Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Supporting Information

Heteroatom-substituted tetra(3,4-pyrido)porphyrazines: A stride toward near-infrared-absorbing macrocycles

Lenka Vachova,[a] Miloslav Machacek,[b] Radim Kučera,[a] Jiri Demuth,[c] Pavel Cermak,[c] Kamil Kopecky,[a] Miroslav Miletin,[a] Adela Jedlickova,[b] Tomas Simunek,[b] Veronika Novakova*[c] and Petr Zimcik*[a]

[a] Department of Pharmaceutical Chemistry and Drug Control, Faculty of Pharmacy in Hradec Kralove, Charles University in Prague, Heyrovskeho 1203, 500 05, Hradec Kralove, Czech Republic, E-mail: petr.zimcik@faf.cuni.cz; Fax: +420 495067167; Tel: +420 495067257

[b] Department of Biochemical Sciences, Faculty of Pharmacy in Hradec Kralove, Charles University in Prague, Heyrovskeho 1203, 500 05, Hradec Kralove, Czech Republic.

[c] Department of Biophysics and Physical Chemistry, Faculty of Pharmacy in Hradec Kralove, Charles University in Prague, Heyrovskeho 1203, 500 05, Hradec Kralove, Czech Republic. E-mail: veronika.novakova@faf.cuni.cz; Fax: +420 495067167; Tel: +420 495067380

Content

Experimental

General

All organic solvents used were of analytical grade. Anhydrous butanol for synthesis was stored over magnesium and distilled prior to use. Tetrahydrofuran for photophysical and photochemical measurements was distilled prior to use. All chemicals for synthesis were obtained from established suppliers (Aldrich, Acros, Merck) and used as received. Zinc phthalocyanine (ZnPc) was purchased from Sigma-Aldrich. TLC was performed on Merck aluminum sheets coated with silica gel 60 F254. Merck Kieselgel 60 (0.040−0.063 mm) was used for column chromatography. Melting points were measured on an Electrothermal IA9200-series digital melting-point apparatus (Electrothermal Engineeering, Southend-on-Sea, Essex, Great Britain). The infrared spectra were measured on a Nicolet 6700 spectrometer in ATR mode. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Mercury Vx BB 300 NMR or VNMR S500 NMR spectrometer. The reported chemical shifts are given relative to $Si(CH_3)_4$ and were locked to the signal of the solvent. Elemental analyses were performed on an Automatic Microanalyser EA1110CE (Fisons Instruments, Milan, Italy). The UV−vis spectra were recorded using a Shimadzu UV-2401PC and UV-2600 spectrophotometers (Shimadzu Europa, GmbH, Duisburg, Germany). The steady-state fluorescence spectra were measured using an AMINCO-Bowman Series 2 luminescence spectrometer (SLM-Aminco, Urbana, Illinois, USA). MALDI-TOF mass spectra were recorded in a positive reflectron mode on a Voyager-DE STR mass spectrometer (Applied Biosystems, Framingham, Massachusetts, USA) in *trans*-2-[3-(4-*tert*butylphenyl)-2-methyl-2-propenylidene]-malononitrile as a matrix. The instrument was calibrated externally with a five-point calibration using a Peptide Calibration Mix1 kit (LaserBio Laboratories, Sophia- Antipolis, France). High resolution mass spectra (HR MS) were measured with the use of UHPLC system Acquity UPLC I-class (Waters, Millford, USA) coupled to high resolution mass spectrometer Synapt G2Si (Waters, Manchester, UK) based on Q-TOF. Chromatography for this HRMS measurement was performed using Acquity UPLC BEH300 C4 (2.1 x 50 mm, 1.7 um) column using isocratic elution with acetonitrile and 10 mM ammonium formate buffer pH 3 (90:10) at flow-rate 0.4 ml/min. Electrospray ionization was operated in positive mode. The ESI spectra were recorded in the range 200 - 2000 m/z using glu-fibrinopeptide B as a lock mass reference and sodium iodide for calibration.

Synthesis of 2-chloro-5,6-dimethylpyridine-3,4-dicarbonitrile (1).

Tetracyanoethylene (1 g, 7.8 mmol) was added to a solution of butan-2-one (675 mg, 9.36 mmol) in 1,4-dioxane (20 mL). Concentrated hydrochloric acid (8 mL) was slowly dropped

into the mixture and the reaction was heated at 65 °C for 2 hours. (Caution! Highly toxic HCN is an elimination product from the reaction!) Subsequently, the mixture was diluted with water (150 mL), the precipitate was collected and the liquid residue was extracted three times with ethyl acetate. The organic layer was dried over anhydrous Na_2SO_4 and the solvent was evaporated. The combined crude product (the precipitate and organic layer from extraction) was purified over the silica with toluene/acetone 20:1 as an eluent (alternatively also chloroform can be used as eluent) and recrystallized from propan-2-ol to yield white needles (763 mg, 51 %). R_f (toluene/acetone 20:1) = 0.49, R_f (hexane/ethyl acetate 5:1) = 0.18, R_f (chloroform) = 0.56, mp 75.7-76.6 °C ; IR (ATR, cm^{-1}): 2236 (CN), 1562, 1540, 1387, 1275, 992, 889 and 806; elemental analysis found (%): C, 56.4; H, 3.3; N, 21.7; $C_9H_6N_3Cl$ requires (%): C, 56.4; H, 3.2; N, 21.9; δ_H ($CDCl_3$, 300 MHz): 2.56 (3H, s, CH_3), 2.66 (3H, s, CH_3); δ_C ($CDCl_3$, 75 MHz): 17.3, 23.7, 109.2, 112.4, 112.7, 125.9, 134.3, 150.1, 164.4. Analytical data corresponded well with those published for this compound.[1]

Synthesis of 2-(*tert*-butylsulfanyl)-5,6-dimethylpyridine-3,4-dicarbonitrile (2)

A solution of 1.77 mL (15.7 mmol) of 2-methylpropane-2-thiol in tetrahydrofurane (30 mL) was stirred at room temperature with 9.12 mL of aq. NaOH (1.0 mol/dm^3) for 30 min. Thereafter, a solution of 600 mg (3.13 mmol) of **1** in 15mL of tetrahydrofurane was added dropwise. The reaction was stirred for another 30 min, evaporated and the solid was washed several times with water. Crude product was purified over the silica with toluene/acetone 20:1 to give **2** as a white solid (707 mg, 92%). R_f (toluene/acetone 20:1) = 0.70, mp 130.1-130.8 °C; IR (ATR, cm^{-1}): 2966, 2929, 2222 (CN), 1552, 1457, 1390, 1362, 1263, 1232, 1163, 1007, 884 and 806; elemental analysis found (%): C, 63.6; H, 6.1; N, 16.9; for $C_{13}H_{15}N_3S$ requires (%): C, 63.6; H, 6.2; N, 17.1; δ_H ($CDCl_3$, 500 MHz): 1.62 (9H, s, *t*Bu), 2.47 (3H, s, CH_3), 2.62 (3H, s, CH_3); δ_C ($CDCl_3$, 125 MHz): 17.0, 23.8, 30.3, 50.4, 106.9, 113.4, 113.5, 124,4, 129.5, 161.9, 162.1.

Synthesis of 2-(2,6-diisopropylphenoxy)-5,6-dimethylpyridine-3,4-dicarbonitrile (3)

Cesium fluoride (2.38 g, 15.7 mmol) was dried at 300 °C under vacuum. After cooling to the room temperature, the flask was filled with argon, and 2,6-diisopropylphenol (1.12 g, 6.26 mmol) in anhydrous DMF (20 mL) was added by a syringe through a septum. Compound **1** (1.00 g, 5.2 mmol) was dissolved in anhydrous DMF (20 mL) under argon atmosphere and added dropwise through the septum to the suspension of cesium fluoride with 2,6-diisoprolyphenol in DMF using a syringe. The solution turned immediately deep purple. The reaction mixture was stirred next 2 hours at room temperature. Then, water (200 mL) was

poured into the mixture, purple precipitate was collected by filtration and washed thoroughly with water. Crude product was purified by column chromatography on silica with hexane/ethyl acetate 5:1 as an eluent and recrystallized from hexane. Yield 1.19 g (68 %) of shiny white crystals. R_f (hexane/ethyl acetate 5:1) = 0.44, mp 152.7-153.8 °C; IR (ATR, cm^{-1}): 2968, 2869, 2232 (CN), 1568, 1408, 1332, 1244, 1169, 1120 and 765; elemental analysis found (%): C, 75.8; H, 7.4; N, 12.7; for $C_{21}H_{23}N_3O$ requires (%): C, 75.7; H, 7.0; N, 12.6; δ_H ($CDCl_3$, 300 MHz): 1.15 (12H, br s, CHC$\underline{H}_3$), 2.38 (3H, s, CH_3), 2.47 (3H, s, CH_3), 2.82 (2H, sep, *J*= 6.9 Hz, CH), 7.18 (1H, d, *J*=1.9 Hz, ArH), 7.24-7.31 (2H, m, ArH); δ_C ($CDCl_3$, 75 MHz): 16.8, 22.7, 23.4, 23.7, 27.6, 94.8, 112.6, 113.5, 124.1, 126.3, 126.7, 128.6, 140.8, 146.8, 161.7, 163.1.

Synthesis of 2-(diethylamino)-5,6-dimethylpyridine-3,4-dicarbonitrile (4)

Diethylamine (2.3 g, 31 mmol) was added dropwise to solution of **1** (850 mg, 4.4 mmol) in tetrahydrofuran (30 mL) and the mixture was refluxed for 30 h. At the end of the reaction the mixture was cooled and precipitated diethylamine hydrochloride was filtered off. The solution was evaporated under reduced pressure and purified by column chromatography on the silica with chloroform. Pure fractions were collected and finally recrystallized from MeOH to give **4** (962 mg, 95%) as yellow solid needles. R_f (hexane/ethyl acetate 5:1) = 0.42, mp 66.2-67.0 °C; IR (ATR, cm^{-1}): 2988, 2941, 2201 (CN), 1565, 1541, 1497, 1471, 1457, 1434, 1376, 1358, 1260, 1171, 810, 758 and 689; δ_H ($CDCl_3$, 500 MHz): 1.25 (6H, t, *J*=7.0 Hz, $CH_2C\underline{H}_3$), 2.35 (3H, s, CH_3), 2.43 (3H, s, CH_3), 3.69 (4H, q, *J*=7.0 Hz, CH_2); δ_C ($CDCl_3$, 125 MHz): 13.4, 16.3, 23.9, 44.2, 86.5, 114.8, 117.0, 122.3, 126.6, 155.5, 162.2.

Synthesis of 2-{[2-(diethylamino)ethyl]sulfanyl}-5,6-dimethylpyridine-3,4-dicarbonitrile (5).

Finelly ground anhydr. K_2CO_3 (4.34 g, 31.4 mmol) was added to solution of **1** (1.50 g, 7.85 mmol) and diethylaminoethanethiol hydrochloride (1.60 g, 9.42 mmol) in DMSO (50 mL) and the mixture was stirred at rt for 1 h. The deep purple solution turned slowly to brown and dark yellow within 10-15 min. The reaction was diluted with water (300 mL) and yellow precipitate was collected and thoroughly washed with water. The crude product was dissolved in water with the use of HCl (to acid pH of the solution) and washed three times with chloroform. The water phase was made basic with the use of KOH, extracted with chloroform three times and the organic phase was dried over sodium sulfate. Evaporation of the solvent gave pure **5** as orange solid (1.27 g, 56%). Another portion of slightly impure **5** can be obtained after evaporation of the first chloroform extract and conversion to free base with KOH (773 mg,

34%). mp 65.7-67.4 °C; IR (ATR, cm^{-1}): 2973, 2936, 2873, 2803, 2220 (CN), 1556, 1438, 1389, 1263; δ_H (CD_3COCD_3, 500 MHz): 1.01 (6H, t, J = 7.1 Hz, $CH_2C\underline{H}_3$), 2.50 (3H, s, CH_3), 2.57 (4H, q, J = 7.1 Hz, $NC\underline{H}_2CH_3$), 2.66 (3H, s, CH_3), 2.79-2.69 (2H, m, $NC\underline{H}_2CH_2$), 3.44-3.33 (2H, m, SCH_2); δ_C (CD_3COCD_3, 125 MHz): 12.7, 17.1, 23.9, 29.1, 47.7, 52.9, 106.1, 114.3, 114.5, 125.0, 131.2, 161.2, 164.2; HRMS *m/z*: found: $[M+H]^+$ 289.1493; $C_{15}H_{21}N_4S$ requires $[M+H]^+$ 289.1481.

General procedure for synthesis of tetra(3,4-pyrido)porphyrazines 6Mg and 7Mg

Magnesium (7 equiv) and a small crystal of iodine were heated to reflux for 2.5 h in anhydrous butanol. Precursor **2** or **3** (1 eq.) was added and reflux continued for the next 20 h. The reaction mixture was left to cool down in the air, the solvent was evaporated under reduced pressure, aqueous acetic acid (50% (v/v)) was added and the suspension was stirred for 30 min at rt. The dark solid was filtered, washed with aqueous acetic acid and water and air-dried. The reaction mixture was purified by column chromatography on the silica (eluents are mentioned below) to obtain magnesium complexes **6Mg** and **7Mg** as green solids.

1,8,15,22-Tetrakis(tert-butylsulfanyl)-3,4,10,11,17,18,24,25-octamethyltetrapyrido[3,4-b:3',4'-g:3'',4''-l:3''',4'''-q]porphyrazinato magnesium (II) (6Mg). Starting amount of **2** (500 mg, 2.0 mmol), eluent: toluene/chloroform/tetrahydrofuran 10:1:0.5, yield: 125 mg (25%) of a green solid, R_f (toluene/chloroform/tetrahydrofuran 10:1:0.5) = 0.48; HPLC (t_R, min): 21.9 (61.5 %), 22.77 (38.5 %); IR (ATR, cm^{-1}): 2957, 2913, 1547, 1264, 1182, 1106, 1018, 982, 913, 791 and 760; δ_H ($CDCl_3$/pyridine-d_5, 300 MHz): 2.09-2.25 (36H, several s, *t*Bu), 2.98-3.19 (12H, several s, CH_3), 3.58-4.20 (12H, several s, CH_3); δ_C ($CDCl_3$/pyridine-d_5, 75 MHz): 156.82, 156.76, 156.15, 155.95, 154.70, 154.63, 154.59, 154.53, 154.14, 153.97, 153.76, 153.71, 153.63, 153.33, 153.11, 152.88, 152.60, 151.98, 151.62, 143.80, 143.76, 143.66, 143.41, 142.76, 128.72, 128.65, 128.24, 128.18, 127.89, 124.15, 124.07, 48.65, 47.88, 47.81, 31.56, 31.06, 31.03, 23.26, 23.20, 23.04, 17.05, 16.86, 16.72, 16.08, 15.92; MS (MALDI-TOF) *m/z*: 1004.4 $[M]^+$, 948.3 $[M\text{-}C_4H_8]^+$, 892.3 $[M\text{-}2\times C_4H_8]^+$; HRMS *m/z*: found: $[M+H]^+$ 1005.3864; $C_{52}H_{61}MgN_{12}S_4$ requires $[M+H]^+$ 1005.3875; UV-Vis λ_{max}/THF; nm (ε/THF; $dm^3mol^{-1}cm^{-1}$): 728 (225 200), 653 (49 700), 465 (12 200), 365 (61 300).

1,8,15,22-Tetrakis(2,6-diisopropylphenoxy)-3,4,10,11,17,18,24,25-octamethyltetrapyrido[3,4-b:3',4'-g:3'',4''-l:3''',4'''-q]porphyrazinato magnesium (II) (7Mg). Starting amount of **3** (1 g, 3.0 mmol), eluent: toluene/chloroform/tetrahydrofuran 10:1:0.2, yield: 195 mg (19%) of a deep green solid, R_f (toluene/chloroform/tetrahydrofuran 10:1:0.5) = 0.48; HPLC (t_R, min): 28.8 (1 %), 29.2 (1.5 %), 30.0 (30.5 %), 30.5 (67 %); IR

(ATR, cm^{-1}): 2965, 2930, 2868, 1734, 1596, 1417, 1322, 1263, 1170, 1096, 1061, 1003, 930, 796 and 766; δ_H ($CDCl_3$/pyridine-d_5, 300 MHz): 0.90-1.62 (m, CHC<u>H</u>$_3$), 2.31-2.44 (m), 2.70 (s, CH_3), 2.76 (s, CH_3), 2.82 (s, CH_3), 2.87 (s, CH_3), 3.27-3.47 (m, CH), 3.60-3.79 (m, CH), 3.82 (s, CH_3), 3.84 (s, CH_3), 3.87 (s, CH_3), 3.89 (s, CH_3), 7.22-7.40 (m, ArH), 7.45-7.57 (m, ArH), number of protons cannot be assigned due to complex mixture of isomers, for details see graphical spectra; δ_C ($CDCl_3$/pyridine-d_5, 75 MHz): 158.50, 158.41, 157.78, 157.54, 157.29, 156.32, 154.48, 153.33, 151.83, 146.88, 146.72, 146.58, 142.11, 142.05, 141.69, 141.64, 125.83, 125.50, 124.09, 123.80, 117.66, 117.28, 29.91, 27.79, 27.71, 24.30, 23.46, 23.35, 23.23, 22.89, 22.73, 15.77, 15.71, 14.19, 14.14; MS (MALDI-TOF) *m/z*: 1356.6 $[M]^+$; HRMS *m/z*: found: $[M+H]^+$ 1357.7270; $C_{84}H_{93}MgN_{12}O_4$ requires $[M+H]^+$ 1357.7290; UV-Vis λ_{max}/THF; nm (ε/THF; $dm^3mol^{-1}cm^{-1}$): 712 (192 500), 639 (43 700), 389 (41 700), 346 (38 100). Despite the complexity of the spectra, the predominant presence of C_s isomers in the mixture is supported by appearance of four signals of roughly same intensity (besides other smaller signals) for both methyls on pyridine ring (see insets of Figure S14). C_s isomer is the only one where four signals of each of the methyls can be expected.

