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Supporting Information

Phenothiazine-Biaryl-Containing Fluorescent RGD Peptides

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Supporting Information

Experimental section

All chemicals were purchased from Sigma Aldrich (Taufkirchen, Germany), Acros (Geel, Belgium), Alfa Aesar (Ward Hill, USA) and VWR (Darmstadt, Germany), Chempur (Karlsruhe, Germany), Iris Biotech (Marktredwitz, Germany), Bachem (Bubendorf, Switzerland) and were employed without additional purification. Moisture- and air-sensitive reactions were conducted in flame-dried glassware and under argon atmosphere. Dichloromethane and toluene were freshly distilled from CaH_2 and Na, respectively.

Analytical RP-HPLC was performed on a Thermo Scientific Accela 600 equipped with a UV-6000 LP detector, a P-4000 pump, a Hypersil Gold C_{18} (3 µm column, 150 × 2.1 mm). Analytical LC-MS and determination of HRMS was performed on a Agilent 6220 TOF-MS with a Dual ESI-soure, 1200 HPLC system with autosampler, degasser, binary pump, column oven, diode array detector and a Hypersil Gold C_{18} column (1.9 µm, 50 × 2.1 mm). External calibration was performed with ESI-L Tuning Mix. MALDI-TOF was conducted with Ultraflex (Bruker), 355 nm Nd:YAG laser, 50 Hz, positive mode, 1000 shots/spectrum using DHB or CHCA as matrix, calibration with PEG 400-1200. Preparative HPLC was performed using a Merck-Hitachi LaChrom HPLC consisting of interface D-7000, pump L-7150, detector L-7420 and a Hypersil Gold C_{18} column (250 × 21.2 mm, 1.9 µM particels) or a Hypersil Gold C_{18} column (250 × 10.0 mm, 7 µM particels).

NMR spectra were recorded on a Bruker DRX-500, an Avance III 500 (^{1}H: 500 MHz, ^{13}C: 126 MHz) and an Avance 600 (^{1}H: 600 MHz, ^{13}C: 151 MHz) spectrometer. Chemical shifts are given in parts per million (ppm) relative to residual solvent peaks for ^{1}H and ^{13}C. For reaction control with TLC silica gel 60 coated aluminum sheets with F254 were used with solvent mixtures specified in the corresponding experiment. Spots were visualized using UV-light (254 or 366 nm). Column chromatography was performed with silica gel 60 (Merck, 40-63 µm).

High-resolution ESI mass spectra are recorded using an Agilent 6220 time-of-flight mass spectrometer (Agilent Technologies, Santa Clara, CA, USA) in extended dynamic range mode equipped with a Dual-ESI source, operating with a spray voltage of 2.5 kV. Nitrogen served both as the nebulizer gas and the dry gas. Nitrogen was generated by a nitrogen generator NGM 11. Samples are introduced with a 1200 HPLC system consisting of an autosampler, degasser, binary pump, column oven and diode array detector (Agilent Technologies, Santa Clara, CA, USA) using a C18 Hypersil Gold column (length: 50 mm, diameter: 2.1 mm, particle size: 1.9 µm) with a short gradient (in 4 min from 0% B to 98% B, back to 0% B in 0.2 min, total run time 7.5 min) at a flow rate of 250 µL/min and column oven temperature of 40°C. HPLC solvent A consists of 94.9% water, 5.0% acetonitrile and 0.1% formic acid, solvent B of 5.0% water, 94.9% acetonitrile and 0.1% formic acid. The mass axis was externally calibrated with ESI-L Tuning Mix (Agilent Technologies, Santa Clara, CA, USA) as calibration standard. The mass spectra are recorded in both profile and centroid mode with the Mass Hunter Workstation Acquisition

B.04.00 software (Agilent Technologies, Santa Clara, CA, USA). Mass Hunter Qualitative Analysis B. 07.00 software (Agilent Technologies, Santa Clara, CA, USA) was used for processing and averaging of several single spectra.

General Procedures

Synthesis procedures and characterization data of new compounds

6-(3-Bromo-10*H*-phenothiazin-10-yl)hexanenitrile (2)

This procedure was modified from the literature [35]pH =Phenothiazine (1.42 g, 5.12 mmol), potassium hydroxide (342 mg, 5.22 mmol), and sodium iodide (78 mg, 0.52 mmol) were combined under argon in 20 mL dry DMF. After 5 min stirring at r.t., 6-bromohexanenitril (2 mL, 15.4 mmol) was added dropwise. The reaction mixture was heated at 50 ºC for 48 h. After the reaction mixture had cooled at r.t. for 30 min, it was diluted with DCM (30 mL) and washed with water (3 × 20 mL). The combined organic layers was dried over magnesium sulfate, filtered, and evaporated to give a yellow liquid. The crude was purified on a flash silica column with EtOAc/hexanes (1 to 5) as eluant to give the final product. Yellow solid, 265 mg (71%), m.p.: 84-86 °C, IR (cm^{-1}): ν 2933, 2863, 2357, 2344, 1454, 1245, 748, ^{1}H NMR (500 MHz, Acetone-d_6) δ 7.32 (dd, *J* = 8.7, 2.3 Hz, 1H, H-Ar), 7.29 (d, *J* = 2.3 Hz, 1H, H-Ar), 7.22 (ddd, *J* = 8.6, 7.4, 1.5 Hz, 1H, H-Ar), 7.15 (dd, *J* = 7.6, 1.5 Hz, 1H, H-Ar), 7.06 (dd, *J* = 8.2, 1.1 Hz, 1H, H-Ar), 6.97 (d, *J* = 8.5 Hz, H-Ar), 6.96 (td, *J* =7.1, 1.1 Hz, H-Ar), 3.97 (t, *J* = 7.0 Hz, 2H, *N*-CH_2), 2.44 (t, *J* = 7.0 Hz, 2H, -CH_2CN), 1.83 (p, *J* = 7.2 Hz, 2H, -CH_2), 1.62–1.72 (m, 2H, -CH_2), 1.53–1.62 (m, 2H, -CH_2). ^{13}C NMR (125 MHz, Acetone-d_6) δ 145.98, 145.80, 131.09, 130.18, 128.79, 128.32, 124.95, 123.88, 120.67 (CN), 118.32, 117.06, 115.01, 47.73, 26.86, 26.80, 26.00, 17.27. HR-MS (EI) = Calc. for $C_{18}H_{17}{}^{79}BrN_2S$ $[M]^+$ 372.02903, Found 372.02770.

6-(3-Bromo-10*H*-phenothiazin-10-yl)hexanoic acid (3)

The nitriles **2** (1.5 mmol) was hydrolyzed in EtOH/MeOH/4M-KOH (25:25:8, 116 mL) at 90 ºC for 3 days. The reaction mixture was poured into water (100 mL) and acidified by slow addition of 10 % citric acid until pH = 2.5. The resulting solution was washed with DCM (3×30 mL). The combined organic layers was dried over magnesium sulfate, filtered, and evaporated to give a yellow liquid. The crude was purified on a flash silica column with hexane/ethyl acetate/AcOH (5:1:0.5) as eluant to give the final product. Pink solid, 282 mg (72%), m.p.: 76-78 °C, IR (cm^{-1}): ν 2936, 2851, 2363, 2338, 1695, 1451, 1245, 742, ^{1}H NMR (500 MHz, Acetone-d_6) δ 10.46 (s, 1H, -COOH), 7.33 (dd, *J* = 8.7, 2.3 Hz, 1H, H-Ar), 7.28 (d, *J* = 2.3 Hz, 1H, H-Ar), 7.22 (ddd, *J* = 8.5, 7.4, 1.5 Hz, 1H,

H-Ar), 7.15 (dd, *J* = 7.7, 1.5 Hz, 1H, H-Ar), 7.05 (dd, *J* = 8.2, 1.1 Hz, 1H, H-Ar), 6.92 – 7.00 (m, 2H, H-Ar), 3.94 (t, *J* = 7.0 Hz, 2H, *N*-CH_2), 2.28 (t, *J* = 7.3 Hz, 2H, -CH_2CO_2), 1.81 (p, *J* = 7.2 Hz, 2H, -CH_2), 1.57 – 1.67 (m, 2H, -CH_2), 1.45 – 1.55 (m, 2H, -CH_2). ^{13}C NMR (125 MHz, Acetone-d_6) δ 174.61 (COO), 145.95, 145.78, 131.04, 130.09, 128.72, 128.23, 128.16, 124.78, 123.77, 118.23, 116.98, 114.88, 47.87, 34.22, 27.30, 27.12, 25.36. HR-MS (ESI) = Calc. for $C_{18}H_{17}{}^{79}BrNO_2S$ $[M-H]^-$ 390.0158, Found 390.016885, Cal. For $C_{36}H_{35}{}^{79}Br_2N_2O_4S_2$ $[2M-H]^-$ 781.0399, Found 781.041047.

6-(3-(4,4,5,5-Tetramethyl-1,3,2-dioxaborolan-2-yl)-10*H*-phenothiazin-10-yl)hexanoic acid (4)

A solution of **3** (784 mg, 2mmol), bis(pinacolato)diborane (B_2Pin_2) (557 mg, 2.2 mmol), potassium acetate (490 mg, 5 mmol) and $PdCl_2(PPh_3)_2$ (14 mg, 0.02 mmol) in dry toluene (40 mL) was refluxed at 110 °C under Argon atmosphere for 18 hr. After cooling to room temperature, the mixture was poured into water (40 mL) and the organic layer was extracted with DCM (3×40 mL) and the combined organic layers were dried over anhydrous Na_2SO_4. The organic solvent was evaporated to dryness and the crude product was purified by preparative HPLC without using TFA in eluents. Pink solid, 307 mg (70%), m.p.: 75-76 ℃, IR (cm^{-1}): ν 3726, 2357, 2344, 1354, 669, ^{1}H NMR (500 MHz, Acetone-d_6) δ 10.32 (s, 1H, -COOH), 7.42 (dd, *J* =8.1, 1.5 Hz, 1H, H-Ar), 7.31 (d, *J* = 1.5 Hz, 1H, H-Ar), 7.06 (ddd, *J* = 8.1, 7.3, 1.5 Hz, 1H, H-Ar), 7.00 (dd, *J* = 7.6, 1.6 Hz, 1H, H-Ar), 6.89-6.93 (m, 2H, H-Ar), 6.82 (td, *J* = 7.5, 1.2 Hz, H-Ar), 3.85 (t, *J* = 7.0 Hz, 2H, *N*-CH_2), 2.14 (t, *J* = 7.4 Hz, 2H, -CH_2COOH), 1.64-1.74 (m, 2H, CH_2), 1.44-1.53 (m, 2H, CH_2), 1.34-1.43 (m, 2H, CH_2), 1.18 (s, 12H, 4 –CH_3); ^{13}C NMR (125 MHz, Acetone-d_6) δ 173.66 (COOH), 147.95, 144.75, 134.22, 133.23, 127.42, 127.13, 124.40, 123.62, 122.7, 115.90, 115.07, 83.52, 46.81, 33.21, 26.36, 26.12, 24.37, 24.26. HR-MS (ESI) = Calc. for $C_{24}H_{30}BNO_4SNa^+$ $[M+Na]^+$ 462.18808, Found 462.1888.

Ethyl 2-(3-bromo-10*H*-phenothiazin-10-yl)acetate (5)

This procedure was modified from the literature [35].pH =Phenothiazine (1.42 g, 5.12 mmol), potassium hydroxide (342 mg, 5.22 mmol), and sodium iodide (78 mg, 0.52 mmol) were combined under argon in 20 mL dry DMF. After 5 min stirring at r.t., bromoethylacetate (1.7 mL, 15.4 mmol) was added dropwise. The reaction mixture was heated at 50 ºC for 48 h. After the reaction mixture had cooled at r.t. for 30 min, it was diluted with DCM (30 mL) and washed with water (3 × 20 mL).pH =The combined organic layers were dried over magnesium sulfate, filtered, and evaporated to give a yellow liquid. The crude was purified on a flash silica column with EtOAc/hexanes (1 to 5) as eluent to give the final product. Yellow oil, 167 mg (46%), IR (cm^{-1}): ν2360,2344, 1746, 1457, 1192, 745, ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.14-7.23 (m, 2H, H-Ar), 7.04-7.13 (m, 2H, H-Ar), 6.93 (t, *J* = 7.5 Hz, 1H, H-Ar), 6.58 (d, *J* = 8.1 Hz, 1H, H-Ar), 6.44 (dd, *J* = 8.6, 1.7 Hz, 1H, H-Ar), 4.47 (s, 2H, *N*-CH_2), 4.31 (q, *J* = 7.2 Hz, 2H, O-CH_2), 1.32 (t, *J* = 7.1 Hz, 3H, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.43 (COO), 143.64, 143.28, 129.77,

129.06, 127.46, 126.95, 125.57, 123.15, 122.48, 115.55, 115.05, 114.52, 61.61, 50.90, 14.10. HR-MS (ESI): Calc. for $[C_{16}H_{14}BrNO_2SH]^+$ 364.00014, Found 363.9992.

Ethyl 2-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-10*H*-phenothiazin-10-yl)acetate (6)

A solution of **5** (724 mg, 2mmol), bis(pinacolato)diborane (B_2Pin_2) (557 mg, 2.2 mmol), potassium acetate (490 mg, 5 mmol) and $PdCl_2(PPh_3)_2$ (14 mg, 0.02 mmol) in dry toluene (40 mL) was refluxed at 110 °C under Argon atmosphere for 18 hr. After being cooled to room temperature, the mixture was poured into water (40 mL) and the organic layer was extracted with DCM (3×40 mL) and the combined organic layers were dried over anhydrous Na_2SO_4. The organic solvent was evaporated to dryness and the crude product was purified on a flash silica column with EtOAc/hexanes (1 to 5) as eluent to give the final product. White solid, 371 mg (90%), m.p.: 104-105 ℃ , IR (cm^{-1}): ν 2357, 2338, 1353, 666, ^{1}H NMR (500 MHz, Acetone-d_6) δ 7.49 (dd, *J* = 8.1, 1.5 Hz, 1H, H-Ar), 7.40 (d, *J* = 1.4 Hz, 1H, H-Ar), 7.14 (ddd, *J* = 8.2, 7.3, 1.6 Hz, 1H, H-Ar), 7.10 (dd, *J* = 7.6, 1.5 Hz, 1H, H-Ar), 6.96 (td, *J* = 7.5, 1.2 Hz, 1H, H-Ar), 7.72-7.79 (m, 2H, H-Ar), 4.66 (s, 2H, *N*-CH_2), 4.28 (q, *J* = 7.1 Hz, 2H, O-CH_2), 1.31 (s, 12H, 4 -CH_3), 1.29 (t, *J* = 7.1 Hz, 3H, -CH_3); ^{13}C NMR (125 MHz, Acetone-d_6) δ 169.78 (COO), 147.58, 144.49, 134.91, 133.40, 128.11, 127.35, 123.85, 123.29, 122.42, 115.91, 115.10, 84.31, 61.76, 50.90, 24.99, 14.33. HR-MS (ESI) = Calc. for $C_{22}H_{26}BNO_4SNa^+$ $[M+Na]^+$ 434.15678, Found 434.1573.

2-(3-(4,4,5,5-Tetramethyl-1,3,2-dioxaborolan-2-yl)-10*H*-phenothiazin-10-yl)acetic acid (7)

The ester **6** (1.5 mmol) was hydrolyzed in EtOH/MeOH/4M-KOH (25:25:8, 116 mL) at 60 ºC for 30 min. The reaction mixture was poured into water (100 mL) and acidified by slow addition of 10 % citric acid until pH=2.5. The resulting solution was washed with DCM (3×30 mL). The combined organic layers was dried over magnesium sulfate, filtered, and evaporated to give a purple liquid. The crude was purified by preparative HPLC without using TFA in eluents. Purple solid, 99 mg (33%), m.p.: 122-124 °C, IR (cm^{-1}): ν 669, 1355, 2341, 2360, ^{1}H NMR (500 MHz, DMSO-d_6) δ 13.14 (s, 1H, -COOH), 7.41 (dd, *J* = 8.2, 1.5 Hz, 1H, H-Ar), 7.27 (d, *J* = 1.5 Hz, 1H, H-Ar), 7.14 (ddd, *J* = 8.4, 7.4, 1.6 Hz, 1H, H-Ar), 7.09 (dd, *J* = 7.6, 1.5 Hz, 1H, H-Ar), 6.94 (td, *J* = 7.5, 1.1 Hz, 1H, H-Ar), 6.64-6.72 (m, 2H, H-Ar), 4.58 (s, 2H, *N*-CH_2), 1.28 (s, 12H, 4 -CH_3); ^{13}C NMR (125 MHz, DMSO-d_6) δ 170.40 (COO), 146.31, 143.00, 133.99, 132.10, 127.40, 126.41, 122.93, 121.04, 120.50, 115.04, 114.25, 83.42, 49.50, 24.46. HR-MS (ESI) = Calc. for $C_{20}H_{21}BNO_4S^-$ $[M-H]^-$ 382.12898, Found 382.1288.

6-(3-Bromo-7-formyl-10*H*-phenothiazin-10-yl)hexanenitrile (8)

Compound **2** (1.43 mg, 3.8 mmol) and DMF (0.5 mL, 4.1 mmol) were dissolved in dry 1,2-dichloroethane (4 mL)and the reaction mixture was cooled to 0 °C. Phosphorus oxychloride (0.4 mL, 4.7 mmol) dissolved in 1,2-

dichloroethane (1 mL) was added dropwise within 4 hr. to the reaction mixture. The reaction was heated to reflux temperature for 48 hr. Then after cooling to room temperature, 24 mL of an aqueous solution of sodium acetate (40%) was added and the resulting biphasic reaction mixture was stirred for 3 hr. The aqueous layer was extracted several times with small amounts of dichloromethane. The combined organic fractions were dried with anhydrous sodium sulfate and the solvents were removed. The residue was chromatographed on silica gel (Petrol ether/Ethyl acetate 5:1). Yellow oil, 184 mg (46%), IR (cm^{-1}): ν 2360, 2341, 1689, 1594, 1457, 1201, 669, ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.79 (s, 1H, -COH), 7.67 (dd, *J* = 8.4, 2.0 Hz, 1H, H-Ar), 7.57 (d, *J* = 1.9 Hz, 1H, H-Ar), 7.25 (dd, *J* = 8.5, 2.3 Hz, 1H, H-Ar), 7.22 (d, *J* = 2.3 Hz, 1H, H-Ar), 6.89 (d, *J* = 8.4 Hz, 1H, H-Ar), 6.70 (d, *J* = 8.6 Hz, 1H, H-Ar), 3.89 (t, *J* = 6.9 Hz, 2H, *N*-CH_2), 2.32 (t, *J* = 6.9 Hz, 2H, CH_2CN), 1.81 (h, *J* = 7.4Hz, 2H, CH_2), 1.61-1.71 (m, 2H, CH_2), 1.52-1.61 (m, 2H, CH_2); ^{13}C NMR (125 MHz, $CDCl_3$) δ 189.69, 150.02, 142.34, 131.28, 130.14, 130.08, 129.77, 128.34, 126.40, 124.75, 119.21, 117.01, 115.83, 114.99, 47.26, 25.76, 25.65, 24.76, 16.94. HR-MS (ESI): Calc. for $[C_{19}H_{17}BrN_2OSH]^+$ 401.03177, Found 401.0308.

6-(3-Bromo-7-formyl-10*H*-phenothiazin-10-yl)hexanoic acid (9)

Prepared according to general procedure used for the synthesis of compound **3**, but this time starting from **8**. Orange solid, 122 mg (29%), m.p.: 155-157 °C, IR (cm^{-1}): ν 669, 675, 2341, 2360, ^{1}H-NMR (500 MHz, Acetone-d_6) δ 9.71 (s, 1H, -CHO), 7.62 (dd, *J* = 8.5, 1.9 Hz, 1H, H-Ar), 7.49 (d, *J* = 1.9 Hz, 1H, H-Ar), 7.24 (dd, *J* = 8.7, 2.3 Hz, 1H, H-Ar), 7.19 (d, *J* = 2.3 Hz, 1H. H-Ar), 7.08 (d, *J* = 8.5 Hz, 1H, H-Ar), 6.92 (d, *J* = 8.7 Hz, 1H, H-Ar), 3.91 (t, *J* = 7.1 Hz, 2H, *N*-CH_2), 2.15 (t, *J* = 7.3 Hz, 2H, CH_2CO_2), 1.71 (p, *J* = 7.4 Hz, 2H, CH_2), 1.43-1.55 (m, 2H, CH_2), 1.33-1.43 (m, 2H, CH_2); ^{13}C-NMR (125 MHz, Acetone-d_6) δ 189.60 (CHO), 173.52 (CO_2H), 150.11, 143.12, 131.83, 130.45, 130.13, 129.28, 127.80, 126.10, 123.96, 117.97, 115.75, 115.15, 47.42, 33.15, 26.15, 25.98, 24.29. HR-MS (ESI): Calc. for $C_{19}H_{18}BrNO_3SH^+$ $[M+H]^+$ 420.02635, Found 420.02682.

6-(3-Formyl-7-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-10*H*-phenothiazin-10yl)hexanoic acid (10)

Prepared according to the general procedure used for the synthesis of compound **4**, but this time starting from **9.** Yellow solid, 434 mg (93%), m.p.: 87-88 °C, IR (cm^{-1}): ν 2363, 2341, 1359, 669, 653, ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.0 (s, 1H, -COOH), 9.82 (s, 1H, -COH), 7.74 (dd, *J* = 8.6, 1.8 Hz, 1H, H-Ar), 7.62 (d, *J* = 1.9 Hz, 1H, H-Ar), 7.52 (dd, *J* = 8.2, 1.5 Hz, 1H, H-Ar), 7.36 (d, *J* = 1.5 Hz, 1H, H-Ar), 7.21 (d, *J* = 8.6 Hz, 1H, H-Ar), 7.10 (d, *J* = 8.2 Hz, 1H, H-Ar), 3.98 (t, *J* = 7.1 Hz, 2H, *N*-CH_2), 2.19 (t, *J* = 7.3 Hz, 2H, CH_2CO_2), 1.71 (p, *J* = 7.1Hz, 2H, CH_2), 1.48-1.59 (m, 2H, CH_2), 1.41 (qd, *J* = 9.5, 9.0, 5.8 Hz, 2H, CH_2), 1.29 (s, 12 H, 4 CH_3); ^{13}C NMR (125 MHz, DMSO-d_6) δ 191.12, 174.80, 149.85, 146.18, 134.93, 33.40, 131.62, 130.51, 128.32, 123.97, 122.40, 116.49, 116.31, 84.15, 47.40, 34.06, 26.27, 26.12, 25.10, 24.51. HR-MS (ESI) = Calc. for $C_{25}H_{30}BNO_5SNa^+$ $[M+Na]^+$ 490.18300, Found 490.1825.

