

Electronic Supplementary Information

Synthetic tetra-acylated lipid As derived from *Porphyromonas gingivalis* are antagonists of human TLR4

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Experimental

Chemical synthesis

Methyl 13-methyl-3-oxo-12-teradecenoate (12). Grubbs 2nd generation catalyst (27.2 mg, 0.032 mmol) was added to a stirred solution of compound **11** (1.0 g, 4.17 mmol) in 2-methyl-2-butene (20 mL) under an atmosphere of nitrogen. After stirring the reaction mixture at room temperature for 24 h, it was concentrated *in vacuo* to 0.5 mL and subjected to purification by silica gel column chromatography (eluent: hexane/ethyl acetate, 30/1, v/v) to afford **12** as a colorless oil (949 mg, 85%). $R_f = 0.50$ (hexane/ethyl acetate, 10/1, v/v); ¹H NMR (300 MHz, CDCl₃): δ 5.08 (t, 1H, $J_{11,12} = 6.9$ Hz, H-12), 3.71 (s, 3H, OCH₃), 3.42 (s, 2H, H-2), 2.50 (t, 2H, $J_{4,5} = 7.5$ Hz, H-4), 1.91 (m, 2H, H-11), 1.66 (s, 3H, H-14), 1.57-1.54 (m, 5H, H-5, H-14), 1.25 [bs, 10H, H-(6-10)]. HR MS (m/z) calcd for C₁₆H₂₈O₃ [M + Na]⁺, 291.1931; found, 291.1965.

Methyl 15-methyl-3-oxo-12-hexadecenoate (13). Grubbs 2nd generation catalyst (13.6 mg, 0.016 mmol) was added to a stirred solution of compound **11** (125 mg, 0.52 mmol) in 4-methyl-1-pentene (2 mL) under an atmosphere of nitrogen. After stirring the reaction mixture at room temperature for 16 h, it was concentrated *in vacuo* to 0.5 mL and subjected to purification by silica gel column chromatography (eluent: hexane/ethyl acetate, 30/1, v/v) to afford **13** as a colorless oil (137 mg, 89%). $R_f = 0.50$ (hexane/ethyl acetate, 10/1, v/v); ¹H NMR (300 MHz, CDCl₃): δ 5.32-5.29 (m, 2H, H-12, H-13), 3.66 (s, 3H, OCH₃), 3.38 (s, 2H, H-2), 2.46 (t, 2H, $J_{4,5} = 7.2$ Hz, H-4), 1.92 (m, 2H, H-14), 1.79 (m, 2H, H-11), 1.55-1.47 (m, 4H, H-5, H-15), 1.22 [bs, 10H, H-(6-10)], 0.80 (d, 6H, $J_{15,16} = 6.9$ Hz, H-16). HR MS (m/z) calcd for C₁₈H₃₂O₃ [M + Na]⁺, 319.2238; found, 319.2607.

Methyl (R)-3-hydroxy-13-methyl-tetradecanoate (14). A solution of **12** (800 mg, 2.99 mmol) in methanol (15 mL) was degassed with nitrogen for 10 min, after which 2 M HCl (0.2 mL) and RuCl₂[(R)-BINAP] (20 mg) were added under an atmosphere of nitrogen. The reaction mixture was shaken under an atmosphere of H₂ (65 psi) at 45 °C for 12 h, after which it was quenched with Et₃N (100 μ L). The solids were filtered off and the filtrate was concentrated *in vacuo*. The residue was purified by silica gel column chromatography (eluent: hexane/ethyl acetate, 6/1, v/v) to afford an alcohol as a colorless oil. The resulting intermediate was shaken with Pd/C (10 mg) in methanol (15 mL) under an atmosphere of H₂ (1 atm) for 12 h, after which

the catalyst was filtered off and the filtrate was concentrated *in vacuo*. The residue was purified by silica gel column chromatography (eluent: hexane/ethyl acetate, 6/1, v/v) to afford **14** as a colorless oil (780 mg, 96%, two steps). $R_f = 0.45$ (hexane/ethyl acetate, 4/1, v/v); $[\alpha]_D^{25} = -7.2^\circ$ ($c = 1.0$, CHCl_3); $^1\text{H NMR}$ (300 MHz, CDCl_3): δ 3.98 (m, 1H, H-3), 3.69 (s, 3H, OCH_3), 2.50 (dd, 1H, $J_{2a,2b} = 13.5$ Hz, $J_{2a,3} = 3.6$ Hz, H-2a), 2.38 (dd, 1H, $J_{2a,2b} = 13.5$ Hz, $J_{2b,3} = 8.7$ Hz, H-2b), 1.53-1.36 (m, 3H, H-4, H-13), 1.23-1.09 [m, 16H, H-(5-12)], 0.84 (d, 6H, $J_{13,14} = 6.9$ Hz, H-14); $^{13}\text{C NMR}$ (75 MHz, CDCl_3): δ 173.51 (C=O), 68.03 (C-3), 51.71 (CH_3O), 41.08 (C-2), 39.03 (C-12), 36.52 (C-4), 22.64 (C-14). HR MS (m/z) calcd for $\text{C}_{16}\text{H}_{32}\text{O}_3$ $[\text{M} + \text{Na}]^+$, 295.2244; found, 295.2194.

Methyl (R)-3-hydroxy-15-methyl-hexadecanoate (15). In a manner similar to the synthesis of compound **14**, compound **13** (100 mg, 0.338 mmol) was reduced by a two step procedure to afford **15** as a colorless oil (93 mg, 92%, two steps). $R_f = 0.50$ (hexane/ethyl acetate, 4/1, v/v); $[\alpha]_D^{25} = -6.0^\circ$ ($c = 1.0$, CHCl_3); $^1\text{H NMR}$ (300 MHz, CDCl_3): δ 3.95 (m, 1H, H-3), 3.66 (s, 3H, OCH_3), 2.47 (dd, 1H, $J_{2a,2b} = 16.2$ Hz, $J_{2a,3} = 3.3$ Hz, H-2a), 2.36 (dd, 1H, $J_{2a,2b} = 16.2$ Hz, $J_{2b,3} = 9.0$ Hz, H-2b), 1.53-1.32 (m, 3H, H-4, H-15), 1.21-1.03 [m, 20H, H-(5-14)], 0.81 (d, 6H, $J_{15,16} = 6.3$ Hz, H-16); $^{13}\text{C NMR}$ (75 MHz, CDCl_3): δ 173.47 (C=O), 67.97 (C-3), 51.67 (CH_3O), 41.08 (C-2), 39.02 (C-12), 36.51 (C-4), 22.62 (C-14). HR MS (m/z) calcd for $\text{C}_{18}\text{H}_{36}\text{O}_3$ $[\text{M} + \text{Na}]^+$, 323.2557; found, 323.1925.

2-(4-Bromophenyl)-2-oxoethyl-(R)-3-hydroxy-13-methyl-tetradecanoate (16). $\text{LiOH}\cdot\text{H}_2\text{O}$ (101 mg, 4.4 mmol) in H_2O (10 mL) was added to a stirred solution of **14** (600 mg, 2.2 mmol) in THF (150 mL). After stirring the reaction mixture at room temperature for 10 h, the THF was removed *in vacuo*. The aqueous residue was neutralized with 1N HCl (4.4 mL) and extracted with ethyl acetate (20 mL). The organic phase was dried (Na_2SO_4) and filtered. The filtrate was concentrated *in vacuo* to afford an acid intermediate. Next, this intermediate was refluxed with dicyclohexaneamine (0.52 mL, 2.64 mmol) in CH_3CN (80 mL) for 2 h. After the reaction mixture cooled down to room temperature, the precipitated solid was collected by filtration to give a salt as a white solid. This product was dissolved in EtOAc (25 mL) and then Et_3N (0.37 mL, 2.64 mmol) and 2,4'-dibromoacetophenone (672 mg, 2.42 mmol) were added. After stirring the reaction mixture at room temperature for 12 h, it was diluted with DCM (50 mL) and washed

with brine (2 x 30 mL). The organic phase was dried (MgSO₄) and filtered. Next, the filtrate was concentrated *in vacuo*. The residue was purified by silica gel column chromatography (eluent: DCM) to afford **16** as an amorphous solid (910 mg, 91%, three steps). $R_f = 0.35$ (DCM); $[\alpha]_D^{25} = -0.8^\circ$ ($c = 1.0$, CHCl₃); ¹H NMR (300 MHz, CDCl₃): δ 7.76 (d, 2H, $J = 8.7$ Hz, aromatic), 7.63 (d, 2H, $J = 8.7$ Hz, aromatic), 5.41 (d, 1H, $J = 16.5$ Hz, CH²_a), 5.29 (d, 1H, $J = 16.5$ Hz, CH²_b), 4.10 (m, 1H, H-3), 2.67 (dd, 1H, $J_{2a,2b} = 15.0$ Hz, $J_{2a,3} = 2.4$ Hz, H-2a), 2.54 (dd, 1H, $J_{2a,2b} = 15.0$ Hz, $J_{2b,3} = 9.0$ Hz, H-2b), 1.60-1.45 (m, 3H, H-4, H-13), 1.24-1.12 [m, 16H, H-(5-12)], 0.84 (d, 6H, $J_{13,14} = 6.6$ Hz, H-14). ¹³C NMR (75 MHz, CDCl₃): δ 191.63 (C=O), 171.95 (C=O), 132.54-129.29 (m, aromatic), 68.45 (C-3), 65.78 (C-2'), 41.99 (C-2), 39.04 (C-12), 36.56 (C-4), 22.65 (C-14). HR MS (m/z) calcd for C₂₃H₃₅BrO₄ [M + Na]⁺, 477.1611; found, 477.1241.

2-(4-Bromophenyl)-2-oxoethyl (R)-3-hydroxy-15-methyl-hexadecanoate (18). In a manner similar to the synthesis of **16**, compound **15** (960 mg, 3.2 mmol) was hydrolyzed with LiOH·H₂O (202 mg, 4.8 mmol), recrystallized by refluxing with DCHA (0.76 mL, 3.84 mmol) and protected by reacting with 2,4'-dibromoacetophenone (979 mg, 3.52 mmol) to afford **18** as an amorphous solid (1.39 g, 90%). $R_f = 0.35$ (DCM); $[\alpha]_D^{25} = -1.2^\circ$ ($c = 1.0$, CHCl₃); ¹H NMR (300 MHz, CDCl₃): δ 7.74 (d, 2H, $J = 8.4$ Hz, aromatic), 7.60 (d, 2H, $J = 8.4$ Hz, aromatic), 5.39 (d, 1H, $J = 16.5$ Hz, CH²_a), 5.29 (d, 1H, $J = 16.5$ Hz, CH²_b), 4.08 (m, 1H, H-3), 2.65 (dd, 1H, $J_{2a,2b} = 15.0$ Hz, $J_{2a,3} = 3.0$ Hz, H-2a), 2.54 (dd, 1H, $J_{2a,2b} = 15.0$ Hz, $J_{2b,3} = 9.0$ Hz, H-2b), 1.58-1.46 (m, 3H, H-4, H-15), 1.24-1.13 [m, 20H, H-(5-14)], 0.83 (d, 6H, $J_{15,16} = 6.6$ Hz, H-14). ¹³C NMR (75 MHz, CDCl₃): δ 191.60 (C=O), 171.86 (C=O), 132.49-129.24 (m, aromatic), 68.38 (C-3), 65.74 (C-2'), 41.96 (C-2), 39.00 (C-14), 36.54 (C-4), 22.61 (C-16). HR MS (m/z) calcd for C₂₅H₃₉BrO₄ [M + Na]⁺, 505.1924; found, 505.1160.

2-(4-Bromophenyl)-2-oxoethyl (R)-3-benzyloxy-13-methyl-tetradecanoate (17). To a cooled (0 °C) solution of **16** (405 mg, 0.89 mmol), benzaldehyde (0.27 mL, 2.67 mmol) and TMS₂O (1.13 mL, 5.34 mmol) in dry THF (20 mL) was added dropwise TMSOTf (77 μ L, 0.445 mmol). After stirring the reaction mixture for 15 min, Et₃SiH (0.50 mL, 3.12 mmol) was added dropwise. The stirring continued at room temperature for another 4 h, after which the reaction mixture was neutralized with Et₃N (60 μ L), diluted with ethyl acetate (40 mL) and washed with brine (2 x 25 mL). The organic phase was dried (MgSO₄) and filtered. Next, the filtrate was

concentrated *in vacuo*. The residue was purified by silica gel column chromatography (eluent: hexane/ethyl acetate, 25/1, v/v) to afford **17** as an amorphous solid (363 mg, 75%). $R_f = 0.55$ (hexane/ethyl acetate, 6/1, v/v); $[\alpha]_D^{25} = -6.2^\circ$ ($c = 1.0$, CHCl_3); $^1\text{H NMR}$ (300 MHz, CDCl_3): δ 7.77-7.23 (m, 9H, aromatic), 5.27 (d, 1H, $J = 16.5$ Hz, CH'_{2a}), 5.21 (d, 1H, $J = 16.5$ Hz, CH'_{2b}), 4.58 (d, 1H, $J = 11.4$ Hz, CHH Ph), 4.53 (d, 1H, $J = 11.4$ Hz, CHHPh), 3.93 (m, 1H, H-3), 2.77 (dd, 1H, $J_{2a,2b} = 15.0$ Hz, $J_{2a,3} = 7.2$ Hz, H-2a), 2.64 (dd, 1H, $J_{2a,2b} = 15.0$ Hz, $J_{2b,3} = 5.4$ Hz, H-2b), 1.66-1.24 (m, 3H, H-4, H-13), 1.24-1.11 [m, 16H, H-(5-12)], 0.84 (d, 6H, $J_{13,14} = 6.9$ Hz, H-14); $^{13}\text{C NMR}$ (75 MHz, CDCl_3): δ 191.36 (C=O), 171.19 (C=O), 138.56-127.56 (m, aromatic), 75.85 (C-3), 71.54 (CH_2Ph), 65.76 (C-2'), 39.54 (C-2), 39.00 (C-12), 34.36 (C-4), 22.62 (C-14). HR MS (m/z) calcd for $\text{C}_{30}\text{H}_{41}\text{BrO}_4$ $[\text{M} + \text{Na}]^+$, 567.2080; found, 567.2116.

2-(4-Bromophenyl)-2-oxoethyl (R)-3-benzyloxy-15-methyl-hexadecanoate (19). In a manner similar to the synthesis of **17**, the hydroxyl of compound **18** (627 mg, 1.30 mmol) was benzylated to afford **19** as an amorphous solid (528 mg, 71%). $R_f = 0.60$ (hexane/ethyl acetate, 6/1, v/v); $[\alpha]_D^{24.4} = -6.7^\circ$ ($c = 1.0$, CHCl_3); $^1\text{H NMR}$ (300 MHz, CDCl_3): δ 7.76-7.24 (m, 10H, aromatic), 5.27 (d, 1H, $J = 16.5$ Hz, CH'_{2a}), 5.21 (d, 1H, $J = 16.5$ Hz, CH'_{2b}), 4.58 (d, 1H, $J = 11.4$ Hz, CHHPh), 4.52 (d, 1H, $J = 11.4$ Hz, CHHPh), 3.92 (m, 1H, H-3), 2.77 (dd, 1H, $J_{2a,2b} = 15.3$ Hz, $J_{2a,3} = 7.2$ Hz, H-2a), 2.64 (dd, 1H, $J_{2a,2b} = 15.3$ Hz, $J_{2b,3} = 5.4$ Hz, H-2b), 1.66-1.36 (m, 3H, H-4, H-13), 1.24-1.12 [m, 20H, H-(5-14)], 0.84 (d, 6H, $J_{15,16} = 6.9$ Hz, H-16). $^{13}\text{C NMR}$ (75 MHz, CDCl_3): δ 191.22 (C=O), 171.15 (C=O), 138.58-127.53 (m, aromatic), 75.95 (C-3), 71.56 (CH_2Ph), 65.79 (C-2'), 39.51 (C-2), 39.06 (C-14), 34.39 (C-4), 22.65 (C-16). HR MS (m/z) calcd for $\text{C}_{32}\text{H}_{45}\text{BrO}_4$ $[\text{M} + \text{Na}]^+$, 595.2393; found, 595.2437.

