Electronic Supplementary Information

N*1*-Linked Melatonin Dimers as Bivalent Ligands Targeting Dimeric Melatonin Receptors

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Pharmacology: competition binding analysis, BRET experiments, BRET donor saturation curves for compounds **11-13**

Chemistry: Experimental procedures, ^{1}H-NMR and ^{13}C-NMR spectra of compounds **3-14**

Pharmacology

Competition binding analysis. All synthesized compounds were tested for their binding affinity and selectivity for each of the melatonin receptor subtypes, MT_1 and MT_2 using competition binding analysis. Briefly, cells expressing the human MT_1 or MT_2 melatonin receptor (MT_1-CHO, MT_2-CHO) were grown to confluence on 10 cm cell culture plates until they reached approximately 80 percent confluence. Next, cells were washed, lifted, and added to tubes containing 80-100 pM 2-[^{125}I]-iodomelatonin in the absence (total binding) or presence of melatonin (1 fM to 1 μM) or the test compounds (1 pM to 1 μM). The reactions were incubated for 1h at 25°C, and then terminated following the addition of cold Tris-HCl solution (50 mM, pH 7.4) and filtered through glass fiber filters (Schleicher and Schuell, Keene, NH) saturated in polyethylenimine 0.5% solution (v/v). Radioactive counts were counted using a gamma counter. Data points were fit by 1-site nonlinear regression analysis based upon the lowest residual sum of squares (GraphPad Prism) and affinity constants (K_i) values were calculated.

BRET experiments. To investigate the effect of the compounds on the bioluminescence resonance energy transfer (BRET), HEK293T cells seeded in a 6-well plates were transiently transfected with 30 ng of MT_1-Rluc or 250 ng of MT_2-Rluc and 0 or 200 ng of yellow fluorescent protein (YFP) plasmids. For BRET donor saturation curves, HEK293T cells seeded in 6-well plates were transiently transfected with 10 ng of MT_1-Rluc or 100 ng of MT_2-Rluc and 10 to 2000 ng of yellow fluorescent protein (YFP) plasmids.

Twenty-four hours after transfection, cells were reseeded in 6-well plates diluted two fold. Twenty-four hours after, cells were transferred into a 96-well white Optiplate (Perkin Elmer Life Sciences) at 100000 cells per well in PBS (+/+). and then the BRET assay has been performed.

The BRET measurement was initiated by adding coelenterazin at a final concentration of 5 µM (diluted in PBS), and incubated for 5 minutes. Then vehicle, melatonin or compounds are added (1nM for the melatonin and 10µM for compounds) and incubated for 10 minutes. BRET measurements were performed with the Mithras lumino/fluorometer (Berthold Technologies). as described previously (Tadagaki K, Tudor D, Gbahou F, Tschische P, Waldhoer M, Bomsel M, Jockers R and Kamal M. Human cytomegalovirus-encoded UL33 and UL78 heteromerize with host CCR5 and CXCR4 impairing their HIV coreceptor activity. (Blood, 119: 4908-4918, 2012).

For BRET donor saturation curves, results are expressed as percentage of the BRET maximum of each saturation curve. For the BRET experiments with a single saturating compound concentration, results are expressed as percentage of the BRET amplitude obtained between the lowest and the highest BRET value for each experiment, and normalized to the vehicle.

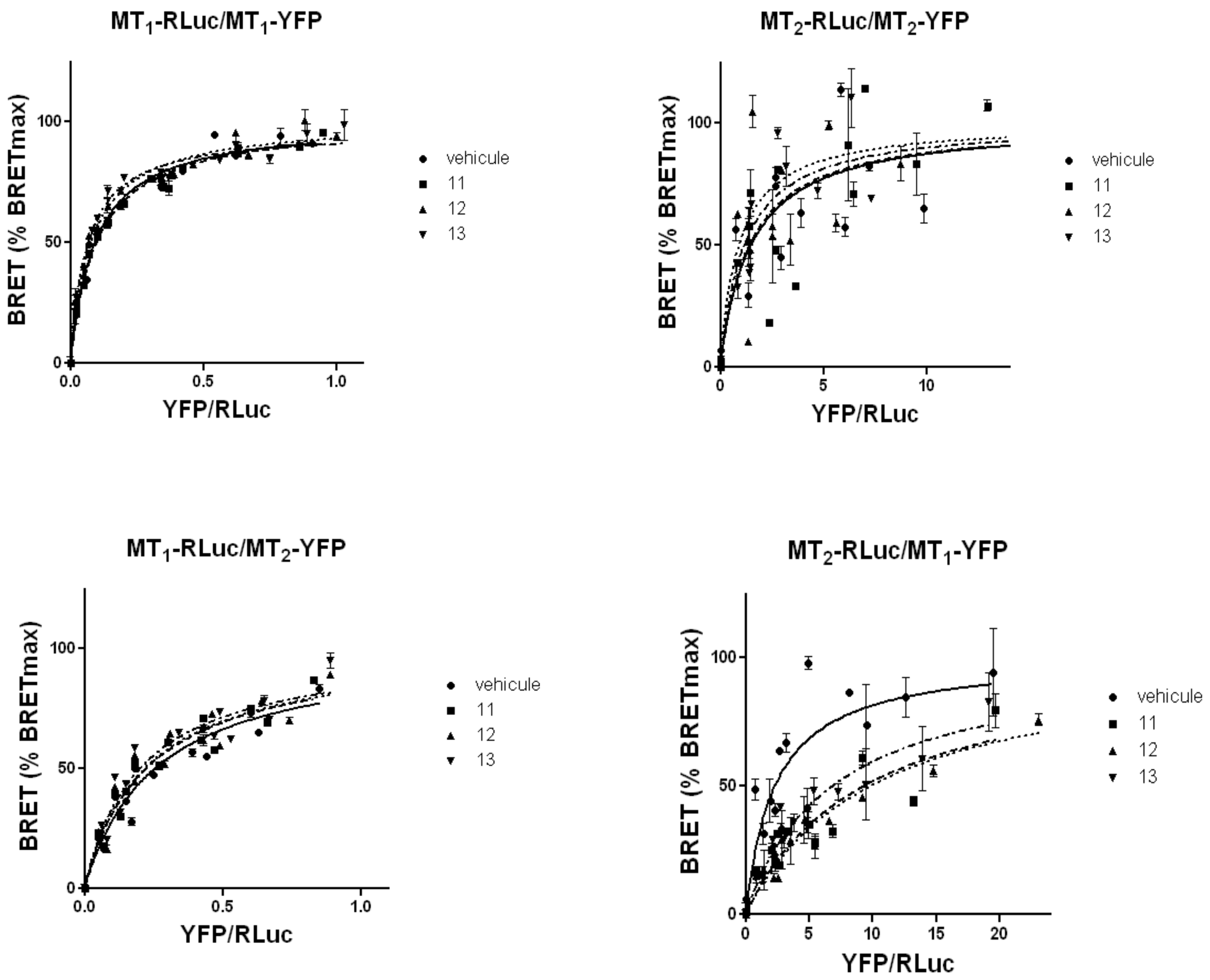


Fig 1. BRET donor saturation curves were generated with cells expressing different ratios of the indicated BRET fusion proteins in the presence of vehicle or compounds **11**, **12** or **13** (10µM).

Chemistry

General Methods. Melting points were determined using a capillary melting point apparatus (Gallenkamp, Sanyo) and are uncorrected. Column chromatography was carried out on silica gel 60 (0.063–0.200 mm) obtained from Merck. A Bruker AV-400 spectrometer was used to obtain ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra respectively. Proton chemical shifts are referred to signals of $CHCl_3$ (7.26 ppm), DMSO-d_6 (2.50 ppm), and methanol d_4 (3.31 ppm). Coupling constants (J values) are given in hertz (Hz). Carbon chemical shifts are referred to $CDCl_3$ (77.16 ppm), DMSO-d_6 (39.52 ppm), and methanol d_4 (49.00 ppm) The NMR resonances were assigned by means of HH-COSY, and HMQC experiments. EI mass spectra were determined on a Finnigan MAT 8200 spectrometer. ESI mass spectra were determined on an Agilent 1100 MS systems. Elemental Analysis were performed by the microanalytical section of the Institute of Inorganic Chemistry, Würzburg University. All reactions were carried out under an argon atmosphere. All chemicals were purchased from commercial suppliers and used directly without any further purification.

Methyl 2-(3-(2-Acetamidoethyl)-5-methoxy-1H-indol-1-yl)acetate (1a)

A solution of melatonin (200 mg, 2.15 mmol) in dry DMF (3 ml) was added dropwise to a stirred suspension of sodium hydride (33 mg of 60% dispersion in mineral oil, 0.83 mmol) in dry DMF (5 ml) at 0 °C. After stirring at 0 °C for 30 min, methyl 2-bromoacetate (0.36 ml, 3.87 mmol) was added, and stirring was continued for 4 hrs at room temperature. The reaction mixture was poured into ice/water and extracted with ethyl acetate (5x10ml). The organic phases were combined, washed with brine, dried over sodium sulphate, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (chloroform/methanol/25% ammonia 100:10:1) to give **1a** (135 mg, 52%) as a pale yellow solid. mp 105 ^{o}C. δ_H (400 MHz, $CDCl_3$) 1.92 (3H, s, Me), 2.91 (2H, t, J 6.7, Ar-CH_2), 3.56 (2H, m, Ar-CH_2-CH_2), 3.72 (3H, s, Me), 3.83 (3H, s, Me), 4.76 (2H, s, N-CH_2), 5.72 (1H, br s, NH), 6.95 (2H, m, H-2, H-6), 7.01 (1H, d, J 2.3, 4-H), 7.09 (1H, d, J 8.9, 7-H). δ_c (100 MHz, $CDCl_3$) 23.3, 25.1, 39.6, 47.7, 52.5, 52.9, 55.9, 101.1, 109.9, 112.49, 112.51, 127.1, 128.5, 132.3, 154.3, 169.1, 170.2. EI-MS m/z 304.2 (M+, 23%), 245.1 (80), 232.1 (100), 186.1 (26).

2-(3-(2-Acetamidoethyl)-5-methoxy-1H-indol-1-yl) acetic acid (2a)

2M aqueous lithium hydroxide solution (10 ml) was added to a stirred solution of **1a** (135 mg, 0.44 mmol) in THF (50 ml). After stirring at room temperature for 48 hrs THF was evaporated under reduced pressure. The residue was diluted with water (20 ml) and the solution was acidified using 2M HCl. The product was extracted with dichloromethane (5x10ml), the organic phases were combined, washed with brine, and dried over Na_2SO_4. Evaporation of the solvent under reduced pressure gave the crude product (brown solid, 125 mg, 97%) that was used for the next steps without further purification. δ_H (400 MHz, MeOH-*d4*) 1.90 (3H, s, Me), 2.88 (2H, t, *J* 6.8, Ar-CH_2), 3.47 (2H, t, *J* 6.8, Ar-CH_2-CH_2), 3.82 (3H, s, Me), 4.69 (2H, s, N-CH_2), 6.79 (1H, dd, *J* 8.8, 2.3, H-6), 6.97 (1H, s, H-2), 7.05 (1H, d, *J* 2.3, H-4), 7.15 (1H, d, *J* 8.8, H-7). δ_c (100 MHz, MeOH-*d4*) 22.6, 26.1, 41.2, 50.1, 56.4, 101.8, 111.1, 112.5, 112.6, 128.8, 129.8, 133.9, 155.2, 175.32, 175.39.