Peptide Syntheses:

Peptide synthesis was conducted with standard protocols using the Fmoc/*t*Bu-strategy in a plastic syringe fitted with a polypropylene porous disk at rt. Washing steps were performed after each reaction with DMF (3×), DCM (2×) and again DMF (3×) with 10 mL/g resin. Solvents and soluble reagents were removed by suction. During couplings the resin was shaken with a horizontal shaker. SPPS was conducted with scales between 0.1-0.5 mmol using 10 mL/g resin for reactions. Reaction control was performed *via* MALDI or LC-MS. Loading of Rink-amide resin, for on-resin side chain cyclization Rink-amide resin was used. At first, resin was swollen using DMF (3×) and each time it was shaken for 10 min. Later the resin was deprotected twice with 20 % piperidine/DMF, 0.1 M 1-hydroxybenzotriazole (HOBt) for 20 min and washed. As a first amino acid in loading step, Fmoc-Trp (7-Br)-OH (1 eq) was loaded on rink amide in the presence of ethyl cyanohydroxyiminoacetate (Oxyma) (1.5 eq), *N,N'*-diisopropylcarbodiimide (DIC) (1.5 eq) and 2,4,6-trimethylpyridine (TMP) (10 eq) and incubated overnight at 400 rpm and rt. Afterwards the resin was filtered, washed with Et_2O and dried under vacuum. Then the loading was measured which is between 95-100 %. Afterward capping was carried out twice with Ac_2O (10 equiv) and pyridine (10 equiv) in DMF. For Fmoc-deprotection the resin was treated twice with a solution of 20 % piperidine/DMF, 0.1 M HOBt for 20 min. coupling steps are repeated twice. Fmoc-Xaa-OH (3 eq) and *O*-(benzotriazol-1-yl)-*N*,*N*,*N'*,*N'*-tetramethyluronium tetrafluoroborate (TBTU) (3 eq) are dissolved in DMF, combined and DIEA (6 eq) is added. The resulted mixture was poured in syringe and incubated for 30 min. Coupling of phenothiazine derivatives the same method used for loading is applied.

On-resin *N*-Methylation:

The Fmoc-group is deprotected and the resin washed with NMP. All steps were repeated twice. For the protection a freshly prepared solution of 2-nitrobenzenesulfonyl chloride (*o*-NBS) (4 equiv) with 2,4,6-collidine (10 equiv) in NMP was added for 15 min. Then a solution of 1, 8-diazabicyclo[5.4.0]undec-7-ene (DBU) (3 equiv) and DMS (10 equiv) in NMP was incubated with the resin for 10 min. To cleave the *o*-NBS group 2-mercaptoethanol (10 equiv) and DBU (5 equiv) in NMP were incubated with the resin for 15 min. The completion of the *N*-methylation was monitored *via* RP-HPLC. The following Fmoc-Xaa-OH (3 equiv) was attached using HATU (3 equiv), HOAt (3 equiv) and DIEA (6 equiv) in NMP for 3 h.

General procedure for on-resin cyclization

A Schlenk tube was charged with the linear peptide precursor (1 equiv), $Pd_2(dba_3)$ (0.2 equiv), sSPhos (0.4 equiv) and KF (4 equiv), evacuated and backfilled with argon (3×). A degassed solution of DME/EtOH/H_2O (9:9:1, 2-3 mL) was added under argon and the mixture was heated using a microwave at 120 °C for 0.5 h. Subsequently, the

resin was washed with DMF (5×), DCM (3×), DMF (5×), EtOH (2×) and Et_2O (1×). The cyclic peptide was cleaved from the resin with a mixture of TFA/H_2O/TIPS (95:2.5:2.5) and DTT (100 mg) for 3 h each. The solvent was evaporated under reduced pressure, the residue dissolved in CH_3CN/H_2O and lyophilized. The crude peptide was purified using preparative RP-HPLC with A flow rate of 10.0 mL/min using Eluent A: H_2O/CH_3CN/TFA (94.9/5.0/0.1) and Eluent B: CH_3CN/H_2O/TFA (94.9/5.0/0.1) Gradient Elution: 0-80 min (80% to 0%), 80-90 min (0%), 90-95 min (0% to 80%).

c(RGDW(7-3PTz^1)) (12a): White solid, 96 mg (58%), HR-MS (ESI): Calc. for $[C_{41}H_{48}N_{10}O_7SH]^+$ 825.35009, Found 825.3474.

c(RGDw(7-3PTz^1)) (12b): White solid, 91 mg (55%), HR-MS (ESI): Calc. for $[C_{41}H_{48}N_{10}O_7SH]^+$ 825.35009, Found 825.3474.

c(RGDW(7-3PTz^2)) (12c): White solid, 72 mg (47%), ^{1}H NMR (DMF-d_7, 500MHz): δ 12.76 (s, 1H, -COOH), 10.23 (d, *J*= 2.5 Hz, 1H, Indol-NH), 8.17 (brs, 1H, W-NH), 8.08 (d, 1H, W-NH), 8.07 (s, 1H, D-NH), 7.90 (brs, 1H, G-NH), 7.82 (d, *J* = 7.9 Hz, 1H, R-NH), 7.59 (dd, *J* = 7.9, 1.2 Hz, 2H, H-Ar), 7.45 (dd, *J* = 8.4, 2.0 Hz, 2H, H-Ar), 7.37 (s, 1H), 7.25-7.34 (m, 5H, H-Ar), 7.23 (d, J =2.2 Hz, 1H), 7.03-7.18 (m, 5H, H-Ar), 7.00 (d, J =8.4 Hz, 1H), 4.80-4.85 (m, 2H, D-C_αH, *N*-CH (PTz)), 4.56 (d, J = 15.6 Hz, 1H, *N*-CH (PTz)), 4.45-4.52 (m, 2H, R-C_αH, W-C_αH), 3.93 (dd, *J* = 17.4, 6.7 Hz, 2H, G-C_αH), 3.57 (m, 1H, W-C_βH), 3.27-3.32 (m, 2H, R-C_δH), 2.78-2.82 (m, 1H, D-C_βH), 2.49 (dd, *J* = 16.5, 6.1 Hz, 1H, D-C_βH), 1.73-1.83 (m, 2H, R-C_βH), 1.61-1.68 (m, 2H, R-C_γH).; ^{13}C NMR (DMF-d_7, 125 MHz): δ 173.8 (-CO_2H), 170.9, 170.5, 168.3, 167.5, 157.8 (guanidine C), 145.9, 142.6, 134.2, 133.9, 127.9, 127.8, 127.6, 127.2, 126.3, 126.2, 124.6, 124.1, 123.2, 119.9, 119.1, 117.4, 116.5, 115.7, 115.6, 110.5, 53.3 (R-C_α), 52.5 (W-C_α), 51.8 (*N*-CPTz), 49.2 (D-C_α), 41.9 (G-C_α), 40.9 (R-C_δ), 36.0 (D-C_β), 30.6 (R-C_β), 27.1 (W-C_β), 25.2 (R-C_γ); HR-MS (ESI): Calc. for $[C_{37}H_{40}N_{10}O_7SH]^+$ 769.28749, Found 769.2872.

c(RGDw(7-3PTz^2)) (12d): White solid, 68 mg (44%), ^{1}H NMR (DMF-d_7, 500MHz): δ 12.89 (s, 1H, -COOH), 10.16 (s, 1H, Indol-NH), 8.24 (d, J = 9.1, 2 Hz, 2H, W-NH, D-NH), 8.09 (s, 1H, G-NH), 7.67 (s, 1H, R-NH), 7.62 (d, *J* = 7.9 Hz, 2H), 7.38 (dd, *J* = 8.2, 2.0 Hz, 1H), 7.29-7.34 (m, 4H), 7.14-7.19 (m, 5H), 7.06-7.10 (m, 4H), 7.03 (dd, J = 8.4, 2.2 Hz, 1H), 4.80-4.85 (m, 2H, w-C_αH, *N*-CH (PTz)), 4.69-4.73 (m, 1H, D-C_αH), 4.59-4.64 (m, 2H, R-C_αH, *N*-CH(PTz)), 4.46-4.54 (m, 2H, G-C_αH), 3.63-3.66 (m, 1H, W-C_βH), 3.25-3.29 (m, 2H, R-C_δH), 2.79-2.83 (m, 1H, D-C_βH), 2.68-2.71 (m, 1H, D-C_βH), 2.66-2.68 (m, 1H, w- C_βH), 1.74-1.83 (m, 2H, R-C_βH), 1.52-1.60 (m, 2H, R-C_γH).; ^{13}C NMR (DMF-d_7, 125 MHz): δ 173.90, 171.22, 170.80, 170.43, 168.41, 168.1, 142.94, 142.42, 135.41, 134.94, 128.44, 128.21, 127.85, 127.74, 126.8, 126.26, 124.84, 124.15, 123.50, 120.43, 119.26, 118.69, 118.58, 116.22, 115.98, 110.1, 52.14, 52.03, 51.89, 50.29, 41.87, 41.06, 35.14, 30.73, 27.83, 27.49.; HR-MS (ESI): Calc. for $[C_{37}H_{40}N_{10}O_7SH]^+$ 769.28749, Found 769.2839.

c(RGD(NMe)W(7-3PTz^1)) (12e): White solid, 54 mg (32%), ^{1}H NMR (DMF-d$_7$, 500MHz): δ 10.37 (d, *J*= 2.7 Hz, Indol-NH), 9.37 (brs, 1H, D-NH), 8.91 (s, 1H, G-NH), 7.77 (d, *J* = 8.3 Hz, 1H, R-NH), 7.55 (dd, *J* = 8.4, 2.1 Hz, 1H, H-Ar), 7.44 (d, *J* = 7.8 Hz, 1H, H-Ar), 7.31 (d, *J* = 2.1 Hz, 2H, H-Ar), 6.96-7.25 (m, 10H, H-Ar), 6.94 (d, *J* = 8.5 Hz, 1H, H-Ar), 6.83 (td, *J* = 7.4, 1.1 Hz, 1H, H-Ar), 6.80 (d, *J* = 2.4 Hz, 1H, Indol-CH), 5.29 (d, *J* = 10.3 Hz, 1H, W-C$_\alpha$H), 5.04 (ddd, *J* = 9.4, 6.6, 3.2 Hz, 1H, D-C$_\alpha$H), 4.56 (td, *J* = 8.5, 3.7 Hz, 1H, R-C$_\alpha$H), 4.23(dd, *J* = 17.8 Hz, 1H, G-C$_\alpha$H), 3.86 (m, 2H, *N*-CH$_2$ (PTz)), 3.45(dd, *J* = 17.1, 4.3 Hz, 1H, G-C$_\alpha$H), 3.11-3.16 (m, 1H, W-C$_\beta$H), 3.04 (d, *J* = 4.2 Hz, 1H, W-C$_\beta$H), 3.02 (S, 3H, *N*-Me), 2.37 (dd, *J* =15.7, 3.1 Hz, 1H, D-C$_\beta$H), 2.24 (m, 1H, D-C$_\beta$H), 2.15-2.23 (m, 2H, R-C$_\delta$H), 2-2.06 (m, 2H, R-C$_\gamma$H), 1.61-1.72 (m, 4H, (2H, R-C$_\beta$H), (2H, -CH$_2$ (PTz)), 1.46-1.52 (m, 4H, 2-CH$_2$(PTz)), 1.33-1.41 (m, 2H, -CH$_2$ (Ptz)).; ^{13}C NMR (DMF-d$_7$, 125 MHz) δ 172.78, 172.64, 172.28, 172.18, 172.08, 167.32, 145.32, 143. 47, 133.47, 133.36, 133.28, 128.70, 128.67, 127.73, 127.45, 127.11, 126.63, 124.76, 124.42, 124.27, 123.86, 123.51, 123.13, 120.58, 117.32, 115.65, 111.22, 56.09 (W-Cα),51.80 (R-Cα), 47.01, 46.22 (D-Cα), 42.48 (G-Cα), 40.48 (W-Cβ), 37.83 (D-Cβ), 34.62 (R-Cγ), 34.53 (R-Cδ), 25.37, 25.08, 24.79 (R-Cβ); HR-MS (ESI): Calc. for [C$_{42}$H$_{50}$N$_{10}$O$_7$SH]$^+$ 839.36574, Found 839.3628.

c(RGD(NMe)W(7-3PTz^2)) (12f): White solid, 45 mg (29%), ^{1}H NMR (DMF-d$_7$, 500MHz): δ 10.33 (d, *J*= 2.6 Hz, Indol-NH), 8.00 (d, *J* = 9.3 Hz, 1H, D-NH), 7.77 (dd, J = 7.5, 3.1 Hz, 1H, G-NH), 7.71 (d, *J* = 7.9 Hz, 1H, R-NH), 7.62 (d, *J* = 7.6 Hz, 1H, H-Ar), 7.48 (dd, *J* = 8.4, 2 Hz, 2H, H-Ar), 7.29-7.34 (m, 5H, H-Ar), 7.11-7.18 (m, 10H, H-Ar), 7.06-7.11 (m, 3H, H-Ar), 6.98 (d, *J* = 8.4 Hz, 1H, H-Ar), 6.96 (s, 1H, Indol-CH), 5.53 (d, *J* = 8.3, 4.2 Hz, 1H, W-C$_\alpha$H), 5.06-5.14 (m, 1H, D-C$_\alpha$H), 4.84 (d, *J* = 15.6 Hz, 1H, *N*-CH (PTz)), 4.50 (d, *J* = 15.4 Hz, 1H, *N*-CH (PTz)),4.40 (q, *J* = 7.2 Hz, 1H, R-C$_\alpha$H), 3.92 (d, *J* = 10.1 Hz, 2H, G-C$_\alpha$H), 3.31 (m, 2H, R-C$_\delta$H), 3.21 (S, 3H, *N*-Me), 3.13 (t, *J* = 12.1 Hz, 1H, W-C$_\beta$H), 2.97 (d, *J* = 12.1 Hz, 1H, W-C$_\beta$H), 7.82-7.87 (m, 1H, D-C$_\beta$H), 2.50 (dd, *J* = 11.2 Hz, 1H, D-C$_\beta$H), 1.71-1.80 (m, 4H, (m, 2H, R-C$_\beta$H), 1.63-1.71 (m, 2H, R-C$_\gamma$H).; ^{13}C NMR (DMF-d$_7$, 125 MHz): δ 174.42 (-CO$_2$H), 173.78, 173.65, 172.01, 169.75, 168.40, 158.92 (guanidine C), 147.16, 143.71, 135.20, 135.14, 129.22, 129.16, 128.63, 128.39, 128.12, 127.48, 126.19, 125.48, 125.47, 124.48, 121.32, 120.27, 118.91, 116.94, 116.79, 111.65, 57.40 (W-Cα), 54.10 (R-Cα), 53.24 (C(PTz)), 45.42 (D-Cα), 42.88 (G-Cα), 42.13 (R-Cδ), 37.83 (D-Cβ), 31.79 (-CH3), 31.71 (R-Cβ), 26.47 (R-Cγ), 24.54 (W-Cβ). HR-MS (ESI): Calc. for [C$_{38}$H$_{42}$N$_{10}$O$_7$SH]$^+$ 783.30314, Found 783.3025.

c(RGDw(7-3PTz^1(CH(CN)$_2$)) (12g): At first the linear pentapeptide using compound **10** was synthesized and then cyclized according to the procedure described. After on-resin cyclization, an on resin-Knoevenagel reaction was done using malononitrile (1eq) and ammonium acetate (1eq) in the mixture of EtOH:DCM (1:1) and shaken overnight and then was washed and cleaved and then purified. Red solid, 24 mg (16%), HR-MS (ESI): Calc. for [C$_{45}$H$_{48}$N$_{12}$O$_7$SH]$^+$ 901.3562, Found 901.3572.

c(RGDw(7-3PTz^2(O))) (12h): Compound **12c** was oxidized using H_2O_2 (5% eq) in acetonitrile (1 mg peptide in 1 mL acetonitrile) and stirred for 3 h at room temperature and then lyophilized and purified. White solid, 23 mg (29%), HR-MS (ESI): Calc. for $[C_{37}H_{40}N_{10}O_8SH]^+$ 785.2824, Found 785.2795.

Spectra

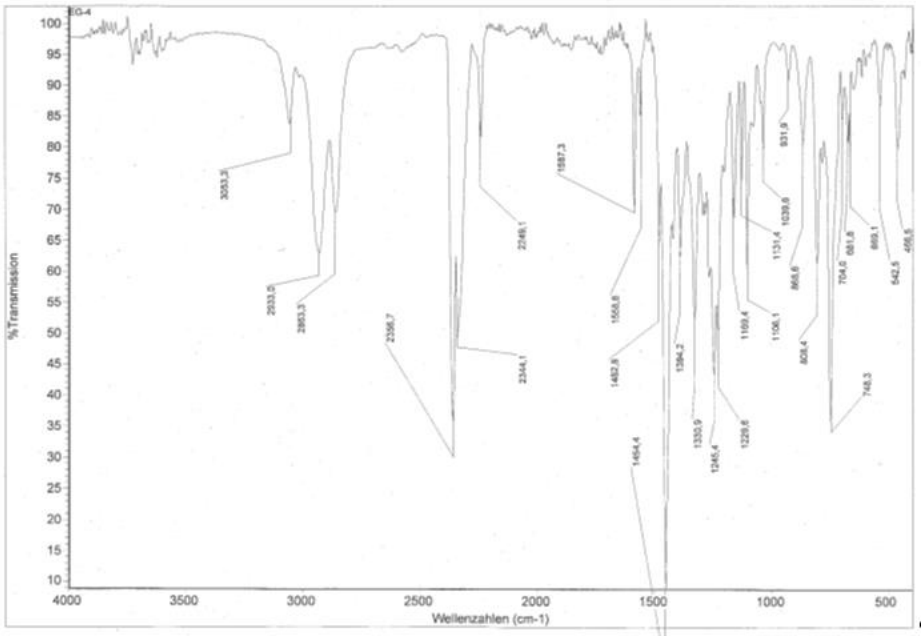

Figure S1. IR spectra of **2**

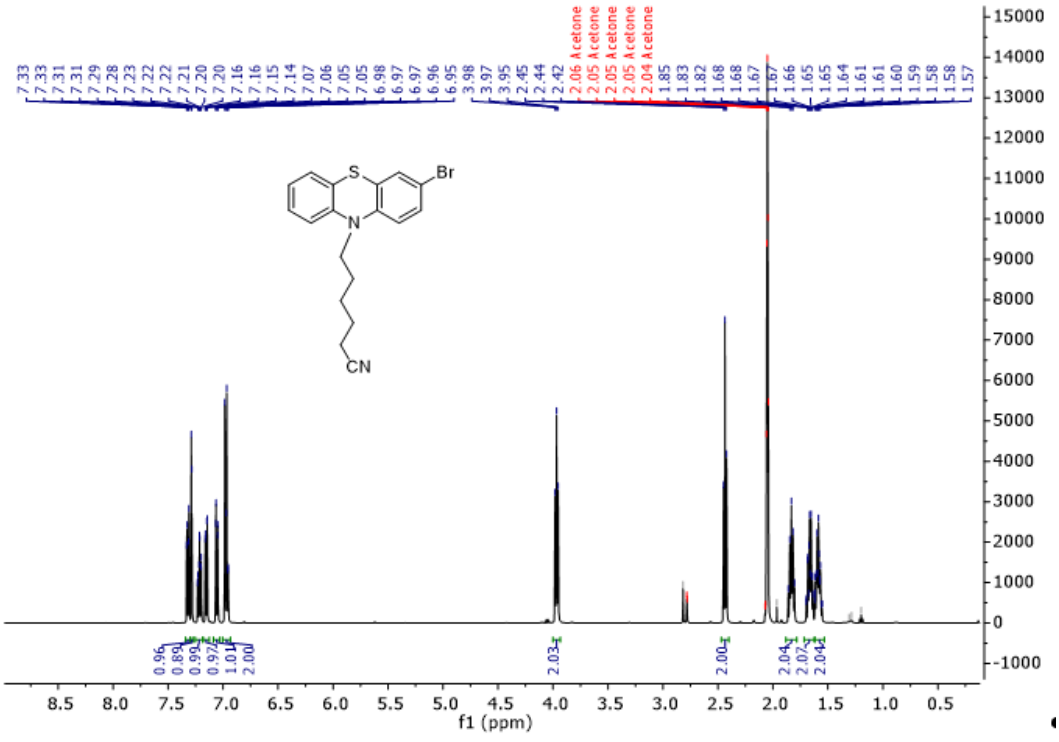

Figure S2. ^{1}H-NMR spectra of **2** measured in Acetone-d_6 at 500 MHz.

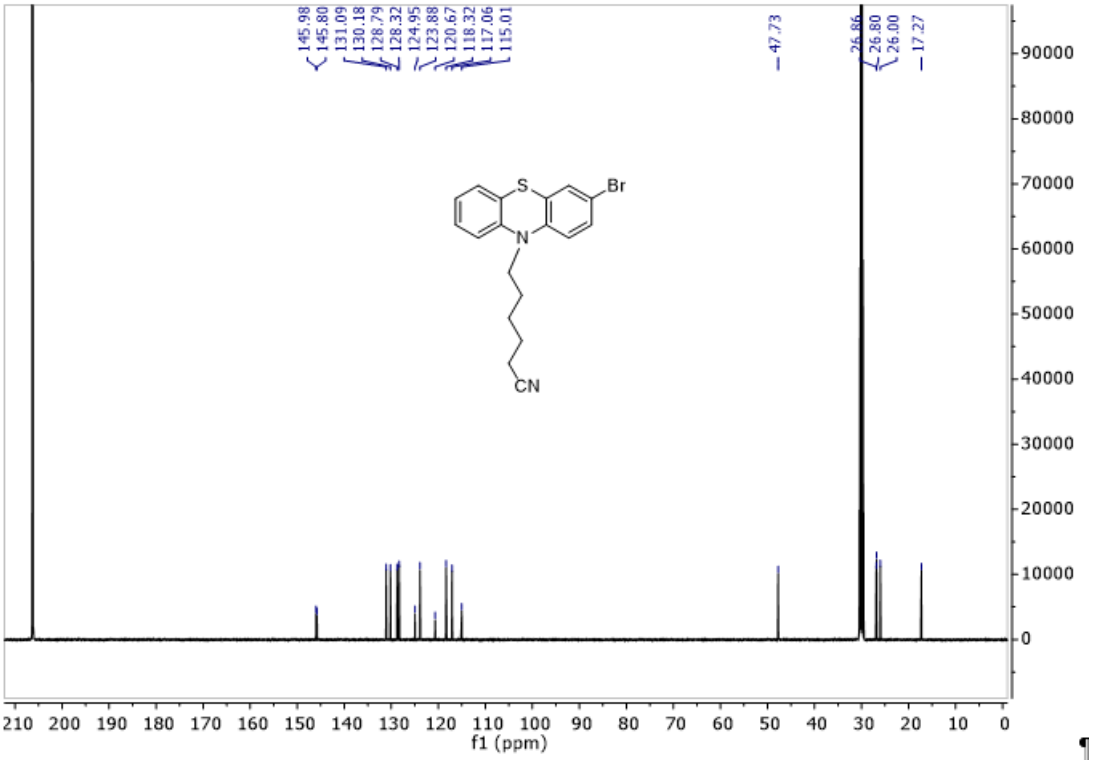

Figure S3. ^{13}C-NMR spectra of **2** measured in Acetone-d_6 at 126 MHz.

