Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Supporting Information

Convenient synthesis and application of versatile nucleic acid lipid membrane anchors in the assembly and fusion of liposomes

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Characterization of oligonucleotides

ON#	Sequence (5'-)	R_t [min][a)]	mass (calc.)	mass (found)
01	T**X** TGT GGA AGA AGT TGG TG	10.1 (A)	6265.65	6267.34
02	CAC CAA CTT CTT CCA CA **X**T	15.2 (B)	6264.90	6267.56
03	T**X** CAC CAA CTT CTT CCA CA	17.8 (C)	5953.75	5951.13
04	TGA AGA AGG TGT **X** TGT GGA AGA AGT	8.8 (A)	8176.04	8178.00
05	ACT TCT TCC ACA **X** ACA CCT TCT TCA	8.8 (A)	7757.82	7759.31
06	T**X** TGT GGA AGA AGT TGG TG **X**T	26.1 (C)	7184.57	7189.23
07	T**X** CAC CAA CTT CTT CCA CA **X**T	29.6 (C)	6874.92	6877.45
08	CAC CAA CTT CTT CCA CA **Y**T	13.4 (B)	6269.97	6271.27
09	T**Y** TGT GGA AGA AGT TGG TG	13.5 (B)	6581.12	6583.11
10	CAC CAA CTT CTT CCA CA **Z**T	16.3 (B)	6062.68	6062.31
11	T**Z** TGT GGA AGA AGT TGG TG	16.2 (B)	6373.83	6373.98

a) HPLC/UPLC method (flow, time [min], solvent gradient 0.05M TEAA, pH7.0 / ACN/H_2O 3:1).
Method A (UPLC): 1mL/min; 0→1, 90:10; →10, →0:100; →13, 0:100.
Method B (HPLC): 1mL/min; 0→4, 90:10; →8, →50:50; →16, →0:100, →19, 0:100.
Method C (HPLC): 0.8mL/min; 0→2, 100:0; →10, →30:70; →30, →0:100; →60, 0:100

Zeta Potential and DLS Experiments

For zeta potential measurements POPC liposomes (1mM, 114nm) were incubated for at least 15min with an appropriate amount of **ON-02** in a total volume of 500µL HEPES buffer ([HEPES] 10mM, [Na^+] 110mM, [Cl^-] 108mM. Zeta potential was measured at 20°C with a Beckman Coulter DelsaMax Pro equipped with a flowcell.

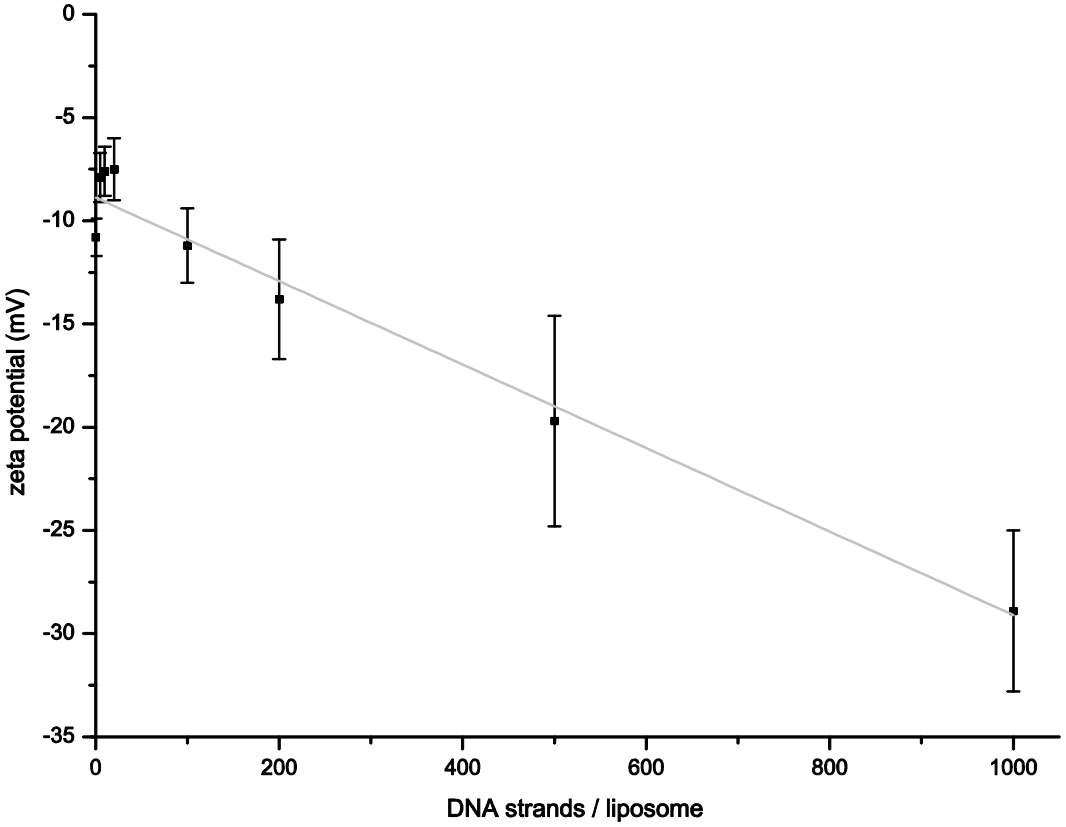


Figure S1: Zeta potential of POPC liposomes (100nm) incubated with different oligonucleotide concentrations

For the experiment two liposome populations, A and B, either consisting of 0.11 mM DOPC/DOPE/cholesterol (2:1:1, molar ratio) and 0.168 µM **ON-10** or 0.44 mM DOPC/DOPE/cholesterol (2:1:1, molar ratio) and 0.336 µM **ON-11** were mixed and measured in DLS for 1 h. Liposomes and lipid-DNA conjugates were incubated prior to mixing for 15 min at room temperature. The hydrodynamic diameters for populations A and B were 121.6 ± 5.6 nm and 118.5 ± 5.8 nm, respectively.

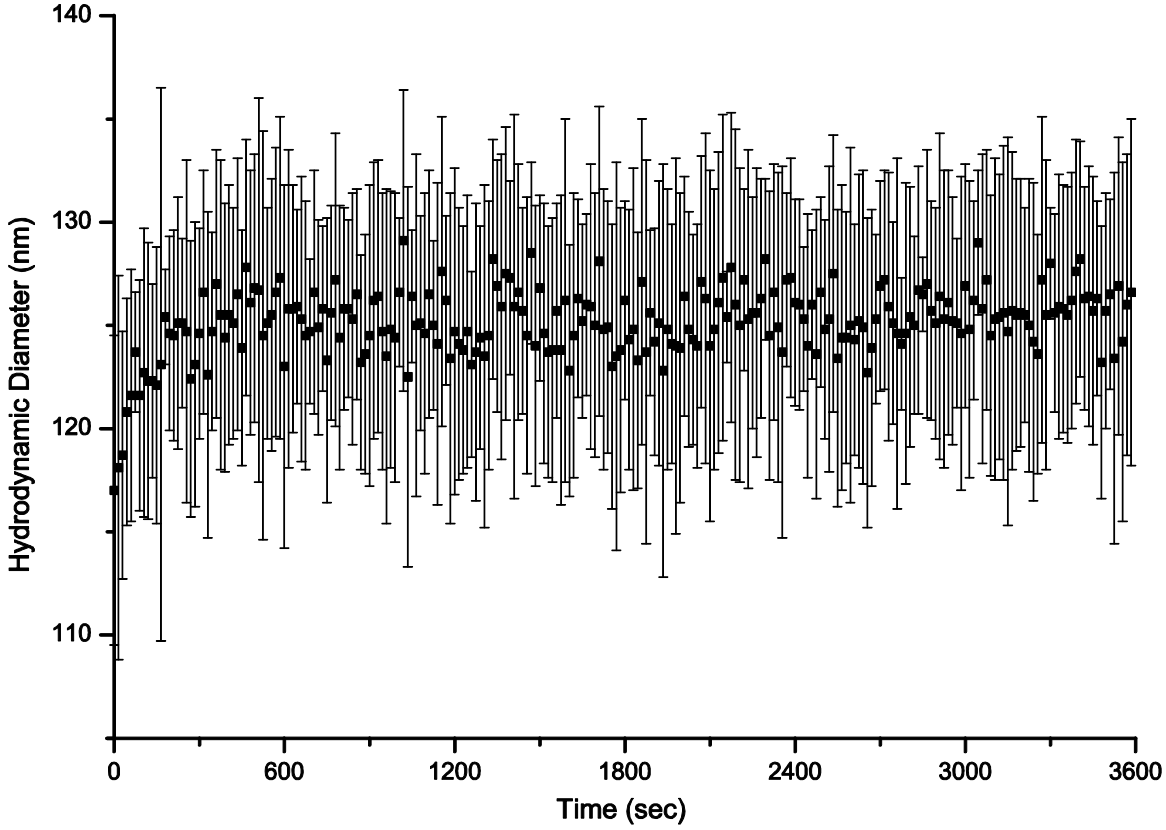
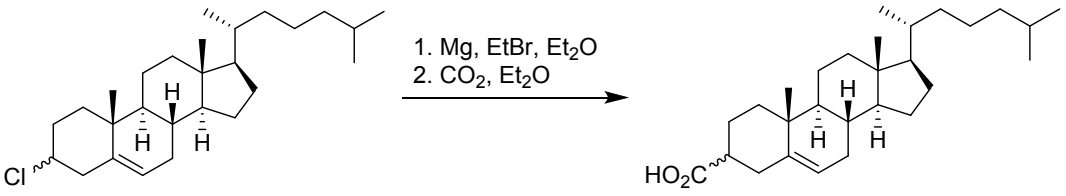


Figure S2: Hydrodynamic diameter over time for a fusion experiment

Syntheses of compounds S1-3 and 2-6a-c

Cholesterylic acid (S1)[1-3]

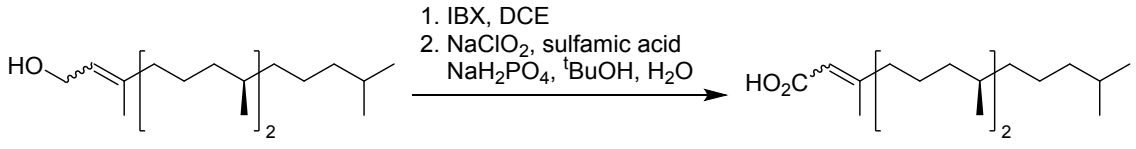


S1

Ethylbromide (4.1 mL, 54.9 mmol, 1.1 eq) was added dropwise to magnesium turnings (2.40 g, 98.7 mmol, 2.0 eq) in dry diethylether (30 mL) under nitrogen atmosphere at room temperature. When gas formation started a solution of cholesteryl chloride (20.0 g, 49.4 mmol, 1.0 eq) in dry diethylether (60 mL) was added slowly over 1 h and the reaction mixture was kept at refluxing. Stirring was continued for 5 h under reflux. The reaction mixture was cooled in an ice-bath and a stream of CO_2 was led through the solution for 7 h at 0 °C. Sulfuric acid (30%, 60 mL) was added slowly and the aqueous layer was extracted with diethylether (3x 200 mL). The combined organic layers were washed with brine (2x 200 mL) and water (2x 100 mL) and dried over sodium sulfate. The solvent was removed under reduced pressure and the crude product was recrystallized from toluene to yield 6.84 g (16.5 mmol, 33%) as a pale yellow solid.

R_f 0.68 (CH_2Cl_2/methanol 9:1); ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.68 (s, 3H, 18-H_3), 0.86 (d, 3H, J = 6.6, 26-H_3), 0.87 (d, 3H, J = 6.6, 27-H_3), 0.91 (d, 3H, J = 6.5, 21-H_3), 0.94-2.10 (m, 29H, chol-CH_2, chol-CH), 1.01 (s, 3H, 19-H_3), 2.21-2.50 (m, 3H, chol-CH_2, chol-CH), 5.33-5.40 (m, 1H, 6-H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 12.02 (C18), 18.88 (C21), 19.48 (C19), 21.03 (chol-CH_2), 22.72 (C26), 22.98 (C27), 24.00, 24.43, 25.26 (chol-CH_2), 28.17 (chol-CH), 28.39 (chol-CH_2), 31.95 (chol-CH), 32.04 (chol-CH_2), 34.84 (chol-CH_2), 35.95 (chol-CH), 36.36 (chol-CH_2), 37.06 (chol-C), 38.80, 39.68, 39.93 (chol-CH_2), 42.46 (chol-C), 44.50, 50.40, 56.33, 56.93 (chol-CH), 121.48 (C5), 140.84 (C-6), 181.54 (3-CO_2H); HRMS (ESI): calcd. for $C_{28}H_{46}NaO_2$ *m/z* 437.3396 $[M+Na]^+$, found 437.3371.

(7*R*,11*R*)-Phytenic acid (S2)[4,5]

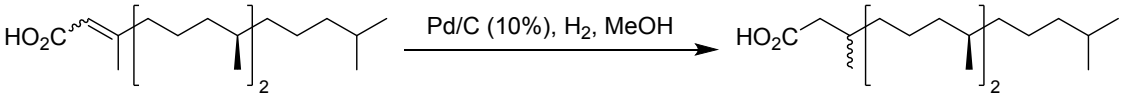


S2

A suspension of phytol (2.00 mL, 5.73 mmol, 1.0 eq) and IBX (4.01 g, 14.3 mmol, 2.5 eq) in 1,2-dichloroethane (20 mL) was stirred under reflux for 2.5 h and afterwards cooled in an ice-bath. The suspension was filtrated and the solvent was removed under reduced pressure. The residue was dissolved in a mixture of *tert*-butanol (10 mL) and water (10 mL) and sodium dihydrogen phosphate dihydrate (1.61 g, 11.5 mmol, 2.0 eq), sulfamic acid (833 mg, 8.58 mmol, 1.5 eq) and sodium chlorite (674 mg, 7.45 mmol, 1.3 eq) were added. The reaction mixture was stirred for 2 h at room temperature and diethylether (50 mL) was added. The organic layer was extracted with water (1x 50 mL) and brine (1x 50 mL) and dried over magnesium sulfate. The solvent was removed under reduced pressure to yield 1.75 g (5.64 mmol, 98 %) of a yellowish oil as mixture of two diastereomers and used without without further purification.

R_f 0.32, 0.24 (petroleum ether/ethyl acetate 4:1); ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.81-0.91 (m, 24H, 2x 7-CH_3, 2x 11-CH_3, 4x 16-H_3), 1.00-1.60 (m, 38H, 2x 5-H_2, 2x 6-H_2, 2x 7-H, 2x 8-H_2-10-H_2, 2x 11-H, 2x 12-H_2-14-H_2, 2x 15-H), 1.92 (s, 3H, 1x 3-CH_3), 2.14 (t, 2H, J = 7.5, 1x 4-H_2), 2.17 (s, 3H, 1x 3-CH_3), 2.61 (t, 2H, J = 7.9, 1 x 4-H_2), 5.67 (s, 1H, 1x 2-H), 5.69 (s, 1H, 1x 2-H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 19.06 (3-CH_3), 19.54, 19.58, 19.60, 19.64, 19.68, 19.75 (3-CH_3, 7-CH_3, 11-CH_3), 22.63, 22.72 (phyt-CH_3), 24.45, 24.82, 24.88 (phyt-CH_2), 25.50 (3-CH_3), 27.99 (phyt-CH), 32.57, 32.59, 32.62, 32.64, 32.77, 32.79 (phyt-CH), 33.74 (1x C4), 37.25, 37.30, 37.36, 37.39, 37.41, 37.44, 39.38 (phyt-CH_2), 41.55 (C4), 115.06, 115.54 (C2), 163.56, 164.06 (C3), 171.86, 172.37 (C1); HRMS (ESI): calcd. for $C_{20}H_{39}O_2$ *m/z* 311.2945 $[M+H]^+$, found 311.2934.

(7*R*,11*R*)-Phytanic acid (S3)



S2 **S3**

A solution of **S2** (8.77 g, 28.2 mmol, 1.0 eq) and Pd/C (10%, 3.00 g, 2.82 mmol, 0.1 eq) in methanol (200 mL) was evaporated 2x until boiling of the solvent and flushed with N_2. Afterwards the suspension was 3x evaporated and flushed with H_2. The reaction mixture was stirred under an atmosphere of H_2 for 3 h (TLC control). The mixture was dried over magnesium sulfate and filtered through a pad of celite (methanol). Solvent was removed under reduced pressure and the crude product was purified by column chromatography (SiO_2, petroleum ether/ethyl acetate 4:1, 1% acetic acid) to yield 7.38 g (23.6 mmol, 84 %) of a clear oil as a mixture of two diastereomers.

R_f 0.20 (petroleum ether/ethyl acetate 4:1); ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.85 (d, 12H, J = 6.3, 2x 7-CH_3, 2x 11-CH_3), 0.87 (d, 12H, J = 6.7, 2x 16-H_3), 0.97 (d, 6H, J = 6.6, 2x 3-CH_3), 1.00-1.44 (m, 40H, 2x 4-H_2-6-H_2, 2x 7-H, 2x 8-H_2-10-H_2, 2x 11-H, 2x 12-H_2-14-H_2), 1.53 (qq, 2H, J = 6.7, 2x 15-H), 1.89-2.03 (m, 2H, 2x 3-H), 2.13 (dd, 1H, J = 14.9, 1.9, 1x 2-H_a), 2.15 (dd, 1H, J = 14.9, 1.9, 1x 2-H_a), 2.35 (dd, 1H, J = 14.9, 1.6, 1x 2-H_b), 2.36 (dd, 1H, J = 14.9, 1.6, 1x 2-H_b); ^{13}C NMR (101 MHz, $CDCl_3$): δ 19.76, 19.82, 19.85, 19.89, 19.91 (3-CH_3, 7-CH_3, 11-CH_3), 22.79, 22.88 (C16), 24.50, 24.62, 24.63, 24.96, 24.98 (phyt-CH_2), 28.14 (C15), 30.33, 30.35 (C3), 32.90, 32.93, 32.96 (phyt-CH), 37.15, 37.18, 37.21, 37.24, 37.31, 37.33, 37.45, 37.48, 37.52, 37.55, 37.61, 39.54 (phyt-CH_2), 41.75, 41.83 (C2), 179.96 (C1); HRMS (ESI): calcd. for $C_{20}H_{39}O_2$ *m/z* 311.2956 $[M-H]^-$, found 311.2948.

GENERAL PROCEDURE FOR ACYLATION (3A-C, 5A-C).

Cholesterylic acid and dihydrophytenic acid have been activated with thionyl chloride prior to the reaction. A solution of the acid in toluene was treated with thionyl chloride (5.0 eq) and stirred under reflux for 2 h. The solvent was removed under reduced pressure and the residue was used without further purification.

The amine (1.0 eq) was dissolved in a mixture of THF/water (3:1) and magnesium oxide (MgO, 5.0 eq) was added. The suspension was cooled to 0 °C and a solution of the acid chloride (1.1 eq) in THF was added dropwise. Stirring was continued at 0 °C under TLC control. Except for **3a** and **5a** the reaction mixture was filtered through a short pad of celite and washed with hot THF. The solvent was removed under reduced pressure and the residue was purified by column chromatography (SiO_2).

(*R*)-*N*-Palmitoyl-3-amino-1,2-propanediol (3a)

2.02 g (22.2 mmol) (*R*)-3-amino-1,2-propanediol, 4.42 g (0.11 mol) MgO in 64 mL THF/water and 7.5 mL (24.7 mmol) palmitoyl chloride in 16 mL THF have been used. Reaction time: 4 h. Celite (20 g) and methanol (80 mL) were added to the reaction mixture and the solvent was removed under reduced pressure. The residue was purified by dry column chromatography (SiO_2, CH_2Cl_2/methanol 9:1). Yield: 5.84 g (17.7 mmol, 80 %) as white powder.

