Supporting Information

Ammonium Ion Recognition:

Luminescent Amino Acid and Peptide Receptors

Modification of Amino Acids:

Artificial Amino Acids, Cyclopeptides and Guanidinium-bis-carboxylates

Dissertation
an der Fakultät für Chemie und Pharmazie
der Universität Regensburg

vorgelegt von
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aus Straubing
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III. Ditopic Crown Ether – Guanidinium Ion Receptors for the Molecular Recognition of Amino Acids and Small Peptides[i]

SUPPORTING INFORMATION

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[i] A. Späth, B. König, *Tetrahedron* **2010**, *66*, 1859-1873.

1. Syntheses

1.1. General methods

Analytical characterization of the synthesized compounds was done by common methods. Melting Points were determined on Büchi SMP or a Lambda Photometrics OptiMelt MPA 100 and are uncorrected. IR Spectra were recorded with a Bio-Rad FT-IR Excalibur FTS 3000. Absorption spectra were recorded on a Varian Cary BIO 50 UV/VIS/NIR spectrometer with temperature control using 1 cm quartz cuvettes (Hellma) and Uvasol solvents (Merck, Baker or Acros). Fluorescence measurements were performed with UV-grade solvents (Baker or Merck) in 1 cm quartz cuvettes (Hellma) and recorded on a Varian 'Cary Eclipse' fluorescence spectrophotometer with temperature control. Electro spray mass spectra were performed on a Finnigan MAT TSQ 7000 ESI-spectrometer. Other Mass Spectra were recorded on Varian CH-5 (EI), Finnigan MAT 95 (CI; FAB and FD); Xenon serves as the ionization gas for FAB.

NMR spectra were recorded on Bruker Avance 600 (^{1}H: 600.1 MHz, ^{13}C: 150.1 MHz, T = 300 K), Bruker Avance 400 (^{1}H: 400.1 MHz, ^{13}C: 100.6 MHz, T = 300 K) or Bruker Avance 300 (^{1}H: 300.1 MHz, ^{13}C: 75.5 MHz, T = 300 K) relative to external standards. NMR spectra were recorded in $CDCl_3$ at 300 MHz (^{1}H) or 75 MHz (^{13}C) unless stated otherwise. Characterization of the signals: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, bs = broad singlet, dd = double doublet, dt = double triplet, ddd = double double doublet. Integration is determined as the relative number of atoms, the coupling constants are given in Hertz [Hz]. The multiplicity of the carbon atoms is given as (+] = CH_3 or CH, (-] = CH_2 and (C_{quat}) for quaternary carbon atoms. Structural assignments are based on DEPT and COSY experiments where applicable. Error of reported values: chemical shift: 0.01 ppm for ^{1}H-NMR, 0.1 ppm for ^{13}C-NMR and 0.1 Hz for coupling constants. The solvent used is reported for each spectrum.

Analytical TLC plates (silica gel 60 F_{254}) and silica gel 60 (70-230 or 230-400 mesh) were used for chromatographic separations. Visualization of the spots was by UV light and/or staining with phosphomolybdate or ninhydrin, both in ethanol. DMF, CH_3CN, $CHCl_3$, THF, and Et_2O were dried by standard procedures and stored over molecular sieves. PE means petrol ether with a boiling range of 70 - 90 °C. All other solvents and chemicals were of reagent grade and used with out further purification.

All test substances were of pro analysi grade, checked by NMR or HPLC and used as purchased with out further purification.

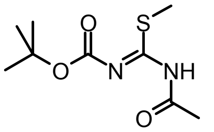
The Solvents for the fluorescence measurements were from special spectroscopic purity purchased from Acros or Baker or Uvasol from Merck. Millipore water (18 MΩ, Milli Q_{Plus}) was used; the HEPES buffer was from according purity, suitable for biochemical optical screenings. As far as not stated differently tetraethylammoniumhydroxide or 0.1 molar hydrochloric acid both analytical grade were used to adjust the pH in the titrations and screenings.

Reagents were used as received. DMF was purchased anhydrous (Fluka) and used as received. Other solvents used were reagent or pro analysi grade, THF was dried over sodium and distilled. Chloroform was dried over $CaCl_2$ and P_2O_5 and distilled. Acetonitrile was dried over CaH_2 and distilled. Pyrene-1-carboxylic acid[1] is commercially available. All reagents were used as received (Merck, Aldrich, Fluka). 1-*N*-^{tert}butoxycarbonyl isothiourea,[2] 1,3-di(^{tert}butoxycarbonyl) isothiourea,[2] 1-*N*-^{tert}butoxycarbonylamino-ethyl-2-isothiocyanate[2] and 1,3-di(^{tert}butoxycarbonyl) guanidine-ethyl-2-amine[3] were prepared after literature known prescriptions.

1.2. Syntheses of building blocks

1.2.1. Simple building blocks and comparison material

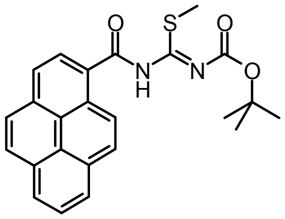
*1-(^{tert}Butyloxycarbonyl)-3-acetyl-2-methyl-2-isothiourea (**9**) (literature known[4], improved synthesis)*



1-*N*-Boc-2-methyl-2-isothiourea (1.90 g, 10.0 mmol) was dissolved in 30 mL of DCM containing 4.0 mL triethylamine (3.03 g, 30.0 mmol). Under cooling in an ice bath and vigorous stirring acetanhydride (1.42 mL, 1.53 g, 15.0 mmol) was added drop wise. The mixture was stirred 2 h at room temperature, then diluted with 70 mL of ethyl acetate, washed with 5 % aqueous acetic acid and three times with water. The organic phase was dried over $MgSO_4$ and evaporated at reduced pressure. The remaining solid was purified by column chromatography with ethyl acetate / petrol ether 1:2 to isolate the product as a white solid (2.04 g, 93 %).

M.p. = 83 – 84 °C (uncorrected); - **^{1}H-NMR** (300 MHz, $CDCl_3$): δ [ppm] = 1.42 (s, 9 H), 2.16 (s, 3 H), 2.31 (s, 3 H), 12.10 (bs, 1 H); - ^{13}C-NMR (75 MHz, $CDCl_3$): δ [ppm] =14.4 (+, 1 C), 24.6 (+, 1 C), 28.0 (+, 3 C), 81.3 (C_{quat}, 1 C), 160.9 (C_{quat}, 1 C), 168.4 (C_{quat}, 1 C), 171.3 (C_{quat}, 1 C); - **MS** (ESI(+), DCM/MeOH + 10 mmol NH_4OAc): m/z (%) = 231.4 (100, MH^+); - **MF:** $C_9H_{16}N_2O_3S$ – **FW:** 232.3 g/mol;

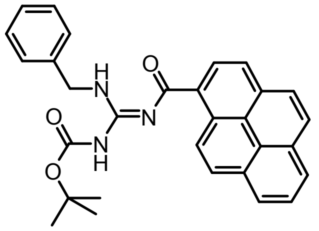
*1-(^{tert}Butyloxycarbonyl)-3-(pyrene-1-carbonyl)-2-methyl-2-isothiourea (**29**) (literature known compound,^{5} alternative procedure)*



Pyrene-1-carboxylic acid[1] (490 mg, 2.0 mmol) was suspended in a 1:1 mixture of DMF and DCM (2.0 mL), triethylamine (0.36 mL, 0.30 g, 3.0 mmol), HOBt (324 mg, 2.4 mmol) and DCC (497 mg, 2.4 mmol) were added and it was stirred for 30 mins in an ice bath. 1-*N*-Boc-2-methyl-2-isothiourea (380 mg, 2.0 mmol) was added in one portion and the mixture was stirred for 2 h at room temperature, and then heated to 40 °C for 4 h under nitrogen. The solvents were evaporated, the residue was suspended in 10 mL of ethyl acetate, the solution was filtered and the filtrate was washed with 5 % ammonium chloride solution once and three times with water (5 mL). The organic phase was dried over $MgSO_4$ and evaporated to dryness. The remaining solid was purified by flash chromatography with ethyl acetate / petrol ether 1:9 to yield 372 mg of a fine yellow powder (1.78 mmol, 89 %).

^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.53 (s, 9 H), 2.74 (s, 3 H), 8.03 – 8.19 (m, 2 H), 8.20 – 8.31 (m, 5 H), 8.93 (m, 1 H), 9.47 (m, 1 H); - **IR** (KBr): ν [cm^{-1}] = 3042 (m), 2980 (m), 2930 (m), 1743 (m), 1707 (m), 1643 (m), 1597 (m), 1537 (s), 1504 (m), 1391 (m), 1362 (m), 1324 (m), 1251 (m), 1229 (m), 1135 (s), 1086 (m), 1052 (m), 845 (m), 767 (m), 711 (m); - **MS** (ESI(+), DCM/MeOH + 10 mmol NH_4OAc): m/z (%) = 419.5 (100, MH^+);$^+$); - **MF:** $C_{24}H_{22}N_2O_3S$ – **FW:** 418.52 g/mol;

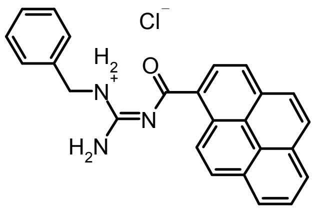
*1-(^{tert}Butyloxycarbonyl)-3-benzyl-2-(pyrene-1-carbonyl)-guanidine (**35**)*



Compound **29** (210 mg, 0.5 mmol) was dissolved in DMF (3 mL), benzylamine (200 µl, 190 mg, 1.6 mmol) and mercury(II)chloride (150 mg, 0.6 mmol) were subsequently added. The mixture was stirred for 6 h, and then warmed to 40 °C for 1 h. It was diluted with ethyl acetate (30 mL), washed twice with 10 % aqueous citric acid and two times with water. The organic phase was dried over $MgSO_4$ and evaporated at reduced pressure. The remaining solid was purified by flash chromatography with ethyl acetate / petrol ether 1:9. The product was isolated as a white solid (218 mg, 0.456 mmol, 91 %).

M.p. = 105 – 107 °C (uncorrected); - **^{1}H-NMR** (300 MHz, $CDCl_3$): δ [ppm] = 1.52 (s, 6 H), 1.53 (s, 3 H), 4.88 (m, 2 H), 7.29 – 7.47 (m, 5 H), 7.96 – 8.29 (m, 7 H), 8.71 (d, 1 H, J = 4.6 Hz), 8.98 (bs, 1 H), 9.32 (d, 1 H, J = 4.6 Hz), 9.68 (bs, 1 H); - **^{13}C-NMR** (75 MHz, $CDCl_3$): δ [ppm] = 28.2 (+, 2 C), 28.3 (+, 1 C), 45.2 (-, 1 C), 82.4 (C_{quat}, 1 C), 124.1 (+, 1 C), 124.6 (C_{quat}, 1 C), 124.7 (+, 1 C), 125.2 (C_{quat}, 1 C), 125.6 (+, 1 C), 125.7 (+, 1 C), 125.9 (+, 1 C), 126.0 (C_{quat}, 1 C), 127.6 (+, 1 C), 127.8 (+, 2 C), 127.9 (+, 1 C), 128.2 (+, 1 C), 128.8 (+, 3 C), 130.5 (C_{quat}, 1 C), 130.6 (C_{quat}, 1 C), 131.1 (C_{quat}, 1 C), 133.1 (C_{quat}, 1 C), 136.7 (C_{quat}, 1 C), 156.3 (C_{quat}, 1 C), 181.1 (C_{quat}, 1 C); - **IR** (KBr): ν [cm^{-1}] = 3317 (bm), 3036 (m), 2968 (m), 2929 (m), 1716 (m), 1599 (s), 1574 (s), 1503 (m), 1396 (m), 1359 (m), 1329 (s), 1257 (m), 1141 (s), 1112 (m), 1046 (m), 994 (m), 910 (m), 842 (m), 812 (m), 731 (m), 707 (m); - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 478.0 (100, MH^+); - **HRMS** (EI-MS 70eV): calc. for $C_{30}H_{27}N_3O_3^{*+}$: 477.2052, found: 477.2046; $^+$); - **MF:** $C_{30}H_{27}N_3O_3$ – **FW:** 477.57 g/mol;

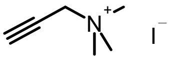
*1-Benzyl-2-(pyrene-1-carbonyl)-guanidine hydrochloride (**36**)*



Compound **35** (192 mg, 0.4 mmol) was dissolved in 2 mL of DCM, 0.5 mL of HCl saturated diethylether was added and the mixture was stirred 2 h at room temperature. Diethylether (3 mL) was added, the product was allowed to settle completely and the solvent was decanted off. After drying at the pump the product was isolated as a faintly yellow, fine powder (74 mg, 0.192 mmol, 96 %).

M.p. = 164 – 169 °C (decomp.); - **^{1}H-NMR** (300 MHz, DMSO-d6): δ [ppm] = 4.72 (m, 2 H), 7.39 – 7.56 (m, 5 H), 8.33 – 8.51 (m, 7 H), 8.67 (d, 1 H, J = 4.6 Hz), 9.11 (d, 1 H, J = 4.6 Hz), 9.19 (bs, 1 H), 9.81 (bs, 1 H); - **^{13}C-NMR** (75 MHz, DMSO-d6): δ [ppm] = 44.3 (-, 1 C), 123.7 (C_{quat}, 1 C), 123.9 (+, 1 C), 124.2 (+, 1 C), 126.3 (+, 1 C), 126.4 (+, 1 C), 126.5 (C_{quat}, 1 C), 126.6 (+, 1 C), 127.0 (+, 2 C), 127.4 (+, 1 C), 127.7 (+, 1 C), 128.0 (+, 1 C), 128.2 (+, 1 C), 128.6 (C_{quat}, 1 C), 128.6 (+, 2 C), 129.4 (+, 1 C), 129.6 (C_{quat}, 1 C), 129.8 (C_{quat}, 1 C), 130.5 (C_{quat}, 1 C), 133.5 (C_{quat}, 1 C), 135.6 (C_{quat}, 1 C), 153.7 (C_{quat}, 1 C), 176.1 (C_{quat}, 1 C); - **IR** (KBr): ν [cm^{-1}] = 3320 (bm), 2972 (m), 2931 (m), 2876 (m), 1719 (m), 1597 (s), 1576 (s), 1505 (m), 1398 (m), 1361 (m), 1327 (m), 1256 (m), 1145 (s), 1113 (m), 1048 (m), 996 (m), 911 (m), 842 (m), 810 (m), 732 (m); - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 478.0 (100, MH^{+}); - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 378.0 (100, MH^{+}), 419.0 (27, (M+MeCN)H^{+}), 755.1 (17, 2MH^{+}); - **HRMS** (EI-MS 70 eV): calc. for $C_{25}H_{19}N_3O^{*+}$: 377.1528, found: 377.1528; - **UV** (MeOH): λ (ε) = 238 (52000), 277 (47000), 238 (68000); - **MF:** $C_{25}H_{20}N_3OCl$ – **FW:** 413.91 g/mol;

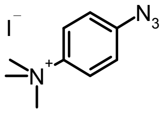
*N,N,N-Trimethyl-propargyl-ammonium iodide (**23**) (literature known,[6] improved synthesis)*



Propargylamine (110 mg, 0.14 mL, 2.0 mmol) was dissolved in 3 mL of acetonitrile. Methyl iodide (1430 mg, 0.63 mL, 10.0 mmol) and potassium carbonate (690 mg, 5.0 mmol) were added and the suspension vigorously stirred over night under light protection and protective gas atmosphere in a water bath. All volatiles were removed at reduced pressure and the remaining salt mixture was suspended in 10 mL of dichloromethane. The inorganic salts were filtered off and washed with small amounts of DCM. The filtrate was evaporated to give the crude material, which was recrystallised from isopropanol to yield the product as fine crystalline, pale brown powder (447 mg, 1.98 mmol, 99 %).

M.p. > 139 °C (decomp.); - **^{1}H-NMR** (300 MHz, MeOD): δ [ppm] = 3.24 (s, 9 H), 4.41 (s, 2 H), 4.76 (s, 1 H); - **^{13}C-NMR** (75 MHz, MeOD): δ [ppm] = 14.2 (+, 1 C), 53.6 (+, 3 C), 57.3 (-, 1 C), 143.2 (C_{quat}, 1 C); - **IR** (KBr): ν [cm^{-1}] = 3170 (s), 3005 (m), 2945 (m), 2123 (m), 1970 (w), 1506 (m), 1472 (s), 1435 (m), 1406 (m), 1363 (m), 1276 (m), 1237 (m), 1131 (m), 1066 (m), 964 (m), 924 (m), 899 (s), 765 (m), 723 (s); - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 97.8 (100 %, M^+); - **MF:** $C_6H_{12}NI$ – **FW:** 225.07 g/mol;

*N,N,N-Trimethyl-4-azido-anilinium iodide (**20**) (literature known,[7] improved synthesis route)*



The 4-iodoaniline (2.19 g, 10.0 mmol) was dissolved in ethanol (25 mL), 1,2-*N,N*-dimethylethylendiamine (0.13 g, 1.42 mmol), three drops 1M aqueous NaOH, ascorbatic acid (0.1g, 0.57 mmol) and sodium azide (1.3 g, 20.0 mmol) were added. The atmosphere in the flask was exchanged by nitrogen and copper(I)iodide (0.25g, 1.31 mmol) was quickly added. The solution was stirred for 90 mins. at room temperature under the protective nitrogen atmosphere and was then added to a vigorously stirred 1:1 mixture of ethyl acetate and brine (100 mL). The organic phase was separated and the aqueous phase was extracted trice with ethyl acetate (20 mL). The combined organic phases were dried over $MgSO_4$, the solvent was evaporated and the residue was purified by column chromatography with ethyl acetate. The product 4-azidoaniline[8] was pale brown oil (1.32 g, 9.62 mmol, 96 %).

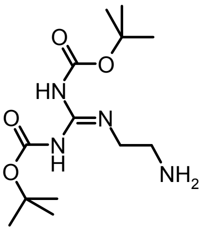
^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 3.58 (bs, 2 H), 6.61 (d, 2 H, J = 5.3 Hz), 6.82 (d, 2 H, J = 5.3 Hz); - **^{13}C-NMR** (75 MHz, $CDCl_3$): δ [ppm] = 116.3 (+, 2 C), 120.0 (+, 2 C), 130.2 (C_{quat}, 1 C), 143.8 (C_{quat}, 1 C); - **MS** (CI-MS, NH_3): m/z (%) = 107.0 (100, ((M-N_2)+H^+)), 135.0 (12, MH^+); - **MF:** $C_6H_6N_4$ – **FW:** 134.14 g/mol;

The above product (1.21 g, 9.0 mmol) was dissolved in 35 mL of methanol. Methyl iodide (5.15 g, 2.28 mL, 36.0 mmol) and potassium carbonate (4.14 g, 30.0 mmol) were added. The reaction mixture was heated to 50 °C over night. After cooling to room temperature, all volatiles were evaporated and the remaining salt mixture was suspended in 60 mL of dichloromethane. The inorganic salts were filtered off, washed with small amounts of DCM.

The filtrate was evaporated to give the crude product, which was purified by recrystallisation from isopropanol / ethyl acetate to yield *N,N,N*-trimethyl-4-azido-anilinium iodide as pale yellow crystal plates (2.41 g, 7.92 mmol, 88 %).

M.p. > 145 °C (decomp.); - **^{1}H-NMR** (300 MHz, MeOD): δ [ppm] = 3.71 (s, 9 H), 7.28 (d, 2 H, J = 5.3 Hz), 7.93 (d, 2 H, J = 5.3 Hz); - **^{13}C-NMR** (75 MHz, MeOD): δ [ppm] = 58.0 (+, 3 C), 121.7 (+, 2 C), 123.2 (+, 2 C), 144.0 (C_{quat}, 1 C), further signals were not detectable; - **IR** (KBr): ν [cm^{-1}] = 3380 (bm), 3010 (m), 2980 (m), 2408 (w), 2254 (w), 2116 (s), 2090 (s), 1602 (m), 1506 (s), 1483 (m), 1456 (m), 1410 (m), 1284 (s), 1202 (m), 1177 (m), 1133 (m), 1120 (m), 1009 (m), 958 (m), 936 (m), 914 (m), 850 (m), 838 (m), 711 (m); - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 176.8 (100, M^+); **$C_9H_{13}N_4I$**: calcd.: C 35.5 H 4.3 N 18.4, found: C 35.2 H 4.1 N 18.2; - **MF:** $C_9H_{13}N_4I$ – **FW:** 304.13 g/mol;

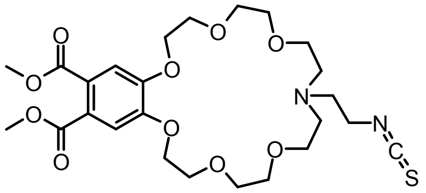
*2-(2,3-di-(^{tert}Butyloxycarbonyl)-guanidino)ethylamine (**18**) (literature known compound,[5] improved procedure)*



Ethylenediamine (6.6 mL, 6.0 g, 0.1 mol) was dissolved in DCM (70 mL), the solution was cooled in an ice bath and 1-*N*-^{tert}butyloxycarbonyl-methyl-isothiourea (1.90 g, 10.0 mmol) in 80 mL DCM was added over 3 h to the vigorously stirred mixture. Stirring was continued for 2 h at room temperature, then the mixture was warmed to 40 °C for 4 h. It was washed four times with water (50 mL) and dried over $MgSO_4$. All volatiles were evaporated at reduced pressure to give the product as a white, oily solid (2.93 g, 9.70 mmol, 97 %).

^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.51 (s, 9 H), 2.86 (t, 2 H, J = 5.9 Hz), 3.47 (m, 2 H), 8.68 (bs, 1 H), 11.52 (bs, 1 H); - **^{13}C-NMR** (75 MHz, $CDCl_3$): δ [ppm] = 28.1 (+, 3 C), 28.3 (+, 3 C), 41.0 (-, 1 C), 43.4 (-, 1 C), 79.2 (C_{quat}, 1 C), 153.2 (C_{quat}, 1 C), 156.4 (C_{quat}, 1 C), 163.6 (C_{quat}, 1 C); - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 303.1 (100, MH^+), 247.0 (8, MH^+-C_4H_8), 203.0 (6, MH^+-CO_2 -C_4H_8); - **MF:** $C_{13}H_{26}N_4O_4$ – **FW:** 302.38 g/mol;

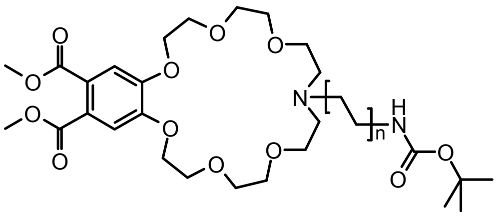
*14-[2-(Isothiocyanato)-ethyl]-6,7,9,10,13,14,15,16,18,19,21,22-dodecahydro-12H-5,8,11,17,20,23-hexaoxa-14-aza-benzocycloheneicosene-2,3-dicarboxylic acid dimethyl ester (**17**)*



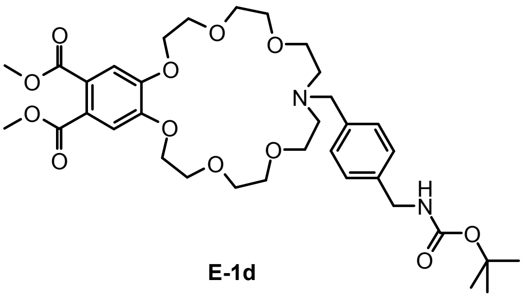
Compound **4a** (230 mg, 0.4 mmol) was dissolved in 1.0 mL of dichloromethane and 1.0 mL of a saturated aqueous sodium hydrogen carbonate solution was added. Under vigorous stirring in an ice bath 5.0 mL of a solution of thiophosgene (0.1 M, 0.5 mmol) in dichloromethane was slowly dropped in the mixture. After 5 h of stirring at room temperature the phases were separated and the organic solution was washed with 2.0 mL of brine and 2.0 mL of water. The solution was dried and all volatiles were evaporated to give the product as clear, yellow oil (205 mg, 0.368 mmol, 92 %).

^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 2.81 (m, 4 H), 2.93 (m, 2 H), 3.56 – 3.71 (m, 12 H), 3.72 – 3.79 (m, 4 H), 3.83 (s, 6 H), 3.88 – 3.93 (m, 4 H), 4.16 – 4.23 (m, 4 H), 7.16 (s, 2 H) - **^{13}C-NMR** (75 MHz, $CDCl_3$): δ [ppm] = 52.6 (+, 2 C), 54.3 (-, 1 C), 54.4 (-, 2 C), 68.9 (-, 1 C), 69.0 (-, 2 C), 69.3 (-, 2 C), 69.4 (-, 2 C), 70.4 (-, 2 C), 70.6 (-, 2 C), 113.6 (+, 2 C), 125.6 (C_{quat}, 2 C), 150.2 (C_{quat}, 2 C), 167.7 (C_{quat}, 2 C), 176.3 (C_{quat}, 1 C); - **IR** (KBr): ν [cm^{-1}] = 2932 (m), 2875 (m), 2188 (m), 2108 (m), 1720 (m), 1598 (m), 1519 (m), 1436 (m), 1350 (m), 1287 (s), 1195 (s), 1136 (s), 1058 (s), 978 (m), 947 (m), 863 (m), 798 (m); - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 557.1 (100, MH^+); - **MF:** $C_{25}H_{36}N_2O_{10}S$ – **FW:** 556.64 g/mol;

1.2.2. General procedures for the Boc deprotection of the crown ether amino acids:



E-1a n = 1
E-1b n = 2
E-1c n = 3



E-1d

The crown ether amino acids were prepared as published.[9] We deprotected their amine functionality by standard conditions[10] with very good to quantitative yields.

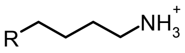
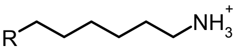
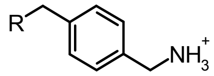
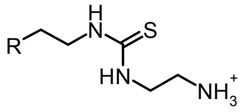
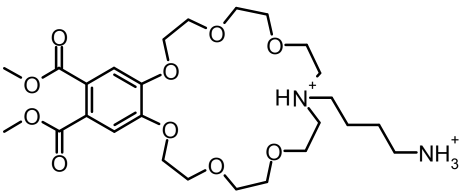
				
Starting material	1.28 g	1.34 g	1.38 g	1.43 g
Yield of product	1.11 g (91 %)	1.11 g (87 %)	1.27 g (96 %)	1.37 g (83 %)
Appearance	Pale yellow powder	Yellow powder	Slightly yellow powder	Pale brown powder
Melting point	63 – 64 °C	66 – 68 °C	70 – 71 °C	74 – 75 °C

Table S-1: Amounts of starting material, yields, appearance and melting points (HCl salts)

General procedures for the Boc deprotection with HCl in diethylether (GP I): The according starting material (2.0 mmol) was dissolved in 10 mL of dry dichloromethane and a saturated solution of hydrochloric acid in diethyl ether (4 mL) was added. After three hours of stirring at room temperature the HCl gas was removed in a N_2 stream. All volatiles were evaporated at reduced pressure. The residue was taken up in a minimum amount of dichloromethane and the product was precipitated carefully by slow addition of diethyl ether. The solution was decanted off the precipitate, it was washed once with diethyl ether, decanted off again and the product was finally dried under reduced pressure to furnish a hygroscopic powder.

General procedures for the Boc deprotection with TFA (GP II): The according starting material (2.0 mmol) was dissolved in 8 mL of dichloromethane and TFA (2 mL) was slowly added. After four hours of stirring at room temperature, all volatiles were evaporated at reduced pressure. The residue was taken up in a minimum amount of dichloromethane and the product was precipitated carefully by slow addition of diethyl ether. The solution was decanted off the precipitate and the product was dried in the vacuum to furnish hygroscopic sticky syrup.

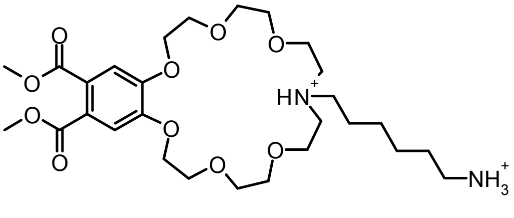
14-[4-Amino-butyl]-6,7,9,10,13,14,15,16,18,19,21,22-dodecahydro-12H-5,8,11,17,20,23-hexaoxa-14-aza-benzocyclohenicosene-2,3-dicarboxylic acid dimethylester hydrochloride ***(4b)***



The according Boc protected compound **E-1b** (1.28 g, 2.0 mmol) was deprotected according to *GP I*. After drying a yellow, hygroscopic solid (1.11 g, 1.81 mmol, 91 %) is obtained.

M.p. = 63 – 64 °C - **^{1}H-NMR** (300 MHz, $CDCl_3$): δ [ppm] = 1.82 (m, 2 H), 1.95 (m, 2 H), 3.03 (m, 2 H), 3.27 (m, 2 H), 3.41 – 3.60 (m, 4 H), 3.60 – 3.75 (m, 8 H), 3.75 – 4.05 (m, 8 H), 3.81 (s, 6 H), 4.16 (m, 4 H), 7.14 (s, 2 H), 8.24 (bs, 3 H, NH_3^+), 10.03 (bs, 1 H, NH^+); - **^{13}C-NMR** (75 MHz, $CDCl_3$): δ [ppm] = 21.2 (-, 1 C), 24.3 (-, 1 C), 39.1 (-, 1 C), 52.6 (+, 2 C), 53.4 (-, 1 C), 54.2 (-, 2 C), 65.5 (-, 2 C), 68.9 (-, 2 C), 69.4 (-, 2 C), 70.4 (-, 2 C), 70.8 (-, 2 C), 113.3 (+, 2 C), 125.4 (Cquat, 2 C), 150.2 (C_{quat}, 2 C), 167.7 (C_{quat}, 2 C); - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 543.4 (100, MH^+), 272.1 (6, $(M+2H^+)^{2+}$); - **MF:** $C_{26}H_{44}N_2O_{10}Cl_2$ – **FW:** 615.55 g/mol;

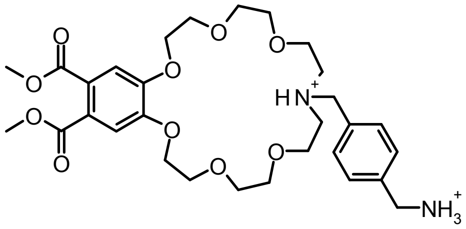
14-[6-Amino-hexyl]-6,7,9,10,13,14,15,16,18,19,21,22-dodecahydro-12H-5,8,11,17,20,23-hexaoxa-14-aza-benzocyclohenicosene-2,3-dicarboxylic acid dimethylester hydrochloride ***(4c)***



Compound **E-1c** (1.34 g, 2.0 mmol) was deprotected according to *GP I.* After drying a yellow, hygroscopic solid (1.11 g, 1.74 mmol, 87 %) is obtained.

M.p. = 66 – 68 °C - **^{1}H-NMR** (300 MHz, $CDCl_3$): δ [ppm] = 1.29 (m, 2 H), 1.39 (m, 2 H), 1.73 (m, 4 H), 2.93 (m, 2 H), 3.19 (m, 2 H), 3.21 – 3.75 (m, 14 H), 3.75 – 4.03 (m, 8 H), 3.81 (s, 6 H), 4.16 (m, 4 H), 7.14 (s, 2 H), 8.16 (bs, 3 H, NH_3^+), 10.15 (bs, 1 H, NH^+); - **^{13}C-NMR** (75 MHz, $CDCl_3$): δ [ppm] = 23.0 (-, 1 C), 25.4 (-, 1 C), 25.6 (-, 1 C), 26.4 (-, 1 C), 39.6 (-, 1 C), 52.7 (+, 2 C), 53.3 (-, 1 C), 54.6 (-, 2 C), 65.4 (-, 2 C), 68.7 (-, 2 C), 69.4 (-, 2 C), 70.4 (-, 2 C), 70.7 (-, 2 C), 113.1 (+, 2 C), 124.5 (Cquat, 2 C), 150.1 (C_{quat}, 2 C), 167.7 (C_{quat}, 2 C); - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 571.3 (100, MH^+), 296.2 (17, $(M+2H^+)^{2+}$); - **MF:** $C_{28}H_{48}N_2O_{10}Cl_2$ – **FW:** 643.60 g/mol;

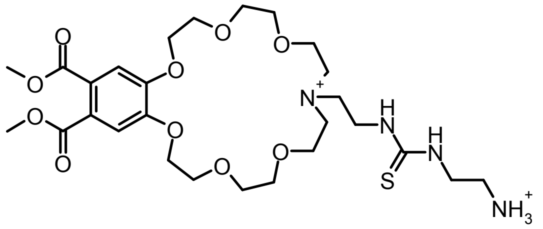
14-[(4-Aminomethyl)phenyl)methyl-amino]-6,7,9,10,13,14,15,16,18,19,21,22-dodecahydro-12H-5,8,11,17,20,23-hexaoxa-14-aza-benzocycloheneicosene-2,3-dicarboxylic acid dimethylester hydrochloride **(4d)**



Compound **E-1d** (1.38 g, 2.00 mmol) was reacted according to *GP I.* The product was dried at reduced pressure to yield **4d** as a yellow, hygroscopic solid (1.27 g, 1.75 mmol, 96 %).

M.p. = 70 – 71 °C - **^{1}H-NMR** (300 MHz, $CDCl_3$): δ [ppm] = 3.32 (m, 4 H), 3.48 – 3.72 (m, 8 H), 3.72 – 4.22 (m, 14 H), 3.79 (s, 6 H), 4.52 (m, 2 H), 6.96 (s, 2 H), 7.35 (m, 2 H), 7.46 (m, 2 H), 8.40 (bs, 1 H), 10.55 (bs, 1 H); - **^{13}C-NMR** (75 MHz, $CDCl_3$): δ [ppm] = 43.1 (-, 1 C), 52.8 (+, 2 C), 57.8 (-, 1 C), 65.7 (-, 2 C), 68.5 (-, 4 C), 69.3 (-, 2 C), 70.2 (-, 2 C), 70.4 (-, 2 C), 112.6 (+, 2 C), 125.1 (C_{quat}, 2 C), 129.9 (C_{quat}, 1 C), 130.1 (+, 2 C), 132.2 (+, 2 C), 134.2 (C_{quat}, 1 C), 149.7 (C_{quat}, 2 C), 167.7 (C_{quat}, 2 C); - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 591.4 (100, MH^+), 296.1 (19, MH_2^{2+}); - **MF:** $C_{30}H_{44}N_2O_{10}Cl_2$ – **FW:** 663.59 g/mol;

*14-[2-(3-(2-Aminoethyl)thioureido)ethyl]-6,7,9,10,13,14,15,16,18,19,21,22-dodecahydro-12H-5,8,11,17,20,23-hexaoxa-14-aza-benzocycloheneicosene-2,3-dicarboxylic acid dimethyl ester hydrochloride **(4e)***



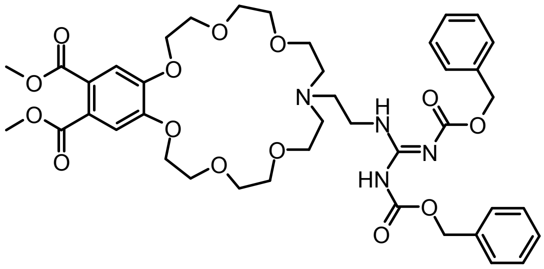
Compound **6** (1.43 g, 1.00 mmol) was deprotected after *GP I.* The product was dried at reduced pressure to furnish a yellow, hygroscopic solid (1.37 g, 1.65 mmol, 83 %).

M.p. (uncorrected) = 74 – 75 °C; - **^{1}H-NMR** (300 MHz, $CDCl_3$): δ [ppm] = 2.95 – 3.05 (m, 2 H), 3.30 (m, 2 H), 3.53 – 3.72 (m, 14 H), 3.77 – 4.01 (m, 10 H), 3.82 (s, 6 H), 4.14 – 4.19 (m, 4 H), 7.16 (s, 2 H), 7.86 (bs, 1 H), 8.23 (bs, 3 H), 9.74 (bs, 1 H); - **^{13}C-NMR** (75 MHz, $CDCl_3$): δ [ppm] = 39.8 (-, 1 C), 41.7 (-, 1 C), 52.7 (+, 2 C), 53.7 (-, 1 C), 53.8 (-, 1 C), 65.5 (-, 2 C), 68.9 (-, 2 C), 69.5 (-, 2 C), 70.4 (-, 4 C), 70.7 (-, 2 C), 113.5 (+, 2 C), 125.4 (C_{quat}, 2 C), 150.3 (C_{quat}, 2 C), 167.8 (C_{quat}, 2 C), 182.8 (C_{quat}, 1 C); - **IR** (KBr): ν [cm^{-1}] = 3226 (bm), 2937 (m), 2882 (m), 2184 (w), 2062 (w), 1935 (w), 1720 (m), 1550 (m), 1521 (m), 1437 (m), 1349 (m), 1288 (s), 1195 (s), 1125 (s), 1062 (m), 977 (m), 918 (m), 782 (m), 729 (m), 643 (m), 602 (m); - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 617.3 (63 %, MH^+), 309.1 (100%, $(M + 2H^+)^{2+}$); - **MF:** $C_{27}H_{46}N_4O_{10}SCl_2$ – **FW:** 689.65 g/mol;

1.3. Syntheses and deprotections of the receptors

1.3.1. Cbz protected guanidines

*14-[2-[2,3-di-(Benzyloxycarbonyl)-guanidino]-ethyl]-6,7,9,10,13,14,15,16,18,19,21,22-dodecahydro-12H-5,8,11,17,20,23-hexaoxa-14-aza-benzocycloheneicosene-2,3-dicarboxylic acid dimethyl ester (**E-2**)*

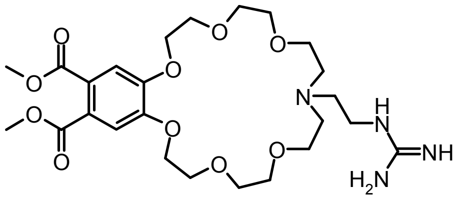


Compound **4a** (0.59 g, 1.0 mmol) was stirred with triethylamine (0.4 g, 4.0 mmol) in DMF (20 mL) for 15 minutes at room temperature. 1,3-Bis(benzyloxycarbonyl)-2-methyl-2-thiopseudourea (0.72 g, 2.0 mmol) and mercury(II)chloride (0.54 g, 2.00 mmol) were subsequently added in several small portions. The mixture was again stirred at room temperature over night. The reaction mixture was poured into 50 mL of water and the aqueous phase was extracted trice with 30 mL of DCM. The combined organic phases were washed two times with 20 mL of brine. After drying over $MgSO_4$, the solvent was evaporated and the residue was purified via column chromatography (ethanol / ethyl acetate 3:1) to yield a clear, yellow oil (710 mg, 0.861 mmol, 86 %) ($R_f = 0.2$).

^{1}H-NMR (400 MHz, $CDCl_3$): δ [ppm] = 2.70 (m, 2 H), 2.78 (m, 4 H), 3.46 (m, 2 H), 3.58 (m, 4H), 3.60 – 3.64 (m, 4 H), 3.72 – 3.76 (m, 4 H), 3.86 (s, 6 H), 3.87 – 3.94 (m, 4 H), 4.16 – 4.21 (m, 4 H), 5.10 (s, 2 H), 5.15 (s, 2 H), 7.18 (s, 2 H), 7.28 – 7.39 (m, 10 H), 8.63 (bs, 1 H), 11.68 (bs, 1 H) - **^{13}C-NMR** (100 MHz, $CDCl_3$): δ [ppm] = 39.0 (-, 1 C), 52.5 (+, 2 C), 53.4 (-, 1 C), 54.0 (-, 2 C), 67.0 (-, 1 C), 67.9 (-, 1 C), 69.4 (-, 2 C), 69.6 (-, 2 C), 70.7 (-, 2 C), 71.1 (-, 2 C), 113.7 (+, 2 C), 125.3 (C_{quat}, 2 C), 127.8 (+, 2 C), 128.1 (+, 2 C), 128.2 (+, 2 C), 128.3 (+, 2 C), 128.4 (+, 2 C), 128.6 (+, 2 C), 134.7 (C_{quat}, 1 C), 136.8 (C_{quat}, 1 C), 150.5 (C_{quat}, 2 C), 153.4 (C_{quat}, 1 C), 155.7 (C_{quat}, 1 C), 163.6 (C_{quat}, 1 C), 167.8 (C_{quat}, 2 C); - **IR** (KBr): ν [cm^{-1}] = 3320 (bm), 2947 (m), 2867 (m), 1723 (m), 1636 (m), 1586 (m), 1520 (m), 1433 (m), 1380 (m), 1346 (m), 1284 (s), 1199 (s), 1123 (s), 1046 (s), 979 (m), 944 (m), 909 (m), 802 (m), 726 (s), 697 (s), 645 (m); - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%)

= 825.5 (100, MH$^+$); - **HRMS** (PI-LSIMS FAB, glycerine): calc. for $C_{41}H_{53}N_4O_{14}^+$: 757.3558, found: 825.3546; - **MF:** $C_{41}H_{52}N_4O_{14}$ – **FW:** 824.89 g/mol;

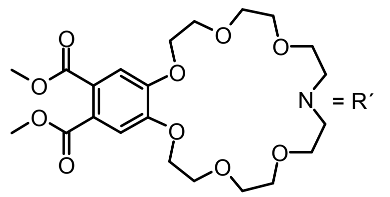
*14-[2-Guanidino-ethyl]-6,7,9,10,13,14,15,16,18,19,21,22-dodecahydro-12H-5,8,11,17,20,23-hexaoxa-14-aza-benzocycloheneicosene-2,3-dicarboxylic acid dimethyl ester (**E-3**)*

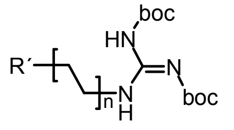


Compound **E-2** (206 mg, 0.25 mmol) were dissolved in methanol (10 mL) and three spatula tips of palladium catalyst on charcoal were added. After stirring for two days under 20 bar of hydrogen the catalyst was filtered off, washed trice with methanol and the solvent was evaporated to give the product **E-3** (140 mg, 0.24 mmol, 97 %) as a clear, faintly yellow glass.

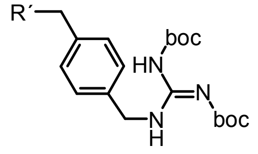
^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 2.60 – 2.65 (m, 6H), 3.16 – 3.21 (m, 2H), 3.48 – 3.53 (m, 4 H), 3.59 – 3.62 (m, 4 H), 3.69 – 3.73 (m, 4 H), 3.81 – 3.92 (m, 4 H), 3.86 (s, 6 H), 4.20 – 4.26 (m, 4 H), 7.17 (s, 2 H); - **^{13}C-NMR** (75 MHz, $CDCl_3$): δ [ppm] = 41.6 (-, 1 C), 52.7 (+, 2 C), 56.0 (-, 2 C), 57.0 (-, 1 C), 68.8 (-, 2 C), 69.1 (-, 2 C), 69.2 (-, 2 C), 70.8 (-, 2 C), 71.3 (-, 2 C), 113.3 (+, 2 C), 125.7 (C_{quat}, 2 C), 149.8 (C_{quat}, 2 C), 159.2 (C_{quat}, 1 C), 167.6 (C_{quat}, 2 C); - **IR** (KBr): ν [cm^{-1}] = 3360 (bm), 2948 (m), 2874 (m), 1719 (m), 1663 (m), 1598 (m), 1519 (m), 1435 (m), 1349 (m), 1285 (s), 1194 (s), 1184 (s), 1122 (s), 1029 (s), 976 (m), 945 (m), 888 (m), 827 (m), 780 (m), 730 (m), 660 (m); - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 557.4 (100, MH$^+$); - **HRMS** (PI-LSIMS FAB, glycerine): calc. for $C_{25}H_{41}N_4O_{10}^+$: 557.2823, found: 557.2825; - **MF:** $C_{25}H_{40}N_4O_{10}$ – **FW:** 556.62 g/mol;

1.3.2. General procedure for synthesis of the bis-Boc-protected guanidine moiety (isothiourea route, GP III)

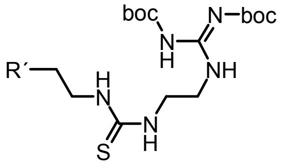




13a n = 1
13b n = 2
13c n = 3



13d

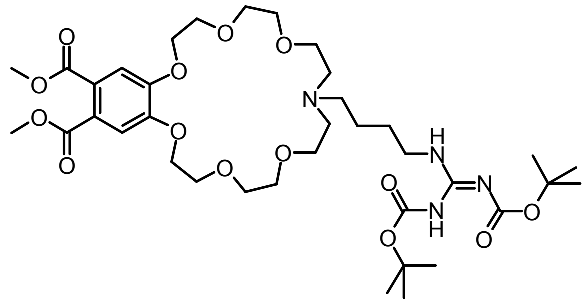


13e

The according crown ether amino acid hydrochloride (0.3 mmol, approx. 200 mg) or TFA salt (0.3 mmol, approx. 300 mg) was dissolved in 5 mL of dried chloroform, freshly distilled triethylamine (1.0 mmol, 101 mg, 0.14 mL) was dropped in and the mixture was stirred for 20 min. at room temperature. After addition of 290 mg of 1,3-bis(Boc)-2-methyl-2-isothiourea (1.0 mmol), the well stirred solution was to heated to reflux under nitrogen atmosphere and held at this temperature for two days.

The solution was cooled to room temperature, diluted with 30 mL of ethyl acetate and filtered. The filtrate was washed with saturated ammonium chloride solution (10 mL) and water (10 mL), dried over magnesium sulfate and the solvent was evaporated. After vacuum drying the crude product was purified by column chromatography on silica gel with ethyl acetate/ethanol or chloroform/methanol as eluent. (*R*f ca. 0.2 to 0.3 in EtOAc / EtOH 3:1).

*14-[4-[2,3-di-(^{tert}Butyloxycarbonyl)-guanidino]-butyl]-6,7,9,10,13,14,15,16,18,19,21,22-dodecahydro-12H-5,8,11,17,20,23-hexaoxa-14-aza-benzocycloheneicosene-2,3-dicarboxylic acid dimethyl ester **(13b)***

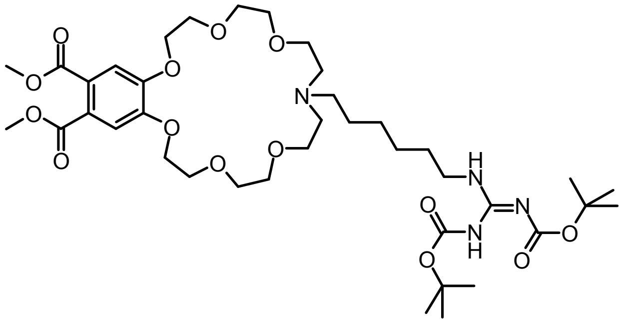


Compound **4b** (185 mg, 0.30 mmol) was reacted according to *GP III* to give the product as a faintly yellow glass (176 mg, 0.224 mmol, 75 %) (*R*f [$CHCl_3$ / MeOH 10:1] = 0.2).

^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.41 (s, 9 H), 1.43 (s, 9 H), 1.45 – 1.49 (m, 4 H), 2.55 (m, 2 H), 2.76 – 2.78 (m, 4 H), 3.33 – 3.35 (m, 2 H), 3.54 - 3.62 (m, 8 H), 3.66 - 3.69 (m, 4 H), 3.81 (s, 6 H), 3.82 – 3.86 (t, 4 H), 4.12 – 4.16 (t, 4 H), 7.13 (s, 2 H), 8.24 (bs, 1 H); -

^{13}**C-NMR** (75 MHz, $CDCl_3$): δ [ppm] = 26.8 (+, 1C), 28.1 (+, 3 C), 28.3 (+, 3 C), 40.6 (-, 1 C), 52.6 (+, 2 C), 53.8 (-, 2 C), 55.1 (-, 1 C), 61.2 (-, 1 C), 69.3 (-, 4 C), 69.5 (-, 2 C), 70.6 (-, 2 C), 71.1 (-, 2 C), 79.2 (C_{quat}, 1 C), 83.1 (C_{quat}, 1 C), 113.6 (+, 2 C), 125.3 (C_{quat}, 2 C), 150.5 (C_{quat}, 2 C), 153.2 (C_{quat}, 1 C), 156.2 (C_{quat}, 1 C), 163.6 (C_{quat}, 1 C), 167.8 (C_{quat}, 2 C); - **IR** (KBr): ν [cm^{-1}] = 3329 (bm), 2937 (m), 2870 (m), 1726 (m), 1631 (m), 1593 (m), 1520 (m), 1433 (m), 1414 (m), 1327 (m), 1284 (s), 1180 (s), 1124 (s), 1051 (m), 981 (m), 915 (m), 876 (m), 803 (m), 778 (m), 730 (m), 650 (m); - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 785.5 (31, MH^+), 393.3 (100, $(M+2H^+)^{2+}$); - **UV** (MeOH): λ (ε) = 267 (7300), 224 (30300); - **HRMS** (PI-LSIMS FAB glycerine): calc. for $C_{37}H_{61}N_4O_{14}^+$: 785.4184, found: 785.4181; - **MF:** $C_{37}H_{60}N_4O_{14}$ – **FW:** 784.91 g/mol;

*14-[6-[2,3-di-(^{tert}Butyloxycarbonyl)-guanidino]-hexyl]-6,7,9,10,13,14,15,16,18,19,21,22-dodecahydro-12H-5,8,11,17,20,23-hexaoxa-14-aza-benzocycloheneicosene-2,3-dicarboxylic acid dimethyl ester (**13c**)*

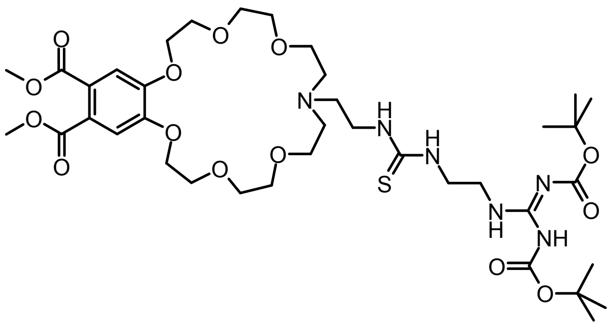


Compound **4c** (195 mg, 0.30 mmol) is submitted to *GP III* to yield a yellow glass (124 mg, 0.153 mmol, 51 %) (R_f [$CHCl_3$ / MeOH 10:1] = 0.26).

^{1}**H-NMR** (300 MHz, $CDCl_3$): δ [ppm] = 1.29 (m, 4 H), 1.48 (s, 9 H), 1.49 (s, 9 H), 1.40 – 1.49 (m, 4 H), 2.51 (m, 2 H), 2.78 (m, 4 H), 3.34 – 3.37 (m, 2 H), 3.56 - 3.70 (m, 8 H), 3.72 - 3.78 (m, 4 H), 3.86 (s, 6 H), 3.88 – 3.91 (t, 4 H), 4.16 – 4.21 (t, 4 H), 7.13 (s, 2 H), 8.24 (bs, 1 H), 11.61 (bs, 1 H); - ^{13}**C-NMR** (75 MHz, $CDCl_3$): δ [ppm] = 26.8 (-, 1 C), 26.9 (-, 1C), 27.1 (-, 1 C), 28.1 (+, 3 C), 28.3 (+, 3 C), 29.0 (-, 1 C), 40.9 (-, 1C), 52.6 (+, 2 C), 53.9 (-, 2 C), 55.6 (-, 1 C), 69.3 (-, 2 C), 69.5 (-, 4 C), 70.7 (-, 2 C), 71.1 (-, 2 C), 79.2 (C_{quat}, 1 C), 83.1 (C_{quat}, 1 C), 113.6 (+, 2 C), 125.4 (C_{quat}, 2 C), 150.5 (C_{quat}, 2 C), 153.3 (C_{quat}, 1 C), 156.1 (C_{quat}, 1 C), 163.7 (C_{quat}, 1 C), 167.8 (C_{quat}, 2 C); - **IR** (KBr): ν [cm^{-1}] = 3334 (bm), 2934 (m), 2868 (m), 1721 (m), 1633 (m), 1591 (m), 1519 (m), 1415 (m), 1328 (m), 1284 (s), 1126 (s), 1053 (s), 982 (m), 916 (m), 805 (m), 779 (m), 731 (m), 646 (m); - **MS** (ESI-MS,

CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 813.5 (72 %, MH^+), 393.2 (100 %, $(M+2H^+)^{2+}$); - **UV** (MeOH): λ (ε) = 267 (7300), 223 (30500); - **HRMS** (PI-LSIMS FAB glycerine): calc. for $C_{39}H_{65}N_4O_{14}^+$: 813.4497, found: 813.4495; - **MF:** $C_{39}H_{64}N_4O_{14}$ – **FW:** 812.96 g/mol;

*14-[2-(3-(2-(2,3-di-(^{tert}Butyloxycarbonyl)-guanidinoethyl)thioureido)ethyl]-6,7,9,10,13,14,15,16,18,19,21,22-dodecahydro-12H-5,8,11,17,20,23-hexaoxa-14-aza-benzocycloheneicosene-2,3-dicarboxylic acid dimethyl ester (**13e**)*



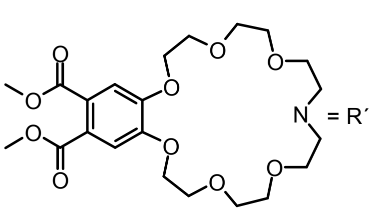
Conversion of compound **4e** (210 mg, 0.30 mmol) according *GP III* yields **13e** as a faintly yellow glass (107 mg, 0.126 mmol, 42 %) (R_f [$CHCl_3$ / MeOH 12:1] = 0.26; R_f [EtOAc / EtOH 3:1] = 0.1).

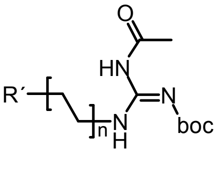
Alternative route:

Compound **17** (170 mg, 0.3 mmol) was stirred with compound **18** (182 mg, 0.6 mmol) in dry ethanol for 1 h at room temperature, then the solution was slowly heated to reflux and held at this temperature over night. The solvent was evaporated and the oily residue was purified by column chromatography with ethyl acetate / ethanol 6:1. The product appears as yellow, sticky oil (166 mg, 0.193 mmol, 65 %).

^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.43 (s, 9 H), 1.46 (s, 9 H), 2.71 – 2.83 (m, 6 H), 3.53 – 3.55 (m, 4 H), 3.56 – 3.64 (m, 8 H), 3.67 – 3.74 (m, 4 H), 3.79 – 3.86 (m, 4 H), 3.84 (s, 6 H), 4.12 – 4.20 (m, 4 H), 7.14 (s, 2 H), 8.53 (bs, 1 H), 9.91 (bs, 1 H), 10.01 (bs, 1 H); - **^{13}C-NMR** (75 MHz, $CDCl_3$): δ [ppm] = 28.1 (+, 3 C), 28.4 (+, 3 C), 29.3 (-, 1 C), 40.2 (-, 1 C), 43.6 (-, 1 C), 52.6 (+, 2 C), 53.8 (-, 1 C), 54.0 (-, 2 C), 69.3 (-, 2 C), 69.6 (-, 2 C), 70.2 (-, 2 C), 70.7 (-, 2 C), 71.2 (-, 2 C), 79.1 (C_{quat}, 1 C), 81.2 (C_{quat}, 1 C), 113.3 (+, 2 C), 125.4 (C_{quat}, 2 C), 150.5 (C_{quat}, 2 C), 153.0 (C_{quat}, 1 C), 163.4 (C_{quat}, 1 C), 166.0 (C_{quat}, 2 C), 167.8 (C_{quat}, 1 C); - **IR** (KBr): ν [cm^{-1}] = 3326 (m), 3299 (m), 2922 (m), 2867 (m), 2132 (m), 1724 (m), 1617 (m), 1577 (m), 1520 (m), 1434 (m), 1411 (m), 1346 (m), 1285 (s), 1196 (s), 1133 (s), 1101 (s), 1052 (s), 1028 (m), 914 (m), 864 (m), 731 (m), 658 (m); - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 859.5 (11, MH^+), 430.1 (100, $(M+2H^+)^{2+}$); - **UV** (MeOH): λ (ε) = 267 (7600), 223 (31600); - **MF:** $C_{38}H_{62}N_6O_{14}S$ – **FW:** 859.01 g/mol;

1.3.3. General procedure for the synthesis of the receptors with acetyl guanidine moiety (isothiourea route, GP IV):





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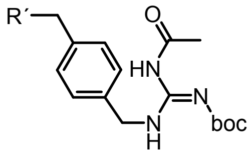
15a n = 2
15a n = 3

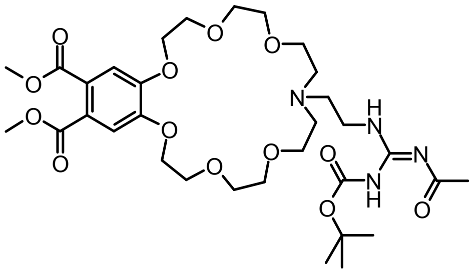


15d

The according crown ether amino acid hydrochloride or tfa salt (0.15 mmol, approx. 100 mg for the hydrochloride or approx. 150 mg for the tfa salt) was dissolved in 3.0 mL of dried chloroform, freshly distilled triethylamine (0.5 mmol, 51 mg, 0.07 mL) was added and the mixture was stirred for 20 min. at room temperature. After addition of 145 mg of 1-Boc-3-acetyl-2-methyl-2-isothiourea (0.5 mmol), the well stirred solution was heated to 40°C under nitrogen atmosphere and held at this temperature over night.

The solution was cooled to room temperature, diluted with 20.0 mL of ethyl acetate and filtered. The filtrate was washed with saturated ammonium chloride solution (5 mL) and water (5 mL), dried over magnesium sulfate and the solvent was evaporated. After vacuum drying the crude product was purified by column chromatography on silica gel with ethyl acetate / ethanol as eluent. (R_f ca. 0.2 to 0.3 in EtOAc / EtOH 3:1).

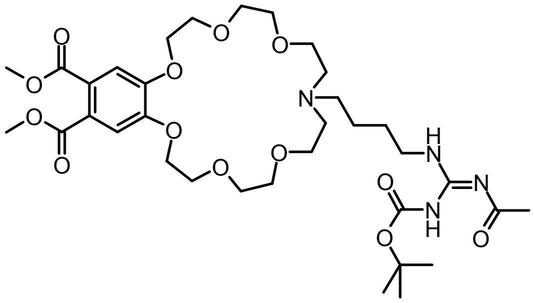
*14-[2-[2-(^{tert}Butyloxycarbonyl)-3-acetyl-guanidino]-ethyl]-6,7,9,10,13,14,15,16,18,19,21,22-dodecahydro-12H-5,8,11,17,20,23-hexaoxa-14-aza-benzocycloheneicosene-2,3-dicarboxylic acid dimethyl ester (**15a**)*



Submitting compound **4a** (176 mg, 0.30 mmol) to *GP IV* gives a yellow glass as the product (145 mg, 0.207 mmol, 69 %).

^{1}H-NMR (300 MHz, CDCl$_3$): δ [ppm] = 1.41 (s, 9 H), 2.11 (s, 3 H), 2.62 (m, 2 H), 2.71 (m, 4 H), 3.36 – 3.42 (m, 2 H), 3.49 – 3.62 (m, 8 H), 3.65 – 3.71 (m, 4 H), 3.84 (s, 6 H), 3.85 – 3.92 (m, 4 H), 4.12 – 4.18 (m, 4 H), 7.12 (s, 2 H), 8.57 (bs, 1 H), 9.11 (bs, 1 H); - **^{13}C-NMR** (75 MHz, CDCl$_3$): δ [ppm] = 25.0 (+, 1 C), 28.3 (+, 3 C), 38.7 (-, 1 C), 52.7 (+, 2 C), 53.4 (-, 1 C), 53.9 (-, 2 C), 69.3 (-, 2 C), 69.5 (-, 2 C), 70.0 (-, 2 C), 71.1 (-, 2 C), 79.4 (C$_{quat}$, 1 C), 113.7 (+, 2 C), 125.3 (C$_{quat}$, 2 C), 150.5 (C$_{quat}$, 2 C), 155.8 (C$_{quat}$, 1 C), 163.7 (C$_{quat}$, 1 C), 167.8 (C$_{quat}$, 2 C), 171.3 (C$_{quat}$, 1 C); - **IR** (KBr): ν [cm-1] = 3296 (bm), 2946 (m), 2872 (m), 1720 (m), 1643 (m), 1605 (m), 1516 (m), 1435 (m), 1346 (m), 1284 (s), 1182 (s), 1128 (s), 1052 (s), 978 (m), 954 (m), 877 (m), 803 (m), 775 (m), 734 (m), 661 (m); - **MS** (ESI-MS, CH$_2$Cl$_2$/MeOH + 10 mmol NH$_4$OAc): m/z (%) = 699.3 (100, MH$^+$), 599.2 (7, MH$^+$-Boc), 350.2 (19, (M+2H$^+$)$^{2+}$); - **UV** (MeOH): λ (ε) = 269 (7500), 222 (30100); - **HRMS** (PI-LSIMS FAB glycerine): calc. for $C_{32}H_{51}N_4O_{13}^+$: 699.3453, found: 699.3442; - **MF:** $C_{32}H_{50}N_4O_{13}$ – **FW:** 698.77 g/mol;

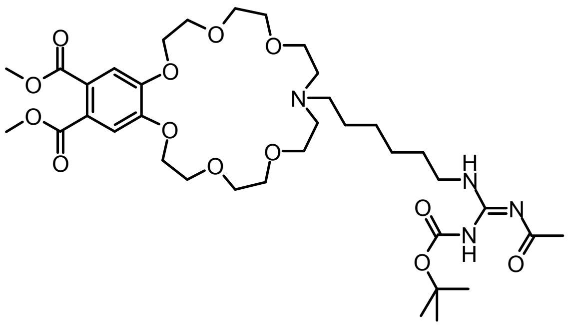
*14-[4-[2-(^{tert}Butyloxycarbonyl)-3-acetyl-guanidino]-butyl]-6,7,9,10,13,14,15,16,18,19,21,22-dodecahydro-12H-5,8,11,17,20,23-hexaoxa-14-aza-benzocycloheneicosene-2,3-dicarboxylic acid dimethyl ester (**15b**)*



Compound **4b** (185 mg, 0.30 mmol) was reacted after *GP IV* to yield the product **15b** as a yellow glass (161 mg, 0.222 mmol, 74 %).

^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.40 (s, 9 H), 1.39 – 1.49 (m, 4 H), 2.16 (s, 3 H), 2.46 (m, 2 H), 2.72 – 2.79 (m, 4 H), 3.21 (m, 2 H), 3.43 (m, 2 H), 3.52 - 3.56 (m, 4 H), 3.59 - 3.64 (m, 4 H), 3.73 - 3.77 (m, 4 H), 3.82 (s, 6 H), 3.84 – 3.86 (t, 4 H), 4.15 – 4.19 (t, 4 H), 7.16 (s, 2 H), 7.72 (bs, 1 H), 8.91 (bs, 1 H); - **^{13}C-NMR** (75 MHz, $CDCl_3$): δ [ppm] = 25.1 (+, 1 C), 26.8 (-, 1 C), 28.3 (+, 3 C), 39.6 (-, 1 C), 40.7 (-, 1 C), 52.6 (+, 2 C), 53.9 (-, 2 C), 54.8 (-, 1C), 69.3 (-, 2 C), 69.5 (-, 4 C), 70.7 (-, 2 C), 71.1 (-, 2 C), 79.6 (C_{quat}, 1 C), 113.7 (+, 2 C), 125.4 (C_{quat}, 2 C), 150.5 (C_{quat}, 2 C), 153.1 (C_{quat}, 1C), 156.0 (C_{quat}, 1 C), 167.8 (C_{quat}, 2 C), 172.0 (C_{quat}, 1 C); - **IR** (KBr): ν [cm^{-1}] = 3320 (bm), 2935 (m), 2872 (m), 1717 (m), 1644 (m), 1601 (m), 1568 (m), 1520 (m), 1436 (m), 1350 (m), 1285 (s), 1245 (s), 1192 (s), 1125 (s), 1056 (m), 978 (m), 945 (m), 877 (m), 778 (m), 732 (m), 642 (m); - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 727.3 (48, MH^+), 365.2 (100, $(M+2H^+)^{2+}$); - **UV** (MeOH): λ (ε) = 268 (7200), 222 (29800); - **HRMS** (PI-LSIMS FAB glycerine): calc. for $C_{34}H_{55}N_4O_{18}^+$: 727.3766, found: 727.3756; - **MF:** $C_{34}H_{54}N_4O_{13}$ – **FW:** 726.83 g/mol;

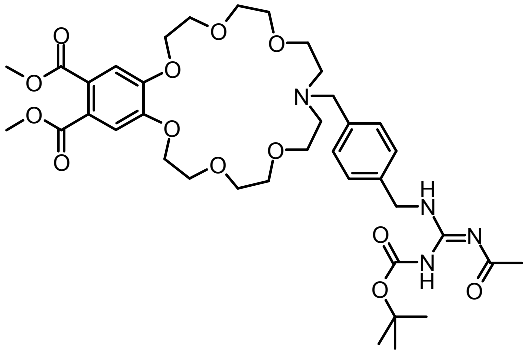
*14-[6-[2-(^{tert}Butyloxycarbonyl)-3-acetyl-guanidino]-hexyl]-6,7,9,10,13,14,15,16,18,19,21,22-dodecahydro-12H-5,8,11,17,20,23-hexaoxa-14-aza-benzocycloheneicosene-2,3-dicarboxylic acid dimethyl ester (**15c**)*



Compound **4c** (195 mg, 0.30 mmol) gives a yellow glass by reaction after *GP IV* (97 mg, 0.129 mmol, 43 %).

^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.28 (m, 4 H), 1.40 (s, 9 H), 1.38 – 1.46 (m, 4 H), 2.16 (s, 3 H), 2.46 (m, 2 H), 2.78 (m, 4 H), 3.33 – 3.38 (m, 2 H), 3.56 - 3.61 (m, 4 H), 3.62 - 3.68 (m, 4 H), 3.70 - 3.78 (m, 4 H), 3.85 (s, 6 H), 3.88 – 3.94 (m, 4 H), 4.15 – 4.22 (m, 4 H), 7.13 (s, 2 H), 8.32 (bs, 1 H), 8.87 (bs, 1 H); - **^{13}C-NMR** (75 MHz, $CDCl_3$): δ [ppm] = 25.1 (+, 1 C), 26.8 (-, 1 C), 27.1 (-, 1 C), 28.1 (+, 3 C), 28.3 (-, 1 C), 28.9 (-, 1 C), 40.8 (-, 2 C), 52.6 (+, 2 C), 53.8 (-, 2 C), 69.3 (-, 2 C), 69.5 (-, 4 C), 70.6 (-, 2 C), 71.0 (-, 2 C), 79.6 (C_{quat}, 1 C), 113.7 (+, 2 C), 125.4 (C_{quat}, 2 C), 150.5 (C_{quat}, 2 C), 156.0 (C_{quat}, 1 C), 163.9 (C_{quat}, 1 C), 167.8 (C_{quat}, 2 C), 172.0 (C_{quat}, 1 C); - **IR** (KBr): ν [cm^{-1}] = 3301 (bm), 2934 (m), 2865 (m), 1720 (m), 1643 (m), 1609 (m), 1572 (m), 1519 (m), 1435 (m), 1323 (m), 1285 (s), 1173 (s), 1126 (s), 1057 (s), 978 (m), 946 (m), 879 (m), 802 (m), 777 (m), 660 (m); - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 755.3 (44, MH^+), 378.9 (100, $(M+2H^+)^{2+}$); - **UV** (MeOH): λ (ε) = 267 (7300), 224 (28900); - **HRMS** (PI-LSIMS FAB glycerine): calc. for $C_{36}H_{59}N_4O_{13}^+$: 755.4079, found: 755.4077; - **MF:** $C_{36}H_{58}N_4O_{13}$ – **FW:** 754.88 g/mol;

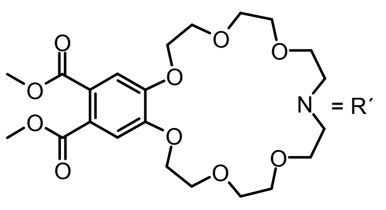
*14-[4-[2-(^{tert}Butyloxycarbonyl)-3-acetyl-guanidino-methyl]-benzyl]-6,7,9,10,13,14,15,16,18,19,21,22-dodecahydro-12H-5,8,11,17,20,23-hexaoxa-14-aza-benzocycloheneicosene-2,3-dicarboxylic acid dimethyl ester (**15d**)*

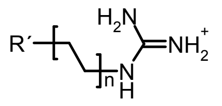


Compound **4d** (203 mg, 0.30 mmol) was converted to the product after *GP IV*. It appears as a yellow glass (170 mg, 0.219 mmol, 73 %).

^{1}H-NMR (300 MHz, CDCl$_3$): δ [ppm] = 1.49 (s, 9 H), 2.18 (s, 3 H), 2.79 (m, 4 H), 3.49 – 3.72 (m, 10 H), 3.70 – 3.77 (m, 4 H), 3.86 (s, 6 H), 3.87 – 3.94 (m, 4 H), 4.17 – 4.22 (m, 4 H), 4.51 (d, 2 H, J = 5.3 Hz), 7.18 (s, 2 H), 7.16 – 7.25 (d, 2 H, J = 4.6 Hz), 7.26 – 7.34 (d, 2 H, J = 4.6 Hz), 8.61 (bs, 1 H), 9.14 (bs, 1 H); - **^{13}C-NMR** (75 MHz, CDCl$_3$): δ [ppm] = 25.1 (+, 1 C), 28.3 (+, 3 C), 44.6 (-, 1 C), 52.6 (+, 2 C), 53.6 (-, 2 C), 58.9 (-, 1 C), 69.2 (-, 2 C), 69.4 (-, 2 C), 69.5 (-, 2 C), 70.5 (-, 2 C), 71.0 (-, 2 C), 79.7 (C$_{quat}$, 1 C), 113.6 (+, 2 C), 125.4 (C$_{quat}$, 3 C), 127.6 (+, 2 C), 129.5 (+, 2 C), 136.9 (C$_{quat}$, 1 C), 138.7 (C$_{quat}$, 1 C), 150.4 (C$_{quat}$, 2 C), 155.9 (C$_{quat}$, 1 C), 163.8 (C$_{quat}$, 1 C), 167.8 (C$_{quat}$, 2 C), 172.0 (C$_{quat}$, 1 C); - **IR** (KBr): ν [cm^{-1}] = 3292 (bm), 2945 (m), 2873 (m), 1719 (m), 1643 (m), 1518 (m), 1434 (m), 1350 (m), 1318 (m), 1286 (s), 1126 (s), 1059 (m), 977 (m), 916 (m), 882 (m), 802 (m), 778 (m), 729 (m), 657 (m); - **MS** (ESI-MS, CH$_2$Cl$_2$/MeOH + 10 mmol NH$_4$OAc): m/z (%) = 775.3 (27, MH$^+$), 388.2 (100, (M+2H$^+$)$^{2+}$); - **UV** (MeOH): λ (ε) = 268 (7600), 224 (30200); - **HRMS** (PI-LSIMS FAB glycerine): calc. for C$_{36}$H$_{59}$N$_4$O$_{13}$$^+$: 775.3766, found: 775.3768; - **MF:** C$_{38}$H$_{54}$N$_4$O$_{13}$ – **FW:** 774.87 g/mol;

1.3.4. General procedure for the Boc deprotection of the crown ether guanidines (GP V)





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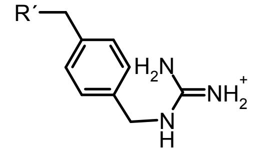
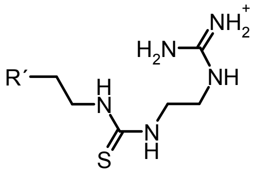
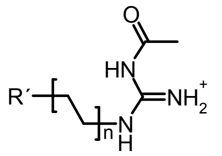


14d



14e



16a n = 1
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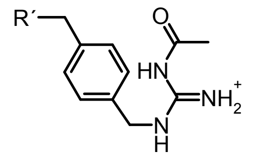
16c n = 3



16d

The according starting material (0.1 mmol) was dissolved in 0.5 mL of dry dichloromethane and a saturated solution of hydrochloric acid in diethyl ether (0.3 mL) was added. After four hours of stirring at room temperature the HCl gas was removed by bubbling N_2 through the solution. All volatiles were evaporated at reduced pressure. The residue was taken up in a minimum amount of dichloromethane and the product was precipitated carefully by slow addition of diethyl ether. The solution was decanted off the precipitate, it was washed once with diethyl ether, decanted off again and the product was dried in the vacuum to furnish a hygroscopic powder.

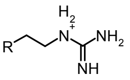
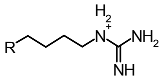
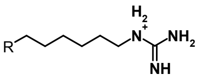
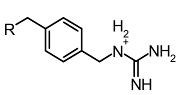
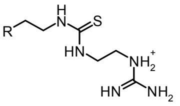
					
Starting material	89 mg	91 mg	94 mg	96 mg	100 mg
Yield of product	96 mg (96 %)	98 mg (94 %)	83 mg (78 %)	103 mg (95 %)	84 mg (76 %)
Appearance	Yellow powder	Yellow powder	Pale yellow powder	Pale yellow powder	Orange powder
Mp. (not corr.)	72 – 74 °C	74 – 75 °C	72 – 73 °C	81 – 83 °C	84 – 88 °C

Table S-2: Amounts of starting material, yields and appearance of the products (hydrochloride salts)

*14-[4-Guanidino-butyl]-6,7,9,10,13,14,15,16,18,19,21,22-dodecahydro-12H-5,8,11,17,20,23-hexaoxa-14-aza-benzocycloheneicosene-2,3-dicarboxylic acid dimethyl ester hydrochloride **(14b)***

Mp. = 74 – 75 °C (uncorrected); - **^{1}H-NMR** (600 MHz, MeOD): δ [ppm] = 1.27 – 1.39 (m, 2 H), 1.42 – 1.58 (m, 2 H), 1.66 – 1.76 (m, 2 H), 3.22 (m, 2 H), 3.38 – 3.54 (m, 4 H), 3.57 – 3.78 (m, 8 H), 3.79 – 3.95 (m, 8 H), 3.84 (s, 6 H), 4.19 – 4.33 (m, 4 H), 7.29 (s, 2 H); - **^{13}C-NMR** (150 MHz, MeOD): δ [ppm] = 20.4 (-, 1 C), 29.7 (-, 1 C), 40.3 (-, 1 C), 52.8 (+, 2 C), 53.1 (-, 2 C), 53.4 (-, 1 C), 64.8 (-, 2 C), 68.4 (-, 2 C), 69.3 (-, 2 C), 70.2 (-, 2 C), 70.6 (-, 2 C), 113.0 (+, 2 C), 125.5 (C_{quat}, 2 C), 149.9 (C_{quat}, 2 C), 157.6 (C_{quat}, 1 C), 167.8 (C_{quat}, 2 C); - **IR** (KBr): ν [cm^{-1}] = 3381 (bm), 2922 (m), 2876 (m), 1682 (m), 1601 (m), 1520 (m), 1437 (m), 1351 (m), 1289 (m), 1197 (s), 1129 (s), 1063 (m), 978 (m), 947 (m), 831 (m), 800 (m), 722 (m), 630 (m), 570 (m); - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 585.2 (53, MH$^+$), 293.1 (100 %, $(M+2H^+)^{2+}$); - **MF:** $C_{29}H_{40}N_4O_{10}Cl_2$ – **FW:** 653.57 g/mol;

*14-[6-Guanidino-hexyl]-6,7,9,10,13,14,15,16,18,19,21,22-dodecahydro-12H-5,8,11,17,20,23-hexaoxa-14-aza-benzocycloheneicosene-2,3-dicarboxylic acid dimethyl ester hydrochloride **(14c)***

Mp. = 72 – 73°C (uncorrected); - **^{1}H-NMR** (300 MHz, MeOD): δ [ppm] = 1.29 – 1.36 (m, 4 H), 1.59 (m, 2 H), 1.68 (m, 2 H), 3.14 (m, 2 H), 3.22 (m, 2 H), 3.69 - 3.74 (m, 4 H), 3.70 - 3.79 (m, 4H), 3.86 (s, 6 H), 3.89 – 3.93 (m, 8 H), 4.23 – 4.29 (m, 4 H), 7.29 (s, 2 H); - **^{13}C-NMR** (75 MHz, MeOD): δ [ppm] = 24.5 (-, 1 C), 27.3 (-, 2 C), 29.7 (-, 1 C), 41.9 (-, 1 C), 53.2 (+, 2 C), 54.4 (-, 2 C), 54.5 (-, 1 C), 61.1 (-, 2C), 67.2 (-, 2 C), 70.2 (-, 2 C), 70.7 (-, 2 C), 71.4 (-, 2 C), 71.8 (-, 2 C), 114.5 (+, 2 C), 126.8 (C_{quat}, 2 C), 151.8 (C_{quat}, 2 C), 163.8 (C_{quat}, 1 C), 169.3 (C_{quat}, 2 C); - **IR** (KBr): ν [cm^{-1}] = 3321 (bm), 3163 (m), 3016 (m), 2940 (m), 2880 (m), 2361 (w), 1691 (m), 1521 (m), 1435 (m), 1356 (m), 1290 (s), 1200 (s), 1129 (s), 1064 (s), 980 (m), 807 (m), 723 (m), 683 (m); - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 613.3 (19, MH$^+$), 307.1 (100, $(M+2H^+)^{2+}$); - **HRMS** (PI-LSIMS FAB glycerine): calc. for $C_{29}H_{49}N_4O_{10}^+$: 613.3437, found: 613.3431; - **MF:** $C_{29}H_{49}N_4O_{10}Cl_2$ – **FW:** 681.62 g/mol;

*14-[2-(3-(2-(2-Guanidino-ethyl)thioureido)ethyl]-6,7,9,10,13,14,15,16,18,19,21,22-dodecahydro-12H-5,8,11,17,20,23-hexaoxa-14-aza-benzocycloheneicosene-2,3-dicarboxylic acid dimethyl ester hydrochloride (**14e**)*

Mp. = 84 – 88 °C (uncorrected); - **^{1}H-NMR** (300 MHz, $CDCl_3$): δ [ppm] = 3.32 – 3.58 (m, 6 H), 3.59 – 3.79 (m, 8 H), 3.80 – 3.99 (m, 10 H), 3.87 (s, 6 H), 4.02 – 4.18 (m, 4 H), 4.19 – 4.37 (m, 6 H), 6.91 (bs, 1 H), 7.16 (s, 2 H), 7.92 (bs, 1 H), 9.51 (bs, 1 H), 9.68 (bs, 1 H), 12.86 (bs, 1 H); - **^{13}C-NMR** (75 MHz, $CDCl_3$): δ [ppm] = 40.2 (-, 1 C), 43.6 (-, 1 C), 52.8 (+, 2 C), 54.8 (-, 1 C), 54.8 (-, 2 C), 65.9 (-, 2 C), 68.6 (-, 2 C), 69.4 (-, 2 C), 70.2 (-, 1 C), 70.3 (-, 2 C), 70.6 (-, 2 C), 113.2 (+, 2 C), 125.4 (C_{quat}, 2 C), 150.9 (C_{quat}, 2 C), 154.2 (C_{quat}, 1 C), 167.8 (C_{quat}, 2 C), 180.2 (C_{quat}, 1 C); - **IR** (KBr): ν [cm^{-1}] = 3320 (m), 2930 (m), 1723 (m), 1677 (m), 1597 (m), 1524 (m), 1470 (m), 1438 (m), 1396 (m), 1355 (m), 1286 (m), 1264 (m), 1176 (s), 1132 (s), 1051 (m), 948 (m), 798 (m), 705 (m); - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 657.2 (23, MH^+), 328.4 (100, $(M+2H^+)^{2+}$); - **MF:** $C_{28}H_{46}N_6O_{14}SCl_2$ – **FW:** 727.67 g/mol;

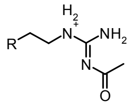
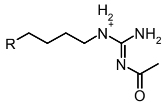
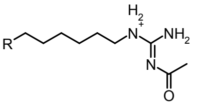
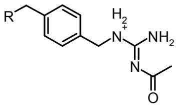
				
Starting material	93 mg	88 mg	98 mg	100 mg
Yield of product	82 mg (91 %)	63 mg (90 %)	82 mg (86 %)	90 mg (93 %)
Appearance	Slightly yellow powder	Yellow powder	Yellow powder	Pale yellow powder
Mp. (not corr.)	69 – 73 °C	71 – 73 °C	70 – 74 °C	78 – 82 °C

Table S-3: Amounts of starting material, yields, appearance and melting points of the products (hydrochloride salts)

*14-[2-[2-Acetyl-guanidino]-ethyl]-6,7,9,10,13,14,15,16,18,19,21,22-dodecahydro-12H-5,8,11,17,20,23-hexaoxa-14-aza-benzocycloheneicosene-2,3-dicarboxylic acid dimethyl ester hydrochloride (**16a**)*

Mp. = 69 – 73 °C (uncorrected); - **^{1}H-NMR** (600 MHz, $CDCl_3$): δ [ppm] = 2.11 (s, 3 H), 3.31 (m, 4 H), 3.45 (m, 2 H), 3.62 – 3.76 (m, 8 H), 3.82 (m, 2 H), 3.86 – 3.93 (m, 8 H), 3.86 (s, 6 H), 4.21 – 4.27 (m, 4 H), 7.18 (s, 2 H), 8.77 (bs, 1 H), 9.63 (bs, 2 H), 10.91 (bs, 1 H),; - **^{13}C-NMR** (150 MHz, $CDCl_3$): δ [ppm] = 24.1 (+, 1 C), 36.1 (-, 1 C), 51.8 (-, 1 C), 52.6 (+, 2 C), 54.0 (-, 2 C), 65.3 (-, 2 C), 68.6 (-, 2 C), 69.0 (-, 2 C), 70.3 (-, 2 C), 70.4 (-, 2 C), 113.2 (+, 2 C), 125.4 (C_{quat}, 2 C), 149.9 (C_{quat}, 2 C), 154.9 (C_{quat}, 1 C), 167.5 (C_{quat}, 2 C), 174.2 (C_{quat}, 1 C); - **IR** (KBr): ν [cm^{-1}] = 3292 (bm), 2948 (m), 2874 (m), 1722 (m), 1673 (m), 1598 (m), 1518 (m), 1435 (m), 1348 (m), 1286 (m), 1193 (s), 1127 (s), 1061 (m), 978 (m), 916 (m), 830 (m), 800 (m), 724 (m), 650 (m); - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 599.2 (43, MH^+), 300.1 (100, $(M+2H^+)^{2+}$); - **MF:** $C_{27}H_{43}N_4O_{11}Cl_2$ – **FW:** 669.56 g/mol;

*14-[4-[2-Acetyl-guanidino]-butyl]-6,7,9,10,13,14,15,16,18,19,21,22-dodecahydro-12H-5,8,11,17,20,23-hexaoxa-14-aza-benzocycloheneicosene-2,3-dicarboxylic acid dimethyl ester hydrochloride (**16b**)*

Mp. = 71 – 73 °C (uncorrected); - **^{1}H-NMR** (300 MHz, $CDCl_3$): δ [ppm] = 1.42 (m, 2 H), 1.69 (m, 2 H), 1.73 (m, 4 H), 2.19 (s, 3 H), 3.25 (m, 2H), 3.38 – 3.47 (m, 4 H), 3.61 – 3.75 (m, 8 H), 3.79 - 3.96 (m, 8 H), 3.87 (s, 6 H), 4.21 – 4.30 (m, 4 H), 7.16 (s, 2 H), 8.56 (bs, 1 H), 9.39 (bs, 1 H), 10.61 (bs, 2 H); - **^{13}C-NMR** (75 MHz, $CDCl_3$): δ [ppm] = 20.4 (-, 1 C), 24.2 (+, 1 C), 26.1 (-, 1 C), 40.3 (-, 1 C), 53.0 (+, 2 C), 53.0 (-, 2 C), 53.5 (-, 1C), 65.1 (-, 2 C), 68.4 (-, 2 C), 69.3 (-, 2 C), 70.1 (-, 2 C), 70.6 (-, 2 C), 113.2 (+, 2 C), 125.5 (C_{quat}, 2 C), 149.9 (C_{quat}, 2 C), 154.4 (C_{quat}, 1 C), 167.8 (C_{quat}, 2 C), 174.1 (C_{quat}, 1 C); -**IR** (KBr): ν [cm^{-1}] = 3320 (bm), 2922 (m), 2870 (m), 1682 (m), 1605 (m), 1520 (m), 1437 (m), 1351 (m), 1289 (s), 1197 (s), 1129 (s), 1063 (m), 975 (m), 948 (m), 831 (m), 800 (m), 722 (m); - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 627.2 (48, MH^+), 314.0 (100, $(M+2H^+)^{2+}$); - **MF:** $C_{29}H_{47}N_4O_{11}Cl_2$ – **FW:** 697.62 g/mol;

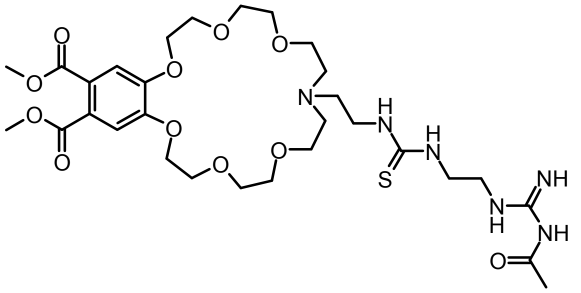
*14-[6-[2-Acetyl-guanidino]-hexyl]-6,7,9,10,13,14,15,16,18,19,21,22-dodecahydro-12H-5,8,11,17,20,23-hexaoxa-14-aza-benzocycloheneicosene-2,3-dicarboxylic acid dimethyl ester hydrochloride (**16c**)*

Mp. = 70 – 74 °C (uncorrected); - **^{1}H-NMR** (300 MHz, $CDCl_3$): δ [ppm] = 1.41 – 1.53 (m, 2 H), 1.54 – 1.86 (m, 6 H), 2.19 (s, 3 H), 3.02 – 3.21 (m, 4 H), 3.38 – 3.56 (m, 4 H), 3.67 – 3.75 (m, 8 H), 3.79 - 3.96 (m, 8 H), 3.88 (s, 6 H), 4.19 – 4.29 (m, 4 H), 7.18 (s, 2 H), 7.91 (bs, 1 H), 10.04 (bs, 1 H); - **^{13}C-NMR** (75 MHz, $CDCl_3$): δ [ppm] = 24.7 (+, 1 C), 27.7 (-, 2 C), 34.8 (-, 2 C), 42.1 (-, 1 C), 52.8 (+, 2 C), 54.8 (-, 2 C), 55.1 (-, 1 C), 66.3 (-, 2 C), 68.6 (-, 2 C), 69.5 (-, 2 C), 70.4 (-, 2 C), 70.6 (-, 2 C), 113.5 (+, 2 C), 125.4 (C_{quat}, 2 C), 149.8 (C_{quat}, 2 C), 153.1 (C_{quat}, 1 C), 167.8 (C_{quat}, 2 C), 176.3 (C_{quat}, 1 C); - **IR** (KBr): ν [cm^{-1}] = 3321 (bm), 2940 (m), 2845 (m), 1720 (m), 1691 (m), 1521 (m), 1435 (m), 1356 (m), 1290 (s), 1200 (s), 1129 (s), 1064 (s), 807 (m), 723 (m), 683 (m); - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 655.3 (12, MH^+), 327.9 (100, $(M+2H^+)^{2+}$); - **MF:** $C_{31}H_{51}N_4O_{11}Cl_2$ – **FW:** 725.67 g/mol;

*14-[4-[2-Acetyl-guanidino-methyl]-benzyl]-6,7,9,10,13,14,15,16,18,19,21,22-dodecahydro-12H-5,8,11,17,20,23-hexaoxa-14-aza-benzocycloheneicosene-2,3-dicarboxylic acid dimethyl ester hydrochloride (**16d**)*

Mp. = 78 – 82 °C (uncorrected); - **^{1}H-NMR** (600 MHz, $CDCl_3$): δ [ppm] = 2.29 (s, 3 H), 3.19 – 3.30 (m, 4 H), 3.68 – 3.76 (m, 8 H), 3.84 – 3.98 (m, 8 H), 3.88 (s, 6 H), 4.02 – 4.12 (m, 4 H), 4.40 (d, 2 H, J = 5.3 Hz), 4.67 (d, 2 H, J = 5.3 Hz), 6.91 (d, 2 H, J = 4.6 Hz), 6.89 (s, 2 H), 7.34 (d, 2 H, J = 4.6 Hz); - **^{13}C-NMR** (150 MHz, $CDCl_3$): δ [ppm] = 24.3 (+, 1 C), 43.8 (-, 1 C), 52.2 (-, 2 C), 52.9 (+, 2 C), 56.6 (-, 1 C), 65.5 (-, 2 C), 68.3 (-, 2 C), 69.2 (-, 2 C), 70.0 (-, 2 C), 70.2 (-, 2 C), 112.2 (+, 2 C), 124.7 (+, 2 C), 126.5 (C_{quat}, 2 C), 129.5 (C_{quat}, 1 C), 132.4 (+, 2 C), 135.6 (C_{quat}, 1 C), 150.0 (C_{quat}, 2 C), 155.1 (C_{quat}, 1 C), 168.2 (C_{quat}, 2 C), 174.7 (C_{quat}, 1 C); - **IR** (KBr): ν [cm^{-1}] = 3320 (bm), 2930 (m), 2870 (m), 1720 (m), 1676 (m), 1599 (m), 1519 (m), 1436 (m), 1352 (m), 1289 (s), 1198 (s), 1131 (s), 1058 (m), 980 (m), 914 (m), 801 (m), 725 (m), 642 (m); - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 675.2 (23, MH^+), 338.1 (100, $(M+2H^+)^{2+}$); - **MF:** $C_{33}H_{47}N_4O_{11}Cl_2$ – **FW:** 745.66 g/mol;

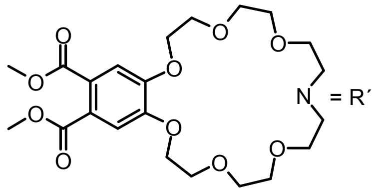
*14-[2-(3-(2-(2-Acetyl-guanidino-ethyl)thioureido)ethyl]-6,7,9,10,13,14,15,16,18,19,21,22-dodecahydro-12H-5,8,11,17,20,23-hexaoxa-14-aza-benzocycloheneicosene-2,3-dicarboxylic acid dimethyl ester (**16e**)*

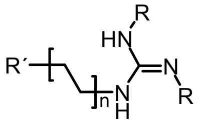


Compound **14e** (88 mg, 0.1 mmol) was dissolved in 1.0 mL of DCM containing 0.04 mL triethylamine (0.03 g, 0.3 mmol). Under cooling in an ice bath and vigorous stirring acetanhydride in DCM (0.1 M, 1.0 mL, 0.1 mmol) was added drop wise. The mixture was stirred for 3 h at 0 °C. The solvent was evaporated at reduced pressure. The remaining solid was purified by column chromatography with chloroform / methanol 6:1. The product was isolated as a colourless solid (63 mg, 90 %).

Mp. = 76 – 78°C (uncorrected); - **^{1}H-NMR** (300 MHz, MeOD): δ [ppm] = 2.18 (s, 3 H), 3.29 – 3.56 (m, 6 H), 3.61 – 3.70 (m, 8 H), 3.72 – 3.95 (m, 8 H), 3.84 (s, 6 H), 4.03 – 4.20 (m, 4 H), 4.22 – 4.33 (m, 6 H), 7.16 (s, 2 H); - **^{13}C-NMR** (75 MHz, MeOD): δ [ppm] = 24.6 (+, 1 C), 39.1 (-, 1 C), 42.8 (-, 1 C), 52.6 (+, 2 C), 53.6 (-, 1 C), 54.7 (-, 2 C), 65.1 (-, 2 C), 68.6 (-, 2 C), 69.7 (-, 2 C), 70.1 (-, 2 C), 70.3 (-, 1 C), 70.6 (-, 2 C), 113.1 (+, 2 C), 125.4 (C_{quat}, 2 C), 150.2 (C_{quat}, 2 C), 154.7 (C_{quat}, 1 C), 164.3 (C_{quat}, 2 C), 167.6 (C_{quat}, 2 C), 179.1 (C_{quat}, 1 C); - **IR** (KBr): ν [cm^{-1}] = 3320 (m), 2930 (m), 1721 (m), 1679 (m), 1598 (m), 1521 (m), 1472 (m), 1436 (m), 1401 (m), 1356 (m), 1286 (m), 1186 (s), 1131 (s), 1055 (m), 946 (m), 798 (m); - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 701.3 (28, MH^+), 350.2 (100, $(M+2H^+)^{2+}$); - **MF:** $C_{30}H_{48}N_6O_{11}S$ – **FW:** 700.67 g/mol;

1.3.5. General procedure for the synthesis of the N,N-dialkyl-substituted guanidine motifs (carbodiimide route, GP VI)





11a n = 1; R = iPr
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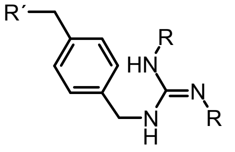
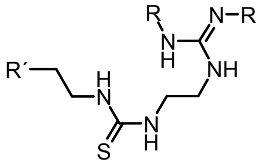
11b n = 2; R = iPr
12b n = 2; R = Cy
11c n = 3; R = iPr
12c n = 3; R = Cy



11d R = iPr
12d R = Cy

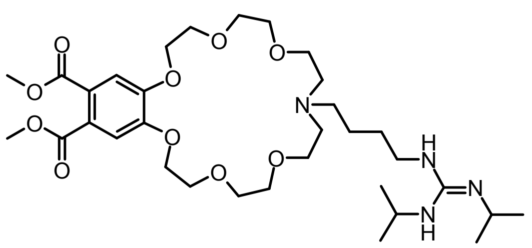


11e R = iPr
12e R = Cy

The according crown ether amino acid hydrochloride (0.3 mmol, approx. 200 mg) were dissolved in 10.0 mL of dry acetonitrile in a protective nitrogen atmosphere, freshly distilled DIPEA (1.0 mmol, 129 mg, 0.17 mL) was dropped in and the mixture was stirred 30 min. While cooling to 2 to 5°C DIC (190 mg) or DCC (310 mg) (1.5 mmol) were added in one portion and the solution was, after 2 hours of stirring in the ice bath, carefully heated to reflux temperature and held under dry conditions at this temperature for four days.

After cooling to room temperature, the reaction mixture was filtered over celite and the filter cake was washed several times with acetonitrile and dichloromethane. The solvent was evaporated; the crude product was thoroughly dried and purified by column chromatography with silica gel and chloroform/methanol as eluent. (R_f ca. 0.2 to 0.3 in chloroform / methanol 4:1).

*14-[4-[2,3-di-isopropyl-guanidino]-butyl]-6,7,9,10,13,14,15,16,18,19,21,22-dodecahydro-12H-5,8,11,17,20,23-hexaoxa-14-aza-benzocycloheneicosene-2,3-dicarboxylic acid dimethyl ester (**11b**)*

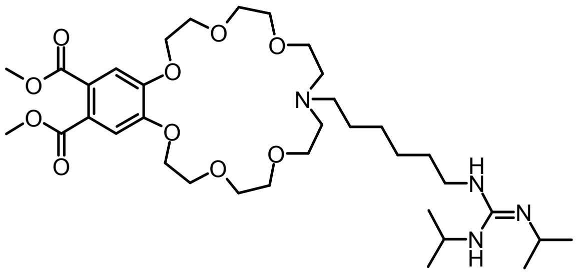


Compound **4b** (185 mg, 0.30 mmol) was reacted according to *GP VI.* The product is a yellow glass (116 mg, 0.174 mmol, 58 %) (R_f [$CHCl_3$ / MeOH 4:1] = 0.16).

^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.29 (d, 12 H, J = 6.3 Hz), 1.69 - 1.76 (m, 4 H), 3.03 (m, 2 H), 3.21 (m, 4 H), 3.40 (m, 2 H), 3.65 – 3.78 (m, 8 H), 3.81 – 3.92 (m, 8 H), 3.87 (s, 6

H), 4.19 – 4.23 (m, 4 H), 6.75 (s, 2 H), 7.20 (s, 2 H), 8.04 (bs, 2 H); - **^{13}C-NMR** (75 MHz, $CDCl_3$): δ [ppm] = 22.9 (+, 4 C), 26.6 (-, 1 C), 42.0 (-, 1 C), 45.7 (+, 2 C), 45.7 (-, 1 C), 52.7 (+, 2 C), 53.4 (-, 2 C), 54.1 (-, 1 C), 68.8 (-, 2 C), 69.4 (-, 2 C), 69.5 (-, 2 C), 70.4 (-, 2 C), 70.8 (-, 2 C), 113.4 (+, 2 C), 125.4 (C_{quat}, 2 C), 150.2 (C_{quat}, 2 C), 154.8 (C_{quat}, 1 C), 167.8 (C_{quat}, 2 C); - **IR** (KBr): ν [cm^{-1}] = 3220 (bm), 2934 (m), 2887 (m), 2197 (w), 1980 (w), 1720 (m), 1614 (m), 1519 (m), 1438 (m), 1350 (m), 1287 (s), 1196 (s), 1124 (s), 1058 (m), 978 (m), 910 (m), 798 (m), 723 (m), 693 (m); - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 669.4 (16, MH^+), 335.2 (100, $(M+2H^+)^{2+}$); - **UV** (MeOH): λ (ε) = 267 (8100), 223 (27800); - **HRMS** (EI-MS 70 eV): calc. for $C_{33}H_{57}N_4O_{10}^+$: 669.4075, found: 669.4086; - **MF:** $C_{33}H_{56}N_4O_{10}$ – **FW:** 668.84 g/mol;

*14-[6-[2,3-Di-isopropyl-guanidino]-hexyl]-6,7,9,10,13,14,15,16,18,19,21,22-dodecahydro-12H-5,8,11,17,20,23-hexaoxa-14-aza-benzocycloheneicosene-2,3-dicarboxylic acid dimethyl ester (**11c**)*

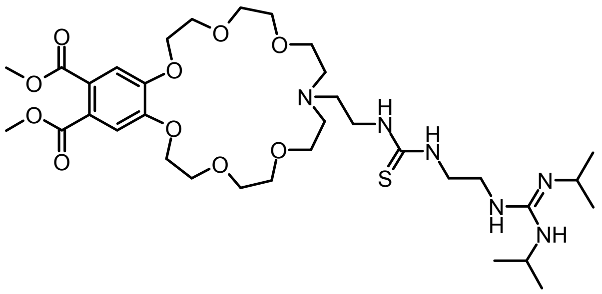


Compound **4c** (195 mg, 0.30 mmol) was reacted after *GP VI* to yield a faintly yellow oil (61 mg, 0.09 mmol, 29 %) (R_f [$CHCl_3$ / MeOH 4:1] = 0.17).

^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.20 – 1.31 (m, 4 H), 1.25 (d, 12 H, J = 6.4 Hz), 1.58 – 1.63 (m, 4 H), 2.98 - 3.04 (m, 2 H), 3.21 – 3.32 (m, 6 H), 3.59 – 3.67 (m, 8 H), 3.77 – 3.91 (m, 8 H), 3.83 (s, 6 H), 4.12 – 4.18 (m, 4 H), 6.78 (d, 2 H, J = 6.4 Hz), 7.15 (s, 2 H), 7.83 (bs, 1 H) - **^{13}C-NMR** (75 MHz, $CDCl_3$): δ [ppm] = 23.0 (+, 4 C), 24.1 (-, 1 C), 25.7 (-, 1 C), 25.8 (-, 1 C), 28.3 (-, 1 C), 42.7 (-, 1 C), 45.9 (+, 2 C), 52.7 (+, 2 C), 53.2 (-, 2 C), 55.1 (-, 1 C), 66.2 (-, 2 C), 68.8 (-, 2 C), 69.5 (-, 2 C), 70.5 (-, 2 C), 70.8 (-, 2 C), 113.3 (+, 2 C), 125.4 (C_{quat}, 2 C), 150.2 (C_{quat}, 2 C), 155.5 (C_{quat}, 1 C), 167.8 (C_{quat}, 2 C); - **IR** (KBr): ν [cm^{-1}] = 3194 (bm), 3071 (m), 2927 (m), 2871 (m), 2200 (w), 1721 (m), 1615 (m), 1519 (m), 1438 (m), 1349 (m), 1288 (s), 1194 (s), 1127 (s), 1060 (m), 978 (m), 925 (m), 804 (m), 730 (m), 644 (m); - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 697.4 (90 %,

MH^{+}), 349.2 (100 %, $(M+2H^{+})^{2+}$); - **UV** (MeOH): λ (ε) = 267 (8400), 224 (27600); - **HRMS** (EI-MS 70 eV): calc. for $C_{35}H_{60}N_4O_{10}^{*+}$: 696.4309, found: 696.4293; - **MF:** $C_{35}H_{60}N_4O_{10}$ – **FW:** 696.89 g/mol;

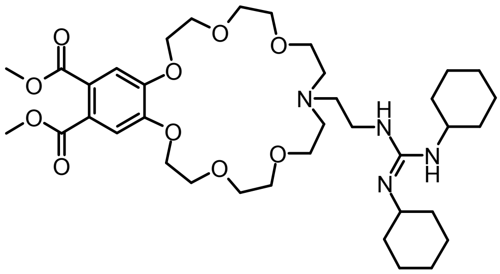
*14-[2-(3-(2-(2,3-Di-isopropyl-guanidino)ethyl)thioureido)ethyl]-6,7,9,10,13,14,15,16,18,19,21,22-dodecahydro-12H-5,8,11,17,20,23-hexaoxa-14-aza-benzocycloheneicosene-2,3-dicarboxylic acid dimethyl ester (**11e**)*



Conversion of **4e** (210 mg, 0.30 mmol) by *GP VI* yields the product appearing as a yellow oil (80 mg, 0.12 mmol, 36 %) (R_f [$CHCl_3$ / MeOH 4:1] = 0.23).

^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.23 (d, 12 H, J = 6.4 Hz), 2.78 (m, 6 H), 3.38 – 3.42 (m, 4 H), 3.48 – 3.62 (m, 8 H), 3.65 – 3.73 (m, 4 H), 3.82 (s, 6 H), 3.84 – 3.89 (m, 4 H), 3.91 – 4.03 (heptett, 2 H, J = 6.4 Hz), 4.17 – 4.22 (m, 4 H), 6.58 (m, 2 H), 7.15 (s, 2 H), 7.88 (bs, 1 H), 8.83 (bs, 1 H) - **^{13}C-NMR** (75 MHz, $CDCl_3$): δ [ppm] = 21.9 (+, 4 C), 40.0 (-, 1 C), 41.0 (-, 1 C), 44.1 (+, 2 C), 51.6 (+, 2 C), 52.3 (-, 1 C), 52.7 (-, 1 C), 53.5 (-, 2 C), 68.1 (-, 2 C), 68.5 (-, 4 C), 69.4 (-, 2 C), 70.0 (-, 2 C), 112.4 (+, 2 C), 124.4 (C_{quat}, 2 C), 149.3 (C_{quat}, 2 C), 152.5 (C_{quat}, 1 C), 166.8 (C_{quat}, 2 C), 181.9 (C_{quat}, 1 C); - **IR** (KBr): ν [cm^{-1}] = 3252 (bm), 3200 (m), 3075 (m), 2931 (m), 2859 (m), 2182 (w), 1722 (m), 1615 (s), 1519 (m), 1439 (m), 1350 (m), 1287 (s), 1196 (s), 1122 (s), 1055 (m), 981 (m), 917 (m), 801 (m), 729 (m), 647 (m); - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 743.3 (16, MH^{+}), 372.0 (100, $(M+2H^{+})^{2+}$); - **UV** (MeOH): λ (ε) = 267 (8400), 223 (29400); - **HRMS** (PI-LSIMS FAB glycerine): calc. for $C_{34}H_{59}N_6O_{10}S^{+}$: 743.4018, found: 743.4027; - **MF:** $C_{34}H_{58}N_6O_{10}S$ – **FW:** 742.94 g/mol;

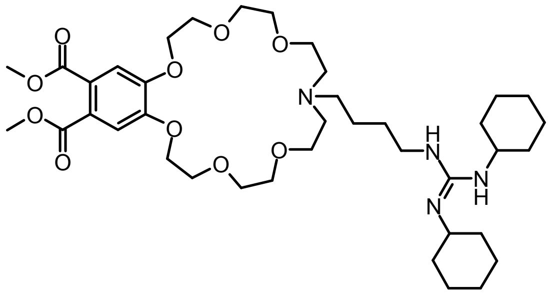
*14-[2-[2,3-Di-cyclohexyl-guanidino]-ethyl]-6,7,9,10,13,14,15,16,18,19,21,22-dodecahydro-12H-5,8,11,17,20,23-hexaoxa-14-aza-benzocycloheneicosene-2,3-dicarboxylic acid dimethyl ester (**12a**)*



Compound **4a** (176 mg, 0.30 mmol) was reacted according to *GP VI* to furnish the product **12a** as a yellow glassy solid (164 mg, 0.228 mmol, 76 %) (*R*$_f$ [EtOAc / MeOH 5:2] = 0.41; *R*$_f$ [EtOAc / EtOH 3:1] = 0.14).

^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.04 – 1.11 (m, 2 H), 1.30 – 1.39 (m, 8 H), 1.47 – 1.55 (m, 2 H), 1.64 (m, 4 H), 1.84 (m, 4 H), 2.76 (m, 4 H), 3.29 (m, 2 H), 3.52 – 3.58 (m, 10 H), 3.61 – 3.66 (m, 4 H), 3.78 – 3.83 (m, 4 H), 3.80 (s, 6 H), 4.12 – 4.17 (m, 4 H), 7.14 (s, 2 H), 7.72 (bs, 2 H);- **^{13}C-NMR** (75 MHz, $CDCl_3$): δ [ppm] = 24.8 (-, 4 C), 25.1 (-, 2 C), 33.1 (-, 2 C), 42.4 (-, 1 C), 51.6 (+, 2 C), 52.6 (+, 2 C), 54.2 (-, 2 C), 55.5 (-, 1 C), 69.1 (-, 4 C), 69.5 (-, 4 C), 70.3 (-, 2 C), 71.0 (-, 2 C), 113.7 (+, 2 C), 125.4 (C_{quat}, 2 C), 150.4 (C_{quat}, 2 C), 154.8 (C_{quat}, 1 C), 167.7 (C_{quat}, 2 C); - **IR** (KBr): ν [cm^{-1}] = 3270 (bm), 3140 (bm), 2929 (m), 2855 (m), 1717 (m), 1617 (m), 1518 (m), 1435 (m), 1347 (m), 1286 (s), 1247 (m), 1182 (s), 1124 (s), 1054 (m), 978 (m), 943 (m), 890 (m), 844 (m), 782 (m), 771 (m), 697 (m), 672 (m); - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 361.3 (8, $(M + 2H^+)^{2+}$), 721.5 (100, MH^+); - **UV** (MeOH): λ (ε) = 267 (8400), 224 (26400); - **HRMS** (PI-LSIMS FAB glycerine): calc. for $C_{37}H_{61}N_4O_{11}^+$: 721.4388, found: 721.4387; - **MF:** $C_{37}H_{60}N_4O_{10}$ – **FW:** 720.91 g/mol;

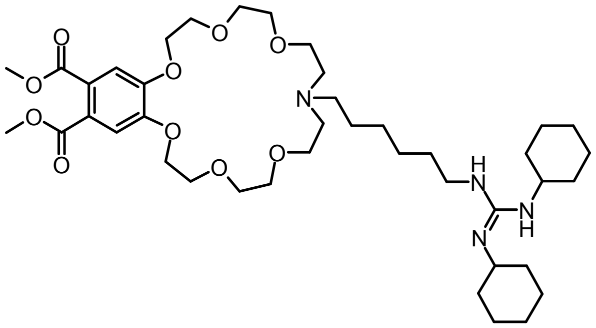
*14-[4-[2,3-Di-cyclohexyl-guanidino]-butyl]-6,7,9,10,13,14,15,16,18,19,21,22-dodecahydro-12H-5,8,11,17,20,23-hexaoxa-14-aza-benzocycloheneicosene-2,3-dicarboxylic acid dimethyl ester (**12b**)*



Compound **4b** (185 mg, 0.30 mmol) was submitted to *GP VI* to give **12b** as the product appearing as a yellow glass (139 mg, 0.186 mmol, 62 %) (R_f [$CHCl_3$ / MeOH 4:1] = 0.21).

^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.23 – 1.54 (m, 10 H), 1.62 – 1.68 (m, 8 H), 1.80 – 1.86 (m, 4 H), 2.88 (m, 2 H), 3.08 (m, 4 H), 3.32 (m, 2 H), 3.47 – 3.54 (m, 2 H), 3.59 – 3.63 (m, 4 H), 3.64 – 3.69 (m, 4 H), 3.71 – 3.76 (m, 4 H), 3.78 – 3.83 (m, 4 H), 3.81 (s, 6 H), 4.12 – 4.16 (m, 4 H), 6.77 (d, 2 H, 6.4 Hz), 7.14 (s, 2 H), 7.93 (bs, 1 H);- **^{13}C-NMR** (75 MHz, $CDCl_3$): δ [ppm] = 8.8 (+, 2C), 22.3 (-, 1 C), 24.9 (-, 4 C), 25.0 (-, 2 C), 26.6 (-, 1 C), 33.1 (-, 4 C), 42.3 (-, 1C), 45.9 (-, 1 C), 52.6 (+, 2 C), 53.4 (-, 2 C), 54.3 (-, 1 C), 67.0 (-, 2 C), 69.0 (-, 2 C), 70.4 (-, 2 C), 70.9 (-, 2 C), 113.4 (+, 2 C), 125.4 (C_{quat}, 2 C), 150.3 (C_{quat}, 2 C), 154.9 (C_{quat}, 1 C), 167.7 (C_{quat}, 2 C); - **IR** (KBr): ν [cm^{-1}] = 3188 (bm), 3080 (bm), 2928 (m), 2860 (m), 2184 (w), 1830 (w), 1721 (m), 1612 (m), 1519 (m), 1438 (m), 1351 (m), 1287 (s), 1195 (m), 1123 (s), 1058 (m), 978 (m), 916 (m), 781 (m), 727 (m), 643 (m); - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 375.1 (100 %, $(M+2H^+)^{2+}$), 749.4 (36 %, MH^+); - **UV** (MeOH): λ (ε) = 268 (8100), 224 (25900); - **HRMS** (PI-LSIMS FAB glycerine): calc. for $C_{39}H_{65}N_4O_{10}^+$: 749.4701, found: 749.4693; - **MF:** $C_{39}H_{64}N_4O_{10}$ – **FW:** 748.97 g/mol;

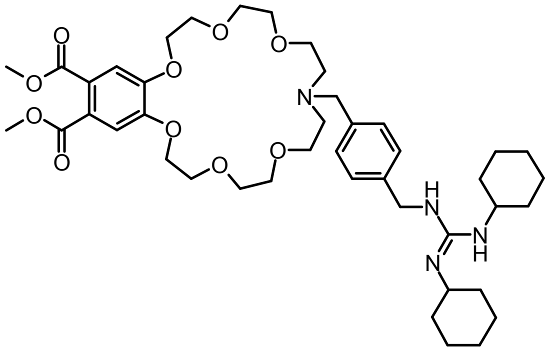
*14-[6-[2,3-Di-cyclohexyl-guanidino]-hexyl]-6,7,9,10,13,14,15,16,18,19,21,22-dodecahydro-12H-5,8,11,17,20,23-hexaoxa-14-aza-benzocycloheneicosene-2,3-dicarboxylic acid dimethyl ester (**12c**)*



Compound **4c** (195 mg, 0.30 mmol) converted to the product by *GP VI.* It appears as yellow, sticky oil (79 mg, 0.102 mmol, 34 %) (R_f [$CHCl_3$ / MeOH 4:1] = 0.18).

^{1}**H-NMR** (600 MHz, $CDCl_3$): δ [ppm] = 1.16 – 1.22 (m, 2 H), 1.31 – 1.39 (m, 6 H), 1.46 – 1.52 (m, 4 H), 1.58 – 1.61 (m, 2 H), 1.66 – 1.69 (m, 4 H), 1.74 – 1.78 (m, 4 H), 1.91 – 1.96 (m, 4 H), 3.02 (m, 2 H), 3.24 (m, 4 H), 3.32 (m, 2 H), 3.51 (m, 2 H), 3.66 – 3.69 (m, 4 H), 3.70 – 3.74 (m, 4 H), 3.82 – 3.85 (m, 4 H), 3.86 (s, 6 H), 3.88 – 3.92 (m, 4 H), 4.21 – 4.24 (m, 4 H), 6.79 (d, 2 H, J = 6.4 Hz), 7.13 (s, 2 H), 7.81 (bs, 1 H) - ^{13}**C-NMR** (150 MHz, $CDCl_3$): δ [ppm] = 24.5 (-, 1 C), 24.8 (-, 4 C), 25.0 (-, 2 C), 25.7 (-, 1 C), 25.9 (-, 1 C), 27.5 (-, 1 C), 33.1 (-, 4 C), 42.9 (-, 1 C), 52.6 (+, 2 C), 52.9 (+, 2 C), 53.2 (-, 2 C), 54.8 (-, 1 C), 66.7 (-, 2 C), 68.9 (-, 2 C), 69.5 (-, 2 C), 70.5 (-, 2 C), 70.8 (-, 2 C), 113.4 (+, 2 C), 125.4 (C_{quat}, 2 C), 150.3 (C_{quat}, 2 C), 155.6 (C_{quat}, 1 C), 167.8 (C_{quat}, 2 C); - **IR** (KBr): ν [cm^{-1}] = 3186 (bm), 3072 (m), 2929 (m), 2870 (m), 2180 (w), 1840 (w), 1722 (m), 1614 (m), 1520 (m), 1435 (m), 1352 (m), 1288 (s), 1196 (s), 1125 (s), 1056 (m), 978 (m), 918 (m), 781 (m), 724 (m), 644 (m); - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 777.5 (23 %, MH^+), 389.3 (100 %, $(M+2H^+)^{2+}$); - **HRMS** (PI-LSIMS FAB glycerine): calc. for $C_{41}H_{69}N_4O_{10}^+$: 777.5014, found: 777.5023; - **MF:** $C_{41}H_{68}N_4O_{10}$ – **FW:** 777.02 g/mol;

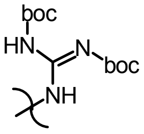
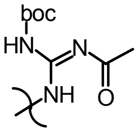
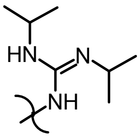
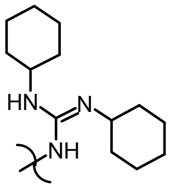
*14-[4-[2,3-Di-cyclohexyl-guanidino-methyl]-benzyl]-6,7,9,10,13,14,15,16,18,19,21,22-dodecahydro-12H-5,8,11,17,20,23-hexaoxa-14-aza-benzocycloheneicosene-2,3-dicarboxylic acid dimethyl ester (**12d**)*



Compound **4d** (203 mg, 0.30 mmol) was reacted according *GP VI.* The product is a yellow glass (163 mg, 0.204 mmol, 68 %) (R_f [$CHCl_3$ / MeOH 6:1] = 0.3).

^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.12 – 1.16 (m, 2 H), 1.33 – 1.44 (m, 8 H), 1.48 – 1.62 (m, 6 H), 1.83 – 1.89 (m, 4 H), 2.95 (m, 4 H), 3.63 – 3.70 (m, 8 H), 3.72 – 3.77 (m, 6 H), 3.88 (s, 6 H), 3.90 – 3.95 (m, 4 H), 4.17 – 4.24 (m, 4 H), 4.56 (d, 2 H, J = 5.3 Hz), 5.67 (m, 2 H), 7.17 (s, 2 H), 7.22 – 7.25 (d, 2 H, J = 4.6 Hz), 7.32 – 7.36 (d, 2 H, J = 4.6 Hz), 7.90 (bs, 1 H) - **^{13}C-NMR** (75 MHz, $CDCl_3$): δ [ppm] = 23.4 (-, 4 C), 23.9 (-, 2 C), 31.8 (-, 4 C), 43.9 (-, 1 C), 50.9 (+, 2 C), 51.8 (+, 2 C), 52.5 (-, 2 C), 57.7 (-, 1 C), 67.8 (-, 2 C), 68.4 (-, 4 C), 69.5 (-, 2 C), 69.8 (-, 2 C), 69.9 (-, 2 C), 112.2 (+, 2 C), 124.3 (C_{quat}, 2 C), 124.4 (+, 2 C), 126.4 (+, 2 C), 129.9 (C_{quat}, 1 C), 146.1 (C_{quat}, 1 C), 149.3 (C_{quat}, 1 C), 152.4 (C_{quat}, 1 C), 166.9 (C_{quat}, 2 C); - **IR** (KBr): ν [cm^{-1}] = 3145 (bm), 2930 (m), 2859 (m), 2193 (w), 1964 (w), 1720 (m), 1610 (m), 1517 (m), 1437 (m), 1350 (m), 1287 (s), 1196 (s), 1122 (s), 1058 (m), 978 (m), 913 (m), 782 (m), 727 (m), 643 (m); - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 797.5 (19, MH^+), 399.1 (100, $(M+2H^+)^{2+}$); - **UV** (MeOH): λ (ε) = 267 (7800), 223 (26900); - **HRMS** (PI-LSIMS FAB glycerine): calc. for $C_{43}H_{65}N_4O_{10}^+$: 797.4731, found: 797.4723; - **MF:** $C_{43}H_{64}N_4O_{10}$ – **FW:** 797.01 g/mol;

*Summary of all compounds for the screening library and their protected precursors: P*urification e.g. 6:1 → 4:1, below yield in mg and in %

	ethyl	***butyl***	***hexyl*[11]**	***p-xylyl***	***bis-ethyl-thioureido***
	*3:1* 195 mg 78 %	*3:1* 176 mg 75 %	*4:1 → 3:1* 124 mg 51 %	*4:1* 205 mg 82 %	*5:1* * 107 mg 42 %
R = boc → R = H	96 mg 96 %	98 mg 94 %	83 mg 78 %	103 mg 95 %	84 mg 76 %
	*3:1* 145 mg 69 %	*3:1* 161 mg 74 %	*3:1 → 5:2* 97 mg 43 %	*4:1* 170 mg 73 %	---
R = boc → R = H	82 mg 91 %	63 mg 90 %	82 mg 86 %	90 mg 93 %	71 mg 74 %
	*8:1 → 6:1* 133 mg 69 %	*8:1 → 6:1* 116 mg 58 %	*9:1 → 5:1* 61 mg 29 %	*6:1* 131 mg 61 %	*8:1 → 5:1* ** 80mg 36 %
	*8:1 → 6:1* 164 mg 76 %	*8:1 → 6:1* 139 mg 62 %	*10:1 → 5:1* 79 mg 34 %	*7:1 → 5:1* 163 mg 68 %	*9:1 → 5:1* ** 96 mg 39 %

A second column chromatography was necessary in the case of… with…: * ethyl acetate/ethanol 3:1; ** ethyl acetate/ethanol 3:2 → 1:1

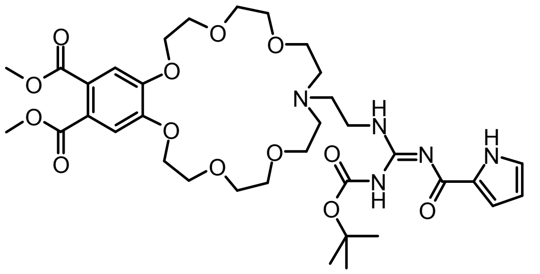
Table S-4: Yields and purification conditions for the small library

1.3.6. General procedure for the Synthesis of the pyrrole- and pyrene substituted motifs (isothiourea route, GP VII)

The according crown ether amino acid hydrochloride or tfa salt (0.2 mmol, approx. 200 mg as the tfa salt) was dissolved in 3.0 mL of dry DMF, freshly distilled triethylamine (1.0 mmol, 101 mg, 0.14 mL) was dropped in and the mixture was stirred for 20 min. at room temperature. After addition of the appropriate 2-methyl-2-isothiourea (0.3 mmol), the well stirred solution was heated to 40 °C under nitrogen atmosphere and held at this temperature over night.

The solution was cooled to room temperature, diluted with 30.0 mL of DCM and filtered. The filtrate was washed with saturated ammonium chloride solution (10 mL) and water (10 mL), dried over magnesium sulfate and the solvent was evaporated. After vacuum drying the crude product was purified by column chromatography on silica gel with ethyl acetate / ethanol as eluent. (R_f ca. 0.3 in EtOAc / EtOH 6:1).

*14-[2-[2-(^{tert}Butyloxycarbonyl)-3-(pyrrole-2-carbonyl)-guanidino]-ethyl]-6,7,9,10,13,14,15,16,18,19,21,22-dodecahydro-12H-5,8,11,17,20,23-hexaoxa-14-aza-benzocycloheneicosene-2,3-dicarboxylic acid dimethyl ester (**37**)*

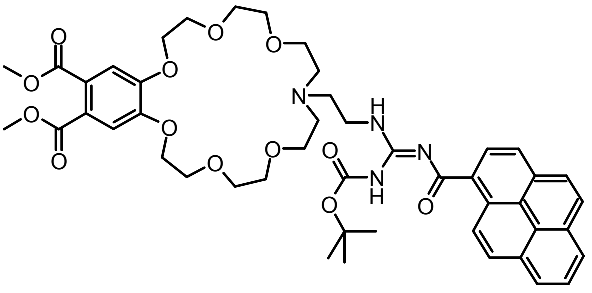


Compound **4a** (120 mg, 0.20 mmol) was submitted to *GP VII* to give the product as a yellow glass (101 mg, 0.135 mmol, 67 %). Ethanol / ethyl acetate 1:6 was used for purification.

^{1}H-NMR (400 MHz, $CDCl_3$): δ [ppm] = 1.49 (s, 6 H), 1.51 (s, 3 H), 2.61 – 2.90 (m, 6 H), 3.41 – 3.70 (m, 10 H), 3.71 – 3.80 (m, 4 H), 3.81 – 3.96 (m, 4 H), 3.86 (s, 6 H), 4.12 – 4.23 (m, 4 H), 6.21 (m, 1 H), 6.88 (m, 1 H), 6.92 (m, 1 H), 7.17 (s, 2 H), 8.59 (bs, 1 H), 9.56 (bs, 1 H); - **^{13}C-NMR** (100 MHz, $CDCl_3$): δ [ppm] = 28.0 (+, 2 C), 28.3 (+, 1 C), 39.0 (-, 1 C), 52.5 (+, 2 C), 53.3 (-, 1C), 54.4 (-, 2 C), 69.2 (-, 2 C), 69.3 (-, 2 C), 69.4 (-, 2 C), 70.5 (-, 2 C), 70.9 (-, 2 C), 82.7 (C_{quat}, 1 C), 110.2 (+, 1 C), 113.8 (+, 1 C), 113.9 (+, 2 C), 121.8 (+, 1 C), 125.3

(C_{quat}, 2 C), 131.2 (C_{quat}, 1 C), 150.4 (C_{quat}, 2 C), 153.1 (C_{quat}, 1 C), 155.9 (C_{quat}, 1 C), 167.8 (C_{quat}, 2 C), 171.0 (C_{quat}, 1 C); - **IR** (KBr): ν [cm^{-1}] = 3296 (bm), 2946 (m), 2874 (m), 1720 (m), 1614 (m), 1571 (m), 1408 (m), 1350 (s), 1287 (s), 1251 (m), 1193 (m), 1125 (s), 1061 (m), 1028 (s), 980 (m), 913 (m), 854 (m), 780 (m), 732 (m); - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 750.2 (100, MH^+), 375.7 (76, $(M+2H^+)^{2+}$); - **UV** (MeOH): λ (ε) = 301 (19100), 224 (28900); - **HRMS** (PI-LSIMS FAB, glycerine): calc. for $C_{38}H_{52}N_5O_{13}^+$: 750.3562, found: 750.3557; - **MF:** $C_{35}H_{51}N_5O_{13}$ – **FW:** 749.82 g/mol;

*14-[2-[2-(^{tert}Butyloxycarbonyl)-3-(pyrene-1-carbonyl)-guanidino]-ethyl]-6,7,9,10,13,14,15,16,18,19,21,22-dodecahydro-12H-5,8,11,17,20,23-hexaoxa-14-aza-benzocycloheneicosene-2,3-dicarboxylic acid dimethyl ester (**38**)*



Compound **4a** (120 mg, 0.20 mmol) was submitted to *GP VII*. The product is a yellow glass (73 mg, 0.082 mmol, 41 %). Ethanol / ethyl acetate 1:9 was used for purification.

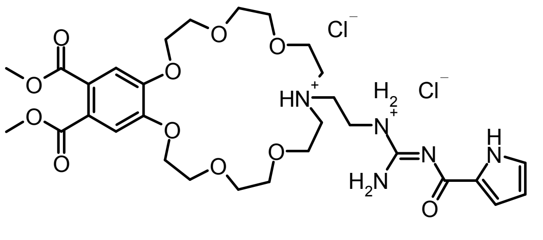
^{1}H-NMR (600 MHz, $CDCl_3$): δ [ppm] = 1.49 (s, 3 H), 1.52 (s, 6 H), 2.78 (m, 2 H), 2.86 (m, 4 H), 3.42 – 3.53 (m, 4 H), 3.55 – 3.69 (m, 4 H), 3.70 – 3.76 (m, 2 H), 3.77 – 3.89 (m, 8 H), 3.87 (s, 6 H), 3.90 – 4.01 (m, 4 H), 4.12 – 4.17 (m, 4 H), 7.14 (s, 2 H), 7.98 – 8.09 (m, 2 H), 8.10 – 8.19 (m, 3 H), 8.19 – 8.27 (m, 2 H), 8.66 (bs, 1 H), 8.81 (bs, 1 H), 9.21 (bs, 1 H), 9.60 (bs, 1 H), 12.56 (s, 1 H); - **^{13}C-NMR** (150 MHz, $CDCl_3$): δ [ppm] = 28.0 (+, 2 C), 28.2 (+, 1 C), 38.7 (-, 1 C), 52.4 (-, 1 C), 52.5 (+, 2 C), 53.7 (-, 2 C), 53.9 (-, 2 C), 54.4 (-, 1 C), 69.7 (-, 2 C), 70.1 (-, 2 C), 70.2 (-, 2 C), 70.5 (-, 2 C), 83.2 (C_{quat}, 1 C), 112.8 (+, 1 C), 114.0 (+, 2 C), 124.1 (+, 1 C), 124.7 (C_{quat}, 1 C), 125.3 (C_{quat}, 2 C), 125.3 (C_{quat}, 1 C), 125.5 (+, 1 C), 125.9 (+, 1 C), 126.1 (+, 1 C), 126.4 (+, 1 C), 126.7 (+, 1 C), 127.0 (C_{quat}, 1 C), 127.3 (+, 1 C), 128.2 (+, 1 C), 128.8 (+, 1 C), 130.5 (C_{quat}, 1 C), 131.2 (C_{quat}, 1 C), 150.2 (C_{quat}, 2 C), 153.3 (C_{quat}, 1 C), 156.0 (C_{quat}, 1 C), 163.8 (C_{quat}, 1 C), 167.6 (C_{quat}, 2 C), 180.8 (C_{quat}, 1 C); - **IR**

(KBr): ν [cm^{-1}] = 3313 (bm), 2947 (m), 2877 (m), 1720 (m), 1599 (m), 1573 (m), 1435 (m), 1342 (m), 1285 (s), 1251 (s), 1191 (m), 1128 (s), 1051 (s), 980 (m), 913 (m), 847 (m), 812 (m), 771 (m), 643 (m); - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 885.5 (100, MH+), 433.1 (56, $(M+2H^+)^{2+}$); - **UV** (MeOH): λ (ε) = 355 (27800), 282 (37100), 225 (74000); - **HRMS** (PI-LSIMS FAB, glycerine): calc. for $C_{47}H_{57}N_4O_{13}$+: 885.3922, found: 885.3919; - **MF:** $C_{47}H_{56}N_4O_{13}$ – **FW:** 884.99 g/mol;

General procedure for the Boc deprotection of the pyrrole- and pyrene substituted motifs *(GP VIII):*

The according starting material (0.1 mmol) was dissolved in 2.0 mL of dry, cold dichloromethane and a cold solution of HCl in diethylether (1.0 mL) was added. After four to six hours of stirring at room temperature under moisture protection, all volatiles were removed at reduced pressure. The residue was dissolved in a minimum amount of dichloromethane and the product was precipitated carefully by slow addition of diethyl ether. The solution was centrifuged, the diethyl ether was decanted off and the process was repeated. The product was vacuum dried to give a fine hygroscopic, faintly yellow powder. The yield is nearly quantitative.

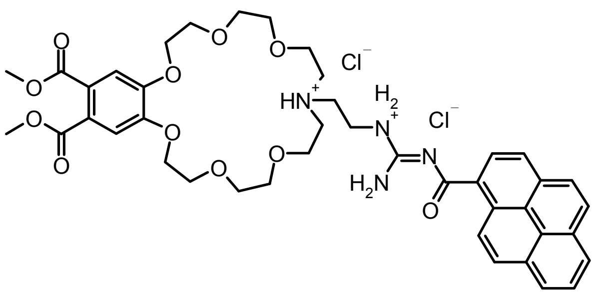
*14-[2-(Pyrrole-2-carbonyl)-guanidino]-ethyl]-6,7,9,10,13,14,15,16,18,19,21,22-dodecahydro-12H-5,8,11,17,20,23-hexaoxa-14-aza-benzocycloheneicosene-2,3-dicarboxylic acid dimethyl ester hydrochloride (**39**)*



Compound **37** (75 mg, 0.10 mmol) was deprotected after *GP VIII.* The product is a yellow, sticky oil (62 mg, 0.095 mmol, 95 %).

^{1}H-NMR (400 MHz, $CDCl_3$): δ [ppm] = 3.31 – 3.70 (m, 6 H), 3.71 – 3.79 (m, 6 H), 3.80 – 4.11 (m, 12 H), 3.84 (s, 6 H), 4.13 – 4.29 (m, 4 H), 6.29 (m, 1 H), 7.07 (m, 1 H), 7.21 (s, 2 H), 7.31 (m, 1 H), 9.89 (m, 1 H), 10.65 (bs, 1 H), 10.71 (bs, 1 H), 12.41 (bs, 1 H); - **^{13}C-NMR** (100 MHz, $CDCl_3$): δ [ppm] = 41.3 (-, 1 C), 51.7 (-, 1 C), 52.5 (+, 2 C), 54.1 (-, 2 C), 65.3 (-, 2 C), 65.8 (-, 2 C), 68.6 (-, 2 C), 69.6 (-, 2 C), 70.4 (-, 2 C), 111.2 (+, 1 C), 113.3 (+, 2 C), 117.8 (C_{quat}, 1 C), 118.8 (+, 1 C), 125.4 (C_{quat}, 2 C), 125.9 (+, 1 C), 149.9 (C_{quat}, 2 C), 155.1 (C_{quat}, 1 C), 160.8 (C_{quat}, 1 C), 167.5 (C_{quat}, 2 C); - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 650.2 (35, MH^+), 325.5 (100, $(M+2H^+)^{2+}$); - **UV** (MeOH): λ (ε) = 294 (19400), 222 (27800); - **HRMS** (PI-LSIMS FAB, glycerine): calc. for $C_{30}H_{44}N_5O_{11}^+$: 650.3037, found: 650.3052; - **MF:** $C_{30}H_{47}N_5O_{11}Cl_2$ – **FW:** 724.64 g/mol;

*14-[2-[2-(Pyrene-1-carbonyl)-guanidino]-ethyl]-6,7,9,10,13,14,15,16,18,19,21,22-dodecahydro-12H-5,8,11,17,20,23-hexaoxa-14-aza-benzocycloheneicosene-2,3-dicarboxylic acid dimethyl ester hydrochloride (**40**)*



Submitting **38** (89 mg, 0.10 mmol) to the deprotection conditions of *GP VII*, the product **40** was obtained as a yellow glass (71 mg, 0.09 mmol, 90 %).

^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 3.39 – 3.51 (m, 4 H), 3.61 (m, 2 H), 3.62 – 3.79 (m, 8 H), 3.82 (s, 6 H), 3.86 – 3.98 (m, 8 H), 4.00 (m, 2 H), 4.22 – 4.29 (m, 4 H), 7.21 (s, 2 H), 8.02 – 8.09 (m, 2 H), 8.16 – 8.21 (m, 3 H), 8.22 – 8.28 (m, 3 H), 8.71 (m, 1 H), 9.31 (bs, 1 H), 10.01 (bs, 1 H), 11.06 (bs, 1 H); - **^{13}C-NMR** (75 MHz, $CDCl_3$): δ [ppm] = 36.2 (-, 1 C), 51.8 (-, 1 C), 52.6 (+, 2 C), 54.1 (-, 2 C), 65.2 (-, 2 C), 68.5 (-, 2 C), 69.4 (-, 2 C), 70.3 (-, 2 C), 70.4 (-, 2 C), 113.2 (+, 2 C), 123.8 (+, 1 C), 124.0 (C_{quat}, 1 C), 124.2 (+, 1 C), 124.7 (C_{quat}, 1 C), 124.9 (C_{quat}, 1 C), 125.3 (+, 1 C), 125.5 (+, 1 C), 125.6 (+, 1 C), 125.6 (+, 1 C), 125.7 (C_{quat}, 2 C), 127.2 (+, 1 C), 129.9 (+, 1 C), 130.2 (C_{quat}, 1 C), 130.7 (+, 1 C), 130.8 (C_{quat}, 1 C), 149.9 (C_{quat}, 2 C), 155.7 (C_{quat}, 1 C), 167.6 (C_{quat}, 2 C), 171.6 (C_{quat}, 1 C); - **IR** (KBr): ν [cm^{-1}] = 3292 (bm), 2940 (m), 2899 (m), 1683 (m), 1596 (m), 1514 (m), 1436 (m), 1350 (m), 1286 (s), 1197 (s), 1131 (s), 1064 (m), 981 (m), 916 (m), 850 (m), 808 (m), 723 (m); - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 785.3 (46, MH^+), 393.1 (100, $(M+2H^+)^{2+}$); - **UV** (MeOH): λ (ε) = 351 (26300), 277 (35300), 222 (71200); - **HRMS** (PI-

LSIMS FAB, glycerine): calc. for $C_{42}H_{49}N_4O_{11}^+$: 785.3398, found: 785.3401; - **MF:** $C_{42}H_{52}N_5O_{11}Cl_2$ – **FW:** 859.81 g/mol;

1.4. Solid phase synthesis of the test peptides

Rink amide resin MBHA (novabiochem), piperidine (iris biotech), ethyl-diisopropyl-amine (DIPEA) (Fluka), dimethylformamide (DMF) (iris biotech), Fmoc amino acids (novabiochem), trifluoroacetic acid (TFA) (Fluka), triisopropylsilane (TIS) (novabiochem), 1-Ethyl-3-(3-dimethyllaminopropyl)carbodiimide (EDC) (Sigma Aldrich), O-Benzotriazole-*N,N,N',N'*-tetramethyl-uronium-hexafluoro-phosphate (HBTU) (novabiochem), 1-hydroxybenzotriazole (HOBt) (Sigma Aldrich), were bought and used without further purification.

All peptides were synthesized on Rink Amide MBHA resin using Fmoc protecting group strategy. Coupling was done by HBTU / HOBt / DIPEA. HOBt and HBTU were used as a 0.45 M solution, DIPEA as a 1.2 M solution in DMF. The Fmoc protected amino acids were dissolved in NMP as 0.5 M solutions. Every peptide was synthesized on 100 mg of resin in standard 5 mL syringe. The lot of the resin used had a loading of 0.72 mmol/g (manufacturer's claims). The resin was allowed to preswell in DMF for 30 min. Each coupling was done twice using 1.0 mL of each solution per step and shaking for 2h each. Fmoc deprotection was done by shaking the resin with 40 % piperidine in DMF for 5 minutes, subsequent washing and addition of 20 % piperidine in DMF followed by shaking for 10 minutes. The progress of the synthesis was monitored by the Kaiser test.[12]

Capping was done for 3 h using 0.5 M of Ac_2O in DMF, containing 1.2 M DIPEA as base. Of the solution 2 mL was used. After completion of the syntheses, the resin was washed with DMF and DCM (5 x 5 mL each).

From the resin was cleaved by shaking for 3 h after addition of 3.0 mL of TFA / TIS / H_2O (90:5:5) (vol/vol). The resin was removed by filtration and washed twice with TFA. Combined filtrates were concentrated under reduced pressure to about 1 mL without heating. It was then transferred to a Falcon tube and precipitated with cold Et_2O. The precipitate was centrifuged at –5 °C for 20 minutes. The solution was then carefully decanted off and the precipitate resuspended in cold Et_2O before being centrifuged again. Finally, the Et_2O was decanted off again and the peptide dried under vacuum. The peptides were analysed by ES-MS and ^{1}H-NMR.

Ac-Glu-Lys-NH$_2$:

^{1}H-NMR (300 MHz, DMSO-d6): δ [ppm] = 1.31 (m, 2 H), 1.51 (m, 1+2 H), 1.68 (m, 2 H), 1.86 (s, 3 H), 1.88 (m, 1 H), 2.22 (t, J = 8 Hz, 2 H), 2.72 (m, 2 H), 4.18 (m, 2 H), 7.04 (s, 1H, NH), 7.31 (s, 1H, NH), 7.70 (bs, 2H, NH), 7.91 (d, J = 8 Hz, 1H, NH), 8.09 (d, J = 8 Hz, 1H, NH); - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 316.9 (100 %, MH^+); - **MF:** $C_{13}H_{25}N_4O_5Cl$ – **FW:** 351.81 g/mol;

Ac-Glu-Gly-Lys-NH$_2$:

^{1}H-NMR (300 MHz, DMSO-d6): δ [ppm] = 1.29 (m, 2 H), 1.51 (m, 1+2 H), 1.71 (m, 2 H), 1.83 (s, 3 H), 1.84 (m, 1 H), 2.27 (t, J = 8 Hz, 2 H), 2.74 (m, 2 H), 3.78 (m, 2 H), 4.17 (m, 2 H), 7.09 (s, 1H, NH), 7.31 (s, 1H, NH), 7.70 (bs, 2H, NH), 7.81 (d, J = 8Hz, 1H, NH), 8.17 (d, J = 8 Hz, 1H, NH), 8.23 (m, 1H, NH); - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 374.0 (100 %, MH^+); - **MF:** $C_{15}H_{28}N_5O_6Cl$ – **FW:** 408.86 g/mol;

Ac-Glu-Gly-Gly-Lys-NH$_2$:

^{1}H-NMR (300 MHz, DMSO-d6): δ [ppm] = 1.30 (m, 2 H), 1.52 (m, 1+2 H), 1.71 (m, 2 H), 1.89 (s, 3 H), 1.90 (m, 1 H), 2.28 (t, J = 8 Hz, 2 H), 2.76 (m, 2 H), 3.78 (m, 4 H), 4.19 (m, 2 H), 7.03 (s, 1H, NH), 7.30 (s, 1H, NH), 7.73 (bs, 2H, NH), 7.89 (d, J = 8Hz, 1H, NH), 8.10 (m, 2H, NH), 8.29 (m, 1H, NH); - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 431.0 (100 %, MH^+); **- MF:** $C_{17}H_{31}N_6O_7Cl$ – **FW:** 465.91 g/mol;

The isomeric glutamic acid tetrapeptide sequences H-E-G-G-G-NH$_2$, H-E-G-G-G-NH$_2$, H-E-G-G-G-NH$_2$ and H-E-G-G-G-NH$_2$ were prepared earlier. Their preparation, purification and analytics were already published.[13] They were properly stored below -5 °C. HPLC analysis showed no decomposition.

