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Water-soluble supramolecular porphyrin dimer: self-organization of mono(imidazolyl)-substituted Zn porphyrin to a special-pair type dimer in water

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ABSTRACT

Tris(4-carboxylphenyl)-mono(*N*-methylimidazolyl)-substituted Zn porphyrin was synthesized as a precursor for a water-soluble supramolecular porphyrin dimer. The dimer formation was performed in a $NaHCO_3$ aq solution (pH 8.4) and phosphate buffer solutions (pH 7.4–9.0). The split Soret bands of Zn porphyrin observed in the absorption spectra clearly showed self-organization to a special-pair type slipped cofacial dimer via metal coordination of imidazole even in water.

Mimicking biological functions using synthetic molecules is a challenging area of biomimetic chemistry.[1] In particular, porphyrins have been widely used to mimic biological functions.[2] For example, imidazole- and other ligand-substituted porphyrins have been reported as models for artificial photosynthesis[3–7] and artificial hemoglobins.[8,9] However, these biomimetic supramolecules have been studied in organic solvents, such as chloroform. We are interested in making water-soluble biomimetic supramolecular systems via the self-organization of porphyrins. Water-soluble supramolecular porphyrins are expected to be used not only as a catalyst[10] that works in water but also in medical applications, such as photodynamic therapy (PDT),[11] in the future. As a first approach, we herein report a tris(4-carboxyphenyl)-mono(*N*-methylimidazolyl)-substituted Zn porphyrin as a precursor for a water-soluble supramolecular porphyrin dimer and its carboxylate salt, which forms a special-pair type slipped cofacial arrangement[12] via metal coordination of the imidazolyl ligand in water.

Scheme 1 shows a synthetic route of the imidazole-substituted porphyrin. Three methyl benzoate- and one *N*-methylimidazole-substituted porphyrin was synthesized by condensation of methyl 4-formylbenzoate and 1-methylimidazole-2-carboxaldehyde with pyrrole in acetic anhydride/propionic acid at 110 °C. Mono(*N*-methylimidazolyl)-substituted porphyrin **1** was isolated by column chromatography (Al_2O_3, activity stage II, CH_2Cl_2/EtOAc = 15/1 as an eluent) in 22% yield. The Zn porphyrin **2** was synthesized by stirring with zinc acetate in MeOH/CH_2Cl_2 at room temperature and isolated by column chromatography (Al_2O_3, activity stage II, CH_2Cl_2/EtOAc = 9/1 as an eluent) in 93% yield. The Zn porphyrin methyl ester **2** was hydrolyzed in a 1 N NaOH/THF solution with stirring at room temperature, and the Zn porphyrin carboxylic acid **3** was obtained by the addition of 1 N HCl as a precipitate in 80% yield (see Supplementary data for details).

The ^{1}H NMR spectrum of the Zn porphyrin methyl ester **2** in $CDCl_3$ elucidated a supramolecular porphyrin dimer formation. As already reported in similar special-pair type slipped cofacial dimers,[3,8] signals for protons in the stacking area of two porphyrin planes were found to shift the up-field. For example, the β-pyrrolic protons of free base porphyrin **1** that appeared at 8.84 ppm (2H) and 8.85 ppm (2H, next to *N*-methylimidazole) shifted the up-field at 8.26 ppm (2H) and 5.59 ppm (2H, next to *N*-methylimidazole) in the case of Zn porphyrin **2**, respectively. Significant up-field shifts were observed at the *N*-methylimidazole moiety of Zn porphyrin **2**. Signals for the imidazole protons of porphyrin **1** that appeared at 7.47 ppm (1H) and 7.66 ppm (1H) shifted the up-field at 2.05 ppm (1H) and 5.57 ppm (1H) in the case of Zn porphyrin **2**, respectively. *N*-Methyl protons (3H) also shifted the up-field from 3.45 ppm (for **1**) to 1.72 ppm (for **2**). These results indicated the shielding effect of porphyrin ring current due to stacking of **2** via coordination. On the other hand, the β-pyrrolic protons of **2** that were expected to be in the non-stacking area did not show such up-field shifts; they appeared at 8.91 ppm (2H) and 8.96 ppm (2H) (see Supplementary data).

