NJC

ROYAL SOCIETY OF CHEMISTRY

PAPER

View Article Online
View Journal | View Issue

Cite this: *New J. Chem.*, 2024, **48**, 11140

Molecular designs with PEG groups for water-solubilization of sparsely substituted porphyrins†

Phuong-Lien Doan Cao,[a] Zhiyuan Wu,[a] Phattananawee Nalaoh [b] and Jonathan S. Lindsey *[a]

Molecular designs that achieve solubility of porphyrins in aqueous media are attractive for diverse applications. The presence of 4-sulfophenyl or 4-*N*-methylpyridinium groups at the four *meso*-positions is effective, but the use of fewer substituents is desirable for custom tailoring. Here, five target porphyrins (along with selected copper or zinc chelates) were prepared bearing PEG groups to understand how distinct designs affect aqueous solubility (where "PEG" refers to an oligoethylene glycol unit). One objective was to employ only one or two pegylated *meso*-aryl groups so that other *meso*-positions would be open for synthetic elaboration while retaining a compact structure. The key design features examined include (i) 2,6- *versus* 3,5-dipegylated aryl groups; (ii) one *versus* two 2,6-dipegylated aryl groups; (iii) a nonpolar *versus* ionizable terminus of the PEG moiety; and (iv) length of the PEG moiety. In each case, the PEG groups were attached on a porphyrin scaffold bearing one or two bis(2-propynyloxy)aryl groups. Assessment entailed octanol–aqueous solution partitioning (log *P* values) and aggregation over the concentration range of 0.2–200 μM. A *trans*-A_2 free base porphyrin bearing two 2,6-dialkoxyphenyl groups equipped with methyl-terminated PEG_6 groups gave ~3 : 1 partitioning in water *versus* octanol but high overall solubility (at least 12 mM) in water alone; the analogous porphyrin with carboxylic acid-terminated PEG_6 groups gave >100 : 1 partitioning in phosphate-buffered saline (PBS, pH 7.4). A *trans*-AB porphyrin bearing a single 2,6-dialkoxyphenyl group equipped with carboxylic acid-terminated PEG_6 groups gave 43 : 1 partitioning in PBS *versus* octanol but was prone to self-aggregation at 20–200 μM in PBS alone. The results point to new molecular designs of potential value in the life sciences.

Received 12th March 2024,
Accepted 30th May 2024

DOI: 10.1039/d4nj01178c

rsc.li/njc

Introduction

The design and synthesis of water-soluble tetrapyrrole macrocycles have been of longstanding interest.[1–4] The chief challenge is to attach groups at the perimeter of the hydrophobic, tetrapyrrole macrocycle so that the entire structure can be dissolved in aqueous solution. The tetrapyrrole macrocycle is disk-shaped and essentially planar, where the core free base porphine macrocycle, lacking any peripheral substituents, has formula $C_{20}N_4H_{14}$. Early designs have relied on incorporation of polar groups at the *meso*-positions of porphyrins. Indeed, *meso*-tetrakis(4-sulfophenyl)porphyrin (**TPS-por**)[5] and the *meso*-tetrakis(4-*N*-methylpyridinium)porphyrin (**TPyr-por**)[6] are water soluble and remain in common use (Chart 1). These designs have attractive features, but the occupancy of all four *meso*-positions leaves diminished opportunities for convenient synthetic elaboration. The uroporphyrins, derived from biosynthesis of tetrapyrrole macrocycles, are water soluble but have been little used for synthetic elaboration or physicochemical studies.[7]

We have pursued several design features over the years in an effort to create compact, water-soluble, bioconjugatable porphyrins. The features include (1) use of a *trans*-AB porphyrin architecture, with A and B substituents at the 5- and 15-positions of the macrocycle and no substituents at any other position, (2) emphasis on substituent types that cause polar groups to project above and below the face of the hydrophobic porphyrin, and ideally, (3) reliance on a "single-junction water-solubilization unit".[8] The work has been intermittent as objectives and synthetic methods have evolved, key examples of which are shown in Chart 2.[9]

• The first design contained a compact, swallowtail substituent, which is a symmetrically branched alkyl group; the preferred conformation contains the C–H unit in the plane of the macrocycle and the two alkyl groups project above and

[a] *Department of Chemistry, North Carolina State University, Raleigh, NC 27695-8204, USA. E-mail: jlindsey@ncsu.edu*
[b] *Department of Chemistry, University of Tennessee, Knoxville, TN 37996, USA*

† Electronic supplementary information (ESI) available: ^{1}H NMR, $^{13}C\{^1H\}$ NMR, and MALDI-MS spectra for new compounds; absorption spectra and photographs of selected compounds in solution; single-crystal X-ray diffraction data. CCDC 2321457 (**Zn5**). For ESI and crystallographic data in CIF or other electronic format see DOI: https://doi.org/10.1039/d4nj01178c

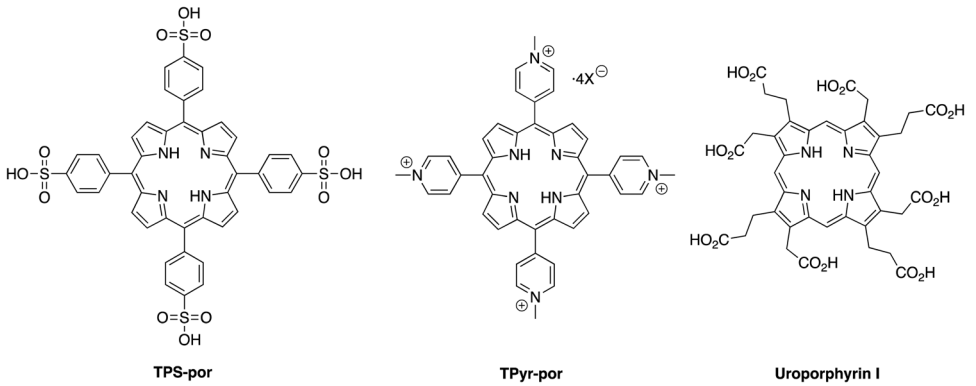


Chart 1 Water-soluble porphyrins.

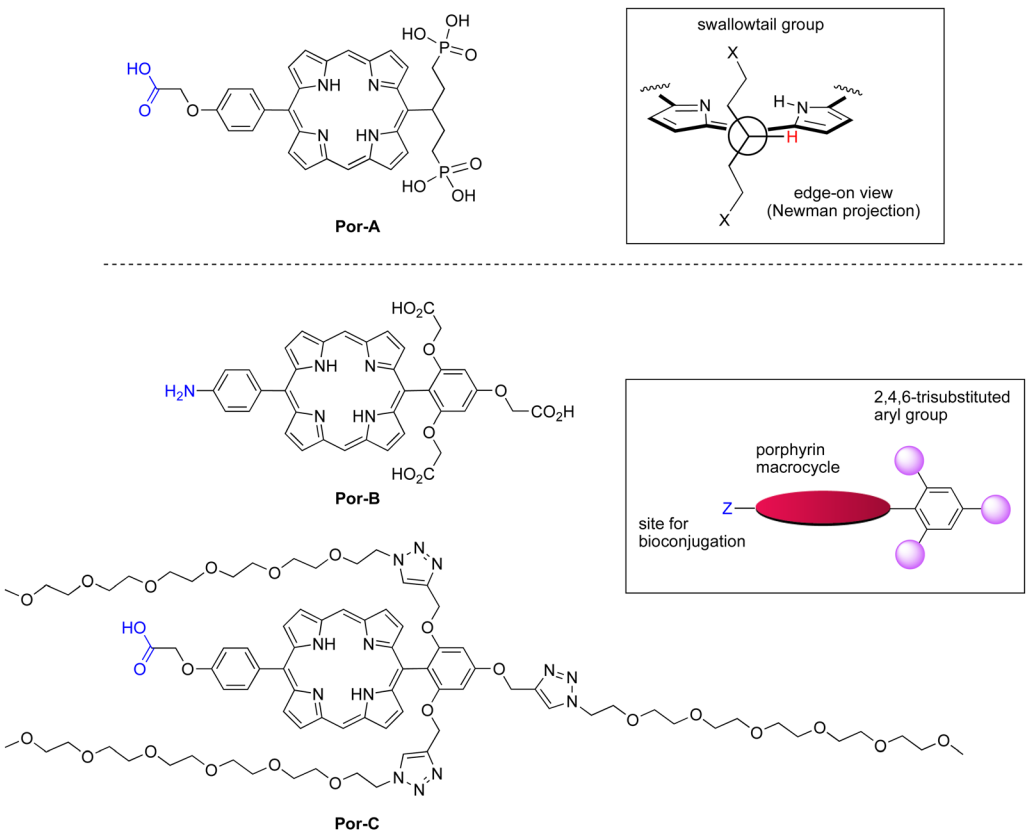


Chart 2 Representative prior *trans*-AB porphyrins.[9]

below the plane of the porphyrin.[10] The porphyrins (*e.g.*, **Por-A**) appeared reasonably soluble in aqueous solution, with the key drawback, however, of the rather lengthy synthesis of the swallowtail units.[11]

• The second design relied on a 2,4,6-trialkoxyaryl unit, which was attractive given the inexpensive phloroglucinaldehyde (2,4,6-trihydroxybenzaldehyde) and ease of synthesis. The earliest set of porphyrins (*e.g.*, **Por-B**) exhibited broadened absorption spectra both in aqueous as well as organic media.[12]

• The third design extended the 2,4,6-trialkoxyaryl unit to include PEG groups (*e.g.*, **Por-C**).[8,9] The resulting porphyrin was water-soluble but in ensuing years uncertainty arose as to how much solubilization was provided by the carboxylic acid in the bioconjugation motif *versus* the three neutral PEG groups.

The use of PEG groups to impart aqueous solubility of hydrophobic compounds has become widespread across the molecular sciences.[13,14] PEG groups have long been used in the pharmaceutical industry[15–17] to increase aqueous solubility of hydrophobic drugs and to prolong the systemic circulation of rapidly excreted drugs. A striking feature of PEG groups is solubility in both aqueous and organic media.[13] The origin of the solubility in water is not merely due to the preponderance

of oxygen atoms, as the polymer with twice the number of oxygen atoms (repeating unit –CH_2O–) is not soluble in water. The solubility likely stems from the preferred *gauche* conformation of the –OCH_2CH_2O– moiety and all of the consequences therefrom including local dipole moments, hydrogen-bonding patterns, and kinked or partially coiled conformations.[18] The PEG groups rarely achieve the fully extended conformation as are shown (for clarity) in Chart 2. The synthetic installation of PEG groups has been aided by the advent of heterotelechelic, monodisperse PEG reagents,[15,17,19,20] which are available from commercial sources.

In this paper, the synthesis and characterization of a dozen porphyrins are described. The motivation for the work has been to revisit and extend studies over the past two decades concerning the role of substitution patterns and substituent composition in engendering aqueous solubility. The issues addressed include the advantages and disadvantages of the following: (i) the pattern of 2,6- *versus* 3,5-dipegylated aryl groups; (ii) the terminus of the PEG moiety (methyl *versus* carboxylic acid); (iii) one *versus* two 2,6-dipegylated aryl groups; and (iv) a very short *versus* intermediate length PEG moiety. Zinc and copper chelates also were prepared given utility in photochemistry[21,22] and radiotherapeutics,[23] respectively. The attributes were assessed by measurements of aqueous–organic partitioning and concentration-dependent self-aggregation in aqueous solution.