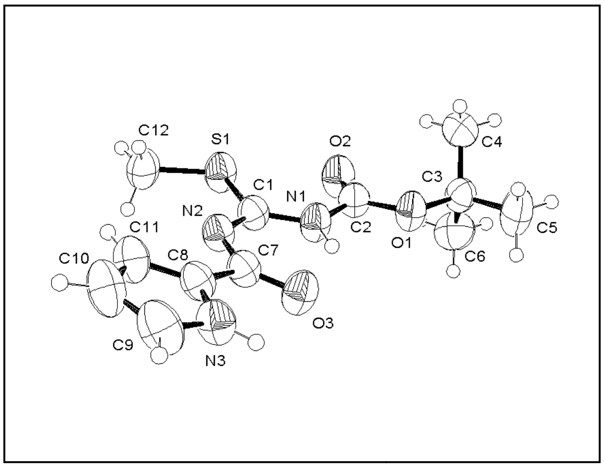
2. Crystal structures of new compounds

X-Ray structure and crystal data of 28:

Triclinic; Space group: P -1; unit cell dimensions: a = 5.8636(9) Å, α = 102.043(17)°, b = 9.7355(15) Å, β = 102.225(18)°, c = 13.7186(19) Å, γ = 93.521(19)°; V = 743.9(2) Å^3; Z = 2, Dx = 1.265 Mg/m^3; μ = 0.225 mm^{-1}; F(000] = 300.

Data collection: T = 296 K; graphite monochromator. A colourless plate with dimensions of 0.320 x 0.280 x 0.120 mm was used to measure 7804 reflections (2975 unique reflections, R$_{int}$ = 0.0414) from 2.35° to 26.86° on a STOE-IPDS diffractometer with the rotation method.

Structure refinement: The F2 value was refined using the full-matrix least squares refinement method, with a goodness-of-fit of 0.88 for all reflections and 176 parameters.



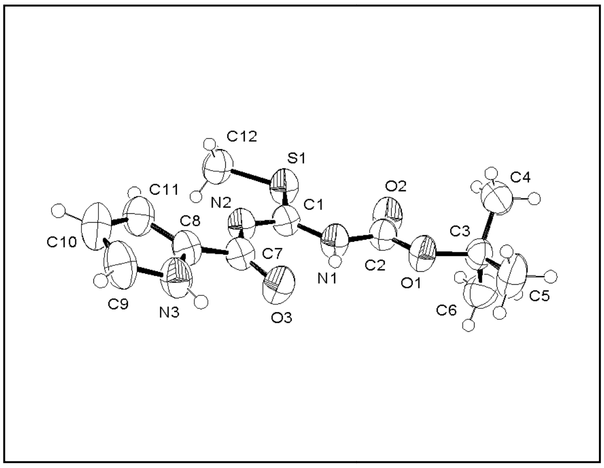


Table of atomic coordinates (x 10^4) and equivalent isotropic displacement parameters (A2 x 10^3) for **28**. U(eq) is defined as one third of the trace of the orthogonalized Uij tensor:

atom	**x**	**y**	**z**	**U(eq)**
S(1)	5412(1)	18(1)	3798(1)	67(1)
O(1)	9832(2)	-3172(1)	2731(1)	62(1)
O(2)	7990(3)	-2274(1)	3990(1)	75(1)
O(3)	9290(2)	-37(1)	1247(1)	70(1)
N(1)	8306(3)	-1176(2)	2688(1)	60(1)
N(2)	6518(2)	749(1)	2188(1)	54(1)
N(3)	7776(3)	1629(2)	-120(1)	66(1)

C(1)	6872(3)	-121(2)	2799(1)	54(1)
C(2)	8651(3)	-2236(2)	3224(1)	56(1)
C(3)	10571(3)	-4417(2)	3131(1)	58(1)
C(4)	8450(4)	-5323(2)	3213(2)	81(1)
C(5)	11672(5)	-5195(2)	2303(2)	89(1)
C(6)	12326(4)	-3915(2)	4142(2)	85(1)
C(7)	7637(3)	674(2)	1386(1)	54(1)
C(8)	6780(3)	1557(2)	686(1)	56(1)
C(9)	6673(5)	2509(2)	-655(2)	84(1)
C(10)	4959(5)	3018(2)	-201(2)	84(1)
C(11)	4999(4)	2417(2)	644(1)	69(1)
C(12)	3721(4)	1451(2)	3588(2)	70(1)

Table of bond lengths [Å] and angles [deg] for **28**:

bond	**length**	**bond**	**angle**
S(1)-C(1)	1.7502(2)	C(1)-S(1)-C(12)	100.40(9)
S(1)-C(12)	1.797(2)	C(2)-O(1)-C(3)	121.15(13)
O(1)-C(2)	1.336(2)	C(1)-N(1)-C(2)	128.48(15)
O(1)-C(3)	1.483(2)	C(1)-N(2)-C(7)	121.20(14)
O(2)-C(2)	1.201(2)	C(8)-N(3)-C(9)	109.41(18)
O(3)-C(7)	1.249(2)	C(2)-N(1)-H(1A)	116.00
N(1)-C(1)	1.372(2)	C(1)-N(1)-H(1A)	116.00
N(1)-C(2)	1.388(2)	C(8)-N(3)-H(3A)	125.00
N(2)-C(1)	1.306(2)	C(9)-N(3)-H(3A)	125.00
N(2)-C(7)	1.387(2)	S(1)-C(1)-N(1)	117.32(12)
N(3)-C(8)	1.367(2)	S(1)-C(1)-N(2)	119.37(13)
N(3)-C(9)	1.349(3)	N(1)-C(1)-N(2)	123.29(15)
N(1)-H(1A)	0.8600	O(1)-C(2)-N(1)	107.38(14)
N(3)-H(3A)	0.8600	O(2)-C(2)-N(1)	125.01(17)
C(3)-C(6)	1.508(3)	O(1)-C(2)-O(2)	127.62(16)
C(3)-C(4)	1.518(3)	C(4)-C(3)-C(6)	112.56(17)
C(3)-C(5)	1.514(3)	C(5)-C(3)-C(6)	112.14(18)
C(7)-C(8)	1.452(2)	O(1)-C(3)-C(5)	102.13(14)
C(8)-C(11)	1.377(3)	O(1)-C(3)-C(6)	108.95(14)
C(9)-C(10)	1.357(4)	O(1)-C(3)-C(4)	110.45(15)
C(10)-C(11)	1.401(3)	C(4)-C(3)-C(5)	110.12(17)
C(4)-H(4A)	0.9600	O(3)-C(7)-N(2)	126.02(15)
C(4)-H(4B)	0.9600	O(3)-C(7)-C(8)	119.72(15)
C(4)-H(4C)	0.9600	N(2)-C(7)-C(8)	114.23(15)
C(5)-H(5A)	0.9600	N(3)-C(8)-C(7)	120.72(16)
C(5)-H(5B)	0.9600	N(3)-C(8)-C(11)	107.22(15)
C(5)-H(5C)	0.9600	C(7)-C(8)-C(11)	132.06(16)
C(6)-H(6A)	0.9600	N(3)-C(9)-C(10)	108.63(19)

C(6)-H(6B)	0.9600	C(9)-C(10)-C(11)	107.5(2)
C(6)-H(6C)	0.9600	C(8)-C(11)-C(10)	107.3(2)
C(9)-H(9)	0.9300	C(3)-C(4)-H(4A)	109.00
C(10)-H(10)	0.9300	C(3)-C(4)-H(4B)	109.00
C(11)-H(11)	0.9300	C(3)-C(4)-H(4C)	109.00
C(12)-H(12A)	0.9600	H(4A)-C(4)-H(4B)	109.00
C(12)-H(12B)	0.9600	H(4A)-C(4)-H(4C)	109.00
C(12)-H(12C)	0.9600	H(4B)-C(4)-H(4C)	109.00
		C(3)-C(5)-H(5A)	110.00
		C(3)-C(5)-H(5B)	110.00
		C(3)-C(5)-H(5C)	109.00
		H(5A)-C(5)-H(5B)	109.00
		H(5A)-C(5)-H(5C)	110.00
		H(5B)-C(5)-H(5C)	109.00
		C(3)-C(6)-H(6A)	110.00
		C(3)-C(6)-H(6B)	109.00
		C(3)-C(6)-H(6C)	109.00
		H(6A)-C(6)-H(6B)	109.00
		H(6A)-C(6)-H(6C)	109.00
		H(6B)-C(6)-H(6C)	109.00
		N(3)-C(9)-H(9)	126.00
		C(10)-C(9)-H(9)	126.00
		C(9)-C(10)-H(10)	126.00
		C(11)-C(10)-H(10)	126.00
		C(8)-C(11)-H(11)	126.00
		C(10)-C(11)-H(11)	126.00
		S(1)-C(12)-H(12A)	109.00
		S(1)-C(12)-H(12B)	109.00
		S(1)-C(12)-H(12C)	109.00
		H(12A)-C(12)-H(12B)	109.00
		H(12A)-C(12)-H(12C)	110.00
		H(12B)-C(12)-H(12C)	109.00

Table of torsion angles [deg] for **28:**

bond	angle
C(12)-S(1)-C(1)-N(1)	-177.52(14)
C(12)-S(1)-C(1)-N(2)	1.01(16)
C(2)-O(1)-C(3)-C(6)	-64.8(2)
C(2)-O(1)-C(3)-C(4)	59.4(2)
C(2)-O(1)-C(3)-C(5)	176.46(16)
C(3)-O(1)-C(2)-O(2)	-2.3(3)
C(3)-O(1)-C(2)-N(1)	177.58(14)
C(2)-N(1)-C(1)-S(1)	4.9(2)
C(1)-N(1)-C(2)-O(1)	168.32(16)
C(1)-N(1)-C(2)-O(2)	-11.8(3)
C(2)-N(1)-C(1)-N(2)	-173.54(17)
C(7)-N(2)-C(1)-S(1)	-179.91(12)
C(7)-N(2)-C(1)-N(1)	-1.5(3)
C(1)-N(2)-C(7)-O(3)	-11.2(3)
C(1)-N(2)-C(7)-C(8)	170.70(15)

C(8)-N(3)-C(9)-C(10)	0.2(2)
C(9)-N(3)-C(8)-C(11)	0.2(2)
C(9)-N(3)-C(8)-C(7)	179.80(17)
O(3)-C(7)-C(8)-N(3)	-0.6(3)
N(2)-C(7)-C(8)-C(11)	-2.9(3)
N(2)-C(7)-C(8)-N(3)	177.65(15)
O(3)-C(7)-C(8)-C(11)	178.88(19)
N(3)-C(8)-C(11)-C(10)	-0.5(2)
C(7)-C(8)-C(11)-C(10)	180.00(19)
N(3)-C(9)-C(10)-C(11)	-0.5(3)
C(9)-C(10)-C(11)-C(8)	0.6(2)

3. ^{1}H – and ^{13}C-NMR spectra of new compounds

For solvents and conditions of measurement, see experimental procedures. NMR spectra were recorded on Bruker Avance spectrometers 300, 400 and 600. Measuring temperature was 300 K. TMS was used as external standard.

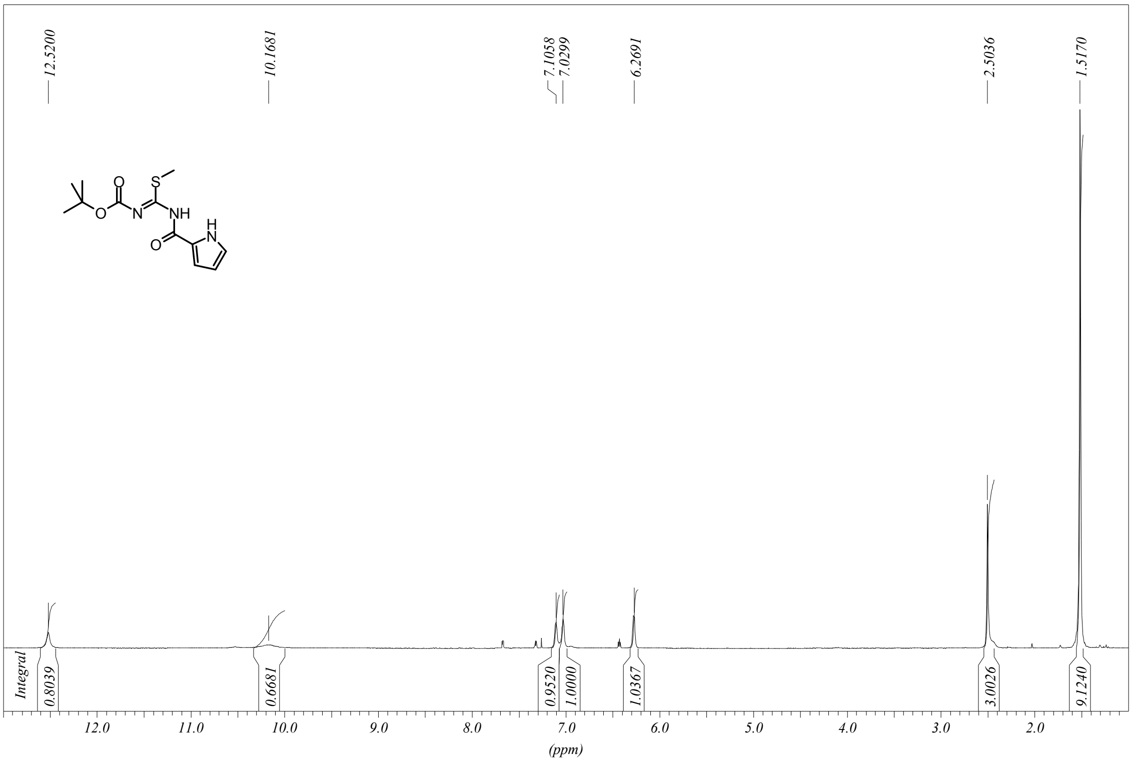


Figure S-1: ^{1}H-NMR spectrum of compound **28**

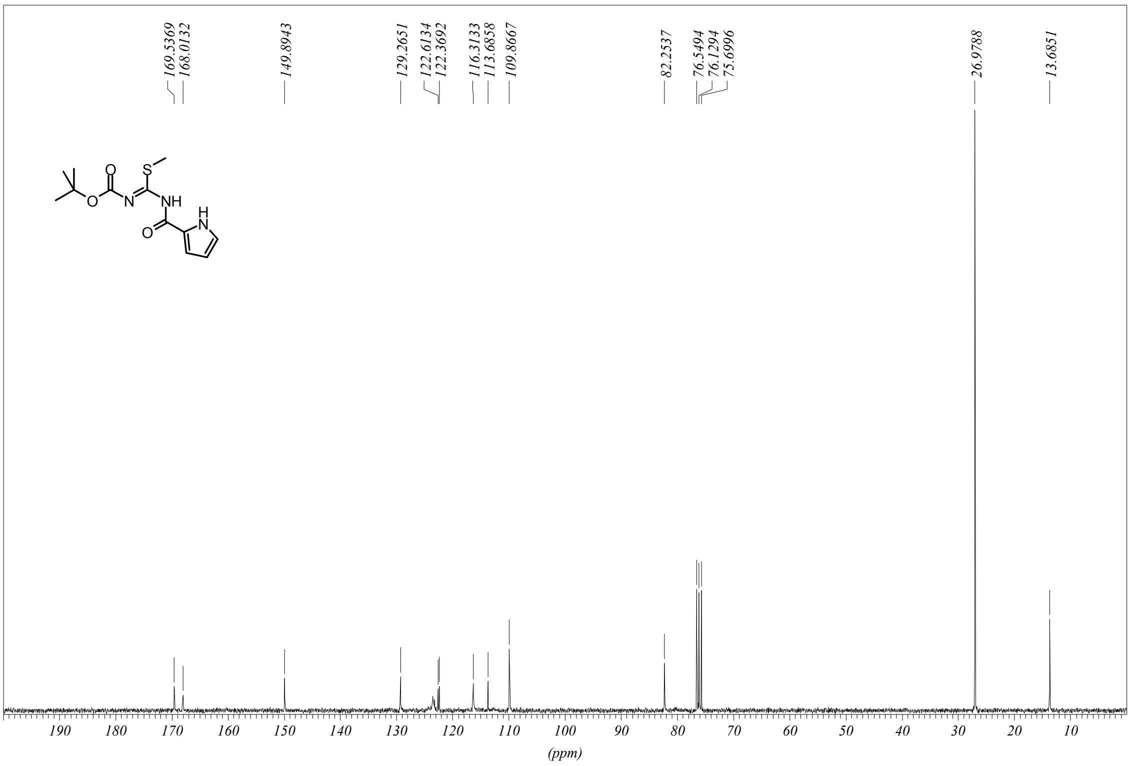


Figure S-2: ^{13}C-NMR spectrum of compound **28**

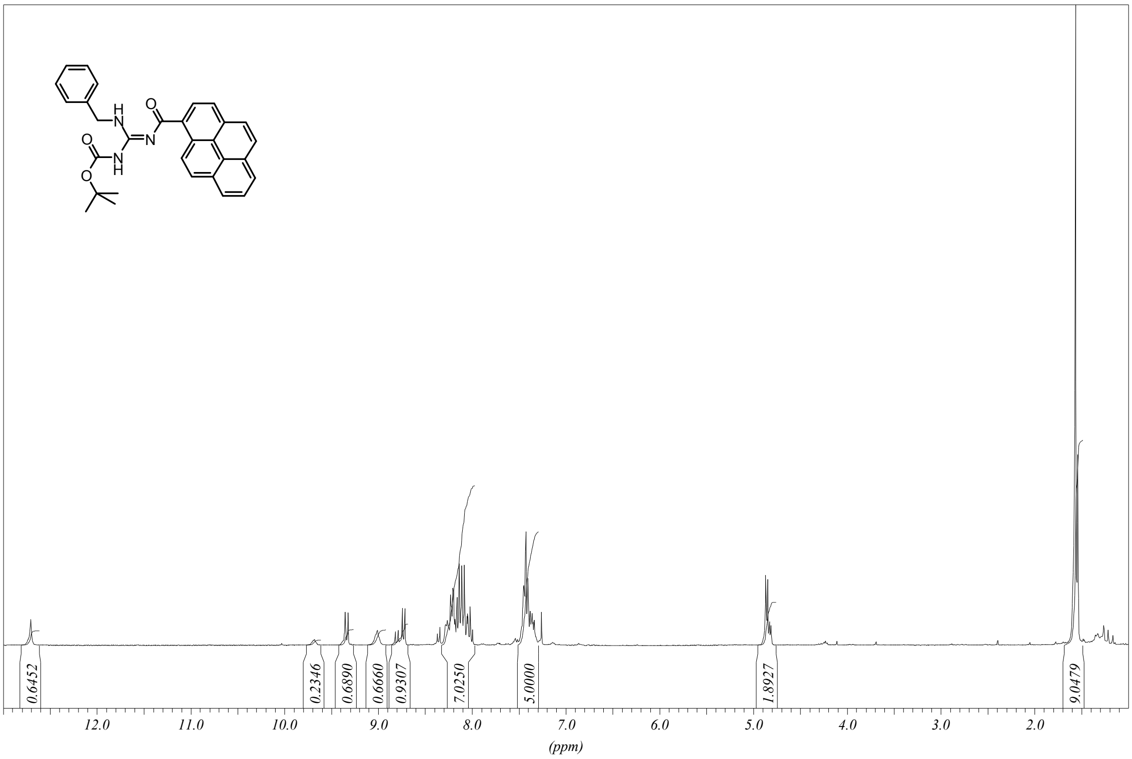


Figure S-3: ^{1}H-NMR spectrum of compound **35**

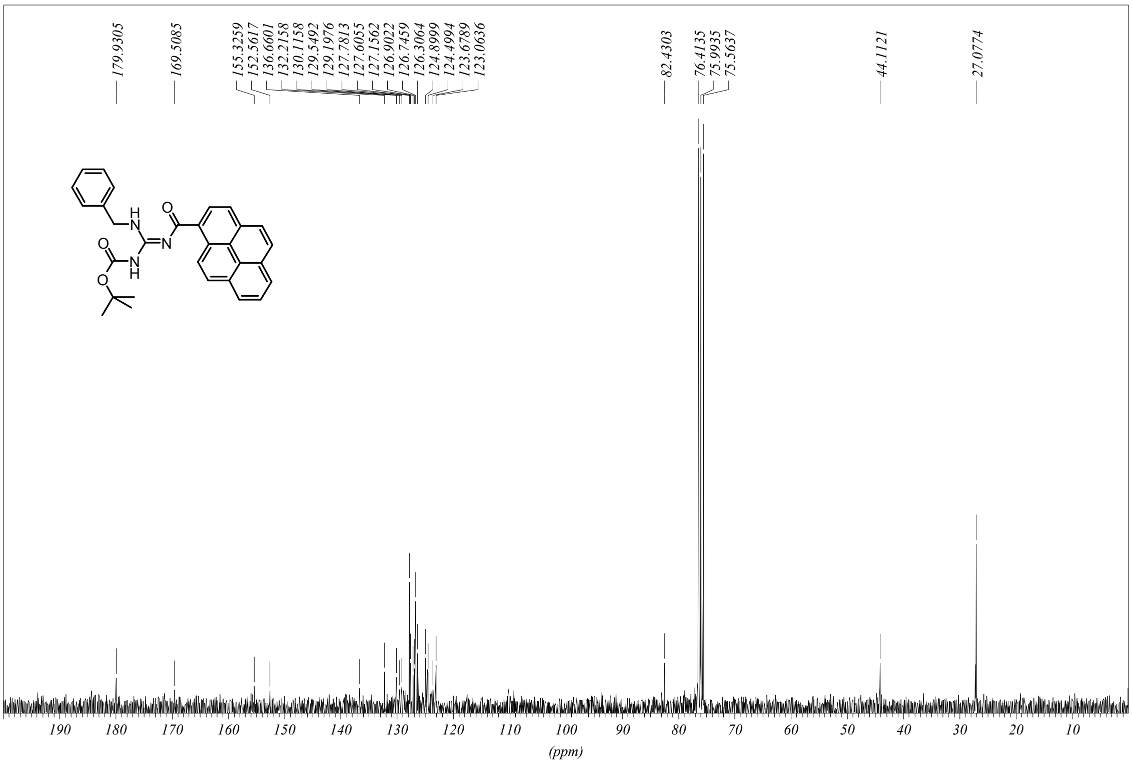


Figure S-4: ^{13}C-NMR spectrum of compound **35**

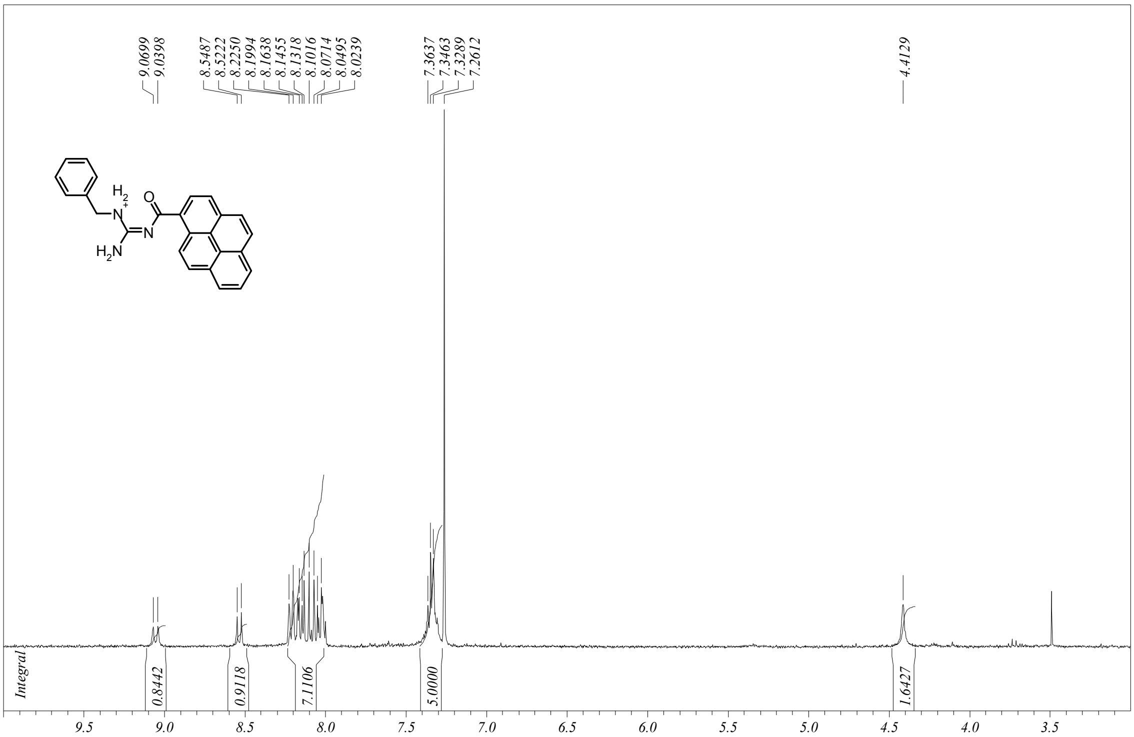


Figure S-5: ^{1}H-NMR spectrum of compound **36**

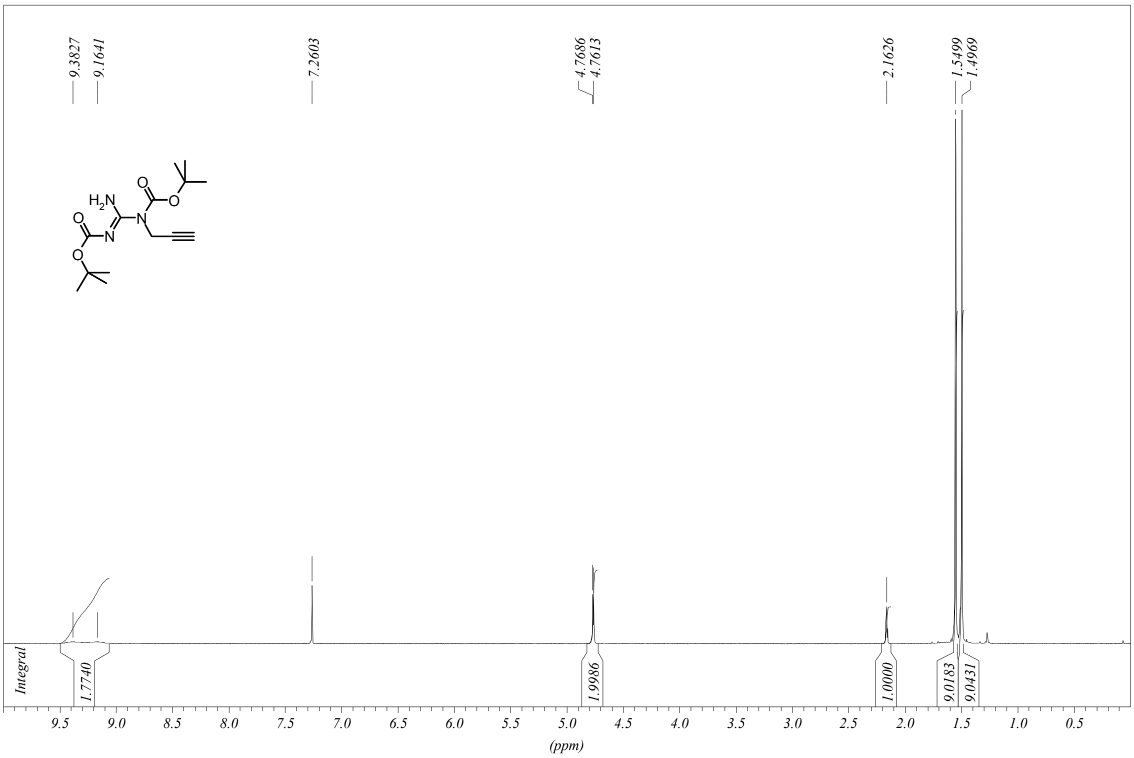


Figure S-6: ^{1}H-NMR spectrum of compound **25**

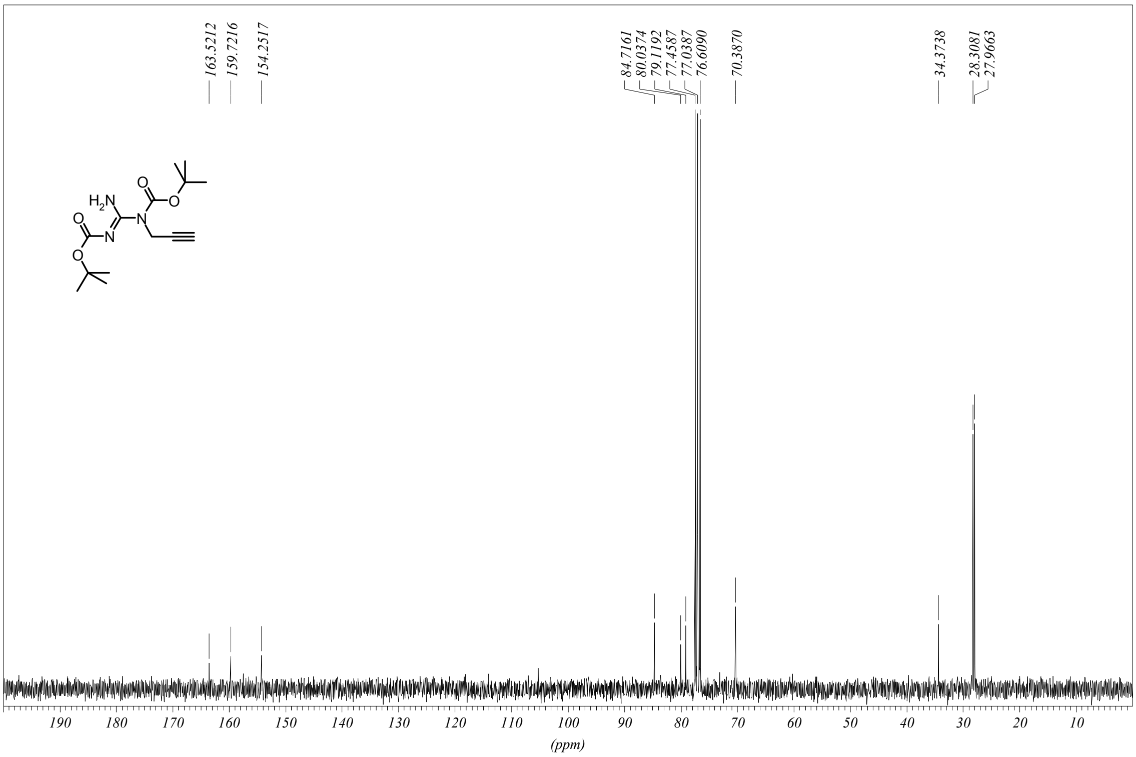


Figure S-7: ^{13}C-NMR spectrum of compound **25**

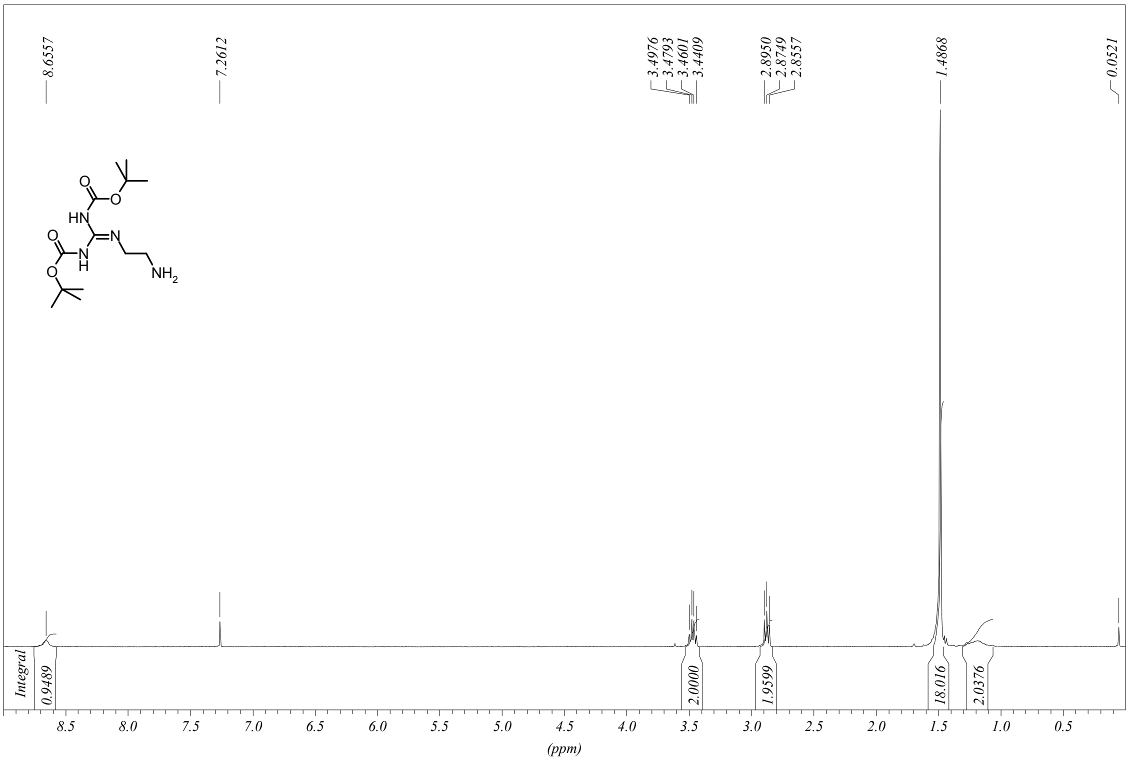


Figure S-8: ^{1}H-NMR spectrum of compound **18**

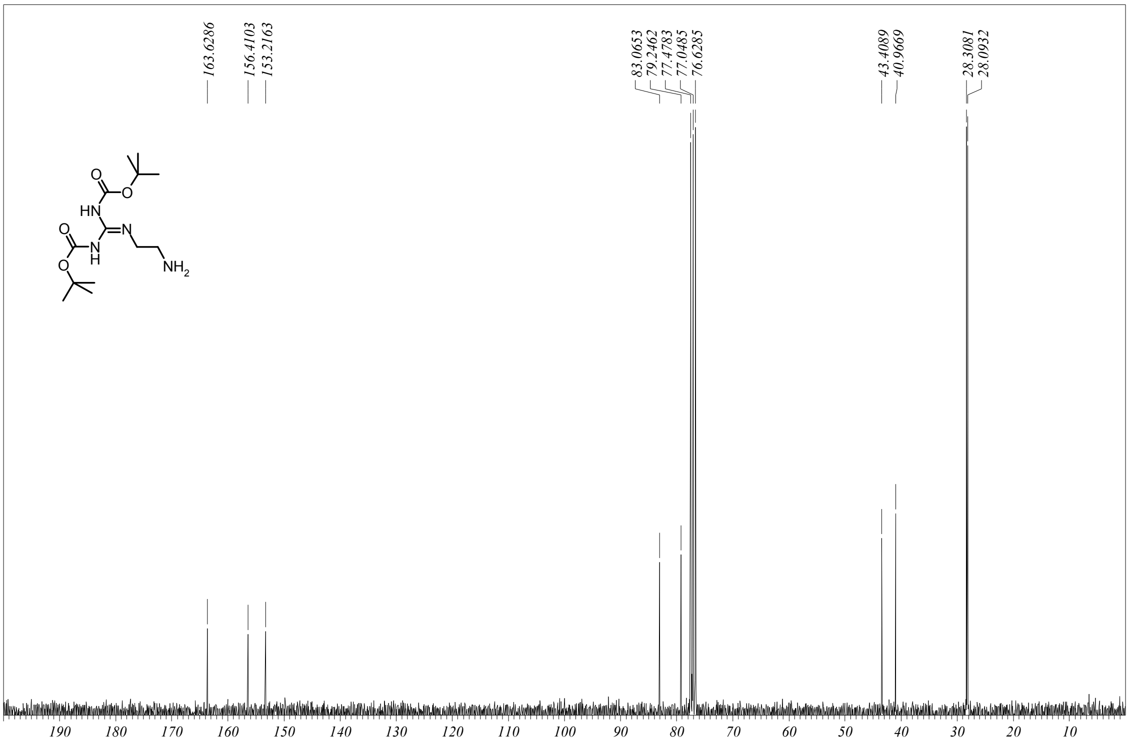


Figure S-9: ^{13}C-NMR spectrum of compound **18**

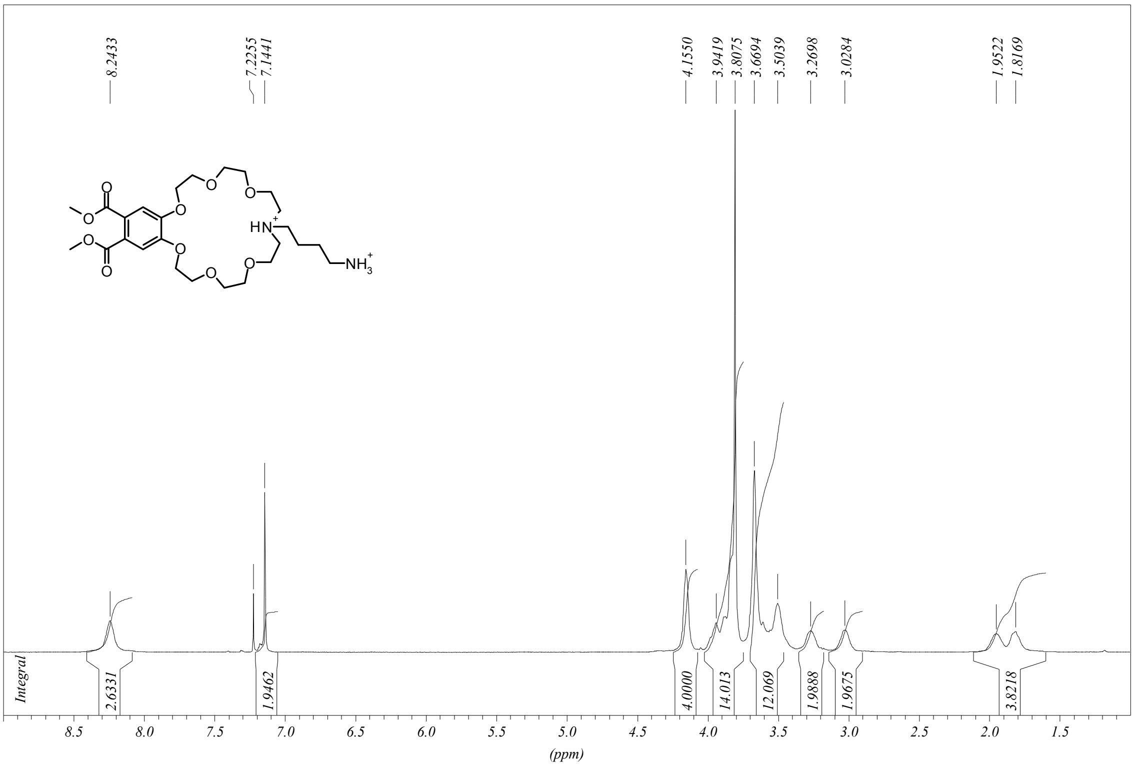


Figure S-10: ^{1}H-NMR spectrum of compound **4b**

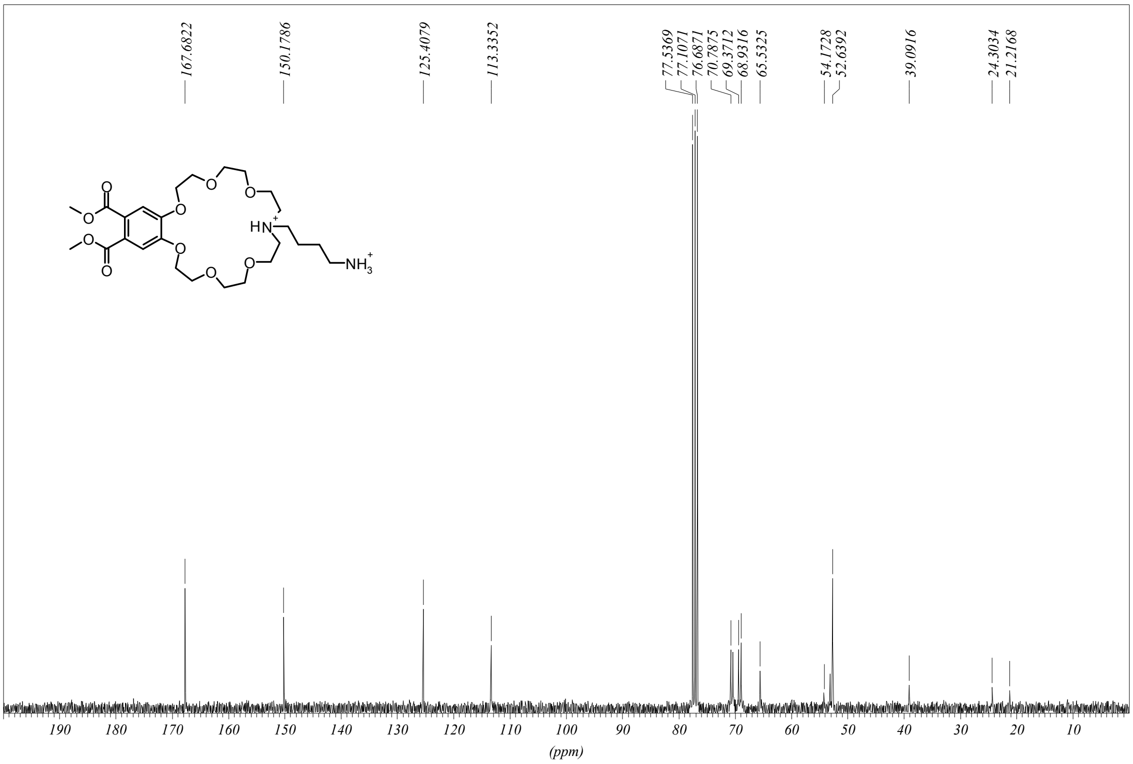


Figure S-11: ^{13}C-NMR spectrum of compound **4b**

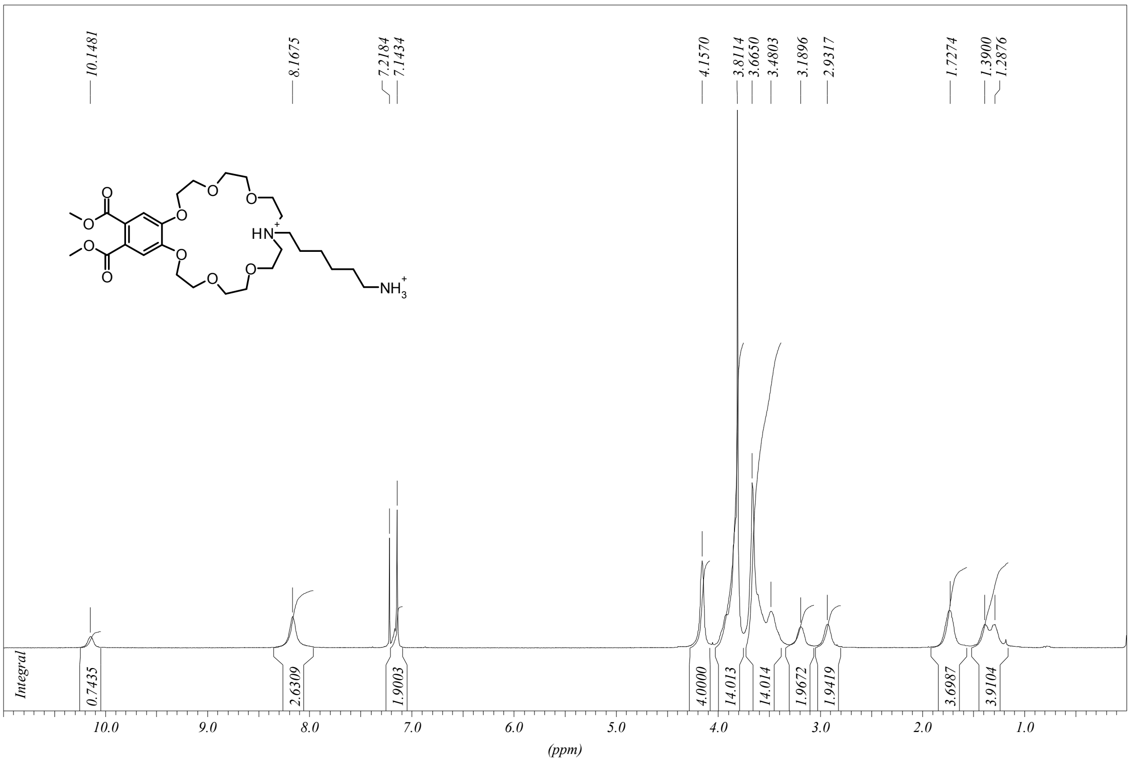


Figure S-12: ^{1}H-NMR spectrum of compound **4c**

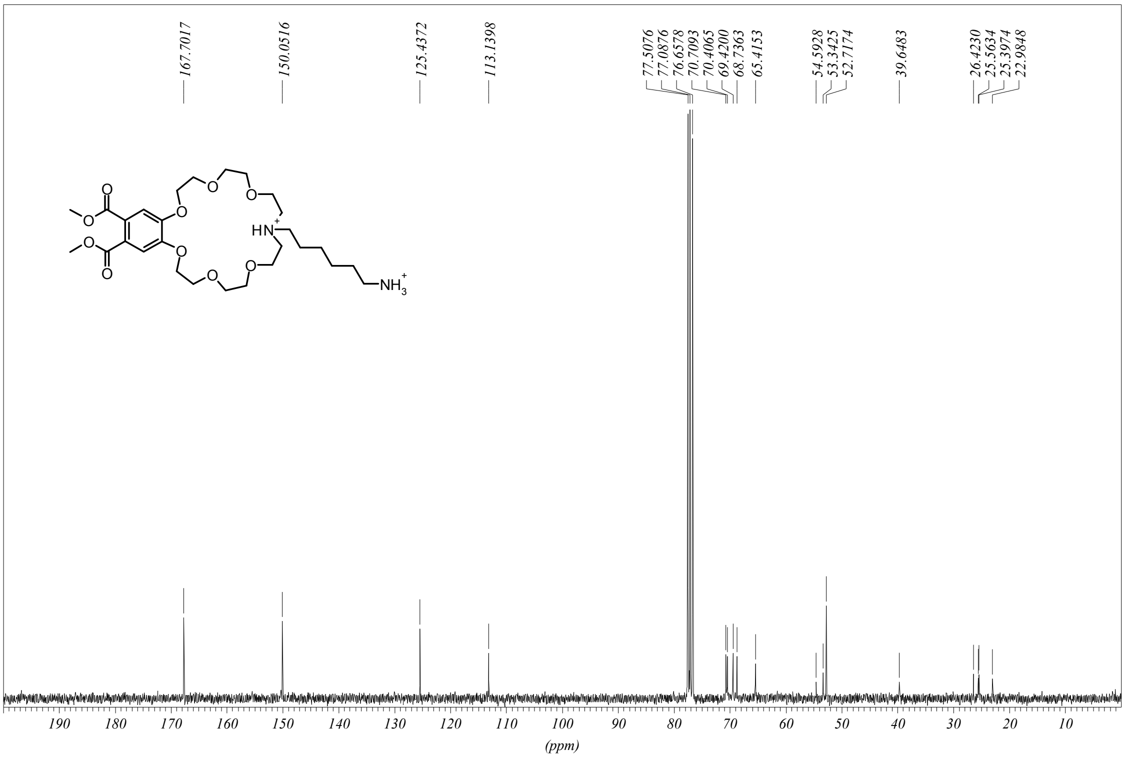


Figure S-13: ^{13}C-NMR spectrum of compound **4c**

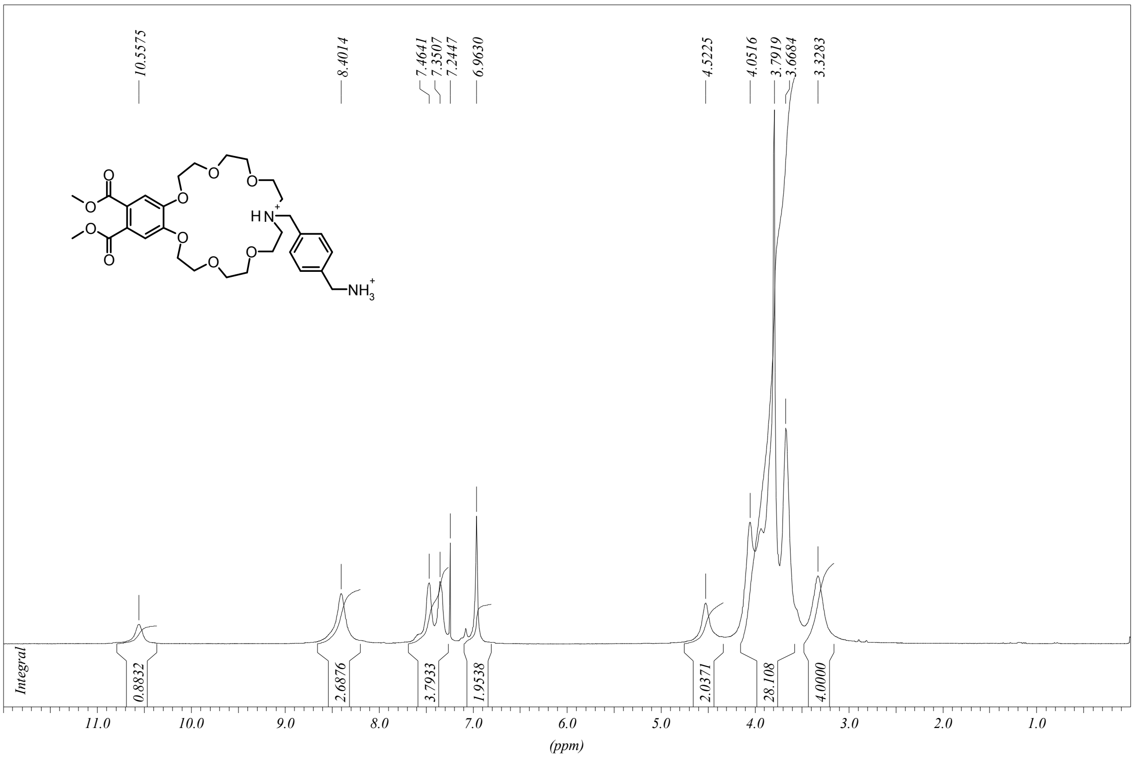


Figure S-14: ^{1}H-NMR spectrum of compound **4d**

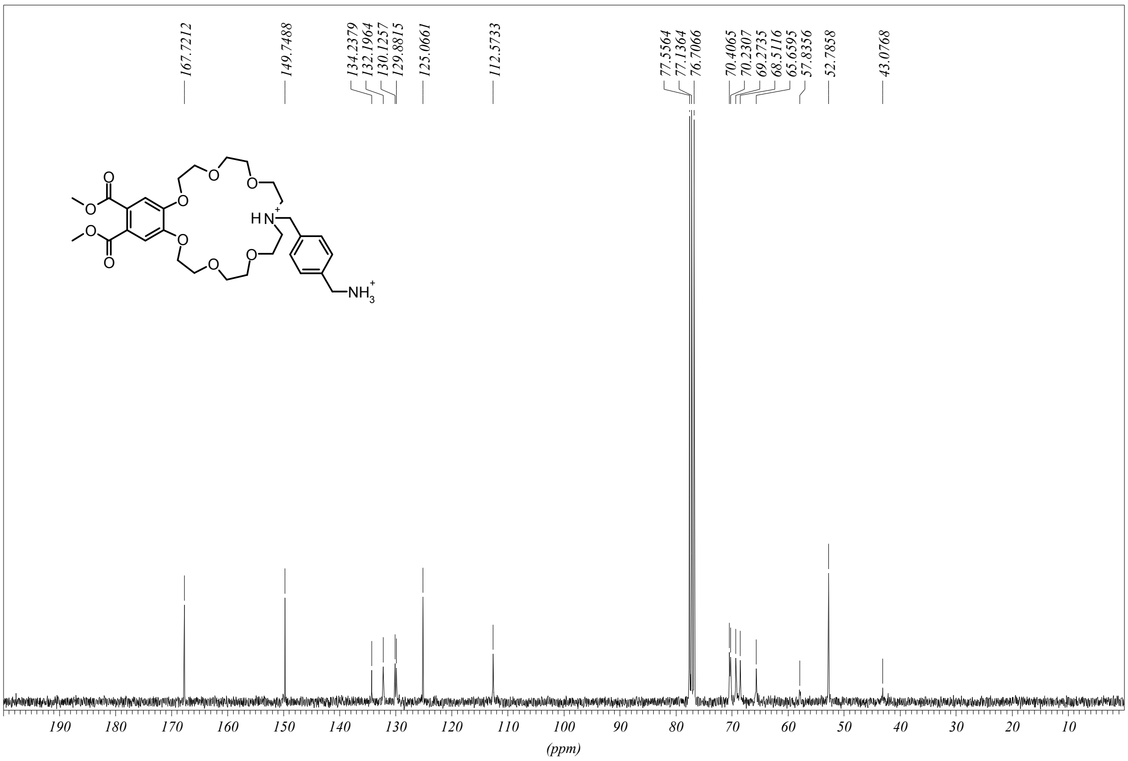


Figure S-15: ^{13}C-NMR spectrum of compound **4d**

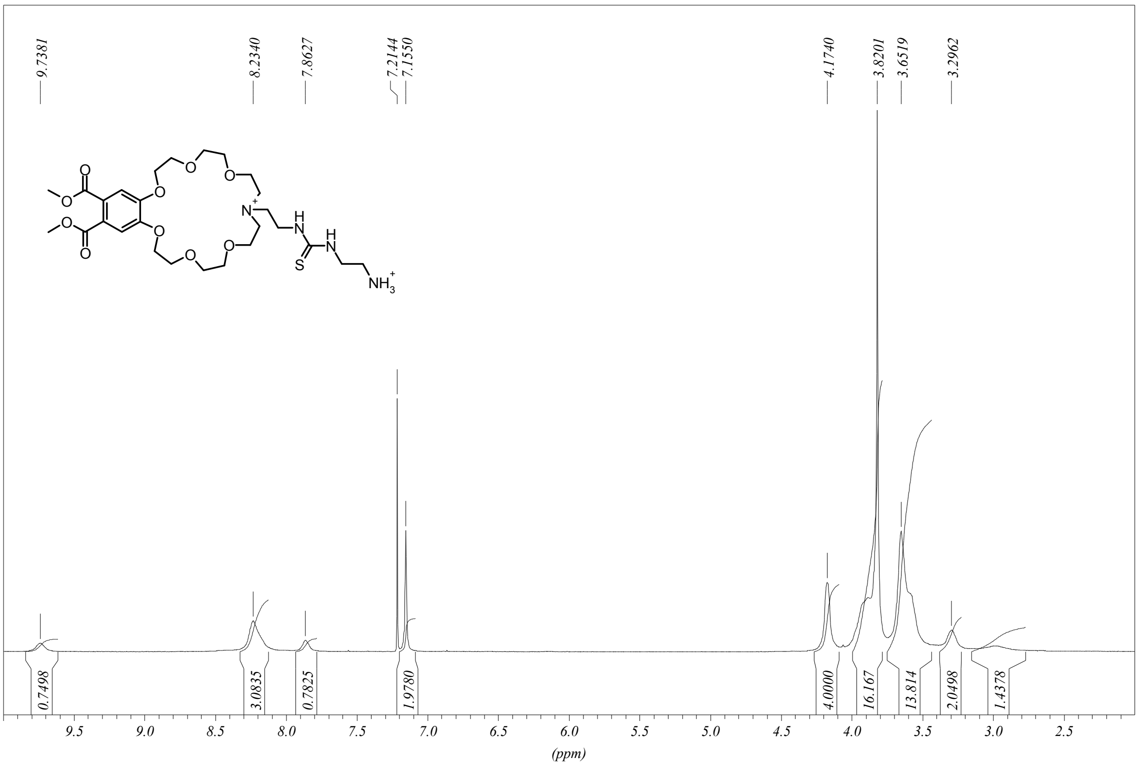


Figure S-16: ^{1}H-NMR spectrum of compound **4e**

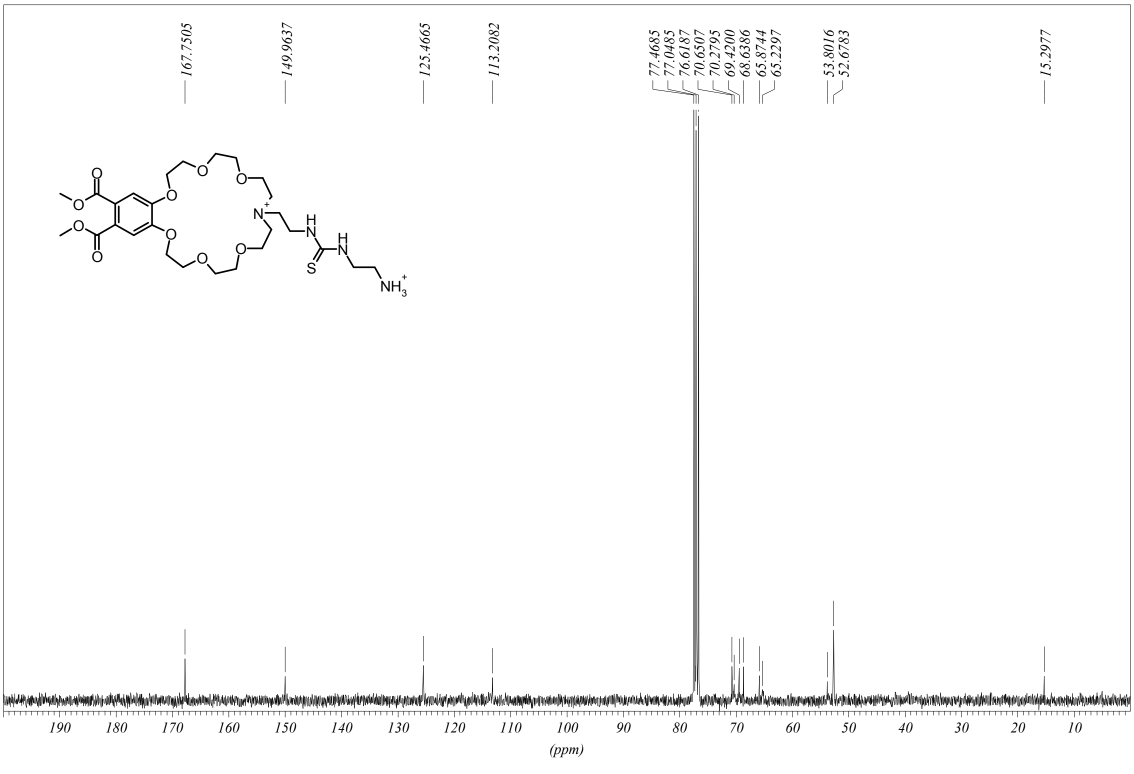


Figure S-17: ^{13}C-NMR spectrum of compound **4e**

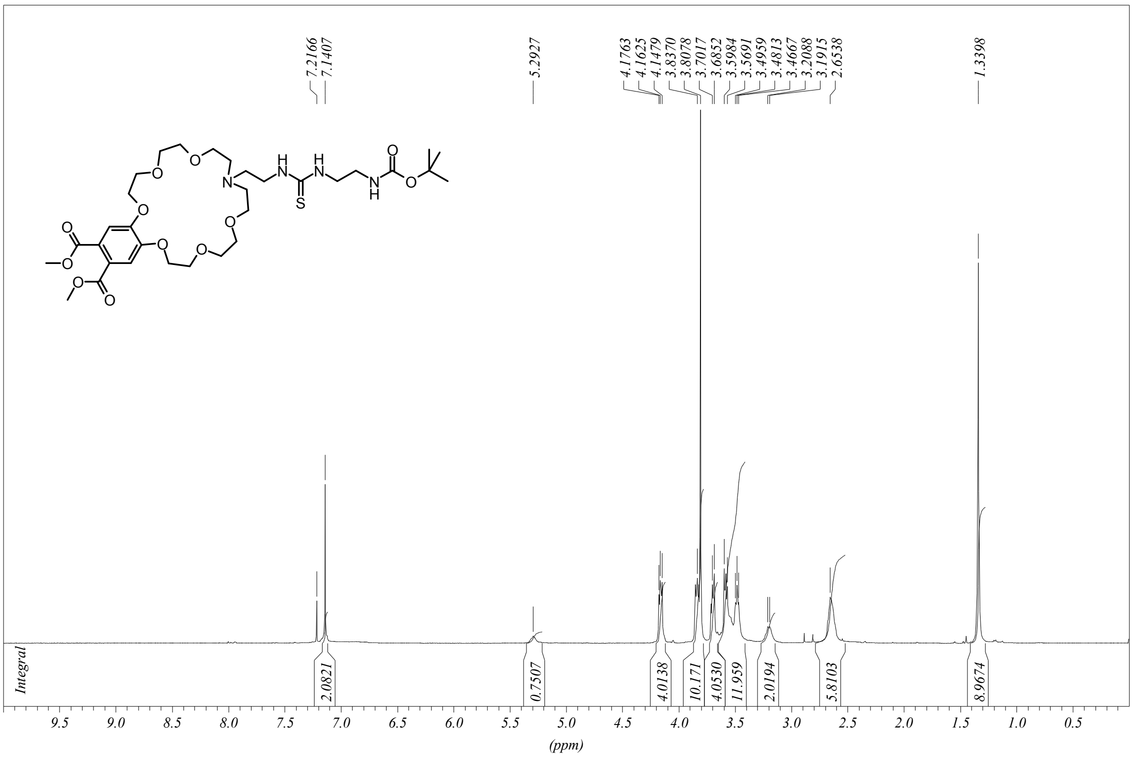


Figure S-18: ^{1}H-NMR spectrum of compound **6**

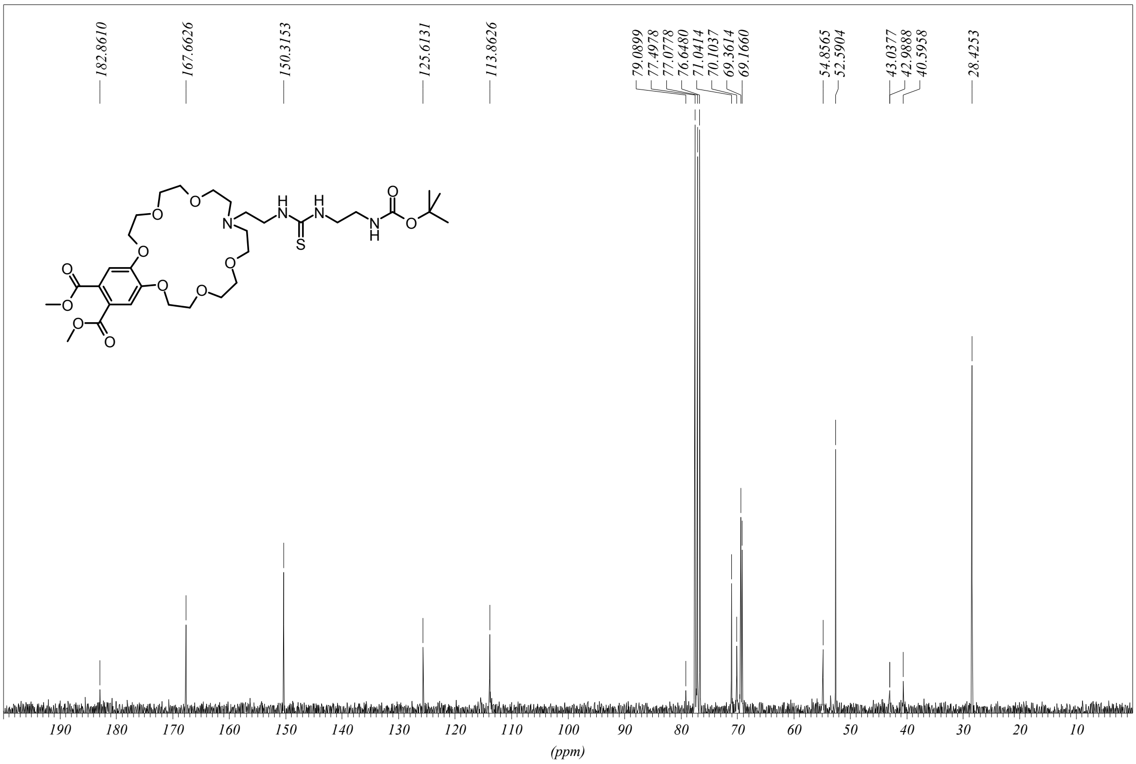


Figure S-19: ^{13}C-NMR spectrum of compound **6**

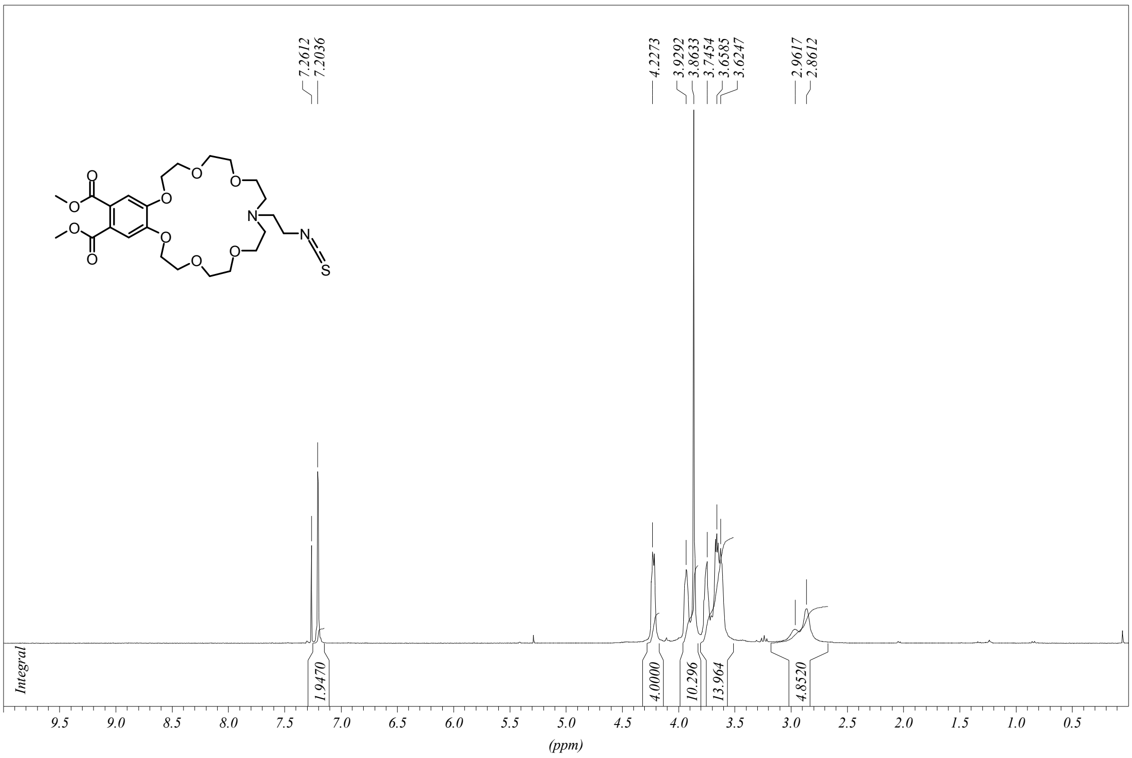


Figure S-20: ^{1}H-NMR spectrum of compound **17**

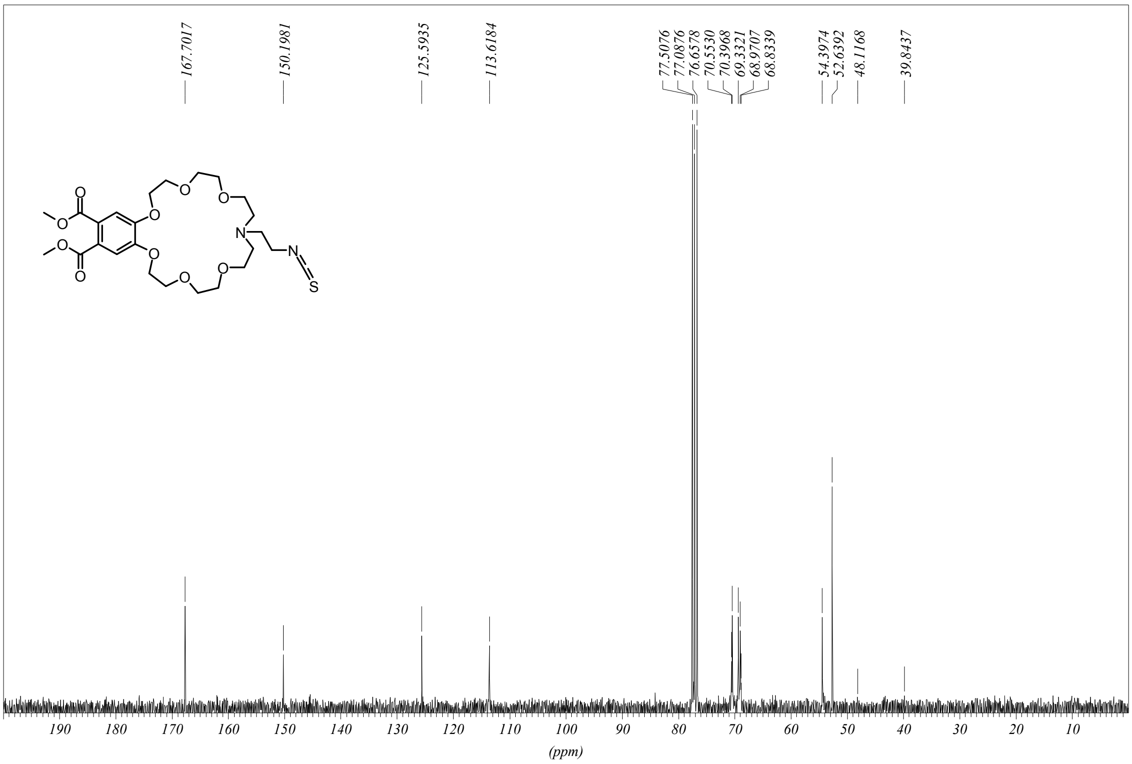


Figure S-21: ^{13}C-NMR spectrum of compound **17**

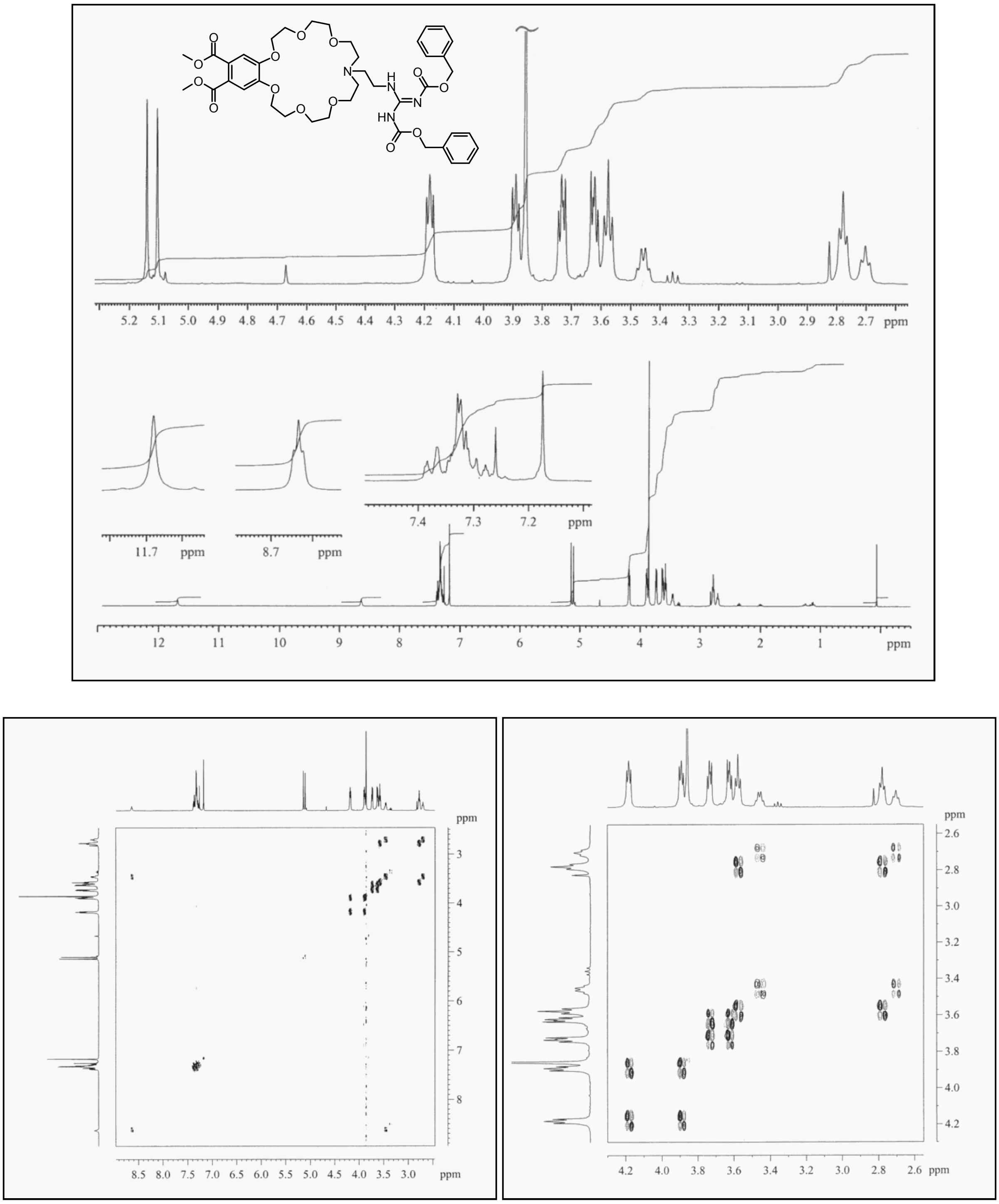


Figure S-22: ^{1}H-NMR and COSY spectra of compound **E-2**

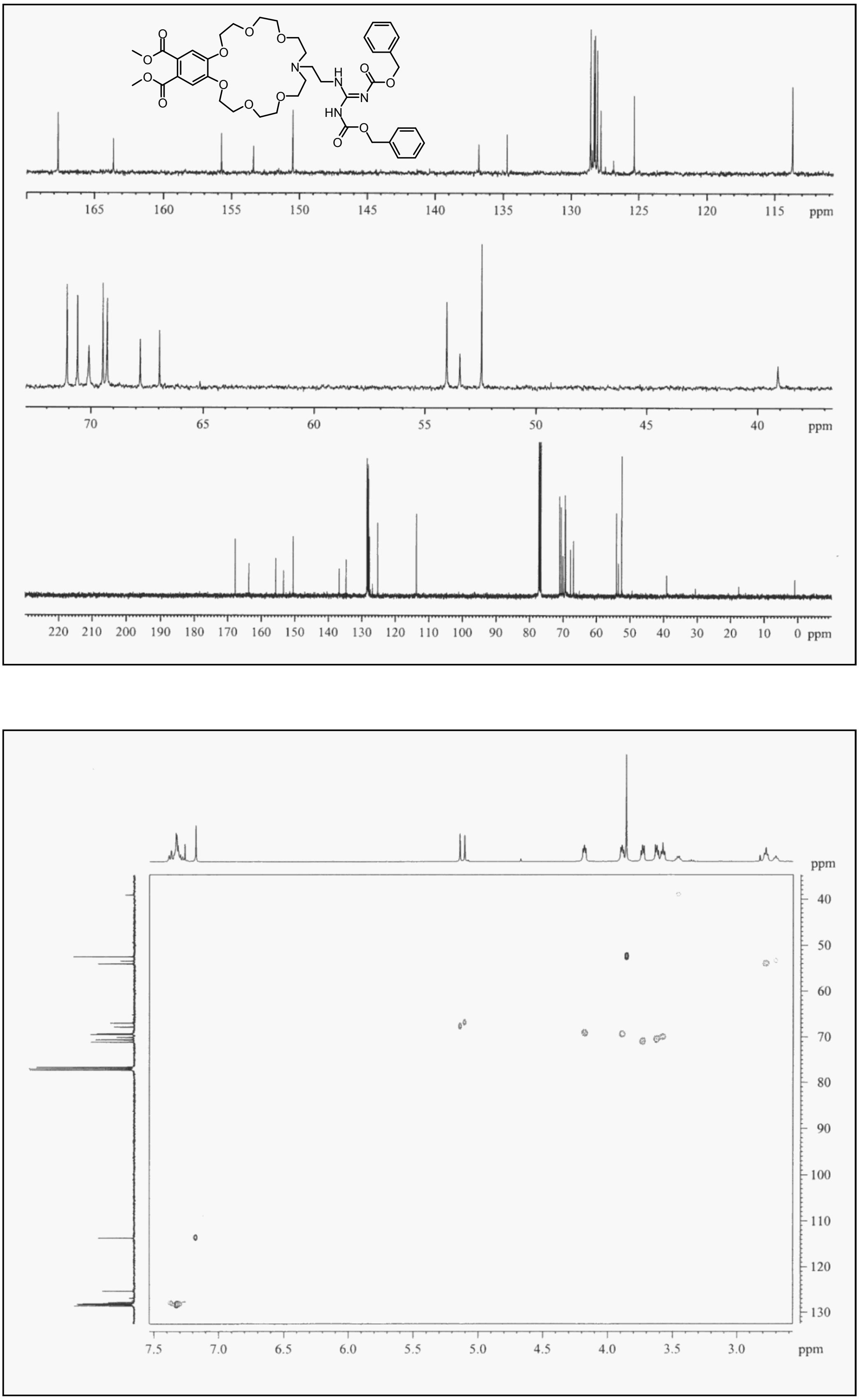


Figure S-23: ^{13}C-NMR and HSQC spectrum of compound **E-2**

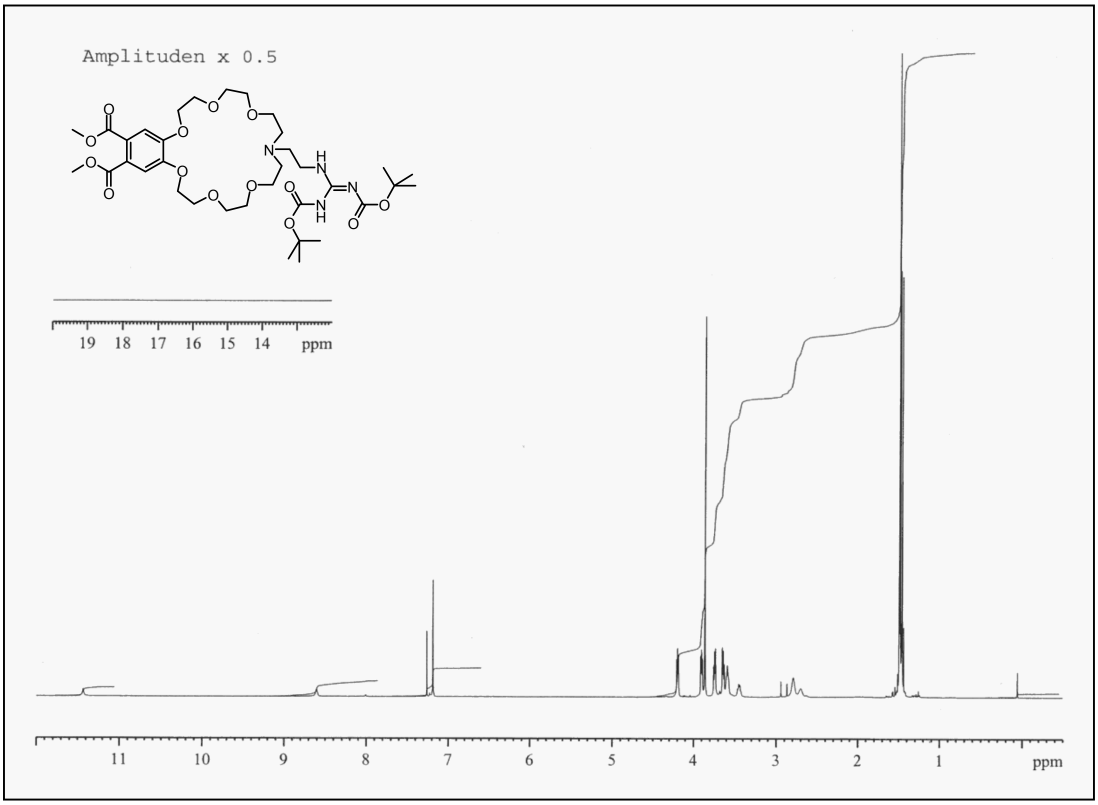


Figure S-24: ^{1}H-NMR spectrum of compound **13a**

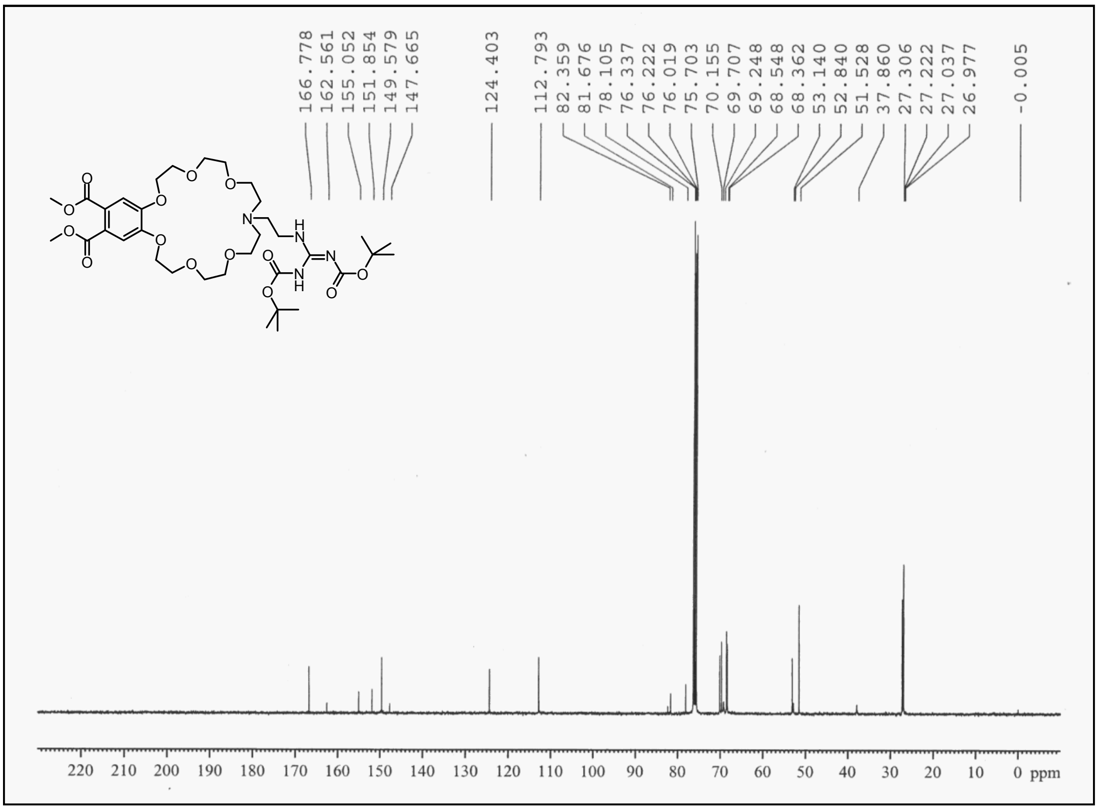


Figure S-25: ^{13}C-NMR spectrum of compound **13a**

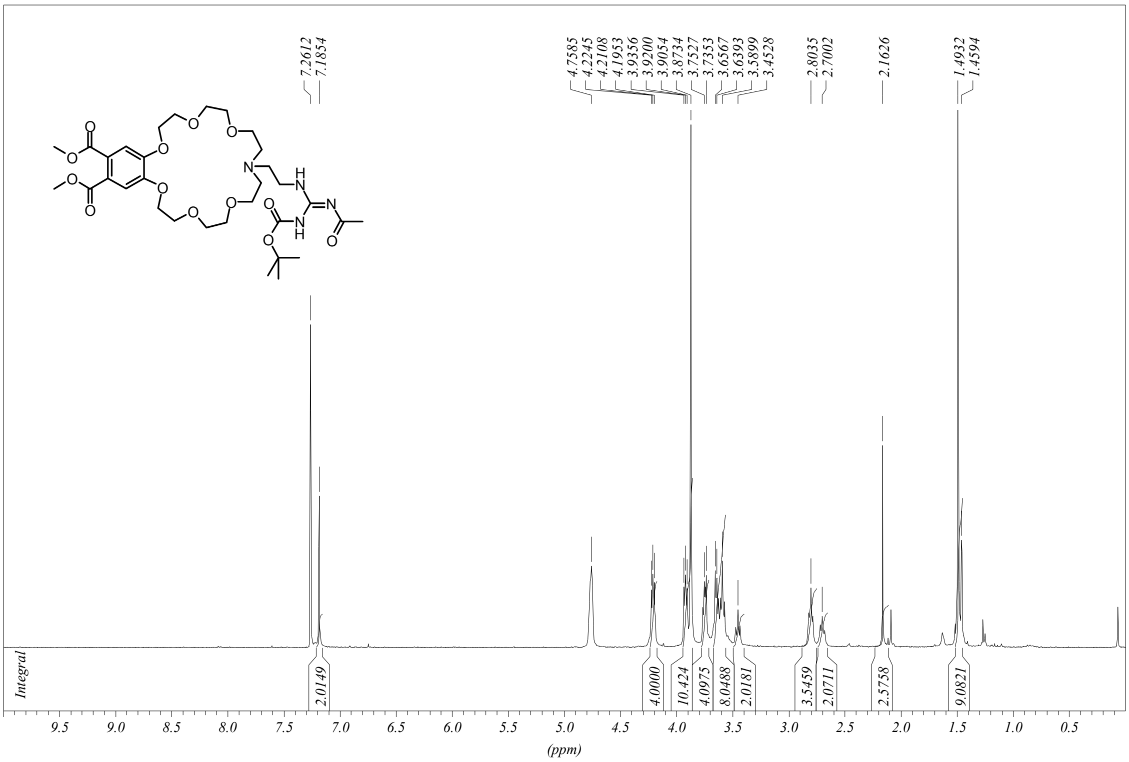


Figure S-26: ^{1}H-NMR spectrum of compound **15a**

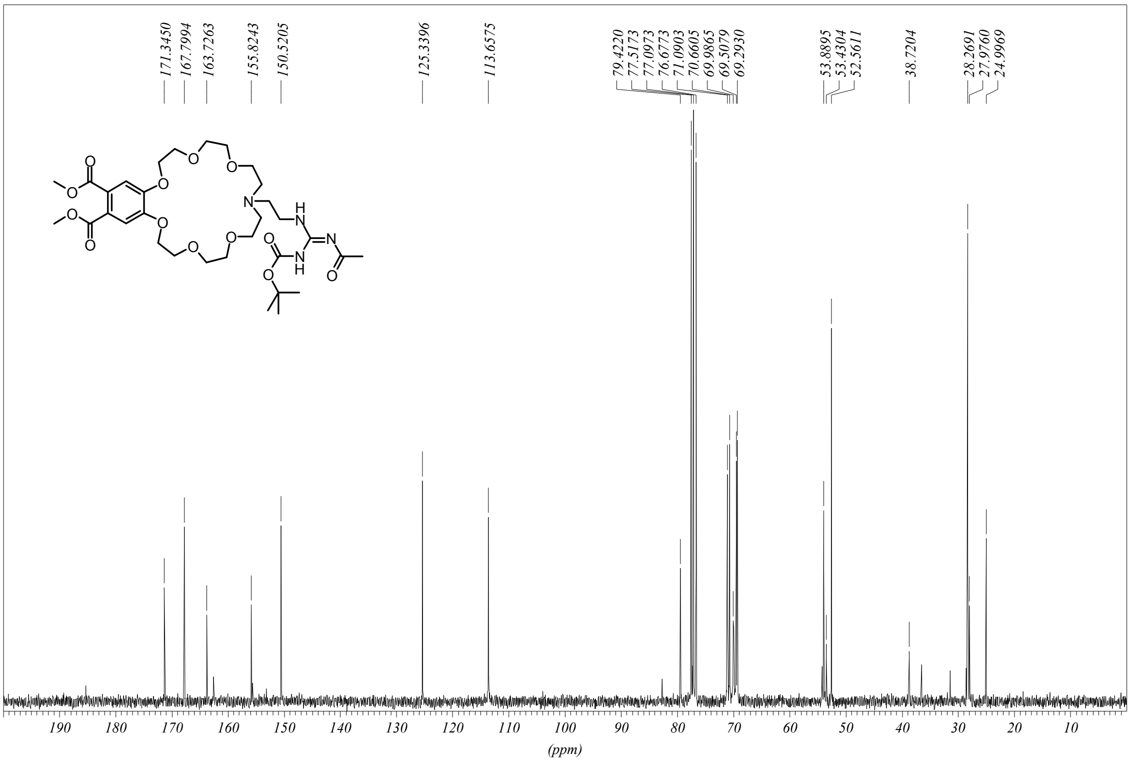


Figure S-27: ^{13}C-NMR spectrum of compound **15a**

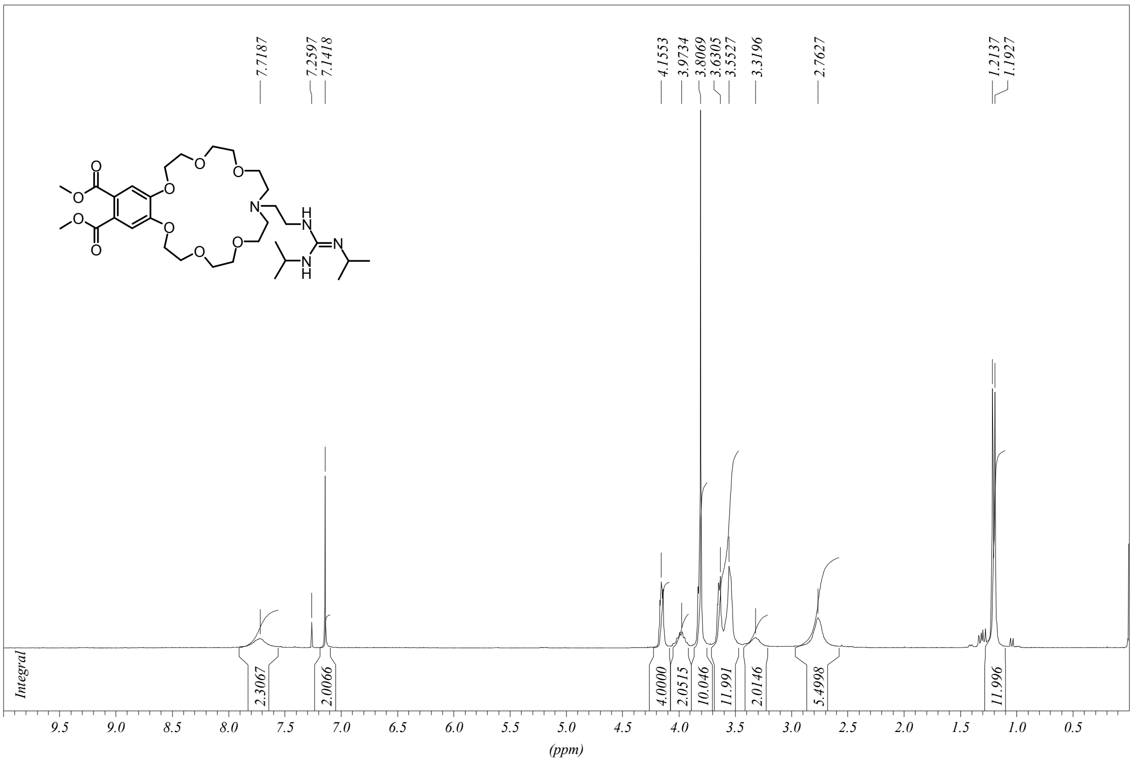


Figure S-28: ^{1}H-NMR spectrum of compound **11a**

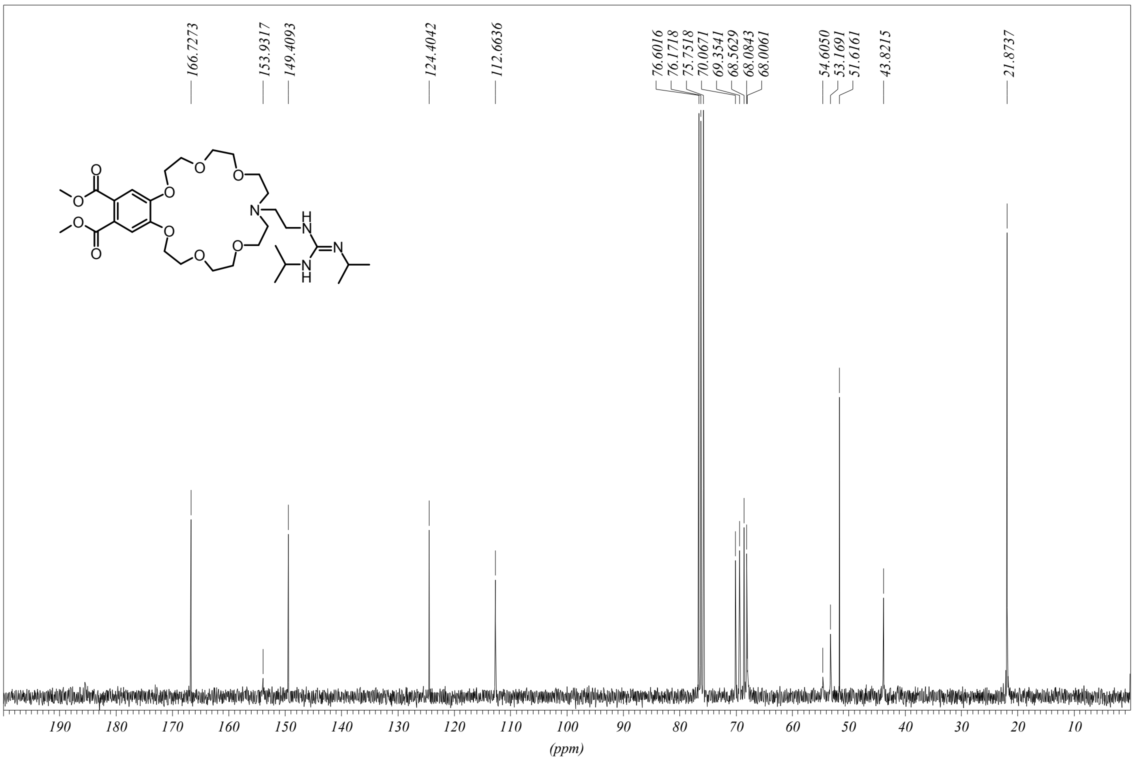


Figure S-29: ^{13}C-NMR spectrum of compound **11a**

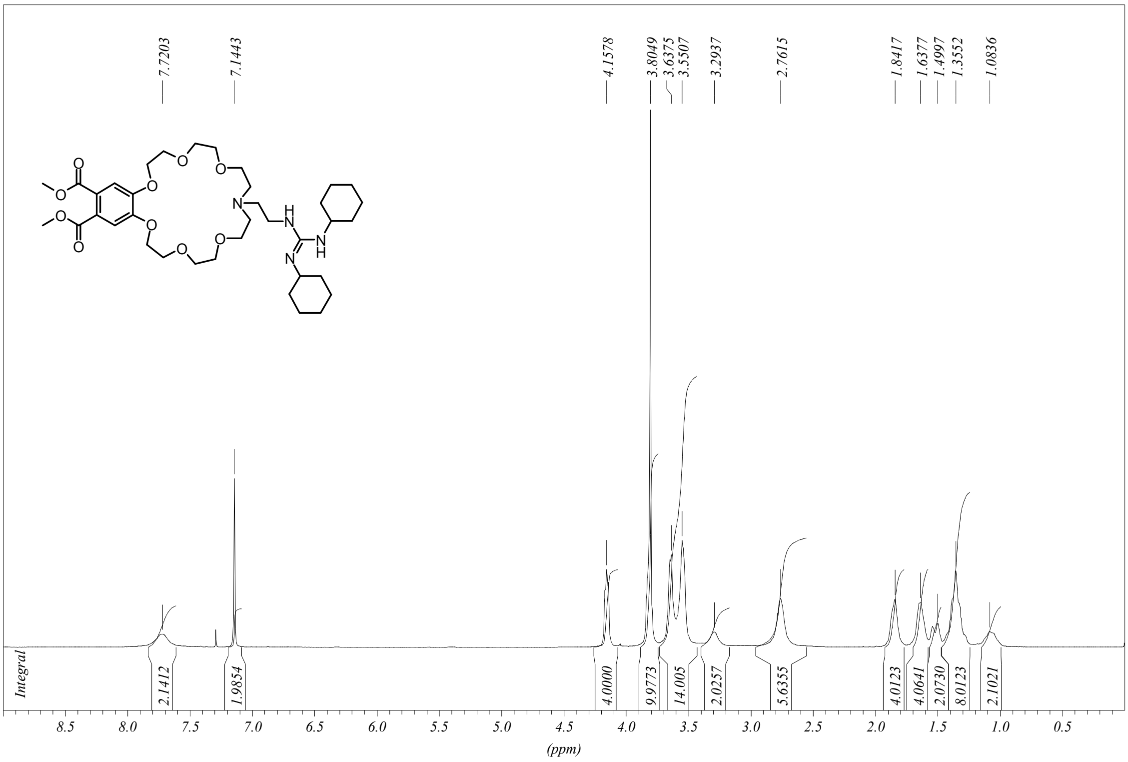


Figure S-30: ^{1}H-NMR spectrum of compound **12a**

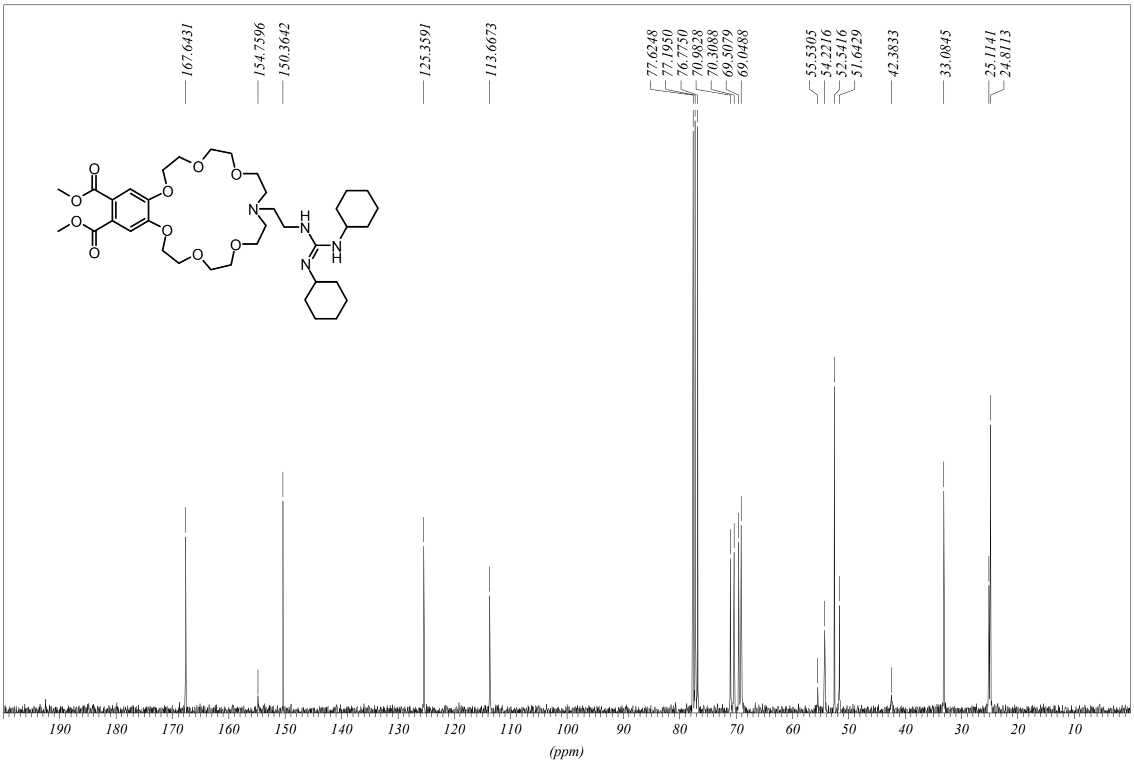


Figure S-31: ^{13}C-NMR spectrum of compound **12a**

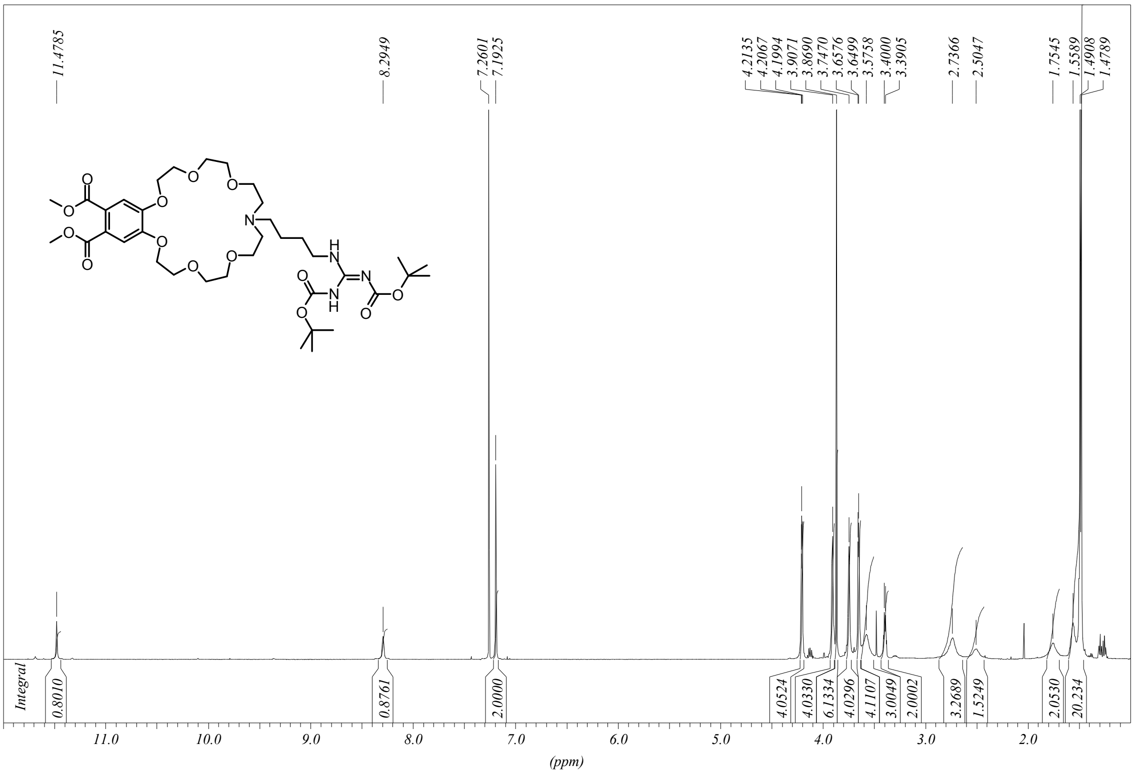


Figure S-32: ^{1}H-NMR spectrum of compound **13b**

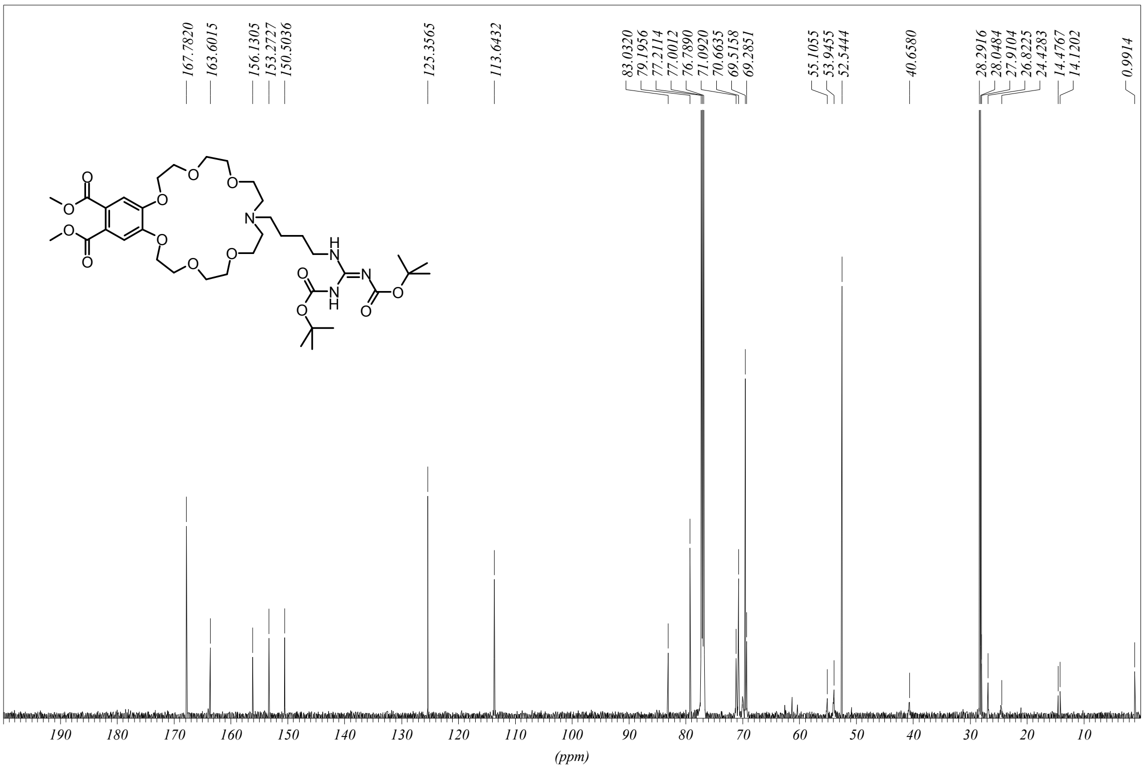


Figure S-33: ^{13}C-NMR spectrum of compound **13b**

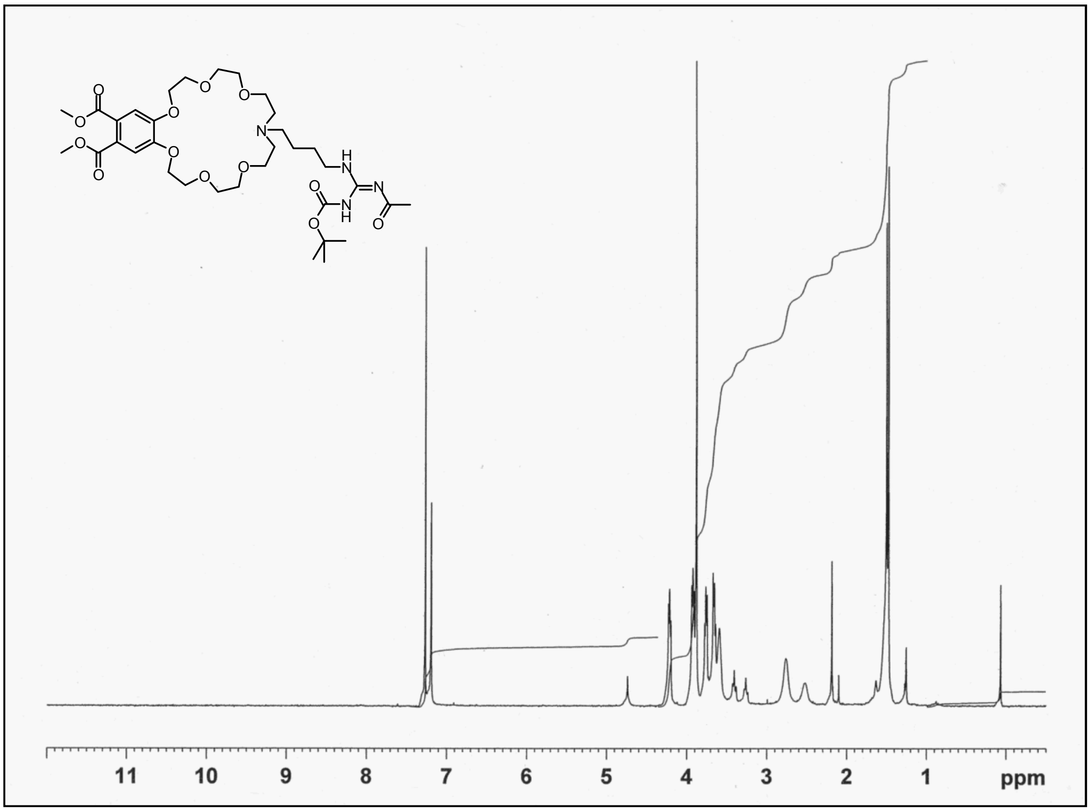


Figure S-34: ^{1}H-NMR spectrum of compound **15b**

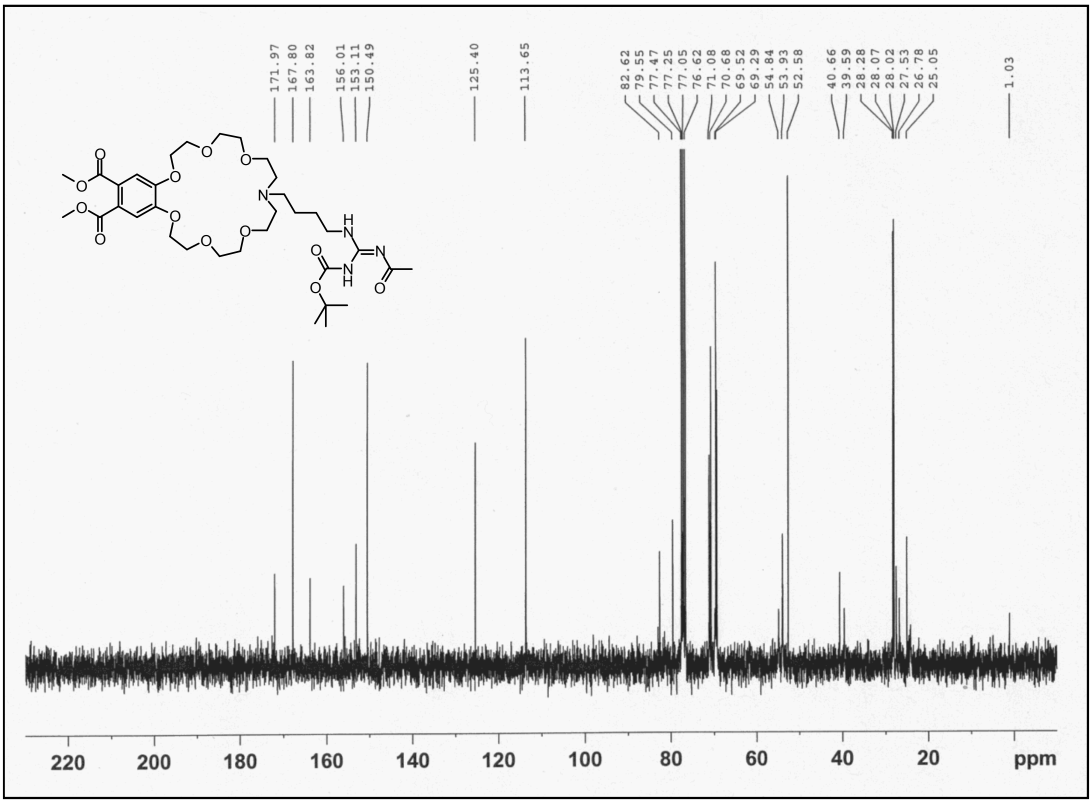


Figure S-35: ^{13}C-NMR spectrum of compound **15b**

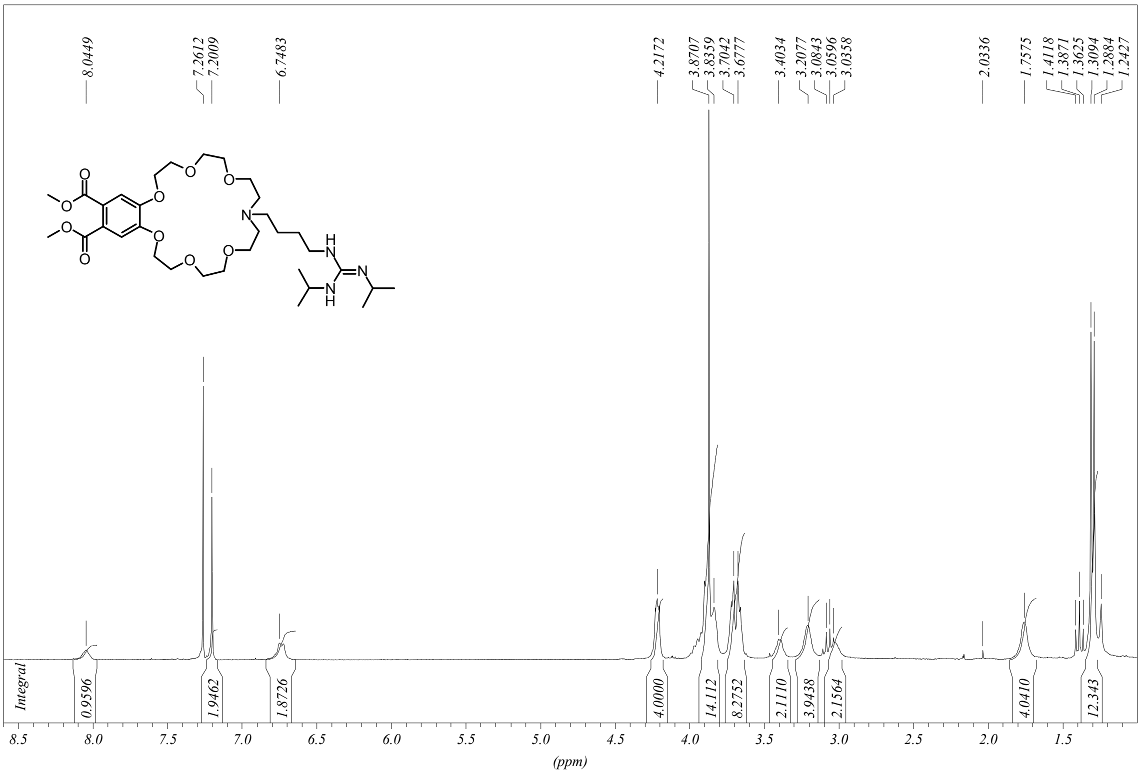


Figure S-36: ^{1}H-NMR spectrum of compound **11b**

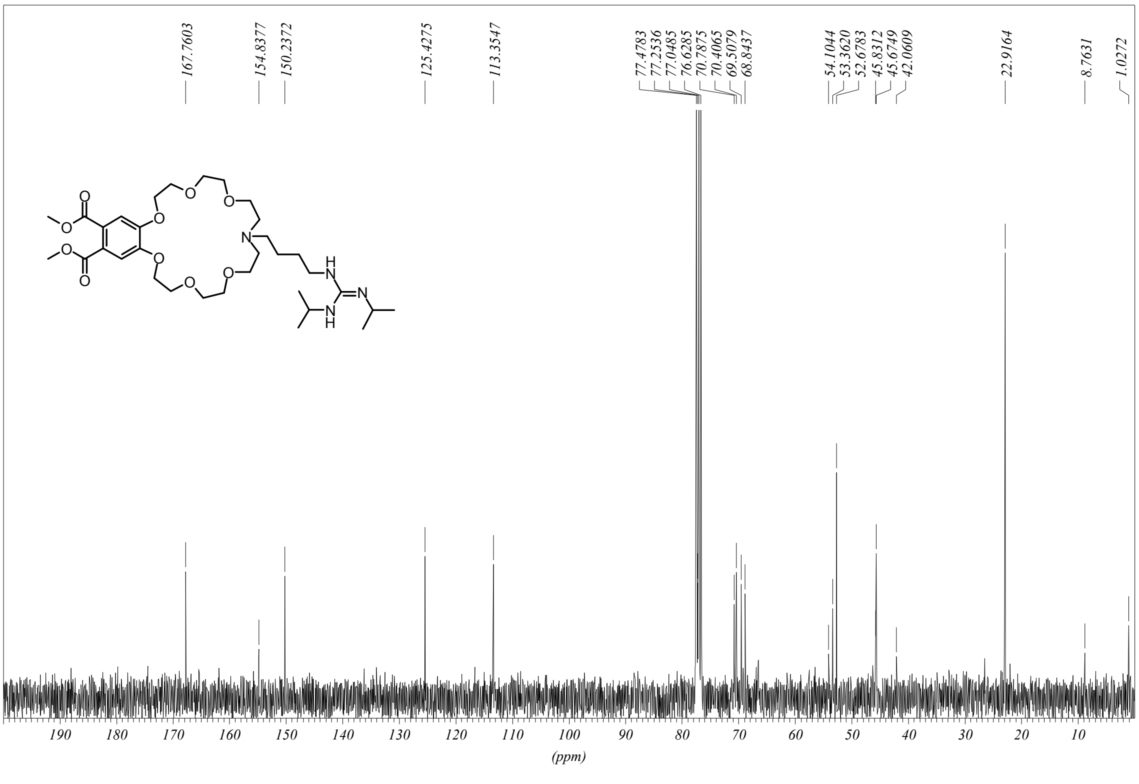


Figure S-37: ^{13}C-NMR spectrum of compound **11b**

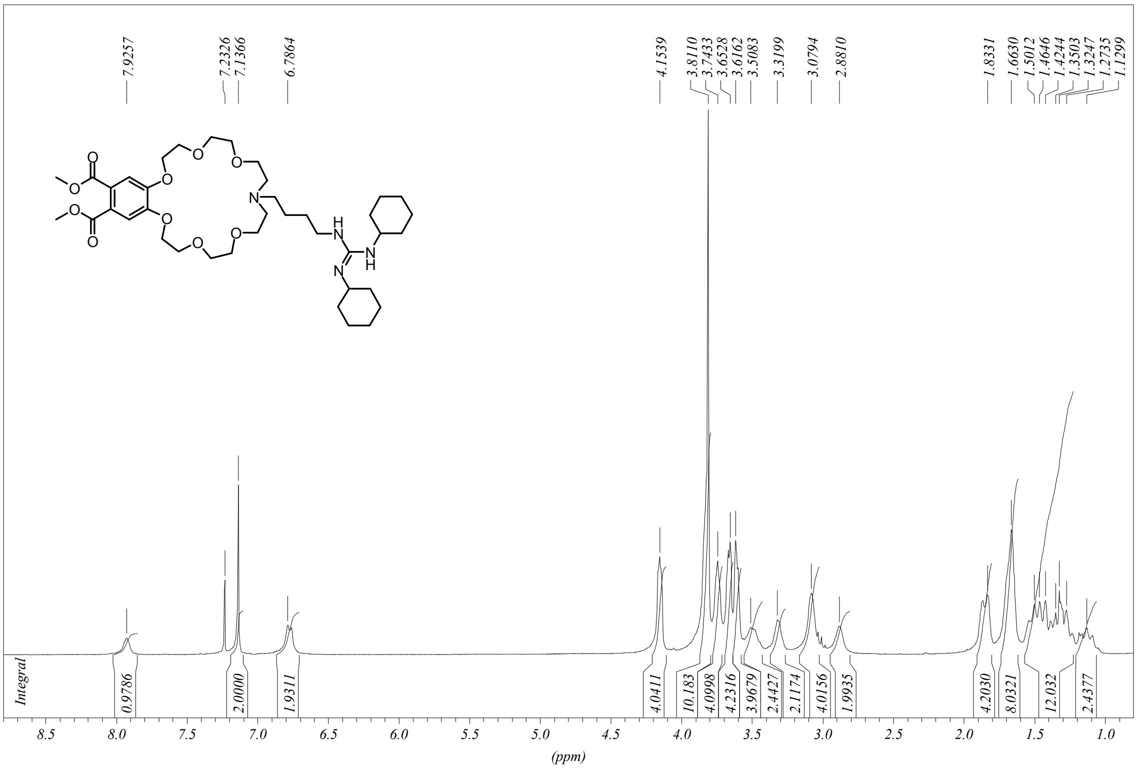


Figure S-38: ^{1}H-NMR spectrum of compound **12b**

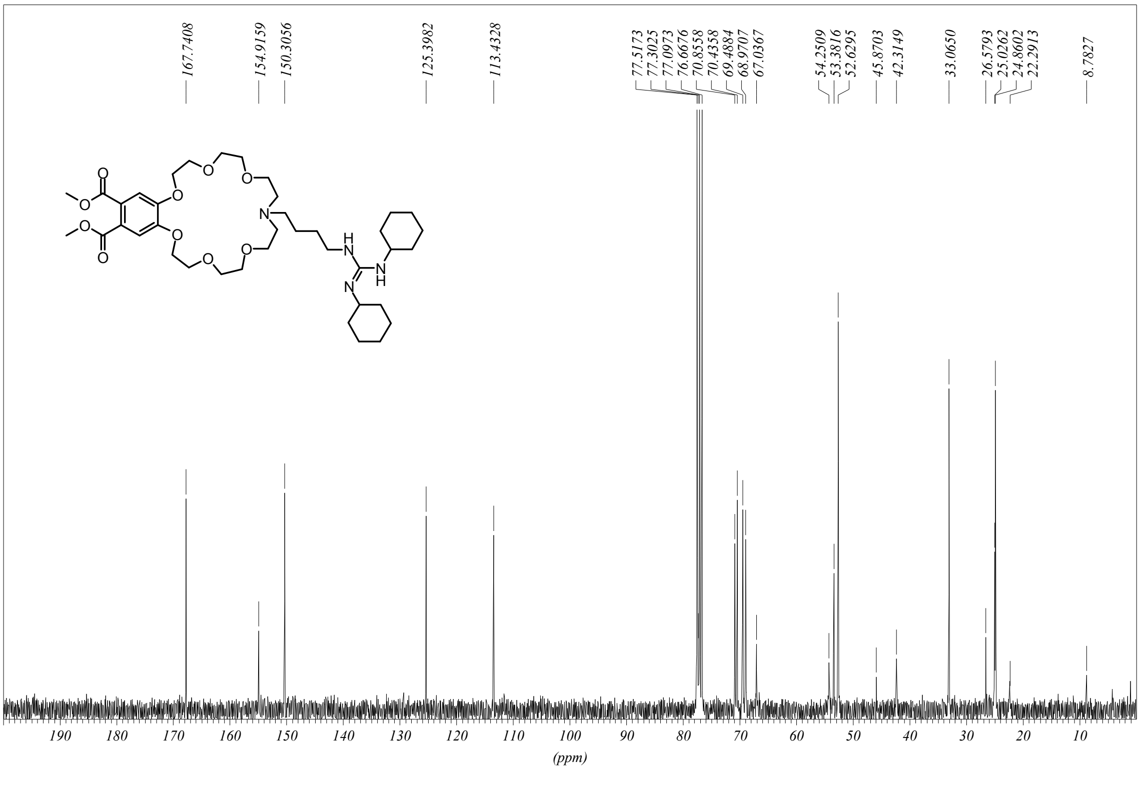


Figure S-39: ^{13}C-NMR spectrum of compound **12b**

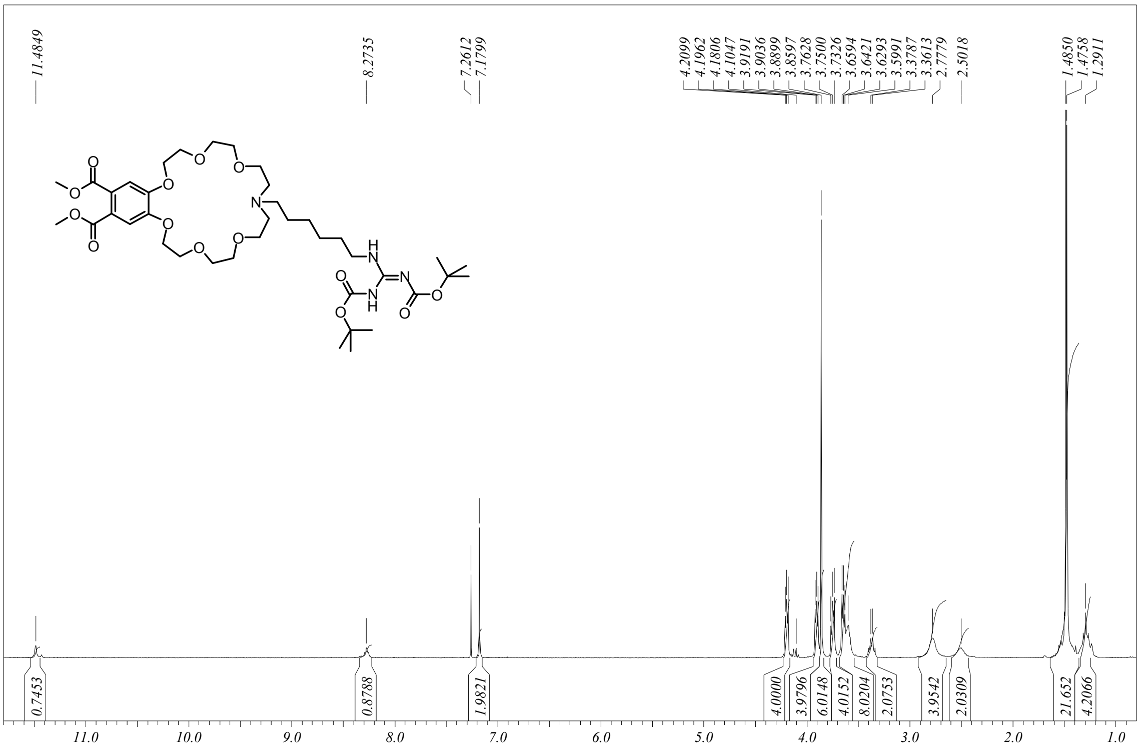


Figure S-40: ^{1}H-NMR spectrum of compound **13c**

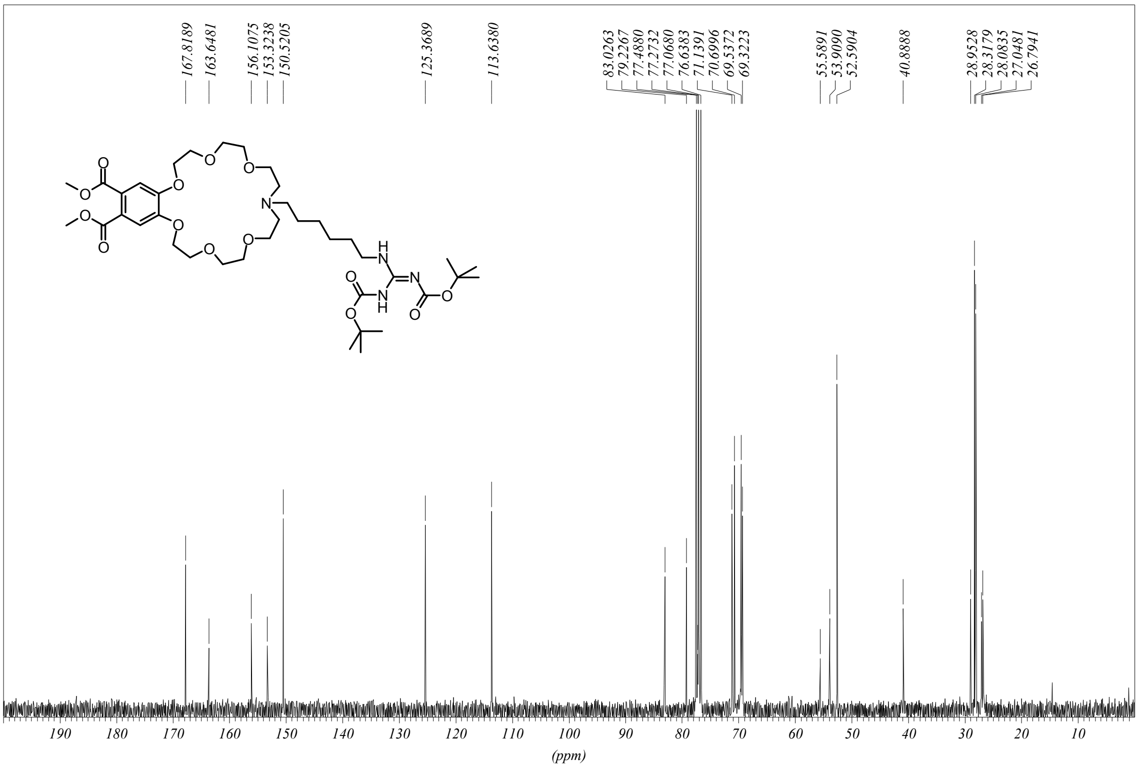


Figure S-41: ^{13}C-NMR spectrum of compound **13c**

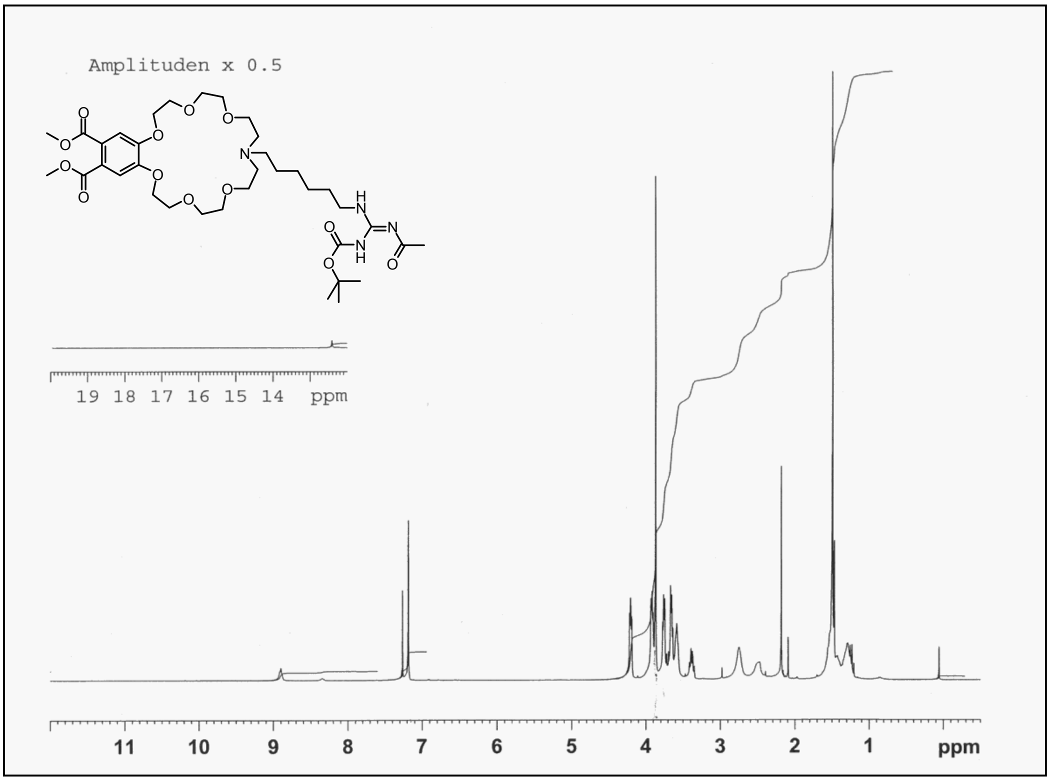


Figure S-42: ^{1}H-NMR spectrum of compound **15c**

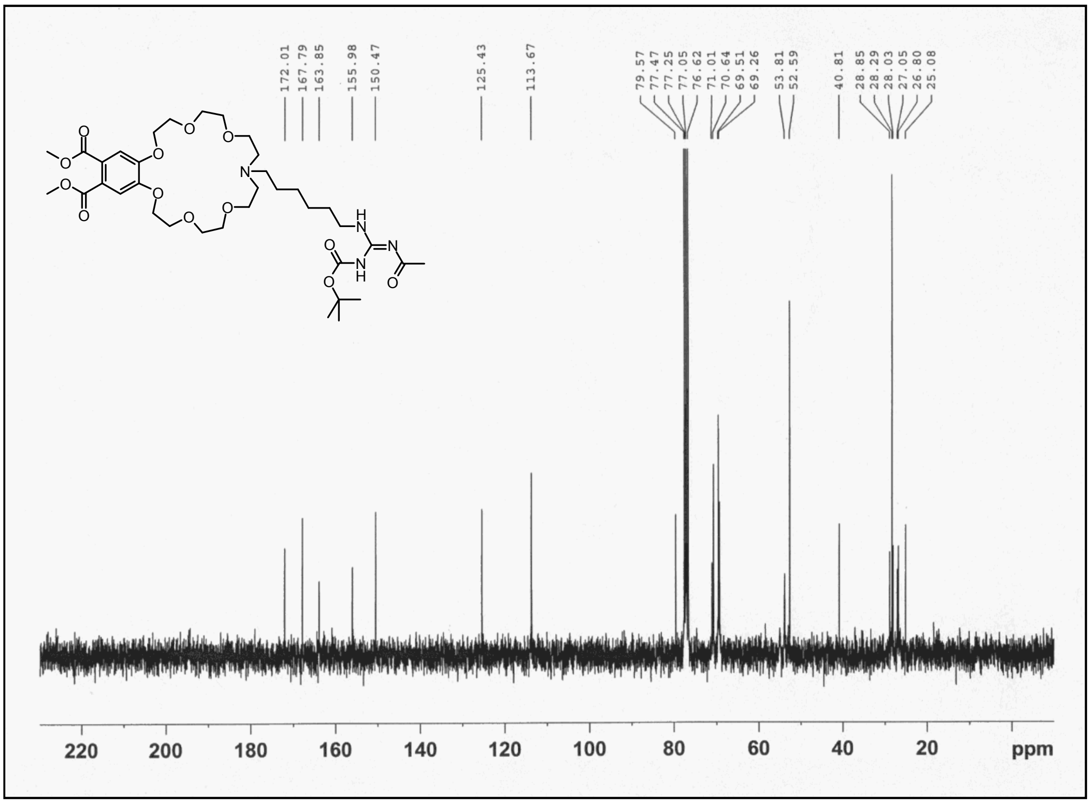


Figure S-43: ^{13}C-NMR spectrum of compound **15c**

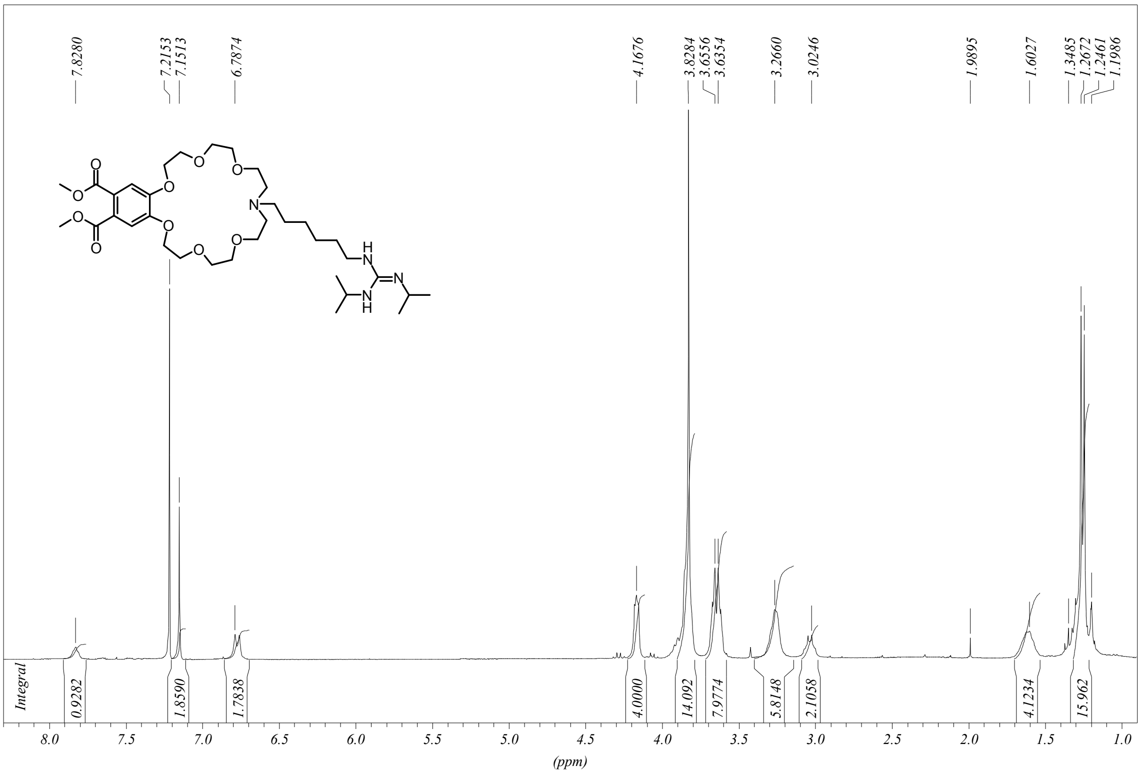


Figure S-44: ^{1}H-NMR spectrum of compound **11c**

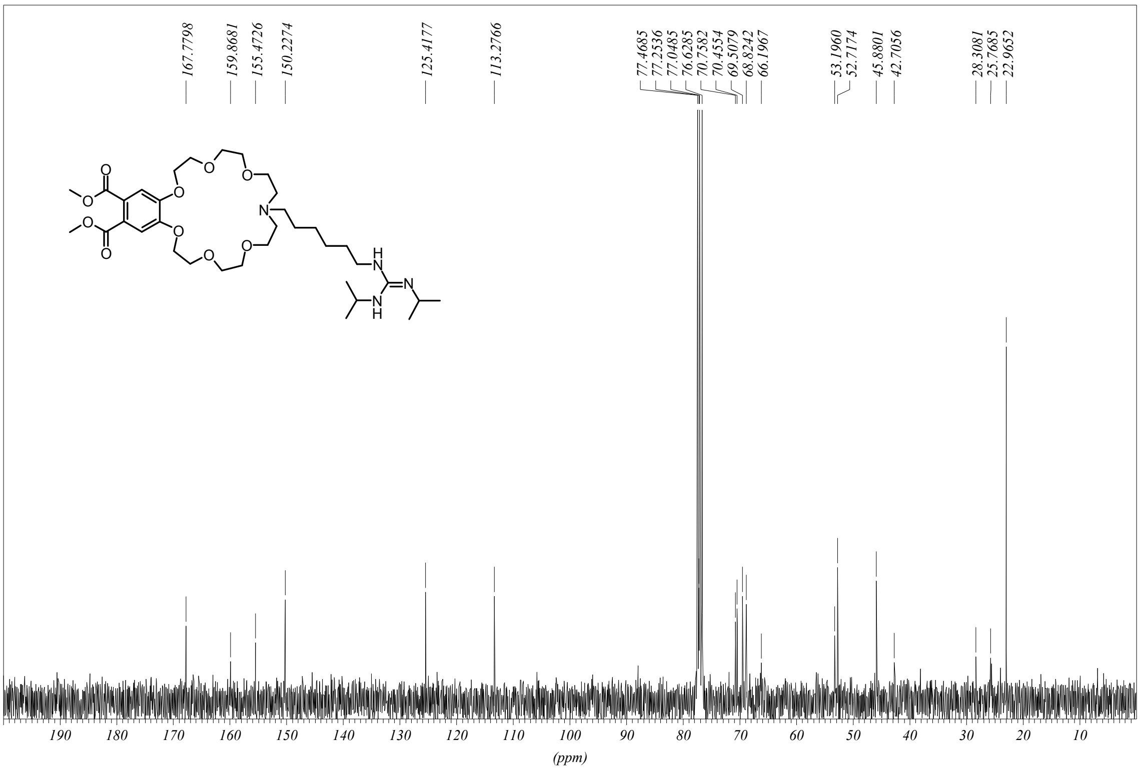


Figure S-45: ^{13}C-NMR spectrum of compound **11c**

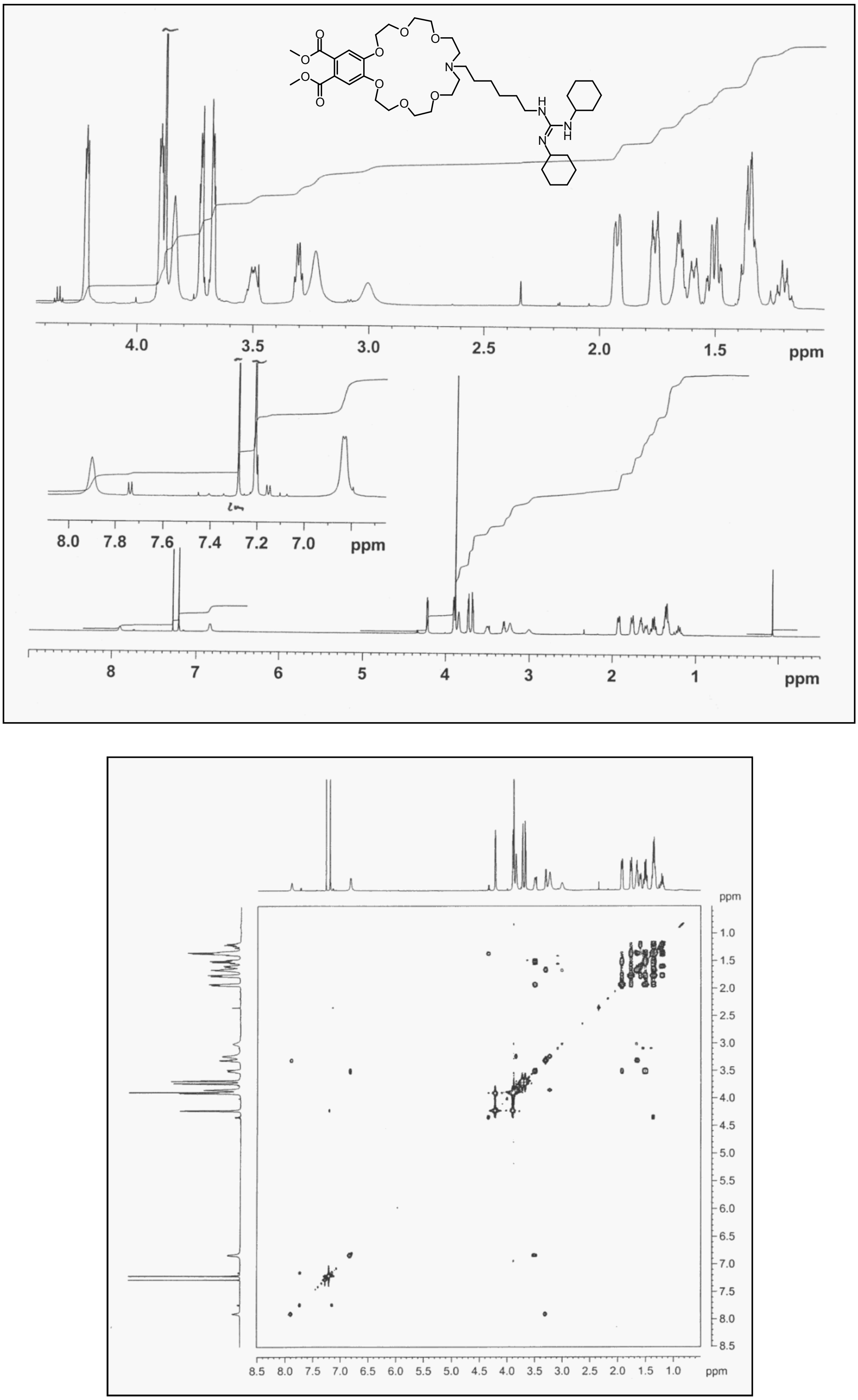
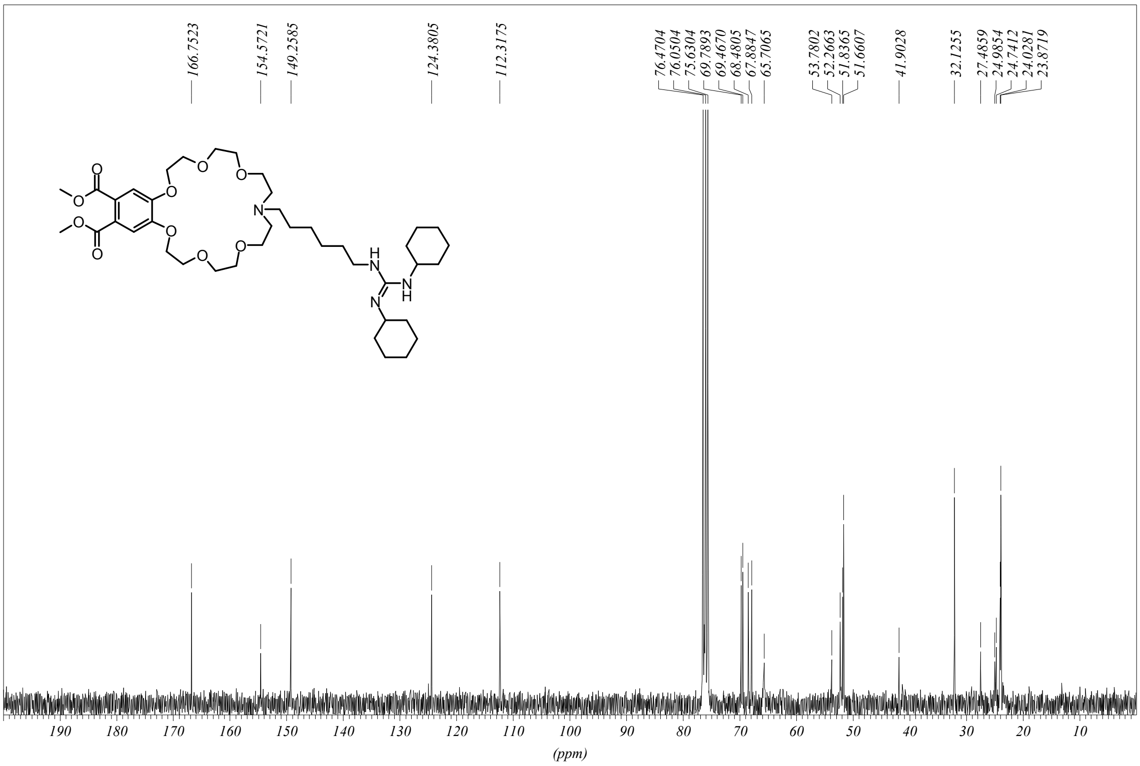


