Electronic Supplementary Material (ESI) for Dalton Transactions.

Synthesis and DNA interaction studies of Ru(II) cell penetrating peptide (CPP) bioconjugates

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Supplementary Information

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Instrument Details

All NMR-spectra were measured at 300 K on a Bruker DPX 200 NMR-spectrometer (^{1}H-NMR*:* 200.13 MHz, ^{13}C-NMR: 50.33 MHz), a Bruker AV III 300 (^{1}H-NMR*:* 300.13 MHz, ^{13}C-NMR: 75.48 MHz), a Bruker DRX 400 NMR-spectrometer (^{1}H-NMR*:* 400.13 MHz, ^{13}C-NMR: 100.62 MHz) or a Bruker AV III 700 NMR-spectrometer (^{1}H-NMR*:* 700.07 MHz, ^{13}C-NMR: 176.05 MHz). Deuterated solvents were purchased from Euriso Top or Deutero and used as received. The chemical shifts were reported in ppm and referenced to the solvent residual peak.

Elementary analysis was performed using an elementar varioMICRO Cube with approximately 2 mg carbon content of each sample. MALDI mass spectra were measured using a Bruker Ultraflex III TOF/TOF mass spectrometer with a ground steel target plate and α-Cyano-4-hydroxycinnamic acid (HCCA) as matrix. ESI mass spectra were recorded on a Bruker Esquire 6000 mass spectrometer in E^{+}-Mode. HRMS-ESI mass spectra were performed using a Waters SYNAPT G2-Si High Definition Mass Spectrometer in ESI^{+} mode. UV/Vis absorption spectra were measured using a Jasco V-770 Spectrophotometer with a double beam setup. Fluorescence spectra were measured using a Jasco FP-8300 Spectrofluorometer with a single excitation wavelength. All solvents were used in spectroscopy grade. Circular Dichroism Spectroscopy measurements were performed on a Jasco J-815 at 25 °C.

HPLC was performed using a Knauer Smartline setup with a four wavelengths detector and a dynamic mixing chamber with reversed phase chromatography columns (*analytical*: Macherey-Nagel Nucleodur C18 Pyramid (5 µm; 125 x 4.6 mm), 1 mL/min) *(semi-preparative*: Macherey-Nagel Nucleodur 100-5 $C18_{ec}$ (5 µm; 125 x 10 mm), 5 mL/min). As eluents MiliQ-water with 0.1% TFA (solvent A) and acetonitrile with 0.1% TFA (solvent B) were used. For the analytical measurements, a linear gradient of solvent B (95% in 20 min) from 95% solvent A with a total run length of 40 min was performed. For the semi-preparative HPLC purifications, the gradient was individually adjusted to achieve an optimal separation for every compound.

Supplementary DNA intercalation studies

Compound 2a

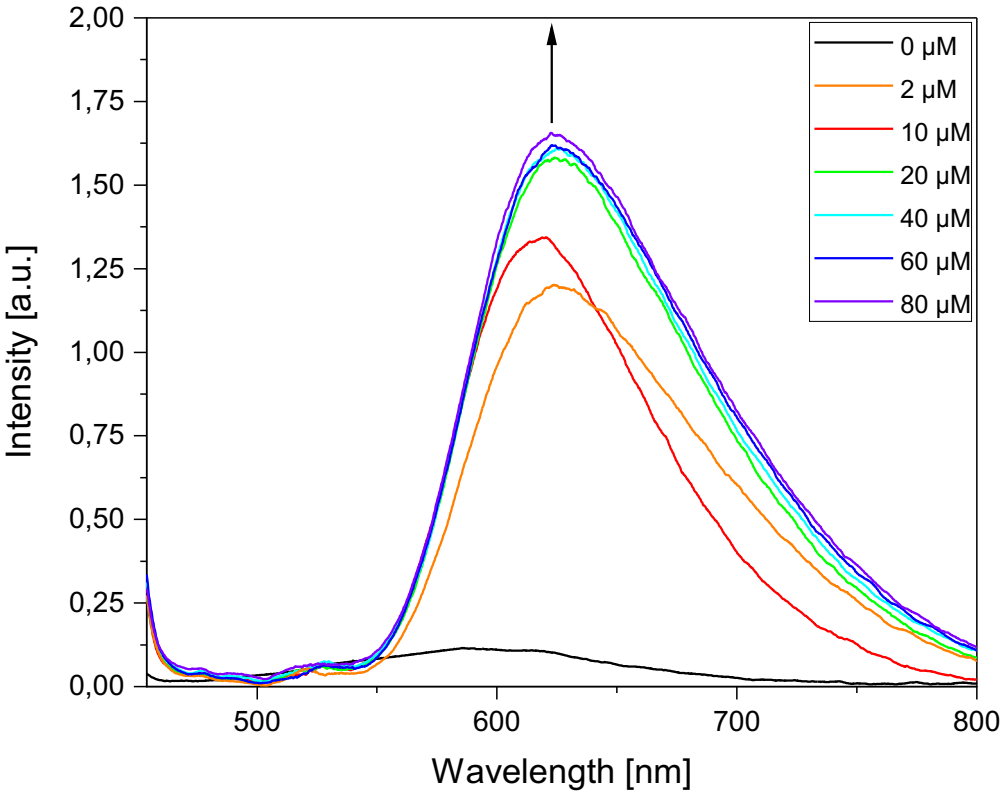


Figure S1: Emission spectra of **2a** in PBS at 25 °C upon addition of increasing concentrations of CT-DNA (excitation at 444 nm).

Supplementary DNA intercalation studies – CD Spectroscopy

Compound 2b

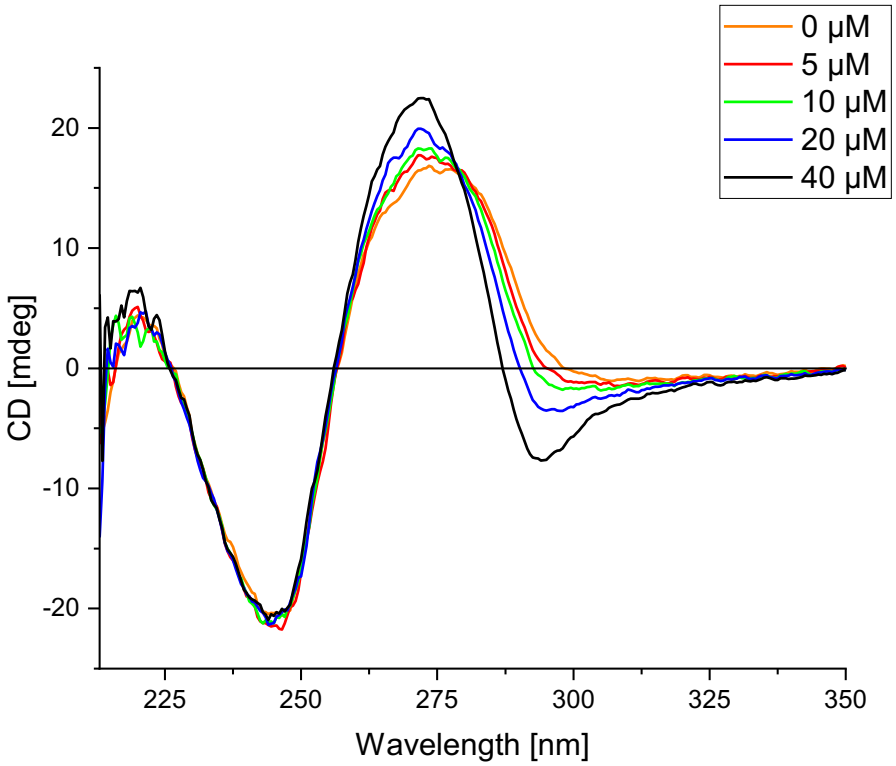


Figure S2: CD spectra 100 µM CT-DNA in PBS (with 1% EDTA) at 25 °C upon addition of increasing concentrations of **2b**.

Peptide 4

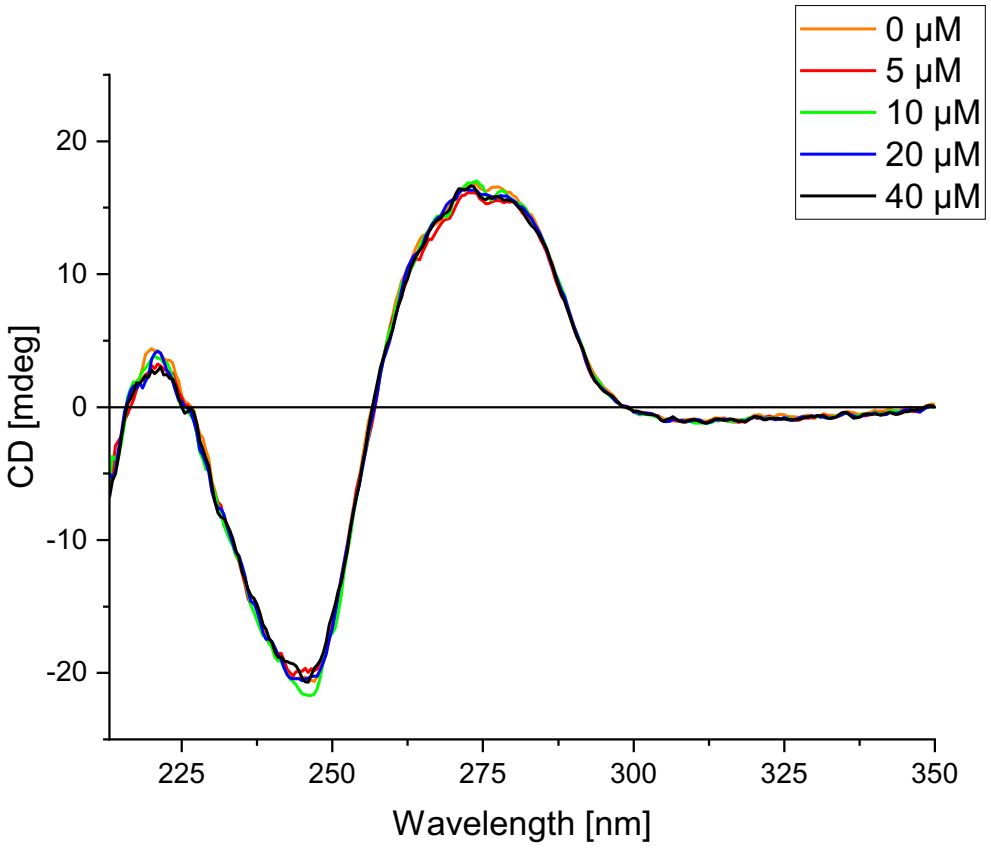


Figure S3: CD spectra 100 µM CT-DNA in PBS (with 1% EDTA) at 25 °C upon addition of increasing concentrations of **4**.

Compound 6b

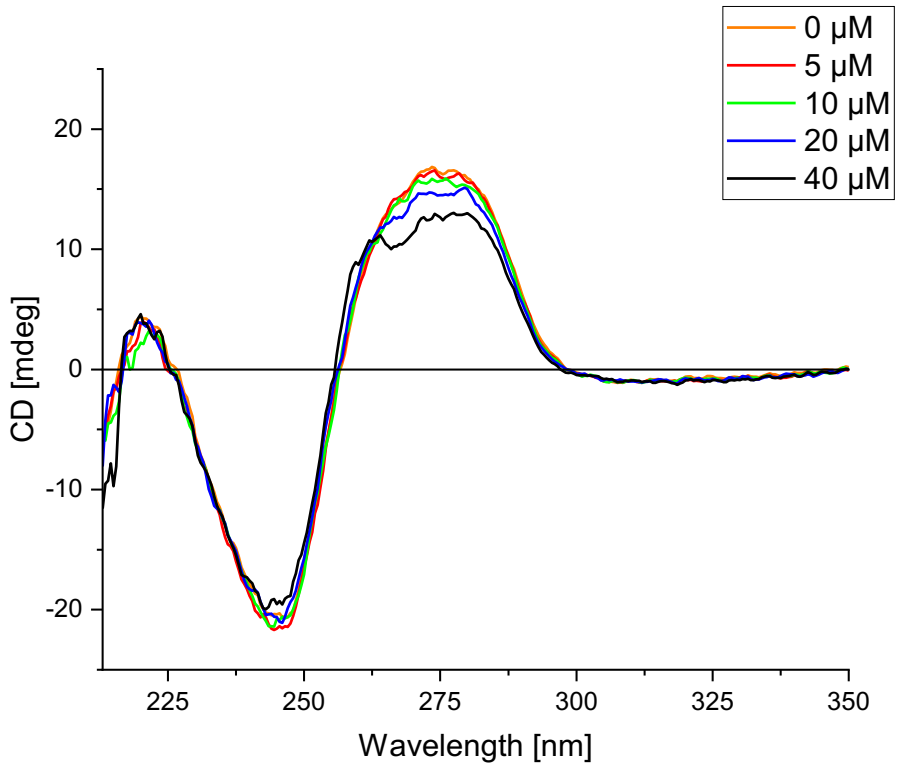


Figure S4: CD spectra 100 µM CT-DNA in PBS (with 1% EDTA) at 25 °C upon addition of increasing concentrations of **6b**.

