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Selective Binding of Glucose-6-phosphate, 3,4-Dihydroxyphenylalanine (DOPA) and Their Analogs with a Boronic-acid-appended Metalloporphyrin

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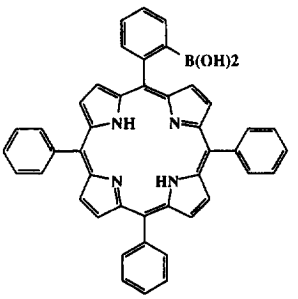
Abstract: A zinc(II) porphyrin (**2**) bearing an intramolecular boronic acid was synthesized. It was shown on the basis of absorption spectroscopy, ^{31}P NMR spectroscopy and circular dichroism (CD) spectroscopy that **2** can bind glucose-6-phosphate (G-6-P) in a two-point interaction manner, one between the 1,2-diol moiety and the boronic acid moiety and the other between the phosphate moiety and Zn(II). This system was successfully applied to facile discrimination of G-6-P from analogous glucose-1-phosphate, which is very difficult by other methods.[1] It was also shown that **2** is applicable to the selective binding of 3,4-dihydroxyphenylalanine and its analogs. In this system the boronic acid moiety in **2** binds the catechol moiety in amino acids and Zn(II) binds the amino group. Thus, the present study demonstrates that combination of metalloporphyrins and boronic acids enables us to design very potential receptors for guests containing a diol group and a ligand group within a molecule.

INTRODUCTION

Saccharides are nature's conveyors of energy and therefore indispensable to cell survival. In particular, conversion of D-glucose to D-glucose-6-phosphate mediated by hexokinase in the presence of ATP and interconversion between D-glucose-6-phosphate (G-6-P) and α-D-glucose-1-phosphate (G-1-P) mediated by phosphoglucomutase are essential steps to produce glycogen stored as an energy source.[2] It occurred to us that if we have some appropriate receptors in our hands which selectively bind one of these three D-glucose derivatives, the equilibria may be controllable by the co-existence of such artificial receptors. We previously developed several diboronic acid derivatives which can selectively bind glucose to form cyclic 1:1 complexes[3-5]: in these complexes each boronic acid forms covalently-bonded cyclic esters with two OH groups. Then, how can we discriminate between G-1-P and G-6-P? After trial-and-errors we reached the molecular design of a porphyrin (**1**) and a metalloporphyrin (**2**) bearing an intramolecular boronic acid. Examination of CPK molecular models reveals that in G-6-P the phosphate group can interact with the central metal when the 1,2-diol is bound to the boronic acid. In G-1-P, on the other hand, the distance between the phosphate group and the 4,6-diol is relatively short and the phosphate group cannot interact with the central metal when the 4,6-diol is bound to the boronic acid. Hence, we expected that **2** would bind G-6-P by the two-point interaction in preference to G-1-P.

Furthermore, we noticed that this concept can be extended to recognition of 3,4-dihydroxyphenylalanine (DOPA) and its derivatives because examination with CPK molecular models suggests that when the catechol moiety in DOPA is bound to the boronic-acid moiety, the amine moiety can intramolecularly coordinate to the central metal. Hence, this system seems useful to discriminate DOPA and

its derivatives which can enjoy the two-points interaction from analogous amino acids which can enjoy only the one-point interaction. With these objects in mind, we synthesized **1** and **2** and characterized their binding properties with various spectroscopic methods.