Results

Synthesis

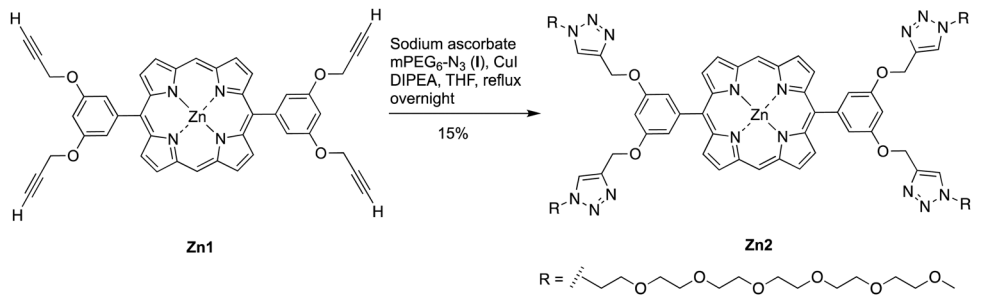
(i) Pattern of 2,6- *versus* 3,5-dipegylated aryl groups. For these architectures, prior synthesis has made available advanced intermediates.[24] Thus, tetraethynylporphyrin **Zn1**[24] and $mPEG_6$-azide (**I**, where the prefix "m" implies a methyl terminal group) were reacted under conditions[25,26] for copper-mediated click chemistry with tetrapyrroles. The conditions entail the use of CuI, sodium ascorbate, and diisopropylethylamine (DIPEA) in tetrahydrofuran (THF) at reflux. The reaction afforded the corresponding tetra-pegylated porphyrin **Zn2** (Scheme 1). The aryl groups are identical and are located at the 5- and 15-positions of the macrocycle, which is denoted as a *trans*-A_2 architecture. The zinc chelate was employed to block adventitious metalation of the free base porphyrin by copper, which if occurred would likely thwart the click reaction.

A facially encumbered porphyrin was prepared by a MacDonald-type condensation[27–31] (Scheme 2). Thus, condensation of 2,6-bis(prop-2-yn-1-yloxy)benzaldehyde (**3**)[32] and dipyrromethane (**4**)[33] was carried out upon catalysis by $BF_3 \cdot O(Et)_2$ followed by oxidation with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) and subsequent neutralization of the Lewis acid with triethylamine (TEA). In this manner, the *trans*-A_2 porphyrin 5,15-bis(2,6-bis(2-propynyloxy)phenyl)-porphyrin (**5**) was obtained in 21% yield. Porphyrin **5** was metalated with $Zn(OAc)_2 \cdot 2H_2O$ in refluxing *N*,*N*-dimethylformamide (DMF)[34] to give the zinc chelate **Zn5**.

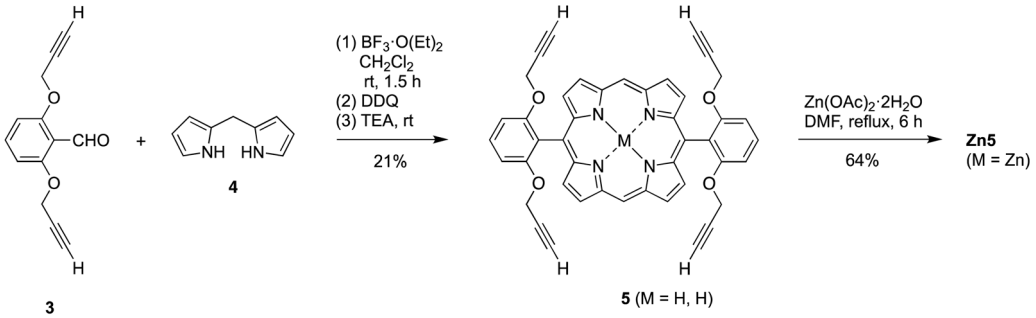
(ii) Terminus of the PEG moiety (methyl *versus* carboxylic acid). Zinc porphyrin **Zn5** and $mPEG_6$-azide were subjected to click reaction to give the corresponding tetra-pegylated zinc porphyrin **Zn6** in 71% yield (Scheme 3). To explore the effect of the nature of the metalation state on aqueous solubility, and to gauge the ease of copper insertion for studies in radiochemistry with copper radionuclides,[32] zinc porphyrin **Zn6** was demetalated with trifluoroacetic acid (TFA) at room temperature to give the free base porphyrin **6** in 96% yield. Free base porphyrin **6** was then treated overnight with $Cu(OAc)_2 \cdot H_2O$ at room temperature, affording the copper chelate **Cu6** in quantitative yield.

Analogues of porphyrins **6**, **Zn6**, and **Cu6** were prepared wherein each PEG group is terminated with an ionizable group. The carboxylic acid was chosen given ionization as the carboxylate at physiological pH as well as commercial availability of the required PEG synthons. Thus, the click reaction of porphyrin **Zn5** with azido-PEG_6-CO_2t-Bu (**II**) gave **Zn7** in 81% yield (Scheme 3). Treatment of the latter with TFA caused removal of the *tert*-butyl protecting groups and the zinc chelate, affording the free base porphyrin bearing four carboxylic acids (**8**) in 58% yield. Finally, exposure to copper acetate gave the copper porphyrin **Cu8** in 62% yield. Zinc porphyrin **Zn8** was prepared in small scale for comparative studies by zincation of free base porphyrin **8**.

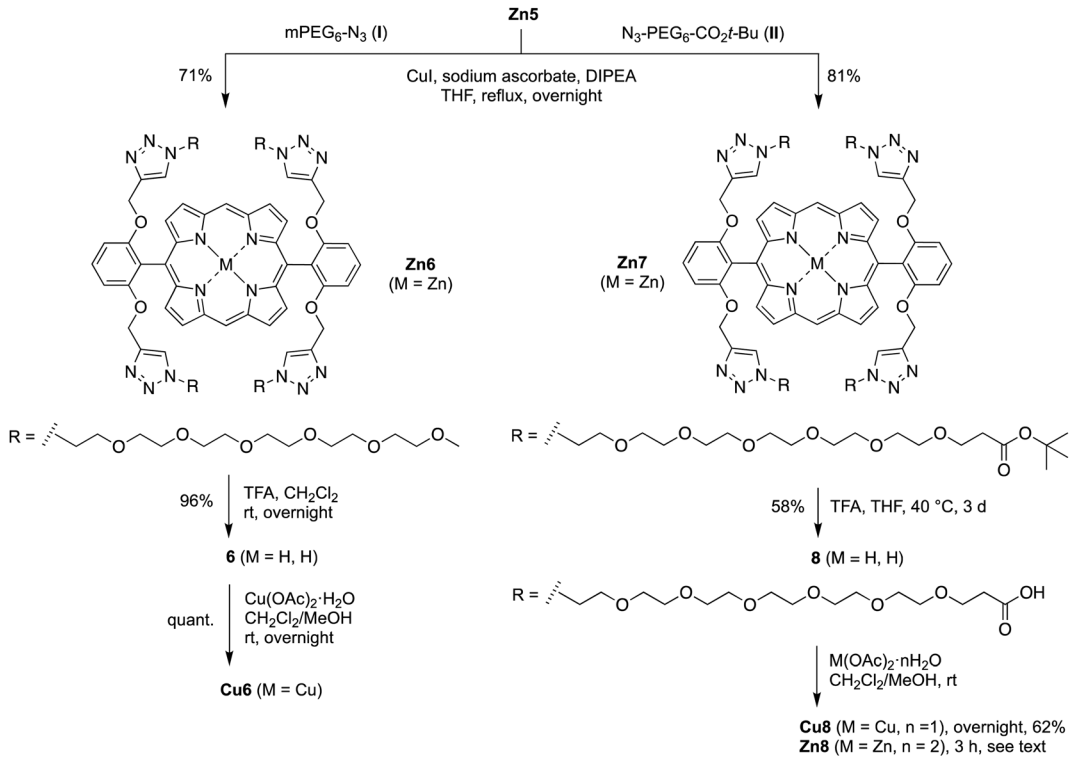
(iii) One 2,6-dipegylated aryl group. A porphyrin scaffold (**Zn9**) containing two propynyloxy groups and one TIPS-protected ethyne was recently prepared for successive elaboration with PEG and bioconjugatable groups.[32] Click chemistry with azido-PEG_6-CO_2t-Bu (**II**), sodium ascorbate, CuBr, and the ligand tris(hydroxypropyltriazolyl)methylamine (THPTA)



Scheme 1 Pegylation of a *meta*-aryl substituted *trans*-A_2 porphyrin.



Scheme 2 Synthesis of a facially encumbered *trans*-A_2 porphyrin scaffold.



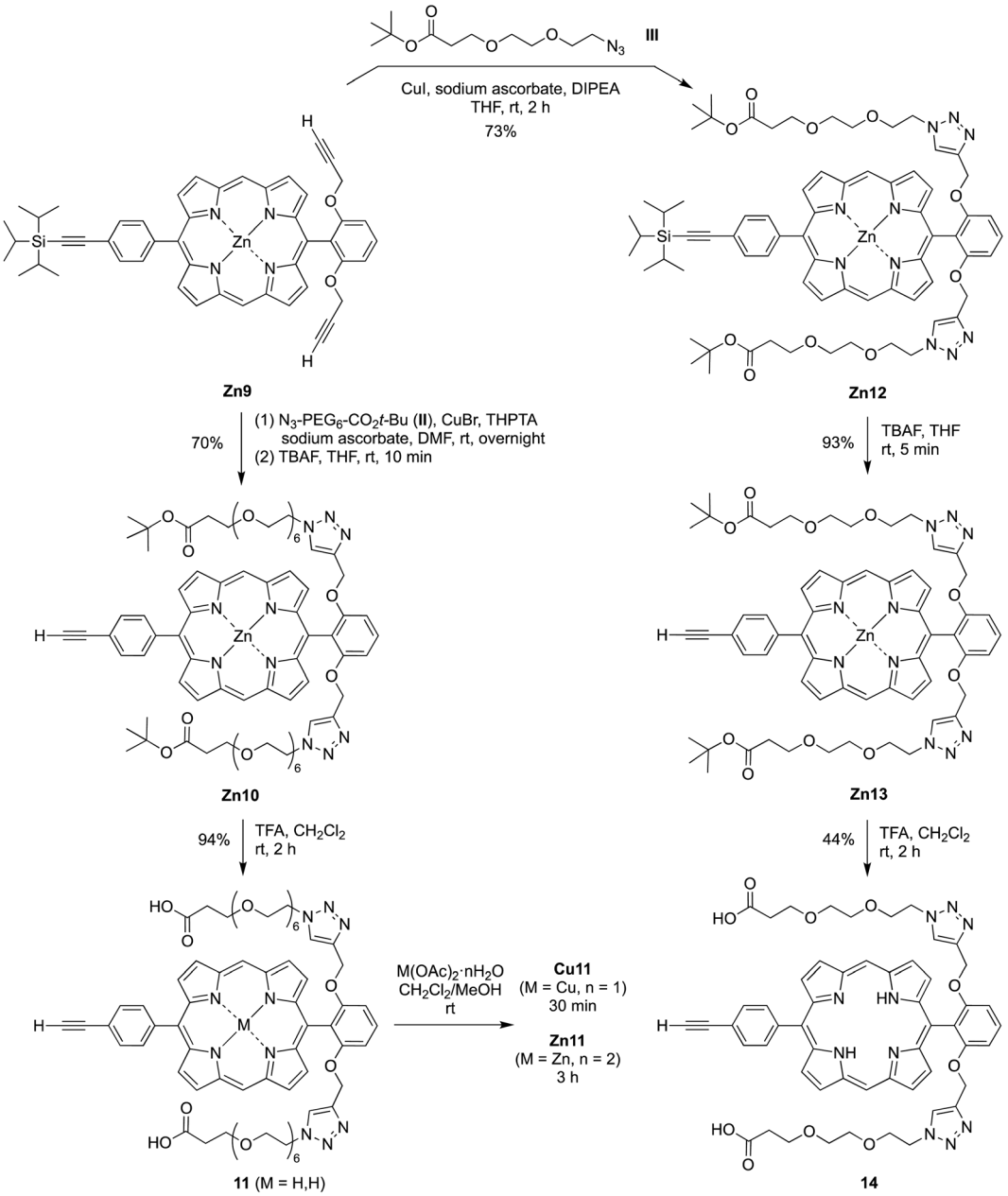
Scheme 3 Synthesis of *trans*-A_2 pegylated porphyrins.