Figure S-46: ^{1}H-NMR and COSY spectrum of compound **12c**



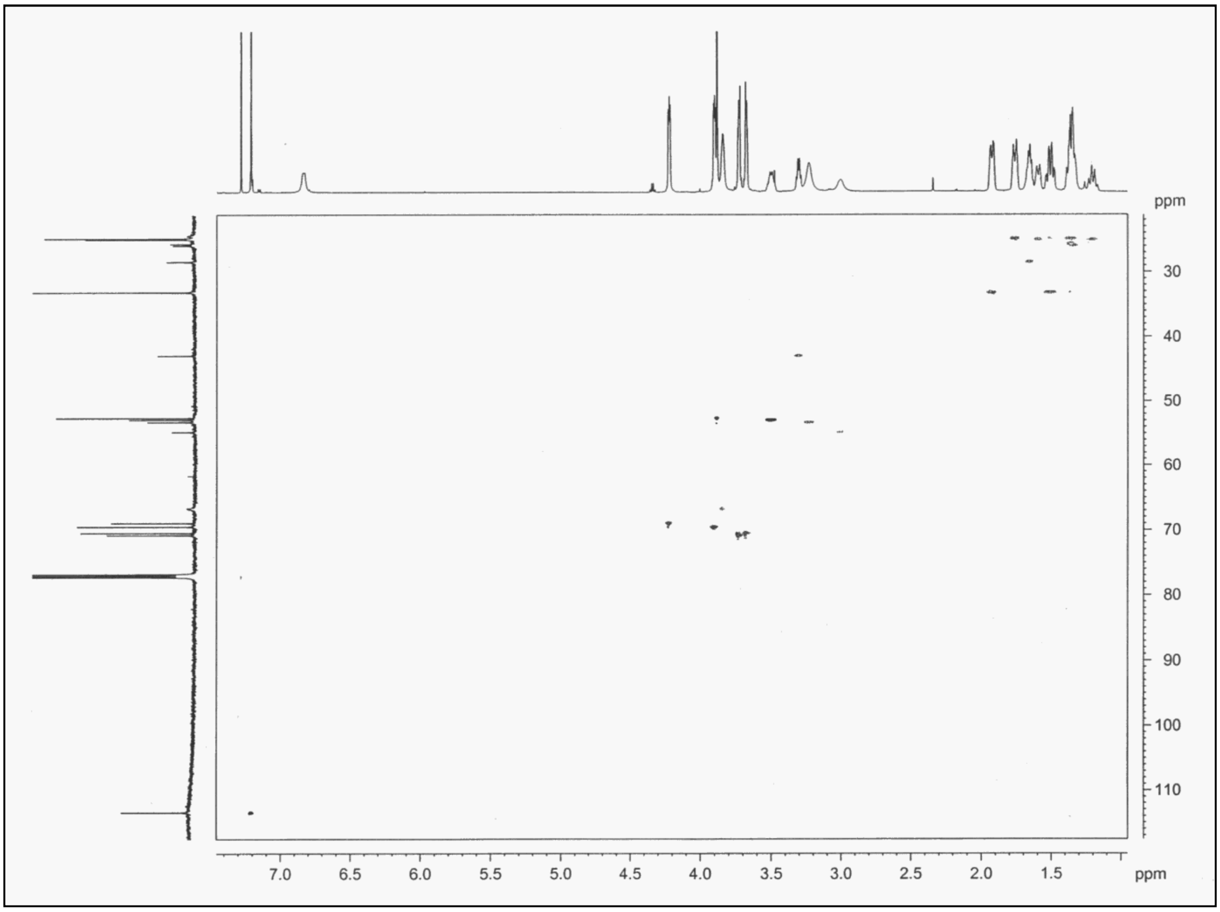


Figure S-47: ^{13}C-NMR spectrum of compound **12c**

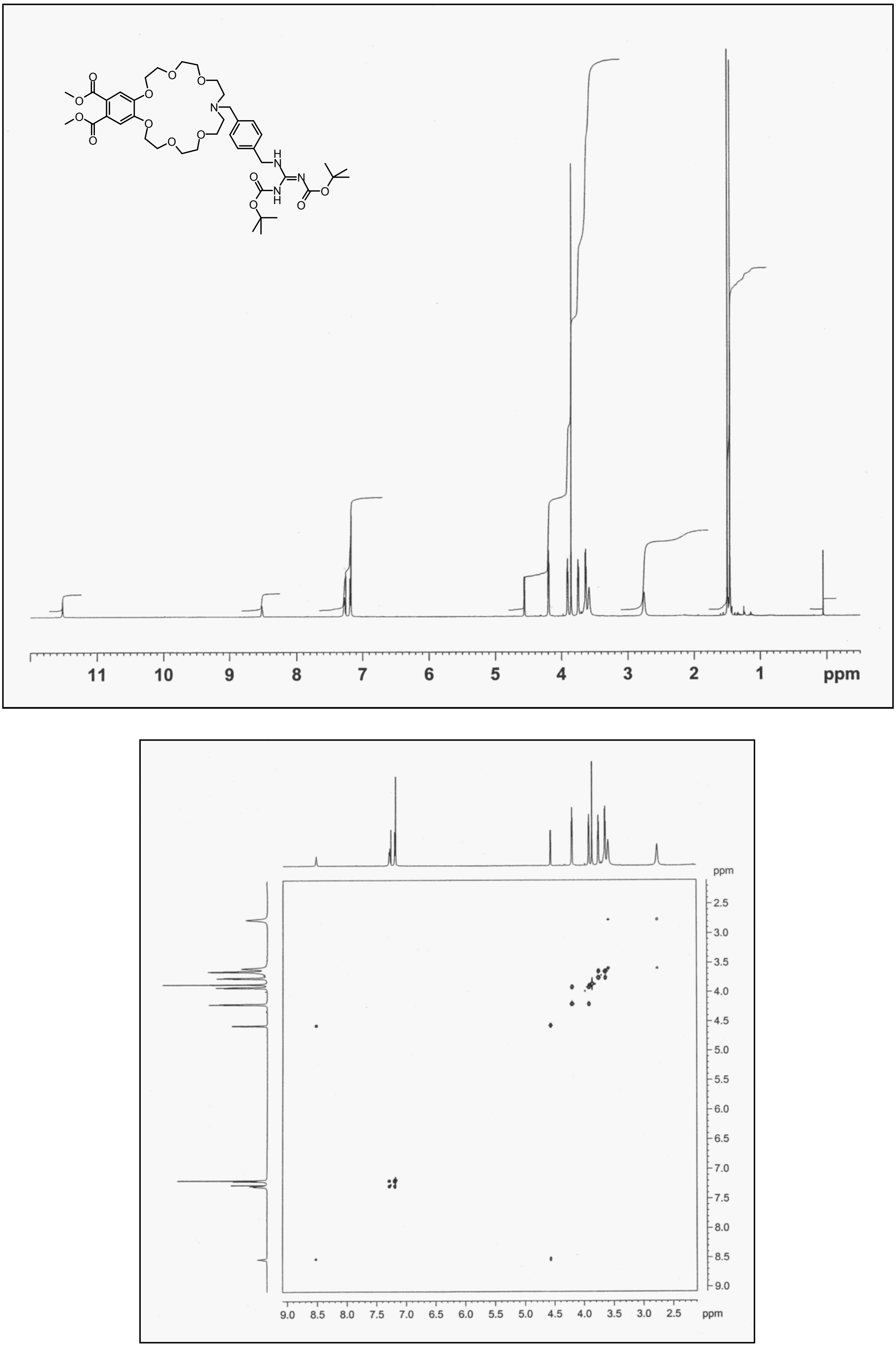


Figure S-48: ^{1}H-NMR spectrum of compound **13d**

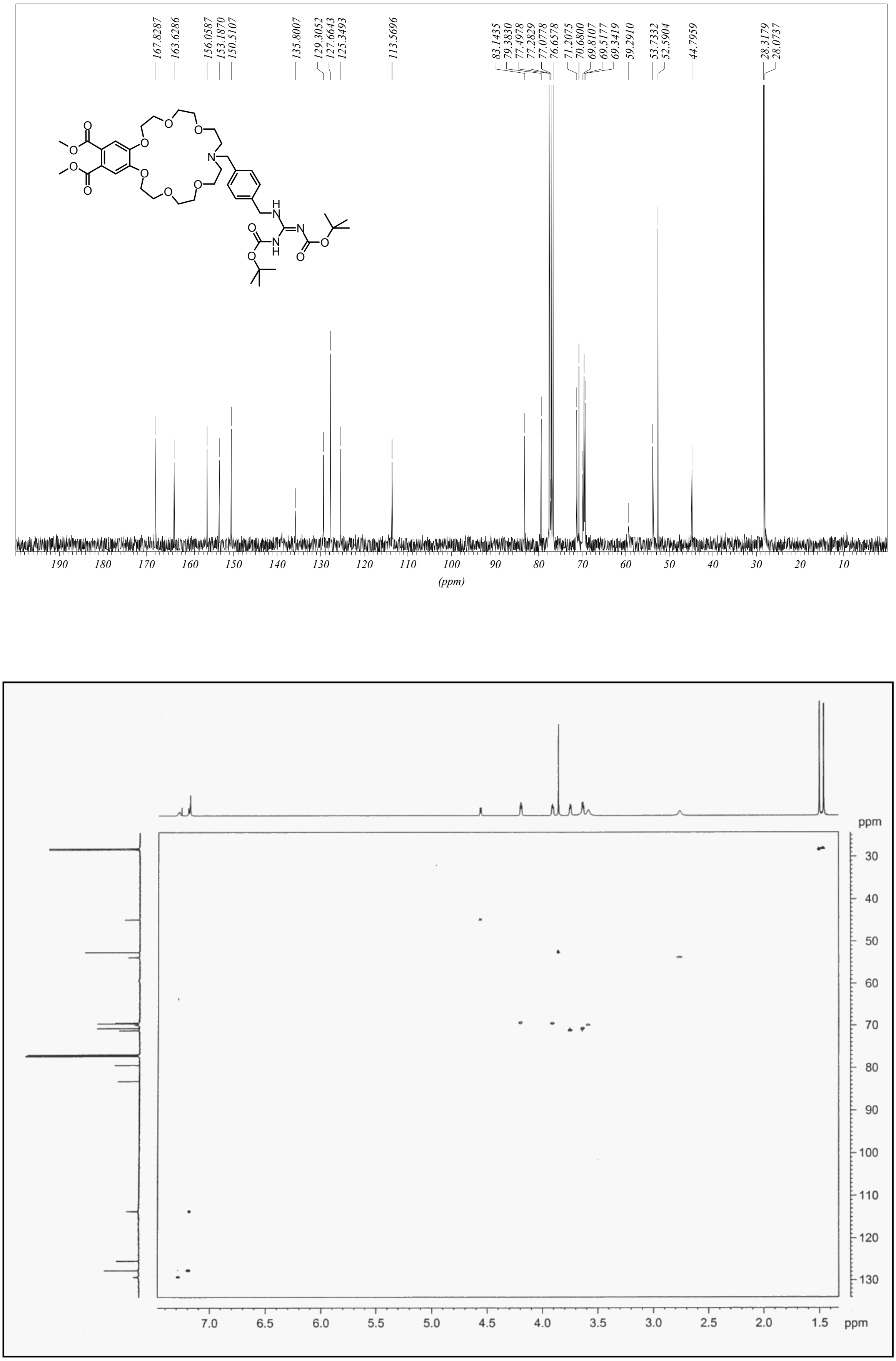


Figure S-49: ^{13}C-NMR and HSQC spectrum of compound **13d**

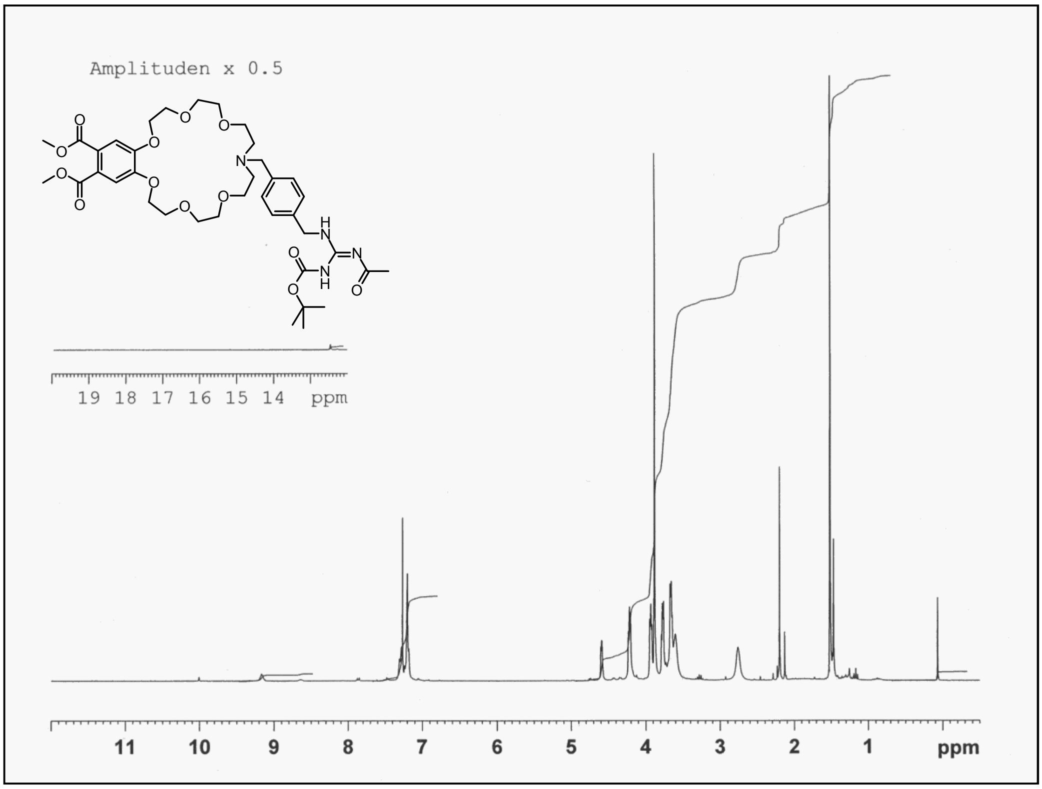


Figure S-50: ^{1}H-NMR spectrum of compound **15d**

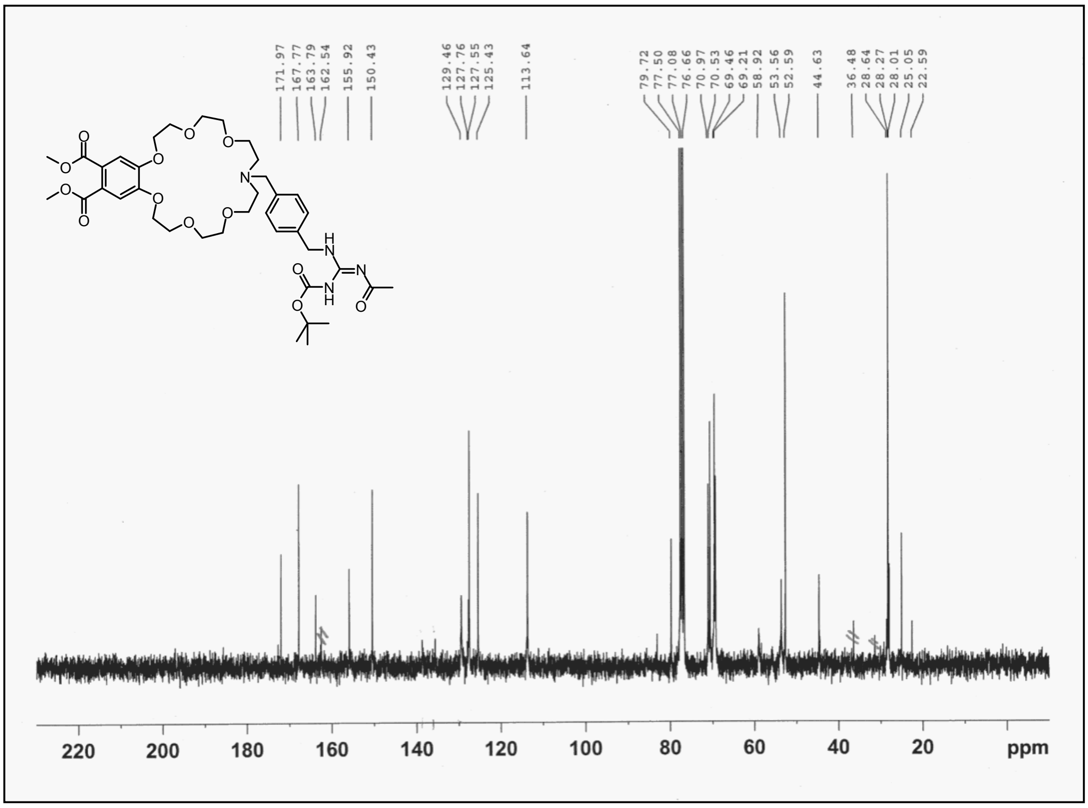


Figure S-51: ^{13}C-NMR spectrum of compound **15d**

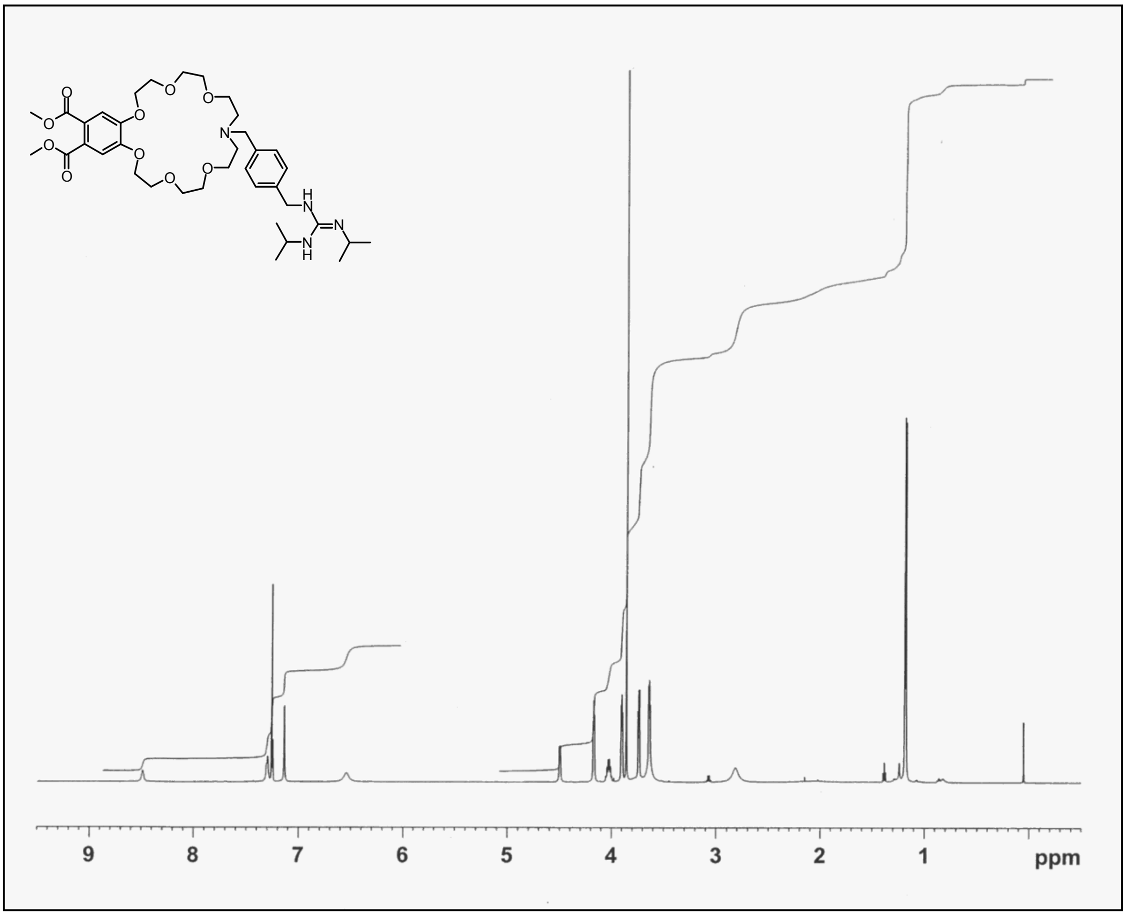
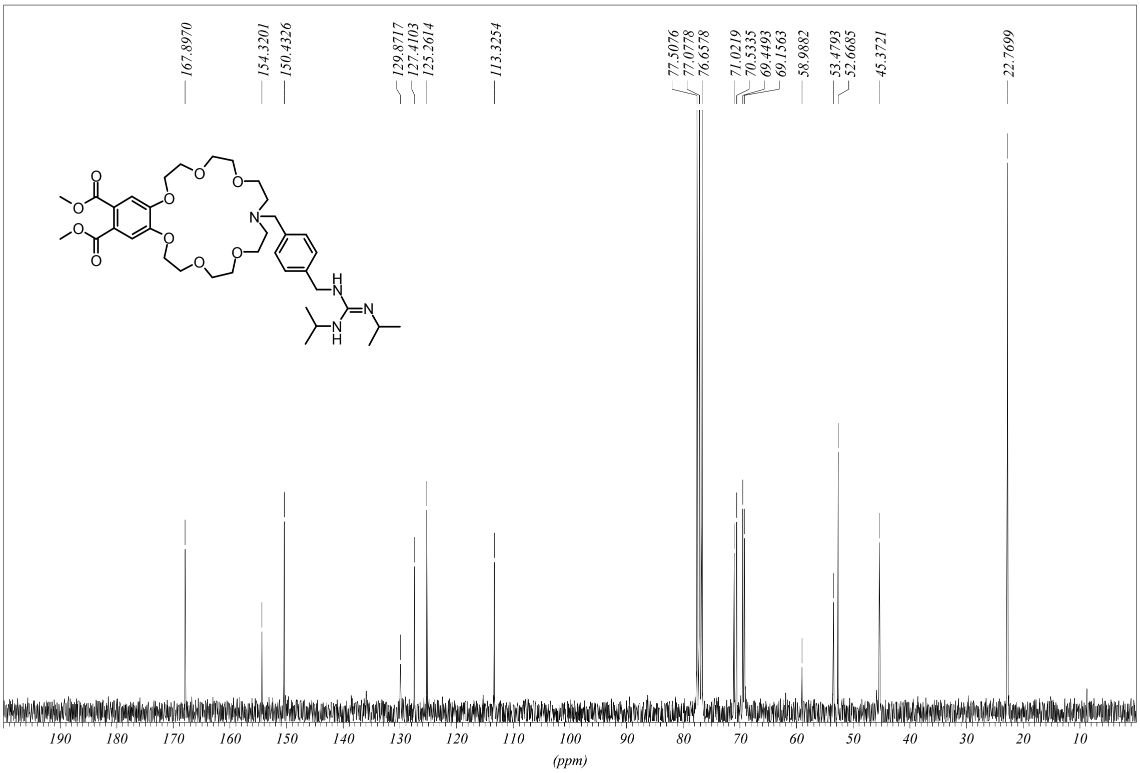


Figure S-52: ^{1}H-NMR spectrum of compound **11d**



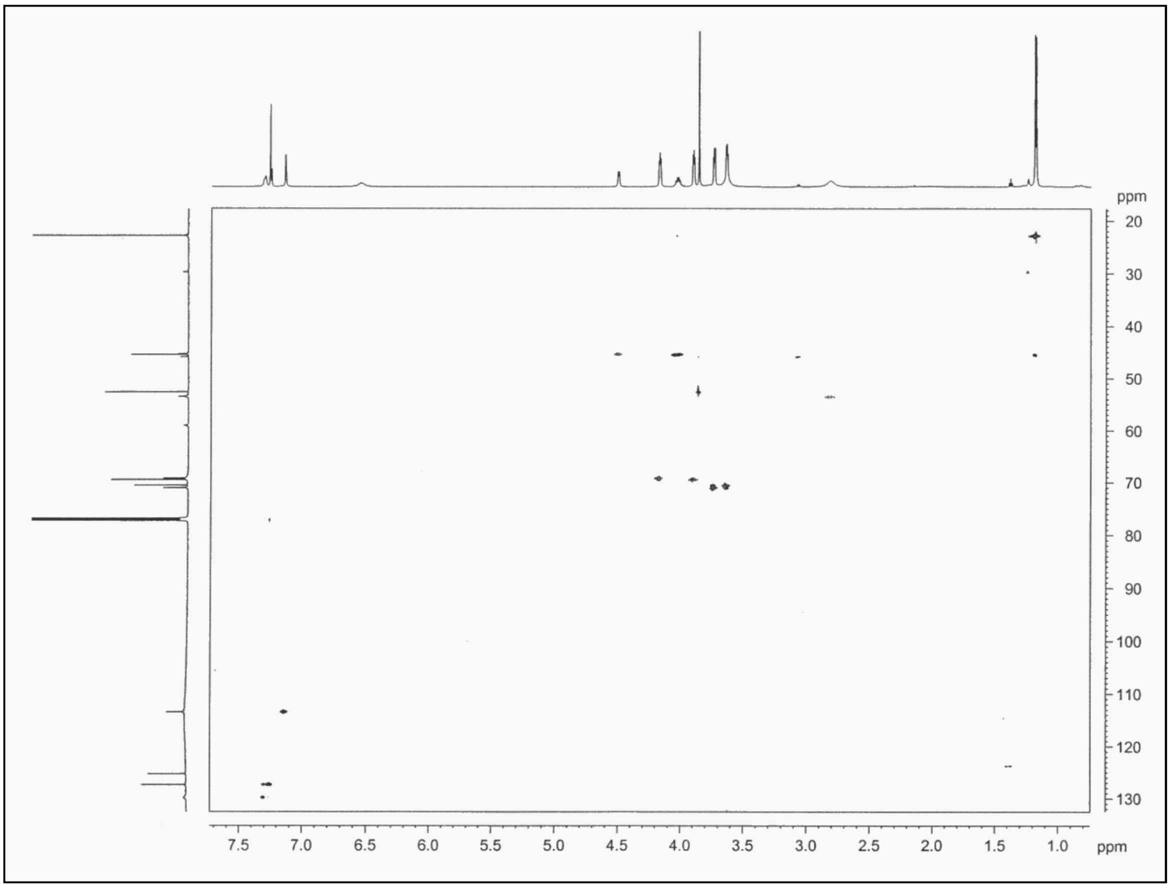


Figure S-53: ^{13}C-NMR spectrum of compound **11d**

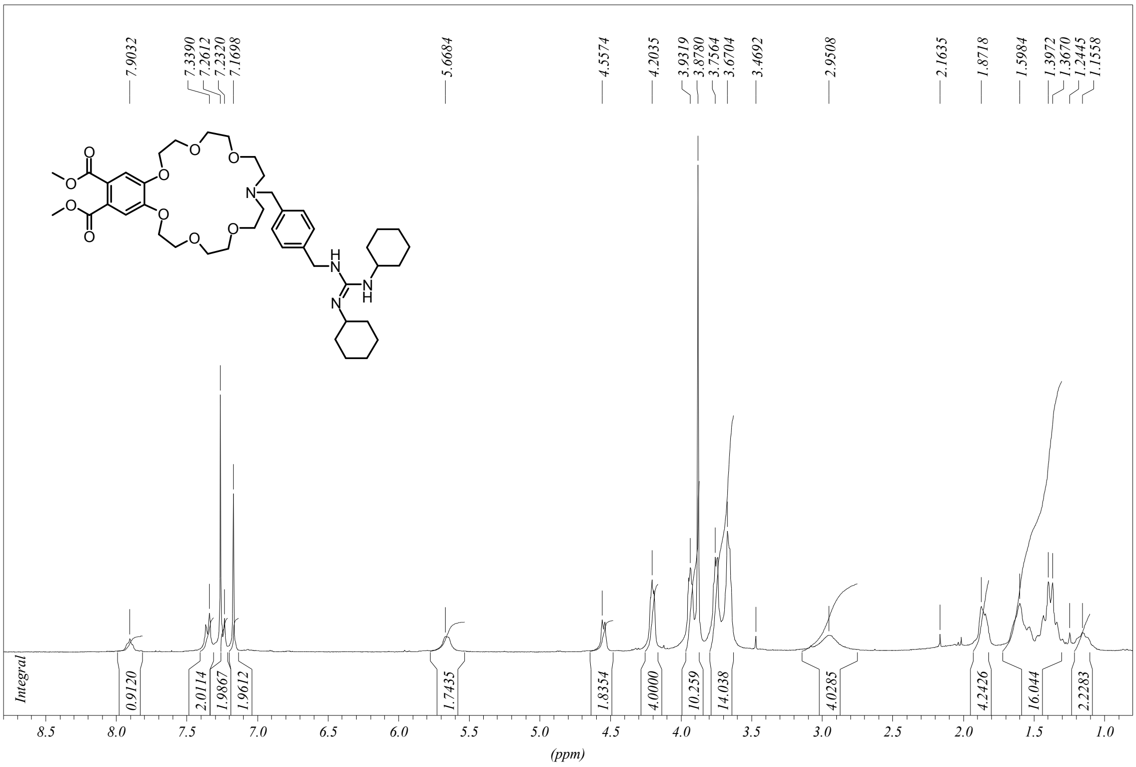


Figure S-54: ^{1}H-NMR spectrum of compound **12d**

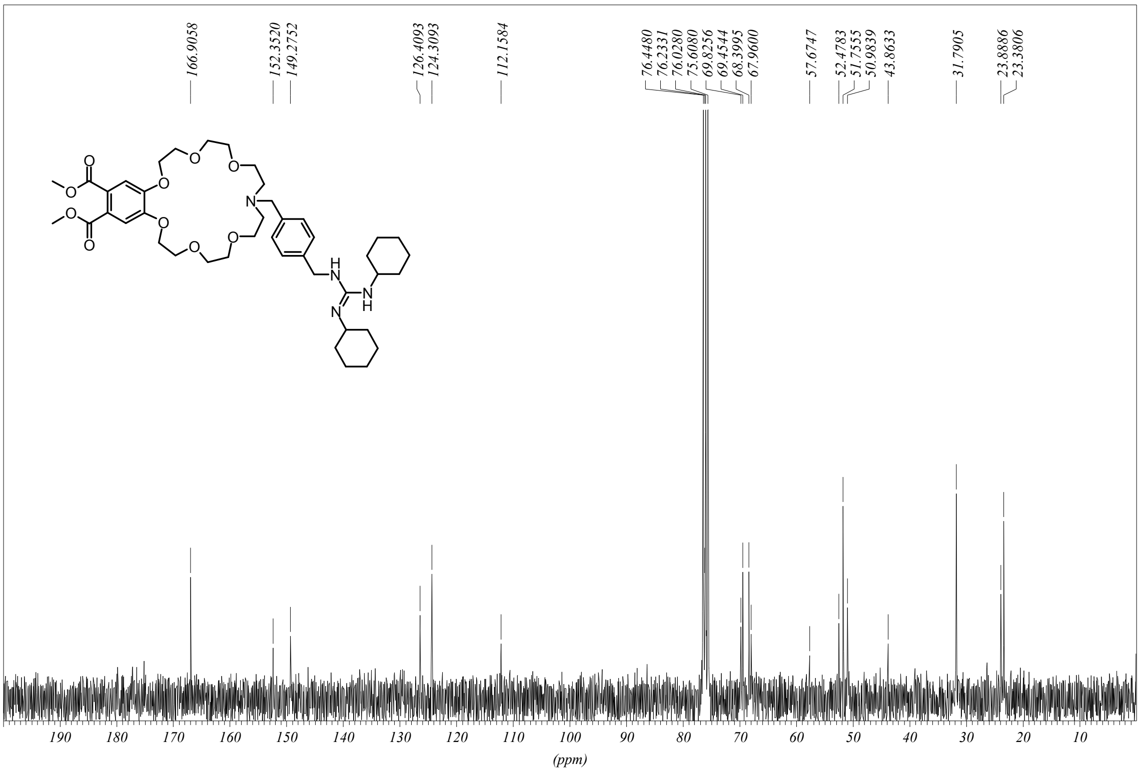


Figure S-55: ^{13}C-NMR spectrum of compound **12d**

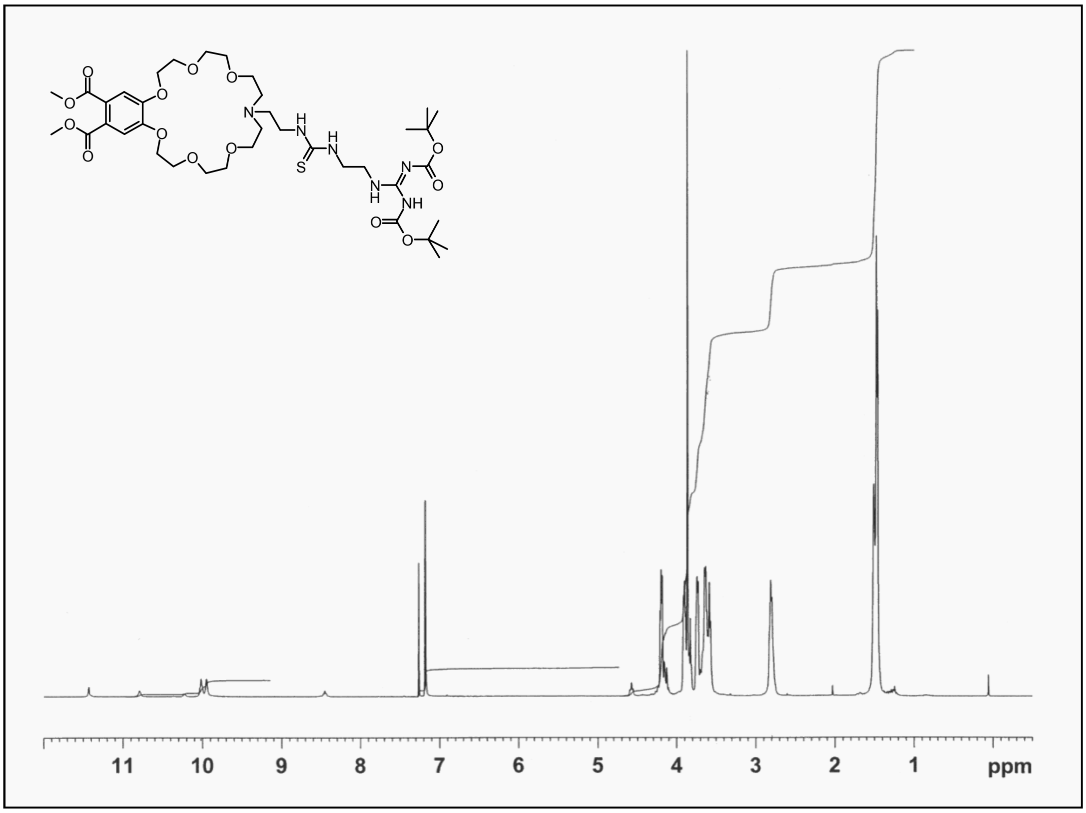


Figure S-56: ^{1}H-NMR spectrum of compound **13e**

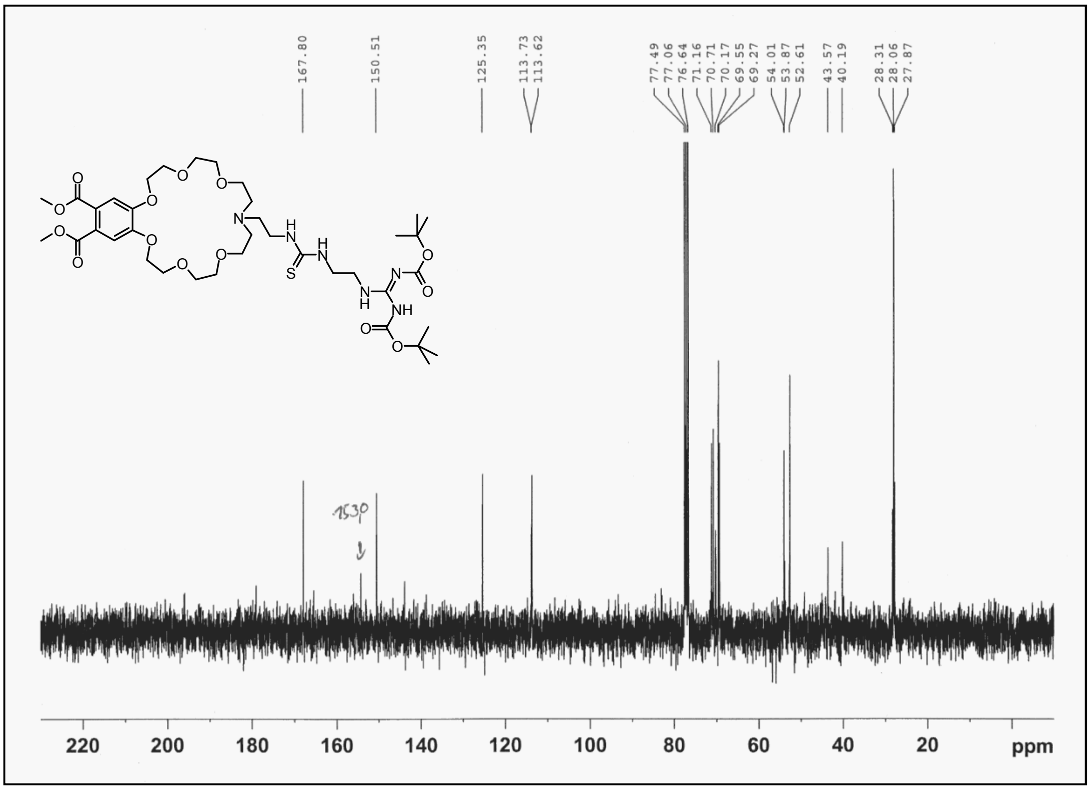


Figure S-57: ^{13}C-NMR spectrum of compound **13e**

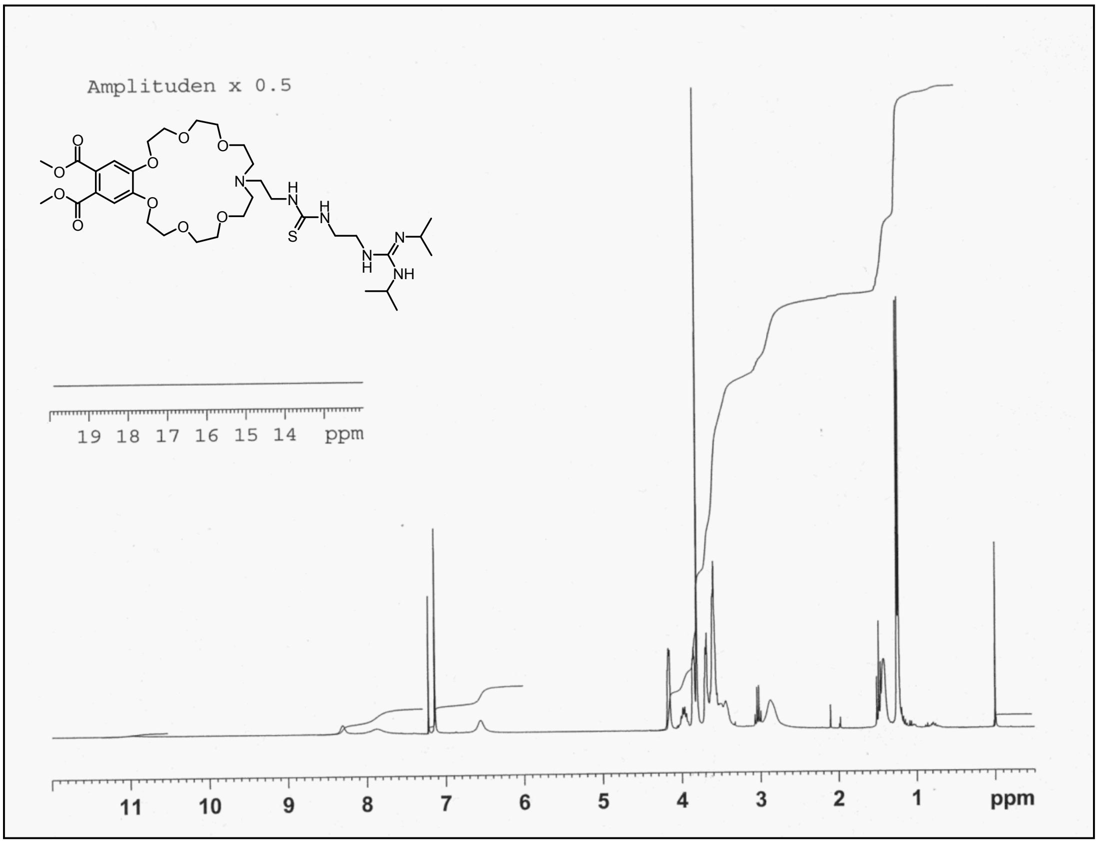


Figure S-58: ^{1}H-NMR spectrum of compound **11e**

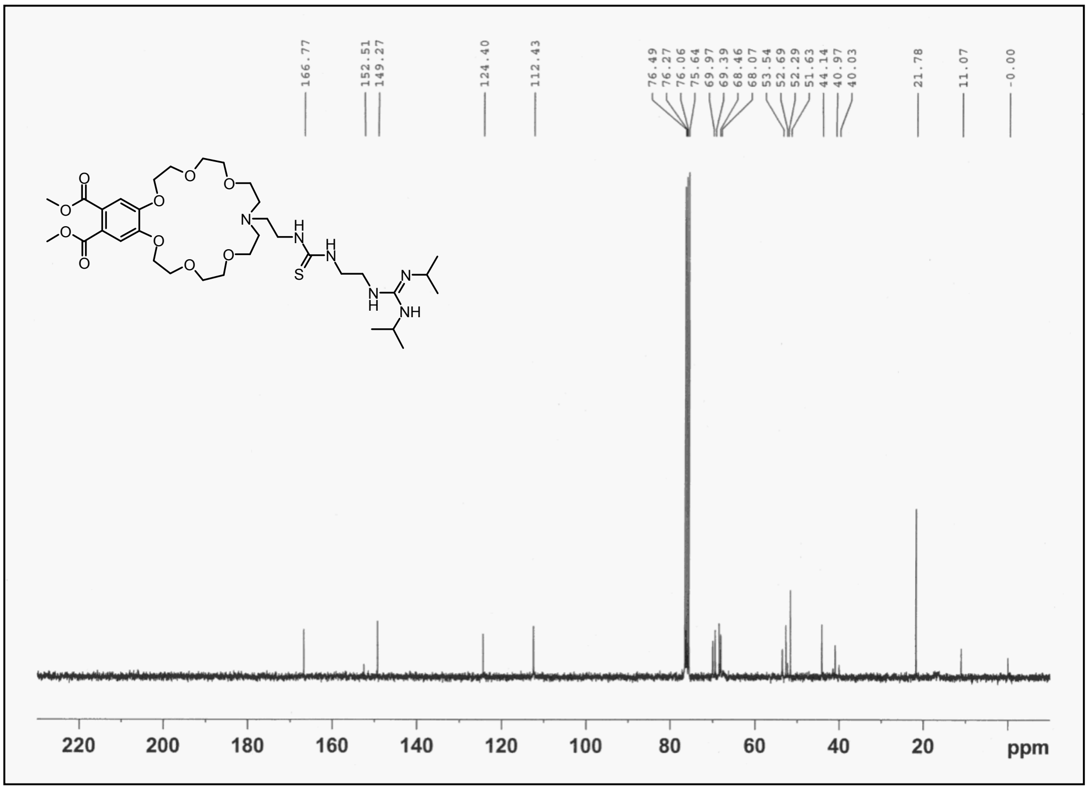


Figure S-59: ^{13}C-NMR spectrum of compound **11e**

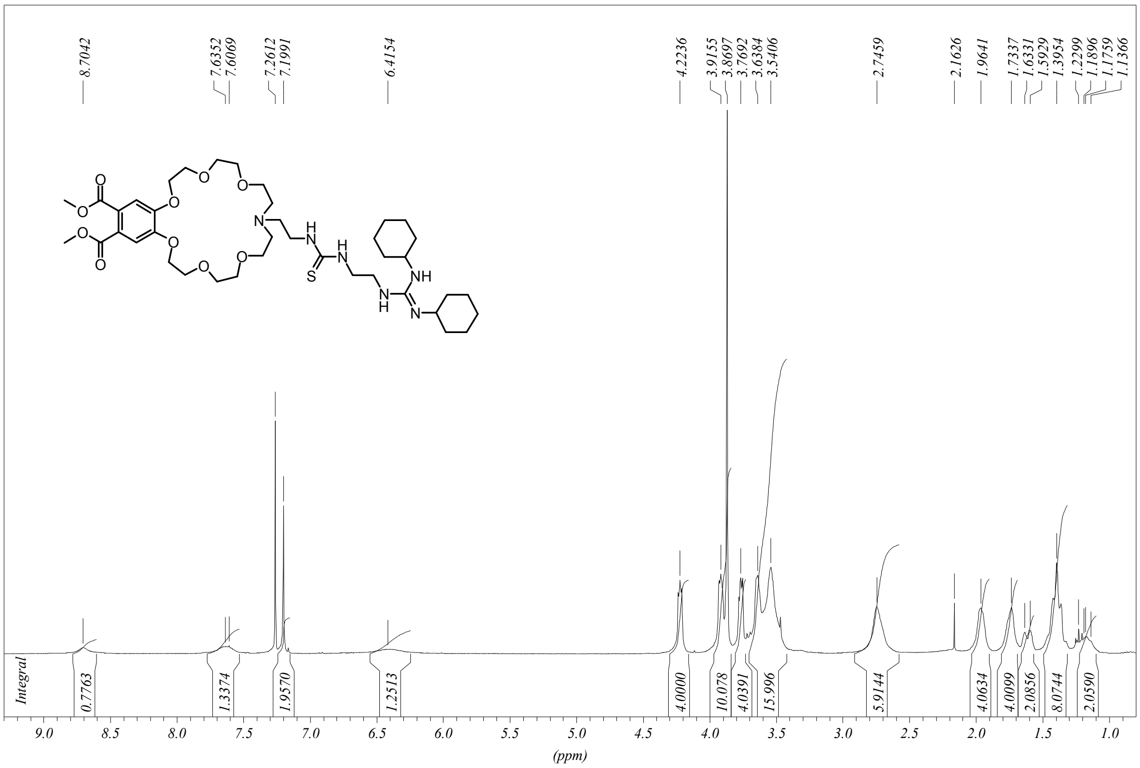


Figure S-60: ^{1}H-NMR spectrum of compound **12e**

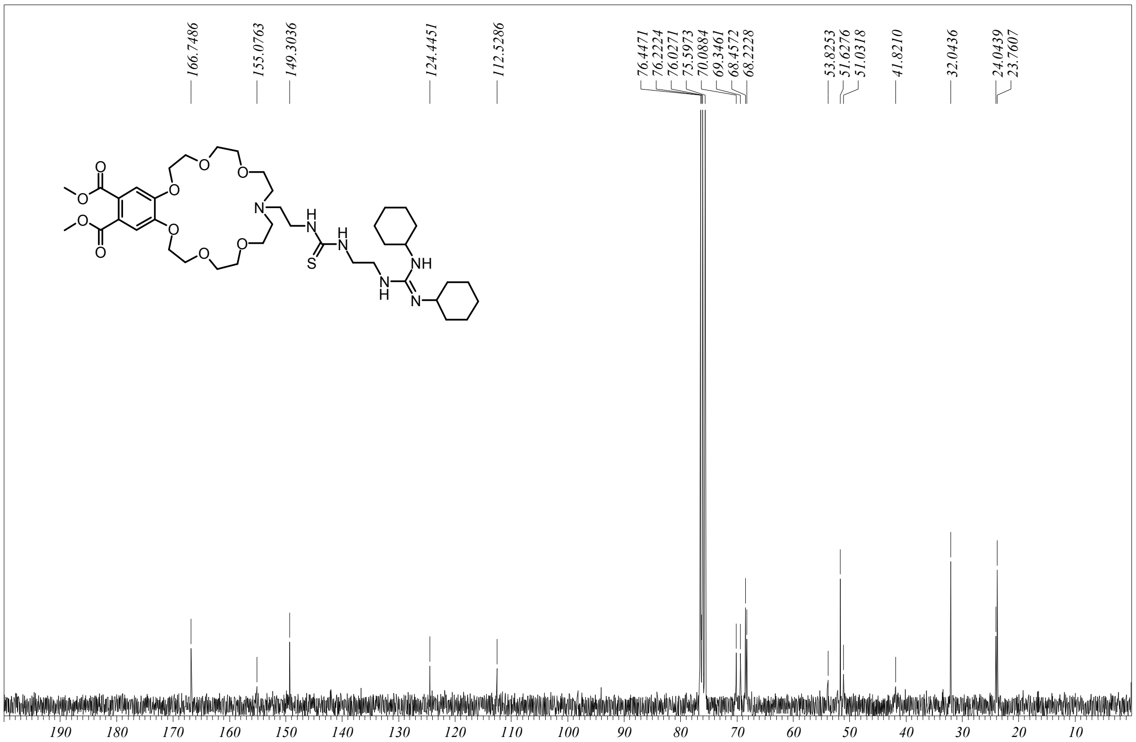


Figure S-61: ^{13}C-NMR spectrum of compound **12e**

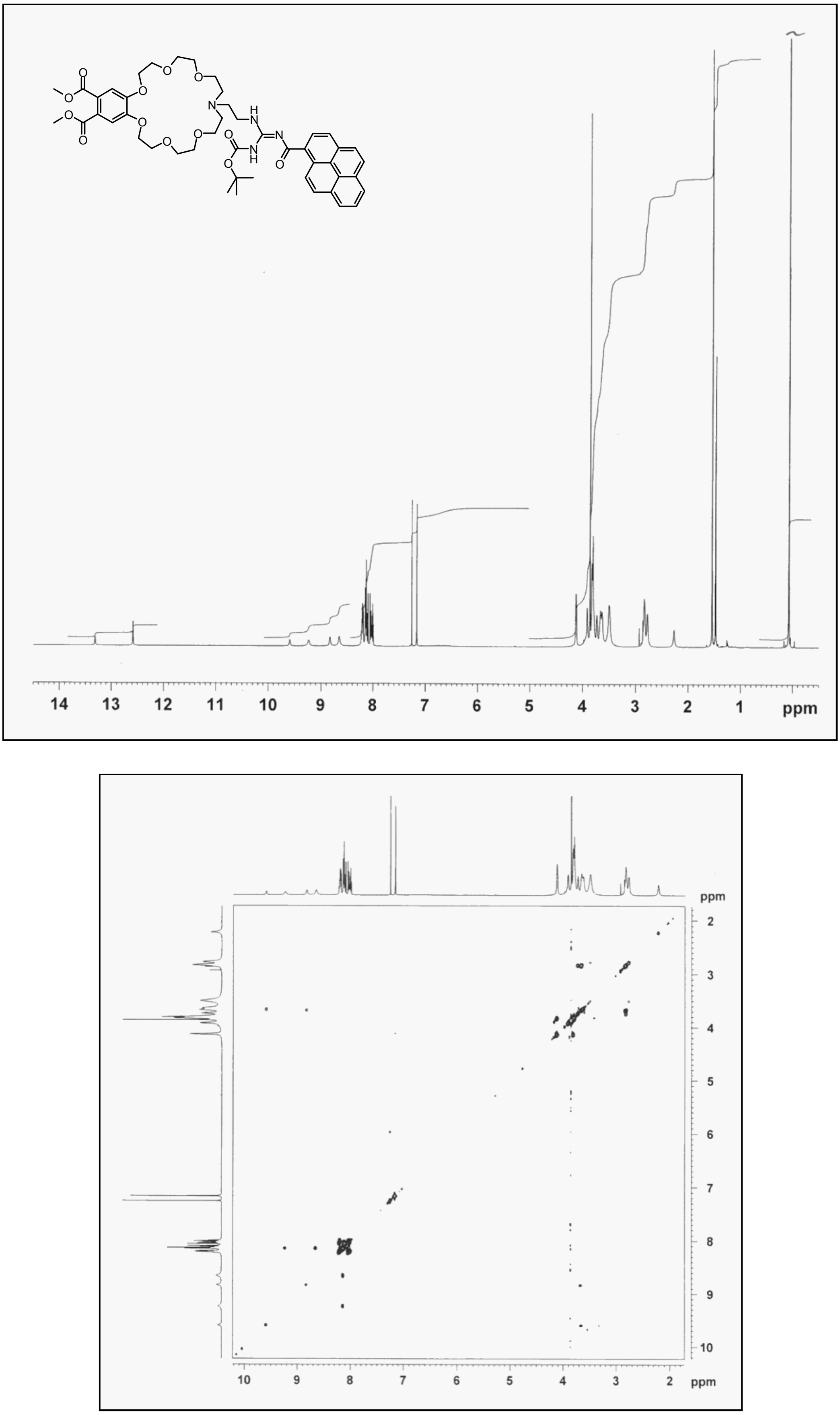
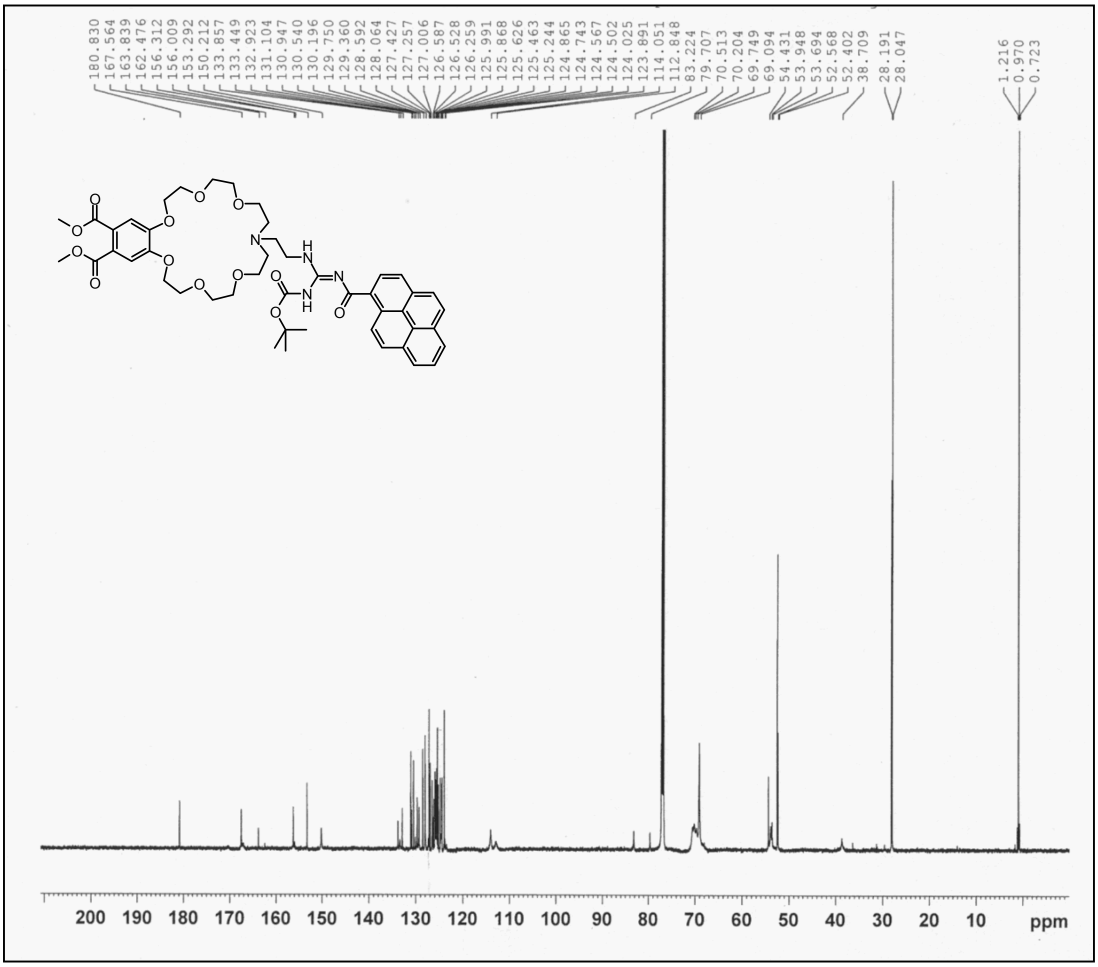


Figure S-62: ^{1}H-NMR spectrum of compound **38**



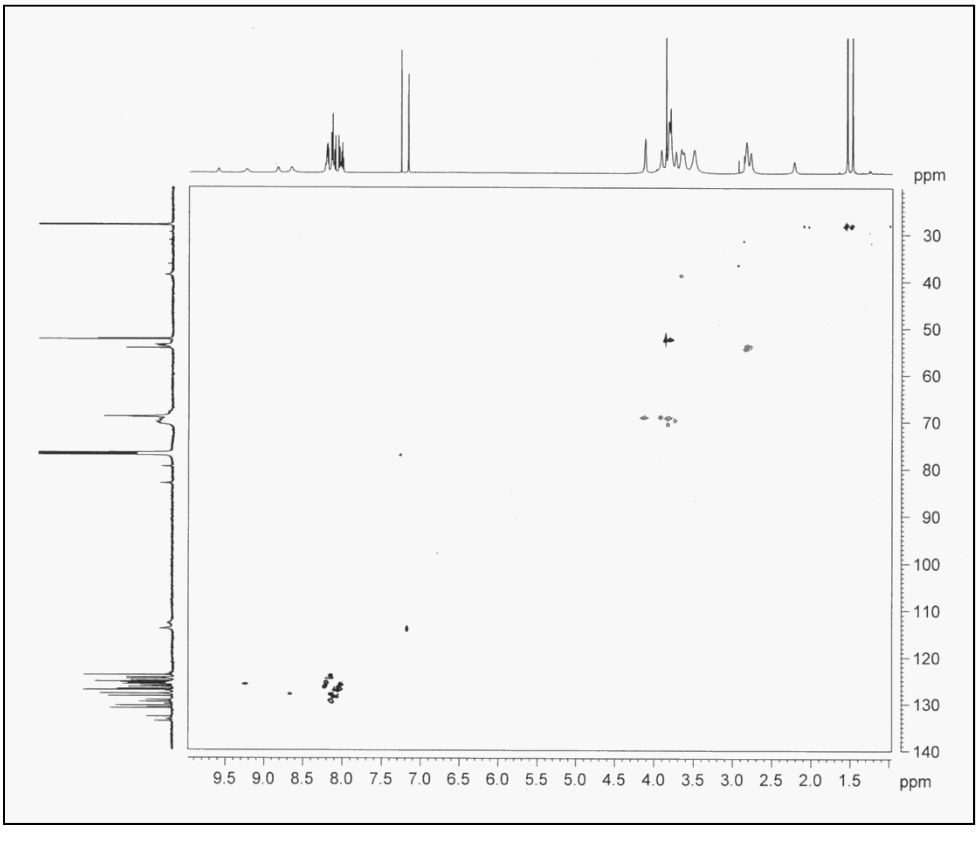


Figure S-63: ^{13}C-NMR spectrum of compound **38**

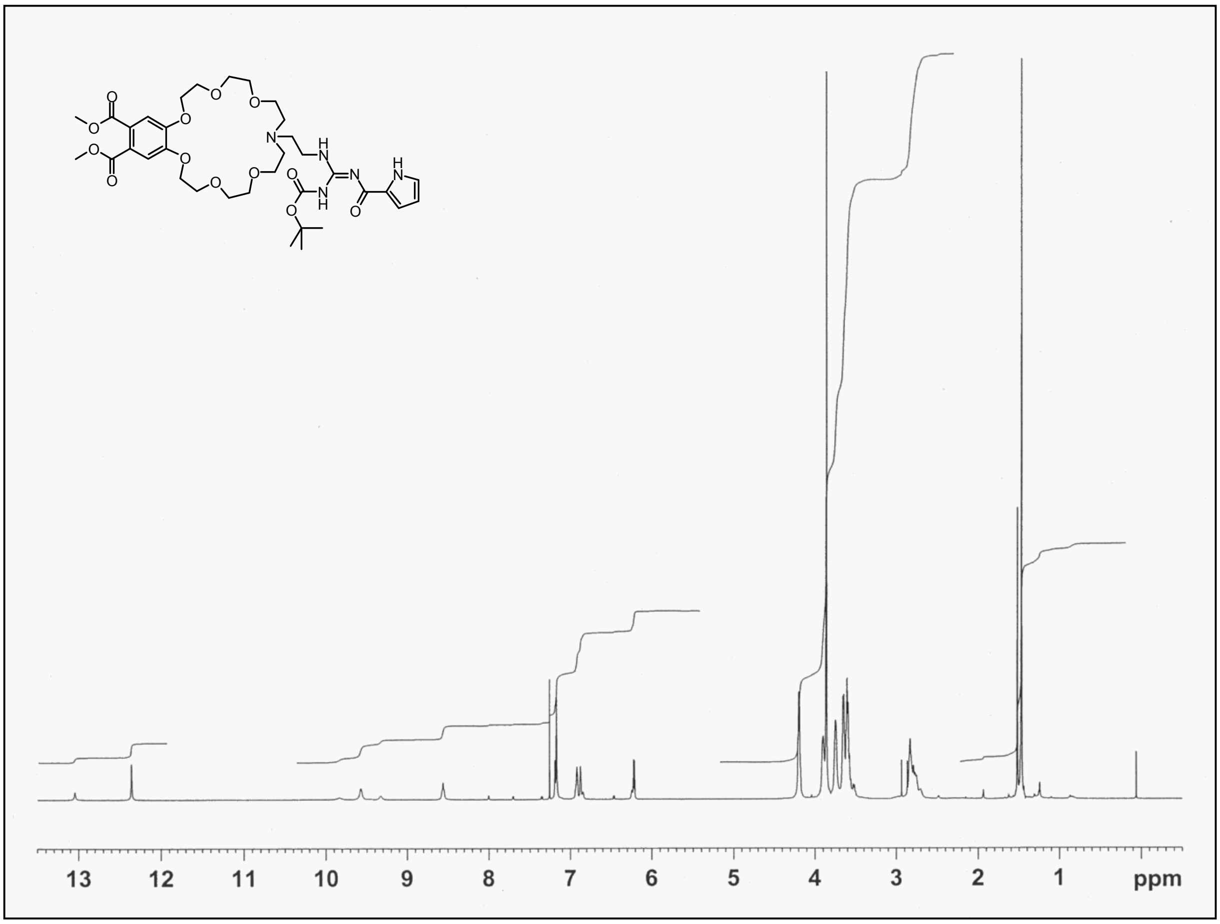


Figure S-64: ^{1}H-NMR spectrum of compound **37**

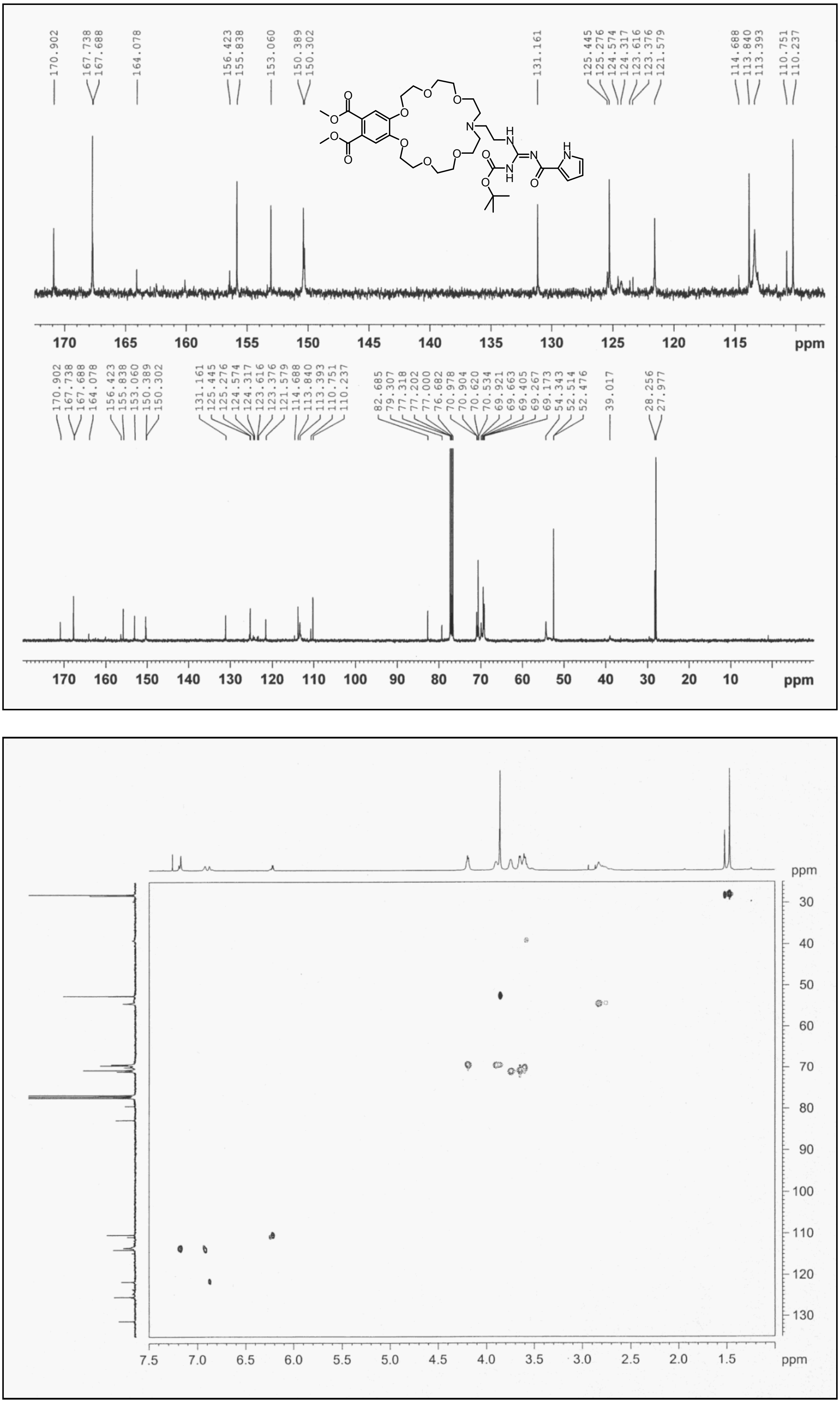


Figure S-65: ^{13}C-NMR spectrum of compound **37**

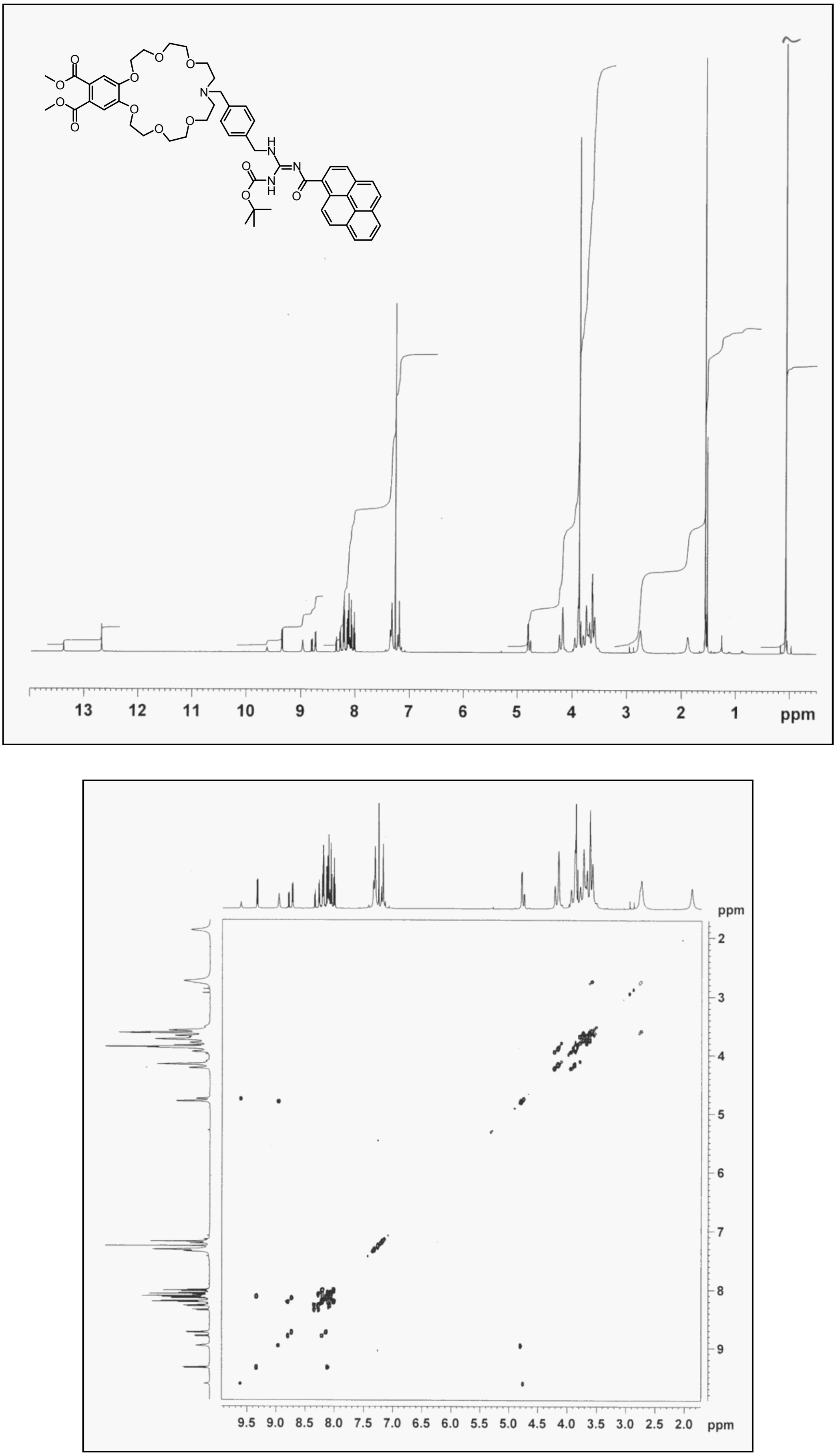


Figure S-66: ^{1}H-NMR spectrum of compound **31**

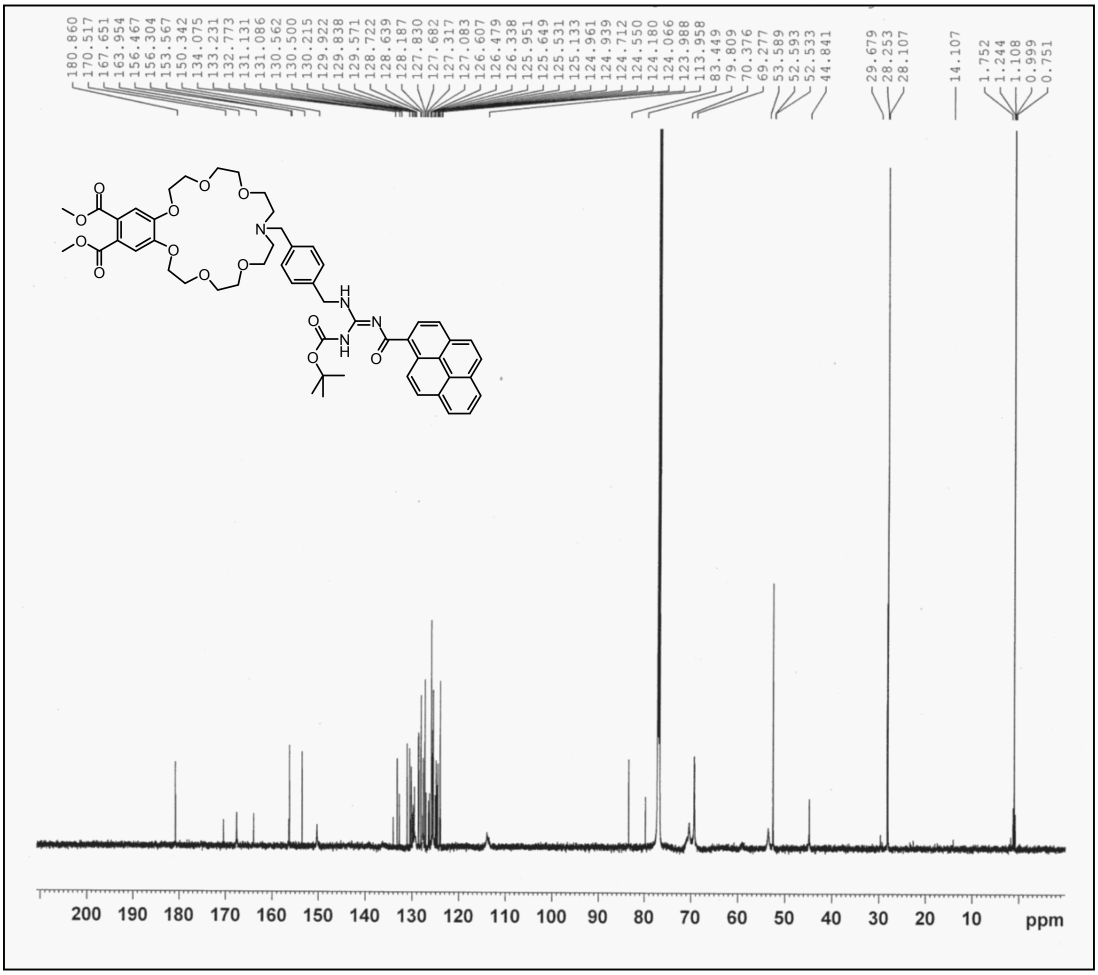
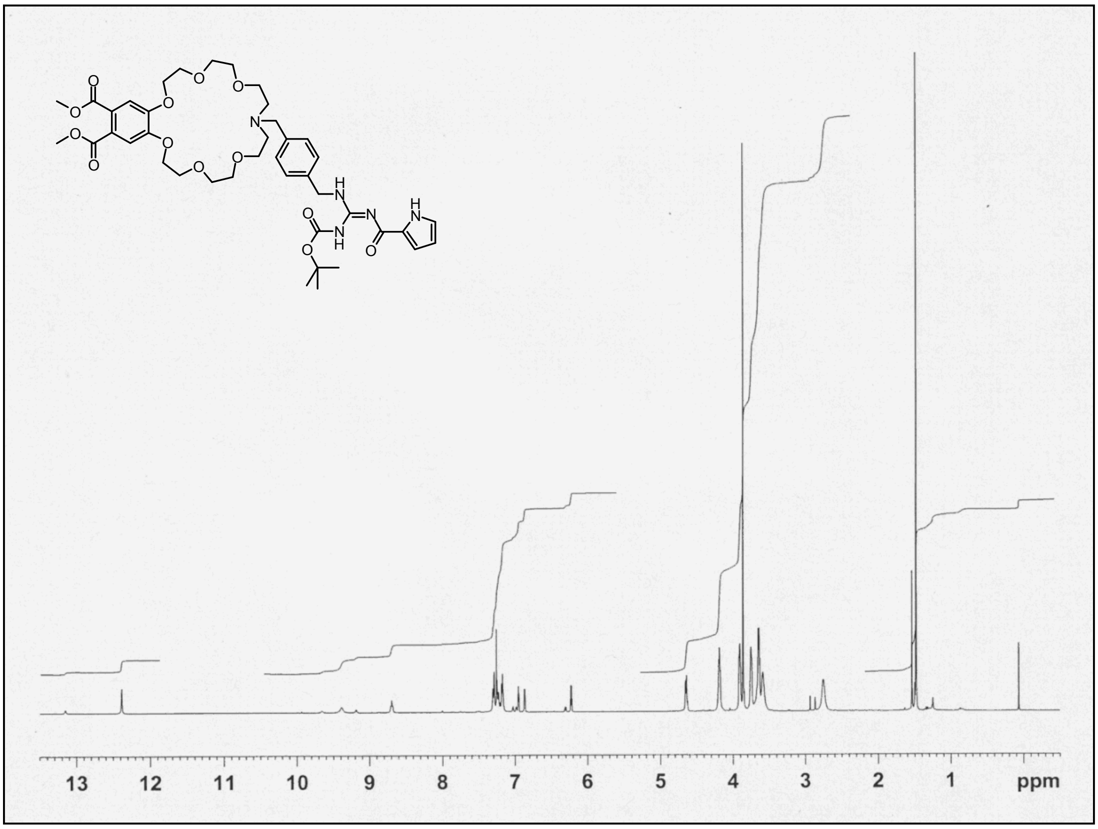


Figure S-67: ^{13}C-NMR spectrum of compound **31**



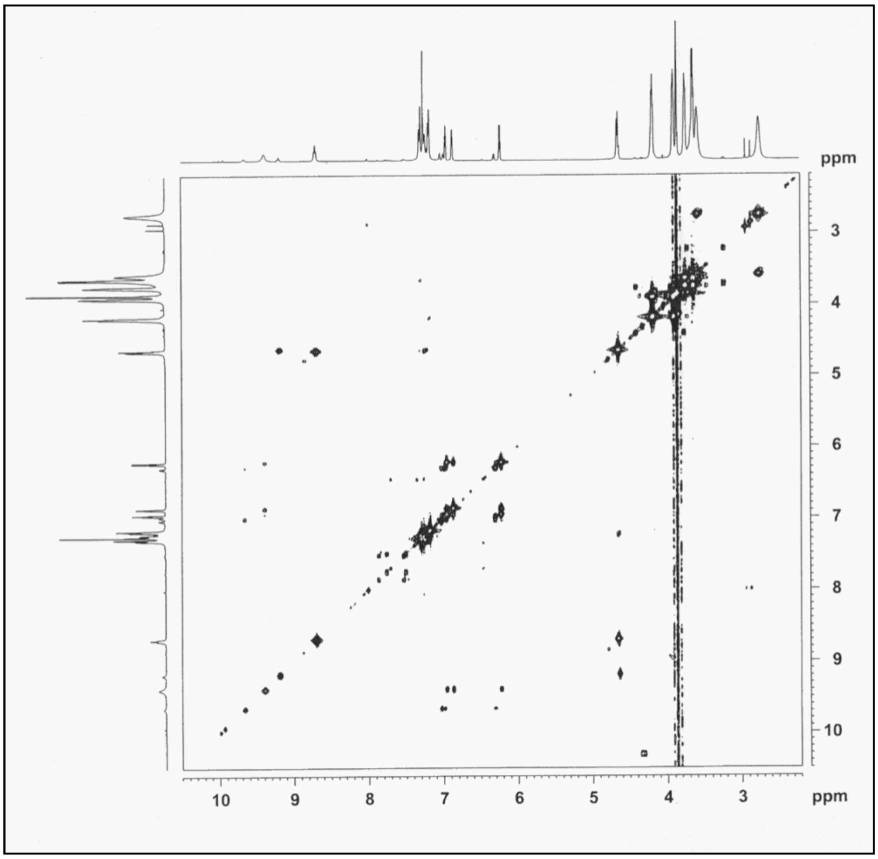
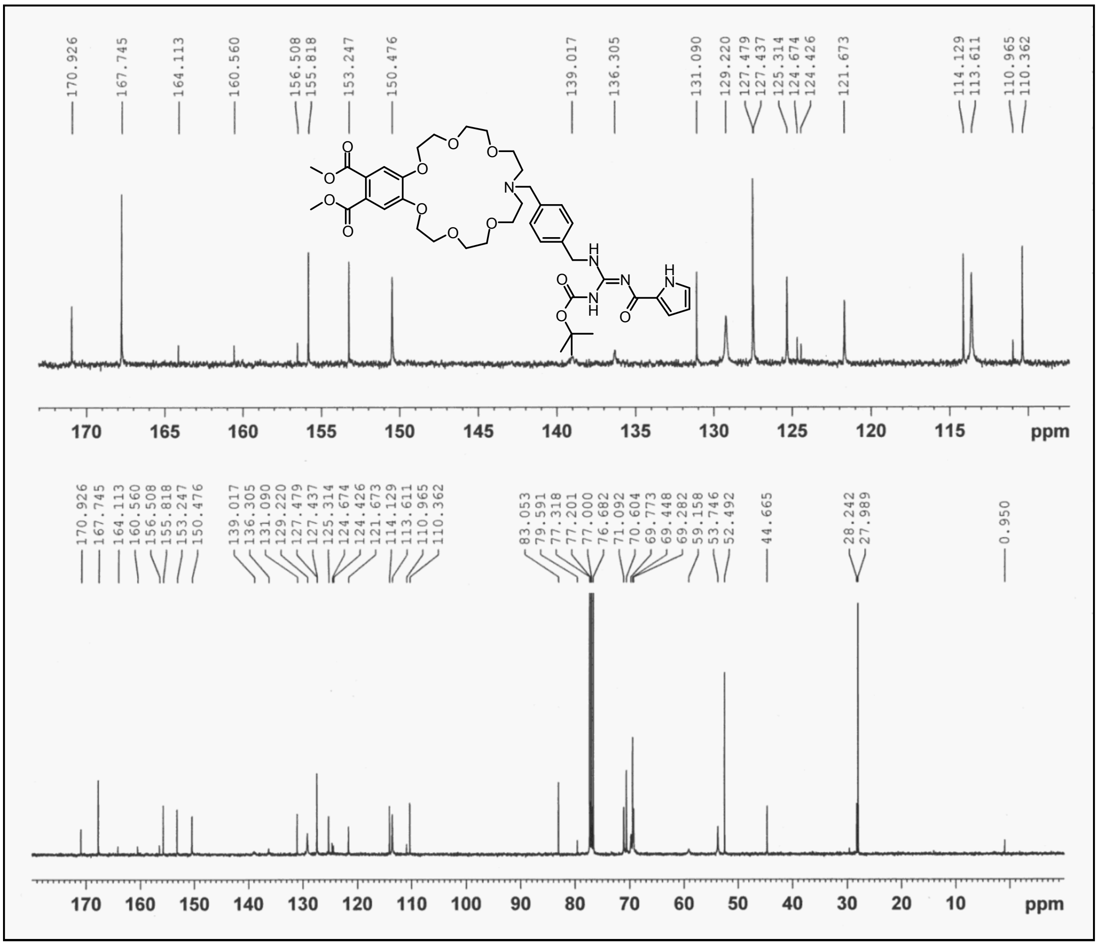


Figure S-68: ^{1}H-NMR spectrum of compound **30**



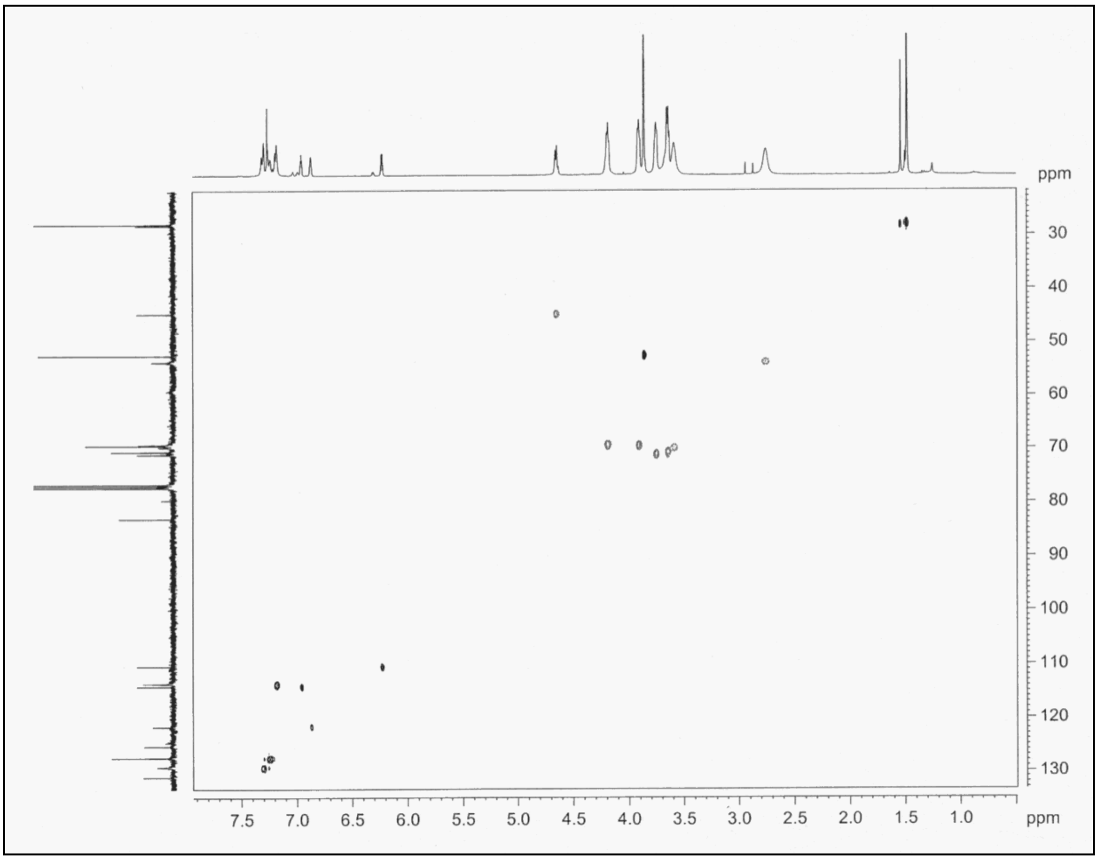


Figure S-69: ^{13}C-NMR spectrum of compound **30**

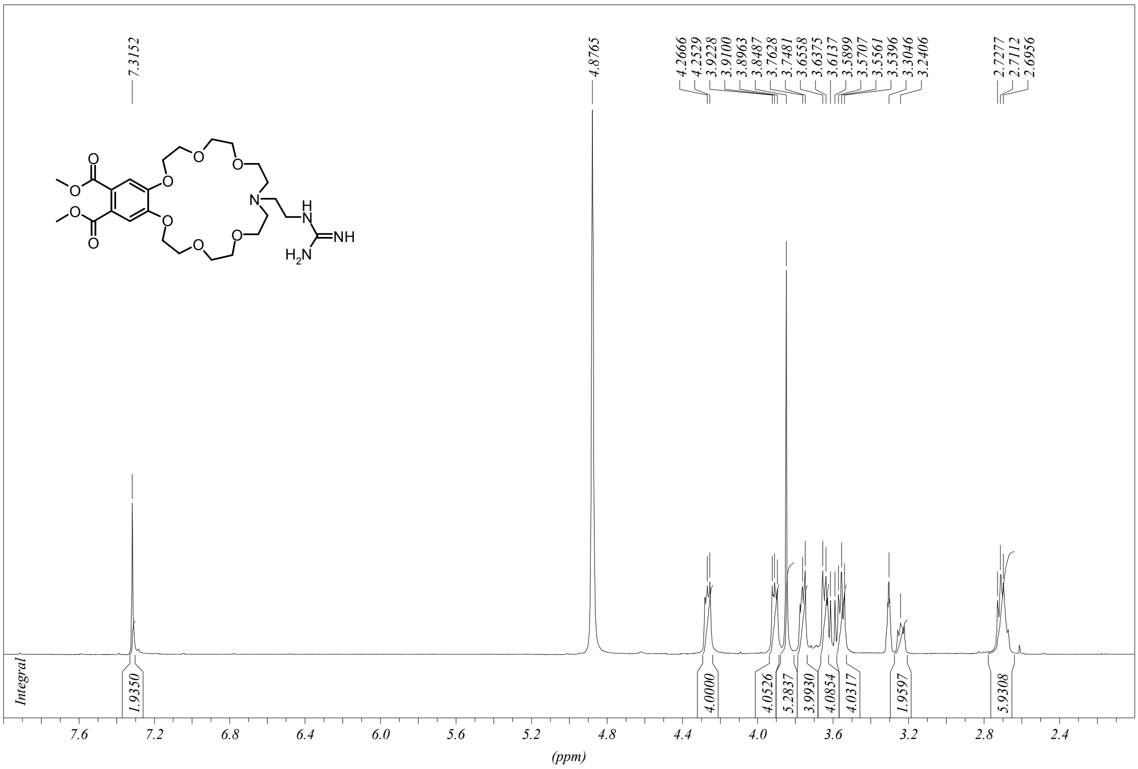


Figure S-70: ^{1}H-NMR spectrum of compound **14a**

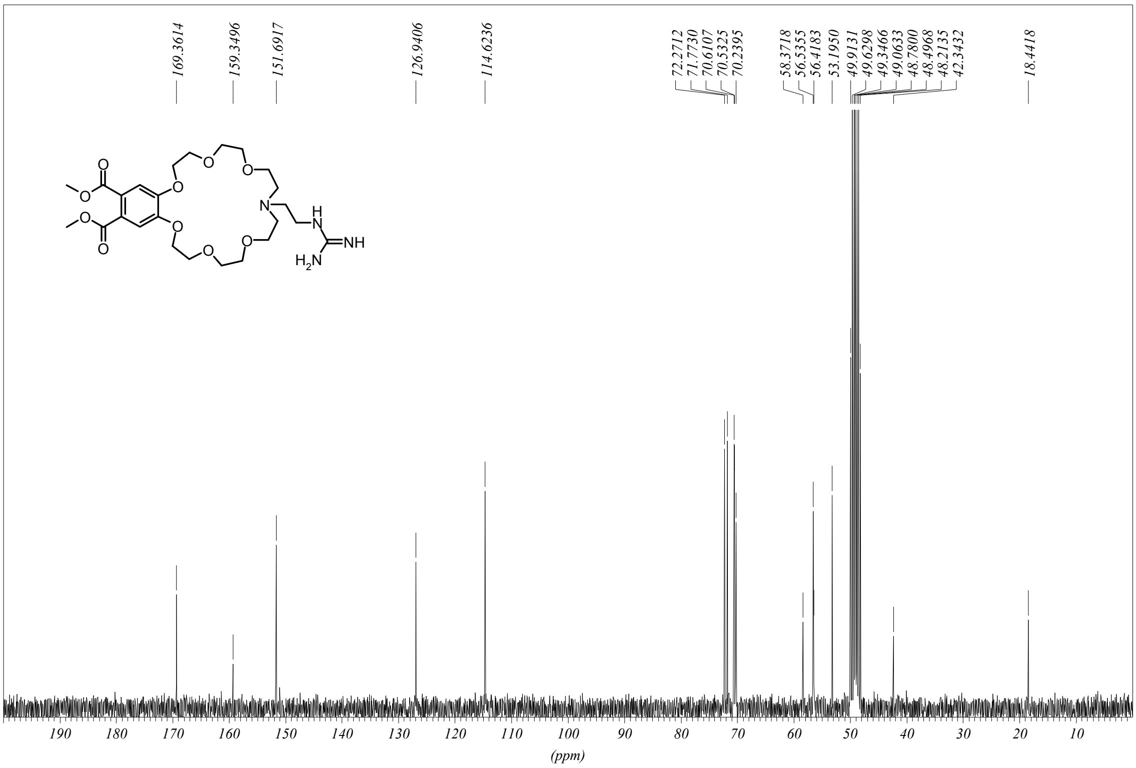


Figure S-71: ^{13}C-NMR spectrum of compound **14a**

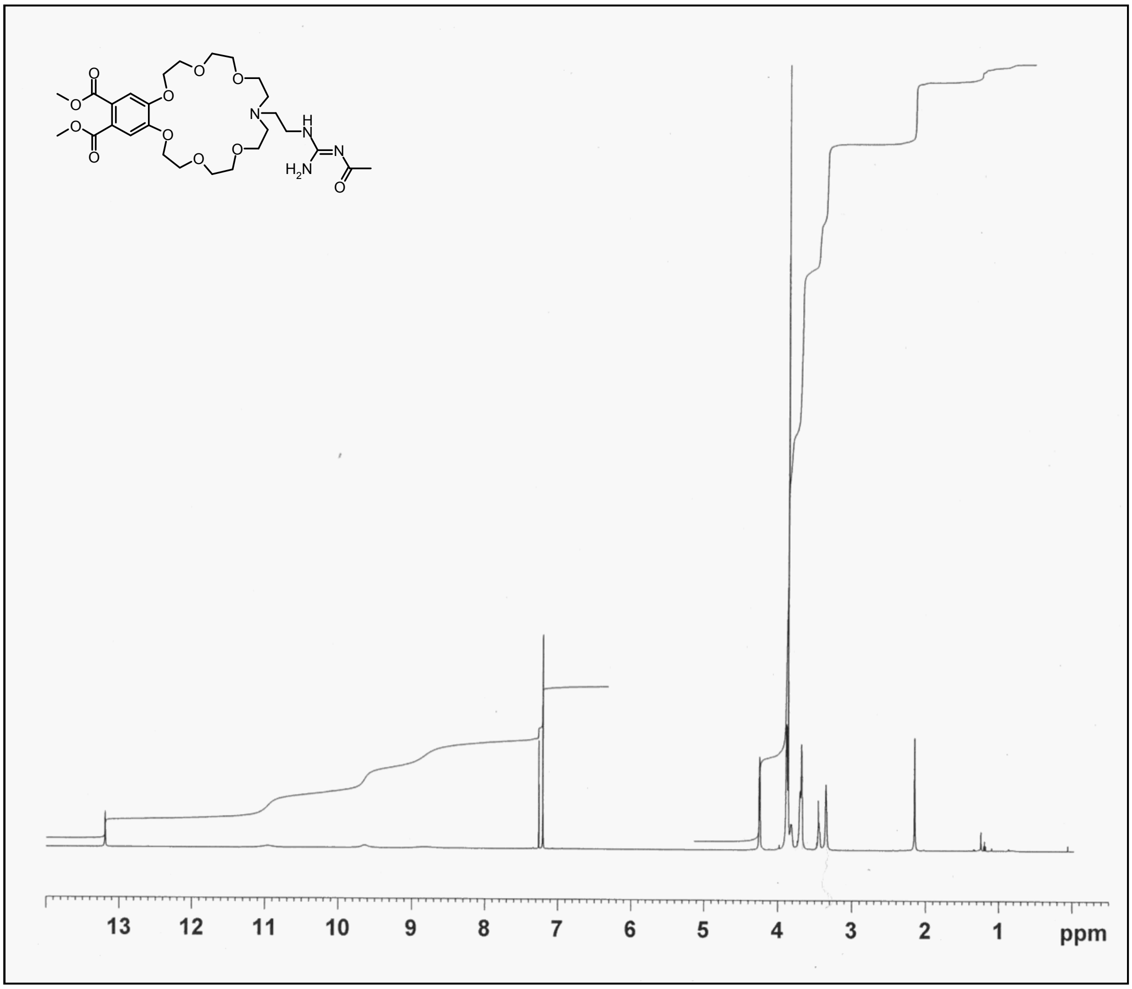


Figure S-72: ^{1}H-NMR spectrum of compound **16a**

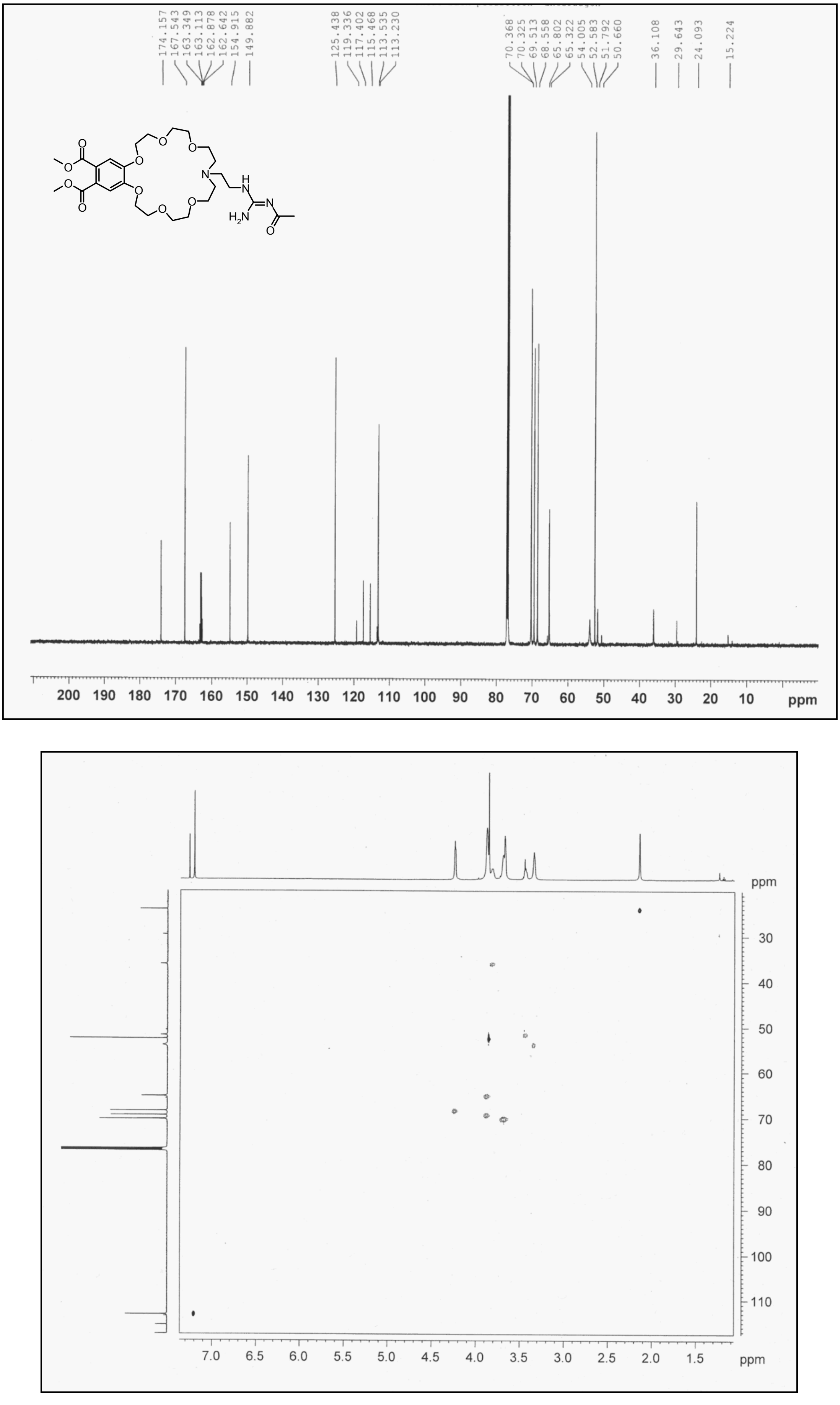


Figure S-73: ^{13}C-NMR spectrum of compound **16a**

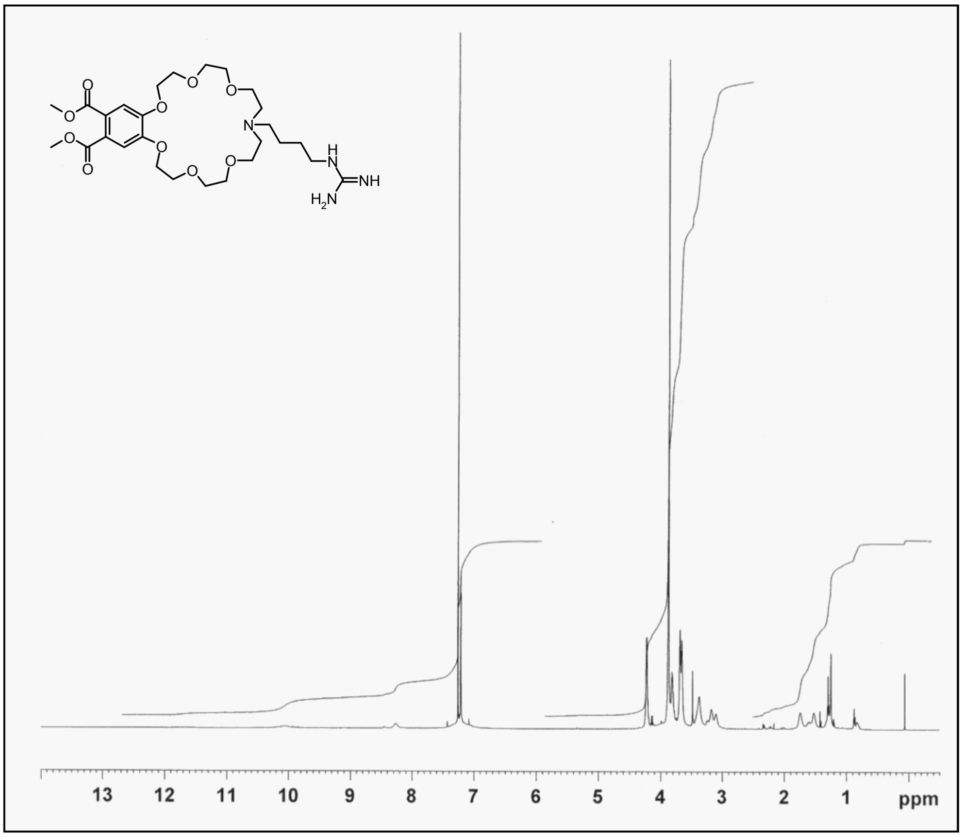


Figure S-74: ^{1}H-NMR spectrum of compound **14b**

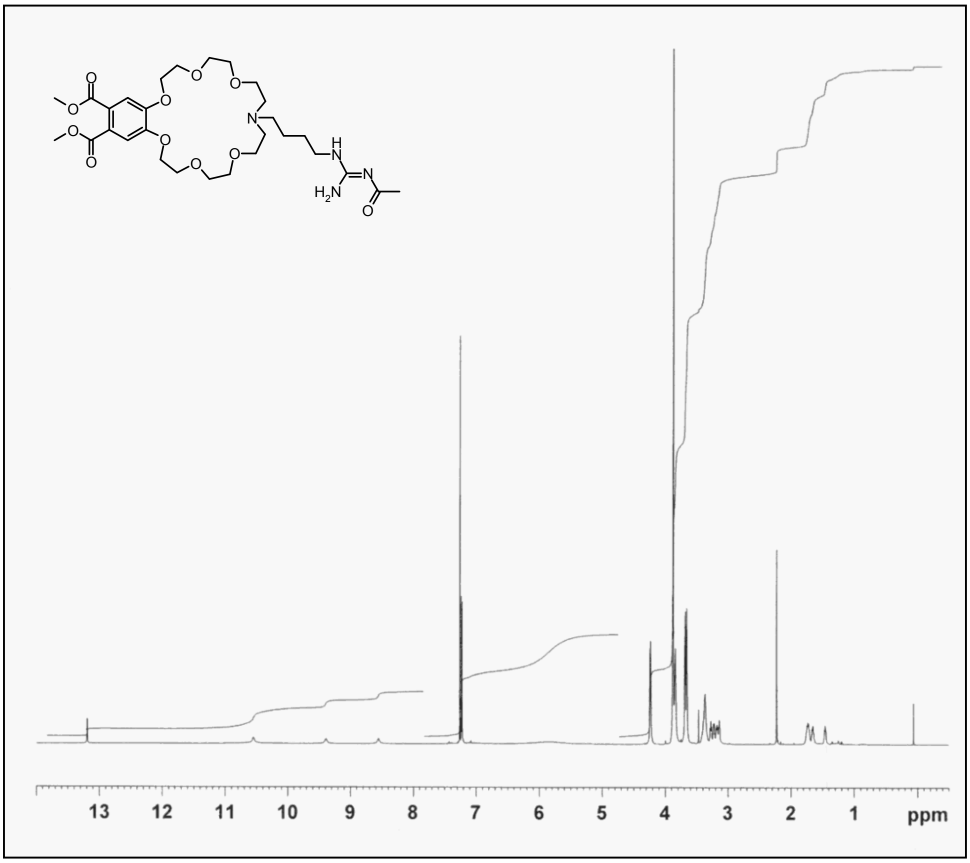


Figure S-75: ^{1}H-NMR spectrum of compound **16b**

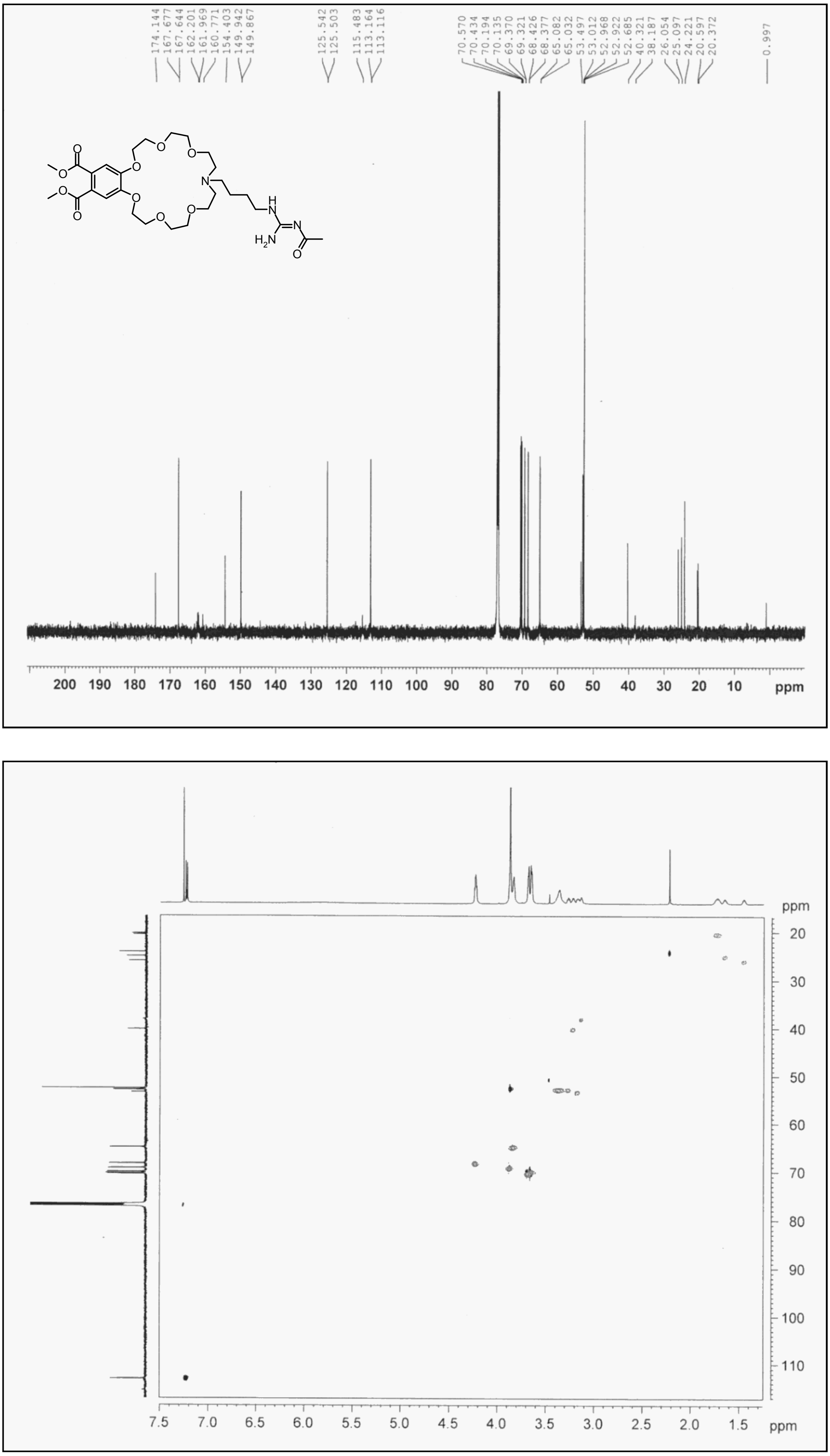


Figure S-76: ^{13}C-NMR spectrum of compound **16b**

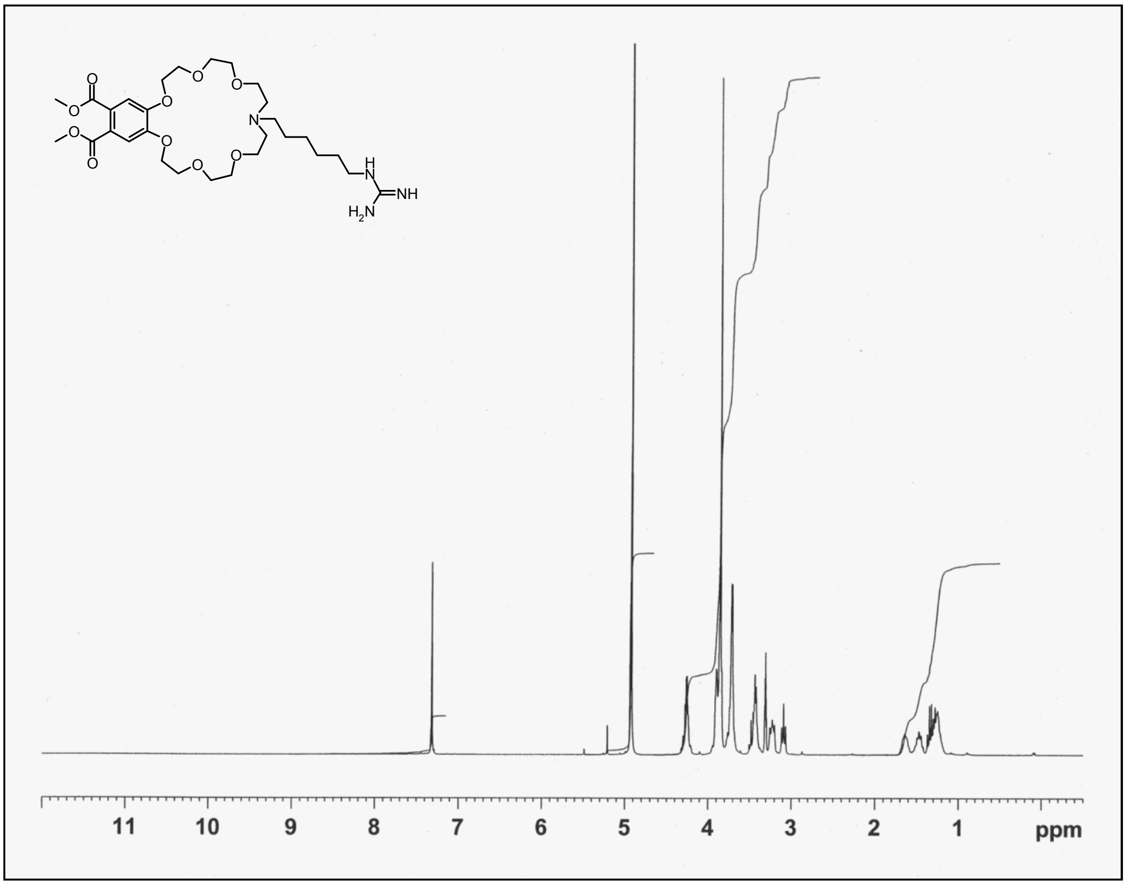


Figure S-77: ^{1}H-NMR spectrum of compound **14c**

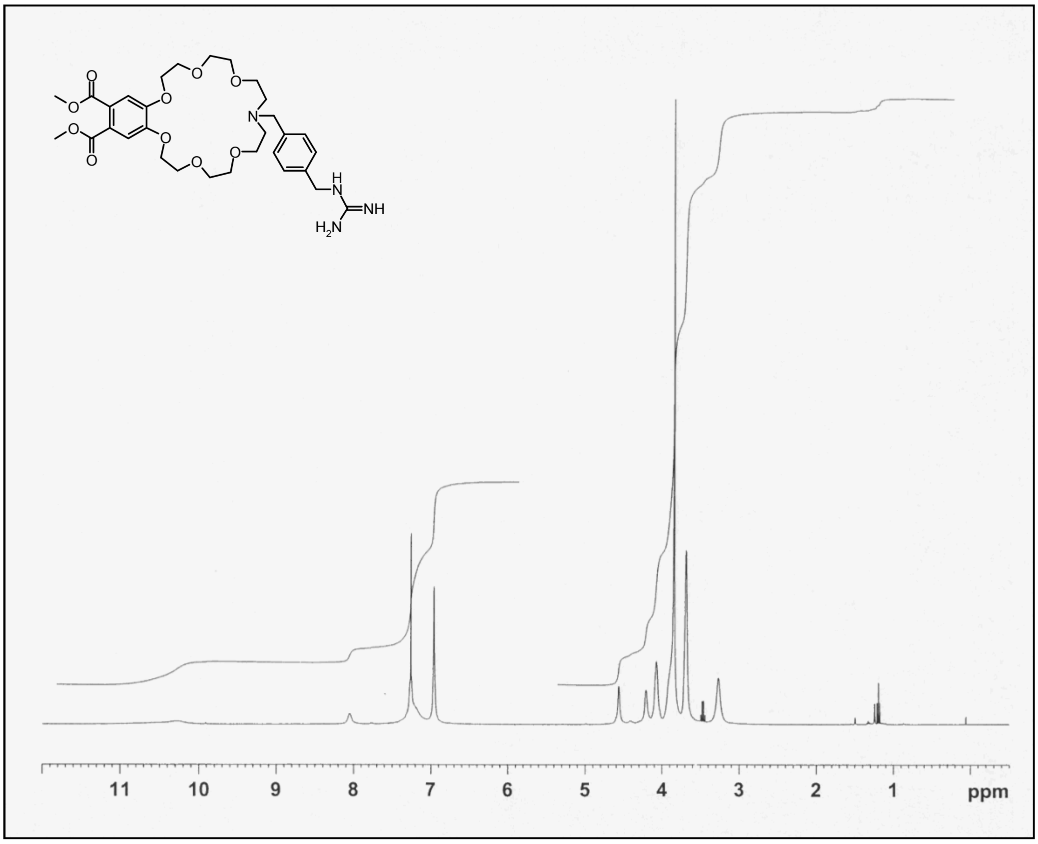


Figure S-78: ^{1}H-NMR spectrum of compound **14d**

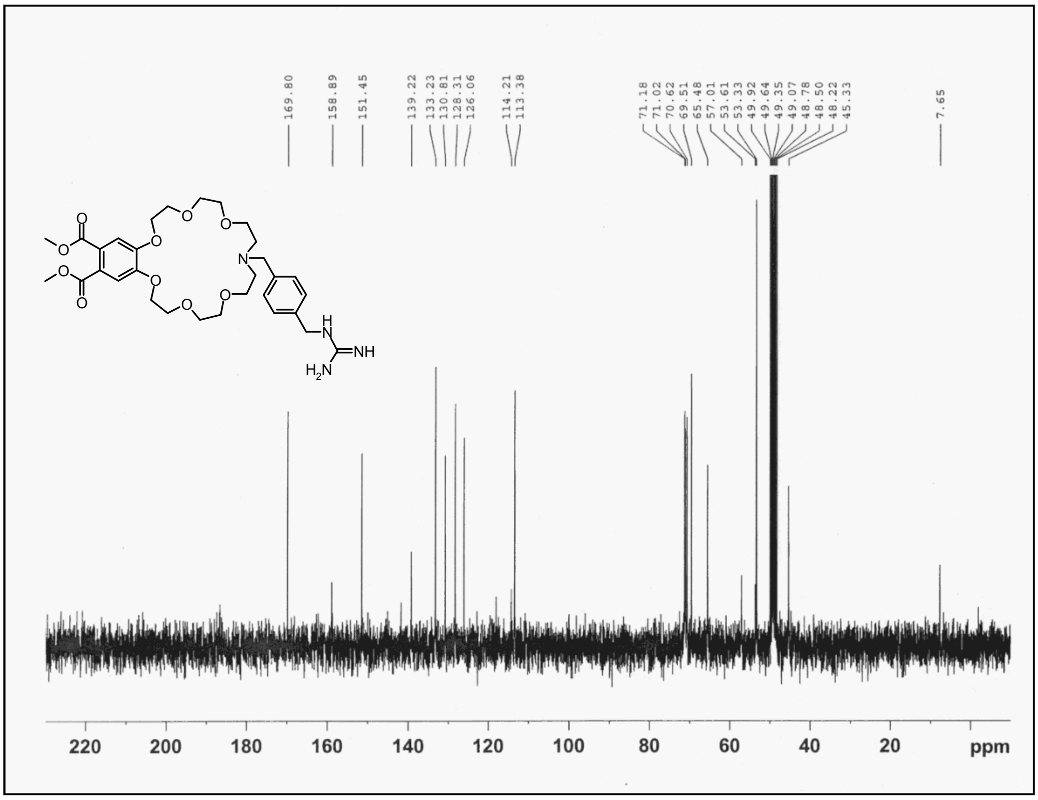
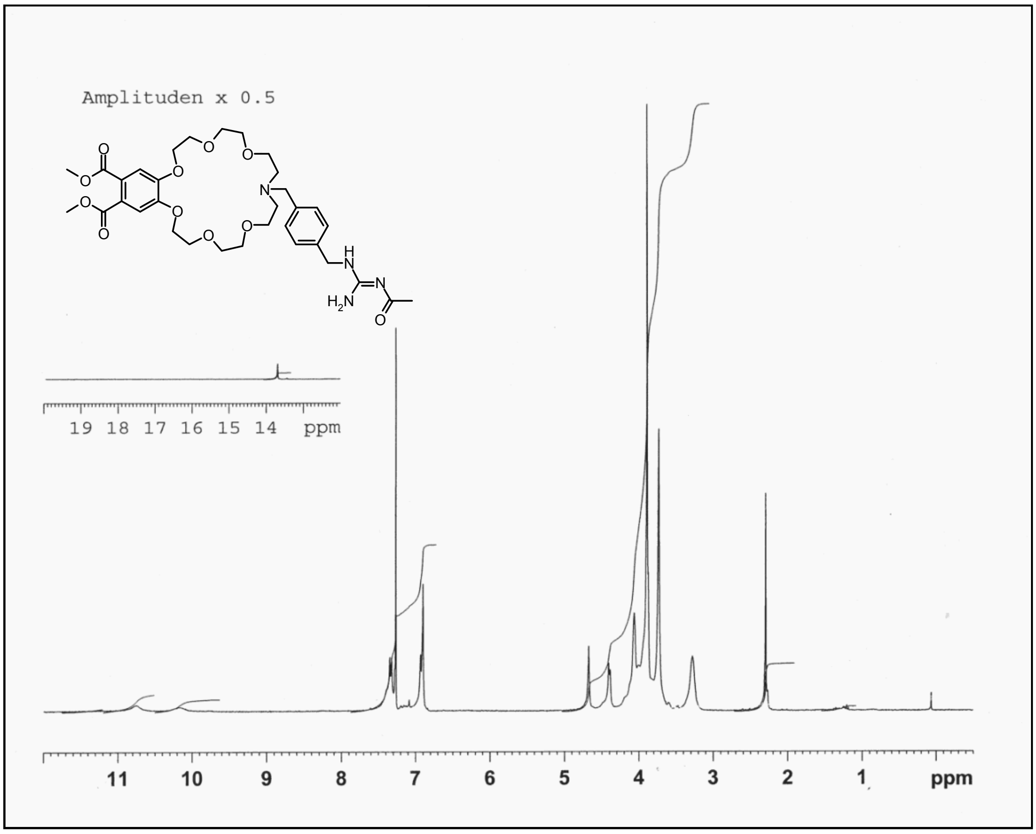


Figure S-79: ^{1}H-NMR spectrum of compound **14d**



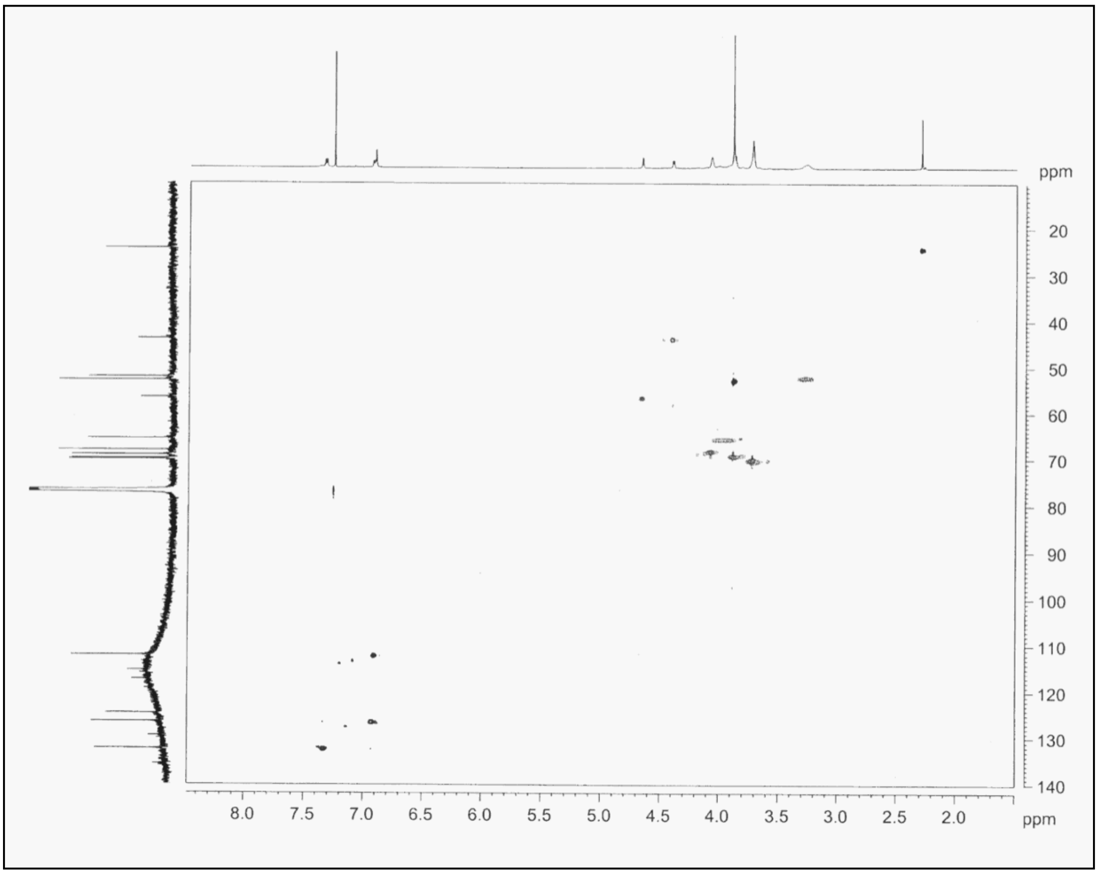


Figure S-80: ^{1}H-NMR and HSQC spectrum of compound **16d**

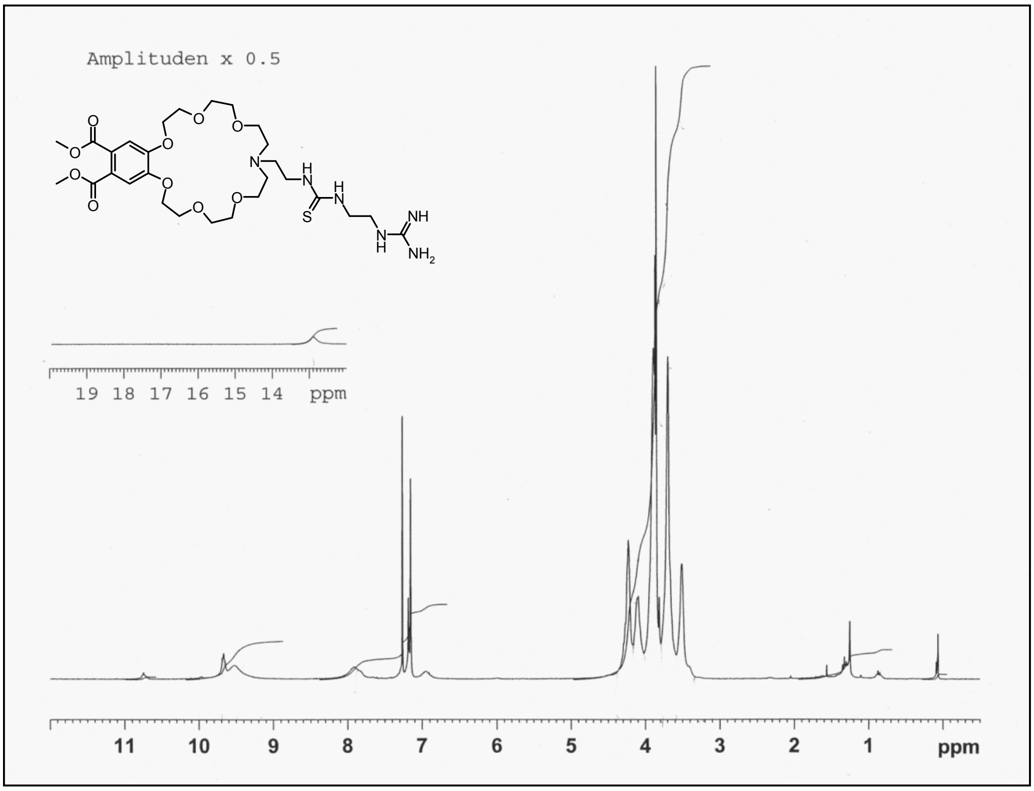


Figure S-81: ^{1}H-NMR spectrum of compound **14e**

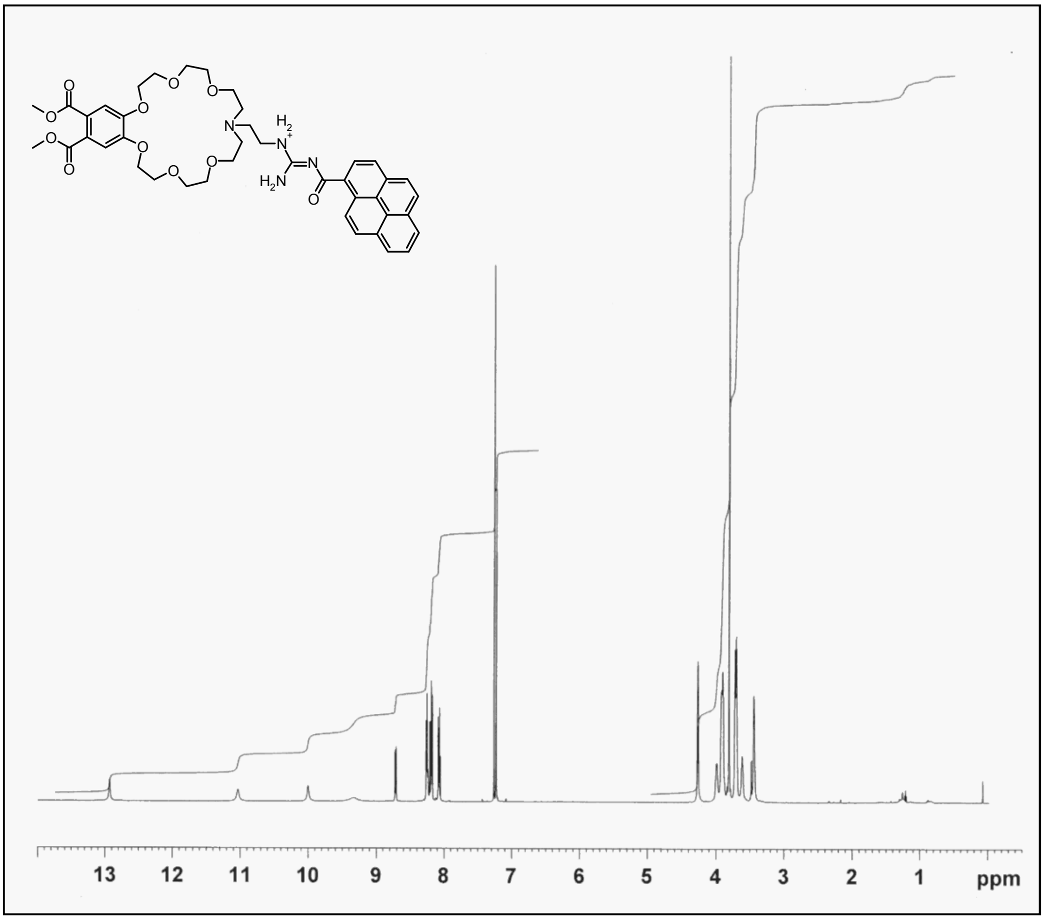
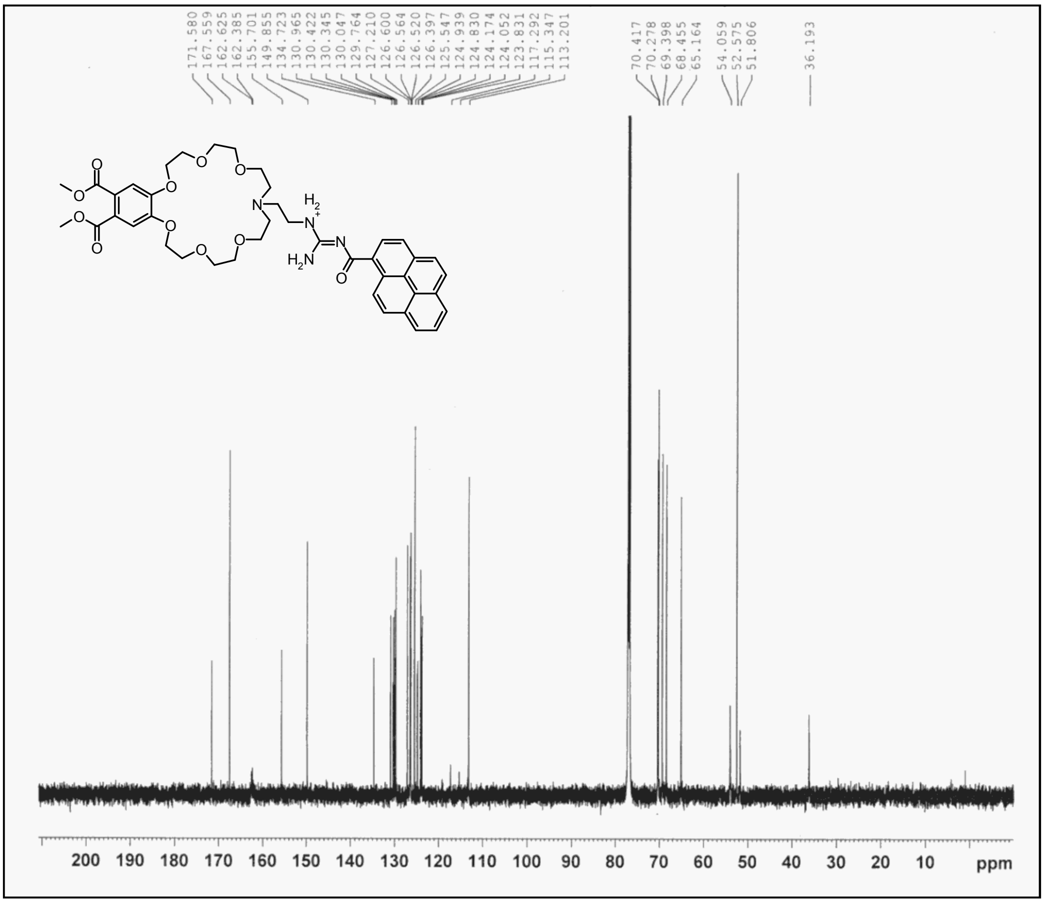