Compound 7b

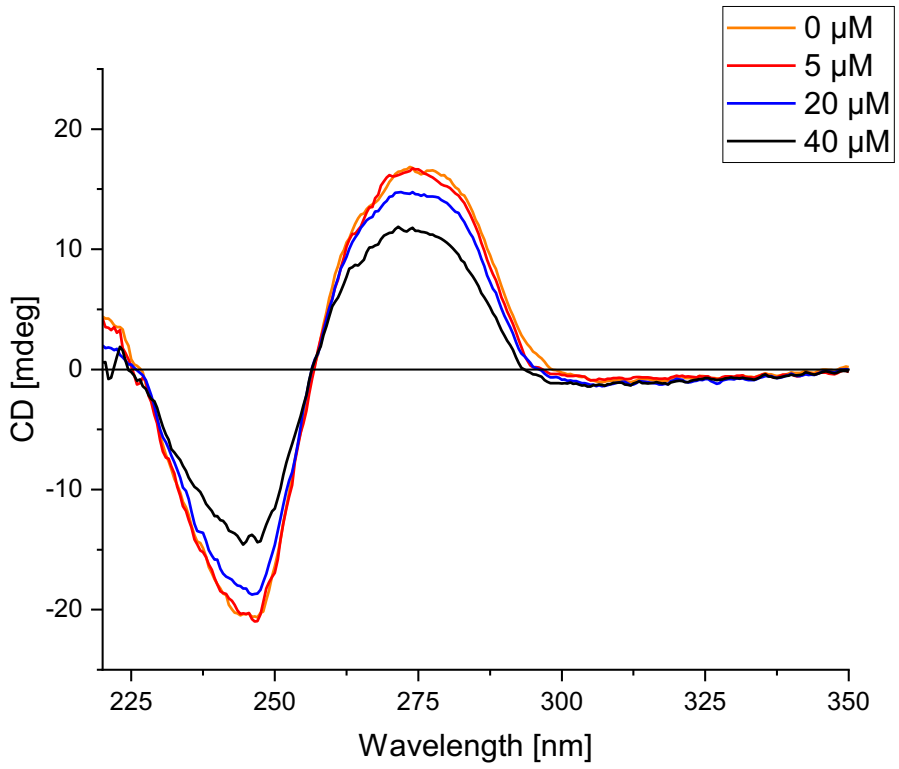


Figure S5: CD spectra 100 µM CT-DNA in PBS (with 1% EDTA) at 25 °C upon addition of increasing concentrations of **7b**.

Supplementary fluorescence microscopy images

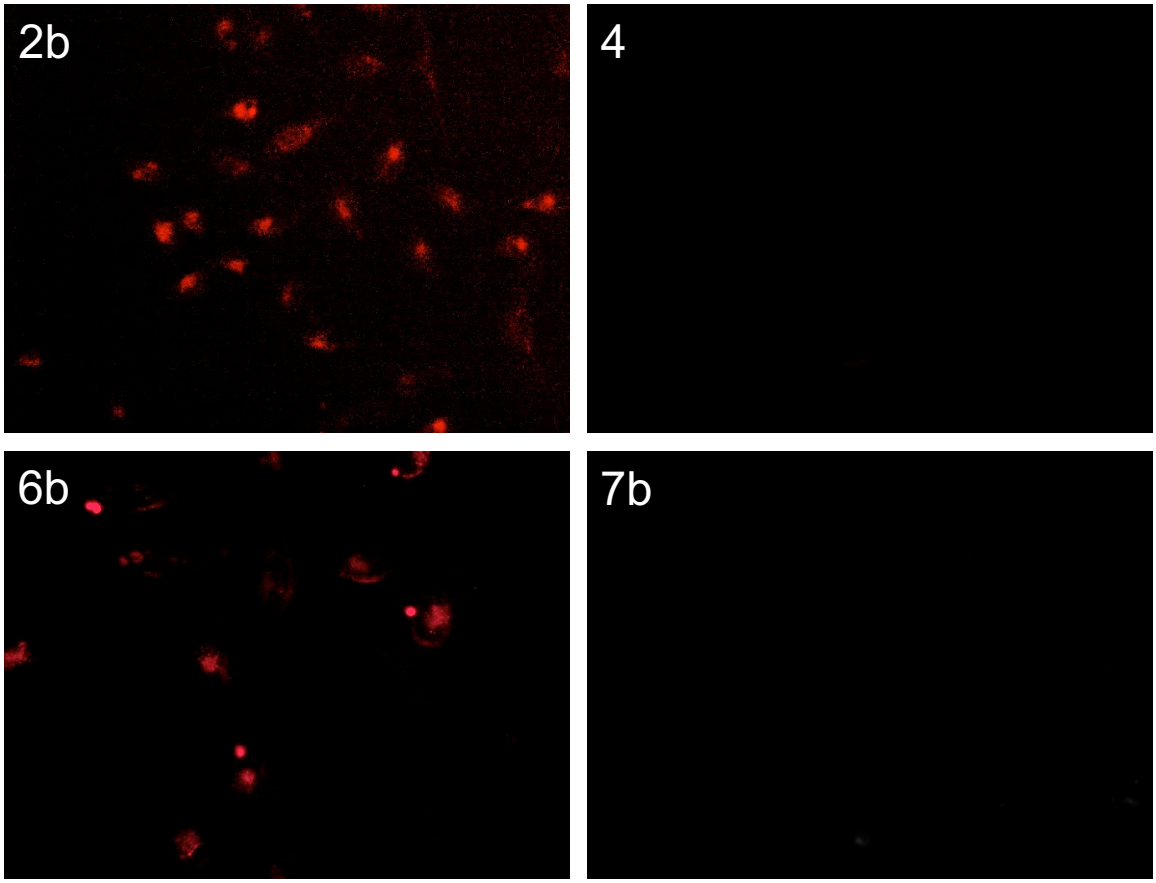


Figure S6: Fluorescence microscopy images of HeLa cell incubated with **4**, **2b, 6b** and **7b** (50 µM, 4 h, 400 x, Ru-Filter) at 37 °C.

Characterization of peptides and ruthenium(II) CPP bioconjugates

Peptide 4

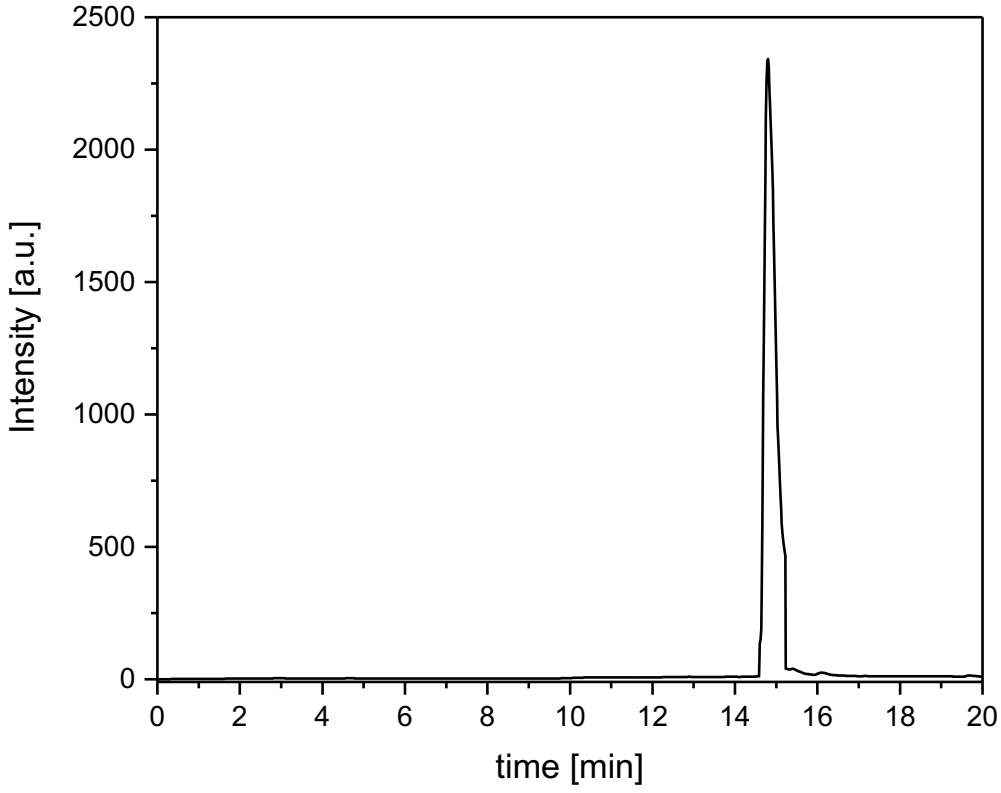


Figure S7: HPLC chromatogram of compound **4**, C_{18}, MeCN / H_2O (0.1% TFA).

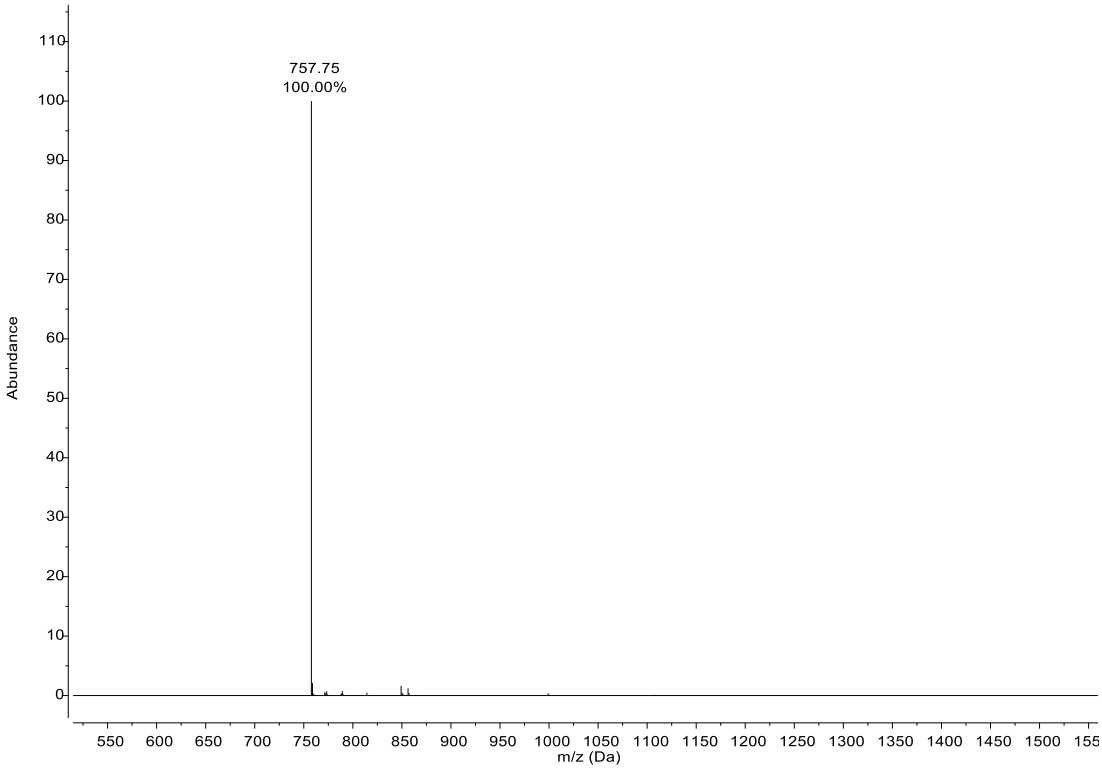


Figure S8: ESI-MS of compound **4**, ESI+-mode.

Peptide 5

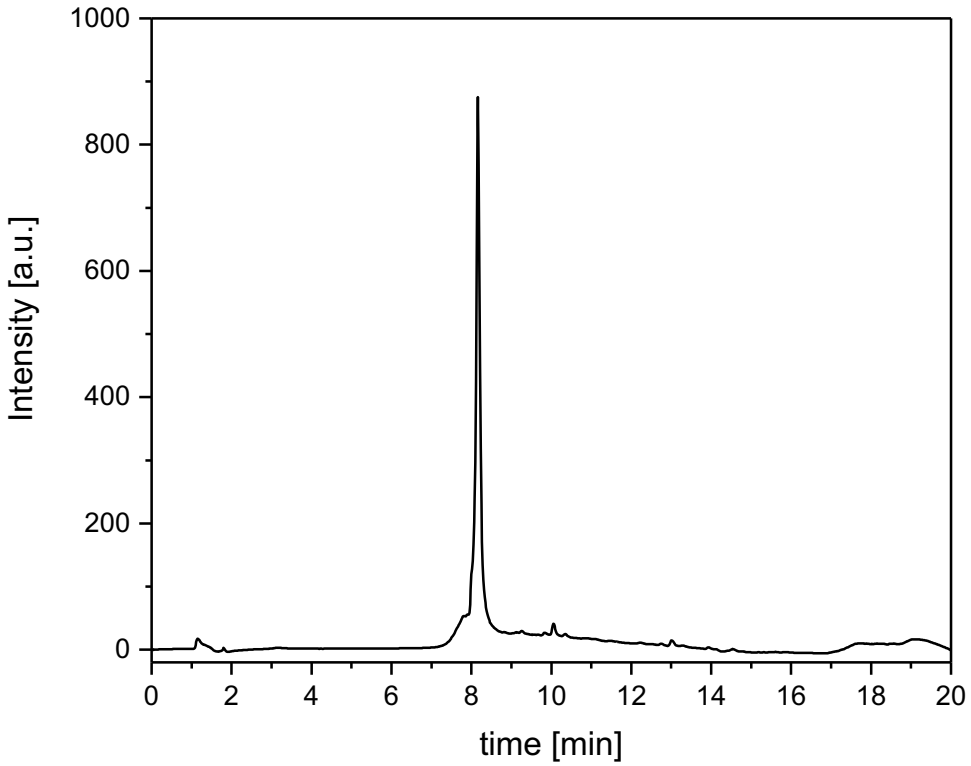


Figure S9: HPLC chromatogram of compound **5**, C_{18}, MeCN / H_2O (0.1% TFA).

