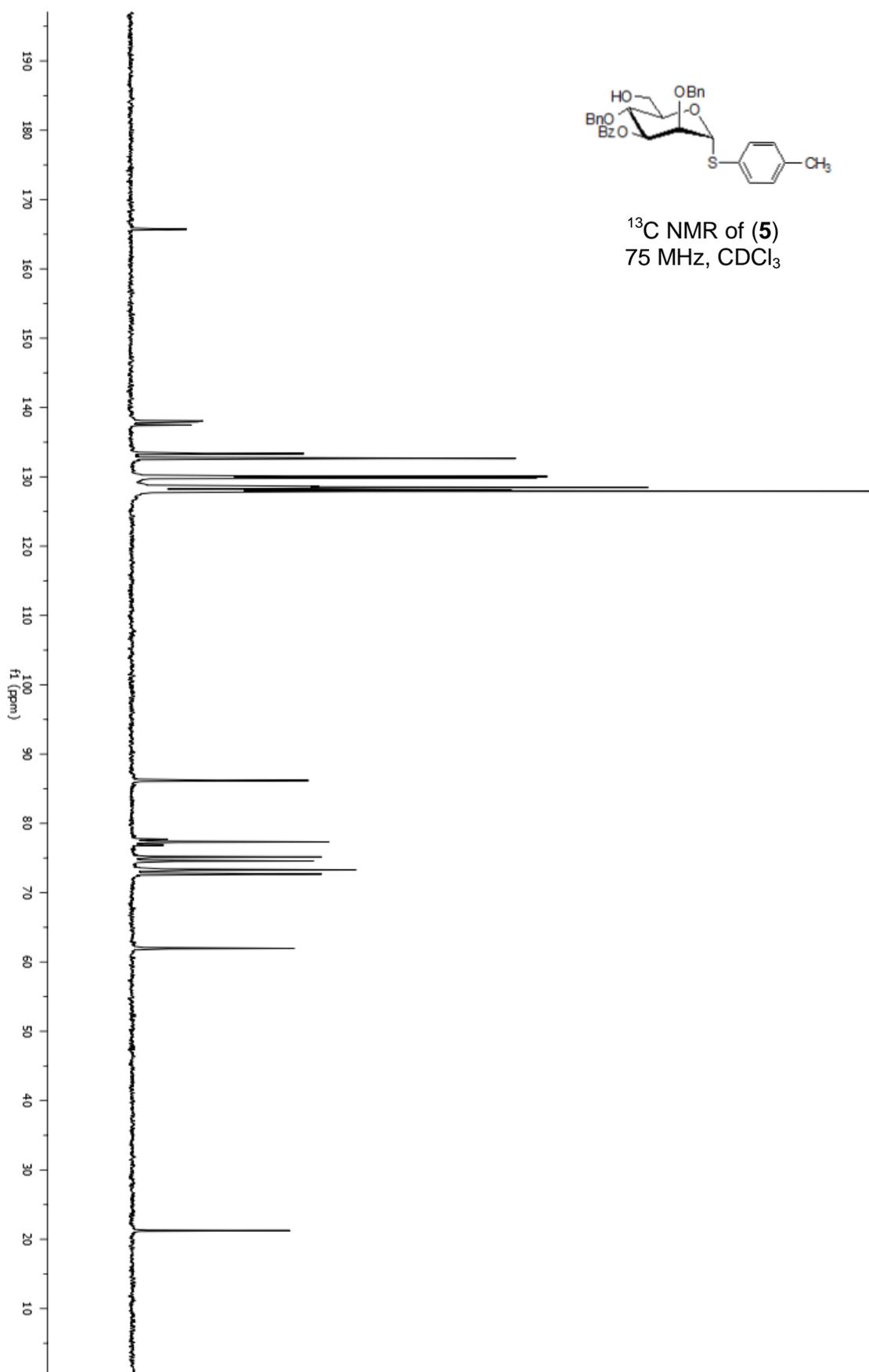
f1 (ppm)

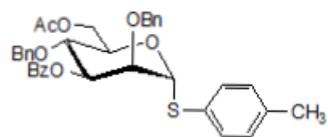
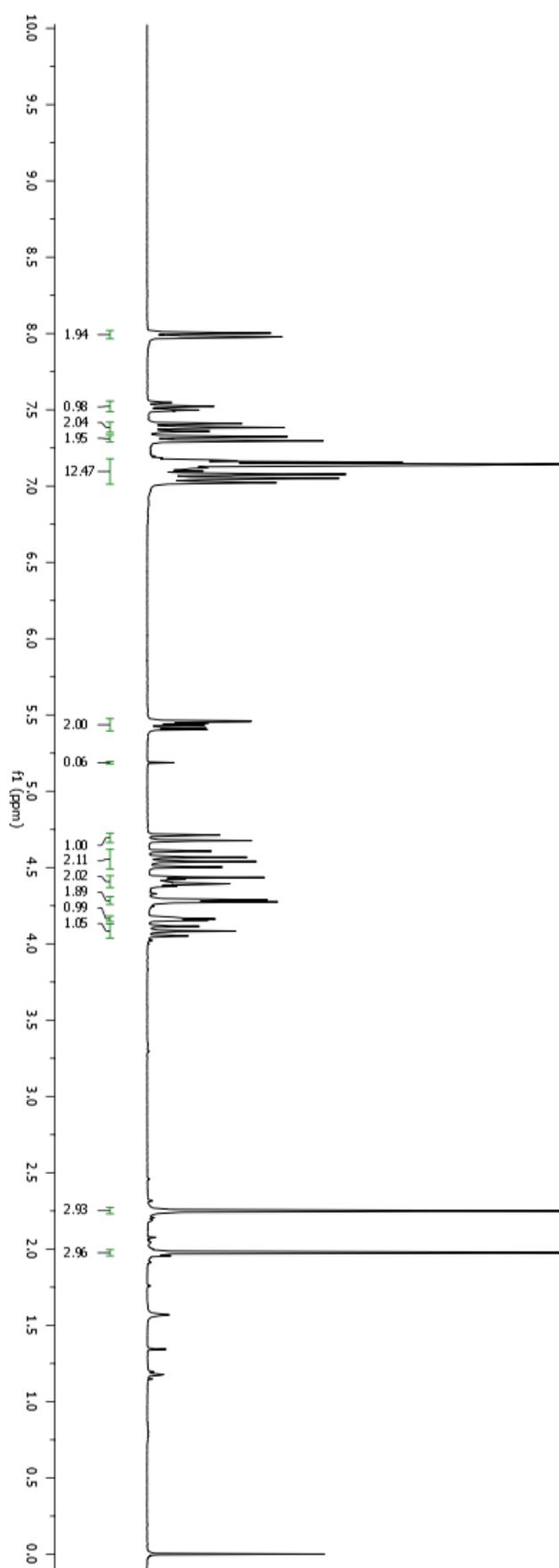