followed by removal of the TIPS group with tetra-*n*-butylammonium fluoride (TBAF) afforded **Zn10**[32] in 70% yield (Scheme 4, left panel). Treatment of **Zn10** with TFA in CH_2Cl_2 for 2 h gave, in 94% yield, the free base porphyrin **11** for examination of aqueous solubility. Finally, **Cu11** and **Zn11** were prepared in small scale by metalation of free base porphyrin **11**.

(iv) Short PEG chains. The porphyrin scaffold **Zn9** also was treated with the short azido-PEG_2-CO_2*t*-Bu (**III**) *via* click chemistry to create porphyrin **Zn12** in 73% yield. Removal of the TIPS group with TBAF gave in 93% yield porphyrin **Zn13**, a homologue of zinc porphyrin **Zn10**,[32] containing PEG_2 *versus* PEG_6 groups. Treatment with TFA in CH_2Cl_2 gave the free base porphyrin **14**, a homologue of **11**, for studies of aqueous solubility (Scheme 4, right panel).

Characterization

The porphyrins were typically characterized by absorption spectroscopy, ^{1}H NMR and $^{13}C\{^1H\}$ NMR spectroscopy, and mass spectrometry. The absorption spectra of *trans*-A_2 and *trans*-AB free base porphyrins closely resemble those of well-known A_4 free base porphyrins, with a strong B (Soret) band and a progression of bands in the visible region typically in a phyllo[35] pattern. The zinc and copper chelates exhibit absorption spectra, particularly in the visible region, that are distinct from the parent free base porphyrin and also distinct from each other. For **6**, for example, the most intense visible band is found at 503 nm *versus* that of **Cu6** at 531 nm and of **Zn6** at 545 nm. The ^{1}H NMR spectrum of each free base or zinc porphyrin exhibited the characteristic resonance of the two



Scheme 4 Synthesis of *trans*-AB pegylated porphyrins.

meso-protons at $\delta \sim 10.12$ ppm, and for the free base porphyrins, the NH resonances near $\delta -3.13$ ppm. The β-pyrrole protons resonated as two doublets in the aromatic region ($\delta \sim 9.29$ and ~ 8.98 ppm) for *trans*-A_2 porphyrins and four doublets ($\delta \sim 9.36$, 9.26, 9.03 and 8.94 ppm) for *trans*-AB porphyrins. The PEG resonances are described below. The copper chelates were not characterized by NMR spectroscopy given the line broadening caused by relaxation from the copper center.[36,37]

The ^{1}H NMR spectrum of the zinc *trans*-A_2 porphyrin with 3,5-dipegylated aryl groups (**Zn2**) is shown in Fig. 1 (top panel). The resonances from the PEG –OCH_2CH_2– units and the terminal methyl group appear in the range $\delta \sim 3.7$–3.0 ppm. The ^{1}H NMR spectrum of the zinc *trans*-A_2 porphyrin with 2,6-dipegylated aryl groups (**Zn6**) is shown in Fig. 1 (bottom panel). Here, the resonances from the PEG –OCH_2CH_2– units and the terminal methyl group encompass a much larger range, spanning δ 3.7–2.0 ppm, *versus* that of **Zn2**. The peaks in the upfield region of **Zn6** are attributed to –OCH_2CH_2– units thrust over the faces of the macrocycle, thereby experiencing the aromatic ring current. The –OCH_2– unit linking the *meso*-aryl and triazole moieties of **Zn6** resonates downfield (δ 5.04 ppm) *versus* that of **Zn2** (δ 4.45 ppm) which is attributed also to the ring current but at the outer edge of the macrocycle. Similarly, the triazolyl-H proton of **Zn6** resonates downfield (δ 5.99 ppm) *versus* that of **Zn2** (δ 5.12 ppm), again shifted by the porphyrin ring current. The minimum conclusions are that the 2,6-disubstitution pattern

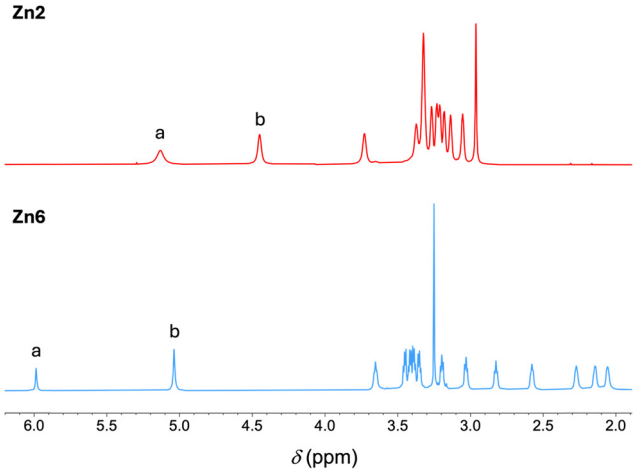


Fig. 1 ^{1}H NMR spectra in $CDCl_3$ at room temperature of zinc porphyrins **Zn2** (top) and **Zn6** (bottom). Peak assignments: (a) the resonance of the triazolyl–H. (b) The resonance of the protons of the $-OCH_2-$ linking the *meso*-aryl and triazole moieties. All other peaks derive from the PEG chains.

(**Zn6**) affords facial encumbrance by the attached PEG chains that is not present in the 3,5-disubstitution pattern (**Zn2**).

The structure of **Zn5** was confirmed by single-crystal X-ray diffraction upon crystallization from THF at −20 °C (Fig. 2). The zinc ion is hexacoordinate with a THF molecule at each apical site. The *meso*-aryl dihedral plane is *ca.* 71° (70.98(4)°). The 2-propynyl groups project over the two faces of the macrocycle. Other analogues include the following substituents on the oxy moieties of 2,6-dialkoxyarylporphyrins: methyl,[38–45] ethyl,[46] butyl,[47–49] octyl,[50] dodecyl,[51] 3,3-dimethylbutyl,[52] 4-hydroxybutyl,[53] 4-oxa-3-oxoheptyl,[54] *tert*-butyldimethylsilyl,[55] and pentafluorophenylmethyl.[56] Strapped porphyrins with 2,6-dialkoxyaryl units include doubly strapped *meso–meso*-linked arrays[57] and basket-handle thiolate Fe(III) porphyrins.[58]

Assessments in aqueous solution

A set of tests was carried out with selected porphyrins to gauge suitability of the designs for use in aqueous solution.

(i) Log *P* measurements. The partitioning of an organic compound between octanol and aqueous solution is a standard proxy to gauge aqueous-membrane partitioning in biological systems.[59] The partition coefficient (*P*) for a given porphyrin was examined by allowing a minute quantity of porphyrin to partition between deionized water (or PBS) and octanol at room temperature ($\sim 10^{-4}$ M total porphyrin concentration).[60] A sample was removed from each phase and examined by absorption spectroscopy in dimethylsulfoxide (DMSO) to determine the concentration. The results are shown in Table 1. Representative photographs are provided in Fig. 3. Two porphyrins can be regarded as benchmarks: **TPS-por** is found entirely in the aqueous layer ($\log P < -2$) whereas the fully *tert*-butyl protected tetraester zinc porphyrin **Zn7** is found entirely in the octanol layer ($\log P > 2$).

Porphyrin **Zn2**, which bears neutral PEG groups at the 3,5-positions of the two aryl rings, exhibited an extensively broadened Soret band (see the ESI†) in water, which is suggestive of

Table 1 Aqueous-organic partitioning of porphyrins

Compd	Type	Projection	PEG *n*	Terminus	Log *P*
TPS-por[b]	A_4	—	0	—	<-2
Zn2[a]	A_2	3,5-	6	–OMe	ND[c]
6[a]	A_2	2,6-	6	–OMe	−0.46
Zn6[a]	A_2	2,6-	6	–OMe	−0.40
Cu6[a]	A_2	2,6-	6	–OMe	−0.37
Zn7[a]	A_2	2,6-	6	$-CO_2t$Bu	>2
8[b]	A_2	2,6-	6	$-CO_2H$	<-2
Zn8[b]	A_2	2,6-	6	$-CO_2H$	<-2
Cu8[b]	A_2	2,6-	6	$-CO_2H$	<-2
11[b]	AB	2,6-	6	$-CO_2H$	−1.6
Zn11[b]	AB	2,6-	6	$-CO_2H$	−1.1
Cu11[b]	AB	2,6-	6	$-CO_2H$	ND[c]
14[b]	AB	2,6-	2	$-CO_2H$	ND[c]

[a] Deionized water as the aqueous phase. [b] PBS as the aqueous phase. [c] Not determined because of insolubility in aqueous solution.

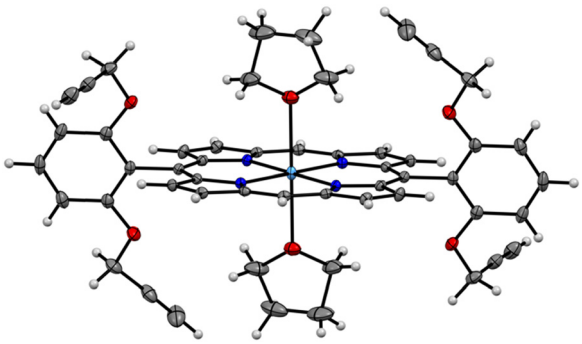


Fig. 2 Crystal structure of **Zn5** (crystallized from THF at −20 °C). Disorder is omitted for clarity. Labels: C = grey, N = blue, O = red, Zn = cyan, and H = white.