R_f 0.34 (CH_2Cl_2/methanol 9:1); ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.90 (t, 3H, J = 6.5, palm-16-H_3), 1.21-1.39 (m, 24H, palm-4-H_2-15-H_2), 1.53-1.67 (m, 2H, palm-3-H_2), 2.21 (t, 2H, J = 7.6, palm-2-H_2), 3.20 (dd, 1H, J = 13.8, 6.6, 3-H_a), 3.34 (dd, 1H, J = 13.8, 5.0, 3-H_b), 3.42-3.54 (m, 2H, 1-H_2), 3.64-3.72 (m, 1H, 2-H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 14.47 (palm-C16), 23.76, 27.05, 30.50, 30.65, 30.75, 30.77, 30.79, 30.82, 33.10 (palm-C4-C15), 26.20 (palm-C3), 37.09 (palm-C2), 43.34 (C-3), 65.02 (C-1), 72.10 (C-2), 176.97 (palm-CO); HRMS (ESI) calcd. for $C_{19}H_{39}NNaO_3$ *m/z* 352.2822 $[M+Na]^+$, found 352.2811.

(2*R*)-*N*-(Cholest-5'-en-3'-carbonyl)-3-amino-1,2-propanediol (3b)

Activation: 2.60 mL (35.8 mmol) thionyl chloride, 3.00 g (7.23 mmol) S1 in 50 mL toluene. 507 mg (5.56 mmol) (*R*)-3-amino-1,2-propanediol, 1.12 g (27.8 mmol) MgO in 40 mL THF/water and crude cholesteroyl chloride in 10 mL THF have been used. Reaction time: 5 h. Column chromatography: CH_2Cl_2/methanol 9:1. Yield: 1.96 g (4.03 mmol, 72 %) as pale yellow solid.

R_f 0.35 (CH_2Cl_2/methanol 9:1); ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.67 (s, 3H, chol-18-H_3), 0.86 (d, 3H, J = 6.6, chol-26-H_3), 0.86 (d, 3H, J = 6.6, chol-27-H_3), 0.89-2.18 (m, 28H, chol-CH_2, chol-CH, chol-CH_a), 0.91 (d, 3H, J = 6.4, chol-21-H_3), 1.01 (s, 3H, chol-19-H_3), 2.45 (t, 1H, J = 13.3, chol-CH_b), 3.28-3.46 (m, 2H, 3-H_2), 3.48-3.60 (m, 2H, 1-H_2), 3.69-3.79 (m, 1H, 2-H), 3.90 (bs, 2H, OH), 5.28-5.36 (m, 1H, chol-6-H), 6.47 (t, 1H, J = 5.6, NH); ^{13}C NMR (101 MHz, $CDCl_3$): δ 12.02 (chol-C18), 18.89 (chol-C21), 19.59 (chol-C19), 21.06 (chol-CH_2), 22.71 (chol-C26), 22.96 (chol-C27), 24.08, 24.42, 26.04 (chol-CH_2), 28.15 (chol-CH), 28.39 (chol-CH_2), 31.92 (chol-CH), 32.05 (chol-CH_2), 35.78 (chol-CH), 35.98, 36.37 (chol-CH_2), 37.06 (chol-C), 38.93, 39.66, 39.96 (chol-CH_2), 42.19 (C3), 42.46 (chol-C), 46.84, 50.44, 56.42, 56.97 (chol-CH), 63.73 (C-1), 71.26 (C2), 121.31 (chol-C6), 141.11 (chol-C5), 177.96 (chol-CO); HRMS (ESI): calcd. for $C_{31}H_{53}NNaO_3$ *m/z* 510.3918 $[M+Na]^+$, found 510.3901.

(*R*)-*N*-Phytanoyl-3-amino-1,2-propanediol (3c)

Activation: 1.60 mL (22.0 mmol) thionyl chloride, 1.78 g (5.70 mmol) **S3** in 8 mL toluene. 404 mg (4.43 mmol) (*R*)-3-amino-1,2-propanediol, 886 mg (22.0 mmol) MgO in 16 mL THF/water and crude phytanoyl chloride in 4 mL THF have been used. Reaction time: 4 h. Column chromatography: CH_2Cl_2/methanol 19:1→9:1. Yield: 1.35 g (3.50 mmol, 79 %).

R_f 0.40 (CH_2Cl_2/methanol 9:1); ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.83 (d, 6H, J = 6.6, phyt-7-CH_3, phyt-11-CH_3), 0.85 (d, 6H, J = 6.6, 2x phyt-16-H_3), 0.91 (d, 3H, J = 6.0, phyt-3-CH_3), 0.97-1.42 (m, 21H, phyt-4-H_2-6-H_2, phyt-7-H, phyt-8-H_2-10-H_2, phyt-11-H, phyt-12-H_2-14-H_2), 1.51 (qqd, 1H, J = 6.6, 6.6, 6.6, phyt-15-H), 1.85-2.01 (m, 2H, phyt-2-H_a, phyt-3-H), 2.16-2.29 (m, 1H, phyt-2-H_b), 3.27-3.48 (m, 2H, 3-H_2), 3.49-3.60 (m, 2H, 1-H_2), 3.71-3.78 (m, 1H, 2-H), 6.38 (t, 1H, J = 6.0, NH); ^{13}C NMR (101 MHz, $CDCl_3$): δ 19.70, 19.76, 19.80, 19.83, 19.87 (phyt-7-CH_3, phyt-11'-CH_3), 22.76, 22.85 (phyt-C16), 24.61, 24.94, 28.10, 32.93, 37.35, 37.40, 37.43, 37.49, 37.53, 37.56, 37.59, 37.63, 39.50 (phyt-C4-C14'), 30.98 (phyt-C3), 42.27 (C-3), 44.44, 44.52 (phyt-C2), 63.78 (C-1), 71.29 (C-2), 175.02 (phyt-CO); HRMS (ESI): calcd. for $C_{23}H_{47}NNaO_3$ *m/z* 408.3448 $[M+Na]^+$, found 408.3461.

(*R*)-*N*-Hexadecyl-*N*-palmitoyl-3-amino-1,2-propanediol (5a)

5.18 g (16.4 mmol) **4a**, 3.31 g (82.1 mmol) MgO in 150 mL THF/water and 5.5 mL (18.1 mmol) palmitoyl chloride in 50 mL THF have been used. Reaction time: 7 h. Dry column chromatography: CH_2Cl_2/methanol 9:1. Yield: 8.14 g (14.7 mmol, 89 %) as white solid.

R_f 0.42 (ethyl acetate); ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.87 (t, 6H, *J* = 6.8, 2x palm-16-H_3), 1.19-1.34 (m, 50H,1x palm-3-H_2, 2x palm-4-H_2-15-H_2), 1.51-1.66 (m, 4H, 1x palm-3-H_2, 1x palm-2-H_2), 2.32 (t, 2H, *J* = 7.6, 1x palm-2-H_2), 3.16-3.36 (m, 2H, 1x palm-1-H_2), 3.40 (dd, 1H, *J* = 14.1, 6.2, 3-H_a), 3.45 (dd, 1H, *J* = 11.9, 3.8, 1-H_a), 3.52 (dd, 1H, *J* = 14.1, 6.0, 3-H_b), 3.55 (dd, 1H, *J* = 11.9, 4.0, 1-H_b), 3.71-3.78 (m, 1H, 2-H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 14.26 (palm-C16), 22.83, 26.92, 29.44, 29.50, 29.57, 29.60, 29.66, 29.69, 29.77, 29.80, 30.03, 32.06 (palm-C4-C15), 25.63, 29.10 (palm-C3), 33.16 (palm-C2), 49.25 (C3), 50.02 (palm-CH_2N), 63.67 (C1), 71.06 (C2), 175.80 (palm-CO); HRMS (ESI): calcd. for $C_{35}H_{71}NNaO_3$ *m/z* 576.5326 $[M+Na]^+$, found 576.5301.

(2*R*)-*N*-(Cholest-5-en-3-carbonyl)-*N*-(cholest-5-en-3-meth-yl)-3-amino-1,2-propanediol (5b)

Activation: 1.35 mL (18.6 mmol) thionyl chloride, 1.54 g (3.71 mmol) **S1** in 25 mL toluene. 1.36 g (2.86 mmol) **4b**, 577 mg (14.3 mmol) MgO in 40 mL THF/water and crude cholesteroyl chloride in 10 mL THF have been used. Reaction time: 4 h. Column chromatography: CH_2Cl_2/methanol 9:1. Yield: 1.36 g (1.56 mmol, 55 %) as pale yellow solid.

R_f 0.53 (CH_2Cl_2/methanol 9:1); ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.68 (s, 6H, 2x chol-18-CH_3), 0.86 (d, 6H, *J* = 6.6, 2x chol-26-H_3), 0.86 (d, 6H, *J* = 6.6, 2x chol-27-H_3), 0.91 (d, 6H, *J* = 6.3, 2x chol-21-H_3), 0.94-1.64 (m, 46H, chol-CH_2, chol-CH), 0.98 (s, 3H, 1x chol-19-H_3), 1.05 (s, 3H, 1x chol-19-H_3), 1.75-2.08 (m, 12H, chol-CH_2), 2.42-2.53 (m, 1H, chol-CH), 2.55-2.67 (m, 1H, chol-CH), 3.15 (dd, 1H, *J* = 14.7, 7.1, chol-CH_aN), 3.24 (dd, 1H, *J* = 14.7, 7.0, chol-CH_bN), 3.37-3.60 (m, 4H, 1-H_2, 3-H_2), 3.67-3.79 (m, 1H, 2-H), 5.24-5.40 (m, 2H, 2x chol-6-H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 12.01 (chol-C18), 18.88 (chol-C21), 19.62, 19.67 (chol-C19), 21.02, 21.07 (chol-CH_2), 22.71 (chol-C26), 22.97 (chol-C27), 23.99, 24.42, 26.08, 26.89 (chol-CH_2), 28.16 (chol-CH), 28.38 (chol-CH_2), 31.93 (chol-CH), 31.98, 32.06, 35.94 (chol-CH_2), 35.35 (chol-CH), 36.35 (chol-CH_2),37.13 (chol-C), 37.22, 37.38 (chol-CH_2), 38.94 (chol-C), 39.18, 39.60, 39.67, 39.91, 39.95 (chol-CH_2), 49.77 (C3), 50.41, 50.46 (chol-CH), 55.24 (chol-NCH_2), 56.30, 56.34, 56.90, 57.00 (chol-CH), 63.69 (C1), 71.00 (C2), 121.04, 121.12 (chol-C6), 141.45, 141.49 (chol-C5), 178.71 (chol-CO); HRMS (ESI): calcd. for $C_{59}H_{100}NO_3$ *m/z* 870.7698 $[M+H]^+$, found 870.7656.

(*R*)-*N*-Phytanyl-*N*-phytanoyl-3-amino-1,2-propanediol (5c)

Activation: 0.47 mL (6.47 mmol) thionyl chloride, 408 mg (1.31 mmol) **S3** in 5 mL toluene. 384 mg (1.00 mmol) **4c**, 203 mg (5.04 mmol) MgO in 8 mL THF/water and crude phytanoyl chloride in 2 mL THF were used. Reaction time: 5 h. Column chromatography: CH_2Cl_2/methanol 19:1. Yield: 506 mg (0.76 mmol, 76 %) as pale yellow oil.

R_f 0.53 (CH_2Cl_2/methanol 9:1); ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.81-0.88 (m, 24H, 2x phyt-7-CH_3, 2x phyt-11-CH_3, 4x phyt-16-CH_3), 0.89-0.96 (m, 6H, 2x phyt-3-CH_3), 0.99-1.66 (m, 43H, 1x phyt-2-H_2, 1x phyt-3-H, 2x phyt-4-H_2-6-H_2, 2x phyt-7-H, 2x phyt-8-H_2-10-H_2, 2x phyt-11-H, 2x phyt-12-H_2-14-H_2), 1.52 (qqd, 2H, *J* = 6.6, 6.6, 6.6, 2x phyt-15-H), 1.96-2.07 (m, 1H, 1x phyt-3-H), 2.08-2.17 (m, 1H, 1x phyt-2-H_a), 2.27-2.35 (m, 1H, 1x phyt-2-H_b), 3.14-3.61 (m, 6H, 1-H_2, 3-H_2, phyt-CH_2N), 3.71-3.80 (m, 1H, 2-H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 19.56, 19.63, 19.69, 19.76, 19.86, 19.90, 19.96 (phyt-C7-CH_3, phyt-C11-CH_3), 22.63, 22.73 (phyt-C16-CH_3), 24.38, 24.48, 24.81, 30.33, 30.59, 30.94, 32.80, 36.01, 36.11, 37.19, 37.30, 37.41, 37.46, 39.38, 40.31, 40.38 (phyt-C2-C14), 27.98

(phyt-C15), 48.26, 49.22 (C-3, phyt-CH_2N), 63.57 (C1), 70.94, 70.98 (C2), 175.11 (phyt-CO); HRMS (ESI): calcd. for $C_{43}H_{88}NO_3$ *m/z* 666.6759 $[M+H]^+$, found 666.6730.

GENERAL PROCEDURE FOR AMIDE REDUCTION (4A-C)

A suspension of the amide (1.0 eq) in dry THF under nitrogen atmosphere was cooled to 0 °C and a solution of lithium aluminium hydride (1M in THF, 4.0 eq) was added dropwise. The solution was stirred for 5 min at 0 °C and subsequently heated under reflux for 4 h. The reaction was diluted with THF (15-30 mL) and cooled to 0 °C. Then 10 % sodium hydroxide solution (10-15 mL) was added very slowly and stirring was continued until the mixture looked cloudy. The suspension was filtered through a short pad of celite and washed with hot THF. The solvent was removed under reduced pressure and the residue was used without further purification.

(*R*)-*N*-Hexadecyl-3-amino-1,2-propanediol (4a)

1.13 g (3.43 mmol) **3a**, 13.5 mL (13.5 mmol) lithium aluminium hydride solution in 30 mL THF were used. Yield: 1.02 g (3.23 mmol, 94 %) as white solid.

R_f 0.59 (CH_2Cl_2/methanol 9:1); ^{1}H NMR (400 MHz, CD_3OD): δ 0.90 (t, 3H, *J* = 6.8, palm-16-H_3), 1.21-1.41 (m, 26H, palm-3-H_2-15-H_2), 1.46-1.58 (m, 2H, palm-2-H_2), 2.56 (dd, 1H, *J* = 12.2, 8.3, 3-H_a), 2.59 (t, 2H, *J* = 8.0, palm-1-H_2), 2.71 (dd, 1H, *J* = 12.2, 3.8, 3-H_b), 3.50 (dd, 2H, *J* = 5.6, 1.6, 1-H_2), 3.71-3.78 (m, 1H, 2-H); ^{13}C NMR (101 MHz, CD_3OD): δ 14.46 (palm-C16), 23.75, 26.97, 28.42, 30.49, 30.56, 30.63, 30.68, 30.73, 30.75, 30.78, 30.80, 30.81, 33.09 (palm-C3-C15), 33.69 (palm-C2), 50.79 (palm-C1), 53.48 (C3), 66.10 (C1), 71.59 (C2); HRMS (ESI): calcd. for $C_{19}H_{42}NO_2$ *m/z* 316.3210 $[M+H]^+$, found 316.3211.

(2*R*)-*N*-(Cholest-5-en-3-methyl)-3-amino-1,2-propanediol (4b)

901 mg (1.85 mmol) 3b, 7.5 mL (7.5 mmol) lithium aluminium hydride solution in 15 mL THF were used. Yield: 870 mg (1.84 mmol, 99 %) as pale yellow solid.

R_f 0.19 (CH_2Cl_2/methanol 9:1); ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.67 (s, 3H, chol-18-H_3), 0.86 (d, 3H, *J* = 6.6, chol-26-H_3), 0.86 (d, 3H, *J* = 6.6, chol-27-H_3), 0.91 (d, 3H, *J* = 6.4, chol-21-H_3), 0.93-1.70 (m, 23H, chol-CH_2, chol-CH), 0.96 (s, 3H, chol-19-H_3), 1.76-2.08 (m, 6H, chol-CH_2), 2.42-2.55 (m, 2H, chol-CH_2N), 2.67 (dd, 1H, *J* =12.0, 6.7, 3-H_a), 2.79 (dd, 1H, *J* = 12.0, 2.8, 3-H_b), 3.60 (dd, 1H, *J* = 11.2, 4.3, 1-H_a), 3.65-3.71 (m, 1H, 1-H_b), 3.72-3.78 (m, 1H, 2-H), 5.26-5.33 (m, 1H, chol-6-H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 12.01 (chol-C18), 18.87 (chol-C21), 19.60 (chol-C19), 21.07 (chol-CH_2), 22.71 (chol-C26), 22.96 (chol-C27), 23.99, 24.43, 27.29 (chol-CH_2), 28.15 (chol-CH), 28.39 (chol-CH_2), 29.55 (chol-CH), 32.02 (chol-CH_2), 32.04, 35.94 (chol-CH), 36.34, 37.51, 37.70, 39.33, 39.66 (chol-CH_2), 39.72 (chol-CH), 39.97 (chol-CH_2), 42.45 (chol-C), 50.58 (chol-CH), 52.73 (chol-C3), 56.32 (chol-CH), 56.55 (chol-CH_2N), 56.97 (chol-CH), 66.08 (C1), 69.48 (C2), 120.05 (chol-C6), 142.54 (chol-C5); HRMS (ESI): calcd. for $C_{31}H_{56}NO_2$ *m/z* 474.4306 $[M+H]^+$, found 474.4305.

(*R*)-*N*-Phytanyl-3-amino-1,2-propanediol (4c)

456 mg (1.18 mmol) **3c**, 4.7 mL (4.7 mmol) lithium aluminium hydride solution in 10 mL were used. Yield: 434 mg (1.16 mmol, 99 %) as clear oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.84 (d, 6H, *J* = 6.6, phyt-7-CH_3, phyt-11-CH_3), 0.86 (d, 6H, *J* = 6.7, phyt-16-H_3), 0.87 (d, 3H, *J* = 6.4, phyt-3-CH_3), 0.98-1.57 (m, 23H, phyt-2-H_2, phyt-3-H, phyt-4-H_2-6-H_2, phyt-7-H, phyt-8-H_2-10-H_2, phyt-11-H, phyt-12-H_2-14-H_2), 1.51 (qqd, 1H, *J* = 6.6, 6.6, 6.6, phyt-15-H), 2.53-2.72 (m, 3H, 3-H_a, phyt-1-H_2), 2.78 (dd, 1H, *J* = 3.4, 3.4, 1x 3-H_b), 2.81 (dd, 1H, *J* = 3.4, 3.4, 1x 3-H_b), 3.10 (bs, 3H, OH, NH), 3.58 (dd, 1H, *J*

= 11.3, 4.9, 1-H_a), 3.70 (dd, 1H, *J* = 11.3, 3.6, 1-H_b), 3.73-3.80 (m, 1H, 2-H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 16.67, 19.70, 19.76 (phyt-7-CH_3, phyt-11-CH_3), 22.63, 22.76 (phyt-C16), 24.43, 24.50, 24.81, 30.33, 30.94, 32.79, 32.81, 37.30, 37.37, 37.40, 37.42, 37.47, 39.38 (phyt-C2-C14), 27.98 (phyt-C15), 47.92, 47.96 (phyt-C1), 52.51, 52.55 (C3), 65.85 (C1), 69.58, 69.61 (C2); HRMS (ESI): calcd. for $C_{23}H_{50}NO_2$ *m/z* 372.3836 $[M+H]^+$, found 372.3825.

GENERAL PROCEDURE FOR DMTR-PROTECTION (6A-C)

A solution of dimethoxytrityl chloride (DMTrCl, 1.5 eq) in dry 1,2-dichloroethane (DCE) was added to a solution of the alcohol (1.0 eq), 4-dimethylamino pyridine (DMAP, 0.1 eq) and triethylamine (TEA, 3.0 eq) in dry DCE at room temperature. The reaction mixture was stirred at 80 °C and the solvent was removed under reduced pressure. The crude product was purified by column chromatography (SiO_2).