Figure S-82: ^{1}H-NMR spectrum of compound **40**



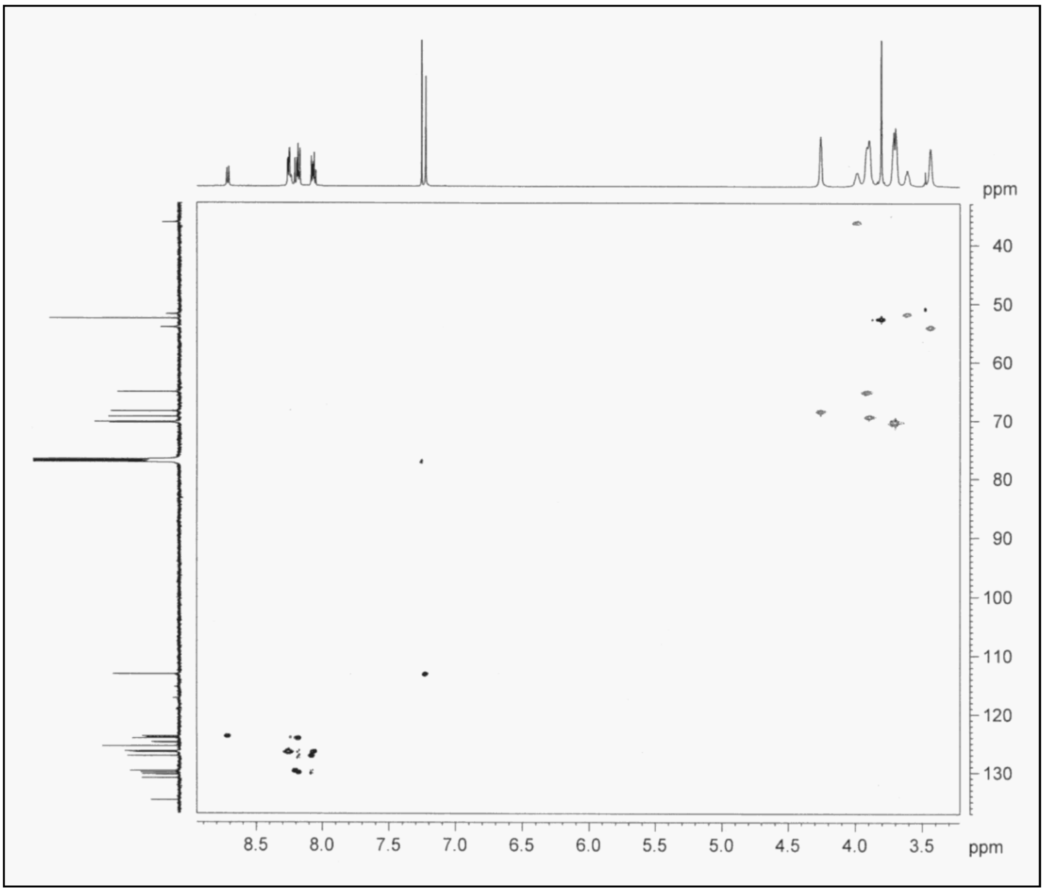


Figure S-83: ^{13}C-NMR and HSQC spectrum of compound **40**

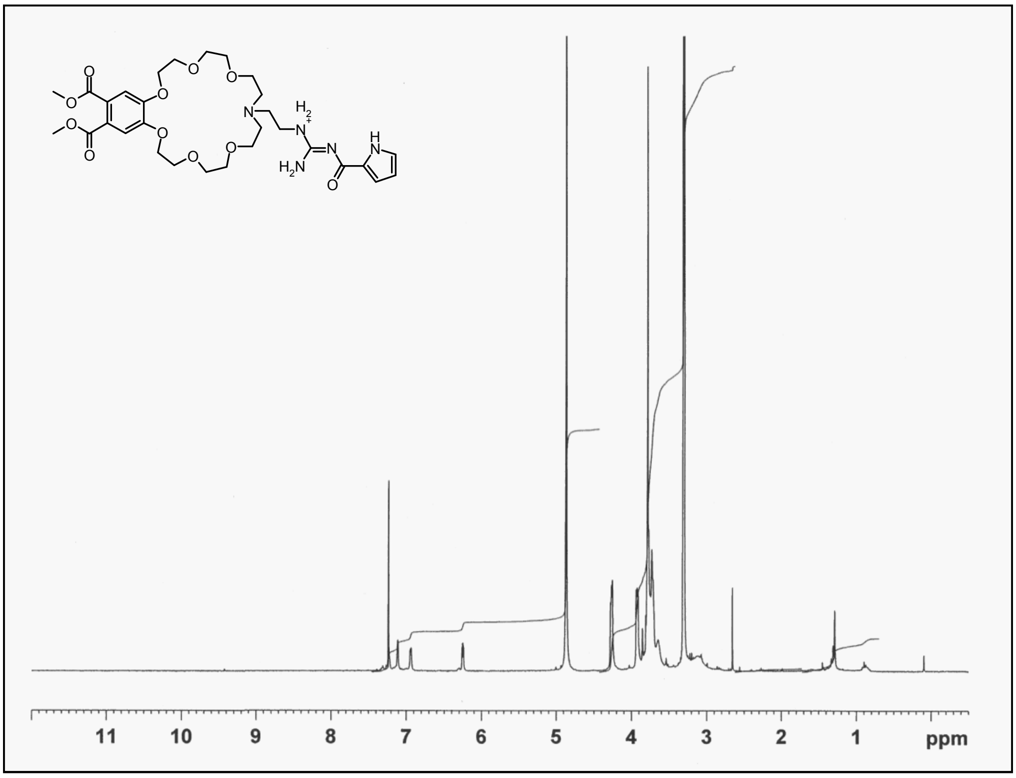


Figure S-84: ^{1}H-NMR spectrum of compound **39**

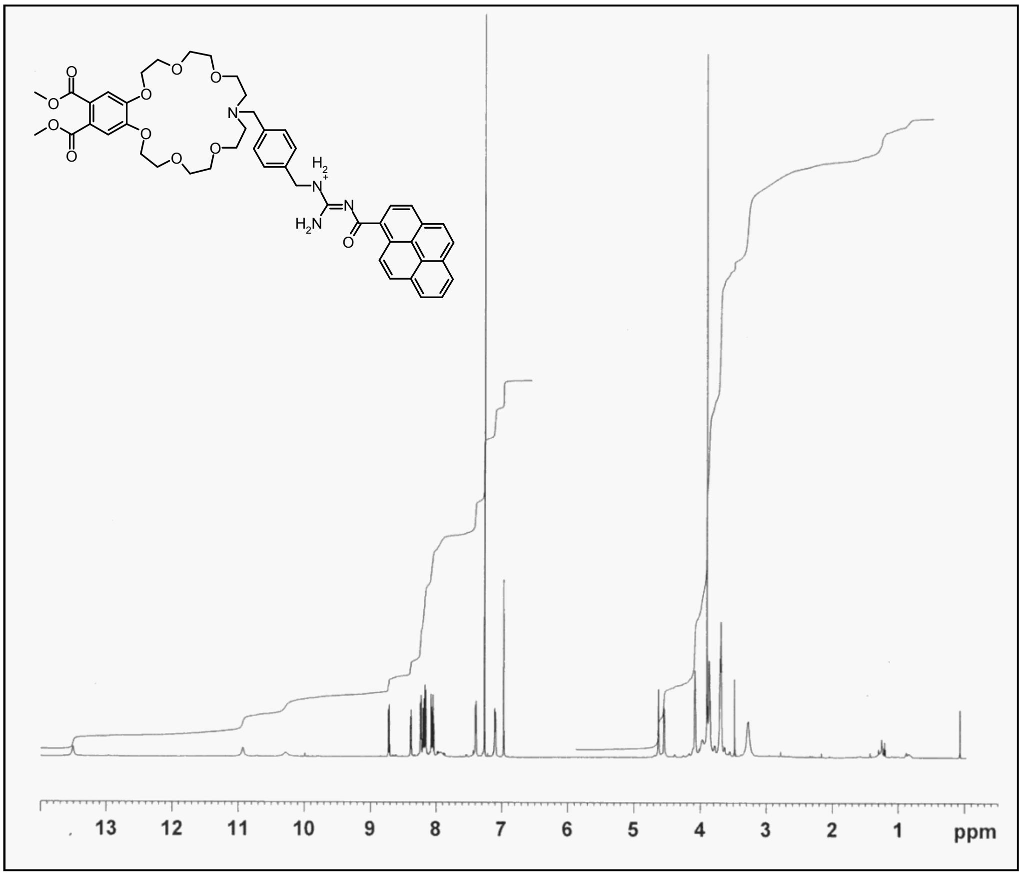


Figure S-85: ^{1}H-NMR spectrum of compound **33**

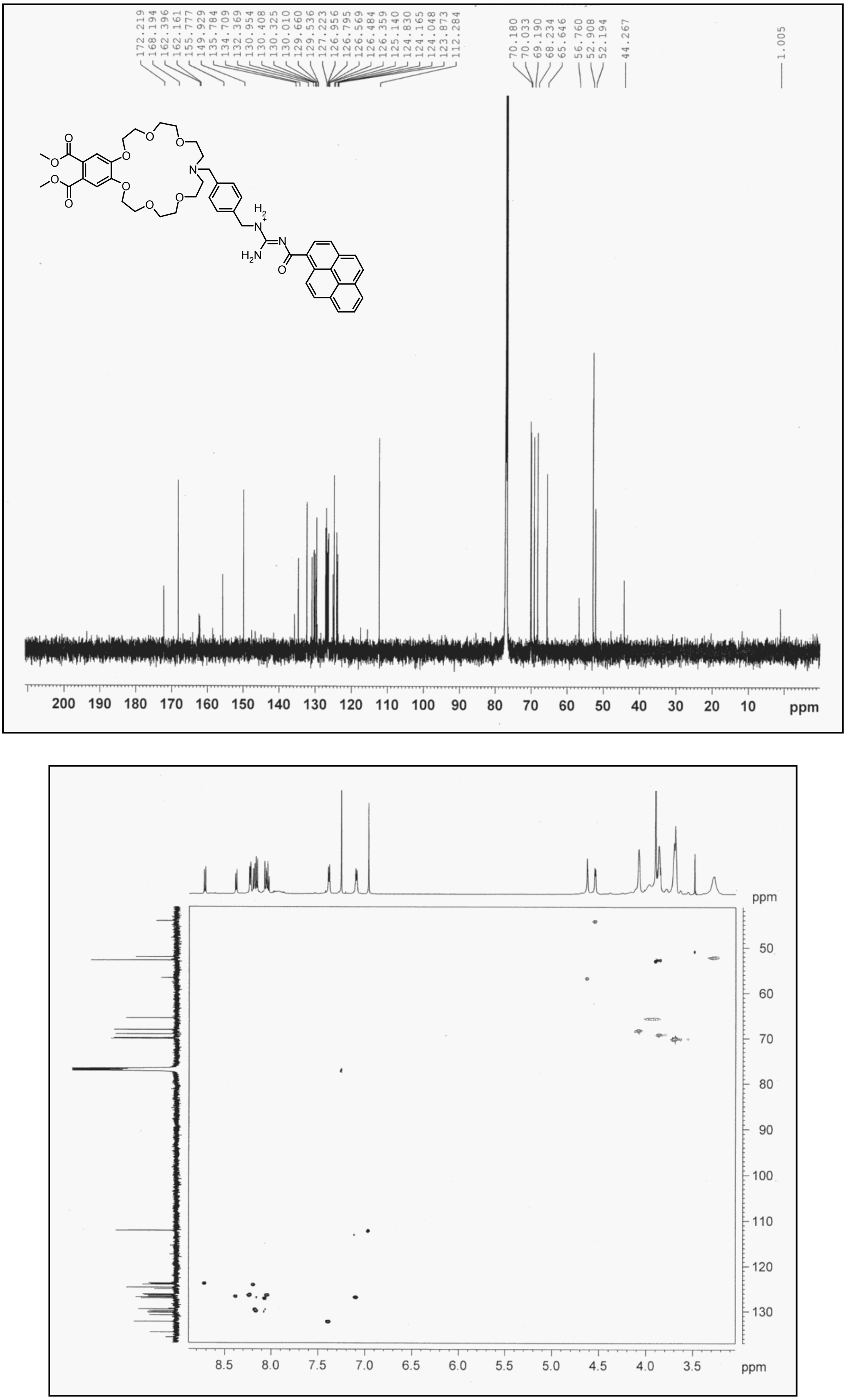


Figure S-86: ^{13}C-NMR and HSQC spectrum of compound **33**

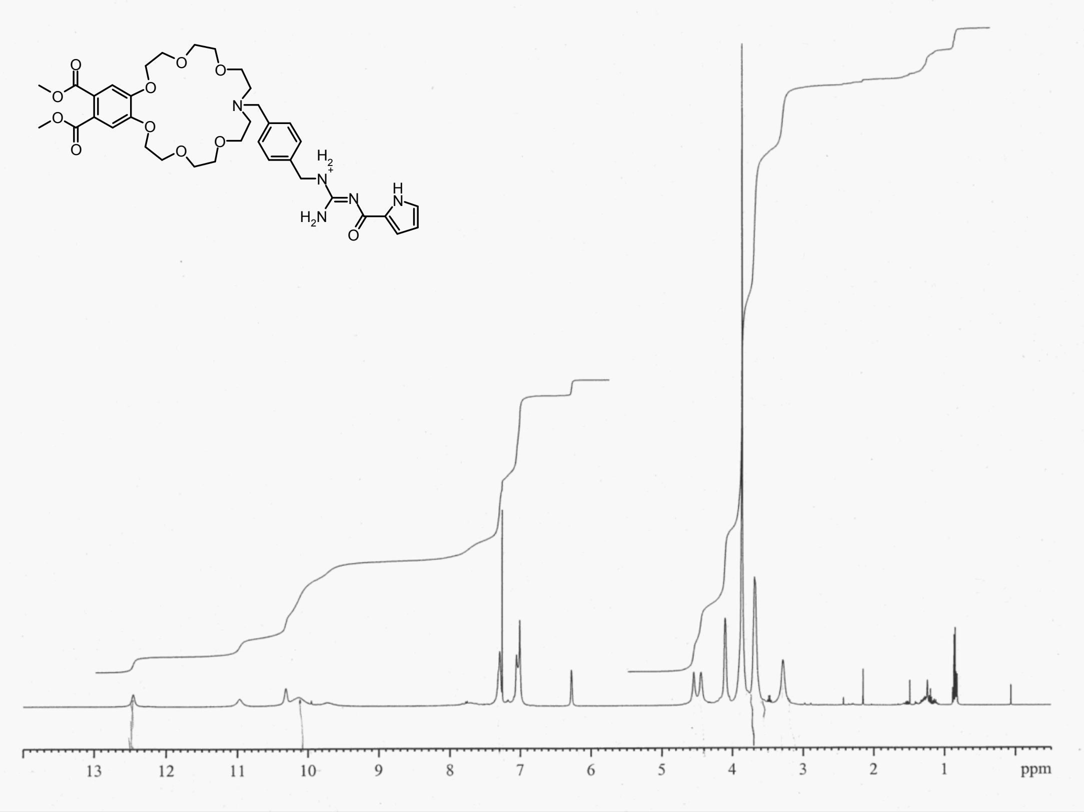


Figure S-87: ^{1}H-NMR spectrum of compound **32**

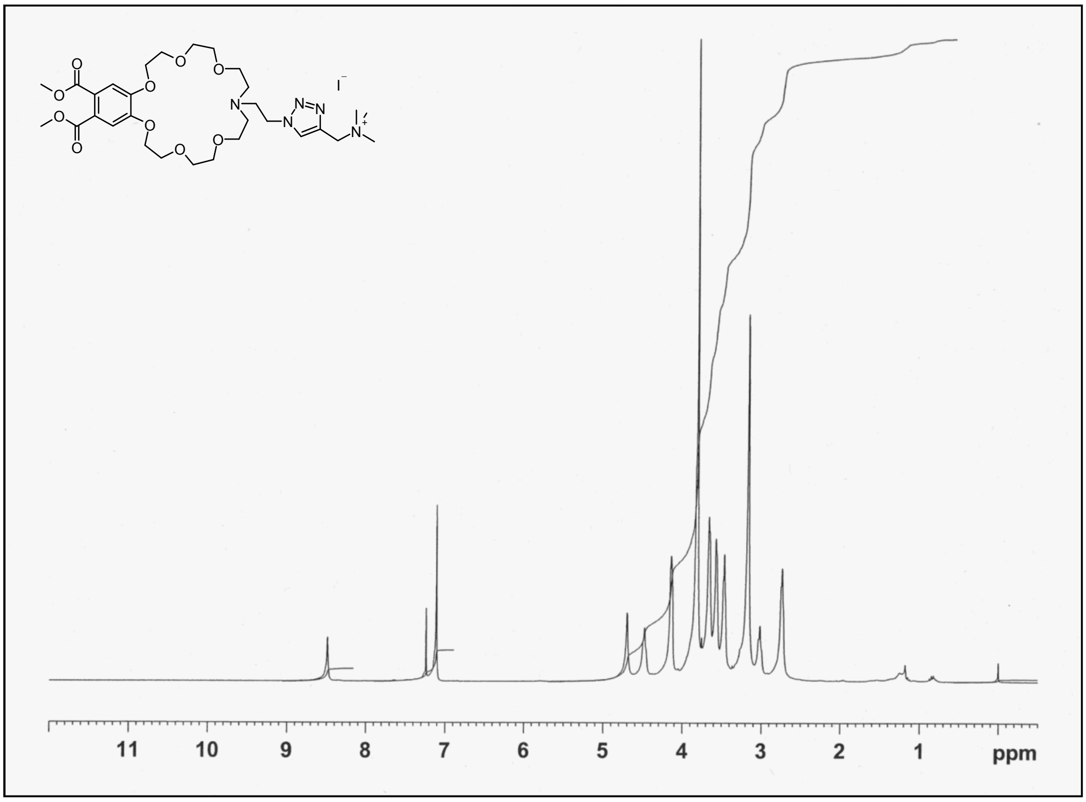


Figure S-88: ^{1}H-NMR spectrum of compound **24**

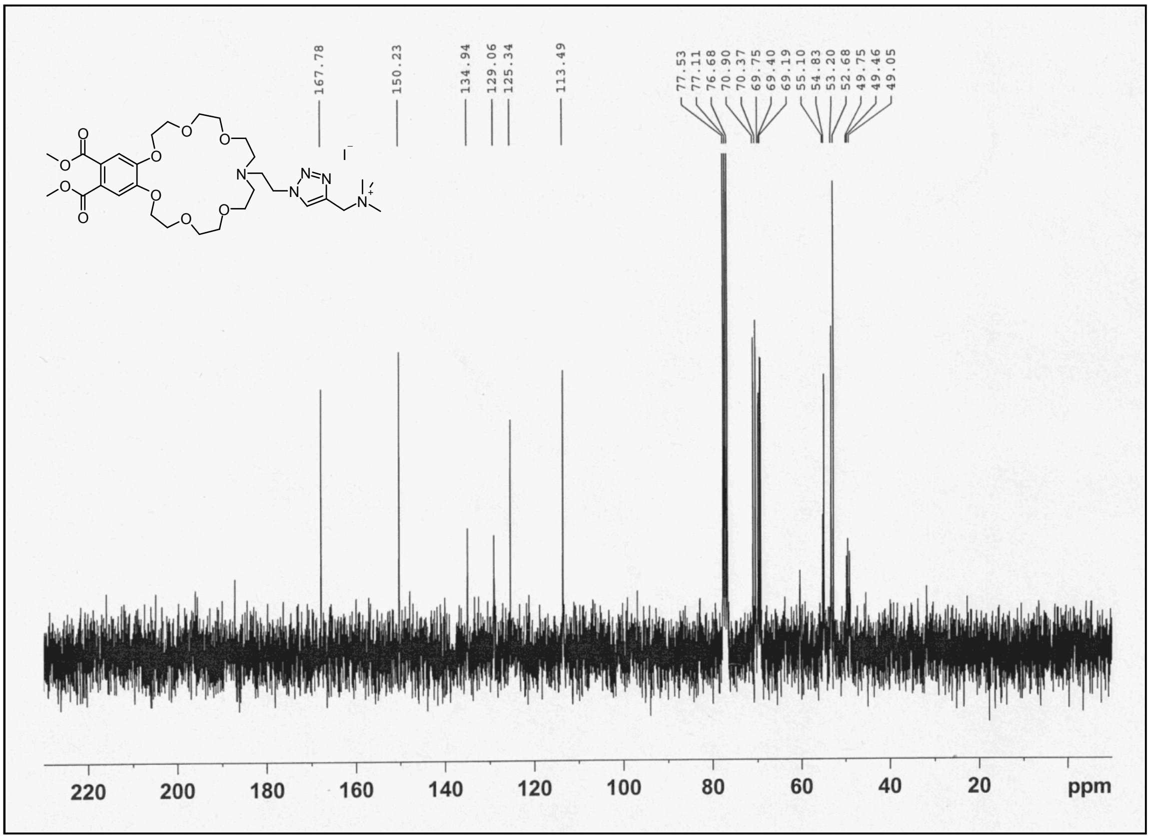


Figure S-89: ^{13}C-NMR spectrum of compound **24**

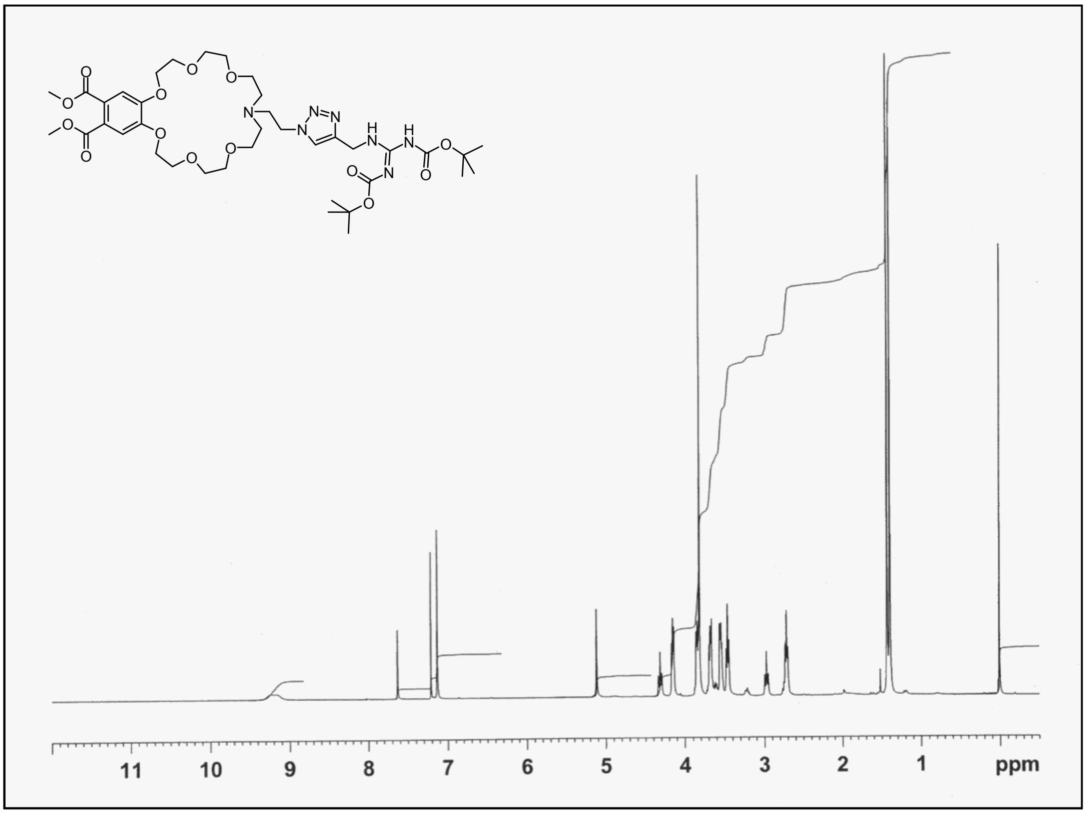


Figure S-90: ^{1}H-NMR spectrum of compound **26**

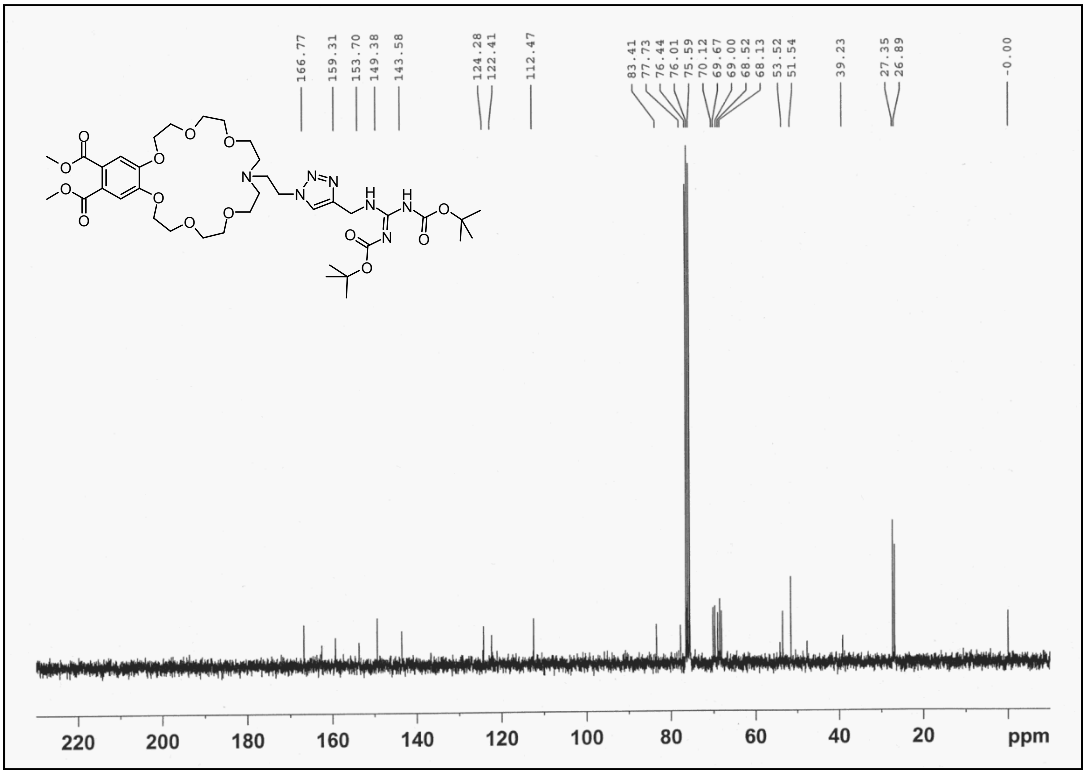


Figure S-91: ^{13}C-NMR spectrum of compound **26**

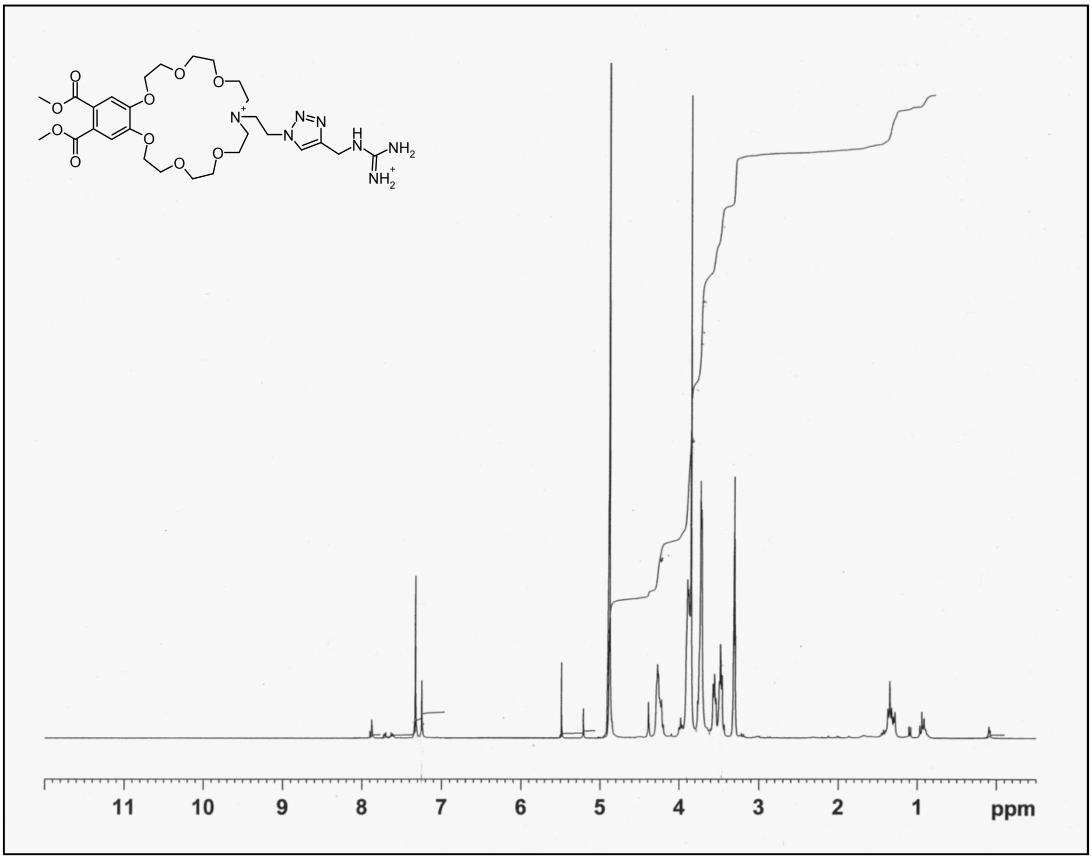


Figure S-92: ^{1}H-NMR spectrum of compound **27**

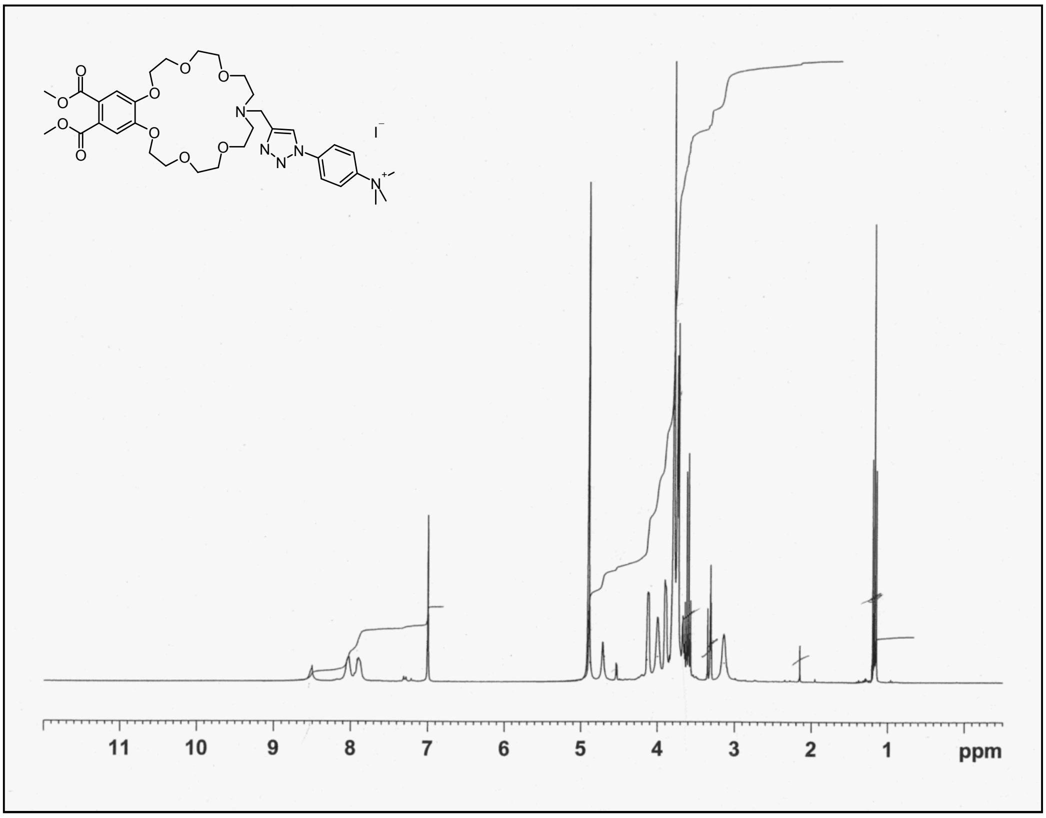


Figure S-93: ^{1}H-NMR spectrum of compound **21**

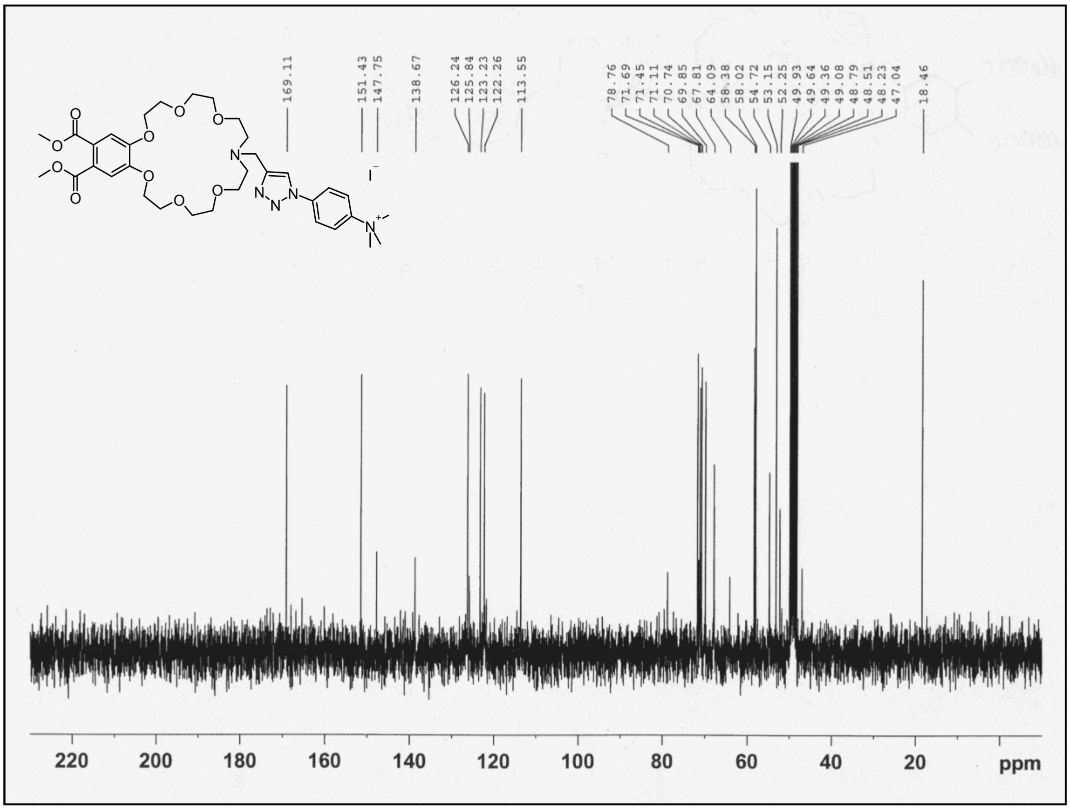


Figure S-94: ^{13}C-NMR spectrum of compound **21**

4. pH dependencies, simulations and pK_a values

4.1. pH titration of selected receptors

The protonation of the crown ether moiety is investigated by pH-titration with perchloric acid[14]. From the binding constants (*K*), the K_S- values can be calculated. Without free guanidine this measurement is eased, but should still give the values in good approximation to the according unprotected species.[15]

$$K = \frac{[XH^+]}{[X]*[H^+]} = \frac{1}{K_S} \qquad \Rightarrow \ \mathbf{\log K = pKa}$$

Equation 1: Relationship between binding constant K and the acidity constant K_S

Compound	Emission (λ_{max})	*K* [M^{-1}]	p*K*a	I_{∞}/I_0
(13a)	389 nm	$3.6 * 10^5$	5.6	1.7
(E-2)	389 nm	$3.5 * 10^5$	5.6	1.7
(13b)	390 nm	$3.9 * 10^5$	5.6	1.6
(13c)	389 nm	$6.9 * 10^5$	5.9	1.6
(13d)	391 nm	$4.1 * 10^6$	6.6	1.7
(13e)	390 nm	$3.2 * 10^5$	5.5	1.5

solvent: water (2 % methanol)

Table S-5: Emission properties of selected protected receptors upon titration with $HClO_4$

Comparison of entry **13a** (Boc) and **E-2** (Cbz), shows, that the Boc group is not affected under the titration conditions with the diluted acid.

4.2. Dependency of the pH value on the methanol content in the mixture

Aqueous HEPES buffer solutions (50 mM, no salt addition, tetraethylammoniumhydroxide or HCl for equibrillation) in the range of pH 2 to pH 11 were mixed in different ratios with methanol (uvasol). The pH values of the mixtures were measured after mixing and standing for five minutes with the aid of a pH electrode for methanolic solutions:

pH of the buffer solution	**pH of the Methanol / Buffer mixture**		
	1:1	**7:3**	**9:1**
3	2.9(7)	2.8(4)	2.5(3)
4	3.9(0)	3.7(3)	3.4(4)
5	4.7(7)	4.7(1)	4.6(1)
6	5.9(0)	5.9(1)	5.7(9)
7	6.8(8)	6.8(4)	6.7(1)
8	7.8(9)	7.8(1)	7.6(4)
9	8.8(7)	8.8(0)	8.5(6)

Table S-6: pH dependency of buffer solutions on the methanol content

4.3. Dependency of the emission on the pH value in methanol / water mixture

Aqueous HEPES buffer solutions (50 mM, no salt addition, tetraethylammoniumhydroxide or HCl for equibrillation) in the range of pH 3 to pH 10 and mixed with $5*10^{-5}$ molar solutions of the receptors in methanol (uvasol) in the ration 1:9. The pH value of the mixtures was checked after mixing with the aid of a pH electrode for methanolic solutions. After standing for five minutes the emission spectrum was recorded (deprotonated sample emission ~ 300 a.u.). Table S-3 summarizes the results (rounded average of three measurements)

pH value	*3*	*4*	*5*	*6*	*7*	***8***	***9***	***10***
11a	190	190	140	70	10	0	0	0
11b	190	190	180	140	90	50	10	0
11c	180	180	180	170	150	90	20	0
11d	200	200	200	190	160	100	20	0
11e	200	200	180	130	40	10	0	0
39	200	150	100	70	30	10	0	0
32	180	180	180	150	100	20	0	0
21	170	170	170	150	80	40	0	0
40	440	430	360	260	200	100	20	0
33	450	450	360	180	50	20	10	0

Table S-7: pH dependency of the luminescent output of representative receptors

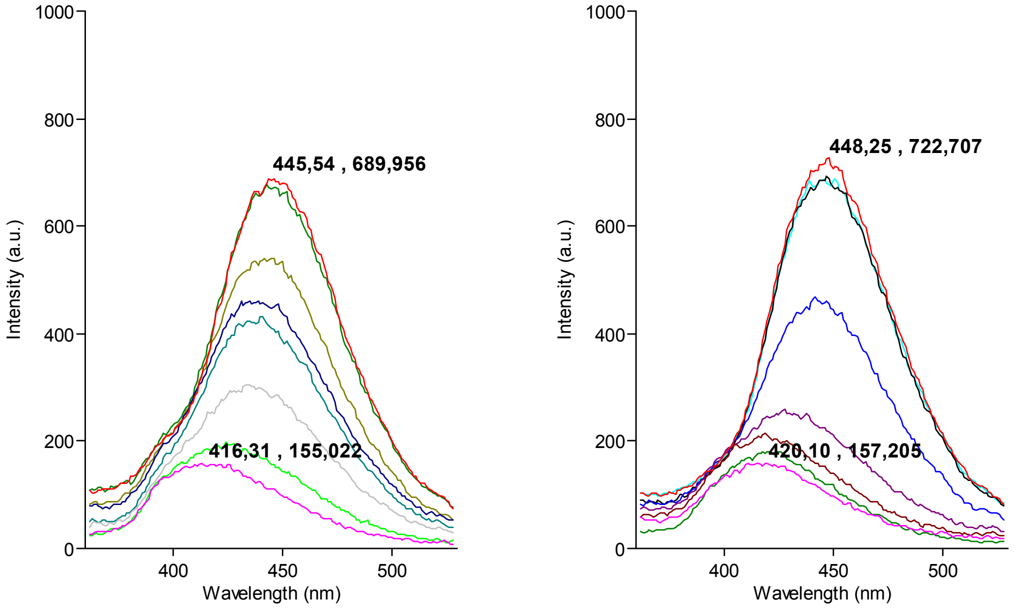


Figure S-95: Shift of the emission maximum of compound **40** (left) and **33** (right) with decreasing pH according to table S-7

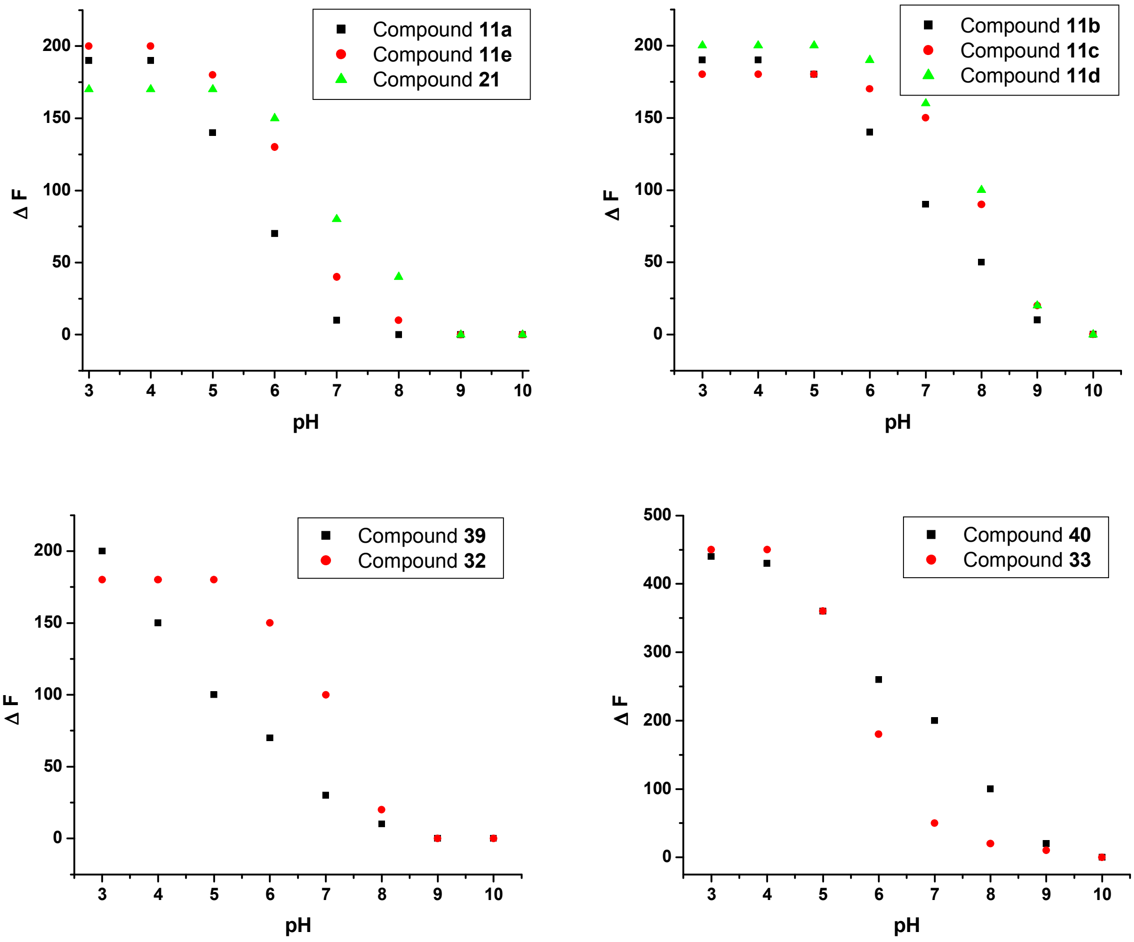
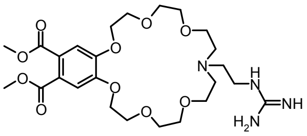


Figure S-96: Fluorescence enhancement of the receptor classes depending on the pH value of the solution (see table S-3)

4.4. Simulation of the properties of selected compounds

Physical properties like solubility, log P values, index of refraction and the expected p*K*a values were simulated with the aid of the structure calculator *Ver.12.1.* of the ACD Labs program package.

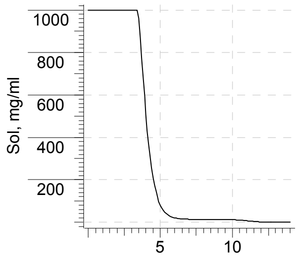


pKa Results

Acidic/Basic	Acidic/Basic	Apparent pKa Value	Error
2	MB	13.21	0.7
21	B	5.82	0.2
5	B	-2.72	0.7

Solubility Results

pH	Sol, mg/ml	Flags
2	1000	B
5.5	35.3	B
6.5	15.38	B
7.4	13.42	B
10	12.25	B



Intrinsic Solubility, mg/ml	0.1314
Intrinsic Solubility, log(S, mol/l)	-3.6269
Solubility in Pure Water at pH = 11.5, mg/ml	4.0454

Single-valued Properties

Name	Value
LogP	0.82
MW	556.61
PSA	175.62
FRB	7
HDonors	4
HAcceptors	14
Rule Of 5	2
Molar Refractivity, cm^3	135.96
Molar Volume, cm^3	431.16
Parachor, cm^3	1127.87
Index of Refraction	1.54
Surface Tension, dyne/cr	46.82
Density, g/cm^3	1.29
Polarizability, 10e-24 cm^	53.9
Boiling Point, °C	701.8

LogD Results

pH	LogD
2	-3.27
5.5	-1.61
6.5	-1.25
7.4	-1.19
10	-1.15

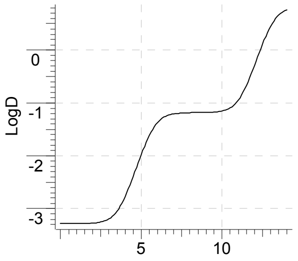
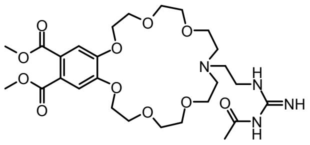


Figure S-97: Simulation of the properties of compound **14a**

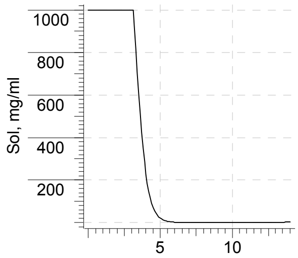


pKa Results

Acidic/Basic	Acidic/Basic	Apparent pKa Value	Error
38	MA	12.57	0.46
36	MB	8.12	0.7
16	B	5.71	0.2
38	B	-1.76	0.7

Solubility Results

pH	Sol, mg/ml	Flags
2	1000	B
5.5	6.53	B
6.5	0.77	BN
7.4	0.23	BN
10	0.15	N



Single-valued Properties

Name	Value
LogP	0.93
MW	598.64
PSA	178.7
FRB	7
HDonors	3
HAcceptors	15
Rule Of 5	2
Molar Refractivity, cm^3	146.98
Molar Volume, cm^3	467.49
Parachor, cm^3	1215.84
Index of Refraction	1.54
Surface Tension, dyne/cr	45.75
Density, g/cm^3	1.28
Polarizability, 10e-24 cm^	58.27

Intrinsic Solubility, mg/ml	0.1522
Intrinsic Solubility, log(S, mol/l)	-3.5948
Solubility in Pure Water at pH = 8.76, mg/ml	0.1557

LogD Results

pH	LogD
2	-3.14
5.5	-0.7
6.5	0.22
7.4	0.75
10	0.93

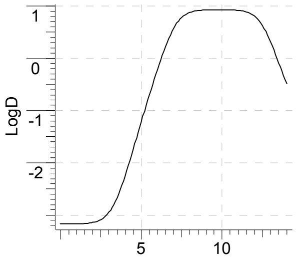
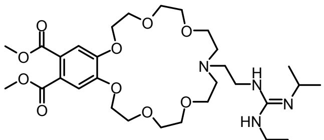


Figure S-98: Simulation of the properties of compound **16a**

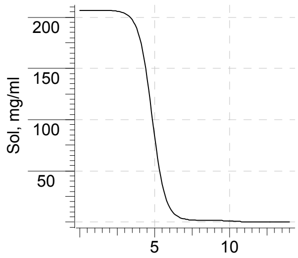


pKa Results

Acidic/Basic	Acidic/Basic	Apparent pKa Value	Error
36	MB	13.92	0.7
16	B	6.21	0.2
39	B	-0.44	0.7

Solubility Results

pH	Sol, mg/ml	Flags
2	206.51	B
5.5	36.99	B
6.5	5.83	B
7.4	2.18	B
10	1.14	B



Single-valued Properties

Name	Value
LogP	3.28
MW	640.77
PSA	147.64
FRB	9
HDonors	2
HAcceptors	14
Rule Of 5	2
Molar Refractivity, cm^3	165.15
Molar Volume, cm^3	539.68
Parachor, cm^3	1354.67
Index of Refraction	1.52
Surface Tension, dyne/cr	39.7
Density, g/cm^3	1.19
Polarizability, 10e-24 cm^	65.47
Boiling Point, °C	708.94

Intrinsic Solubility, mg/ml	1.6429e-2
Intrinsic Solubility, log(S, mol/l)	-4.5911
Solubility in Pure Water at pH = 10.64, mg/ml	0.5619

LogD Results

pH	LogD
2	-0.82
5.5	-7.12e-2
6.5	0.73
7.4	1.16
10	1.44

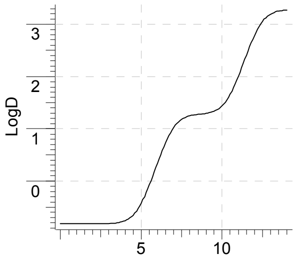
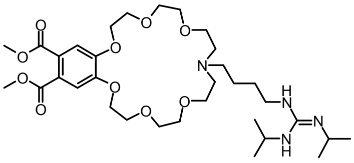


Figure S-99: Simulation of the properties of compound **11a**

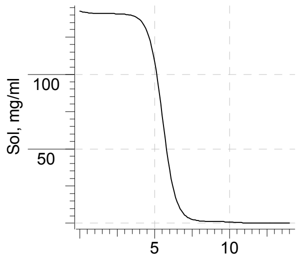


pKa Results

Acidic/Basic	Acidic/Basic	Apparent pKa Value	Error
2	MB	14.26	0.7
27	B	7.62	0.2
5	B	0.99	0.7

Solubility Results

pH	Sol, mg/ml	Flags
2	140.79	B
5.5	72.56	B
6.5	14.35	B
7.4	2.93	B
10	0.81	B



Single-valued Properties

Name	Value
LogP	3.84
MW	668.82
PSA	147.64
FRB	11
HDonors	2
HAcceptors	14
Rule Of 5	2
Molar Refractivity, cm^3	174.37
Molar Volume, cm^3	571.72
Parachor, cm^3	1431.89
Index of Refraction	1.52
Surface Tension, dyne/cr	39.35
Density, g/cm^3	1.17
Polarizability, 10e-24 cm^	69.12
Boiling Point, °C	730.06

Intrinsic Solubility, mg/ml	1.1185e-2
Intrinsic Solubility, log(S, mol/l)	-4.7767
Solubility in Pure Water at pH = 10.61, mg/ml	0.4399

LogD Results

pH	LogD
2	-0.26
5.5	3.12e-2
6.5	0.73
7.4	1.42
10	1.98

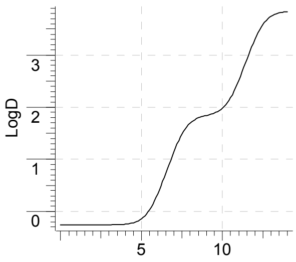
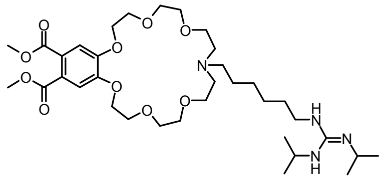


Figure S-100: Simulation of the properties of compound **11b**

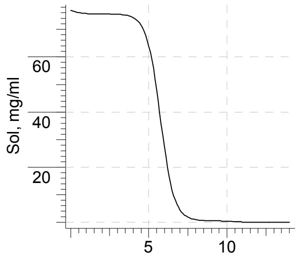


pKa Results

Acidic/Basic	Acidic/Basic	Apparent pKa Value	Error
2	MB	14.31	0.7
28	B	7.84	0.2
5	B	1.21	0.7

Solubility Results

pH	Sol, mg/ml	Flags
2	75.61	B
5.5	48.24	B
6.5	11.72	B
7.4	2.21	B
10	0.46	B



Single-valued Properties

Name	Value
LogP	4.64
MW	696.87
PSA	147.64
FRB	13
HDonors	2
HAcceptors	14
Rule Of 5	2
Molar Refractivity, cm^3	183.59
Molar Volume, cm^3	603.74
Parachor, cm^3	1509.11
Index of Refraction	1.52
Surface Tension, dyne/cr	39.04
Density, g/cm^3	1.15
Polarizability, 10e-24 cm^	72.78
Boiling Point, °C	747.5

Intrinsic Solubility, mg/ml	6.006e-3
Intrinsic Solubility, log(S, mol/l)	-5.0646
Solubility in Pure Water at pH = 10.53, mg/ml	0.2901

LogD Results

pH	LogD
2	0.54
5.5	0.73
6.5	1.35
7.4	2.07
10	2.76

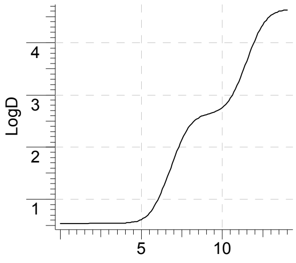
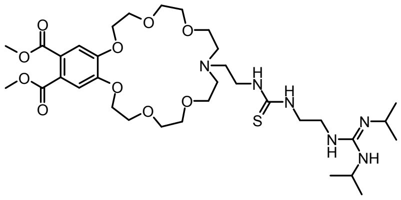


Figure S-101: Simulation of the properties of compound **11c**

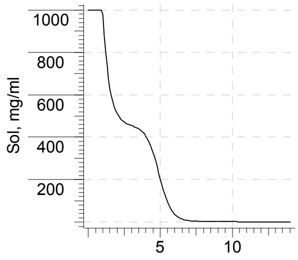


pKa Results

Acidic/Basic	Acidic/Basic	Apparent pKa Value	Error
13	A	15.98	0.7
2	MB	14.98	0.7
13	MA	13.23	0.7
29	B	7	0.2

Solubility Results

pH	Sol, mg/ml	Flags
2	511.95	B
5.5	94.34	B
6.5	14.69	B
7.4	5.03	B
10	2.67	B



Single-valued Properties

Name	Value
LogP	2.89
MW	742.92
PSA	203.79
FRB	12
HDonors	4
HAcceptors	16
Rule Of 5	2
Molar Refractivity, cm^3	192.41
Molar Volume, cm^3	605.61
Parachor, cm^3	1538.82
Index of Refraction	1.55
Surface Tension, dyne/cr	41.69
Density, g/cm^3	1.23
Polarizability, 10e-24 cm^	76.28
Boiling Point, °C	791.43

Intrinsic Solubility, mg/ml	3.6086e-2
Intrinsic Solubility, log(S, mol/l)	-4.3136
Solubility in Pure Water at pH = 10.72, mg/ml	1.2582

LogD Results

pH	LogD
2	-1.26
5.5	-0.53
6.5	0.28
7.4	0.74
10	0.89

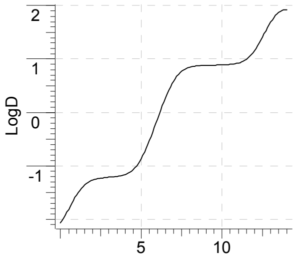
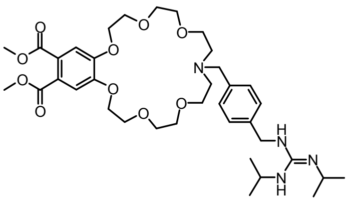


Figure S-102: Simulation of the properties of compound **11e**

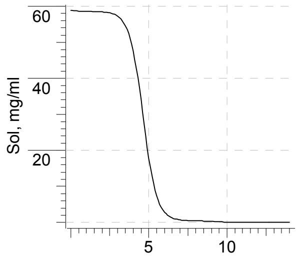


pKa Results

Acidic/Basic	Acidic/Basic	Apparent pKa Value	Error
2	MB	13.27	0.7
23	B	6.75	0.2
5	B	0.24	0.7

Solubility Results

pH	Sol, mg/ml	Flags
2	58.43	B
5.5	7.39	B
6.5	1.24	B
7.4	0.56	B
10	7.61e-2	BN



Single-valued Properties

Name	Value
LogP	4.53
MW	716.86
PSA	147.64
FRB	10
HDonors	2
HAcceptors	14
Rule Of 5	2
Molar Refractivity, cm^3	190.25
Molar Volume, cm^3	607.24
Parachor, cm^3	1531.53
Index of Refraction	1.54
Surface Tension, dyne/cr	40.46
Density, g/cm^3	1.18
Polarizability, 10e-24 cm^	75.42
Boiling Point, °C	765.03

Intrinsic Solubility, mg/ml	4.6519e-3
Intrinsic Solubility, log(S, mol/l)	-5.1878
Solubility in Pure Water at pH = 9.76, mg/ml	0.1159

LogD Results

pH	LogD
2	0.43
5.5	1.33
6.5	2.11
7.4	2.45
10	3.32

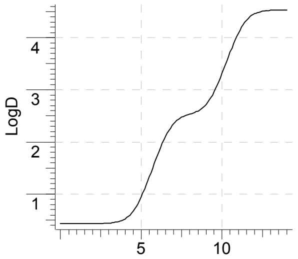
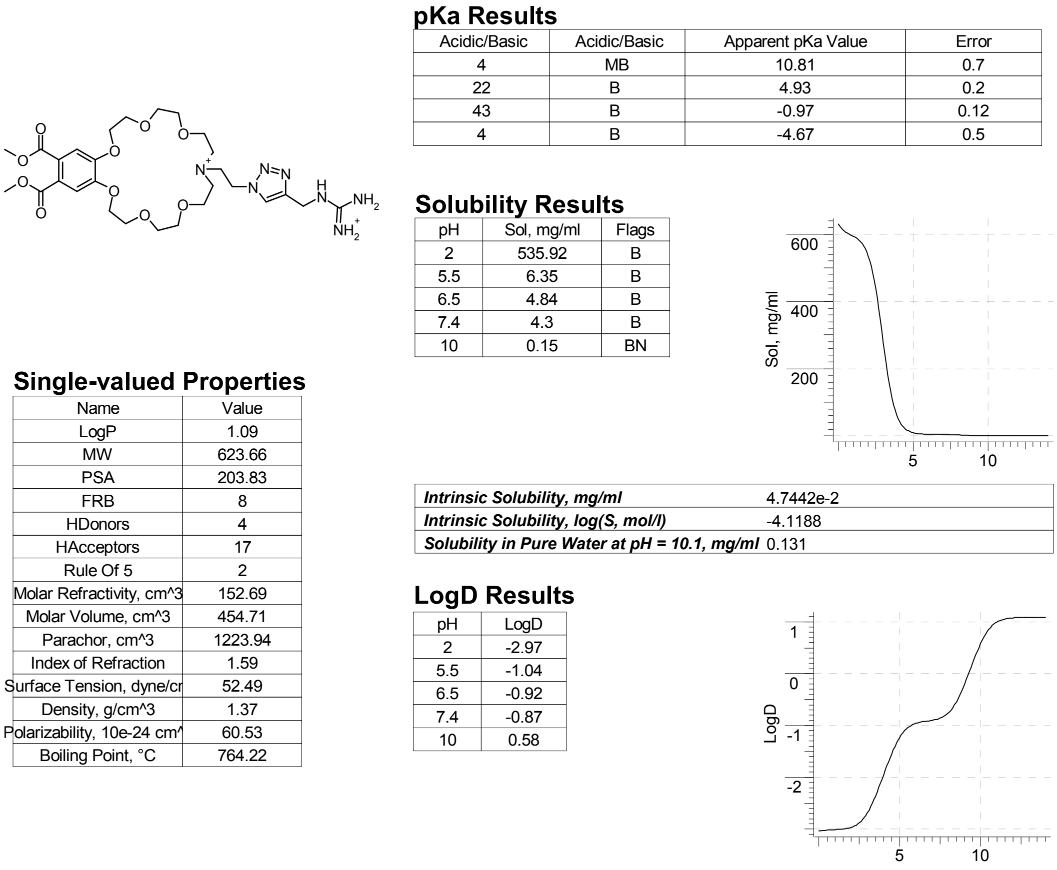


Figure S-103: Simulation of the properties of compound **11d**



pKa Results

Acidic/Basic	Acidic/Basic	Apparent pKa Value	Error
4	MB	10.81	0.7
22	B	4.93	0.2
43	B	-0.97	0.12
4	B	-4.67	0.5

Solubility Results

pH	Sol, mg/ml	Flags
2	535.92	B
5.5	6.35	B
6.5	4.84	B
7.4	4.3	B
10	0.15	BN

Single-valued Properties

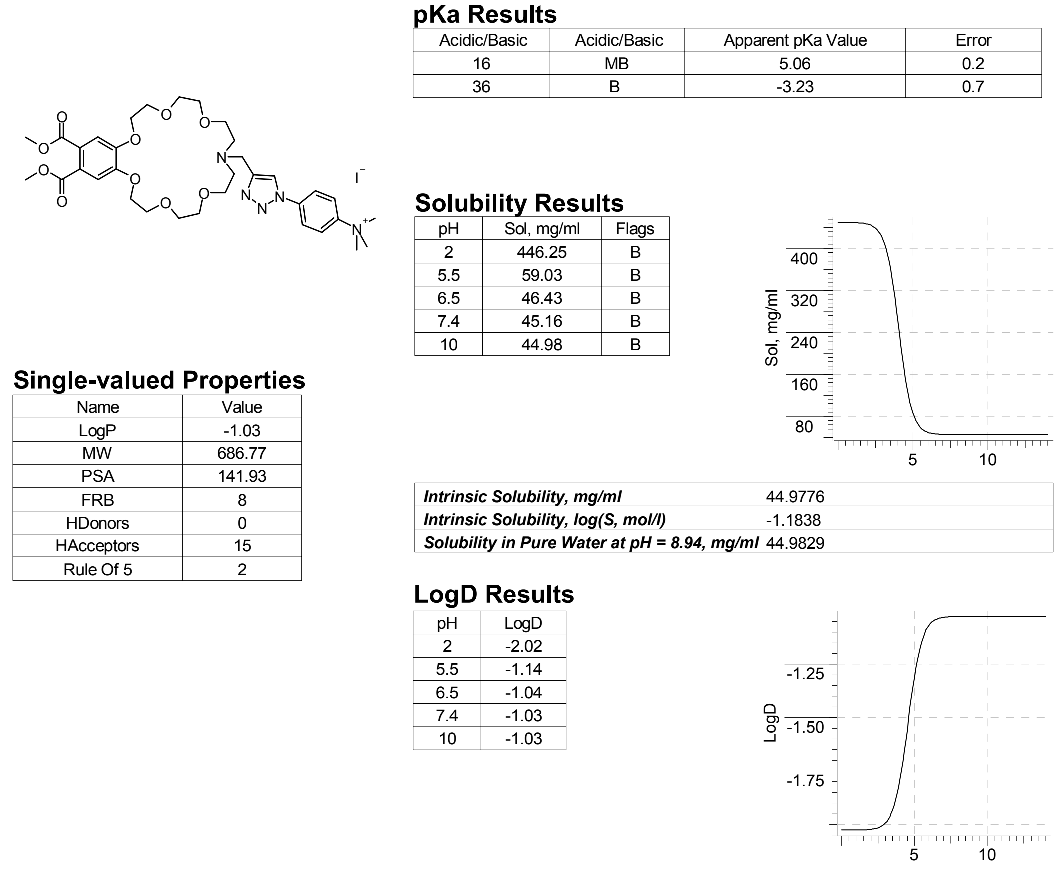
Name	Value
LogP	1.09
MW	623.66
PSA	203.83
FRB	8
HDonors	4
HAcceptors	17
Rule Of 5	2
Molar Refractivity, cm^3	152.69
Molar Volume, cm^3	454.71
Parachor, cm^3	1223.94
Index of Refraction	1.59
Surface Tension, dyne/cr	52.49
Density, g/cm^3	1.37
Polarizability, 10e-24 cm^	60.53
Boiling Point, °C	764.22

Intrinsic Solubility, mg/ml	4.7442e-2
Intrinsic Solubility, log(S, mol/l)	-4.1188
Solubility in Pure Water at pH = 10.1, mg/ml	0.131

LogD Results

pH	LogD
2	-2.97
5.5	-1.04
6.5	-0.92
7.4	-0.87
10	0.58

Figure S-104: Simulation of the properties of compound **27**



pKa Results

Acidic/Basic	Acidic/Basic	Apparent pKa Value	Error
16	MB	5.06	0.2
36	B	-3.23	0.7

Solubility Results

pH	Sol, mg/ml	Flags
2	446.25	B
5.5	59.03	B
6.5	46.43	B
7.4	45.16	B
10	44.98	B

Single-valued Properties

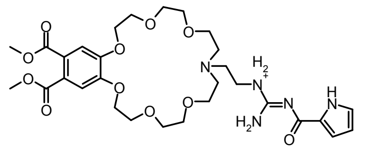
Name	Value
LogP	-1.03
MW	686.77
PSA	141.93
FRB	8
HDonors	0
HAcceptors	15
Rule Of 5	2

Intrinsic Solubility, mg/ml	44.9776
Intrinsic Solubility, log(S, mol/l)	-1.1838
Solubility in Pure Water at pH = 8.94, mg/ml	44.9829

LogD Results

pH	LogD
2	-2.02
5.5	-1.14
6.5	-1.04
7.4	-1.03
10	-1.03

Figure S-105: Simulation of the properties of compound **21**



Single-valued Properties

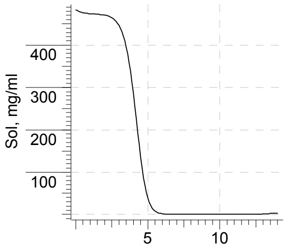
Name	Value
LogP	1.63
MW	649.69
PSA	191.99
FRB	8
HDonors	4
HAcceptors	16
Rule Of 5	2
Molar Refractivity, cm^3	161.5
Molar Volume, cm^3	492.54
Parachor, cm^3	1304.16
Index of Refraction	1.57
Surface Tension, dyne/cr	49.15
Density, g/cm^3	1.32
Polarizability, 10e-24 cm^	64.02

pKa Results

Acidic/Basic	Acidic/Basic	Apparent pKa Value	Error
5	MA	16.39	0.5
11	MB	11.77	0.7
11	B	7.86	0.7
11	B	5.99	0.7

Solubility Results

pH	Sol, mg/ml	Flags
2	470.59	B
5.5	7.92	B
6.5	0.46	BN
7.4	8.45e-2	BN
10	3.94e-2	AN



Intrinsic Solubility, mg/ml	3.7597e-2
Intrinsic Solubility, log(S, mol/l)	-4.2375
Solubility in Pure Water at pH = 8.62, mg/ml	4.0441e-2

LogD Results

pH	LogD
2	-2.47
5.5	-0.69
6.5	0.55
7.4	1.28
10	1.61

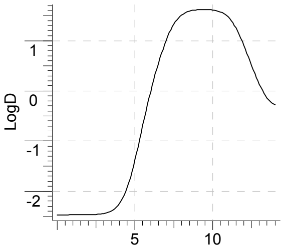
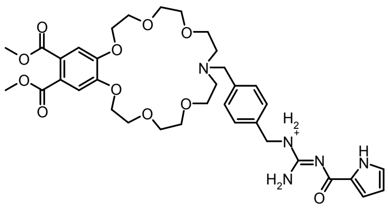


Figure S-106: Simulation of the properties of compound **39**



Single-valued Properties

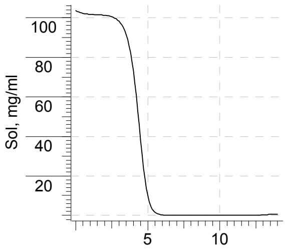
Name	Value
LogP	2.91
MW	725.79
PSA	194.49
FRB	9
HDonors	4
HAcceptors	16
Rule Of 5	2
Molar Refractivity, cm^3	186.6
Molar Volume, cm^3	560.1
Parachor, cm^3	1481.03
Index of Refraction	1.58
Surface Tension, dyne/cr	48.89
Density, g/cm^3	1.3
Polarizability, 10e-24 cm^	73.98

pKa Results

Acidic/Basic	Acidic/Basic	Apparent pKa Value	Error
5	A	16.25	0.5
1	MA	11.26	0.46
9	MB	7.22	0.7
27	B	6.66	0.2

Solubility Results

pH	Sol, mg/ml	Flags
2	101.2	B
5.5	1.57	B
6.5	5.34e-2	BN
7.4	1.22e-2	BN
10	8.51e-3	AN



Intrinsic Solubility, mg/ml	8.0631e-3
Intrinsic Solubility, log(S, mol/l)	-4.9543
Solubility in Pure Water at pH = 8.07, mg/ml	8.9064e-3

LogD Results

pH	LogD
2	-1.19
5.5	0.62
6.5	2.09
7.4	2.73
10	2.88

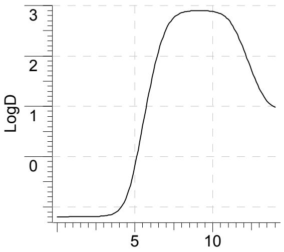
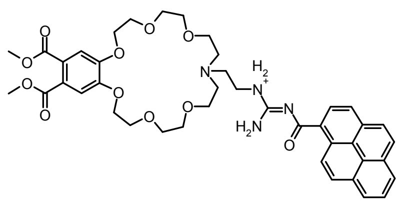


Figure S-107: Simulation of the properties of compound **32**

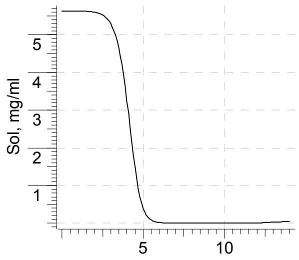


pKa Results

Acidic/Basic	Acidic/Basic	Apparent pKa Value	Error
1	MA	10.9	0.46
7	MB	7.06	0.7
7	B	5.19	0.7
1	B	-2.78	0.7

Solubility Results

pH	Sol, mg/ml	Flags
2	5.6	B
5.5	8.32e-2	B
6.5	5.49e-3	BN
7.4	1.03e-3	BN
10	5.24e-4	AN



Single-valued Properties

Name	Value
LogP	5.03
MW	784.85
PSA	176.2
FRB	8
HDonors	3
HAcceptors	15
Rule Of 5	3
Molar Refractivity, cm^3	206.1
Molar Volume, cm^3	594.46
Parachor, cm^3	1583.34
Index of Refraction	1.61
Surface Tension, dyne/cr	50.33
Density, g/cm^3	1.32
Polarizability, 10e-24 cm^	81.71

Intrinsic Solubility, mg/ml	4.4749e-4
Intrinsic Solubility, log(S, mol/l)	-6.244
Solubility in Pure Water at pH = 7.65, mg/ml	7.7698e-4

LogD Results

pH	LogD
2	0.93
5.5	2.76
6.5	3.94
7.4	4.67
10	4.96

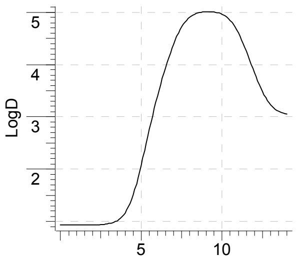
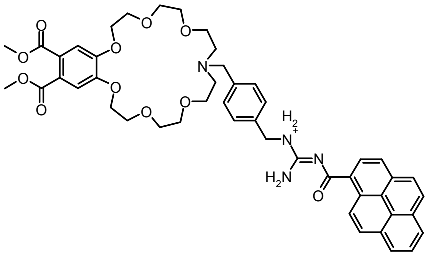


Figure S-108: Simulation of the properties of compound **40**

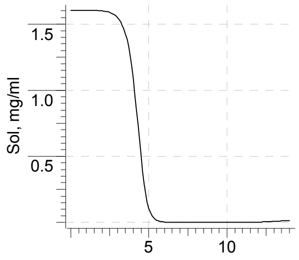


pKa Results

Acidic/Basic	Acidic/Basic	Apparent pKa Value	Error
1	MA	10.71	0.46
23	MB	6.86	0.2
5	B	6.1	0.7
1	B	-2.75	0.7

Solubility Results

pH	Sol, mg/ml	Flags
2	1.6	B
5.5	1.61e-2	B
6.5	6.75e-4	BN
7.4	1.82e-4	BN
10	1.53e-4	AN



Single-valued Properties

Name	Value
LogP	6.46
MW	860.95
PSA	178.7
FRB	9
HDonors	3
HAcceptors	15
Rule Of 5	3
Molar Refractivity, cm^3	231.21
Molar Volume, cm^3	661.93
Parachor, cm^3	1760.21
Index of Refraction	1.62
Surface Tension, dyne/cr	50
Density, g/cm^3	1.3
Polarizability, 10e-24 cm^	91.66

Intrinsic Solubility, mg/ml	1.276e-4
Intrinsic Solubility, log(S, mol/l)	-6.8291
Solubility in Pure Water at pH = 7.22, mg/ml	2.1228e-4

LogD Results

pH	LogD
2	2.36
5.5	4.35
6.5	5.73
7.4	6.3
10	6.38

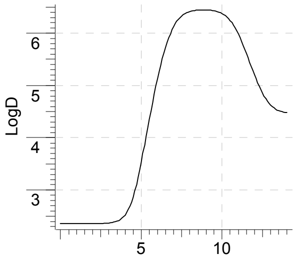


Figure S-109: Simulation of the properties of compound **33**

5. Screenings and binding studies via fluorescence and UV measurements

5.1. General methods, material and sample preparation

Emission Spectroscopy. Fluorescence measurements were performed with UV-grade solvents (Merck, Baker or Acros) in 1 cm quartz cuvettes (Hellma) and recorded on a Varian 'Cary Eclipse' fluorescence spectrophotometer with temperature control. To determine the binding constants and Job´s plots fluorescence titration experiments were carried out.

Absorption Spectroscopy. Absorption were recorded on a Varian Cary BIO 50 UV/VIS/NIR Spectrometer with temperature control by use of a 1 cm quartz cuvettes (Hellma) and Uvasol solvents or of quality for spectrophotometrical investigations (Merck, Baker or Acros).

Sample preparation. As a standard solvent mixture 10 % water in methanol was used. No buffer or salt was added. The exact solutions have been prepared by adding to a weighed amount of receptor or substrate, the calculated amount of solvent necessary by means of microliter precision pipettes. This method is more accurate than to determine exactly the weighed amount (to 10^{-5} g) and admit a certain volume, since the volume measurement has a higher accuracy than the weight determination. The receptor was first dissolved in the methanol content of the solvent mixture, then the water content was added lacking 100 µl, to have room to adjust the pH. For pH adjusting 1 M tetraethylammoniumhydroxide or hydrochloric acid of pro analysi quality in high-purity water was used. Thereafter, the water difference filled up to the full volume.

For measurements with pyrene substituted compounds, all solvents used were degassed prior to the titration by treatment with ultrasound in the already filled cells.

Receptor solutions were stored properly stoppered and closed with parafilm at maximum for one week in the refrigerator at -4 °C. They were freshly prepared every week from deep frozen aliquots of the receptor material. Amino acid solutions were kept at -4 °C properly stoppered and closed with parafilm for maximal one week. Peptide solutions were always freshly prepared from the properly dried and carefully stored material (nitrogen, -20 °C).

5.2. Fluorescence data

All fluorescence experiments were performed on a Varian Cary Eclipse Fluorimeter. To determine the binding constants, fluorescence titration experiments were carried out. The screening of the amino acids and the synthesized peptide library was conducted in a microtiter plate (96 wells half area, UV star material). The fluorescence intensity was found to be constant after 5 min.

Screening conditions:

Solvent: methanol / water 9:1 (vol/vol)
Well volume: 200 µL
Concentration [crown ether guanidine]: 0.03 - 0.05 mM
Concentration [amino acid or peptide]: 10.0 - 50.0 mM

Instrument Parameters:

Excitation wavelength: λ_{ex} = 300 nm
Detection wavelength: λ = 320 – 470 nm and 360 – 530 nm
Temperature: T = 298 K
PMT voltage: 500 - 600 volts
Excitation slit: 10 nm
Emission slit: 10 nm

Procedure: To each column in the microtiter plate samples of the receptor with 150 to 500 eq. of the corresponding amino acid or peptide and the receptor without added peptide but volume corrected as reference were given. Emission spectra for all wells were recorded. Every solution was pH adjusted before to pH 7.5 - 8 or pH 6.5, depending on the receptor used (see table above)

For amino acids and peptides inducing a significant emission increase over the parent compound (> 30 %) the binding constant and stoichiometry of the respective guest with selected receptors were determined by emission titration in a cuvette

Emission titrations:

Titration conditions (amino acids and related guests):

Solvent: MeOH / H_2O 9:1 (vol/vol), pH 6.5 or pH 7.5 - 8.5
Starting volume: 1.2 mL
Concentration [crown ether guanidine]: 0.02 mM
Concentration [amino acid or peptide]: 30.0 mM

Instrument Parameters

Excitation wavelength: $\lambda_{ex} = 300$ nm
Detection wavelength: $\lambda = 320 – 470$ nm and 360 – 530 nm
Temperature: T = 298 K
PMT voltage 700 volts

Titration conditions (peptides):

Solvent: 90 % aqueous methanol, pH 7.5 - 8.5 or 6.5
Starting volume: 1.2 mL
Concentration [receptor]: $2.0 * 10^{-5}$ M
Concentration [guest]: $1 * 10^{-2}$ M or $5 * 10^{-3}$ M

Instrument Parameters

Excitation wavelength: $\lambda_{ex} = 300$ nm
Detection wavelength: $\lambda = 320 – 500$ nm or 360 – 550 nm
Temperature: T = 298 K
PMT voltage: 600 volts

Procedure: To a cuvette with 1.2 mL of the receptor in 90% aqueous methanol (pH adjusted, no salt added) were added 10 µL (≙ 1 eq.) aliquots of the amino acid or peptide solution (pH adjusted) or it was titrated stepwise with small amounts 5 – 100 µL, depending on the initial emission response, of the substrate solution. After each addition the solution was allowed to equilibrate for 5 min before the fluorescence intensity or the UV spectrum (see Figure 18 for a representative example) were recorded. The stoichiometry was determined by Job´s plot analysis extracted from titration data[16].

The pH value was checked before and after every titration with the aid of a pH electrode suitable for methanol/water mixtures. Measurements with the pH differing more than 0.5 units before and after the titrations were not used.

To determine the binding constant the obtained fluorescence intensities were volume corrected, plotted against the concentration of peptide and evaluated by non linear fitting methods.

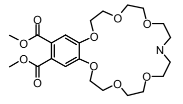
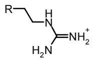
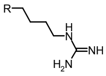
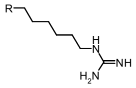
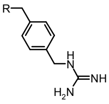
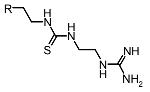
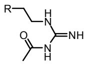
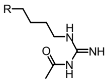
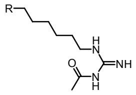
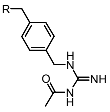
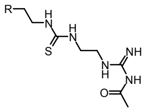
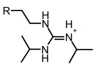
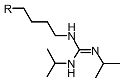
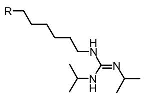
5.2.1. *Titrations of the crown ether part with ammonium guests*:

Receptor	**n-Butylammonium hydrochloride**		**Acetyl-lysine methylester hydrochloride**	
	K [M^{-1}]	***I_{∞}/I_0***	***K [M^{-1}]***	***I_{∞}/I_0***
(13a)	260	3.7	250	3.4
(14a)	280	3.6	260	3.2
(E-3)	280	3.7	220	3.3
(11a)	230	3.4	190	3.2
(11c)	250	3.3	230	3.4
(11d)	290	3.4	270	3.4

Table S-8: Binding properties of selected receptors in comparison to their protected precursors with different ammonium targets in methanol

In methanol / water 9:1 the same couples were investigated. All binding strengths were weaker than 100 M^{-1} in this mixture.

5.2.2. Summary of all fluorescence enhancement factors screened:

Receptor	**Amino Acid**										**Peptide**	
	unpolar					**polar**	**basic**		**acidic**			
	*glycine*	*β-alanine*	*GABA*	*AHX*	*phenylalanine*	*serine*	*lysine*	*histidine*	*glutaminic acid*	*aspartic acid*	*glycyl glycine*	*glycyl glycyl glycine*
	1	**1.25**	**1.2**	**1.1**	1	1	1	1	1	1	1	1
	1	**1.2**	**1.3**	**1.35**	1	1	1	1	1	1	**1.1**	1
	**1.1**	**1.15**	**1.25**	**1.2**	1	**1.1**	**1.1**	**1.1**	1	1	**1.22**	1
	1	**1.1**	**1.45**	**1.3**	1	1	1	1	1	1	**1.12**	1
	1	**1.1**	**1.22**	**1.25**	1	1	**1.1**	1	1	1	**1.25**	1.2
	1	**1.28**	**1.2**	**1.1**	1	1	1	1	1	1	1	1
	1	**1.2**	**1.32**	**1.38**	1	1	1	1	1	1	**1.1**	1
	**1.1**	**1.15**	**1.28**	**1.22**	1	**1.1**	**1.1**	**1.1**	1	1	**1.25**	1
	1	**1.12**	**1.48**	**1.28**	1	1	1	1	1	1	**1.12**	1
	1	**1.1**	**1.25**	**1.3**	1	1	**1.1**	1	1	1	**1.28**	**1.22**
	1	**1.3**	**1.2**	**1.1**	1	1	1	1	1	1	1	1
	1	**1.2**	**1.35**	**1.4**	1	1	1	1	1	1	**1.1**	1
	**1.1**	**1.18**	**1.3**	**1.25**	1	**1.1**	**1.1**	**1.1**	1	1	**1.28**	1

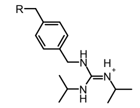
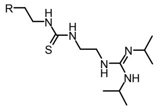
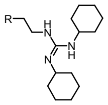
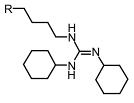
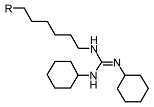
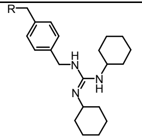
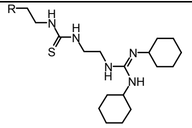
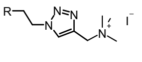
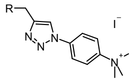
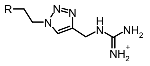
	1	**1.15**	**1.5**	**1.3**	1	1	1	1	1	1	**1.15**	1
	1	**1.1**	**1.28**	**1.32**	1	1	**1.12**	1	1	1	**1.3**	**1.25**
	1	**1.3**	**1.2**	**1.1**	1	1	1	1	1	1	1	1
	1	**1.2**	**1.35**	**1.4**	1	1	1	1	1	1	**1.1**	1
	**1.1**	**1.18**	**1.3**	**1.25**	1	**1.1**	**1.1**	**1.1**	1	1	**1.28**	1
	1	**1.15**	**1.5**	**1.3**	1	1	1	1	1	1	**1.13**	1
	1	**1.1**	**1.28**	**1.3**	1	1	**1.12**	1	1	1	**1.3**	1.22
	1	1	**1.22**	**1.15**	1	1	**1.1**	1	1	1	**1.2**	1
	1	1	1	**1.22**	1	1	**1.1**	1	1	1	**1.22**	1.3
	1	1	**1.32**	**1.2**	1	1	**1.1**	1	1	1	**1.25**	1

Table S-9: Fluorescence screening results of all receptors with selected amino acids and glycine peptides; average of two measurements, rounded values (fluorescence enhancement factor of 1 means no binding event detected)

5.2.3. Titrations of amino acids and peptides with selected receptors:

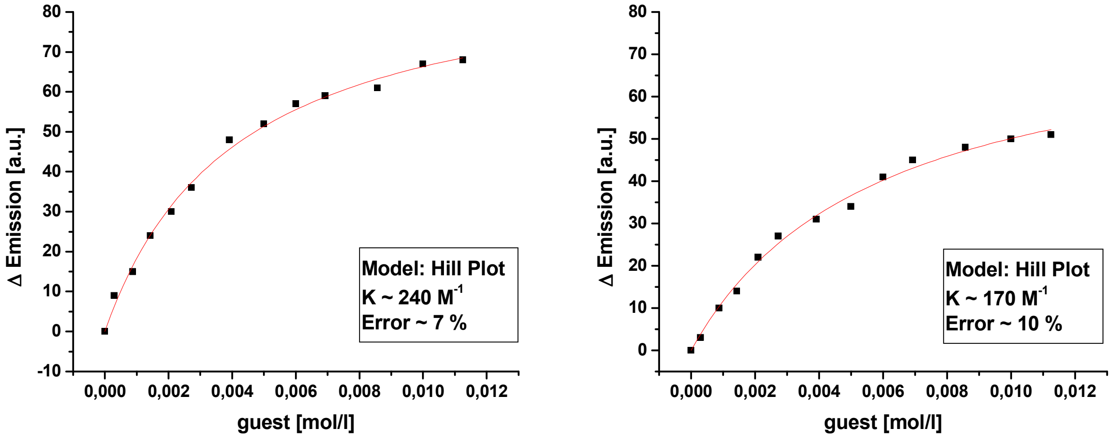


Figure S-110: Emission titration of compound **11a** with β-Ala (left) and GABA (right)

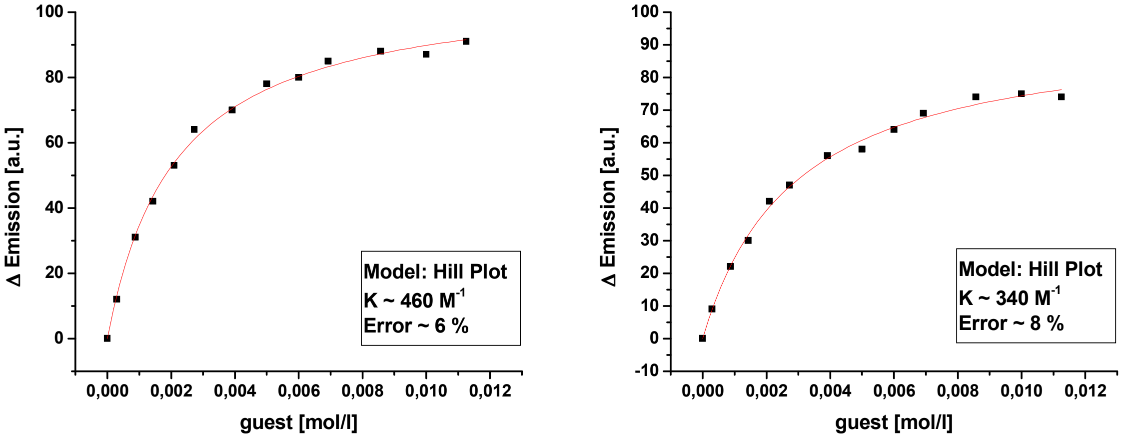


Figure S-111: Emission titration of compound **11d** with GABA (left) and AHX (right)

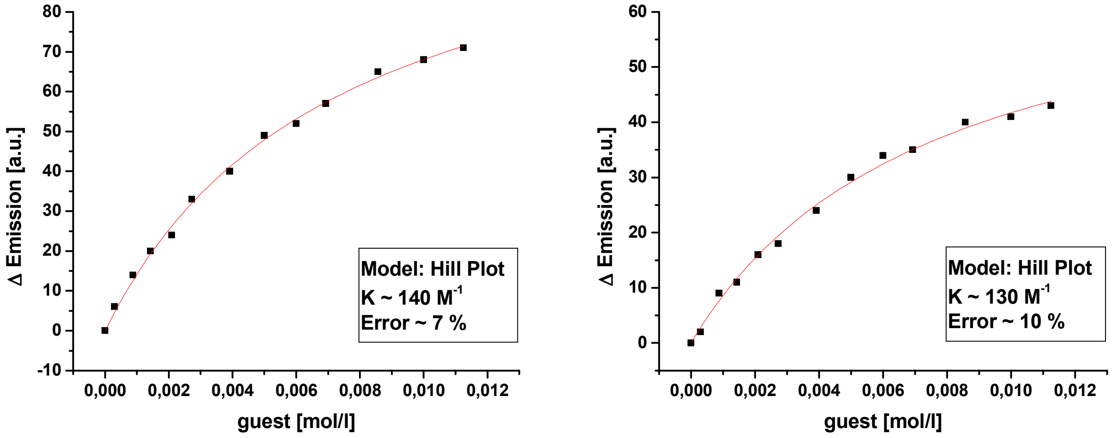


Figure S-112: Emission titration of compound **27** with GABA (left) and AHX (right)

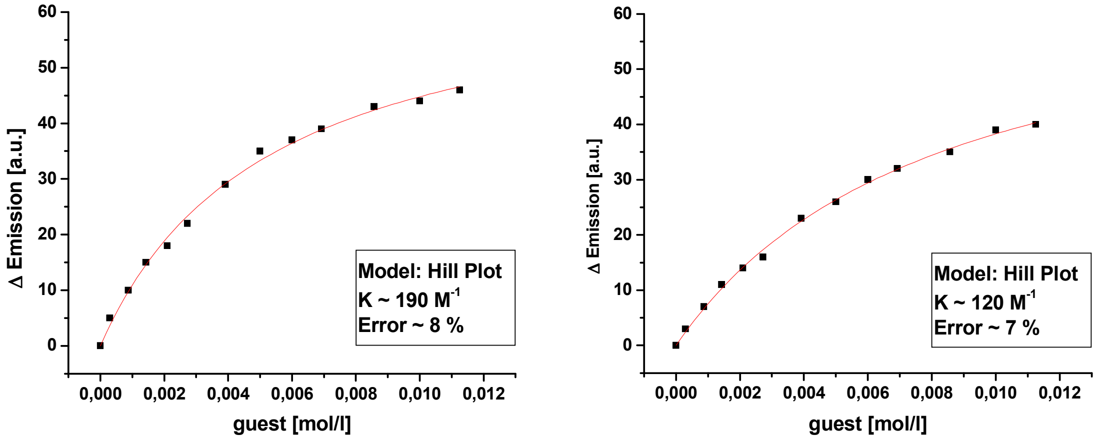


Figure S-113: Emission titration of compound **24** with glycyl glycine (left) and compound **21** with glycyl glycine (right)

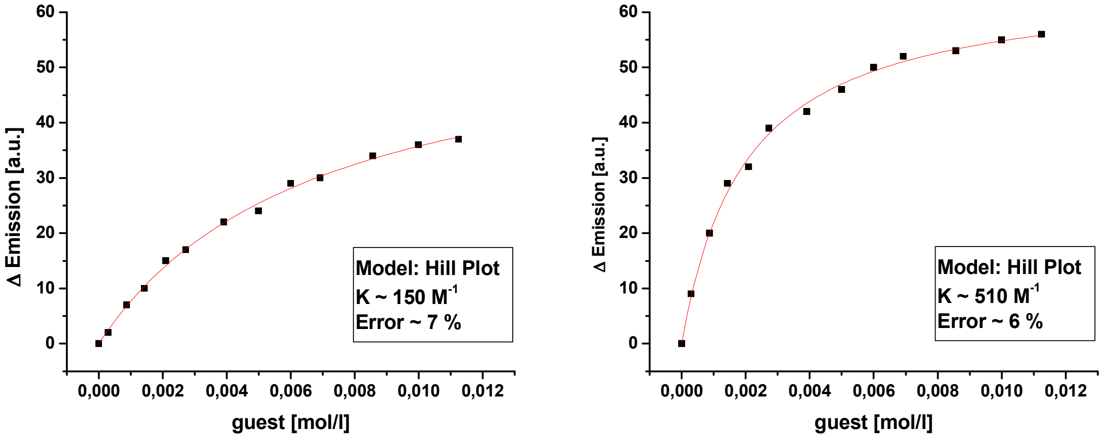


Figure S-114: Emission titration of compound **21** with AHX (left) and glycyl glycyl glycine (right)

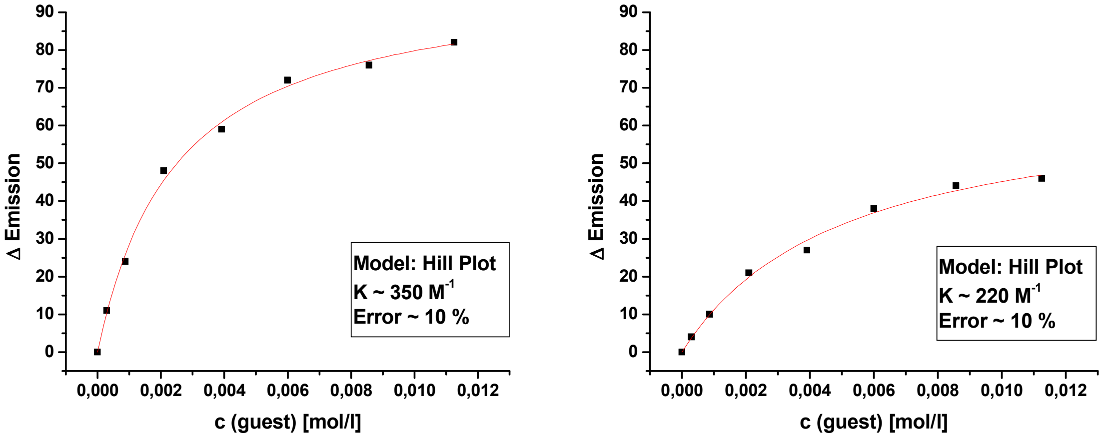


Figure S-115: Emission titration of compound **11d** with H-E-G-G-G-amide (left) and H-G-E-G-G-amide (right)

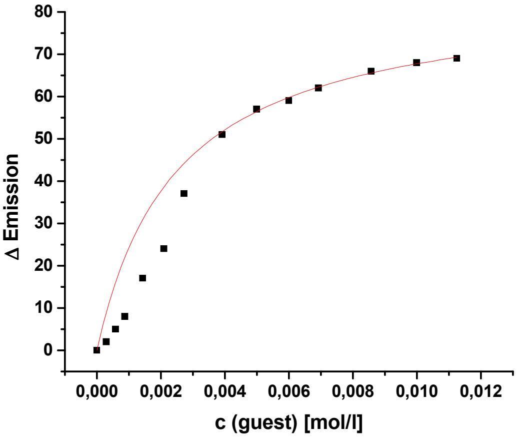


Figure S-116: Emission titration of compound **11d** with Ac-Glu-Lys-amide

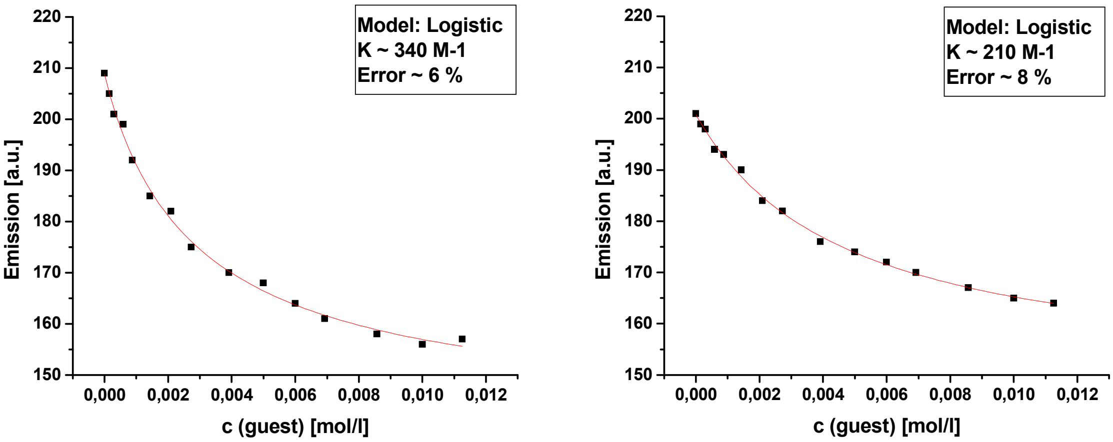


Figure S-117: Emission titration of compound **40** with β-Ala (left) and GABA (right)

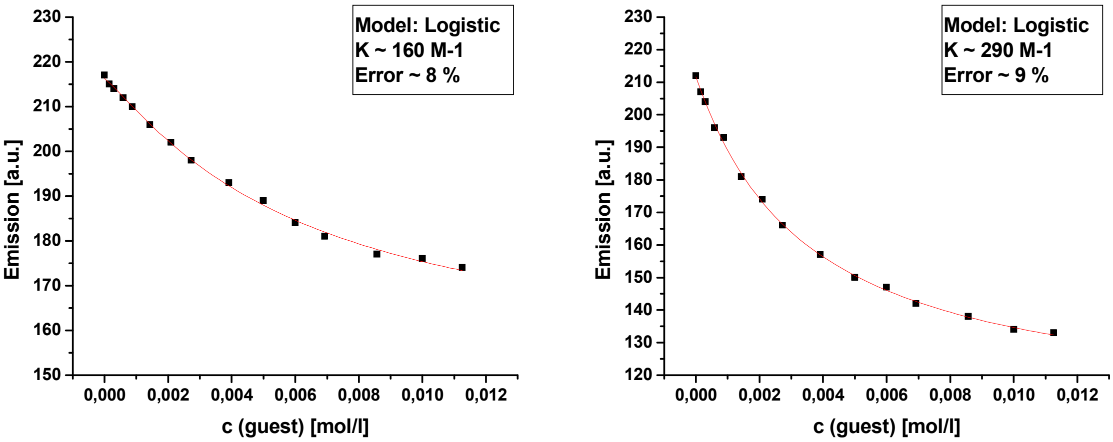


Figure S-118: Emission titration of compound **33** with β-Ala (left) and GABA (right)

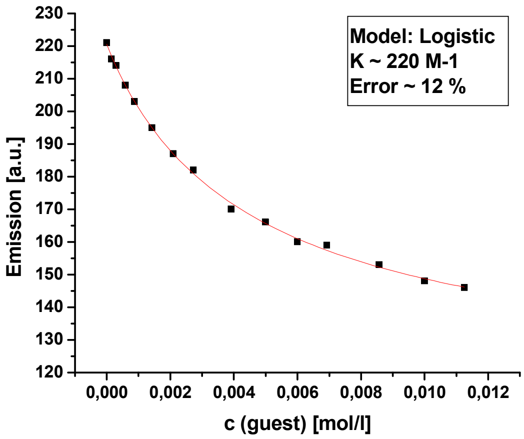


Figure S-119: Emission titration of compound **33** with AHX

5.3. Explanation for the fluorescence decrease in pyrene receptor systems:

The quenching observed can be rationalized in terms of the change in redox properties of guanidinium. Due to occupancy of the lone pair by protonation, the guest-free guanidinium has a relative lower HOMO level below that of an excited pyrene (pyr*), therefore its influence to fluorescent intensity is negligible. However, the formation of the complex with carboxylate by hydrogen bonds weakens the N-H bonds of guanidinium; hence the HOMO level increases to be above that of pyr* such that the electron transfer from the former to the half filled later one occurs, interrupting the fluorescence process of pyr*. Consequently, the quenching of fluorescence is observed.[17]

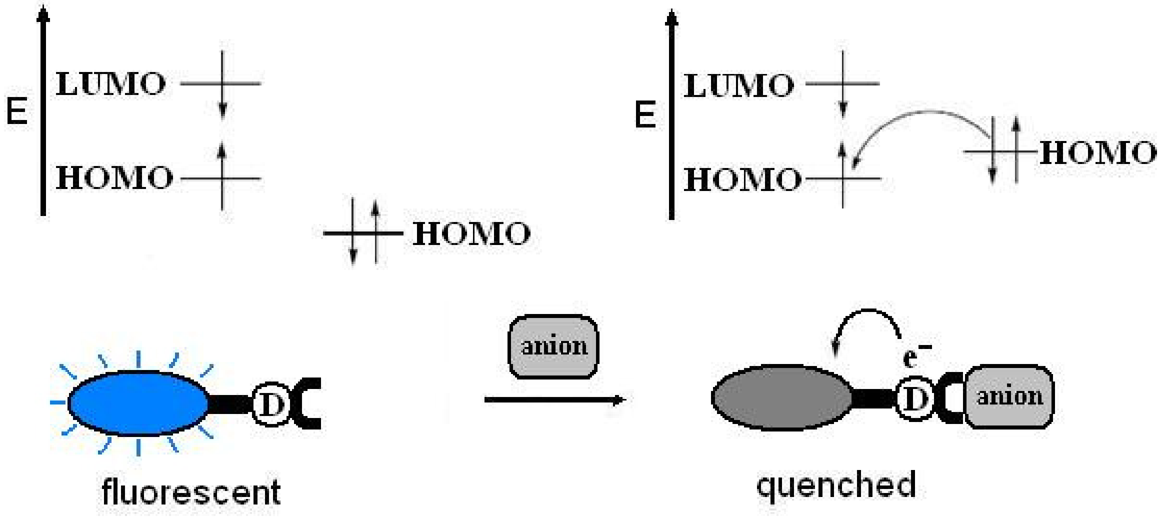


Figure S-120: Principle of the fluorescence quenching induced by anion recognition of the fluorescent pyrene-guanidinium PET sensor.

Interestingly, the pyrene systems **33** and **40** show a recognition and distinction of aspartic and glutamic acid signalled by a differently strong fluorescence output. Although this can be attributed mainly to a pH effect – it is not seen in buffered medium above pH 6.5 – it is remarkably in view of the small difference of both structures.

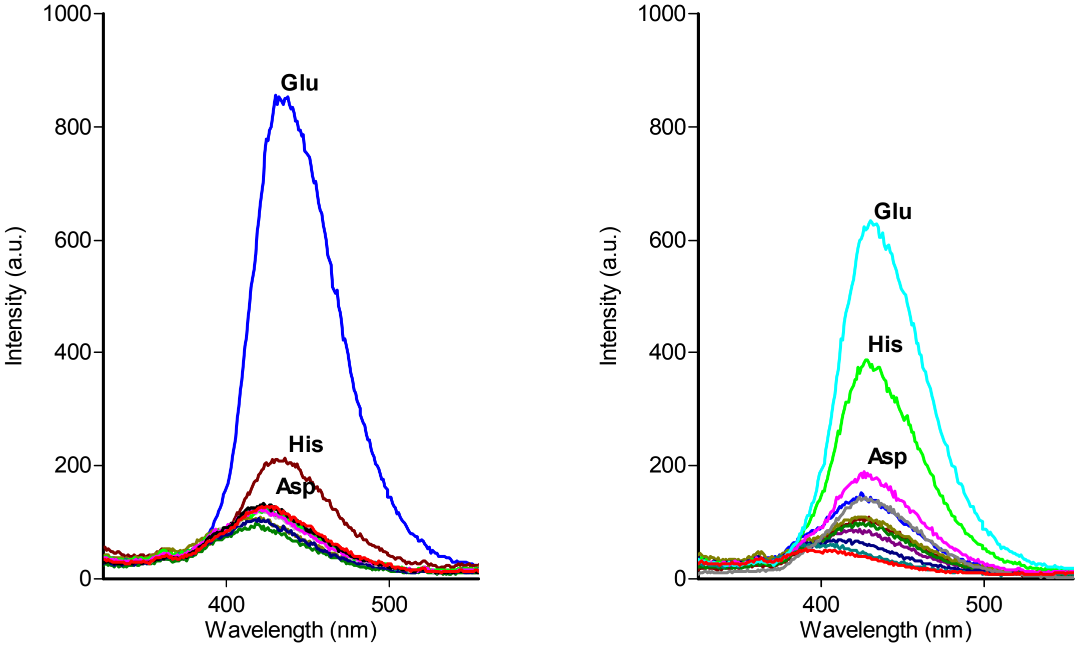


Figure S-121: Recognition of aspartic, glutaminic acid and histidine in aqueous methanol at pH < 6 (ethylene spacered system left, with xylylene spacer right)

The difference can be even seen with the naked eye and underlines the good spectroscopic properties of the pyrene systems.

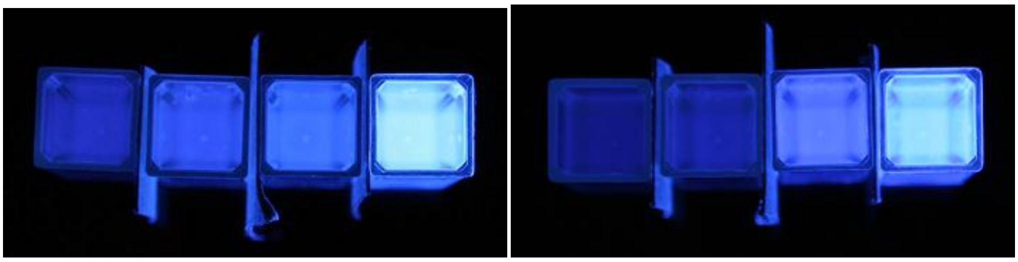


Figure S-122: Fluorescence of crown ether guanidine systems with acyl-pyrene substituent; from left to right: Blanc sample, aspartic acid, histidine and glutaminic acid in aqueous methanol at pH < 6 (ethylene spacered system left, with xylylene spacer right)

5.4. UV measurements

The cuvettes have a thickness of 1 cm and were filled with 2 mL solution. As blanc sample the pure solvent mixture, 10 % water in methanol, was used. The cell was temperated to 25 ° C. The additions were performed using precision microliter pipettes. After each addition the solution was given three minutes to reach the new equilibrium before the measurement was started. From each titration step UV spectrum in the wavelength range from 250 to 340 nm was recorded. Prior to the acquisition of spectra a baseline correction was performed. At the end of a titration, the pH was checked to exclude protonation reactions.

For the evaluation of the validity of the Lambert-Beer's a measurement of dilution series was conducted. For this a receptor and substrate solution was titrated with aliquots of the solvent (pH-adjusted value) under identical experimental conditions.

Titration conditions:

Solvent:	MeOH / H_2O 9:1 (vol/vol), pH 6.5
Starting volume:	2.0 mL
Concentration [crown ether guanidine]:	0.05 mM
Concentration [amino acid or peptide]:	10.0 mM

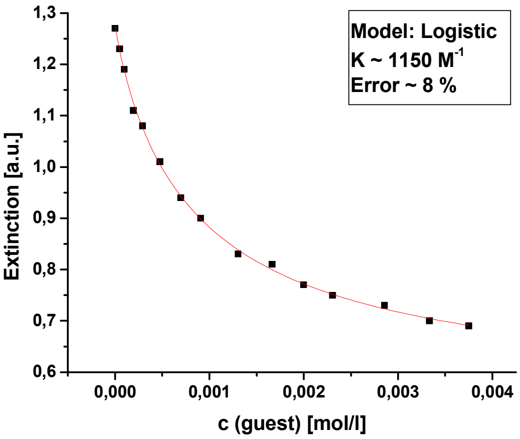


Figure S-123: UV titration of compound **39** with β-alanine

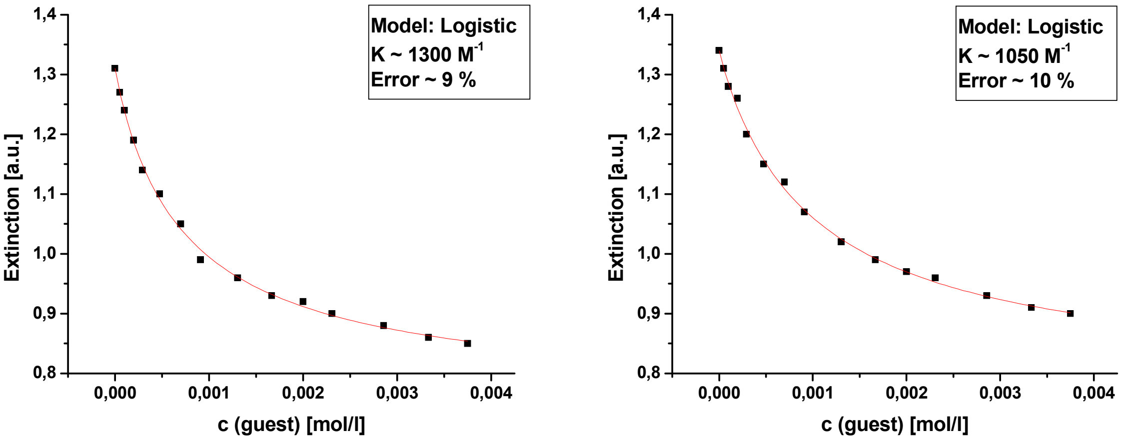


Figure S-124: UV titration of compound **32** with GABA (left) and AHX (right)

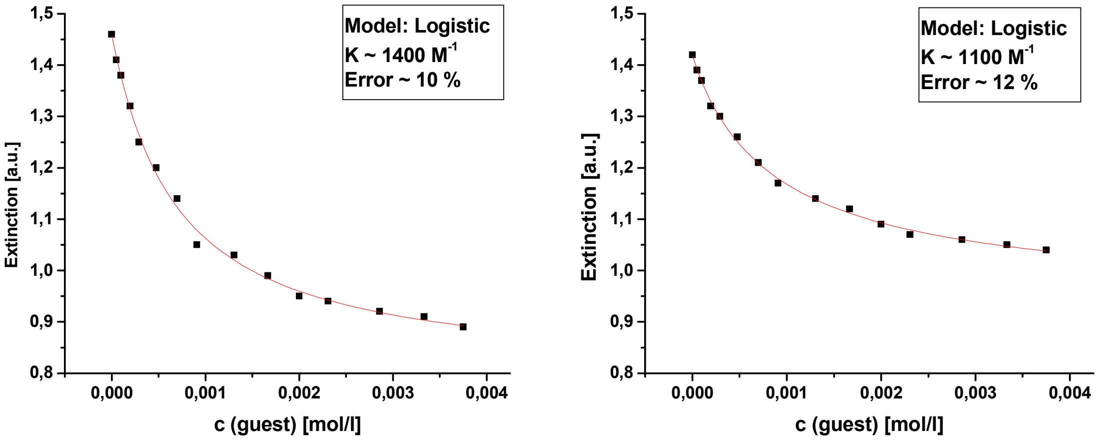


Figure S-125: UV titration of compound **32** with H-E-G-G-G-amide (left) and H-G-E-G-G-amide (right)

6. Energy minimization calculation pictures with Spartan´06 V.112

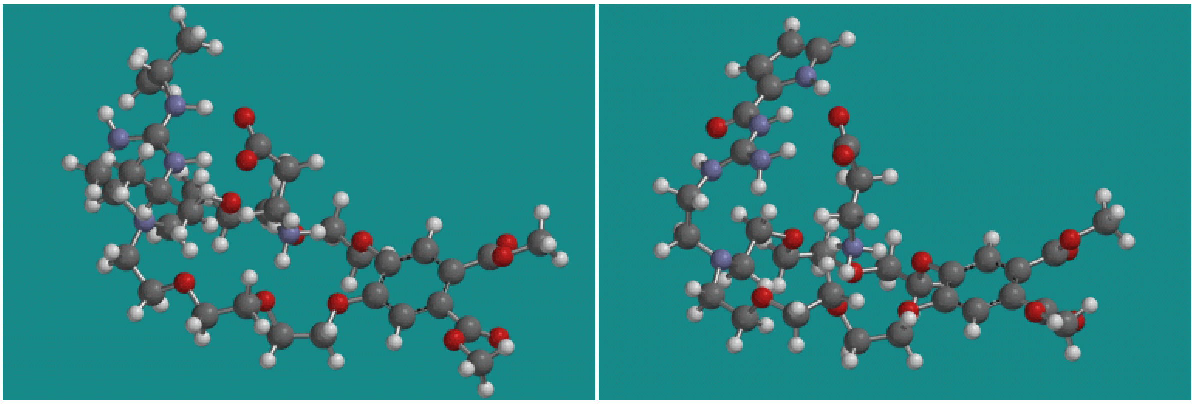


Figure S-126: Receptor **11a** (left) and **39** (right) binding to β-alanine

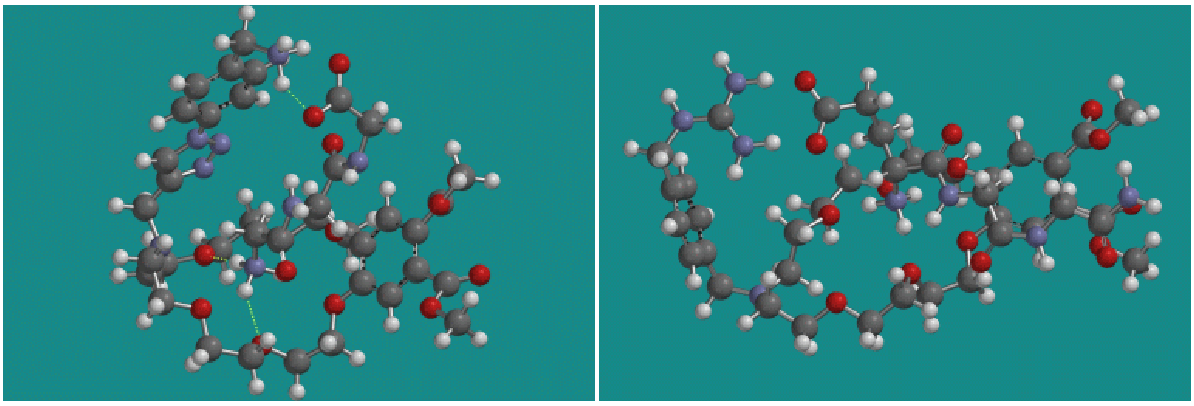


Figure S-127: Receptor **21** (left) binding to glycyl glycyl glycine and **11d** (right) to the peptide E-G-G-G

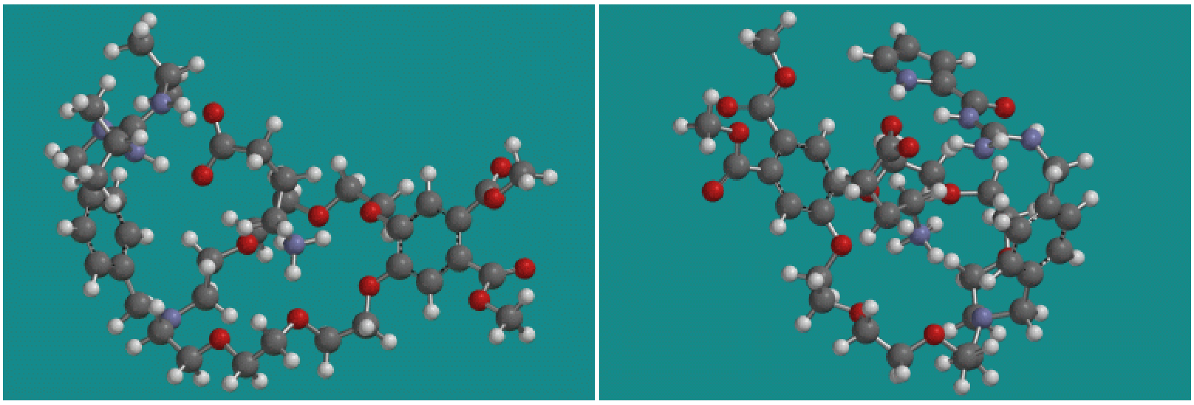


Figure S-128: Receptor **11d** (left) and **32** (right) binding to GABA

7. References

[1] H. Vollmann, H. Becker, M. Corell, H. Streeck, *Liebigs Ann. Chem.* **1937**, *531*, 108.

[2] This material is commercially available. It is easily prepared by Boc protection at room temperature with 1.2 equivalents Boc anhydride in THF using sodium hydroxide as base. Colum chromatography with ethyl acetate / petrolether 4:1 gives the product in high purity and nearly quantitative yield.

[3] M. Carmignani, A.R. Volpe, B.Botta, R.Espinal, S.C. de Bonnevaux, C.de Luca, M. Botta, F. Corelli, A. Tafi, R. Sacco, G. Delle Monache, *J. Med. Chem.* **2001**, *44*, 2950–2958.

[4] R.M. Williams, C. Yuan, V.J. Lee, S. Chamberland, *J. Antibiot.* **1998**, *51(2)*, 189-201.

[5] S. Weiss, M. Keller, G. Bernhard, A. Buschauer, B. König, *Bioorg. Med. Chem.* ***2008,*** *16,* 9858–9866.

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[10] Either 20% TFA in dichloromethane or HCl in dry diethylether can be used without changes in yield and reactivity of the resulting amine salt.

[11] It became clearly apparent, that derivatives of the material with hexyl spacer chain give the lowest yields. Its quite low reactivity can be explained by potential self aggregation of the starting material. A higher amount of side products is formed in its reactions, making also the purification more complicated. In progression to the first fact this results in an even stronger decreased yield.

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[14] This acid is extremely strong and, therefore, completely dissociated under the present concentration conditions.

[15] The guanidine moiety is separated by at least two methylene groups from the aza nitrogen, so avoiding any conjugation. It´s direct influence is assumed to be rather small.

[16] P. MacCarthy *Anal. Chem.* **1978**, *50*, 2165.; C. Schmuck, P. Wich, *Angew. Chem. Int. Ed.* **2006**, *45*, 4277-4281.

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IV. Crown Ether Guanidinium Ion Synthetic Receptor for a Hemoregulatory Tetrapeptide[i]

SUPPORTING INFORMATION

Page

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[i] A. Späth, B. König, *Tetrahedron* **2010**, accepted.

1. Syntheses

1.1. General methods

Melting Points were determined on Büchi SMP or a Lambda Photometrics OptiMelt MPA 100 and are uncorrected. IR Spectra were recorded with a Bio-Rad FT-IR Excalibur FTS 3000. UV spectra were recorded on a Cary 50 BIO spectrometer with temperature control at 25 °C. Electro spray mass spectra were performed on a Finnigan MAT TSQ 7000 ESI-spectrometer. Other Mass Spectra were recorded on Varian CH-5 (EI), Finnigan MAT 95 (CI; FAB and FD); Xenon serves as the ionization gas for FAB.

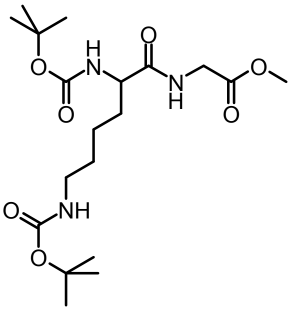
NMR spectra were recorded on Bruker Avance 600 (^{1}H: 600.1 MHz, ^{13}C: 150.1 MHz, T = 300 K), Bruker Avance 400 (^{1}H: 400.1 MHz, ^{13}C: 100.6 MHz, T = 300 K) or Bruker Avance 300 (^{1}H: 300.1 MHz, ^{13}C: 75.5 MHz, T = 300 K) relative to external standards. NMR spectra were recorded in $CDCl_3$ at 300 MHz (^{1}H) or 75 MHz (^{13}C) unless stated otherwise. Characterization of the signals: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, bm = broad multiplet, bs = broad singlet, dd = double doublet, dt = double triplet, ddd = double double doublet. Integration is determined as the relative number of atoms, the coupling constants are given in Hertz [Hz]. The multiplicity of the carbon atoms is given as (+) = CH_3 or CH, (-) = CH_2 and (C_{quat}) for quaternary carbon atoms. Structural assignments are based on DEPT, COSY and HSQC experiments where applicable. Error of reported values: chemical shift: 0.01 ppm for ^{1}H-NMR, 0.1 ppm for ^{13}C-NMR and 0.1 Hz for coupling constants. The solvent used is reported for each spectrum.

Analytical TLC plates (silica gel 60 F_{254}) and silica gel 60 (70-230 or 230-400 mesh) were used for chromatographic separations. Visualization of the spots was by UV light and/or staining with phosphomolybdate or ninhydrin, both in ethanol. DMF, CH_3CN, $CHCl_3$, THF, and Et_2O were dried by standard procedures and stored over molecular sieves. PE means petrol ether with a boiling range of 70 - 90 °C, EA means ethyl acetate. All other solvents and chemicals were of reagent grade and used with out further purification.

All test substances were of pro analysi grade, checked by NMR or HPLC and used as purchased without further purification. The Solvents for the fluorescence measurements were from special spectroscopic purity purchased from Acros or Baker or Uvasol grade from Merck. Millipore water (18 MΩ, Milli Q_{Plus}) was used; the HEPES buffer was from according purity, suitable for biochemical optical screenings. As far as not stated differently tetraethylammoniumhydroxide or 0.1 molar hydrochloric acid, both of analytical grade, were used to adjust the pH. Ac-Ser-Asp-Lys-Pro (**1**) was purchased from GENSCRIPT in a purity > 95 % and used as is.

1.2. Syntheses of test peptides

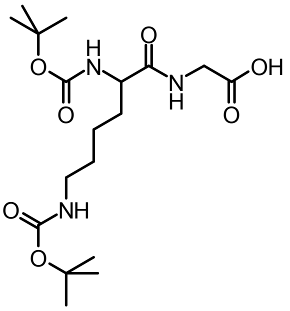
Boc-Lys(Boc)-Gly-OMe



Boc-Lys(Boc)-OH dicyclohexylamine salt (2.64 g, 5.00 mmol) was suspended with glycine methylester hydrochloride (630 mg, 5.00 mmol) in a mixture of dichloromethane (20 mL) and DMF (5 mL) at 0 °C. HOBt (750 mg, 5.50 mmol) and DCC (1.14 g, 5.50 mmol) were added and the mixture was stirred over night at room temperature.
The precipitated solid was filtered off and washed with dichloromethane. The organic phase was washed with 20 mL of water, dried over $MgSO_4$ and evaporated in vacuo. The residue was purified by column chromatography (ethyl acetate, $R_f = 0.4$) to obtain a colourless solid (2.23 g, 4.79 mmol, 96 %).

^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.42 (s, 9 H), 1.43 (s, 9 H), 1.38 – 1.49 (m, 4 H), 1.51 – 1.68 (m, 1 H), 1.72 – 1.81 (m, 1 H), 3.07 (m, 2 H), 3.67 (s, 3 H), 3.99 (dd, 2 H, 2.8, 5.4 Hz), 4.08 – 4.12 (m, 1 H), 4.89 (bs, 1 H), 5.48 (bs, 1 H), 7.23 (bs, 1 H); - **^{13}C-NMR** (75 MHz, $CDCl_3$): δ [ppm] = 22.5 (-, 1 C), 28.3 (+, 3 C), 28.5 (+, 3 C), 29.5 (-, 1 C), 32.0 (-, 1 C), 39.9 (-, 1 C), 41.1 (-, 1 C), 52.2 (+, 1 C), 54.1 (+, 1 C), 78.9 (C_{quat}, 1 C), 79.8 (C_{quat}, 1 C), 155.8 (C_{quat}, 1 C), 156.2 (C_{quat}, 1 C), 170.2 (C_{quat}, 1 C), 172.8 (C_{quat}, 1 C); - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 418.3 (100, MH^+), 362.2 (15, MH^+ - C_4H_8), 318.2 (2, MH^+ - CO_2 - C_4H_8); - **MF:** $C_{19}H_{35}N_3O_7$ – **FW:** 417.51 g/mol;

Boc-Lys(Boc)-Gly-OH

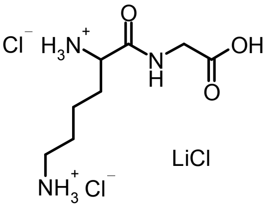


To a solution of Boc-Lys(Boc)-Gly-OMe (1.40 g, 3.36 mmol) in methanol (25 mL) was added an aqueous solution of LiOH (2 M, 3.36 mL, 6.71 mmol). The mixture was stirred over night at room temperature and the solvent was evaporated.

The remaining substance was taken up in chloroform (120 mL) and washed with aqueous $KHSO_4$ solution (5 %, 60 mL). The aqueous phase was extracted two times with chloroform (50 mL). The combined organic phases were dried over $MgSO_4$ and the solvent was evaporated to yield a colourless solid (1.30 g, 3.22 mmol, 96 %).

^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.41 (s, 9 H), 1.43 (s, 9 H), 1.34 – 1.50 (m, 4 H), 1.58 – 1.67 (m, 1 H), 1.73 – 1.88 (m, 1 H), 3.08 (m, 2 H), 3.80 (m, 1 H), 4.01 (m, 1 H), 4.25 (m, 1 H), 4.81 (bs, 1 H), 5.51 (m, 1 H), 6.72 (m, 1 H); - **^{13}C-NMR** (75 MHz, $CDCl_3$): δ [ppm] = 22.7 (-, 1 C), 28.3 (+, 3 C), 28.4 (+, 3 C), 29.5 (-, 1 C), 32.1 (-, 1 C), 33.5 (-, 1 C), 41.2 (-, 1 C), 54.2 (+, 1 C), 80.0 (C_{quat}, 2 C), 156.1 (C_{quat}, 1 C),156.5 (C_{quat}, 1 C), 173.1 (C_{quat}, 1 C); - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 404.3 (100, MH^+), 348.3 (12, MH^+ - C_4H_8); - **HRMS** (PI-LSIMS FAB, MeOH/glycerine): calc. for $C_{18}H_{34}N_2O_7$*H^+: 404.2397, found: 404.2394; - **MF:** $C_{18}H_{33}N_3O_7$ – **FW:** 403.48 g/mol;

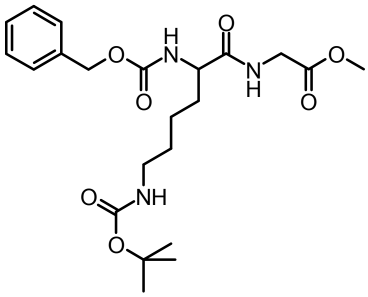
Lys-Gly-OH hydrochloride



A solution of Boc-Lys(Boc)-Gly-OH lithium salt (0.41 g, 1.00 mmol) in methanol (2 mL) was stirred with aqueous hydrochloric acid (1.0 M, 3.0 mL, 3.00 mmol) until TLC indicated complete deprotection (3 h). The methanol was evaporated at reduced pressure and the residue was lyophilized to yield a colourless solid (0.29 g, 0.99 mmol, 99 %).

^{1}H-NMR (300 MHz, DMSO): δ [ppm] = 1.36 – 1.48 (m, 4 H), 1.52 – 1.63 (m, 1 H), 1.65 – 1.77 (m, 1 H), 2.72 (m, 1 H), 2.89 (m, 2 H), 3.71 – 3.98 (m, 4 H), 6.61 (bs, 1 H), 8.03 (bs, 3 H), 8.21 (bs, 1 H), 9.03 (bs, 1 H); - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 204.3 (100, MH^+), 148.3 (19, MH^+ - C_4H_8); - **MF:** $C_8H_{19}N_3O_3Cl_2$ – **FW:** 276.161 g/mol;

Cbz-Lys(Boc)-Gly-OMe

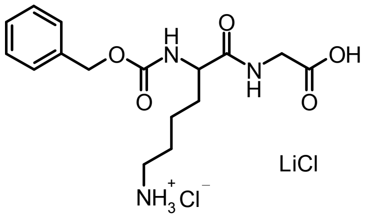


Cbz-Lys(Boc)-OH (700 mg, 1.84 mmol) and glycine methylester hydrochloride (230 mg, 1.84 mmol) were suspended in dichloromethane (10 mL) and cooled to 0 °C. After addition of DIPEA (0.40 mL, 300 mg, 2.21 mmol) a clear solution was obtained. HOBt (280 mg, 2.02 mmol) and DCC (420 mg, 2.02 mmol) were successively added. The mixture was stirred over night at room temperature, filtered over celite and the filter cake was washed with dichloromethane.

The filtrate was washed with 30 mL of a 1/1 mixture of brine and water. The aqueous phase was re-extracted once with dichloromethane. The combined organic phases were dried over $MgSO_4$ and the solvent was evaporated. The residue is purified by column chromatography (ethyl acetate, $R_f = 0.5$) to give 828 mg colourless solid (1.83 mmol, 98 %).

^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.41 (s, 9 H), 1.32 – 1.48 (m, 4 H), 1.57 – 1.67 (m, 1 H), 1.84 – 1.95 (m, 1 H), 3.09 (m, 2 H), 3.74 (s, 3 H), 4.01 (m, 2 H), 4.20 (m, 1 H), 4.68 (bs, 1 H), 5.10 (s, 2 H), 5.55 (m, 1 H), 6.72 (bs, 1 H), 7.33 (m, 5 H); - **^{13}C-NMR** (75 MHz, $CDCl_3$): δ [ppm] = 22.3 (-, 1 C), 28.4 (+, 3 C), 29.6 (-, 1 C), 31.9 (-, 1 C), 33.9 (-, 1 C), 49.2 (+, 1 C), 52.4 (+, 1 C), 67.1 (C_{quat}, 1 C), 79.2 (C_{quat}, 1 C), 128.1 (+, 2 C), 128.2 (+, 1 C), 128.5 (+, 2 C), 136.2 (C_{quat}, 1 C), 156.3 (C_{quat}, 1 C), 170.1 (C_{quat}, 1 C), 172.1 (C_{quat}, 1 C); - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 469.2 (100, $M+NH_4^+$), 452.1 (64, MH^+), 413.1 (76, $M+NH_4^+$ - C_4H_8), 369.2 (36, MH^+ - C_4H_8); - **HRMS** (PI-LSIMS FAB, MeOH/glycerine): calc. for $C_{22}H_{33}N_3O_7{}^{*+}$: 451.2319, found: 451.2315; - **MF:** $C_{22}H_{32}N_2O_7$ – **FW:** 451.52 g/mol;

Cbz-Lys-Gly-OH hydrochloride



To a solution of Cbz-Lys(Boc)-Gly-OMe (230 mg, 0.5 mmol) in methanol (3 mL) was added an aqueous solution of LiOH (2 M, 0.25 mL, 0.5 mmol). The mixture was stirred over night at room temperature and the solvents were evaporated. The residue was taken up in dichloromethane (5 mL), a saturated solution of HCl in diethylether (0.5 mL) was added and the mixture was stirred for 2 h. The solvents were evaporated and the residue was dried to give 190 mg of a colourless solid containing 1 eq. of LiCl (0.487 mmol, 97 %).

^{1}H-NMR (300 MHz, CD_3OD): δ [ppm] = 1.30 – 1.45 (m, 4 H), 1.55 – 1.64 (m, 1 H), 1.69 – 1.82 (m, 1 H), 3.06 (m, 2 H), 3.98 (m, 2 H), 4.23 (m, 1 H), 4.68 (bs, 1 H), 5.13 (s, 2 H), 5.48 (m, 1 H), 7.27 – 7.44 (m, 5 H); - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 338.2 (100, MH^+); - **MF:** $C_{16}H_{24}N_3O_7Cl$ – **FW:** 373.84 g/mol;

1.3. Solid phase synthesis of the test peptides

Rink amide resin MBHA (novabiochem), piperidine (iris biotech), ethyl-diisopropyl-amine (DIPEA) (Fluka), dimethylformamide (DMF) (iris biotech), Fmoc amino acids (novabiochem), trifluoroacetic acid (TFA) (Fluka), triisopropylsilane (TIS) (novabiochem), 1-Ethyl-3-(3-dimethyllaminopropyl)carbodiimide (EDC) (Sigma Aldrich), O-Benzotriazole-*N,N,N',N'*-tetramethyl-uronium-hexafluoro-phosphate (HBTU) (novabiochem), 1-hydroxybenzotriazole (HOBt) (Sigma Aldrich), were bought and used without further purification.

All peptides were synthesized on Rink Amide MBHA resin using Fmoc protecting group strategy. Coupling was done by HBTU / HOBt / DIPEA. HOBt and HBTU were used as a 0.45 M solution, DIPEA as a 1.2 M solution in DMF. The Fmoc protected amino acids were dissolved in NMP as 0.5 M solutions. Every peptide was synthesized on 100 mg of resin in standard 5 mL syringe. The lot of the resin used had a loading of 0.72 mmol/g (manufacturer's claims). The resin was allowed to preswell in DMF for 30 min. Each coupling was done twice using 1.0 mL of each solution per step and shaking for 2 h each.

Fmoc deprotection was done by shaking the resin with 40 % piperidine in DMF for 5 minutes, subsequent washing and addition of 20 % piperidine in DMF followed by shaking for 10 minutes. The progress of the synthesis was monitored by the Kaiser test.[1]

Capping was done for 3 h using 0.5 M of Ac_2O in DMF, containing 1.2 M DIPEA as base. Of the solution 2 mL was used. After completion of the syntheses, the resin was washed with DMF and DCM (5 x 5 mL each).

From the resin was cleaved by shaking for 3 h after addition of 3.0 mL of TFA / TIS / H_2O (90:5:5) (vol/vol). The resin was removed by filtration and washed twice with TFA. Combined filtrates were concentrated under reduced pressure to about 1.0 mL without heating. It was then transferred to a Falcon tube and precipitated with cold Et_2O. The precipitate was centrifuged at –5 °C for 20 minutes. The solution was then carefully decanted off and the precipitate resuspended in cold Et_2O before being centrifuged again. Finally, the Et_2O was decanted off again and the peptide dried under vacuum. The peptides were analysed by ES-MS and ^{1}H-NMR.

Analytical control of Ac-Glu-Lys-NH_2: **MF:** $C_{13}H_{25}N_4O_5Cl$ – **FW:** 351.81 g/mol;

^{1}H-NMR (300 MHz, DMSO-d6): δ [ppm] = 1.31 (m, 2 H), 1.51 (m, 1+2 H), 1.68 (m, 2 H), 1.86 (s, 3 H), 1.88 (m, 1 H), 2.22 (t, J = 8 Hz, 2 H), 2.72 (m, 2 H), 4.18 (m, 2 H), 7.04 (s, 1 H, NH), 7.31 (s, 1 H, NH), 7.70 (bs, 2 H, NH), 7.91 (d, J = 8 Hz, 1H, NH), 8.09 (d, J = 8 Hz, 1 H, NH); - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 316.9 (100 %, MH^+);

The isomeric glutamic acid tetrapeptide sequences H-E-G-G-G-NH_2, H-E-G-G-G-NH_2, H-E-G-G-G-NH_2 and H-E-G-G-G-NH_2 were prepared according to a similar protocol earlier. Their preparation, purification and analytics were already published.[2] They were properly stored below -5 °C. HPLC analysis showed no decomposition.

2. ^{1}H – / ^{13}C-NMR and LC-MS spectra of new compounds

For solvents and conditions of measurement, see experimental procedures. NMR spectra were recorded on Bruker Avance spectrometers 300, 400 and 600. Measuring temperature was 300 K. TMS was used as external standard.