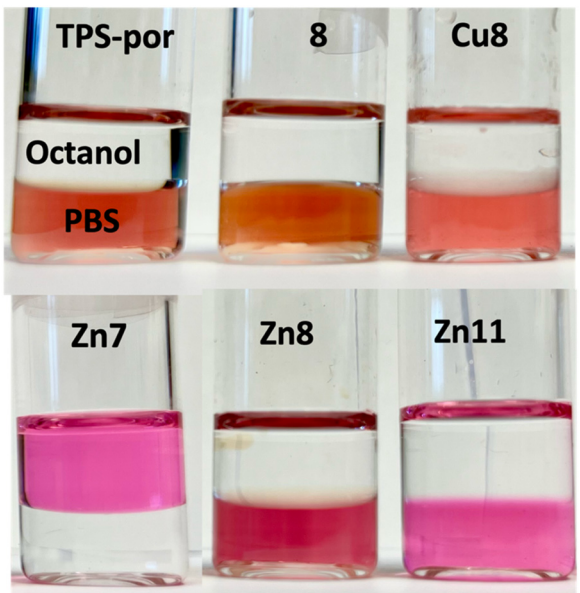


Fig. 3 Photographs of results upon log *P* evaluations with free base porphyrins (**TPS-por**, **8**), copper porphyrin **Cu8**, and zinc porphyrins (**Zn7**, **Zn8**, **Zn11**).

aggregation; hence, a log *P* value was unobtainable. All of the other porphyrins examined bear PEG groups at the 2,6-positions and gave sharp absorption bands at very dilute concentration (2 μM) in aqueous media. The three porphyrins **6**, **Zn6**, and **Cu6** each showed preferential partitioning in water *versus* octanol by a ratio of ~2.5 : 1. No significant difference was observed as a function of the metalation state: zinc(II), copper(II), or free base. Similar phenomena were observed for the series of **8**, **Zn8**, and **Cu8**; however, the aqueous partitioning decreased along the series **11** (log *P* = −1.6), **Zn11** (−1.1), and **Cu11** (insoluble in aqueous media). The lack of solubility of the copper chelate **Cu11** *versus* the zinc chelate **Zn11** and free base **11** was surprising and may indicate beneficial solubilization interactions in the latter two cases that are not available in the copper chelate. Such interactions could include aqueous (or PEG carboxylate/carboxylic acid) oxygenic coordination to the apical zinc site of **Zn11**, or aqueous (or PEG carboxylate/carboxylic acid) hydrogen-bonding with the free base N–H moieties of **11**.

Porphyrin **8**, which bears four PEG groups terminated with carboxylic acid moieties (as opposed to methyl groups), was not detected in the organic layer. We denote the ratio as >100 : 1 and the log *P* value as <−2. For porphyrins with only two PEG groups, the PEG_6 groups imparted preference for aqueous solution (**11**, log *P* −1.6) whereas the PEG_2 groups (**14**) resulted in aggregation in PBS; hence, a log *P* value could not be determined.

(ii) Self-aggregation in aqueous solution. The second test examined self-aggregation as a function of concentration in aqueous solution. The occurrence of self-aggregation can be examined by absorption spectroscopy upon reciprocal change of porphyrin concentration (0.2–200 μM) and cuvette path-length (100–0.1 mm).[60] The results with porphyrin **6**, **Cu6**, **8** and **11** in aqueous solution over the 1000-fold concentration range are shown in Fig. 4. Porphyrins **6**, **Cu6** and **8** showed no change in absorption spectral features consistent with absence of self-aggregation over this range. On the other hand, **11** showed slight spectral broadening at 20 μM and extensive broadening at 200 μM, indicative of self-aggregation. The inclusion of 3% bovine serum albumin (BSA), a protein that can solubilize organic molecules in aqueous solution,[60] resulted in little or no spectral broadening of porphyrin **11** even at 200 μM (see the ESI†).

During the course of experimentation, stock solutions of porphyrins **6** and **11** were prepared in aqueous solution (see ESI†). Porphyrin **6** in water (12 mM) gave an optically clear, bright red solution. Porphyrin **11** (1 mM) in PBS gave a transparent albeit muddy red solution. A muddy appearance is consistent with broadened absorption bands,[61] and the aforementioned concentration-dependent studies indicated aggregation even at substantially lower concentrations.

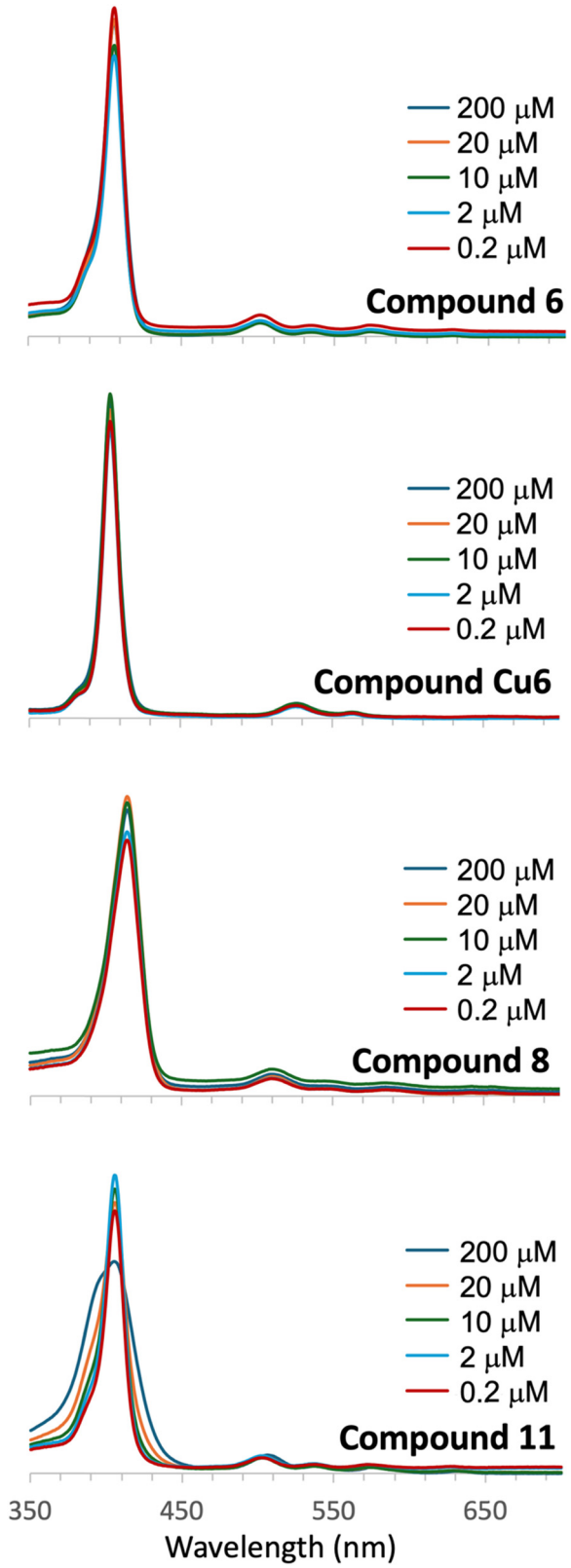


Fig. 4 Absorption spectra of porphyrins in PBS at room temperature.

Discussion

The development of water-soluble porphyrins has been an ongoing activity since the 1950s. Early studies required only a simple porphyrin devoid of substituents other than those that impart aqueous solubility, which could be satisfied by uroporphyrins and the synthetic porphyrins shown in Chart 1. In the decades since, designs have been sought that achieve aqueous

solubilization while maintaining sites on the porphyrin macrocycle for other substituents such as auxochromes and bioconjugatable groups. In some cases, very compact designs are desired for biomedical applications.

PEG groups are widely used in seemingly *ad hoc* fashion to impart aqueous solubility without studies to explore the effects of the number of PEGs, the length of PEGs, and the terminal groups of PEGs. Here, three porphyrin scaffolds bearing 2-propynyloxy groups (**Zn1**, **Zn5**, **Zn9**) have been derivatized with PEG groups *via* click chemistry. It may be little appreciated beyond the aficionado that PEG molecules can afford both organic and aqueous solubility, although a core text states that "PEG will partition in favor of water in a water–benzene system and in favor of methylene chloride in a water–methylene chloride system."[13]

Assessments pertaining to aqueous solubilization entailed partitioning between octanol and aqueous solution and examination of the absorption spectra as a function of concentration. Methods for calculation of $c\log P$ values often give wildly disparate results with porphyrins;[62] hence, measured values are essential. It warrants emphasis that a $\log P$ value is not an indication of solubility *per se* but rather a partitioning in lipophilic *versus* aqueous media. Aqueous–organic partitioning and solubility are related yet distinct phenomena. The former represents competition between solubilization in two distinct liquid phases, whereas the latter represents competition between homogeneous dispersion in a liquid phase *versus* the affinity for the aggregated solid state. Aqueous–organic partitioning is given by a dimensionless value whereas solubility has units such as g cm^{-3}. The overall solubility in water could be low or high but give the same ratio for aqueous–organic partitioning. The key findings are as follows:

(i) 2,6-diaryl substitution (**Zn6**) is superior to 3,5-diaryl substitution (**Zn2**) for aqueous solubilization. An interpretation is provided in Fig. 5. The 2,6-dialkoxyaryl group has constrained motion causing the groups to project above and below the plane, whereas the 3,5-dialkoxyaryl group is not so constrained and can rotate toward planarity with the macrocycle.[63]

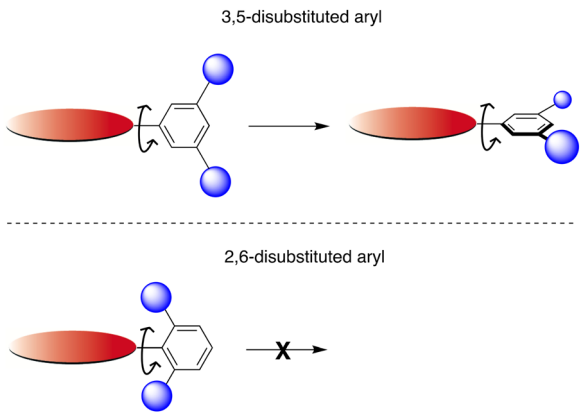


Fig. 5 Wide conformational motion is available in the 3,5-disubstituted aryl porphyrin (top) *versus* limited motion for the 2,6-disubstituted aryl porphyrin (bottom).

The latter motion opens the possibility of intermolecular hydrophobic interactions leading to self-aggregation and therefore limited solubility. We had surmised that facial encumbrance was invaluable for solubilization but had heretofore not done a direct test in aqueous solution with non-ionizable substituents.

(ii) PEG groups terminated with carboxylic acid groups provide enhanced aqueous solubility *versus* simple methyl termination at physiological pH, where the former are predominantly in the carboxylate form. Both *trans*-A_2 free base porphyrins **6** (methyl termini) and **8** (carboxylate termini) are aqueous soluble, as evidenced by the $\log P$ values (Table 1) and the retention of sharp absorption spectra over a 1000-fold concentration range (Fig. 4). The $\log P$ values show a key distinction, however the aqueous:octanol ratio is 100 : 35 for **6** *versus* > 100 : 1 for **8**.