(*R*)-*N*-Hexadecyl-*N*-palmitoyl-3-amino-1-(dimethoxytriphenylmethyloxy)-2-propanol (6a)

1.20 g (3.54 mmol) DMTr chloride in 10 mL DCE and 1.31 g (2.36 mmol) **5a**, 29 mg (0.24 mmol) DMAP and 0.99 mL (7.1 mmol) TEA in 25 mL DCE were used. Reaction time: 5 h. Column chromatography: petroleum ether/ethyl acetate 4:1→1:1, 0.1% TEA. Yield: 1.48 g (1.73 mmol, 73 %) as yellow oil.

R_f 0.19 (petroleum ether/ethyl acetate 4:1); ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.88 (t, 6H, *J* = 6.8, 2x palm-16-H_3), 1.19-1.35 (m, 50H, 1x palm-3-H_2, 2x palm-4-H_2-15-H_2), 1.49-1.66 (m, 4H, 1x palm-3-H_2, 1x palm-2-H_2), 2.27 (t, 2H, *J* = 7.5, 1x palm-2-H_2) 2.95 (dd, 1H, *J* = 9.3, 7.6, 3-H_a), 3.21 (t, 2H, *J* = 8.1, palm-1-H_2), 3.25 (dd, 1H, *J* = 9.3, 5.0, 3-H_b), 3.48 (dd, 1H, *J* = 14.4, 2.4, 1-H_a), 3.59 (dd, 1H, *J* = 14.4, 7.0, 1-H_b), 3.79 (s, 6H, DMTr-OCH_3), 3.92-4.00 (m, 1H, 2-H), 4.62 (bs, 1H, OH), 6.79-6.87 (m, 4H, DMTr-CH), 7.15-7.34 (m, 7H, DMTr-CH), 7.38-7.45 (m, 2H, DMTr-CH); ^{13}C NMR (101 MHz, $CDCl_3$): δ 14.27 (palm-C16), 22.84, 27.02, 29.51, 29.58, 29.60, 29.67, 29.73, 29.75, 29.81, 29.83, 29.85, 32.07 (palm-C3, palm-C4-C15), 25.56, 29.02 (palm-C3, palm-C2), 33.24 (palm-C2), 50.27 (palm-C1), 51.46 (C1), 55.33 (DMTr-OCH_3), 64.97 (C3), 71.75 (C2), 86.23 (DMTr-CAr_3), 113.22, 113.25, 113.36, 126.92, 127.93, 128.22, 130.12 (DMTr-CH), 136.07, 136.20, 144.98, 158.62 (DMTr-C), 176.14 (palm-CO); HRMS (ESI): calcd. for $C_{56}H_{89}NNaO_5$ m/z 878.6633 $[M+Na]^+$, found 878.6595.

(2*R*)-*N*-(Cholest-5-en-3-carbonyl)-*N*-(cholest-5-en-3-methyl)-3-amino-1-(dimethoxytriphenyl-methyloxy)-2-propanol (6b)

587 mg (1.73 mmol) DMTr chloride in 10 mL DCE and 1.00 g (1.15 mmol) **5b**, 15 mg (0.12 mmol) DMAP and 0.48 mL (3.44 mmol) TEA in 15 mL DCE were used. Reaction time: 6 h. Column chromatography: petroleum ether/ethyl acetate 3:1, 0.1% TEA. Yield: 1.09 g (0.93 mmol, 81 %) as pale yellow foam.

R_f 0.23 (petroleum ether/ethyl acetate 3:1); ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.68 (s, 3H, chol-18-H_3), 0.68 (s, 3H, chol-18-H_3), 0.86 (d, 6H, *J* = 6.6, 2x chol-26-H_3), 0.87 (d, 6H, *J* = 6.6, 2x chol-27-H_3), 0.92 (d, 6H, *J* = 6.4, 2x chol-21-H_3), 0.94-1.65 (m, 46H, chol-CH_2, chol-CH), 0.99 (s, 3H, 1x chol-19-H_3), 1.02 (s, 3H, 1x chol-19-H_3), 1.75-2.08 (m, 12H, chol-CH_2), 2.32-2.44 (m, 1H, chol-CH), 2.48-2.61 (m, 1H, chol-CH), 2.90 (dd, 1H, J = 9.1, 8.4, 1-H_a), 3.06 (dd, 1H, *J* = 14.8, 7.4, palm-CH_aN), 3.16 (dd, 1H, *J* = 14.8, 6.7, palm-CH_bN), 3.24 (dd, 1H, *J* = 9.1, 4.9, 1-H_b), 3.50-3.58 (m, 1H, 3-H_a), 3.62 (dd, 1H, *J* = 14.2, 6.3, 3-H_b), 3.78 (s, 3H, DMTr-OCH_3), 3.79 (s, 3H, DMTr-OCH_3), 3.94-4.04 (m, 1H, 2-H), 4.71 (bs, 1H, OH), 5.27-5.37 (m, 2H, 2x chol-6-H), 6.78-6.86 (m, 4H, DMTr-CH), 7.23-7.34 (m,

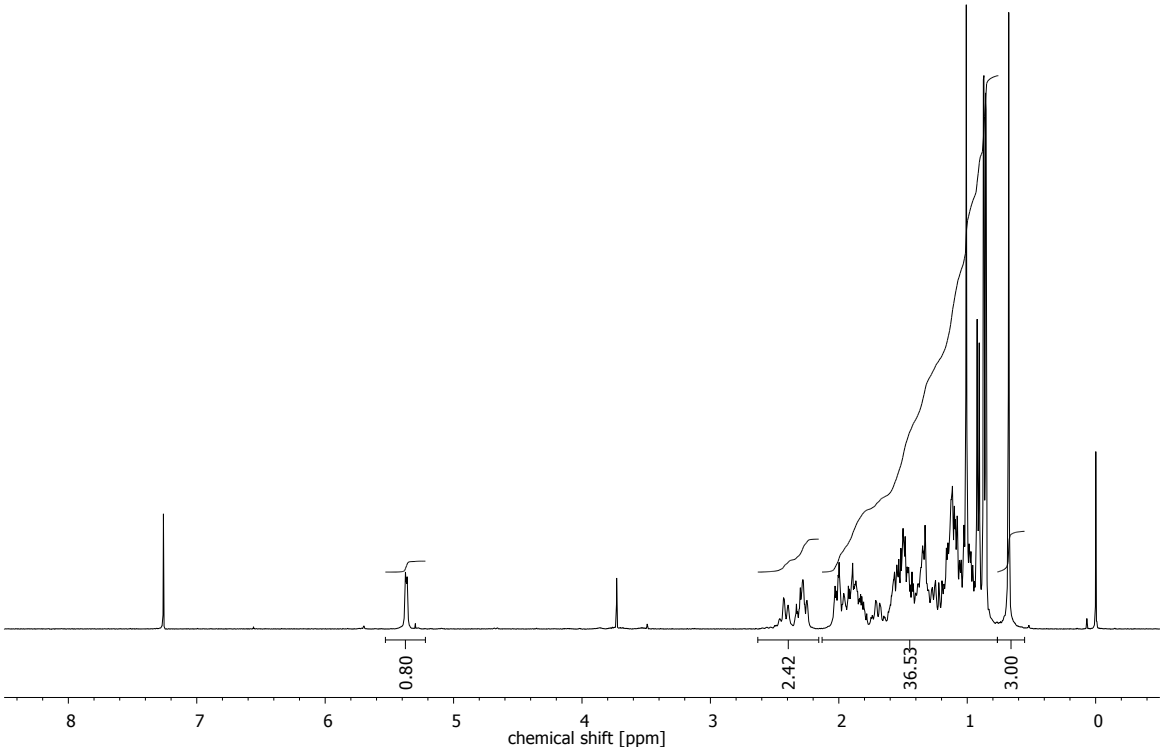
7H, DMTr-CH), 7.37-7.44 (m, 2H, DMTr-CH); ^{13}C NMR (101 MHz, $CDCl_3$): δ 11.99, 12.01 (chol-C18), 18.86 (chol-C21), 19.59, 19.65 (chol-C19), 20.99, 21.04 (chol-CH2), 22.71 (chol-C26), 22.97 (chol-C27), 23.96, 24.41 (chol-CH_2), 25.95, 26.96 (chol-C), 28.15 (chol-CH), 28.37 (chol-CH_2), 31.90, 31.96, 31.99, 32.04 (chol-CH_2, chol-CH), 35.46 (chol-CH_2), 35.92 (chol-CH), 36.32, 37.08 (chol-CH_2), 37.16, 37.36 (chol-C), 38.96 (chol-CH_2), 39.14 (chol-CH), 39.54, 39.65, 39.89, 39.95 (chol-CH_2), 42.20 (chol-CH), 42.42, 42.44 (chol-C), 50.38, 50.45 (chol-CH), 52.07 (C3), 55.31 (DMTr-OCH_3), 55.58 (chol-CH_2N), 56.26, 56.31, 56.89, 56.98 (chol-CH), 60.52 (chol-C), 64.67 (C1), 71.60 (C2), 68.18 (DMTr-CAr_3), 113.18, 113.22, 113.27 (DMTr-CH), 120.86, 120.92 (chol-C6), 127.89, 127.94, 127.96, 128.18, 129.25, 130.13 (DMTr-CH), 135.93, 136.11 (DMTr-C), 141.64 (chol-C5), 144.91, 158.60, 158.73 (DMTr-C), 179.01 (chol-CO); HRMS (ESI): calcd. for $C_{80}H_{117}NNaO_5$ *m/z* 1194.8824 $[M+Na]^+$, found 1194.8780.

(*R*)-*N*-Phytanyl-*N*-phytanoyl-3-amino-1-(dimethoxytriphenylmethyloxy)-2-propanol (6c)

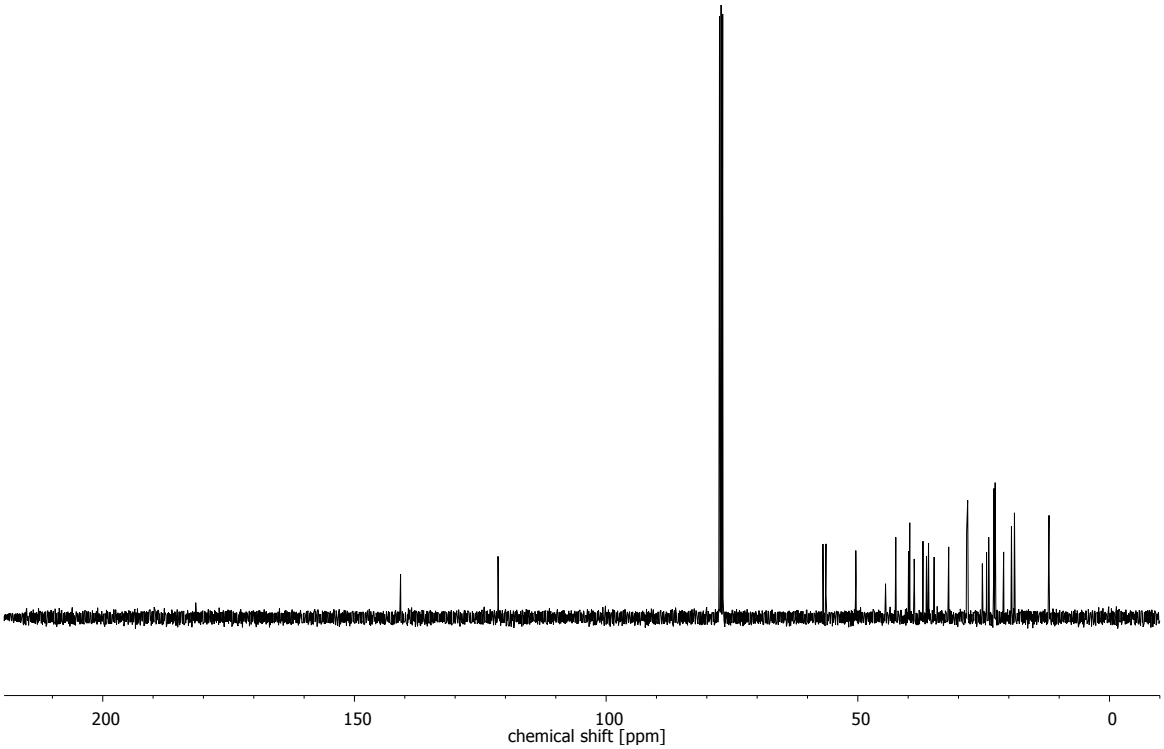
351 mg (1.04 mmol) DMTr chloride in 5 mL DCE and 459 mg (0.69 mmol) **5c**, 8 mg (0.07 mmol) DMAP and 0.29 mL (2.08 mmol) TEA in 10 mL DCE were used. Reaction time: 5 h. Column chromatography: petroleum ether/ethyl acetate 4:1, 0.1% TEA). Yield: 509 mg (0.53 mmol, 76%) as pale yellow oil.

R_f 0.35 (petroleum ether/ethyl acete 3:1); ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.80-0.92 (m, 30H, phyt-3-CH_3, phyt-7-CH_3, phyt-11-CH_3, phyt-16-H_3), 0.98-1.44 (m, 42H, 1x phyt-2-H_a, 1x phyt-3-H, 2x phyt-4-H_2-6-H_2, 2x phyt-7-H, 2x phyt-8-H_2-10-H_2, 2x phyt-11-H, 2x phyt-12-H_2-14-H_2), 1.46-1.68 (m, 1H, 1x phyt-2-H_b), 1.52 (qqt, 2H, *J* = 6.6, 6.6, 6.6, 2x phyt-15-H), 1.86-2.11 (m, 2H, 1x phyt-2-H_a, 1x phyt-3-H), 2.19-2.31 (m, 1H, 1x phyt-2-H_b), 2.88-2.97 (m, 1H, 1-H_a), 3.09-3.33 (m, 3H, 1-H_b, 1x phyt-1-H_2), 3.44-3.64 (m, 2H, 3-H_2), 3.78 (s, 3H, DMTr-OCH_3), 3.79 (s, 3H, DMTr-OCH_3), 3.92-4.03 (m, 1H, 2-H), 4.71 (d, 0.5H, *J* = 3.8, OH), 4.73 (d, 0.5H, *J* = 3.8, OH), 6.77-6.86 (m, 4H, DMTr-CH), 7.12-7.34 (m, 8H, DMTr-CH), 7.38-7.44 (m, 1H, DMTr-CH); ^{13}C NMR (101 MHz, $CDCl_3$): δ 19.62, 19.70, 19.77 (phyt-3-CH_3, phyt-7-CH_3, phyt-11-CH_3), 22.65, 22.74 (phyt-C16), 24.52, 24.83, 30.98, 32.82, 37.32, 37.43, 37.45, 37.49, 39.40 (phyt-C2, phyt-C3, phyt-C4-C14), 28.00 (phyt-C15), 30.65 (phyt-C3), 40.37 (phyt-C2), 48.56 (phyt-C1), 51.43 (C3), 55.21, 55.25 (DMTr-OCH_3), 64.73 (C1), 71.58 (C2), 81.44, 86.09 (DMTr-CAr_3), 113.11, 113.14, 113.19, 113.61, 126.80, 127.08, 127.79, 127.82, 127.86, 128.08, 129.15, 130.01 (DMTr-CH), 136.04, 139.50, 144.86, 147.37, 158.51, 158.66 (DMTr-C), 175.56 (phyt-CO); HRMS (ESI): calcd. for $C_{64}H_{105}NNaO_5$ *m/z* 990.7885 $[M+Na]^+$, found 990.7839.

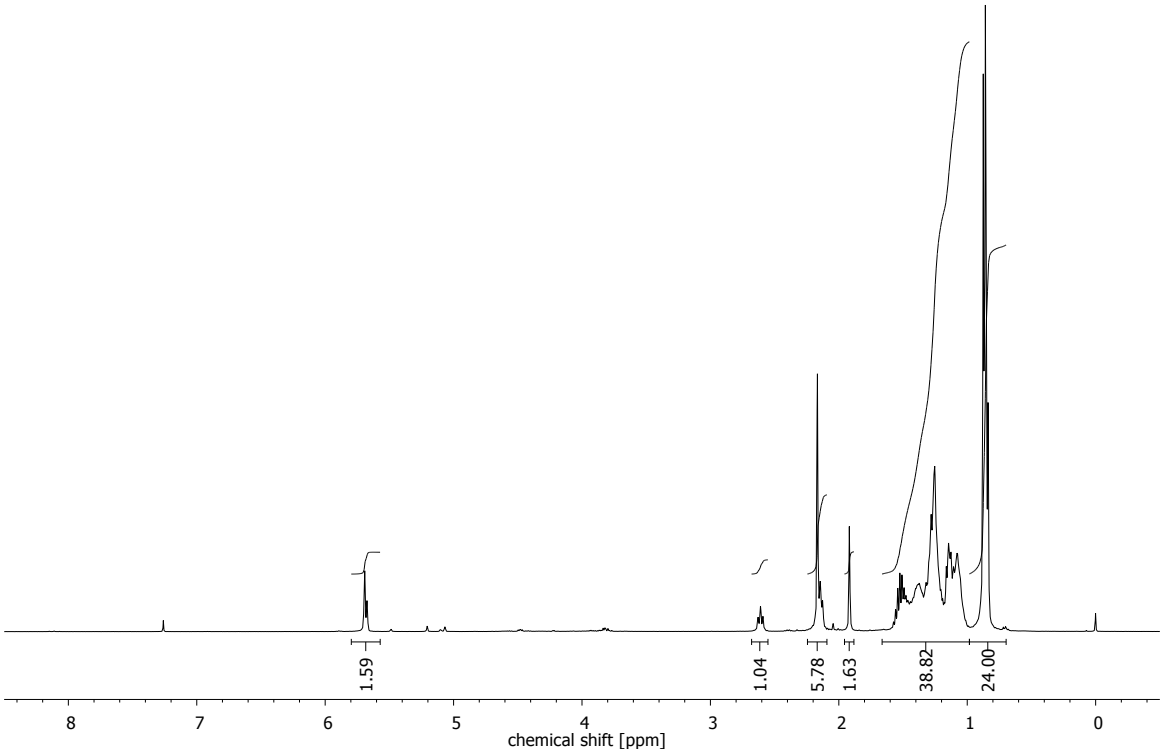
^{1}H, ^{13}C, ^{31}P NMR spectra of compounds S1-3 and 1-6a-c



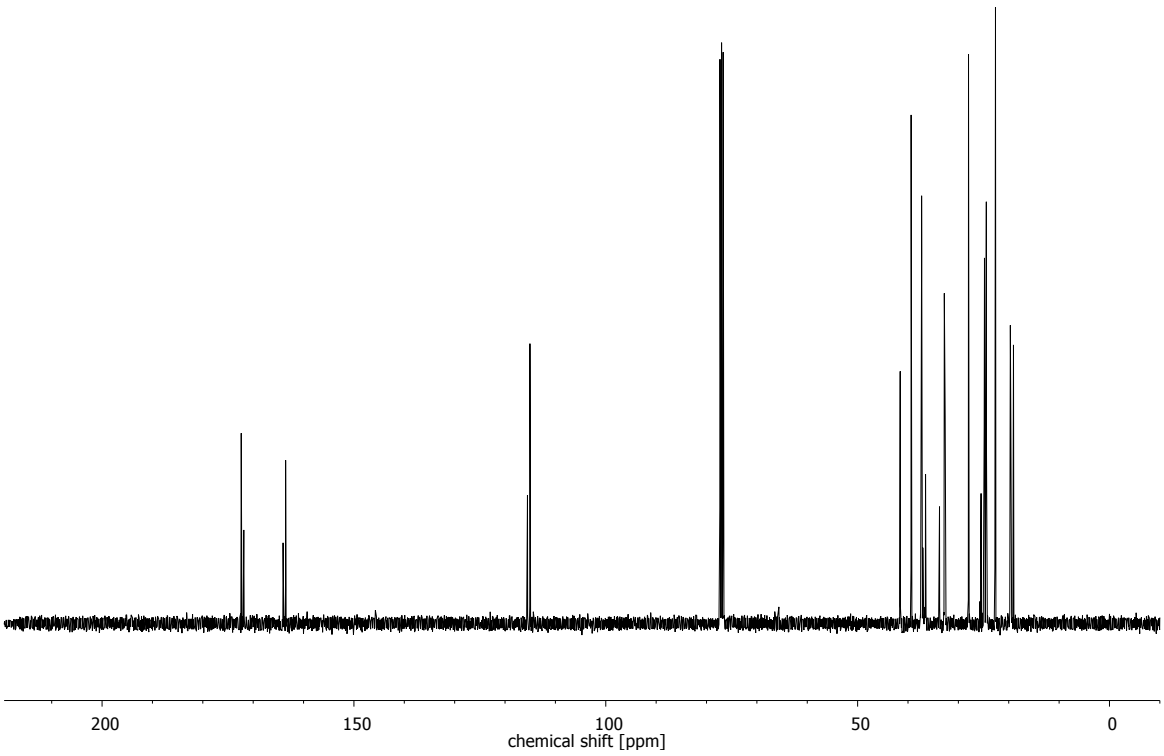
^{1}H-NMR spectrum of **S1** (400 MHz, $CDCl_3$, 25°C)