General procedure for synthesis of metal-free tetra(3,4-pyrido)porphyrazines 6H and 7H

The magnesium complex (**6Mg** or **7Mg**, 1 eq.) was dissolved in chloroform and stirred at rt for 1 h with *p*-toluenesulfonic acid (10 eq.). Thereafter, the solvent was evaporated, the crude product was washed with water and purified by column chromatography with hexane/ethyl acetate 20:1 as an eluent to obtain a dark green solid **6H** or **7H**.

1,8,15,22-Tetrakis(tert-butylsulfanyl)-3,4,10,11,17,18,24,25-octamethyltetrapyrido[3,4-b:3',4'-g:3'',4''-l:3''',4'''-q]porphyrazine (6H). Starting amount of **6Mg** (100 mg, 0.1 mmol), yield: 39 mg (41%), R_f (hexane/ethyl acetate 20:1) = 0.55; HPLC was not performed due to low solubility; IR (ATR, cm^{-1}): 3296 (NH), 2922, 2853, 1739, 1575, 1457, 1262, 1171, 1030, 973 and 761; δ_H ($CDCl_3$/pyridine-d_5, 300 MHz): 1.77 (9H, s, *t*Bu), 1.93-2.32 (27H, several s, *t*Bu), 2.52-4.11 (24H, several s, CH_3); δ_C ($CDCl_3$/pyridine-d_5, 75 MHz): no signals were detected; MS (MALDI-TOF) *m/z*: 982.3 $[M]^+$; UV-Vis λ_{max}/THF; nm (ε could not be properly determined due to low solubility): 743, 695, 331.

1,8,15,22-Tetrakis(2,6-diisopropylphenoxy)-3,4,10,11,17,18,24,25-octamethyltetrapyrido[3,4-b:3',4'-g:3'',4''-l:3''',4'''-q]porphyrazine (7H). Starting amount of **7Mg** (41 mg, 0.03 mmol), yield: 22 mg (54%), R_f (hexane/ethyl acetate 20:1) = 0.38; HPLC (t_R, min): 26.9, 28.0; IR (ATR, cm^{-1}): 3257 (NH), 2960, 2925, 2855, 1734, 1594, 1438, 1311, 1270, 1165, 1097. 1080, 991, 913 and 766; δ_H ($CDCl_3$/pyridine-d_5, 500 MHz): 1.82-1.55 (m,

CHC<u>H</u>$_3$), 1.62-1.79 (m), 2.00-2.44 (m), 2.66 (s, CH_3), 2.73 (s, CH_3), 2.78 (s, CH_3), 2.84 (s, CH_3), 3.24-3.38 (m, CH), 3.57-3.72 (m, CH), 3.76 (s, CH_3), 3.78 (s, CH_3), 3.81 (s, CH_3), 7.20-7.32 (m, ArH), 7.42-7.52 (m, ArH), number of protons cannot be assigned due to complex mixture of isomers, for details see graphical spectra; δ_C ($CDCl_3$/pyridine-d_5, 125 MHz): 159.72, 159.49, 158.97, 158.23, 158.03, 157.77, 157.50, 157.34, 148.84, 142.04, 141.95, 141.62, 126.08, 125.55, 124.18, 124.00, 122.39, 122.27, 115.52, 115.10, 37.58, 33.90, 32.94, 32.11, 30.36, 29.90, 29.56, 27.86, 27.83, 27.72, 24.30, 23.42, 23.27, 23.17, 22.99, 22.89, 15.73, 14.40, 14.33, 14.23; MS (MALDI-TOF) *m/z*: 1334.6 $[M]^+$; HRMS *m/z*: found: $[M+H]^+$ 1335.7566; $C_{84}H_{95}N_{12}O_4$ requires $[M+H]^+$ 1335.7599; UV-Vis λ_{max}/THF; nm (ε/THF; $dm^3mol^{-1}cm^{-1}$): 741 (88 700), 714 (82 600), 674 (27 300), 646 (26 000), 325 (32 500).

General procedure for synthesis of zinc tetra(3,4-pyrido)porphyrazines 6Zn and 7Zn

A metal-free TPyPzs **6H** or **7H** (1 eq.) was dissolved in pyridine and anhydrous zinc acetate (7 eq) was added. The mixture was refluxed for 2.5 h. Pyridine was evaporated and the product was washed thoroughly with water to yield a crude TPyPzs **6Zn** or **7Zn** that were purified by column chromatography on the silica (eluents see below).

1,8,15,22-Tetrakis(tert-butylsulfanyl)-3,4,10,11,17,18,24,25-octamethyltetrapyrido[3,4-b:3',4'-g:3'',4''-l:3''',4'''-q]porphyrazinato zinc (II) (6Zn). Starting amounts: **6H** (20 mg, 0.02 mmol), eluent: toluene/chloroform/tetrahydrofuran 10:1:0.5, yield: 19 mg (90%) of a green solid, R_f (toluene/chloroform/tetrahydrofuran 10:1:0.5) = 0.58; HPLC (t_R, min): 24.6, 25.5; IR (ATR, cm^{-1}): 2914, 2853, 1544, 1472, 1456, 1265, 1185, 1104, 1007, 984 and 752; δ_H ($CDCl_3$/pyridine-d_5, 300 MHz): 1.99-2.35 (36H, several s, *t*Bu), 2.85-4.28 (24H, several s, CH_3); δ_C ($CDCl_3$/pyridine-d_5, 75 MHz): 156.73, 156.67, 156.20, 155.78, 154.59, 154.25, 153.71, 153.64, 153.55, 153.42, 153.05, 151.93, 151.13, 151.08, 150.95, 143.08, 142.54, 141.42, 128.87, 128.35, 128.31, 127.68, 126.81, 125.25, 48.90, 48.79, 48.01, 47.97, 47.82, 32.09, 31.69, 31.65, 31.14, 31.06, 23.65, 23.41, 23.37, 23.10, 22.95, 22.87, 17.49, 16.98, 16.76, 16.45, 16.13; MS (MALDI-TOF) *m/z*: 1044.2 $[M]^+$, 988.9 $[M-C_4H_8]^+$; HRMS *m/z*: found: $[M+H]^+$ 1045.3306; $C_{52}H_{61}N_{12}S_4Zn$ requires $[M+H]^+$ 1045.3316; UV-Vis λ_{max}/THF; nm (ε/THF; $dm^3mol^{-1}cm^{-1}$): 730 (154 200), 653 (37 400), 461 (9 800), 359 (45 000).

1,8,15,22-Tetrakis(2,6-diisopropylphenoxy)-3,4,10,11,17,18,24,25-octamethyltetrapyrido[3,4-b:3',4'-g:3'',4''-l:3''',4'''-q]porphyrazinato zinc (II) (7Zn). Starting amounts: **6H** (19.5 mg, 0.015 mmol), eluent: toluene/chloroform/tetrahydrofuran 10:1:0.2, yield: 19.6 mg (96%) of a green solid, R_f (toluene/chloroform/tetrahydrofuran 10:1:0.5) = 0.69; HPLC (t_R, min): 22.0, 22.2; IR (ATR, cm^{-1}): 2960, 2924, 2854, 1593, 1577, 1438, 1314, 1272, 1170, 1128, 1096, 1002, 928 and 767; δ_H ($CDCl_3$/pyridine-d_5, 300 MHz):

0.83-1.64 (m, CHC$\underline{H}_3$), 2.00-2.26 (m), 2.72-2.50 (m), 2.67 (s, CH_3), 2.74 (s, CH_3), 2.79 (s, CH_3), 2.85 (s, CH_3), 3.27-3.45 (m, CH), 3.58-3.78 (m, CH), 3.77- 3.99 (m, CH_3), 7.20-7.37 (m, ArH), 7.40-7.57 (m, ArH), number of protons cannot be assigned due to complex mixture of isomers, for details see graphical spectra; δ_C ($CDCl_3$/pyridine-d_5, 75 MHz): 158.25, 157.83, 157.70, 157.58, 157.49, 157.39, 157.20, 157.02, 156.33, 156.27, 156.18, 155.42, 154.80, 154.65, 154.12, 154.01, 153.78, 153.38, 152.71, 151.35, 146.53, 146.24, 142.02, 141.70, 131.07, 125.83, 125.30, 124.03, 122.08, 117.53, 117.45, 117.25, 38.87, 34.10, 32.07, 29.86, 29.51, 27.72, 24.25, 23.52, 23.41, 23.34, 23.07, 22.84, 22.70, 15.71, 14.30, 14.19, 14.10; MS (MALDI-TOF) *m/z*: 1396.5 $[M]^+$; HRMS *m/z*: found: $[M+H]^+$ 1397.6729; $C_{84}H_{93}N_{12}O_4Zn$ requires $[M+H]^+$ 1397.6734; UV-Vis λ_{max}/THF; nm (ε/THF; $dm^3mol^{-1}cm^{-1}$): 712 (125 900), 639 (31 800), 314 (88 000).

1,8,15,22-Tetrakis(diethylamino)-3,4,10,11,17,18,24,25-octamethyltetrapyrido[3,4-b:3',4'-g:3'',4''-l:3''',4'''-q]porphyrazine (8H). Compound **4** (700 mg, 3.1 mmol) was heated to reflux in dry butanol (10 mL) and then lithium metal (149 mg, 21.5 mmol) was added. The mixture was refluxed for 15 h. After this time, the solution was evaporated to dryness, aqueous acetic acid (50% v/v, 50 ml) were poured in and the suspension was collected and washed with aqueous acetic acid and water. The product was purified by column chromatography on silica with toluene/chloroform/tetrahydrofuran 5:1:1 as an eluent. Yield 97 mg (14%) of a purple solid, R_f (toluene/chloroform/tetrahydrofuran 10:1:0.5) = 0.57; HPLC (t_R, min): 26.5 (16 %), 28.5 (71 %), 29.0 (13 %); IR (ATR, cm^{-1}): 3292 (NH), 2968, 2931, 2867, 1560, 1442, 1424, 1254, 1237, 1180, 1060, 1037, 986, 909 and 760; δ_H ($CDCl_3$/pyridine-d_5, 300 MHz): 1.06-1.49 (24H, m, $CH_2C\underline{H}_3$), 3.00 (12H, br s, CH_3), 3.66 (5H, br s, CH_3), 3.78 (7H, br s, CH_3), 4.24-4.52 (16H, m, CH_2, overlapped with signal of residual H_2O); δ_C ($CDCl_3$/pyridine-d_5, 75 MHz): 158.58, 158.43, 157.64, 157.53, 156.03, 155.07, 154.28, 154.02, 46.36, 45.38, 23.48, 14.25, 13.50, 13.10; MS (MALDI-TOF) *m/z*: 914.5 $[M]^+$; HRMS *m/z*: found: $[M+H]^+$ 915.5716; $C_{52}H_{67}N_{16}$ requires $[M+H]^+$ 915.5735; UV-Vis λ_{max}/THF; nm (ε/THF; $dm^3mol^{-1}cm^{-1}$): 806 (85 700), 718sh, 537 (9 600), 339 (38 500).

1,8,15,22-Tetrakis(diethylamino)-3,4,10,11,17,18,24,25-octamethyltetrapyrido[3,4-b:3',4'-g:3'',4''-l:3''',4'''-q]porphyrazinato magnesium (II) (8Mg). Metal-free **8H** (30 mg, 0.033 mmol) was dissolved in pyridine (5mL) and excess of anhydrous magnesium acetate (33 mg, 0.23 mmol) was added. Reaction mixture was heated at reflux for 24 h. After this time, pyridine was removed under reduced pressure and dark solid washed thoroughly with water. A crude product was purified by column chromatography with

toluene/chloroform/tetrahydrofuran 10:1:0.2 as an eluent. Yield 16 mg (53%) of a purplish brown solid. R_f (toluene/chloroform/tetrahydrofuran 10:1:0.5) = 0.29; HPLC ($t_{R,}$ min): 18.4, 18.6, 18.8; IR (ATR, cm^{-1}): 2973, 2933, 2869, 1559, 1444, 1253, 1173, 1083, 1060, 992, 934 and 764; δ_H ($CDCl_3$/pyridine-d_5, 300 MHz): 1.10-1.68 (24H, m, $CH_2C\underline{H}_3$), 2.86-3.27 (12H, m, CH_3), 3.56-3.97 (12H, m, CH_3), 4.13-4.70 (16H, m, CH_2); δ_C ($CDCl_3$/pyridine-d_5, 75 MHz): 47.79, 45.62, 23.20, 13.18, aromatic signals were not detected; MS (MALDI-TOF) *m/z*: 936.4 $[M]^+$, 908.4 $[M\text{-}C_2H_4]^+$; HRMS *m/z*: found: $[M+H]^+$ 937.5400; $C_{52}H_{65}MgN_{16}$ requires $[M+H]^+$ 937.5429; UV-Vis λ_{max}/THF; nm (ε/THF; $dm^3mol^{-1}cm^{-1}$): 771 (94 800), 690 (32 900), 505 (10 400), 367 (48 200).

1,8,15,22-Tetrakis(diethylamino)-3,4,10,11,17,18,24,25-octamethyltetrapyrido[3,4-b:3',4'-g:3'',4''-l:3''',4'''-q]porphyrazinato zinc (II) (8Zn). Metal-free **8H** (30 mg, 0.033 mmol) was dissolved in pyridine (5 mL), anhydrous zinc acetate (42 mg, 0.23 mmol) was added and the reaction mixture was heated at reflux for 4 h. After this time, pyridine was removed under reduced pressure and dark solid washed thoroughly with water. A crude product was purified by column chromatography on silica with toluene/chloroform/tetrahydrofuran 10:1:0.5 as an eluent. Yield 24 mg (76%) of a purple solid. R_f (toluene/chloroform/tetrahydrofuran 10:1:0.5) = 0.41; HPLC ($t_{R,}$ min): 17.7, 18, 20, 21; IR (ATR, cm^{-1}): 2967, 2930, 2867, 1559, 1480, 1442, 1253, 1172, 1124, 1087, 1061, 991, 937 and 755; δ_H ($CDCl_3$/pyridine-d_5, 300 MHz): 1.33 (24H, br s, $CH_2C\underline{H}_3$), 3.01 (12H, br s, CH_3), 3.78 (5H, br s, CH_3), 3.87 (7H, br s, CH_3), 4.22-4.66 (16H, m, CH_2); δ_C ($CDCl_3$/pyridine-d_5, 75 MHz): 156.50, 155.19, 153.98, 46.62, 45.28, 23.35, 15.63, 14.37, 13.47, 12.85; MS (MALDI-TOF) *m/z*:. 976.4 $[M]^+$, HRMS *m/z*: found: $[M+H]^+$ 977.4854; $C_{52}H_{64}N_{16}Zn$ requires $[M+H]^+$ 977.4870; UV-Vis λ_{max}/THF; nm (ε/THF; $dm^3mol^{-1}cm^{-1}$): 772 (94 000), 697 (29 400), 504 (54 000), 359 (37 400).

1,8,15,22-Tetrakis{[2-(diethylamino)ethyl]sulfanyl}-3,4,10,11,17,18,24,25-octamethyltetrapyrido[3,4-b:3',4'-g:3'',4''-l:3''',4'''-q]porphyrazinato magnesium (II) (9Mg).

Magnesium turnings (708 mg, 29.1 mmol) and a small crystal of iodine were refluxed in freshly distilled butanol (30 mL) for 3 h. Compound **5** (1.20 g, 4.16 mmol) was added and the reflux continued for next 4 h. Solvent was evaporated and the green product was carefully extracted from the mixture with tetrahydrofuran. The crude product was adsorbed on silica and washed with MeOH. The silica with product was dried under reduced pressure and the product was purified by column chromatography on silica with pyridine/MeOH 5:1. The pure product was further repurified by precipitation of the concentrated chloroform solution (5 mL) with hexane. Yield 743 mg (61%) of green solid. R_f (pyridine/MeOH 5:1) = 0.5-0.1; IR (ATR, cm^{-1}): 2968,

2930, 2871, 2810, 1553, 1468, 1406, 1330, 1266, 1230, 1184, 1107; δ_H ($CDCl_3$/pyridine-d_5, 300 MHz): 1.27-1.58 (24H, m, $NCH_2C\underline{H}_3$), 2.57-3.93 (56H, m, CH_2+CH_3); δ_C ($CDCl_3$/pyridine-d_5, 75 MHz): 156.70, 155.93, 152.91, 151.97, 142.85, 127.60, 124.51, 124.25, 53.17, 47.75, 47.38, 26.51, 26.28, 23.51, 23.36, 23.27, 16.86, 12.84, 12.76; MS (MALDI-TOF) *m/z*:. 1177.5 $[M+H]^+$, 1104.45 $[M\text{-}NC_4H_{10}]^+$; UV-Vis λ_{max}/THF; nm (ε/THF; $dm^3mol^{-1}cm^{-1}$): 728 (232 000), 654 (50 900), 365 (62 400).

