Articles

Conformation Changes of Dimeric Zinc(II) Porphyrins Induced by Binding of Bidentate Ligands

Yoshio Uemori, Akiko Nakatsubo, Hiroyasu Imai, Shigeo Nakagawa, and Eishin Kyuno*

School of Pharmacy, Hokuriku University, 3-Ho, Kanagawa machi, Kanazawa, Japan

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Three zinc(II) porphyrin dimers have been prepared and characterized by ^{1}H NMR spectroscopy. Their conformations in solution were evaluated on the basis of a calculation of the ring current shifts. The conformation of Zn_2-**1**, in which the linking groups between two *meso*-tetrakis(2-aminophenyl)porphyrins are propanediamido groups, was found to be a "screwed down" conformation in $CDCl_3$. The ligand binding properties of the Zn(II) porphyrins were analyzed by UV–vis and NMR spectroscopy. The ligands employed were 4,4′-bipyridyl (bpy), 1,2-bis(4-pyridyl)-ethane (bpyEt), and 1,3-bis(4-pyridyl)propane (bpyPr). The formation constants and the thermodynamic parameters for the ligand adducts were calculated and compared with those for the corresponding monomers. The formation constant for the bpy adducts of Zn_2-**1** was small compared with that of Zn_2-**2**, in which the linking groups between two *meso*-tetrakis(2-aminophenyl)porphyrins were phenylenediamido groups. The small formation constant for the bpy adduct of Zn_2-**1** can be ascribed to the free energy costs for breaking the screwed-down conformation of Zn_2-**1**. On the other hand, Zn_2-**2** has a complementary structure for bpy binding and binds bpy with small conformational changes.

Introduction

It is well-known that enzymes having more than one interacting site for substrate binding can recognize a suitable substrate among others. Such a recognition mechanism of enzymes for substrates is an important function in biological systems. The recognition mechanism between dioxygen and carbon monoxide binding to hemoproteins has been studied extensively. Many investigations[1] using varieties of iron(II) porphyrins have been performed to elucidate the structure–function relationships between O_2 and CO affinities and the superstructures near O_2 and CO binding sites. Also, porphyrin derivatives have been employed as model systems for the recognition of molecules.[2-4]

In order to address the recognition mechanism in enzymes, we have prepared dimeric Zn(II) porphyrins as a model system for an enzyme which offers one binding site for a substrate having two interactive groups. The ligand adducts for the Zn(II) porphyrins with bidentate ligands were analyzed using UV–vis and NMR spectroscopies. In comparison of the formation constants, thermodynamic parameters, and the conformations of the monodentate ligand adducts with those of the bidentate ligand adducts, the effects of size of the binding pocket on the formation of the bidentate ligand adducts are discussed herein.

The binding of ligands to Zn(II) porphyrins occurs at both sides of the porphyrin planes, so the analysis for the binding of ligands should be complicated in dimeric Zn(II) porphyrins. Thus, a heptamethylene chain was strapped over one side of the porphyrin planes to prevent ligand binding. The linking groups connecting two porphyrins were propanediamido in Zn_2-**1** and phenylenediamido in Zn_2-**2** as shown in Figure 1. Because the effects of the superstructures near the ligand binding sites on the ligand binding are significant,[5,6] reference monomers with superstructures similar to those of the dimers have been prepared (Figure 1).

Experimental Section

Measurements. UV–vis spectra were recorded on a Hitachi 340 spectrophotometer. Equilibrium constants for the ligand bindings to the Zn(II) complexes (ca. 5 μM) in $CHCl_3$ were determined by spectrophotometric titration as described previously.[6] Proton NMR spectra were recorded on a JEOL GSX-400 spectrometer. The concentrations of Zn(II) complexes were ca. 0.5 mM. Variable-temperature NMR spectra were obtained on a JEOL GSX-400 spectrometer with the use of a standard JEOL constant-temperature controller. Each NMR sample was allowed to equilibrate for 15 min in the spectrometer before the collection of data.

For the calculation of K_B, the following equations were used.

(1) Monodentate ligand (L) binding to Zn(II) porphyrin monomer {Zn(P)}:

$$\mathrm{Zn(P)} + \mathrm{L} \overset{K_B}{\rightleftharpoons} \mathrm{Zn(P)L}$$

$$K_B = \frac{[\mathrm{Zn(P)L}]}{[\mathrm{Zn(P)}][\mathrm{L}]} \tag{1}$$

(2) Bidentate ligand (L-L) binding to Zn(II) porphyrin monomer:

$$\mathrm{Zn(P)} + \mathrm{L\text{-}L} \overset{K_B}{\rightleftharpoons} \mathrm{Zn(P)L\text{-}L}$$

$$K_B = \frac{[\mathrm{Zn(P)L\text{-}L}]}{2[\mathrm{Zn(P)}][\mathrm{L\text{-}L}]} \tag{2}$$

(3) Monodentate ligand binding to Zn(II) porphyrin dimer {Zn_2(P)}:

(1) For review, see: (a) Collman, J. P.; Halbert, T. R.; Suslick, K. S. *Metal ions in biology*; Spiro, T. G., Ed.; John Wiley & Sons, Inc.: New York, 1980; Vol. 2, p 1. (b) Traylor, T. G. *Acc. Chem. Res.* **1981**, *14*, 102. (c) David, D.; Dolphin, D.; James, B. R. *Frontiers in Bioinorganic Chemistry*; Xavier, A. V., Ed.; VCH: Weinheim, 1986; p 163. (d) Momenteau, M. *Pure Appl. Chem.* **1986**, *58*, 1493. (e) Smith, T. D.; Pilbrow, J. R. *Coord. Chem. Rev.* **1981**, *39*, 295. (f) Jameson, G. B.; Ibers, J. A. *Comments Inorg. Chem.* **1983**, *2*, 97.

