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Photodynamic activity of meso-substituted cationic pyridyl porphyrin Zn-TOEt4PyP and its non-covalent interaction product with folic acid

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Supplementary Data

Methods for the MS and NMR

^{1}H NMR Spectroscopy

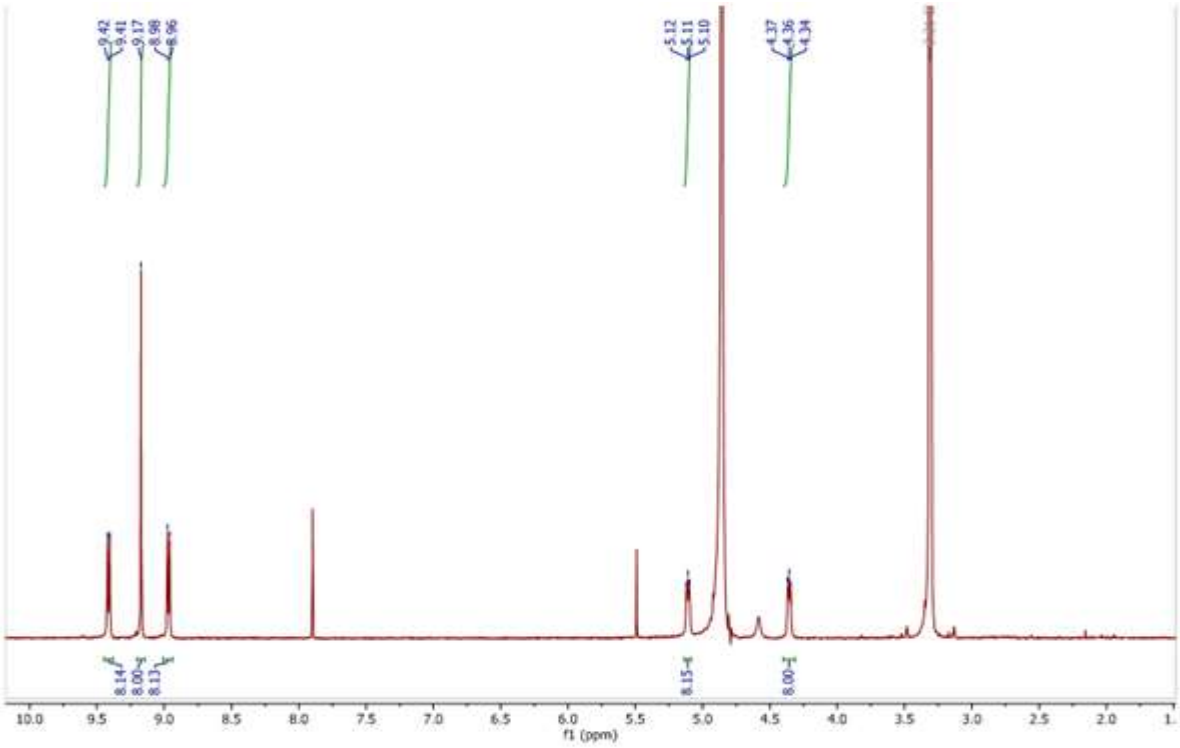
^{1}H NMR spectra were recorded at room temperature on a Bruker Avance III 500 MHz spectrometer at Chimie ParisTech – PSL, in the laboratory of Prof. Gilles Gasser. For Zn-TOEt$_4$PyP, 5 mg of sample was dissolved in 0.5 mL of deuterated methanol (CD_3OD). For folic acid, DMSO-d_6 was used as the solvent. Chemical shifts (δ) are reported in parts per million (ppm), and multiplicities are denoted as s (singlet), d (doublet), t (triplet), etc. Integration reflects relative proton counts. Spectra were processed using Bruker TopSpin software. This setup and referencing approach are consistent with published methods from the Gasser laboratory for characterizing metal–polypyridyl complexes (1).

High-Resolution Electrospray Ionization Mass Spectrometry (HR-ESI-MS)