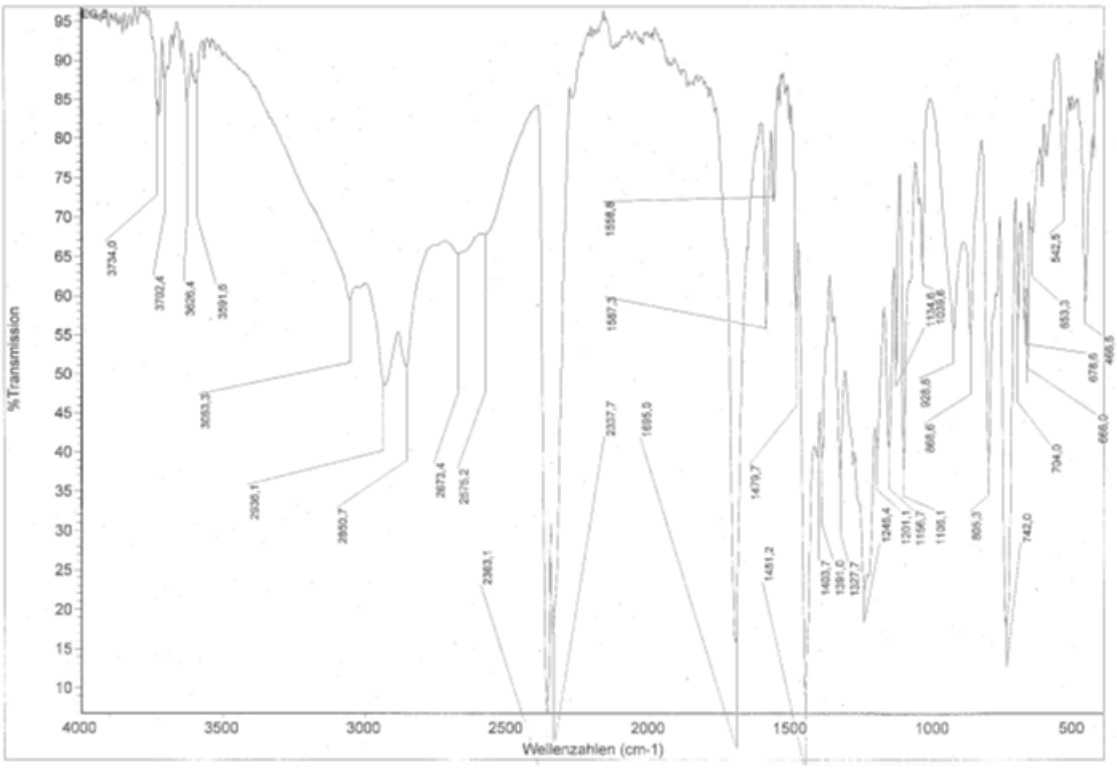

Figure S4. IR spectra of **3**

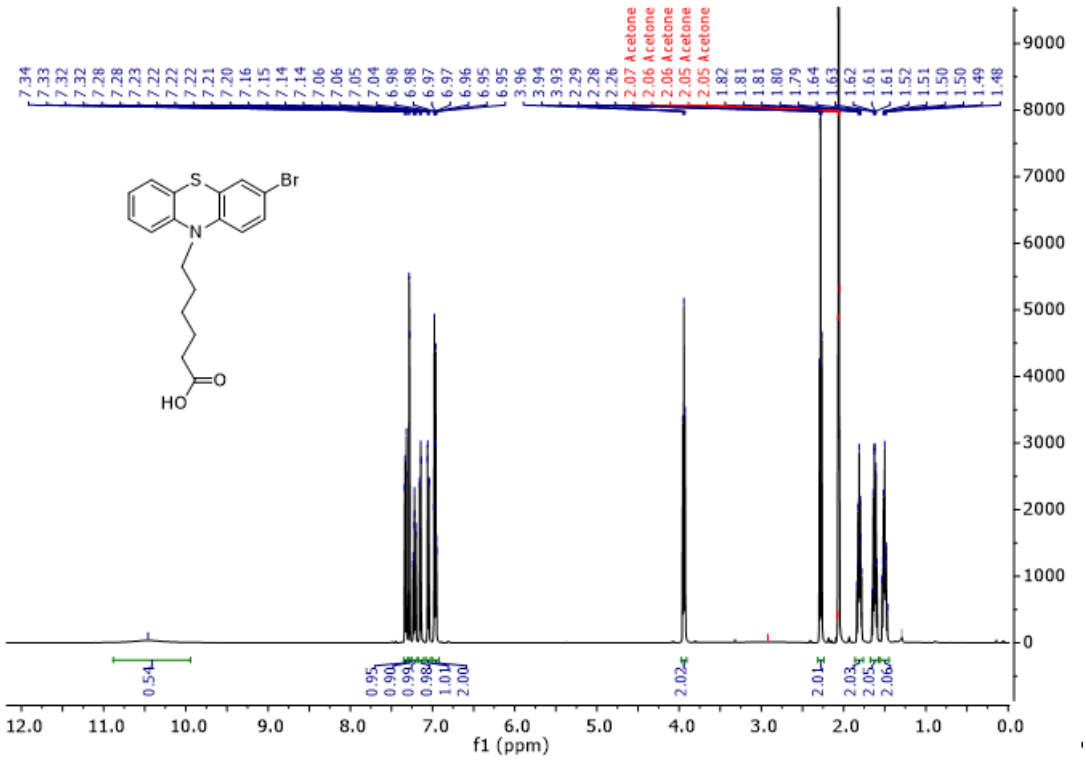

Figure S5. ^{1}H-NMR spectra of **3** measured in Acetone-d_6 at 500 MHz.

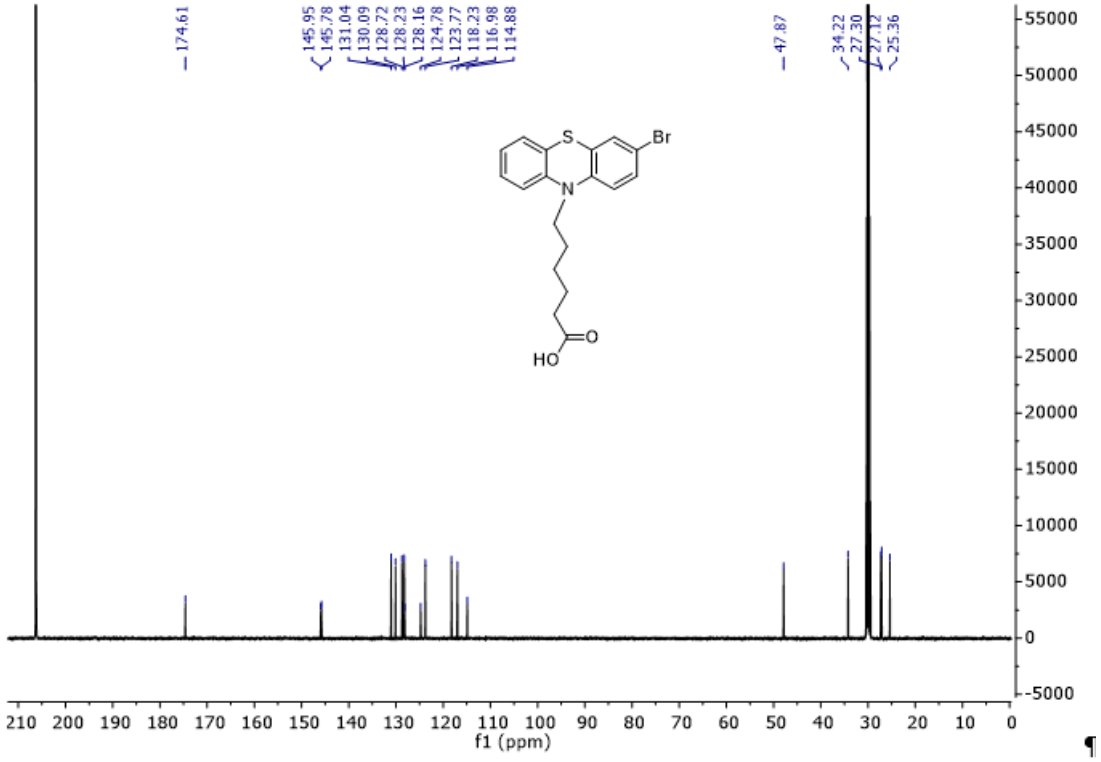

Figure S6. ^{13}C-NMR spectra of **3** measured in Acetone-d_6 at 126 MHz.

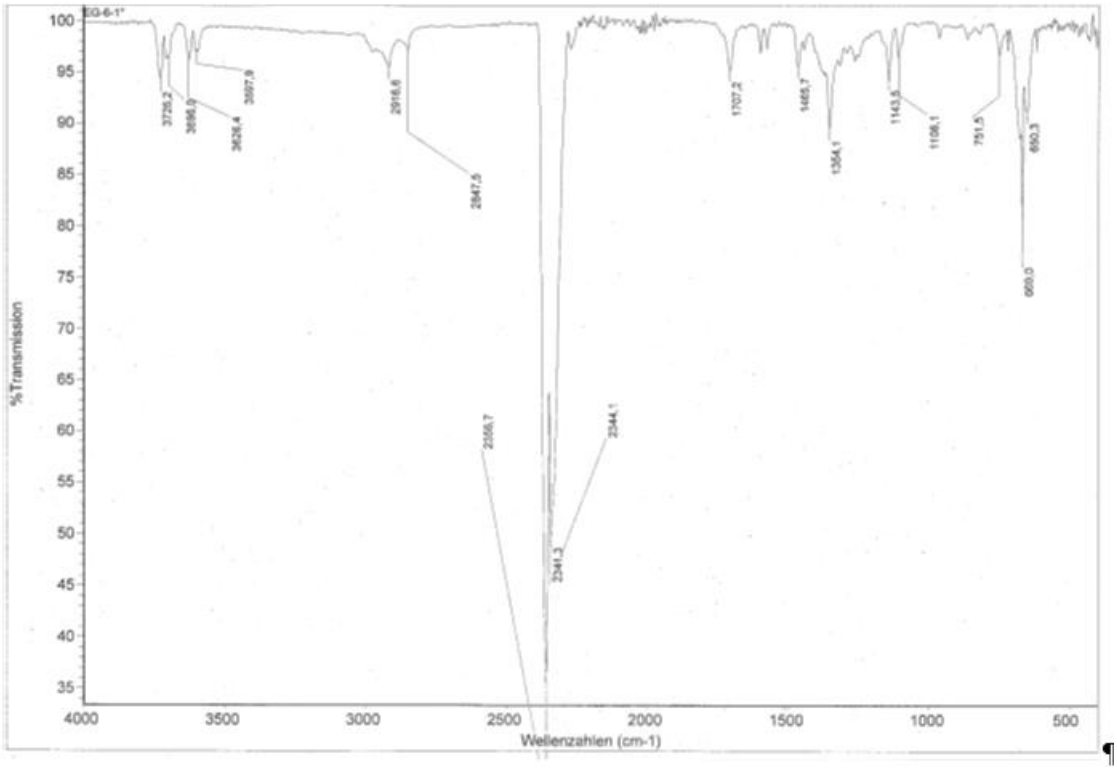

Figure S7. IR spectra of **4**

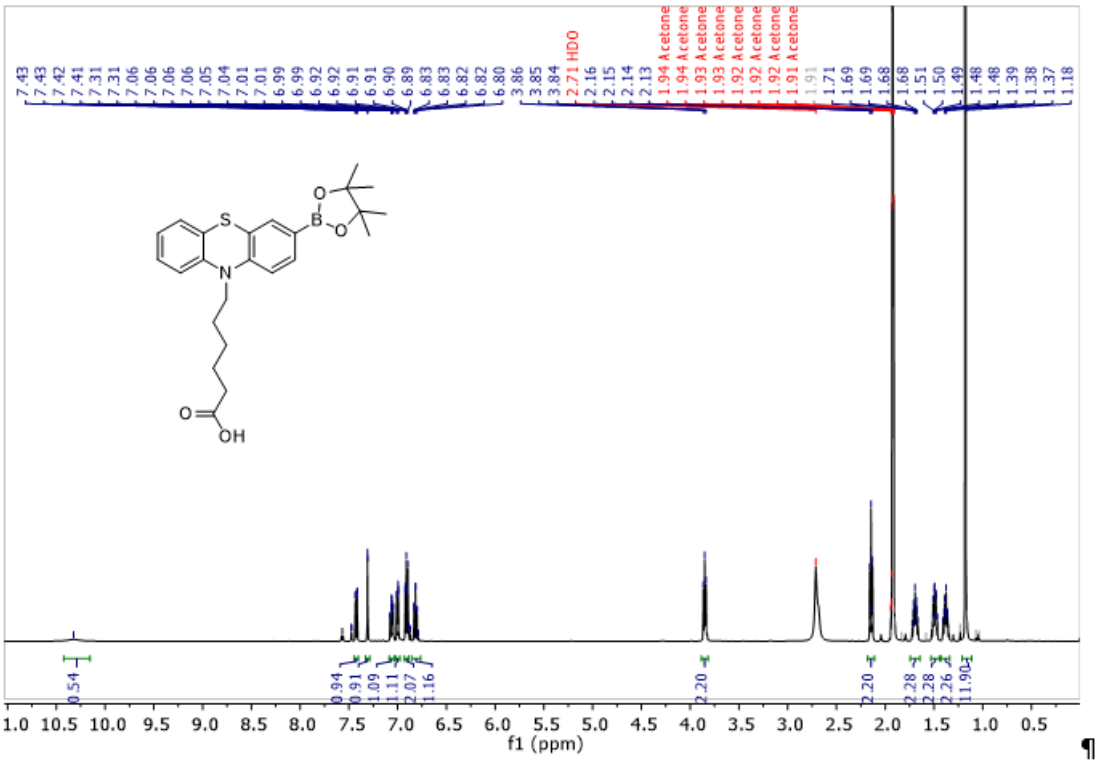

Figure S8. ^{1}H-NMR spectra of **4** measured in Acetone-d_6 at 500 MHz.

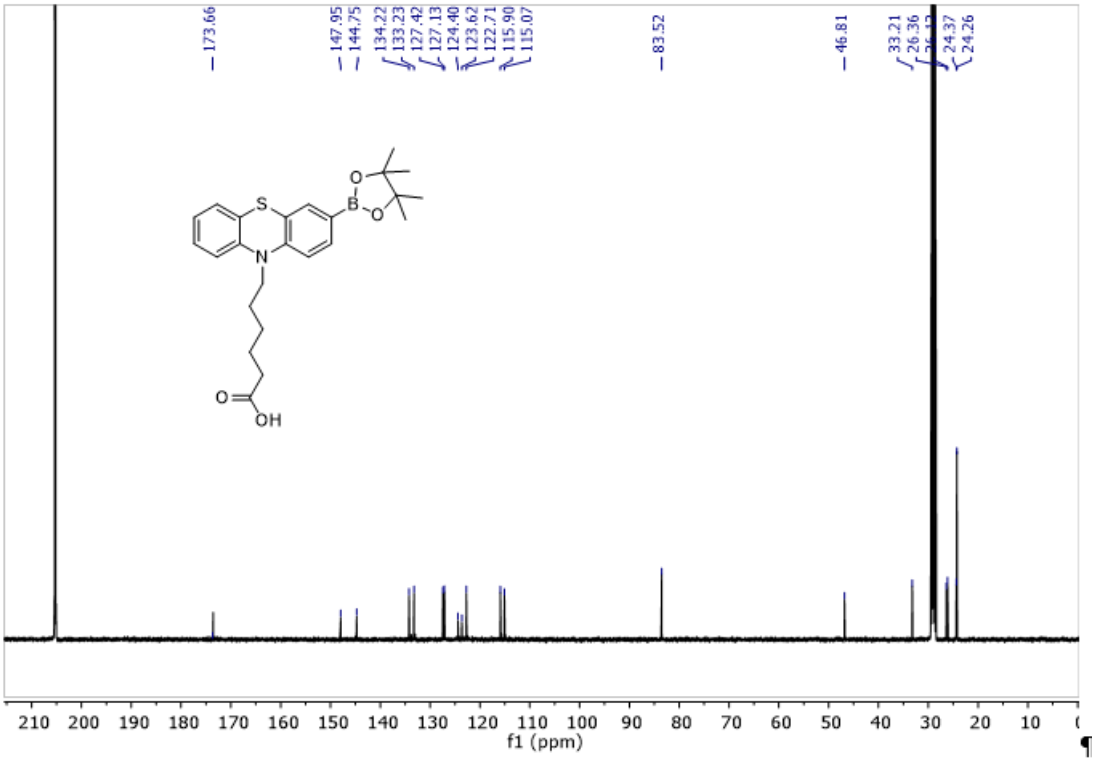

Figure S9. ^{13}C-NMR spectra of **4** measured in Acetone-d_6 at 126 MHz.

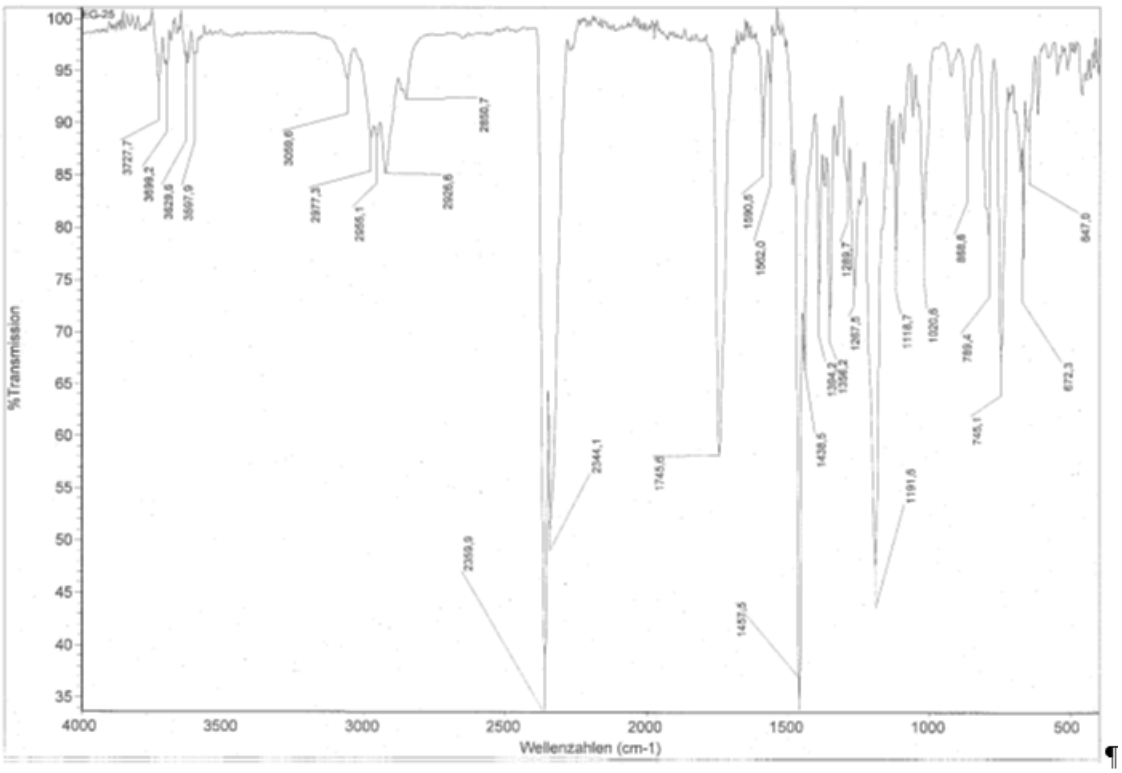

Figure S10. IR spectra of **5**

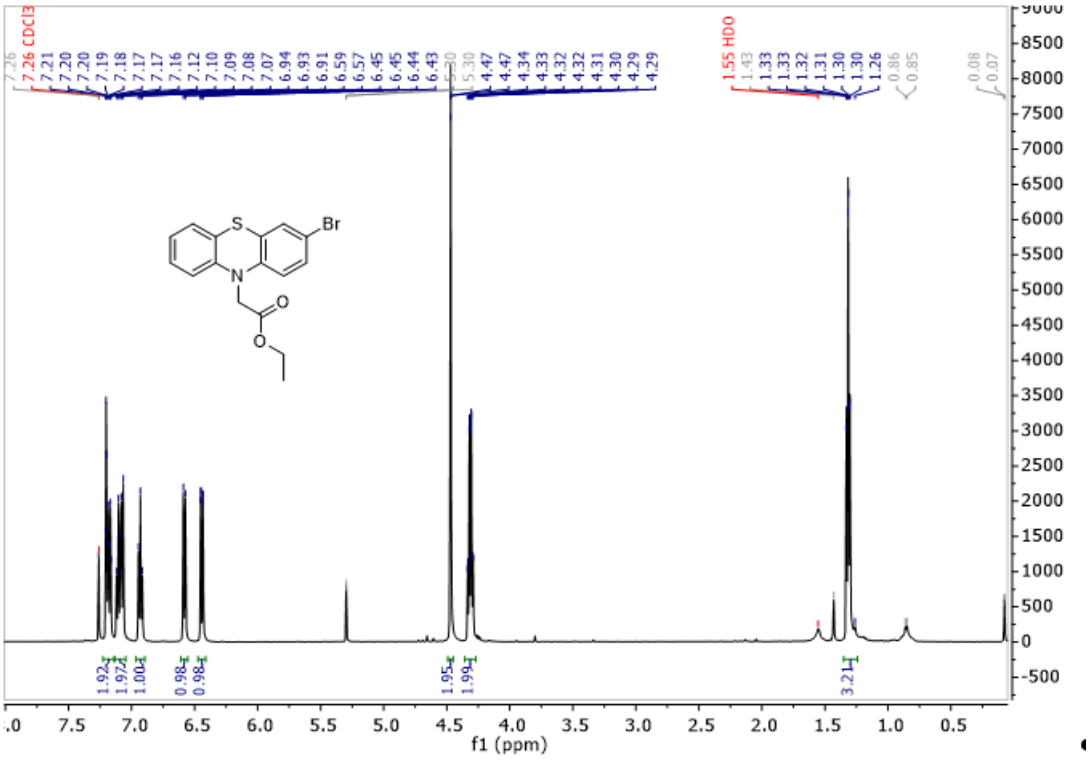

Figure S11. ^{1}H-NMR spectra of **5** measured in $CDCl_3$ at 500 MHz.

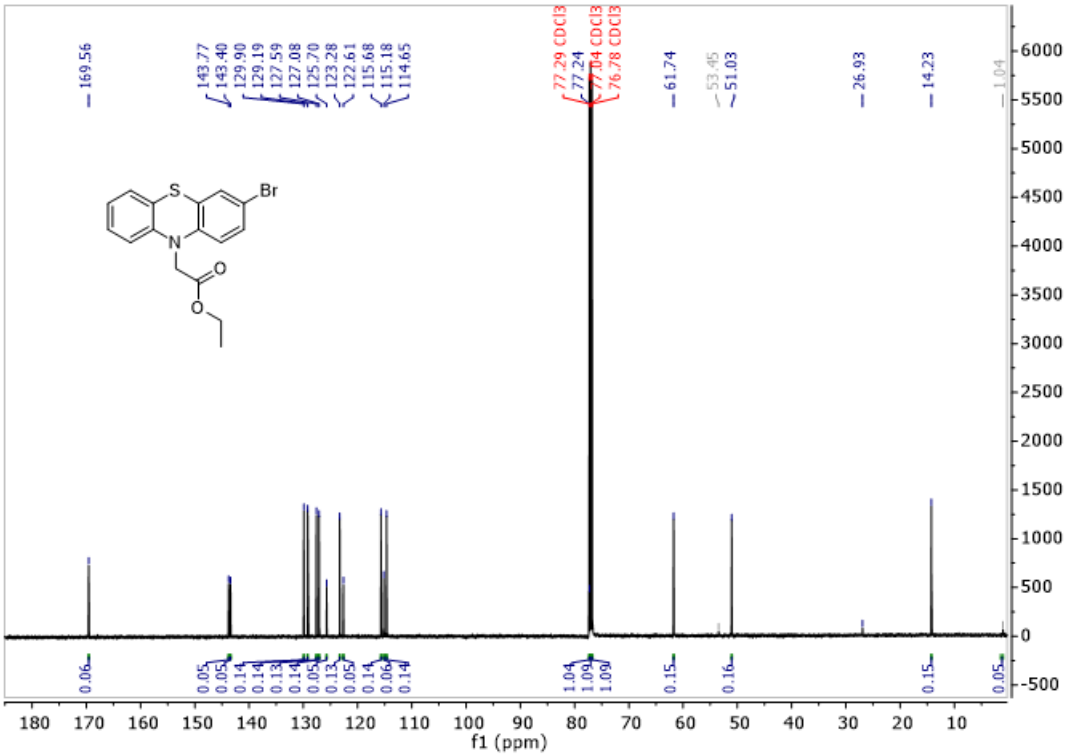

Figure S12. ^{13}C-NMR spectra of **5** measured in $CDCl_3$ at 126 MHz.