¹³C NMR of (7)
75 MHz, CDCl₃

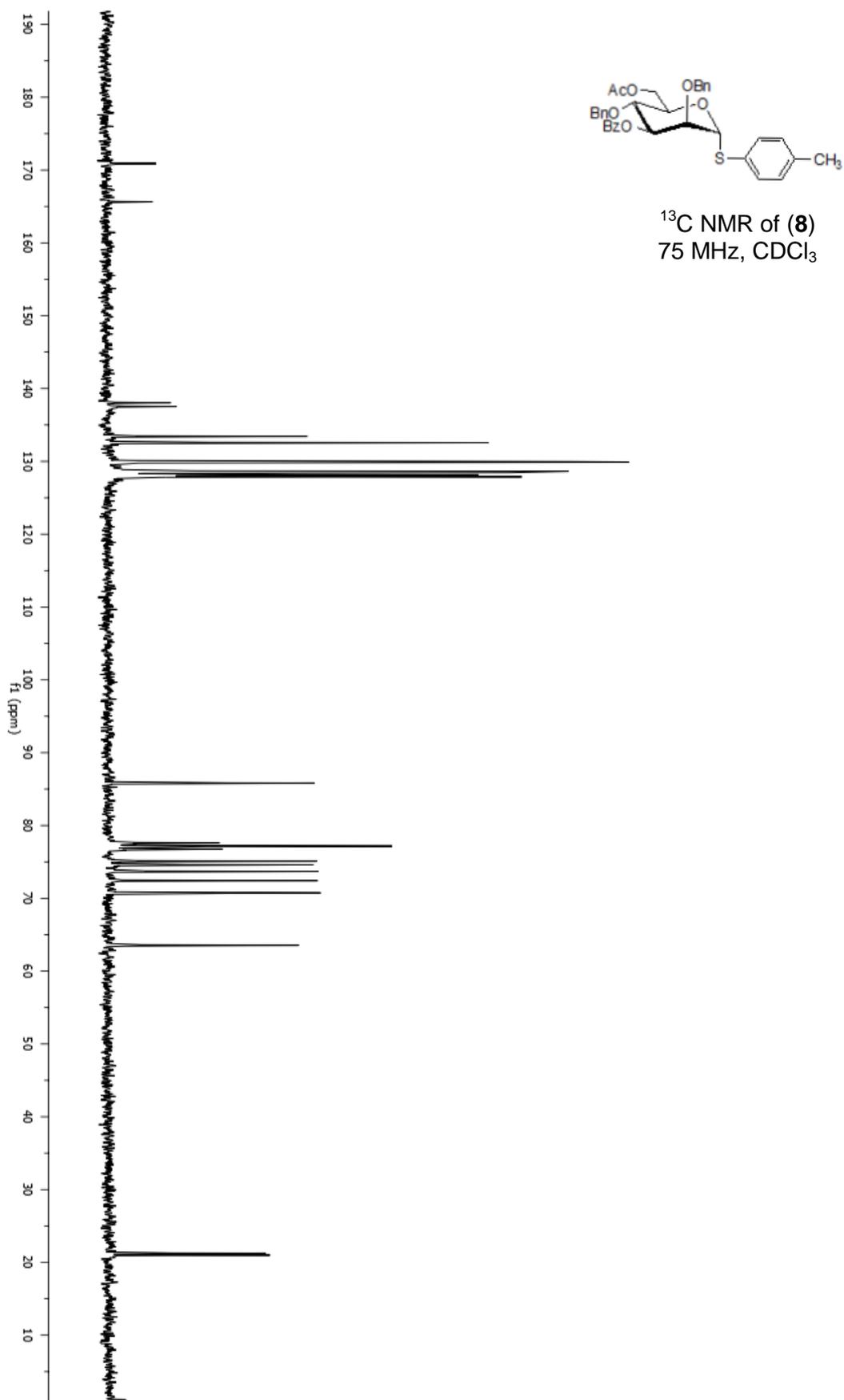


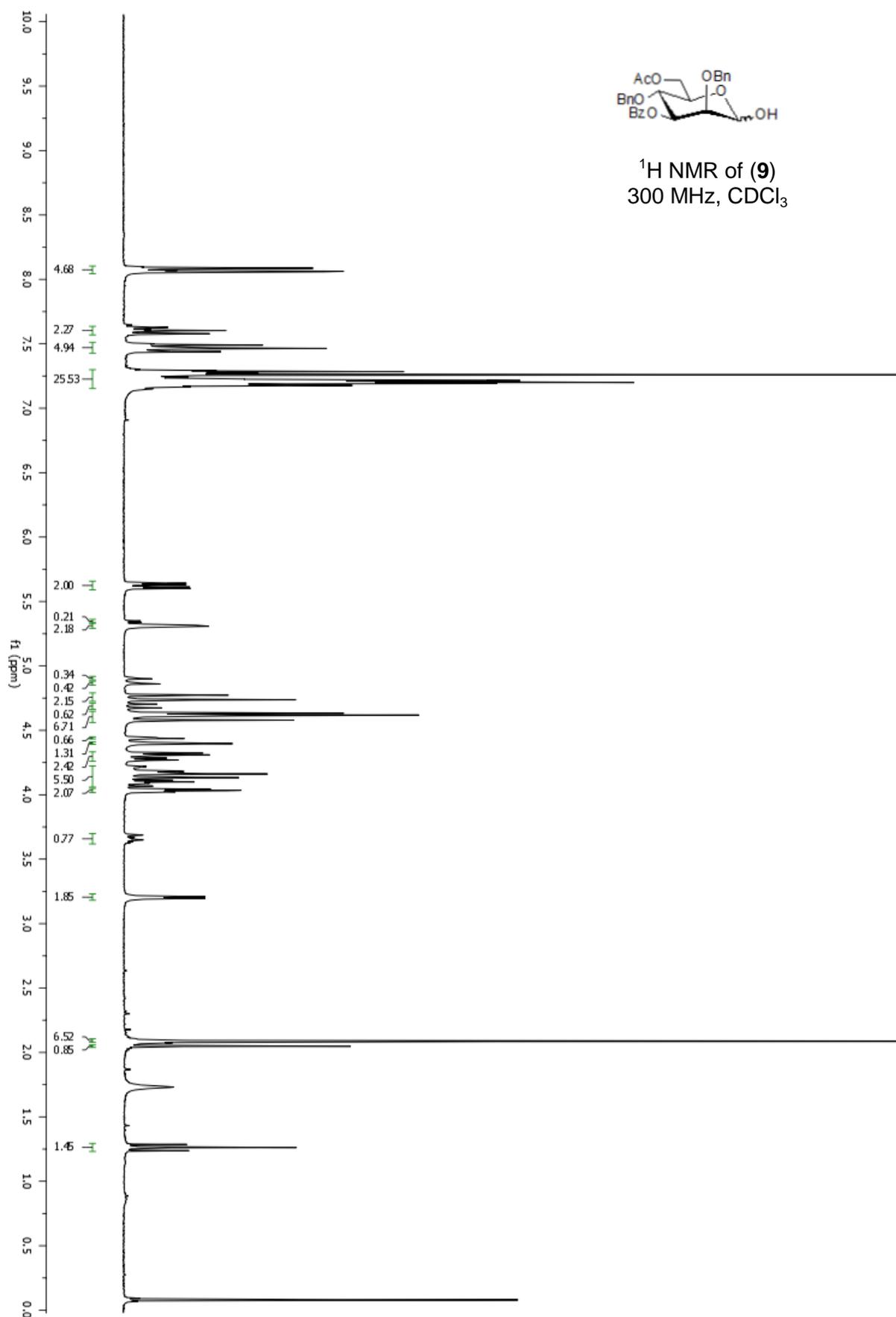


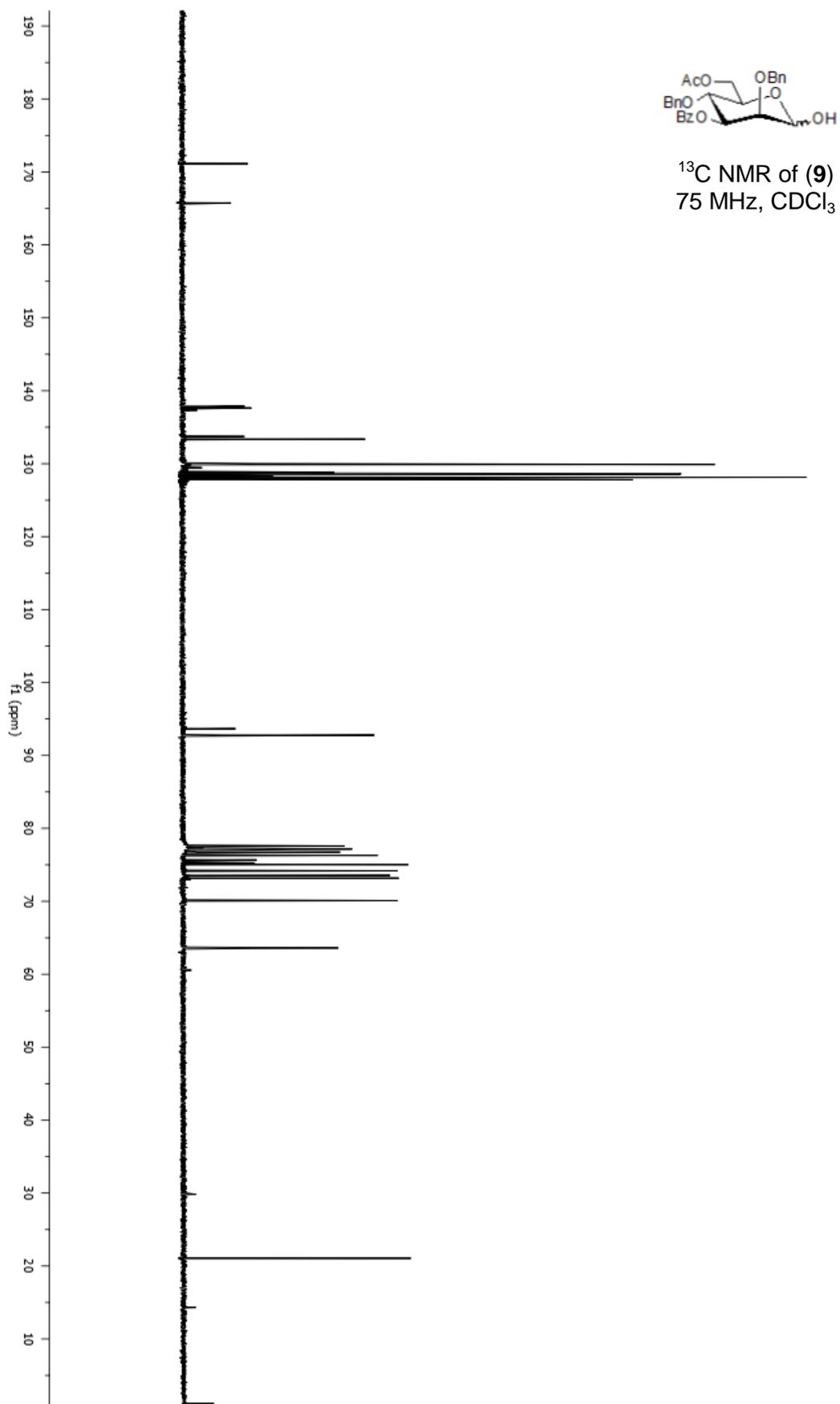


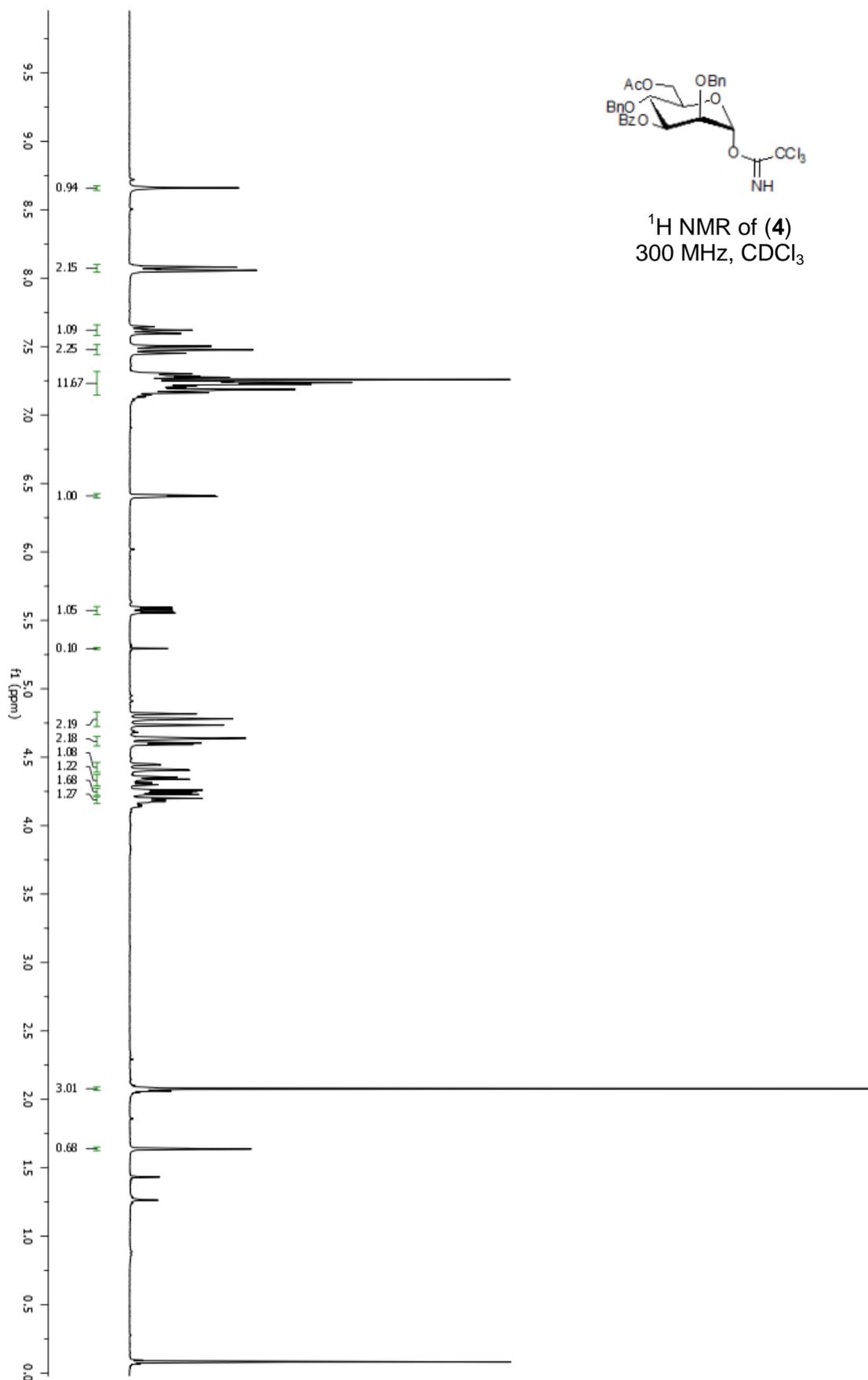