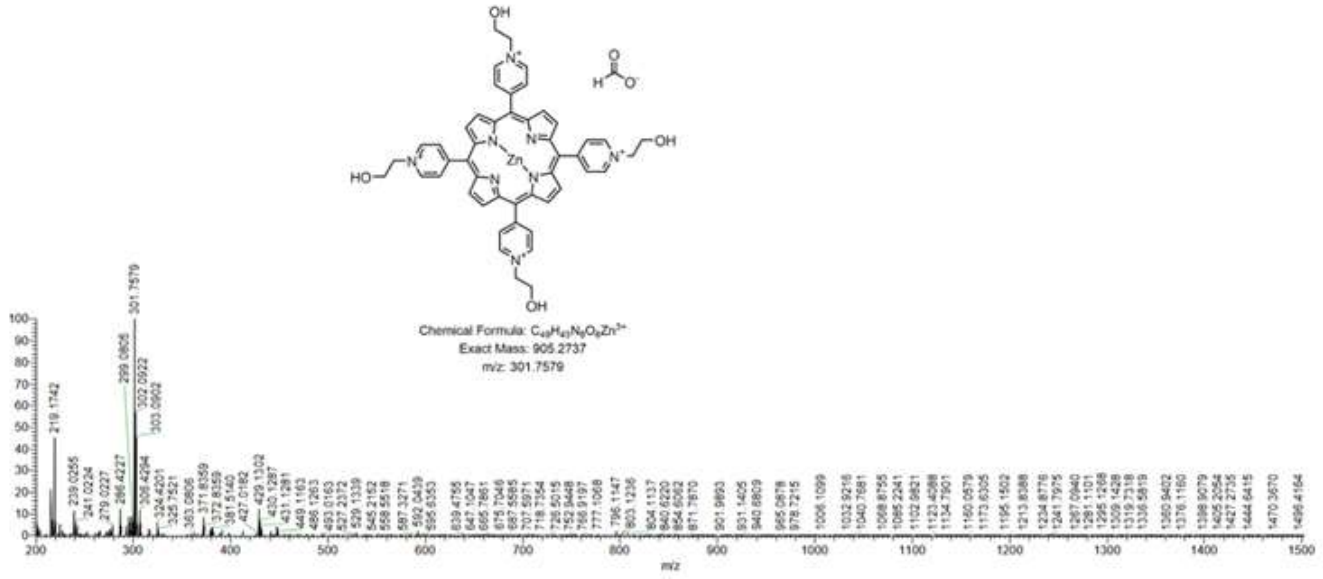
HRMS analysis was performed using a Thermo Scientific Exactive Orbitrap mass spectrometer operated in positive ion mode at the analytical platform of the Gasser laboratory (Chimie ParisTech – PSL). The sample was prepared as a 1 mg/mL solution of Zn-TOEt$_4$PyP in Milli-Q water. Data were acquired in full scan mode over the *m/z* range 200–1500 and processed using ThermoXcalibur software (1).

Results and Discussion

HRMS (ESI$^+$) *m/z*: $[M]^{3+}$ calculated for $C_{48}H_{44}N_8O_4Zn$ 301.7579, found 301.7579 (error: 0.0 ppm). The observed isotopic pattern was in excellent agreement with the theoretical distribution calculated for the zinc-containing triply charged porphyrin, confirming both the molecular composition and high purity of Zn-TOEt$_4$PyP.



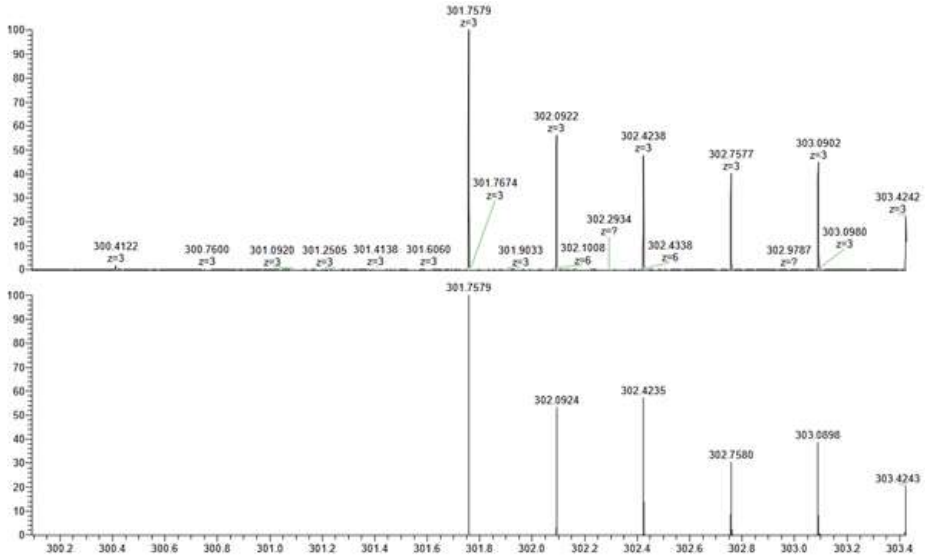
Suppl. Fig. S1 — ^{1}H NMR spectrum of Zn-TOEt4PyP (500 Mz, CD_3OD) δ9.415 (8H, d), 9.17 (8H, s), 8.97 (8H, d), 5.11 (8H, t), 4.36 (8H, t); (Relative Intensity (%) 100)



Suppl. Fig. S2 — High-resolution ESI-MS characterization of Zn-TOEt4PyP

Full Scan Spectrum

Full scan ESI-MS spectrum of Zn-TOEt4PyP acquired over the *m/z* range 200-1500. The base peak at *m/z* 301.7579 corresponds to the $[M]^{3+}$ molecular ion of the zinc porphyrin complex. Additional peaks represent fragment ions, adduct species, and multiply charged states of the parent compound.