(iii) One rather than two water-solubilization motifs suffices to impart a degree of aqueous solubility, which enables use of a *trans*-AB porphyrin (*e.g.*, **11**, two PEG-carboxylates) rather *trans*-A_2 porphyrins (*e.g.*, **8**, four PEG-carboxylates). Both **8** and **11** exhibit quite negative $\log P$ values (< −2, −1.6; *i.e.*, high aqueous partitioning) but **11** does show signs of self-aggregation at concentrations in the range of 20–200 μM.

(iv) The porphyrin bearing two carboxylate-terminated PEG_2 groups (**14**) rather than PEG_6 groups (**11**) was insufficiently soluble to obtain a $\log P$ measurement. Despite the self-aggregation of **11** in the 20–200 μM range, a derivative thereof that contains a folic acid moiety tethered *via* a PEG_5 group (clicked onto the 4-ethynylphenyl moiety) did not show signs of self-aggregation across the concentration range of 4.8–480 μM in PBS at room temperature.[32] Such results indicate that incorporation of a polar motif with **11** substantially increases the aqueous solubility.

In summary, the studies reported herein point to molecular designs for achieving aqueous solubilization with porphyrins. The use of only one or two *meso*-substituents leaves other sites available for substitution. More extensive comparisons beyond the pairwise evaluations reported herein are required to explore the generality of the molecular design heuristics.

Experimental section

General methods

^1H and $^{13}C\{^1H\}$ NMR spectra were recorded (Bruker Ascend™ 500 and Bruker Ascend™ 700 MHz instruments) in CD_2Cl_2 or $CDCl_3$ at room temperature unless noted otherwise. Absorption spectra were collected in toluene or DMSO at room temperature. Matrix-assisted laser desorption ionization mass spectrometry (MALDI-MS) was recorded using a Bruker autoflex® max with the matrix α-cyano-4-hydroxycinnamic acid (α-CHCA). Electrospray ionization mass spectrometry (ESI-MS) data were recorded using a Thermo Fisher Scientific Exactive Plus MS, a benchtop full-scan orbitrap mass spectrometer using heated electrospray ionization. Data are reported for the molecular ion or cationized molecular ion. Single crystal X-ray diffraction

(SCXRD) data were collected using a Bruker D8 Venture, running at 100 K with MoKα (λ = 0.71073 Å). All crystal data and graphics were refined and generated by OLEX2 and Mercury software, respectively. THF was freshly distilled from sodium/benzophenone ketyl and used immediately. Commercially available compounds were used as received. Silica gel (40 μm average particle size) was used for column chromatography. PBS buffer (1×) was employed at pH 7.4. Compounds **Zn1**,[24] **4**,[33] **Zn9**,[32] and **Zn10**[32] were prepared as described in the literature.

Determination of log *P* values

Values were determined at room temperature following a reported method.[60] The quantity of porphyrin employed was ~0.2–0.4 mg in a total octanol–aqueous solution of 2 mL giving concentrations of 40–150 μM (*i.e.*, $\sim 10^{-4}$ M). In each case, octanol refers to 1-octanol.

Examination of concentration-dependent self-aggregation

Values were determined by reciprocal change of concentration and cuvette pathlength in aqueous media at room temperature following a reported method.[60]

Synthesis procedures and characterization data

Zn(II) 5,15-bis{3,5-bis[1-(1-(3,6,9,12,15,18-hexaoxanonadecyl)-1*H*-1,2,3-triazol-4-yl)methoxy]phenyl}porphyrin (Zn2). Following a reported method[26] with some modifications, a solution of zinc porphyrin **Zn1** (14 mg, 0.019 mmol) and $mPEG_6$-azide (**I**, 101 mg, 0.314 mmol) in dry THF (8.0 mL) was treated sequentially with sodium ascorbate (9.3 mg, 0.047 mmol), DIPEA (200 μL, 1.1 mmol), and CuI (5.7 mg, 0.030 mmol). The reaction mixture was allowed to reflux overnight (oil bath). The solution was concentrated under reduced pressure and then diluted in CH_2Cl_2. The organic phase was washed with water and brine, dried (Na_2SO_4), and concentrated to dryness. The crude product was chromatographed using a gradient [silica, CH_2Cl_2/MeOH (25 : 1) to (19 : 1) to (15 : 1)] to afford a red oil (5.4 mg, 15%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.21 (s, 2H), 9.32 (d, J = 4.4 Hz, 4H), 9.08 (d, J = 4.4 Hz, 4H), 7.81 (s, 4H), 7.45 (s, 4H), 7.04 (s, 2H), 5.13 (s, 4H), 4.45 (s, 8H), 3.73 (s, 8H), 3.37–2.86 (m, 96H); ^{13}C{^{1}H} NMR (175 MHz, $CDCl_3$) δ 156.3, 148.6, 148.5, 144.0, 142.4, 131.2, 130.6, 123.2, 118.0, 114.5, 104.9, 100.2, 70.4, 69.3–68.2, 61.0, 57.6, 53.2, 49.3; λ_{abs} (toluene) 416, 544, 580 nm; MALDI-MS obsd 2024.02, calcd 2024.90 [M^+]; ESI-MS obsd 1035.4384, calcd 1035.4393 [$(M + 2Na)^{2+}$], M = $C_{96}H_{136}N_{16}O_{28}Zn$.

5,15-Bis(2,6-bis(2-propynyloxy)phenyl)porphyrin (5). Following a reported method[24] with some modifications, a solution of aldehyde **3** (2.2 g, 10 mmol) and dipyrromethane **4** (1.5 g, 10 mmol) in CH_2Cl_2 (500 mL) was treated dropwise with $BF_3{\cdot}O(Et)_2$ (190 μL, 1.54 mmol) over 1 minute. The mixture was stirred at room temperature. Aliquots of the solution were analyzed by absorption spectroscopy to monitor the progress of the reaction. After 1.5 h, the reaction mixture was oxidized by the addition of DDQ (2.3 g, 10 mmol), stirred at room temperature for 15 min, and then neutralized by the addition of triethylamine (2.0 mL, 14 mmol). After 10 min, the resulting mixture was poured onto a silica pad (5 cm × 30 cm) and eluted with CH_2Cl_2. The filtrate was concentrated to dryness under reduced pressure. The crude product was triturated with hexanes/$CHCl_3$ to afford a dark-red solid (0.72 g, 21%): ^{1}H NMR (700 MHz, CD_2Cl_2) δ 10.25 (s, 2H), 9.37 (d, J = 4.4 Hz, 4H), 8.99 (d, J = 4.4 Hz, 4H), 7.84 (t, J = 8.8 Hz, 2H), 7.29 (d, J = 8.8 Hz, 4H), 4.43 (s, 8H), 2.37 (t, J = 2.6 Hz, 4H), −3.17 (s, 2H); ^{13}C{^{1}H} NMR (175 MHz, CD_2Cl_2) δ 158.5, 131.5, 130.3, 130.1, 120.3, 110.3, 106.8, 104.5, 78.5, 75.1, 56.3; λ_{abs} (toluene) 408, 501, 534, 579 nm; MALDI-MS obsd 678.13, calcd 678.23 [M^+]; ESI-MS obsd 679.2335, calcd 679.2339 [$(M + H)^+$], M = $C_{44}H_{30}N_4O_4$.

Zinc(II) 5,15-bis(2,6-bis(2-propynyloxy)phenyl)porphyrin (Zn5). A sample of $Zn(OAc)_2{\cdot}2H_2O$ (1.3 g, 5.8 mmol) was added to a solution of porphyrin **5** (78 mg, 0.12 mmol) in DMF (20.0 mL). The reaction mixture was placed in an oil bath to reflux for 6 h. The mixture was concentrated under reduced pressure. The crude product was dissolved in CH_2Cl_2, washed (with saturated aqueous $NaHCO_3$, water, and brine), and then dried (Na_2SO_4) and concentrated to give a red solid (55 mg, 64%): ^{1}H NMR (500 MHz, CD_2Cl_2) δ 10.29 (s, 2H), 9.43 (d, J = 4.4 Hz, 4H), 9.06 (d, J = 4.4 Hz, 4H), 7.84 (t, J = 8.6 Hz, 2H), 7.29 (d, J = 8.6 Hz, 4H), 4.41 (d, J = 2.4 Hz, 8H), 2.33 (t, J = 2.4 Hz, 4H); ^{13}C{^{1}H} NMR (125 MHz, CD_2Cl_2) δ 158.5, 150.2, 149.4, 131.8, 131.5, 129.9, 121.6, 111.0, 106.8, 105.5, 78.5, 75.0, 56.3; λ_{abs} (toluene) 414, 539, 575 nm; MALDI-MS obsd 740.05, calcd 740.15 [M^+]; ESI-MS obsd 741.1453, calcd 741.1475 [$(M + H)^+$], M = $C_{44}H_{28}N_4O_4Zn$. Porphyrin **Zn5** upon slow evaporation from tetrahydrofuran at −20 °C afforded a crystalline sample that was then examined by single-crystal X-ray diffraction.

Zn(II) 5,15-bis{2,6-bis[1-(1-(3,6,9,12,15,18-hexaoxanonadecyl)-1*H*-1,2,3-triazol-4-yl)methoxy]phenyl}porphyrin (Zn6). Following a reported method[26] with some modifications, a solution of zinc porphyrin **Zn5** (15 mg, 0.020 mmol) and $mPEG_6$-azide (**I**, 93 mg, 0.29 mmol) in dry THF (8.0 mL) was treated sequentially with sodium ascorbate (10 mg, 0.050 mmol), DIPEA (200 μL, 1.10 mmol), and CuI (5.6 mg, 0.030 mmol) under an argon atmosphere. The reaction mixture was refluxed overnight (oil bath). The solution was concentrated under reduced pressure and then dissolved in CH_2Cl_2. The organic layer was washed with water and brine, dried (Na_2SO_4), and concentrated to dryness. The crude product was chromatographed using a gradient [silica, CH_2Cl_2/MeOH (25 : 1) to (15 : 1) to (9 : 1)] to afford a red oil (29 mg, 71%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.12 (s, 2H), 9.29 (d, J = 4.4 Hz, 4H), 8.97 (d, J = 4.4 Hz, 4H), 7.79 (t, J = 8.5 Hz, 2H), 7.25 (d, J = 8.5 Hz, 4H), 5.99 (s, 4H), 5.04 (s, 8H), 3.66 (t, J = 5.2 Hz, 8H), 3.47–3.35 (m, 32H), 3.26 (s, 12H), 3.20 (t, J = 5.0 Hz, 8H), 3.05–3.03 (m, 8H), 2.83 (t, J = 5.0 Hz, 8H), 2.58 (t, J = 5.0 Hz, 8H), 2.28 (t, J = 5.0 Hz, 8H), 2.15 (t, J = 4.0 Hz, 8H), 2.06 (t, J = 4.5 Hz, 8H); ^{13}C{^{1}H} NMR (175 MHz, CD_2Cl_2) δ 159.5, 150.4, 149.2, 143.5, 131.7, 131.6, 130.3, 122.9, 121.9, 111.6, 106.9, 105.1, 72.0–63.2, 58.7, 58.6, 50.9, 49.5; λ_{abs} (toluene) 414, 545, 581 nm; MALDI-MS obsd 2025.40, calcd 2024.90 [M^+]; ESI-MS obsd 2047.8895, calcd 2047.8894 [$(M + Na)^+$], M = $C_{96}H_{136}N_{16}O_{28}Zn$.

