

Electronic Supplementary Information

Synthesis and mass spectrometric analysis of disaccharides from methanolysis of heparan sulfate

Qi Qi He,^a Paul Trim,^b Marten Snel,^b John J. Hopwood^b and Vito Ferro^{a,*}

^a*School of Chemistry and Molecular Biosciences, The University of Queensland, Brisbane, QLD 4072, Australia*

^b*Hopwood Centre for Neurobiology, South Australian Health and Medical Research Institute, Adelaide, SA 5000, Australia*

Contents

Experimental details for compounds 13, 17-24, 29, 37, 42, 44, 46.	S3-S12
LC-MS/MS data for compounds 1-12.	S13-S37
¹ H and ¹³ C NMR spectra for compounds 1-14, 17-30, 32, 34-48.	S38-S133

4'-Methylphenyl 2-azido-2-deoxy-1-thio- β -D-glucopyranoside (13)^{1,2}

Off white solid; m.p. 114 – 115.5 °C; $[\alpha]_D -25.7$ (*c* 3.7, CHCl₃); ¹H NMR (500 MHz, CDCl₃): δ 7.44-7.38 (m, 2H, Ar), 7.15-7.11 (m, 2H, Ar), 4.44 (d, 1H, *J*_{1,2} = 10.0 Hz, H-1), 3.90 (dd, 1H, A part of ABX, *J*_{5,6a} = 3.4 Hz, *J*_{6a,6b} = 11.9 Hz, H-6a), 3.80 (dd, 1H, B part of ABX, *J*_{5,6b} = 4.6 Hz, H-6b), 3.52 (dd, 1H, *J*_{3,4} = *J*_{4,5} = 9.1 Hz, H-4), 3.46 (dd, 1H, *J*_{2,3} = *J*_{3,4} = 9.1 Hz, H-3), 3.35 – 3.32 (m, 1H, H-5), 3.23 (dd, 1H, *J*_{1,2} = *J*_{2,3} = 9.6 Hz, 1H, H-2), 2.33 (s, 3H, CH₃); ¹³C NMR (100 MHz, CDCl₃): δ 138.9, 133.8, 129.9, 127.2 (Ar), 86.5 (C-1), 79.1 (C-5), 77.2 (C-3), 70.1 (C-4), 64.9 (C-2), 62.4 (C-6), 21.1 (CH₃); LRMS: *m/z* = 334.0 [M+Na]⁺; HRMS: *m/z* calcd for C₁₃H₁₇N₃O₄SNa [M+Na]⁺: 334.0837, found: 334.0826.

4'-Methylphenyl 3-*O*-benzyl-4,6-*O*-isopropylidene-1-thio- α -L-idoside (29)²

White solid; m.p. 89 – 92.0 °C; $[\alpha]_D -85.8$ (*c* 1.1, CHCl₃); ¹H NMR (500 MHz, CDCl₃): δ 7.44 – 7.32 (m, 7 H, Ar), 7.11-7.08 (m, 2H, Ar), 5.57 (s, 1H, H-1), 4.85, 4.57 (ABq, 2H, *J*_{A,B} = 11.7 Hz, CH₂Ph), 4.31 (ddd, 1H, *J*_{4,5} = 1.5 Hz, *J*_{5,6a} = 1.9 Hz, *J*_{5,6b} = 1.7 Hz, H-5), 4.12 (dd, 1H, A part of ABX, *J*_{6a,6b} = 12.9 Hz, H-6a), 4.07 (ddd, 1H, *J*_{1,2} = 1.5 Hz, *J*_{2,3} = 2.7 Hz, *J*_{2,OH} = 11.5 Hz, H-2), 4.04 (dd, 1H, *J*_{3,4} = 2.7 Hz, H-4), 4.00 (d, 1H, OH), 3.94 (dd, 1H, B part of ABX, H-6b), 3.66 (dd, 1H, *J*_{2,3} = *J*_{3,4} = 2.7 Hz, H-3), 2.31 (s, 3H, ArCH₃), 1.45 (s, 6H, 2 × CH₃); ¹³C NMR (100 MHz, CDCl₃): δ 137.4, 136.7, 133.5, 131.2, 130.6, 129.6, 128.5, 127.9, 127.7, 127.6 (C-Ar), 99.2 (C(CH₃)₂), 89.2 (C-1), 74.0 (C-3), 72.4 (CH₂Ph), 67.8 (C-2, C-4), 63.4 (C-6), 60.2 (C-5), 29.5 (CH₃), 21.0 (ArCH₃), 18.2 (CH₃); LRMS: *m/z* = 439.2 [M+Na]⁺, 455.1 [M+K]⁺; HRMS: *m/z* calcd for C₂₃H₂₈O₅SNa [M+Na]⁺: 439.1550; found: 439.1547.

Methyl 4,6-*O*-(4'-methoxybenzylidene)- α -D-glucoside (17)

Methyl α -D-glucopyranoside **15** (6 g, 30.9 mmol) was dissolved in anhydrous DMF (30 mL) and anisaldehyde dimethyl acetal (7.9 mL, 46.4 mmol) and camphor-10-sulfonic acid (360 mg,

1.55 mmol) were added (to pH 3). The reaction mixture was stirred at r.t. under Ar for 18 h and was then neutralized by addition of Et₃N (to pH 8). The resulting solution was then diluted with EtOAc, washed with H₂O, dried (MgSO₄), filtered and concentrated to dryness. The residue was then recrystallized from MeOH/*n*-hexane to give the 4'-methoxybenzylidene **17** as colourless crystals (3.75 g, 39 %); *R*_f = 0.76 (EtOAc/MeOH, 9:1); m.p. 200 – 201 °C (lit.³ 201 – 202 °C); [α]_D +87.5 (*c* 2.8, CHCl₃; lit.³ +88, *c* 1.0, MeOH); ¹H NMR (500 MHz, CDCl₃): δ 7.46–7.40 (m, 2H, Ar), 6.93–6.88 (m, 2H, Ar), 5.52 (s, 1H, CHAr), 4.83 (d, 1H, *J*_{1,2} = 3.8 Hz, H-1), 4.30 (dd, 1H, A part of ABX, *J*_{5,6a} = 4.5, *J*_{6a,6b} = 9.9 Hz, H-6a), 3.95 (dd, 1H, *J*_{2,3} = *J*_{3,4} = 9.1 Hz, H-3), 3.85 – 3.80 (m, 1H, H-5), 3.78 (s, 3H, ArOCH₃), 3.75 (dd, 1H, B part of ABX, *J*_{6a,6b} = *J*_{5,6b} = 10.1 Hz, H-6b), 3.65 (dt, 1H, *J*_{1,2} = 4.0, *J*_{2,3} = *J*_{2,OH} = 9.1 Hz, H-2), 3.51 (dd, 1H, *J*_{4,5} = *J*_{3,4} = 9.3 Hz, H-4), 3.45 (s, 3H, OCH₃). 2.65 (s, 1H, OH-3), 2.24 (d, 1H, *J*_{2,OH} = 9.4 Hz, OH-2); ¹³C NMR (125 MHz, CDCl₃): δ 160.3, 129.5, 127.6, 113.7 (C-Ar), 101.9 (ArCH), 99.7 (C-1), 80.8 (C-4), 72.9 (C-2), 71.9 (C-3), 68.9 (C-6), 62.4 (C-5), 55.6 (OCH₃), 55.3 (ArOCH₃); LRMS: *m/z* = 313.07 [M+H]⁺, 335.04 [M+Na]⁺, 350.96 [M+K]⁺.

Methyl 2,3-di-*O*-benzyl-4,6-*O*-(4'-methoxybenzylidene)- α -D-glucoside (**19**)

The diol **17** (2.0 g, 6.41 mmol) dissolved in anhydrous DMF (7 mL) was slowly added to a suspension of NaH (616 mg, 15.4 mmol, pre-washed with *n*-hexane) in anhydrous DMF (7 mL). The mixture was stirred at r.t. for 10 min, and BnBr (1.83 mL, 15.4 mmol) was added dropwise under ice-cooling. The stirring was continued for 10 min under ice-cooling, and for an additional 1.5 h at r.t. MeOH (10 mL) was added slowly to quench the reaction. The resulting mixture was diluted with EtOAc, washed with H₂O, dried (MgSO₄), filtered and concentrated. The residue was recrystallized from EtOAc/*n*-hexane to give the dibenzyl ether **19** as colourless crystals (3.15 g, 100%); *R*_f = 0.17 (EtOAc/*n*-hexane, 1:4); m. p. 141 – 142 °C (lit.⁴ 143 – 144 °C); [α]_D -42.9 (*c* 1.11, CHCl₃; lit.⁴ -22); ¹H NMR (500 MHz, CDCl₃): δ 7.39 – 7.23 (m, 12H,

Ar), 6.90-6.86 (m, 2H, Ar), 5.49 (s, 1H, CHAr), 4.88, 4.81 (ABq, 2H, $J_{A,B} = 11.2$ Hz, PhCH₂), 4.83, 4.67 (ABq, 2H, $J_{A,B} = 12.2$ Hz, PhCH₂), 4.75 (d, 1H, $J_{1,2} = 3.6$ Hz, H-1), 4.22 (dd, 1H, A part of ABX, $J_{5,6a} = 4.8$ Hz, $J_{6a,6b} = 10.3$ Hz, H-6a), 4.02 (dd, 1H, $J_{2,3} = J_{3,4} = 9.3$ Hz, H-3), 3.80 (ddd, 1H, $J_{4,5} = J_{5,6b} = 9.9$ Hz, H-5), 3.79 (s, 3H, ArOCH₃), 3.67 (dd, 1H, B part of ABX, $J_{5,6b} = J_{6a,6b} = 10.3$ Hz, H-6b), 3.57 (dd, 1H, $J_{3,4} = J_{4,5} = 9.3$ Hz, H-4), 3.53 (dd, 1H, H-2), 3.38 (s, 3H, OCH₃); ¹³C NMR (125 MHz, CDCl₃): δ 159.9, 138.7, 138.1, 129.9, 128.4, 128.2, 128.1, 128.0, 127.8, 127.5, 127.3, 113.5 (C-Ar), 101.2 (ArCH), 99.2 (C-1), 82.0 (C-4), 79.1 (C-2), 78.6 (C-3), 75.3, 73.7 (2 × CH₂Ph), 69.0 (C-6), 62.3 (C-5), 55.3 (OCH₃), 55.2 (ArOCH₃); LRMS: $m/z = 493.1$ [M+Na]⁺, 515.1 [M+K]⁺, 531.1 [M+K]⁺; HRMS: m/z calcd for C₂₉H₃₂O₇Na [M+Na]⁺: 515.2040, found: 515.2020.

Methyl 2,3-di-O-benzyl- α -D-glucopyranoside (21)

The 4'-methoxybenzylidene **19** (3.0 g, 6.09 mmol) was dissolved in a solution of 1:1 TFA/H₂O (24 mL), and stirred for 21 h at r.t. The solution was then diluted with H₂O (20 mL) and extracted with EtOAc (2 × 20 mL). The combined organic layers were neutralized with Et₃N to pH 8, then washed with sat. aq. NaHCO₃ solution (20 mL), brine (20 mL), dried (MgSO₄), filtered and concentrated. The residue was purified by flash chromatography (EtOAc/n-hexane, 1:1) to give the diol **21** as a colourless oil (2.09 g, 91%); $R_f = 0.1$ (EtOAc/n-hexane, 1:1); $[\alpha]_D +23.8$ (*c* 5.6, CHCl₃; lit.⁴ +18.5, *c* 2.1, CHCl₃); ¹H NMR (500 MHz, CDCl₃): δ 7.36 – 7.27 (m, 10H, Ph), 5.00, 4.69 (ABq, 2H, $J_{A,B} = 11.5$ Hz, CH₂Ph), 4.74, 4.63 (ABq, 2H, $J_{A,B} = 12.1$ Hz, CH₂Ph), 4.58 (d, 1H, $J_{1,2} = 3.6$ Hz, H-1), 3.77 (dd, 1H, $J_{2,3} = J_{3,4} = 9.3$ Hz, H-3), 3.76 – 3.72 (m, 2H, H-6), 3.58 (ddd, 1H, $J_{4,5} = J_{5,6b} = 9.7$ Hz, $J_{5,6a} = 3.9$ Hz, H-5), 3.49 (ddd, 1H, $J_{3,4} = J_{4,5} = 9.7$ Hz, $J_{4,OH} = 2.7$ Hz, H-4), 3.47 (dd, 1H, H-2), 2.43 (d, 1H, $J_{4,OH} = 2.7$ Hz, OH-4), 2.02 (dd, 1H, $J_{OH,6a} = 5.6$ Hz, $J_{OH,6b} = 7.0$ Hz, OH-6); ¹³C NMR (125 MHz, CDCl₃): δ 138.7, 137.9, 128.6, 128.5, 128.0, 127.9, 127.8 (Ph), 98.1 (C-1), 81.3 (C-3), 79.7 (C-2), 75.3, 73.1 (2 ×

CH_2Ph), 70.6 (C-5), 70.3 (C-4), 62.3 (C-6), 55.2 (OCH_3); LRMS: $m/z = 397.1$ [$\text{M}+\text{Na}]^+$; HRMS: m/z calcd for $\text{C}_{21}\text{H}_{26}\text{O}_6\text{Na}$ [$\text{M}+\text{Na}]^+$: 397.1622, found: 397.1625.

Methyl (methyl 2,3-di-*O*-benzyl- α -D-glucopyranosid)uronate (23)

The diol **21** (2.0 g, 5.34 mmol) was dissolved in 3:1 DCM/H₂O (32 mL) and to this solution, TEMPO (167 mg, 1.07 mmol) and BAIB (3.4 g, 10.7 mmol) were added. The resulting mixture was stirred vigorously at r.t. for 2 h. The resulting solution was washed with sat. aq. $\text{Na}_2\text{S}_2\text{O}_3$ solution, dried (MgSO_4), filtered and concentrated. The crude carboxylic acid (5.34 mmol) was used in the next step without further purification, and it was dissolved in anhydrous DMF (32 mL), and to this mixture, iodomethane (0.5 mL, 8.02 mmol) and KHCO_3 (1.07 g, 10.7 mmol) were added. The resulting solution was stirred at r.t. for 24 h under Argon in the dark. The reaction mixture was then concentrated to dryness and the residue was purified by flash chromatography (EtOAc/*n*-hexane, 1:4) to give the ester **23** as yellow oil (1.1 g, 52 %); $R_f = 0.4$ (EtOAc/*n*-hexane, 1:1); $[\alpha]_D + 15.0$ (*c* 1.91, CHCl_3). The NMR and MS data were in accord with the literature.⁵ ¹H NMR (500 MHz, CDCl_3): δ 7.32 – 7.24 (m, 10H, Ph), 4.89, 4.79 (ABq, 2H, $J_{\text{A},\text{B}} = 11.3$ Hz, CH_2Ph), 4.78, 4.63 (ABq, 2H, $J_{\text{A},\text{B}} = 12.1$ Hz, CH_2Ph), 4.64 (d, 1H, $J_{1,2} = 3.4$ Hz, H-1), 4.13 (d, 1H, $J_{4,5} = 8.9$ Hz, H-5), 3.81 (dd, 1H, $J_{2,3} = J_{3,4} = 8.6$ Hz, H-3), 3.78 (dd, 1H, $J_{3,4} = J_{4,5} = 8.5$ Hz, H-4), 3.77 (s, 3H, CO_2CH_3), 3.52 (dd, 1H, H-2), 3.41 (s, 3H, OCH_3); ¹³C NMR (125 MHz, CDCl_3): δ 170.6 (C=O), 138.6, 137.9, 128.5, 128.1, 128.0, 127.9, 127.8 (Ph), 98.7 (C-1), 80.4 (C-3), 78.4 (C-2), 75.4, 73.6 (2 \times CH_2Ph), 71.8 (C-4), 70.5 (C-5), 55.8 (OCH_3), 52.6 (CO_2CH_3); LRMS: $m/z = 425.1$ [$\text{M}+\text{Na}]^+$, 441.0 [$\text{M}+\text{K}]^+$; HRMS: m/z calcd for $\text{C}_{22}\text{H}_{26}\text{O}_7\text{Na}$ [$\text{M}+\text{Na}]^+$: 425.1571, found: 425.1570.

Methyl 4,6-*O*-(4'-methoxybenzylidene)- β -D-glucoside (18)

Methyl β -D-glucopyranoside hemihydrate **16** (6.5 g, 33.4 mmol) was dried under high vacuum overnight. It was then dissolved in anhydrous DMF (60 mL), and anisaldehyde dimethyl acetal (8.5 mL, 50.2 mmol) and freshly dried AW300 mol sieves (3 g) were added. Camphor-10-sulfonic acid (388 mg, 1.67 mmol) was added to pH 3. The reaction mixture was stirred at r.t. under argon for 18 h. The solution was neutralized by addition of Et₃N (to pH 8) and then diluted with EtOAc, washed with H₂O, dried (MgSO₄), filtered and concentrated. The residue was then purified by flash chromatography (EtOAc/*n*-hexane, 1:1) to give the *p*-methoxybenzylidene **18** as white solid (5.21 g, 52%); *R*_f = 0.47 (EtOAc/MeOH, 9:1); m.p. 176 °C; lit.⁶ 176 – 177 °C; [α]_D +10.5 (*c* 4.5, MeOH); ¹H NMR (500 MHz, CDCl₃): δ 7.41–7.37 (m, 2H, Ar), 6.89–6.85 (m, 2H, Ar), 5.47 (s, 1H, CHAr), 4.32 (dd, 1H, *J*_{5,6a} = 5.0 Hz, *J*_{6a,6b} = 10.3 Hz, H-6a), 4.30 (d, 1H, *J*_{1,2} = 7.8 Hz, H-1), 3.82 – 3.76 (m, 1H, H-3), 3.78 (s, 3H, ArOCH₃), 3.74 (dd, 1H, *J*_{5,6b} = *J*_{6a,6b} = 10.3 Hz, H-6b), 3.56 (s, 3H, OCH₃), 3.51 (dd, *J*_{3,4} = *J*_{4,5} = 9.3 Hz, H-4), 3.47 – 3.46 (m, 1H, H-2), 3.43 (ddd, 1H, H-5), 2.81 (d, 1H, *J*_{3,OH} = 2.4 Hz, OH-3), 2.68 (d, 1H, *J*_{2,OH} = 2.5 Hz, OH-2); ¹³C NMR (125 MHz, CDCl₃): δ 160.3, 129.4, 127.6, 113.7 (C-Ar), 104.1 (C-1), 101.8 (CHAr), 80.5 (C-4), 74.5 (C-2), 73.2 (C-3), 68.6 (C-6), 66.4 (C-5), 57.5 (OCH₃), 55.3 (ArOCH₃); LRMS: *m/z* = 313.06 [M+H]⁺, 335.01 [M+Na]⁺, 350.93 [M+K]⁺; HRMS: *m/z* calcd for C₁₅H₂₀O₇Na [M+Na]⁺: 335.1101, found: 335.1091.

Methyl 2,3-di-*O*-benzyl-4,6-*O*-(4'-methoxybenzylidene)- β -D-glucoside (**20**)

The diol **18** (5.2 g, 16.6 mmol) was dissolved in anhydrous DMF (15 mL). A suspension of NaH (1.6 g, 39.7 mmol, pre-washed with *n*-hexane × 3) in anhydrous DMF (15 mL) was added. The mixture was stirred at r.t. for 10 min, and then BnBr (4.73 mL, 39.7 mmol) was added dropwise under ice-cooling. The stirring was continued for 10 min under ice-cooling, and for an additional 3 h at r.t. MeOH (20 mL) was slowly added to quench the reaction. The resulting mixture was diluted with EtOAc, washed with H₂O, dried (MgSO₄), filtered and concentrated.

The residue was recrystallised from EtOAc/*n*-hexane to give the dibenzyl ether **20** as white crystals (7.96 g, 98%); R_f = 0.28 (EtOAc/*n*-hexane, 1:4); m.p. 148 – 150 °C, lit.⁶ 151 °C; $[\alpha]_D$ –46.7 (*c* 0.4, CHCl₃), lit.⁶ –39.0 (*c* 1.19, CHCl₃); ¹H NMR (500 MHz, CDCl₃): δ 7.42–7.24 (m, 12H, Ar), 6.91–6.87 (m, 2H, Ar), 5.52 (s, 1H, CHAr), 4.88, 4.77 (ABq, 2H, $J_{A,B}$ = 11.4 Hz, CH₂Ph), 4.85, 4.74 (ABq, 2H, $J_{A,B}$ = 11.0 Hz, CH₂Ph), 4.40 (d, 1H, $J_{1,2}$ = 7.7 Hz, H-1), 4.33 (dd, 1H, $J_{5,6a}$ = 5.0 Hz, $J_{6a,6b}$ = 10.3 Hz, H-6a), 3.80 (s, 3H, ArOCH₃), 3.76 (dd, 1H, $J_{5,6b}$ = $J_{6a,6b}$ = 10.3 Hz, H-6b), 3.73 (dd, 1H, $J_{2,3}$ = 8.6 Hz, $J_{3,4}$ = 9.3 Hz, H-3), 3.65 (dd, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.3 Hz, H-4), 3.57 (s, 3H, OCH₃), 3.42 (dd, 1H, H-2), 3.38 (ddd, 1H, H-5); ¹³C NMR (125 MHz, CDCl₃): δ 160, 138.5, 138.4, 129.8, 128.3, 128.2, 128.0, 127.6, 127.5, 127.3, 113.6 (C-Ar), 105.2 (C-1), 101.0 (CHAr), 82.2 (C-2), 81.4 (C-4), 80.8 (C-3), 75.2, 75.0 (2 × CH₂Ph), 68.7 (C-6), 66.0 (C-5), 57.4 (OCH₃), 55.3 (ArOCH₃); LRMS: *m/z* = 493.2 [M+H]⁺, 515.2 [M+Na]⁺, 531.1 [M+K]⁺; HRMS: *m/z* calcd for C₂₉H₃₂O₇Na [M+Na]⁺: 515.2040, found: 515.2051.

Methyl 2,3-di-*O*-benzyl- β -D-glucopyranoside (22)

The 4'methoxybenzylidene **20** (2.5 g, 5.08 mmol) was dissolved in a solution of TFA/H₂O (1:1, 24 mL) and stirred for 18 h at r.t. The solution was diluted with H₂O (20 mL) and then extracted with EtOAc (2 × 20 mL). The combined organic layers were neutralized with Et₃N to pH 8, then washed with sat. aq. NaHCO₃ solution (20 mL), brine (20 mL), dried (MgSO₄), filtered and concentrated. The residue was then purified by flash chromatography (EtOAc/*n*-hexane, 1:1) to give the diol **22** as white solid (1.79 g, 94%); R_f = 0.08 (EtOAc/*n*-hexane, 1:1); m.p. 104 – 105 °C, lit.⁷ 104 – 105 °C; $[\alpha]_D$ –12.5 (*c* 5.2, CHCl₃), lit.⁷ –7 (*c* 3.0, CHCl₃); ¹H NMR (500 MHz, CDCl₃): δ 7.33 – 7.24 (m, 10 H, Ph), 4.94, 4.66 (ABq, 2H, $J_{A,B}$ = 11.5 Hz, CH₂Ph), 4.91, 4.68 (ABq, 2H, $J_{A,B}$ = 11.1 Hz, CH₂Ph), 4.35 (d, 1H, $J_{1,2}$ = 7.6 Hz, H-1), 3.89 – 3.85 (m, 1H, H-6a), 3.78 – 3.73 (m, 1H, H-6b), 3.56 (s, 3H, OCH₃), 3.54 (ddd, 1H, $J_{4,\text{OH}}$ = 2.0 Hz, $J_{3,4}$ = $J_{4,5}$ = 9.0 Hz, H-4), 3.44 (dd, 1H, $J_{2,3}$ = $J_{3,4}$ = 9.0 Hz, H-3), 3.37 (dd, 1H, H-2), 3.33

– 3.30 (m, 1H, H-5), 2.40 (d, 1H, $J_{4,\text{OH}} = 2.0$ Hz, OH-4), 2.12 (t, 1H, $J_{6,\text{OH}} = 6.7$ Hz, OH-6); ^{13}C NMR (125 MHz, CDCl_3): δ 138.5, 138.3, 128.6, 128.4, 128.1, 128.0, 127.9, 127.7 (Ph), 104.9 (C-1), 83.8 (C-3), 81.9 (C-2), 75.2 (C-5), 74.8, 74.6 ($2 \times \text{CH}_2\text{Ph}$), 70.3 (C-4), 62.6 (C-6), 57.3 (OCH_3); LRMS: $m/z = 397.1$ [M+Na] $^+$, 413.1 [M+K] $^+$; HRMS: m/z calcd for $\text{C}_{21}\text{H}_{26}\text{O}_6\text{Na}$ [M+Na] $^+$: 397.1622, found: 397.1630.

Methyl (methyl 2,3-di-O-benzyl- β -D-glucopyranosid)uronate (24)

The diol **22** (509 mg, 1.36 mmol) was dissolved in a mixture of DCM/H₂O (3:1, 8 mL), and to this mixture, TEMPO (43 mg, 0.27 mmol) and BAIB (876 mg, 2.7 mmol) were added. The resulting mixture was stirred vigorously at r.t. for 2 h. The solution was then washed with sat. aq. $\text{Na}_2\text{S}_2\text{O}_3$ solution, dried (MgSO_4), filtered and concentrated. The residue was dissolved in anhydrous DMF (8 mL), and to this mixture, iodomethane (0.13 mL, 2.04 mmol) and KHCO_3 (272 mg, 2.72 mmol) were added. The resulting solution was stirred at r.t. for 18 h in the dark under argon. The reaction mixture was then concentrated to dryness and the residue was purified by flash chromatography (EtOAc/n-hexane, 1:4) to give the ester **24** as a colourless oil (441 mg, 80 %); $R_f = 0.33$ (EtOAc/n-hexane, 1:1); $[\alpha]_D -15.7$ (*c* 3.6, CHCl_3). The NMR data were in accord with the literature.⁸ ^1H NMR (500 MHz, CDCl_3): δ 7.30 – 7.24 (m, 10H, Ph), 4.87, 4.78 (ABq, 2H, $J_{\text{A,B}} = 11.4$ Hz, CH_2Ph), 4.86, 4.69 (ABq, 2H, $J_{\text{A,B}} = 11.0$ Hz, CH_2Ph), 4.35 (d, 1H, $J_{1,2} = 7.6$ Hz, H-1), 3.85 (ddd, 1H, $J_{4,\text{OH}} = 2.1$ Hz, $J_{3,4} = J_{4,5} = 9.7$ Hz, H-4), 3.82 (d, 1H, H-5), 3.80 (s, 3H, CO_2CH_3), 3.57 (s, 3H, OCH_3), 3.50 (dd, 1H, $J_{2,3} = 9.1$ Hz, H-3), 3.42 (dd, 1H, H-2), 2.82 (d, 1H, OH-4); ^{13}C NMR (125 MHz, CDCl_3): δ 169.7 (C=O), 138.4, 138.2, 128.4, 128.3, 128.0, 127.9, 127.8, 127.7 (Ph), 104.9 (C-1), 83.0 (C-3), 81.1 (C-2), 75.3, 74.8 ($2 \times \text{CH}_2\text{Ph}$), 74.1 (C-5), 71.7 (C-4), 57.4 (OCH_3), 52.7 (CO_2CH_3); LRMS: $m/z = 425.1$ [M+Na] $^+$; HRMS: m/z calcd for $\text{C}_{22}\text{H}_{26}\text{O}_7\text{Na}$ [M+Na] $^+$: 425.1571, found: 425.1570.

Methyl (methyl 2,3-di-*O*-benzyl- β -L-idopyranosid)uronate (37)

The diol **34** (680 mg, 1.8 mmol) was dissolved in a mixture of DCM/H₂O (3:1, 12 mL), and to this mixture, TEMPO (57 mg, 0.4 mmol) and BAIB (1.17 g, 3.6 mmol) were added. The mixture was stirred vigorously at r.t. for 3 h. The solution was then washed with sat. aq. Na₂S₂O₃ solution, dried (MgSO₄), filtered and concentrated. The residue was dissolved in anhydrous DMF (12 mL), and to this mixture, iodomethane (0.17 mL, 2.73 mmol) and KHCO₃ (364 mg, 3.6 mmol) were added. The solution was stirred in the dark at r.t. for 24 h under argon. The mixture was then concentrated and the residue purified by flash chromatography (EtOAc/*n*-hexane, 1:4) to give the ester **37** as yellow oil (486 mg, 67 %); *R*_f = 0.43 (EtOAc/*n*-hexane, 1:1); [α]_D +90.1 (*c* 1.4, CHCl₃), lit.⁹ +109 (*c* 2.7, CHCl₃); ¹H NMR (500 MHz, CDCl₃): δ 7.33 – 7.21 (m, 10H, Ph), 4.85, 4.59 (ABq, 2H, *J*_{A,B} = 12.1 Hz, CH₂Ph), 4.66 (d, 1H, *J*_{1,2} = 1.2 Hz, H-1), 4.54, 4.49 (ABq, 2H, *J*_{A,B} = 11.8 Hz, CH₂Ph), 4.48 (d, 1H, *J*_{4,5} = 1.7 Hz, H-5), 3.97 (dd, 1H, *J*_{3,4} = 3.6 Hz, H-4), 3.80 (dd, 1H, *J*_{2,3} = *J*_{3,4} = 3.6 Hz, H-3), 3.79 (s, 3H, CO₂CH₃), 3.59 (dd, 1H, H-2), 3.57 (s, 3H, OCH₃); ¹³C NMR (125 MHz, CDCl₃): δ 169.5 (C=O), 137.4, 137.2, 128.5, 128.4, 128.1, 128.0, 127.9, 127.7 (Ph), 101.1 (C-1), 75.7 (C-3), 74.9 (C-5), 74.3 (C-2), 74.1, 72.3 (2 × CH₂Ph), 68.1 (C-4), 57.3 (OCH₃), 52.2 (CO₂CH₃); LRMS: *m/z* = 420.2 [M+NH₄]⁺, 425.2 [M+Na]⁺, 441.1 [M+K]⁺; HRMS: *m/z* calcd for C₂₂H₂₆O₇Na [M+Na]⁺: 425.1571, found: 425.1580.

Methyl (methyl 2-*O*-benzoyl-3-*O*-benzyl- α -L-idopyranosiduronate)-(1→4)-2-azido-3-*O*-benzyl-6-*O*-benzoyl-2-deoxy- α -D-glucopyranoside (42)²

White solid; m.p.: 62 – 65 °C; $[\alpha]_D$ +47.9 (*c* 3.0, CHCl₃), lit.¹⁰ +44; lit.¹¹ +31.8; The NMR spectra were in accord with the literature.^{10, 12} ¹H NMR (500 MHz, CDCl₃): δ 8.05 – 7.24 (m, 20H, Ph), 5.37 (s, 1H, H-1'), 5.21 – 5.20 (m, 1H, H-2'), 4.97 (d, 1H, $J_{4',5'} = 2.4$ Hz, H-5'), 4.82, 4.67 (ABq, 2H, $J_{A,B} = 10.7$ Hz, CH₂Ph), 4.80, 4.69 (ABq, 2H, $J_{A,B} = 12.0$ Hz, CH₂Ph), 4.81 (d, 1H, $J_{1,2} = 2.9$ Hz, H-1), 4.78 (dd, 1H, A part of ABX, $J_{5,6a} = 1.8$ Hz, $J_{6a,6b} = 12.2$ Hz, H-6a), 4.48 (dd, 1H, B part of ABX, $J_{5,6b} = 4.2$ Hz, H-6b), 4.05 – 3.97 (m, 3H, H-4, H-4', H-5), 3.92 – 3.86 (m, 2H, H-3', H-3), 3.47 (dd, 1H, $J_{2,3} = 6.6$ Hz, H-2), 3.49 (s, 3H, CO₂CH₃), 3.45 (s, 3H, OCH₃); ¹³C NMR (100 MHz, CDCl₃): δ 169.4, 166.0, 165.0 (3 × C=O), 137.7, 137.2, 133.6, 133.0, 129.7, 129.7, 129.6, 128.7, 128.5, 128.4, 128.3, 128.1, 128.0, 127.6, 127.4 (Ph), 98.4 (C-1), 98.0 (C-1'), 78.7 (C-3), 75.3 (C-4), 75.0 (C-3'), 74.8 (CH₂Ph), 72.6 (CH₂Ph), 69.2 (C-5), 68.8 (C-5'), 68.1 (C-4'), 67.9 (C-2'), 63.8 (C-2), 62.7 (C-6), 55.4 (OCH₃), 52.0 (CO₂CH₃); LRMS: *m/z* = 820.2 [M+Na]⁺.

Methyl (methyl 1-thio-β-D-glucopyranosid)uronate (44)

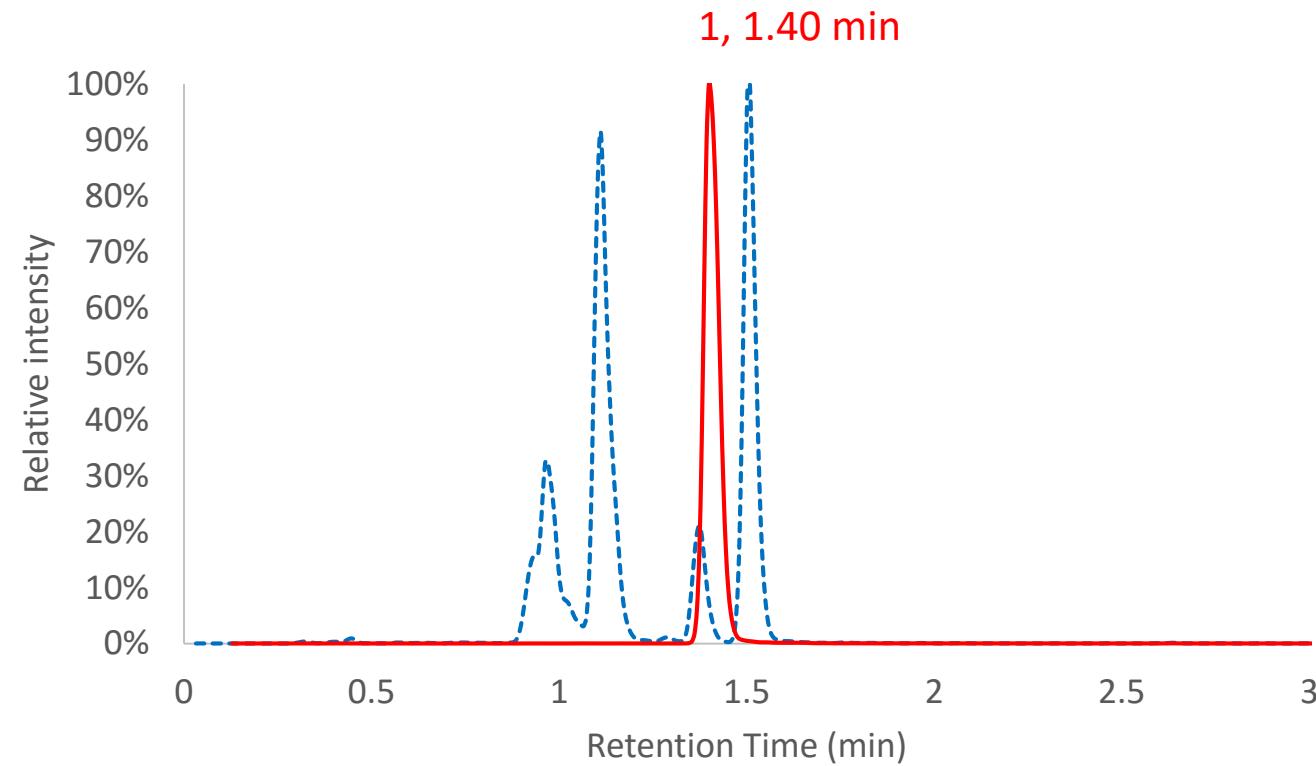
White solid; m.p.: 121 - 122 °C; $[\alpha]_D$ –43.2 (*c* 2.1, MeOH); ¹H NMR (500 MHz, CDCl₃): δ 4.36 (d, 1H, $J_{1,2} = 9.5$ Hz, H-1), 4.30 (br. s, 1H, OH), 4.05 (br. s, 1H, OH), 3.91 (d, 1H, $J_{4,5} = 9.5$ Hz, H-5), 3.82 (s, 3H, CO₂CH₃), 3.79 (dd, 1H, $J_{4,5} = J_{3,4} = 9.5$ Hz, H-4), 3.63 (dd, 1H, $J_{2,3} = J_{3,4} = 9.5$ Hz, H-3), 3.61 (br. s, 1H, OH), 3.50 (dd, 1H, H-2), 2.23 (s, 3H, SCH₃); ¹³C NMR (100 MHz, CDCl₃): δ 169.6 (C=O), 86.4 (C-1), 77.9 (C-5), 76.9 (C-3), 71.4 (C-2), 71.2 (C-4), 52.9 (CO₂Me), 12.3 (SMe); LRMS: *m/z* = 261.0 [M+Na]⁺; HRMS: *m/z* calcd for C₈H₁₄O₆SNa [M+Na]⁺: 261.0409, found: 261.0405.

Methyl 2-azido-6-O-benzoyl-3-O-benzyl-2-deoxy-α-D-glucopyranoside (46)²

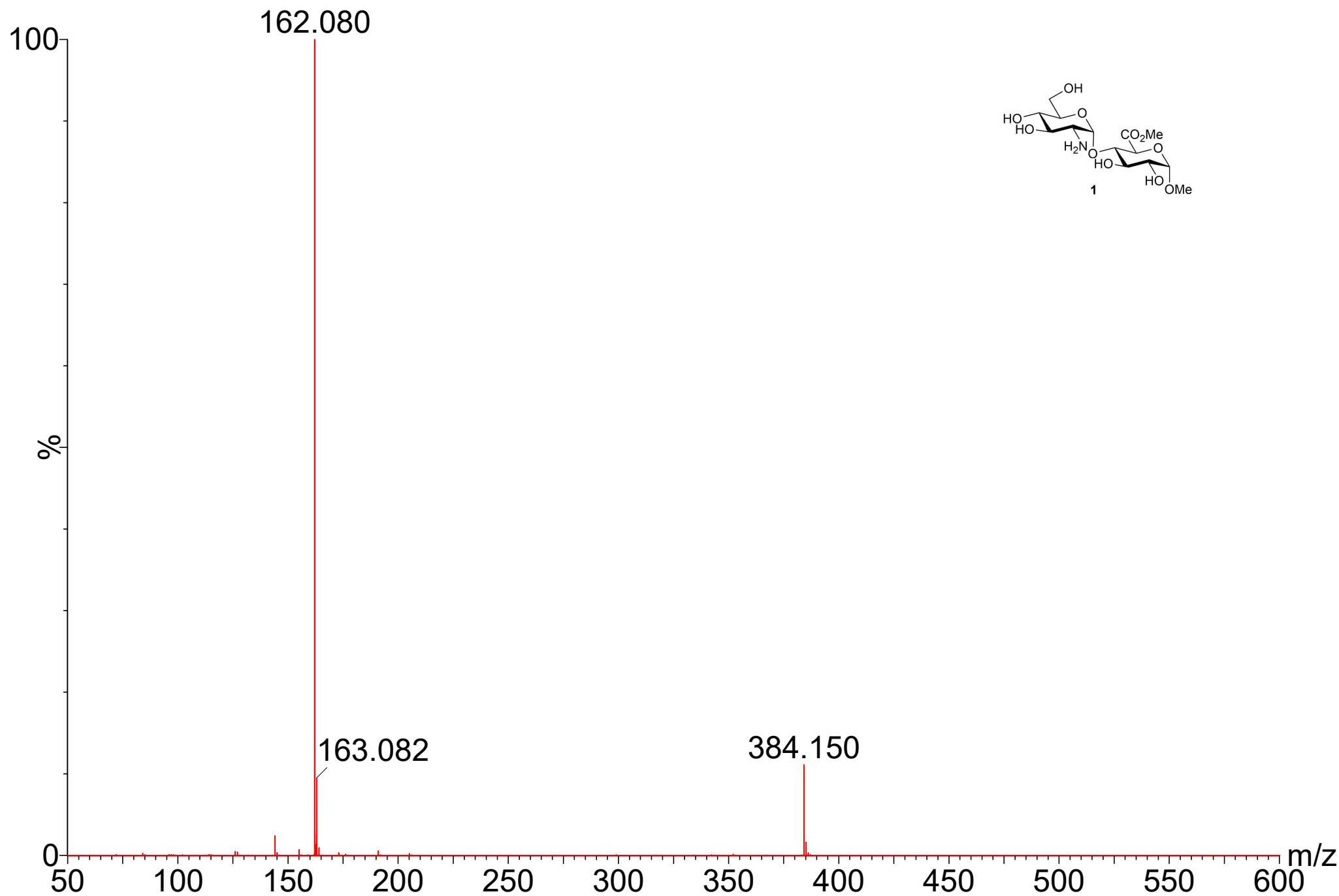
White solid; m.p.: 55 – 58 °C; $[\alpha]_D +78.3$ (c 3.65, CHCl_3), lit. $^{13} +73.8$; lit. $^{14} +60.5$; The NMR data were in accord with the literature. $^{13, 14}$ ^1H NMR (500 MHz, CDCl_3): δ 8.06-8.02 (m, 2H, Ph), 7.57– 7.30 (m, 8H, Ph), 4.93, 4.83 (ABq, 2H, $J_{\text{A},\text{B}} = 11.1$ Hz, CH_2Ph), 4.81 (d, 1H, $J_{1,2} = 3.6$ Hz, H-1), 4.74 (dd, 1H, A part of ABX, $J_{5,6\text{a}} = 4.2$ Hz, $J_{6\text{a},6\text{b}} = 12.2$ Hz, H-6a), 4.47 (dd, 1H, B part of ABX, $J_{5,6\text{b}} = 2.2$ Hz, H-6b), 3.87 (ddd, 1H, $J_{4,5} = 9.1$ Hz, H-5), 3.85 (dd, 1H, $J_{3,4} = 9.1$ Hz, $J_{2,3} = 10.1$ Hz, H-3), 3.57 (dd, 1H, H-4), 3.45 (s, 3H, OCH_3), 3.38 (dd, 1H, H-2), 2.79 (br s, 1H, OH-4); ^{13}C NMR (125 MHz, CDCl_3): δ 167.1 (C=O), 137.8, 133.3, 129.7, 129.4, 128.6, 128.4, 128.2, 128.1 (Ph), 98.8 (C-1), 79.7 (C-3), 75.3 (PhCH_2), 70.6 (C-4), 70.0 (C-5), 63.3 (C-6), 63.1 (C-2), 55.3 (OMe); LRMS: $m/z = 436.1$ [$\text{M}+\text{Na}]^+$, 849.2 [2 $\text{M}+\text{Na}]^+$.

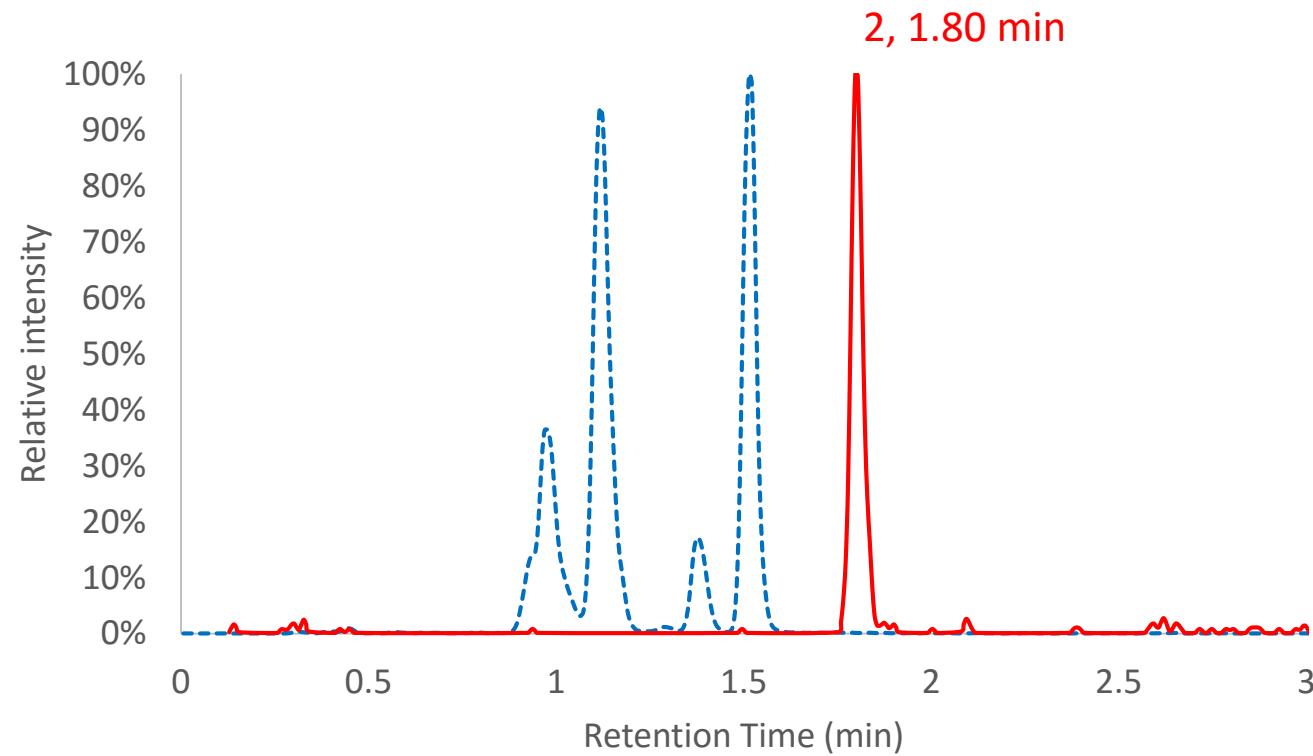
References

1. L. Singh, A. Lam, R. Premraj and J. Seifert, *Tetrahedron Lett.*, 2008, **49**, 4854-4856.
2. M. L. West, N. B. Drinnan, T. E. Ramsdale, L. Singh and J. Seifert, *Australian Patent AU 2002/331426 B2 Pat.*, Synthetic heparin pentasaccharides, 2008.
3. D. Joniak and B. Košíková, *Chem. Zvesti*, 1974, **28**, 110-114.
4. R. Johansson and B. Samuelsson, *J. Chem. Soc., Perkin Trans. 1*, 1984, 2371-2374.
5. C. Puchner, T. Eixelsberger, B. Nidetzky and L. Brecker, *Carbohydr. Res.*, 2017, **437**, 50-58.
6. M. B. Carter, P. A. Petillo, L. Anderson and L. E. Lerner, *Carbohydr. Res.*, 1994, **258**, 299-306.
7. V. Pedretti, J.-M. Mallet and P. Sinay, *Carbohydr. Res.*, 1993, **244**, 247-257.
8. N. J. Davis and S. L. Flitsch, *Tetrahedron Lett.*, 1993, **34**, 1181-1184.
9. T. Chiba, J.-C. Jacquinot and P. Sinay, *Carbohydr. Res.*, 1988, **174**, 253-264.
10. P. Xu, W. Xu, Y. Dai, Y. Yang and B. Yu, *Org. Chem. Front.*, 2014, **1**, 405-414.
11. F. Lin, G. Lian and Y. Zhou, *Carbohydr. Res.*, 2013, **371**, 32-39.
12. L. Tiehai, Y. Hui, C. Xuefeng, W. Jiajia, L. Yonghui, Z. Lifei, L. Qiang, W. Wenjun, S. Jie, Z. Wei and W. Peng, *ChemMedChem*, 2014, **9**, 1071-1080.
13. S.-C. Hung, X.-A. Lu, J.-C. Lee, M. D.-T. Chang, S.-I. Fang, T.-c. Fan, M. M. L. Zulueta and Y.-Q. Zhong, *Org. Biomol. Chem.*, 2012, **10**, 760-772.
14. Y. Zhou, F. Lin, J. Chen and B. Yu, *Carbohydr. Res.*, 2006, **341**, 1619-1629.

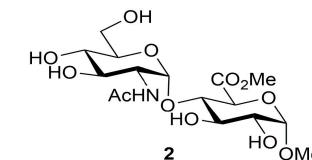
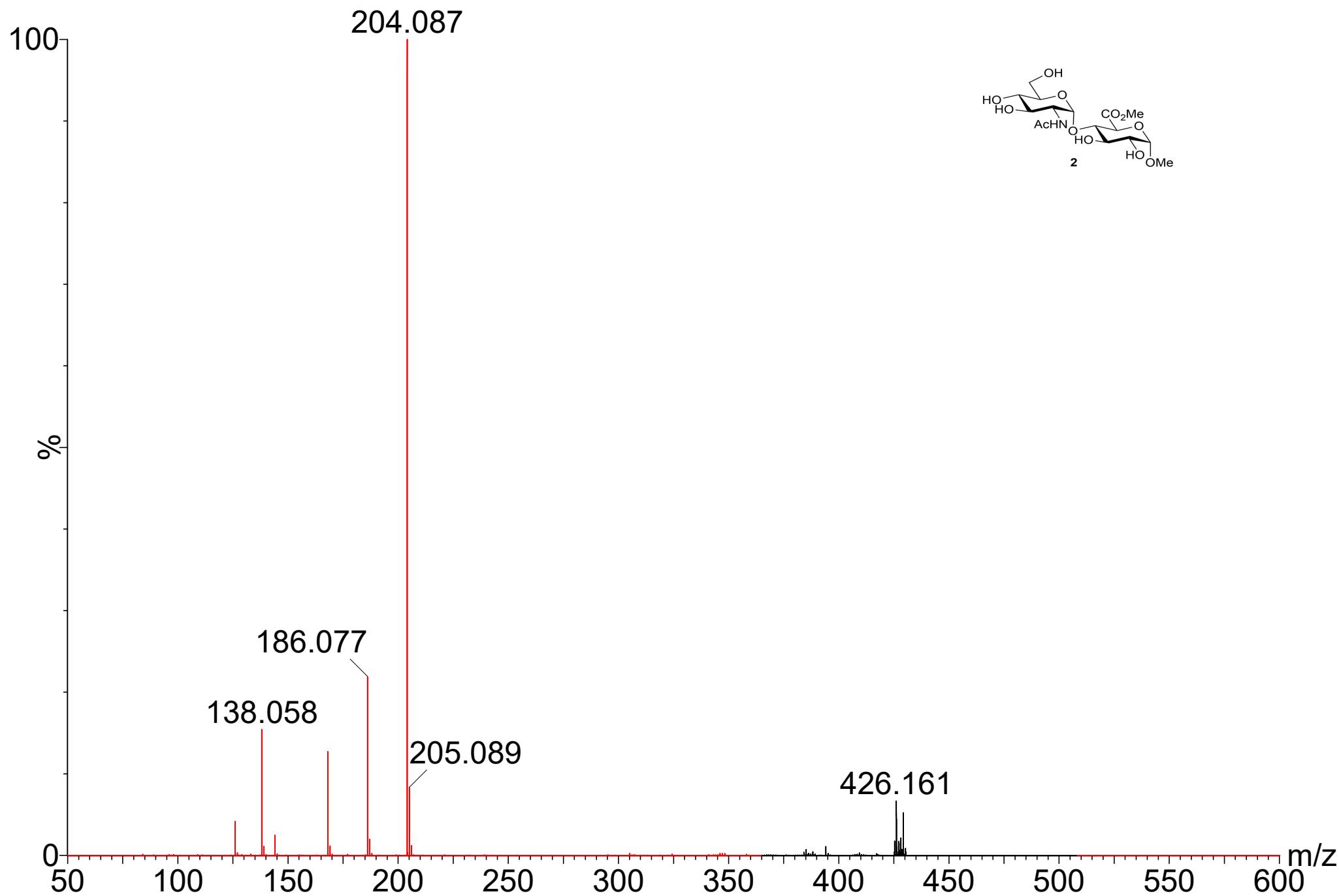


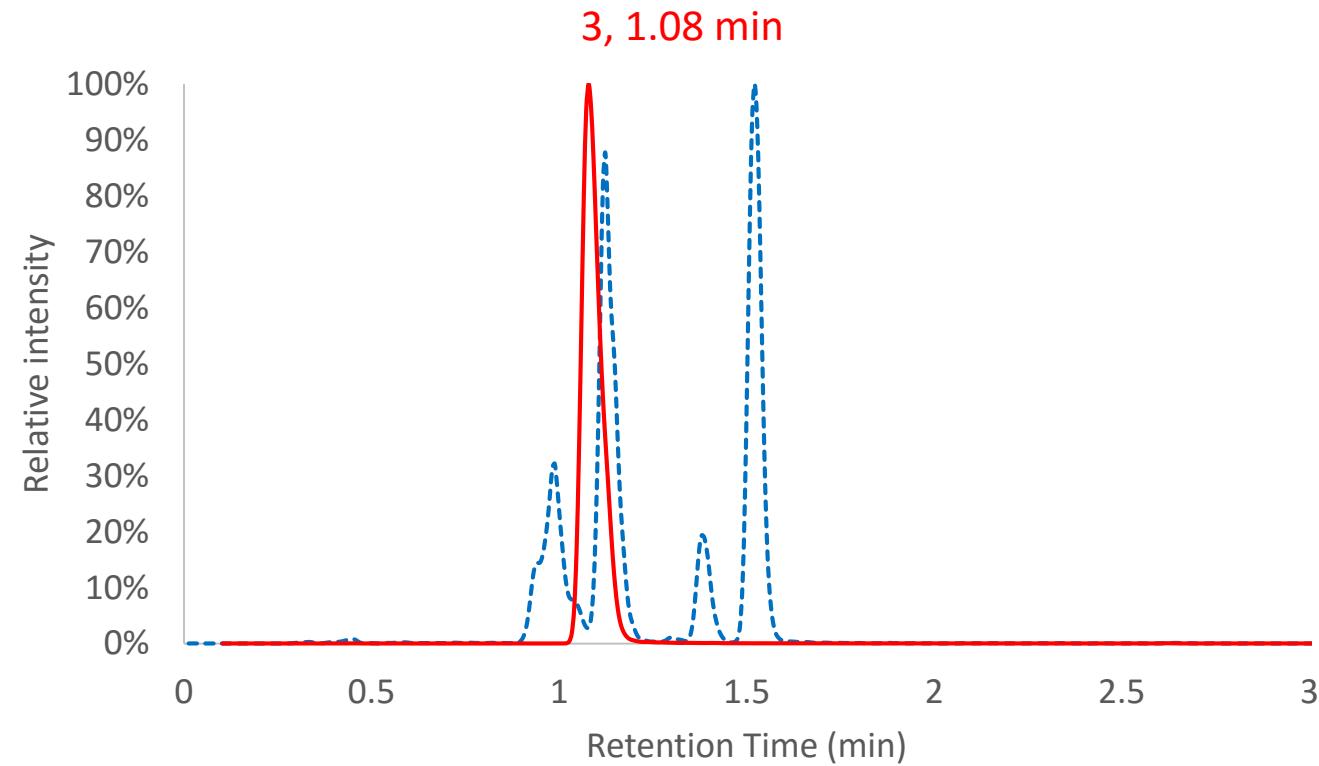
HS d6 internal standard (blue dotted trace) MRM transition m/z 390-162
Disaccharide 1 (Red solid trace) MRM transition m/z 384-162



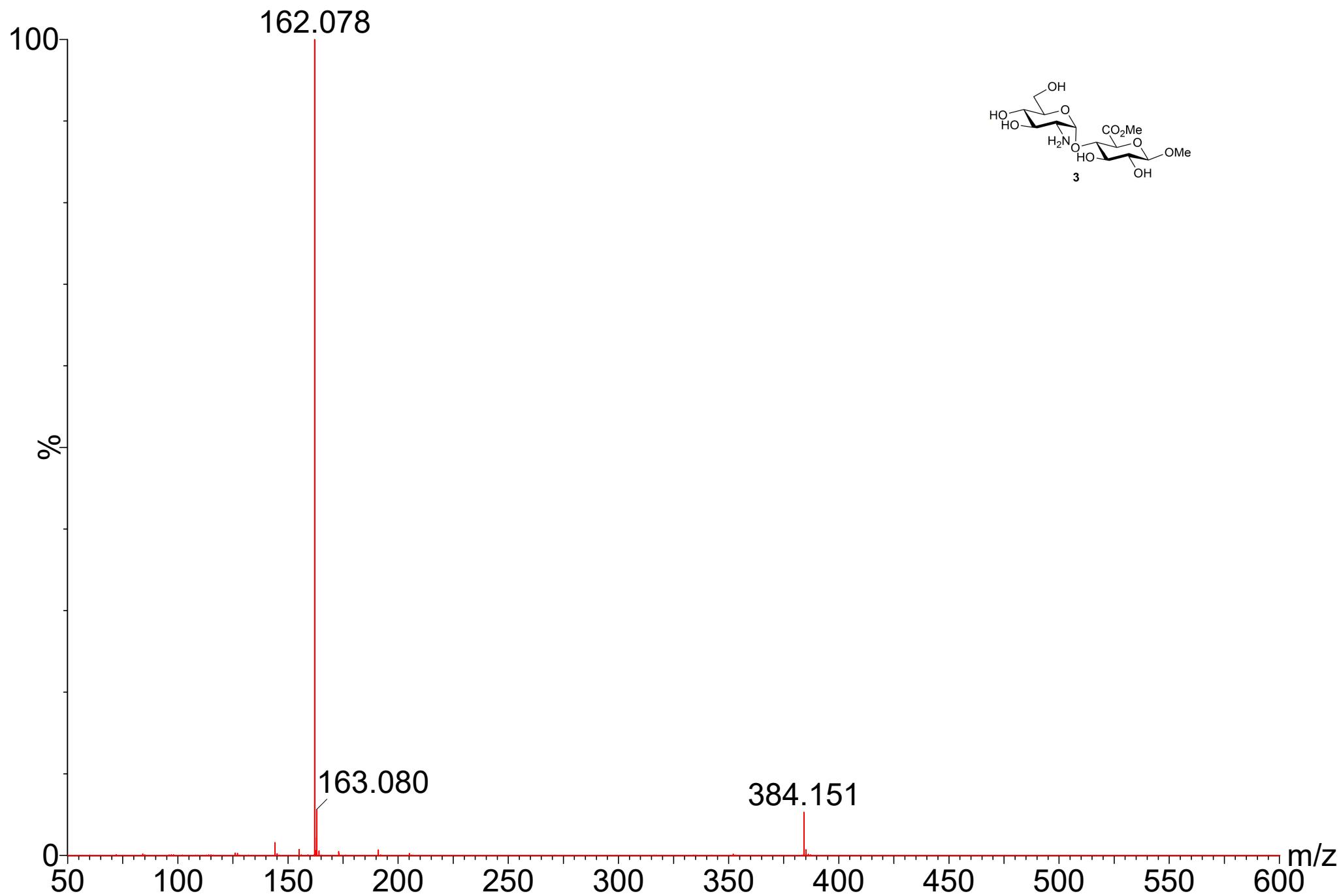


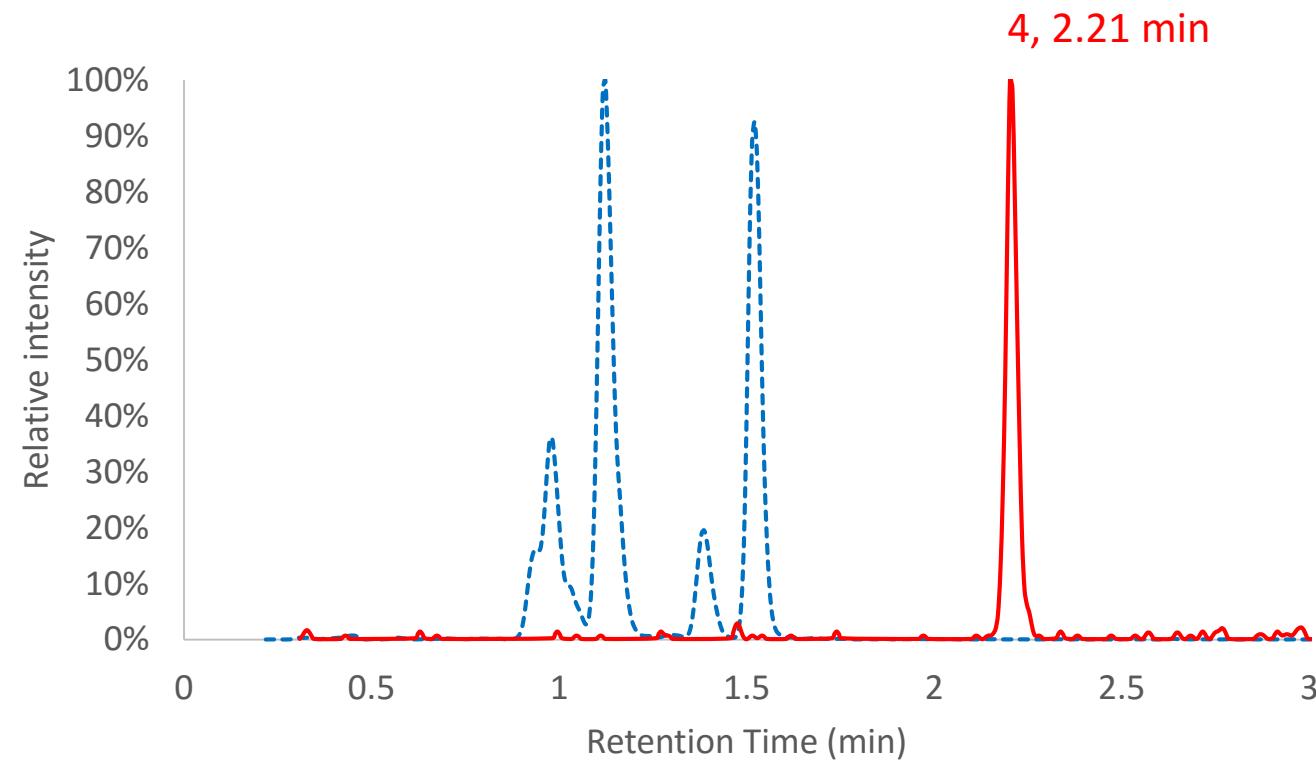
HS d6 internal standard (blue dotted trace) MRM transition m/z 390-162
Disaccharide 2 (Red solid trace) MRM transition m/z 426-204