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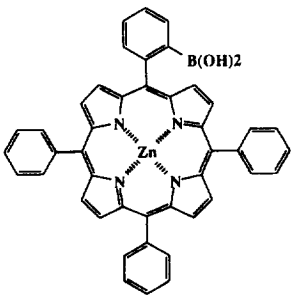

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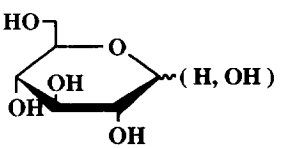
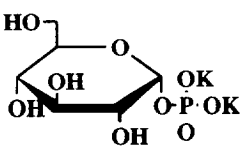
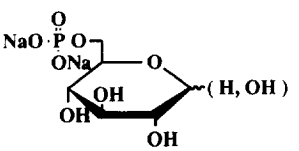


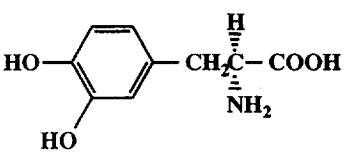
D - Glucose



α-D-Glucose-1-phosphate dipotassium salt (G-1-P)



D-Glucose-6-phosphate disodium salt (G-6-P)



L-DOPA

RESULTS AND DISCUSSION

Discrimination between G-1-P and G-6-P. To find unequivocal evidence for the metal-phosphate interaction, we measured ^{31}P NMR spectra in DMSO-d_6. As shown in Fig. 1, well-resolved ^{31}P NMR spectra were obtained after integration of 30,000 scans. An equimolar mixture (2.68 mmol dm^{-3} each) of **1** and G-6-P gave a sharp peak at 57.0 ppm assignable to free G-6-P and a broad peak at 54.6 ppm assignable to a **1**·G-6-P complex. The shift of the δ_p to higher magnetic field (by 2.4 ppm) indicates that the phosphate group of bound G-6-P exists on the free base porphyrin plane.* An equimolar mixture of **2** and G-6-P gave the similar splitting peaks but the broad peak assignable to a **2**·G-6-P complex shifted further to higher magnetic field (47.9 ppm). The large shift of the δ_p (by 9.1 ppm) implies that the phosphate group in the **2**·G-6-P complex exists on the

* The coordination of the phosphate group to Zn(II) also induces the chemical shift change in ^{31}P NMR spectroscopy. In this case the chemical shift should move to lower magnetic field. The observed up-field shift implies that the shielding effect of the porphyrin ring is predominant over the coordination effect.

porphyrin plane in the probability much higher than that in the **1**·G-6-P complex. The difference between **1**·G-6-P and **2**·G-6-P is reasonably attributed to the interaction between the phosphate group and the central metal in **2**·G-6-P. In an equimolar mixture of **2** and G-1-P, on the other hand, the δ_P assignable to the **2**·G-1-P complex appears at 56.7 ppm, only 0.3 ppm higher magnetic field from free G-1-P. The foregoing results would allow us to propose the following complexation modes: (i) in **2**+G-6-P the phosphate group coordinates to the central metal to allow the two-points recognition, (ii) in **1**+G-6-P the phosphate group is adsorbed onto the free base porphyrin probably because of the formation of hydrogen-bonds and (iii) as predicted from the CPK molecular model the phosphate group in **2**+G-1-P cannot enjoy the metal-phosphate interaction. We tried to estimate the association constants (K) from ^{1}H NMR spectroscopy. As shown in Fig. 2, the peak at 5.45 ppm for G-1-P and that at 5.44 ppm for G-6-P decreased with increasing **2** concentration and a new peak appeared at 5.32 ppm for **2** plus G-1-P and at 5.32 ppm for **2** plus G-6-P. Judging from the chemical shift, these peaks are assignable to the hemiacetal C-1 proton. We tried to confirm this assignment by ^{1}H-^{1}H COSY but could not obtain a good correlation spectrum in DMSO-d_6 because of the low solubility of G-1-P and G-6-P. To enhance the concentration (10 mmol dm^{-3}), we used DMSO-d_6:D_2O = 4:1 v/v mixed solvent. Although the chemical shifts were slightly changed, the basic spectral pattern was scarcely affected by the change in the medium. From the correlation with the neighboring C-2 proton, these peaks

