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Supporting Information

A Route to Lipid ALC-0315: a Key Component of a COVID-19 mRNA Vaccine

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Supporting Information

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Experimental Protocols. Unless otherwise noted, all reactions were carried under ordinary atmosphere in sealed (septum) glassware. Dry THF was freshly distilled from Na/benzophenone; dry CH_2Cl_2 and triethylamine were freshly distilled from CaH_2. All other commercial solvents and compounds were used as received. Thin layer chromatography (TLC) was carried out with aluminum plates coated with silica gel (Merck 60 F254 plates) and column chromatography was performed with Merck silica gel 60 (230-400 mesh; 40−63 μm). All NMR spectra were recorded at room temperature from $CDCl_3$ solutions at 300 MHz for ^1H and 75 MHz for ^{13}C. Solvent signals were used as references to calibrate spectra (residual $CHCl_3$ in $CDCl_3$: δ_H = 7.26 ppm; $CDCl_3$: δ_C = 77.1 ppm; residual CH_3OH in CD_3OD: δ_H = 3.31 ppm; CD_3OD: δ_C = 49.8 ppm;). Low- and high-resolution mass spectra (HRMS) were recorded in the ESI mode.

6-Hydroxyhexyl 2-hexyldecanoate (2). Prepared as described in ref. 2. **^{1}H NMR** (300 MHz, $CDCl_3$) δ = 4.05 (t, 2H, *J* = 7.4 Hz), 3.63 (t, 2H, *J* = 7.7 Hz), 2.29 (m, 1H), 1.67-1.17 (c m, 33H), 0.86 (br t, 6H, *J* = 7.6 Hz); **^{13}C NMR** (75 MHz, $CDCl_3$) δ = 176.8, 64.1, 62.9, 45.9, 32.7, 32.6 (2 peaks), 31.9, 31.8, 29.7, 29.5, 29.3 (2 peaks), 28.8, 27.6 (2 peaks), 25.9, 25.5, 22.7 (2 peaks), 14.1 (2 peaks); **LRMS** m/z = 379 $[M+Na]^+$.

6-Oxohexyl 2-hexyldecanoate (3). A mixture of 10 mL each of saturated aqueous $NaHCO_3$ and commercial bleach was added over 1 min at room temperature to a vigorously stirred solution of 6-hydroxyhexyl 2-hexyldecanoate (3.0 g, 8.4 mmol) and TEMPO (263 mg, 1.7 mmol, 0.2 equiv) in CH_2Cl_2 (17 mL). After completion of the addition, more bleach (10 mL) was added dropwise over 20 min with continued stirring. After 2 h the reaction was complete (^{1}H NMR). Aqueous saturated $Na_2S_2O_3$ solution (20 mL) was added to quench excess bleach. The CH_2Cl_2 phase was

separated and the aqueous phase was extracted with more CH_2Cl_2 (3 x 30 mL). The combined organic phases were washed with aqueous saturated $NaHCO_3$ (15 mL), dried ($MgSO_4$), and concentrated, to afford 2.7 g (90%) of crude aldehyde as a red oil. The red color, of unknown origin, could not be removed by chromatography or by treatment with charcoal. Accordingly, the aldehyde, which appeared to be of very good quality by ^{1}H and ^{13}C NMR, was used in crude form for the reductive amination step. **^{1}H NMR** (300 MHz, $CDCl_3$) δ = 9.77 (t, *J* = 1.6 Hz, 1H), 4.11 (t, *J* = 6.6 Hz, 2H), 2.49 (dt, J_1 = 6.9 Hz, J_2 = 1.6 Hz, 2H), 2.35 (m, 1H), 1.8-1.2 (c m, 30H), 0.92 (t, *J* = 6.3 Hz, 6H). **^{13}C NMR** (75 MHz, $CDCl_3$) δ = 201.3, 175.7, 62.7, 44.8, 42.8, 31.6 (2 peaks), 30.9, 30.7, 28.6, 28.5, 28.3 (2 peaks), 27.5, 26.5 (2 peaks), 24.6, 21.7, 21.6, 20.7, 13.2 (2 peaks); **LRMS** (FD) m/z = 355 $[M+H]^+$.

4-Aminobutanol, *tert*-butyldiphenylsilyl ether (5). A solution of *tert*-butyl(chloro)-diphenylsilane (TBDPSCl; 6.8 g, 24.7 mmol, 1.1 equiv) in CH_2Cl_2 (4 mL) was added dropwise during 15 min to a well-stirred solution of 4-amino-1-butanol (2.0 g, 22.4 mmol, 1.0 equiv) and imidazole (3.4 g, 49.3 mmol, 2.2 equiv) in DCM (5 mL). The mixture was stirred overnight at room temperature. The reaction mixture was sequentially washed with sat. aq. $NaHCO_3$ solution (2×5 mL), water (2×5 mL), and sat. aq. NaCl chloride solution (2×5 mL), then dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure to furnish **7** (6.72 g, 92 %) as a yellow oil. **^{1}H NMR** (300 MHz, $CDCl_3$) δ = 7.71 – 7.68 (m, 4H), 7.40 – 7.36 (m, 6H), 3.70 (t, *J*=6.0 Hz, 2H), 2.67 (t, *J*=6.6 Hz, 2H), 1.86 (s, 2H), 1.65 – 1.48 (m, 4H), 1.09 (s, 9H); **^{13}C NMR** (75 MHz, $CDCl_3$) δ = 135.4, 133.8, 129.4, 127.5, 63.6, 41.8, 29.9, 29.8, 26.7, 19.0.

Sodium tri(propionyloxy)borohydride. A solution of propionic acid (17.8 mL, 17.6 g, 237.6 mmol, 3.0 equiv) in CH_2Cl_2 (10 mL) was added by syringe pump over 2h to a cold (0 °C) suspension of $NaBH_4$ (3.0 g, 78.9 mmol, 1.0 equiv) in dry CH_2Cl_2 (70 mL) in a sealed (septum) flask fitted with a balloon (**CAUTION**: H_2 evolution). The resulting mixture was stirred at room temperature for 30 min. The solvent was removed under reduced pressure and the residue was poured into 50 mL of pentane and cooled to –20 ºC in a freezer. After 15 minutes, the white precipitate that formed was collected by filtration and washed with more pentane, then it was dried under vacuum. The white solid thus obtained (11.0 g, 55%) was used without further purification. **^{1}H NMR** (300 MHz, $CDCl_3$) δ = 2.38 (q, *J*=7.5 Hz, 6H), 1.14 (t, *J*=7.6 Hz, 9H); **^{13}C NMR** (75 MHz, $CDCl_3$) δ = 179.9, 29.7, 9.4.

Compound 6 by reductive alkylation of 5 with 3. A solution of aldehyde **2** (1.00 g, 2.82 mmol, 2.3 equiv) in CH_2Cl_2 (1.5 mL) and a solution of sodium tripropionyloxy borohydride (937 mg, 3.7 mmol, 3 equiv) in CH_2Cl_2 (2 mL) were added simultaneously via syringe pump over 2 h to a cold (0º C), vigorously stirred solution of protected aminoalcohol **5** (402 mg, 1.23 mmol, 1 equiv) and AcOH (1 drop) in CH_2Cl_2 (900 μL), under Ar. After completion of the addition, the mixture was stirred for 1 h at rt, then cooled back to 0º C, diluted with more CH_2Cl_2 (5 mL), and treated with aqueous saturated $NaHCO_3$ (6 mL). The organic phase was separated, sequentially washed with more aqueous saturated $NaHCO_3$ (5 mL) and water (2 x 5 mL), dried (Na_2SO_4), and evaporated. The oily residue was purified by silica gel column chromatography (1% MeOH in CH_2Cl_2) to furnish 600 mg (49%) of **6** as a colorless oil. **^{1}H NMR** (300 MHz, $CDCl_3$) δ = 7.68 – 7.65 (m, 4H), 7.43 – 7.33 (m, 6H), 4.06 (t, *J*=6.6 Hz, 4H), 3.67 (t, *J*=5.9 Hz, 2H), 2.40 – 2.34 (m, 6H), 2.33 – 2.26 (m, 2H), 1.64 – 1.25 (m, 68H), 1.05 (s, 9H), 0.87 (t, *J*=6.4 Hz, 12H); **^{13}C NMR** (75 MHz,

$CDCl_3$) δ = 176.8, 135. 7, 134.2, 129.6, 127.7, 64.2, 63.9, 54.2, 53.9, 45.9, 32.7, 31.9, 31.8, 30.8, 29.7, 29.6, 29.4, 29.3, 28.9, 27.6, 27.5, 27.4, 27.1, 26.9, 26.1, 23.4, 22.8, 22.7, 19.3, 14.2, 14.2; **HRMS:** Calcd for $C_{64}H_{114}N_2O_5Si^+$ $[M+H]^+$ 1004.8466, found 1004.8461.

ALC-0315 (1). Pyridine-HF complex (70 % w/w, 0.16 mL, 4 equiv) and pyridine (0.14 mL) were added to a cold (0 °C) solution of **6** (1.47 g, 1.4 mmol, 1.0 equiv) in 2 mL of anhydrous THF. The mixture was allowed to warm to ambient temperature and stirred overnight. Saturated aq. $NaHCO_3$ solution (2 mL) and tert-butyl methyl ether (2 mL; *t*BuOMe reduces the extent of formation of a gelatinous precipitate and greatly facilitates the extraction of the product) were added to the reaction mixture at 0 °C. The aqueous phase was extracted with diethyl ether (2×5 mL), the combined extracts were dried over Na_2SO_4, filtered and evaporated, and the residue was purified by flash chromatography (2% MeOH/DCM) to give ALC-0315 as a colorless oil (920 mg, 82 %). **^{1}H NMR** (300 MHz, $CDCl_3$) δ = 4.04 (t, *J*=6.6 Hz, 4H), 3.61 (t, *J*=5.2 Hz, 2H), 2.73 – 2.40 (b, 6H), 2.330 (m, 2H), 1.76 – 1.24 (m, 68H), 0.87 (br t, *J*=6.4 Hz, 12H); **^{13}C NMR** (75 MHz, $CDCl_3$) δ = 176.8, 63.9, 62.3, 53.8, 53.2, 45.9, 32.6, 31.9, 31.8, 29.7, 29.6, 29.4, 29.3, 28.7, 27.6, 27.5, 27.1, 25.9, 24.9, 22.8, 22.7, 14.2 (2 peaks). **LRMS** 766 $[M+H]^+$.

6-Acetoxy-1-hexanol (8). A solution of 1,6-hexanediol (40 g, 339.0 mmol, 1.0 equiv), DMAP (413 mg, 3.9 mmol, 1 mol%) and Et_3N (51.3 g, 70.6 mL, 507.5 mmol, 1.5 equiv) in dry CH_2Cl_2 (120 mL) was stirred at room temperature for 15 min prior to the dropwise addition (addition funnel) of a solution of acetic anhydride (22.4 mL, 24.2 g, 237.2 mmol, 0.7 equiv) in dry CH_2Cl_2 (30 mL) over 40 min. The mixture was stirred at room temperature overnight, then it was quenched with saturated aqueous sodium bicarbonate solution (30 mL). The aqueous phase was removed

and the organic layer was washed with saturated aqueous sodium chloride solution (2×20 mL) and concentrated under reduced pressure. The residue was dissolved in diethyl ether (50 mL) and filtered through Celite® to remove some suspended material. The filtrate was evaporated and the residue was washed with hexanes (3×10 mL). The hexanes-insoluble material was filtered through a pad of silica gel (15 g) with 5% EtOAc/hexanes to remove residual diacetate. Further elution with 20% EtOAc/hexanes provided the desired monoacetate, which was Kugelrohr distilled (75 ^{o}C) to afford pure **5** (29 g, 76%) as a colorless oil. **^{1}H NMR** (300 MHz, $CDCl_3$) δ = 4.05 (t, *J*=6.7 Hz, 2H), 3.63 (t, *J*=6.5 Hz, 2H), 2.03 (s, 3H), 1.67 – 1.52 (m, 4H), 1.39 – 1.34 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ = 171.4, 64.6, 62.9, 32.7, 28.7, 25.8, 25.5, 21.1; **LRMS:** 161 $[M+H]^+$, 183 $[M+Na]^+$.

6-Acetoxyhexanal (9). The procedure described above for the synthesis of **3** was repeated starting with 4.8 g of **8** (25 mmol, 1.0 equiv) and 390 mg of TEMPO (2.5 mmol, 10 mol %). Crude **9** thus obtained was purified by Kugelrohr distillation (60 ^{o}C) to afford pure material (3.6 g, 91%) as a pale-yellow oil. **^{1}H NMR** (300 MHz, $CDCl_3$) δ = 9.75 (t, *J*=1.7 Hz, 1H), 4.04 (t, *J*=6.6 Hz, 2H), 2.43 (td, *J*=7.3, 1.7 Hz, 2H), 2.02 (s, 3H), 1.69 – 1.57 (m, 4H), 1.44-1.31 (m, 2H). **^{13}C NMR** (75 MHz, $CDCl_3$); δ = 202.4, 171.3, 64.3, 43.8, 28.5, 25.6, 21.8, 21.0; **LRMS:** 159 $[M+H]^+$, 181 $[M+Na]^+$.