Table 1 lists the absorption and fluorescence spectral data of the free-base porphyrin **1**, the Zn porphyrin methyl ester **2**, **2** with

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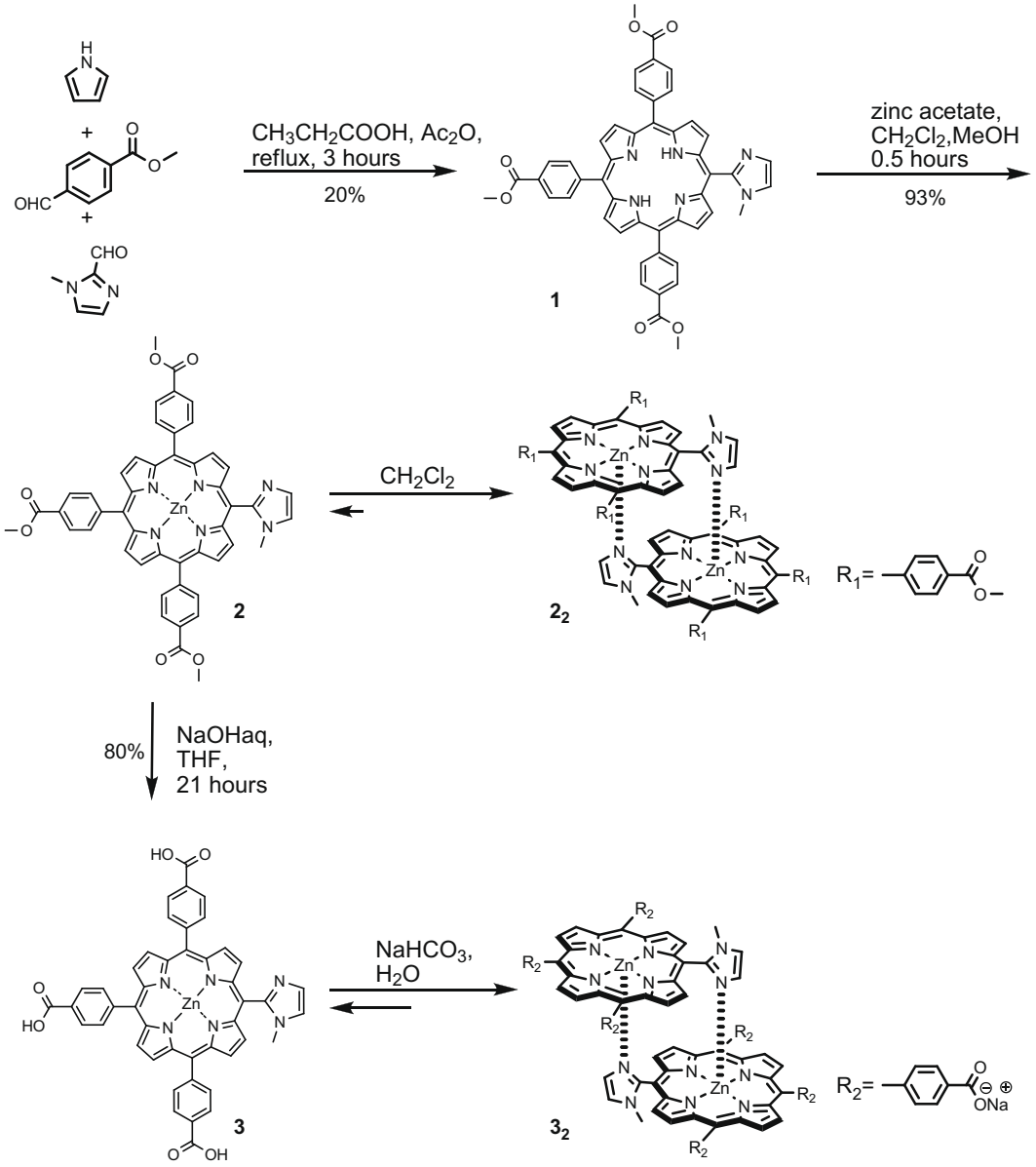

Scheme 1. Synthetic route of mono(*N*-methylimidazolyl)-substituted porphyrin dimer.