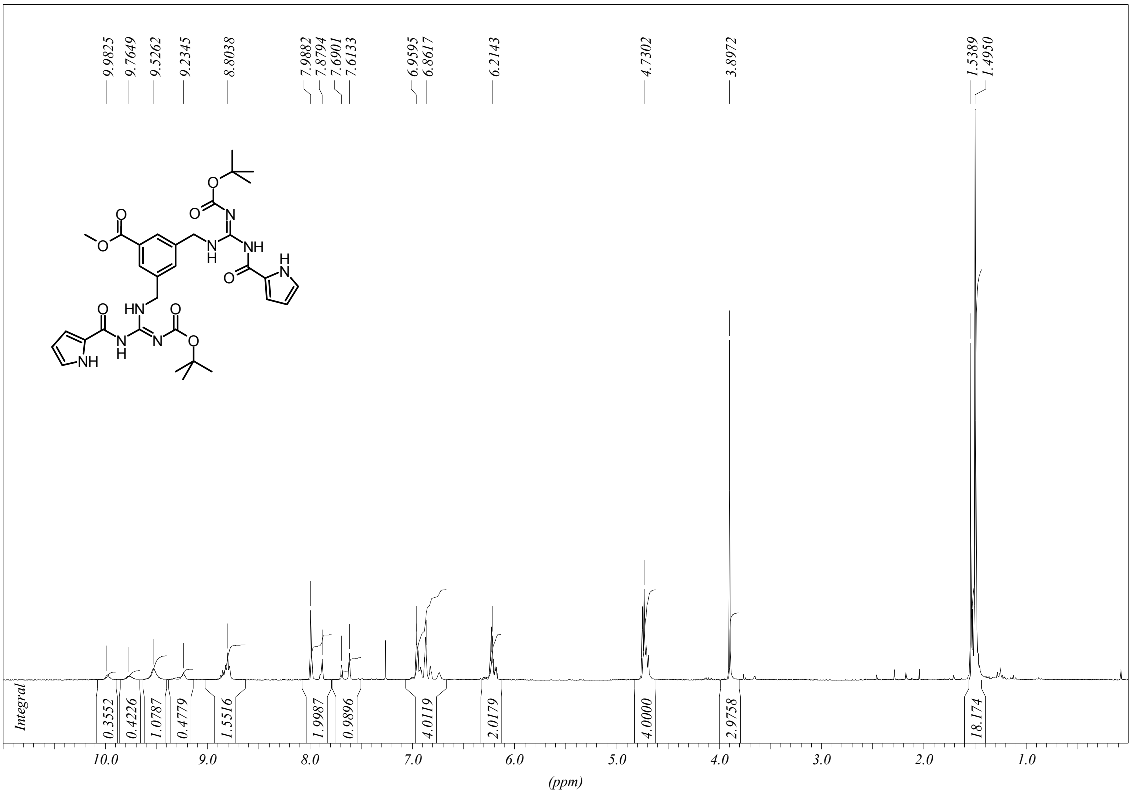


Figure S-1: ^{1}H-NMR spectrum of compound **5a**

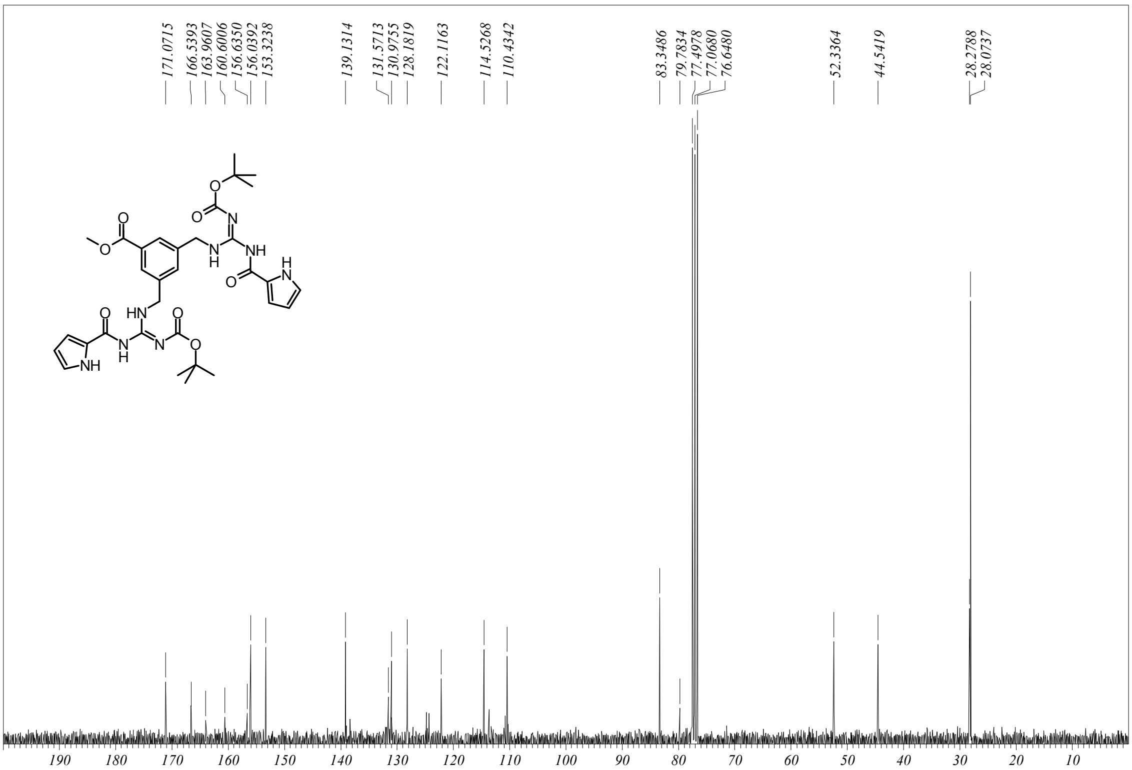


Figure S-2: ^{13}C-NMR spectrum of compound **5a**

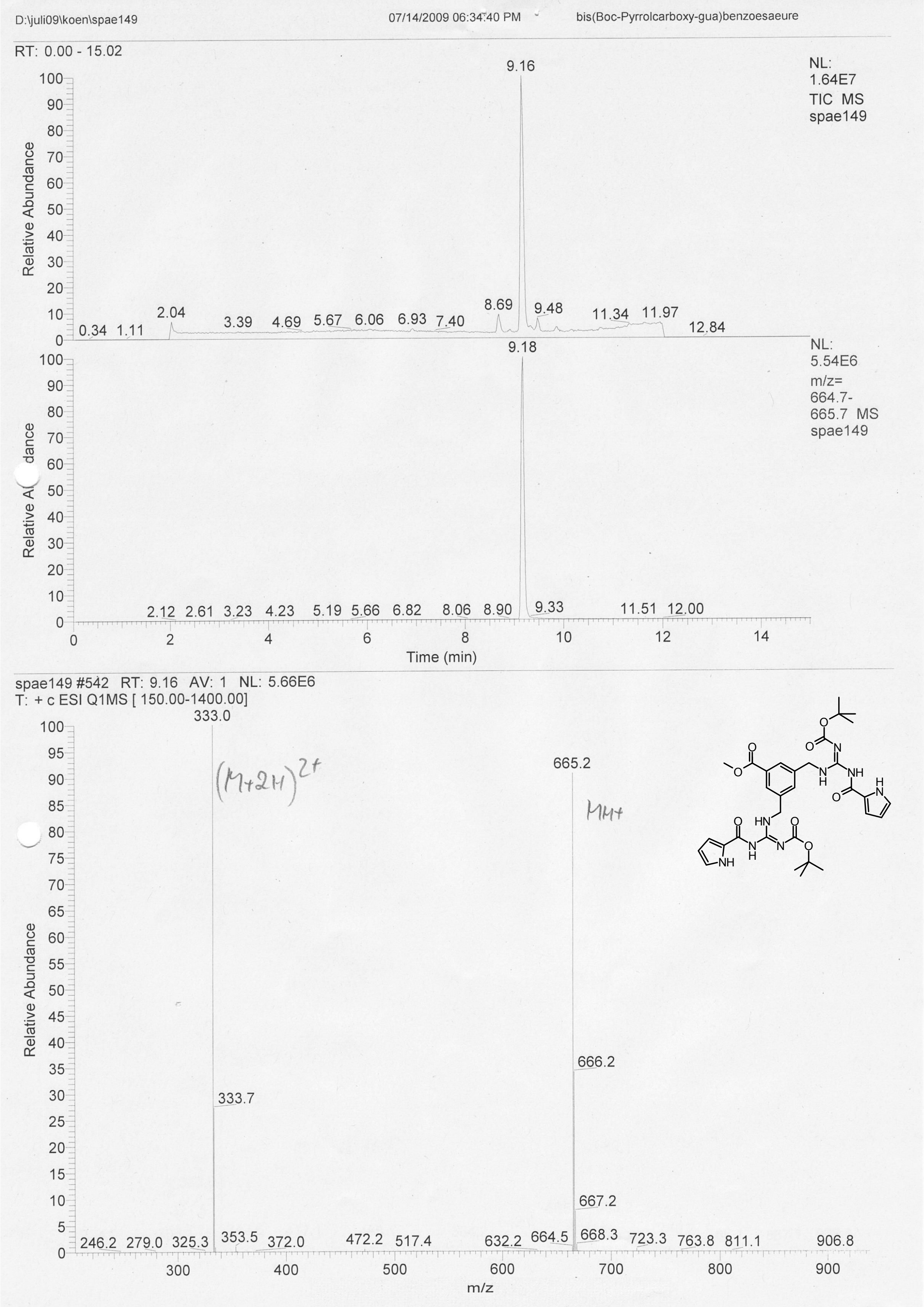


Figure S-3: LC-MS spectrum of compound **5a**

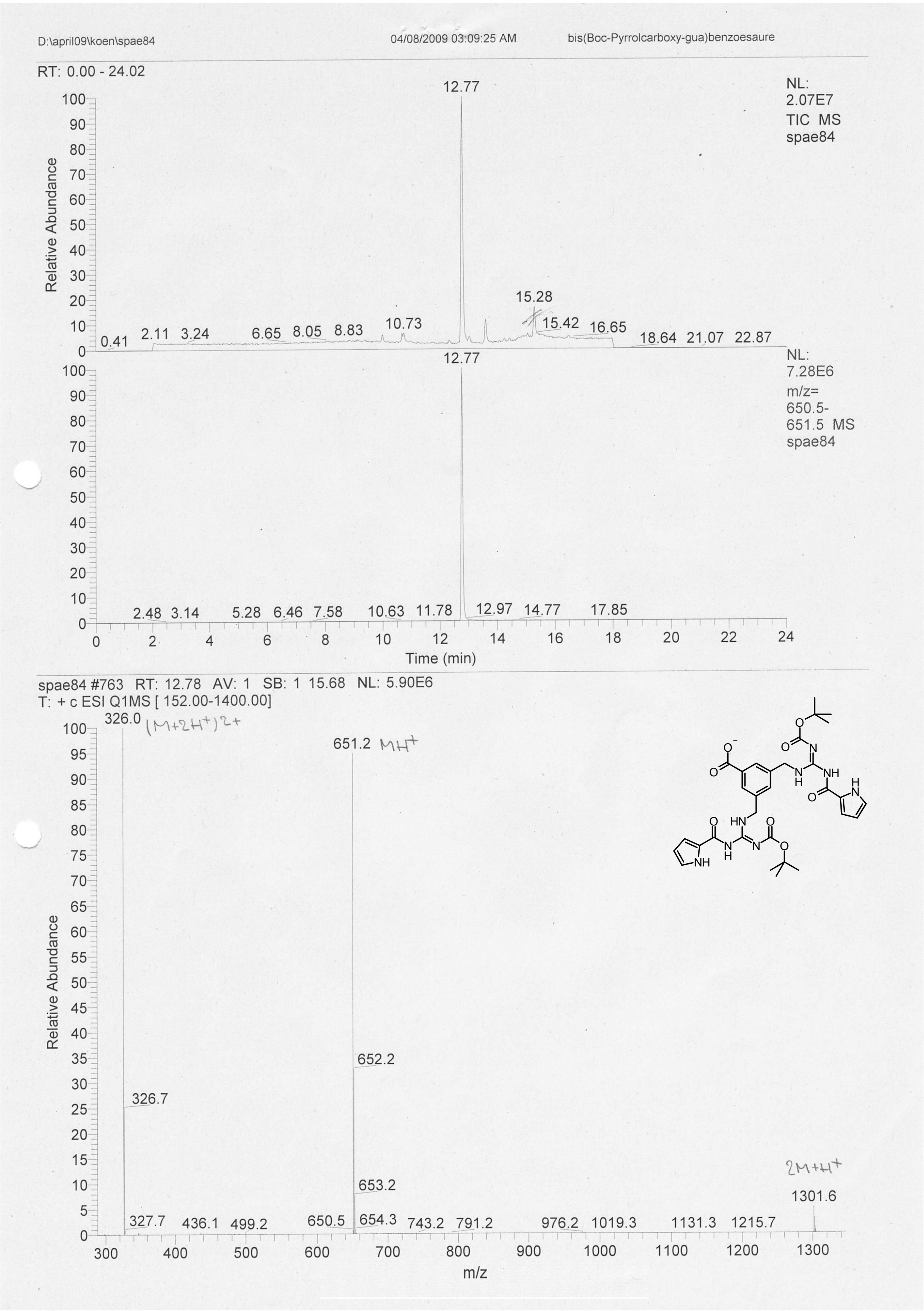


Figure S-4: LC-MS spectrum of compound **5b**

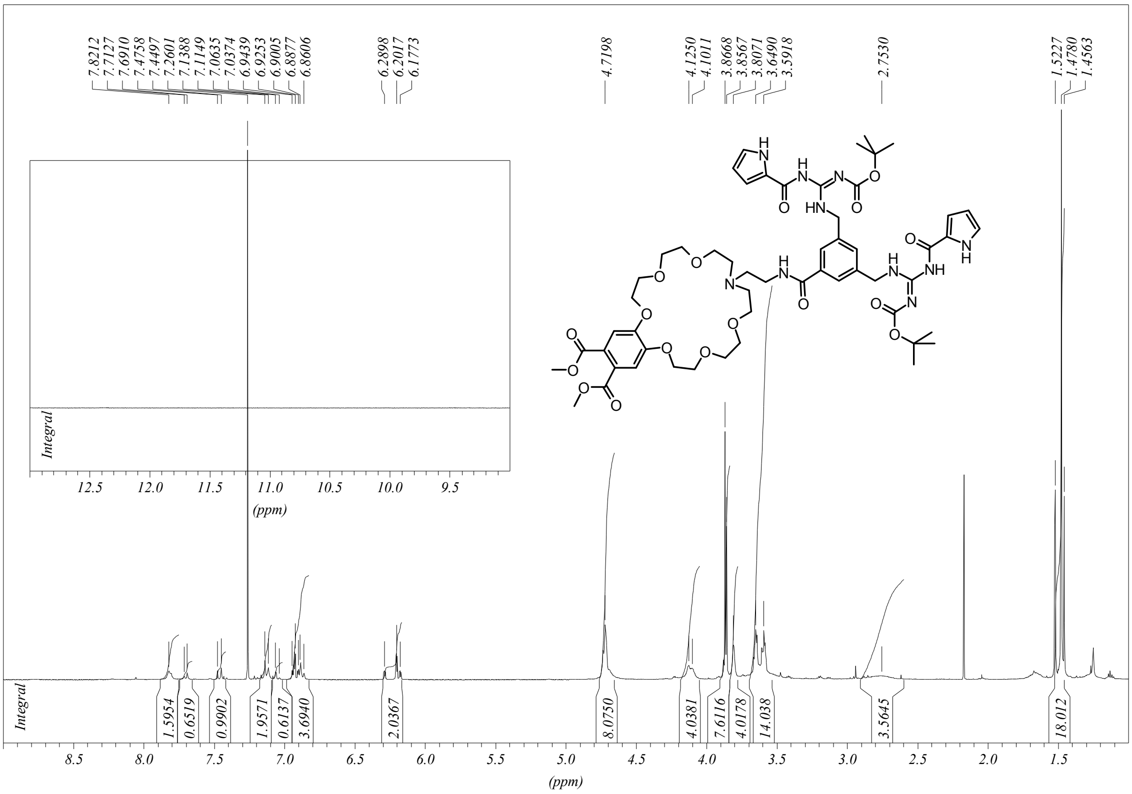


Figure S-5: ^{1}H-NMR spectrum of compound **7**

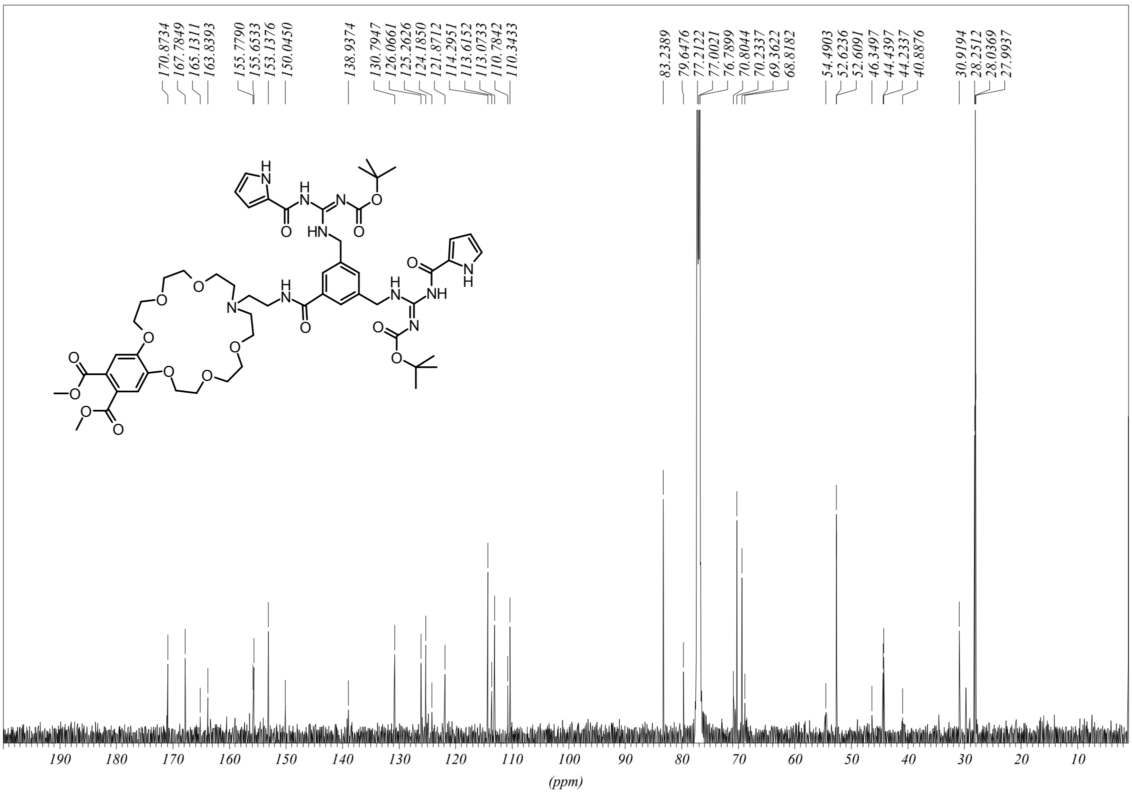


Figure S-6: ^{13}C-NMR spectrum of compound **7**

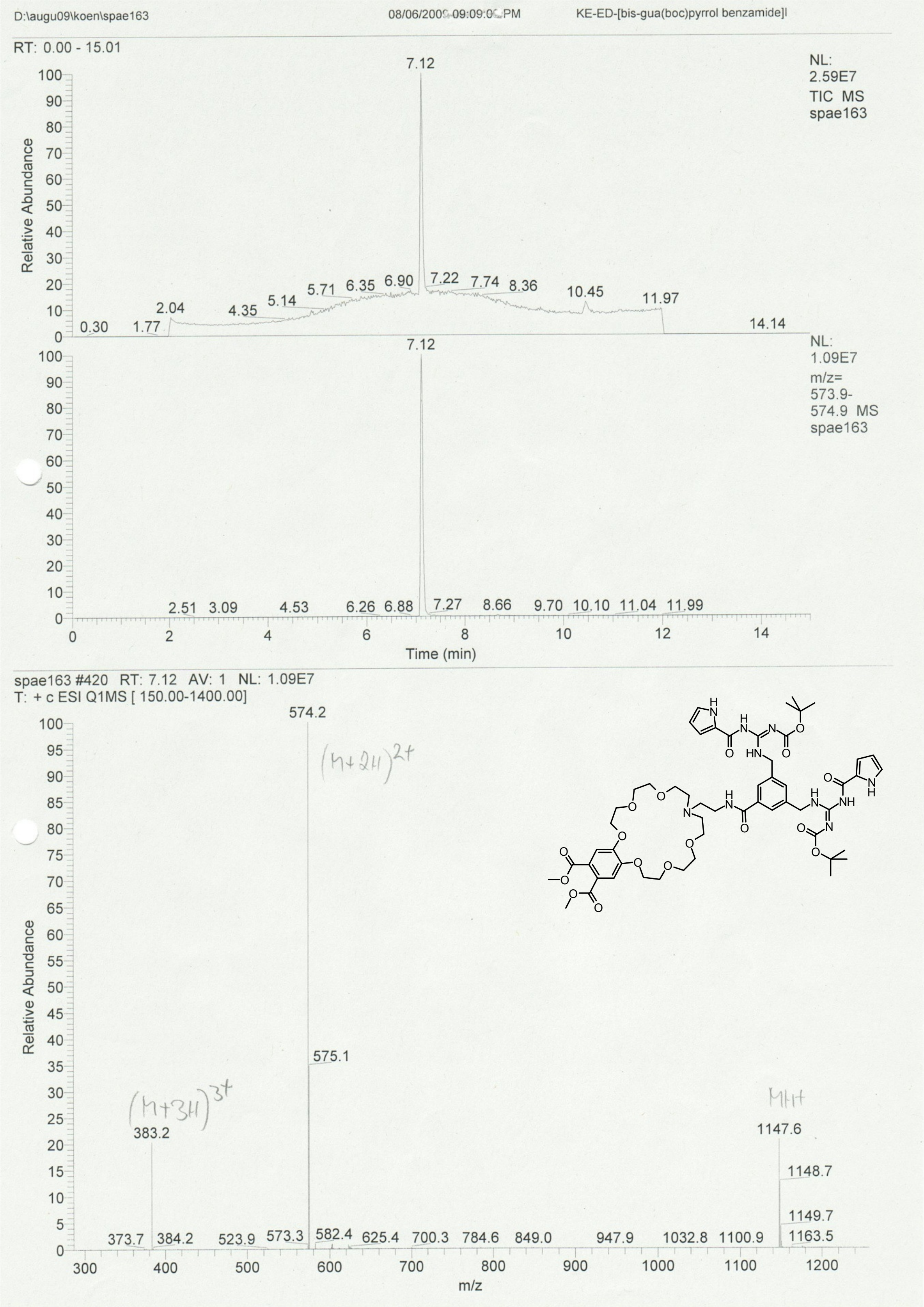


Figure S-7: LC-MS spectrum of compound **7**

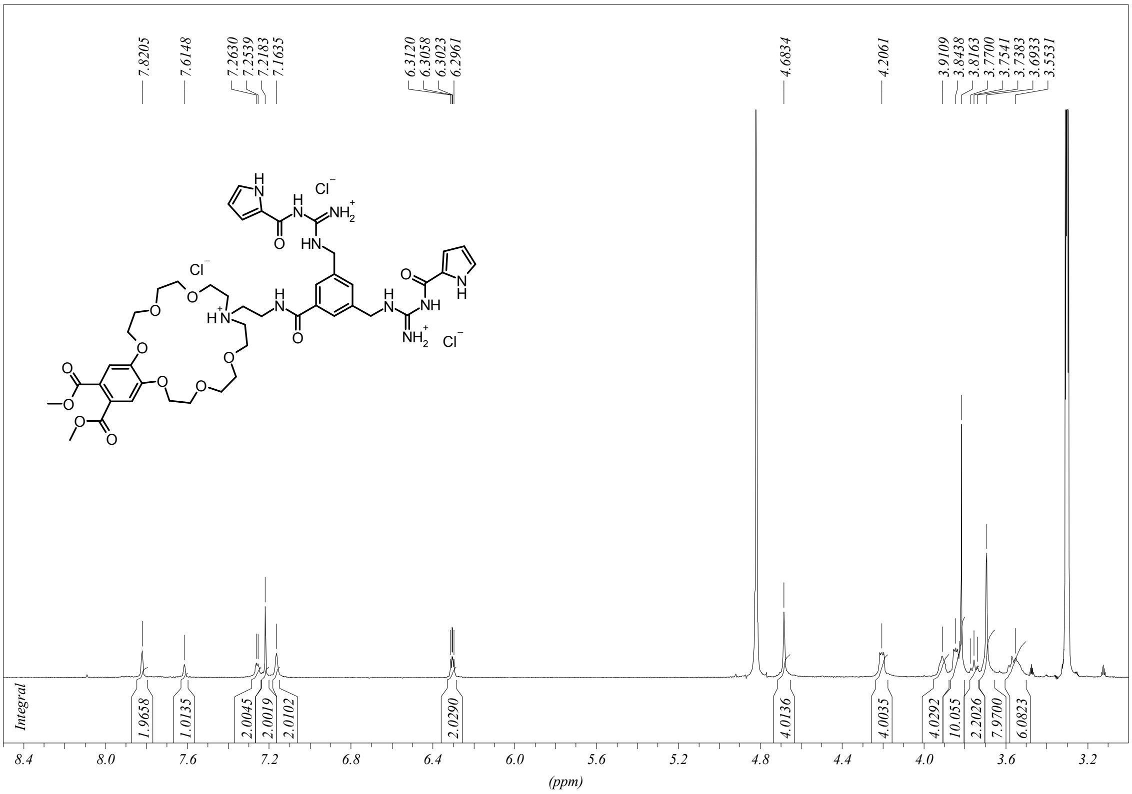


Figure S-8: ^{1}H-NMR spectrum of compound **2**

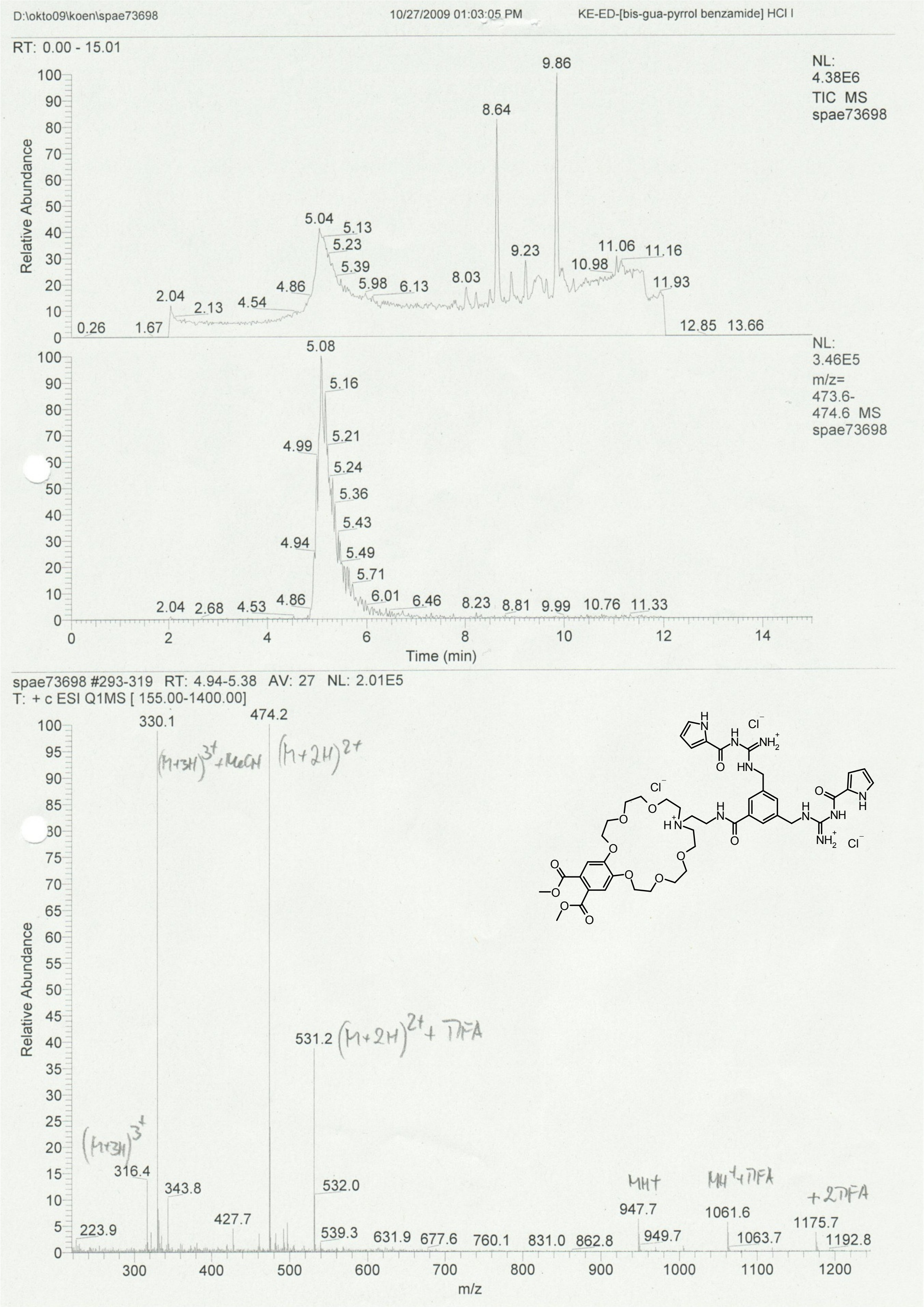


Figure S-9: LC-MS spectrum of compound **2**[3]

3. Fluorescence data and selected emission titrations

All fluorescence experiments were performed on a Varian Cary Eclipse fluorimeter. To determine the binding constants and Job´s plots fluorescence titration experiments were carried out.

Instrument Parameters

Excitation wavelength: $\lambda_{ex} = 310$ nm
Detection wavelength: $\lambda =$ 330 – 500 nm
Excitation slit: 10 nm
Emission slit: 10 nm
Temperature: T = 298 K
PMT voltage 560 volts

The thoroughly dried substance **2** was dissolved in methanol ($1*10^{-3}$ mol/L); this stock solution was kept properly closed in the fridge at -4 °C. The solutions needed for the measurements were always freshly prepared from this stock solution, diluting the necessary amount with methanol and buffer accordingly. The pH value of the solution was measured before use, and if necessary, adjusted with hydrochloric acid or tetrabutyl ammonium hydroxide.

All screenings and titrations were performed in a 1.0 mL quarz cuvette using methanol / buffer mixtures (HEPES, 10 mM, pH 6.3, no salt addition).

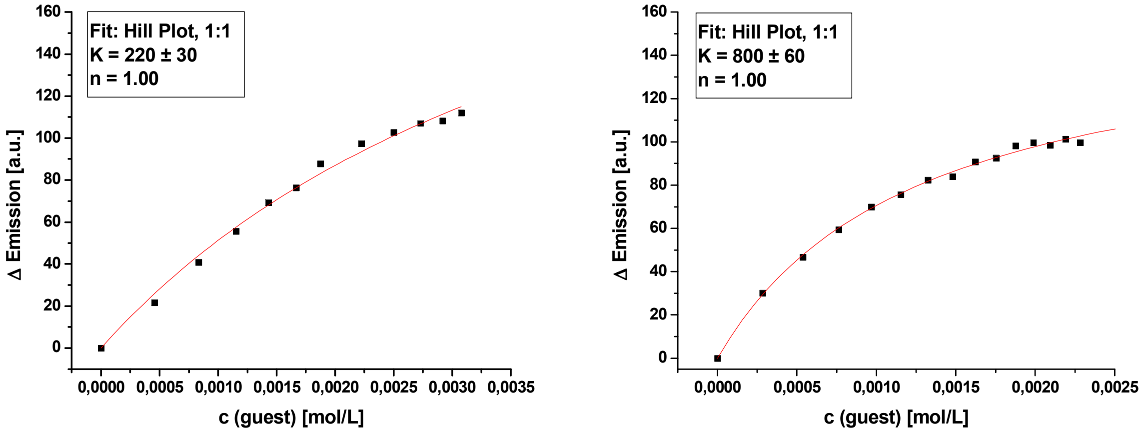


Figure S-10: Emission titration of compound **2** with Gly-Glu-Gly-Gly-NH_2 (left) and Gly-Gly-Glu-Gly-NH_2 (right) in methanol / buffer 4:1 (10 mM, pH 6.3)

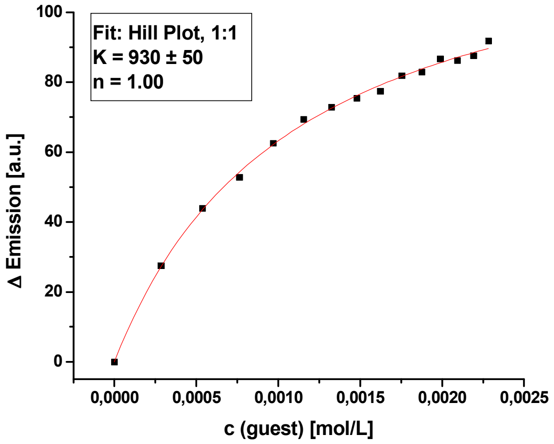


Figure S-11: Emission titration of compound **2** with Lys-Gly in methanol / buffer 4:1 (10 mM, pH 6.3)

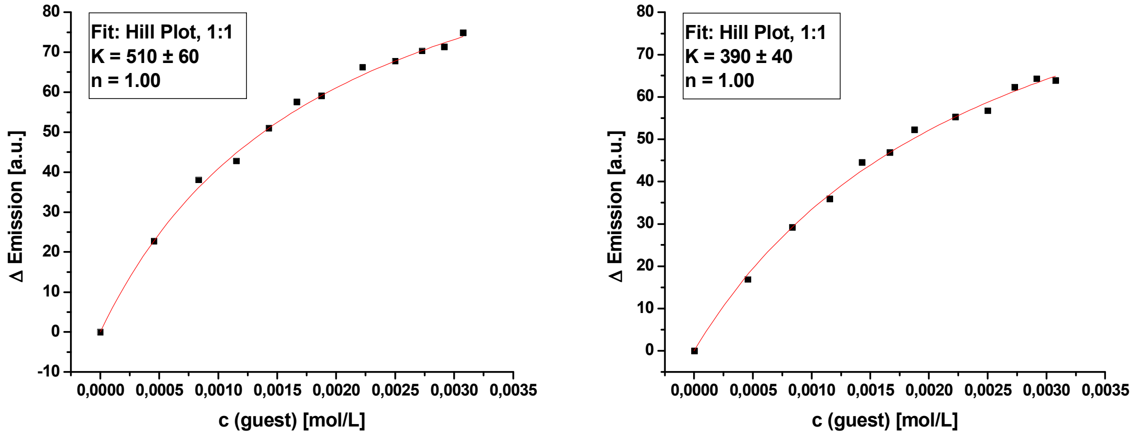


Figure S-12: Emission titration of compound **2** with Gly-Gly-Gly (left) and Gly-Gly-Gly-Gly (right) in methanol / buffer 4:1 (10 mM, pH 6.3)

4. pK_a-values and simulation of fundamental properties

4.1. pH titration of 2 and 6b

The protonation of the crown ether moiety is investigated by pH-titration with perchloric acid.[4] From the binding constants (*K*), the K_S- values can be calculated. Without free guanidine this measurement is eased, but should still give the values in good approximation to the according unprotected species.[5]

$$K = \frac{[XH^+]}{[X]*[H^+]} = \frac{1}{K_A} \qquad \Rightarrow \ \log K = pK_a$$

Equation S-1: Relationship between binding constant K and the acidity constant K_S

Compound	Emission (λ_{max})	K [M^{-1}]	pK_a	I_∞/I_0
(2)	391 nm	$6.3 * 10^5$	5.8	1.9
(6b)	389 nm	$3.7 * 10^5$	5.6	1.7

solvent: water (2 % methanol)

Table S-1: Emission properties of selected protected receptors upon titration with $HClO_4$

4.2. pH dependent emission of 2

Aqueous HEPES buffer solutions (50 mM, no salt addition, tetraethylammoniumhydroxide or HCl for equibrillation) in the range of pH 3 to pH 10 were mixed in a 1:4 ratio with a methanol solution of compound **2** ($3*10^{-5}$ M). After mixing and standing for five minutes an emission spectrum was recorded:

pH of the buffer solution	Emission [a.u.]	pH of the buffer solution	Emission [a.u.]
10	151,02	6	193,27
9,5	152,58	5,5	216,74
9	152,61	5	287,17
8,5	153,36	4,5	313,77
8	155,71	4	320,81
7,5	158,41	3,5	323,16
7	162,10	3	322,38
6,5	170,75		

Table S-2: pH dependent luminescence of compound 2

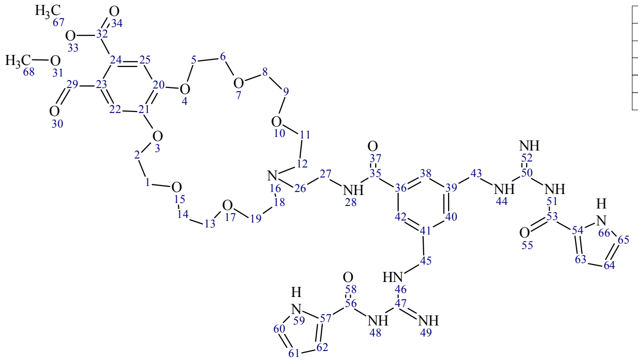
4.3. Simulation of fundamental properties

Physical properties like solubility, log P values, index of refraction and the expected p*K*$_a$ values for all representative structures were simulated by computational methods with the aid of the ACD Labs program package structure calculator *Ver.12.1.* They are in good accordance with the measurements and typical literature data[6].

pKa Results

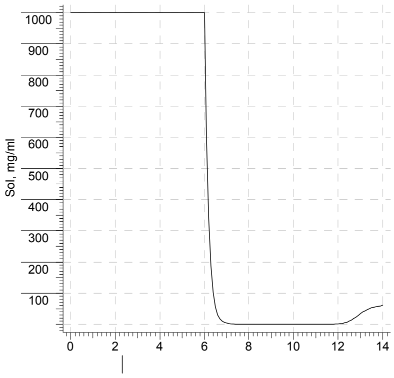
Diss. Atom	Acidic/Basic	Apparent pKa Value	Error
66	A	16.58	0.5
59	A	15.98	0.5
28	A	14.93	0.46
51	A	11.64	0.46
48	MA	11.05	0.46
16	MB	8.16	0.2
52	B	7.37	0.7
49	B	6.76	0.7
66	B	-0.33	0.7
59	B	-0.93	0.7

Diss. Atom	Acidic/Basic	Apparent pKa Value	Error
28	B	-2.22	0.7
51	B	-3.74	0.7
48	B	-4.35	0.7



Solubility Results

pH	Sol, mg/ml	Flags	%
2	1000	B	100
5.5	1000	B	100
6.5	55.75	B	100
7.4	0.67	BN	92 / 8
10	6.04e-2	AN	5 / 94



Single-valued Properties

Name	Value	Error
LogP	1.74	1
MW	947	-
PSA	301.86	-
FRB	14	-
HDonors	9	-
HAcceptors	23	-
Rule Of 5	3	-
Molar Refractivity, cm^3	241.37	0.5
Molar Volume, cm^3	685.14	7
Parachor, cm^3	1871.28	8
Index of Refraction	1.62	4.67e-2
Surface Tension, dyne/cm	55.65	7
Density, g/cm^3	1.38	0.14
Polarizability, 10e-24 cm^3	95.69	0.5
C ratio	0.66	-

Name	Value	Error
N ratio	0.15	-
NO ratio	0.34	-
Hetero ratio	0.34	-
Halogen ratio	0	-
Num Rings	5	-
Num Aromatic Rings	4	-
Num Rings 3	0	-
Num Rings 4	0	-
Num Rings 5	2	-
Num Rings 6	2	-

LogD Results

pH	LogD
2	-3.36
5.5	-3.19
6.5	-1.25
7.4	0.67
10	1.71

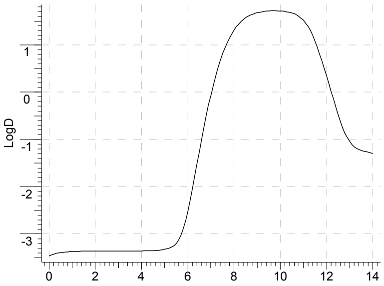


Figure S-13: Simulation of the properties of compound **2**

5. Energy minimization calculations with Spartan V.6.01

Energy minimizations in the gas phase; they may supply an illustrative picture of the receptor-guest aggregate.

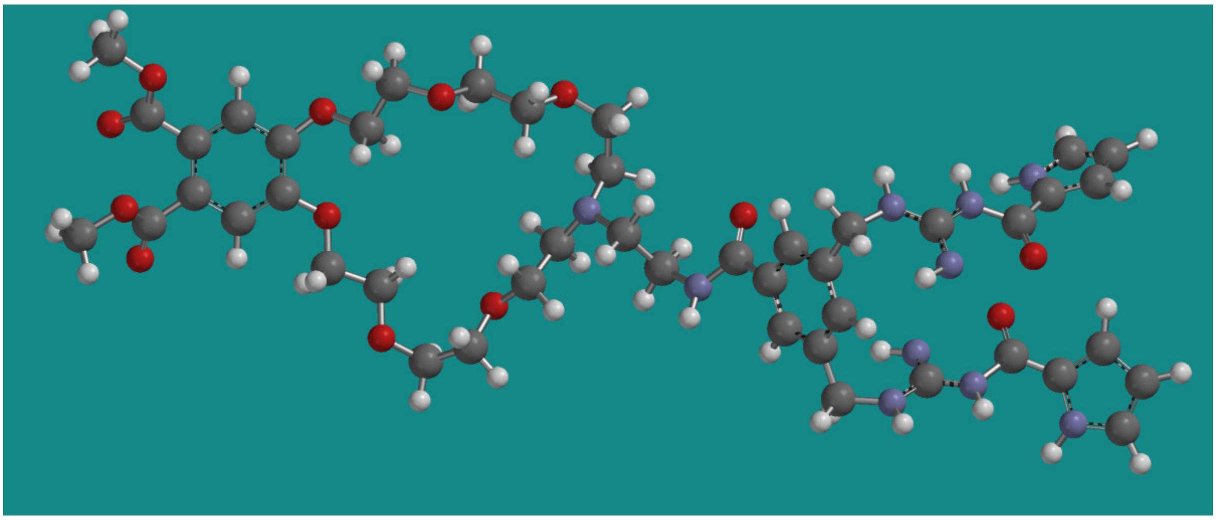


Figure S-14: Energy minimized structure of compound **2**

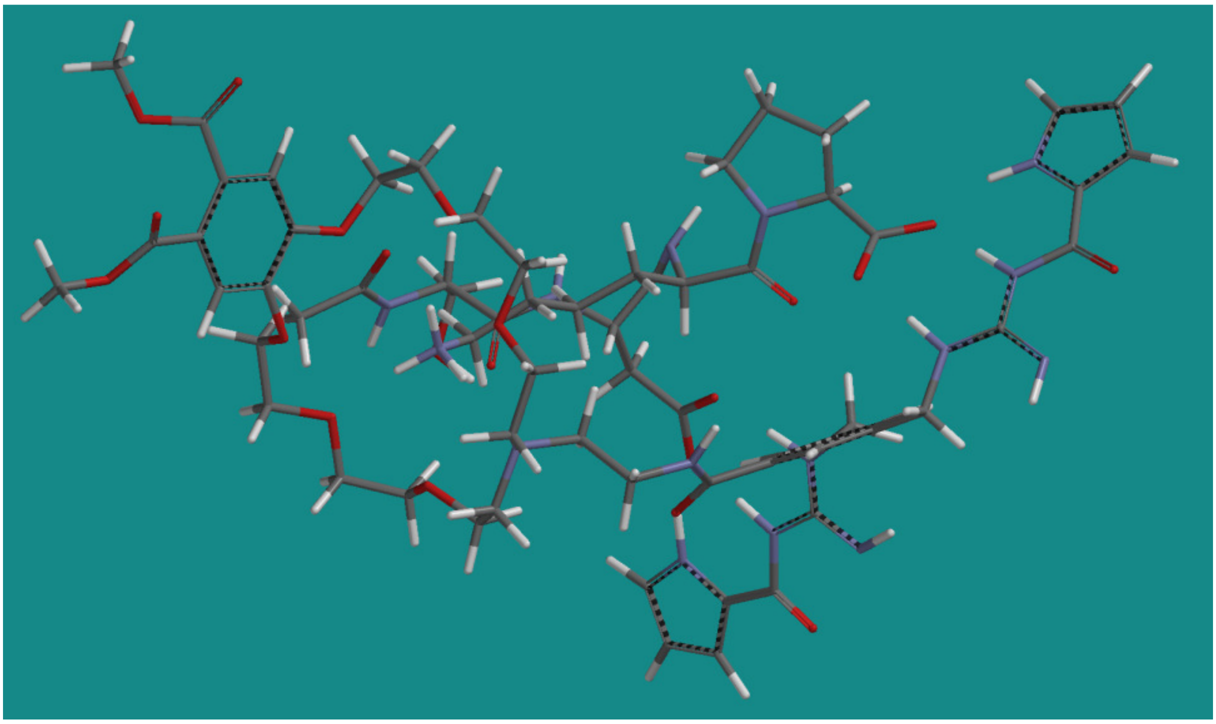


Figure S-15: Simulation of compound **1@2** (front view)

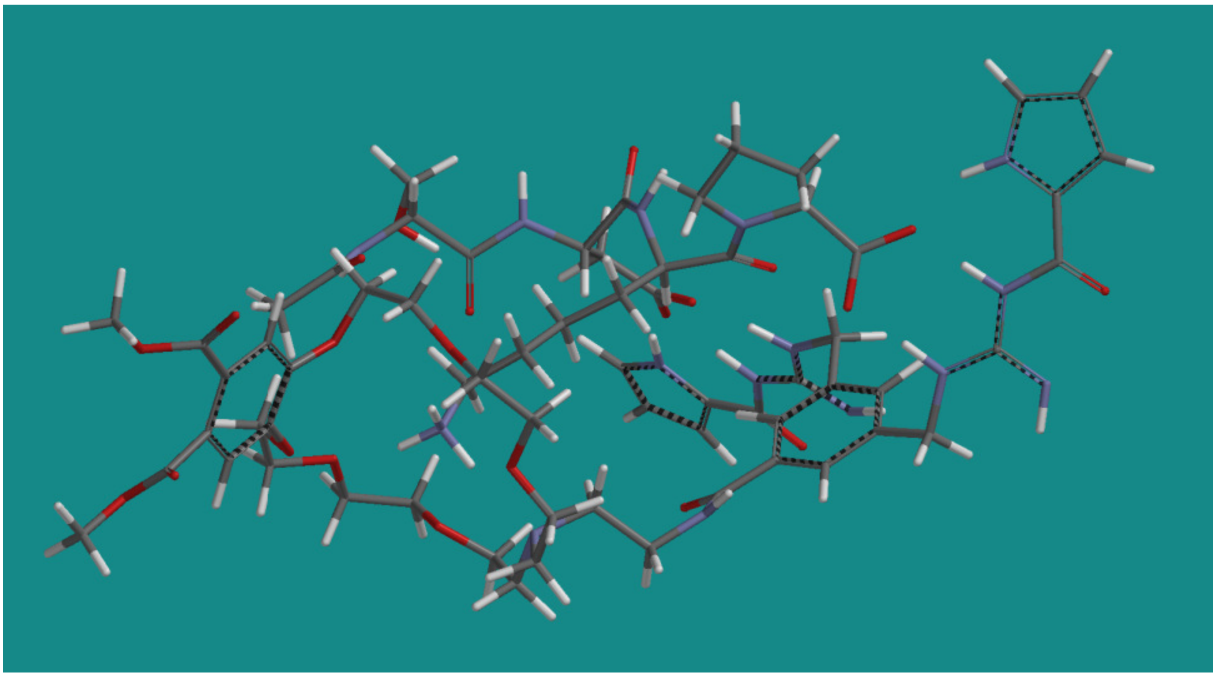


Figure S-16: Simulation of compound **1@2** (backside view)

6. References and Notes

[1] E. Kaiser, R. L. Colescott, C. D. Bossinger, P. I. Cook, *Anal. Biochem.* **1970**, *34*, 595-598.

[2] S. Stadlbauer, A. Riechers, A. Späth, B. König; *Chem. Eur. J.* **2008**, *14*, 2536–2541.

[3] The peaks at 8.64 min and 9.86 min retention time show the same mass trace as the main product peak at 5.08 min. They reflect fractions being less protonated e.g. having only one protonated guanidinium unit. (the pK_a of the guanidine is 6.5).

[4] This acid is extremely strong and, therefore, completely dissociated under the present concentration conditions.

[5] The guanidine moiety is separated by at least two methylene groups from the aza nitrogen, so avoiding any conjugation. It´s direct influence is assumed to be rather small.

[6] G.W. Gokel, Crown Ethers and Cryptands – Monographs in Supramolecular Chemistry, The Royal Society of Chemistry **1991.;** J.S. Bradshaw, R.M. Izatt, A.V. Bordunov, C.Y. Zhu, J.K. Hathaway, *Comprehensive Supramolecular Chemistry* **1996**, *Vol. 1*, 35-95; G.W. Gokel, E. Abel, *Comprehensive Supramolecular Chemistry* **1996**, *Vol. 1*, 511-535.

V. Luminescent Metal Complex - Crown Ether Hybrid Receptors bind Phosphate and Ammonium Ions simultaneously[i]

SUPPORTING INFORMATION

Page

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[i] A. Späth, C. Koch, B. König, *Eur. J. Inorg. Chem.* **2010**, accepted.

1. Syntheses

1.1. General methods

Compound **7**[1], **8**[2], **10a**[3], **10c**[4], **16**[3], **17**[5], **18**[6], **20**[7] and **24**[8] were prepared following literature known procedures. The PKG substrate (pSer 4) was purchased from Genscript (> 95 %) and used as is.

Analytical control of the synthesized compounds was done by common methods. Melting points were determined on Büchi SMP or a Lambda Photometrics OptiMelt MPA 100 and are uncorrected. IR Spectra were recorded with a Bio-Rad FT-IR Excalibur FTS 3000. UV spectra were recorded on a Cary 50 BIO spectrometer with temperature control at 25 °C. Electro spray mass spectra were performed on a Finnigan MAT TSQ 7000 ESI-spectrometer. Other Mass Spectra were recorded on Varian CH-5 (EI), Finnigan MAT 95 (CI; FAB and FD); Xenon serves as the ionization gas for FAB.

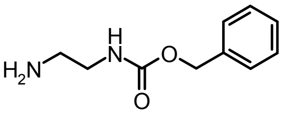
NMR spectra were recorded on Bruker Avance 600 (^{1}H: 600.1 MHz, ^{13}C: 150.1 MHz, T = 300 K), Bruker Avance 400 (^{1}H: 400.1 MHz, ^{13}C: 100.6 MHz, T = 300 K) or Bruker Avance 300 (^{1}H: 300.1 MHz, ^{13}C: 75.5 MHz, T = 300 K) relative to external standards. NMR spectra were recorded in $CDCl_3$ at 300 MHz (^{1}H) or 75 MHz (^{13}C) unless stated otherwise. Characterization of the signals: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, bs = broad singlet, dd = double doublet, dt = double triplet, ddd = double double doublet. Integration is determined as the relative number of atoms, the coupling constants are given in Hertz [Hz]. The multiplicity of the carbon atoms is given as (+) = CH_3 or CH, (-) = CH_2 and (C_{quat}) for quaternary carbon atoms. Structural assignments are based on DEPT and COSY experiments where applicable. Error of reported values: chemical shift: 0.01 ppm for ^{1}H-NMR, 0.1 ppm for ^{13}C-NMR and 0.1 Hz for coupling constants. The solvent used is reported for each spectrum.

Analytical TLC plates (silica gel 60 F_{254}) and silica gel 60 (70 - 230 or 230 - 400 mesh) were used for chromatographic separations. Visualization of the spots was by UV light and/or staining with phosphomolybdate or ninhydrin, both in ethanol. Solvents used were reagent or pro analysi grade, CH_3CN, $CHCl_3$, THF, and Et_2O were dried by standard procedures and stored over molecular sieves. DMF was purchased anhydrous (Fluka) and used as received. PE means petrol ether with a boiling range of 70 - 90 °C. All reagents were reagent grade and used as received (Merck, Aldrich, Fluka). All test substances were of pro analysi grade, checked by NMR or HPLC and used as purchased without further purification.

The Solvents for the fluorescence measurements were from special spectroscopic purity purchased from Acros or Baker or Uvasol from Merck. Millipore water (18 MΩ, Milli Q_{Plus}) was used; the HEPES buffer was from according purity, suitable for biochemical optical screenings. As far as not stated differently tetraethylammoniumhydroxide or hydrochloric acid of analytical grade were used to adjust the pH in the titrations and screenings.

1.2. Syntheses of building blocks

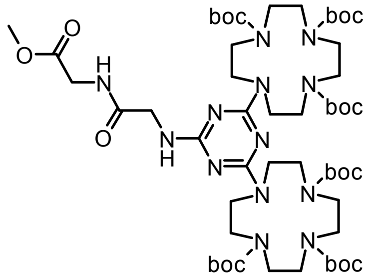
*(2-Amino-ethyl)-carbamic acid benzyl ester (**8**) (literature known[9] but improved procedure)*



To a solution of 1,2-diaminoethane (30.0 g, 0.50 mmol) in DCM (500 mL) benzylorthochloroformiate (8.53 g, 50 mmol) in DCM (250 mL) was added drop wise at 0 °C over a period of 8 h. After stirring at ambient temperature over night, the mixture was washed five times with brine (100 mL) and then with water (100 mL). The combined organic phases were dried over $MgSO_4$ and concentrated in vacuo to afford colourless oil, which solidifies in the refrigerator as a white solid (7.58 g, 39 mmol, 78 %).

M.p. (uncorrected) = 80 – 82°C; - **^{1}H-NMR** (300 MHz, $CDCl_3$): δ [ppm] = 1.21 (bs, 2 H), 2.81 (app. t, 2 H, 5.8 Hz), 3.23 (dd, 2 H, 11.3 Hz & 5.8 Hz), 5.10 (s, 2 H), 5.29 (bs, 1 H), 7.29 - 7.38 (m, 5 H); - **^{13}C-NMR** (75 MHz, $CDCl_3$): δ [ppm] = 41.8 (-, 1 C), 43.8 (-, 1 C), 66.8 (-, 1 C), 128.4 (+, 2 C), 128.6 (+, 2 C), 128.7 (+, 1 C), 136.6 (C_{quat}, 1 C), 156.7 (C_{quat}, 1 C); - **MS** (CI-MS, NH_3): m/z (%) = 195.2 (100, MH^+), 104.2 (17, MH^+ - C_7H_7); - **MF:** $C_{10}H_{14}N_2O_2$ – **FW:** 194.24 g/mol;

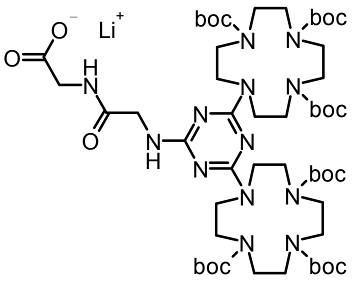
{2-[4,6-Bis-(4,7,10-tri-(^{tert}butyloxycarbonyl)-1,4,7,10-tetraaza-cyclododec-1-yl)-[1,3,5]triazin-2-ylamino]-acetylamino}-acetic acid methyl ester (**13b**)



Glycyl glycine methyl ester hydrochloride (0.91 g, 5.0 mmol) and potassium carbonate (1.04 g, 7.5 mmol) were suspended in 10 mL of dioxane. The solution was stirred for one hour, then compound **7** (2.11 g, 2.0 mmol) was added and the solution was stirred for two days at 130 °C. The precipitated solids were filtered off and the solvent was removed at reduced pressure. The crude product was purified by column chromatography on silica gel with petrol ether / ethyl acetate (1:1, $R_f = 0.3$) as eluent to obtain a colourless solid (1.65 g. 1.41 mmol, 71 %).

M.p. (uncorrected) = 124 – 126 °C; - **^{1}H-NMR** (300 MHz, $CDCl_3$): δ [ppm] = 1.37 - 1.51 (m, 54 H), 2.28 (m, 1 H), 3.08 - 3.79 (m, 32 H), 3.67 (s, 3 H), 3.99 (d, 4 H, 5.2 Hz), 5.30 (bs, 1 H); - **^{13}C-NMR** (75 MHz, $CDCl_3$): δ [ppm] = 28.5 (+, 18 C), 40.9 (-, 1 C), 44.9 (-, 1 C), 50.2 (-, 16 C), 52.1 (+, 1 C), 79.9 (C_{quat}, 6 C), 156.5 (C_{quat}, 6 C), 165.7 (C_{quat}, 1 C), 170.1 (C_{quat}, 1 C), 171.1 (C_{quat}, 1 C), further signals were not detectable; - **IR** (KBr): ν [cm^{-1}] = 3349 (bm), 2976 (m), 2932 (m), 2876 (m), 2248 (w), 1753 (m), 1677 (s), 1537 (m), 1469 (m), 1407 (m), 1364 (m), 1247 (m), 1158 (s), 1107 (m), 1029 (m), 972 (m), 913 (m), 858 (m), 814 (m), 776 (m), 727 (m), 646 (m); - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 1167.0 (100, MH^+), 584.1 (7, $(M + 2 H^+)^{2+}$); - **MF:** $C_{54}H_{95}N_{13}O_{15}$ – **FW:** 1166.44 g/mol;

{2-[4,6-Bis-(4,7,10-tri-(^{tert}butyloxycarbonyl)-1,4,7,10-tetraaza-cyclododec-1-yl)-[1,3,5]triazin-2-ylamino]-acetylamino}-acetic acid lithium salt (**14b**)

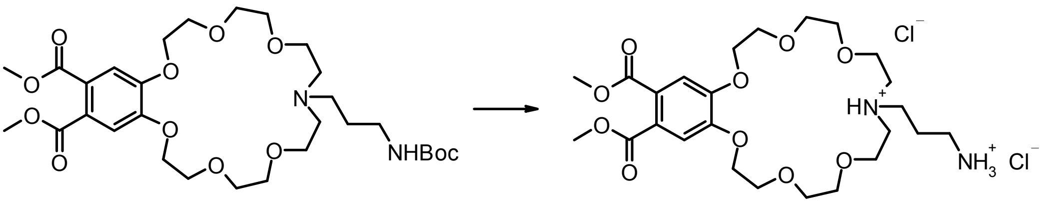


Compound **13b** (1.17 g, 1.0 mmol) was dissolved in 30 mL of acetone. A freshly prepared aqueous solution of lithium hydroxide (2 M, 0.5 mL) was added and the mixture was stirred for two days at ambient temperature. The solvent was evaporated and the residue was lyophilised to yield **14b** as colourless solid (1.11 g. 0.96 mmol, 96 %).

M.p. (uncorrected) = 132 – 134 °C (decomp.); - **^{1}H-NMR** (300 MHz, DMSO-d6): δ = 1.29 - 1.53 (m, 54 H), 3.02 - 3.64 (m, 32 H), 3.74 (d, 2 H, 5.5 Hz), 3.84 (m, 2 H), 6.87 (bs, 1 H), 7.96 (bs, 1 H); - **^{13}C-NMR** (75 MHz, DMSO-d6): δ = 27.9 (+, 18 C), 40.5 (-, 1 C), 49.2 (-, 16

C), 78.8 (C_{quat}, 6 C), 155.2 (C_{quat}, 6 C), 171.0 (C_{quat}, 1 C), further signals were not detectable; - **IR** (KBr): ν [cm^{-1}] = 3410 (bm), 2977 (m), 2935 (m), 2876 (m), 2052 (w), 1686 (s), 1538 (m), 1471 (m), 1408 (m), 1363 (m), 1247 (m), 1157 (s), 1106 (m), 1029 (m), 971 (m), 941 (m), 858 (m), 813 (m), 776 (m), 620 (m); - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 1153.0 (100, MH^+), 577.0 (13, $(M + 2 H^+)^{2+}$); - **MF:** $C_{53}H_{92}N_{13}O_{15}Li$ – **FW:** 1158.34 g/mol;

*14-[3-Amino-propyl]-6,7,9,10,13,14,15,16,18,19,21,22-dodecahydro-12H-5,8,11,17,20,23-hexaoxa-14-aza-benzocyclohenicosene-2,3-dicarboxylic acid dimethylester hydrochloride **(10b)***

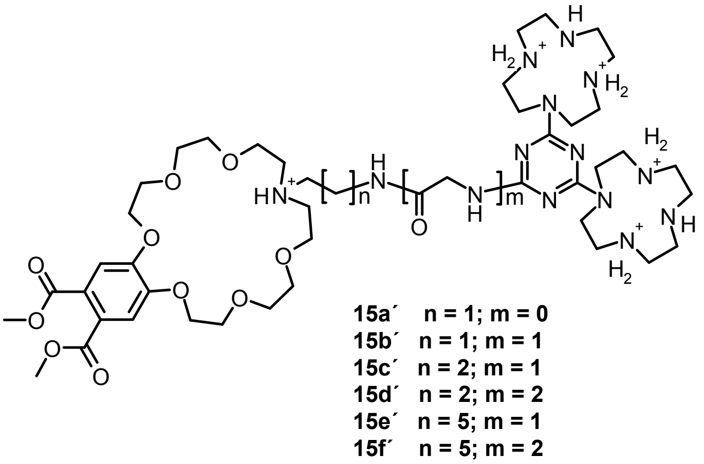


The respective N-terminal protected crown ether[5] (1.25 g, 2.0 mmol) was dissolved in 20 mL of dry dichloromethane and a saturated solution of hydrochloric acid in diethyl ether (4 mL) was added. After three hours of stirring at room temperature the HCl gas was removed in a N_2 stream. All volatiles were evaporated at reduced pressure. The residue was taken up in a minimum amount of dichloromethane and the product was precipitated carefully by slow addition of diethyl ether. The solution was decanted off the precipitate, it was washed once with diethyl ether, decanted off again and the product was dried in the vacuum to furnish a yellow, hygroscopic solid (1.12 g, 1.876 mmol, 94 %).

M.p. (uncorrected) = 66 – 67 °C - **^{1}H-NMR** (400 MHz, $CDCl_3$): δ [ppm] = 2.38 (m, 2 H), 3.21 (m, 2 H), 3.48 – 3.61 (m, 6 H), 3.61 – 3.75 (m, 8 H), 3.81 – 4.07 (m, 8 H), 3.85 (s, 6 H), 4.20 (m, 4 H), 7.21 (s, 2 H), 8.43 (bs, 3 H), 10.35 (bs, 1 H); - **^{13}C-NMR** (100 MHz, $CDCl_3$): δ [ppm] = 22.0 (-, 1 C), 37.4 (-, 1 C), 52.1 (-, 1 C), 52.6 (+, 2 C), 53.4 (-, 1 C), 65.4 (-, 2 C), 69.0 (-, 2 C), 69.4 (-, 2 C), 70.4 (-, 2 C), 70.7 (-, 2 C), 113.4 (+, 2 C), 125.5 (C_{quat}, 2 C), 150.2 (C_{quat}, 2 C), 167.7 (C_{quat}, 2 C); - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 529.3 (100, MH^+); - **MF:** $C_{25}H_{42}N_2O_{10}$ – **FW:** 601.52 g/mol;

1.3. Deprotection and complexation of the ligands

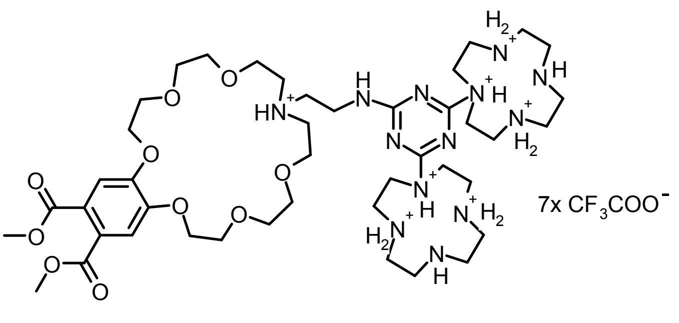
General procedure for the deprotection of crown ether cyclenes to the free ligand salts (GP II)[10]



15a′ n = 1; m = 0
15b′ n = 1; m = 1
15c′ n = 2; m = 1
15d′ n = 2; m = 2
15e′ n = 5; m = 1
15f′ n = 5; m = 2

To a well stirred solution of the Boc protected ligand (0.1 mmol) in dichloromethane (2 mL) a solution of TFA in dichloromethane (2 mL, 20 % vol/vol) was added. Stirring was continued for 4 h (TLC control), then the solvent was evaporated. The residue was redissolved in a minimum amount of dichloromethane and the product was precipitated by drop wise addition of diethyl ether. The solvent mixture was decanted off carefully, the precipitate was suspended in diethyl ether, allowed to settle completely and the solvent was decanted off again. After drying a yellow under reduced pressure, a yellow, deliquescent solid is obtained in nearly quantitative yield.

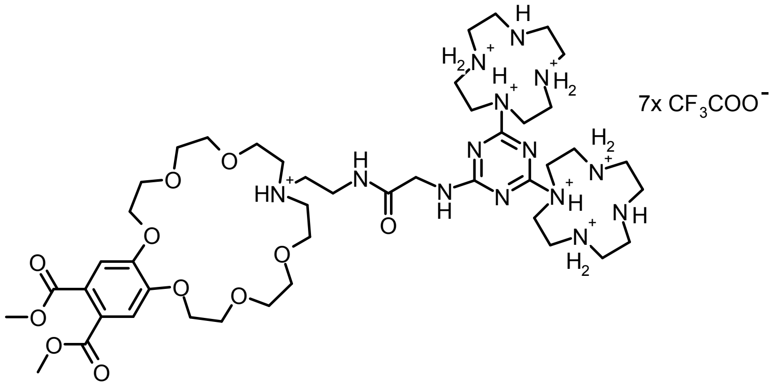
*14-[4,6-Bis-(1,4,7,10-tetraaza-cyclododec-1-yl)-[1,3,5]triazinyl-amino-ethyl]-6,7,9,10,13,14,15,16,18,19,21,22-dodecahydro-12H-5,8,11,17,20,23-hexaoxa-14-aza-benzocyclohenicosene-2,3-dicarboxylic acid dimethyl ester TFA salt (**15a′**)*



Compound **11a** (154 mg, 0.1 mmol) was deprotected according to *GP II.* After drying a colourless, hygroscopic solid (158 mg, 0.091 mmol, 91 %) is obtained.

M.p. (uncorrected) = 42 – 46 °C; - **^{1}H-NMR** (600 MHz, MeOD): δ [ppm] = 2.98 – 3.37 (m, 36 H), 3.51 – 3.58 (m, 4 H), 3.62 – 3.74 (m, 8 H), 3.82 (s, 6 H), 3.83 – 3.95 (m, 8 H), 4.17 – 4.25 (m, 4 H), 7.23 (s, 2 H) - **^{13}C-NMR** (150 MHz, MeOD): δ [ppm] = 36.2 (-, 1 C), 43.2 – 45.1 (-, 4 C), 45.6 – 48.0 (-, 16 C), 53.2 (+, 2 C), 54.6 (-, 1 C), 54.8 (-, 2 C), 65.7 (-, 2 C), 70.2 (-, 2 C), 70.6 (-, 2 C), 71.3 (-, 2 C), 71.7 (-, 2 C), 114.2 (+, 2 C), 114.2 & 116.1 & 118.1 & 120.0 (C_{quat}, 7 C), 126.6 (C_{quat}, 2 C), 151.7 (C_{quat}, 2 C), 162.7 & 162.9 (C_{quat}, 7 C), 167.0 (C_{quat}, 2 C), 168.5 (C_{quat}, 1 C), 169.3 (C_{quat}, 2 C); - **IR** (KBr): ν [cm^{-1}] = 3000 (bm), 2800 (bm), 2360 (m), 2331 (m), 1715 (m), 1650 (m), 1600 (s), 1519 (m), 1433 (m), 1348 (m), 1286 (s), 1194 (s), 1123 (s), 1055 (m), 972 (m), 939 (m), 778 (m) - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 467.9 (21, $(M+2H^+)^{2+}$), 524.9 (100, $(M+TFA+2H^+)^{2+}$), 934.6 (6, MH^+), 1048.6 (17, $((M+TFA+H^+)^+)$); - **HRMS** (PI-LSIMS FAB, glycerine): calc. for $C_{43}H_{76}N_{13}O_{10}^+$: 934.5838, found: 934.5808; - **MF:** $C_{57}H_{90}N_{13}O_{24}$ – **FW:** 1740.43 g/mol;

*14-[4,6-Bis-(1,4,7,10-tetraaza-cyclododec-1-yl)-[1,3,5]triazin-2-yl-amino-N-(2-methylamino)-acetamido-3-ethyl]-6,7,9,10,13,14,15,16,18,19,21,22-dodecahydro-12H-5,8,11,17,20,23-hexaoxa-14-aza-benzocyclohenicosene-2,3-dicarboxylic acid dimethyl ester TFA salt (**15b′**)*

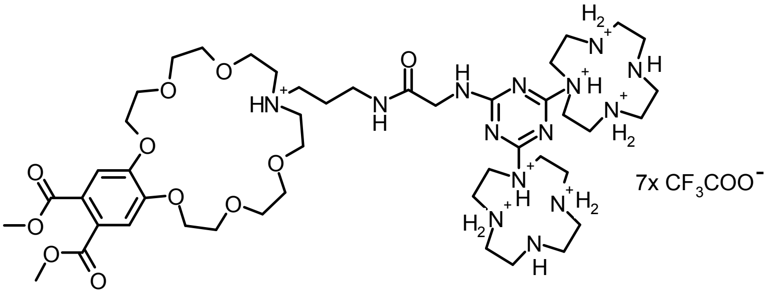


Compound **11b** (160 mg, 0.1 mmol) was deprotected according to *GP II* to give a yellow, hygroscopic solid (166 mg, 0.093 mmol, 93 %).

M.p. (uncorrected) = 49 – 52 °C; - **^{1}H-NMR** (600 MHz, MeOD): δ [ppm] = 2.97 – 3.38 (m, 38 H), 3.48 – 3.53 (m, 4 H), 3.56 – 3.61 (m, 2 H), 3.62 – 3.73 (m, 8 H), 3.82 (s, 6 H), 3.83 – 3.95 (m, 6 H), 4.17 – 4.26 (m, 4 H), 7.21 (s, 2 H) - **^{13}C-NMR** (150 MHz, MeOD): δ [ppm] = 35.7 (-, 1 C), 43.1 – 44.8 (-, 4 C), 45.4 – 48.0 (-, 17 C), 53.1 (+, 2 C), 53.9 (-, 1 C), 54.8 (-, 1 C), 55.0 (-, 2 C), 65.7 (-, 2 C), 66.8 (-, 2 C), 70.2 (-, 2 C), 70.6 (-, 2 C), 71.2 (-, 2 C), 71.7 (-, 2 C), 114.2 (+, 2 C), 115.0 & 116.9 & 118.9 & 120.8 (C_{quat}, 7 C), 126.6 (C_{quat}, 2 C), 151.6

(C_{quat}, 2 C), 162.5 & 162.7 (C_{quat}, 7 C), 167.1 (C_{quat}, 1 C), 168.7 (C_{quat}, 1 C), 168.8 (C_{quat}, 1 C), 169.3 (C_{quat}, 2 C), 174.9 (C_{quat}, 1 C); - **IR** (KBr): ν [cm^{-1}] = 3000 (bm), 2800 (bm), 2360 (m), 2331 (m), 1715 (m), 1650 (m), 1600 (s), 1519 (m), 1433 (m), 1348 (m), 1286 (s), 1194 (s), 1123 (s), 1055 (m), 972 (m), 939 (m), 778 (m) - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 331.2 (50, $(M+3H^+)^{3+}$), 494.4 (100, $(M+2H^+)^{2+}$), 553.4 (82, $(M+TFA+2H^+)^{2+}$), 667.3 (42, $(M+3TFA+2H^+)^{2+}$), 991.7 (9, MH^+), 1105.7 (21, $((M+TFA+H^+)^+$); - **MF:** $C_{59}H_{92}N_{14}O_{25}F_{21}$ – **FW:** 1796.48 g/mol;

*14-[4,6-Bis-(1,4,7,10-tetraaza-cyclododec-1-yl)-[1,3,5]triazin-2-yl-amino-N-(2-methylamino)-acetamido-3-propyl]-6,7,9,10,13,14,15,16,18,19,21,22-dodecahydro-12H-5,8,11,17,20,23-hexaoxa-14-aza-benzocyclohenicosene-2,3-dicarboxylic acid dimethyl ester TFA salt (**15c′**)*

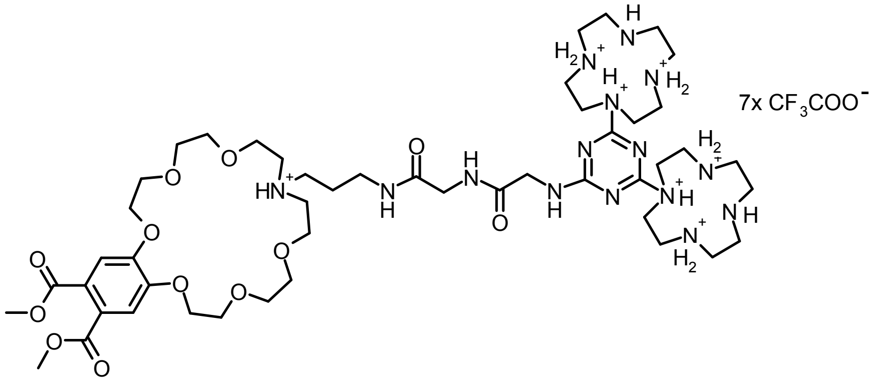


Compound **11c** (161 mg, 0.1 mmol) was deprotected after *GP II.* A faintly yellow, hygroscopic solid (150 mg, 0.083 mmol, 83 %) is obtained.

M.p. (uncorrected) = 46 – 49 °C; - **^{1}H-NMR** (600 MHz, MeOD): δ [ppm] = 1.91 (m, 2 H), 3.00 – 3.12 (m, 8 H), 3.13 – 3.26 (m, 8 H), 3.27 – 3.35 (m, 16 H), 3.36 – 3.45 (m, 4 H), 3.68 – 3.79 (m, 10 H), 3.81 – 3.92 (m, 8 H), 3.87 (s, 6 H), 3.95 – 3.97 (m, 2 H), 4.19 – 4.32 (m, 4 H), 7.28 (s, 2 H) - **^{13}C-NMR** (150 MHz, MeOD): δ [ppm] = 25.0 (-, 1 C), 37.3 (-, 1 C), 43.1 – 44.8 (-, 5 C), 46.2 – 48.6 (-, 12 C), 43.5 (-, 1 C), 53.1 (+, 2 C), 54.4 (-, 2 C), 65.7 (-, 2 C), 70.1 (-, 2 C), 70.7 (-, 2 C), 71.4 (-, 2 C), 71.8 (-, 2 C), 114.3 (+, 2 C), 115.0 & 116.9 & 118.9 & 120.8 (C_{quat}, 7 C), 126.7 (C_{quat}, 2 C), 151.8 (C_{quat}, 2 C), 162.0 & 162.2 (C_{quat}, 7 C), 167.1 (C_{quat}, 1 C), 168.9 (C_{quat}, 1 C), 169.0 (C_{quat}, 1 C), 169.4 (C_{quat}, 2 C), 173.9 (C_{quat}, 1 C); - **IR** (KBr): ν [cm^{-1}] = 3300 (bm), 2953 (bm), 2750 (bm), 2640 (bm), 1716 (m), 1649 (m), 1598 (s), 1520 (m), 1432 (m), 1350 (m), 1284 (s), 1261 (m), 1195 (m), 1107 (s), 1055 (m), 972 (m), 944 (m), 882 (m), 779 (m); - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 335.9 (47, $(M+3H^+)^{3+}$), 503.4 (43, $(M+2H^+)^{2+}$), 560.5 (100, $(M+TFA+2H^+)^{2+}$), 674.4 (52,

$(M+3TFA+2H^{+})^{2+}$), 1005.7 (6, MH^{+}), 1119.7 (20, $((M+TFA+H^{+})^{+}$); - **MF:** $C_{60}H_{94}N_{14}O_{25}F_{21}$ – **FW:** 1810.50 g/mol;

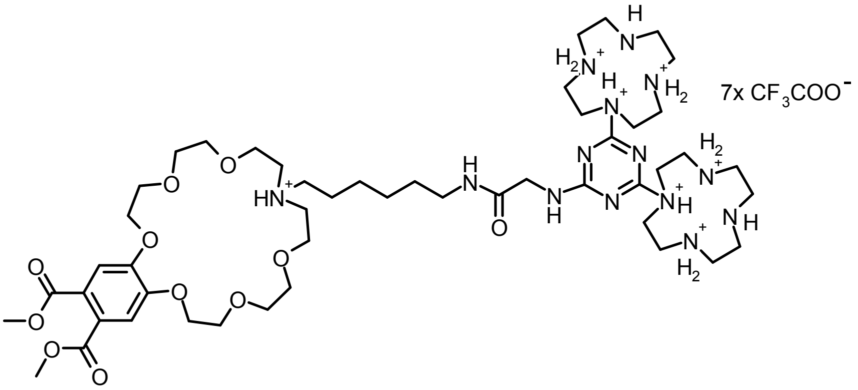
*14-[4,6-Bis-(1,4,7,10-tetraaza-cyclododec-1-yl)-[1,3,5]triazin-2-yl-amino-N-(2-methylamino)-acetamido-N-(2-methylamino)-acetamido-3-propyl]-6,7,9,10,13,14,15,16,18,19,21,22-dodecahydro-12H-5,8,11,17,20,23-hexaoxa-14-aza-benzocyclohenicosene-2,3-dicarboxylic acid dimethyl ester TFA salt (**15d′**)*



Compound **11d** (167 mg, 0.1 mmol) was deprotected as given in *GP II* to yield a colourless, hygroscopic solid (181 mg, 0.097 mmol, 97 %).

M.p. (uncorrected) = 51 – 54 °C; - ^{1}**H-NMR** (600 MHz, MeOD): δ [ppm] = 1.89 (m, 2 H), 3.01 – 3.13 (m, 8 H), 3.15 – 3.26 (m, 10 H), 3.27 – 3.36 (m, 16 H), 3.38 – 3.45 (m, 4 H), 3.68 – 3.77 (m, 8 H), 3.79 – 3.92 (m, 10 H), 3.86 (s, 6 H), 3.95 – 3.97 (m, 2 H), 4.02 – 4.07 (m, 2 H), 4.19 – 4.32 (m, 4 H), 7.30 (s, 2 H) - ^{13}**C-NMR** (150 MHz, MeOD): δ [ppm] = 25.1 (-, 1 C), 37.1 (-, 1 C), 43.2 – 44.6 (-, 5 C), 45.5 (-, 1 C), 45.3 – 48.4 (-, 12 C), 52.9 (-, 1 C), 53.1 (+, 2 C), 54.2 (-, 2 C), 65.7 (-, 2 C), 70.1 (-, 2 C), 70.7 (-, 2 C), 71.5 (-, 2 C), 71.9 (-, 2 C), 114.3 (+, 2 C), 114.9 & 116.8 & 118.8 & 120.7 (C_{quat}, 7 C), 126.7 (C_{quat}, 2 C), 151.8 (C_{quat}, 2 C), 161.9 & 162.1 (C_{quat}, 7 C), 167.2 (C_{quat}, 1 C), 168.9 (C_{quat}, 1 C), 169.1 (C_{quat}, 1 C), 169.4 (C_{quat}, 2 C), 172.2 (C_{quat}, 1 C), 174.1 (C_{quat}, 1 C); - **IR** (KBr): ν [cm^{-1}] = 3377 (bm), 2950 (bm), 2932 (m), 2793 (bm), 2659 (m), 2362 (m), 1720 (m), 1650 (m), 1600 (s), 1519 (m), 1432 (m), 1349 (m), 1287 (s), 1193 (m), 1116 (s), 1056 (m), 974 (m), 950 (m), 890 (m), 771 (m), 611 (m), 529 (m); - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 354.9 (66, $(M+3H^{+})^{3+}$), 532.0 (23, $(M+2H^{+})^{2+}$), 588.9 (100, $(M+TFA+2H^{+})^{2+}$), 702.9 (16, $(M+3TFA+2H^{+})^{2+}$), 1062.7 (8, MH^{+}), 1176.8 (19, $((M+TFA+H^{+})^{+}$);- **MF:** $C_{62}H_{97}N_{15}O_{26}$ – **FW:** 1867.56 g/mol;

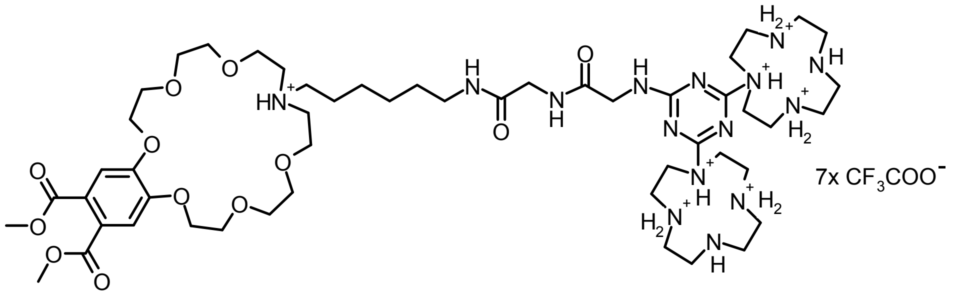
*14-[4,6-Bis-(1,4,7,10-tetraaza-cyclododec-1-yl)-[1,3,5]triazin-2-yl-amino-N-(2-methylamino)-acetamido-6-hexyl]-6,7,9,10,13,14,15,16,18,19,21,22-dodecahydro-12H-5,8,11,17,20,23-hexaoxa-14-aza-benzocyclohenicosene-2,3-dicarboxylic acid dimethyl ester TFA salt (**15e′**)*



Compound **11e** (165 mg, 0.1 mmol) was deprotected after *GP II.* A colourless, hygroscopic solid (177 mg, 0.096 mmol, 96 %) is obtained.

M.p. (uncorrected) = 42 – 46 °C; - **^{1}H-NMR** (600 MHz, MeOD): δ [ppm] = 1.21 (m, 4 H), 1.42 (m, 2 H), 1.63 (m, 2 H), 3.00 – 3.12 (m, 10 H), 3.14 – 3.25 (m, 8 H), 3.27 – 3.36 (m, 16 H), 3.38 – 3.44 (m, 4 H), 3.68 – 3.78 (m, 10 H), 3.82 – 3.92 (m, 10 H), 3.88 (s, 6 H), 4.20 – 4.31 (m, 4 H), 7.31 (s, 2 H) - **^{13}C-NMR** (150 MHz, MeOD): δ [ppm] = 24.5 (-, 1 C), 27.2 (-, 1 C), 27.5 (-, 1 C), 30.1 (-, 1 C), 40.2 (-, 1 C), 43.4 – 44.7 (-, 5 C), 45.7 (-, 1 C), 45.8 – 48.1 (-, 12 C), 53.1 (+, 2 C), 54.1 (-, 2 C), 54.9 (-, 1 C), 65.7 (-, 2 C), 70.2 (-, 2 C), 70.8 (-, 2 C), 71.4 (-, 2 C), 71.9 (-, 2 C), 114.3 (+, 2 C), 114.9 & 116.8 & 118.8 & 120.7 (C_{quat}, 7 C), 126.7 (C_{quat}, 2 C), 151.8 (C_{quat}, 2 C), 161.9 & 162.2 (C_{quat}, 7 C), 167.2 (C_{quat}, 1 C), 168.9 (C_{quat}, 1 C), 169.0 (C_{quat}, 1 C), 169.4 (C_{quat}, 2 C), 173.3 (C_{quat}, 1 C); - **IR** (KBr): ν [cm^{-1}] = 3300 (bm), 2945 (bm), 2753 (bm), 2640 (bm), 2340 (m), 1716 (m), 1649 (m), 1599 (s), 1519 (m), 1432 (m), 1349 (m), 1287 (s), 1194 (m), 1123 (s), 1107 (s), 1056 (m), 972 (m), 944 (m), 885 (m), 779 (m), 655 (m); - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 349.9 (96, $(M+3H^+)^{3+}$), 524.4 (31, $(M+2H^+)^{2+}$), 581.4 (100, $(M+TFA+2H^+)^{2+}$), 695.4 (37, $(M+3TFA+2H^+)^{2+}$), 1047.7 (5, MH^+), 1161.8 (12, $((M+TFA+H^+)^+$); - **MF:** $C_{63}H_{101}N_{14}O_{25}F_{21}$ – **FW:** 1853.59 g/mol;

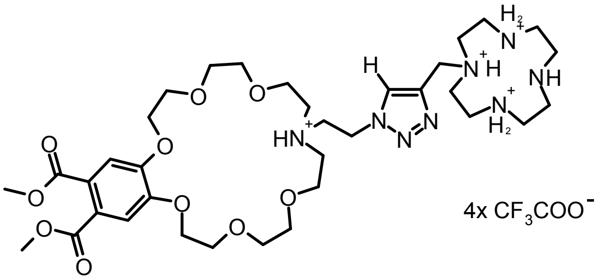
*14-[4,6-Bis-(1,4,7,10-tetraaza-cyclododec-1-yl)-[1,3,5]triazin-2-yl-amino-N-(2-methylamino)-acetamido-N-(2-methylamino)-acetamido-6-hexyl]-6,7,9,10,13,14,15,16,18,19,21,22-dodecahydro-12H-5,8,11,17,20,23-hexaoxa-14-aza-benzocyclohenicosene-2,3-dicarboxylic acid dimethyl ester TFA salt **(15f')***



Compound **11f** (171 mg, 0.1 mmol) was submitted to *GP II* to yield a faintly yellow, hygroscopic solid (174 mg, 0.092 mmol, 92 %) is obtained.

M.p. (uncorrected) = 47 – 50 °C; - **^{1}H-NMR** (600 MHz, MeOD): δ [ppm] = 1.22 (m, 4 H), 1.41 (m, 2 H), 1.64 (m, 2 H), 3.01 – 3.13 (m, 10 H), 3.14 – 3.27 (m, 8 H), 3.29 – 3.37 (m, 16 H), 3.38 – 3.47 (m, 4 H), 3.66 – 3.78 (m, 12 H), 3.78 – 3.91 (m, 8 H), 3.86 (s, 6 H), 4.03 (m, 2 H), 4.19 – 4.31 (m, 4 H), 7.29 (s, 2 H) - **^{13}C-NMR** (150 MHz, MeOD): δ [ppm] = 24.5 (-, 1 C), 27.1 (-, 1 C), 27.3 (-, 1 C), 30.1 (-, 1 C), 37.9 (-, 1 C), 40.2 (-, 1 C), 43.5 – 44.7 (-, 5 C), 45.3 – 48.6 (-, 12 C), 53.2 (+, 2 C), 54.1 (-, 2 C), 54.8 (-, 1 C), 65.7 (-, 2 C), 70.1 (-, 2 C), 70.8 (-, 2 C), 71.4 (-, 2 C), 71.9 (-, 2 C), 114.3 (+, 2 C), 115.0 & 117.0 & 119.0 & 120.9 (C_{quat}, 7 C), 126.7 (C_{quat}, 2 C), 151.8 (C_{quat}, 2 C), 162.4 & 162.6 (C_{quat}, 7 C), 167.3 (C_{quat}, 1 C), 168.9 (C_{quat}, 1 C), 169.1 (C_{quat}, 1 C), 169.4 (C_{quat}, 2 C), 171.2 (C_{quat}, 1 C), 174.0 (C_{quat}, 1 C); - **IR** (KBr): ν [cm^{-1}] = 3300 (bm), 2944 (bm), 2790 (m), 2667 (bm), 2362 (m), 1715 (m), 1648 (m), 1599 (s), 1518 (m), 1432 (m), 1349 (m), 1287 (s), 1262 (s), 1188 (m), 1108 (s), 1054 (m), 971 (m), 945 (m), 889 (m), 779 (m), 579 (m); - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 369.0 (100, $(M+3H^+)^{3+}$), 552.9 (19, $(M+2H^+)^{2+}$), 609.9 (77, $(M+TFA+2H^+)^{2+}$), 723.9 (12, $(M+3TFA+2H^+)^{2+}$), 1104.7 (5, MH^+), 1218.8 (6, $((M+TFA+H^+)^+$); - **MF:** $C_{65}H_{103}N_{15}O_{26}F_{21}$ – **FW:** 1909.64 g/mol;

14-[3-(1,4,7,10-Tetraaza-cyclododec-1-yl)-[1,4,5]triazol-amino-ethyl]-6,7,9,10,13,14,15,16,18,19,21,22-dodecahydro-12H-5,8,11,17,20,23-hexaoxa-14-aza-benzocyclohenicosene-2,3-dicarboxylic acid dimethyl ester TFA salt (***19b´***)



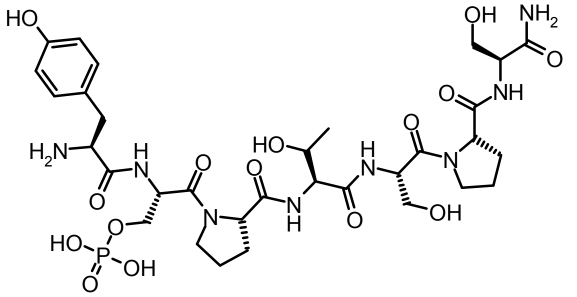
The Boc-protected ligand **19a** (106 mg, 0.1 mmol) was dissolved in dichloromethane (2.0 mL). To the well stirred solution was given TFA (0.6 mL) and it was stirred for 3 h at room temperature.

The dichloromethane and the TFA were evaporated, the product was taken up in a minimum volume of dichloromethane containing 5 % methanol and was then carefully precipitated by addition of diethyl ether. The product was allowed to settle and the supernatant solution was decanted off. The product was suspended in diethyl ether and centrifuged. It was again decanted. The residue was taken up in a minimum volume of water and lyophilized to give the free ligand salt as colourless to slightly yellow hygroscopic solid in quantitative yield (108 mg, 99 %).

M.p. (uncorrected) = 47 - 48 °C; - **^{1}H-NMR** (300 MHz, MeOD): δ [ppm] = 2.78 (m, 4 H), 2.96 (m, 4 H), 3.12 (m, 4 H), 3.20 (m, 4 H), 3.54 (m, 8 H), 3.71 (m, 8 H), 3.85 (m, 6 H), 3.89 (m, 6 H), 3.96 (m, 2 H), 4.19 (m, 4 H), 4.94 (m, 4 H), 7.22 (s, 2 H), 7.84 (s, 1 H) – **^{13}C-NMR** (75 MHz, MeOD): δ [ppm] = 43.4 (-, 2 C), 43.6 (-, 4 C), 46.0 (-, 4 C), 48.0 (-, 1 C), 53.3 (+, 2 C), 53.4 (-, 1 C), 56.0 (-, 2 C), 65.7 (-, 2 C), 70.2 (-, 2 C), 70.8 (-, 2 C), 71.4 (-, 2 C), 71.5 (-, 2 C), 114.1 (+, 2 C), 116.2 (C_{quat}, 3 C), 120.0 (+, 1 C), 126.4 (+, 1 C), 126.6 (C_{quat}, 2 C), 143.9 (C_{quat}, 1 C), 151.6 (C_{quat}, 2 C), 162.5 & 162.9 (C_{quat}, 3 C), 169.5 (C_{quat}, 2 C) - **MS** (ESI-MS, H_2O/MeCN): m/z (%) = 264.8 (57, $(M + 3H^+)^{3+}$ + MeOH), 376.2 (100, $(M + 2H^+)^{2+}$), 751.3 (18, (MH^+)); - **MF:** $C_{43}H_{67}N_8O_{18}F_{12}$ – **FW:** 1213.06 g/mol;

1.4. Preparation of peptide 1

H_2N-Tyr-Ser(PO_3H_2)-Pro-Thr-Ser-Pro-Ser-$CONH_2$ (**1**):[11]



The heptapeptide was synthesized according to the following Fmoc based SPPS on rink amide resin after the standard protocol:

MBHA resin and Fmoc protecting group strategy were used throughout all syntheses. Coupling was achieved by TBTU / HOBt / DIPEA. HOBt was used as a 0.45 M solution, TBTU as a 0.44 M solution and DIPEA as a 1.2 M solution, all in DMF. The Fmoc protected amino acids were dissolved in NMP as 0.4 M solutions. The peptide was synthesized on 50 mg resin. The lot of the resin used had a loading of 0.72 mmol/g (manufacturer's claims). Before each synthesis the resin was allowed to preswell in DMF for 30 min. Each coupling was done twice using a 5 fold excess of HOBt and slightly less than 5 fold excess of TBTU. DIPEA was used in 10 fold excess. Fmoc deprotection was done by shaking the resin with 40 % piperidine in DMF for 3 minutes, subsequent washing and addition of 20 % piperidine in DMF followed by shaking for 10 minutes. After completion, the resin was washed with MeOH, DCM and Et_2O (5 x 2 mL each). Where acylation was necessary, it was conducted using 5 eq. of Ac_2O and 5 eq. of DIPEA in DMF for 30 minutes.
Phosphorylated serine was introduced by using Fmoc-Ser(PO(OBzl)OH)-OH as the phosphorylated building block. As the cleavage mixture for the peptide, TFA / Phenol / H_2O / Thioanisole / EDT (82.5:5:5:2.5) was used. After filtering off the resin, the TFA solution was reduced in volume to about 0.5 mL. It was then transferred to a Falcon tube and precipitated with cold Et_2O. The precipitate was centrifuged at -4 °C for 10 minutes. The solution was then carefully decanted off and the precipitate resuspended in cold Et_2O before being centrifuged again. This resuspending/centrifuging step was repeated five times. Finally, the Et_2O was decanted off again and the peptide dried under vacuum. The peptides were analysed by ES-MS and LC-MS or HPLC or both.

Work-up and Purification:

The precipitate was pelletized and washed four times with cold diethyl ether, before it was solubilised in H_2O and freeze-dried. After analytical HPLC and LC-MS, a preparative HPLC run was performed to obtain the pure heptapeptide (11.73 mg, 14.32 µmol, 4 %).

HPLC system: Agilent 1100
Column: Phenomenex Luna 10 C18(2) 250 x 21.2 mm
Column temperature: 25°C
Injection volume: 100 µL
Solvent A: H_2O (0.0059 % TFA)
Solvent B: MeCN
Gradient: 0 min – 3 % MeCN / H_2O (0.0059 % TFA)
19 min – 33 % MeCN / H_2O (0.0059 % TFA)
20 min – 98 % MeCN / H_2O (0.0059 % TFA)
30 min – 98 % MeCN / H_2O (0.0059 % TFA)
Flow: 22 mL/min
Elution time: 13.2 min

MS (ESI-MS, H_2O/MeOH + 10 mmol/l NH_4OAc): m/z (%) = 815.4 (100, MH^+), 817.4 (100, MH^+), 837.4 (6, $M+ 2H^+ + Na^+$), 839.4 (8, MNa^+); - **MF:** $C_{32}H_{49}N_8O_{15}P$ – **FW:** 816.77 g/mol;

2. ^{1}H – / ^{13}C-NMR and mass spectra of new compounds

For solvents and conditions of measurement, see experimental procedures. NMR spectra were recorded on Bruker Avance spectrometers 300, 400 and 600. Measuring temperature was 300 K. TMS was used as external standard.