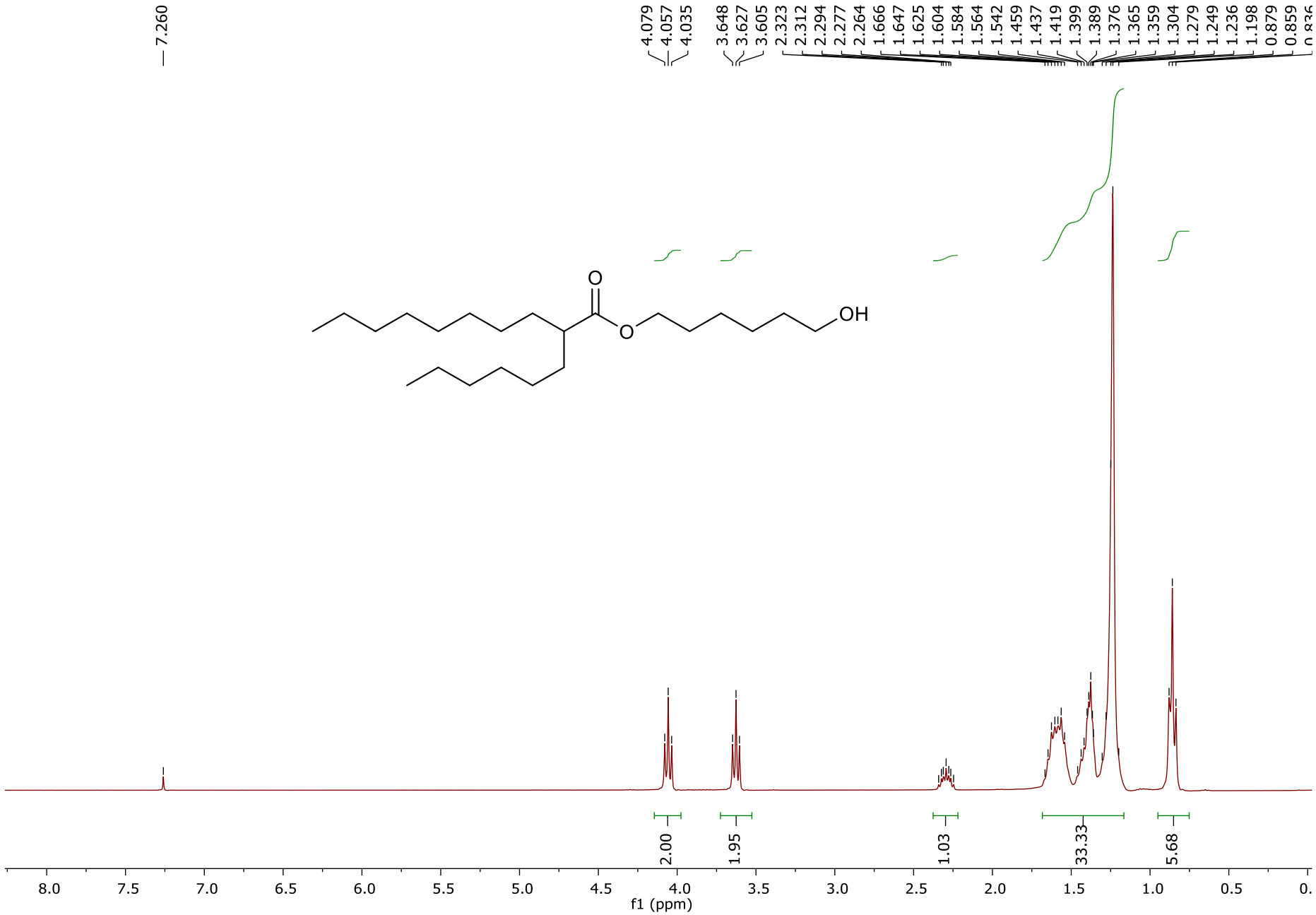
Compound 10. A solution of **9** (1.0 g, 6.3 mmol, 2.3 equiv) in CH_2Cl_2 (3 mL) and one of $NaBH(OCOC_2H_5)_3$ (2.1 g, 8.3 mmol, 3.1 equiv) in CH_2Cl_2 (6 mL), placed in two distinct syringe pumps, were simultaneously added over 2 h to a cold (0 ^{o}C) solution of **5** (0.9 g, 2.7 mmol, 1.0 equiv) and glacial AcOH (16 mg, 17 uL, 0.3 mmol, 0.1 equiv) in 2 mL of anhydrous CH_2Cl_2, under

Ar. After completion of the addition, the mixture was allowed to warm to ambient temperature and stirred for another 15 min, then it was quenched with sat. aq. $NaHCO_3$ solution (10 mL) and extracted with CH_2Cl_2 (2×5 mL). The combined extracts were washed with brine (2×5 mL), dried (Na_2SO_4) filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography on silica gel with 2% MeOH in CH_2Cl_2 to afford 1.3 g (79%) of **10** as a colorless oil. **^{1}H NMR** (300 MHz, $CDCl_3$) δ = 7.67 – 7.64 (m, 4H), 7.43 – 7.33 (m, 6H), 4.04 (t, *J*=6.7 Hz, 4H), 3.67 (t, *J*=6 Hz, 2H), 2.43 – 2.37 (m, 6H), 2.02 (s, 6H), 1.65 – 1.25 (m, 20H), 1.04 (s, 9H). **^{13}C NMR** (75 MHz, $CDCl_3$) δ = 171.2, 135.6, 134.1, 129.6, 127.7, 64.6, 63.8, 54.0, 53.9, 30.6, 28.7, 27.3, 26.9, 26.9, 25.9, 23.2, 21.1, 19.3. **HRMS:** Calcd for $C_{36}H_{58}NO_5Si^+$ $[M+H]^+$ 612.4079, found 612.4084.

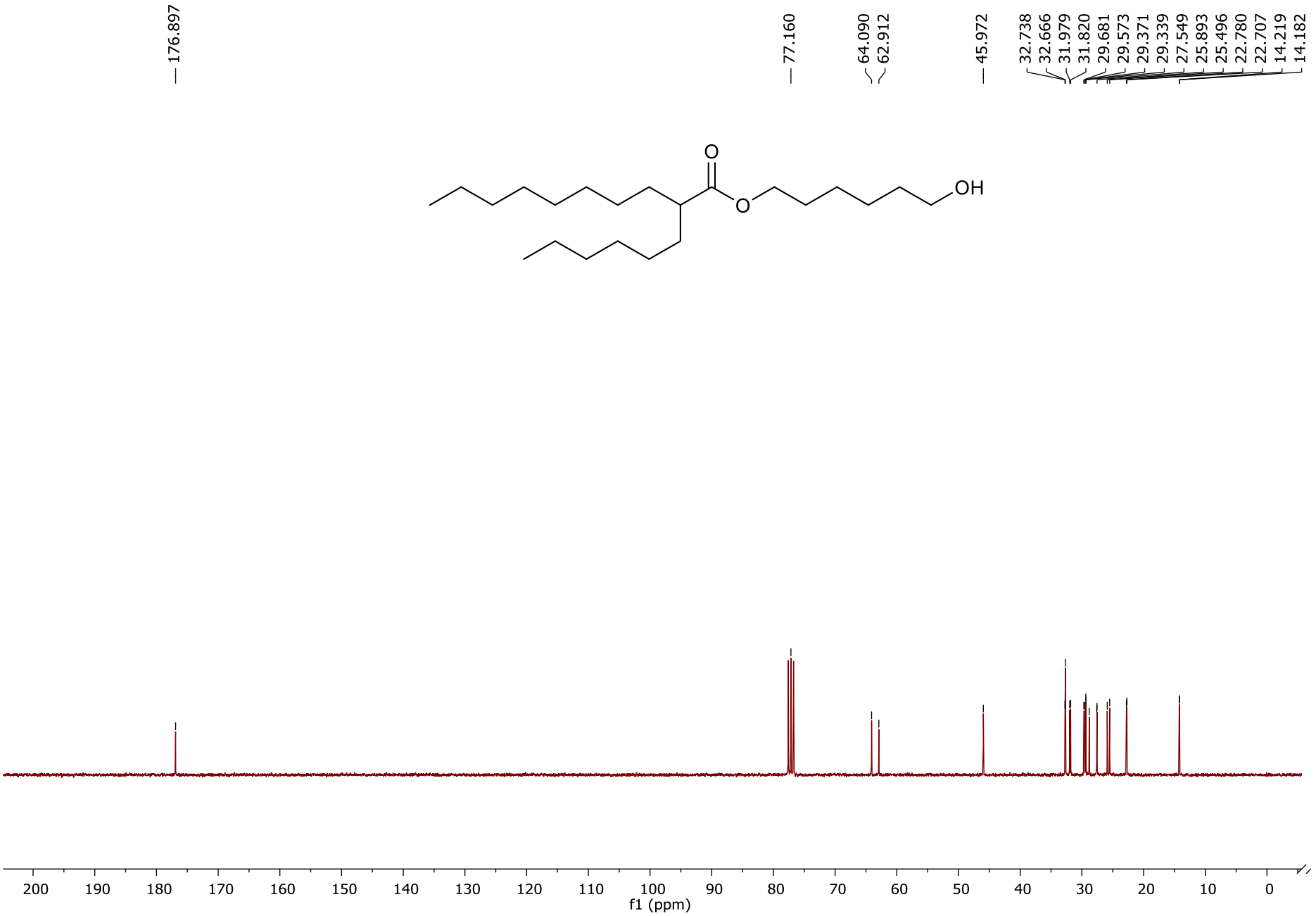
Compound 11. A solution of **10** (1.3 g, 2.1 mmol, 1.0 equiv) in 2 mL of MeOH containing suspended K_2CO_3 (120 mg, 0.9 mmol, 0.4 equiv) was stirred at room temperature overnight. The mixture was filtered and the filtrate was concentrated under reduced pressure. The residue was dissolved in DCM containing 5% v/v of MeOH (5 mL), and sequentially washed with sat. aq. NH_4Cl solution (2×3 mL) and sat. aq. NaCl solution (3 mL). Concentration under reduced pressure afforded 1.1 g of **11** (quantitative) as a yellow oil that was used without purification. **^{1}H NMR** (300 MHz, CD_3OD) δ = 7.67 – 7.64 (m, 4H), 7.46 – 7.36 (m, 6H), 3.76 (t, *J*=5.8 Hz, 2H), 3.55 (t, *J*=6.4 Hz, 4H), 3.12 – 3.02 (m, 6H), 1.85 – 1.31 (m, 20H), 1.05 (s, 9H); **^{13}C NMR** (75 MHz, CD_3OD) δ = 136.6, 134.7, 130.9, 128.9, 64.2, 62.6, 54.2, 53.9, 33.3, 30.5, 27.5, 27.4, 26.5, 25.0, 21.9, 20.0. **HRMS:** Calcd for $C_{32}H_{54}NO_3Si^+$ $[M+H]^+$ 528.3867, found 528.3873.

Compound 6 by esterification of 11. A solution of 2-hexyldecanoic acid (1.6 g, 6.3 mmol, 3.0 equiv) in CH_2Cl_2 (1 mL) was added to a solution of **9** (1.1 g, 2.1 mmol, 1.0 equiv), DMAP (128 mg, 1.1 mmol, 0.5 equiv), *N*,*N*-diisopropylethylamine (540 mg, 727 uL, 4.2 mmol, 2.0 equiv), and EDCI·HCl (1.2 g, 6.3 mmol, 3.0 equiv) in CH_2Cl_2 (3 mL), under Ar. The resulting mixture was stirred for 24 h at room temperature, then it was diluted with CH_2Cl_2 (10 mL), sequentially washed with sat. aq. $NaHCO_3$ solution (2×5 mL), H_2O (2×5 mL), sat. aq. NaCl solution (2×5 mL), dried (Na_2SO_4) and concentrated under reduced pressure. This residue was purified by silica gel column chromatography with 2% MeOH/ DCM to provide 1.47 g (70% yield) of desired product as a colorless oil. **^{1}H NMR** (300 MHz, $CDCl_3$) δ = 7.68 – 7.65 (m, 4H), 7.43 – 7.33 (m, 6H), 4.06 (t, *J*=6.6 Hz, 4H), 3.67 (t, *J*=5.9 Hz, 2H), 2.40 – 2.34 (m, 6H), 2.33 – 2.26 (m, 2H), 1.64 – 1.25 (m, 68H), 1.05 (s, 9H), 0.87 (t, *J*=6.4 Hz, 12H); **^{13}C NMR** (75 MHz, $CDCl_3$) δ = 176.8, 135. 7, 134.2, 129.6, 127.7, 64.2, 63.9, 54.2, 53.9, 45.9, 32.7, 31.9, 31.8, 30.8, 29.7, 29.6, 29.4, 29.3, 28.9, 27.6, 27.5, 27.4, 27.1, 26.9, 26.1, 23.4, 22.8, 22.7, 19.3, 14.2, 14.2; **HRMS:** Calcd for $C_{64}H_{114}N_2O_5Si^+$ $[M+H]^+$ 1004.8466, found 1004.8461.

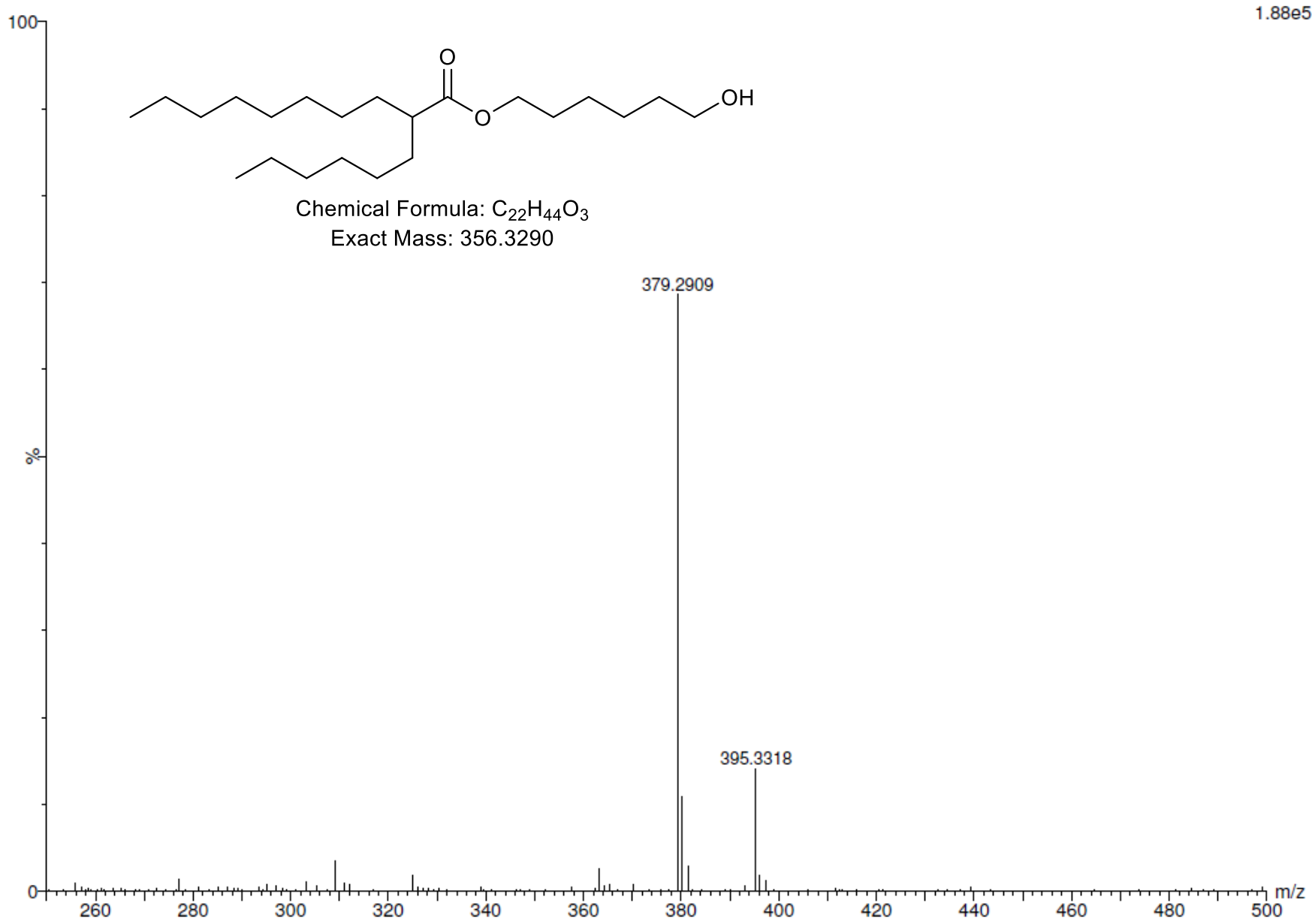
^{1}H NMR Spectrum of 6-hydroxyhexyl 2-hexyldecanoate (2) (300 MHz, $CDCl_3$)