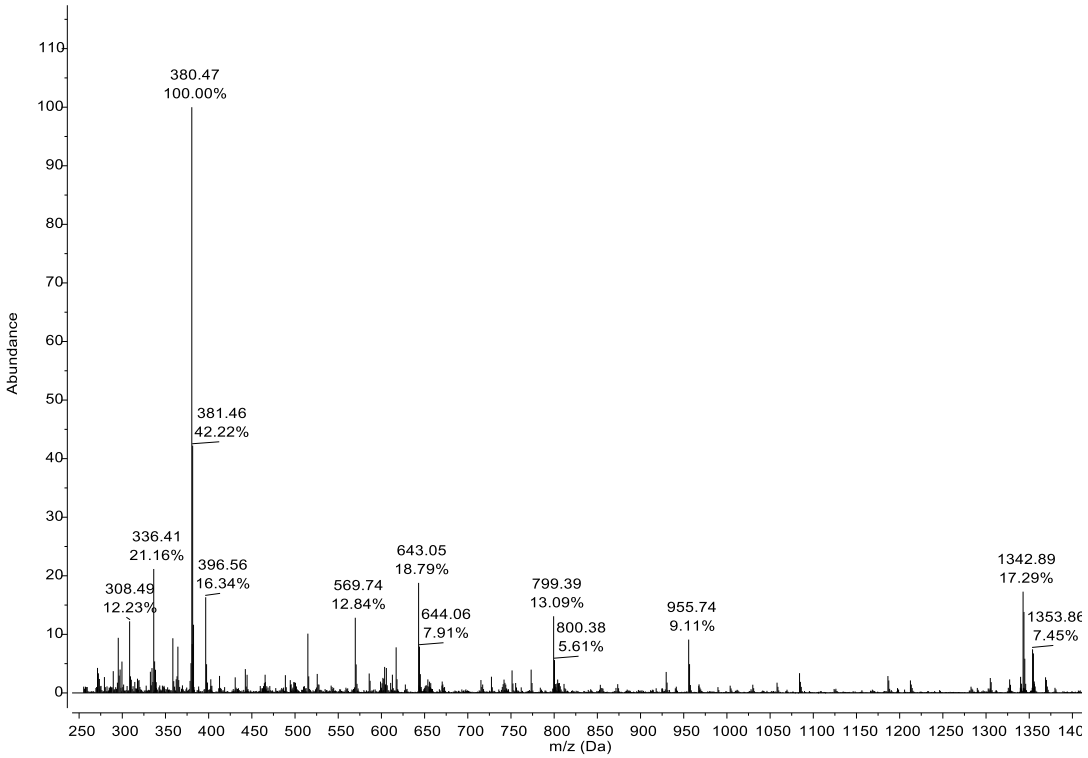


Figure S10: MALDI-MS of compound **5**.

Compound 6a

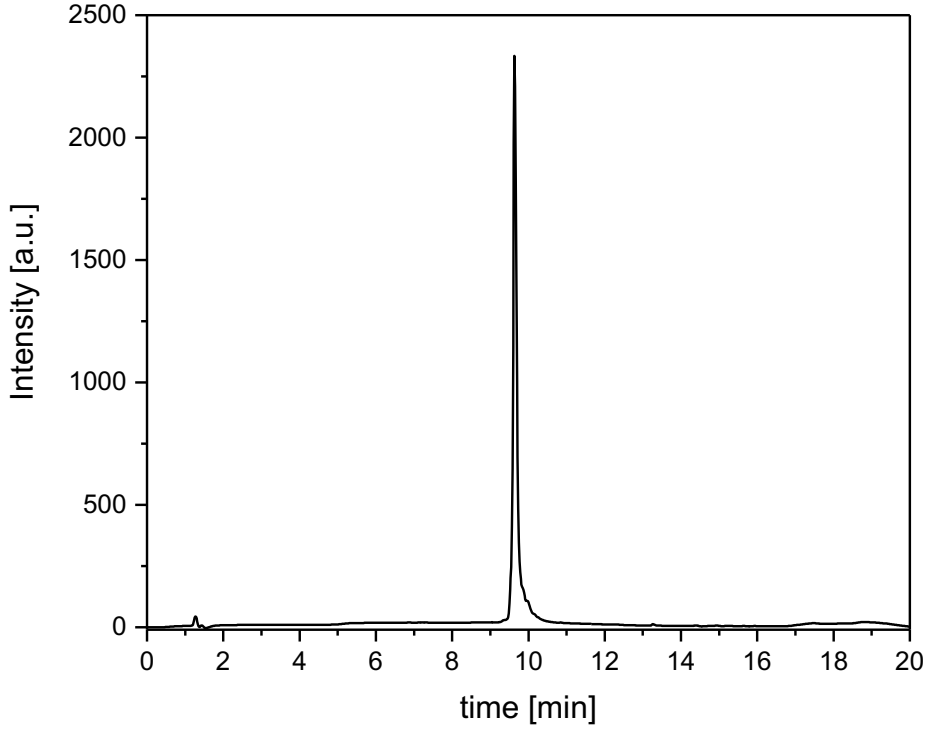


Figure S11: HPLC chromatogram of compound **6a**, C_{18}, MeCN / H_2O (0.1% TFA).

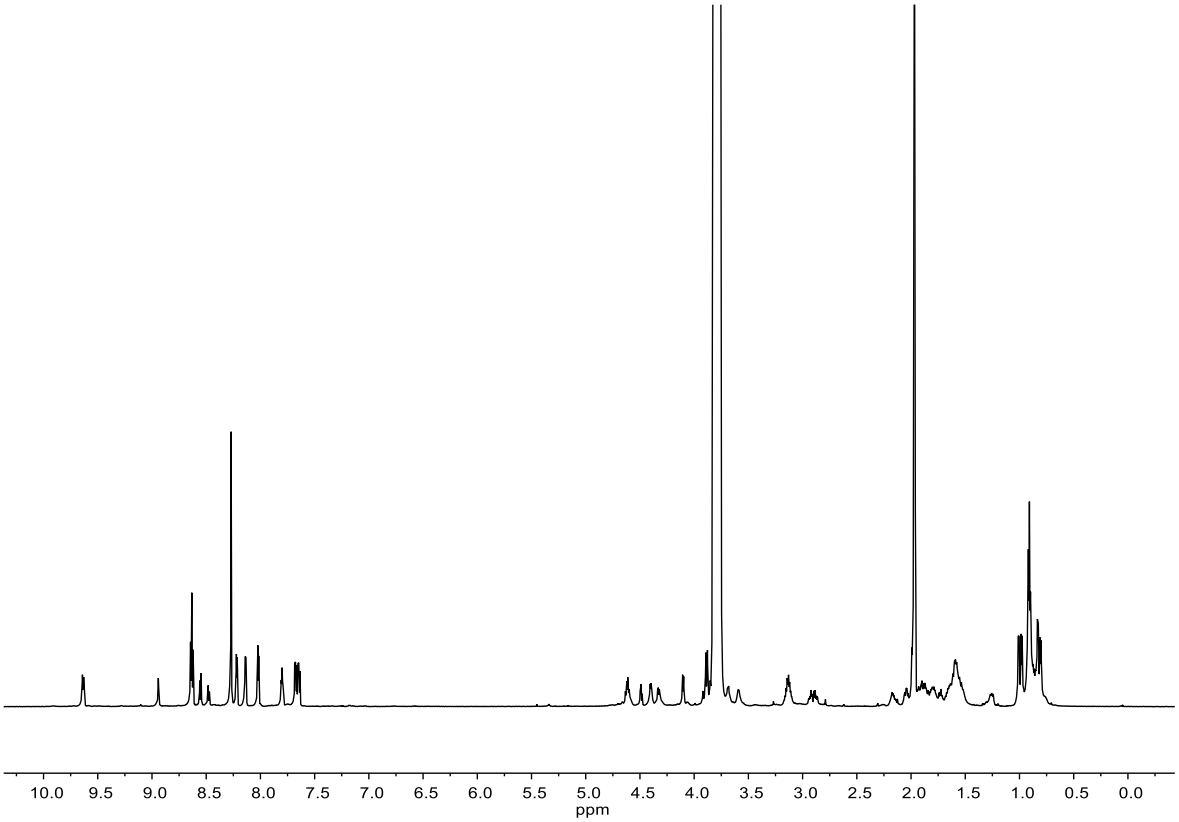


Figure S12: ^{1}H-NMR of compound **6a**, 700 MHz, Acetonitrile-*d3.*

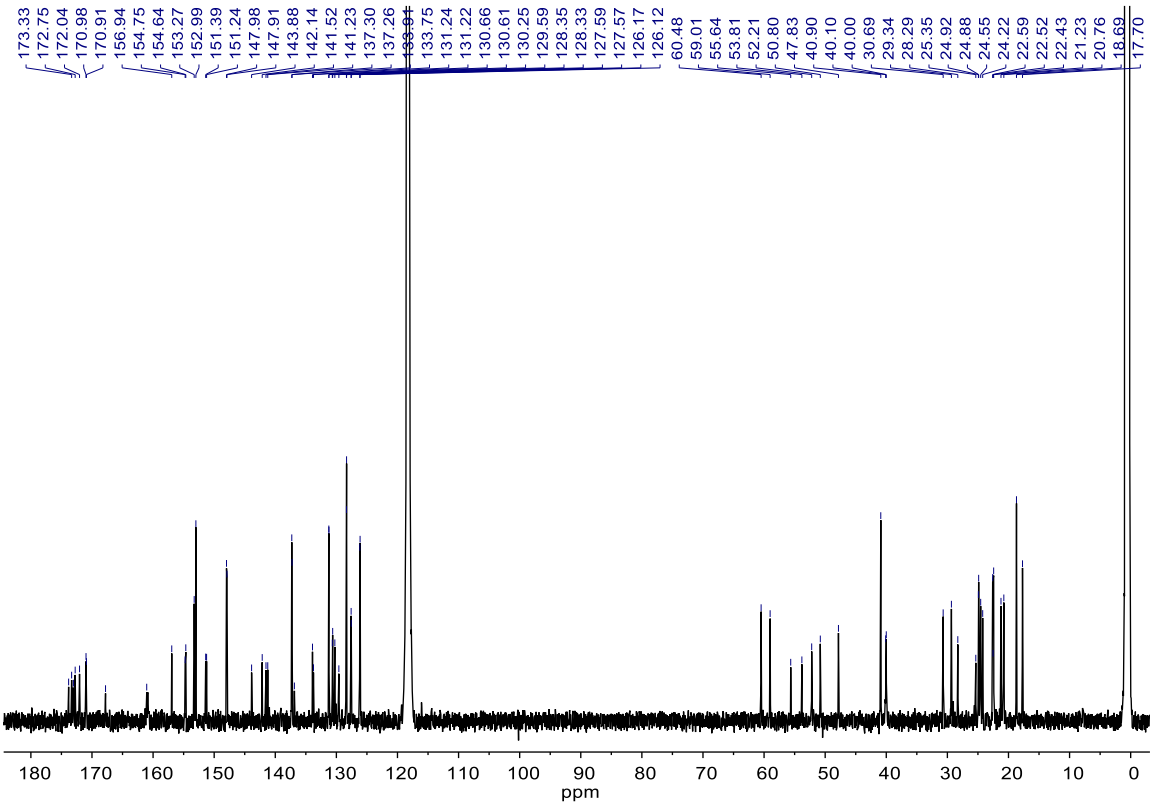


Figure S13: ^{13}C-NMR of compound **6a**, 176 MHz, Acetonitrile-*d3.*

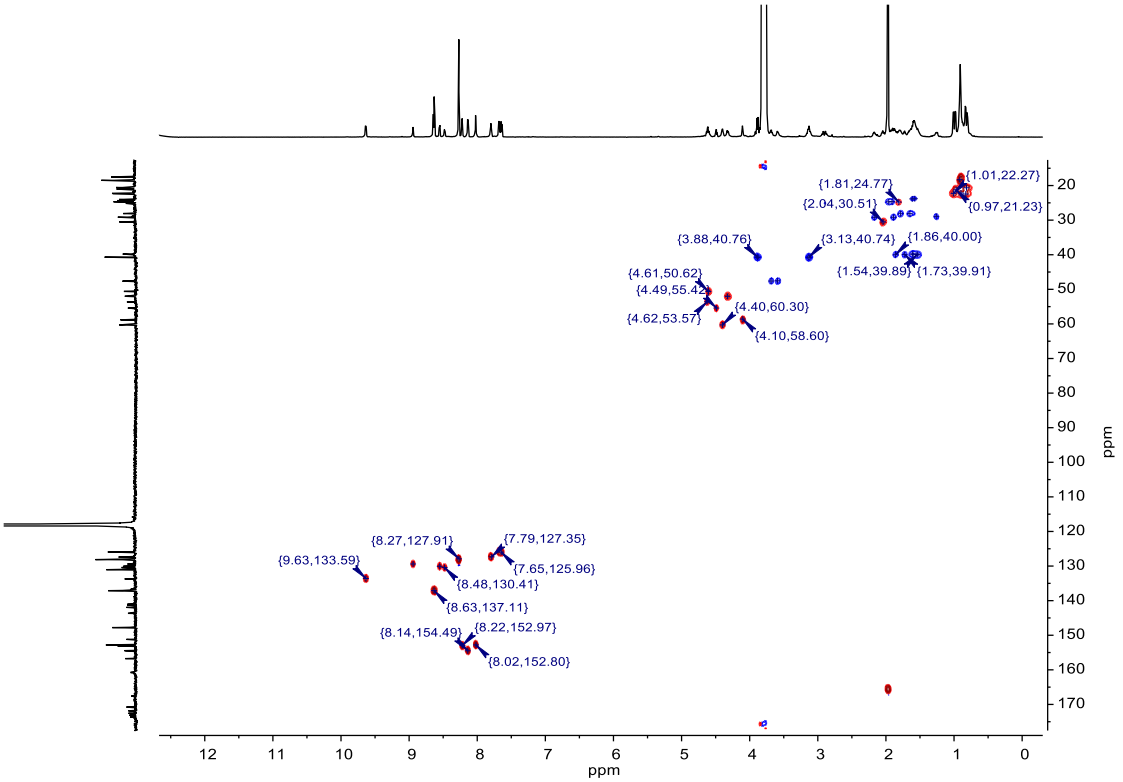


Figure S14: ^{1}H-^{13}C HSQC of compound **6a**, 176 MHz (^{13}C) and 700 MHz (^{1}H), Acetonitrile-*d3*

