SUPPLEMENTARY INFORMATION

Unusual *cis*-diprotonated forms and fluorescent aggregates of non-peripherally alkoxy-substituted metallophthalocyanines

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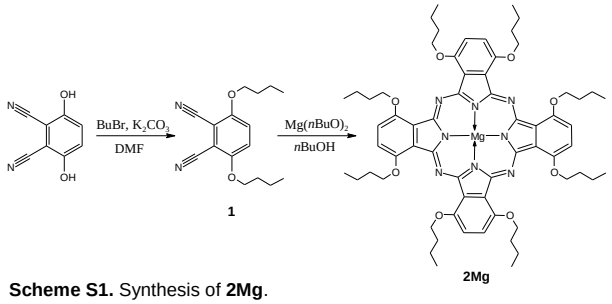
Supplementary Information

Experimental details

Sample preparation

[1,4,8,11,15,18,22,25-octakis(1,4,7-trioxanonyl)phthalocyanine]magnesium(II) (**1Mg**) and [1,4,8,11,15,18,22,25-octakis(1,4,7-trioxanonyl)phthalocyanine]zinc(II) (**1Zn**) were synthesized using procedures reported previously.[1,2] Samples for spectroscopy experiments were prepared by diluting stock solutions with appropriate solvents. Diluted solutions were vortexed for at least one hour prior to measurements. Their purity and concentrations were verified by measuring steady-state absorption spectra with Perkin Elmer Lambda 35 UV/Vis spectrophotometer. All measurements were carried out in aerated conditions, but it has been verified that differences of fluorescence decay times of **1Mg** and **1Zn** measured in air-saturated dimethylsulfoxide and in solutions purged with argon for 60 minutes were smaller than experimental errors. The following solvents available commercially (CHEMPUR, Sigma Aldrich, POCh) were used in reported experiments as supplied: dimethylformamide (DMF) – 99.8%, dimethyl sulfoxide (DMSO) – 99%, chloroform - 99.8%, tetrahydrofuran – 99%, pyridine – anhydrous, 99.8%.

Phthalocyanine **2Zn** was purchased from Aldrich, whereas the known compound **2Mg**[3] was synthetized according to a two-step procedure (Scheme 1). First, 3,6-dihydroxyphthalonitrile was treated with 1-bromobutane in the presence of potassium carbonate as a base, adapting a literature method, to give phthalonitrile derivative **1**.[4] Resulting compound **1** was subjected to the Linstead macrocyclization reaction to give phthalocyanine derivative **2Mg**. Magnesium turnings (48 mg, 2 mmol) and a small crystal of iodine were refluxed for 3 h in *n*-butanol (16 mL). After the mixture was cooled down to room temperature, phthalonitrile **1** (544 mg, 2 mmol) was added and the reaction mixture was refluxed for 22 h. *n*-Butanol was evaporated with toluene and a dark green residue was dissolved in dichloromethane, Celite filtered and chromatographed (dichloromethane : methanol, 50 : 1, v/v) to give phthalocyanine **2Mg** as a green solid (243 mg, 44%);. 1H NMR (400 MHz, pyridine–d5): δ 7.88, 5.14, 2.30, 1.70, 1.01; 13C NMR (101 MHz, pyridine–d5): δ 153.6, 152.7, 130.5, 119.5, 72.8, 32.7, 20.2, 14.7; MS (MALDI) m/z Found: [M+H]+ 1113.6092; C64H81MgN8O8 requires [M+H]+ 1113.6028.



Scheme S1. Synthesis of **2Mg**.

Analytical HPLC was carried out with an Agilent 1200 instrument equipped with a DAD detector. The chromatographic separation was achieved on octadecylsilane coated column, 150 mm × 4.6 mm, 5 µm (Eclipse XDB-C18, Agilent) using an isocratic elution at a flow rate of 1.0 mL/min in different configurations. Separations were performed at 25°C. Assessed HPLC purity: 99.58 – 100.00%.

Steady-state absorption spectroscopy

Steady-state absorption spectra of **1Zn** and **1Mg** solutions in DMF during titration with sulphuric acid were recorded at room temperature using Perkin Elmer Lambda 35 UV/Vis spectrophotometer in 1 cm quartz cuvettes. The spectra were recorded at approximately equal time intervals after each addition of the acid. Sets of spectra reflecting consecutive protonation steps were selected and each set was analyzed with help of the Hill equation of the form:[5]

$$\log\frac{A_o - A_i}{A_f - A_o} = \log K_i + n_H \log c_a$$

where K_i - formation constant of the i^{th} protonated species, A_o - observed absorbance of the sample, A_i – absorbance of the substrate ((i-1)th protonated form, i = 1..4), A_f – absorbance of the product of the protonation reaction (i^{th} protonated form), n_H – Hill coefficient, c_a – acid concentration. Experimental values of $\log[(A_o\text{-}A_i)/(A_f\text{-}A_o)]$ were plotted against log c_a (Hill plots) and fitted with the above function. The procedure was verified with zinc phthalocyanine and zinc 2,9,16,23-tetra-*tert*-butyl-phthalocyanine. Very high values of n_H, exceeding the limits of the Hill model, were needed to fit the data for the studied trioxanonyl and *n*-butoxy derivatives. Because n_H = 1 was expected for each single protonation step, formation constants K_i were taken as x-intercepts of fitted linear plots (for reference

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zinc phthalocyanine and zinc 2,9,16,23-tetra-*tert*-butyl-phthalocyanine $n_H \approx 1$ were obtained).