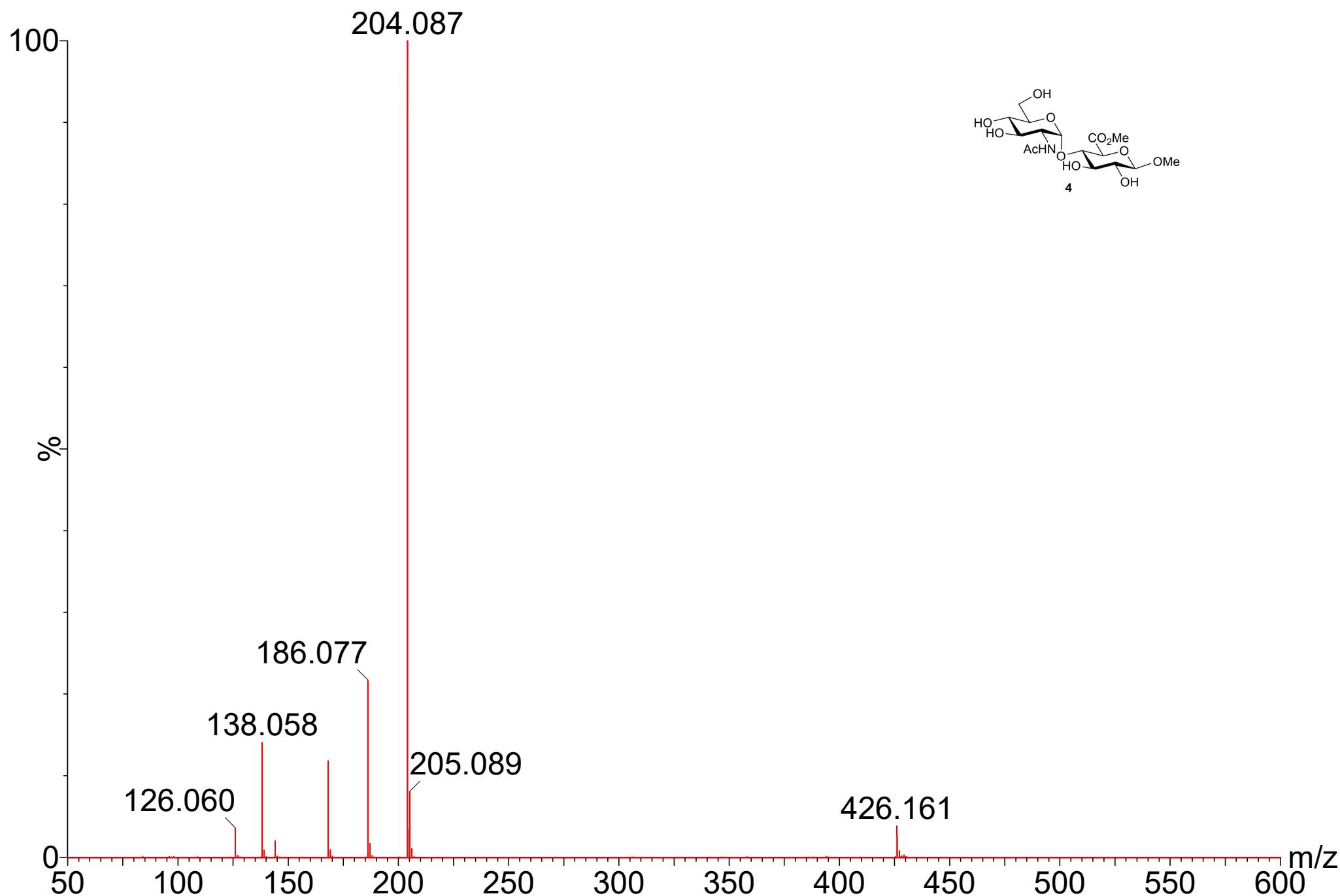


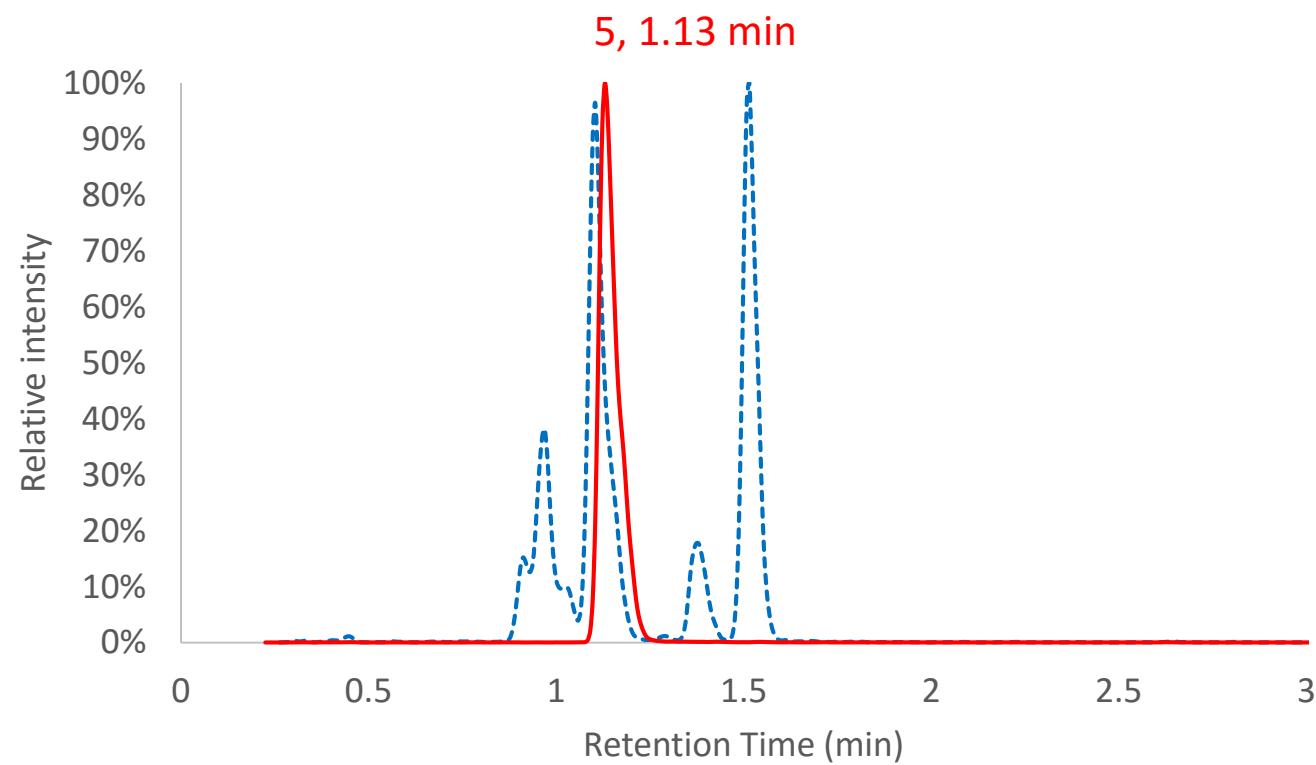
HS d6 internal standard (blue dotted trace) MRM transition m/z 390-162
Disaccharide 3 (Red solid trace) MRM transition m/z 384-162



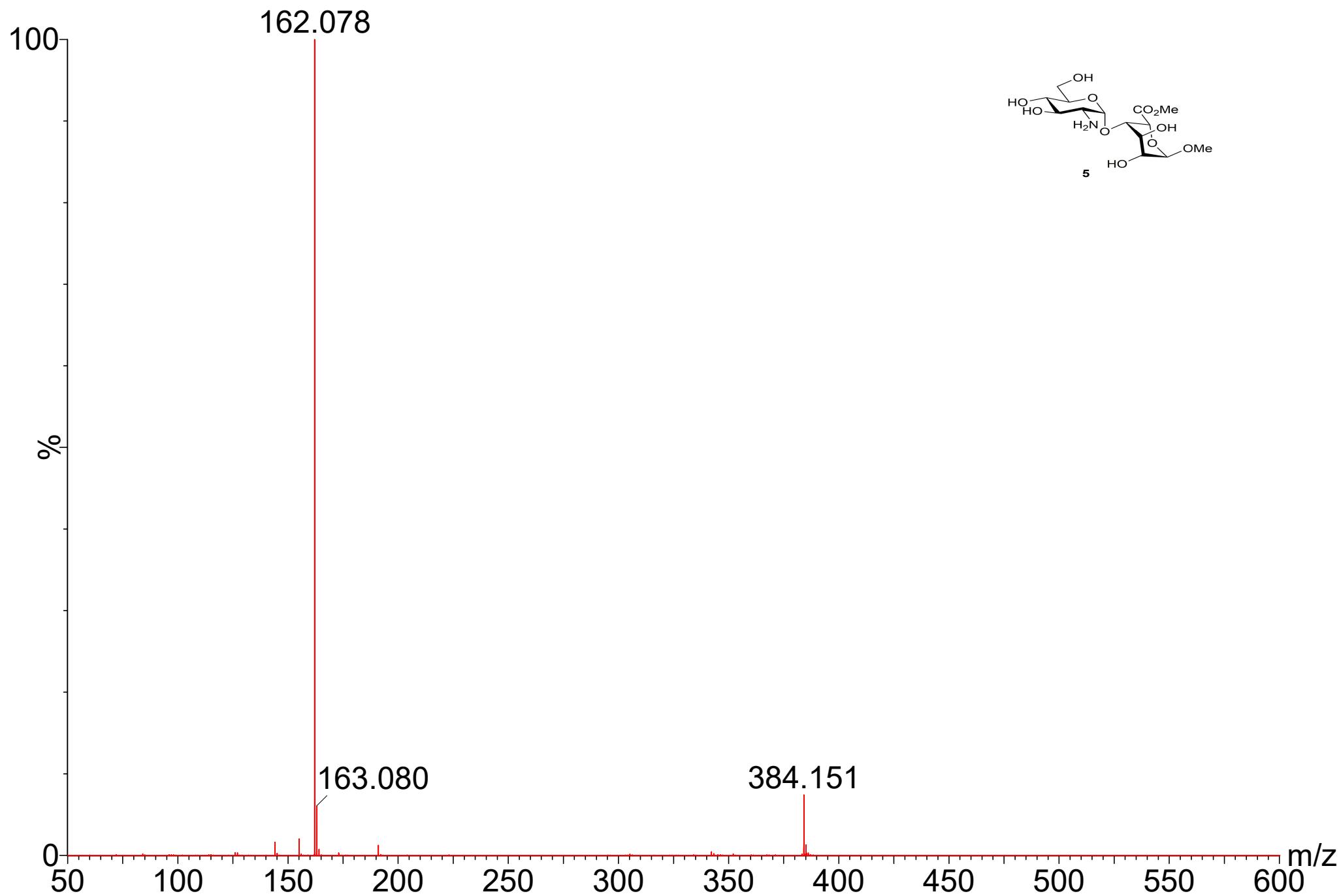


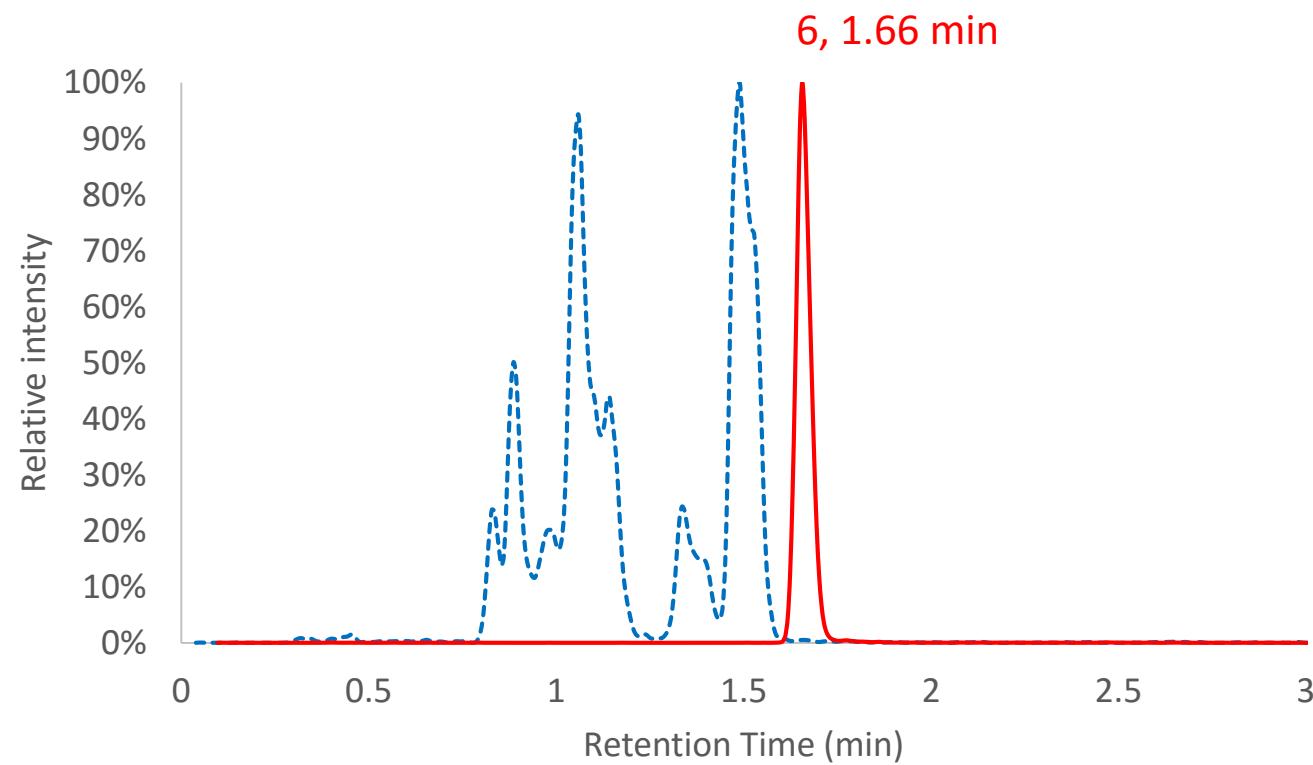
HS d6 internal standard (blue dotted trace) MRM transition m/z 390-162
Disaccharide 4 (Red solid trace) MRM transition m/z 426-204



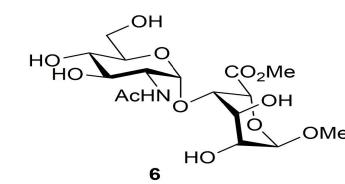
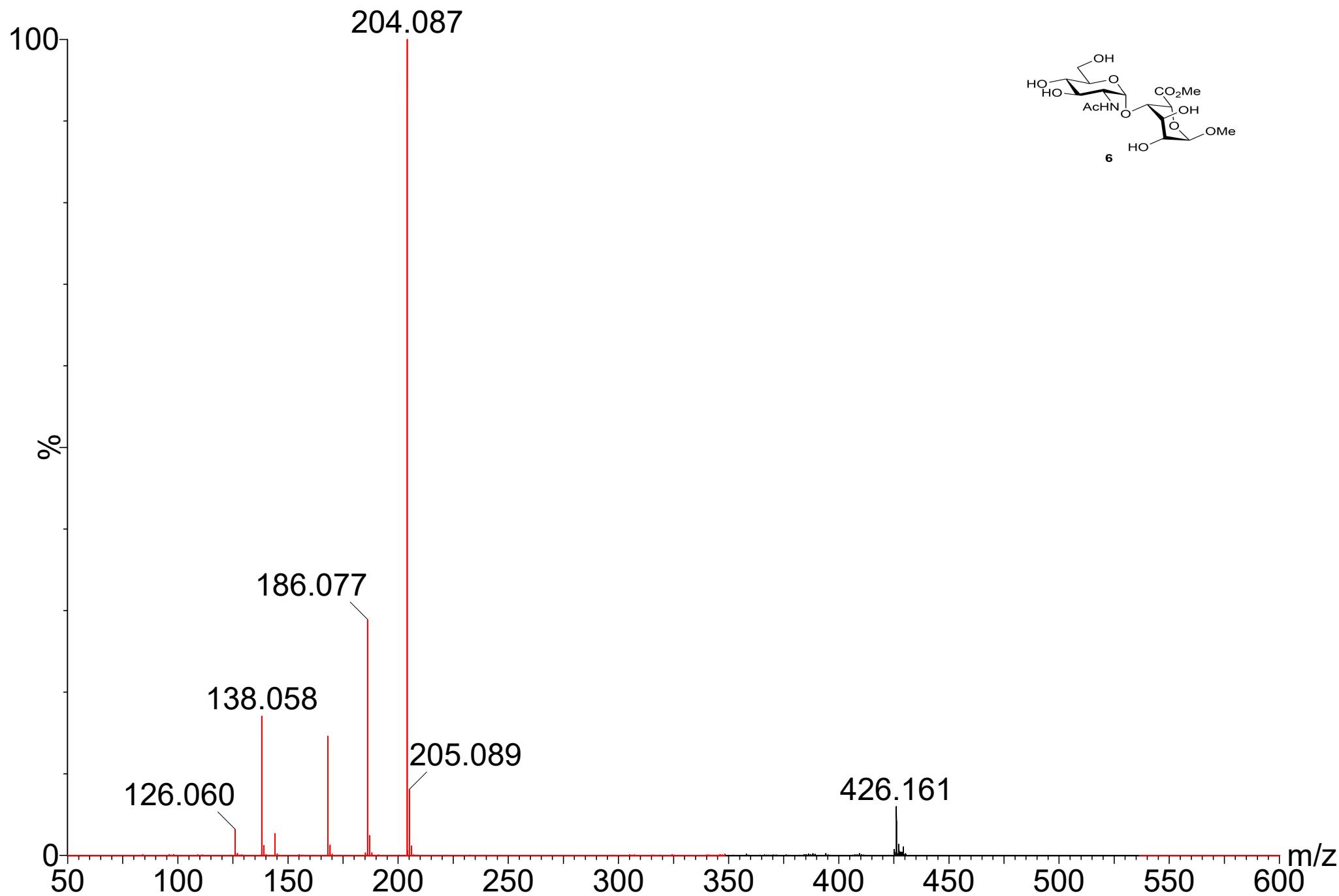


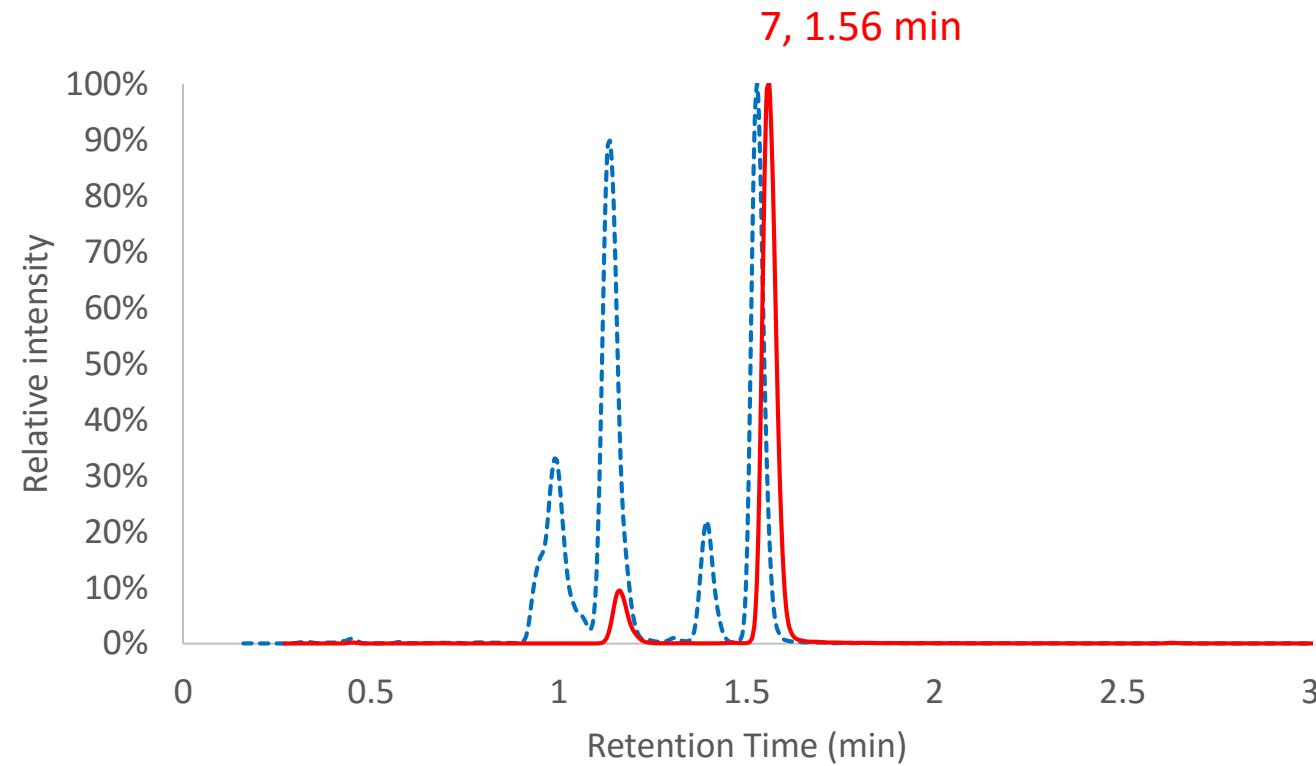
HS d6 internal standard (blue dotted trace) MRM transition m/z 390-162
Disaccharide 5 (Red solid trace) MRM transition m/z 384-162



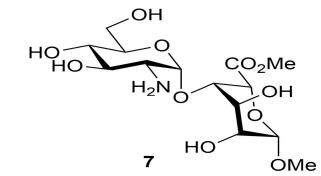
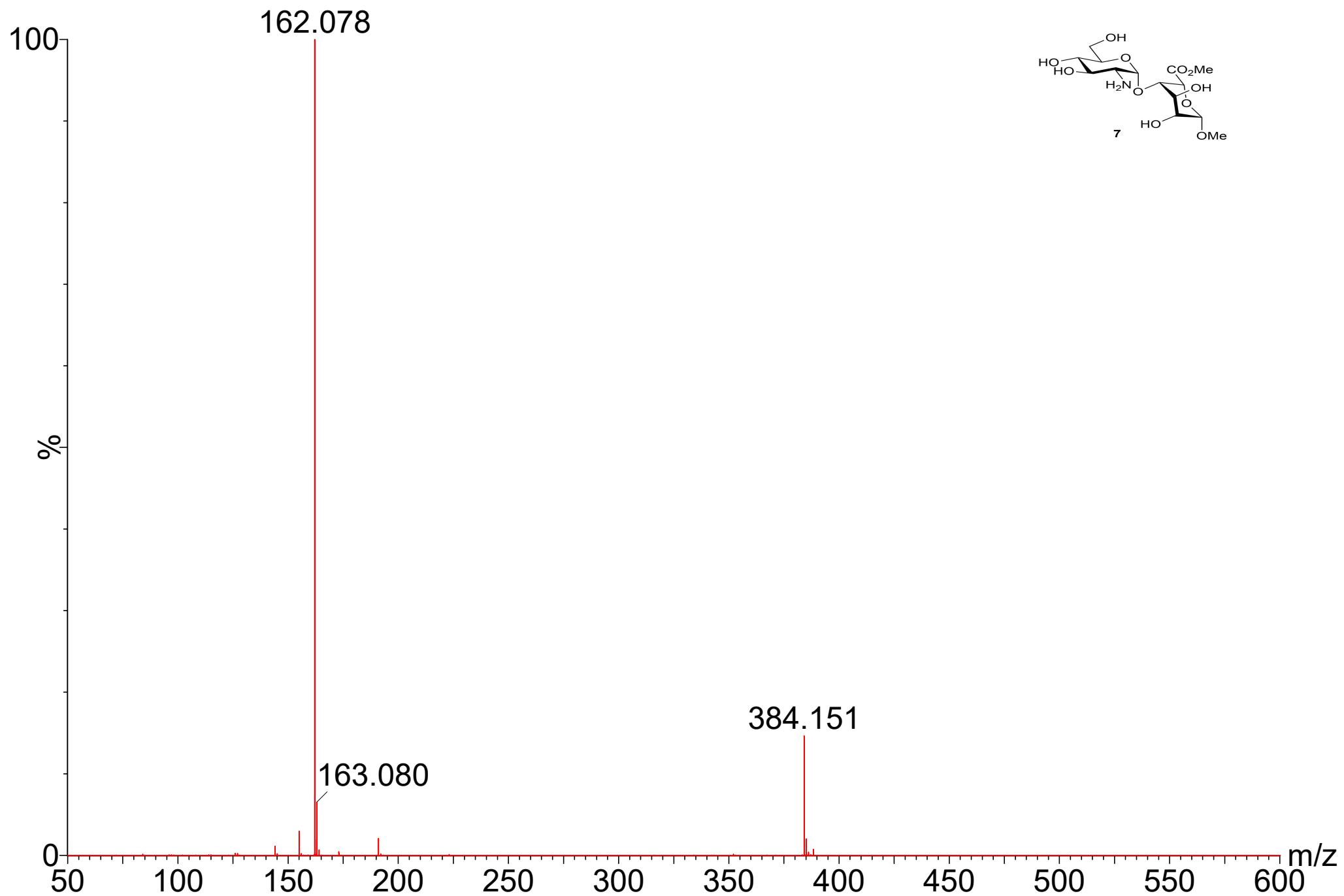


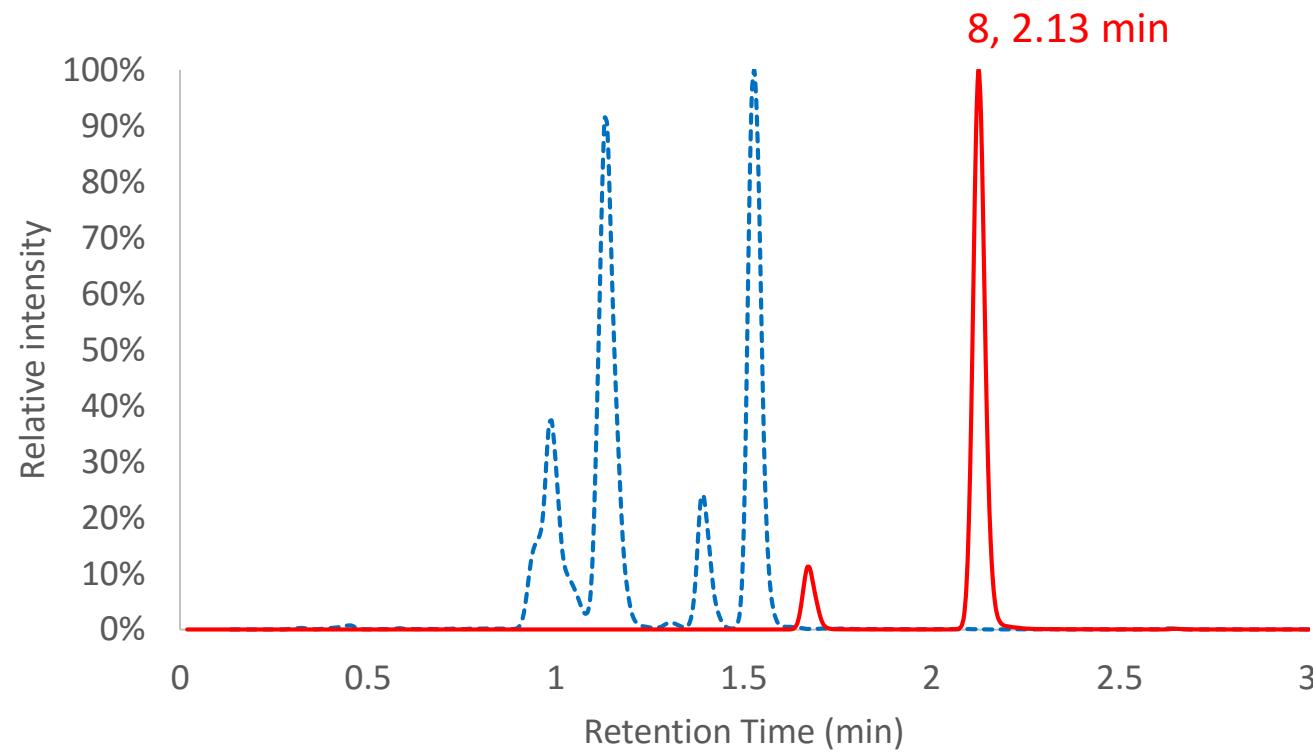
HS d6 internal standard (blue dotted trace) MRM transition m/z 390-162
Disaccharide 6 (Red solid trace) MRM transition m/z 426-204



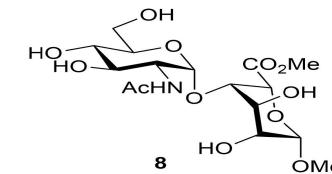
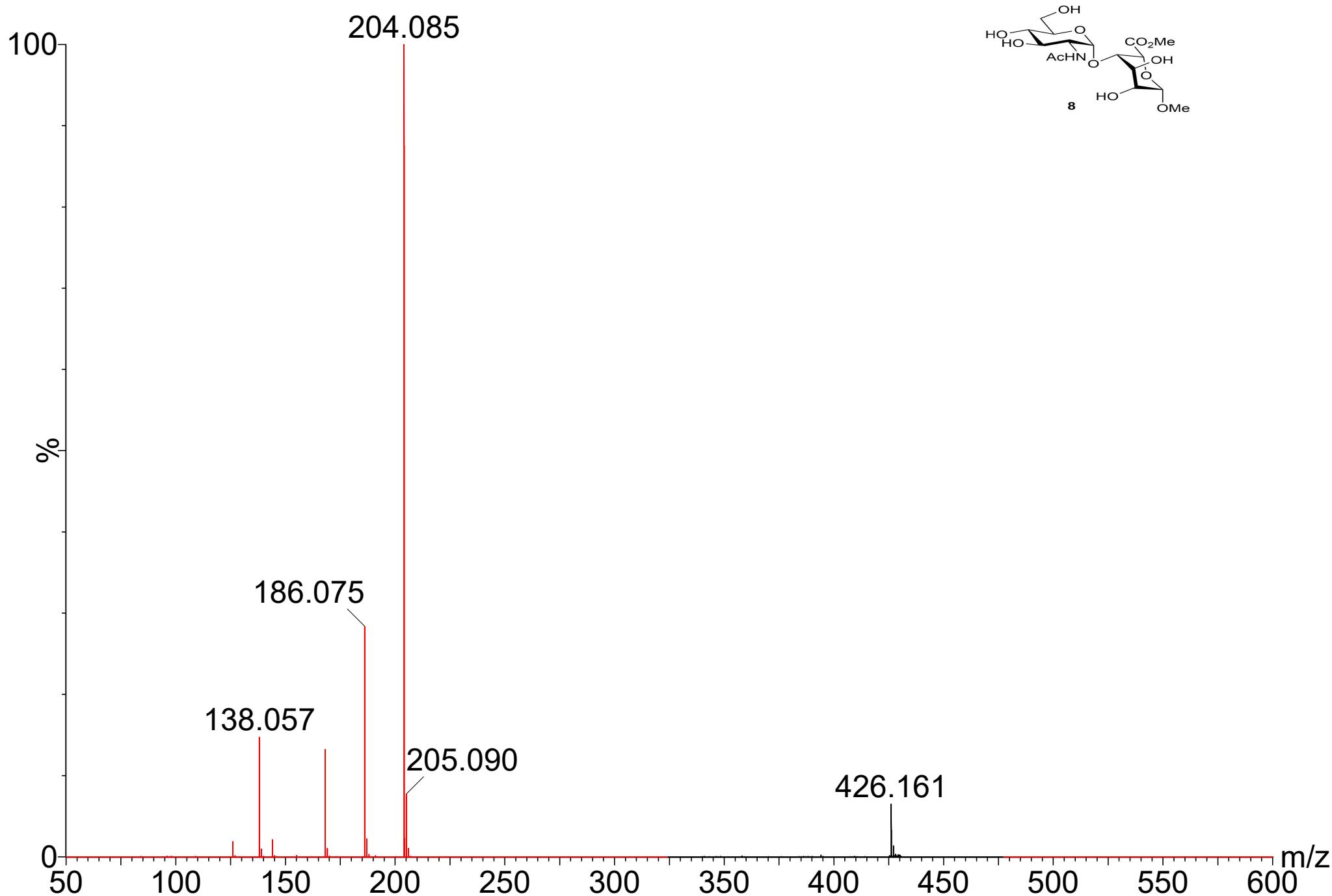


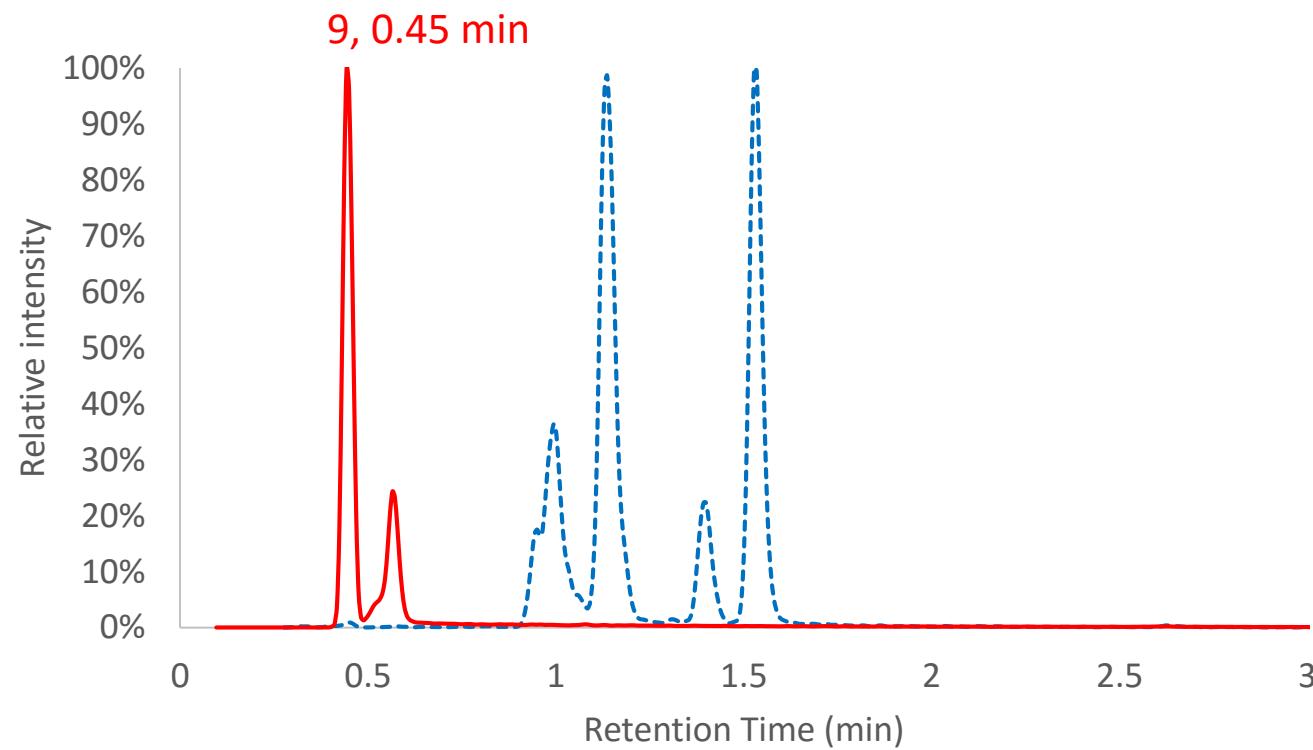
HS d6 internal standard (blue dotted trace) MRM transition m/z 390-162
Disaccharide 7 (Red solid trace) MRM transition m/z 384-162



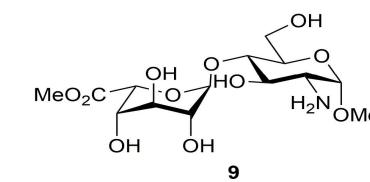
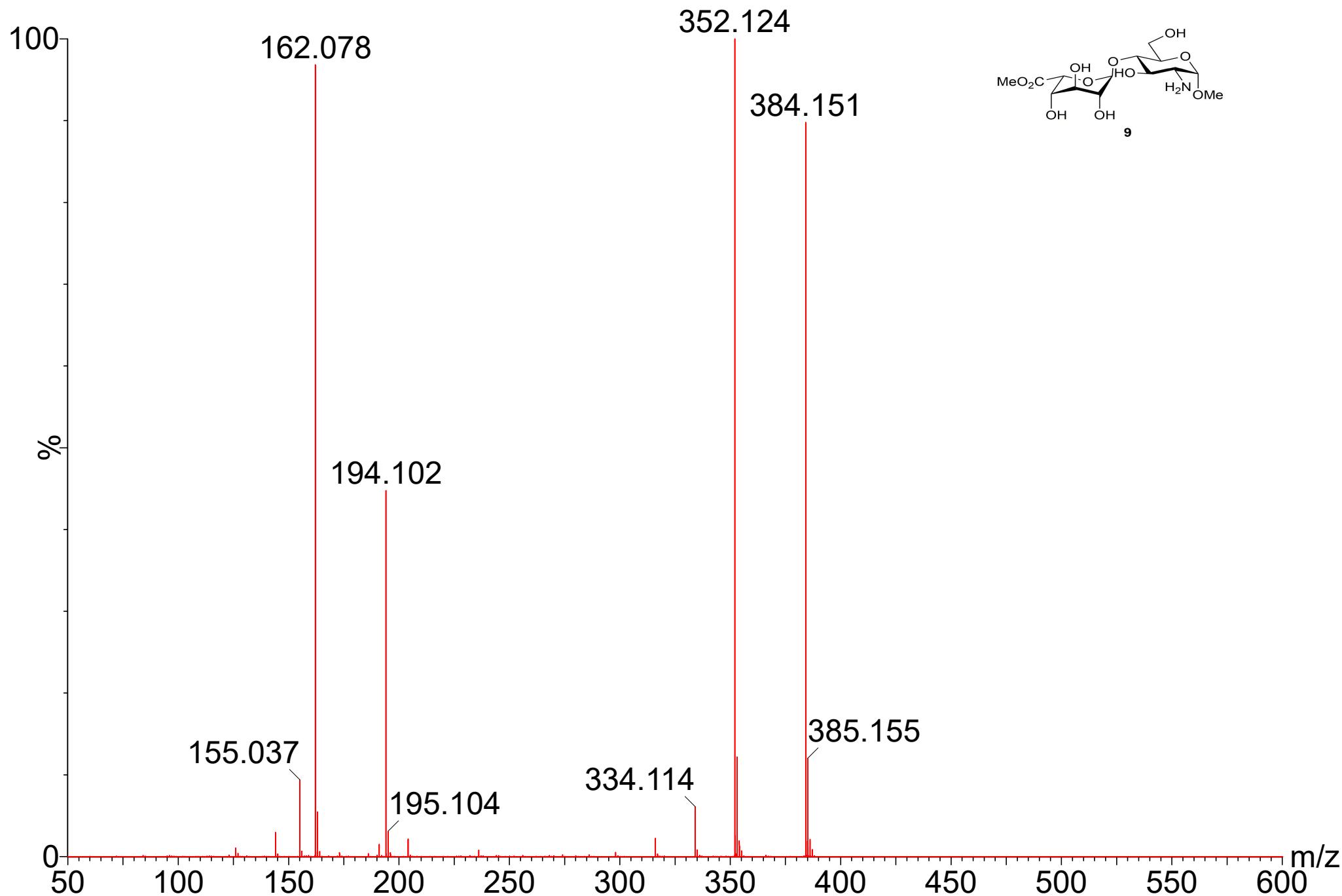


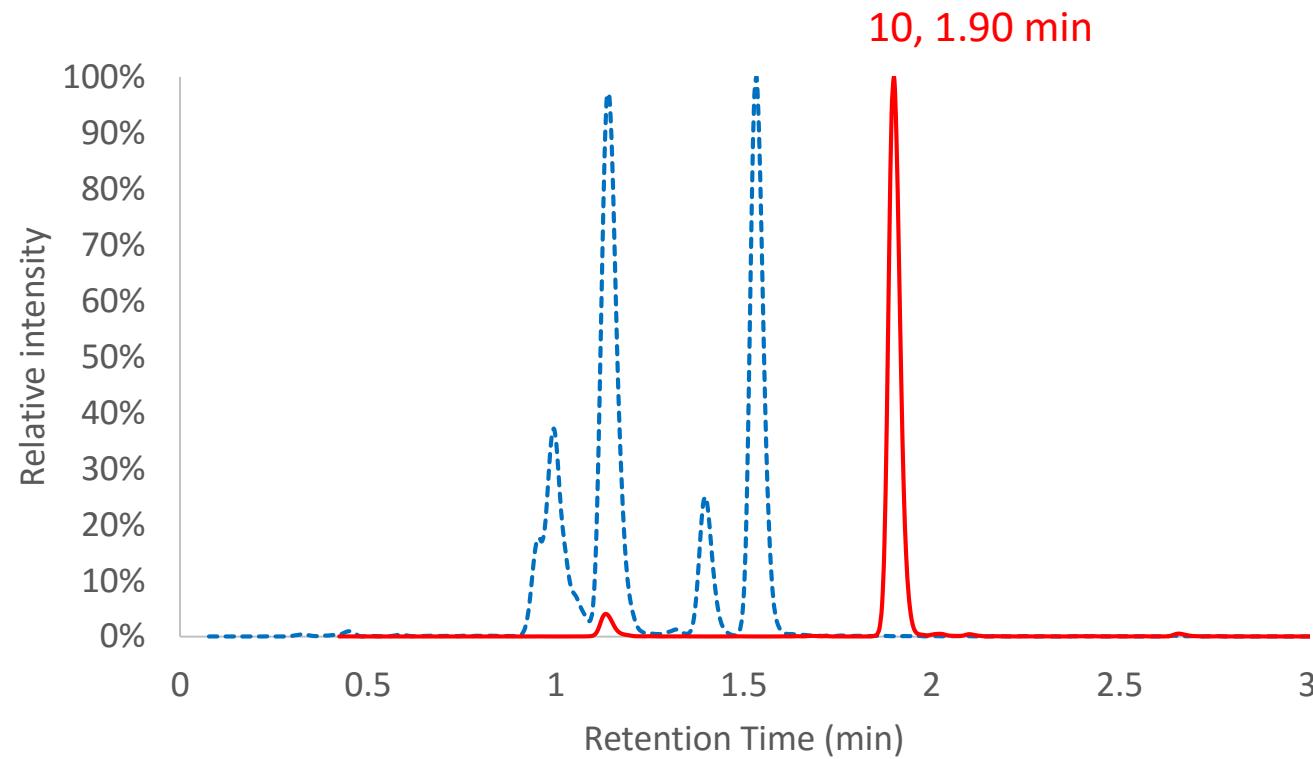
HS d6 internal standard (blue dotted trace) MRM transition m/z 390-162
Disaccharide 8 (Red solid trace) MRM transition m/z 426-204



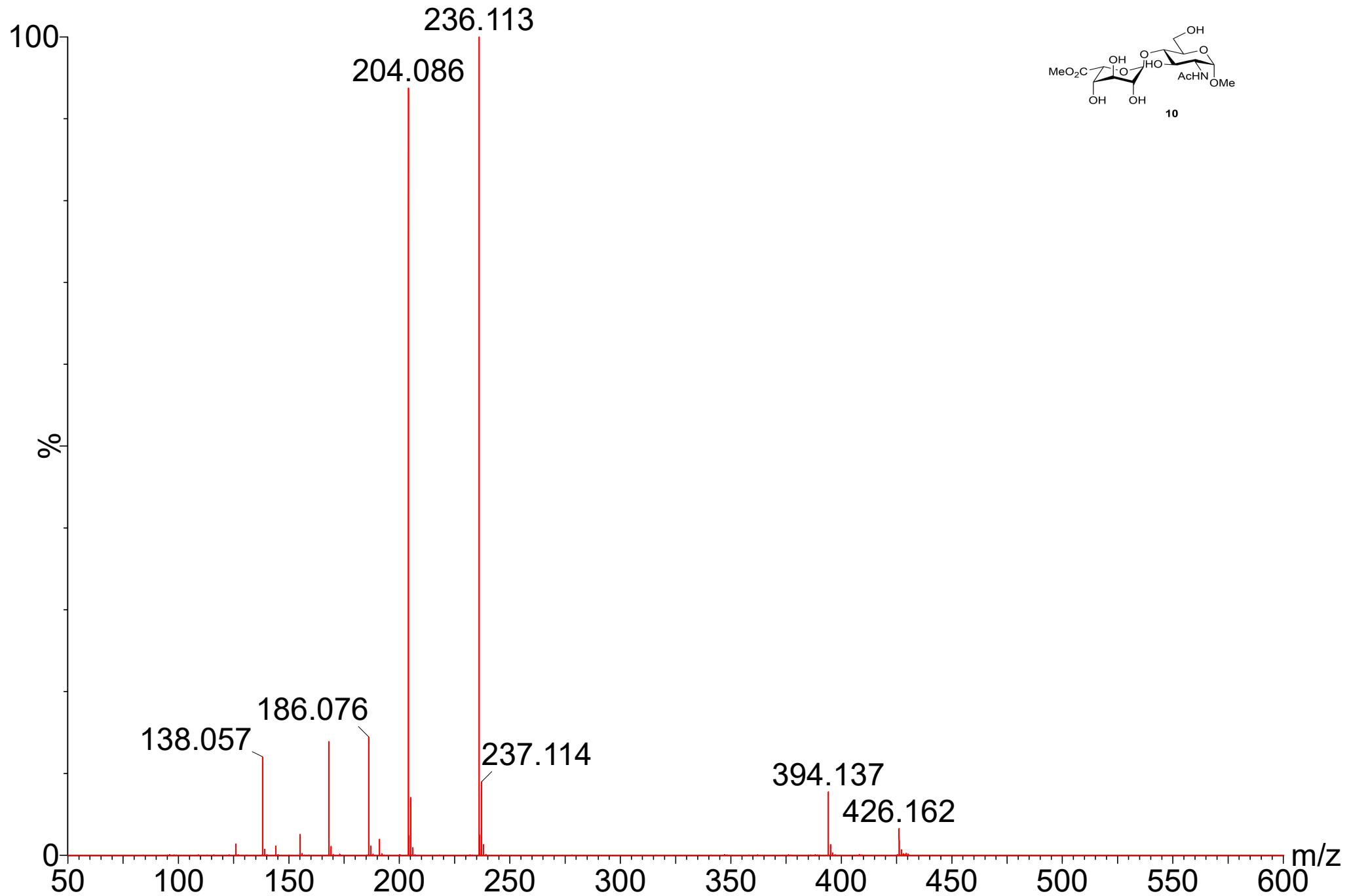


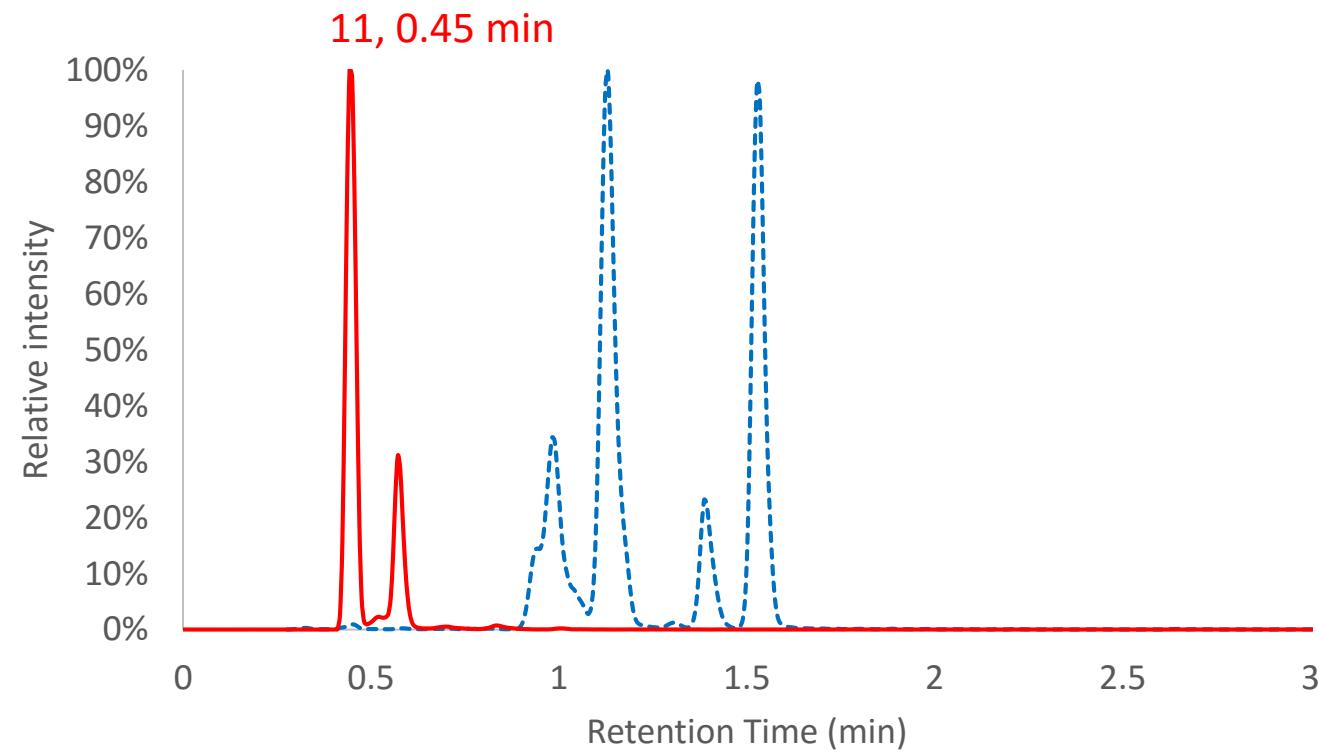
HS d6 internal standard (blue dotted trace) MRM transition m/z 390-162
Disaccharide 9 (Red solid trace) MRM transition m/z 384-352



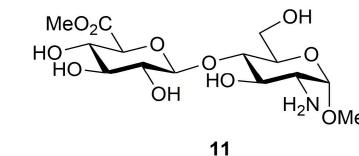
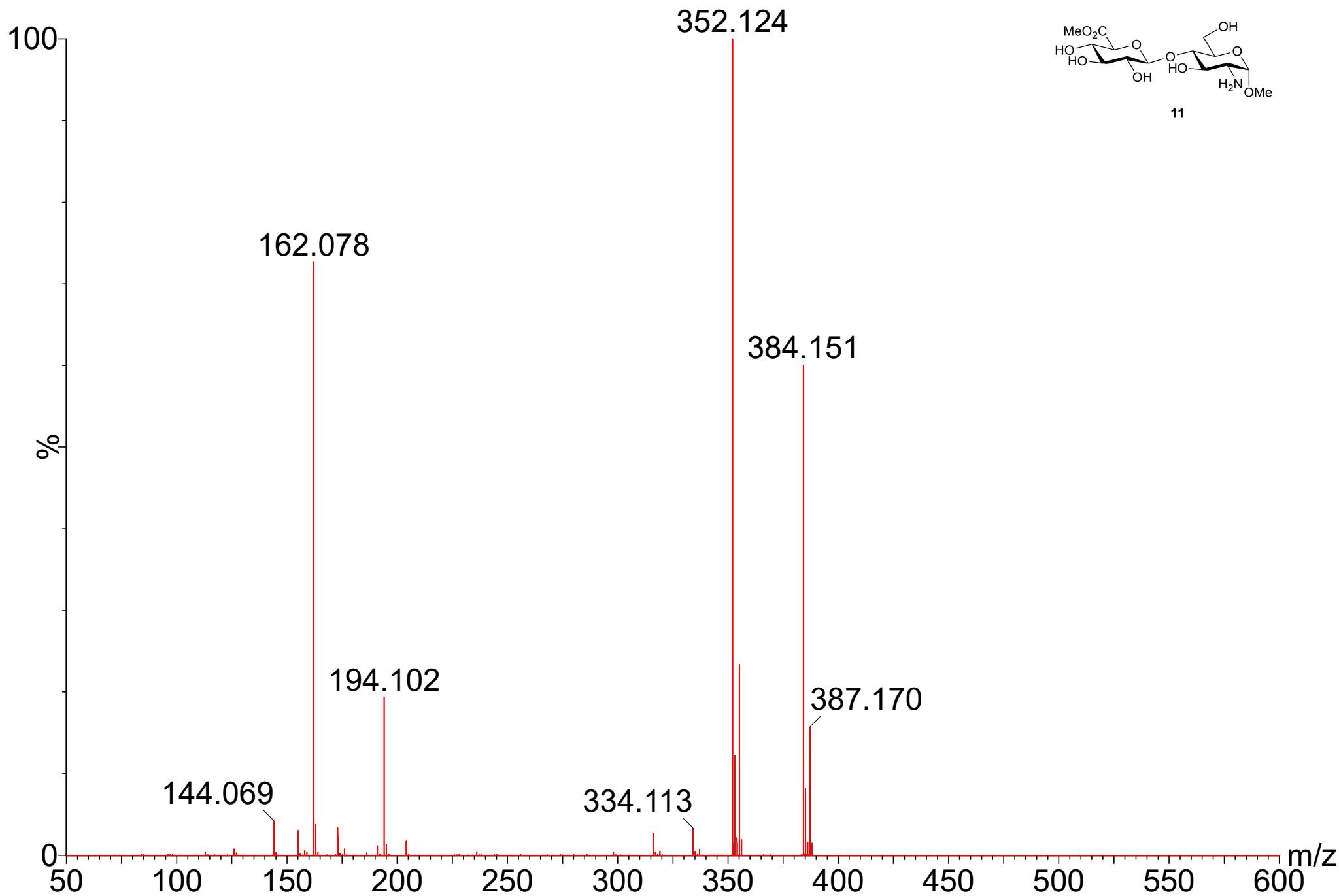


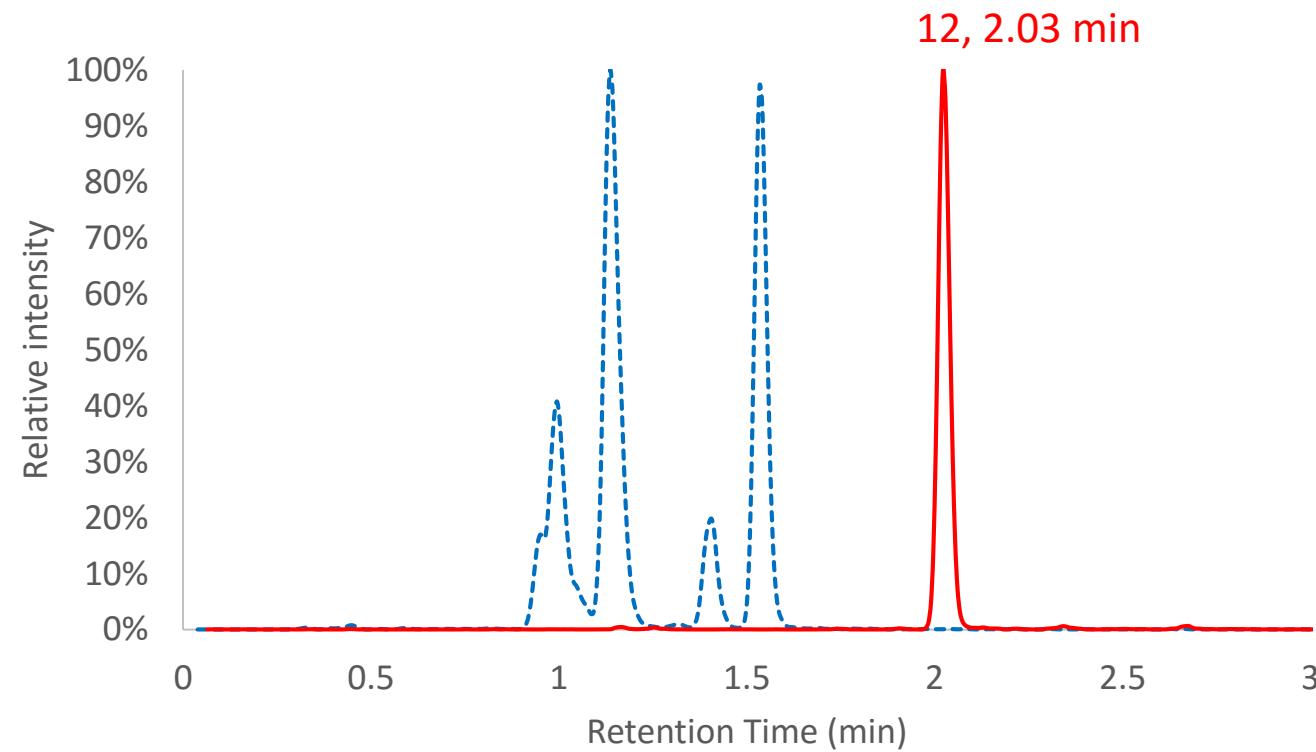
HS d6 internal standard (blue dotted trace) MRM transition m/z 390-162
Disaccharide 10 (Red solid trace) MRM transition m/z 426-394



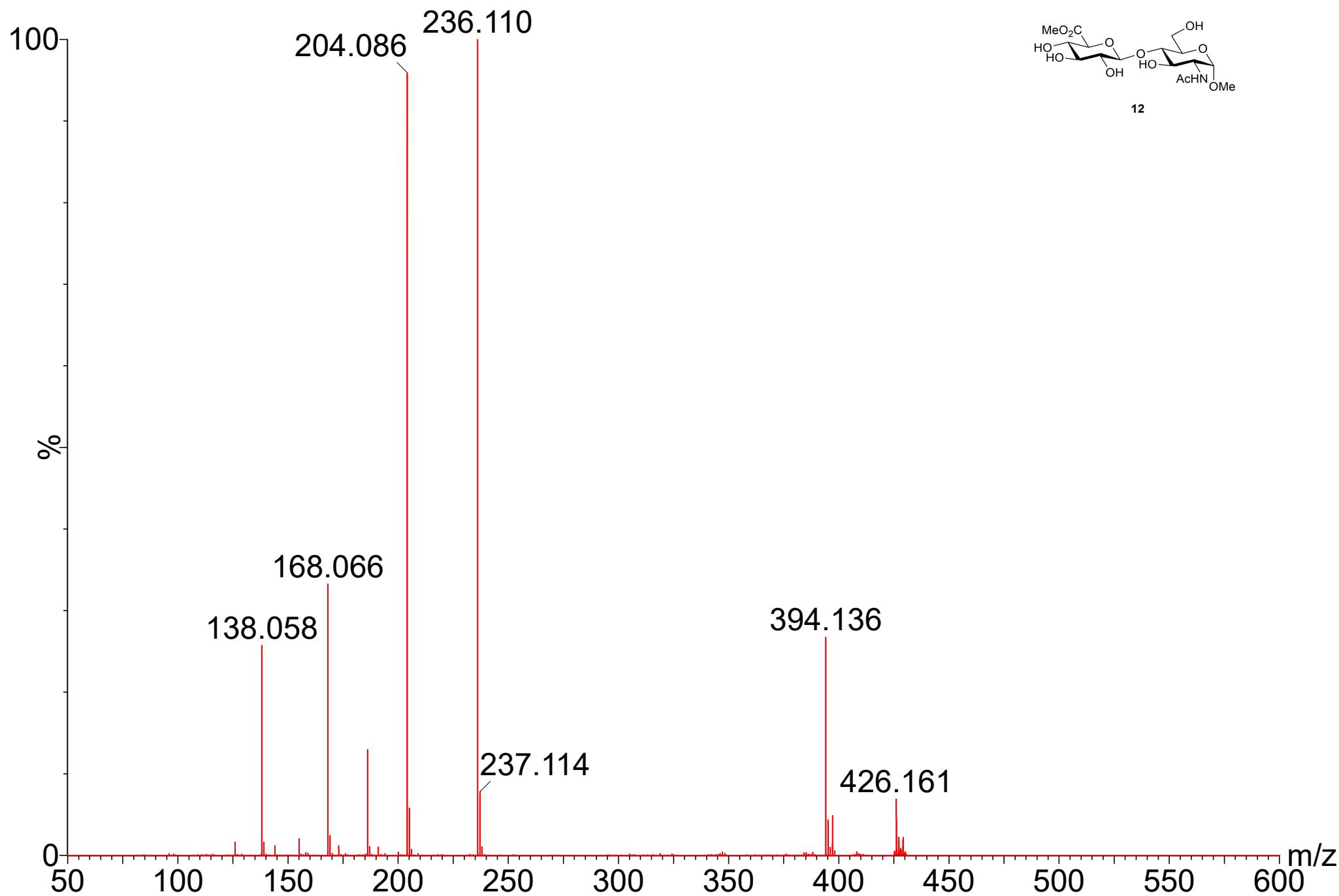


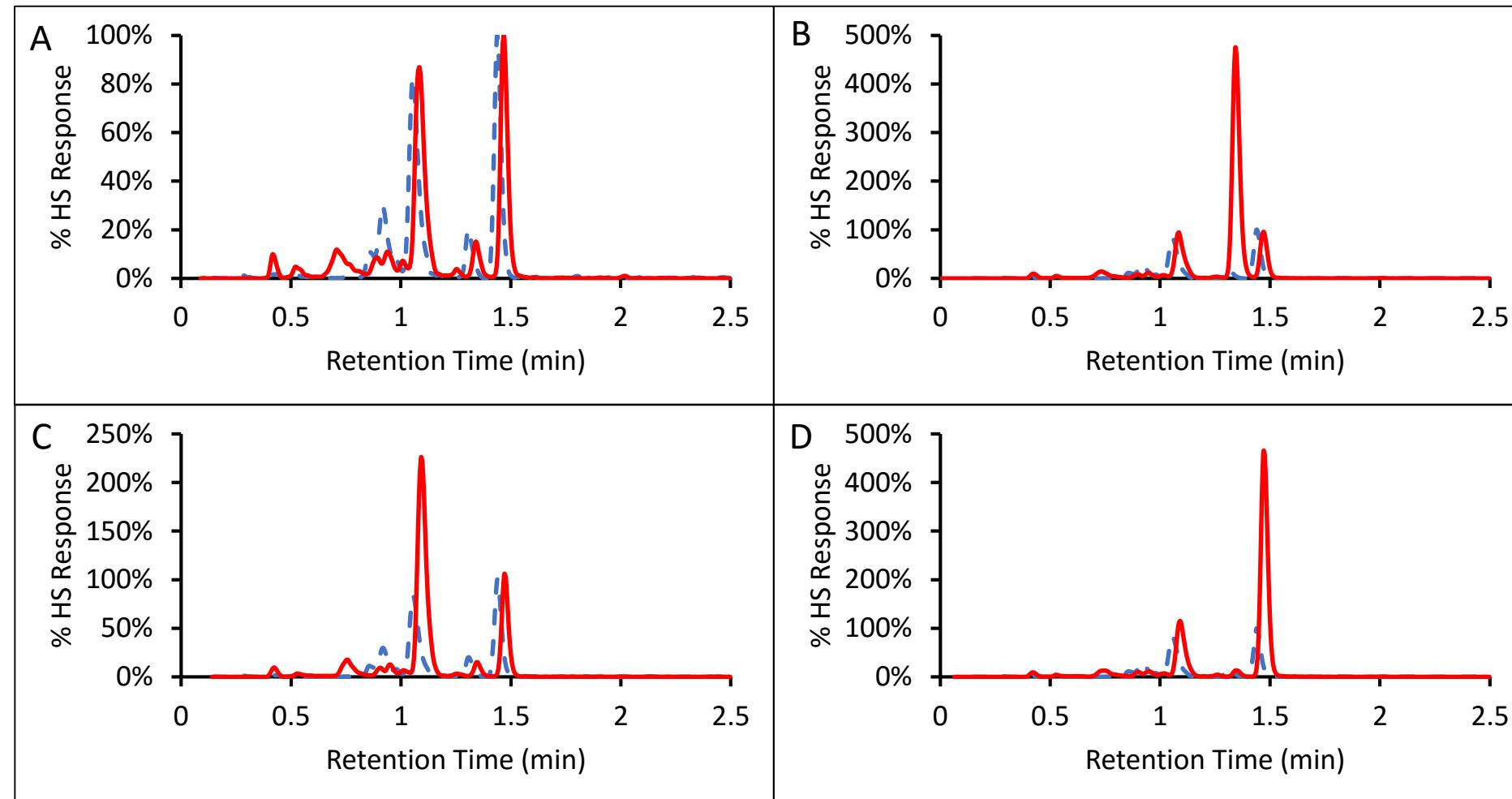
HS d6 internal standard (blue dotted trace) MRM transition m/z 390-162
Disaccharide 11 (Red solid trace) MRM transition m/z 384-352