(R)-3-Benzyloxy-13-methyl-tetradecanoic acid (7). Zinc dust (< 10 micron, 382 mg, 5.87 mmol) was added portionwise to a solution of **17** (320 mg, 0.587 mmol) in acetic acid (15 mL). The reaction mixture was stirred at 60 °C for 2 h and then diluted with DCM (20 mL). The solids were filtered off through a pad of Celite and the residue was washed with DCM (3 x 5 mL). The combined filtrates were concentrated *in vacuo* and the residue was purified by silica gel column chromatography (eluent: DCM/methanol, 100/1, v/v) to afford **7** as an amorphous solid (198 mg, 97%). $R_f = 0.40$ (toluene/ethyl acetate, 3/1, v/v); $[\alpha]_D^{25} = -2.3^\circ$ ($c = 1.0$, CHCl_3); $^1\text{H NMR}$ (300 MHz, CDCl_3): δ 7.35-7.25 (m, 5H, aromatic), 4.55 (s, 2H, CH_2Ph), 3.86 (m, 1H, H-3), 2.62 (dd,

1H, $J_{2a,2b} = 15.6$ Hz, $J_{2a,3} = 6.9$ Hz, H-2a), 2.53 (dd, 1H, $J_{2a,2b} = 15.6$ Hz, $J_{2b,3} = 5.1$ Hz, H-2b), 1.66-1.45 (m, 3H, H-4, H-13), 1.38-1.10 [m, 16H, H-(5-12)], 0.85 (d, 6H, $J_{15,16} = 6.9$ Hz, H-14); ^{13}C NMR (75 MHz, CDCl_3): δ 176.76 (C=O), 138.04-127.73 (aromatic), 75.70 (C-3), 71.53 (CH_2Ph), 39.42 (C-2), 39.03 (C-12), 34.09 (C-4), 22.66 (C-14); HR MS (m/z) calcd for $\text{C}_{22}\text{H}_{36}\text{O}_3$ $[\text{M} + \text{Na}]^+$, 371.2557; found, 371.1906.

(R)-3-Benzoyloxy-15-methyl-hexadecanoic acid (8). In a manner similar to the synthesis of **7**, compound **19** (350 mg, 0.611 mmol) was treated with zinc (< 10 micron, 397 mg, 6.11 mmol) to afford **8** as an amorphous solid (207 mg, 97%). $R_f = 0.45$ (toluene/ethyl acetate, 3/1, v/v); $[\alpha]_D^{25} = -2.5^\circ$ ($c = 1.0$, CHCl_3); ^1H NMR (300 MHz, CDCl_3): δ 7.34-7.26 (m, 5H, aromatic), 4.57 (s, 2H, CH_2Ph), 3.87 (m, 1H, H-3), 2.64 (dd, 1H, $J_{2a,2b} = 15.6$ Hz, $J_{2a,3} = 6.9$ Hz, H-2a), 2.55 (dd, 1H, $J_{2a,2b} = 15.6$ Hz, $J_{2b,3} = 5.1$ Hz, H-2b), 1.68-1.38 (m, 3H, H-4, H-15), 1.26-1.14 [m, 20H, H-(5-14)], 0.86 (d, 6H, $J_{15,16} = 6.9$ Hz, H-16); ^{13}C NMR (75 MHz, CDCl_3): δ 176.95 (C=O), 138.08-127.71 (aromatic), 75.71 (C-3), 71.53 (CH_2Ph), 39.47 (C-2), 39.05 (C-12), 34.12 (C-4), 22.65 (C-14). HR MS (m/z) calcd for $\text{C}_{24}\text{H}_{40}\text{O}_3$ $[\text{M} + \text{Na}]^+$, 399.2870; found, 399.2552.

2-(4-Bromophenyl)-2-oxoethyl-(R)-3-hexadecanoyloxy-15-methyl-hexadecanoate (20). Palmitoyl chloride (0.41 mL, 1.34 mmol) was added dropwise to a stirred solution of **18** (540 mg, 1.12 mmol), pyridine (0.22 mL, 2.68 mmol) and DMAP (13 mg, 0.11 mmol) in DCM (10 mL). After stirring the reaction mixture at room temperature for 10 h, it was diluted with DCM (20 mL) and then washed with saturated aqueous NaHCO_3 (2 x 20 mL) and brine (2 x 20 mL). The organic phase was dried (MgSO_4) and filtered. Next, the filtrate was concentrated *in vacuo*. The residue was purified by silica gel column chromatography (eluent: toluene) to afford **20** as an amorphous solid (767 mg, 95%). $R_f = 0.70$ (DCM); $[\alpha]_D^{25} = -0.1^\circ$ ($c = 1.0$, CHCl_3); ^1H NMR (300 MHz, CDCl_3): δ 7.73 (d, 2H, $J = 8.4$ Hz, aromatic), 7.59 (d, 2H, $J = 8.4$ Hz, aromatic), 5.29-5.24 (m, 3H, H-3, $\text{OCH}_2\text{COPhBr}$), 2.70 (m, 2H, H-2), 2.28 (t, 2H, $J_{2,3} = 7.5$ Hz, H-2'), 1.64-1.42 (m, 5H, H-4, H-15, H-3'), 1.23-1.11 [m, 44H, H-(5-14), H-(4'-15')], 0.84-0.82 (m, 9H, H-16, H-16'); ^{13}C NMR (75 MHz, CDCl_3): δ 190.78 (C=O), 173.20 (C=O), 169.80 (C=O), 132.83-129.06 (m, aromatic), 70.08 (C-3), 65.84 ($\text{OCH}_2\text{COPhBr}$). HR MS (m/z) calcd for $\text{C}_{41}\text{H}_{69}\text{BrO}_5$ $[\text{M} + \text{Na}]^+$, 743.4221; found, 745.4365.

(R)-3-Hexadecanoyloxy-15-methyl-hexadecanoic acid (9). In a manner similar to the synthesis of **7**, compound **20** (500 mg, 0.666 mmol) was treated with zinc (<10 micron, 430 mg, 6.66 mmol) to afford **9** as an amorphous solid (335 mg, 96%). $R_f = 0.35$ (toluene/ethyl acetate, 4/1, v/v); $[\alpha]_D^{25} = -0.6^\circ$ ($c = 1.0$, CHCl_3); $^1\text{H NMR}$ (300 MHz, CDCl_3): δ 5.19 (m, 1H, H-3), 2.58 (m, 2H, H-2), 2.25(t, 2H, $J = 7.5$ Hz, H-2'), 1.60-1.43 (m, 5H, H-4, H-15, H-3'), 1.23-1.14 [m, 44H, H-(5-14), H-(4'-15')], 0.85-0.83 (m, 9H, H-16, H-16'); $^{13}\text{C NMR}$ (75 MHz, CDCl_3): δ 176.24 (C=O), 173.27 (C=O), 69.95 (C-3). HR MS (m/z) calcd for $\text{C}_{33}\text{H}_{64}\text{O}_4$ $[\text{M} + \text{Na}]^+$, 547.4697; found, 547.5009.

Dimethylhexylsilyl 4,6-O-benzylidene-2-deoxy-2-(9-fluorenylmethoxycarbonylamino)- β -D-glucopyranoside (22). A suspension of compound **21** (1.02 g, 2.34 mmol) and zinc (<10 micron, 1.52 g, 23.4 mmol) in a mixture of acetic acid (250 μL) and DCM (12 mL) was stirred at room temperature for 4 h, after which it was diluted with ethyl acetate (40 mL). The solids were removed by filtration and the residue was washed with ethyl acetate (2 x 4 mL). The combined filtrates were washed with saturated aqueous NaHCO_3 (2 x 30 mL) and brine (2 x 25 mL). The organic phase was dried (MgSO_4) and filtered. The filtrate was concentrated *in vacuo* to afford an amine as a pale yellow oil. The resulting amine was dissolved in DCM (12 mL) and then FmocCl (664 mg, 2.57 mmol) and DIPEA (447 μL , 2.57 mmol) were added. The reaction mixture was stirred at room temperature for 3 h, after which it was diluted with DCM (20 mL) and washed with brine (2 x 30 mL). The organic phase was dried (MgSO_4) and filtered. Next, the filtrate was concentrated *in vacuo*. The residue was purified by silica gel column chromatography (eluent: hexane/ethyl acetate, 3/1, v/v) to yield **22** as an amorphous solid (1.38 g, 90%, two steps). $R_f = 0.55$ (hexane/ethyl acetate, 3/2, v/v); $[\alpha]_D^{25} = -13.9^\circ$ ($c = 1.0$, CHCl_3); $^1\text{H NMR}$ (300 MHz, CD_3COCD_3): δ 7.86-7.23 (m, 13H, aromatic), 6.64 (d, 1H, $J_{\text{NH},2} = 9.0$ Hz, NH), 5.61 (s, 1H, $>\text{CHPh}$), 4.92 (d, 1H, $J_{1,2} = 7.8$ Hz, H-1), 4.32-4.19 (m, 4H, H-6a, OCH_2CH of Fmoc), 3.88 (m, 1H, H-3), 3.78 (t, 1H, $J_{5,6b} = J_{6a,6b} = 9.9$ Hz, H-6b), 3.56 (t, 1H, $J_{3,4} = J_{4,5} = 9.3$ Hz, H-4), 3.54 (m, 1H, H-2), 3.44 (m, 1H, H-5), 1.61 (m, 1H, CH of TDS), 0.86-0.84 [m, 12H, $\text{SiC}(\text{CH}_3)_2\text{CH}(\text{CH}_3)_2$], 0.15 (s, 3H, SiCH_3), 0.14 (s, 3H, SiCH_3). $^{13}\text{C NMR}$ (75 MHz, CDCl_3): δ 156.96 (C=O), 144.99-120.57 (m, aromatic), 101.93 ($>\text{CHPh}$), 97.80 (C-1), 82.77 (C-4), 71.75 (C-3), 69.09 (C-6), 67.11 (C-5), 66.86 (OCH_2 of Fmoc), 61.28 (C-2), 47.84 (OCH_2CH of Fmoc),

34.59 (CH of TDS), -1.83 (SiCH₃), -3.23 (SiCH₃). HR MS (m/z) calcd for C₃₆H₄₅NO₇Si[M+Na]⁺, 654.2857; found, 654.2962.

Dimethylhexylsilyl 4,6-O-benzylidene-2-deoxy-2-(9-fluorenylmethoxycarbonylamino)-3-O-levulinoyl-β-D-glucopyranoside (23). A solution of levulinic acid (234 mg, 2.02 mmol) and 1,3-dicyclohexylcarbodiimide (DCC) (499 mg, 2.42 mmol) in DCM (8 mL) was stirred at room temperature for 10 min, after which compound **22** (1.16 g, 1.84 mmol) and DMAP (12 mg, 0.1 mmol) were added and the stirring was continued for another 10 h. The insoluble materials were removed by filtration and the residue was washed with DCM (2 x 1 mL). The combined filtrates were concentrated *in vacuo* and the residue was purified by silica gel column chromatography (eluent: DCM/CH₃OH, 60/1, v/v) to give **23** as an amorphous solid (1.16g, 86%). *R_f* = 0.55 (hexane/ethyl acetate, 2/1, v/v); [α]_D²⁵ = -14.6° (c = 1.0, CHCl₃); ¹H NMR (300 MHz, CD₃COCD₃): δ 7.86-7.29 (m, 13H, aromatic), 6.62 (d, 1H, *J*_{NH,2} = 9.6 Hz, *NH*), 5.63 (s, 1H, >CHPh), 5.31 (t, 1H, *J*_{2,3} = *J*_{3,4} = 9.9 Hz, H-3), 5.09 (d, 1H, *J*_{1,2} = 7.8 Hz, H-1), 4.32-4.19 (m, 4H, H-6a, OCH₂CH of Fmoc), 3.83 (t, 1H, *J*_{5,6b} = *J*_{6a,6b} = 9.9 Hz, H-6b), 3.78 (t, 1H, *J*_{3,4} = *J*_{4,5} = 9.3 Hz, H-4), 3.68 (m, 1H, H-2), 3.54 (m, 1H, H-5), 2.64 (t, 2H, *J* = 6.9 Hz, CH₂ of Lev), 2.49 (t, 2H, *J* = 6.9 Hz, CH₂ of Lev), 2.01 (s, 3H, CH₃ of Lev), 1.62 (m, 1H, CH of TDS), 0.86-0.84 (m, 12H, SiC(CH₃)₂CH(CH₃)₂), 0.17 (s, 6H, Si(CH₃)₂). ¹³C NMR (75 MHz, CDCl₃): 172.49 (C=O), 156.73 (C=O), 145.07-120.64 (m, aromatic), 101.67 (>CHPh), 97.55 (C-1), 79.80 (C-4), 72.64 (C-3), 69.03 (C-6), 67.11 (C-5, OCH₂ of Fmoc), 59.33 (C-2), 47.83 (OCH₂CH of Fmoc), 38.13 (CH₂ of Lev), 34.66 (CH of TDS), -1.85 (SiCH₃), -3.25 (SiCH₃). HR MS (m/z) calcd for C₄₁H₅₁NO₉Si[M+Na]⁺, 752.3225; found 752.2672.

4,6-O-Benzylidene-2-deoxy-2-(9-fluorenylmethoxycarbonylamino)-3-O-levulinoyl-D-glucopyranosyl trichloroacetimidate (5). A mixture of Bu₄NF (1 M in THF, 5 mL) and acetic acid (500 μl) was added dropwise to a stirred solution of **23** (800 mg, 1.10 mmol) in THF (15 mL). After stirring the reaction mixture at room temperature for 24 h, it was diluted with DCM (20 mL) and then washed with saturated aqueous NaHCO₃ (2 x 30 mL) and brine (2 x 30 mL). The organic phase was dried (MgSO₄) and filtered. Next, the filtrate was concentrated *in vacuo*. The residue was purified by silica gel column chromatography (eluent: DCM/CH₃OH, 30/1, v/v) to afford a lactol as a pale yellow solid (606 mg, 94%). *R_f* = 0.60 (hexane/ethyl acetate, 3/5, v/v).

^1H NMR (300 MHz, CDCl_3): δ 7.88-7.31 (m, 13H, aromatic), 6.62 (d, 1H, $J_{\text{NH},2} = 9.6$ Hz, NH), 5.64 (s, 1H, $>\text{CHPh}$), 5.39 (t, 1H, $J_{2,3} = J_{3,4} = 9.9$ Hz, H-3), 5.09 (bs, 1H, H-1), 4.43-4.17 (m, 4H, H-6a, OCH_2CH of Fmoc), 4.13-3.97 (m, 2H, H-2, H-5), 3.81 (t, 1H, $J_{5,6b} = J_{6a,6b} = 9.9$ Hz, H-6b), 3.80 (t, 1H, $J_{3,4} = J_{4,5} = 9.6$ Hz, H-4), 2.65 (t, 2H, $J = 6.6$ Hz, CH_2 of Lev), 2.50 (t, 2H, $J = 6.6$ Hz, CH_2 of Lev), 2.00 (s, 3H, CH_3 of Lev). HR MS (m/z) calcd for $\text{C}_{33}\text{H}_{33}\text{NO}_9[\text{M}+\text{Na}]^+$, 610.2048; found, 610.2293. The resulting lactol (606 mg, 1.03 mmol) was dissolved in a mixture of trichloroacetonitrile (2.0 mL) and DCM (10 mL) and then Cs_2CO_3 (163 mg, 0.50 mmol) was added. The reaction mixture was stirred at room temperature for 1 h, after which it was diluted with DCM (20 mL) and then washed with saturated aqueous NaHCO_3 (2 x 30 mL) and brine (2 x 30 mL). The organic phase was dried (Na_2SO_4) and filtered. Next, the filtrate was concentrated *in vacuo*. The residue was purified by silica gel column chromatography (eluent: hexane/ethyl acetate, 4/3, v/v) to yield **5** as a pale yellow solid (700 mg, 93%). $R_f = 0.45$ (hexane/ethyl acetate, 3/2, v/v).