1,8,15,22-Tetrakis{[2-(diethylamino)ethyl]sulfanyl}-3,4,10,11,17,18,24,25-octamethyltetrapyrido[3,4-b:3',4'-g:3'',4''-l:3''',4'''-q]porphyrazine (II) (9H)

Magnesium TPyPz **9Mg** (680 mg, 0.58 mmol) was dissolved in tetrahydrofuran (50 mL) and *p*-toluenesulfonic acid (1.65 g, 8.65 mmol) was added. The mixture was stirred at rt for 15 min and the green precipitate was formed. The precipitate dissolved after addition of MeOH (50 mL) and the reaction was further stirred at rt for next 30 min. The solvents were evaporated under reduced pressure, water was added and the green solution was neutralized by $NaHCO_3$. The green precipitate that was formed was collected and washed thoroughly with water. The product (green solid, 650 mg, 97%) was not further analyzed and was used directly in the next reaction to synthesize **9Zn**.

1,8,15,22-Tetrakis{[2-(diethylamino)ethyl]sulfanyl}-3,4,10,11,17,18,24,25-octamethyltetrapyrido[3,4-b:3',4'-g:3'',4''-l:3''',4'''-q]porphyrazinato zinc (II) (9Zn)

Metal-free **9H** (650 mg, 0.56 mmol) and anhydrous zinc acetate (720 mg, 3.94 mmol) were dissolved in pyridine (20 mL) and refluxed for 1 h. Pyridine was evaporated under reduced pressure, water was added and the green product was extracted three times with chloroform. Part of the product still remained in the water phase, for this reason, the water was acidified with HCl and then again neutralized with $NaHCO_3$. Very fine precipitate was formed and was extracted to chloroform three times. The chloroform extracts were combined, evaporated under reduced pressure and washed with water and acetone. The product was purified by column chromatography on silica with step gradient elution with pyridine/MeOH 20:1 changing to 5:1. The pure product was dissolved in very small amount of chloroform, precipitated by hexane, the fine precipitate was collected and dried. Yield 331 mg (48%) of green solid. R_f (pyridine/MeOH 5:1) = 0.62-0.26; IR (ATR, cm^{-1}): 2969, 2932, 2810, 1555, 1473, 1407, 1330, 1267, 1231, 1108; δ_H ($CDCl_3$/pyridine-d_5, 300 MHz): 1.27-1.59 (24H, m, $NCH_2C\underline{H}_3$), 2.73-3.99 (56H, m, CH_2+CH_3); δ_C ($CDCl_3$/pyridine-d_5, 75 MHz): 52.63, 47.30, 46.99, 26.23, 23.14, 22.99, 12.31, 12.19 (aromatic signals were not detected); MS (MALDI-TOF) *m/z*:. 1217.4

$[M+H]^+$, 1144.4 $[M-NC_4H_{10}]^+$; UV-Vis λ_{max}/THF; nm (ε/THF; $dm^3mol^{-1}cm^{-1}$): 729 (115 600), 658sh, 357 (45 200).

1,8,15,22-Tetrakis{[2-(triethylammonio)ethyl]sulfanyl}-3,4,10,11,17,18,24,25-octamethyltetrapyrido[3,4-b:3',4'-g:3'',4''-l:3''',4'''-q]porphyrazinato zinc (II) tetraiodide (10Zn)

TPyPz **9Zn** (100 mg, 82 μmol) was dissolved in ethyliodide (5 mL) and stirred at rt for 2 days. The green precipitate that was formed was dissolved after addition of *N*-methylpyrrolidinon (5 mL) and the reaction continued at rt for next 4 days. Then, the product was precipitated by addition of diethylether (100 mL), collected and washed with diethylether. The product was purified by repeated (three times) dissolution in MeOH, precipitation by diethylether and collection of the precipitate. The alkylated product **10Zn** was obtained as green solid (72 mg, 48%). Under these conditions, only the aliphatic tertiary nitrogens are alkylated (not the one in pyridine). This fact was confirmed by alkylation of **6Zn** under the same conditions where no reaction proceeded as detected on TLC. IR (ATR, cm^{-1}): 2978, 2943, 1555, 1475, 1404, 1330, 1265, 1229, 1186, 1111; δ_H (DMSO-d_6/pyridine-d_5, 500 MHz): 1.28-1.64 (36H, m, $NCH_2C\underline{H}_3$), 2.91-4.01 (64H, m,CH_2+ CH_3, overlapped with signal of water); δ_C (DMSO-d_6/pyridine-d_5, 125 MHz): 56.74, 53.11, 23.38, 21.49, 17.13, 8.13; elemental analysis found (%): C, 42.81; H, 5.47; N, 11.67; $C_{68}H_{100}I_4N_{16}S_4Zn$ + $3H_2O$ requires (%): C, 43.06; H, 5.63; N, 11.81; UV-Vis λ_{max}/DMF; nm (ε/DMF; $dm^3mol^{-1}cm^{-1}$): 722 (207 500), 649 (41 700), 365 (40 300).

NMR spectra

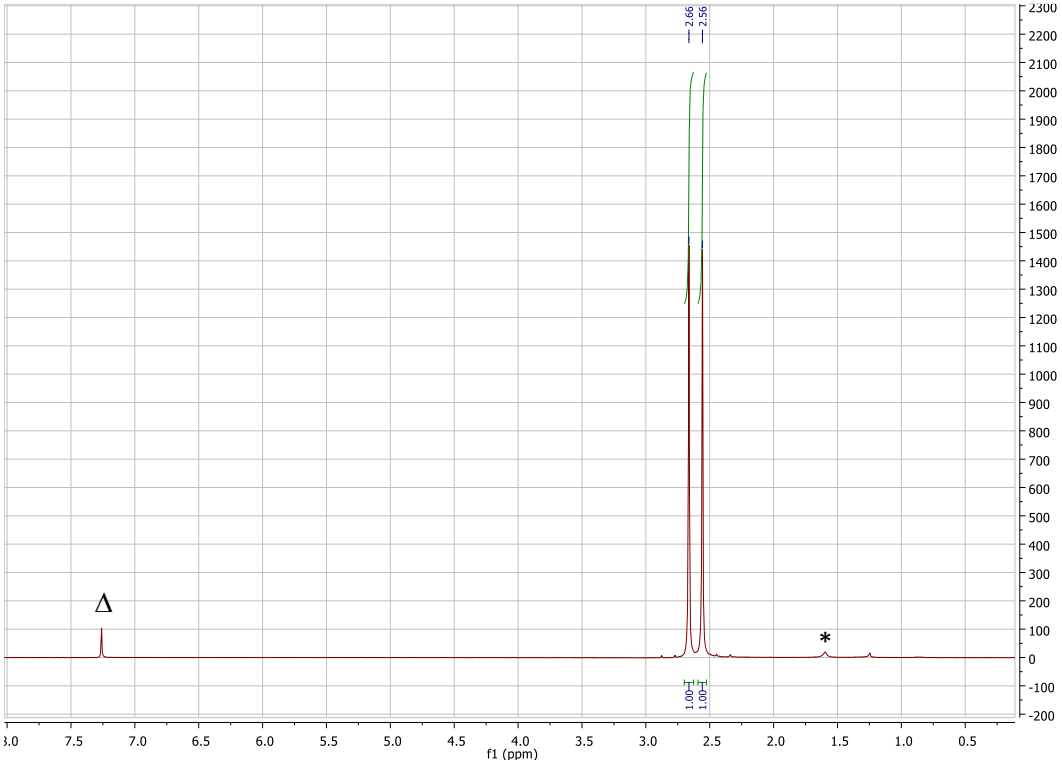

Figure S1. ^{1}H NMR spectrum of **1** in $CDCl_3$. Asterisk = signal of residual water. Triangle = residual signal of non-deuterated solvent.

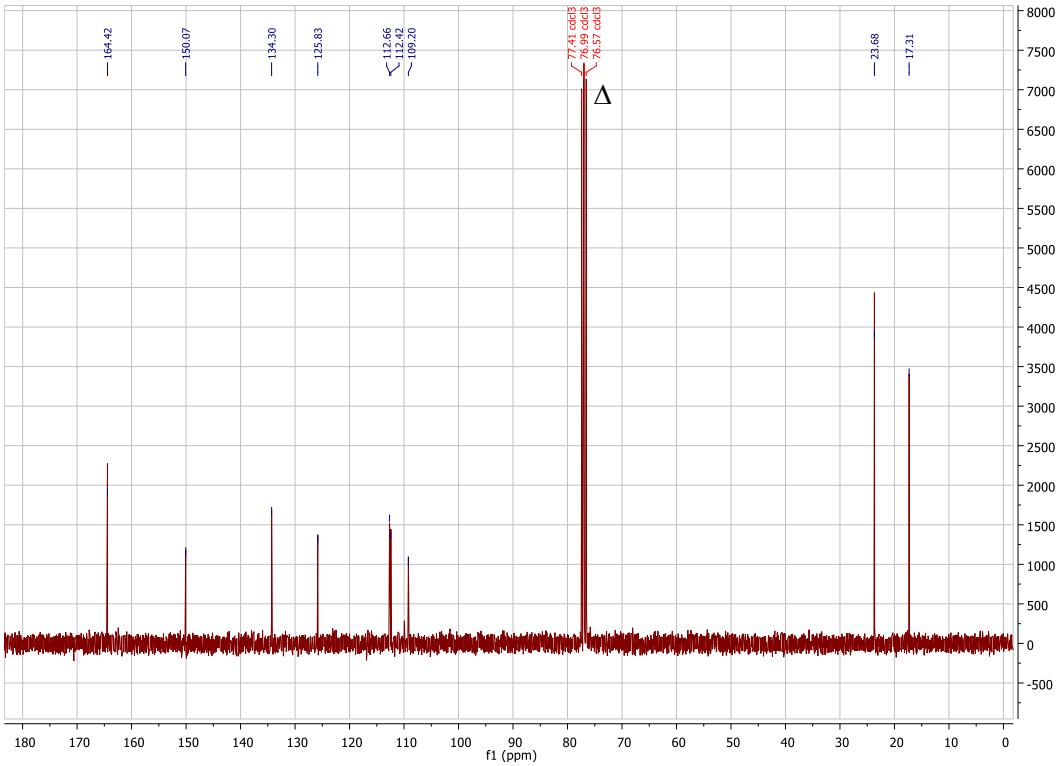

Figure S2. ^{13}C NMR spectrum of **1** in $CDCl_3$. Triangle = signal of solvent.

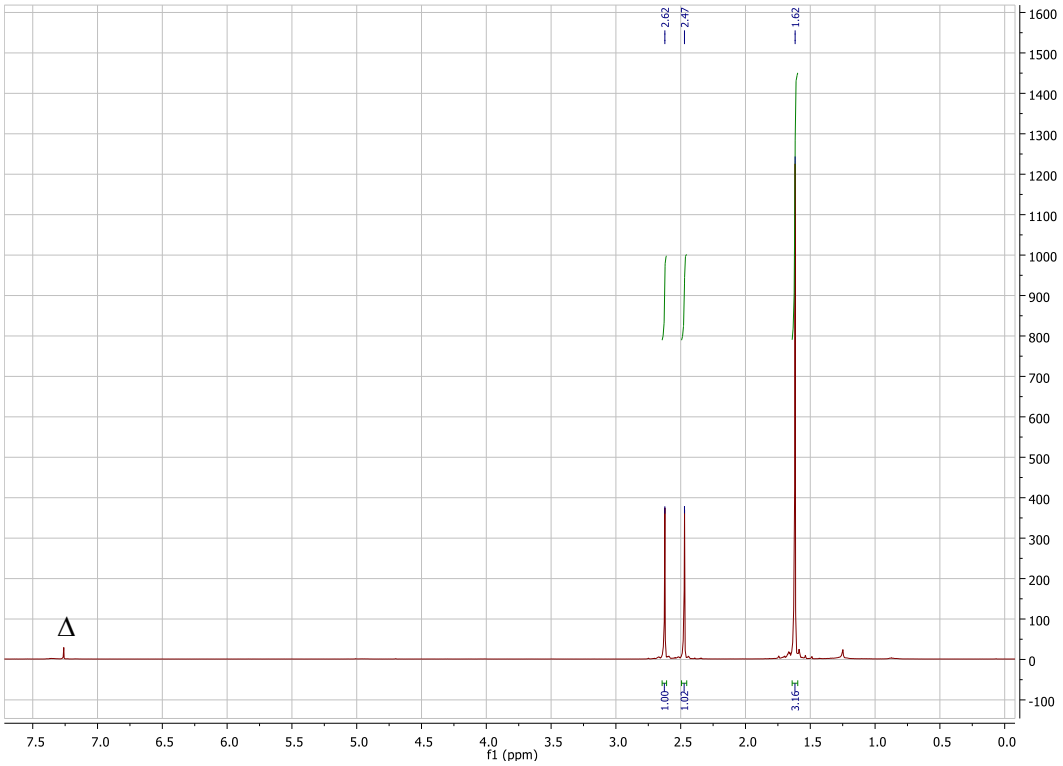

Figure S3. ^{1}H NMR spectrum of **2** in $CDCl_3$. Triangle = residual signal of non-deuterated solvent.

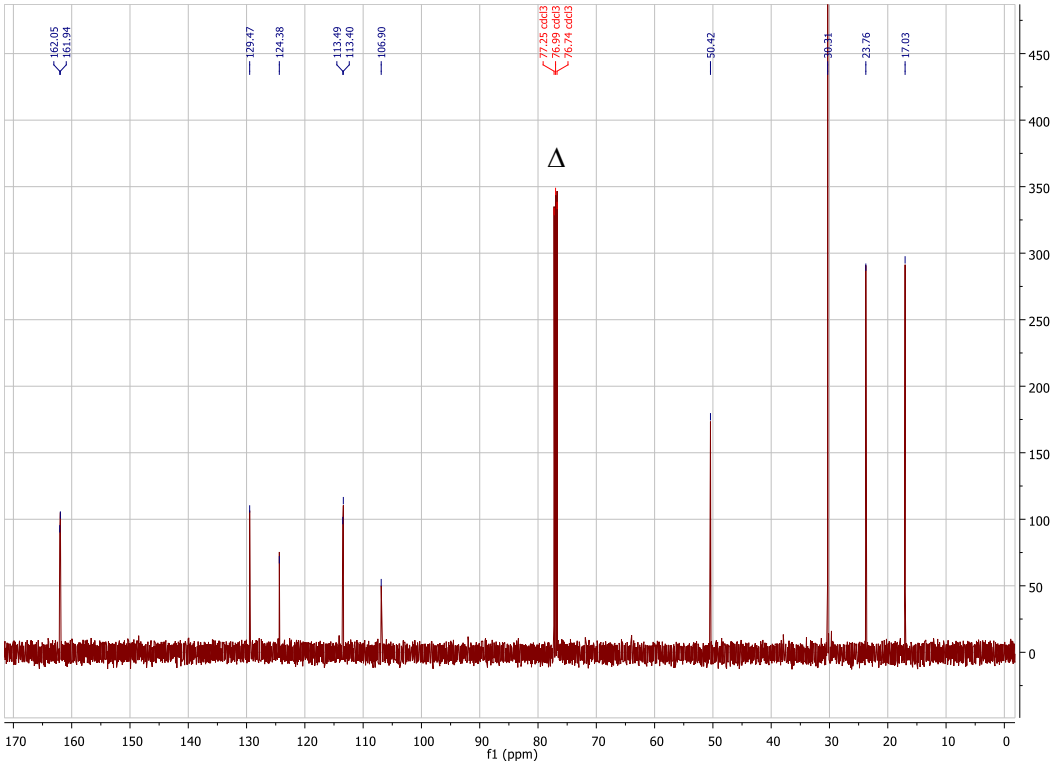

Figure S4 ^{13}C NMR spectrum of **2** in $CDCl_3$. Triangle = signal of solvent.