Steady-state fluorescence spectroscopy

Fluorescence spectra of **1Zn** and **1Mg** in DMF solutions titrated with sulphuric acid and in chloroform solutions were recorded using a HORIBA Jobin-Yvon Fluorolog 3-2-IHR320 spectrofluorometer equipped with a 1024x256 Symphony Solo CCD camera.

The same spectrofluorometer equipped with a photomultiplier tube was used to record emission spectra for fluorescence quantum yields measurements. Unsubstituted zinc phthalocyanine dissolved in DMSO (fluorescence quantum yield 0.20) and in DMF (quantum yield 0.17) was used as a reference.[6] The samples were excited at 650 nm. The data were analyzed with the comparative method proposed by Williams *et al*.[7] Both reference solutions were cross-calibrated and the calculated correction factor was applied to the measured fluorescence quantum yields of **1Zn** and **1Mg**.

Time-resolved fluorescence measurements

Time-resolved fluorescence measurements were carried out using a home-built setup based on a standard Multichannel Picoseconds Event Timer and Time-Correlated Single Photon Counting (TCSPC) module HydraHarp400 from PicoQuant GmbH. The excitation source was a commercial titanium-sapphire laser (MaiTai from Spectra Physics) tunable in the 690-1020 nm range with over 2.5 W of average power and a pulse width of less than 100 fs at 80 MHz repetition rate. The laser beam was sent through an acousto-optic TeO_2 Bragg cell (Coherent Pulse Picker) to reduce the pulse train repetition rate down to 5 MHz. At this repetition rate the time window was much longer than the excited state relaxation time of any investigated molecule. The laser beam was either directly used for the excitation at the Q-bands of investigated molecules or sent through a second harmonic generation crystal (BBO) for the excitation in the Soret band. In the latter configuration an absorption filter and a set of dichroic mirrors were used to eliminate the remaining fundamental beam. The excitation beam was focused in the sample cell (1x1 cm quartz cuvette). The average power used for the excitation at any wavelength never exceeded 3 mW. The emitted light was collected in the direction perpendicular to the excitation beam. Fluorescence decays were measured at the magic angle (54.7 deg) to eliminate polarization artefacts. The emitted light passed through a collecting lens and an edge absorption filter so that the scattered excitation light was completely eliminated. The collected fluorescence was coupled into a Czerny-Turner monochromator (SpectraPro-150, Acton Research Corporation) to select a narrow band of the emitted light. The output focal-plane slit of the monochromator was imaged on the 50 µm active area of a single photon avalanche photodiode (Micro Photon Devices). The instrument response function (IRF) was measured by scattering the excitation light in a dilute suspension of colloidal titanium dioxide in acetone. The full width at a half-maximum of IRF never exceeded 150 ps. Data analysis was carried out using the nonlinear least squares method with a home-designed reconvolution software implemented in the Python programming language. In certain cases the reconvolution procedure required an additional negative component of the multi-exponential fit function with a time constant shorter than the IRF width. The presence of this component was attributed to imperfectly measured IRF and was not taken into account in the interpretation of the results.

The reported errors of the fluorescence decay times for **1Mg** and **1Zn** in DMF were calculated as mean standard deviations of results of multiple measurements. The errors calculated by this method were generally not greater than three times the fitting errors based on χ^2 calculation. Therefore the errors reported for other fluorescence decay times are three times the fitting errors returned by the reconvolution algorithm.

1 L. Sobotta, M. Wierzchowski, M. Mierzwicki, Z. Gdaniec, J. Mielcarek, L. Persoons, T. Goslinski and J. Balzarini, *J. Inorg. Biochem.*, 2016, **155**, 76–81.

2 M. Wierzchowski, L. Sobotta, P. Skupin-Mrugalska, J. Kruk, W. Jusiak, M. Yee, K. Konopka, N. Düzgüneş, E. Tykarska, M. Gdaniec, J. Mielcarek and T. Goslinski, *J. Inorg. Biochem.*, 2013, **127**, 62–72.

3 Patent no. EP0573201 A1, https://www.google.com/patents/EP0573201A1?cl=en

4 M. J. Cook, A. J. Dunn, S. D. Howe, A. J. Thomson and K. J. Harrison, *J. Chem. Soc. Perkin Trans. 1*, 1988, 2453.

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Additional experimental results