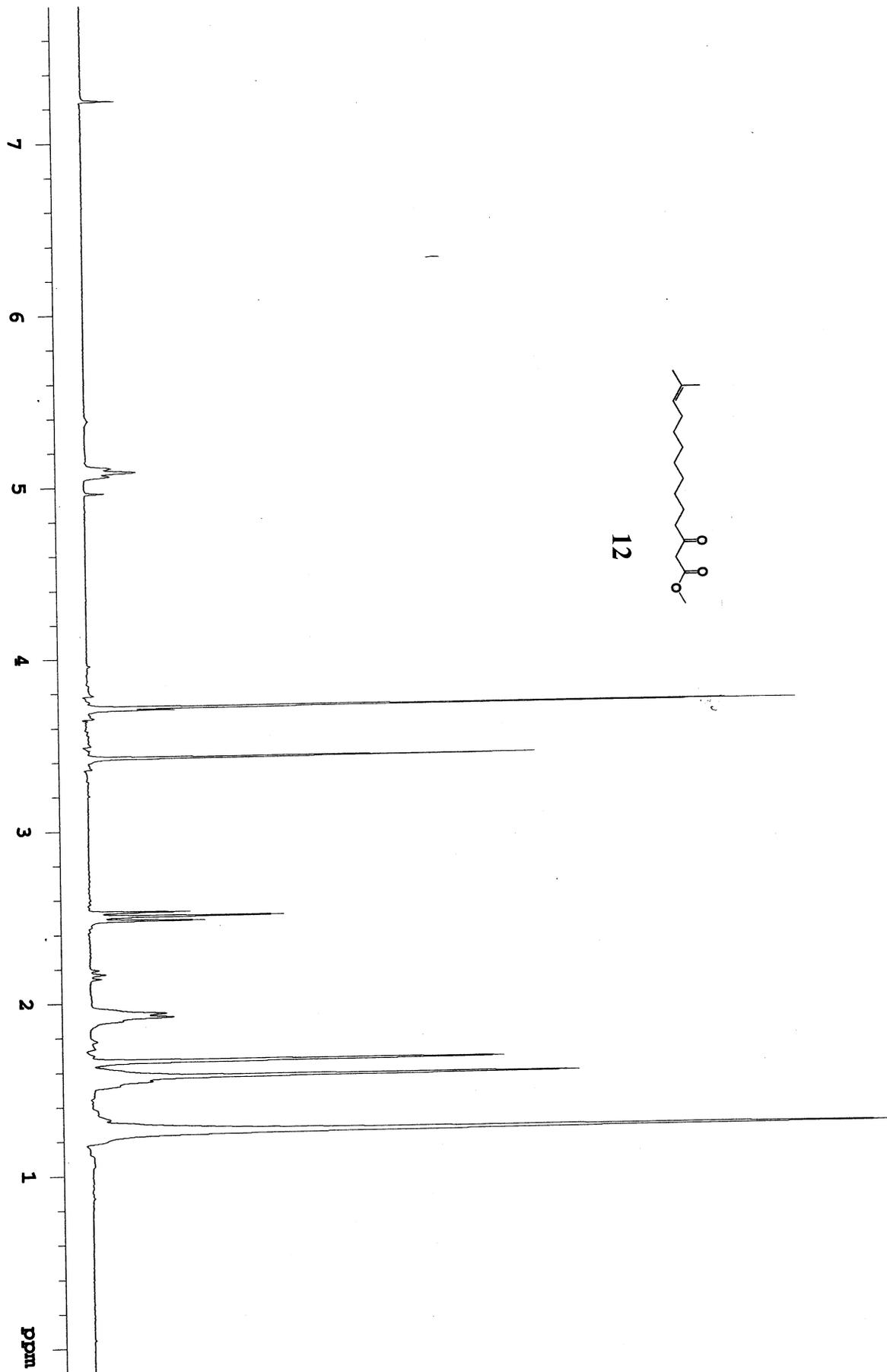
Biological experiments

Cell maintenance. Mono Mac 6 (MM6) cells, provided by Dr. H.W.L. Ziegler-Heitbrock (Institute for Inhalationbiology, Munich, Germany), were cultured in RPMI 1640 medium with L-glutamine (BioWhittaker) supplemented with penicillin (100 u mL^{-1})/streptomycin ($100 \mu\text{g mL}^{-1}$; Mediatech, OPI supplement (1%; Sigma; containing oxaloacetate, pyruvate and bovine insulin) and fetal calf serum (FCS; 10%; HyClone). New batches of frozen cell stock were grown up every 2 months and growth morphology evaluated. Before each experiment, MM6 cells were incubated with calcitriol (10 ng mL^{-1} ; Sigma) for 2 days to differentiate into macrophage like cells. RAW 264.7 $\gamma\text{NO}(-)$ cells, derived from the RAW 264.7 mouse monocyte/macrophage cell line, were obtained from ATCC. The cells were maintained in RPMI 1640 medium (ATCC) with L-glutamine (2 mM), adjusted to contain sodium bicarbonate (1.5 g L^{-1}), glucose (4.5 g L^{-1}), HEPES (10 mM) and sodium pyruvate (1.0 mM) and supplemented with penicillin (100 u mL^{-1}) / streptomycin ($100 \mu\text{g mL}^{-1}$) and FBS (10%). Human embryonic kidney (HEK) 293T cells were grown in Dulbecco's modified Eagle's medium (ATCC) with L-glutamine (4 mM), glucose (4.5 g L^{-1}) and sodium bicarbonate (1.5 g L^{-1}) supplemented with penicillin (100 u mL^{-1}) / streptomycin ($100 \mu\text{g mL}^{-1}$), Normocin ($100 \mu\text{g mL}^{-1}$; InvivoGen) and FBS (10%). Stably transfected HEK 293T cells with human and murine TLR4/MD2/CD14 and human and murine

TLR2 were obtained from InvivoGen and grown in the same growth medium as for HEK 293T cells supplemented with the appropriate selective agents HygroGold (50 $\mu\text{g mL}^{-1}$; InvivoGen) and blasticidin (10 $\mu\text{g mL}^{-1}$; InvivoGen). All cells were maintained in a humid 5% CO_2 atmosphere at 37°C.

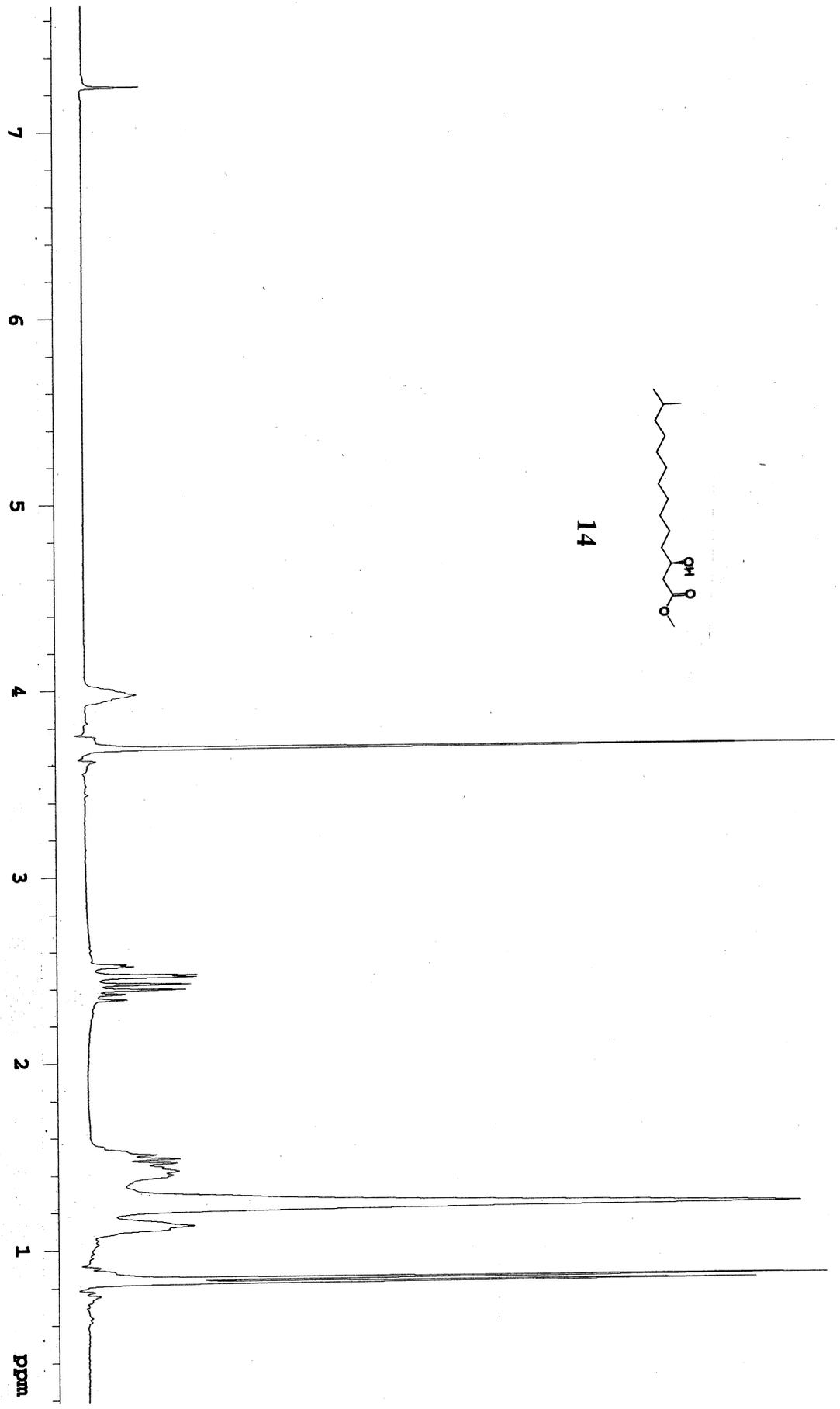


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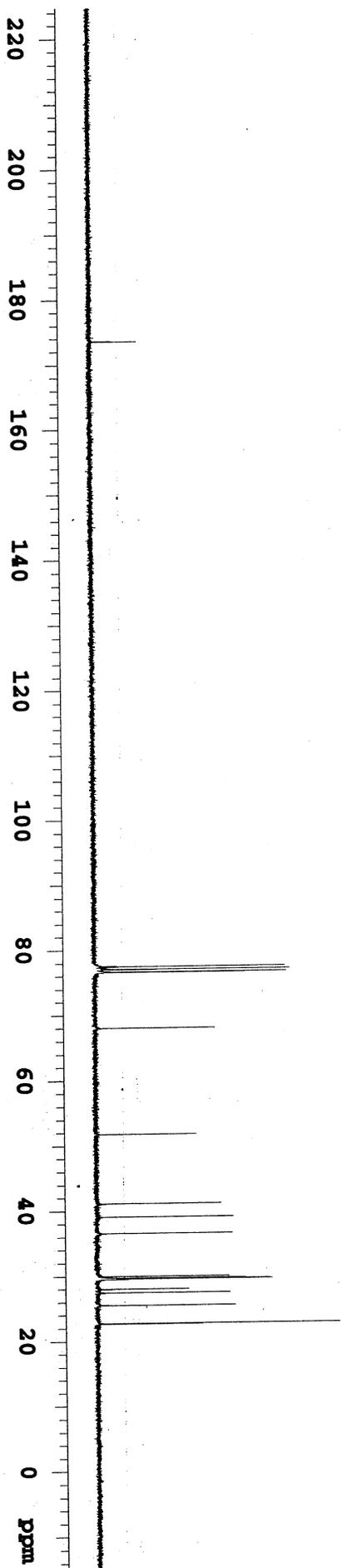


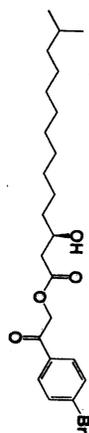
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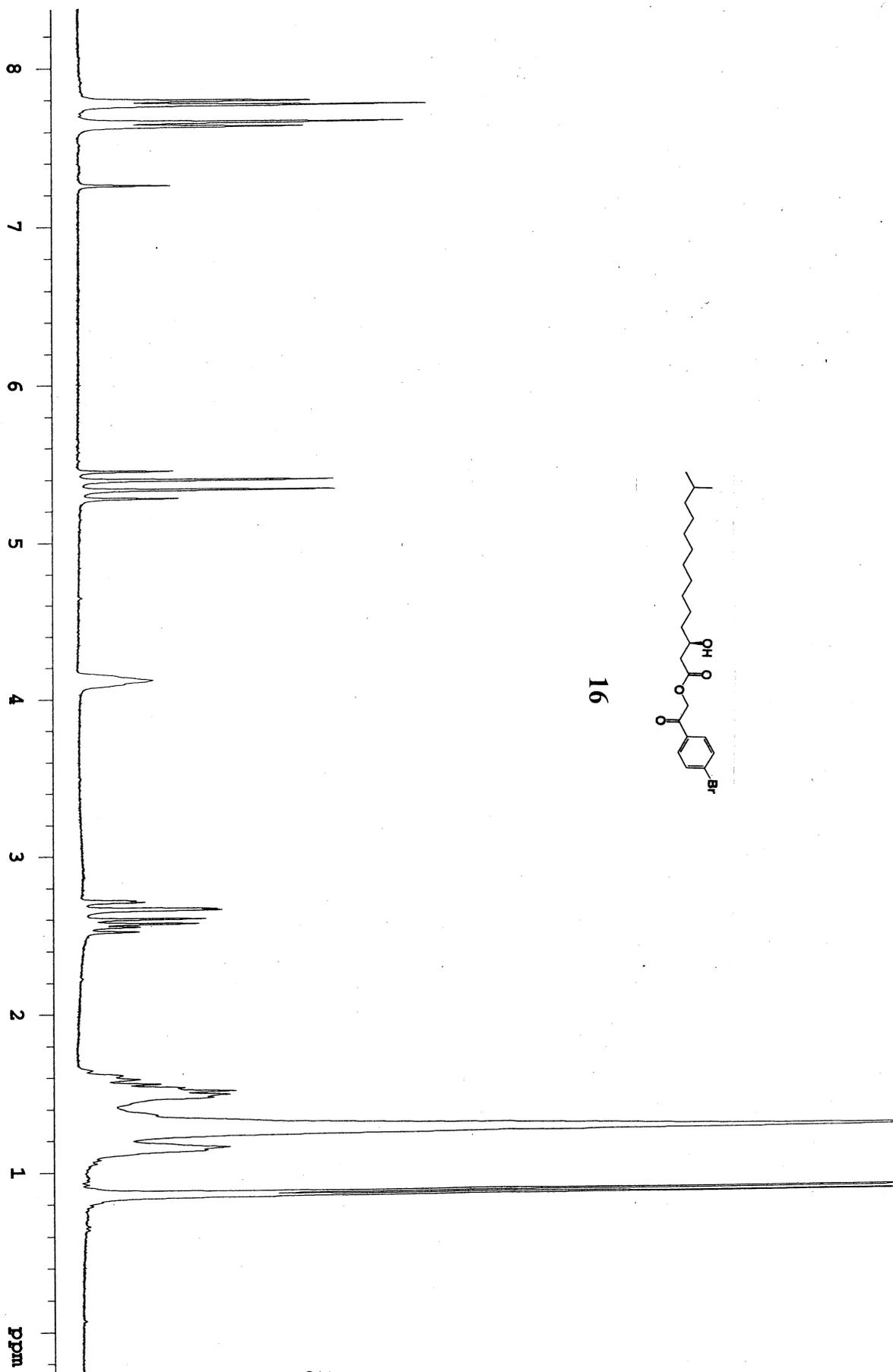