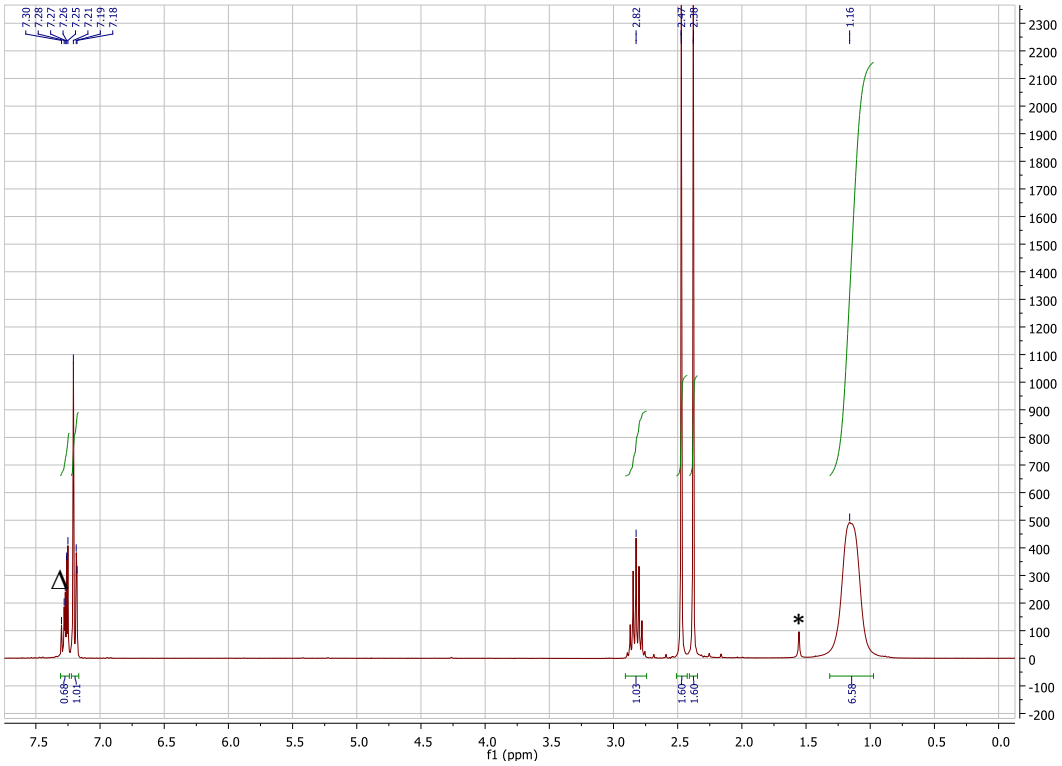

Figure S5. ^{1}H NMR spectrum of **3** in $CDCl_3$. Asterisk = signal of residual water. Triangle = residual signal of non-deuterated solvent.

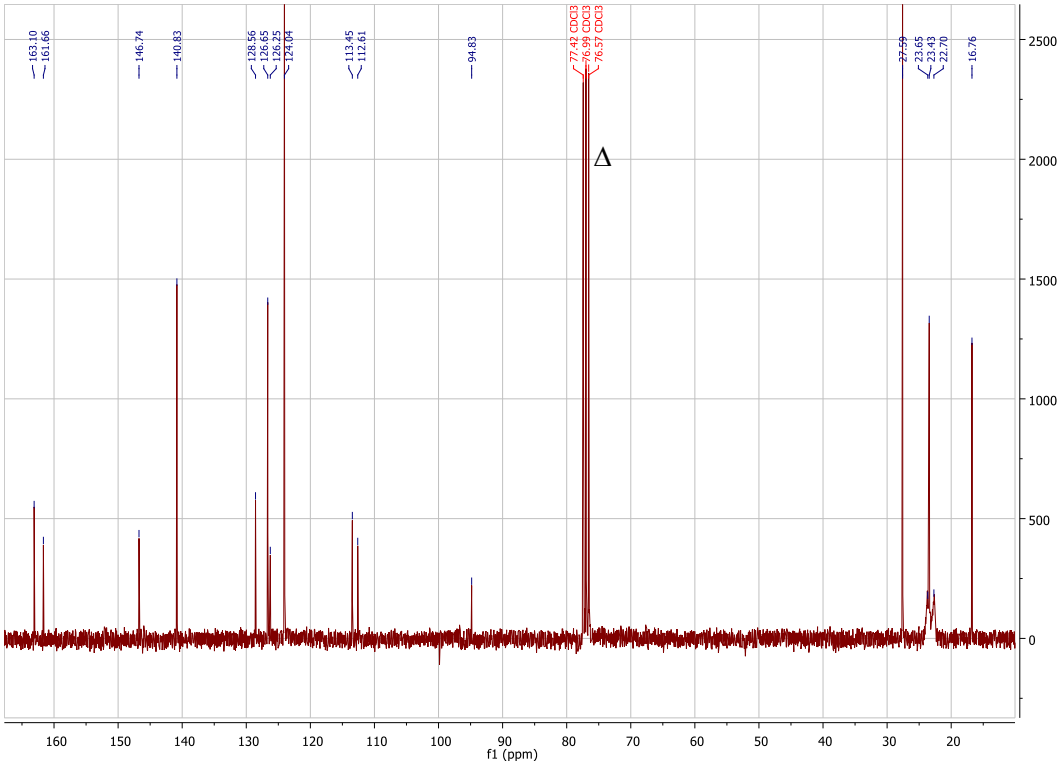

Figure S6. ^{13}C NMR spectrum of **3** in $CDCl_3$. Triangle = signal of solvent.

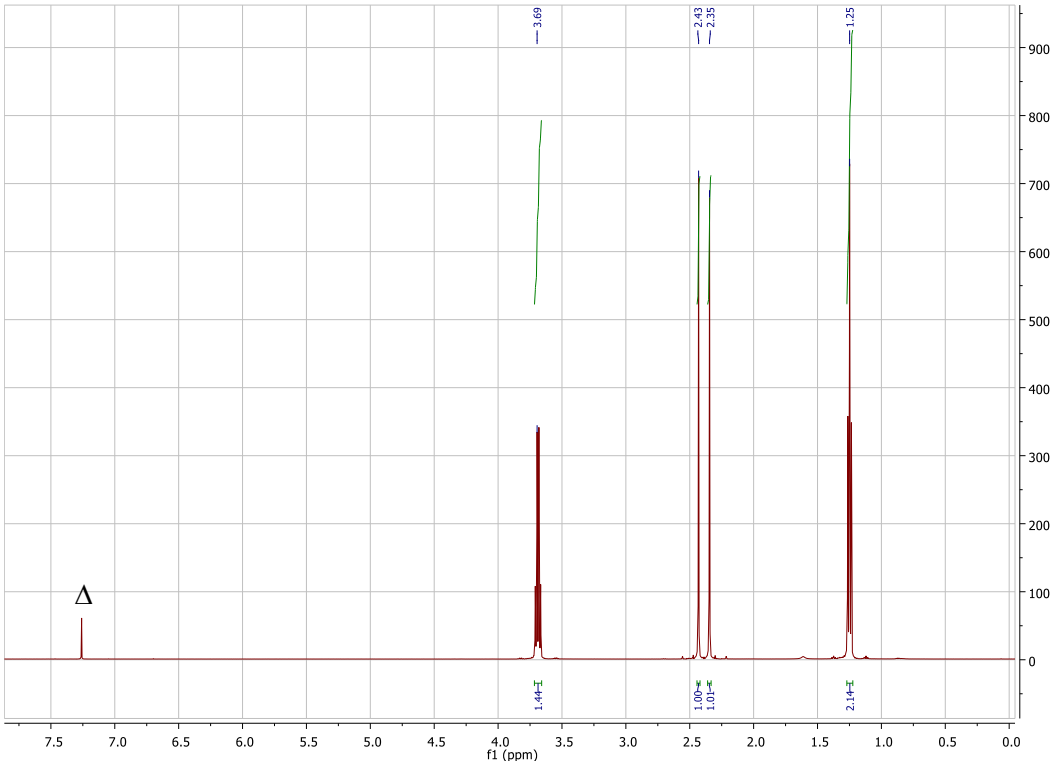

Figure S7. ^{1}H NMR spectrum of **4** in $CDCl_3$. Triangle = residual signal of non-deuterated solvent.

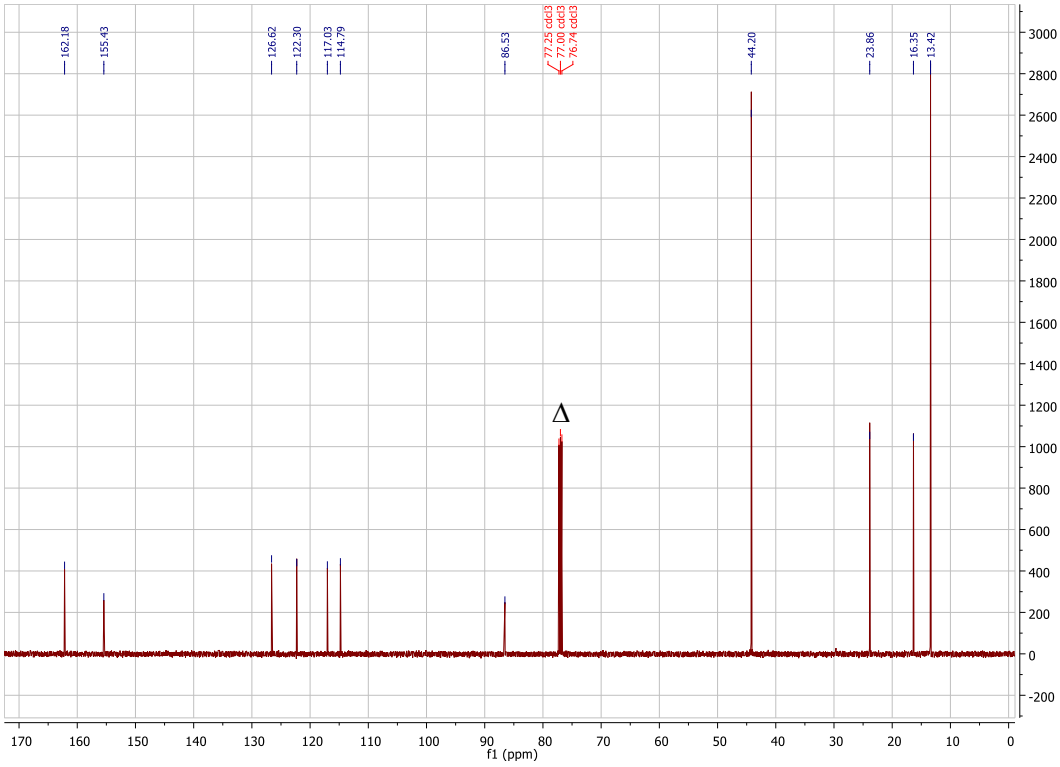

Figure S8. ^{13}C NMR spectrum of **4** in $CDCl_3$. Triangle = signal of solvent.

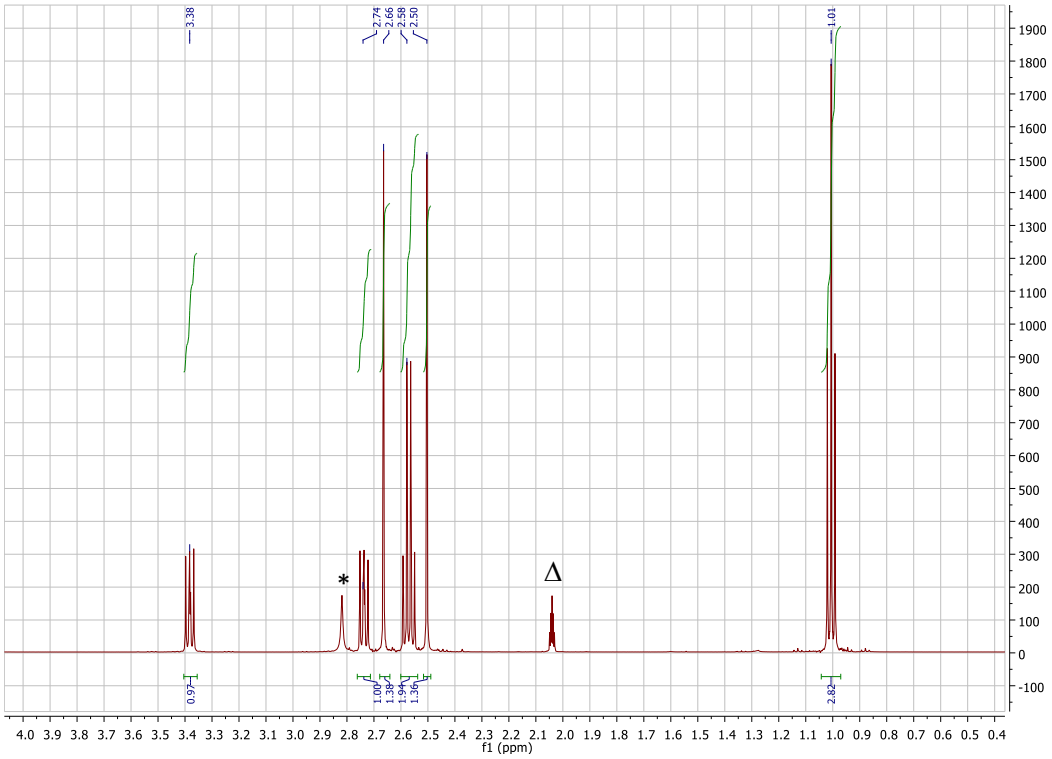

Figure S9. ^{1}H NMR spectrum of **5** in CD_3COCD_3. Asterisk = signal of residual water. Triangle = residual signal of non-deuterated solvent.

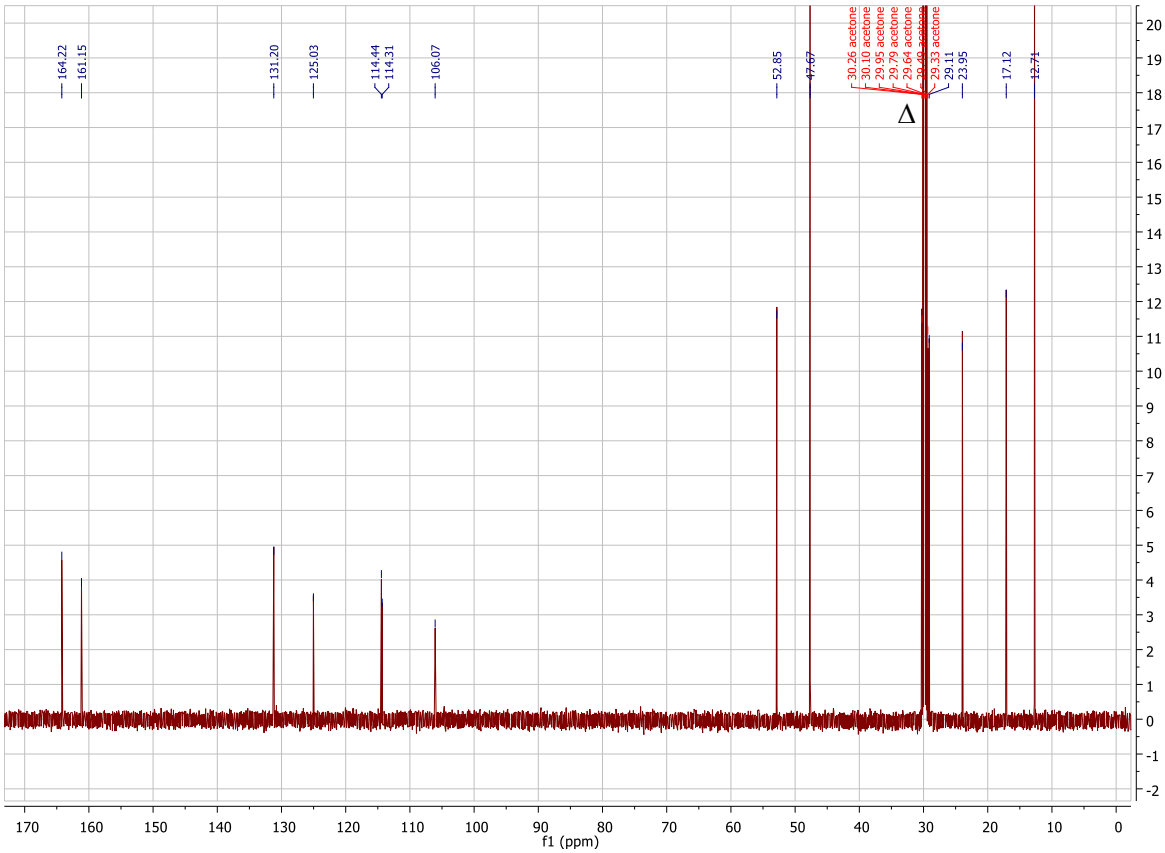

Figure S10. ^{13}C NMR spectrum of **5** in CD_3COCD_3. Triangle = signal of solvent.

