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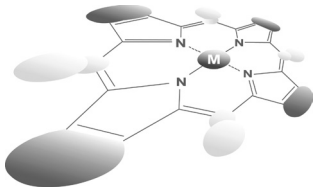

Hydrophilic bioconjugatable *trans*-AB-porphyrins and peptide conjugates

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ABSTRACT: Porphyrins bearing a single bioconjugatable group and a single water-solubilization motif in a *trans*-AB-architecture (with no other substituents) provide a compact design of value for studies in diverse disciplines. Established synthetic methods have been employed to prepare four new free base porphyrins and one Mn(III) chelate. The hydrophilic motif includes 4-*N*-methylpyridinium, 2,4,6-tris(carboxymethoxy)phenyl, 2,6-bis(phosphonomethoxy)phenyl, and carboxy; the bioconjugatable unit includes carboxy, maleimido, and *N*-hydroxysuccinimido (NHS) ester. Bioconjugation experiments with a protected porphyrin-diphosphate or unprotected porphyrin-diphosphonate were examined in organic solution or water, respectively. Both approaches were employed to conjugate to the ε-amino group of Lys^{11} in AcKPV-NH_2, a tripeptide fragment [Ac-α-MSH(11-13)-NH_2] of melanocyte stimulating hormone, yielding porphyrin-peptide conjugates.

KEYWORDS: water-soluble, manganese, dipyrromethane, molecular design, facial encumbrance.

INTRODUCTION

The synthesis of *trans*-AB-porphyrins has distant origins in the seminal work of Gunter and Mander, who reacted *o*-nitrobenzaldehyde with 2,3,7,8-tetramethyldipyrromethane in a two-step one-flask procedure of acid catalysis followed by oxidation [1]. The resulting *trans*-A_2-porphyrin bears eight β-methyl groups and two linearly disposed *meso*-aryl groups, with two *meso*-positions unsubstituted (Scheme 1). The only other approach at that time for gaining access to such *trans*-disubstituted porphyrins relied on reaction of two aryl aldehydes with pyrrole, thereby forming a statistical distribution of *meso*-tetraarylporphyrins with six types of substitution patterns: A_4, A_3B, *cis*-A_2B_2, *trans*-A_2B_2, AB_3 and B_4. The chromatographic separation of the mixture (particularly to isolate the *cis*-A_2B_2 and *trans*-A_2B_2 isomers in pure form) is often difficult, and the *trans*-A_2B_2-porphyrin constitutes only 12.5% (on a statistical basis) of the total set of porphyrins [2]. The Gunter and Mander approach thus represented a significant advance in liberating the field from the tyranny of statistical reactions. On the other hand, the advance was beset with two limitations: first, the presence of two β-methyl groups flanking each of the *meso*-aryl groups results in steric deformation of the porphyrin macrocycle [3]. Second, access to a *trans*-AB-porphyrin (with or without flanking β-alkyl groups) was only achievable by statistical formation of a mixture of the *trans*-A_2-porphyrin, *trans*-AB-porphyrin, and *trans*-B_2-porphyrin (*e.g.* by reaction of two dipyrromethanes and trimethyl orthoformate [4]).

Methodology for the rational synthesis of *trans*-A_2-porphyrins and *trans*-AB-porphyrins that lack flanking β-alkyl groups was developed over the course of a generation following the work of Gunter and Mander [5–9]. One route is shown in Scheme 1. A dipyrromethane [10] is subjected to Vilsmeier formylation and then imination with an alkylamine (*e.g.* propylamine); subsequent condensation of the bis(imino)dipyrromethane with a dipyrromethane in the presence of zinc acetate gives the *trans*-AB-porphyrin [7]. Other synthetic routes also can be employed [8, 9].

◊ SPP full member in good standing

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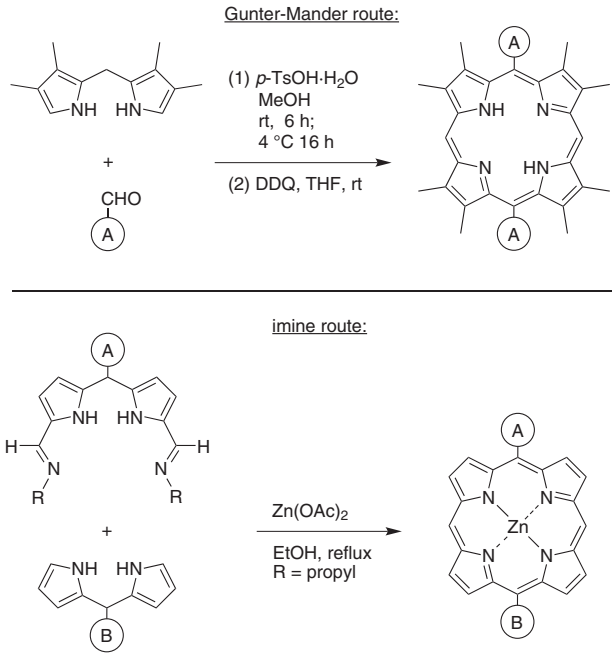


Scheme 1. Routes to *trans*-substituted porphyrins

Compound	Bioconjugatable group (A)	Hydrophilic group (B)
P1a		
P1b		
P1c		
P2a		
P2b		
P3a		
P3b		
P4a		
P4b		
P4c		
P4d		

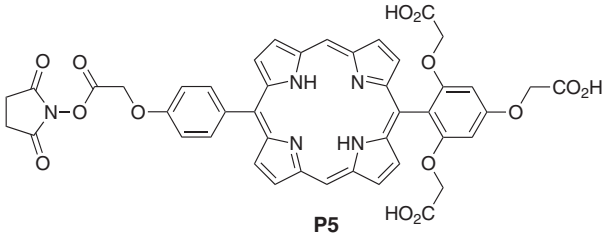
Chart 1. Prior relevant *trans*-AB-porphyrins

The imine route has been exploited to gain access to a variety of *trans*-AB-porphyrins [11–15]. *trans*-AB-porphyrins that bear a hydrophilic substituent and a bioconjugatable group are particularly attractive given the sparsely substituted molecular design, which enables the porphyrin chromophore to be utilized in diverse applications wherein a compact architecture is desirable. The applications range from medicine (*e.g.* photodynamic therapy, free radical scavenging) to clinical diagnostics (*e.g.* imaging, flow cytometry) to energy sciences (*e.g.* light-harvesting, charge separation). A common theme in these diverse studies is the ability to (1) attach the porphyrin to a particle, macromolecule or surface, and (2) achieve a resulting conjugate that does not aggregate in aqueous solution, either with itself or with other entities in the sample (*e.g.* non-specific binding with proteins or cells).

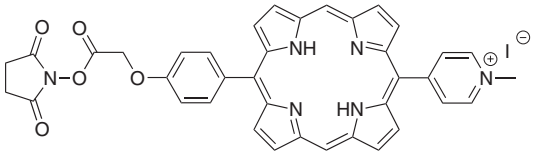
Motifs for bioconjugation can be incorporated with tetrapyrrole macrocycles in a fairly straightforward manner [16]. On the other hand, satisfactory approaches for water-solubilization of the intrinsically hydrophobic, disk-like tetrapyrrole macrocycle are less developed despite extensive work [17, 18]. Most such work concerns *meso*-tetrasubstituted porphyrins rather than the more compact disubstituted porphyrins bearing a single hydrophilic motif. Motifs that have been employed with *trans*-AB-porphyrins are shown in Chart 1. *N*-pyridinium moieties (**P1a**, **P1b**) and the *p*-(*N,N,N*-trimethylammonium)-phenyl motif (**P1c**) place the charged moiety at sites removed from the porphyrin macrocycle (such porphyrins were prepared *via* statistical methods [4]). By contrast, the 2,4,6-tris(carboxymethoxy)phenyl unit (**P2**) [15] positions ionizable functional groups above and below the face (as well as at the perimeter) of the macrocycle, as do the swallowtail motifs bearing phosphate (**P3**) [11] or phosphonate (**P4**) [11, 14] termini.

The groups distal to the hydrophilic motif include those often used for bioconjugation (amino, α-iodoacetamido, isothiocyanato, carboxy) and groups used rarely if at all (formyl, bromo). Other notable examples of *trans*-AB-porphyrins, while not containing both a bioconjugatable group and a hydrophilic group, include the following substituent pairs: *p*-decyloxyphenyl/PEG-dendrimer [19]; *p*-aminophenyl/formyl [20]; protected imidazole/ester [12]; hydroxymethyl/ethyne [21]; and hydroxymethyl/ethoxycarbonyl [21].

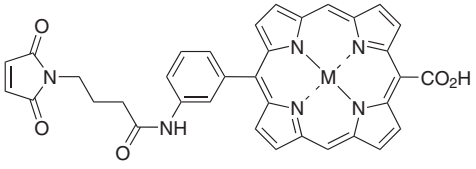
In this paper, we present the design and synthesis of a set of *trans*-AB-porphyrins that build from the prior examples yet are more versatile in the nature of both the bioconjugatable handle and the water-solubilization motif (Chart 2). The bioconjugatable handles include the NHS ester (*e.g.* for amidation with a protein amine) and the maleimide group (for Michael addition with a cysteinyl thiol), which are common motifs for bioconjugation [22]. The hydrophilic substituents include carboxylates, phosphonates, and quaternized pyridinium moieties. Three examples of bioconjugation also are reported.



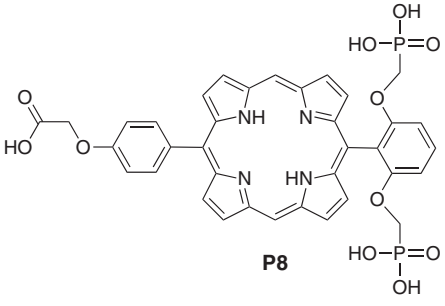
P5



P6

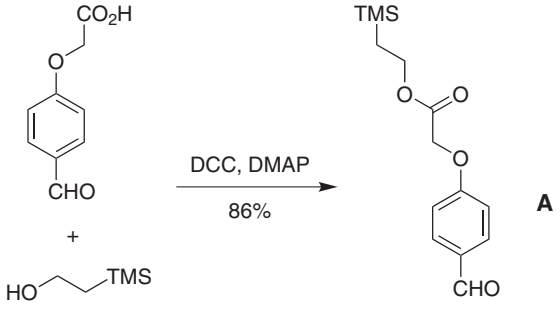


P7: M = H, H
MnP7: M = ClMn(III)

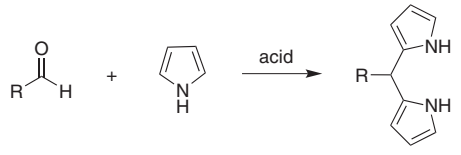


P8

Chart 2. Target *trans*-AB-porphyrins



Scheme 2. Synthesis of an aryl aldehyde



R	Cmpd	Acid	Yield, %
TMSCH$_2$CH$_2$O$_2$C—	**B**	$InCl_3$	33 (here)
t-BuO$_2$C—	**C**	$InCl_3$	35 (ref 15)
	D	heat	58 (ref 26)
O$_2$N—	**E**	TFA	74 (ref 27)
t-BuO$_2$C—	**F**	TFA CH_2Cl_2	18 (ref 21)
EtO$_2$C—	**G**	TFA CH_2Cl_2	35 (ref 28)
t-BuO$_2$C—	**H**	$InCl_3$	88 (ref 11)
	I	$InCl_3$	88 (ref 29)

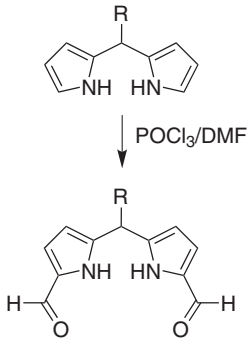
Scheme 3. Synthesis of aryl aldehydes

RESULTS AND DISCUSSION

Synthesis of porphyrins

The overall synthesis proceeds along the series aldehydes → dipyrromethanes → α-disubstituted dipyrromethanes → porphyrins → derivatized porphyrins. All but one of the aldehydes required herein have been made previously or are commercially available. The reaction of (4-formylphenoxy)acetic acid [23] and (2-trimethylsilyl)ethanol was carried out in the presence of *N,N*-dicylohexylcarbodiimide (DCC) [24] and 4-*N,N*-dimethylaminopyridine (DMAP) in 86% yield to give aryl aldehyde **A** (Scheme 2).