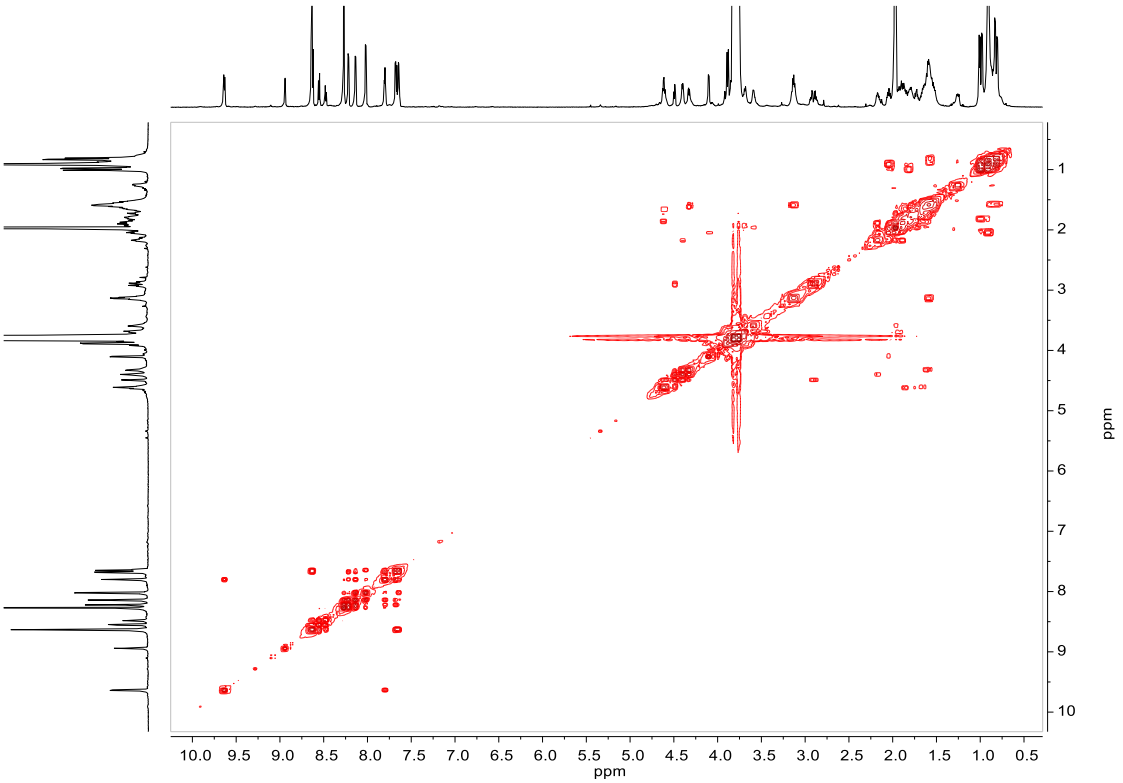


Figure S15: ^{1}H-^{1}H-COSY of compound **6a**, 700 MHz (^{1}H, ^{1}H), Acetonitrile-*d3.*

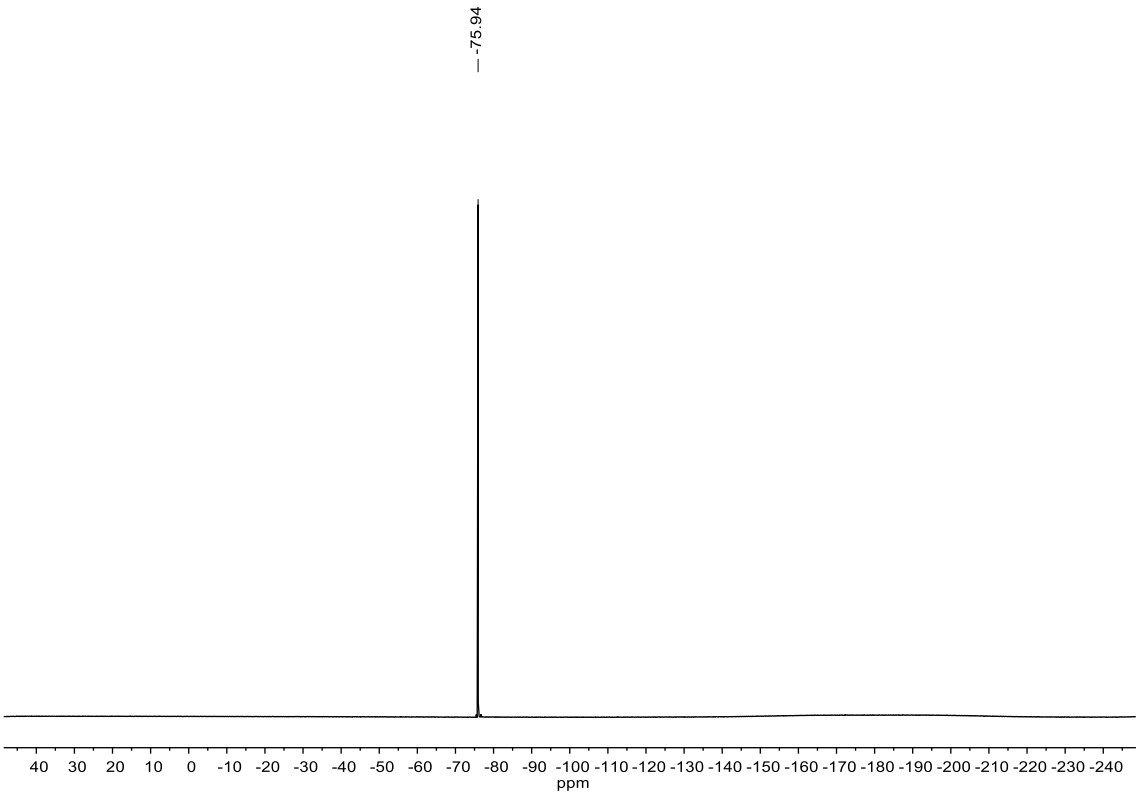


Figure S16: ^{19}F-NMR of compound **6a**, 250 MHz, Acetonitrile-*d3.*

Compound 6b

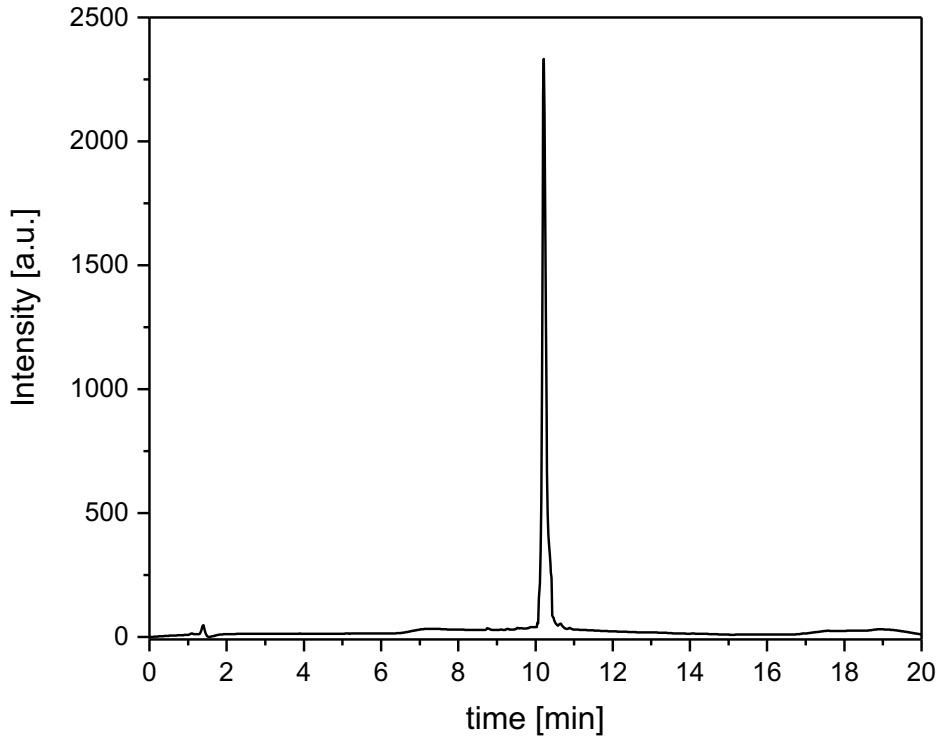


Figure S17: HPLC chromatogram of compound **6b**, C_{18}, MeCN / H_2O (0.1% TFA).

