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Article

Molecular Recognition of Inositol 1,4,5-Trisphosphate and Model Compounds in Aqueous Solution by Ditopic Zn^{2+} Complexes Containing Chiral Linkers

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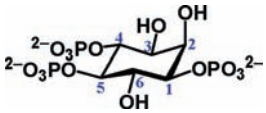
We report on molecular recognition of inositol 1,4,5-trisphosphate (Ins(1,4,5)P_3), an important intracellular second messenger, and some related model compounds, cyclohexanediol bisphosphate derivatives (CDP_2), by ditopic Zn^{2+} complexes containing chiral linkers ((*S*,*S*)- and (*R*,*R*)-**11**) in aqueous solution at physiological pH. A crystal structure analysis of (*S*,*S*)-**11** indicated that the distance between two Zn^{2+} ions (6.8 Å) is suitable for accommodating two phosphate groups at the 4- and 5-positions of Ins(1,4,5)P_3 and two phosphate groups of *trans*-1,2-CDP_2. ^{1}H NMR, ^{31}P NMR, potentiometric pH, and isothermal calorimetric titration data indicate that (*S*,*S*)-**11** forms 1:1 complexes with (*S*,*S*)- and (*R*,*R*)-1,2-CDP_2 at pH 7.4 and 25 °C. The apparent 1:1 complexation constants (log K_{app}) for (*S*,*S*)-**11**−(*S*,*S*)-1,2-CDP_2 and (*S*,*S*)-**11**−(*R*,*R*)-1,2-CDP_2 (K_{app} = [(*S*,*S*)-**11**−1,2-CDP_2 complex]/[(*S*,*S*)-**11**][1,2-CDP_2] (M^{-1})) were determined to be 7.6 ± 0.1 and 7.3 ± 0.1, respectively, demonstrating that both enantiomers of **11** bind to chiral *trans*-1,2-CDP_2 to almost the same extent. The log K_{app} value of 6.3 was obtained for a 1:1 complex of (*S*,*S*)-**11** with *cis*-1,3-CDP_2, while a small amount of 2:1 (*S*,*S*)-**11**−*cis*-1,3-CDP_2 was detected, as evidenced by electrospray ionization mass spectrometry (ESI-MS). In contrast, **11** formed several complexes with *trans*-1,4-CDP_2. On the basis of isothermal titration calorimetry data for (*S*,*S*)- and (*R*,*R*)-**11** with Ins(1,4,5)P_3, it was concluded that **11** forms a 2:1 complex with Ins(1,4,5)P_3, in which the first molecule of **11** binds to the 4- and 5-phosphates of Ins(1,4,5)P_3 and the second molecule of **11** binds to the 1- and 5-phosphates.

Introduction

It is well-known that D-*myo*-inositol 1,4,5-trisphosphate (Ins(1,4,5)P_3) is one of the important second messengers in intracellular signal transduction, which induces the release of Ca^{2+} from intracellular Ca^{2+} stores such as the endoplasmic reticulum (ER).[1] It has been reported that Ins(1,4,5)P_3 receptors ($InsP_3R$) are intracellular channel proteins that mediate the release of Ca^{2+} from ER[2] and regulate a number of processes, including cell proliferation and cell death.[3]



The intracellular concentration of Ins(1,4,5)P_3 is generally in the nanomolar range,[2c,4,5] and $InsP_3R$ cooperatively responds to subtle changes (nanomolar) in its concentrations.[6] In addition, the intracellular Ins(1,4,5)P_3 is rapidly converted to derivatives that are unable to activate the Ca^{2+}

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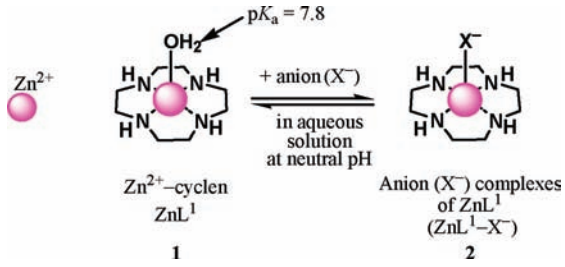
channel.[7] Therefore, development of novel receptors that bind tightly and rapidly to Ins(1,4,5)P_3 is highly required.

Biological sensing systems for Ins(1,4,5)P_3 and its analogues have been extensively studied during the past decade.[4,8] However, only a few reports of chemical sensing systems for Ins(1,4,5)P_3 have appeared to date,[9−11] despite the many studies on chemical receptors for phosphates.[12] A representative example of chemical systems for the detection of Ins(1,4,5)P_3 is an indicator-displacement assay that involves the use of a synthetic receptor having six guanidinium groups developed by Anslyn and colleagues.[9] Ahn recently exploited a tridentate Zn^{2+} complex as a synthetic receptor for Ins(1,4,5)P_3 with an indicator displacement method, as well.[10]

It has been established that Zn^{2+}−1,4,7,10-tetraazacyclododecane (Zn^{2+}−cyclen) complexes, such as **1** (ZnL^1), are good anion receptors and form 1:1 complexes **2** with biorelevant anions (X^-) such as phosphate monoester dianions,[13] carboxylates,[14] imidates,[15] and thiolates[16] in aqueous solution at neutral pH (Scheme 1).[17] We previously examined the complexation of an achiral tris(Zn^{2+}−cyclen) complex **3** (Zn_3L^2)[18] with *cis,cis*-1,3,5-cyclohexanetriol trisphosphate (CTP_3) (Chart 1).[11] CTP_3 was chosen as a model compound for Ins(1,4,5)P_3 because it is readily available and is able to activate an $InsP_3R$ of *Neurospora crassa*.[19] The findings revealed that **3** forms the 1:1 complex **4**, the dissociation constant (K_d) of which was 10 nM at pH 7.4 (Chart 1).[11] In addition, a luminescent Ins(1,4,5)P_3 sensor **5** ($Ru(Zn_2L^3)_3$) was designed and synthesized as the first chemical sensor to directly respond to Ins(1,4,5)P_3 by the Ru-templated assembly of bis(Zn^{2+}−cyclen) containing 2,2′-bipyridine (bpy) linker (Zn_2L^3).[11] The findings indicate that **5** forms a 2:1 complex, **6**, with CTP_3, resulting in a considerable enhancement in its luminescent emission in neutral aqueous solution. However, luminescent titrations of **5** with chiral Ins(1,4,5)P_3 suggested that **5**−Ins(1,4,5)P_3 complexation is rather weaker than that for **5** with achiral CTP_3.