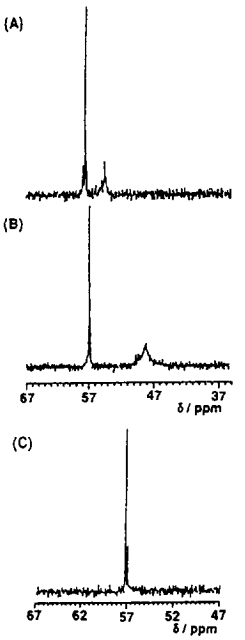


Fig. 1. ^{31}P NMR spectra of D-glucose derivatives (2.68 mmol dm^{-3}) in the presence of **1** or **2** (2.68 mmol dm^{-3}) at 25 °C in DMSO-d_6: (A) **1** plus G-6-P, (B) **2** plus G-6-P, (C) **2** plus G-1-P.

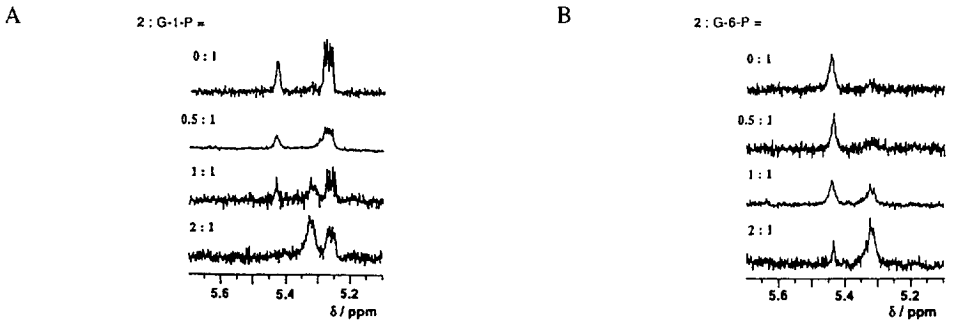


Fig. 2. Partial ^{1}H NMR spectra for the C-1 proton of D-glucose derivatives (2.68 mmol dm^{-3}) in the absence and the presence of **2** at 25 °C in DMSO-d_6: (A) **2** plus G-1-P, (B) **2** plus G-6-P.

have been assigned to the C-1 proton of α-glucopyranoside derivatives. From the ratio of the C-1 proton peak intensities one can now compute the K values: K = 250 dm^3 mol^{-1} for **2**+G-1-P and 1,500 dm^3 mol^{-1} for **2**+G-6-P. The result, $K_{G\text{-}1\text{-}P} < K_{G\text{-}6\text{-}P}$, is compatible with the proposed two-point interaction between **2** and G-

6-P.** To obtain concrete evidence for the two-point interaction we measured the circular dichroism (CD) spectra of these complexes. Our previous studies on the saccharide-binding to boronic-acid-based receptors have established that when a saccharide interacts with a diboronic acid receptor at two sites to form an intramolecular 1:1 complex, the resultant saccharide-containing macrocycle becomes CD-active.[3-6] Mizutani *et al.*[7] also found that when an amino acid derivative interacts with a metalloporphyrin at two sites (the amino group with the central metal through a coordination bond and the ester carbonyl group with the aromatic hydroxyl group through a hydrogen bond), the resultant 1:1 complex becomes CD-active. These findings are all rationalized in terms of chiral orientation of achiral chromophoric receptors by chiral guest molecules, which is effected by macrocyclization. As shown in Fig. 3, an equimolar mixture (2.68 mmol dm^{-3}) of **1** and G-6-P gave a weak positive exciton-coupling band (λ_{max} 424 nm ($[\theta]_{max}$ 16,300) and λ_{min} 418 nm ($[\theta]_{min}$ -11,300)) with $\lambda_{[\theta]=0}$ 419 nm near the absorption maximum (421 nm). The results, together with the ^{31}P NMR spectral data, suggest that G-6-P, primarily bound to the boronic acid moiety, is partially immobilized on **1** by the secondary effect of the hydrogen-bonding interaction between the NH protons in the free base porphyrin and the phosphate anion in the bound G-6-P. An equimolar mixture of **2** and G-6-P gave a very strong positive exciton-coupling band (λ_{max} 435 nm ($[\theta]_{max}$ 165,000) and λ_{min} 426 nm ($[\theta]_{min}$ -44,300); $\lambda_{[\theta]=0}$ 428 nm). The unsymmetrical spectral shape is very similar to that observed for metalloporphyrin-amino acid complexes.[7] In contrast, the G-1-P complex gave a very weak, single Cotton effect (λ_{max} 428 nm ($[\theta]_{max}$