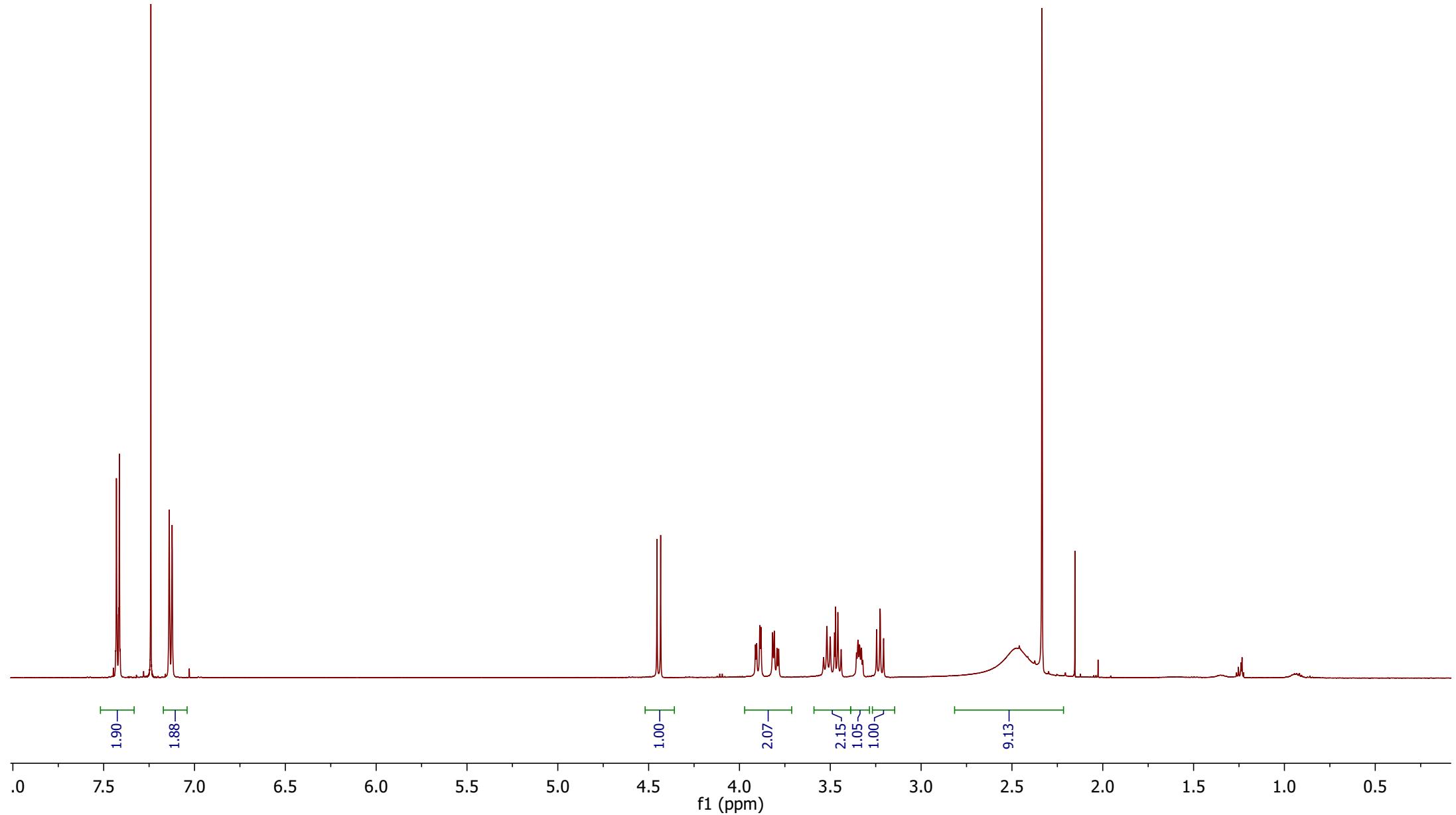
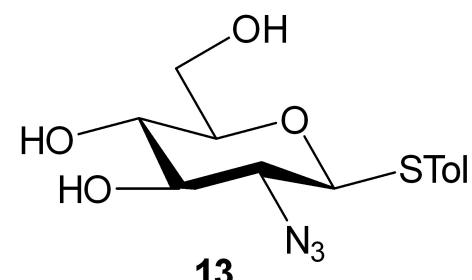


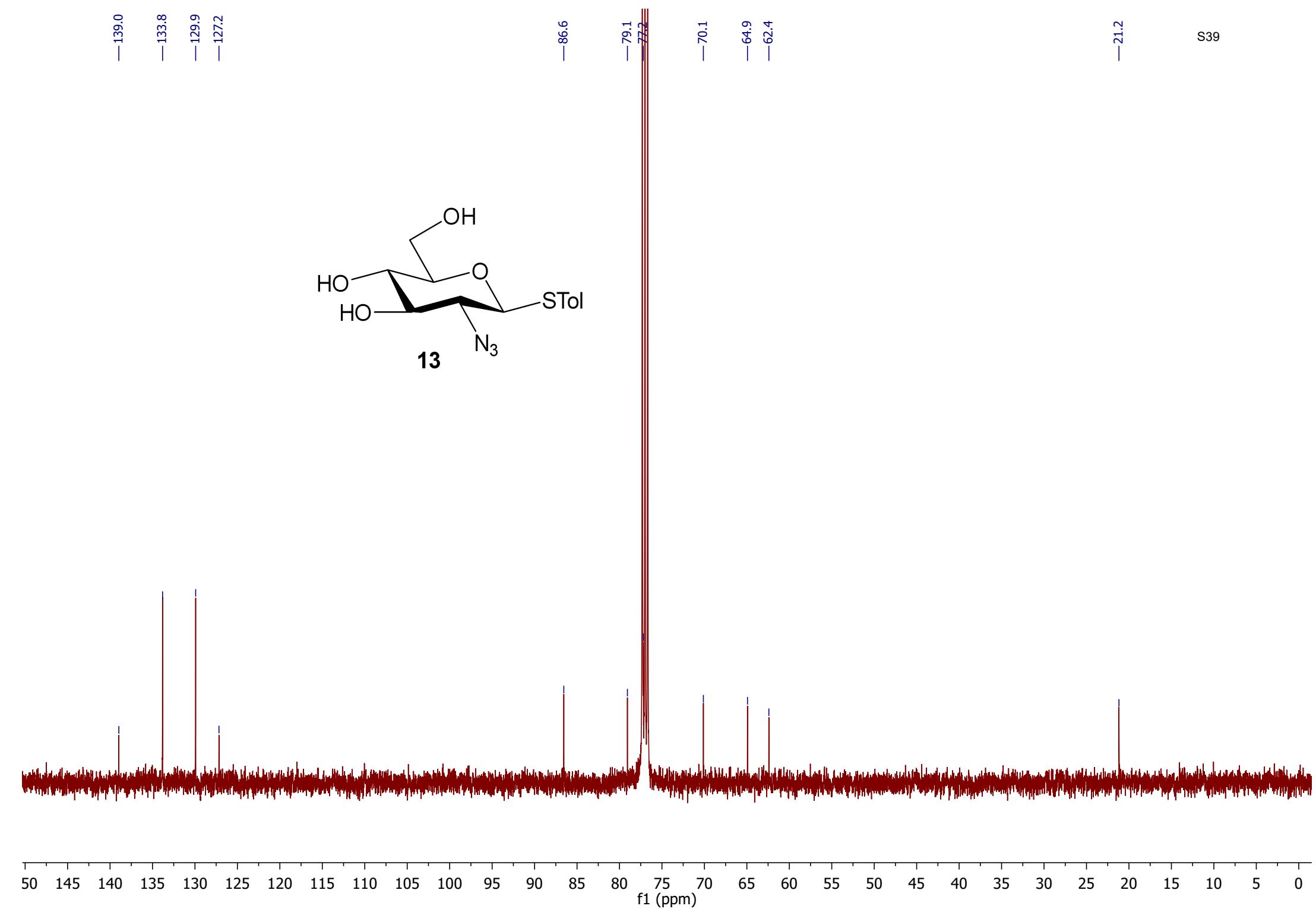
HS d6 internal standard (blue dotted trace) MRM transition m/z 390-162
Disaccharide 12 (Red solid trace) MRM transition m/z 426-394

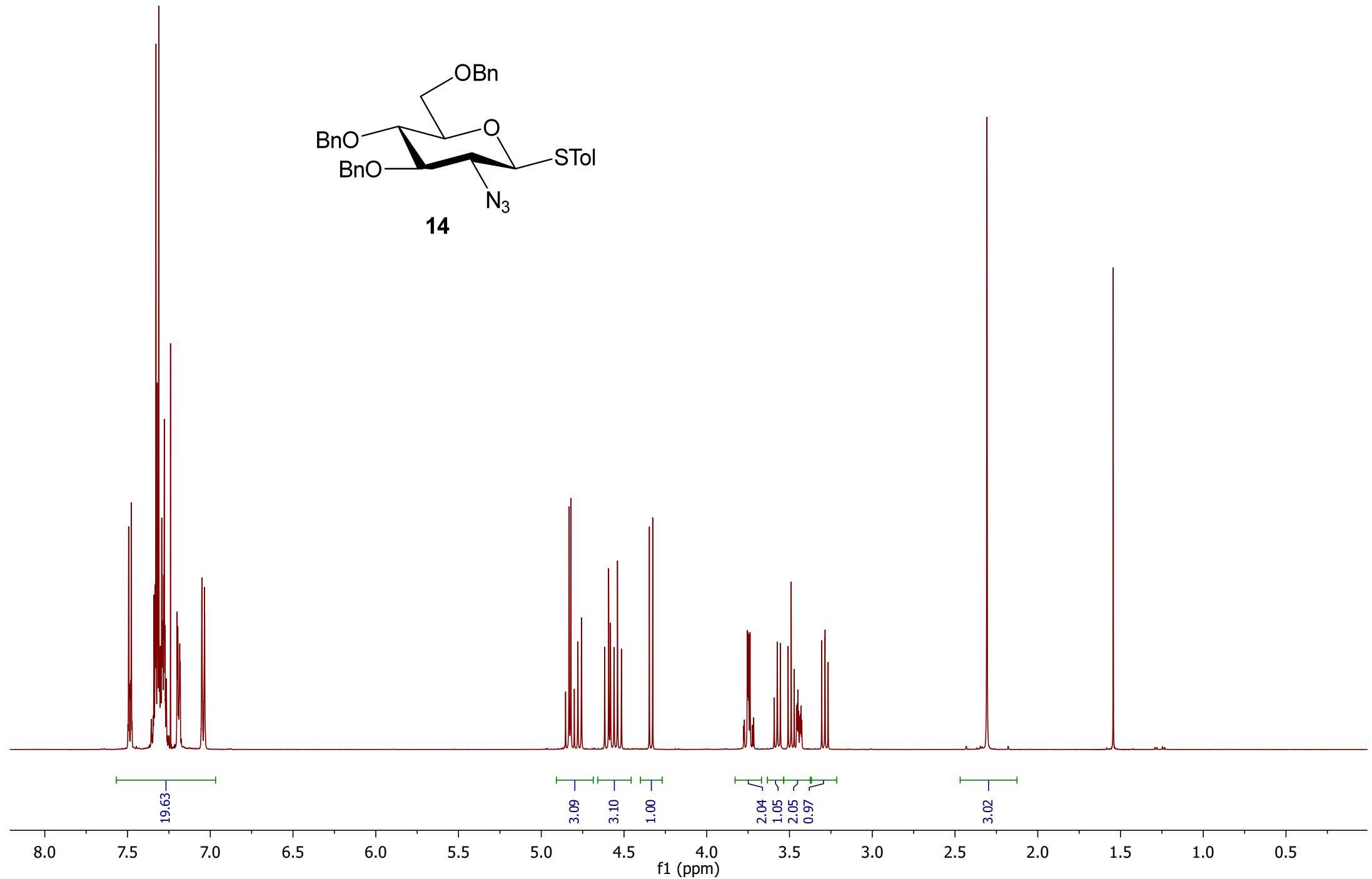
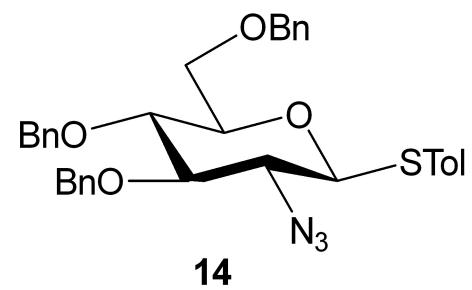


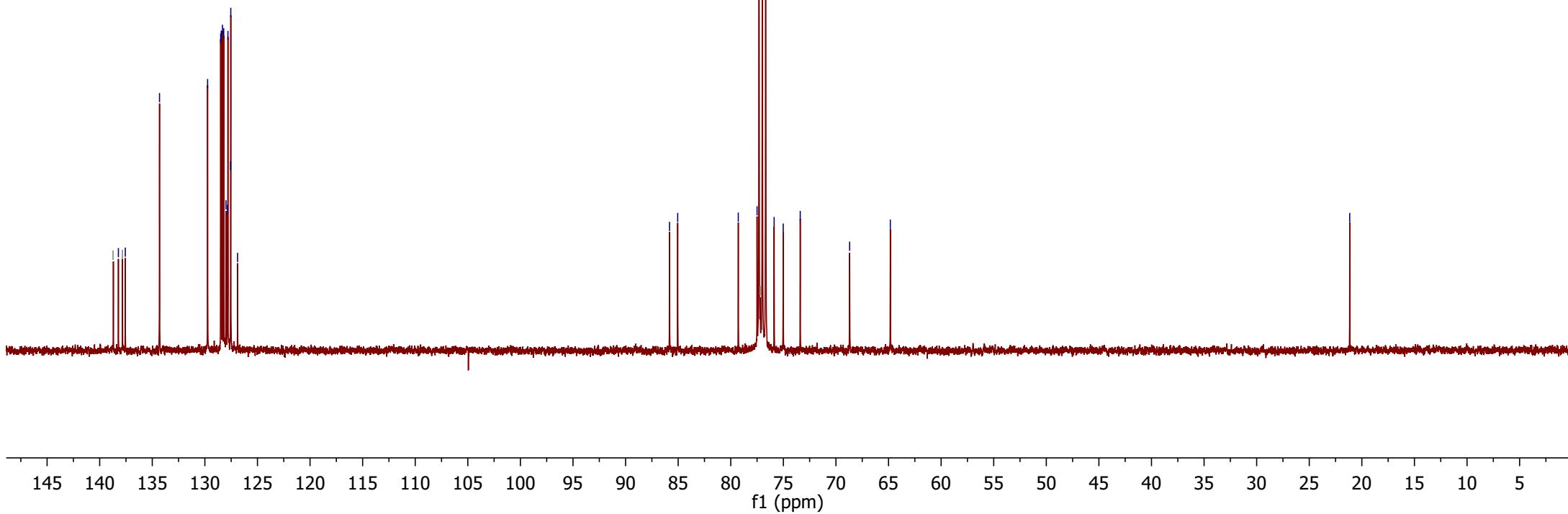
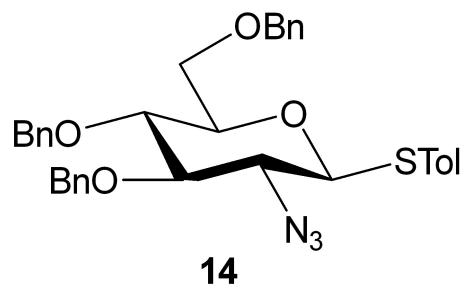
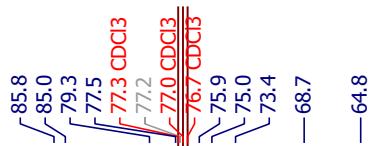
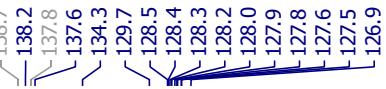


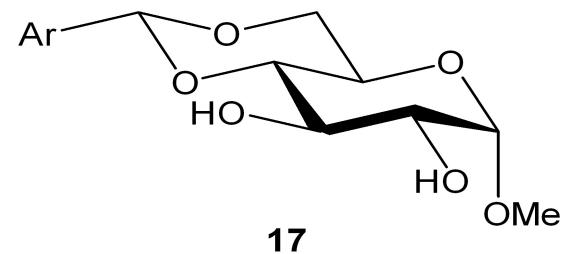
Confirmation of methanolysis digestion products of HS shown by the addition of known synthesised disaccharide standards. An internal standard of HS d6 digest is shown (blue dotted trace, MRM m/z 390-162) compared to the primary HS digestion products (Red solid trace, MRM m/z 384-162). For comparison purpose all signal intensities are displayed as % response normalised to the signal intensity of the digestion products of 10 µg HS (A), with the standard addition of disaccharide **1** (B), disaccharide **5** (C) and disaccharide **7** (D).



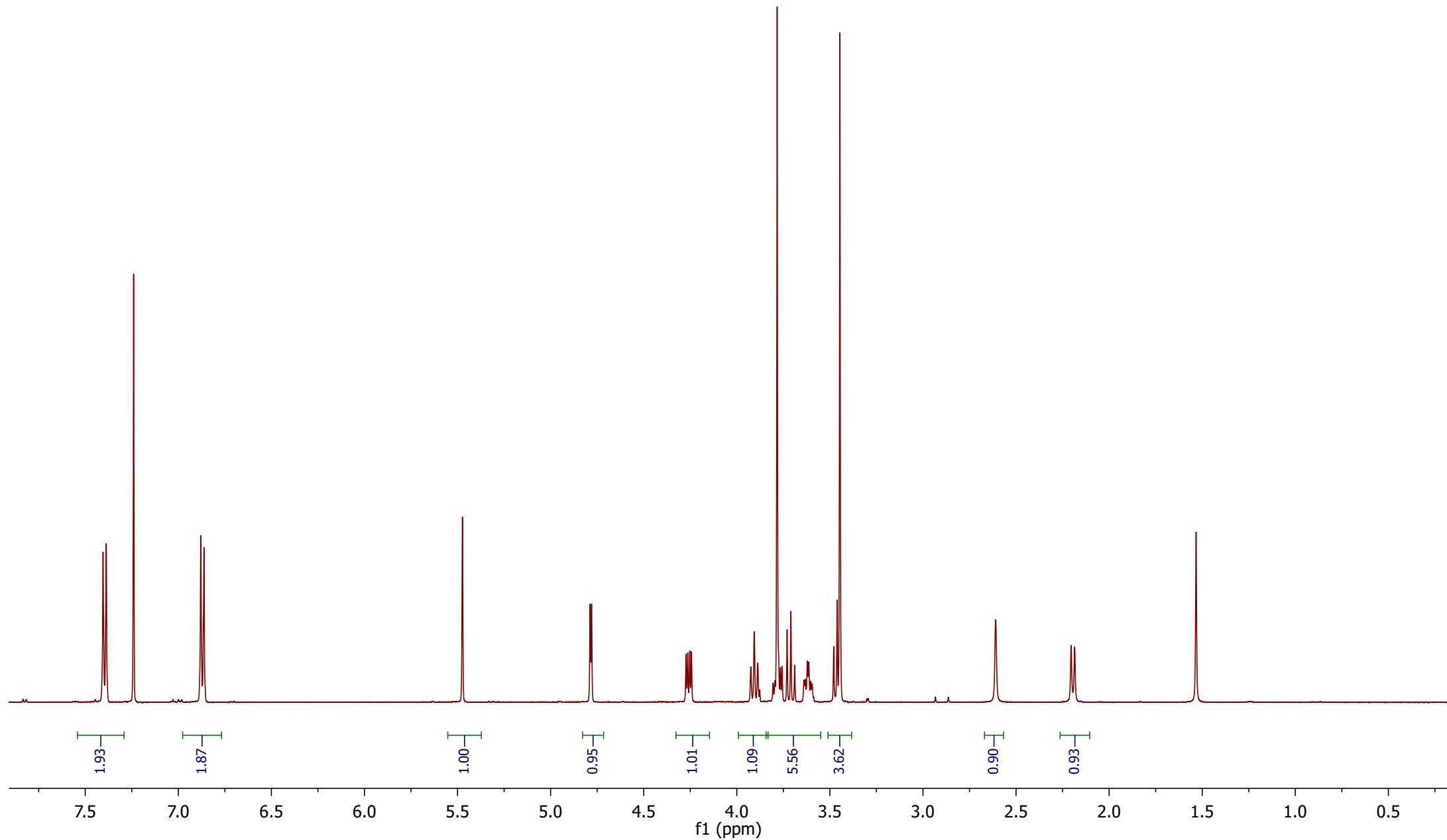








S42



—160.2

—129.5

—127.6

—113.7

—101.9

—99.7

—80.8

—72.9

~71.9

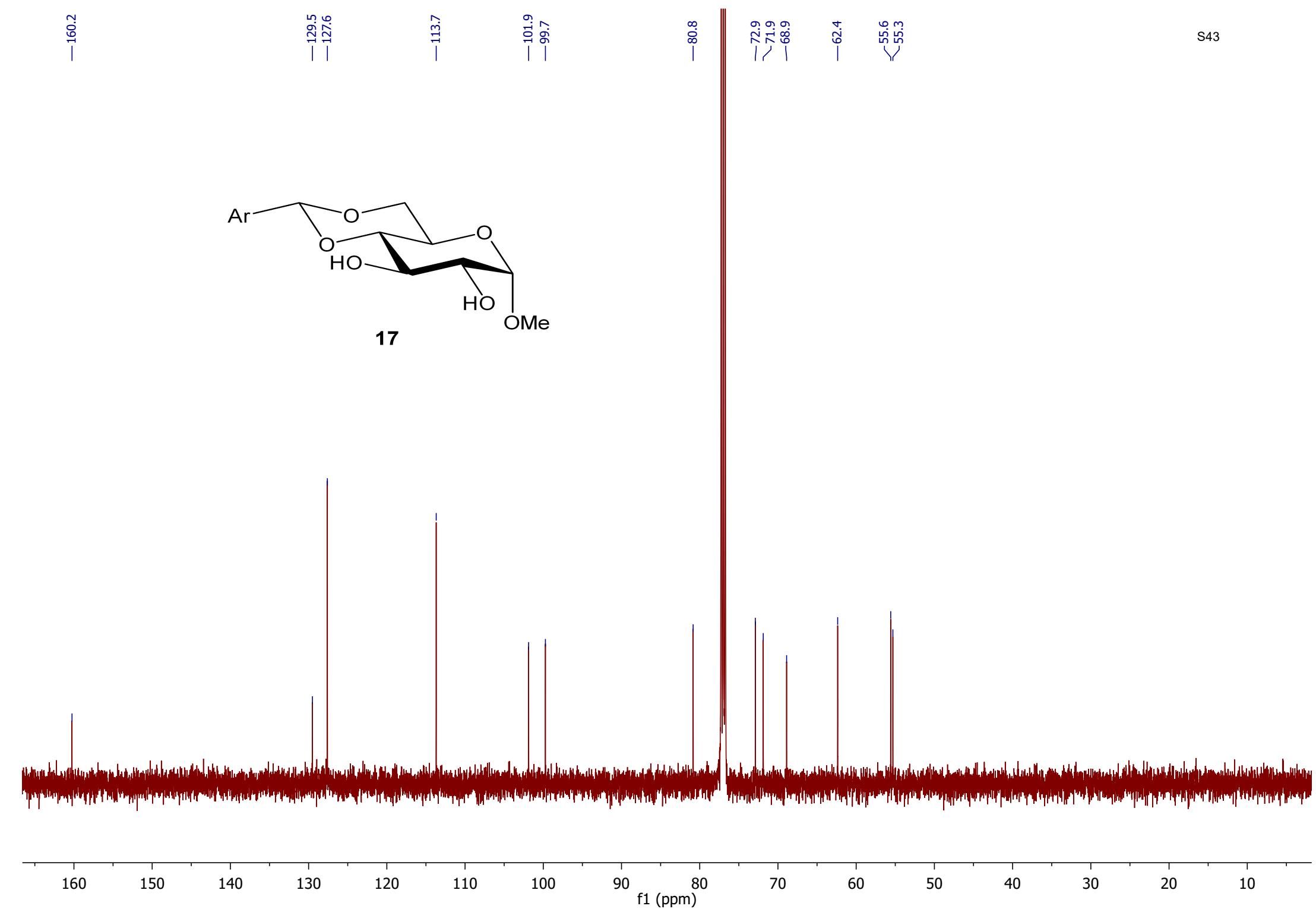
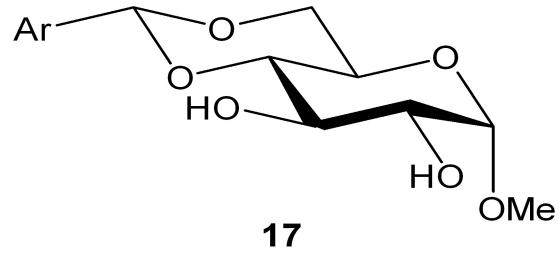
—68.9

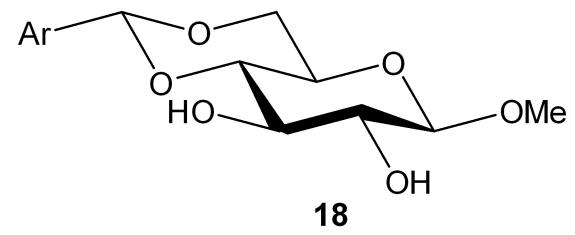
—62.4

—55.6

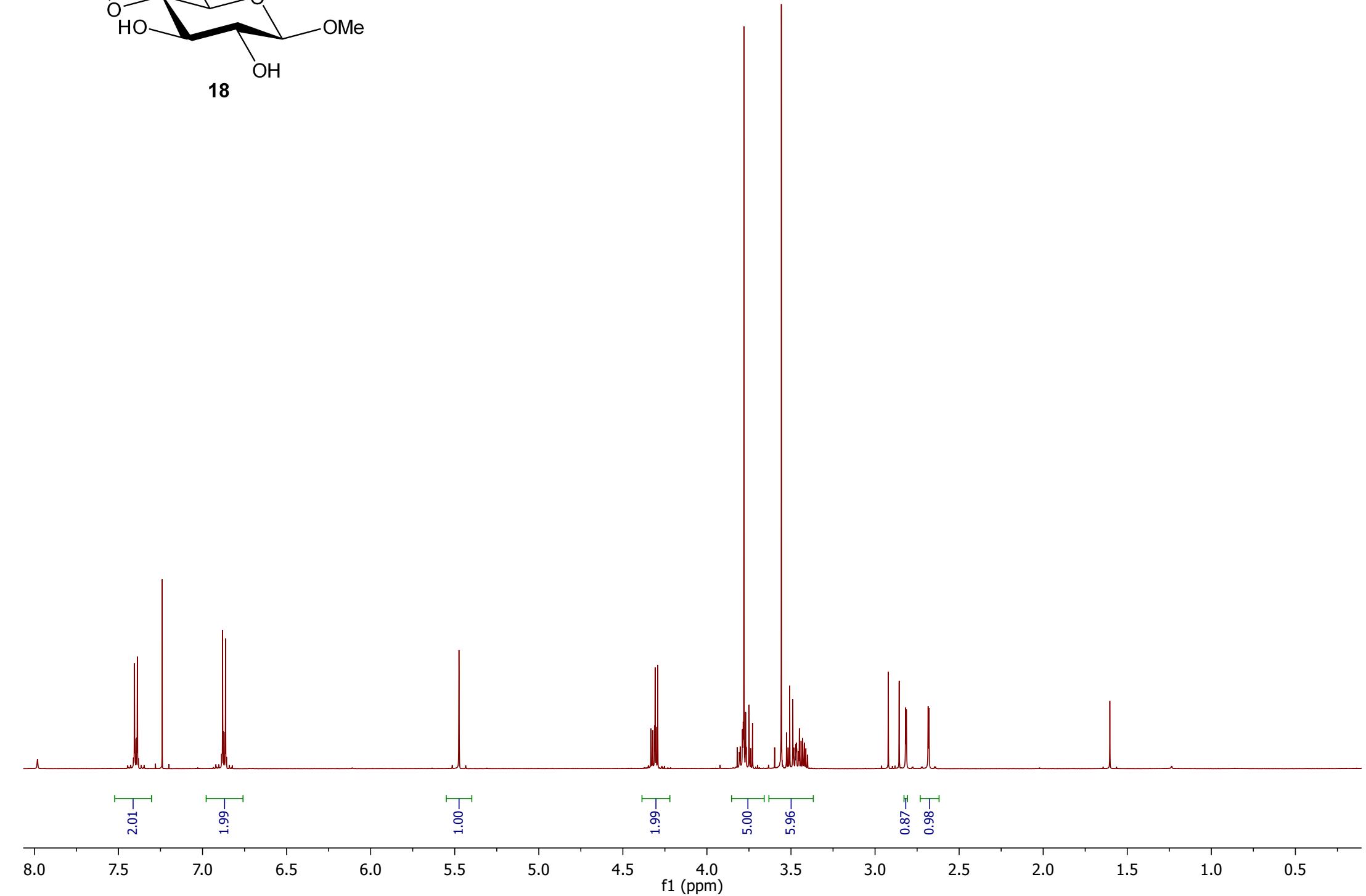
~55.3

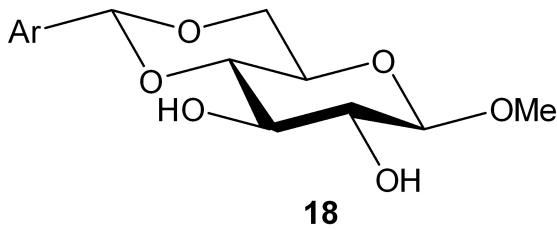
S43





S44





—129.4

—127.6

—113.7

—104.1

—101.8

—80.5

—74.5

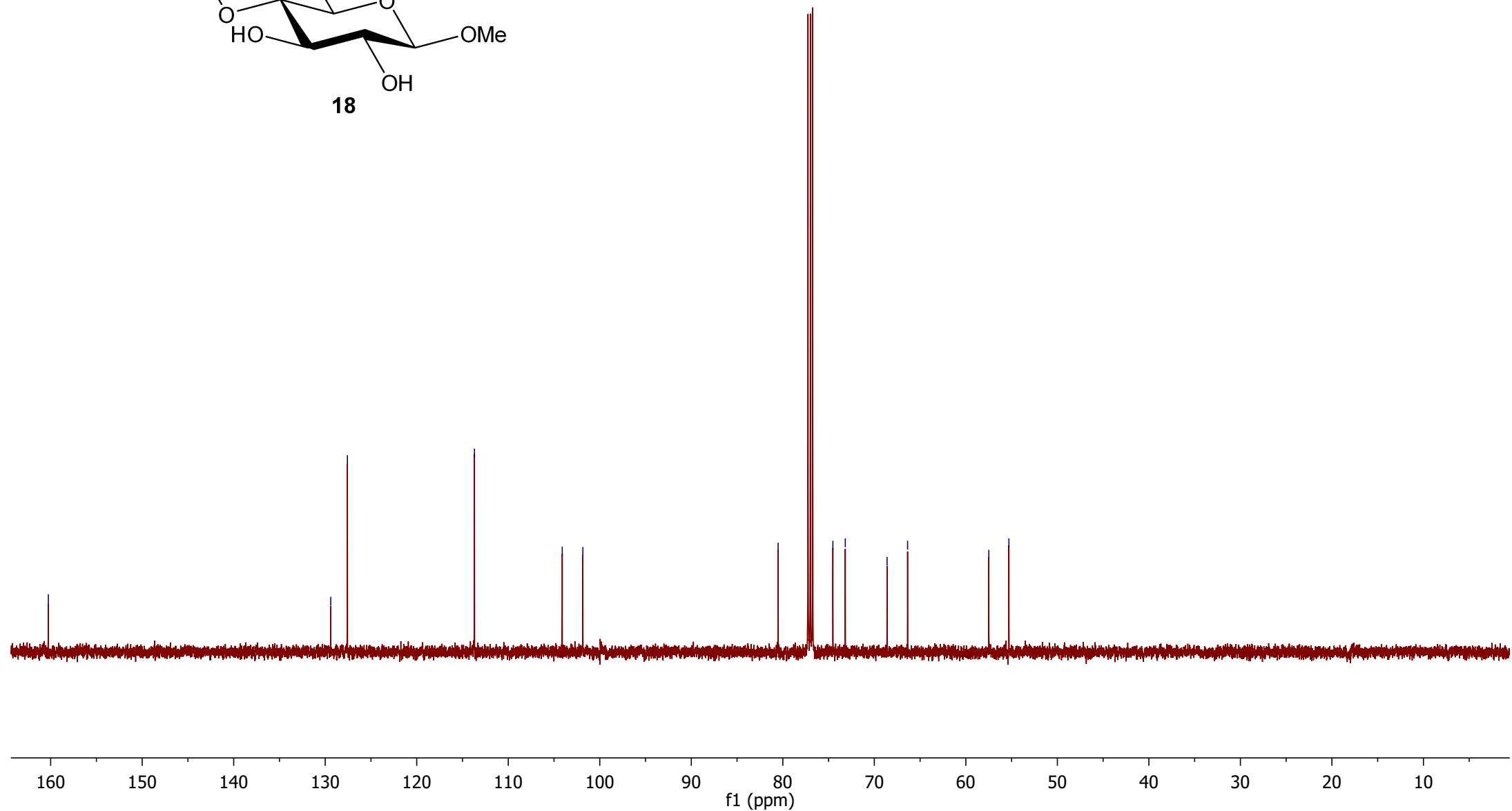
—73.2

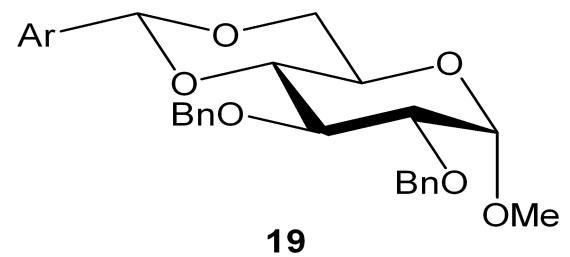
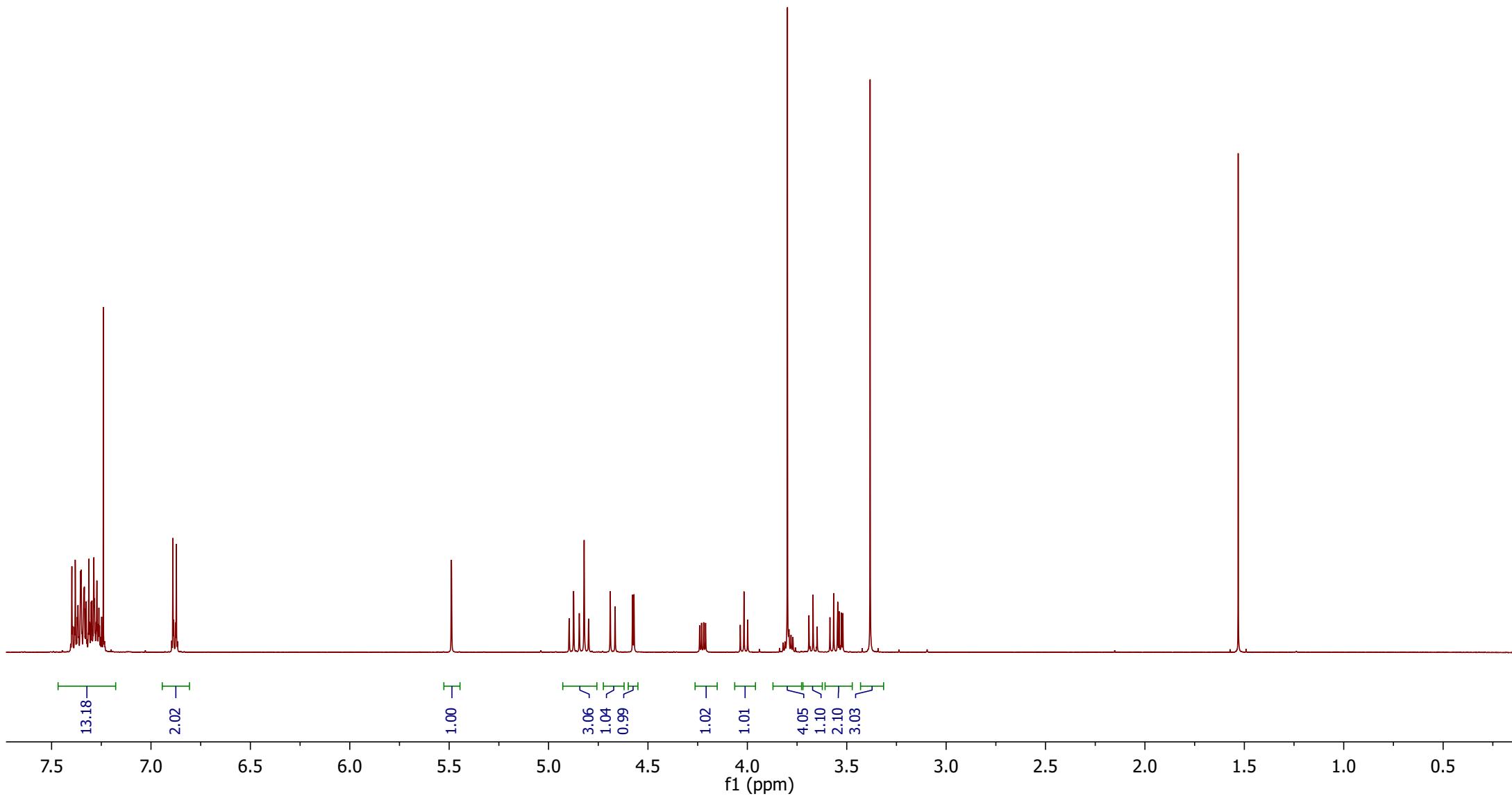
—68.6

—66.4

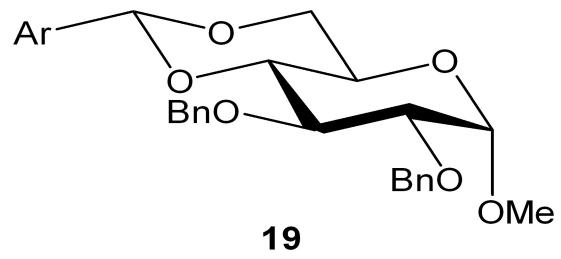
—57.5

—55.3



**19**

-160.0



~138.7
~138.2
129.9
128.4
128.3
128.1
~128.0
~127.9
127.5
127.3

-113.5

-101.2
-99.2

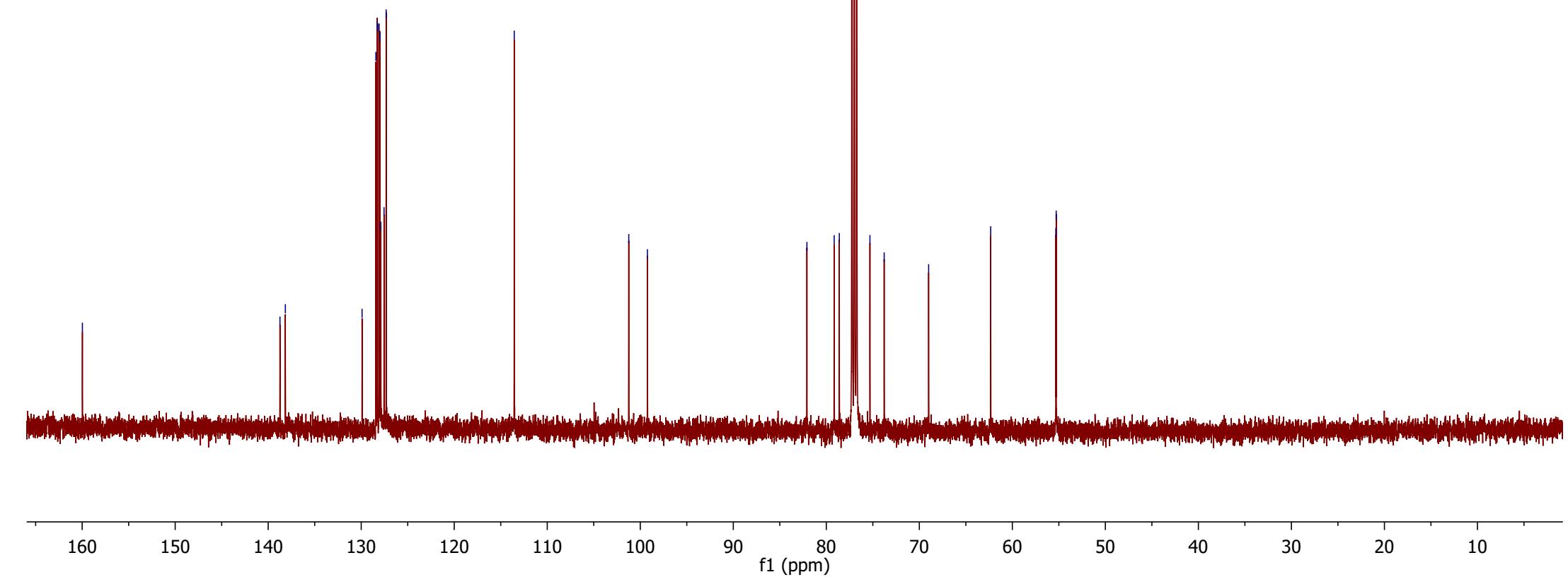
~82.1
~79.2
~78.6
77.2
~75.3
~73.8

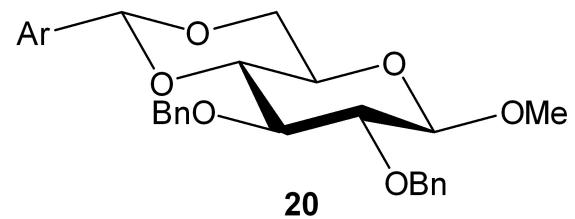
-69.0

-62.3

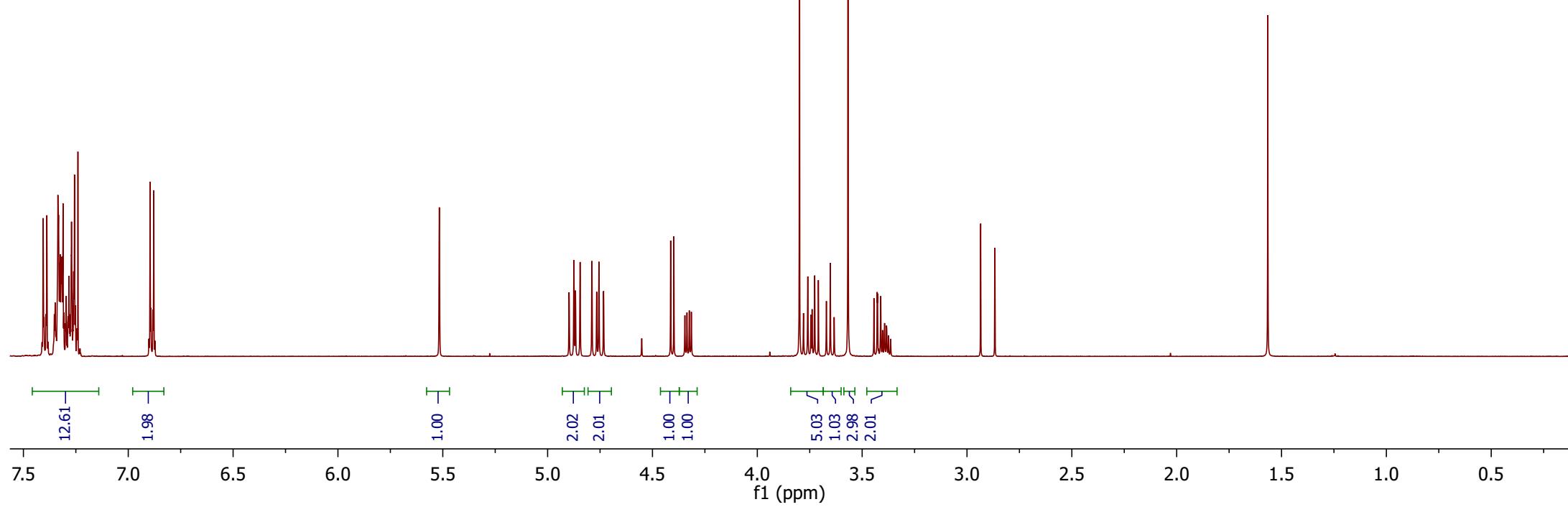
55.3
55.3

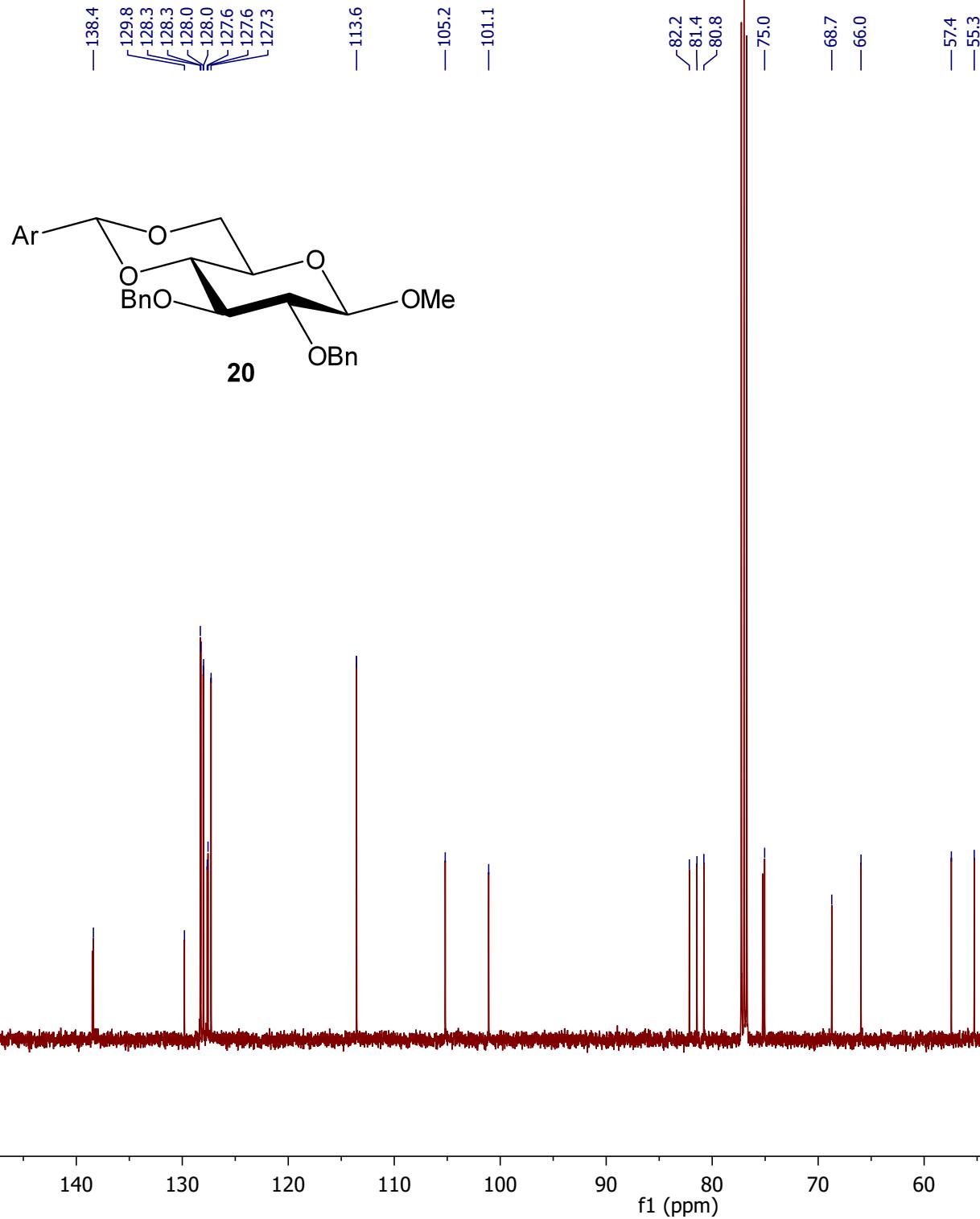
S47

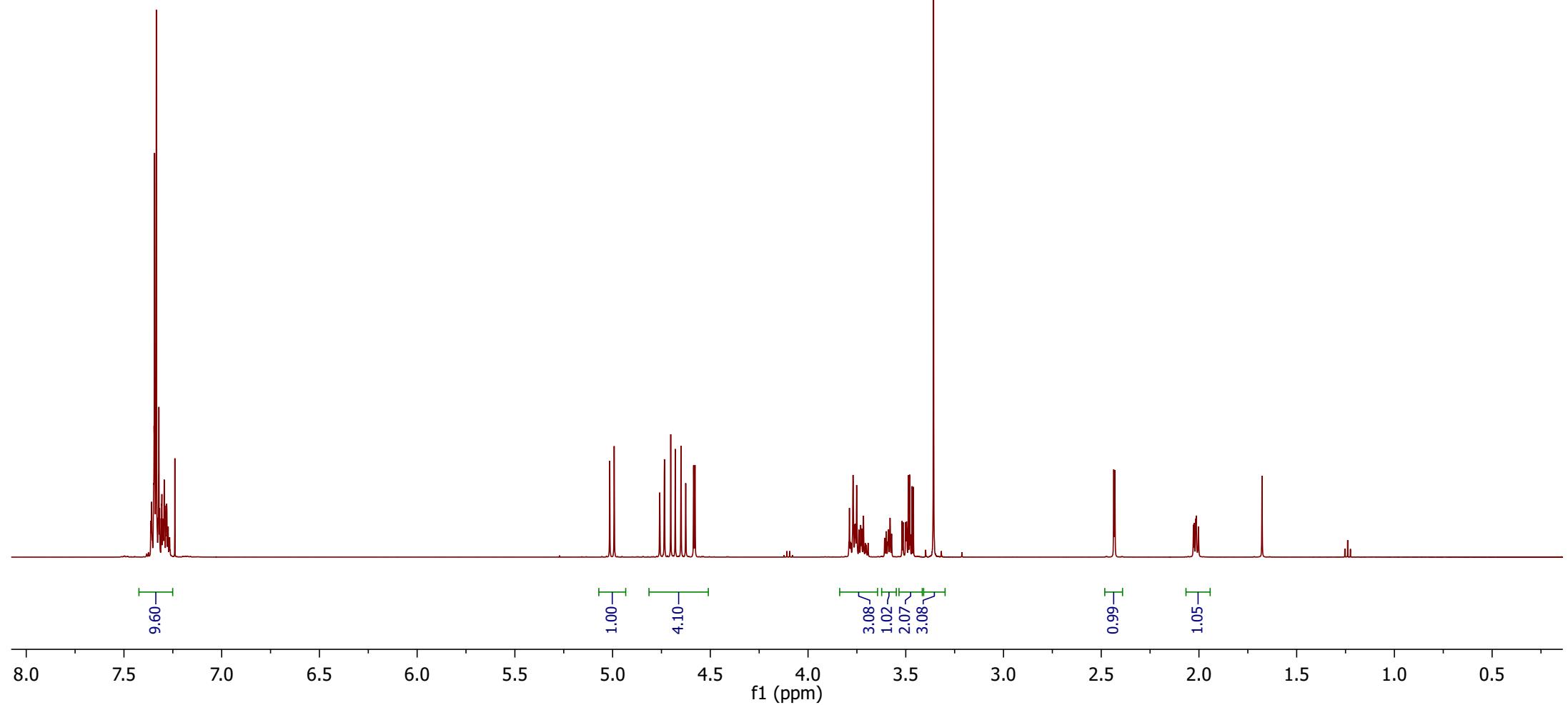
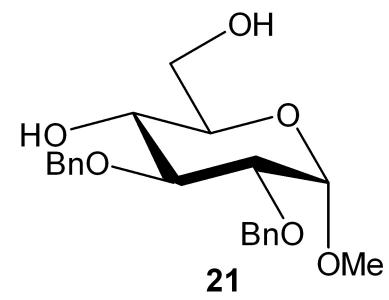


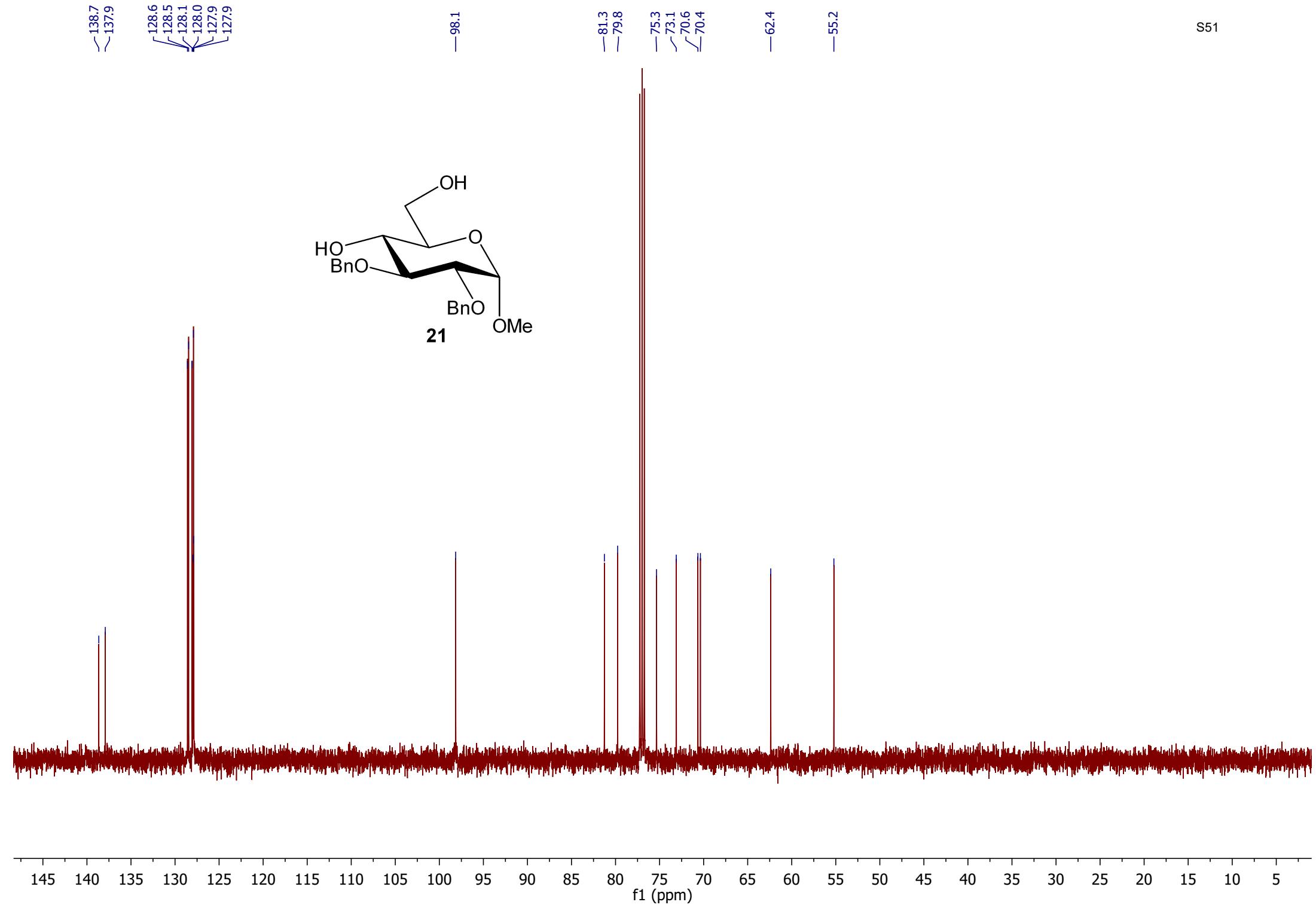


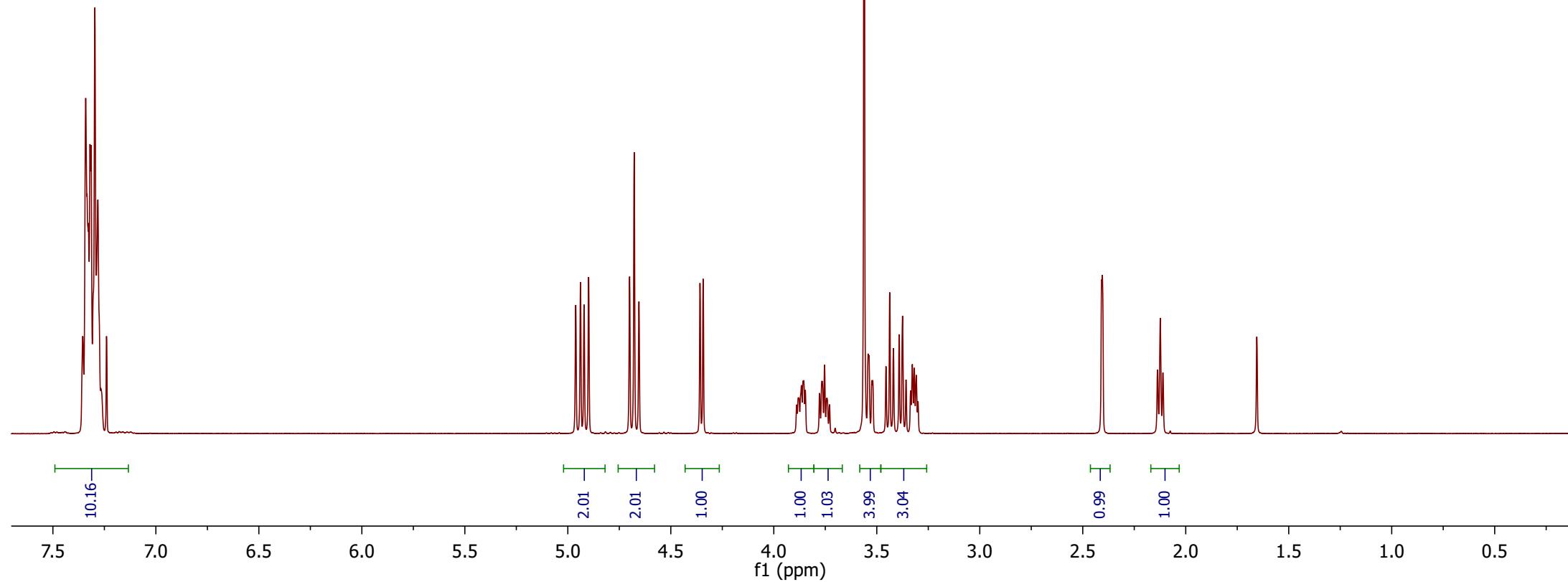
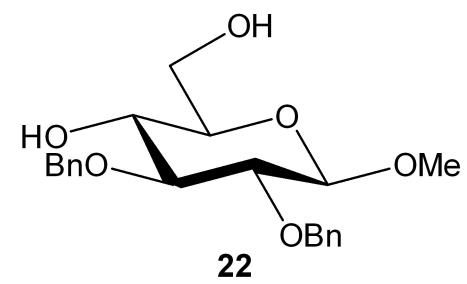
S48