Scheme 1



We later performed isothermal titration calorimetry (ITC) of Ins(1,4,5)P_3 with **3**, but the analysis was complicated, as described in the first part of this manuscript, possibly because of a symmetrical mismatch between **3** and Ins(1,4,5)P_3. We next attempted the optical resolution of racemic **5** (isolation of Δ and Λ forms) for the enantioselective recognition of Ins(1,4,5)P_3, because Ins(1,4,5)P_3 is a chiral molecule that contains six asymmetric carbons. However, this has not been successful, as of this writing.

The crystal structures of Ins(1,4,5)P_3 complexed with the $InsP_3R$ binding core[20] and the binding site of Ins(1,4,5)P_3 3-kinase (IPK)[21] have been reported. These findings suggest that two phosphates at the 4- and 5-positions (P4 and P5) of Ins(1,4,5)P_3 are important for complexation at the binding sites of these proteins, and the 1-phosphate (P1) and

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Chart 1

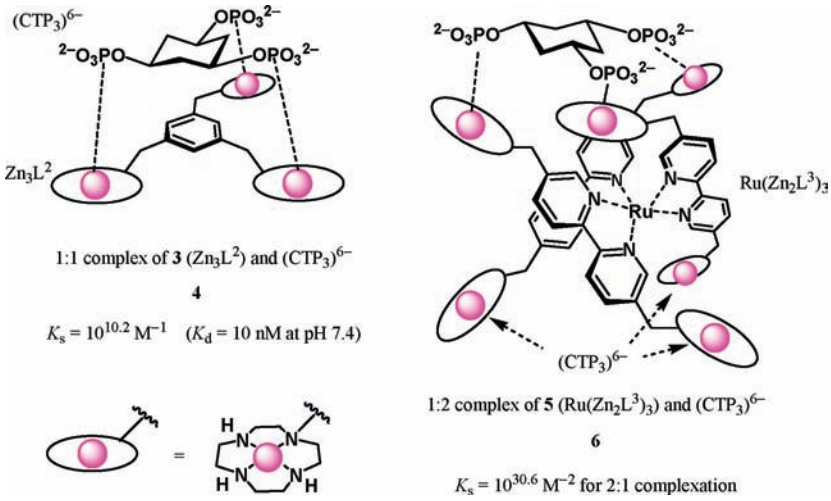


Chart 2

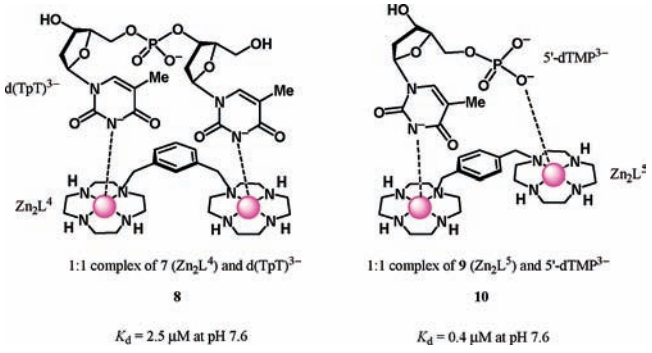
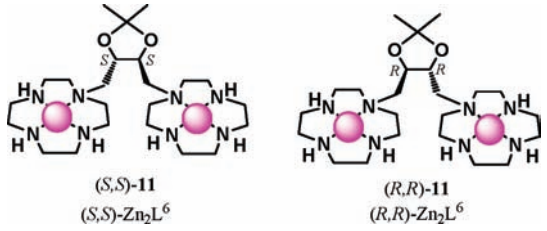


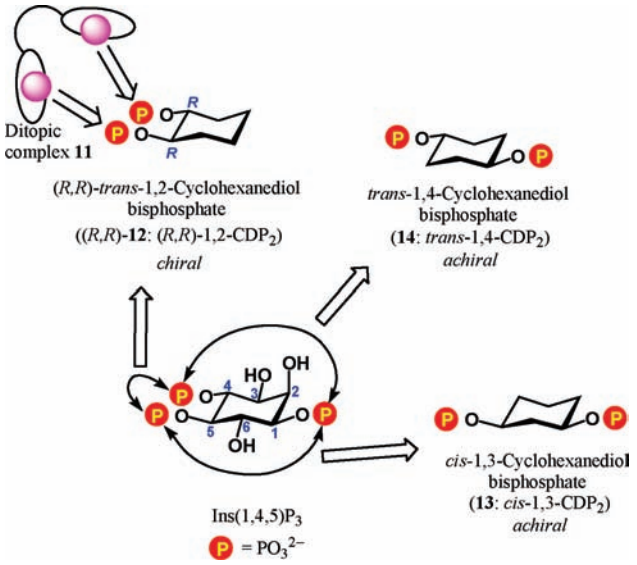
Chart 3



other hydroxyl groups are less important for complexation.[20,22]

We thus hypothesized that chiral ditopic Zn^{2+} complexes would be effective in terms of cooperatively and selectively recognizing the P4 and P5 of Ins(1,4,5)P_3. It was previously reported that achiral bis(Zn^{2+}−cyclen) complexes having *m*- and *p*-xylene linkers, **7** (Zn_2L^4) and **9** (Zn_2L^5), are potent receptors for thymidinyl(3′−5′)thymidine (d(TpT)) and dT-containing nucleotides including thymidine 5′-monophosphate (5′-dTMP) and yield 1:1 complexes such as **8** and **10** because of coordination of Zn^{2+} with T^- anion and phosphate anion, the dissociation constants (K_d) of which are in the submicromolar order (Chart 2).[23,24] On the basis of this background information, we attempted to design and synthesize chiral ditopic Zn^{2+} complexes (*S,S*)- and (*R,R*)-**11** (Chart 3), whose chiral linkers are readily available from L- and D-tartaric acid or D-mannitol, for the recognition of the P4 and P5 of Ins(1,4,5)P_3.