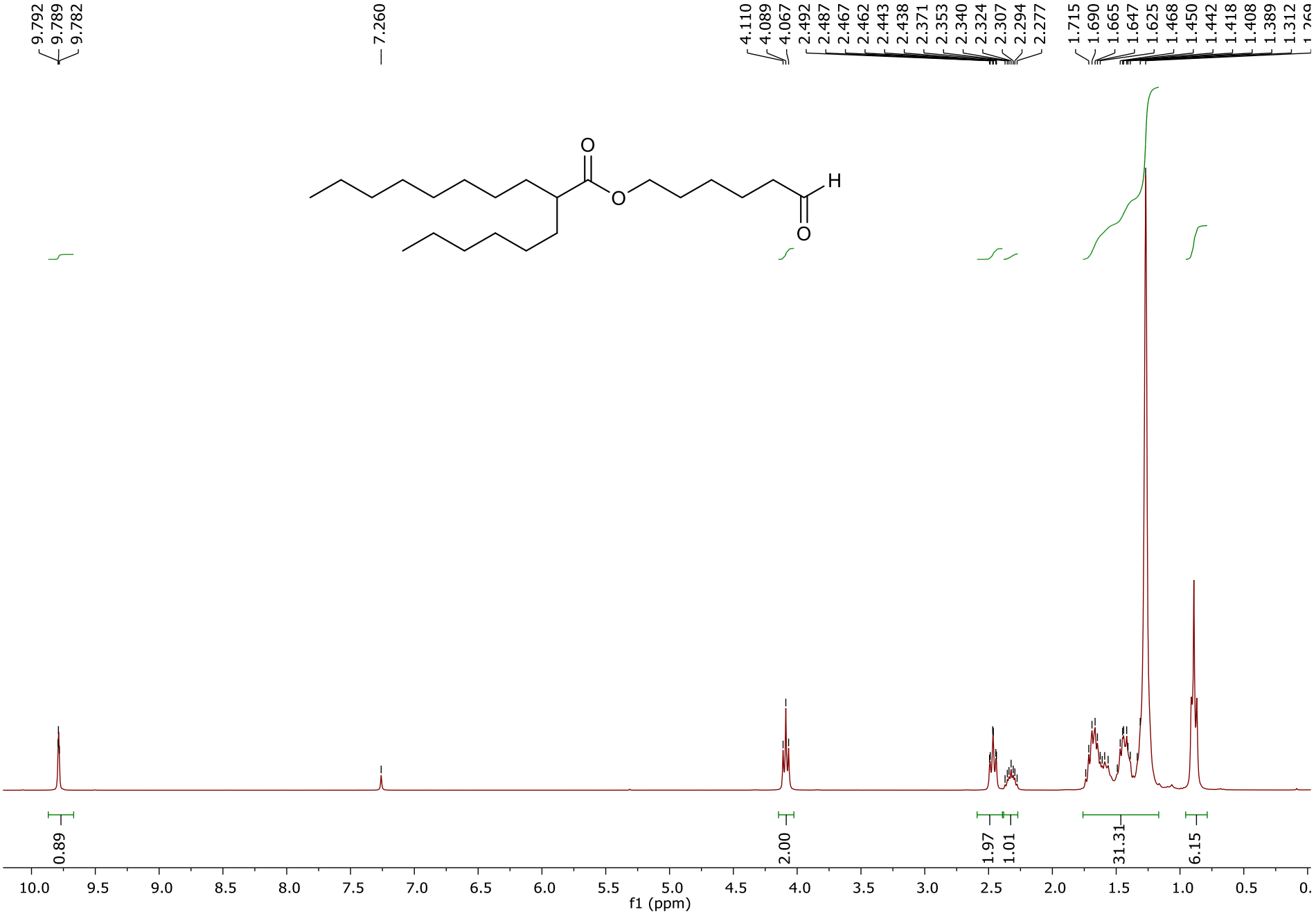
^{13}C NMR Spectrum of 6-hydroxyhexyl 2-hexyldecanoate (2) (75 MHz, $CDCl_3$)



Low resolution mass spectrum of 6-hydroxyhexyl 2-hexyldecanoate (2) (ESI)



^{1}H NMR Spectrum of 6-(2-hexyldecanoyloxy)-1-hexanal (3) (300 MHz, $CDCl_3$)



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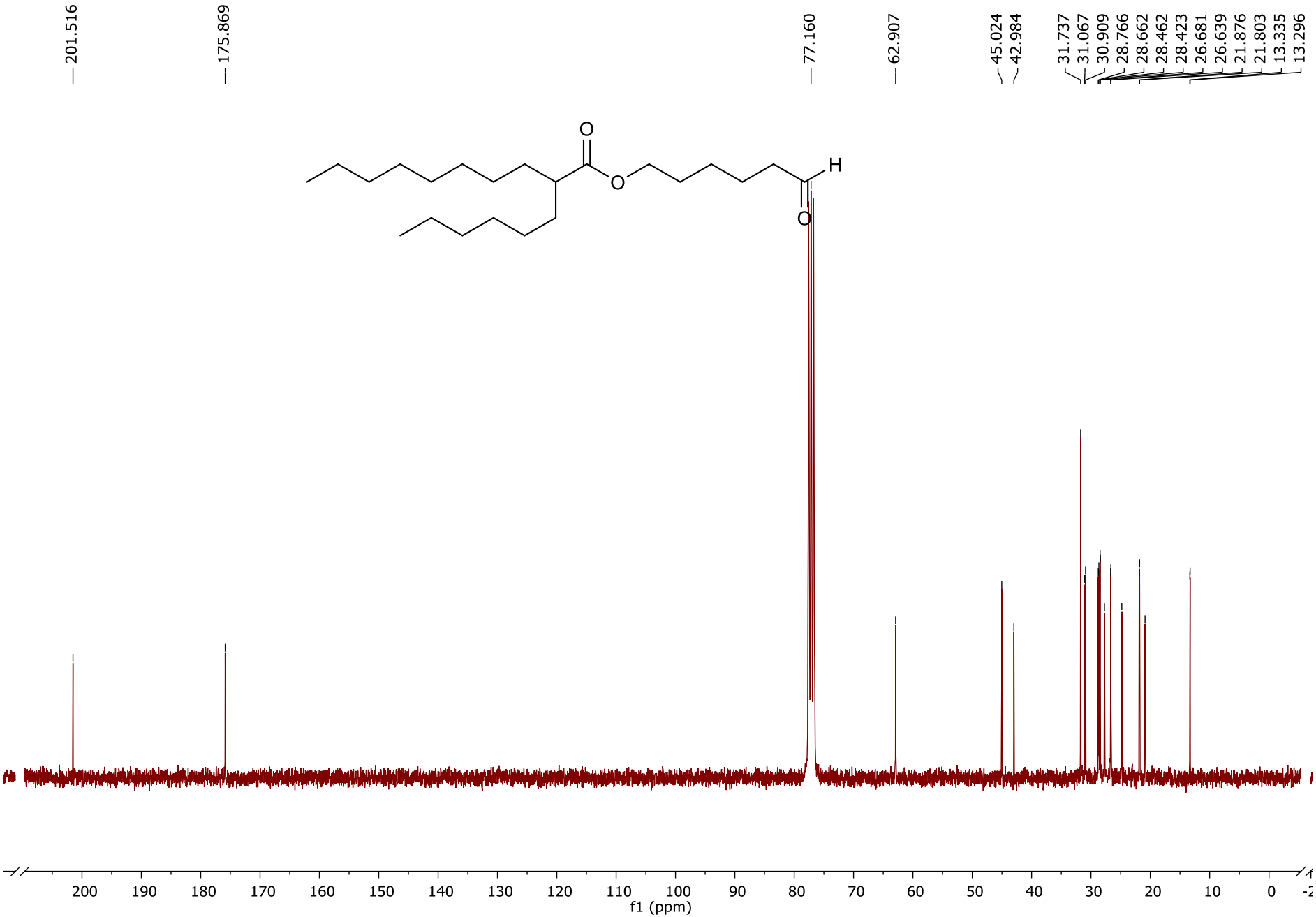
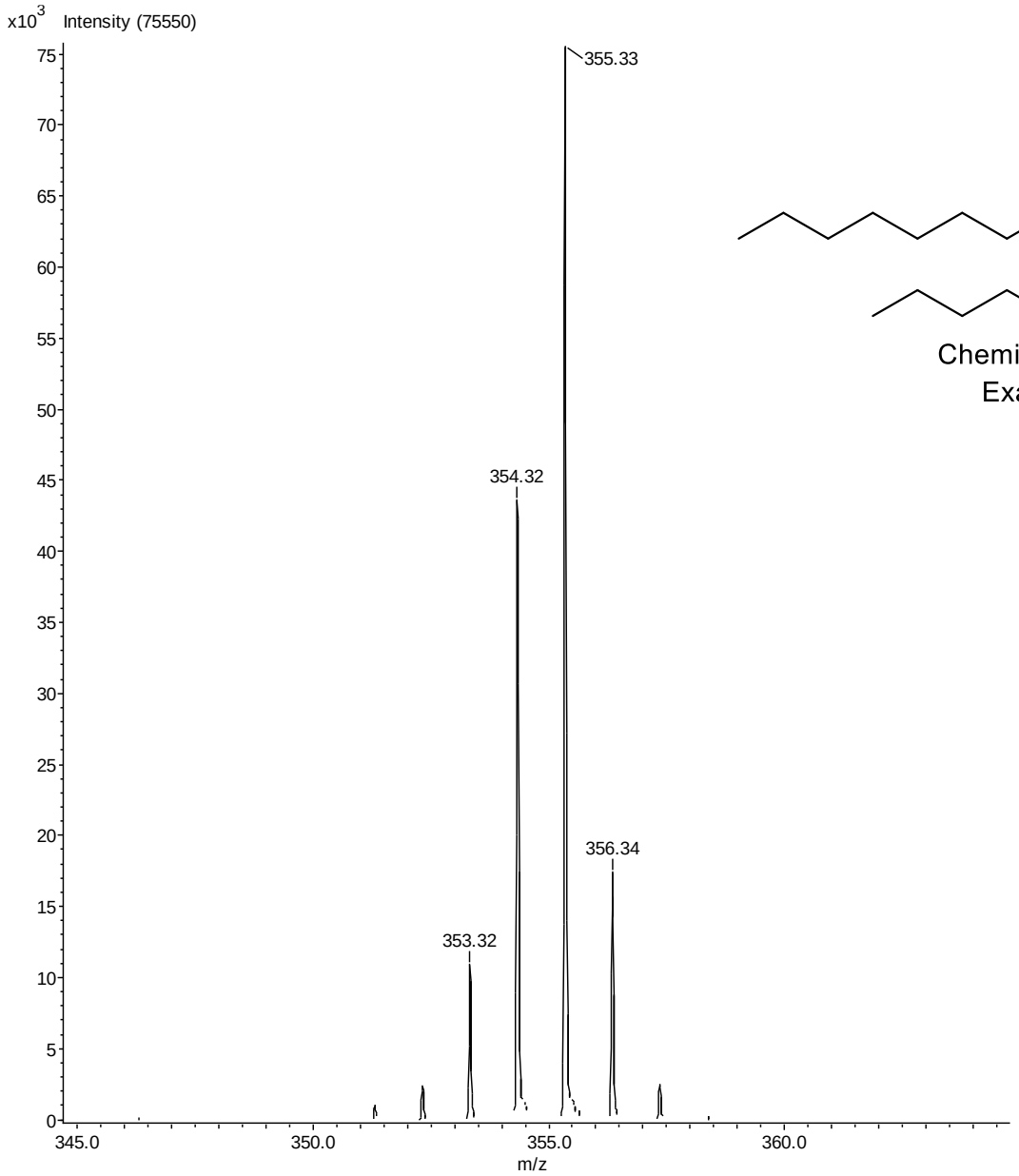
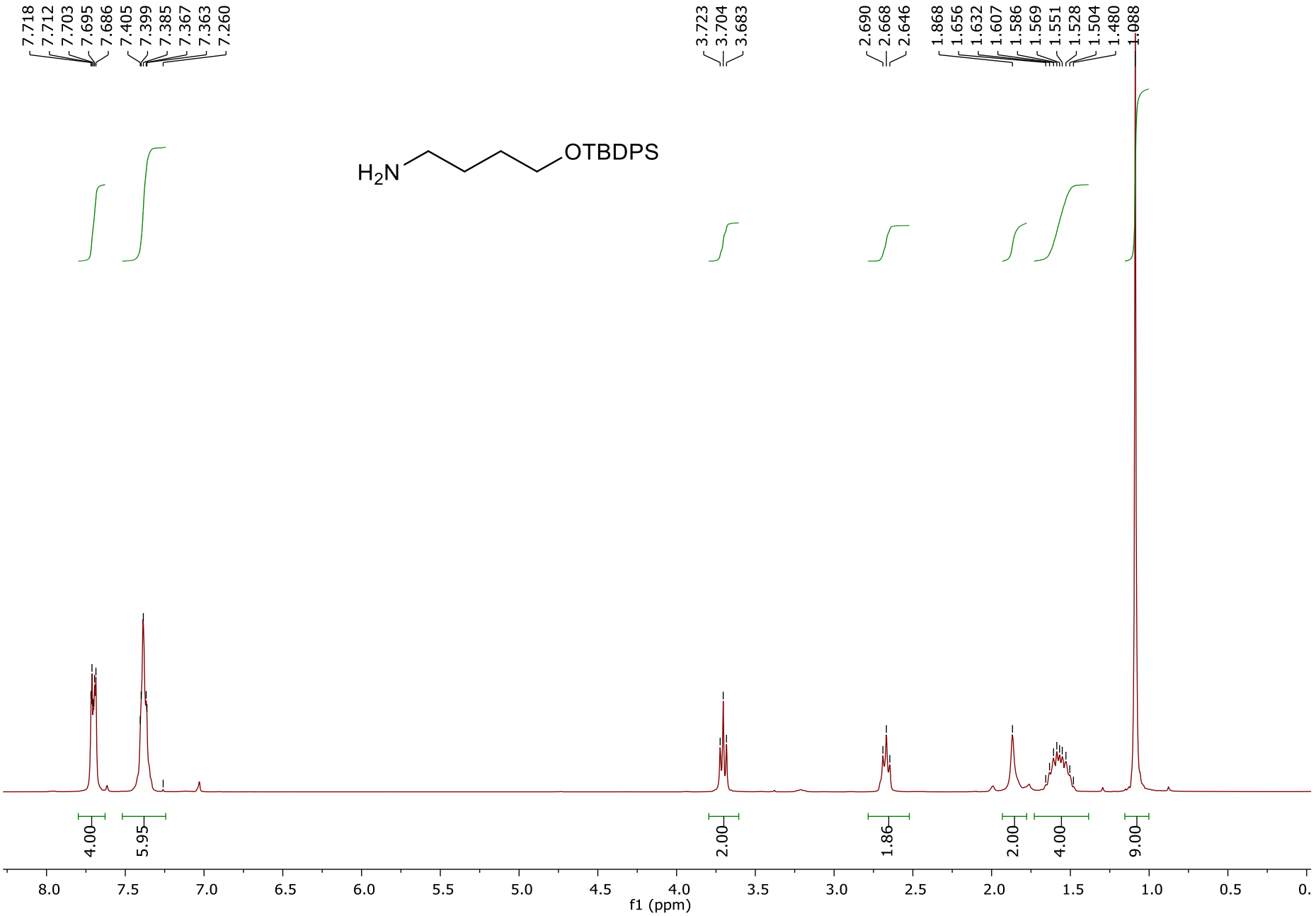