Suppl. Fig. S3 — Isotopic Pattern for ESI-MS spectrum of Zn-TOEt4Py: Expanded view of the molecular ion region showing the experimental isotopic pattern (top) and calculated theoretical distribution (bottom) for $[C_{48}H_{44}N_8O_4Zn+HCOO]^{3+}$. The perfect match between experimental and theoretical patterns (error: 0.0 ppm) confirms the molecular composition $C_{49}H_{45}N_8O_6Zn$

MS spectrum explanation

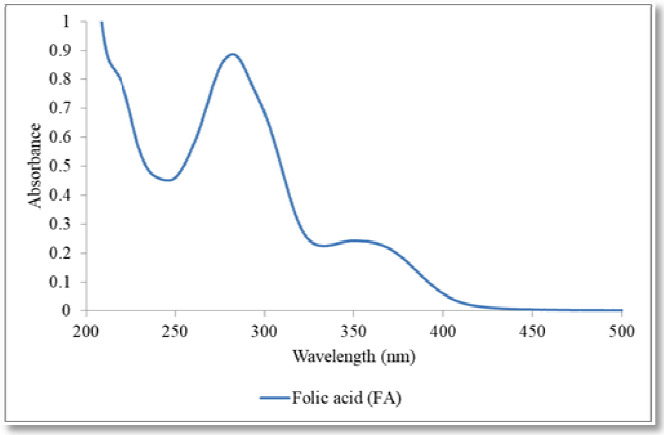
High-resolution electrospray ionization mass spectrometry (ESI-MS) was used to confirm the structure of Zn-TOEt4PyP (Fig. S2 & S3). The spectrum displays a dominant peak at m/z = 301.7579, which corresponds to the $[M]^{3+}$ species of the molecular ion $C_{35}H_{45}N_8O_2Zn^{3+}$. This value is in excellent agreement with the calculated m/z of 301.7579 for the triply charged ion of a molecule with a monoisotopic mass of 905.2737 Da.

The observed charge state (+3) is typical for porphyrin derivatives carrying multiple protonated or quaternized pyridyl substituents, and the absence of additional high-intensity peaks from other charge states (e.g., 2+ or 4+) suggests that the +3 form is the most stable and abundant under the ionization conditions used.

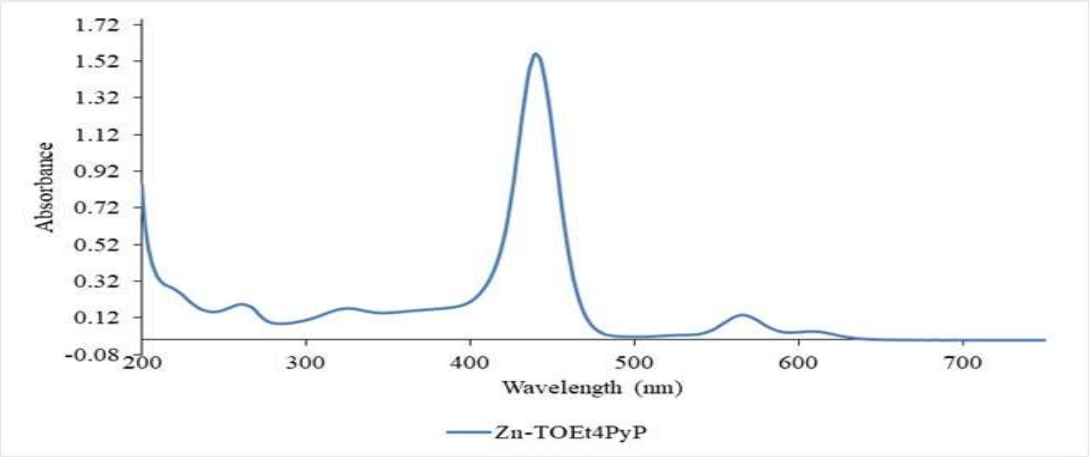
No significant fragmentation or impurity signals were detected, and the isotopic distribution of the 301.76 m/z peak matches the theoretical pattern for a Zn-containing triply charged ion. These results confirm both the successful synthesis and high purity of Zn-TOEt4PyP.