Scheme 2



As shown in Scheme 2, the absolute configurations of Ins(1,4,5)P_3 at C4 and C5 are *R*, allowing us to assume that (*R,R*)-*trans*-1,2-cyclohexanediol bisphosphate, (*R,R*)-**12** or (*R,R*)-1,2-CDP_2 could serve as a simple model for Ins(1,4,5)P_3.[25] We expected that the chiral linkers of **11** would fix the position of its two Zn^{2+}−cyclen units in appropriate positions for binding with the two phosphate groups of chiral (*R,R*)-1,2-CDP_2, as depicted in the top left of Scheme 2. In addition, we envisioned that (*S,S*)- or (*R,R*)-**11** might be able to discriminate *trans*-1,2-CDP_2 from its regioisomers such as achiral *cis*-1,3-CDP_2 (**13**), which resembles the P1 and P5 of Ins(1,4,5)P_3, and achiral *trans*-1,4-CDP_2 (**14**), which stands for P1 and P4 of Ins(1,4,5)P_3.

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In this paper, we report on the selective recognition of CDP_2 derivatives by (*S,S*)- and (*R,R*)-**11**, as studied by ^{1}H NMR, ^{31}P NMR, potentiometric pH and isothermal calorimetric titrations, and mass spectrometry. The molecular recognition of Ins(1,4,5)P_3 by both enantiomers of **11** were also examined. The findings show that **11** and Ins(1,4,5)P_3 form a 2:1 complex, in which the first molecule of **11** binds to P4 and P5, and the second molecule binds to P1 and P5, respectively.

Experimental Section

General Information. All reagents and solvents were purchased at the highest commercial quality and were used without further purification. $Zn(NO_3)_2\cdot 6H_2O$ was purchased from Kanto Chemical Co. Ltd. Acetonitrile (CH_3CN) and dichloromethane (CH_2Cl_2) were distilled from calcium hydride. All aqueous solutions were prepared using deionized and distilled water. Buffer solutions (HEPES, pH 7.4) were used, and the ionic strengths were adjusted with $NaNO_3$. Melting points were measured on a YANACO Micro Melting Point Apparatus and are uncorrected. IR spectra were recorded on a JASCO FTIR-410 spectrophotometer at room temperature (rt). ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR spectra at 25 ± 0.1 °C were recorded on a JEOL Lambda 400 spectrometer. ^{1}H (300 MHz) and ^{13}C (75 MHz) NMR spectra were recorded on a JEOL Always 300 spectrometer. Chemical shifts (δ) in $CDCl_3$ were determined relative to an internal reference of tetramethylsilane (TMS) for ^{1}H NMR and $CDCl_3$ for ^{13}C NMR. Chemical shifts (δ) in D_2O were determined relative to an external reference of 3-(trimethylsilyl)propionic-2,2,3,3-d_4 acid (TSP) sodium salt for ^{1}H NMR and 1,4-dioxane for ^{13}C NMR. 85% H_3PO_4 was used as an external reference for the ^{31}P NMR measurements. The pD values in D_2O were corrected for a deuterium isotope effect using pD = (pH-meter reading) + 0.40. Elemental analyses were performed on a Perkin-Elmer CHN 2400 analyzer. Electrospray ionization (ESI) mass spectra were recorded on a JEOL JMS-T100CS. Optical rotations were determined using a JASCO P-1030 digital polarimeter in 50 mm cells using the D line of sodium (589 nm). Thin-layer (TLC) and silica gel column chromatographies were performed using a Merck 5554 (silica gel) TLC plate and Fuji Silysia Chemical FL-100D, respectively. (*R,R*)-**15**[26] and (*S,S*)-**15**[27] were prepared from L-tartaric acid and D-mannitol according to literature procedures.

(*S,S*)-17. A mixture of 3Boc-cyclen **16** (960 mg, 2.03 mmol), diiodide ((*R,R*)-**15**)[26] (358 mg, 0.94 mmol), and Na_2CO_3 (591 mg, 5.8 mmol) in CH_3CN (0.5 mL) was refluxed for 8 days. The mixture was filtered through a Celite pad, passed through a short silica gel column using ethyl acetate as the eluant, and evaporated. To a $CHCl_3$ solution (6 mL) of the resulting residue and benzyl chloroformate (0.30 mL, 2.1 mmol) was added *i*Pr_2NEt (0.39 mL, 2.2 mmol) at 0 °C. The mixture was stirred for 30 min at rt and evaporated to dryness. The resulting residue was purified by silica gel column chromatography (hexane/AcOEt = 3:1) to afford (*S,S*)-**17** as a colorless solid (533 mg, 53% yield). Mp 96−98 °C. $[\alpha]_D^{24} = -75.8$ (c = 1.00 in $CHCl_3$). Anal. Calcd (%) for $C_{55}H_{104}N_8O_{15}$: C, 58.18; H, 9.41; N, 9.87. Found: C, 57.73; H, 9.28; N, 9.78. IR (KBr): ν = 2976, 2931, 2872, 1686, 1458, 1415, 1365, 1316, 1249, 1158, 1105, 977, 859, 771, 754 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$/TMS): δ = 1.33 (6H, s), 1.44 (36H, s), 1.47 (18H, s), 2.63−3.72 ppm (38H, br). ^{13}C NMR (100 MHz, $CDCl_3$/TMS): δ = 27.09, 28.34, 28.56, 47.12, 47.55, 48.13, 48.39, 49.39, 49.84, 55.47, 55.94, 56.32, 76.80, 78.96, 79.13, 79.25, 109.18, 155.25, 155.57, 156.04 ppm.

(*S,S*)-18·4.5TFA. To a CH_2Cl_2 solution (16 mL) of (*S,S*)-**17** (201 mg, 0.19 mmol) was added trifluoroacetic acid (8 mL) at 0 °C. The mixture was stirred for 2.5 h at rt, filtered, and the filtrate was concentrated under reduced pressure. Reprecipitation from EtOH/Et_2O gave (*S,S*)-**18**·4.5TFA as a colorless powder (158 mg, 73% yield). Mp 199−200 °C (dec.). $[\alpha]_D^{25} = -74.9$ (c = 1.00 in H_2O). Anal. Calcd (%) for $C_{32}H_{54.5}F_{13.5}N_8O_{11}$: C, 39.07; H, 5.58; N, 11.39. Found: C, 38.99; H, 5.48; N, 11.50. IR (KBr): ν = 3435, 3293, 3103, 2984, 2856, 1681, 1459, 1418, 1203, 1128, 1088, 918, 833, 799, 776, 721 cm^{-1}. ^{1}H NMR (300 MHz, D_2O/TSP): δ = 1.48 (6H, s), 2.76−3.18 (36H, m), 3.98 ppm (2H, d, J = 7.3 Hz). ^{13}C NMR (100 MHz, D_2O/dioxane): δ = 26.64, 41.10 (br), 42.82 (br), 44.85 (br), 47.49 (br), 49.51 (br), 53.47, 76.63, 110.52, 116.80 (q, J_{C-F} = 292 Hz), 163.33 ppm (q, J_{C-F} = 35 Hz).

