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Design and synthesis of water-soluble bioconjugatable *trans*-AB-porphyrins

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Abstract

Three free base porphyrins have been prepared that bear a polar and facially encumbering 2,4,6-tris (carboxymethoxy)phenyl motif at one meso (5-) position. The only other substituent (15-position) comprises phenyl, formyl, or *p*-aminophenyl. The porphyrins exhibit solubility in water (or aqueous buffer solutions) at pH ≥ 7 and concentrations > 1 mM at room temperature. The concise syntheses, water-solubility, and bioconjugatable handle make these porphyrin constructs suitable for biological applications.

Keywords

Porphyrin; Water; Hydrocarbon; Water-soluble; Facial encumbrance; Bioconjugation

1. Introduction

Tetrapyrrolic molecules have distinct chemical and photophysical properties that are attractive for a wide range of diagnostic and therapeutic applications in the field of photomedicine. The applications encompass optical imaging of diseased tissue,^{1–3} fluorescent labeling in flow cytometry,^{4–7} photodynamic inactivation of microbial infections,^{8–10} and photodynamic therapy of solid tumors.^{11–15} A key challenge to the implementation of tetrapyrrolic molecules in photomedicine entails tailoring the molecules to exhibit appropriate solubility. In particular, synthetic motifs that impart water solubility are important for a number of biological studies. Tetrapyrrolic macrocycles (porphyrins, chlorins, and bacteriochlorins) present a particular challenge in this regard owing to the large size of the planar hydrocarbon macrocycle. In recent years, approaches for solubilizing related carbon-rich molecules such as fullerenes and nanotubes have relied on grafting polar substituents to create a polar layer or sheath that encompasses the hydrophobic molecule.^{16–20} For biological applications of tetrapyrrolic macrocycles, a typical requirement is to introduce substituents to achieve both water-solubility and facility for conjugation to biomolecules.²¹

Water solubilization of porphyrins has traditionally been achieved by use of uroporphyrin, which bears eight β -substituted alkylcarboxylic acids,^{22–26} or by attachment of small aryl motifs such as *N*-alkyl pyridinium or phenylsulfonic acid units to the meso sites of synthetic porphyrins²⁷ (Chart 1). In the latter case, four polar substituents (one at each of the meso sites)

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traditionally were introduced to impart water solubility. The use of four substituents stemmed from the synthetic methods of the day,²⁸ which only readily supported the synthesis of such A₄-porphyrins. Regardless, the most easily synthesized members – the pyridinium- or sulfoaryl-derivatized porphyrins – exhibit only limited solubility in aqueous media. Moreover, the sense that four such groups were essential also presented an impediment to further functionalization of the macrocycle, as required for applications where bioconjugation is required. The advent of versatile and rational synthetic routes to compact porphyrins that bear less than four meso substituents^{29,30} (e.g., *trans*-AB-porphyrins) has opened the door to consideration of new molecular designs for water solubilization that are both potent and compatible with features required for bioconjugation.

In parallel with the development of new synthetic methods, new water-solubilization motifs have been developed to serve a variety of objectives. Representative examples of such solubilization motifs include oligoethylene glycol monomers³¹ and dendrimers,³² alkyl polyamines,³³ and polycarboxy chains.³⁴ While each such group has merit, most are quite large and are not well suited for our goals in developing compact bioconjugatable molecules for use in photomedical applications.

We initiated a program to develop water-solubilization motifs that ideally adhere to the following design guidelines: (1) ionic groups are more potent than non-ionic groups for solubilization, (2) anionic groups are generally preferred over cationic groups to minimize non-specific binding to cellular structures, (3) the projection of polar substituents above and below the plane of the macrocycle is expected to suppress cofacial aggregation of the porphyrins, and (4) solubilization is desired with a single group so as to achieve a compact molecular architecture. The emphasis on groups that project over the porphyrin macrocycle stemmed from our finding that the mesityl group and homologues (e.g., 2,4,6-triethylphenyl) impart increased organic solubility (versus phenyl) to porphyrins.^{35,36} The results from this program include a 1,5-diphosphonopent-3-yl group (swallowtail moiety, **I**),^{7,37} a *N,N*-diethylimidazolium-2-yl unit (**II**),³⁸ and a 2,6-bis(phosphonomethoxy)phenyl moiety (**III**)³⁹ (Chart 2). The first two solubilization moieties (**I**, **II**) have been incorporated as one substituent in *trans*-AB-porphyrins wherein the distal substituent constituted a bioconjugatable handle (carboxylic acid, iodoacetamide).^{7,37,39} The diphosphonate solubilization moieties (**I**, **III**) have been incorporated in a synthetic chlorin.³⁹ Jux also has developed facially encumbering motifs for porphyrins by reaction of a 2,6-bis(bromomethyl)aryl group located at the porphyrin meso-position with 4-*tert*-butylpyridine or with diethyl malonate.^{40–43}

Here we describe an additional water-solubilization motif that provides facial encumbrance, the 2,4,6-tris(carboxymethoxy)phenyl unit. This motif is incorporated as one substituent in a *trans*-AB-porphyrin. The other substituent is a handle suitable for bioconjugation. Typical groups for bioconjugation include isothiocyanate and imidate esters (for amines) and iodoacetamide (for sulfhydryls).⁴⁴ To perform bioconjugation in aqueous solution, and therefore avoid the solubility problems that can arise with mixed aqueous-organic media, the carboxy moieties must be deprotected. Hence, the conjugatable groups should be compatible with the carboxylic motif to allow for selective bioconjugation. We have elected to focus on aldehyde and amine groups, which are suitable for reductive amination in aqueous media. Reductive amination has been used successfully for derivatization of tetrapyrrolic macrocycles.^{45–48} This design complements prior examples of *trans*-AB-porphyrins that bear a bioconjugatable handle including isothiocyanate,²¹ carboxylic acid/ester,^{7,38} and iodoacetamide.³⁷ Taken together, the work described herein provides an additional approach for the design and synthesis of compact, water-soluble, bioconjugatable porphyrins.

2. Results and discussion

2.1. Molecular design and synthesis strategy

Each target molecule (**H₂P-1-3**) is a free base *trans*-AB-porphyrin (Chart 3). The *trans*-AB-porphyrin architecture, wherein the porphyrin contains only two meso substituents, was chosen to maintain a compact size. Each of the porphyrins is tailored with a water-solubilizing 2,4,6-tris(carboxymethoxy)phenyl substituent at one meso position. The 2,4,6-tris(carboxymethoxy)phenyl unit is relatively compact. The mass of this water-solubilizing moiety (C₁₂H₁₁O₉, 299 Da) is comparable to that of the core porphine unit (C₂₀H₁₂N₄, 308 Da) in the *trans*-AB-porphyrin.

The 2,4,6-tris(carboxymethoxy)phenyl unit is an *o,o'*-disubstituted meso-aryl motif, whereby the polar groups are (i) in close proximity to the porphyrin macrocycle and (ii) are thrust in an orientation that encumbers the faces of porphyrin, thereby impeding aggregation. The introduction of 2,6-disubstituted aryl units to porphyrins has presented challenges over the years; however, the *o*-benzyloxy motif is compatible with the steric constraints encountered upon formation of the pyrromethane intermediate (e.g., dipyrromethane, porphyrinogen) on the path to the porphyrin.⁴⁹ Indeed, we previously synthesized porphyrins that bear 2,6-bis(benzyloxy) and 2,4,6-tris(benzyloxy) substituents at the meso positions wherein a number of substituents were introduced on the arene moiety of the benzyloxy group.^{36,50,51}

The conjugatable groups should be compatible with carboxylic acids to allow for selectivity during the bioconjugation step. Hence, conjugatable groups include a formyl group for porphyrin **H₂P-2** and a *p*-aminophenyl group for **H₂P-3**. Porphyrin **H₂P-1**, which has a meso-phenyl substituent in lieu of a bioconjugatable handle, was prepared as a benchmark for the solubility studies.

The synthesis relied on two methods for the preparation of *trans*-AB-porphyrins. One method employs condensation of a 1,9-bis(*N,N*-dimethylaminomethyl)dipyrromethane with a dipyrromethane in refluxing ethanol containing zinc acetate followed by DDQ oxidation.²⁹ The second method requires the 1,9-diformylation of a dipyrromethane and subsequent imination of the 1,9-diformyldipyrromethane by treatment with *n*-propylamine. Reaction of the resulting 1,9-bis(imino)dipyrromethane with a dipyrromethane in the presence of zinc acetate and air leads to the corresponding *trans*-AB-porphyrin.³⁰ In both routes the zinc porphyrin is obtained. Both routes typically afford a single porphyrin product, albeit in rather low yield (5–30%).

The *tert*-butyl group was chosen as the protective moiety for each of the three carboxylic acids of the water-solubilization motif. The water-solubilization motif to be introduced thus contains three phenolic ethers and three esters. Accordingly, the 1,9-functionalization (imination or aminoalkylation) was performed on the complementary dipyrromethane.