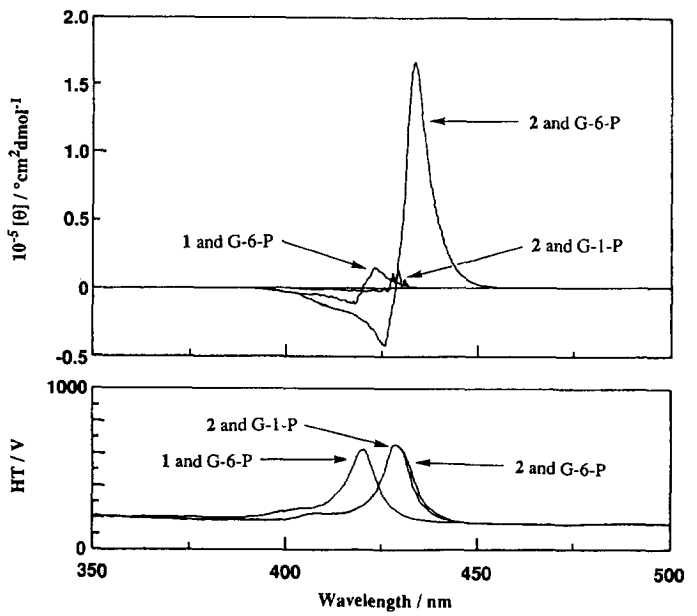


Fig. 3 HT voltages and CD spectra of **1** and **2** in the presence of D-glucose derivatives: 25 °C, DMSO, [**1** or **2**] = [D-glucose derivative] = 2.68 mmol dm^{-3}: lower figure shows voltages of the CD spectra because the measurement of the absorption spectra under the identical conditions was impossible. We confirmed, however, HT voltages showed a close correlation with the absorption spectra.

** Previously, we estimated the *K* values from the ratio of the peak integral intensity in ^{31}P NMR spectra. [1] This estimation method may not be necessarily correct if the peak integral intensity is not linearly correlated with the concentration because of the slow relaxation time.

11,000)) and the D-glucose complex is CD-silent. Thus, the results obtained from the CD spectroscopic studies are all in line with those obtained from the ^{31}P NMR spectroscopic studies.

The foregoing findings consistently support the view that compound **2** is capable of discriminating G-6-P from G-1-P through the two-point interaction. Figure 4 is a proposed structure for the two-point binding mode between **2** and G-6-P.

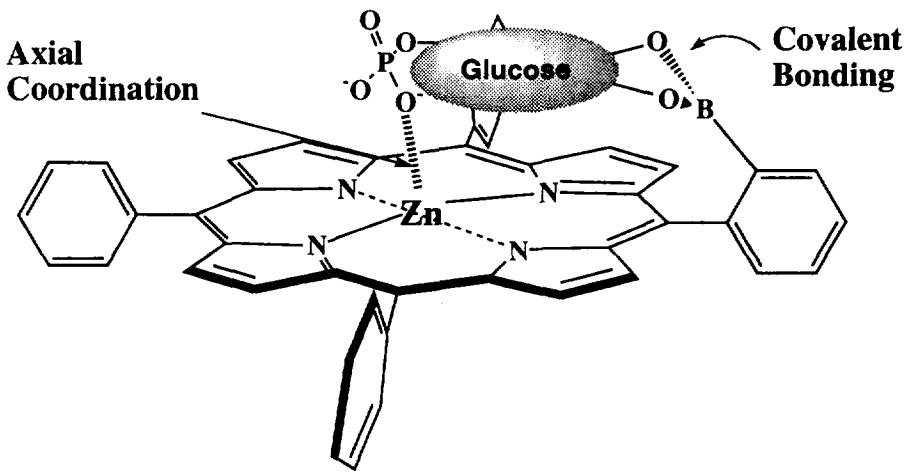


Fig. 4 Complex structure proposed for the **2**·G-6-P complex.