(*S,S*)-11·$(NO_3)_4$·0.5H_2O ((*S,S*)-$Zn_2L^6\cdot(NO_3)_4\cdot 0.5H_2O$). A H_2O solution of (*S,S*)-**18**·4.5TFA (150 mg, 0.13 mmol) was passed through a column of ion-exchange resin (IRA-400J, HO^- form) to afford the acid-free ligand (*S,S*)-**18** as a pale yellow oil. To a solution of the free ligand (*S,S*)-**18** in EtOH (4 mL) was added $Zn(NO_3)_2\cdot 6H_2O$ (124 mg, 0.42 mmol) in EtOH (4 mL), and the resulting mixture was stirred overnight. The precipitate that formed during the reaction was isolated on a filter, and then recrystallized from EtOH/H_2O, afforded colorless crystals (110 mg, quant) which were suitable for X-ray crystal structure analysis. Mp 295−300 °C (dec.). $[\alpha]_D^{20} = -5.0$ (c = 0.5 in H_2O). Anal. Calcd (%) for $C_{23}H_{51}N_{12}O_{14.5}Zn_2$: C, 32.18; H, 5.99; N, 19.58. Found: C, 32.45; H, 6.18; N, 19.61. IR (KBr): ν = 3434, 3240, 2932, 2882, 1627, 1480, 1459, 1379, 1305, 1256, 1240, 1224, 1134, 1090, 1050, 992, 904, 858, 834, 812, 794, 748 cm^{-1}. ^{1}H NMR (300 MHz, D_2O/TSP): δ = 1.47 (6H, s), 2.75−3.25 (36H, m), 3.77 (2H, br), 4.02 (4H, br), 4.21 ppm (2H, d, J = 7.1 Hz). ^{13}C NMR (100 MHz, D_2O/dioxane): δ = 25.74, 41.94, 41.99, 42.06, 43.04, 43.47, 44.15, 44.27, 44.40, 50.29, 50.59, 54.92, 74.50, 111.65 ppm. HRMS (ESI^+): calcd for $[M-NO_3^-]^+$, 784.2274; found, 784.2274.

(*R,R*)-11·$(NO_3)_4$·H_2O ((*R,R*)-$Zn_2L^6\cdot(NO_3)_4\cdot H_2O$). The (*R,R*)-**11** was synthesized from (*S,S*)-**15**[27] in a similar manner as for (*S,S*)-**11**. Mp 295−300 °C (dec.). $[\alpha]_D^{19} = +4.3$ (c = 0.5 in H_2O). Anal. Calcd (%) for $C_{23}H_{52}N_{12}O_{15}Zn_2$: C, 31.84; H, 6.04; N, 19.38. Found: C, 31.99; H, 5.83; N, 19.33.

Crystallographic Study of (*S,S*)-11·$(NO_3)_4$ ((*S,S*)-$Zn_2L^6\cdot(NO_3)_4$). (*S,S*)-**11**·$(NO_3)_4$ was recrystallized from EtOH/H_2O at rt. All measurements were made on a Rigaku Saturn CCD area detector with graphite monochromated Mo−Kα radiation at 123 K. The structure was solved by direct methods[28] and refined by full-matrix least-squares techniques. All calculations were performed using the CrystalStructure crystallographic software package except for refinements, which were performed with SHELXL-97.[29] $C_{23}H_{50}N_{12}O_{15}Zn_2$, M_r = 865.53, a colorless block crystal, crystal size 0.20 × 0.15 × 0.10 mm, orthorhombic, space group $P2_12_12_1$ (#19), a = 8.8633(18), b = 12.378(3), c = 31.719(6) Å, V = 3480.0(12) Å^3, Z = 4, D_{calc} = 1.652 g·cm^{-3}, 28446 measured reflections, 7970 unique reflections, $2\theta_{max}$ = 57.4°, $R1$ (w$R2$) = 0.0854 (0.2036), GOF = 1.153.

Potentiometric pH Titrations. The preparation of the test solutions and the method used for calibration of the electrode system (Potentiometric Automatic Titrator AT-400 and Auto Piston Buret APB-410, Kyoto Electronics Manufacturing, Co. Ltd.) with a Kyoto Electronics Manufacturing Co. Combination pH Electrode 98100C171 have been described previously.[18] All of the test solutions (50 mL) were maintained under an argon (> 99.999% purity) atmosphere. The potentiometric pH titrations were performed with I = 0.1 ($NaNO_3$) at 25.0 ± 0.1 °C (0.1 M aqueous NaOH was used as the base). The deprotonation constants were determined using the "BEST" software program.[30] The K_W