2.2. Synthesis

2.2.1. Porphyrin H₂P-1—The *O*-alkylation^{51,52} of 2,4,6-trihydroxybenzaldehyde with *tert*-butyl bromoacetate in the presence of K₂CO₃ gave the alkylated product **1** in 82% yield. Condensation⁵³ of aldehyde **1** with pyrrole (40 equiv) in the presence of InCl₃ followed by crystallization gave dipyrromethane **2a** in 35% yield (Scheme 1). Condensation of dipyrromethane **2a** with 1,9-bis(imino)dipyrromethane **3a**³⁰ in refluxing ethanol containing excess Zn(OAc)₂ with exposure to air gave the corresponding protected zinc ester-porphyrin **ZnP-1'** in 18% yield. Treatment of **ZnP-1'** with aqueous KOH (2 M) in THF at 60 °C for 8 h afforded cleavage of the esters. The porphyrin was extracted in the aqueous layer and concentrated to give the zinc porphyrin with free carboxylic acids. Upon adjusting an aqueous solution of the latter compound to pH = 3 (by addition of 2 M aqueous HCl), the zinc porphyrin

was demetalated and the resulting free base porphyrin **H₂P-1** was isolated as a precipitate in 82% yield (overall from **ZnP-1'**). The porphyrins were characterized by ¹H NMR spectroscopy, ESI-MS or LD-MS (laser-desorption mass spectrometry in the absence of a matrix),⁵⁴ absorption spectroscopy, and fluorescence spectroscopy.

2.2.2. Porphyrin H₂P-2—Condensation of the 1,9-bis(*N,N*-dimethylaminomethyl)-5-acetaldipyrromethane **4**²⁹ and dipyrromethane **2a** in refluxing ethanol containing Zn(OAc)₂ followed by oxidation with DDQ gave the corresponding zinc porphyrin **ZnP-2'** in 10% yield (Scheme 2). Attempts to simultaneously cleave both the *tert*-butyl and the acetal protective groups with a biphasic solution⁵⁵ of TFA/H₂O/CH₂Cl₂ gave a mixture of porphyrins. Treatment of **ZnP-2'** with neat TFA at room temperature for 3 days unveiled the carboxylic and formyl groups, giving **H₂P-2** in 77% yield. The structure of **H₂P-2** was confirmed by LD-MS, ESI-MS and ¹H NMR analyses. The purity of the porphyrin **H₂P-2** was estimated to be 90% according to the ¹H NMR analysis. Attempts to purify porphyrin **H₂P-2** employing reversed-phase (RP) chromatography were not successful.

To achieve greater purity, stepwise deprotection conditions were employed. The carboxylic acid units were unveiled by treatment of porphyrin **ZnP-2'** with aqueous KOH (2 M) in THF at 60 °C for 12 h. Aqueous-organic workup and subsequent treatment of the crude product with neat TFA at 50 °C for 24 h resulted in deprotection of the formyl group. The crude reaction mixture was diluted with H₂O to pH 3 to induce precipitation of the porphyrin. Sonication, centrifugation, decanting and drying of the resulting solid pellet gave porphyrin **H₂P-2** (overall 65% yield) in pure form without chromatography. Porphyrin **H₂P-2** was characterized by ¹H NMR and ¹³C NMR spectroscopy, absorption and fluorescence spectroscopy, and LD-MS and ESI-MS analyses.

2.2.3. Porphyrin H₂P-3—Dipyrromethane **2b**⁵⁶ was subjected to Vilsmeier formylation to give the corresponding 1,9-diformyldipyrromethane **5** in 68% yield. Condensation of **5** with excess *n*-propylamine gave the corresponding 1,9-bis(imino)dipyrromethane **3b** quantitatively. The resulting 1,9-bis(imino)dipyrromethane **3b** was employed promptly for porphyrin formation without further purification. The condensation of 1,9-bis(imino)dipyrromethane **3b** and dipyrromethane **2a** in refluxing toluene for 12 h gave porphyrin **ZnP-3'** in 28% yield (Scheme 3). No other porphyrin products (e.g., derived from scrambling) were detectable by LD-MS analysis.

The deprotection of **ZnP-3'** was examined under various conditions, including TFA in CH₂Cl₂ (6 days), neat TFA (2 days), concentrated aqueous HCl/CH₂Cl₂ (5 days), TMSBr/triethylamine in CHCl₃ (2 days), and LiOH in aqueous THF (1 day), all of which gave incomplete reaction or mixtures of products. Treatment with KOH (2 M) and THF for 28 h at 60 °C gave a porphyrin that lacked the *tert*-butyl esters but retained the *tert*-BOC protective group on the amino moiety, as indicated by LD-MS and ESI-MS data. Treatment with TFA at room temperature for 11 h gave the target porphyrin **H₂P-3** in 83% overall yield. Porphyrin **H₂P-3** was characterized by ¹H NMR and ¹³C NMR spectroscopy, absorption and fluorescence spectroscopy, and LD-MS and ESI-MS analyses.

2.3. Characterization

2.3.1. Molecular modeling—A molecular model of porphyrin **H₂P-1** is shown in Figure 1. The figure shows the projection of the carboxymethoxy substituents above and below the plane of the porphyrin macrocycle. A considerable range of motion is available to the 2,6-carboxymethoxy units owing to rotation about the single bonds of the carboxymethoxy moiety, and the torsion of the aryl unit with respect to the plane of the porphyrin (by rotation about the single bond between the aryl carbon and the porphyrin meso carbon). On account of the

combined motions, the hydroxy moiety of the carboxylic acid can extend as far as the pyrrolic NH and pyrrolyl units (and contact the π -chromophore of the porphyrin), project almost normal to the plane of the porphyrin macrocycle, or project back over the aryl unit.

2.3.2. Chemical characterization—All porphyrins were characterized by ^1H NMR and ^{13}C NMR spectroscopy, mass spectrometry (LD-MS, ESI-MS and/or FAB-MS), and absorption and fluorescence spectroscopy. Analytical RP-HPLC was also employed to confirm the purity of the hydrophilic porphyrins (**H₂P-1-3**). The hydrophobic porphyrins (**ZnP-1'-3'**) were examined by ^1H NMR spectroscopy in CDCl_3 , and by absorption and emission spectroscopy in toluene. The hydrophilic porphyrins (**H₂P-1-3**) were examined by ^1H NMR spectroscopy in DMSO, and by absorption and emission spectroscopy in water.

The hydrophilic porphyrins did not give observable NH and COOH signals upon ^1H NMR spectroscopic analysis in DMSO- d_6 due to broadening/exchange with solvent. The ^1H NMR spectra were typically characteristic of meso-substituted porphyrins, with the exception of a splitting in the peak attributed to the inner NH of the free base porphyrins; this phenomenon occurred with each of **H₂P-1-3**. A variable temperature experiment with **H₂P-2** over the range 20 °C to 80 °C showed that the peaks coalesced upon heating to 80 °C, with a coalescence temperature estimated to be ~60 °C (Figure 2). The origin of the splitting at room temperature is not known but likely stems from different conformations of the 2,6-bis(carboxymethoxy) phenyl unit (*vide supra*).

The hydrophilic porphyrins were examined by absorption spectroscopy in a range of solvents, including THF, DMSO, water, and aqueous buffer solutions. In each case, the Soret band (~410 nm) was broadened in all solvents examined versus that of a benchmark *trans*-A₂-porphyrin (5,15-diphenylporphyrin,⁵⁷ **H₂P-4**) in toluene or DMSO. The absorption spectrum of **H₂P-3** is displayed in three solvents (water, THF, DMSO) in Figure 3.

The breadth of the Soret band was assessed by the full width at half maximum (fwhm) for the hydrophilic porphyrins in a range of solvents (THF, MeOH, DMSO, water, aqueous buffer solutions of pH 7 and 10) and for the hydrophobic porphyrins in toluene and DMSO. Regardless of solvent, the hydrophilic porphyrins (**H₂P-1-3**) exhibited broader Soret bands (~20–30 nm or greater versus ~11–14 nm) than those of the hydrophobic porphyrins (**ZnP-1'-3'**). This difference does not stem from the different metalation states (the former are free base porphyrins whereas the latter are zinc porphyrins) but must be due to the different conformations of the polar, ionizable carboxylic acid moieties (at the 2,6-positions of the meso-aryl unit) and resulting range of electronic interaction with the π -chromophore of the porphyrin. Indeed, the benchmark porphyrin (**H₂P-4**) lacks the solubilizing feature and exhibits a sharp Soret band (fwhm = 13 nm). The results from an extensive study are summarized in Table 1.

The only notable absorption spectral distinction observed in water versus other solvents among the hydrophilic porphyrins occurred for **H₂P-2**, the porphyrin bearing a meso-formyl group. In DMSO, examination of the visible region showed peaks (declining order of intensity) at 561, 641, 584 (shoulder) and 518 nm. In water the peaks in the visible region were substantially broader, which remained unchanged upon dilution (*vide infra*), suggesting that these features are characteristic of the meso-formyl porphyrin in aqueous media rather than due to aggregation. Finally, upon increasing the pH to 10, the Soret band broadened to a fwhm of 63 nm (entry 19, Table 1).