Ethyl 5-(3-(2-Acetamidoethyl)-5-methoxy-1H-indol-1-yl) pentanoate (1b)

A solution of melatonin (300 mg, 1.29 mmol) in dry DMF (2.5 ml) was added dropwise to a stirred suspension of sodium hydride (51.6 mg of a 60% dispersion in mineral oil, 1.29 mmol) in dry DMF (5 ml) at 0 °C. The mixture was stirred at 0 °C for 30 min, ethyl 5-bromovalerate (0.368 ml, 2.32 mmol) was added, and the resulting mixture was stirred at room temperature for 6 hrs. The reaction mixture was poured into ice/water and extracted with ethyl acetate (5x10ml). The organic phases were combined, washed with brine, dried over sodium sulphate, and concentrated under reduced pressure. The excess of the alkylating agent was removed by extraction with hexane (5x10ml), and the residue was purified by silica gel column chromatography (chloroform/methanol/25% ammonia 100:10:1) to give **1b** (270 mg, 58%) as a pale brown solid. mp 60 ^oC. δ_H (400 MHz, $CDCl_3$) 1.17 (3H, t, *J* 7.2, Me), 1.55 (2H, m, N-CH_2-CH_2-CH_2-), 1.78 (2H, m, N-CH_2-CH_2-CH_2-), 1.87 (3H, s, Me), 2.22 (2H, t, *J* 7.3, N-CH_2-CH_2-CH_2-CH_2-), 2.86 (2H, t, *J* 6.8, Ar-CH_2-), 3.49 (2H, m, Ar-CH_2-CH_2-), 3.79 (3H, s, Me), 3.98 (2H, t, *J* 6.9, N-CH_2-CH_2-CH_2-), 4.03 (2H, q, *J* 7.2, O-CH_2-CH_3), 6.05 (1H, br s, NH), 6.84 (1H, dd, *J* 8.8, 2.3, H-6), 7.13 (1H, d, *J* 8.8, H-7), 6.85 (1H, s, H-2), 6.98 (1H, d, *J* 2.3, H-4).). δ_c (100 MHz, $CDCl_3$) 14.0, 22.1, 23.0, 25.0, 29.4, 33.5, 39.7, 45.7, 55.7, 60.2, 100.5, 110.0, 111.0, 111.7, 126.0, 128.0, 131.5, 153.6, 170.1. 173.0

5-(3-(2-Acetamidoethyl)-5-methoxy-1H-indol-1-yl) pentanoic acid (2b)

2M aqueous lithium hydroxide solution (10 ml) was added to a stirred solution of **1b** (272 mg, 0.75 mmol) in THF (50 ml). After stirring at room temperature for 48 hrs the solvent was evaporated under reduced pressure. The residue was diluted with water (20 mL) and the solution was acidified using 2M HCl. The precipitated solid was extracted using ethyl acetate (5x10 ml). The organic phases were combined and dried over Na_2SO_4 followed by evaporation of the solvent under reduced pressure to give the crude product (245 mg, 97%, mp 106 ^oC) that was used directly for the next steps without further purification. δ_H (400 MHz, $CDCl_3$) 1.57 (2H, m, N-CH_2-CH_2-C*H*$_2$-),1.83 (2H, m, N-CH_2-C*H*$_2$-CH_2-), 1.90 (3H, s, Me), 2.27 (2H, t, *J* 7.2, N-CH_2-CH_2-CH_2-C*H*$_2$-), 2.88 (2H, m, Ar-CH_2-), 3.53 (2H, m, Ar-CH_2-C*H*$_2$-), 3.82 (3H, s, Me), 4.02 (2H, t, *J* 6.5, N-C*H*$_2$-CH_2-CH_2-), 5.90 (1H, br s, NH), 6.84 (1H, dd, *J* 8.8, 1.8, H-6), 6.88 (1H, s, H-2), 6.99 (1H, d, *J* 1.8, H-4), 7.17 (1H, d, *J* 8.8, H-7). δ_c (100 MHz, $CDCl_3$) 22.1, 23.1, 25.0, 29.5, 33.4, 40.0, 45.9, 55.9, 100.7, 110.2, 110.9, 111.9, 126.3, 128.1, 131.7, 153.8, 170.1, 173.1.

General procedure for the synthesis of the bivalent ligands 3-13.

EDCI HCl (1.2 -1.7 equivalents) was added to a stirred solution of **2a** or **2b** (1 equivalent) in in dry CH_2Cl_2 (10 mL). After 10 minutes, the reaction mixture was cooled using water-ice bath, and a solution of the appropriate diaminoalkane (033-0.5 equivalents) in dry CH_2Cl_2 (5 mL) was dropwise added. After 1 hour, the cooling bath was removed, and stirring was continued for 24 hours. The solvent was evaporated under reduced pressure and the residue was purified by silica gel column chromatography using ethyl acetate/methanol 10:1.

***N,N'*-(Nonane-1,9-diyl)-bis{2-[3-(2-acetamidoethyl)-5-methoxy-1H-indol-1-yl]-acetamide} (3).** Compound **3** (45 mg, 19%) was obtained from **2a** (200 mg, 0.69 mmol), EDCI HCl (230 mg, 1.2 mmol), and 1,9-diaminononane (54 mg, 0.34 mmol) as a pale yellow solid. mp 159 ^oC. Found: C, 66.41; H, 7.50; N, 12.05. $C_{39}H_{54}N_6O_6$ requires C, 66.64; H, 7.74; N, 11.96. δ_H (400 MHz, DMSO-*d*$_6$) 1.14 – 1.26 (10H, m, 2 x HN-CH_2-CH_2-C*H*$_2$-C*H*$_2$-, HN-CH_2-CH_2-C*H*$_2$-CH_2-CH_2- C*H*$_2$), 1.37 (4H, m, 2 x HN-CH_2-CH_2-C*H*$_2$-), 1.79 (6H, s, 2 x Me), 2.75 (4H, t, *J* 7.4, 2 x Ar-CH_2-), 3.03 (4H, m, 2 x HN-C*H*$_2$-), 3.28 (4H, m, 2 x Ar-CH_2-C*H*$_2$-), 3.76 (6H, s, 2 x Me), 4.66 (4H, s, 2 x N-C*H*$_2$-CO-), 6.74 (2H, dd, *J* 8.8, 2.4, 2 x H-6), 7.04 (2H, d, *J* 2.4, 2 x H-4), 7.07 (2H, s, 2 x H-2), 7.19 (2H, d, *J* 8.8, 2 x H-7), 7.87 – 7.99 (4H, m, 4 x NH). δ_c (100 MHz, DMSO-*d*$_6$) 22.6 (2 x Me), 25.1 (2 x CH_2), 26.3 (2 x CH_2), 28.6 (2 x CH_2), 28.87 (1 x CH_2), 28.95 (2 x CH_2), 38.3 (2 x CH_2), 39.1 (2 x CH_2), 48.9 (2 x CH_2), 55.4 (2 x Me), 100.6 (2 x CH), 110.4 (2 x CH), 111.0 (2 x CH), 111.3 (2 x C), 127.8 (2 x CH), 128.1 (2 x C), 131.8 (2 x C), 153.3 (2 x C), 167.4 (2 x CO), 169.0 (2 x CO). ESI-MS m/z 704.1 $(M+H)^+$, 726.0 $(M+Na)^+$.

***N,N'*-(Decane-1,10-diyl)-bis{2-[3-(2-acetamidoethyl)-5-methoxy-1H-indol-1-yl]-acetamide} (4).** Compound **4** (40 mg, 16%) was obtained from **2a** (200 mg, 0.69 mmol), EDCI HCl (230 mg, 1.2 mmol), and 1,10-diaminodecane (59 mg, 0.34 mmol) as a pale yellow solid. mp 156 ^{o}C. Found: C, 66.69; H, 7.62; N, 11.60 . $C_{40}H_{56}N_6O_6$ requires C, 67.01; H, 7.87; N, 11.72. δ_H (400 MHz, MeOH-*d₄*) 1.21 – 1.51 (16H, m, 2 x HN-CH_2-CH_2-CH_2-CH_2- CH_2-CH_2-), 1.92 (6H, s, 2 x Me), 2.92 (4H, t, *J* 6.8, 2 x Ar-CH_2-), 3.20 (4H, t, *J* 6.7, 2 x HN-CH_2-), 3.50 (4H, t, *J* 6.8, 2 x Ar-CH_2-CH_2-), 3.85 (6H, s, 2 x Me), 4.75 (4H, s, 2 x N-CH_2-CO-), 6.84 (2H, dd, *J* 8.8, 2.4, 2 x H-6), 7.04 (2H, s, 2 x H-2), 7.12 (2H, s, 2 x H-4), 7.19 (2H, d, *J* 8.8, 2 x H-7). δ_c (100 MHz, MeOH-d_4) 22.7 (2 x Me), 26.2 (2 x CH_2), 27.8 (2 x CH_2), 30.3 (2 x CH_2), 30.3 (2 x CH_2), 30.5 (2 x CH_2), 30.8 (2 x CH_2), 40.4 (2 x CH_2), 41.3 (2 x CH_2), 50.3 (2 x CH_2), 56.3 (2 x Me), 102.0 (2 x CH), 111.0 (2 x CH), 113.1 (2 x CH), 113.8 (2 x C), 128.6 (2 x CH), 133.6 (2 x C), 155.6 (2 x C), 171.0 (2 x CO), 173.3 (2 x CO). ESI-MS m/z 718.6 $(M+H)^+$, 739.1 $(M+Na)^+$.

***N,N'*-(Undecane-1,11-diyl)-bis{2-[3-(2-acetamidoethyl)-5-methoxy-1H-indol-1-yl]-acetamide} (5).** Compound **5** (30 mg, 38%) was obtained from **2a** (200 mg, 0.69 mmol), EDCI HCl (230 mg, 1.2 mmol), and 1,11-diaminoundecane (20 mg, 0.11 mmol) as a pale yellow solid. mp 156.5 ^{o}C. Found: C, 67.30; H, 7.87; N, 11.38; $C_{41}H_{58}N_6O_6$ requires C, 67.37; H, 8.00; N, 11.50. δ_H (400 MHz, MeOH-d_4) 1.17 – 1.34 (16H, m, 2 x HN-CH_2-CH_2-CH_2-CH_2- CH_2-), 1.39 – 1.48 (4H, m, , 2 x HN- CH_2-CH_2-), 1.89 (6H, s, 2 x Me), 2.89 (4H, t, *J* 6.8, 2 x Ar-CH_2-), 3.17 (4H, t, *J* 6.7, 2 x HN-CH_2-), 3.46 (4H, t, *J* 6.8, 2 x Ar-CH_2-CH_2-), 3.82 (6H, s, 2 x Me), 4.72 (4H, s, 2 x N-CH_2-CO-), 6.81 (2H, dd, *J* 8.8, 2.3, 2 x H-6), 7.01 (2H, s, 2 x H-2), 7.08 (2H, d, *J* 2.3, 2 x H-4), 7.15 (2H, d, *J* 8.8, 2 x H-7). δ_c (100 MHz, MeOH-d_4) 22.7 (2 x Me), 26.1 (2 x CH_2), 27.8 (2 x CH_2), 30.3 (4 x CH_2), 30.5 (1 x CH_2), 30.6 (2 x CH_2), 40.4 (2 x CH_2), 41.3 (2 x CH_2), 50.3 (2 x CH_2), 56.3 (2 x Me), 102.0 (2 x CH), 111.1 (2 x CH), 113.1 (2 x CH), 113.8 (2 x C), 128.6 (2 x CH), 130.3 (2 x C), 133.6 (2 x C), 155.5 (2 x C), 171.0 (2 x CO), 173.3 (2 x CO). ESI-MS m/z 732.1 $(M+H)^+$, 754 $(M+Na)^+$.

***N,N'*-(Dodecane-1,11-diyl)-bis{2-[3-(2-acetamidoethyl)-5-methoxy-1H-indol-1-yl]-acetamide} (6).** Compound **6** (30 mg, 16%) was obtained from **2a** (150 mg, 0.52 mmol), EDCI HCl (173 mg, 0.9 mmol), and 1,12-diaminododecane (50 mg, 0.25 mmol) as a pale yellow solid. mp 159 ^{o}C. Found: C, 67.38; H, 7.88; N, 10.99; $C_{42}H_{60}N_6O_6$ requires C, 67.72; H, 8.12; N, 11.28. δ_H (400 MHz, $CDCl_3$) 1.06 – 1.34 (20H, m, 2 x HN-CH_2-*CH_2*-CH_2-CH_2-CH_2- CH_2), 1.90 (6H, s, 2 x Me), 2.92 (4H, t, *J* 6.8, 2 x Ar-CH_2-), 3.14 (4H, m, 2 x HN-CH_2-), 3.55 (4H, m, 2 x Ar-CH_2-CH_2-), 3.85 (6H, s, 2 x Me), 4.68 (4H, s, 2 x N-CH_2-CO-), 6.87 (2H, s, 2 x H-2), 6.90 (2H, dd, *J* 8.9, 2.4, 2 x H-6), 7.04 (2H, d, *J* 2.3, 2 x H-4), 7.13 (2H, d, *J* 8.9, 2 x H-7). δ_c (100 MHz, $CDCl_3$) 23.3 (2 x Me), 25.4 (2 x CH_2), 26.6 (2 x CH_2), 29.1 (2 x CH_2), 29.3 (2 x CH_2), 29.35 (2 x CH_2), 29.36 (2 x CH_2), 39.4 (2 x CH_2), 39.9 (2 x CH_2), 50.1 (2 x CH_2), 55.9 (2 x Me), 101.1 (2 x CH), 110.2 (2 x CH), 112.9 (2 x CH), 113.6 (2 x C), 126.6 (2 x CH), 128.8 (2 x C), 131.8 (2 x C), 154.6 (2 x C), 168.2 (2 x CO), 170.1 (2 x CO). MS (MALDI-TOF) m/z 746 $(M+H)^+$, 768 $(M+Na)^+$.

_N,N'_-(Hexane-1,6-diyl)-bis{5-[3-(2-acetamidoethyl)-5-methoxy-1H-indol-1-yl]-pentanamide} (7).