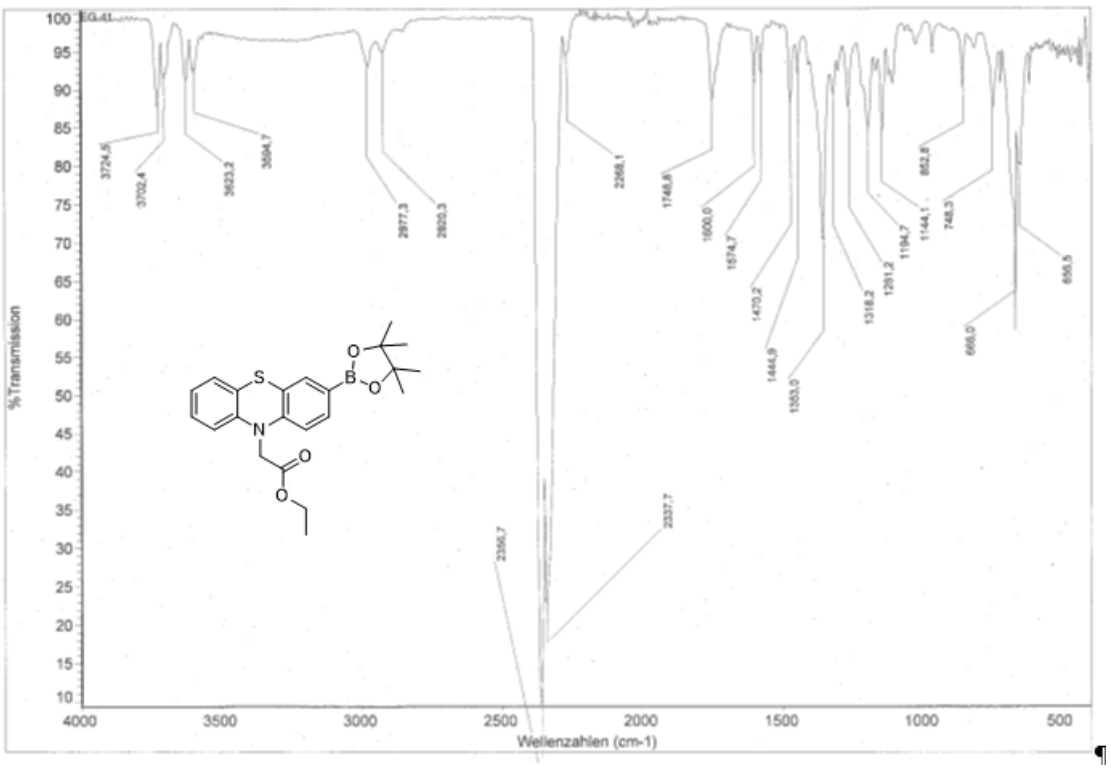

Figure S13. IR spectra of **6**

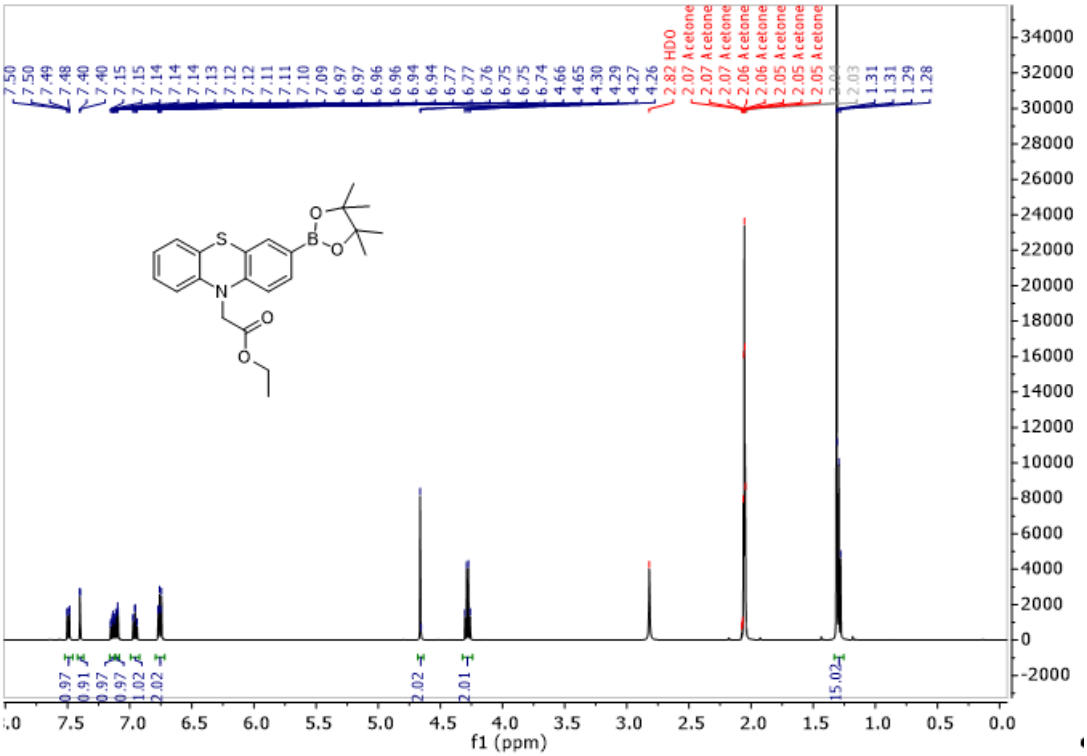

Figure S14. ^{1}H-NMR spectra of **6** measured in Acetone-d_6 at 500 MHz.

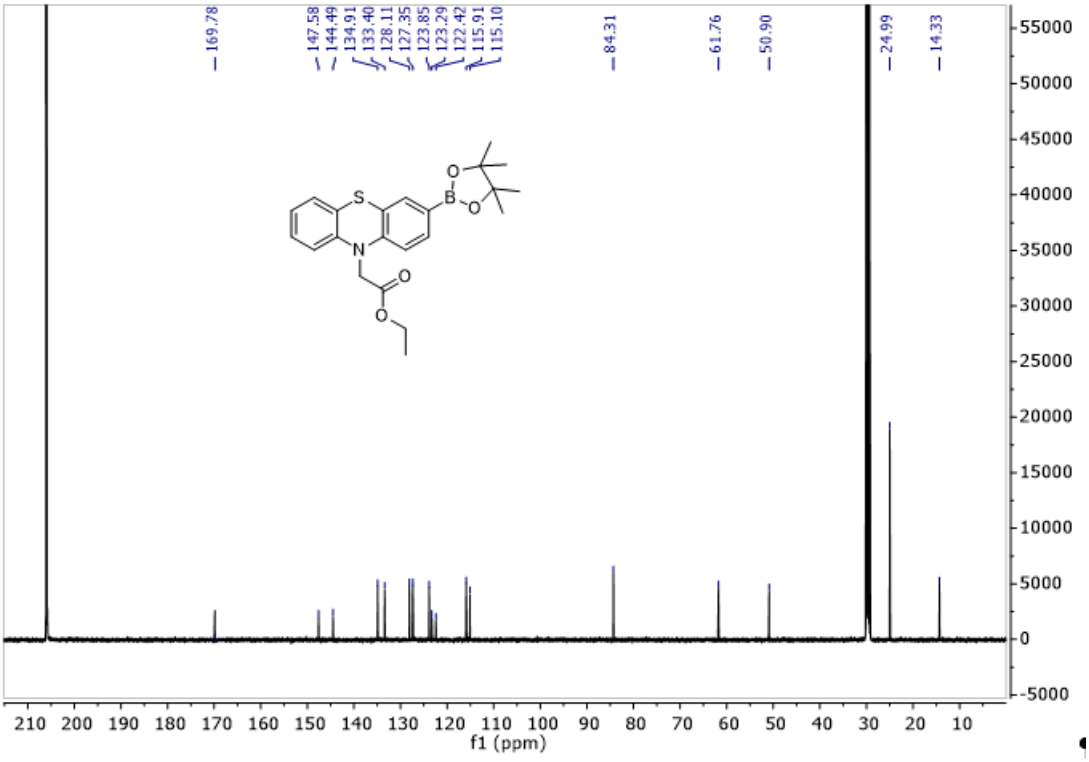

Figure S15. ^{13}C-NMR spectra of **6** measured in Acetone-d_6 at 126 MHz.

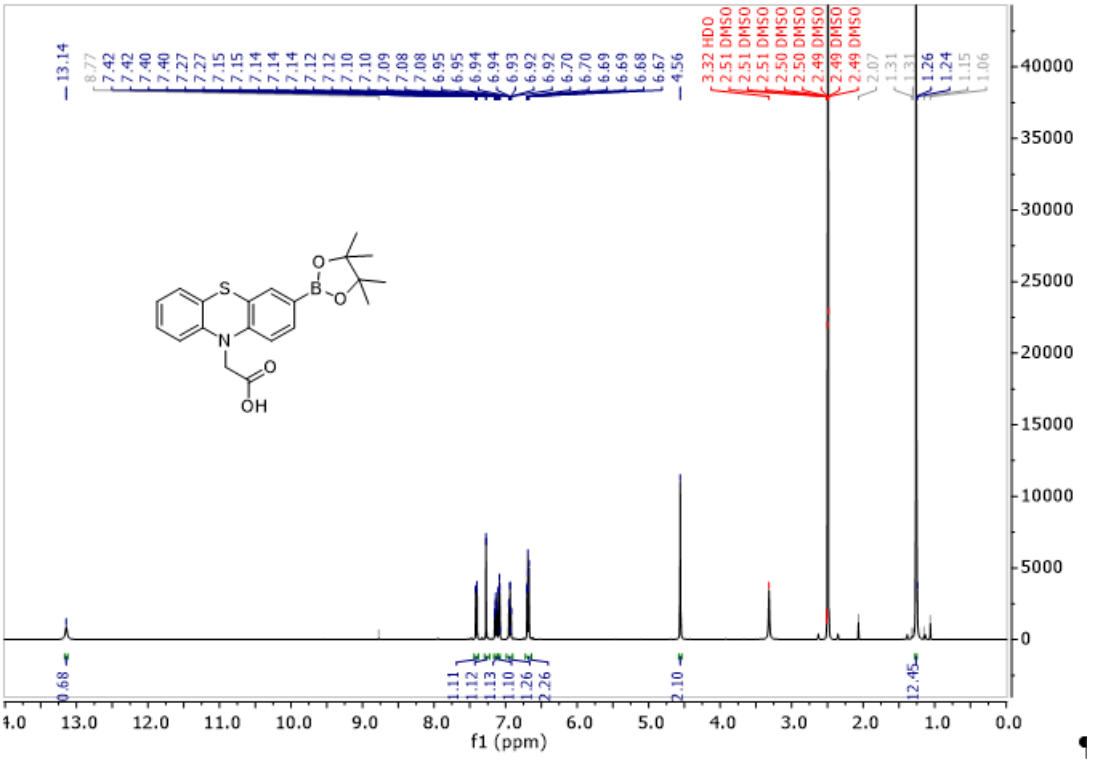

Figure S16. ^{1}H-NMR spectra of **7** measured in DMSO-d_6 at 500 MHz.

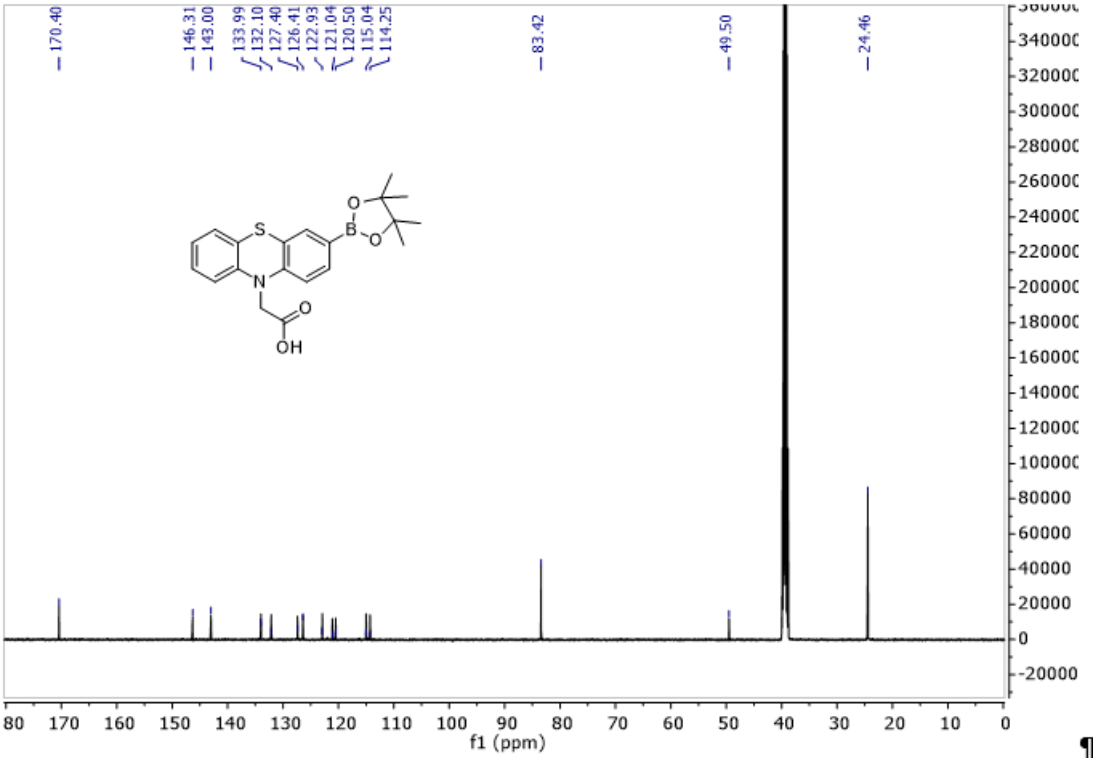

Figure S17. ^{13}C-NMR spectra of **7** measured in DMSO-d_6 at 126 MHz.

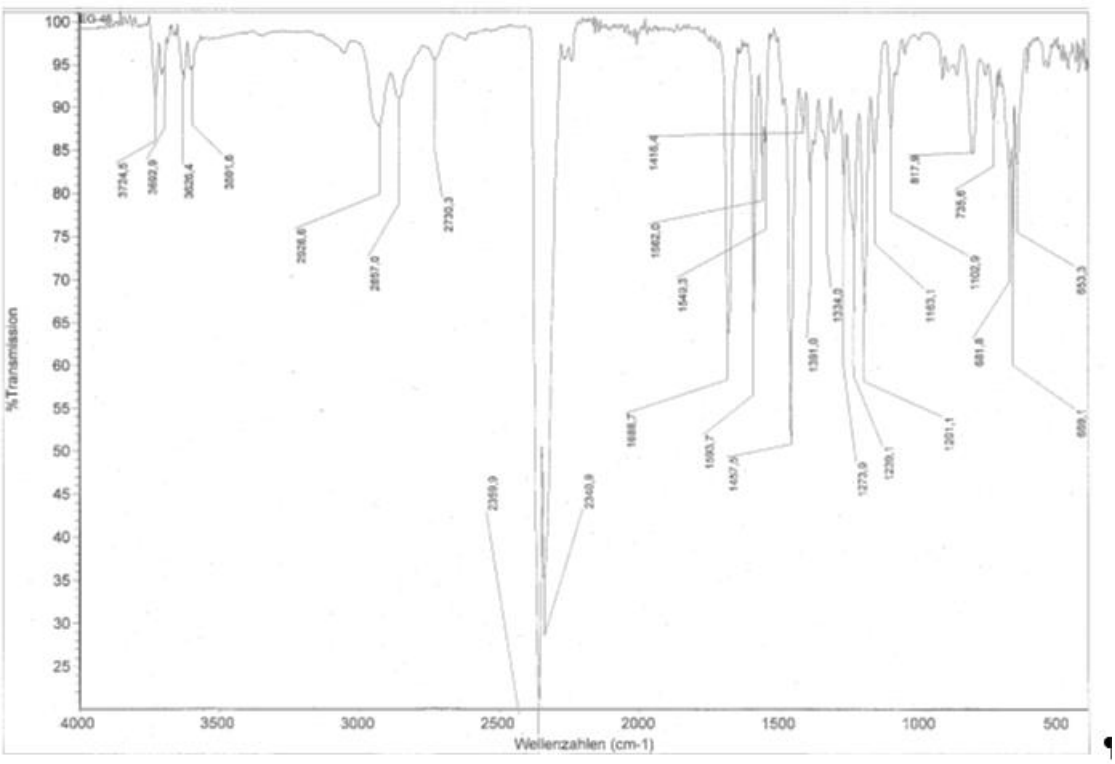

Figure S18. IR spectra of **8**

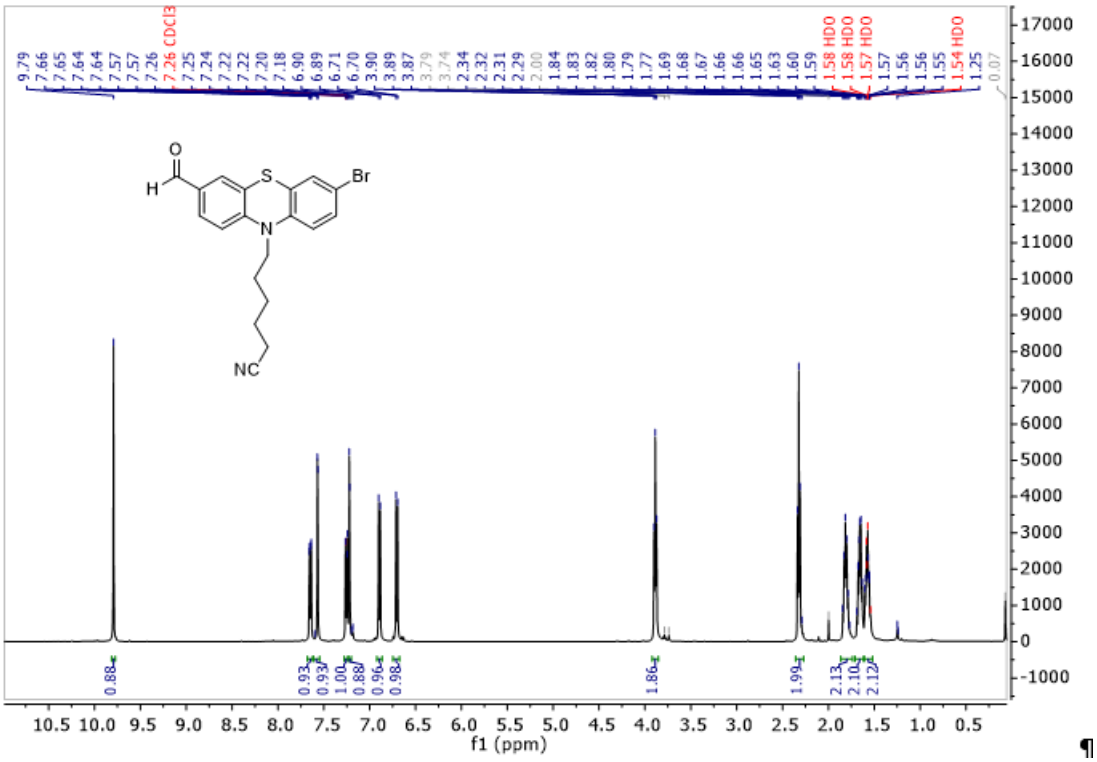

Figure S19. ^{1}H-NMR spectra of **8** measured in $CDCl_3$ at 500 MHz.

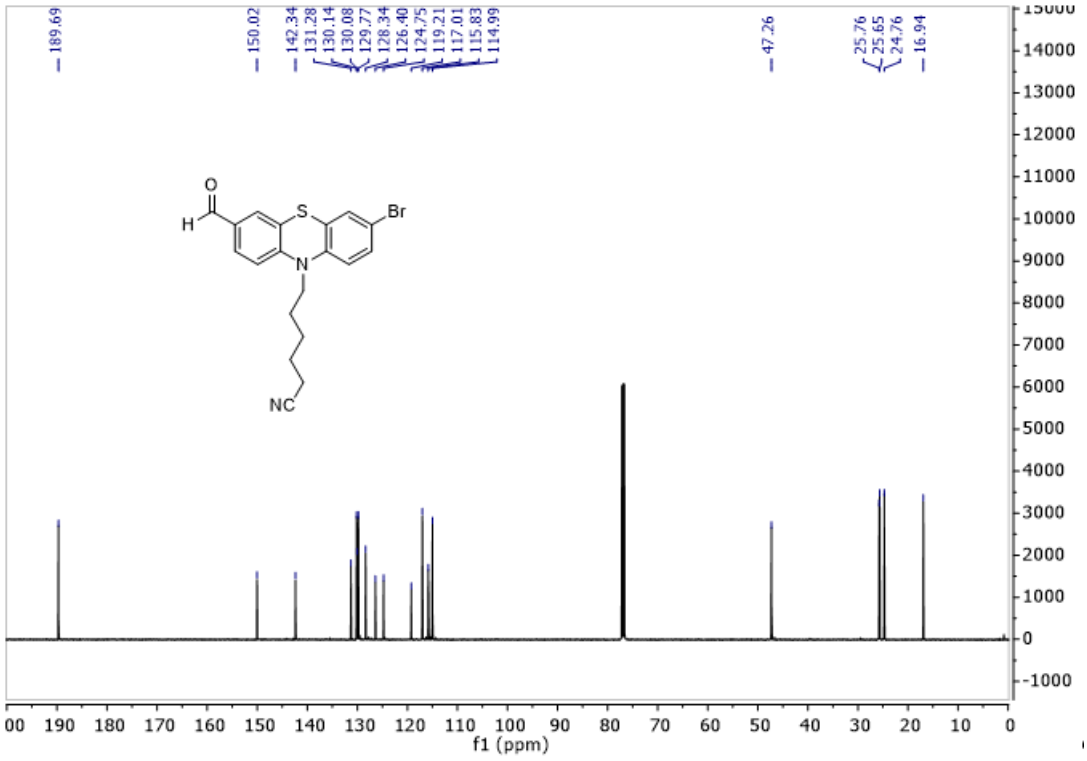

Figure S20. ^{13}C-NMR spectra of **8** measured in $CDCl_3$ at 126 MHz.