5,15-Bis{2,6-bis[1-(1-(3,6,9,12,15,18-hexaoxanonadecyl)-1*H*-1,2,3-triazol-4-yl)methoxy]phenyl}porphyrin (6). A solution of zinc porphyrin **Zn6** (18 mg, 8.8 μmol) in CH_2Cl_2 (6.0 mL) was

treated with TFA (40 μmol, 0.52 mmol). The reaction mixture was stirred overnight at room temperature under an argon atmosphere. The solution was neutralized by the addition of saturated aqueous $NaHCO_3$, then washed with water and brine, and dried (Na_2SO_4). The combined organic extract was concentrated under reduced pressure to give a red oil (17 mg, 96%): ^{1}H NMR (700 MHz, $CDCl_3$) δ 10.18 (s, 2H), 9.31 (d, J = 4.4 Hz, 4H), 8.96 (d, J = 4.4 Hz, 4H), 7.80 (t, J = 8.7 Hz, 2H), 7.27 (s, 4H), 6.10 (s, 4H), 5.07 (s, 8H), 3.71 (t, J = 5.2 Hz, 8H), 3.56–3.43 (m, 32H), 3.37–3.35 (m, 8H), 3.31 (s, 12H), 3.27–3.26 (m, 8H), 3.07–3.05 (m, 8H), 2.93 (t, J = 4.4 Hz, 8H), 2.80 (t, J = 4.4 Hz, 8H), 2.47–2.43 (m, 16H), −3.15 (s, 2H); ^{13}C{^{1}H} NMR (175 MHz, $CDCl_3$) δ 159.2, 143.5, 131.4, 130.8, 122.8, 119.9, 111.3, 106.9, 104.2, 77.2–69.5, 68.4, 63.2, 59.0, 50.7, 49.5; λ_{abs} (toluene) 410, 503, 537, 578, 632 nm; MALDI-MS obsd 1963.56, calcd 1963.99 [(M + H)$^+$]; ESI-MS obsd 1985.9752, calcd 1985.9759 [(M + Na)$^+$], M = $C_{96}H_{138}N_{16}O_{28}$.

Cu(II) 5,15-bis{2,6-bis[1-(1-(3,6,9,12,15,18-hexaoxanonadecyl)-1*H*-1,2,3-triazol-4-yl)methoxy]phenyl}porphyrin (Cu6). A solution of free base porphyrin **6** (5.2 mg, 2.7 μmol) in CH_2Cl_2/MeOH (3.0 mL, 1 : 2) was treated with $Cu(OAc)_2{\cdot}H_2O$ (54 mg, 0.27 mmol). The reaction mixture was stirred overnight at room temperature under an argon atmosphere. The mixture was concentrated under reduced pressure, diluted in CH_2Cl_2, and neutralized by the addition of saturated aqueous $NaHCO_3$. The organic phase was washed with water and brine, and then dried (Na_2SO_4). The combined organic extract was concentrated to give a red oil (5.4 mg, 100%) λ_{abs} (toluene) 407, 531, 563 nm; MALDI-MS obsd 2046.98, calcd 2046.90 [(M + Na)$^+$]; ESI-MS obsd 2046.8931, calcd 2046.8898 [(M + Na)$^+$], M = $C_{96}H_{136}CuN_{16}O_{28}$.

Zn(II)-5,15-bis{2,6-bis[1-(1-(21-*tert*-butyloxy-21-oxo-3,6,9,12,15,18-hexaoxaheneicosanyl)-1*H*-1,2,3-triazol-4-yl)methoxy]phenyl}-porphyrin (Zn7). Following a reported method[26] with some modifications, a solution of zinc porphyrin **Zn5** (15 mg, 0.020 mmol) and azido-PEG$_6$-CO$_2$*t*-Bu (**II**, 70 mg, 0.16 mmol) in dry THF (8.0 mL) was treated with sodium ascorbate (10 mg, 0.050 mmol), DIPEA (200 μL, 1.10 mmol), and CuI (6.0 mg, 0.032 mmol). The reaction mixture was allowed to reflux overnight (oil bath). The mixture was concentrated under reduced pressure and then dissolved in CH_2Cl_2. The organic phase was washed with water and brine, dried (Na_2SO_4), and concentrated to dryness under reduced pressure. The crude product was chromatographed using a gradient [silica, CH_2Cl_2/MeOH (19 : 1) to (16 : 1)] to afford a red oil (40 mg, 81%): ^{1}H NMR (700 MHz, $CDCl_3$) δ 10.13 (s, 2H), 9.29 (d, J = 4.3 Hz, 4H), 8.98 (d, J = 4.3 Hz, 4H), 7.78 (t, J = 8.7 Hz, 2H), 7.27–7.17 (m, 4H), 5.99 (s, 4H), 5.05 (s, 8H), 3.69–3.64 (m, 16H), 3.53–3.49 (m, 16H), 3.44 (t, J = 4.9 Hz, 8H), 3.34 (t, J = 4.9 Hz, 8H), 3.18 (t, J = 4.9 Hz, 8H), 2.99 (t, J = 4.9 Hz, 8H), 2.81 (t, J = 4.9 Hz, 8H), 2.47–2.45 (m, 16H), 2.13 (t, J = 4.9 Hz, 8H), 2.04 (t, J = 4.9 Hz, 8H), 1.92 (t, J = 4.9 Hz, 8H), 1.43 (s, 36H); ^{13}C{^{1}H} NMR (175 MHz, $CDCl_3$) δ 170.9, 159.3, 150.3, 149.0, 143.5, 131.8, 131.5, 130.3, 122.8, 121.7, 111.5, 106.8, 104.9, 80.5, 70.4–68.8, 68.1, 66.8, 63.3, 49.2, 36.2, 28.1; λ_{abs} (toluene) 414, 544, 582 nm; MALDI-MS obsd 2481.87, calcd 2481.17 [M$^+$]; ESI-MS obsd 2504.1593, calcd 2504.1617 [(M + Na)$^+$], M = $C_{120}H_{176}N_{16}O_{36}Zn$.

5,15-Bis{2,6-bis[1-(1-(21-hydroxy-21-oxo-3,6,9,12,15,18-hexaoxaheneicosanyl)-1*H*-1,2,3-triazol-4-yl)methoxy]phenyl}porphyrin (8). A solution of zinc porphyrin **Zn7** (19 mg, 7.7 μmol) in THF (2.0 mL) was treated with TFA (300 μL). The reaction mixture was stirred at 40 °C (oil bath) for 3 days, then concentrated under reduced pressure. The crude residue was dissolved in CH_2Cl_2 (25 mL), and water (25 mL) was added followed by triethylamine until pH = 9. The aqueous phase was washed once with CH_2Cl_2. The aqueous phase was acidified with aqueous 1 M HCl until pH = 2, then extracted with CH_2Cl_2 (2 × 25 mL). The combined organic extract was washed with water (25 mL) and brine (25 mL), dried (Na_2SO_4), and concentrated to give a red oil (9.9 mg, 58%): ^{1}H NMR (700 MHz, $CDCl_3$) δ 10.21 (s, 2H), 9.32 (d, J = 4.4 Hz, 4H), 8.96 (d, J = 4.4 Hz, 4H), 7.79 (t, J = 8.6, 2H), 7.27 (m, 4H), 6.14 (s, 4H), 5.09 (s, 8H), 3.73–3.36 (m, 64H), 3.28–2.89 (m, 16H), 2.69–2.68 (m, 8H), 2.54–2.48 (m, 8H), 2.39–2.32 (m, 16H), −3.21 (s, 2H); ^{13}C{^{1}H} NMR (175 MHz, $CDCl_3$) δ 175.0, 159.1, 143.5, 131.5, 130.9, 130.8, 123.0, 119.8, 111.3, 106.9, 104.3, 70.3–69.3, 68.3, 66.8, 63.1, 49.6, 35.5; λ_{abs} (toluene) 410, 503, 531, 574, 630 nm; ESI-MS obsd 2193.9992, calcd 2194.0013 [(M − H)$^-$], M = $C_{104}H_{146}N_{16}O_{36}$.

Cu(II)-5,15-bis{2,6-bis[1-(1-(21-hydroxy-21-oxo-3,6,9,12,15,18-hexaoxaheneicosanyl)-1*H*-1,2,3-triazol-4-yl)methoxy]phenyl}-porphyrin (Cu8). A solution of free base porphyrin **8** (4.1 mg, 1.9 μmol) in CH_2Cl_2/MeOH (3.0 mL, 1 : 2) was treated with $Cu(OAc)_2{\cdot}H_2O$ (54 mg, 0.27 mmol). The mixture was stirred overnight at room temperature, then concentrated under reduced pressure. The crude residue was dissolved in CH_2Cl_2 and neutralized by the addition of saturated aqueous $NaHCO_3$. The organic layer was washed with water and brine, dried (Na_2SO_4), and concentrated to give a red oil (2.6 mg, 62%): λ_{abs} (toluene) 408, 530, 568 nm; ESI-MS obsd 2254.9144, calcd 2254.9152 [(M − H)$^-$], M = $C_{104}H_{144}CuN_{16}O_{36}$.

5-{2,6-Bis[1-(1-(21-hydroxy-21-oxo-3,6,9,12,15,18-hexaoxaheneicosanyl)-1*H*-1,2,3-triazol-4-yl)methoxy]phenyl}-5-(4-ethynylphenyl)porphyrin (11). A solution of zinc porphyrin **Zn10** (3.0 mg, 2.0 μmol) in CH_2Cl_2 (200 μL) was treated with TFA (200 μL). The reaction mixture was stirred at room temperature for 2 h, then concentrated under reduced pressure. The crude residue was dissolved in CH_2Cl_2 (15 mL), and water (15 mL) was added followed by saturated aqueous $NaHCO_3$/NaOH (20 : 1) until pH = 11. The aqueous phase was washed once with CH_2Cl_2. The aqueous phase was acidified with aqueous 1 M HCl until pH = 2, then extracted with CH_2Cl_2 (2 × 15 mL). The combined organic extract was dried (Na_2SO_4) and concentrated to give a red solid (2.5 mg, 94%): ^{1}H NMR (700 MHz, $CDCl_3$) δ 10.28 (s, 2H), 9.41 (d, J = 4.4 Hz, 2H), 9.34 (d, J = 4.4 Hz, 2H), 9.05 (d, J = 4.4 Hz, 2H), 8.99 (d, J = 4.4 Hz, 2H), 8.23 (d, J = 7.8 Hz, 2H), 7.96 (d, J = 7.8 Hz, 2H), 7.80 (t, J = 8.7 Hz, 1H), 7.28 (d, J = 8.7 Hz, 2H), 5.91 (s, 2H), 5.11 (s, 4H), 3.66 (t, J = 5.2 Hz, 4H), 3.61 (t, J = 6.0 Hz, 4H), 3.50–3.37 (m, 12H), 3.36 (s, 1H), 3.32–3.27 (m, 4H), 3.21–3.14 (m, 4H), 3.05–2.99 (m, 4H), 2.87 (t, J = 5.1 Hz, 4H), 2.76 (t, J = 4.7 Hz, 4H), 2.48–2.44 (m, 8H), 2.29–2.24 (m, 4H), 2.15–2.7 (m, 4H), −3.11 (s, 2H); ^{13}C{^{1}H} NMR (175 MHz, $CDCl_3$) δ 174.0, 159.1, 143.7, 142.0, 134.7, 131.9, 131.6, 131.1, 131.0, 130.8, 130.7, 122.8, 121.8, 119.8, 118.1, 111.4, 107.0, 105.0, 83.6, 70.4, 70.2, 70.1,

70.0, 70.0, 69.6, 69.4, 69.3, 69.3, 68.4, 66.5, 63.2, 49.5, 35.1; ESI-MS obsd 1375.5843, calcd 1375.5857 [(M + Na)$^+$], M = $C_{70}H_{84}N_{10}O_{18}$.

