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Supporting Information

Total Synthesis of Dansylated Park's Nucleotide for High-Throughput MraY Assays

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Syntheses of precursor compounds

General methods. Compounds **8**,[S1] **9**,[S2] **22**[S3] and **S3**[S4] were prepared according to established procedures. All other chemicals were purchased from standard suppliers. High pressure hydrogenation reactions were carried out with a Parr hydrogenation apparatus. Reactions involving oxygen- and/or moisture-sensitive reagents were carried out under an atmosphere of argon using anhydrous solvents. Anhydrous solvents were obtained in the following manner: THF was dried over sodium/benzophenone and distilled, CH_2Cl_2 was dried over CaH_2 and distilled, MeOH was dried over activated molecular sieves (3 Å) and degassed, MeCN was dried over P_2O_5 and distilled, pyridine was dried over CaH_2 and distilled, DMF was dried over activated molecular sieves (4 Å) and degassed. All other solvents were of technical quality and distilled prior to their use, and deionized water was used throughout. Column chromatography was carried out on silica gel 60 (0.040-0.063 mm, 230-400 mesh ASTM, VWR) under flash conditions. TLC was performed on aluminium plates precoated with silica gel 60 F_{254} (VWR). Visualization of the spots was carried out using UV light (254 nm and 366 nm) and/or staining under heating (H_2SO_4 staining solution: 4 g vanillin, 25 mL conc. H_2SO_4, 80 mL AcOH and 680 mL MeOH; $KMnO_4$ staining solution: 1 g $KMnO_4$, 6 g K_2CO_3 and 1.5 mL 1.25 M NaOH solution, all dissolved in 100 mL H_2O; ninhydrin staining solution: 0.3 g ninhydrin, 3 mL AcOH and 100 mL 1-butanol). 300 MHz- and 500 MHz-^{1}H and 75 MHz- and 126 MHz-^{13}C as well as 282 MHz-^{19}F NMR and 121 MHz-^{31}P NMR spectra were recorded on Varian MERCURY 300, UNITY 300, INOVA 500, Bruker AVANCE 300 and AVANCE 500 spectrometers. All ^{13}C, ^{19}F and ^{31}P NMR spectra are ^{1}H-decoupled. All spectra were recorded at room temperature except of samples in DMSO-d_6 and D_2O (standard 35°C) and where indicated otherwise and were referenced internally to solvent reference frequencies wherever possible. Chemical shifts (δ) are quoted

in ppm, and coupling constants (*J*) are reported in Hz. Assignment of signals was carried out using ^{1}H,^{1}H-COSY, HSQC and HMBC spectra obtained on the spectrometers mentioned above. Low resolution ESI mass spectrometry was performed on a Varian MAT 311 A spectrometer operating in positive ionization mode. High resolution (HR) ESI mass spectrometry was carried out on a Bruker microTOF spectrometer or a Bruker 7 T FTICR APEX IV spectrometer. Melting points (mp) were measured on a Büchi instrument and are not corrected. Optical rotations were recorded on a Perkin-Elmer polarimeter 241 with a Na source using a 10 cm cell (concentrations in g/100 mL). Infrared spectroscopy (IR) was performed on a Perkin-Elmer Vektor 22 spectrometer with solids being measured as KBr pills or on a Jasco FT/IR-4100 spectrometer equipped with an integrated ATR unit (GladiATR™, PIKE Technologies). Wavenumbers (ν) are quoted in cm^{-1}. UV spectroscopy was carried out on a Perkin-Elmer Lambda 2 or on a Jasco V-630 spectrometer. Wavelengths of maximum absorption (λ_{max}) are reported in nm with the corresponding logarithmic molar extinction coefficient (log ε, $\varepsilon/dm^3\ mol^{-1}\ cm^{-1}$) given in parenthesis.

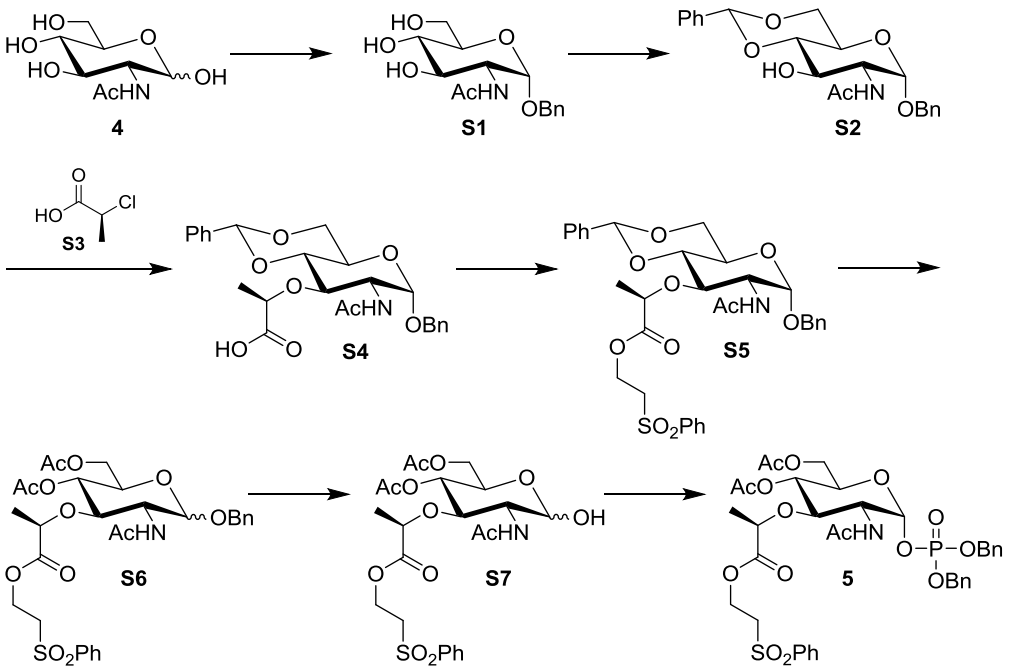
HPLC methods. Analytical HPLC was performed on a VWR-Hitachi system equipped with an L-2300 pump, an L-2200 autosampler, an L-2300 column oven (24 °C), an L-2455 Diode Array Detector (DAD) and a LiChroCart™ column (4 x 125 mm) containing reversed phase silica gel Purospher™ RP18e (5 µm) purchased from VWR. Method: eluent A water (50 mM HOAc-NEt_3 (1:1)); eluent B MeCN; 0-5 min gradient of B (10-30%), 5-25 min gradient of B (30-70%), 25-30 min gradient of B (70-100%), 30-35 min 100% B, 35-40 min gradient of B (100-10%), 40-45 min 10% B; flow 0.5 mL/min.

Semi-preparative HPLC was carried out on a VWR-Hitachi system equipped with an L-2300 pump, an L-2200 autosampler, an L-2300 column oven (24 °C), an L-2455 Diode Array Detector (DAD), an L-2485 Fluorescence Detector (FLD), and a LiChroCart™ column

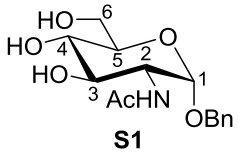
(10 x 250 mm) containing reversed phase silica gel Purospher™ RP18e (5 µm) purchased from VWR. Method: eluent A water (10 mM HOAc-NEt_3 (1:1)), eluent B MeCN; 0-20 min gradient of B (10-30%), 20-20.1 min gradient of B (30-100%), 20.1-25 min 100% B, 25-25.1 min gradient of B (100-10%), 25.1-35 min 10% B; flow 5 mL/min. This method was used for the purification of the crude product of semi-synthetically obtained **3**.

Preparative HPLC was carried out on a Jasco system equipped with a DG-2080-53 degasser, two PU-2080 Plus pumps, a UV-2075 Plus UV/Vis detector (detection at 260 nm) and a LiChroCart™ column (20 x 250 mm) containing reversed phase silica gel Purospher™ RP18e (10 µm) purchased from VWR. Method: eluent A water (50 mM HOAc-NEt_3 (1:1)), eluent B MeCN; 0-20 min 20% B, 20-25 min gradient of B (20-100%), 25-35 min 100% B, 35-40 min gradient of B (100-10%); flow 15 mL/min. This method was used for a first purification of the crude product of synthetically obtained **3**. Subsequently, pure **3** was obtained by preparative HPLC carried out on a Hitachi system equipped with an L-7150 pump, an L-7614 mixer, an L-7400 detector, and a column (21 x 250 mm) containing reversed phase silica gel Nucleodur™ 100-10 C18ec (10 µm) purchased from Macherey-Nagel. Method: eluent A water (50 mM HOAc-NEt_3 (1:1)), eluent B MeCN; 0-5 min gradient of B (10-30%), 5-25 min gradient of B (30-70%), 25-30 min gradient of B (70-100%), 30-35 min 100% B, 35-40 min gradient of B (100-10%), 40-45 min 10% B; flow 10 mL/min.

Synthesis of Mur*N*Ac phosphate derivative 5. The synthesis of **5** from *N*-acetylglucosamine **4**, based on the route reported by Hitchcock et al.,[S5] is summarized in the scheme given below.



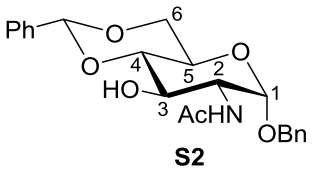
1-*O*-Benzyl-*N*-acetyl-α-D-glucosamine (S1)



To a suspension of *N*-acetylglucosamine **4** (10.0 g, 45.2 mmol, 1.0 eq.) in benzyl alcohol (125 mL), acetyl chloride (10.9 mL, 12.0 g, 152 mmol, 3.4 eq.) was added dropwise at 0 °C. The reaction mixture was stirred for 30 min at rt and further stirred for 4 h at 70 °C. At 0 °C, $NaHCO_3$ was added until pH 7 was achieved. The suspension was filtered through a short pad of Celite™ and further washed with MeOH (200 mL). The solvent was removed under reduced pressure and diethyl ether (200 mL) was added to the residue. The precipitate was filtered and dried *in vacuo*. Recrystallization from EtOH yielded **S1** (12.1 g, 86%) as a

colorless solid. $[\alpha]_D^{25}$ = +115.5 (c = 0.53, MeOH); ^{1}H NMR (300 MHz, CD_3OD): δ = 7.39-7.24 (m, 5H, Ar-H), 4.84 (d, *J* = 3.6 Hz, 1H, H-1), 4.74 (d, *J* = 12.0 Hz, 1H, Bn-CH_2), 4.49 (d, *J* = 12.0 Hz, 1H, Bn-CH_2), 3.89 (dd, *J* = 10.7 Hz, *J* = 3.6 Hz, 1H, H-2), 3.84-3.81 (m, 1H, H-3), 3.74-3.63 (m, 3H, H-4, H-5, H-6_a), 3.40-3.34 (m, 1H, H-6_b), 1.94 (s, 3H, acetyl-CH_3) ppm; ^{13}C NMR (75 MHz, CD_3OD): δ = 173.65 (acetyl-C=O), 138.98 (Ar-C), 129.36 (Ar-CH), 129.23 (Ar-CH), 128.80 (Ar-CH), 97.44 (C-1), 74.05 (C-4), 72.63 (C-3), 72.36 (C-6), 70.07 (Bn-CH_2), 62.69 (C-5), 55.36 (C-2), 22.53 (acetyl-CH_3) ppm; IR: *v* = 3297, 3029, 2934, 1650, 1636, 1552, 1454, 1377, 1122, 1091, 1038, 734, 695 cm^{-1}; MS (HR-ESI): *m/z*: calcd for 334.1261 $[M+Na]^+$; found: 334.1266.

1-*O*-Benzyl-4,6-*O*-benzylidene-*N*-acetyl-α-D-glucosamine (S2)[S6]

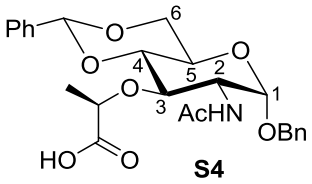


S2

1-*O*-benzyl-*N*-acetyl-α-D-glucosamine **S1** (1.03 g, 3.21 mmol, 1.0 eq.) was coevaporated with dry EtOH (2 mL) and dry toluene (6.5 mL). Starting material **S1** (998 mg, 3.20 mmol) was then dissolved in dry DMF (3 mL) and dry dioxane (3 mL), and triethyl orthoformate (1.60 mL, 1.24 g, 9.60 mmol, 3.0 eq.), benzaldehyde (1.30 mL, 1.37 g, 12.9 mmol, 4.0 eq.) and *p*-toluenesulfonic acid (166 mg, 0.96 mmol, 0.3 eq.) were added subsequently. The reaction mixture was stirred for 20 h at rt. Et_2O (8 mL) was added to the suspension and stirred for 1 h at 0 °C. The colorless solid was filtered off, washed with diethyl ether (20 mL) and dried *in vacuo* to yield **S2** (1.03 g, 80%) as a colorless solid. $[\alpha]_D^{25}$ = +86.0 (c = 1.1, DMSO); ^{1}H NMR (300 MHz, DMSO-d_6): δ = 7.95 (d, *J* = 8.2 Hz, 1H, NH), 7.47-7.28 (m, 10H, Ar-H), 5.62 (s, 1H, Ph-CH), 5.15 (d, *J* = 5.6 Hz, 1H, 3-OH), 4.83 (d, *J* = 3.2 Hz, 1H, H-1), 4.71 (d, *J* = 12.5 Hz, 1H, Bn-CH_2), 4.50 (d, *J* = 12.5 Hz, 1H, Bn-CH_2), 4.15 (d,

J = 4.9 Hz, 1H, H-6_a), 3.93-3.84 (m, 1H, H-2), 3.80-3.67 (m, 3H, H-3, H-5, H-6_b), 3.55-3.50 (m, 1H, H-4), 1.87 (s, 3H, acetyl-CH_3) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): δ = 169.17 (acetyl-C=O), 137.52 (Ar-C), 137.46 (Ar-C), 128.61 (Ar-CH), 128.00 (Ar-CH), 127.76 (Ar-CH), 127.41 (Ar-CH), 127.32 (Ar-CH), 126.16 (Ar-CH), 100.71 (Ph-CH), 96.83 (C-1), 81.98 (C-4), 68.53 (Bn-CH_2), 67.91 (C-6), 67.18 (C-3), 62.75 (C-5), 54.15 (C-2), 22.47 (acetyl-CH_3) ppm; IR: ν = 3425, 3292, 1650, 1556, 1453, 1372, 1130, 1090, 1040, 1022, 1001, 749, 734, 696 cm^{-1}; MS (HR-ESI): *m/z*: calcd for 422.1574 $[M+Na]^+$; found: 422.1583.

1-*O*-Benzyl-4,6-*O*-benzylidene-3-*O*-((*R*)-propion-2-yl)-*N*-acetyl-α-D-glucosamine (S4)[S7]

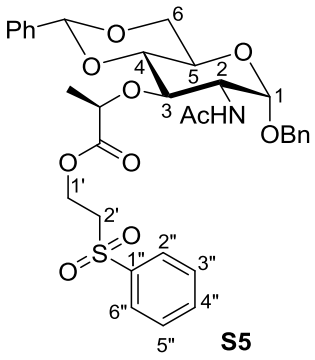


S4

The reaction was carried out under an inert atmosphere of argon. To a solution of 1-*O*-benzyl-4,6-*O*-benzylidene-*N*-acetyl-α-D-glucosamine **S2** (3.32 g, 8.31 mmol, 1.0 eq.) in dry dioxane (200 mL) was added NaH (60% dispersion in oil, 588 mg, 24.5 mmol, 2.9 eq.) at 60 °C. The reaction mixture was then heated under reflux for 5 min. (*S*)-2-chloropropionic acid **S3**[S4] (4.98 g, 41.7 mmol, 5.7 eq.) was added at 60 °C and the reaction mixture was stirred for 30 min. A second portion of NaH (60 % dispersion in oil, 2.35 g, 98.1 mmol, 12 eq.) was added and the mixture was stirred for 16 h at 60 °C. The reaction was quenched by adding ice water (8.3 mL). At 0 °C, the reaction mixture was acidified with ice-cold aq. HCl (6 M) until pH 2 and poured into ice water (400 mL). The precipitate was filtered off, washed with water (3 x 50 mL) and petroleum ether (2 x 50 mL) and dried *in vacuo* to yield **S4** (3.70 g, 94%) as a colorless solid. $[\alpha]_D^{25}$ = +101.7 (c = 1.2, DMSO); ^{1}H NMR (300 MHz, DMSO-d_6): δ = 12.79 (s_{br}, 1H, COOH), 7.94 (d, J = 6.0 Hz, 1H, NH), 7.45-7.26 (m, 10H, Ar-CH), 5.69 (s, 1H, Ph-CH), 5.07 (d, J = 3.3 Hz, 1H, H-1), 4.70 (d, J = 12.4 Hz, 1H, Bn-CH_2), 4.50 (d,

J = 12.4 Hz, 1H, Bn-CH_2), 4.30 (q, *J* = 6.9 Hz, 1H, propionyl-CH), 4.15 (d, *J* = 7.3 Hz, 1H, H-6_a), 3.87-3.73 (m, 5H, H-2, H-3, H-4, H-5, H-6_b), 1.86 (s, 3H, acetyl-CH_3), 1.29 (d, *J* = 6.9 Hz, 3H, propionyl-CH_3) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): δ = 174.94 (acetyl-C=O), 169.07 (COOH), 137.38 (Ar-C), 137.35 (Ar-C), 128.53 (Ar-CH), 128.02 (Ar-CH), 127.91 (Ar-CH), 127.41 (Ar-CH), 127.35 (Ar-CH), 125.60 (Ar-CH), 100.14 (Ph-CH), 96.67 (C-1), 81.46 (C-4), 74.94, 74.92 (C-3, propionyl-CH), 68.90 (Bn-CH_2), 67.77 (C-6), 62.77 (C-5), 53.44 (C-2), 22.53 (acetyl-CH_3), 18.60 (propionyl-CH_3) ppm; IR: ν = 3299, 3035, 2926, 1658, 1561, 1123, 1091, 1080, 1055, 1023, 696 cm^{-1}; MS (HR-ESI): *m/z*: calcd. for 472.1966 $[M+H]^+$; found: 472.1962.

1-*O*-Benzyl-4,6-*O*-benzylidene-3-*O*-((*R*)-phenylsulfonylethyl-propion-2-yl)-*N*-acetyl-α-D-glucosamine (S5)

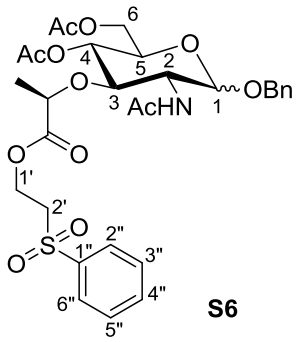


S5

The reaction was carried out under an inert atmosphere of argon. To a suspension of 1-*O*-benzyl-4,6-*O*-benzylidene-3-*O*-((*R*)-propion-2-yl)-*N*-acetyl-α-D-glucosamine **S4** (1.77 g, 3.75 mmol, 1.0 eq.) in dry CH_2Cl_2 (40 mL) was added HOBt (554 mg, 4.10 mmol, 1.1 eq.) and EDC·HCl (787 mg, 4.10 mmol, 1.1 eq.). The mixture was stirred for 45 min at rt. Subsequently, 2-phenylsulfonyl ethanol (764 mg, 4.10 mmol, 1.1 eq.) was added dropwise and the reaction mixture was stirred for 16 h at rt. The reaction mixture was washed with water (50 mL), aq. HCl (10%, 50 mL), water (50 mL) and sat. aq. Na_2CO_3 (50 mL), dried over Na_2SO_4 and concentrated *in vacuo*. Purification by column chromatography

(CH_2Cl_2:MeOH 98:2 → 80:20) yielded **S5** (1.52 g, 63%) as a colorless solid. $[\alpha]_D^{25}$ +86.3 (c = 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.85-7.82 (m, 2H, Ar-CH), 7.49-7.27 (m, 14H, Ar-CH, NH), 5.56 (s, 1H, Ph-CH), 5.36 (d, *J* = 3.5 Hz, 1H, H-1), 4.69 (d, *J* = 11.9 Hz, 1H, Bn-CH_2), 4.53 (d, *J* = 11.9 Hz, 1H, Bn-CH_2), 4.50-4.45 (m, 2H, H-1'), 4.22 (dd, *J* = 9.8 Hz, *J* = 4.3 Hz, 1H, H-6_a), 4.16 (q, *J* = 7.1 Hz, 1H, propionyl-CH), 3.94-3.80 (m, 2H, H-2, H-3), 3.77-3.65 (m, 3H, H-4, H-5, H-6_b), 3.45 (t, *J* = 6.6 Hz, 2H, H-2'), 2.01 (s, 3H, acetyl-CH_3), 1.18 (d, *J* = 7.1 Hz, 3H, propionyl-CH_3) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ = 174.10 (acetyl-C=O), 170.56 (propionyl-C=O), 138.97 (Ar-C), 137.19 (Ar-C), 134.06 (Ar-C), 129.28 (Ar-C), 129.16 (Ar-CH), 128.95 (Ar-CH), 128.27 (Ar-CH), 128.19 (Ar-CH), 127.82 (Ar-CH), 127.80 (Ar-CH), 127.78 (Ar-CH), 127.73 (Ar-CH), 125.79 (Ar-CH), 101.26 (Ph-CH), 97.20 (C-1), 83.21 (C-4), 74.85 (propionyl-CH), 74.81 (C-3), 70.32 (Bn-CH_2), 68.91 (C-6), 62.86 (C-5), 58.32 (C-1'), 54.92 (C-2'), 54.08 (C-2), 23.13 (acetyl-CH_3), 18.40 (propionyl-CH_3) ppm; IR: ν = 3313, 1755, 1653, 1373, 1305, 1145, 1119, 1085, 1053, 732, 694 cm^{-1}; MS (HR-ESI): *m/z*: calcd. for 662.2030 $[M+H]^+$; found: 662.2038.

1-*O*-Benzyl-4,6-*O*-diacetyl-3-*O*-((*R*)-phenylsulfonylethyl-propion-2-yl)-*N*-acetyl-α-D-glucosamine (S6)[S5]

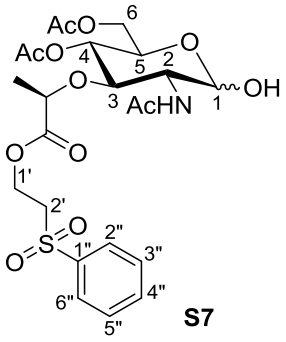


S6

1-*O*-Benzyl-4,6-*O*-benzylidene-3-*O*-((*R*)-phenylsulfonylethyl-propion-2-yl)-*N*-acetyl-α-D-glucosamine **S5** (4.16 g, 6.51 mmol, 1.0 eq.) was dissolved in a mixture of glacial AcOH (70 mL) and water (45 mL) and stirred for 45 min at 110 °C. The reaction mixture was cooled

to 0 °C and the solvent was removed *in vacuo*. The residue was dissolved in dry pyridine (70 mL) under an inert atmosphere of argon, then Ac_2O (25 mL, 27.9 g, 27.3 mmol, 4.2 eq.) and DMAP (16 mg, 0.13 mmol, 0.02 eq.) were added and the mixture was stirred for 16 h at rt. The solvent was removed *in vacuo*, and purification by column chromatography (PE:EtOAc 1:3) yielded **S6** (3.38 g, 82%) as a colorless oil (mixture of α,β-anomers, α:β 6:1). α-anomer: ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.87 (d, *J* = 7.5 Hz, 2H, Ph-H-2, Ph-H-6), 7.63 (t, *J* = 7.5 Hz, 1H, Ph-H-4), 7.51 (t, *J* = 7.5 Hz, 2H, Ph-H-3, Ph-H-5), 7.41 (d, *J* = 7.4 Hz, 1H, NH), 7.31-7.24 (m, 5H, Ar-H), 5.33 (d, *J* = 3.0 Hz, 1H, H-1), 5.03 (t, *J* = 9.4 Hz, 1H, H-3), 4.61 (d, *J* = 11.8 Hz, 1H, Bn-CH_2), 4.49 (d, *J* = 11.8 Hz, 1H, Bn-CH_2), 4.53-4.40 (m, 2H, H-1'), 4.12 (dd, *J* = 12.4 Hz, *J* = 4.5 Hz, 1H, H-6_a), 4.05 (q, *J* = 7.0 Hz, 1H, propionyl-CH), 3.94-3.70 (m, 4H, H-2, H-4, H-5, H-6_b), 3.44 (t, *J* = 5.9 Hz, 2H, H-2'), 2.05 (s, 6H, 2 x *O*-acetyl-CH_3), 1.97 (s, 3H, *N*-acetyl-CH_3), 1.19 (d, *J* = 7.0 Hz, 3H, propionyl-CH_3) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ = 173.76 (*N*-acetyl-C=O), 170.55 (propionyl-C=O), 170.49 (*O*-acetyl-C=O), 168.93 (*O*-acetyl-C=O), 138.87 (Ar-C), 137.08 (Ar-C), 134.01 (Ph-C-4), 129.30 (Ph-C-3, Ph-C-5), 128.26 (Ph-C-2, Ph-C-4), 127.87 (Ar-CH), 127.81 (Ar-CH), 127.76 (Ar-CH), 96.49 (C-1), 75.97 (C-4), 75.03 (propionyl-CH), 71.88 (C-3), 70.34 (Bn-CH_2), 68.10 (C-5), 62.02 (C-6), 58.17 (C-1'), 54.72 (C-2'), 53.76 (C-2), 23.10 (*N*-acetyl-CH_3), 20.87 (*O*-acetyl-CH_3), 20.76 (*O*-acetyl-CH_3), 18.52 (propionyl-CH_3) ppm; α,β-anomeric mixture: IR: ν = 3360, 1747, 1662, 1253, 1146, 1124, 1045, 732, 693 cm^{-1}; MS (HR-ESI): *m/z*: calcd. for 658.1929 $[M+Na]^+$; found: 658.1927.

4,6-*O*-Diacetyl-3-*O*-((*R*)-phenylsulfonylethyl-propion-2-yl)-*N*-acetyl-α,β-D-glucosamine (S7)

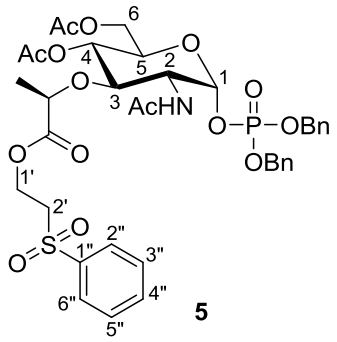


S7

The reaction was carried out under an inert atmosphere of argon. To a solution of 1-*O*-benzyl-4,6-*O*-diacetyl-3-*O*-((*R*)-phenylsulfonylethyl-propion-2-yl)-*N*-acetyl-α-D-glucosamine **S6** (48 mg, 76 µmol, 1.0 eq.) in dry EtOAc (0.95 mL) and dry MeOH (1.95 mL) were added glacial AcOH (0.15 mL) and $Pd(OH)_2$ (20% on charcoal, 50 mg, 0.38 mmol, 5.0 eq.). The reaction mixture was stirred under H_2 atmosphere (3 bar) for 48 h at rt. Pyridine (0.3 mL) and silica were added and the solvent was removed *in vacuo*. Purification by column chromatography (CH_2Cl_2:MeOH 97:3 → 95:5) yielded **S7** (34 mg, 80%) as a colorless oil (mixture of α,β-anomers, α:β 6:1). α-anomer: ^{1}H NMR (300 MHz, CD_3OD): δ = 7.98-7.95 (m, 2H, Ph-H-2, Ph-H-6), 7.79-7.74 (m, 1H, Ph-H-4), 7.69-7.64 (m, 2H, Ph-H-3, Ph-H-5), 5.24 (d, *J* = 2.4 Hz, 1H, H-1), 4.95-4.88 (m, 1H, H-3), 4.55-4.41 (m, 2H, H-1'), 4.17 (dd, *J* = 11.7 Hz, *J* = 4.6 Hz, 1H, H-6_a), 4.12-3.98 (m, 3H, H-5, H-6_b, propionyl-CH), 3.82-3.80 (m, 2H, H-2, H-4), 3.67 (t, 2H, *J* = 5.2 Hz, H-2'), 2.10 (s, 3H, *O*-acetyl-CH_3), 2.03 (s, 3H, *O*-acetyl-CH_3), 1.97 (s, 3H, *N*-acetyl-CH_3), 1.14 (d, *J* = 7.0 Hz, 3H, propionyl-CH_3) ppm; ^{13}C NMR (75 MHz, CD_3OD): δ = 174.47 (*N*-acetyl-C=O), 173.27 (propionyl-C=O), 172.32 (*O*-acetyl-C=O), 171.16 (*O*-acetyl-C=O), 140.68 (Ph-C-1), 135.22 (Ph-C-4), 130.49 (Ph-C3, Ph-C-5), 129.10 (Ph-C2, Ph-C6), 92.03 (C-1), 77.82 (C-4), 76.69 (propionyl-CH), 72.86 (C-3), 68.62 (C-5), 63.76 (C-6), 59.54 (C-1'), 55.53 (C-2'), 55.36 (C-2), 23.04 (*N*-acetyl-CH_3), 21.07 (*O*-acetyl-CH_3), 20.76 (*O*-acetyl-CH_3), 19.09 (propionyl-CH_3) ppm; α,β-anomeric

mixture: IR: ν = 3429, 2992, 1752, 1733, 1662, 1260, 1154, 1133, 1037, 732 cm^{-1}; MS (HR-ESI): *m/z*: calcd. for 568.1459 $[M+Na]^+$; found: 568.1460.