Table 1
Absorption and fluorescence spectral data of the free-base and the Zn complex of mono(*N*-methylimidazolyl)porphyrin and ZnTCPP

Porphyrin	Solvent	Absorption			
		Soret band (nm)	Half-band width (nm)	Q band (nm)	Fluorescence (nm)
1	CH_2Cl_2	420	16	515, 550, 587, 543	650, 712
2	CH_2Cl_2	418, 435	35	565, 612	616, 667
2(MeIm)	CH_2Cl_2	431	12	565, 605	614, 666
3	$NaHCO_3$ aq (pH 8.4)	413, 430	32	565, 610	615, 661
3(MeIm)	$NaHCO_3$ aq (pH 8.4)	427	15	564, 606	613, 660
3	Phosphate buffer (pH 7.4)	413, 430	32	564, 609	611, 656
3(MeIm)	Phosphate buffer (pH 7.4)	427	19	564, 606	612, 664
ZnTCPP	Phosphate buffer (pH 7.4)	422	12	556, 596	610, 661
ZnTCPP (MeIm)	Phosphate buffer (pH 7.4)	427	12	562, 603	617, 667

excess amounts of *N*-methylimidazole, **2**(MeIm) in CH_2Cl_2,[13] the Zn porphyrin carboxylic acid **3** in a saturated $NaHCO_3$ aq solution (pH 8.4), **3** with excess amounts of *N*-methylimidazole, **3**(MeIm) in a saturated $NaHCO_3$ aq solution (pH 8.4), **3** in a phosphate buffer solution (pH 7.4),[14] **3** with excess amounts of *N*-methylimidazole, **3**(MeIm) in a phosphate buffer solution (pH 7.4) along with Zn tetrakis(4-carboxyphenyl)porphyrin, ZnTCPP,[15] as the reference compound with or without the axial coordination from MeIm in a phosphate buffer solution (pH 7.4). The free-base porphyrin **1** gave a normal Soret band and four Q bands. After Zn was introduced

(shown as **2**) to the free-base porphyrin, the Soret band was split by 17 nm, and the Q-bands changed to two peaks. The split Soret bands are explained by exciton coupling theory,[16] i.e., interactions of two transition dipoles (face to face and head to tail) between two porphyrin rings arising from the slipped cofacial dimer formation $\mathbf{2_2}$.

Figure 1a shows the absorption spectra of **2** at various concentrations (dilution experiments) in CH_2Cl_2. As we expected, the slipped cofacial dimer $\mathbf{2_2}$ was very stable in CH_2Cl_2. The split Soret bands remained until the lowest concentration (0.1 μM) of the absorption spectra.[17] After the addition of MeIm (Fig. 1b), the equilibrium between dimer $\mathbf{2_2}$ and monomer **2**(MeIm) was observed. Furthermore, after the addition of excess amounts of MeIm, most of the dimer $\mathbf{2_2}$ dissociated, and a new peak appeared at 427 nm (**2**(MeIm), a coordination species of MeIm as an axial ligand).