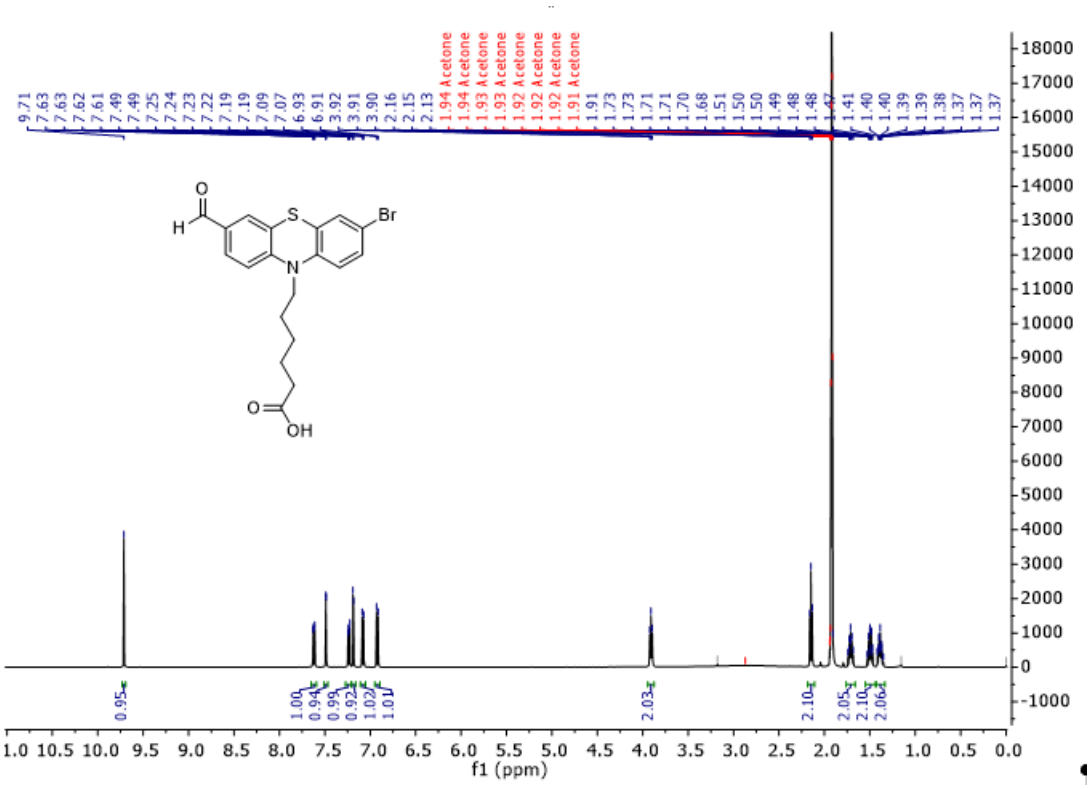

Figure S21. ^{1}H-NMR spectra of **9** measured in Acetone-d_6 at 500 MHz

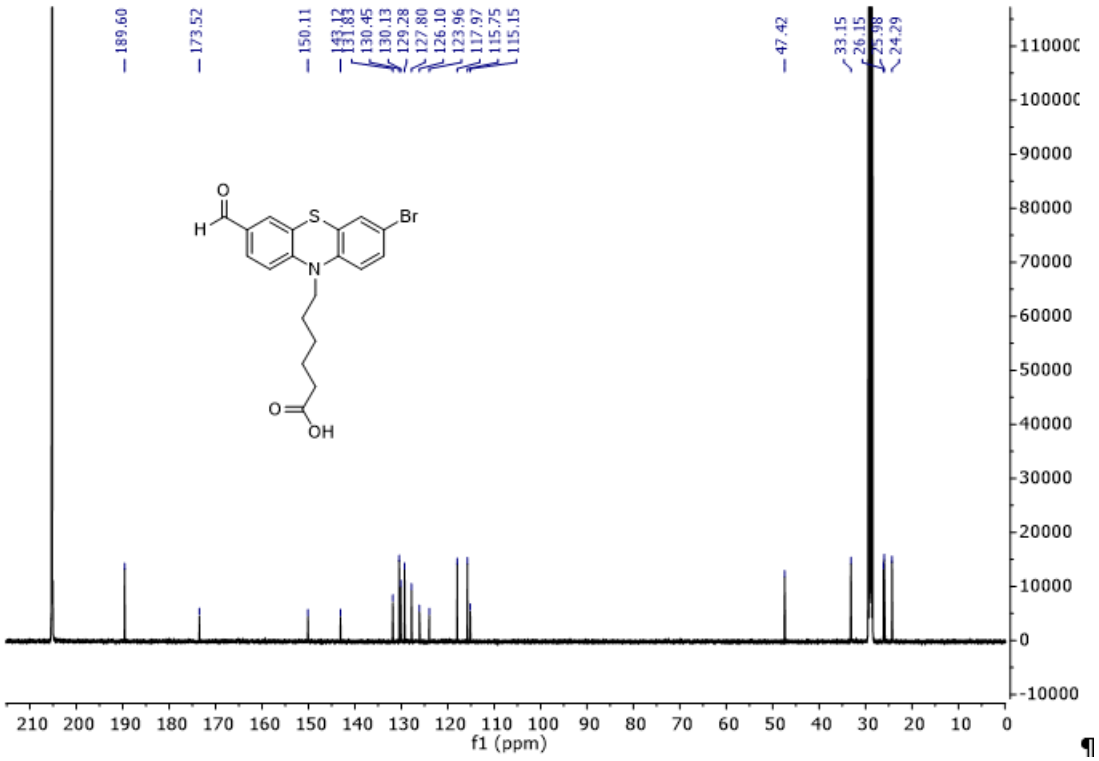

Figure S22. ^{13}C-NMR spectra of **9** measured in Acetone-d_6 at 126 MHz.

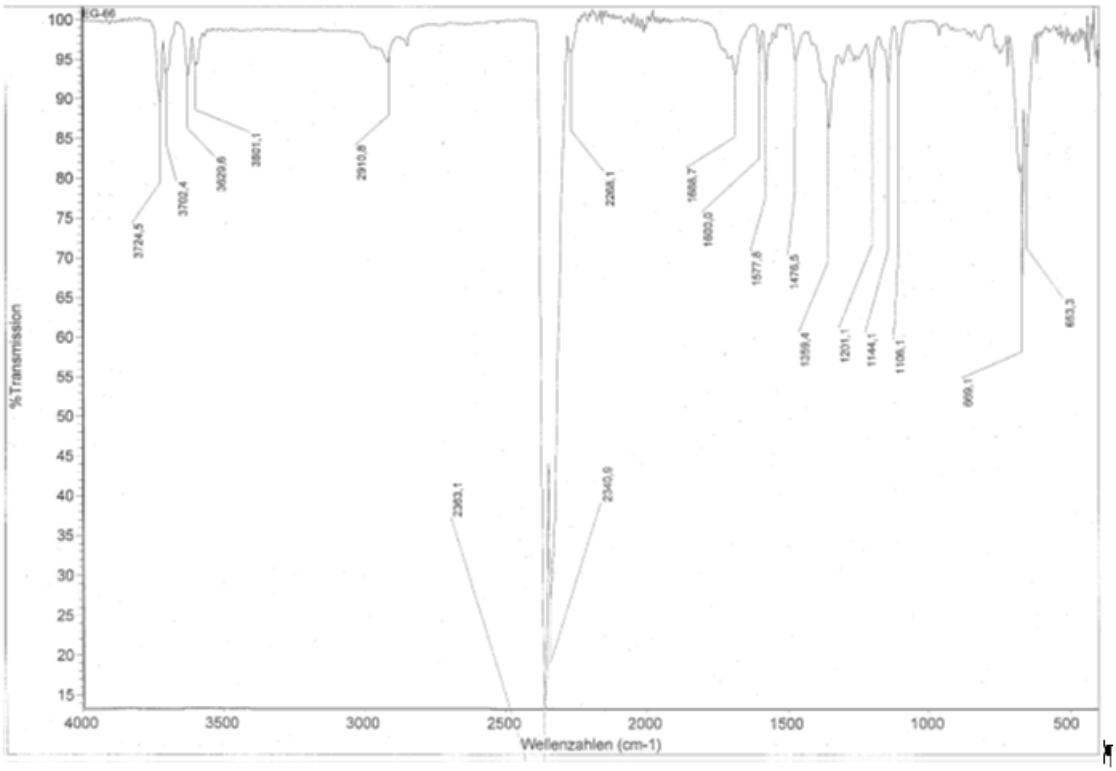

Figure S23. IR spectra of **10**

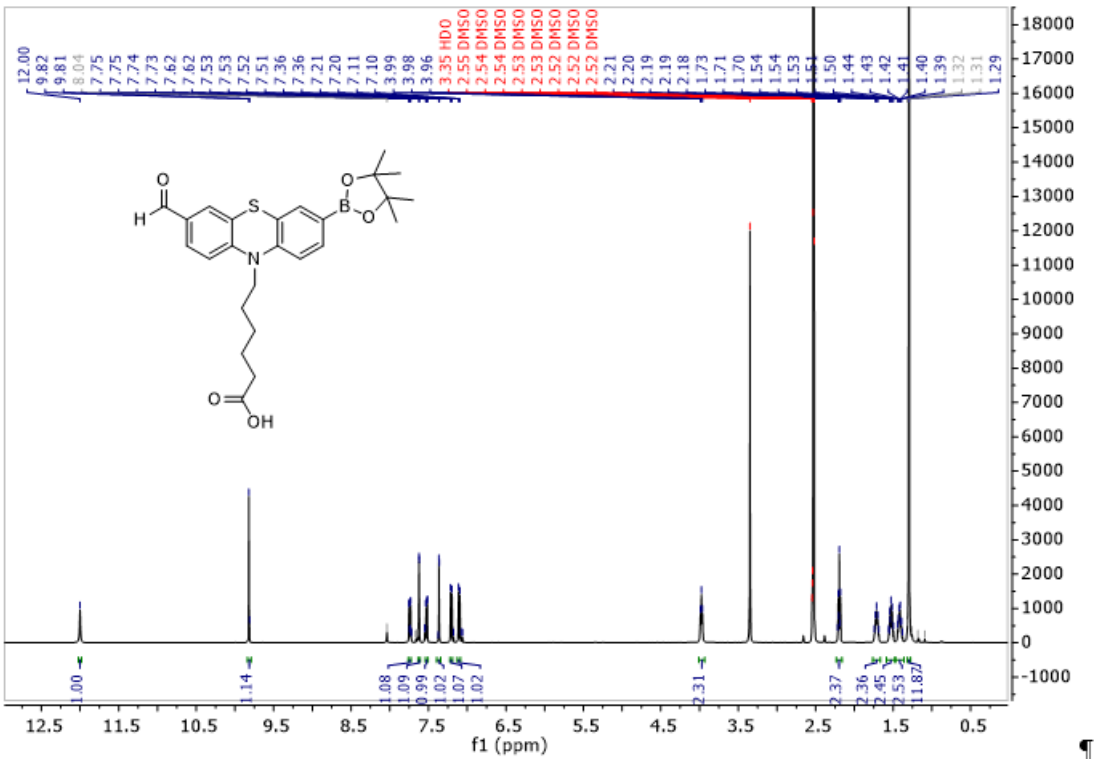

Figure S24. ^{1}H-NMR spectra of **10** measured in DMSO-d_6 at 500 MHz

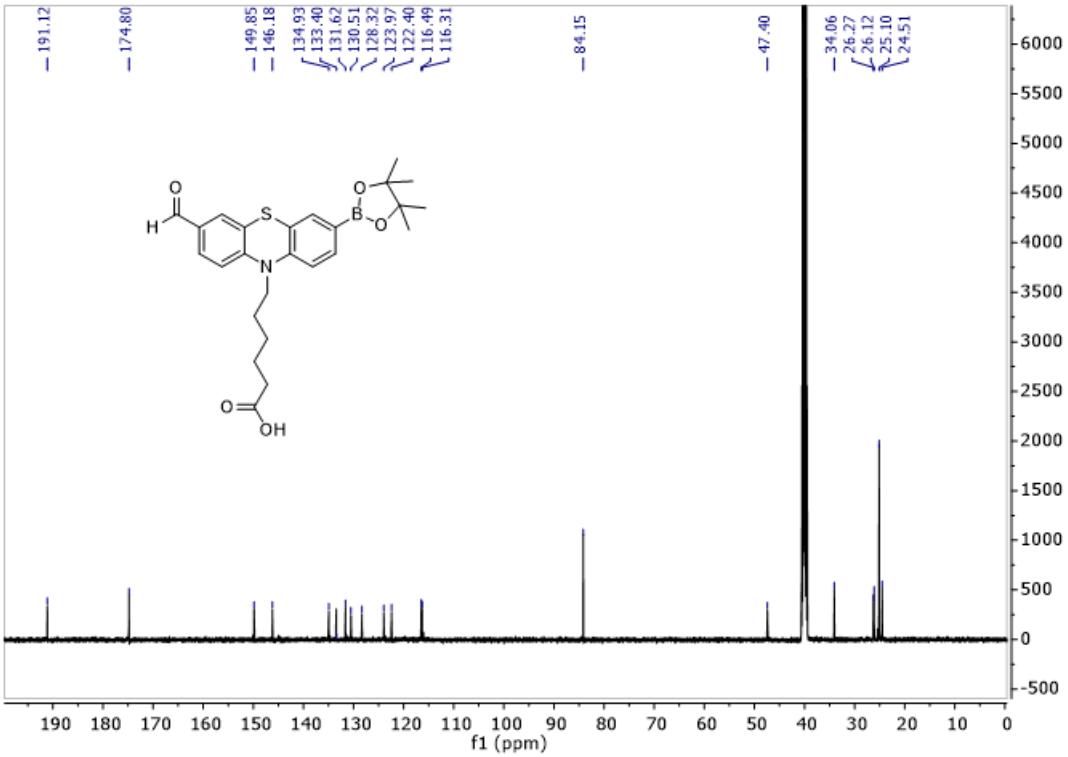

Figure S25. ^{13}C-NMR spectra of **10** measured in DMsO-d_6 at 126 MHz.

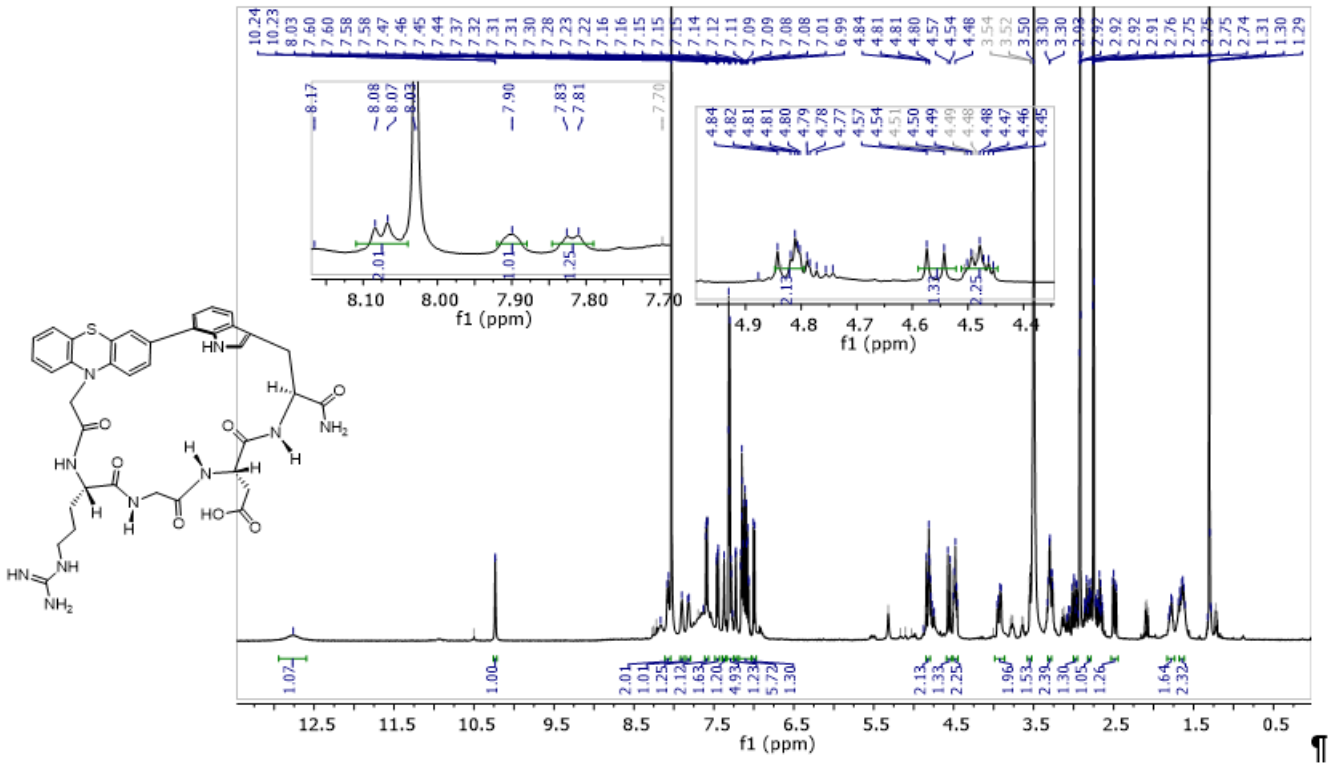

Figure S26. ^{1}H-NMR spectra of **12c** measured in DMF-d$_7$ at 500 MHz

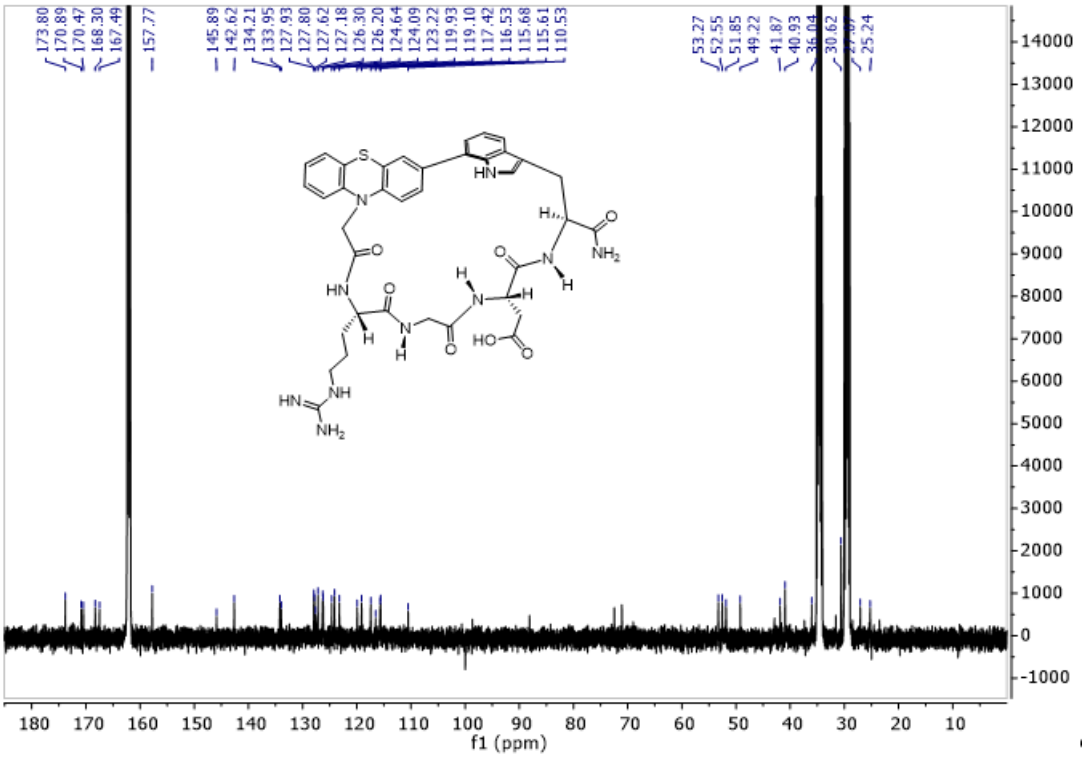

Figure S27. ^{13}C-NMR spectra of **12c** measured in DMF-d$_7$ at 126 MHz.

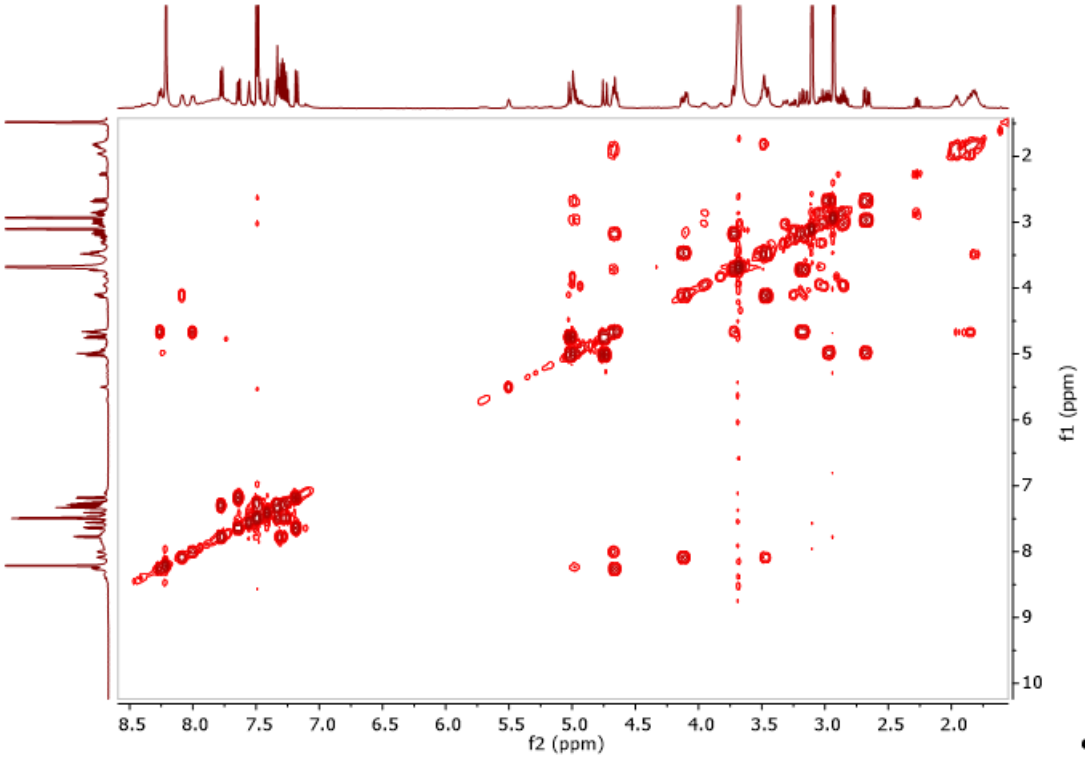

Figure S28. ^{1}H-^{1}H Cosy spectra of **12c** measured in DMF-d$_7$ at 500 MHz.

