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

Ana Z. Muresan, Jonathan S. Lindsey *

Department of Chemistry, North Carolina State University, Raleigh, NC 27695-8204, USA

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ABSTRACT

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

1. Introduction

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

Water solubilization of porphyrins has traditionally been achieved by use of uroporphyrin, which bears eight β-substituted alkylcarboxylic acids,[22–26] or by attachment of small aryl motifs such as *N*-alkyl pyridinium or phenylsulfonic acid units to the *meso* sites of synthetic porphyrins[27] (Chart 1). In the latter case, four polar substituents (one at each of the *meso* sites) traditionally were introduced to impart water solubility. The use of four substituents stemmed from the synthetic methods of the day,[28] which only readily supported the synthesis of such A_4-porphyrins. Regardless, the most easily synthesized members—the pyridinium- or sulfoaryl-derivatized porphyrins—exhibit only limited solubility in aqueous media. Moreover, the sense that four such groups were essential also presented an impediment to further functionalization of the macrocycle, as required for applications where bioconjugation is required. The advent of versatile and rational synthetic routes to compact porphyrins that bear less than four *meso* substituents[29,30] (e.g., *trans*-AB-porphyrins) has opened the door to consideration of new molecular designs for water solubilization that are both potent and compatible with features required for bioconjugation.

In parallel with the development of new synthetic methods, new water-solubilization motifs have been developed to serve a variety of objectives. Representative examples of such solubilization motifs include oligoethylene glycol monomers[31] and dendrimers,[32] alkyl polyamines,[33] and polycarboxy chains.[34] While each such group has merit, most are quite large and are not well suited for our goals in developing compact bioconjugatable molecules for use in photomedical applications.

We initiated a program to develop water-solubilization motifs that ideally adhere to the following design guidelines: (1) ionic

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* Corresponding author. Tel.: +1 919 5156406; fax: +1 919 5132830.
E-mail address: jlindsey@ncsu.edu (J.S. Lindsey).

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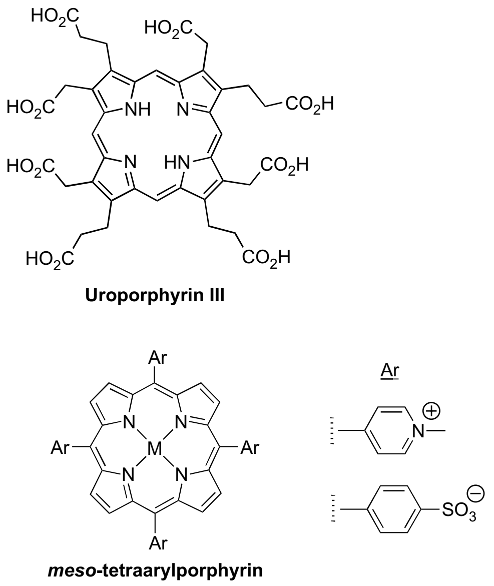

Chart 1.

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

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

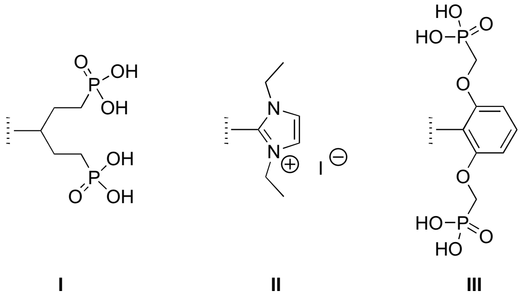

Chart 2.

2. Results and discussion

2.1. Molecular design and synthesis strategy

Each target molecule (**H$_2$P-1–3**) is a free base *trans*-AB-porphyrin (Chart 3). The *trans*-AB-porphyrin architecture, wherein the porphyrin contains only two *meso* substituents, was chosen to maintain a compact size. Each of the porphyrins is tailored with a water-solubilizing 2,4,6-tris(carboxymethoxy)phenyl substituent at one *meso* position. The 2,4,6-tris(carboxymethoxy)phenyl unit is relatively compact. The mass of this water-solubilizing moiety ($C_{12}H_{11}O_9$, 299 Da) is comparable to that of the core porphine unit ($C_{20}H_{12}N_4$, 308 Da) in the *trans*-AB-porphyrin.

The 2,4,6-tris(carboxymethoxy)phenyl unit is an *o*,*o*′-disubstituted *meso*-aryl motif, whereby the polar groups are (i) in close proximity to the porphyrin macrocycle and (ii) are thrust in an orientation that encumbers the faces of porphyrin, thereby impeding aggregation. The introduction of 2,6-disubstituted aryl units

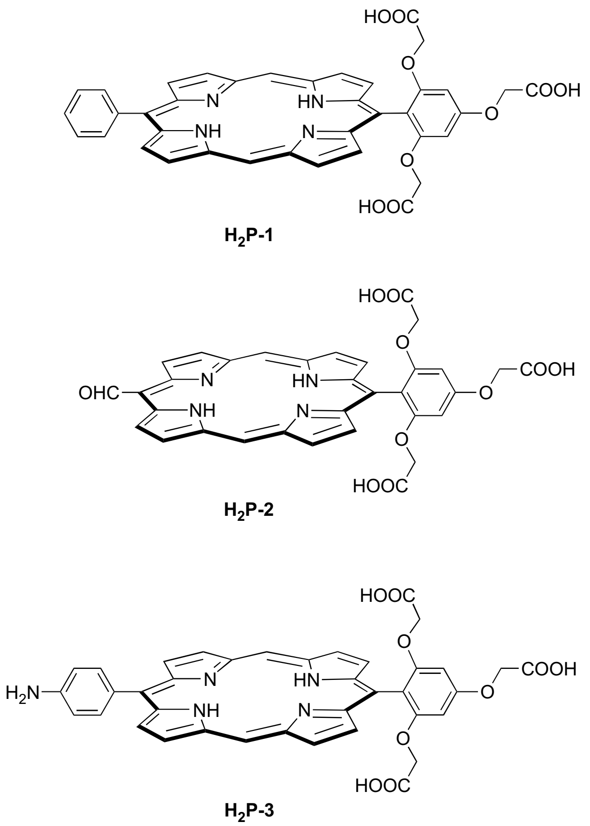

Chart 3.