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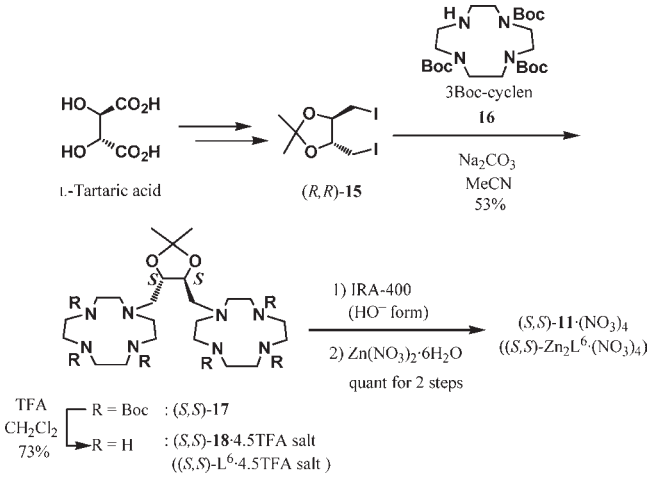
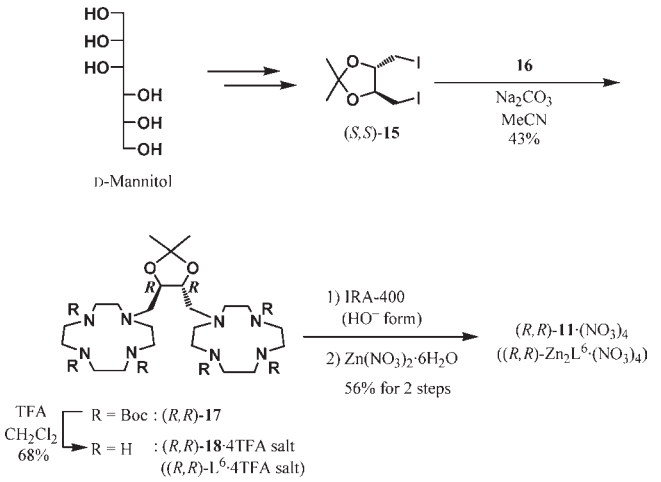
Scheme 3



Scheme 4



(equivalent to $a_{H+}a_{OH-}$), K_W' (equivalent to $[H^+][HO^-]$), and f_{H+} values used at 25 °C were $10^{-14.00}$, $10^{-13.79}$, and 0.825, respectively. The corresponding mixed constants K_2 (= [HO^--bound species]a_{H+}/[H_2O-bound species]), were derived using $[H^+] = a_{H+}/f_{H+}$. The percentage species distribution values against pH (= $-\log[H^+] + 0.084$) were obtained using the "SPE" software program.[30]

ITC.[31] The heat of complexation were recorded on a MicroCal VP-ITC at 25.0 °C and pH 7.4 (50 mM HEPES buffer with $I = 0.10$ ($NaNO_3$)). In a typical experiment, the solution (1.4 mL) of 0.1 mM (*S*,*S*)-1,2-CDP_2 in 50 mM HEPES buffer with I=0.10 ($NaNO_3$) was placed in a calorimeter cell, to which a solution of 1 mM (*S*,*S*)-**11** in 50 mM HEPES was loaded. The obtained calorimetric data were used to determine the ΔH value and apparent complexation constants, K_{app}, using the "Origin 7" software program provided by OriginLab Corporation.

Results and Discussion

Complexation of 3 (Zn_3L^2) with Ins(1,4,5)P_3, as Examined by ITC. We first performed ITC experiments to examine the complexation of **3** (Zn_3L^2) with Ins(1,4,5)P_3. A solution of **3** (1 mM) was titrated into Ins(1,4,5)P_3 (50 μM) at 25 °C and pH 7.4 (50 mM HEPES with $I = 0.1$ ($NaNO_3$)). A typical titration curve is shown in the Supporting Information (Figure S1) indicates that the complexation of these two molecules was exothermic and that it continued over the addition of one equivalent of Zn_3L^2. Unfortunately, an analysis of a typical titration curve (Supporting Information, Figure S1) based on the assumption that Zn_3L^2 binds to Ins(1,4,5)P_3 in a 1:1 ratio was not successful.

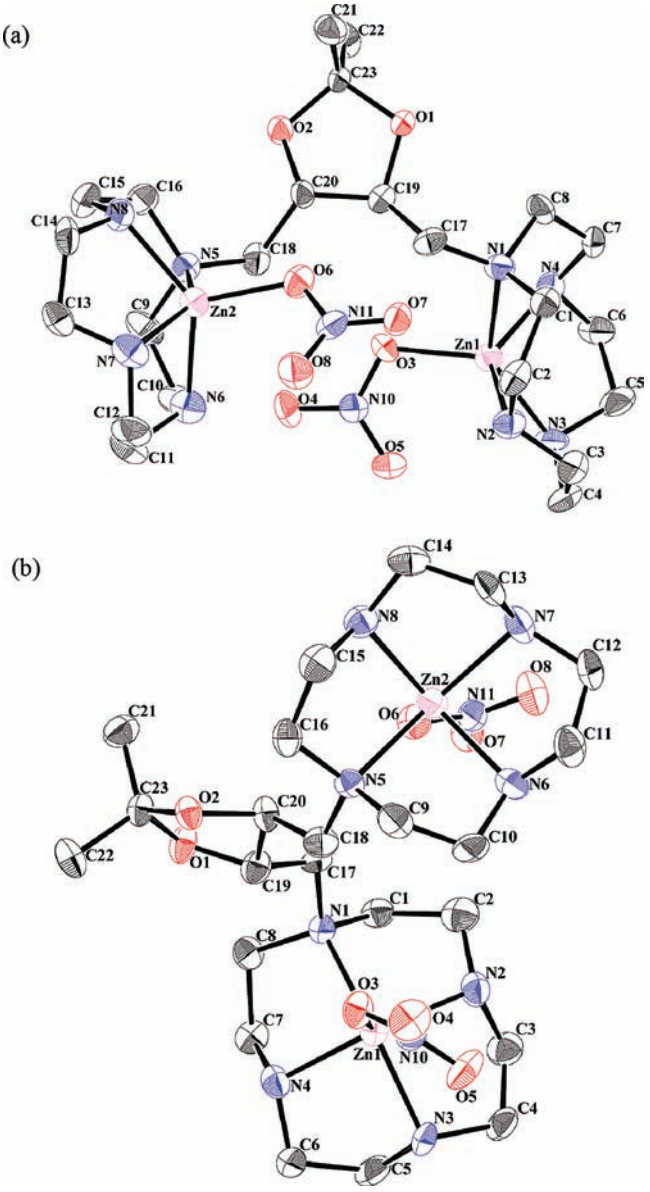


Figure 1. ORTEP drawing of (*S*,*S*)-**11**·$(NO_3)_4$ ((*S*,*S*)-Zn_2L^6·$(NO_3)_4$) (50% ellipsoids). (a) Top view and (b) side view. Selected bond lengths [Å]: Zn(1)−O(3) 1.991(5), Zn(1)−N(1) 2.210(6), Zn(1)−N(2) 2.098(6), Zn(1)−N(3) 2.163(6), Zn(1)−N(4) 2.093(6), Zn(2)−O(6) 1.989(5), Zn(2)−N(5) 2.201(6), Zn(2)−N(6) 2.100(7), Zn(2)−N(7) 2.152(6), Zn(2)−N(8) 2.132(6). Two external nitrate anions (not Zn^{2+}-bound nitrates), hydrogen atoms, and water have been omitted for clarity.