Compound **7** (59 mg, 16%) was obtained from **2b** (100 mg, 0.30 mmol), EDCI HCl (100 mg, 0.52 mmol), and 1,6-diaminohexane (17 mg, 0.15 mmol) as a white solid. mp 153 ^oC. Found: C, 67.48; H, 7.92; N, 11.12. $C_{42}H_{60}N_6O_6$ requires C, 67.72; H, 8.12; N, 11.28. δ_H (400 MHz, MeOH-d_4) 1.20 – 1.34 (4H, m, 2 x HN-CH_2-CH_2-CH_2-), 1.36 – 1.46 (4H, m, 2 x HN-CH_2-CH_2-), 1.50 – 1.63 (4H, m, 2 x CO-CH_2-CH_2-), 1.72 – 1.84 (4H, m, 2 x CO- CH_2-CH_2-CH_2-), 1.91 (6H, s, 2 x Me), 2.15 (4H, t, *J* 7.3, CO-CH_2-), 2.87 (4H, t, *J* 7.3, 2 x Ar-CH_2-), 3.09 (4H, t, *J* 6.9, 2 x HN-CH_2-), 3.43 (4H, m, 2 x Ar-CH_2-CH_2-), 3.81 (6H, s, 2 x Me), 4.07 (4H, t, *J* 6.8, 2 x N-CH_2-), 6.78 (2H, dd, *J* 8.8, 2.3, 2 x H-6), 6.99 (2H, s, 2 x H-2), 7.05 (2H, d, *J* 2.3, 2 x H-4), 7.22 (2H, d, *J* 8.8, 2 x H-7). δ_c (100 MHz, MeOH-d_4) 22.7 (2 x Me), 24.4 (2 x CH_2), 26.1 (2 x CH_2), 27.5 (2 x CH_2), 30.3 (2 x CH_2), 31.0 (2 x CH_2), 36.6 (2 x CH_2), 40.2 (2 x CH_2), 41.5 (2 x CH_2), 46.8 (2 x CH_2), 56.3 (2 x Me), 101.9 (2 x CH), 111.2 (2 x CH), 112.4 (2 x CH), 112.6 (2 x C), 127.6 (2 x CH), 129.8 (2 x C), 133.3 (2 x C), 155.1 (2 x C), 173.2 (2 x CO), 175.7 (2 x CO). ESI-MS: m/z 768 $(M+Na)^+$.

_N,N'_-(Heptane-1,7-diyl)-bis{5-[3-(2-acetamidoethyl)-5-methoxy-1H-indol-1-yl]pentanamide} (8).

Compound **8** (46 mg, 47%) was obtained from **2b** (88 mg, 0.26 mmol), EDCI HCl (86 mg, 0.45 mmol), and 1,7-diaminoheptane (17 mg, 0.13 mmol) as a pale brown solid. mp 86 ^oC. Found: C, 67.93; H, 8.16; N, 11.01. $C_{43}H_{62}N_6O_6$ requires C, 68.05; H, 8.23; N, 11.07. δ_H (400 MHz, MeOH-d_4) 1.23 – 1.29 (6H, m, 2 x HN-CH_2-CH_2-CH_2-, HN-CH_2-CH_2- CH_2-CH_2-), 1.38 – 1.46 (4H, m, 2 x HN-CH_2-CH_2-), 1.52 – 1.62 (4H, m, 2 x CO-CH_2-CH_2-),1.72 – 1.82 (4H, m, 2 x CO- CH_2-CH_2-CH_2-), 1.91 (6H, s, 2 x Me), 2.14 (4H, t, *J* 7.3, CO-CH_2-), 2.87 (4H, t, *J* 7.3, 2 x Ar-CH_2-), 3.09 (4H, t, *J* 7.0, 2 x HN-CH_2-), 3.43 (4H, , t, *J* 7.3, 2 x Ar-CH_2-CH_2-), 3.81 (6H, s, 2 x Me), 4.07 (4H, t, *J* 6.9, 2 x N-CH_2-), 6.78 (2H, dd, *J* 8.8, 2.3, 2 x H-6), 6.99 (2H, s, 2 x H-2), 7.05 (2H, d, *J* 2.3, 2 x H-4), 7.22 (2H, d, *J* 8.8, 2 x H-7). δ_c (100 MHz, MeOH-d_4) 22.7 (2 x Me), 24.4 (2 x CH_2), 26.1 (2 x CH_2), 27.8 (2 x CH_2), 29.9 (1 x CH_2), 30.3 (2 x CH_2), 31.0 (2 x CH_2), 36.6 (2 x CH_2), 40.3 (2 x CH_2), 41.5 (2 x CH_2), 46.8 (2 x CH_2), 56.3 (2 x Me), 101.8 (2 x CH), 111.2 (2 x CH), 112.4 (2 x CH), 112.6 (2 x C), 127.6 (2 x CH), 129.8 (2 x C), 133.3 (2 x C), 155.1 (2 x C), 173.2 (2 x CO), 175.6 (2 x CO). ESI-MS: m/z 782 $(M+Na)^+$.

***N,N'*-(Octane-1,7-diyl)-bis{5-[3-(2-acetamidoethyl)-5-methoxy-1H-indol-1-yl]pentanamide} (9).**

Compound **9** (59 mg, 51%) was obtained from **2b** (100 mg, 0.30 mmol), EDCI HCl (100 mg, 0.52 mmol), and 1,8-diaminooctane (22 mg, 0.15 mmol) as a white solid. mp 110 ^oC. Found: C, 67.99; H, 8.02; N, 10.48. $C_{44}H_{64}N_6O_6$ requires C, 68.37; H, 8.35; N, 10.87. δ_H (400 MHz, DMSO-*d6*) 1.17 – 1.37 (8H, m, 2 x HN-CH_2-CH_2-C*H2*- C*H2*), 1.39 – 1.46 (4H, m, 2 x HN-CH_2-*CH2*-), 1.62 – 1.70 (4H, m, 2 x CO-CH_2-*CH2*-), 1.77 – 1.81 (10H, m, 2 x CO- CH_2-CH_2-*CH2*-, 2 x Me), 2.04 (4H, t, *J* 7.3, CO-*CH2*-), 2.75 (4H, t, *J* 7.3, 2 x Ar-CH_2-), 2.98 (4H, m, 2 x HN-C*H2*-), 3.28 (4H, , m, 2 x Ar-CH_2-C*H2*-), 3.76 (6H, s, 2 x Me), 4.04 (4H, t, *J* 6.7, 2 x N-C*H2*-), 6.74 (2H, dd, *J* 8.8, 2.0, 2 x H-6), 7.02 (2H, d, *J* 2.0, 2 x H-4), 7.10 (2H, s, 2 x H-2), 7.28 (2H, d, *J* 8.8, 2 x H-7), 7.70 (2H, m, 2 x NH), 7.92 (2H, m, 2 x NH). δ_c (100 MHz, DMSO-*d6*) 22.66 (2 x Me), 22.68 (2 x CH_2), 25.1 (2 x CH_2), 26.4 (2 x CH_2), 28.7 (2 x CH_2), 29.1 (2 x CH_2), 29.5 (2 x CH_2), 34.9 (2 x CH_2), 38.3 (2 x CH_2), 39.1 (2 x CH_2), 45.1 (2 x CH_2), 55.4 (2 x Me), 100.5 (2 x CH), 110.3 (2 x CH), 110.8 (2 x CH), 111.0 (2 x C), 126.5 (2 x CH), 127.9 (2 x C), 131.2 (2 x C), 153.0 (2 x C), 169.0 (2 x CO), 171.6 (2 x CO). MALDI-MS: m/z 773.5 (M^+), 796.4 $(M+Na)^+$.

***N,N'*-(Nonane-1,7-diyl)-bis{5-[3-(2-acetamidoethyl)-5-methoxy-1H-indol-1-yl]pentanamide} (10).**

Compound **10** (60 mg, 34%) was obtained from **2b** (150 mg, 0.45 mmol), EDCI HCl (150 mg, 0.78 mmol), and 1,9-diaminononane (35 mg, 0.22 mmol) as a white solid. mp 100 ^oC. Found: C, 68.35; H, 8.52; N, 10.38. $C_{45}H_{66}N_6O_6$ requires C, 68.67; H, 8.45; N, 10.68. δ_H (400 MHz, DMSO-*d6*) 1.16 – 1.36 (10H, m, 2 x HN-CH_2-CH_2-C*H2*- C*H2*-, HN-CH_2-CH_2-CH_2-CH_2- C*H2*-), 1.38 – 1.46 (4H, m, 2 x HN-CH_2-*CH2*-), 1.62 – 1.70 (4H, m, 2 x CO-CH_2-*CH2*-), 1.80 (6H, 2 x Me), 2.04 (4H, t, *J* 6.7, CO-*CH2*-), 2.76 (4H, t, *J* 6.7, 2 x Ar-CH_2-), 2.98 (4H, m, 2 x HN-C*H2*-), 3.28 (4H, , m, 2 x Ar-CH_2-C*H2*-), 3.76 (6H, s, 2 x Me), 4.04 (4H, t, *J* 6.7, 2 x N-C*H2*-), 6.74 (2H, d, *J* 7.9, 2 x H-6), 7.02 (2H, s, 2 x H-4), 7.10 (2H, s, 2 x H-2), 7.28 (2H, d, *J* 8.7, 2 x H-7), 7.71 (2H, m, 2 x NH), 7.92 (2H, m, 2 x NH). δ_c (100 MHz, DMSO-*d6*) 22.66 (2 x Me), 22.68 (2 x CH_2), 25.1 (2 x CH_2), 26.4 (2 x CH_2), 28.7 (2 x CH_2), 28.9 (1 x CH_2), 29.1 (2 x CH_2), 29.5 (2 x CH_2), 34.9 (2 x CH_2), 38.3 (2 x CH_2), 39.1 (2 x CH_2), 45.1 (2 x CH_2), 55.4 (2 x Me), 100.5 (2 x CH), 110.3 (2 x CH), 110.8 (2 x CH), 111.0 (2 x C), 126.5 (2 x

CH), 127.9 (2 x C), 131.2 (2 x C), 153.0 (2 x C), 169.0 (2 x CO), 171.6 (2 x CO). MALDI-MS: m/z 787.5 (M^+), 809.5 $(M+Na-1)^+$

***N,N'*-(Decane-1,7-diyl)-bis{5-[3-(2-acetamidoethyl)-5-methoxy-1H-indol-1-yl]pentanamide} (11).**

Compound **11** (60 mg, 33%) was obtained from **2b** (150 mg, 0.45 mmol), EDCI HCl (150 mg, 0.78 mmol), and 1,9-diaminodecane (38 mg, 0.22 mmol) as a white solid. mp 126 ^oC. Found: C, 68.82; H, 8.41; N, 10.32. $C_{46}H_{68}N_6O_6$ requires C, 68.97; H, 8.56; N, 10.49. δ_H (400 MHz, MeOH-*d4*) 1.26 – 1.30 (12H, m, 2 x HN-CH_2-CH_2-C*H2*-C*H2*-C*H2*-), 1.39 – 1.47 (4H, m, 2 x HN-CH_2-*CH2*-), 1.52 – 1.62 (4H, m, 2 x CO-CH_2-*CH2*-), 1.73 – 1.83 (4H, m, 2 x CO-CH_2-CH_2-*CH2*-), 1.91 (6H, s, 2 x Me), 2.15 (4H, t, *J* 7.4, CO-*CH2*-), 2.87 (4H, t, *J* 7.3, 2 x Ar-CH_2-), 3.10 (4H, t, *J* 7.0, 2 x HN-C*H2*-), 3.43 (4H, , t, *J* 7.3, 2 x Ar-CH_2-C*H2*-), 3.81 (6H, s, 2 x Me), 4.08 (4H, t, *J* 6.9, 2 x N-C*H2*-), 6.78 (2H, dd, *J* 8.8, 2.3, 2 x H-6), 7.00 (2H, s, 2 x H-2), 7.05 (2H, d, *J* 2.3, 2 x H-4), 7.22 (2H, d, *J* 8.8, 2 x H-7). δ_c (100 MHz, MeOH-*d4*) 22.7 (2 x Me), 24.4 (2 x CH_2), 26.2 (2 x CH_2), 27.9 (2 x CH_2), 30.3 (2 x CH_2), 30.4 (2 x CH_2), 30.5 (2 x CH_2), 30.9 (2 x CH_2), 36.6 (2 x CH_2), 40.3 (2 x CH_2), 41.5 (2 x CH_2), 46.8 (2 x CH_2), 56.3 (2 x Me), 101.8 (2 x CH), 111.2 (2 x CH), 112.4 (2 x CH), 112.6 (2 x C), 127.6 (2 x CH), 129.8 (2 x C), 133.3 (2 x C), 155.1 (2 x C), 173.2 (2 x CO), 175.6 (2 x CO). ESI-MS: m/z 823.7 $(M+Na)^+$.