The Zn porphyrin carboxylic acid **3** was first dissolved in a saturated $NaHCO_3$ aq solution (pH 8.4). The $NaHCO_3$ aq solution was chosen in order to avoid a protonation of the imidazole moiety and to form carboxylate salts in water. As we expected, **3** was dissolved in the aqueous solution, and the Soret band of the absorption spectrum was split by 17 nm, as we observed in $\mathbf{2_2}$. The split Soret bands show the formation of a slipped cofacial dimer $\mathbf{3_2}$ in the aqueous solution. We also tested dissolving **3** (2 μM) in various buffer solutions. When **3** was dissolved in basic solutions, such as phosphate buffer solutions (pH 7.4 and 8.0) and pH 9–12 solutions, similar split Soret bands were observed.[14] At pH values higher than 12, a new peak seemed to be overlapping at 430 nm due to the coordination of OH^-. When **3** was dissolved in acidic solutions, such as a phosphate buffer solution with pH 6.8, a new peak appeared at 421 nm. At pH 6.8, dimer $\mathbf{3_2}$ seemed to dissociate (or to exist in equilibrium between the dimer and monomer) due to the protonation of imidazole (see Supplementary data). In a tris buffer with pH 8.0 and pH 7.5, the equilibria between the dimer and monomer seemed to exist, probably due to coordination of the buffer.

To dissolve **3** in a physiologically similar pH condition, the phosphate buffer with pH 7.4 was chosen as a solvent. Figure 2a shows the absorption spectra of **3** at various concentrations (dilution experiments) in a phosphate buffer solution (pH 7.4). Differently from **2** in Figure 1a, the split Soret bands of **3** appeared at 413 and 430 nm were difficult to discern at concentrations lower than 0.5 μM, and a new peak appeared at 421 nm. This result indicated the concentration dependence between the dimer and the monomer. The monomer seemed to be **3**(H_2O), a coordination species of H_2O as an axial ligand in the aqueous solution. At concentrations higher than 0.5 μM, the slipped cofacial dimer $\mathbf{3_2}$ predominantly existed in the solutions, but, at lower concentrations,

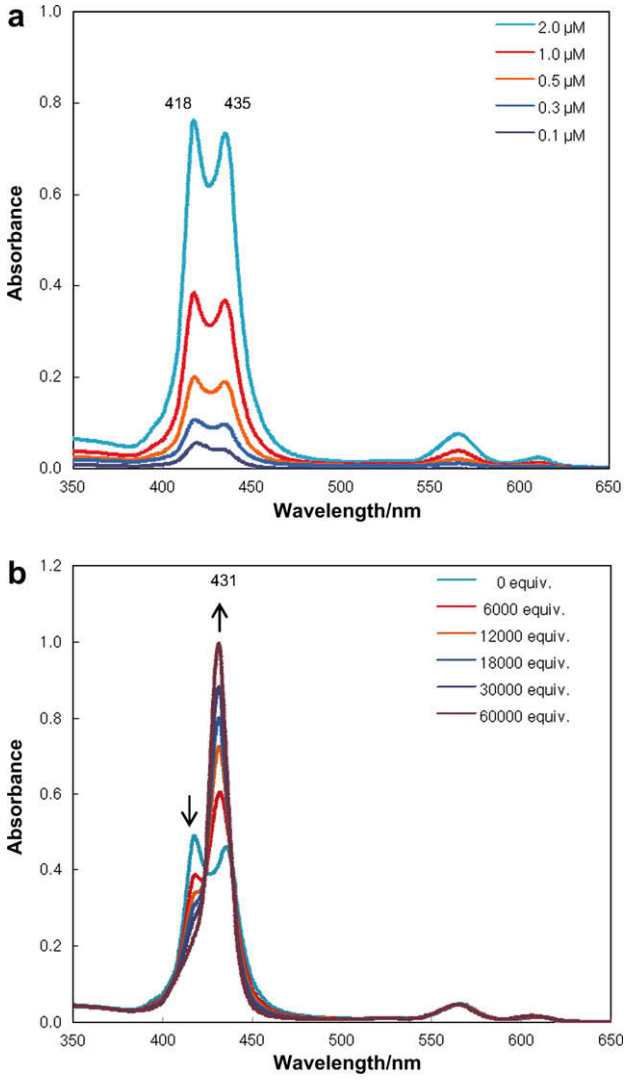

Figure 1. Absorption spectra of the Zn porphyrin **2** recorded in CH_2Cl_2, (a) at various concentrations, (b) before and after the addition of *N*-methylimidazole (MeIm).

