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Synthesis of water-soluble porphyrin and the corresponding highly planar benzoporphyrin without *meso*-substituents

Takashi Murashima,[a,b] Satoshi Tsujimoto,[a] Takashi Yamada,[a] Toshifumi Miyazawa,[a] Hidemitsu Uno,[c] Noboru Ono[d] and Naoki Sugimoto[a,b,*]

[a]*Department of Chemistry, Faculty of Science and Engineering, Konan University, Japan*
[b]*Frontier Institute for Biomolecular Engineering Research (FIBER), Konan University, 8-9-1 Okamoto, Higashinada-ku, Kobe 685-8501, Japan*
[c]*Department of Chemistry, Faculty of Science, Ehime University, Japan*
[d]*Integrated Center for Science, Ehime University, 2-5 Bunkyo-cho, Masuyama 790-8577, Japan*

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Abstract—Water-soluble bicycloporphyrin and benzoporphyrin having octacarboxyl groups are synthesized from the corresponding octaester porphyrin by simple hydrolyzing and heating procedures. These compounds are characterized by UV–vis, NMR and mass spectroscopies. The benzoporphyrin octacarboxylic acid exhibits extremely high planarity in spite of its poly-substitution.
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Naturally occurring porphyrins play essential roles in photosynthesis, cellular respiration and electron transfer.[1] In these biological processes, the characteristics of porphyrin such as large planar π-conjugation and high oxygen affinity[2] are the intrinsically important properties. These two characteristics are also required when porphyrins and related compounds are used as DNA cleavage catalysts[3] and photosensitizers for photodynamic therapy (PDT)[4] in in vivo studies. For example, general features of molecules binding to G-quartet[5] include a large planar aromatic surface.[6]

Water-solubility is another required property to use porphyrins for such medicinal purposes. The study of artificial water-soluble porphyrins is currently under active research with regard to the design, synthesis and especially biological assay.[7] Most of naturally occurring porphyrins are *meso*-unsubstituted ones, however, DNA–porphyrin interactions were studied using only a few kinds of *meso*-substituted water-soluble porphyrins such as 5,10,15,20-tetra(*N*-methyl-4-pyridyl)porphyrin (TPyP),[8] 5,10,15,20-tetrakis(*p*-sulfonatophenyl)porphyrin (TSPP)[9] and their derivatives[10] because of their simple preparation[11] or commercial availability. In contrast, both preparation and purification of *meso*-unsubstituted water-soluble porphyrins are quite difficult.[12] Furthermore, *meso*-unsubstituted porphyrins are more easily oxidized than *meso*-substituted ones.

Here we report the synthesis and purification of *meso*-unsubstituted water-soluble porphyrins, which have highly planar frameworks. The preparation is based on a hydroxylation and aromatization strategy using previously reported tetra(bicyclo)porphyrin with octaester groups.[13]

The parent compound **1** in Scheme 1a was prepared from 4,7-dihydro-5,6-dimethoxycarbonyl-4,7-ethano-2*H*-isoindole in 37% yield.[13] There were three strategies for the preparation of the target compound, octacarboxyl tetrabenzoporphyrin **6** or **7** as shown in Schemes 1b and c. Route 1 is two-step methodology consisting of hydrolysis and aromatization by retro Diels–Alder reaction. Route 2 is a modified one-pot process of route 1. Route 3 is a reversal process of route 1, thus aromatization is carried out before hydrolysis.

According to route 1, hydrolysis of **1** was conducted using aqueous tetramethylammonium hydroxide in DMF at 60 °C for 16 h and a water-soluble porphyrin

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* Corresponding author. Tel.: +81 784352502; fax: +81 784352539; e-mail: sugimoto@konan-u.ac.jp

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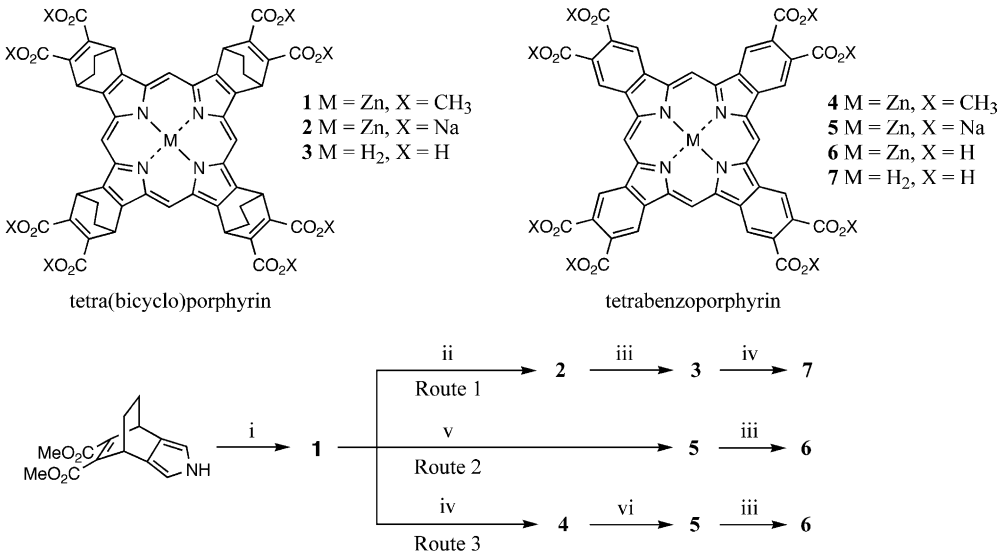