***N,N'*-(Undecane-1,7-diyl)-bis{5-[3-(2-acetamidoethyl)-5-methoxy-1H-indol-1-yl]pentanamide} (12).**

Compound **12** (60 mg, 43%) was obtained from **2b** (95 mg, 0.29 mmol), EDCI HCl (96 mg, 0.50 mmol), and 1,9-diaminoundecane (27 mg, 0.14 mmol) as a pale brown solid. mp 102 ^oC. Found: C, 69.12; H, 8.52; N, 10.30. $C_{47}H_{70}N_6O_6$ requires C, 69.26; H, 8.66; N, 10.31. δ_H (400 MHz, MeOH-*d4*) 1.26 – 1.30 (14H, m, 2 x HN-CH_2-CH_2-C*H2*-C*H2*-C*H2*- C*H2*-),1.40 – 1.48 (4H, m, 2 x HN-CH_2-*CH2*-), 1.53 – 1.62 (4H, m, 2 x CO-CH_2-*CH2*-), 1.75 – 1.85 (4H, m, 2 x CO- CH_2-CH_2-*CH2*-), 1.93 (6H, s, 2 x Me), 2.17 (4H, t, *J* 7.4, CO-*CH2*-), 2.89 (4H, t, *J* 7.3, 2 x Ar-CH_2-), 3.13 (4H, t, *J* 7.0, 2 x HN-C*H2*-), 3.45 (4H, , t, *J* 7.3, 2 x Ar-CH_2-C*H2*-), 3.83 (6H, s, 2 x Me), 4.10 (4H, t, *J* 6.9, 2 x N-C*H2*-), 6.80 (2H, dd, *J* 8.9, 2.4, 2 x H-6), 7.02 (2H, s, 2 x H-2), 7.07 (2H, d, *J* 2.3, 2 x H-4), 7.24 (2H, d, *J* 8.8, 2 x H-7). δ_c (100 MHz, MeOH-*d4*) 22.7 (2 x Me), 24.5 (2 x CH_2), 26.2 (2 x CH_2), 27.9 (2 x CH_2), 30.1 (1 x CH_2), 30.35 (2 x CH_2), 30.48 (2 x CH_2), 30.6 (2 x CH_2), 30.9 (2 x CH_2), 36.6 (2 x CH_2), 40.3 (2 x CH_2), 41.5 (2 x CH_2), 46.8 (2 x CH_2), 56.3 (2 x Me), 101.8 (2 x CH), 111.2 (2 x CH), 112.4 (2 x CH), 112.6 (2 x C), 127.6 (2 x CH), 129.8 (2 x C), 133.3 (2 x C), 155.1 (2 x C), 173.2 (2 x CO), 175.6 (2 x CO). ESI-MS: m/z 837.4 $(M+Na)^+$.

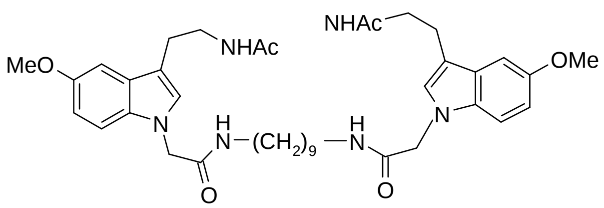
_N,N'_-(Dodecane-1,7-diyl)-bis{5-[3-(2-acetamidoethyl)-5-methoxy-1H-indol-1-yl]pentanamide} (13).

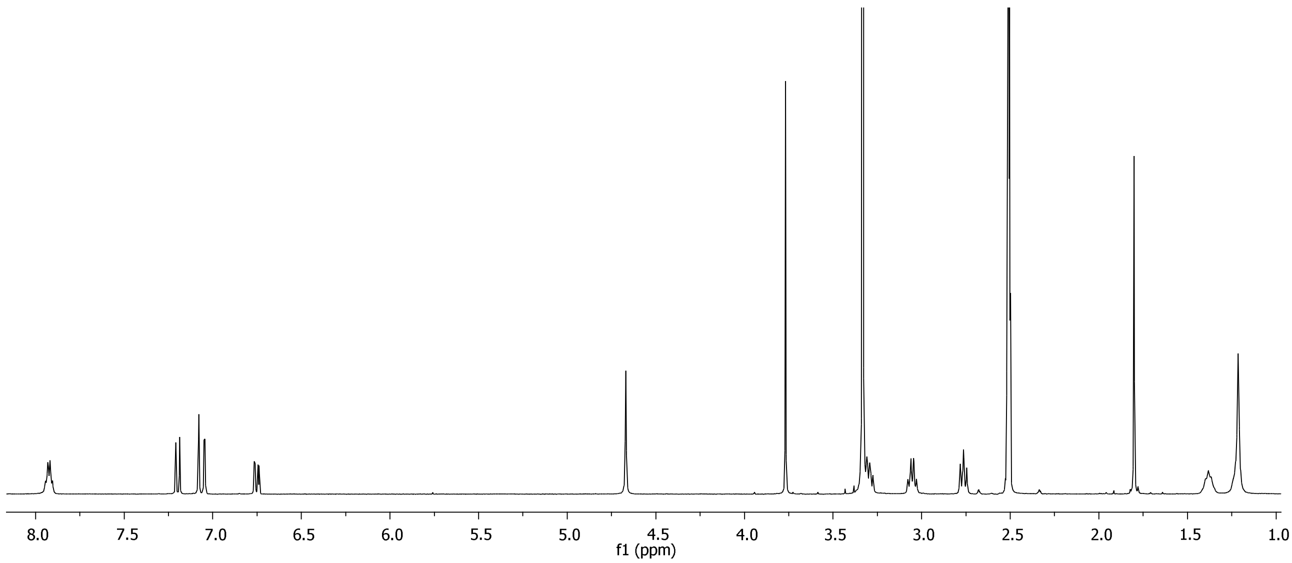
Compound **13** (80 mg, 44%) was obtained from **2b** (150 mg, 0.45 mmol), EDCI HCl (150 mg, 0.78 mmol), and 1,9-diaminododecane (45 mg, 0.22 mmol) as a pale brown solid. mp 97 ^oC. Found: C, 69.43; H, 8.66; N, 10.13. $C_{48}H_{72}N_6O_6$ requires C, 69.53; H, 8.75; N, 10.14. δ_H (400 MHz, $CDCl_3$) 1.18 – 1.26 (16H, m, 2 x HN-CH_2-CH_2-C*H₂*-C*H₂*-C*H₂* -C*H₂*-), 1.34 – 1.45 (4H, m, 2 x HN-CH_2-CH_2-), 1.52 – 1.61 (4H, m, 2 x CO-CH_2-CH_2-), 1.74 – 1.82 (4H, m, 2 x CO-CH_2-CH_2-CH_2-), 1.91 (6H, s, 2 x Me), 2.06 (4H, t, *J* 7.4, CO-CH_2-), 2.88 (4H, t, *J* 6.7, 2 x Ar-CH_2-), 3.13 (4H, m, 2 x HN-CH_2-), 3.51 (4H, , q, *J* 6.6, 2 x Ar-CH_2-CH_2-), 3.82 (6H, s, 2 x Me), 4.01 (4H, t, *J* 6.7, 2 x N-CH_2-), 5.89 (2H, t, *J* 5.5, 2 x NH), 6.12 (2H, t, *J* 5.4, 2 x NH), 6.83 (2H, dd, *J* 8.9, 2.4, 2 x H-6), 6.87 (2H, s, 2 x H-2), 6.99 (2H, d, *J* 2.3, 2 x H-4), 7.15 (2H, d, *J* 8.9, 2 x H-7). δ_c (100 MHz, $CDCl_3$) 23.1 (2 x CH_2), 23.3 (2 x Me), 25.3 (2 x CH_2), 26.9 (2 x CH_2), 29.2 (2 x CH_2), 29.46 (4 x CH_2), 29.6 (2 x CH_2), 29.7 (2 x CH_2), 36.0 (2 x CH_2), 39.6 (2 x CH_2), 40.0 (2 x CH_2), 46.1 (2 x CH_2), 56.0 (2 x Me), 100.8 (2 x CH), 110.3 (2 x CH), 111.2 (2 x C), 111.9 (2 x CH), 126.4 (2 x CH), 128.3 (2 x C), 131.7 (2 x C), 155.8 (2 x C), 172.5 (2 x CO), MALDI-MS: m/z 829.8 $(M)^+$, 852.8 $(M+Na)^+$

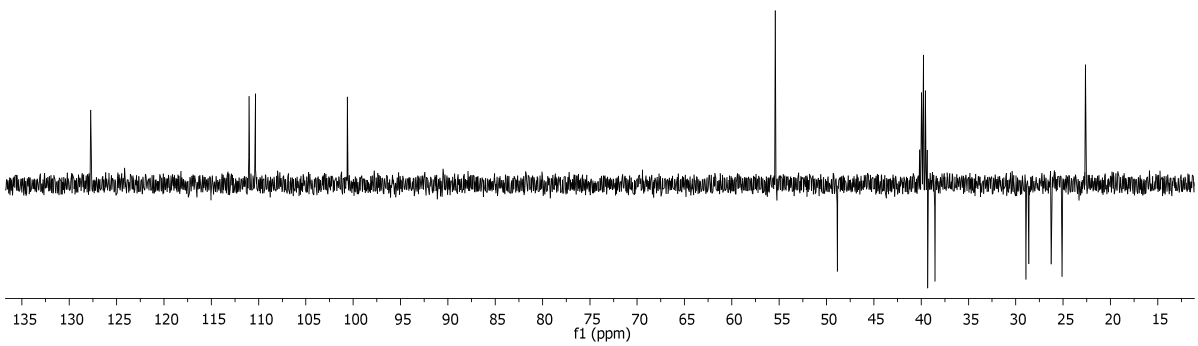
5-[3-(2-Acetylamino-ethyl)-5-methoxy-indol-1-yl]-pentanoic acid hexylamide (14)

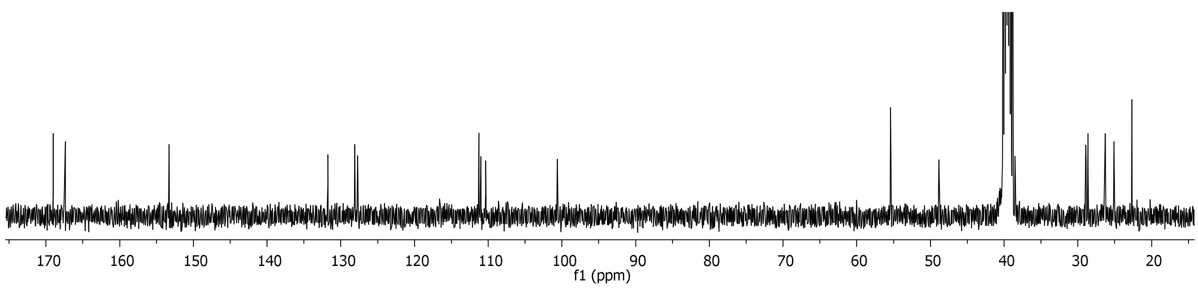
Hexylamine (0.03ml, 0.23 mmol) was added dropwise to a mixture of **2b** (77.5mg, 0.23 mmol), PyBop(182 mg, 0.350 mmol), HOBT(47 mg, 0.350 mmol), DIPEA (0.16 ml, 0.933 mmol) in dry DMF (8 ml). After stirring for 12 hours, the reaction mixture was extracted with ethyl acetate (3 x 20 ml). The combined organic layers were washed with water, dried over sodium sulphate and the solvent was removed under reduced pressure. The residue was purified twice by column chromatography (silica gel, chloroform/MeOH/ammonia, 100:10:1; silica gel, AcOEt:MeOH 10:1) to give **14** (56 mg, 58%) as a white solid, mp 98 ^oC. Found: C, 69.46; H, 8.69; N, 10.26 . $C_{24}H_{37}N_3O_3$ requires C, 69.36; H, 8.97; N, 10.11. δ_H (400 MHz, $CDCl_3$) 0.86 (3H, t, *J* 7.0, -CH_2-CH_3), 1.23 – 1.30 (6H, m, HN-CH_2-CH_2- CH_2-CH_2-CH_2-CH_3), 1.38 – 1.45 (2H, m, HN-CH_2-CH_2-), 1.54 – 1.64 (2H, m, CO-CH_2-CH_2-), 1.72 – 1.87 (2H, m, CO- CH_2-CH_2-CH_2-), 1.92 (3H, s, Me), 2.15 (2H, t, *J* 7.3, CO-CH_2-), 2.90 (2H, t, *J* 6.6, Ar-CH_2-), 3.16 (2H, m, HN-CH_2-), 3.43 (4H, m, 2 x Ar-CH_2-CH_2-), 3.85 (3H, s, Me), 4.05 (2H, t, *J* 6.8, N-CH_2-), 5.50 (1H, m, NH), 5.84 (1H, m, NH), 6.86 (1H, dd, *J* 8.9, 2.4, H-6), 6.90 (1H, s, H-2), 7.01 (2H, d, *J* 2.4, H-4), 7.18 (2H, d, *J* 8.9, H-7). δ_c (100 MHz, $CDCl_3$) 14.1 (Me), 22.6 (CH_2), 23.2 (CH_2), 23.4 (Me), 25.3 (CH_2), 26.7