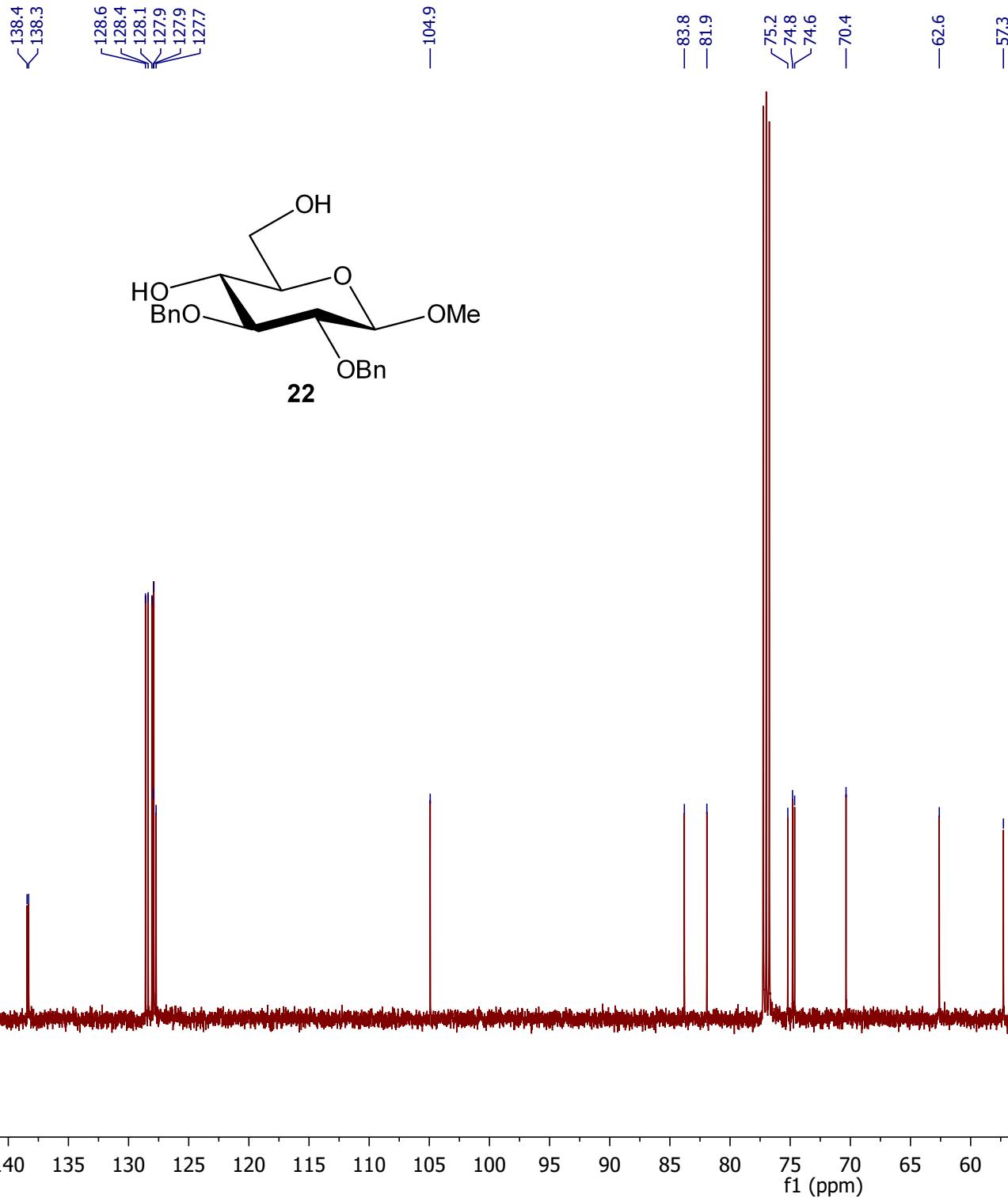


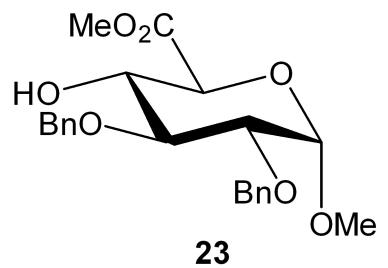




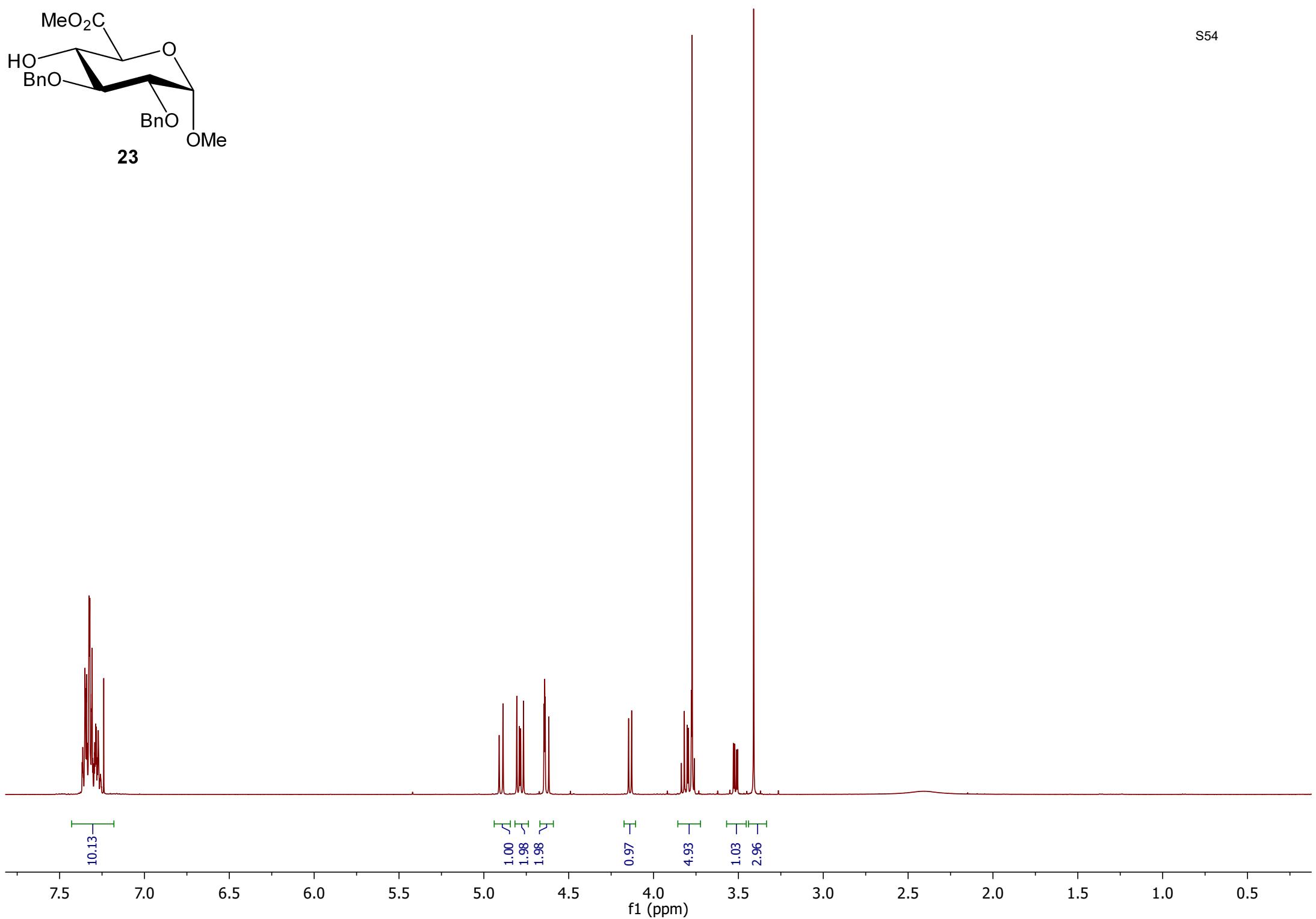


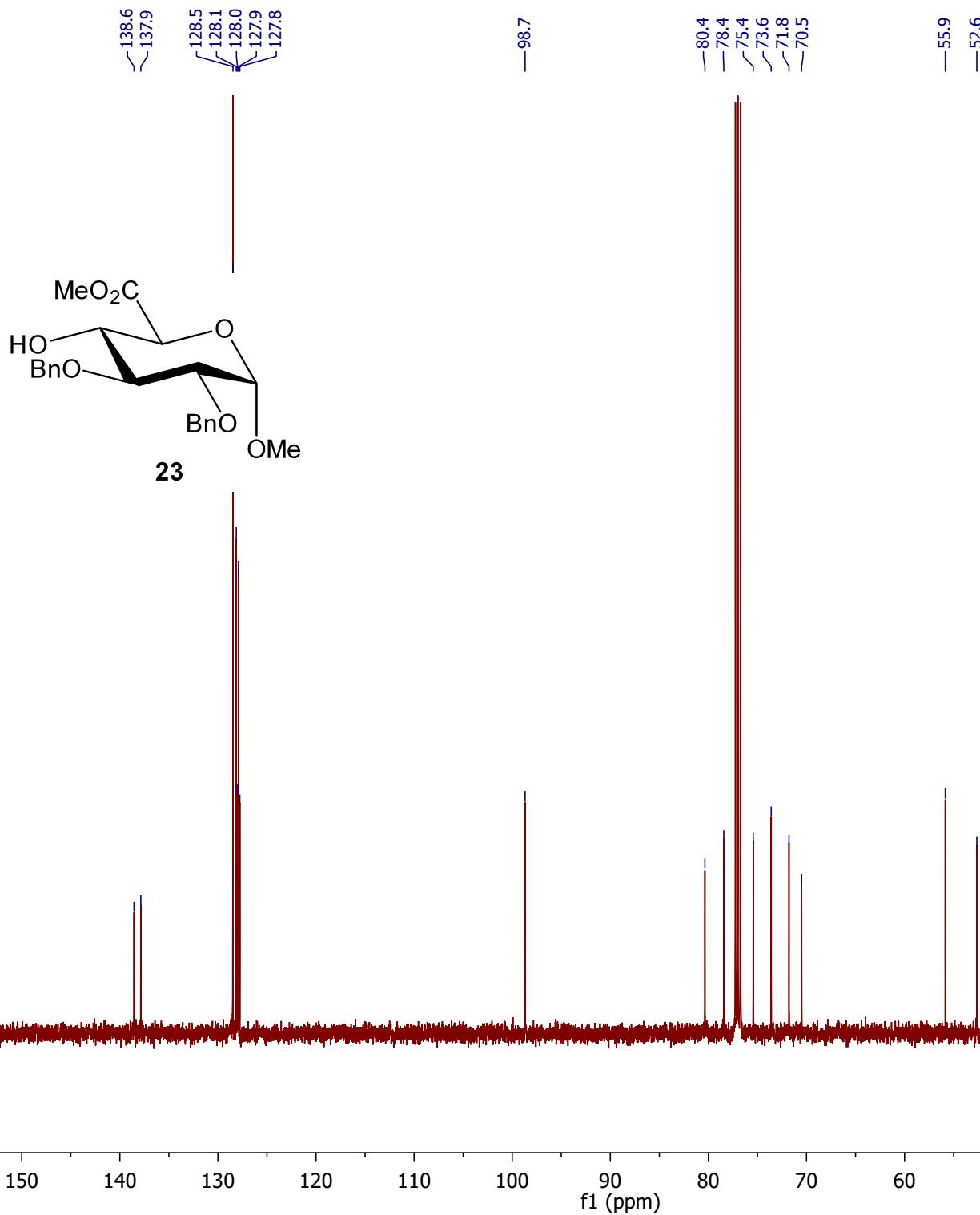


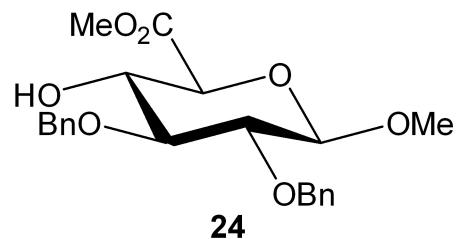




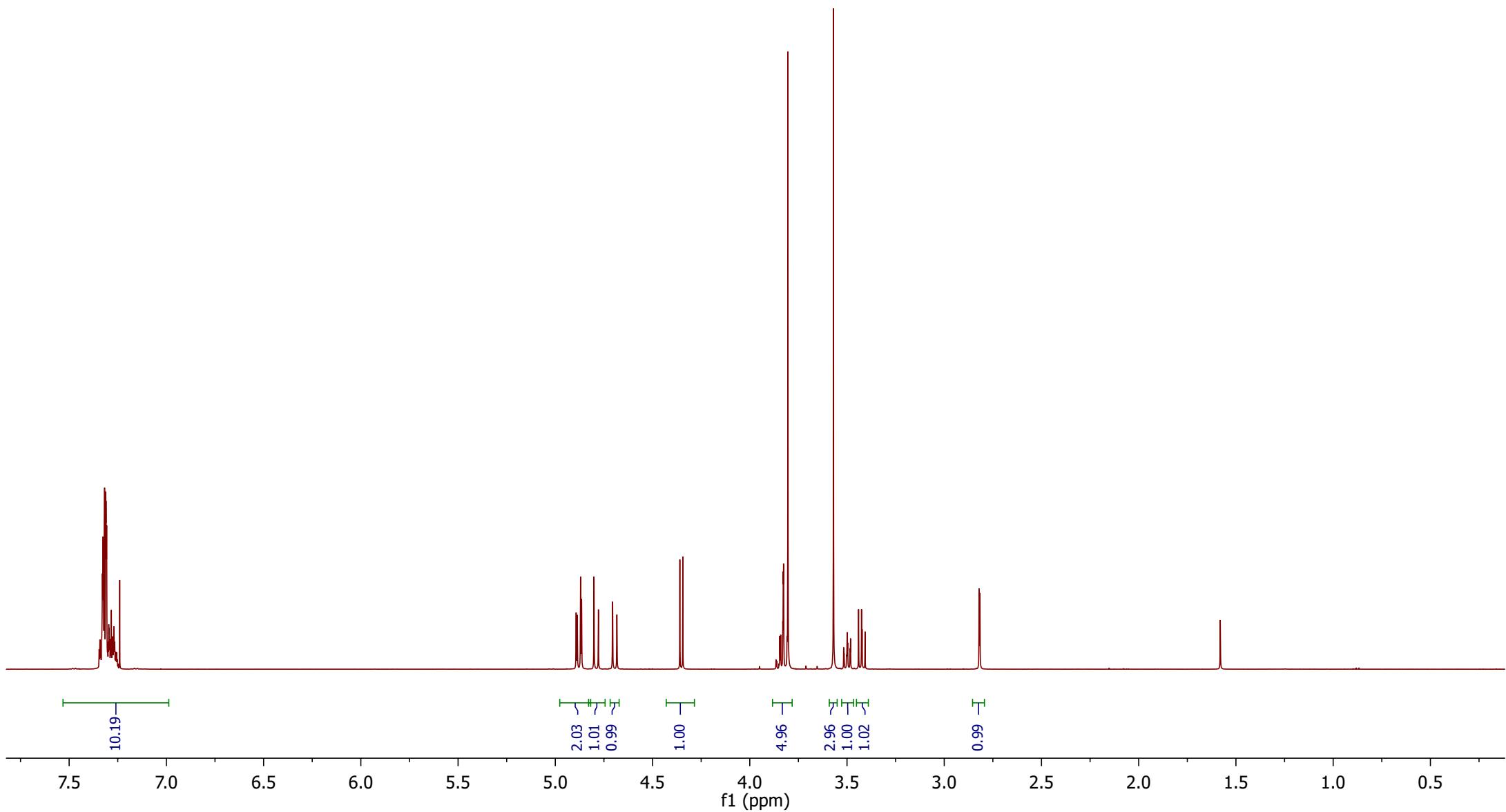
S54

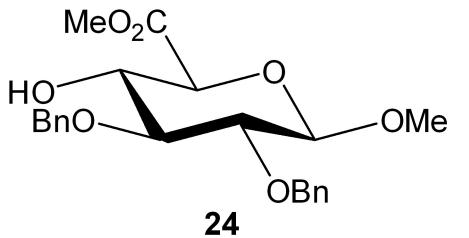






S56





—138.2

128.4
128.3
128.0
127.9
127.8
127.7

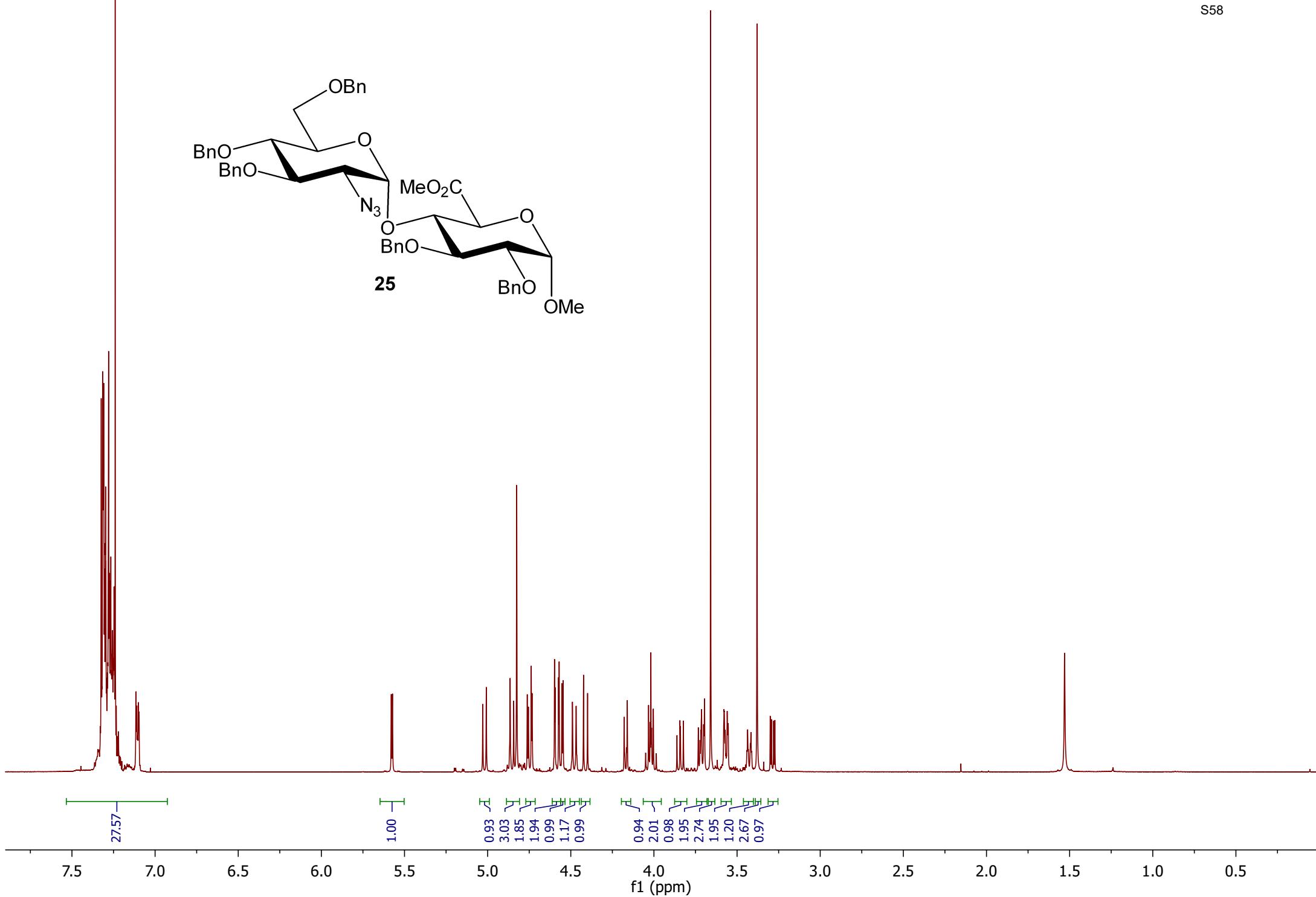
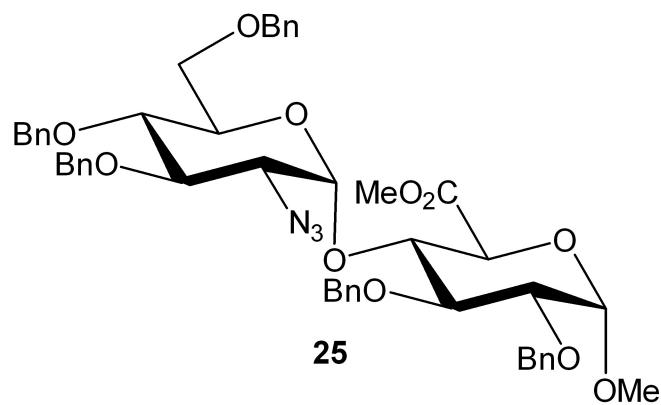
—105.0

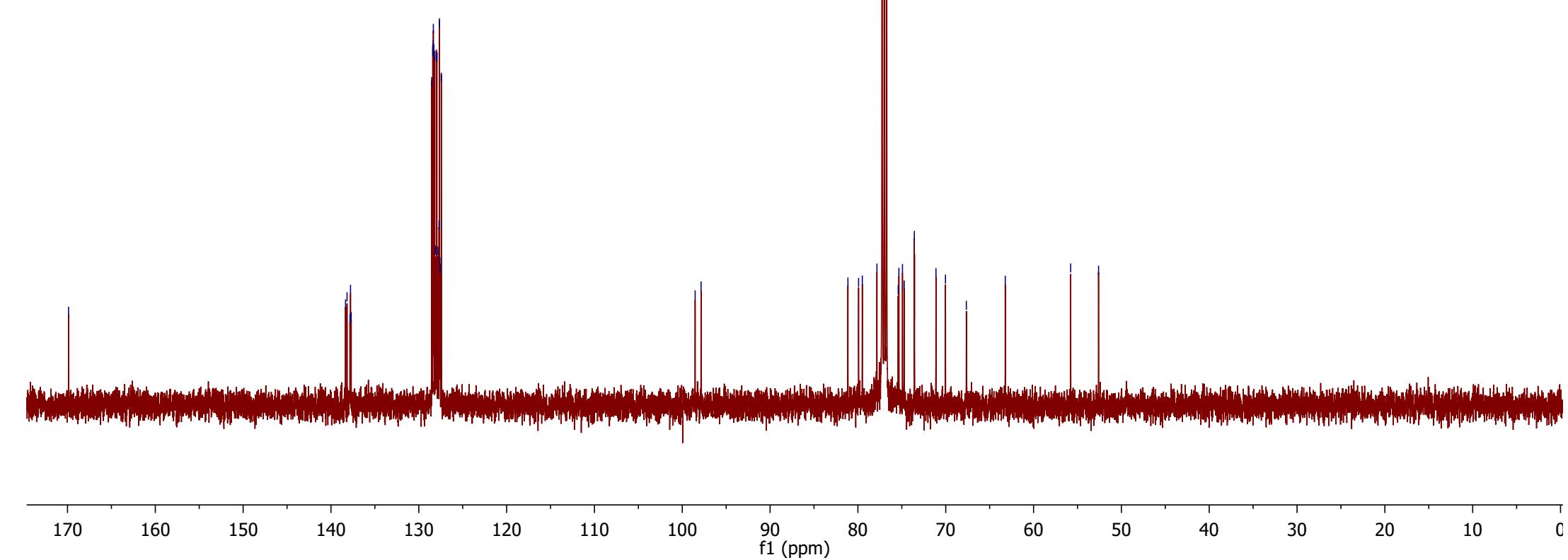
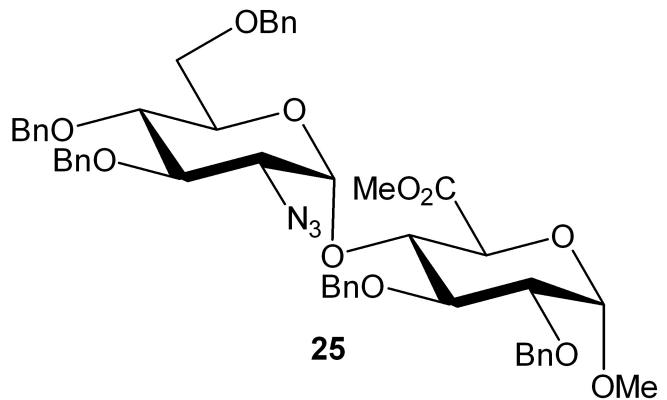
—83.1
—81.1

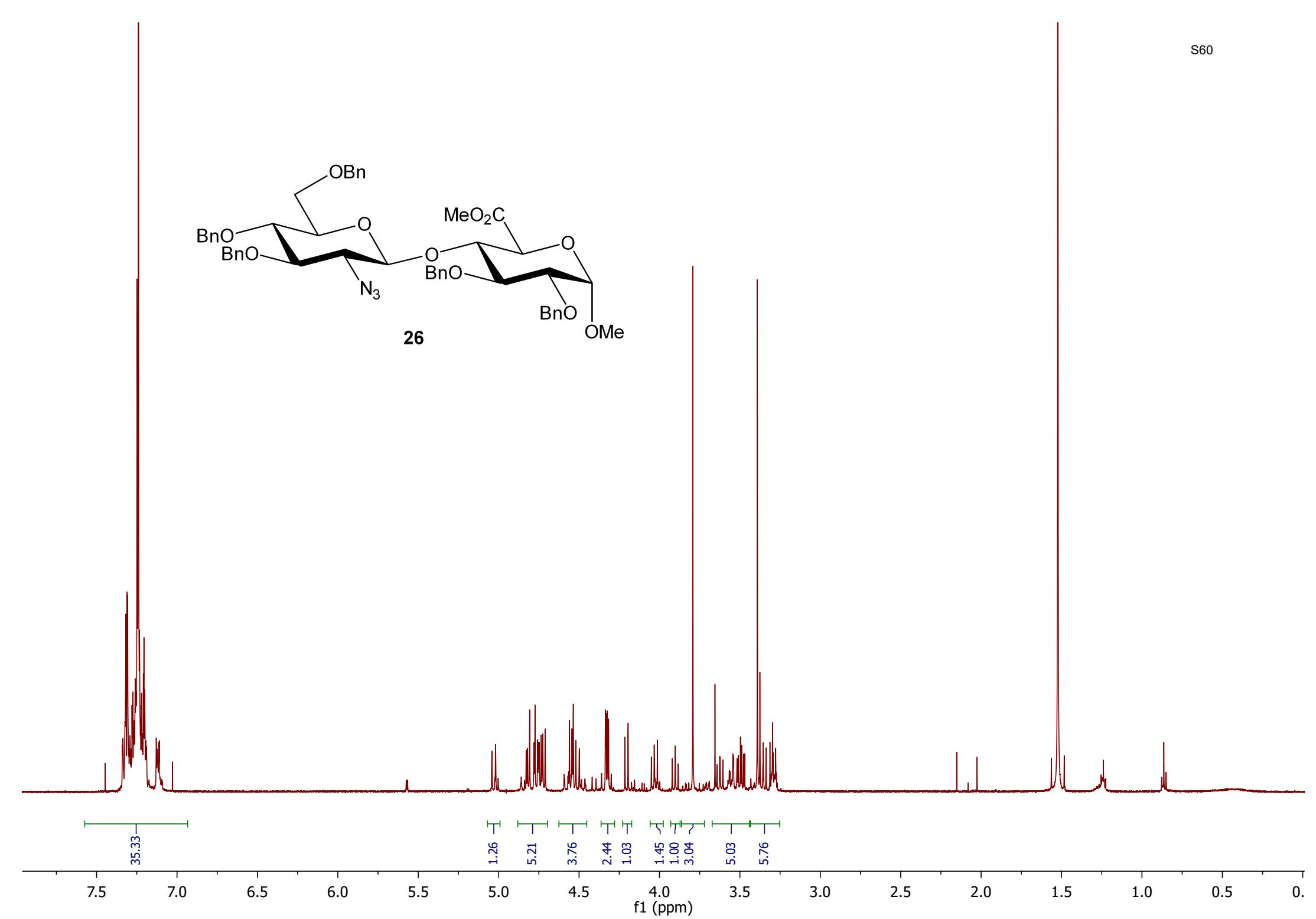
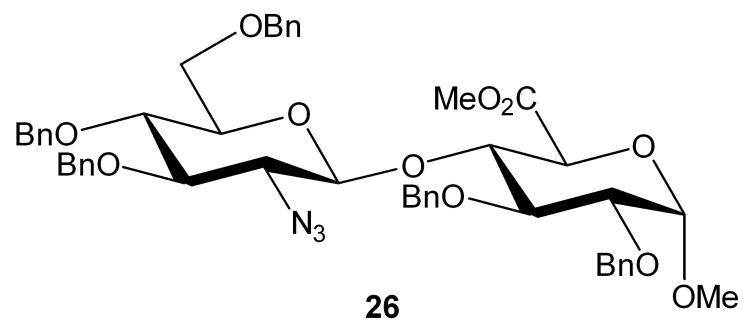
75.4
74.8
74.2
71.7

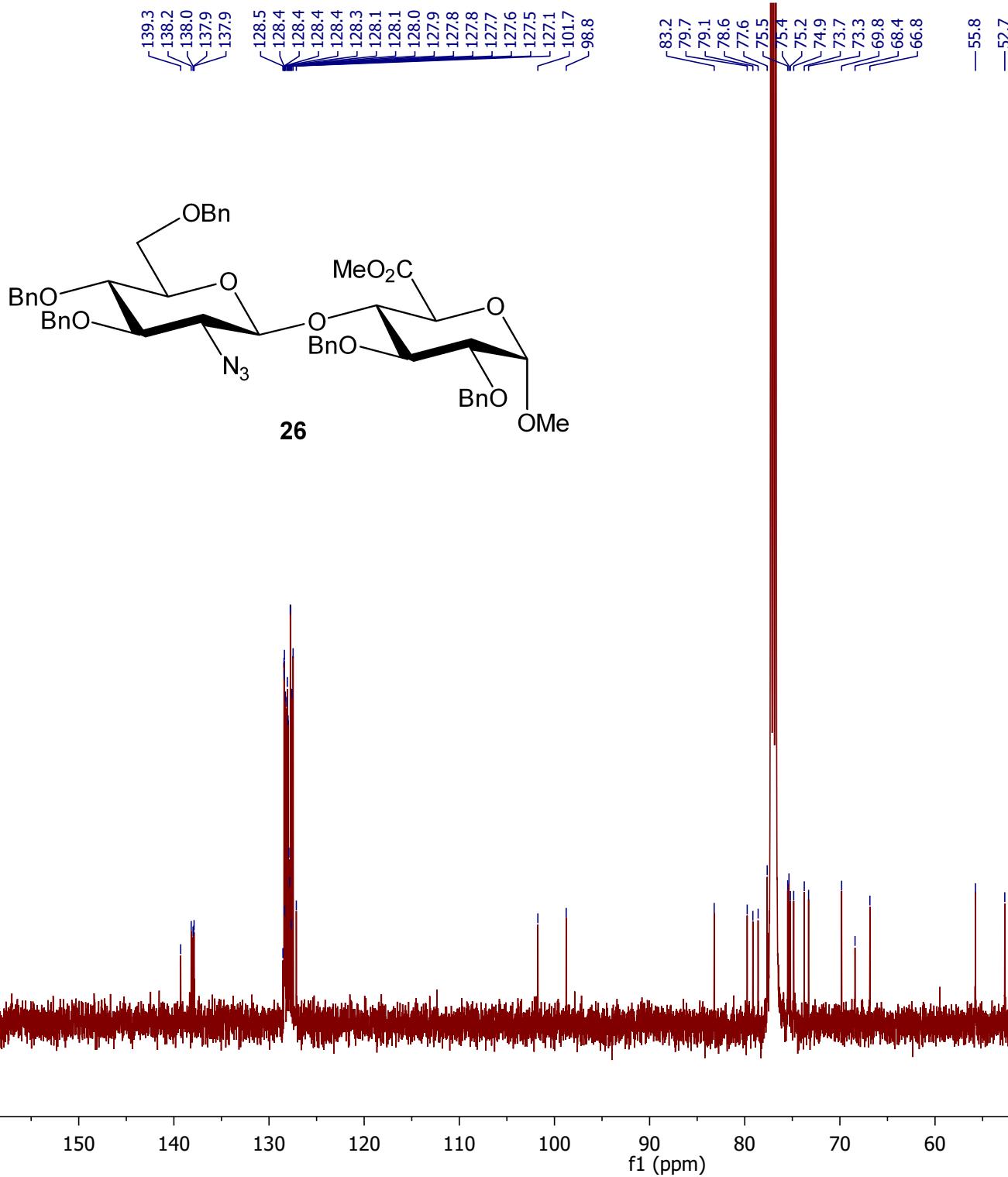
—57.4

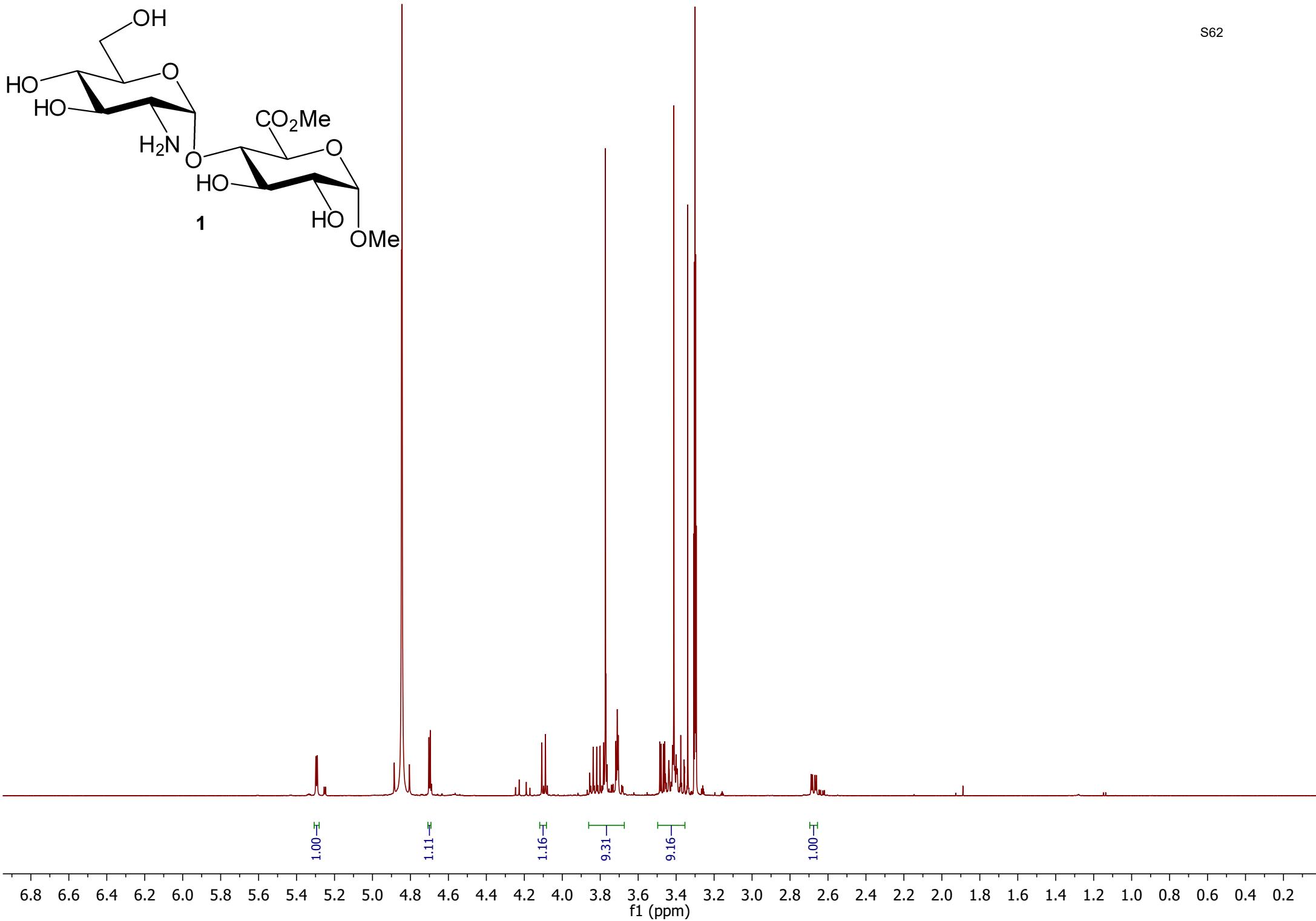
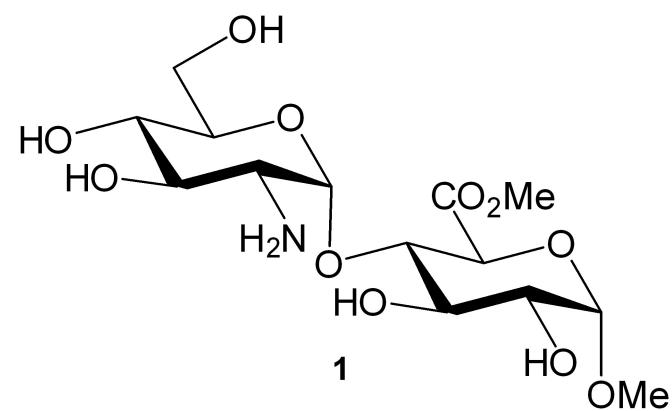
—52.7











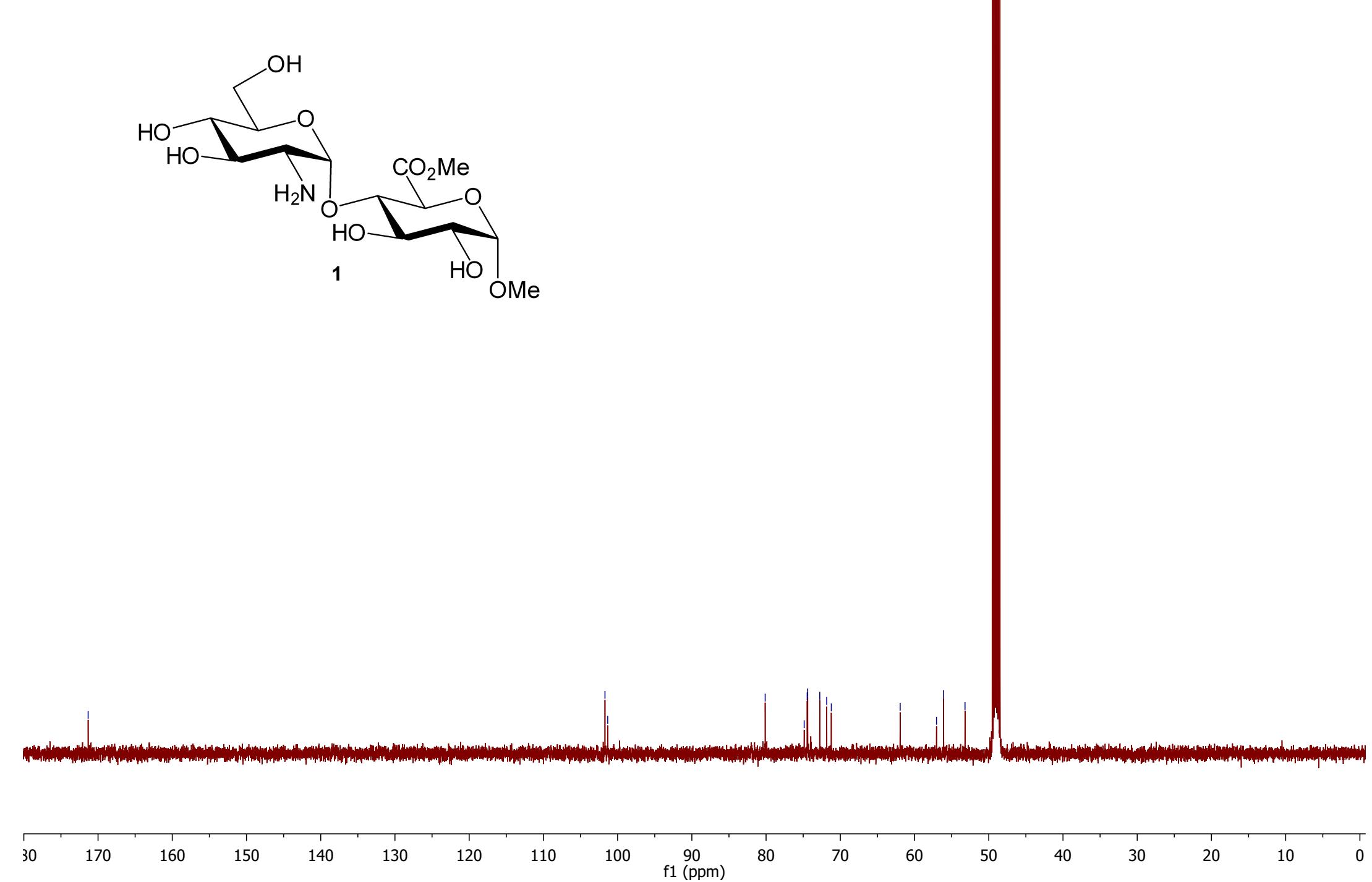
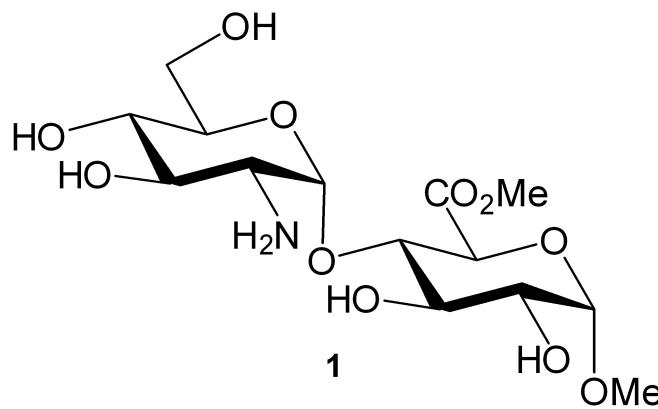
—171.3

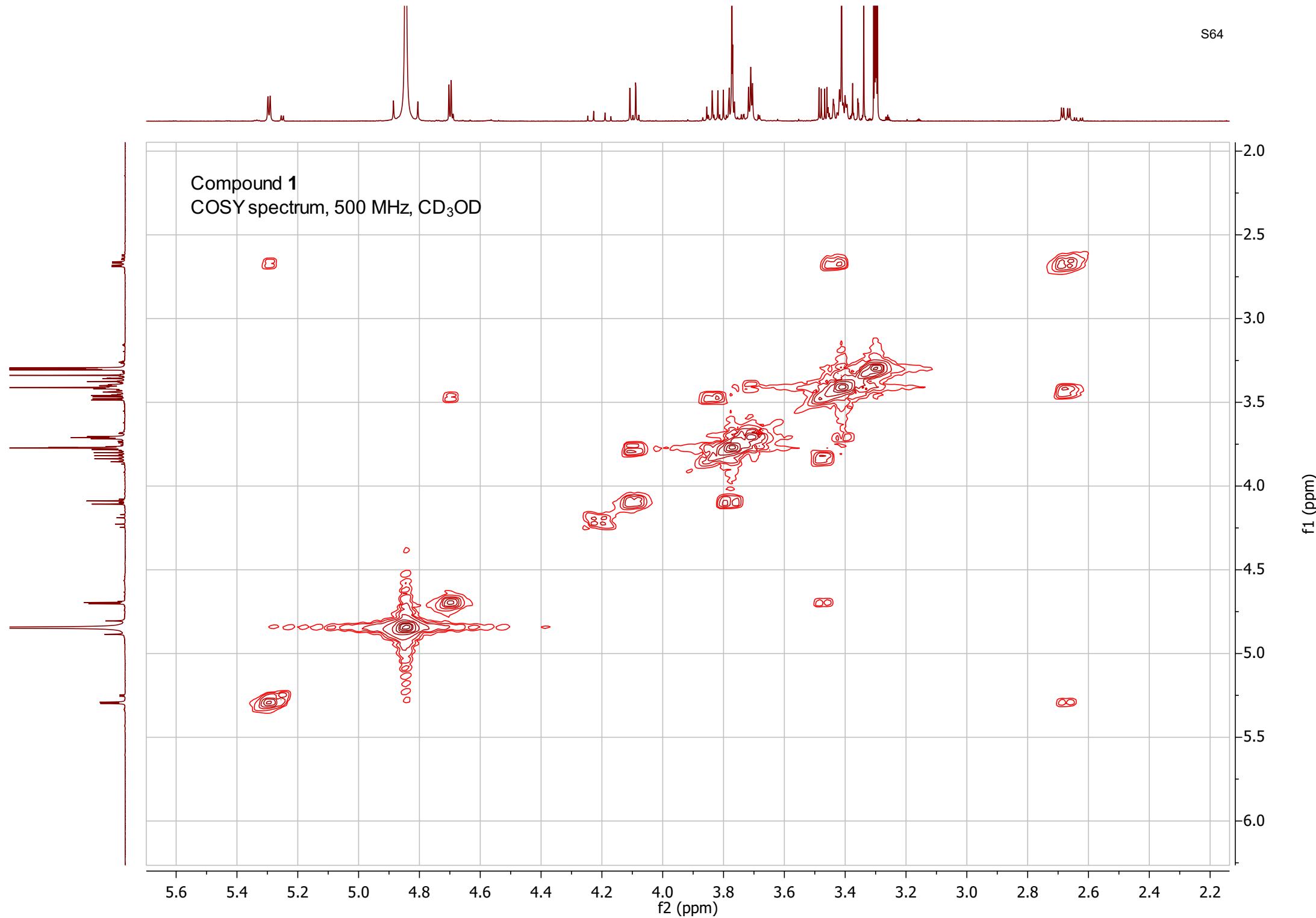
<101.7
<101.3

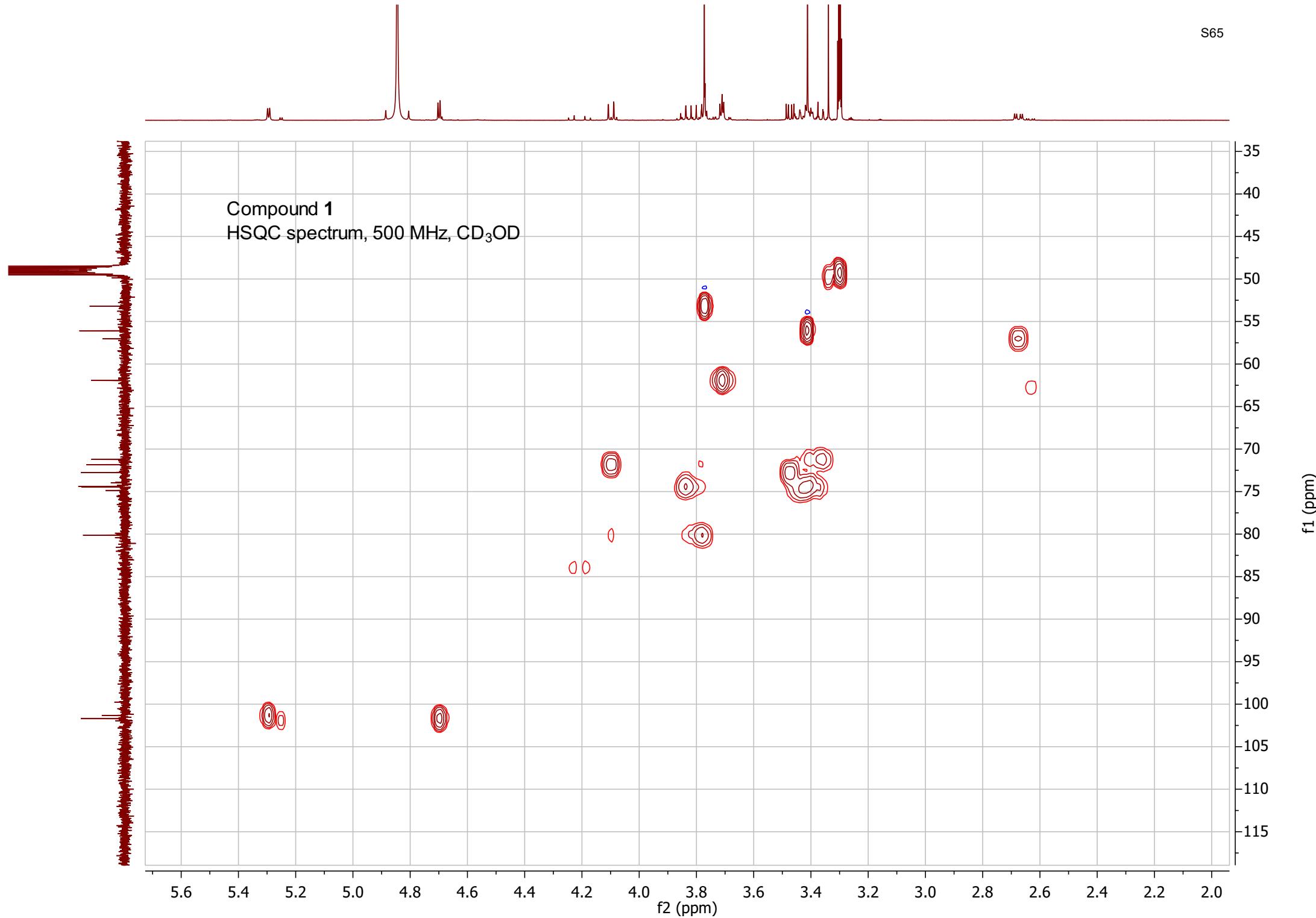
—80.1
—74.9
—74.5
—74.4
—72.8
—71.8
—71.2

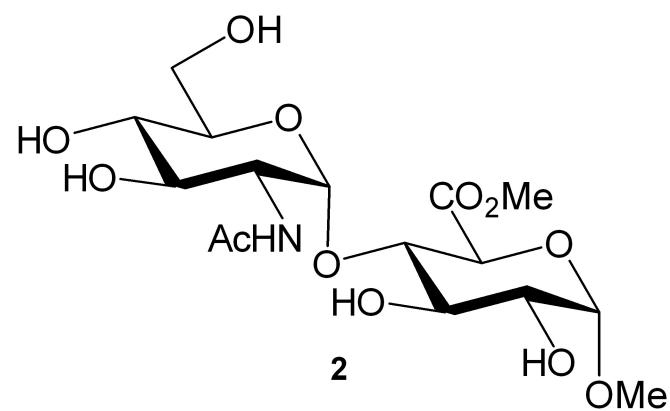
—61.9
—57.0
—56.1
—53.2

S63

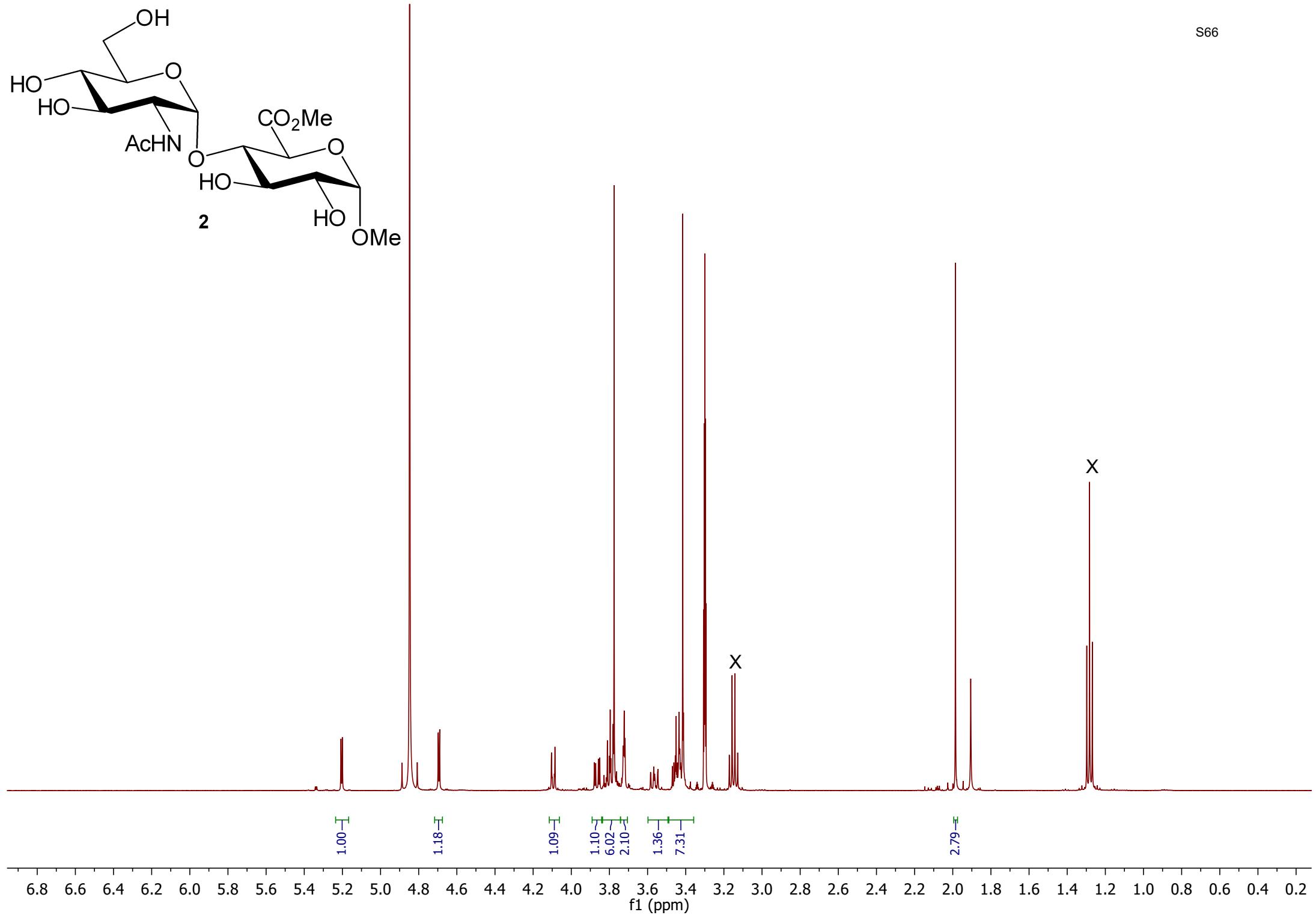








2



—173.9

—171.2

—101.7

—100.2

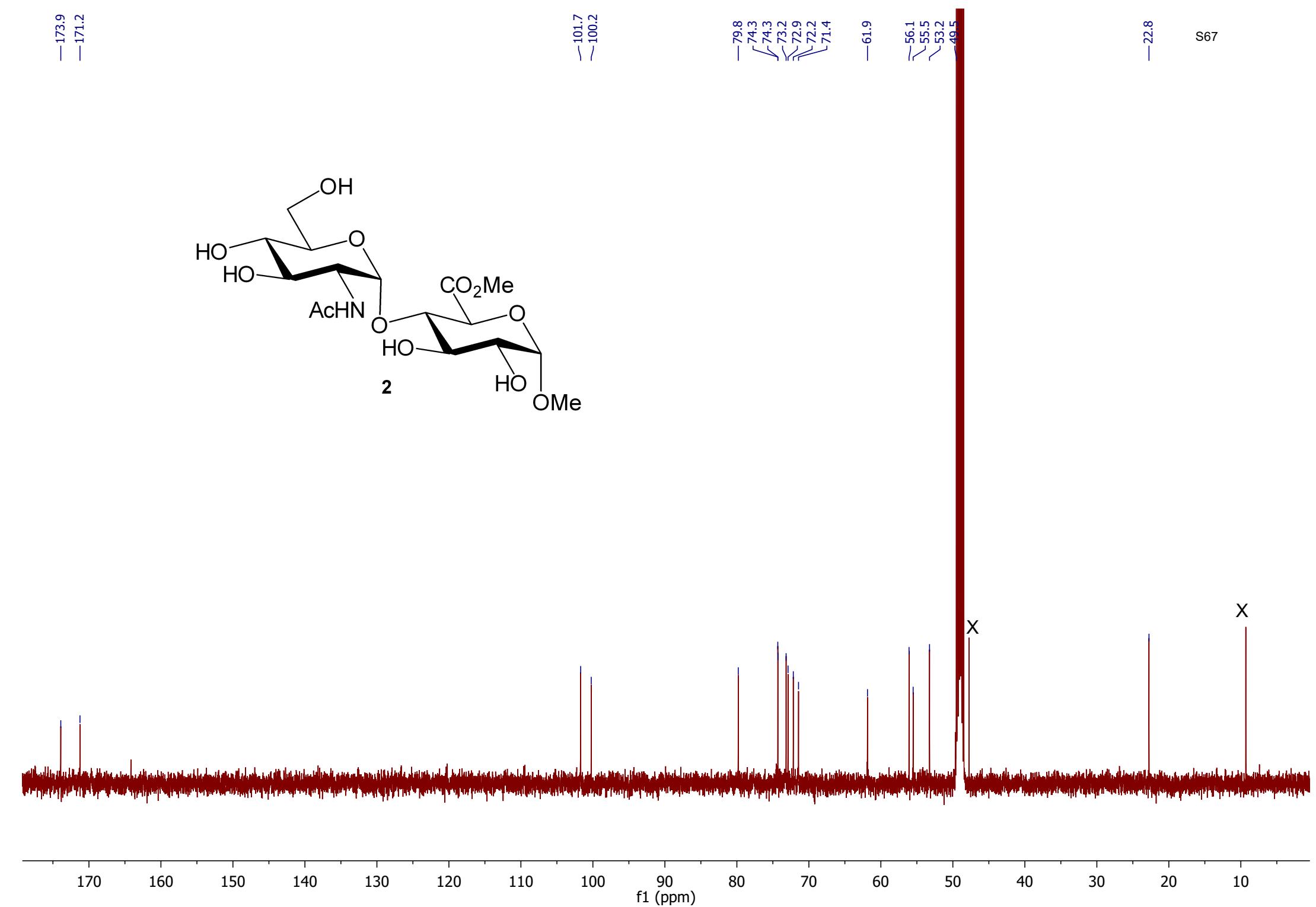
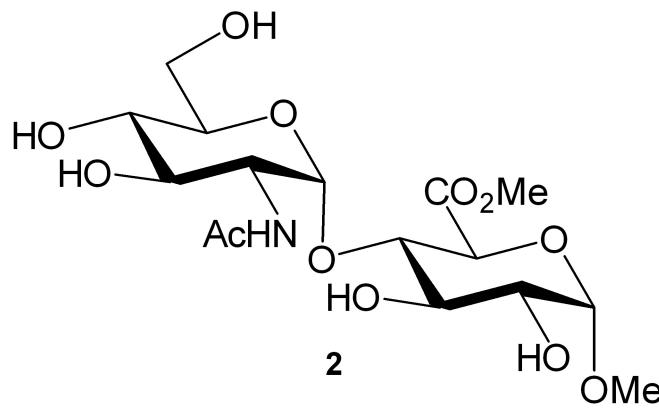
—79.8
74.3
74.3
73.2
72.9
72.2
71.4

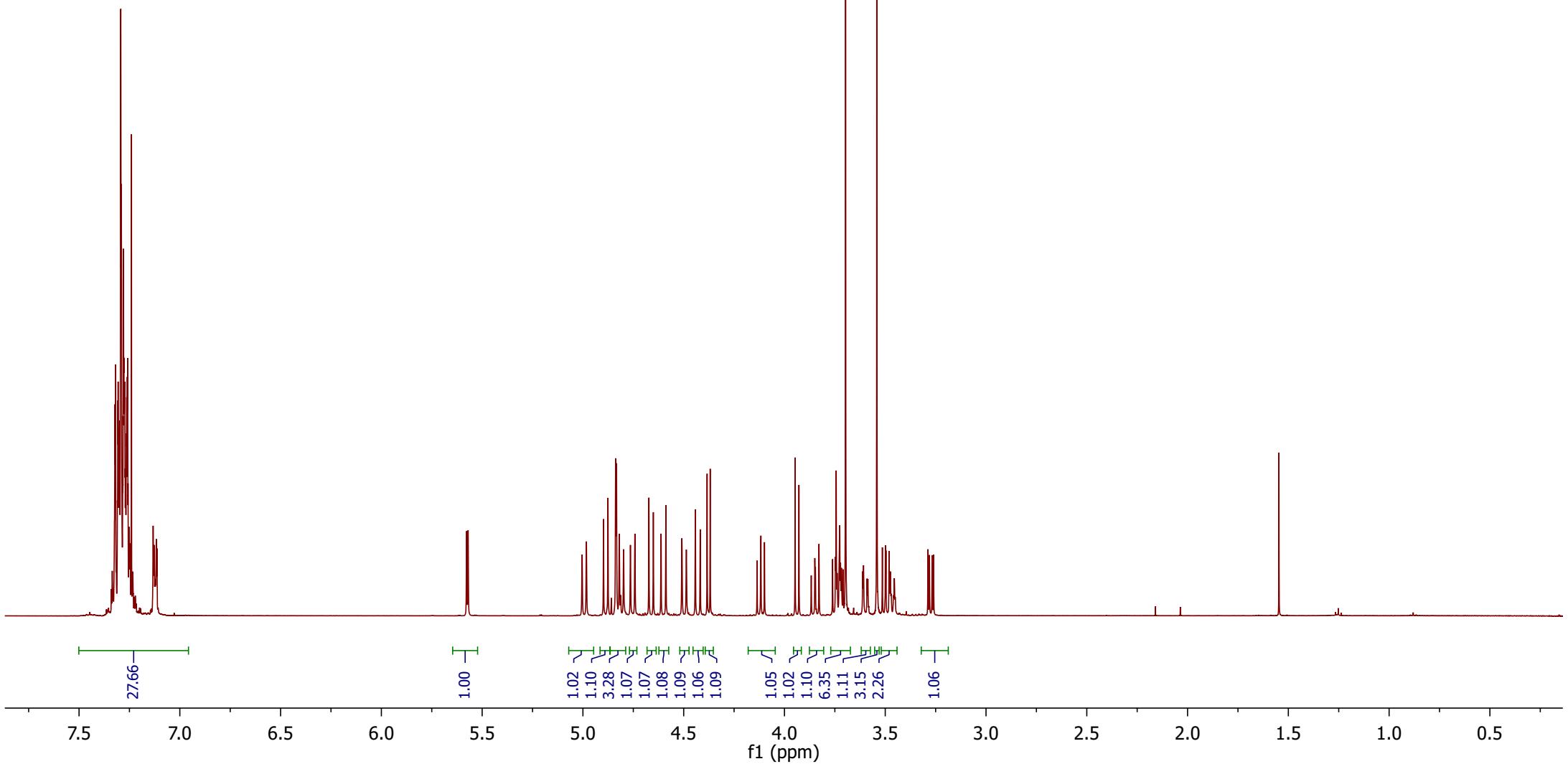
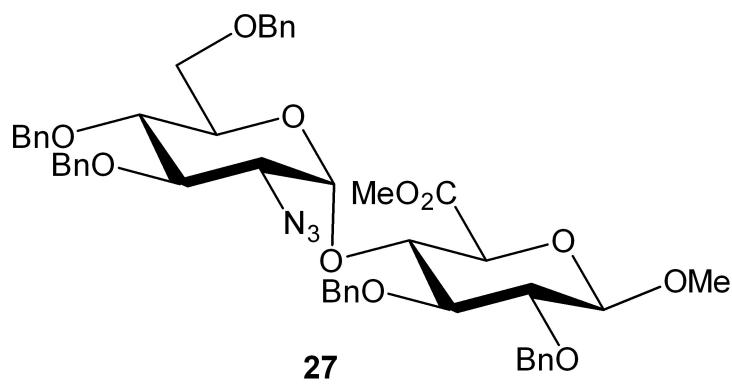
—61.9

—56.1
55.5
53.2
49.5

—22.8

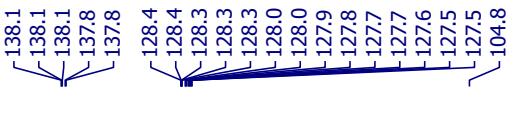
S67



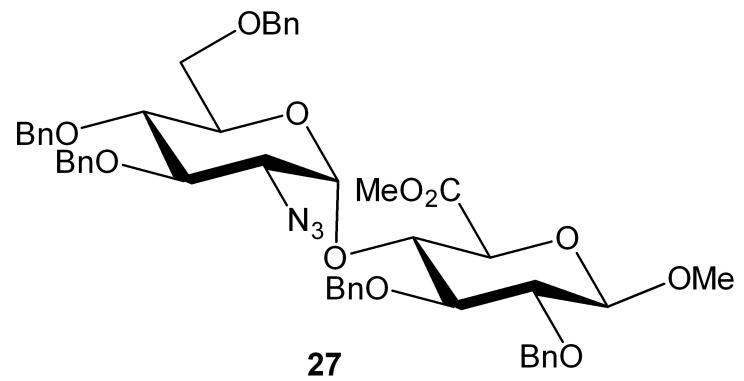
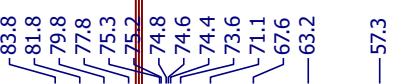