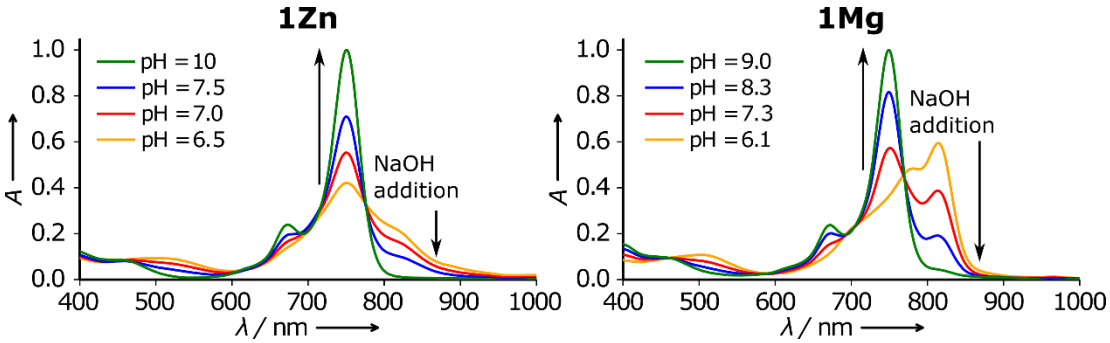


Fig. S1. Absorption changes illustrating the conversion of monoprotonated **1Zn** (left) and **1Mg** (right) created in water acidified with sulphuric acid back to the nonprotonated form upon addition of sodium hydroxide.

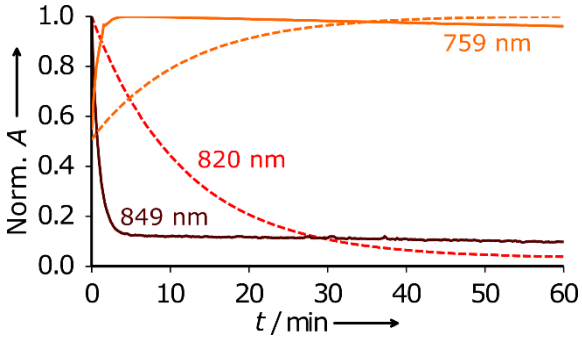


Fig. S2. Absorption changes illustrating the conversion of monoprotonated (absorption at 820 nm) and triprotonated (absorption at 849 nm) forms of **1Mg** to the diprotonated form (759 nm) in 2.6 μM DMF solution at 60 μM (dashed line) and 2.6 mM (solid line) concentrations of sulphuric acid, respectively.

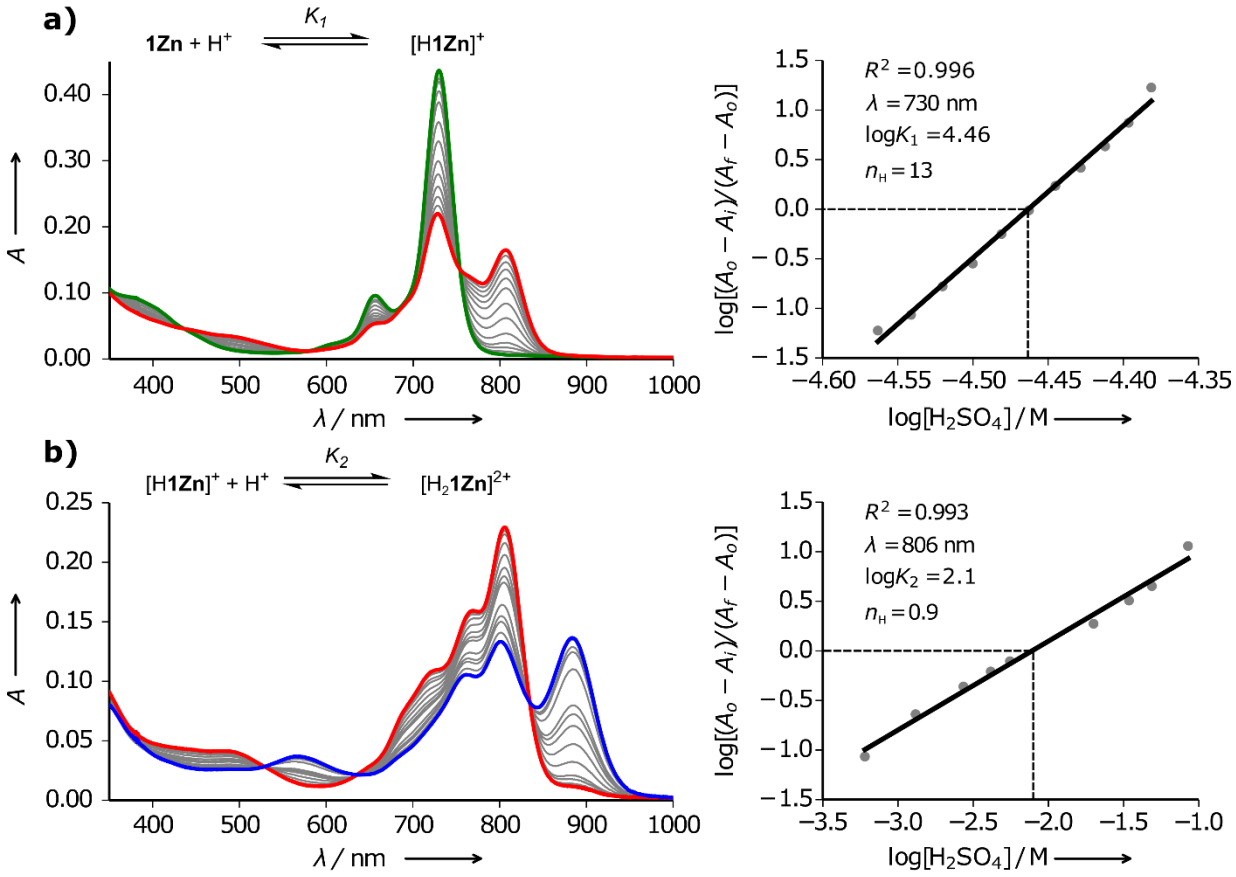


Fig. S3. Spectral evolution (left) and corresponding Hill plots (right) for the first (a) and second (b) protonation steps of **1Zn**.