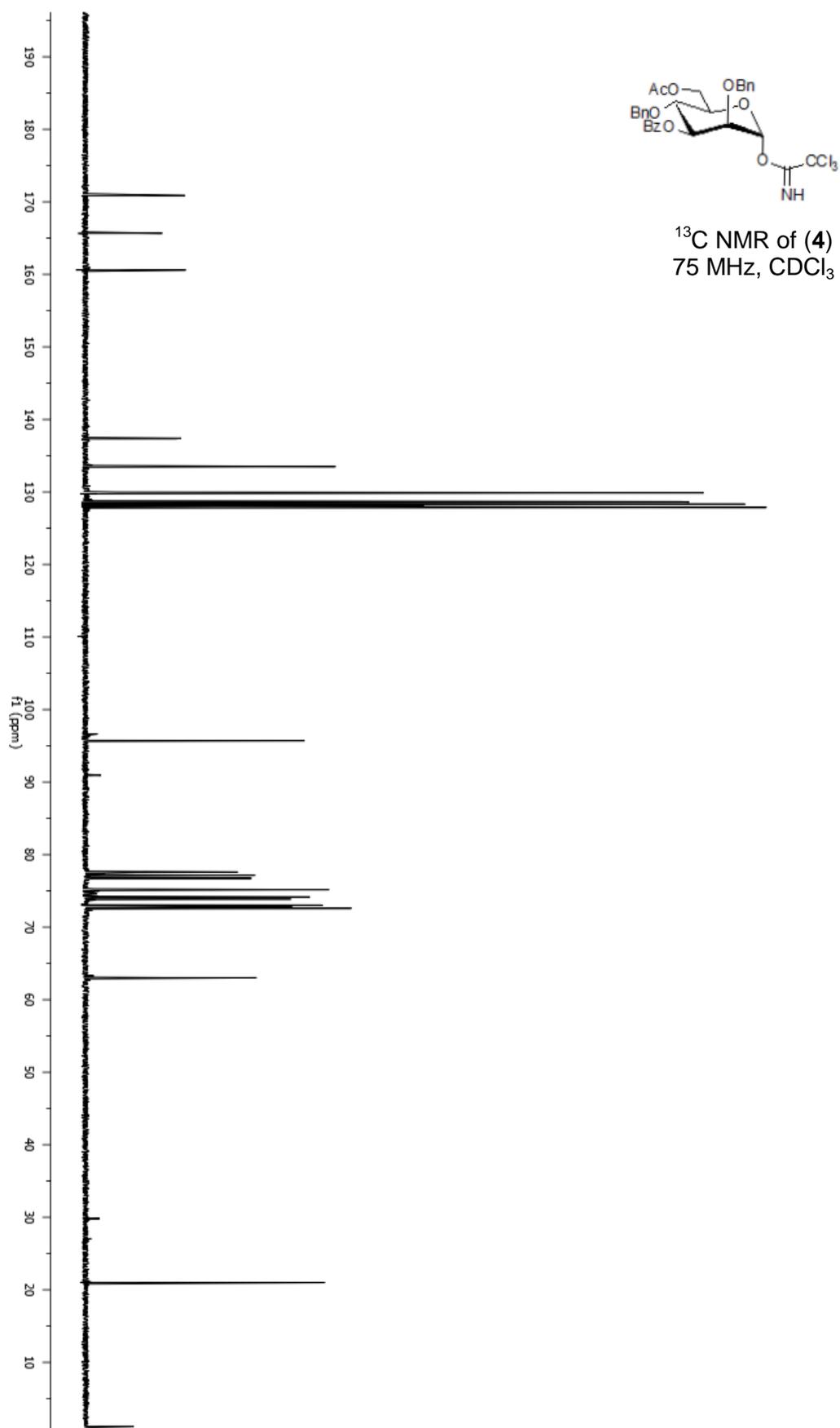
^1H NMR of **(8)**
300 MHz, CDCl_3

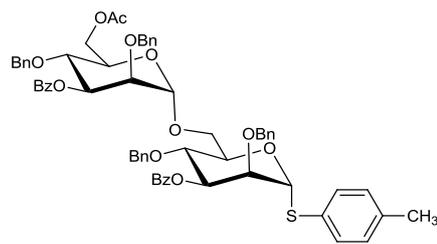
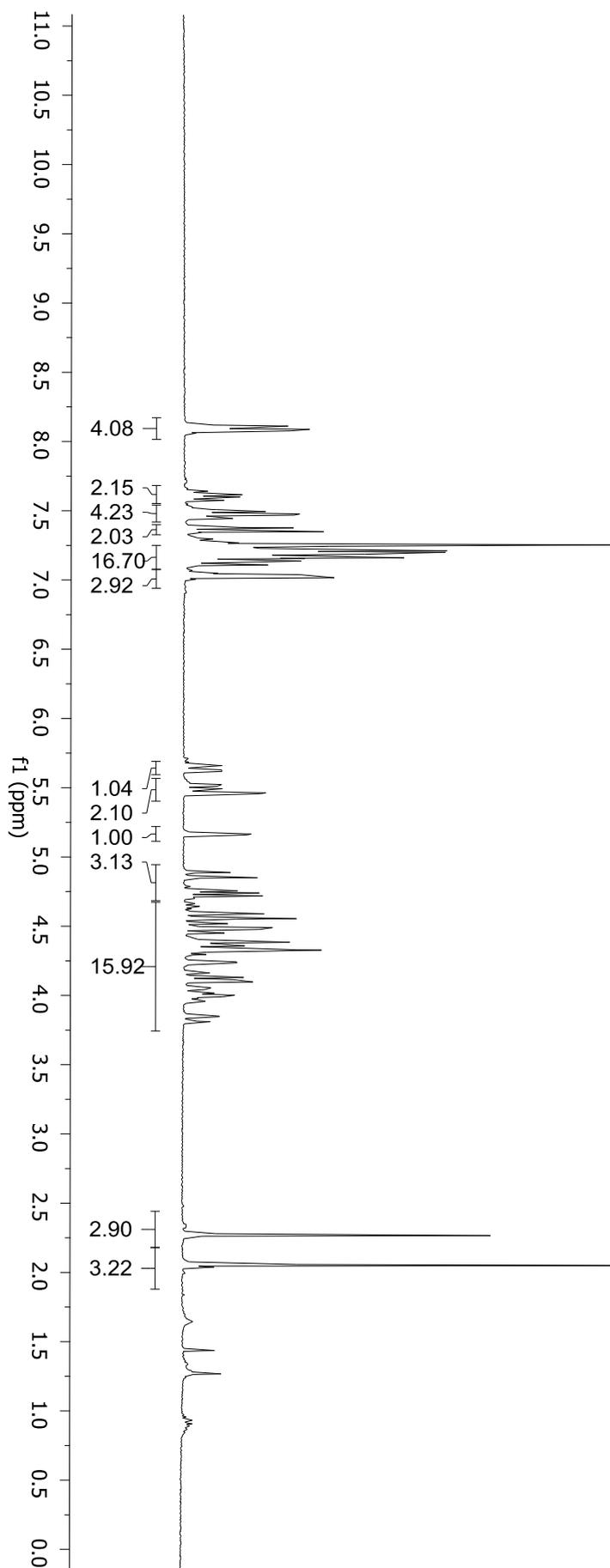