(CH_2), 29.7 (CH_2), 29.8 (CH_2), 31.6 (CH_2), 36.1 (CH_2), 39.7 (CH_2), 40.0 (CH_2), 46.2 (CH_2), 56.1 (Me), 100.8 (CH), 110.4 (CH), 111.3 (C), 112.1 (CH), 126.5 (CH), 128.4 (C), 131.8 (C), 153.9 (C), 170.3 (CO), 172.3 (CO). ESI-MS: m/z 438.3 $(M+Na)^+$

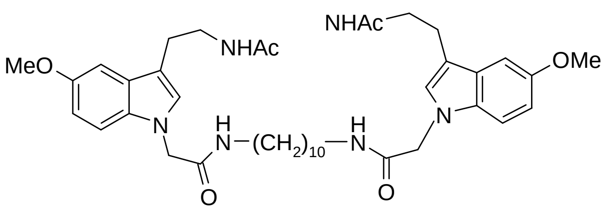


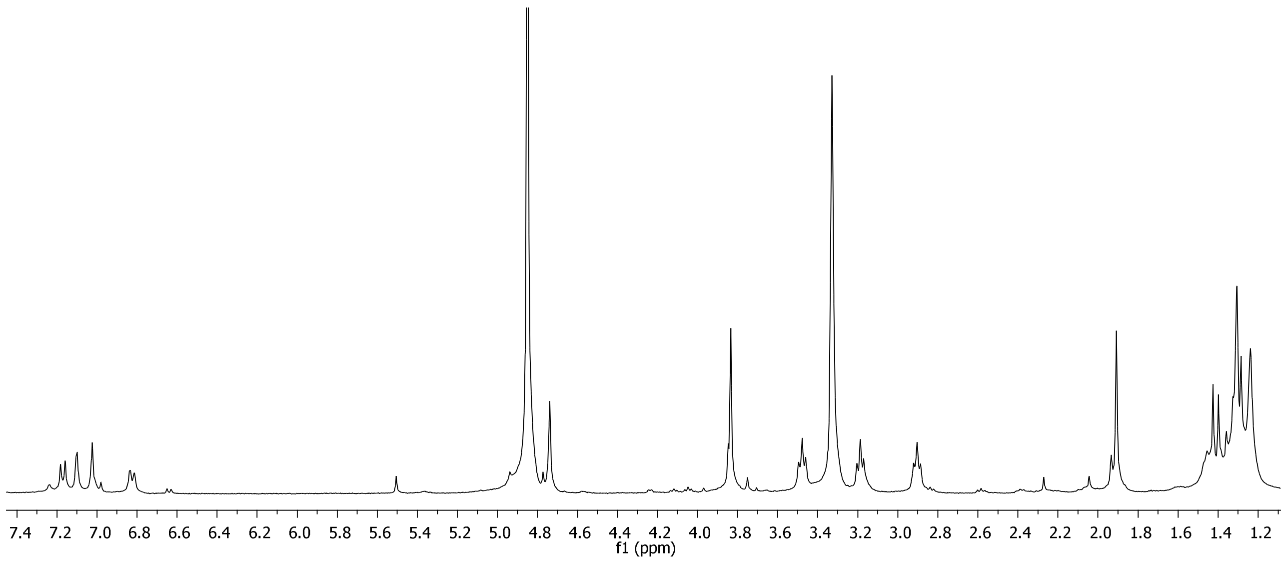


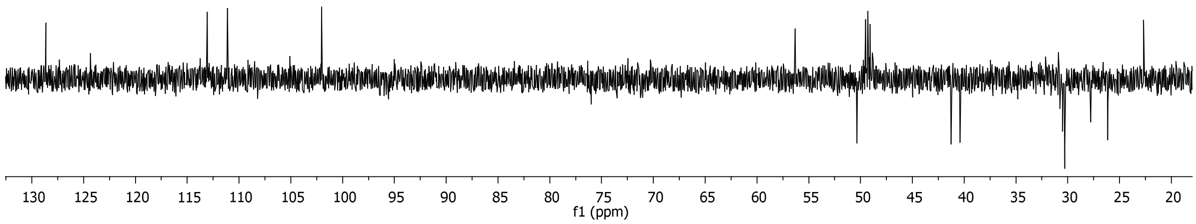


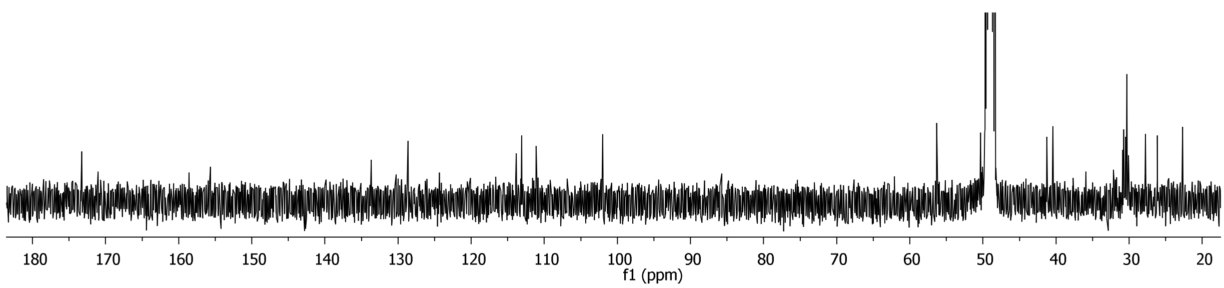


^{1}H (400 MHz), DEPT-135, and ^{13}C (100 MHz) spectra of compound **3** (DMSO-d_6)

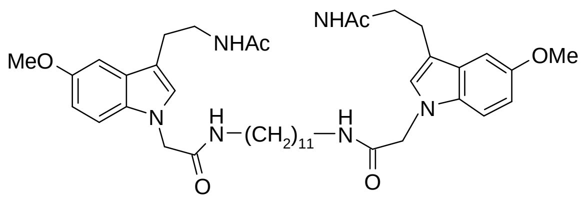


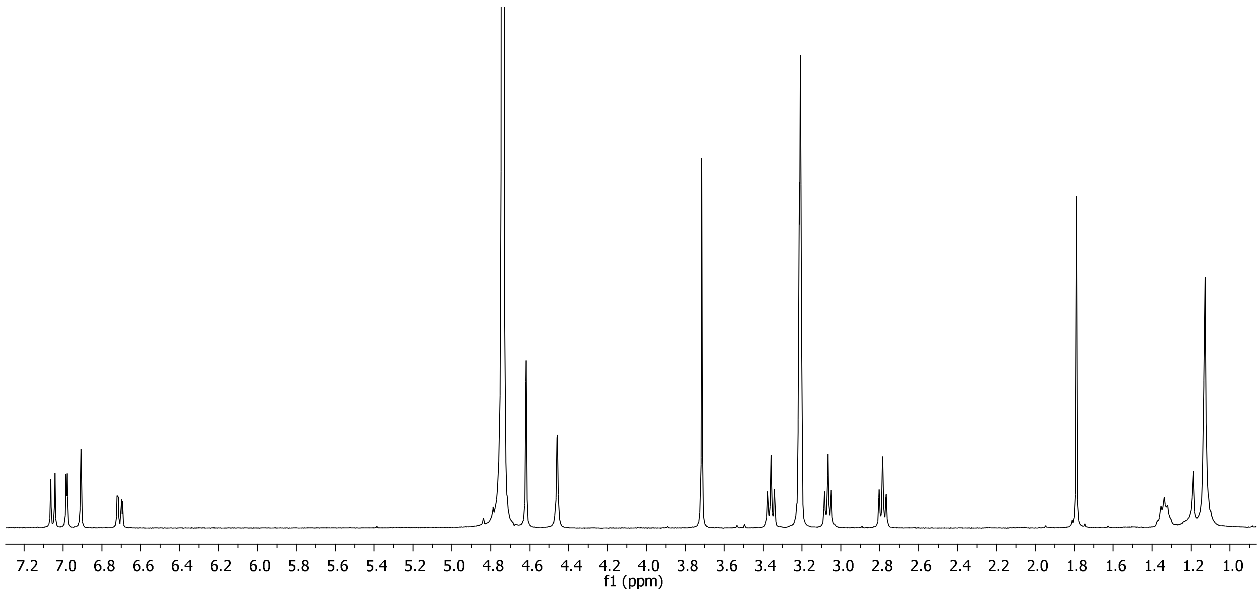


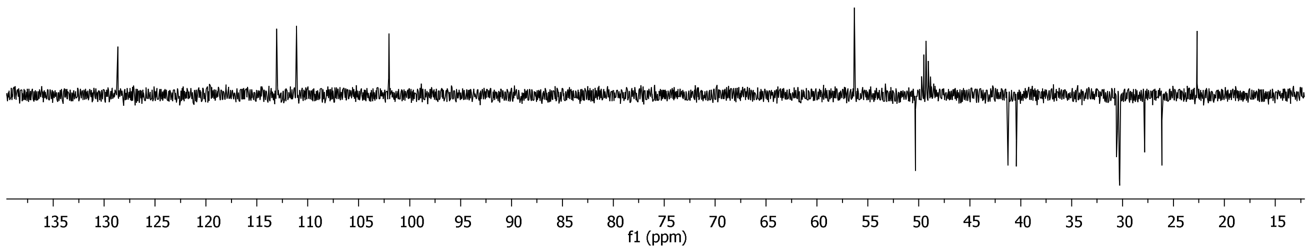


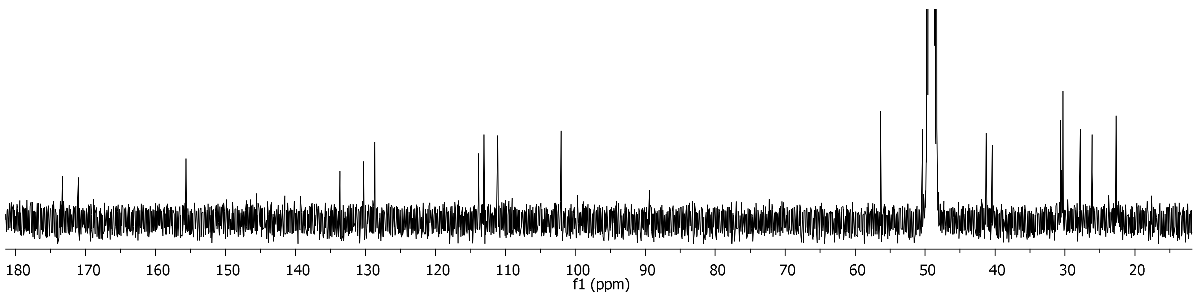


^{1}H (400 MHz), DEPT-135, and ^{13}C (100 MHz) spectra of compound **4** (MeOH-d_4)