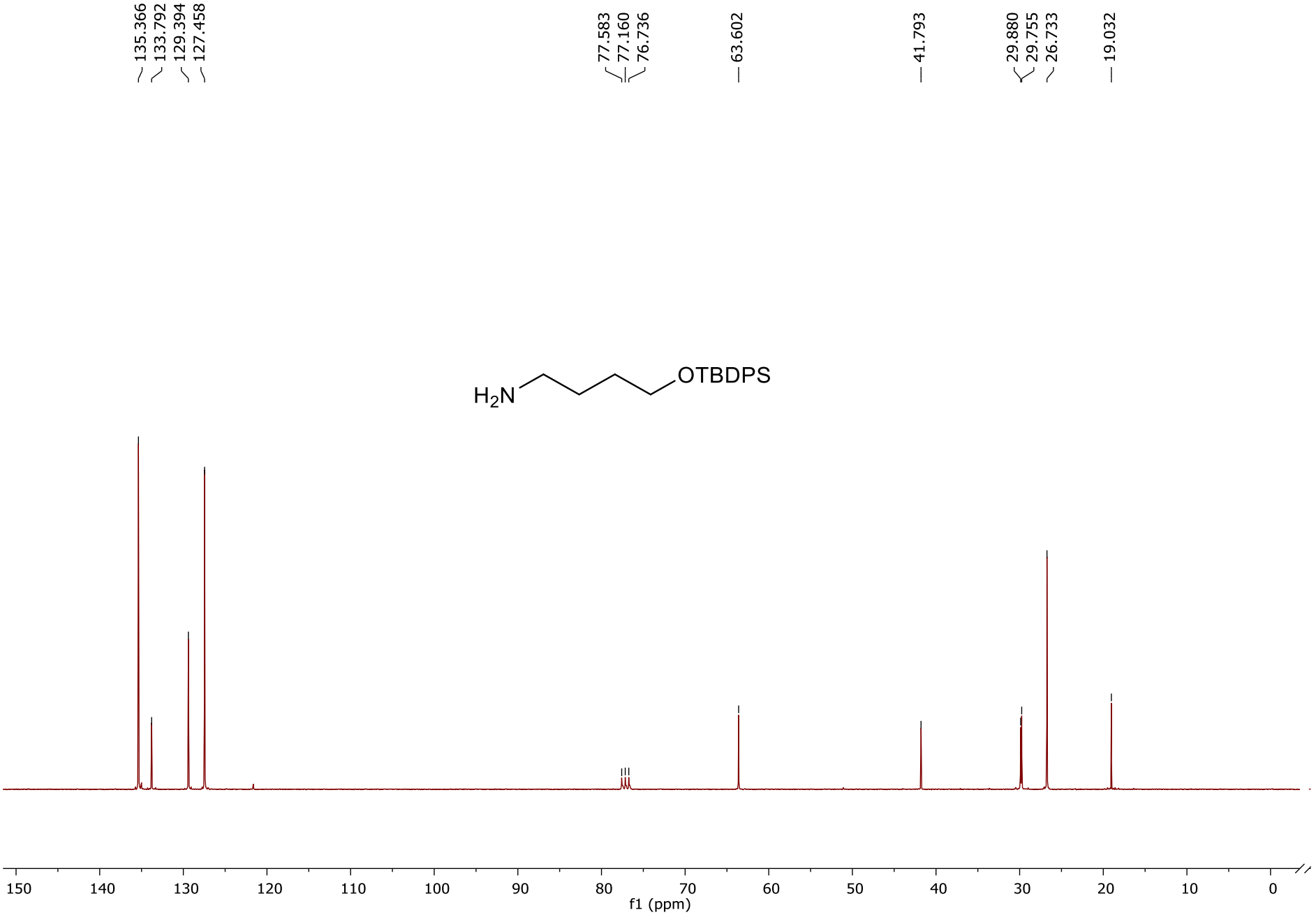
Low resolution mass spectrum of 6-(2-hexyldecanoyloxy)-1-hexanal (3) (FD)



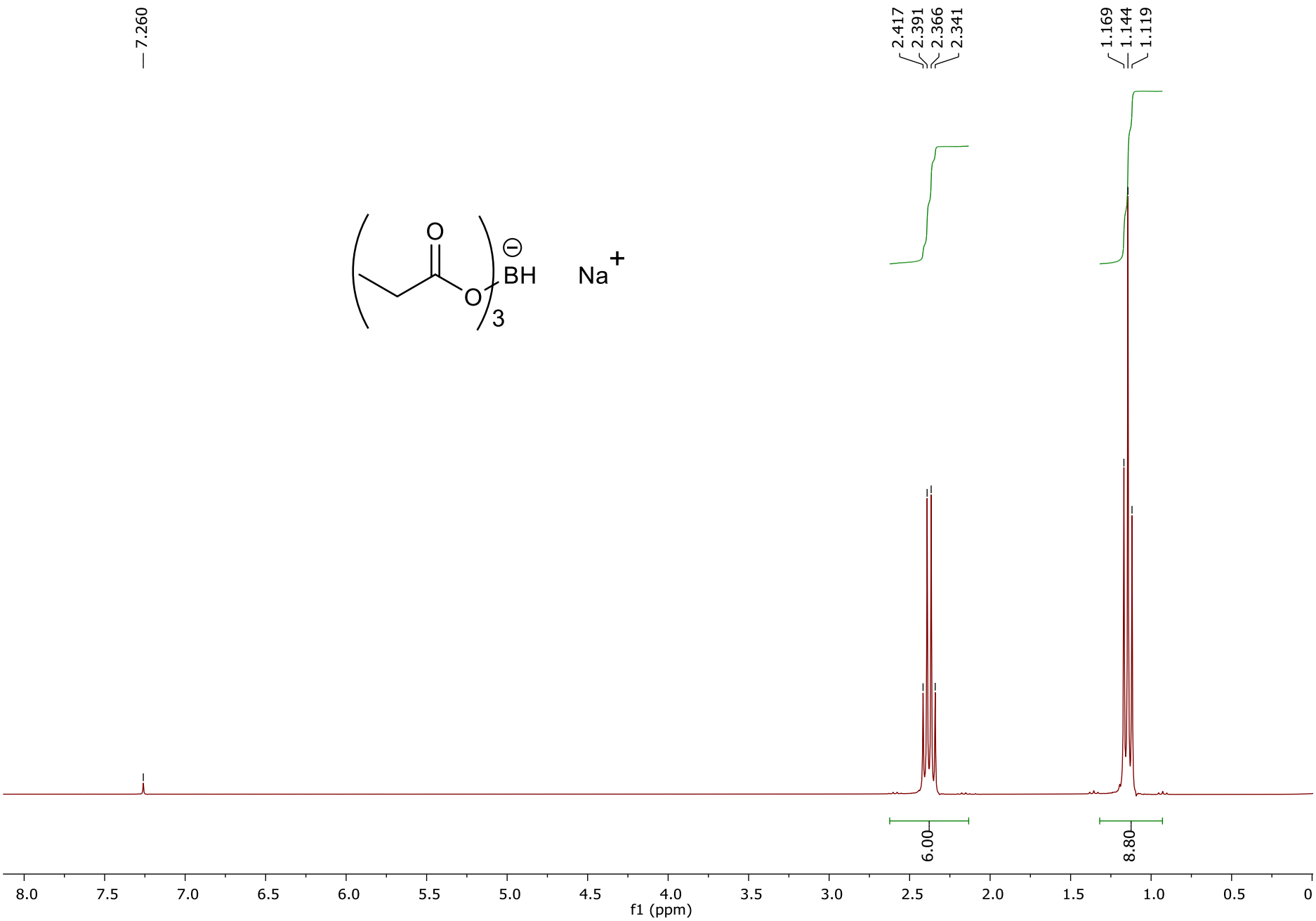
^{1}H-NMR Spectrum of 4-aminobutanol, tert-butyldiphenylsilyl ether (4) (300 MHz, $CDCl_3$)



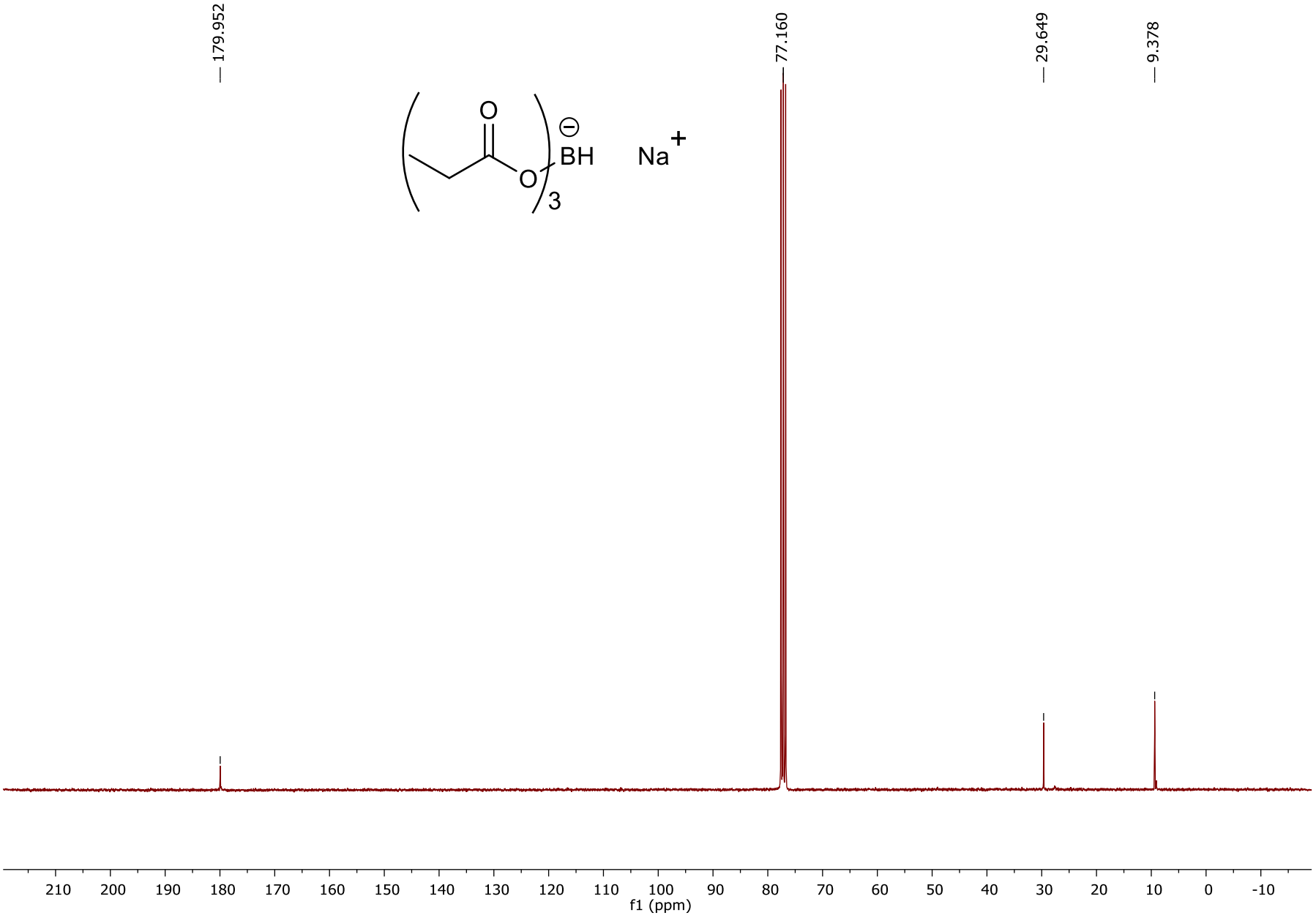
^{13}C-NMR Spectrum of 4-aminobutanol, tert-butyldiphenylsilyl ether (4) (75MHz, $CDCl_3$)



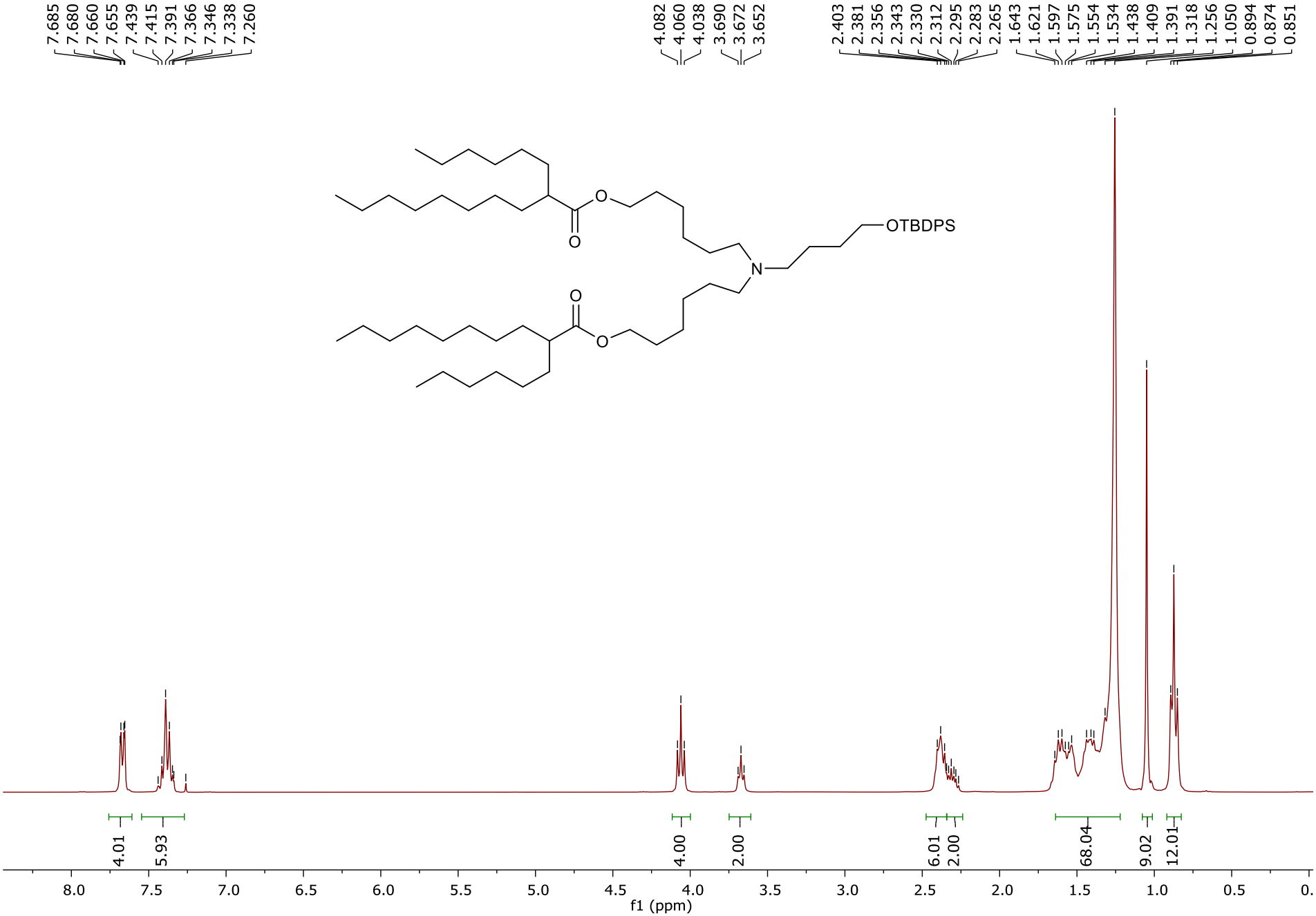
^{1}H-NMR Spectrum of sodium tri(propionyloxy)borohydride (300 MHz, $CDCl_3$)