Di-*O*-benzyl-4,6-*O*-diacetyl-3-*O*-((*R*)-phenylsulfonylethyl-propion-2-yl)-*N*-acetyl-α-D-glucosamine-1-phosphate (5)

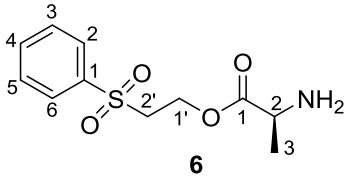


5

The reaction was carried out under an inert atmosphere of argon. To a solution of dibenzyl-*N*,*N*-diethylphosphoramidite (0.82 mL, 845 mg, 2.45 mmol, 3.6 eq.) and 1*H*-tetrazole (0.45 M in MeCN, 8.18 mL, 315 mg, 3.68 mmol, 5.3 eq.) in dry CH_2Cl_2 (4 mL), a solution of 4,6-*O*-diacetyl-3-*O*-((*R*)-phenylsulfonylethyl-propion-2-yl)-*N*-acetyl-α-D-glucosamine **S7** (376 mg, 0.69 mmol, 1.0 eq.) in dry CH_2Cl_2 (4 mL) was added dropwise over 10 min and stirred for 17.5 h at rt. CH_2Cl_2 (30 mL) was added and the solution was washed with sat. aq. Na_2CO_3, water and brine (30 mL each), dried over Na_2SO_4 and concentrated *in vacuo*. The crude product was recrystallized from Et_2O:hexane (1:1, 18 mL) and dried *in vacuo*. The resultant material was dissolved in dry THF (14 mL), and at -10 °C, *tert*-butylhydroperoxide (5.5 M in decane, 0.88 mL, 4.84 mmol, 7.0 eq.) was added. The reaction mixture was stirred for 1.5 h at -10 °C and then stirred for 18.5 h at rt. Et_2O was added (30 mL) and the solution was washed with sat. aq. $Na_2S_2O_3$, sat. aq. $NaHCO_3$ and brine (20 mL each), dried over Na_2SO_4 and concentrated *in vacuo*. Recrystallization from Et_2O:hexane (1:1, 18 mL) yielded **5** (337 mg, 61%) as a colorless solid. $[\alpha]_D^{25}$ = +46.8 (c = 1.0, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$): δ = 7.94-7.91 (m, 2H, Ph-H-2, Ph-H-6), 7.70-7.65 (m, 1H, Ph-H-4), 7.61-7.56 (m, 2H, Ph-H-3,

Ph-H-5), 7.38-7.30 (m, 10H, Ar-H), 6.10 (dd, J_{HP} = 5.9 Hz, J = 3.0 Hz, 1H, H-1), 5.14-5.09 (m, 1H, H-3), 5.06-5.01 (m, 4H, Bn-CH_2), 4.56 (t, J = 6.2 Hz, 2H, H-1'), 4.12-4.05 (m, 2H, propionyl-CH, H-6_a), 4.01-3.95 (m, 2H, H-5, H-6_b), 3.92-3.87 (m, 1H, H-2), 3.77-3.71 (m, 1H, H-4), 3.53-3.41 (m, 2H, H-2'), 2.10 (s, 3H, *N*-acetyl-CH_3), 1.98 (s, 3H, *O*-acetyl-CH_3), 1.86 (s, 3H, *O*-actyl-CH_3), 1.25 (d, J = 7.1 Hz, 3H, propionyl-CH_3) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ = 174.09 (*N*-acetyl-C=O), 171.09 (propionyl-C=O), 170.62 (*O*-acetyl-C=O), 168.97 (*O*-acetyl-C=O), 139.08 (Ph-C-1), 135.50 (d, J_{CP} = 7.5 Hz, Ar-C), 135.40 (d, J_{CP} = 7.5 Hz, Ar-C), 134.22 (Ph-C-4), 129.53 (Ph-C-3, Ph-C-5), 128.64 (Ar-CH), 128.61 (Ar-CH), 128.03 (Ar-CH), 127.97 (Ph-C-2), 127.93 (Ph-C-6), 95.54 (d, J_{CP} = 7.4 Hz, C-1), 75.21 (propionyl-CH), 74.93 (C-4), 71.05 (C-5), 69.91 (C-3), 69.56 (d, J_{CP} = 5.7 Hz, 2 x Bn-CH_2), 61.47 (C-6), 58.33 (C-1'), 54.72 (C-2'), 53.77 (d, J_{CP} = 8.6 Hz, C-2), 22.79 (*O*-acetyl-CH_3), 20.80 (*N*-acetyl-CH_3), 20.61 (*O*-acetyl-CH_3), 18.44 (propionyl-CH_3) ppm; ^{31}P NMR: (121 MHz, $CDCl_3$): δ -2.98 ppm; IR: ν = 3483, 2937, 1746, 1660, 1376, 1297, 1241, 1145, 1044, 733 cm^{-1}; MS (HR-ESI): *m/z*: calcd. for 828.2061 $[M+Na]^+$; found: 828.2061.

L-alanine phenylsulfonylethyl ester (6)



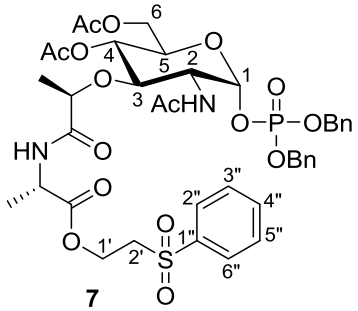
6

The reaction was carried out under an inert atmosphere of argon. To a solution of *N*-Cbz-L-alanine **22** (250 mg, 1.12 mmol, 1.0 eq.) in dry CH_2Cl_2 (12.5 mL), HOBt (227 mg, 1.68 mmol, 1.5 eq.) and EDC·HCl (322 mg, 1.68 mmol, 1.5 eq.) were added. After 30 min, 2-phenylsulfonyl ethanol (230 mg, 1.23 mmol, 1.1 eq.) was added and the reaction mixture was stirred for 39 h at rt. It was then washed with water, aq. HCl (0.5 M), water and sat. aq. Na_2CO_3 (10 mL each). The combined aqueous layers were extracted with CH_2Cl_2

(3 x 10 mL). The combined organics were dried over Na_2SO_4 and concentrated *in vacuo*. Purification by column chromatography (petroleum ether:EtOAc 3:2) yielded *N*-Cbz-protected L-alanine phenylsulfonylethyl ester (286 mg) as a colorless oil.

To a solution of the thus obtained *N*-Cbz-L-alanine phenylsulfonylethyl ester (102 mg, 0.26 mmol, 1.0 eq.) in EtOAc (10 mL), Pd (10% on charcoal, 70 mg, 0.06 mmol, 0.2 eq.) was added. The reaction mixture was stirred for 2 h under an H_2 atmosphere (1 bar) at rt and then filtered through a short pad of Celite™. The solvent of the filtrate was removed *in vacuo* to yield **6** (72 mg, 65% over 2 steps from **22**), which was used without further purification. $[\alpha]_D^{25}$ = -9.7 (c = 1.1, MeOH); ^{1}H NMR (300 MHz, DMSO-d_6): δ = 7.90-7.86 (m, 2H, Ph-H-2, Ph-H-6), 7.75-7.70 (m, 1H, Ph-H-4), 7.70-7.63 (m, 2H, Ph-H-3, Ph-H-5), 4.07 (q, *J* = 7.2 Hz, 1H, H-2), 3.65, (t, *J* = 6.5 Hz, 2H, H-1'), 3.43 (t, *J* = 6.5 Hz, 2H, H-2'), 1.36 (d, *J* = 7.2 Hz, 3H, CH_3) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): δ = 170.56 (C-1), 139.88 (Ph-C-1), 133.52 (Ph-C-4), 129.17 (Ph-C-3, Ph-C-6), 127.93 (Ph-C-2, Ph-C-5), 57.54 (C-2'), 54.89 (C-1'), 47.89 (C-2), 15.83 (CH_3) ppm; IR: ν = 1859, 1411, 1308, 1016, 904, 630, 561 cm^{-1}; MS (HR-ESI): *m/z*: calcd. for 258.0795 $[M+H]^+$; found: 258.0798.

Di-*O*-benzyl-4,6-*O*-diacetyl-3-*O*-((*R*)-propion-2-yl-alanyl-phenylsulfonylethyl ester)-*N*-acetyl-α-D-glucosamine-1-phosphate (7)

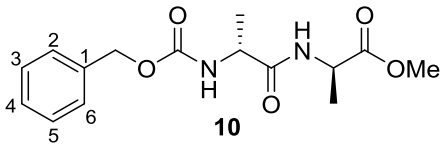


The reaction was carried out under an inert atmosphere of argon. To a solution of Di-*O*-benzyl-4,6-*O*-diacetyl-3-*O*-((*R*)-phenylsulfonylethyl-propion-2-yl)-*N*-acetyl-α-D-

glucosamine-1-phosphate **5** (292 mg, 0.36 mmol, 1.0 eq.) in dry CH_2Cl_2 (10 mL), DBU (54 μL, 55 mg, 0.36 mmol, 1.0 eq.) was added, and the resultant mixture was stirred for 15 h at rt. Aq. HCl (1 M, 9 mL) was added and the organic layer was washed with water and brine (10 mL each), dried over Na_2SO_4 and the solvent was removed *in vacuo*. The crude product was dissolved in dry CH_2Cl_2 (9 mL), and HOBt (99 mg, 0.73 mmol, 2.0 eq.) and EDC·HCl (139 mg, 0.73 mmol, 2.0 eq.) were added. A solution of L-alanine phenylsulfonylethyl ester **6** (102 mg, 0.40 mmol, 1.1 eq.) in dry THF (9 mL) and DIPEA (0.2 mL, 1.08 mmol, 3.0 eq.) were added. The reaction mixture was stirred for 15.5 h at rt. CH_2Cl_2 (50 mL) was then added and the solution was washed with water, aq. HCl (1 M), sat. aq. $NaHCO_3$ and water (50 mL each). The combined organics were dried over Na_2SO_4 and concentrated *in vacuo*. Purification by column chromatography (EtOAc) yielded **7** (208 mg, 66%) as a colorless oil. $[\alpha]_D^{25}$ = +115.5 (c = 0.53, MeOH); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.88 (d, *J* = 7.9 Hz, 2H, Ph-H-2, Ph-H-6), 7.64 (t, *J* = 7.9 Hz, 1H, Ph-H-4), 7.55 (t, *J* = 7.9 Hz, 2H, Ph-H-3, Ph-H-5), 7.35-7.29 (m, 10H, Ar-H), 6.74 (d, *J* = 7.1 Hz, 1H, Ala-NH), 6.41 (d, *J* = 8.9 Hz, 1H, acetyl-NH), 5.60 (dd, J_{HP} = 5.8 Hz, *J* = 3.2 Hz, 1H, H-1), 5.09-4.98 (m, 5H, H-3, Bn-CH_2), 4.40 (t, *J* = 6.2 Hz, 2H, H-1'), 4.32-4.27 (m, 1H, H-2), 4.16 (q, *J* = 7.0 Hz, 1H, Ala-H-2), 4.08-4.05 (m, 1H, H-6_a), 3.94-3.87 (m, 3H, propionyl-H-2, H-5, H-6_b), 3.50-3.47 (m, 1H, H-2'), 3.45-3.38 (m, 2H, H-4), 2.04 (s, 3H, *N*-acetyl-CH_3), 1.97 (s, 3H, *O*-acetyl-CH_3), 1.72 (s, 3H, *O*-acetyl-CH_3), 1.25 (d, *J* = 6.7 Hz, 3H, propionyl-CH_3), 1.24 (d, *J* = 7.0 Hz, 3H, Ala-H-3) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ = 171.94 (*N*-acetyl-C=O), 171.43 (Ala-C=O), 170.54 (propionyl-C=O), 170.50 (*O*-acetyl-C=O), 169.04 (*O*-acetyl-C=O), 139.04 (Ph-C-1), 135.33 (d, J_{CP} = 7.1 Hz, Ar-C), 135.09 (d, J_{CP} = 7.1 Hz, Ar-C), 134.03 (Ph-C-4), 129.40 (Ph-C-3, Ph-C-5), 128.93 (Ar-CH), 128.71 (Ar-CH), 128.03 (Ar-CH), 128.00 (Ph-C-2), 127.98 (Ph-C-6), 96.74 (d, J_{CP} = 7.7 Hz, C-1), 78.21 (propionyl-CH), 76.85 (C-4), 70.08 (C-5), 69.90 (d, J_{CP} = 6.9 Hz, 2 x Bn-CH_2), 68.69 (C-3), 61.46 (C-6), 58.05 (C-1'), 54.78 (C-2'), 52.90 (d,

J_{CP} = 8.6 Hz, C-2), 47.85 (Ala-C-2), 22.87 (*O*-acetyl-CH_3), 20.72 (*O*-acetyl-CH_3), 20.57 (*N*-acetyl-CH_3), 18.58 (propionyl-CH_3), 16.95 (Ala-C-3) ppm; ^{31}P-NMR (121 MHz, $CDCl_3$): δ = -2.56 ppm; IR: ν = 1742, 1214, 1141, 1034, 1010, 950, 730, 629, 505 cm^{-1}; MS (HR-ESI): *m/z*: calcd. for 899.2433 $[M+Na]^+$; found: 899.2432.

_N_-Cbz-D-alanine-D-alanine methyl ester (10)

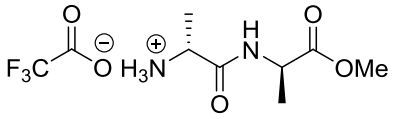


10

The reaction was carried out under an inert atmosphere of argon. To a solution of *N*-Cbz-D-alanine **9**[S2] (160 mg, 0.720 mmol, 1.0 eq.) in dry THF (4 mL), HOBt (97 mg, 0.72 mmol, 1.0 eq.) and EDC·HCl (138 mg, 0.720 mmol, 1.0 eq.) were added. The reaction mixture was stirred for 30 min at rt. Then D-alanine methyl ester hydrochloride **8**[S1] (100 mg, 0.720 mmol, 1.0 eq.) and DIPEA (0.25 mL, 190 mg, 1.4 mmol, 1.9 eq.) were added and the solution was stirred for 21 h at rt. EtOAc (10 mL) was added and the solution was washed with water, aq. HCl (0.5 M), sat. aq. $NaHCO_3$ and water (10 mL each). The organic layer was dried over Na_2SO_4 and the solvent was removed *in vacuo*. Purification by column chromatography (CH_2Cl_2:MeOH 95:5) yielded **10** (186 mg, 88%) as a colorless solid. $[\alpha]_D^{25}$ = +11.3 (c = 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.31-7.24 (m, 5H, Cbz-CH), 6.84 (d, *J* = 6.9 Hz, 1H, NH), 5.60 (d, *J* = 7.5 Hz, 1H, NH), 5.07 (s, 2H, Cbz-CH_2), 4.52 (dq, *J* = 7.5 Hz, *J* = 7.2 Hz, 1H, H-2), 4.30 (dq, *J* = 7.0 Hz, *J* = 6.9 Hz, 1H, H-2), 3.70 (s, 3H, OCH_3), 1.35 (d, *J* = 7.0 Hz, 3H, H-3), 1.34 (d, *J* = 7.2 Hz, 3H, H-3) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ = 172.95 (C=O), 171.78 (C=O), 155.74 (Cbz-C=O), 136.05 (Cbz-C), 128.35 (Cbz-CH), 127.99 (Cbz-CH), 127.84 (Cbz-CH), 66.88 (Cbz-CH_2), 52.41 (OCH_3), 50.33 (C-2), 48.01 (C-2), 18.74 (C-3), 18.13 (C-3) ppm; IR: ν = 3299, 1739, 1687, 1648, 1540, 1455, 1327, 1261, 1233,

1069, 1057, 695, 672 cm^{-1}; MS (HR-ESI): *m/z*: calcd. for 331.1264 $[M+Na]^+$; found: 331.1266.

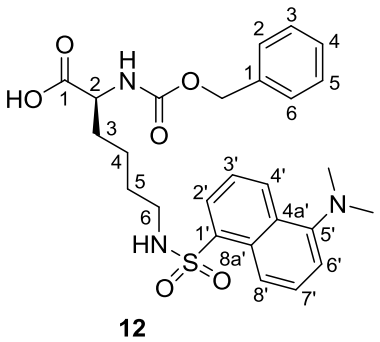
D-alanine-D-alanine methyl ester trifluoroacetate (11)



11

To a suspension of $Pd(OH)_2$ (20% on charcoal, 56 mg, 0.11 mmol) and TFA (32 µL, 48 mg, 0.49 mmol) in degassed MeOH (1.5 mL), *N*-Cbz-D-alanine-D-alanine methyl ester **10** (150 mg, 0.49 mmol, 1.0 eq.) was added. The reaction mixture was stirred for 2 h under an H_2 atmosphere (1 bar) at rt, was then filtered through a short pad of Celite™ and the Celite™ were washed with MeOH (25 mL). Evaporation of the solvent of the combined filtrates *in vacuo* yielded **11** (202 mg, quant.) as a colorless oil. $[\alpha]_D^{25}$ = +24.3 (c = 1.1, MeOH); ^1H NMR (300 MHz, CD_3OD): δ = 4.45 (q, *J* = 7.3 Hz, 1H, H-2), 3.93 (q, *J* = 7.0 Hz, 1H, H-2), 3.72 (s, 3H, OCH_3), 1.52 (d, *J* = 7.0 Hz, 3H, H-3), 1.41 (d, *J* = 7.3 Hz, 3H, H-3) ppm; ^{13}C NMR (75 MHz, CD_3OD): δ = 173.96 (C=O), 170.74 (C=O), 58.91 (OCH_3), 50.07 (C-2), 49.85 (C-2), 17.53 (C-3), 17.25 (C-3) ppm; ^{19}F NMR (282 MHz, CD_3OD): δ = -77.07 ppm; IR: ν = 1662, 1556, 1198, 1179, 1130, 1054, 836, 799, 721 cm^{-1}; MS (HR-ESI): *m/z*: calcd. for 175.1077 $[M-TFA]^+$; found: 175.1078.

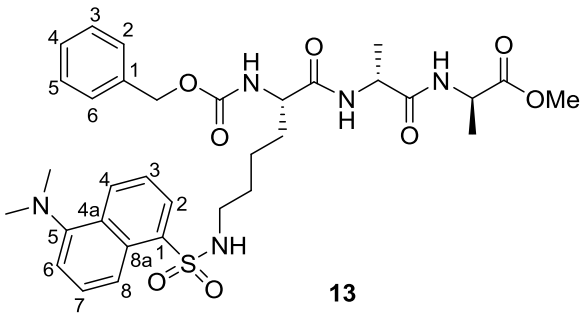
***N*$^{\alpha}$-Cbz-*N*$^{\varepsilon}$-dansyl-L-lysine (12)**[S8]



12

To a solution of *N*-Cbz-L-lysine hydrochloride (8.6 g, 30.7 mmol, 1.0 eq.) in water (276 mL) and MeOH (696 mL), $NaHCO_3$ (7.45 g, 88.7 mmol, 2.9 eq.) and dansyl chloride (12.0 g, 44.5 mmol, 1.5 eq.) were added. The reaction mixture was stirred for 19 h at rt, then acidified until pH 2 with aq. HCl (1 M) and extracted with CH_2Cl_2 (3 x 1.2 L). The combined organics were washed with brine, dried over Na_2SO_4 and concentrated *in vacuo*. Purification by column chromatography (CH_2Cl_2:MeOH 10:0 → 9:1) yielded **12** (10.0 g, 64%) as a greenish solid. $[\alpha]_D^{25}$ = +10.4 (c = 0.5, $CHCl_3$); ^{1}H NMR (300 MHz, DMSO-d_6): δ = 8.45 (d, *J* = 8.5 Hz, 1H, dansyl-H-2), 8.30 (d, *J* = 8.6 Hz, 1H, dansyl-H-4), 8.08 (dd, *J* = 7.3 Hz, *J* = 1.1 Hz, 1H, dansyl-H-8), 7.81 (t, *J* = 5.9 Hz, 1H, N$^{\varepsilon}$H), 7.63-7.54 (m, 2H, dansyl-H-3, dansyl-H-7), 7.36-7.29 (m, 5H, Ar-H), 7.24 (d, *J* = 7.5 Hz, 1H, dansyl-H-6), 5.01 (s, 2H, Cbz-CH_2), 3.83-3.76 (m, 1H, H-2), 2.83 (s, 6H, N(CH_3)$_2$), 2.75 (q, *J* = 5.9 Hz, 2H, H-6), 1.53-1.20 (m, 6H, H-3, H-4, H-5) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): δ = 174.06 (C-1), 155.73 (Cbz-C=O), 151.16 (dansyl-C-5), 136.92 (dansyl-C-1), 136.05 (Cbz-C), 129.13 (dansyl-C-2), 128.99 (dansyl-C-8$_a$), 128.95 (dansyl-C-4$_a$), 128.13 (C-4), 127.97 (Cbz-CH), 127.59 (C-7), 127.55 (Cbz-CH), 127.47 (Cbz-CH), 123.36 (C-3), 119.02 (dansyl-C-8), 114.95 (dansyl-C-6), 65.22 (Cbz-CH_2), 54.19 (C-2), 45.00 (N(CH_3)$_2$), 42.30 (C-6), 30.77 (C-3), 28.91 (C-5), 22.59 (C-4) ppm; IR: ν = 1698, 1308, 1139, 1059, 788, 697, 622, 568, 536 cm^{-1}; MS (HR-ESI): *m/z*: calcd. for 512.1861 [M-H]$^-$; found: 512.1864.

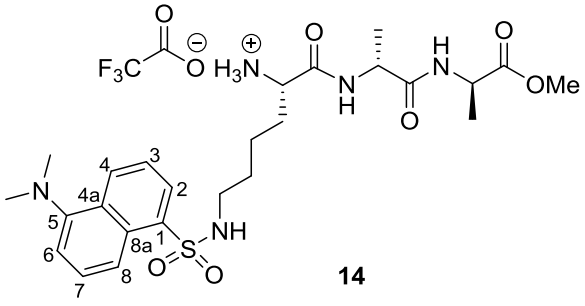
***N*-Cbz-L-lysine-(N^{ε}-dansyl)-D-alanine-D-alanine methyl ester (13)**



The reaction was carried out under an inert atmosphere of argon. To a solution of N^{α}-Cbz-N^{ε}-dansyl-L-lysine **12** (20 mg, 0.039 mmol, 1.0 eq.) in dry CH_2Cl_2 (1 mL) and dry THF (1 mL), PyBOP (20 mg, 0.039 mmol, 1.0 eq.) was added. Then D-alanine-D-alanine-methyl ester trifluoroacetate **11** (6.8 mg, 0.039 mmol, 1.0 eq.) and DIPEA (7 µL, 5 mg, 0.078 mmol, 2.0 eq.) were added. The reaction mixture was stirred for 24 h at rt. Then a second portion of PyBOP (10 mg, 0.02 mmol, 0.5 eq.) and DIPEA (4 µL, 3 mg, 0.02 mmol, 0.5 eq.) were added and the reaction mixture was stirred for an additional 24 h at rt. CH_2Cl_2 (10 mL) was added and the solution was washed with aq. HCl (0.5 M, 10 mL), sat. aq. $NaHCO_3$ (10 mL) and water (2 x 10 mL). The combined aqueous layers were extracted with CH_2Cl_2 (3 x 10 mL). The combined organics were dried over Na_2SO_4 and the solvent was removed *in vacuo*. Purification by column chromatography (CH_2Cl_2:MeOH 98:2) yielded **13** (221 mg, 85%) as a greenish solid. $[\alpha]_D^{25}$ = +13.2 (c = 0.5, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.52 (d, *J* = 8.5 Hz, 1H, dansyl-H-2), 8.30 (d, *J* = 8.6 Hz, 1H, dansyl-H-8), 8.21 (dd, *J* = 7.3 Hz, *J* = 1.3 Hz, 1H, dansyl-H-4), 7.53-7.47 (m, 2H, dansyl-H-3, dansyl-H-7), 7.32-7.28 (m, 5H, Cbz-CH), 7.16 (d, *J* = 7.5 Hz, 1H, dansyl-H-6), 7.00 (d, *J* = 7.6 Hz, 1H, Ala-NH), 5.69 (d, *J* = 8.0 Hz, 1H, Lys-N$^{\alpha}$H), 5.48 (t, *J* = 5.8 Hz, 1H, Lys-N$^{\varepsilon}$H), 5.07 (d, *J* = 2.6 Hz, 2H, Cbz-CH_2), 4.57-4.47 (m, 2H, Ala-H-2), 4.09 (q, *J* = 5.6 Hz, 1H, Lys-H-2), 3.68 (s, 3H, OCH_3), 2.88 (s, 6H, $N(CH_3)_2$), 2.84-2.80 (m, 2H, Lys-H-6), 1.72-1.60 (m, 2H, Lys-H-3), 1.58-1.50 (m, 2H, Lys-H-5), 1.43-1.22 (m, 8H, Lys-H-4, 2 x Ala-H-3) ppm; ^{13}C NMR (75 MHz,

$CDCl_3$): δ = 173.06 (Lys-C=O), 171.73 (Ala-C=O), 171.69 (Ala-C=O), 156.33 (Cbz-C=O) 151.77 (dansyl-C-5), 136.08 (dansyl-C-1), 134.82 (Cbz-C), 130.25 (dansyl-C-2), 129.77 (dansyl-C-8_a), 129.57 (dansyl-C-4_a), 129.46 (dansyl-C-4), 128.47 (Cbz-CH), 128.26 (dansyl-C-7), 128.15 (Cbz-CH), 128.03 (Cbz-CH), 123.21 (dansyl-C-3), 118.88 (dansyl-C-8), 115.21 (dansyl-C-6), 67.09 (Cbz-CH_2), 54.79 (Lys-C-2), 52.41 (OCH_3), 48.85 (Ala-C-2), 48.11 (Ala-C-2), 45.40 ($N(CH_3)_2$), 42.67 (Lys-C-6), 31.79 (Lys-C-3), 28.89 (Lys-C-5), 22.17 (Lys-C-4), 18.08 (Ala-C-3), 17.89 (Ala-C-3) ppm; IR: ν = 1647, 1521, 1453, 1308, 1139, 1048, 789, 622, 568 cm^{-1}; MS (HR-ESI): *m/z*: calcd. for 692.2725 $[M+Na]^+$; found: 692.2725.

L-Lysine-(N^{ε}-dansyl)-D-alanine-D-alanine methyl ester (14)

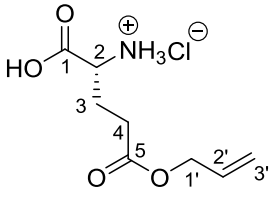


14

The reaction was carried out under an inert atmosphere of argon. To a solution of *N*-Cbz-L-lysine-(N^{ε}-dansyl)-D-alanine-D-alanine methyl ester **13** (50 mg, 0.075 mmol, 1.0 eq.) in degassed MeOH (1 mL), TFA (6 μL, 9 mg, 0.075 mmol, 1.0 eq.) and $Pd(OH)_2$ (20% on charcoal, 10 mg, 0.02 mmol, 3.8 eq.) were added. The reaction mixture was stirred under an H_2 atmosphere (1 bar) at rt for 4.5 h. Then a second portion of $Pd(OH)_2$ (20% on charcoal, 10 mg, 0.02 mmol, 3.8 eq.) and MeOH (0.5 mL) were added and the reaction mixture was stirred for further 16 h at rt. It was then filtered through a short pad of Celite™ and the Celite™ were washed with MeOH. Evaporation of the solvent of the combined filtrates *in vacuo* yielded **14** (61 mg, quant.) as a greenish oil. $[\alpha]_D^{25}$ = +35.2 (c = 1.0, MeOH); ^{1}H NMR (300 MHz, CD_3OD): δ = 8.56 (d, *J* = 8.5 Hz, 1H, dansyl-H-2), 8.33 (d, *J* = 8.6 Hz, 1H,

dansyl-H-8), 8.17 (d, *J* = 7.4 Hz, 1H, dansyl-H-4), 7.61-7.55 (m, 2H, dansyl-H-3, dansyl-H-7), 7.28 (d, *J* = 7.5 Hz, 1H, dansyl-H-6), 4.40-4.35 (m, 2H, Ala-H-2), 3.71-3.66 (m, 1H, Lys-H-2), 3.71 (s, 3H, OCH_3), 2.88 (s, 6H, $N(CH_3)_2$), 2.82 (t, *J* = 6.5 Hz, 2H, Lys-H-6), 1.74-1.64 (m, 2H, Lys-H-3), 1.48-1.37 (m, 4H, Lys-H-4, Lys-H-5), 1.39 (d, *J* = 7.2 Hz, 6H, 2 x Ala-H-3) ppm; ^{13}C NMR (75 MHz, CD_3OD): δ = 175.17 (Lys-C=O), 174.64 (Ala-C=O), 174.28 (Ala-C=O), 153.20 (dansyl-C-5), 137.08 (dansyl-C-1), 131.22 (dansyl-C-2), 131.11 (dansyl-C-8_a), 130.97 (dansyl-C-4_a), 130.12 (dansyl-C-4), 129.07 (dansyl-C-7), 124.29 (dansyl-C-3), 120.53 (dansyl-C-8), 116.43 (dansyl-C-6), 59.28 (Lys-C-2), 55.15 (OCH_3), 54.44 (Ala-C-2), 52.68 (Ala-C-2), 45.81 ($N(CH_3)_2$), 43.52 (Lys-C-6), 32.69 (Lys-C-3), 32.51 (Lys-C-5), 23.75 (Lys-C-4), 17.88 (Ala-CH_3), 17.23 (Ala-CH_3) ppm; IR: ν = 1735, 1666, 1241, 1200, 1138, 1044, 790, 623, 569 cm^{-1}; MS (HR-ESI): *m/z*: calcd. for 536.2537 $[M\text{-}TFA]^+$; found: 536.2544.