—168.9

S69

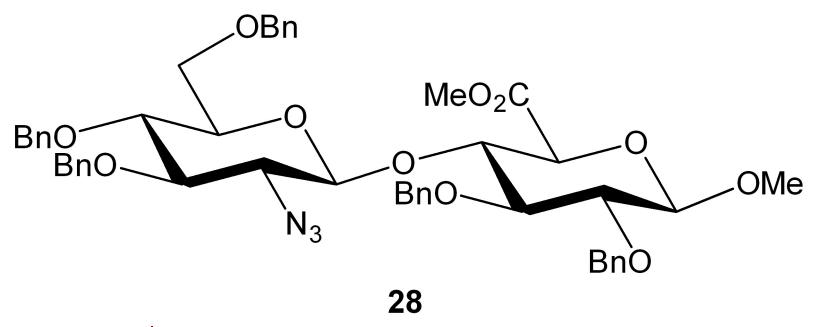


—97.8

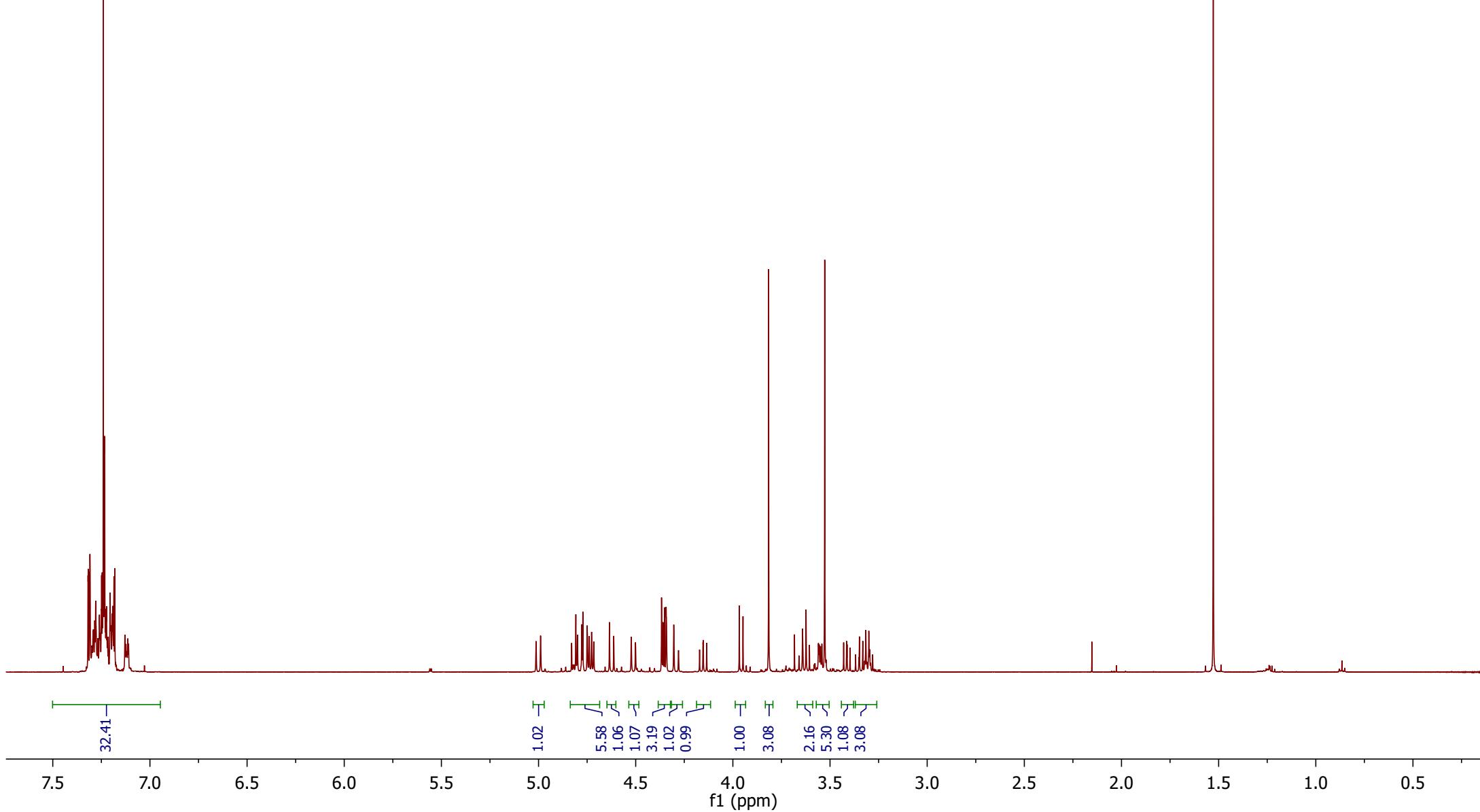


170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10

f1 (ppm)

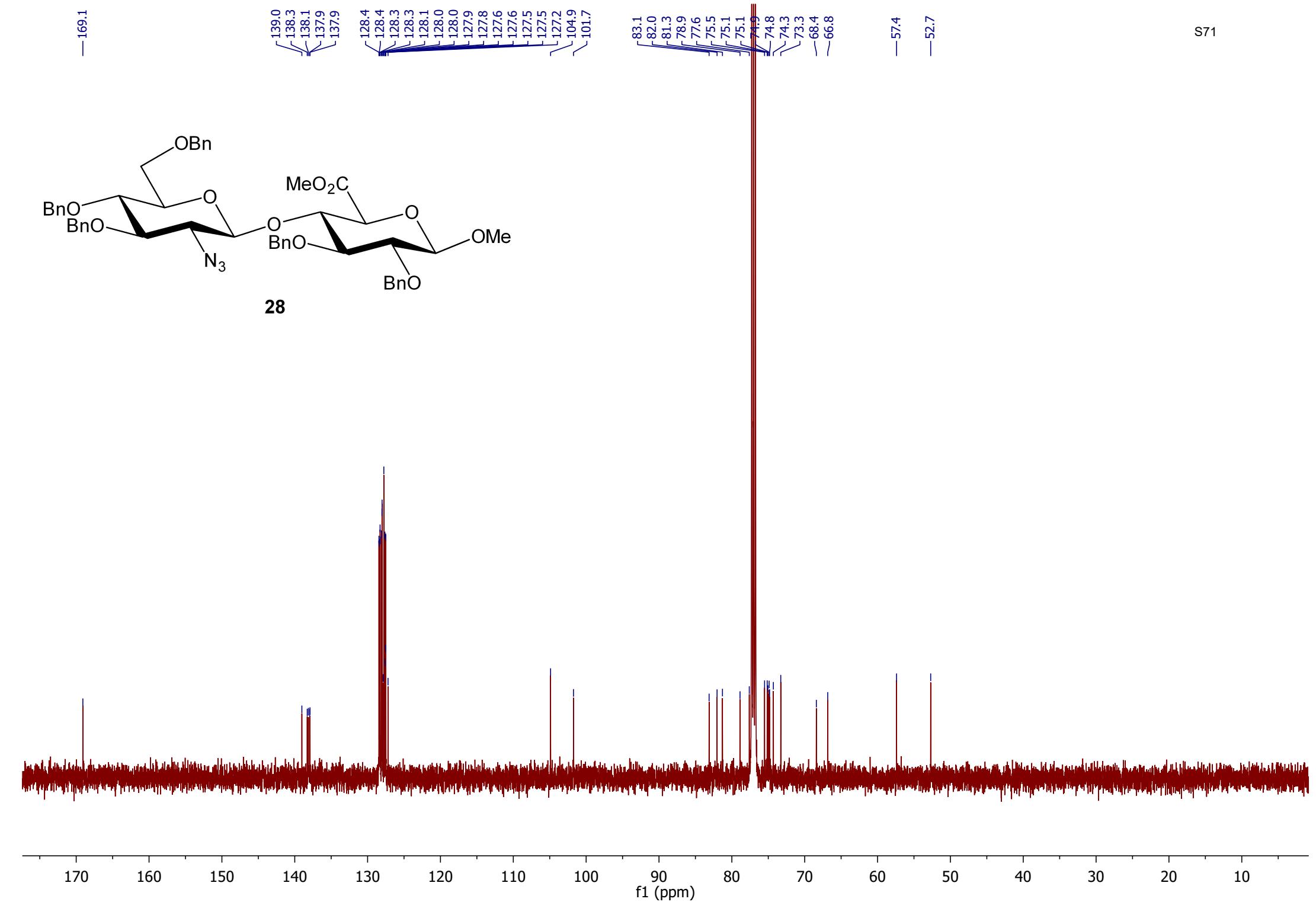
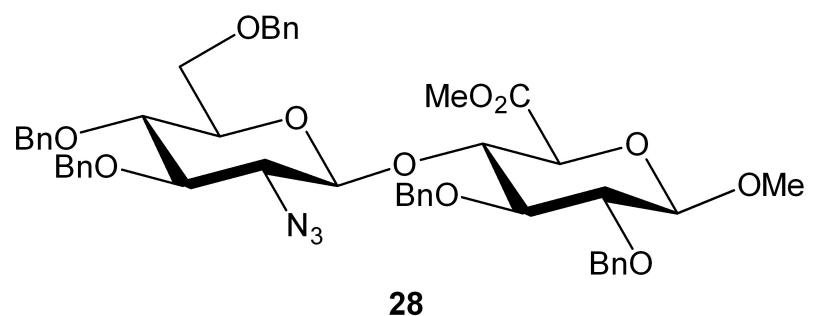


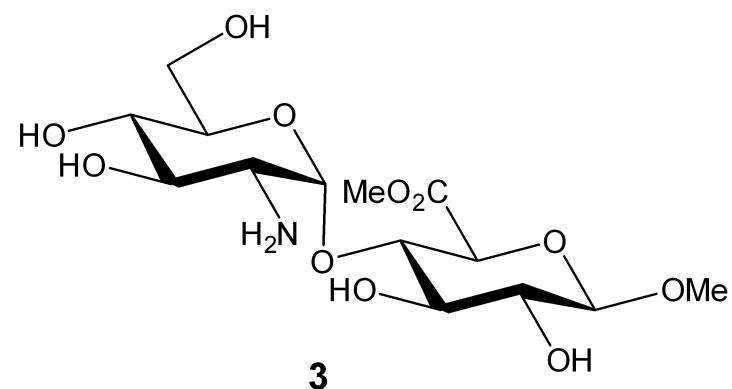
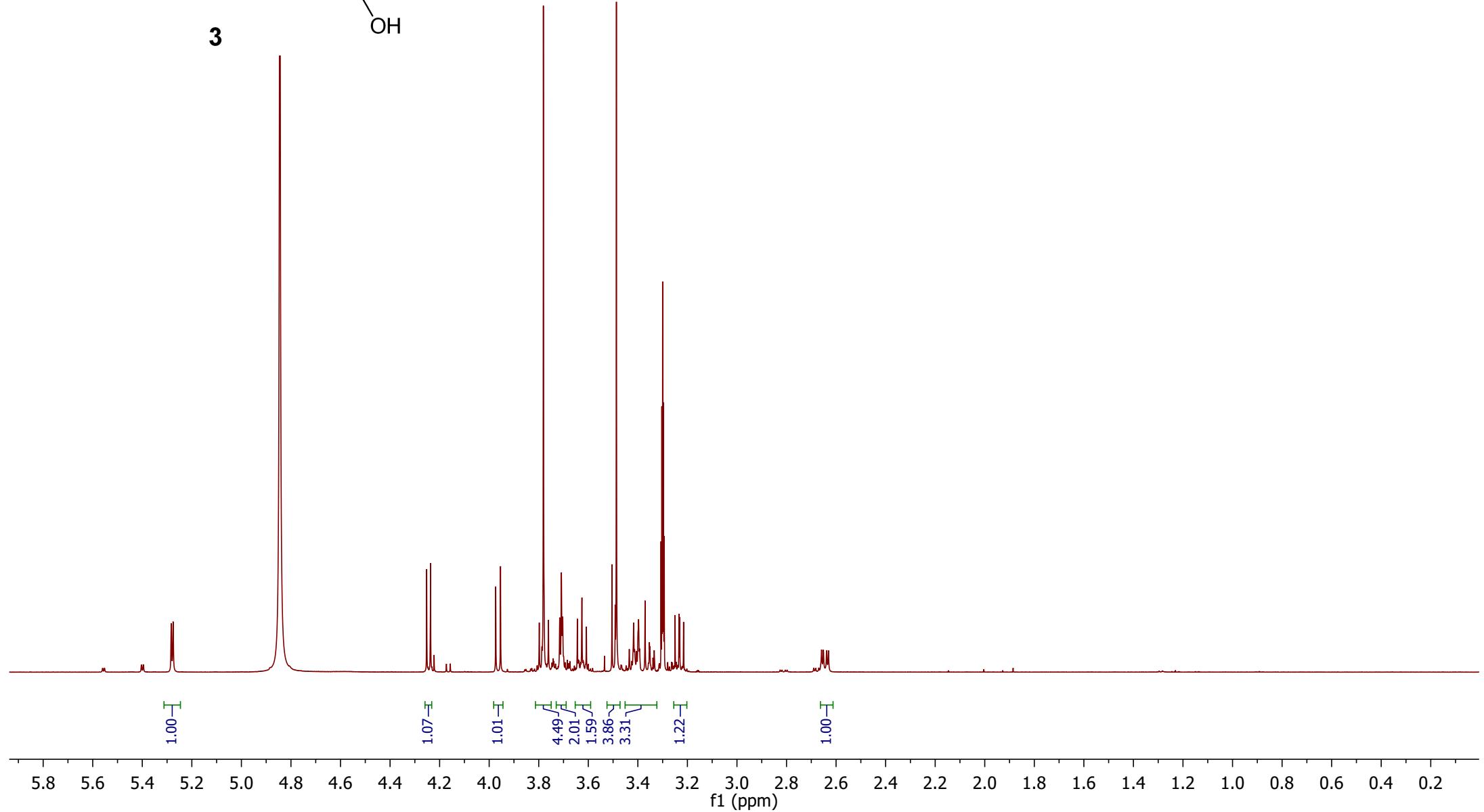
S70



—169.1

S71



**3**

—170.7

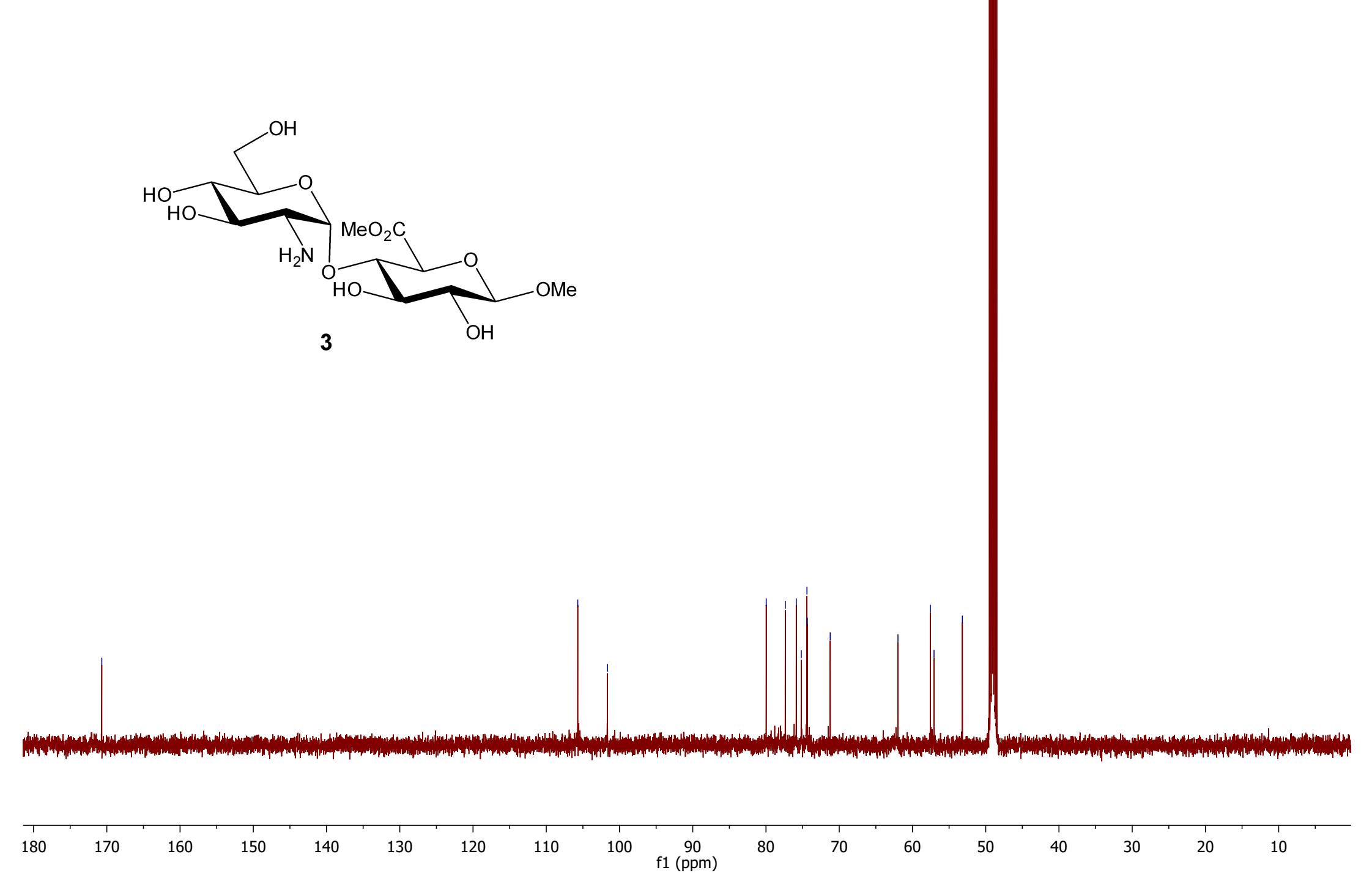
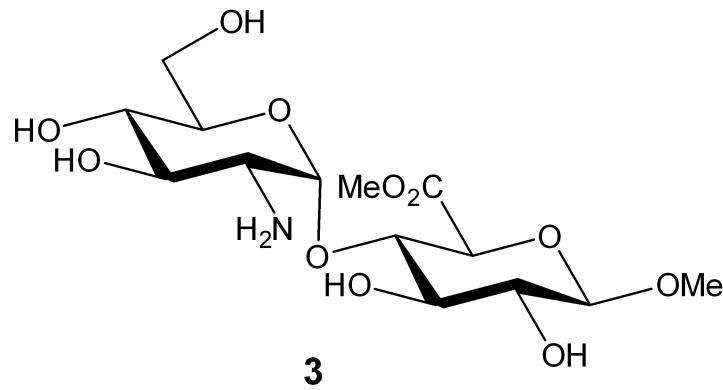
—105.7

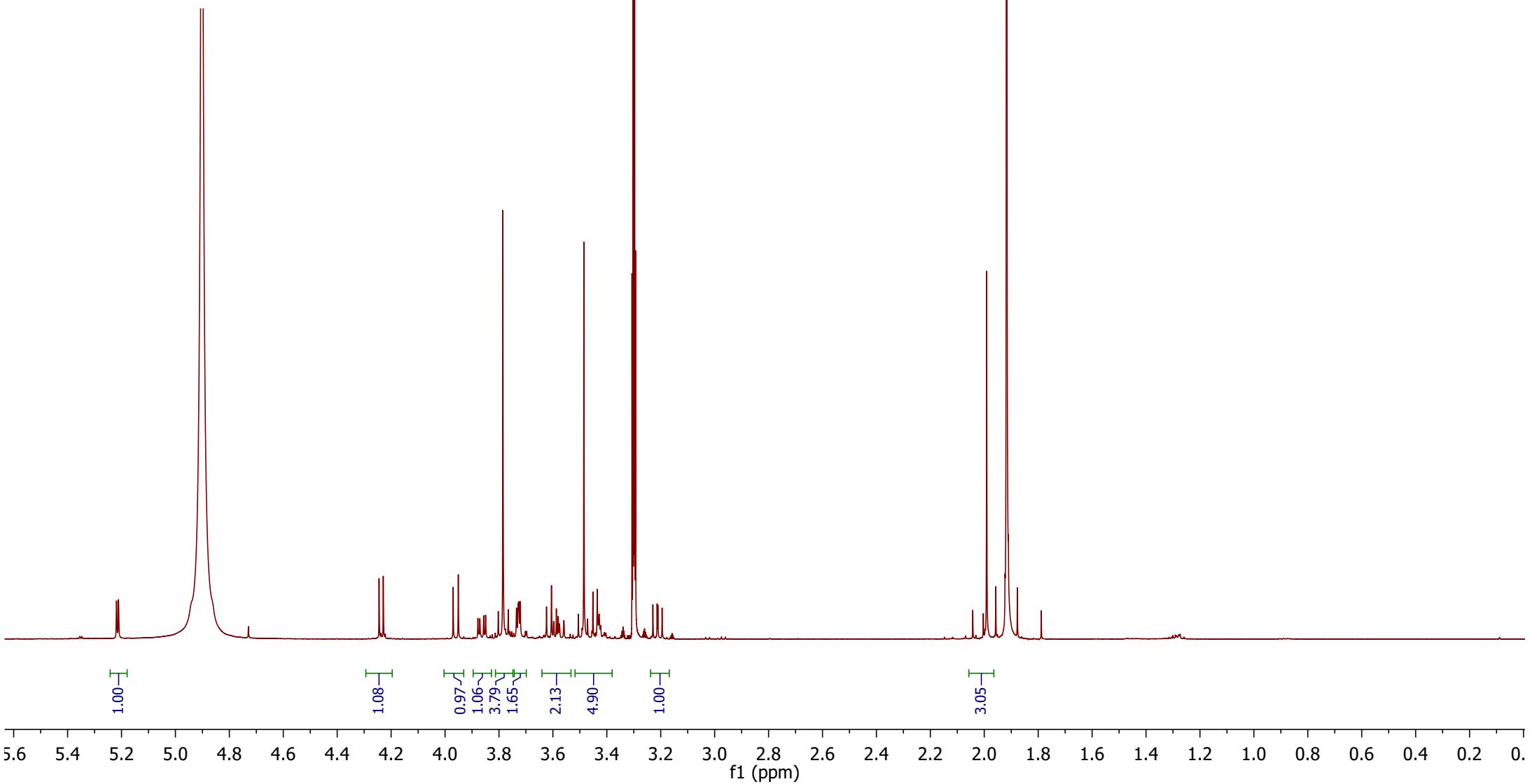
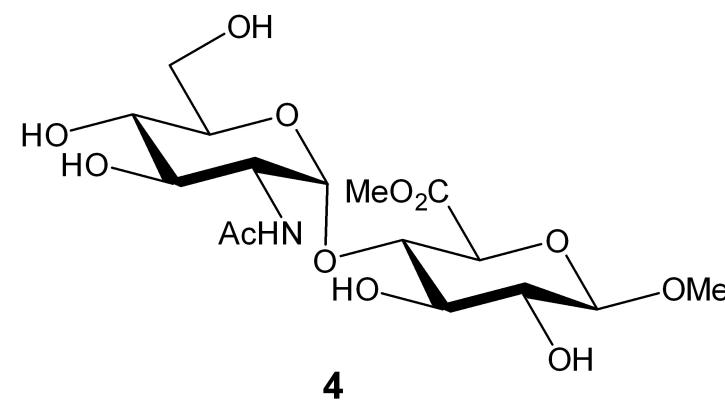
—101.6

80.0
77.4
75.9
75.2
74.4
74.4
71.2

—62.0
57.6
57.1
53.2

S73





—173.9
—170.6

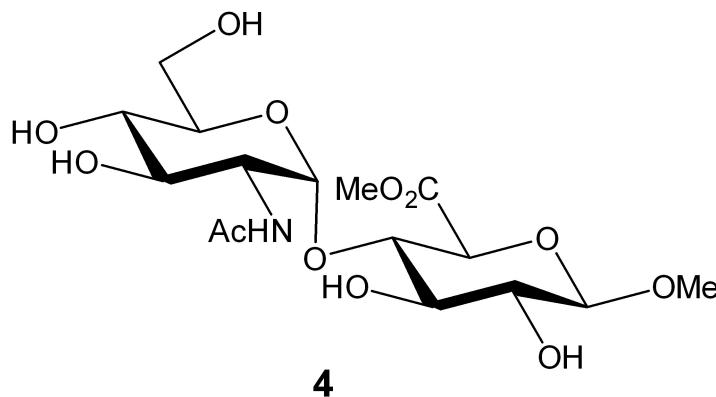
—105.7
—100.2

✓79.6
✓77.2
✓76.2
✓74.9
✓74.3
✓72.8
✓71.4

—61.8
—57.6
—55.5
—53.3

✓23.0
✓22.8

S75

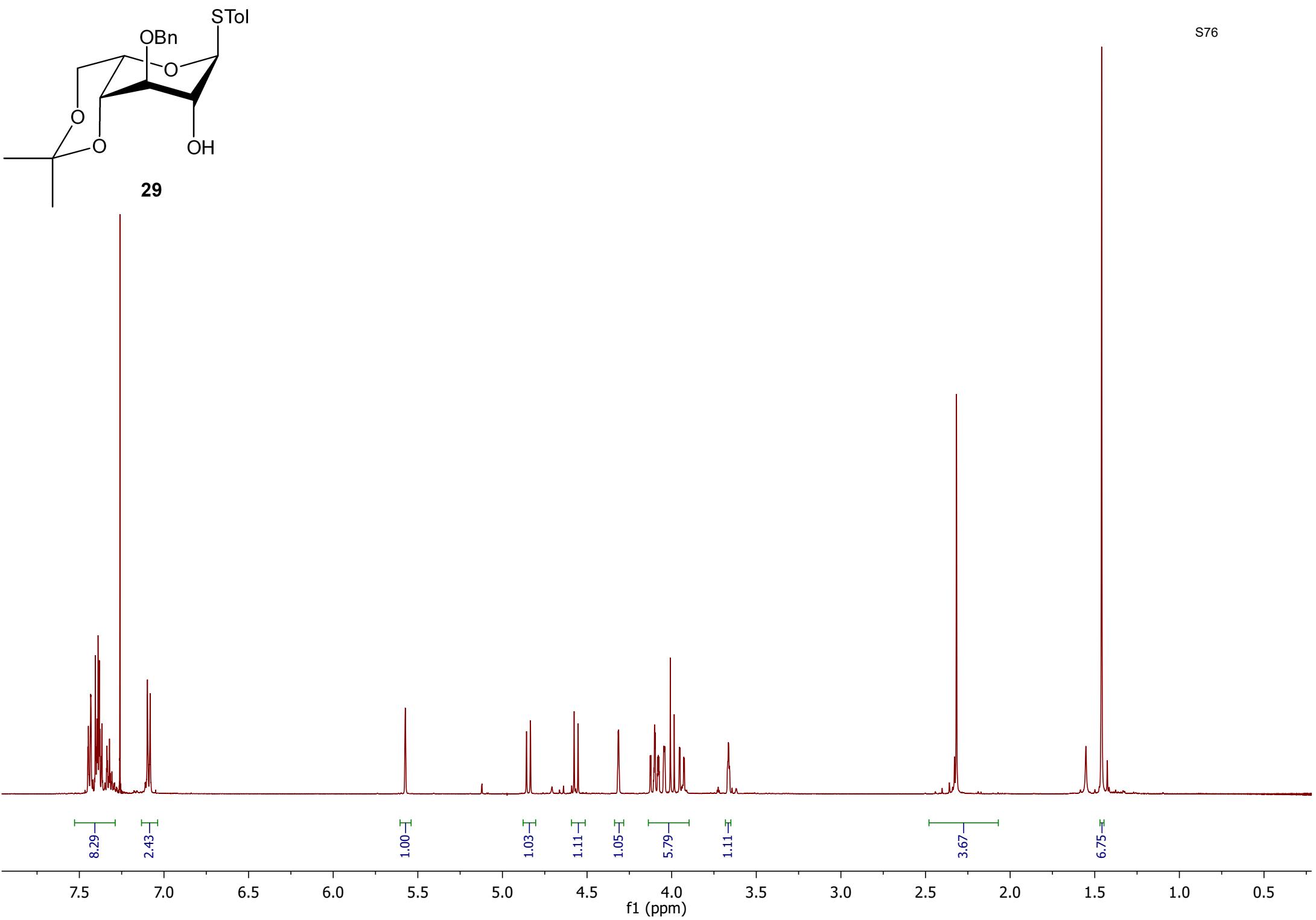


X

X

180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10

f1 (ppm)



✓ 137.4
✓ 136.7
✓ 133.6
✓ 130.6
✓ 129.6
✓ 128.5
✓ 128.0
✓ 127.8

—99.2

—89.3

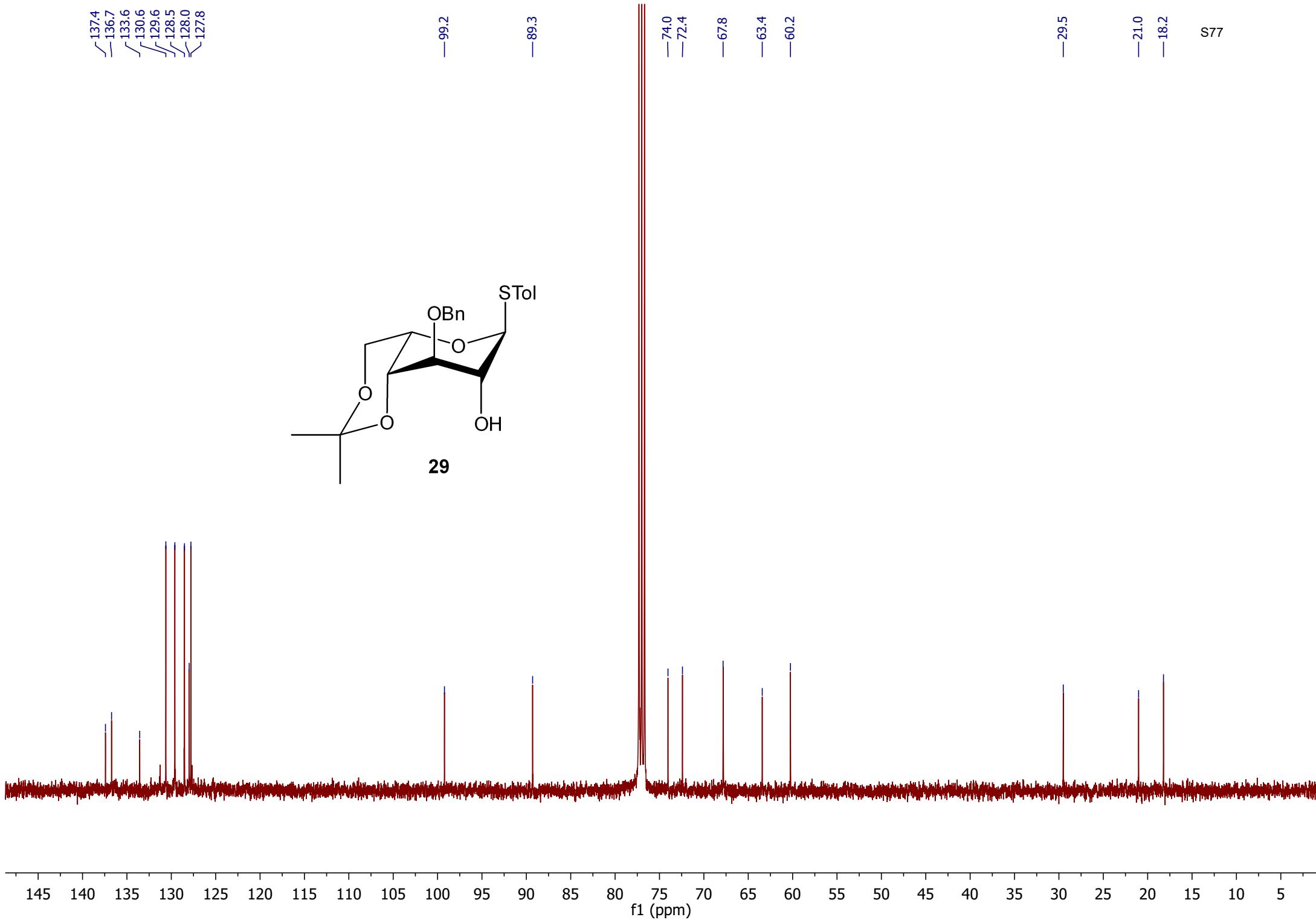
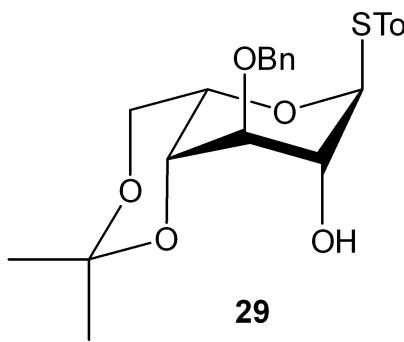
—74.0
—72.4

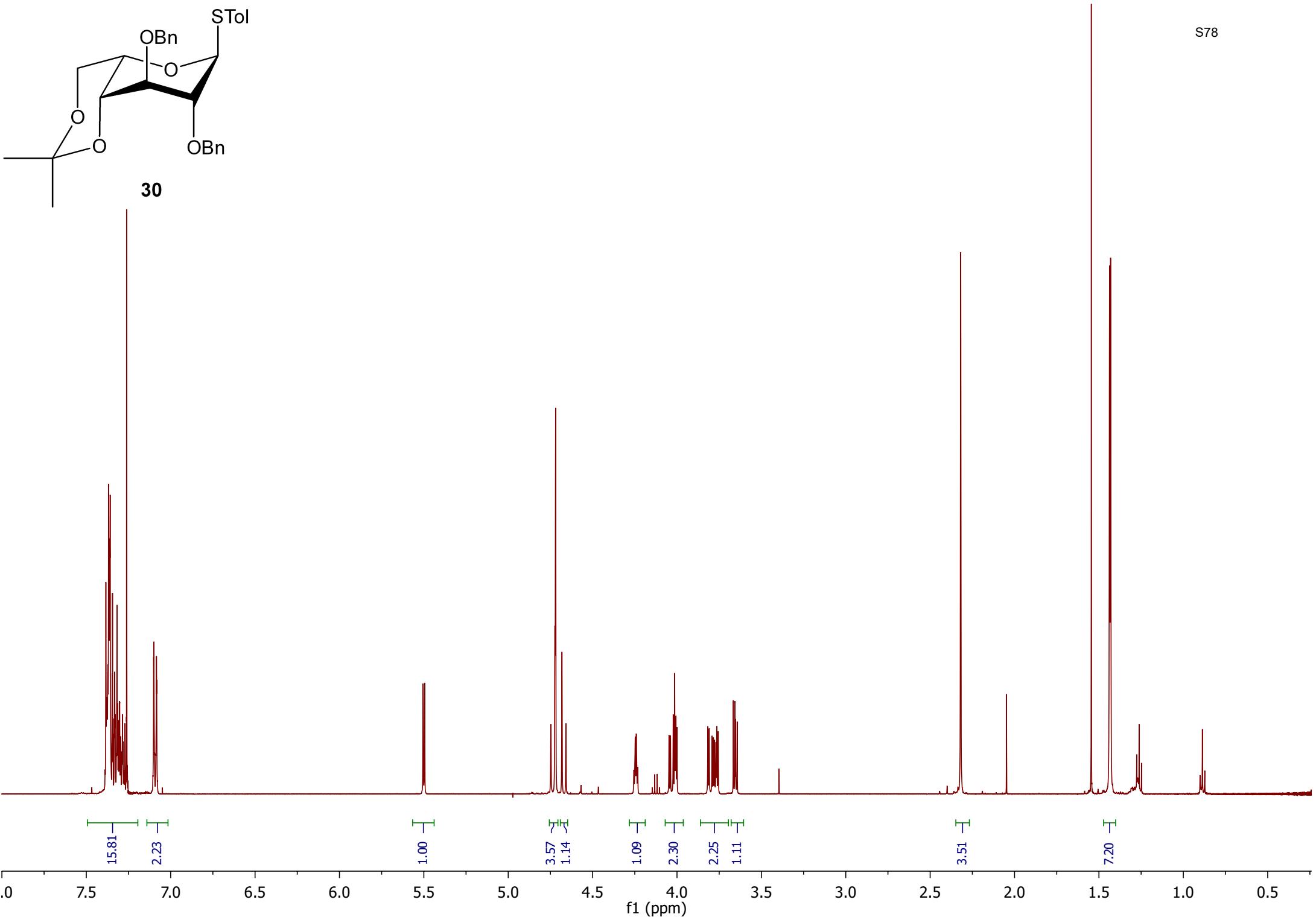
—67.8
—63.4
—60.2

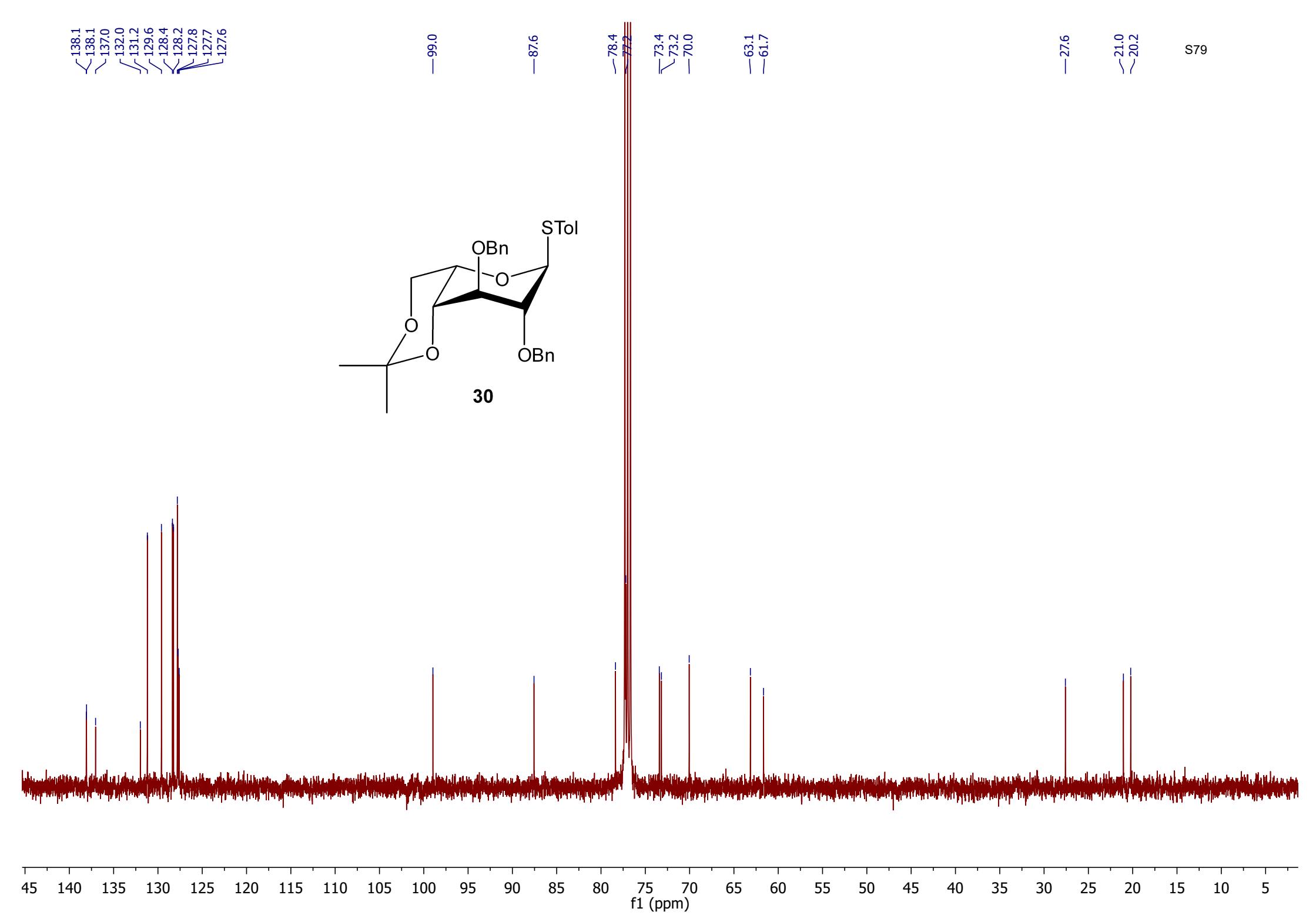
—29.5

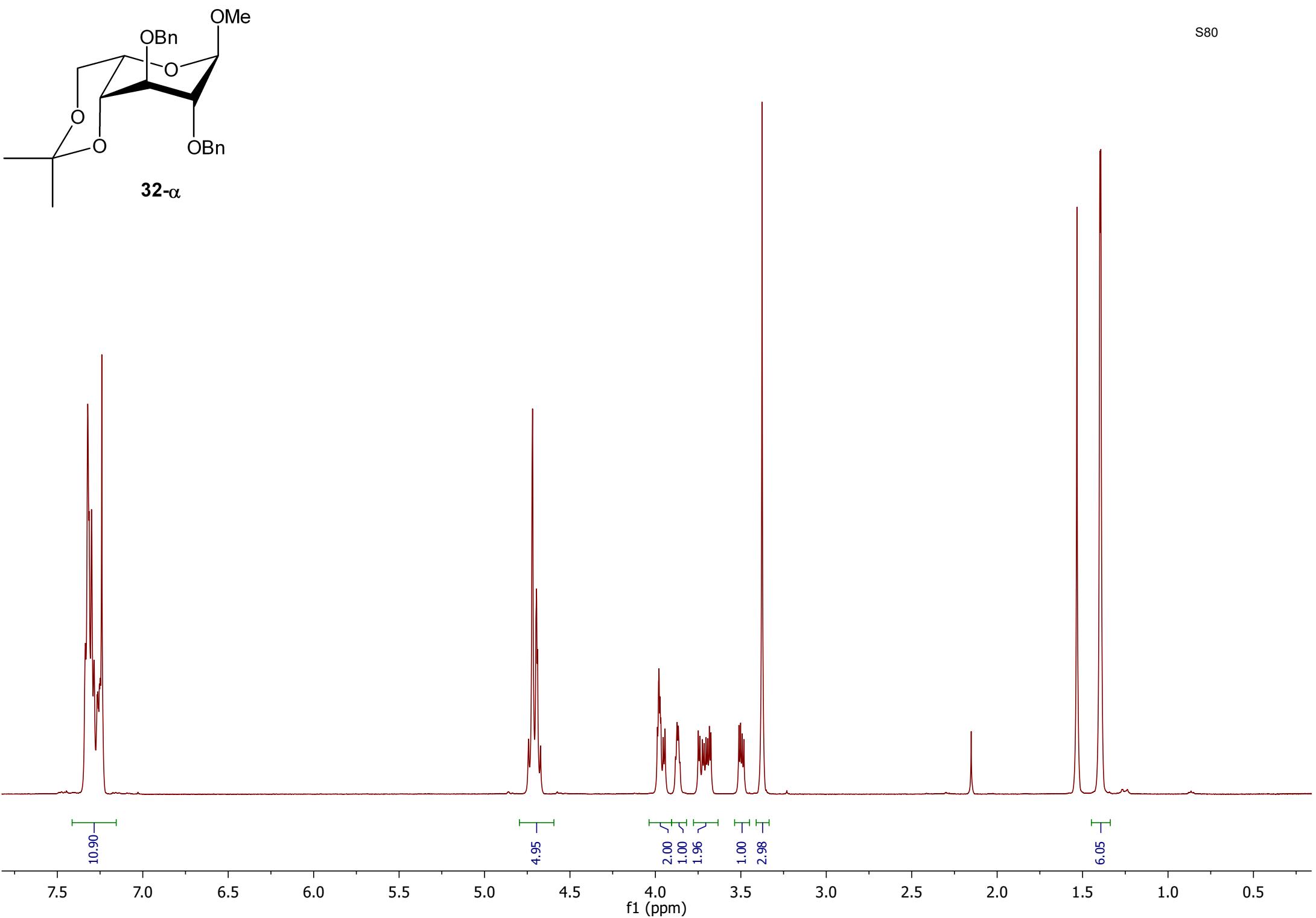
—21.0
—18.2

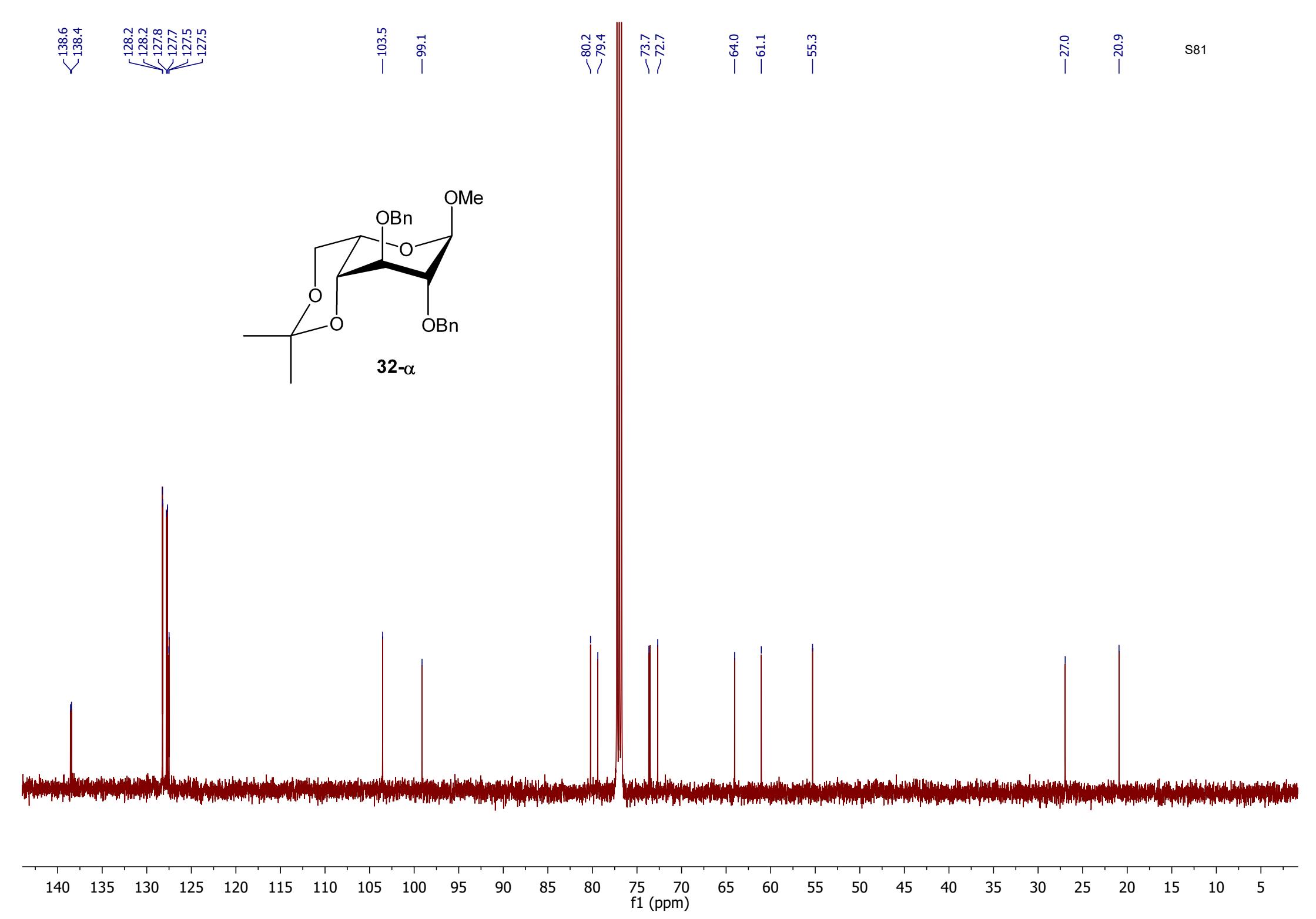
S77

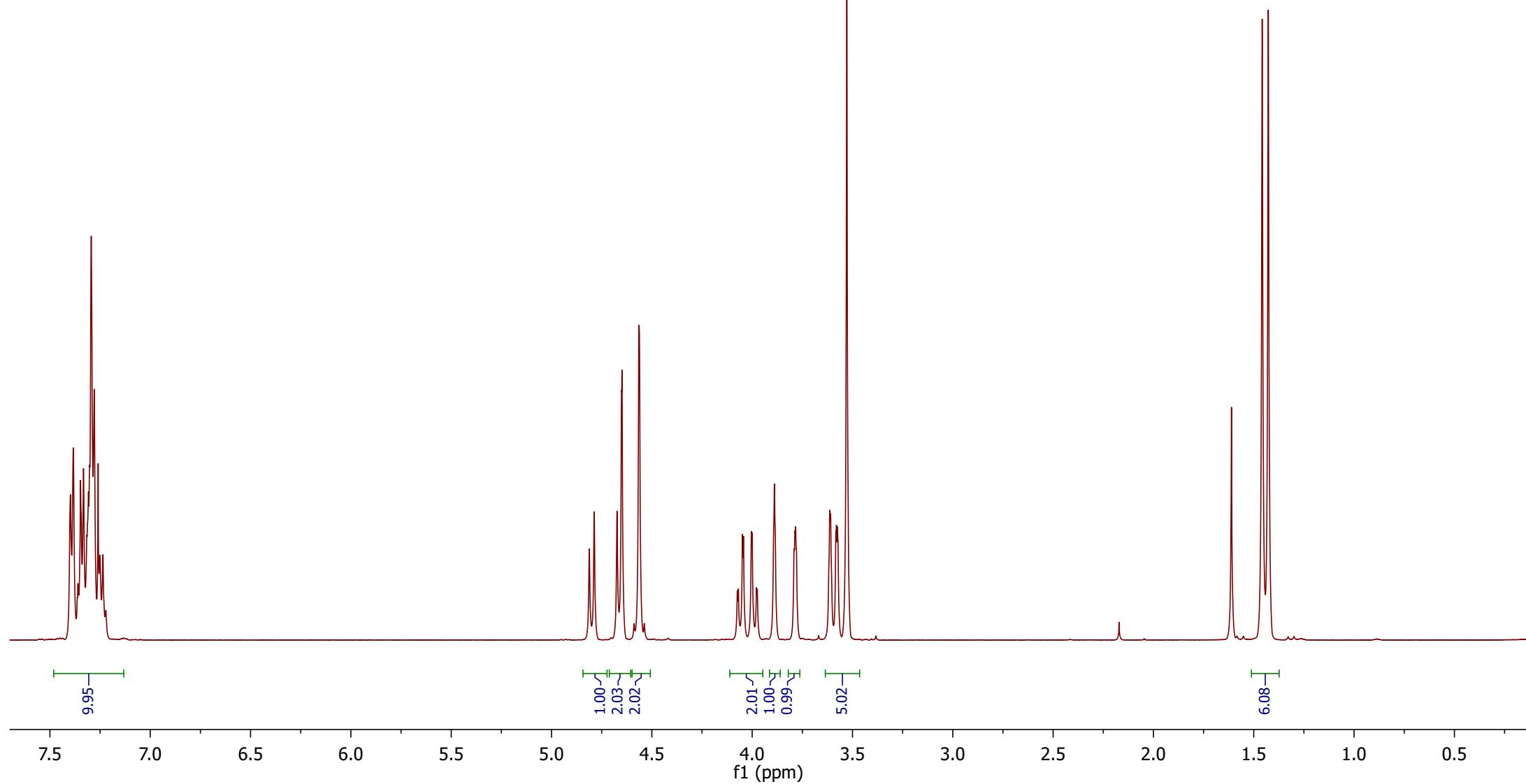
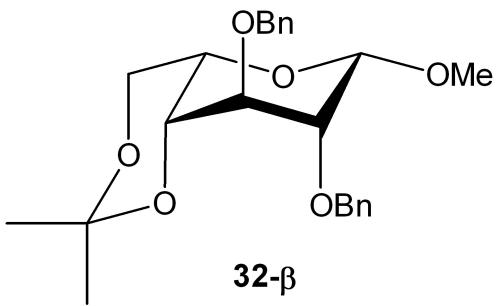


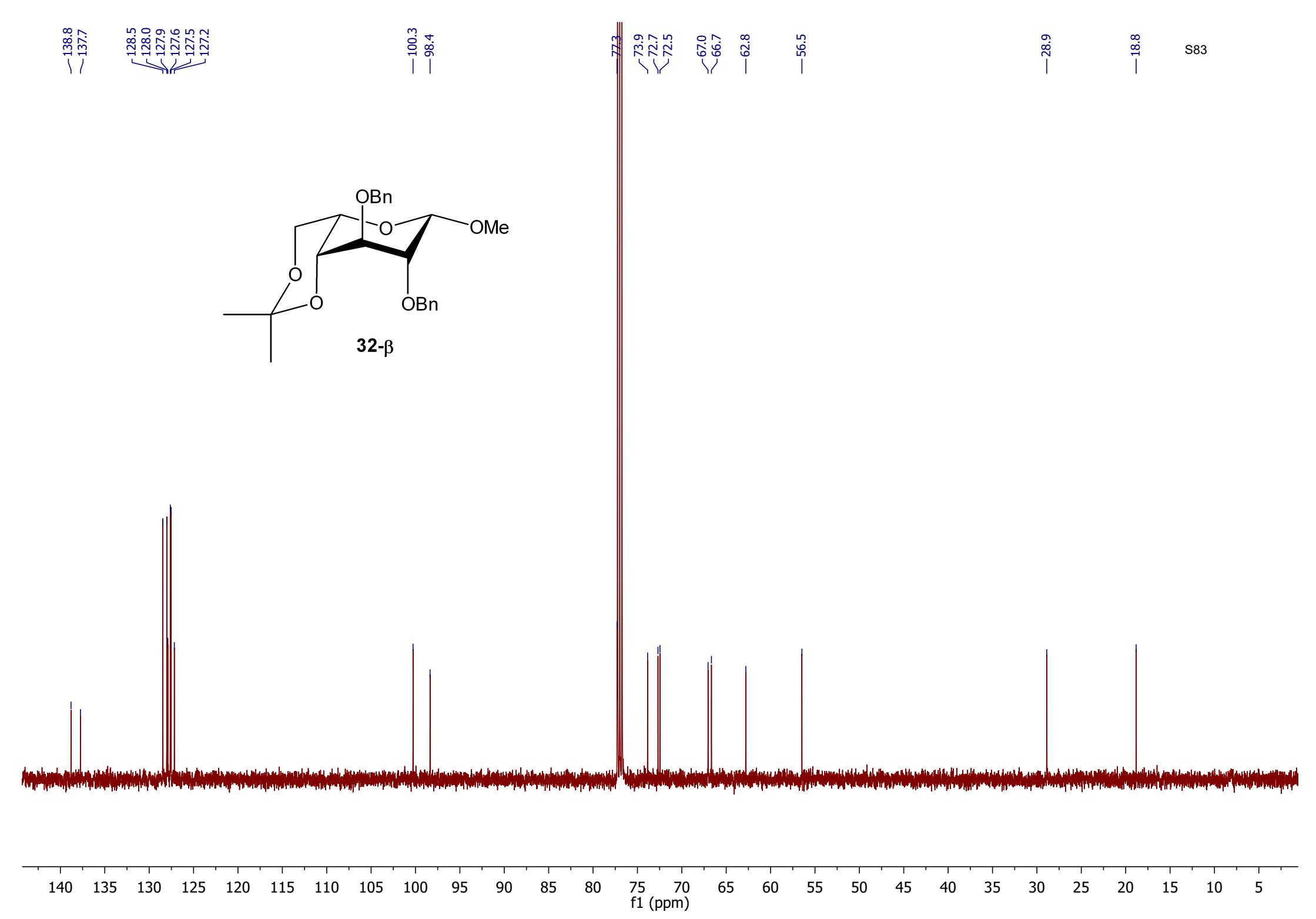


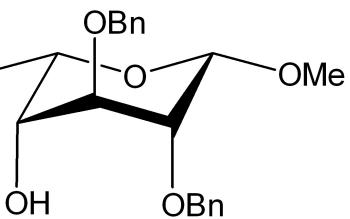






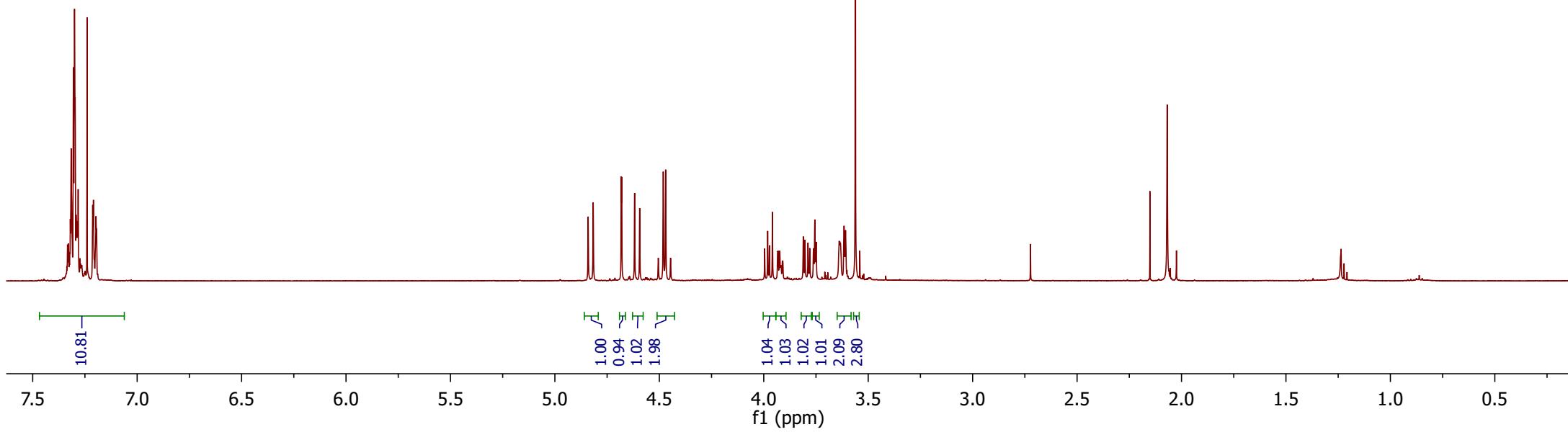






34

S84

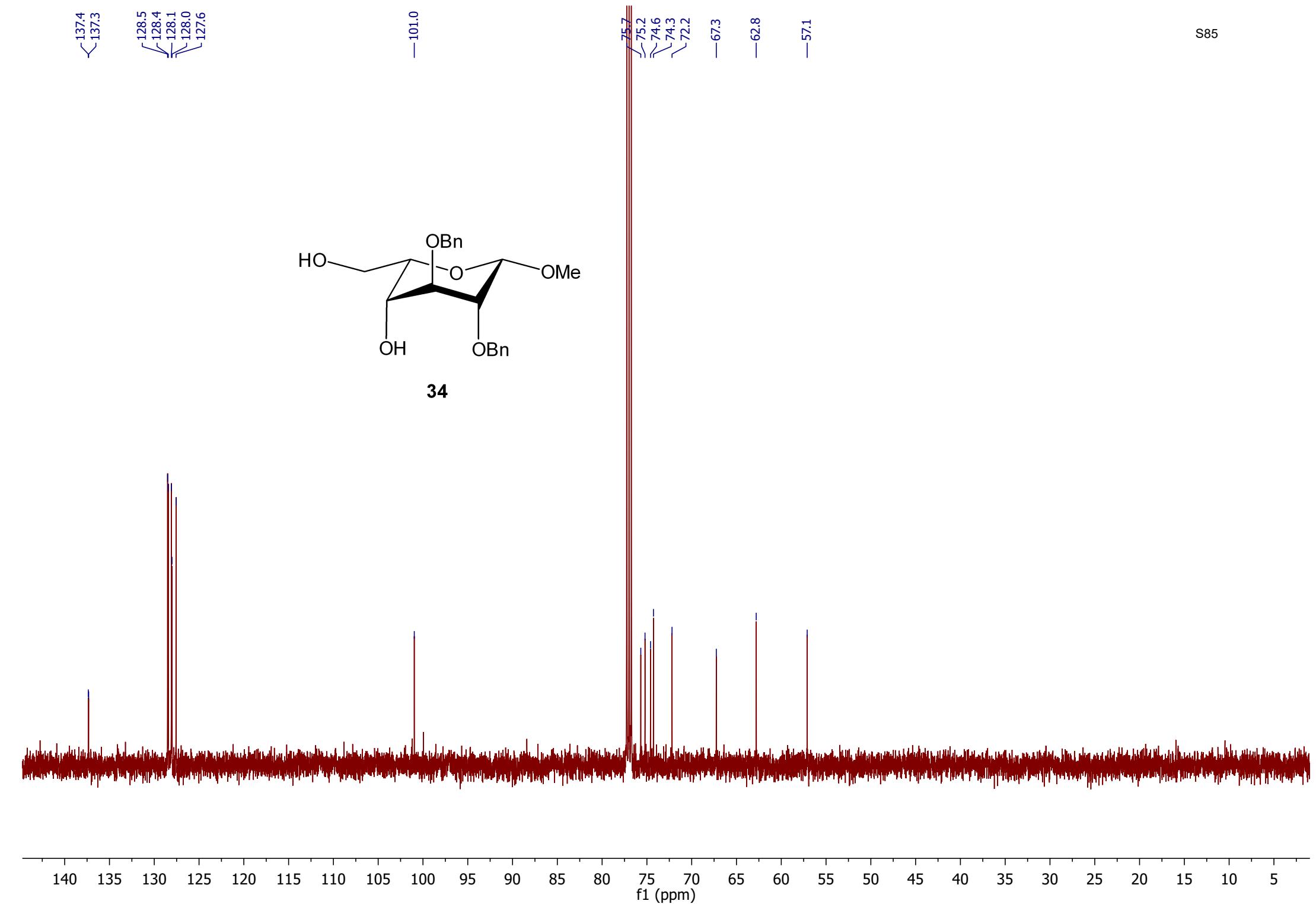
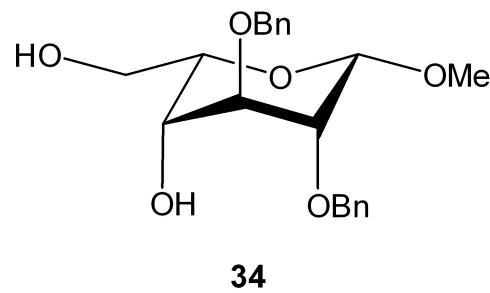