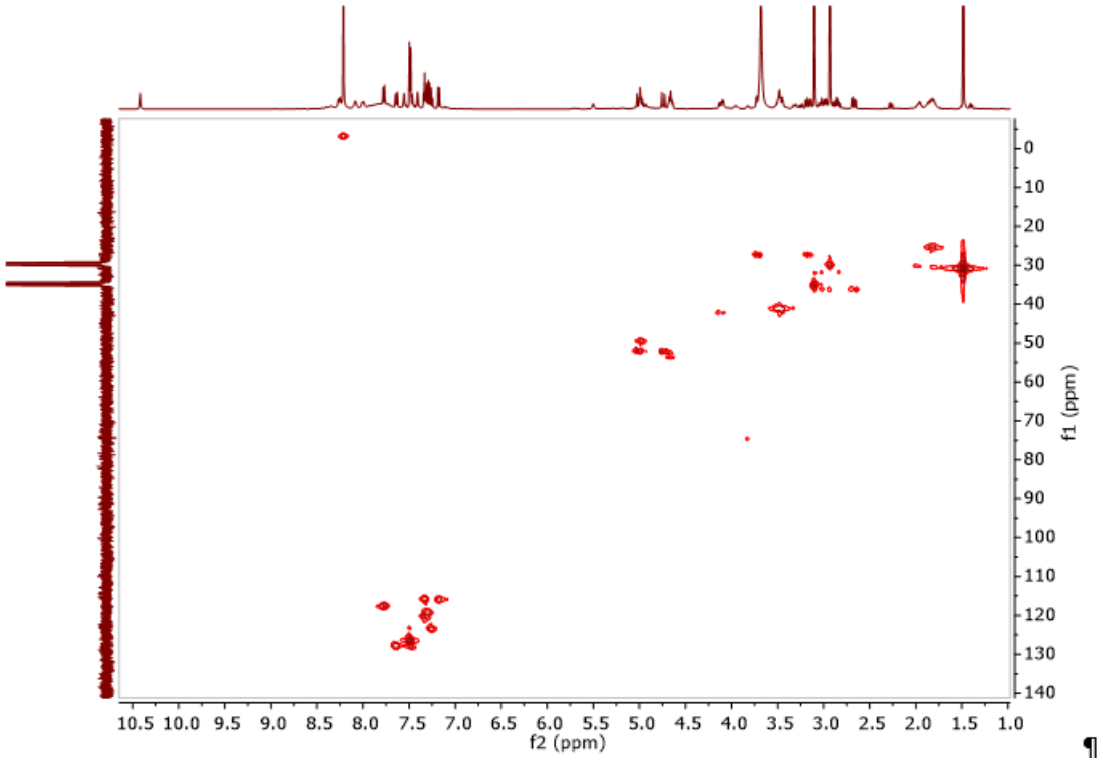

Figure S29. ^{1}H-^{13}C HMQC spectra of **12c** measured in DMF-d$_7$

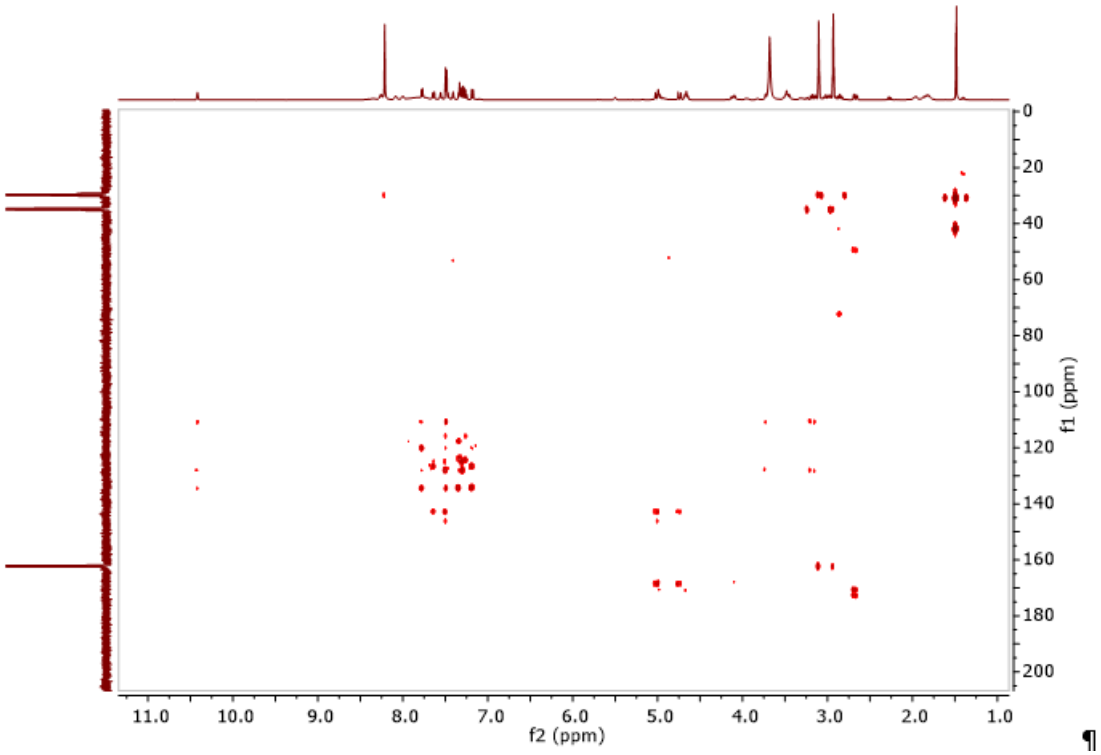

Figure S30. ^{1}H-^{13}C HMBC spectra of **12c** measured in DMF-d_7

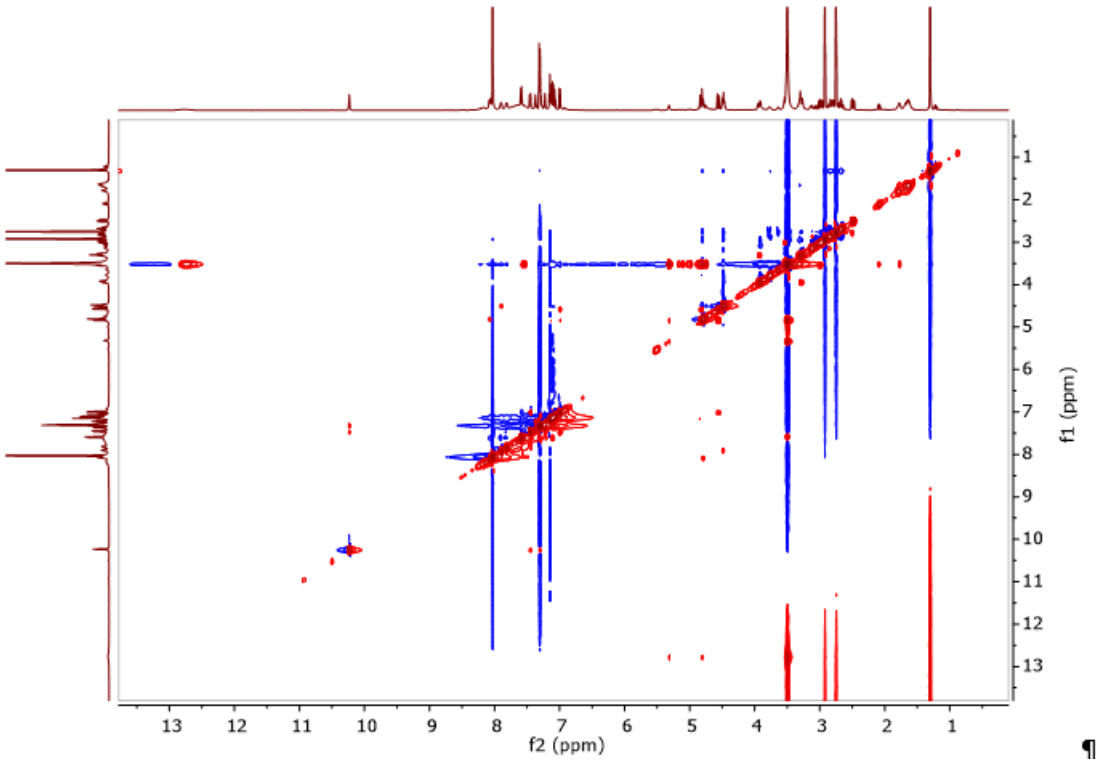

Figure S31. NOESY spectra of **12c** measured in DMF-d_7

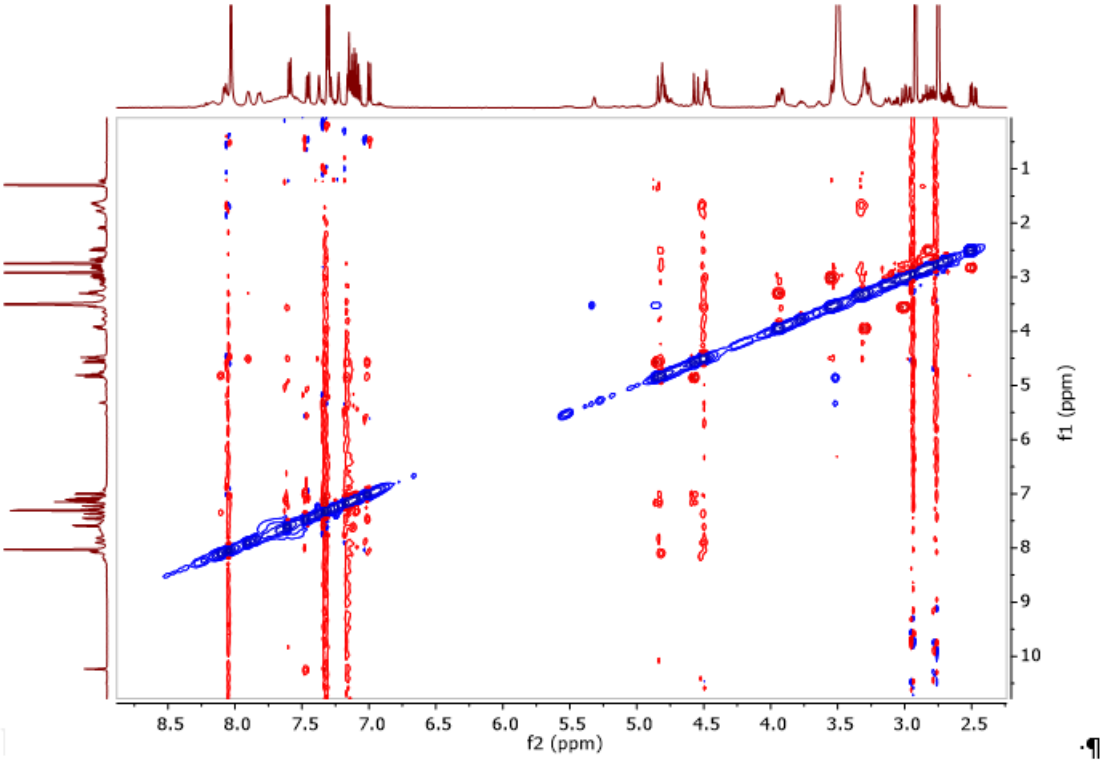

Figure S32. ROESY spectra of **12c** measured in DMF-d_7

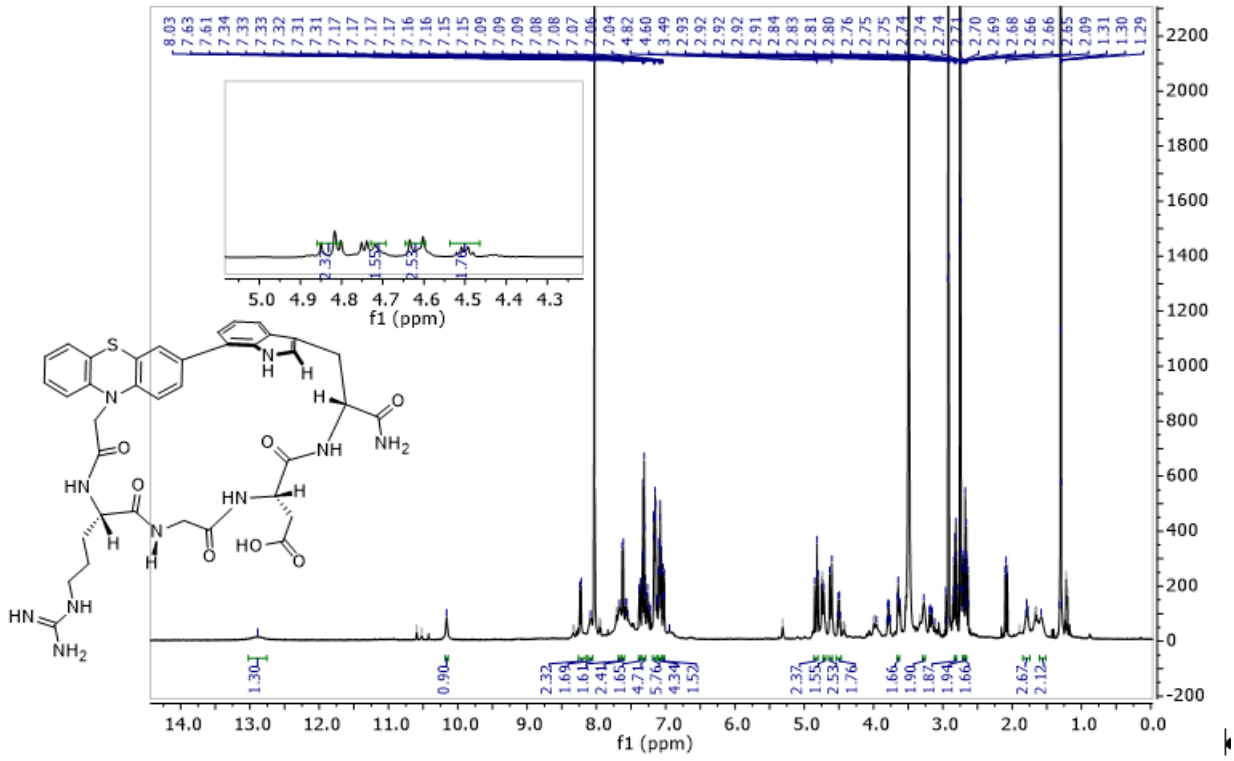

Figure S33. ^{1}H-NMR spectra of **12d** measured in DMF-d$_7$ at 500 MHz

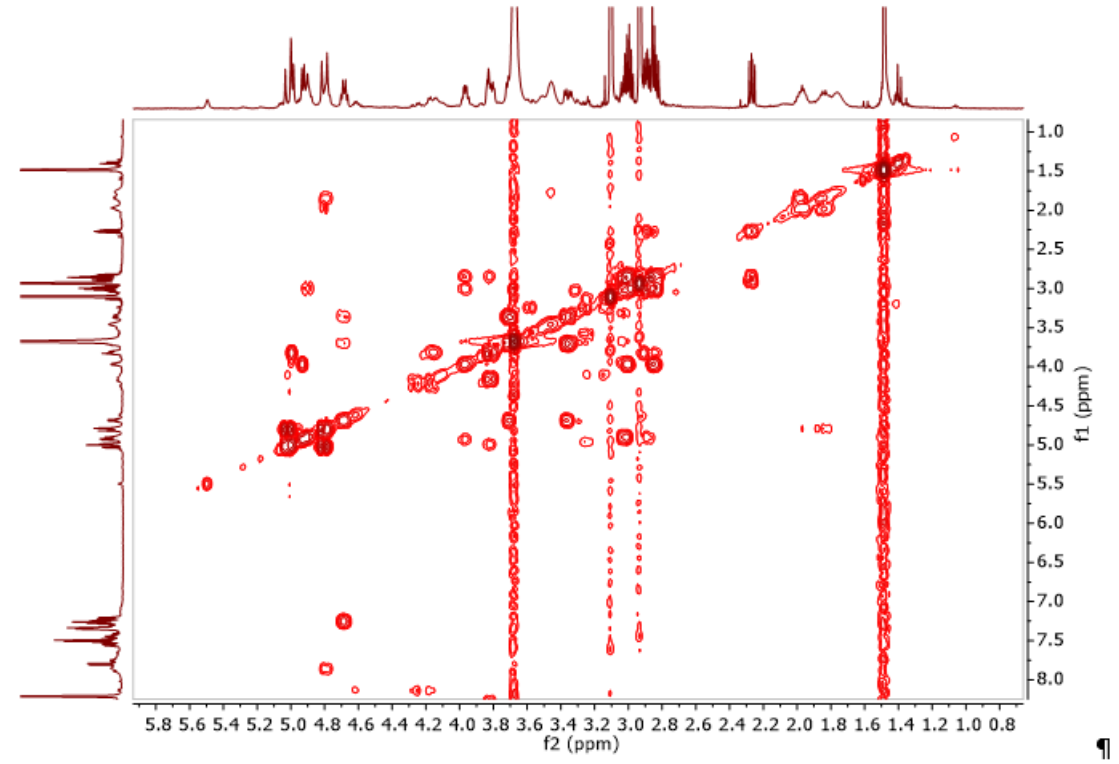

Figure S34. ^{1}H-^{1}H Cosy spectra of **12d** measured in DMF-d$_7$ at 500 MHz.