Molecular Recognition of DOPA and Its Derivatives. It is known that catechol shows a very high affinity (probably the highest among diols) with boronic acids.[8] One can expect, therefore, that guest molecules bearing a catechol moiety and a ligand group within a molecule would be selectively bound to **2** by the two-point interaction if the distance between two functional groups is appropriate for the binding.[9] Examination of the CPK molecular model suggests that the catechol group and the amino group in DOPA can satisfy this requirement to form an intramolecular 1:1 complex. Provided that this binding mode is correct, the resultant **2**·DOPA complex should be CD-active as seen for the **2**·G-6-P complex.

The association constants (K) were estimated by an absorption spectroscopic method in two different solvents, methanol:water (pH 9.0 with 50 mmol dm^{-3} carbonate buffer) and methanol. In the former medium the boron atom in the boronic acid-catechol complex is the sp^3-hybridized anion whereas in the latter medium it is sp^2-hybridized and neutral. Typical absorption spectral changes are illustrated in Figs. 5 and 6. When catechol was added, the Soret band at 422 nm decreased with increasing catechol concentration (Fig. 5). On the other hand, when L-DOPA methyl ester was added, the Soret band shifted to 427 nm (Fig. 6). Since the absorbance of **1** and **2** satisfied the Lambert-Beer's law, the porphyrins should exist discretely in this medium. One can thus regard that the spectral change is induced by the complex formation but not by the aggregation-deaggregation equilibrium of porphyrins. It is known that when the fifth axial ligand coordinates to Zn(II), the Soret band moves to longer wavelength.[10] Figures 5 and 6 suggest that catechol is simply bound to the boronic acid site in **2** whereas L-DOPA methyl ester is bound with both the boronic acid and the central metal.

The absorbance change in the 422 nm was analyzed by a Benesi-Hildebrand equation for a 1:1 complex. We observed a good linear relationship for the wide concentration range (10^{-5} ~ 10^{-2} mol dm^{-3}).[11] The similar spectral change was also observed in methanol. The K values thus determined are summarized in Table 1.

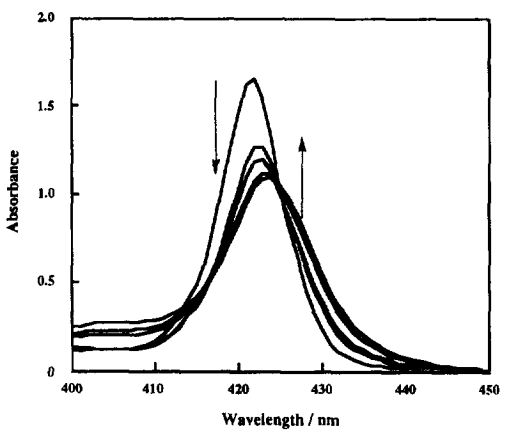


Fig. 5. Spectral change of **2** (3.00×10^{-6} mol dm^{-3}) induced by catechol: 25 °C, methanol:water (pH 9.0 with 50 mmol dm^{-3} carbonate) = 3:1 v/v, [catechol] = 5.00×10^{-5} ~ 1.00×10^{-2} mol dm^{-3}.