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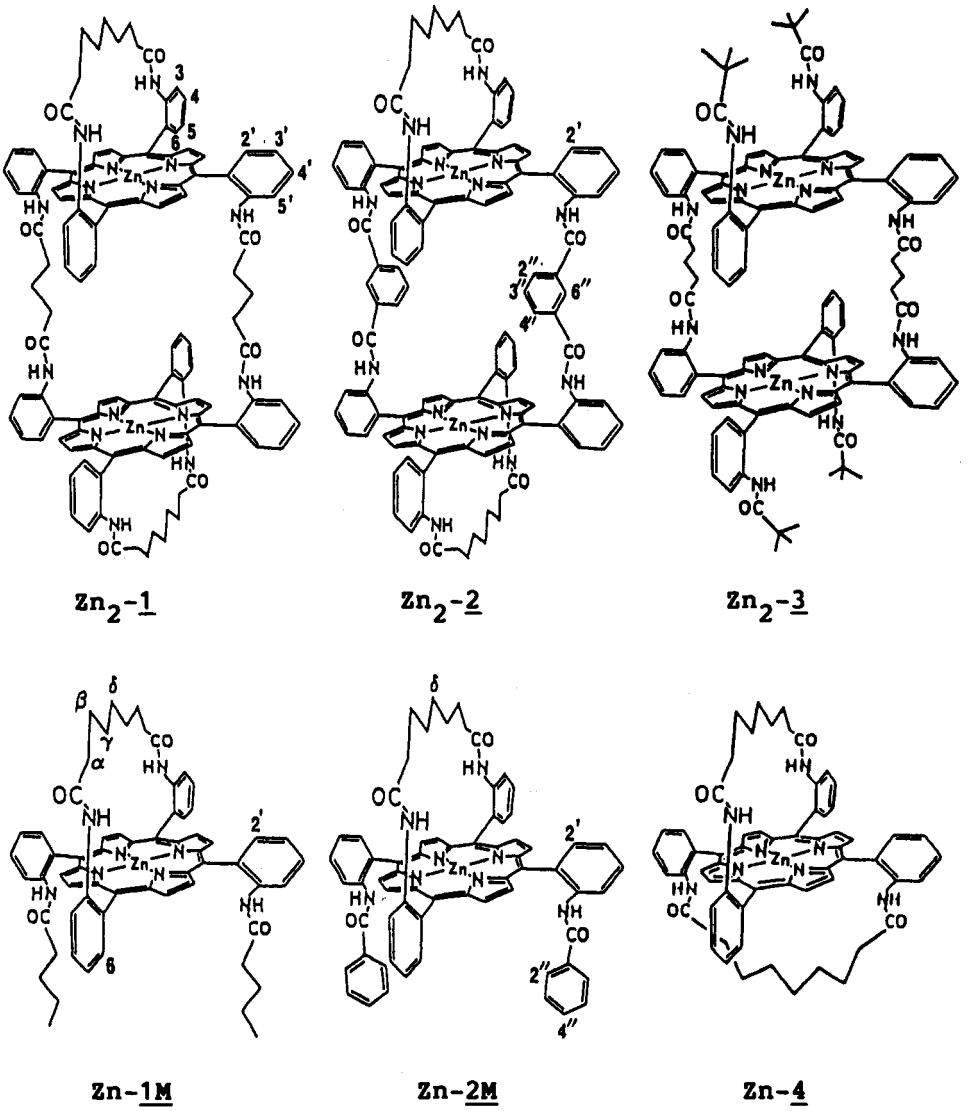

Figure 1. Zn(II) porphyrins and labeling scheme.

$$Zn_2(P) + L \overset{K1_{ap}}{\rightleftharpoons} Zn_2(P)L$$

$$Zn_2(P)L + L \overset{K2_{ap}}{\rightleftharpoons} Zn_2(P)L_2$$

$$K1_{ap} = \frac{[Zn_2(P)L]}{[Zn_2(P)][L]} \qquad K2_{ap} = \frac{[Zn_2(P)L_2]}{[Zn_2(P)L][L]}$$

The change in absorbance (ΔA) at any wavelength upon addition of the ligands is given by

$$\Delta A = [Zn_2(P)L](A1) + [Zn_2(P)L_2](A2)$$

and can be expressed by

$$\Delta A = \frac{(K1_{ap})[L](A1) + (K1_{ap})(K2_{ap})[L](A2)}{1 + (K1_{ap})[L] + (K1_{ap})(K2_{ap})[L]} \qquad (3)$$

where $A1$ is the difference of molar absorptivity between $Zn_2(P)$ and $Zn_2(P)L$ and $A2$ is the difference of molar absorptivity between $Zn_2(P)$ and $Zn_2(P)L_2$. We assumed "$A2 = A1 \times 2$". This is reasonable where the degree of excitation coupling is negligible.[7,8] The data were analyzed by using a least-squares curve-fitting program. Other equations follow.

(4) Bidentate ligand binding inside the two porphyrins in a Zn(II) porphyrin dimer:

$$Zn_2(P) + L\text{-}L \overset{K_B}{\rightleftharpoons} Zn_2(P)(L\text{-}L)$$

$$K_B = \frac{[Zn_2(P)(L\text{-}L)]}{[Zn_2(P)][L\text{-}L]} \qquad (4)$$

(5) Bidentate ligand binding outside the two porphyrins in a Zn(II) porphyrin dimer:

$$Zn_2(P) + L\text{-}L \rightleftharpoons Zn_2(P)(L\text{-}L)$$

$$Zn_2(P)(L\text{-}L) + L\text{-}L \rightleftharpoons Zn_2(P)(L\text{-}L)_2$$

$$K_B = \frac{[\sim Zn(P)L\sim]}{[\sim ZnP][L\sim]} \qquad (5)$$

where $[\sim ZnP]$ is the concentration of one porphyrin unit in the dimer, $[L\sim]$ is the concentration of one pyridyl unit in the bidentate ligand, and $[\sim Zn(P)L\sim]$ is the concentration of the ligand adduct for one porphyrin unit.

The ring current shifts were calculated using the double-loop model of Abraham et al.[9] The ring current of the porphyrin was treated as the sum of four pentagons, four hexagons, and four phenyl rings. The ring current shift ($\Delta\delta$) of a proton is given by eq 6, where Z_R ($Z_{R'}$) is the

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$$\Delta\delta = 17.1\sum_{i=1}^{8}(1 - 3(Z_R \pm 0.64)^2/r_{iR}^2)/r_{iR}^3 + 19.3\sum_{j=1}^{8}(1 - 3(Z_R \pm 0.64)^2/r_{jR}^2)/r_{jR}^3 + 27.6\sum_{k=1}^{8}(1 - 3(Z_{R'} \pm 0.64)^2/r_{kR}^2)/r_{kR}^3 \ldots \quad (6)$$

distance of a proton along the z (x or y) axis to the center of each polygon and r_{iR}, r_{jR}, and r_{kR} are the distances between a proton and the center of each polygon. The coordinates for the pyrrole and phenyl protons were calculated on the basis of the X-ray crystallographic data for ZnTPP[10] and CoTPP,[11] respectively.

Materials. All chemicals were reagent grade and were used without further purification, except as noted below. 4,4'-Bipyridyl (bpy), 1,2-Bis(4-pyridyl)ethane (bpyEt), 1,3-bis(4-pyridyl)propane (bpyPr), and 4-phenylpyridine (phpy) were recrystallized from benzene/hexane. For the measurements of UV–vis spectra, chloroform (stabilized with amylene, HPLC grade) was used without purification. Pyridine (py) was vacuum distilled from KOH. Silica gel (Wakogel C-200) was used for column chromatography. Zn-**1M**, H_4-**1**, H_4-**3**, and H_2-**4** were prepared by the method reported previously.[6,12]

H_2-2M. A CH_2Cl_2 solution (100 mL) containing 90 mg (0.4 mmol) of 5β,15β-bis(2-aminophenyl)-10α,20α-(((nonanediyldicarbonyl)(diamino)di-*o*-phenylene)porphyrin (H_2-Azam$\beta\beta$)[12] was treated with pyridine (0.5 mL) and benzoyl chloride (0.3 mL, 2.6 mmol)) in an ice bath. The solution was stirred for 1 h, and then 10% aqueous ammonia (100 mL) was added and the solution stirred for 0.5 h. The organic layer was separated and stripped to dryness. The resultant solid was dissolved in $CHCl_3$ and chromatographed on a silica gel column ($CHCl_3$, 3 × 20 cm). The column was eluted with 1:1 $CHCl_3$/ether. The product was recrystallized from benzene/hexane. The yield was 92 mg (72%). Anal. Calcd for $C_{67}H_{56}N_8O_4\cdot 2H_2O$: C, 76.41; H, 5.36; N, 10.64. Found: C, 76.07; H, 5.28; N, 10.63. ^{1}H NMR ($CDCl_3$): δ = –2.56 (2 H, s, pyrrole NH), –2.53 (2 H, m, δ-CH_2), –1.26 (4 H, m, β-CH_2), –0.52 (4 H, m, γ-CH_2), 1.20 (4 H, m, α-CH_2), 5.97 (2 H, s, amide H), 6.50 {4 H, t, H(3″) and H(5″)}, 6.57 {4 H, d, H(2″) and H(6″)}, 6.83 {2 H, t, H(4″)}, 7.51 (2 H, s, amide H), 7.63 {2 H, t, H(3′)}, 7.65 {2 H, t, H(4)}, 7.85 {2 H, t, H(5)}, 7.91 {2 H, t, H(4′)}, 8.10 {2 H, d, H(2′)}, 8.32 {2 H, d, H(6)}, 8.36 {2 H, d, H(3)}, 8.85 (4 H, d, pyrrole), 8.88 {2 H, d, H(5′)}, 8.93 (4 H, d, pyrrole).