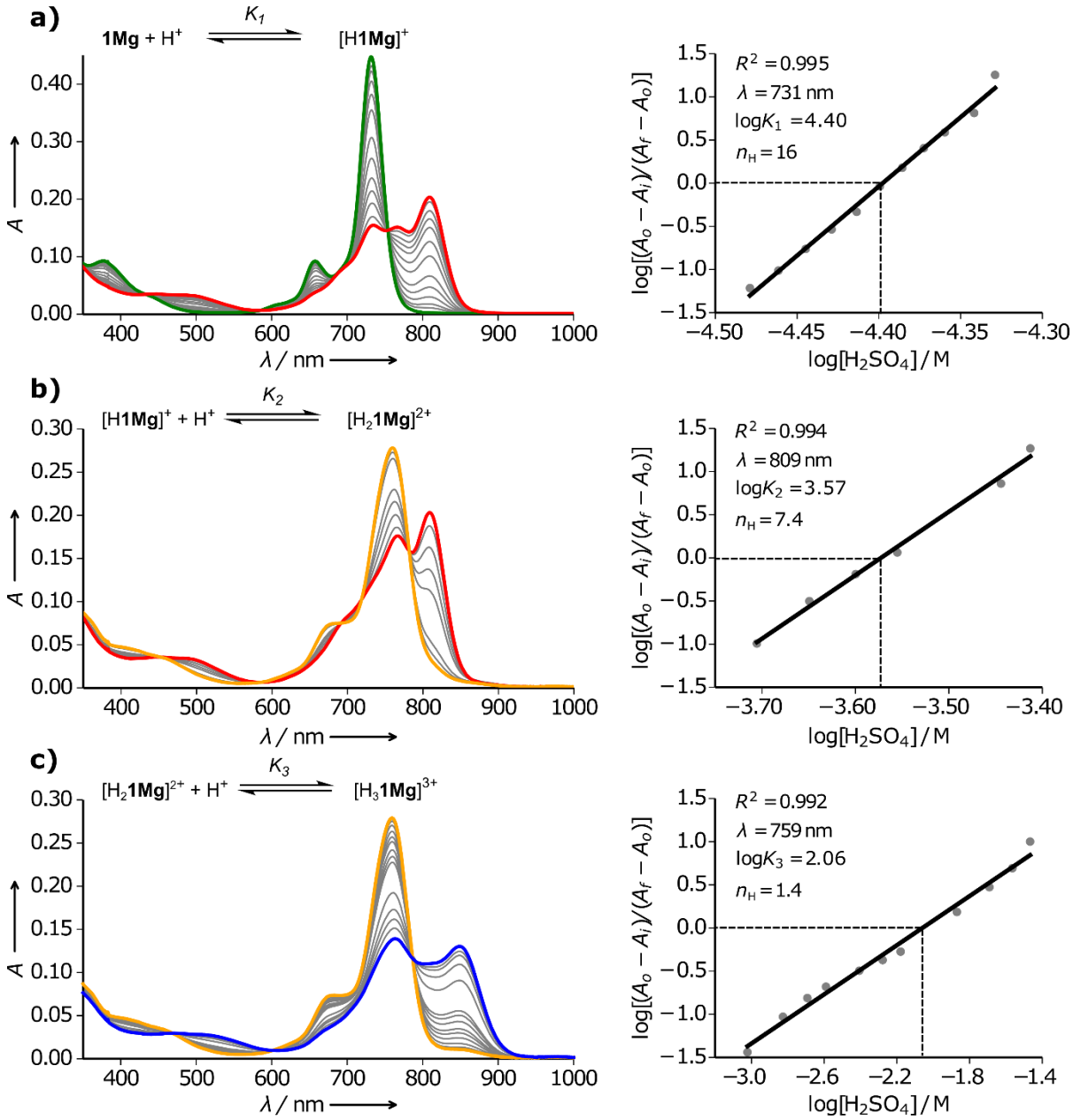


Fig. S4. Spectral evolution (left) and corresponding Hill plots (right) for the first (a), second (b) and third (c) protonation steps of **1Mg**.