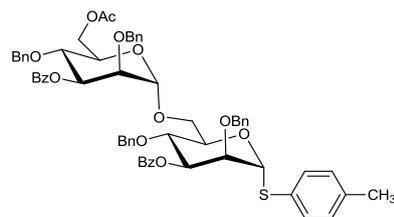
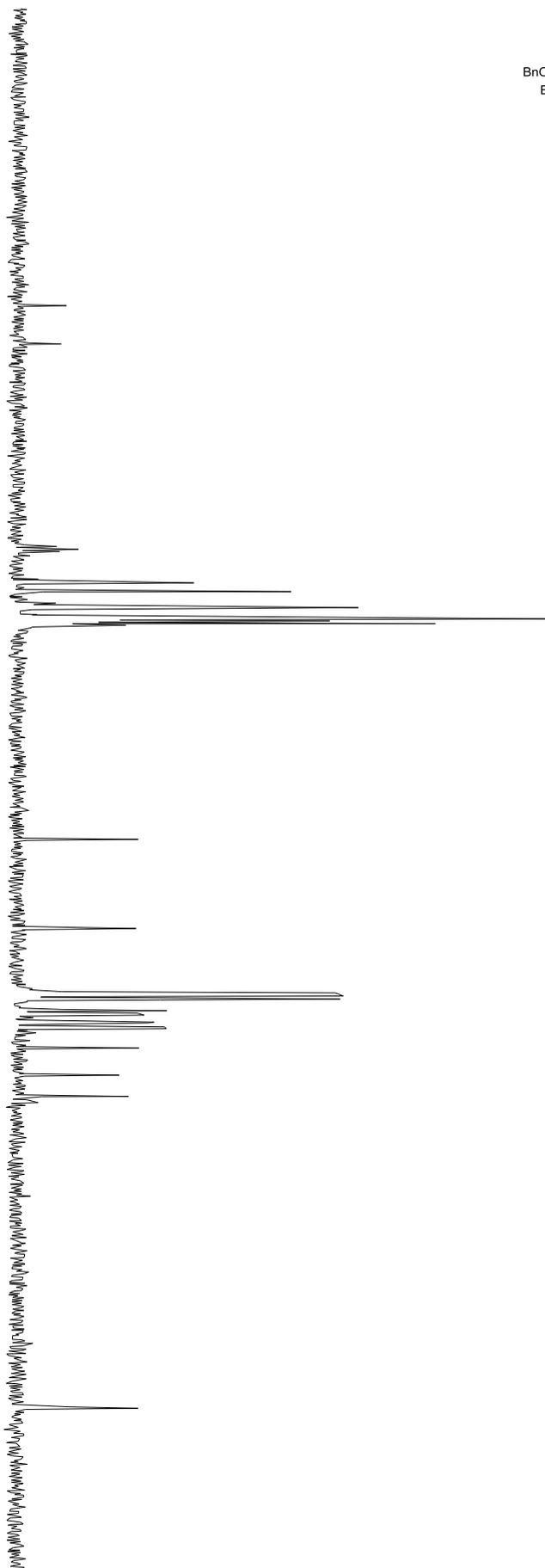