H_4-2. This porphyrin was prepared form H_2-Azam$\beta\beta$ (200 mg, 0.8 mmol) and isophthaloyl dichloride (49 mg, 0.8 mmol) in the same manner as described above for H_2-**2M**, in a yield of 15%. Anal. Calcd for $C_{124}H_{96}N_{16}O_8\cdot 2CH_2Cl_2$: C, 71.46; H, 4.84; N, 10.75. Found: C, 71.30; H, 4.75; N, 10.55. ^{1}H NMR ($CDCl_3$): δ = –3.08 (4 H, s, pyrrole NH), –2.67 (4 H, m, δ-CH_2), –1.38 (8 H, m, β-CH_2), –0.62 (8 H, q, γ-CH_2), 1.12 (8 H, t, α-CH_2), 5.51 {2 H, t, H(3″)}, 5.73 {4 H, d, H(2″) and H(4″)}, 5.79 (4 H, s, amide H), 7.36 {4 H, t, H(5)}, 7.58 (4 H, s, amide H), 7.61 {2 H, s, H(6″)}, 7.67 {4 H, t, H(3′)}, 7.82 {4 H, t, H(4)}, 7.79 {4 H, t, H(4′)}, 8.03 {4 H, d, H(6)}, 8.05 {4 H, d, H(2′)}, 8.26 {4 H, d, H(3)}, 8.68 {4 H, d, H(5′)}, 8.73 (8 H, d, pyrrole), 8.84 (8 H, d, pyrrole).

Zn_2-1. The methanol solution (400 mL) containing H_4-**1** (40 mg, 0.02 mmol) and $Zn(CH_3COO)_2\cdot 2H_2O$ (40 mg, 0.18 mmol) was heated at 50 °C for 15 min. The solution was stripped to dryness, the residue was dissolved in $CHCl_3$, and the solution was washed three times with 10% aqueous ammonia and with water. The organic layer was separated and dried with Na_2SO_4. The solution was stripped to dryness, and the residue was crystallized from hot $CHCl_3$/hexane. The yield was quantitative. Anal. Calcd for $Zn_2C_{116}H_{96}N_{16}O_8$: C, 70.62; H, 4.90; N, 11.36. Found: C, 71.26; H, 5.08; N, 11.53. ^{1}H NMR data are shown in Table I. UV/vis [λ_{max} ($CHCl_3$)]: 400 (sh), 421, 434, 518, 556, 593, 625 nm.

Zn_2-2. The acetic acid solution (100 mL) containing H_4-**2** (40 mg, 0.019 mmol) and $Zn(CH_3COO)_2\cdot 2H_2O$ (40 mg, 0.18 mmol) was heated at 50 °C for 10 min. The solution was stripped to dryness, the residue was dissolved in $CHCl_3$, and the solution was washed three times with 10% aqueous ammonia and with water. The organic layer was chromatographed on a silica gel column ($CHCl_3$, 1 × 20 cm). The column was eluted with 95:5 $CHCl_3$/MeOH. The product was recrystallized from $CHCl_3$/hexane. The yield was 33 mg (80%). Anal. Calcd for $Zn_2C_{122}H_{92}N_{16}O_8\cdot CHCl_3\cdot H_2O$: C, 67.82; H, 4.88; N, 10.29. Found: C, 67.55; H, 4.39; N, 10.53. ^{1}H NMR data are given in Table VII. UV/vis [λ_{max} ($CHCl_3$)]: 419, 430 (sh), 515, 550, 584 nm.

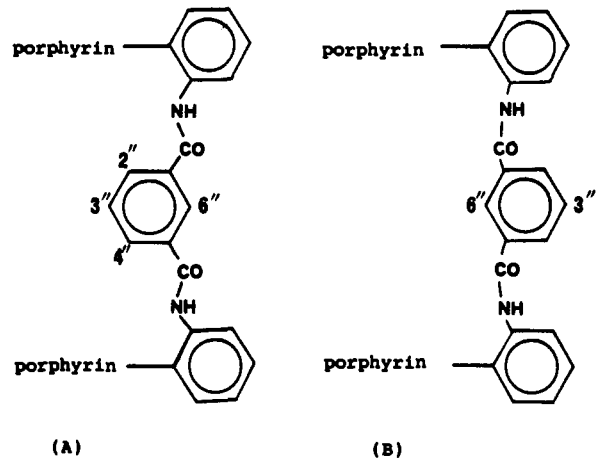

Figure 2. Two possible conformations for the phenyl groups linking two porphyrins in H_4-**2**.

The following three Zn(II) porphyrins were prepared from porphyrins and $Zn(CH_3COO)_2\cdot 2H_2O$ in the same manner as described above for Zn_2-**2**.

Zn-2M. Anal. Calcd for $ZnC_{67}H_{54}N_8O_4\cdot H_2O$: C, 71.94; H, 5.05; N, 10.02. Found: C, 71.98; H, 5.05; N, 9.96. ^{1}H NMR ($CDCl_3$): δ = –2.64 (2 H, m, δ-CH_2), –1.36 (4 H, m, β-CH_2), –0.54 (4 H, m, γ-CH_2), 1.11 (4 H, m, α-CH_2), 5.95 (2 H, s, amide H), 6.40 {8 H, m, H(2″), H(3″), H(5″), and H(6″)}, 6.74 {2 H, m, H(4″)}, 7.52 (2 H, s, amide H), 7.61 {2 H, t, H(3′)}, 7.63 {2 H, t, H(4)}, 7.82 {2 H, t, H(5)}, 7.87 {2 H, t, H(4′)}, 8.11 {2 H, d, H(6)}, 8.27 {2 H, m, H(2′)}, 8.32 {2 H, d, H(3)}, 8.76 {2 H, m, H(5′)}, 8.9 (4 H, d, pyrrole), 8.97 (4 H, m, pyrrole). UV/vis [λ_{max} ($CHCl_3$)]: 400, 422, 510, 547, 585 nm.

Zn_2-3. Anal. Calcd for $Zn_2C_{118}H_{100}N_{16}O_8\cdot 2H_2O$: C, 69.58; H, 5.15; N, 11.00. Found: C, 69.19; H, 5.15; N, 10.99. ^{1}H NMR ($CDCl_3$): δ = –1.5 (2 H, m, –CH_2–), –0.52 (4 H, m, –CH_2–), –0.25 (36 H, s, –CH_3), 0.71 (1 H, m, –CH_2–), 1.52 (1 H, m, –CH_2–), 1.89 (1 H, m, –CH_2–), 5.94 {2 H, d, H(6)}, 6.37 (4 H, s, amide H), 6.87 (4 H, s, amide H), 7.26 {2 H, m, H(3′)}, 7.54 {2 H, t, H(4)}, 7.62 {2 H, t, H(5)}, 7.64 {2 H, t, H(5)}, 7.89 {2 H, t, H(4)}, 8.17 (2 H, m, pyrrole), 8.23 {2 H, d, H(6)}, 8.39 (2 H, m, pyrrole), 8.50 {2 H, d, H(3)}, 9.03 (2 H, m, pyrrole). The signals at ca. 7.6, 7.8, 8.4, 8.5, 8.7, and 9.0 ppm were not assigned. UV/vis [λ_{max} ($CHCl_3$)]: 400 (sh), 421, 435, 519, 556, 595 nm.