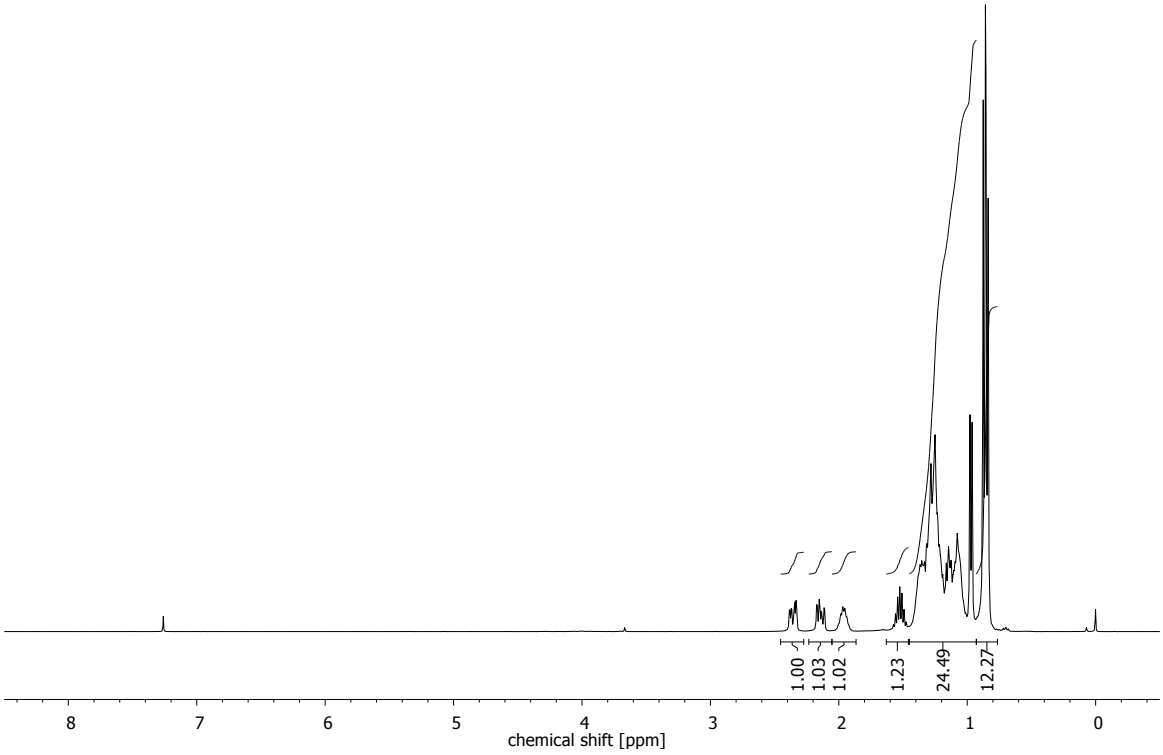
^{13}C-NMR spectrum of **S1** (101 MHz, $CDCl_3$, 25°C)



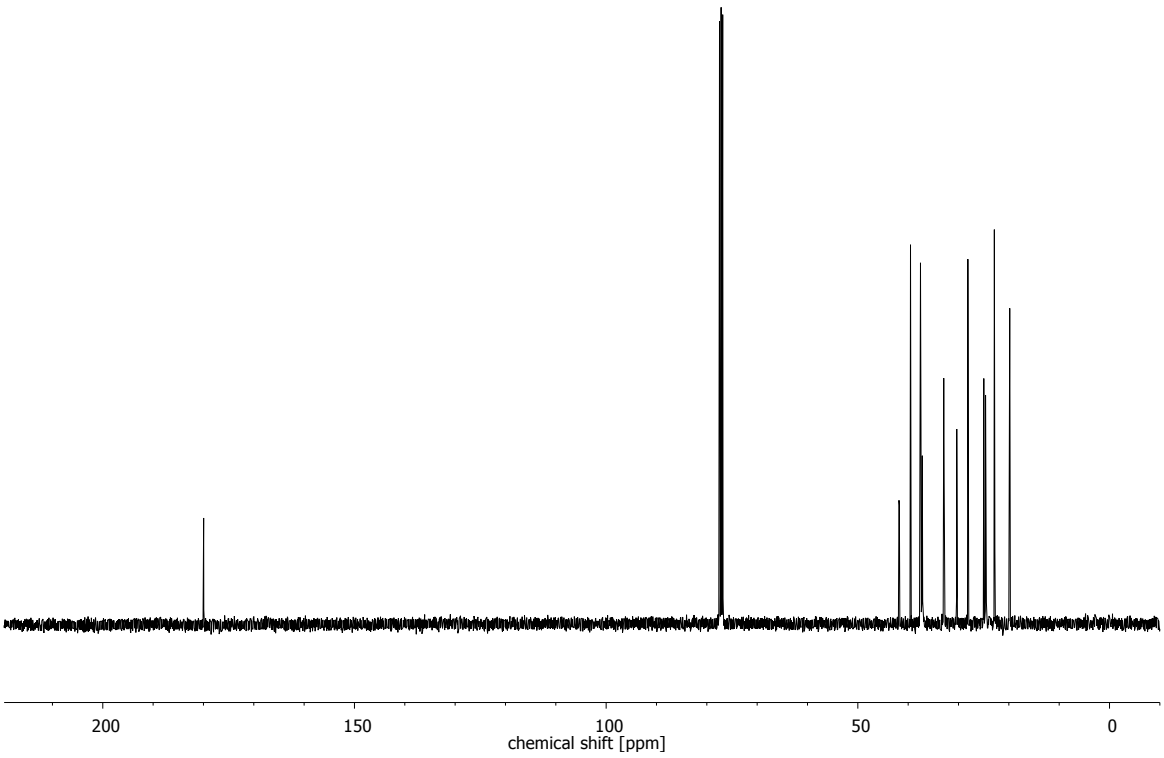
^{1}H-NMR spectrum of **S2** (400 MHz, $CDCl_3$, 25°C)



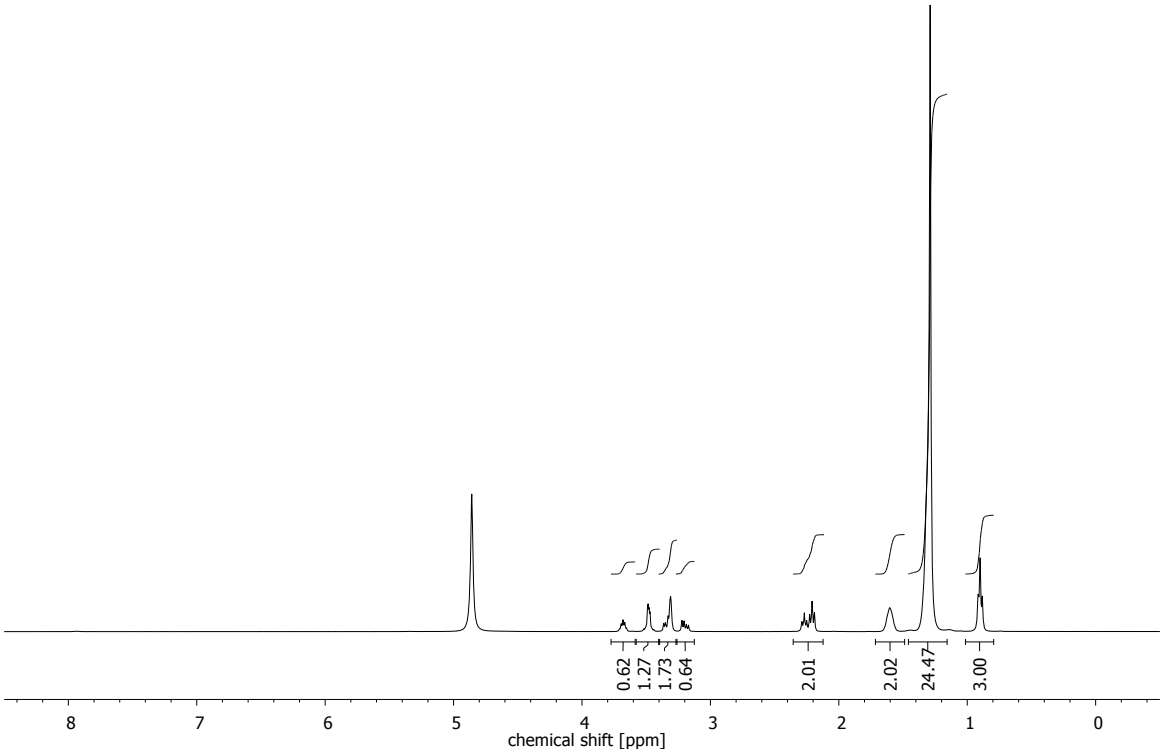
^{13}C-NMR spectrum of **S2** (101 MHz, $CDCl_3$, 25°C)



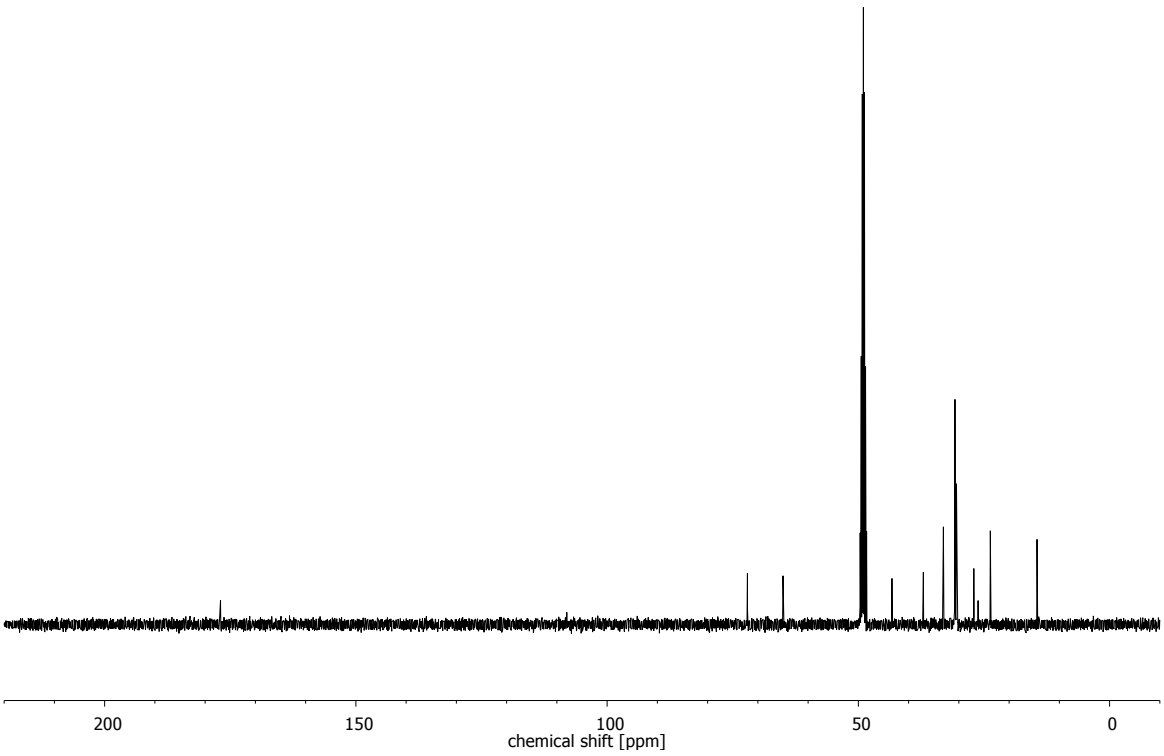
^{1}H-NMR spectrum of **S3** (400 MHz, $CDCl_3$, 25°C)



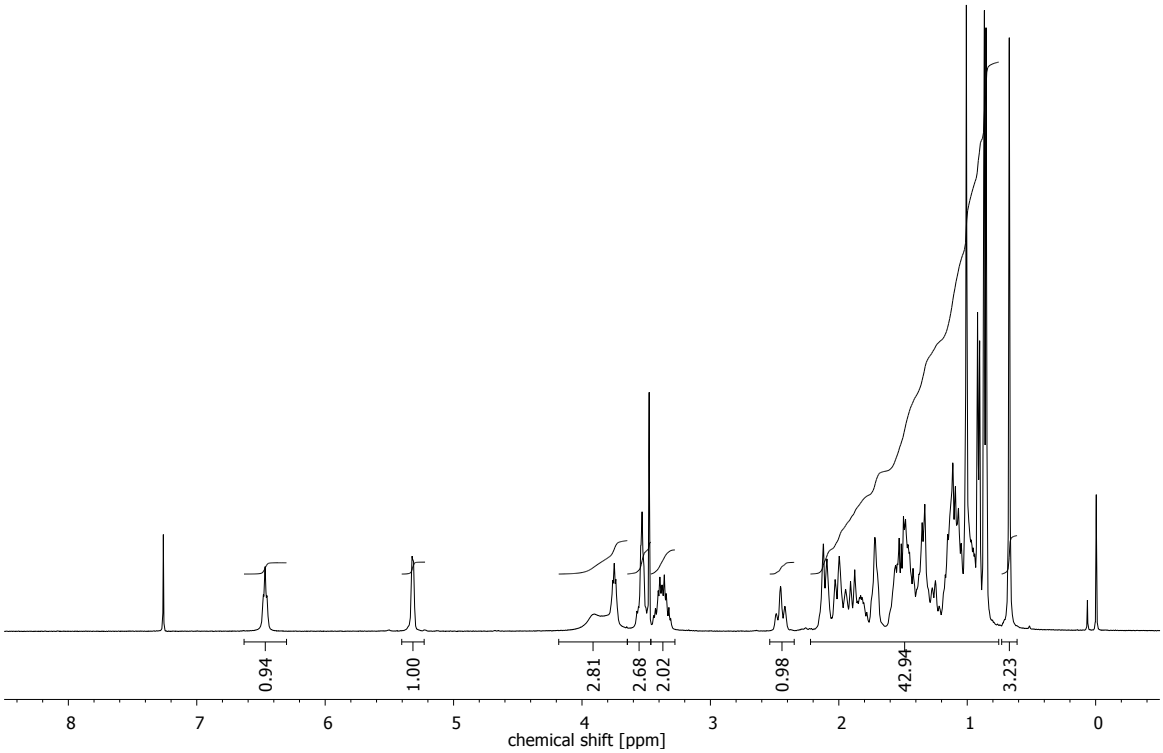
^{13}C-NMR spectrum of **S3** (101 MHz, $CDCl_3$, 25°C)



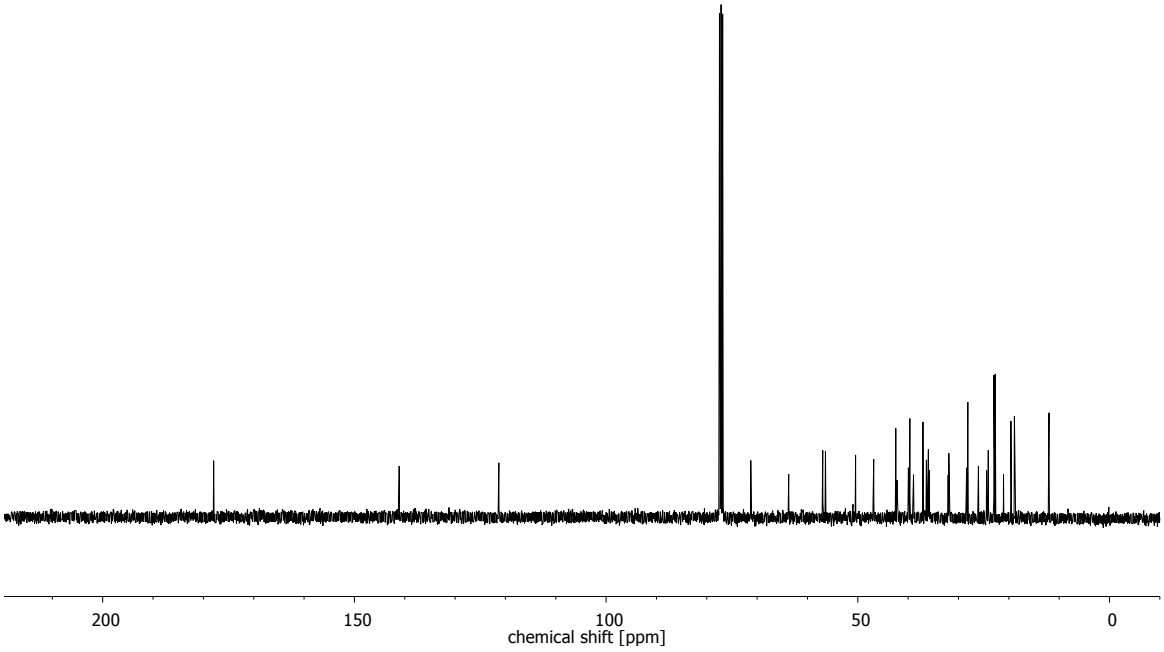
^{1}H-NMR spectrum of **3a** (400 MHz, CD_3OD, 25°C)



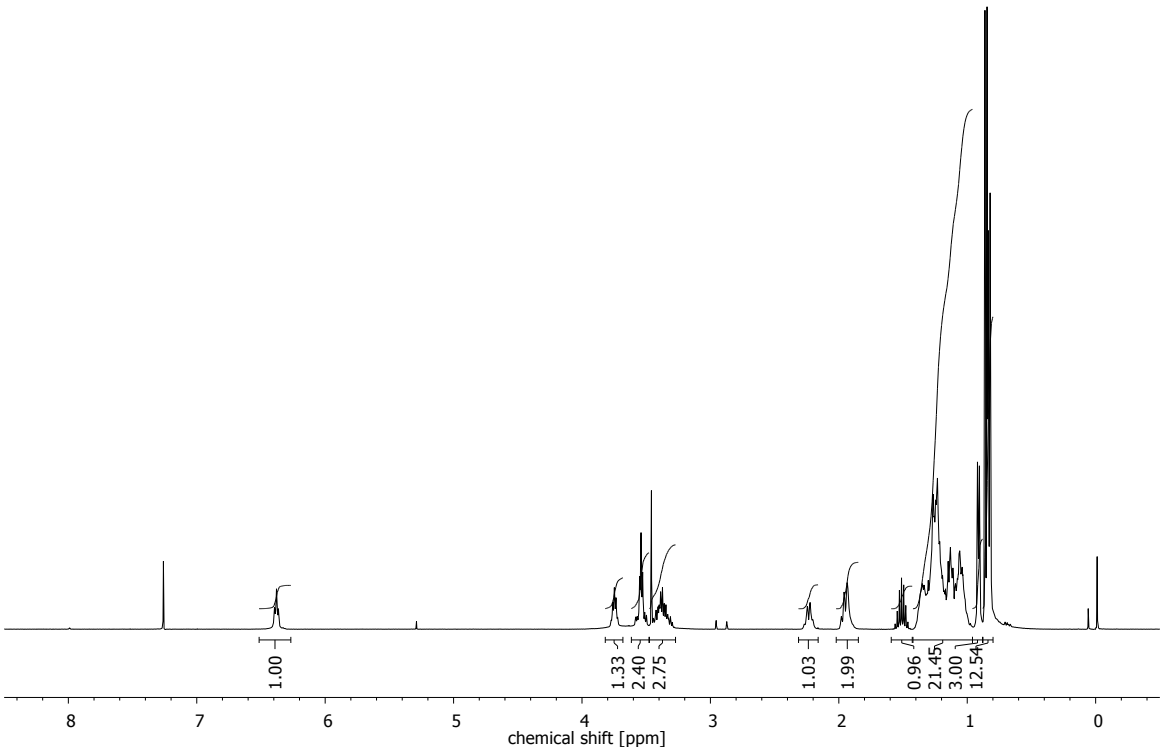
^{13}C-NMR spectrum of **3a** (101 MHz, CD_3OD, 25°C)