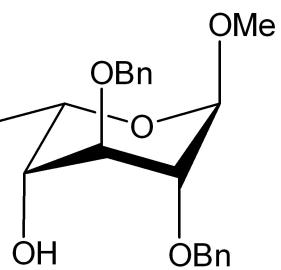
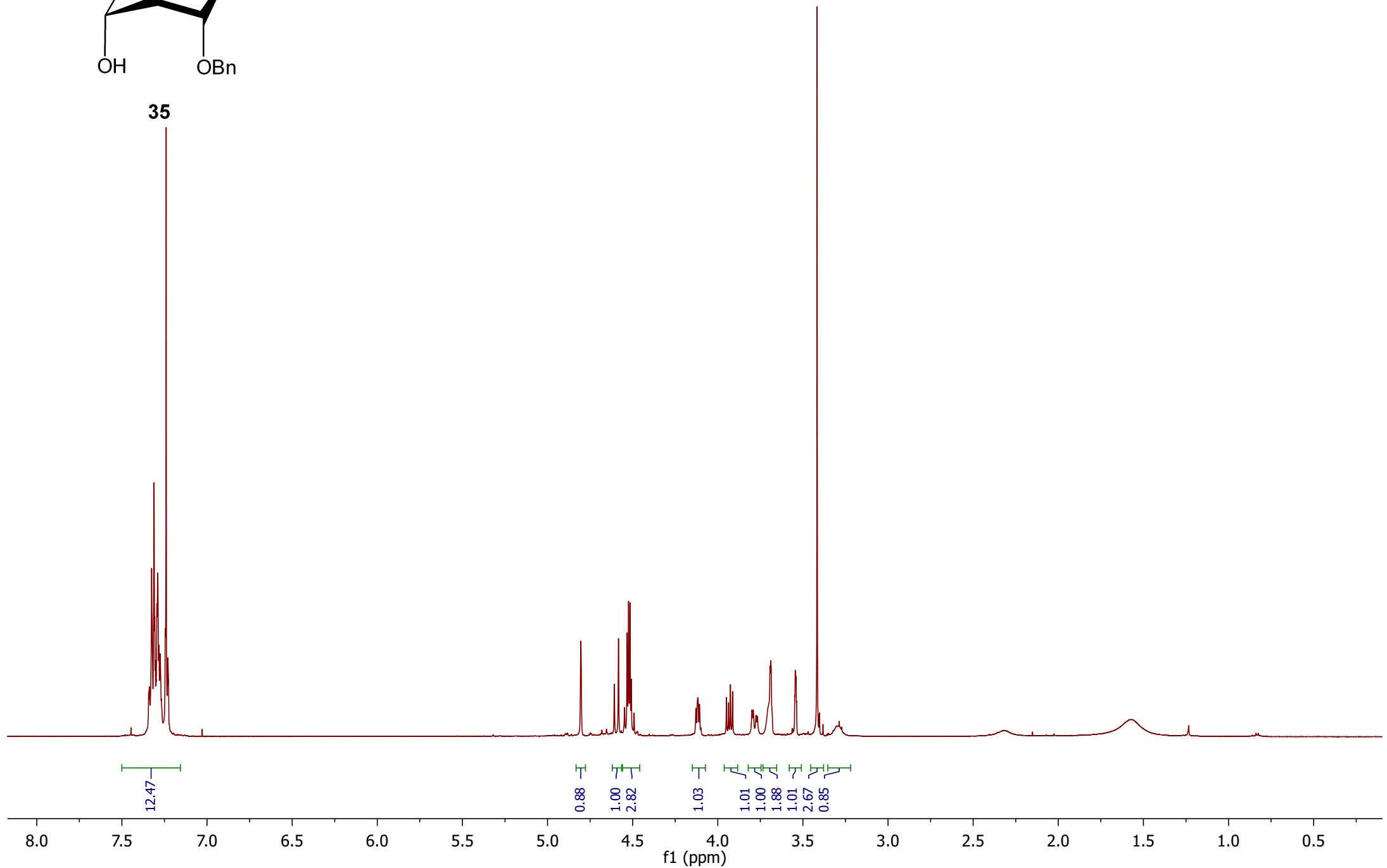
137.4
137.3

128.4
128.5
128.1
128.0
127.6

—101.0

S85



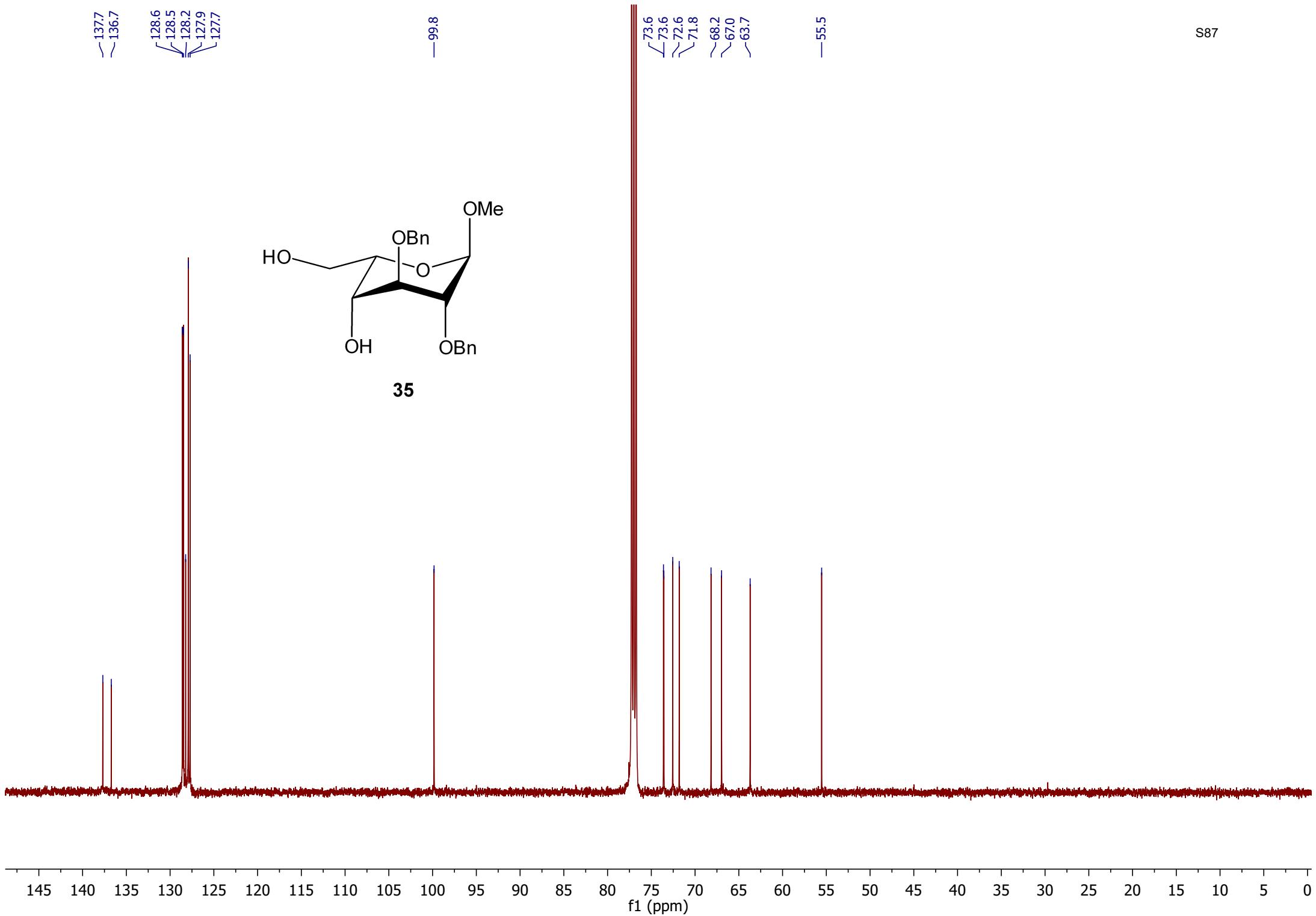
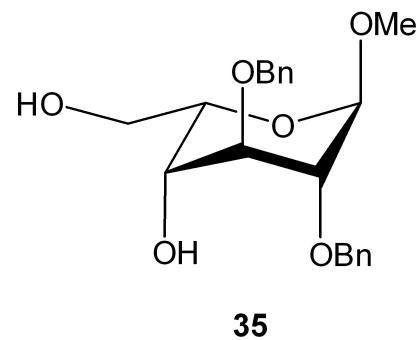
**35**

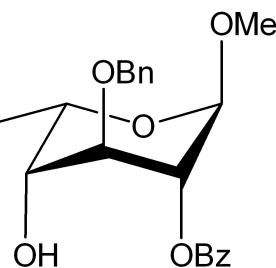
—137.7
—136.7
—128.6
—128.5
—128.2
—127.9
—127.7

—99.8

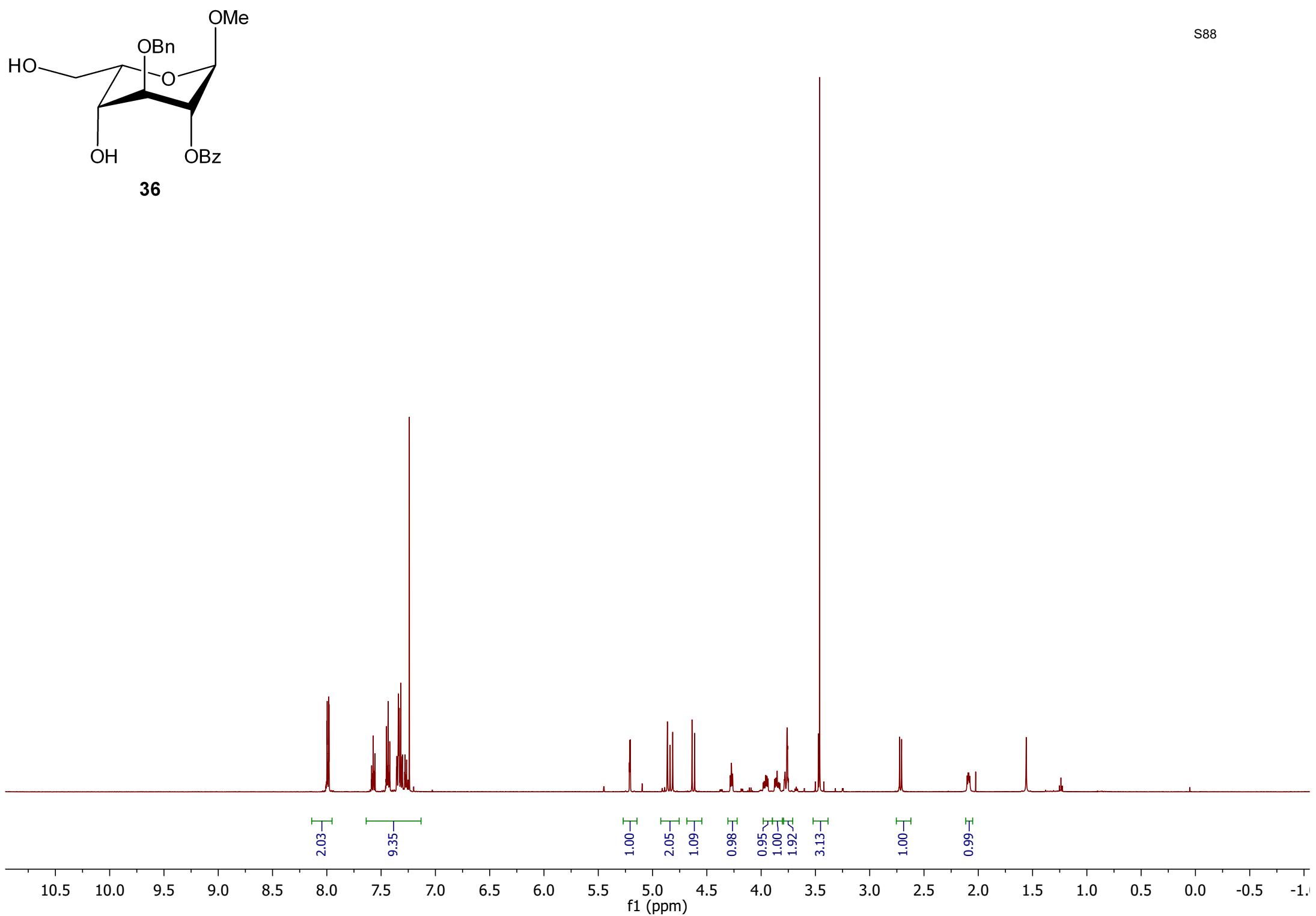
—55.5

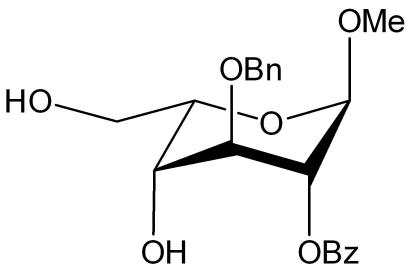
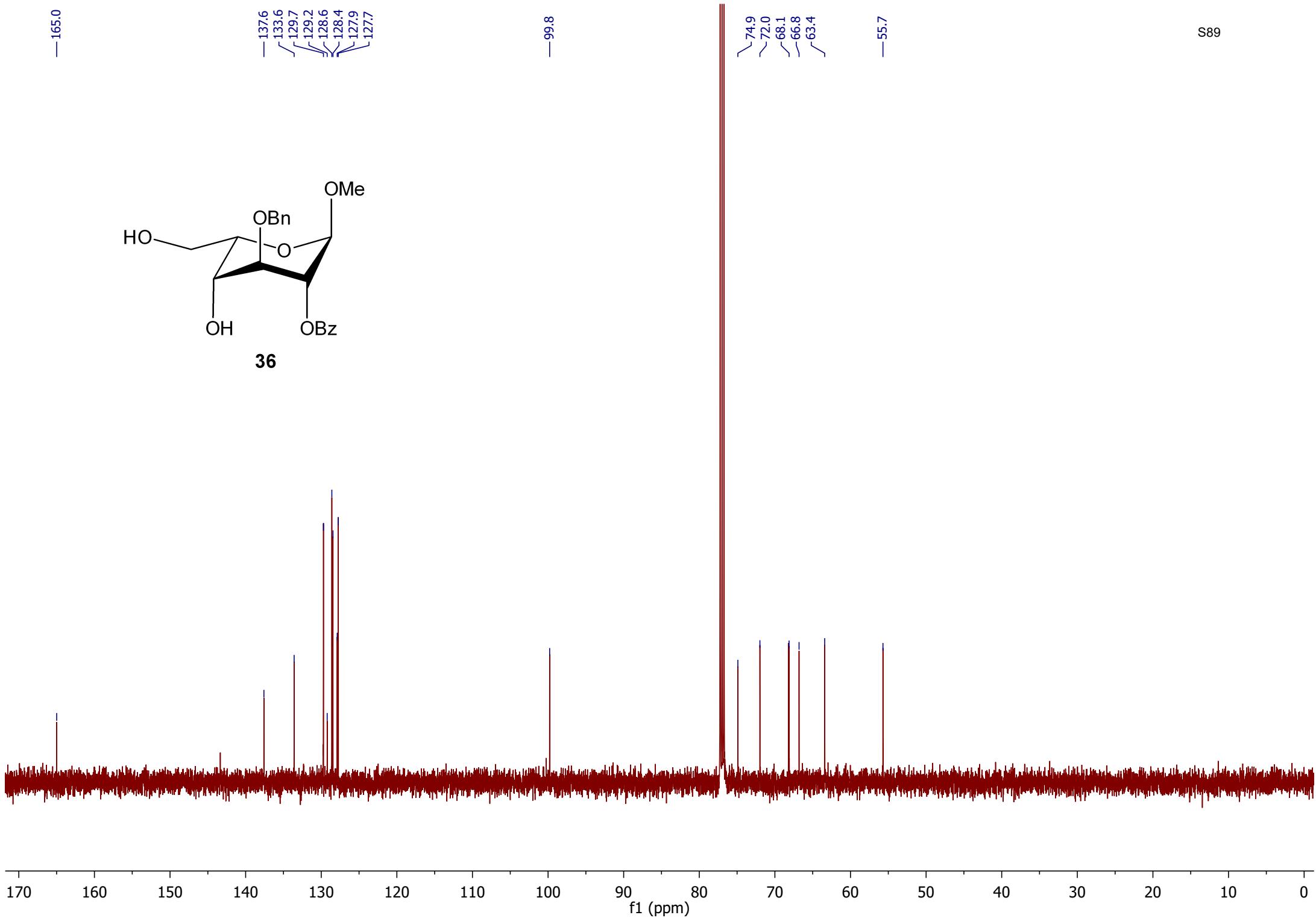
—73.6
—73.6
—72.6
—71.8
—68.2
—67.0
—63.7

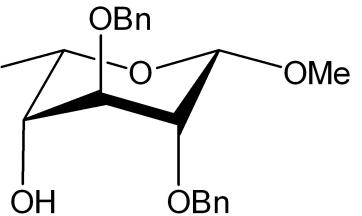




36

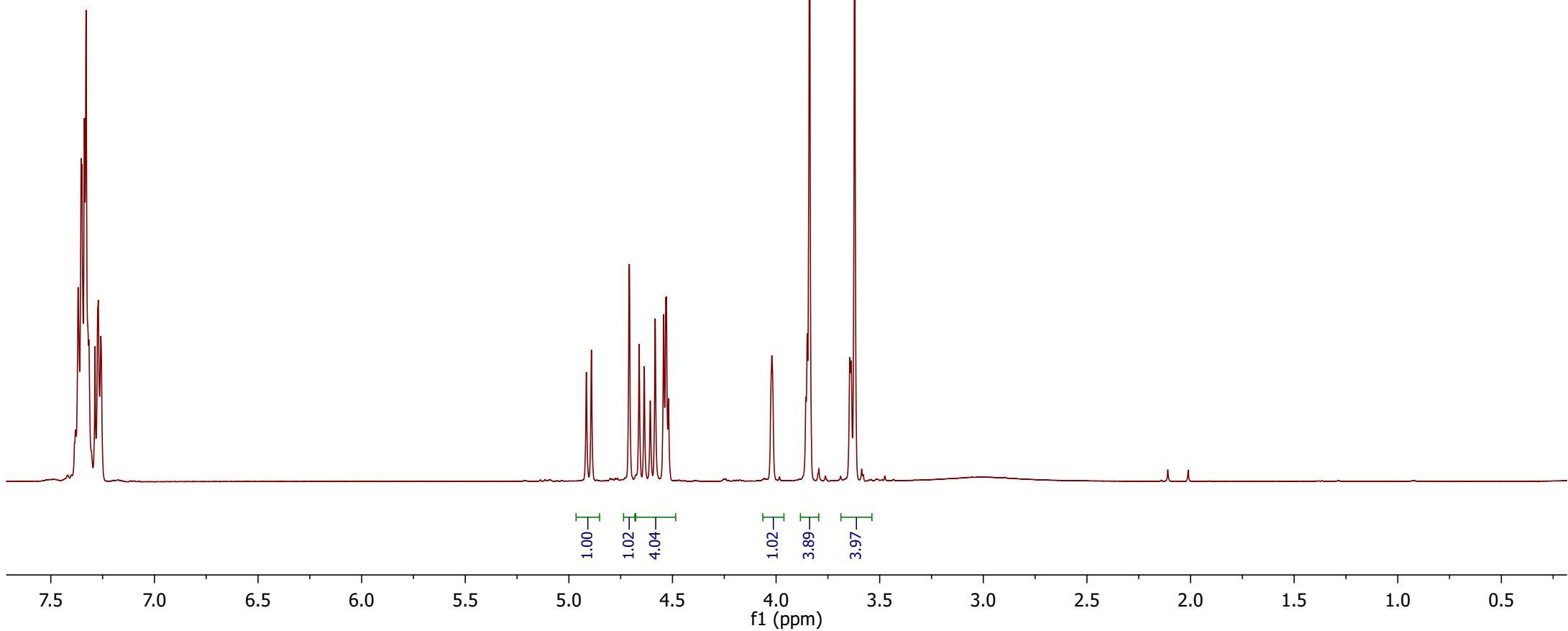


**36**



37

S90



—169.5

—137.5
—137.2
[128.6
/ 128.4
/ 128.1
/ 128.0
/ 127.7

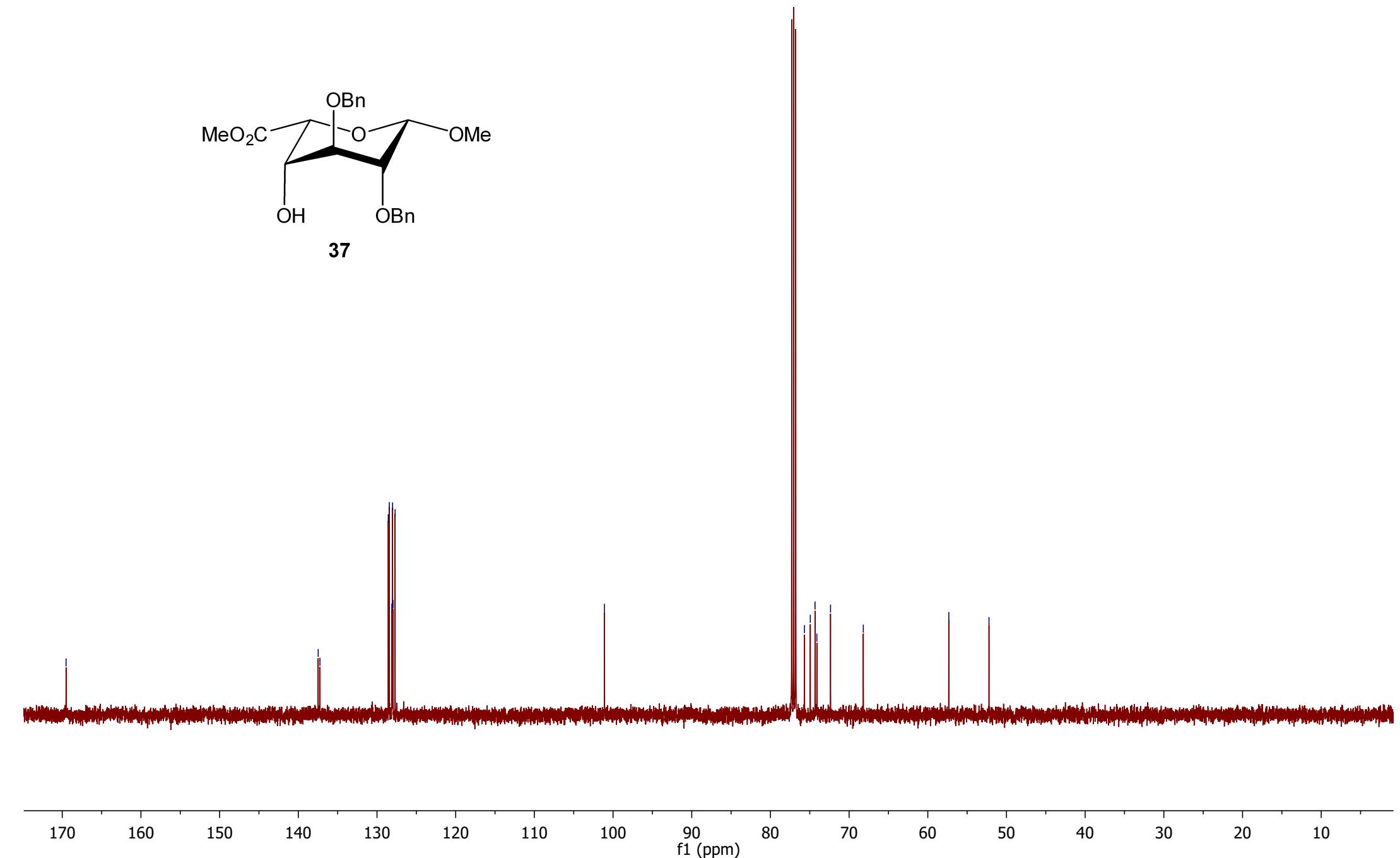
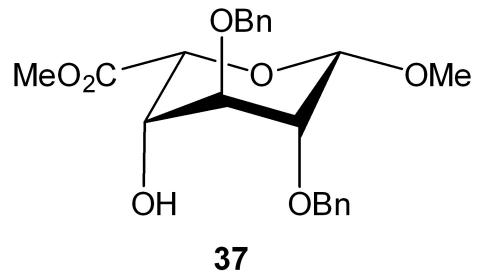
—101.1

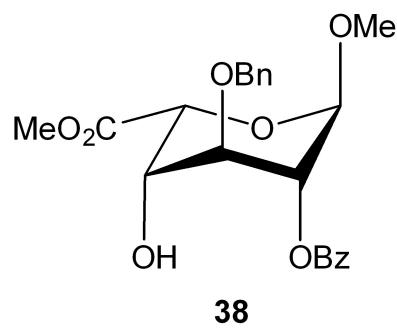
✓ 75.7
✓ 74.9
✓ 74.3
✓ 74.1
✓ 72.4
—68.2

—57.3

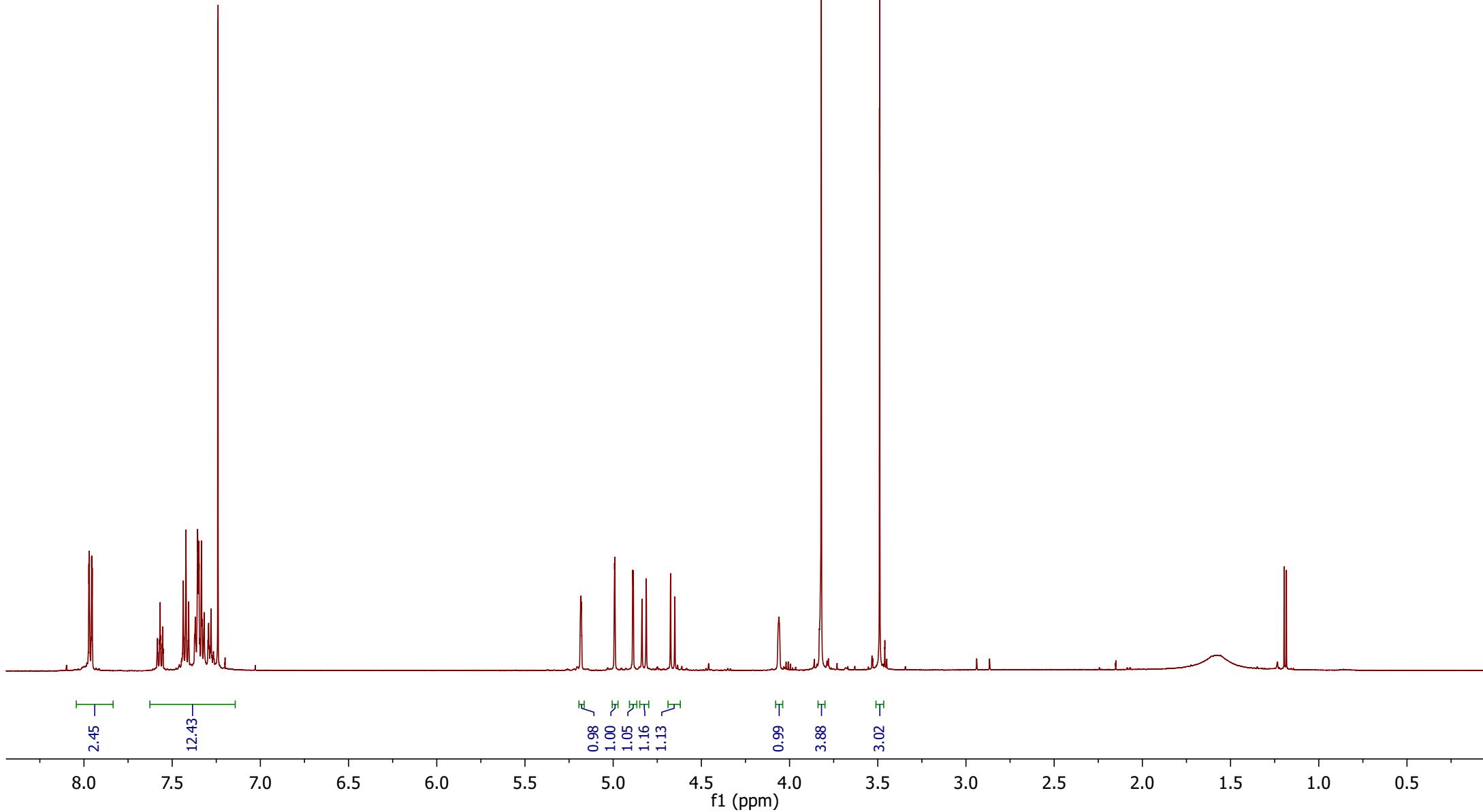
—52.2

S91





S92



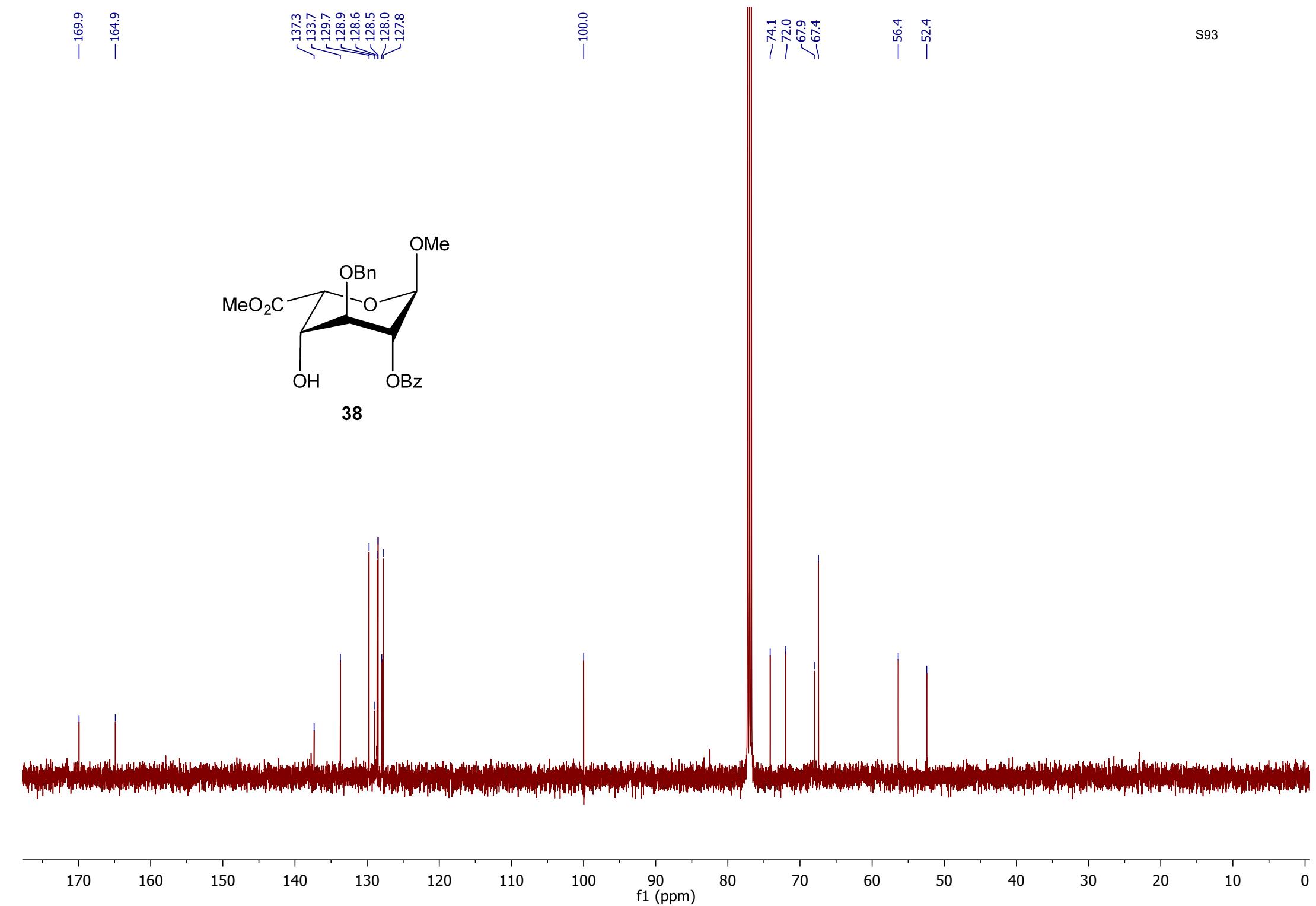
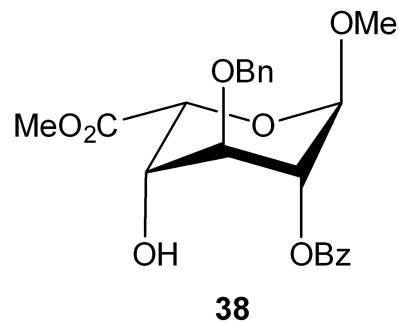
—169.9
—164.9

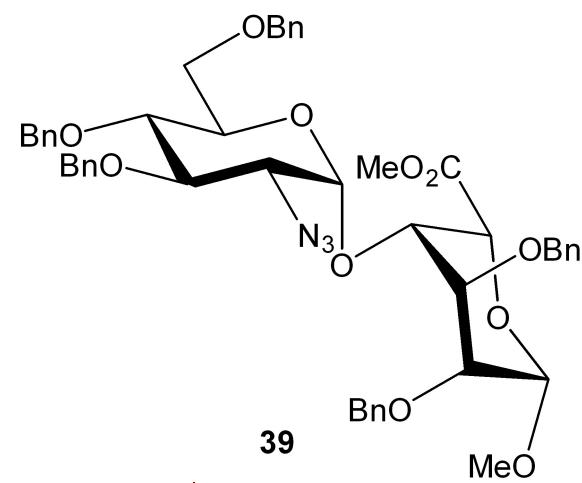
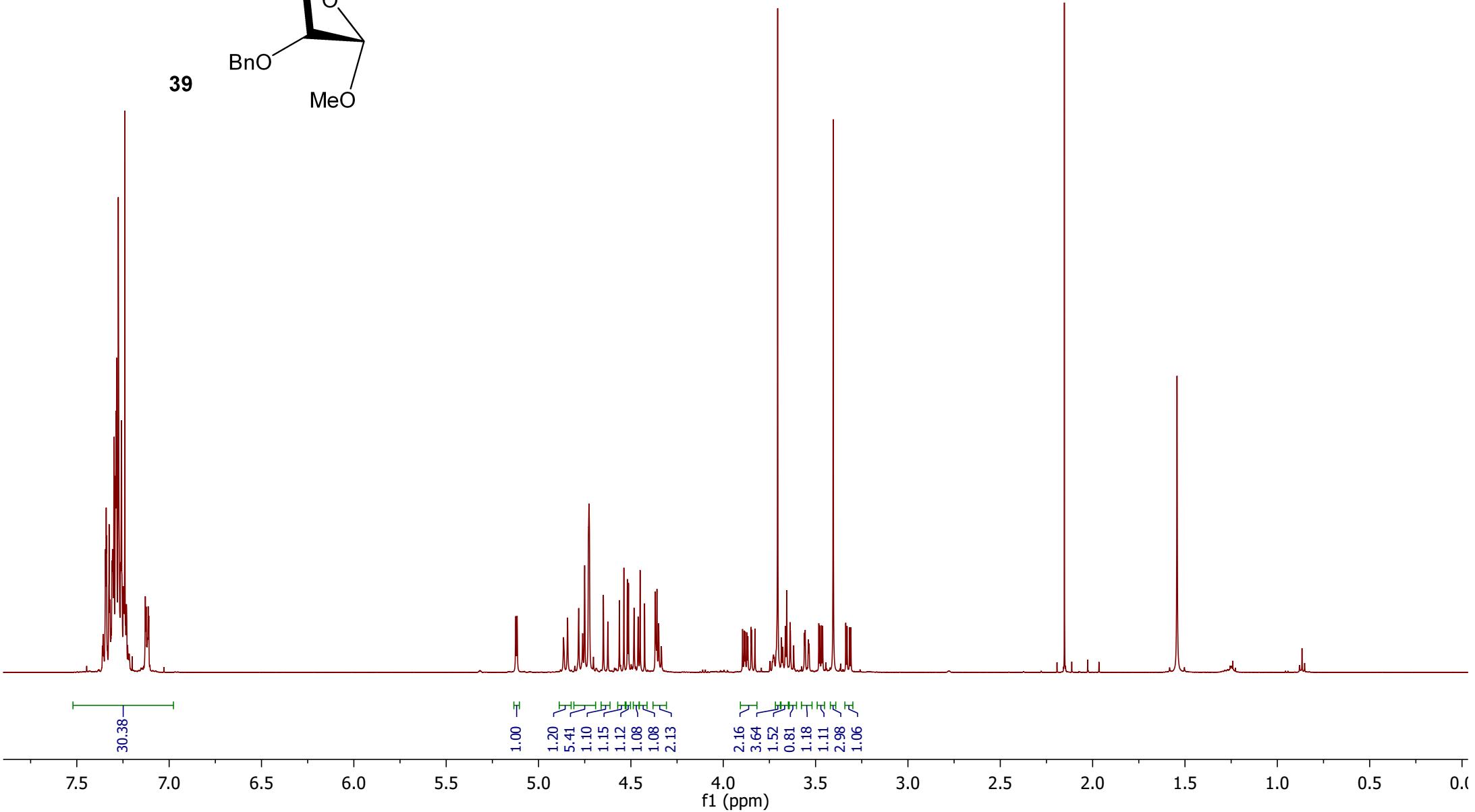
137.3
133.7
129.7
128.9
128.6
128.5
128.0
127.8

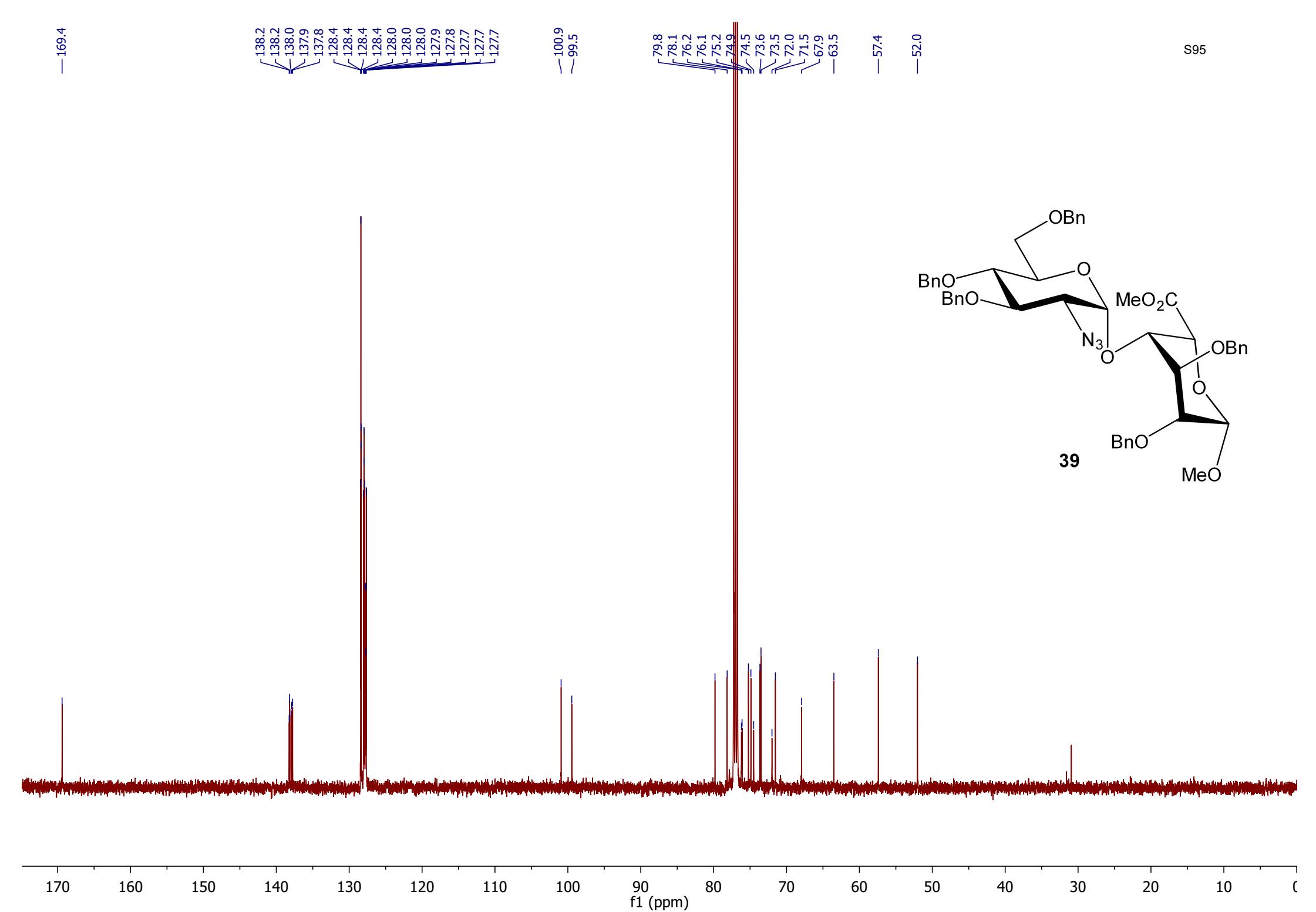
—100.0

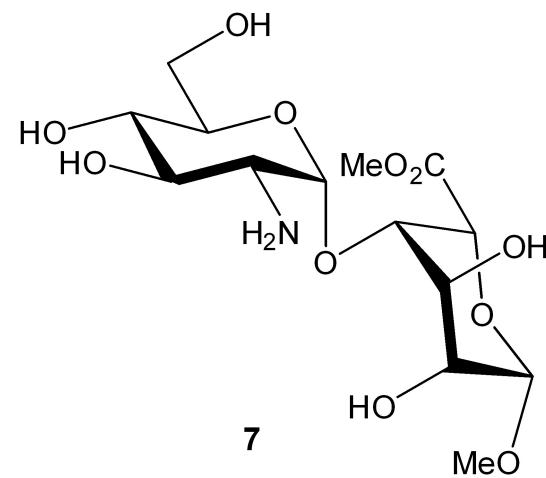
—74.1
—72.0
—67.9
—67.4

—56.4
—52.4

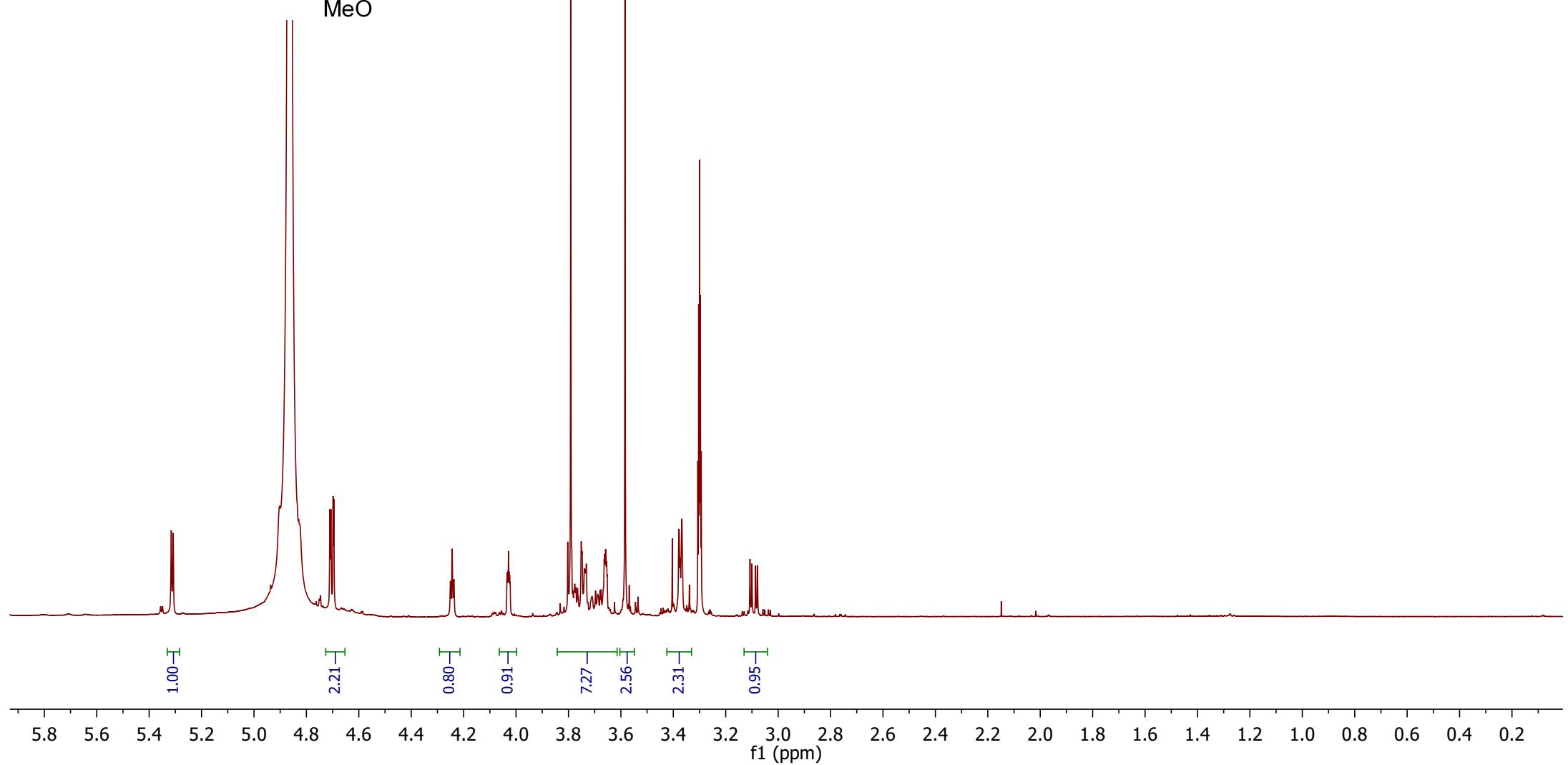


**39**

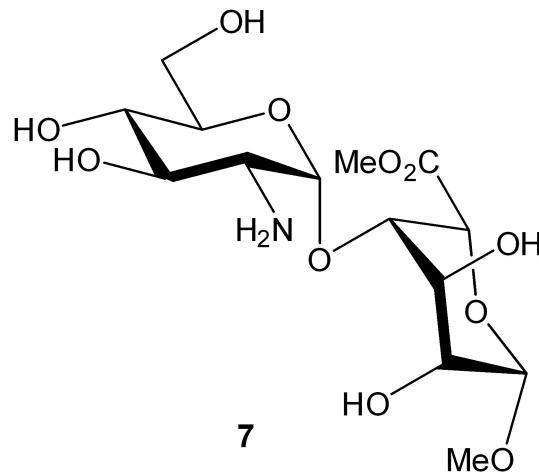




7



—171.0



—101.9

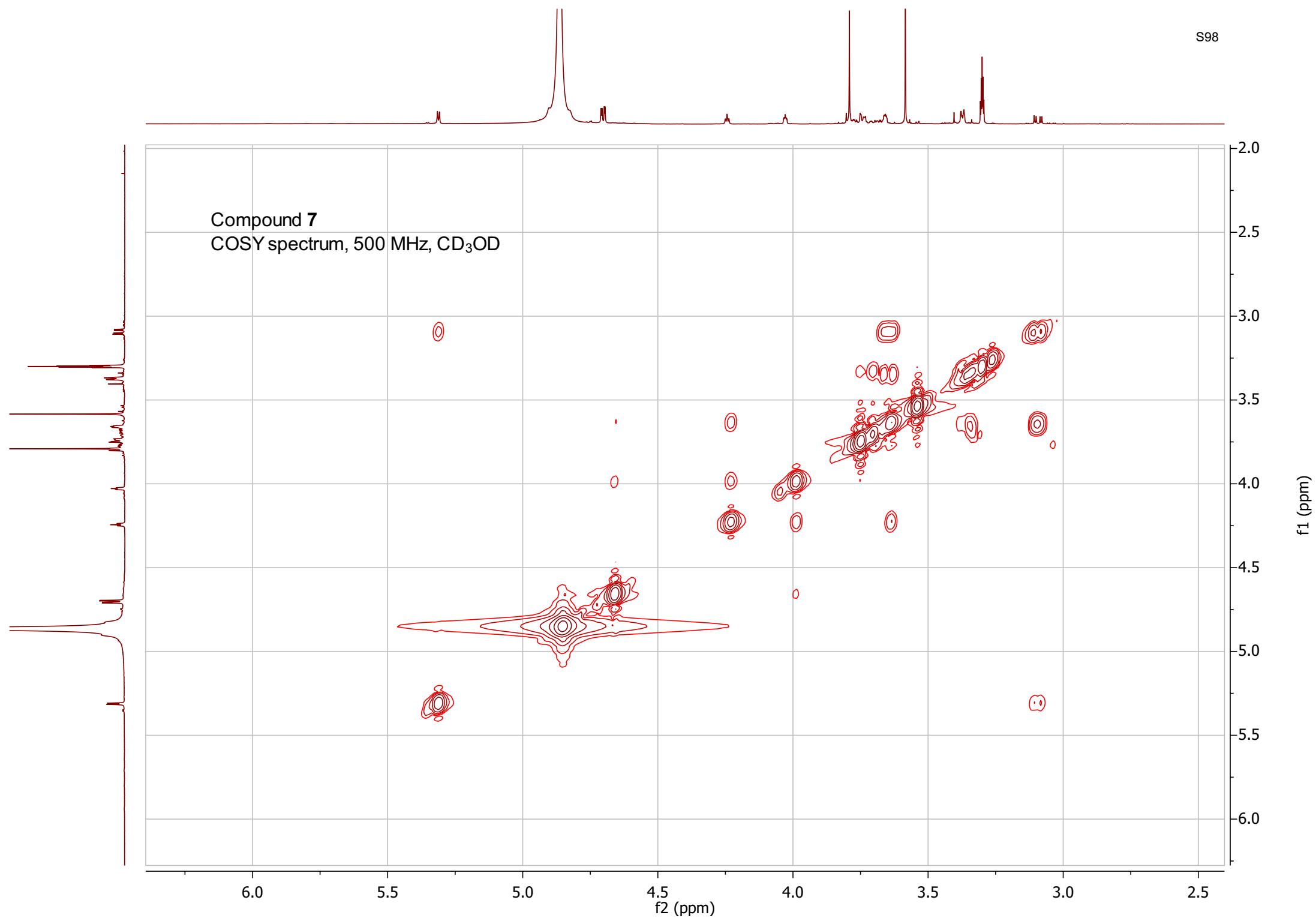
—93.8

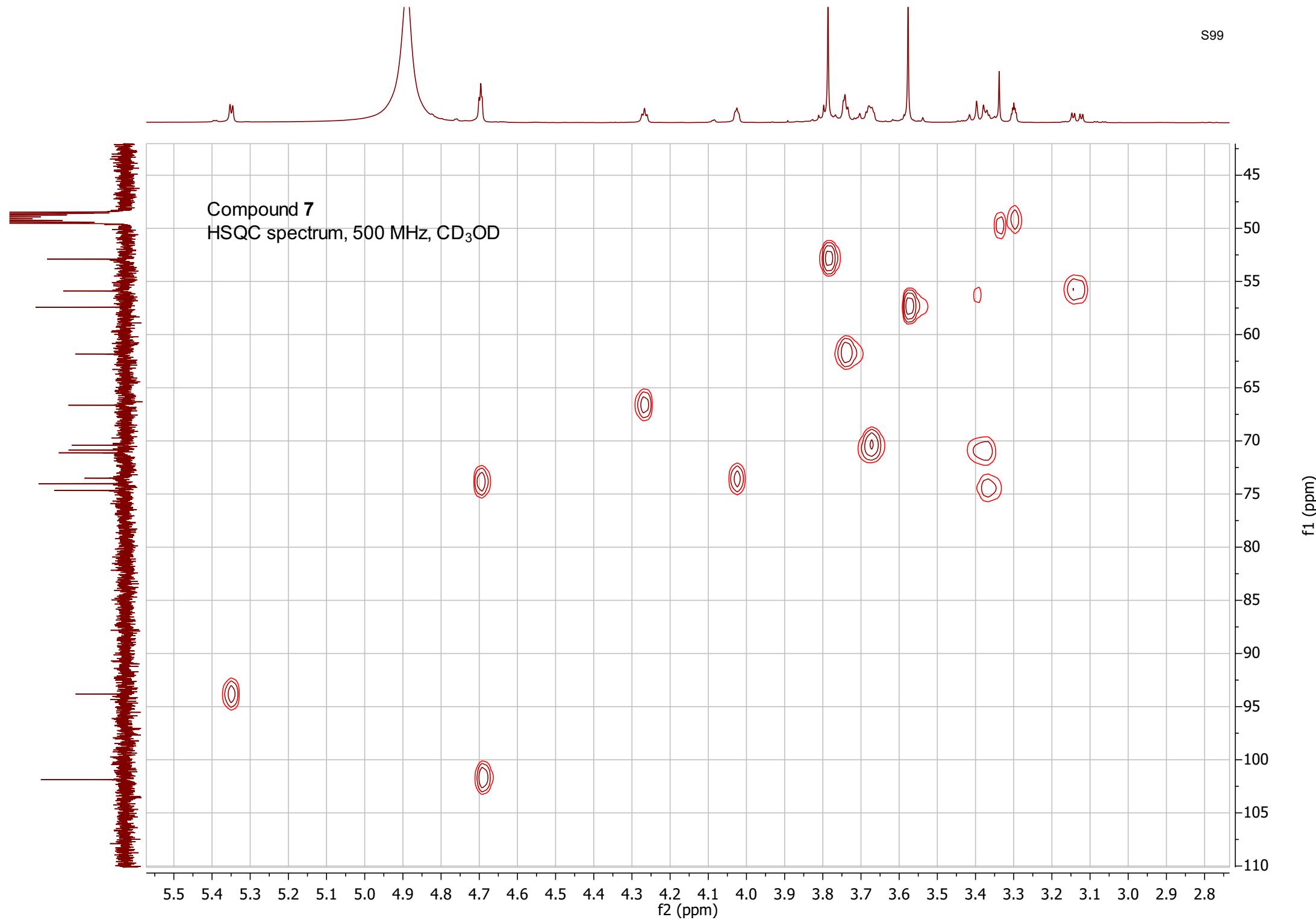
74.7
74.0
73.5
71.1
70.9
70.4
66.6
61.8
57.4
55.9
52.9

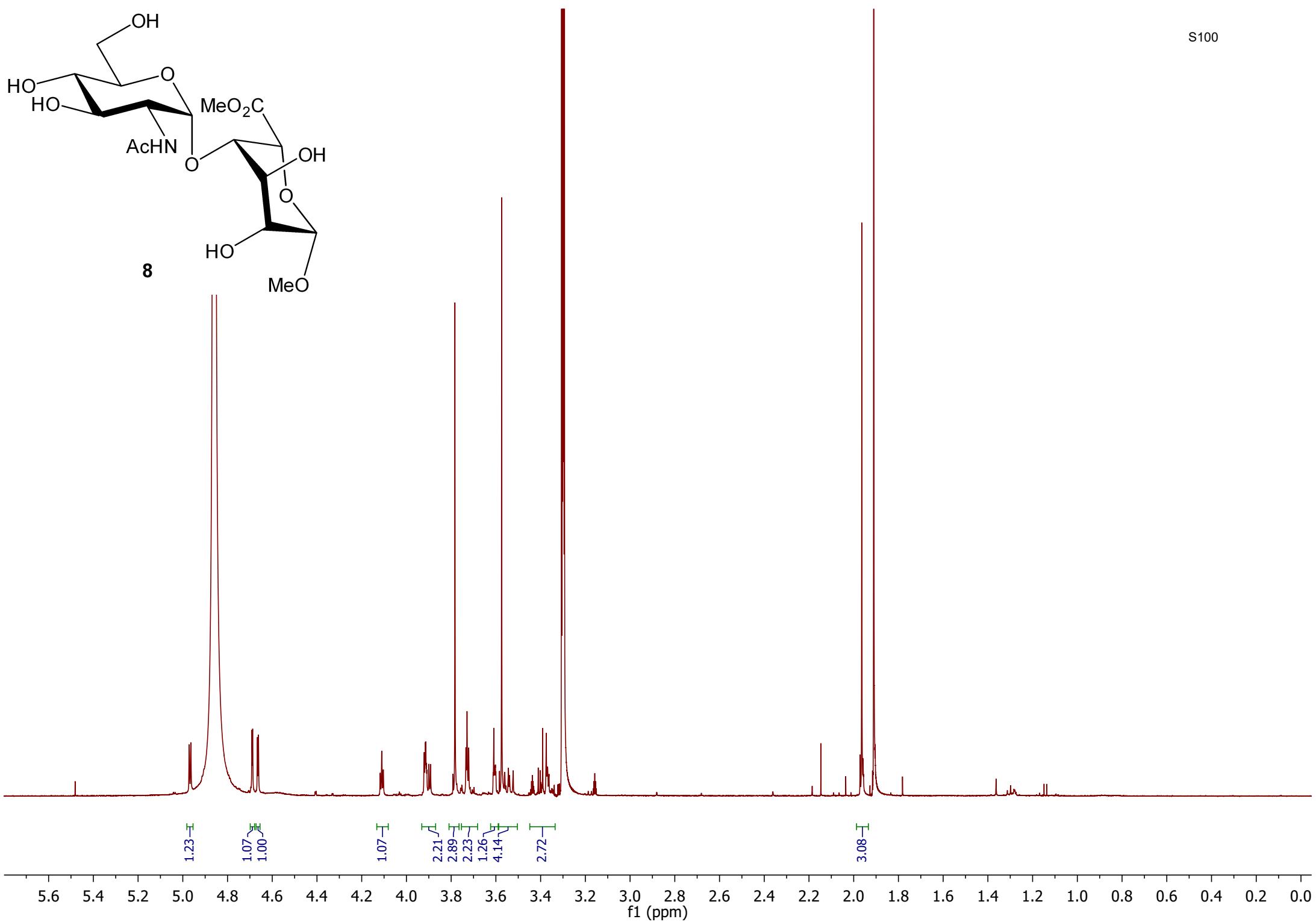
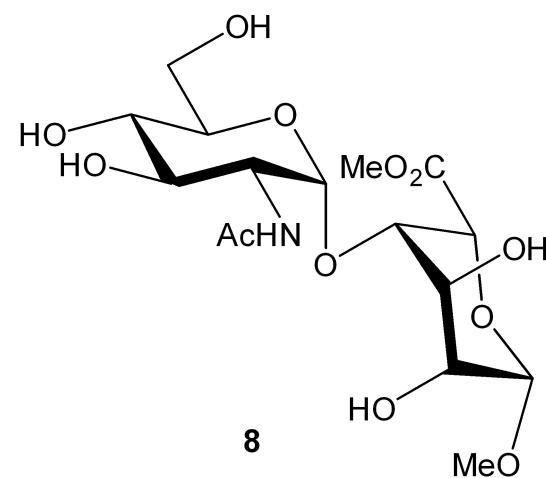
S97

f1 (ppm)

170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0







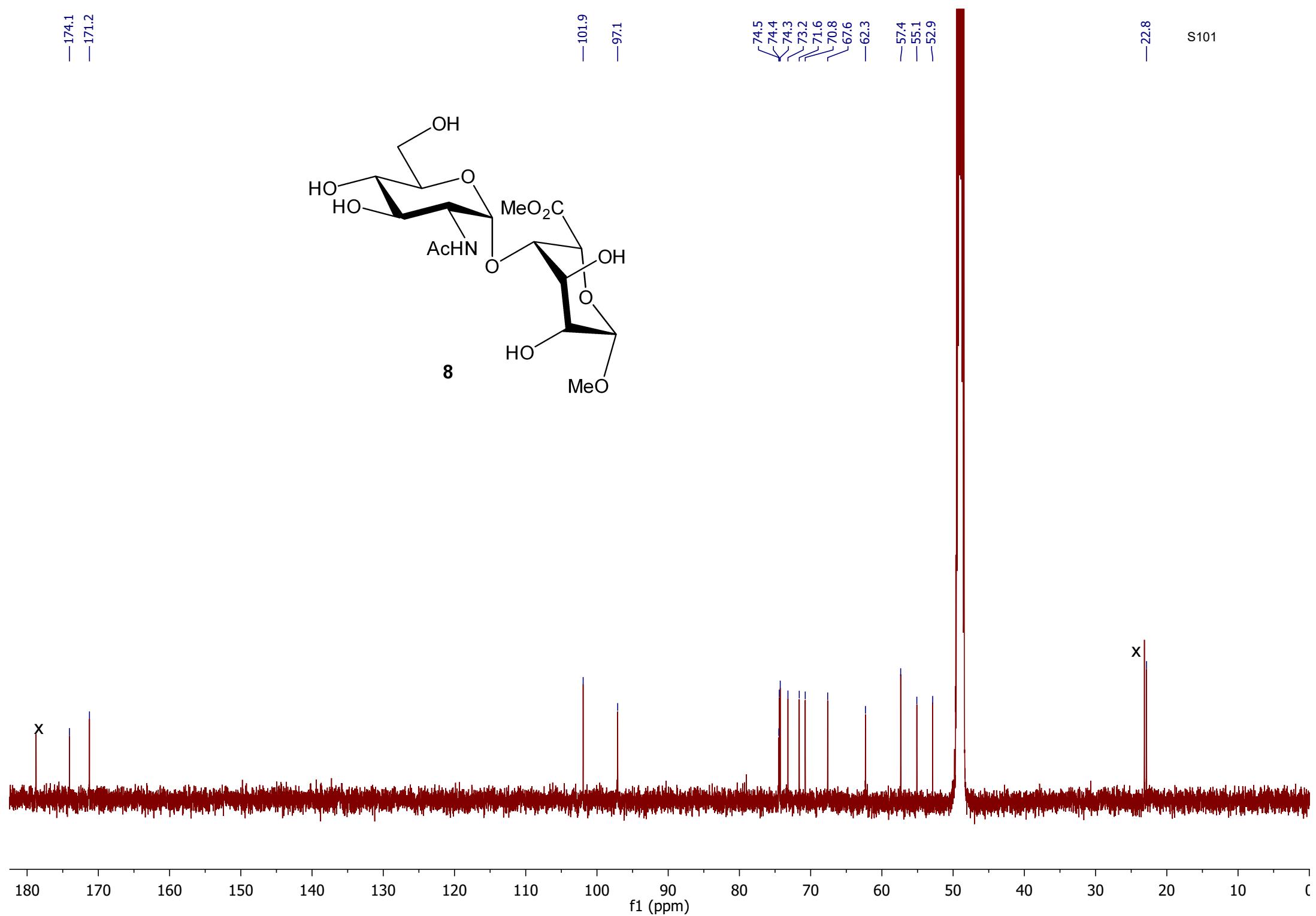
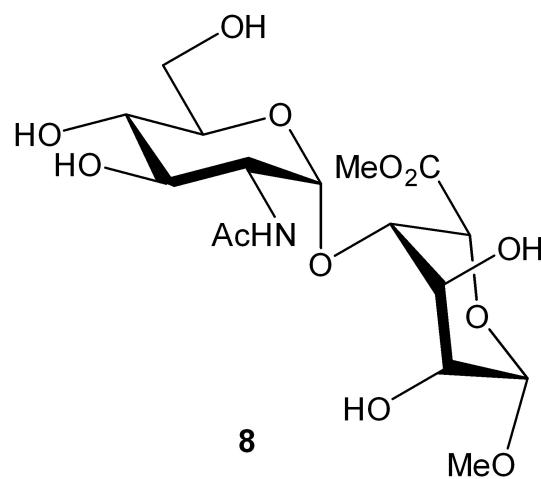
—174.1
—171.2