D-Glutamic acid 5-allyl ester hydrochloride (16)

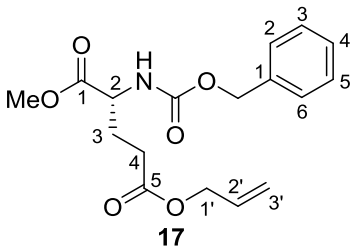


16

The reaction was carried out under an inert atmosphere of argon. To a suspension of D-glutamic acid **15** (500 mg, 2.87 mmol, 1.0 eq.) in dry allyl alcohol (13.4 mL), trimethylsilyl chloride (1.15 mL, 988 mg, 9.10 mmol, 3.2 eq.) was added dropwise. The solution was stirred for 18 h at rt. Then Et_2O was added at 0 °C, the precipitate was filtered off, washed with Et_2O and dried *in vacuo* to yield **16** (531 mg, 80%) as a colorless solid. $[\alpha]_D^{25}$ = -20.3 (c = 1.2, MeOH); ^1H NMR (300 MHz, CD_3OD): δ = 5.95 (ddt, *J* = 17.2 Hz, *J* = 10.5 Hz, *J* = 5.7 Hz, 1H, H-2'), 5.32 (dq, *J* = 17.2 Hz, *J* = 1.5 Hz, 1H, H-3_a'), 5.23 (dq, *J* = 10.5 Hz, *J* = 1.5 Hz, 1H, H-3_b'), 4.62 (dt, *J* = 5.7 Hz, *J* = 1.5 Hz, 2H, H-1'), 4.06 (t, *J* = 7.0 Hz, 1H, H-2), 2.63 (dt,

J = 7.0 Hz, *J* = 2.7 Hz, 2H, H-4), 2.29-2.16 (m, 2H, H-3) ppm; ^{13}C NMR (75 MHz, CD_3OD): δ = 173.10 (C-5), 171.16 (C-1), 133.34 (C-2'), 118.40 (C-3'), 66.42 (C-1'), 53.15 (C-2), 30.57 (C-4), 26.59 (C-3) ppm; IR: ν = 3019, 2938, 1740, 1651, 1512, 1488, 1216, 1179 cm^{-1}; MS (HR-ESI): *m/z*: calcd. for 188.0917 $[M-Cl]^+$; found: 188.0917.

_N_-Cbz-D-glutamic acid 5-allyl-1-methyl ester (17)



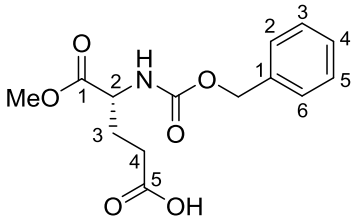
17

To a solution of D-glutamic acid 5-allyl ester hydrochloride **16** (8.93 g, 40.0 mmol, 1.0 eq.) in water (400 mL), Na_2CO_3 (33.6 g, 400 mmol, 10 eq.) was added. At 0 °C, benzyl chloroformate (5.70 mL, 6.90 g, 40.0 mmol, 1.0 eq.) was added dropwise over 5 min. The reaction mixture was stirred for 2 h at 0 °C and stirred for 36 h at rt. It was then washed with Et_2O (3 x 300 mL). The aqueous layer was acidified until pH 1 with aq. HCl (10%) and was extracted with EtOAc (3 x 300 mL). The combined organics were washed with water (300 mL), dried over Na_2SO_4 and evaporated *in vacuo* to yield *N*-Cbz-D-glutamic acid 5-allyl ester (11.3 g) as a colorless solid.

To a solution of the thus obtained *N*-Cbz-D-glutamic acid 5-allyl ester (11.2 g, 34.9 mmol, 1.0 eq.) in dry DMF (220 mL), $NaHCO_3$ (5.86 g, 69.7 mmol, 2.0 eq.) was added. Methyl iodide (10.9 mL, 24.7 g, 174 mmol, 5.0 eq.) was slowly added and the mixture was stirred for 2 d at rt. EtOAc (200 mL) was added, the resultant precipitate was filtered off, and the solvent was removed under reduced pressure. The residue was dissolved in EtOAc (1.5 L) and washed with water (500 mL). The aqueous layer was extracted with EtOAc (3 x 150 mL). The combined organics were washed with sat. aq. $Na_2S_2O_3$ (2 x 400 mL) and sat. aq. $NaHCO_3$

(2 x 400 mL), dried over Na_2SO_4 and concentrated *in vacuo*. Purification by column chromatography (petroleum ether:EtOAc 4:1) yielded **17** (9.15 g, 69% over 2 steps from **16**) as a colorless oil. $[\alpha]_D^{25}$ = -7.0 (c = 0.7, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.36-7.31 (m, 5H, Cbz-CH), 5.89 (ddt, *J* = 17.3 Hz, *J* = 10.4 Hz, *J* = 5.7 Hz, 1H, H-2'), 5.44 (d, *J* = 8.1 Hz, 1H, NH), 5.30 (dq, *J* = 17.3 Hz, *J* = 1.5 Hz, 1H, H-3_a'), 5.23 (dq, *J* = 10.4 Hz, *J* = 1.5 Hz, 1H, H-3_b'), 5.10 (s, 2H, Cbz-CH_2), 4.56 (dt, *J* = 5.7 Hz, *J* = 1.5 Hz, 2H, H-1'), 4.45-4.38 (m, 1H, H-2), 3.74 (s, 3H, OCH_3), 2.47-2.40 (m, 2H, H-4), 2.28-2.17 (m, 1H, H-3_a), 2.06-1.98 (m, 1H, H-3_b) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ = 172.24 (C-1, C-5), 155.85 (Cbz-C=O), 136.07 (Cbz-C), 131.91 (C-2'), 128.48 (Cbz-CH), 128.15 (Cbz-CH), 128.06 (Cbz-CH), 118.40 (C-3'), 67.03 (Cbz-CH_2), 65.35 (C-1'), 53.26 (C-2), 52.50 (OCH_3), 30.06 (C-4), 27.55 (C-3) ppm; IR: ν = 1717, 1521, 1207, 1171, 1048, 985, 738, 697 cm^{-1}; MS (HR-ESI): *m/z*: calcd. for 358.1261 $[M+Na]^+$; found: 358.1261.

_N_-Cbz-D-glutamic acid 1-methyl ester (18)

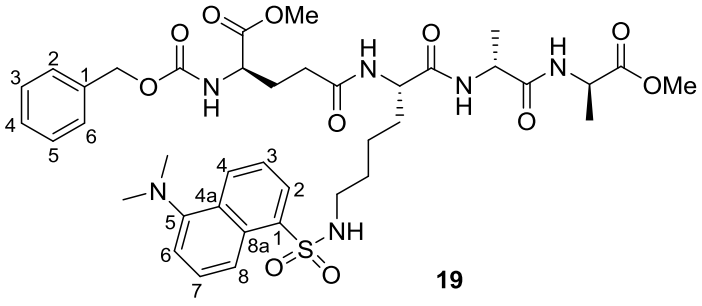


18

The reaction was carried out under an inert atmosphere of argon. To a solution of *N*-Cbz-D-glutamic acid 5-allyl-1-methyl ester **17** (503 mg, 1.5 mmol, 1.0 eq.) in dry CH_2Cl_2 (45 mL), phenylsilane (0.35 mL, 308 mg, 2.85 mmol, 1.9 eq.) was added dropwise. Tetrakis(triphenyl-phosphine)palladium (35 mg, 0.03 mmol, 0.02 eq.) was added and the reaction mixture was stirred for 2.5 h at rt. CH_2Cl_2 (150 mL) was added and the solution was extracted with sat. aq. $NaHCO_3$ (3 x 150 mL). The combined aqueous layers were washed with diethyl ether, acidified with aq. HCl (2 M) until pH 2 and extracted with CH_2Cl_2 (3 x 150 mL). The

combined organics were dried over Na_2SO_4. Evaporation of the solvent *in vacuo* yielded **18** (452 mg, quant.) as a colorless oil. $[\alpha]_D^{25}$ = -3.3 (c = 0.5, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.37-7.31 (m, 5H, Cbz-CH), 5.44 (d, *J* = 8.2 Hz, 1H, NH), 5.11 (s, 2H, Cbz-CH_2), 4.48-4.40 (m, 1H, H-2), 3.75 (s, 3H, OCH_3), 2.49-2.42 (m, 2H, H-4), 2.28-2.17 (m, 1H, H-3_a), 2.03-1.91 (m, 1H, H-3_b) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ = 177.85 (C-5), 172.39 (C-1), 156.00 (Cbz-C=O), 136.01 (Cbz-C), 128.50 (Cbz-CH), 128.20 (Cbz-CH), 128.06 (Cbz-CH), 67.14 (Cbz-CH_2), 53.12 (C-2), 52.55 (OCH_3), 29.85 (C-4), 27.41 (C-3) ppm; IR: ν = 1699, 1524, 1210, 1175, 1050, 1027, 737, 697, 576 cm^{-1}; MS (HR-ESI): *m/z*: calcd. for 294.0983 $[M-H]^-$; found: 294.0982.

_N_-Cbz-D-γ-glutamic acid-(1-methyl ester)-L-lysine-(N^{ε}-dansyl)-D-alanine-D-alanine methyl ester (19)

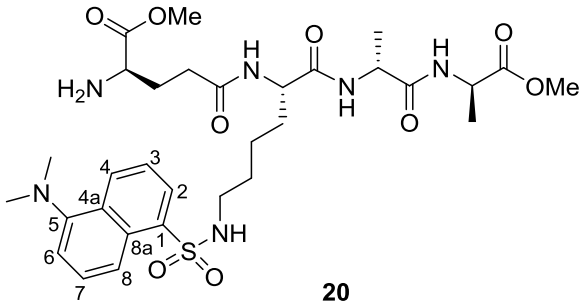


19

The reaction was carried out under an inert atmosphere of argon. To a solution of *N*-Cbz-D-glutamic acid 1-methyl ester **18** (235 mg, 0.80 mmol, 1.2 eq.) in dry THF (10 mL), HOBt (90 mg, 0.66 mmol, 1.0 eq.) and EDC·HCl (127 mg, 0.66 mmol, 1.0 eq.) were added and the mixture was stirred for 20 min at rt. Then L-lysine-(N^{ε}-dansyl)-D-alanine-D-alanine methyl ester **14** (414 mg, 0.66 mmol, 1.0 eq.) and DIPEA (0.23 mL, 171 mg, 1.30 mmol, 2.0 eq.) were added and the reaction mixture was stirred for 20 h at rt. Then a second portion of HOBt (45 mg, 0.33 mmol, 0.5 eq.) and EDC·HCl (63 mg, 0.33 mmol, 0.5 eq.) were added and the reaction mixture was further stirred for 24 h at rt. EtOAc was added and the solution was washed with water, aq. HCl (0.5 M), water and sat. aq. Na_2CO_3 (50 mL each). The combined

aqueous layers were extracted with EtOAc (3 x 50 mL), and the combined organics were dried over Na_2SO_4 and concentrated *in vacuo*. Purification by column chromatography (CH_2Cl_2:MeOH 95:5) yielded **19** (381 mg, 72%) as a greenish solid. $[\alpha]_D^{25}$ = +2.4 (c = 0.5, $CHCl_3$); ^{1}H NMR (300 MHz, DMSO-d_6): δ = 8.45 (d, *J* = 8.4 Hz, 1H, dansyl-H-2), 8.31 (d, *J* = 8.7 Hz, 1H, dansyl-H-8), 8.13 (d, *J* = 7.3 Hz, 1H, Ala-NH), 8.08 (d, *J* = 7.8 Hz, 1H, dansyl-H-4), 8.04 (d, *J* = 7.3 Hz, 1H, Ala-NH), 7.91 (d, *J* = 7.3 Hz, 1H, NH), 7.68 (d, *J* = 7.3 Hz, 1H, NH), 7.63-7.55 (m, 2H, dansyl-H-3, dansyl-H-7), 7.35-7.26 (m, 5H, Cbz-CH), 7.25 (d, *J* = 6.7 Hz, 1H, dansyl-H-6), 5.03 (s, 2H, Cbz-CH_2), 4.31-4.21 (m, 2H, Ala-H-2), 4.13-4.00 (m, 2H, Lys-H-2, Glu-H-2), 3.59 (s, 3H, OCH_3), 3.18 (s, 3H, OCH_3), 2.83 (s, 6H, $N(CH_3)_2$), 2.74 (t, *J* = 6.8 Hz, 2H, Lys-H-6), 2.31-2.17 (m, 2H, Glu-H-4), 1.98-1.86 (m, 1H, Glu-H-3_a), 1.81-1.72 (m, 1H, Glu-H-3_b), 1.51-1.33 (m, 6H, Lys-H-3, Lys-H-4, Lys-H-5), 1.28 (d, *J* = 7.2 Hz, 3H, Ala-H-3), 1.18 (d, *J* = 7.2 Hz, 3H, Ala-H-3) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): δ = 172.80 (Lys-C=O), 172.58 (Glu-C=O), 172.02 (Glu-C=O), 171.43 (Ala-C=O), 171.29 (Ala-C=O), 156.02 (Cbz-C=O), 151.30 (dansyl-C-5), 136.83 (dansyl-C-1), 136.09 (Cbz-C), 129.26 (dansyl-C-8_a), 129.08 (dansyl-C-4_a), 129.03 (dansyl-C-2), 128.32 (Cbz-CH), 128.28 (Cbz-CH), 128.12 (Cbz-CH), 127.74 (dansyl-C-7), 127.67 (dansyl-C-4), 123.54 (dansyl-C-3), 119.12 (dansyl-C-8), 115.07 (dansyl-C-6), 65.53 (Cbz-CH_2), 53.48 (Lys-C-2), 52.69 (Glu-C-2), 51.78 (OCH_3), 48.58 (OCH_3), 47.53 (Ala-C-2), 45.04 ($N(CH_3)_2$), 42.28 (Lys-C-6), 31.26 (Glu-C-4), 31.19 (Lys-C-3), 28.91 (Lys-C-5), 26.68 (Glu-C-3), 22.34 (Lys-C-4), 17.97 (Ala-C-3), 16.72 (Ala-C-3) ppm; IR: ν = 3281, 1688, 1630, 1536, 1218, 1140, 1020, 622, 569 cm^{-1}; MS (HR-ESI): *m/z*: calcd. for 811.3342 $[M+H]^-$; found: 811.3355.

D-γ-glutamic acid-(1-methyl ester)-L-lysine-(N^{ε}-dansyl)-D-alanine-D-alanine methyl ester (20)

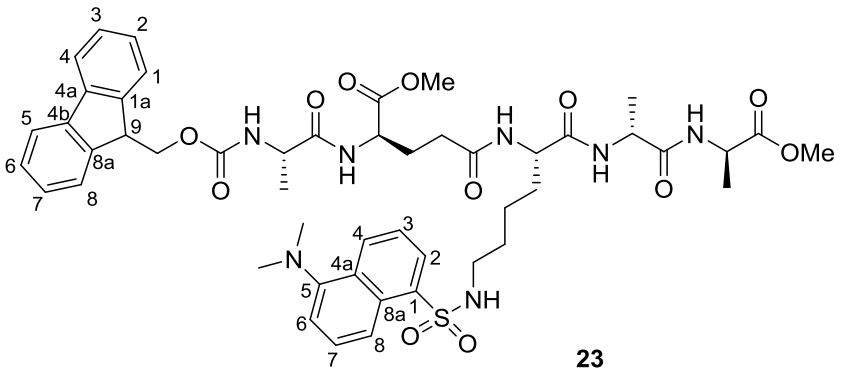


20

The reaction was carried out under an inert atmosphere of argon. To a solution of *N*-Cbz-D-γ-glutamic acid-(1-methyl ester)-L-lysine-(N^{ε}-dansyl)-D-alanine-D-alanine methyl ester **19** (251 mg, 0.30 mmol, 1.0 eq.) in degassed MeOH (25 mL), Pd (10% on charcoal, 100 mg, 0.09 mmol, 0.3 eq.) was added and the reaction mixture was stirred under an H_2 atmosphere for 2 h at rt. The suspension was filtered through a short pad of Celite^{TM} and the Celite^{TM} were washed with MeOH. Evaporation of the solvent of the combined filtrates *in vacuo* yielded **20** (188 mg, 92%) as a greenish oil. $[\alpha]_D^{25}$ = +18.5 (c = 1.0, MeOH); ^{1}H NMR (300 MHz, CD_3OD): δ = 8.55 (d, *J* = 8.6 Hz, 1H, dansyl-H-2), 8.34 (d, *J* = 8.7 Hz, 1H, dansyl-H-8), 8.17 (dd, *J* = 7.3 Hz, *J* = 1.2 Hz, 1H, dansyl-H-4), 7.60-7.54 (m, 2H, dansyl-H-3, dansyl-H-7), 7.27 (d, *J* = 7.7 Hz, 1H, dansyl-H-6), 4.40-4.32 (m, 2H, Ala-H-2), 4.12-4.02 (m, 2H, Lys-H-2, Glu-H-2), 3.74 (s, 3H, OCH_3), 3.68 (s, 3H, OCH_3), 2.87 (s, 6H, $N(CH_3)_2$), 2.81 (t, *J* = 6.5 Hz, 2H, Lys-H-6), 2.41-2.35 (m, 2H, Glu-H-4), 2.20-2.01 (m, 2H, Glu-H-3), 1.64-1.27 (m, 6H, Lys-H-3, Lys-H-4, Lys-H-5), 1.40 (d, *J* = 7.3 Hz, 3H, Ala-H-3), 1.33 (d, *J* = 7.3 Hz, 3H, Ala-H-3) ppm; ^{13}C NMR (75 MHz, CD_3OD): δ = 175.41 (Lys-C=O), 175.17 (Glu-C=O), 174.64 (Glu-C=O), 174.40 (Ala-C=O), 174.28 (Ala-C=O), 153.20 (dansyl-C-5), 137.08 (dansyl-C-1), 131.27 (dansyl-C-8_a), 131.11 (dansyl-C-4_a), 130.97 (dansyl-C-2), 130.12 (dansyl-C-7), 129.07 (dansyl-C-4), 124.29 (dansyl-C-3), 120.53 (dansyl-C-8), 116.43 (dansyl-C-6), 59.28 (Lyc-C-2), 55.15 (Ala-C-2), 54.44 (OCH_3), 52.69 (Glu-C-2), 52.56 (OCH_3),

50.03 (Ala-C-2), 45.81 ($N(CH_3)_2$), 43.52 (Lys-C-6), 32.51 (Glu-C-4), 31.92 (Lys-C-3), 31.02 (Lys-C-5), 30.27 (Glu-C-3), 23.75 (Lys-C-4), 17.88 (Ala-C-3), 17.23 (Ala-C-3) ppm; IR: ν = 3338, 2943, 2874, 1740, 1658, 1537, 1316, 1208, 1146, 795, 627, 571 cm^{-1}; MS (HR-ESI): *m/z*: calcd. for 679.3120 $[M+H]^+$; found: 679.3125.

_N_-Fmoc-L-alanine-D-γ-glutamic acid-(1-methyl ester)-L-lysine-(N^{ε}-dansyl)-D-alanine-D-alanine methyl ester (23)

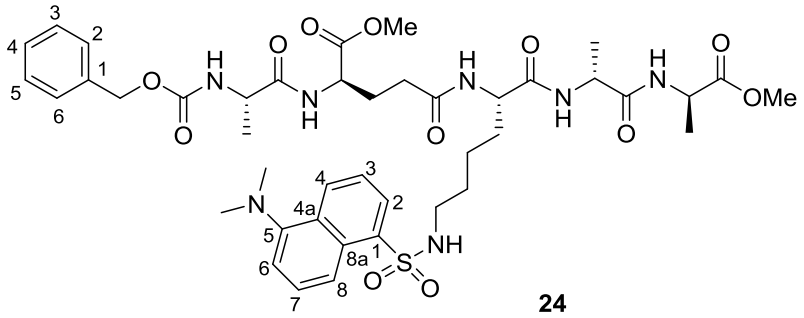


23

The reaction was carried out under an inert atmosphere of argon. To a solution of *N*-Fmoc-L-alanine **21** (68 mg, 0.22 mmol, 2.0 eq.) in dry THF (3 mL), HOBt (29 mg, 0.22 mmol, 2.0 eq.) and EDC·HCl (42 mg, 0.22 mmol, 2.0 eq.) were added and the mixture was stirred for 20 min at rt. Then D-γ-glutamic acid-(1-methyl ester)-L-lysine-(N^{ε}-dansyl)-D-alanine-D-alanine methyl ester **20** (74 mg, 0.11 mmol, 1.0 eq.) and DIPEA (0.4 mL, 28 mg, 0.22 mmol, 2.0 eq.) were added and the reaction mixture was stirred for 20 h at rt. Then a second portion of HOBt (45 mg, 0.33 mmol, 0.5 eq.) and EDC·HCl (63 mg, 0.33 mmol, 0.5 eq.) were added and the reaction mixture was further stirred for 4 d at rt. EtOAc was added and the solution was washed with water, aq. HCl (0.5 M), water and sat. aq. Na_2CO_3 (50 mL each). The combined aqueous layers were extracted with EtOAc (3 x 50 mL), and the combined organics were dried over Na_2SO_4 and concentrated *in vacuo.* Purification by column chromatography (CH_2Cl_2:MeOH 95:5) yielded **19** (53 mg, 55%) as a greenish solid. $[\alpha]_D^{25}$ = -1.5 (c = 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, DMSO-d_6): δ = 8.45 (d, *J* = 8.5 Hz, 1H, dansyl-H-2), 8.30 (d,

J = 8.7 Hz, 1H, dansyl-H-8), 8.23 (d, *J* = 7.6 Hz, 1H, NH), 8.12 (d, *J* = 7.1 Hz, 1H, NH), 8.09-8.04 (m, 2H, NH, dansyl-H-4), 7.92-7.86 (m, 3H, Fmoc-H-4, Fmoc-H-5, NH), 7.80 (dd, *J* = 5.9 Hz, *J* = 5.9 Hz, 1H, Lys-N$^{\varepsilon}$H), 7.73-7.70 (m, 2H, Fmoc-H-1, Fmoc-H-8), 7.61-7.53 (m, 2H, dansyl-H-3, dansyl-H-7), 7.41-7.36 (m, 3H, Fmoc-H-3, Fmoc-H-6, NH), 7.31 (dd, *J* = 7.4 Hz, *J* = 7.4 Hz, 2H, Fmoc-H-2, Fmoc-H-7), 7.24 (d, *J* = 7.2 Hz, 1H, dansyl-H-6), 4.30-4.05 (m, 8H, Ala-H-2, Lys-H-2, Glu-H-2, Fmoc-H-9, Fmoc-CH_2), 3.59 (s, 6H, 2 x OCH_3), 2.82 (s, 6H, $N(CH_3)_2$), 2.77-2.70 (m, 2H, Lys-H-6), 2.18-2.13 (m, 2H, Glu-H-4), 1.98-1.76 (m, 2H, Glu-H-3), 1.51-1.13 (m, 6H, Lys-H-3, Lys-H-4, Lys-H-5), 1.28 (d, *J* = 7.3 Hz, 3H, Ala-H-3), 1.24 (d, *J* = 7.1 Hz, 3H, Ala-H-3), 1.18 (d, *J* = 7.1 Hz, 3H, Ala-H-3) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): δ = 172.50 (Lys-C-1), 172.37 (Glu-C-1), 171.85 (Glu-C-5), 171.73 (Ala-C-1), 171.18 (Ala-C-1), 171.05 (Ala-C-1), 155.34 (Fmoc-C=O), 151.10 (dansyl-C-5), 143.66 (dansyl-C-1), 143.55 (Fmoc-C-1a, Fmoc-C-8a), 140.47 (Fmoc-C-4a, Fmoc-C-4b), 135.94 (dansyl-C-8a), 129.07 (dansyl-C-4a), 128.92 (dansyl-C-2), 128.88 (dansyl-C-7), 127.90 (Fmoc-C-3, Fmoc-C-6), 127.38 (dansyl-C-4), 126.84 (Fmoc-C-2, Fmoc-C-7), 125.06 (Fmoc-C-1, Fmoc-C-8), 123.32 (dansyl-C-3), 119.85 (dansyl-C-8), 118.95 (Fmoc-C-4, Fmoc-C-5), 114.90 (dansyl-C-6), 65.55 (Fmoc-CH_2), 54.76 (Lys-C-2), 52.69 (Glu-C-2), 51.69 (OCH_3), 51.66 (OCH_3), 49.76 (Ala-C-2), 47.51 (Ala-C-2), 47.45 (Ala-C-2), 46.58 (Fmoc-C-9), 44.96 ($N(CH_3)_2$), 42.22 (Lys-C-6), 31.15 (Glu-C-4), 31.07 (Lys-C-3), 28.87 (Lys-C-5), 27.00 (Glu-C-3), 22.32 (Lys-C-4), 18.52 (Ala-C-3), 17.89 (Ala-C-3), 16.71 (Ala-C-3) ppm; IR: *v* = 3293, 2957, 1654, 1632, 1536, 1448, 1229, 1143, 791, 740 cm^{-1}; MS (HR-ESI): *m/z*: calcd. 994.3991 $[M+Na]^+$; found: 994.3998.

***N*-Cbz-L-alanine-D-glutamic acid-(1-methyl ester)-L-lysine-(N^{ε}-dansyl)-D-alanine-D-alanine methyl ester (24)**

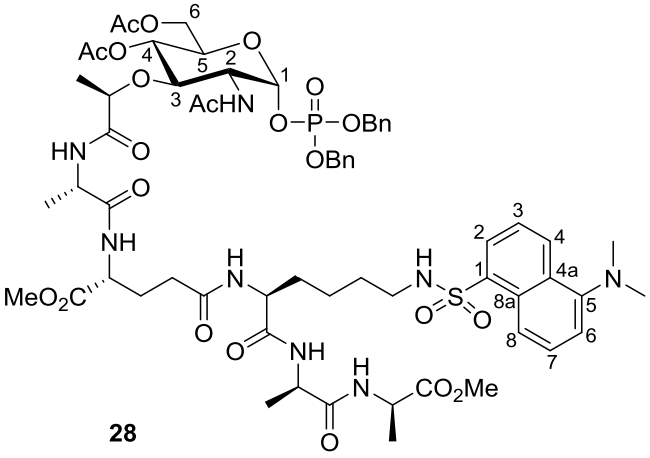


24

The reaction was carried out under an inert atmosphere of argon. To a solution of *N*-Cbz-L-alanine **22**[S3] (124 mg, 0.55 mmol, 2.0 eq.) in dry THF (7 mL), HOBt (75 mg, 0.55 mmol, 2.0 eq.) and EDC·HCl (106 mg, 0.55 mmol, 2.0 eq.) were added and the mixture was stirred for 20 min at rt. Then D-γ-glutamic acid-(1-methyl ester)-L-lysine-(N^{ε}-dansyl)-D-alanine-D-alanine methyl ester **20** (188 mg, 0.28 mmol, 1.0 eq.) and DIPEA (0.9 mL, 72 mg, 0.55 mmol, 2.0 eq.) were added and the reaction mixture was stirred for 20 h at rt. Then a second portion of HOBt (45 mg, 0.33 mmol, 0.5 eq.) and EDC·HCl (63 mg, 0.33 mmol, 0.5 eq.) were added and the reaction mixture was further stirred for 44 h at rt. EtOAc was added and the solution was washed with water, aq. HCl (0.5 M), water and sat. aq. Na_2CO_3 (50 mL each). The combined aqueous layers were extracted with EtOAc (3 x 50 mL), and the combined organics were dried over Na_2SO_4 and concentrated *in vacuo*. Purification by column chromatography (CH_2Cl_2:MeOH 95:5) yielded **24** (145 mg, 59%) as a greenish oil. $[\alpha]_D^{25}$ = +3.6 (c = 0.42, MeOH); ^{1}H NMR (300 MHz, DMSO-d_6): δ = 8.45 (d, *J* = 8.5 Hz, 1H, dansyl-H-2), 8.30-8.26 (m, 2H, dansyl-H-8, NH), 8.18 (d, *J* = 7.1 Hz, Ala-NH), 8.13 (d, *J* = 7.9 Hz, 1H, dansyl-H-4), 8.08 (d, *J* = 7.3 Hz, Ala-NH), 7.96 (d, *J* = 7.4 Hz, 1H, NH), 7.86 (dd, *J* = 5.6 Hz, *J* = 5.6 Hz, 1H, Lys-N$^{\varepsilon}$H), 7.64-7.55 (m, 2H, dansyl-H-3, dansyl-H-7), 7.39 (d, *J* = 7.9 Hz, 1H, NH), 7.35-7.26 (m, 5H, Cbz-CH), 7.25 (d, *J* = 7.3 Hz, 1H, dansyl-H-6), 5.01 (d, *J* = 2.8 Hz, 2H, Cbz-CH_2), 4.29-4.17 (m, 3H, 3 x Ala-H-2), 4.12-4.05 (m, 2H, Lys-H-2, Glu-H-2), 3.59 (s,

3H, OCH_3), 3.34 (s, 3H, OCH_3), 2.82 (s, 6H, $N(CH_3)_2$), 2.75-2.69 (m, 2H, Lys-H-6), 2.18-2.11 (m, 2H, Glu-H-4), 1.99-1.70 (m, 2H, Glu-H-3), 1.50-1.22 (m, 6H, Lys-H-3, Lys-H-4, Lys-H-5), 1.28 (d, J = 7.3 Hz, 3H, Ala-H-3), 1.19 (d, J = 7.1 Hz, 3H, Ala-H-3), 1.19 (d, J = 7.1 Hz, 3H, Ala-H-3) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): δ = 172.66 (Lys-C-1), 172.52 (Glu-C-1), 171.99 (Glu-C-5), 171.89 (Ala-C-1), 171.34 (Ala-C-1), 171.21 (Ala-C-1), 155.46 (Cbz-C=O), 151.24 (dansyl-C-5), 136.91 (dansyl-C-1), 136.07 (Cbz-C-1), 129.17 (dansyl-C-8a), 129.02 (dansyl-C-4a), 128.99 (dansyl-C-2), 128.18 (Cbz-C-2, Cbz-C-3, Cbz-C-5, Cbz-C-6), 128.00 (Cbz-C-4), 127.62 (dansyl-C-7), 127.54 (dansyl-C-4), 123.42 (dansyl-C-3), 119.04 (dansyl-C-8), 114.99 (dansyl-C-6), 65.27 (Cbz-CH_2), 52.68 (Lys-C-2), 51.67 (Glu-C-2), 51.65 (OCH_3), 51.43 (OCH_3), 49.81 (Ala-C-2), 47.50 (Ala-C-2), 47.44 (Ala-C-2), 44.95 ($N(CH_3)_2$), 42.21 (Lys-C-6), 31.11 (Glu-C-4), 31.03 (Lys-C-3), 28.83 (Lys-C-5), 26.93 (Glu-C-3), 22.27 (Lys-C-4), 18.41 (Ala-C-3), 17.83 (Ala-C-3), 16.66 (Ala-C-3) ppm; IR: ν = 3279, 2917, 1649, 1630, 1536, 1458, 1257, 1022, 797, 626 cm^{-1}; MS (HR-ESI): *m/z*: calcd. for 906.3678 $[M+Na]^+$; found: 906.3679.