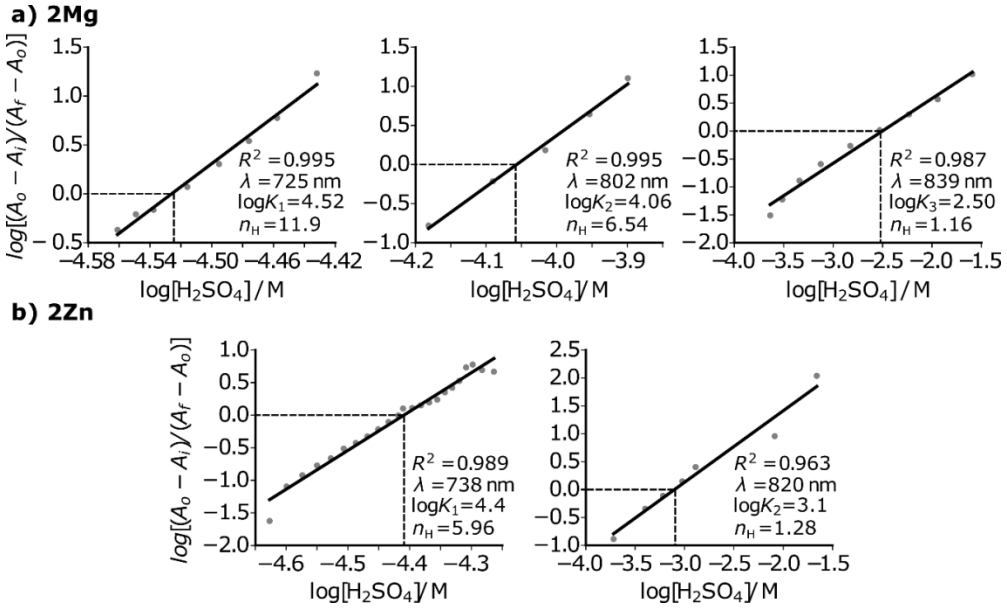


Fig. S5. Hill plots for protonation of all non-peripherally butoxy-substituted magnesium **2Mg** (a) and zinc **2Zn** (b) phthalocyanines.

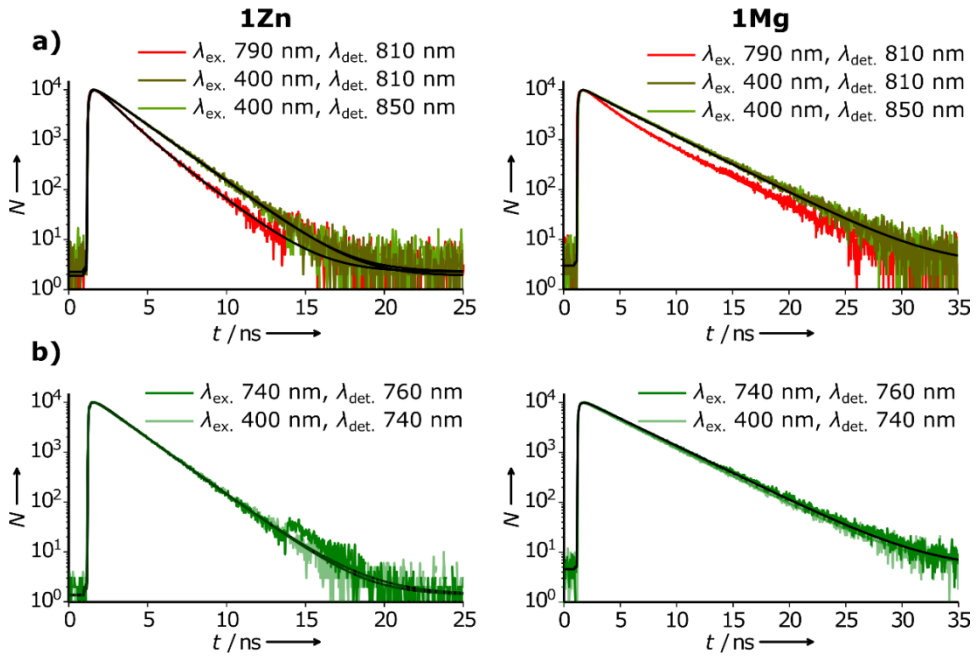


Fig. S6. a, b) Fluorescence decays of **1Zn** (left) and **1Mg** (right) in chloroform/pyridine mixtures recorded at selected excitation (λ_{ex}) and detection (λ_{det}) wavelengths