Observed m/z	Calculated m/z	Ion Species	Charge	Δ (ppm)
301.7579	301.7579	$[M]^{3+}$	+3	0.3

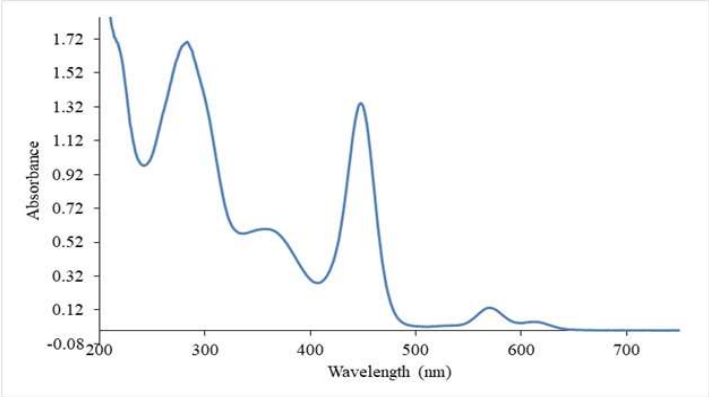
UV–Vis absorption spectra of the compounds



Suppl. Fig. S4 — UV-Vis spectrum of FA in 0.1 M PBS, 20 % glycerol, with 326,6 μM concentration, in 1 mm quartz cuvette



Suppl. Fig. S5 — UV-Vis spectrum of Zn-TOEt4PyP in 0.1 M PBS, 20 % glycerol, with 2704.5 μM concentration, in 0.1 mm quartz cuvette, 25 times diluted



Suppl. Fig. S6 — UV-Vis spectrum of Zn-TOEt4PyP+FA in 0.1 M PBS, 20 % glycerol, with 2046.72 μM concentration for Zn-TOEt4PyP, and 16886.24, in 0.1 mm quartz cuvette, 25 times diluted ([FA]/[Zn-TOEt4PyP]~8.25/1)

Synthesis of porphyrins and metalloporphyrins

5,10,15,20-tetra[4-N-(2/-oxyethylpyridyl)]porphinetetrachloride, H_2TOEt4PyP. The synthesis of H_2TOEt4PyP was carried out by boiling the mixture of H_2T4PyP (60 mg, 0.097 mmol) and 2-chloroethanol (4.5 mL, 67.2 mmol) in N,N-dimethylformamide (1.8 mL) for 4.5 h at reflux. Reaction completeness was controlled by thin-layer chromatography (chloroform:methanol – 6:1 system). The solvent was removed until the minimum possible volume remained. The final product was precipitated by acetone, filtered and washed in turn with acetone, hot propane l-2, and acetone. The obtained porphyrin was dried under reduced pressure. Yield: 90 mg (98.6%). Anal. calcd. for $C_{48}H_{46}Cl_4N_8O_4$: C, 61.28; H, 4.93; N, 11.91%. Found: C, 61.03; H, 5.35; N, 12.14%. UVvis (H_2O): λmax, nm (log ε) 424 (5.32), 519 (4.176), 556 (3.763), 585 (3.806), 640 (3.176). 1H NMR (300MHz; DMSO-d6; Me4Si): δH, ppm -3.06 (2H, s, pyrrole-NH), 4.21 (8H, dt, J1 = 6.0, J2 = 4.1, -OCH2-), 5.04 (8H, t, J = 4.1, -NCH2-), 5.86 (4H, t, J = 6.0,-OH), 9.03 (8H, m, C5H4N), 9.29 (8H, s, pyrrole-H), 9.52 (8H, m, C5H4N). 13C NMR (300 MHz; DMSO-d6; Me4Si): δC, ppm 60.120 (-CH_2OH), 63.307 (-CH_2N+),115.847 (meso-C-porphine), 132.131 (2-C-pyridyl), 132.616 br. (β-C-pyrrole), 143.820 (3-C-pyridyl), 156.552 (1-C-pyridyl).

5,10,15,20-tetra (4-N-pyridyl) porphine, H_2T4PyP and 5,10,15,20-tetra (3-N-pyridyl) porphine, H_2T3PyP. The initial porphyrins H_2T4PyP and H_2T3PyP were synthesized by the Adler-Longo method of condensation (3) via interaction of appropriate 3- or 4-pyridinecarboxaldehydes with pyrrolein propionic acid. The reaction mediums were evaporated under high vacuum and the products were purifiedby flash column chromatography. The results of 1H NMR and electronic absorption spectroscopy were in agreement with literature data (4).

The zinc(II) metallocomplex of the 5,10,15,20-tetra[4-N-(2/-oxyethylpyridyl)]porphinetetrachloride, ZnTOEt4PyP, was prepared by refluxing the mixture of zinc(II) chloride ($ZnCl_2$) and H_2TOEt4PyP similarly, to that described in this article in the same way that as for the complexes 5,10,15,20-tetra(4-N-allylpyridyl)porphinato Zn(II) tetrabromide, ZnTAll4PyP (2).

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