Protected dansylated 1-(dibenzylphospho)-muramic acid pentapeptide (28)



28

The reaction was carried out under an inert atmosphere of argon. To a solution of di-*O*-benzyl-4,6-*O*-diacetyl-3-*O*-((*R*)-propion-2-yl-alanyl-phenylsulfonylethyl ester)-*N*-acetyl-α-D-glucosamine-1-phosphate **7** (18 mg, 20 μmol, 1.0 eq.) in dry CH_2Cl_2 (1 mL), DBU (3 μL,

3 mg, 21 μmol, 1.0 eq.) was added. The reaction mixture was stirred for 16 h at rt. Aq. HCl (1 M, 0.7 mL) and CH_2Cl_2 (5 mL), was subsequently added. The organic layer was washed with water (2 x 5 mL) and brine (5 mL), dried over Na_2SO_4 and concentrated *in vacuo* to yield **27** (16 mg, quant.) which was used without further purification.

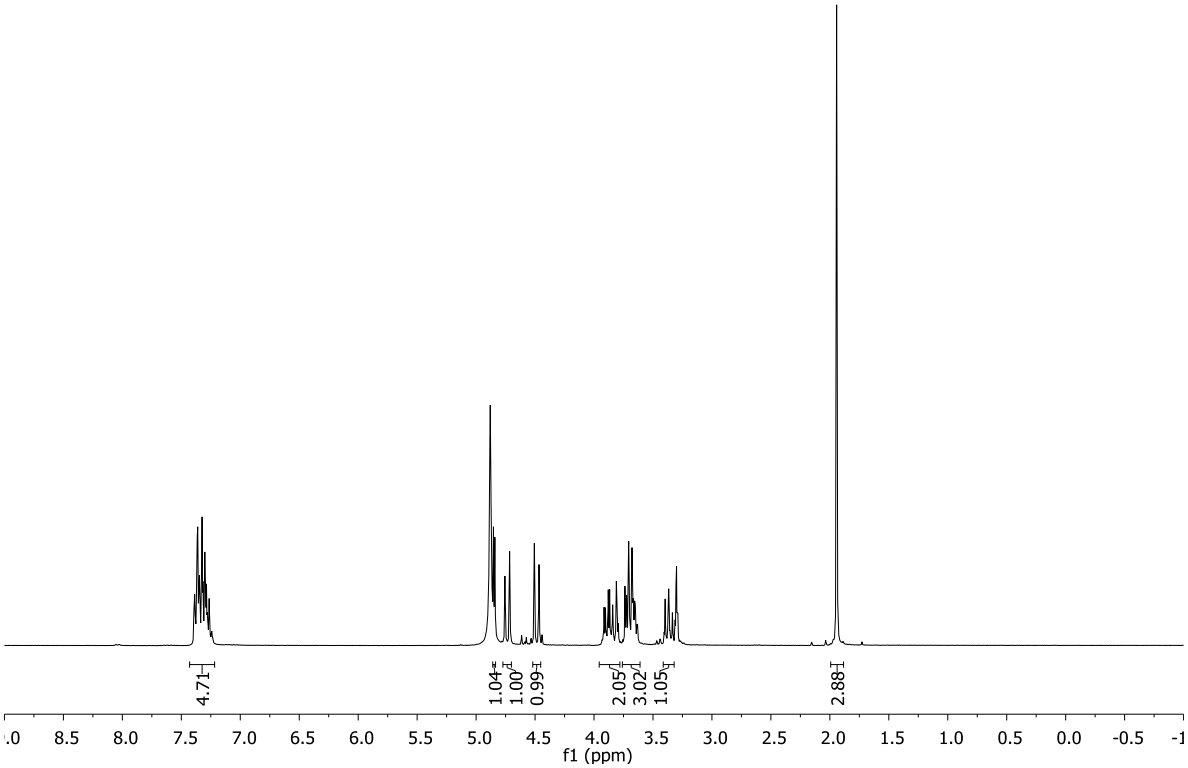
To a solution of the thus obtained carboxylic acid **27** (16 mg) in dry DMF (1 mL), HATU (11 mg, 30 μmol, 1.5 eq.) and DIPEA (30 μL, 23 mg, 0.17 mmol, 8.5 eq.) were added. The reaction mixture was stirred for 25 h at rt, then concentrated *in vacuo* and dissolved in CH_2Cl_2 (5 mL). The resultant solution was washed with sat. aq. $NaHCO_3$ (5 mL) and the aqueous layer was extracted with CH_2Cl_2 (2 x 5 mL). The combined organics were dried over Na_2SO_4 and concentrated *in vacuo*. Purification by column chromatography (CH_2Cl_2:MeOH 96:4) yielded **28** (20 mg, 73% over 2 steps from **7**) as a greenish solid. $[\alpha]_D^{25}$ = +21.5 (c = 1.1, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.50 (d, *J* = 8.4 Hz, 1H, dansyl-H-2), 8.22 (d, *J* = 8.6 Hz, 1H, dansyl-H-8), 8.10 (dd, *J* = 7.3 Hz, *J* = 1.1 Hz, 1H, dansyl-H-4), 7.71 (d, *J* = 8.1 Hz, 1H, NH), 7.51-7.40 (m, 2H, dansyl-H-3, dansyl-H-7), 7.31-7.20 (m, 11H, Ar-H, NH), 7.16 (d, *J* = 7.5 Hz, 1H, dansyl-H-6), 7.11-7.05 (m, 1H, NH), 6.88 (d, *J* = 9.8 Hz, 1H, acetyl-NH), 6.84-6.76 (m, 1H, NH), 5.86 (t, *J* = 6.4 Hz, 1H, Lys-N$^{\varepsilon}$H), 5.62 (dd, J_{HP} = 5.7 Hz, *J* = 3.2 Hz, 1H, Glc-H-1), 5.10-4.94 (m, 5H, Bn-CH_2, Glc-H-5), 4.55-4.44 (m, 4H, Glc-H-2, Lys-H-2, 2 x Ala-H-2), 4.32-4.25 (m, 1H, Ala-H-2), 4.15-4.10 (m, 2H, Glu-H-2, Glc-H-6_a), 3.93-3.86 (m, 3H, propionyl-H-2, Glc-H-4, Glc-H-6_b), 3.71 (s, 3H, OCH_3), 3.70-3.65 (m, 1H, Glc-H-3), 3.64 (s, 3H, OCH_3), 2.86 (s, 6H, $N(CH_3)_2$), 2.84-2.78 (m, 2H, Lys-H-6), 2.52-2.43 (m, 1H, Glu-H-4_a), 2.32-2.14 (m, 2H, Glu-H-3_a, Glu-H-4_b), 2.07 (s, 3H, *N*-acetyl-CH_3), 2.00 (s, 3H, *O*-acetyl-CH_3), 1.89 (s, 3H, *O*-acetyl-CH_3), 1.74-1.69 (m, 1H, Glu-H-3_b), 1.52 (d, *J* = 7.2 Hz, 3H, Ala-H-3), 1.42-1.23 (m, 6H, Lys-H-3, Lys-H-4, Lys-H-5), 1.41 (d, *J* = 8.7 Hz, 3H, Ala-H-3), 1.32 (d, *J* = 6.6 Hz, 3H, Ala-H-3), 1.25 (d, *J* = 10.3 Hz, 3H, propionyl-CH_3) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ = 175.31 (C=O), 173.38 (C=O), 173.10 (C=O), 172.43

(C=O), 127.24 (C=O), 171.89 (C=O), 171.29 (C=O), 170.55 (C=O), 169.49 (C=O), 152.19 (dansyl-C-5), 135.31 (d, J_{CP} = 7.0 Hz, Ar-C), 135.24, (d, J_{CP} = 7.0 Hz, Ar-C), 134.32 (dansyl-C-1), 130.62 (dansyl-C-2), 129.82 (dansyl-C-4), 129.42 (dansyl-C-8a), 128.76 (Ar-CH), 128.66 (Ar-CH), 128.57 (Ar-CH), 128.54 (Ar-CH), 128.14 (dansyl-C-7), 128.01 (Ar-CH), 127.88 (Ar-CH), 127.40 (dansyl-C-4a), 123.23 (dansyl-C-3), 118.15 (dansyl-C-8), 115.22 (dansyl-C-6), 96.61 (d, J_{CP} = 6.4 Hz, Glc-C-1), 78.81 (propionyl-C-2), 78.79 (Glc-C-3), 69.99 (Glc-C-4), 69.81, (d, J_{CP} = 5.5 Hz, Bn-CH_2), 69.73 (d, J_{CP} = 5.5 Hz, Bn-CH_2), 68.53 (Glc-C-6), 61.34 (Glc-C-5), 54.97 (Lys-C-2), 52.84 (d, J_{CP} = 8.3 Hz, Glc-C-2), 52.59 (OCH_3), 52.17 (OCH_3), 51.39 (Glu-C-2), 49.92 (Ala-C-2), 49.40 (Ala-C-2), 47.93 (Ala-C-2), 45.33 ($N(CH_3)_2$), 41.41 (Lys-C-6), 31.36 (Glu-C-4), 30.36 (Lys-C-3), 28.28 (Glu-C-3), 27.73 (Lys-C-5), 22-95 (*N*-acetyl-CH_3), 21.42 (Lys-C-4), 20.88 (*O*-acetyl-CH_3), 20.61 (*O*-acetyl-CH_3), 19.06 (Ala-C-3), 18.18 (Ala-C-3), 17.21 (propionyl-CH_3), 16.89 (Ala-C-3) ppm; ^{31}P NMR (121 MHz, $CDCl_3$): δ = -1.33 ppm; IR: ν = 3297, 3029, 2934, 1650, 1636, 1552, 1454, 1377, 1122, 1091, 1038, 734, 695 cm^{-1}; MS (HR-ESI): *m/z*: calcd. for 1391.5129 $[M+Na]^+$; found: 1391.5136.

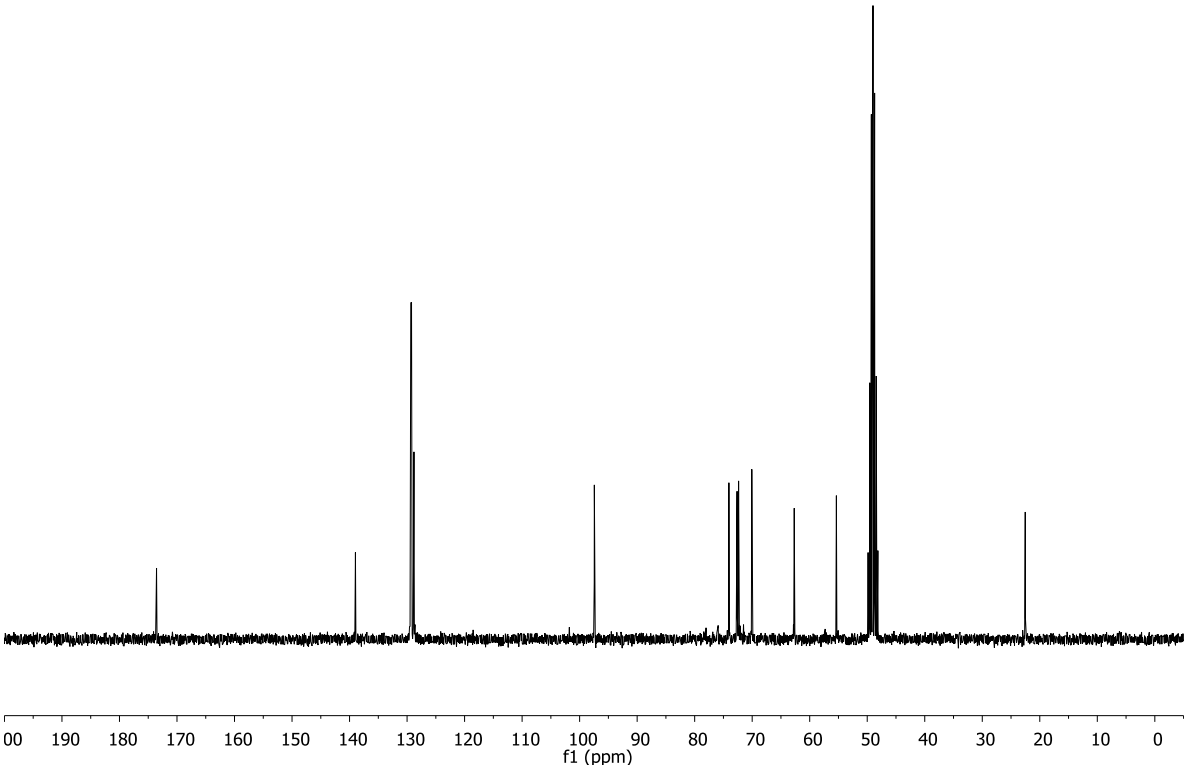
Sequence of the *mraY* gene

5'-G**CCATG**G<u>TTTTTGTATATGCGTTATTAGCGCTAGTGATTACATTTGTTTTGGTA
CCTGTTTTAATACCTACATTAAAAAGGATGAAATTTGGTCAAAGTATTCGAGAAG
AAGGCCCACAAAGCCATATGAAGAAGACTGGTACACCAACGATGGGTGGACTAA
CATTTCTATTAAGTATTGTGATAACGTCTTTGGTGGCTATTATATTTGTAGATCAA
GCTAATCCAATCATACTGTTATTATTTGTGACGATTGGTTTTGGGTTAATTGGTTT
TATAGATGATTATATTATTGTTGTTAAAAAGAATAACCAAGGTTTAACAAGTAAA
CAGAAGTTTTTGGCGCAAATTGGTATTGCGATTATTTTCTTTGTTTTAAGTAATGT
ATTTCATTTGGTGAATTTTTCTACGAGCATACATATTCCATTTACGAATGTAGCAA
TCCCACTATCATTTGCATATGTTATTTTCATTGTTTTTTGGCAAGTAGGTTTTTCTA
ATGCGGTAAATTTAACAGATGGTTTAGATGGATTAGCAACTGGACTGTCAATTAT
CGGATTTACAATGTATGCCATCATGAGCTTTGTGTTAGGAGAAACGGCGATTGGT
ATTTTCTGTATCATTATGTTGTTTGCACTTTTAGGATTTTTACCATATAACATTAAC
CCTGCTAAAGTGTTTATGGGAGATACAGGTAGCTTAGCTTTAGGTGGTATATTTG
CTACGATTTCAATCATGCTTAATCAGGAATTATCATTAATTTTTATAGGTTTAGTA
TTCGTAATTGAAACATTATCTGTTATGTTACAAGTCGCTAGCTTTAAATTGACTGG
AAAGCGTATATTTAAAATGAGTCCGATTCATCATCATTTTGAATTGATAGGTTGG
AGTGAATGGAAAGTAGTTACAGTATTTTGGGCTGTTGGTCTGATTTCAGGTTTAAT
CGGTTTATGGATTGGAGTGCAT</u>**CTCGAG**-3' (NcoI/XhoI)

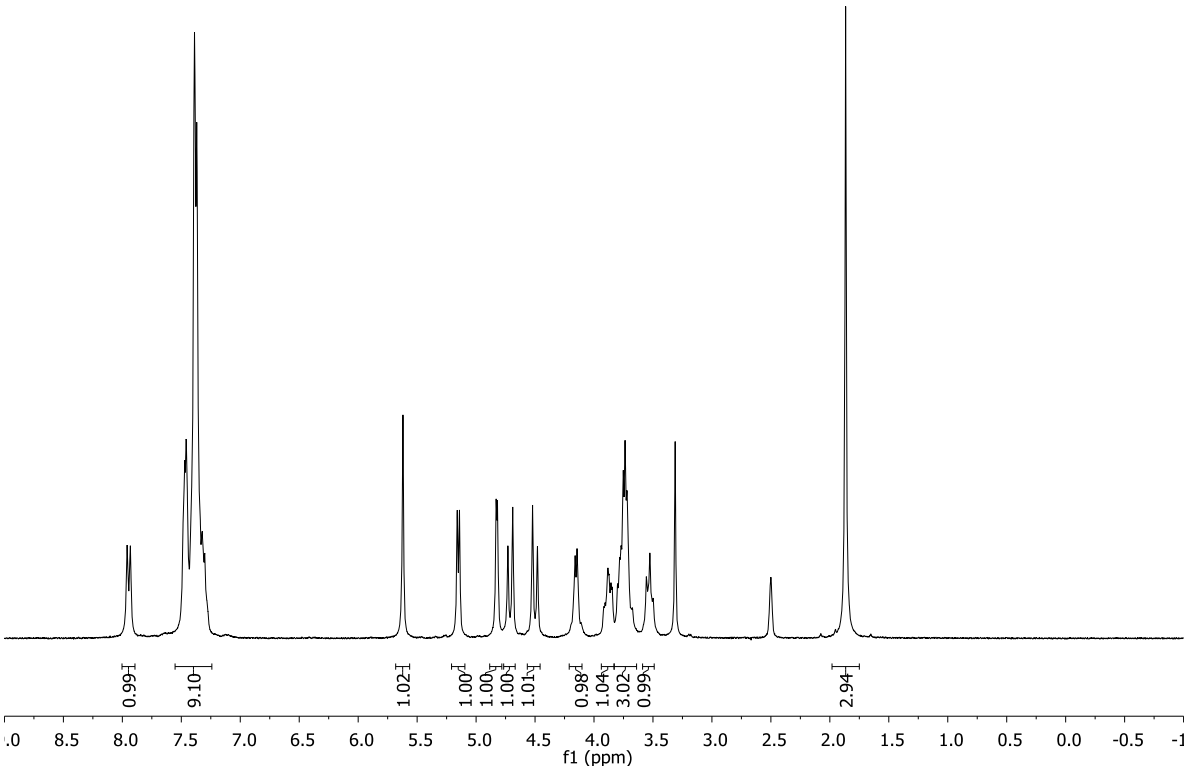
This sequence is identical to the *mray* gene of *S. aureus* subsp. *aureus* MRSA252 (underlined) except of the G (in red) at position 4, which was introduced to create the NcoI restriction site. Additional restriction sites (bold, named in parentheses) were also introduced.



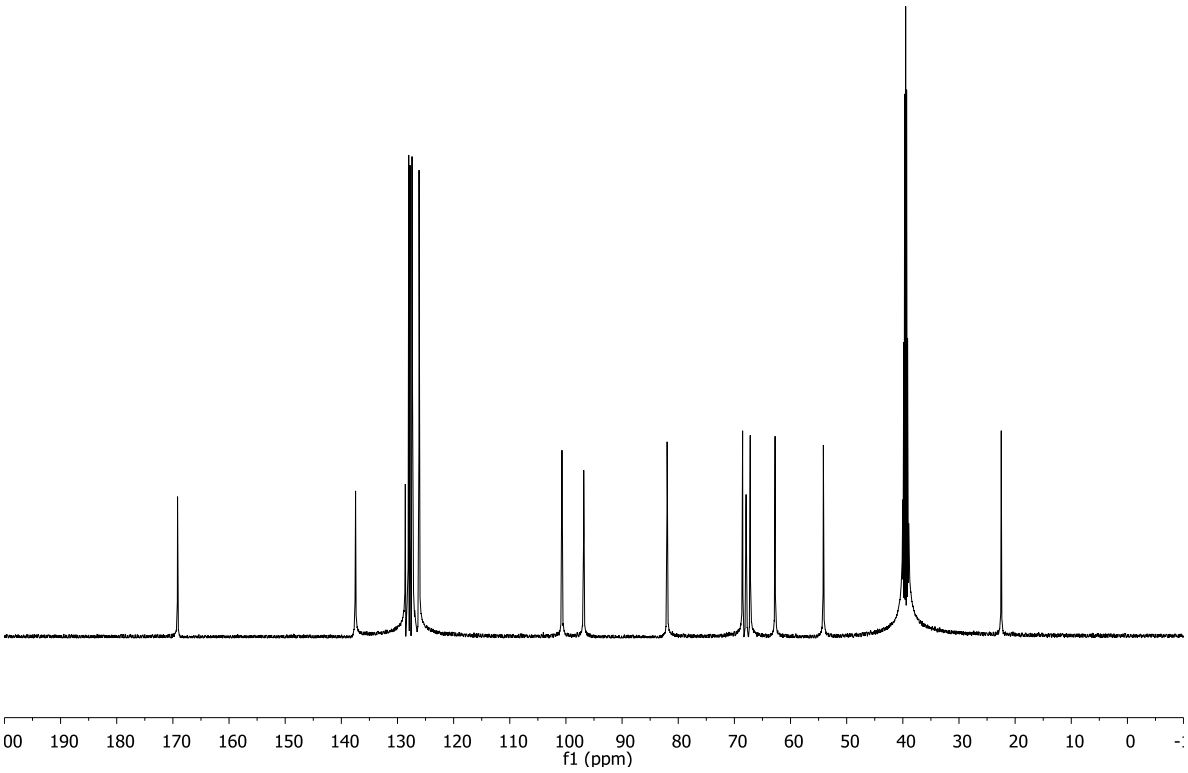
^{1}H NMR spectrum of **S1** (300 MHz, CD_3OD)



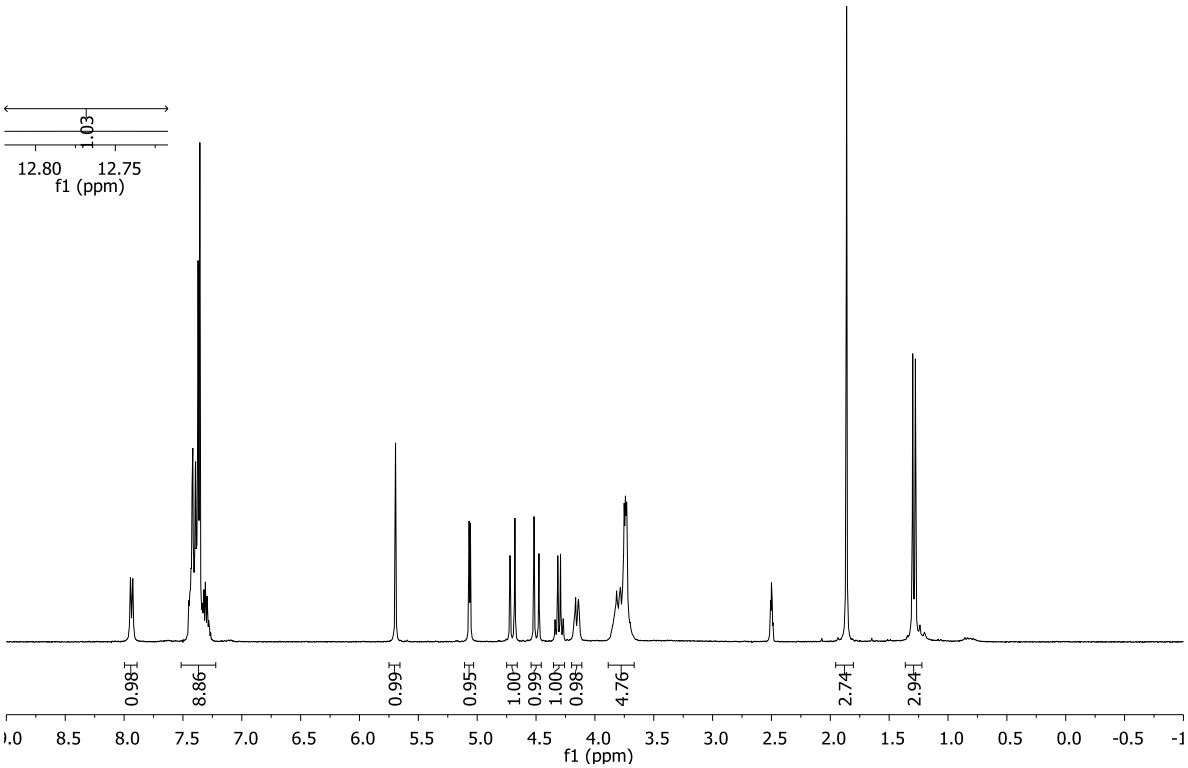
^{13}C NMR spectrum of **S1** (75 MHz, CD_3OD)



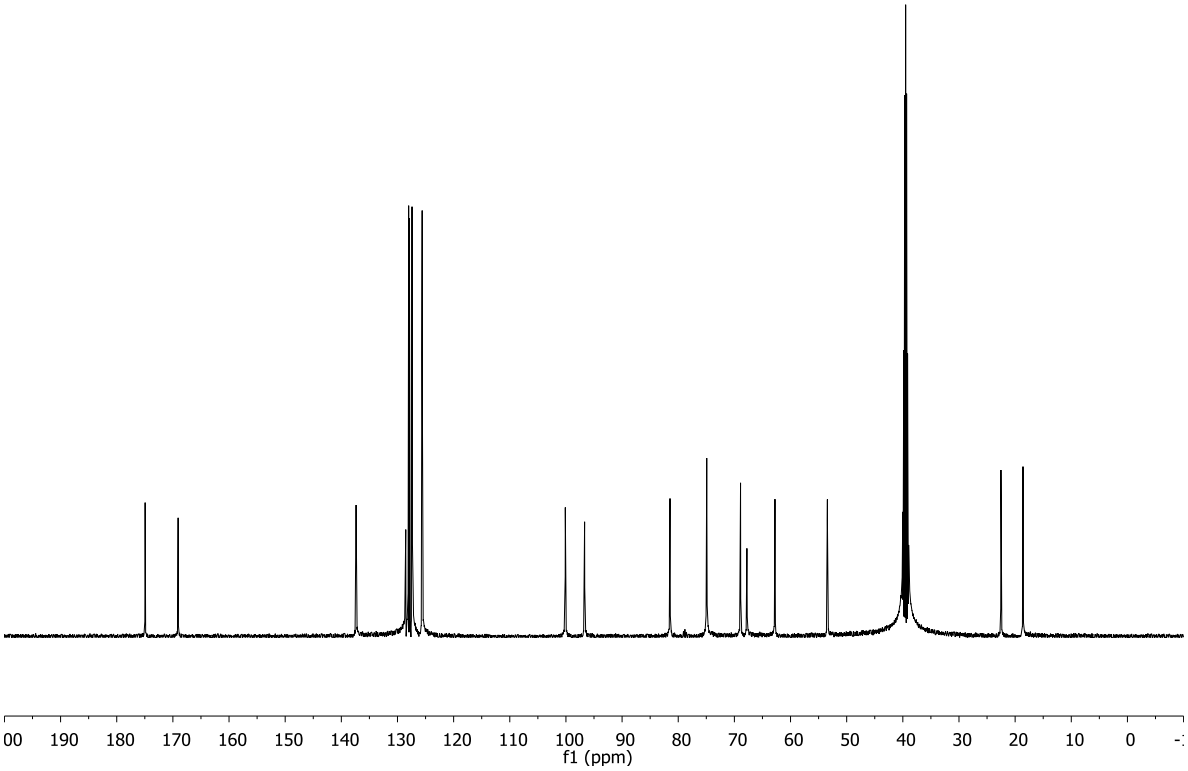
^{1}H NMR spectrum of **S2** (300 MHz, DMSO-d_6)



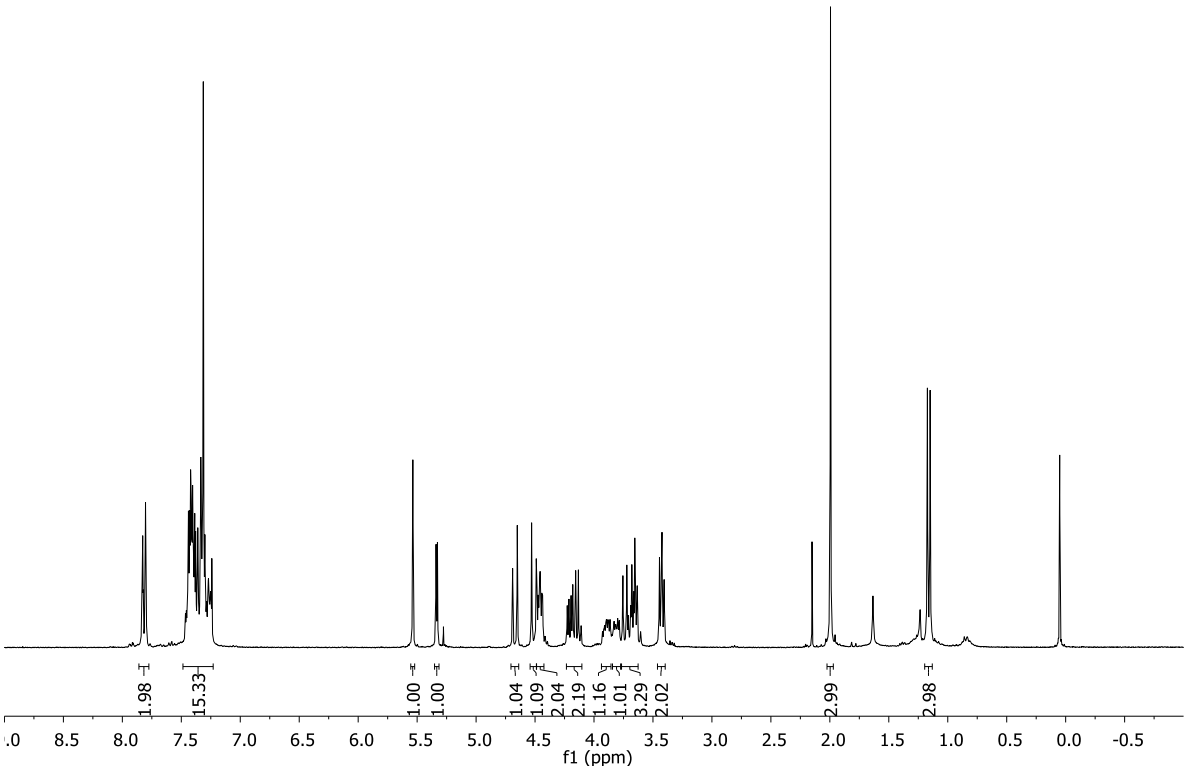
^{13}C NMR spectrum of **S2** (75 MHz, DMSO-d_6)