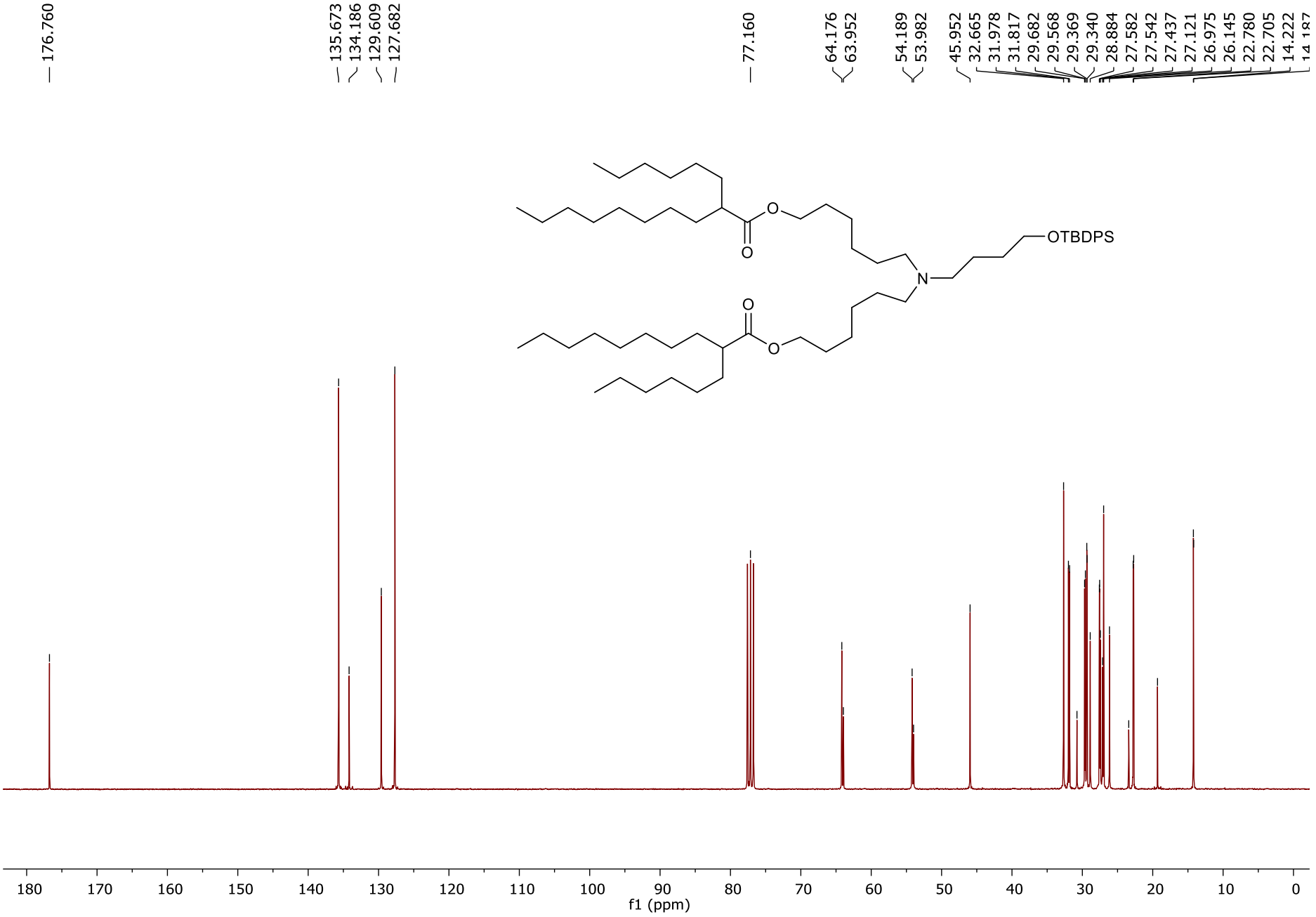
^{13}C-NMR Spectrum of sodium tri(propionyloxy)borohydride (75MHz, $CDCl_3$)



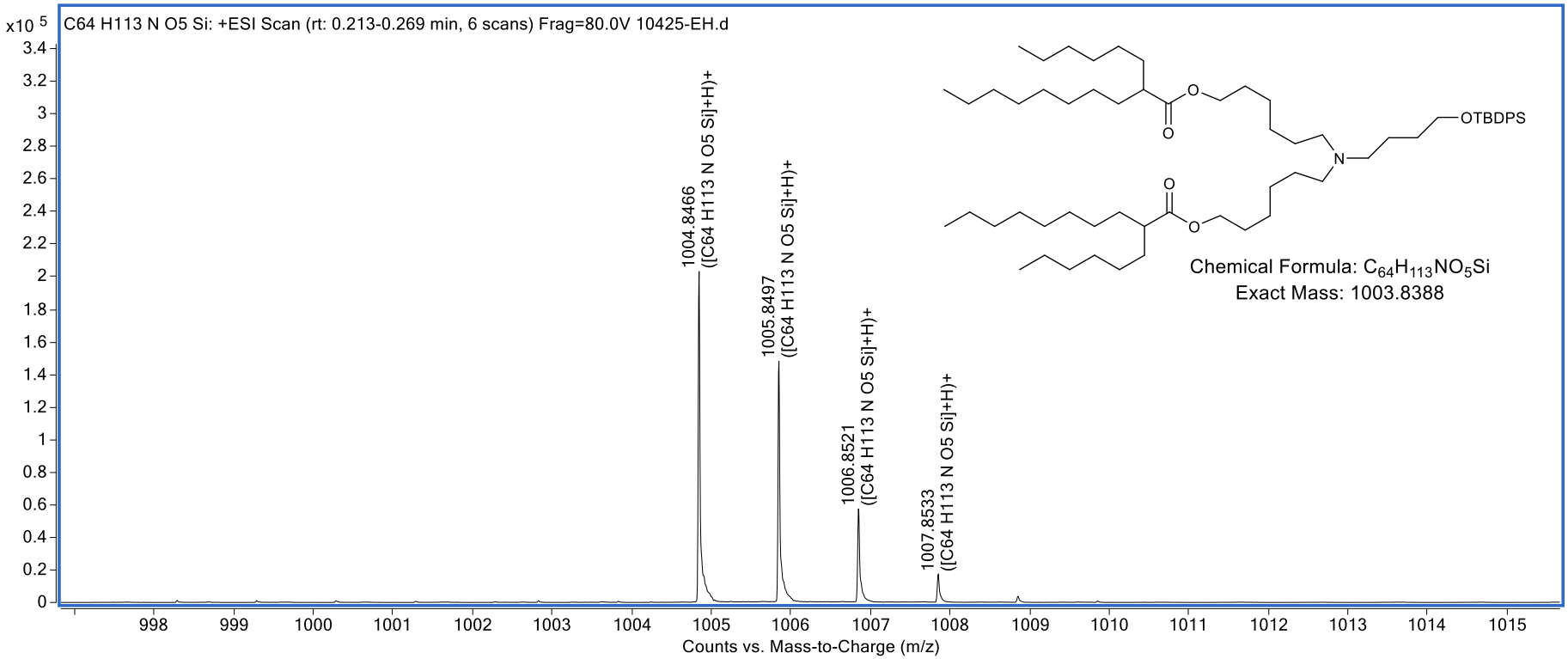
^{1}H-NMR Spectrum of compound 6 (300 MHz, $CDCl_3$)



^{13}C-NMR Spectrum of compound 6 (75 MHz, $CDCl_3$)



High resolution mass spectrum of compound 6 (ESI)



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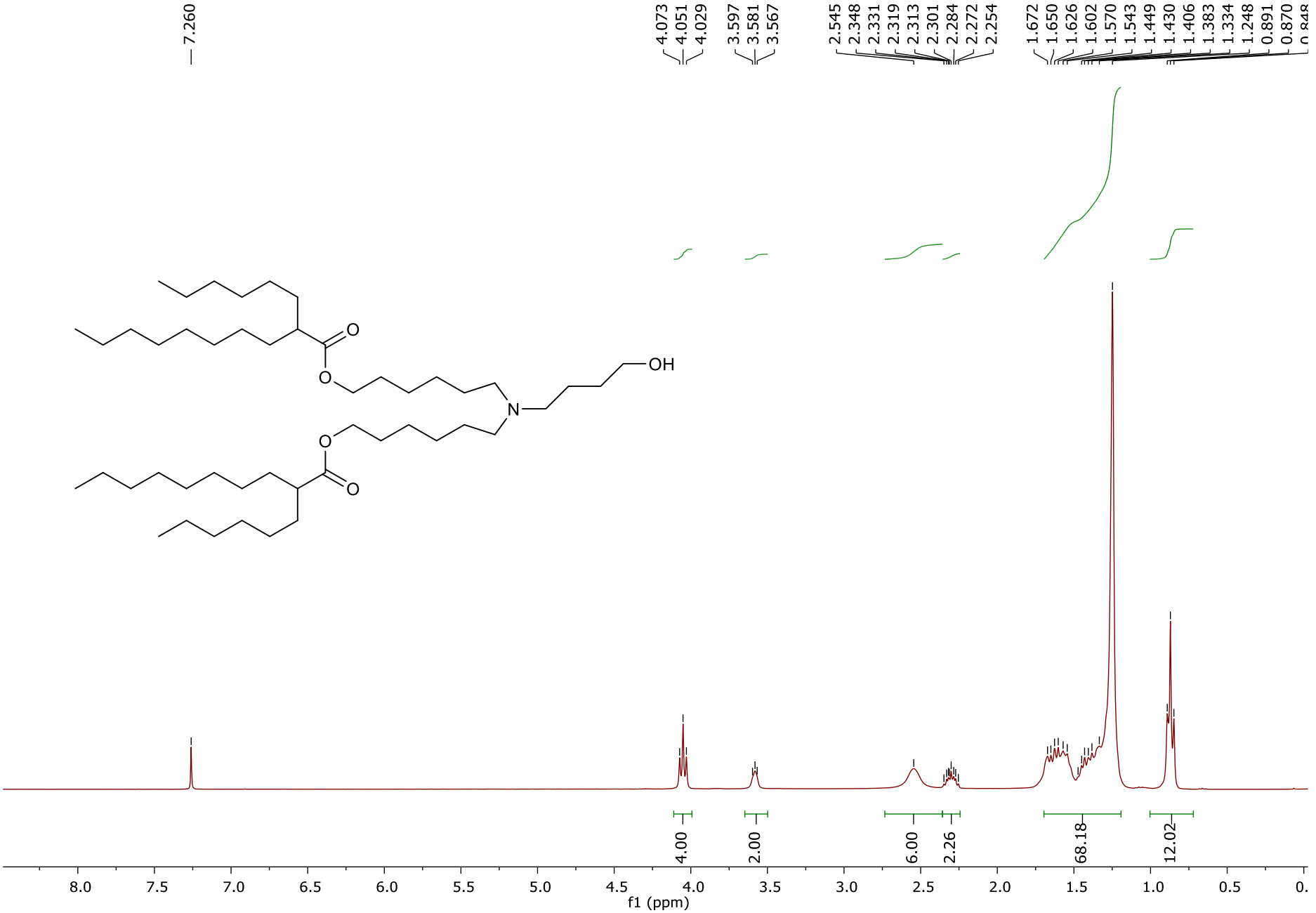
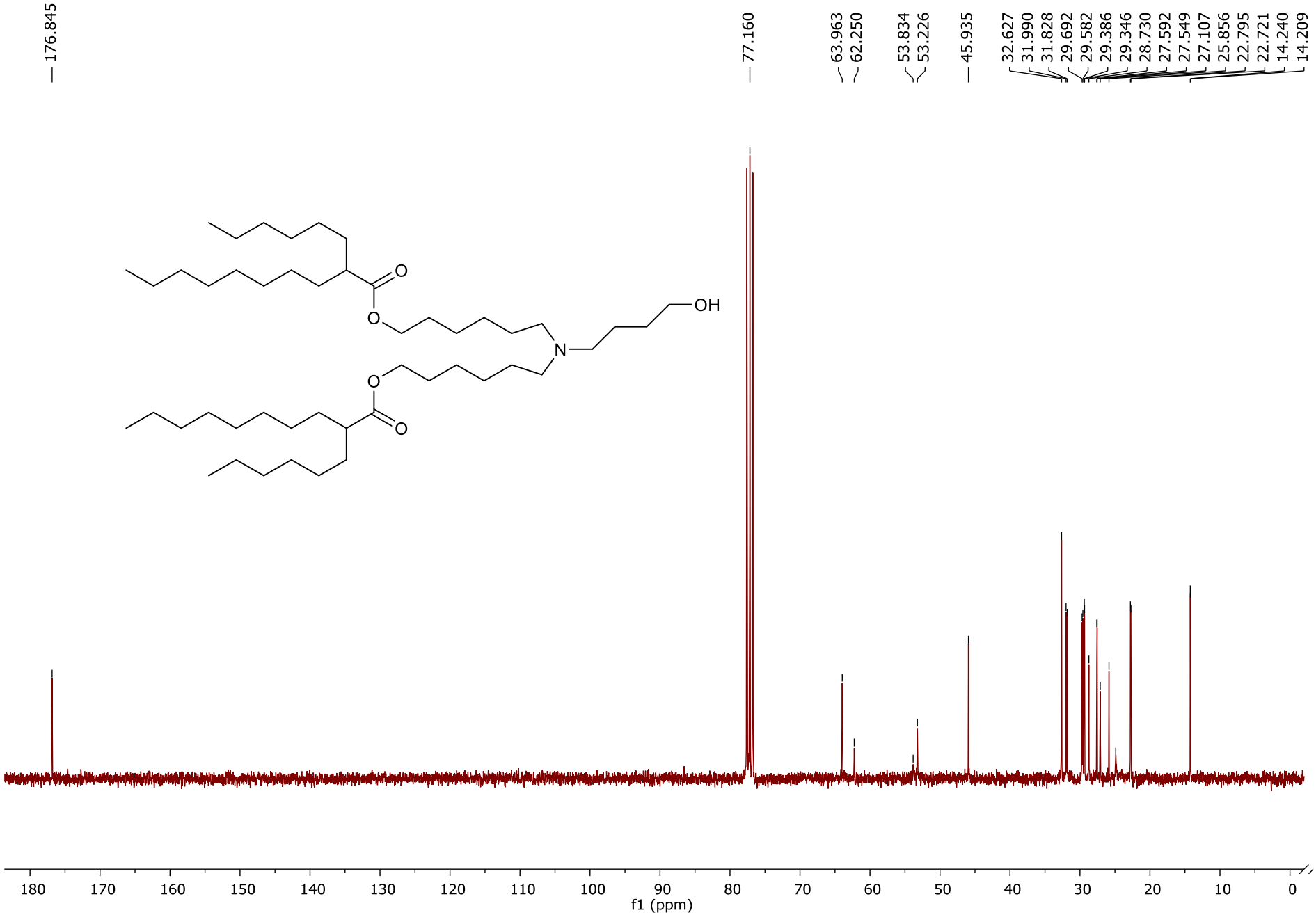
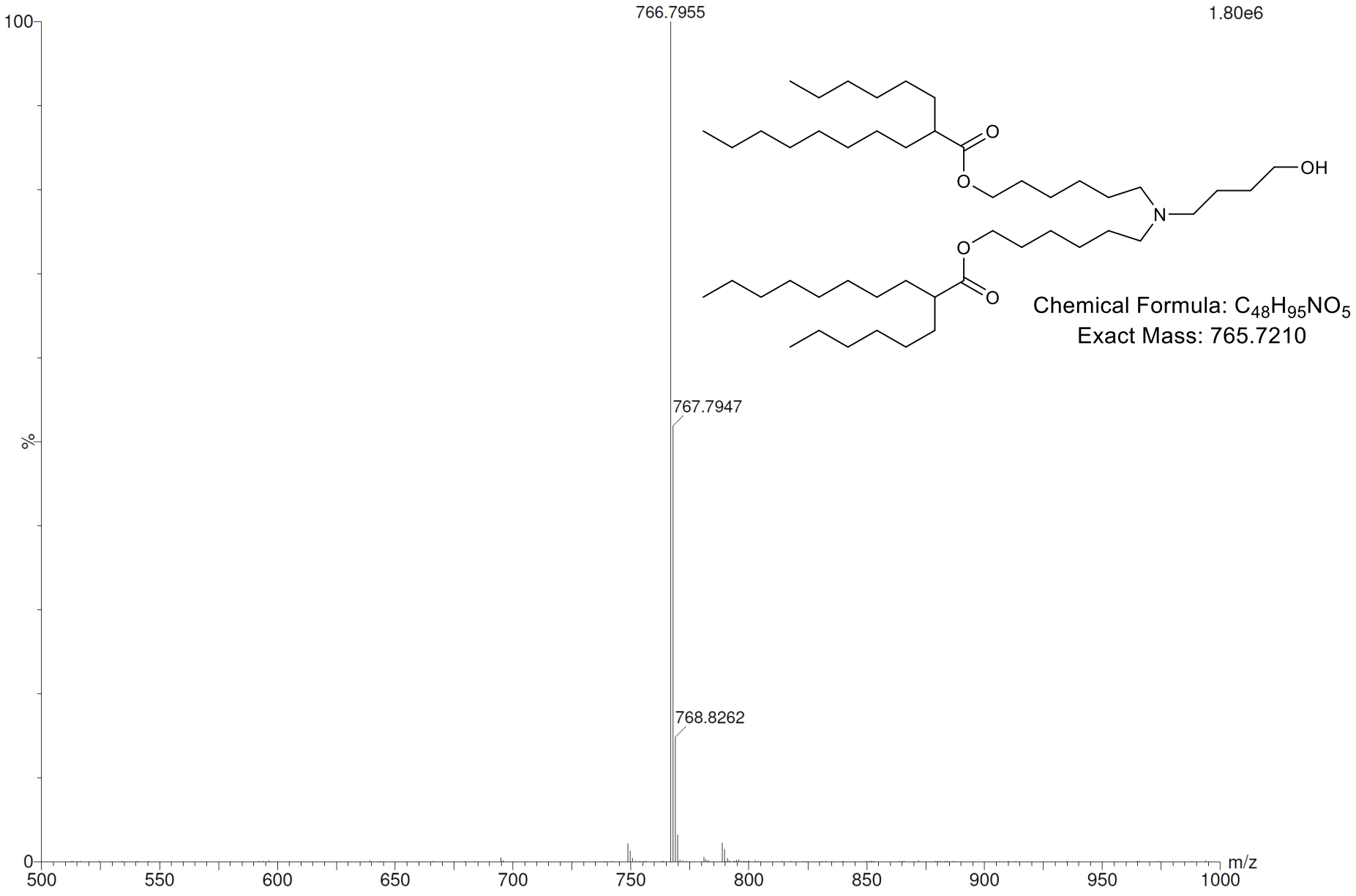