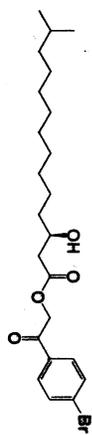
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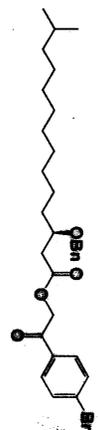
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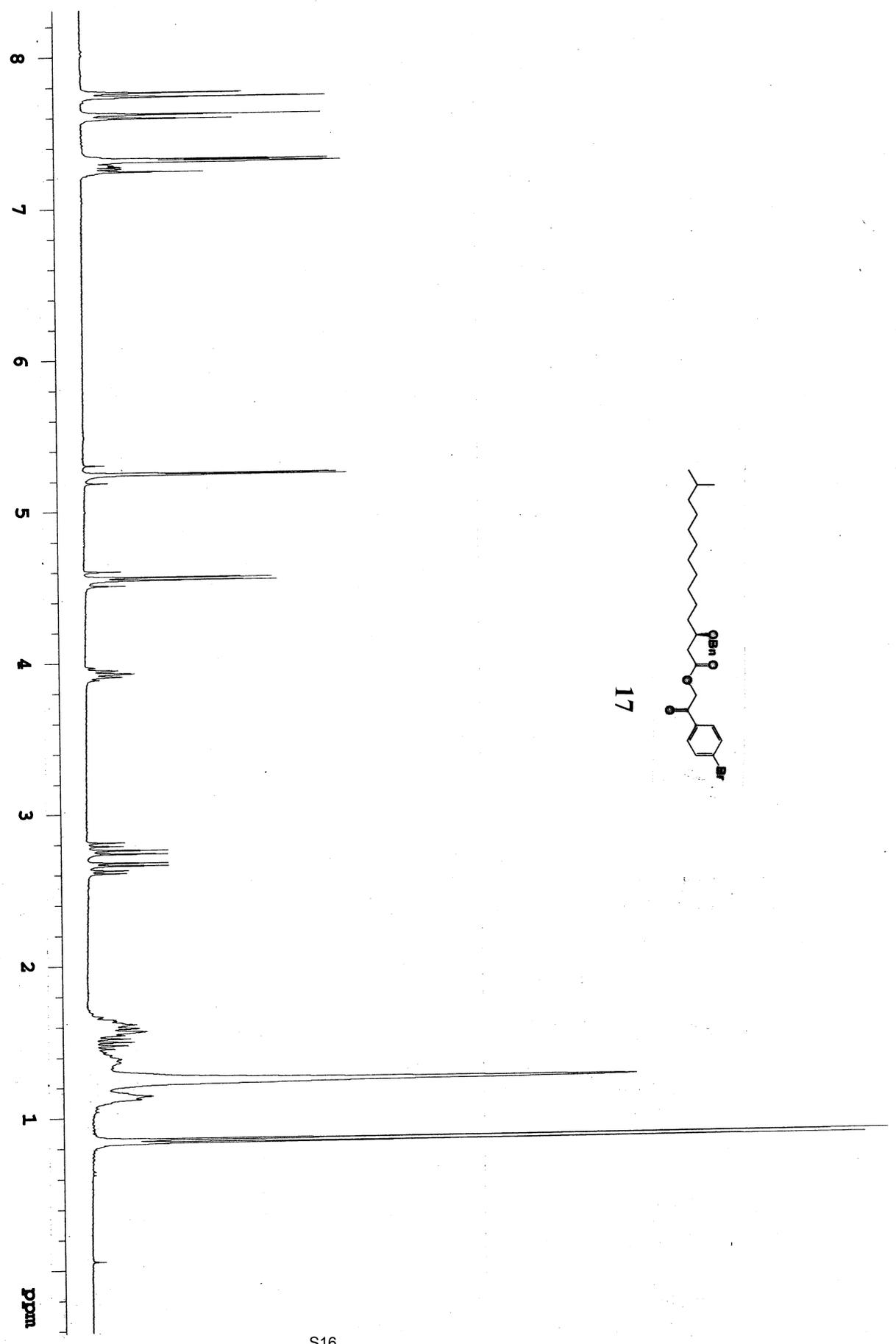


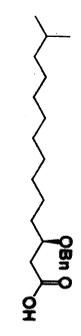
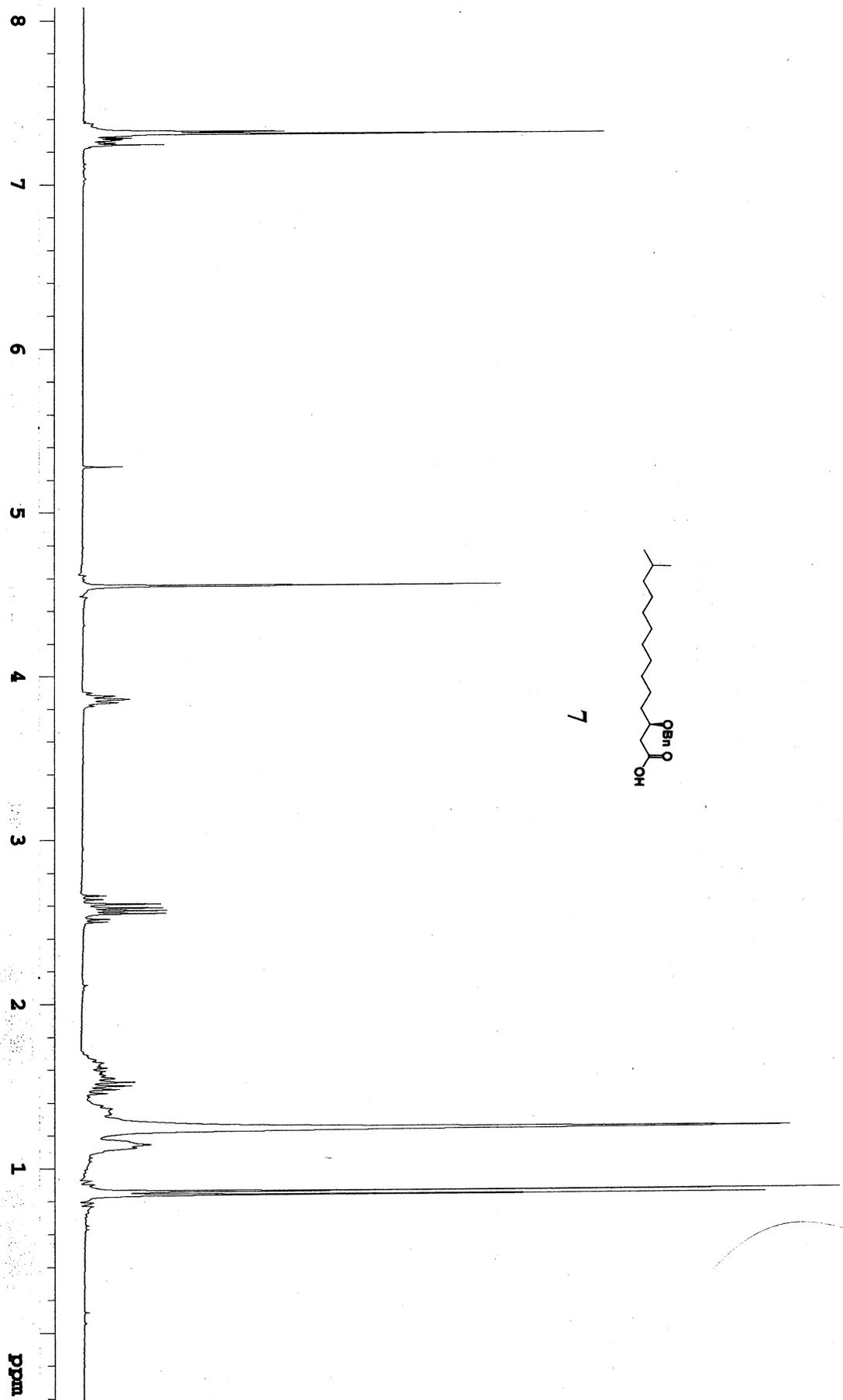
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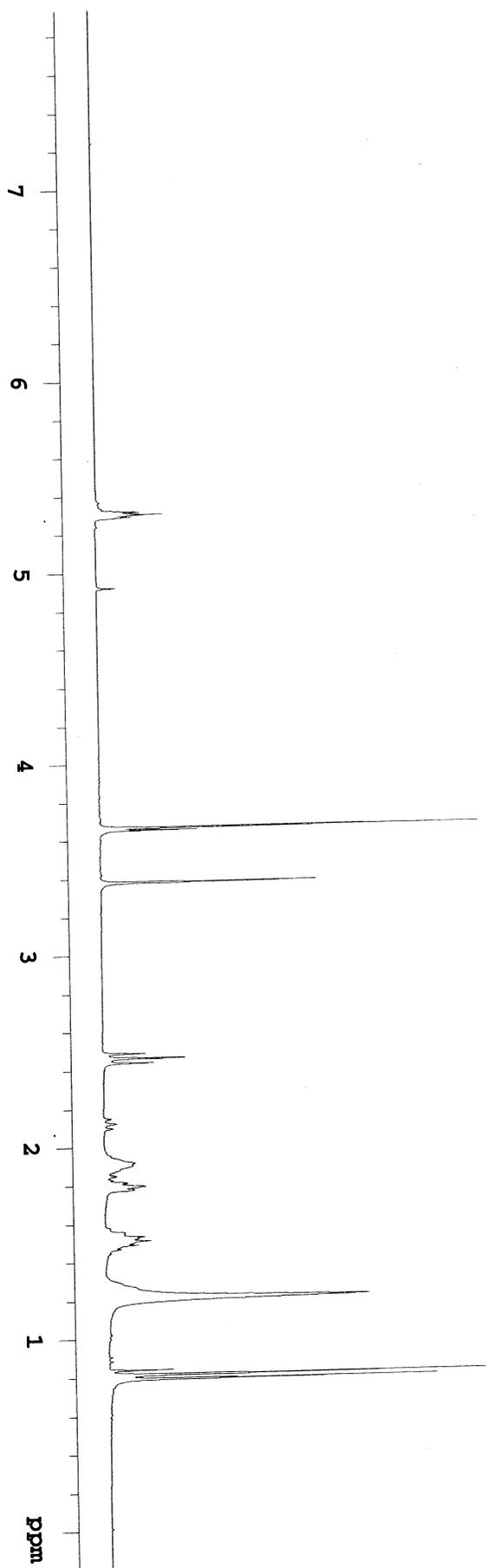




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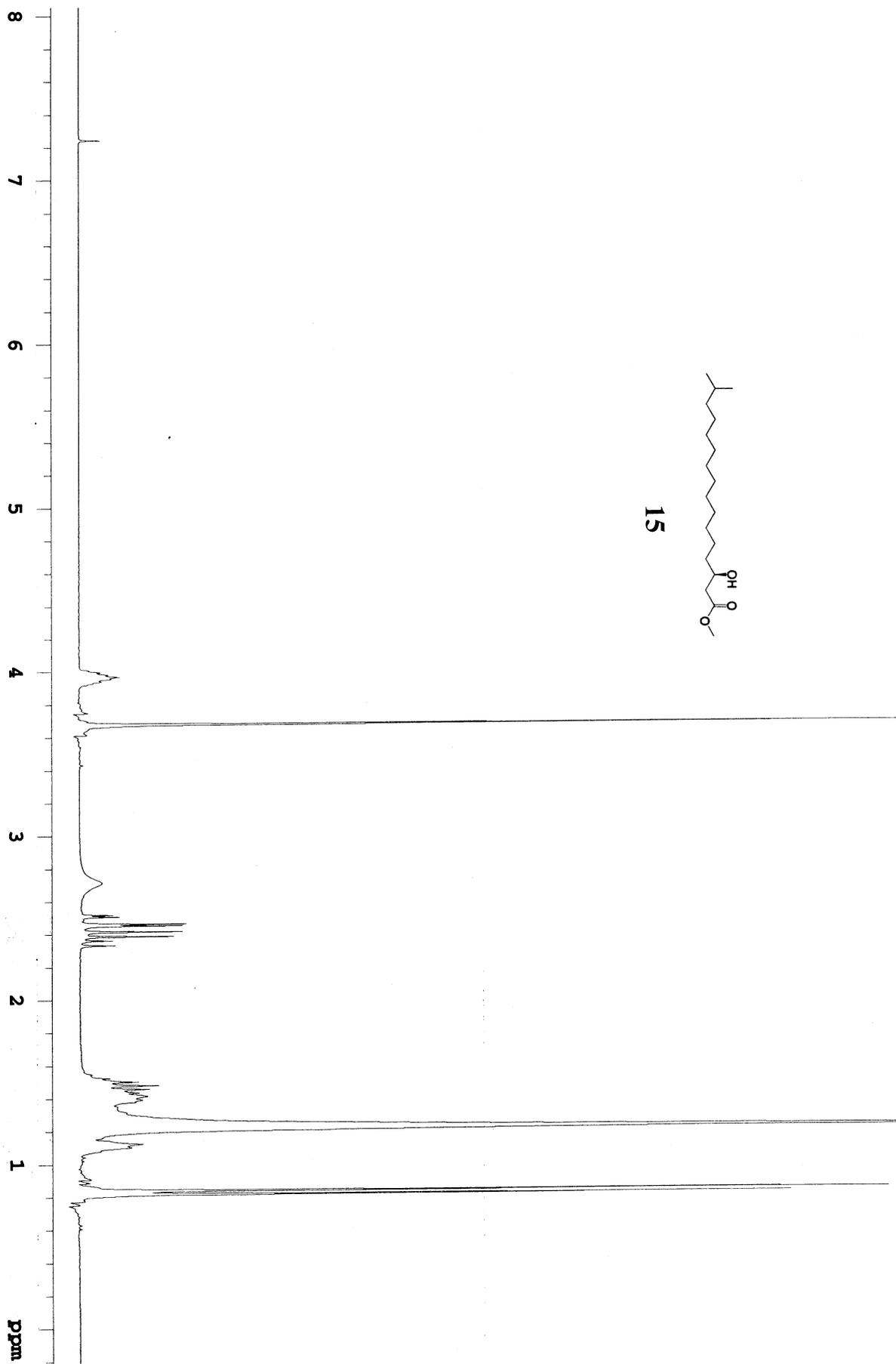


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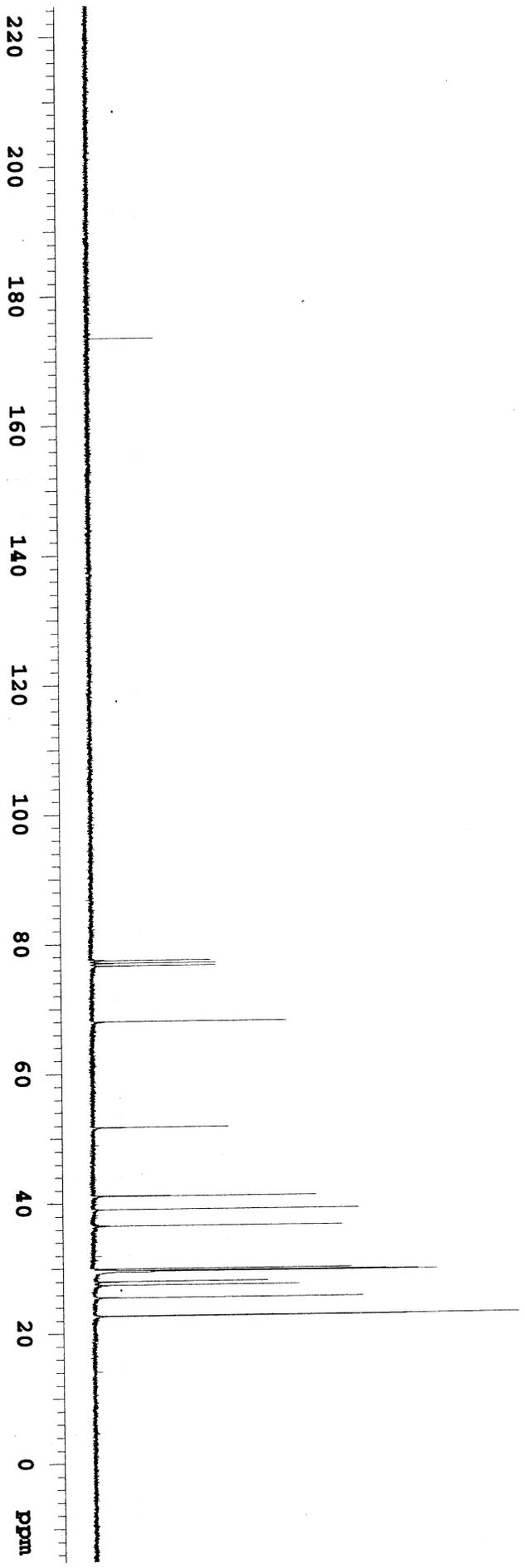