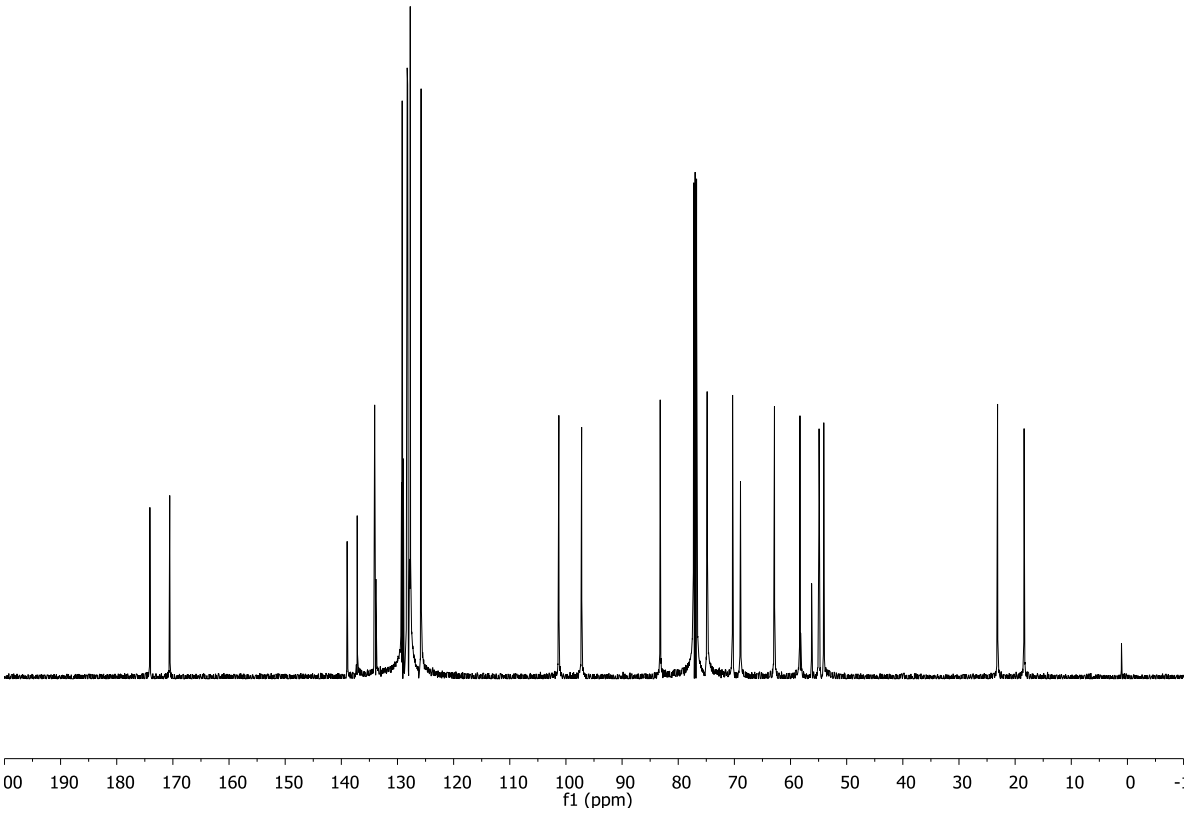
^{1}H NMR spectrum of **S4** (300 MHz, DMSO-d_6)



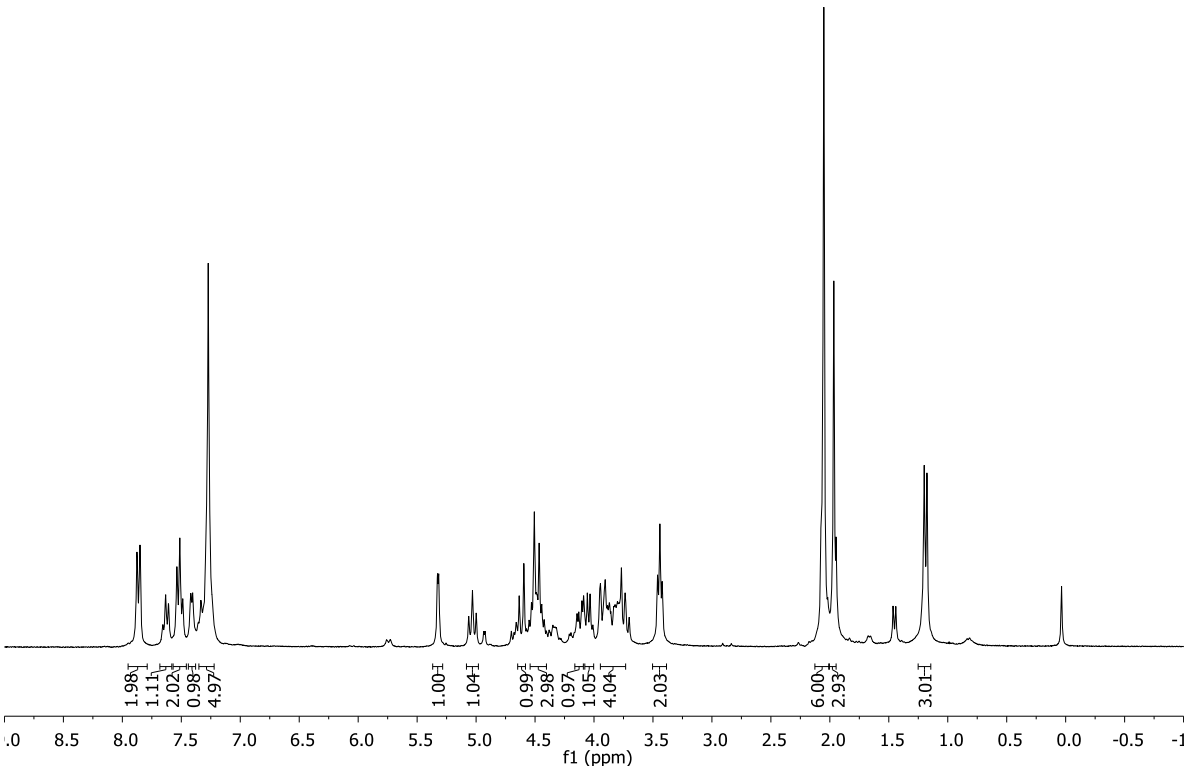
^{13}C NMR spectrum of **S4** (75 MHz, DMSO-d_6)



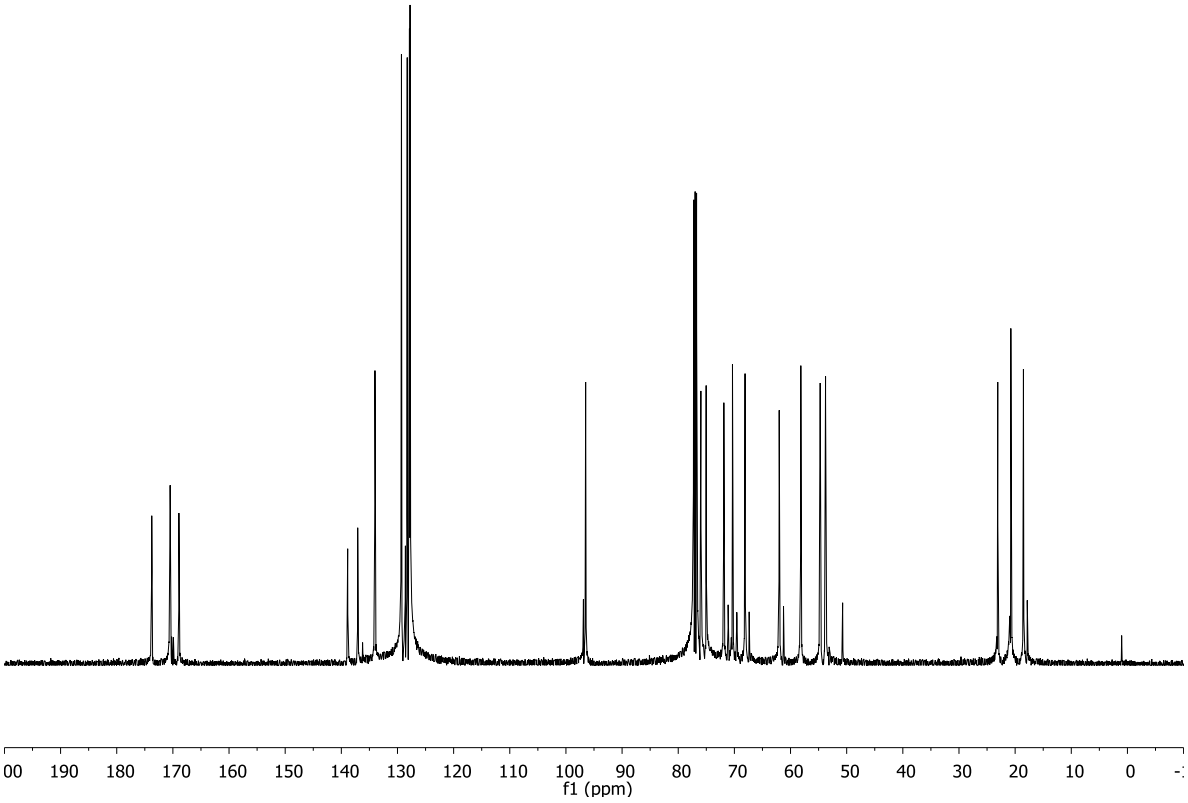
^{1}H NMR spectrum of **S5** (300 MHz, $CDCl_3$)



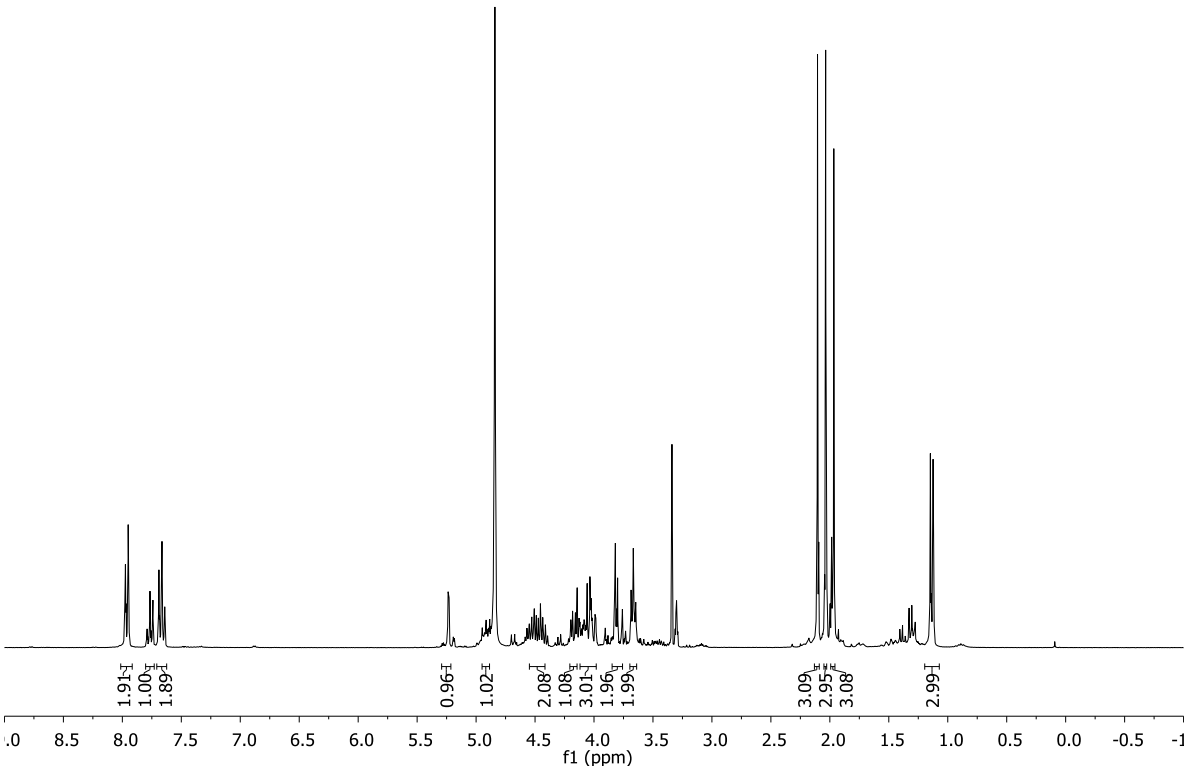
^{13}C NMR spectrum of **S5** (75 MHz, $CDCl_3$)



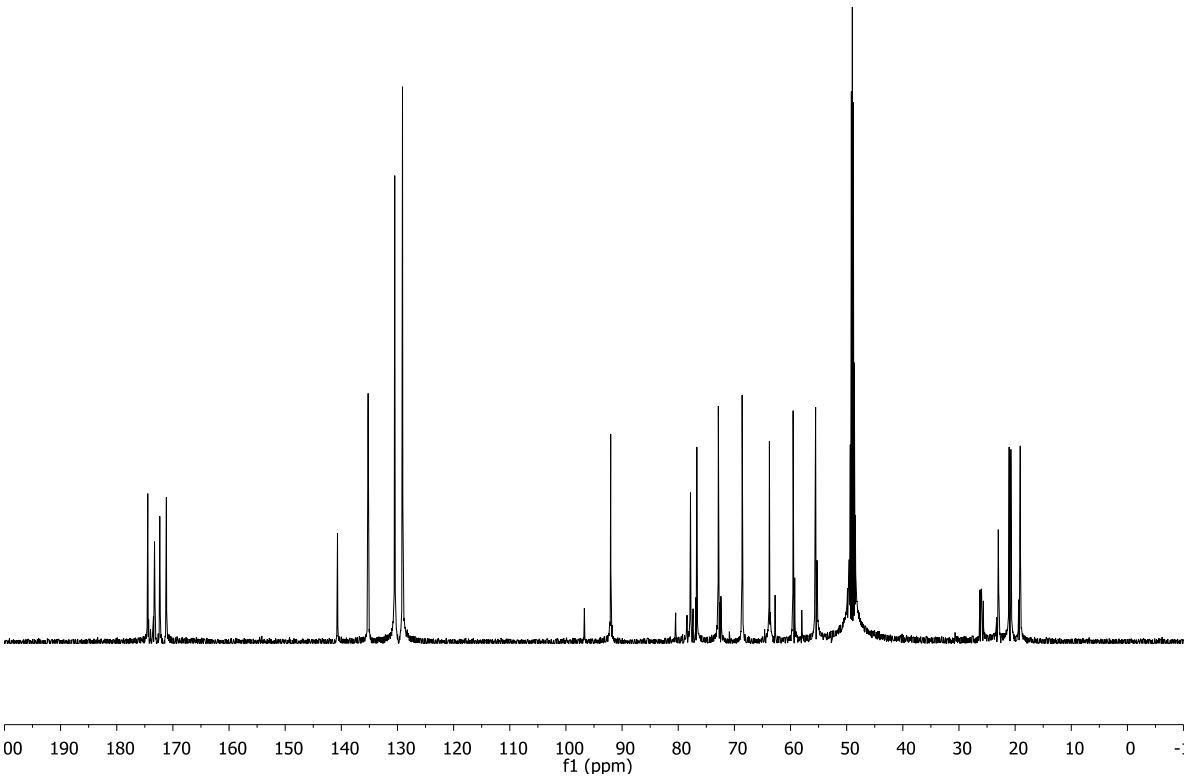
^{1}H NMR spectrum of **S6** (300 MHz, $CDCl_3$)



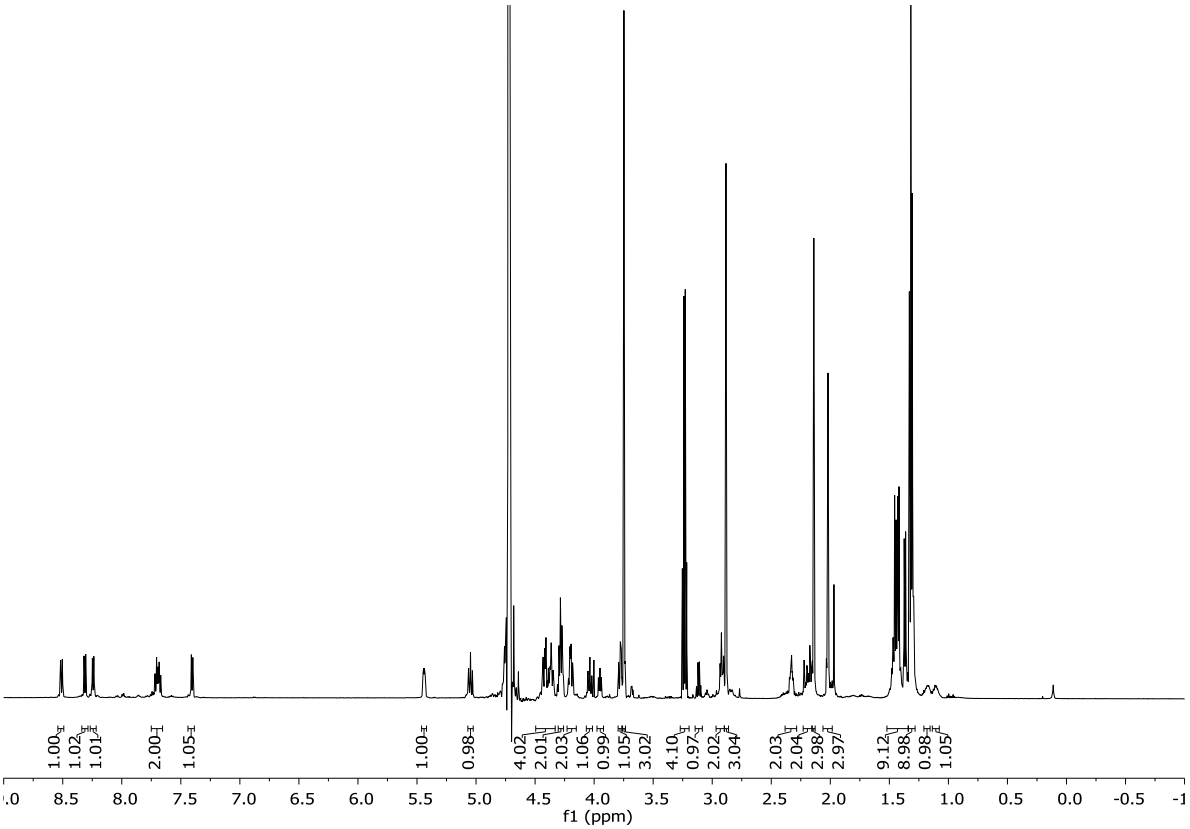
^{13}C NMR spectrum of **S6** (75 MHz, $CDCl_3$)



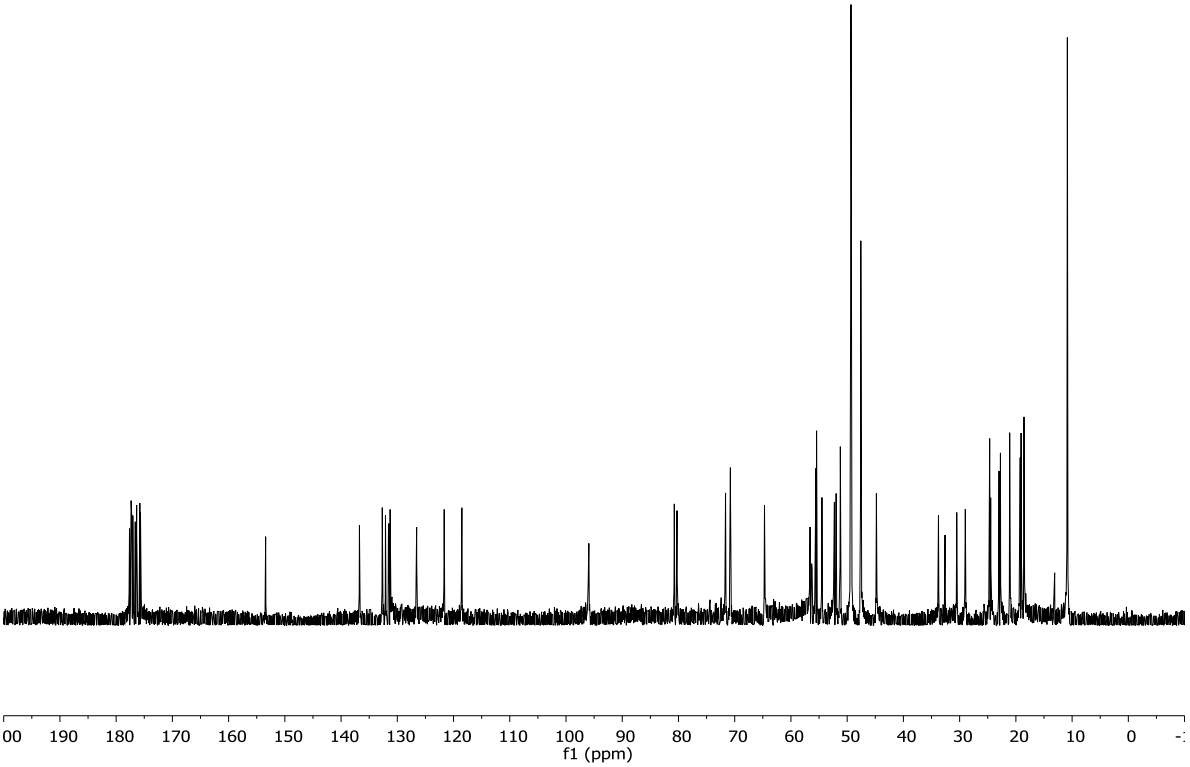
^{1}H NMR spectrum of **S7** (300 MHz, CD_3OD)



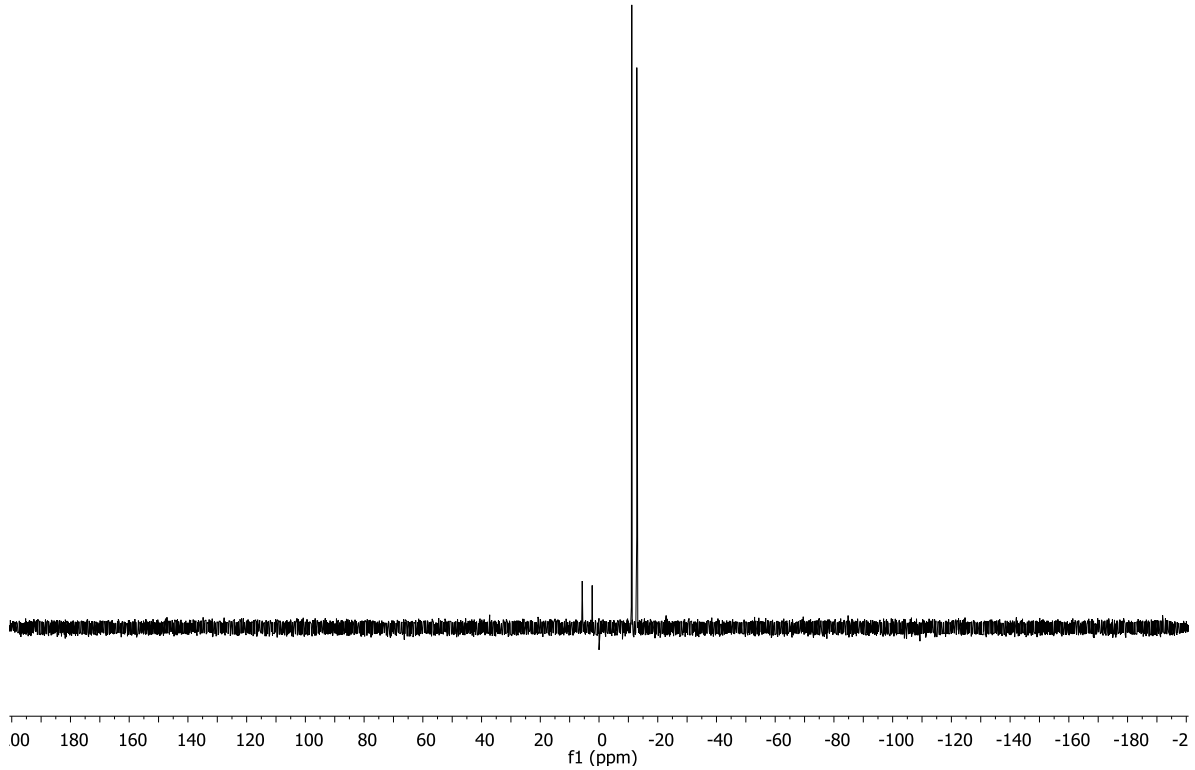
^{13}C NMR spectrum of **S7** (75 MHz, CD_3OD)



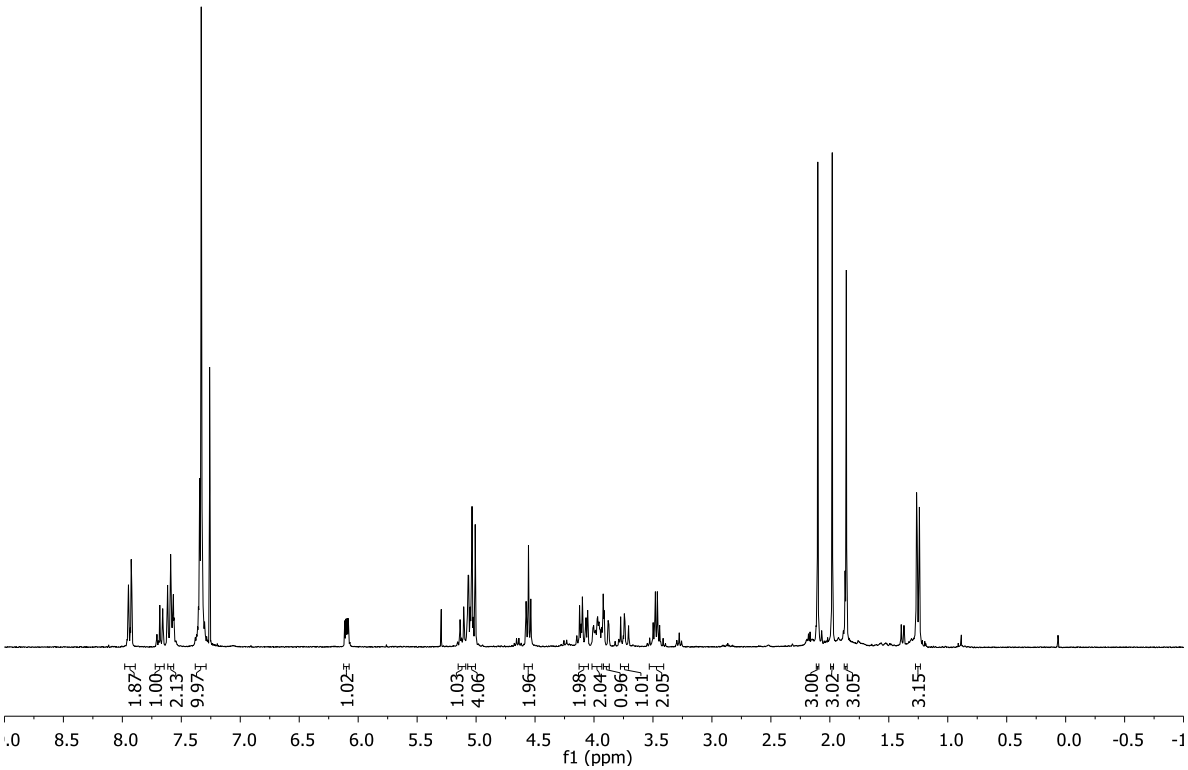
^{1}H NMR spectrum of **3** (500 MHz, D_2O)