Meso-substituted dipyrromethanes are readily synthesized in a solventless process from the corresponding aldehyde in a solution of excess pyrrole containing an acid catalyst (Scheme 3) [25]. Thus, reaction of aldehyde **A**



R	Yield, %	Cmpd
$TMSCH_2CH_2O_2C$—O—	91	**B-(CHO)$_2$**
O_2N—	53	**E-(CHO)$_2$**
O, O, O—	45	**H-(CHO)$_2$**

Scheme 4. Diformylation of dipyrromethanes

with pyrrole in the presence of $InCl_3$ gave dipyrromethane **B** in 33% yield. Dipyrromethanes **C** [15], **D** [26], **E** [27], **F** [21], **G** [28], **H** [11] and **I** [29] were synthesized as described in the literature. Dipyrromethane **D** was prepared without added acid, whereas **F** was prepared from an ester precursor.

The imine route was chosen for the synthesis of the *trans*-AB-porphyrins given the presence of aryl groups at both of the *meso*-positions. To prepare the requisite bis(imino)dipyrromethanes, dipyrromethanes **B** and **E** were formylated under Vilsmeier conditions to give **B-(CHO)$_2$** and **E-(CHO)$_2$**, respectively (Scheme 4). Diformyldipyrromethane **H-(CHO)$_2$** has been prepared previously [11].

Formation of zinc porphyrins starts with conversion of a dipyrromethane-1,9-dicarboxaldehyde to the corresponding bis(imine) as shown in Scheme 5. A dipyrromethane is treated with excess alkylamine (*e.g.* propylamine or butylamine) to give the corresponding bis(imine) [7], which without further purification is reacted with the appropriate choice of dipyrromethane in the presence of $Zn(OAc)_2$ and air to give the zinc(II) *trans*-AB-porphyrin. Thus, **B-(CHO)$_2$** was treated with propylamine to give the corresponding bis(imine) **B-(imine)$_2$**, and the latter was reacted with dipyrromethane **C** to give **ZnP5-1** (the first porphyrin precursor to the target **P5**) in 25% yield. In a similar manner, four other porphyrins were prepared in yields ranging from 8–32%.

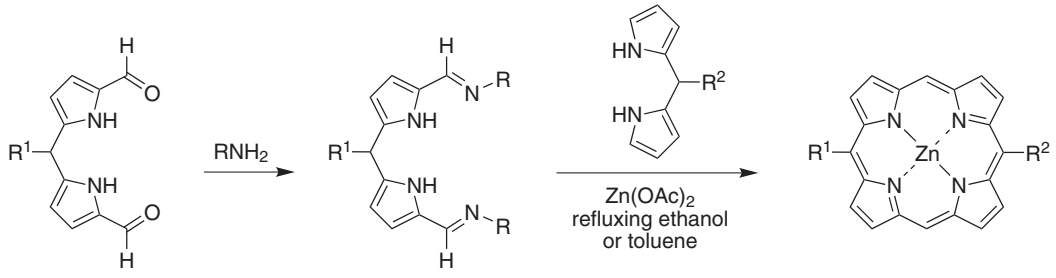
The porphyrins were subjected to functional group transformations as required to prepare the target compounds. The transformations are as follows:

ZnP5-1 was treated with tetrabutylammonium fluoride (TBAF) to remove the trimethylsilylethyl protecting group (giving **ZnP5-2**), whereupon the unmasked carboxylic acid was reacted with *N,N′*-disuccinimidyl carbonate [30] to install the activated NHS bioconjugatable handle, giving **ZnP5-3**. The latter was treated with trifluoroacetic acid (TFA) to both cleave the *tert*-butyl groups and demetalate the porphyrin while leaving the NHS ester unaffected (Scheme 6).

ZnP6-1 was treated with CH_3I at 60 °C to quaternize the pyridine unit. The crude product was treated with TFA to remove the trimethylsilylethyl group and the central zinc, affording the porphyrin **P6-2** in 94% yield. The latter was reacted with *N*-hydroxysuccinimide in the presence of (3-dimethylaminopropyl)carbodiimide (EDC) to afford the target porphyrin in 79% yield (Scheme 7).

Manganese porphyrins exhibit multiple stable redox states [31]. The electrochemical properties of manganese hematoporphyrin IX in water have been characterized for the 2+, 3+ and 4+ oxidation states [32–34]. Manganese porphyrins have been examined as superoxide dismutase mimics [28, 35, 36], catalase mimics [37], oxygen-atom transfer catalysts [38], magnetic resonance imaging contrast agents [19], and in studies of electron transfer across lipid bilayers [39–41].

To prepare a bioconjugatable manganese porphyrin, **ZnP7-1a** or **ZnP7-1b** was treated with Zn/NH_4Cl [42] to reduce the nitro group (Scheme 8). In each case, the crude product was treated with 3N HCl to give the free base porphyrin **P7-2a** or **P7-2b**, respectively. Other reagent systems such as Sn/HCl or Pd/C/ammonium formate did not result in the amino product but led to decomposition of the starting material. For the porphyrin ethyl ester (**P7-2b**), saponification of the ester with NaOH in a mixed solvent (H_2O/MeOH/THF) afforded putative **P7-3b**, but attempts to couple the latter with 4-maleimidobutyric acid failed to give the expected product. On the other hand, the porphyrin *tert*-butyl ester (**P7-2a**) was coupled with 4-maleimidobutyric acid in the presence of DCC to install the bioconjugatable handle, affording **P7-3a** in 52% yield. The *tert*-butyl group was then removed with 40% TFA/CH_2Cl_2 to unmask the hydrophilic substituent of the target porphyrin, affording **P7** in 80% yield. For the synthesis of the manganese porphyrin (**MnP7**), first metalation [36] was performed with **P7-3a** using $MnCl_2{\cdot}4H_2O$ and a hindered pyridine base (here 2,6-di-*tert*-butylpyridine instead of 2,6-dimethylpyridine) in $CHCl_3$/MeOH at 50 °C to give **MnP7-3a** in 62% yield. The metalation begins with the Mn(II) reagent but affords the Mn(III) chelate [43]. The apical counterion is assumed to be chloride. The protected manganese porphyrin was treated with 20%



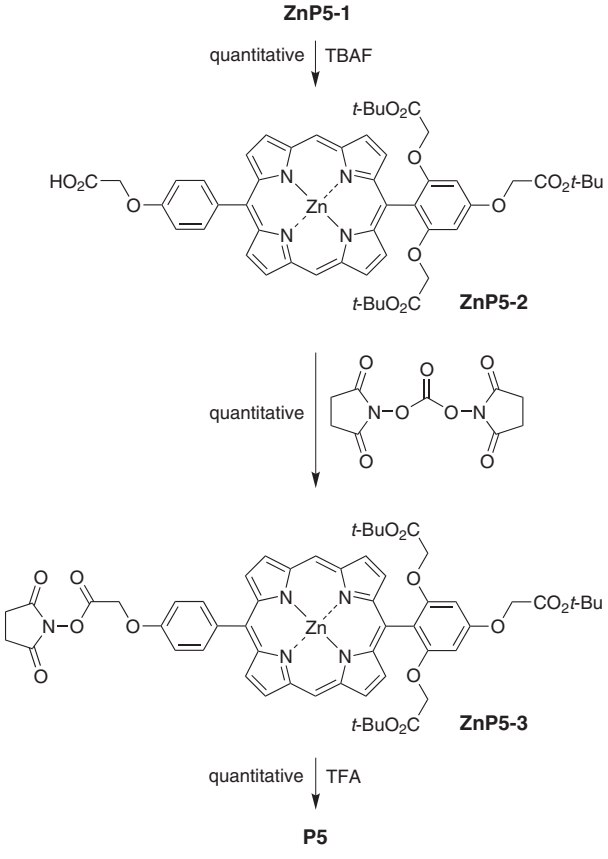
Reactants	R	R^1	R^2	Yield, %	Porphyrin
B-(imine)$_2$ + C	Pr	$TMSCH_2CH_2O_2C$–O–	CO_2t-Bu; O; CO_2t-Bu; O; CO_2t-Bu	25	**ZnP5-1**
B-(imine)$_2$ + D	Pr	$TMSCH_2CH_2O_2C$–O–	N	8	**ZnP6-1**
E-(imine)$_2$ + F	Bu	O_2N–	CO_2t-Bu	32	**ZnP7-1a**
E-(imine)$_2$ + G	Bu	O_2N–	CO_2Et	26	**ZnP7-1b**
H-(imine)$_2$ + I	Pr	t-BuO_2C–O–	OEt; EtO–P=O; O; O; EtO–P=O; EtO	19	**ZnP8-1**

Scheme 5. Porphyrin formation

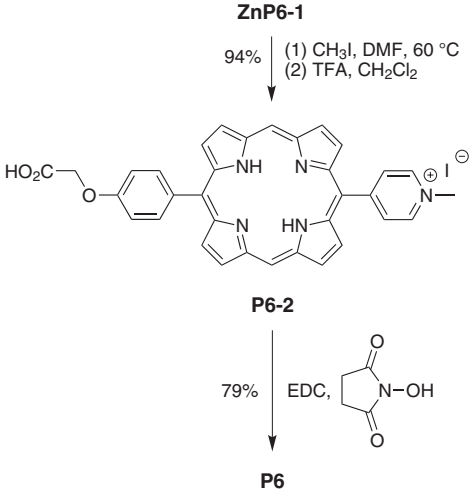
TFA/CH_2Cl_2 to remove the *tert*-butyl group affording **MnP7** in 87% yield.

The manganese porphyrin **MnP7** was characterized by absorption and fluorescence spectroscopy (in aqueous solution) and by mass spectrometry (MALDI-MS, ESI-MS). The absorption spectrum of **MnP7** in aqueous phosphate buffer at neutral pH (Fig. 1) shows the characteristic bands [31] of a Mn(III)porphyrin. The characteristic weak absorption feature at 765 nm also was observed (inset in Fig. 1). A nearly identical spectrum was observed in DMF at room temperature. The free base porphyrin (**P7-3a** or **P7**) exhibits a characteristically strong Soret band at 407 nm (toluene); such a band is missing in the spectrum of **MnP7**. Fluorescence emission also was absent, as expected for the Mn(III)porphyrin. The MALDI-MS spectrum showed the expected molecular ion upon loss of the anionic apical ligand. The manganese chelates were not characterized by NMR spectroscopy given their paramagnetic character.