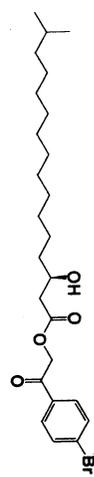
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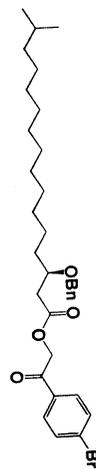
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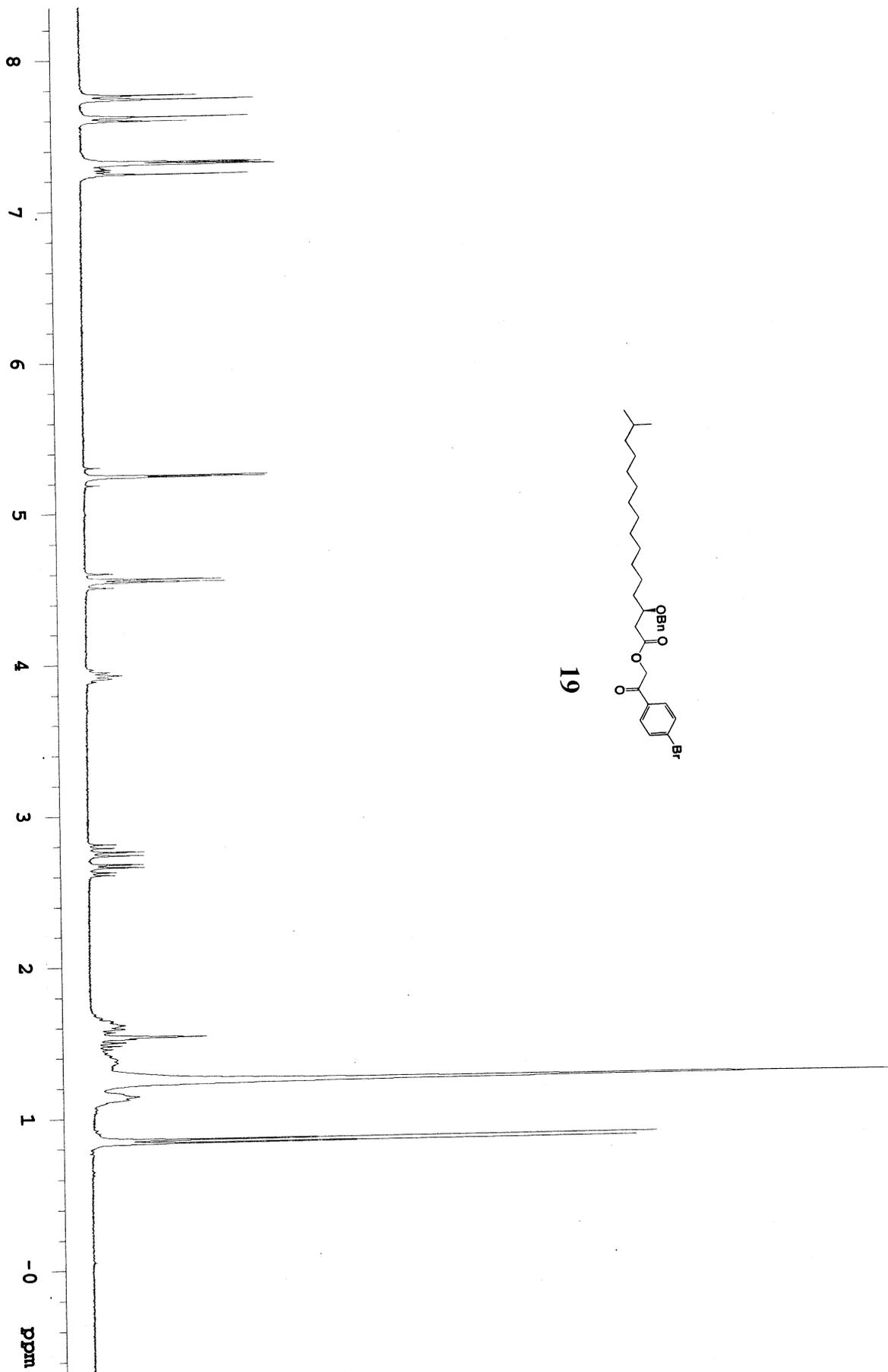


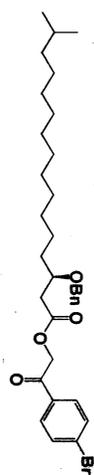
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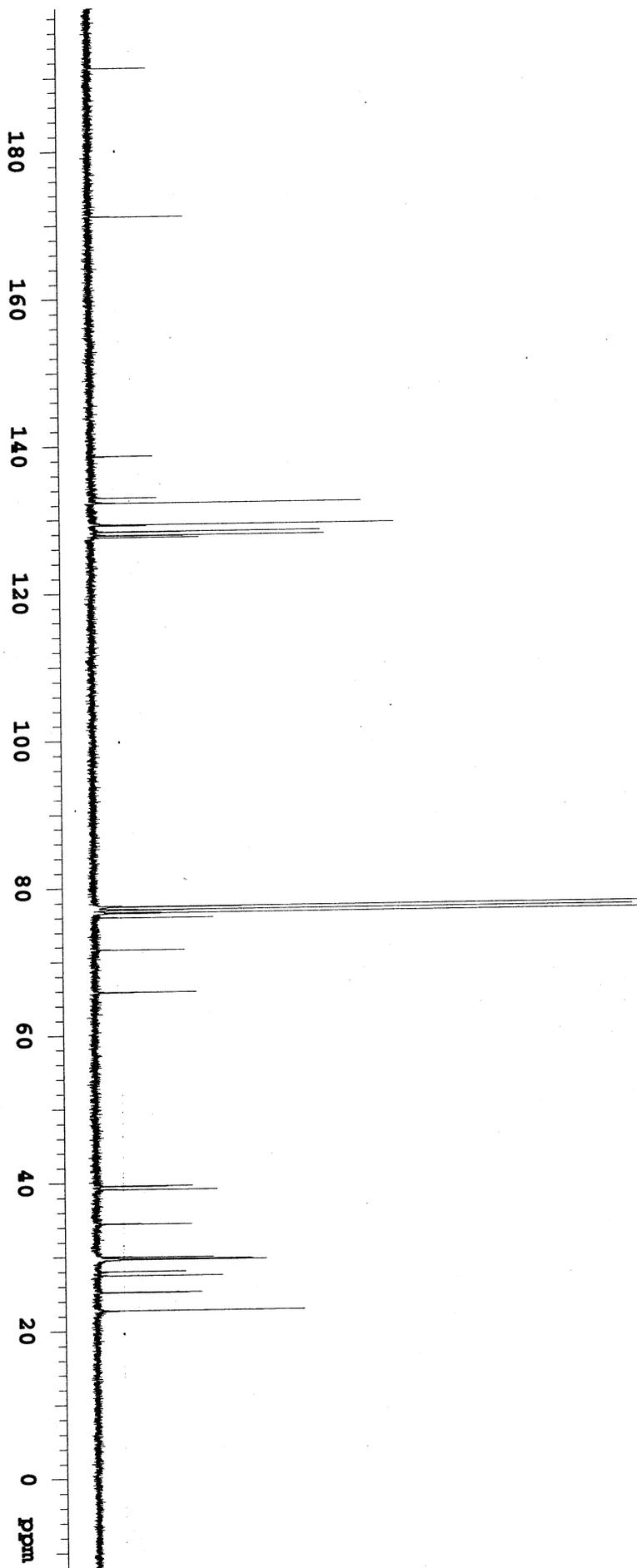


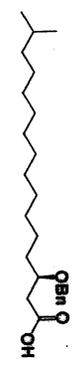
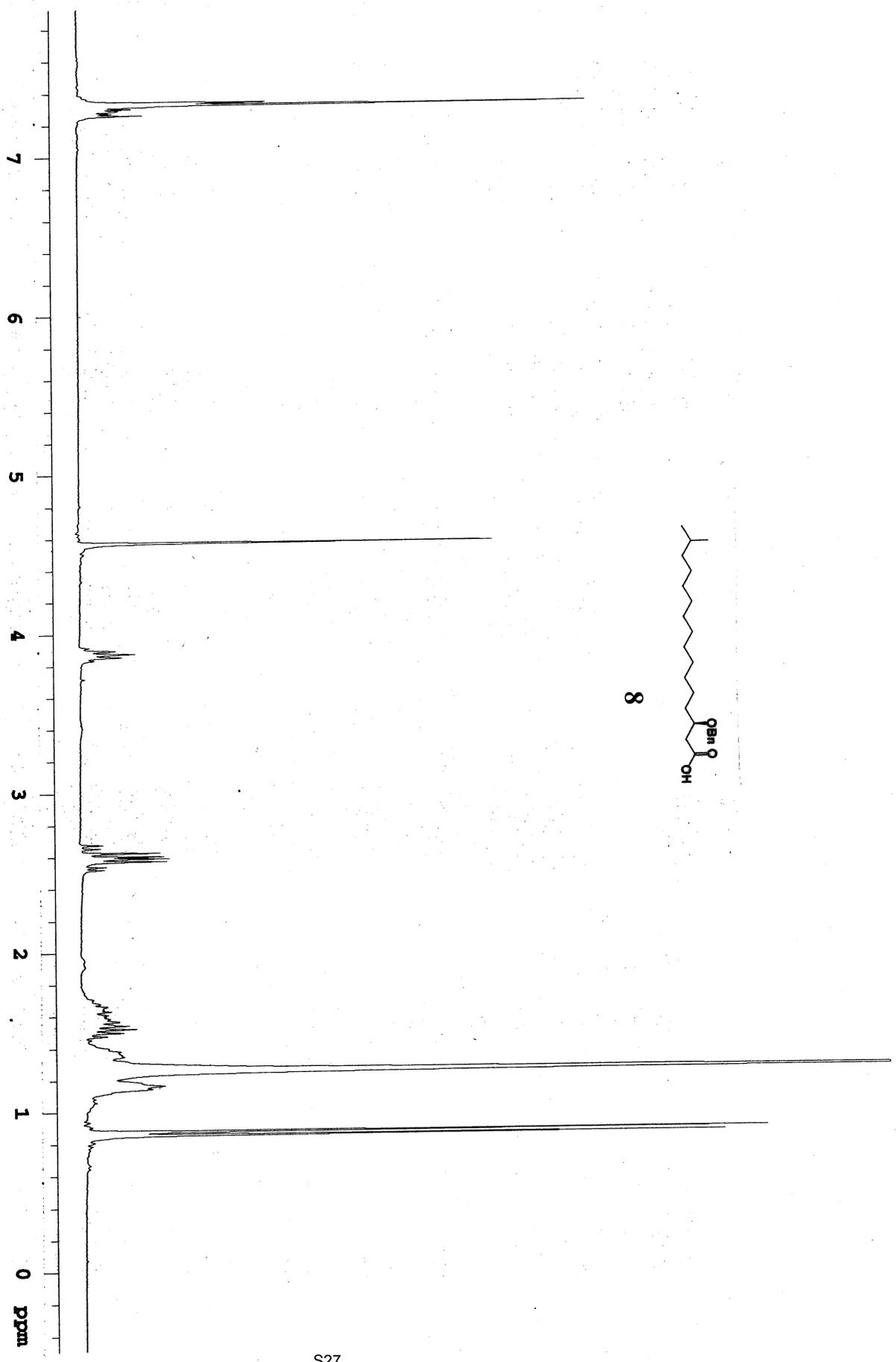
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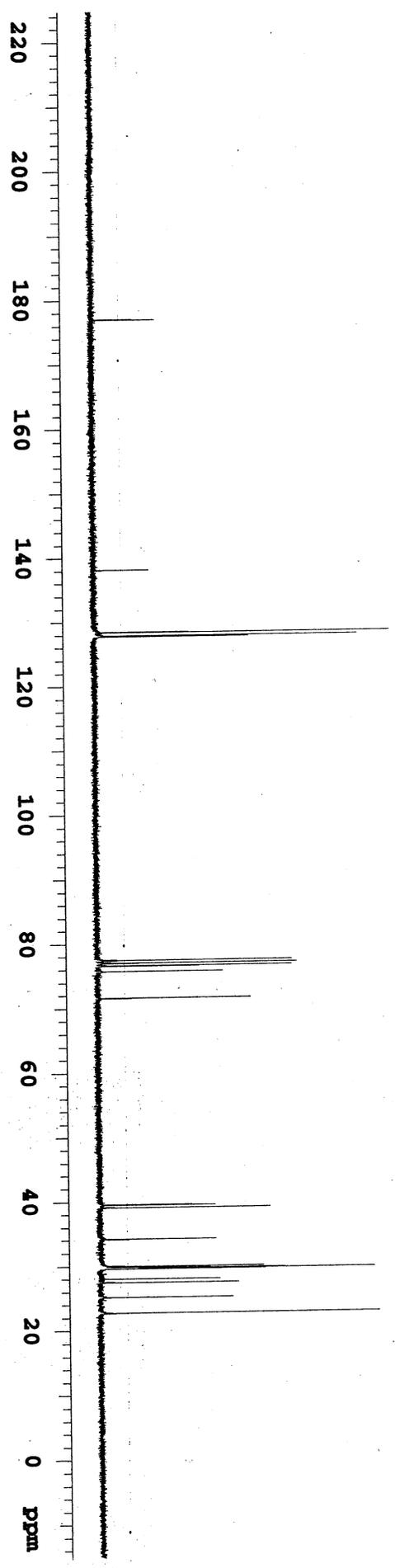




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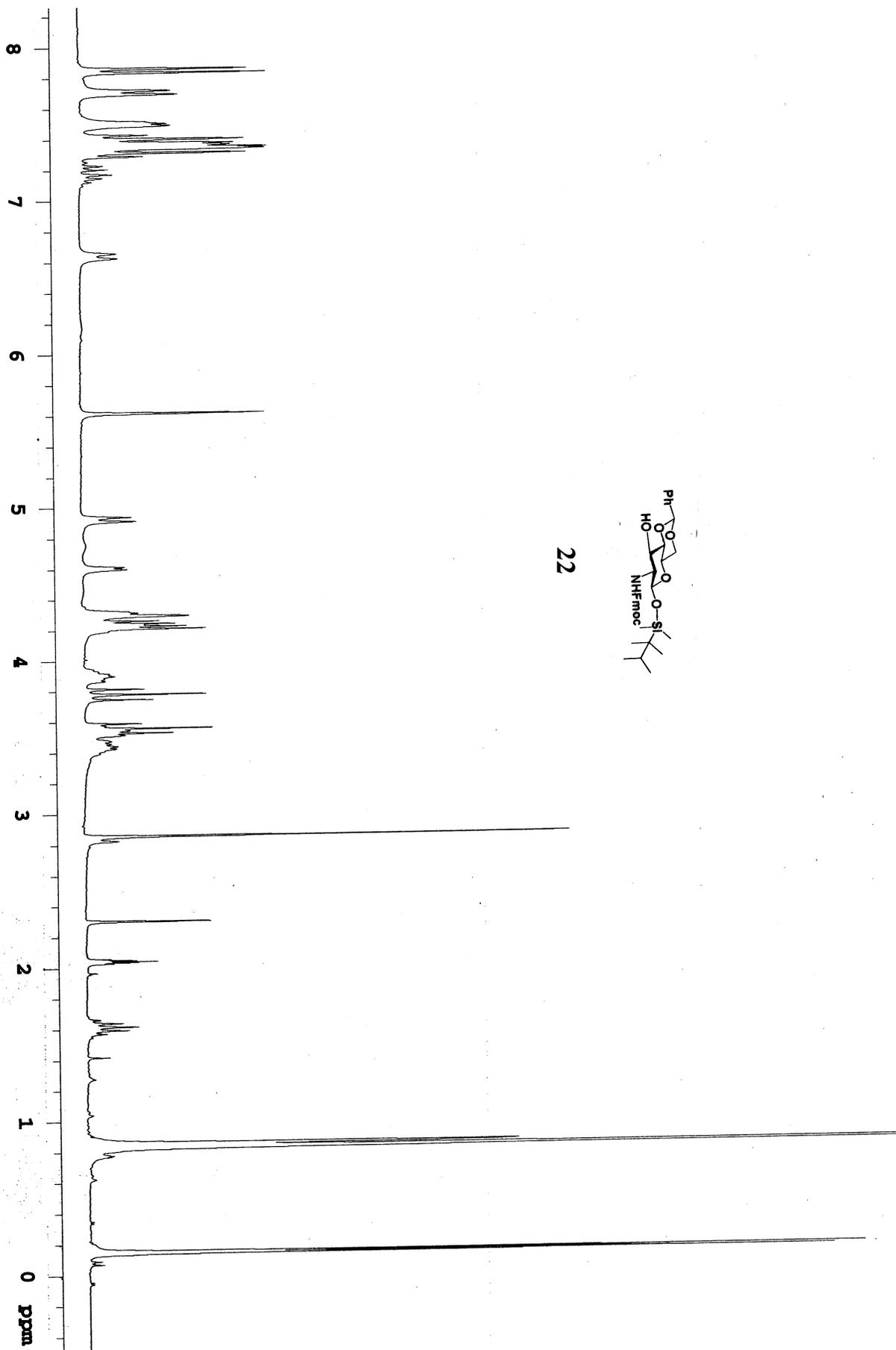