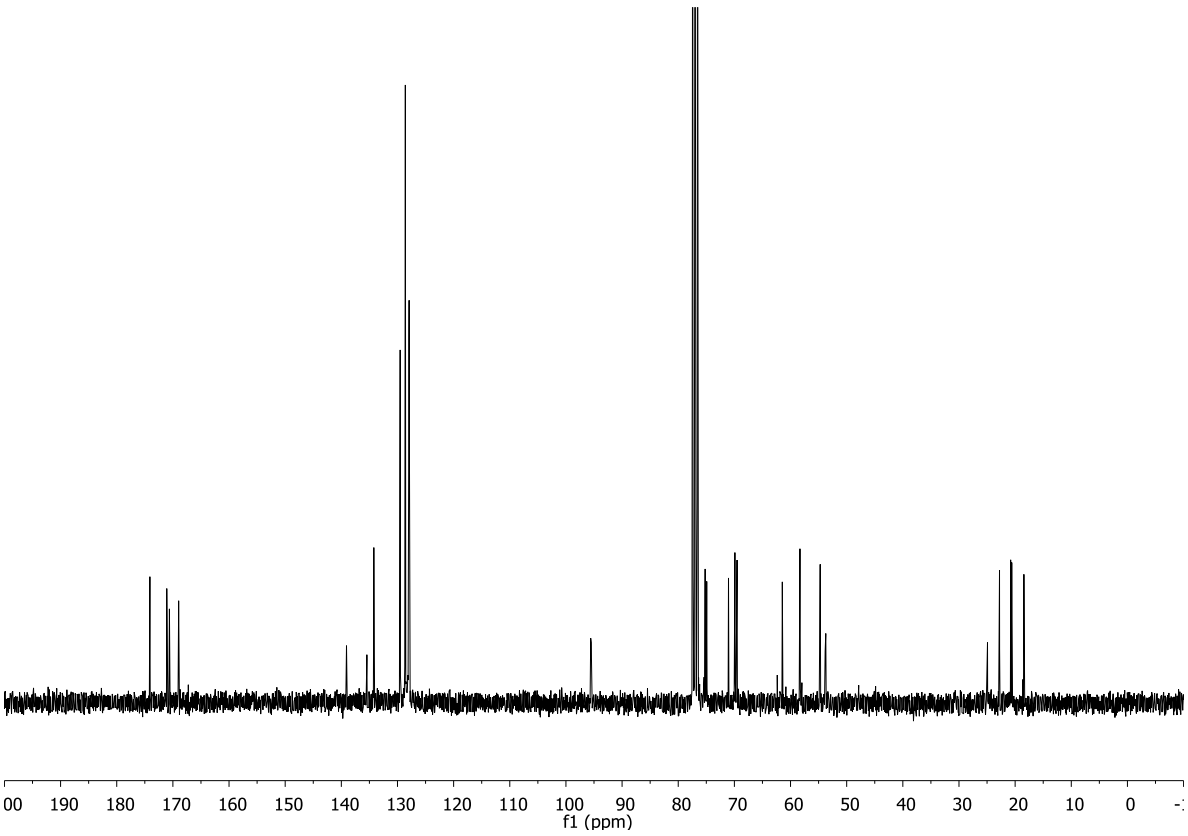
^{13}C NMR spectrum of **3** (126 MHz, D_2O)



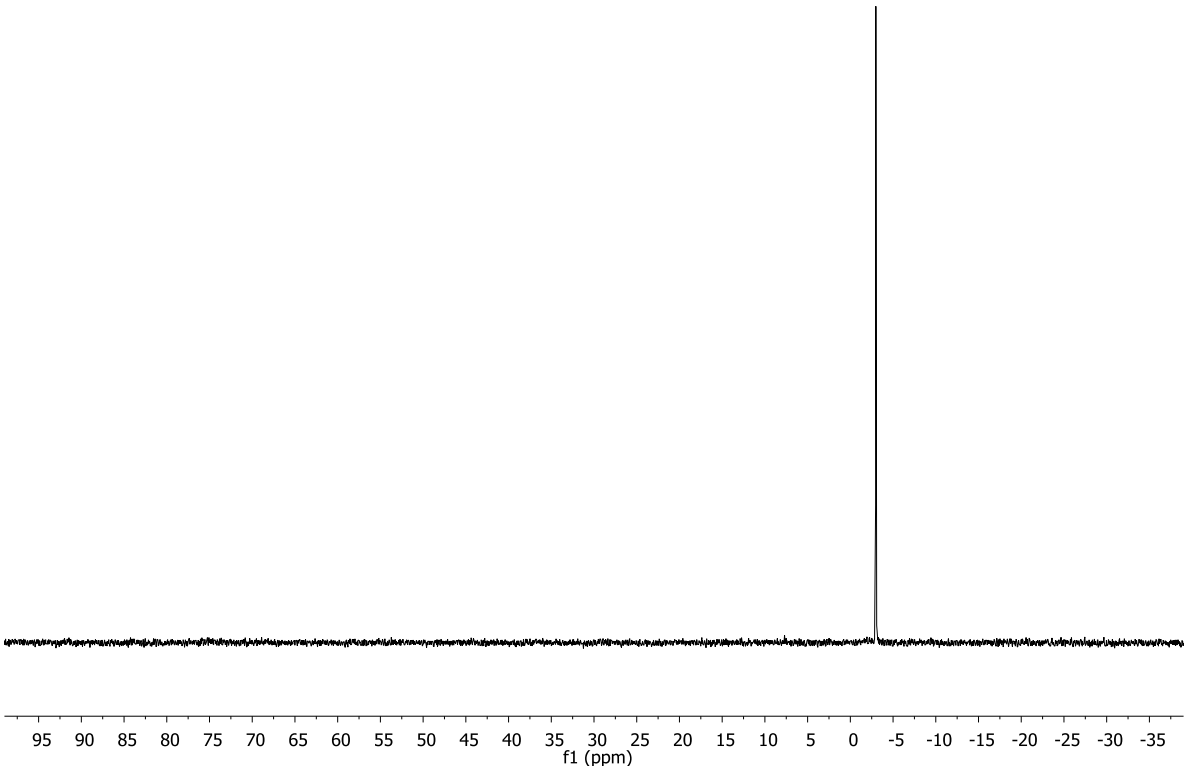
^{31}P NMR spectrum of **3** (121 MHz, D_2O)



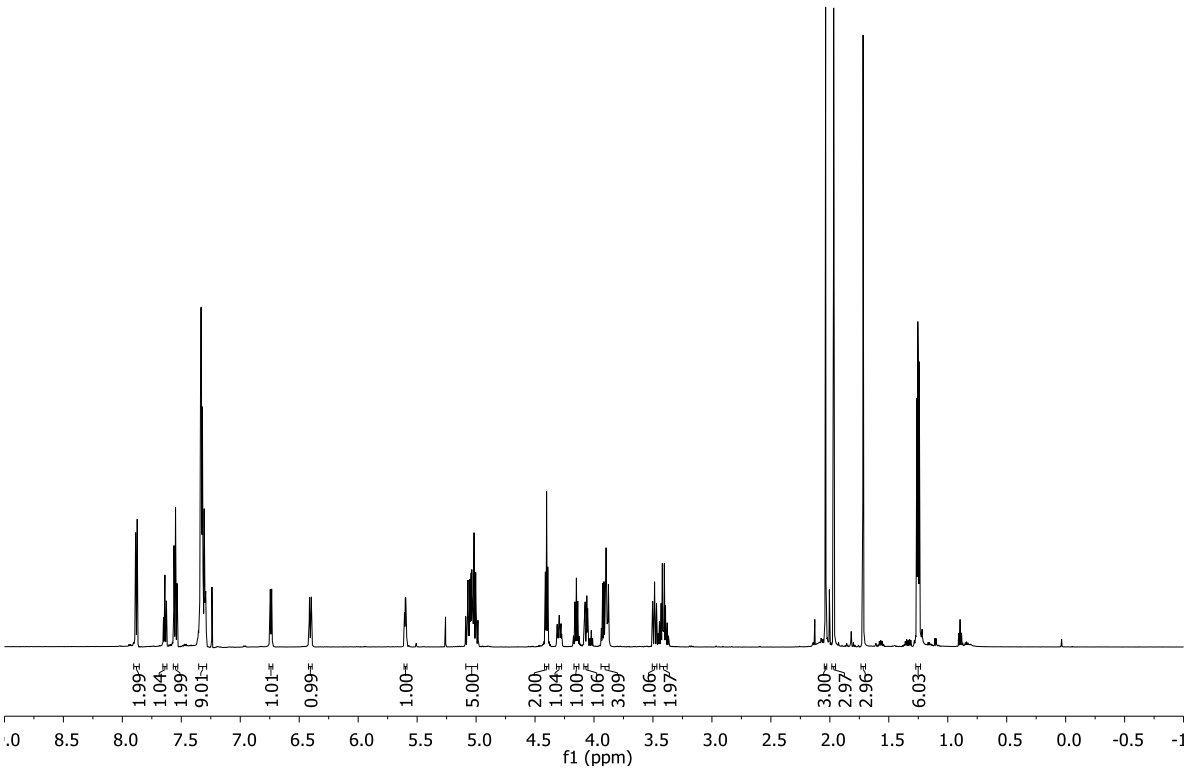
^{1}H NMR spectrum of **5** (300 MHz, $CDCl_3$)



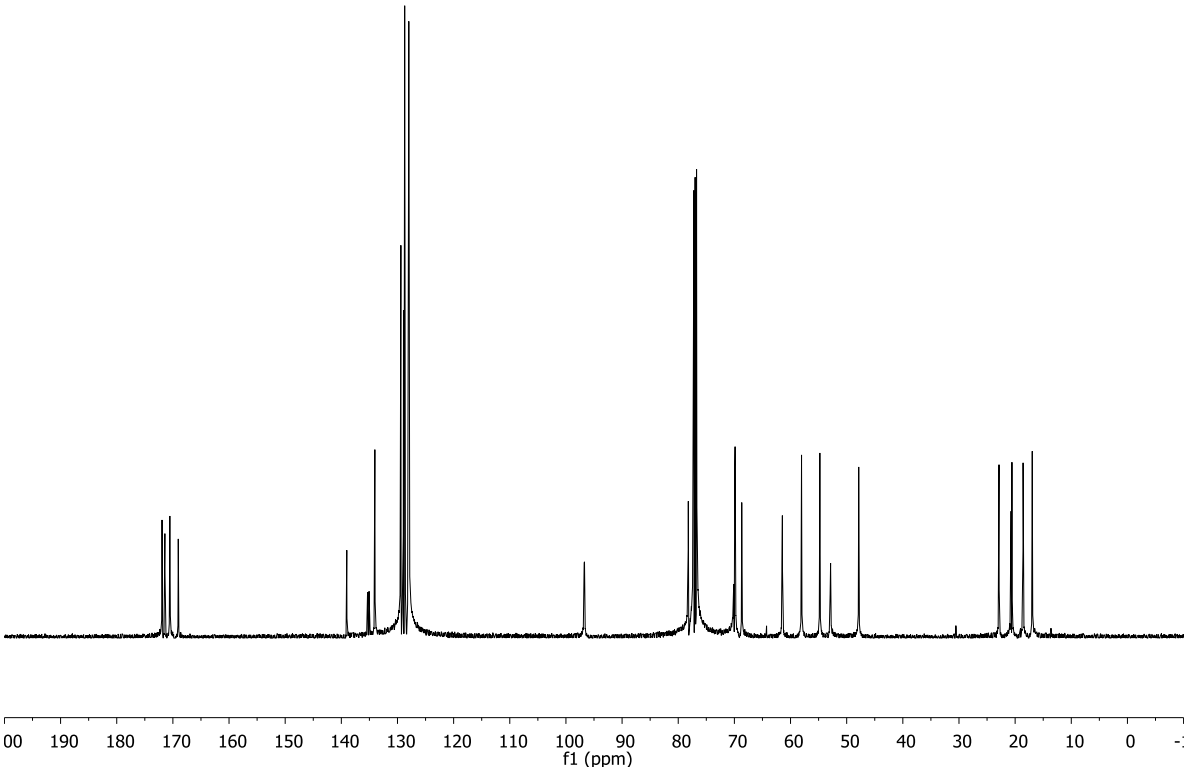
^{13}C NMR spectrum of **5** (75 MHz, $CDCl_3$)



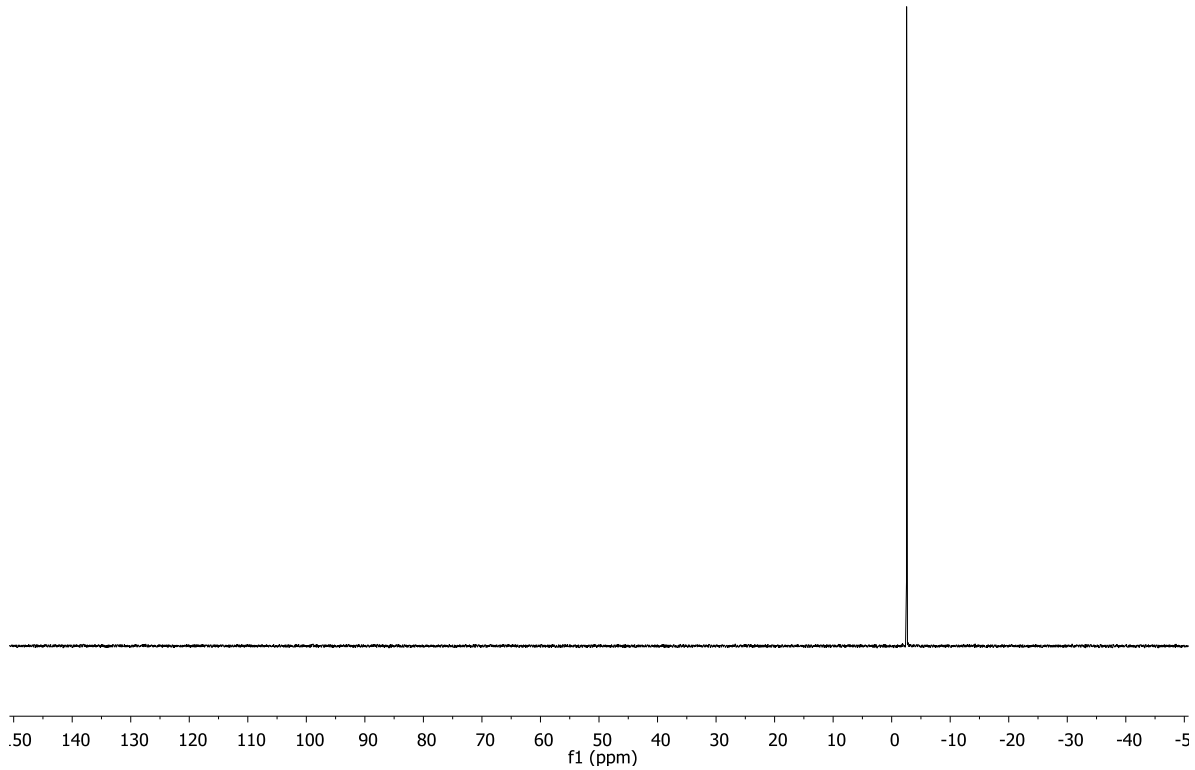
^{31}P NMR spectrum of **5** (121 MHz, $CDCl_3$)



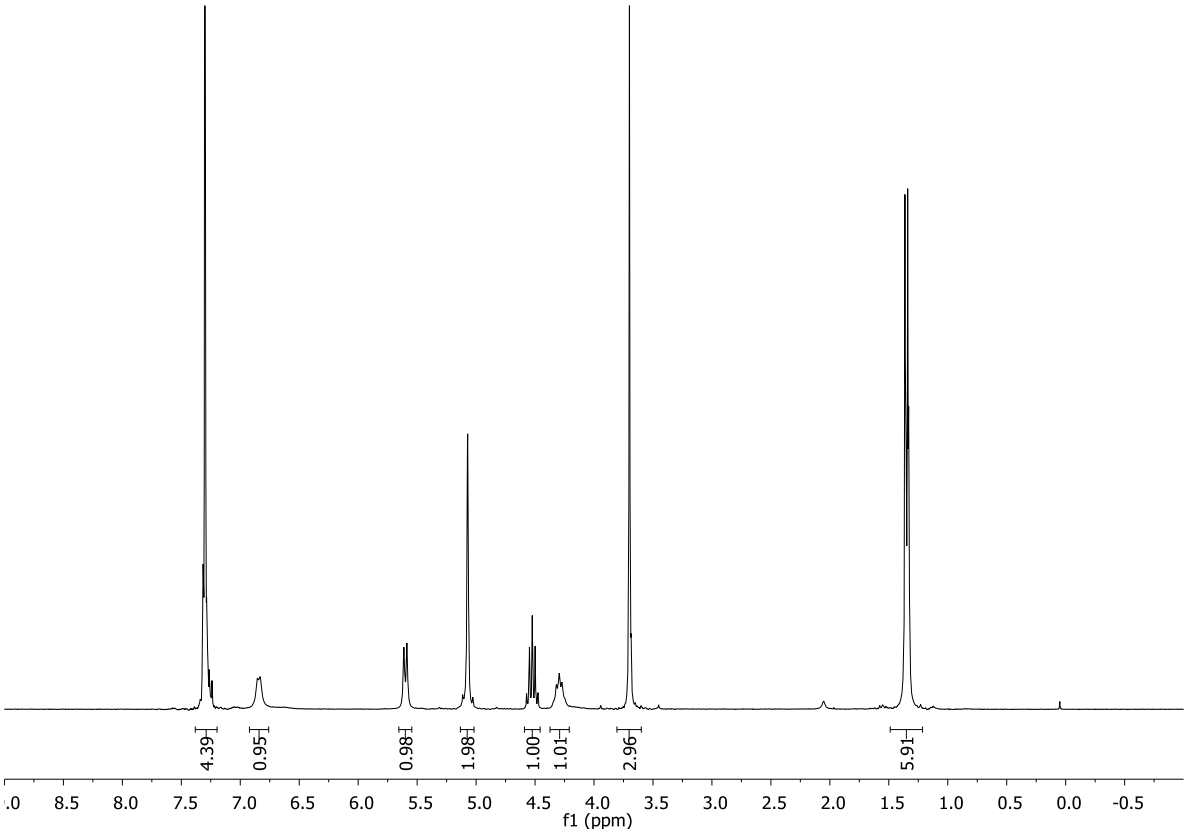
^{1}H NMR spectrum of **7** (300 MHz, $CDCl_3$)



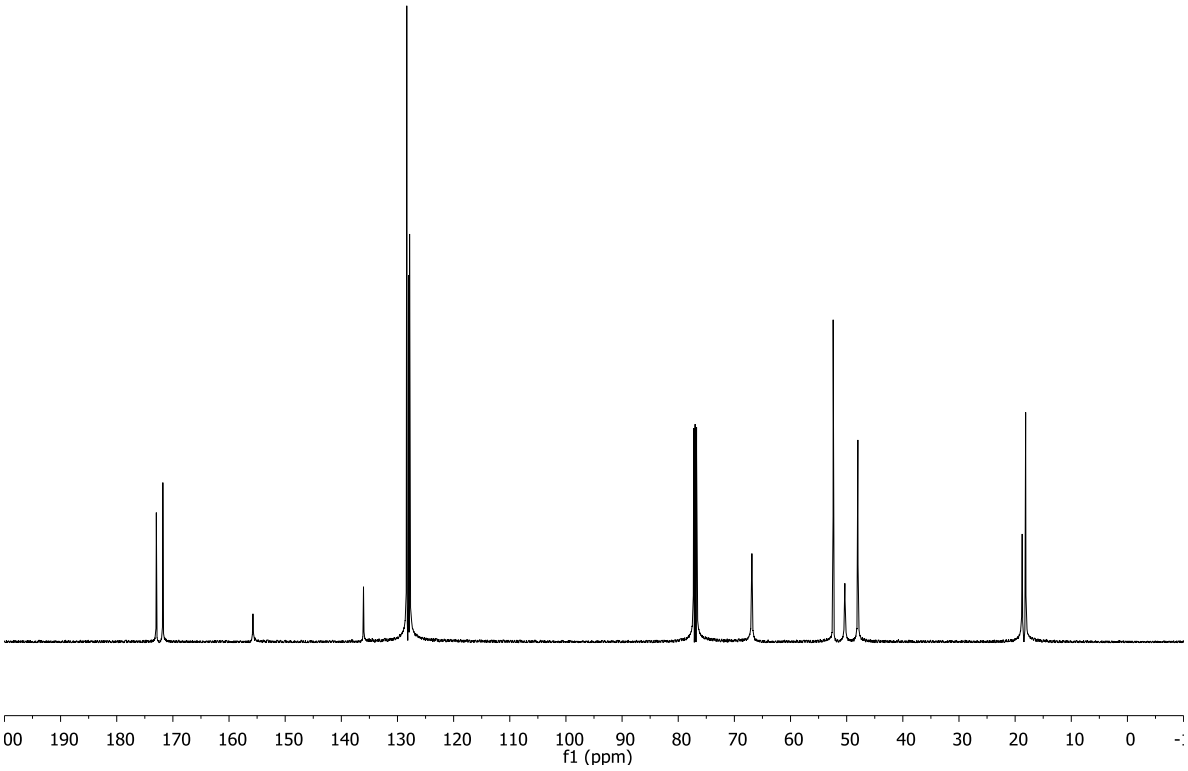
^{13}C NMR spectrum of **7** (75 MHz, $CDCl_3$)



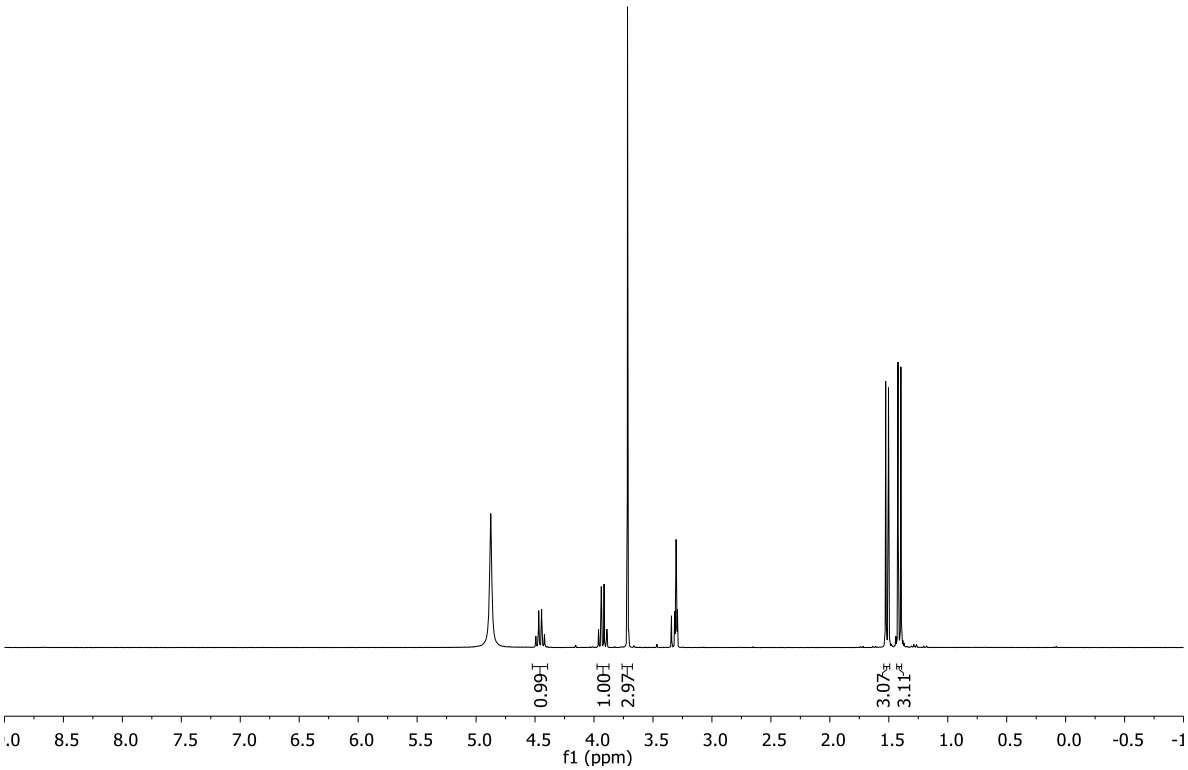
^{31}P NMR spectrum of **7** (121 MHz, $CDCl_3$)



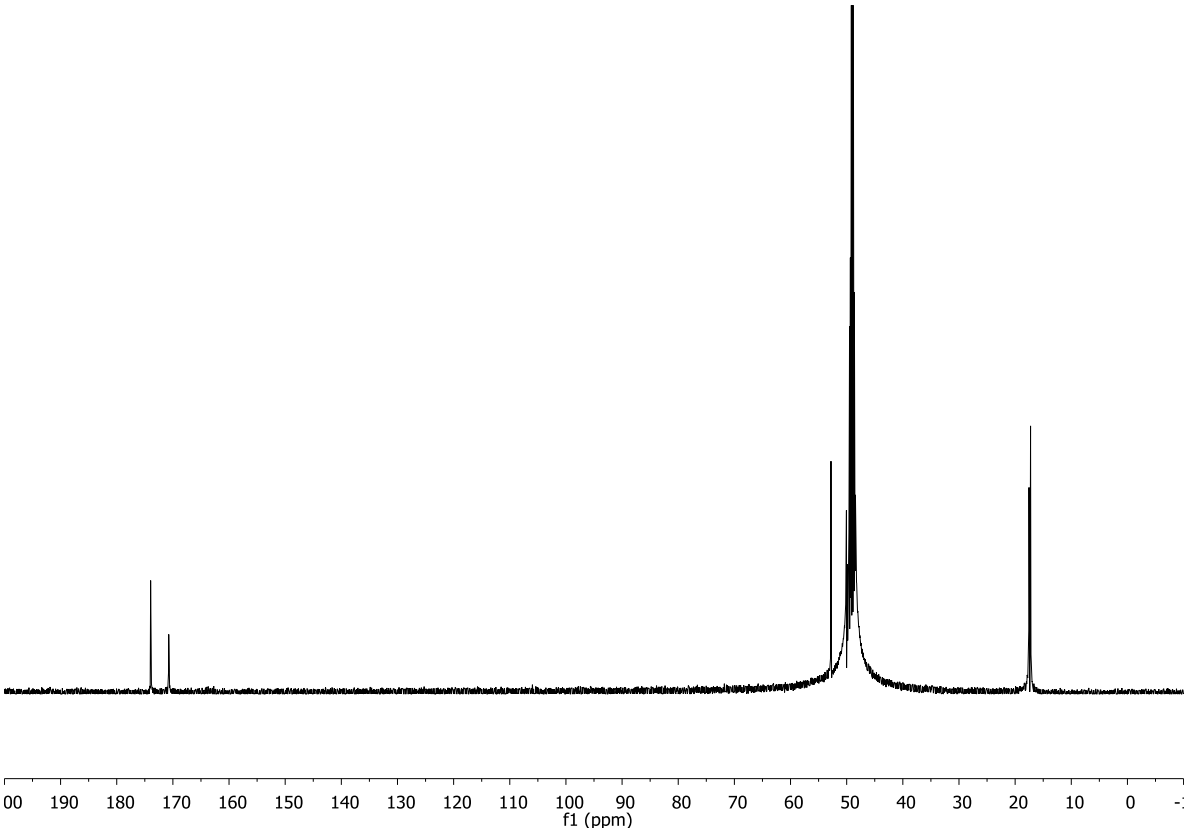
^{1}H NMR spectrum of **10** (300 MHz, $CDCl_3$)



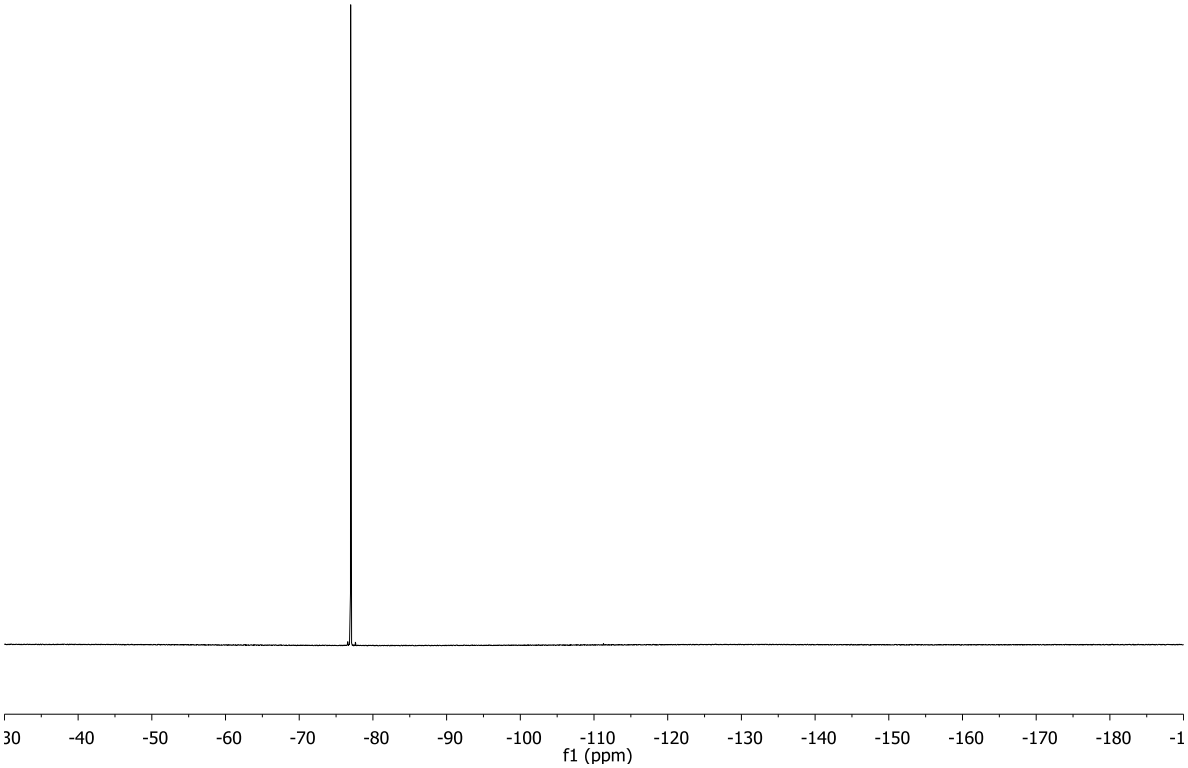
^{13}C NMR spectrum of **10** (75 MHz, $CDCl_3$)