The porphyrins also were characterized by fluorescence emission spectroscopy with excitation into the Soret band. The hydrophobic, zinc ester-porphyrins were analyzed in toluene and exhibited emission characteristic of zinc *trans*-AB-porphyrins, which consisted of two bands at ~578 and 632 nm. The hydrophilic, deprotected free base porphyrin analogues were analyzed

in water and also exhibited two emission bands. The emission maxima were typical for free base *trans*-AB-porphyrins bearing *meso*-aryl substituents for **H₂P-1** (625 and 686 nm) and **H₂P-3** (629 and 688 nm), but were shifted bathochromically for **H₂P-2** (641 and 707 nm). The shift in the emission spectrum of **H₂P-2** stems from the presence of the *meso*-formyl substituent, which is known to be a potent auxochrome.^{58,59}

2.3.3. Water solubility—The hydrophilic porphyrins (**H₂P-1–3**) were examined for solubility at room temperature in aqueous media of physiologic pH 7.4 and higher. Solubility was assessed by visual inspection using pure water or aqueous buffer solutions (pH 7, 7.4, 8, and 10) at porphyrin concentrations of 10 to 14 mM (using short-pathlength cells). Each of the porphyrins **H₂P-1–3** gave an optically clear solution in an aqueous buffer solution at pH 7 or higher for porphyrin concentrations in this regime.

One method to detect the aggregation of porphyrins entails the comparison of absorption spectra of various concentrations. In the case of no aggregation, the shape of the absorption spectra remain constant upon change of concentration of the sample. For this determination, stock solutions of porphyrins **H₂P-1** (14 mM), **H₂P-2** (10 mM) and **H₂P-3** (14 mM) were prepared in an aqueous buffer at pH 7. The absorption spectra were collected in the same buffer solution at concentrations obtained by 1, 5 and 10 fold-dilution using cuvettes of different path lengths ($l = 1, 5$ and 10 mm), thereby in principle affording equal absorbancies. The spectra of porphyrins **H₂P-1–3** are shown in Figure 4. A very slight change was observed in the spectra of **H₂P-1** (over the concentration of 2.8, 0.56 and 0.28 mM) and **H₂P-2** (2.5, 0.5 and 0.25 mM), whereas the three spectra of porphyrin **H₂P-3** (3, 0.6 and 0.3 mM) were superimposable with each other. The spectra suggest that any aggregation across this concentration regime is at most very slight.

3. Outlook

The molecular design of water-soluble tetrapyrrolic molecules generally begins with a non-polar, carbon-rich macrocycle that must be functionalized to achieve water solubility (and bioconjugatability). The use of tetrapyrrolic molecules in aqueous media might seem unnecessarily complex, given the availability of numerous organic dyes that are soluble in water. However, such dyes typically bear an intrinsic charge; consequently, the synthetic chemistry and purification of elaborate analogues of such dyes can be quite cumbersome.⁶⁰ The tetrapyrrole family presents the opportunity to carry out all synthetic manipulations on neutral, non-ionic compounds in organic solution, and then unveil the groups for water-solubilization and bioconjugation in the last step of the synthetic process. In addition, few organic dyes present the opportunities for molecular tuning that are available with the tetrapyrrolic macrocycles by virtue of chelation of different metals, reduction of the macrocycle (porphyrin, chlorin, bacteriochlorin), and introduction of diverse substituents.

The *trans*-AB-porphyrins described herein provide a compact architecture with controlled substitution pattern. The presence of a single conjugatable group enables formation of a single product upon bioconjugation, as opposed to a mixture that results with porphyrins that have multiple reactive functional groups. It is noteworthy that chromatography was not employed in the purification of the water-soluble porphyrins upon unveiling the water-solubilizing carboxylic acid moieties. As anticipated, introduction of the polar and facially encumbering 2,4,6-tris(carboxymethoxy)phenyl motif on the macrocyclic perimeter increased the hydrophilicity of the porphyrins. The hydrophilic porphyrins proved to be soluble in aqueous buffer solutions at $\text{pH} \geq 7$ and concentrations of at least 1 mM at room temperature. The target molecules are amenable to conjugation with biologically active molecules.

We now have described four motifs for imparting water solubility to tetrapyrrolic macrocycles, ^{7,37–39} all of which rely on facial encumbrance of polar groups. The potency of water-solubilization appears to decline in the order of diphosphonate-bearing groups (swallowtail, 2,6-aryl) > 2,4,6-tris(carboxymethoxy)phenyl > *N,N*-dialkylimidazolium-2-yl. One distinction between the three types of groups is that the diphosphonate-derivatized group is expected to be ionized (and hence to impart solubility) at essentially all physiological pH ranges, the carboxy-derivatized group would be expected to be ionized only at neutral to basic pH ranges, and the imidazolium group is permanently charged. Accordingly, the use of the carboxy-containing motif described herein may be expected to enable ready penetration of lipid membranes and also selective precipitation upon exposure to acidic environments. The former feature may be essential for bioavailability whereas the latter may be attractive for localization in sub-cellular domains, as required for staining or for targeted molecular brachytherapy.⁶¹

4. Experimental section

4.1. General methods

All ¹H NMR spectra (400 MHz) and ¹³C NMR spectra (100 MHz) were collected in CDCl₃ unless noted otherwise. The DMSO-*d*₆ employed was 99.9% pure. Absorption spectra were collected in toluene at room temperature unless noted otherwise. Mass spectra of porphyrins were obtained via laser desorption mass spectrometry (LD-MS) without a matrix,⁵⁴ by high-resolution fast atom bombardment mass spectrometry (FAB-MS) using a matrix of nitrobenzyl alcohol and polyethylene glycol, and by electrospray ionization mass spectrometry (ESI-MS). Positive ions were detected unless noted otherwise. Silica gel (40 μm average particle size) was used for column chromatography. Molecular modeling was carried out using Pmodel version 7.50.00 (Serena Software).

The aqueous buffer solutions employed were the following: (i) potassium phosphate (0.05 M, pH 7); (ii) potassium phosphate monobasic (0.05 M, pH 7) was titrated with sodium hydroxide to give pH 7.4 or pH 8; and (iii) potassium carbonate/potassium borate/potassium hydroxide (0.05 M, pH 10).

Analytical RP-HPLC in all cases was carried out using a reversed-phase, C-18 column (5 μm, 125 mm × 4 mm) with the following elution program: flow rate = 1.5 mL/min; 0–2 min, 0% B; 2–40 min 95% B; 40–50 min 100% A; A = water (0.1% TFA), B = methanol (0.1% TFA); detection at 405 nm. In all cases, the void volume corresponded to t = 2 min.

4.2. Noncommercial compounds

Dipyrromethanes **2b**,⁵⁶ **3a**,³⁰ and **4**²⁹ were prepared following reported procedures.

4.3. Synthetic procedures

4.3.1. 2,4,6-Tris(*tert*-butoxycarbonylmethoxy)benzaldehyde (1)—Following the K₂CO₃ alkylation method,^{51,52} a solution of 2,4,6-trihydroxybenzaldehyde (2.0 g, 13 mmol) in anhydrous DMF (43 mL, 0.3 M) was treated with K₂CO₃ (17.8 g, 129 mmol, dried *in vacuo* at 140 °C overnight), and the mixture was heated at 60 °C. After 30 min, *tert*-butyl bromoacetate (7.70 mL, 51.6 mmol) was added dropwise to the reaction mixture, and the temperature was set at 80 °C. After stirring for 1 h at 80 °C, the reaction mixture was allowed to reach ambient temperature. The reaction mixture was treated with water (20 mL) and extracted with ethyl acetate. The organic layer was washed (brine and water), separated, dried (Na₂SO₄) and concentrated to a red oil. Purification by column chromatography [silica, hexanes/ethyl acetate (1:1)] gave a red-brown viscous oil that solidified upon standing for 1 day at room temperature (5.2 g, 82%): mp 55–60 °C; ¹H NMR δ 1.48 (s, 18H), 1.49 (s, 9H), 4.49 (s, 2H), 4.57 (s, 4H), 6.01 (s, 2H), 10.43 (s, 1H); ¹³C NMR δ 28.2, 65.8, 66.8, 82.9, 83.2,

93.5, 110.6, 162.3, 163.7, 166.9, 167.1, 187.5; FAB-MS obsd 497.2396, calcd 497.2387 (C₂₅H₃₆O₁₀); Anal. Calcd for C₂₅H₃₆O₁₀: C, 60.47; H, 7.31; Found: C, 60.03; H, 7.33.

4.3.2. 5-(2,4,6-Tris(*tert*-butoxycarbonylmethoxyphenyl)dipyrromethane (2a)—

Following a procedure for dipyrromethane formation,⁵³ a sample of aldehyde **1** (1.53 g, 3.08 mmol) and pyrrole (8.4 mL, 0.12 mol) was purged with argon for 10 min. Then InCl₃ (66 mg, 0.30 mmol) was added, and the mixture was stirred at room temperature under argon. After 1 h, the reaction mixture was treated with powdered NaOH (360 mg, 6.00 mmol), and stirring was continued for 30 min. The reaction mixture was filtered through a Buchner funnel, and the filtrate was concentrated. The resulting solid was treated with hexanes, and the suspension was sonicated. The volatile components were evaporated. This sequence of operations was repeated thrice in order to remove traces of pyrrole. Crystallization from ethanol-water afforded an ash-grey solid (659 mg, 35%): mp 107–112 °C; ¹H NMR δ 1.45 (s, 18H), 1.50 (s, 9H), 4.39 (s, 6H), 5.94–5.97 (m, 2H), 6.01–6.06 (m, 2H), 6.09 (s, 2H), 6.23 (s, 1H), 6.64–6.68 (m, 2H), 9.50 (br, 2H); ¹³C NMR δ 28.26, 28.29, 32.8, 66.1, 66.8, 82.8, 106.3, 107.4, 116.2, 116.6, 132.7, 158.1, 167.8, 168.3; FAB-MS obsd 612.3082, calcd 612.3047 (C₃₃H₄₄N₂O₉).