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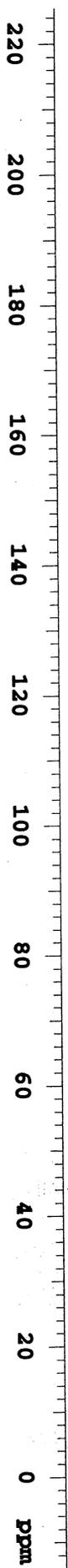


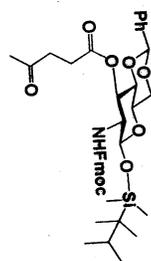
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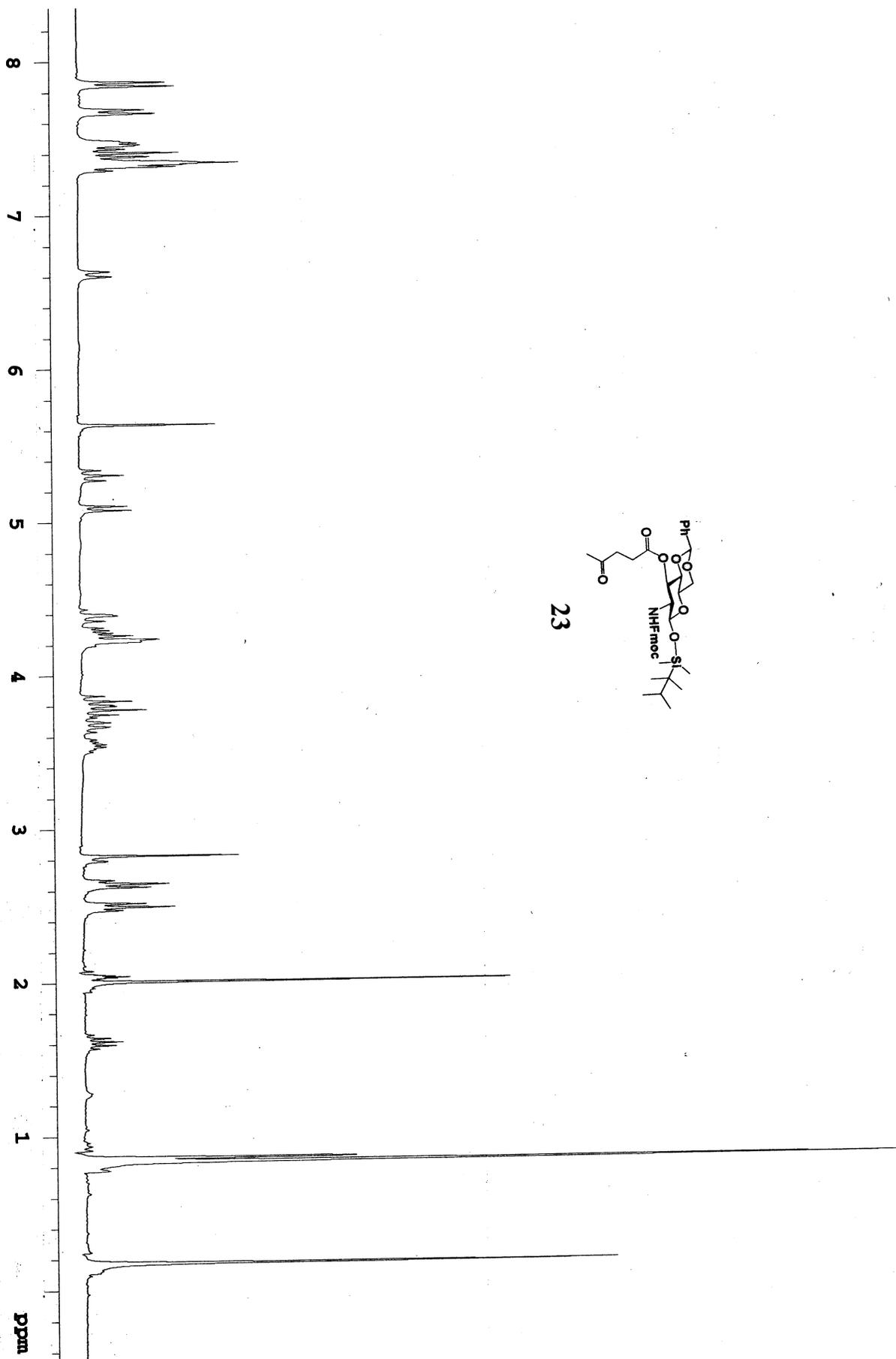


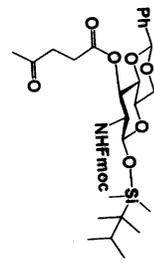
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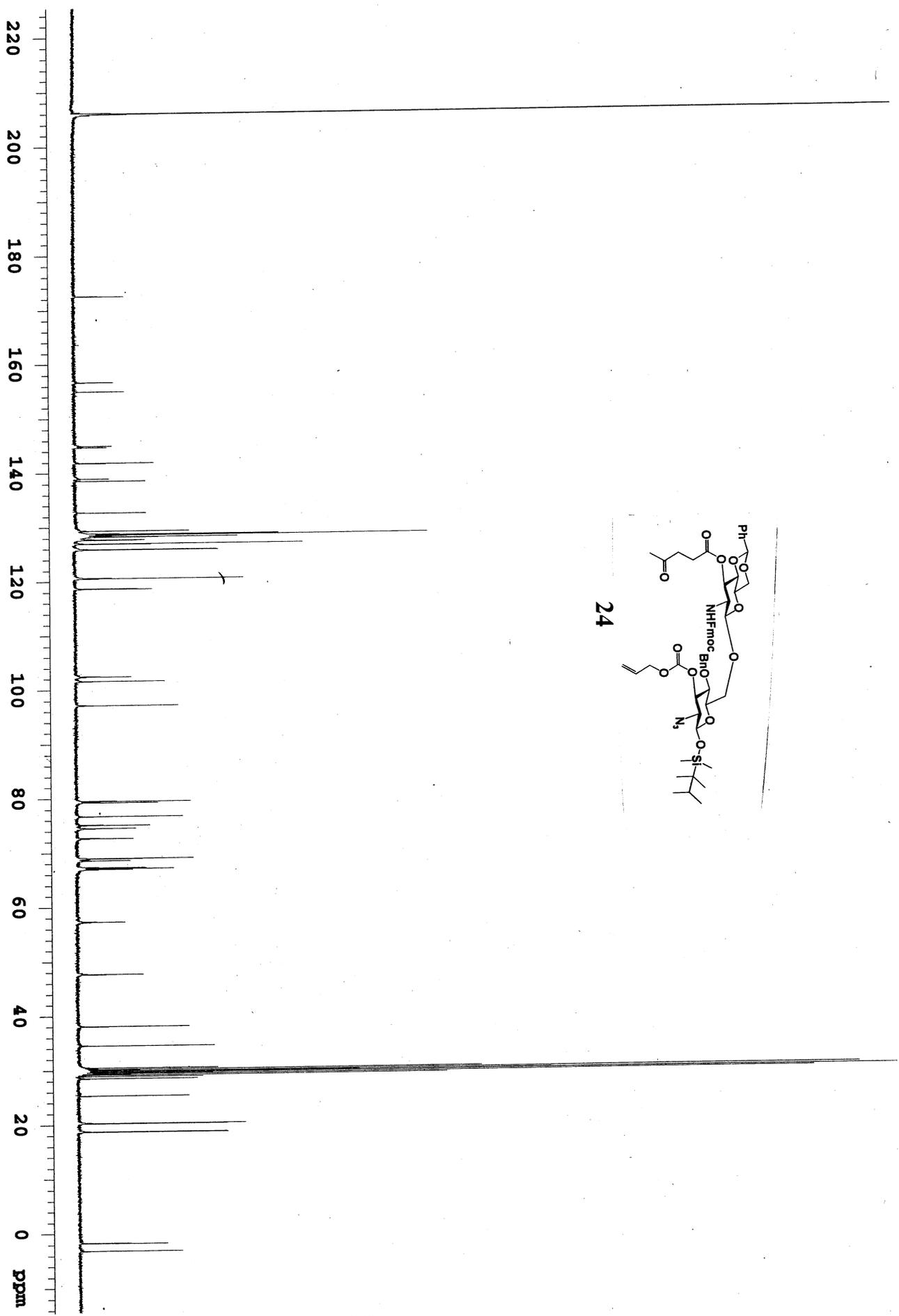
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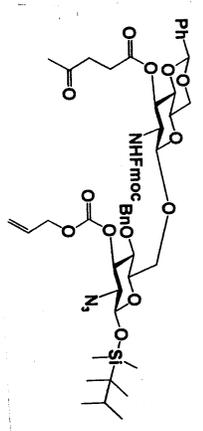


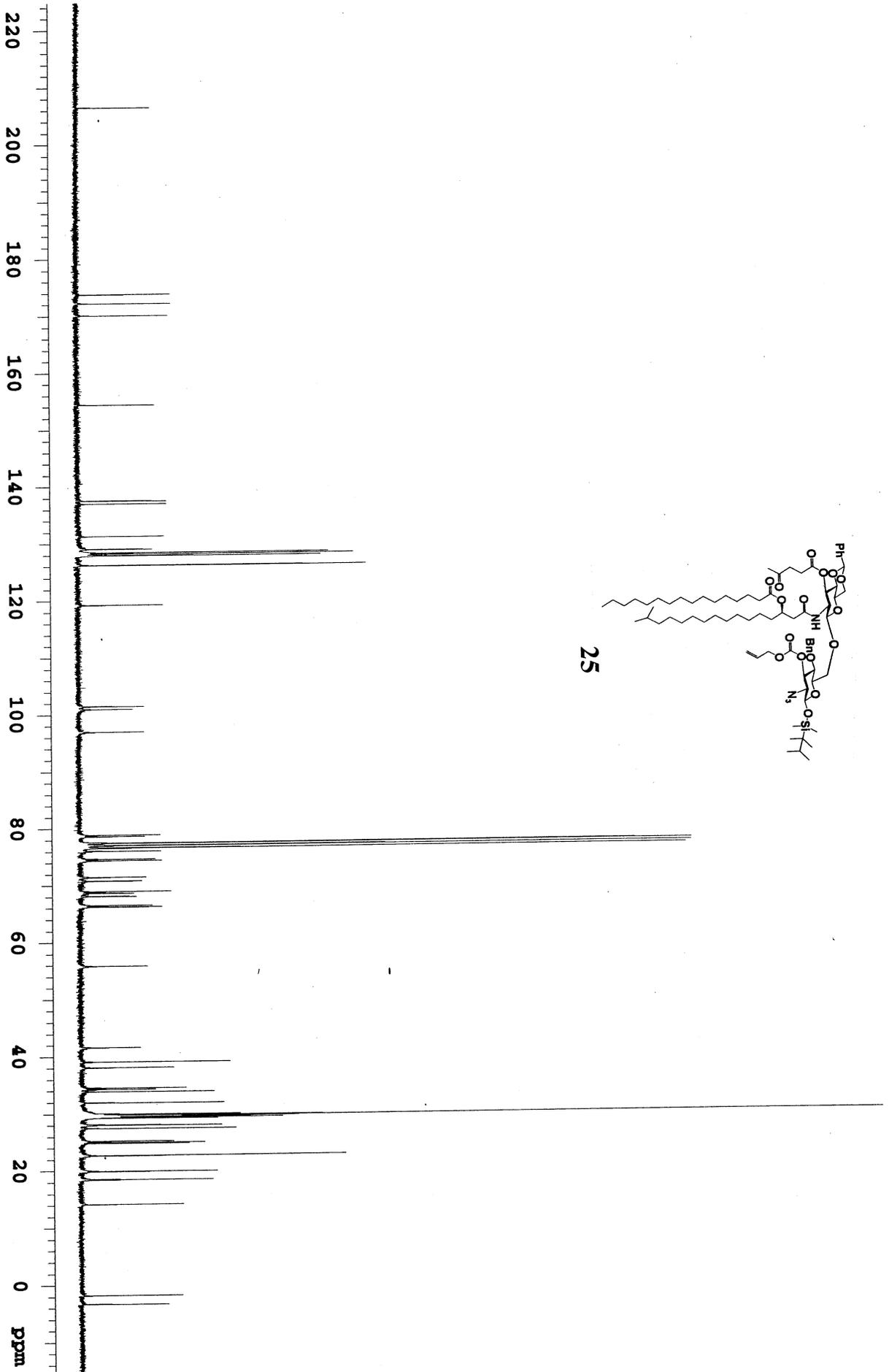
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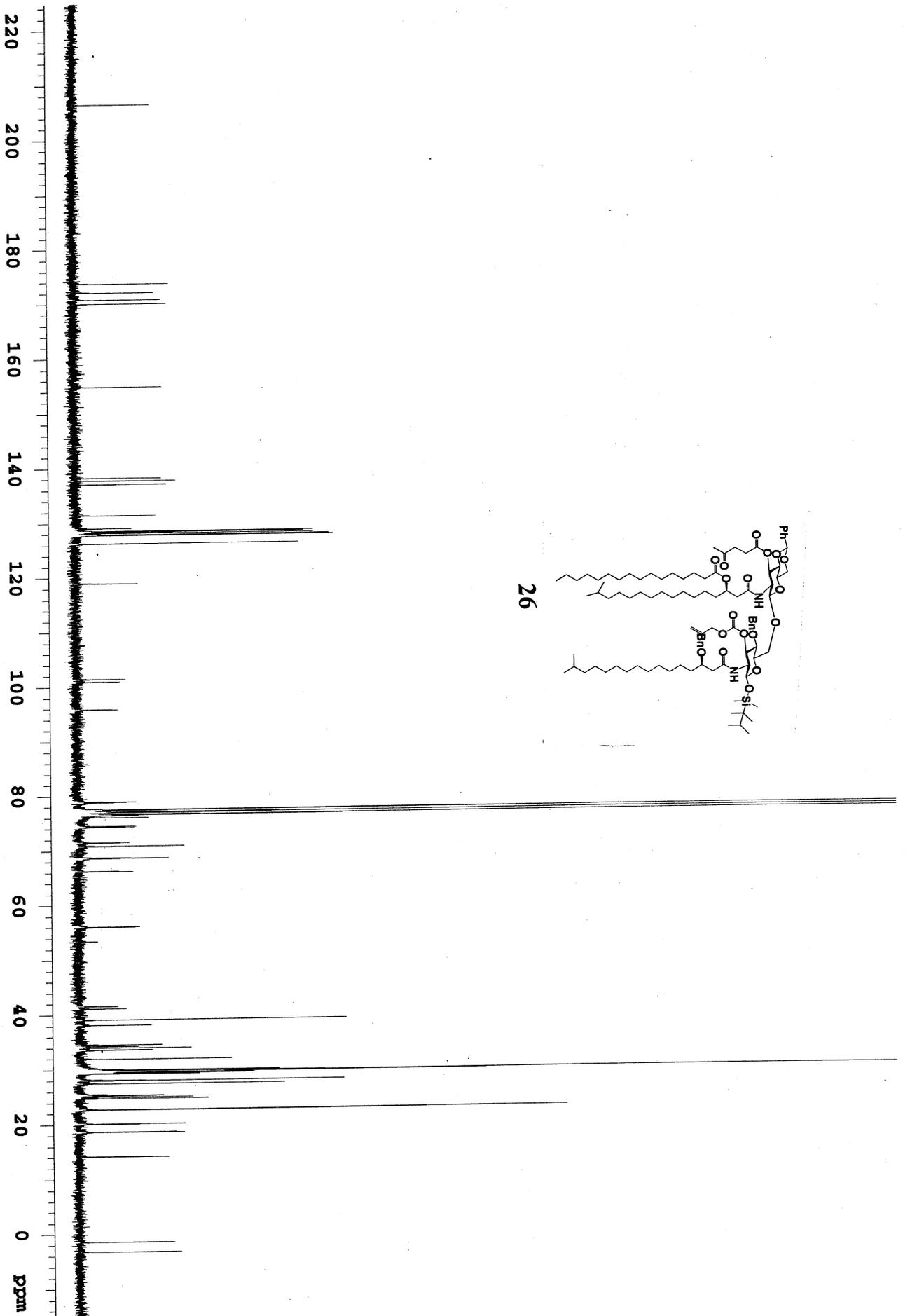