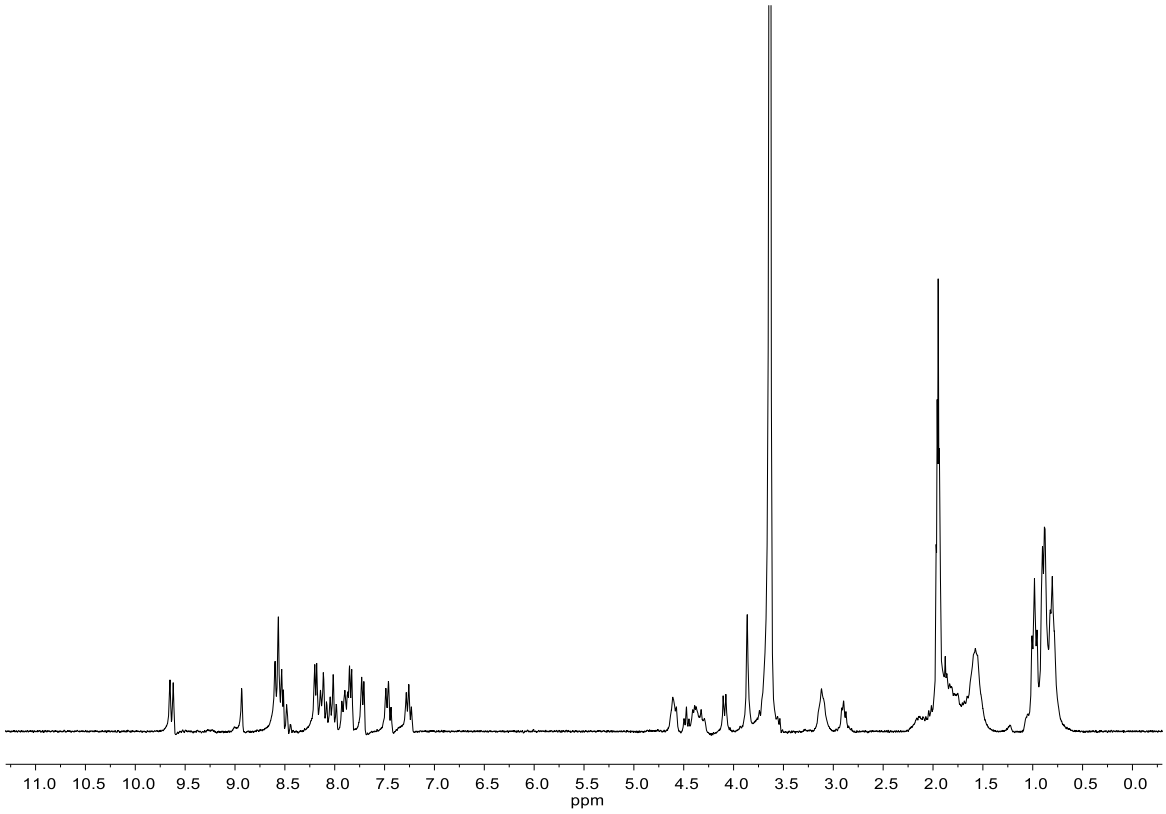


Figure S18: ^{1}H-NMR of compound **6b**, 700 MHz, Acetonitrile-*d3.*

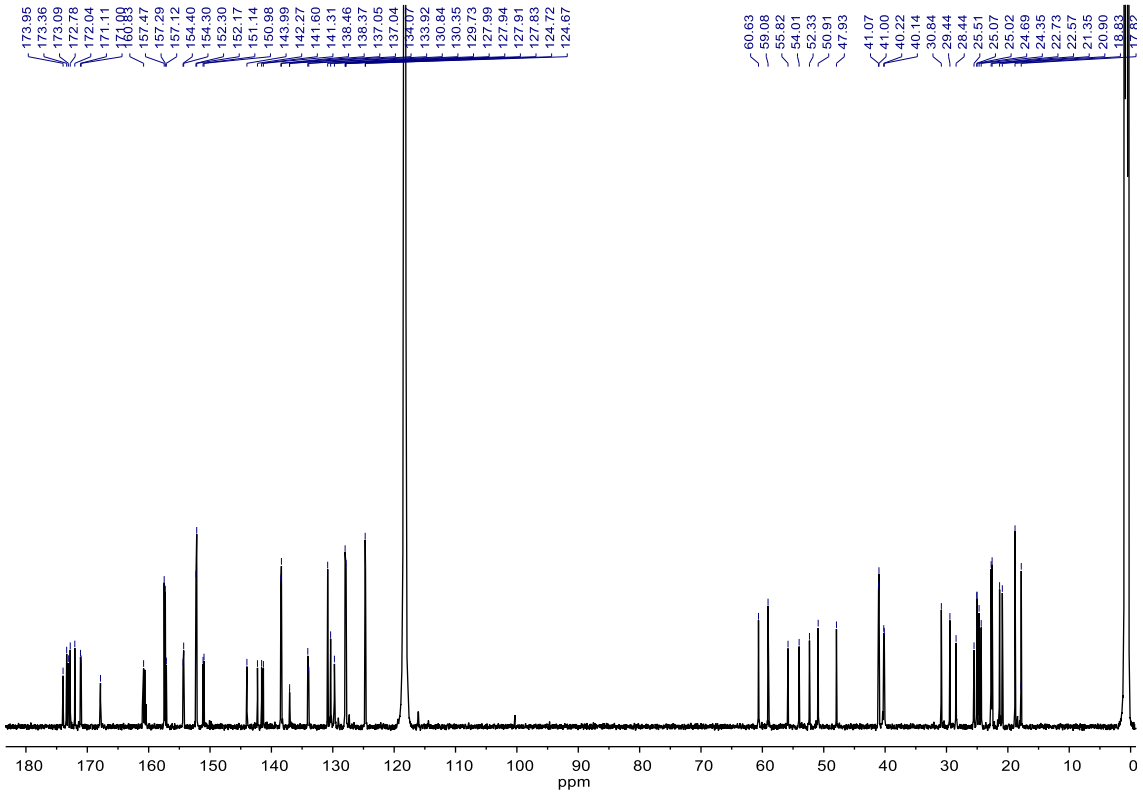


Figure S19: ^{13}C-NMR of compound **6b**, 176 MHz, Acetonitrile-*d3.*

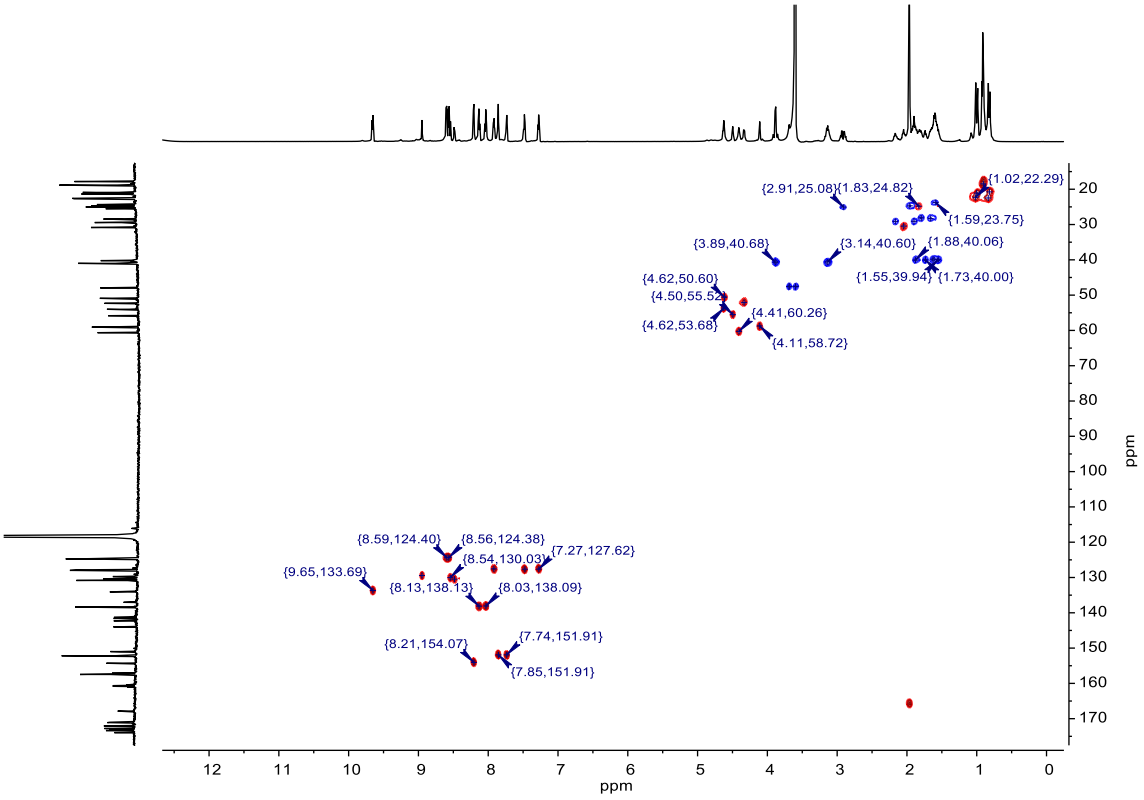


Figure S20: ^{1}H-^{13}C HSQC of compound **6b**, 176 MHz (^{13}C) and 700 MHz (^{1}H), Acetonitrile-*d3.*

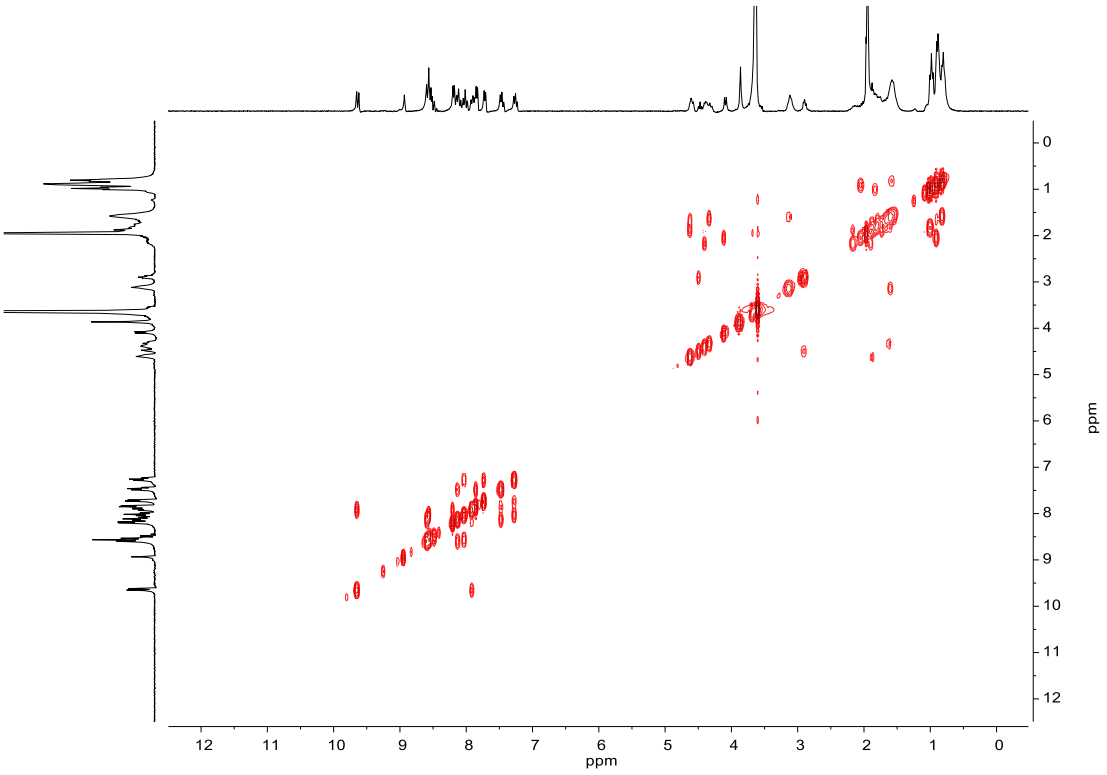


Figure S21: ^{1}H-^{1}H-COSY of compound **6b**, 700 MHz (^{1}H, ^{1}H), Acetonitrile-*d3.*

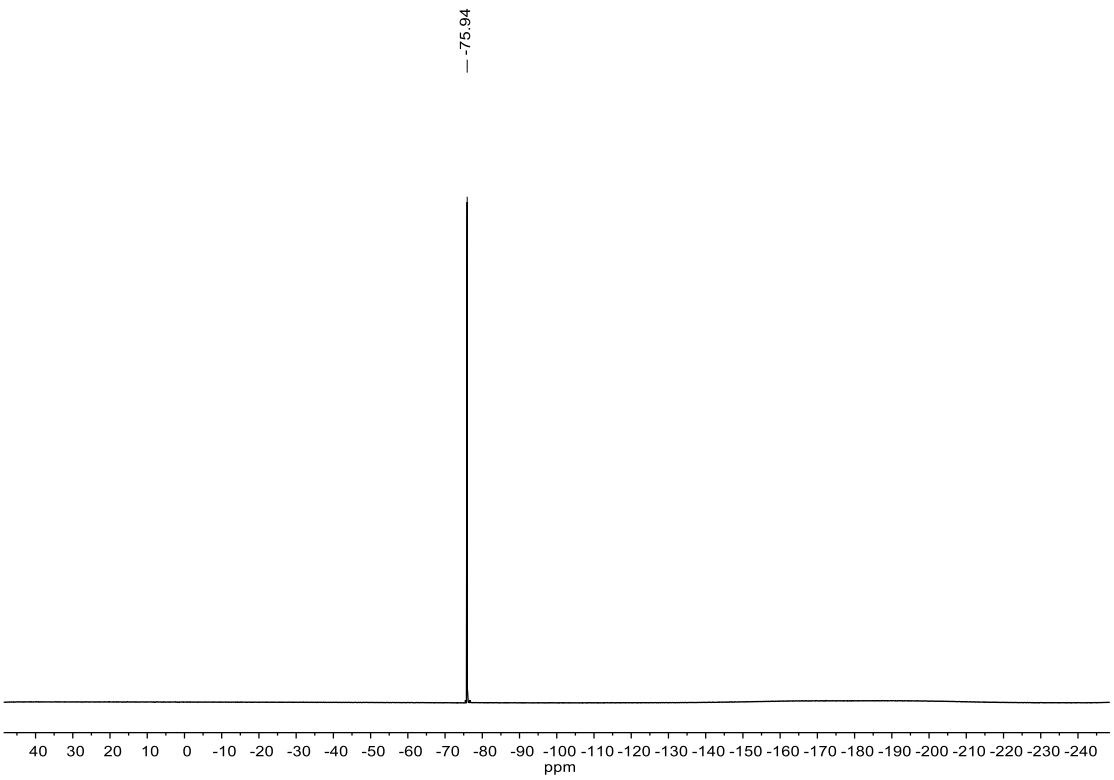


Figure S22: ^{19}F-NMR of compound **6b**, 250 MHz, Acetonitrile-*d3.*

Compound 7b

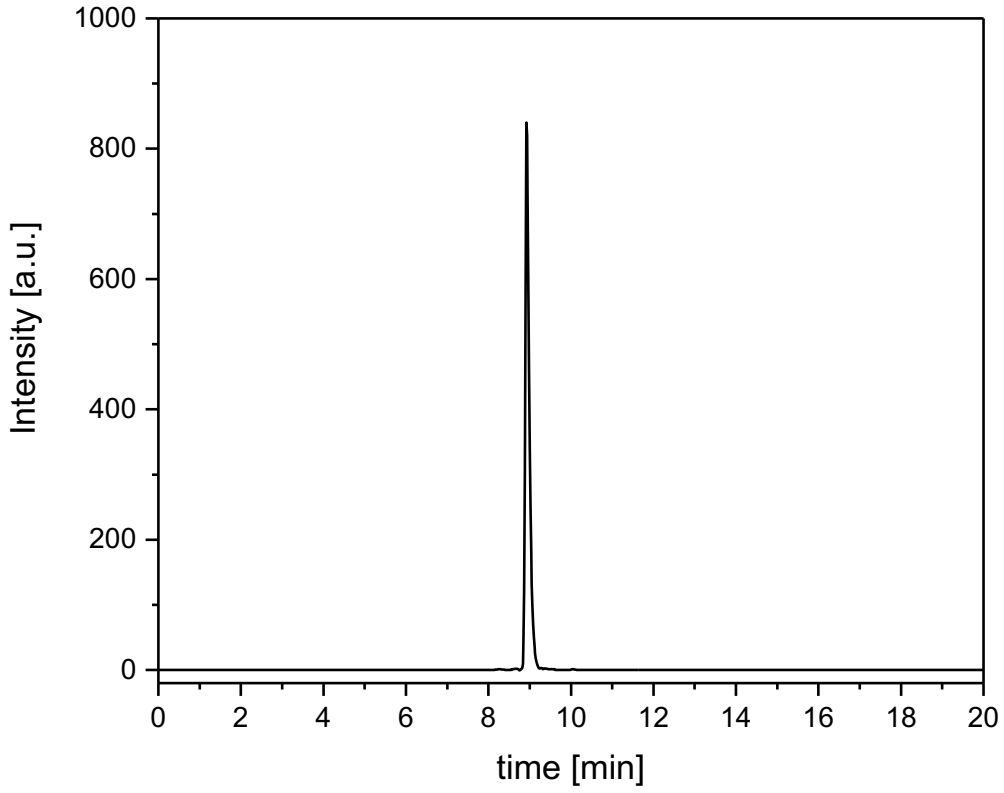


Figure S23: HPLC chromatogram of compound **7b**, C_{18}, MeCN / H_2O (0.1% TFA).