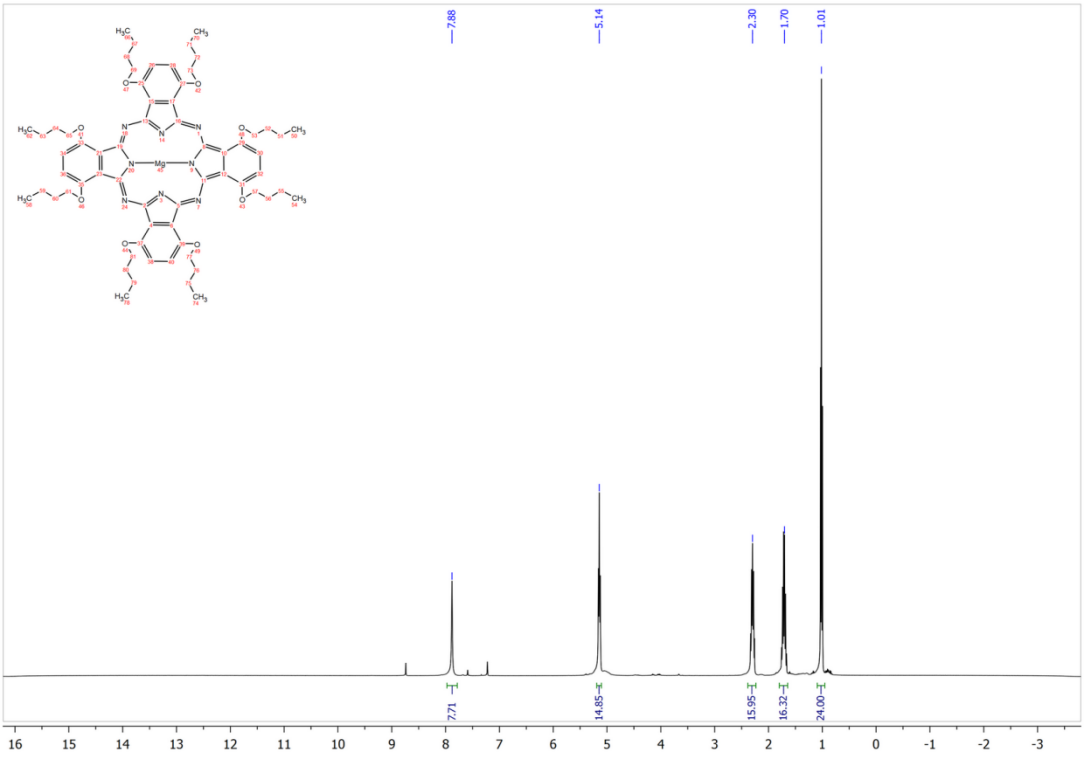


Fig. S7. ^{1}H-NMR spectrum of **2Mg** in pyridine-d_5

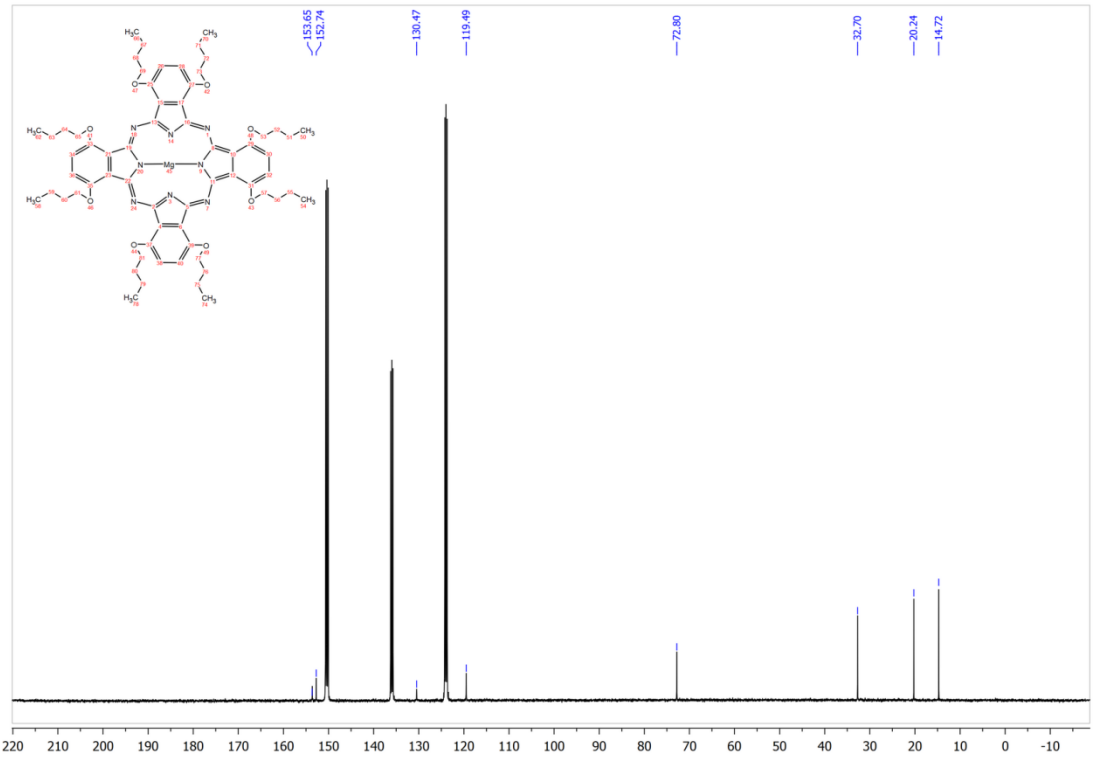
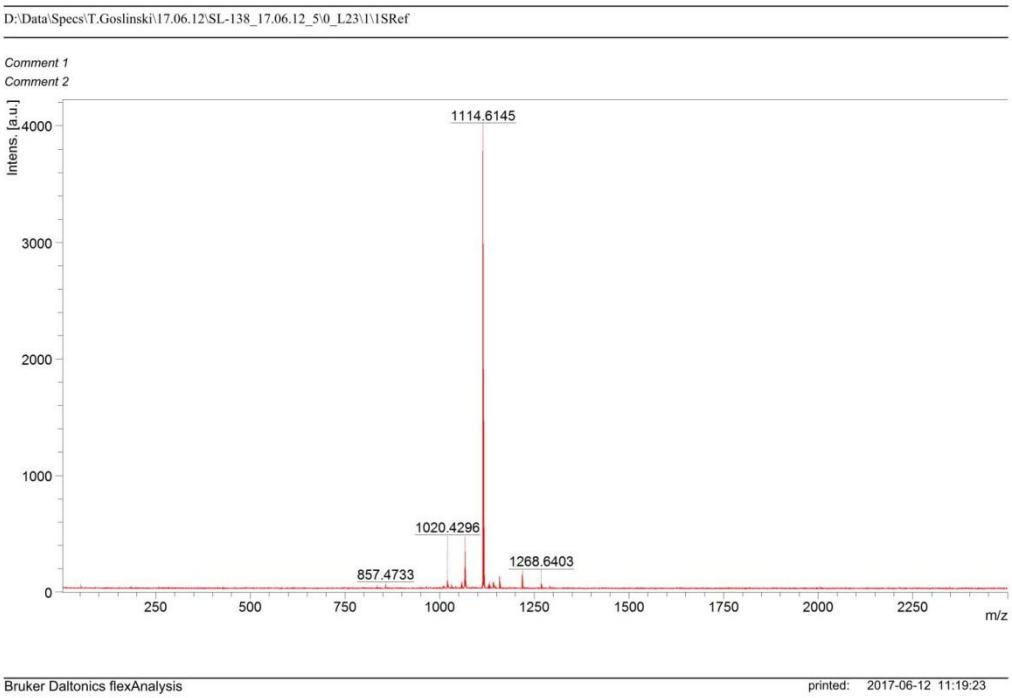