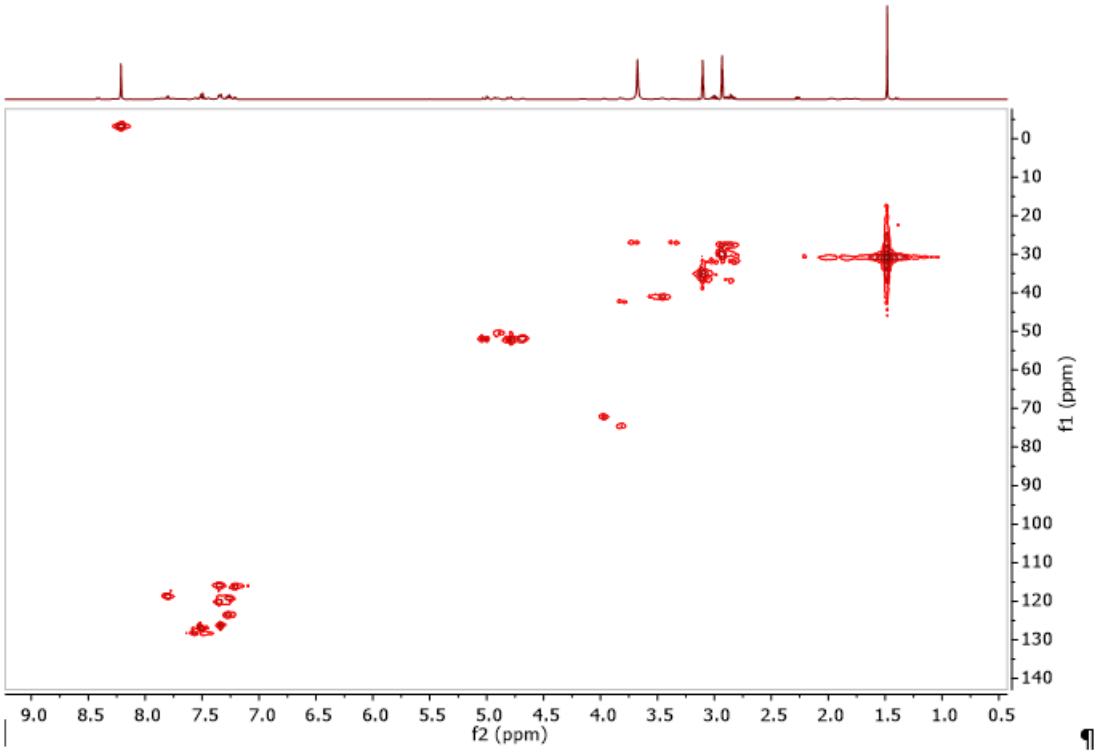

Figure S35. ^{1}H-^{13}C HMQC spectra of **12d** measured in DMF-d_7

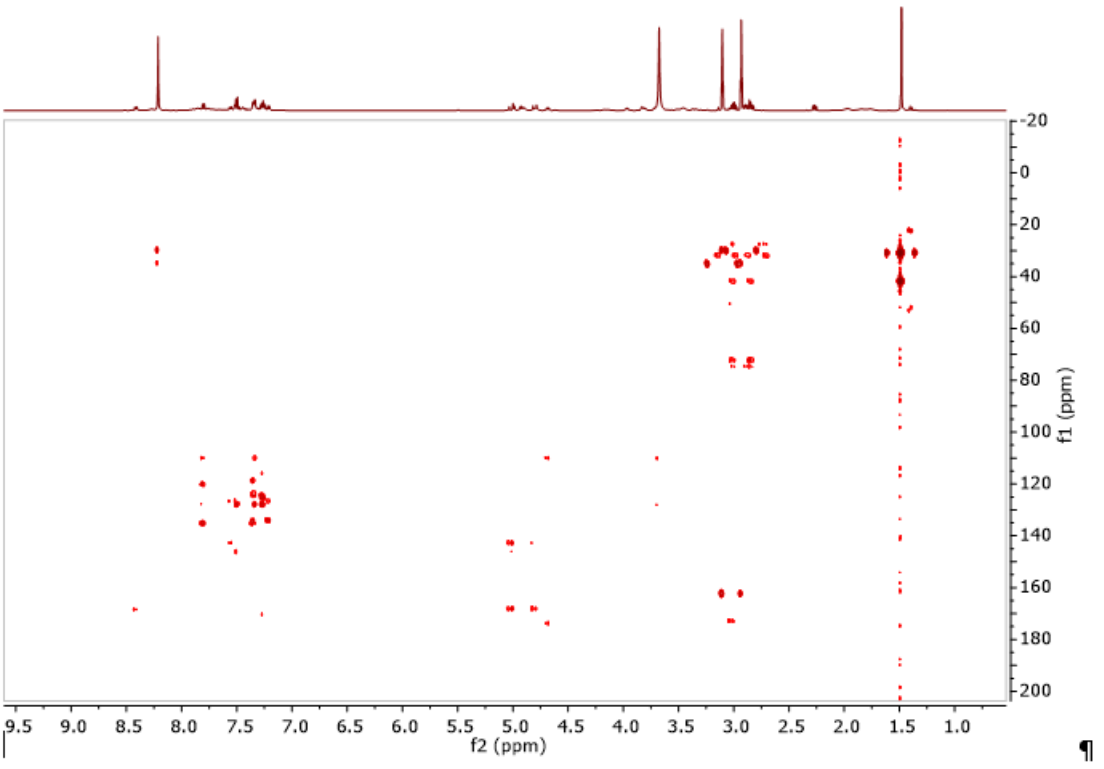

Figure S36. ^{1}H-^{13}C HMBC spectra of **12d** measured in DMF-d_7

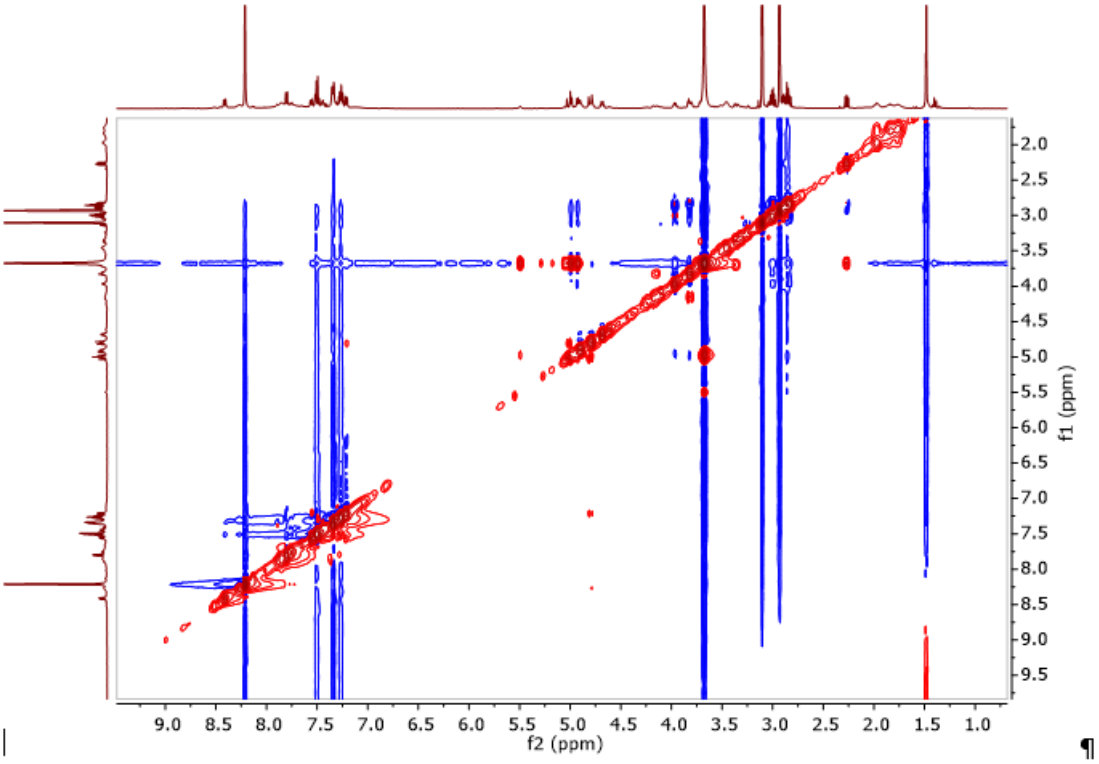

Figure S37. NOESY spectra of **12d** measured in DMF-d_7

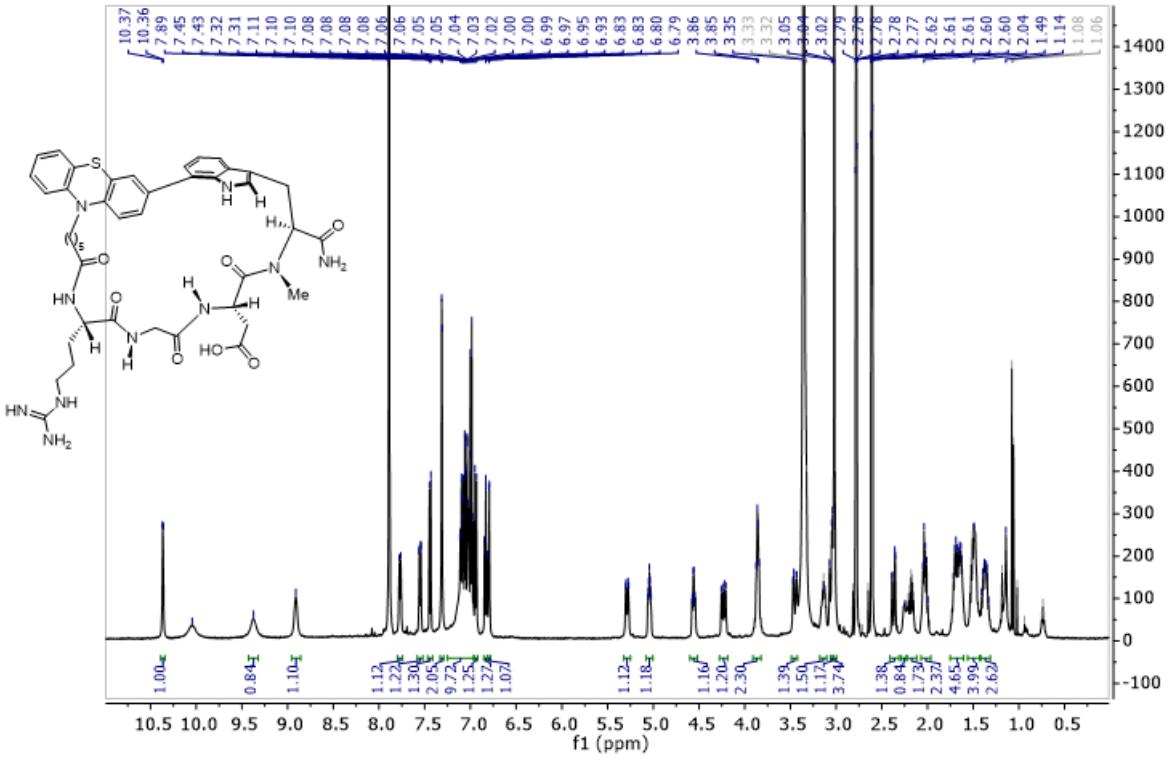

Figure S38. ^{1}H-NMR spectra of **12e** measured in DMF-d$_7$ at 500 MHz

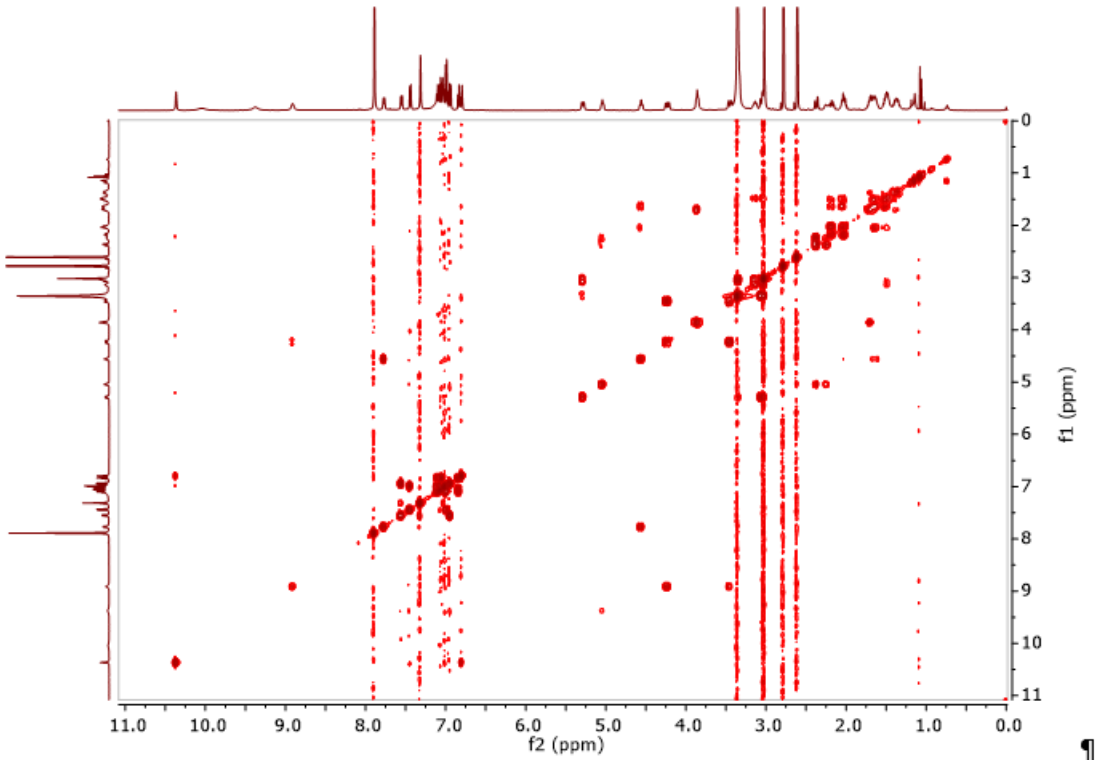

Figure S39. ^{1}H-^{1}H Cosy spectra of **12e** measured in DMF-d$_7$ at 500 MHz.

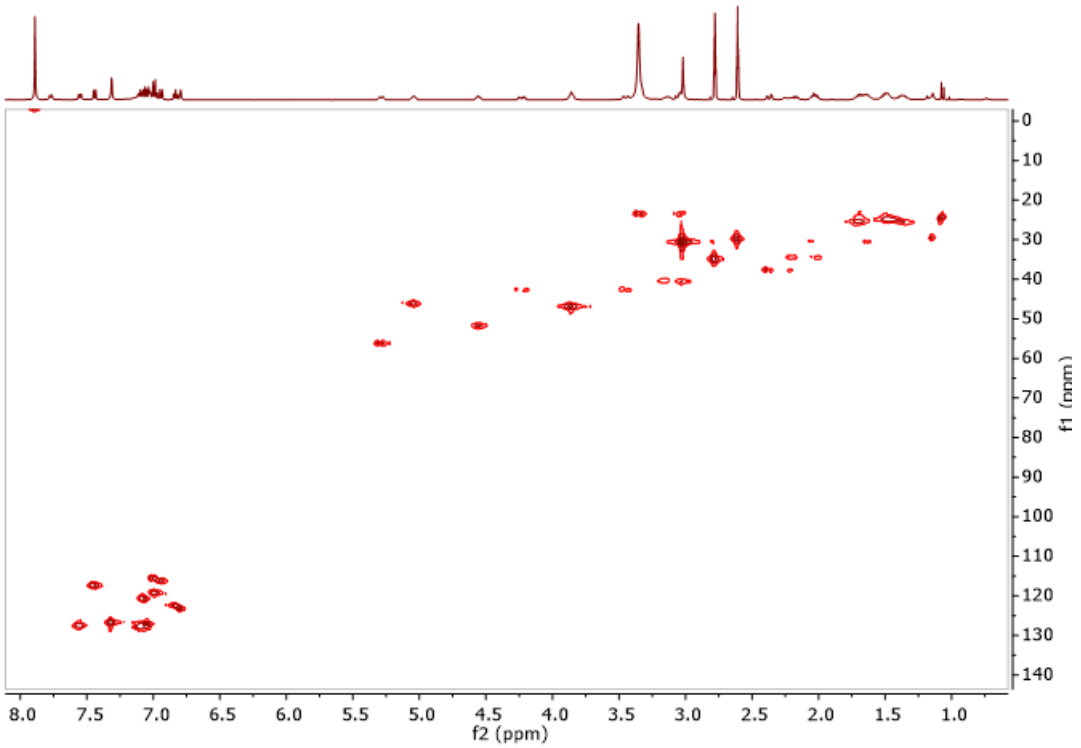

Figure S40. HMQC spectra of **12e** measured in DMF-d_7

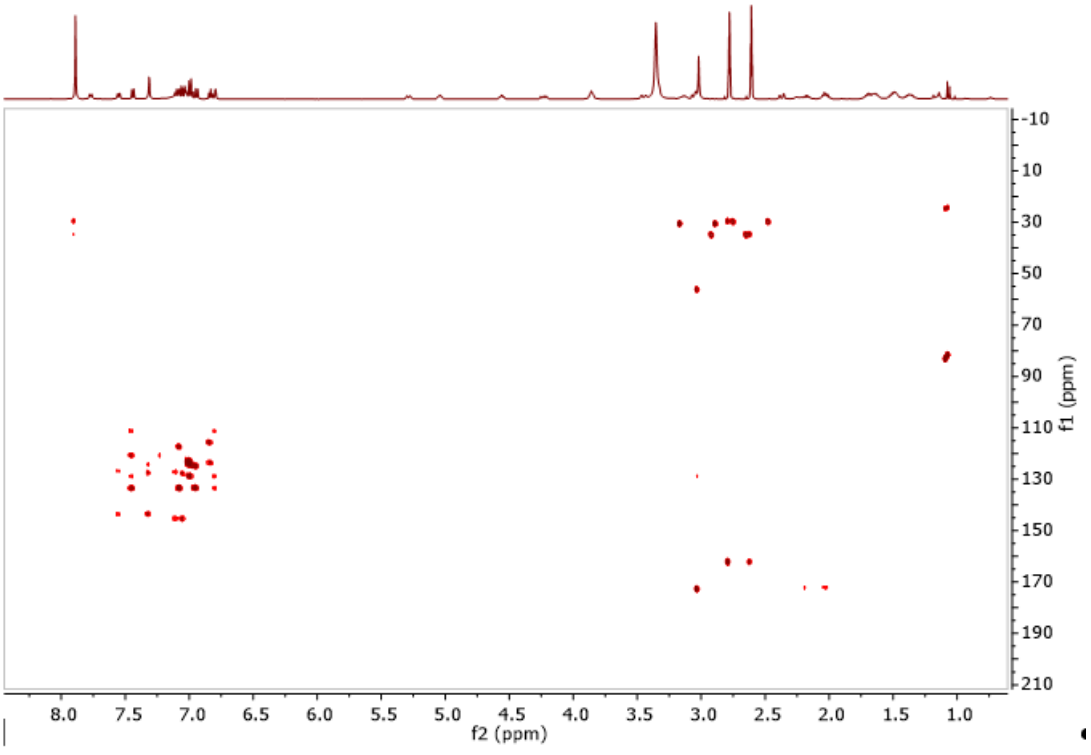

Figure S41. HMBC spectra of **12e** measured in DMF-d_7

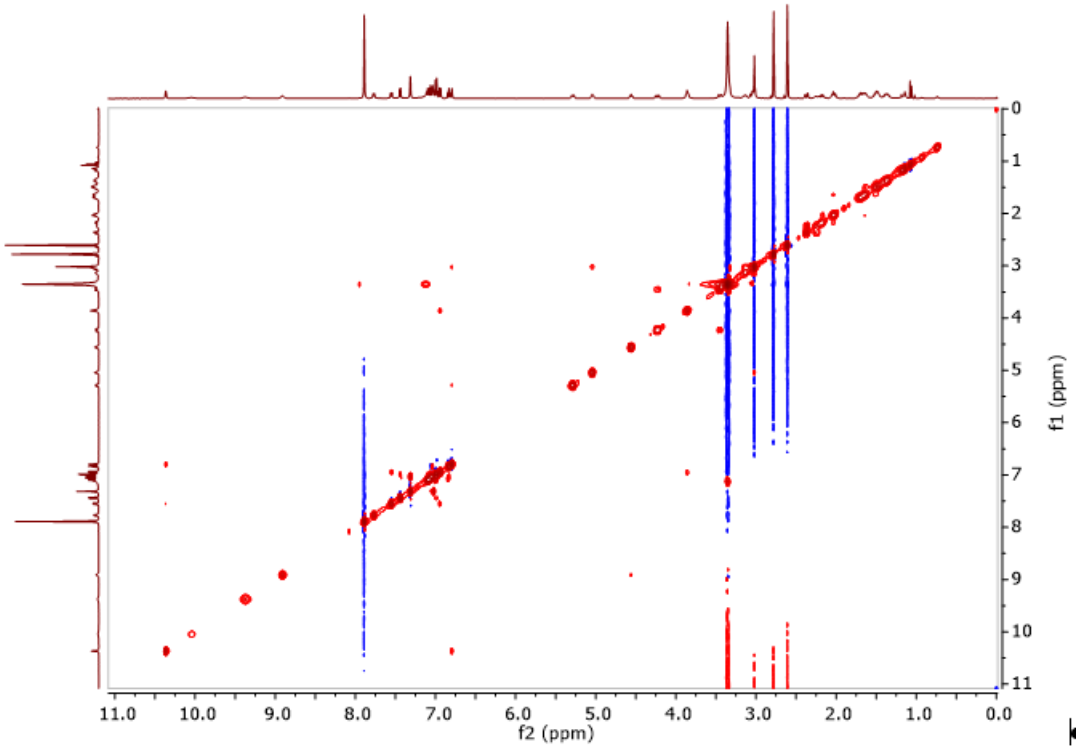

Figure S42. NOESY spectra of **12e** measured in DMF-d_7

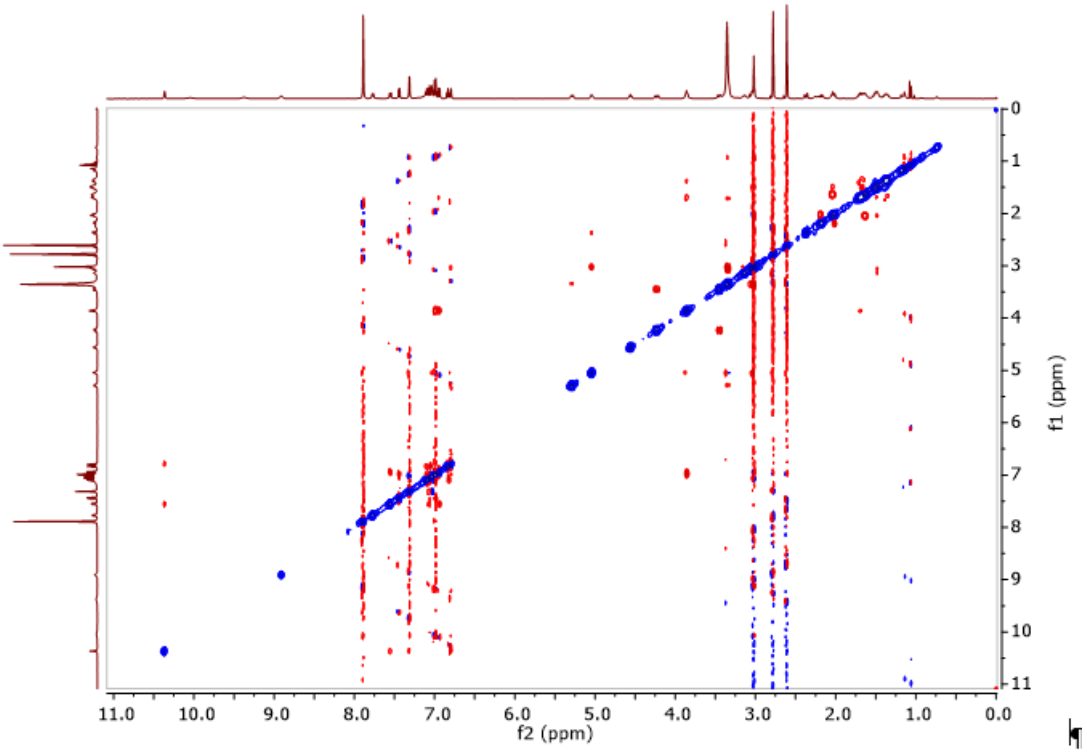

Figure S43. ROESY spectra of **12e** measured in DMF-d_7

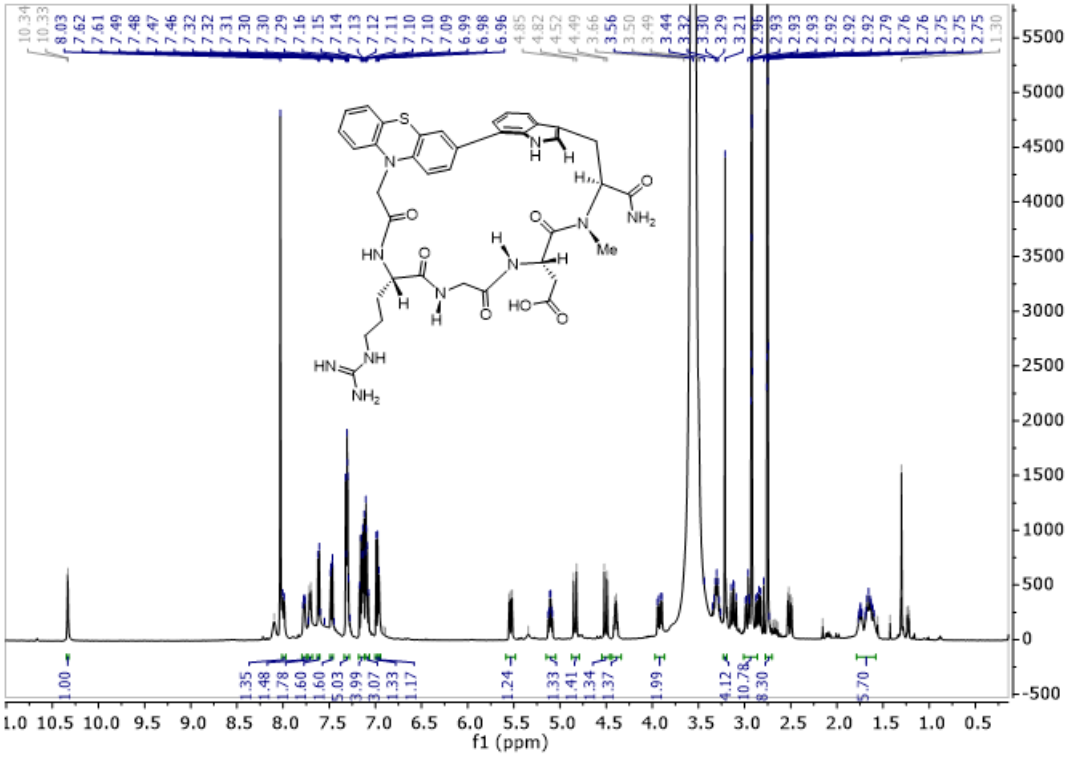

Figure S44. ^{1}H-NMR spectra of **12f** measured in DMF-d_7 at 500 MHz

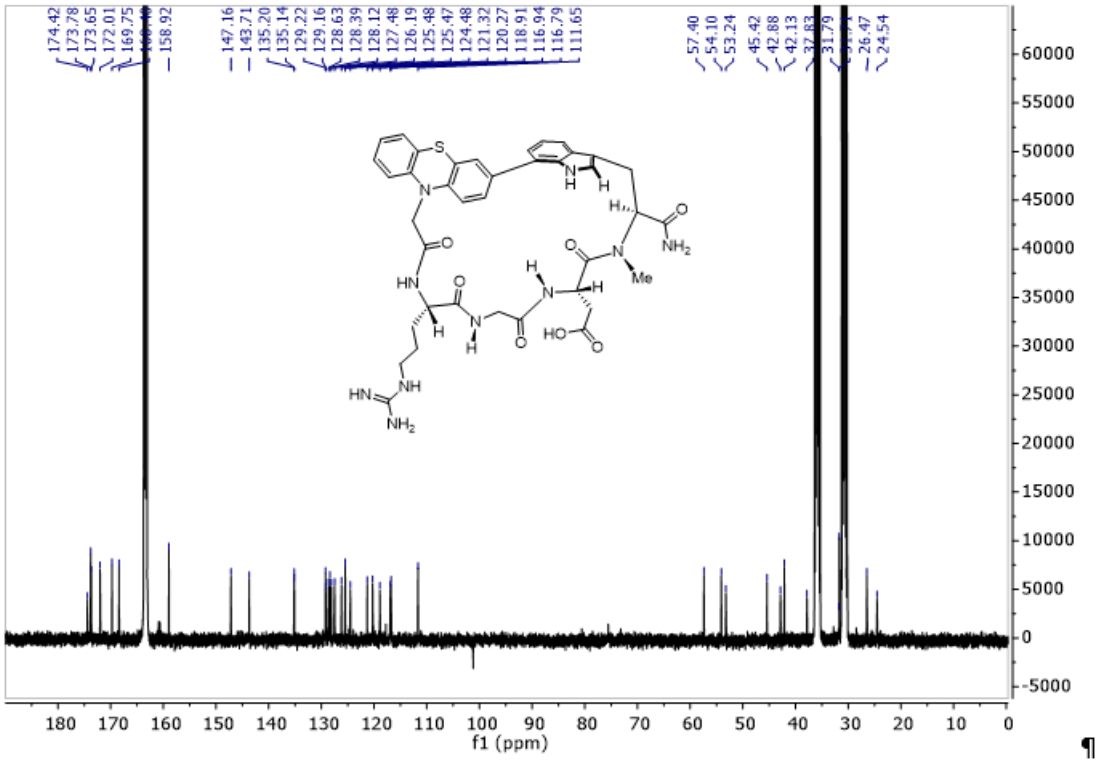

Figure S45. ^{13}C-NMR spectra of **12f** measured in DMF-d_7 at 126 MHz.