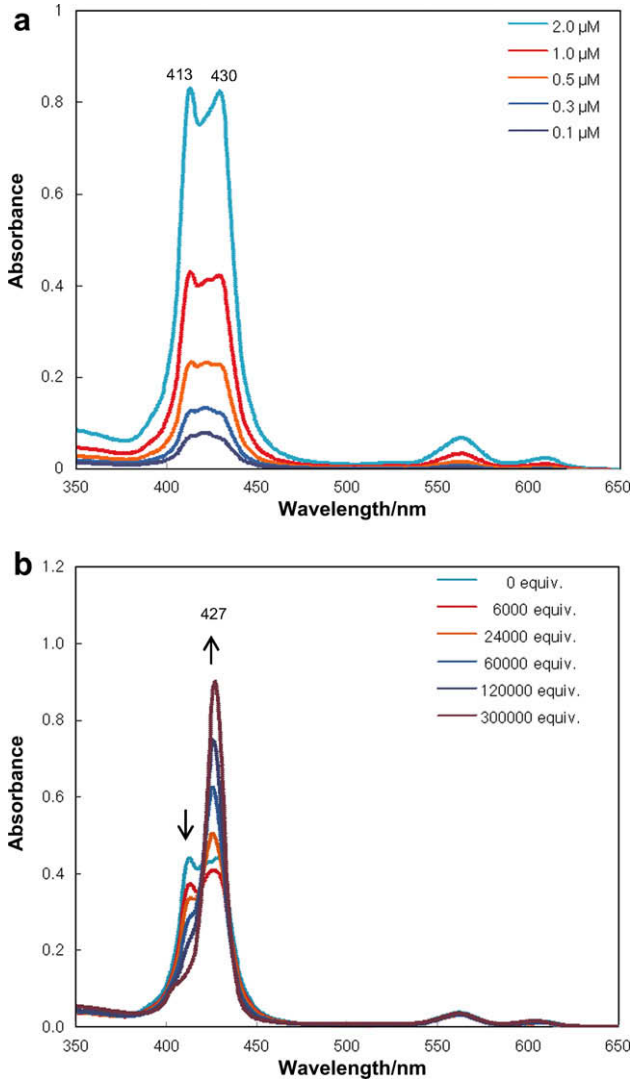

Figure 2. Absorption spectra of the Zn porphyrin **3** recorded in phosphate buffer solution (pH 7.4), (a) at various concentrations, (b) before and after the addition of *N*-methylimidazole (MeIm).

equilibria between dimer $\mathbf{3_2}$ and monomer $\mathbf{3}(H_2O)$ seemed to exist in the solutions. After the addition of MeIm (Fig. 2b), the equilibrium between the dimer $\mathbf{3_2}$ and monomer $\mathbf{3}(H_2O)$ was found to shift to the monomer, **3**(MeIm). Upon the addition of excess amounts of MeIm, most of the dimer $\mathbf{3_2}$ dissociated, and a new peak appeared at 427 nm (**3**(MeIm), a coordination species of MeIm as an axial ligand). The fluorescence emission maxima also shifted from λ_{em} = 611 and 656 nm to 612 and 664 nm upon the addition of excess amounts of MeIm, and the fluorescence intensity increased five times.[18] These results also supported the dissociation of dimer $\mathbf{3_2}$ (including $\mathbf{3}(H_2O)$) to monomer, **3**(MeIm) by the addition of excess amounts of MeIm.[19]