Zn(II)-5-(2,6-bis[1-(1-(9-*tert*-butyloxy-9-oxo-3,6-dioxanonyl)-1*H*-1,2,3-triazol-4-yl)methoxy]phenyl)-15-(4-(2-(triisopropylsilyl)ethynyl)phenyl)porphyrin (Zn12). Following a reported method[26] with some modifications, a solution of zinc porphyrin **Zn9** (20 mg, 25 μmol), *tert*-butyl 1-azido-3,6-dioxanonan-9-oate (**III**, 52 mg, 0.10 mmol), sodium ascorbate (20 mg, 0.10 mmol), and CuI (9.5 mg, 50 μmol) in dry THF (5.0 mL) was treated with DIPEA (100 μL, 0.57 mmol) under an argon atmosphere. The reaction mixture was stirred at room temperature for 2 h then eluted through a silica pad (2.5 cm × 1 cm) with ethyl acetate. The filtrate was concentrated and chromatographed [silica, CH_2Cl_2/ethyl acetate (9 : 1) to (1 : 9) then pure ethyl acetate] to afford a red non-crystalline solid (24 mg, 73%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.05 (s, 2H), 9.28 (d, J = 4.5 Hz, 2H), 9.13 (d, J = 4.4 Hz, 2H), 8.99 (d, J = 4.4 Hz, 2H), 8.78–8.65 (m, 2H), 8.14 (d, J = 7.5 Hz, 2H), 7.89 (d, J = 7.5 Hz, 2H), 7.60 (t, J = 8.5 Hz, 1H), 6.79–6.61 (m, 2H), 4.88 (s, 2H), 3.91 (s, 4H), 2.99 (s, 4H), 2.36–2.11 (m, 8H), 1.75–1.50 (m, 8H), 1.39–1.31 (m, 4H), 1.28 (s, 21H), 1.16 (s, 18H); ^{13}C{^{1}H} NMR (125 MHz, $CDCl_3$) δ 170.3, 158.6, 150.3, 149.4, 149.1, 149.0, 143.4, 143.1, 134.5, 131.8, 131.7, 131.4, 130.2, 122.5, 121.6, 121.3, 119.0, 111.1, 107.3, 106.4, 105.3, 91.6, 80.3, 68.5, 68.4, 67.4, 65.6, 62.2, 48.6, 35.0, 27.8, 18.9, 11.5; ESI-MS obsd 1331.5653, calcd 1331.5662 [(M + H)$^+$], M = $C_{71}H_{86}N_{10}O_{10}SiZn$.

Zn(II)-5-(2,6-bis[1-(1-(9-*tert*-butyloxy-9-oxo-3,6-dioxanonyl)-1*H*-1,2,3-triazol-4-yl)methoxy]phenyl)-15-(4-ethynylphenyl)porphyrin (Zn13). A solution of zinc porphyrin **Zn12** (24 mg, 18 μmol) in THF (3.6 mL) was treated with TBAF (1.0 M solution in THF, 36 μL, 36 μmol) for 5 min. The reaction mixture was concentrated and dissolved in CH_2Cl_2 (20 mL). The solution was washed with brine (30 mL × 2), saturated aqueous $NaHCO_3$ (30 mL × 2), and then brine (30 mL). The organic phase was then dried (Na_2SO_4), concentrated, and chromatographed [silica, CH_2Cl_2/ethyl acetate (9 : 1 to 1 : 2)] to afford a dark red solid (20 mg, 93%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.04 (s, 2H), 9.28 (d, J = 4.4 Hz, 2H), 9.11 (d, J = 4.4 Hz, 2H), 8.98 (d, J = 4.4 Hz, 2H), 8.70 (d, J = 4.4 Hz, 2H), 8.20–8.14 (m, 2H), 7.94–7.87 (m, 2H), 7.57 (t, J = 8.5 Hz, 1H), 6.65 (d, J = 8.6 Hz, 2H), 4.88 (s, 2H), 3.74 (s, 4H), 3.34 (s, 1H), 2.95 (t, J = 5.4 Hz, 4H), 2.37–2.24 (m, 8H), 1.81–1.67 (m, 8H), 1.45 (t, J = 6.5 Hz, 4H), 1.18 (s, 18H); ^{13}C{^{1}H} NMR (125 MHz, $CDCl_3$) δ 170.4, 158.7, 150.4, 149.5, 149.3, 149.1, 144.1, 143.1, 134.7, 131.9, 131.8, 131.6, 130.4, 130.2, 121.5, 121.4, 121.2, 118.8, 111.2, 106.3, 105.5, 84.0, 80.4, 78.2, 68.8, 68.6, 67.5, 65.8, 62.1, 48.7, 35.2, 28.0; ESI-MS obsd 1175.4308, calcd 1175.4328 [(M + H)$^+$], M = $C_{62}H_{66}N_{10}O_{10}Zn$.

5-(2,6-Bis[1-(1-(9-hydroxy-9-oxo-3,6-dioxanonyl)-1*H*-1,2,3-triazol-4-yl)methoxy]phenyl)-15-(4-ethynylphenyl)porphyrin (14). A solution of zinc porphyrin **Zn13** (11 mg, 10 μmol) in CH_2Cl_2 (500 μL) was treated with TFA (500 μL). The reaction mixture was stirred at room temperature for 2 h, then concentrated under reduced pressure. The crude residue was dissolved in CH_2Cl_2 (15 mL), then water (15 mL) was added followed by saturated aqueous $NaHCO_3$/NaOH (20 : 1) until pH = 11. The aqueous phase was washed once with CH_2Cl_2. The aqueous phase was acidified with aqueous 1 M HCl until pH = 2, then extracted with CH_2Cl_2 (2 × 15 mL). The combined organic extract was dried (Na_2SO_4) and concentrated to give a red solid (4.4 mg, 44%): ^{1}H NMR (500 MHz, CD_2Cl_2) δ 10.29 (s, 2H), 9.41 (d, J = 4.5 Hz, 2H), 9.36 (d, J = 4.5 Hz, 2H), 9.05 (d, J = 4.4 Hz, 2H), 8.95 (d, J = 4.4 Hz, 2H), 8.24 (d, J = 7.9 Hz, 2H), 7.96 (d, J = 7.9 Hz, 2H), 7.78 (t, J = 8.7 Hz, 1H), 7.25 (d, J = 8.7 Hz, 2H), 6.16 (s, 2H), 5.06 (s, 4H), 3.72 (t, J = 5.0 Hz, 4H), 3.42 (s, 1H), 2.95 (t, J = 5.0 Hz, 4H), 2.82 (t, J = 6.1 Hz, 4H), 2.49–2.42 (m, 4H), 2.39–2.33 (m, 4H), 2.03 (t, J = 6.1 Hz, 4H); ^{13}C{^{1}H} NMR (125 MHz, CD_2Cl_2) δ 175.4, 159.0, 143.5, 142.0, 134.8, 132.0, 131.6, 130.8, 122.9, 121.6, 119.7, 118.2, 111.2, 106.8, 105.0, 83.5, 78.2, 69.3, 69.2, 68.4, 65.6, 63.0, 49.6, 34.4; ESI-MS obsd 1023.3746, calcd 1023.3760 [(M + Na)$^+$], M = $C_{54}H_{52}N_{10}O_{10}$.

For log$P$ measurements, the following porphyrins were prepared in small scale by zincation or cupration of the corresponding free base porphyrin. The products were characterized by absorption spectroscopy, fluorescence spectroscopy (for the zinc chelates), and mass spectrometry prior to examination in log$P$ measurements.

Zn(II)-5,15-bis{2,6-bis[1-(1-(21-hydroxy-21-oxo-3,6,9,12,15,18-hexaoxaheneicosanyl)-1*H*-1,2,3-triazol-4-yl)methoxy]phenyl}-porphyrin (Zn8). Following a reported method,[24] $Zn(OAc)_2\cdot 2H_2O$ (34.5 mg) was added to a solution of free base porphyrin **8** (1.0 mg) in CH_2Cl_2/MeOH (570 μL, 9 : 1). The reaction mixture was stirred at room temperature for 3 h. The mixture was diluted with CH_2Cl_2 (10 mL). The organic phase was washed with water (10 mL) and brine, dried (Na_2SO_4), and concentrated to dryness: λ_{abs} (DMSO) 427, 556, 571 nm; λ_{em} 600, 652 nm; MALDI-MS obsd 2257.65, calcd 2257.92 [(M + H)$^+$], M = $C_{104}H_{144}N_{16}O_{36}Zn$.

Zn(II)-5-{2,6-bis[1-(1-(21-hydroxy-21-oxo-3,6,9,12,15,18-hexaoxaheneicosanyl)-1*H*-1,2,3-triazol-4-yl)methoxy]phenyl}-5-(4-ethynylphenyl)porphyrin (Zn11). Following a reported method,[24] $Zn(OAc)_2\cdot 2H_2O$ (60.0 mg) was added to a solution of free base porphyrin **11** (1.0 mg) in CH_2Cl_2/MeOH (570 μL, 9 : 1). The reaction mixture was stirred at room temperature for 3 h. The mixture was diluted with CH_2Cl_2 (10 mL). The organic phase was washed with water (10 mL) and brine, dried (Na_2SO_4), and concentrated to dryness: λ_{abs} (DMSO) 419, 549, 584 nm; λ_{em} 591, 643 nm; MALDI-MS obsd 1415.46, calcd 1415.51 [(M + H)$^+$], M = $C_{70}H_{82}N_{10}O_{18}Zn$.

Cu(II)-5-{2,6-bis[1-(1-(21-hydroxy-21-oxo-3,6,9,12,15,18-hexaoxaheneicosanyl)-1*H*-1,2,3-triazol-4-yl)methoxy]phenyl}-5-(4-ethynylphenyl)porphyrin (Cu11). A sample of $Cu(OAc)_2\cdot H_2O$ (45.0 mg) was added to a solution of free base porphyrin **11** (1.0 mg) in CH_2Cl_2/MeOH (570 μL, 9 : 1). The reaction mixture was stirred at room temperature for 30 min. The mixture was diluted with CH_2Cl_2 (10 mL). The organic phase was washed with water (10 mL) and brine, dried (Na_2SO_4), and concentrated to dryness: λ_{abs} (DMSO) 407, 528, 559 nm; MALDI-MS obsd 1436.66, calcd 1436.51 [(M + Na)$^+$], M = $C_{70}H_{82}N_{10}O_{18}Cu$.