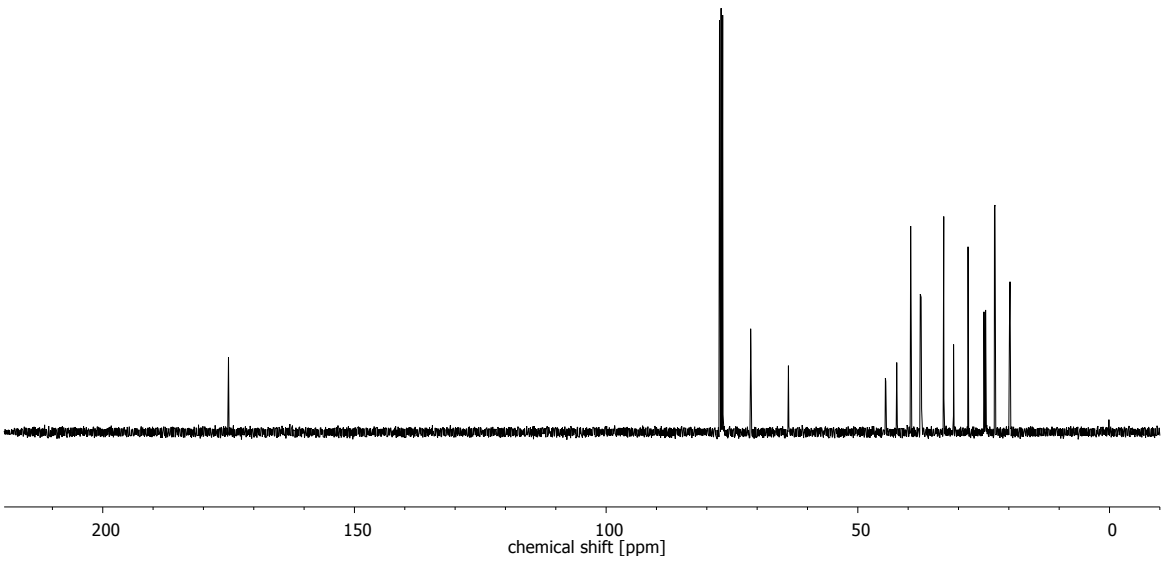
^{1}H-NMR spectrum of **3b** (400 MHz, $CDCl_3$, 25°C)



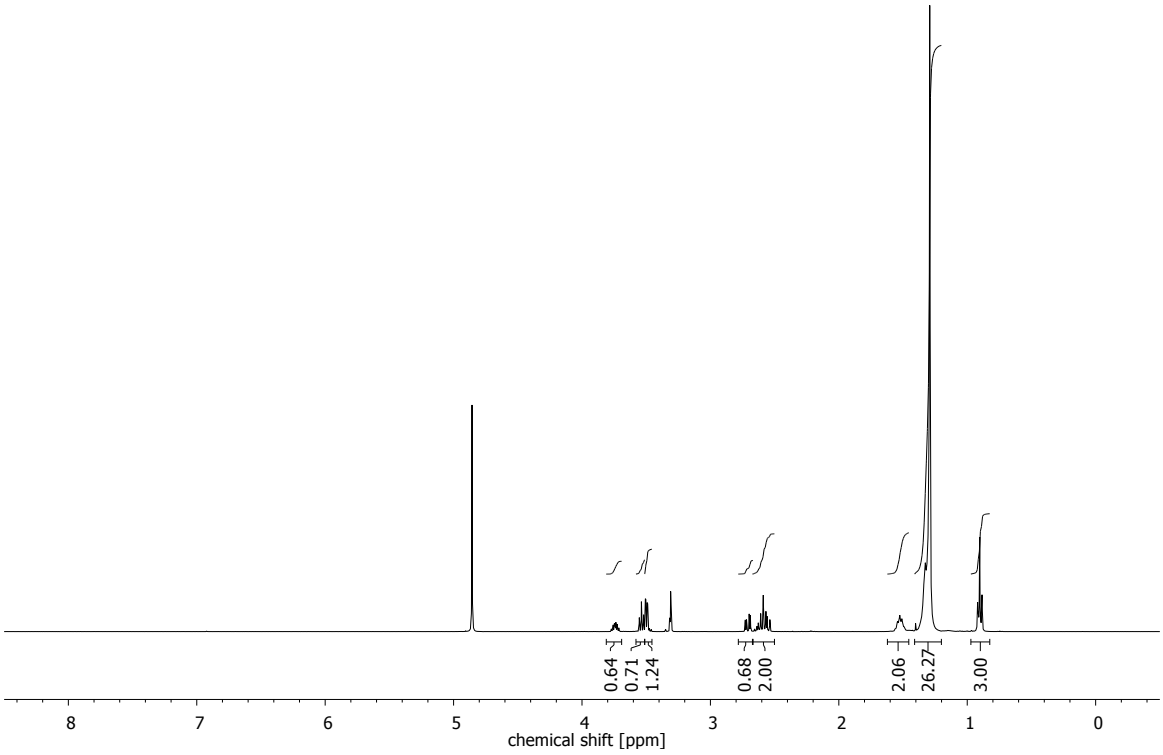
^{13}C-NMR spectrum of **3b** (101 MHz, $CDCl_3$, 25°C)



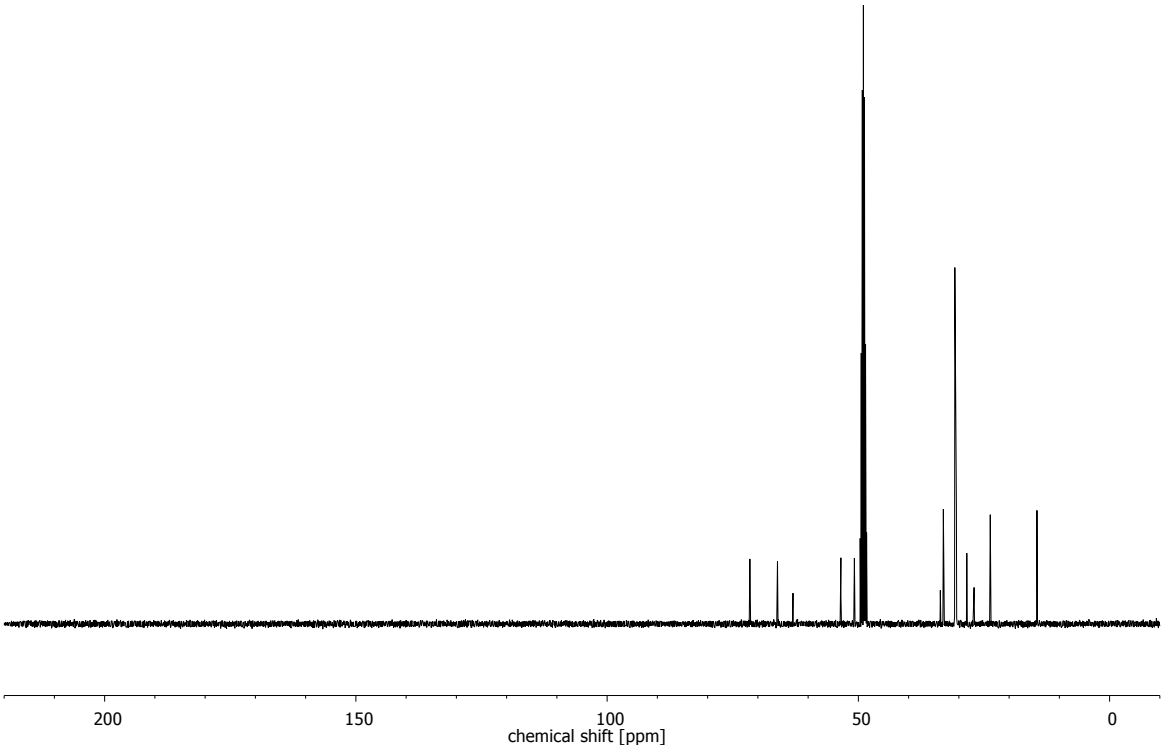
^{1}H-NMR spectrum of **3c** (400 MHz, $CDCl_3$, 25°C)



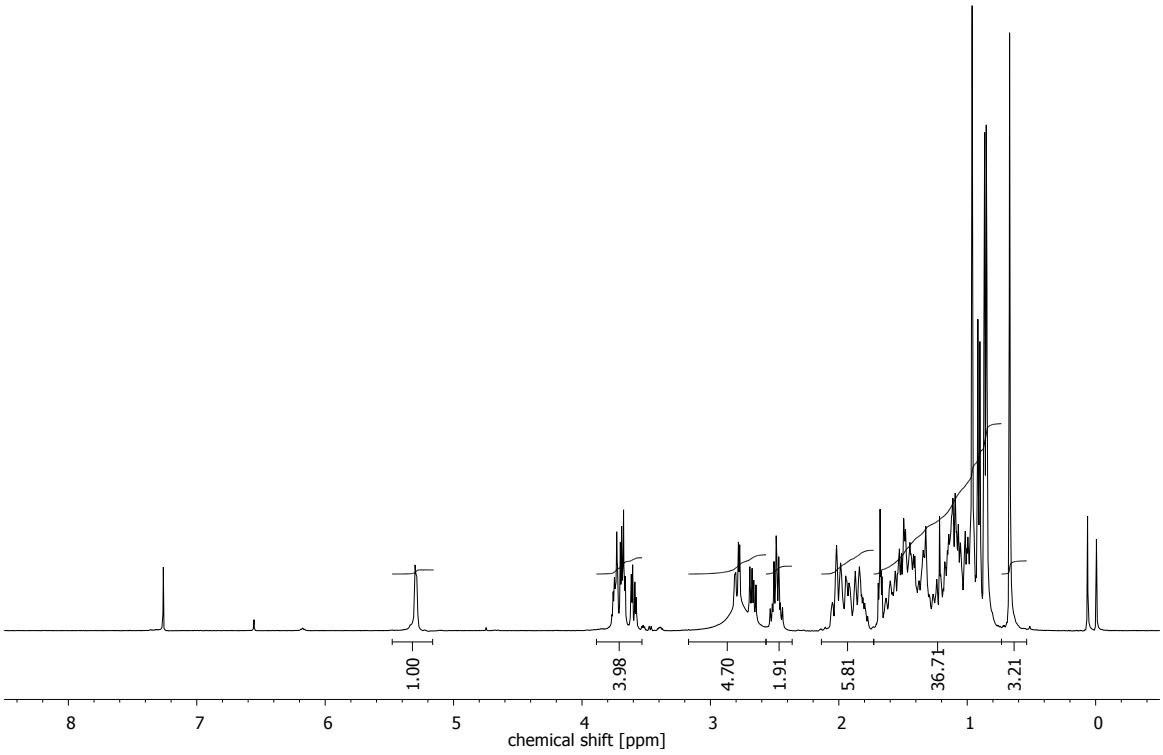
^{13}C-NMR spectrum of **3c** (101 MHz, $CDCl_3$, 25°C)



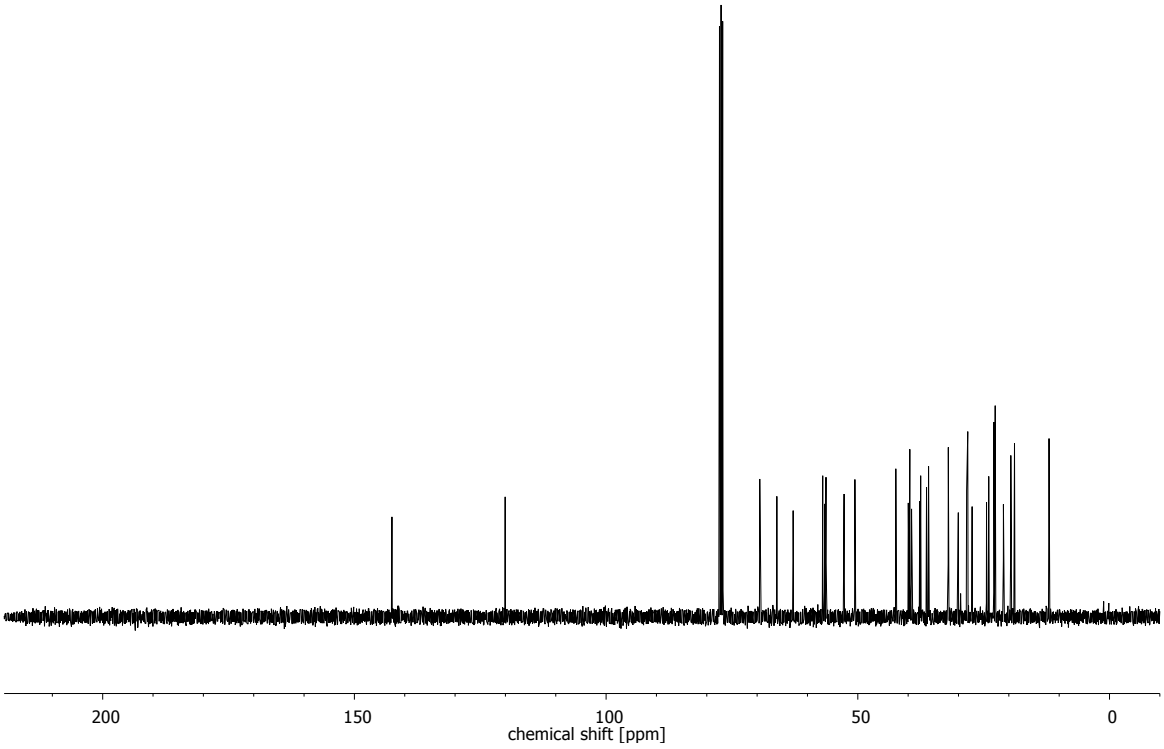
^{1}H-NMR spectrum of **4a** (400 MHz, CD_3OD, 25°C)



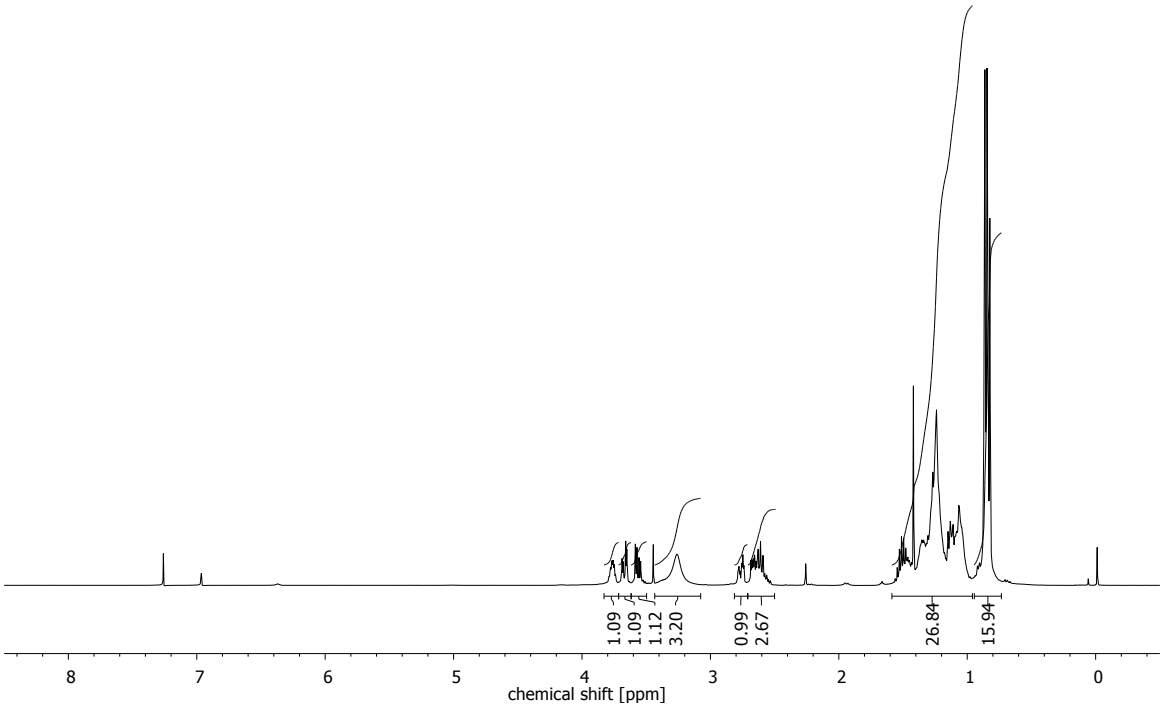
^{13}C-NMR spectrum of **4a** (101 MHz, CD_3OD, 25°C)



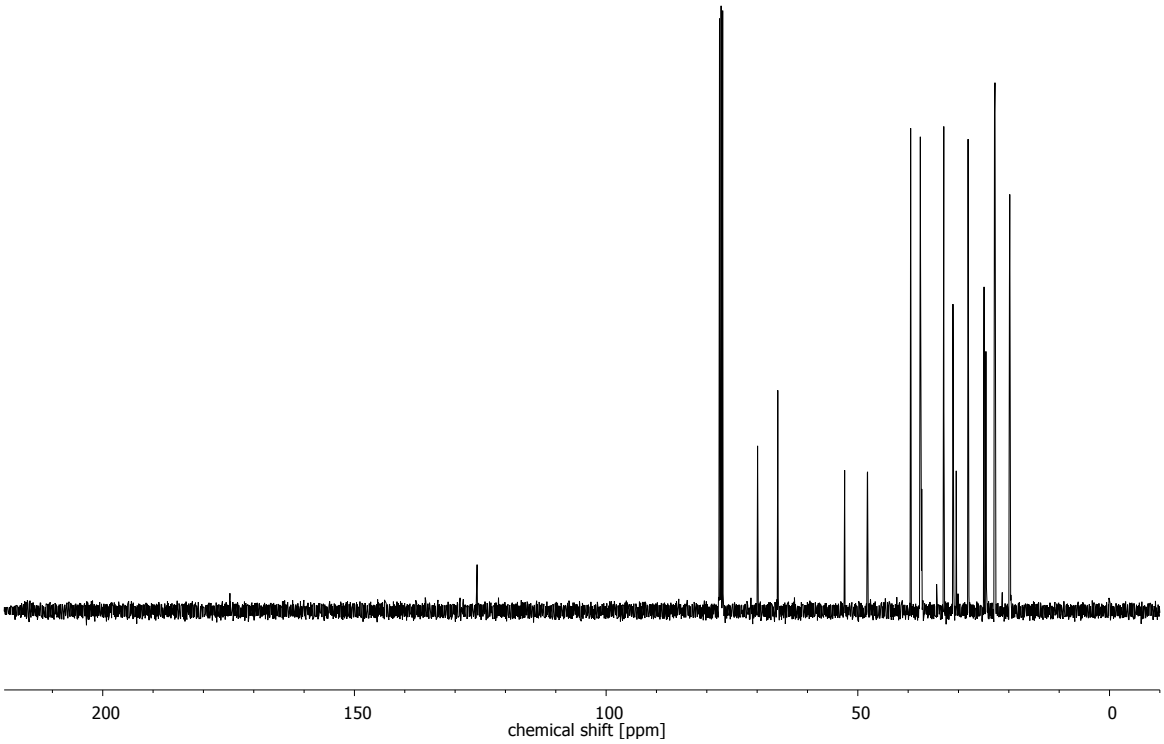
^{1}H-NMR spectrum of **4b** (400 MHz, $CDCl_3$, 25°C)