Zn-4. Anal. Calcd for $ZnC_{62}H_{58}N_8O_4\cdot H_2O$: C, 70.21; H, 5.51; N, 10.57. Found: C, 70.12; H, 5.37; N, 10.52. ^{1}H NMR ($CDCl_3$): δ = –2.41 (2 H, m, δ-CH_2), –1.18 (4 H, m, β-CH_2), –0.46 (4 H, m, γ-CH_2), 1.19 (4 H, m, α-CH_2), 5.98 (4 H, s, amide H), 7.70 {4 H, t, H(4)}, 7.87 {4 H, t, H(5)}, 8.34 {4 H, d, H(6)}, 8.41 {4 H, d, H(3)}, 8.98 (8 H, s, pyrrole). UV/vis [λ_{max} ($CHCl_3$)]: 400, 421, 510, 546, 582 nm.

Results and Discussion

Conformation of Free Base Porphyrins. The conformation of H_4-**2** was characterized by its ^{1}H NMR spectrum. For the conformation of the phenylenediamido groups linking two porphyrins in H_4-**2**, there are two possible conformations as shown in Figure 2. In the case of conformation A in Figure 2, the ring current shift for H(3″) is estimated to be greater than that for H(6″), while that for H(3″) is estimated to be smaller than that for H(6″) in the conformation B. The signals of the phenylenediamido groups in H_4-**2** appeared at 5.51 {triplet, H(3″)}, 5.74 {doublet, H(2″) and H(4″)}, and 7.61 ppm {singlet, H(6″)}, while the protons in isophthalic acid appeared at 7.61 (triplet), 8.25 (doublet), and 8.7 (singlet). The ring current shifts for the phenylenediamido groups in H_4-**2** were estimated by subtracting the chemical shift values for each proton in isophthalic acid from those for the corresponding protons in H_4-**2** and are –2.1, –2.51, and –1.1 ppm for H(3″), H(2″), H(4″), and H(6″), respectively. Because the ring current shift for H(3″) is greater than that for H(6″), the conformation of the phenylenediamido groups in H_4-**1** is concluded to be (A) in Figure 2.

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(11) Madura, P.; Scheidt, W. R. *Inorg. Chem.* **1976**, *15*, 3182.
(12) Uemori, Y.; Nakatsubo, A.; Imai, H.; Nakagawa, S.; Kyuno, E. *Inorg. Chim. Acta* **1986**, *124*, 153.

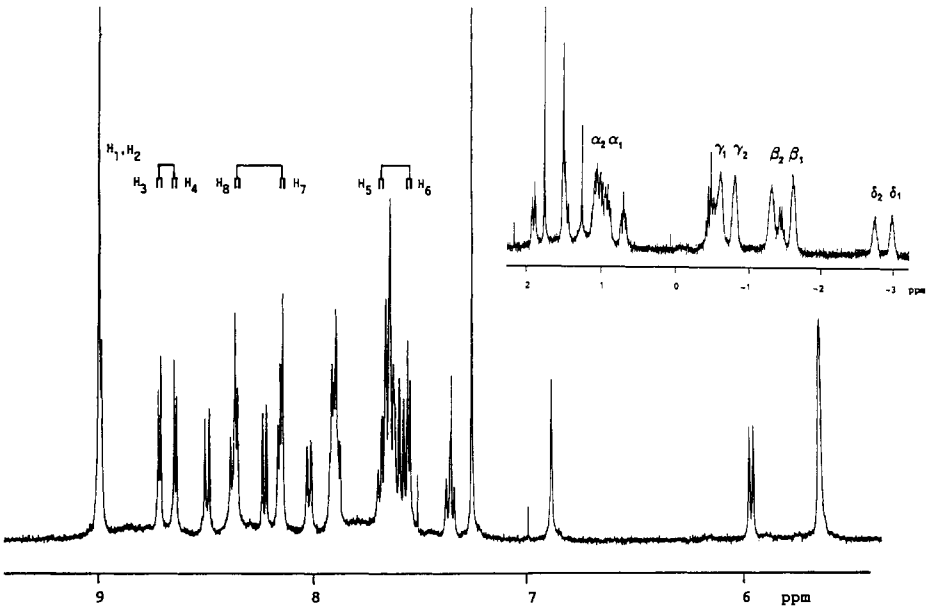

Figure 3. ^{1}H NMR spectrum of Zn_2-**1** in $CDCl_3$.

Table I. ^{1}H NMR Data for Zn(II) Porphyrins[a]

	Zn_2-**1**	Zn_2-**1** (bpy)		Zn_2-**1**	Zn_2-**1** (bpy)
δ	−2.98, −2.73	−2.49	3′	7.36, 7.90	7.74
γ	−0.60, −0.81	−0.51	4′	7.67, 7.67	7.69
β	−1.62, −1.32	−1.18	5′	8.03, 8.38	8.38
α	+1.04, +0.90	+1.08	pyrrole	8.91	8.75, 8.64
amide	5.64	5.82		8.72, 8.65	
	6.89, 8.98	5.42		8.38, 8.16	
3	8.49, 7.91	8.30		7.68, 7.58	
4	7.67, 7.60	7.74	linking[c]	−1.47, −0.47	−0.80
5	7.90, 7.69	7.69		+0.69, +1.43	+1.0
6	8.24, 5.97	8.21		+1.90	
2′	*b*	8.11	ligand		2.17, 4.84

[a] Chemical shifts (δ) in $CDCl_3$ at 24 °C. For the labeling system, see Figure 1. [b] Signals were not assigned. [c] Protons in the linking groups.

The signal for the pyrrole protons in H_4-**2** appeared at −3.08 ppm and shifted to high field by 0.52 ppm compared with that of H_2-**2M**. The high-field shift is a characteristic feature in dimeric porphyrins in a face-to-face conformation in which two porphyrin rings lie parallel to each other. The shift of 0.52 ppm agreed with the calculated ring current shift (0.52 ppm) assuming that the distance between the two porphyrin planes is 9.2 Å, although a CPK model experiment indicated that the distance is ca. 12 Å. Therefore, the ^{1}H NMR results suggest that the distances between the two porphyrin planes in H_4-**2** are shorter than that in the CPK experiment. Similar results[12] have been observed for both H_4-**1** and H_4-**3**: the signals for pyrrole N–H in both H_4-**1** and H_4-**3** shifted to high field by ca. 0.4 ppm relative to the corresponding monomers. The shifts were comparable to the calculated ring current shift (0.41 ppm) assuming that the distance between the two porphyrin planes is 10 Å, although a CPK model experiment indicated that the distance is ca. 12 Å. Therefore, the two porphyrins in the dimers were found to lie close to each other in solution.