4.3.3. Zn(II)-5-Phenyl-15-[2,4,6-tris(*tert*-butoxycarbonylmethoxy)phenyl]porphyrin (ZnP-1')—

Following a reported procedure,³⁰ a solution of **2a** (150 mg, 0.245 mmol) and **3a** (88.0 mg, 0.245 mmol) in EtOH (25 mL, 0.01 M) was treated with Zn(OAc)₂ (450 mg, 2.45 mmol) and heated to reflux for 16 h. The heat source was removed, and the reaction mixture was concentrated. Purification by column chromatography [alumina, hexanes/ethyl acetate (1:3)] gave a pink solid (40 mg, 18%): ¹H NMR δ 1.24 (s, 18H), 1.25 (s, 9H), 4.11 (s, 4H), 4.75 (s, 2H), 6.51 (s, 2H), 7.77–7.80 (m, 3H), 8.25–8.28 (m, 2H), 9.11 (d, *J* = 4.4 Hz, 2H), 9.21 (d, *J* = 4.7 Hz, 2H), 9.40 (d, *J* = 4.4, 4H), 10.26 (s, 2H); ¹³C NMR δ 28.1, 28.4, 66.4, 66.7, 82.2, 82.9, 93.7, 105.9, 126.8, 127.6, 131.5, 132.26, 132.39, 132.54, 134.9, 143.0, 149.5, 149.8, 151.4, 159.83, 159.99, 167.9; LD-MS obsd 914.5; ESI-MS obsd 915.2927, calcd 915.2942 [(M + H)⁺; M = C₅₀H₅₁N₄O₉Zn]; λ_{abs} 412, 538 nm; λ_{em} (λ_{ex} = 412 nm) 578, 632 nm.

4.3.4. 5-Phenyl-15-[2,4,6-tris(carboxymethoxy)phenyl]porphyrin (H₂P-1)—

A solution of **ZnP-1'** (20 mg, 0.022 mmol) in THF (4 mL, 5 mM) was treated with 1 mL of aqueous KOH (2 M) and heated at 60 °C. After 8 h, CH₂Cl₂ (15 mL) and H₂O (15 mL) were added to the reaction mixture. The aqueous layer, which contained the porphyrin, was separated and concentrated to a pink solid. The solid was dissolved in H₂O (3 mL) and treated with aqueous HCl (2 M) to obtain pH = 3, whereupon the porphyrin precipitated. The mixture was sonicated, centrifuged, and decanted to afford a solid pellet. Water was added followed by sonication, centrifugation, and decanting; this treatment was performed four times, whereupon the supernatant was colorless. The pellet was dried *in vacuo* to afford a red-brown solid (12 mg, 80% yield): ¹H NMR (DMSO-*d*₆) δ -3.32 (s, 1H), -3.24 (s, 1H), 4.46 (s, 4H), 4.95 (s, 2H), 6.66 (s, 2H), 7.87–7.90 (m, 3H), 8.28–8.31 (m, 2H), 9.01 (d, *J* = 4.9 Hz, 2H), 9.10 (d, *J* = 4.4 Hz, 2H), 9.53 (d, *J* = 4.4, 2H), 9.63 (d, *J* = 4.9, 2H), 10.53 (s, 2H); ¹³C NMR δ 64.7, 65.2, 92.8, 105.1, 110.9, 127.3, 130.5, 131.3, 132.1, 134.6, 159.1, 169.7, 170.1; ESI-MS obsd 684.1866, calcd 684.1856 [(M - H)⁺; M = C₃₈H₂₈N₄O₉]; λ_{abs} (H₂O) 401, 504, 563 nm; λ_{em} (λ_{ex} = 401 nm) 625, 686 nm; RP-HPLC *t*_R = 30.05 min. In one case, the deprotection was carried out with isolation of the zinc ester-porphyrin: A solution of **ZnP-1'** (9 mg, 0.009 mmol) in THF (2.0 mL, 4.5 mM) was treated with 0.5 mL of aqueous KOH (2 M) and heated at 60 °C. After 8 h, CH₂Cl₂ (10 mL) and H₂O (10 mL) were added to the reaction mixture. The aqueous layer, which contained the porphyrin, was separated and concentrated to give Zn(II)-5-phenyl-15-[2,4,6-tris(carboxymethoxy)phenyl]porphyrin as a pink solid: ¹H NMR (methanol-*d*₄) δ 4.09 (s, 4H), 4.69 (s, 2H), 6.78 (s, 2H), 7.80–7.82 (m, 3H), 8.22–8.25 (m, 2H), 8.98 (d, *J* = 4.4 Hz, 2H), 9.17 (d, *J* = 4.4 Hz, 2H), 9.36–9.40 (m, 4H), 10.23 (s, 2H); ESI-MS obsd

745.0917, calcd 745.0918 [(M - H)⁺]; M = C₃₈H₂₆N₄O₉Zn; λ_{abs} (H₂O) 409, 543 nm; λ_{em} (λ_{ex} = 409 nm) 587, 638 nm.

4.3.5. Zn(II)-5-(5,5-Dimethyl-1,3-dioxan-2-yl)-15-[2,4,6-tris(*tert*-butoxycarbonylmethoxy)phenyl]-porphyrin (ZnP-2')—Following a reported procedure,²⁹ a solution of **2a** (0.163 g, 0.267 mmol) and **4** (0.100 g, 0.267 mmol) in ethanol (26 mL, 10 mM) was treated with anhydrous Zn(OAc)₂ (0.489 mg, 2.67 mmol) and heated to reflux. After 16 h, DDQ (0.181 g, 0.801 mmol) was added, and the mixture was stirred for 15 min. Triethylamine (187 μL, 1.33 mmol) was added, and the reaction mixture was concentrated to dryness. Column chromatography [alumina, hexanes/ethyl acetate, (1:3)] afforded a purple solid (26 mg, 10%): ¹H NMR δ 1.23 (s, 27H), 1.64 (s, 6H), 4.11 (s, 4H), 4.38–4.39 (m, 4H), 4.74 (s, 2H), 6.49 (s, 2H), 8.10 (s, 1H), 9.19 (d, *J* = 4.3 Hz, 2H), 9.36 (d, *J* = 4.3 Hz, 2H), 9.50 (d, *J* = 4.3 Hz, 2H), 10.16 (d, *J* = 4.7 Hz, 2H), 10.24 (s, 2H); ¹³C NMR δ 22.9, 25.4, 28.0, 28.2, 29.9, 31.2, 66.4, 66.6, 67.6, 80.5, 82.2, 82.9, 93.5, 106.0, 107.0, 130.4, 131.8, 132.43, 132.58, 149.3, 149.5, 149.7, 150.7, 159.7, 159.9, 167.8; LD-MS obsd 952.3; FAB-MS obsd 952.3222; calcd 952.3237 (C₅₀H₅₆N₄O₁₁Zn); λ_{abs} 411, 543, 578 nm; λ_{em} (λ_{ex} = 411 nm) 578, 632 nm.

4.3.6. 5-Formyl-15-[2,4,6-tris(carboxymethoxy)phenyl]porphyrin (H₂P-2)—A sample of porphyrin **ZnP-2'** (15 mg, 0.016 mmol) in THF (1.6 mL, 0.01M) was treated with aqueous KOH (0.75 mL, 2 M) at 60 °C for 10 h. The reaction mixture was allowed to reach ambient temperature. The reaction mixture was treated with H₂O (10 mL) and CH₂Cl₂ (10 mL). The aqueous layer, which contained the porphyrin, was separated and concentrated to a pink solid. The solid was treated with neat TFA (1 mL) and stirred at 50 °C for 24 h. The mixture was diluted with H₂O to adjust the pH to 3. The resulting suspension was sonicated and centrifuged. Decanting afforded a solid pellet. Water was added followed by sonication, centrifugation, and decanting; this treatment was performed three times, whereupon the supernatant was colorless. The pellet was dried *in vacuo* to a green solid (6.5 mg, 65%): ¹H NMR (DMSO-*d*₆) δ -2.58 (s, 1H), -2.52 (s, 1H), 4.44 (s, 4H), 4.91 (s, 2H), 6.65 (s, 2H), 9.09 (d, *J* = 4.7 Hz, 2H), 9.46 (d, *J* = 4.3 Hz, 2H), 9.78 (d, *J* = 4.7 Hz, 2H), 10.32 (d, *J* = 4.7 Hz, 2H), 10.59 (s, 2H), 12.52 (s, 1H); ¹³C NMR δ 64.6, 65.1, 92.7, 104.9, 116.6, 128.8, 131.87, 132.04, 134.9, 143.7, 145.6, 147.4, 148.9, 158.8, 160.1, 170.0; LD-MS obsd 637.0; ESI-MS obsd 636.1479, calcd 636.1492 (C₃₃H₂₄N₄O₁₀); λ_{abs} (H₂O) 409, 562, 633 nm; λ_{em} (H₂O, λ_{ex} = 409 nm) 641, 707; RP-HPLC *t*_R = 28.94 min.