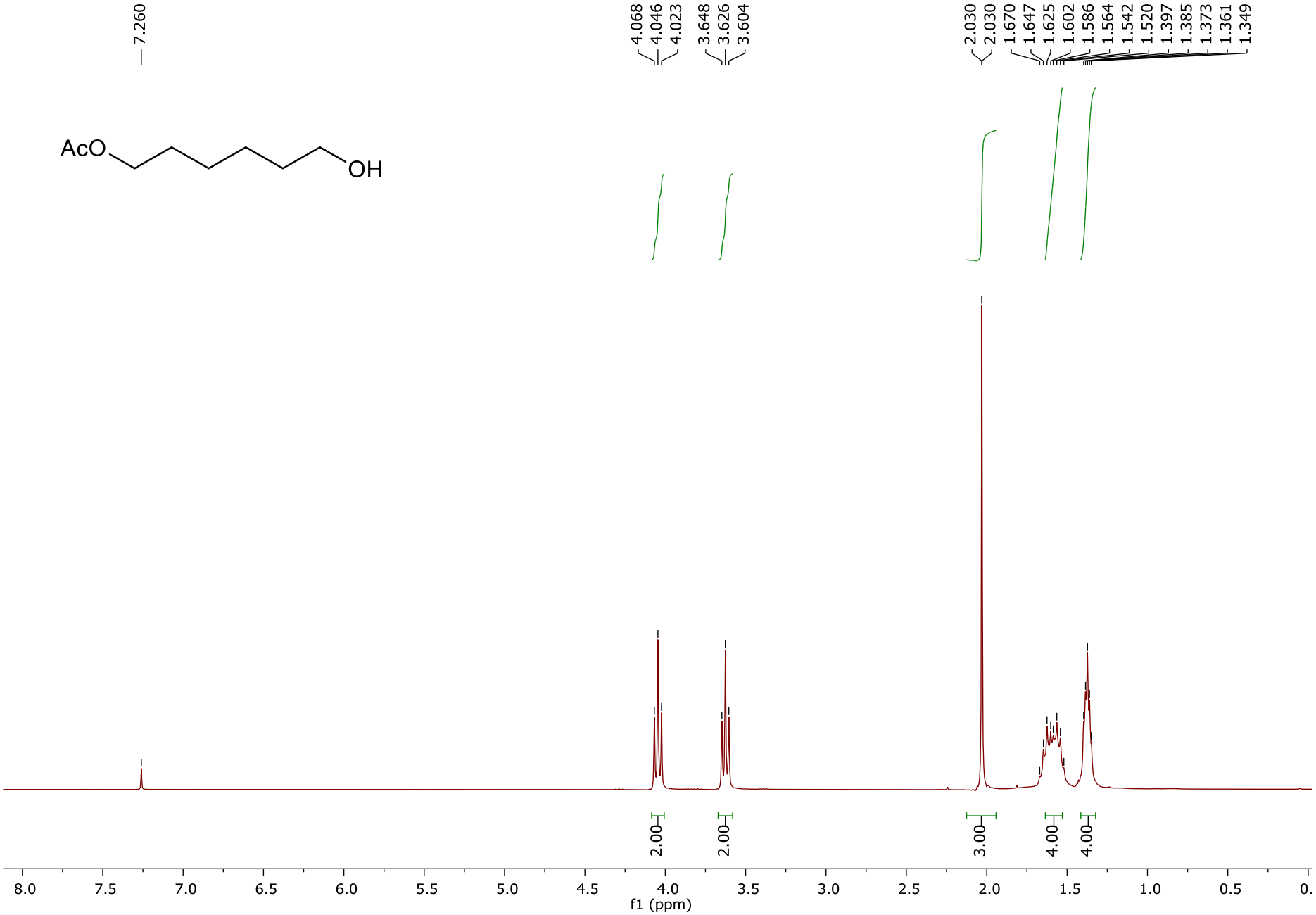
^{13}C-NMR Spectrum of ALC-0315 (1) (75 MHz, $CDCl_3$)



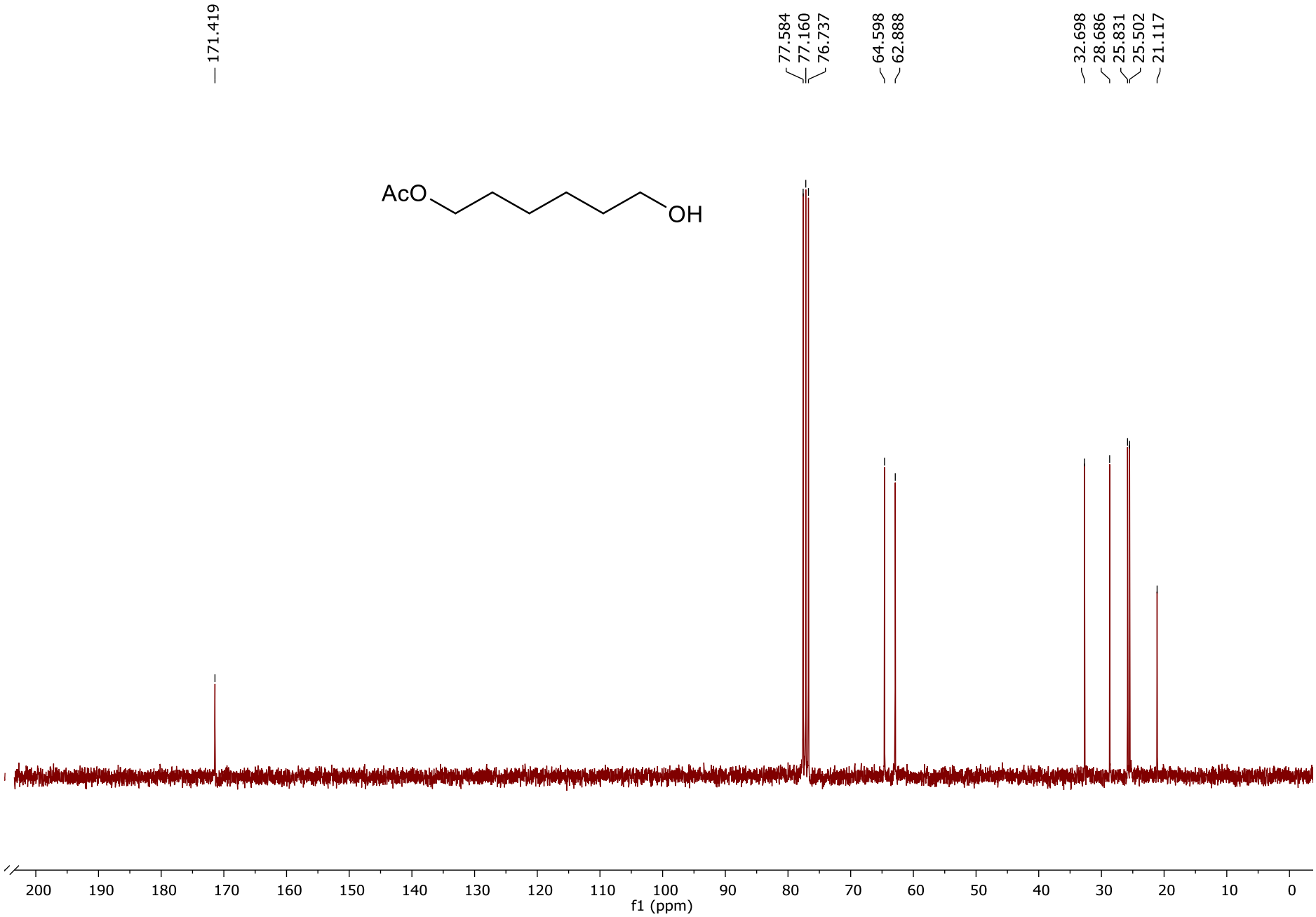
Low resolution mass spectrum of ALC-0315 (1) (ESI)



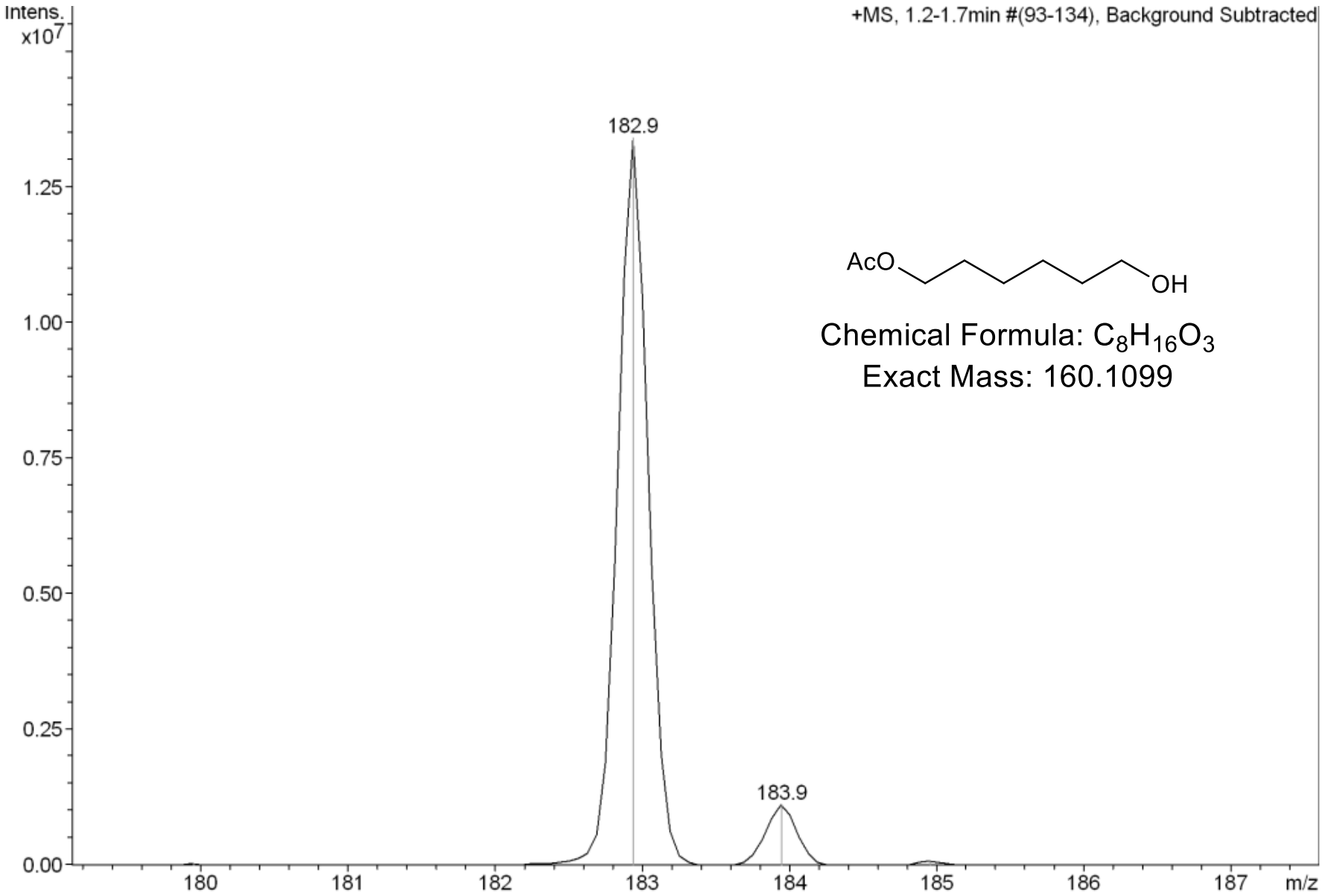
^{1}H-NMR Spectrum of 6-acetoxy-1-hexanol (8) (300 MHz, $CDCl_3$)