Conformation of Zinc(II) Porphyrins. Figure 3 shows the ^{1}H NMR spectrum of Zn_2-**1** in $CDCl_3$. The proton signals of the heptamethylene chains were split into two sets of signals, and four sets of signals appeared for the pyrrole and phenyl groups as shown in Table I. Furthermore, the proton signals of the propane chains linking two porphyrins shifted to high field relative to those for H_4-**1**. Contrary to this, only one set of signals was observed for the protons in the heptamethylene chains and pyrrole and phenyl groups in H_4-**1** in the temperature range from −60 to 23 °C in $CDCl_3$. From decoupling experiments, it was found

Table II. Experimental and Calculated Δδ Values of Zn_2-**1** in $CDCl_3$[a]

proton[b]	$\Delta\delta_{exptl}$[c]	$\Delta\delta_{calcd}$[d]	proton[b]	$\Delta\delta_{exptl}$[c]	$\Delta\delta_{calcd}$[d]
1	0.09	0.08	5	−1.23	−1.14
2	0.09	0.12	6	−1.33	−1.45
3	−0.19	−0.14	7	−0.75	−0.74
4	−0.26	−0.15	8	−0.53	−0.45

[a] Displacement coordinates, 2.8, −0.8, and 5.4 Å, rotated 36°. [b] For numbering system, see Figure 4. [c] $\Delta\delta_{exptl}$ = δ(proton in Zn_2-**1**) − δ(proton in Zn-**1M**). [d] The root-mean-square error is 0.07 ppm.

that two protons bound to each carbon atom of the heptamethylene chain in Zn_2-**1** appear at two signals. This should be the explanation for the movements of the heptamethylene chains being slow relative to the NMR time scale upon formation of the Zn(II) complexes.[5,13,14] On the other hand, the splitting of the signals for the pyrrole and phenyl groups in Zn_2-**1** implies that the four pyrroles and four phenyl groups each have different magnetic environments.

To evaluate the conformation for Zn_2-**1**, the ring current shifts were calculated using eq 6 with the assumption that the four sets of pyrrole proton signals are split by the ring current of the porphyrin. In the calculation of eq 6, two porphyrin planes are assumed to be in parallel with each other and the four meso-phenyl rings are assumed to lie perpendicular to the porphyrin plane. The chemical shift value (8.91 ppm) of the pyrrole proton for Zn-**1M** was used as the reference value in the absence of the ring current shift due to another porphyrin ring. The procedure for the changing of the conformation of the two porphyrin planes was as follows: the porphyrin plane containing the pyrrole protons rotates θ degree around Zn as a center (the *z* axis); then the center of the porphyrin was translated to (*x*, *y*, *z*) with two porphyrin planes parallel to each other. Computer simulations using a simplex routine were carried out to minimize the difference between the observed and calculated ring current shifts; the results are listed in Table II.

The calculated conformation for Zn_2-**1** is that the two Zn(II) porphyrin rings are screwed down as shown in Figure 4. The signal for H(6) of the phenyl groups linked by the heptamethylene chain in Zn_2-**1** shifted to high field by 2.3 ppm relative to that in Zn-**1M**. In the conformation of Figure 4, the ring current shift

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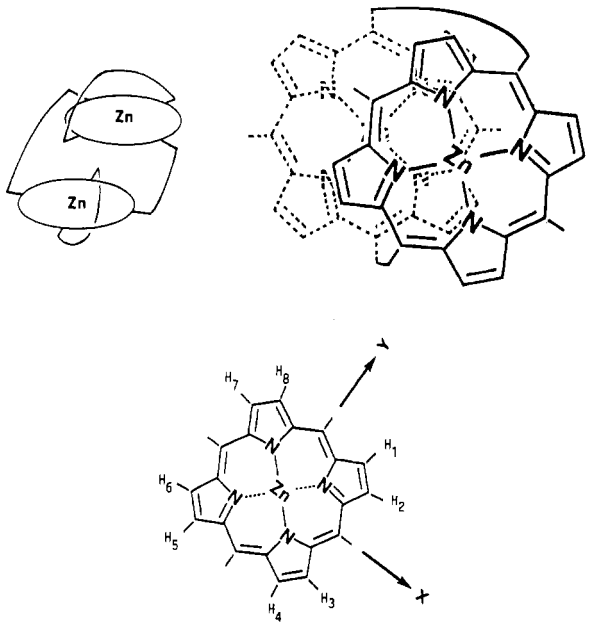

Figure 4. Speculated conformation of Zn_2-**1** in $CDCl_3$.

Table III. ^{13}C NMR Data for Zn(II) Porphyrins[a]

	Zn_2-**1**	Zn_2-**1** + D_2O		Zn_2-**1**	Zn_2-**1** + D_2O
C3 chain[b]	17.87	18.20	α	36.72, 36.61	36.65
β	24.23, 24.23	24.29	amide[c]	168.53	168.77
γ	26.40	26.40		170.03	170.25
δ	26.87	26.90		170.28	
C3 chain[b]	29.32	29.02		171.48	171.83
C3 chain[b]	34.33	34.96			

[a] Chemical shifts (δ) in $CDCl_3$ at 24 °C. For the labeling system, see Figure 1. [b] Carbons in $-(CH_2)_3-$. [c] Carbons in –NHCO–.

Table IV. Experimental and Calculated $\Delta\delta$ Values of Zn_2-**1**$(D_2O)_2$ in $CDCl_3$[a]

proton[b]	$\Delta\delta_{exptl}$[c]	$\Delta\delta_{calcd}$[d]	proton[b]	$\Delta\delta_{exptl}$[c]	$\Delta\delta_{calcd}$[d]
1	–0.08	–0.02	5	–0.91	–0.84
2	–0.11	–0.03	6	–0.36	–0.37
3	–0.36	–0.35	7	–0.11	0.05
4	–0.91	–0.86	8	–0.08	0.03

[a] Displacement coordinates, 3.1, 4.8, and 6.5 Å, rotated 34°. [b] For numbering system, see Figure 4. [c] $\Delta\delta_{exptl} = \delta$(proton in Zn_2-**1**$(D_2O)_2$) – δ(proton in Zn-**1M**). [d] The root-mean-square error is 0.08 ppm.

for H(6) was estimated to be –3.62 ppm. If the phenyl groups rotate 30° clockwise on the bond between the meso-carbon and the phenyl group, the calculated shift (–2.13 ppm) agrees with the observed shift (–2.3 ppm). Because the changes in the ring current shift due to the rotation of the phenyl rings were small, the ring current shifts for the pyrrole protons were not recalculated.

On addition of D_2O to Zn_2-**1** in $CDCl_3$, the pyrrole proton signals appeared at 8.83, 8.80, 8.55, and 8.0 ppm and the signal for H(6′) in the phenyl groups linked by the heptamethylene chain appeared at 6.10 ppm. For comparison of the ^{13}C NMR spectrum for Zn_2-**1** and that after addition of D_2O, major changes in the chemical shifts values were observed for the propanediamido groups linking two porphyrins as shown in Table III. These spectral changes are due to the binding of D_2O to the complexes; therefore, D_2O should bind inside of Zn_2-**1**. The ring current shifts for the pyrrole proton signals for the D_2O adduct of Zn_2-**1** were calculated, and the conformation was evaluated as in Zn_2-**1**. The results are listed in Table IV; the calculated shifts agreed with the observed shifts when the porphyrin is translated (3.1, 4.8, 6.5 Å) and rotated 34°. Furthermore, the ring current shifts for H(6) were calculated assuming that the phenyl groups rotate 40° counterclockwise. The calculated shift (–2.17 ppm) agreed well with the observed shift (–2.17 ppm = 6.10 – 8.37 ppm). The separation of the two porphyrin planes in the D_2O adduct of Zn_2-**1** is more distant than in Zn_2-**1**; however, both complexes are concluded to be in a similar screwed-down conformation.