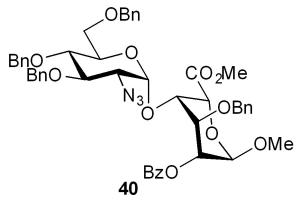
—101.9
—97.1

74.5
74.4
74.3
73.2
71.6
70.8
67.6
62.3
—57.4
—55.1
—52.9

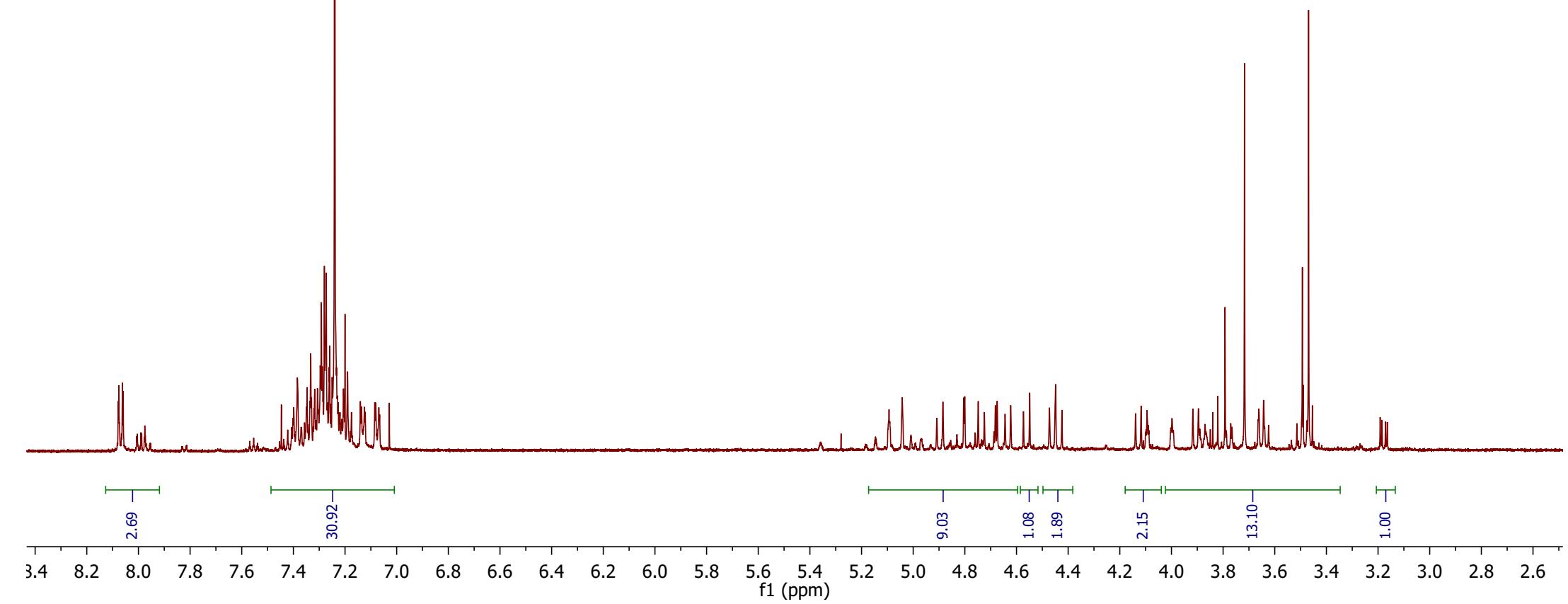
—22.8

S101





S102



—169.6

—165.4

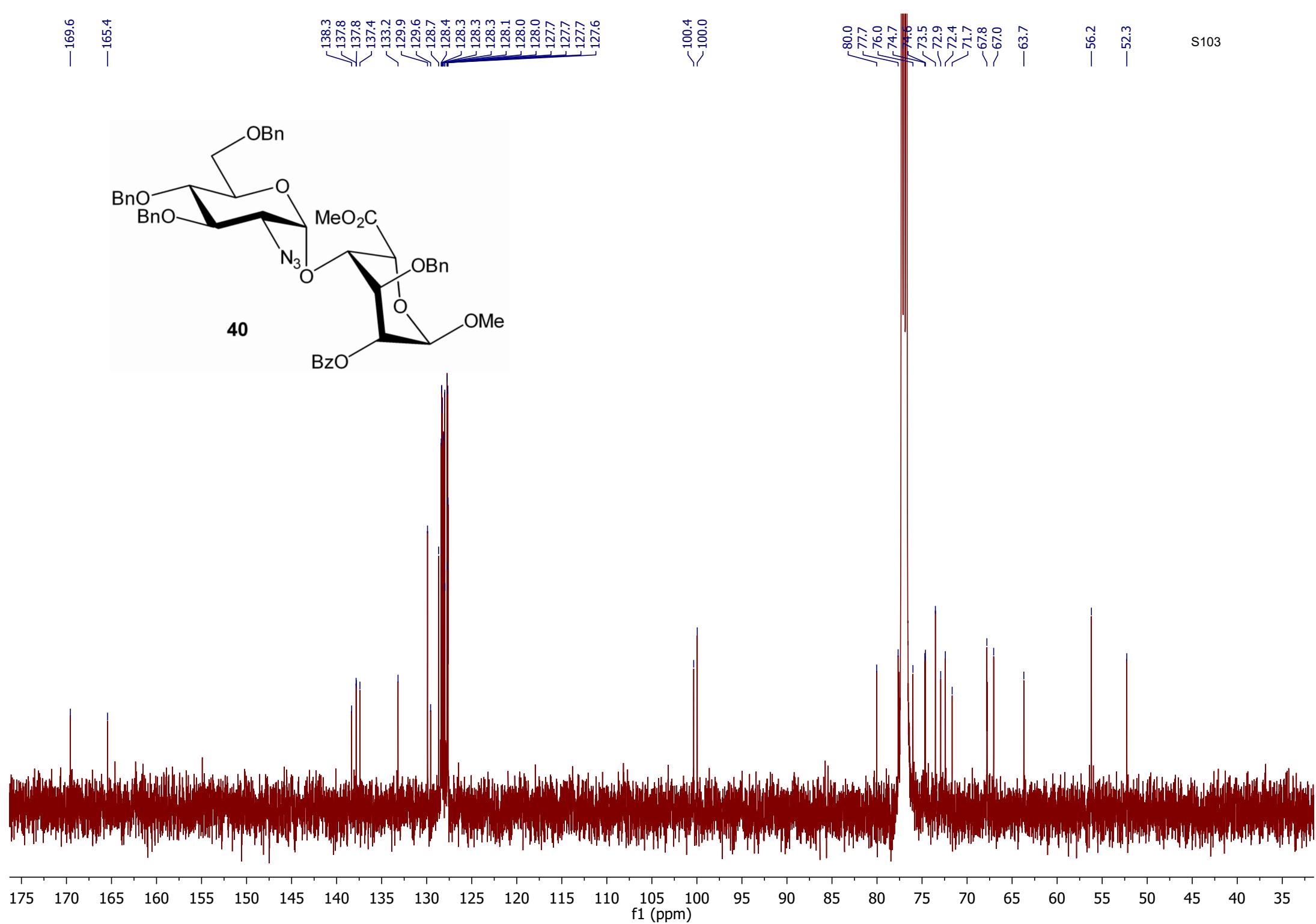
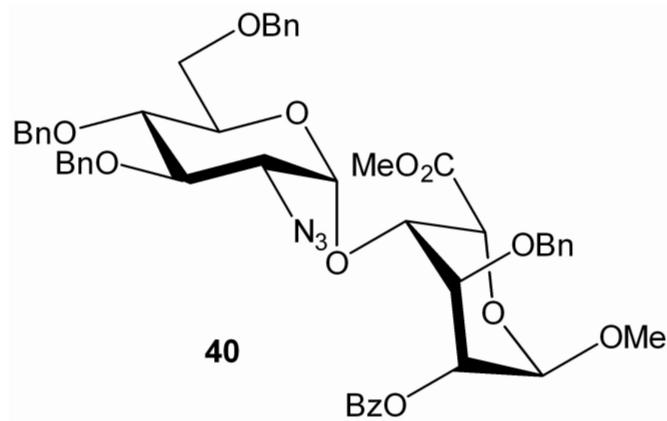
138.3
137.8
137.8
137.4
133.2
129.9
129.6
128.7
128.4
128.3
128.3
128.3
128.1
128.0
127.7
127.7
127.7
127.6

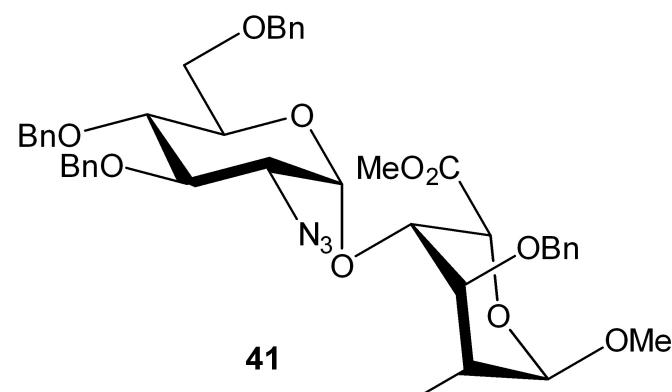
~100.4
~100.0

80.0
77.7
76.0
74.7
74.6
73.5
72.9
72.4
71.7
67.8
~67.0
~63.7

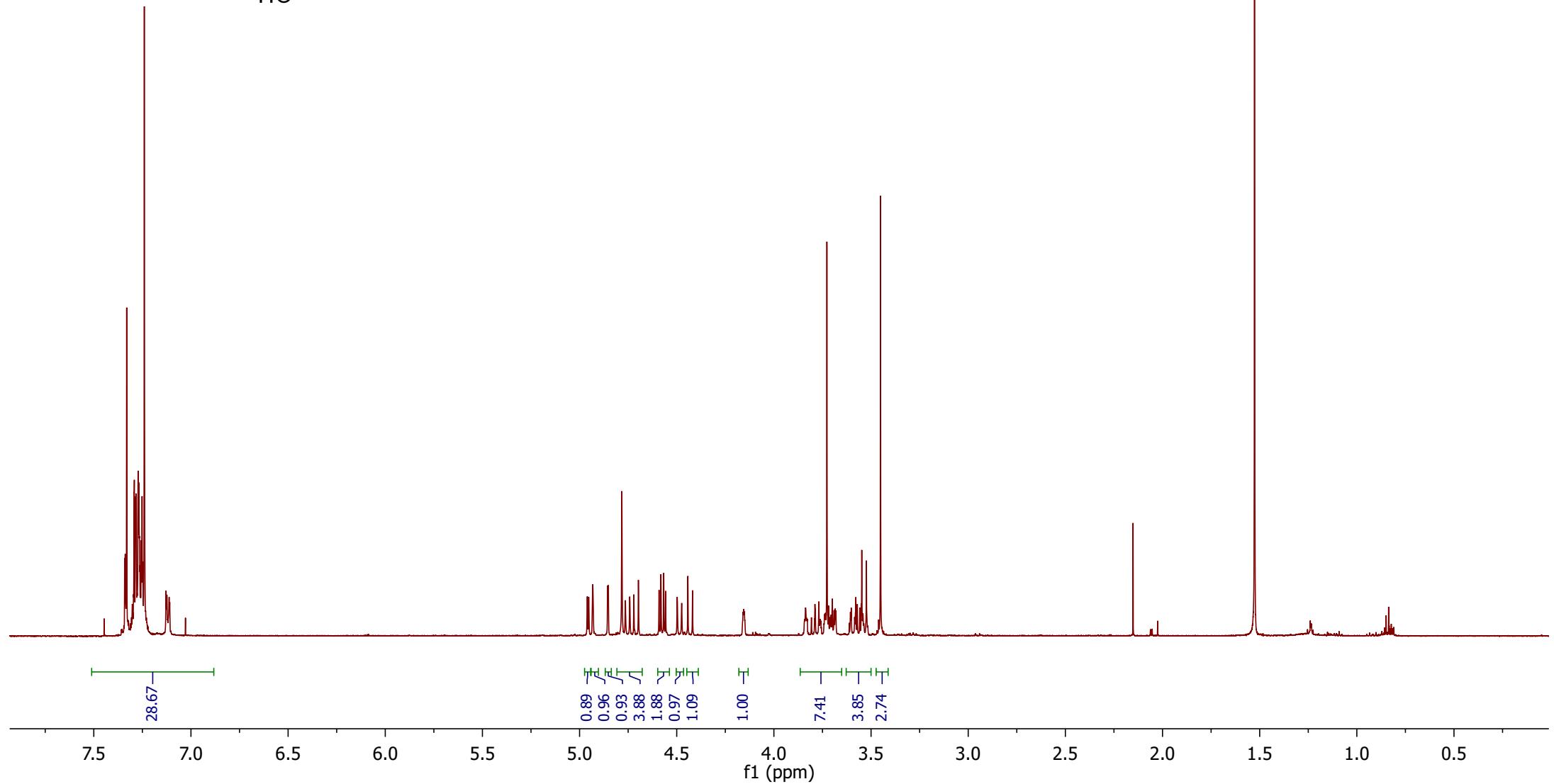
—56.2
—52.3

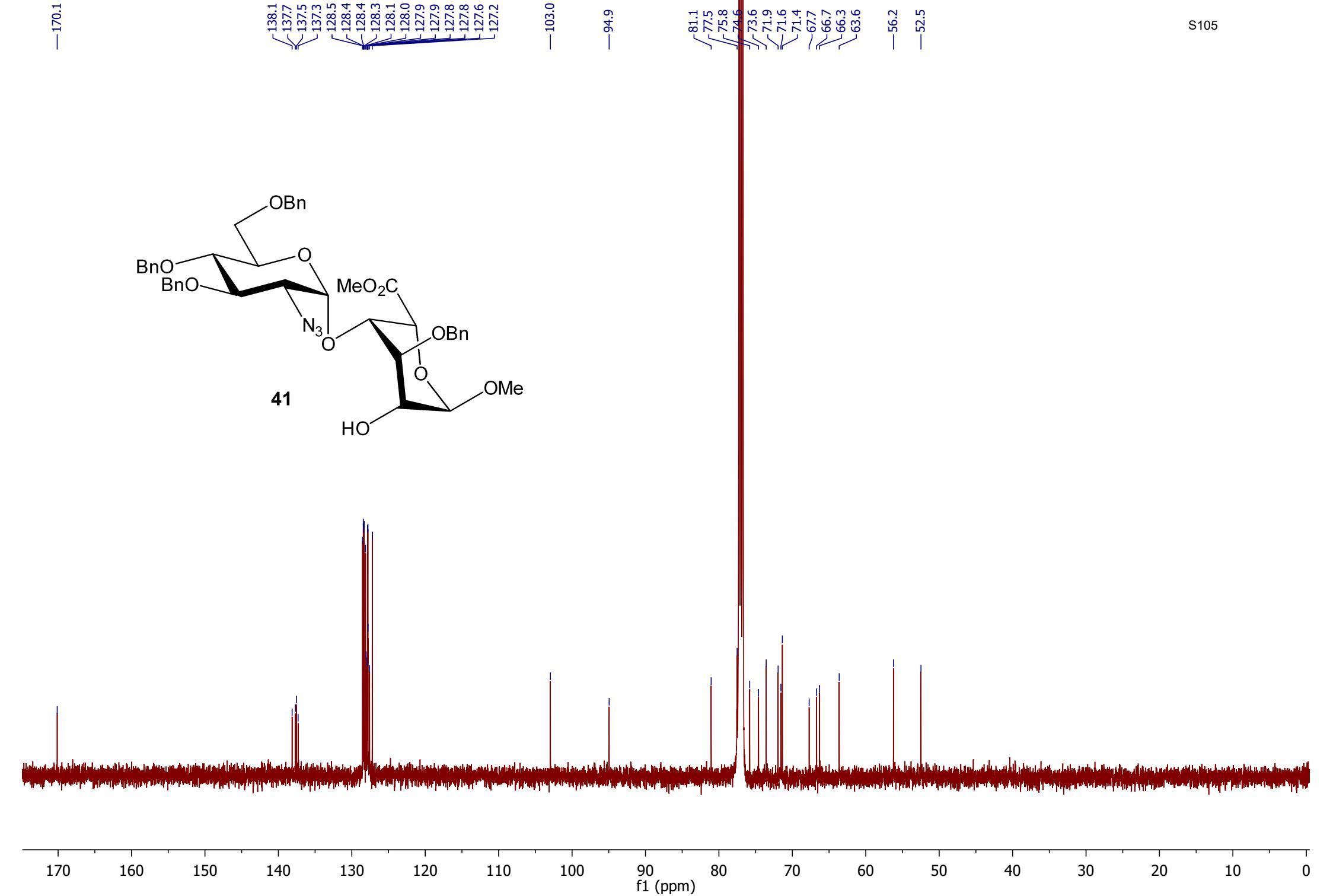
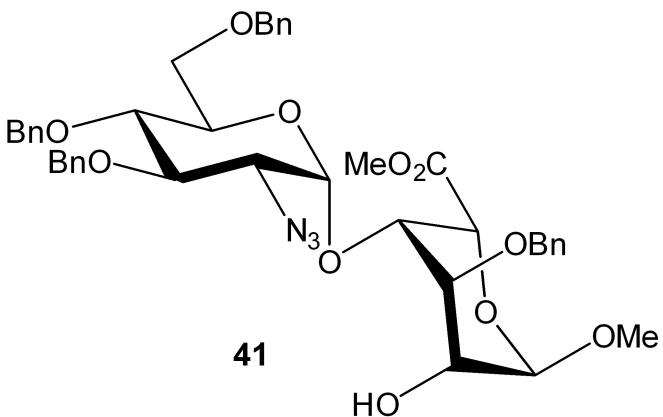
S103

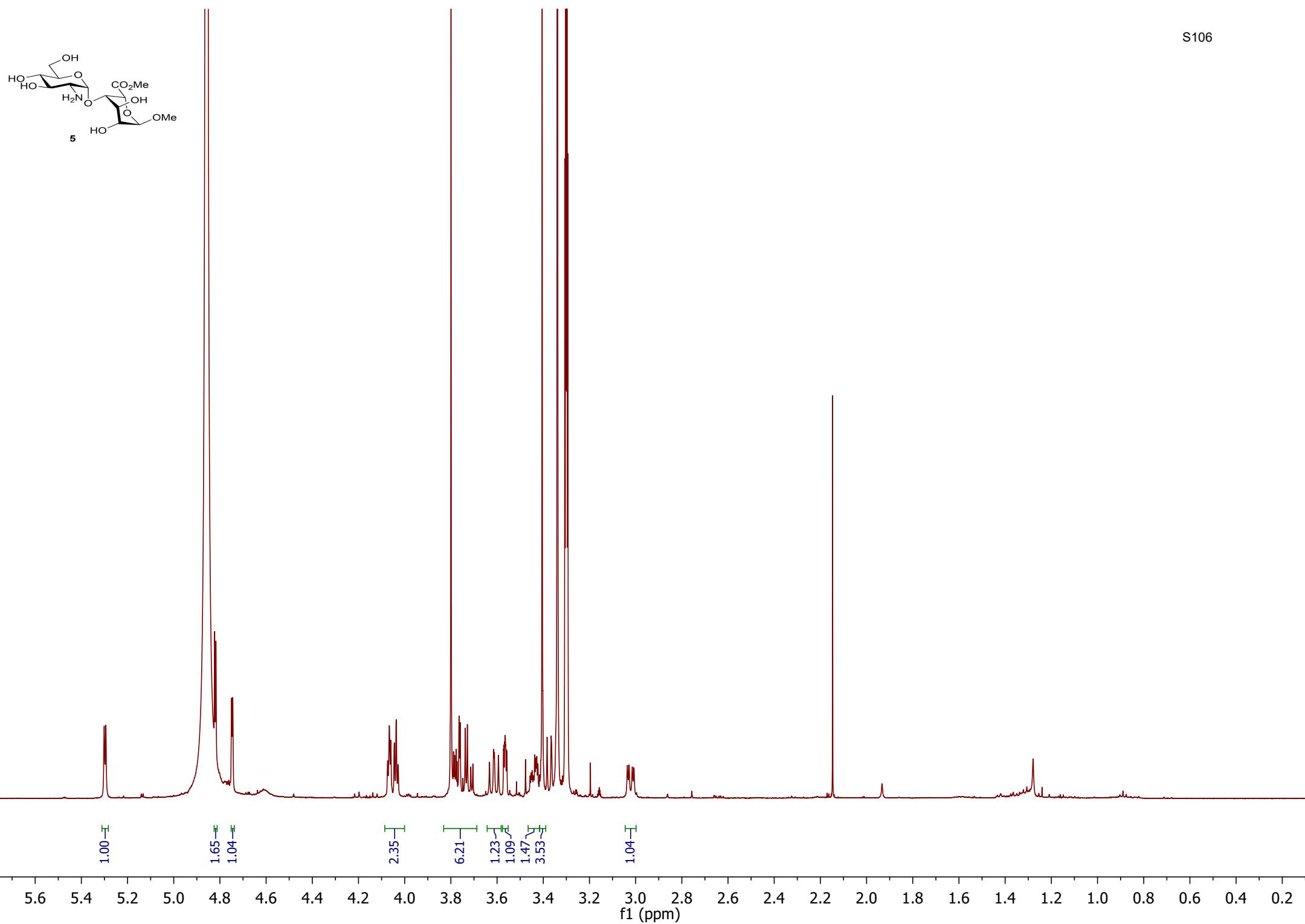


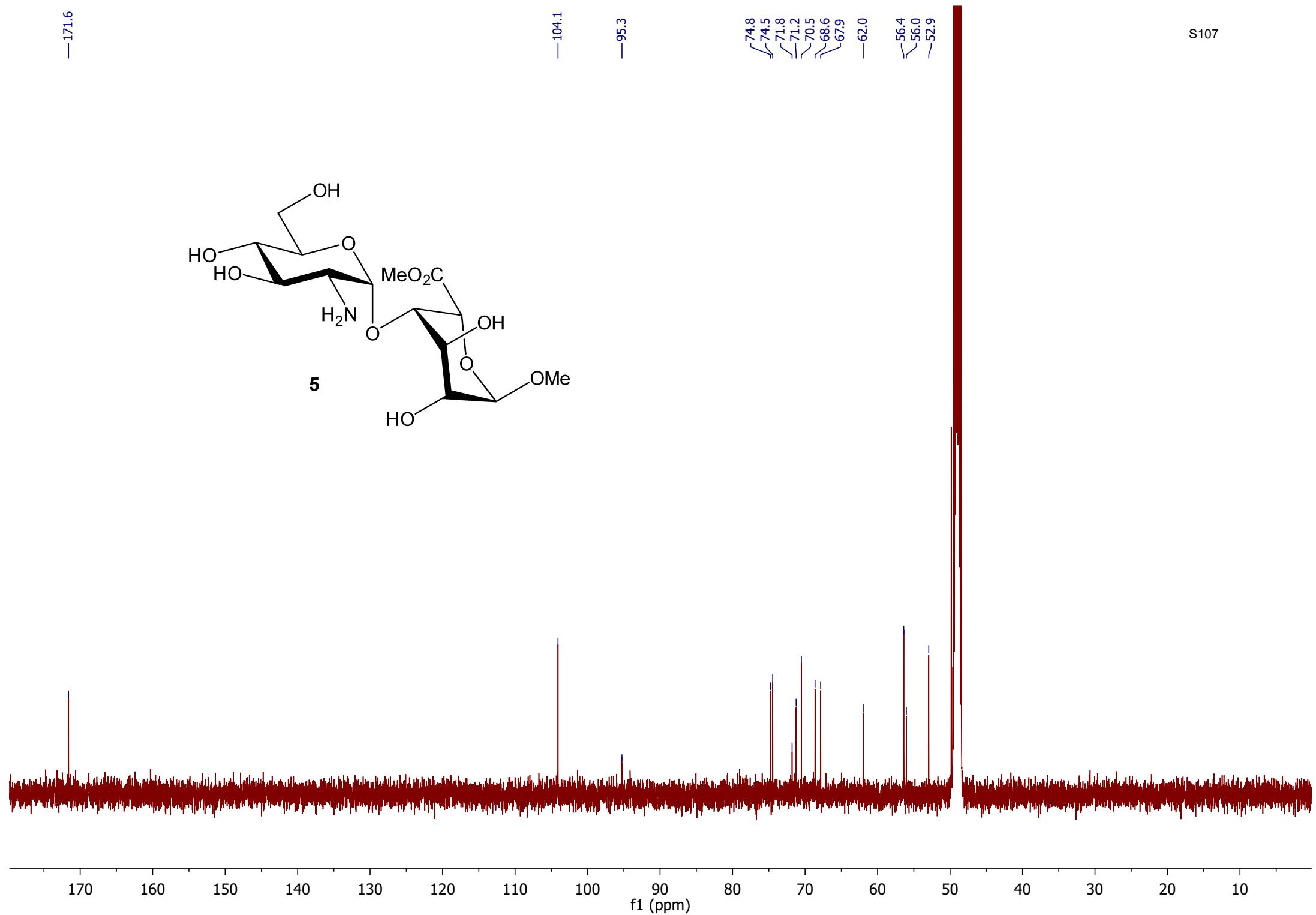
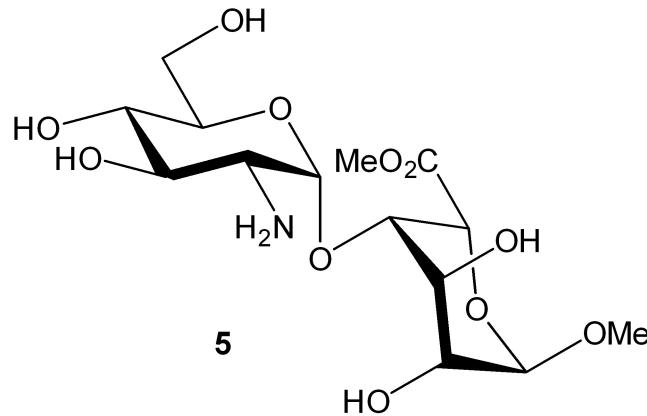


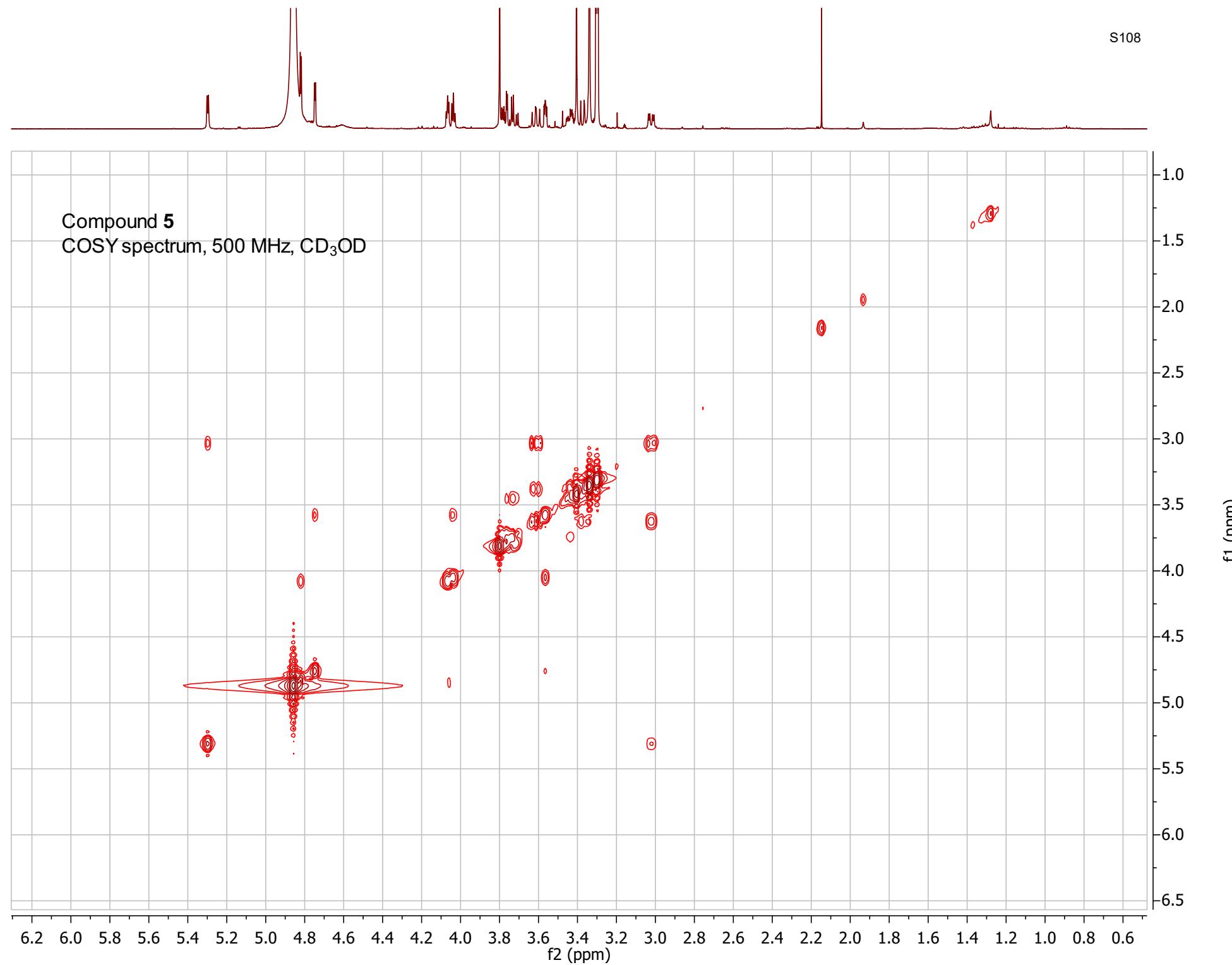
S104

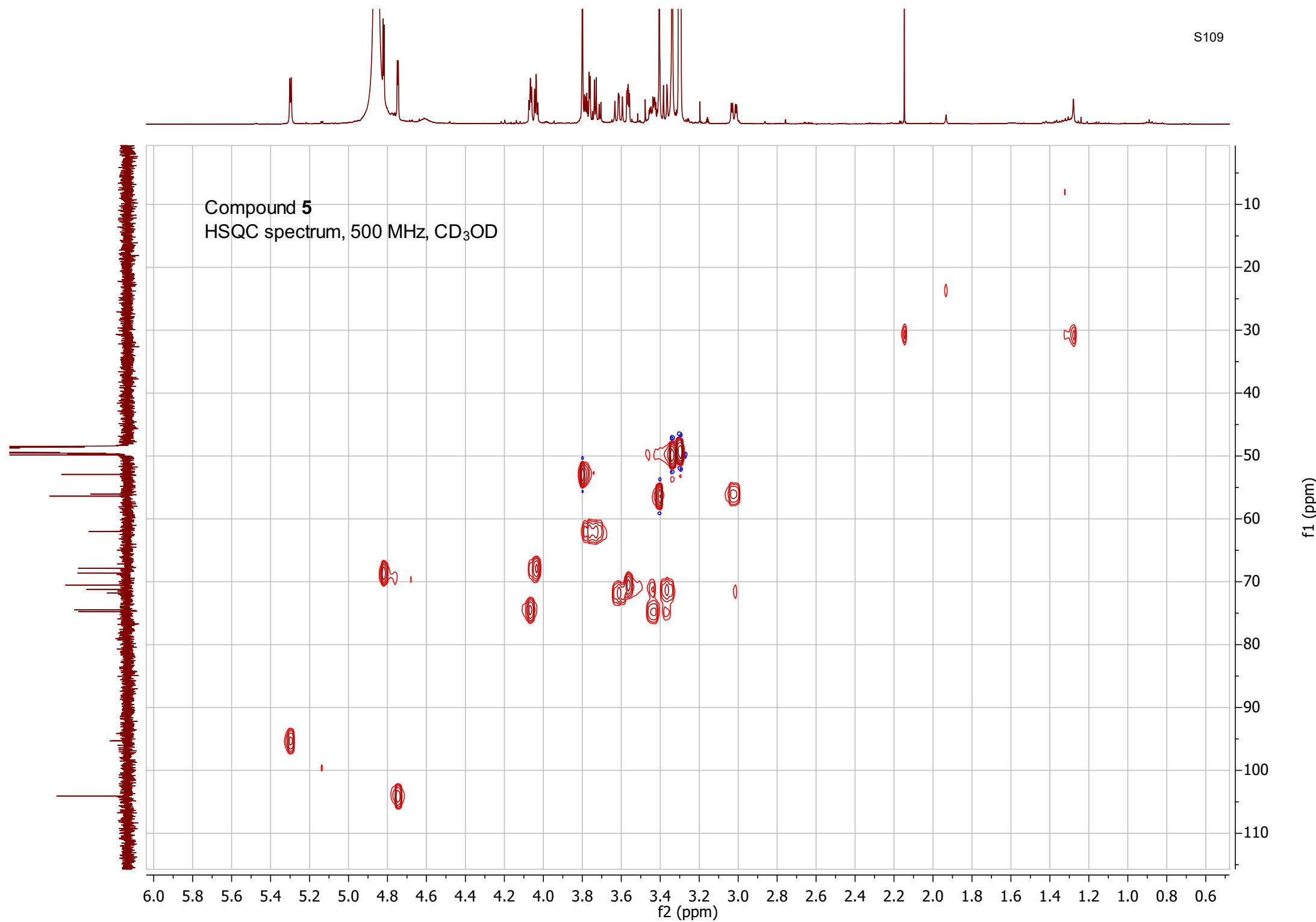


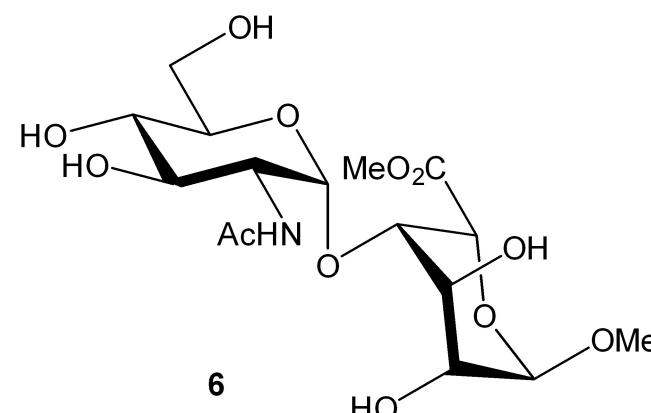
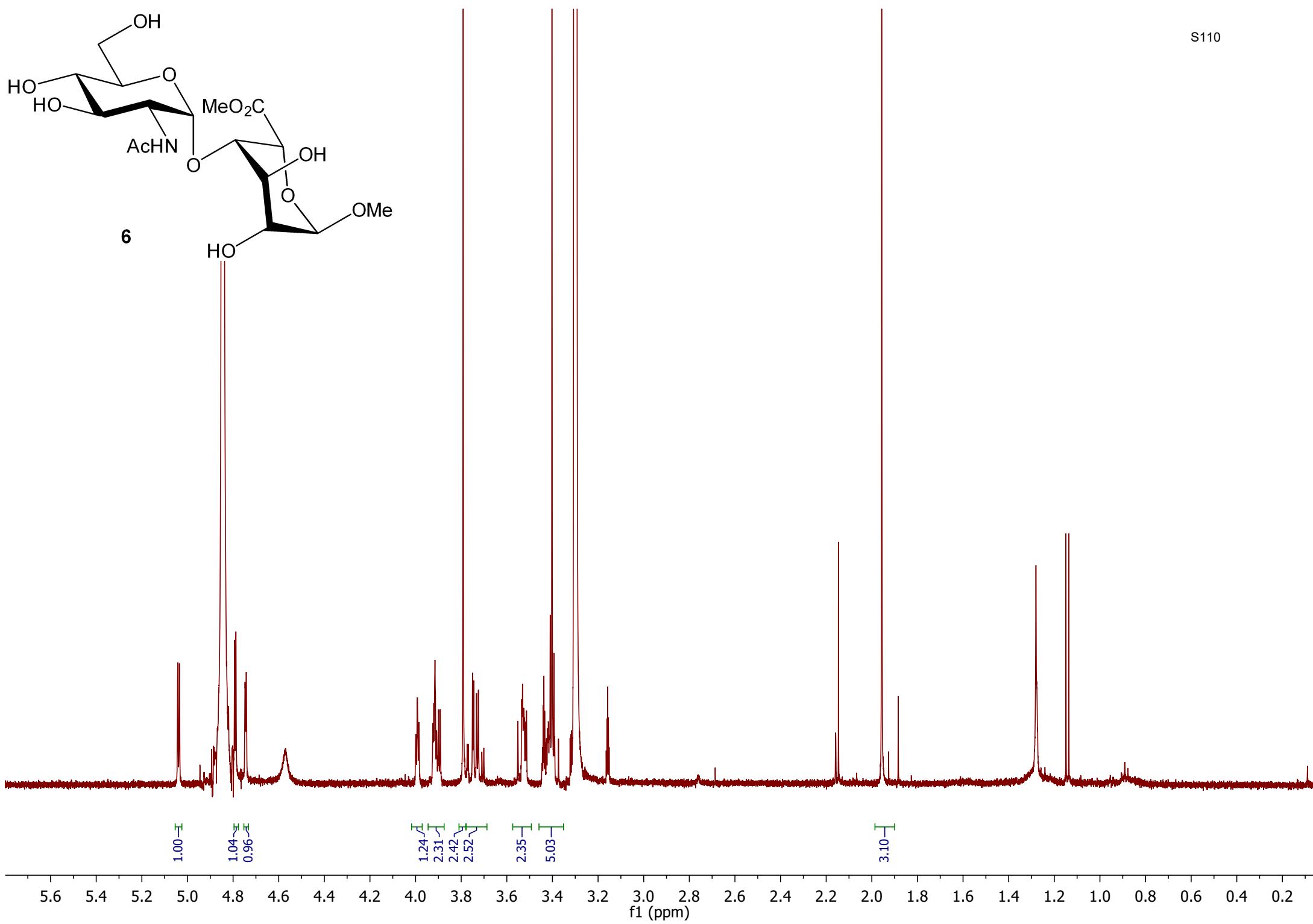




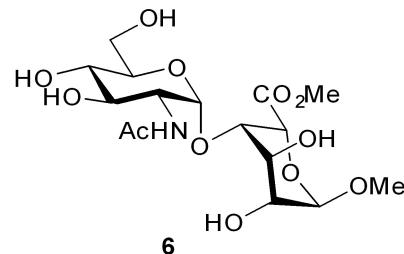






**6**

—174.0
—171.7



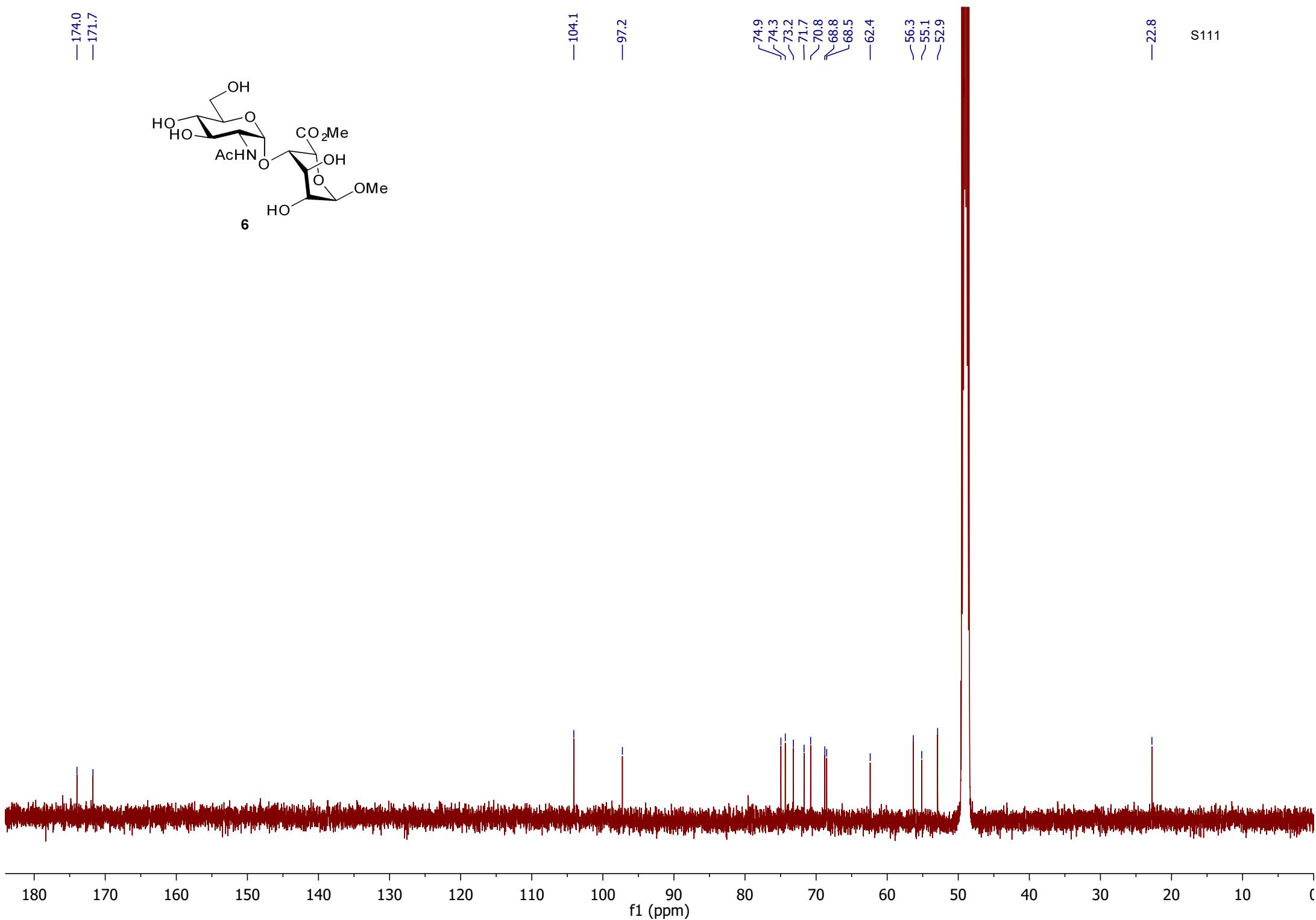
—104.1

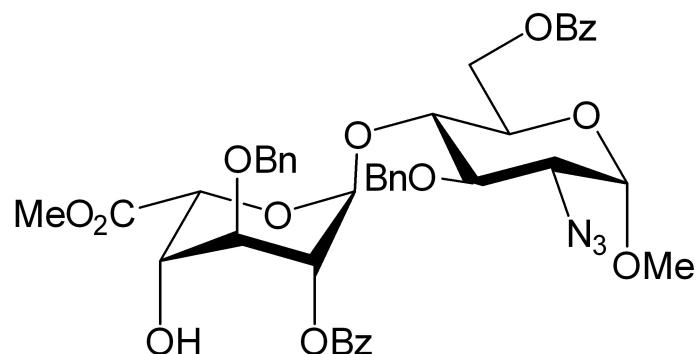
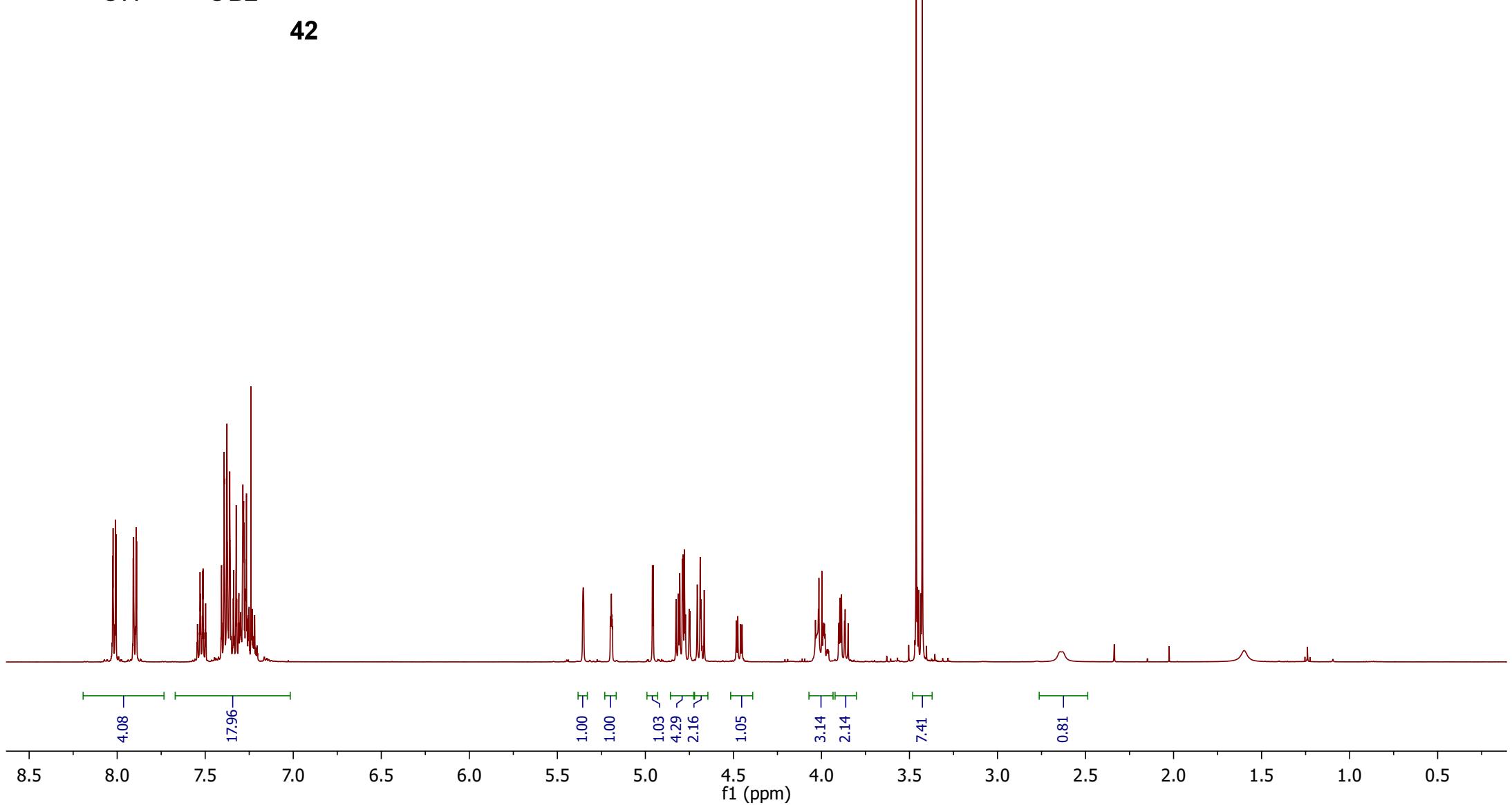
—97.2

74.9
74.3
73.2
—71.7
—70.8
68.8
68.5
—62.4
—56.3
—55.1
—52.9

—22.8

S111



**42**

-169.5
-166.0
-165.0

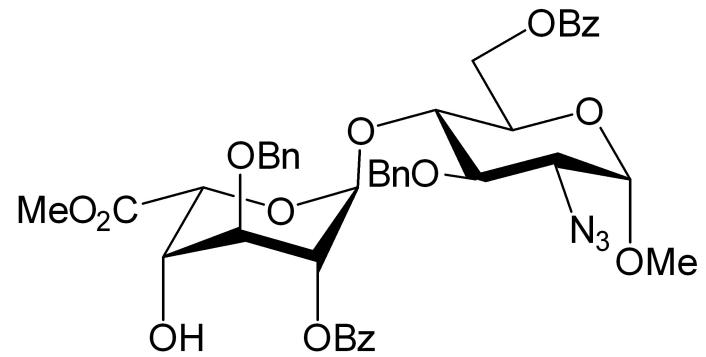
137.7
137.2
133.7
133.1
129.8
129.8
129.7
128.8
128.5
128.5
128.4
128.2
128.1
127.6
127.4

<98.5
<98.1

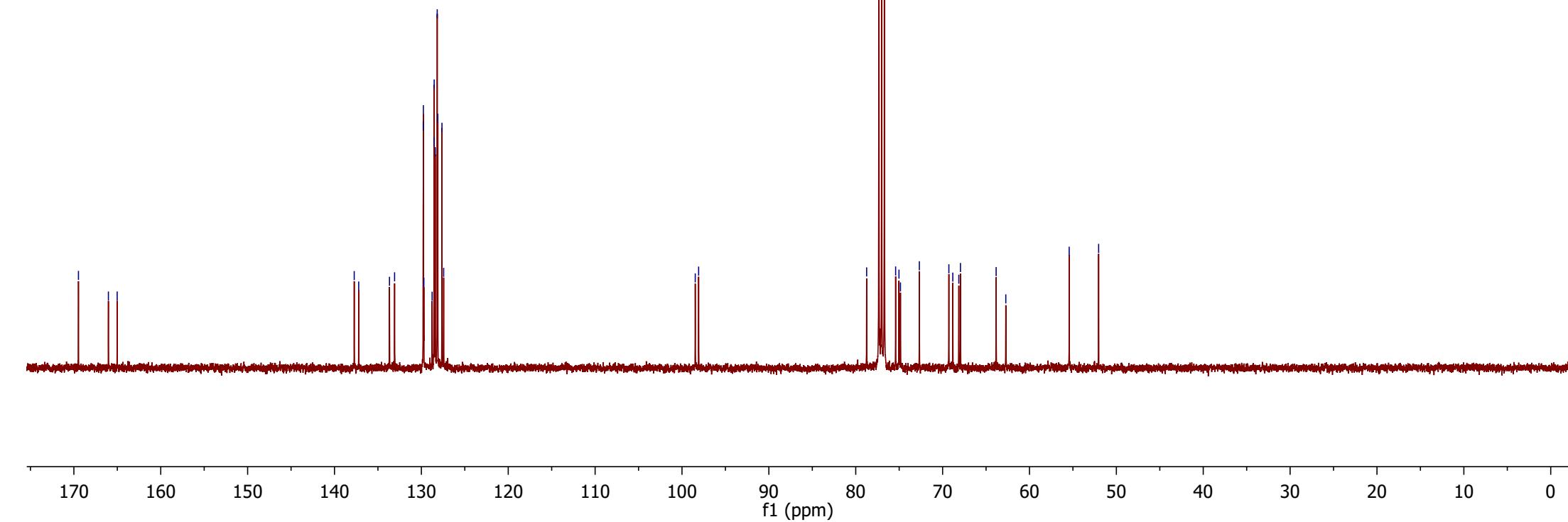
78.7
75.4
75.0
74.9
72.7
69.3
68.8
68.1
67.9
63.8
~62.7

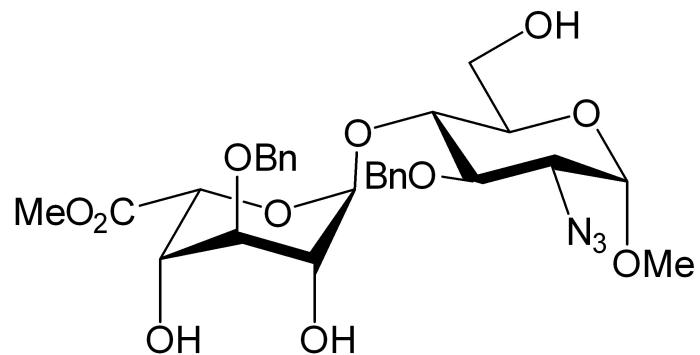
-55.4
-52.0

S113

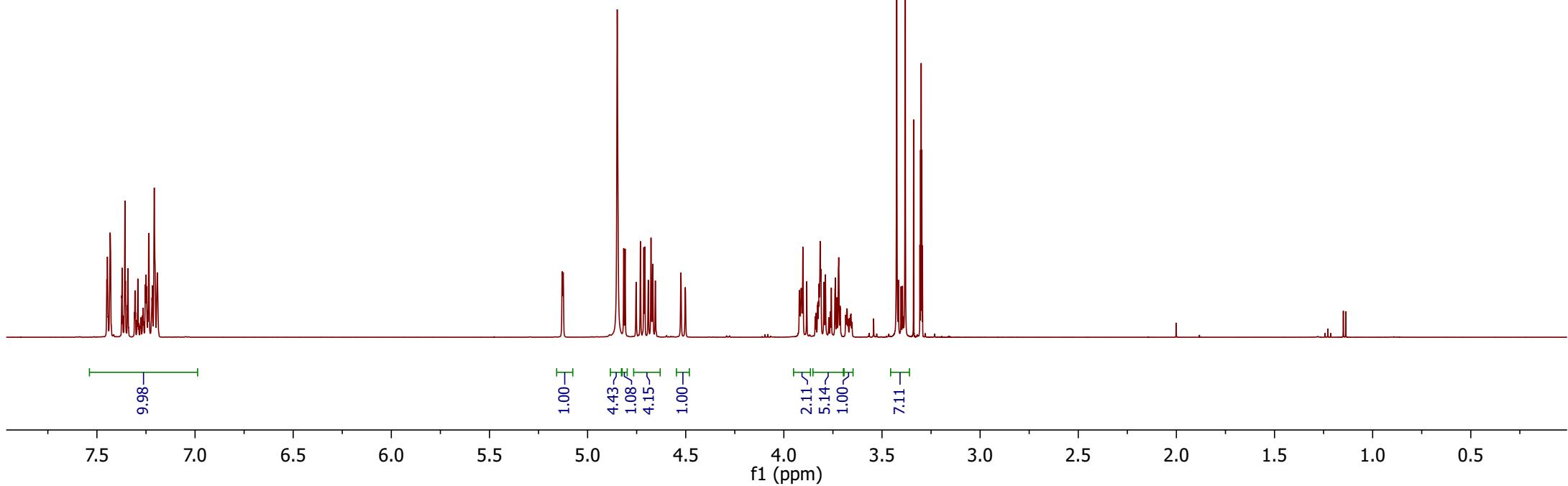


42





43



—170.5

—138.2

128.0
127.7
127.5
126.9
126.8

—100.8

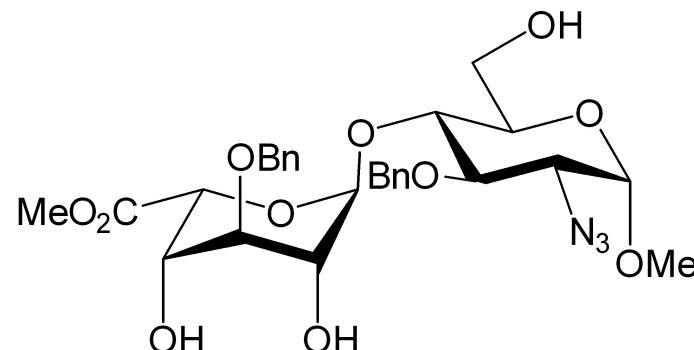
—98.7

78.3
76.6
74.4
73.7
72.0
71.9
69.4
68.7
67.1
63.5
60.2

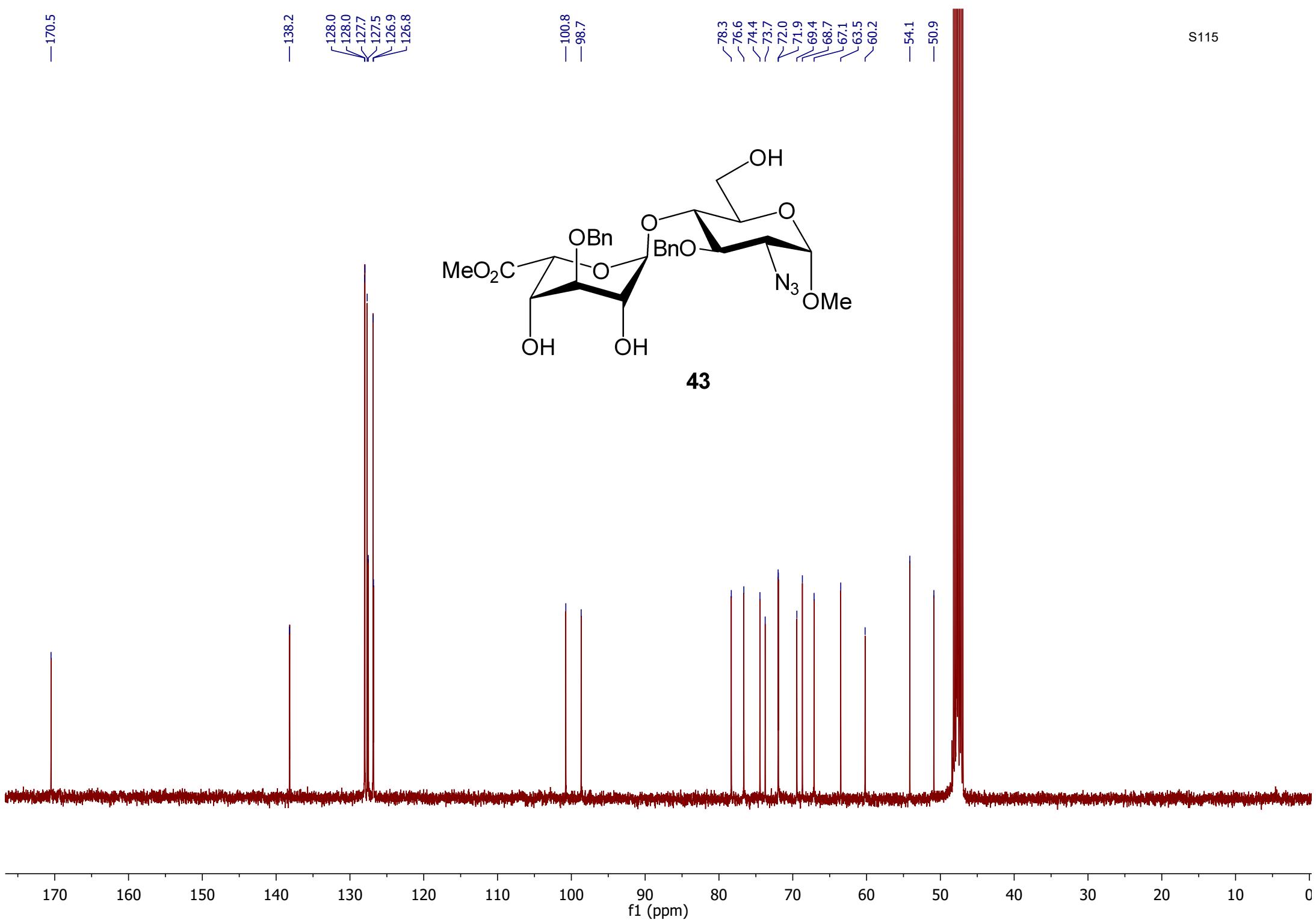
—54.1

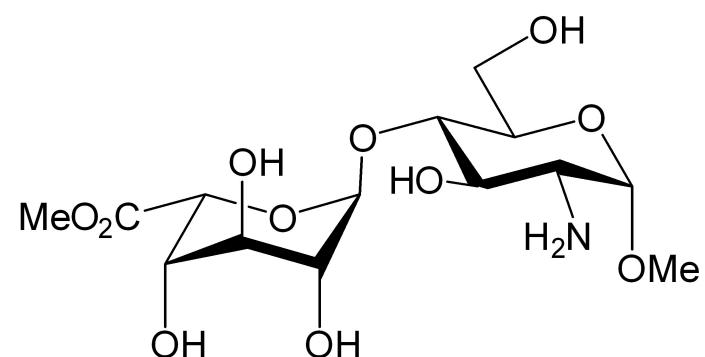
—50.9

S115

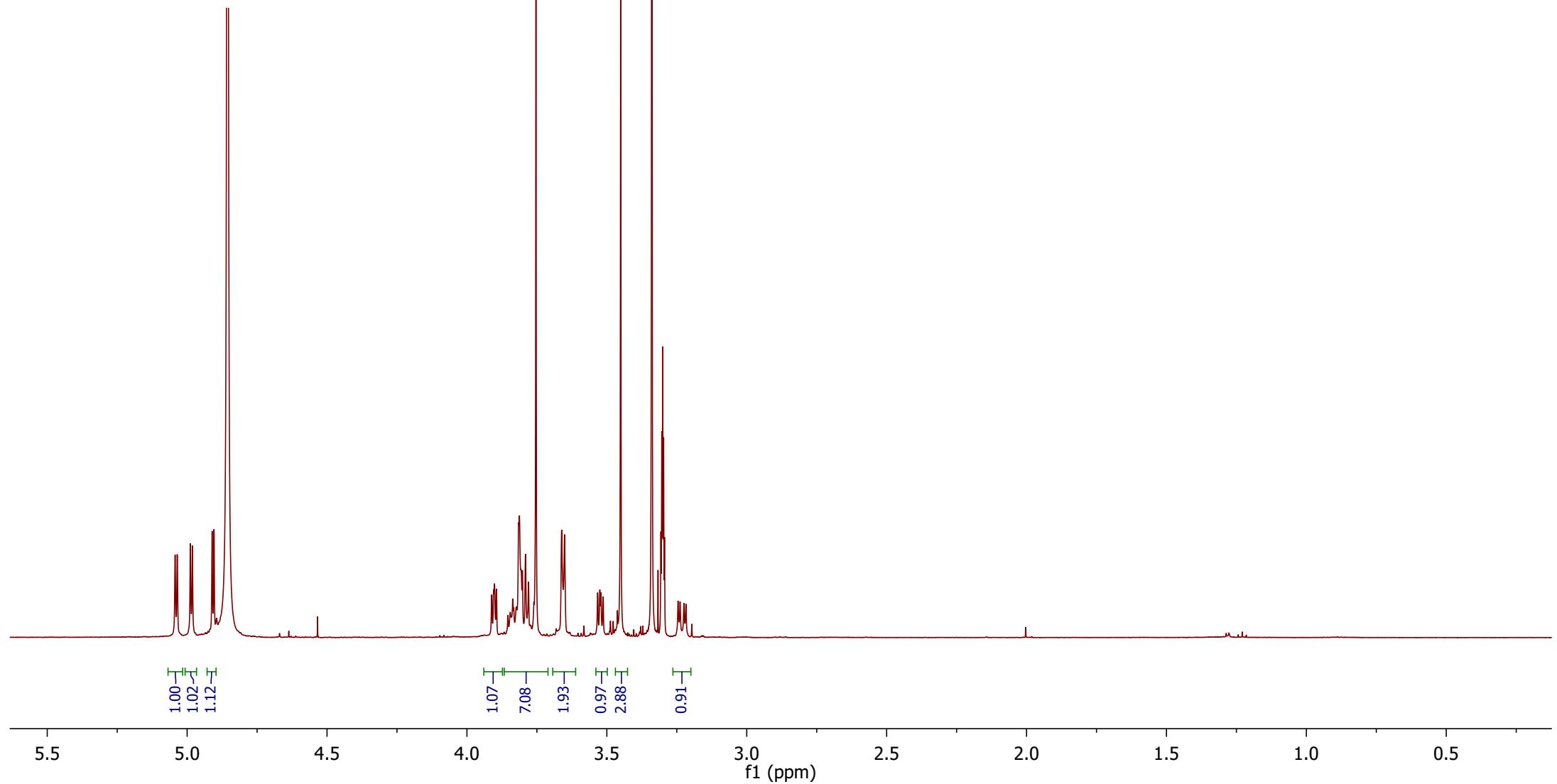


43



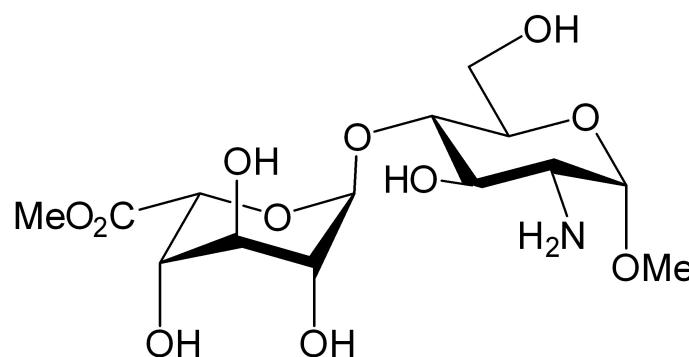


9



-172.1

S117

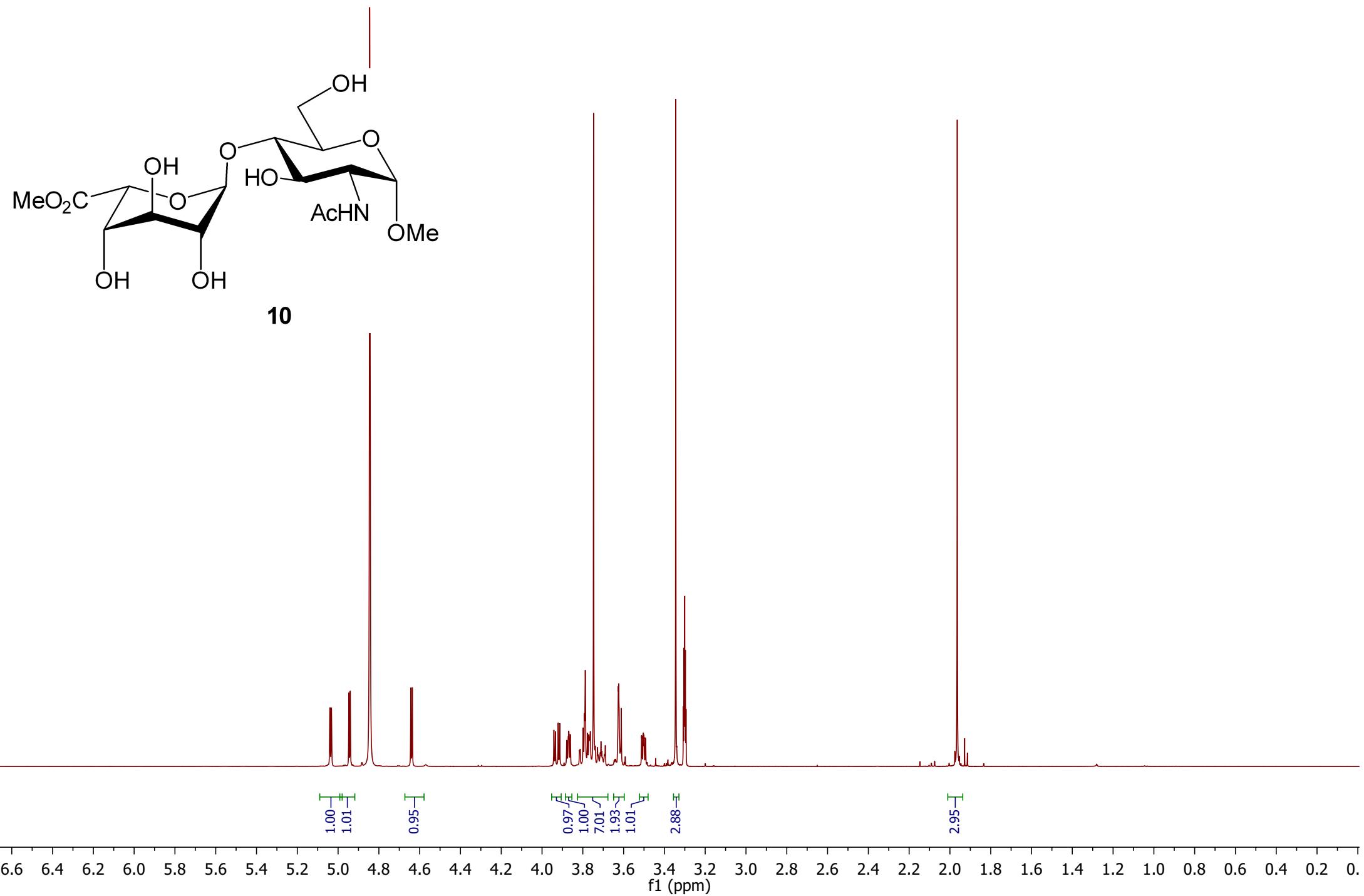


9

-103.5
-97.6
-79.3
72.7
72.4
71.8
70.3
-61.4
-55.8
-52.6

f1 (ppm)