Synthesis of (*S*,*S*)- and (*R*,*R*)-11·$(NO_3)_4$ ((*S*,*S*)- and (*R*,*R*)-Zn_2L^6·$(NO_3)_4$). We next synthesized chiral ditopic Zn^{2+} complexes (*S*,*S*)- and (*R*,*R*)-**11**·$(NO_3)_4$ ((*S*,*S*)- and (*R*,*R*)-Zn_2L^6·$(NO_3)_4$), which can be readily prepared from L-tartaric acid or D-mannitol (Scheme 3 and 4). For the synthesis of (*S*,*S*)-**11** ((*S*,*S*)-Zn_2L^6), (*R*,*R*)-**15** was prepared from L-tartaric acid[26] and reacted with

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3Boc-cyclen **16**[18] to give (*S,S*)-**17** (Scheme 3). The six Boc groups of (*S,S*)-**17** were selectively deprotected by treatment with trifluoroacetic acid (TFA) in CH_2Cl_2 to give (*S,S*)-**18** ((*S,S*)-L^6) as the TFA salt. Acid-free (*S,S*)-**18** was obtained by treatment of (*S,S*)-**18**·4.5TFA with IRA-400 (HO^- form) and reacted with $Zn(NO_3)_2 \cdot 6H_2O$ to afford (*S,S*)-**11**. To prepare enantiomeric (*R,R*)-**11**, D-mannitol was converted to (*S,S*)-**15** according to a literature procedure[27] and was then reacted with 3Boc-cyclen **16** to obtain (*R,R*)-**17** (Scheme 4). The (*R,R*)-**17** was converted into (*R,R*)-**11**·$(NO_3)_4$ in a manner similar to that for (*S,S*)-**11**.

Crystal Structure Analysis of (*S,S*)-11·$(NO_3)_4$ ((*S,S*)-$Zn_2L^6 \cdot (NO_3)_4$). Figure 1 shows the crystal structure of (*S,S*)-**11**·$(NO_3)_4$ ((*S,S*)-$Zn_2L^6 \cdot (NO_3)_4$).[32] Representative crystallographic parameters are listed in the Supporting Information, Table S1. The distance between the two Zn^{2+} ions (Zn(1)−Zn(2) in Figure 1) was determined to be 6.8 Å and the two nitrates coordinated to Zn^{2+} ions. The crystal structures of Ins(1,4,5)P_3 complexed with the InsP_3R binding core[20] and the binding site of the Ins(1,4,5)P_3 3 kinase (IPK)[21] complex disclosed that the cyclohexane ring of Ins(1,4,5)P_3 has a chair conformation with three phosphate groups in equatorial positions. In addition, the distances of three sets of two phosphorus atoms in Ins(1,4,5)P_3 are 5.0 Å for P4−P5, 7.2 Å for P1−P5, and 8.4 Å for P1−P4, as shown in Chart 4.

In an earlier study, Hiskey's group calculated the distances between two phosphorus atoms of *trans*-1,2-CDP_2 and *cis*-1,3-CDP_2 to be 6.01 and 7.82 Å (for diequatrial forms).[25b] For a more accurate calculation, we used density functional theory at the MPW1PW91/6-31++G level,[33] which gave the distances between two phosphorus atoms in *trans*-1,2-CDP_2, *cis*-1,3-CDP_2, and *trans*-1,4-CDP_2 of 6.7, 8.4, and 8.9 Å, respectively, as indicated in Chart 4 (a semiempirical PM3 calculation method[33] also afforded similar results). Thus, it was expected that the distance between two Zn^{2+} ions in (*S,S*)-**11** could accommodate the two phosphate groups at P4 and P5 of Ins(1,4,5)P_3 as well as the two phosphate groups of *trans*-1,2-CDP_2.

Deprotonation Constants for Zn^{2+}-Bound Water Molecules of (*S,S*)-11 ((*S,S*)-Zn_2L^6). The plain curve (a) in Figure 2 shows a typical potentiometric pH titration curve for 0.5 mM (*S,S*)-**11** ((*S,S*)-Zn_2L^6) in aqueous solution with I = 0.1 ($NaNO_3$) at 25 °C, from which the deprotonation constants (pK_a) for two Zn^{2+}-bound water molecules of (*S,S*)-**11** defined by eqs 1−2 were determined to be 7.1 ± 0.1 and 7.8 ± 0.1 using the "BEST" software program.[30] Previously, two pK_a values for Zn^{2+}-bound waters of **7** (Zn_2L^4) were reported to be 6.7 and 8.5 in aqueous solution with I = 0.1 ($NaNO_3$) at 25 °C,[18,34] in which the separate pK_a values was explained by strong intramolecular hydrogen bonding between the Zn^{2+}−(HO^-) and Zn^{2+}−OH_2. In contrast, the pK_a values for the two Zn^{2+}-bound waters in **9** (Zn_2L^5) were 7.2 and 7.9,[18,35] suggesting that they are involved in less interactions compared to those in **7**. Considering these collective facts, the two close pK_a values of (*S,S*)-**11** suggest weak interactions between the two Zn^{2+}−bound waters. Similarly, the pK_a values