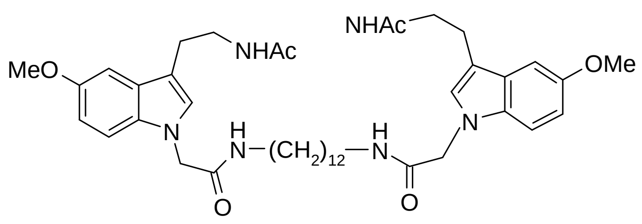


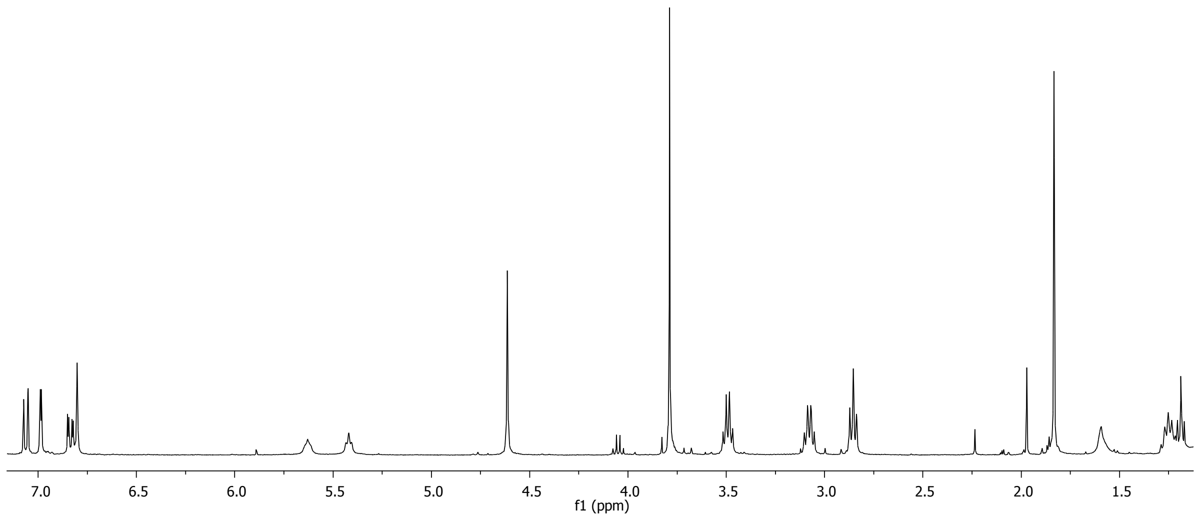


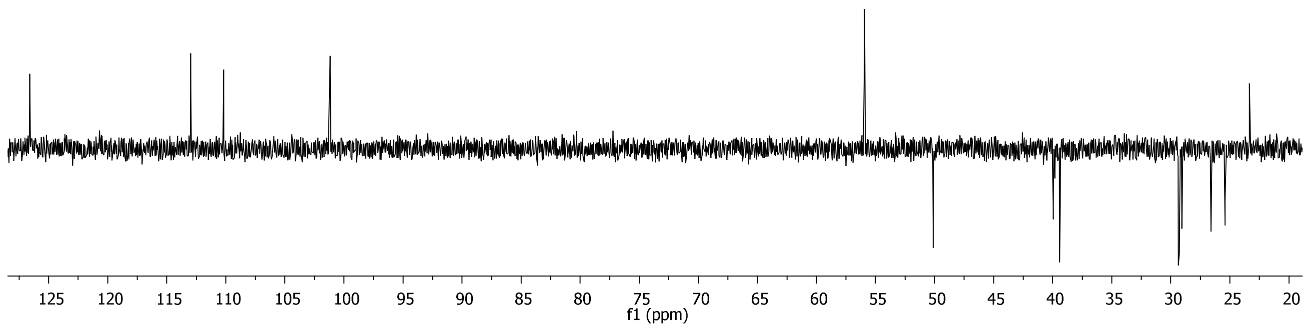


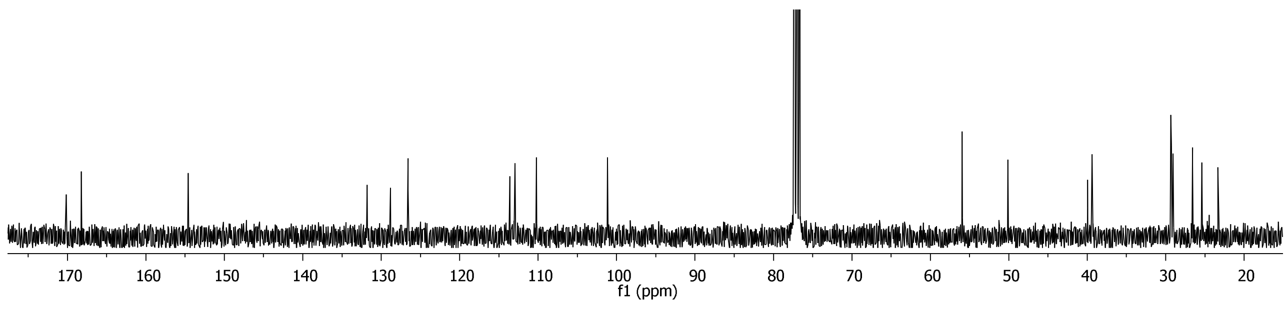


^{1}H (400 MHz), DEPT-135, and ^{13}C (100 MHz) spectra of compound **5** (MeOH-d_4)

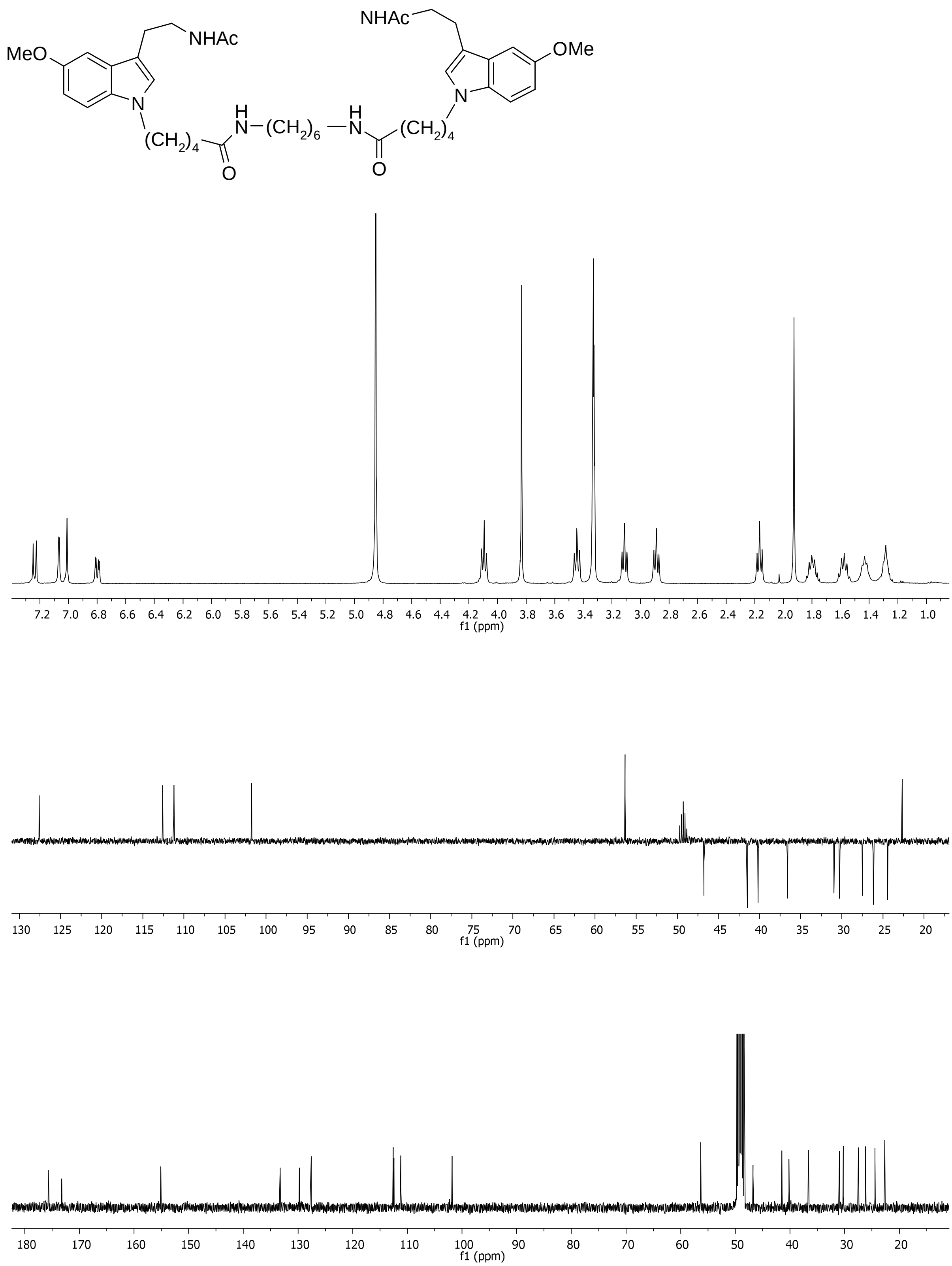




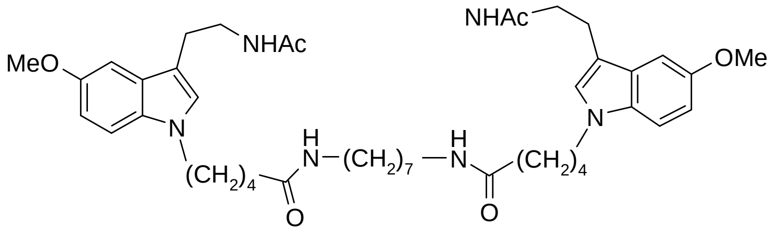


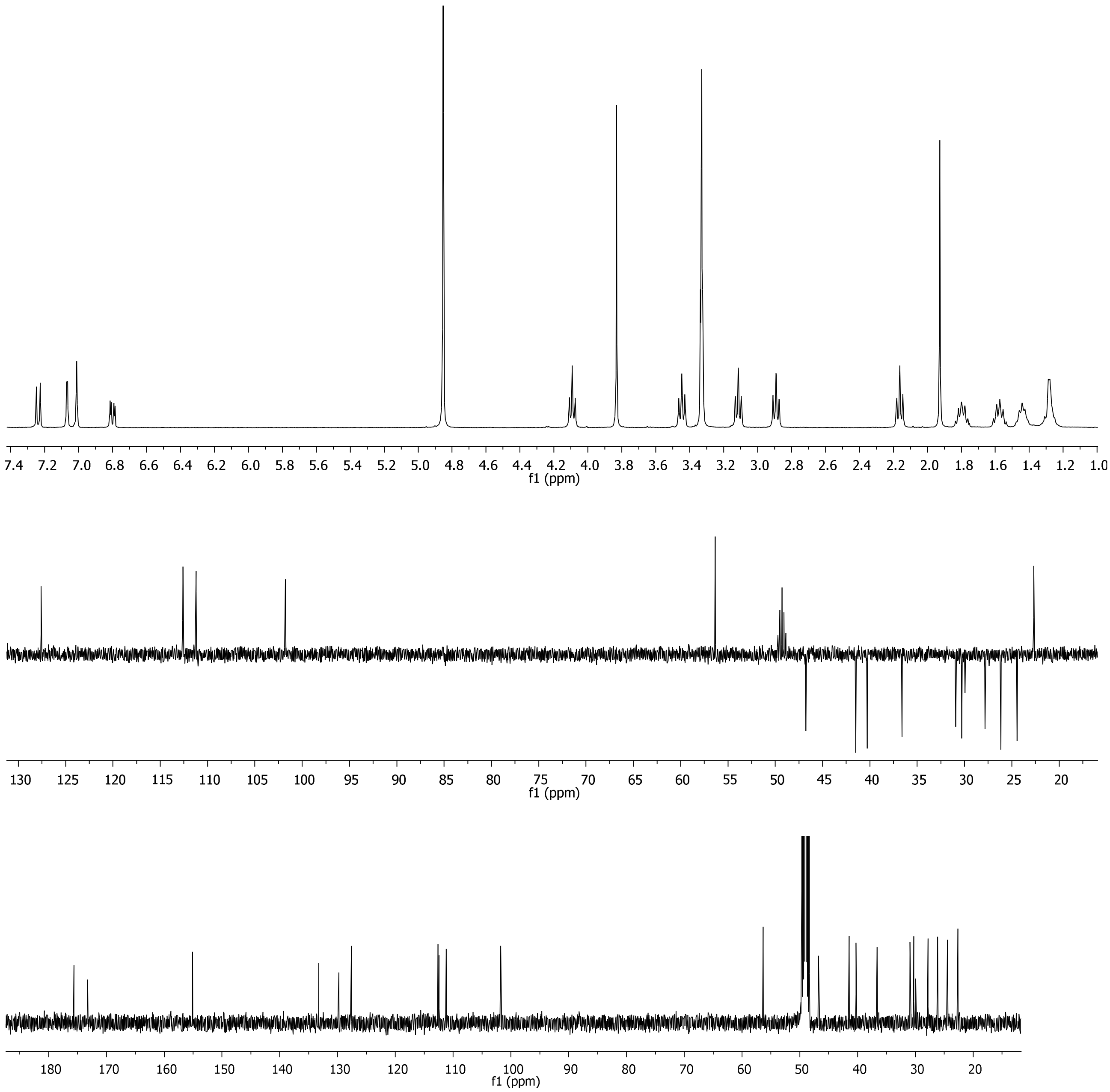


^{1}H (400 MHz), DEPT-135, and ^{13}C (100 MHz) spectra of compound **6** ($CDCl_3$)