Scheme 1. Reagents and conditions: (i) $(HCHO)_n$, $(AcO_2)Zn$, TFA, $MeOH/CHCl_3$, rt, 13 h, then DDQ, rt, 17 h, (ii) $NaOH/H_2O$, DMF, rt, 12 h, (iii) 1 M HCl, (iv) 200 °C, 10 min, (v) $NaOH/H_2O$, DMF, 110 °C, 16 h, (vi) $NaOH/H_2O$, pyridine/DMF, rt, 20 h.

was obtained. Under these mild conditions a part of the ester groups were not hydrolyzed and the product obtained here is a mixture of many kinds of porphyrins. Hydrolysis of **1** in the acidic media was unsuccessful because of the limited solubility of porphyrins with carboxylic acid groups. On the other hand, the use of excess amounts of aqueous sodium hydroxide in DMF at room temperature gave a satisfactory result to obtain the expected octacarboxyl porphyrin sodium salt **2**. After the addition of enough 1 M HCl to acidify the reaction mixture, a red precipitate was formed. Because of the strong acidic conditions, the demetallation of metalloporphyrin **2** occurred and the resulting precipitate was free-base octacarboxyl tetra(bicyclo)porphyrin **3**. Demetallation was confirmed by the UV–vis absorption at 496, 528, 564, 616 nm corresponding to Q-band (Fig. 1a) and the ^{1}H NMR signal of NH broad singlet at −3.98 ppm.[19] The sodium salt of porphyrin **3** has a solubility of 638 g/L (= 0.555 mol/L) in water. In contrast to the high solubility of sodium salt, porphyrin **3** is slightly soluble in neutral water.

In general, retro Diels–Alder reaction requires 200 °C or higher temperature for a facilitate conversion.[13–15] Therefore, the aromatization step of route 1 was conducted at 200 °C. Unfortunately, this step was not a clean reaction as the case of other tetra(bicyclo)porphyrins reported[13–15] mainly because of the decarboxylation of adjacent carboxyl groups.

In contrast to the above-mentioned unsatisfactory reaction, one-pot reaction has been successfully carried out at relatively low temperature, 110 °C to give octacarboxyl benzoporphyrin sodium salt **5**. Salt **5** was converted into a free acid form **6** by the addition of 1 M HCl. The outstanding characteristic of benzoporphyrin is linearly π-extended structures and it causes strong stacking which prevents dissolution. Thus, the acidic conditions used here was milder than those used in the case of **2**.

Porphyrin **6** was insoluble in most of organic solvents, but its sodium salt **5** was soluble in water with the solubility of 122 g/L (= 0.111 mol/L). Therefore, **6** could be

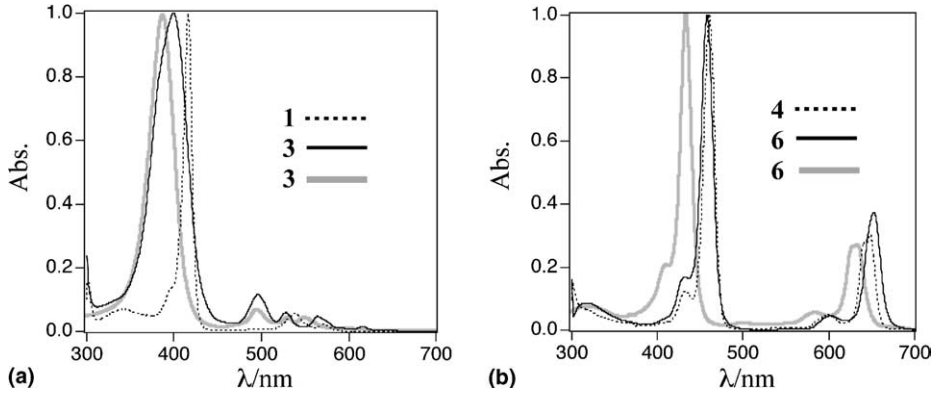

Figure 1. UV–vis spectra of (a) tetra(bicyclo)porphyrin octaester **1**, octacarboxylic acid **3** and (b) tetrabenzoporphyrin octaester **4**, octacarboxylic acid **6** in pyridine at 298 K. The gray lines represent the spectra of **3** and **6** in basic water (containing 3 equiv of NaOH). All spectra were normalized in absorbance of Soret-band for comparison.

purified by taking advantage of the differences in its solubility in organic solvents (as **6**) and in basic water (as **5**). After acidified the reaction mixture, green precipitate was filtered and washed thoroughly with chloroform and hexane. Then the filter cake was dissolved in aqueous sodium hydroxide. The duplicate operations of these purification procedures followed by addition of 1 M HCl gave pure **6** without column chromatography.