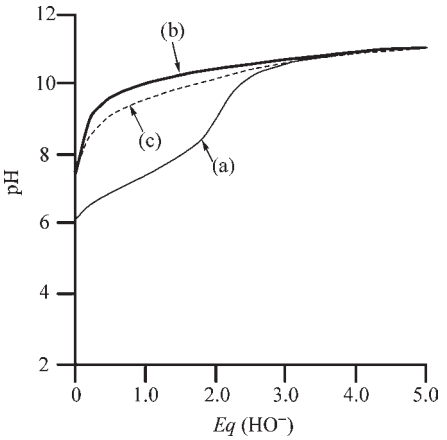
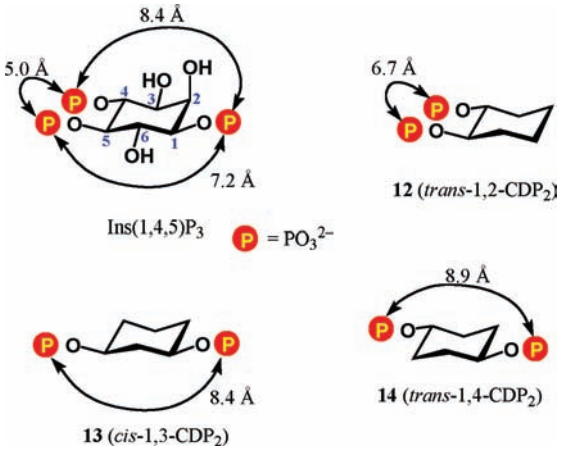


Figure 2. Typical potentiometric pH titration curves for 0.5 mM (*S,S*)-**11** ((*S,S*)-Zn_2L^6) (a plain curve (a)) and a mixture of 0.5 mM (*S,S*)-**11** + 0.5 mM $Na_4 \cdot$(*S,S*)-1,2-CDP_2 (a bold curve (b)) in aqueous solution with I = 0.1 ($NaNO_3$) at 25 °C. *Eq* (HO^-) is the number of equivalents of base (NaOH) added.

Chart 4. Phosphorus−Phosphorus Distances of Ins(1,4,5)P_3 in the Crystal Structure of Ins(1,4,5)P_3−InsP_3R[20] and Phosphorus−Phosphorus Distances of **12** (*trans*-1,2-CDP_2), **13** (*cis*-1,3-CDP_2), and **14** (*trans*-1,4-CDP_2) Calculated by the DFT Method in This Study



(32) CCDC 767554 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

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Scheme 5

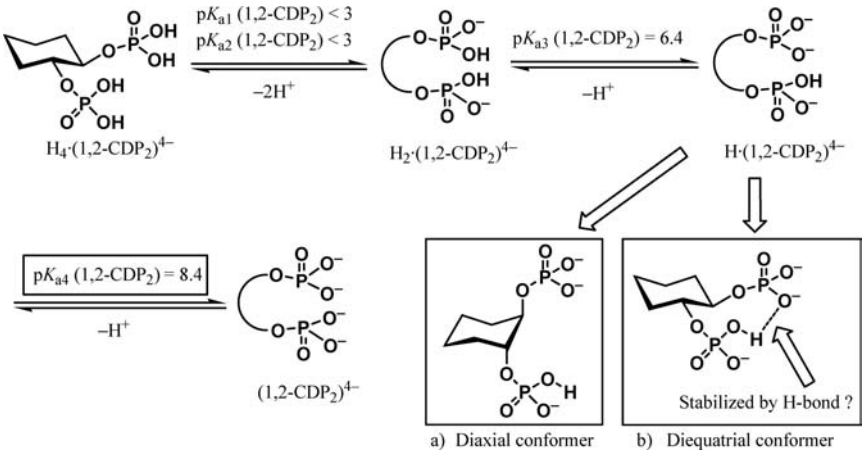


Table 1. pK_a Values for CDP_2 and Relevant Phosphate Compounds

	pK_{a1}	pK_{a2}	pK_{a3}	pK_{a4}
trans-1,2-CDP_2	< 3	< 3	6.4 (6.42)[a]	8.4 (8.32)[a]
cis-1,3-CDP_2	< 3	< 3	6.6 (6.80)[a]	7.5 (7.86)[a]
trans-1,4-CDP_2	< 3	< 3	6.6 (7.10)[b]	7.3 (7.10)[b]
Glu-1-P[c,d]	1.10	6.13		
PP[d]	< 3	5.8		
4-NPP[d]	< 3	5.2		

[a] From ref 25b. [b] pK_a values for a mixture of *cis*- and *trans*-1,4-CDP_2 reported in ref 25b. [c] From ref 36. [d] From ref 18.

for the enantiomer (*R,R*)-**11** were determined to be 7.1 ± 0.1 and 7.9 ± 0.1, which are identical to those for (*S,S*)-**11** under the same conditions.

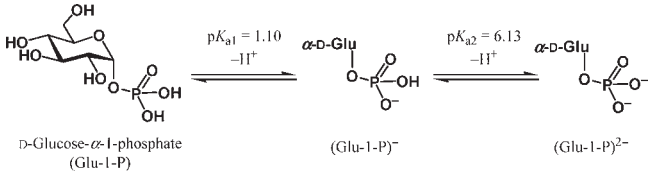
$$Zn_2L^6(H_2O)_2 \rightleftarrows Zn_2L^6(H_2O)(HO^-) + H^+ : K_{a1}(Zn_2L^6) = [Zn_2L^6(H_2O)(HO^-)]\cdot a_{H+} / [Zn_2L^6(H_2O)_2] \quad (1)$$

$$Zn_2L^6(H_2O)(HO^-) \rightleftarrows Zn_2L^6(HO^-)_2 + H^+ : K_{a2}(Zn_2L^6) = [Zn_2L^6(HO^-)_2]\cdot a_{H+} / [Zn_2L^6(H_2O)(HO^-)] \quad (2)$$

pK_a Values Determined by Potentiometric pH Titration for 12 (*trans*-1,2-CDP_2) and Its Conformation in Aqueous Solution. The synthesis of (*S,S*)- and (*R,R*)-**12** ((*S,S*)- and (*R,R*)-1,2-CDP_2), **13** (*cis*-1,3-CDP_2), and **14** (*trans*-1,4-CDP_2) was carried out as described in the Supporting Information. The plain curve (a) in Supporting Information, Figure S2 shows a typical potentiometric pH titration for (*S,S*)-1,2-CDP_2. An analysis of this curve gave four pK_{ai} values ($i = 1-4$) defined by eq 3 as < 3 (pK_{a1}), < 3 (pK_{a2}), 6.4 ± 0.1 (pK_{a3}), and 8.4 ± 0.1 (pK_{a4}), which are summarized in Scheme 5 and Table 1.