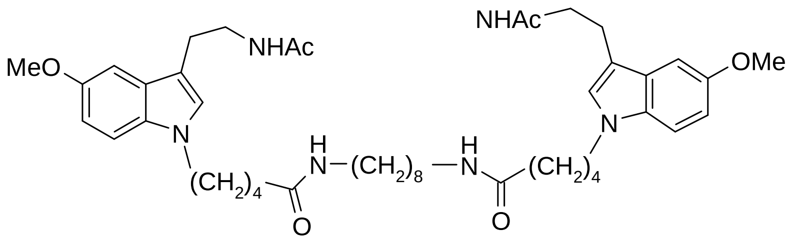


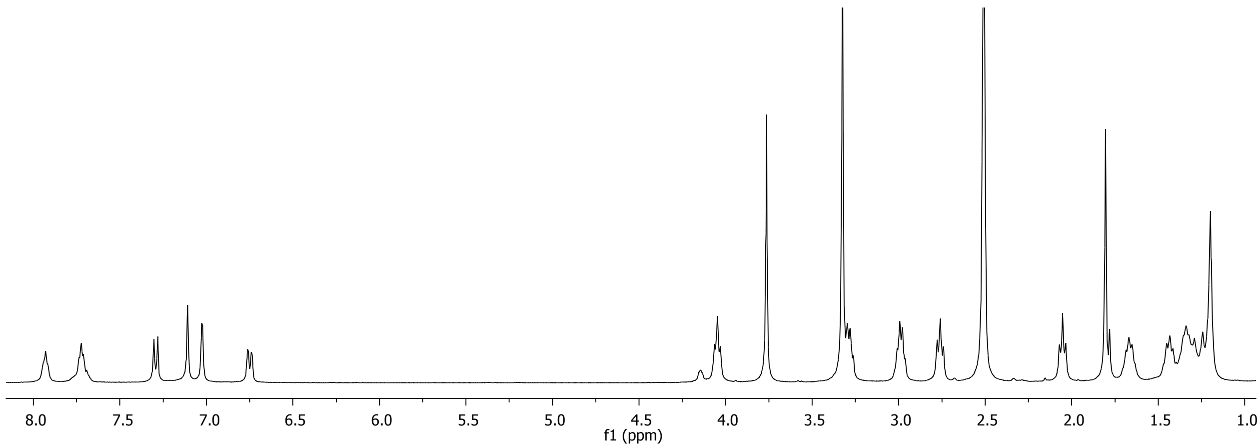
[1]H (400 MHz), DEPT-135, and [13]C (100 MHz) spectra of compound **7** (MeOH-d_4)

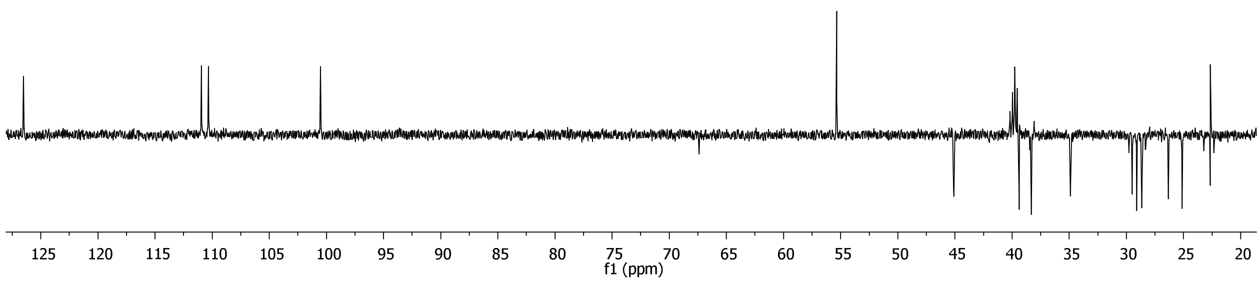


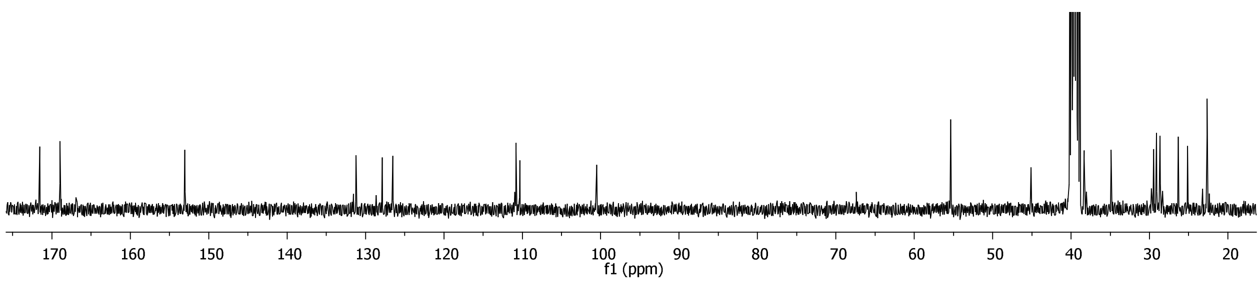


[1]H (400 MHz), DEPT-135, and [13]C (100 MHz) spectra of compound **8** (MeOH-d_4)

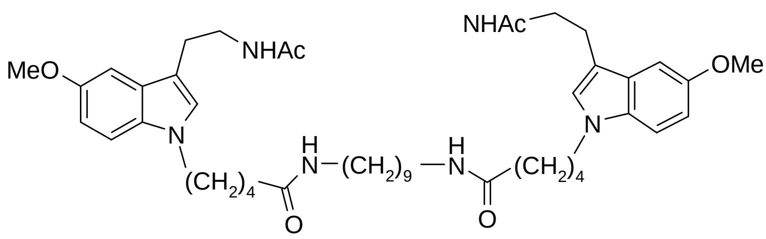


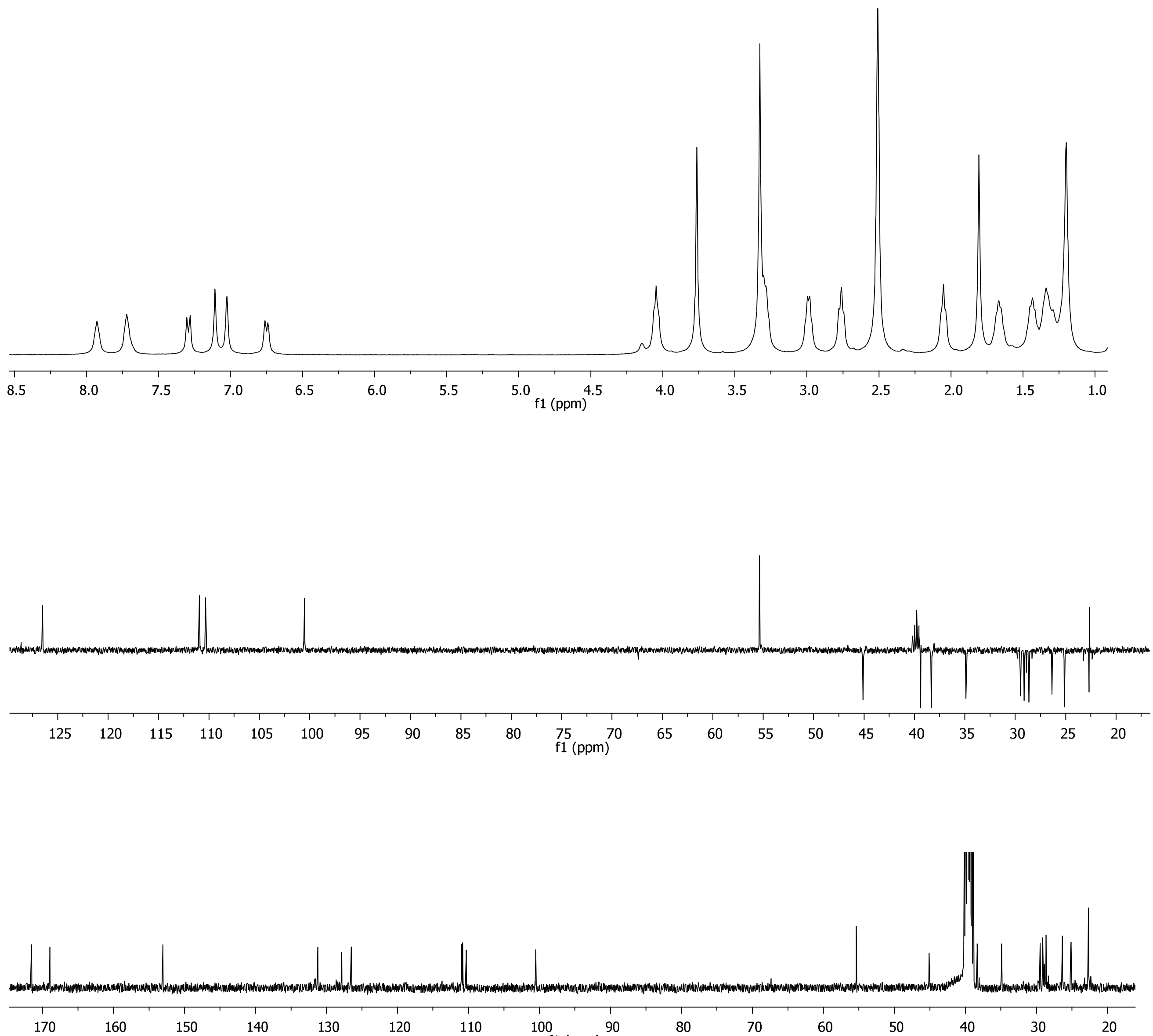




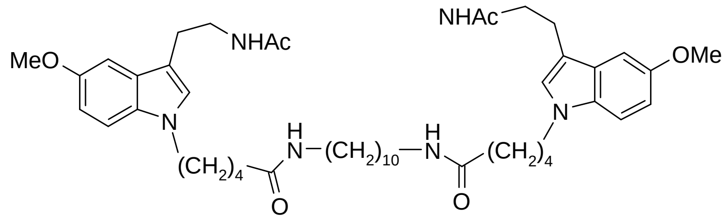


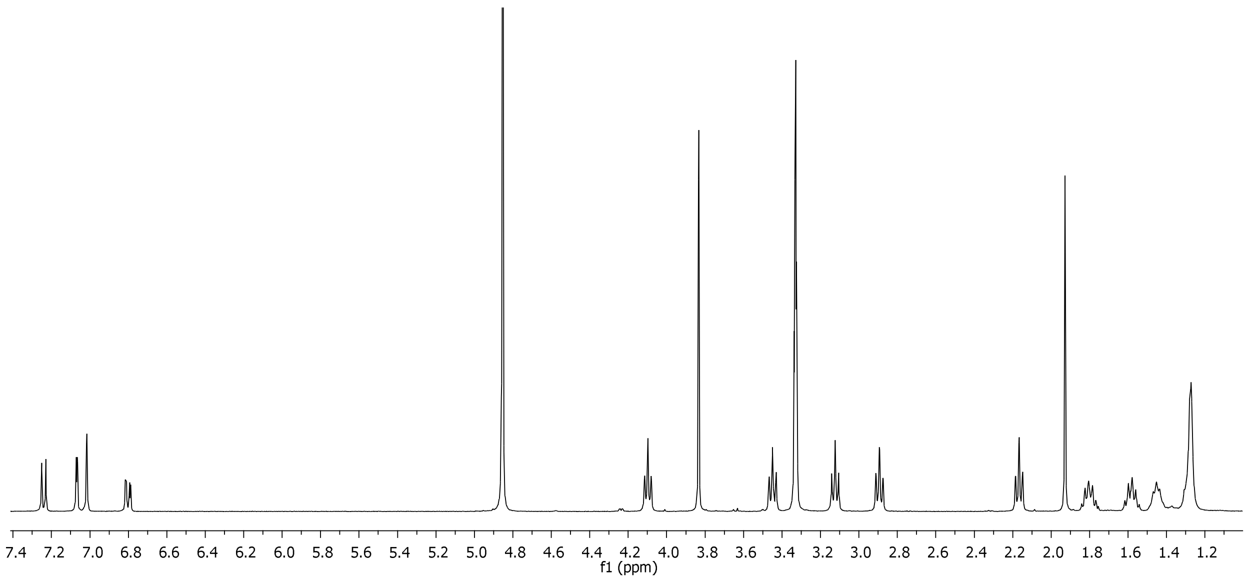
^{1}H (400 MHz), DEPT-135, and ^{13}C (100 MHz) spectra of compound **9** (DMSO-d_6)

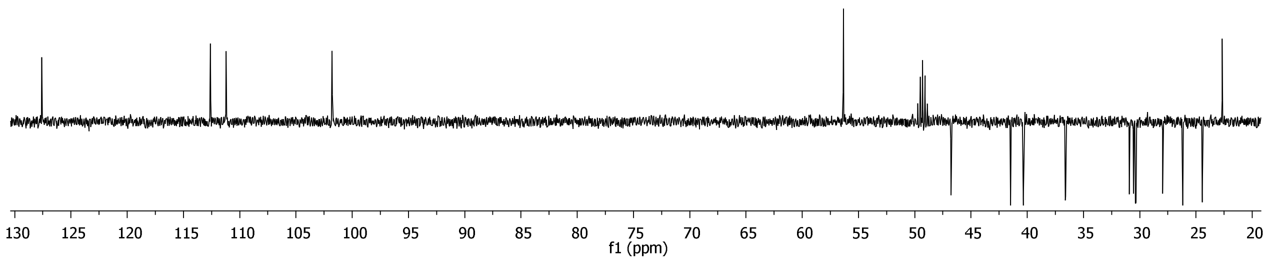


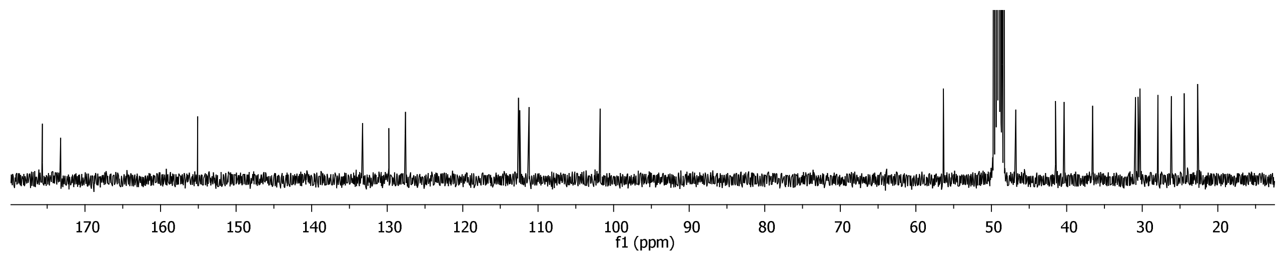


^{1}H (400 MHz), DEPT-135, and ^{13}C (100 MHz) spectra of compound **10** (DMSO-d_6)