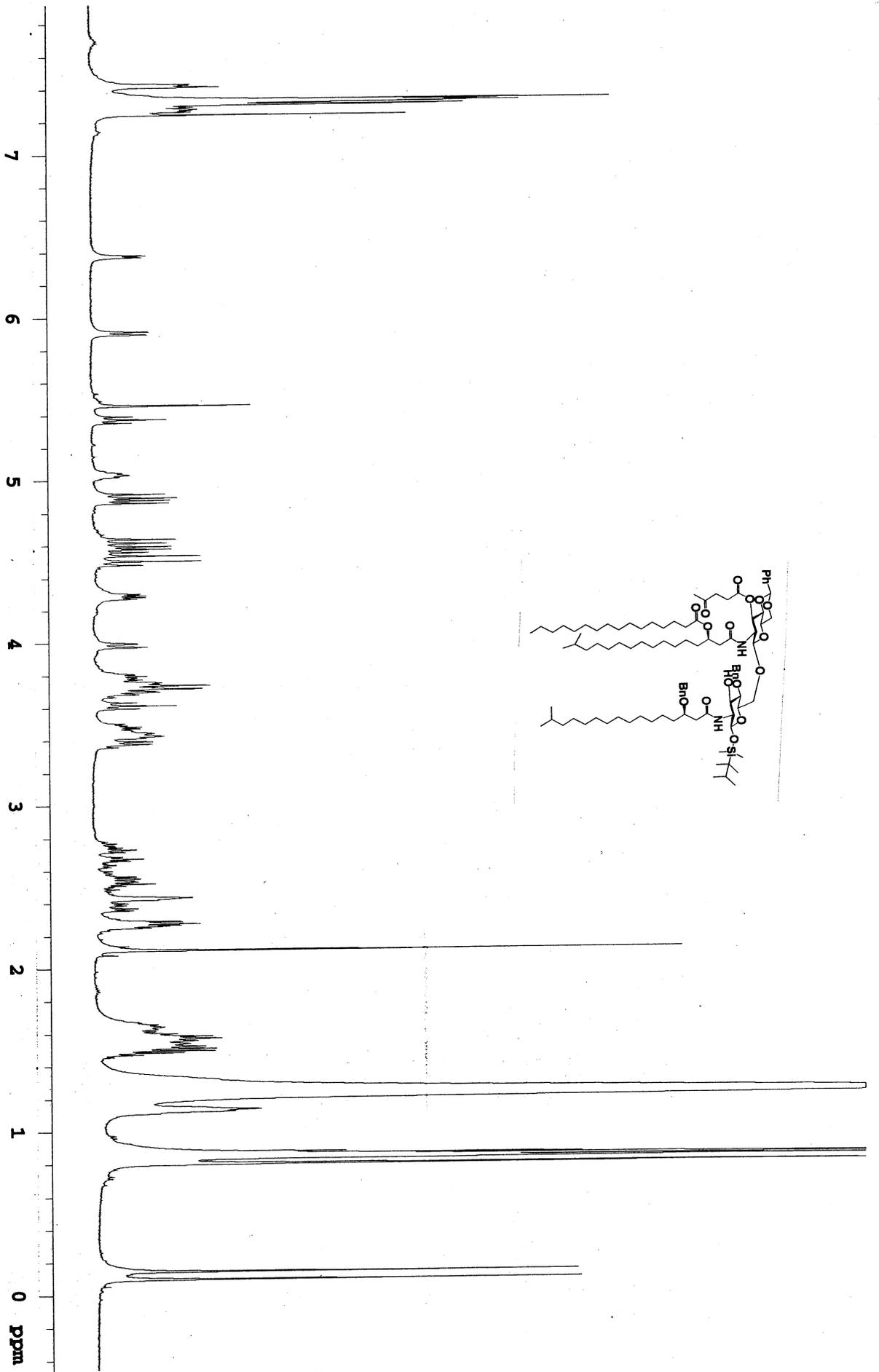


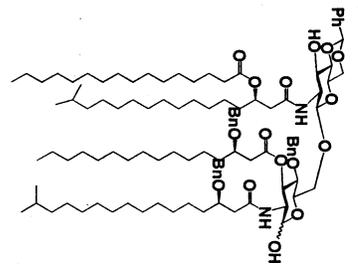
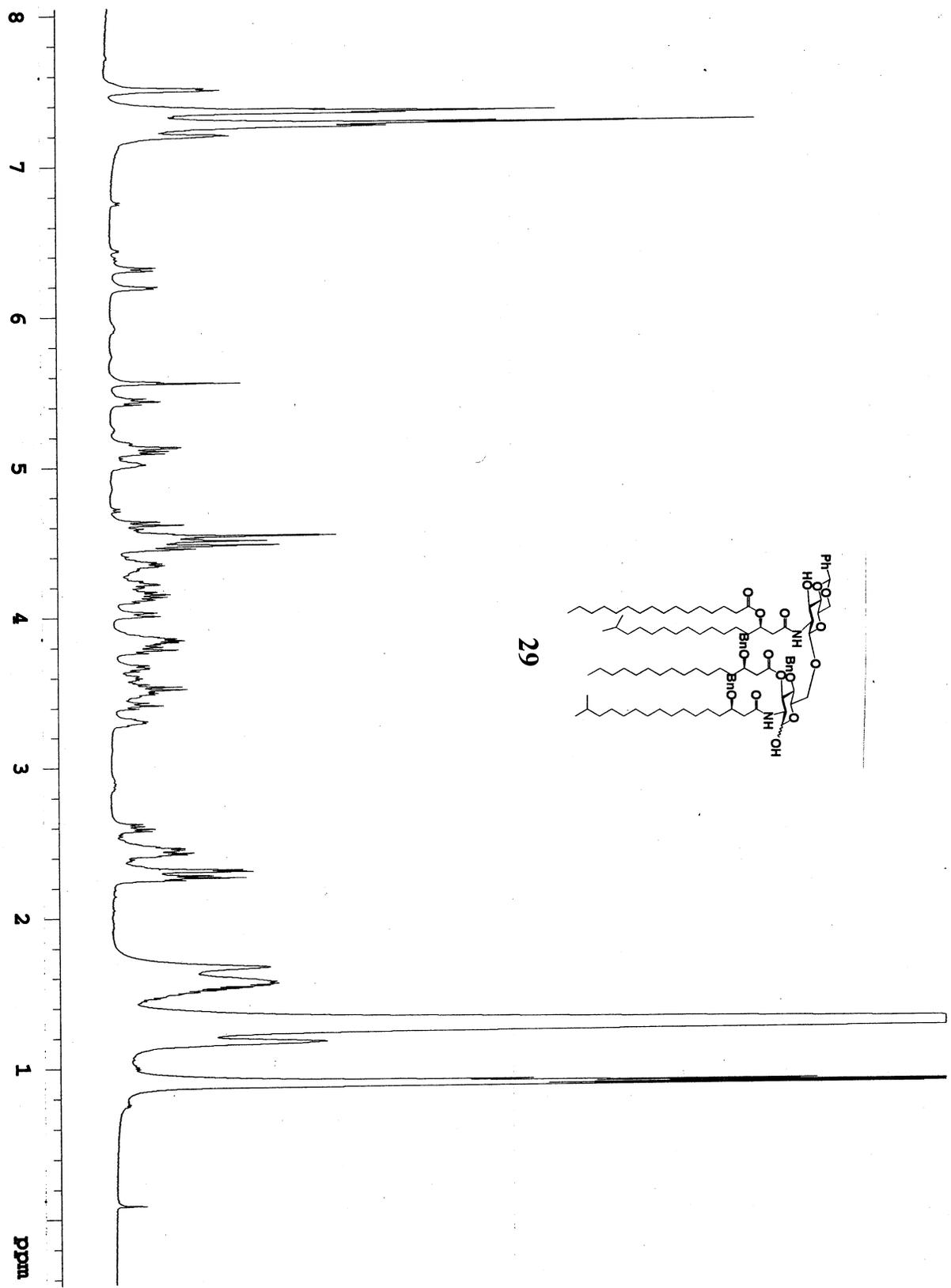
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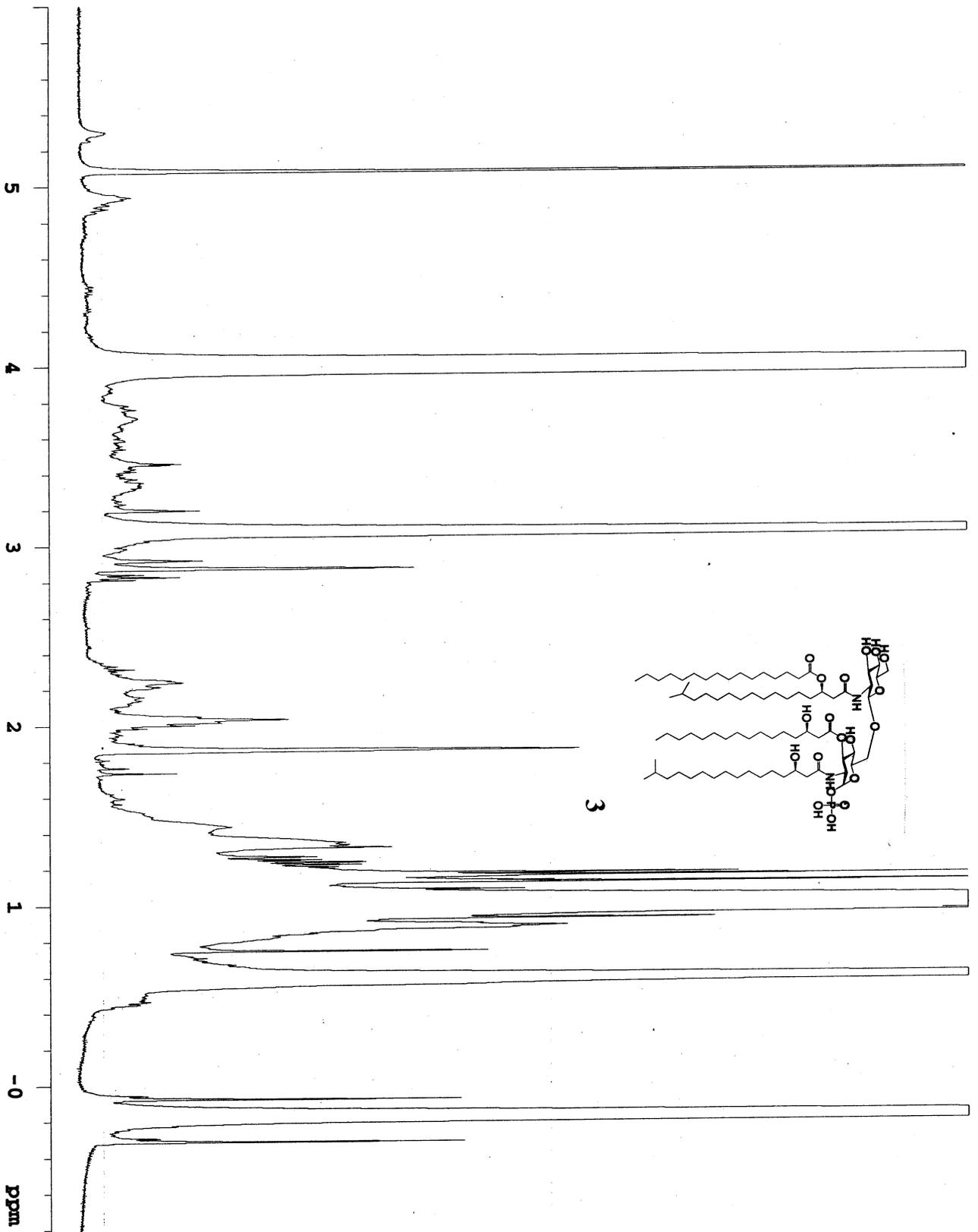