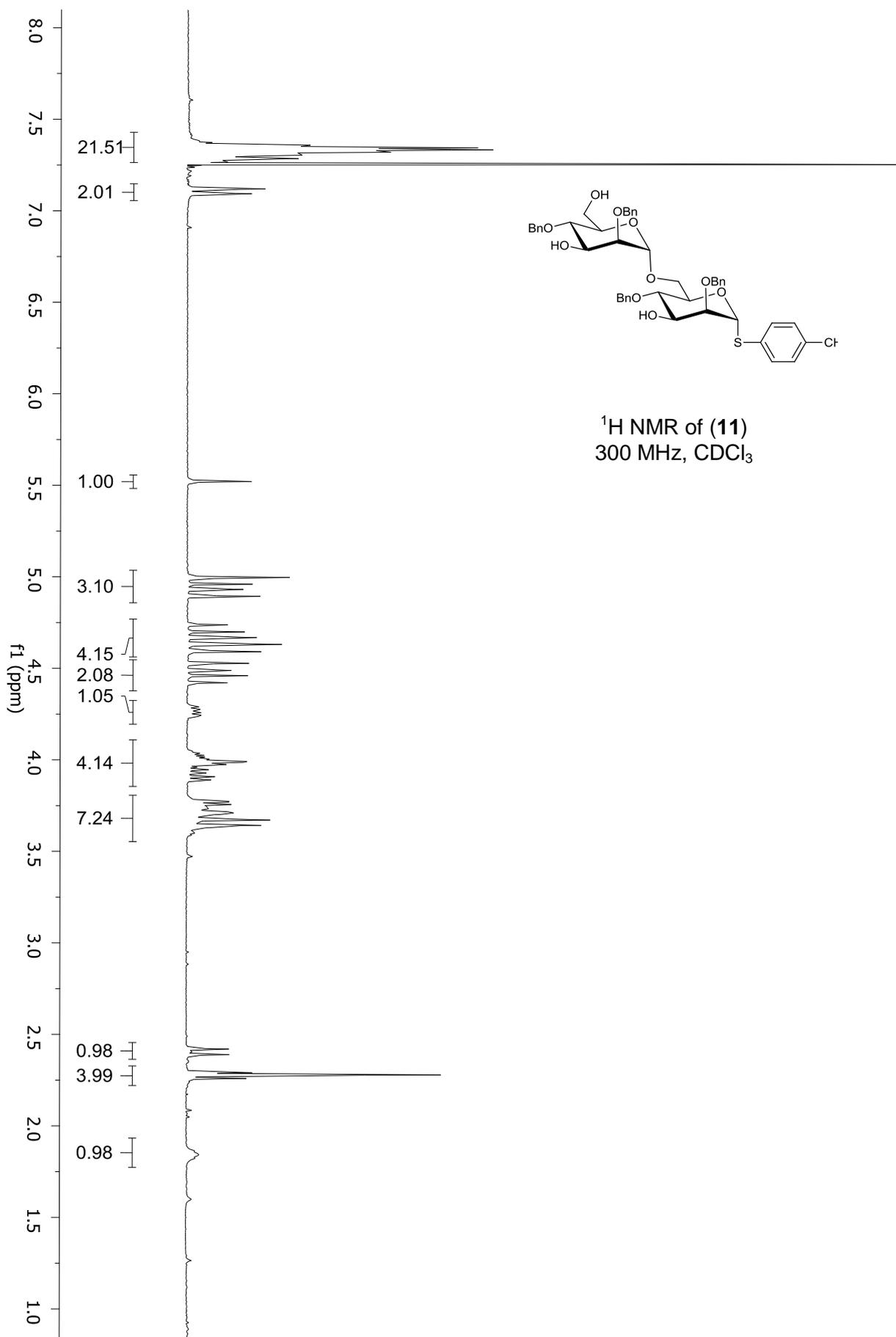
^1H NMR of (10)
300 MHz, CDCl_3

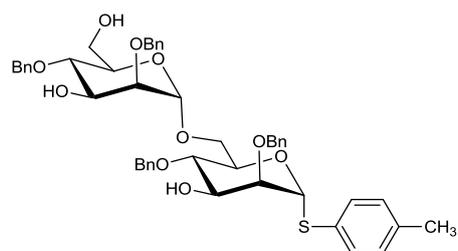
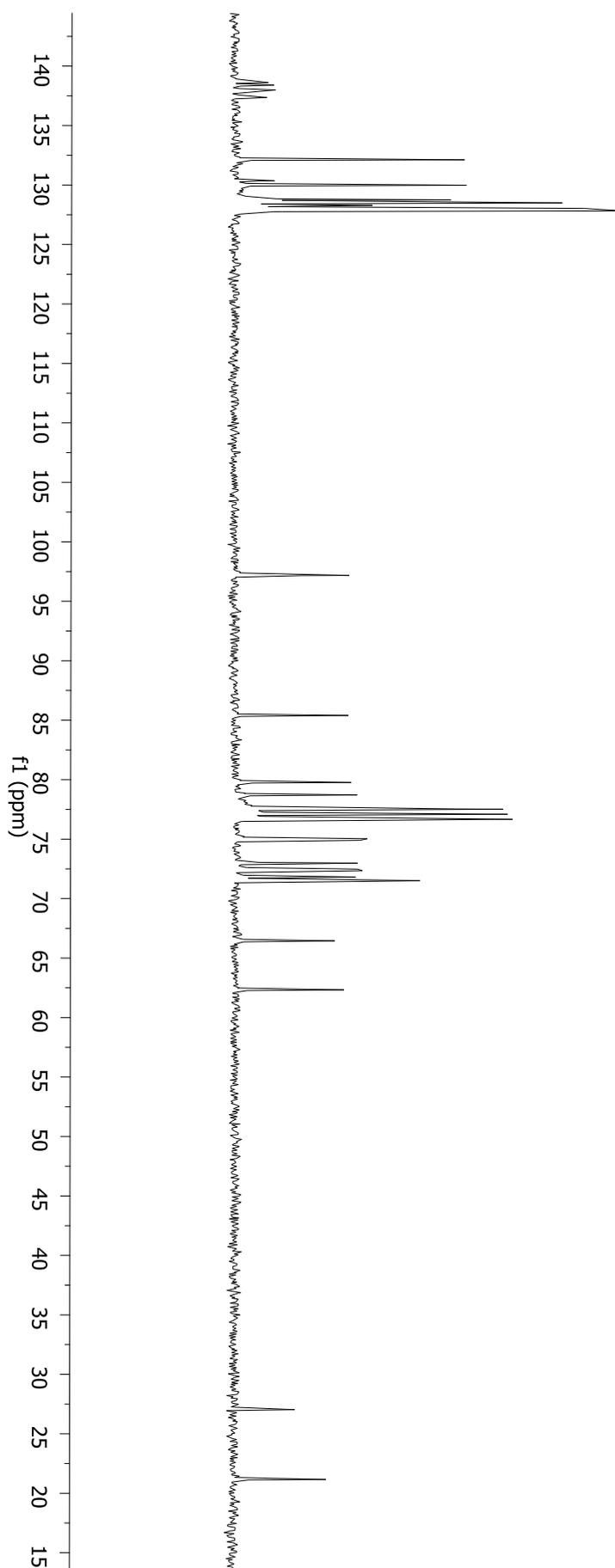
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f1 (ppm)

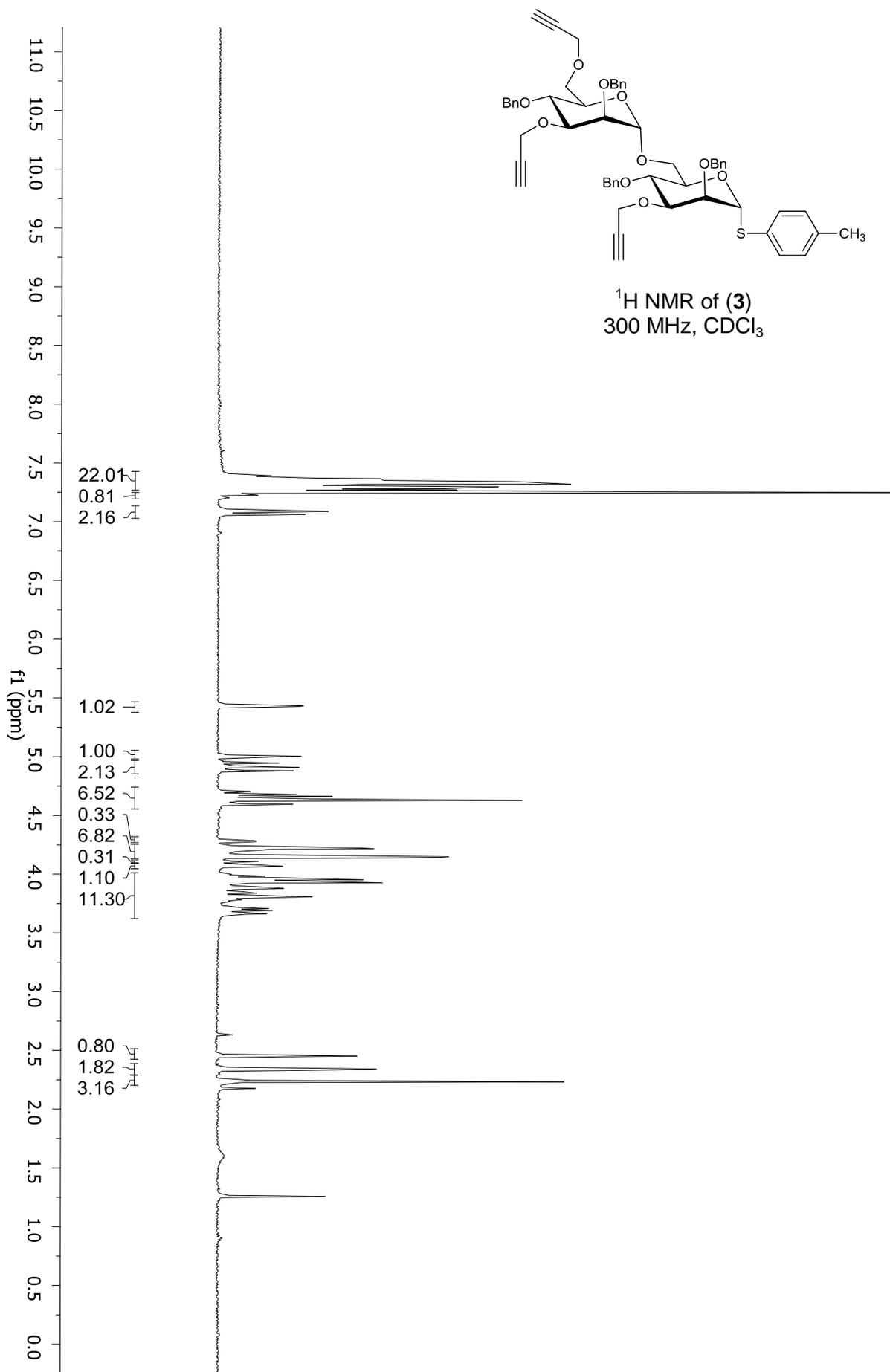


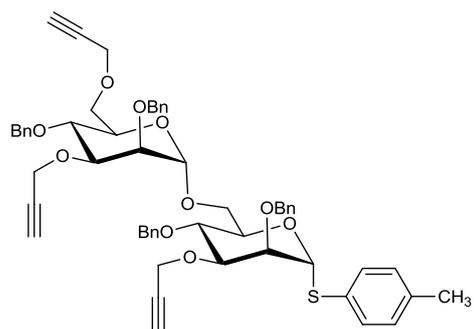
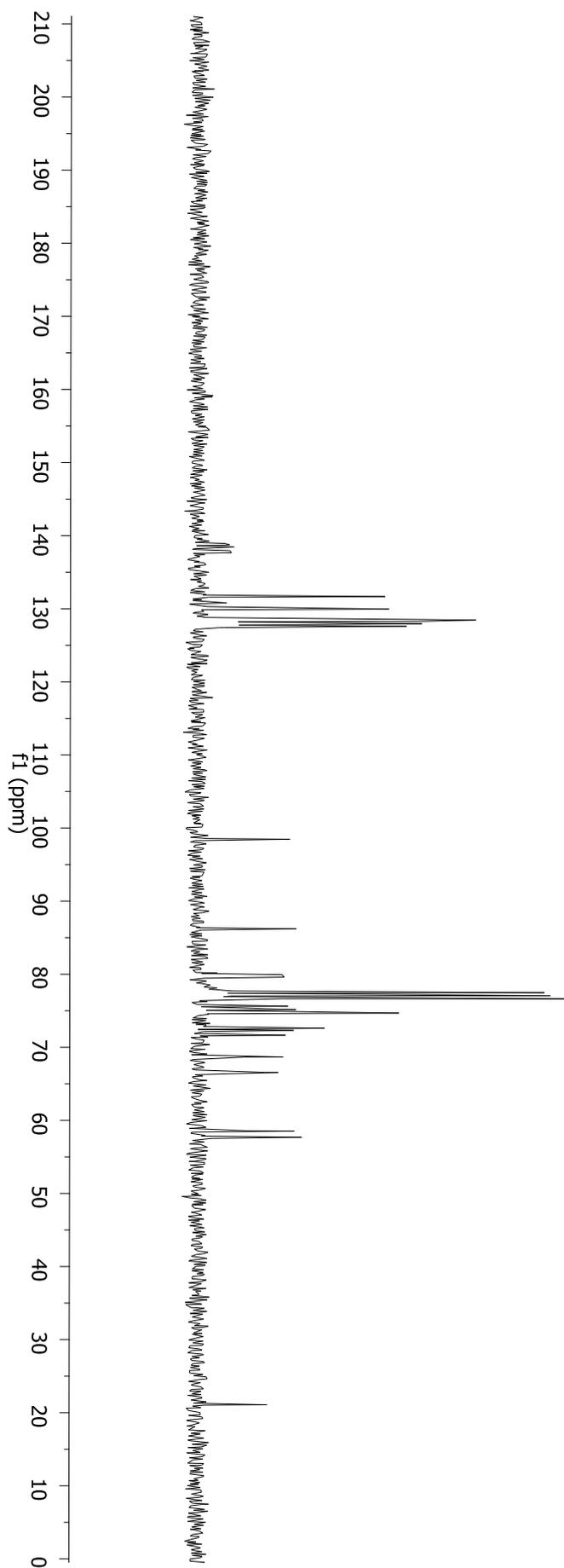
^{13}C NMR of (10)
75 MHz, CDCl_3