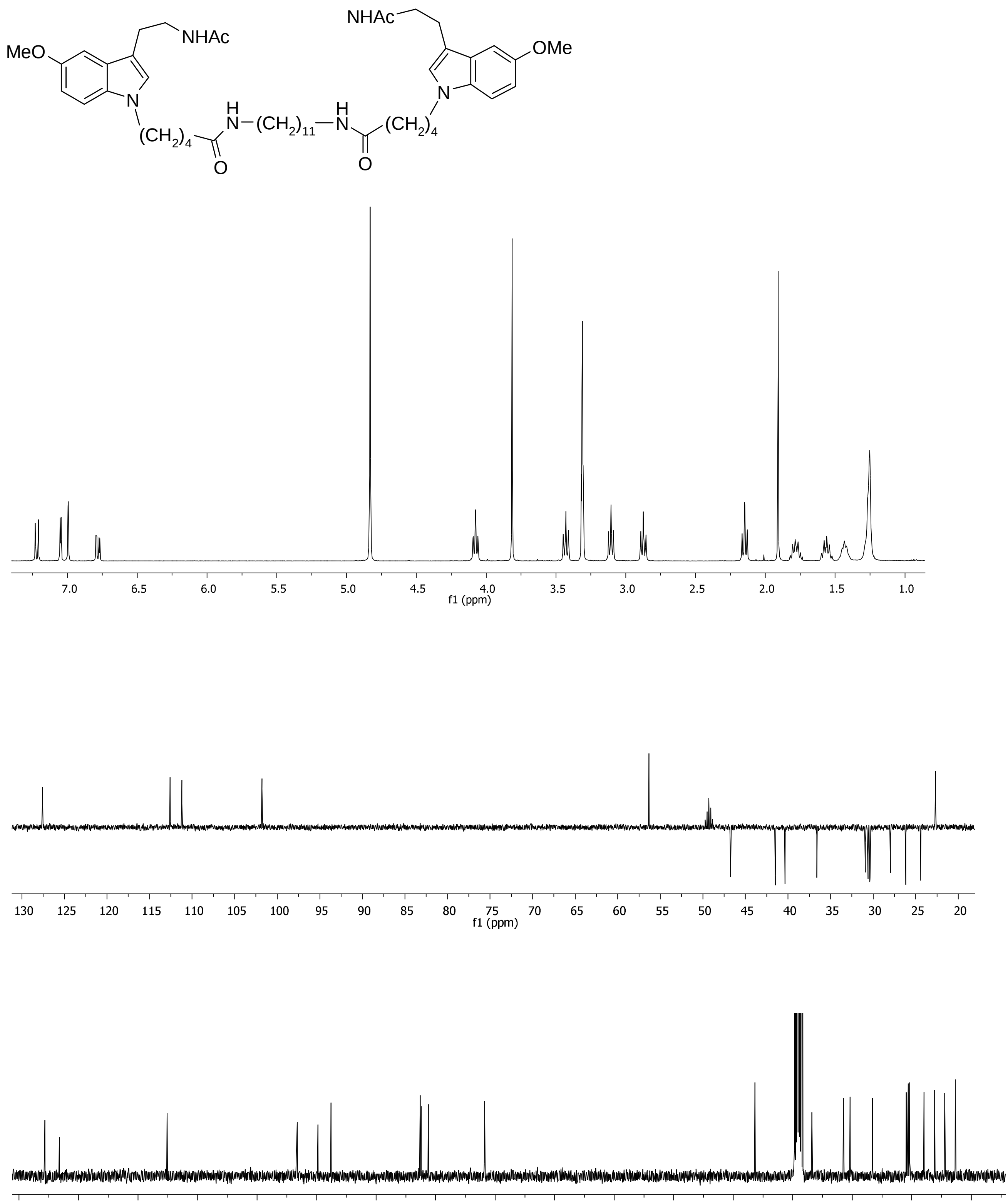




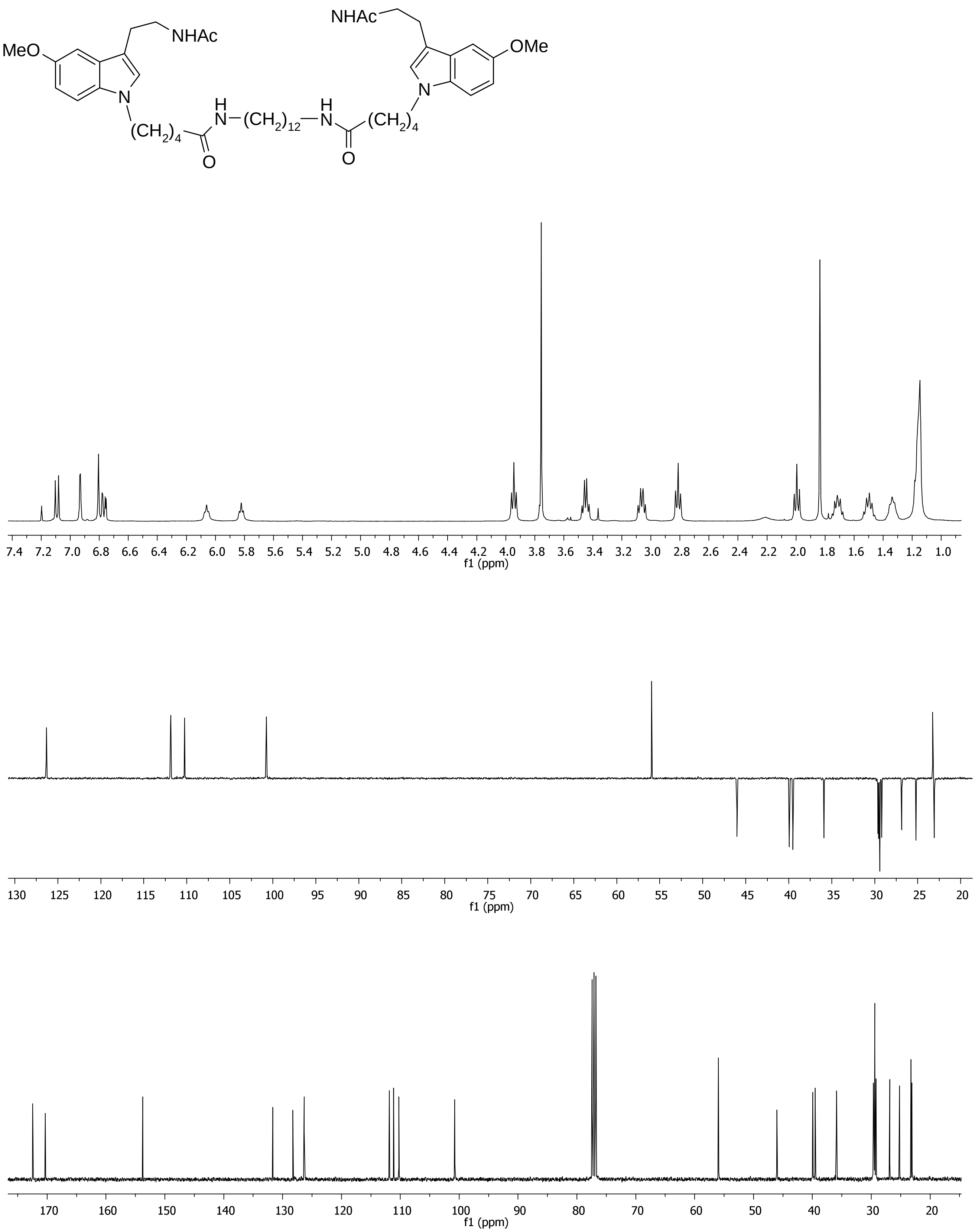




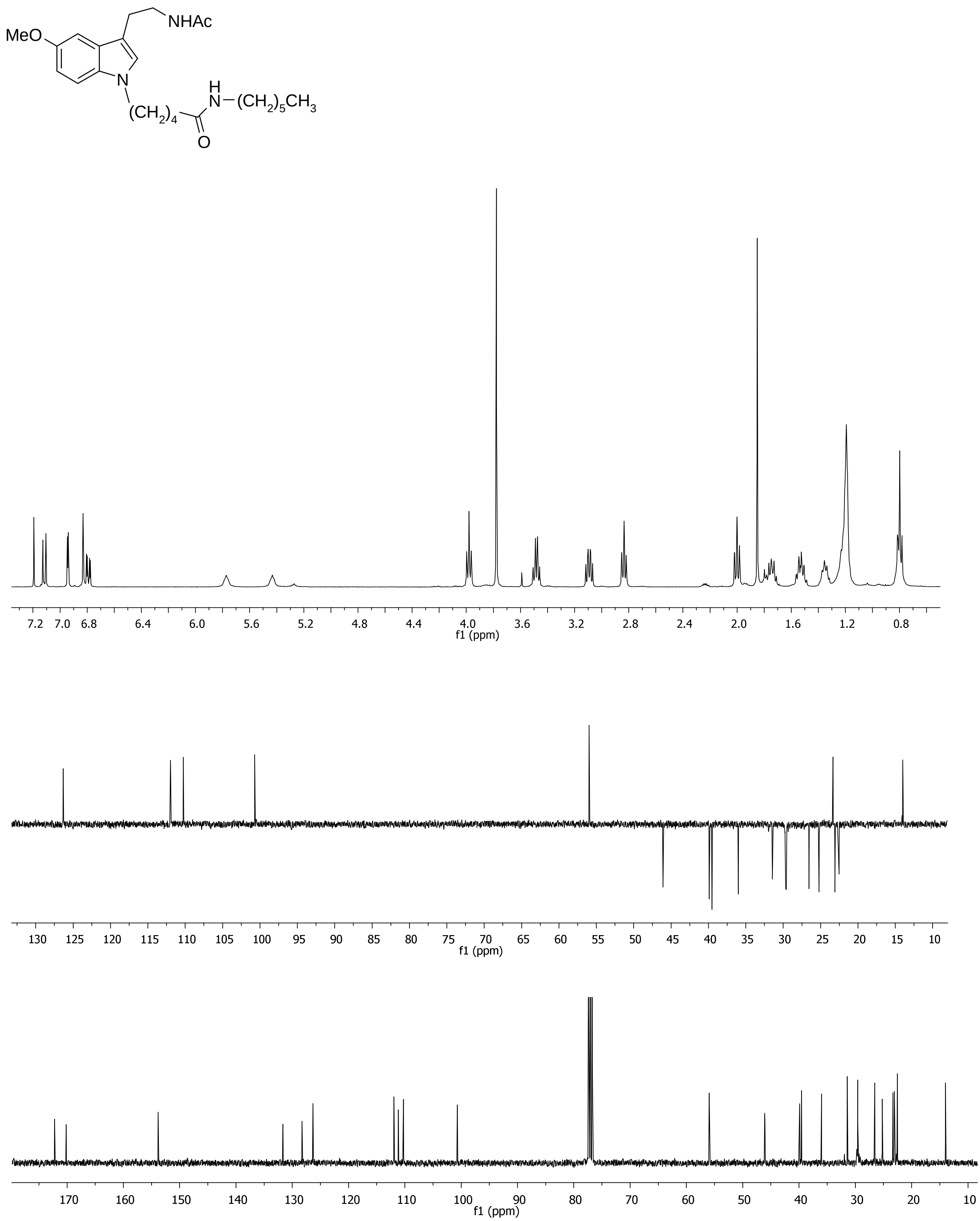
^{1}H (400 MHz), DEPT-135, and ^{13}C (100 MHz) spectra of compound **11** (MeOH-d_4)



^{1}H (400 MHz), DEPT-135, and ^{13}C (100 MHz) spectra of compound **12** (MeOH-d_4)



[1]H (400 MHz), DEPT-135, and [13]C (100 MHz) spectra of compound **13** ($CDCl_3$)



^{1}H (400 MHz), DEPT-135, and ^{13}C (100 MHz) spectra of compound **14** ($CDCl_3$)