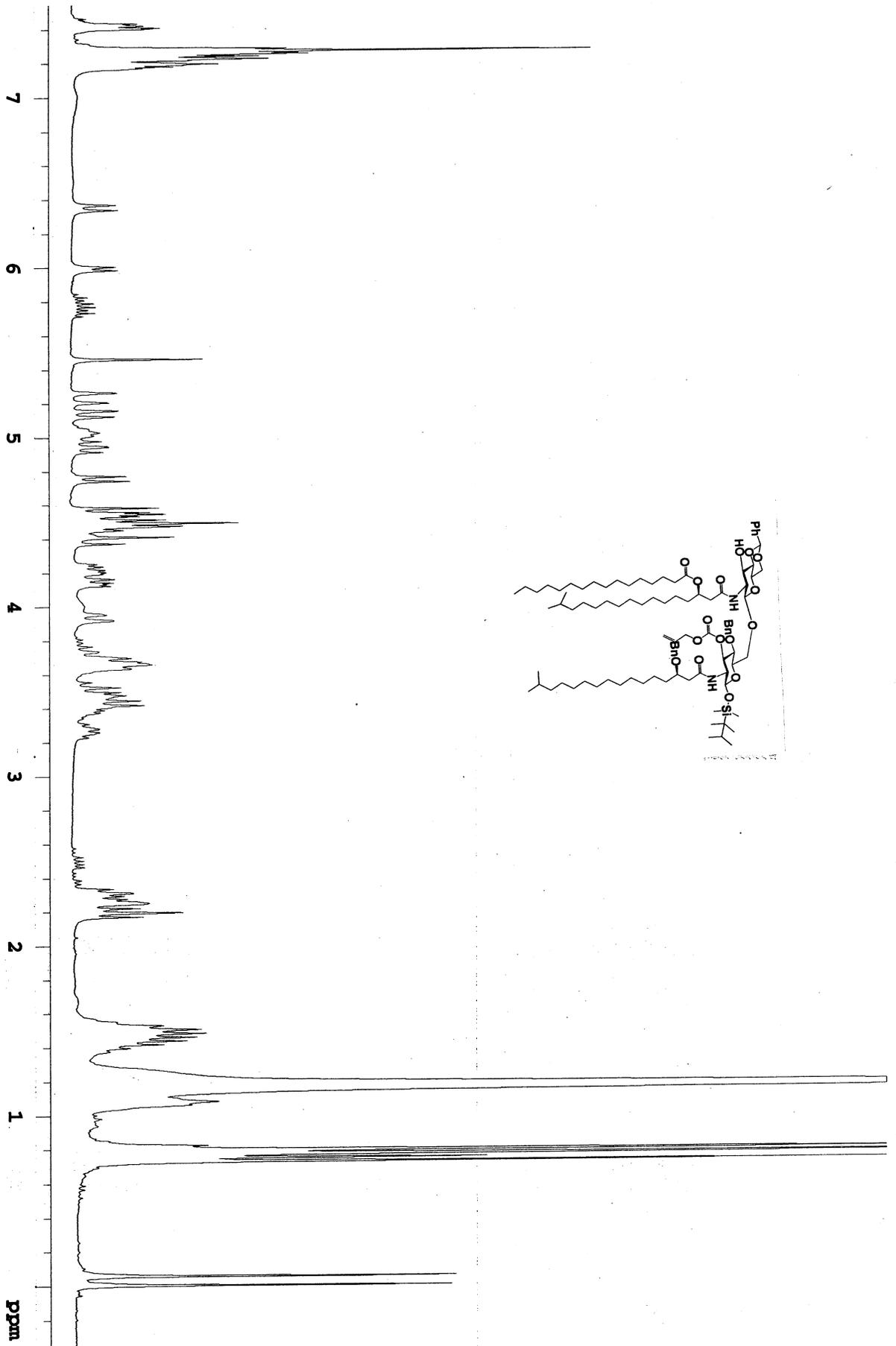


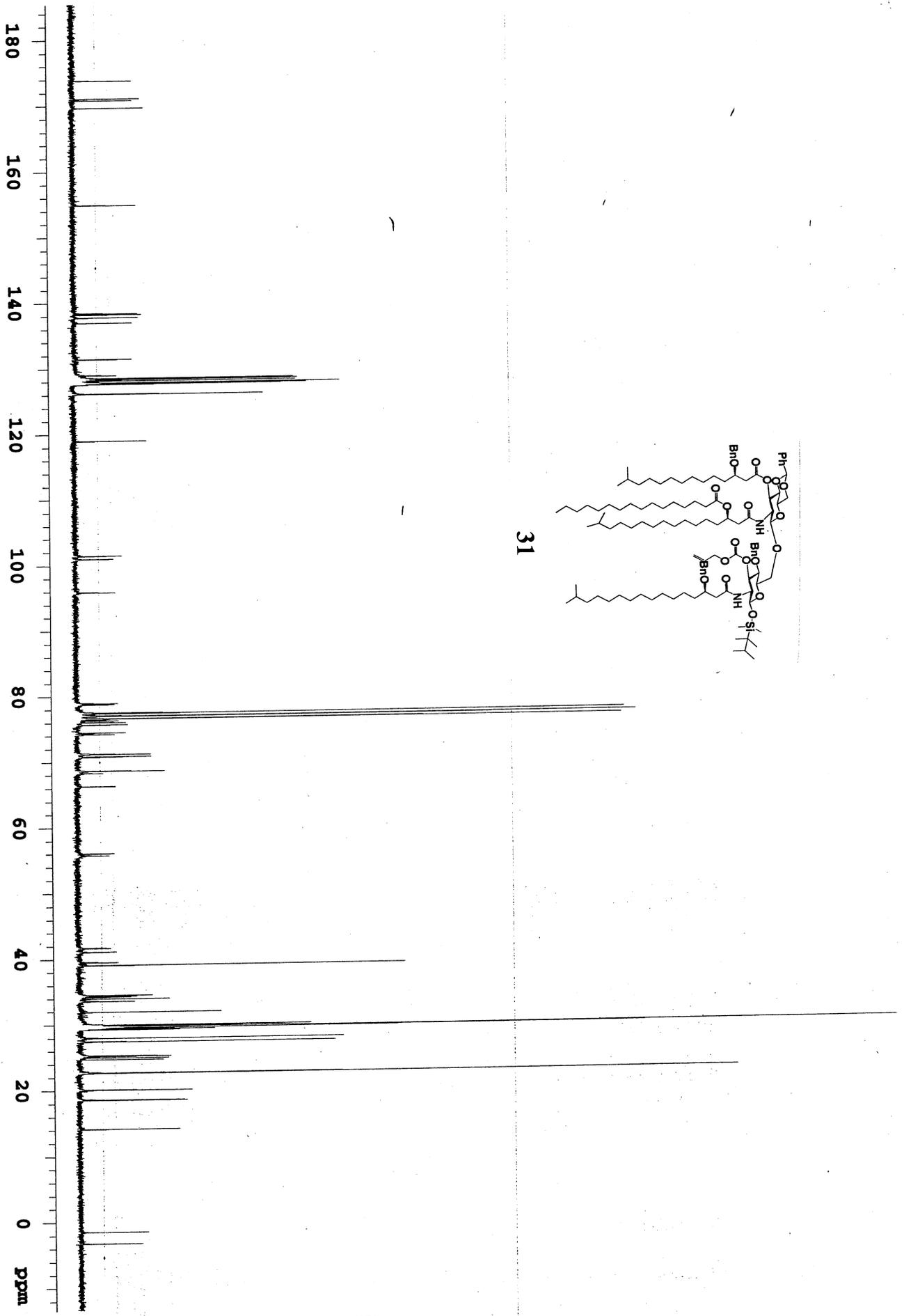




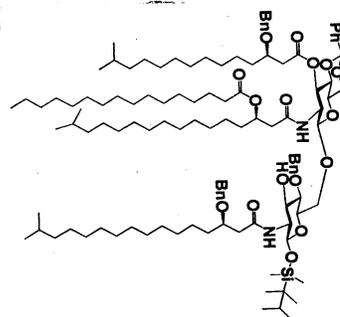




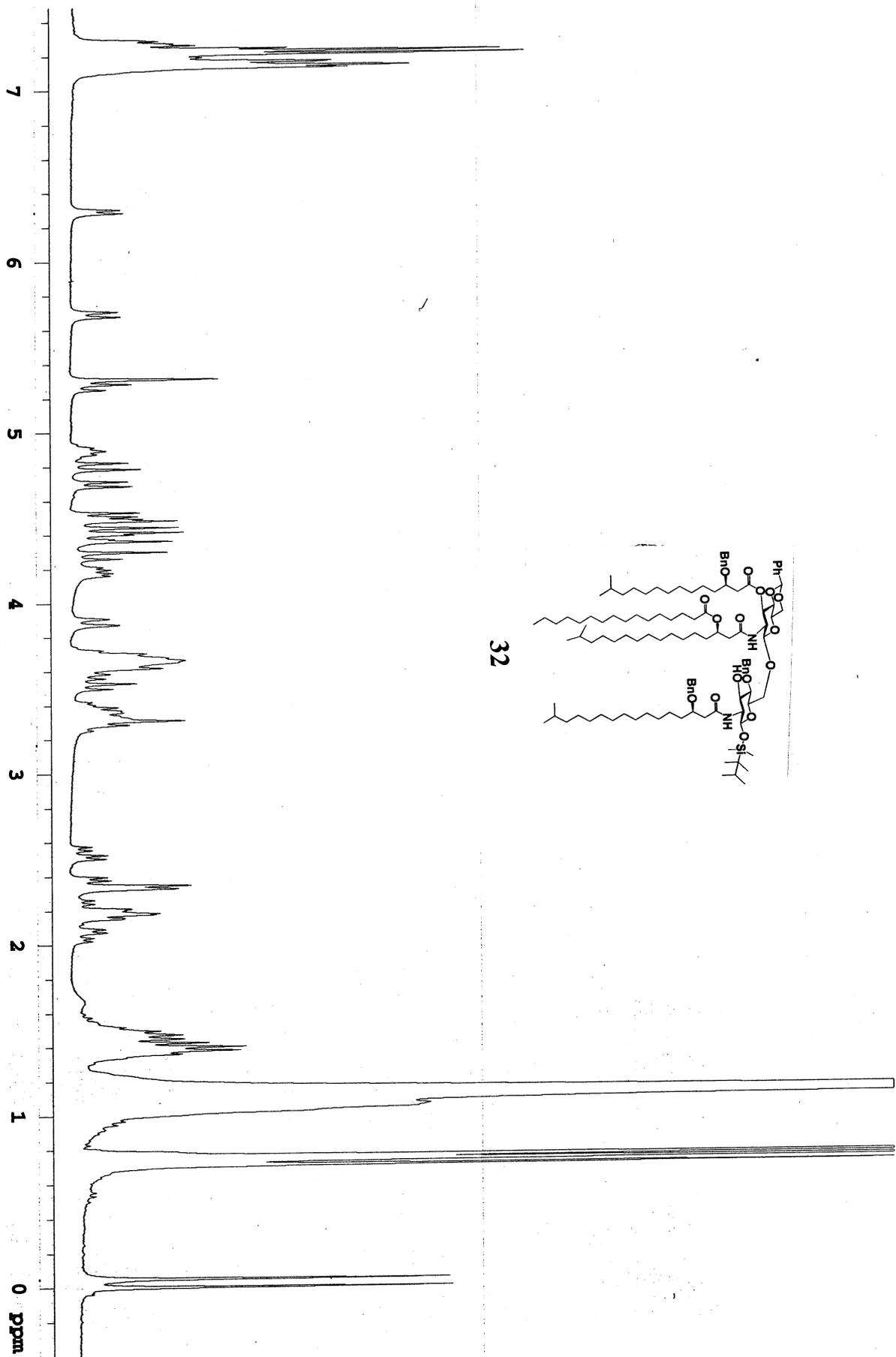


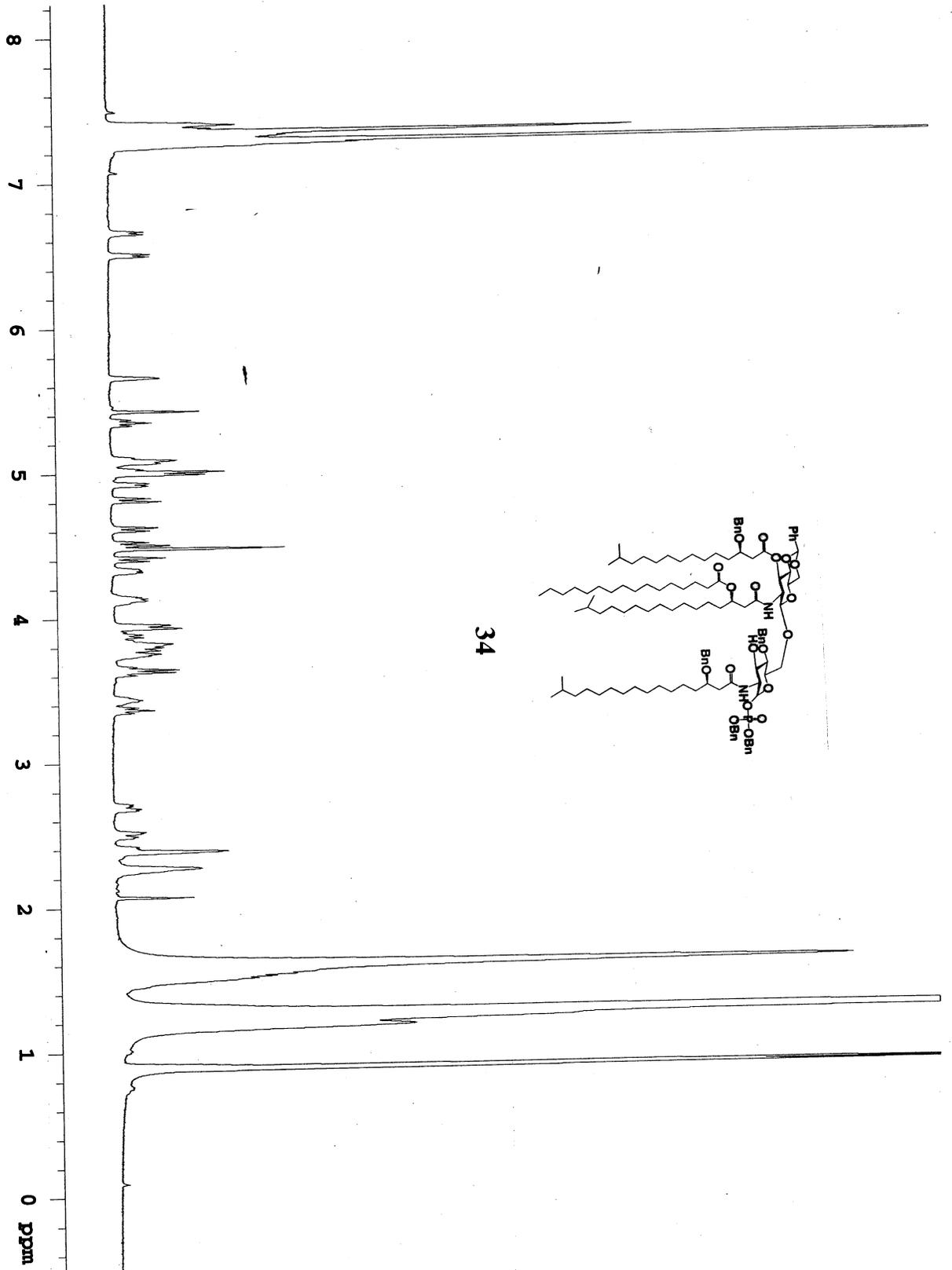


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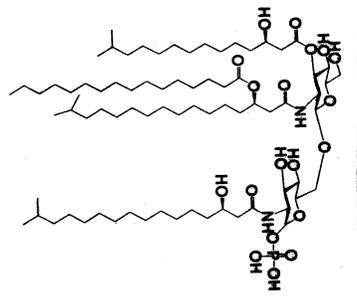
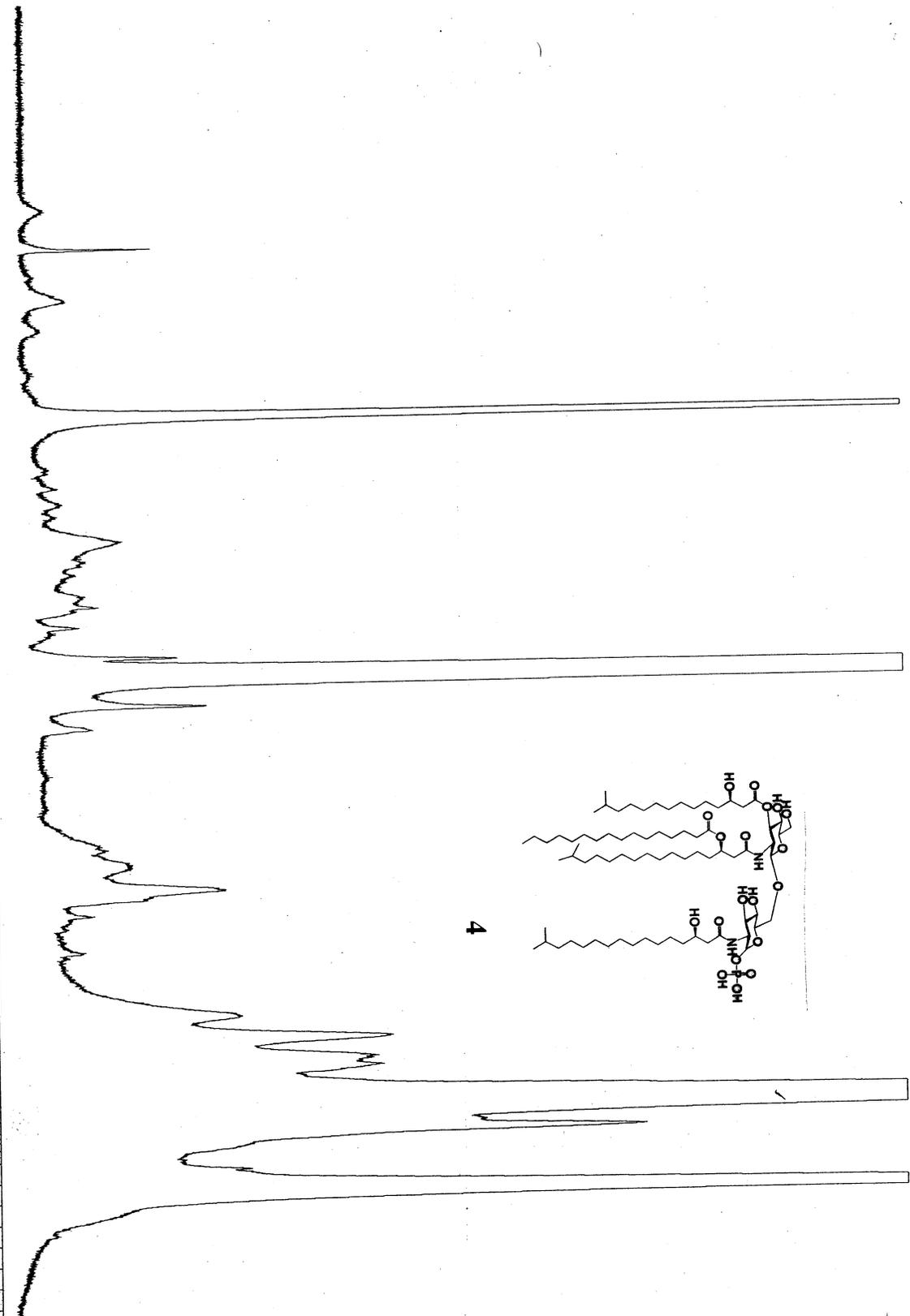


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