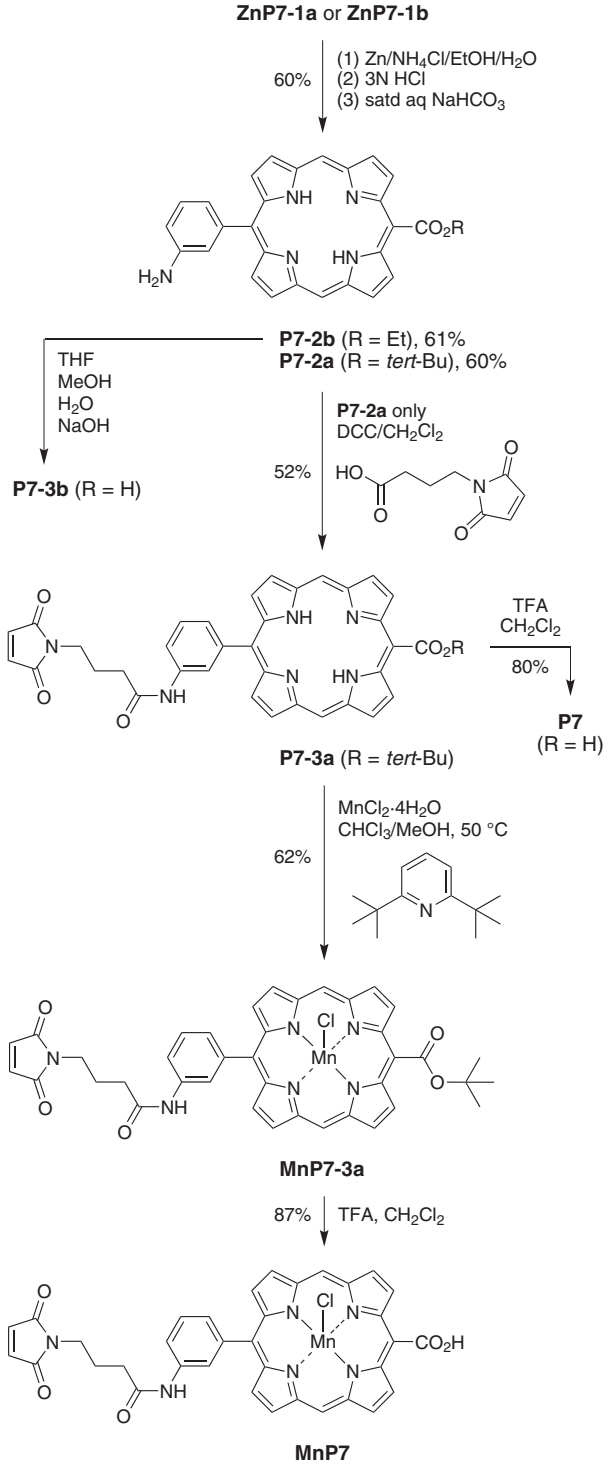
Treatment of **ZnP8-1** with *p*-TsOH·H_2O in CH_2Cl_2 at room temperature [13] resulted in the free base porphyrin **P8-1** in 90% yield. The *tert*-butyl ester remained intact under these conditions. Porphyrin **P8-1** was treated with excess KOH in aqueous THF at room temperature for 24 h (Scheme 9). The resulting porphyrin salt was liberated



Scheme 6. An NHS ester-porphyrin-tricarboxylate



Scheme 7. An NHS ester-porphyrin-pyridinium



Scheme 8. A maleimido-porphyrin-carboxylate

with a small amount of acetic acid, whereupon the porphyrin was treated with excess trimethylsilyl bromide (TMSBr) in CH_2Cl_2 [29] at room temperature for 4 h. Quenching with aqueous methanol, concentration and trituration with CH_2Cl_2 afforded the desired porphyrin **P8** as a green solid in quantitative yield.

Bioconjugation

Bioconjugation of porphyrins can be carried out in aqueous or organic solution [4, 16, 44, 45]. To gain information about handling of porphyrin-diphosphates in bioconjugation processes, the tetramethyl-protected porphyrin-diphosphate **P3a-1** [11] was reacted with

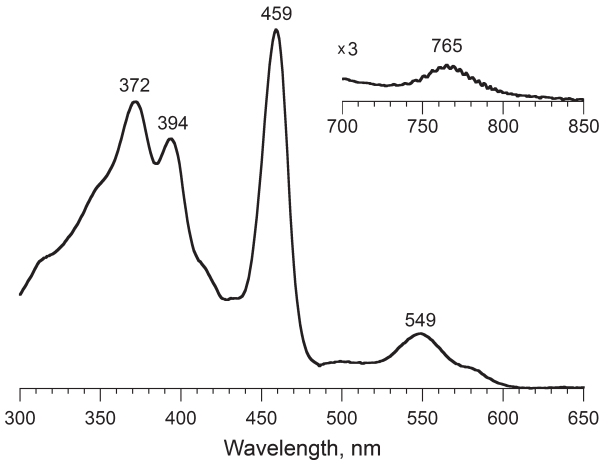
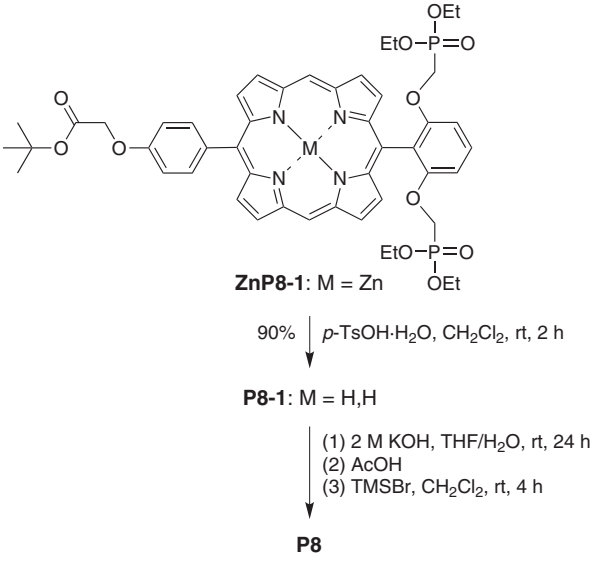
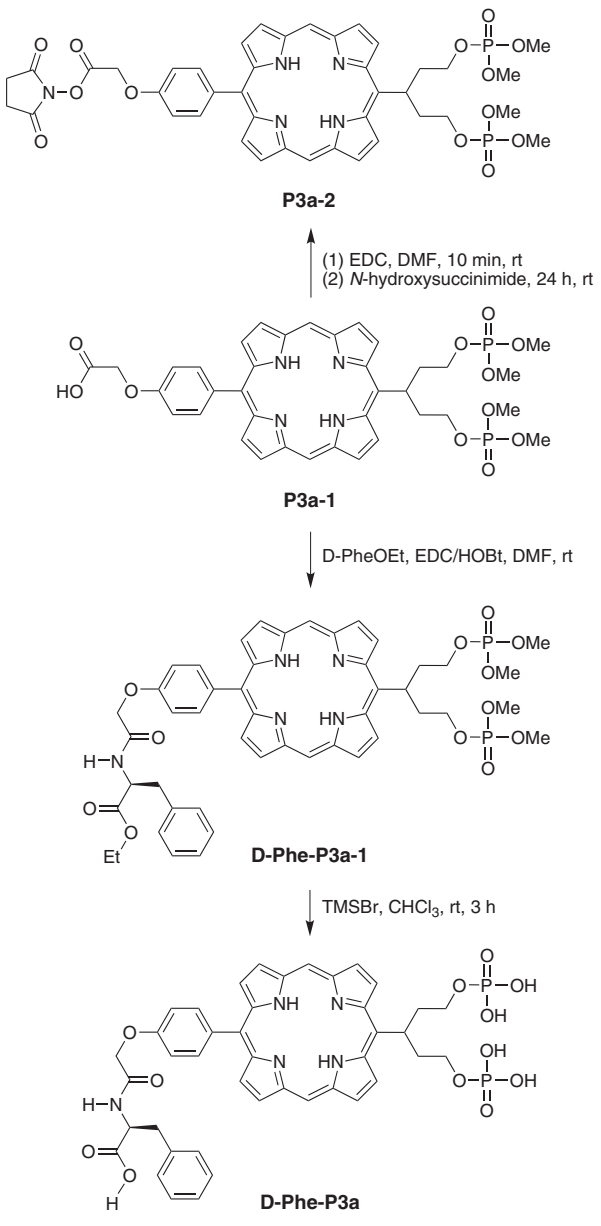


Fig. 1. Absorption spectrum of **MnP7** in aqueous phosphate buffer (pH 7) at room temperature. The inset shows the weak absorption at long wavelength



Scheme 9. A carboxylic acid-porphyrin-diphosphonate



Scheme 10. Phe-porphyrin-diphosphate: bioconjugation in DMF

D-PheOEt in DMF containing the coupling reagents EDC and 1-hydroxybenzotriazole (HOBt) (Scheme 10). The product **D-Phe-P3a-1** was isolated after conventional aqueous-organic workup and silica column chromatography. The use of $CHCl_3$ containing TMSBr [46] gave the best results to afford the target porphyrin **D-Phe-P3a**. ESI-MS analysis indicated that the starting porphyrin (m/z = 953.1) disappeared after ~3 h. The major products were the fully deprotected **D-Phe-P3a** (m/z = 868.0) and a compound with a mass spectral signal at 28 mass units higher. The latter could arise from either a dimethyl-protected molecule with the cleaved ethyl moiety, or, more likely, the free diphosphate with an uncleaved phenylalanine ethyl ester. Concentration of the sample, followed by neutralization with dilute aqueous NaOH and reverse-phase column chromatography yielded **D-Phe-P3a** as a purple solid. The sample was homogeneous by reverse-phase HPLC but ESI-MS showed peaks attributed to monomethyl and dimethyl/ethyl esters. Increasing the reaction time or the amount of TMSBr resulted in decomposition of the porphyrin moiety.

Porphyrin **P3a-1** also was converted to the corresponding NHS ester (**P3a-2**) by treatment with EDC and *N*-hydroxysuccinimide. The NHS ester was isolated along with the free carboxylic acid in a 4:1 ratio. The mixture was characterized by ^{1}H NMR spectroscopy and mass spectrometry and used as such in conjugation reactions.

A next objective was to conjugate porphyrin-diphosphate **P3a-1** to Ac-KPV-NH_2, the tripeptide carboxamide-terminal

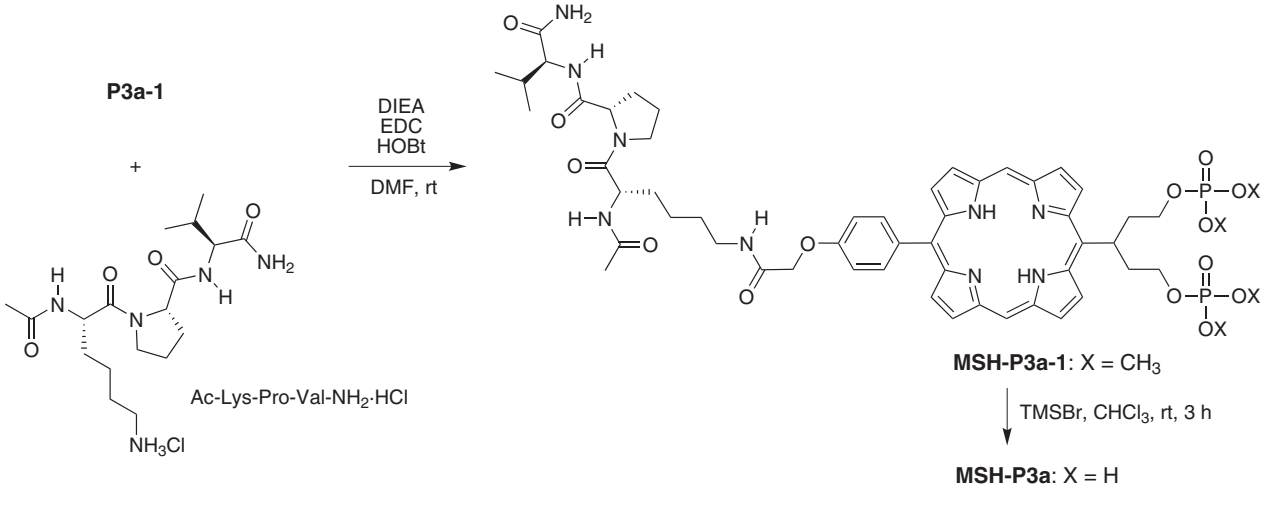
segment of α-melanocyte stimulating hormone (α-MSH) [47, 48]. α-MSH is a tridecapeptide that is produced by a wide variety of mammalian cell types, is widely distributed in the central nervous system, exhibits enhanced uptake by melanoma cells, and exhibits immunomodulatory effects, particularly anti-inflammatory activity [49–51]. Extensive studies of truncated segments of α-MSH have been carried out [52]. One finding is that Ac-KPV-NH_2 also exhibits anti-inflammatory activity; moreover, within this peptide, L-Pro^{12} is essential for such activity whereas L-Lys^{11} can be replaced with D-Lys^{11} without loss of activity [48]. The sole site of nucleophilicity is the lysine ε-NH_2 group (given the protected C- and N-termini), which was expected to result in selective reaction with the carboxylic acid in the porphyrin-diphosphate **P3a-1**. Derivatives of α-MSH itself have been prepared; representative examples include those that contain an *N*-terminal fatty acid (for receptor localization studies) [51] or a metal chelating ligand (for melanoma uptake studies) [49, 53]. In studies of the latter, amidation at the ε-amino group of Lys^{11} dramatically reduced kidney uptake and thereby afforded increased retention for radiolabeling studies of melanoma uptake. Accordingly, an analogous porphyrin-containing construct might prove viable for biomedical imaging studies to identify likely sites of uptake or localization of α-MSH including for melanoma diagnoses.