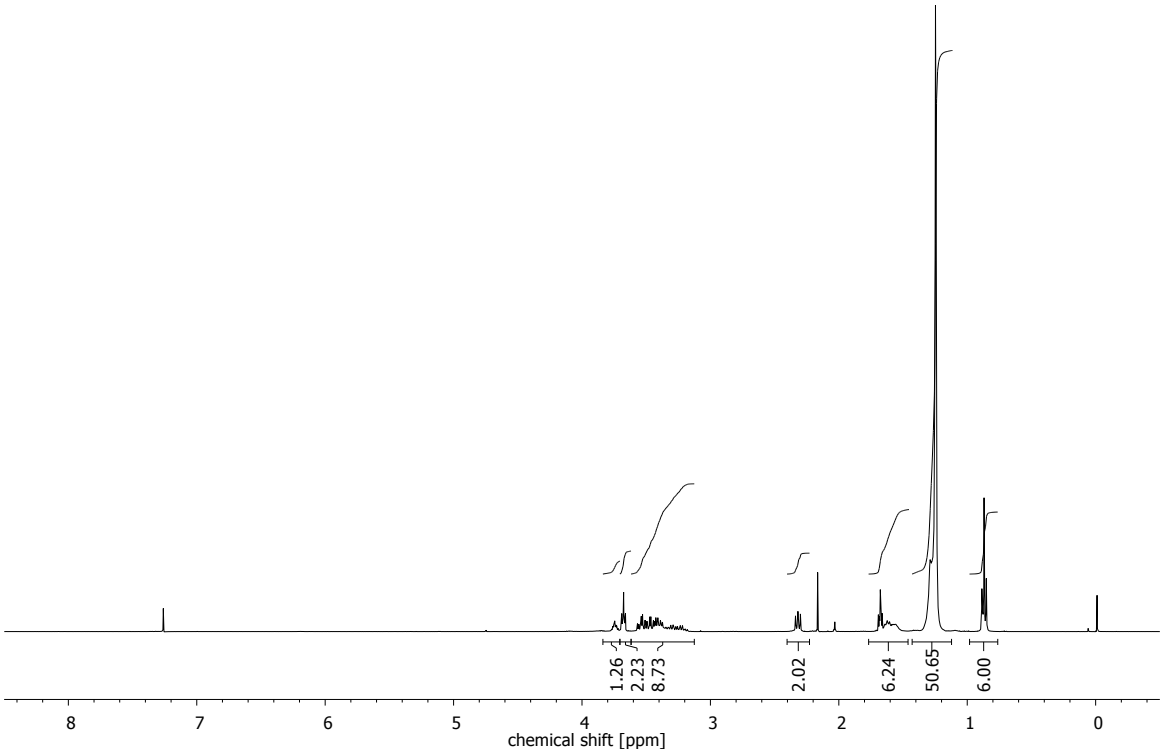
^{13}C-NMR spectrum of **4b** (101 MHz, $CDCl_3$, 25°C)



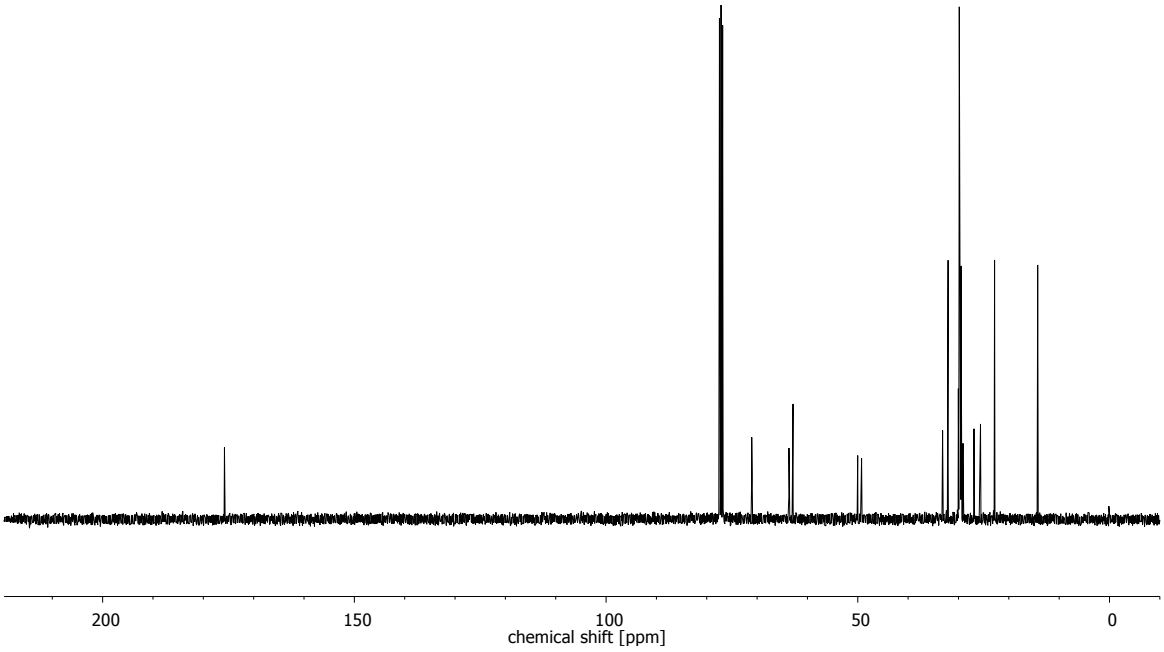
^{1}H-NMR spectrum of **4c** (400 MHz, CD_3OD $CDCl_3$, 25°C)



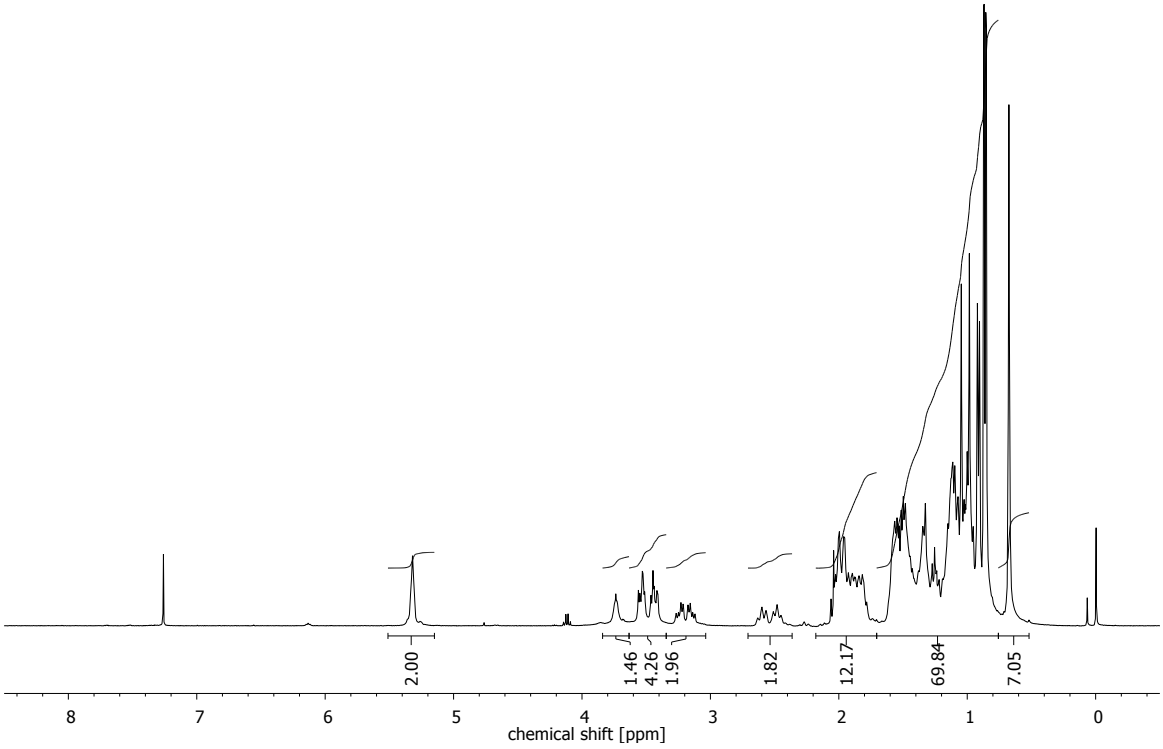
^{13}C-NMR spectrum of **4c** (101 MHz, CD_3OD $CDCl_3$, 25°C)



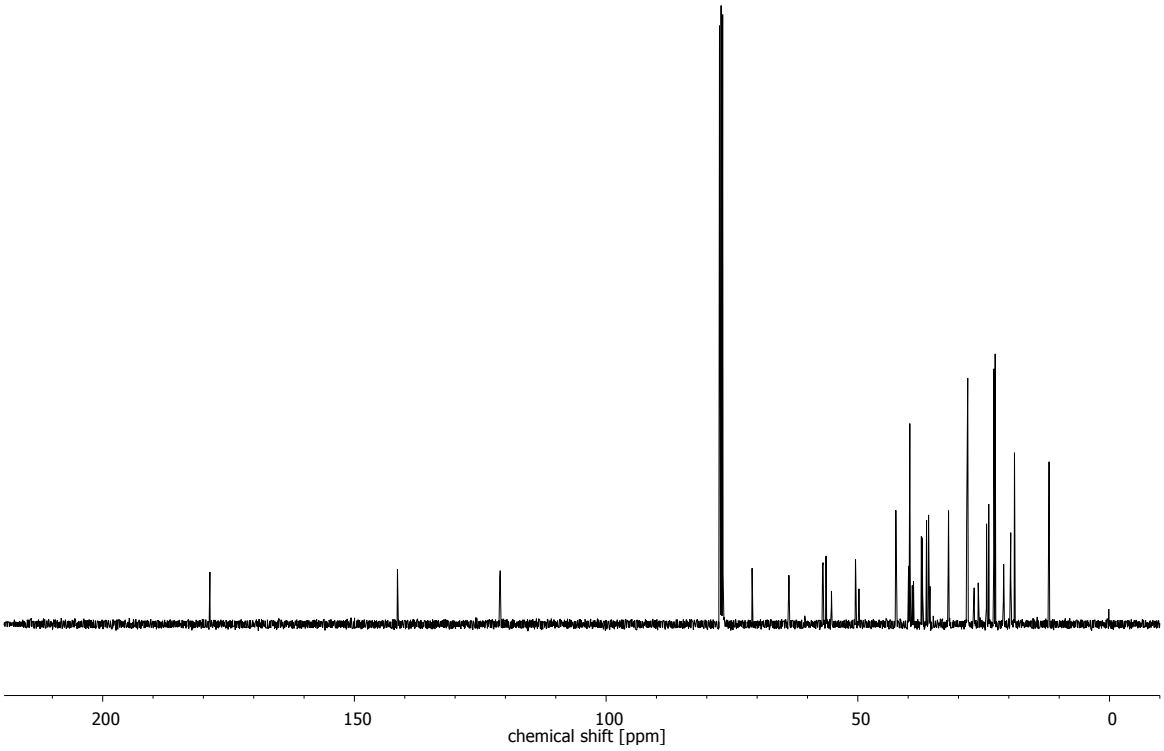
^{1}H-NMR spectrum of **5a** (400 MHz, $CDCl_3$, 25°C)



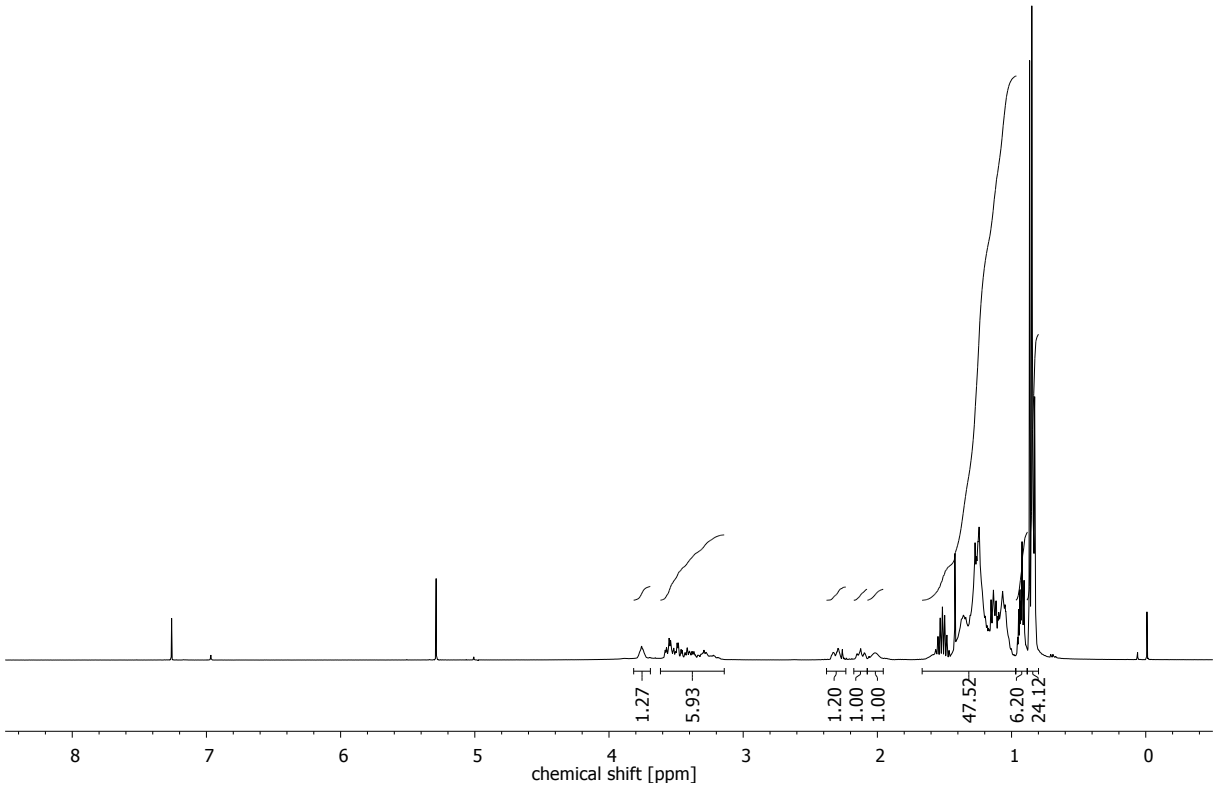
^{13}C-NMR spectrum of **5a** (101 MHz, $CDCl_3$, 25°C)



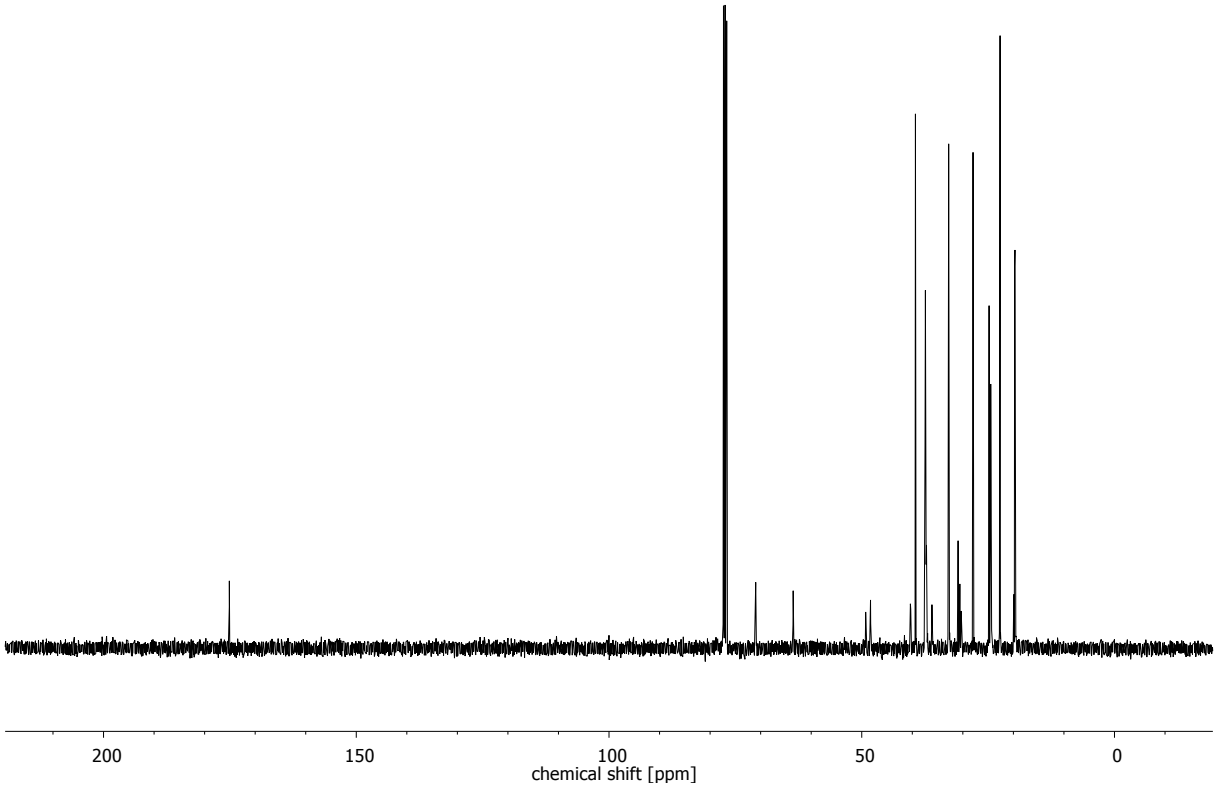
^{1}H-NMR spectrum of **5b** (400 MHz, $CDCl_3$, 25°C)



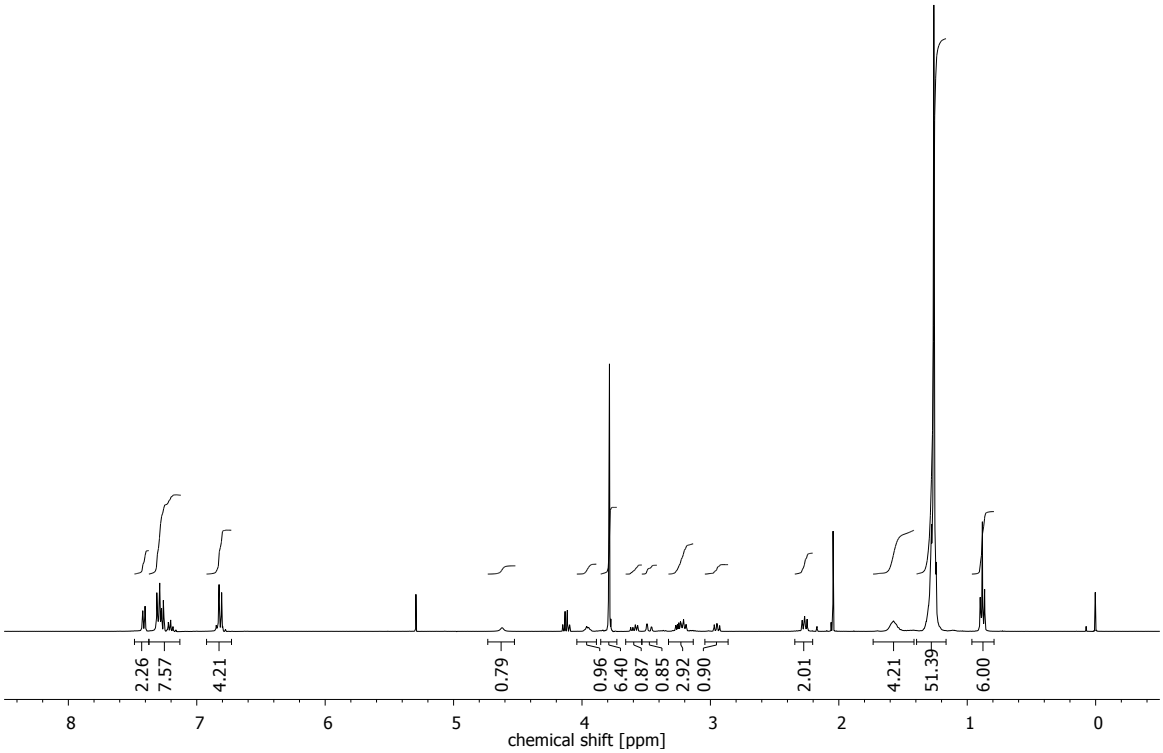
^{13}C-NMR spectrum of **5b** (101 MHz, $CDCl_3$, 25°C)