Porphyrin **6** was also prepared according to route 3. Initial aromatization was achieved at 200 °C and the resulting porphyrin **4** was hydrolyzed with aqueous sodium hydroxide in pyridine/DMF at room temperature for 20 h. The expected porphyrin **6** was isolated from the reaction mixture consisting the fully hydrolyzed and partially hydrolyzed products by following extraction-precipitation procedures. After the solution was evaporated to dryness, the resulting solid was extracted with pyridine and the insoluble precipitate was filtered and dissolved in 1 M NaOH. Fully hydrolyzed product **5** was extracted into the aqueous phase and partially hydrolyzed products were recovered from the pyridine solution. To the aqueous solution was added an excess amount of 1 M HCl, and the resulting precipitate was rinsed with water to give pure **6**.

Compounds **3** and **6** were characterized by ^{1}H NMR, UV–vis and mass spectroscopies.[19] The absorption spectra of **3** and **6** together with their parent compounds **1** and **4** are shown in Figure 1. The Soret-band of **6** was slightly blue-shifted by 2.5 nm compared to that of **4**, while the Q-band was red-shifted by 4 nm. Judging from the intensity of the peak at the longest wavelength of **6**, π-conjugation is more extended than the parent porphyrin octaester **4**.

The ^{1}H NMR spectra of **3** showed characteristic peaks, which were attributed to bicycloalkyl structure. The ^{1}H NMR spectral signals of **6** were two sharp singlets at 10.63 ppm (fused aromatic-H) and 11.51 ppm (*meso*-H). Generally, poly-substitution causes the distortion of porphyrin frameworks,[13,16] and the ^{1}H NMR spectral signals of ester **4** were broad.[13] However, the ^{1}H NMR spectrum of **6** exhibit sharp signals suggest the porphyrin ring does not distort and the framework is extremely planar.[13,16] One of the most widely investigated porphyrins, TPyP is also planar molecule,[17] but once the compound complexed with DNA duplex, the porphyrin showed a highly nonplanar conformation.[18] This fact means the structure of porphyrin does NOT control the structure of DNA, but the structure of DNA control the structure of porphyrin. To the contrary, the use of the rigid and planar porphyrin **6** should affect the structure of DNA.

In conclusion, new water-soluble benzoporphyrin having eight carboxylic acid groups at the fused benzene rings was successfully synthesized. This compound exhibits highly planar structure and well-expanded π-conjugation. Application towards DNA binding and PDT studies are expected by using octacarboxylated benzoporphyrin **6** and its free base form **7**.

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19. Typical procedures and selected spectroscopic data for compounds **3** and **6**; **3** (mixture of four isomers): To a solution of compound **1** (0.050 g, 0.04 mmol) in DMF (10 mL) was added 2 N NaOH (20 mL, 40 mmol) under a nitrogen atmosphere and the resulting mixture was stirred for 16 h at 60 °C in the dark. After the solvent was removed under the reduced pressure, the residue was dissolved in water and washed with $CHCl_3$, ethyl acetate, then distilled hexane. Compound **3** was precipitated from the aqueous solution by the addition of 1 N HCl. The resulting precipitate was centrifuged and washed with water to give porphyrin **3** as a red powder. Yield 98.8% from **1**; ^{1}H NMR (500 MHz, pyridne-d_6) δ: −3.98 (br s, 2H), 1.96–2.16 (m, 8H), 2.34–2.49 (m, 8H), 7.09 (s, 8H), 11.09 (s, 4H); *m*/*z* (ESI) 975 ($M^+ + 1$); λ_{max}/nm (ε) (water) 389 (194,500), 497 (12,500), 533 (7000), 551 (7000), 599 (1000); **6**: To a solution of compound **4** (0.081 g, 0.078 mmol) in pyridine (10 mL) and DMF (10 mL) was added 2 N NaOH (10 mL, 20 mmol) under a nitrogen atmosphere and the resulting mixture was stirred for 16 h at 80 °C in the dark. The product was treated by similar procedures to those adopted in the preparation of **3** to give pure porphyrin **6** as a green powder. Yield 51.6% from **1**; ^{1}H NMR (500 MHz, pyridne-d_6) δ : 10.63 (s, 8H), 11.51 (s, 4H), 12.36 (br s, 8H); *m*/*z* (ESI) 925 ($M^+ + 1$); λ_{max}/nm (ε) (water) 437 (218,500), 588 (11,500), 638 (58,500).