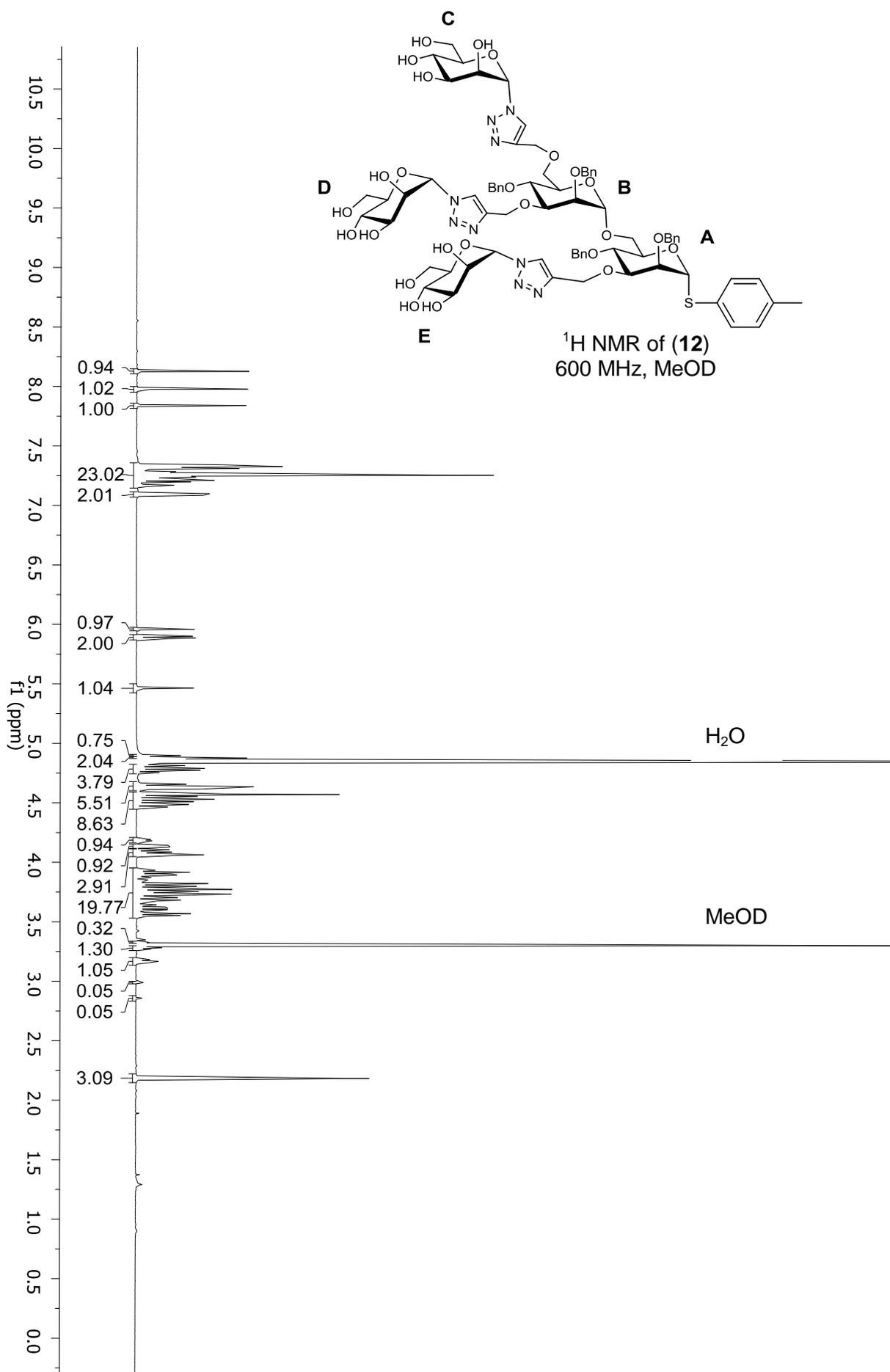


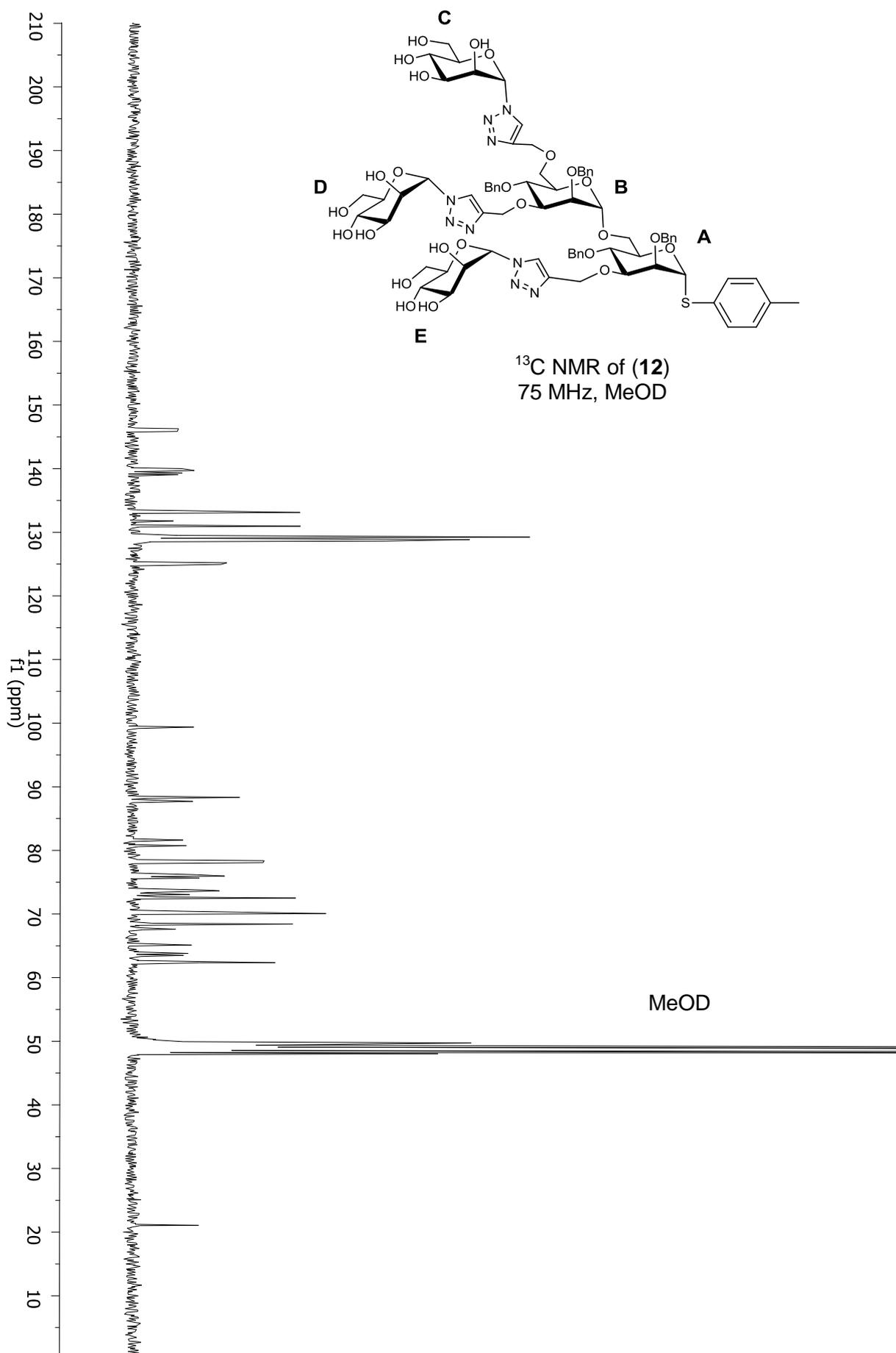
^{13}C NMR of (11)
75 MHz, CDCl_3

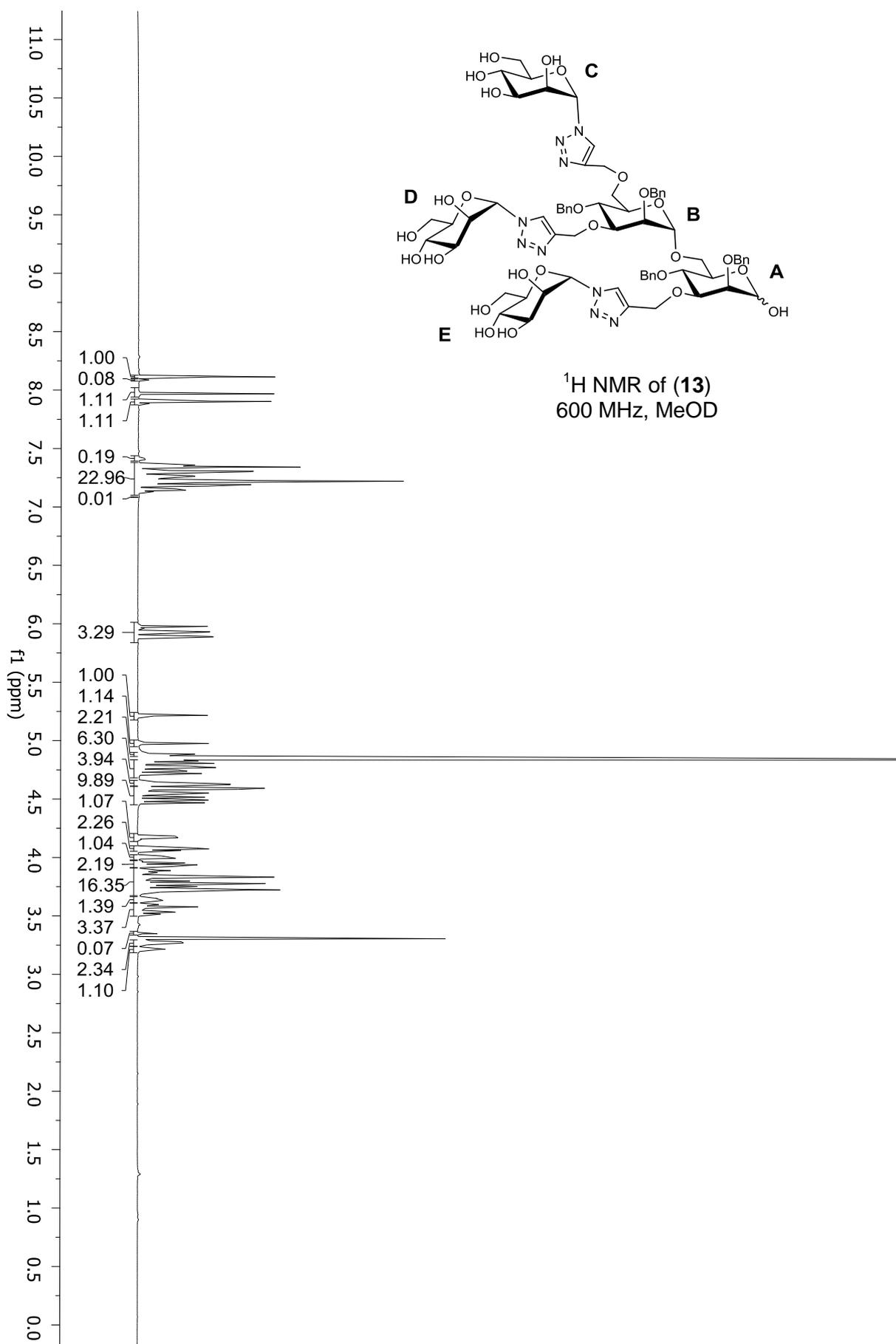


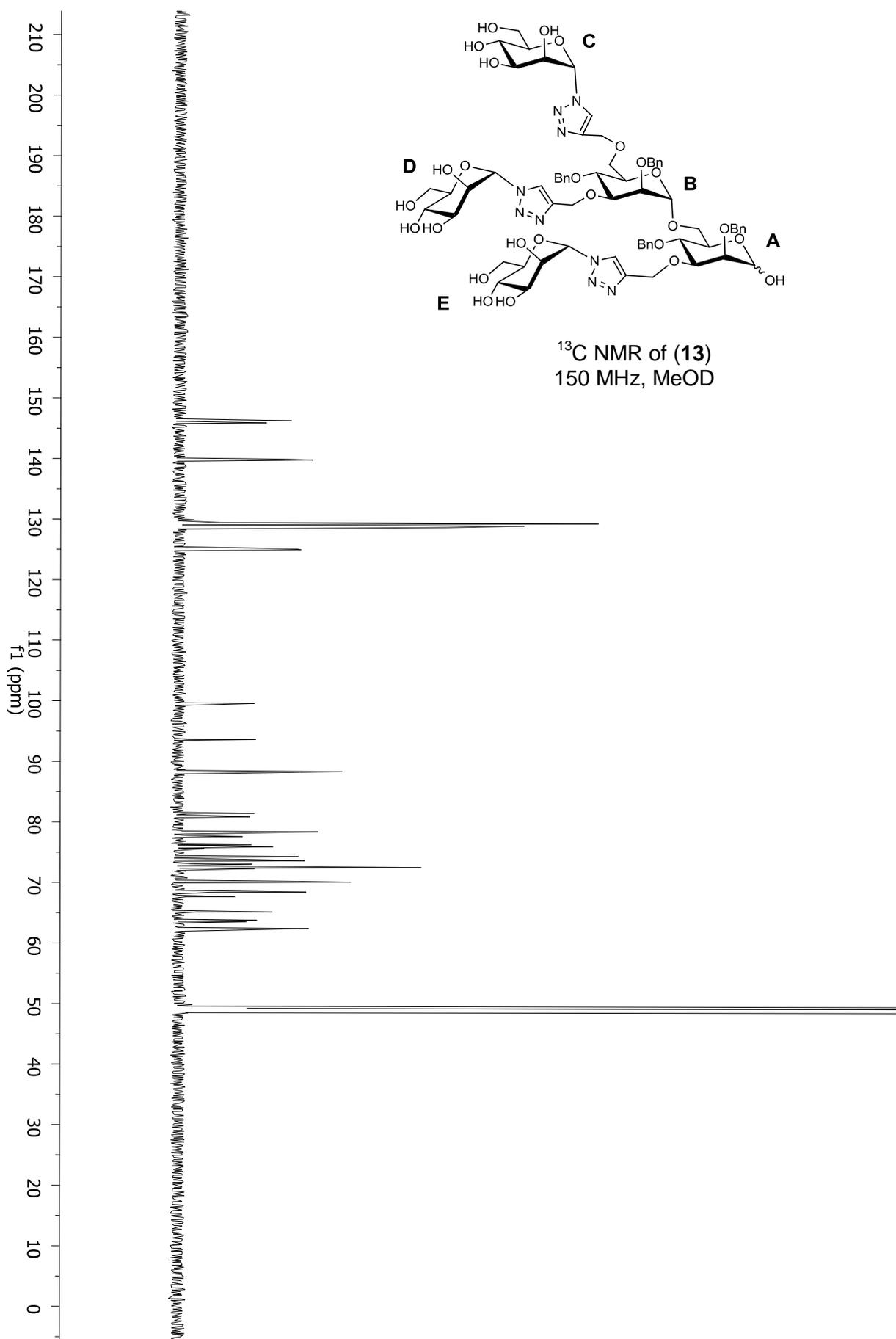