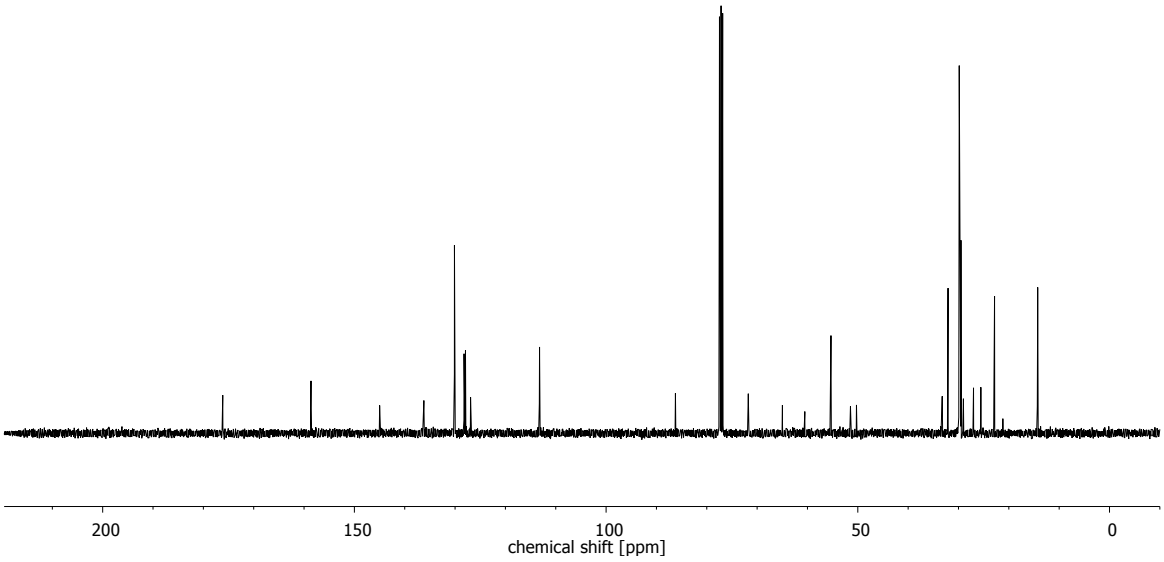
^{1}H-NMR spectrum of **5c** (400 MHz, $CDCl_3$, 25°C)



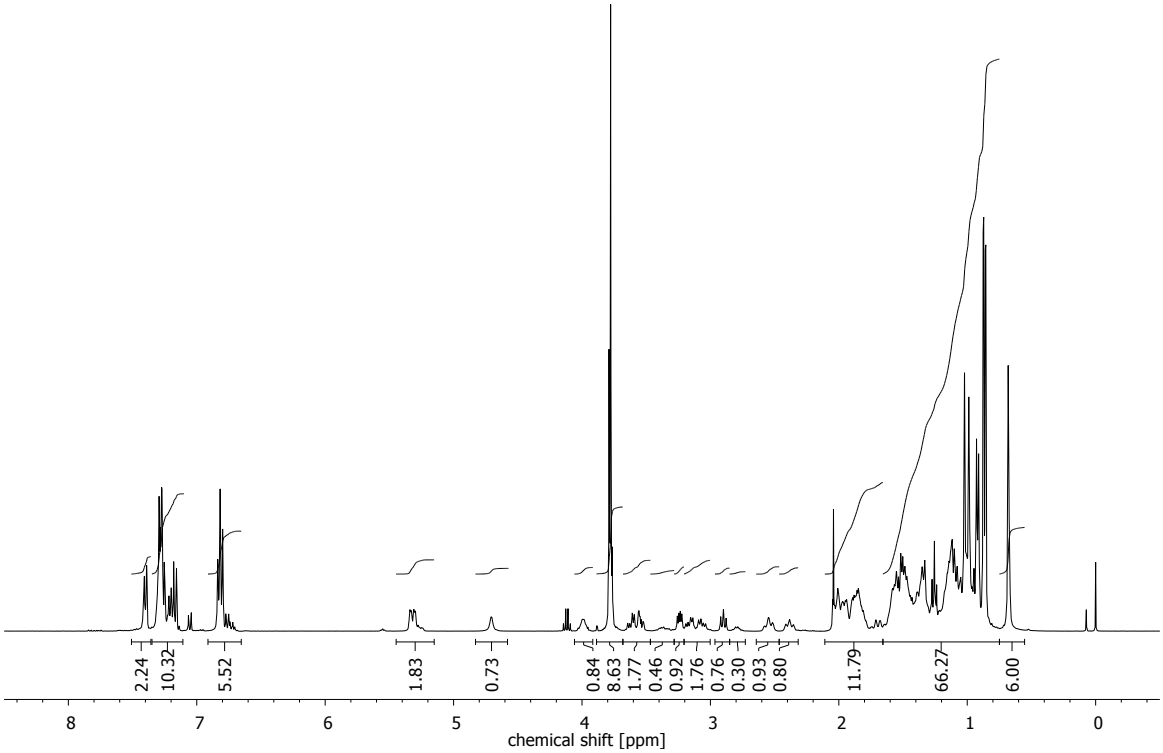
^{13}C-NMR spectrum of **5c** (101 MHz, $CDCl_3$, 25°C)



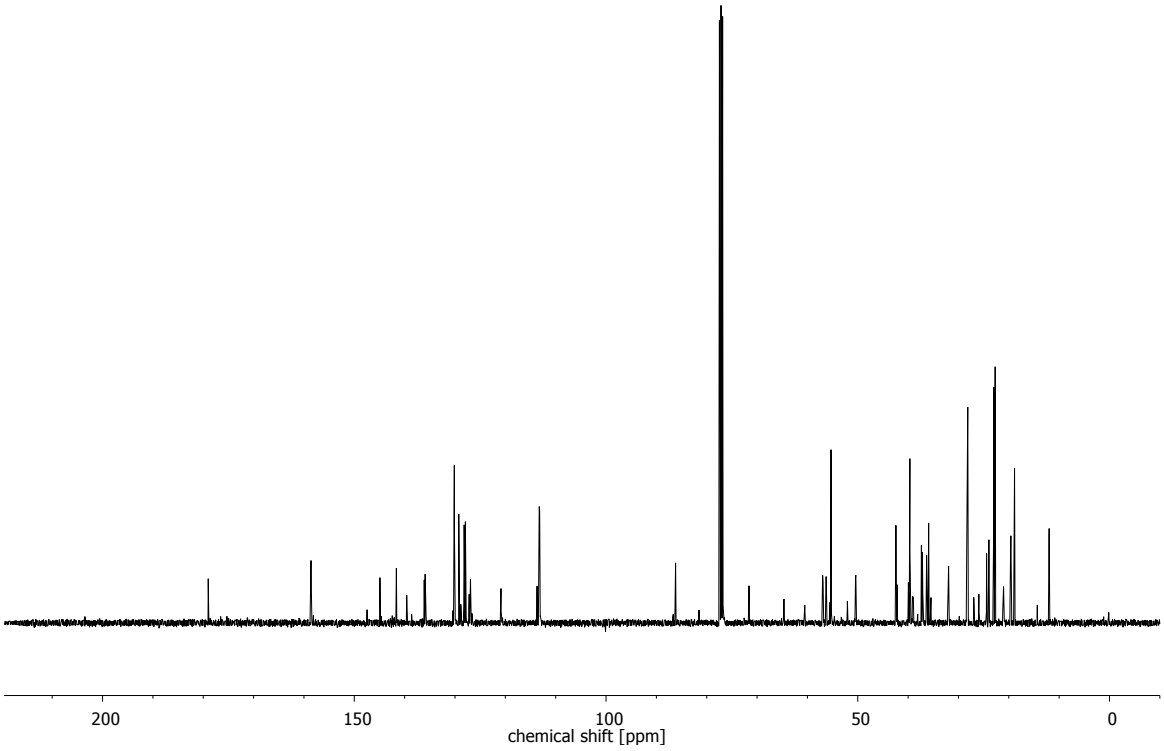
^{1}H-NMR spectrum of **6a** (400 MHz, $CDCl_3$, 25°C)



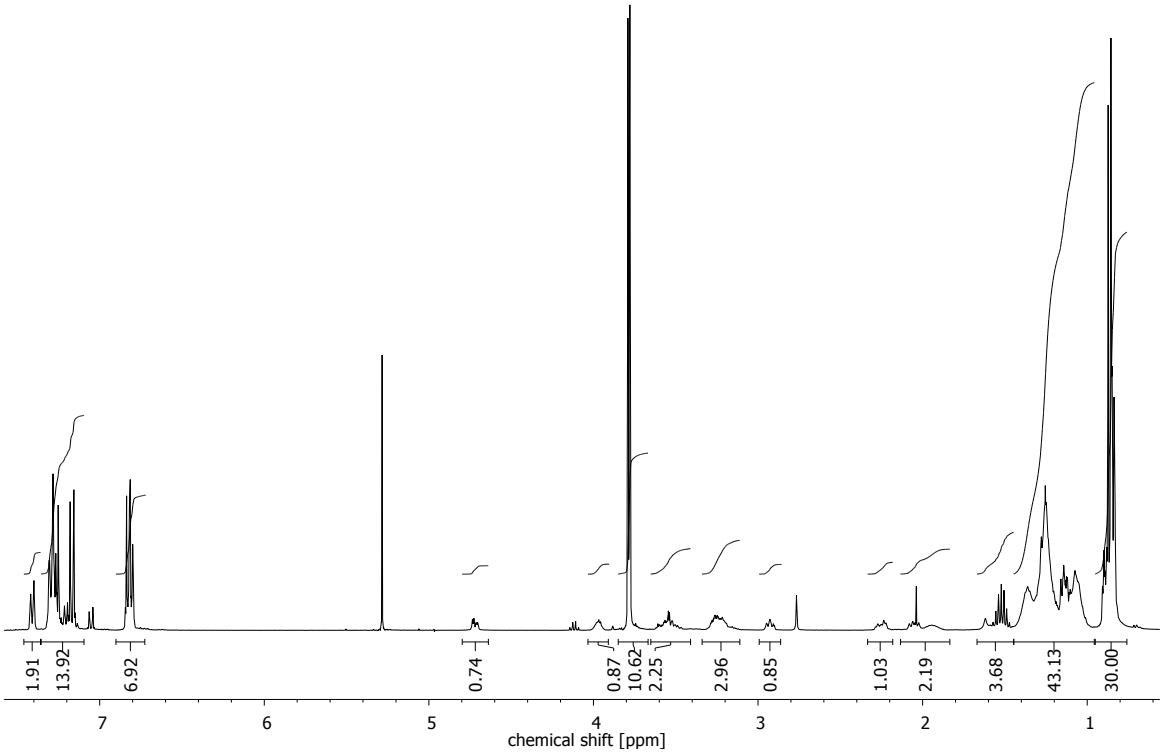
^{13}C-NMR spectrum of **6a** (101 MHz, $CDCl_3$, 25°C)



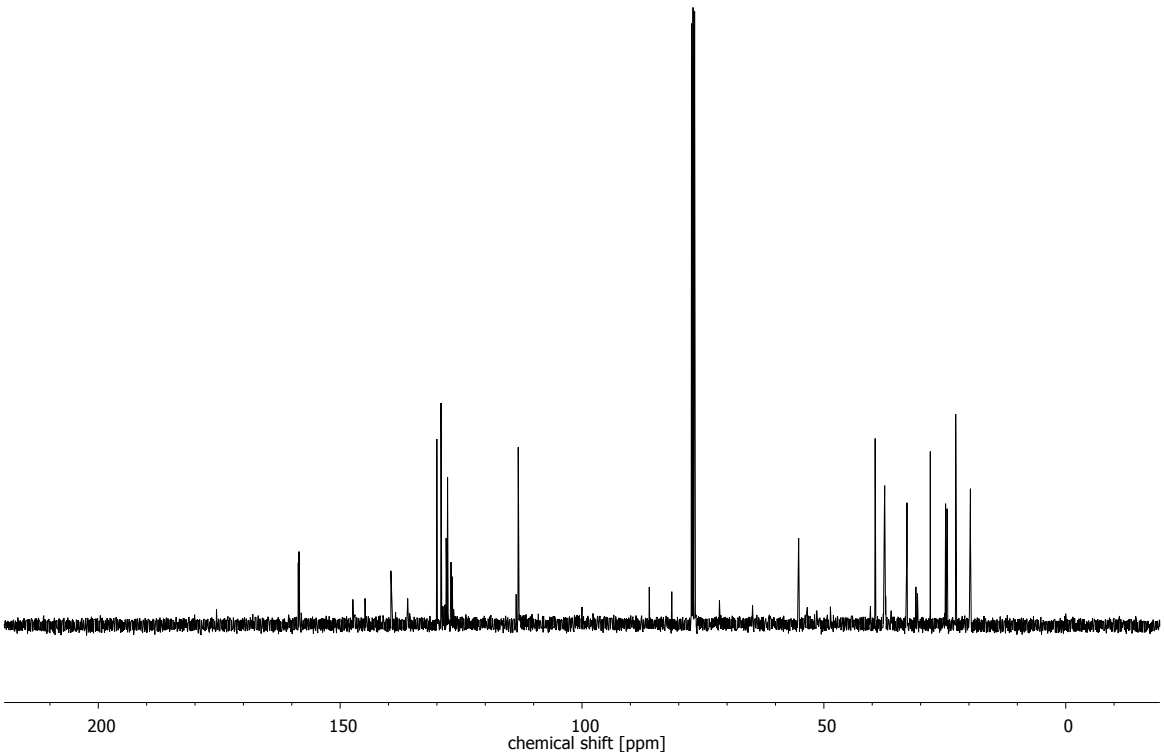
^{1}H-NMR spectrum of **6b** (400 MHz, $CDCl_3$, 25°C)



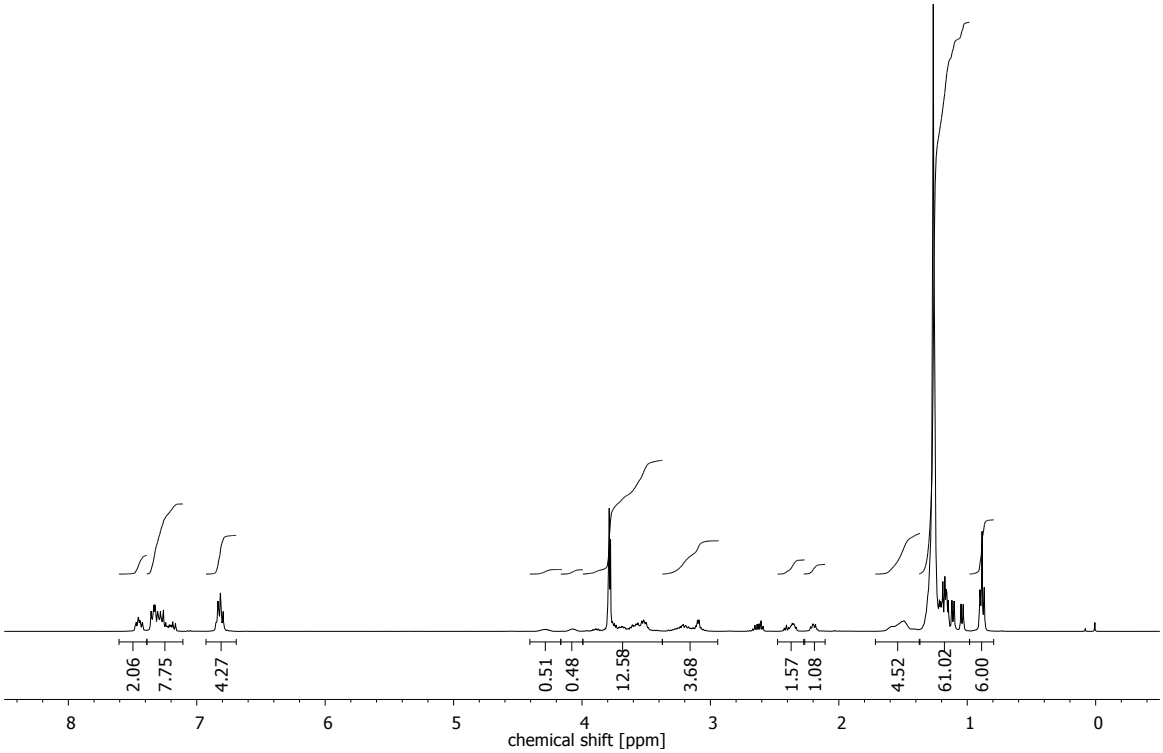
^{13}C-NMR spectrum of **6b** (101 MHz, $CDCl_3$, 25°C)



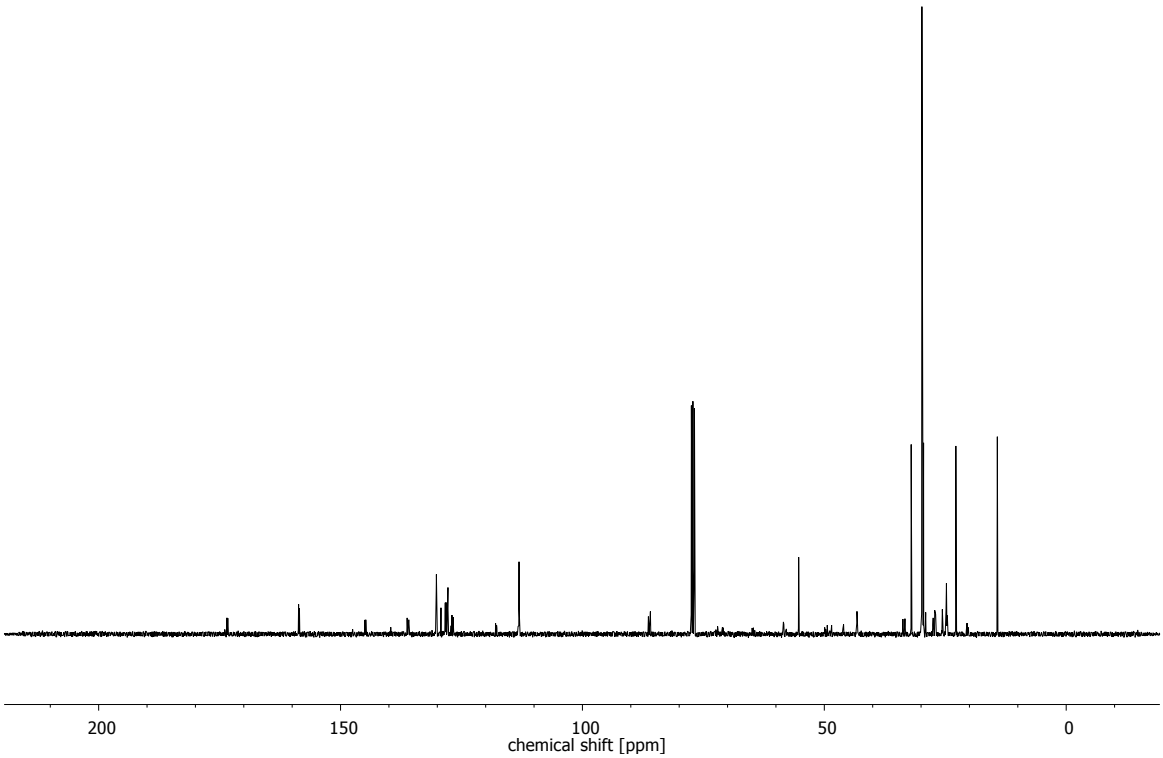
^{1}H-NMR spectrum of **6c** (400 MHz, CD_3OD $CDCl_3$, 25°C)