Zn_2-**3** was prepared to determine the contribution of the heptamethylene chain to the screwed-down conformation in Zn_2-**1**. Figure 5 shows the low-field part of the ^1H NMR spectrum for Zn_2-**3**. The calculation of the ring current shifts for the pyrrole proton signals could not be performed, because the pyrrole proton signals were not well resolved. Nevertheless, among the four sets of the phenyl signals in Zn_2-**3**, two sets of the signals for the phenyl groups could be assigned. As in the case of Zn_2-**1**, the signal for H(6) resonated at 5.94 ppm, suggesting that Zn_2-**3** has a conformation similar to that of Zn_2-**1**. Upon addition of D_2O to Zn_2-**3**, the signal of H(6) at 5.94 ppm shifted to 6.09 ppm, and the signals for the pyrrole protons appeared at 8.84, 8.81, 8.51, and 7.98 ppm. Within experimental error, the chemical shift values for the signals were in agreement with those in the D_2O adduct of Zn_2-**1**. Therefore, the conformation for the D_2O adduct of Zn_2-**3** was concluded to be identical with that of Zn_2-**1**, and the heptamethylene chains did not appear responsible for the screwed-down conformation.

The ^1H NMR spectrum for Zn_2-**2** was less resolved than that for the free base porphyrin (H_4-**2**); however, the chemical shift values for the each proton in Zn_2-**2** were essentially identical with those in H_4-**2**. Thus, the conformation of Zn_2-**2** was concluded to be similar to that of H_4-**2**. Furthermore, the ^1H NMR spectrum for Zn-**2M** was essentially identical with those for H_2-**2M**, except for the line broadening of the signals of both the phenylenediamido groups and the meso-phenyl groups appending the phenylamido groups. Thus, the conformation of Zn-**2M** should be identical to that of H_2-**2M**.

Porphyrins tend to aggregate in solution,[15-18] and Hunter and Sanders[19] have realized the aggregated conformation of two porphryins in terms of π–π interaction. The magnitude of the π–π interaction between two porphyrins is enhanced by metalation.[19] According to this, the screwed-down conformation of the complexes may be due to the π–π interaction, because Zn_2-**1** and Zn_2-**3** assume a screwed-down conformation upon the formation of Zn(II) complexes. Of the three Zn(II) dimeric porphyrin complexes, both Zn_2-**1** and Zn_2-**3** have screwed-down conformations, while Zn_2-**2** does not. This may be responsible for the observation that the propanediamido chains linking the two porphyrins in both Zn_2-**1** and Zn_2-**3** are more flexible than the phenylenediamido groups in Zn_2-**2**.

Ligand Binding to Zn_2-1. Because the binding of ligands to Zn(II) porphyrins occurs at both sides of the porphyrin, analyses of the ligand binding become more complicated in the Zn(II) porphyrin dimers. Thus, a heptamethylene chain was strapped over one side of the porphyrin to prevent the binding of ligands to that side in both Zn_2-**1** and Zn_2-**2**. It has been established that the binding of ligands to the metalloporphyrin is reduced to $^1/_{260}$ by the strapped heptamethylene chain.[5,12] In this study, the formation constants (K_B) for the ligand adducts were measured for the Zn(II) porphyrins except for Zn_2-**3**.

The UV–vis spectral changes upon addition of bpy to $CHCl_3$ solution of Zn_2-**1** showed isosbestic points. This indicates that only one type of bpy adduct is formed. Figure 6 shows the ^1H NMR spectrum of Zn_2-**1** with 1 equiv of bpy; a further change in the signals for Zn_2-**1**(bpy) was not observed upon addition of excess bpy up to 50 equiv. The bound bpy signals appeared at

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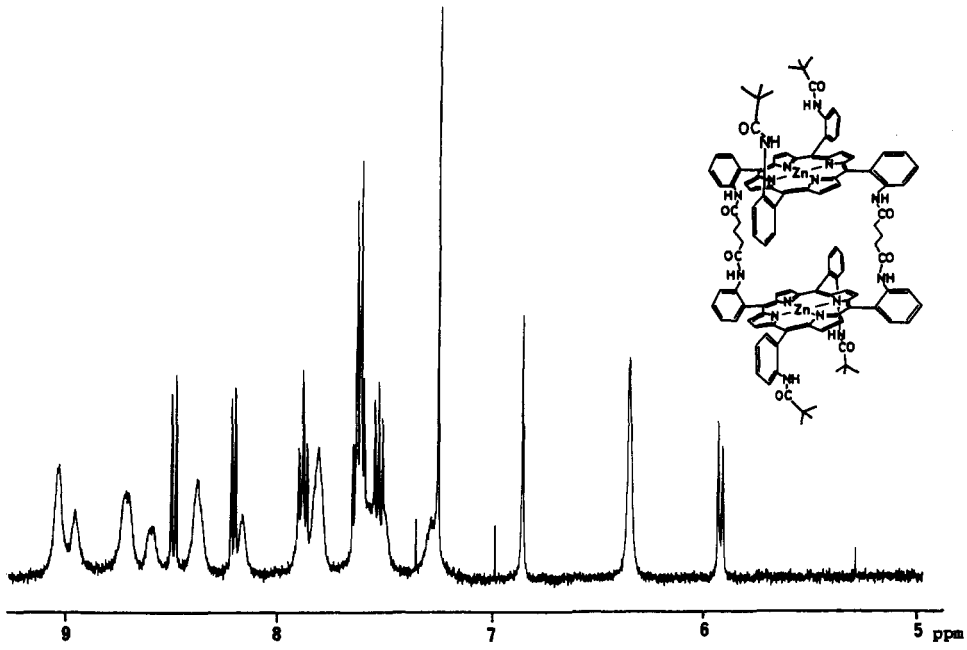

Figure 5. ^{1}H NMR spectrum (lower part) of Zn_2-**3** in $CDCl_3$.

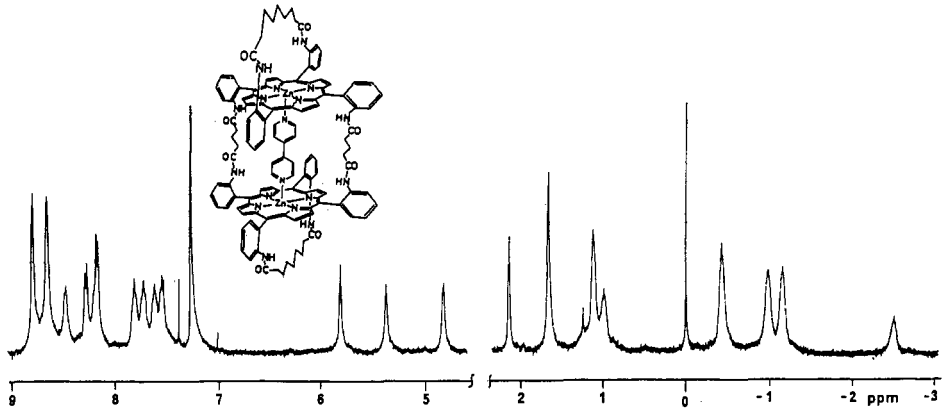

Figure 6. ^{1}H NMR spectrum of Zn_2-**1** with 1 equiv of bpy at −20 °C in $CDCl_3$.