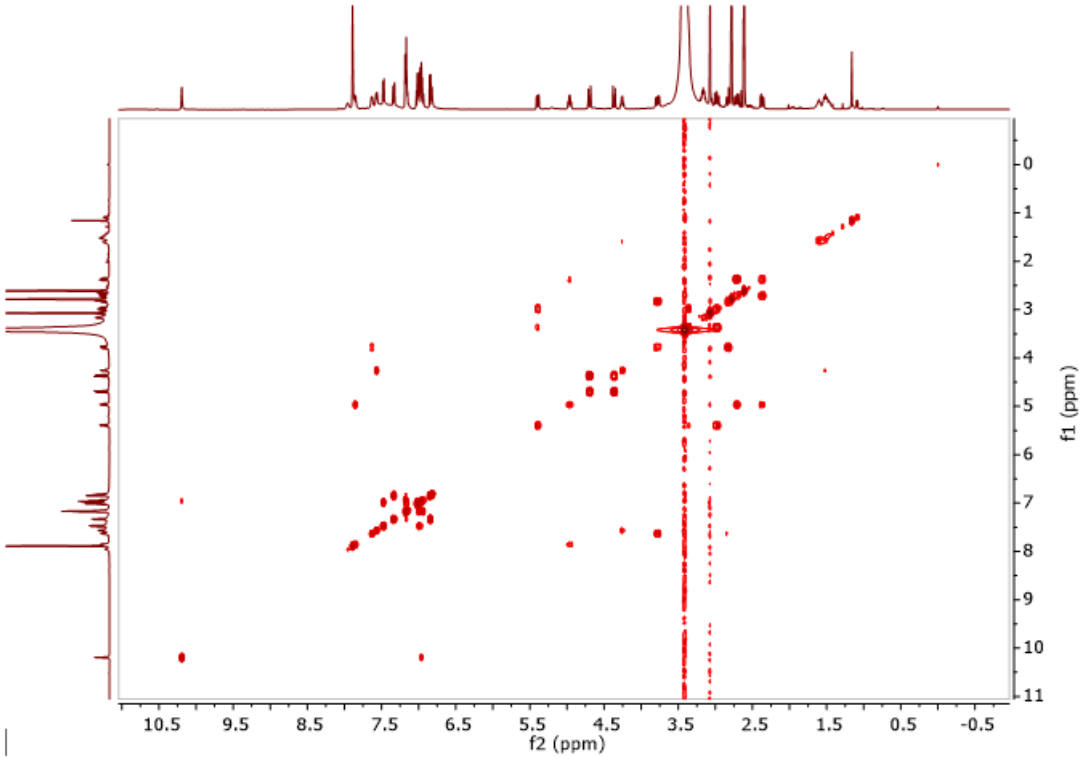

Figure S46. ^{1}H-^{1}H Cosy spectra of **12f** measured in DMF-d$_7$ at 500 MHz.

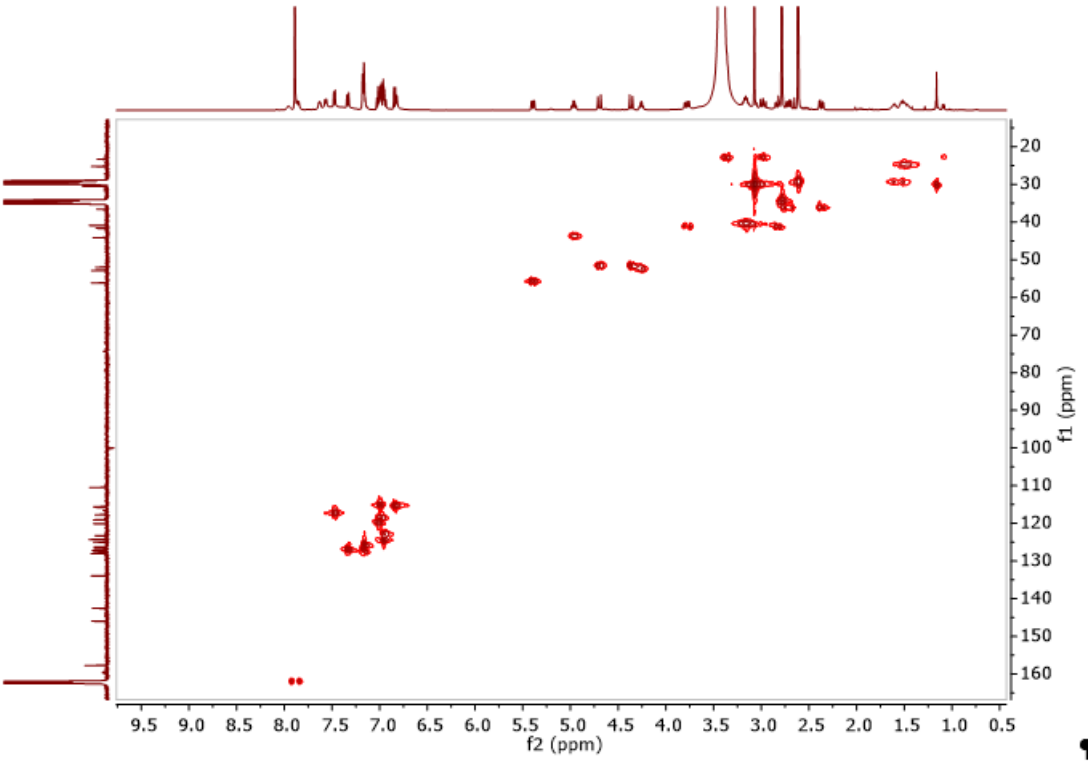

Figure S47. HMQC spectra of **12f** measured in DMF-d$_7$

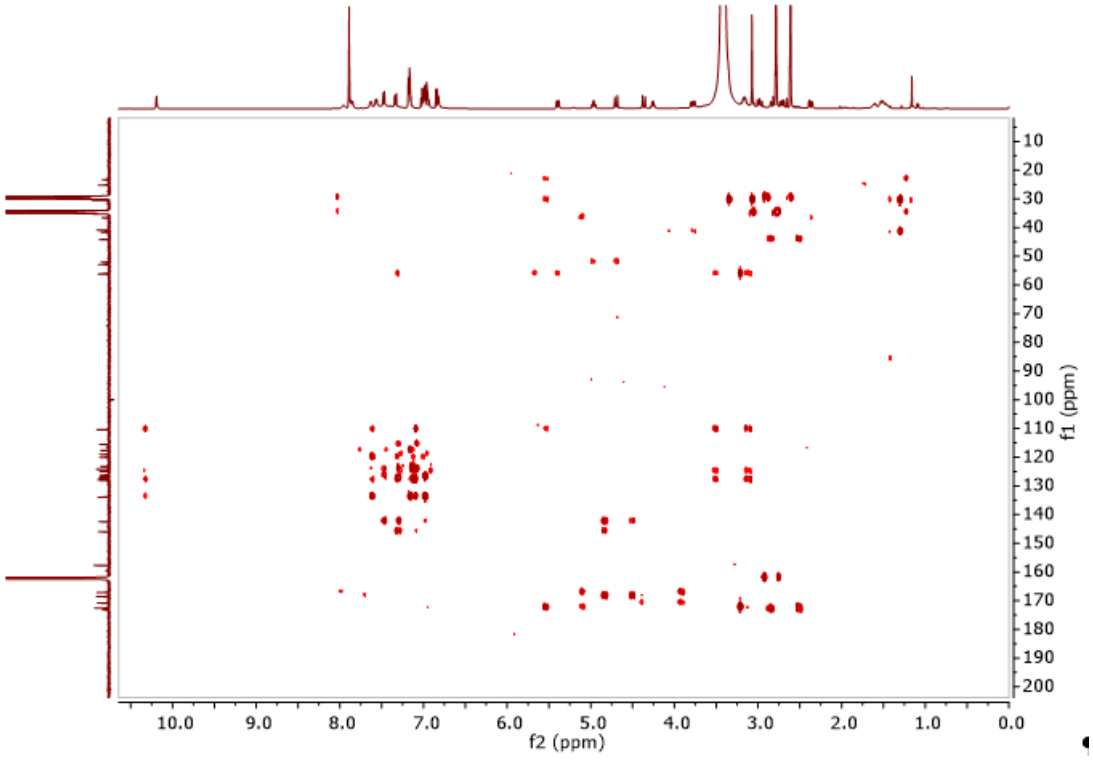

Figure S48. HMBC spectra of **12f** measured in DMF-d_7

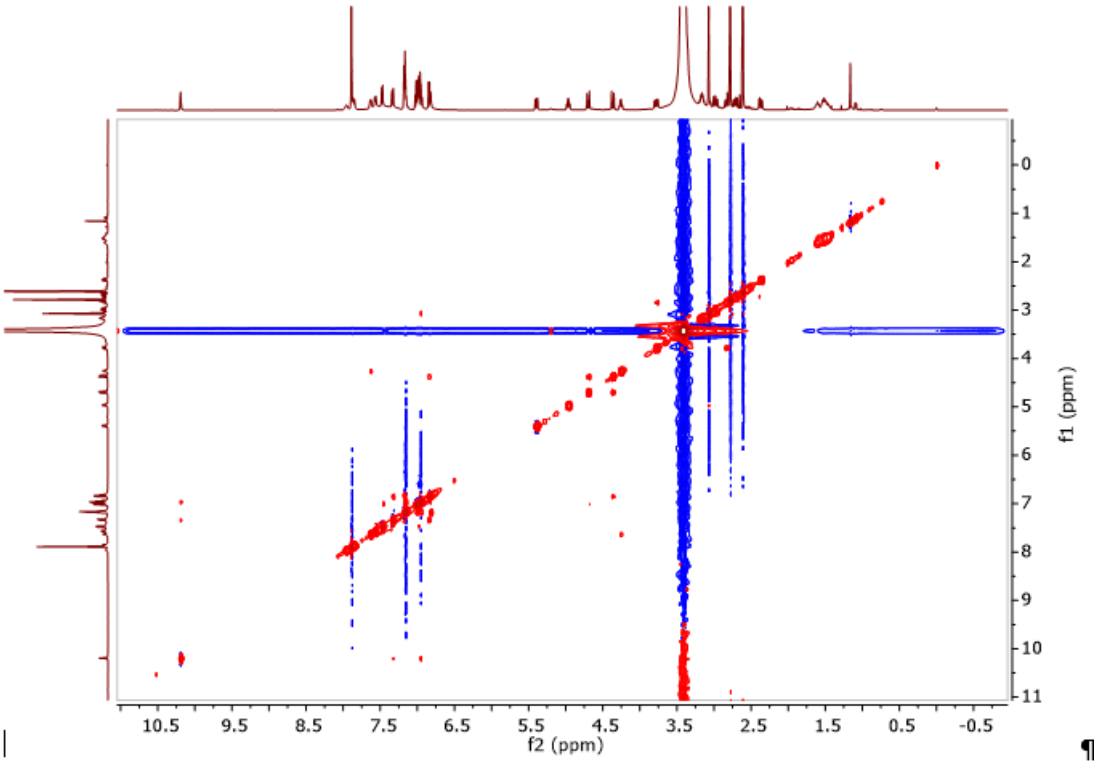

Figure S49. NOESY spectra of **12f** measured in DMF-d_7

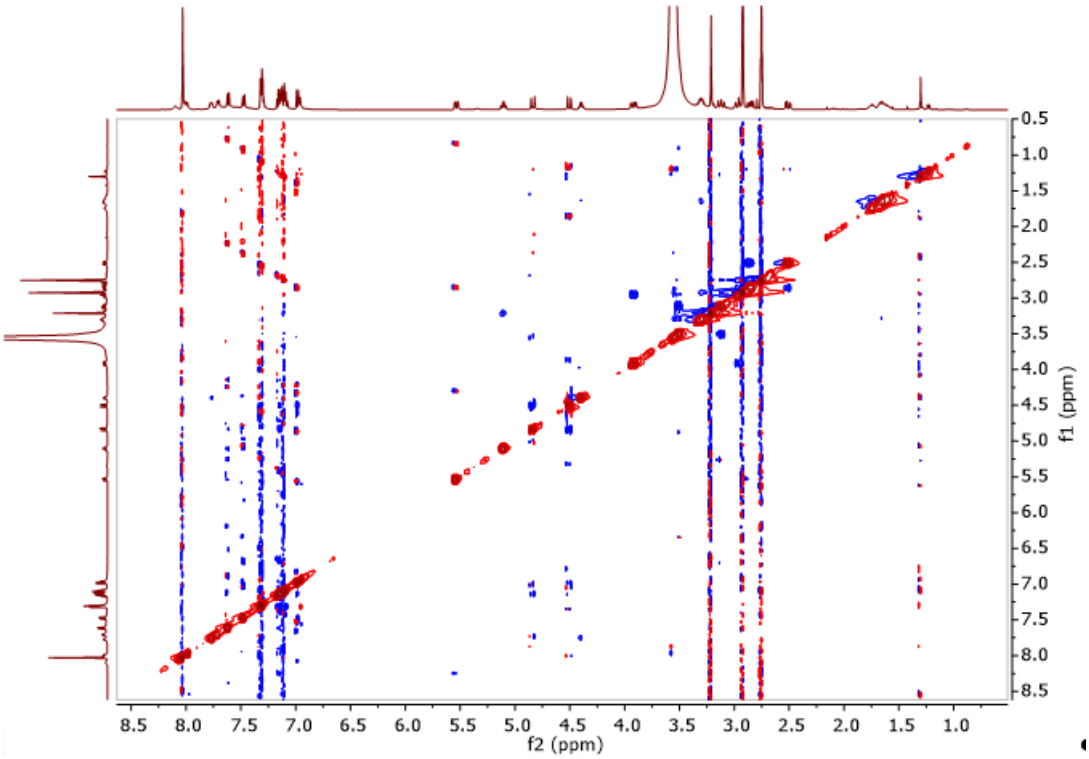

Figure S50 . ROESY spectra of **12f** measured in DMF-d_7

Integrin Binding Assay

To determine activity an ELISA-like assay using isolated integrins 26 and cell-adhesion assays with WM-115 cells was performed. As standard Cilengitide (*αvβ3*: 0.54 nM, *α5β1*: 15.4 nM) was used. All wells of flat-bottom 96-well Immuno Plates (BRAND) were coated overnight at 4 °C with 100 µL protein (*1*) in carbonate buffer (15 mM Na_2CO_3, 35 mM $NaHCO_3$, pH 9.6). Each well was then washed with PBS-T-buffer (phosphate-buffered saline/Tween20, 137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 2 mM KH_2PO_4, 0.01 % Tween20, pH 7.4; 3 x 200 µL) and blocked for 1 h at room temperature with TS-B-buffer (Tris-saline/BSA buffer, 150 µL/well, 20 mM Tris-HCl, 150 mM NaCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 1 mM $MnCl_2$, pH 7.5, 1 % BSA). Meanwhile, a dilution series of the compound and internal standard was prepared in an extra plate in 1:5 dilution steps using TS-B-buffer. After washing the assay plate with PBS-T (3×200 µL), 50 µl of the dilution series was transferred to each well from B–G. For a negative control well A was filled with 100 µl TS-B-solution and for a positive control well H was filled with 50 µl TS-B-solution. 50 µL of human integrin (*2*) in TS-B-buffer was transferred to wells H-B and incubated for 1h at r. t. After washing the plates (3×200 µL) with PBS-T buffer, 100 µL primary antibody (*3*) was added to all wells and incubated for 1 h at r.t. The plate was washed (3×200 µL) with PBS-T buffer and 100 µL of secondary antibody (*4*) was added to all wells and incubated for 1 h. at room temperature. The plate was washed (3×200 µL) with PBS-T buffer and 50 µL SeramunBlau (Seramun Diagnostic GmbH) was added to all wells. The development was stopped with 3 M H_2SO_4 (50 µL/well) when a blue color gradient from well A to H was visible (*αvβ3*: ~1 min, *α5β1*: < 1 min). The absorbance was measured with a plate reader at 450 nM (Tecan, Infinite M200). The resulting curves were analyzed with OriginPro 2017G with the inflection point describing the IC_{50} value. Each compound was tested in duplicates or, if enough available, twice in duplicates and referenced to the internal standard.

	αvβ3	α5β1
(*1*)	1.0 µg/mL human fibronectin, R&D	0.5 µg/mL human fibronectin, R&D
(*2*)	2.0 µg/mL, human αvβ3 integrin, R&D	2.0 µg/mL, human α5β1 integrin, R&D
(*3*)	2.0 µg/mL, mouse anti-human CD51/61, BD Bioscience	1.0 µg/mL, mouse anti-human CD49e, BD Bioscience
(*4*)	1.0 µg/mL, anti-mouse IgG-POD goat, Sigma Aldrich	2.0 µg/mL, anti-mouse IgG-POD goat, Sigma Aldrich

Docking experiments

αVβ3 integrin structure was acquired from protein data bank (PDB: 1l5g). the structure was modified by adding the missing hydrogens and the ligand (cilengitide) was removed. Structures of the different tested cyclic RGD pseudopeptide derivatives were built using Yasara structure with the correct stereochemistry. The cyclic RGD pseudopeptide derivatives were energy minimized using Yasara2 force field before using in docking experiments. The modified αVβ3 integrin structure was optimized by running MD simulation for up to 2ns, different snapshots were energy minimized using AMBER14 force field, 10Å for cut off and PME was used for long range electrostatic. The simulated snapshots were tested for the docking study compared to the cilengitide as reference, 200ps was selected for proceeding experiments.

Molecular dynamic simulation was done using Yasara structure; the modified αVβ3 integrin structure was used for simulation. Simulation cell was extended 10Å around the whole structure and filled with randomized oriented water molecule with density of 0.99 g/mL. AMBER14, 10 Å for cut off and PME was used for long range electrostatic as force field for running the simulation up to 2 ns. The root mean square deviation (RMSD) of the protein structure was monitored throughout the simulation time to assure the stability of the whole structure.

Docking was performed by Yasara structure using Autodock, simulation cell was defined around the binding domain of cilengitide at the interface between subunits αv and β3 of the optimized integrin structure for docking of RGD pseudopeptide derivatives. Docking results were analysed based on the B-factor (binding energy) calculated by Yasara structure and interaction with the amino acid residues of the target binding pocket. (Reference: Automated Docking Using a Lamarckian Genetic Algorithm and and Empirical Binding Free Energy Function. Morris GM, Goodsell DS, Halliday RS, Huey R, Hart WE, Belew RK and Olson AJ (1998), J. Comput. Chem. 19, 1639-1662)

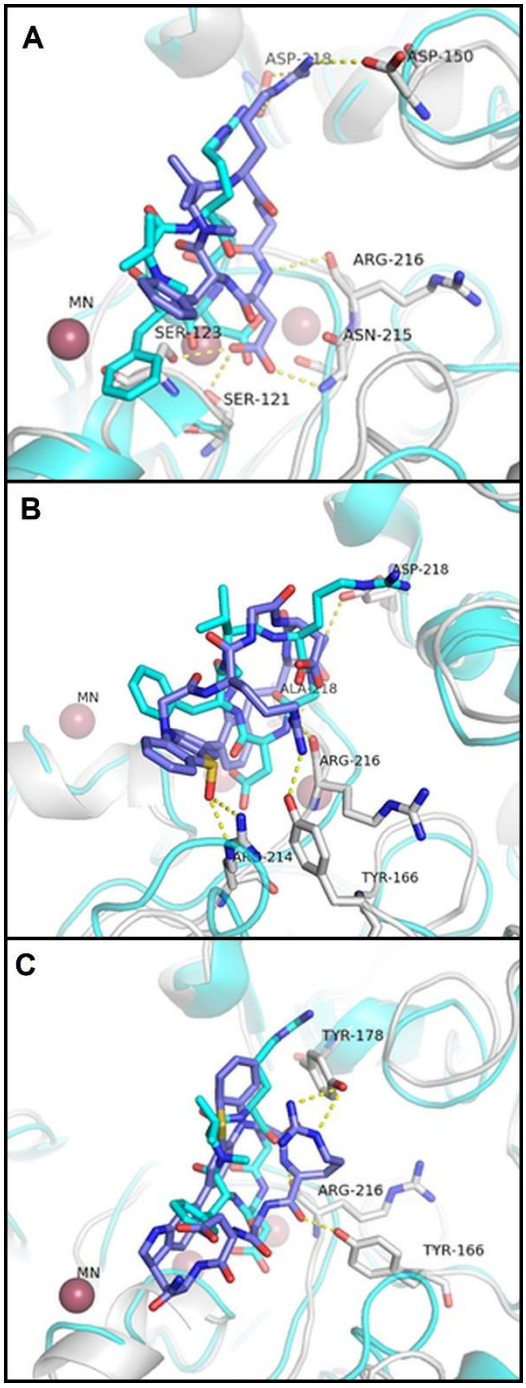

Fig S51. Superimposition of Docking and X-ray Cilengitide:integrin complex and compounds 12h and 12c. The subunits are represented as follow: α- integrin (pink) and β-integrin (light-blue). All the inhibitors interact in the RGD-binding pocket at the interface between α-and β-subunits. (A) Cilengitide:integrin complex (blue and grey, PDB: 1l5g) with docked Cilengitide_integrin complex (cyan), showing that Cilengitide in the docking experiment has similar binding interactions and orientation as in the X-ray structure. B), R-12h (blue) and Cilengitide (cyan). C) 12c (blue) and Cilengitide (cyan) (D), and αvβ3 integrin.

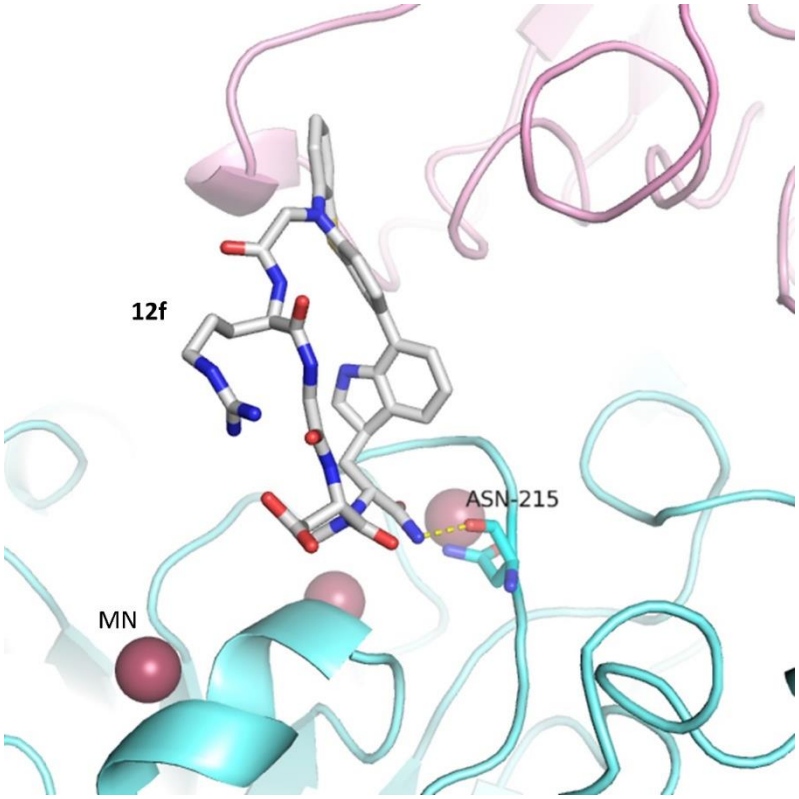

Fig S52. Model for the interaction between **12f**, and $\alpha_v\beta_3$ integrin. The subunits are represented as follow: α-integrin (pink) and β-integrin (light-blue).

Table S1. Comparison of binding affinity/energy of the cyclic RGD-peptides to integrin $\alpha_V\beta_3$ obtained by Yasara.

Cyclopeptide	B. Aff[b]($\alpha_V\beta_3$) [kJ/mol]
c(RGDW(7-3Ptz[1])) **(12c)**	17.16
c(RGD(NMe)W(7-3Ptz[1])) **(12f)**	21.68
c(RGDW(7-3Ptz[1](O))) (**R-12h**)	31.81
c(RGDf(NMe)V) EMD (Cilengitide)	33.53