Attempts to react the NHS ester-porphyrin **P3a-2** with Ac-KPV-NH_2·HCl were unsuccessful, as only starting materials were observed by ESI-MS even after 24 h under rigorously anhydrous conditions. Therefore, the conjugation was carried out in the same manner as for **D-Phe-P3a-1**, with *in situ* activation of the carboxy-porphyrin **P3a-1** by use of EDC/HOBt in DMF (Scheme 11). *N,N*-Diisopropylethylamine (DIEA) was included to liberate the peptide from its HCl-salt. The product **MSH-P3a-1** was isolated after silica column chromatography in good yield as a purple solid. Porphyrin **MSH-P3a-1** exhibited the molecular ion peak upon mass spectral analysis. No unconjugated porphyrin or tripeptide was observed. Upon examination by ^{1}H NMR spectroscopy, the peptidyl NH-protons, the characteristic amino acid CH-resonances, and the porphyrin aromatic protons were readily identified by gCOSY experiments. The integrations of the signals were of the expected ratios. On the other hand, as expected, the ^{1}H NMR spectrum was rather complicated in the aliphatic region and unambiguous assignment of the signals in this region was not possible.

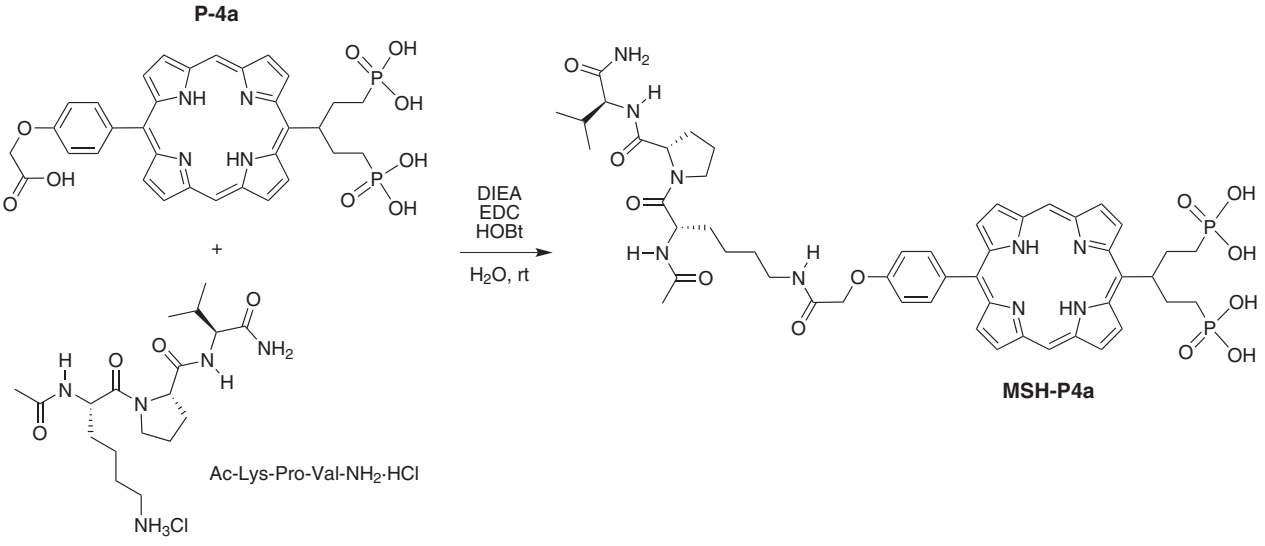
The complexity of the ^{1}H NMR spectrum of **MSH-P3a-1** was expected given our prior studies of swallowtail porphyrins [11, 13, 14, 54]. The following structural features are relevant: (1) the $C^{1'}H$ is more or less in the plane of the porphyrin macrocycle, which results in two sets of β-protons and two different *meso*-proton signals, and (2) the swallowtail alkyl groups (which preferentially occupy positions out-of-plane with the porphyrin ring) are hindered in their rotation around the carbon–carbon single bond between the porphyrin *meso*-position and the swallowtail branching site (*i.e.* C^5–$C^{1'}H$).

Removal of the protecting groups of **MSH-P3a-1** was achieved with TMSBr in $CHCl_3$. Purification by reverse-phase column chromatography yielded a purple solid, which upon ESI-MS analysis (*m/z* = 1088, $[M + H]^+$) proved to be consistent with the desired deprotected peptide-porphyrin-diphosphate conjugate (**MSH-P3a**), yet also containing a trace of monomethyl-**MSH-P3a**. Both porphyrin conjugates **D-Phe-P3a** and **MSH-P3a** displayed excellent solubility in water.

The initial porphyrin conjugates **D-Phe-P3a-1** and **MSH-P3a-1** were prepared with the phosphate moiety in protected form, leaving the unveiling of the water-solubilization motif to the last step of the overall synthesis. For conjugation to water-soluble macromolecules, however, this approach is not feasible. Therefore, the water-soluble porphyrin-diphosphonate **P4a** was reacted with Ac-KPV-NH_2·HCl in the presence of EDC/HOBt



Scheme 11. Synthesis of a peptide-porphyrin-diphosphate: bioconjugation in DMF



Scheme 12. A peptide-porphyrin-diphosphonate: bioconjugation in water

in water containing DIEA as shown in Scheme 12. (Porphyrin-diphosphonate **P4a** is highly water-soluble, given that 8–10 mg completely dissolved in 500 µL of distilled water, consistent with a concentration of ≥ 20 mM.) The reaction was monitored by ESI-MS: the peak corresponding to the protonated porphyrin (*m/z* = 691) was very weak after 24 h, while that of the desired peptide-porphyrin conjugate (*m/z* = 1056.4) was observed. The sample was then diluted with a small volume of water and purified on a short reverse-phase silica column. The peptide-porphyrin-diphosphonate conjugate (**MSH-P4a**) was isolated as a deep purple solid. The conjugate was found to be homogeneous upon analysis by RP-HPLC, and upon ESI-MS analysis gave the protonated molecular ion (*m/z* = 1056.3) and a doubly charged cationized molecular ion [*m/z* = 539.3, $(M + H + Na)^{2+}$] in the positive ion mode, *vs*. the deprotonated molecular ion (*m/z* = 1054.3) in the negative ion mode.

EXPERIMENTAL

General methods

^{1}H NMR (300 or 400 MHz) or ^{13}C NMR (75 or 100 MHz) spectroscopy was performed at room temperature in $CDCl_3$ unless noted otherwise. All solvents were reagent grade and were used as received unless noted otherwise. THF was freshly distilled from sodium/benzophenone ketyl. Anhydrous CH_2Cl_2 was used as received. Laser-desorption mass spectrometry (LD-MS) was performed in the absence of a matrix. Matrix-assisted laser-desorption mass spectrometry (MALDI-MS) was performed with the matrix 1,4-bis(5-phenyl-2-oxaxol-2-yl)benzene (POPOP) [55] or α-cyano-4-hydroxycinnamic acid. LD-MS, MALDI-MS and electrospray ionization mass spectrometry (ESI-MS) data are reported for the molecular ion or cationized molecular ion unless noted otherwise. Noncommercial compounds (4-formylphenoxy)acetic acid [23], **C** [15], **D** [26], **E** [27], **F** [21], **G** [28], **H** [11], **I** [29], **P3a-1** [11], and **P4a** [11] were obtained following literature procedures. The tripeptide Ac-KPV-NH_2·HCl was obtained from Bachem California, Inc. The coupling reagent (3-dimethylaminopropyl)carbodiimide (EDC) was employed as the hydrochloride salt. All other compounds were used as received from commercial sources.

Chromatography

Silica gel (40 µm average particle size) was used for column chromatography. Preparative size-exclusion chromatography (SEC) was performed using BioRad Bio-Beads SX-1 (200–400 mesh) beads. Reverse-phase preparative column chromatography was carried out using C-18-coated silica and eluants based on water admixed with methanol. Analytical RP-HPLC was carried out using an ODS C-18 column (5 µm, 125 mm × 4 mm); flow rate = 1 mL/min; detection at 254, 410 and 417 nm; and the following elution program with solvents A (water containing 0.1% TFA) and B (acetonitrile containing 0.1% TFA): 0-2 min, 0% B; 2-20 min, 90% B; 20-23 min, 90% B.

Synthesis of porphyrin precursors

4-[2-(Trimethylsilyl)ethoxycarbonylmethoxy] benzaldehyde (A). Following a reported procedure [24], a solution of (4-formylphenoxy)acetic acid (1.00 g, 5.55 mmol) in DMF/CH_2Cl_2 (30 mL, 1:1) was treated with 2-(trimethylsilyl)ethanol (1.19 mL, 8.33 mmol) and DMAP (0.135 g, 1.11 mmol), and the mixture was

cooled to 0 °C. DCC (1.26 g, 6.11 mmol) was added, and the mixture was stirred and allowed to warm overnight to room temperature. The precipitate was filtered. The filtrate was concentrated and chromatographed (silica, CH_2Cl_2) to afford a yellow oil (1.34 g, 86%). ^{1}H NMR (300 MHz, $CDCl_3$): δ, ppm 0.05 (s, 9H), 0.99–1.06 (m, 2H), 4.29–4.34 (m, 2H), 4.69 (s, 2H), 7.01 (d, J = 8.4 Hz, 2H), 7.85 (d, J = 8.7 Hz, 2H), 9.90 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ, ppm -1.4, 17.4, 64.3, 65.5, 115.0, 130.8, 132.1, 162.8, 168.3, 190.9. ESI-MS: *m/z* obsd. 281.1210; calcd. 281.1204 ([M + H]$^+$, M = $C_{14}H_{20}O_4Si$).

5-[4-(2-(Trimethylsilyl)ethoxycarbonylmethoxy) phenyl]dipyrromethane (B). Following a reported procedure [25], a solution of aldehyde **A** (2.44 g, 8.70 mmol) in pyrrole (60 mL, 870 mmol) was degassed with a stream of argon for 10 min. $InCl_3$ (192 mg, 0.868 mmol) was added, and the mixture was stirred under argon at room temperature for 1.5 h. NaOH (1 g, powdered) was added to quench the reaction, and the mixture was stirred for 45 min. The mixture was filtered. The filtrate was concentrated and chromatographed [silica, hexanes/ CH_2Cl_2/ethyl acetate (7:2:1)] to afford a yellow viscous oil (1.13 g, 33%). ^{1}H NMR (400 MHz, $CDCl_3$): δ, ppm 0.09 (s, 9H), 1.05–1.09 (m, 2H), 4.31–4.35 (m, 2H), 4.58 (s, 2H), 5.39 (s, 1H), 5.89–5.90 (m, 2H), 6.15–6.17 (m, 2H), 6.65–6.66 (m, 2H), 6.84 (d, J = 8.4 Hz, 2H), 7.12 (d, J = 8.8 Hz, 2H), 7.96 (brs, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ, ppm -1.5, 17.4, 43.1, 63.9, 65.5, 107.1, 108.3, 114.7, 117.3, 129.5, 132.8, 135.5, 156.7, 169.2. ESI-MS: *m/z* obsd. 397.1940; calcd. 397.1942 ([M + H]$^+$, M = $C_{22}H_{28}N_2O_3Si$).