4.3.7. 1,9-Diformyl-5-{4-[*N*-(*tert*-butoxycarbonyl)amino]phenyl}dipyrrromethane (5)—Following a reported procedure,³⁰ a solution of **2b** (500 mg, 1.48 mmol) in anhydrous DMF (1.50 mL) at 0 °C under argon was treated dropwise with POCl₃ (0.28 mL, 3.1 mmol). The mixture was stirred at room temperature. After 1 h the reaction mixture was poured into a saturated aqueous solution of NaOAc (50 mL). The milky reaction mixture was extracted with CH₂Cl₂. The organic extract was washed with water, dried (Na₂SO₄), and filtered. The filtrate was concentrated to dryness. The crude residue was chromatographed [silica, CH₂Cl₂/ethyl acetate (3:1)] to afford a beige solid (400 mg, 68%): mp 196–202 °C; ¹H NMR δ 1.51 (s, 9H), 5.51 (s, 1H), 6.05–6.08 (m, 2H), 6.84–6.90 (m, 2H), 7.15–7.18 (m, 2H), 7.34–7.32 (m, 2H), 9.24 (s, 2H), 10.94 (br, 2H); ¹³C NMR δ 28.5, 43.9, 111.7, 119.2, 122.7, 129.2, 132.8, 133.8, 138.1, 142.2, 179.3; FAB-MS obsd 394.1773, calcd 394.1767 [(M + H)⁺]; M = C₂₂H₂₃N₃O₄].

4.3.8. 1,9-Bis(propylimino)-5-{4-[*N*-(*tert*-butoxycarbonyl)amino]phenyl}dipyrrromethane (3b)—Following a reported procedure,³⁰ a solution of **5** (250 mg, 0.630 mmol) and *n*-propylamine (1.00 mL, 12.7 mmol) in THF (2 mL) was stirred at room temperature for 1 h. Removal of the solvent and excess *n*-propylamine gave a beige solid (300 mg, quantitative): mp 65–68 °C; ¹H NMR δ 0.88 (t, *J* = 7.2 Hz, 6H), 1.49 (s, 9H), 1.57–1.63

(m, 4H), 1.99 (s, 2H), 3.38 (t, $J = 7.2$ Hz, 4H), 5.40 (s, 1H), 5.92 (d, $J = 3.6$ Hz, 2H), 6.41 (d, $J = 3.6$ Hz, 2H), 7.07–7.10 (m, 2H), 7.23–7.25 (m, 2H), 7.82 (s, 2H); ^{13}C NMR δ 11.9, 24.3, 28.5, 43.9, 61.5, 109.8, 118.81, 118.84, 129.14, 129.20, 135.2, 137.7, 139.0, 151.6; FAB-MS obsd 476.3026, calcd 476.3056 [(M + H)⁺; M = C₂₈H₃₇N₅O₂].

4.3.9. Zn(II)-5-[4-[N-(*tert*-butyloxycarbonyl)amino]phenyl]-15-[2,4,6-tris(*tert*-butoxycarbonylmethoxy) phenyl]porphyrin (ZnP-3')—Following a reported procedure,³⁰ a solution of **2a** (123 mg, 0.200 mmol) and **3b** (95 mg, 0.20 mmol) in toluene (20 mL, 0.01 M) was treated with anhydrous Zn(OAc)₂ (367 mg, 2.00 mmol) and refluxed for 12 h with exposure to air. The reaction mixture was concentrated to dryness. Column chromatography [alumina, hexanes/ethyl acetate (1:1)] afforded a purple solid (58 mg, 28%): ^1H NMR δ 1.15 (s, 9H), 1.62 (s, 27H), 4.15 (s, 4H), 4.79 (s, 2H), 6.49 (s, 2H), 7.76–7.78 (m, 2H), 8.17 (d, $J = 8.0$ Hz, 2H), 9.18 (d, $J = 4.0$ Hz, 2H), 9.10 (d, $J = 4.0$ Hz, 2H), 9.16 (d, $J = 4.0$ Hz, 2H), 9.37 (dd, $J = 4.3$ Hz, $J = 4.3$ Hz, 2H), 10.20 (s, 2H); ^{13}C NMR δ 28.0, 28.4, 28.7, 66.4, 66.6, 82.2, 82.9, 90.7, 105.9, 110.3, 114.9, 116.8, 119.7, 131.4, 132.21, 132.34, 135.4, 137.84, 137.96, 149.4, 149.7, 149.9, 151.3, 153.3, 159.7, 159.9, 163.2, 167.8, 168.0, 204.21, 204.25, 211.1; LD-MS obsd 1029.2; FAB-MS obsd 1029.3558, calcd 1029.3503 (C₅₅H₅₉N₅O₁₁Zn); λ_{abs} 414, 545, 580 nm; λ_{em} ($\lambda_{\text{ex}} = 414$ nm) 579, 634 nm.

4.3.10. 5-(4-Aminophenyl)-15-[2,4,6-tris(carboxymethoxy)phenyl]porphyrin (H₂P-3)—A solution of **ZnP-3'** (20 mg, 0.019 mmol) in THF (1.9 mL, 0.01 M) was treated with aqueous KOH (0.5 mL, 2 M) at 60 °C for 28 h. The reaction mixture was treated with water and CH₂Cl₂. The aqueous layer, which contained the porphyrin, was separated and concentrated to a pink solid. The solid was diluted with water. The solution was acidified with aqueous HCl (2 M) to pH 3, whereupon the porphyrin precipitated. The suspension was sonicated, centrifuged, and decanted to afford a solid pellet; this procedure was repeated four times, whereupon the supernatant was colorless. The solid pellet was dried and treated with neat TFA (1 mL). The resulting solution was stirred at room temperature for 11 h. The mixture was diluted with water (1 mL). The suspension was sonicated and centrifuged. Decanting afforded a solid pellet. Water was added followed by sonication, centrifugation, and decanting; this treatment was performed three times, whereupon the supernatant was colorless. The resulting solid was dried *in vacuo* to afford a dark-purple solid (11 mg, 83%): ^1H NMR (DMSO-*d*₆) δ -3.21 (s, 1H), -3.11 (s, 1H), 4.45 (s, 4H), 4.94 (s, 2H), 6.65 (s, 2H), 7.07 (d, $J = 8.0$ Hz, 2H), 7.95 (d, $J = 8.4$ Hz, 2H), 9.06 (d, $J = 4.4$ Hz, 2H), 9.13 (d, $J = 4.4$ Hz, 2H), 9.49 (d, $J = 4.8$ Hz, 2H), 9.58 (d, $J = 4.4$ Hz, 2H), 10.46 (s, 2H); ^{13}C NMR δ 64.8, 65.2, 92.7, 104.8, 110.1, 110.8, 112.9, 120.4, 127.66, 127.68, 130.8, 131.0, 131.6, 132.0, 135.9, 144.4, 146.5, 148.1, 148.7, 159.1, 159.8, 169.8, 170.1; LD-MS obsd 700.3, ESI-MS obsd 700.2032, calcd 700.2038 [(M + H)⁺; M = C₃₈H₂₉N₅O₉]; λ_{abs} (H₂O) 404, 505, 544, 567, 622 nm; λ_{em} (H₂O, $\lambda_{\text{ex}} = 407$ nm) 629, 688; RP-HPLC $t_{\text{R}} = 22.25$ min.

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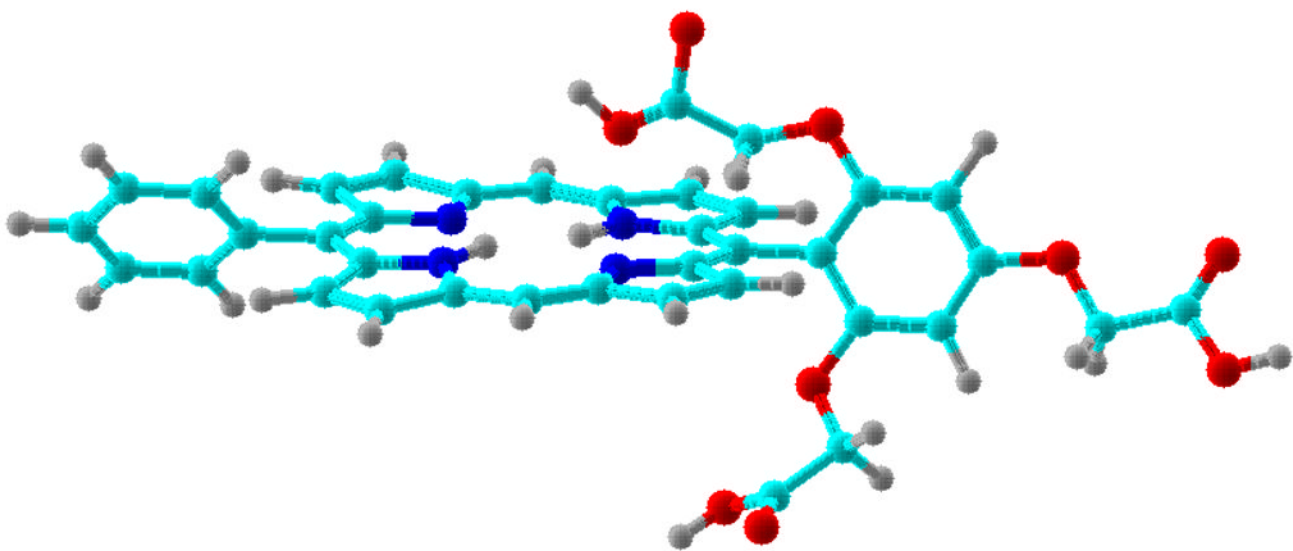


Figure 1. Molecular model of porphyrin **H₂P-1** showing conformations (not energy-minimized) of the 2,6-carboxymethoxy units attached to the aryl moiety.