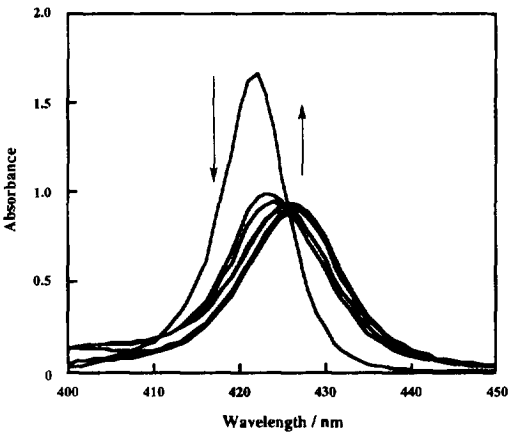


Fig. 6. Spectral change of **2** (3.00×10^{-6} mol dm^{-3}) induced by L-DOPA methyl ester.
The measurement conditions are similar to those described in the caption to Fig. 5.

Table 1 Association constants (in log K) for **2** at 25 °C

Guest	MeOH:water = 3:1 v/v[a]	MeOH[b]
L-DOPA	4.29	c
L-DOPA methyl ester	5.46	5.03
Dopamine	4.28	4.17
Catechol	4.64	3.33
Phenylalanine methyl ester	< 1.0	1.94
Tyrosine methyl ester	3.99	2.86

[a] Water was buffered to pH 9.0 with 50 mmol dm^{-3} carbonate.

[b] Guests with the amino group were added as their hydrochloride salts.

[c] L-DOPA was not soluble in methanol.

Examination of Table 1 reveals that catechol and catechol-containing guests have the K values larger than 10^3 dm^3 mol^{-1} whereas guests without the catechol moiety show the small K values or do not show any perceptible affinity with **2**. In phenylalanine methyl ester, for example, coordination of the amino group to Zn(II) results in $K = 10^{1.94}$ dm^3 mol^{-1}. Catechol itself possesses $K = 10^{4.64}$ dm^3 mol^{-1} in methanol:water = 3:1

imply that in aqueous solution the coordination of the amino group to Zn(II) scarcely contributes to the guest-binding or even if it does, the relative contribution is much smaller than that of the catechol complexation. Expecting the greater contribution of the amino group, we chose methanol as the measurement medium. In fact, although the significant association between phenylalanine methyl ester and **2** was not observed in methanol:water = 3:1 v/v, $K = 10^{1.94}$ dm^3 mol^{-1} was obtained in methanol which is based on the coordination of the amino group to Zn (II). As expected, the K values for L-DOPA methyl ester and dopamine are greater by 51-fold and 7-fold, respectively, than that for catechol. Obviously, the increase is ascribed to the two-point interaction between **2** and these guests (as in Fig. 7).

Table 2 CD spectral parameters for **2**[a]

	MeOH:water = 3:1 v/v[b]	MeOH
Guest	λmax or λmin (nm) ([θ] / ° cm^2 dmol^{-1})	
L-DOPA	425 (-6.7x10^4)	c
D-DOPA	426 (6.4x10^4)	c
L-DOPA methyl ester	425 (-5.1x10^4)	424 (-1.8x10^4)
Tyrosine methyl ester	423 (-3.6x10^4)	422 (-2.8x10^4)

[a] [**2**]=3.00x10^{-6} mol dm^{-3}, [guest]=1.00x10^{-2} mol dm^{-3}, 25°C

[b] Water was buffered to pH 9.0 with 50 mmol dm^{-3} carbonate.

[c] D- and L-DOPA were not soluble in methanol.