1,9-Diformyl-5-[4-(2-(trimethylsilyl) ethoxycarbonylmethoxy)phenyl]dipyrromethane (B-(CHO)$_2$). Following a reported procedure [7] with slight modification, the Vilsmeier reagent was prepared by treatment of dry DMF (3.63 mL) with $POCl_3$ (560 µL, 5.98 mmol) at 0 °C and stirring of the resulting mixture for 10 min. In a separate flask, a solution of dipyrromethane **B** (1.13 g, 2.85 mmol) was treated with the freshly prepared Vilsmeier reagent at 0 °C. The resulting mixture was stirred at 0 °C for 1 h. Then the reaction mixture was allowed to warm to room temperature upon which a mixture of saturated aqueous NaOAc/CH_2Cl_2 [50 mL (1:1)] was added, and the reaction mixture was stirred for 1 h. The aqueous phase was separated and extracted with CH_2Cl_2. The combined organic extract was washed with brine, dried (Na_2SO_4), concentrated and chromatographed [silica pretreated with 1% triethylamine in hexanes, CH_2Cl_2/ethyl acetate (4:1)] to afford a brown viscous oil (1.18 g, 91%). ^{1}H NMR (400 MHz, $CDCl_3$): δ, ppm 0.03 (s, 9H), 0.99–1.03 (m, 2H), 4.26–4.30 (m, 2H), 4.55 (s, 2H), 5.52 (s, 1H), 5.99–6.01 (m, 2H), 6.81–6.83 (m, 4H), 7.17–7.19 (m, 2H), 9.12 (s, 2H), 10.86 (brs, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ, ppm -1.5, 17.4, 43.6, 63.9, 65.5, 111.6, 115.0, 122.4, 129.7, 132.6, 132.6, 142.2, 157.2, 169.0, 179.1. ESI-MS: *m/z* obsd. 453.1845; calcd. 453.1840 ([M + H]$^+$, M = $C_{24}H_{28}N_2O_5Si$).

1,9-Diformyl-5-(3-nitrophenyl)dipyrromethane (E-(CHO)$_2$). The Vilsmeier reagent was prepared by treatment of dry DMF (20.0 mL) with $POCl_3$ (3.73 mL, 40.0 mmol) at 0 °C and stirring of the resulting mixture for 10 min. In a separate flask, a solution of dipyrromethane **E** (4.01 g, 15.0 mmol) in DMF (75 mL) was treated with the freshly prepared Vilsmeier reagent at 0 °C. The resulting mixture was stirred at 0 °C for 15 min and then for 1 h at room temperature. The reaction mixture was diluted with CH_2Cl_2 (200 mL) and then treated with saturated aqueous sodium acetate. After stirring for 1 h, the aqueous phase was separated and extracted with CH_2Cl_2 (3 × 50 mL). The combined organic extract was washed (water), dried (Na_2SO_4), concentrated and chromatographed [silica, CH_2Cl_2/ethyl acetate (19:1)] to afford a pale yellow solid (2.57 g, 53%): mp 189–190 °C. ^{1}H NMR (300 MHz, $CDCl_3$/CD_3OD): δ, ppm 5.73 (s, 1H), 6.08 (d, J = 3.9 Hz, 2H), 6.96 (d, J = 3.9 Hz, 2H), 7.53–7.62 (m, 2H), 8.11 (brs, 1H), 8.17 (dt, J_1 = 3.9 Hz, J_2 = 1.8 Hz, 1H), 9.35 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$/CD_3OD): δ, ppm 43.6, 111.2, 122.7, 123.3, 129.9, 132.9, 134.5, 140.1, 141.9, 148.5, 179.6. ESI-MS: *m/z* obsd. 324.0938, calcd. 324.0979 ([M + H]$^+$, M = $C_{17}H_{13}N_3O_4$).

Formation of porphyrins

Zn(II)-5-[4-(2-(trimethylsilyl)ethoxycarbonyl-methoxy)phenyl]-15-[2,4,6-tris(*tert*-butoxycarbonyl-methoxy)phenyl]porphyrin (ZnP5-1). Following a reported procedure [7], a mixture of **B-(CHO)$_2$** (1.18 g, 2.61 mmol) and propylamine (4.00 mL, 52.2 mmol) in THF (15 mL) was stirred at room temperature for 1 h. Removal of the solvent and the excess propylamine afforded **B-(imine)$_2$** as a brown viscous oil, which was used without further purification in the next step. A solution of **B-(imine)$_2$** (60 mg, 0.11 mmol) and dipyrromethane **C** (69 mg, 0.11 mmol) in toluene (11 mL, 0.01 M) was treated with anhydrous $Zn(OAc)_2$ (202 mg, 1.10 mmol) and refluxed for 15 h with exposure to air. The reaction mixture was concentrated and chromatographed [basic alumina, hexanes/ethyl acetate (3:1)] to afford a purple solid (30 mg, 25%). ^{1}H NMR (400 MHz, $CDCl_3$): δ, ppm 0.15 (s, 9H), 1.16 (s, 18H), 1.19–1.25 (m, 2H), 1.67 (s, 9H), 3.95 (s, 4H), 4.43–4.47 (m, 2H), 4.74 (s, 2H), 4.88 (s, 2H), 6.43 (s, 2H), 7.30 (d, J = 8.4 Hz, 2H), 8.14 (d, J = 8.4 Hz, 2H), 9.06 (d, J = 4.4 Hz, 2H), 9.16 (d, J = 4.4 Hz, 2H), 9.34 (d, J = 4.4 Hz, 2H), 9.37 (d, J = 4.4 Hz, 2H), 10.19 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ, ppm -1.3, 17.6, 27.9, 28.4, 64.1, 66.0, 66.4, 82.2, 82.9, 93.5, 105.8, 110.3, 112.9, 114.7, 119.5, 131.3, 132.1, 132.2, 132.3, 135.7, 136.5, 149.3, 149.6, 149.8, 151.2, 157.6, 159.6, 159.8, 167.8, 168.0, 169.5. ESI-MS: *m/z* obsd. 1089.3652; calcd. 1089.3654 ([M + H]$^+$, M = $C_{57}H_{64}N_4O_{12}SiZn$). UV-vis (toluene): λ_{abs}, nm 413, 539, 574; (λ_{exc} = 413 nm, toluene): λ_{em}, nm 579, 633.

Zn(II)-5-[4-(2-(trimethylsilyl)ethoxycarbonyl-methoxy)phenyl]-15-(4-pyridyl)porphyrin (ZnP6-1).

Following a reported procedure [7], a solution of **B-(imine)$_2$** (1.92 g. 3.58 mmol) and dipyrromethane **D** (0.800 g, 3.58 mmol) in toluene (358 mL, 0.01 M) was treated with anhydrous $Zn(OAc)_2$ (6.57 g, 35.8 mmol) and refluxed for 15 h with exposure to air. The reaction mixture was concentrated and chromatographed [silica, ethyl acetate/CH_2Cl_2 (1:4)] to afford a purple solid (200 mg, 8%). ^{1}H NMR (400 MHz, THF-d_8): δ, ppm 0.19 (s, 9H), 1.13–1.17 (m, 2H), 4.39–4.43 (m, 2H), 4.95 (s, 2H), 7.35 (d, J = 8.4 Hz, 2H), 7.98 (d, J = 5.5 Hz, 2H), 8.15 (d, J = 8.8 Hz, 2H), 8.28 (brs, 2H), 8.86 (d, J = 4.4 Hz, 2H), 9.07 (d, J = 4.4 Hz, 2H), 9.39 (d, J = 2.6 Hz, 2H), 9.41 (d, J = 2.6 Hz, 2H), 10.28 (s, 2H). ^{13}C NMR (100 MHz, THF-d_8): δ, ppm -1.2, 18.3, 63.9, 66.6, 106.9, 113.8, 120.9, 121.4, 128.9, 130.6, 132.0, 132.5, 132.9, 133.2, 136.6, 137.2, 149.9, 150.7, 150.9, 151.5, 152.3, 159.3, 169.6. ESI-MS: *m/z* obsd. 700.17150; calcd. 700.17169 ([M + H]$^+$, M = $C_{38}H_{33}N_5O_3SiZn$). UV-vis (toluene): λ_{abs}, nm 413, 539; (λ_{exc} = 413 nm, toluene): λ_{em}, nm 580, 631.

***tert*-Butyl Zn(II)-15-(3-nitrophenyl)porphyrin-5-carboxylate (ZnP7-1a).** Following a reported procedure [7] with slight modification, a sample of **E-(CHO)$_2$** (360 mg, 1.11 mmol) was treated with butylamine (2.00 mL) at room temperature for 3 h. The mixture was filtered through a short pad of Na_2SO_4, which was washed with CH_2Cl_2. The filtrate was concentrated and dried to obtain a brown residue. The residue was treated with dipyrromethane **F** (250 mg, 1.01 mmol) and $Zn(OAc)_2{\cdot}2H_2O$ (2.20 g, 10.0 mmol) in ethanol (100 mL) under argon. The resulting pink solution was stirred under reflux for 18 h open to the atmosphere. The mixture was filtered. The filtrate was concentrated and chromatographed (silica, CH_2Cl_2) to obtain a dark red solid (192 mg, 32%). ^{1}H NMR (400 MHz, THF-d_8): δ, ppm 2.10 (s, 9H), 8.03 (t, J = 8.0 Hz, 1H), 8.60 (d, J = 7.2 Hz, 1H), 8.71 (dd, J_1 = 8.0 Hz, J_2 = 1.2 Hz, 1H), 8.97 (d, J = 4.4 Hz, 2H), 9.08 (t, J = 2.0 Hz, 1H), 9.44 (d, J = 4.8 Hz, 2H), 9.51 (d, J = 4.4 Hz, 2H), 9.67 (d, J = 4.8 Hz, 2H), 10.34 (s, 2H). ^{13}C NMR (100 MHz, THF-d_8): δ, ppm 28.9, 83.4, 107.2, 112.0, 118.8, 123.2, 128.4, 129.0, 131.9, 132.3, 132.7, 133.4, 140.8, 145.8, 148.0, 149.6, 149.8, 150.8, 150.9, 171.7. ESI-MS: *m/z* obsd. 594.1114, calcd. 594.1114 ([M + H]$^+$, M = $C_{31}H_{23}N_5O_4Zn$). UV-vis (toluene): λ_{abs}, nm 413.

Ethyl Zn(II)-15-(3-nitrophenyl)porphyrin-5-carboxylate (ZnP7-1b). Following a reported procedure [7] with slight modification, a sample of **E-(CHO)$_2$** (323 mg, 1.00 mmol) was treated with butylamine (3.00 mL) at room temperature for 2 h. The mixture was filtered through a short pad of Na_2SO_4 and washed with CH_2Cl_2. The filtrate was concentrated and dried to obtain a brown residue. The residue was treated with dipyrromethane **G** (218 mg, 1.00 mmol) and $Zn(OAc)_2{\cdot}2H_2O$ (2.20 g, 10.0 mmol) in ethanol (200 mL) under argon. The resulting pink solution was stirred under reflux for 15 h at open atmosphere. The mixture was filtered. The filtrate was concentrated and chromatographed [silica, hexanes/ethyl acetate (9:1)] to obtain a dark red solid (145 mg, 26%). ^{1}H NMR (400 MHz, $CDCl_3/CD_3OD$): δ, ppm 1.87 (t, J = 7.2 Hz, 3H), 5.15 (q, J = 7.2 Hz, 3H), 7.34 (s, 2H), 7.97 (t, J = 8.0 Hz, 1H), 8.57 (dt, J_1 = 8.0 Hz, J_2 = 1.2 Hz, 1H), 8.69 (ddd, J_1 = 8.4 Hz, J_2 = 2.4 Hz, J_3 = 0.8 Hz, 1H), 8.94 (d, J = 4.4 Hz, 2H), 9.10 (t, J = 2.0 Hz, 1H), 9.39 (d, J = 4.4 Hz, 2H), 9.49 (d, J = 4.8 Hz, 2H), 9.62 (d, J = 4.4 Hz, 2H), 10.29 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3/CD_3OD$): δ, ppm 14.9, 63.3, 107.0, 108.0, 118.4, 122.7, 127.6, 128.4, 131.1, 131.9, 132.1, 133.3, 140.0, 144.8, 146.9, 148.8, 148.9, 150.0, 150.1, 173.5. ESI-MS: *m/z* obsd. 565.0727, calcd. 565.0723 ([M]$^+$, M = $C_{29}H_{19}N_5O_4Zn$). UV-vis (toluene): λ_{abs}, nm 413.