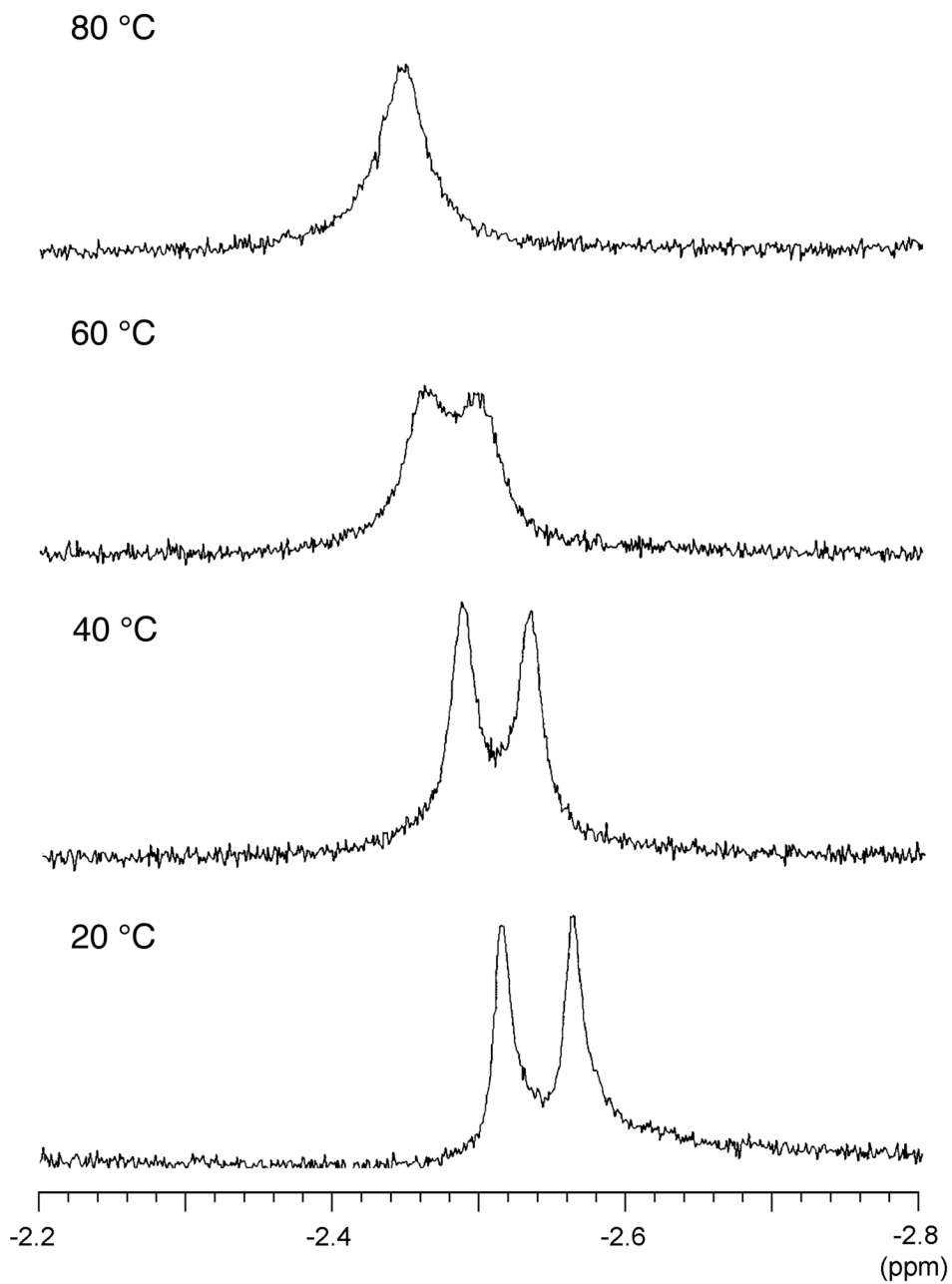


Figure 2. Variable temperature ¹H NMR spectra for porphyrin **H₂P-2** in DMSO-*d*₆.

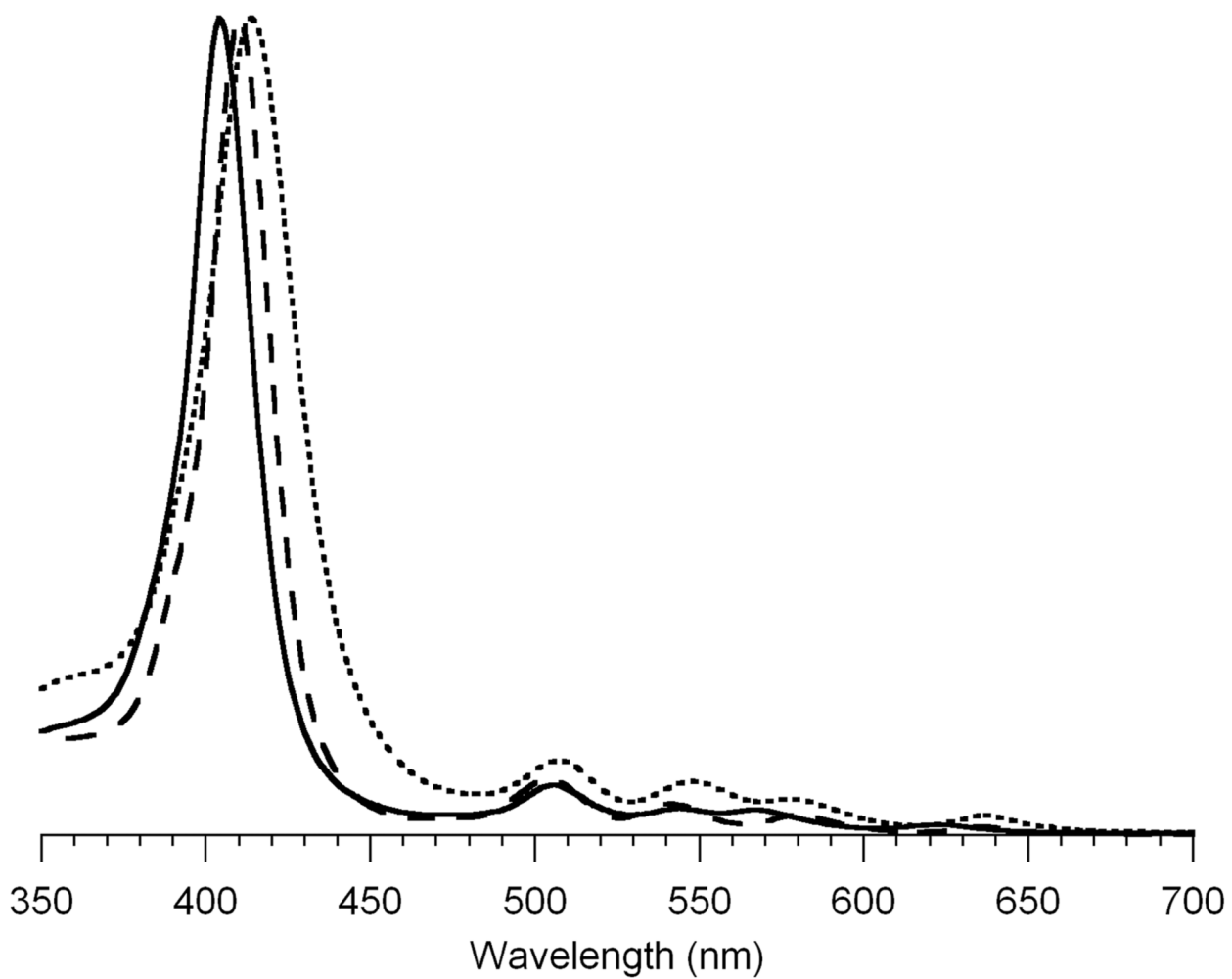


Figure 3. Absorption spectra of porphyrin **H₂P-3** at room temperature in water (solid line), THF (dashed line) and DMSO (dotted line).