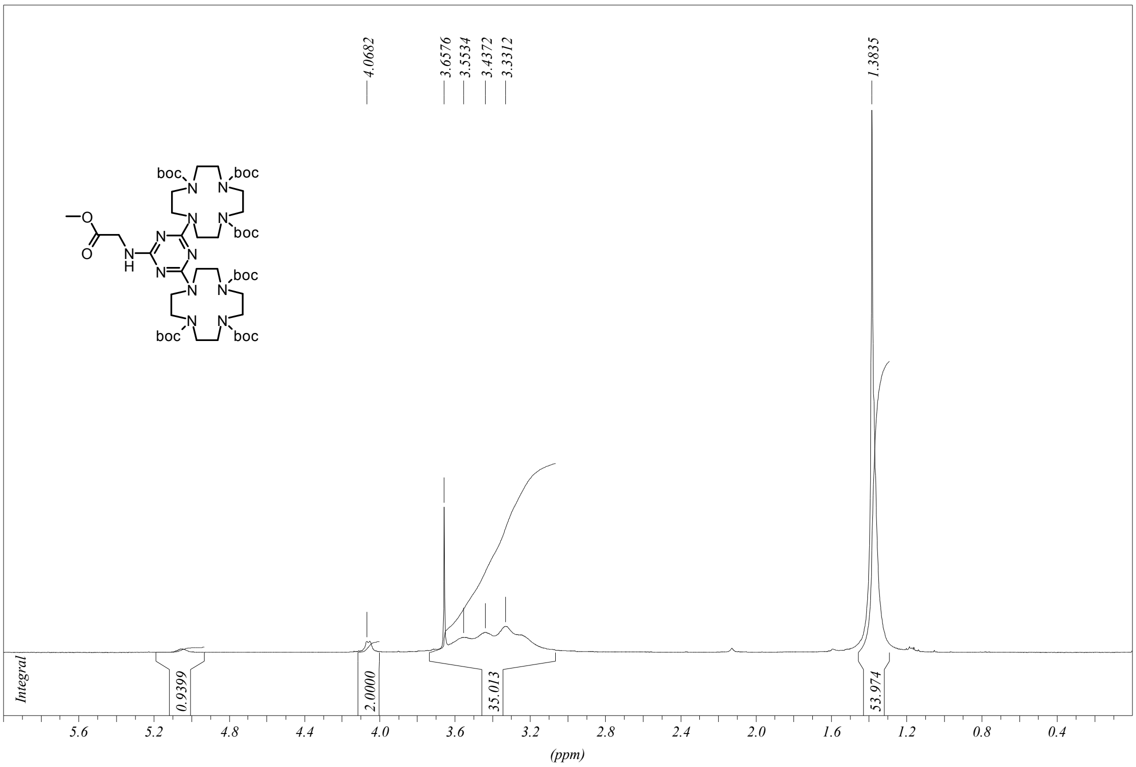


Figure S-1: ^{1}H-NMR spectrum of compound **13a**

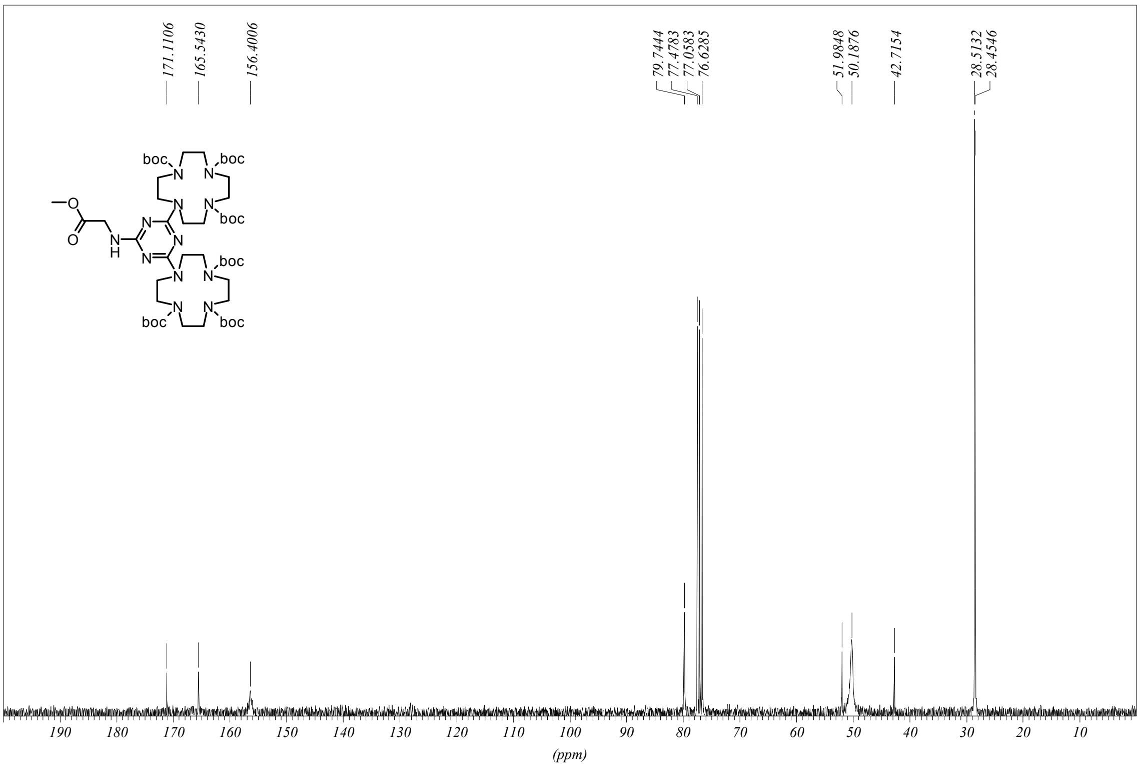


Figure S-2: ^{13}C-NMR spectrum of compound **13a**

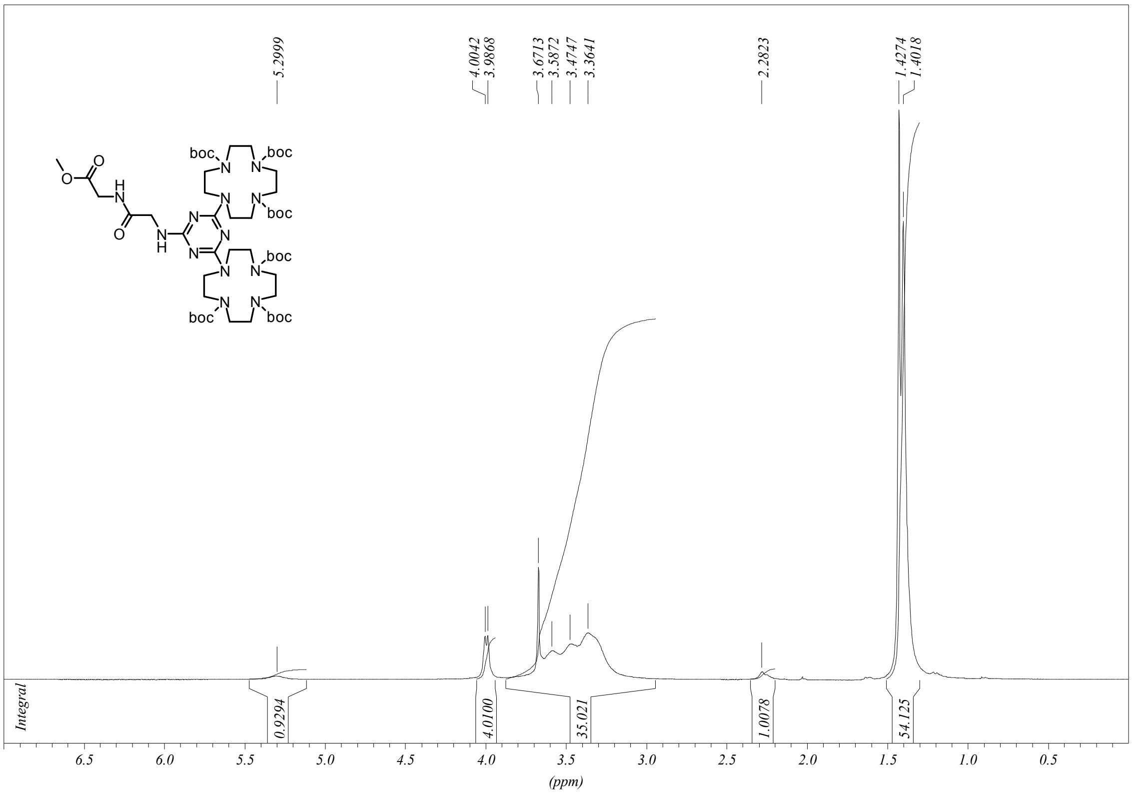


Figure S-3: ^{1}H-NMR spectrum of compound **13b**

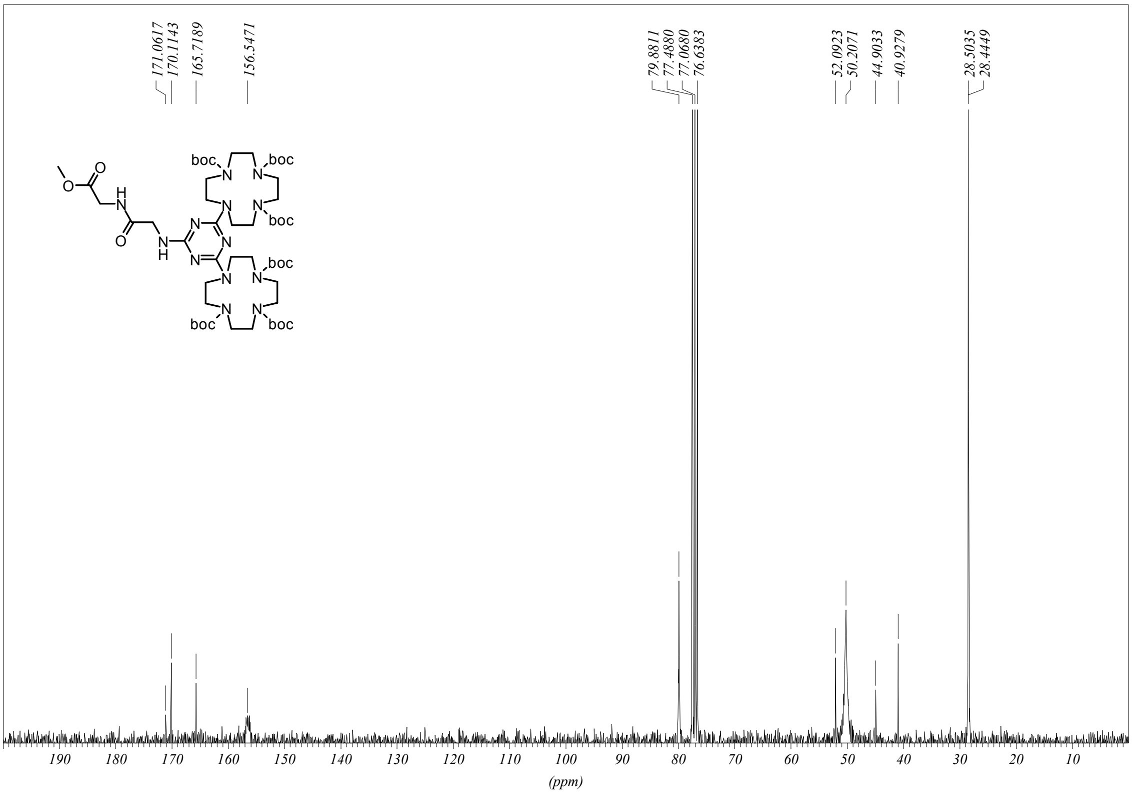


Figure S-4: ^{13}C-NMR spectrum of compound **13b**

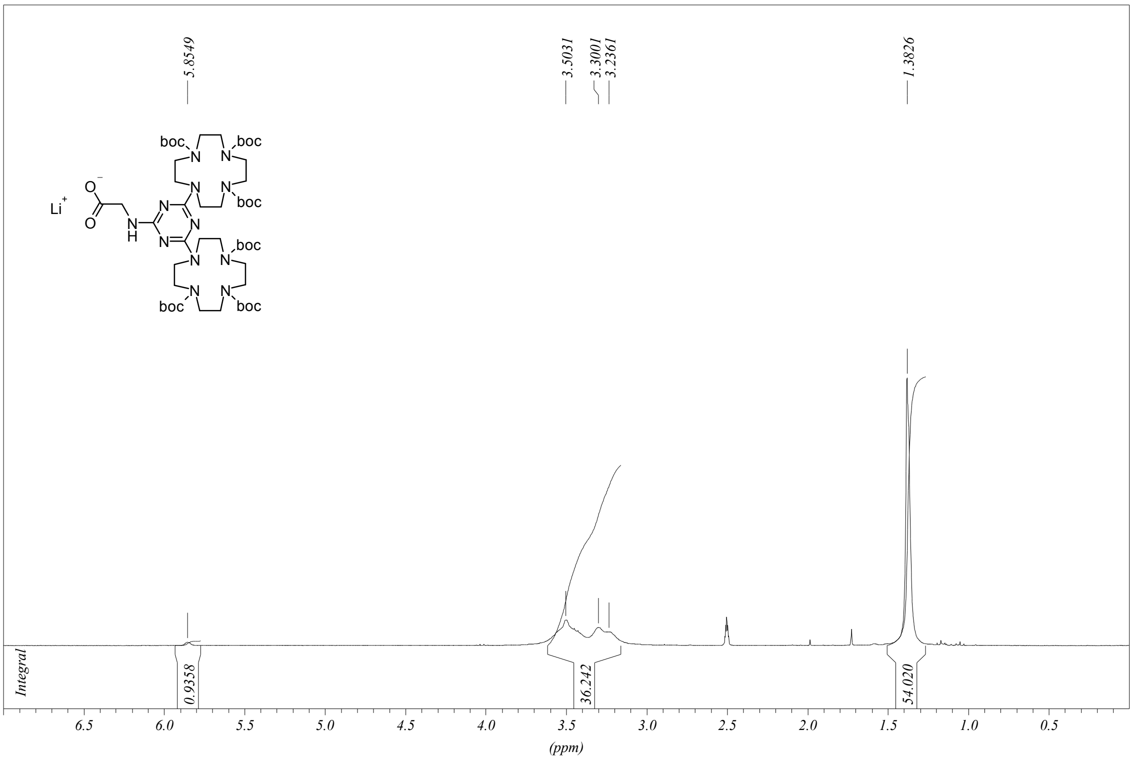


Figure S-5: ^{1}H-NMR spectrum of compound **14a**

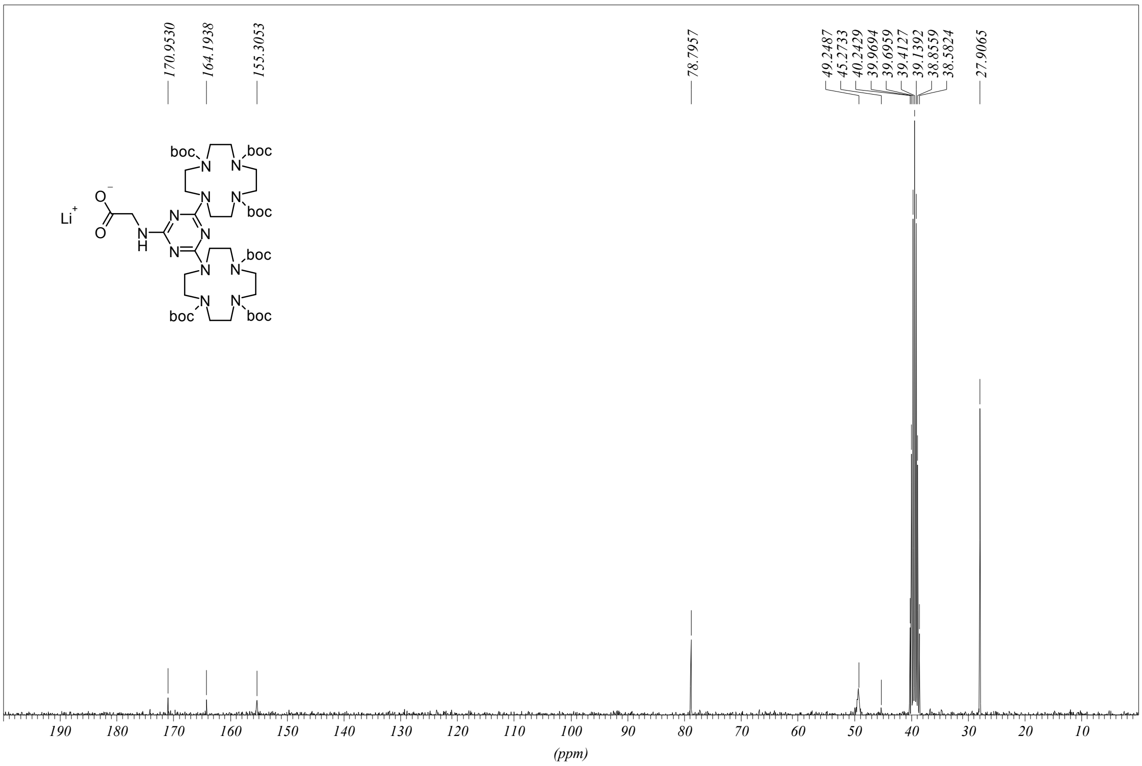


Figure S-6: ^{13}C-NMR spectrum of compound **14a**

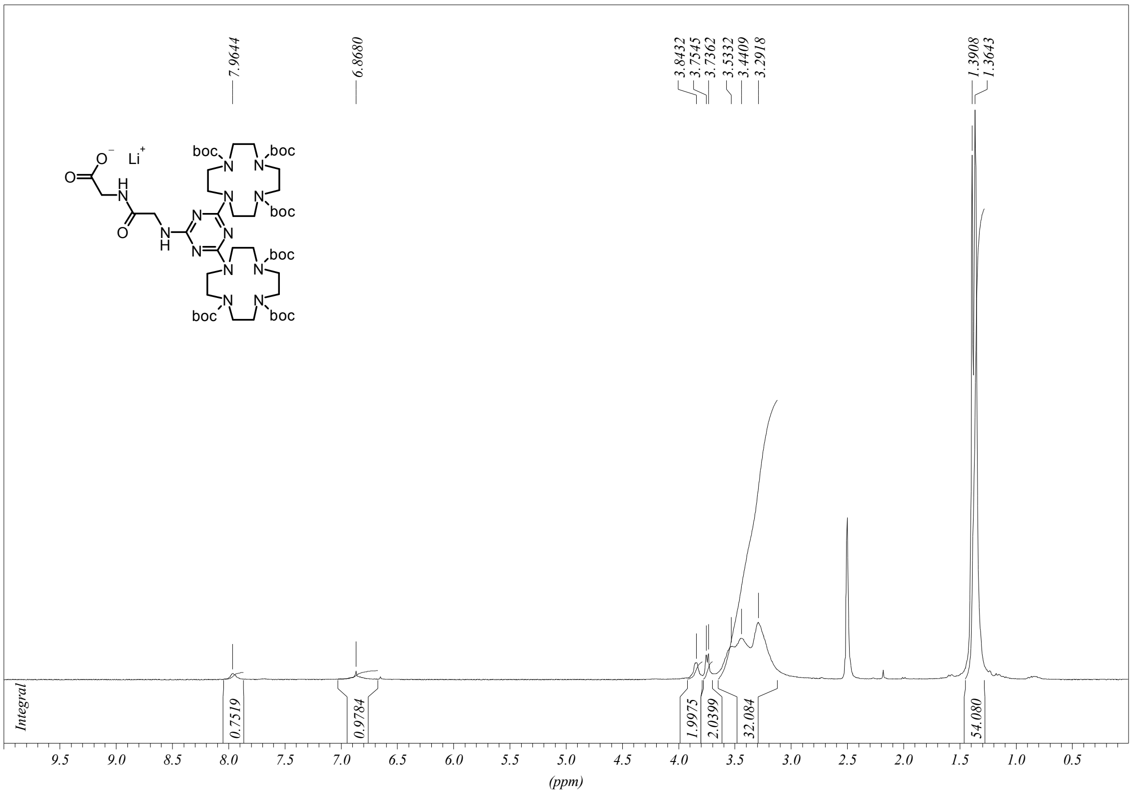


Figure S-7: ^{1}H-NMR spectrum of compound **14b**

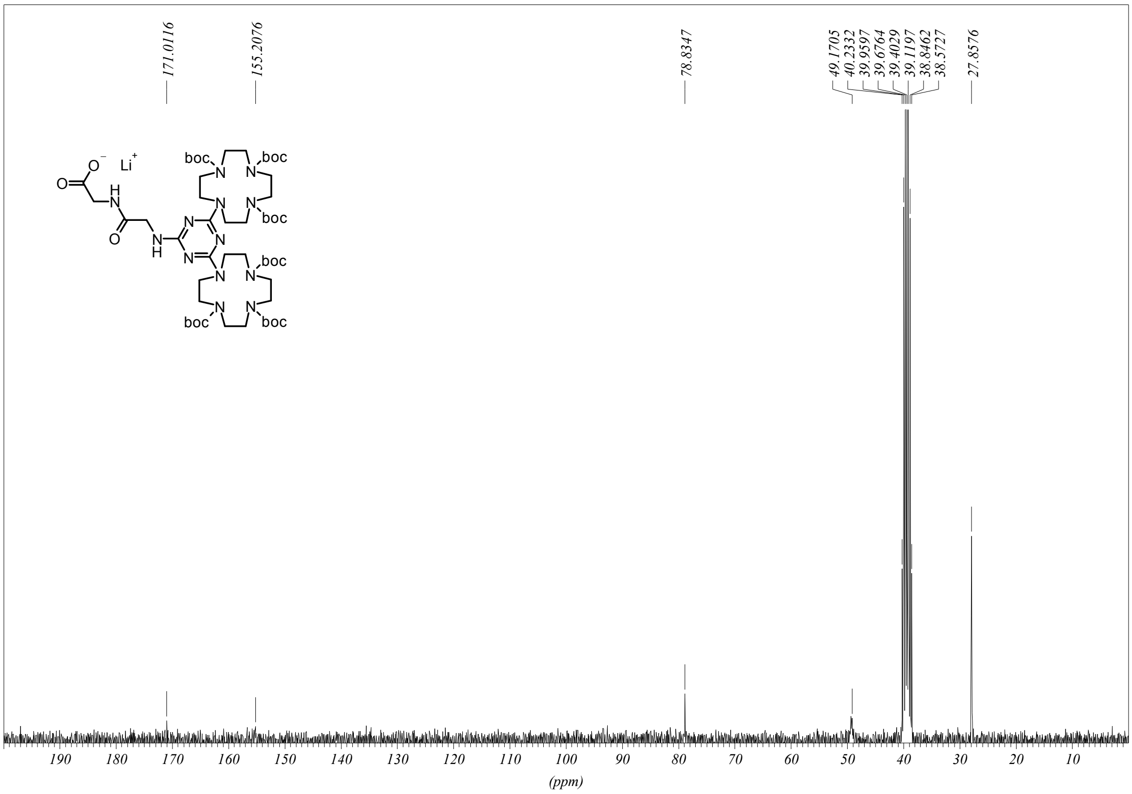


Figure S-8: ^{13}C-NMR spectrum of compound **14b**

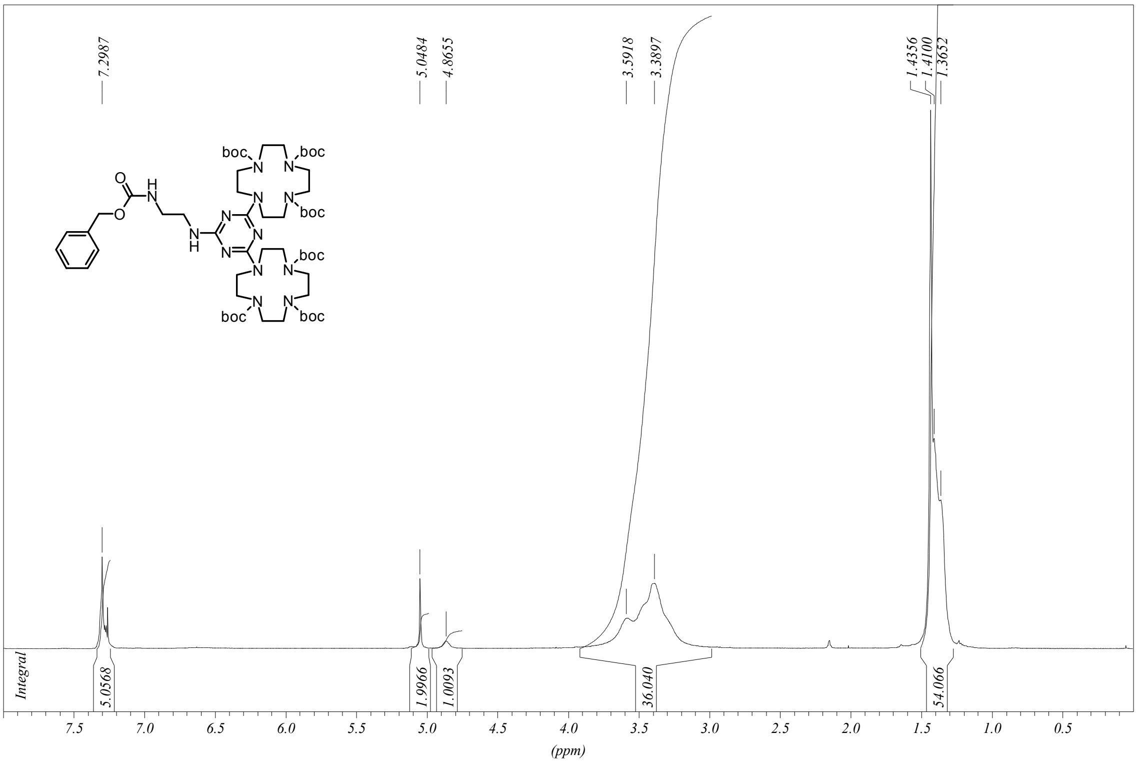


Figure S-9: ^{1}H-NMR spectrum of compound **9a**

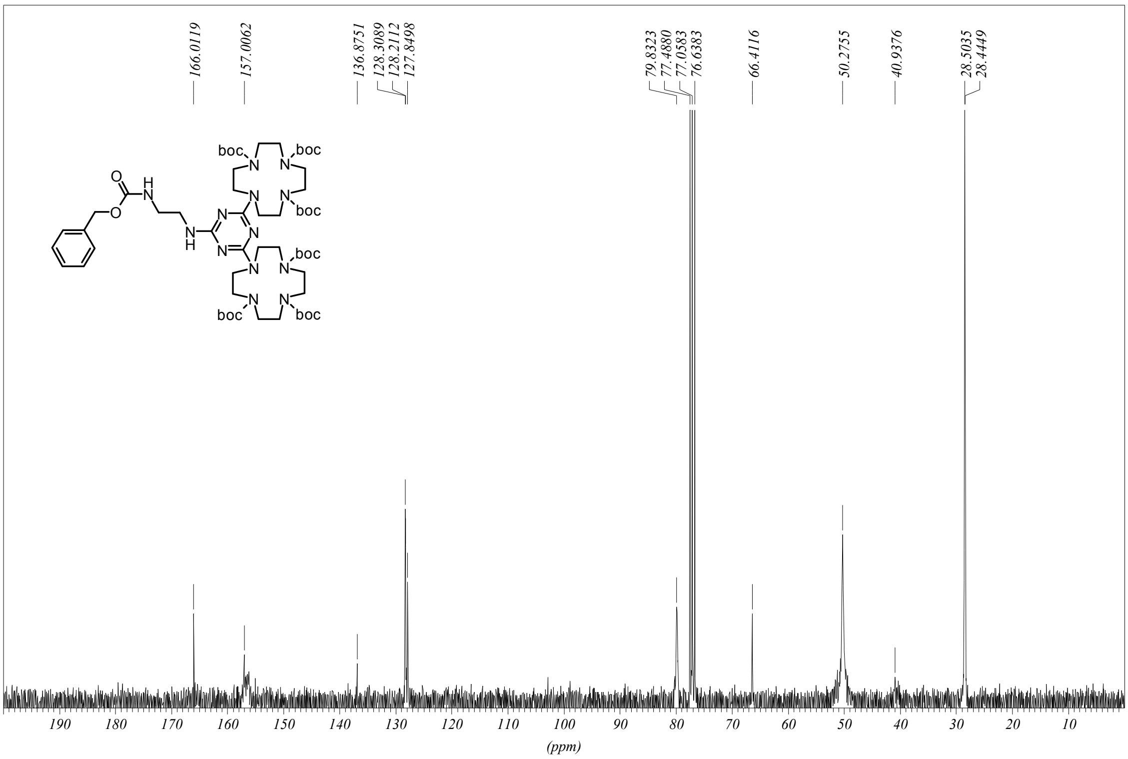


Figure S-10: ^{13}C-NMR spectrum of compound **9a**

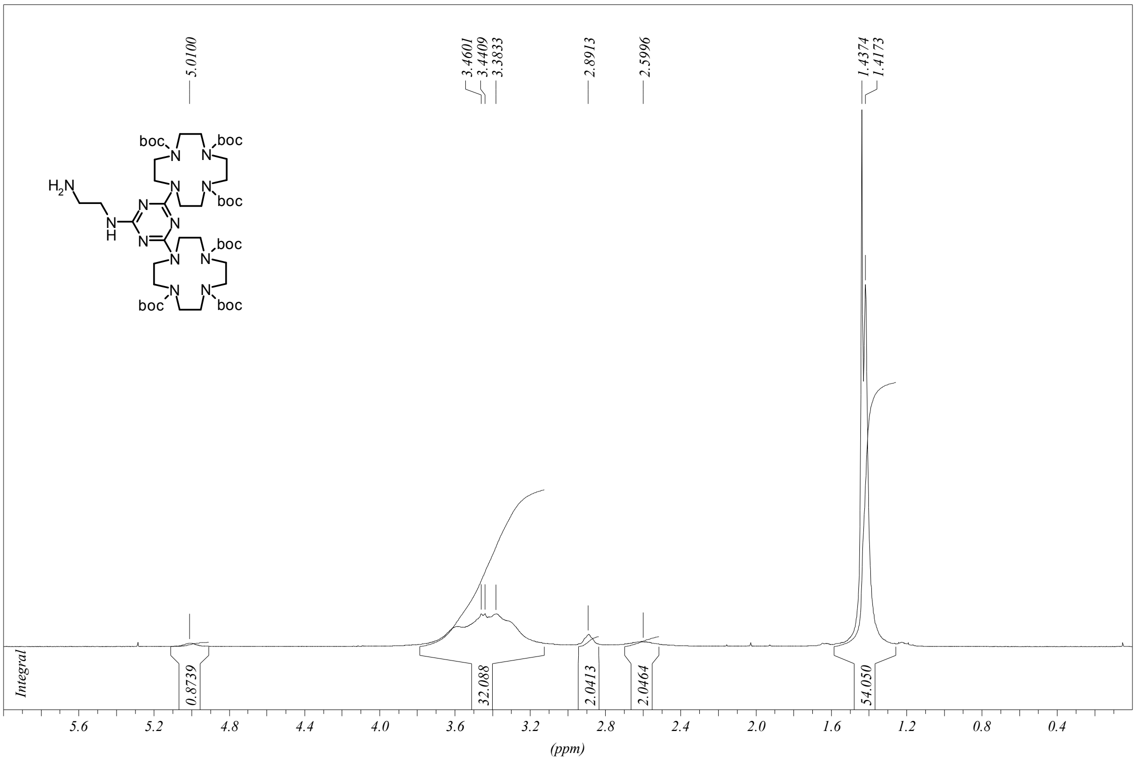


Figure S-11: ^{1}H-NMR spectrum of compound **9b**

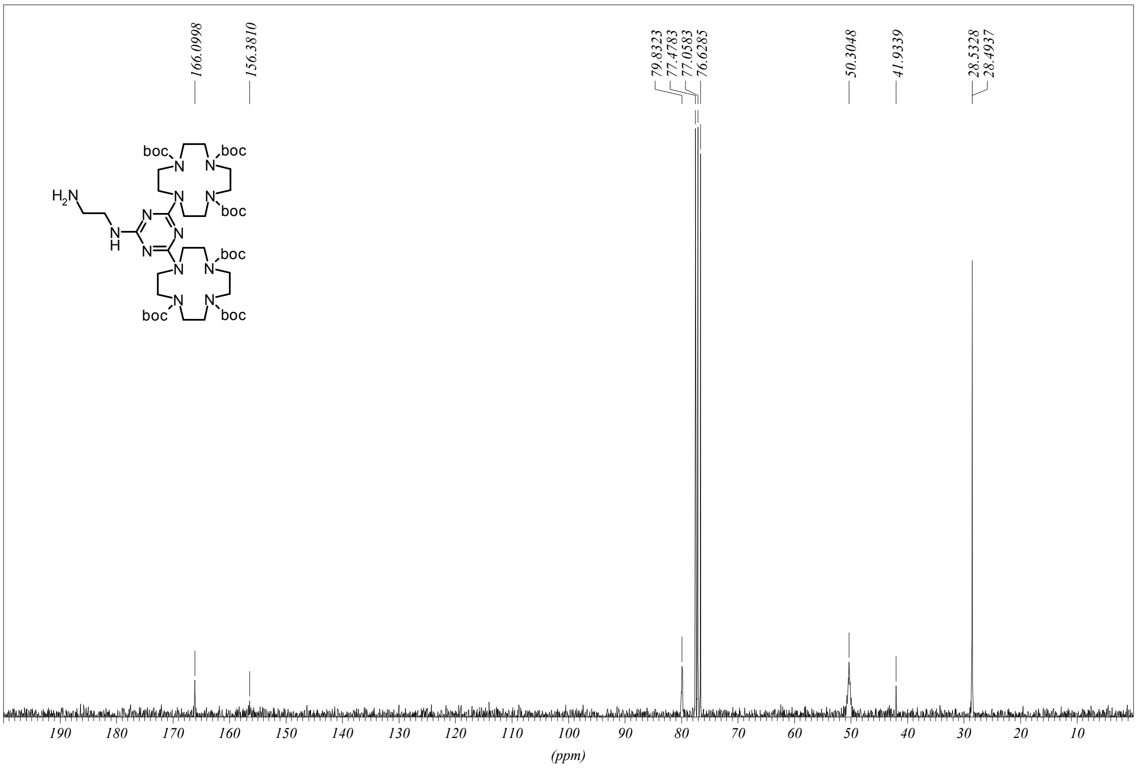


Figure S-12: ^{13}C-NMR spectrum of compound **9b**

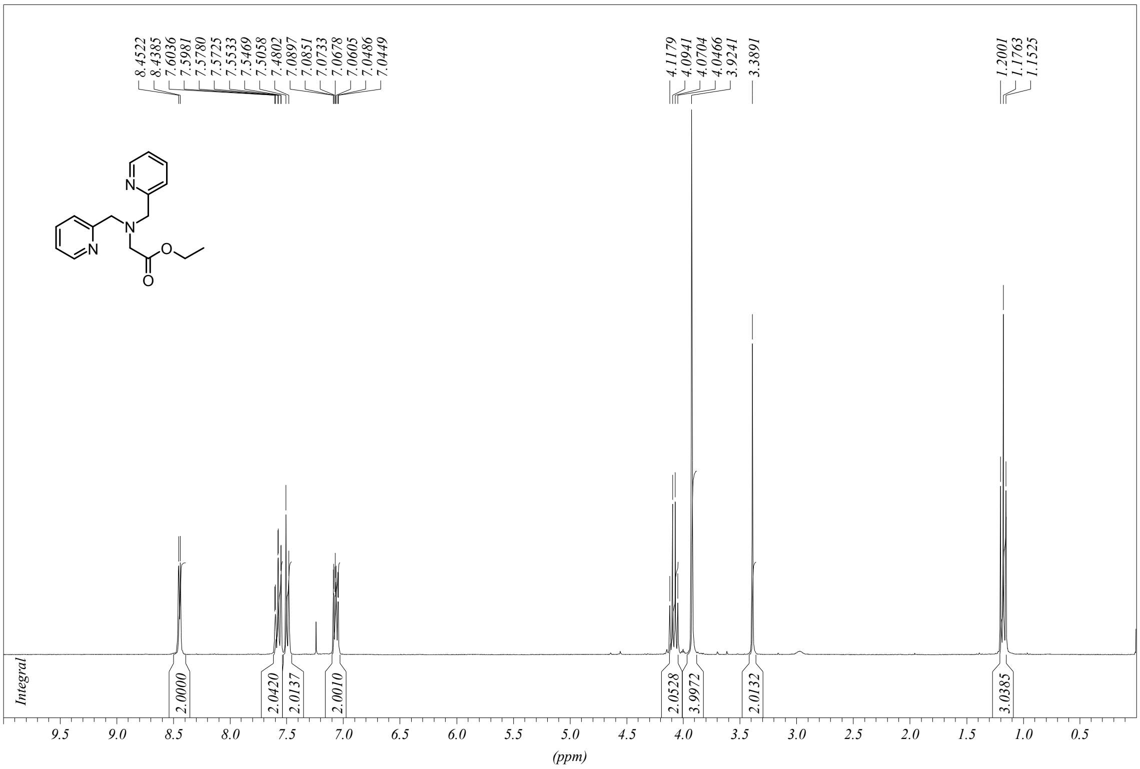


Figure S-13: ^{1}H-NMR spectrum of compound **22a**

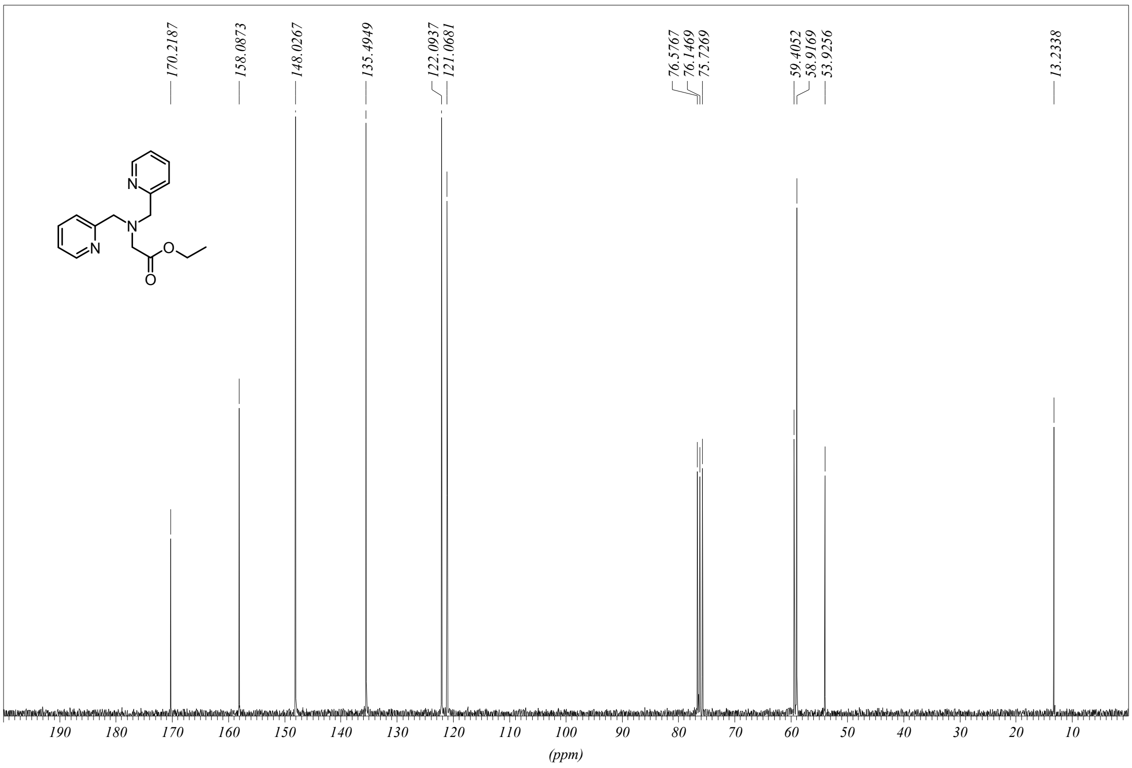


Figure S-14: ^{13}C-NMR spectrum of compound **22a**

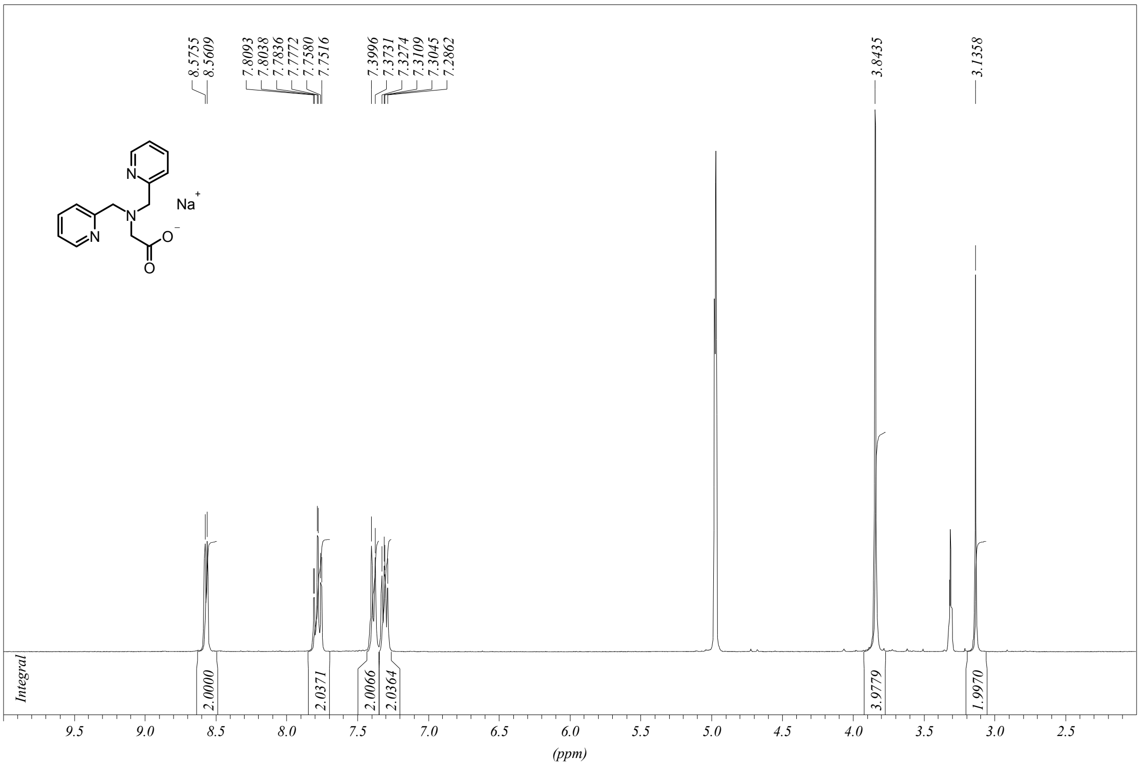


Figure S-15: ^{1}H-NMR spectrum of compound **22b**

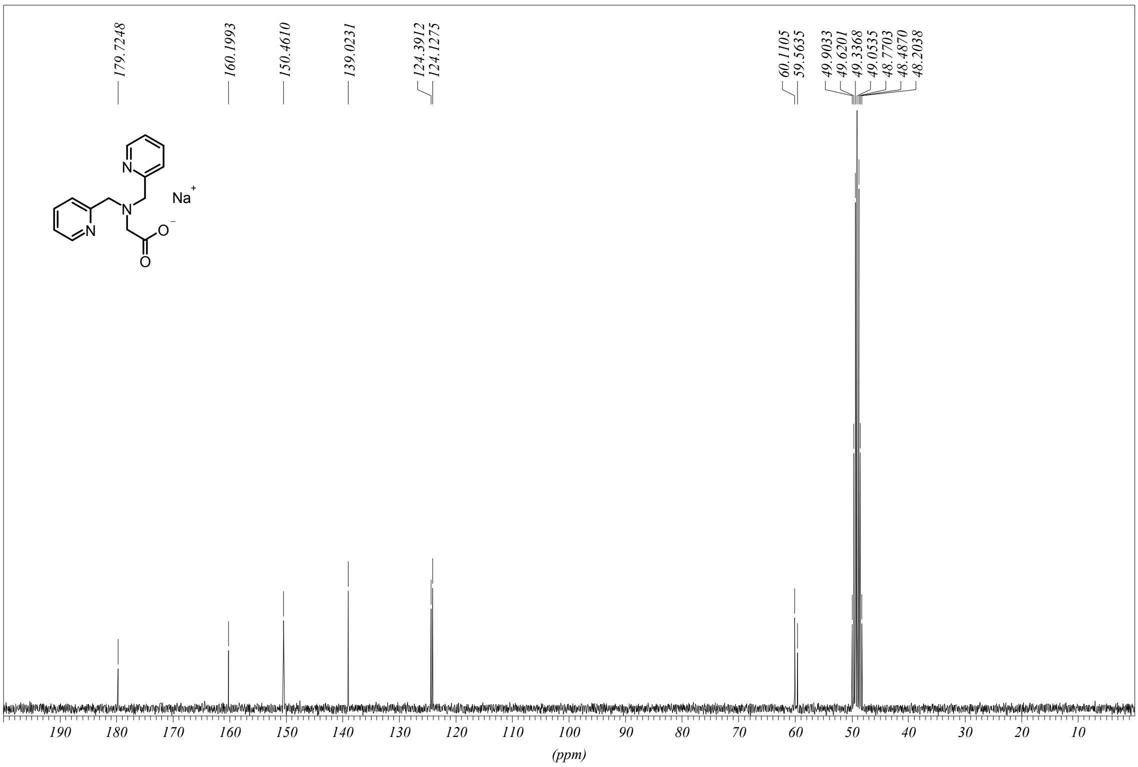


Figure S-16: ^{13}C-NMR spectrum of compound **22b**

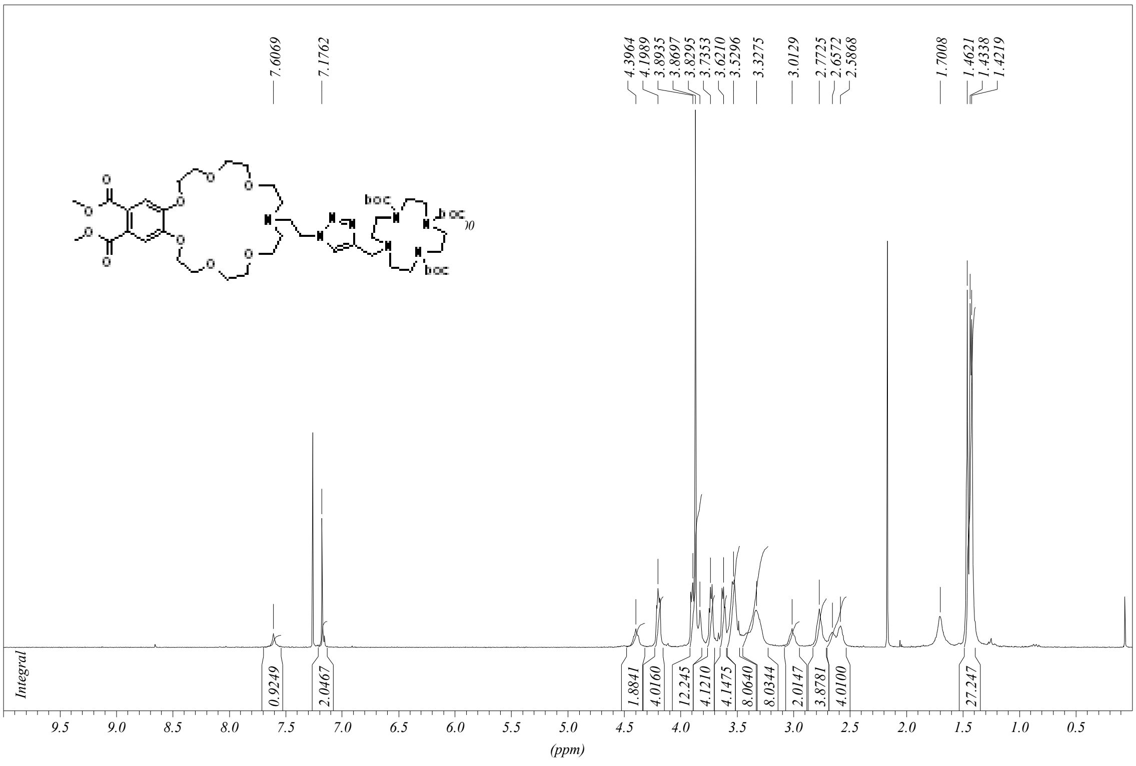


Figure S-17: ^{1}H-NMR spectrum of compound **19a**

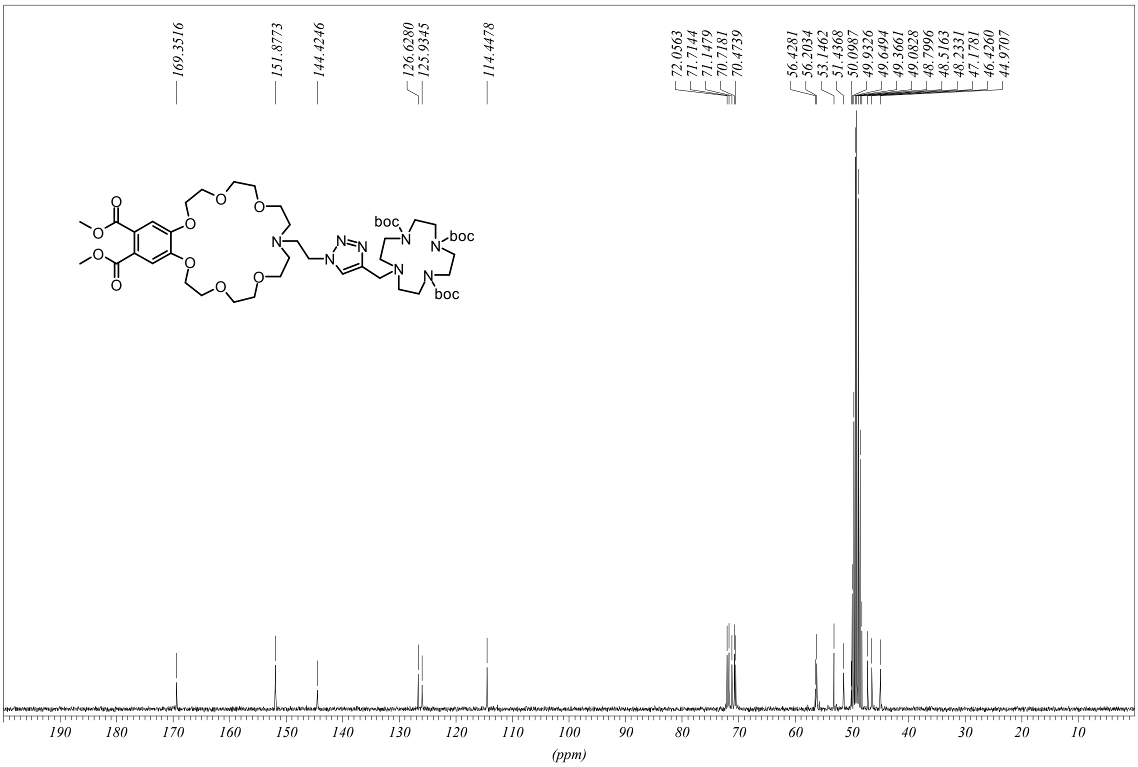


Figure S-18: ^{13}C-NMR spectrum of compound **19a**

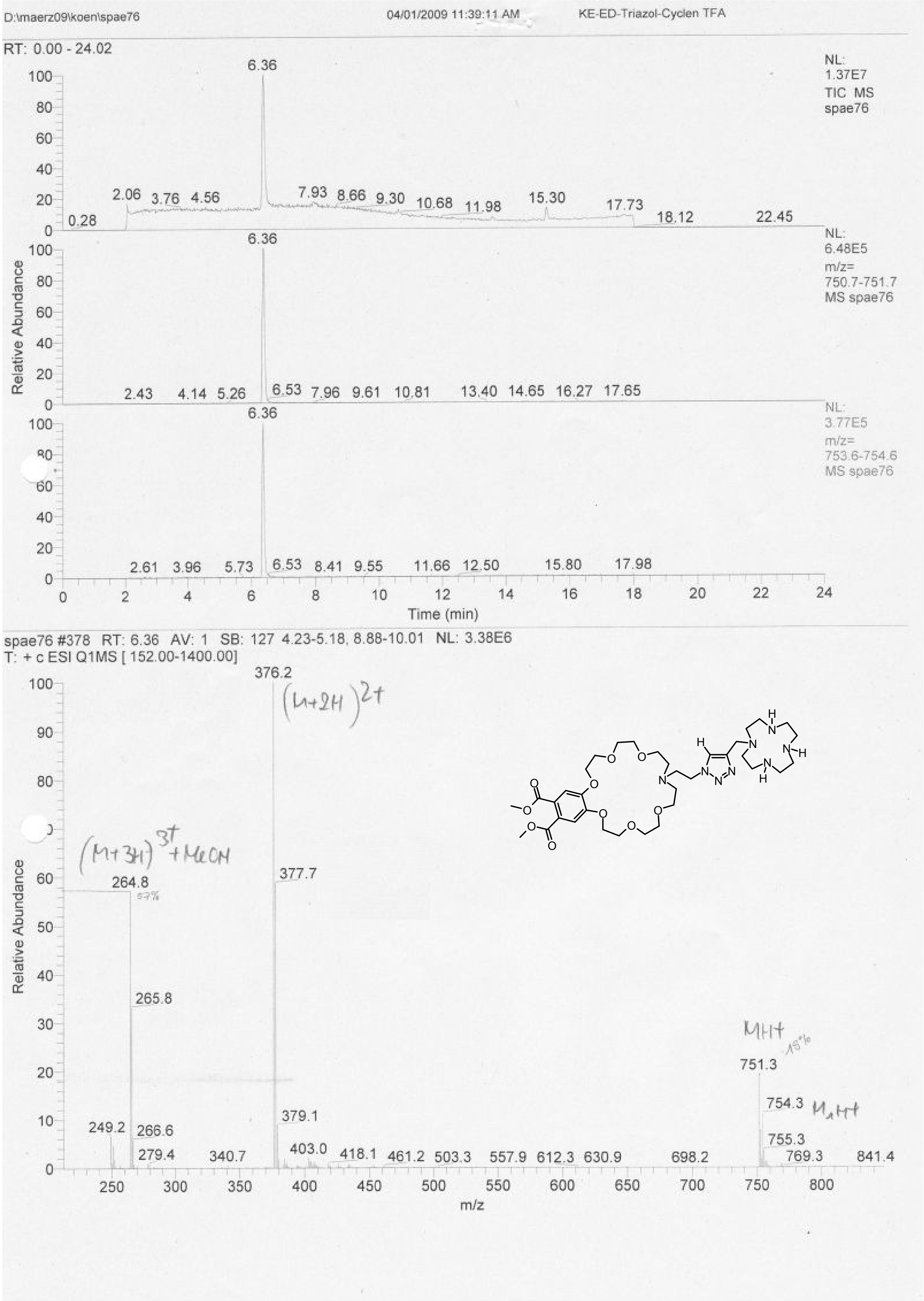


Figure S-19: Mass spectrum of compound **19b**

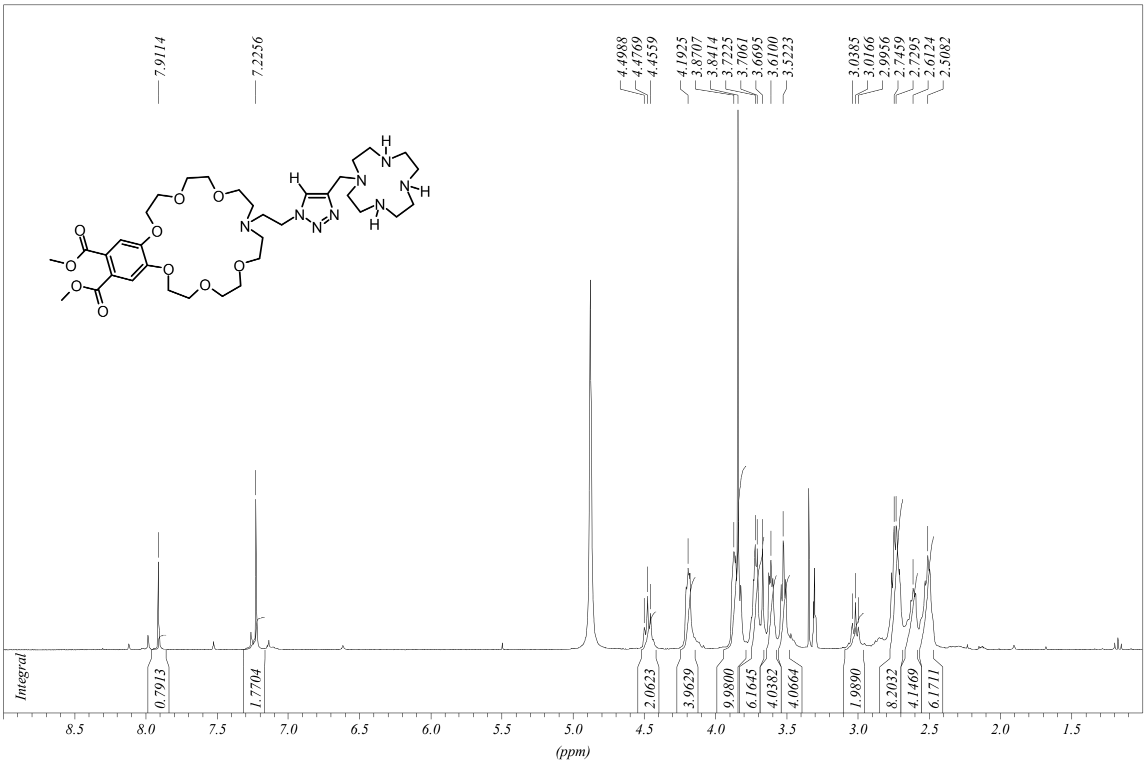


Figure S-20: ^{1}H-NMR spectrum of compound **19b**

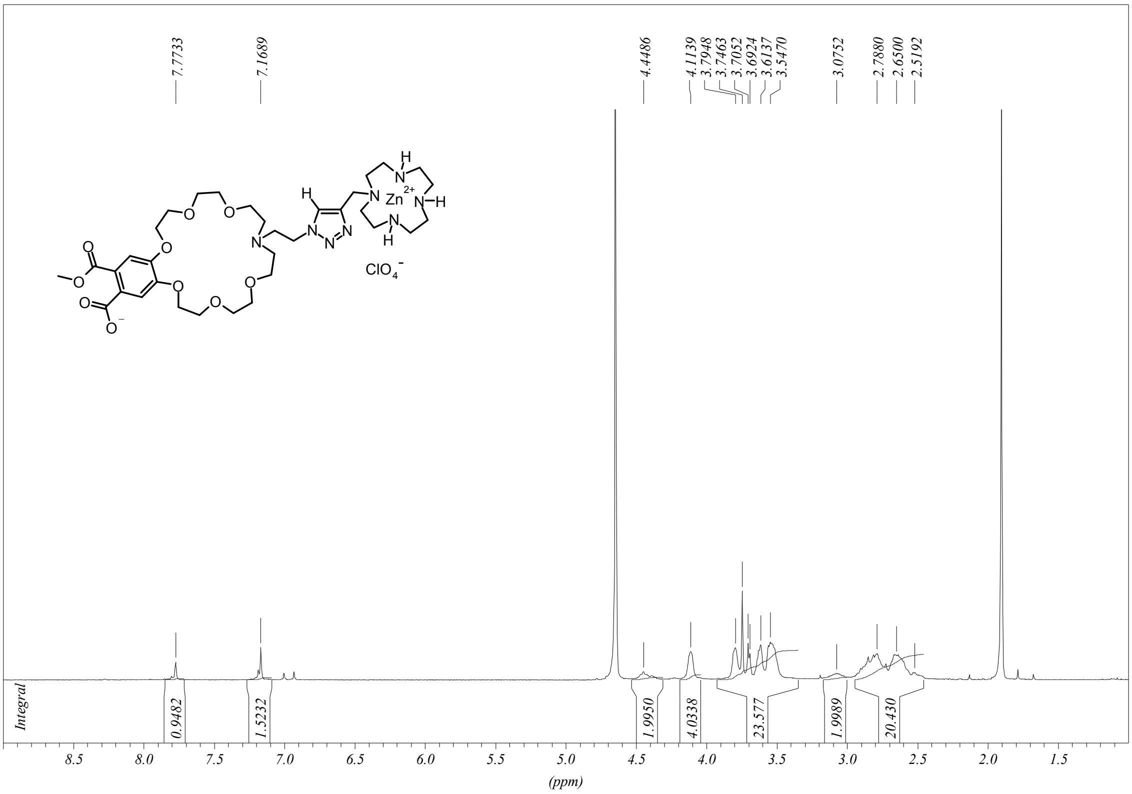


Figure S-21: ^{1}H-NMR spectrum of compound **5**

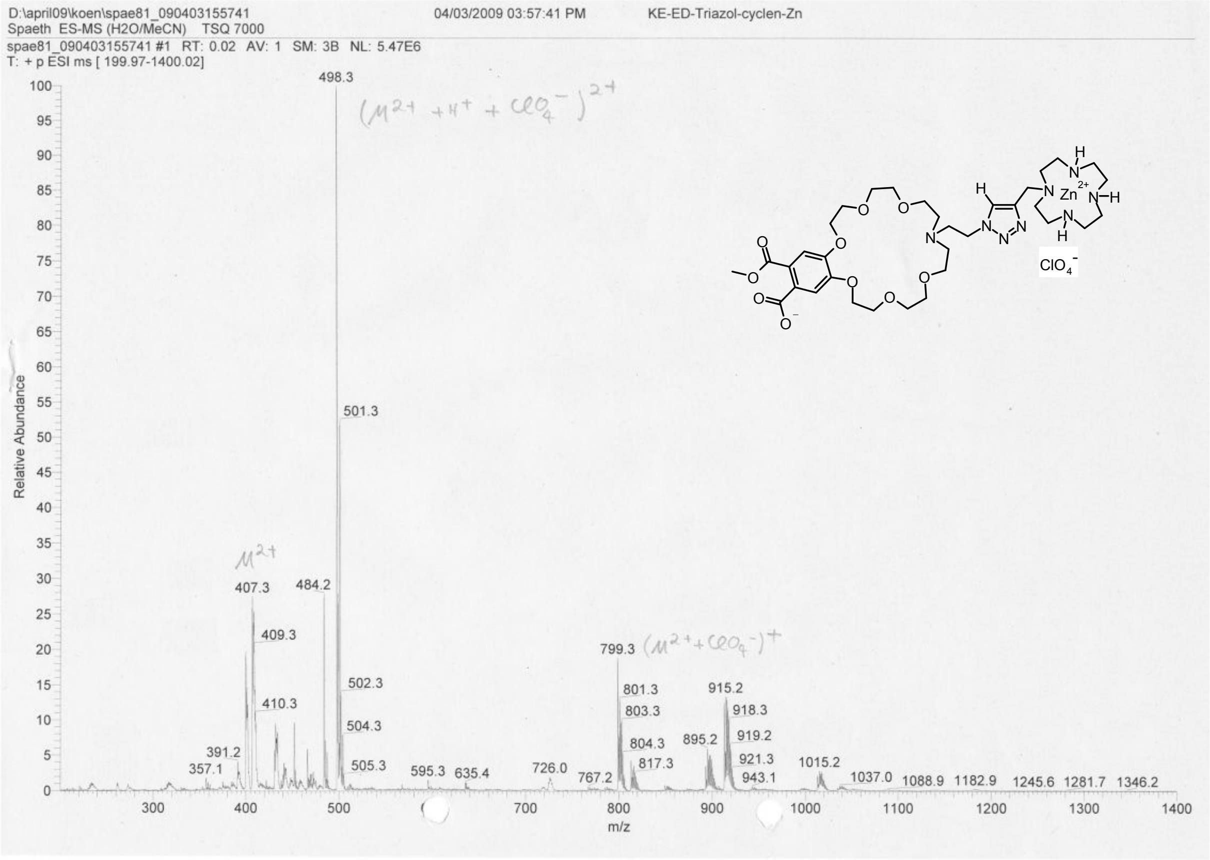


Figure S-22: Mass spectrum of compound **5**

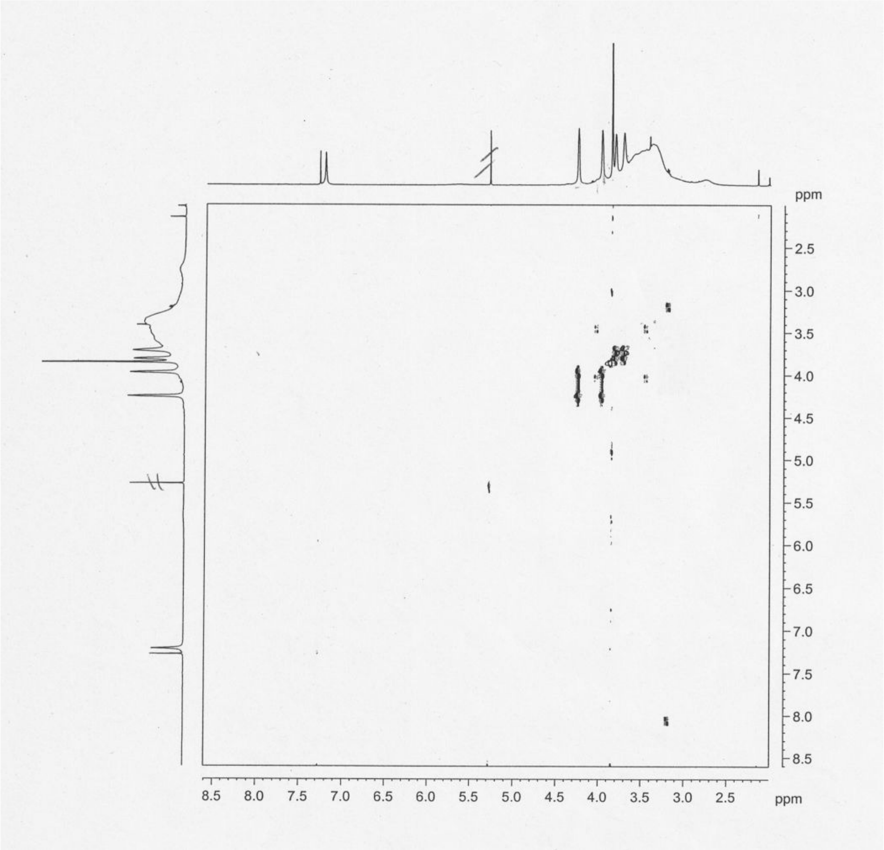


Figure S-23: COSY spectrum of compound **11a**

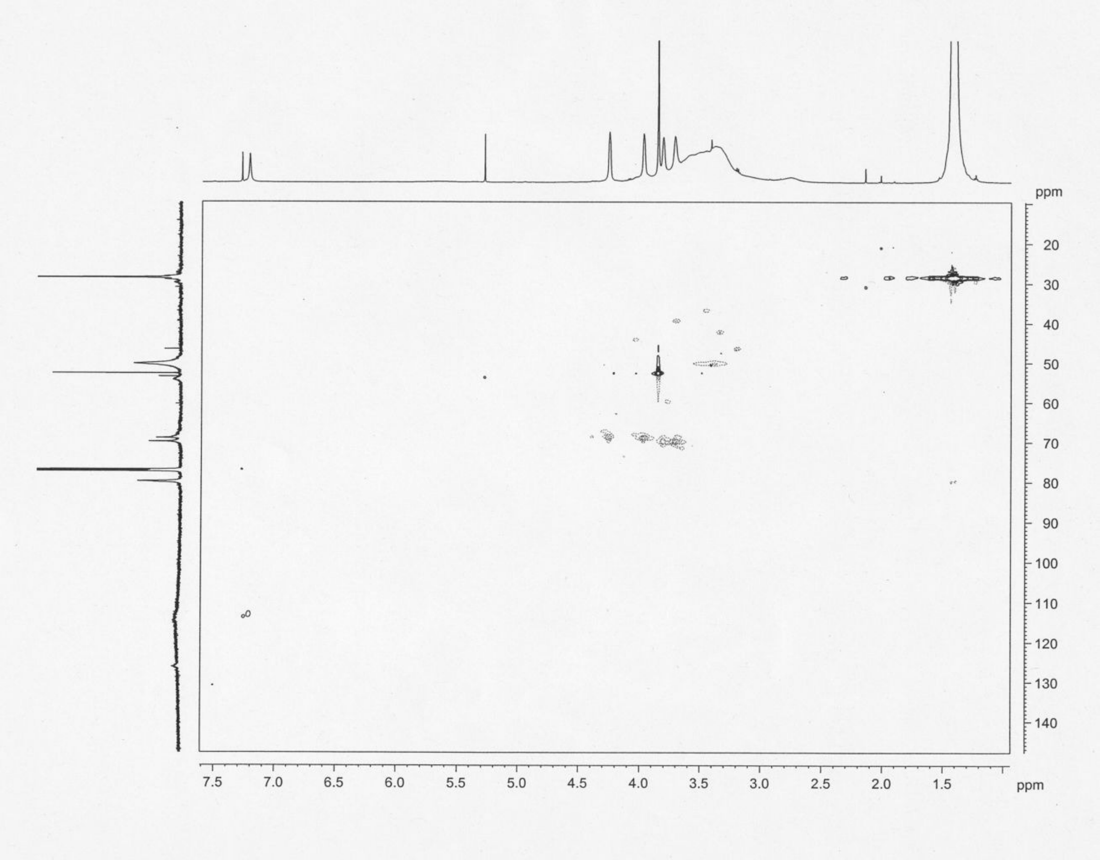


Figure S-24: HSQC spectrum of compound **11a**

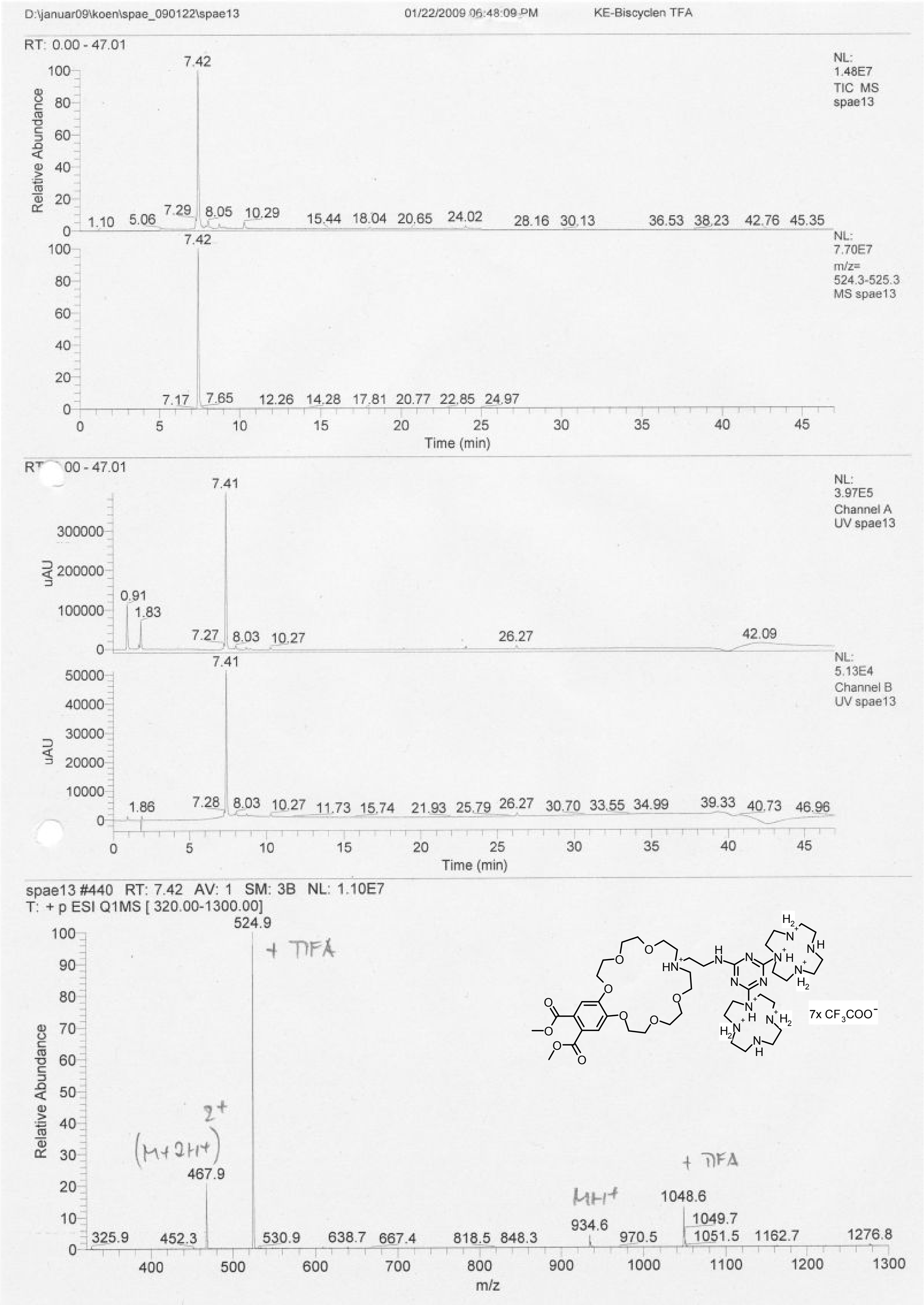


Figure S-25: Mass spectrum of compound **15a´**

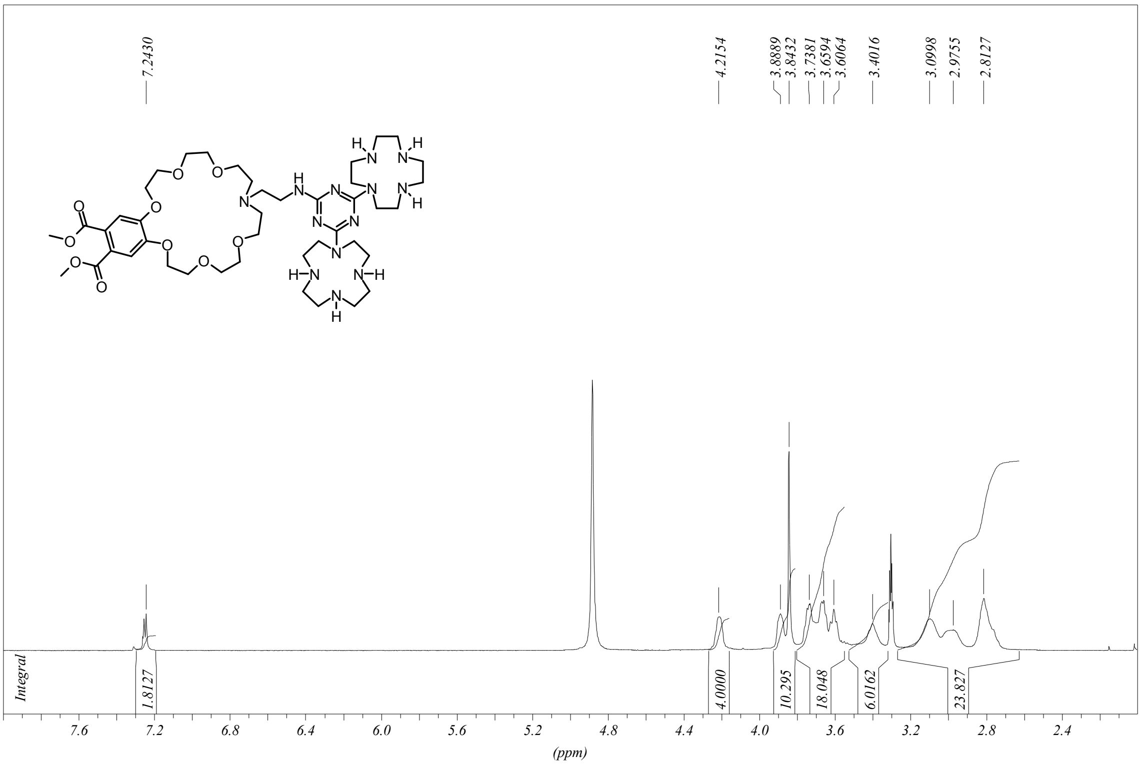


Figure S-26: ^{1}H-NMR spectrum of compound **15a**

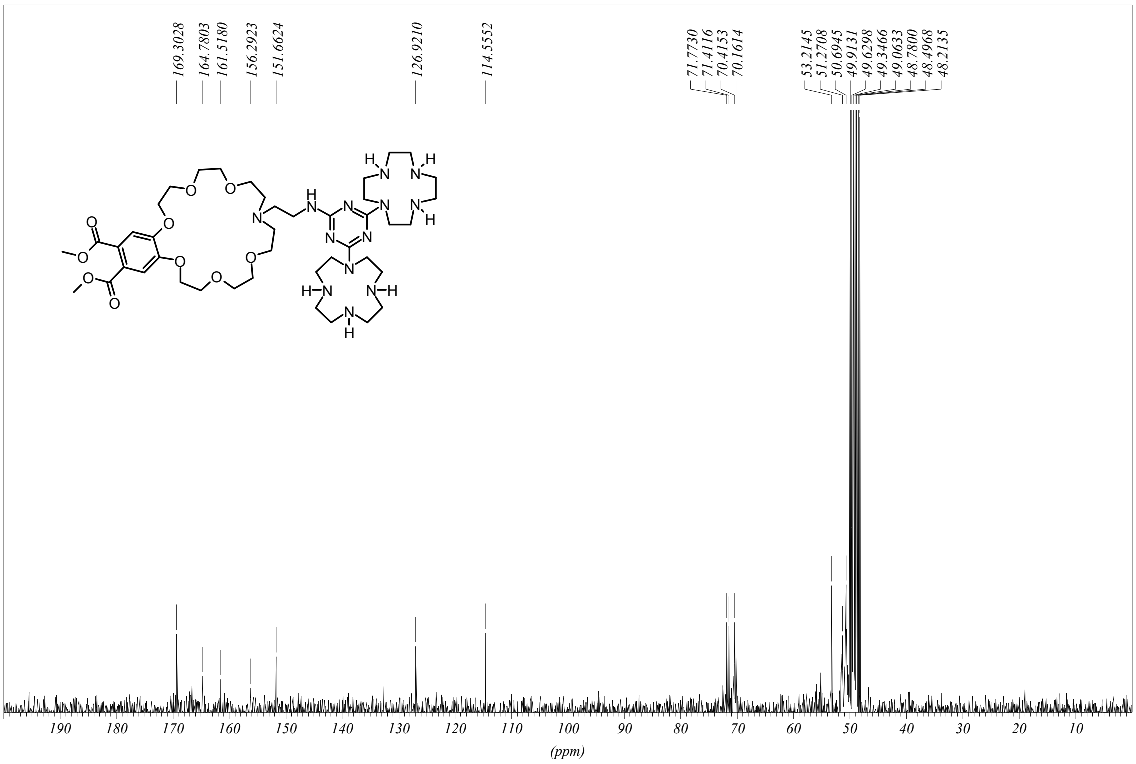


Figure S-27: ^{13}C-NMR spectrum of compound **15a**

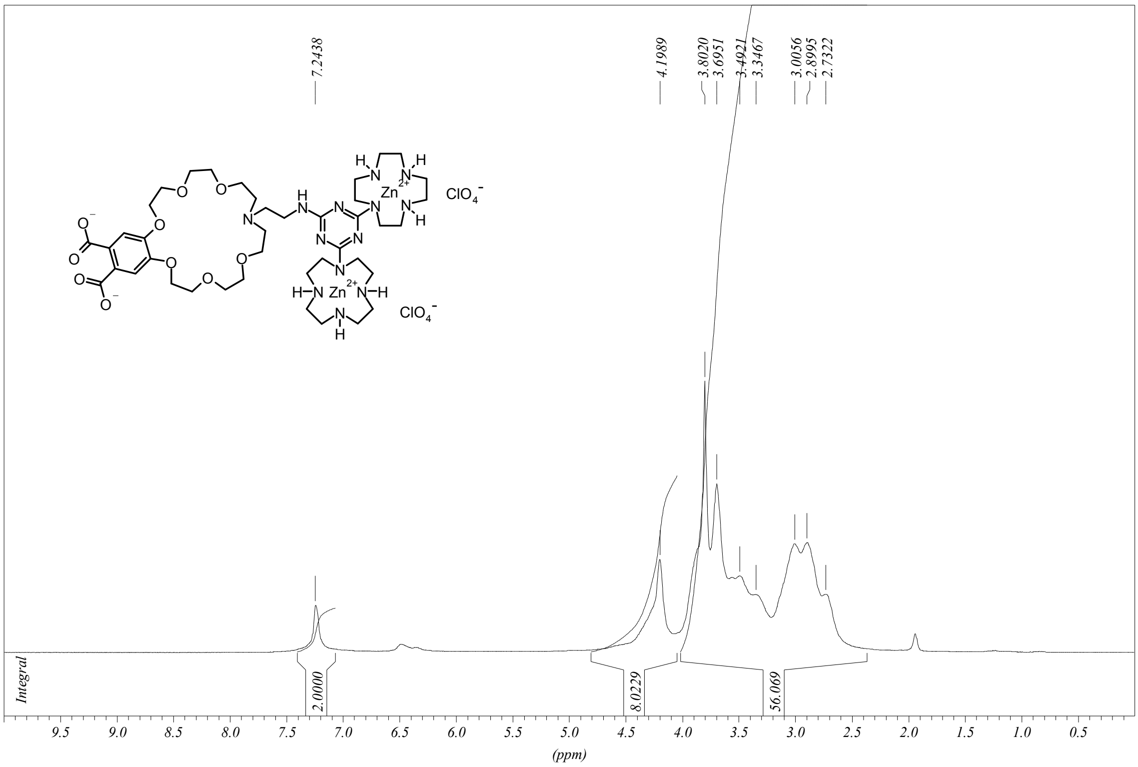


Figure S-28: ^{1}H-NMR spectrum of compound **3a**

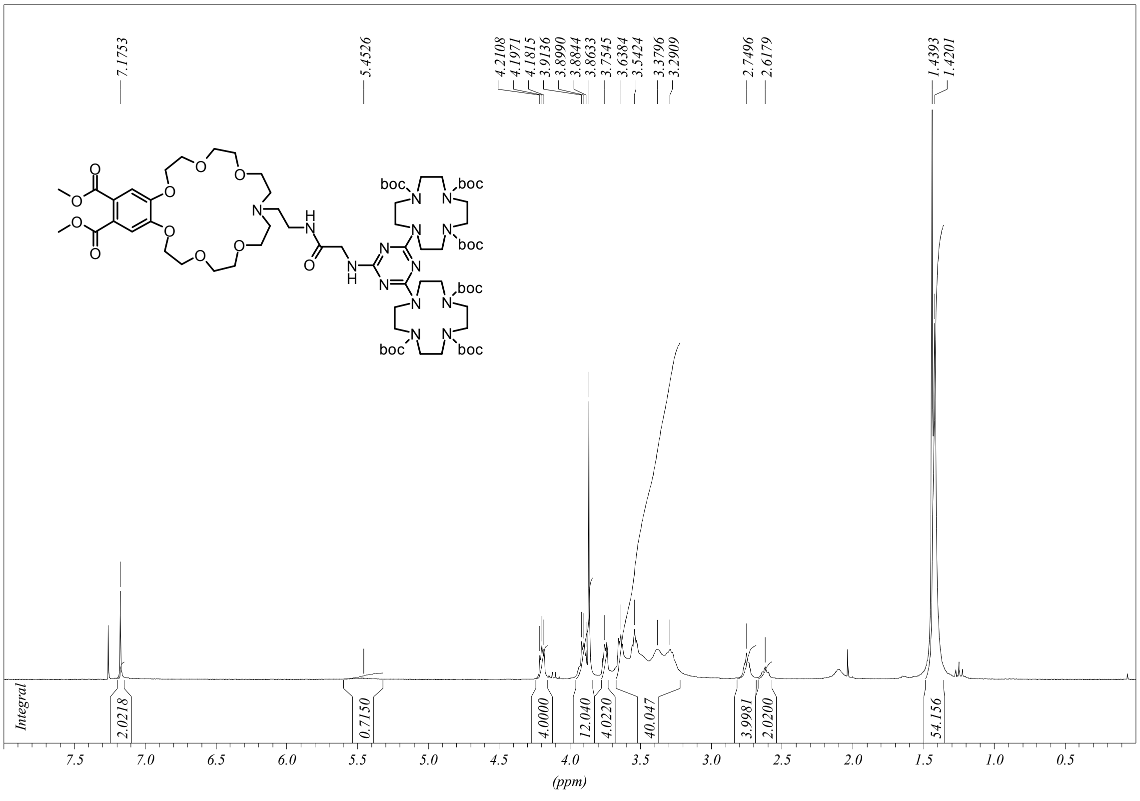


Figure S-29: ^{1}H-NMR spectrum of compound **11b**

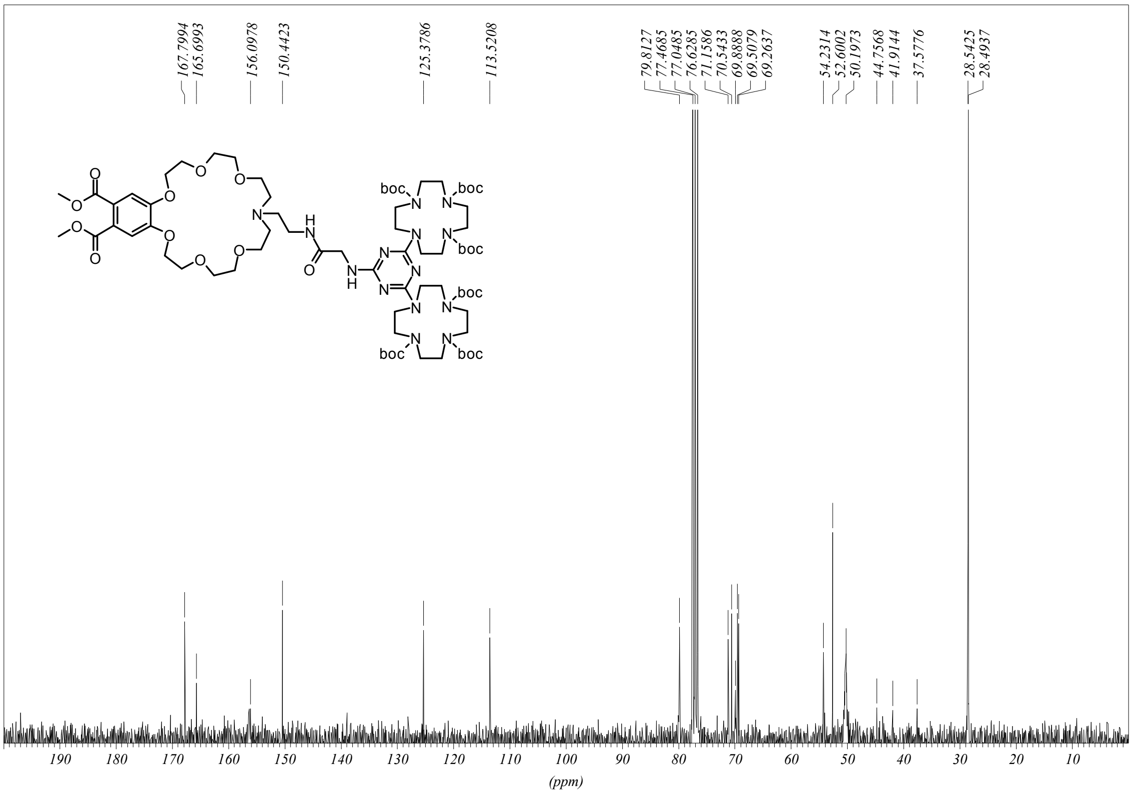


Figure S-30: ^{13}C-NMR spectrum of compound **11b**

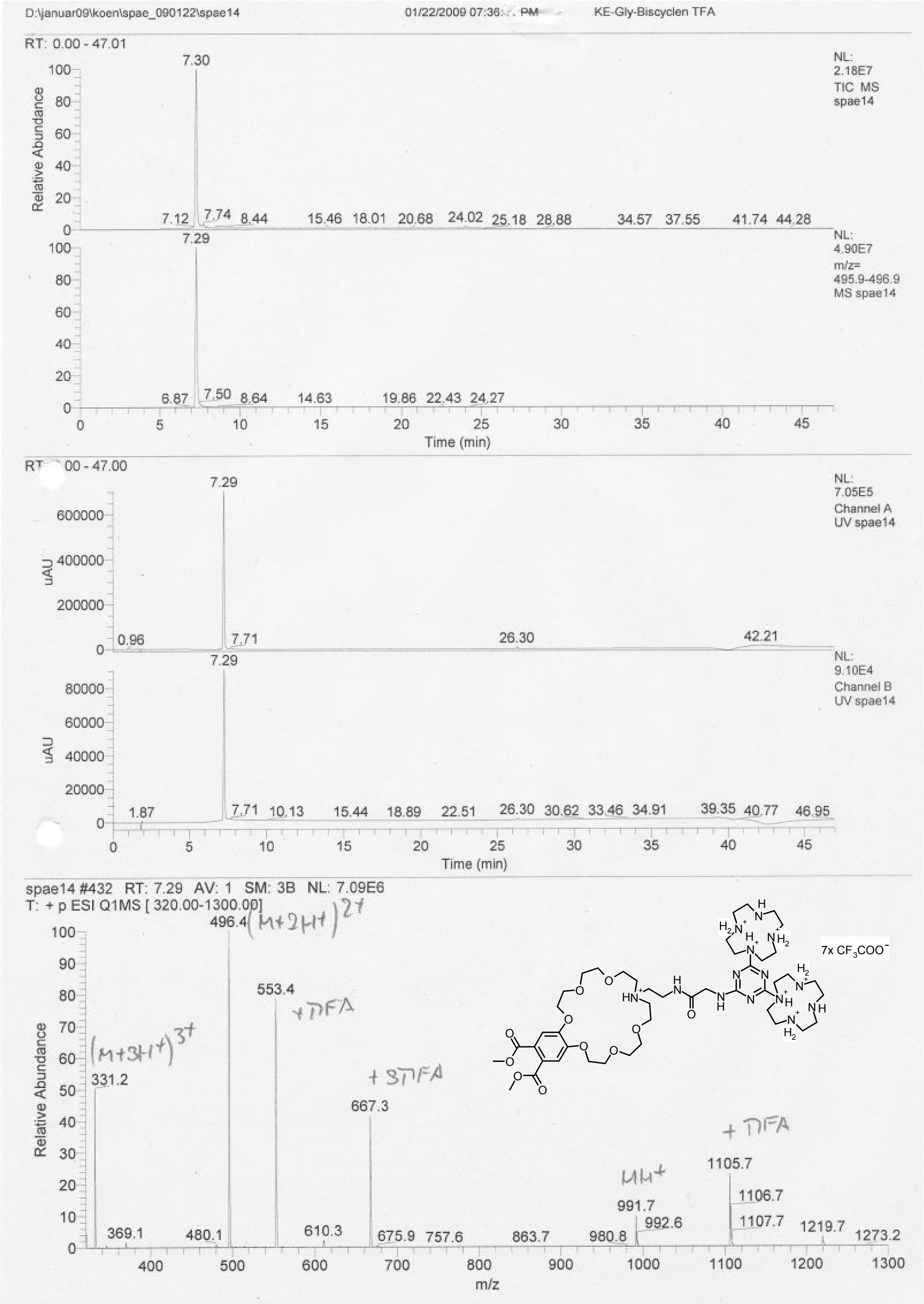


Figure S-31: Mass spectrum of compound **15b´**

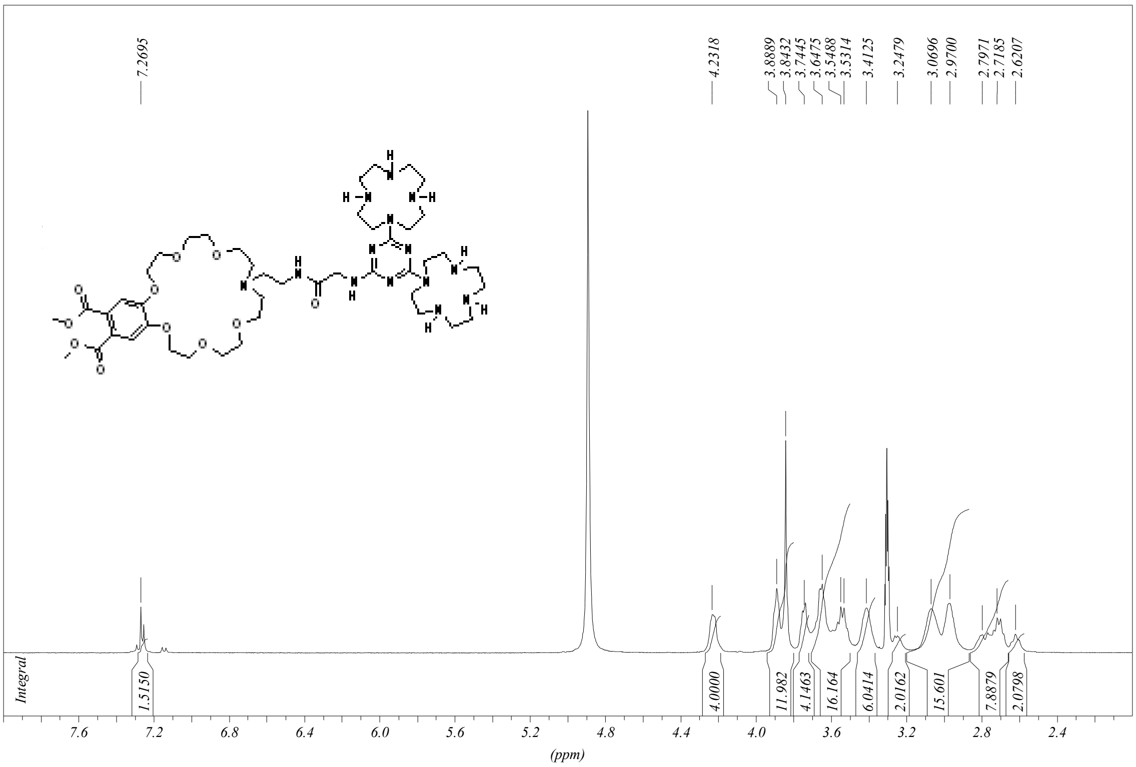


Figure S-32: ^{1}H-NMR spectrum of compound **15b**

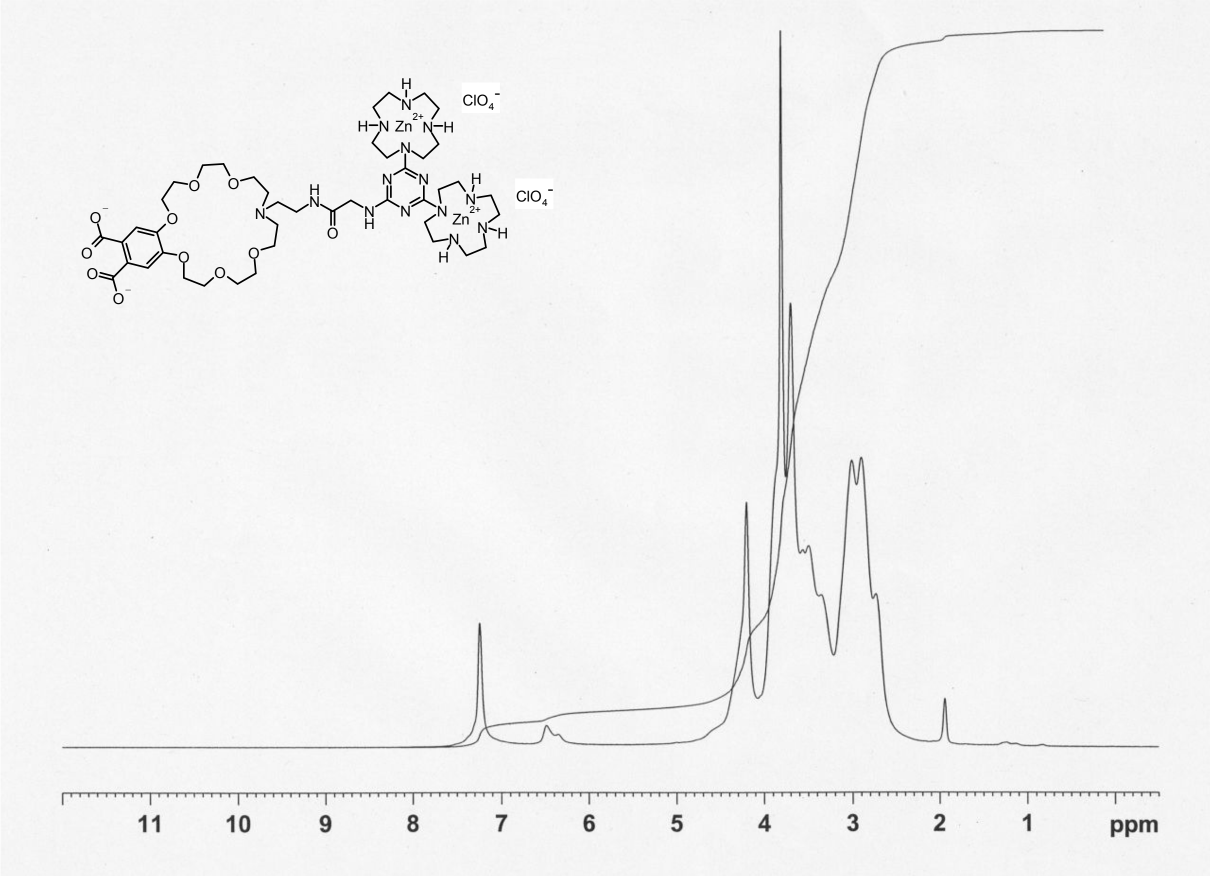


Figure S-33: ^{1}H-NMR spectrum of compound **3b**

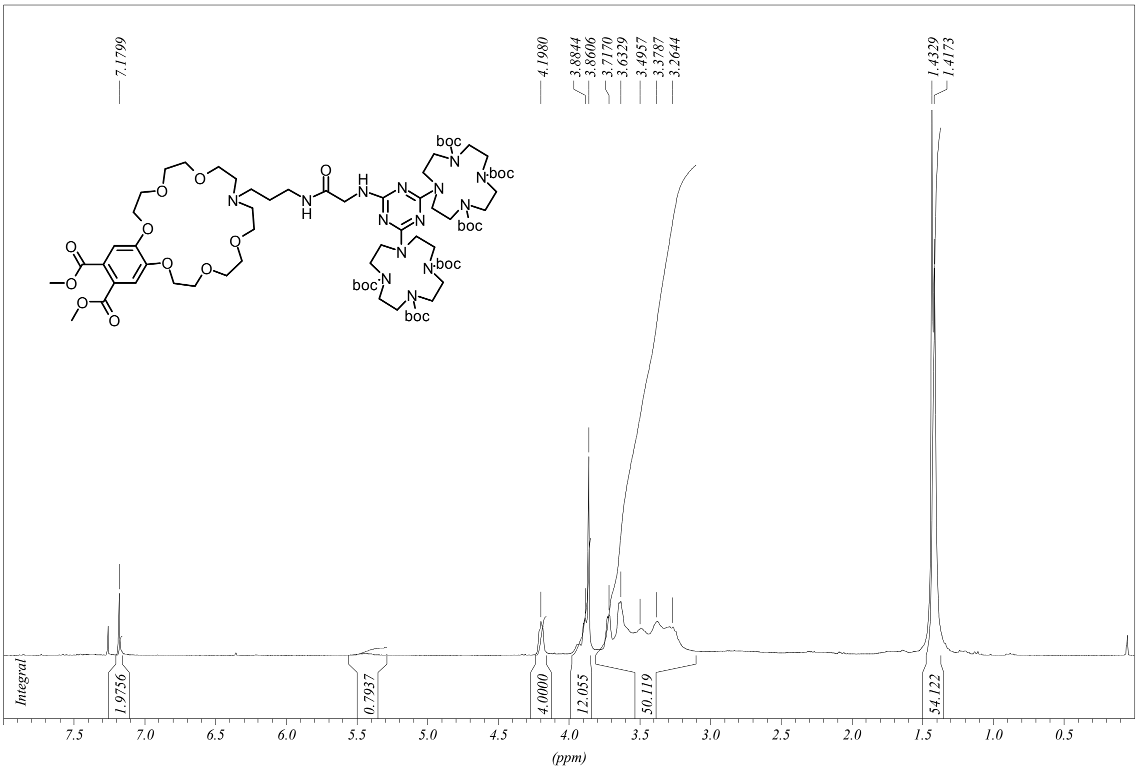


Figure S-34: ^{1}H-NMR spectrum of compound **11c**

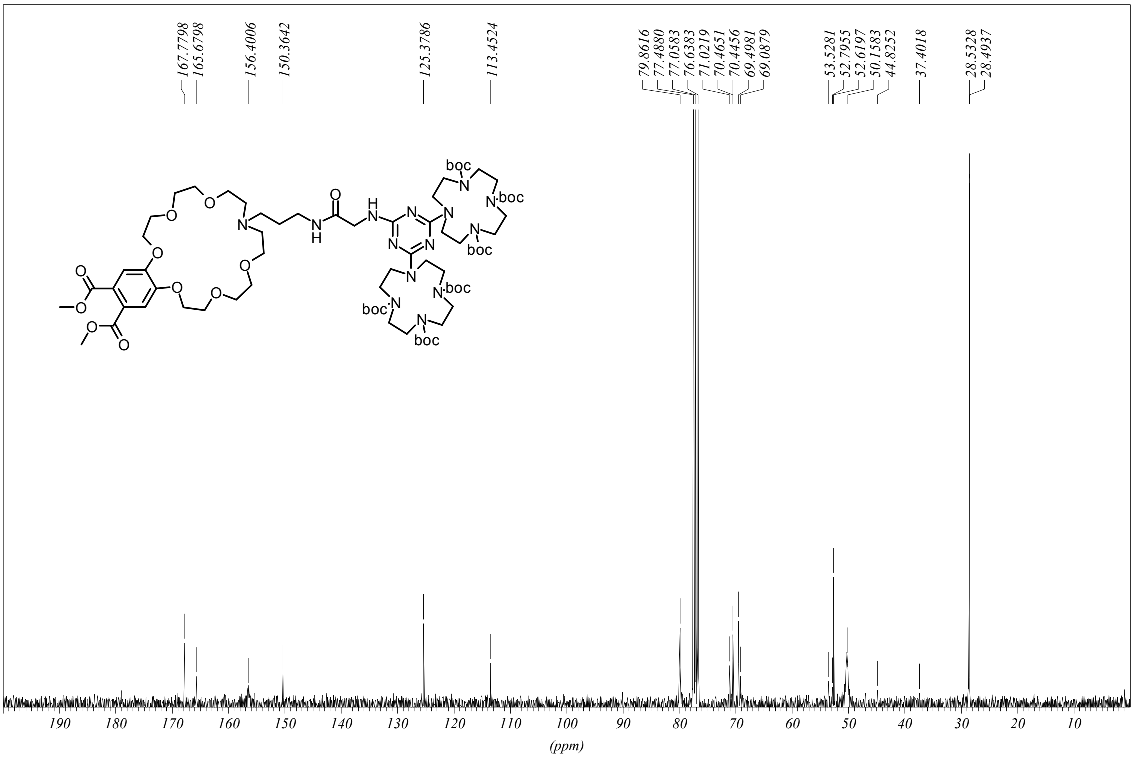


Figure S-35: ^{13}C-NMR spectrum of compound **11c**

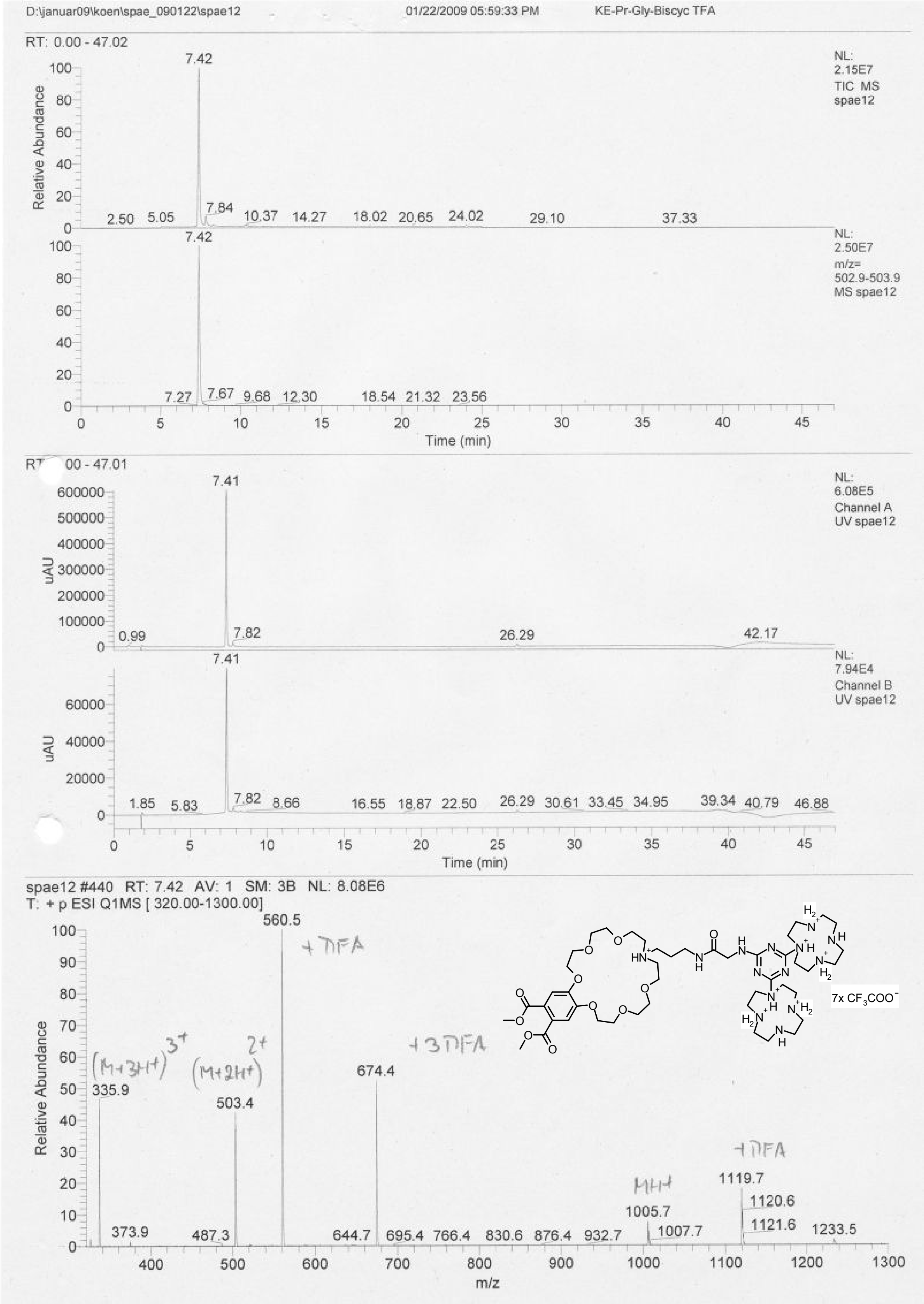


Figure S-36: Mass spectrum of compound **15c´**

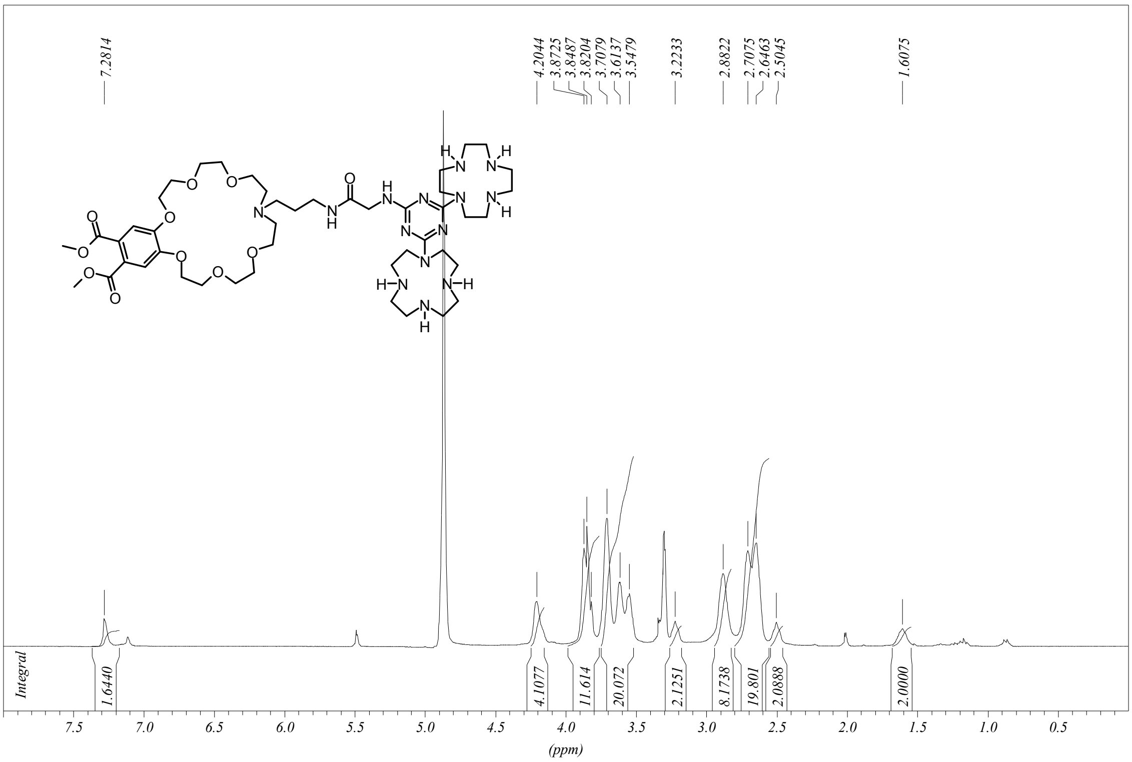


Figure S-37: ^{1}H-NMR spectrum of compound **15c**

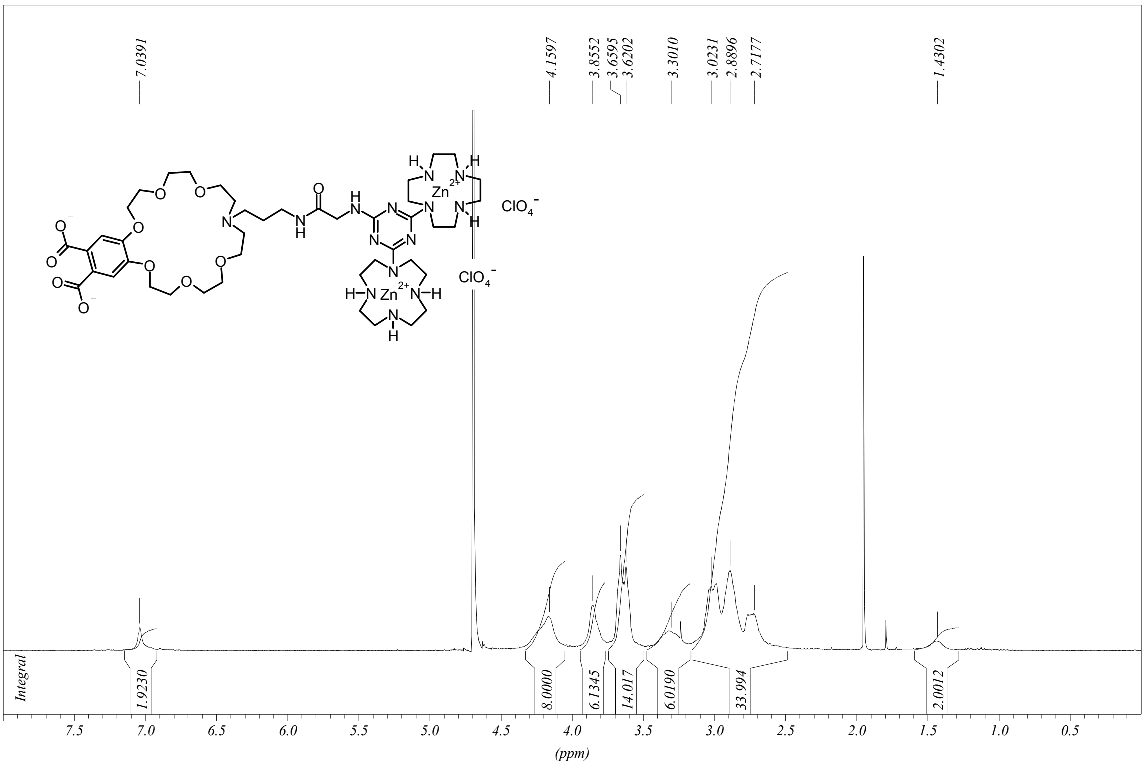


Figure S-38: ^{1}H-NMR spectrum of compound **3c**

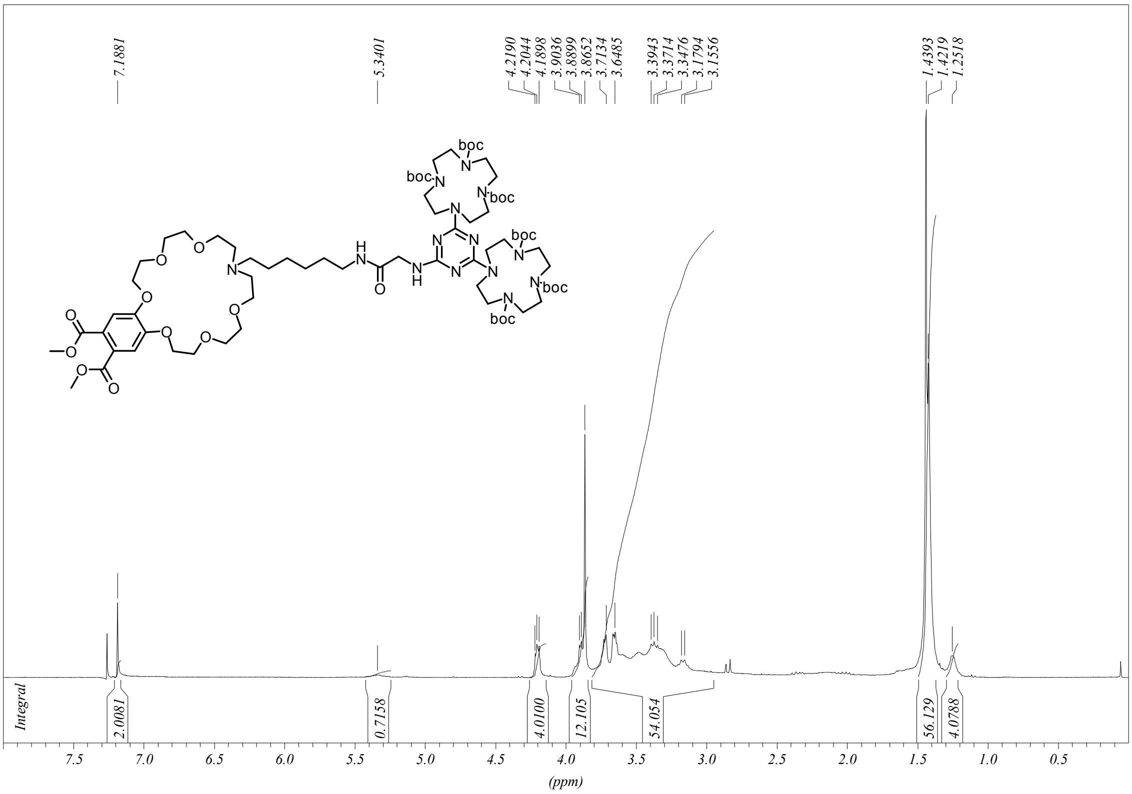


Figure S-39: ^{1}H-NMR spectrum of compound **11e**

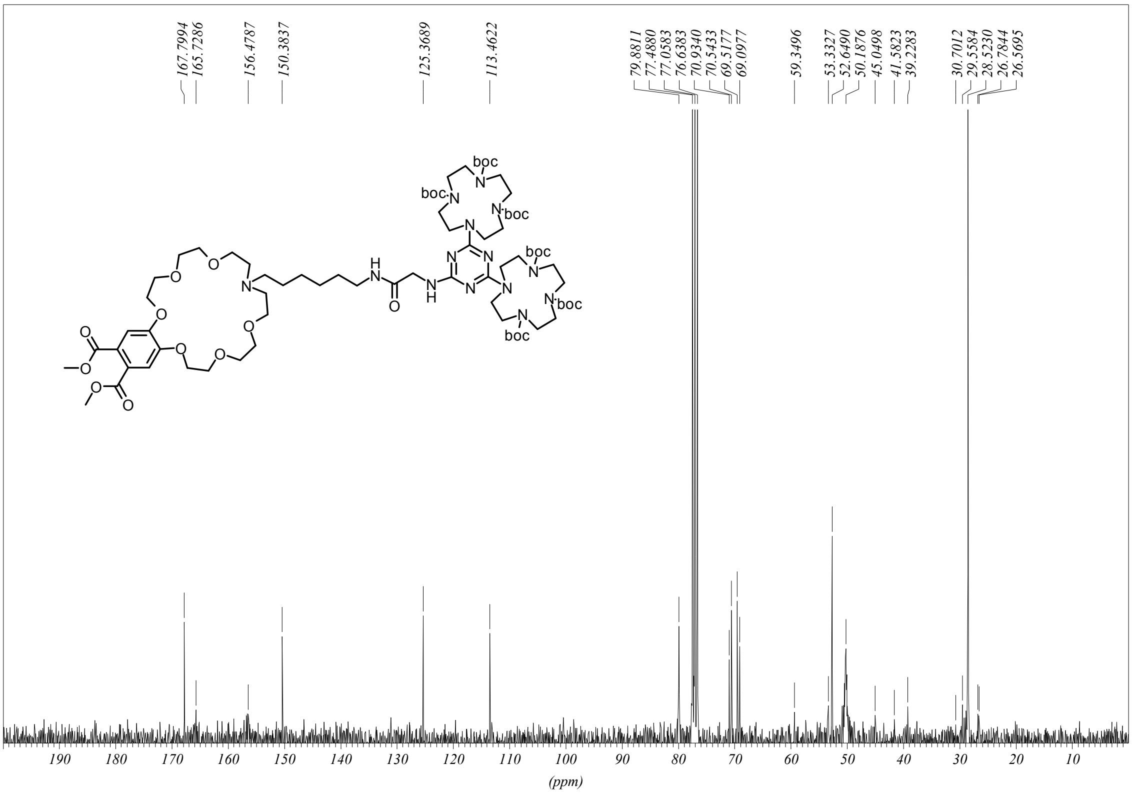
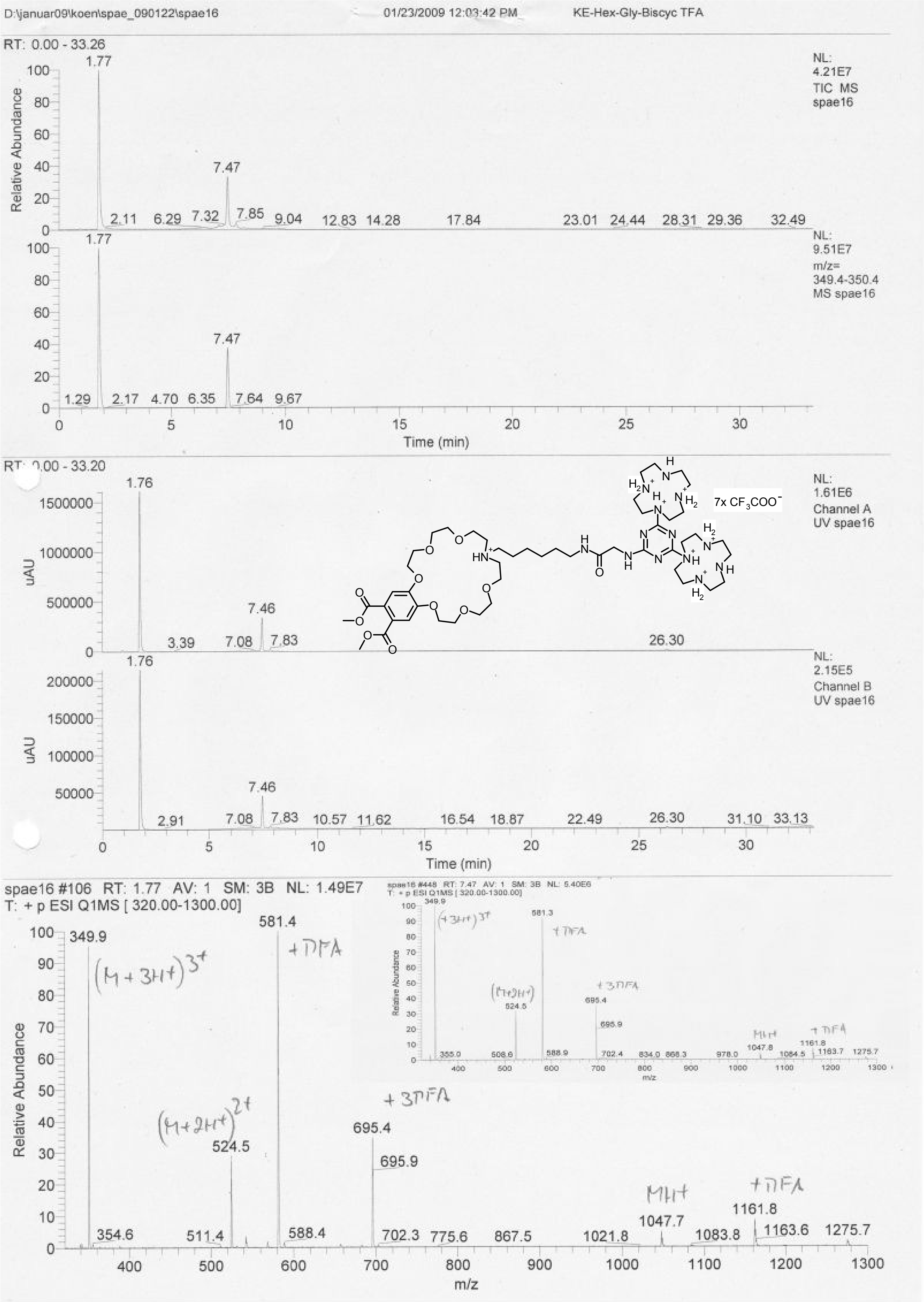


Figure S-40: ^{13}C-NMR spectrum of compound **11e**



D:\januar09\koen\spae_090122\spae16 01/23/2009 12:03:42 PM KE-Hex-Gly-Biscyc TFA

spae16 #106 RT: 1.77 AV: 1 SM: 3B NL: 1.49E7
T: + p ESI Q1MS [320.00-1300.00]

spae16 #448 RT: 7.47 AV: 1 SM: 3B NL: 5.40E6
T: + p ESI Q1MS [320.00-1300.00]

Figure S-41: Mass spectrum of compound **15e´**

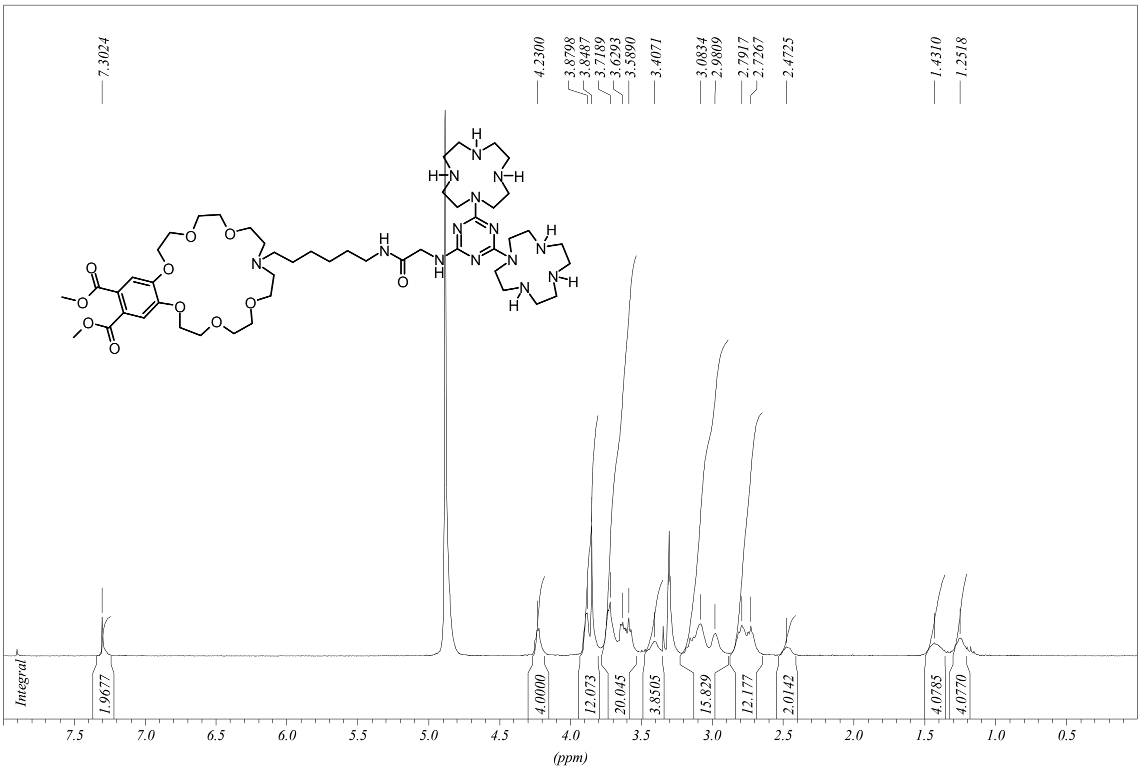


Figure S-42: ^{1}H-NMR spectrum of compound **15e**

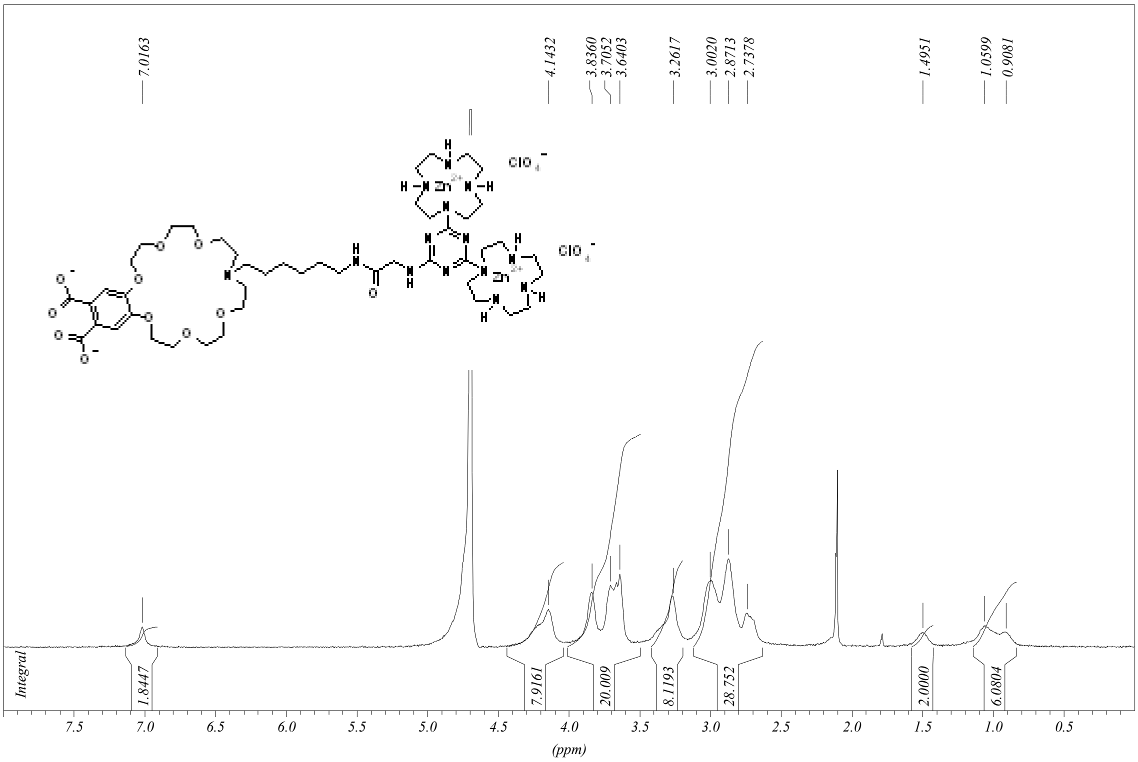


Figure S-43: ^{1}H-NMR spectrum of compound **3e**

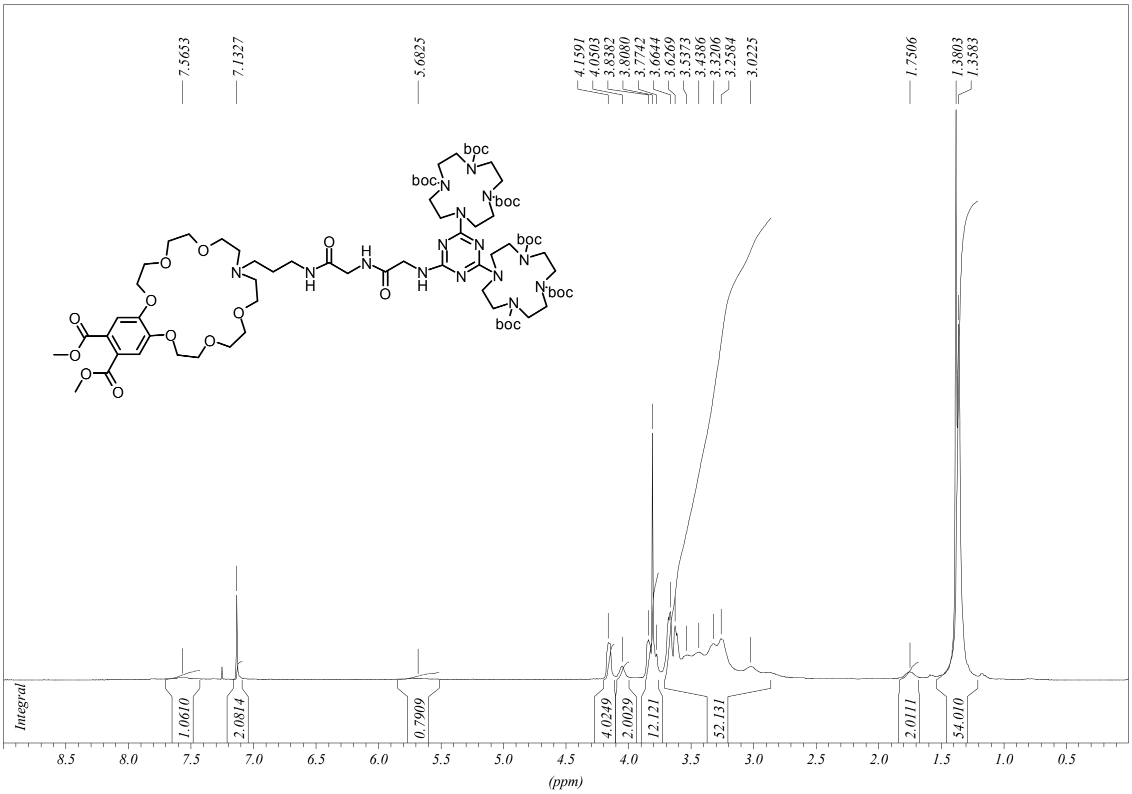


Figure S-44: ^{1}H-NMR spectrum of compound **11d**

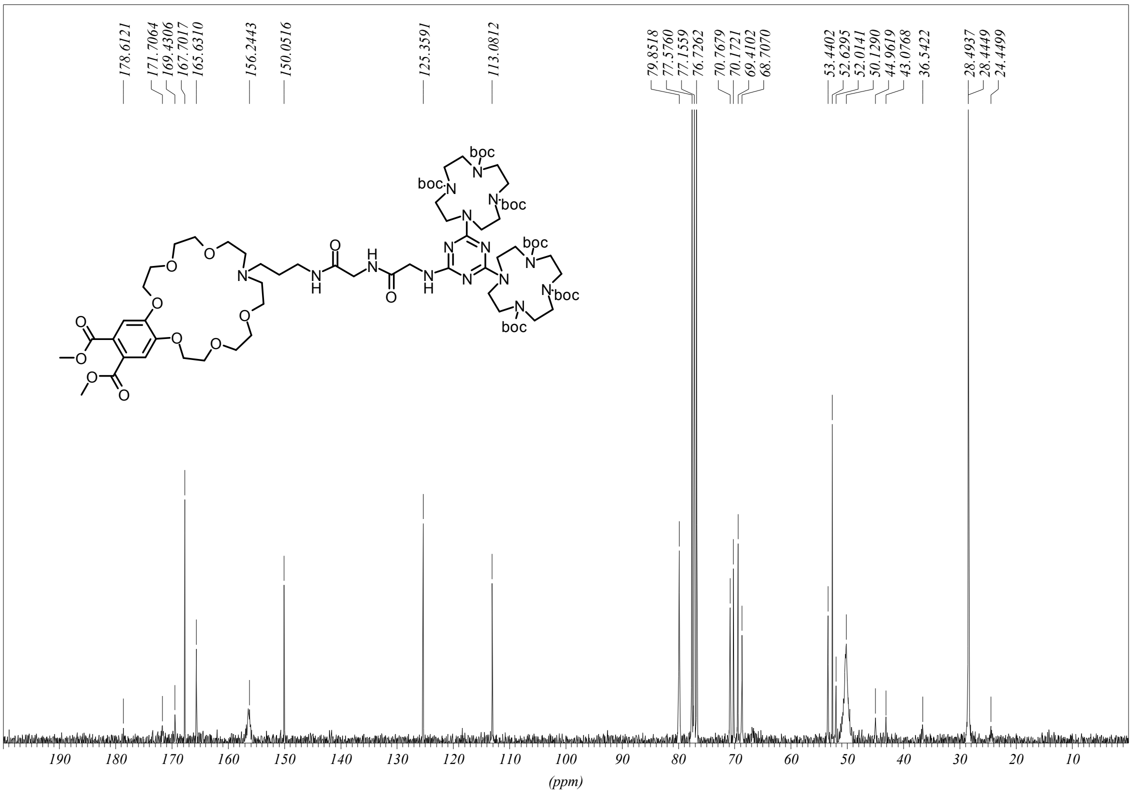


Figure S-45: ^{13}C-NMR spectrum of compound **11d**

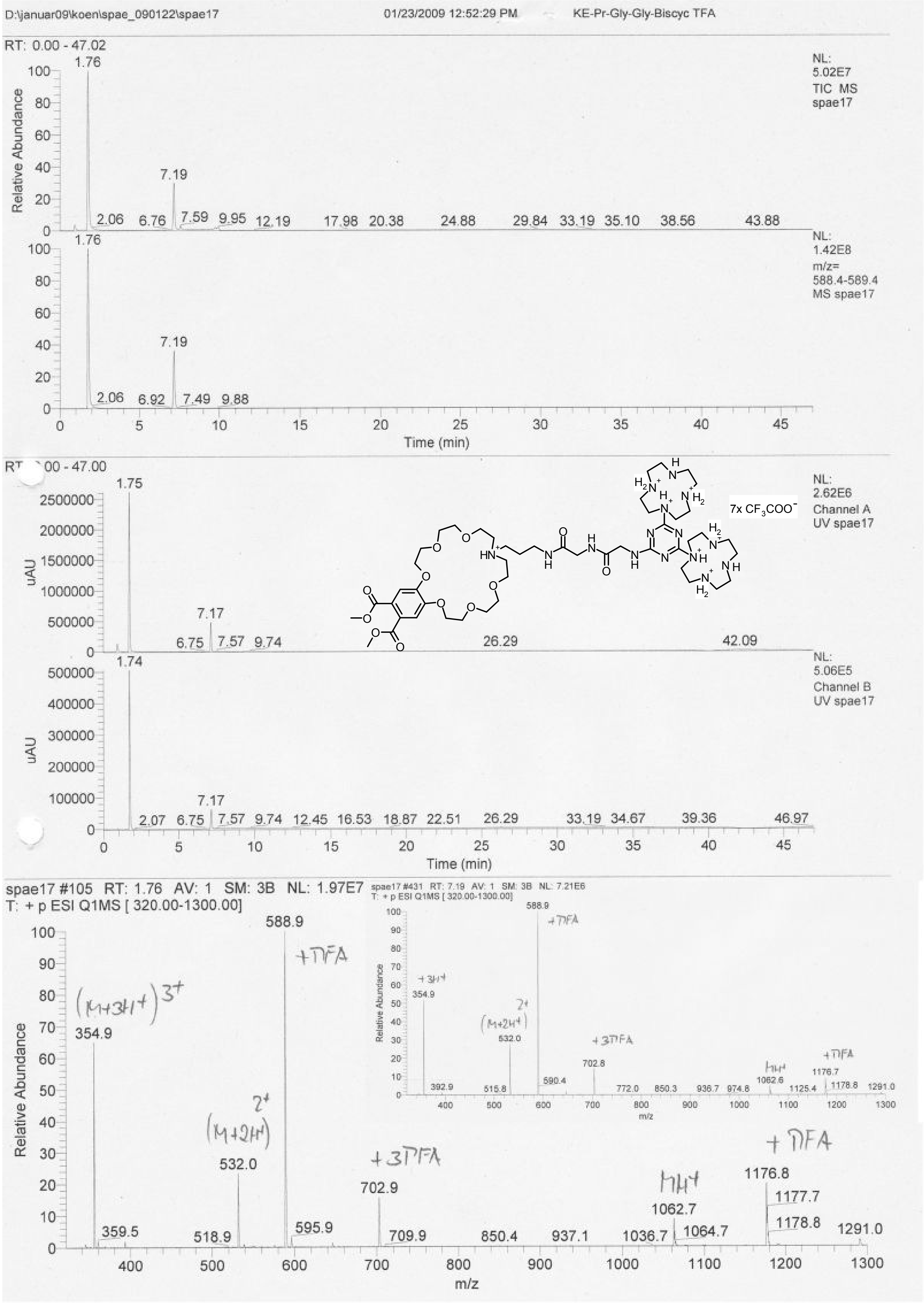


Figure S-46: Mass spectrum of compound **15d´**

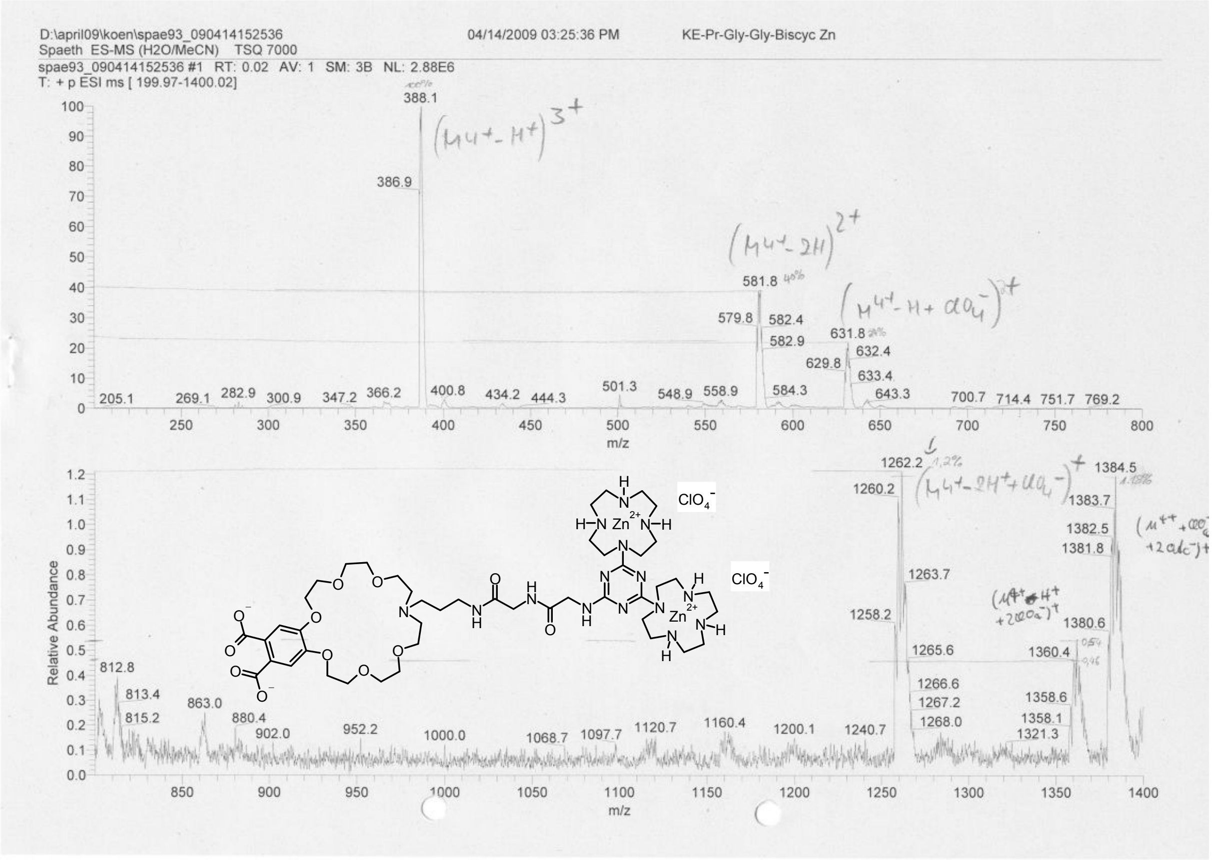


Figure S-47: Mass spectrum of compound **3d**

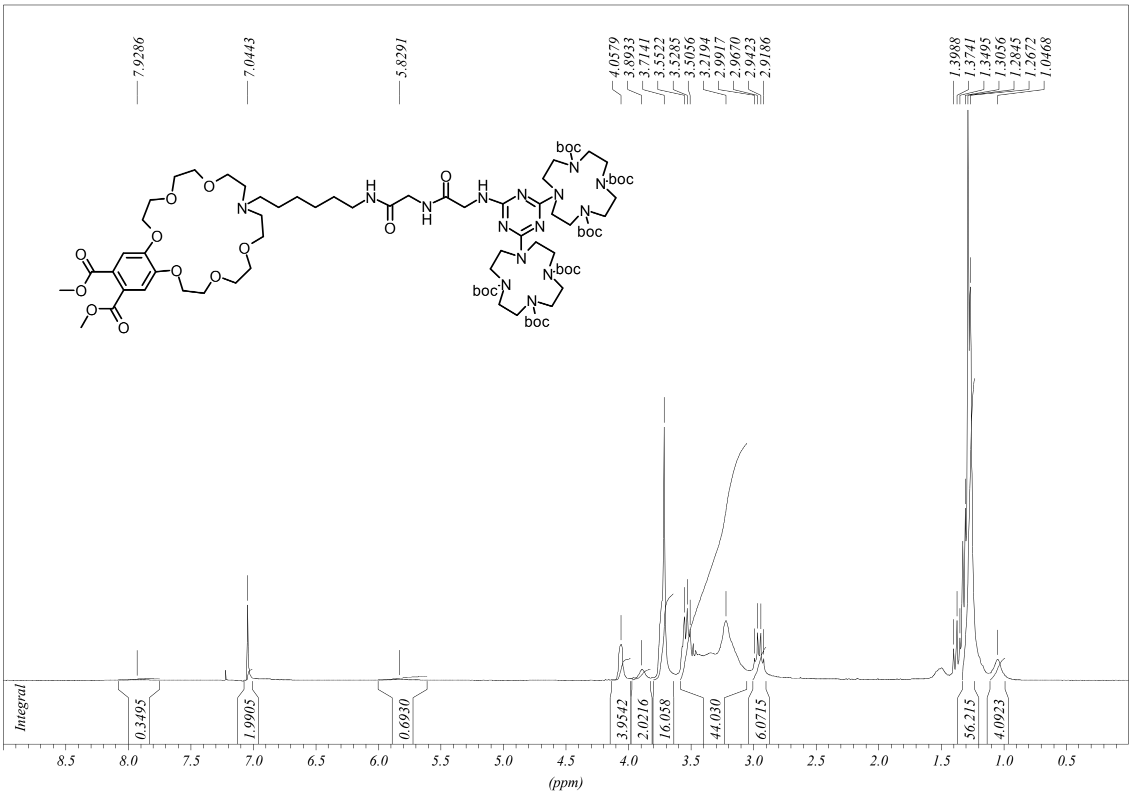


Figure S-48: ^{1}H-NMR spectrum of compound **11f**

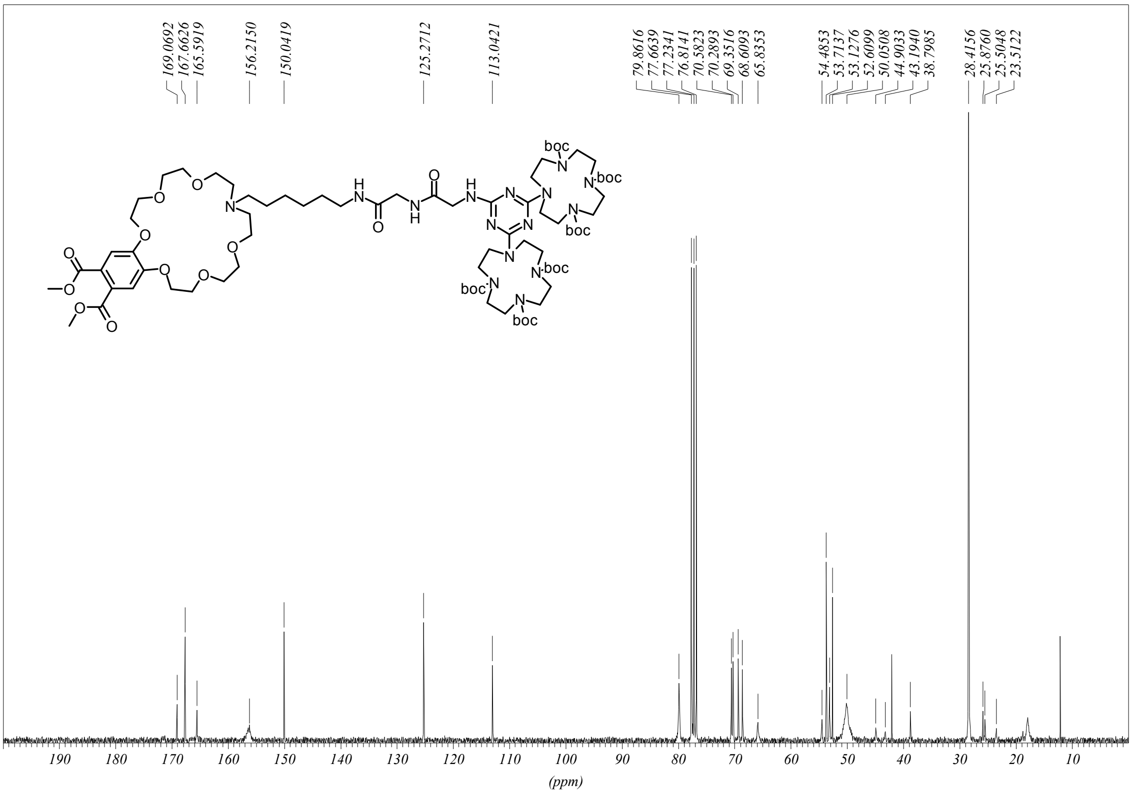


Figure S-49: ^{13}C-NMR spectrum of compound **11f**

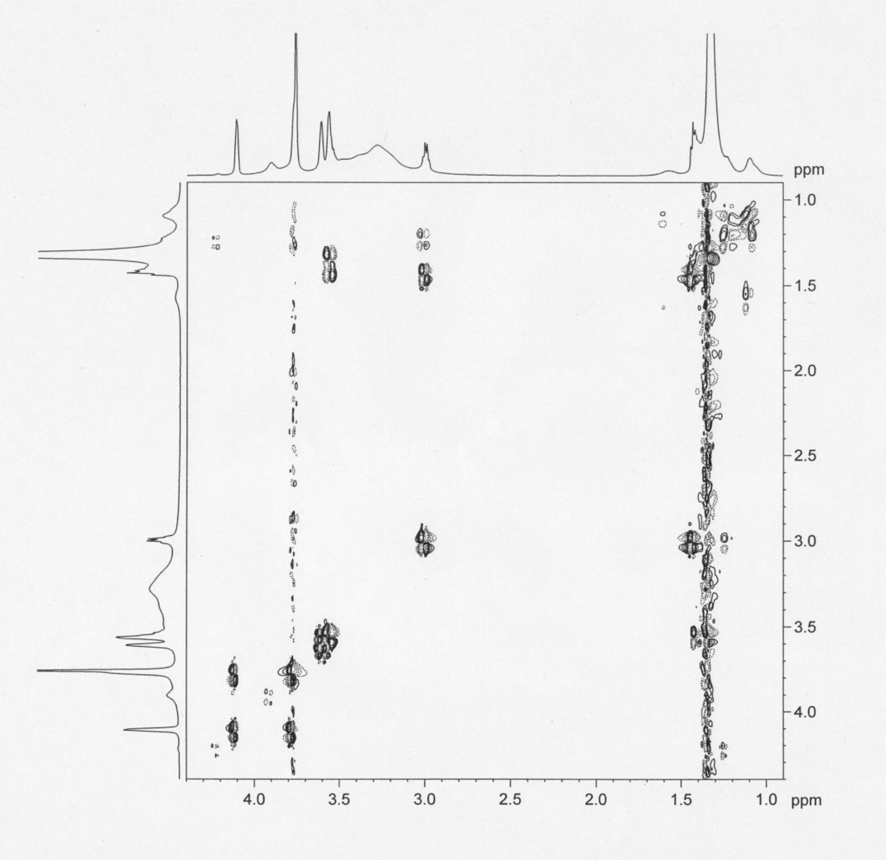


Figure S-50: COSY spectrum of compound **11f**

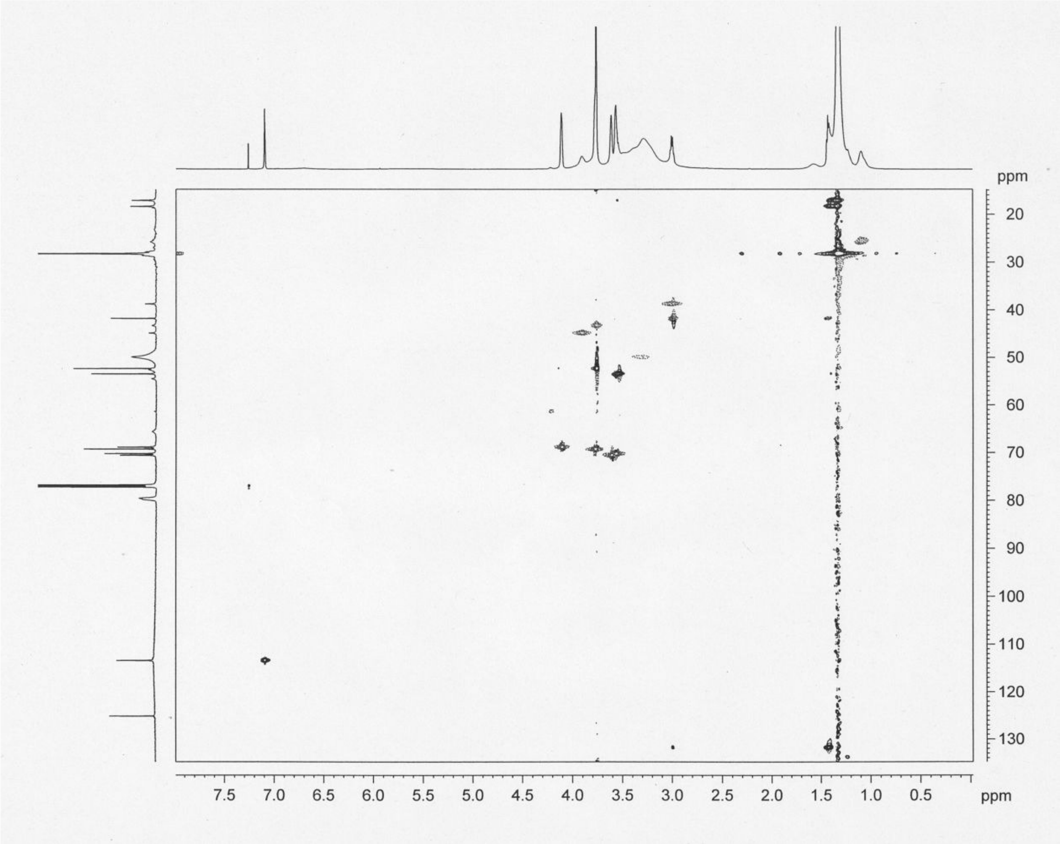


Figure S-51: HSQC spectrum of compound **11f**

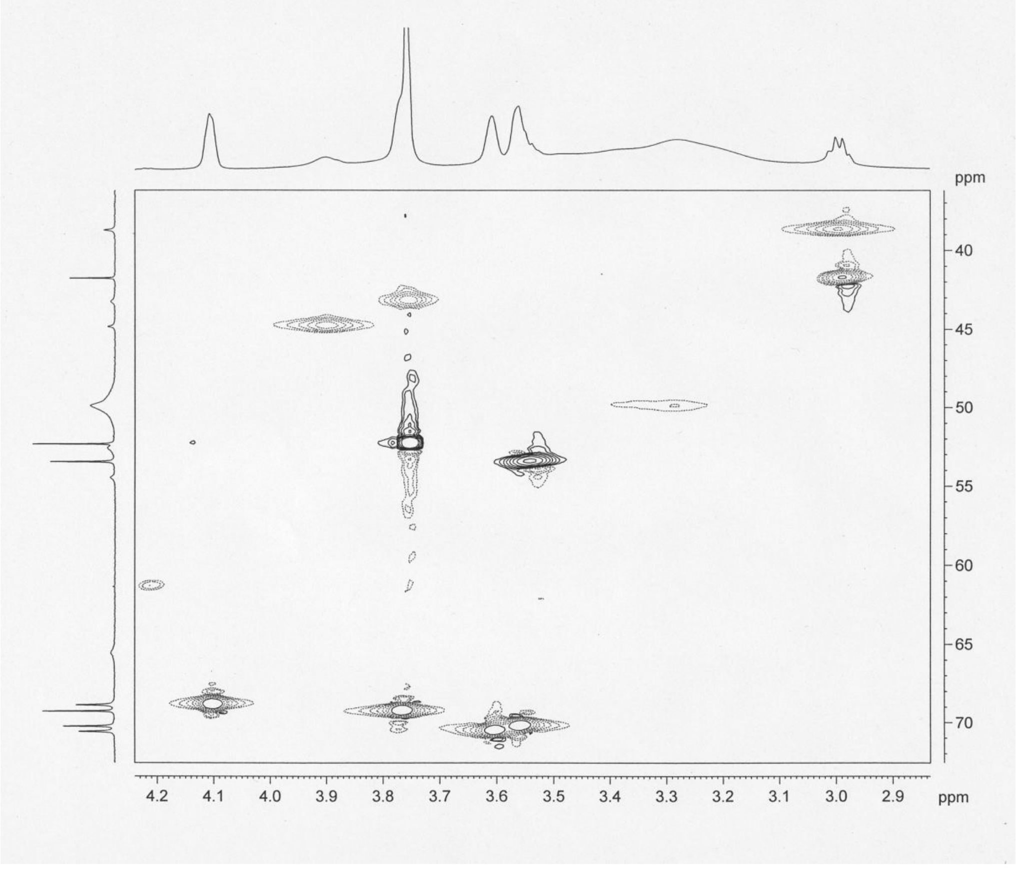


Figure S-52: HSQC spectrum (enlarged) of compound **11f**

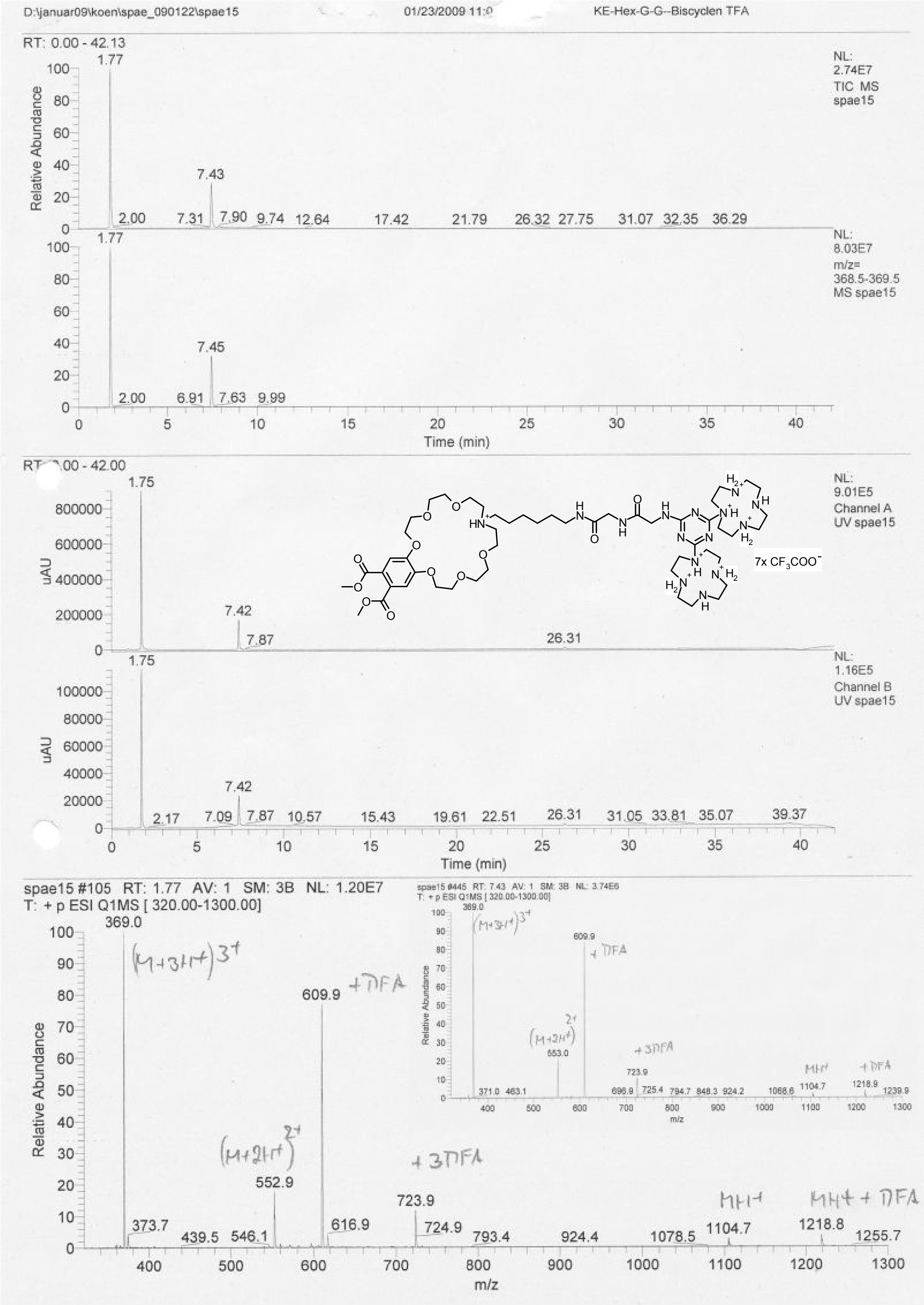


Figure S-53: Mass spectrum of compound **15f′**

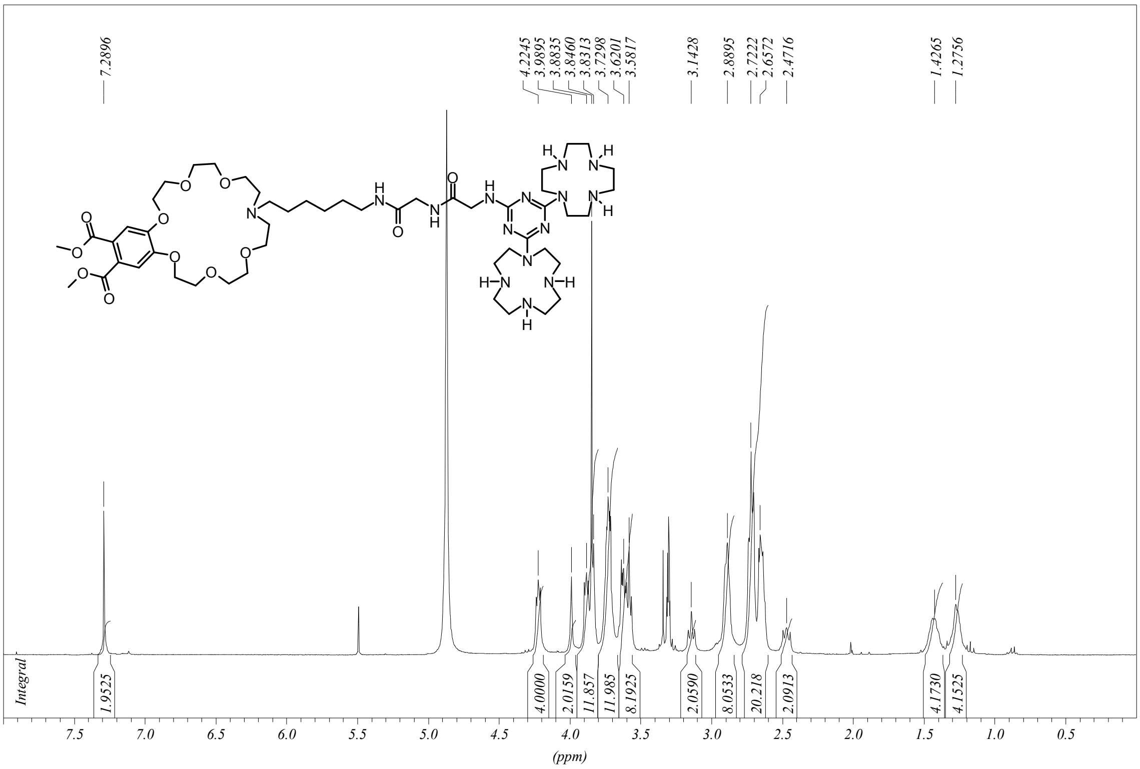


Figure S-54: ^{1}H-NMR spectrum of compound **15f**

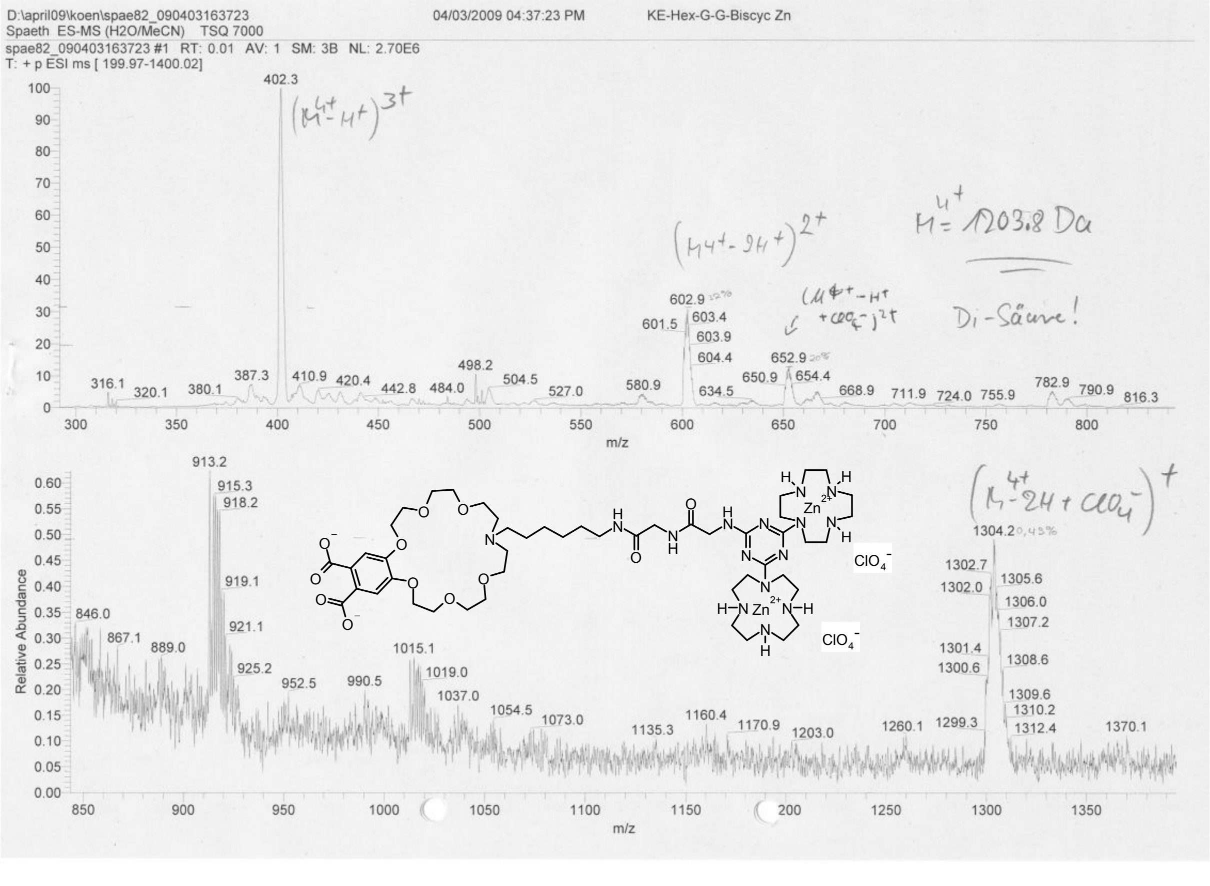


Figure S-55: Mass spectrum of compound **3f**

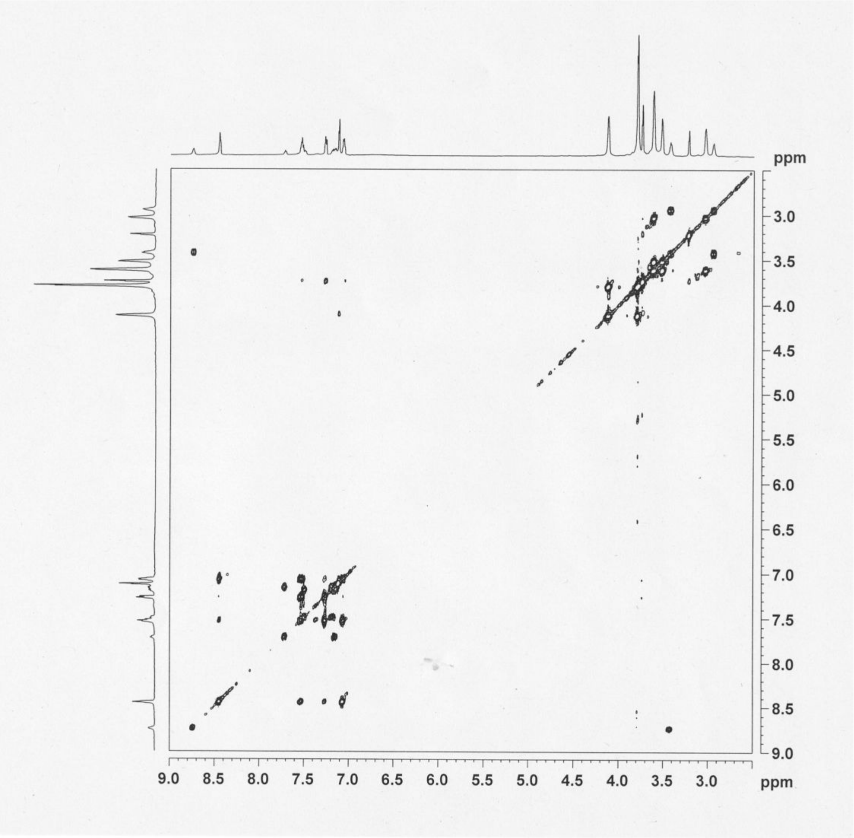


Figure S-56: COSY spectrum of compound **23**

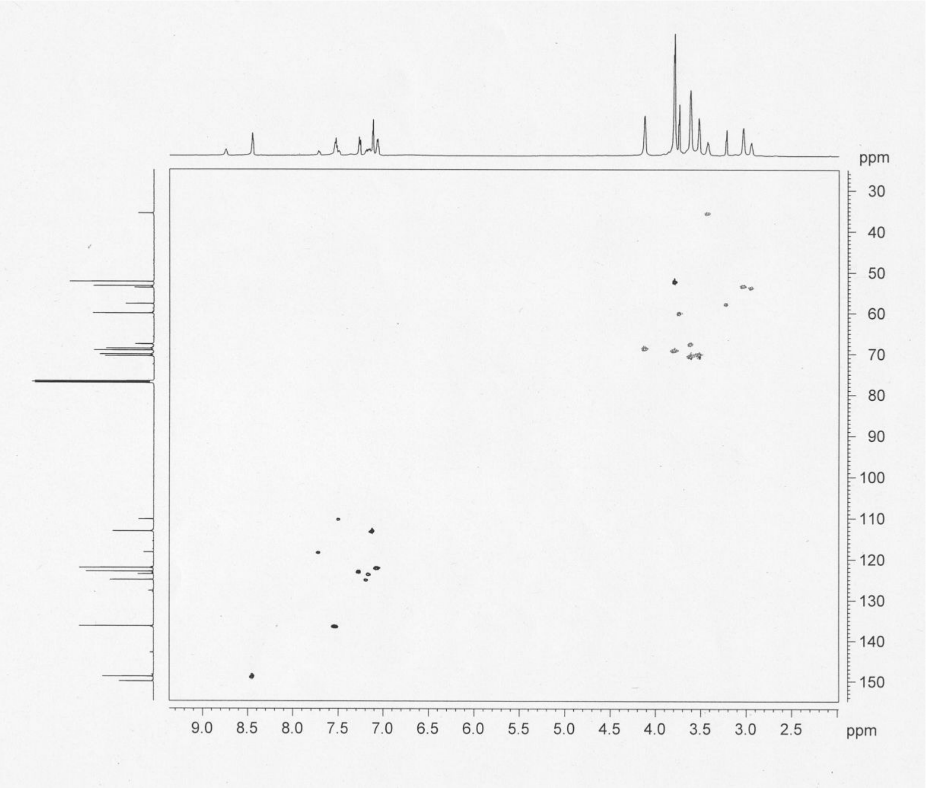


Figure S-57: HSQC spectrum of compound **23**

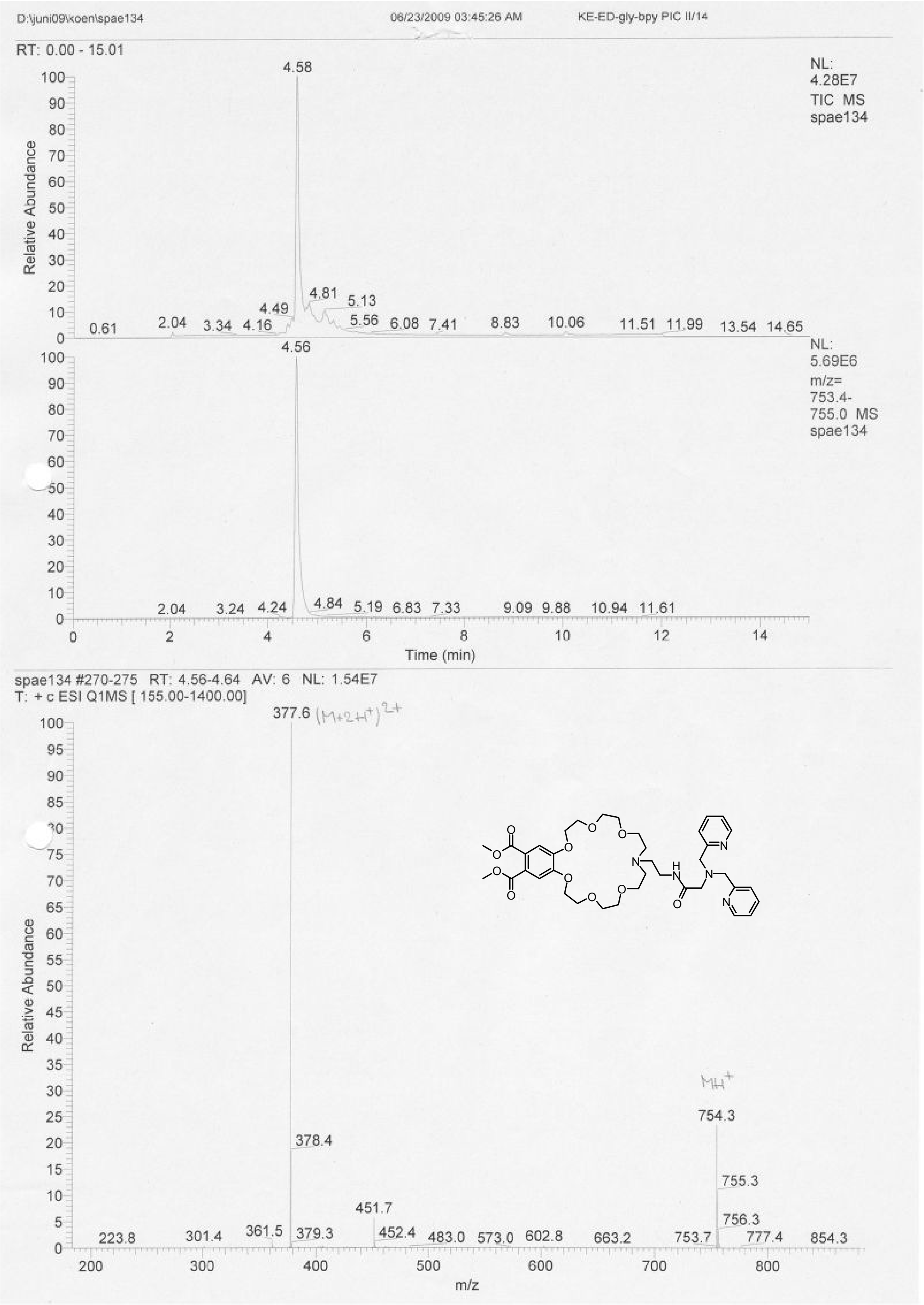


Figure S-58: Mass spectrum of compound **23**

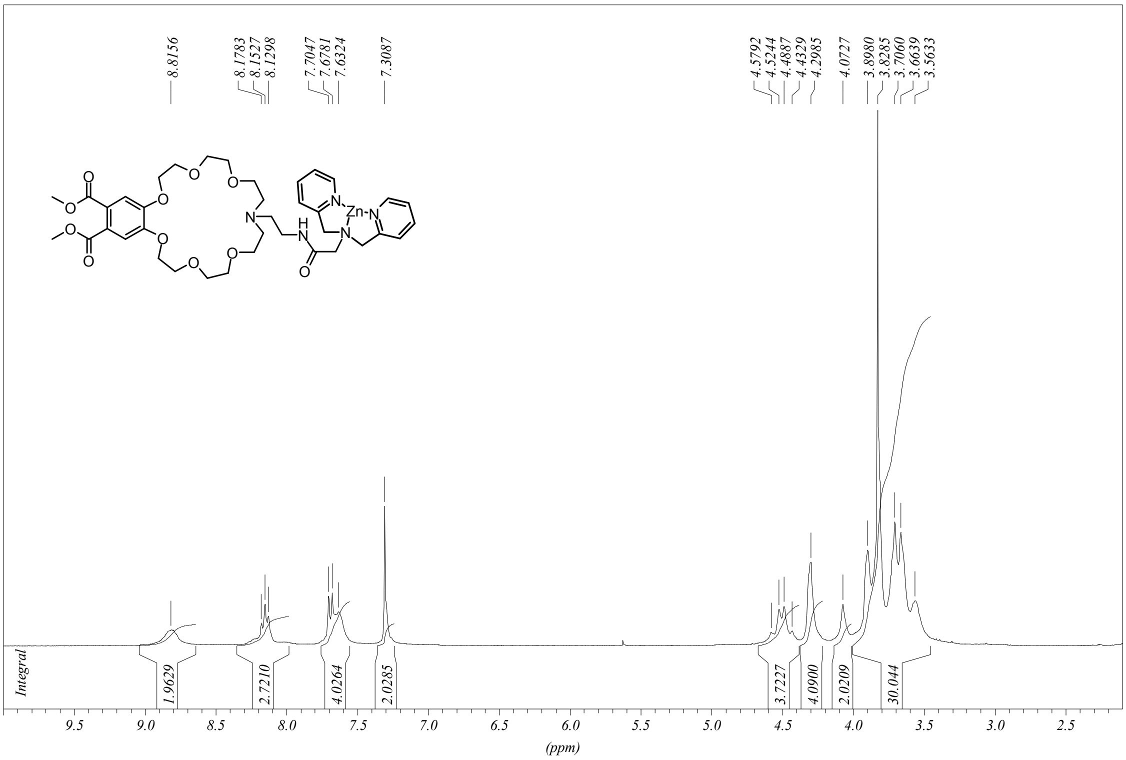


Figure S-59: ^{1}H-NMR spectrum of compound **6a**

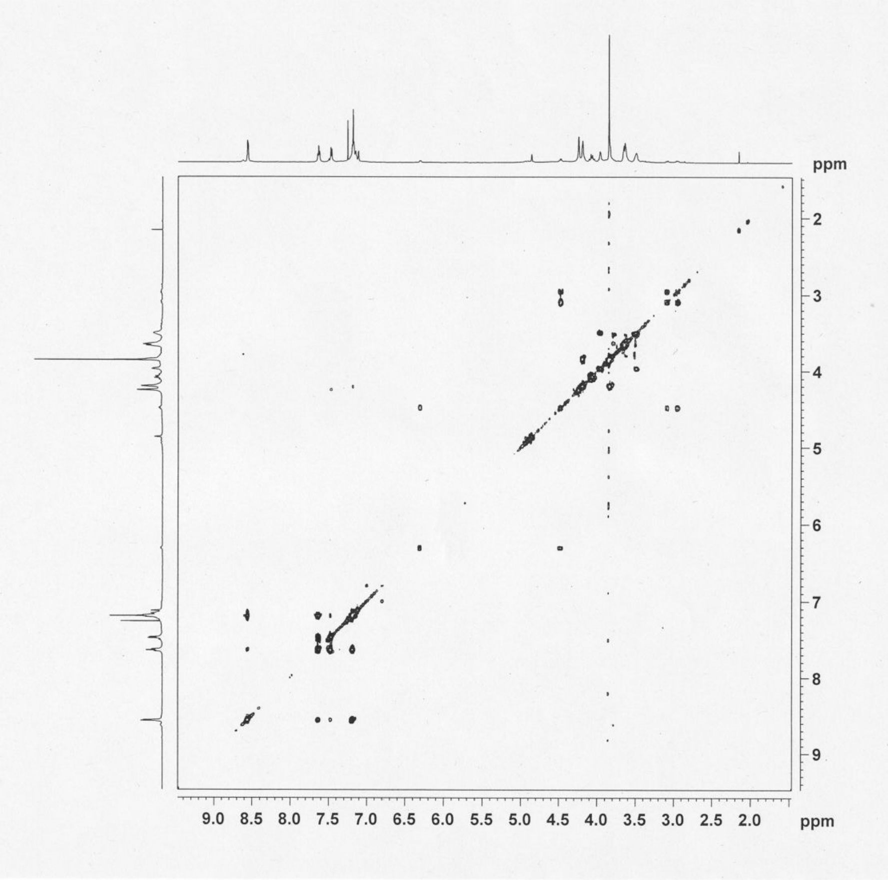


Figure S-60: COSY spectrum of compound **25**

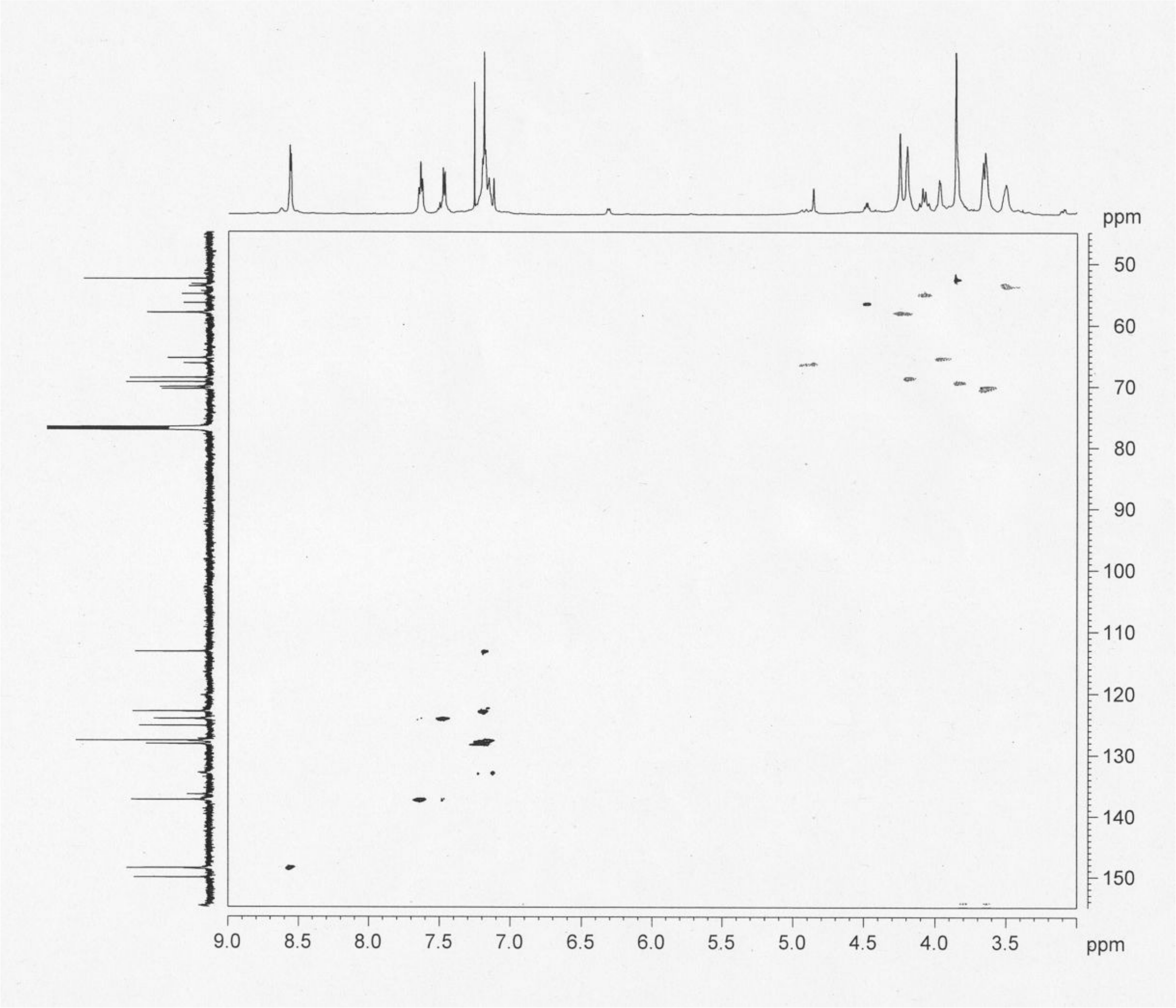


Figure S-61: HSQC spectrum of compound **25**

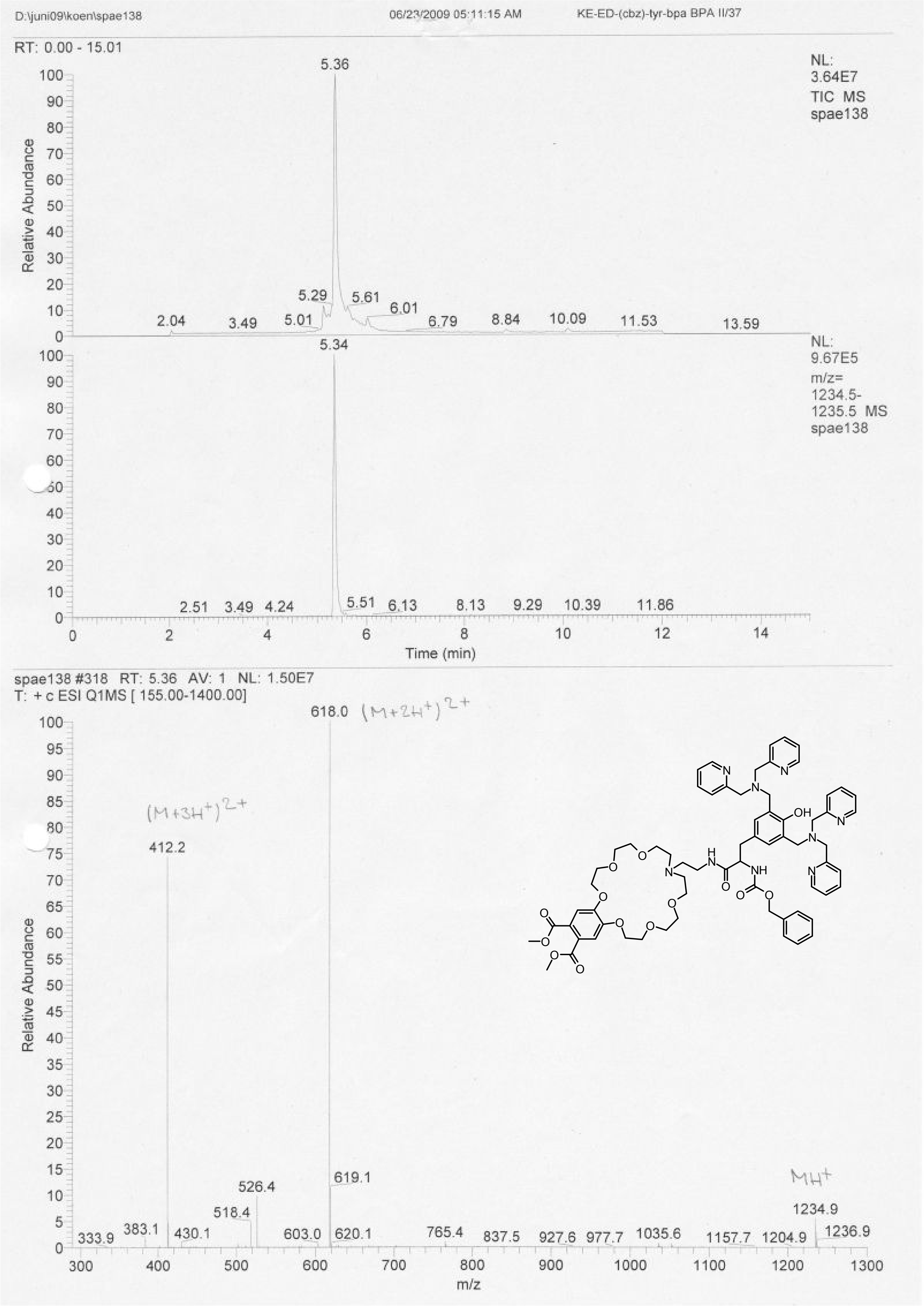


Figure S-62: Mass spectrum of compound **25**

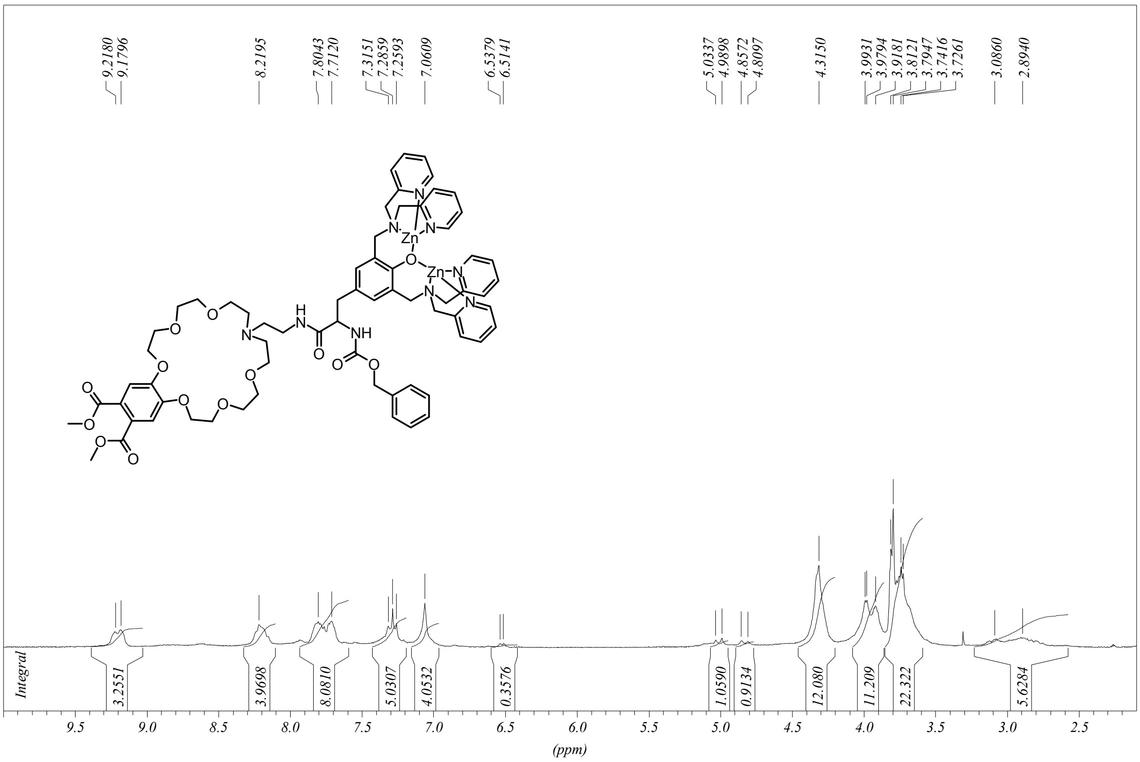
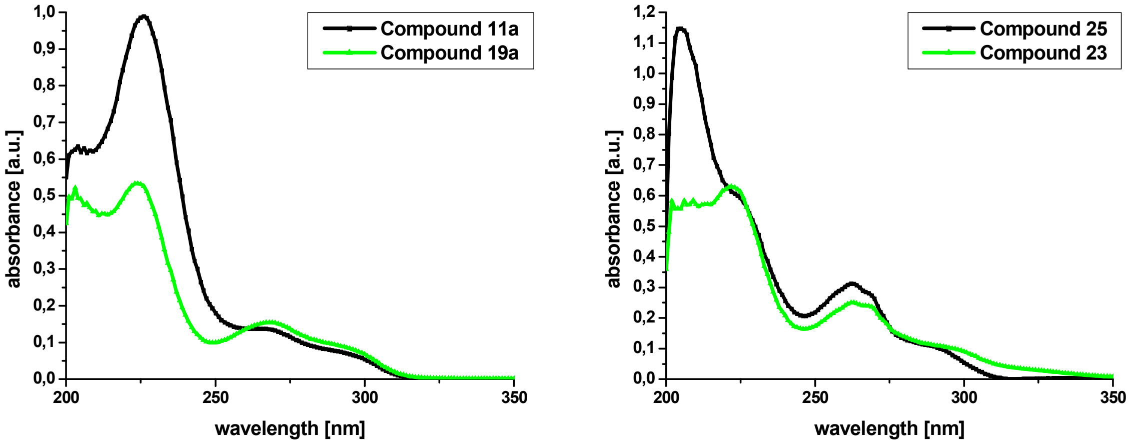


Figure S-63: ^{1}H-NMR spectrum of compound **4a**

3. UV and emission spectra of selected compounds

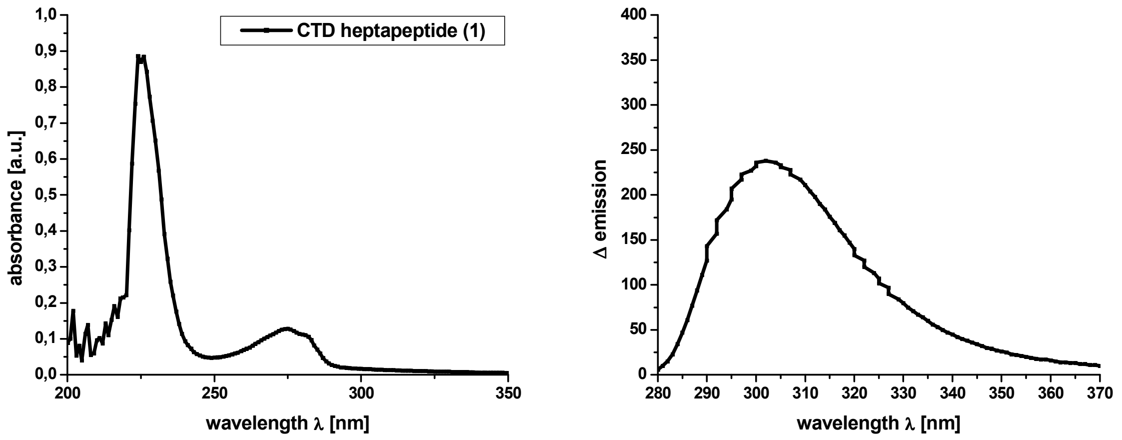
Absorption Spectroscopy. Absorption were recorded on a Varian Cary BIO 50 UV/VIS/NIR Spectrometer with temperature control by use of a 1 cm quartz cuvettes (Hellma) and Uvasol solvents or of quality for spectrophotometrical investigations (Merck, Baker or Acros).

Emission Spectroscopy. Fluorescence measurements were performed with UV-grade solvents (Merck, Baker or Acros) in 1 cm quartz cuvettes (Hellma) and recorded on a Varian 'Cary Eclipse' fluorescence spectrophotometer with temperature control. To determine the binding constants and Job´s plots fluorescence titration experiments were carried out.



Figures S-64: Absorption spectra of compound **11a** and **19a** (left, c = 2*10^{-5} mol/L) and compound **23** and **25** (right, c = 2*10^{-5} mol/L)

The UV spectra of the free ligands (**15a-f**) and their protected precursors (**11a-f**) or the free bpy ligands (**23** and **25**) are similar to the spectra of the different zinc-biscyclene (**3a-f**) and zinc or manganese bpy crown ether receptors (**4** and **6**), respectively, only their maxima are slightly shifted from 260 to 270 nm and from 220 to 225 nm.