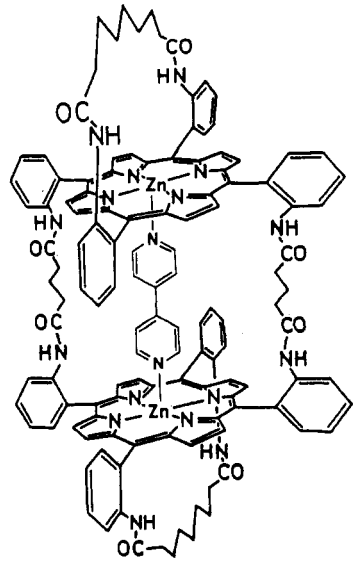

Figure 7. Speculated conformation for Zn_2-**1**(bpy) in $CDCl_3$.

2.17 and 4.84 ppm. The ring current shifts for the bound bpy signals were calculated using eq 6. The calculated $\Delta\delta$ values agreed well with the observed values assuming that bpy binds inside Zn_2-**1** (Figure 7, Table V). From these results, the K_B value was calculated using eq 4 and is given in Table VI.

Table V. Experimental and Calculated $\Delta\delta$ Values for the Protons of 4,4′-Bipyridine in Zn_2-**1**(bpy)[a]

	$\Delta\delta_{calcd}$[b]				
proton[b]	porphyrin A	porphyrin B	tot.	rotationally avgd	$\Delta\delta_{exptl}$[c]
2, 6	−6.29	−0.45	−6.74	−6.16	−6.59
2′, 6′	−5.11	−0.46	−5.57		
3, 5	−1.72	−0.93	−2.65	−2.73	−2.71
3′, 5′	−1.76	−0.94	−2.80		

[a] Coordination geometry is the same with the case of Zn_2-**1**. The bond length of Zn–N(bpy) and the displacement of Zn from the porphyrin plane are assumed to be 2.2 and 0.3 Å, respectively.[23] From CPK model analysis, the distance between two porphyrins is assumed to be 12 Å. [b] The calculated contributions are for bpy in the *xz* plane (upper set) and for rotation by 45° (lower set). Porphyrin A means the contribution of porphyrin A to which the pyridine moiety is binding, and porphyrin B means the contribution of porphyrin B to which the pyridine moiety is not binding. [c] $\Delta\delta_{exptl}$ = δ(proton in Zn_2-**1**(bpy)) − δ(proton in bpy).

The UV–vis spectral changes upon addition of bpyEt or bpyPr to Zn_2-**1** were similar to that for Zn_2-**1**(bpy), except that the ligand concentrations required to form the ligand adducts were ca. 2 orders of magnitude higher than that for Zn_2-**1**(bpy). In accordance with this, the ^{1}H NMR spectrum of Zn_2-**1** was virtually unchanged from that of Zn_2-**1** upon the addition of 1 equiv of bpyEt or bpyPr, and only broad signals were observed even in the presence of 100 equiv of the ligands. Therefore, K_B values were calculated using eq 5 with the assumption that the ligands bind

Table VI. K_B Values and Thermodynamic Values for Ligand Binding to Zn(II) Porphyrins in $CHCl_3$

		K_B,[a] L/mol	$\Delta H°$, kcal/mol	$\Delta S°$, cal/(mol·K)
Zn-**1M**	py	1.4×10^4	-10 ± 0.1	-16 ± 0.3
	bpy	8.2×10^3	-9.4 ± 0.4	-14 ± 0.1
Zn_2-**1**	py	7, 20[b]		
	bpy	1.1×10^4	-8.6 ± 0.6	-10 ± 1.9
	bpyEt	1.1×10^2		
	bpyPr	23		
Zn-**2M**	py	3.8×10^4	-10 ± 0.1	-13 ± 0.1
	bpy	3.3×10^4	-9.9 ± 0.1	-13 ± 0.1
	bpyEt	3.2×10^4	-9.6 ± 0.1	-12 ± 0.1
	bpyPr	4.3×10^4	-9.7 ± 0.7	-12 ± 0.1
Zn_2-**2**	py	*c*		
	phpy	51		
	bpy	2.8×10^7	-10 ± 0.9	-0.4 ± 0.2
	bpyEt	9.3×10^6	-14 ± 0.8	-15 ± 0.2
	bpyPr	3.5×10^5	-11 ± 0.4	-12 ± 0.1

[a] At 25 °C. [b] Cooperative. [c] Isosbestic points were not observed.

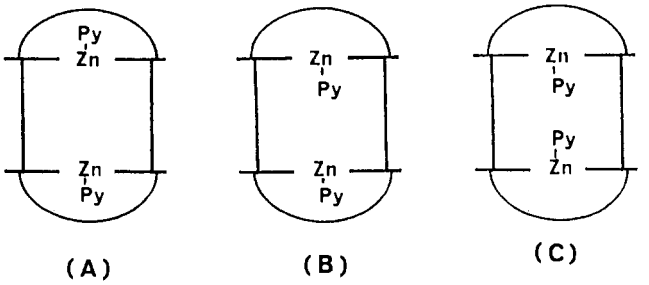

Figure 8. Binding mode of py to the Zn(II) porphyrin dimer.

to the strapped side of porphyrins. As listed in Table VI, the K_B values are comparable to those for the ligand adducts of Zn-**4**, and our assumption is confirmed to be true.

The isosbestic points were observed in the py titration of Zn_2-**1**, and the K_B value was calculated using eq 3. As shown in Figure 8, py can bind inside two porphyrins, and both $K1_{ap}$ and $K2_{ap}$ values for the py adducts of Zn_2-**1** are small compared with the K_B value for the bpy adduct of Zn_2-**1**. Along with the results, broad signals were observed for the ^1H NMR spectrum of Zn_2-**1** in py-d_5. The ^1H NMR results indicate that py adducts of Zn_2-**1** are a mixture of more than two binding modes as shown in Figure 8.

Ligand Binding to Zn_2-2. On the addition of bidentate ligands to Zn_2-**2**, the UV–vis spectral changes showed isosbestic points, and the K_B values were calculated by using eq 4 (Table VI). Because the K_B values for the bpy, bpyEt, or bpyPr adducts are large compared with those for these ligand adducts of Zn-**2M**, these ligands are suggested to bind inside of Zn_2-**2** as in Zn_2-**1**(bpy). In accordance with the K_B results, the changes in the ^1H NMR spectra of Zn_2-**2** upon the addition of the bidentate ligands confirm this suggestion. Table VII contains the selected ^1H NMR data for the bidentate ligand adducts of Zn_2-**2**. The ^1H NMR spectra of Zn_2-**2** with 1 equiv of bpy, bpyEt, or bpyPr revealed that the ligand adducts formed selectively, but those with 0.5 equiv of bpy or bpyEt were a mixture of Zn_2-**2** and its ligand adducts. Upon the addition of 1 equiv of bpyPr to Zn_2-**2**, the δ proton signal in the heptamethylene chain shifted from −2.74 to −2.61 ppm and no further change was observed up to the addition of 5 equiv of bpyPr. Similar results were observed for bpy or bpyEt addition to Zn_2-**2**. Therefore, bpy, bpyEt, and bpyPr are concluded to bind inside Zn_2-**2** as in Zn_2-**1**(bpy).