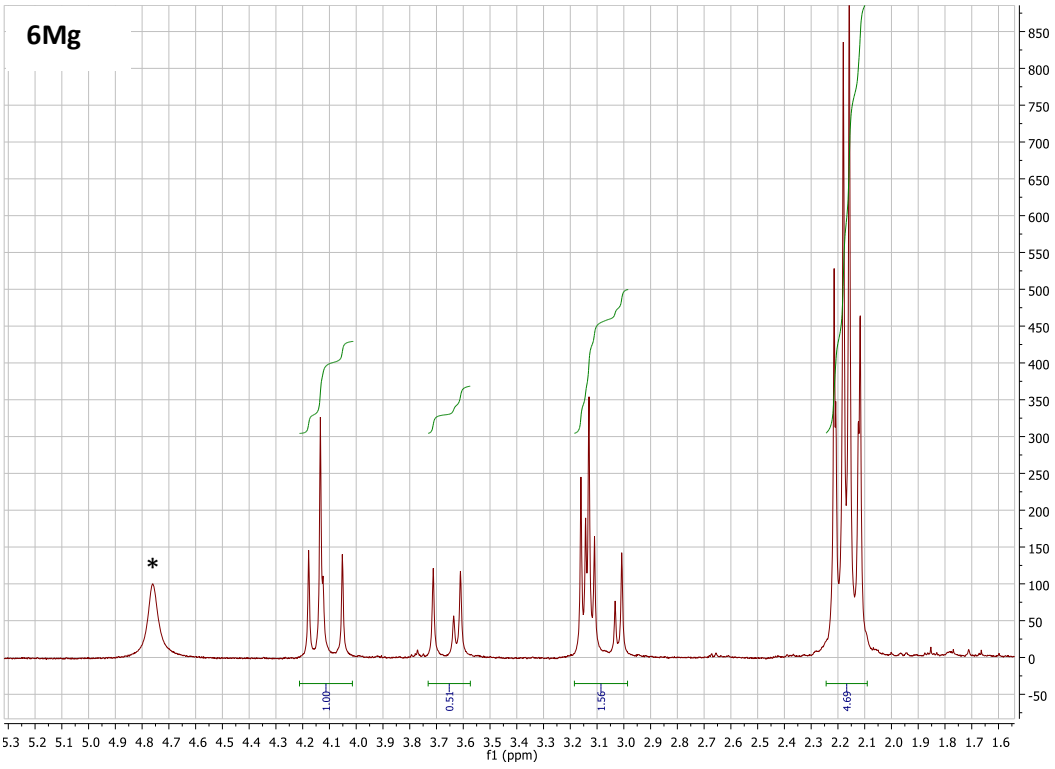

Figure S11. ^{1}H NMR spectrum of **6Mg** in $CDCl_3$/pyridine-d_5. Asterisk = signal of residual water.

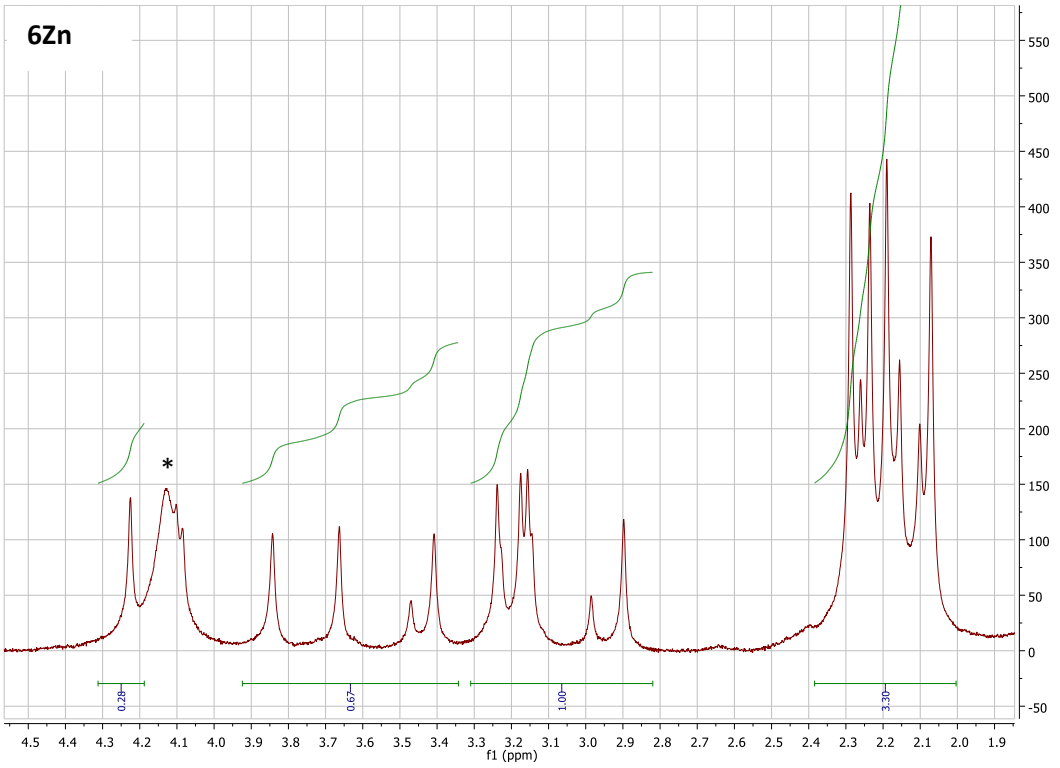

Figure S12. ^{1}H NMR spectrum of **6Zn** in $CDCl_3$/pyridine-d_5. Asterisk = signal of residual water.

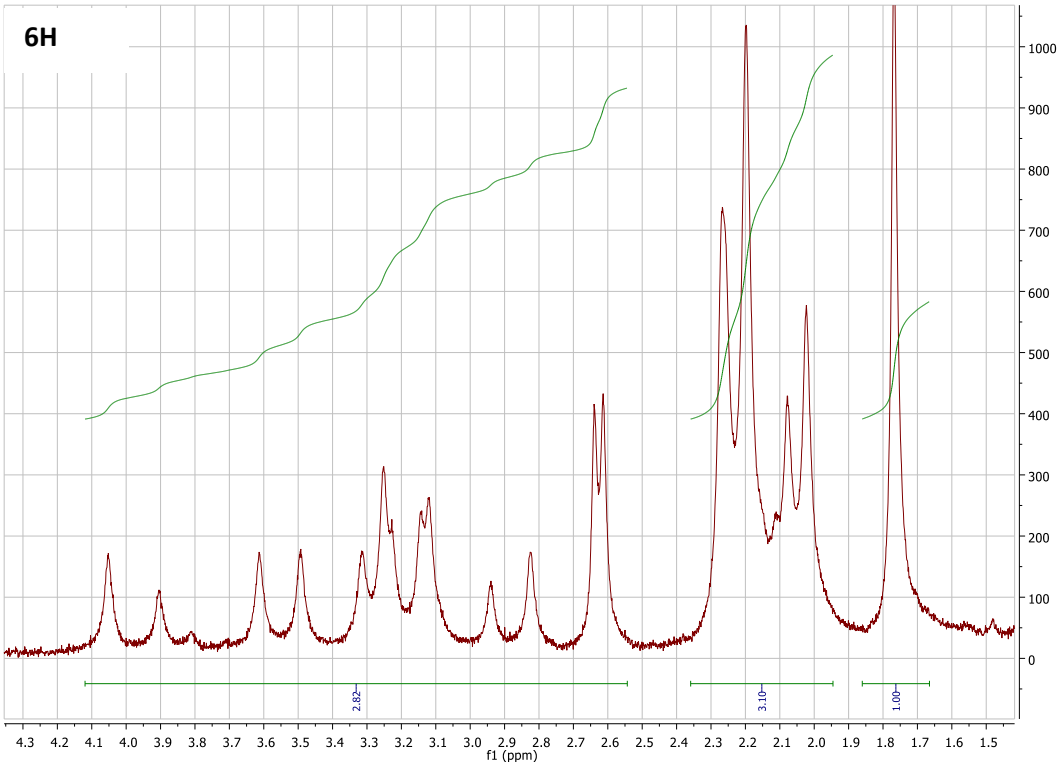

Figure S13. ^{1}H NMR spectrum of **6H** in $CDCl_3$/pyridine-d_5.

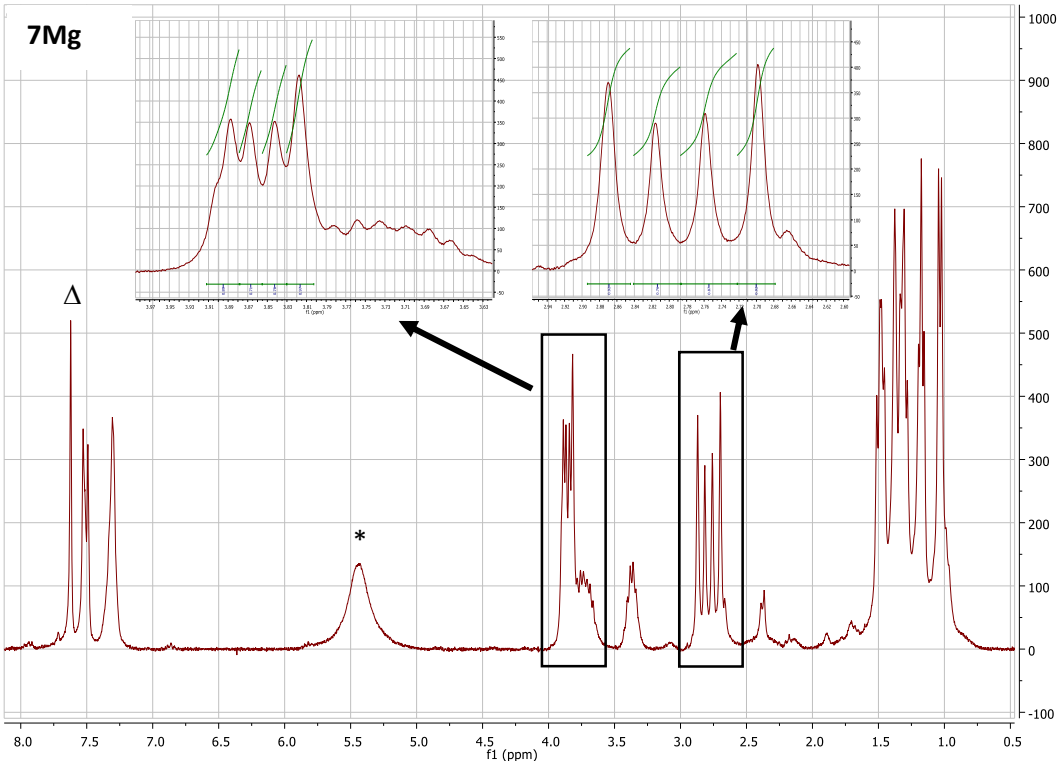

Figure S14. ^{1}H NMR spectrum of **7Mg** in $CDCl_3$/pyridine-d_5. Asterisk = signal of residual water. Triangle = residual signal of non-deuterated solvent.

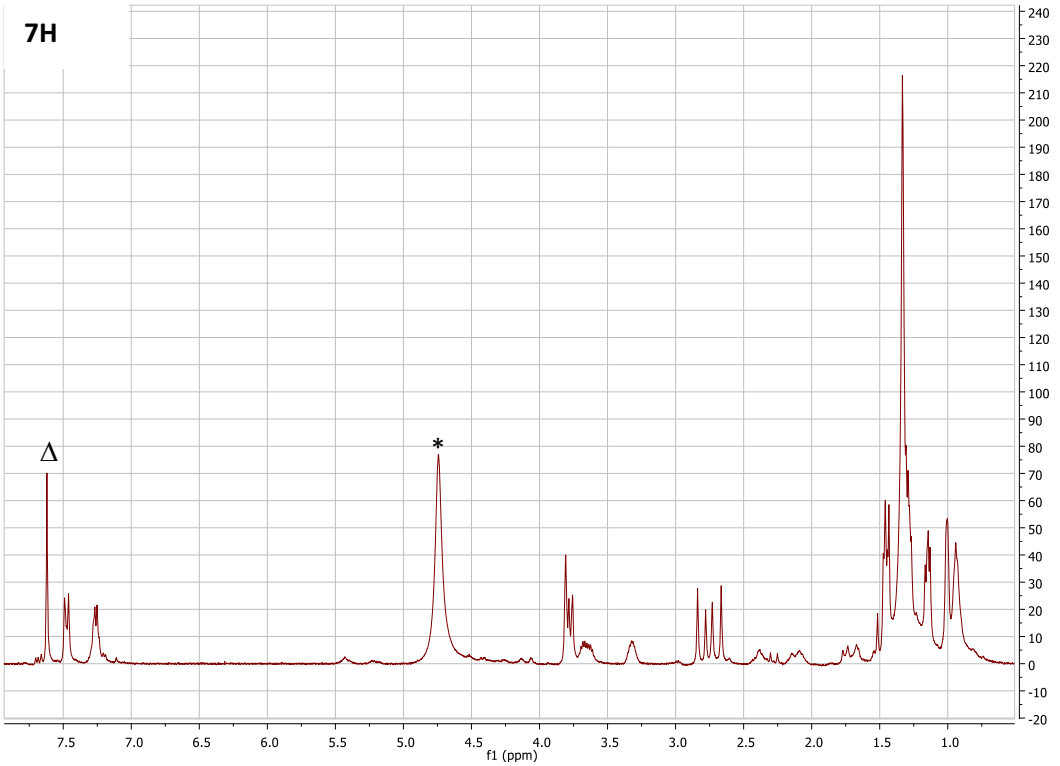

Figure S15. ^{1}H NMR spectrum of **7H** in $CDCl_3$/pyridine-d_5. Asterisk = signal of residual water. Triangle = residual signal of non-deuterated solvent.

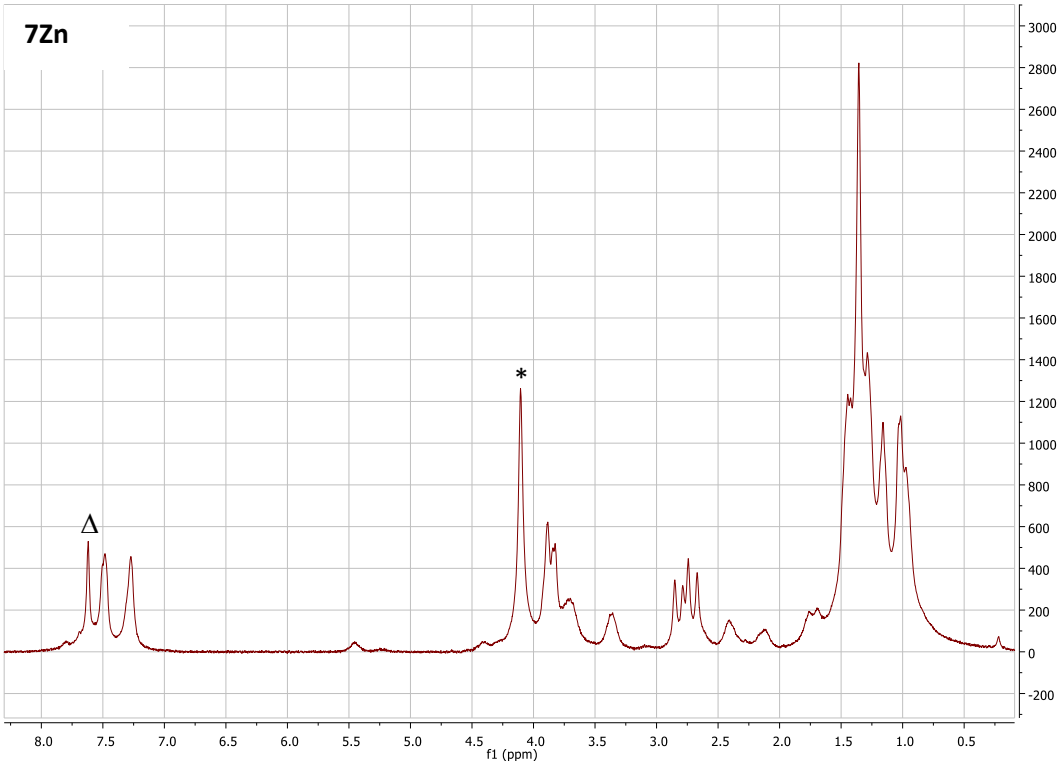

Figure S16. ^{1}H NMR spectrum of **7Zn** in $CDCl_3$/pyridine-d_5. Asterisk = signal of residual water. Triangle = residual signal of non-deuterated solvent.