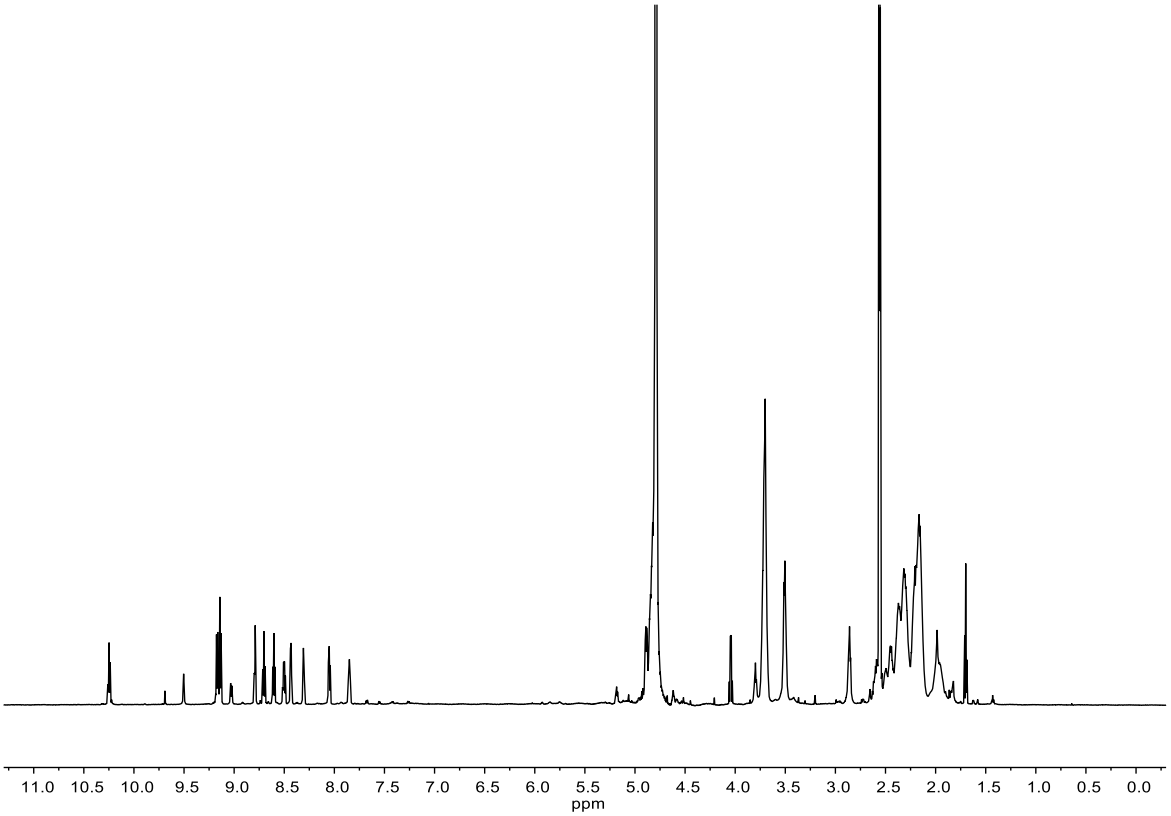


Figure S24: ^{1}H-NMR of compound **7b**, 700 MHz, Acetonitrile-*d3.*

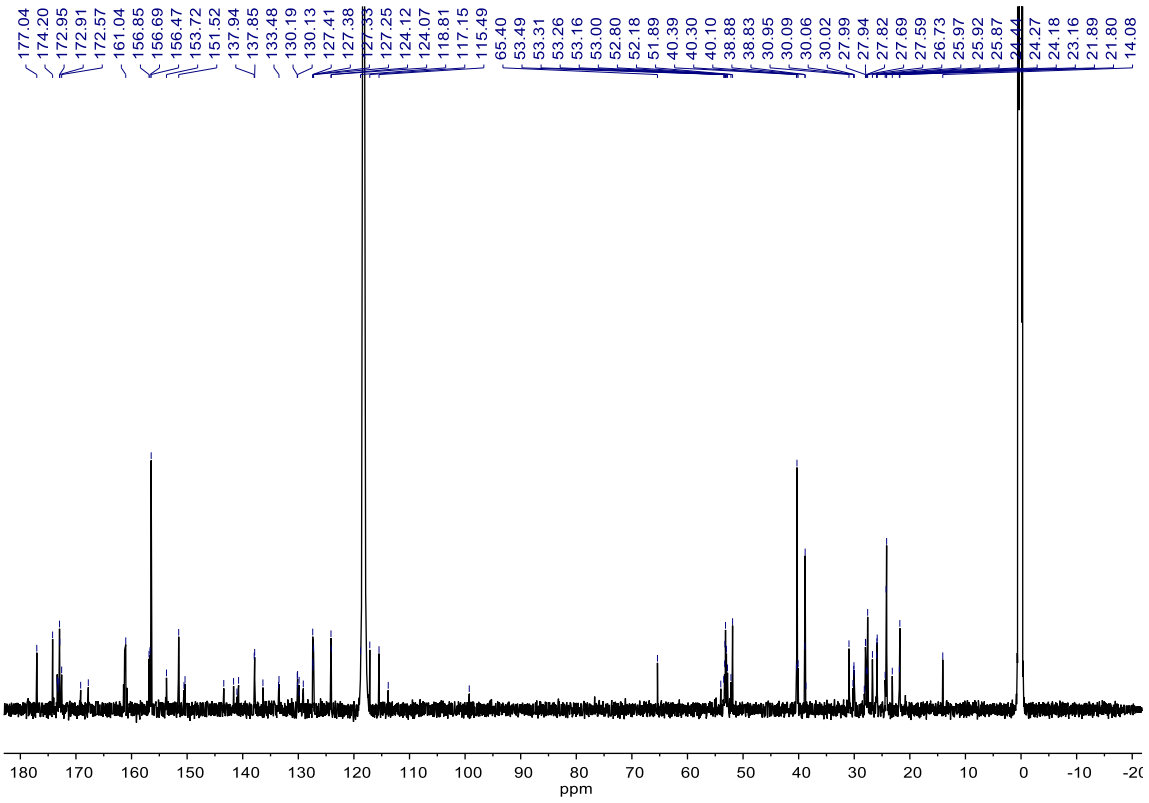


Figure S25: ^{13}C-NMR of compound **7b**, 176 MHz, Acetonitrile-*d3.*

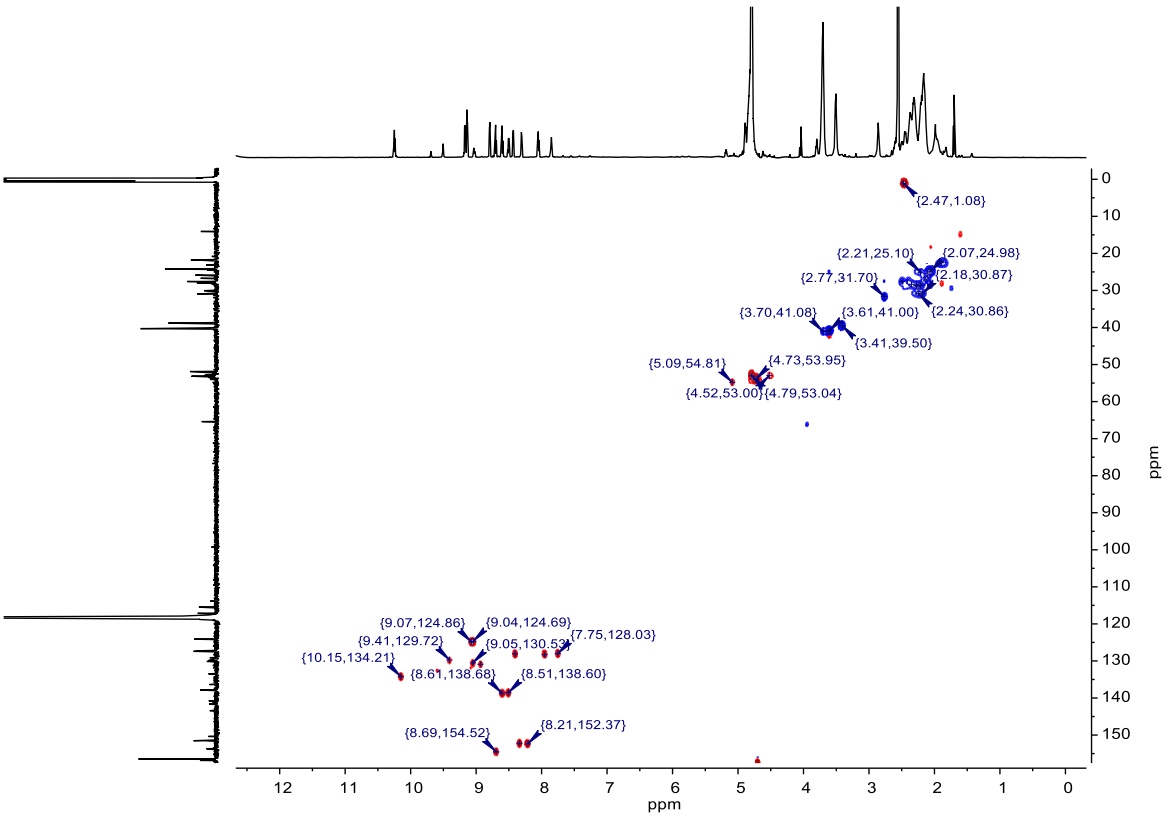


Figure S26: ^{1}H-^{13}C HSQC of compound **7b**, 176 MHz (^{13}C) and 700 MHz (^{1}H), Acetonitrile-*d3.*

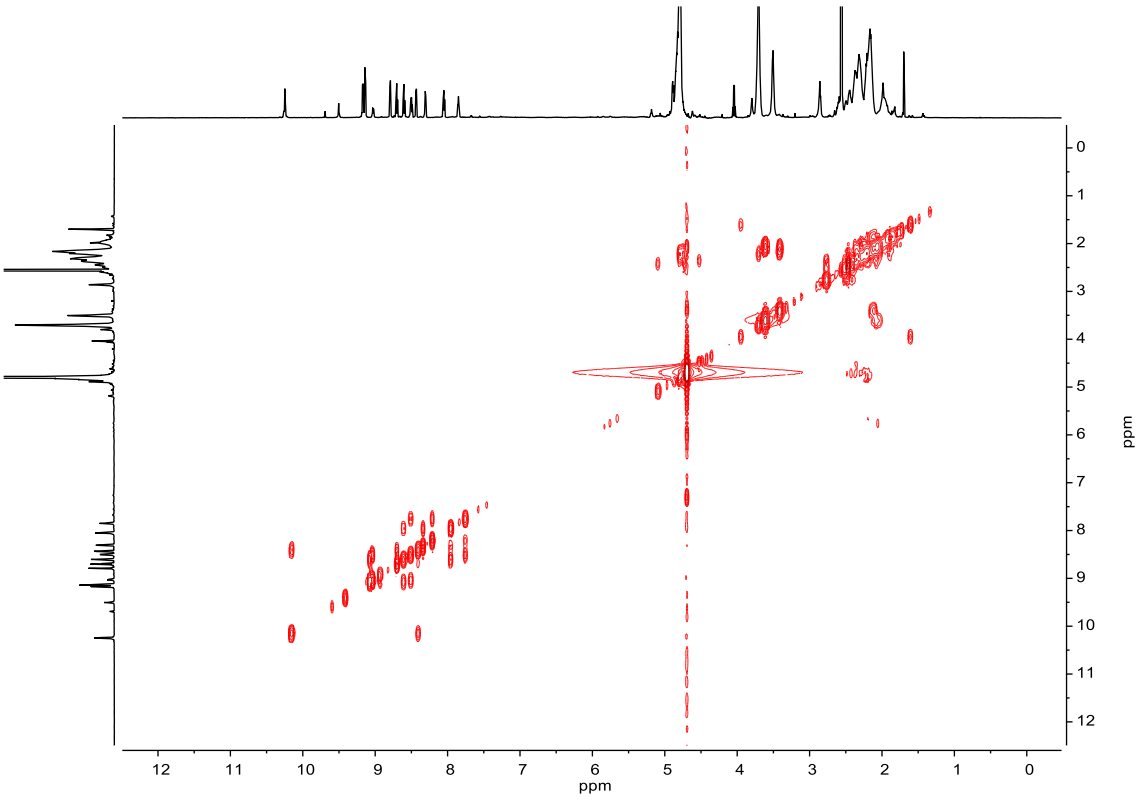


Figure S27: ^{1}H-^{1}H-COSY of compound **7b**, 700 MHz (^{1}H, ^{1}H), Acetonitrile-*d3.*

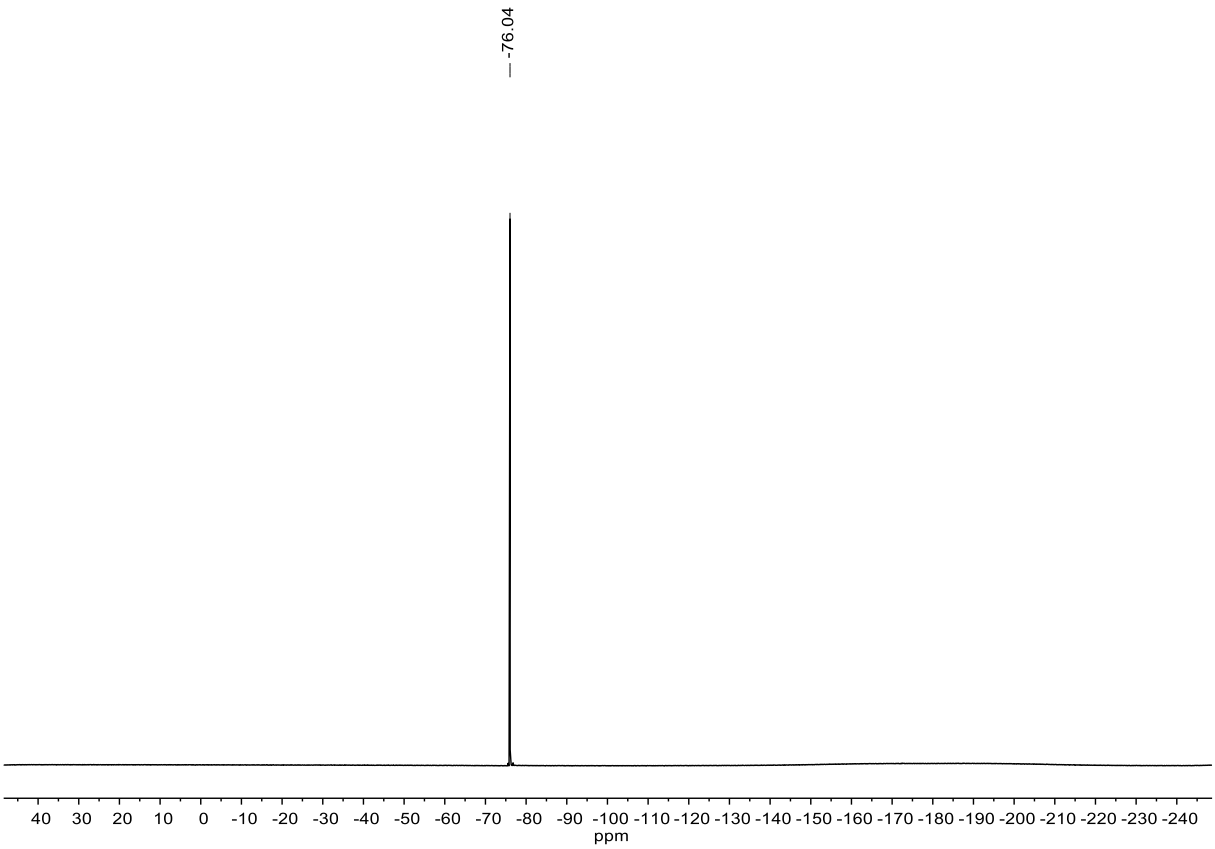
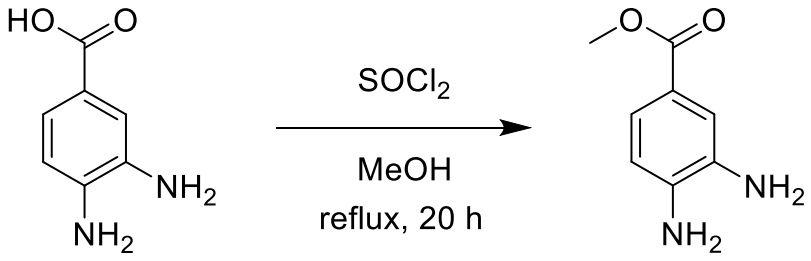


Figure S28: ^{19}F-NMR of compound **7b**, 250 MHz, Acetonitrile-*d3.*

Supplementary synthetic details

Synthesis of 3,4-diamino methyl benzoate ($C_8H_{10}N_2O_2$)



Scheme S29: Synthesis of 3,4-diamino methyl benzoate.