The K_B values for Zn-**2M**(bidentate ligands) are ca. 4-fold larger than those for Zn-**1M**(bpy), and this is due to the effect of linking phenyl groups on the ligand binding. Such an effect is observed frequently for complex formation in aqueous solution and is termed as a stacking effect.[20-22] The K_B value for Zn_2-**2**(phpy)$_2$ is comparable with that for Zn-**4**(py); it is confirmed that phpy binds to the strapping side in Zn_2-**2**. Therefore, the stacking interactions between the bidentate ligands and the linking phenyl planes are not the main driving force for the binding of ligands inside of Zn_2-**2**. We speculate that the main driving force is the complementary structure of Zn_2-**2** with bpy. Assuming that the Zn–N(py) bond length is ca. 2.2 Å and the displacement

Table VII. ^1H NMR Data for Zn_2-**2** and Its Ligand Adducts[a]

	Zn_2-**2**	bpy	bpyEt	bpyPr
δ	−2.74	−2.53	−2.64	−2.60
γ	−0.61	−0.53	−0.58	−0.55
β	−1.45	−1.22	−1.25	−1.25
α	+1.09	+1.12	+1.10	+1.14
amide	5.82	5.84	5.76	5.88
		6.94	*b*	*b*
3	8.28	8.27	8.38	8.41
4	7.80	7.81	7.85	7.83
5	7.37	7.58	7.53	7.62
6	8.06	8.11	8.06	8.13
2′	8.08	8.23	8.29	8.25
3′	7.95	7.53	7.49	7.62
4′	7.68	7.77	7.90	7.85
5′	8.59	8.31	8.53	8.56
pyrrole	8.91, 8.82	8.78	8.87	8.90
		8.75	8.82	8.82
linking[c]	5.7	8.19	5.9	5.2
		4.47		
ligand		1.82	1.55	2.04
		3.83	3.15	3.88
			0.88	0.18
				0.10

[a] Chemical shifts (δ) in $CDCl_3$ at 24 °C. For the labeling system, see Figure 1. [b] Signals were not assigned. [c] Protons in the linking groups.

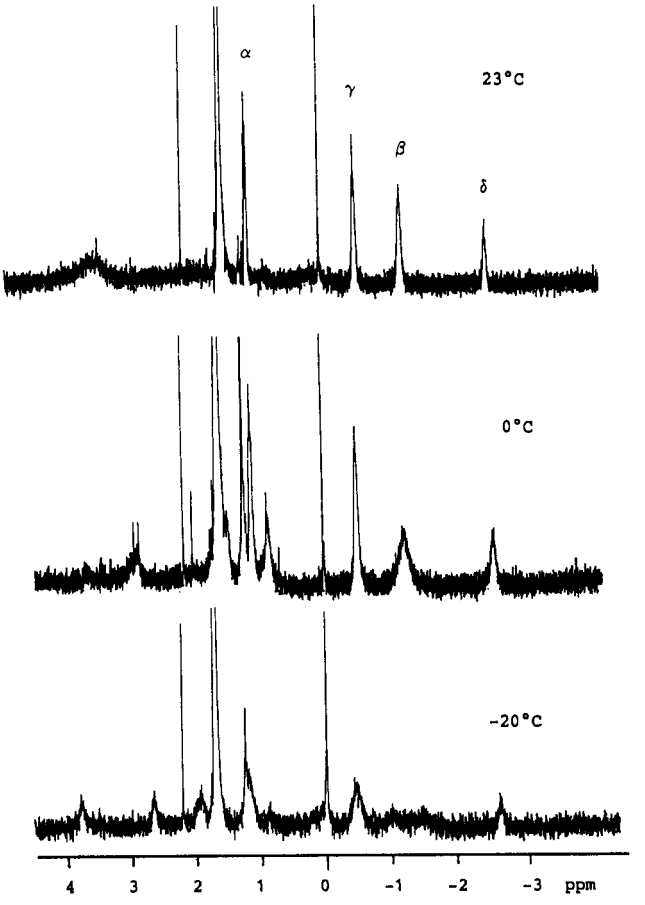

Figure 9. ^1H NMR spectra for Zn_2-**2** with 1 equiv of bpyEt in $CDCl_3$ at various temperatures.

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of the Zn atom from the mean porphyrin plane is ca. 0.3 Å,[23] the distances between two Zn atoms required to form bidentate ligand adducts are 12, 14, and 14.2 Å for the bpy, bpyEt, and bpyPr adducts, respectively. The distance between two Zn atoms in Zn_2-**2** is ca. 12 Å and is close to that for the binding of bpy. The β proton signal in the heptamethylene chain split into two at −20 °C in Zn_2-**2**(bpyEt) (Figure 9) and Zn_2-**2**(bpyPr), while such splitting was not observed in the ^{1}H NMR spectra of Zn_2-**2**(bpy) and Zn_2-**1**(bpy). These results suggest that there are strains in the Zn(II) porphyrin core in Zn_2-**2**(bpyEt) and Zn_2-**2**(bpyPr). In accordance with this result, CPK model experiments showed that there are strains in the Zn(II) porphyrin core in Zn_2-**2**(bpyEt) and Zn_2-**2**(bpyPr). Therefore, it is suggested that such strains are responsible for the reduced K_B values for Zn_2-**2**(bpyEt or bpyPr) compared with that of Zn_2-**2**(bpy).

Comparison of Binding Behavior between Zn_2-1 and Zn_2-2. In comparison of the K_B values for the monomer, the K_B values for Zn_2-**1**(bpy) and Zn_2-**2**(bpy) are 1.3-fold and 850-fold larger, respectively. According to the thermodynamic parameters for the ligand adducts of the Zn(II) porphyrins in Table VI, the large K_B value for Zn_2-**2**(bpy) is due mainly to the ΔS value, which is less negative than those for other adducts. From the ^{1}H NMR results, there are no detectable strains or stresses in the Zn(II) porphyrin core in both Zn_2-**1**(bpy) and Zn_2-**2**(bpy). Therefore, the difference in K_B values should be due to the conformational differences between Zn_2-**1** and Zn_2-**2**: the screwed-down conformation of Zn_2-**1** must be broken in the formation of Zn_2-**1**(bpy). The small K_B value for Zn_2-**1**(bpy) compared with that for Zn_2-**2**(bpy) can be ascribed to the free energy costs for breaking the screwed-down conformation of Zn_2-**1**. On the other hand, Zn_2-**2** has a complementary structure for bpy binding and binds bpy with small conformational changes. Therefore, the large K_B value for Zn_2-**2**(bpy) compared with that for Zn_2-**1**(bpy) can be ascribed to the small free energy costs for the conformational change compared with that for Zn_2-**1**(bpy).

On the binding of bpy inside Zn(II) porphyrin dimers, two reports have appeared: The K_B value for the bpy adduct of a Zn(II) porphyrin dimer (3×10^7 M^{-1} in CH_2Cl_2 at 25 °C)[24] is comparable to that of Zn_2-**2**(bpy), and the K_B value for the bpy adduct of a Zn(II) porphyrin dimer (1.3×10^3 M^{-1} in CH_2Cl_2 at 25 °C)[25] is smaller than that of Zn_2-**1**(bpy). From our results, it is speculated that the reason for the two extreme findings is due to the clear difference in the structures of the Zn(II) porphyrin dimers. In the latter case, the conformation of the Zn(II) porphyrin dimer is found to change upon bpy binding. We suggest that the conformational change is a main reason for the reduced K_B value in the latter case. No conformational information on the Zn(II) porphyrin dimer is available in the former case. However, we speculate, on the basis of our work, that conformational changes might not be required for bpy binding to the Zn(II) porphyrin dimer, and this results in the large K_B value in the former case.

Both the conformations of enzymes and substrates are known to change in the binding of substrates to enzymes. An X-ray crystallographic study has shown that the conformation of hexokinase A changes upon the binding of glucose.[26] Our results demonstrate that the energy cost for the conformational change of the Zn(II) porphyrin dimer reduces the free energy for the binding of a bidentate ligand to the enzymes and suggest that the energy cost is one of the important factors in the recognition mechanisms for the binding of substrates to enzymes.

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