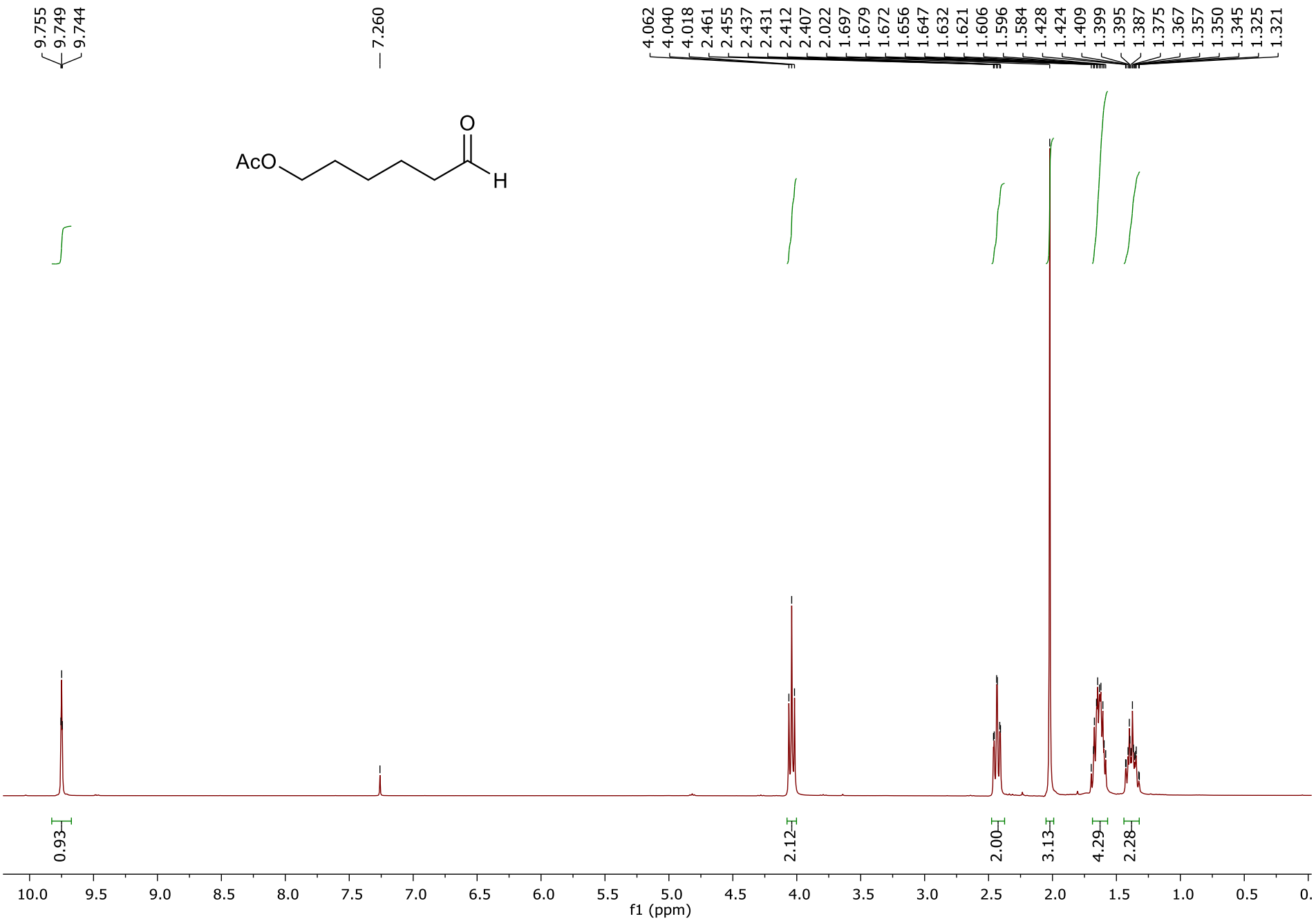
^{13}C-NMR Spectrum of 6-acetoxy-1-hexanol (8) (75 MHz, $CDCl_3$)



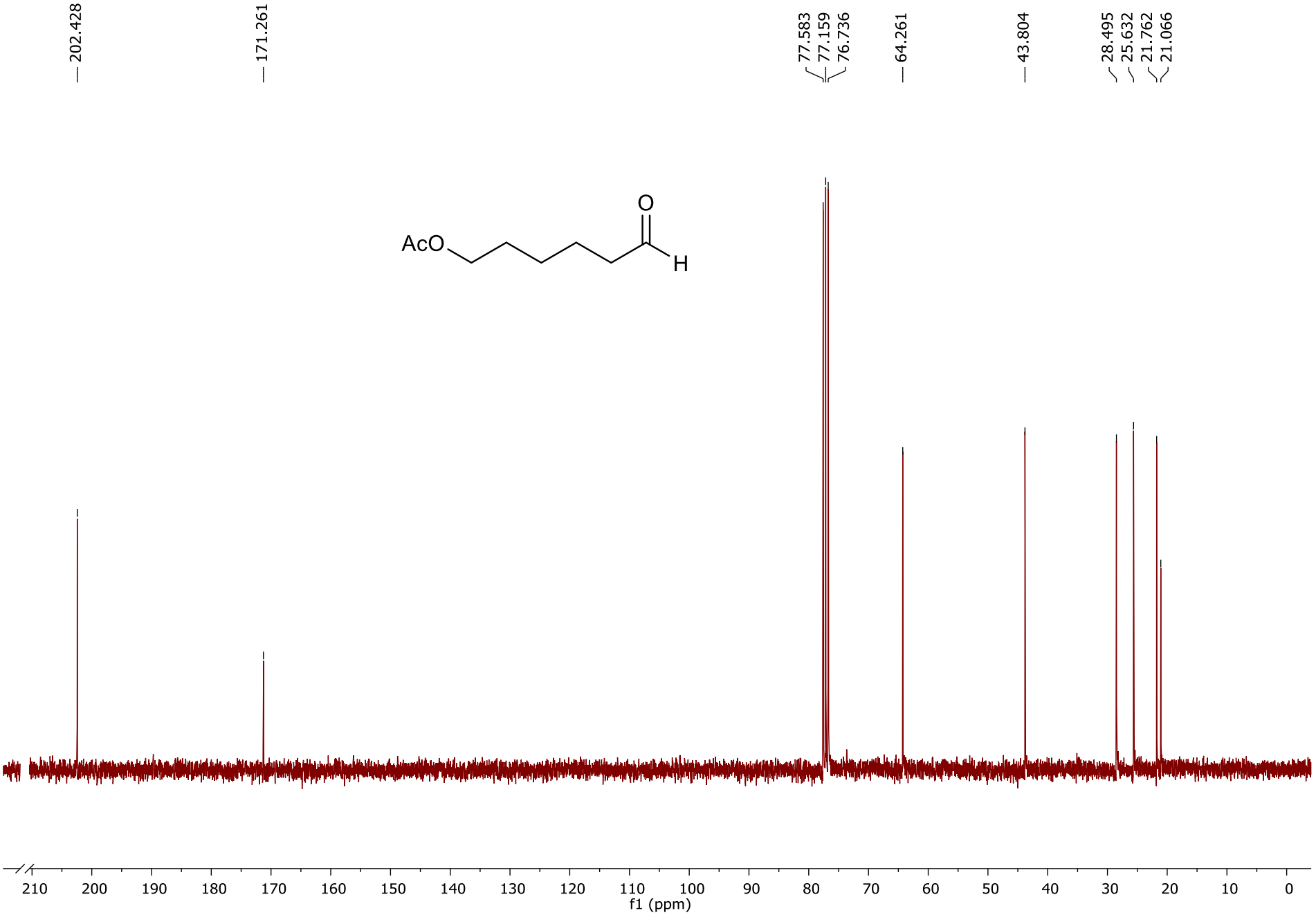
Low resolution mass spectrum of 6-acetoxy-1-hexanol (8) (ESI)



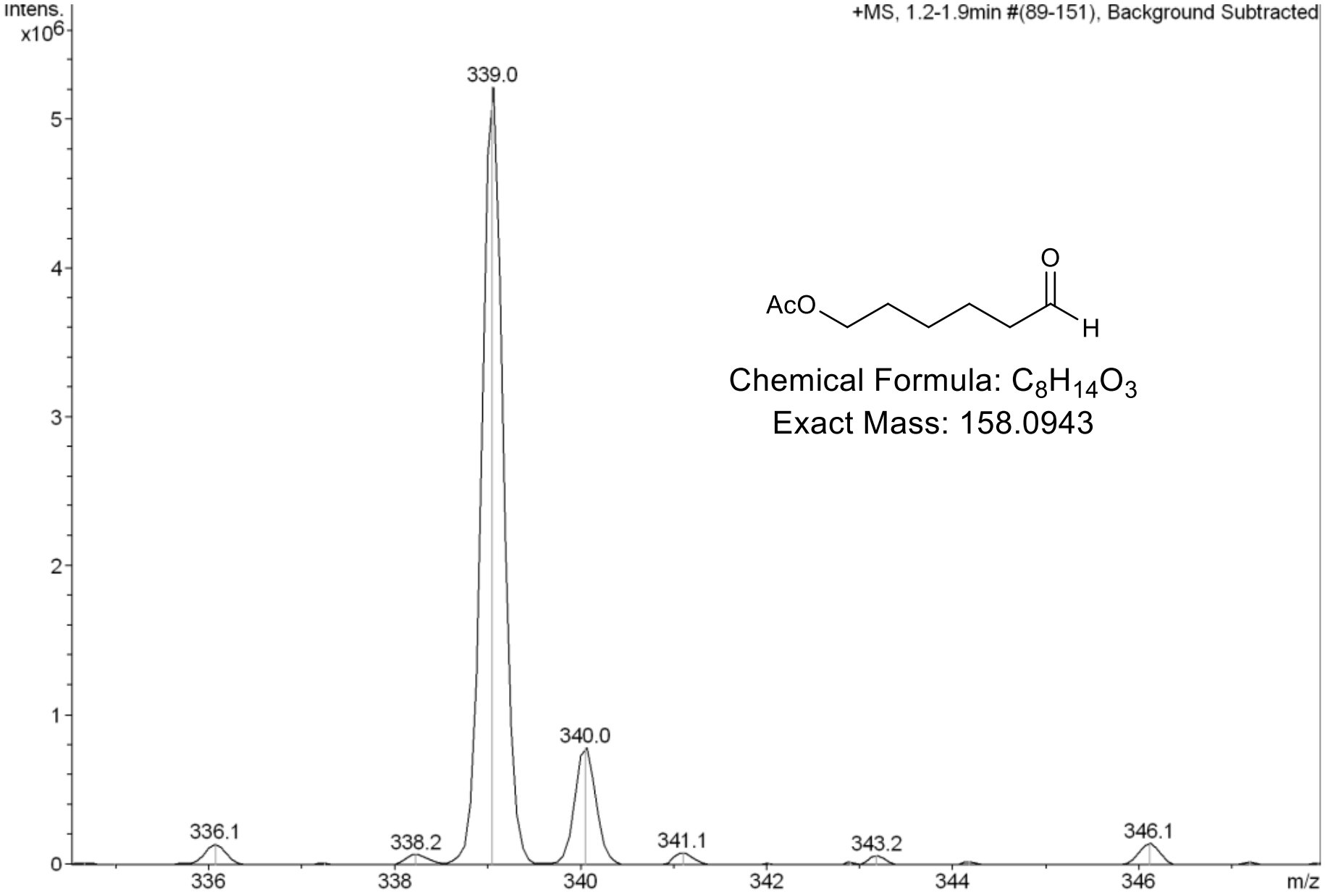
^{1}H-NMR Spectrum of 6-acetoxyhexanal (9) (300 MHz, $CDCl_3$)



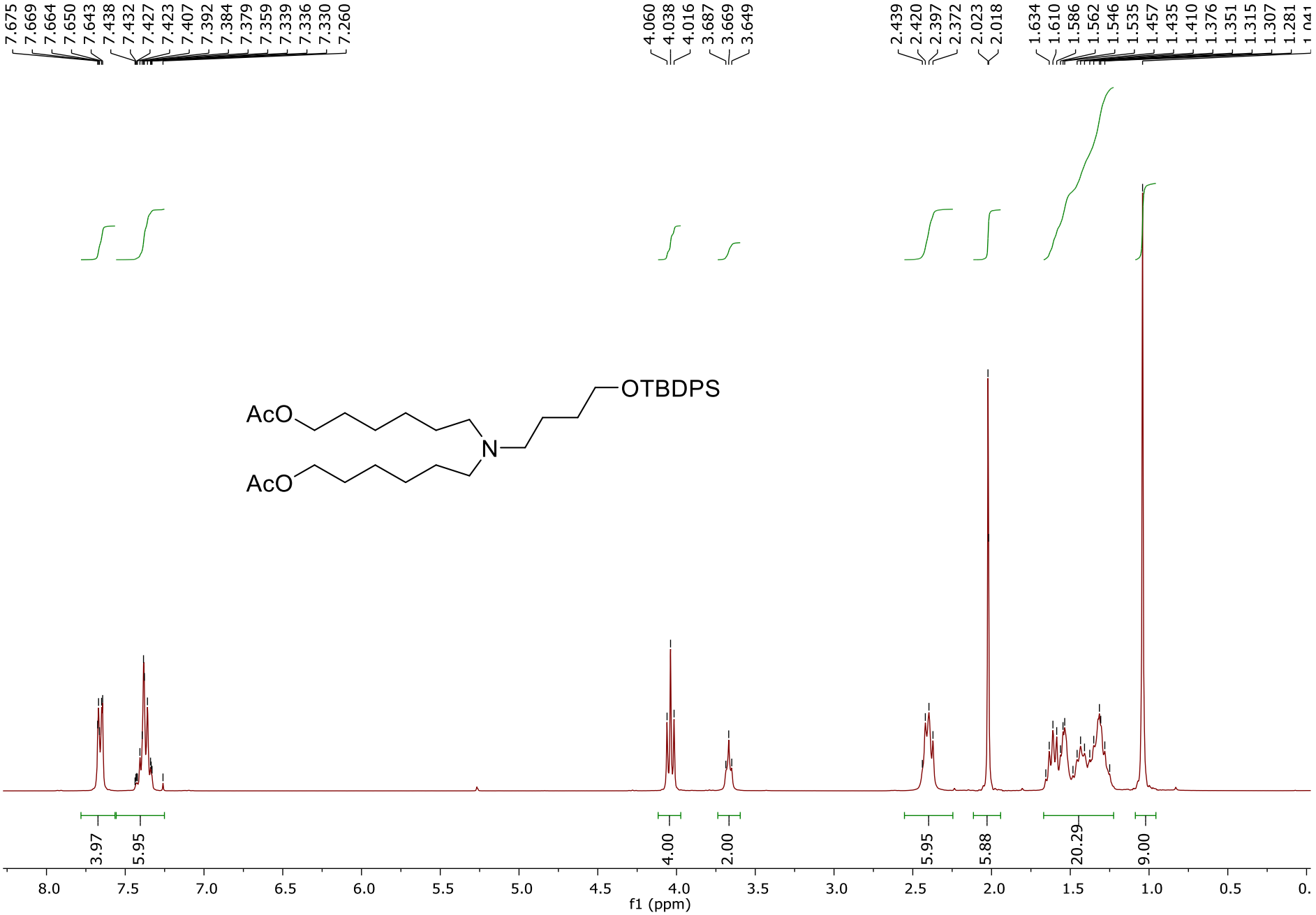
^{13}C-NMR Spectrum of 6-acetoxyhexanal (9) (75 MHz, $CDCl_3$)