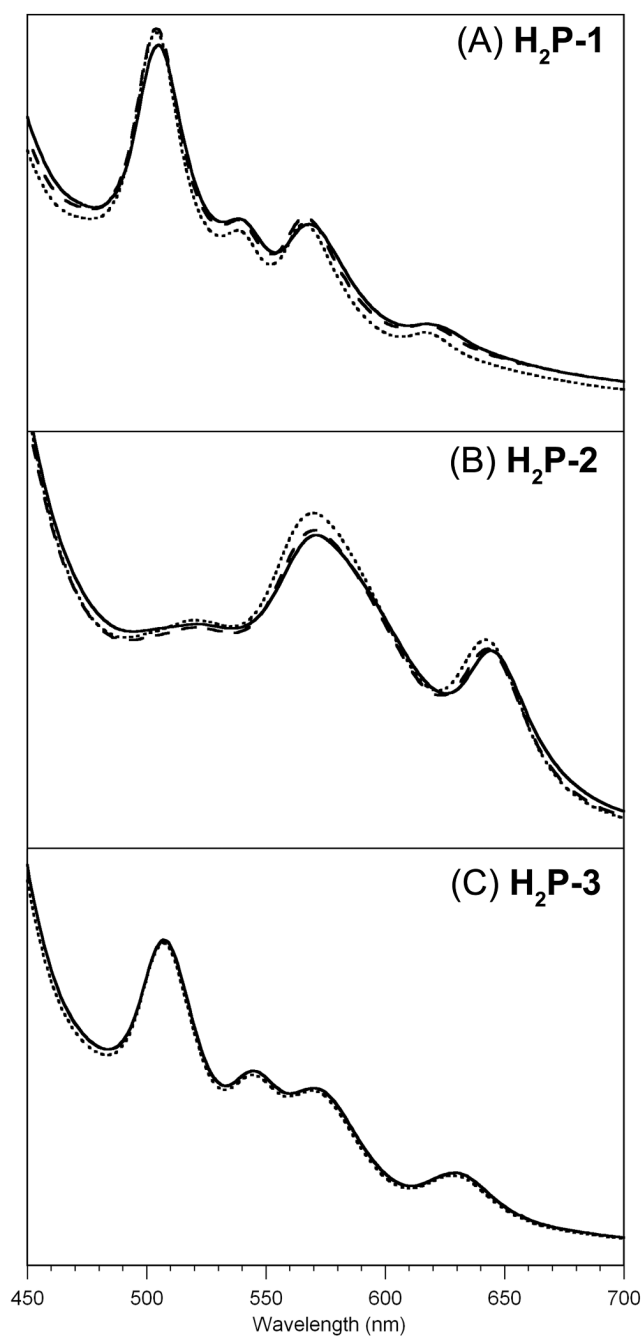
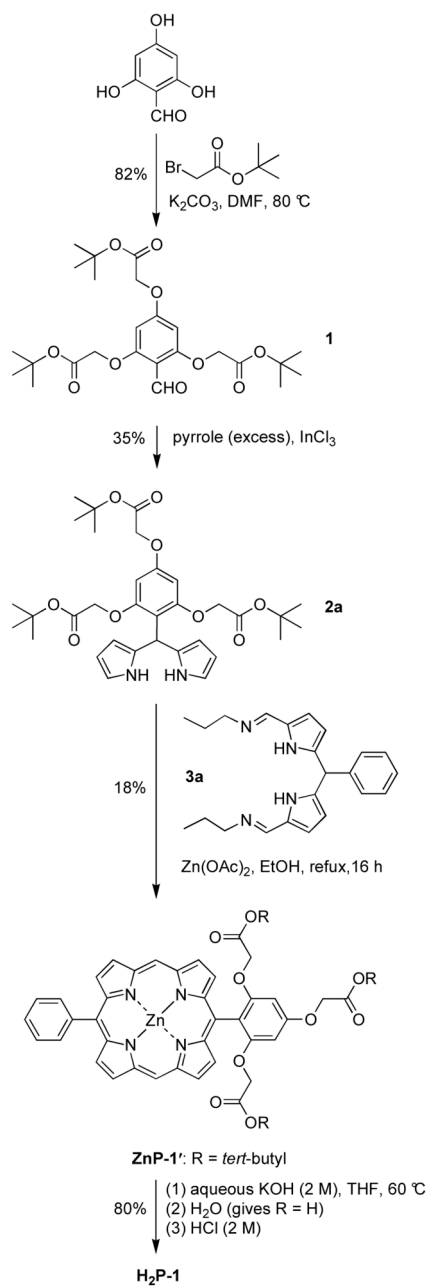
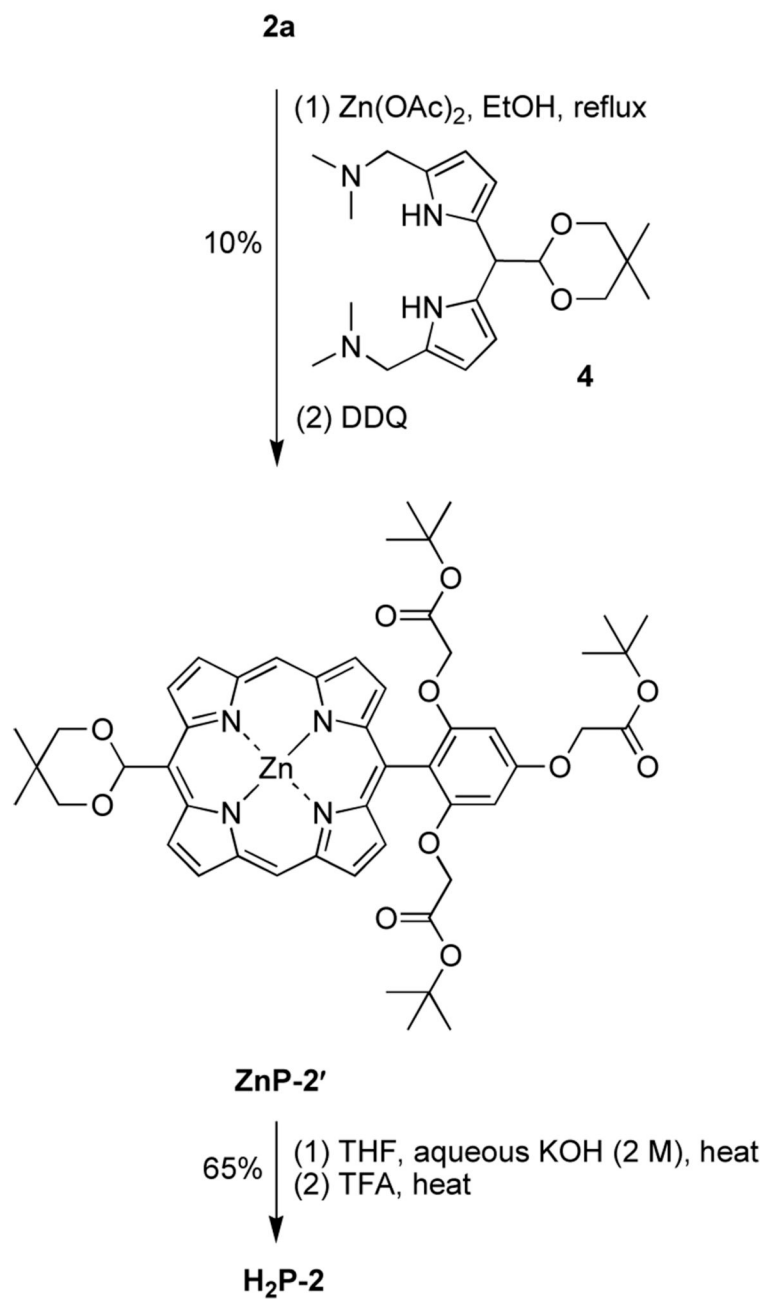


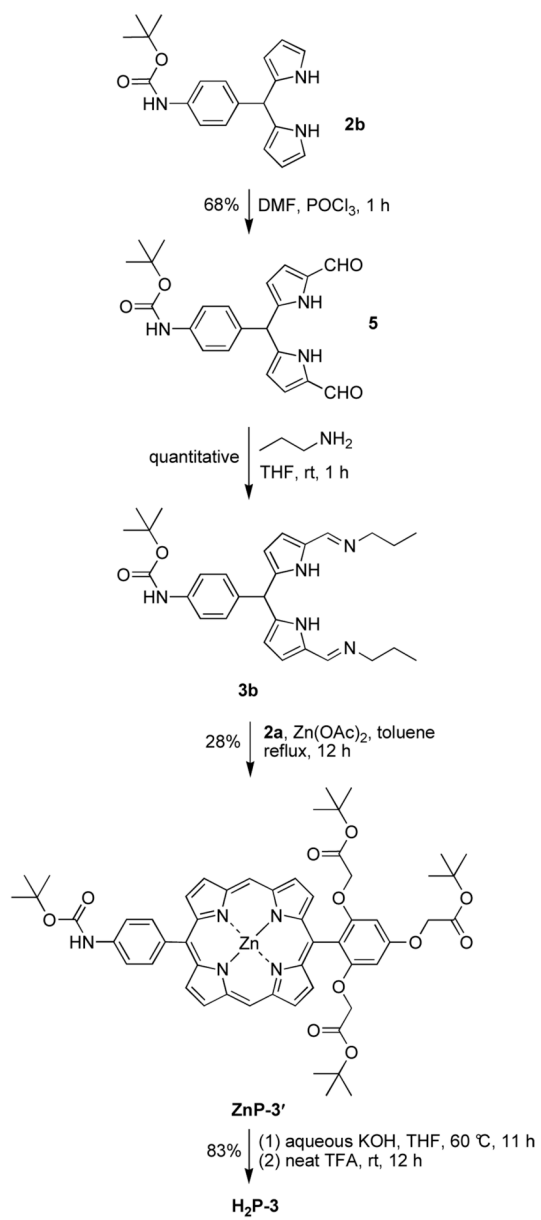
Figure 4. Absorption spectra of porphyrins in aqueous buffer solution (pH = 7) at room temperature as a function of concentration. (A) Porphyrin **H₂P-1**: (solid line) $c = 2.8$ mM, $l = 1$ mm; (dotted line) $c = 0.56$ mM, $l = 5$ mm; (dashed line) $c = 0.28$ mM, $l = 10$ mm. (B) Porphyrin **H₂P-2**: (solid line) $c = 2.5$ mM, $l = 1$ mm; (dotted line) $c = 0.5$ mM, $l = 5$ mm; (dashed line) $c = 0.25$ mM, $l = 10$ mm. (C) Porphyrin **H₂P-3**: (solid line) $c = 3$ mM, $l = 1$ mm; (dotted line) $c = 0.6$ mM, $l = 5$ mm; (dashed line) $c = 0.3$ mM, $l = 10$ mm.