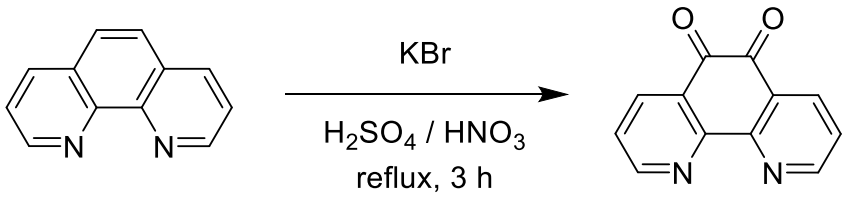
In a 100 ml twin-neck round-bottom flask 4 g (26.29 mmol) 3,4-diamino benzoic acid were dissolved in 30 ml methanol and the solution was cooled down to 0 °C. Afterwards 3.85 ml (6.29 g, 52.91 mmol) thionyl chloride were added at 0 °C under vigorous stirring. After complete addition, the reaction mixture was heated to reflux for 20 h and then cooled down to room temperature. The solution was dumped on 150 ml ice and neutralized with potassium carbonate. The pale brown solution was extracted with chloroform (3 x 100 ml). Afterwards, the combined organic solution was washed with brine (1 x 100 ml) and dried with Mg_2SO_4. The solvent was removed under reduced pressure and the remaining pale-brown solid was recrystallized in 15 ml ethanol. After recrystallisation an off-white solid was obtained after washing with 20 ml dieethylether. The product was obtained as an off-white solid in 51% yield, 2.23 g, 13.42 mmol.

^{1}H NMR (300 MHz, DMSO-d_6) δ 7.15 (d, J = 2.0 Hz, 1H), 7.08 (dd, J = 8.1, 2.0 Hz, 1H), 6.50 (d, J = 8.1 Hz, 1H), 5.26 (s, 2H), 4.64 (s, 2H), 3.71 (s, 3H).

^{13}C NMR (75 MHz, DMSO-d_6) δ 206.6, 166.8, 140.6, 133.8, 120.2, 114.9, 112.7, 51.1

Elemental analysis: found: C 57.50%, H 5.64%, N 16.80% (Calculated: C 57.82%, H 6.07%, N 16.86%)

Synthesis of 1,10-phenanthroline-5,6-dione ($C_{12}H_6N_2O_2$)



Scheme S30: Synthesis of 1,10-phenanthroline-5,6-dione.

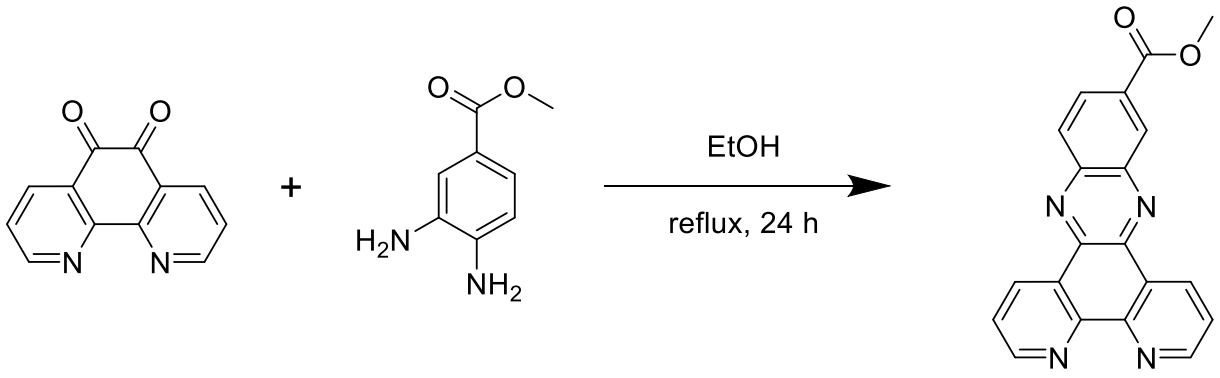
2.5 g (16.43 mmol) 1,10-phenanthroline and 2.6 g (21.85 mmol) potassium bromide were combined in a 250 ml twin-neck round-bottom flask. Afterwards, an ice-cooled mixture of 40 ml H_2SO_4 (95%) and 20 ml HNO_3 (65%) was slowly added to the solids. The mixture turned brown immediately and bromine gas evolved. The solution was heated to reflux for 3 h while exhausting bromine gas was pipelined to a gas washing bottle and neutralized with a concentrated $Na_2S_2O_3$ solution. Then the mixture was cooled down to room temperature and the bromine was pushed out with nitrogen and neutralized. The solution was dumped on 400 ml ice and the resulting aqueous solution was neutralized to pH 6 with NaOH. During the neutralization the mixture turns from dark brown to yellow. The aqueous solution was extracted with $CHCl_3$ (6 x 100 ml) and the combined organic solutions were washed with brine (1 x 100 ml). Afterwards the solution was dried over $MgSO_4$ and the solvent was removed under reduced pressure to obtain a yellow residue. The residue was recrystallized in 150 ml EtOH and yellow needle-shaped crystals were obtained. The product was obtained in 74% yield, 2.56 g, 12.18 mmol.

^{1}H NMR (300 MHz, DMSO-d_6) δ 8.99 (dd, J = 4.7, 1.8 Hz, 2H), 8.39 (dd, J = 7.8, 1.8 Hz, 2H), 7.67 (dd, J = 7.8, 4.6 Hz, 2H).

^{13}C NMR (75 MHz, DMSO-d_6) δ 177.8, 154.3, 152.3, 135.6, 129, 125.2.

Elemental analysis: found: C 68.22%, H 2.64%, N 13.15% (Calculated: C 68.57%, H 2.88%, N 13.33%)

Synthesis of methyl dipyrido[3,2-a:2',3'-c]phenazine-11-carboxylate ($C_{20}H_{12}N_4O_2$)



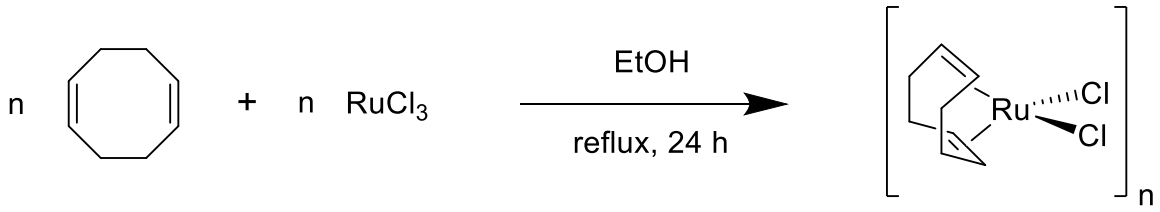
Scheme S31: Synthesis of methyl dipyrido[3,2-a:2',3'-c]phenazine-11-carboxylate.

In a 100 ml round bottom flask, 0.58 g (2.76 mmol) 1,10-phenanthroline-5,6-dione and 0.46 g (2.76 mmol) 3,4-diamino methyl benzoate were dissolved in 50 ml ethanol. The yellow solution was refluxed for 24 h and during the reaction a pale-yellow solid precipitated. The suspension was passed through a glass sintered funnel and the remaining solid was washed with ethanol (2 x 20 ml) and diethylether (2 x 20 ml). The product was obtained as a yellow solid in 91% yield, 0.85 g, 2.50 mmol.

^{1}H NMR (200 MHz, Chloroform-*d*) δ 9.60 (d, *J* = 8.1 Hz, 2H), 9.31 (d, *J* = 4.5 Hz, 2H), 9.03 (d, *J* = 1.8 Hz, 1H), 8.60 – 8.22 (m, 2H), 7.82 (dd, *J* = 8.3, 4.3 Hz, 2H), 4.08 (s, 3H).

Elemental analysis: found: C 70.16%, H 3.24%, N 16.27% (Calculated: C 70.58%, H 3.55%, N 16.46%)

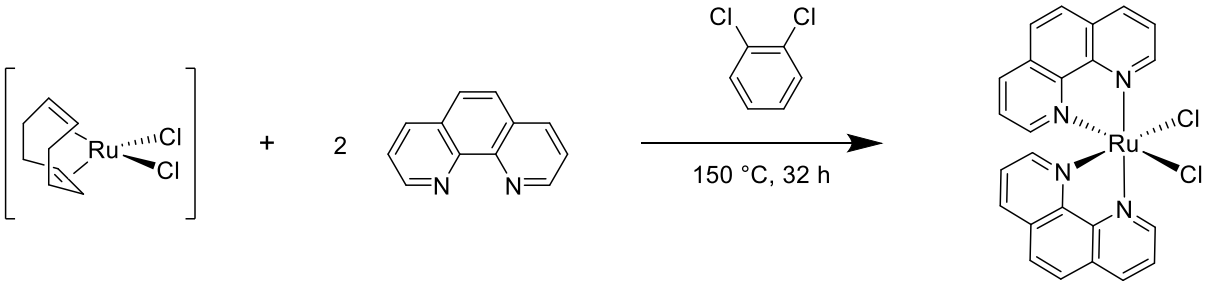
Synthesis of [(1,2,5,6-η)-1,5-cyclooctadienedichlororuthenium(II)] ($[C_8H_{12}Cl_2Ru]_n$)



Scheme S32: Synthesis of Dichloro[(1,2,5,6-η)-1,5-cyclooctadiene]ruthenium(II).

2.95 g (12.24 mmol) ruthenium(III) chloride x-hydrate (41.95% Ru) was dissolved in 50 ml ethanol in a 100 ml round bottom flask, forming a dark-brown to black solution. Afterwards, 17 ml cyclooctadiene (19.87 g, 183.67 mmol) was added to the solution and the mixture was heated to reflux. The solution was refluxed under vigorous stirring for 24 h and a red-brown powder precipitated. The suspension was passed through a glass sintered funnel and the remaining solid was washed with ethanol (2 x 20 ml) and dried under reduced pressure overnight. The product was obtained as a red-brown powder in 95% yield, (3.25 g, 11.60 mmol). The product was used without further purification for the next step.

Synthesis of cis-[bis-(1,10-phenanthroline)dichlororuthenium(II)] ($C_{24}H_{16}Cl_2N_4Ru$) [1a]



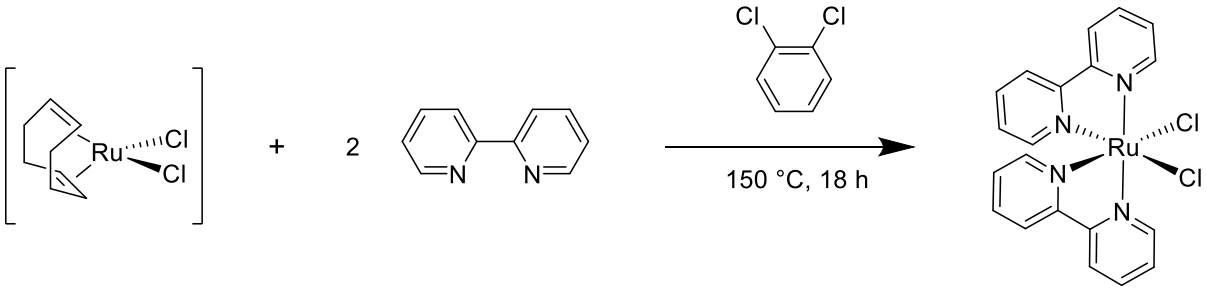
Scheme S33: Synthesis of cis-[bis (1,10-phenanthroline) dichlororuthenium(II) [1a].

In a 100 ml round bottom flask, 1.5 g (5.35 mmol) [(1,2,5,6-η)-1,5-cyclooctadiene]dichlororuthenium(II) and 1.93 g (10.71 mmol) 1,10-phenanthroline were suspended in 50 ml 1,2-dichlorobenzene. The reaction mixture was stirred at 150 °C for 32 h and turns violet during the reaction. Afterwards, the solution was cooled down to room temperature and filtered to remove the remaining educts. The filtrate was slowly added to 250 ml *n*-hexane and a violet powder precipitated. The suspension was passed through a glass sintered funnel and the remaining solid was washed with *n*-hexane (2 x 100 ml). The powder was dried under reduced pressure overnight. cis-[bis-(1,10-phenanthroline)dichlororuthenium(II)] was obtained as a violet powder in 95% yield, (2.88 g, 5.07 mmol).

^{1}H NMR (200 MHz, DMSO-d_6) δ 10.28 (dd, *J* = 5.3, 1.3 Hz, 2H), 8.71 (dd, *J* = 8.1, 1.4 Hz, 2H), 8.43 – 7.99 (m, 8H), 7.75 (dd, *J* = 5.4, 1.2 Hz, 2H), 7.33 (dd, *J* = 7.4, 4.7 Hz, 2H).

MS (ESI+): *m / z* 532.42 $[M+H]^+$.

Synthesis of cis-[bis-(2,2'-bipyridine)dichlororuthenium(II)] ($C_{20}H_{16}Cl_2N_4Ru$) [1b]



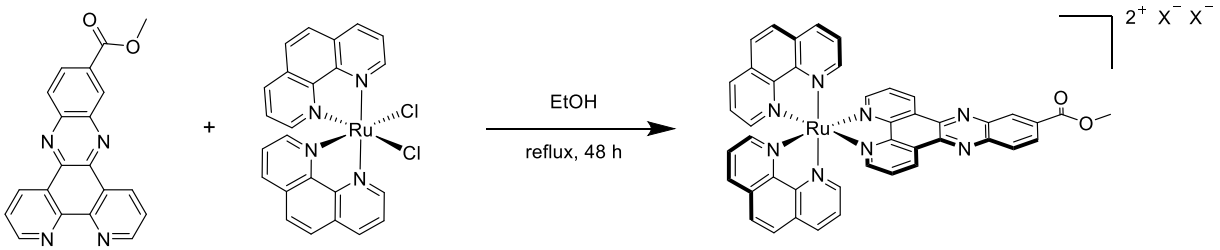
Scheme S34: Synthesis of cis-[bis-(2.2',bipyridine)dichlororuthenium(II)] [1b].