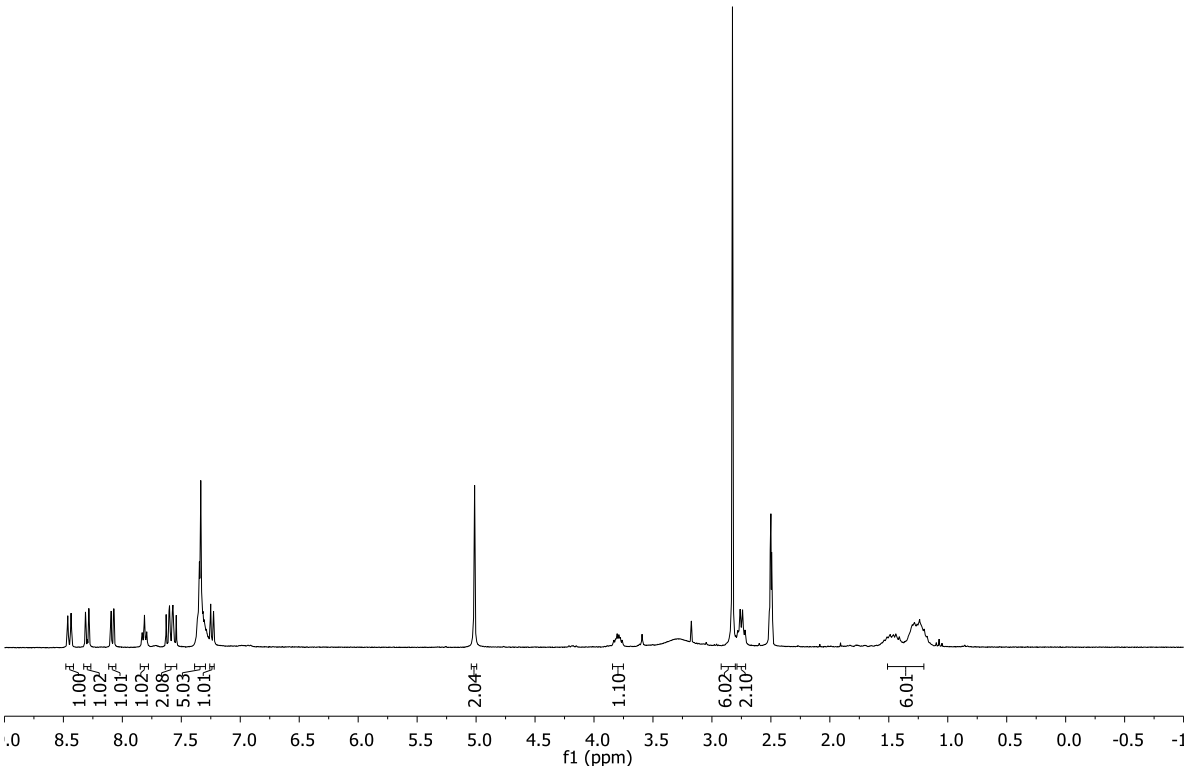
^{1}H NMR spectrum of **11** (300 MHz, CD_3OD)



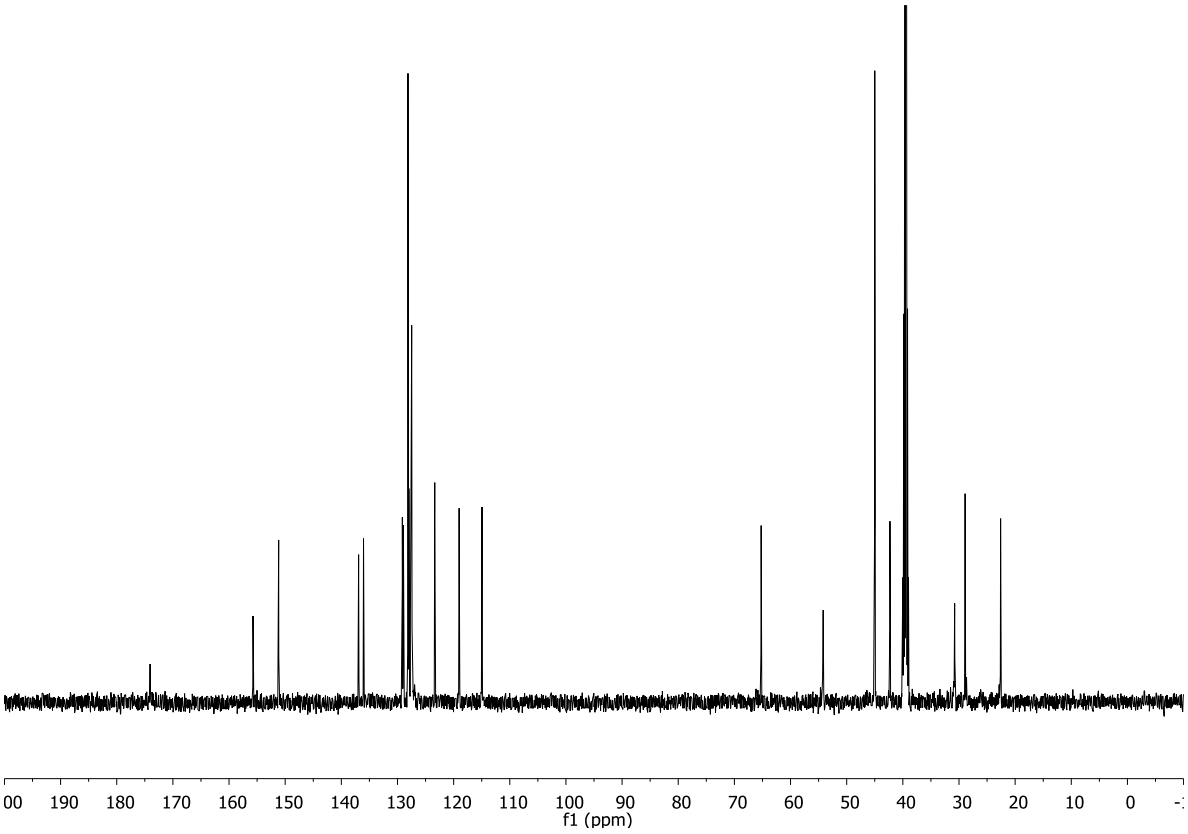
^{13}C NMR spectrum of **11** (75 MHz, CD_3OD)



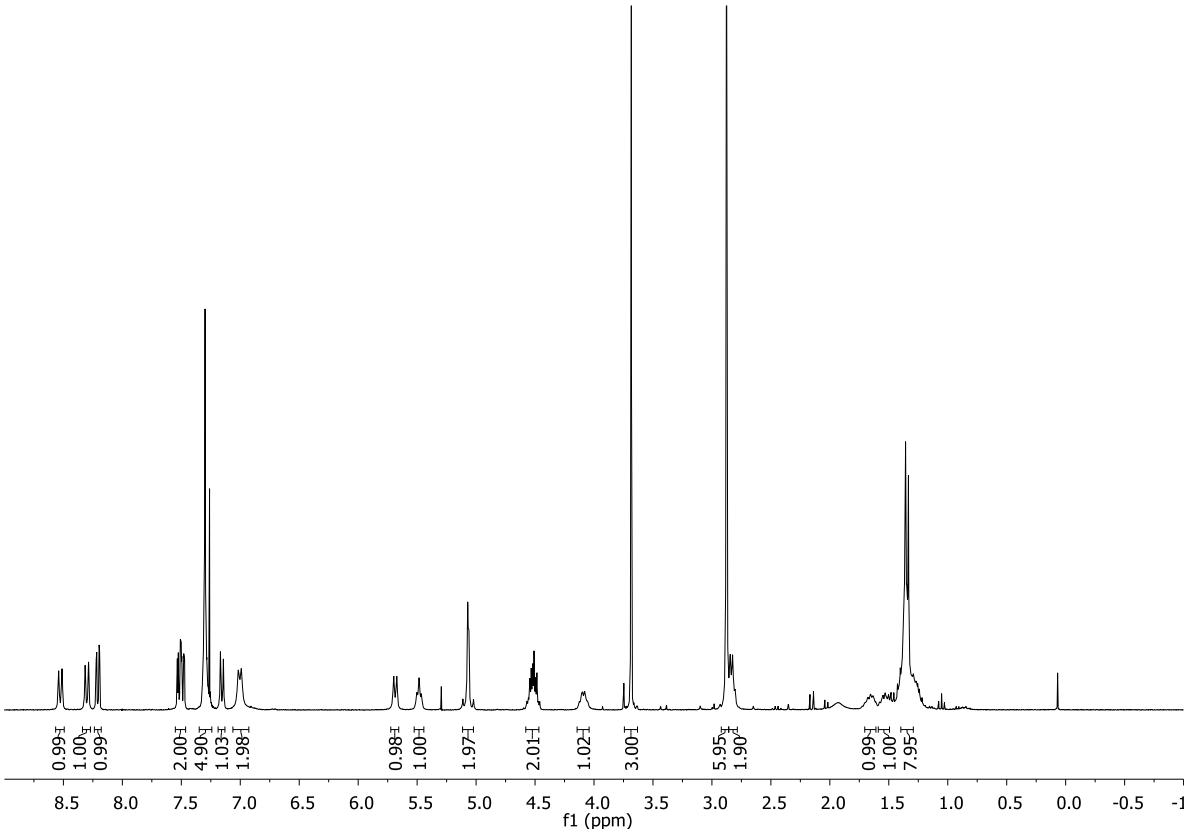
^{19}F NMR spectrum of **11** (282 MHz, CD_3OD)



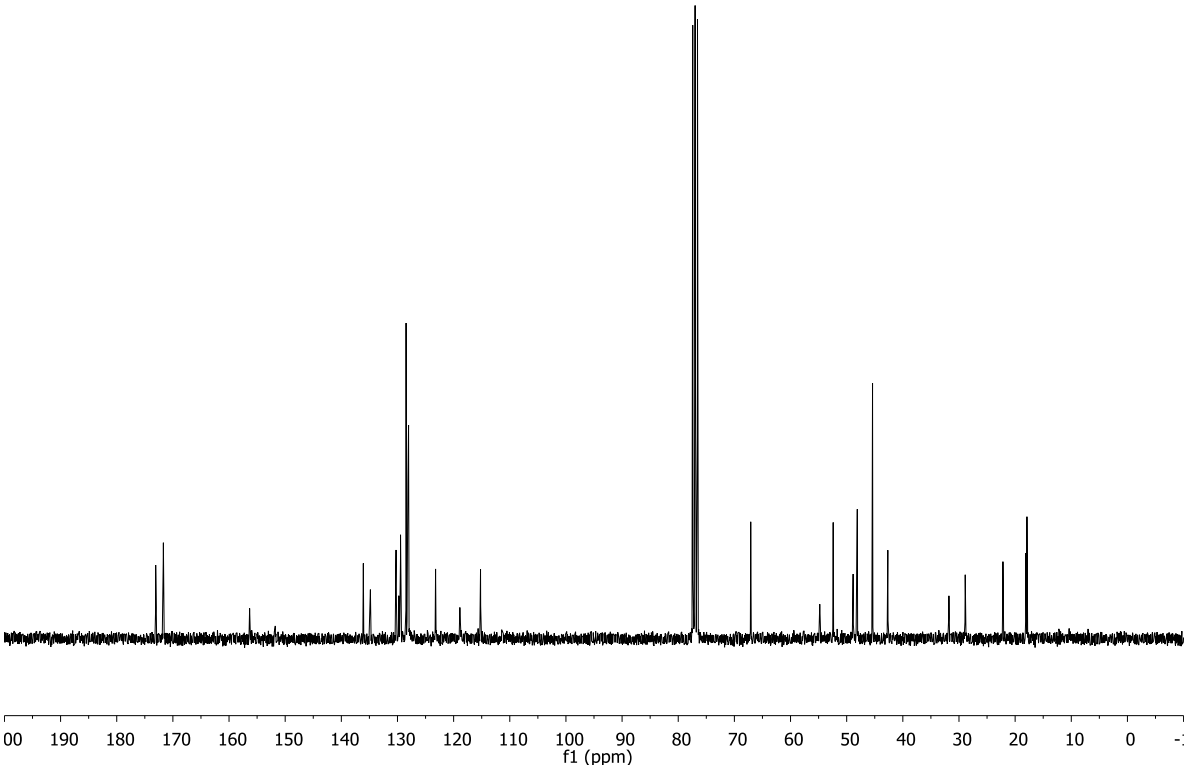
^{1}H NMR spectrum of **12** (300 MHz, DMSO-d_6)



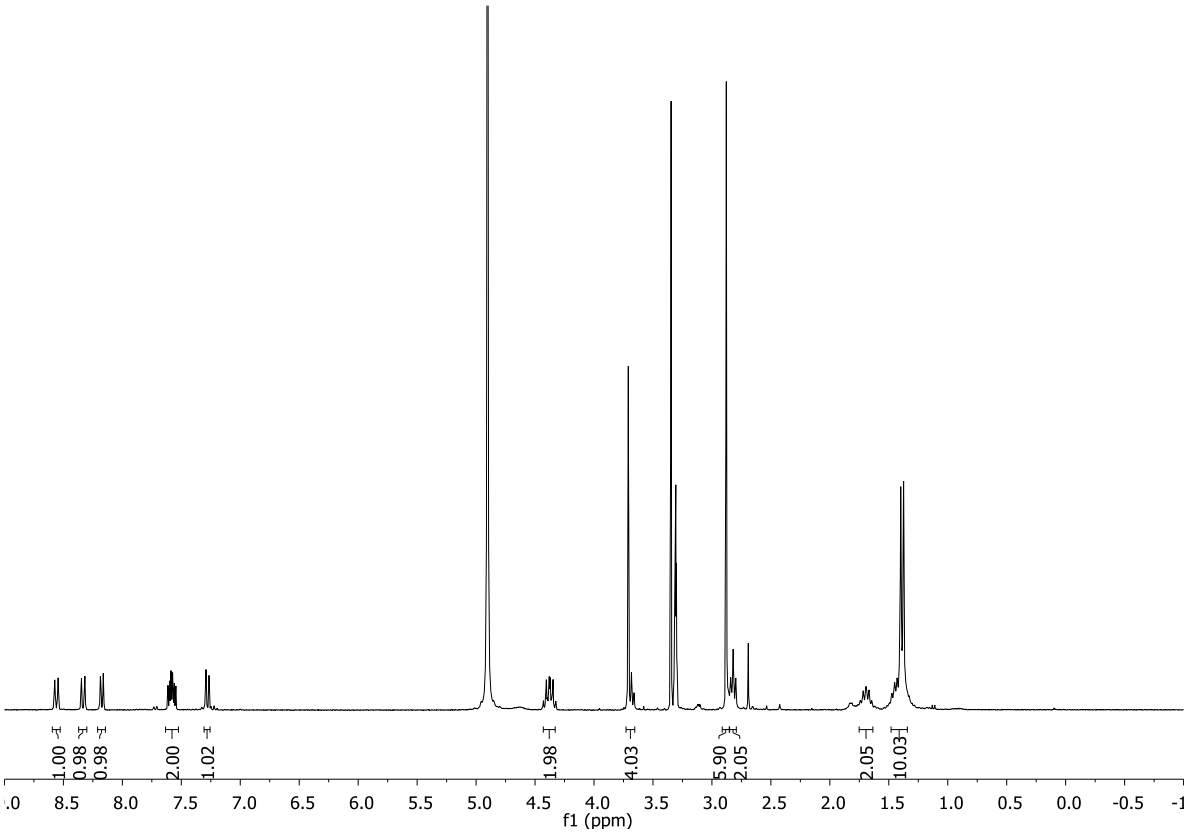
^{13}C NMR spectrum of **12** (75 MHz, DMSO-d_6)



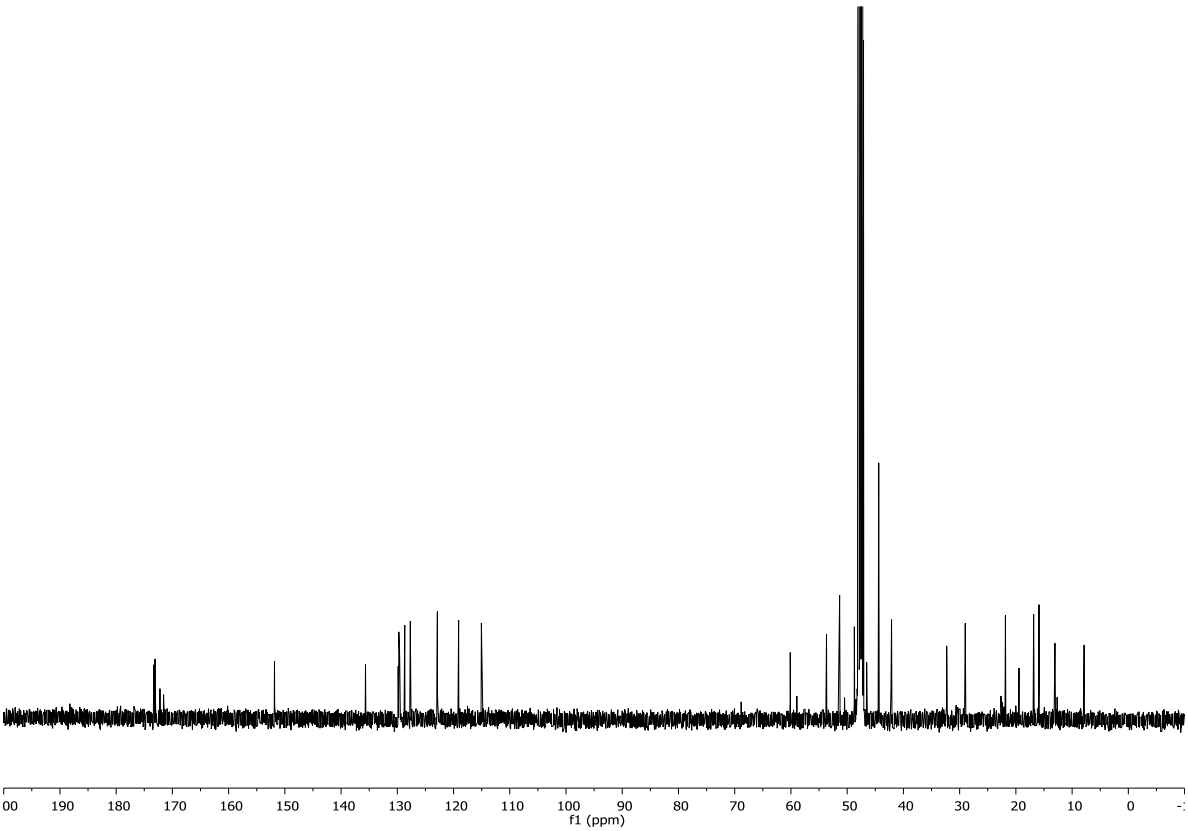
^{1}H NMR spectrum of **13** (300 MHz, $CDCl_3$)



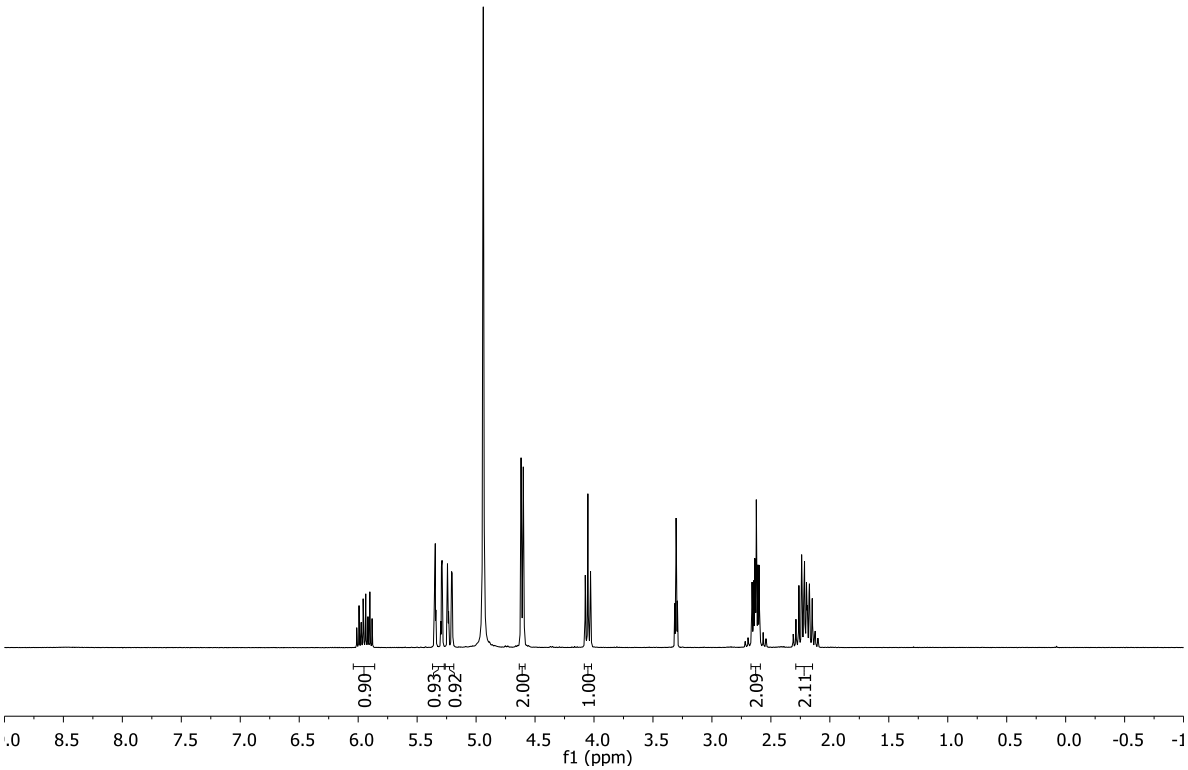
^{13}C NMR spectrum of **13** (75 MHz, $CDCl_3$)



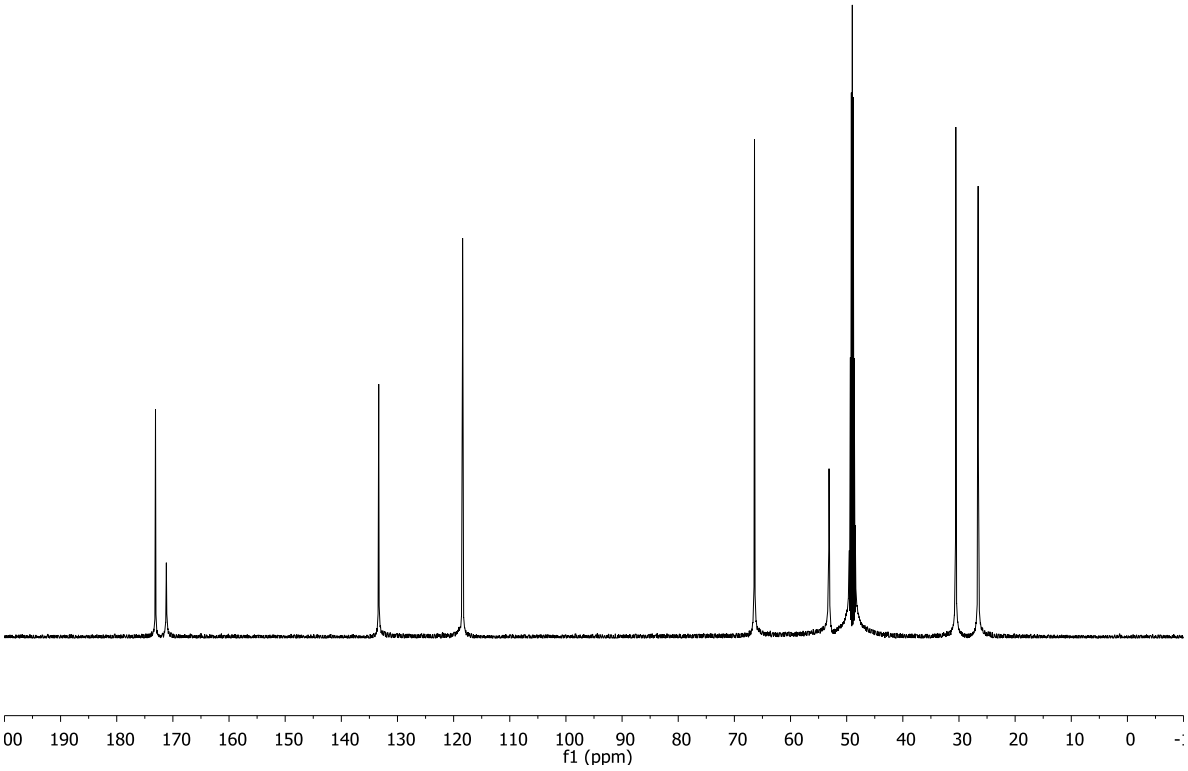
^{1}H NMR spectrum of **14** (300 MHz, CD_3OD)



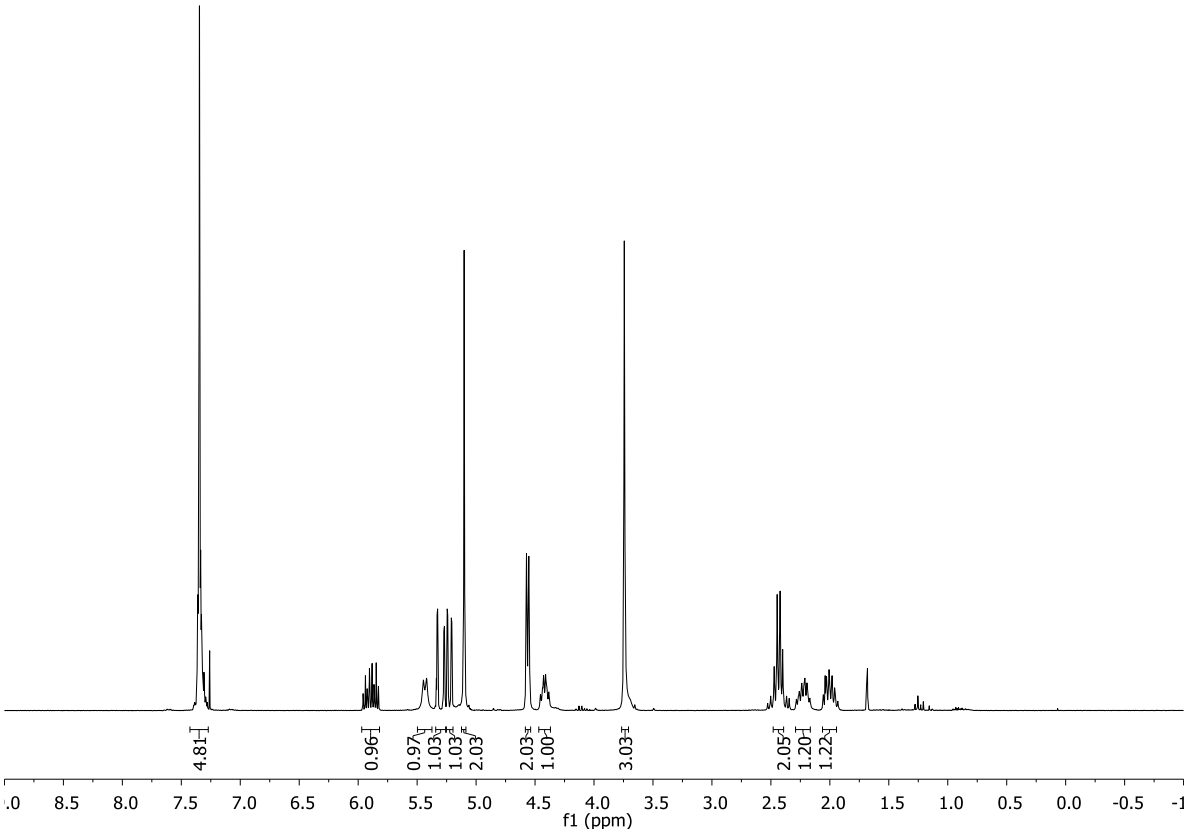
^{13}C NMR spectrum of **14** (75 MHz, CD_3OD)



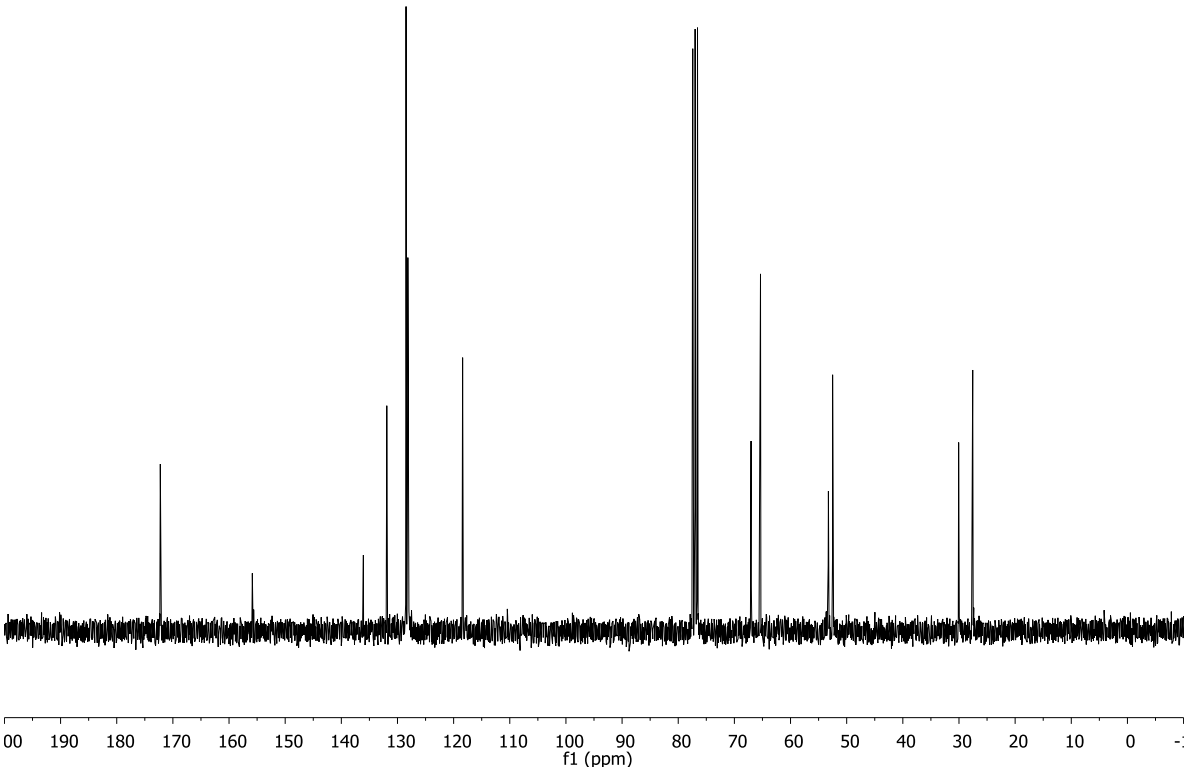
^{1}H NMR spectrum of **16** (300 MHz, DMSO-d_6)