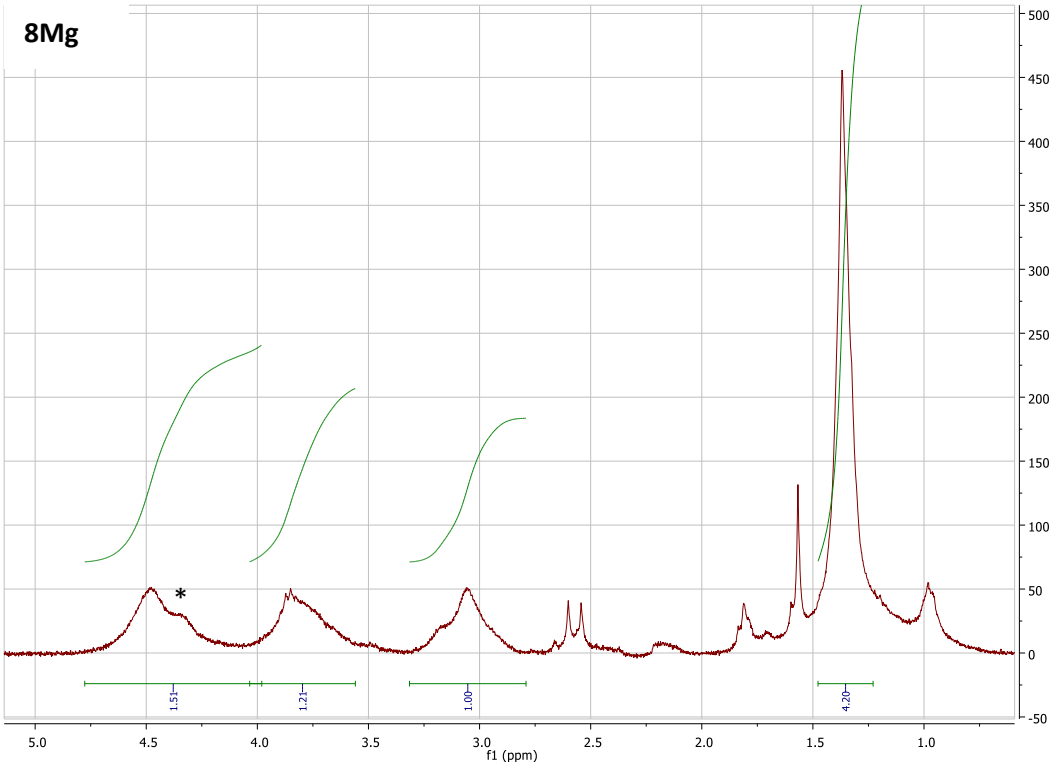

Figure S17. ^{1}H NMR spectrum of **8Mg** in $CDCl_3$/pyridine-d_5. Asterisk = signal of residual water.

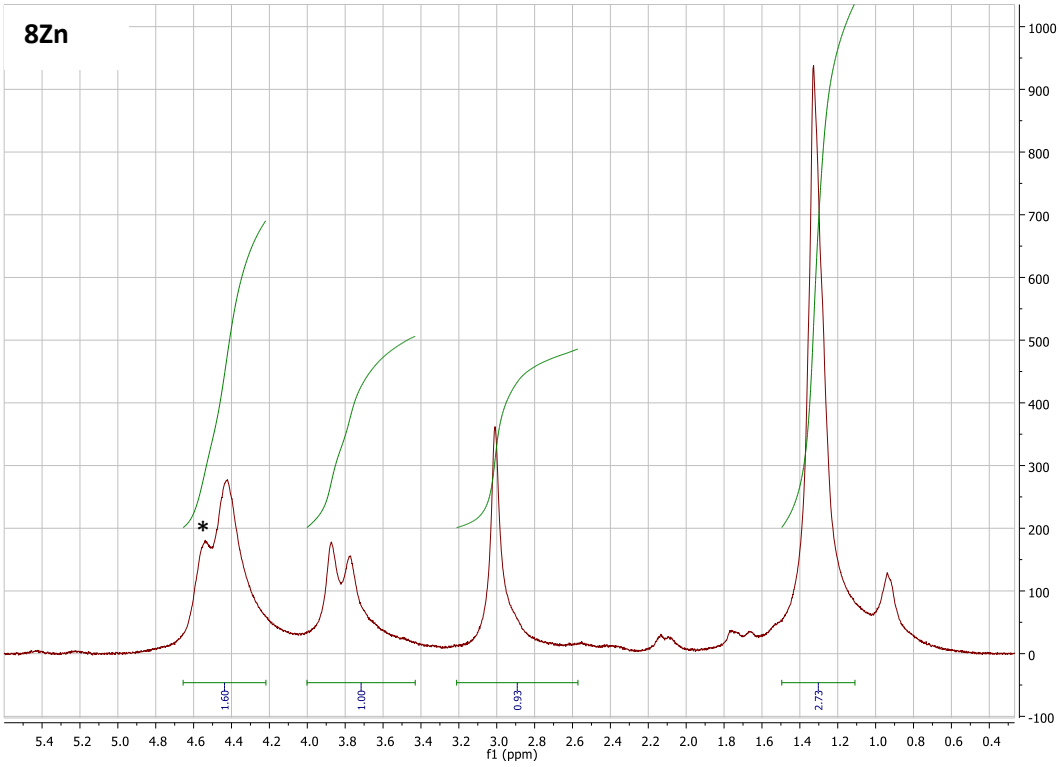

Figure S18. ^{1}H NMR spectrum of **8Zn** in $CDCl_3$/pyridine-d_5. Asterisk = signal of residual water.

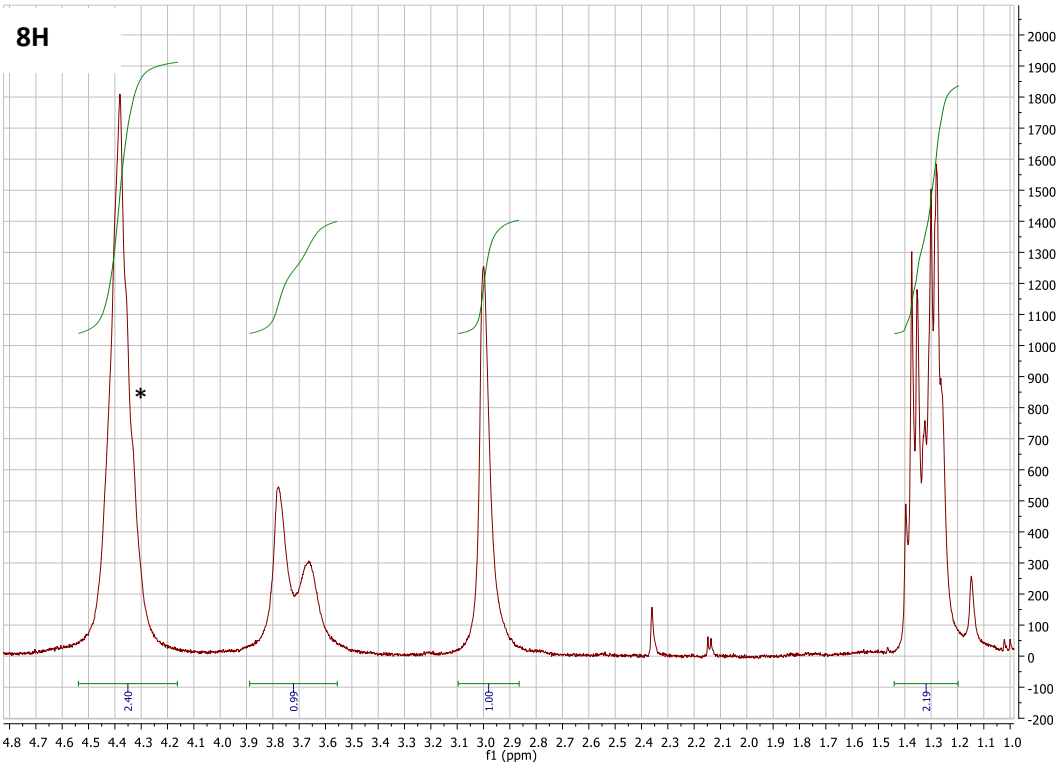

Figure S19. ^{1}H NMR spectrum of **8H** in $CDCl_3$/pyridine-d_5. Asterisk = signal of residual water.

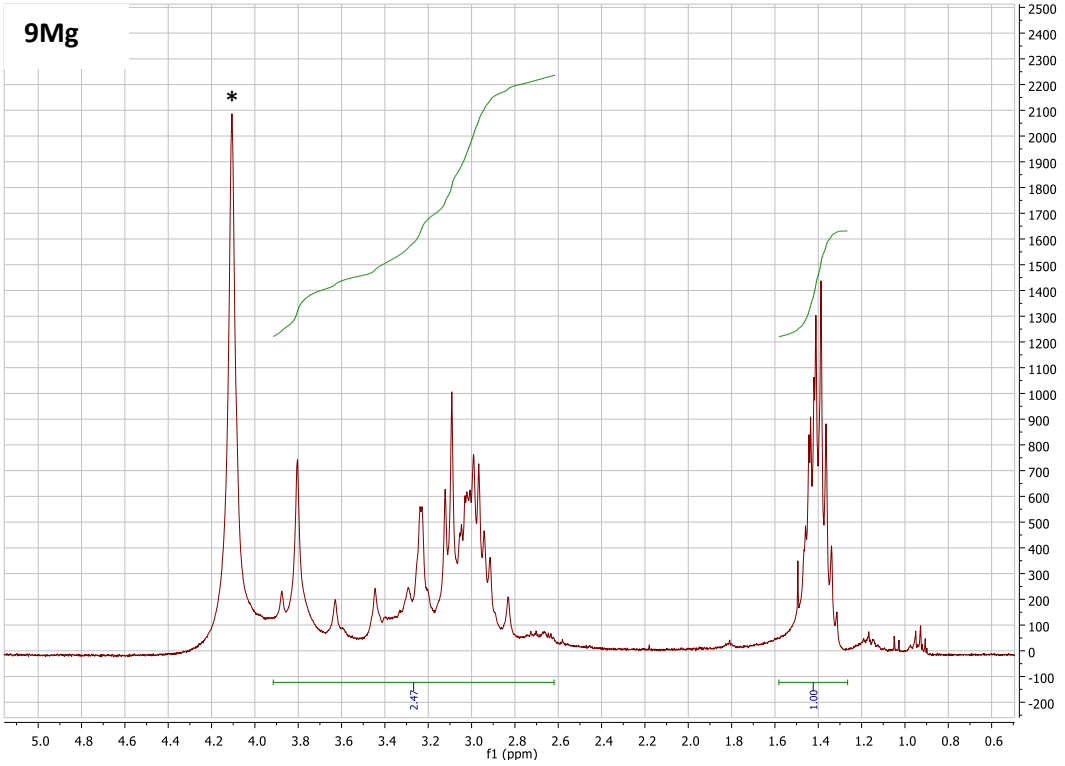

Figure 20. ^{1}H NMR spectrum of **9Mg** in $CDCl_3$/pyridine-d_5. Asterisk = signal of residual water.

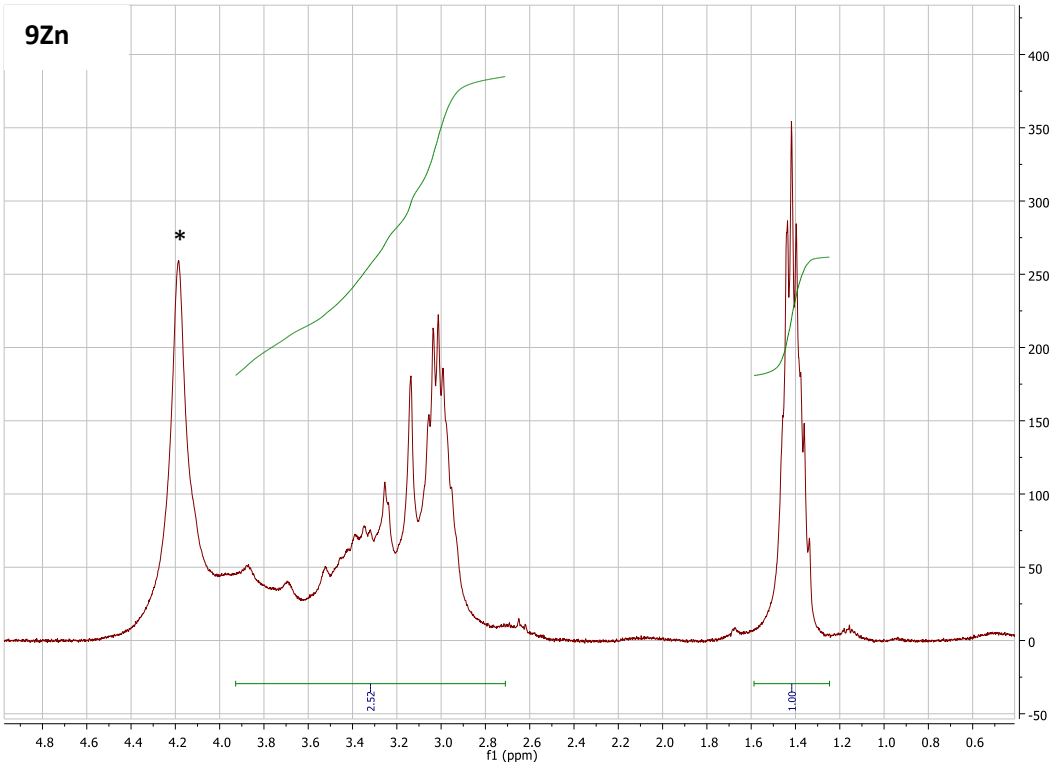

Figure S21. ^{1}H NMR spectrum of **9Zn** in $CDCl_3$/pyridine-d_5. Asterisk = signal of residual water.

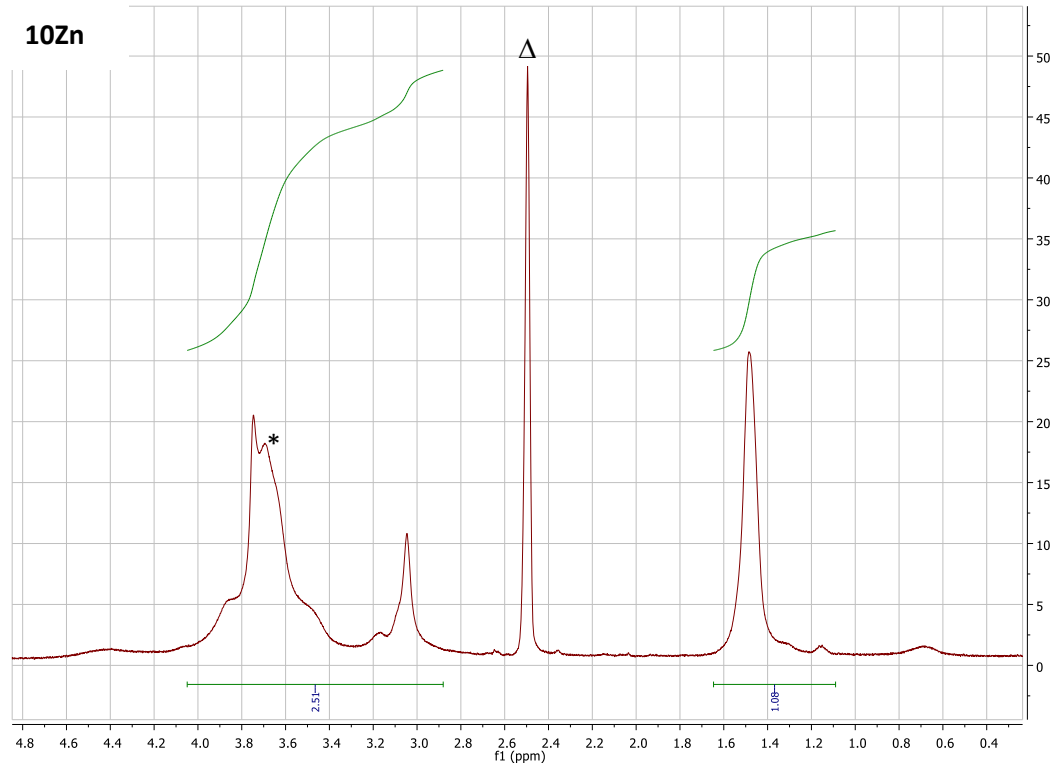

Figure S22. ^{1}H NMR spectrum of **10Zn** in DMSO-d_6/pyridine-d_5. Asterisk = signal of residual water. Triangle = residual signal of non-deuterated solvent.

MS spectra

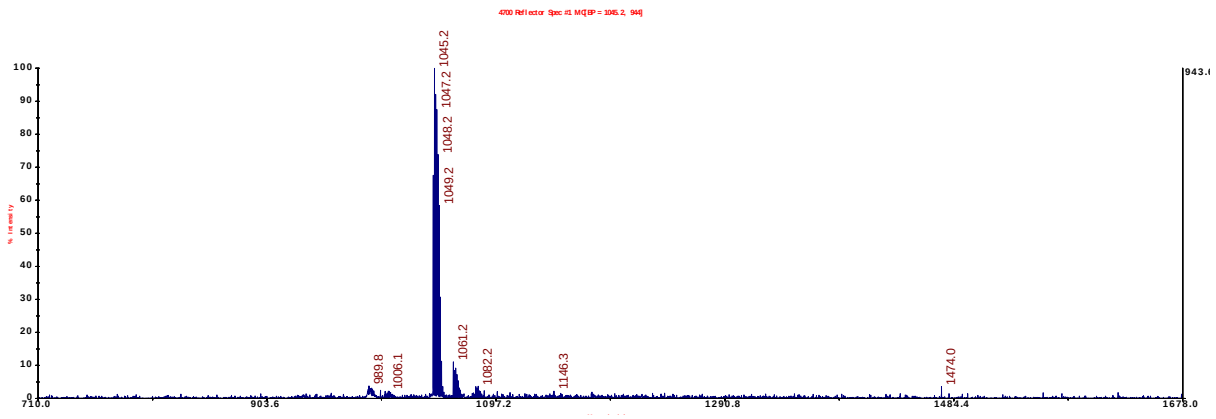

Figure S23. MALDI-TOF mass spectrum of **6Zn**.