Figures S-65: Absorption- and emission-spectrum of compound **1** (c = 4*10^{-5} mol/L)

4. Screenings and binding studies via fluorescence measurements

The chromophore-containing aza-crown ether amino acids are able to participate in photoinduced electron transfer (PET), where, upon excitation an electron of the lone pair on the nitrogen is able to travel to the chromophore, quenching the fluorescence.[12] Such a photo-induced electron transfer (PET) can occur over a distance of 10 Å.[13] Yoshida et al. showed, chromophore containing aza-crown ether compounds maintain their absorption and emission wavelengths and absorption intensity, while their fluorescence intensities are significantly altered upon cation complexation.[14]

Cation complexation disrupts the electrostatic field of the amine and thereby the PET process, causing an increase in fluorescence intensity proportional to the amount of complexation and therefore the concentration of cation present. However, such crown ethers suffer pH dependence problems, where protonation of the amine mimics cation complexation at low pH, so the pH has to be maintained by an base like tetraethylammoniumhydroxide or all studies have to be conducted in a buffered medium (the system can be used at pH values ≥ 6). The pH effects on the luminescence[15] and cation selectivity[16] of aza-crown-ethers based on this signalling principle have been studied.

4.1. General methods, material and sample preparation

Sample preparation. HEPES buffer 50 mM with pH 7.4 was used. The exact solutions have been prepared by adding to a weighed amount of receptor or substrate, the calculated amount of solvent necessary by means of microliter precision pipettes. This method is more accurate than to determine exactly the weighed amount (to 10^{-5} g) and admit a certain volume, since the volume measurement has a higher accuracy than the weight determination.

Receptor solutions were stored properly stoppered and closed with parafilm at maximum for one week in the refrigerator at -4 °C. They were freshly prepared every week from deep frozen aliquots of the receptor material. Amino acid and peptide solutions were kept at -4 °C properly stoppered and closed with parafilm not longer than for one day or over night. Peptide and nucleotide solutions were always freshly prepared from the properly dried and carefully stored material (nitrogen, -20 °C).

4.2. Fluorescence data

All fluorescence experiments were performed on a Varian Cary Eclipse Fluorimeter. To determine the binding constants, fluorescence titration experiments were carried out. The screening of the amino acids and the synthesized peptide library was conducted in a microtiter plate (384 wells, UV star material). The fluorescence intensity was found to be constant after 5 min.

Screening conditions:

Solvent: HEPES buffer 50 mM pH 7.4
Well volume: 130 μL
Concentration [crown ether host complex]: 0.01 mM
Concentration [amino acid / peptide]: 1.00 mM / 0.20 mM

Instrument Parameters:

Excitation wavelength: $\lambda_{ex} = 300$ nm
Detection wavelength: $\lambda = 330 – 500$ nm
Temperature: $T = 298$ K
PMT voltage: 500 - 600 volts
Excitation slit: 10 nm
Emission slit: 10 nm

Procedure:

To each column in the microtiter plate samples of the receptor (65 μL) and the amino acid or peptide (65 μL) and the receptor without added peptide but volume corrected as reference were given. The mixtures were quickly pipetted row by row, mixed with the aid of the pipette and allowed to equilibrate for 10 minutes. Emission spectra ($\lambda_{ex} = 300$ nm) for all wells were recorded. All measurements were repeated twice.

For amino acids and peptides inducing a sufficient emission increase over the parent compound (> 10 %) the binding constant and stoichiometry of the respective guest with selected receptors were determined by emission titration in a cuvette

Emission titrations:

Titration conditions (amino acids and related guests):

Solvent:	HEPES buffer 50 mM pH 7.4
Starting volume:	1.0 mL
Concentration [receptor]:	0.02 mM
Concentration [amino acid or peptide]:	0.20 – 1.00 mM

Instrument Parameters:

Excitation wavelength:	λ_{ex} = 300 nm
Detection wavelength:	λ = 320 – 500 nm
Temperature:	T = 298 K
PMT voltage	560 - 600 volts

Procedure:

A cuvette with 1.0 mL of the receptor solution was titrated stepwise with small amounts (10 - 50 µL ≙ 0.1 – 1.0 eq.) of the peptide solution. After each addition the solution was allowed to equilibrate for 10 min before the fluorescence intensity (λ_{ex} = 300 nm) was recorded. The stoichiometry was determined by Job´s plot analysis extracted from titration data.**Fehler! Textmarke nicht definiert.** To determine the binding constant the obtained fluorescence intensities were volume corrected, plotted against the concentration of peptide and evaluated by non linear fitting methods.

4.2.1. Titrations of the crown ether part with ammonium guests:

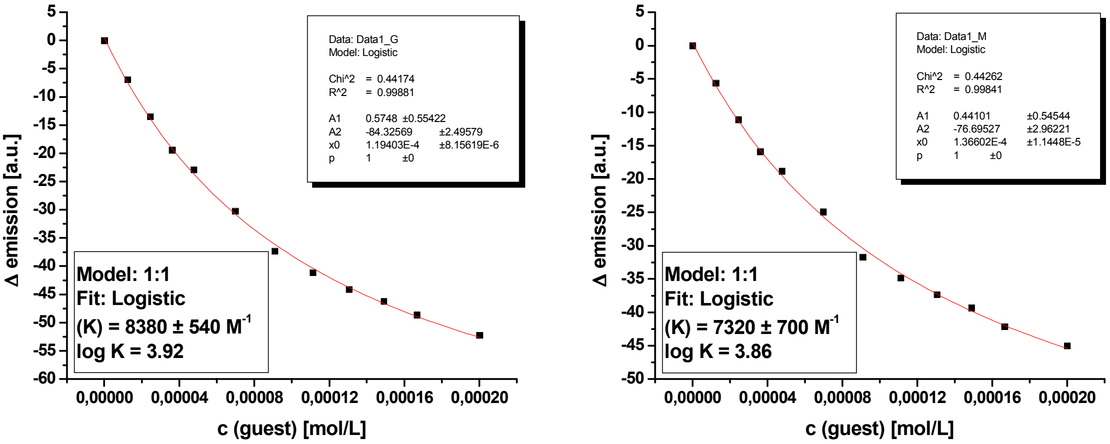
In preparation of the measurements, the binding constants of the crown ether unit alone were determined in a cuvette. To 1.0 mL of the receptor in methanol were added small (≙ 0.5 to 10 eq.) aliquots of the guest solution. After each addition the solution was allowed to equilibrate and the emission intensity was recorded. To determine the binding constant the obtained fluorescence intensities were volume corrected, plotted against the concentration of peptide and evaluated by non linear fitting methods. In all measurements the receptor was employed as $2*10^{-5}$ molar solutions, the guest were added as $1*10^{-3}$ molar solutions at 25 °C. The system was excitated at 300 nm. Within the limits of error the values show no difference to the literature known ones of the single CEAAs:[17]

Crown ether zinc biscyclene motif	**Log *K* ($n\text{-BuNH}_3^+$)**	**Log *K* (KSCN)**
3b	2.15 (4 %)	3.38 (9 %)
3c	2.21 (5 %)	3.31 (14 %)
3d	2.07 (3 %)	3.17 (11 %)
3e	2.12 (5 %)	3.23 (13 %)
5	2.39 (5 %)	3.71 (10 %)
3a	2.28 (4 %)	3.66 (11 %)

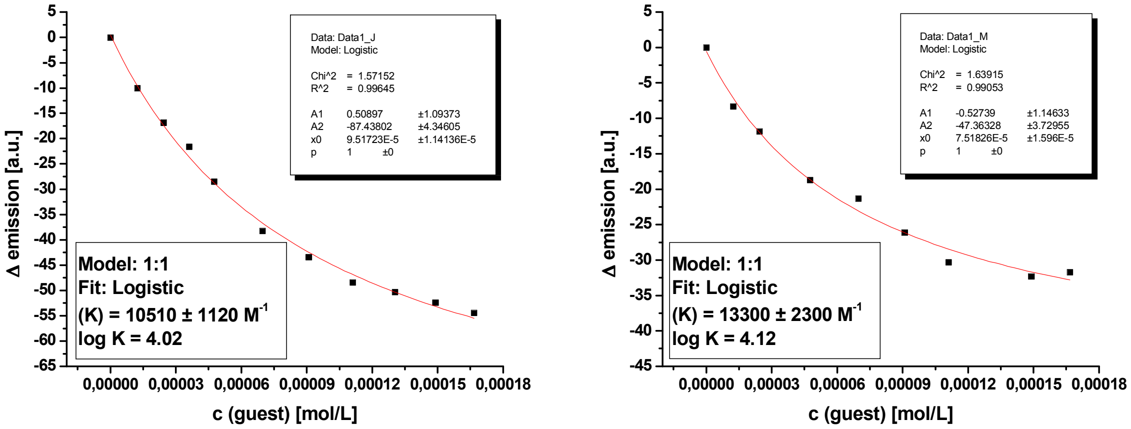
Table S-1: Binding constants for the zinc-biscyclenes in MeOH against n-butylamine and KSCN as guests (error in % in brackets)

Neither n-butylamine nor potassium isothiocyanate cause a difference of the fluorescence output in aqueous HEPES buffer. Even if 2000 equivalents of the salts are added, they are not bound in this medium by the crown ether moiety.

4.2.2. Titrations with phosphoserine as guest:

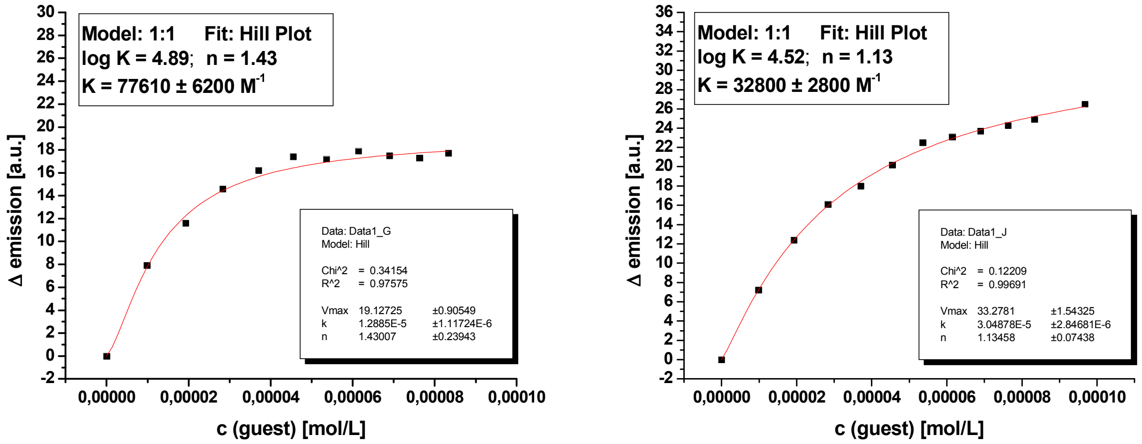


Figures S-66: Emission titration of compound **6b** (left) and compound **6a** (right) (c = 2*10^{-5} mol/L) with phosphoserine (c = 1*10^{-3} mol/L)

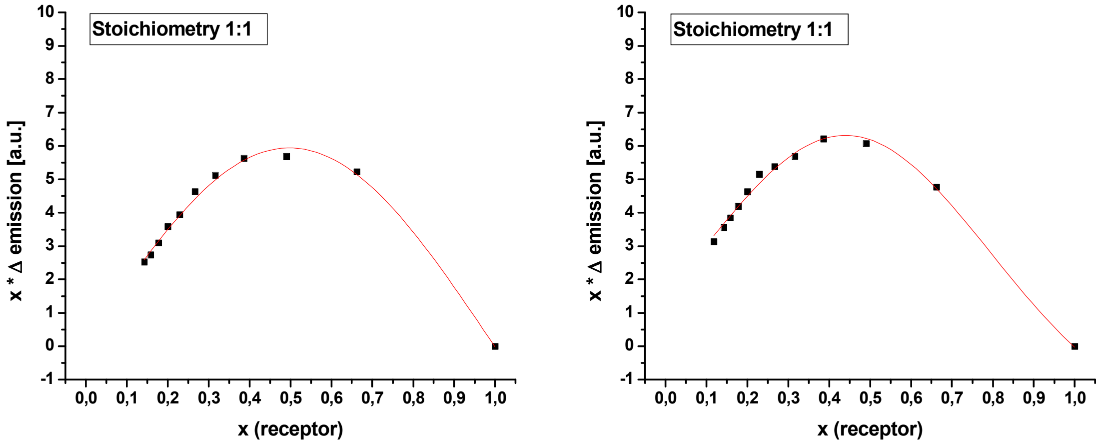


Figures S-67: Emission titration of compound **4b** (left) and compound **4a** (right) (c = 2*10^{-5} mol/L) with phosphoserine (c = 2*10^{-4} mol/L)

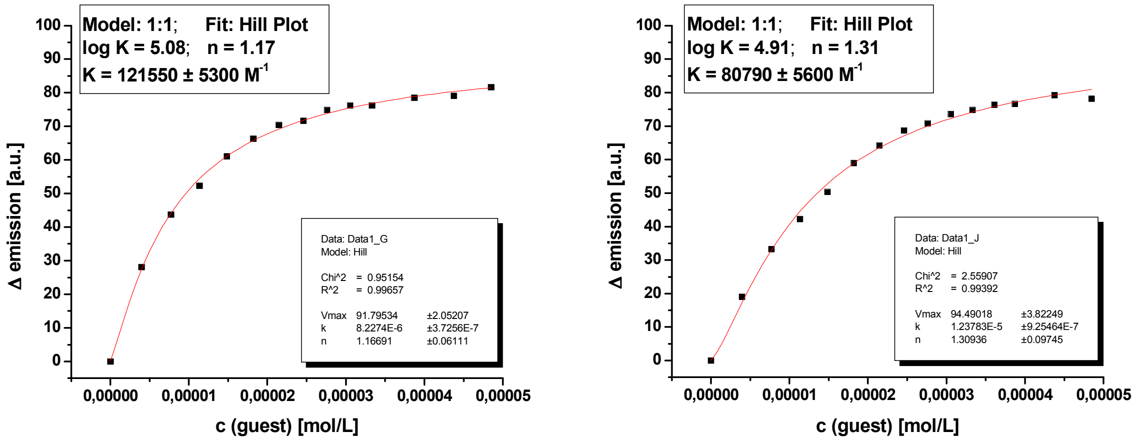
4.2.3. Titrations with the PKG substrate (2) as guest:



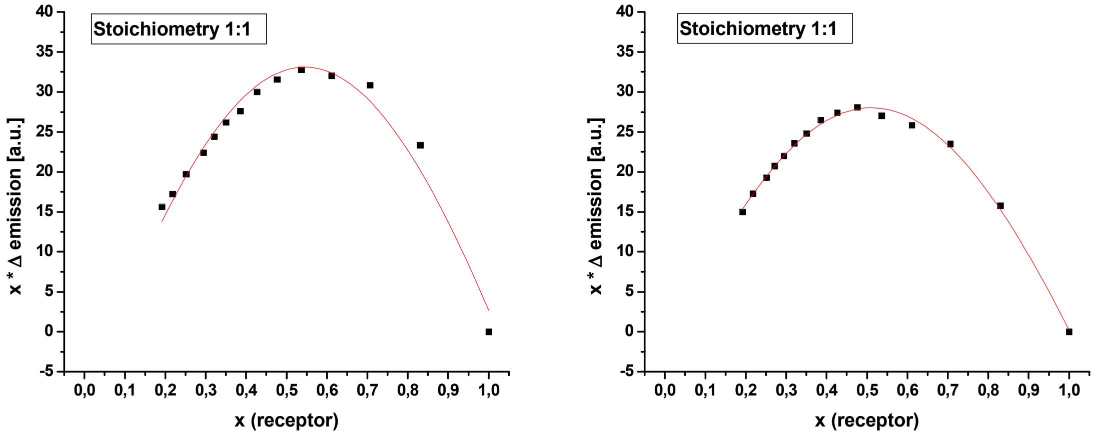
Figures S-68: Emission titration of compound **6b** (left) and compound **6a** (right) ($c = 2*10^{-5}$ mol/L) with **2** ($c = 5*10^{-4}$ mol/L)



Figures S-69: Job´s Plot of compound **6b** (left) and compound **6a** (right) ($c = 2*10^{-5}$ mol/L) binding to **2** ($c = 5*10^{-4}$ mol/L)

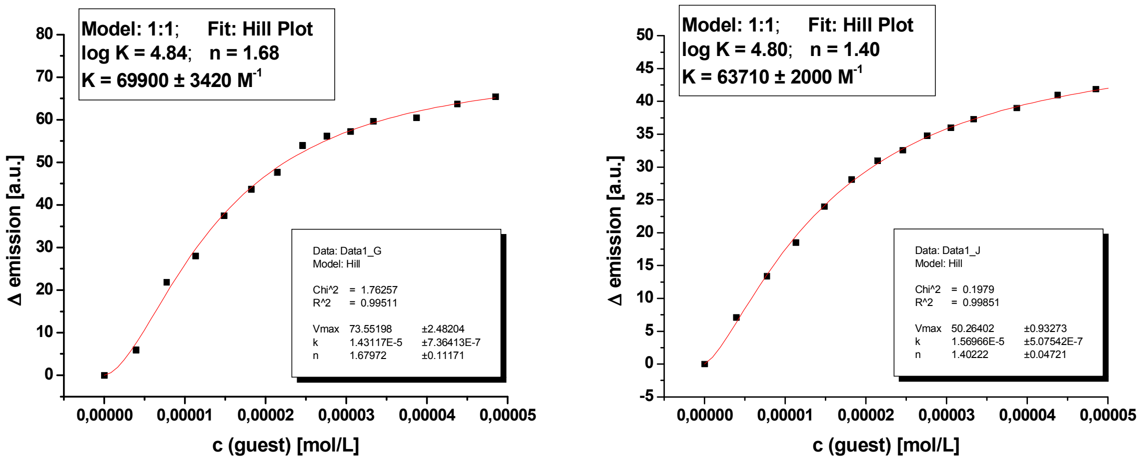


Figures S-70: Emission titration of compound **4b** (left) and compound **4a** (right) (c = 2*10^{-5} mol/L) with **2** (c = 2*10^{-4} mol/L)

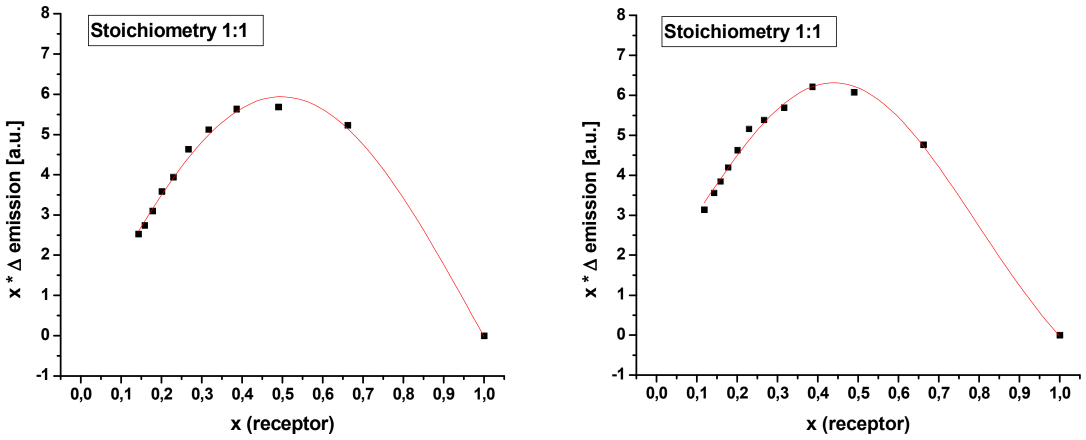


Figures S-71: Job´s Plot of compound **4b** (left) and compound **4a** (right) (c = 2*10^{-5} mol/L) binding to **2** (c = 2*10^{-4} mol/L)

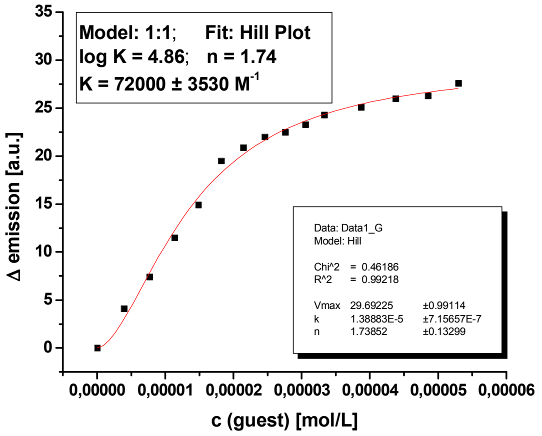
4.2.4. Titrations with the CTD heptapeptide (1) as guest:

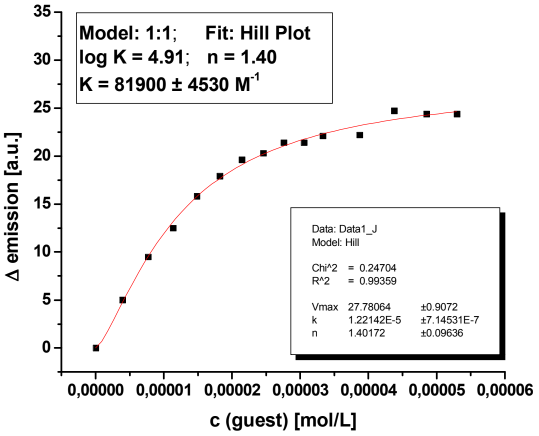


Figures S-72: Emission titration of compound **6b** (left) and compound **6a** (right) ($c = 2*10^{-5}$ mol/L) with **1** ($c = 2*10^{-4}$ mol/L)

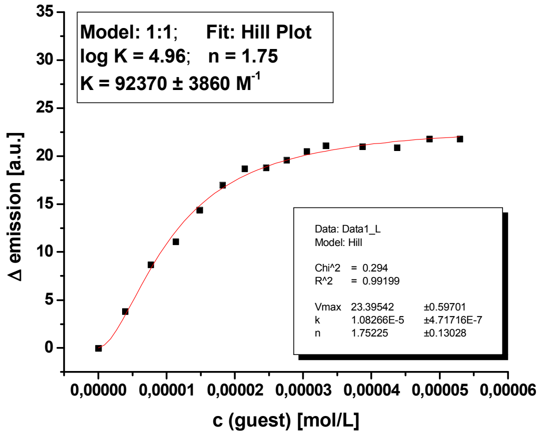


Figures S-73: Job´s Plot of compound **6b** (left) and compound **6a** (right) ($c = 2*10^{-5}$ mol/L) binding to **1** ($c = 2*10^{-4}$ mol/L)

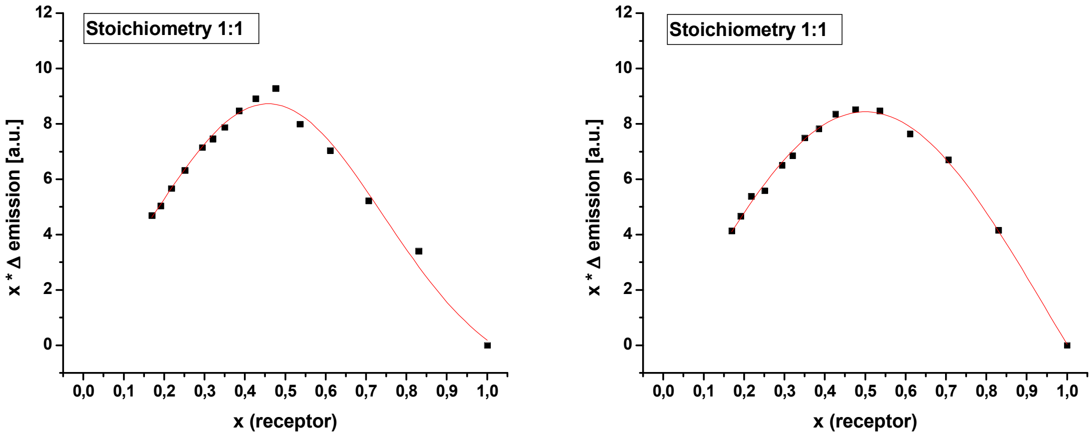




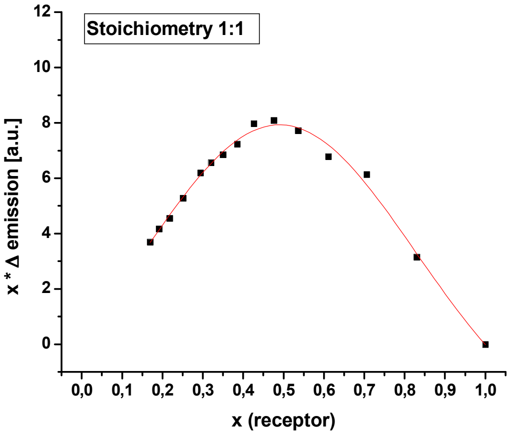
Figures S-74: Emission titration of compound **3a** (left) and compound **3b** (right) (c = 2*10^{-5} mol/L) with **1** (c = 2*10^{-4} mol/L)



Figures S-75: Emission titration of compound **3c** (c = 2*10^{-5} mol/L) with **1** (c = 2*10^{-4} mol/L)



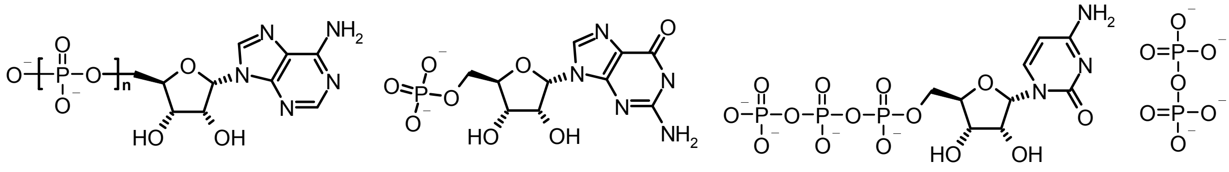
Figures S-76: Job´s Plot of compound **3a** (left) and compound **3b** (right) (c = 2*10^{-5} mol/L) binding to **1** (c = 2*10^{-4} mol/L)



Figures S-77: Job´s Plot of compound **3c** (c = 2*10^{-5} mol/L) binding to **1** (c = 2*10^{-4} mol/L)

4.3. Fluorescence measurements with Nucleotides.[18]

Zn(II)cyclene or bis-[Zn(II)cyclene] complexes can bind imide-containing nucleotides like uridine (U) and thymidine (T) or their derivatives by specific Zn(II)-imide N-coordination.[19] Such Zn(II)cyclene complexes with appended aromatic ring systems were shown to selectively bind to T- or U-rich regions in double stranded DNA or RNA.[20] They do not interact with the other DNA nucleosides (G, A and C), making the reversible coordination of the metal complex highly selective.



E-1a n = 1
E-1b n = 1
E-1c n = 1

E-2

E-3

E-4

Figure S-78: Nucleotides AMP (**E-1a**), ADP (**E-1b**), ATP (**E-1c**), GMP (**E-2**), CTP (**E-3**) and the pyrophosphate anion (PPi, **E-4**)

Especially these three nucleotides moved in our focus, when observing the ester-cleavage upon complexation of compounds **3a** to **3f.** The combination of the strong and selective phosphate coordination of the metal complex together with the two-fold negative charge of the phthalic acid favourably oriented by the bowl shape of the crown ether pushed us towards studies with these encouraging guests for a quick assay on the basis of the discussed recognition motif. We selected these representative targets to measure also the phosphate binding abilities of the systems towards mono-, di- and triphosphates.

All bases exist mainly in the tautomere of the amino- and ketoforms (more then 99.99 %) and are under physiological conditions uncharged ($5 < pH < 9$). A protonation to an only weak extent promoted by the intramolecular interaction is absolutely sufficient for binding. In water adenine has its absorption maximum at 261 nm with a molar extinction coefficient of 13400 $M^{-1}cm^{-1}$, cytosine with 6100 $M^{-1}cm^{-1}$ at 220 nm, guanine behaves similar[21] Due to the expected low fluorescence yield ($\phi < 0.01$) a disturbing fluorescence signal of the base is not expected.[22]

In all measurements, the intensity decreases with the increase of the guest concentration (Figure S-79). A representative titration with AMP (**E-1a**) is shown:

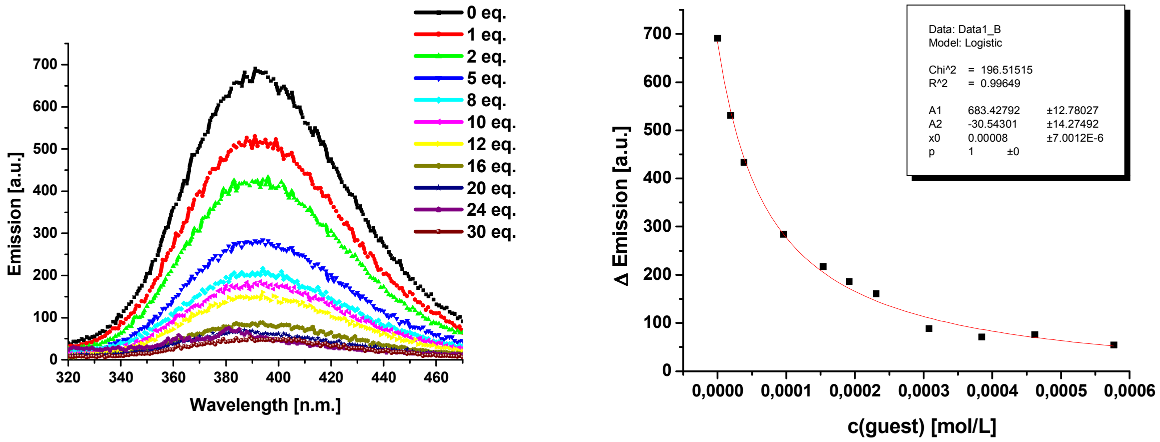


Figure S-79: Intensity decrease of compound **3e** with rising amount of AMP (left) and the according titration curve (right); in red: logistic fit

The data was interpreted with the aid of the origin program package. The falling curves were fitted by a logistic plot (equation 1), the result was checked by fitting of the inverted curves with the hill plot (equation 2):

(1) $y = A / [1 + (x/x_0)^p]$; $p = 1$

(2) $y = (V_{max} * x^n) / (k^n + x^n)$; $n = 1$; no cooperativity assumed

Equation S-1: Calculation of the binding constants

Both calculations gave comparable results. Representative values are given for receptor compound **3e** with all guests and for all receptors with the same guest, AMP, in table S-2.

Guest	**Log *K* (logistic)**	**Log *K* (Hill plot)**
AMP	4.22 (7 %)	4.15 (8 %)
ADP	4.52 (11 %)	4.70 (12 %)
ATP	4.70 (13 %)	4.70 (10 %)
CTP	4.70 (6 %)	4.70 (5 %)
GMP	4.30 (8 %)	4.26 (10 %)
PPi	4.36 (10 %)	4.22 (11 %)

Host	**Log *K* (logistic)**	**Log *K* (Hill plot)**
3a	4.05 (10 %)	4.05 (8 %)
3b	4.05 (10 %)	3.89 (8 %)
3c	4.22 (12 %)	4.00 (10 %)
3d	4.10 (11 %)	3.95 (13 %)
3e	4.05 (11 %)	4.20 (12 %)
3f	4.22 (7 %)	4.15 (7 %)
5	4.05 (10 %)	3.89 (7 %)

Table S-2: Binding constants for compound **3e** in with the phosphorylated guests and for all biscyclene receptors with AMP (HEPES-buffer, 50 mM, pH 7.4; error in % in brackets)

An increase in binding strength with increasing number of phosphate residues and therefore of the negative charge in the anion is obvious: ATP, CTP and ADP have the highest negative charge and thus show the highest binding constants, whereas for AMP with one negative charge a slightly weaker association was found.

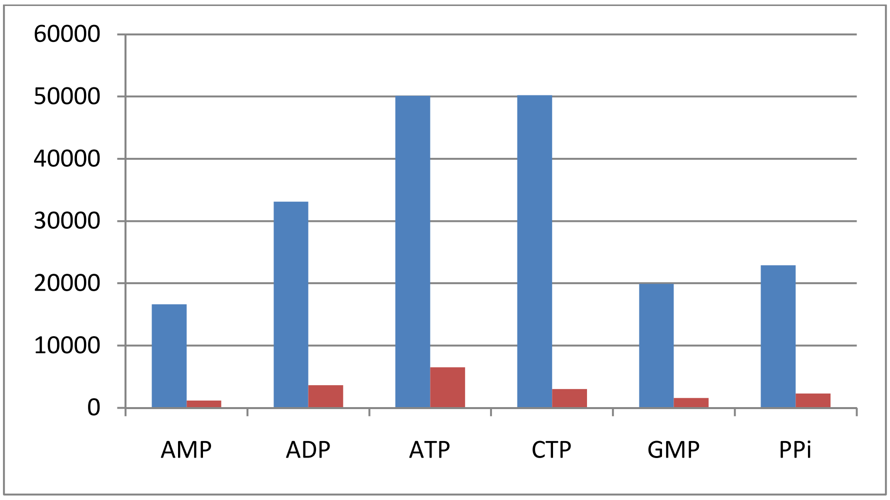


Figure S-80: Receptor **3e** with all used guests, a tendency is observable (blue = binding value, red, error).

As found in earlier investigations with such crown ether receptors,[23] an increase in emission is expected if a guest molecule is bound in the crown ether ring. The opposite effect is observed, which reflects a binding only by the metal complex. Here, the crown ether part acts as a signalling beacon without switching off the PET upon binding. This is underlined by binding constants corresponding approximately to each other. Consequently the binding is just between the cyclene complexed Zn(II)-ions and the phosphate group. The decrease of the fluorescence is clearly visible and can be distinguished with the naked eye.

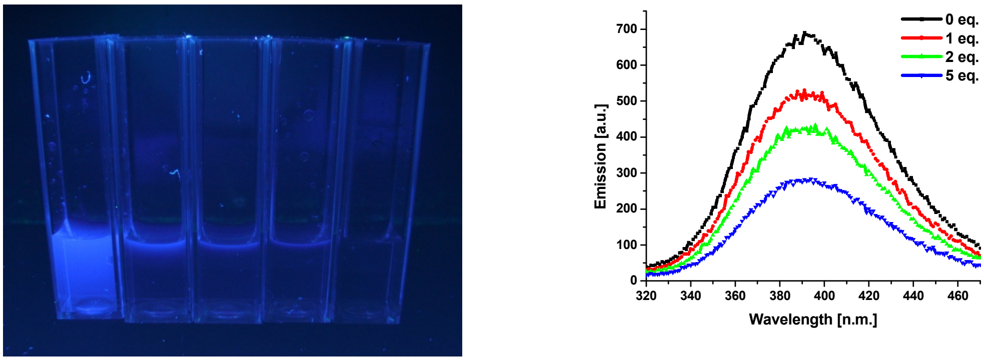


Figure S-81: On the left: Luminescence of a solution of **3e** in the absence and presence of AMP. From left to right: 0, 1, 2, 3, 5 equivalents of AMP were given to a solution of **3e**. On the right: The corresponding graph.

Unfortunately, the ditopic mode of binding could not be confirmed. Binding constants are in the normal range as expected for zinc-complex-phosphate interaction, a shift in emission of the phthalic acid is not observed.[24] The biscyclene-crowns feature a weak selectivity between triphosphates over diphospates and monophosphates of nucleotides. The binding is strong in buffered aqueous solution or under rather physiological conditions. The corresponding luminescence decrease can be observed clearly with the naked eye. Despite the weak selectivity, this could be used as a quick test for the presence of any nucleotides in aqueous solution.

5. Energy minimization calculation pictures with Spartan´06 V.112

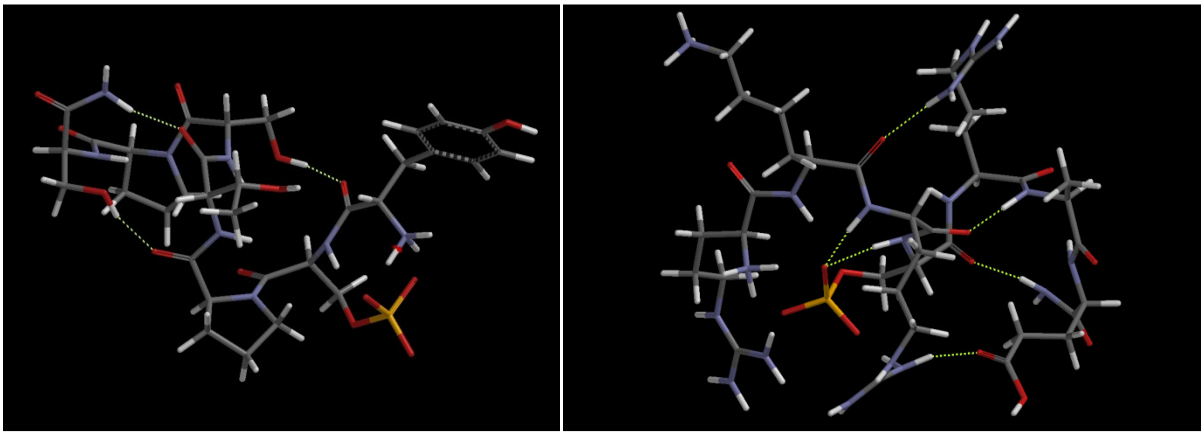


Figure S-82: The stabilizing H-bond network in the CTD heptapeptide **1** (left) and the PKG substrate **2** (right) simulated in the gas phase

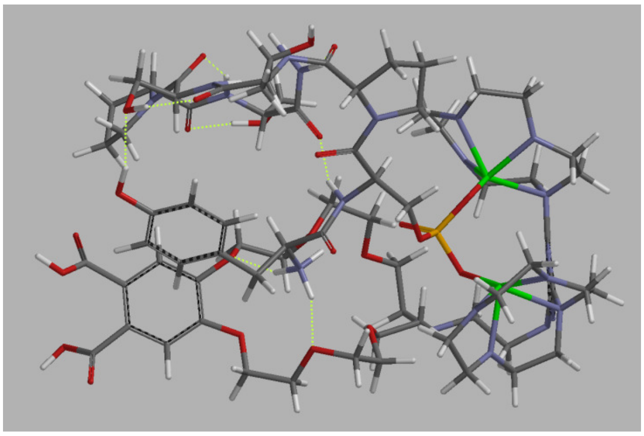


Figure S-83: Structure of the stable aggregate of the CTD heptapeptide (**1**) with **3a** simulated in the gas phase

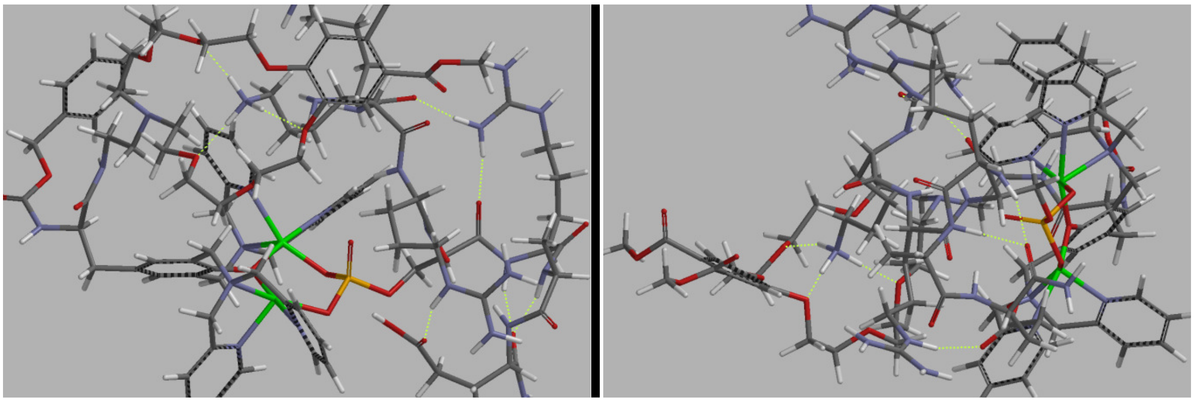


Figure S-84: Structure of the stable aggregate of the PGK substrate (**2**) with **4a** simulated in the gas phase

6. References

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[10] *Alternative procedure for the Boc deprotection with HCl in diethylether:* The respective starting material (0.1 mmol) was dissolved in 2 mL of dry dichloromethane and a saturated solution of hydrochloric acid in diethyl ether (0.4 mL) was added. After three hours of stirring at room temperature the HCl gas and the solvent was removed in a N_2 stream. The residue was taken up in a minimum amount of dichloromethane and the product was precipitated carefully by slow addition of diethyl ether. The solution was decanted off the precipitate, it was washed once with diethyl ether, decanted off again and the product was dried in the vacuum to furnish a hygroscopic powder. *Caution! Care has been taken to work under anhydrous conditions, else the ligands will decompose!*

[11] The peptide was prepared by F. Schmidt in his diploma thesis (University of Regensburg, **2006**) under the supervision of Prof. Dr. B. König.

[12] The thermodynamics of Photoinduced Electron Transfer are described by a simplified Rehm-Weller equation ($\Delta G_{PET} = E_{ox} - E_{red} - e^2/\varepsilon r - E_{oo}$). ΔG_{PET} is calculated to determine the spontaneity of the PET process for chemosensors with a fluorophore-spacer-receptor system. Here, ΔG_{PET} is the free energy for the PET process, E_{ox} is the oxidation potential of the amine, E_{red} is the reduction potential of the chromophore, and ε the dielectric constant of the solvent. To optically detect cation concentrations, the molecule must be able to participate in photo-induced electron transfer (PET) (H.-F. Ji, R. Dabestani, G.M. Brown, R.L. Hettich, *Photochem. Photobiol.* **1999**, *69*, 513.). PET requires that an electron donor is able to travel to the fluorophore and thus quench fluorescence upon excitation (R.A. Bissell, A.P. de Silva, H.Q.N. Gunaratne, P.L.M. Lynch, G.E.M. Maguire, *Chem. Soc. Rev.* **1992**, 187.)

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Am. Chem. Soc. **1999**, *121*, 5426.; E. Kimura, H. Kitamura, K. Ohtani, T. Koike, *J. Am. Chem. Soc.* **2000**, *122*, 4668.; E. Kikuta, S. Aoki, E. Kimura, *J. Biol. Inorg. Chem.* **2002**, *7*, 473.

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[24] The phthalic acid emits at 380 nm if it is protonated. This emission gradually changes with rising pH to nearly 360 nm (A. Späth, B. König, unpublished results). Upon coordination the emission should shift in either the one or the other direction, which is not the case.

VI. Synthesis and Binding Properties of Guanidinium Bis-carboxylates[i]

SUPPORTING INFORMATION

Page

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[i] A. Späth, N. Gonschor, B. König, *Supramol. Chem.* **2010**, accepted.

1. Syntheses

1.1. General methods

Analytical control of the synthesized compounds was done by common methods. Melting Points were determined on Büchi SMP or a Lambda Photometrics OptiMelt MPA 100 and are uncorrected. IR Spectra were recorded with a Bio-Rad FT-IR Excalibur FTS 3000. UV spectra were recorded on a Cary 50 BIO spectrometer with temperature control at 25°C. Electro spray mass spectra were performed on a Finnigan MAT TSQ 7000 ESI-spectrometer. Other Mass Spectra were recorded on Varian CH-5 (EI), Finnigan MAT 95 (CI; FAB and FD), Xenon serves as the ionization gas for FAB.

NMR spectra were recorded on Bruker Avance 600 (^{1}H: 600.1 MHz, ^{13}C: 150.1 MHz, T = 300 K), Bruker Avance 400 (^{1}H: 400.1 MHz, ^{13}C: 100.6 MHz, T = 300 K) or Bruker Avance 300 (^{1}H: 300.1 MHz, ^{13}C: 75.5 MHz, T = 300 K) relative to external standards. NMR spectra were recorded in $CDCl_3$ at 300 MHz (^{1}H) or 75 MHz (^{13}C) unless stated otherwise. Characterization of the signals: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, bs = broad singlet, dd = double doublet, dt = double triplet, ddd = double double doublet. Integration is determined as the relative number of atoms, the coupling constants are given in Hertz [Hz]. The multiplicity of the carbon atoms is given as (+) = CH_3 or CH, (-) = CH_2 and (C_{quat}) for quaternary carbon atoms. Structural assignments are based on DEPT and COSY experiments where applicable. Error of reported values: chemical shift: 0.01 ppm for ^{1}H-NMR, 0.1 ppm for ^{13}C-NMR and 0.1 Hz for coupling constants. The solvent used is reported for each spectrum.

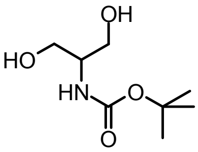
Analytical TLC plates (silica gel 60 F_{254}) and silica gel 60 (70-230 or 230-400 mesh) were used for chromatographic separations. Visualization of the spots was by UV light and/or staining with phosphomolybdate or ninhydrin, both in ethanol. DMF, CH_3CN, $CHCl_3$, THF, and Et_2O were dried by standard procedures and stored over molecular sieves. PE means petrol ether with a boiling range of 70 - 90 °C, EA means ethyl acetate. All other solvents and chemicals were of reagent grade and used with out further purification.

All test substances were of pro analysi grade, checked by NMR or HPLC and used as purchased with out further purification. The Solvents for the fluorescence measurements were from special spectroscopic purity purchased from Acros or Baker or Uvasol from Merck. Millipore water (18 MΩ, Milli Q_{Plus}) was used; the HEPES buffer was from according purity, suitable for biochemical optical screenings. As far as not stated differently tetraethylammoniumhydroxide or 0.1 molar hydrochloric acid both analytical grade were used to adjust the pH in the titrations and screenings.

1.2. Syntheses and protection of amino alcohols

The according *N*-Boc-protected amino acid methyl ester (2.00 g)[1] was dissolved in dry THF (30.0 mL) in a nitrogen atmosphere and $NaBH_4$ and LiCl were subsequently added in small portions. The mixture was stirred over night at room temperature under moisture protection. After having added 20 mL of water slowly, acetic acid (1.2 mL, 20.0 mmol) was dropped in. After short stirring the THF was removed under reduced pressure. A 1:1 mixture of water and diethyl ether (50 mL; 50 mL) was added; the solid was completely dissolved. The organic phase was separated and the aqueous layer was extracted with diethyl ether (3 x 50 mL). The combined ether phases were dried over $MgSO_4$ and the solvent was evaporated under reduced pressure. The residue was purified by column chromatography (ethyl acetate / petrol ether 1:2).

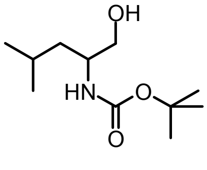
N-(^{tert}*Butyloxycarbonyl)-2-aminopropane-1,3-diol*



N-Boc-L-serine methyl ester (2.00 g, 8.23 mmol) was employed together with 0.69 g $NaBH_4$ (18.1 mmol) and 0.77 g LiCl (18.1 mmol). The reaction yielded 0.99 g colourless oil (4.6 mmol, 59 %).

^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.41 (s, 9 H), 3.54 (m, 1 H), 3.61 (m, 2 H), 3.83 (bs, 2 H,), 4.11 (m, 2 H), 5.04 (bs, 1 H); - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 192.2 (100, MH^+); - **MW** = 191.23; - **MF** = $C_8H_{17}NO_4$.

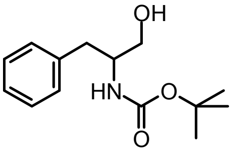
N-(^{tert}*Butyloxycarbonyl)-2-amino-4-methyl-pentan-1-ol*



N-Boc-L-leucine methyl ester (2.00 g, 7.72 mmol) was reacted with 0.65 g $NaBH_4$ (17 mmol) and 0.72 g LiCl (17 mmol), to obtain 1.66 g colourless oil (7.2 mmol, 87 %).

^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 0.86 (d, 6 H, *J* = 6.0 Hz), 1.22 (m, 2 H), 1.40 (s, 9 H), 1.61 (m, 1 H), 3.46 (m, 1 H), 3.68 (bs, 2 H), 4.78 (bs, 2 H); - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 218.2 (100, MH^+); - **MW** = 217.31; - **MF** = $C_{11}H_{23}NO_3$.

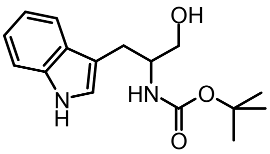
N-(^{tert}Butyloxycarbonyl)-2-amino-3-phenyl-propan-1-ol



N-Boc-L-phenylalanine methyl ester (2.00 g, 6.6 mmol) was reduced with 0.55 g $NaBH_4$ (14.5 mmol) and 0.62 g LiCl (14.5 mmol), to give 1.71 g of an off white solid (6.2 mmol, 94 %).

M.p. (uncorrected) = 88 – 89 °C; - **^{1}H-NMR** (300 MHz, $CDCl_3$): δ [ppm] = 1.40 (s, 9 H), 2.76 (m, 1 H), 2.88 (d, 2 H, *J* = 6.0 Hz), 3.49 – 3.70 (m, 2 H), 3.87 (bs, 1 H), 4.86 (bs, 1 H), 7.19 – 7.28 (m, 3 H), 7.28 – 7.36 (m, 2 H); - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 252.3 (100, MH^+); - **MW** = 251.33; - **MF** = $C_{14}H_{21}NO_3$.

N-(^{tert}Butyloxycarbonyl) 2-amino-3-(1H-indol-3-yl)propan-1-ol

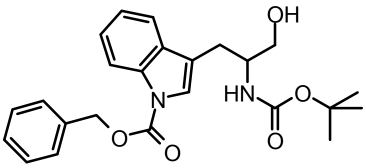


N-Boc-L-tryptophan methyl ester (2.00 g, 5.85 mmol) was reacted with 0.49 g $NaBH_4$ (12.9 mmol) and 0.55 g LiCl (12.9 mmol), to obtain 1.74 g of a white solid (5.5 mmol, 93 %).

M.p. (uncorrected) = 119 – 120 °C; - **^{1}H-NMR** (300 MHz, $CDCl_3$): δ [ppm] = 1.40 (s, 9 H), 2.79 (m, 1 H), 2.99 (d, 2 H, *J* = 6.0 Hz), 3.51 – 3.72 (m, 2 H), 4.00 (bs, 1 H), 4.89 (bs, 1 H), 7.01 (m, 1 H), 7.06 – 7.24 (m, 2H), 7.34 (app. d, 1 H), 7.68 (app. d, 1 H), 8.29 (bs, 1 H); - **MS**

(ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 291.2 (100, MH^+); - **MW** = 290.37; - **MF** = $C_{16}H_{22}N_2O_3$.

3-(N-(^{tert}Butyloxycarbonyl)-2-amino-3-hydroxy-propyl)-1-N-(benzyloxycarbonyl)-indole

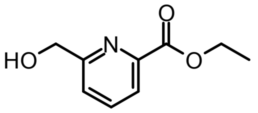


Powdered sodium hydroxide (120 mg, 3.0 mmol) was added to a solution of *N*-Boc-L-tryptophanol (290 mg, 1.0 mmol) and tetrabutylammonium hydrogen sulfate (290 mg, 0.6 mmol) in dried CH_2Cl_2 (5 mL) and the mixture was stirred for two and a half hours at room temperature. Benzylchloroformate (412 mg, 2.4 mmol) was then added and the mixture was allowed to stir for 20 h. After dilution with ethyl acetate (10 mL) and stirring for a half hour, it was washed three times with water (5 mL). After being dried over $MgSO_4$ the solvent was removed under reduced pressure. The residue was purified by flash chromatography (silica gel, ethyl acetate / petrolether 1:3) to afford the title compound as a white solid (380 mg, 0.921 mmol, 92 %).

M.p. (uncorrected) = 76 – 78 °C; - **^{1}H-NMR** (300 MHz, $CDCl_3$): δ [ppm] = 1.47 (s, 9 H), 3.03 (m, 2 H), 4.16 (m, 2 H), 4.25 (m, 1 H), 4.70 (m, 1 H), 4.94 (bs, 1 H), 5.20 (s, 2 H), 6.95 (m, 1 H), 7.12 (m, 1 H), 7.21 (m, 1 H), 7.28 – 7.46 (m, 6 H), 7.65 (m, 1 H); - **IR** (FT-IR, film): ν [cm^{-1}] = 3346 (bm), 2976 (m), 2886 (m), 1726 (m), 1689 (s), 1528 (m), 1454 (m), 1397 (m), 1367 (m), 1254 (s), 1162 (s), 1067 (m), 1019 (m), 941 (m), 896 (m), 852 (m), 791 (m), 739 (s), 697 (s), 660 (m), 593 (m), 556 (m); - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 425.1 (48, MH^+), 442.0 (25, MNH_4^+), 849.3 (100, $2M+H^+$), 866.4 (11, $2M+NH_4^+$); - **MW** = 424.50; - **MF** = $C_{24}H_{28}N_2O_5$.

1.3. Syntheses and deprotections of the building blocks

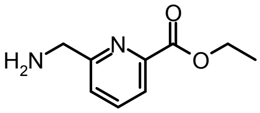
6-(hydroxymethyl)pyridine-2-carboxylic acid ethyl ester (literature known[2], improved)



The 2,6-pyridine carboxylic acid diethyl ester (27.9 g, 0.125 mol) and $NaBH_4$ (3.8 g, 0.1 mol) were dissolved in 250 mL of dry THF and refluxed for 2 h under moisture protection. The solvent was removed and 50 mL water was added. After stirring for 10 mins, the mixture was extracted with $CHCl_3$ (3 x 50 mL). The organic phases were combined, dried over $MgSO_4$ and the solvent was evaporated under reduced pressure. The residue was purified by flash chromatography on silica gel with ethyl acetate / ethanol 6:1 → 3:1 to give the product as colourless solid (15.39 g, 85.02 mmol, 85 %).

^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.33 (t, 3 H, *J* = 7.2 Hz), 4.12 (bs, 1 H), 4.39 (q, 2 H, *J* = 7.2 Hz), 4.81 (s, 2 H), 7.49 (d, 1 H, *J* = 4.6 Hz), 7.78 (app. t, 1 H), 7.92 (d, 1 H, *J* = 4.6 Hz); - **^{13}C-NMR** (75 MHz, $CDCl_3$): δ [ppm] = 14.3 (+, 1 C), 61.9 (-, 1 C), 64.6 (-, 1 C), 123.6 (+, 1 C), 123.9 (+, 1 C), 137.6 (+, 1 C), 147.2 (C_{quat}, 1 C), 160.5 (C_{quat}, 1 C), 165.1 (C_{quat}, 1 C); - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 182.0 (100, MH^+); - **MW** = 181.19; - **MF** = $C_9H_{11}NO_3$.

6-(aminomethyl)pyridine-2-carboxylic acid ethyl ester (literature known, improved)



Thoroughly dried 6-hydroxymethyl-pyridine-2-carboxylic acid ethyl ester (5.8 g, 35 mmol) was dissolved in small portions in well stirred $SOCl_2$ (11.5 mL) under moisture protection not allowing the temperature to rise over 0°C. After 90 min the solution was allowed to reach room temperature and the excess of $SOCl_2$ was removed under reduced pressure without heating. Toluene (20 mL) was added to the oily residue, the solution was washed with cold aqueous 1 M $NaHCO_3$ (2 x 10 mL) and dried over $MgSO_4$. Evaporation of the solvent afforded 6.5 g of the product (32.5 mmol, 83 %) as orange oil (analogue to the literature[3]).

^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.31 (t, 3 H, *J* = 7.2 Hz), 4.39 (q, 2 H, *J* = 7.2 Hz), 4.70 (s, 2 H), 7.62 (d, 1 H, *J* = 4.6 Hz), 7.81 (app. t, 1 H), 7.97 (d, 1 H, *J* = 4.6 Hz); - **^{13}C-**

NMR (75 MHz, $CDCl_3$): δ [ppm] = 14.2 (+, 1 C), 46.3 (-, 1 C), 62.0 (-, 1 C), 124.3 (+, 1 C), 126.1 (+, 1 C), 138.1 (+, 1 C), 147.6 (C_{quat}, 1 C), 157.1 (C_{quat}, 1 C), 164.7 (C_{quat}, 1 C); - **IR** (FT-IR, film): ν [cm^{-1}] = 2982 (m), 2936 (m), 2884 (m), 1716 (s), 1589 (m), 1454 (m), 1369 (m), 1313 (s), 1257 (s), 1226 (m), 1175 (s), 1123 (s), 1086 (m), 1021 (m), 945 (m), 863 (m), 834 (m), 746 (s), 695 (s), 593 (m); - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 202.1 (31, MH^+), 200.1 (100, MH^+); - **MW** = 199.64; - **MF** = $C_9H_{10}ClNO_2$.

2-Chloromethyl-pyridine-6-carboxylic acid ethyl ester (6.0 g, 30 mmol) in anhydrous DMF (10 mL) was slowly added to a dry DMF (10 mL) sodium phthalimide solution (6.12 g, 36 mmol). After stirring for 2 h at room temperature the reaction mixture was centrifuged, the solvent was removed under reduced pressure and the residue was dissolved in $CHCl_3$ (100 mL). The resulting solution was washed with 0.2 M NaOH (2 x 100 mL), then with water and dried. Removal of the solvent yielded a solid residue, which was dissolved in warm ethanol (500 mL). Hydrazine (1.13 g, 35 mmol) was added and the mixture was refluxed until disappearance (monitored by TLC) of the starting material. The mixture was cooled to 5 °C, filtered and the solvent was removed to finally receive 4.05 g of yellow syrup (22.5 mmol, 75 %).

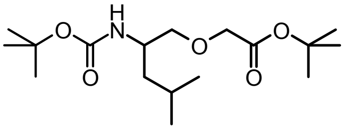
^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.27 (t, 3 H, *J* = 7.2 Hz), 4.28 (q, 2 H, *J* = 7.2 Hz), 4.49 (s, 2 H), 7.69 (d, 1 H, *J* = 4.6 Hz), 7.77 (m, 1 H), 7.87 (d, 1 H, *J* = 4.6 Hz), 8.61 (bs, 1 H); - **^{13}C-NMR** (75 MHz, $CDCl_3$): δ [ppm] = 14.2 (+, 1 C), 44.4 (-, 1 C), 62.1 (-, 1 C), 124.5 (+, 1 C), 126.5 (+, 1 C), 138.2 (+, 1 C), 147.2 (C_{quat}, 1 C), 154.4 (C_{quat}, 1 C), 164.8 (C_{quat}, 1 C); - **IR** (FT-IR, film): ν [cm^{-1}] = 3402 (bm), 2979 (m), 2901 (m), 2722 (m), 2631 (m), 1720 (s), 1663 (m), 1591 (s), 1526 (s), 1449 (s), 1388 (m), 1369 (m), 1300 (s), 1253 (s), 1230 (s), 1180 (s), 1143 (s), 1091 (m), 1020 (m), 997 (s), 894 (m), 862 (m), 831 (m), 760 (s), 655 (m), 621 (m); - **MS** (CI-MS, NH_3): m/z (%) = 181.0 (100, MH^+); - **MW** = 180.21; - **MF** = $C_9H_{12}N_2O_2$.

1.4. Ether syntheses

1.4.1. Phase transfer catalytic etherification of substituted amino alcohols (GP 1)

The *N*-Boc-amino alcohol was dissolved with tetrabutylammonium hydrogensulfate (0.4 mmol, 98.8 mg) in CH_2Cl_2 (6 mL). Aqueous NaOH (5.0 M, 6.0 mL) was added, bromoacetic acid ^{tert}butyl ester (1.2 mmol, 233 mg) in CH_2Cl_2 (1 mL) was dropped in and the reaction mixture was vigorously stirred for 3 h at room temperature. The same amount bromoacetic acid ^{tert}butyl ester was added again and the heterogeneous solution was stirred over night. The mixture was poured on ice (10 g) in a separation funnel, the organic phase was separated and the aqueous layer was extracted with dichloromethane (3 x 10 mL). The organic phases were combined, dried over $MgSO_4$ and the solvent was removed under reduced pressure. The crude product was purified by column chromatography with ethyl acetate / petrol ether 1:4 to obtain the pure compound.

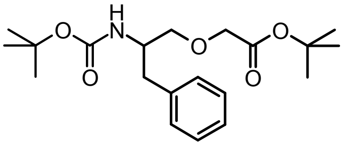
2-(N-(^{tert}Butyloxycarbonyl)-2-amino-4-methyl-pentoxy)-acetic acid ^{tert}butylester **(7e)***:*



The *N*-Boc-L-leucinol (217 mg, 1.0 mmol) was reacted to obtain 256 mg of a white oily solid (0.77 mmol, 77 %).

^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 0.86 (d, 6 H, *J* = 7.2 Hz), 1.36 (m, 2 H), 1.38 (s, 9 H), 1.41 (s, 9 H), 1.61 (m, 1 H), 3.43 (m, 2 H), 3.70 (m, 1 H), 3.89 (s, 2 H), 4.83 (bs, 1 H); - **^{13}C-NMR** (75 MHz, $CDCl_3$): δ [ppm] = 22.4 (+, 1 C), 22.9 (+, 1 C), 24.8 (+, 1 C), 28.1 (+, 3 C), 28.4 (+, 3 C), 41.0 (-, 1 C), 48.5 (+, 1 C), 68.4 (-, 1 C), 73.8 (-, 1 C), 78.9 (C_{quat}, 1 C), 82.8 (C_{quat}, 1 C), 155.6 (C_{quat}, 1 C), 166.2 (C_{quat}, 1 C); - **IR** (FT-IR, film): ν [cm^{-1}] = 3354 (bm), 2959 (m), 2934 (m), 2874 (m), 1748 (m), 1710 (s), 1503 (m), 1457 (m), 1387 (m), 1367 (s), 1295 (m), 1228 (s), 1164 (s), 1128 (s), 1049 (m), 941 (m), 846 (m), 778 (m), 751 (m); - **MS** (CI-MS, NH_3): m/z (%) = 219.1 (13, MH^+ - 2 C_4H_8), 237.0 (20, MNH_4^+ - $2C_4H_8$), 275.2 (48, MH^+ - C_4H_8), 293.2 (69, MNH_4^+ - C_4H_8), 332.2 (100, MH^+), 349.2 (83, MNH_4^+); - **MW** = 331.46; - **MF** = $C_{17}H_{33}NO_5$.

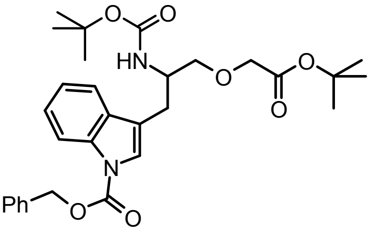
2-(N-(^{tert}Butyloxycarbonyl)-2-amino-3-phenyl-propoxy)-acetic acid ^{tert}butylester **(7g)**:



The *N*-Boc-L-phenylalaninol (250 mg, 1.0 mmol) was employed to yield 328 mg of **7g** as a colourless wax (0.89 mmol, 89 %).

^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.33 (s, 9 H), 1.39 (s, 9 H), 2.82 (m, 2 H), 3.37 (m, 2 H), 3.65 (m, 1 H), 3.87 (s, 2 H), 5.17 (bs, 1 H), 7.18 – 7.30 (m, 5 H); - **^{13}C-NMR** (75 MHz, $CDCl_3$): δ [ppm] = 28.1 (+, 3 C), 28.4 (+, 3 C), 37.1 (-, 1 C), 51.8 (+, 1 C), 68.5 (-, 1 C), 71.6 (-, 1 C), 79.2 (C_{quat}, 1 C), 82.9 (C_{quat}, 1 C), 126.3 (+, 1 C), 128.4 (+, 2 C), 129.5 (+, 2 C), 138.3 (C_{quat}, 1 C), 155.5 (C_{quat}, 1 C), 166.3 (C_{quat}, 1 C); - **IR** (FT-IR, film): ν [cm^{-1}] = 3375 (bm), 2978 (m), 2932 (m), 2880 (m), 1746 (m), 1710 (s), 1497 (m), 1456 (m), 1389 (m), 1367 (m), 1294 (m), 1230 (m), 1163 (s), 1130 (s), 1058 (m), 943 (m), 917 (m), 846 (m), 742 (m), 701 (m); - **MS** (CI-MS, NH_3): m/z (%) = 253.1 (14, MH^+ - 2 C_4H_8), 271.0 (21, MNH_4^+ - 2 C_4H_8), 309.1 (82, MH^+ - C_4H_8), 327.1 (63, MNH_4^+ - C_4H_8), 366.2 (100, MH^+), 383.2 (78, MNH_4^+); - **HRMS** (EI-MS 70 eV): calc. for $C_{20}H_{31}NO_5^{*+}$: 365.2202, found: 365.2197; - **MW** = 365.47; - **MF** = $C_{20}H_{31}NO_5$.

2-((N-(^{tert}Butyloxycarbonyl)-2-amino-3-hydroxy-propyl)-3-(1-N-(benzyloxycarbonyl)-indole))-acetic acid ^{tert}butylester **(7i)**



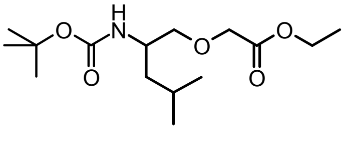
The 3-(*N*-Boc-L-2-amino-3-hydroxy-propyl)-1-*N*-Cbz-indole (298 mg, 0.7 mmol) was used to give 236 mg as colourless oil (0.441 mmol, 63 %).

^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.41 (s, 9 H), 1.43 (s, 9 H), 3.06 (m, 2 H), 3.46 (m, 1 H), 3.98 (s, 2 H), 4.11 (m, 2 H), 4.61 (m, 2 H), 5.19 (bs, 1 H), 6.98 (m, 1 H), 7.10 (m, 1 H), 7.19 (m, 1 H), 7.29 – 7.41 (m, 7 H); - **^{13}C-NMR** (75 MHz, $CDCl_3$): δ [ppm] = 28.1 (+, 3 C), 28.4 (+, 3 C), 48.5 (-, 1 C), 51.0 (+, 1 C), 67.7 (-, 1 C), 68.5 (-, 1 C), 68.9 (-, 1 C), 79.1 (C_{quat}, 1 C), 81.8 (C_{quat}, 1 C), 108.9 (+, 1 C), 111.9 (C_{quat}, 1 C), 119.4 (+, 1 C), 121.9 (+, 1 C), 127.4 (+, 1 C), 127.9 (-, 1 C), 128.1 (+, 2 C), 128.4 (+, 2 C), 128.6 (+, 1 C), 136.8 (C_{quat}, 1 C), 137.4 (C_{quat}, 1 C), 155.6 (C_{quat}, 1 C), 167.6 (C_{quat}, 1 C), 169.0 (C_{quat}, 1 C), 169.6 (C_{quat}, 1 C); - **IR** (FT-IR, film): ν [cm^{-1}] = 3061 (bm), 2977 (m), 2933 (m), 1745 (s), 1491 (m), 1468 (m), 1389 (m), 1367 (m), 1299 (m), 1227 (s), 1189 (s), 1128 (s), 1050 (m), 943 (m), 847 (m), 803 (m), 740 (m), 699 (m), 584 (m); - **MS** (CI-MS, NH_3): m/z (%) = 539.1 (33, MH^+), 556.1 (36, MNH_4^+), 577.1 (100, MK^+), 599.1 (64, $M+HOAc+H^+$); - **MW** = 538.65; - **MF** = $C_{30}H_{38}N_2O_7$.

1.4.2. Sodium hydride catalysed ether synthesis (GP 2)

In an nitrogen flushed Schlenck-flask the *N*-Boc-aminoalcohol (5.0 mmol) was added to a suspension of NaH (60 % susp., 380 mg, 8.0 mmol) and KI (150 mg, 0.8 mmol) in 30.0 mL of dry THF at 0°C. After drop wise addition of ethyl bromoacetate (1.67 g, 1.11 mL, 10.0 mmol) in 10 mL of dry THF, the reaction mixture was stirred for 4 h at room temperature. The NaH suspension and precipitated solids were settled by a centrifuge. The THF solution was decanted off and evaporated to give the crude product. The residue was purified by column chromatography (petrol ether / ethyl acetate, 9:1 → 4:1) to give the according glycol-δ- amino acid ester.

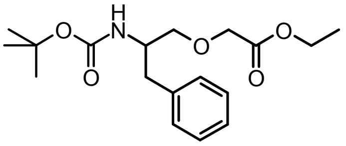
2-(N-(^{tert}Butyloxycarbonyl)-2-amino-4-methyl-pentoxy)-acetic acid ethyl ester **(7f)**:



The *N*-Boc-L-leucinol (1.09 g, 5.0 mmol) was reacted to obtain 790 mg as a white oily solid (2.62 mmol, 52 %).

^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 0.84 (d, 6 H, *J* = 7.1 Hz), 1.23 (t, 3 H, *J* = 7.1 Hz), 1.38 (s, 9 H), 1.39 (m, 2 H), 1.61 (m, 1 H), 3.48 (m, 2 H), 3.72 (m, 1 H), 4.01 (s, 2 H), 4.16 (q, 2 H, *J* = 7.1 Hz), 4.81 (bs, 1 H); - **^{13}C-NMR** (75 MHz, $CDCl_3$): δ [ppm] = 14.1 (+, 1 C), 22.1 (+, 1 C), 22.9 (+, 1 C), 24.7 (+, 1 C), 25.7 (+, 1 C), 28.4 (+, 3 C), 40.6 (-, 1 C), 47.7 (-, 1 C), 61.1 (-, 1 C), 68.2 (-, 1 C), 71.4 (-, 1 C), 79.5 (C_{quat}, 1 C), 155.4 (C_{quat}, 1 C), 167.2 (C_{quat}, 1 C); - **IR** (FT-IR, film): ν [cm^{-1}] = 3354 (bm), 2958 (m), 2921 (m), 2874 (m), 1739 (m), 1690 (s), 1517 (m), 1454 (m), 1388 (m), 1366 (m), 1277 (s), 1251 (m), 1163 (s), 1115 (m), 1049 (m), 1003 (m), 870 (m), 780 (m); - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 304.2 (100, MH^+); - **MW** = 303.40; - **MF** = $C_{15}H_{29}NO_5$.

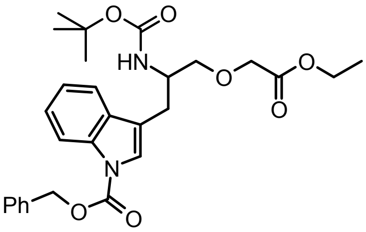
2-(N-(^{tert}Butyloxycarbonyl)-2-amino-3-phenyl-propoxy)-acetic acid ethyl ester **(7h)**



The *N*-Boc-L-phenylalaninol (1.25 g, 5.0 mmol) was employed to yield 820 mg of the product as a faintly yellow waxy solid (2.44 mmol, 49 %).

^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.27 (t, 3 H, *J* = 7.1 Hz), 1.41 (s, 9 H), 2.86 (m, 2 H), 3.42 (m, 2 H), 3.81 (s, 2 H), 4.07 (m, 1 H), 4.22 (q, 2 H, *J* = 7.1 Hz), 5.17 (bs, 1 H), 7.13 – 7.31 (m, 5 H); - **^{13}C-NMR** (75 MHz, $CDCl_3$): δ [ppm] = 14.2 (+, 1 C), 28.3 (+, 3 C), 37.7 (-, 1 C), 50.8 (+, 1 C), 61.0 (-, 1 C), 66.6 (-, 1 C), 68.5 (-, 1 C), 79.4 (C_{quat}, 1 C), 126.8 (+, 1 C), 128.4 (+, 2 C), 129.3 (+, 2 C), 138.2 (C_{quat}, 1 C), 155.5 (C_{quat}, 1 C), 167.1 (C_{quat}, 1 C); - **IR** (FT-IR, film): ν [cm^{-1}] = 3366 (bm), 2978 (m), 2933 (m), 1743 (m), 1703 (s), 1498 (s), 1453 (m), 1388 (m), 1366 (m), 1276 (m), 1247 (m), 1208 (m), 1163 (s), 1132 (s), 1057 (m), 1026 (m), 851 (m), 742 (m), 701 (m); - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 338.1 (100, MH^+); - **MW** = 337.42; - **MF** = $C_{18}H_{27}NO_5$.

2-((N-(^{tert}Butyloxycarbonyl)-2-amino-3-hydroxy-propyl)-3-(1-N-(benzyloxycarbonyl)-indole))-acetic acid-ethylester



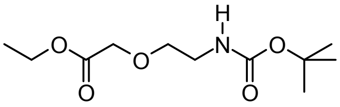
The 3-(*N*-Boc-L-2-amino-3-hydroxy-propyl)-1-N-Cbz-indole (**8e**) (298 mg, 0.7 mmol) was reacted to give 64 mg as colourless waxy solid (0.126 mmol, 18 %).

^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.23 (t, 3 H, *J* = 7.1 Hz), 1.39 (s, 9 H), 3.08 (m, 2 H), 3.47 (m, 1 H), 3.96 (s, 2 H), 4.11 (m, 2 H), 4,15 (q, 2 H, *J* = 7.1 Hz), 4.63 (m, 2 H), 5.17

(bs, 1 H), 6.97 (m, 1 H), 7.12 (m, 1 H), 7.18 (m, 1 H), 7.26 – 7.43 (m, 7 H); - **IR** (FT-IR, film): ν [cm^{-1}] = 3060 (bm), 2981 (m), 2936 (m), 2889 (m), 1743 (s), 1493 (m), 1470 (m), 1386 (m), 1368 (m), 1301 (m), 1225 (s), 1189 (s), 1123 (s), 1054 (m), 944 (m), 846 (m), 805 (m), 743 (m), 699 (m); - **MW** = 510.59; - **MF** = $C_{28}H_{34}N_2O_7$.

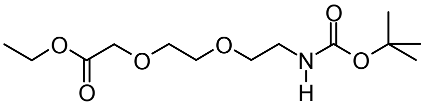
1.4.3. Alternative prescriptions for the ether synthesis

Catalyzed by potassium ^{tert}butylate



A 100 mL Schlenck-flask was evacuated and then filled by nitrogen several times. Into the solution of *N*-Boc-protected ethanolamine (0.80 g, 5.0 mmol) in dry THF (10 mL) was given ethyl bromoacetate (1.14 mL, 10.0 mmol) and potassium ^{tert}butylate (1.39 g, 12.0 mmol) in small portions. The mixture changed its colour to orange. The mixture was stirred for 3 hours at 0 °C. The base suspension was settled by a centrifuge, the THF solution was decanted off and the solvent was evaporated. The crude orange oil was purified by column chromatography (ethyl acetate / petrol ether – 1:1). The product (EtOAc / PE 1:1 R_f ~ 0.5) is isolated as a slightly yellowish oil (0.58 g, 2.34 mmol, 47 %).

2-(2-(2-^{tert}Butoxycarbonylaminoethoxy)ethoxy)acetic acid ethyl ester

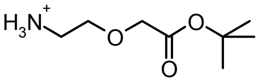


A solution of *N*-Boc-ethoxy-ethanolamine (500 mg, 2.5 mmol) in DCM (10 mL) was cooled to 5 °C in a nitrogen atmosphere. Copper(II)triflate (90 mg, 0.25 mmol, 10 mol%) and one drop of phenyl hydrazine were added, followed by TMEDA (40 mg, 0.25 mmol, 10 mol%). After stirring for 30 minutes 2.2 mL of 15 % (weight/weight) azo acetic acid ethyl ester in DCM (0.43 g, 3.8 mmol) were dropped in over a period of 3 h at 5 °C. The mixture was allowed to warm up slowly to room temperature and was stirred over night. The solution was filtered over alumina N, the filter cake was washed with DCM and the solvent was evaporated. The raw material was purified by column chromatography (ethyl acetate / petrol ether 2:1 → 1:1) to yield a yellow oil (468 mg, 1.69 mmol, 68 %) (EtOAc / PE 1:2 R_f = 0.2).

^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.28 (t, 3 H, *J* = 7.1 Hz), 1.43 (s, 9 H), 3.31 (m, 2 H), 3.54 (t, 2 H, *J* = 5.2 Hz), 3.65 (m, 2 H), 3.71 (m, 2 H), 4.13 (s, 2 H), 4.21 (q, 2 H, *J* = 7.1 Hz), 5.03 (bs, 1 H); - **^{13}C-NMR** (75 MHz, $CDCl_3$): δ [ppm] = 14.2 (+, 1 C), 28.4 (+, 3 C), 40.4 (-, 1 C), 61.0 (-, 1 C), 61.2 (-, 1 C), 61.3 (-, 1 C), 68.7 (-, 1 C), 70.8 (-, 1 C), 79.1 (C_{quat}, 1 C), 156.0 (C_{quat}, 1 C), 170.4 (C_{quat}, 1 C); - **IR** (FT-IR, film): ν [cm^{-1}] = 3379 (bm), 2977 (m), 2953 (m), 1749 (m), 1705 (s), 1515 (m), 1455 (m), 1366 (m), 1248 (m), 1202 (s), 1117 (s), 907 (m), 863 (m), 779 (m), 718 (m), 579 (m), 530 (m); - **MS** (CI-MS, NH_3): m/z (%) = 192.2 (8, MH^+ - CO_2 -C_4H_8), 235.2 (100, MH^+ -C_4H_8), 253.1 (16, MNH_4^+ - C_4H_8), 292.2 (4, MH^+), 309.2 (6, MNH_4^+); - **MW** = 291.35; - **MF** = $C_{13}H_{25}NO_6$.

1.4.4. Deprotection of glycol amino acids

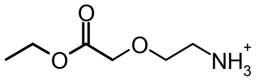
*Example procedure for hydrogenolytic deprotection: 2-(2-Aminoethyloxy)acetic acid ethyl ester acetate (**10c**)*



Compound **7c** (1.55 g, 5.0 mmol) was dissolved in methanol (20 mL), three spatula tips of palladium on charcoal (10 % Pd) and acetic acid (0.3 g, 0.3 mL, 5.0 mmol) were added and the mixture was stirred for two days in a hydrogen atmosphere (20 bar). The solution was filtered over celite, the filter cake was washed with small amounts of methanol and the solvent was removed under reduced pressure. The product appears as clear, colourless oil (1.14 g, 4.84 mmol, 94 %).

^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.45 (s, 9 H), 2.00 (s, 3 H), 3.15 (t, 2 H, *J* = 5.2 Hz), 3.77 (t, 2 H, *J* = 5.2 Hz), 3.99 (s, 2 H), 9.13 (bs, 3 H); - **^{13}C-NMR** (75 MHz, $CDCl_3$): δ [ppm] = 22.0 (+, 1 C), 28.1 (+, 3 C), 39.5 (-, 1 C), 68.0 (-, 1 C), 68.4 (-, 1 C), 82.6 (C_{quat}, 1 C), 170.3 (C_{quat}, 1 C), 176.9 (C_{quat}, 1 C); - **IR** (FT-IR, film): ν [cm^{-1}] = 3373 (bm), 2977 (m), 2929 (m), 1737 (m), 1666 (m), 1556 (m), 1398 (m), 1369 (m), 1241 (m), 1128 (s), 1014 (m), 839 (m), 721 (m), 652 (m); - **MS** (CI-MS, NH_3): m/z (%) = 120.1 (13, MH^+ - C_4H_8), 176.1 (100, MH^+); - **MW** = 235.29; - **MF** = $C_{10}H_{21}NO_5$.

*(2-amino-ethoxy)-acetic acid ethyl ester hydrochloride (**10b**)*



To a solution of **7b** (0.52 g, 2.0 mmol) in dried DCM (10 mL) was slowly added 4 mL of HCl saturated diethylether. After 3 h of stirring at room temperature the solvent was evaporated. The residue was taken up in a little of DCM, and the solvent was removed under reduced pressure again. This process was repeated once. The remaining sticky solid was dried in the vacuum over night. The yield was 0.38 g of a yellow, deliquescent solid (1.96 mmol, 98 %).

The same result is achieved, if instead of HCl in diethylether TFA is used. The yields and the appearance of the product are comparable. Both products show no difference in reactivity.

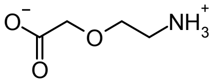
Hydrochloric Acid Salt:

^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.26 (t, 3 H, *J* = 7.1 Hz), 3.32 (m, 2 H), 3.91 (m, 2 H), 4.09 – 4.21 (q, 2 H, *J* = 7.1 Hz), 4.18 (s, 2 H), 6.50 (bs, 3 H); - **^{13}C-NMR** (75 MHz, $CDCl_3$): δ [ppm] = 14.1 (+, 1 C), 39.4 (-, 1 C), 61.3 (-, 1 C), 67.4 (-, 1 C), 68.2 (-, 1 C), 171.0 (C_{quat}, 1 C); - **IR** (FT-IR, film): ν [cm^{-1}] = 3470 (bm), 2937 (m), 2916 (m), 1726 (m), 1669 (s), 1516 (m), 1430 (m), 1183 (s), 1127 (s), 1020 (m), 965 (m), 836 (m), 799 (s), 721 (m), 673 (m), 517 (m); - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 147.8 (100, MH^+), 294.9 ($2M+H^+$, 5%); - **MW** = 183.63; - **MF** = $C_6H_{14}ClNO_3$.

TFA Salt:

^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.28 (t, 3 H, *J* = 7.1 Hz), 3.32 (m, 2 H), 3.81 (t, 2 H, *J* = 5.2 Hz), 4.14 (s, 2H), 4.21 (q, 2 H, *J* = 7.1 Hz), 7.78 (bs, 3 H); - **IR** (FT-IR, film): ν [cm^{-1}] = 3460 (bm), 2984 (m), 2937 (m), 1720 (m), 1671 (s), 1519 (m), 1428 (m), 1378 (m), 1184 (s), 1127 (s), 1021 (m), 966 (m), 836 (m), 799 (s), 721 (m), 677 (m), 596 (m), 517 (m), 495 (m); - **MS** (CI-MS, NH_3, +Q1MS): m/z (%) = 148.1 (100, MH^+); **MS** (CI-MS, NH_3, -Q1MS): m/z (%) = 146.0 (100, $M-H^+$); - **MW** = 261.20; - **MF** = $C_8H_{14}F_3NO_5$.

*(2-Amino-ethoxy)-acetic acid (**10a**)*



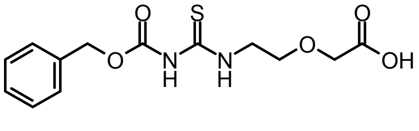
Compound **7a** or **7b** (270 mg, 1.0 mmol) was dissolved in 0.5 mL of methanol, 1.0 mL of 1 N aqueous HCl were added, and the mixture was stirred for 3 h. The solvent was evaporated and the residue was lyophilised to yield **10a** as colourless sticky oil (118 mg, 1.0 mmol, quant.).

^{1}H-NMR (300 MHz, MeOD): δ [ppm] = 3.19 (t, 2 H, *J* = 5.2 Hz), 3.81 (t, 2 H, *J* = 5.2 Hz), 4.26 (s, 2 H), 4.86 (bs, 3 H), 8.08 (bs, 1 H); - **^{13}C-NMR** (75 MHz, MeOD): δ [ppm] = 40.8 (-, 1 C), 68.4 (-, 1 C), 68.8 (-, 1 C), 173.2 (C_{quat}, 1 C); - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 119.8 (100, MH^+); - **MW** = 119.12; - **MF** = $C_4H_9NO_3$.

1.4.5. Thiourea synthesis with free acids in dioxane and water (GP 3)

Benzyloxycarbonyl-isothiocyanate (250 mg, 1.3 mmol) in dioxane (4.0 mL) was added to a solution of the amine compound (1.0 mmol) in aqueous NaOH (1 M; 2.2 mL). The mixture was stirred at room temperature for 12 h. The organic solvent was removed under reduced pressure. The aqueous phase was acidified with 5 % $KHSO_4$ solution to pH 2. After addition of ethyl acetate (10 mL) the phases were separated and the water phase was extracted twice with ethyl acetate (20 mL). The combined organic phases were dried over Na_2SO_4 and the solvent was removed under reduced pressure. The residue was suspended in diethyl ether (10 mL), the ether was decanted off and the remaining precipitate was dried. The crude product was purified by column chromatography on silica gel (ethyl acetate).

2-(2-[3-N-(benzyloxycarbonyl)thioureido]ethyloxy)acetic acid **(12a)***:*

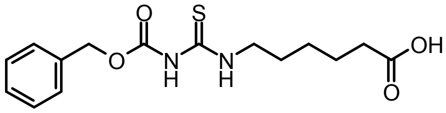


Amino ethoxy acetic acid (118 mg, 1.00 mmol) was used to give 262 mg of yellow oil (0.839 mmol, 84 %).

^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 3.78 (t, 2 H, *J* = 5.2 Hz), 3.91 (t, 2 H, *J* = 5.2 Hz), 4.14 (s, 2 H), 5.19 (s, 2 H), 7.28 – 7.43 (m, 5 H), 8.10 (bs, 1 H), 9.95 (bs, 1 H); - **^{13}C-NMR**

(75 MHz, $CDCl_3$): δ [ppm] = 44.5 (-, 1 C), 67.2 (-, 1 C), 67.2 (-, 1 C), 68.0 (-, 1 C), 127.3 (+, 2 C), 127.7 (+, 2 C), 127.8 (+, 1 C), 133.5 (C_{quat}, 1 C), 151.2 (C_{quat}, 1 C), 169.8 (C_{quat}, 1 C), 178.2 (C_{quat}, 1 C); - **IR** (FT-IR, film): ν [cm^{-1}] = 3285 (bm), 2950 (m), 1719 (s), 1512 (s), 1452 (m), 1396 (m), 1315 (m), 1218 (s), 1128 (s), 1082 (m), 1023 (m), 974 (m), 838 (m), 738 (m), 694 (s), 580 (m), 490 (m); - **MS** (EI-MS, 70 eV): m/z (%) = 312.1 (73, M^{*+}), 221.0 (6, M^{*+}), 91.1 (100, $C_7H_7^{*+}$); - **MW** = 312.35; - **MF** = $C_{13}H_{16}N_2O_5S$.

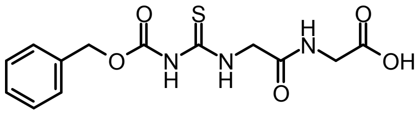
*6-[3-N-(benzyloxycarbonyl)thioureido]hexanoic acid **(13a)***



ε-Aminohexanoic acid (131 mg, 1.0 mmol) was used to obtain 261 mg of an oily, colourless solid (0.806 mmol, 81 %).

^{1}H-NMR (300 MHz, MeOD): δ [ppm] = 1.37 (m, 2 H), 1.62 (m, 4 H), 2.39 (t, 2 H, *J* = 7.2 Hz), 3.61 (t, 2 H, *J* = 7.2 Hz), 5.17 (s, 2 H), 7.21 – 7.47 (m, 5 H); - **^{13}C-NMR** (75 MHz, MeOD): δ [ppm] = 25.6 (-, 1 C), 27.5 (-, 1 C), 29.1 (-, 1 C), 34.9 (-, 1 C), 46.0 (-, 1 C), 68.8 (-, 1 C), 128.9 (+, 1 C), 129.5 (+, 2 C), 129.7 (+, 2 C), 136.9 (C_{quat}, 1 C), 154.9 (C_{quat}, 1 C), 177.6 (C_{quat}, 1 C), 181.2 (C_{quat}, 1 C); - **IR** (FT-IR, film): ν [cm^{-1}] = 3390 (bm), 3040 (m), 2989 (m), 2937 (m), 2870 (m), 1770 (m), 1706 (s), 1654 (s), 1462 (m), 1397 (m), 1341 (m), 1215 (m), 1044 (m), 744 (m), 666 (m), 591 (m); - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 324.9 (100, MH^+), 649.1 (46, 2M + H^+), 666.1 (46, 2M + NH_4^+); - **MW** = 324.40; - **MF** = $C_{15}H_{20}N_2O_4S$.

*2-(2-[3-N-(benzyloxycarbonyl)thioureido]acetamido)acetic acid **(14a)***



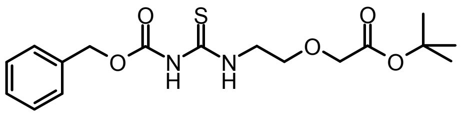
Glycyl glycine (132 mg, 1.0 mmol) was employed to give 253 mg of an oily product (0.778 mmol, 78 %).

^{1}H-NMR (300 MHz, MeOD): δ [ppm] = 3.95 (s, 2 H), 4.38 (s, 2 H), 5.19 (s, 2 H), 7.22 – 7.48 (m, 5 H); - **^{13}C-NMR** (75 MHz, MeOD): δ [ppm] = 41.8 (-, 1 C), 67.4 (-, 1 C), 68.8 (-, 1 C), 128.9 (+, 1 C), 129.5 (+, 2 C), 129.7 (+, 2 C), 137.0 (C_{quat}, 1 C), 154.8 (C_{quat}, 1 C), 170.9 (C_{quat}, 1 C), 173.0 (C_{quat}, 1 C), 182.1 (C_{quat}, 1 C); - **IR** (FT-IR, film): ν [cm^{-1}] = 3299 (bm), 3236 (m), 3057 (m), 2900 (m), 2456 (m), 2332 (m), 1711 (s), 1656 (m), 1538 (s), 1398 (m), 1360 (m), 1216 (s), 1136 (m), 1034 (m), 976 (m), 877 (m), 802 (m), 765 (m), 731 (m), 693 (m), 662 (m), 606 (m); - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 326.0 (86, MH^+), 343.0 (26, MNH_4^+), 651.0 (100, 2M + H^+), 668.0 (39, 2M + NH_4^+), 976.1 (17, 3M + H^+), 993.2 (30, 3M + NH_4^+), 1301.3 (9, 4M + H^+), 1318.4 (16, 4M + NH_4^+); - **MW** = 325.35; - **MF** = $C_{13}H_{15}N_3O_5S$.

1.4.6. Preparation of benzyloxycarbonylthioureas from amino acid esters (GP 4)

Benzyloxycarbonylisothiocyanat (500 mg, 2.6 mmol) in dichloromethane (10 mL) was added slowly to a solution of the according amine compound (2.0 mmol) and triethylamine (300 mg, 0.4 mL, 3.0 mmol or 500 mg, 0.68 mL, 5.0 mmol if the amine salt is employed) in dichloromethane (10 mL) at 2 – 5 °C. The solution was stirred at room temperature until TLC indicated no further reaction (2 – 6 h). All volatiles were removed under reduced pressure. Ethyl acetate (30 mL) was added, the organic phase was washed with saturated ammonium chloride solution (5 mL) and twice with water (10 mL). After drying over $MgSO_4$, the solvent was evaporated and the residue was suspended in diethyl ether / petrol ether 1:4 (5 mL). The product was allowed to settle completely, the ether mixture was decanted off, and after drying the precipitate, it was purified by column chromatography if necessary (ethyl acetate / petrol ether 1:1 if not stated otherwise).

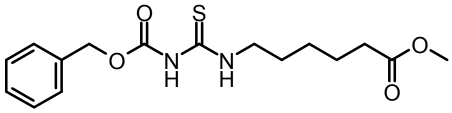
*2-(2-[3-N-(benzyloxycarbonyl)thioureido]ethyloxy)acetic acid ^{tert}butylester (**12c**)*



The acetate salt of 2-(2-Amino-ethoxy)-acetic acid ^{tert}butyl ester (470 mg, 2.0 mmol) was reacted according to *GP 4* to yield **12c** as a colourless oil (640 mg, 1.82 mmol, 91 %).

^{1}**H-NMR** (300 MHz, $CDCl_3$): δ [ppm] = 1.47 (s, 9 H), 3.76 (t, 2 H, *J* = 5.2 Hz), 3.90 (t, 2 H, *J* = 5.2 Hz), 4.00 (s, 2 H), 5.17 (s, 2 H), 7.30 – 7.41 (m, 5 H), 8.30 (bs, 1 H), 9.93 (bs, 1 H); - ^{13}**C-NMR** (75 MHz, $CDCl_3$): δ [ppm] = 28.1 (+, 1 C), 45.6 (-, 1 C), 68.1 (-, 1 C), 68.8 (-, 1 C), 68.9 (-, 1 C), 81.9 (C_{quat}, 1 C), 128.4 (+, 2 C), 128.7 (+, 1 C), 128.8 (+, 2 C), 134.6 (C_{quat}, 1 C), 152.3 (C_{quat}, 1 C), 169.4 (C_{quat}, 1 C), 179.3 (C_{quat}, 1 C); - **IR** (FT-IR, film): ν [cm^{-1}] = 3290 (bm), 2977 (m), 2937 (m), 2883 (m), 1720 (s), 1515 (s), 1453 (m), 1368 (m), 1319 (m), 1220 (s), 1187 (s), 1123 (s), 1023 (s), 961 (m), 913 (m), 840 (m), 758 (m), 735 (m), 696 (m), 583 (m); - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 369.0 (100, MH^+), 386.0 (14, MNH_4^+), 737.1 (19, 2M + H^+), 754.1 (29, 2M + NH_4^+); **- $C_{17}H_{24}N_2O_5S$:** calc. C 55.4, H 6.6, N 7.6, S 8.7, found. C 55.3, H 6.5, N 7.3, S 8.2; - **MW** = 368.46; - **MF** = $C_{17}H_{24}N_2O_5S$.

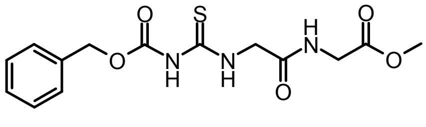
*6-[3-N-(benzyloxycarbonyl)thioureido]hexanoic acid methylester (**13b**)*



ε-Aminohexanoic acid methylester hydrochloride (170 mg, 1.0 mmol) was used to obtain 281 mg of an oily, colourless solid (0.831 mmol, 83 %).

^{1}H-NMR (300 MHz, MeOD): δ [ppm] = 1.39 (m, 2 H), 1.67 (m, 4 H), 2.40 (t, 2 H, *J* = 7.2 Hz), 3.62 (t, 2 H, *J* = 7.2 Hz), 3.65 (s, 3 H), 5.18 (s, 2 H), 7.23 – 7.45 (m, 5 H); - **^{13}C-NMR** (75 MHz, MeOD): δ [ppm] = 25.4 (-, 1 C), 26.9 (-, 1 C), 28.3 (-, 1 C), 34.4 (-, 1 C), 46.1 (-, 1 C), 52.1 (+, 1 C), 68.9 (-, 1 C), 128.8 (+, 1 C), 129.4 (+, 2 C), 129.5 (+, 2 C), 136.6 (C_{quat}, 1 C), 155.2 (C_{quat}, 1 C), 175.7 (C_{quat}, 1 C), 181.1 (C_{quat}, 1 C); - **IR** (FT-IR, film): ν [cm^{-1}] = 3040 (m), 2986 (m), 2936 (m), 2874 (m), 1772 (m), 1705 (s), 1656 (s), 1464 (m), 1398 (m), 1342 (m), 1216 (m), 1045 (m), 746 (m), 665 (m), 592 (m); - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 339.2 (100, MH^+); - **MW** = 338.43; - **MF** = $C_{16}H_{22}N_2O_4S$.

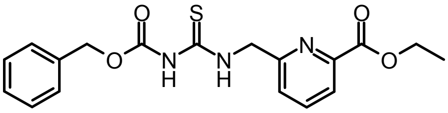
*2-(2-[3-N-(benzyloxycarbonyl)thioureido]acetamido)acetic acid methylester (**14b**)*



Glycyl glycine methyl ester hydrochloride (364 mg, 2.0 mmol) was converted by *GP 4* to the according thiourea. The product is as a colourless solid (540 mg, 0.839 mmol, 84 %).

M.p. (uncorrected) = 137 – 138 °C; - **^{1}H-NMR** (300 MHz, acetone-d6): δ [ppm] = 3.68 (s, 3 H), 4.04 (d, 2 H, $J_{1,1}$ = 6.0 Hz), 4.40 (d, 2 H, $J_{1,1}$ = 6.0 Hz), 5.23 (s, 2 H), 7.29 – 7.45 (m, 5 H), 7.83 (bs, 1 H), 9.98 (bs, 1 H), 10.32 (bs, 1 H); - ^{13}**C-NMR** (75 MHz, acetone-d6): δ [ppm] = 41.6 (-, 1 C), 48.9 (-, 1 C), 52.3 (+, 1 C), 68.2 (-, 1 C), 129.1 (+, 2 C), 129.2 (+, 2 C), 129.4 (+, 1 C), 136.6 (C_{quat}, 1 C), 154.1 (C_{quat}, 1 C), 154.2 (C_{quat}, 1 C), 168.6 (C_{quat}, 1 C), 170.6 (C_{quat}, 1 C), 180.6 (C_{quat}, 1 C); - **IR** (FT-IR, film): ν [cm^{-1}] = 3258 (bm), 3032 (m), 2955 (m), 1719 (s), 1513 (s), 1437 (m), 1371 (s), 1196 (s), 1131 (m), 1031 (m), 970 (m), 905 (m), 796 (m), 778 (m), 697 (m), 603 (m); - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 339.3 (31, MH^+), 357.0 (46, MH^+ + MeCN), 679.2 (69, 2M + H^+), 696.2 (100, 2M + NH_4^+); - **HRMS** (EI-MS 70 eV): calc. for $C_{14}H_{17}N_3O_5S^{*+}$: 339.0889, found: 339.0884; - **MW** = 339.37; - **MF** = $C_{14}H_{17}N_3O_5S$.

*5-([3-N-(benzyloxycarbonyl)thioureido]methyl)pyrrol-2-carboxylic acid methylester (**16**)*

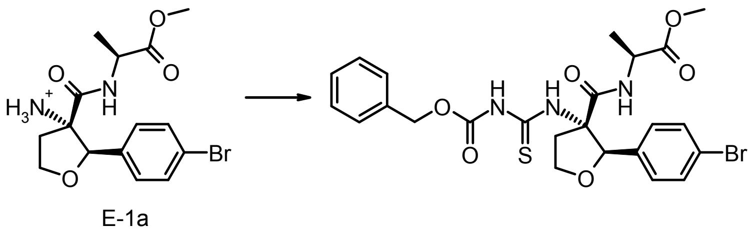


The 2-Aminomethyl-pyridine-6-carboxylic acid ethyl ester (360 mg, 2.0 mmol) was submitted to *GP 4* to give its according thiourea as orange oil (604 mg, 1.62 mmol, 81 %).

^{1}**H-NMR** (300 MHz, $CDCl_3$): δ [ppm] = 1.49 (t, 3 H, *J* = 7.2 Hz), 4.09 (q, 2 H, *J* = 7.2 Hz), 5.06 (d, 2 H, *J* = 4.7 Hz), 5.23 (s, 2 H), 7.30 – 7.46 (m, 5H), 7.52 (d, 1 H, *J* = 7.7 Hz), 7.85 (dd, 1 H, *J* = 7.7 Hz), 8.06 (d, 1 H, *J* = 7.7 Hz), 8.12 (bs, 1 H), 10.81 (bs, 1 H); - ^{13}**C-NMR** (75 MHz, $CDCl_3$): δ [ppm] = 13.2 (+, 1 C), 49.4 (-, 1 C), 61.0 (-, 1 C), 67.2 (-, 1 C), 122.2 (+, 1 C), 123.1 (+, 1 C), 124.1 (+, 1 C), 127.3 (+, 2 C), 127.7 (+, 2 C), 127.9 (+, 1 C), 133.5 (C_{quat}, 2 C), 136.8 (C_{quat}, 1 C), 146.7 (C_{quat}, 1 C), 151.2 (C_{quat}, 1 C), 154.2 (C_{quat}, 1 C), 178.0 (C_{quat}, 1 C); - **IR** (FT-IR, film): ν [cm^{-1}] = 3425 (bm), 3348 (bm), 2960 (m), 2929 (m), 1709 (s), 1591 (m), 1495 (m), 1453 (m), 1394 (m), 1305 (m), 1220 (s), 1163 (s), 1086 (m), 1053 (m), 1024 (m), 910 (m), 861 (m), 761 (m), 735 (m), 697 (m), 581 (m); - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 373.9 (100, MH^+), 747.1 (31, 2M+H^+); - **HRMS** (PI-LSIMS FAB glycerine): calc. for $C_{18}H_{20}N_3O_4S^+$: 374.1175, found: 374.1165; - **MW** = 373.43; - **MF** = $C_{18}H_{19}N_3O_4S$.

2-{[2-(4-Bromo-phenyl)-3-(3-benzyloxycarbonyl-thioureido)-tetrahydro-furan-3-carbonyl]-amino}-propionic acid methyl ester

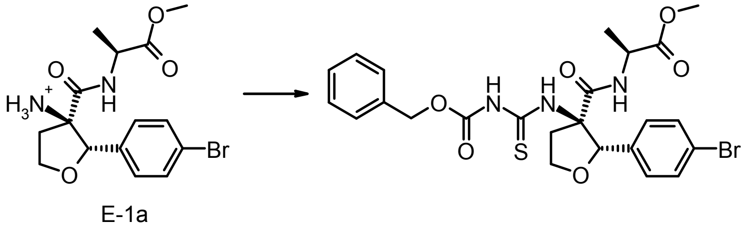
Diastereomer A (**E-2a**)



The reaction was conducted in half scale after *GP 4*. Compound **E-1a** (410 mg, 1.0 mmol) was converted to its according thiourea and purified with ethyl acetate / petrol ether 1:2. The product is as a pale yellow solid (388 mg, 0.709 mmol, 71 %).

M.p. (uncorrected) = 131 - 132 °C; - **^{1}H-NMR** (600 MHz, CDCl$_3$): δ [ppm] = 0.91 (d, 3 H, J = 7.0 Hz), 2.65 (m, 1 H), 3.48 (m, 1 H), 3.72 (s, 3 H), 4.29 (m, 1 H), 4.38 (m, 1 H), 4.50 (m, 1 H), 5.24 (d, 1 H, *J* = 11.8 Hz), 5.28 (d, 1 H, *J* = 11.8 Hz), 6.24 (bs, 1 H), 6.46 (bs, 1 H), 7.15 (d, 2 H, *J* = 8.2 Hz), 7.34 – 7.43 (m, 7 H); - **^{13}C-NMR** (150 MHz, CDCl$_3$): δ [ppm] = 17.5 (+, 1 C), 34.2 (-, 1 C), 48.3 (-, 1 C), 52.6 (+, 1 C), 67.0 (+, 1 C), 68.5 (-, 1 C), 70.5 (+, 1 C), 121.6 (C$_{quat}$, 1 C), 126.8 (+, 2 C), 128.7 (+, 2 C), 128.8 (+, 2 C), 129.0 (+, 1 C), 131.3 (+, 2 C), 134.4 (C$_{quat}$, 1 C), 135.6 (C$_{quat}$, 1 C), 152.1 (C$_{quat}$, 1 C), 170.1 (C$_{quat}$, 1 C), 173.0 (C$_{quat}$, 1 C), 176.8 (C$_{quat}$, 1 C); - **IR** (FT-IR, film): ν [cm^{-1}] = 3371 (bm), 3171 (bm), 2957 (m), 2936 (m), 2886 (m), 2361 (m), 2348 (m), 1728 (m), 1677 (m), 1500 (s), 1452 (m), 1358 (m), 1312 (m), 1258 (m), 1217 (s), 1177 (s), 1069 (m), 1010 (s), 975 (m), 909 (m), 798 (m), 730 (s), 697 (m); - **MS** (ESI-MS, CH$_2$Cl$_2$/MeOH + 10 mmol NH$_4$OAc): m/z (%) = 563.9/565.9 (100, MH$^+$), 582.9/584.0 (90, MNH$_4^+$), 1146.2/1148.3 (27, (2M+NH$_4^+$)$^+$); - **MW** = 564.46; - **MF** = C$_{24}$H$_{26}$BrN$_3$O$_6$S.

Diastereomer B (**E-2b**)



Compound **E-1b** (410 mg, 1.0 mmol) was converted by *GP 4* to its according thiourea. The reaction was conducted in half scale. Ethyl acetate / petrolether 1:2 was used for purification. The product is as a pale yellow solid (350 mg, 0.640 mmol, 64 %).

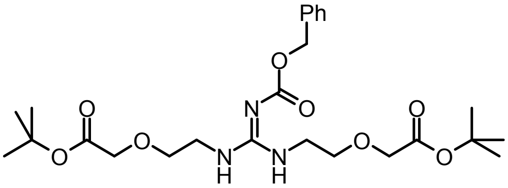
M.p. (uncorrected) = 143 - 145 °C; - **^{1}H-NMR** (600 MHz, $CDCl_3$): δ [ppm] = 1.22 (d, 3 H, *J* = 7.0 Hz), 2.66 (m, 1 H), 3.45 (m, 1 H), 3.70 (s, 3 H), 3.91 (m, 1 H), 4.09 (m, 1 H), 4.43 (m, 1 H), 5.25 (s, 2 H), 6.06 (bs, 1 H), 6.70 (bs, 1 H), 7.14 (d, 2 H, *J* = 8.2 Hz), 7.37 (d, 2 H, *J* = 8.2 Hz), 7.38 – 7.42 (m, 5 H), 8.03 (bs, 1 H), 9.36 (bs, 1 H); - **^{13}C-NMR** (150 MHz, $CDCl_3$): δ [ppm] = 18.3 (+, 1 C), 34.1 (-, 1 C), 48.4 (-, 1 C), 52.6 (+, 1 C), 67.0 (+, 1 C), 68.5 (-, 1 C), 70.0 (+, 1 C), 121.8 (C_{quat}, 1 C), 127.1 (+, 2 C), 128.7 (+, 2 C), 128.8 (+, 2 C), 129.0 (+, 1 C), 131.1 (+, 2 C), 135.1 (C_{quat}, 1 C), 135.6 (C_{quat}, 1 C), 152.2 (C_{quat}, 1 C), 170.1 (C_{quat}, 1 C), 172.7 (C_{quat}, 1 C), 177.0 (C_{quat}, 1 C); - **IR** (FT-IR, film): ν [cm^{-1}] = 3363 (bm), 3195 (bm), 2956 (m), 2932 (m), 2889 (m), 2360 (m), 2348 (m), 1728 (m), 1675 (m), 1500 (s), 1451 (m), 1358 (m), 1305 (m), 1258 (m), 1214 (s), 1177 (s), 1070 (m), 1010 (s), 975 (m), 910 (m), 800 (m), 732 (s), 697 (m); - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 563.9/565.9 (100, MH^+), 583.0/584.0 (50, MNH_4^+), 1127.4/1129.4 (12, $(2M+H^+)^+$), 1147.4/1149.4 (23, $(2M+NH_4^+)^+$); - **MW** = 564.46; - **MF** = $C_{24}H_{26}BrN_3O_6S$.

1.5. Syntheses and deprotections of the receptors

1.5.1. Preparation of sym. 1,3-substituted benzyloxycarbonyl-guanidines (GP 5)

The benzyloxycarbonylthiourea (0.5 mmol) was dissolved together with its according amine compound (0.6 mmol) and triethylamine (130 mg, 0.17 mL, 1.3 mmol) in DCM (5 mL). EDC hydrochloride (110 mg, 0.6 mmol) was added at 2 – 5 °C in one portion.[4] The mixture was allowed to reach room temperature in 1 hour and was then stirred over night. In the case were TLC indicated thiourea still being present at this point, another portion of triethylamine (30 mg, 0.04 mL, 0.3 mmol) and EDC hydrochloride (54 mg, 0.3 mmol) was added and stirring was continued for 4 h. It was diluted with DCM (25 mL), the organic solution was washed with saturated ammonium chloride solution (5 mL) and twice with water (10 mL). After drying over $MgSO_4$, the solvent was evaporated and the oily residue was purified by column chromatography with ethyl acetate / petrol ether 4:1 to yield the guanidine.

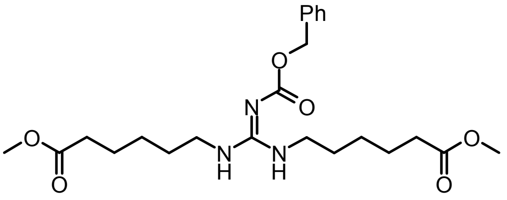
*(2-[N-(2-^{tert}Butyloxycarbonylmethyloxyethyl)-N-(benzyloxycarbonyl)guanidine]ethyloxy)acetic acid ^{tert}butylester (**17a**)*



The thiourea compound **12c** (176 mg, 0.5 mmol) was reacted with the TFA salt of 2-(2-Amino-ethoxy)-acetic acid ^{tert}butyl ester (**10c**) (158 mg, 0.6 mmol) following *GP 5* to yield a clear, faintly yellow oil (198 mg, 0.389 mmol, 78 %).

^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.46 (s, 18 H), 3.52 (m, 4 H), 3.67 (m, 4 H), 3.99 (s, 4 H), 5.11 (s, 2 H), 7.28 – 7.41 (m, 5 H), 9.23 (bs, 2 H); - **^{13}C-NMR** (75 MHz, $CDCl_3$): δ [ppm] = 28.1 (+, 6 C), 41.6 (-, 2 C), 48.8 (-, 2 C), 66.4 (-, 2 C), 70.8 (-, 1 C), 82.0 (C_{quat}, 2 C), 127.5 (+, 1 C), 128.0 (+, 2 C), 128.3 (+, 2 C), 137.8 (C_{quat}, 1 C), 161.1 (C_{quat}, 2 C), 164.1 (C_{quat}, 1 C), further signals were not detectable; - **IR** (FT-IR, film): ν [cm^{-1}] = 3343 (bm), 2977 (m), 2929 (m), 1743 (s), 1633 (s), 1599 (s), 1455 (m), 1386 (m), 1368 (m), 1303 (m), 1228 (m), 1129 (s), 1052 (m), 942 (m), 845 (m), 801 (m), 743 (m), 699 (m); - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 510.2 (100, MH^+); - **HRMS** (PI-LSIMS FAB, glycerine): calc. for $C_{25}H_{40}N_3O_8^+$: 510.2815, found: 510.2808; - **MW** = 509.60; - **MF** = $C_{25}H_{39}N_3O_8$.

*(2-[N-(2-Ethyloxycarbonylmethyloxyethyl)-N-(benzyloxycarbonyl)guanidine]ethyloxy)acetic acid ethylester (**18**)*

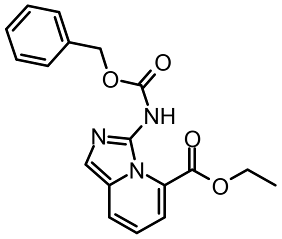


Thiourea **13b** (161 mg, 0.5 mmol) was reacted with ε-aminohexanoic acid methylester hydrochloride (103 mg, 0.6 mmol) after *GP 5* to give a colourless oil (175 mg, 0.391 mmol, 78 %).

^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.38 (m, 4 H), 1.66 (m, 8 H), 2.42 (m, 4 H), 3.63 (m, 4 H), 3.68 (s, 6 H), 5.17 (s, 2 H), 7.25 – 7.43 (m, 5 H); - **^{13}C-NMR** (75 MHz, $CDCl_3$): δ

[ppm] = 25.5 (-, 2 C), 27.1 (-, 2 C), 28.2 (-, 2 C), 34.5 (-, 2 C), 46.2 (-, 2 C), 52.0 (+, 2 C), 68.4 (-, 1 C), 128.0 (+, 1 C), 128.2 (+, 2 C), 128.4 (+, 2 C), 136.9 (C_{quat}, 1 C), 160.1 (C_{quat}, 2 C), 161.7 (C_{quat}, 1 C), 172.1 (C_{quat}, 1 C); - **IR** (FT-IR, film): ν [cm^{-1}] = 3280 (bm), 2986 (m), 2938 (m), 1743 (s), 1666 (m), 1597 (s), 1519 (m), 1452 (m), 1384 (m), 1276 (m), 1213 (s), 1134 (s), 1056 (m), 1027 (m), 913 (m), 854 (m), 801 (m), 732 (m), 693 (m); - **MW** = 449.55; - **MF** = $C_{23}H_{35}N_3O_6$.

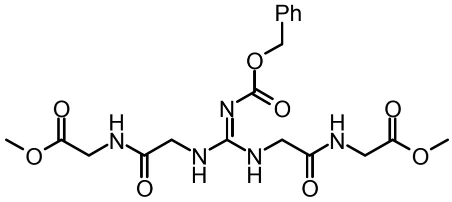
3-Benzyloxycarbonylamino-1,5-dihydro-imidazo[1,5-a]pyridine-5-carboxylic acid ethyl ester (21)



The thiourea compound **16** (187 mg, 0.5 mmol) was submitted to the conditions of *GP 5* to give the product **21** as a red glass (112 mg, 0.328 mmol, 66 %).

^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.39 (t, 3 H, *J* = 7.2 Hz), 4.31 (q, 2 H, *J* = 7.2 Hz), 5.18 (s, 2 H), 6.67 (t, 1 H, *J* = 7.2 Hz), 7.27 – 7.42 (m, 6 H), 7.58 (d, 2 H, *J* = 4.6 Hz), 8.61 (bs, 1 H); - **^{13}C-NMR** (75 MHz, $CDCl_3$): δ [ppm] = 14.1 (+, 1 C), 62.5 (-, 1 C), 67.5 (-, 1 C), 116.4 (+, 1 C), 120.7 (+, 1 C), 121.6 (+, 1 C), 123.6 (+, 1C), 124.9 (C_{quat}, 1 C), 128.2 (+, 2 C), 128.3 (+, 2 C), 128.7 (+, 1 C), 130.7 (C_{quat}, 1 C), 135.9 (C_{quat}, 1 C), 153.7 (C_{quat}, 1 C), 162.8 (C_{quat}, 1 C); - **IR** (FT-IR, film): ν [cm^{-1}] = 3255 (bm), 2981 (m), 2908 (m), 1722 (s), 1531 (m), 1454 (m), 1408 (m), 1372 (m), 1266 (s), 1235 (s), 1212 (s), 1171 (m), 1141 (m), 1106 (m), 1036 (m), 913 (m), 872 (m), 810 (m), 743 (m), 698 (m), 595 (m), 495 (m); - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 340.0 (100, MH^+), 679.1 (11, $2M+H^+$); - **UV** (MeOH): λ (ε) = 380 (1300), 280 (3200), 230 (13200); - **MW** = 339.35; - **MF** = $C_{18}H_{17}N_3O_4$.

*(2-[N-(2-Ethyloxycarbonylmethylacetamido)-N-(benzyloxycarbonyl)guanidine]acetamido)acetic acid ethylester (**19**)*



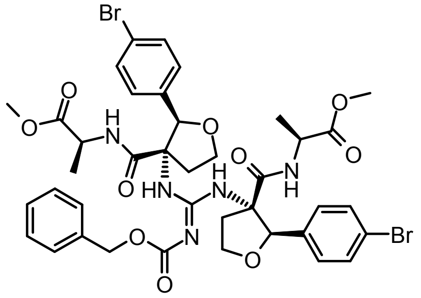
Compound **14b** (161 mg, 0.5 mmol) was reacted with glycyl glycine hydrochloride (110 mg, 0.6 mmol) analogue to *GP 5* (10 % NMP was added to the reaction mixture) to give a pale yellow solid (172 mg, 0.381 mmol, 76 %).

M.p. (uncorrected) = 92 – 94°C; - **^{1}H-NMR** (300 MHz, DMSO-d6): δ [ppm] = 3.66 (s, 6 H), 3.96 (m, 4 H), 4.08 (m, 4 H), 5.05 (s, 2 H), 7.28 – 7.42 (m, 5 H), 7.90 (bs, 1 H), 9.05 (bs, 2 H); - **^{13}C-NMR** (75 MHz, DMSO): δ [ppm] = 41.5 (-, 2 C), 44.9 (-, 2 C), 52.2 (+, 2 C), 66.8 (-, 1 C), 128.3 (+, 1 C), 128.7 (+, 2 C), 129.0 (+, 2 C), 139.2 (C_{quat}, 1 C), 161.6 (C_{quat}, 2 C), 170.9 (C_{quat}, 1 C), 175.7 (C_{quat}, 2 C), further signals were not detectable; - **IR** (FT-IR, film): ν [cm^{-1}] = 3352 (m), 3290 (bm), 3069 (m), 2951 (m), 1744 (m), 1639 (s), 1572 (m), 1530 (m), 1434 (m), 1378 (m), 1330 (m), 1283 (m), 1259 (m), 1203 (s), 1171 (s), 1094 (s), 1030 (m), 1009 (m), 983 (m), 941 (m), 794 (m), 719 (m), 672 (m), 603 (m), 539 (m), 456 (m); - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 452.1 (100, MH^+), 903.5 (5, (2 MH^+); - **HRMS** (PI-LSIMS FAB, glycerine): calc. for $C_{19}H_{26}N_5O_8^+$: 452.1781, found: 452.1782; - **MW** = 451.44; - **MF** = $C_{19}H_{25}N_5O_8$.

1.5.2. Bis-TAA-guanidines

2-[(2-(4-Bromo-phenyl)-3-{N'-[2-(4-bromo-phenyl)-3-(1-methoxycarbonyl-ethylcarbamoyl)-tetrahydro-furan-3-yl]-N''-benzyloxycarbonyl-guanidino}-tetrahydro-furan-3-carbonyl)-amino]-propionic acid methyl ester

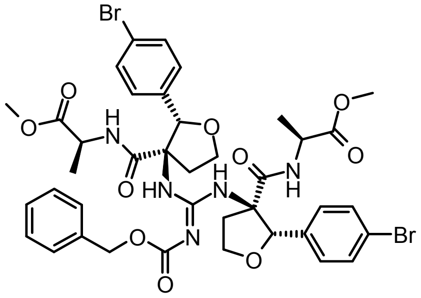
Diastereomer A (**E-3a**)



Compound **E-2a** (273 mg, 0.5 mmol) was reacted with its according diastereomer of the TAA-Ala methylester hydrochloride (**E-1a**) (224 mg, 0.6 mmol) after *GP 5* and purified with ethyl acetate / petrol ether 1:2 to yield the guanidine as a pale yellow solid (414 mg, 0.459 mmol, 31 %).

^{1}H-NMR (600 MHz, $CDCl_3$): δ [ppm] = 0.94 (d, 6 H, *J* = 7.5 Hz), 2.40 (m, 1 H), 2.63 (m, 1 H), 3.64 (s, 6 H), 3.67 (m, 2 H), 4.12 (m, 1 H), 4.19 (m, 1 H), 4.48 (m, 2 H), 4.68 (m, 2 H), 4.78 (s, 1 H), 5.15 (d, 1 H, *J* = 12.5 Hz), 5.20 (d, 1 H, *J* = 12.5 Hz), 7.18 (d, 4 H, *J* = 8.2 Hz), 7.36 – 7.43 (m, 9 H), 8.91 (bs, 2 H); - **^{13}C-NMR** (150 MHz, $CDCl_3$): δ [ppm] = 13.3 (+, 2 C), 35.1 (-, 2 C), 48.5 (+, 2 C), 52.6 (+, 2 C), 67.4 (-, 2 C), 67.9 (+, 2 C), 70.3 (-, 1 C), 122.8 (C_{quat}, 1 C), 128.3 (+, 4 C), 127.7 (+, 1 C), 128.3 (+, 2 C), 128.5 (+, 2 C), 131.5 (+, 4 C), 133.5 (C_{quat}, 2 C), 136.0 (C_{quat}, 1 C), 159.9 (C_{quat}, 1 C), 163.6 (C_{quat}, 1 C), 169.1 (C_{quat}, 2 C), 172.0 (C_{quat}, 1 C), further signals were not detectable; - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 900.1/903.9 (100, MH^+); - **MW** = 901.61; - **MF** = $C_{32}H_{43}Br_2N_5O_{10}$.

Diastereomer B (**E-3b**)



Compound **E-2b** (273 mg, 0.5 mmol) was reacted with its according diastereomer of the TAA-Ala methylester hydrochloride (**E-1b**) (224 mg, 0.6 mmol) after *GP 5* to give a pale yellow solid (369 mg, 0.410 mmol, 24 %) after column chromatography with ethyl acetate / petrol ether 1:2.

^{1}H-NMR (600 MHz, $CDCl_3$): δ [ppm] = 1.21 (d, 6 H, *J* = 7.5 Hz), 2.40 (m, 2 H), 2.63 (m, 2 H), 3.49 (s, 6 H), 4.17 (m, 2 H), 4.09 (m, 1 H), 4.48 (m, 2 H), 4.62 (m, 2 H), 4.82 (s, 2 H), 5.14 (d, 1 H, *J* = 12.3 Hz), 5.19 (d, 1 H, *J* = 12.3 Hz), 7.20 (d, 4 H, *J* = 8.2 Hz), 7.33 – 7.45 (m, 9 H), 8.83 (bs, 2 H); - **^{13}C-NMR** (150 MHz, $CDCl_3$): δ [ppm] = 14.1 (+, 2 C), 36.1 (-, 2 C), 48.1 (+, 2 C), 52.6 (+, 2 C), 67.2 (-, 2 C), 67.9 (+, 2 C), 70.5 (-, 1 C), 122.7 (C_{quat}, 2 C), 127.7 (+, 2 C), 128.1 (+, 2 C), 128.2 (+, 4 C), 128.5 (+, 2 C), 131.4 (+, 4 C), 133.6 (C_{quat}, 2 C), 136.2 (C_{quat}, 2 C), 159.6 (C_{quat}, 1 C), 163.6 (C_{quat}, 1 C), 169.0 (C_{quat}, 2 C), 171.7 (C_{quat}, 2 C) further signals were not detectable; - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 900.1/903.9 (100, MH^+); - **MW** = 901.61; - **MF** = $C_{32}H_{43}Br_2N_5O_{10}$.

1.6. Deprotection of the benzyloxycarbonylguanidine esters

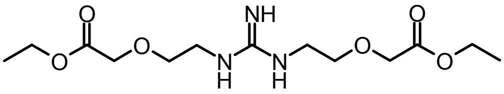
1.6.1. Deprotection of the Cbz-guanidine (GP 6a)

The symmetric 1,3-substituted benzyloxycarbonylguanidine ester (0.4 mmol) was dissolved, depending on the ester in the molecule, in ethanol or methanol (3 mL). Three spatula tips of palladium on charcoal (10 % Pd) were added. It was stirred in a hydrogen atmosphere (30 bar) for two days at room temperature. The reaction mixture was diluted with the according alcohol (10 mL) and filtered over celite. The filter cake was washed with small portions of this alcohol. The clear filtrate was evaporated to dryness to give the guanidine free base.

1.6.2. Synthesis of the guanidinium hexafluorophosphate salts (GP 6b):

The guanidine free base (0.2 mmol) was dissolved in 6 mL of methanol or ethanol, according to its esters. Ammonium hexafluorophosphate (38 mg, 0.24 mmol) was added and the mixture was warmed to 40 °C for three hours. The solvent was evaporated and the residue was extracted with DCM (1 mL) trice. The solvent was distilled off and the remaining guanidinium salt was properly dried in vacuo. The products appear as colourless solids in nearly quantitative yield.

*(2-[N-(2-Ethyloxycarbonylmethyloxyethyl)-N-guanidine]ethyloxy)acetic acid ethyl ester hexafluorophosphate (**22b**)*



Compound **17b** (181 mg, 0.4 mmol) was deprotected after *GP 6a* to give a colourless, sticky oil (97 mg, 0.369 mmol, 92 %).

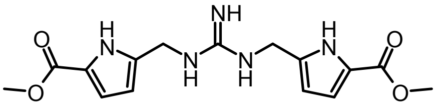
Free Base:

^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.22 (t, 6 H, *J* = 7.1 Hz), 3.33 (m, 4 H), 3.62 (m, 4 H), 4.06 (s, 4 H), 4.12 (q, 2 H, *J* = 7.1 Hz), 7.76 (bs, 1 H), 9.71 (bs, 1 H), 10.43 (bs, 1 H); - **IR** (FT-IR, film): ν [cm^{-1}] = 3308 (bm), 3183 (bm), 2971 (m), 2927 (m), 2882 (m), 1749 (m), 1630 (s), 1586 (m), 1404 (m), 1317 (m), 1208 (s), 1134 (s), 1049 (m), 881 (m), 704 (m), 587 (m); - **MW** = 319.36; - **MF** = $C_{13}H_{25}N_3O_6$.

Hexafluorophosphate Salt:

MW = 465.33; - **MF** = $C_{13}H_{26}PF_6N_3O_6$.

*5-[(N'-[(2-Methoxycarbonyl-1H-pyrrole)-5-methyl-guanidino)-methyl]-1H-pyrrole-2-carboxylic acid methyl ester hexafluorophosphate (**23**)*



Compound **20** (187 mg, 0.4 mmol) was reacted according to *GP 6a* to yield a yellow solid (117 mg, 0.384 mmol, 96 %).

Free Base:

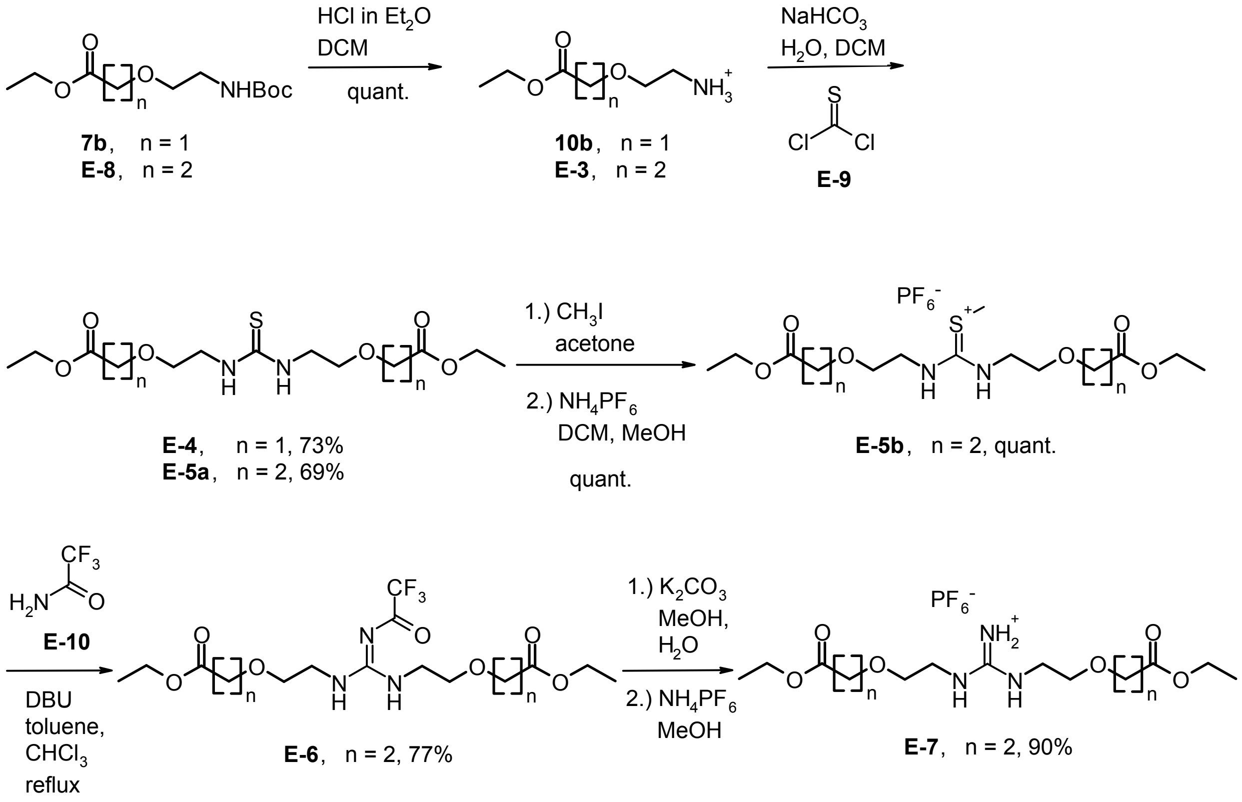
^{1}H-NMR (300 MHz, MeOD): δ [ppm] = 3.81 (s, 6 H), 4.41 (s, 4 H), 6.12 (m, 2 H), 6.79 (m, 2 H); - **^{13}C-NMR** (75 MHz, MeOD): δ [ppm] = 39.5 (-, 2 C), 51.8 (+, 2 C), 110.2 (+, 2 C), 117.0 (+, 2 C), 124.1 (C_{quat}, 2 C), 133.5 (C_{quat}, 2 C), 157.3 (C_{quat}, 1 C), 163.0 (C_{quat}, 2 C); - **IR** (FT-IR, film): ν [cm^{-1}] = 3179 (bm), 2953 (m), 1660 (s), 1627 (s), 1489 (m), 1439 (m), 1320 (s), 1279 (m), 1218 (s), 1132 (m), 1003 (s), 930 (m), 800 (m), 762 (s), 656 (m); - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 334.0 (100, MH$^+$), 375.0 (38, MH$^+$ + MeCN), 713.3 (13, 2M + H$^+$ + HCOOH); - **HRMS** (EI-MS, 70 eV): calc. for $C_{15}H_{19}N_5O_4^{\bullet +}$: 333.1437, found: 333.1439; - **MW** = 333.35; - **MF** = $C_{15}H_{19}N_5O_4$.

Hexafluorophosphate Salt:

MW = 479.32; - **MF** = $C_{15}H_{20}PF_6N_5O_4$.

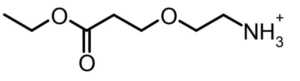
1.7. An alternative route to the receptors

Alternatively, 1,3-substituted symmetric guanidines can be prepared via their according thiourea, which is obtained from the amine compound (**7b** or **E-8**) and thiophosgene.[5] The thiourea (**10b** or **E-3**) can be transformed in the desired guanidine using trifluoroacetamide (**E-10**) as reagent[6]. This route is working well, but is more time consuming, laborious and is involving more steps than the reaction sequence via Cbz-NCS. Consequently, the overall yield is lower. However, this was investigated for the conversion of **10b** and 3-(2-*N*-^{tert}Butyloxycarbonyl-aminoethoxy)propionic acid ethyl ester[7] and should be presented here:



Scheme S-1: Thiourea synthesis with glycol-amino acids and alkylation route to the tweezers using trifluoroacetamide for conversion

3-(2-amino-ethoxy)propionic acid ethyl ester hydrochloride



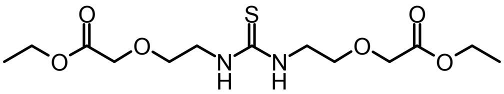
To a solution of 3-(2-*N*-^{tert}Butyloxycarbonyl-aminoethoxy)propionic acid ethyl ester (**43**) (0.52 g, 2.0 mmol) in DCM (10 mL) was slowly added 4 mL of HCl saturated diethylether. After 3 h of stirring at room temperature the solvent was evaporated. The residue was taken up in a little of DCM, and the solvent was removed under reduced pressure again. The remaining sticky solid was dried under vacuum over night. The yield was 0.38 g of a yellow, hygroscopic solid (1.96 mmol, 98 %).

^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.94 (t, 3 H, *J* = 7.1 Hz), 2.56 (t, 2 H, *J* = 6.8 Hz), 3.21 (m, 2 H), 3.68 – 3.81 (m, 4 H), 4.08 (q, 2 H, *J* = 7.1 Hz), 8.11 (bs, 3 H); - **^{13}C-NMR** (75 MHz, $CDCl_3$): δ [ppm] = 14.2 (+, 1 C), 34.7 (-, 1 C), 39.5 (-, 1 C), 60.9 (-, 1 C), 65.6 (-, 1 C), 66.2 (-, 1 C), 172.1 (C_{quat}, 1 C); - **IR** (FT-IR, film): ν [cm^{-1}] = 3468 (bm), 2976 (m), 2934 (m), 1716 (m), 1672 (s), 1521 (m), 1425 (m), 1379 (m), 1183 (s), 1123 (s), 1024 (m), 968 (m), 834 (m), 799 (s), 722 (m), 678 (m), 598 (m); - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 162.1 (100, MH^+); - **MW** = 197.66; - **MF** = $C_7H_{16}ClNO_3$.

1.7.1. Preparation of symmetric thioureas from glycol-amino acid esters (GP 7)

To an ice-cold solution of the amine compound (3.5 mmol) in 20 mL of chloroform containing triethylamine (727 mg, 0.96 mL, 7.2 mmol) thiophosgene (0.13 mL, 1.7 mmol) in 5 mL of chloroform was added drop wise and the mixture was stirred for 2 d at room temperature. The solvent was evaporated and all volatiles were removed under reduced pressure. The residue was dissolved in 10 mL of ethanol and cooled to 0 °C for 3 h. It is filtered with suction and the crystals are washed with a little ice-cold ethanol. The filtrate is reduced to about 2 mL and the thiourea is precipitated by slow addition of diethylether. The solvent is decanted off, the residue is suspended in diethylether and after the solid has settled completely, the ether is decanted off again. The product is dried in the vacuum. If necessary, the material can be purified by column chromatography with ethyl acetate/ petrol ether 1:2.

{2-[3-(2-Ethoxycarbonylmethoxy-ethyl)-thioureido]-ethoxy}-acetic acid ethyl ester[8]***(E-4)***

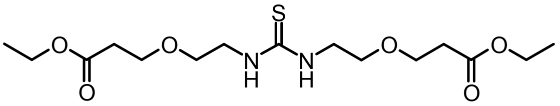


The 2-(2-amino-ethoxy)acetic acid ethyl ester hydrochloride (640 mg, 3.5 mmol) was submitted to *GP 7*. The clean product is a clear, yellow sticky oil (417 mg, 1.24 mmol, 73 %).

^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.26 (t, 6 H, *J* = 7.1 Hz), 3.81 (m, 4 H), 3.98 – 4.13 (m, 8 H), 4.14 – 4.29 (q, 4 H, *J* = 7.1 Hz), 6.61 (bs, 2 H); - **^{13}C-NMR** (75 MHz, $CDCl_3$): δ

[ppm] = 14.2 (+, 2 C), 40.8 (-, 2 C), 46.9 (-, 2 C), 54.2 (-, 2 C), 60.9 (-, 2 C), 169.0 (C_{quat}, 2 C), 181.7 (C_{quat}, 1 C); - **IR** (FT-IR, film): ν [cm^{-1}] = 2979 (m), 2944 (m), 2870 (m), 1740 (s), 1496 (m), 1418 (m), 1350 (m), 1261 (m), 1206 (s), 1123 (s), 1026 (m), 861 (m), 805 (m), 661 (m), 579 (m); - **MS** (CI-MS, NH_3: m/z (%) = 337.2 (100, MH^+), 354.2 (19, MH_4^+); - **MW** = 336.41; - **MF** = $C_{13}H_{24}N_2O_6S$.

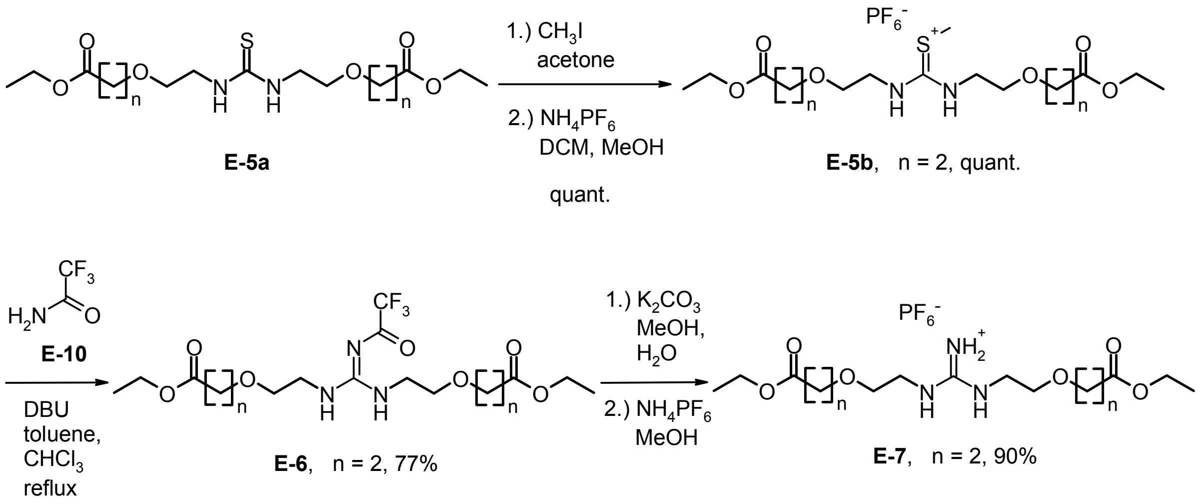
3-(2-{3-[2-(2-Ethoxycarbonyl-ethoxy)-ethyl]-thioureido}-ethoxy)-propionic acid ethyl ester ***(E-5a)***



The 3-(2-amino-ethoxy)propionic acid ethyl ester hydrochloride (690 mg, 3.5 mmol) was reacted after *GP 7* to yield the thiourea as a clear, yellow oil (439 mg, 1.21 mmol, 69 %).

^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.20 (t, 6 H, *J* = 7.1 Hz), 2.51 (t, 4 H, *J* = 6.0 Hz), 3.51 – 3.70 (m, 8 H), 3.67 (t, 4 H, *J* = 6.3 Hz), 4.08 (q, 4 H, *J* = 7.1 Hz), 6.61 (bs, 2 H); - **^{13}C-NMR** (75 MHz, $CDCl_3$): δ [ppm] = 14.2 (+, 2 C), 34.9 (-, 2 C), 44.3 (-, 2 C), 60.7 (-, 2 C), 66.2 (-, 2 C), 69.5 (-, 2 C), 171.8 (C_{quat}, 2 C), 182.8 (C_{quat}, 1 C); - **IR** (FT-IR, film): ν [cm^{-1}] = 2978 (m), 2942 (m), 2874 (m), 1728 (s), 1546 (m), 146 (m), 1373 (m), 1261 (m), 1189 (s), 1124 (s), 1064 (m), 860 (m), 802 (m), 659 (m), 603 (m); - **MS** (CI-MS, NH_3: m/z (%) = 364.9 (100, MH^+), 381.9 (4, MH_4^+); - **MW** = 364.46; - **MF** = $C_{15}H_{28}N_2O_6S$.

1.7.2. (2-[N-(2-Ethyloxycarbonylmethyloxyethyl)-N-(2,2,2-trifluoroacetyl)guanidine]ethyloxy)acetic acid ethyl ester (E-6)



To a solution of compound **E-5a** (364 mg, 1.0 mmol) in acetone (20 mL) methyl iodide (0.2 mL, 2.0 mmol) was added and the reaction mixture was stirred at room temperature over night under light protection. All volatiles were removed under reduced pressure without heating. The residue was dissolved in 30 mL of methanol and dichloromethane (1:1). Ammonium hexafluorophosphate (424 mg, 2.6 mmol) was added and the solution was stirred over night at room temperature. The solvents were distilled off, the remaining yellow oil was taken up in 100 mL of dichloromethane and washed with two 20 mL portions of water. After drying over $MgSO_4$, the solvent was evaporated to give the according hexafluorophosphate in nearly quantitative yield.

It was re-dissolved in a mixture of toluene (20 mL) and chloroform (5 mL), DBU (0.45 mL, 3.0 mmol) and trifluoroacetamide (540 mg, 4.8 mmol) were added. The mixture was refluxed over night under vigorous stirring. After cooling to room temperature the solvents were distilled off at reduced pressure. The oily raw material was purified by column chromatography with ethyl acetate / petrol ether 1:2 to yield the guanidine as clear, yellow oil (279 mg, 0.773 mmol, 77 %).

^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.25 (t, 6 H, *J* = 7.1 Hz), 2.61 (m, 4 H), 3.61 (m, 4 H), 3.72 (m, 4 H), 4.16 (q, 4 H, *J* = 7.1 Hz), 6.59 (bs, 2 H), 9.67 (bs, 1 H); - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 444.0 (100, M+H$^+$), 909.3 (6, 2M+Na$^+$);

Deprotection to the free guanidine:

The above material (270 mg, 0.75 mmol) was dissolved in methanol (2.0 mL) and a solution of potassium carbonate (138 mg, 1.0 mmol) in water (0.5 mL) was added. After warming the well stirred mixture to 50 °C for 4 h, the solvents were evaporated. The residue was dissolved in a 1:1 mixture of DCM and water (10 mL). The phases were separated and the aqueous layer was extracted two times with DCM (5 mL). The combined organic phases were washed with brine, dried over $MgSO_4$ and evaporated to dryness. The product **E-7** appears as yellow oil (232 mg, 0.68 mmol, 90 %).