Zn(II)-5-[4-(*tert*-butoxycarbonylmethoxy)phenyl-15-[3,5-bis(diethylphosphonomethoxy)phenyl]-porphyrin (ZnP8-1). Following a reported procedure [7], a sample of **H-(CHO)$_2$** (200 mg, 0.490 mmol) in propylamine (3.00 mL) was stirred at room temperature. After 1 h, the excess propylamine was removed under reduced pressure. A solution of the resulting solid and dipyrromethane **I** (271 mg, 0.490 mmol) in ethanol (45.0 mL) was treated with zinc acetate (917 mg, 5.00 mmol) under reflux for 18 h. The solvent was removed *in vacuo*, affording a dark pink residue. The residue was chromatographed [silica, CH_2Cl_2/methanol (0–10%)] to afford a pink solid (90.0 mg, 19%). ^{1}H NMR (CD_3OD): δ, ppm -0.22 (t, J = 6.8 Hz, 12H), 1.65 (s, 9H), 2.28–2.5 (m, 8H), 4.28 (s, 2H), 4.31 (s, 2H), 5.05 (s, 2H), 7.30 (d, J = 8.8 Hz, 2H), 7.38 (m, 2H), 7.88 (t, J = 8.4 Hz, 1H), 8.18 (d, J = 8.0 Hz, 2H), 8.92 (d, J = 4.8 Hz, 2H), 9.04 (d, J = 4.0 Hz, 1H), 9.37 (d, J = 4.4 Hz, 2H), 9.40 (d, J = 4.4 Hz, 2H), 10.22 (s, 2H). ESI-MS: *m/z* obsd. 986.23973, calcd. 986.23993 ([M]$^+$, M = $C_{48}H_{52}N_4O_{11}P_2Zn$). UV-vis ($CH_2Cl_2$): λ_{abs}, nm 409, 538.

Modification of porphyrins

Zn(II)-5-[4-(carboxymethoxy)phenyl]-15-[2,4,6-tris(*tert*-butoxycarbonylmethoxy)phenyl]porphyrin (ZnP5-2). A solution of **ZnP5-1** (29 mg, 0.027 mmol) in anhydrous THF (2.7 mL) was treated with TBAF solution (40 μL, 0.04 mmol, 1 M in THF) at 0 °C. The solution was allowed to reach room temperature and was stirred for 1.5 h. Ethyl acetate, H_2O and saturated aqueous NH_4Cl were added, and the mixture was extracted with ethyl acetate. The organic phase was dried (Na_2SO_4), concentrated and chromatographed [silica, ethyl acetate/MeOH (9:1)] to afford a purple solid (26 mg, quantitative). ^{1}H NMR (400 MHz, $CDCl_3/CD_3OD$): δ, ppm 1.26 (s, 18H), 1.66 (s, 9H), 4.16 (s, 4H), 4.82 (s, 2H), 5.12 (s, 2H), 6.58 (s, 2H), 7.38 (d, J = 8.2 Hz, 2H), 8.18 (d, J = 8.2 Hz, 2H), 9.07 (d, J = 4.3 Hz, 2H), 9.11 (d, J = 4.6 Hz, 2H), 9.34 (d, J = 4.6 Hz, 2H), 9.36 (d, J = 4.6 Hz, 2H), 10.18 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3/CD_3OD$): δ, ppm 29.3, 29.7, 31.5, 57.5, 68.0, 68.3, 84.2, 84.8, 95.8, 107.0, 108.0, 111.2, 114.6, 117.4, 121.1, 132.9, 133.6, 137.5, 138.4, 151.2, 151.4, 151.7, 152.8, 159.6, 161.5, 161.6, 170.1, 170.5.

ESI-MS: *m/z* obsd. 989.2935; calcd. 989.2946 ([M + H]$^+$, M = $C_{52}H_{52}N_4O_{12}Zn$). UV-vis (toluene): λ_{abs}, nm 414, 540, 575; (λ_{exc} = 414 nm, toluene): λ_{em}, nm 579, 632.

Zn(II)-5-[4-(*N*-succinimidyloxycarbonylmethoxy) phenyl]-15-[2,4,6-tris(*tert*-butoxycarbonylmethoxy)-phenyl]porphyrin (ZnP5-3). A sample of **ZnP5-2** (50 mg, 0.050 mmol) was dissolved in a mixture of dry DMF (770 µL) and dry pyridine (39 µL). Disuccinimidyl carbonate (19 mg, 0.075 mmol) was added, and the reaction mixture was stirred at room temperature for 5 h. Water and ethyl acetate were added to the reaction mixture. The organic phase was separated and washed (water and brine), dried (Na_2CO_3) and concentrated. The crude product was treated with 2% CH_2Cl_2 in hexanes, sonicated, and centrifuged. The supernatant was decanted to give the title product as a purple solid (38 mg, 70% yield). MALDI-MS: *m/z* (POPOP) 1085.6; calcd. 1086.3 ([M+H]$^+$, M=$C_{56}H_{55}N_5O_{14}Zn$). UV-vis (toluene): λ_{abs}, nm 413, 540; (λ_{exc} = 413 nm, toluene): λ_{em}, nm 579, 633.

5-[4-(*N*-succinimidyloxycarbonylmethoxy) phenyl]-15-[2,4,6-tris(carboxymethoxy)phenyl] porphyrin (P5). A solution of **ZnP5-3** (26 mg, 0.024 mmol) in CH_2Cl_2 (3.8 mL) was treated with TFA (960 µL), and the mixture was stirred for 4 h at room temperature. Water was added, and the aqueous layer was extracted with ethyl acetate. The organic layer was dried (Na_2SO_4) and concentrated to give a purple solid (20 mg, quantitative). MALDI-MS: *m/z* 856.2; calcd. 856.2 ([M + H]$^+$, M = $C_{44}H_{33}N_5O_{14}$). UV-vis (0.5 M phosphate buffer, pH 7.0): λ_{abs}, nm 404, 504, 542, 573; (λ_{exc} = 404 nm, 0.5 M phosphate buffer, pH 7.0): λ_{em}, nm 626, 687.

5-[4-(Carboxymethoxy)phenyl]-15-(*N*-methyl-pyridinium-4-yl)porphyrin iodide (P6-2). A sample of **ZnP6-1** (38 mg, 0.054 mmol) in DMF (1 mL) containing iodomethane (300 µL, 4.8 mmol) in a capped vial was heated overnight at 60 °C with stirring. The reaction mixture was concentrated, and the resulting crude solid was treated with anhydrous diethyl ether followed by sonication in a benchtop sonication bath. The resulting suspension was centrifuged. The supernatant was removed leaving the desired product as a solid (45 mg, quantitative, assuming an iodide counterion). MALDI-MS: *m/z* obsd. 713.8. ESI-MS: *m/z* obsd. 714.1865, calcd. 714.1873 ([M – I]$^+$, M = $C_{39}H_{36}N_5O_3SiIZn$]. A solution of this product (1.5 mL, CH_2Cl_2) was treated with TFA (0.5 mL) at room temperature for 5 h. The reaction mixture was concentrated to dryness. Water was added followed by sonication, centrifugation and decantation, which was performed twice (each time retaining the pellet). The final pellet was dried in vacuum to afford a black-purple solid (34 mg, 94%, assuming an iodide counterion). ^{1}H NMR (400 MHz, TFA-*d*): δ, ppm 4.82 (s, 3H), 5.14 (s, 2H), 7.63 (d, *J* = 8.8 Hz, 2H), 8.49 (d, *J* = 8.4 Hz, 2H), 9.06–9.08 (m, 4H), 9.17 (d, *J* = 6.6 Hz, 2H), 9.31 (d, *J* = 6.2 Hz, 2H), 9.58 (d, *J* = 4.8 Hz, 2H), 9.69 (d, *J* = 4.8 Hz, 2H), 11.11 (s, 2H). MALDI-MS: *m/z* obsd. 552.5, ESI-MS: *m/z* obsd. 552.2032; calcd. 552.2030 ([M – I]$^+$, M = $C_{34}H_{26}IN_5O_3$). UV-vis (DMF): λ_{abs}, nm 414, 504, 548, 576, 637; (λ_{exc} = 414 nm, DMF): λ_{em}, nm 642, 704.

5-[4-(*N*-succinimidyloxycarbonylmethoxy) phenyl]-15-(*N*-methylpyridinium-4-yl)porphyrin iodide (P6). A solution of **P6-2** (38 mg, 0.057 mmol) and *N*-hydroxysuccinimide (66 mg, 0.57 mmol) in DMF (1 mL) was treated overnight with EDC (109 mg, 0.568 mmol) at room temperature. Diethyl ether was added. The mixture was sonicated and centrifuged whereupon the supernatant was decanted. The same procedure was repeated twice with water then once with THF. Drying *in vacuo* afforded the product as a purple solid (35 mg, 79%, assuming an iodide counterion): ^{1}H NMR spectra were recorded in two different solvents given that the CD_3OD signal overlapped with the methyl and methylene protons, and the DMF-d_7 signal overlapped with the NHS ester protons. ^{1}H NMR (400 MHz, DMF-d_7): δ, ppm -3.03 (brs, 1H), -3.08 (brs, 1H), 5.02 (s, 3H), 5.16 (s, 2H), 7.56 (d, *J* = 8.8 Hz, 2H), 8.32 (d, *J* = 8.3 Hz, 2H), 9.21 (d, *J* = 4.4 Hz, 2H), 9.24 (d, *J* = 6.8 Hz, 2H), 9.29 (d, *J* = 4.9 Hz, 2H), 9.75–9.77 (m, 4H), 9.89 (d, *J* = 4.4 Hz, 2H), 10.81 (s, 2H). ^{1}H NMR (400 MHz, CD_3OD): δ, ppm 2.68 (s, 4H), 7.47 (d, *J* = 8.3 Hz, 2H), 8.20 (d, *J* = 8.8 Hz, 2H), 8.99 (d, *J* = 6.9 Hz, 2H), 9.15 (d, *J* = 4.4 Hz, 2H), 9.18 (d, *J* = 4.4 Hz, 2H), 9.37 (d, *J* = 6.3 Hz, 2H), 9.55 (d, *J* = 4.9 Hz, 2H), 9.67 (d, *J* = 4.4 Hz, 2H), 10.56 (s, 2H). MALDI-MS: *m/z* obsd. 649.4, ESI-MS: *m/z* obsd. 649.2178; calcd. 649.2939 ([M – I]$^+$, M = $C_{38}H_{29}IN_6O_5$). UV-vis (DMF): λ_{abs}, nm 414, 505, 548, 579, 635; (λ_{exc} = 414 nm, DMF): λ_{em}, nm 641, 703.