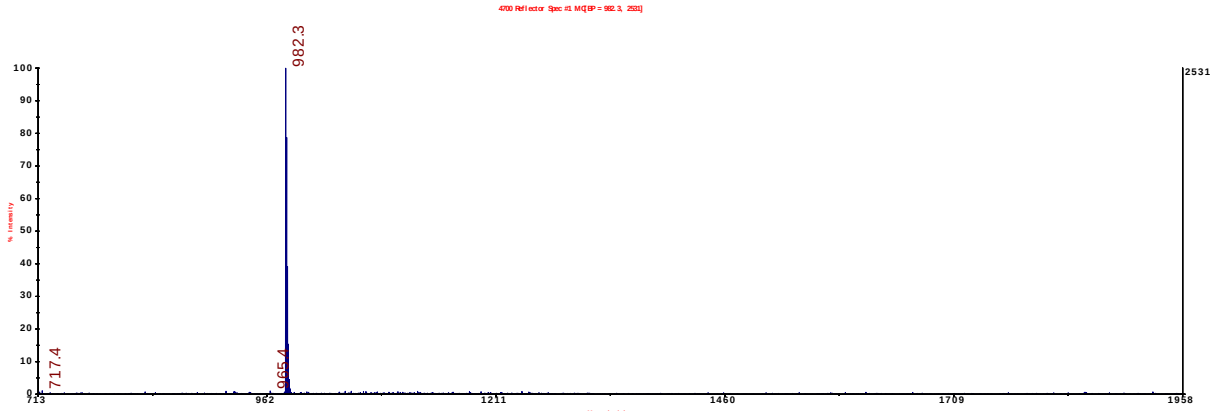

Figure S24. MALDI-TOF mass spectrum of **6H**.

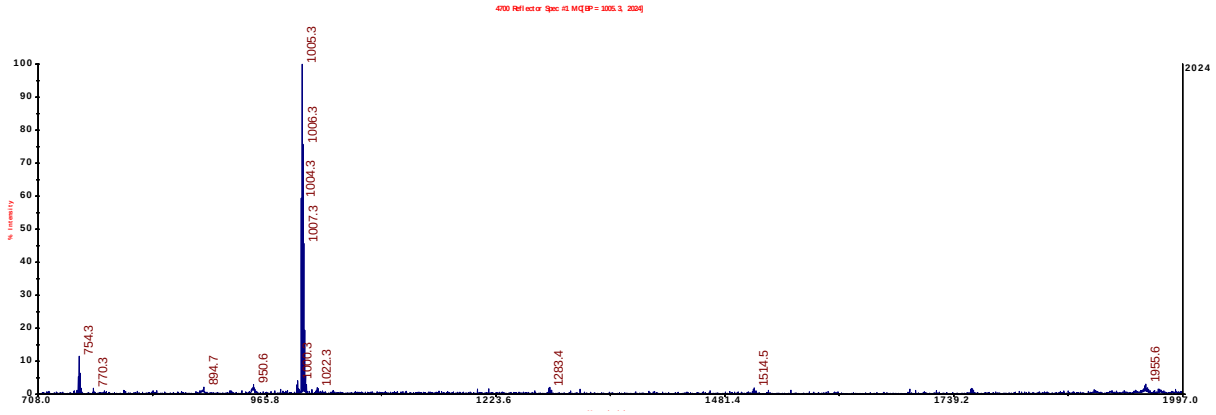

Figure S25. MALDI-TOF mass spectrum of **6Mg**.

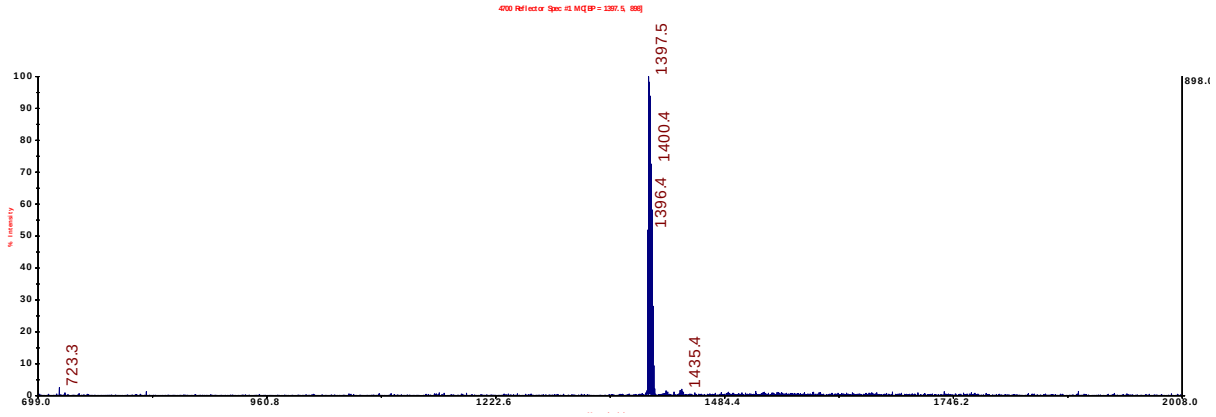

Figure S26. MALDI-TOF mass spectrum of **7Zn**.

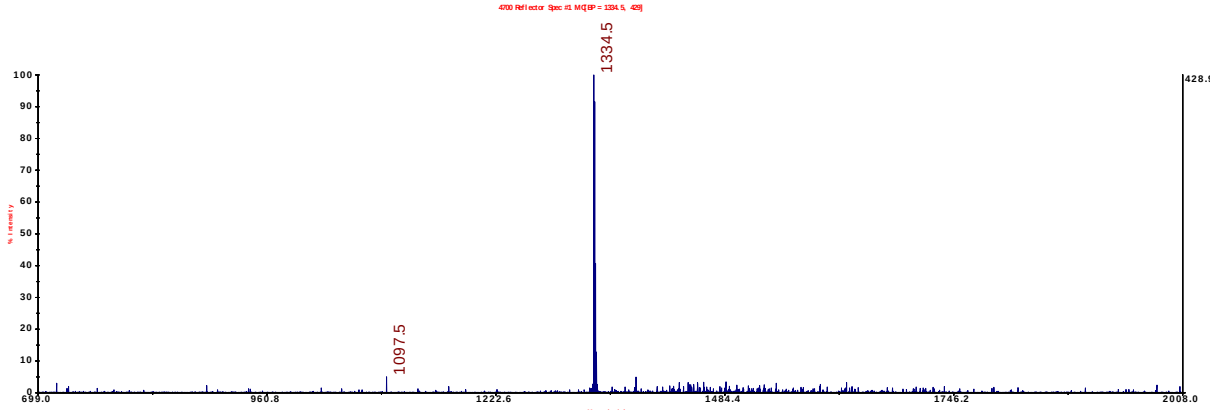

Figure S27. MALDI-TOF mass spectrum of **7H**.

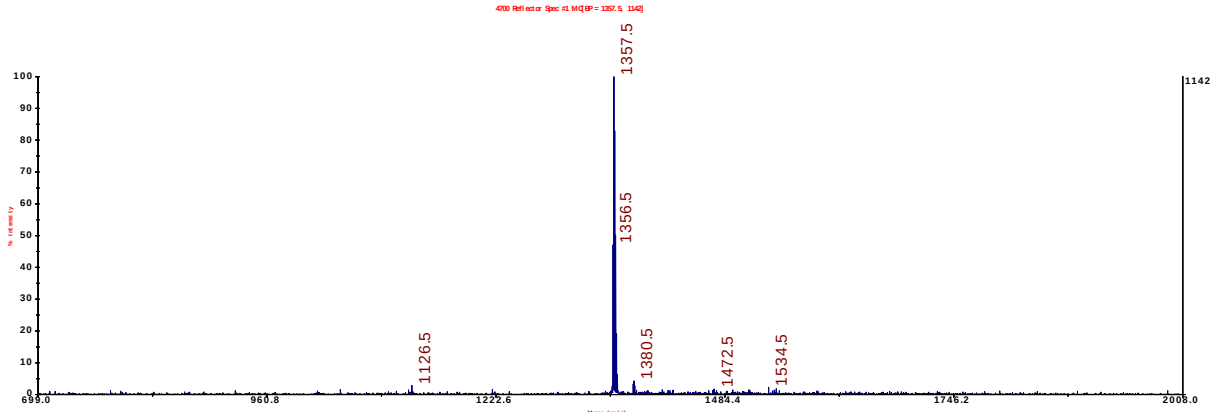

Figure S28. MALDI-TOF mass spectrum of **7Mg**.

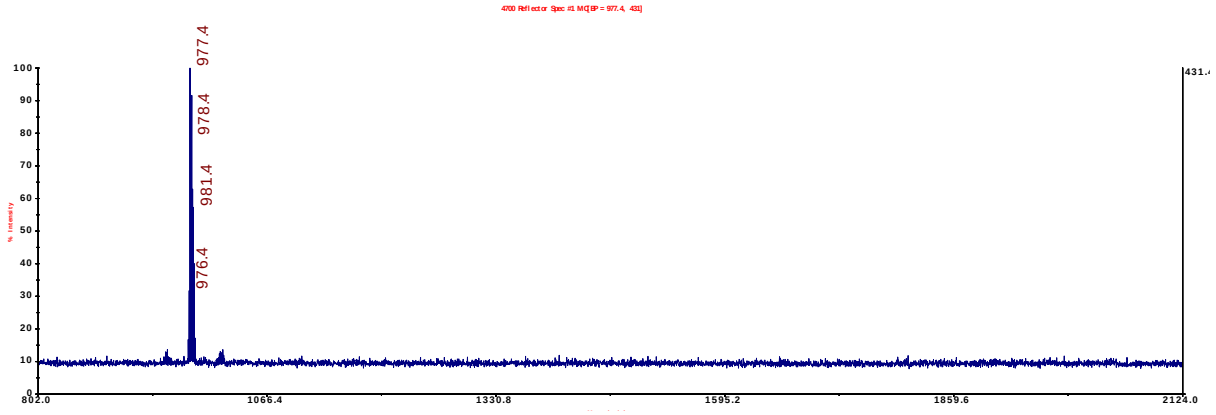

Figure S29. MALDI-TOF mass spectrum of **8Zn**.

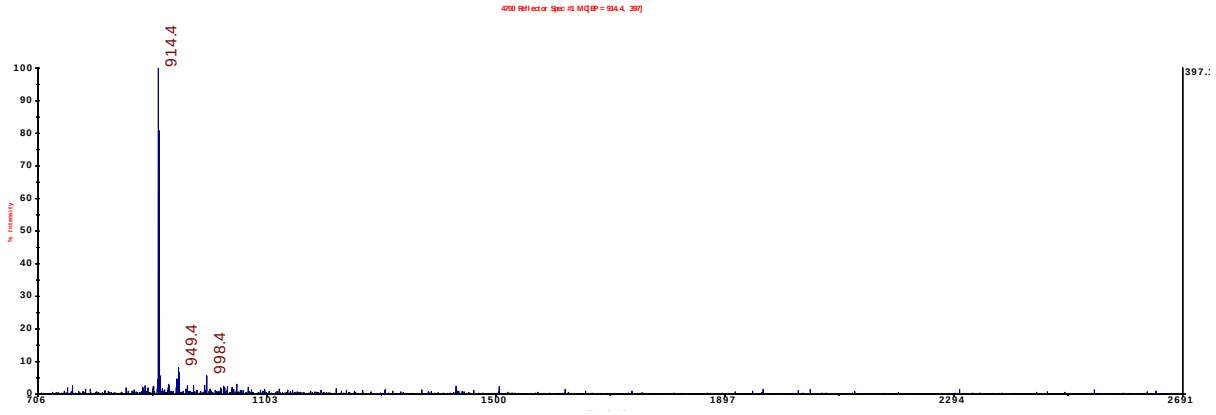

Figure S30. MALDI-TOF mass spectrum of **8H**.

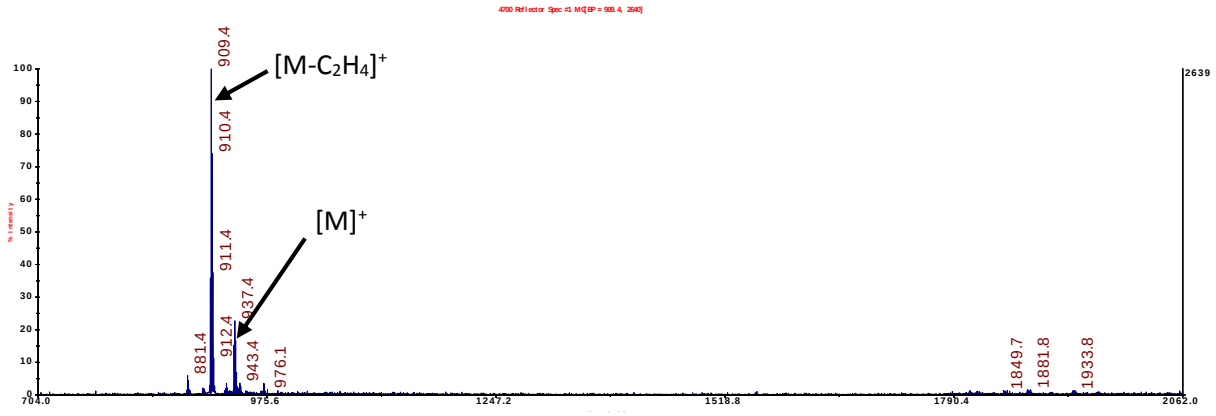

Figure S31. MALDI-TOF mass spectrum of **8Mg**.

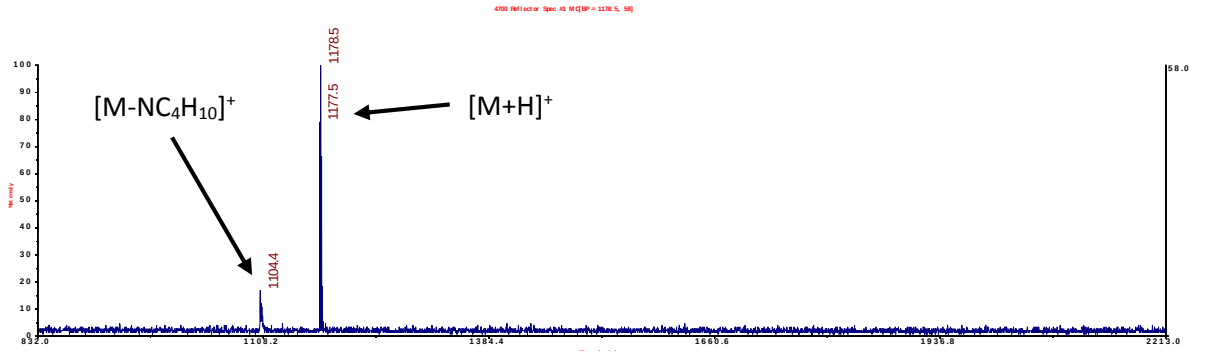

Figure S32. MALDI-TOF mass spectrum of **9Mg**.

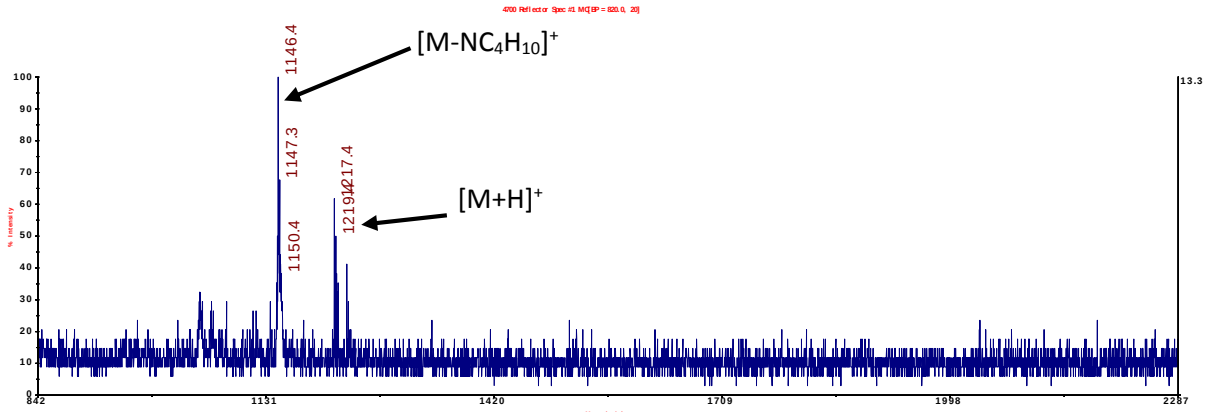

Figure S33. MALDI-TOF mass spectrum of **9Zn**.