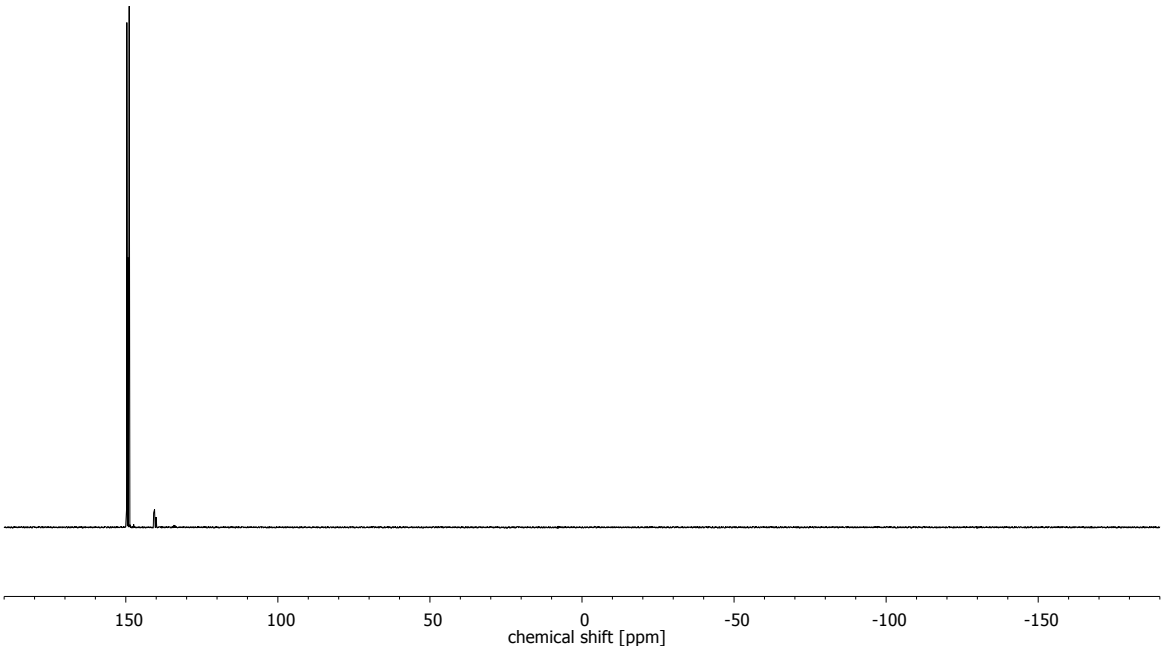
^{13}C-NMR spectrum of **6c** (101 MHz, CD_3OD $CDCl_3$, 25°C)



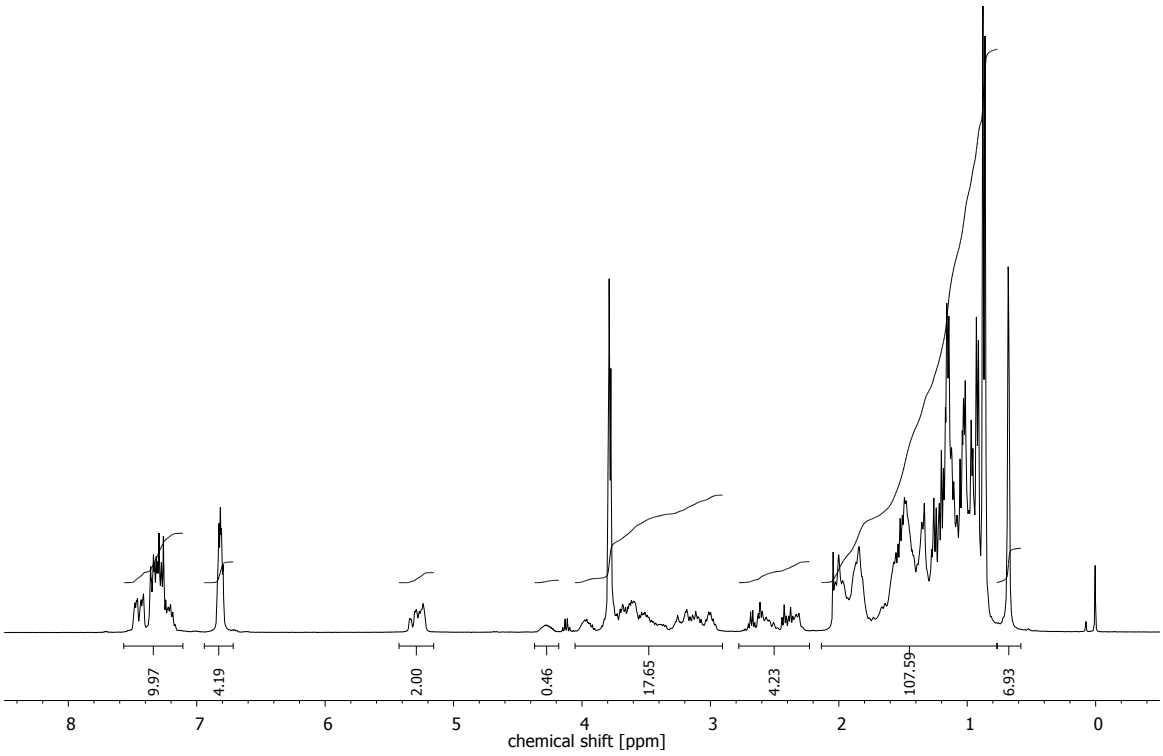
^{1}H-NMR spectrum of **1a** (400 MHz, $CDCl_3$, 25°C)



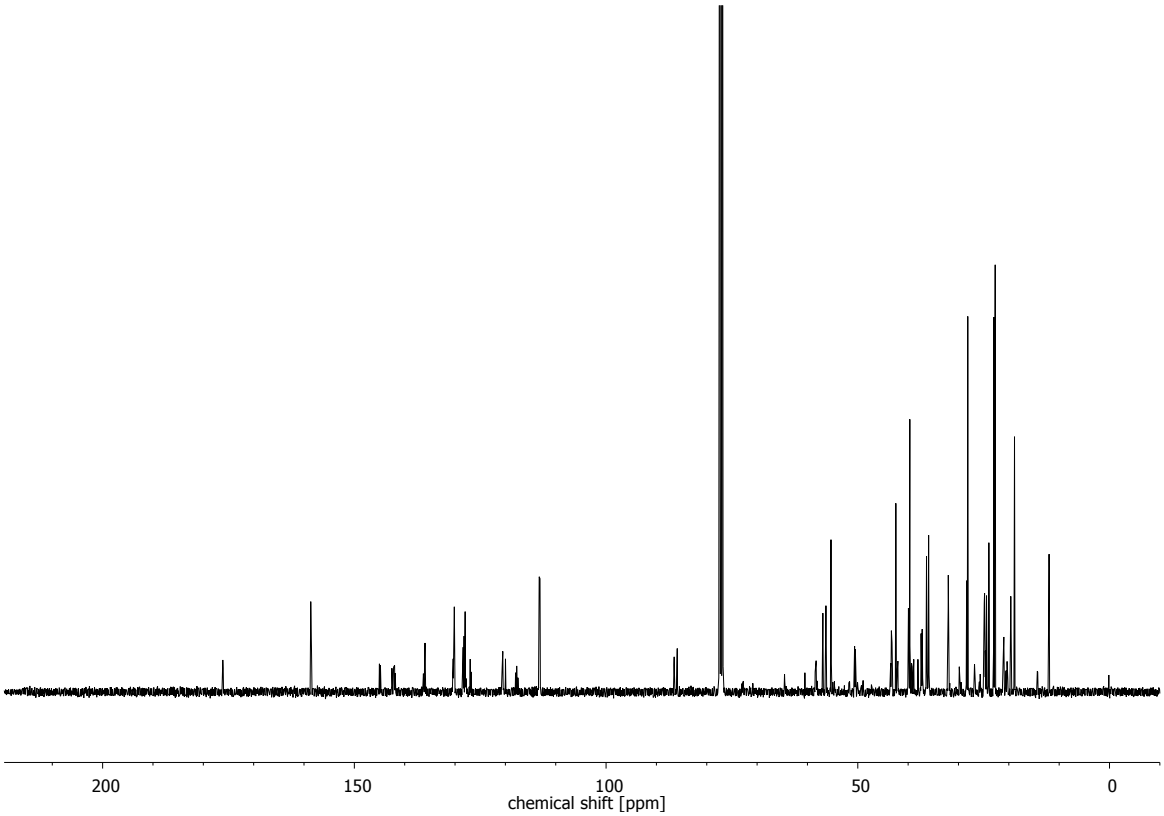
^{13}C-NMR spectrum of **1a** (101 MHz, $CDCl_3$, 25°C)



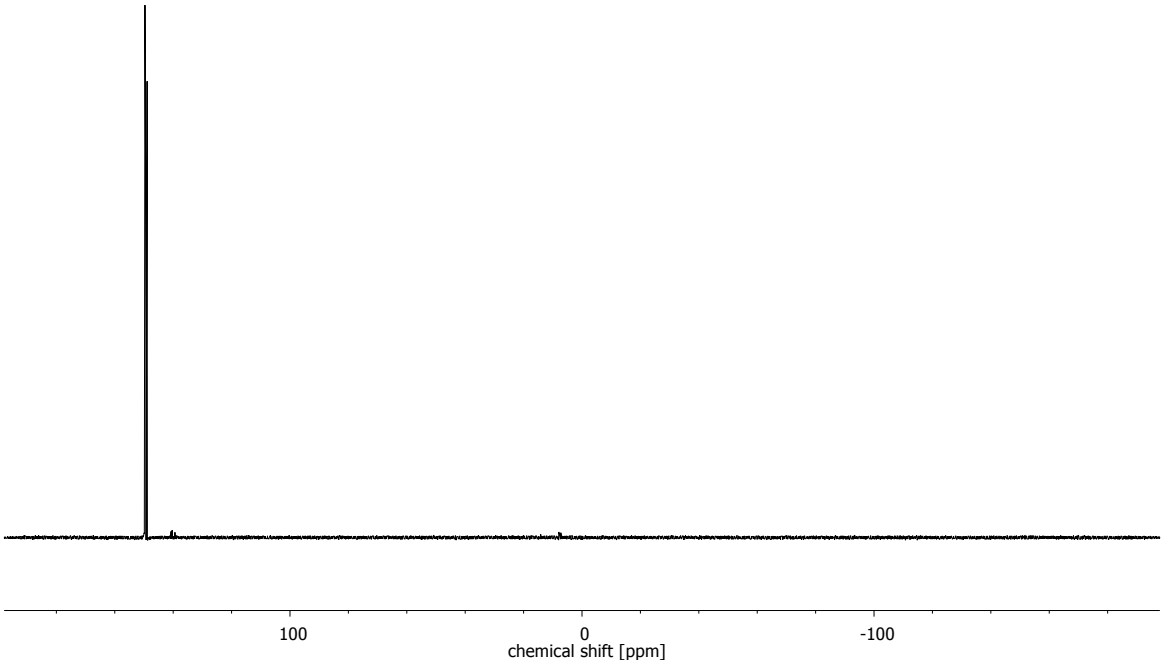
^{31}P-NMR spectrum of **1a** (162 MHz, $CDCl_3$, 25°C)



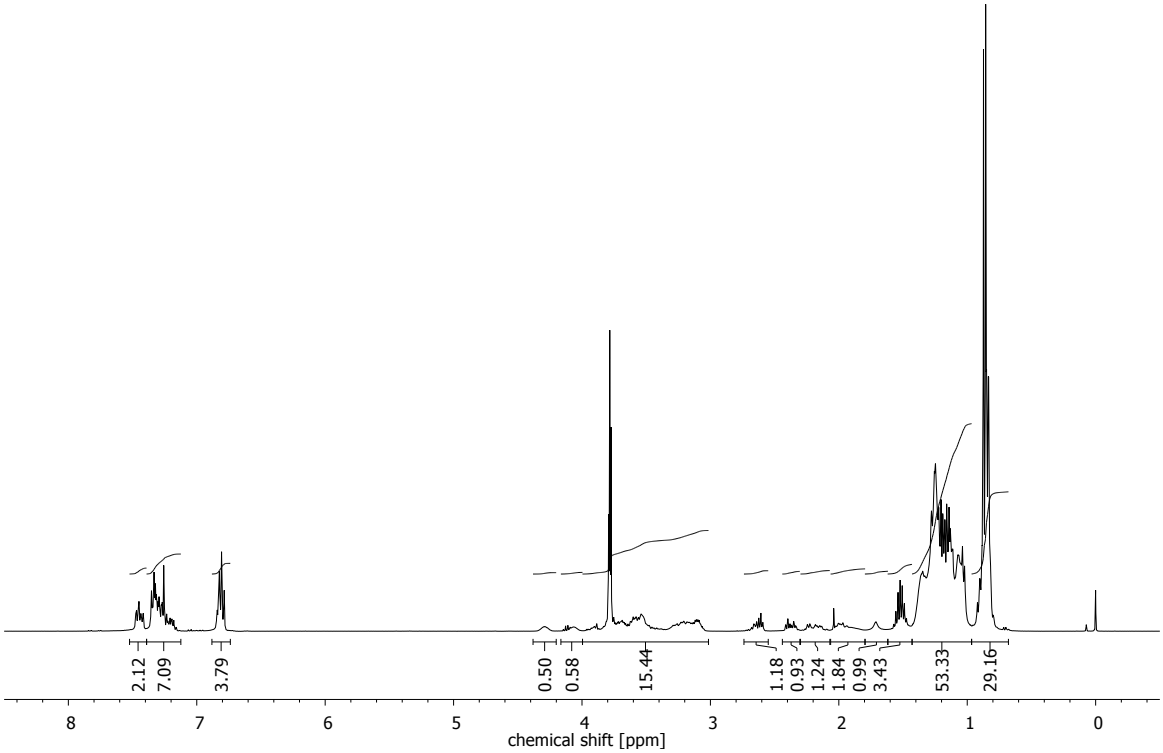
^{1}H-NMR spectrum of **1b** (400 MHz, $CDCl_3$, 25°C)



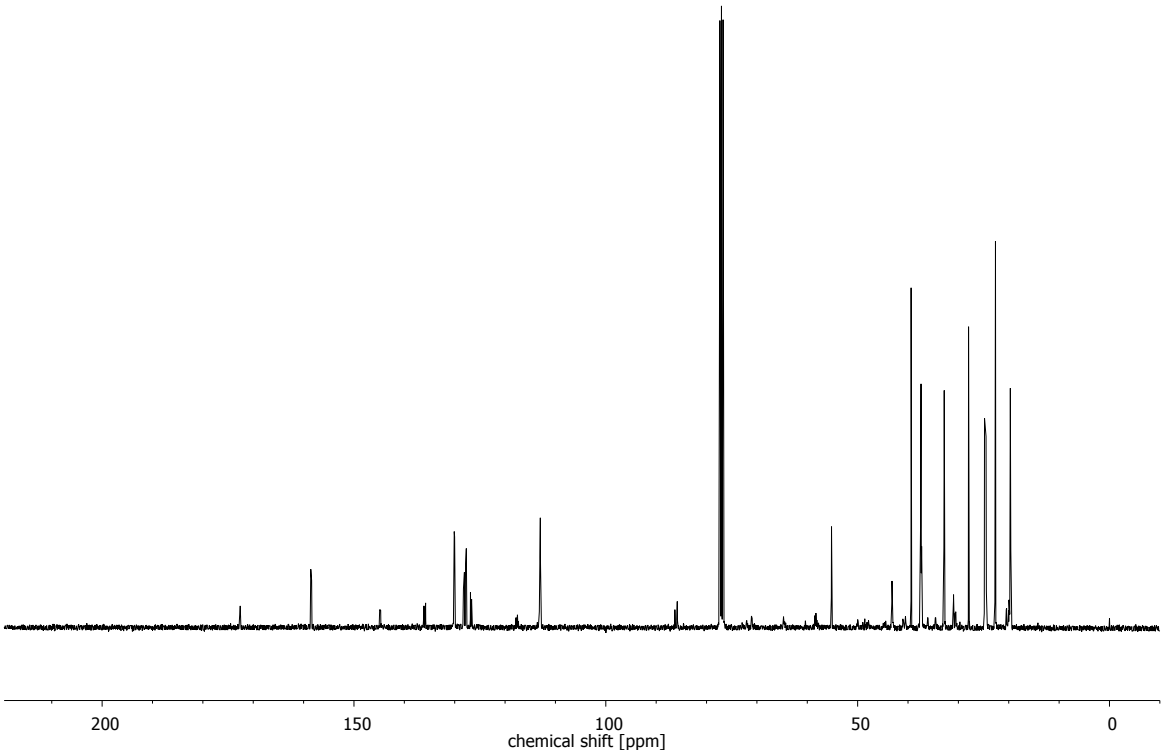
^{13}C-NMR spectrum of **1b** (101 MHz, $CDCl_3$, 25°C)



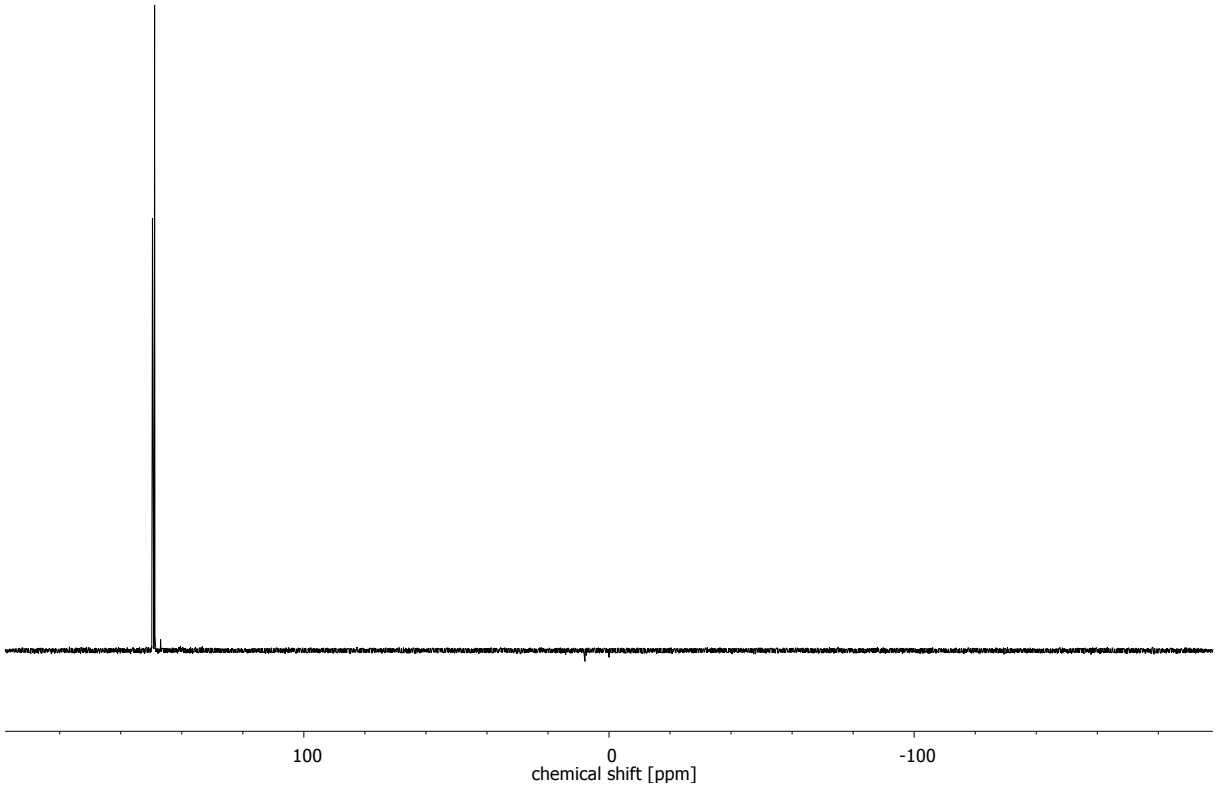
^{31}P-NMR spectrum of **1b** (162 MHz, $CDCl_3$, 25°C)



^{1}H-NMR spectrum of **1c** (400 MHz, CD_3OD $CDCl_3$, 25°C)



^{13}C-NMR spectrum of **1c** (101 MHz, CD_3OD $CDCl_3$, 25°C)



^{31}P-NMR spectrum of **1c** (162MHz, $CDCl_3$, 25°C)

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