***tert*-Butyl 15-(3-aminophenyl)porphyrin-5-carboxylate (P7-2a).** Following a reported procedure [42], a suspension of **ZnP7-1a** (150 mg, 0.252 mmol), Zn dust (817 mg, 12.5 mmol) and NH_4Cl (1.34 g, 25.0 mmol) in ethanol (40.0 mL) and water (10.0 mL) was stirred under reflux for 4 h. The mixture was filtered, and the filtrate was concentrated. The resulting residue was treated with 3 N HCl, stirred for 1 h at room temperature, and then washed with ethyl acetate (colorless). The acidic aqueous solution (green) was neutralized with saturated aqueous $NaHCO_3$ solution followed by extraction with ethyl acetate. The resulting organic solution (purple) was concentrated and chromatographed [silica, CH_2Cl_2/ethyl acetate (9:1)] to obtain a dark purple solid (75.9 mg, 60%). ^{1}H NMR (300 MHz, THF-d_8): δ, ppm -3.10 (s, 1H) -2.95 (s, 1H), 2.09 (s, 9H), 4.90 (s, 2H), 7.01–7.05 (m, 1H), 7.44–7.45 (m, 2H), 7.50–7.51 (m, 1H), 9.16 (d, *J* = 4.8 Hz, 2H), 9.37 (d, *J* = 4.5 Hz, 2H), 9.49 (d, *J* = 4.2 Hz, 2H), 9.67 (d, *J* = 4.8 Hz, 2H), 10.36 (s, 2H). ^{13}C NMR (100 MHz, THF-d_8): δ, ppm 28.9, 83.8, 106.6, 109.7, 114.5, 122.3, 123.3, 125.0, 128.2, 130.7, 131.9, 132.3, 133.5, 142.6, 146.1, 146.6, 147.3, 147.8, 148.2, 170.4. MALDI-MS: *m/z* (POPOP) obsd. 501.1, calcd. 501.2165 ([M]$^+$, M = $C_{31}H_{27}N_5O_2$). UV-vis (toluene): λ_{abs}, nm 408.

***tert*-Butyl 15-[3-(4-(*N*-maleimido)-1-oxobutylamino) phenyl]porphyrin-5-carboxylate (P7-3a).** A mixure of **P7-2a** (25.0 mg, 50.0 µmol), DCC (51.5 mg,

0.250 mmol) and 4-maleimidobutyric acid (45.8 g, 0.250 mmol) in CH_2Cl_2 (1.00 mL) was stirred at room temperature under argon for 15 h. The mixture was diluted with CH_2Cl_2 (20 mL) and washed with water. The organic phase was separated, washed (brine), dried (Na_2SO_4), concentrated and chromatographed [silica, CH_2Cl_2/MeOH (99.5:0.5)] to obtain a mixture containing the title compound and 4-maleimidobutyric acid. Further chromatography [basic alumina, CH_2Cl_2/ethyl acetate (2:1)] afforded a dark brown solid (17.3 mg, 52%). ^{1}H NMR (400 MHz, $CDCl_3/CD_3OD$): δ, ppm 1.91–1.97 (m, 2H), 2.10 (s, 9H), 2.32 (t, J = 7.2 Hz, 2H), 3.51 (t, J = 6.8 Hz, 2H), 6.53 (s, 2H), 7.67 (t, J = 8.0 Hz, 1H), 7.96 (d, J = 7.6 Hz, 1H), 8.02 (m, 1H), 8.29 (t, J = 1.6 Hz, 1H), 9.07 (d, J = 4.8 Hz, 2H), 9.32 (d, J = 4.4 Hz, 2H), 9.45 (d, J = 4.8 Hz, 2H), 9.56 (d, J = 4.4 Hz, 2H), 10.22 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3/CD_3OD$): δ, ppm 25.2, 29.2, 34.9, 37.7, 84.5, 107.0, 110.1, 119.6, 121.6, 126.7, 127.5, 130.9, 131.3, 132.0, 133.2, 133.3, 134.6, 137.1, 144.1, 148.7, 150.0, 150.1, 150.4, 171.7, 172.1, 173.5. ESI-MS: *m/z* obsd. 667.2649, calcd. 667.2663 ([M + H]$^+$, M = $C_{39}H_{34}N_6O_5$). UV-vis (toluene): λ_{abs}, nm 407.

15-[3-(4-(*N*-maleimido)-1-oxobutylamino)phenyl] porphyrin-5-carboxylic acid (P7). A solution of **P7-3a** (16.6 mg, 25.0 μmol) in CH_2Cl_2 (1.20 mL) was treated with TFA (800 μL) for 4 h at room temperature. The volatile components (solvent and TFA) were removed by purging with argon. The resulting residue was washed with water and chromatographed [silica, CH_2Cl_2/MeOH (19:1)]. The resulting solid was dissolved in CH_2Cl_2/MeOH (5.0 mL, 19:1) and filtered to remove insoluble matter. The filtrate was added to hexanes (200 mL). The resulting precipitate was separated by filtration and dried to obtain a dark brown solid (12.2 mg, 80%). ^{1}H NMR (400 MHz, $CDCl_3/CD_3OD$): δ, ppm 2.03 (qt, J = 7.2 Hz, 2H), 2.44 (t, J = 7.2 Hz, 2H) 3.56 (t, J = 6.8 Hz, 2H), 6.64 (s, 2H), 7.72 (t, J = 8.0 Hz, 1H), 7.90 (d, J = 7.6 Hz, 1H), 8.08 (d, J = 8.0 Hz, 1H), 8.38 (s, 1H), 9.05 (d, J = 4.4 Hz, 2H), 9.28 (d, J = 4.4 Hz, 2H), 9.37 (brs, 2H), 9.76 (brs, 2H), 10.20 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3/CD_3OD$): δ, ppm 25.1, 34.8, 37.8, 106.5, 109.6, 120.2, 121.3, 127.0, 128.0, 131.0, 131.4, 131.9, 132.0, 133.1, 133.3, 134.7, 137.8, 142.0, 146.0, 146.9, 171.9, 172.7. ESI-MS: *m/z* obsd. 611.2035, calcd. 611.2037 ([M + H]$^+$, M = $C_{35}H_{26}N_6O_5$). UV-vis (toluene): λ_{abs}, nm 407.

Ethyl 15-(3-aminophenyl)porphyrin-5-carboxylate (P7-2b). Following a reported procedure [42], a suspension of **ZnP7-1b** (85 mg, 0.15 mmol), Zn dust (0.13 g, 2.0 mmol) and NH_4Cl (0.11 g, 2.0 mmol) in ethanol (50 mL) and water (5.0 mL) was stirred under reflux for 4 h. The content was filtered, and the filtrate was concentrated. The resulting residue was treated with 3N HCl, stirred for 1 h at room temperature, and then washed with ethyl acetate (colorless). The acidic aqueous solution (green) was neutralized with saturated aqueous $NaHCO_3$ solution and then extracted with ethyl acetate. The resulting organic solution (purple) was concentrated and chromatographed [silica, CH_2Cl_2/ethyl acetate (9:1)] to obtain a dark purple solid (43 mg, 61%). ^{1}H NMR (400 MHz, $CDCl_3/CD_3OD$): δ, ppm -3.10 (s, 1H) -2.95 (s, 1H), 1.88 (t, J = 7.6 Hz, 3H), 5.13 (q, J = 7.6 Hz, 2H), 7.13 (d, J = 1.2 Hz, 1H), 7.57 (brs, 2H), 7.61–7.67 (m, 1H), 9.16 (d, J = 4.4 Hz, 2H), 9.34 (d, J = 4.8 Hz, 2H), 9.46 (d, J = 4.8 Hz, 2H), 9.69 (d, J = 4.8 Hz, 2H), 10.32 (s, 2H). ^{13}C NMR (100 MHz, THF-d_8): δ, ppm 14.2, 62.5, 106.0, 113.8, 121.6, 123.0, 124.4, 125.2, 127.5, 130.3, 131.2, 131.6, 133.0, 137.6, 141.7, 145.4, 145.8, 147.0, 147.4, 170.3. MALDI-MS: *m/z* (POPOP) obsd. 473.5, calcd. 473.2 ([M]$^+$, M = $C_{29}H_{23}N_5O_2$). UV-vis (toluene): λ_{abs}, nm 408.

ClMn(III) 5-(*tert*-Butoxycarbonyl)-15-[3-(4-(*N*-maleimido)-1-oxobutylamino)phenyl]porphyrin (MnP7-3a). Following a literature procedure [36] with slight modification, a sample of **P7-3a** (29 mg, 0.043 mmol) in $CHCl_3$/MeOH (2:1, 18 mL) was treated with $MnCl_2{\cdot}4H_2O$ (273 mg, 1.38 mmol) and 2,6-di-*tert*-butylpyridine (8 drops). The reaction mixture was stirred at 50 °C for 4 days. The reaction mixture was diluted with $CHCl_3$ and extracted with water. The organic extract was separated, dried (Na_2SO_4), concentrated and chromatographed [silica, $CHCl_3$ → $CHCl_3$/MeOH (9:1)] to afford a red-brown solid (20.0 mg, 62% assuming a chloride counterion): MALDI-MS: *m/z* (α-cyano-4-hydroxycinnamic acid) obsd. 719.1, ESI-MS: *m/z* obsd. 719.1809; calcd. 719.1809 ([M – Cl]$^+$, M = $C_{39}H_{32}ClMnN_6O_5$). UV-vis (DMF): λ_{abs}, nm 368, 390, 411, 458, 552.

ClMn(III) 5-Carboxy-15-[3-(4-(*N*-maleimido)-1-oxobutylamino)phenyl]porphyrin (MnP7). A sample of **MnP7-3a** (10 mg, 0.014 mmol) in CH_2Cl_2 (2.4 mL) was treated with TFA (0.6 mL), and the mixture was stirred for 4 h at room temperature. Hexanes was added, and the resulting mixture was sonicated in a benchtop sonication bath. After centrifuging the suspension, the supernatant was carefully decanted and this process was repeated with diethyl ether to obtain the title product as a red-brown solid (8.5 mg, 87% assuming a chloride counterion): MALDI-MS: *m/z* (α-cyano-4-hydroxycinnamic acid) obsd. 663.1, ESI-MS: *m/z* obsd. 663.1159; calcd. 663.1183 ([M – Cl]$^+$, M = $C_{35}H_{24}ClMnN_6O_5$). UV-vis (0.5 M phosphate buffer, pH 7.0): λ_{abs}, nm 372, 394, 459, 549.

5-[4-(*tert*-Butoxycarbonylmethoxy)phenyl-15-[3,5-bis(diethylphosphonomethoxy)phenyl]porphyrin (P8-1). Following an established procedure for de-zincation in the presence of phosphono esters [13], a solution of **ZnP8-1** (30. mg, 0.030 mmol) in CH_2Cl_2 (10 mL) was treated with *p*-TsOH·H_2O (480 mg, 2.52 mmol) at room temperature for 1 h. The reaction mixture was diluted with CH_2Cl_2 and washed with saturated aqueous $NaHCO_3$ solution. The organic layer was separated, dried (Na_2SO_4) and filtered. The filtrate was concentrated to a dark pink solid. The solid was chromatographed [silica, CH_2Cl_2/methanol (0–10%)] to afford a pink solid (25.3 mg,

90%). ^{1}H NMR: δ, ppm -3.15 (brs, 2H), -0.38 (t, *J* = 7.2 Hz, 12H), 1.64 (s, 9H), 2.27–2.44 (m, 8H), 4.18 (s, 2H), 4.20 (s, 2H), 4.85 (s, 1H), 4.95 (s, 1H), 7.14 (d, *J* = 8.8 Hz, 2H), 7.36 (m, 2H), 7.82 (t, *J* = 8.8 Hz, 1H), 8.21 (m, 2H), 8.95 (d, *J* = 4.4 Hz, 2H), 9.10 (m, 2H), 9.33 (d, *J* = 4.8 Hz, 2H), 9.38 (d, *J* = 4.8 Hz, 2H), 10.24 (s, 2H). ESI-MS: *m/z* obsd. 924.32462, calcd. 924.32643 ($[M]^+$, M = $C_{48}H_{54}N_4O_{11}P_2$). UV-vis (CH_2Cl_2): λ_{abs}, nm 406, 501, 534, 575.