HPLC separation

The analysis of the TPyPzs samples was accomplished by HPLC-DAD. The separation was performed on a Hypersil BDS C18 column (100 × 4.6 mm, particle size 2.4 μm) using a mobile phase consisting of acetonitrile, THF and water. The mobile phase A was water and mobile phase B contained acetonitrile-THF (1:1, v/v), both with addition of 0.025% triethylamine. The gradient program (except for **7Mg**) was set as follows: 0-20 min 60→90% B; 20-40 min 90% B; 40-40.5 min 90→60% B. The column was then equilibrated for 5 min under initial conditions (60% B). In order to achieve better separation of **7Mg** isomers the final concentration of mobile phase B in the gradient program was lowered to 83%. The column temperature was maintained at 40 °C and the flow rate was set at 1.0 mL/min. The chromatograms were recorded by a diode array detector at the wavelength corresponding to the absorbance maximum for each compound: **6Mg**, **6Zn** 730 nm; **7Mg**, **7Zn** 710 nm; **7H** 715 nm; **8Mg** 770 nm; **8Zn** 775 nm; **8H** 800 nm. Compound **6H** was not sufficiently soluble for HPLC analysis.

Quantitative HPLC analysis (*i.e.* percentual distribution of the isomers in the mixture) was performed only for the **6Mg**, **7Mg** and **8H** (Table S1), *i.e.* the compounds that originated directly from cyclotetramerization reaction and not from insertion/removal of the central metal. The reason is that some of the isomers could be removed or their amount reduced during next reaction steps. Nevertheless, the qualitative parameter, the retention time (t_R) of the isomers, is also mentioned in Experimental section for other complexes besides **6H**. Very low solubility of this compound did not allow HPLC analysis.

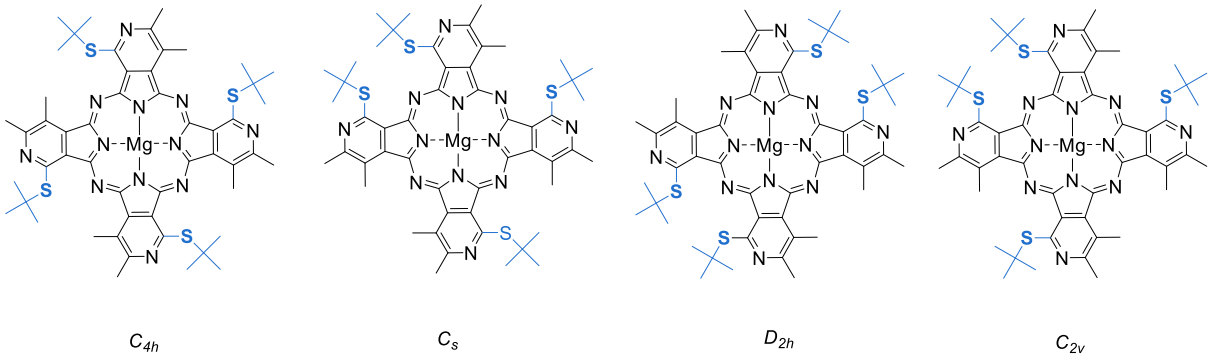

Figure S34. The possible positional isomers of synthesized TPyPz. Example for **6Mg**.

Table S1. Retention times (t_R, min) and percentual representation (in parentheses) of TPyPz isomers.

	Isomer A	Isomer B	Isomer C	Isomer D
5Mg	21.9 (61.5%)	22.77 (38.5%)	-	-
6Mg	28.8 (1%,) C_{2v}	29.2 (1.5%), D_{2h}	30.0 (30.5%), C_{4h}	30.5 (67%) C_s
7H	26.5 (16%)	28.5 (71%)	29.0 (13%)	-

Photophysical characterization

Fluorescence measurements

The samples were repurified using preparative TLC (except **10Zn**) before the photophysical measurements (both Φ_F and Φ_Δ) to ensure that they were highly pure. The fluorescence spectra were obtained using an AMINCO Bowman Series 2 luminescence spectrometer. All emission spectra were corrected for instrument response. The fluorescence quantum yields (Φ_F) were determined in THF (DMF for **10Zn**) *via* the comparative method using unsubstituted zinc phthalocyanine (ZnPc, Sigma-Aldrich) as a reference (Φ_F = 0.32 in THF[2]). Both the reference and sample were excited at 360 nm. The absorbance at the excitation wavelength and at the Q-band maximum was held below 0.05 to limit the inner filter effect. The value of Φ_F was calculated using Eq. S1[3]:

$$\Phi_F^S = \Phi_F^R \frac{F^S}{F^R}\left(\frac{1-10^{-A^R}}{1-10^{-A^S}}\right)\left(\frac{n^S}{n^R}\right)^2 \qquad \text{(Eq. S1)}$$

where F is the integrated area under the emission spectrum, n is refractive index of the solvent and A is the absorbance at the excitation wavelength. The superscripts R and S correspond to the reference and sample, respectively. All experiments were performed in triplicate with the data representing the mean (estimated error ±15%, for **8Mg**, **8Zn** and **8H** ±50% due to detection limit of the instrument).

Determination of the singlet oxygen production

The quantum yields of the singlet oxygen (Φ_Δ) were determined in THF (in DMF for **10Zn**) according to a previously published procedure[4] using the decomposition of a chemical trap 1,3-diphenylisobenzofuran (DPBF) with ZnPc as a reference (Φ_Δ = 0.53 in THF[5], Φ_Δ = 0.56 in DMF[6]) The detailed procedure was as follows: 2.5 mL of a DPBF stock solution in THF (or DMF) (5×10^{-5} M) was transferred into a 10 mm × 10 mm quartz optical cell and was saturated with oxygen for 1 min. A stock solution of the tested compound in THF (or DMF) (typically 20 µL) was then added to achieve an absorbance of the final solution in the Q-band

maximum of approximately 0.1. The solution was stirred and irradiated using a xenon lamp (100 W, ozone-free XE DC short-arc lamp, Newport). The incident light was filtered through a water filter (6 cm) and an OG530 cut-off filter (Newport) to remove the heat and the light below 523 nm, respectively. Decrease of DPBF in solution with irradiation time was monitored at 414 nm. All experiments were performed in triplicate, and the data presented herein represent the mean of the three experiments (estimated error: ±15%).

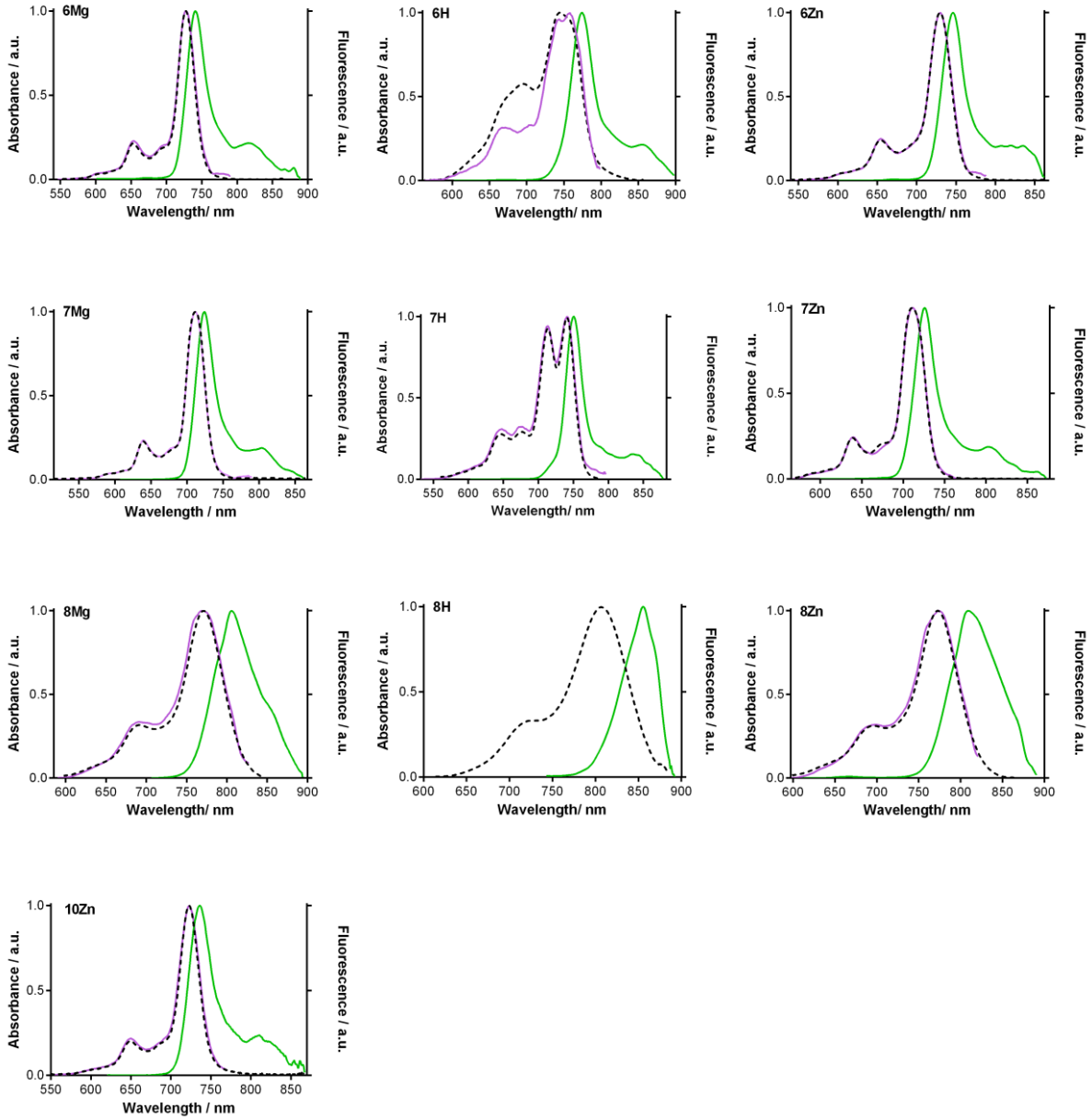

Figure S35. Absorption (black dashed), emission (green) and excitation (pink) spectra of **6M**-**8M** (M=Mg, 2H, Zn) in THF and **10Zn** in DMF. Absorption spectra of **6H** indicated aggregation. Fluorescence of **8H** was too weak to collect excitation spectra of sufficient quality. Emission spectra of compounds **8M** were influenced by fluorescence detector limit of the instrument (900 nm).

In vitro cytotoxicity assessments

Cell cultures

The HeLa cell line was obtained from the American Type Cell Culture Collection (ATCC, U.S.A.). Cells were cultured in Dulbecco's modified Eagle's medium (DMEM) without Phenol Red (Lonza, Belgium) supplemented with 10 % heat-inactivated fetal bovine serum (FBS, Lonza), 1 % penicillin/streptomycin solution (Lonza), 10 mM HEPES buffer (Sigma, Germany) and 4 mM L-glutamine (Lonza). The cells were cultured in 75 cm^2 tissue culture flasks (TPP, Switzerland) at 37 °C in a humidified atmosphere of 5% CO_2. Sub-confluent cells were subcultured every 3-4 days. For dark toxicity and photodynamic therapy experiments, cells were seeded in 96-well plates (TPP) at density of 7,500 cells per well (HeLa) 24 h prior an addition of the studied compound.

Uptake to the cells

To establish the time profile of intracellular accumulation of compound **10Zn**, HeLa cells were seeded in 6 cm petri dishes at density of 45,000 cells per dish. Cells had been left to attach for 24 h and then the medium was removed and 10 μM compound **10Zn** was added in 5 ml of cultivation medium in selected times prior harvesting (see Figure S36). After incubations, the cells were washed three times with 5 ml of phosphate buffered saline (PBS, Sigma) followed by addition of 5 ml of medium. Cells were scraped and transferred to 15 ml centrifugation tubes (TPP) and centrifuged 5 min at 70 × g. Supernatant was replaced with 2 ml of fresh medium, cell pellet was gently resuspended and centrifuged again. After the centrifugation, the medium was replaced with DMF (500 μL). Lysis of cells was performed overnight in -20°C. Trifluoracetic acid (10 μl of its 10% solution in DMF) was added and the fluorescence of **10Zn** (for spectrum see Figure S35) was measured (λ_{exc} = 354 nm and λ_{em} = 736 nm) using AMINCO-Bowman Series 2 luminescence spectrometer. Uptake experiments were performed in duplicate.

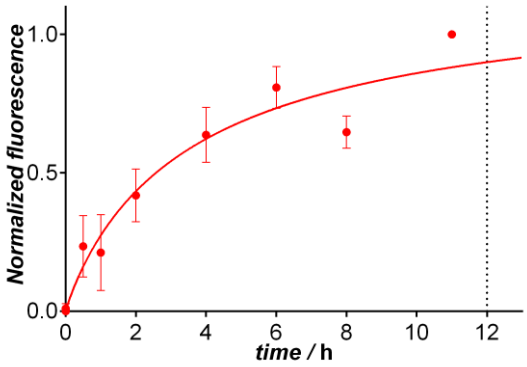

Figure S36. Uptake of compound **10Zn** by HeLa cells. Points represent mean of two independent experiments, curve is the best non-linear fit.

Subcellular localization.

Subcellular localization was performed by fluorescent microscopy method. Cells were seeded on glass-bottom 3 cm Petri dishes suitable for confocal microscopy (WillCo Wells B.V., Netherlands) at the concentration of 15 000 cells per dish. After 24 h medium was replaced with 10 μM solution of **10Zn** in fresh medium. Incubation time was set to 12 h. LysoTracker Blue DND-22 (2 μM, Molecular Probes) and MitoTracker Green FM (2 μM, Molecular Probes) were added for another 15 min to stain lysosomes and mitochondria, respectively. Cells were rinsed two times with pre-heated PBS (37°C) and 1 ml of medium was added. Photographs were obtained using Nikon Eclipse Ti (Nikon, Japan) fluorescence microscope equipped with CoolLED pE-300white fluorescence source (CoolLED Ltd., United Kingdom), cooled digital sCMOS camera Andor Zyla 5.5 (Andor Technology, United Kingdom) and NIS Elements AR 4.20 software (Laboratory Imaging, Czech Republic).

Dark toxicity experiments and photodynamic treatment.

The own toxicity of the **10Zn** without the presence of light (dark toxicity) was assayed at wide concentration range from 1 μM to 500 μM after 24 h incubations. Viabilities of HeLa cells were determined using neutral red (NR) uptake assay (Sigma) based on ability of living cells to incorporate NR in their intact lysosomes. Soluble NR was measured as its optical density at λ = 540 nm using Tecan Infinite 200M plate reader (Tecan, Austria). The viability of experimental groups was expressed as percentage of untreated controls (100%).

For photodynamic treatment experiments, HeLa cells were first loaded for 12 h with various concentrations (0.01 – 10 μM) of compound **10Zn**. After loading, the cells were washed with PBS and fresh medium was added. Irradiation of HeLa cells with light was performed using 450 W ozone-free Xe lamp (Newport) with intensity reduced to 400 W (λ > 570 nm, 12.4

mW/cm^2, 15 min, 11.2 J/cm^2), with long pass filter (Newport OG570) and water filter (8 cm) to eliminate undesirable wavelengths. After irradiation, the cells were incubated another 24 h before assaying their viability by NR as described before. At least five independent experiments, each in quadruplicate, were performed.

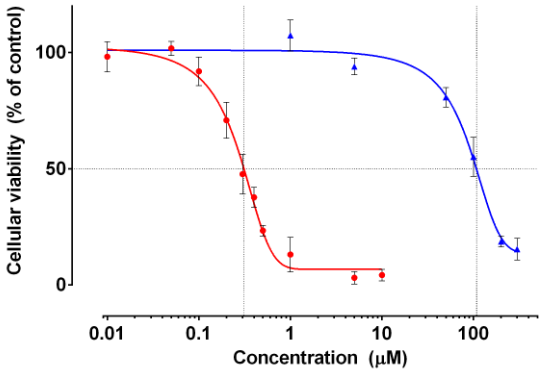

Figure S37. Phototoxicity (λ > 570 nm, 12.4 mW/cm^2, 15 min, 11.2 J/cm^2, red line) and dark toxicity (blue line) of **10Zn** on HeLa cells

References

1. K. V. Lipin, V. N. Maksimova, O. V. Ershov, A. V. Eremkin, Y. S. Kayukov and O. E. Nasakin, *Russ. J. Org. Chem.*, 2010, **46**, 617-618.
2. P. Zimcik, V. Novakova, K. Kopecky, M. Miletin, R. Z. Uslu Kobak, E. Svandrlikova, L. Váchová and K. Lang, *Inorg. Chem.*, 2012, **51**, 4215-4223.
3. J. R. Lakowicz, *Principles of fluorescence spectroscopy*, 3rd edn., Springer, New York, 2006.
4. V. Novakova, M. Miletin, T. Filandrová, J. Lenčo, A. Růžička and P. Zimcik, *J Org. Chem.*, 2014, **79**, 2082-2093..
5. L. Kaestner, M. Cesson, K. Kassab, T. Christensen, P. D. Edminson, M. J. Cook, I. Chambrier and G. Jori, *Photochem. Photobiol. Sci.*, 2003, **2**, 660-667.
6. U. Michelsen, H. Kliesch, G. Schnurpfeil, A. K. Sobbi and D. Wöhrle, *Photochem. Photobiol.*, 1996, **64**, 694-701.