The association constant for dimer $\mathbf{3_2}$ in a phosphate buffer solution (pH 7.4) was estimated by the reported method.[8] First, the association constant K_a for the control compound, ZnTCPP with MeIm in a phosphate buffer solution (pH 7.4), was determined by a UV–vis. titration experiment ($K_a = 1.1 \times 10^2\ M^{-1}$). Second, the association constant K_b for **3**(MeIm) was determined from the titration of dimer $\mathbf{3_2}$ in a phosphate buffer solution (pH 7.4) with MeIm ($K_b = 1.9\ M^{-1}$). The association constant K_c ($=K_a^2/K_b$) for dimer $\mathbf{3_2}$ in a phosphate buffer solution (pH 7.4) was obtained as $6.4 \times 10^3\ M^{-1}$. This value was 58 times larger than K_a.[20] Dimer $\mathbf{3_2}$ seemed to be stabilized by cooperative coordination and π-stacking. The pH dependence of the association constant for dimer $\mathbf{3_2}$ was evaluated in phosphate buffer solutions with pH 8.0 and 9.0. The association constants K_c for dimer $\mathbf{3_2}$ in phosphate buffer solutions with pH 8.0 and 9.0 were obtained as $4.0 \times 10^3\ M^{-1}$ and $3.0 \times 10^3\ M^{-1}$, respectively. At pH values higher than pH 7.4, the association constants for dimer $\mathbf{3_2}$ were found to decrease due to the influence of OH^-.

In conclusion, tris(4-carboxylphenyl)-mono(*N*-methylimidazolyl)-substituted Zn porphyrin was synthesized. Zn porphyrin was dissolved in a $NaHCO_3$ aq solution (pH 8.4) and phosphate buffer solutions (pH 7.4–9.0). Though equilibria between the dimer and the monomer were observed at the μM range in such aqueous solutions,[21] the splitting of the Soret bands of Zn porphyrin clearly showed self-organization to a special-pair type slipped cofacial dimer even in water. Further applications using the Zn porphyrin and other central metals (e.g., Mn, Fe, Co) of porphyrin will be reported elsewhere.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2010.03.115.

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13. Spectroscopic-grade dichloromethane (containing amylene as a stabilizer) purchased from Aldrich was used instead of chloroform because chloroform contains small amounts of acid and methanol as a stabilizer.
14. Phosphate buffer solutions (pH 7.4 and pH 8.0) were purchased from TCI. These buffer solutions consisted of KH_2PO_4–NaOH. Solutions with pH 9.0 and higher were prepared by the addition of NaOH into the pH 8.0 phosphate buffer solution.
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17. Further dilution experiments until the detection limit of the fluorescence (10^{-9} M) also supported the stability of dimer $\mathbf{2_2}$ in CH_2Cl_2.
18. Control experiments using ZnTCPP also supported the formation of dimer $\mathbf{3_2}$ in a phosphate buffer solution (pH 7.4). Although red shifts of the absorption and fluorescence maxima (Table 1) were observed by the coordination of MeIm, no increase in the fluorescence intensity was observed in the case of ZnTCPP(MeIm). The excitation wavelength (λ_{ex}) was set to the isosbestic point of the Soret bands of the absorption spectra. Self-quenching of the fluorescence seemed to occur in the case of dimer $\mathbf{3_2}$ in a phosphate buffer solution (pH 7.4). See Supplementary data S-2 and S-3.
19. Excess amounts (more than 300,000 equiv) of MeIm are required to dissociate dimer $\mathbf{3_2}$; this result also supported the stability of dimer $\mathbf{3_2}$ in water due to cooperative coordination and π-stacking.
20. The association constant $K_{c'}$ for dimer $\mathbf{2_2}$ in CH_2Cl_2 was determined in the same way ($K_{c'} = K_{a'}^2/K_{b'} = 2.1 \times 10^{10}\ M^{-1}$). $K_{a'}$ was obtained as $5.2 \times 10^5\ M^{-1}$ from the titration of Zn tetrakis(4-methoxycarbonylphenyl)porphyrin in CH_2Cl_2 with MeIm. $K_{b'}$ was obtained as $13\ M^{-1}$.
21. A similar equilibrium between dimer $\mathbf{3_2}$ and monomer $\mathbf{3}(H_2O)$ was observed in the case of dilution experiments in a $NaHCO_3$ aq solution (pH 8.4). See Supplementary data S-4 and S-5.