Conflicts of interest

The authors declare competing financial interests.

Acknowledgements

Funding was provided by the National Science Foundation to Oncurie, Inc. (NSF 2136700), and by NC State University. NMR spectroscopy and mass spectrometry measurements were carried out in the Molecular Education, Technology, and Research Innovation Center (METRIC) at NC State University.

References

1 P. Hambright, in *The Porphyrin Handbook*, ed. K. M. Kadish, K. M. Smith and R. Guilard, Academic Press, San Diego, CA, 2000, vol. 3, pp. 129–210.
2 F. Dumoulin, M. Durmuş, V. Ahsen and T. Nyokong, *Coord. Chem. Rev.*, 2010, **254**, 2792–2847.
3 S. Pisarek, K. Maximova and D. Gryko, *Tetrahedron*, 2014, **70**, 6685–6715.
4 M. Luciano and C. Brückner, *Molecules*, 2017, **22**, 980.
5 J. Winkelman, *Cancer Res.*, 1962, **22**, 589–596.
6 P. Hambright and E. B. Fleischer, *Inorg. Chem.*, 1970, **9**, 1757–1761.
7 P. A. Carapellucci and D. Mauzerall, *Ann. N. Y. Acad. Sci.*, 1975, **244**, 214–238.
8 N. Matsumoto, M. Taniguchi and J. S. Lindsey, *J. Porphyrins Phthalocyanines*, 2020, **24**, 362–378.
9 A. K. Mandal, T. Sahin, M. Liu, J. S. Lindsey, D. F. Bocian and D. Holten, *New J. Chem.*, 2016, **40**, 9648–9656.
10 P. Thamyongkit, M. Speckbacher, J. R. Diers, H. L. Kee, C. Kirmaier, D. Holten, D. F. Bocian and J. S. Lindsey, *J. Org. Chem.*, 2004, **69**, 3700–3710.
11 K. E. Borbas, P. Mroz, M. R. Hamblin and J. S. Lindsey, *Bioconjugate Chem.*, 2006, **17**, 638–653.
12 A. Z. Muresan and J. S. Lindsey, *Tetrahedron*, 2008, **64**, 11440–11448.
13 J. M. Harris, *Poly(ethylene glycol) Chemistry: Biotechnical and Biomedical Applications*, Plenum Press, New York, NY, 1992, pp. 1–14.
14 D. Sato, Z. Wu, H. Fujita and J. S. Lindsey, *Organics*, 2021, **2**, 161–273.
15 N. Larson and H. Ghandehari, *Chem. Mater.*, 2012, **24**, 840–853.
16 A. Kolate, D. Baradia, S. Patil, I. Vhora, G. Kore and A. Misra, *J. Controlled Release*, 2014, **192**, 67–81.
17 J. Herzberger, K. Niederer, H. Pohlit, J. Seiwert, M. Worm, F. R. Wurm and H. Frey, *Chem. Rev.*, 2016, **116**, 2170–2243.
18 R. Begum and H. Matsuura, *J. Chem. Soc., Faraday Trans.*, 1997, **93**, 3839–3848.
19 S. Zalipsky and J. M. Harris, in *Introduction to Chemistry and Biological Applications of Poly(ethylene glycol)*; In Poly(ethylene glycol) Chemistry and Biological Applications; ACS Symposium Series, American Chemical Society, Washington, DC, 1997, vol. 680, pp. 1–13.
20 M. S. Thompson, T. P. Vadala, M. L. Vadala, Y. Lin and J. S. Riffle, *Polymer*, 2008, **49**, 345–373.
21 M. Taniguchi, J. S. Lindsey, D. F. Bocian and D. Holten, *J. Photochem. Photobiol., C*, 2021, **46**, 100401.
22 Y. Kuramochi and A. Satake, *Catalysts*, 2023, **13**, 282.
23 M. Shokeen and T. J. Wadas, *Med. Chem.*, 2011, **7**, 413–429.
24 P.-L. D. Cao, Z. Wu, J. Rong and J. S. Lindsey, *J. Porphyrins Phthalocyanines*, 2023, **27**, 1049–1058.
25 F. Dumoulin and V. Ahsen, *J. Porphyrins Phthalocyanines*, 2011, **15**, 481–504.
26 R. Liu, M. Liu, D. Hood, C.-Y. Chen, C. J. MacNevin, D. Holten and J. S. Lindsey, *Molecules*, 2018, **23**, 130.
27 G. P. Arsenault, E. Bullock and S. F. MacDonald, *J. Am. Chem. Soc.*, 1960, **82**, 4384–4389.
28 C. Brückner, J. J. Posakony, C. K. Johnson, R. W. Boyle, B. R. James and D. Dolphin, *J. Porphyrins Phthalocyanines*, 1998, **2**, 455–465.
29 B. J. Littler, Y. Ciringh and J. S. Lindsey, *J. Org. Chem.*, 1999, **64**, 2864–2872.
30 G. R. Geier III, B. J. Littler and J. S. Lindsey, *J. Chem. Soc., Perkin Trans. 2*, 2001, 701–711.
31 C. K. Chang, *Isr. J. Chem.*, 2016, **56**, 130–143.
32 H. A. Houson, Z. Wu, P.-L. D. Cao, J. S. Lindsey and S. E. Lapi, *Mol. Pharm.*, 2024, **21**, 2441–2445.
33 J. K. Laha, S. Dhanalekshmi, M. Taniguchi, A. Ambroise and J. S. Lindsey, *Org. Process Res. Dev.*, 2003, **7**, 799–812.
34 A. D. Adler, F. R. Longo, F. Kampas and J. Kim, *J. Inorg. Nucl. Chem.*, 1970, **32**, 2443–2445.
35 J. E. Falk, *Porphyrins and Metalloporphyrins*, Elsevier Publishing Co., Amsterdam, 1964, p. 74.
36 G. M. Godziela and H. M. Goff, *J. Am. Chem. Soc.*, 1986, **108**, 2237–2243.
37 G. N. La Mar and F. A. Walker (Jensen), in *The Porphyrins*, ed. D. Dolphin, Academic Press, New York, 1979, vol. 4, pp. 61–157.
38 K. W. Gold, D. J. Hodgson, A. Gold, J. E. Savrin and G. E. Toney, *J. Chem. Soc., Chem. Commun.*, 1985, 563–564.
39 M. V. Peters, R. Goddard and S. Hecht, *J. Org. Chem.*, 2006, **71**, 7846–7849.
40 D. Conrad, J. DeCoskey, C. Yeisley, M. Zeller, A. D. Hunter and E. P. Zovinka, *Acta Crystallogr.*, 2007, **E63**, m2824.
41 D. Conrad, J. DeCoskey, S. Mock, B. C. Noll, J. Petrovic and E. P. Zovinka, *Acta Crystallogr.*, 2007, **63**, m3058.
42 S. Maji and S. Sarkar, *Inorg. Chim. Acta*, 2010, **363**, 2778–2785.
43 S. Nowakowska, F. Mazzola, M. N. Alberti, F. Song, T. Voigt, J. Nowakowski, A. Wäckerlin, C. Wäckerlin, J. Wiss, W. B. Schweizer, M. Broszio, C. Polley, M. Leandersson, S. Fatayer, T. Ivas, M. Baljozovic, S. F. Mousavi, A. Ahsan, T. Nijs, O. Popova, J. Zhang, M. Muntwiler, C. Thilgen, M. Stöhr, I. A. Pasti, N. V. Skorodumova, F. Diederich, J. Wells and T. A. Jung, *ACS Nano*, 2018, **12**, 768–778.
44 C. Li, K. Lang, H. Lu, Y. Hu, X. Cui, L. Wojtas and X. P. Zhang, *CCDC 1894934: Experimental Crystal Structure Determination*, 2019.
45 R. Inoue, M. Yokoyama, I. Maruyama and Y. Morisaki, *Chem. – Eur. J.*, 2023, **29**, e202301717.
46 S. Choi, S. H. Chae, J. H. Shin, Y. Kim, S.-J. Kim, D. H. Choi and S. J. Lee, *Chem. Commun.*, 2013, **49**, 3994–3996.

47 C. Arunkumar, P. Bhyrappa and B. Varghese, *Tetrahedron Lett.*, 2006, **47**, 8033–8037.
48 S. J. Lee, C. D. Malliakas, M. G. Kanatzidis, J. T. Hupp and S. T. Nguyen, *Adv. Mater.*, 2008, **20**, 3543–3549.
49 S. H. Chae, K. Y. Lee, S.-J. Kim, S. J. Lee and Y. Kim, *Inorg. Chem. Commun.*, 2016, **69**, 40–44.
50 A. Meindl, S. Plunkett, A. A. Ryan, K. J. Flanagan, S. Callaghan and M. O. Senge, *Eur. J. Org. Chem.*, 2017, 3565–3583.
51 S. Choi, C. H. Kim, J.-O. Baeg, H.-J. Son, C. Pac and S. O. Kang, *ACS Appl. Energy Mater.*, 2020, **3**, 11581–11596.
52 M. P. Nikiforov, U. Zerweck, P. Milde, C. Loppacher, T.-H. Park, H. T. Uyeda, M. J. Therien, L. Eng and D. Bonnell, *Nano Lett.*, 2008, **8**, 110–113.
53 A. Zingg, B. Felber, V. Gramlich, L. Fu, J. P. Collman and F. Diederich, *Helv. Chim. Acta*, 2002, **85**, 333–351.
54 P. J. Dandliker, F. Diederich, M. Gross, C. B. Knobler, A. Louati and E. M. Sanford, *Angew. Chem., Int. Ed. Engl.*, 1994, **33**, 1739–1742.
55 A. Sen and K. S. Suslick, *J. Am. Chem. Soc.*, 2000, **122**, 11565–11566.
56 R. W. Wagner, J. S. Lindsey, I. Turowska-Tyrk and W. R. Scheidt, *Tetrahedron*, 1994, **50**, 11097–11112.
57 T. Ikeda, J. M. Lintuluoto, N. Aratani, Z. S. Yoon, D. Kim and A. Osuka, *Eur. J. Org. Chem.*, 2006, 3193–3204.
58 P. Li, K. Alenezi, S. K. Ibrahim, J. A. Wright, D. L. Hughes and C. J. Pickett, *ChemSusChem*, 2012, **5**, 2361–2375.
59 A. Leo, C. Hansch and D. Elkins, *Chem. Rev.*, 1971, **71**, 525–616.
60 Q. Liu, M. Taniguchi, S. Goel and J. S. Lindsey, *Dyes Pigm.*, 2024, **223**, 111914.
61 S. F. Mason, in *The Chemistry of Synthetic Dyes*, ed. K. Venkataraman, Academic Press, NY, 1970, vol. III, pp. 169–221.
62 A. R. M. Soares, Y. Thanaiah, M. Taniguchi and J. S. Lindsey, *New J. Chem.*, 2013, **37**, 1087–1097.
63 J. S. Lindsey, *Chem. Rev.*, 2015, **115**, 6534–6620.