In a 100 ml round bottom flask, 0.9 g (3.21 mmol) [(1,2,5,6-η)-1,5-cyclooctadiene]dichlororuthenium(II) and 1 g (6.42 mmol) 2.2',bipyridine were suspended in 30 ml 1,2-dichlorobenzene. The reaction mixture was stirred at 150 °C for 18 h and turns violet during the reaction. Afterwards, the solution was cooled down to room temperature and filtered to remove the remaining educts. The filtrate was slowly added to 200 ml *n*-hexane and a violet powder precipitated. The suspension was passed through a glass sintered funnel and the remaining solid was washed with *n*-hexane (2 x 60 ml). The powder was dried under reduced pressure overnight. cis-[bis-(2.2',bipyridine)dichlororuthenium(II)] was obtained as a violet powder in 90% yield, (1.5 g, 2.89 mmol).

^{1}H NMR (200 MHz, DMSO-d_6) δ 9.97 (dd, *J* = 5.8, 1.5 Hz, 2H), 8.64 (d, *J* = 8.1 Hz, 2H), 8.48 (d, *J* = 8.0 Hz, 2H), 8.06 (td, *J* = 7.8, 1.6 Hz, 2H), 7.90 – 7.61 (m, 4H), 7.51 (dd, *J* = 5.9, 1.3 Hz, 2H), 7.10 (ddd, *J* = 7.3, 5.7, 1.3 Hz, 2H).

^{13}C NMR (50 MHz, DMSO-d_6) δ 160.2, 158.2, 153.1, 151.9, 134.5, 133.3, 125.3, 125.2, 122.8, 122.5.

Synthesis of rac-[(phen)$_2$(dppz-COOMe)ruthenium(II)] ($C_{44}H_{28}N_8O_2RuX_2$) [2a]



Scheme S35: Synthesis of rac-[(phen)$_2$(dppz-COOMe)ruthenium(II)] [2a].

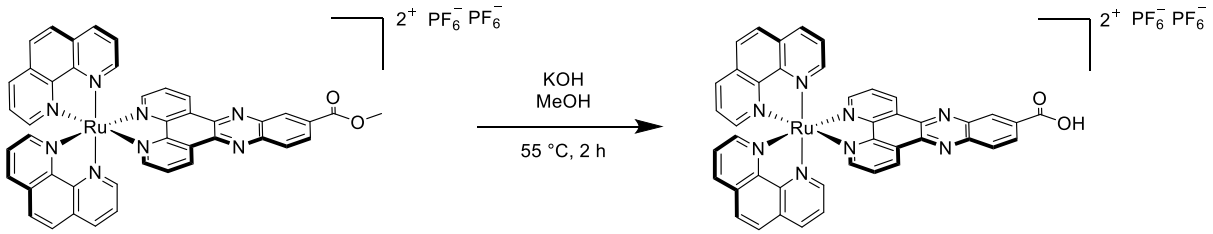
In a 250 ml round bottom flask, 0.7 g (1.23 mmol) of compound 1a and 0.42 g (1.23 mmol) of compound L3 were suspended in 100 ml ethanol. The reaction mixture was refluxed for 48 h until no visible solids remained in the solution. During the reaction the suspension turns to an orange solution. After cooling down to room temperature the suspension was filtered to obtain an orange filtrate. The solvent was removed under reduced pressure from the filtrate to obtain a dark-orange solid. The powder is consisted of the chloride salt which was obtained in 89% yield, (1 g, 1.15 mmol) and was used for the DNA and Fluorescence experiments, but it contains minor impurities. For all other experiments, 0.8 g (0.92 mmol) of the solid was purified on silica gel, using CH_3CN / H_2O / $KNO_{3(sat.\ aq.)}$ (40 : 4 : 1) (product R_f = 0.78) as eluent. After removal of the solvent the compound was redissolved in 100 ml MiliQ-Water and NH_4PF_6 was added until an orange solid precipitated. The suspension was passed through a glass-sintered funnel and washed with MiliQ-Water (2 x 50 ml). The obtained solid was dried under reduced pressure. The PF_6 salt was obtained as an orange solid in 48% yield, (0.38 g, 0.35 mmol).

^{1}H NMR (400 MHz, DMSO-d_6) δ 10.15 (ddd, *J* = 8.3, 4.2, 1.4 Hz, 2H), 9.47 – 9.40 (m, 1H), 9.33 –9.18 (m, 4H), 9.08 (dd, *J* = 5.4, 1.3 Hz, 2H), 8.99 (q, *J* = 3.0, 2.3 Hz, 3H), 8.90 – 8.81 (m, 7H), 8.42 (ddd, *J* = 7.9, 5.4, 2.3 Hz, 2H), 8.28 (ddd, *J* = 13.6, 6.8, 4.1 Hz, 4H), 4.49 (s, 3H).

^{13}C NMR (101 MHz, Acetone-d_6) δ = 166.2, 155.8, 155.7, 154.5, 154, 152.5, 152.3, 149, 148.9, 148.8, 144.9, 142.6, 142.1, 138, 137.8, 134.8, 134.6, 134, 132.6, 132.1, 132, 131.8, 131.5, 131.4, 131.1, 129.2, 129.1, 128.5, 128.4, 127.2, 127.1, 53.3.

MS (ESI+): *m / z* 946.65 $[M+PF_6]^+$.

Synthesis of rac-[(phen)$_2$(dppz-COOH)ruthenium(II)] ($C_{44}H_{28}F_{12}N_8O_2P_2Ru$) [3a]



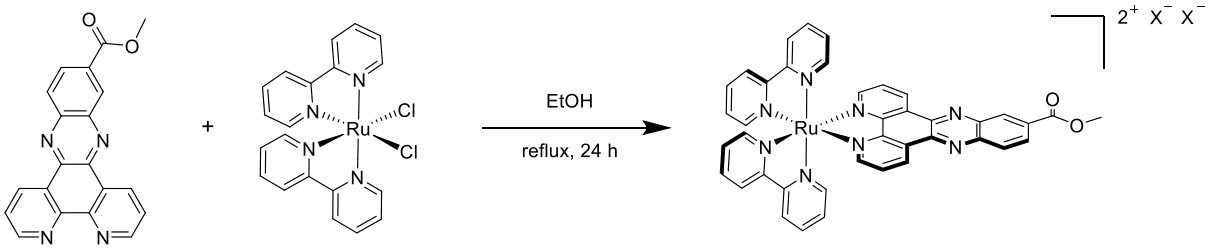
Scheme S36: Synthesis of rac-[(phen)$_2$(dppz-COOH)ruthenium(II)] [3a].

In a 25 ml round bottom flask, 300 mg (0.27 mmol) of compound M5a were dissolved in 4 ml methanol. Afterwards 127 mg (1.92 mmol) KOH was added to the solution which was stirred afterwards at 55 °C for 2 h. Then 1.54 ml (1.93 mmol) 1.25 M HCl in methanol were slowly added to the solution at 0 °C. All volatiles were removed under reduced pressure and the remaining orange residue was redissolved in 15 ml MiliQ-water. NH_4PF_6 was slowly added to the solution until an orange solid precipitated. The suspension was passed through a glass-sintered funnel and the remaining solid was washed with MiliQ-water (2 x 10 ml). The solid was dried under reduced pressure overnight. The free carboxylic acid was obtained as an orange solid in 96% yield with minor impurities, (285 mg, 0.26 mmol).

^{1}H NMR (300 MHz, DMSO-d_6) δ 9.66 – 9.57 (m, 2H), 8.95 (s, 1H), 8.78 (ddd, *J* = 7.1, 5.7, 4.3 Hz, 4H), 8.63 – 8.50 (m, 2H), 8.40 (s, 4H), 8.29 (dd, *J* = 5.3, 1.2 Hz, 2H), 8.24 – 8.16 (m, 2H), 8.10 – 8.02 (m, 2H), 7.92 (ddd, *J* = 8.5, 5.4, 3.3 Hz, 2H), 7.79 (ddd, *J* = 10.6, 8.3, 5.3 Hz, 4H).

MS (ESI+): *m / z* 932.42 $[M+PF_6]^+$.

Synthesis of rac-[(bpy)$_2$(dppz-COOMe)ruthenium(II)] ($C_{40}H_{28}N_8O_2RuX_2$) [2b]



Scheme S37: Synthesis of rac-[(bpy)$_2$(dppz-COOMe)]ruthenium(II) [2b].

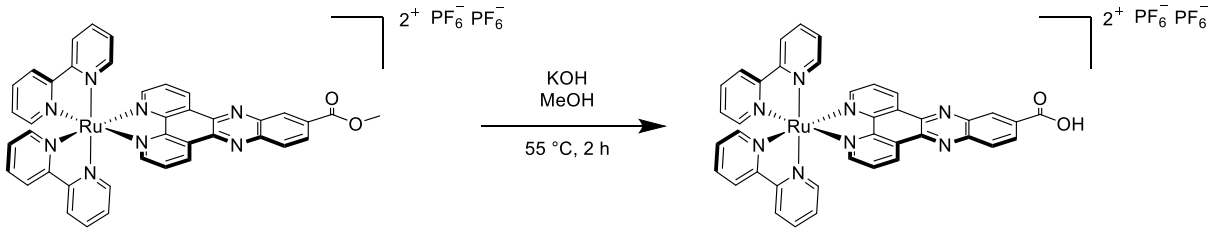
In a 250 ml round bottom flask, 0.7 g (1.23 mmol) of compound 1b and 0.42 g (1.23 mmol) of compound L3 were suspended in 100 ml ethanol. The reaction mixture was refluxed for 24 h until no visible solids remained in the solution. During the reaction the suspension turns to an orange solution. After cooling down to room temperature the suspension was filtered to obtain an orange filtrate. The solvent was removed under reduced pressure from the filtrate to obtain a dark-orange solid. The powder is consisted of the chloride salt which was obtained in 90% yield, (1.4 g, 1.7 mmol) and was used for the DNA and Fluorescence experiments, but it contains minor impurities. For all other experiments, 1 g (1.21 mmol) of the solid was purified on silica gel, using CH_3CN / H_2O / $KNO_{3(sat.\ aq.)}$ (40 : 4 : 1) (product R_f = 0.80) as eluent. After removal of the solvent the compound was redissolved in 150 ml MiliQ-Water and NH_4PF_6 was added until an orange solid precipitated. The suspension was passed through a glass-sintered funnel and washed with MiliQ-water (2 x 50 ml). The obtained solid was dried under reduced pressure. The PF_6 salt was obtained as an orange solid in 56% yield, (0.71 g, 0.68 mmol).

^{1}H NMR (400 MHz, Acetone-d_6) δ 8.98 (ddd, *J* = 8.2, 5.9, 1.4 Hz, 2H), 8.24 (d, *J* = 1.6 Hz, 1H), 8.08 (dd, *J* = 12.5, 8.2 Hz, 4H), 7.80 (dt, *J* = 6.3, 2.2 Hz, 4H), 7.49 (td, *J* = 7.9, 1.5 Hz, 2H), 7.40 (dd, *J* = 5.2, 3.4 Hz, 4H), 7.33 (ddt, *J* = 8.0, 5.5, 2.7 Hz, 4H), 6.87 (ddd, *J* = 7.3, 5.6, 1.4 Hz, 2H), 6.72 – 6.58 (m, 2H), 3.28 (s, 3H).

^{13}C NMR (101 MHz, Acetone-d_6) δ 166.3, 158.5, 158.2, 155.4, 155.3, 153.3, 153.1, 152.3, 152.1, 145.1, 142.7, 142.6, 142.2, 139.3, 139.2, 135, 134.8, 134.2, 132.8, 131.9, 131.7, 131.6, 131.2, 128.9, 128.8, 128.8, 128.7, 125.5, 125.4, 53.4.

MS (ESI+): *m / z* 898.6 $[M+PF_6]^+$.

Synthesis of rac-[(bpy)$_2$(dppz-COOH)ruthenium(II)] ($C_{39}H_{26}F_{12}N_8O_2P_2Ru$) [3b]



Scheme S38: Synthesis of rac-[(bpy)$_2$(dppz-COOH)]ruthenium(II) [3b].

In a 25 ml round bottom flask, 300 mg (0.29 mmol) of compound M6a were dissolved in 4 ml methanol. Afterwards 123 mg (2.01 mmol) KOH was added to the solution which was then stirred at 55 °C for 2 h. Afterwards, 1.62 ml (2.02 mmol) 1.25 M HCl in methanol were slowly added to the solution at 0 °C. All volatiles were removed under reduced pressure and the remaining orange residue was redissolved in 15 ml MiliQ-Water. NH_4PF_6 was slowly added to the solution until an orange solid precipitated. The suspension was passed through a glass-sintered funnel and the remaining solid was washed with MiliQ-water (2 x 10 ml). The solid was dried under reduce pressure overnight. The free carboxylic acid was obtained as an orange solid in 95% yield, (280 mg, 0.27 mmol).

^{1}H NMR (400 MHz, DMSO-d_6) δ 9.64 (t, J = 8.9 Hz, 2H), 8.99 – 8.78 (m, 5H), 8.57 (dd, J = 8.8, 1.8 Hz, 1H), 8.44 (d, J = 8.8 Hz, 1H), 8.23 (dd, J = 11.3, 6.2 Hz, 4H), 8.14 (t, J = 7.9 Hz, 2H), 8.03 (dt, J = 8.1, 5.6 Hz, 2H), 7.81 (dd, J = 17.4, 5.6 Hz, 4H), 7.60 (t, J = 6.7 Hz, 2H), 7.39 (t, J = 6.7 Hz, 2H).

MS (ESI+): m / z 884.55 $[M+PF_6]^+$.