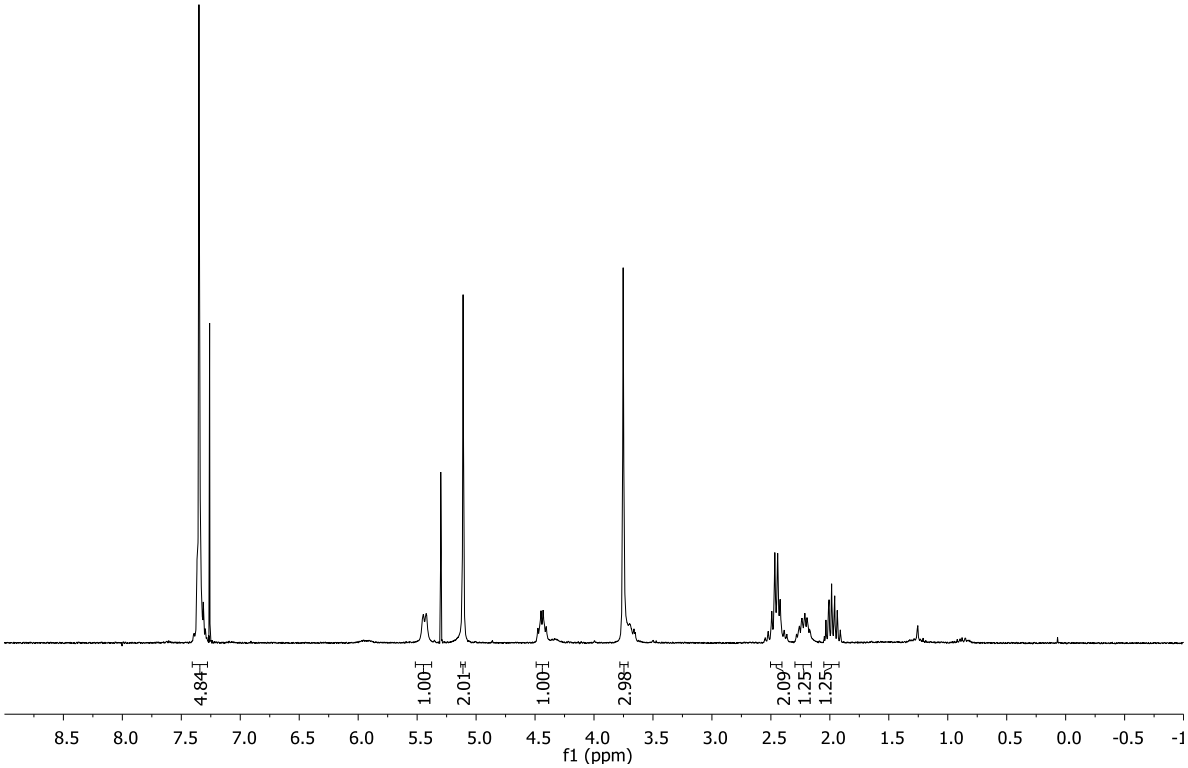
^{13}C NMR spectrum of **16** (75 MHz, DMSO-d_6)



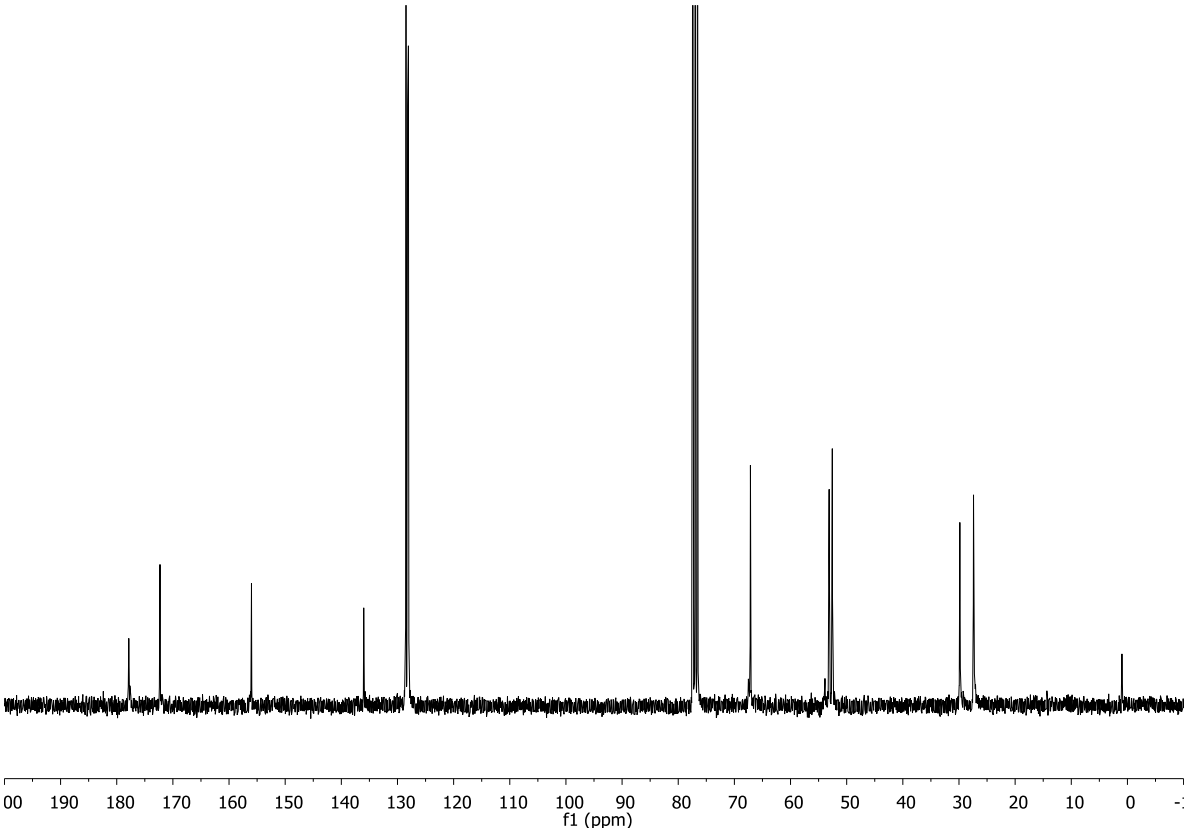
^{1}H NMR spectrum of **17** (300 MHz, $CDCl_3$)



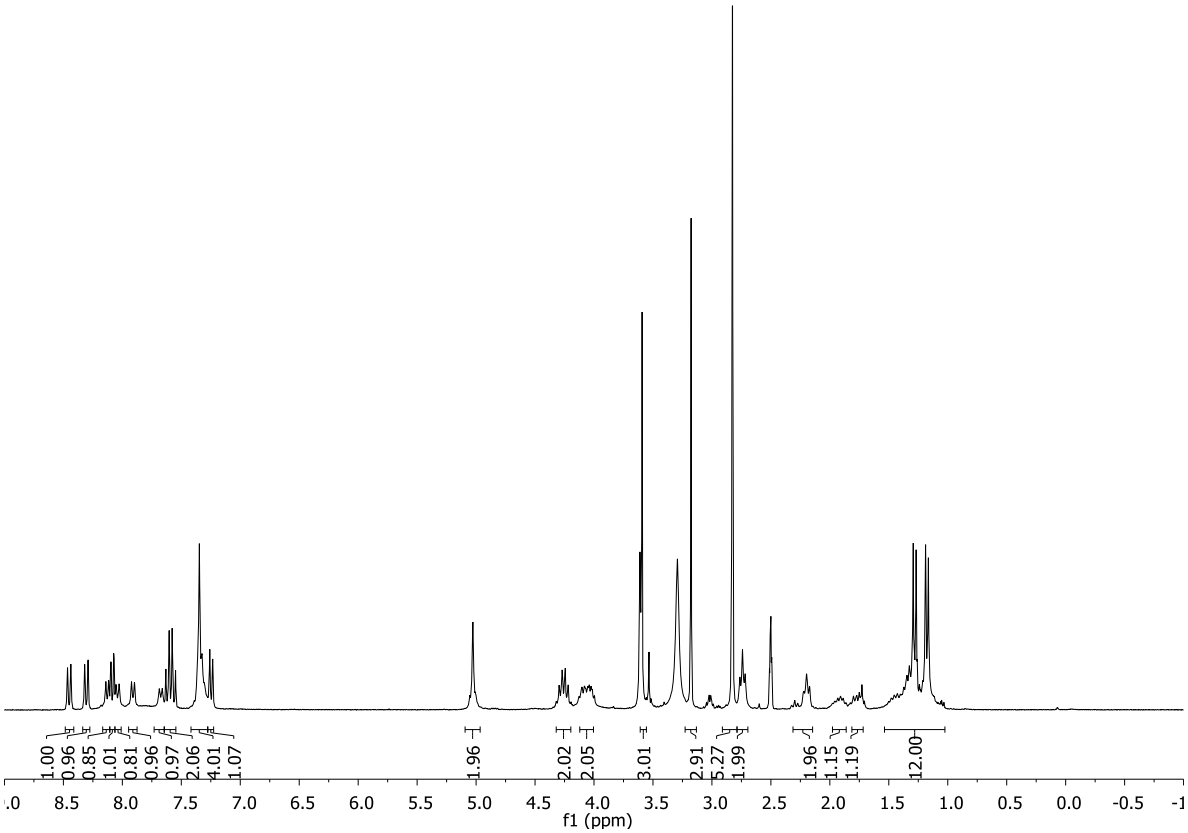
^{13}C NMR spectrum of **17** (75 MHz, $CDCl_3$)



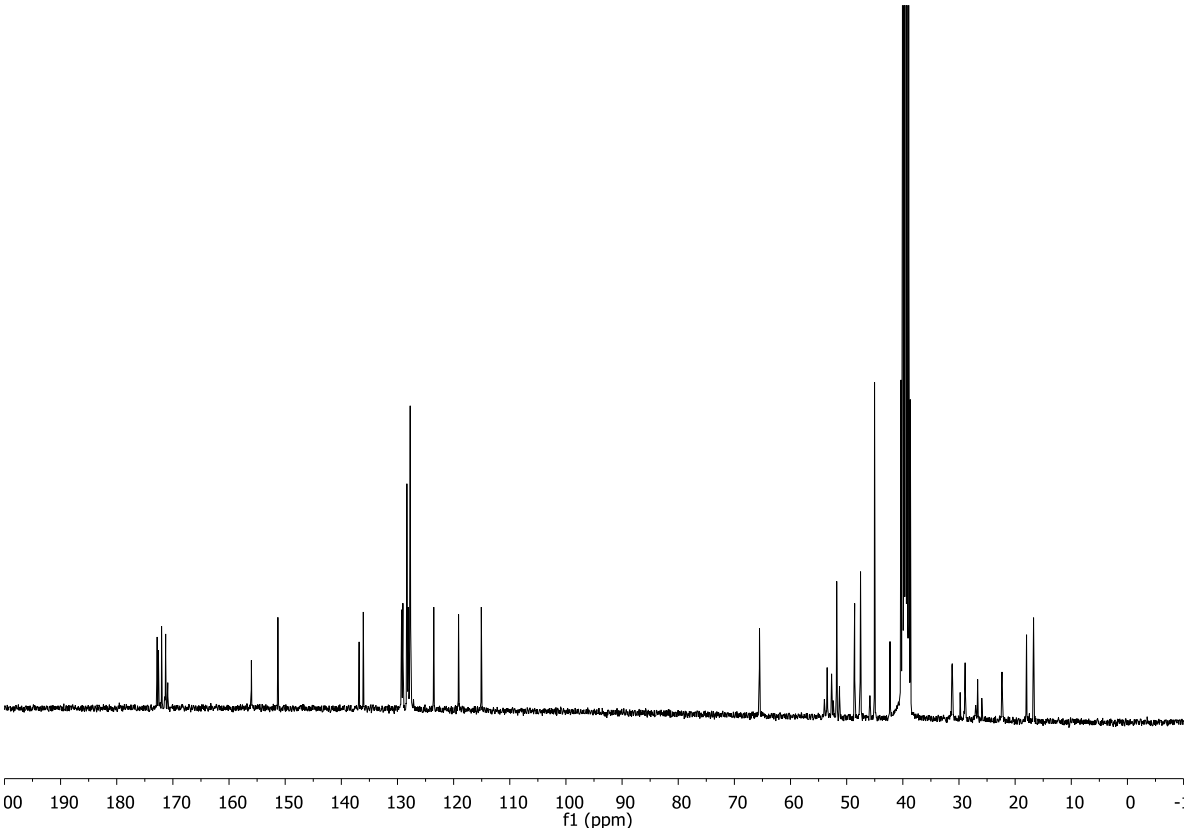
^{1}H NMR spectrum of **18** (300 MHz, $CDCl_3$)



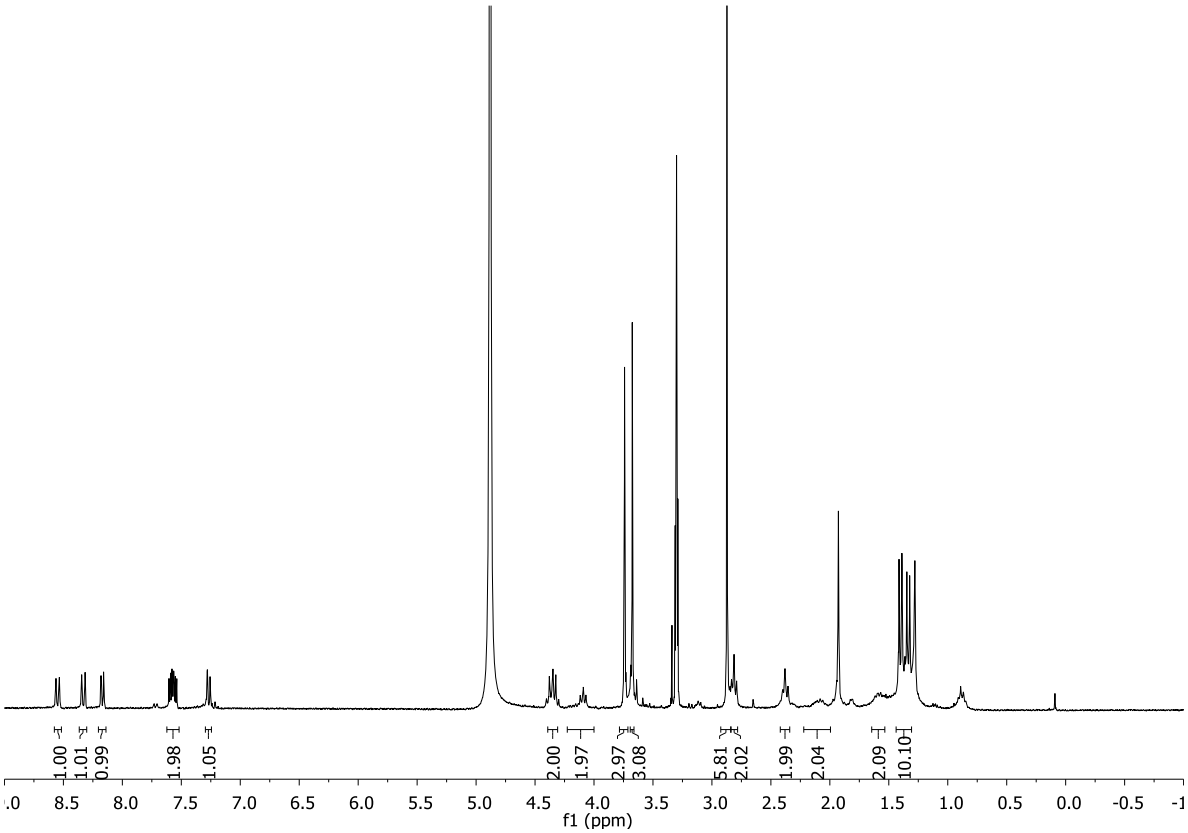
^{13}C NMR spectrum of **18** (75 MHz, $CDCl_3$)



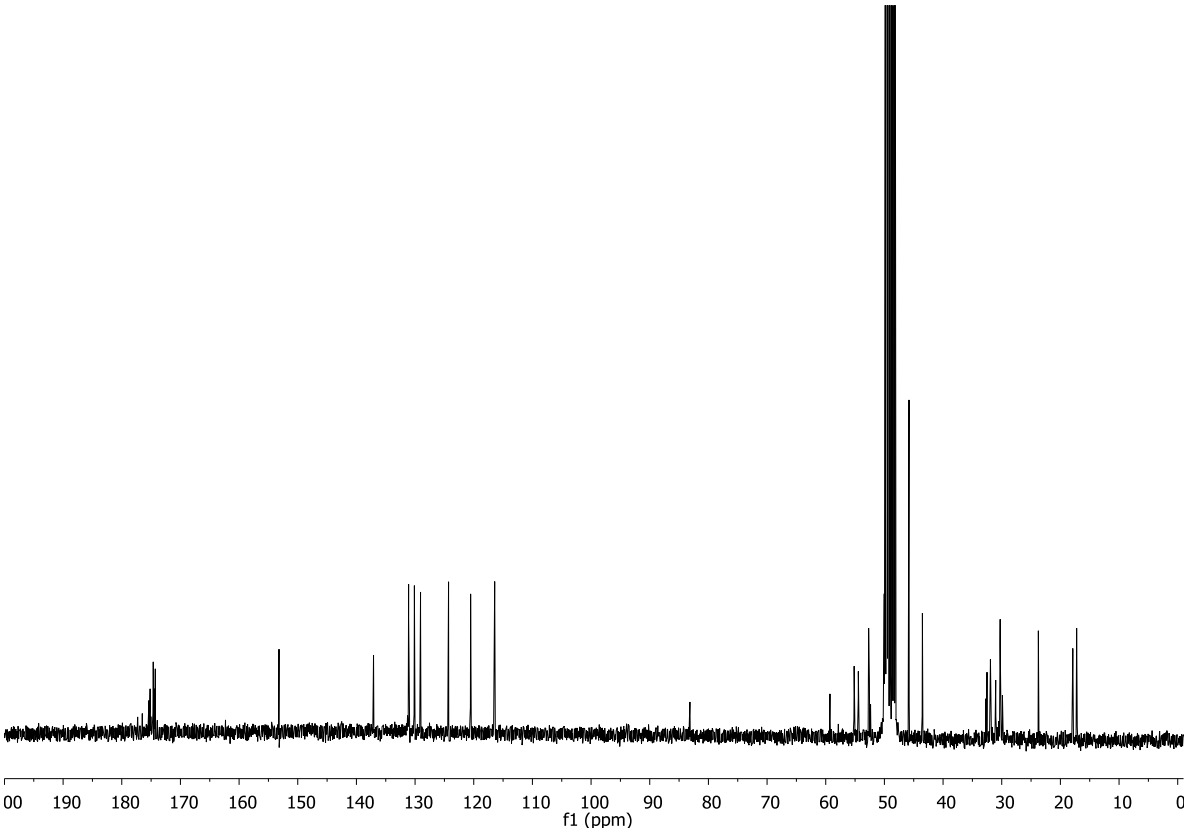
^{1}H NMR spectrum of **19** (300 MHz, DMSO-d_6)



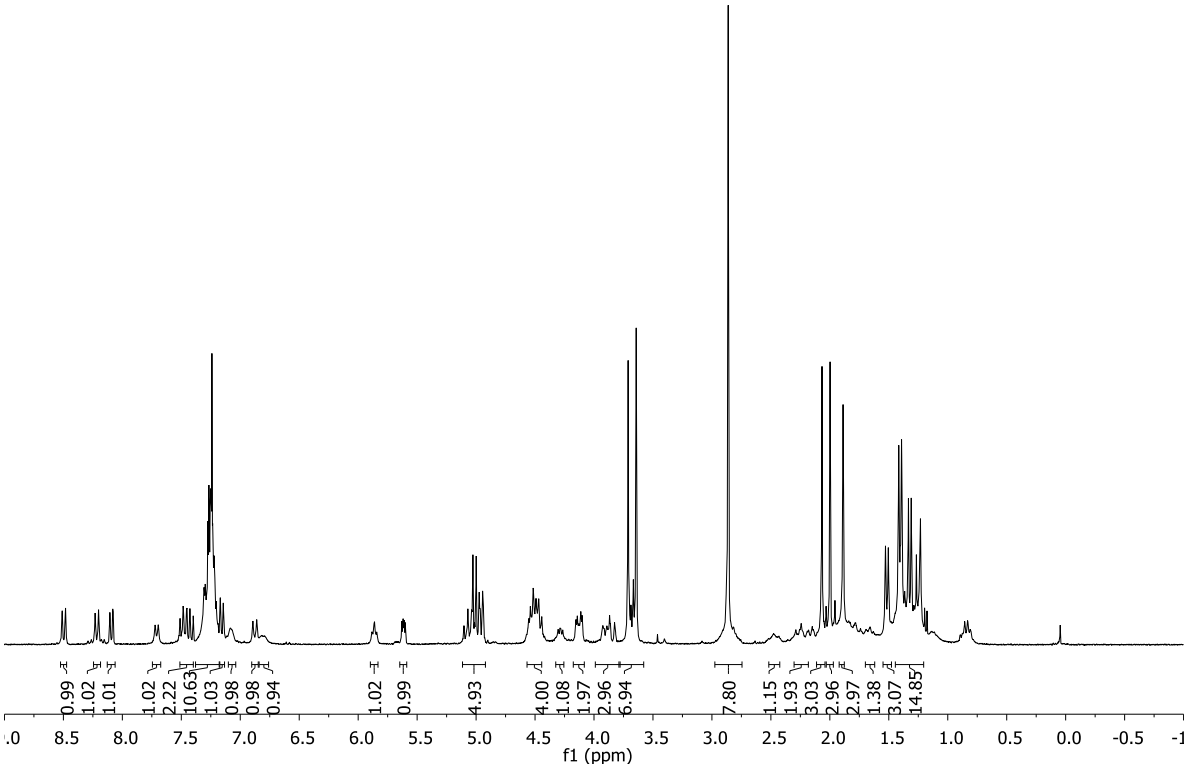
^{13}C NMR spectrum of **19** (75 MHz, DMSO-d_6)



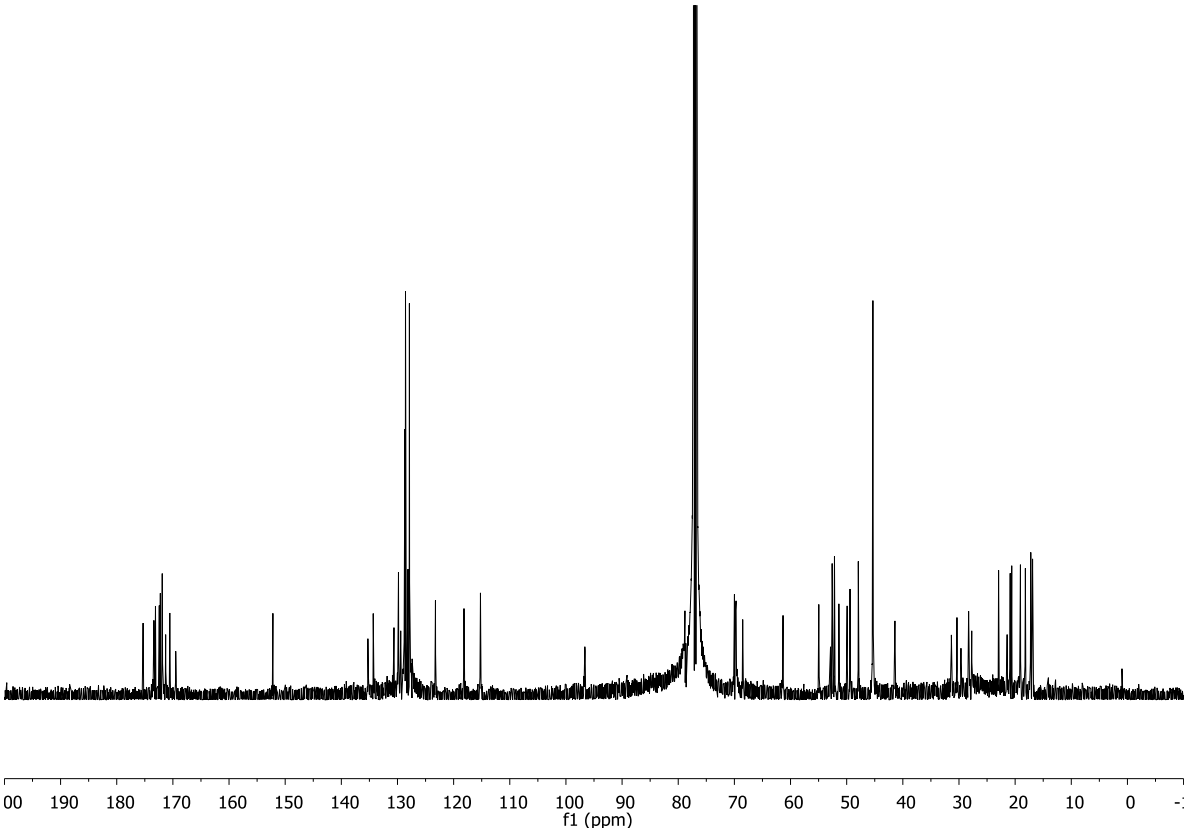
^{1}H NMR spectrum of **20** (300 MHz, CD_3OD)



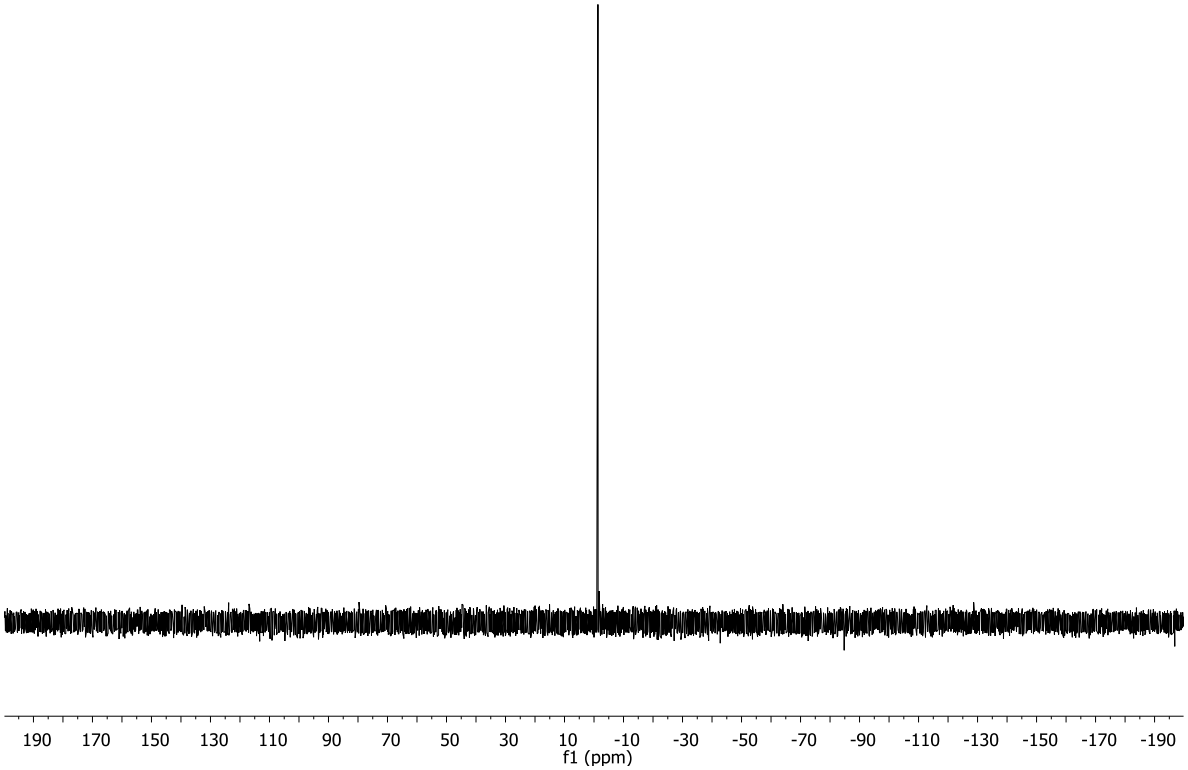
^{13}C NMR spectrum of **20** (75 MHz, CD_3OD)



^{1}H NMR spectrum of **28** (300 MHz, $CDCl_3$)



^{13}C NMR spectrum of **28** (75 MHz, $CDCl_3$)



^{31}P NMR spectrum of **28** (121 MHz, $CDCl_3$)

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