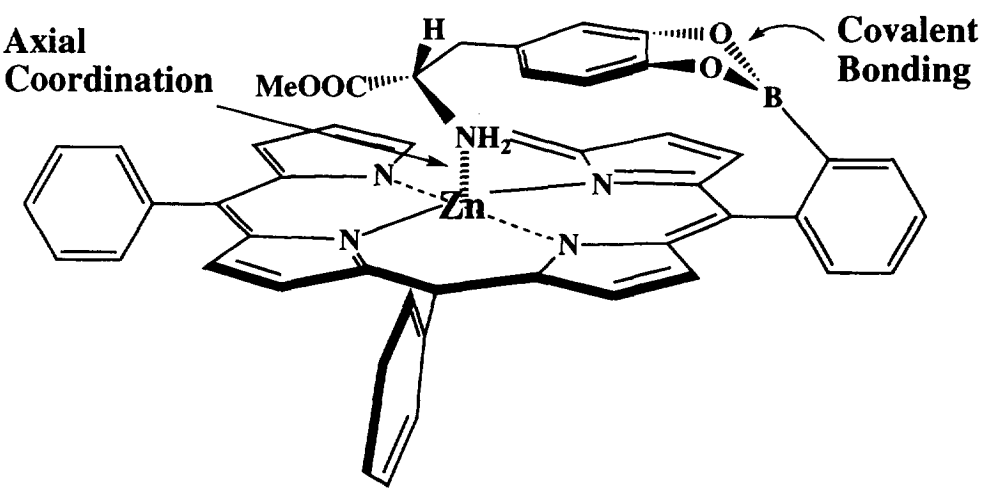


Fig. 7 Complex structure proposed for the **2**·L-DOPA methyl ester complex.

To obtain further evidence for the two-point binding mode we measured the CD spectra in methanol:water = 3:1 v/v and methanol (L- and D-DOPA were measured only in methanol:water = 3:1 v/v because they were scarcely soluble in methanol). We confirmed that (i) **2** becomes CD-active in the presence of L-DOPA, D-DOPA, L-DOPA methyl ester or tyrosine methyl ester, (ii) L- and D-DOPA give the symmetrical CD spectra and (iii) the CD band appears at 425 nm, the same wavelength as the absorption maximum. The typical CD spectra are illustrated in Fig. 8 and the spectral parameters are summarized in Table 2. On the other hand, metal-free **1** was CD-silent even in the presence of L-DOPA and L-DOPA methyl ester. Also, **2** was CD-silent in the presence of phenylalanine methyl ester. The results consistently support the view that L- and D-DOPA, L-DOPA methyl ester and tyrosine methyl ester can be immobilized on **2** as CD-active species through the two-point interaction, one between the catechol moiety (the phenol moiety in tyrosine methyl ester) and the boronic acid moiety and the other between the amino group and Zn(II). In tyrosine methyl ester, the phenol moiety can form a covalent bond with the boronic acid moiety as **2**-$B^-(OH)_2$-OC_6H_4-(tyrosine). Although this bond is not so stable as the cyclic ester formed from catechol and boronic acid, it seems strong enough to immobilize tyrosine methyl ester as a CD-active species. As shown in Table 1, the K values for L-DOPA and L-DOPA methyl ester are not or only slightly enhanced from that for catechol. The CD spectroscopic data indicate that the amino group surely coordinates to Zn(II) even though the interaction does not distinctly improve the K value. This is ascribed to the very high affinity of catechol with boronic acid which primarily governs the K values.

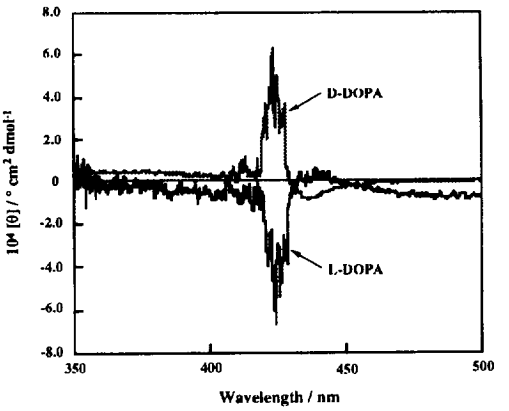


Fig. 8 .CD spectra of **2** in the presence of L-DOPA or D-DOPA: 25 °C, methanol:water (pH 9.6 with 100 mmol dm^{-3} carbonate) = 3:1 v/v, [2] = 1.00×10^{-5} mol dm^{-3}, [L-DOPA or L-DOPA methyl ester] = 1.00×10^{-2} mol dm^{-3}.