^{1}H-NMR (300 MHz, MeOD): δ [ppm] = 1.25 (t, 6 H, *J* = 7.1 Hz), 2.62 (t, 4 H, *J* = 6.0 Hz), 3.11 (t, 4 H, *J* = 4.9 Hz), 3.67 (t, 4 H, *J* = 4.9 Hz), 3.76 (t, 4 H, *J* = 6.0 Hz), 4.16 (q, 4 H, *J* = 7.1 Hz); - **^{13}C-NMR** (75 MHz, MeOD): δ [ppm] = 14.5 (+, 2 C), 35.5 (-, 2 C), 40.5 (-, 2 C), 61.8 (-, 2 C), 67.5 (-, 2 C), 67.6 (-, 2 C), 160.4 (C_{quat}, 1 C), 173.7 (C_{quat}, 2 C); - **IR** (FT-IR, film): ν [cm^{-1}] = 3419 (bm), 2986 (m), 2934 (m), 2891 (m), 1670 (s), 1438 (m), 1379 (m), 1276 (m), 1189 (s), 1123 (s), 1029 (m), 840 (m), 800 (m), 721 (m), 606 (m); - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 348.0 (100, MH^+);

2. ^{1}H – and ^{13}C-NMR spectra of selected new compounds

For solvents and conditions of measurement, see experimental procedures. NMR spectra were recorded on Bruker Avance spectrometers 300, 400 and 600. Measuring temperature was 300 K. TMS was used as external standard.

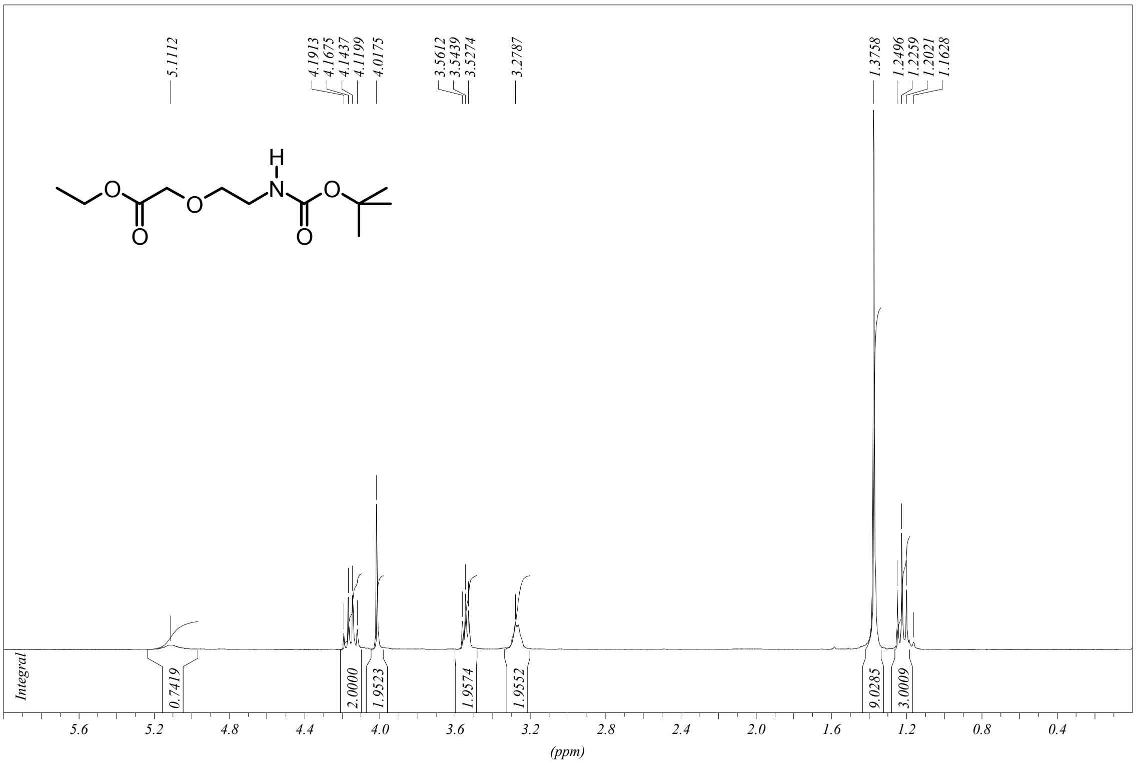


Figure S-1: ^{1}H-NMR spectrum of compound **7b**

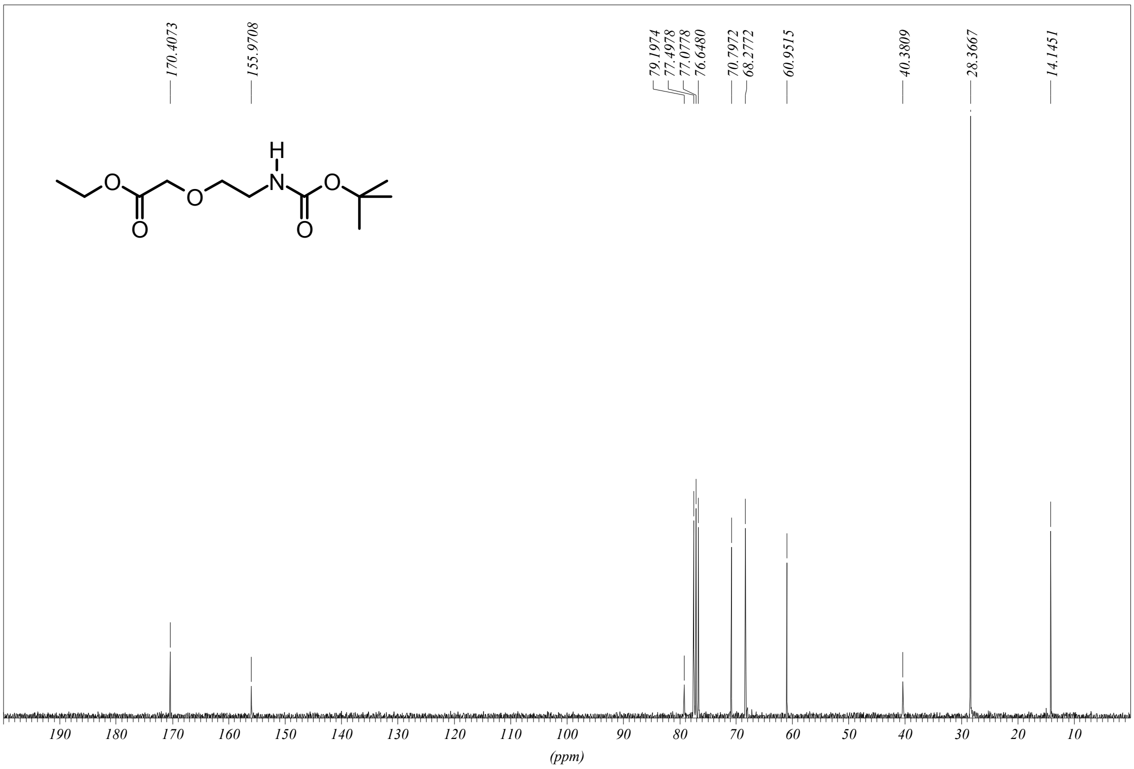


Figure S-2: ^{13}C-NMR spectrum of compound **7b**

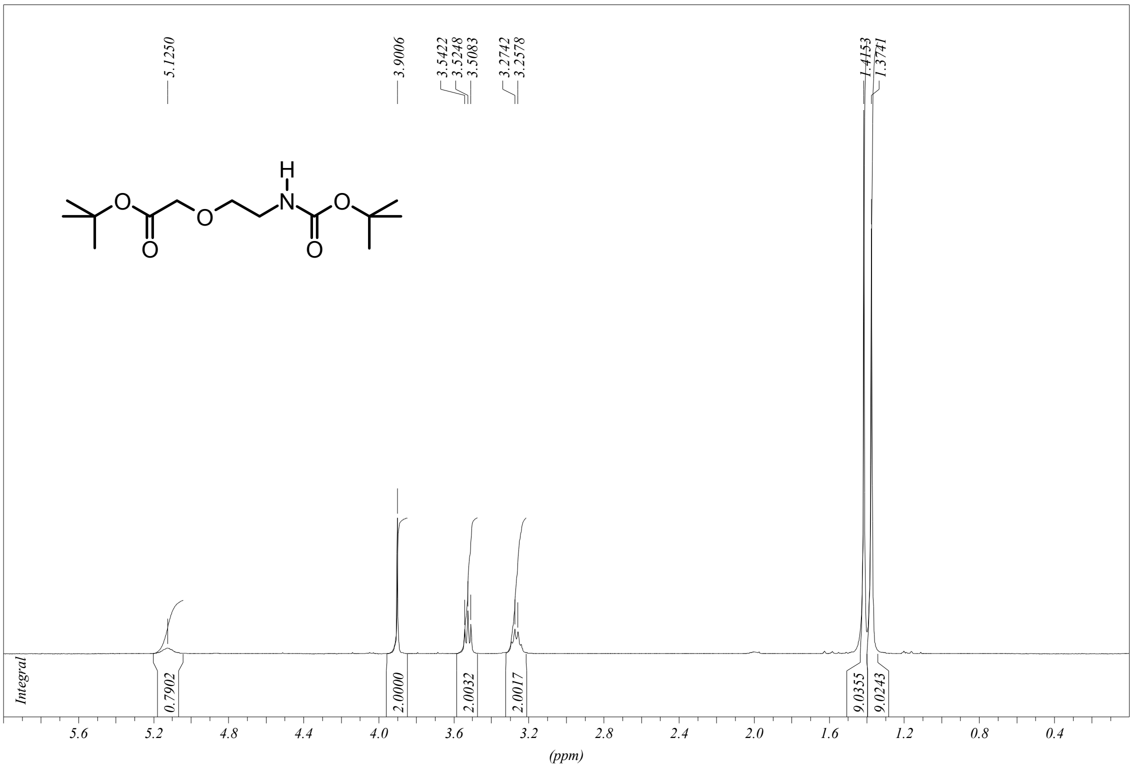


Figure S-3: ^{1}H-NMR spectrum of compound **7a**

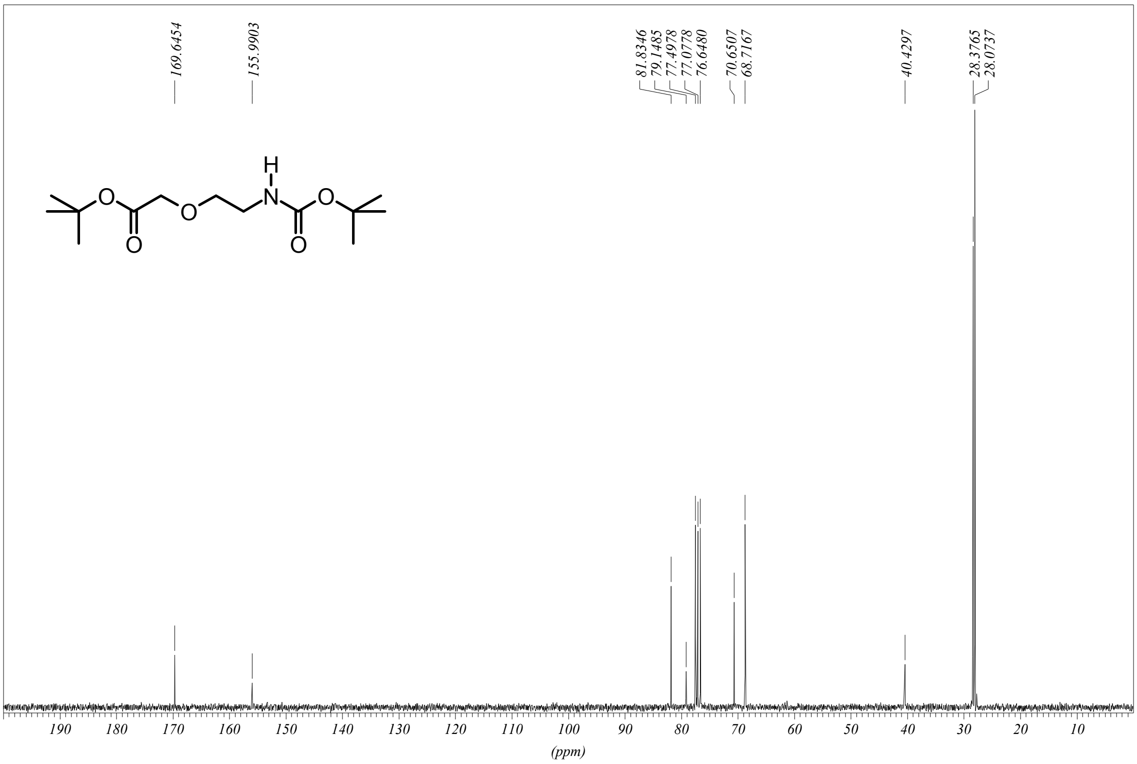


Figure S-4: ^{13}C-NMR spectrum of compound **7a**

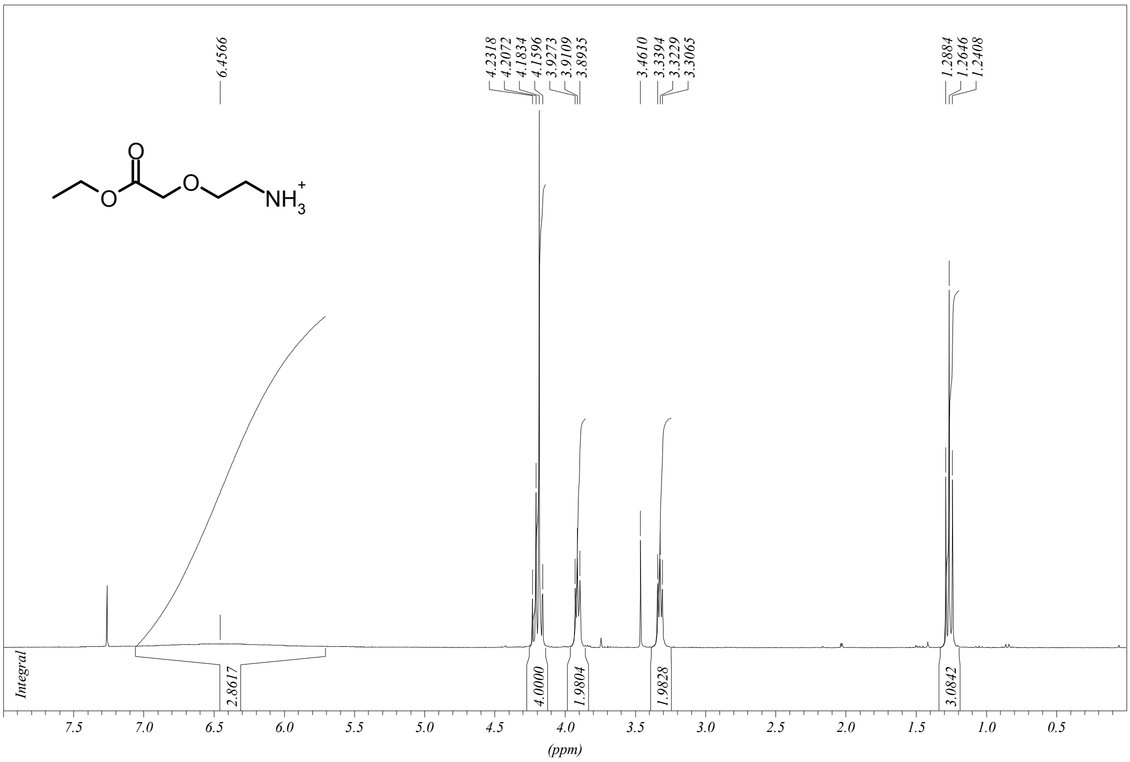


Figure S-5: ^{1}H-NMR spectrum of compound **10b**

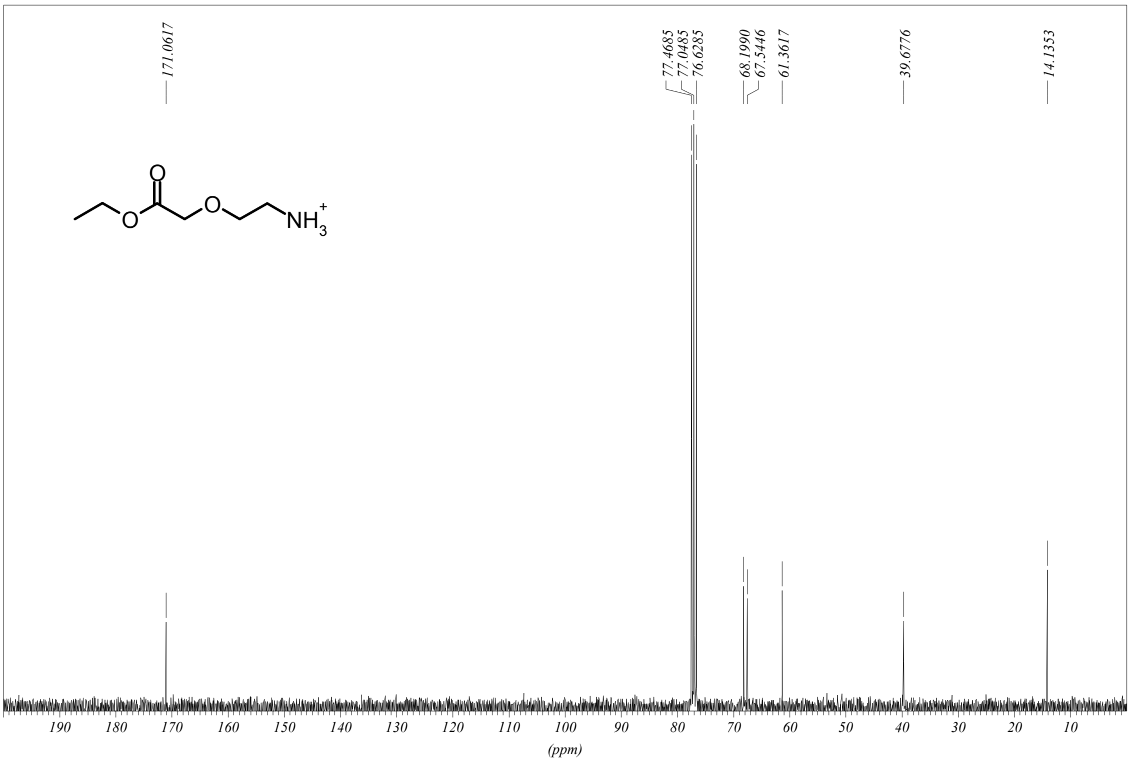


Figure S-6: ^{13}C-NMR spectrum of compound **10b**

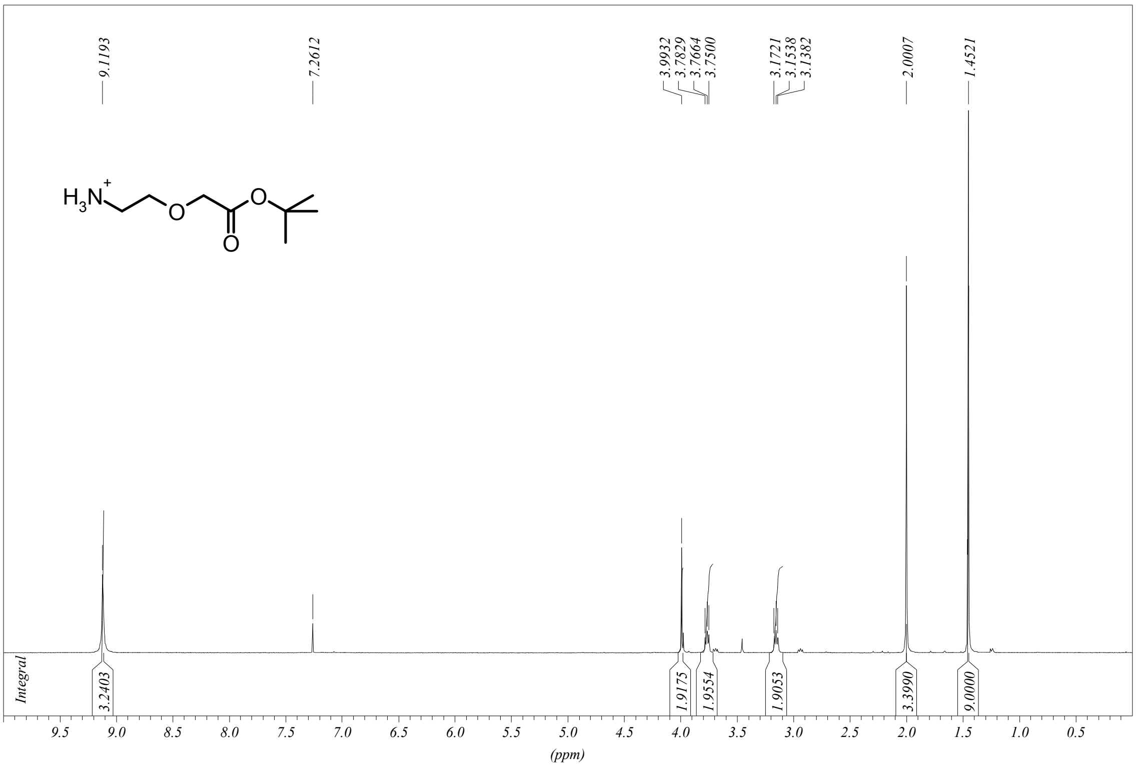


Figure S-7: ^{1}H-NMR spectrum of compound **10a**

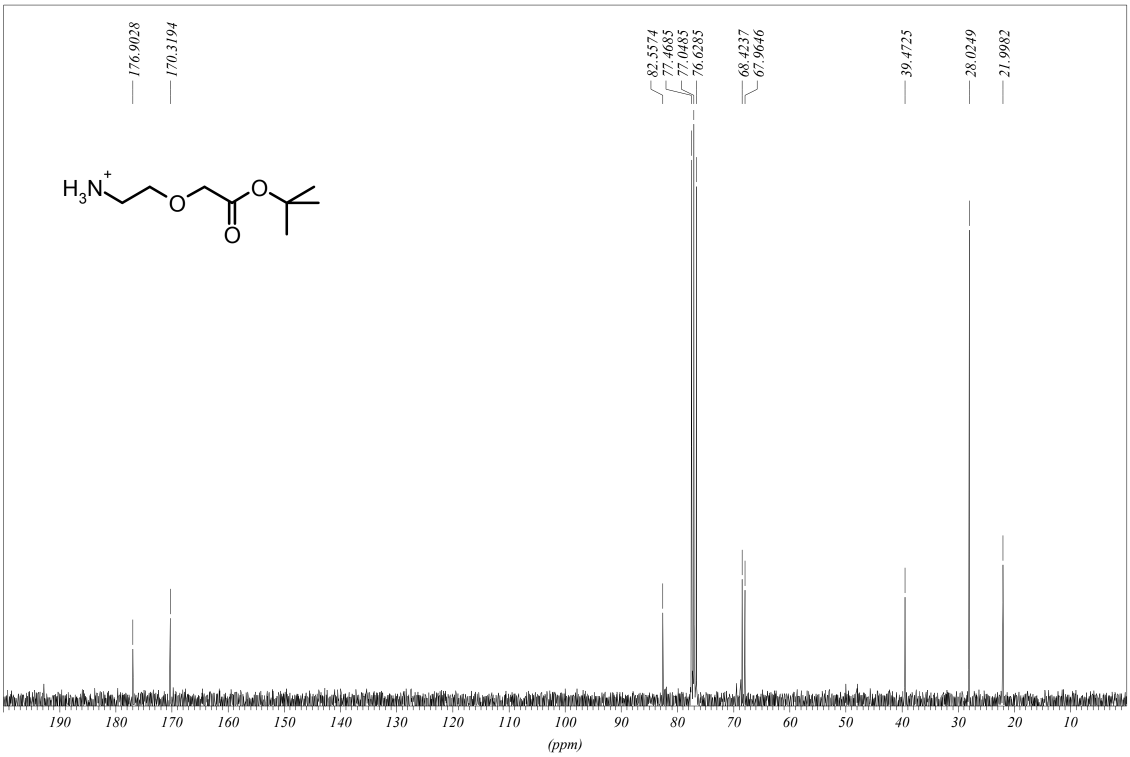


Figure S-8: ^{13}C-NMR spectrum of compound **10a**

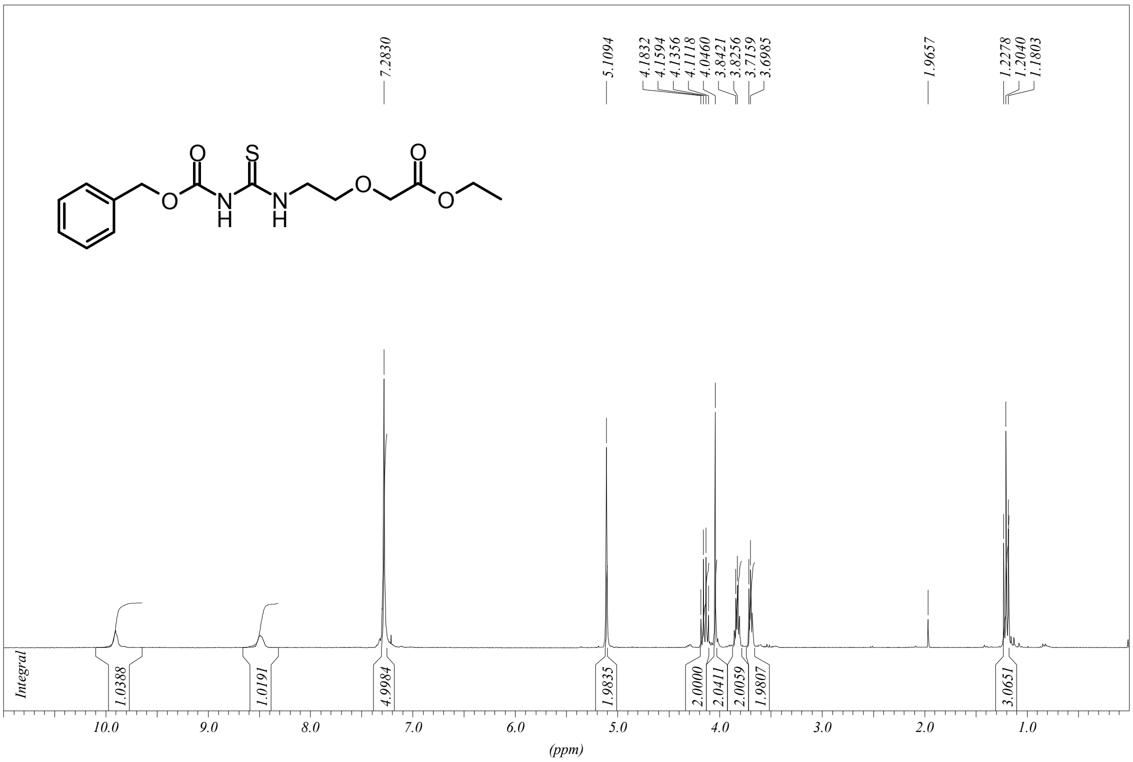


Figure S-9: ^{1}H-NMR spectrum of compound **12b**

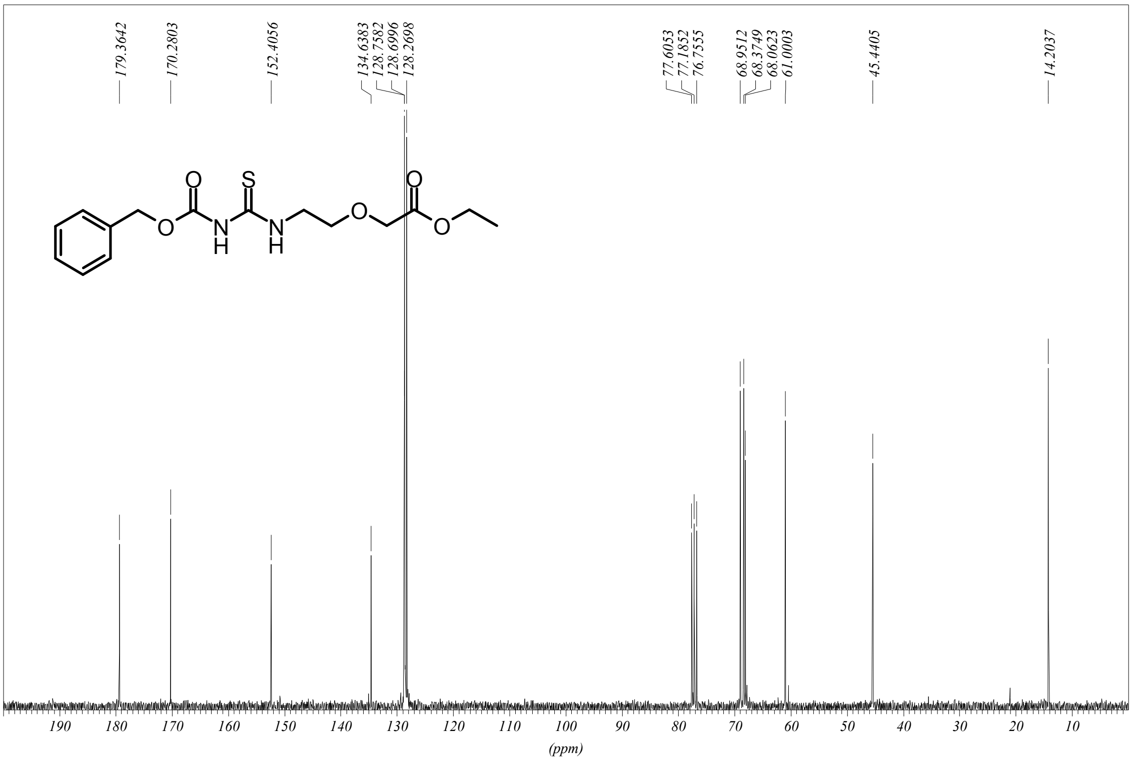


Figure S-10: ^{13}C-NMR spectrum of compound **12b**

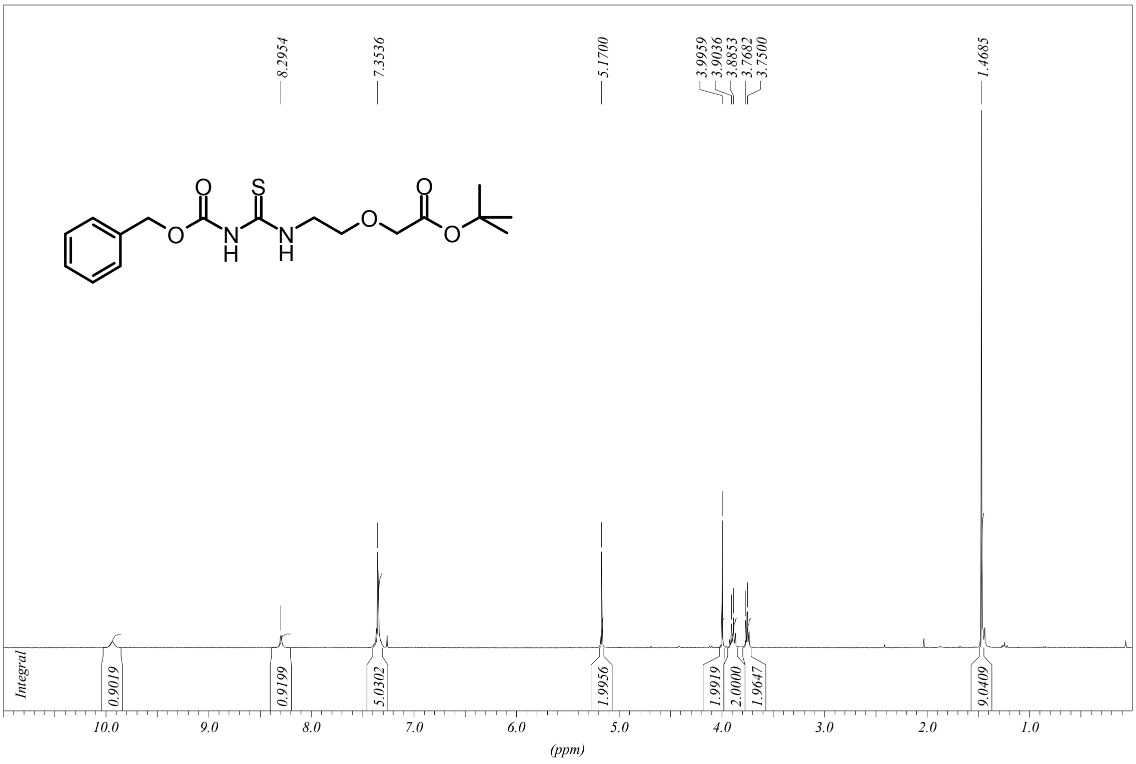


Figure S-11: ^{1}H-NMR spectrum of compound **12c**

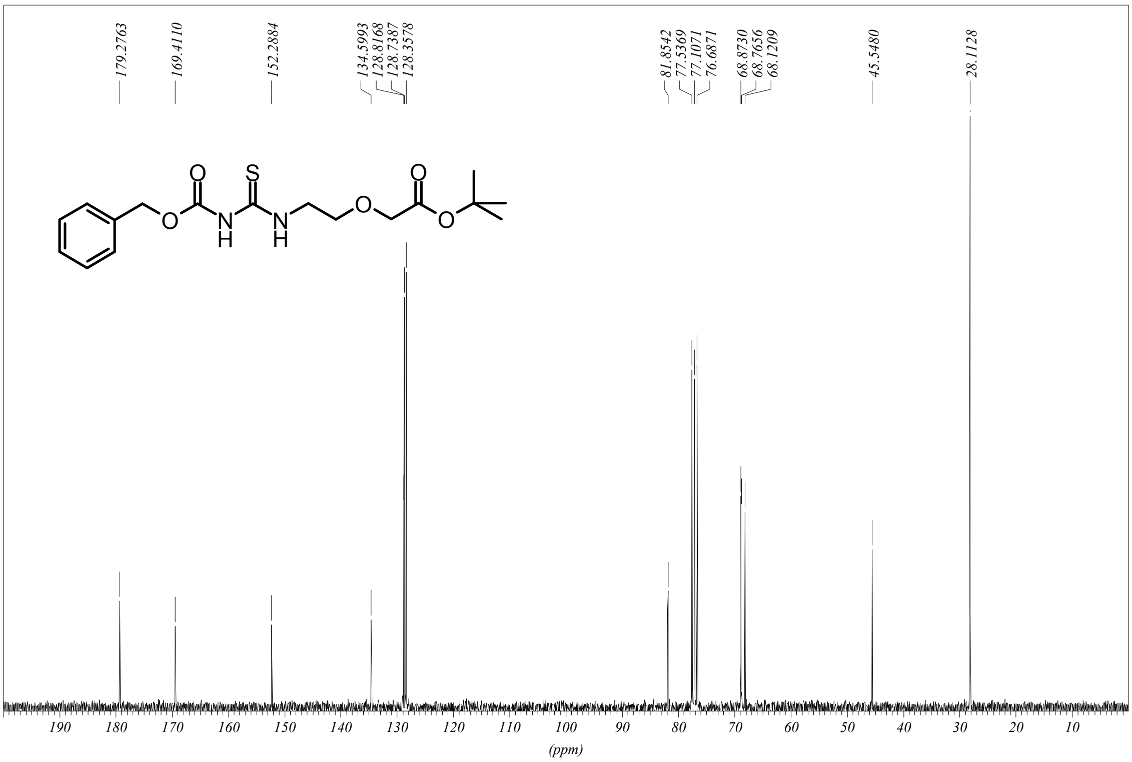


Figure S-12: ^{13}C-NMR spectrum of compound **12c**

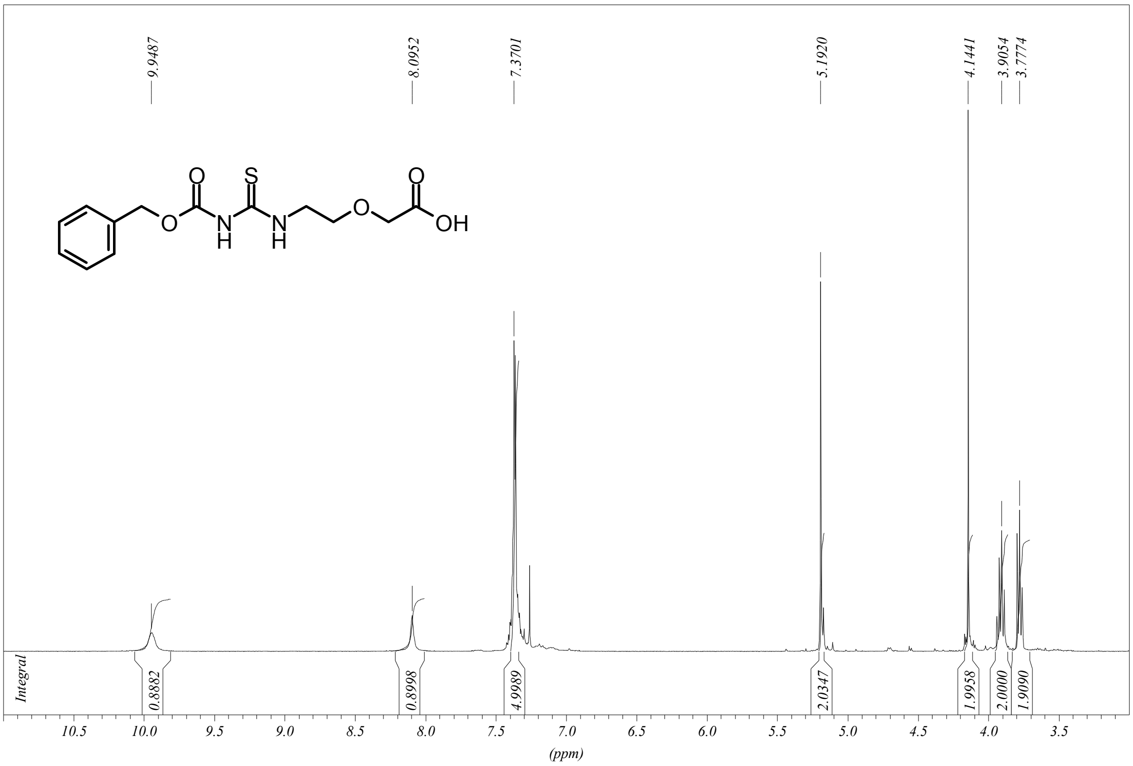


Figure S-13: ^{1}H-NMR spectrum of compound **12a**

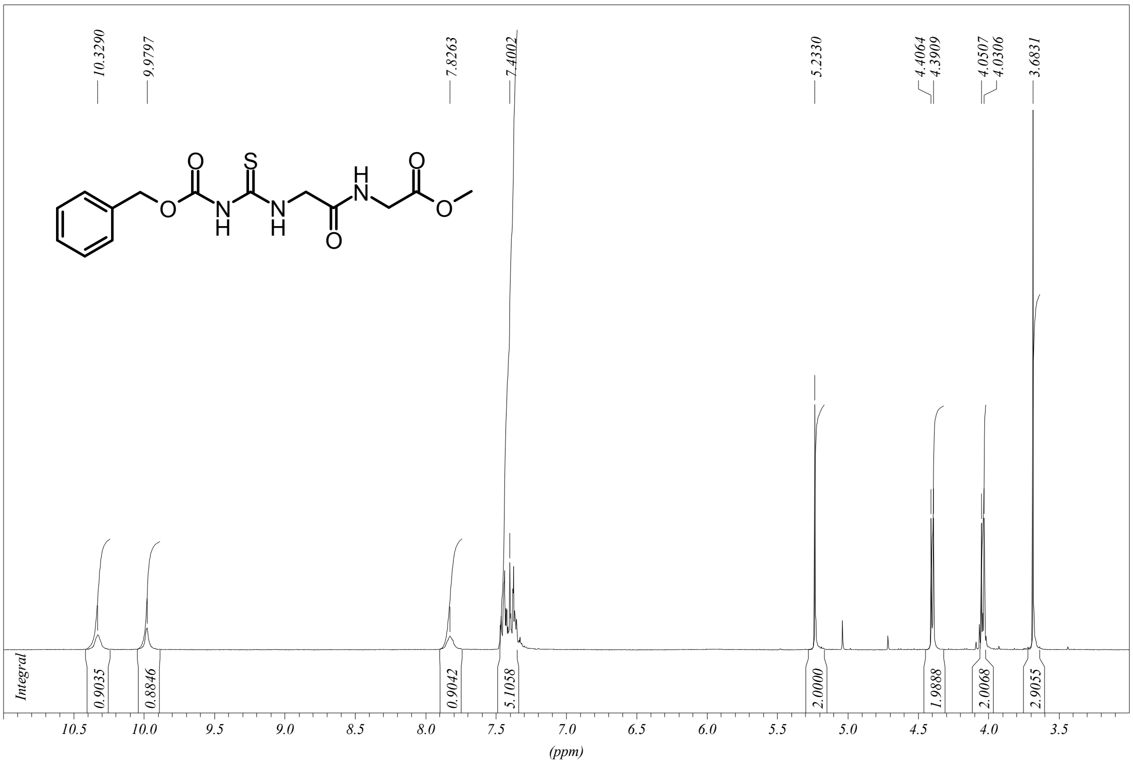


Figure S-14: ^{1}H-NMR spectrum of compound **14b**

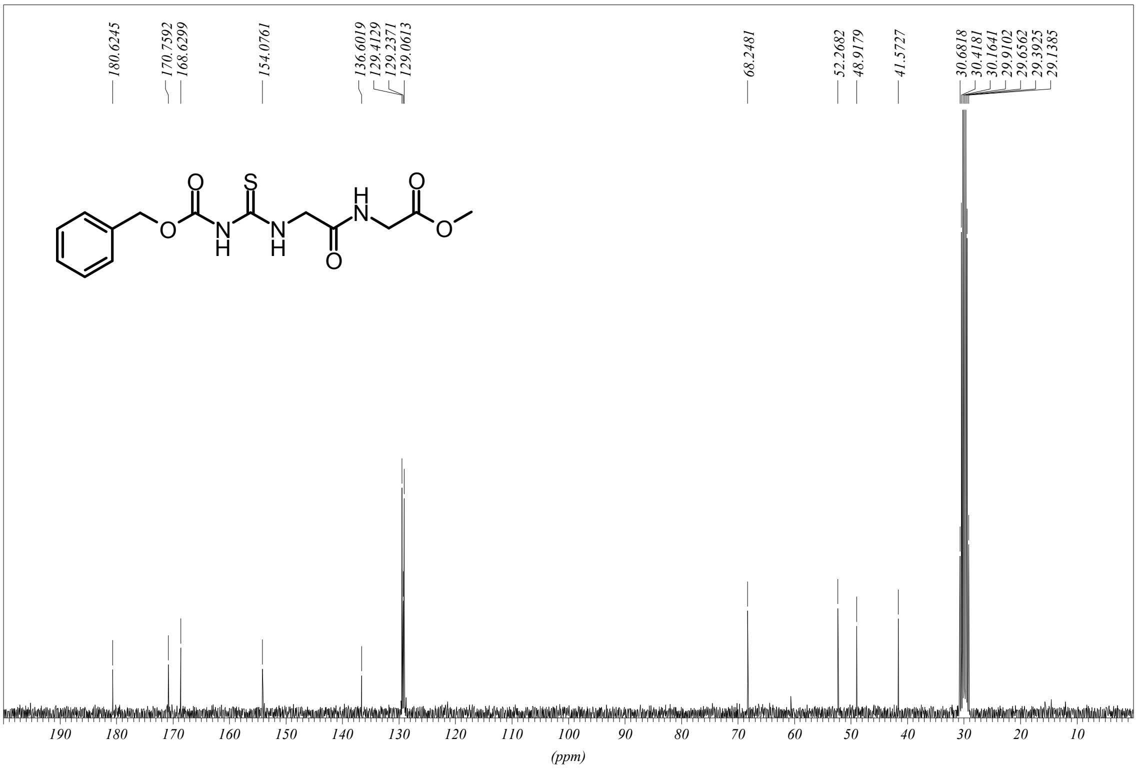


Figure S-15: ^{13}C-NMR spectrum of compound **14b**

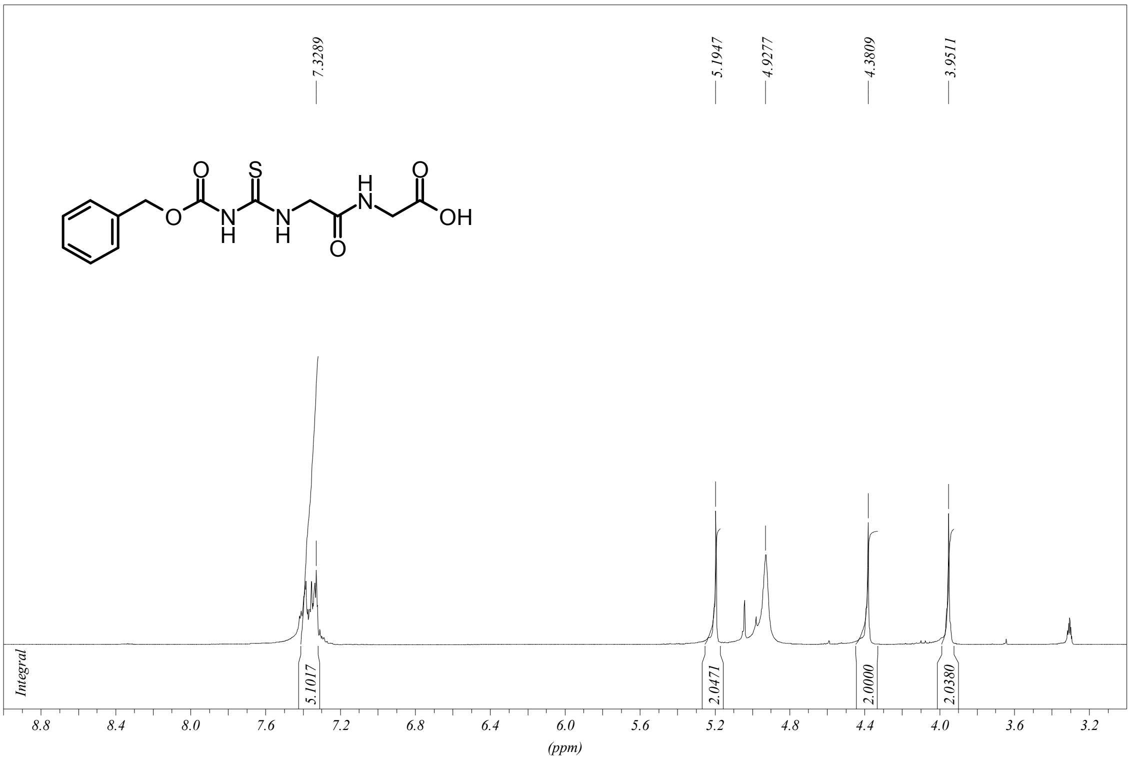


Figure S-16: ^{1}H-NMR spectrum of compound **14a**

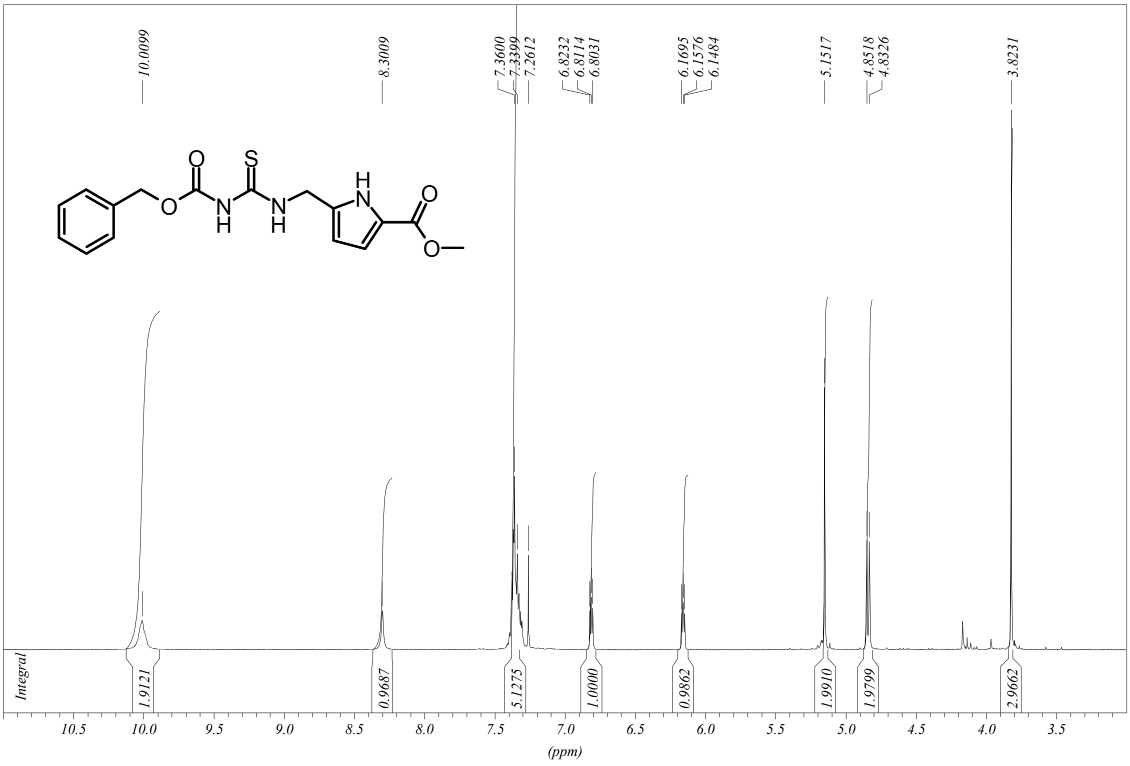


Figure S-17: ^{1}H-NMR spectrum of compound **15**

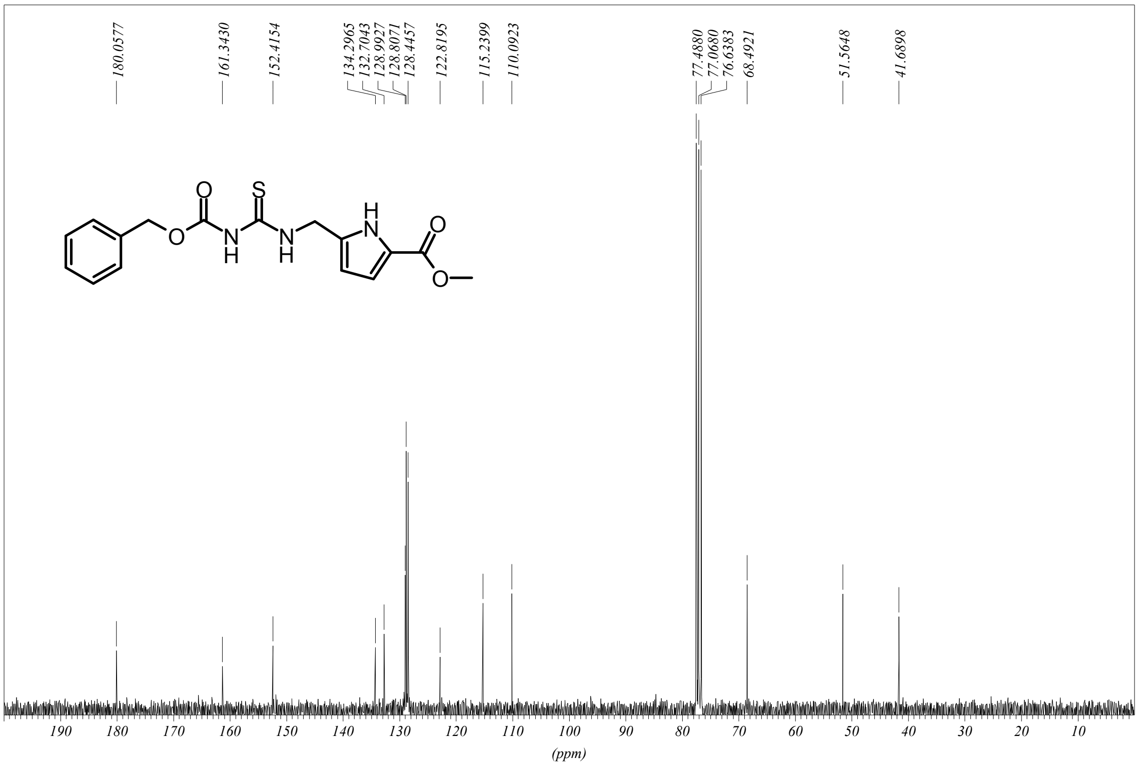


Figure S-18: ^{13}C-NMR spectrum of compound **15**

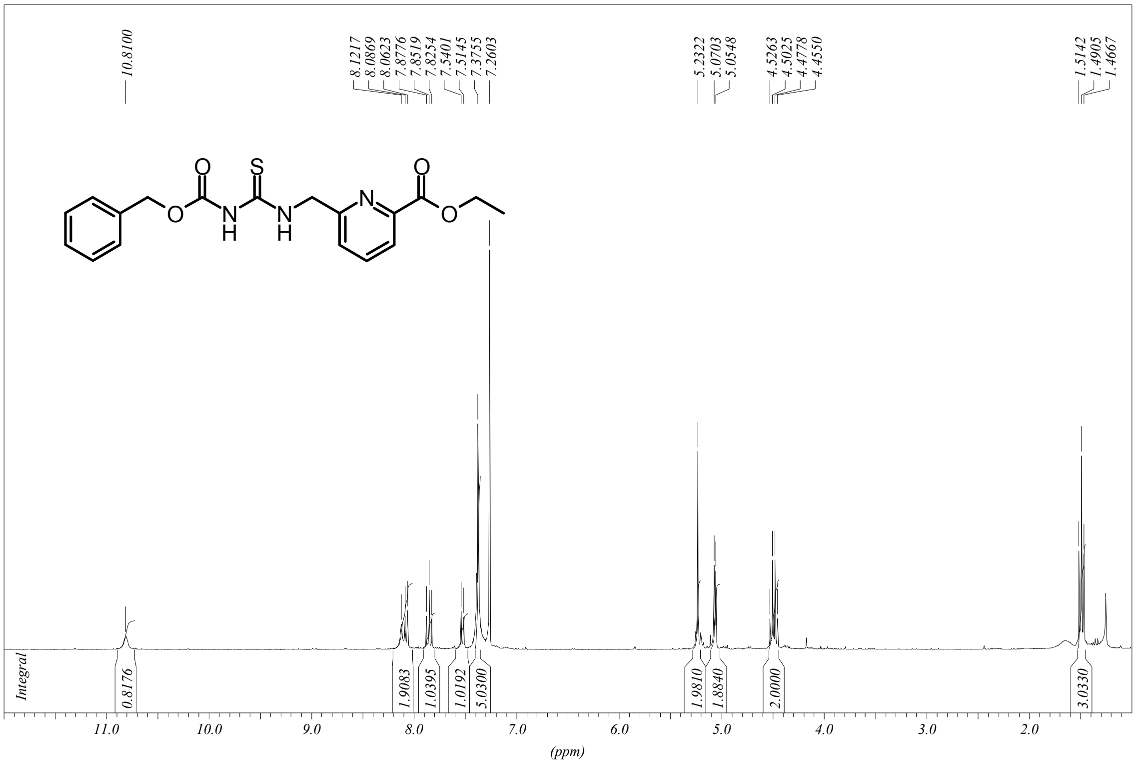


Figure S-19: ^{1}H-NMR spectrum of compound **16**

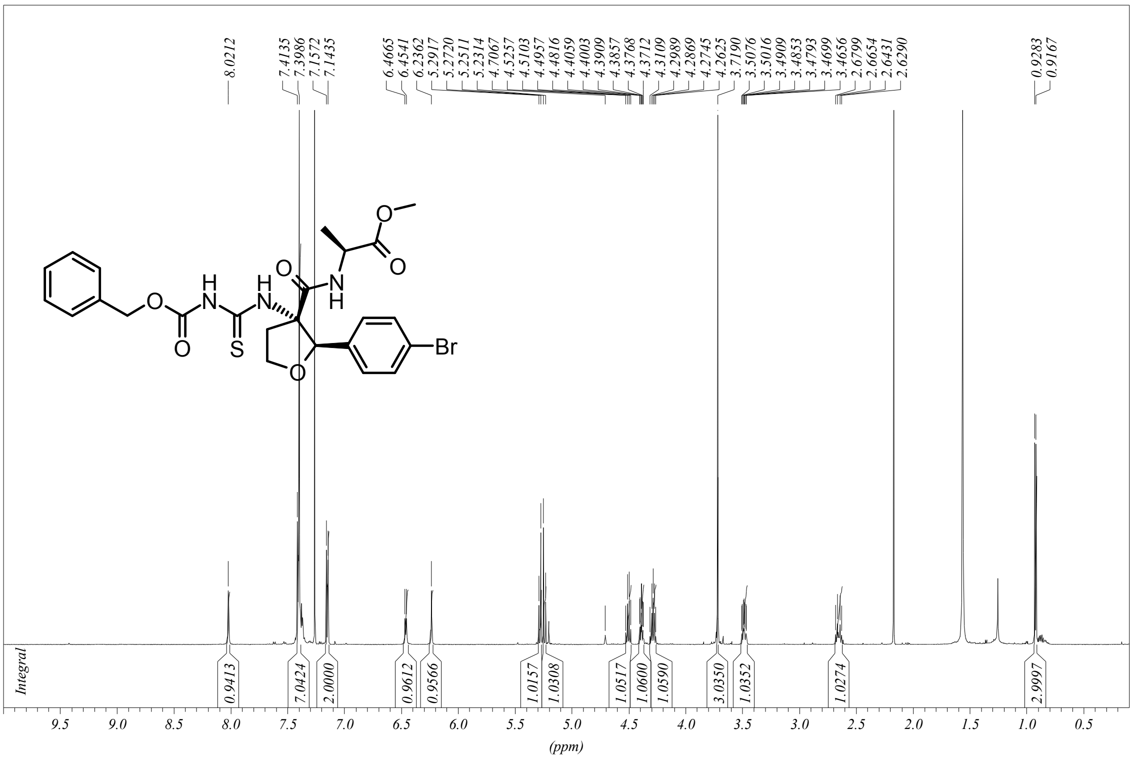


Figure S-20: ^{1}H-NMR spectrum of compound **E-2a**

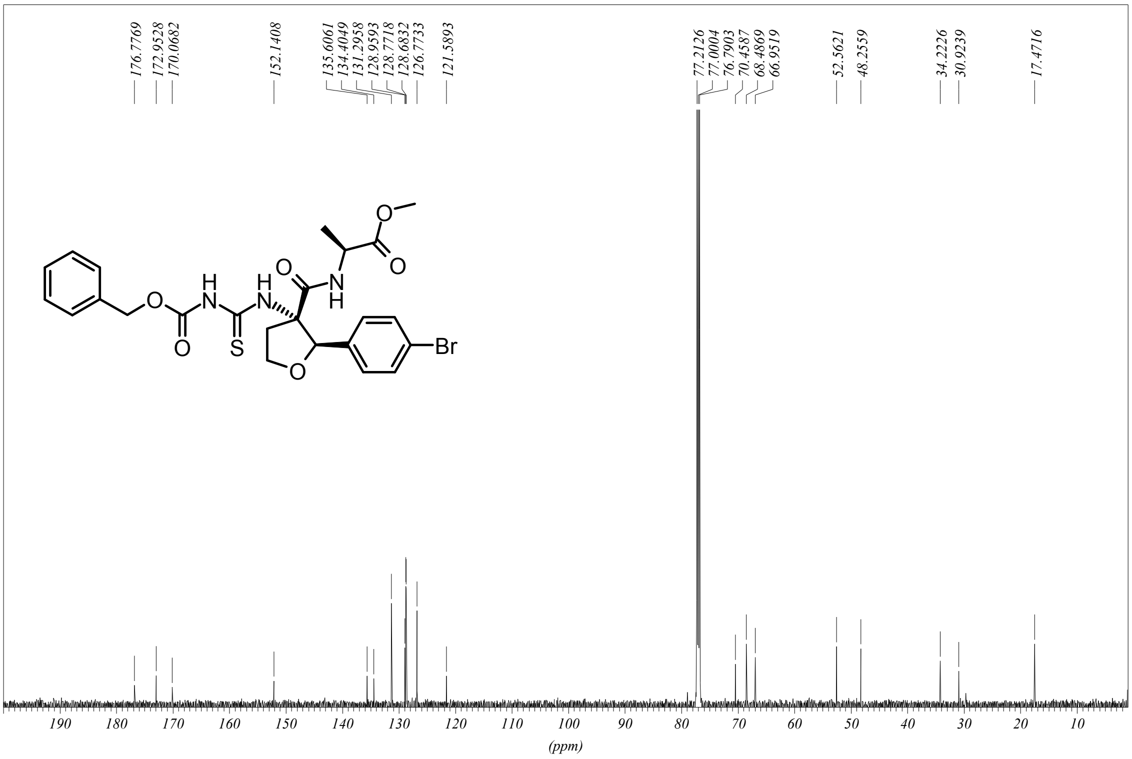


Figure S-21: ^{13}C-NMR spectrum of compound **E-2a**

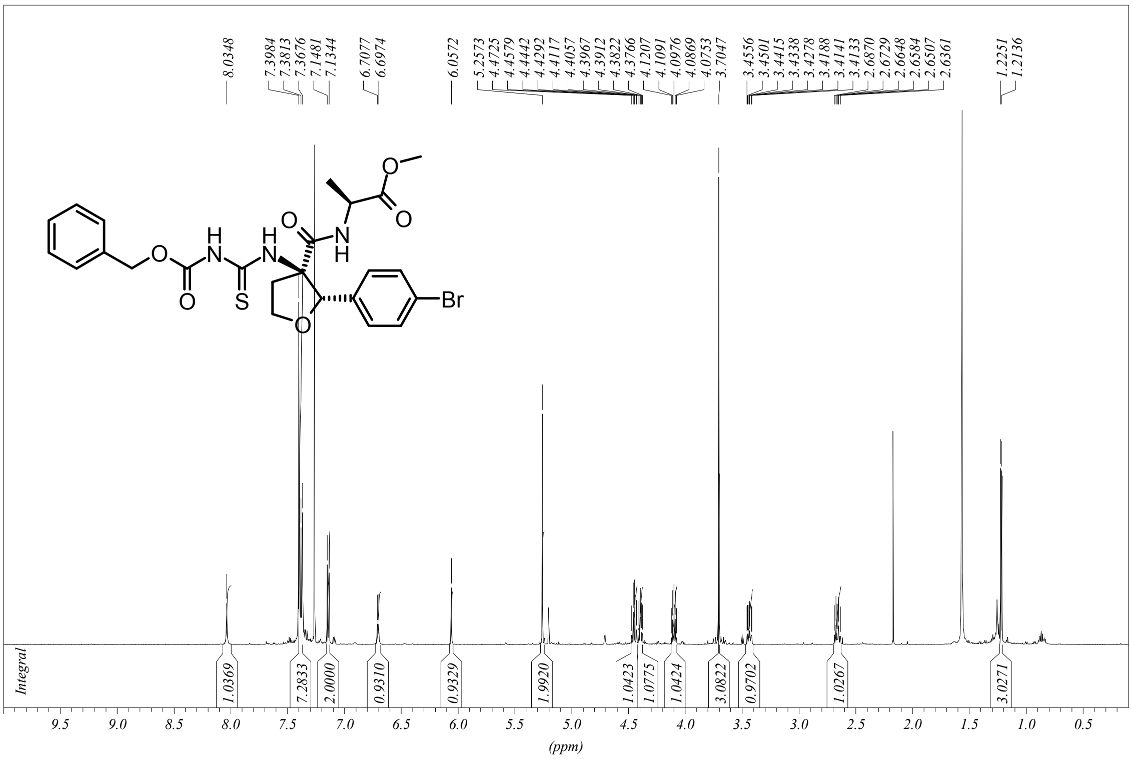


Figure S-22: ^{1}H-NMR spectrum of compound **E-2b**

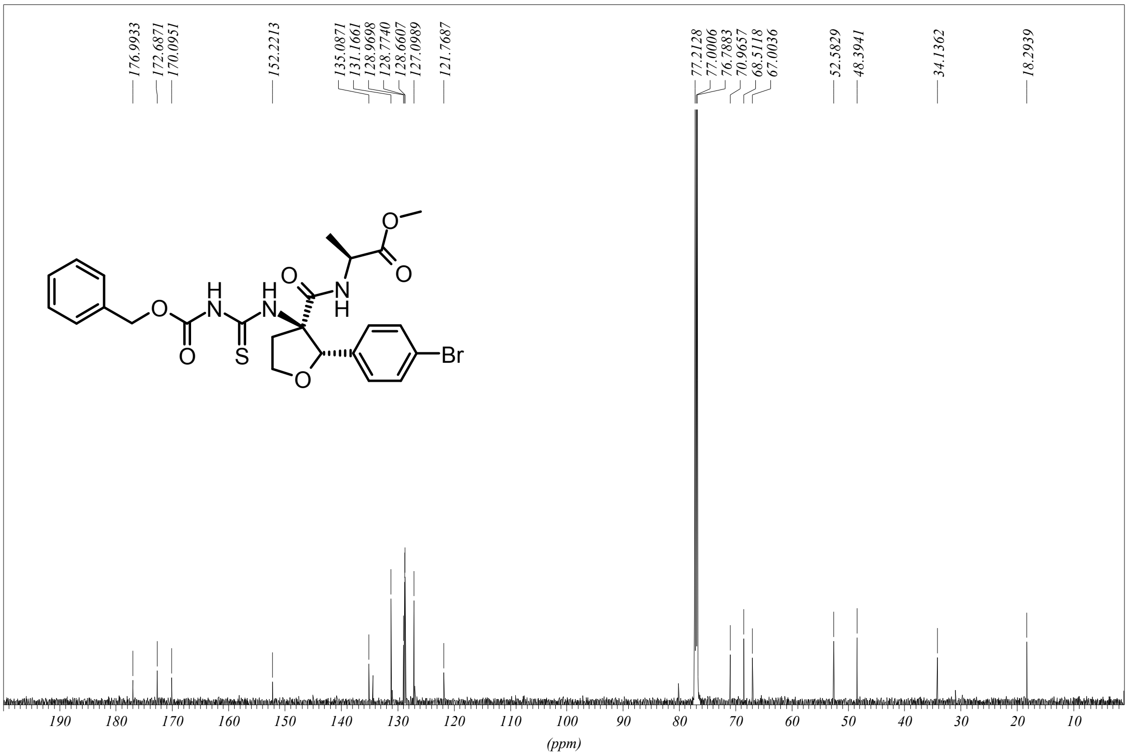


Figure S-23: ^{13}C-NMR spectrum of compound **E-2b**

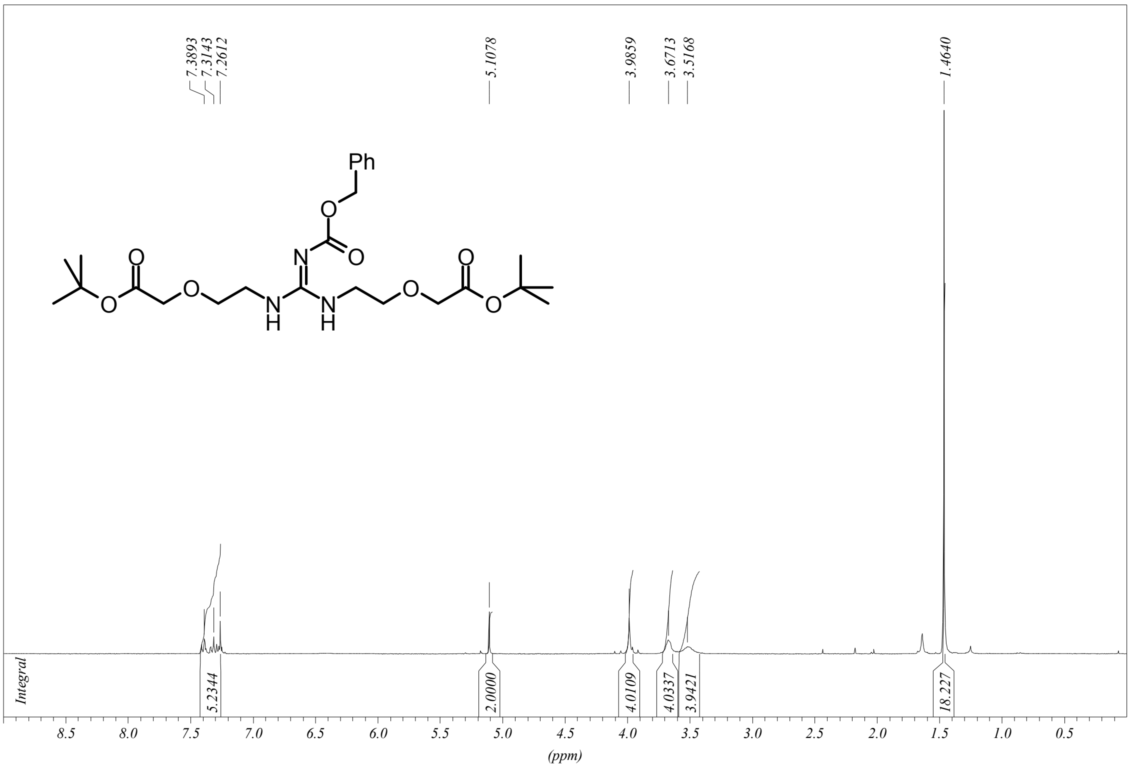


Figure S-24: ^{1}H-NMR spectrum of compound **17a**

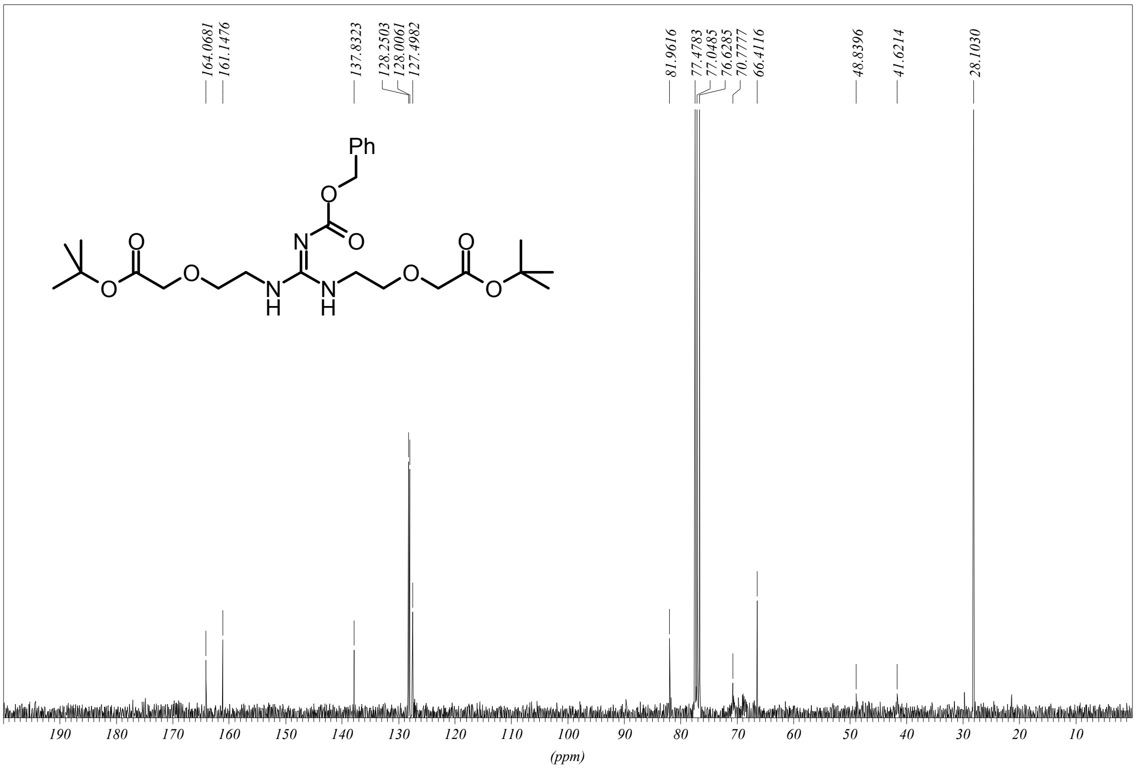


Figure S-25: ^{13}C-NMR spectrum of compound **17a**

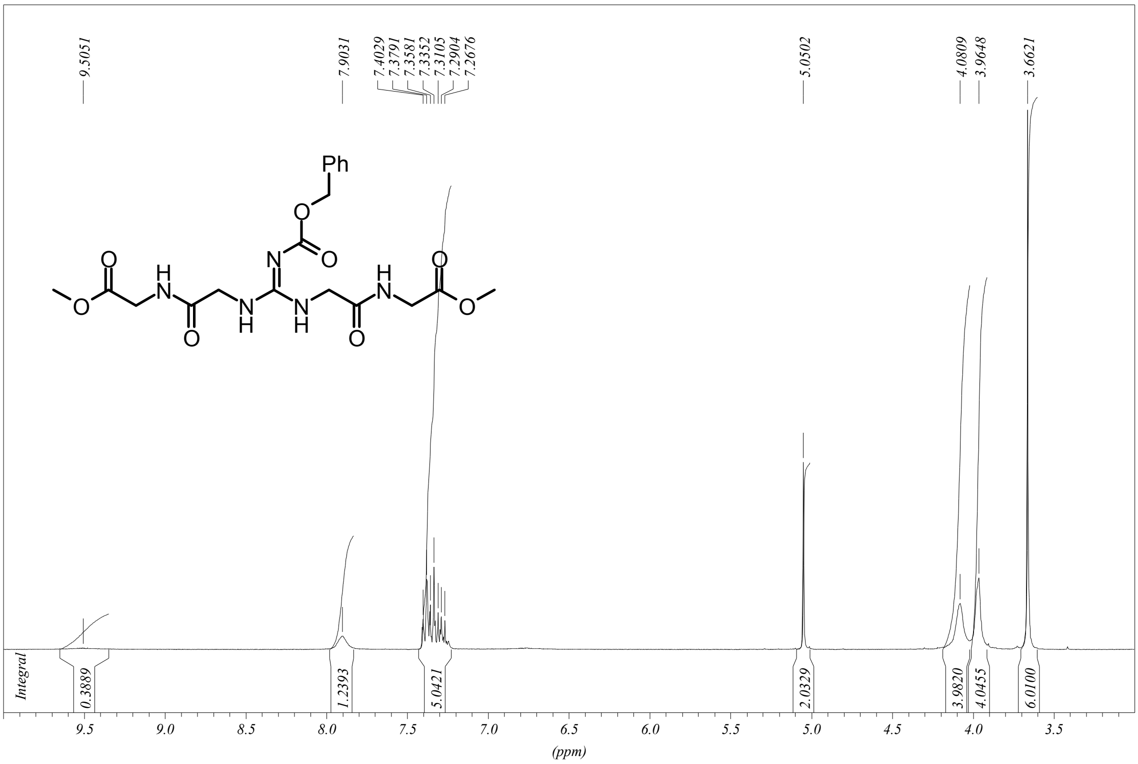


Figure S-26: ^{1}H-NMR spectrum of compound **19**

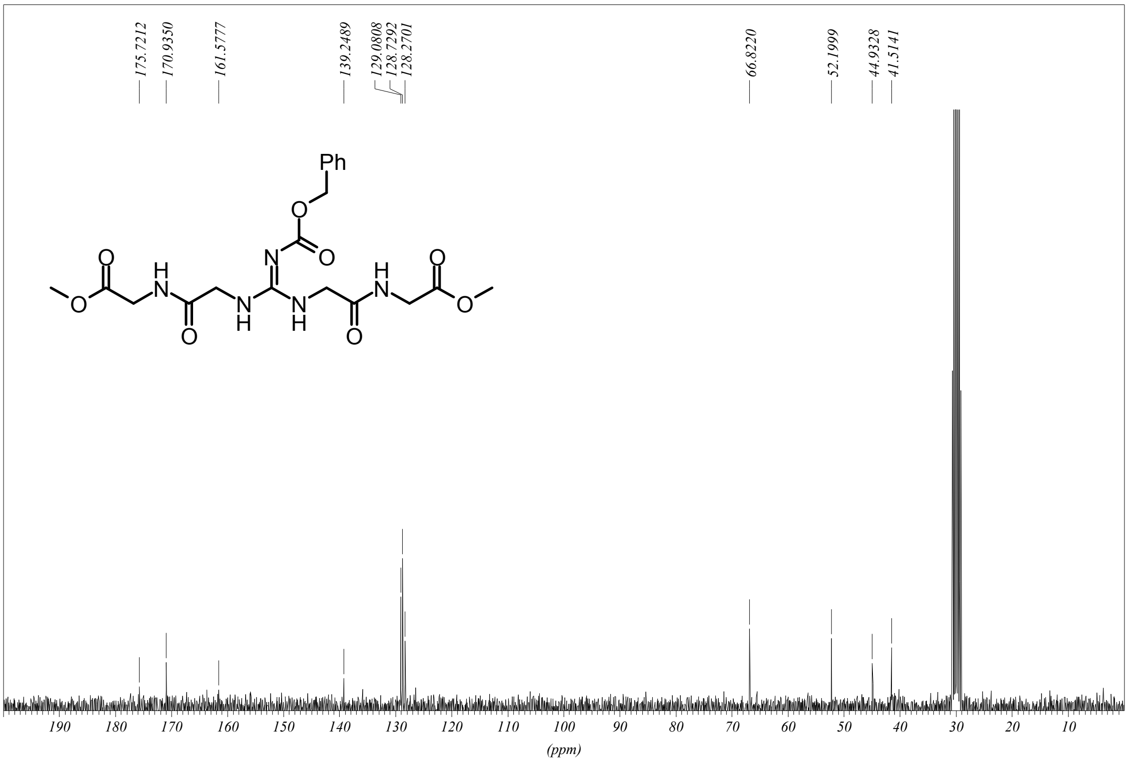


Figure S-27: ^{13}C-NMR spectrum of compound **19**

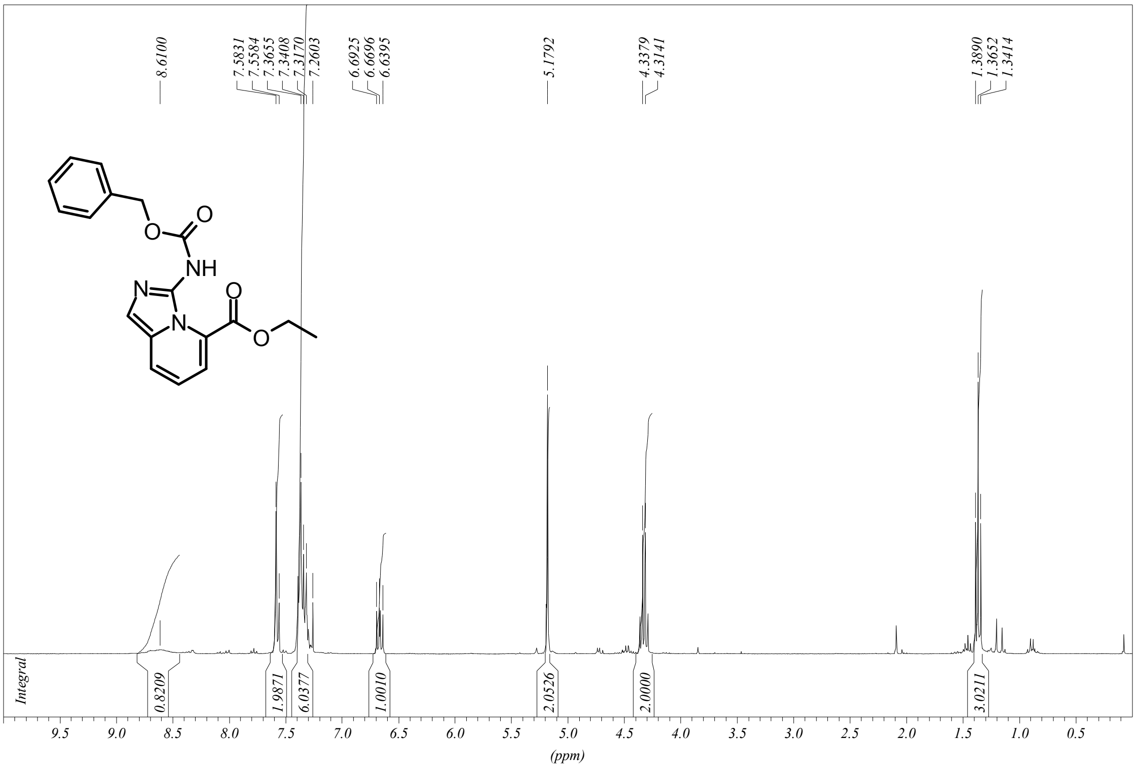


Figure S-28: ^{1}H-NMR spectrum of compound **21**

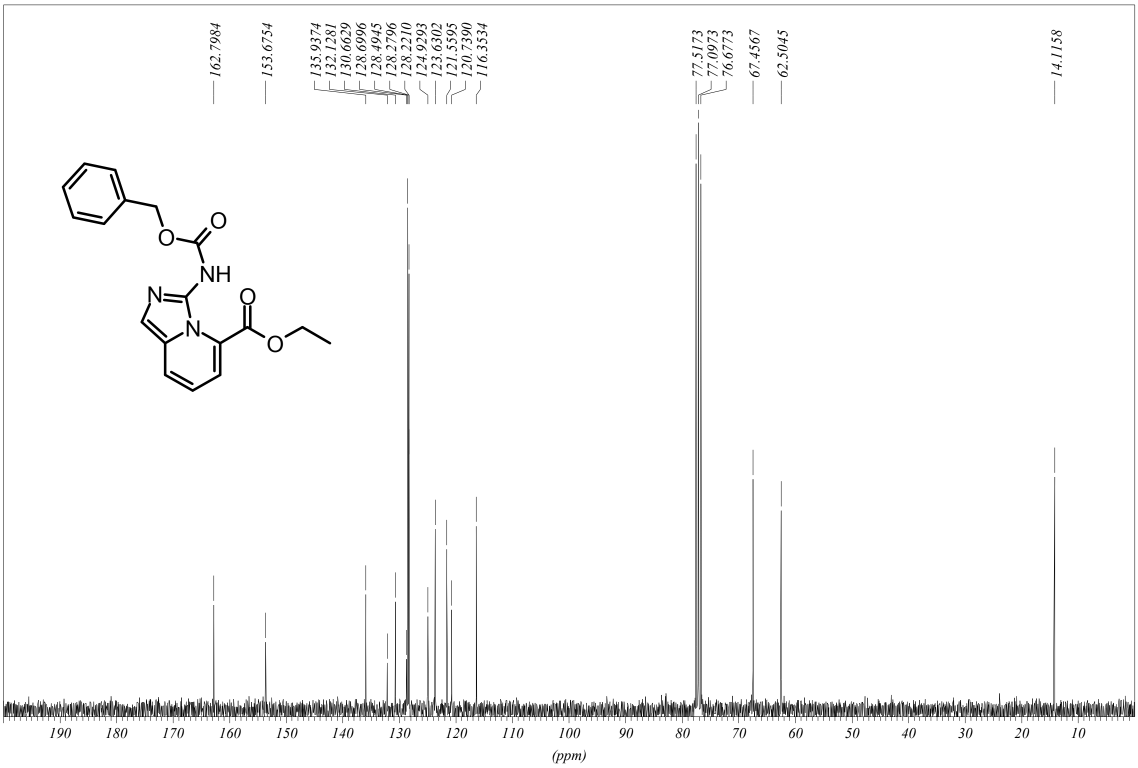


Figure S-29: ^{13}C-NMR spectrum of compound **21**

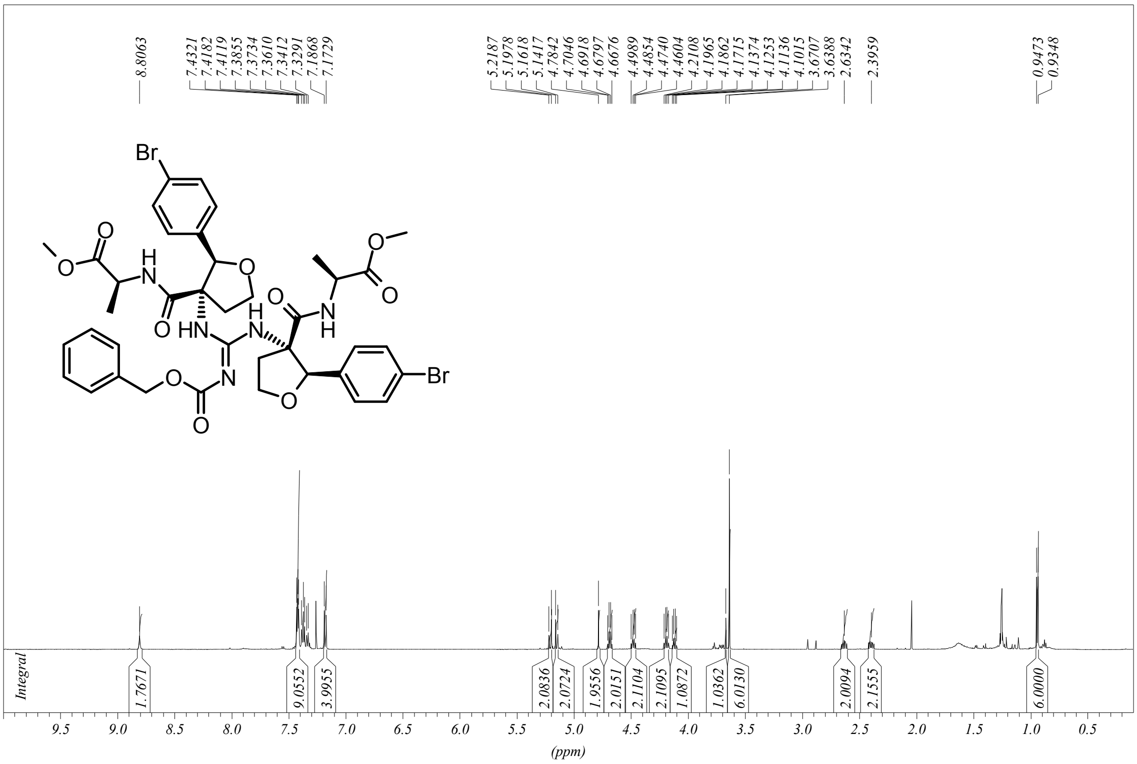


Figure S-30: ^{1}H-NMR spectrum of compound **E-3a**

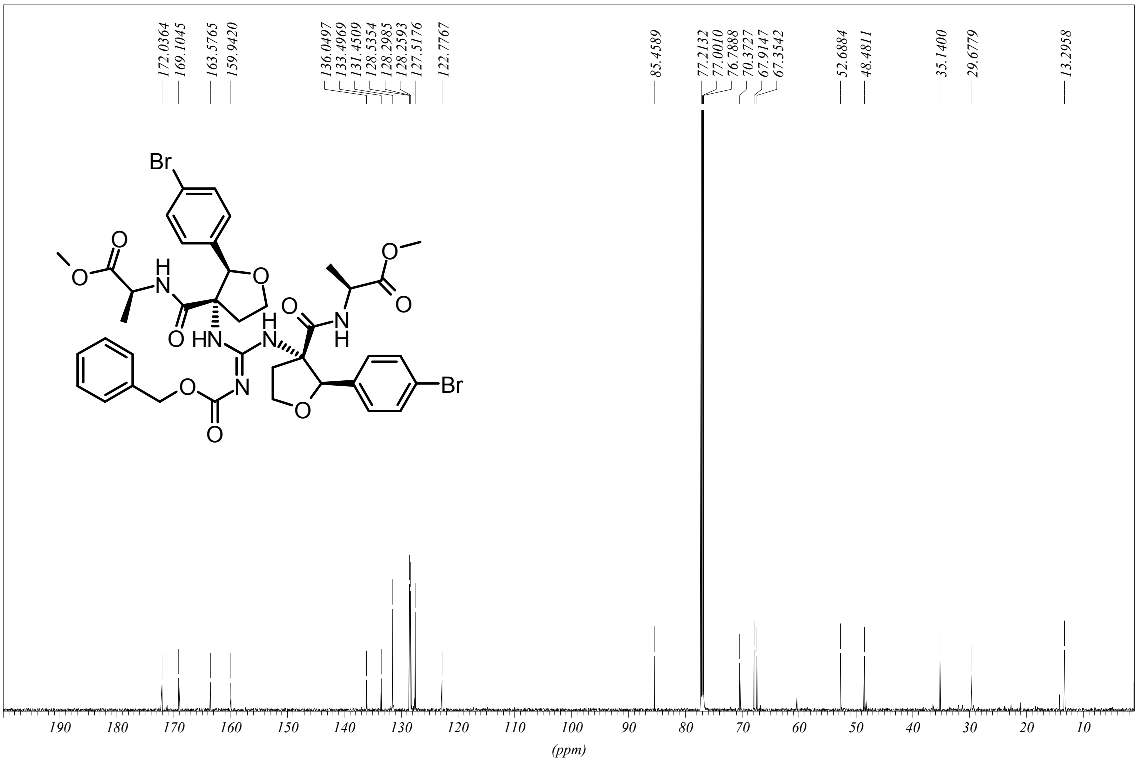


Figure S-31: ^{13}C-NMR spectrum of compound **E-3a**

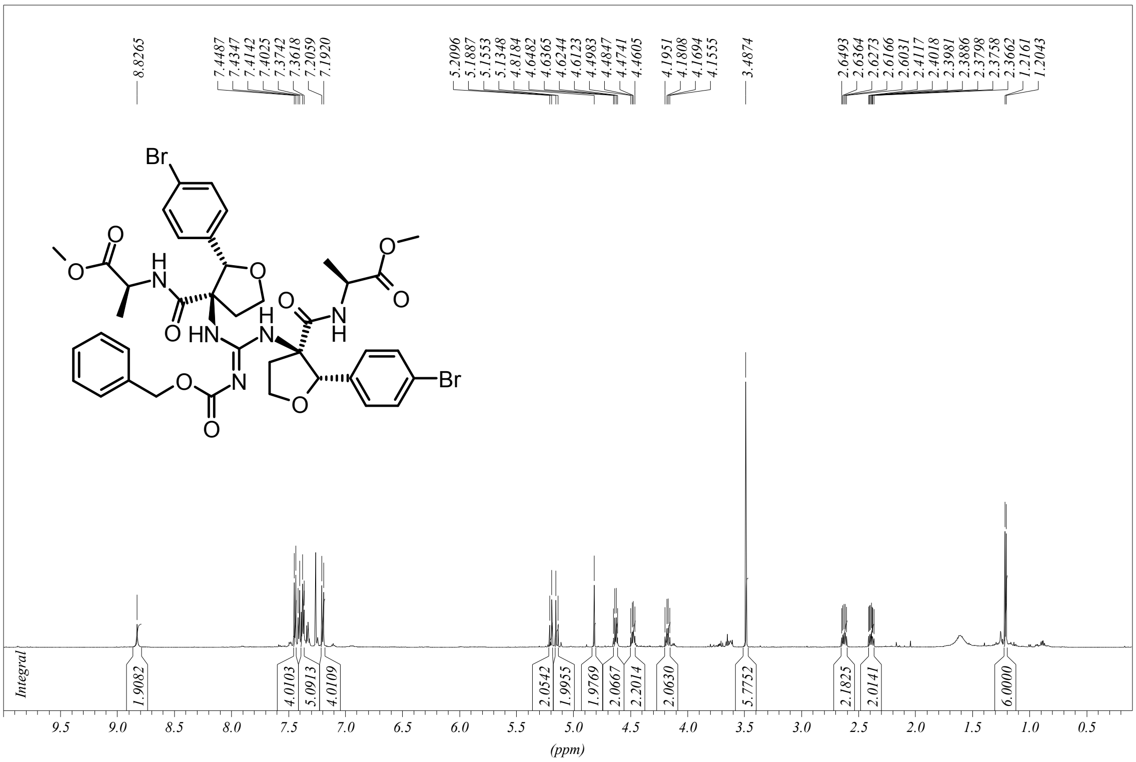


Figure S-32: ^{1}H-NMR spectrum of compound **E-3b**

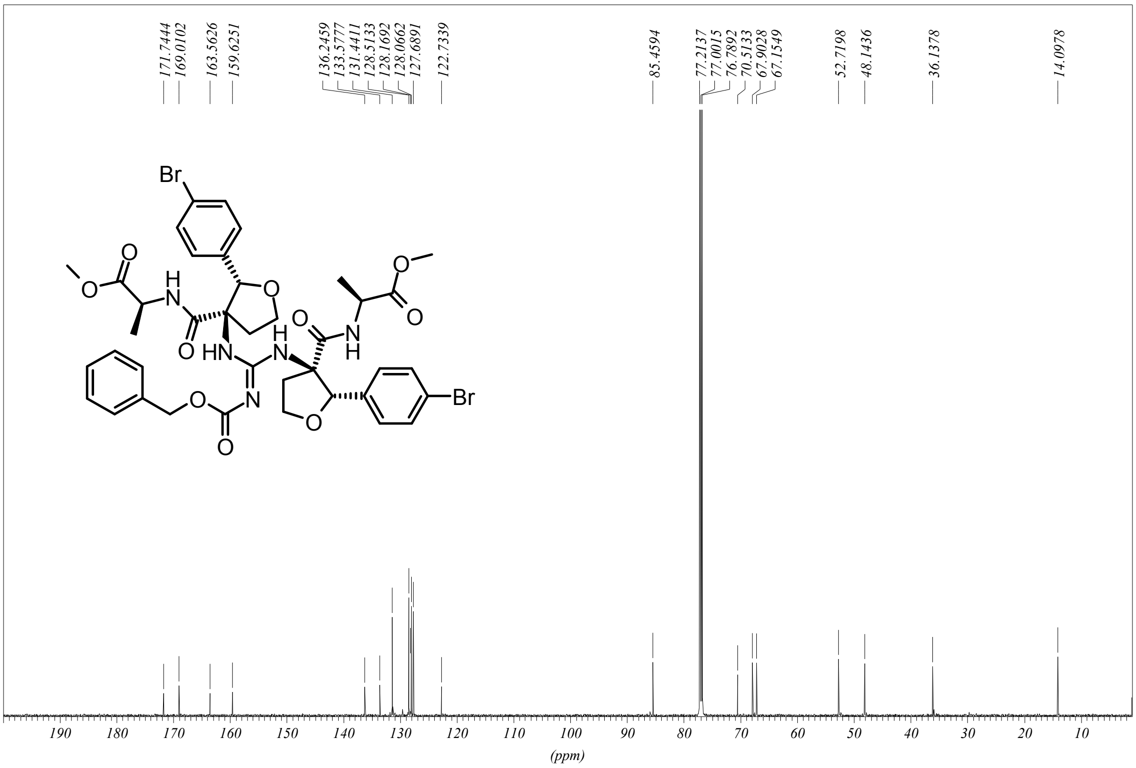


Figure S-33: ^{13}C-NMR spectrum of compound **E-3b**

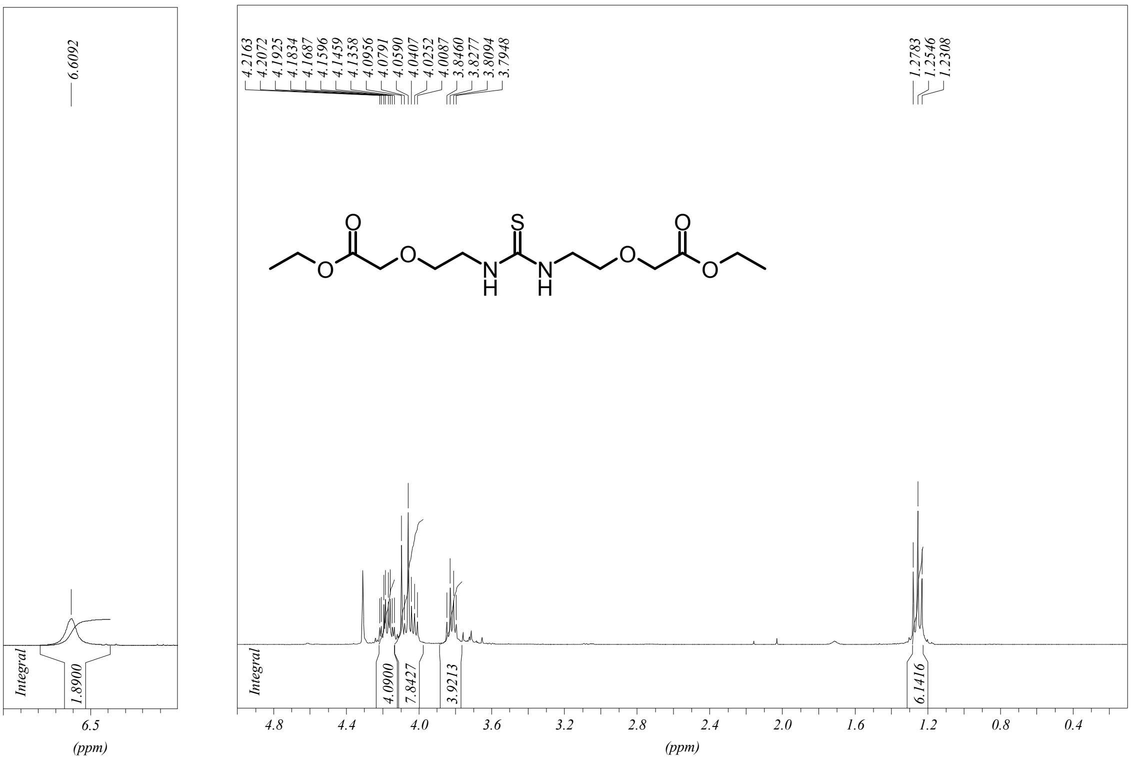


Figure S-34: ^{1}H-NMR spectrum of compound **E-4** (left part x2)

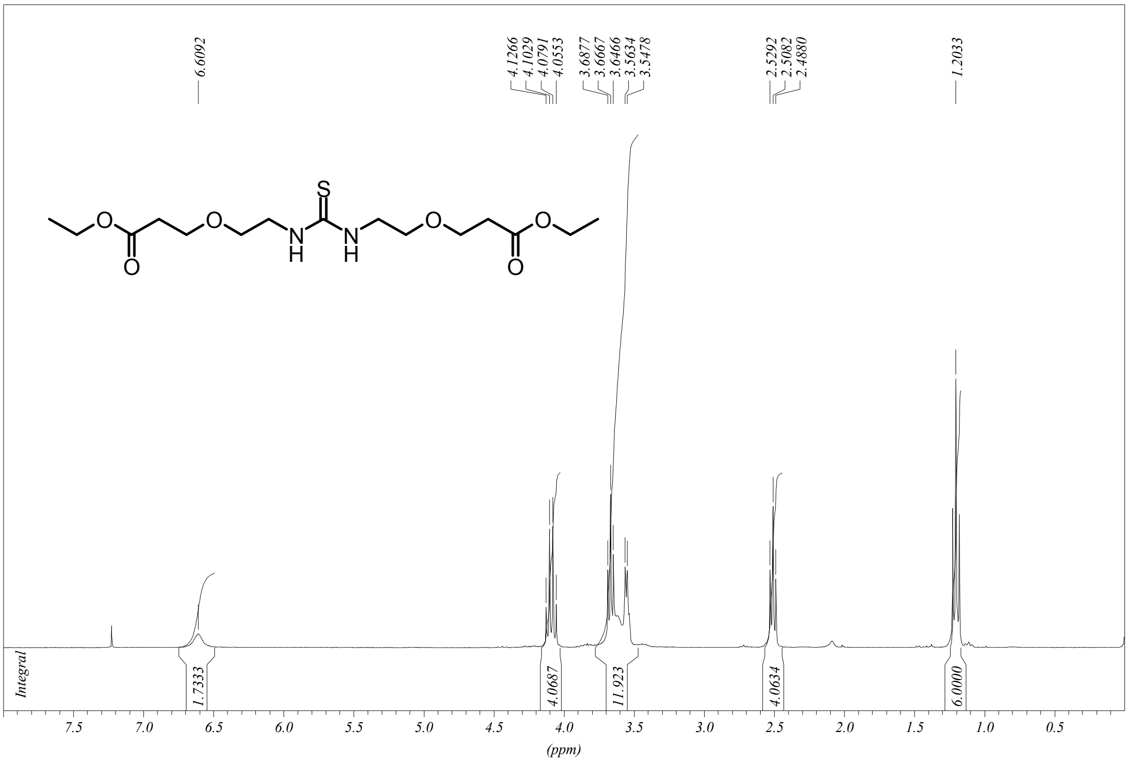


Figure S-35: ^{1}H-NMR spectrum of compound **E-5a**

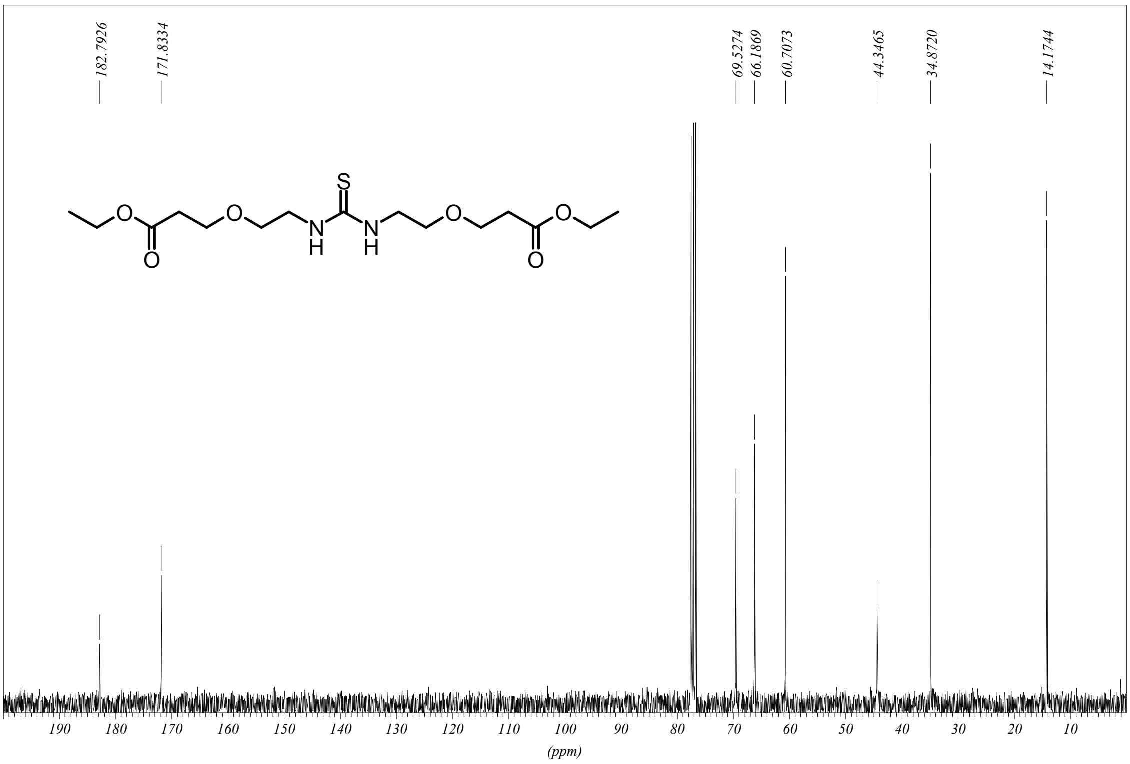


Figure S-36: ^{13}C-NMR spectrum of compound **E-5a**

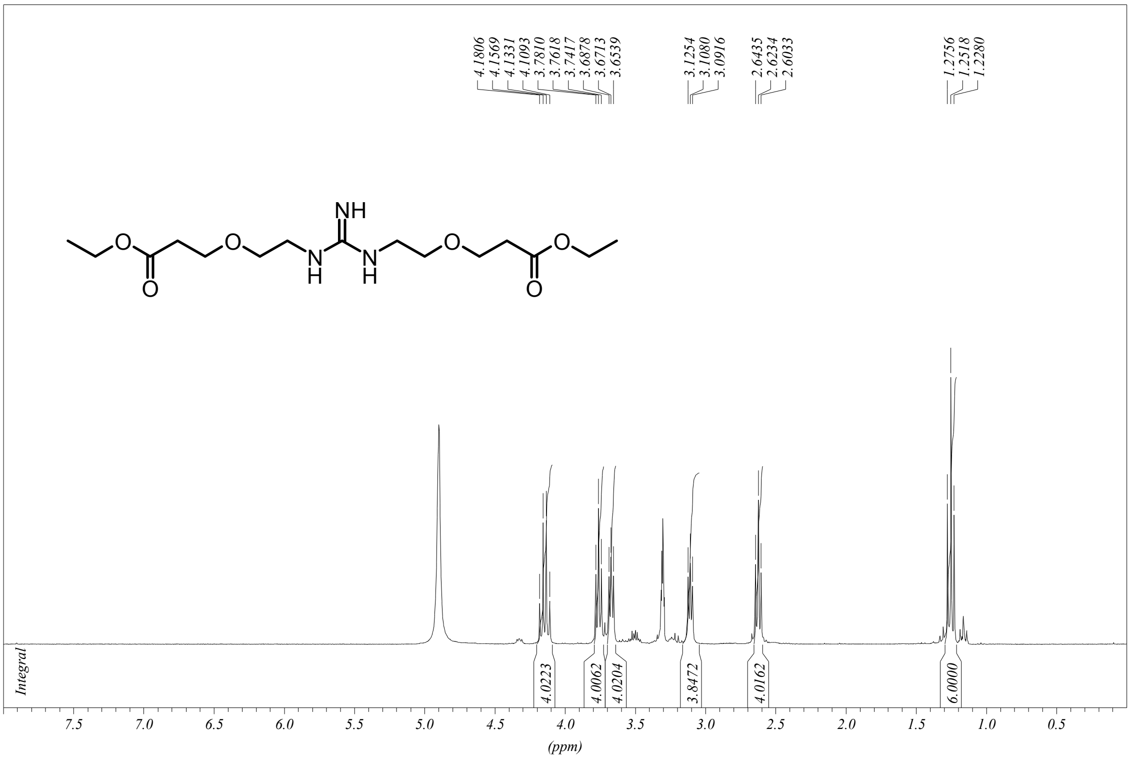


Figure S-37: ^{1}H-NMR spectrum of compound **E-7**

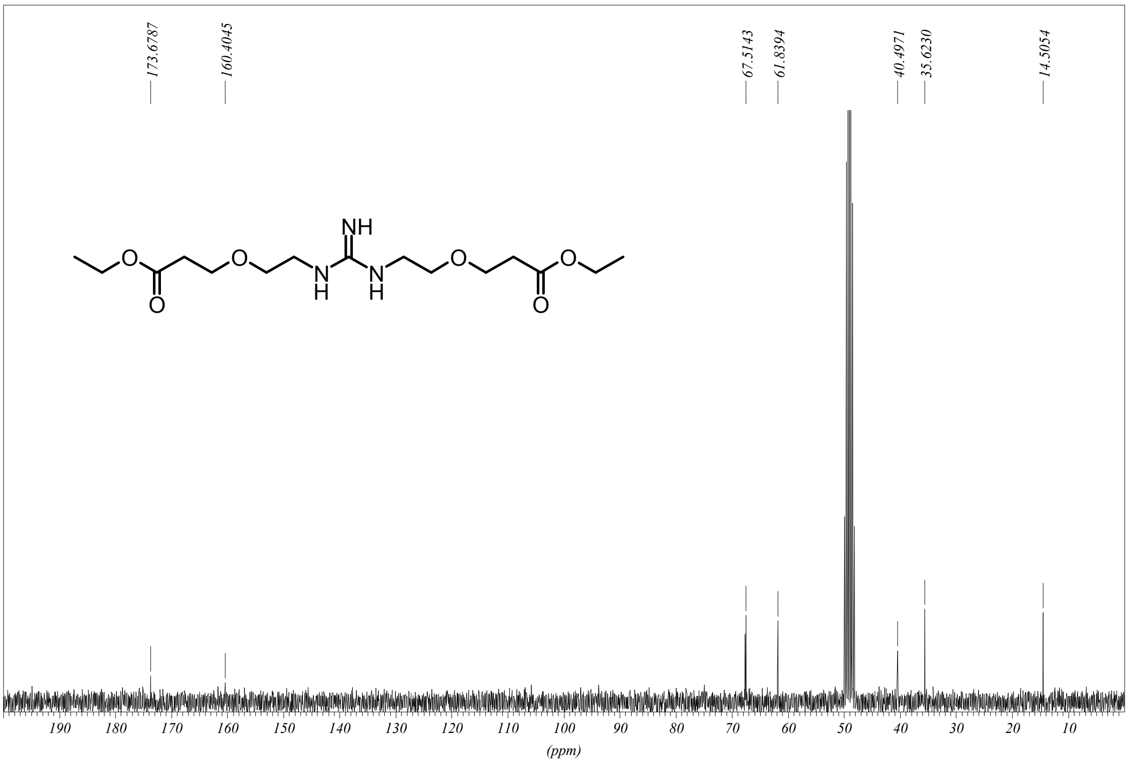


Figure S-38: ^{13}C-NMR spectrum of compound **E-7**

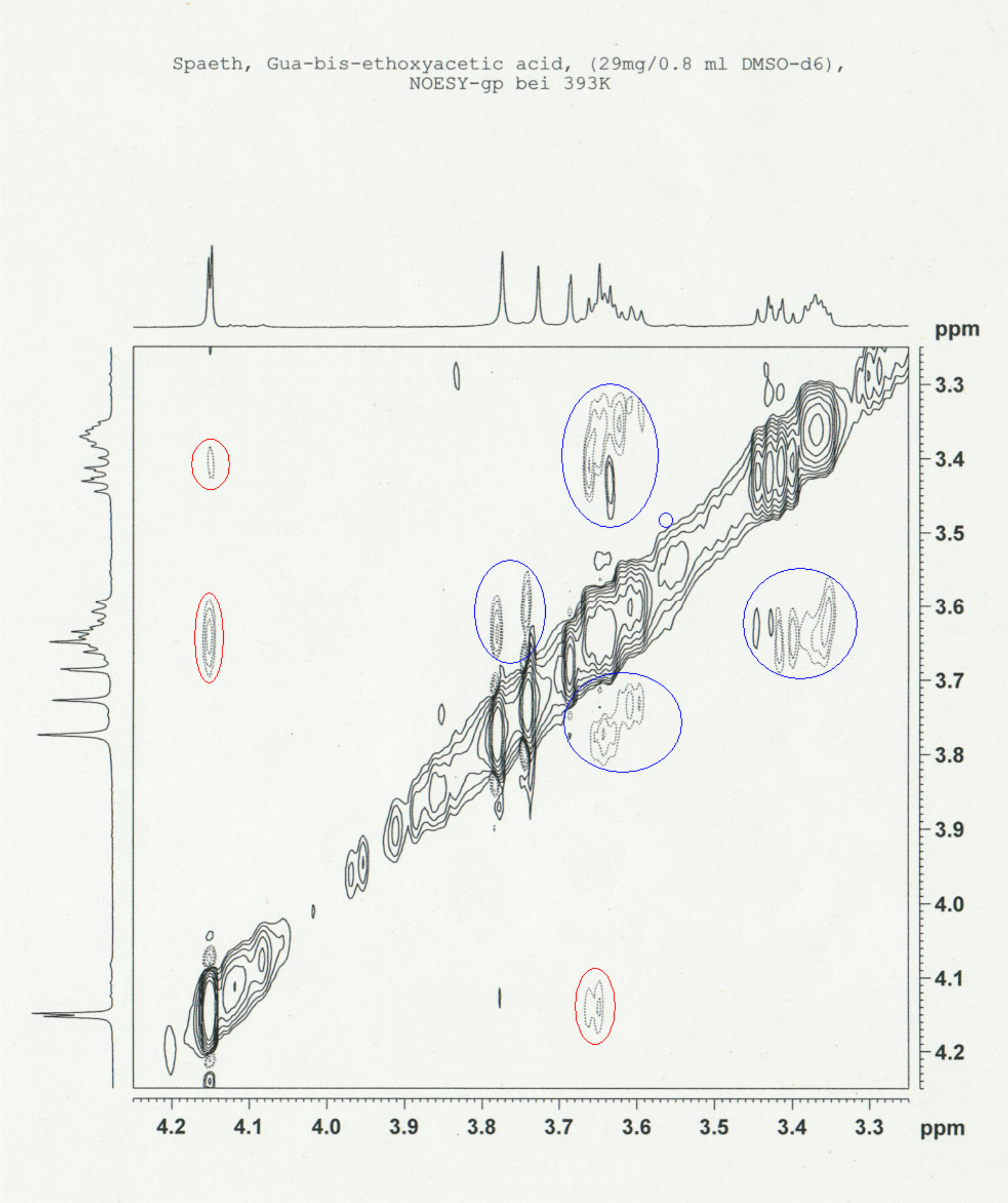


Figure S-39: ^{1}H-NMR NOESY of compound **6,** through-the-space contacts marked in red

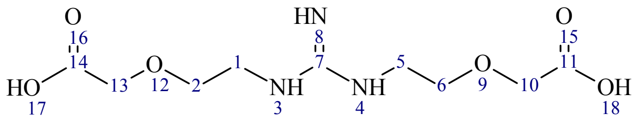
3. Determination of pK_a values, simulation of fundamental properties

The pK_a values of the tweezer were determined by pH-titration of 1 mM solutions in water. The values for compound **6** were determined with the aid of dilute sodium hydroxide solution, for **24** perchloric acid[9] was used.

Compound	*pK_a (guanidine)*	*pK_a (carboxylic acids)*
6	11.5	3.5
24	~ 12	4.0

Table S-1: pK_a values of the ligands

Fundamental properties like solubility and polarity, also including the p*K*a values were simulated with ACD Labs program package structure calculator *Ver.12.1.* The later ones are in good accordance to the experimental data.



pKa Results

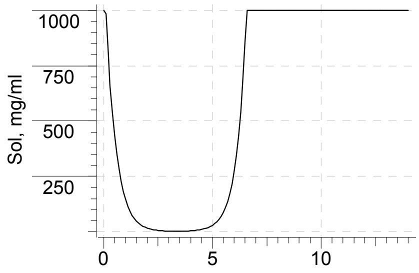
Diss. Atom	Acidic/Basic	Apparent pKa Value	Error
8	MB	11.86	0.7
18	A	3.7	0.1
18	MA	3.1	0.1
4	B	-2.39	0.7

Single-valued Properties

Name	Value	Error
LogP	-1.27	0.73
MW	263.25	-
PSA	140.97	-
FRB	10	-
HDonors	5	-
HAcceptors	9	-
Rule Of 5	1	-
Molar Refractivity, cm^3	58.23	0.5
Molar Volume, cm^3	185.9	7
Parachor, cm^3	509.52	8
Index of Refraction	1.54	4.67e-2
Surface Tension, dyne/cm	56.43	7
Density, g/cm^3	1.42	0.14
Polarizability, 10e-24 cm^3	23.08	0.5
Boiling Point, °C	500.58	60
C ratio	0.5	-
N ratio	0.17	-
NO ratio	0.5	-
Hetero ratio	0.5	-
Halogen ratio	0	-
Num Rings	0	-
Num Aromatic Rings	0	-
Num Rings 3	0	-
Num Rings 4	0	-
Num Rings 5	0	-
Num Rings 6	0	-

Solubility Results

pH	Sol, mg/ml	Flags	%
2	15.96	BZ	92 / 8
5.5	86.9	A	99
6.5	854.17	A	100
7.4	1000	A	100
10	1000	A	100



LogD Results

pH	LogD
2	-3.3
5.5	-4.72
6.5	-4.77
7.4	-4.77
10	-4.78

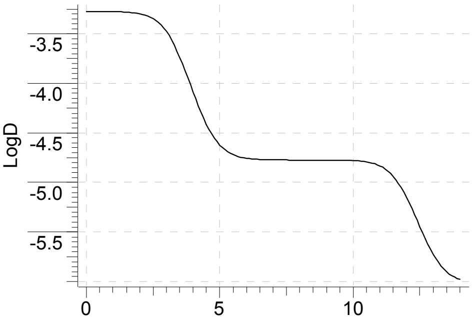
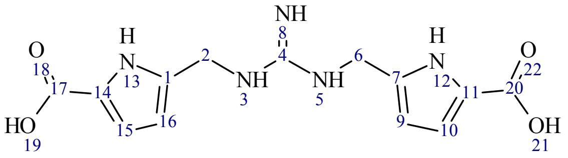


Figure S-40: Simulation of the properties of compound **6**



pKa Results

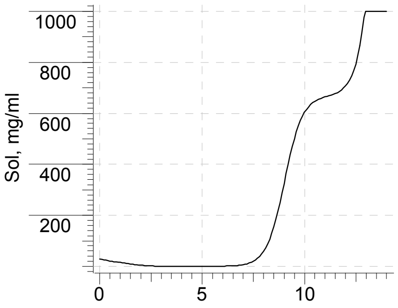
Diss. Atom	Acidic/Basic	Apparent pKa Value	Error
8	MB	20.25	0.7
12	A	17.33	0.5
13	A	16.7	0.5
19	A	4.74	0.1
21	MA	4.14	0.1
12	B	-0.38	0.7
13	B	-0.99	0.7

Single-valued Properties

Name	Value	Error
LogP	1.18	0.85
MW	305.29	-
PSA	154.09	-
FRB	6	-
HDonors	7	-
HAcceptors	9	-
Rule Of 5	1	-
Molar Refractivity, cm^3	74.9	0.5
Molar Volume, cm^3	189.65	7
Parachor, cm^3	555.57	8
Index of Refraction	1.72	4.67e-2
Surface Tension, dyne/cm	73.65	7
Density, g/cm^3	1.61	0.14
Polarizability, 10e-24 cm^3	29.69	0.5
Boiling Point, °C	679.87	65
C ratio	0.59	-
N ratio	0.23	-
NO ratio	0.41	-
Hetero ratio	0.41	-
Halogen ratio	0	-
Num Rings	2	-
Num Aromatic Rings	2	-
Num Rings 3	0	-
Num Rings 4	0	-
Num Rings 5	2	-
Num Rings 6	0	-

Solubility Results

pH	Sol, mg/ml	Flags	%
2	3.97	B	99
5.5	0.24	AZ	85 / 15
6.5	2.04	A	98
7.4	15.62	A	100
10	603.84	A	100



LogD Results

pH	LogD
2	-0.83
5.5	-1.92
6.5	-2.26
7.4	-2.31
10	-2.32

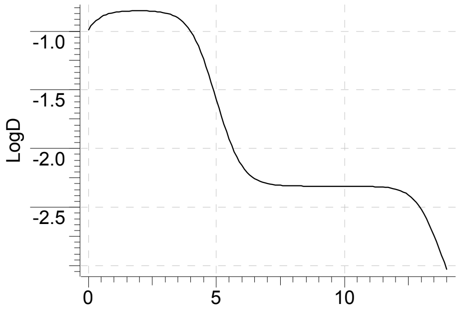
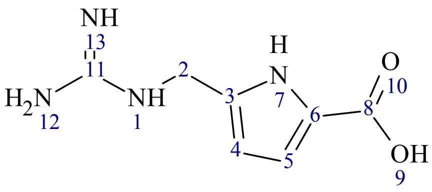


Figure S-41: Simulation of the properties of compound **24**



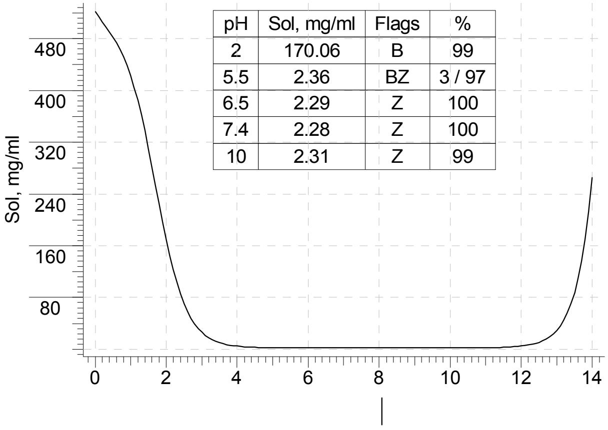
pKa Results

Diss. Atom	Acidic/Basic	Apparent pKa Value	Error
7	A	17.62	0.5
13	MB	13.35	0.7
9	MA	4.41	0.1
7	B	-0.7	0.7
12	B	-2.94	0.7

Single-valued Properties

Name	Value	Error
LogP	7.23e-2	0.56
MW	182.18	-
PSA	114.99	-
FRB	3	-
HDonors	6	-
HAcceptors	6	-
Rule Of 5	1	-
Molar Refractivity, cm^3	43.89	0.5
Molar Volume, cm^3	114.28	7
Parachor, cm^3	335	8
Index of Refraction	1.69	4.67e-2
Surface Tension, dyne/cm	73.84	7
Density, g/cm^3	1.59	0.14
Polarizability, 10e-24 cm^3	17.4	0.5
Boiling Point, °C	460.9	55
C ratio	0.54	-
N ratio	0.31	-
NO ratio	0.46	-
Hetero ratio	0.46	-
Halogen ratio	0	-
Num Rings	1	-
Num Aromatic Rings	1	-
Num Rings 3	0	-
Num Rings 4	0	-
Num Rings 5	1	-
Num Rings 6	0	-

Solubility Results



pH	Sol, mg/ml	Flags	%
2	170.06	B	99
5.5	2.36	BZ	3 / 97
6.5	2.29	Z	100
7.4	2.28	Z	100
10	2.31	Z	99

LogD Results

pH	LogD
2	-1.93
5.5	-2.4
6.5	-2.42
7.4	-2.43
10	-2.43

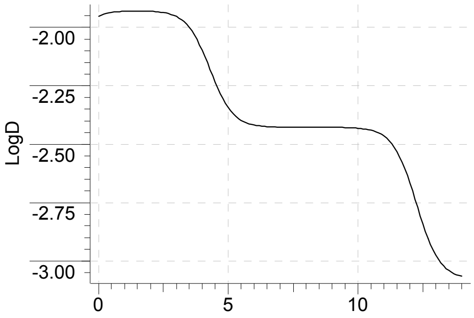


Figure S-42: Simulation of the properties of a fragment of compound **24**

4. Photophysical properties of glycol-δ-amino acids

In methanol compounds **7g** or **7h** have an absorption maximum at 260 nm, the indole substituted compounds (**7i)** at 210 nm and 280. Compounds **7g** or **17h** (λ_{em} = 280 nm; ϕ = 0.05[10]) and **7i** (λ_{em} = 350 nm; ϕ = 0.15[10]) show emission spectra very similar to the amino acids phenylalanine[11] and tryptophan[11,12] The absorption and emission properties are only marginally affected by the nature of the protection group.

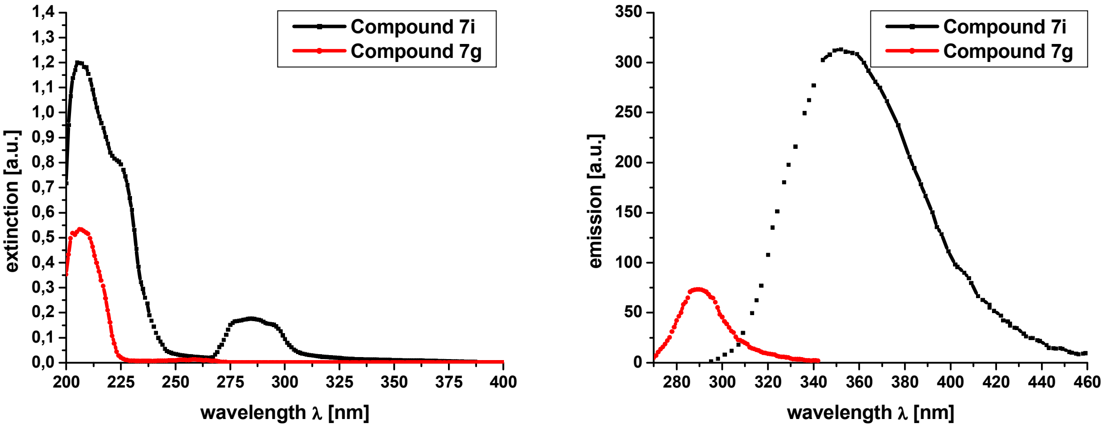


Figure S-43: Absorption spectra of compounds **7g**, **7i** (c = 8.1*10^{-5} and 8.4*10^{-5} mol/L) and emission spectra of compounds **7g**, **7i** (c = 4.1*10^{-5} and 4.0*10^{-5} mol/L)

The imidazo[1,5-a]pyridine derivative **21** shows absorption maxima in methanol at 230 nm, 280 nm and 380 nm and emits upon excitation at 510 nm with a quantum yield of about ϕ = 0.15.[10]

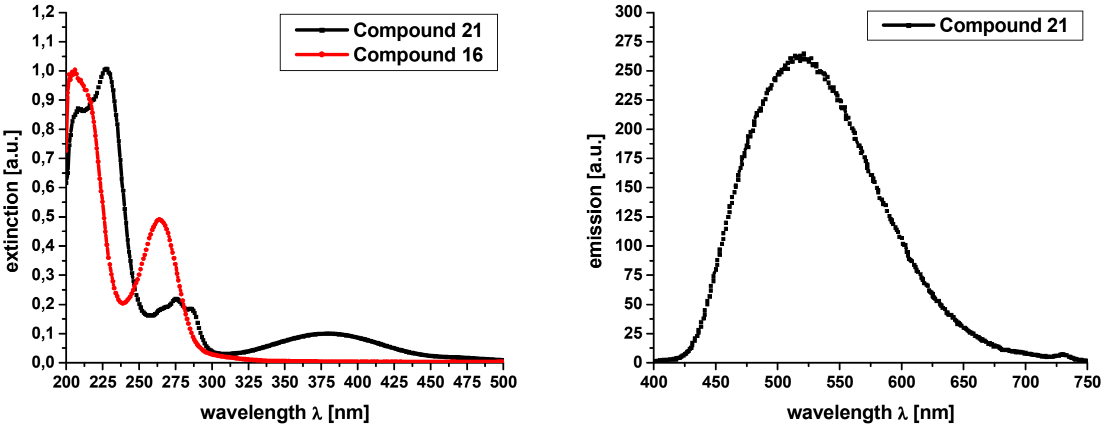


Figure S-44: Absorption spectra of compounds **21** and **16** in comparison (c = 8.3*10^{-5} and 8.1*10^{-5} mol/L) and the emission spectrum of compound **21** (c = 2.2*10^{-5} mol/L)

5. Sample preparation, Fluorescence and UV-vis titrations

Sample preparation. The exact solutions have been prepared by adding to a weighed amount of receptor or substrate, the calculated amount of solvent necessary by means of microliter precision pipettes. This method is more accurate than to determine exactly the weighed amount (to 10^{-5} g) and admit a certain volume, since the volume measurement has a higher accuracy than the weight determination.

If a solvent mixture 20 % water in methanol was used, the receptor was first dissolved in the methanol content of the solvent mixture, then the water content was added lacking 100 µl, to have room to adjust the pH. For pH adjusting 0.1 M LiOH or hydrochloric acid of pro analysi quality in high-purity water were used. No buffer or salt was added. Thereafter, the water difference is filled up to the full volume.

Receptor solutions were stored properly stoppered and closed with parafilm at maximum for one week in the refrigerator at -4 °C. They were freshly prepared every week from deep frozen aliquots of the receptor material. Amino acid solutions were kept at -4 °C properly stoppered and closed with parafilm for maximal one week. Peptide solutions were always freshly prepared from the properly dried and carefully stored material (nitrogen, -20 °C).

Absorption spectroscopy. Absorption spectra were recorded on a Varian Cary BIO 50 UV/VIS/NIR Spectrometer with temperature control by use of a 1 cm quartz cuvettes (Hellma) and Uvasol solvents or of quality for spectrophotometrical investigations (Merck, Baker or Acros).

Absorbtion-titrations:

Solvent:	MeOH or MeOH / H_2O 4:1 (vol/vol), pH 6.5 - 7
Starting volume:	2.0 mL
Concentration [**24**]:	0.03 mM
Concentration [guest]:	20.0 mM

(Amino acids in 80 % aqueous methanol (pH adjusted, no salt added), n-butylammonium chloride and tetrabutylammonium acetate in 80% aqueous methanol (pH adjusted, no salt added), DMSO, MeOH)

Procedure. The cuvettes have a thickness of 1 cm and were filled with 2 ml solution. As blanc sample the pure solvent mixture, 20 % water in methanol, was used. The cell was temperated to 25 °C. The additions were performed using precision microliter pipettes. After each addition the solution was given three minutes to reach the new equilibrium before the measurement was started. From each titration step UV spectrum in the wavelength range from 250 to 400 nm was recorded. Prior to the acquisition of spectra a baseline correction was performed. At the end of a titration, the pH was checked to exclude protonation reactions.

For the evaluation of the validity of the Lambert-Beer's a measurement of dilution series was conducted. For this a receptor and substrate solution was titrated with aliquots of the solvent (pH-adjusted value) under identical experimental conditions.

Emission spectroscopy. Fluorescence measurements were performed with UV-grade solvents (Merck, Baker or Acros) in 1 cm quartz cuvettes (Hellma) and recorded on a Varian 'Cary Eclipse' fluorescence spectrophotometer with temperature control. To determine the binding constants and Job´s plots fluorescence titration experiments were carried out.

Emission-titrations:

Solvent:	MeOH or MeOH / H_2O 4:1 (vol/vol), pH 6.5 - 7
Starting volume:	1.5 mL
Concentration [**24**]:	0.03 mM
Concentration [guest]:	20.0 mM

(Amino acids in 90 % aqueous methanol (pH adjusted, no salt added), n-butylammonium chloride and tetrabutylammonium acetate in 90 % aqueous methanol (pH adjusted, no salt added), DMSO, MeOH)

Instrument Parameters.

Excitation wavelength:	$\lambda_{ex} = 270$ nm
Detection wavelength:	$\lambda = 290 – 510$ nm
Temperature:	T = 298 K
PMT voltage	700 volts

Procedure: To a cuvette with 1.5 mL of the receptor in 80 % aqueous methanol (pH adjusted, no salt added), DMSO or MeOH were added 10 µL (≙ 1 eq.) aliquots of the amino acid or peptide solution (pH adjusted) or it was titrated stepwise with small amounts 5 – 100 µL, depending on the initial emission response, of the substrate solution. After each addition the solution was allowed to equilibrate for 5 min before the fluorescence intensity was recorded. The stoichiometry was determined by Job´s plot analysis extracted from titration data.[13]

To determine the binding constant the obtained fluorescence intensities were volume corrected, plotted against the concentration of peptide and evaluated by non linear fitting methods.

The pH value of aqueous methanol solvent was checked before and after every titration with the aid of a suitable pH electrode. Measurements with the pH differing more than one units before and after the titrations were not used.

5.1. Titrations with tetrabutylammonium acetate

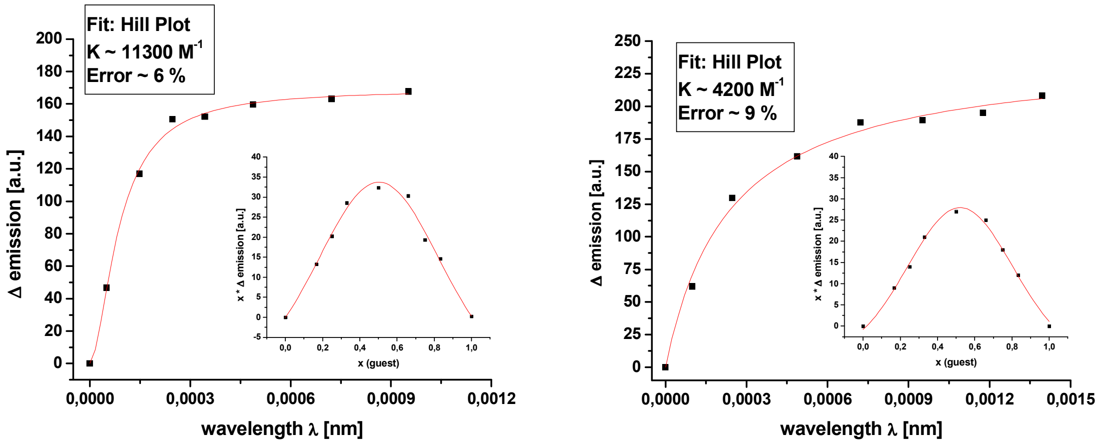


Figure S-46: Emission titration of compound **23** with tetrabutylammonium acetate in methanol (left) and methanol / water 4:1 (right); Inserts: Job´s Plots

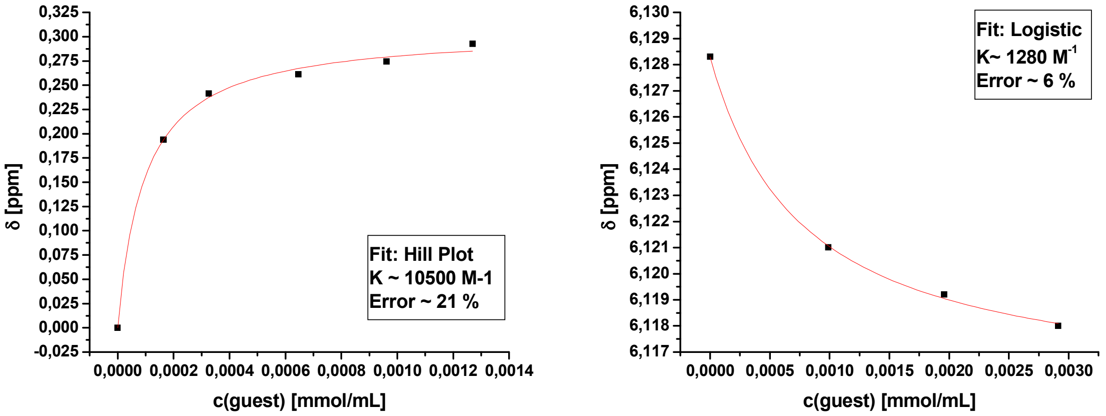


Figure S-47: ^{1}H-NMR titration of compound **22b** (left, guanidinium proton traced) and **23** (right, pyrrole proton in 4-position traced) with tetrabutylammonium acetate in DMSO

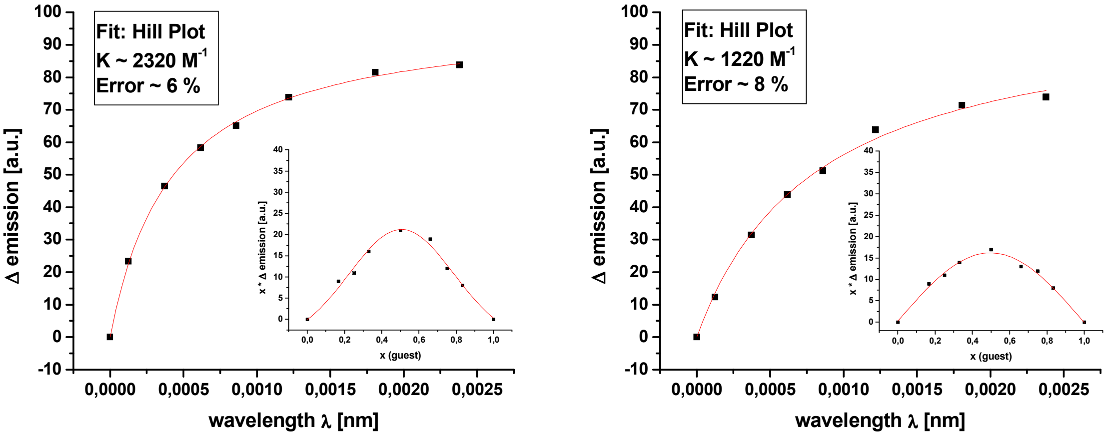


Figure S-48: Emission titration of compound **24** with tetrabutylammonium acetate in methanol (left) and methanol / water 4:1 (right); Inserts: Job´s Plots

5.2. Titrations with amino acids

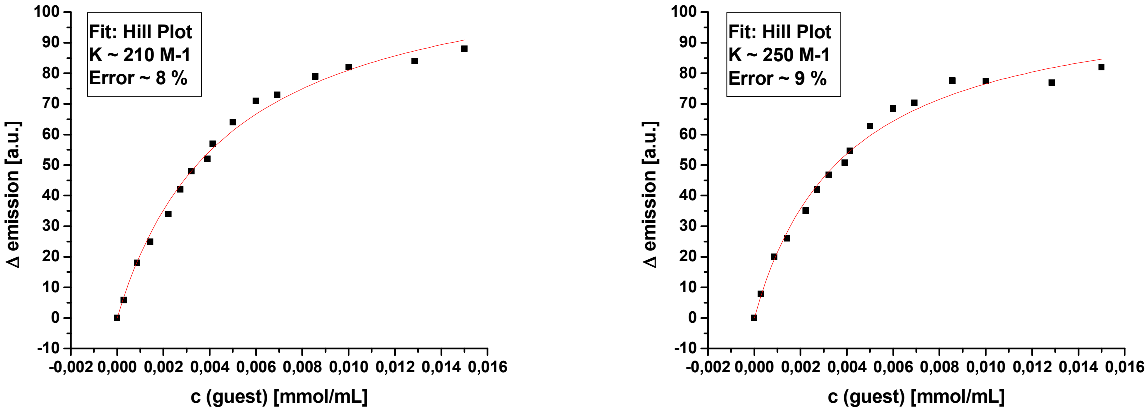


Figure S-49: Emission titration of compound **24** with glycine (left) and β-alanine (right) in Methanol / water 4:1

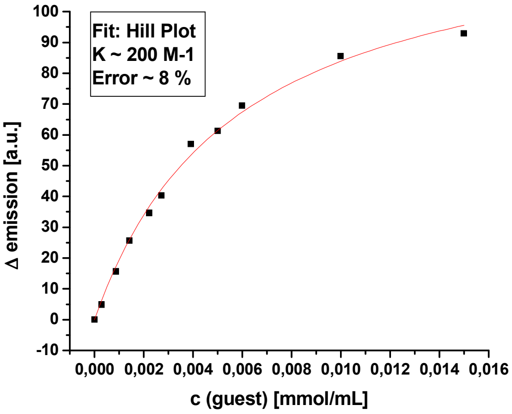


Figure S-50: Emission titration of compound **24** with γ-aminobutyric acid in methanol / water 4:1

6. References and Notes

[1] This amount of reactant proved to be very suitable: Depending on the amino acid employed, the solution gets very thick and viscous during the reaction; the larger the residue the more viscosity. These concentrations allowed good stirring and a smooth reaction.

[2] T.H. Fife, T.J. Przystas, *J. Am. Chem. Soc*. **1982**, *104,* 2251-2257.

[3] R. Fornasier, D. Milani, P. Scrimin, U. Tonellato, *J. Chem. Soc. Perkins Trans II*, **1986,** 233-237.

[4] Instead of EDC, mercury(II)chloride can be used in this reaction. DCM has to be replaced by DMF then. For example: The benzyloxycarbonylthiourea (0.5 mmol) was added to a solution of its according amine compound (0.6 mmol) and triethylamine (130 mg, 0.17 mL, 1.3 mmol) in DMF (5 mL). Mercury(II)-chloride (170 mg, 0.62 mmol) was added and the reaction mixture was stirred for 15 h at room temperature. The mixture was filtered over Celite and the solvent was removed under reduced pressure. The crude product was dissolved in H_2O and acidified with 5 % $KHSO_4$ to pH = 3. The aqueous layer was extracted three times with EtOAc. The combined organic phases were dried over $MgSO_4$ and the solvent removed under reduced pressure. The crude product was purified by column chromatography.

[5] N.J.A. Martin, L. Ozores, B. List, *J. Am. Chem. Soc.* **2007**, *129*, 8976-8977.

[6] S. Bartoli, K.B. Jensen, J.D. Kilburn*, J. Org. Chem.* **2003**, *68*, 9416-9422

[7] D. Srinivasa Reddy, D. Vander Velde, J. Aube, *J. Org. Chem.* **2004**, *69*, 1716–1719.

[8] Similar prescriptions for other interesting thioureas can be found in: D. Mansuy, M. Fontecave, J. F. Bartoli*, J. Am. Chem. Soc*., *Chem. Commun.* **2003**, *68(24*), 9419.

[9] This acid is extremely strong and, therefore, completely dissociated under the present concentration conditions.

[10] All quantum yields were determined with quinine disulfate in 1 N H_2SO_4 as the reference compound (Φ = 0.546).

[11] G.D. Fasnan, Handbook of biochemistry and molecular biology, Proteins I, CRC press, **1976**, 3rd ed., 183-203.; D.F. Eaton, *Pure Appl. Chem.* **1988**, *60*, 1107-1114.

[12] E.P. Kirby, R.F. Steiner, *J. Phys. Chem.* **1970**, *74*, 4480-4490.

[13] P. MacCarthy *Anal. Chem.* **1978**, *50*, 2165.; C. Schmuck, P. Wich, *Angew. Chem. Int. Ed.* **2006**, *45*, 4277-4281.