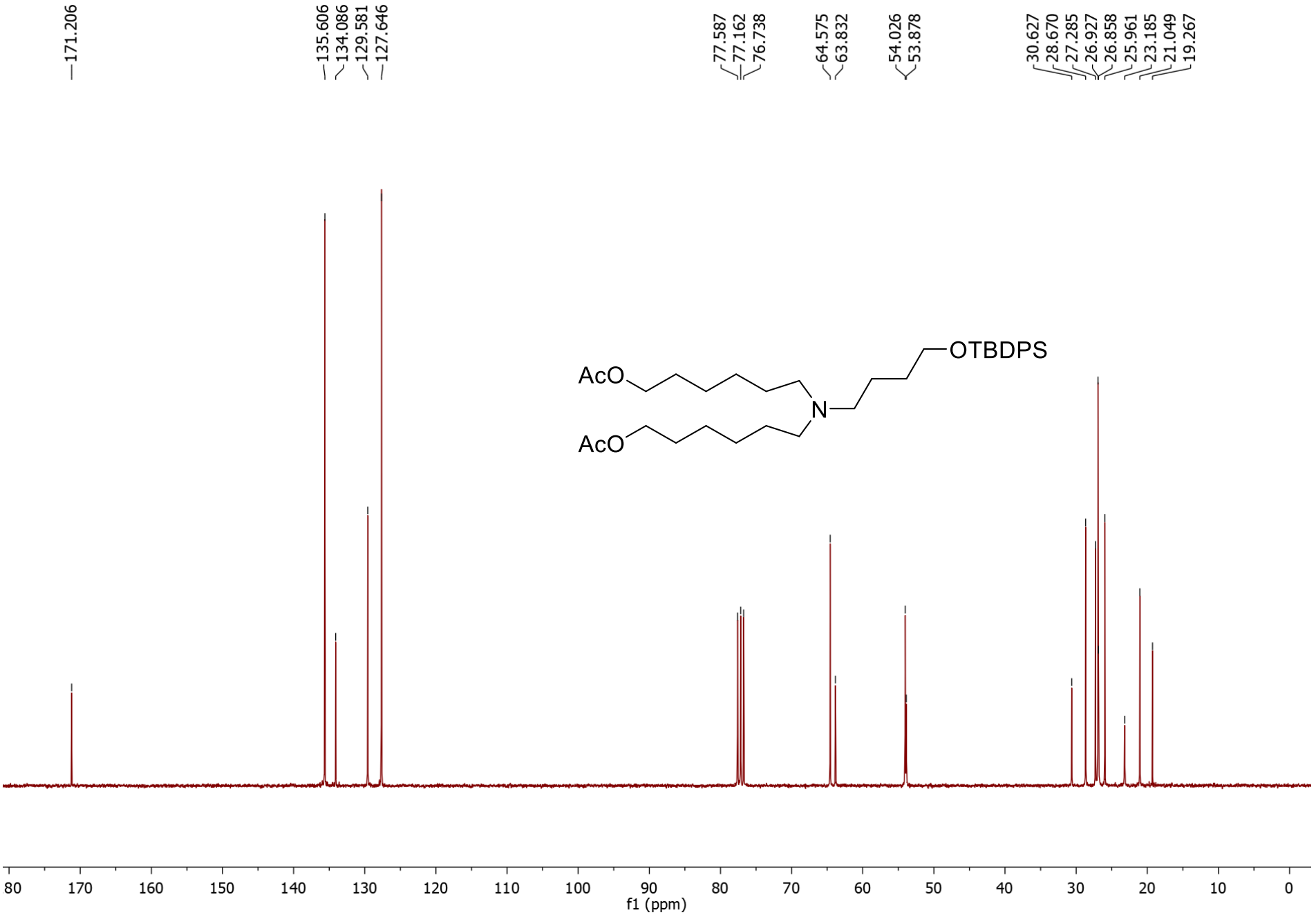
Low resolution mass spectrum of 6-acetoxyhexanal (9) (ESI)



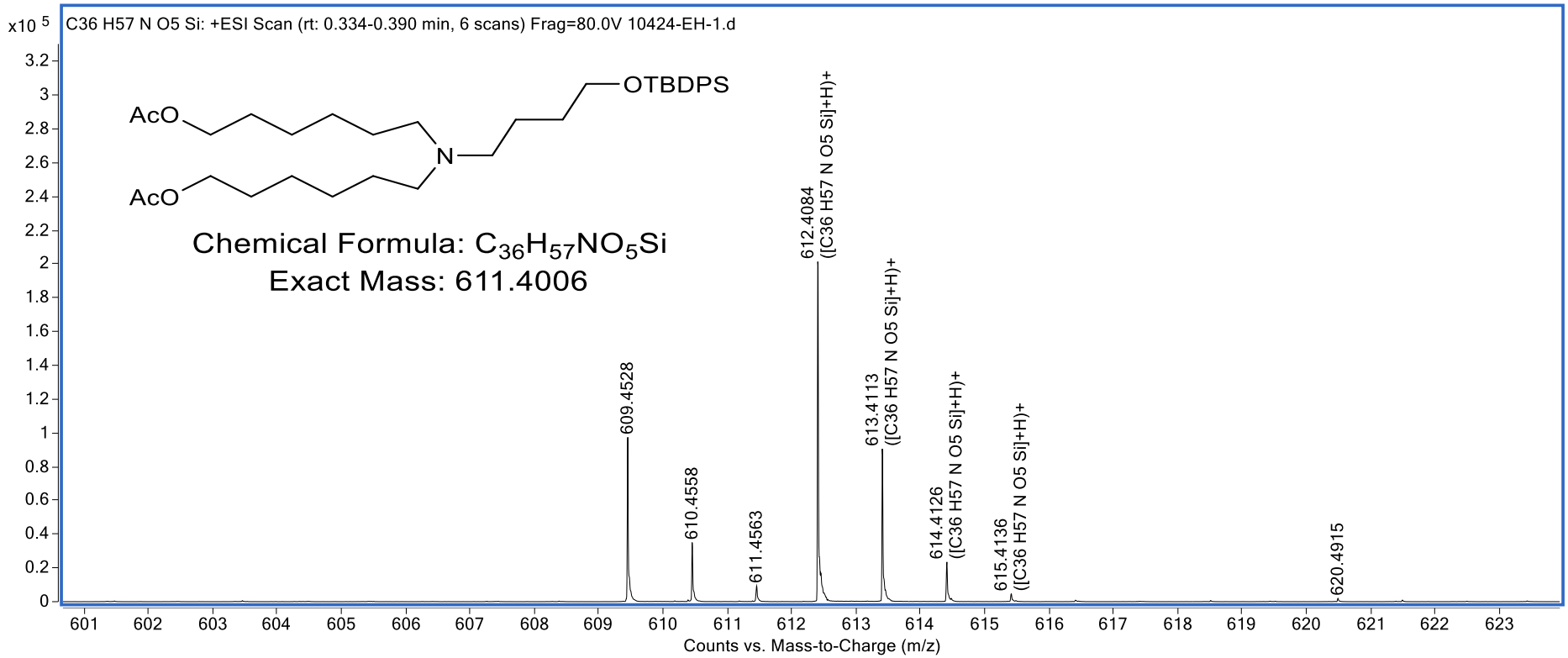
^{1}H-NMR Spectrum of compound 10 (300 MHz, $CDCl_3$)



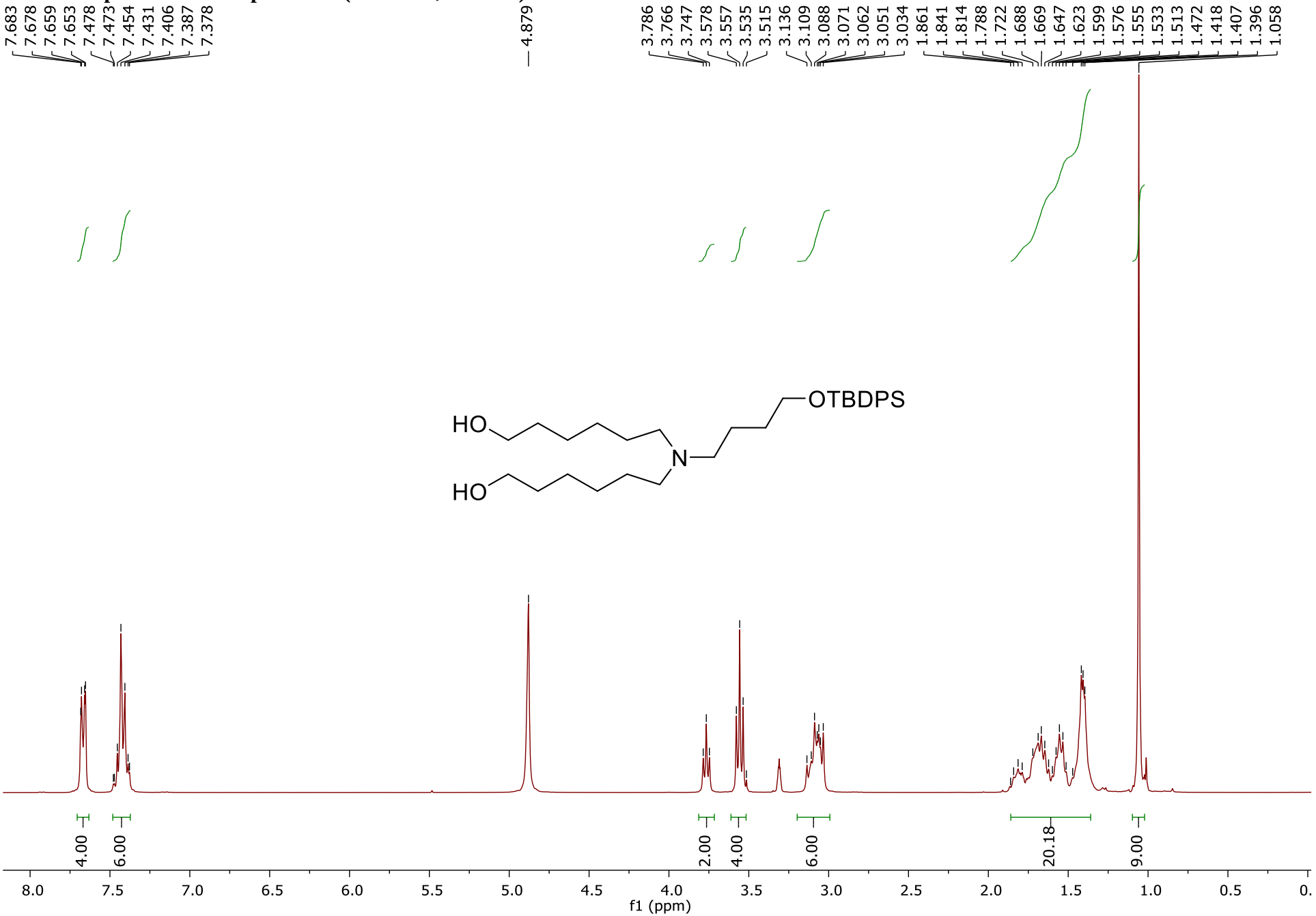
^{13}C-NMR Spectrum of compound 10 (75 MHz, $CDCl_3$)



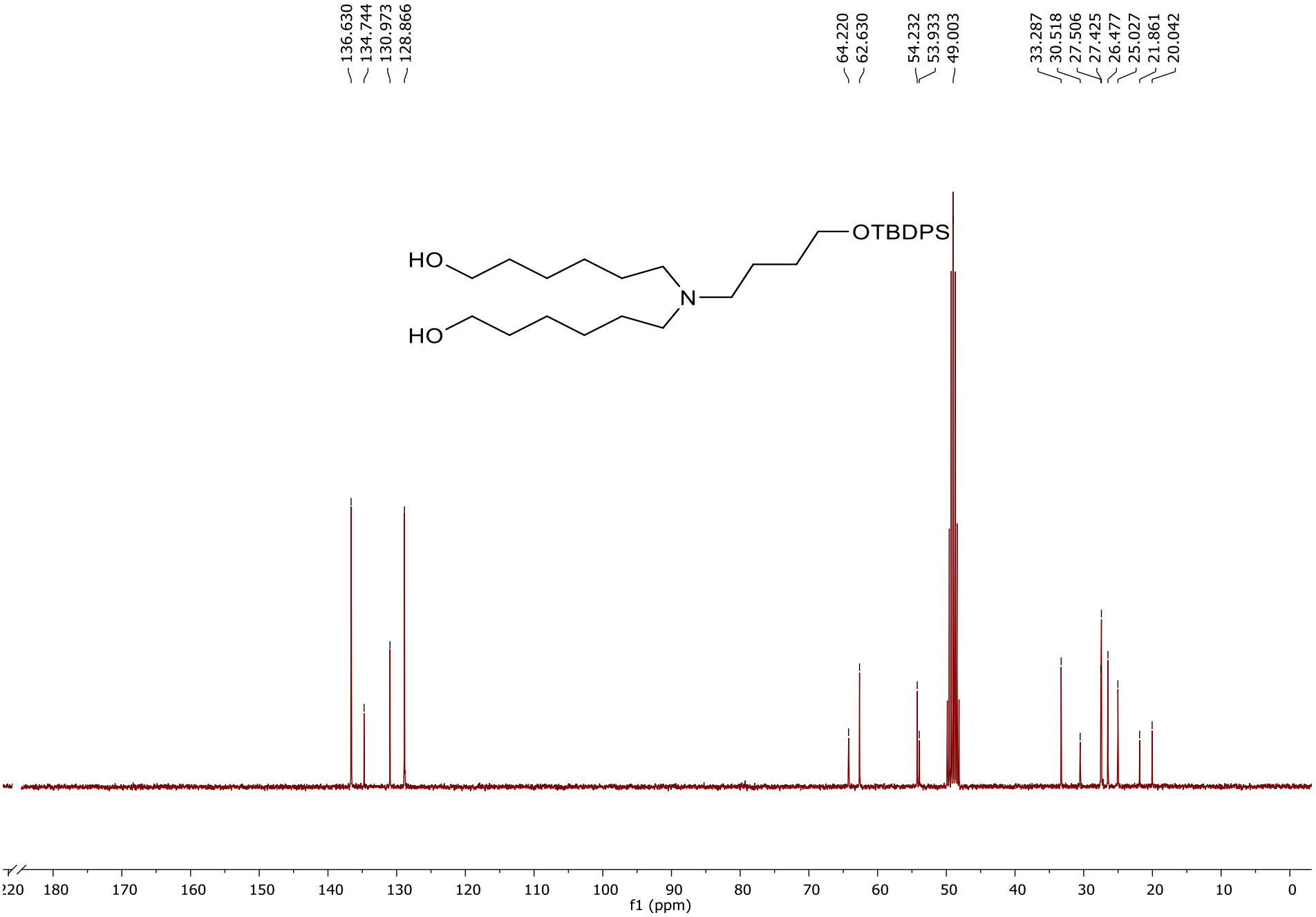
High resolution mass spectrum of compound 10 (ESI)



^{1}H-NMR Spectrum of compound 11 (300 MHz, CD_3OD)



^{13}C-NMR Spectrum of compound 11 (75 MHz, CD_3OD)



High resolution mass spectrum of compound 11 (ESI)