Scheme 1.



Scheme 2.



Scheme 3.

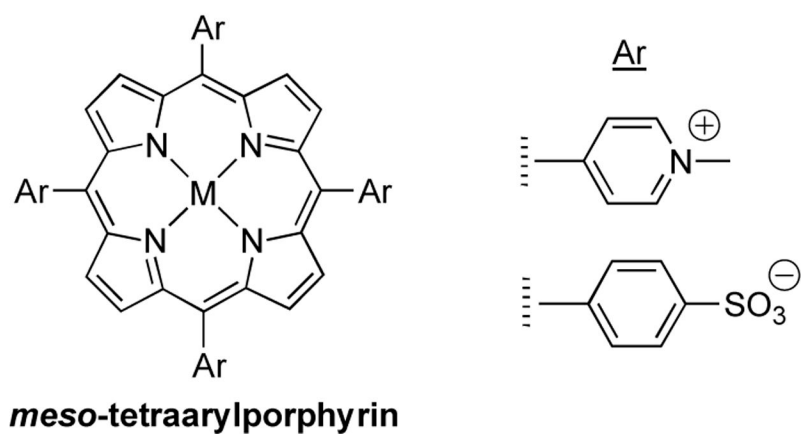
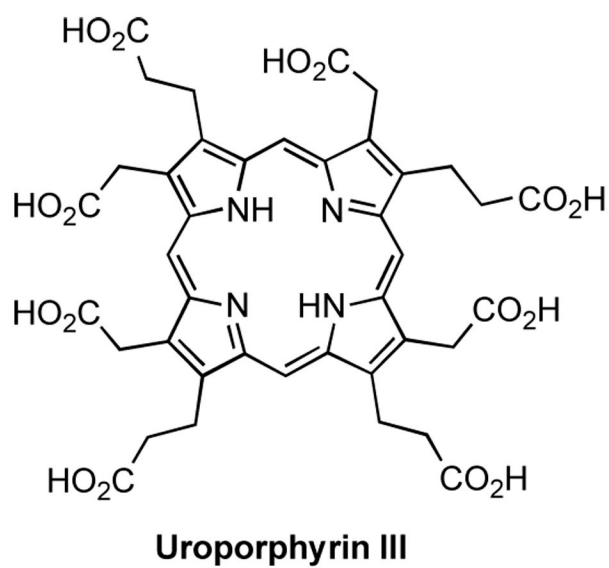


Chart 1.

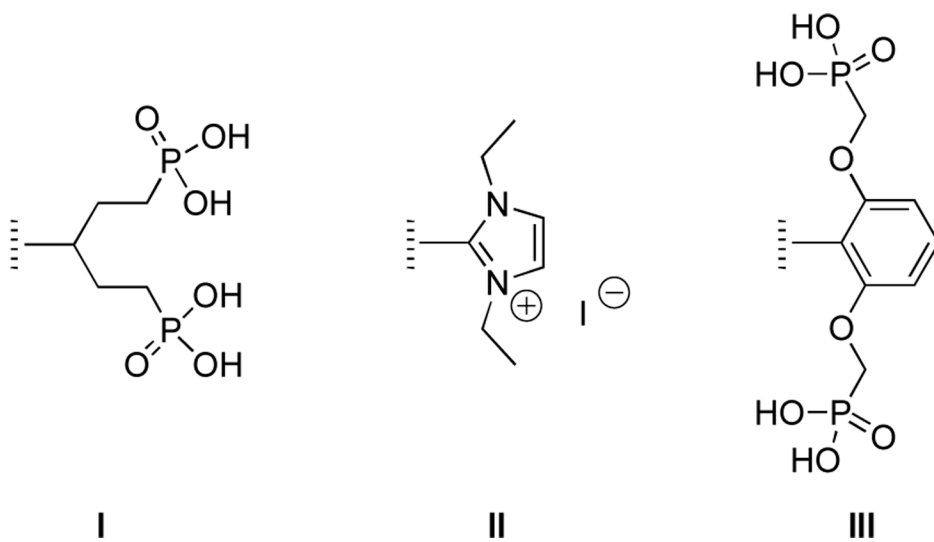


Chart 2.

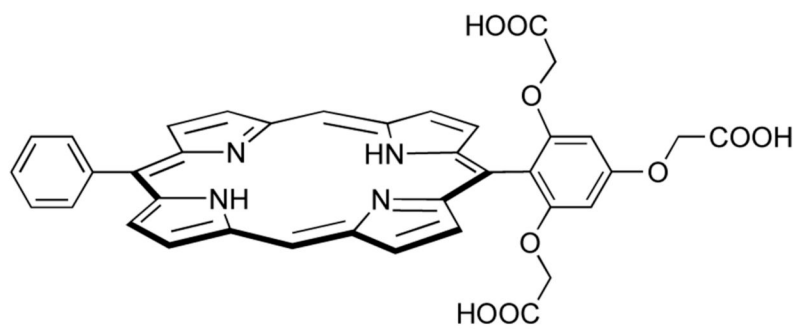
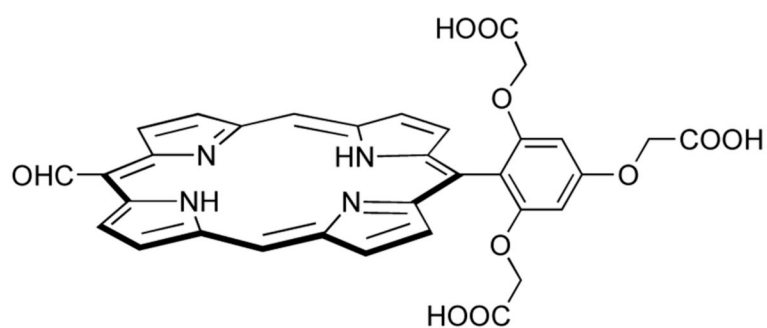
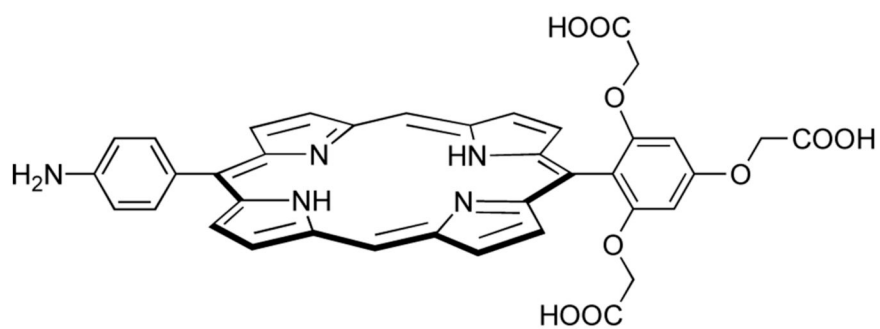
**H₂P-1****H₂P-2****H₂P-3****Chart 3.**

Table 1

Absorption spectra of porphyrins in various solvents

Entry	Cmpd	M	Solvent	λ_{Soret} (nm)	fwhm (nm)	λ_{Q} (nm)
1	ZnP-1'	Zn	toluene	413	13	539, 573
2	ZnP-1'	Zn	MeOH	410	10	544, 579
3	ZnP-1'	Zn	DMSO	417	10	548, 583
4	ZnP-2'	Zn	toluene	411	14	502, 539, 574
5	ZnP-2'	Zn	MeOH	408	10	542, 577
6	ZnP-2'	Zn	DMSO	414	11	502, 545, 580
7	ZnP-3'	Zn	toluene	414	13	539, 575
8	ZnP-3'	Zn	THF	414	11	545, 580
9	ZnP-3'	Zn	DMSO	418	11	548, 583
10	H ₂ P-1	H, H	DMSO	416	27	506, 547, 577, 632
11	H ₂ P-1	H, H	MeOH	406	21	501, 542, 574, 629
12	H ₂ P-1	H, H	H ₂ O	402	30	505, 564, 620
13	H ₂ P-1	H, H	pH 7	402	20	504, 541, 617
14	H ₂ P-1	H, H	pH 10	403	20	504, 541, 617
15	H ₂ P-2	H, H	DMSO	415	31	518, 560, 641
16	H ₂ P-2	H, H	MeOH	411	33	516, 559, 642
17	H ₂ P-2	H, H	H ₂ O	407	33	519, 561, 637
18	H ₂ P-2	H, H	pH 7	408	41	518, 562, 633
19	H ₂ P-2	H, H	pH 10	395	63	519, 565, 637
20	H ₂ P-3	H, H	DMSO	414	34	507, 548, 579, 647
21	H ₂ P-3	H, H	THF	409	22	504, 541, 579, 636
22	H ₂ P-3	H, H	H ₂ O	404	24	505, 543, 568, 623
23	H ₂ P-3	H, H	pH 7	405	23	506, 544, 567, 622
24	H ₂ P-3	H, H	pH 10	405	24	506, 544, 567, 622
25	H ₂ P-4	H, H	toluene	408	13	503, 535, 576, 633
26	H ₂ P-4	H, H	DMSO	407	13	502, 537, 574, 629