Fig. S8. ^{13}C-NMR spectrum of **2Mg** in pyridine-d_5



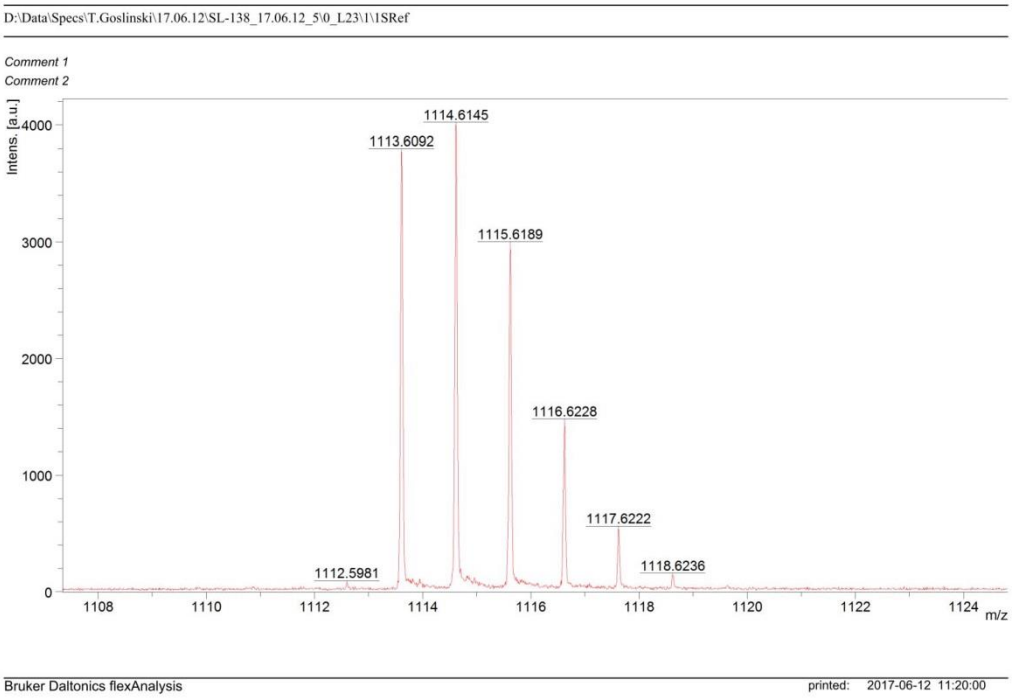
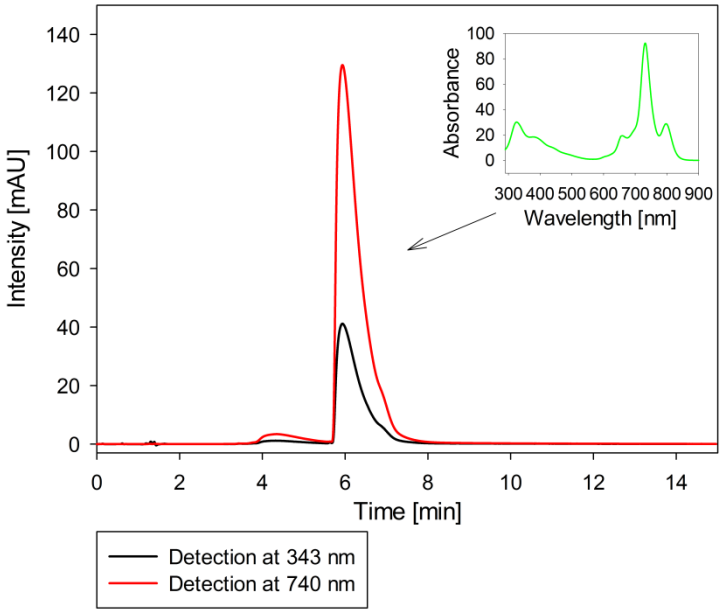