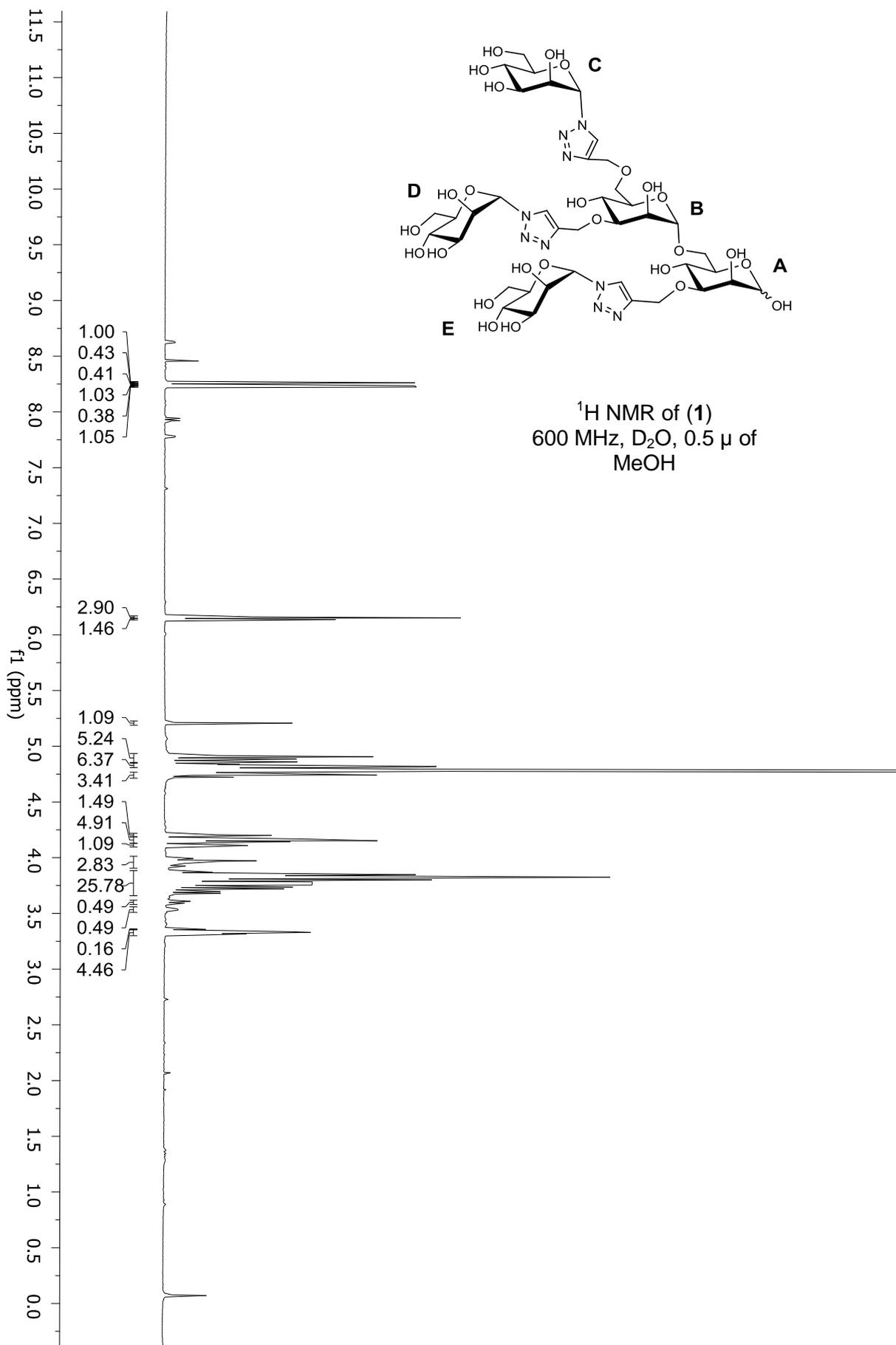
^{13}C NMR of **(3)**
75 MHz, CDCl₃

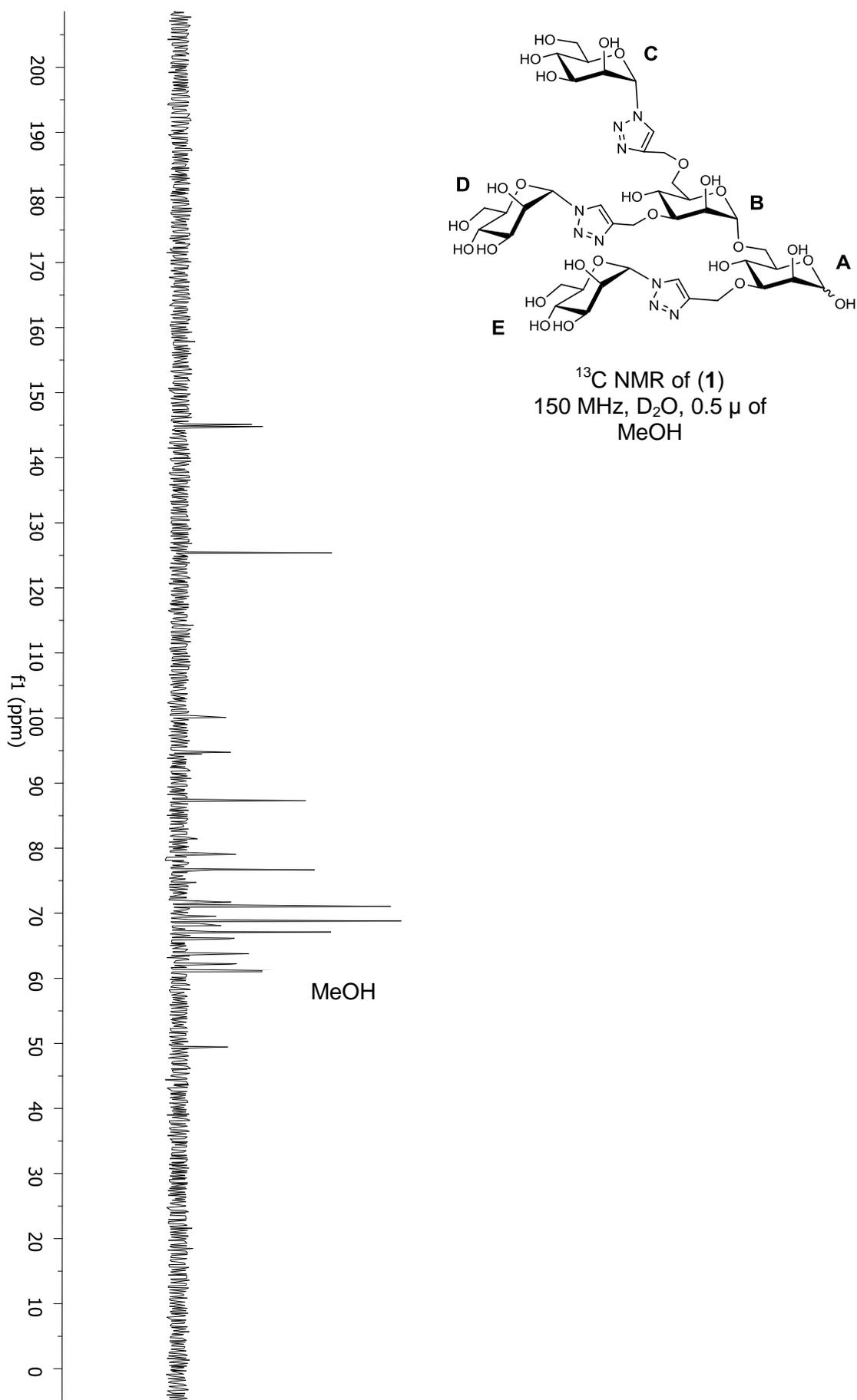




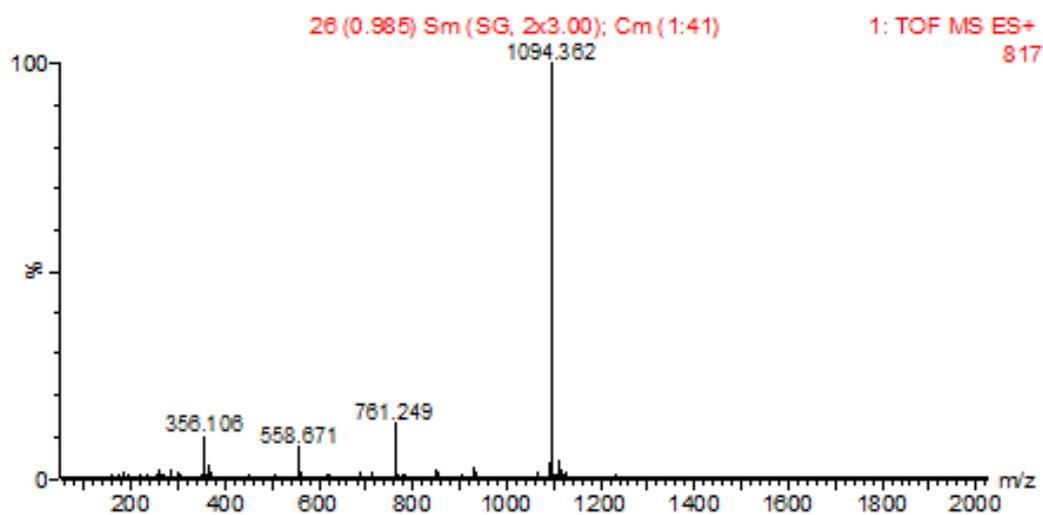




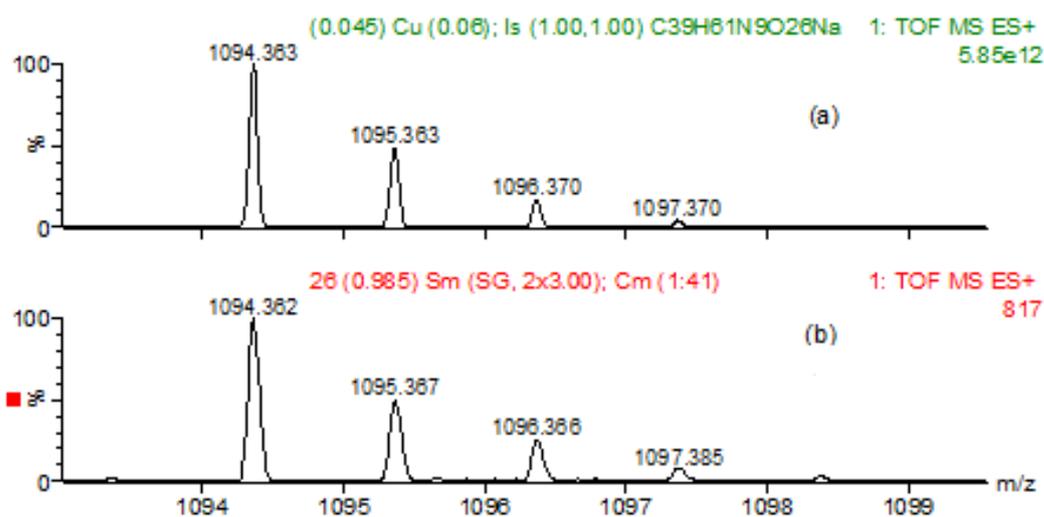




5 : Theoretical and experimental isotopic patterns for the MNa^+ ion of (1)



ESI-MS spectra for the MNa^+ ion of (1)



Theoretical (a) and experimental (b) isotopic patterns for the MNa^+ ion of (1)