170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0



-173.6

-172.1

-103.3

-99.6

-80.3

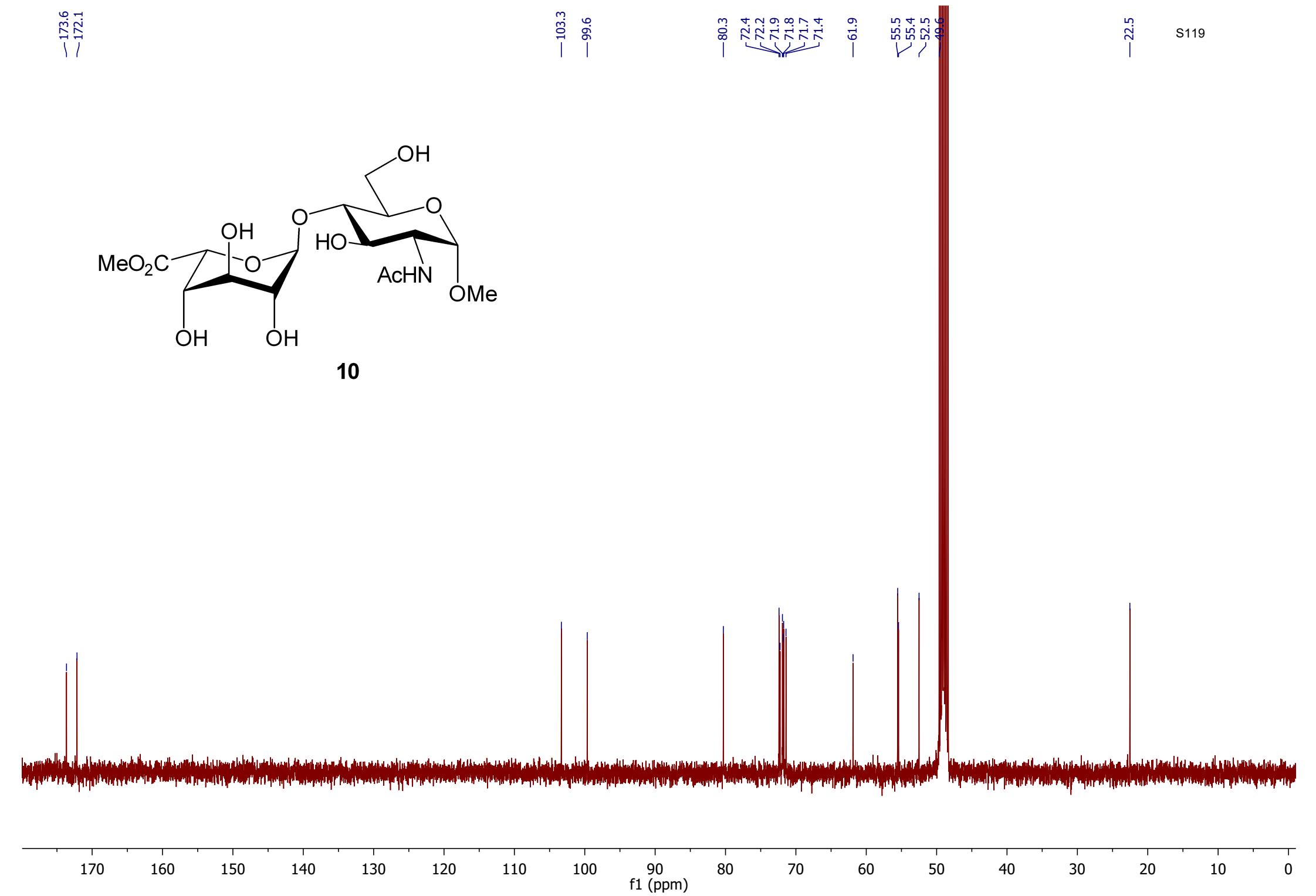
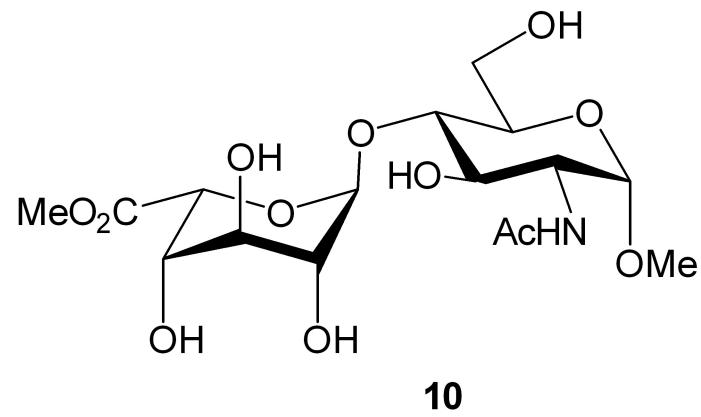
72.4
72.2
71.9
71.8
71.7
71.4

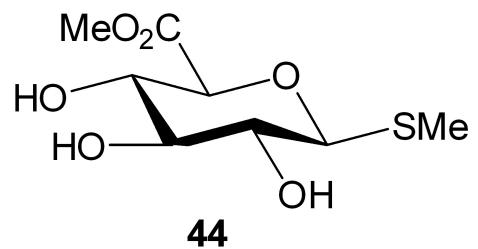
-61.9

55.5
55.4
52.5
49.6

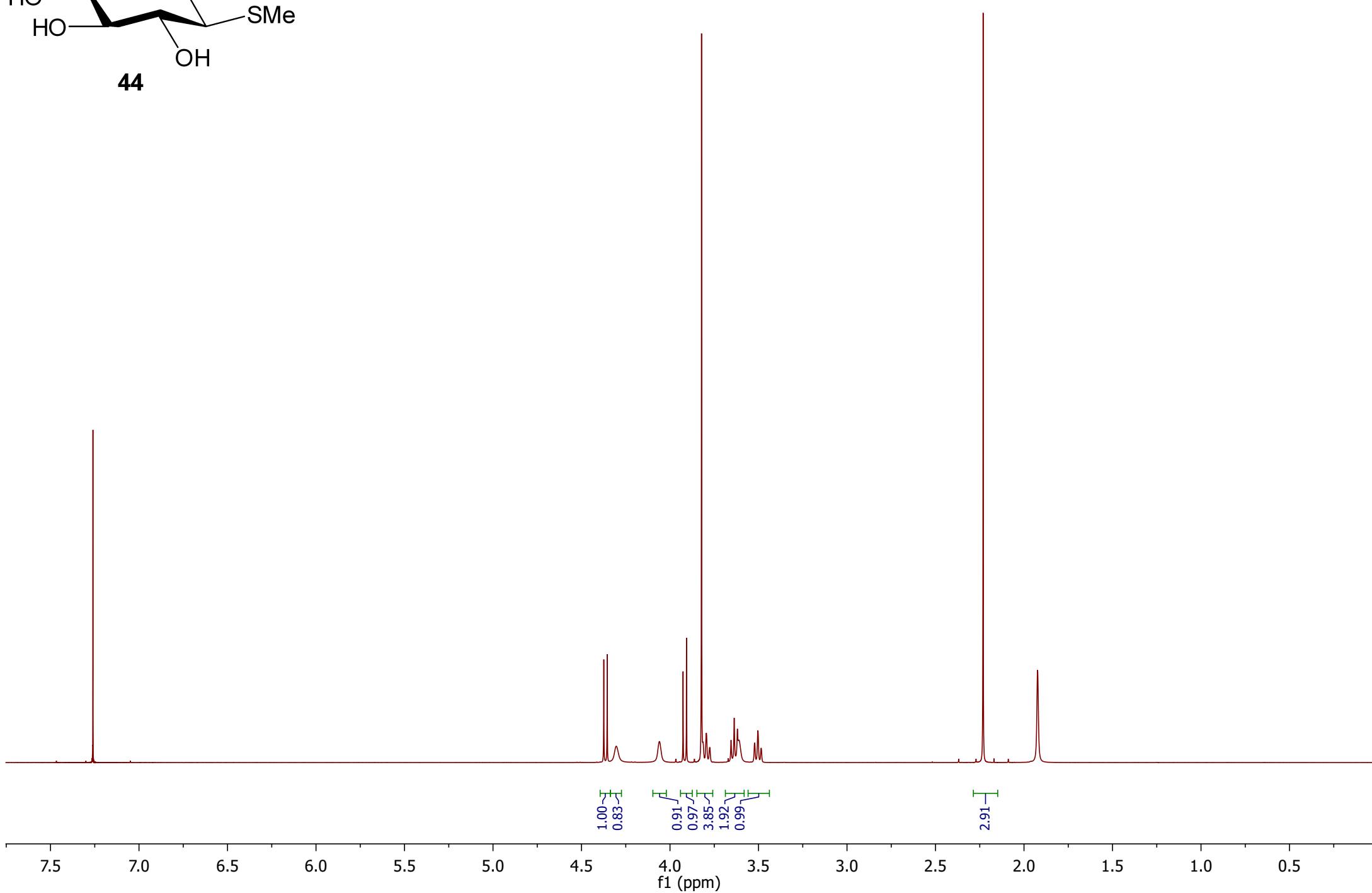
-22.5

S119

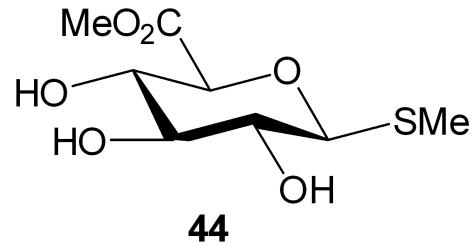




S120



—169.6



S12.3

—52.9

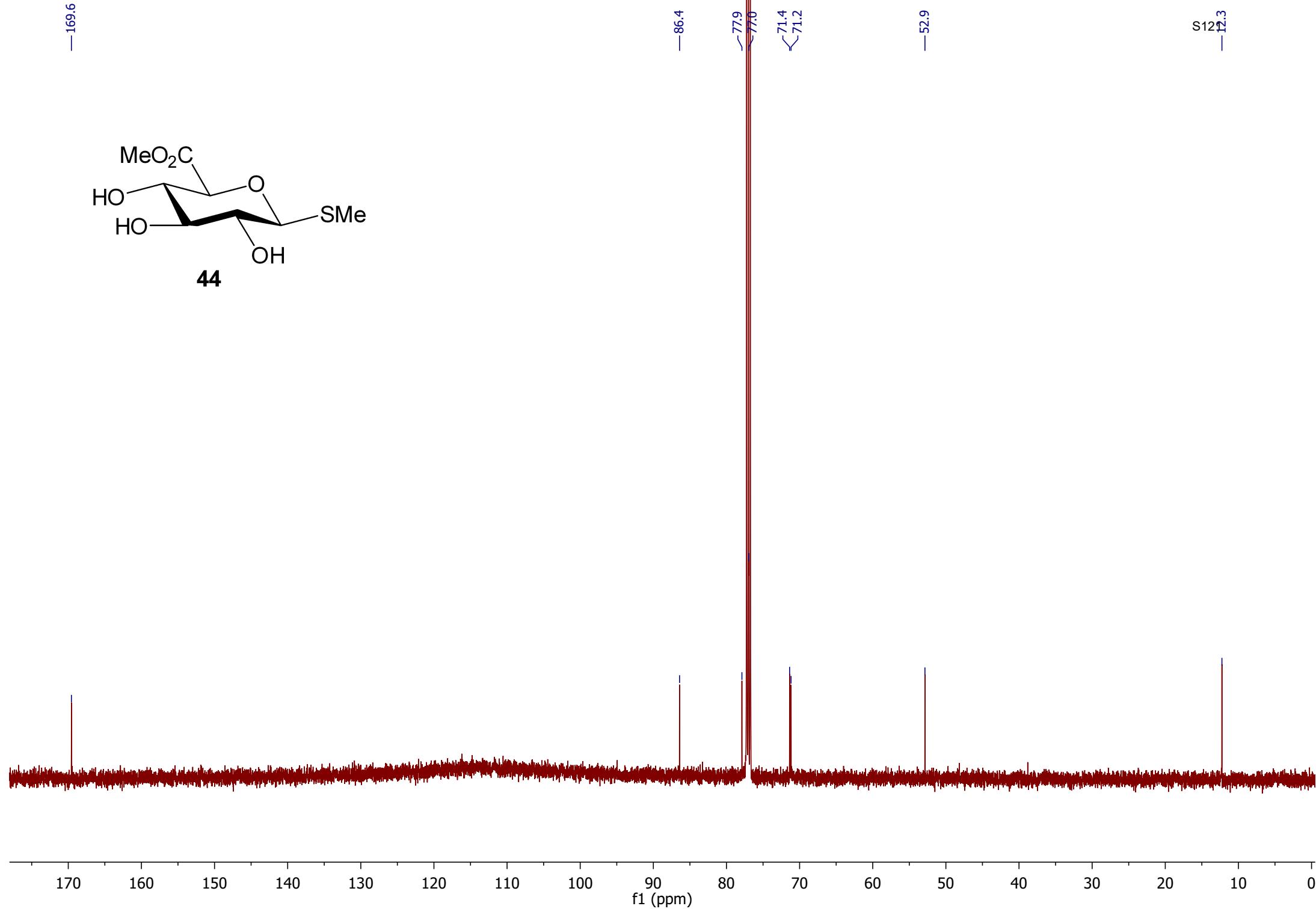
<71.4

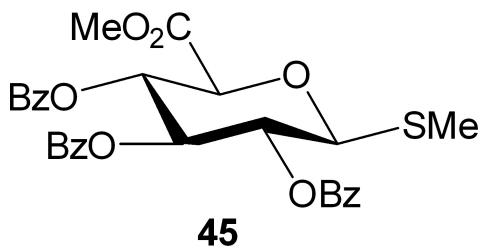
<71.2

—86.4

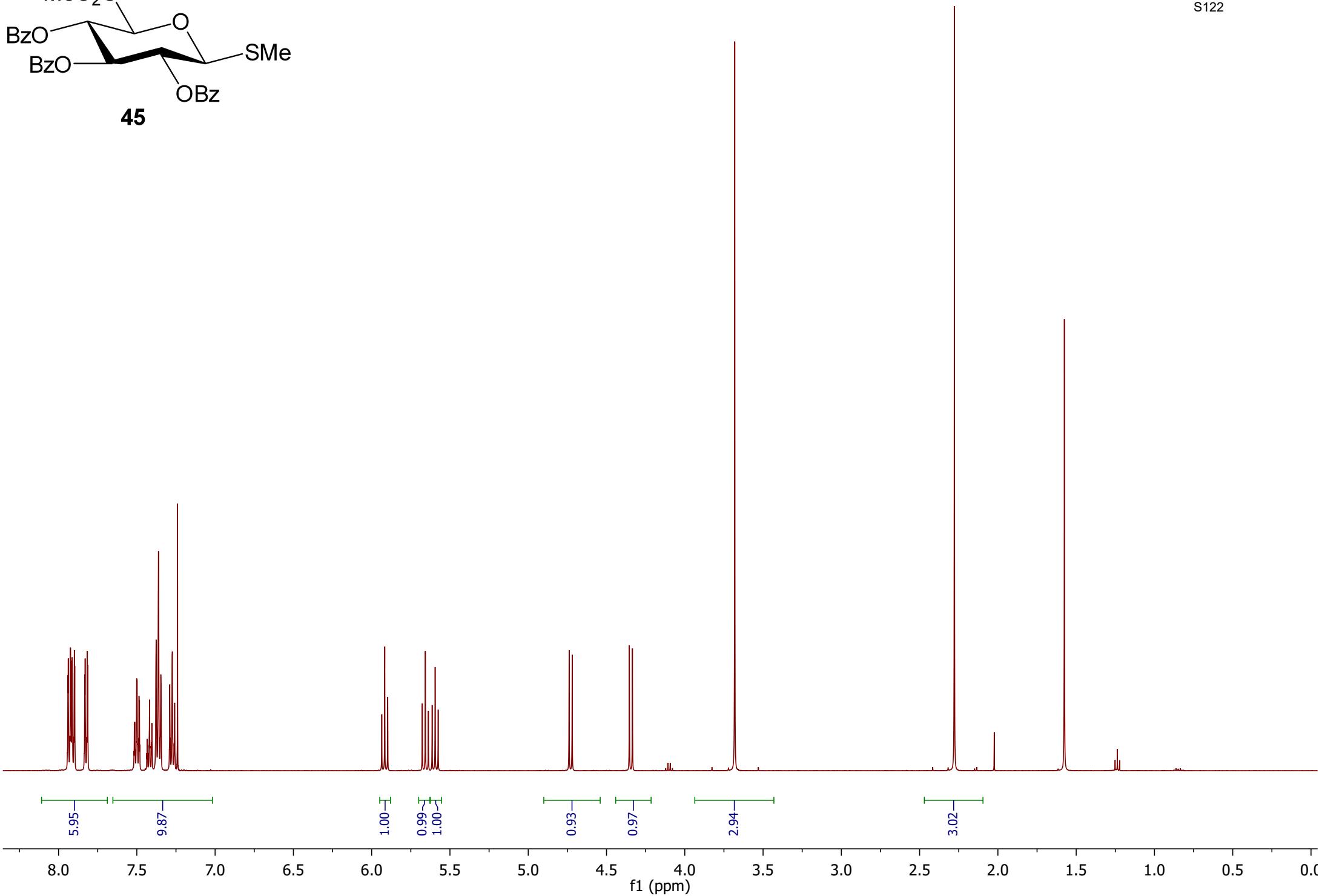
<77.0

<77.9





S122



167.1
165.7
165.2
165.2

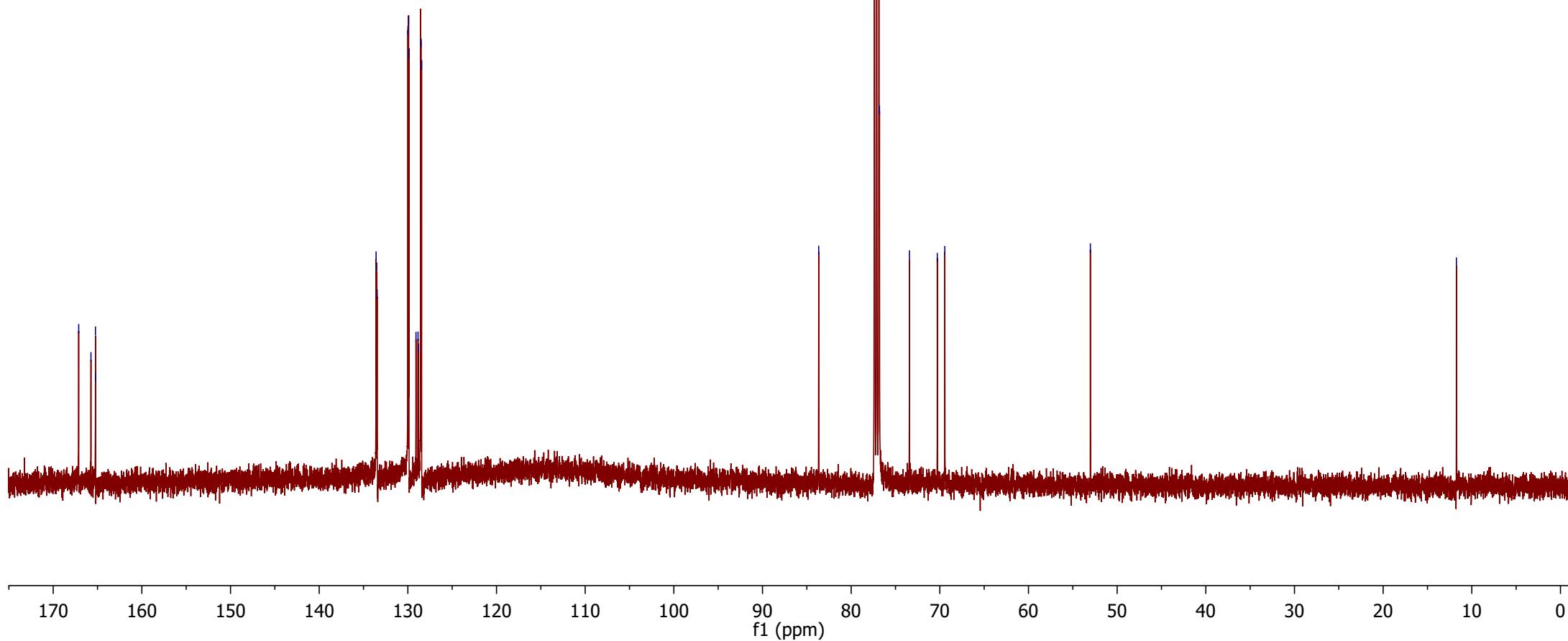
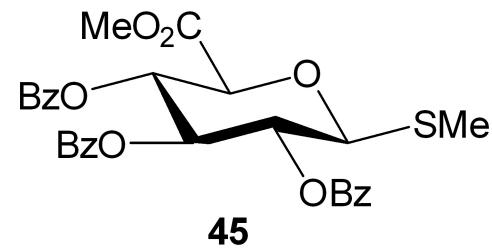
133.6
133.5
133.5
130.0
130.0
129.9
129.9
129.1
128.9
128.8
128.6
128.5
128.5

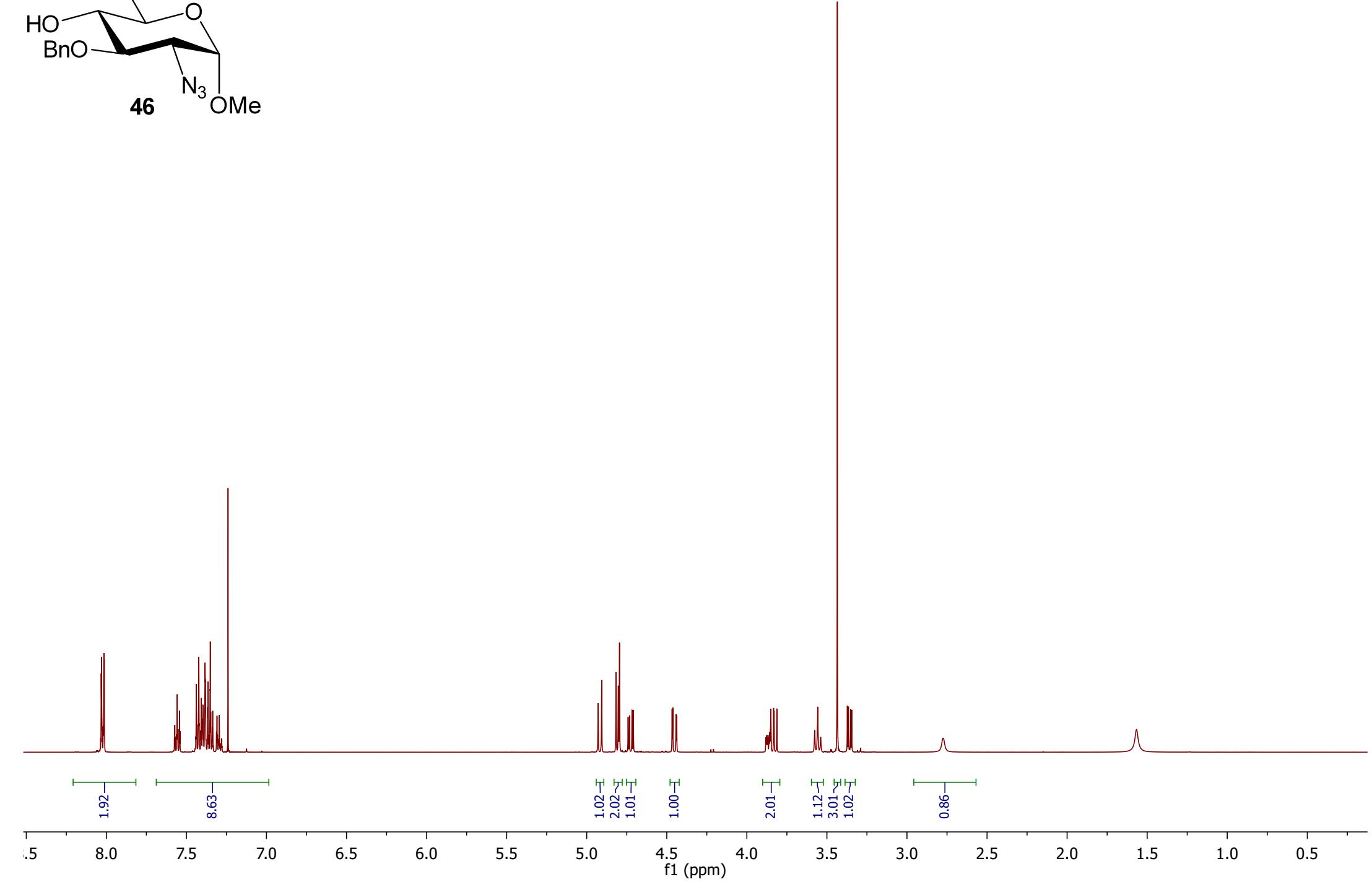
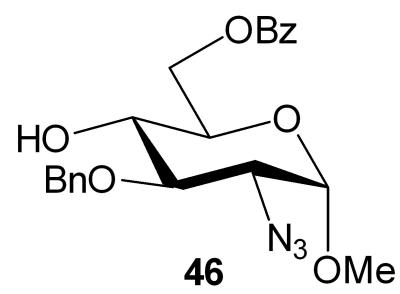
-83.7

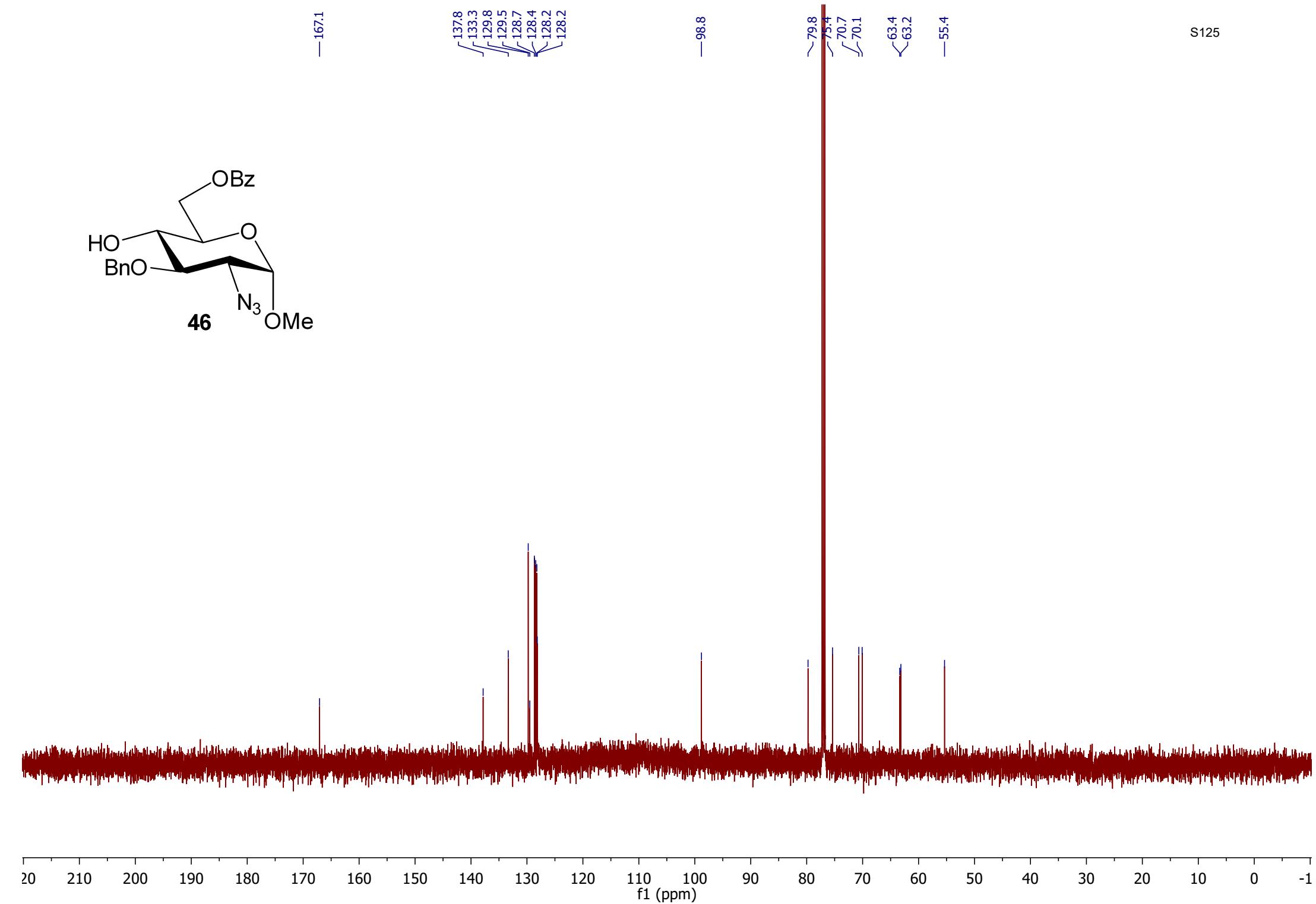
76.8
73.4
70.3
69.4

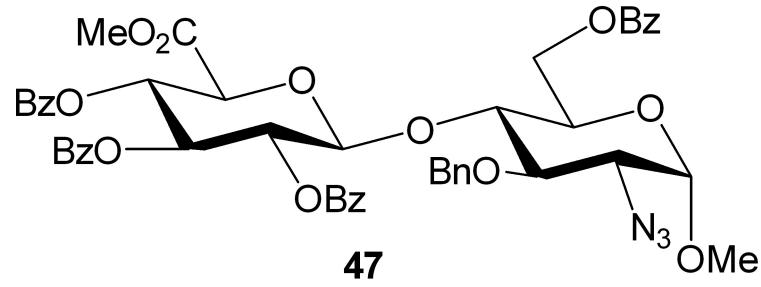
-53.0

12.7



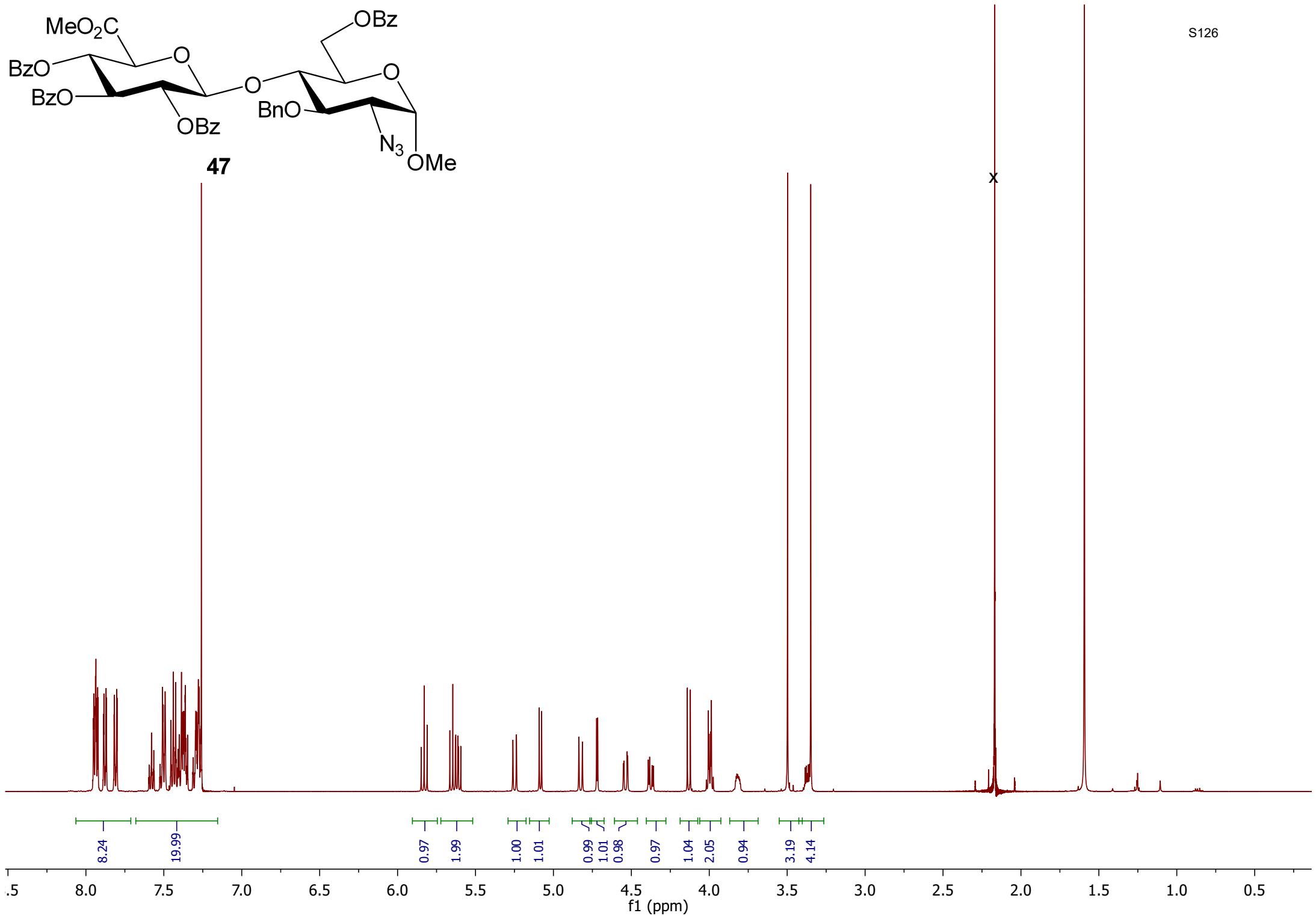


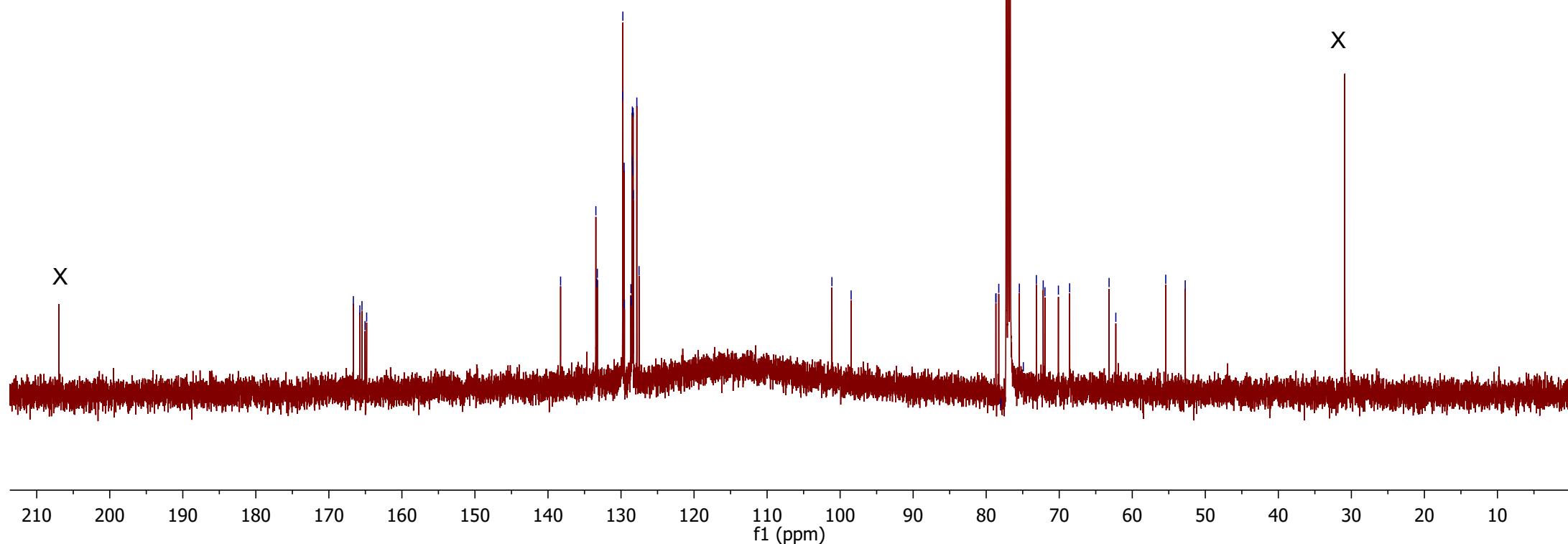
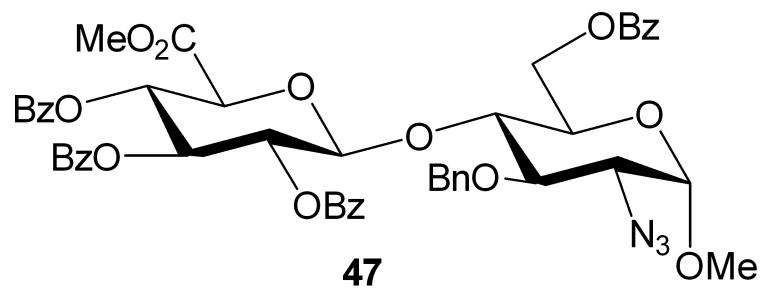


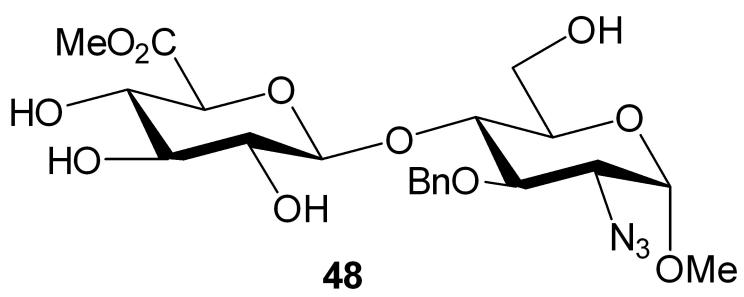


47

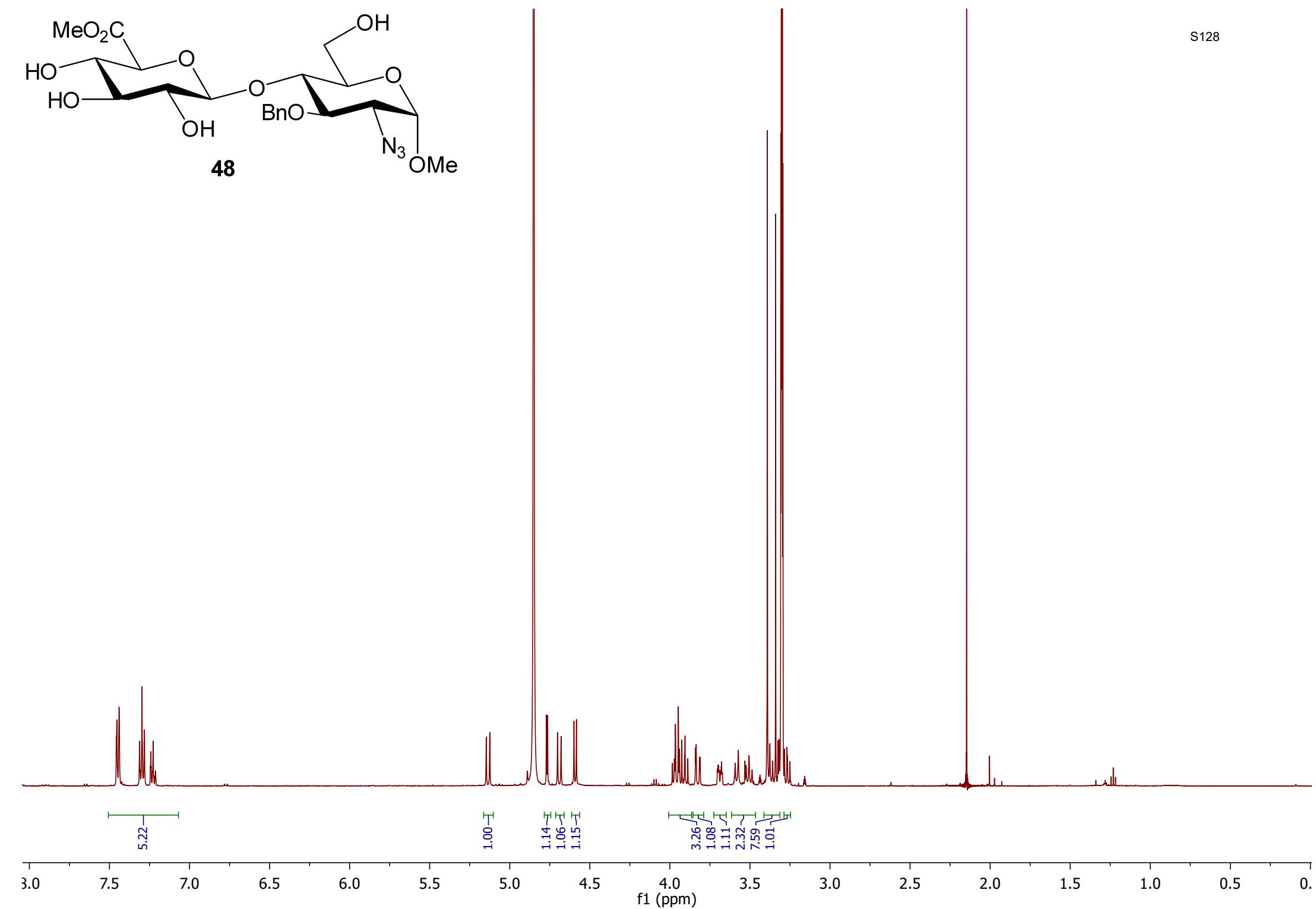
S126







S128



—139.9

✓129.6
✓129.2
✓128.5

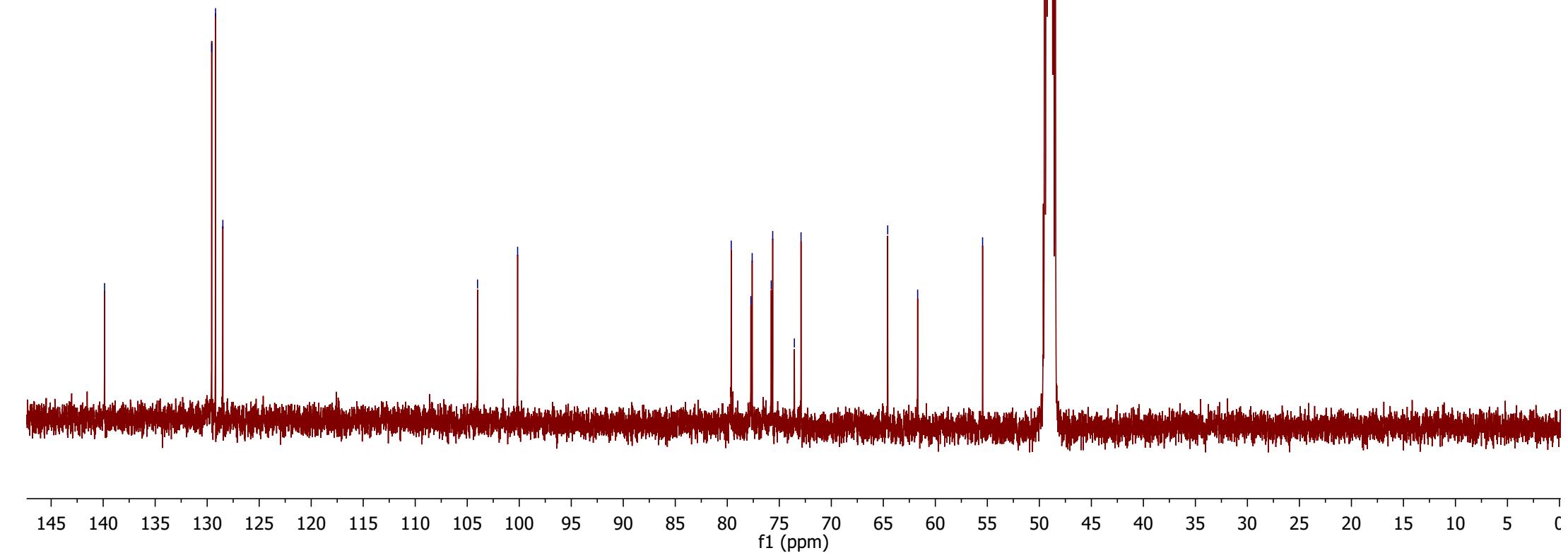
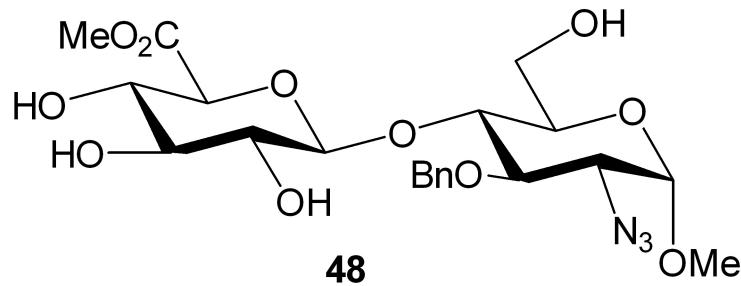
—104.0
—100.2

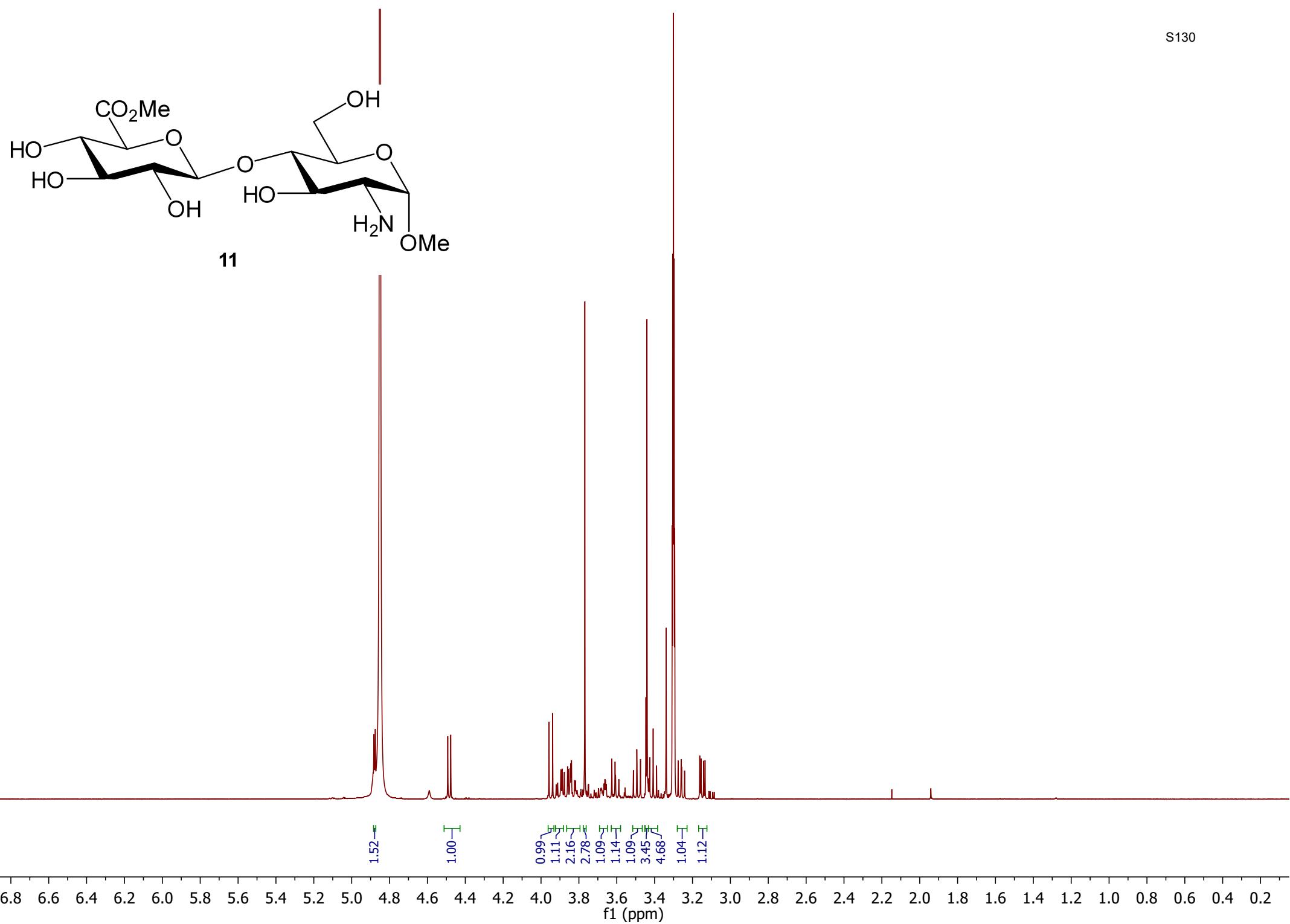
✓79.6
✓77.7
✓77.6
✓75.8
✓75.6
✓73.6
✓72.9

—64.6
—61.7

—55.5

S129





—171.2

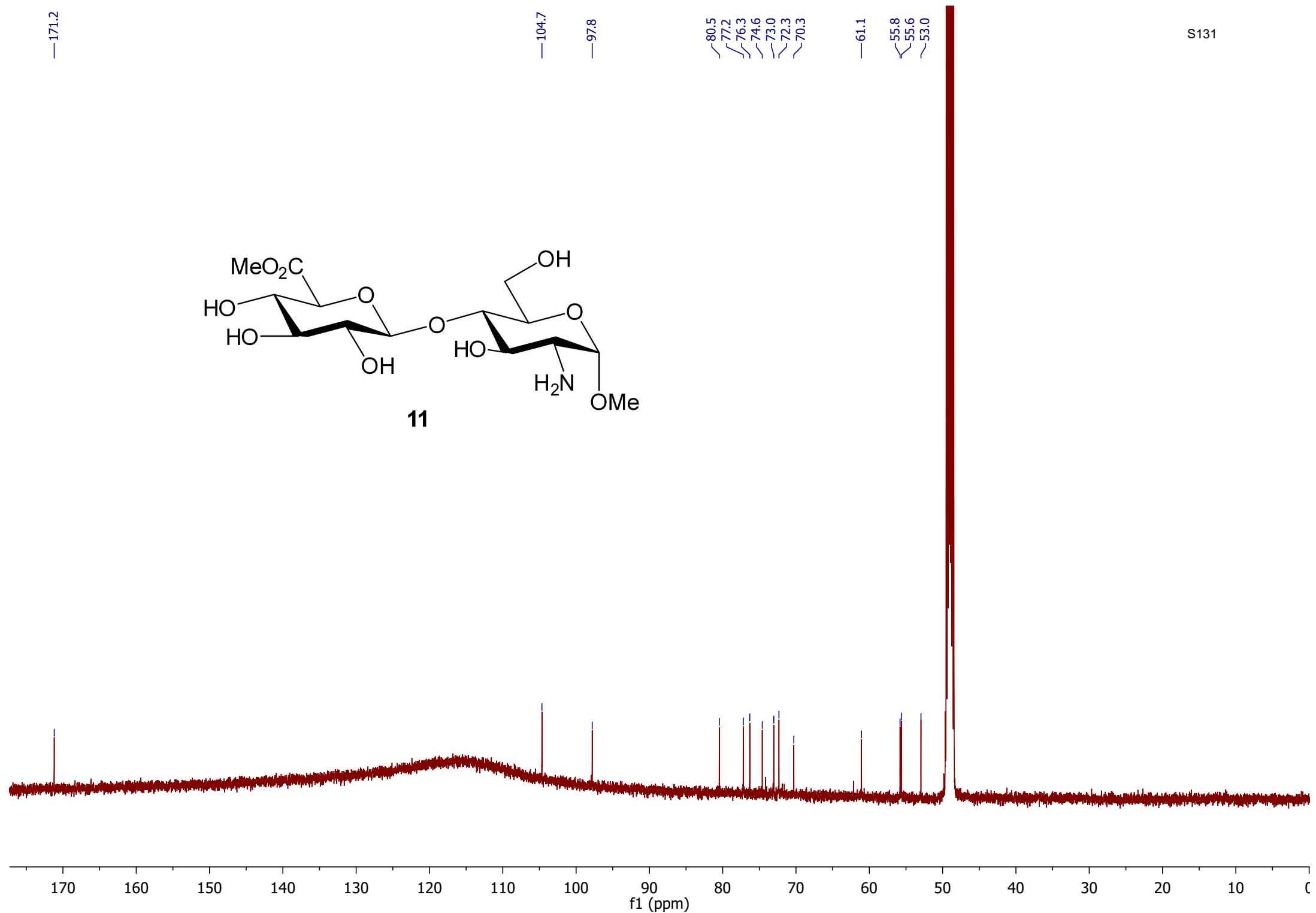
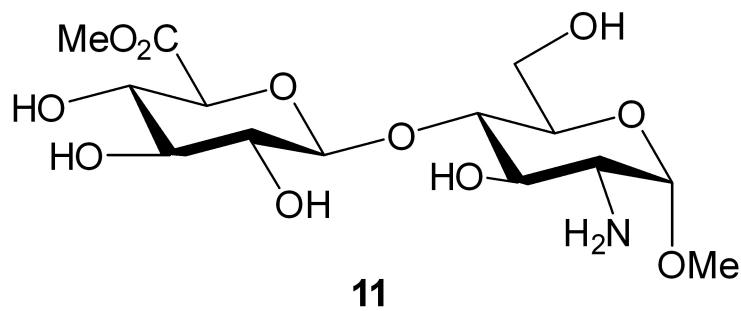
S131

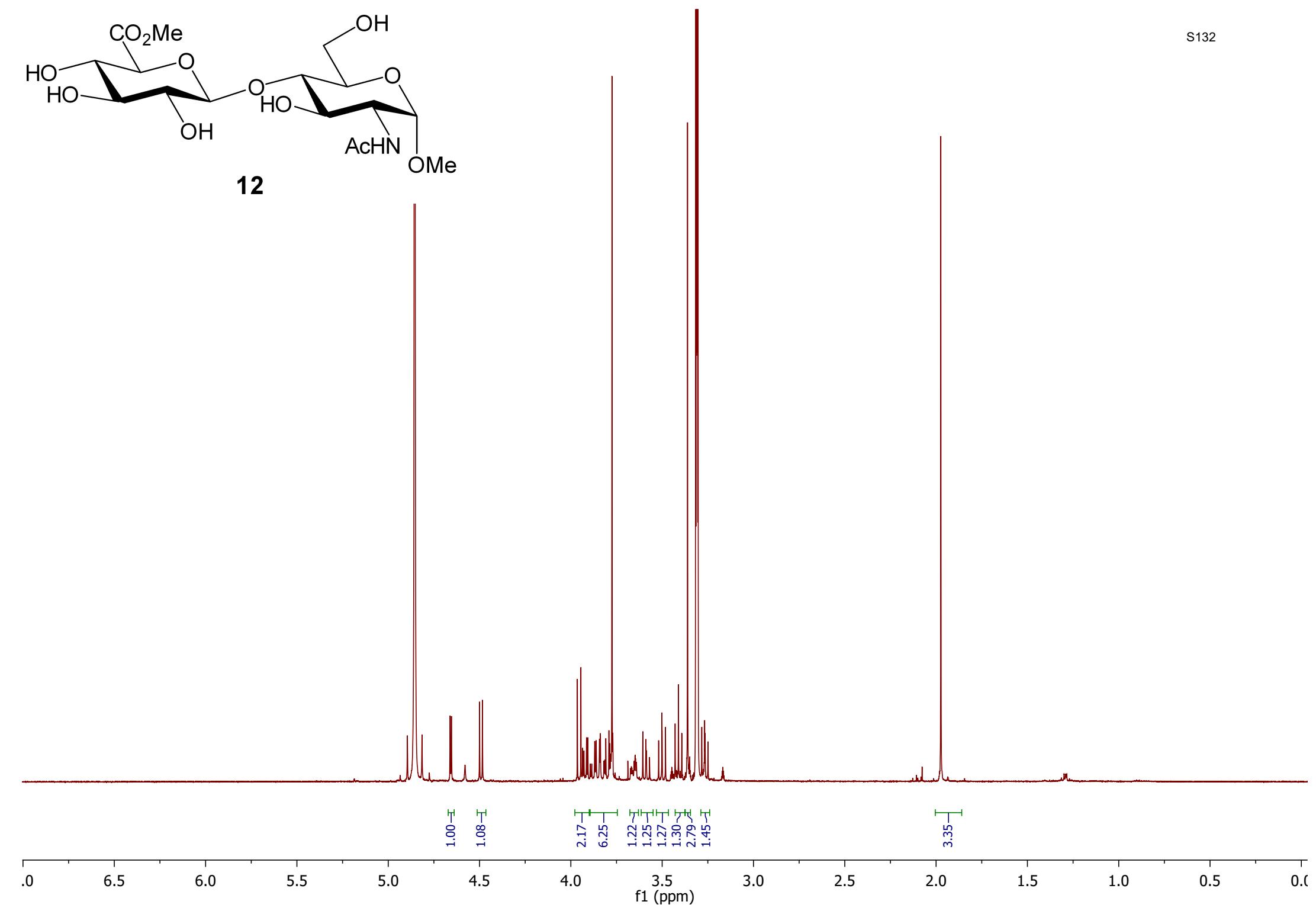
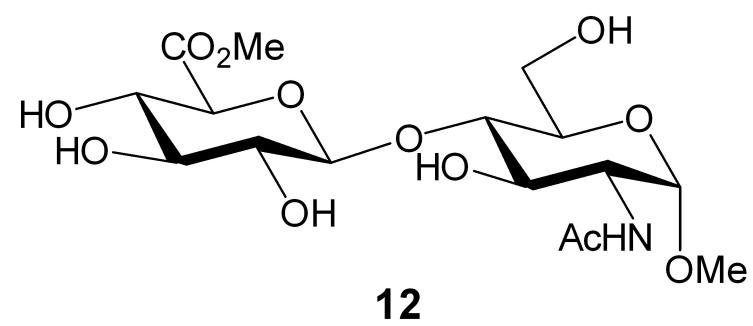
—104.7

—97.8

✓80.5
✓77.2
✓76.3
✓74.6
✓73.0
✓72.3
✓70.3

—61.1
✓55.8
✓55.6
✓53.0





—173.6

—171.0

—104.7

—99.6

—81.6

✓77.2

✓76.3

✓74.6

—73.1

✓71.9

✓71.0

—61.7

✓55.6

✓55.0

✓52.9

—22.5

S133