CONCLUSION

The present study shows that **2**, a Zn(II) porphyrin bearing an intramolecular boronic acid group, can selectively bind guest molecules bearing both a diol group and a metal ligand group in an appropriate distance. When the guest is optically-active, the resultant complex is turned into the CD-active species. From the sign of the CD spectra one can predict the absolute configuration. We have demonstrated that **2** is useful to recognize glucose-phosphates, DOPA and its analogs. The findings reveal that combination of metalloporphyrins and boronic acids is very potential for molecular design of novel receptor molecules.[12,13]

EXPERIMENTAL SECTION

Materials

Compound **1** was synthesized from pyrrole, benzaldehyde and 1,3-dioxa-2-(2-formylphenyl)-5,5-dimethylborinane according to Lindsey's method.[14]

5-(2-Hydroxyborinanylphenyl)-10,15,20-triphenylporphyrin (1). Benzaldehyde (1.16 g, 15 mmol) and 1,3-dioxa-2-(2-formylphenyl)-5,5-dimethylborinane (1.10 g, 5.0 mmol) were dissolved in a chloroform (2 l)-ethanol (15 ml) mixed solvent. After the reaction system was kept under the anaerobic (N_2) conditions, pyrrole (1.38 ml, 20 mmol) was added and the resultant solution was stirred at room temperature for 10 min. Then, $BF_3{\cdot}OEt_2$ (245 μl, 2.0 mmol) was added and the reaction was continued at room temperature for 1 h. After addition of DDQ (2.0 g, 8.81 mmol), the solution was stirred at room temperature for 8 h. The solution was concentrated to 50 ml and washed with water three times. The organic phase was subjected to column chromatography (silica gel, chloroform-ethyl acetate) for purification: m.p. > 300 °C, yield 0.8 %; δ_H ($CDCl_3$, TMS standard, 25°C, 250 MHz) -2.71 (2H, s, pyrrole NH), 2.57 (2H, s, $B(OH)_2$), 7.75 (11H, m, ArH), 8.20 (8H, m, ArH), 8.80 (8H, m, pyrrole β-H) (Found: C, 78.31; H, 5.30; N, 7.59. $C_{44}H_{31}BN_4O_2{\cdot}0.7MeCOOEt$ requires C, 78.05; H, 5.12; N, 7.78%).

1·Zn Complex (2). Compound **1** (100 mg, 0.13mmol) was dissolved in DMF (5.0 ml) and then treated with zinc acetate (100 mg, excess) at room temperature for 1 h under a nitrogen stream. The aliquot of the reaction mixture was withdrawn and the progress of the reaction was monitored by absorption spectroscopy. After the typical spectrum for zinc porphyrin appeared, the reaction mixture was diluted with water (30 ml) and stirred for 30 min. The precipitate was collected by filtration and dissolved in chloroform. The chloroform solution was dried over $MgSO_4$. The concentration of the solution gave the product: m.p. > 300 °C, yield 88%; δ_H ($CDCl_3$, TMS standard, 25°C, 250 MHz) 3.24 (2H, s, $B(OH)_2$), 7.77 (11H, m, ArH), 8.20 (8H, m, ArH), 8.93 (8H, m, pyrrol β-H) (Found: C, 70.24; H, 4.73; N, 7.52. $C_{44}H_{29}BN_4O_2Zn{\cdot}2H_2O$ requires C, 69.72; H, 4.39; N, 7.39%).

Miscellaneous

^1H NMR and ^{31}P NMR spectra were measured with a JEOL GSX-400 spectrometer using tetramethylsilane as reference. CD spectra were measured with a JASCO J-720 CD spectrometer. In order to obtain reproducible CD spectra at the Soret band which has a strong extinction coefficient, we used a 0.1mm width cell. UV-Vis spectra were measured with a SHIMADZU UV-160A.

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