5-[4-(Carboxymethoxy)phenyl-15-[3,5-bis-(phosphonomethoxy)phenyl]porphyrin (P8). A solution of **P8-1** (30 mg, 0.032 mmol) in THF/H_2O (10 mL, 4:1) was treated with aqueous 2M KOH (200 µL, 0.400 mmol). The mixture was stirred for 24 h at room temperature. Then, THF was removed under vacuum. The residue was partioned between water (5 mL) and CH_2Cl_2 (5 mL). The aqueous layer was freeze-dried to obtain the potassium salt of the porphyrin. The solid was suspended in CH_2Cl_2 (5 mL), whereupon a few drops of acetic acid were added. The mixture was filtered. The filtrate was concentrated. The resulting solid was dried under high vacuum. The solid was dissolved in CH_2Cl_2 (5 mL), whereupon TMSBr (300 µL, excess) was added. The solution was stirred for 4 h at room temperature. The solvent and excess TMSBr were removed *in vacuo*, then methanol/water (5 mL, 4:1) was added. Stirring was continued for 1 h, whereupon the volatile components were removed under high vacuum. The resulting green solid was dried under high vacuum, then triturated with CH_2Cl_2 (10 mL × 3) and finally dried to obtain a green solid (25 mg, quantitative). ^{1}H NMR (CD_3OD): δ, ppm 4.18 (s, 2H), 4.20 (s, 2H), 5.12 (s, 2H), 7.41 (d, *J* = 7.6 Hz, 2H), 7.69 (d, *J* = 8.8 Hz, 2H), 8.02 (t, *J* = 8.8 Hz, 1H), 8.59 (d, *J* = 7.6 Hz, 2H), 9.34 (m, 4H), 9.89 (d, *J* = 5.2 Hz, 2H), 9.93 (d, *J* = 4.8 Hz, 2H), 11.34 (s, 2H). ESI-MS: *m/z* (–) obsd. 756.14070, calcd. 756.13863 ($[M]^-$, M = $C_{36}H_{30}N_4O_{11}P_2$). UV-vis (H_2O): λ_{abs}, nm 404, 504, 541, 565.

Bioconjugation of porphyrins

5-[4-(*N*-succinimidyloxycarbonylmethoxy) phenyl]-15-[1,5-bis(dimethoxyphosphoryl)pent-3-yl] porphyrin (P3a-2). A solution of **P3a-1** (10.1 mg, 13.0 µmol) in anhydrous DMF (400 µL) was treated with EDC (4.88 mg, 25.5 µmol). The solution was stirred at room temperature for 10 min. *N*-hydroxysuccinimide (2.94 mg, 25.6 µmol) was added. Stirring was continued for 24 h. The mixture was diluted with CH_2Cl_2 and washed with water. The aqueous phase was extracted with CH_2Cl_2. The combined organic extract was washed with water. The organic layer was dried (Na_2SO_4). Concentration of the sample gave a mixture of **P3a-1** and **P3a-2** (approximately 1:4 ratio). ^{1}H NMR: δ, ppm 1.09 (t, *J* = 6.9 Hz, 3H), 2.66–3.68 (m, approximately 22H), 5.30 (s, 2H), 5.82–5.93 (m, 1H), 7.39–7.42 (m, 2H), 8.19–8.21 (m, 2H), 9.07 (app s, 2H), 9.38–9.48 (m, 2H), 9.61–9.76 (m, 4H), 10.28 (s, 2H). LD-MS: *m/z* obsd. 877.2, calcd. 875.2 ($C_{41}H_{43}N_5O_{13}P_2$); also obsd. 778.9 (**P3a-1**). This crude material was used in pilot conjugation experiments without further characterization.

(D-Phe-P3a-1). A solution of **P3a-1** (5.41 mg, 6.95 µmol) in anhydrous DMF (400 µL) was treated with EDC (6.25 mg, 32.7 µmol) and HOBt (5.60 mg, 41.5 µmol). The solution was stirred at room temperature for 10 min. D-Phe-OEt (*p*-toluenesulfonic acid salt, 7.61 mg, 20.9 µmol) and DIEA (5.6 µL, 31 µmol) were added. Stirring was continued for 24 h. The mixture was diluted with CH_2Cl_2 and washed with water. The aqueous phase was extracted with CH_2Cl_2. The combined organic extract was washed (water), dried (Na_2SO_4) and chromatographed [silica, CH_2Cl_2/MeOH (97:3)] to afford a deep purple solid (4.70 mg, 71%). ^{1}H NMR: δ, ppm -2.89 (s, 1H), -2.84 (s, 1H), 1.32 (t, *J* = 6.9 Hz, 3H), 3.20–3.49 (m, 18H), 3.91 (m, 2H), 4.06 (m, 2H), 4.28 (q, *J* = 6.9 Hz, 2H), 4.82 (s, 2H), 5.09–5.11 (m, 1H), 5.80 (m, 1H), 7.30–7.38 (m, 7H), 8.19 (d, *J* = 7.5 Hz, 2H), 9.06 (s, 2H), 9.38–9.40 (m, 2H), 9.49 (s, 2H), 9.66 (s, 1H), 9.83 (s, 1H), 10.28 (s, 1H), 10.31 (s, 1H); LD-MS: *m/z* obsd. 955.6; FAB-MS: *m/z* obsd. 976.3107, calcd. 976.3064 [(M + Na)$^+$, M = $C_{48}H_{53}N_5O_{12}P_2$). UV-vis: λ_{abs}, nm 406, 504; λ_{em}, nm (λ_{exc} 406 nm) 633, 700.

(D-Phe-P3a). A solution of **D-Phe-P3a-1** (5.15 mg, 5.40 µmol) in anhydrous $CHCl_3$ (0.5 mL) was treated with TMSBr (5.7 µL, 4.3 µmol). The solution was stirred at room temperature for 3 h. The solution was concentrated, and the resulting residue was dissolved in dilute aqueous NaOH (1 mL, 0.1 M). The mixture was stirred for 1 h. Chromatography [C-18 silica, water/MeOH (0 → 70%)] yielded a deep purple solid (6.30 mg): ESI-MS: *m/z* obsd. (–) 868.0 (M – H), also obsd. 881.0 (M + methylene – H)$^-$. ESI-MS: *m/z* obsd. 870.2 $[M + H]^+$, 891.2 $[M + Na]^+$, calcd. 869.2 (M = $C_{42}H_{41}N_5O_{12}P_2$). UV-vis: λ_{abs}, nm 402, 505; λ_{em}, nm (λ_{exc} 402 nm) 627, 686. HPLC t_R = 9.60 min.

(MSH-P3a-1). A solution of **P3a-1** (4.76 mg, 6.12 µmol) in anhydrous DMF (400 µL) was treated with EDC (11.7 mg, 61.2 µmol) and HOBt (9.09 mg, 67.3 µmol) and stirred at room temperature for 10 min. Samples of Ac-α-MSH(11-13)-NH_2·HCl (3.75 mg, 8.94 µmol) and DIEA (2.4 µL, 13 µmol) were added. Stirring was continued for 24 h. The solution was diluted with CH_2Cl_2 and washed with water. The aqueous phase was extracted with CH_2Cl_2. The combined organic extract was washed (water), dried (Na_2SO_4) and chromatographed [silica, CH_2Cl_2/MeOH (0 → 12%)] to afford a deep purple solid (5.30 mg, 76%). ^{1}H NMR: δ, ppm -2.89 (s, 1H), -2.84 (s, 1H), 0.93–0.98 (m, 6H), 1.42–1.76 (m, 12H), 3.20–3.22 (m, 2H), 3.40–3.49 (s, 14H), 3.65–4.06 (m, 7H), 4.26–4.31 (m, 1H), 4.55 (m, 1H), 4.81 (s, 2H), 5.78 (br, 2H), 6.17 (s, 1H), 6.72 (d, *J* = 7.5 Hz, 1H), 6.92 (d, *J* = 8.4 Hz, 1H), 7.08 (s, 1H), 7.37–7.40 (m, 2H), 8.19–8.22 (m, 2H), 9.06 (s, 2H), 9.37–9.40 (m, 2H), 9.49 (m, 2H), 9.65 (m, 1H), 9.81 (m, 1H), 10.28 (s, 1H), 10.30 (s, 1H). ESI-MS: *m/z* obsd. (–) 1142.3 $[M - H]^-$, 1179.3

$[M + Cl - H]^-$, 1188.3 $[M + Na + Cl - H]^-$. ESI-MS: *m/z* obsd. 1144.4 $[M + H]^+$, 572.7 $[M + 2H]^{2+}$, calcd. 1143.5 ($M = C_{55}H_{71}N_9O_{14}P_2$). LD-MS: *m/z* obsd. 1146.6, 1169.3 $[M + Na]^+$. UV-vis: λ_{abs}, nm 406, 503, 538, 577; λ_{em}, nm (λ_{exc} 406 nm) 634, 699.

(MSH-P3a). A solution of **MSH-P3a-1** (3.18 mg, 2.78 μmol) in anhydrous $CHCl_3$ (0.5 mL) was treated with TMSBr (2.9 μL, 22 μmol) and stirred at room temperature for 3 h. The mixture was concentrated, and the resulting residue was dissolved in MeOH (3 mL). The resulting solution was stirred for 1 h and then concentrated. The resulting dark green solid was treated with dilute aqueous NaOH (0.5 mL of 0.05 M solution). Chromatography [C-18 silica, water/MeOH (0 → 70%)] yielded a deep purple solid (4.24 mg). ESI-MS: *m/z* obsd. (–) 1086.3 $[M - H]^-$, calcd. 1087.4 ($M = C_{51}H_{63}N_9O_{14}P_2$). ESI-MS: *m/z* obsd. 1100.2 [M + methylene – H]$^+$, 1115.3 [M + 2 methylene – H]$^+$. UV-vis: λ_{abs}, nm 402, 505; λ_{em}, nm (λ_{exc} 402 nm) 626, 687. HPLC t_R = 11.76 min.

(MSH-P4a). A solution of **P4a** (1.04 mg, 1.51 μmol), EDC (4.36 mg, 22.8 μmol) and HOBt (3.00 mg, 22.2 μmol) in water (180 μL) was treated with Ac-α-MSH(11-13)-NH_2 (1.65 mg, 3.93 μmol) and DIEA (3.2 μL, 34.5 μmol). Stirring was continued for 24 h. The solution was diluted with water (1 mL). Chromatography [C-18 silica, water/MeOH (0 → 70%)] afforded a deep purple solid (1.68 mg). ESI-MS: *m/z* obsd. 539.3 $[M + Na + H]^{2+}$, 1056.3 $[M + H]^+$. ESI-MS: *m/z* obsd. (–) 1054.3 $[M - H]^-$, 573.3 $[M - H + 2Cl + Na]^{2-}$, 609.3 $[M - H + Cl + DIEA]^{2-}$, calcd. 1055.4 ($M = C_{51}H_{63}N_9O_{12}P_2$), also obsd. 691.1 (**P4a** + H)$^+$. UV-vis: λ_{abs}, nm 403, 507; λ_{em}, nm (λ_{exc} 403 nm) 628, 688. HPLC t_R = 10.58 min.

CONCLUSION

The synthesis of porphyrins has advanced substantially over the years since the classic work of Gunter and Mander. The *trans*-AB-porphyrins described herein contain only two substituents — the A and B groups. The absence of extraneous substituents affords a compact, sparsely substituted architecture that may be ideal for a number of biological applications. Despite the advances in methodology for construction of such porphyrins, their extension to water-soluble porphyrin-peptide bioconjugates presents a number of challenges, chief among which remains the nature of the hydrophilic motif. The *trans*-AB-porphyrins described herein contain a single bioconjugatable motif and a single hydrophilic group. The bioconjugations examined herein were carried out with the hydrophilic moiety in protected form (phosphate) in DMF or in unprotected form (phosphonate) in aqueous solution. In general, the porphyrins were constructed in a form wherein the hydrophilic motif was not ionized by means of protection (carboxylic acid ester, phosphono ester) or not yet quaternized (pyridine). Such porphyrins were fully characterized. As the hydrophilicity of the porphyrins increased upon elaboration, characterization became more challenging. Excellent characterization of the protected porphyrin conjugates was generally obtained whereas the partial characterization of the deprotected conjugates (**D-Phe-P3a**, **MSH-P3a**, and **MSH-P4a**) causes their assignments to remain provisional.

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