Fig. S9. Mass spectrum of **2Mg**

HPLC analysis of 2Mg

Phases configuration 1



Mobile phase
THF [%]
100

Flow	1.0 ml/min
Temperature	25°C
Column	Agilent, Eclipse XDB-C18
	150 mm · 4.6 mm, 5 µm

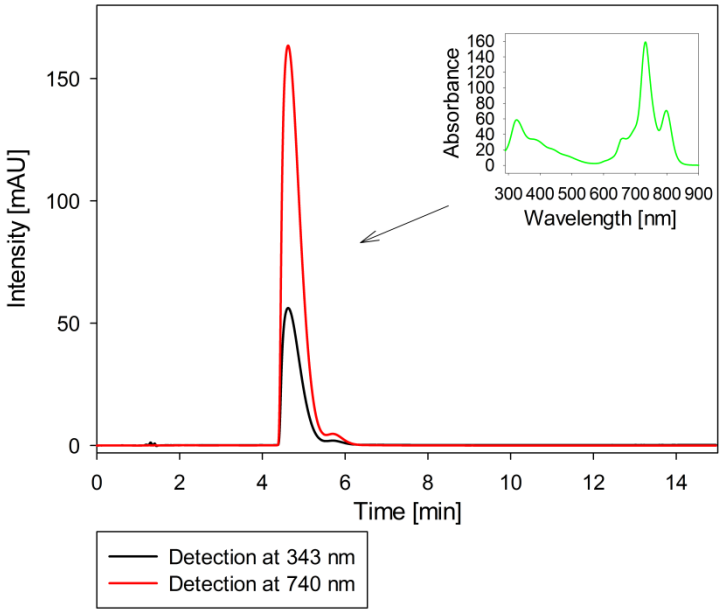
Detection at λ = 343 nm

Signal	Retention time [min]	Area	Content [%]
1	1.30	6.65	0.42
2	5.93	1572.67	99.58

Detection at λ = 740 nm

Signal	Retention time [min]	Area	Content [%]
1	5.93	5010.62	100.00

Phases configuration 2



Mobile phase

THF [%]	CH_2Cl_2 [%]
95	5

Flow	1.0 ml/min
Temperature	25°C
Column	Agilent, Eclipse XDB-C18
	150 mm · 4.6 mm, 5 µm

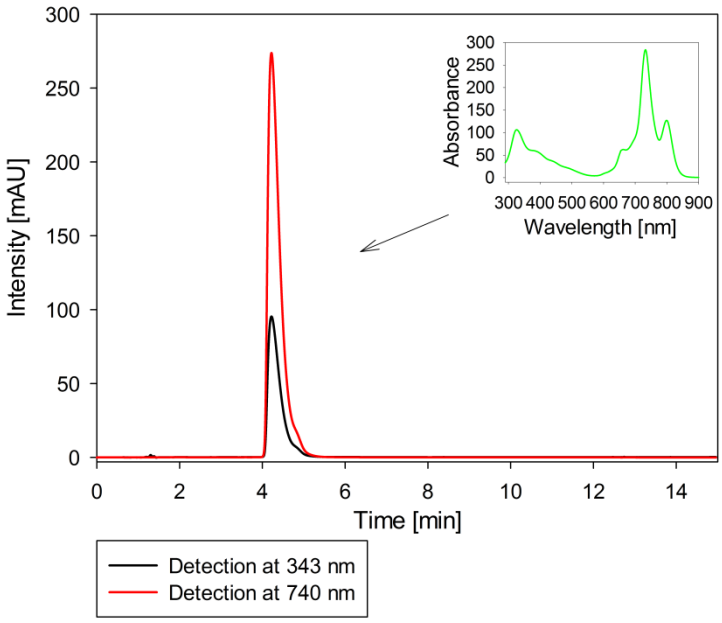
Detection at λ = 343 nm

Signal	Retention time [min]	Area	Content [%]
1	1.30	6.85	0.41
2	4.62	1665.00	99.59

Detection at λ = 740 nm

Signal	Retention time [min]	Area	Content [%]
1	4.62	4821.54	100.00

Phases configuration 3



Mobile phase

THF [%]	MeOH [%]
95	5

Flow	1.0 ml/min
Temperature	25°C
Column	Agilent, Eclipse XDB-C18
	150 mm · 4.6 mm, 5 µm

Detection at λ = 343 nm

Signal	Retention time [min]	Area	Content [%]
1	1.30	7.68	0.38
2	4.22	2016.63	99.62

Detection at λ = 740 nm

Signal	Retention time [min]	Area	Content [%]
1	4.22	5753.22	100.00