For comparison, the pK_{ai} values (i = 1−2) for D-glucose-α-1-phosphate (Glu-1-P) are 1.10 and 6.13 as shown in Scheme 6,[18,36] and the pK_{a2} values for phenyl phosphate (PP) and 4-nitrophenyl phosphate (4-NPP) are 5.8 ± 0.1 and 5.2 ± 0.1, respectively (Table 1).[18] Similarly, the four pK_{ai} values (i = 1−4) for *cis*-1,3-CDP_2 were determined to be < 3 (pK_{a1}), < 3 (pK_{a2}), 6.6 ± 0.1 (pK_{a3}), 7.5 ± 0.1 (pK_{a4}) by potentiometric pH titration (bold curve (b) in Supporting Information, Figure S2) and the pK_a values for *trans*-1,4-CDP_2 were determined to be < 3 (pK_{a1}), < 3 (pK_{a2}), 6.6 ± 0.1 (pK_{a3}), 7.3 ± 0.1 (pK_{a4}) (dashed curve (c) in Supporting Information, Figure S2). These pK_a values are nearly identical to those reported by Hiskey and co-workers (Table 1). The pK_{a4} value for *trans*-1,2-CDP_2 of 8.4 is higher than typical pK_{a2} values for phosphates, an observation that suggests that the diequatorial conformation is preferred over the diaxial conformation at neutral pH, which leads to the conclusion that *trans*-1,2-CDP_2 has the potential for serving a good model for Ins(1,4,5)P_3, as previously described by Hiskey and co-workers.[25b]

Scheme 6



$$(H_{(5-i)}CDP_2)^{(i-1)-} \rightleftarrows (H_{(4-i)}CDP_2)^{i-} + H^+ : \quad K_{ai}(CDP_2) = [(H_{(4-i)}CDP_2)^{i-}]\cdot a_{H+} / [(H_{(5-i)}CDP_2)^{(i-1)-}] \quad (i = 1-4) \quad (3)$$

Recognition of 12 (*trans*-1,2-CDP_2) by (*S,S*)-11 ((*S,S*)-Zn_2L^6), as Evidenced by ^{1}H NMR and ^{31}P NMR. A ^{1}H NMR titration of (*S,S*)-**11** ((*S,S*)-Zn_2L^6) (1 mM) was performed with increasing concentrations of optically pure **12** (*trans*-1,2-CDP_2) in D_2O at pD 7.4 and 25 °C. The methyl signal of (*S,S*)-**11** was shifted from 1.469 ppm to 1.490 ppm upon the addition of (*S,S*)-1,2-CDP_2, reaching a plateau at [(*S,S*)-1,2-CDP_2]/[(*S,S*)-**11**] > 1.0 (closed circles in Figure 3a and in the Supporting Information, Figure S3). A Job's plot for (*S,S*)-**11** and (*S,S*)-1,2-CDP_2 shows a 1:1 stoichiometry for the complexation (closed circles in Figure 3b), and the log K_{app} for (*S,S*)-**11** and (*S,S*)-1,2-CDP_2 was estimated to be > 6 at pD 7.4 and 25 °C by nonlinear least-squares curve fitting of the titration data in Figure 3a. A shift in the ^{31}P NMR signal (peak) ($\Delta\delta$) for (*S,S*)-1,2-CDP_2 (5 mM) upon the

(36) (a) Cori, C. F.; Colowick, S. P.; Cori, G. T. *J. Biol. Chem.* **1937**, *121*, 465–477. (b) Kumler, W. D.; Eiler, J. J. *J. Am. Chem. Soc.* **1943**, *65*, 2355–2361.

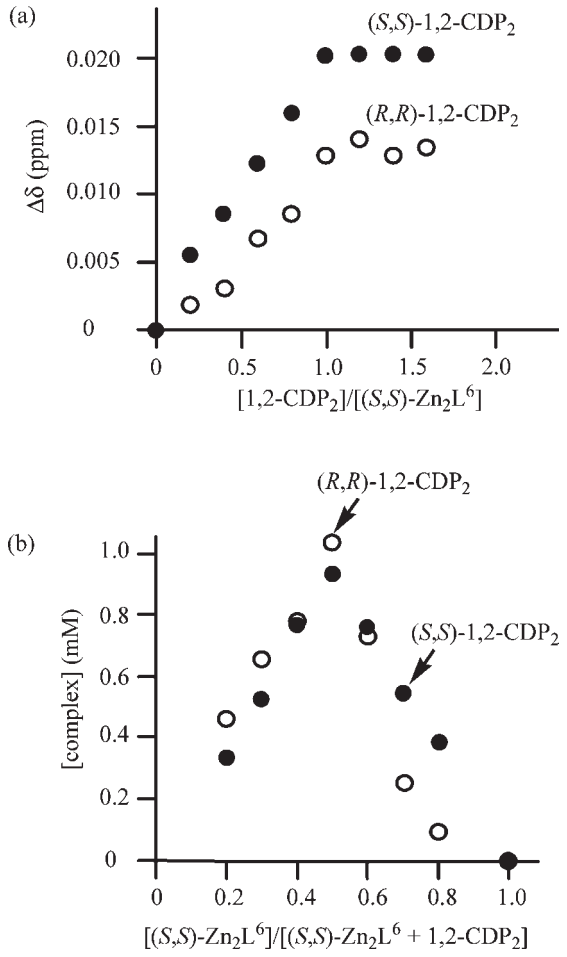


Figure 3. (a) Results of ^{1}H NMR titrations for (*S*,*S*)-**11** ((*S*,*S*)-Zn$_2$L^6) (1 mM) with increasing concentration of (*S*,*S*)-**12** ((*S*,*S*)-1,2-CDP$_2$) (closed circles) and (*R*,*R*)-**12** ((*R*,*R*)-1,2-CDP$_2$) (open circles) in D$_2$O at pD 7.4 and 25 °C. (b) Job plots of (*S*,*S*)-**11** with (*S*,*S*)-1,2-CDP$_2$ (closed circles) and (*R*,*R*)-1,2-CDP$_2$ (open circles) in D$_2$O at pD 7.4 and 25 °C. [(*S*,*S*)-**11** + *trans*-1,2-CDP$_2$] = 2 mM ([complex] = [[(*S*,*S*)-**11**]$_{total}$ × (Δδ/Δδ$_{max}$)]. The methyl signal of (*S*,*S*)-**11** was used in these NMR titration experiments.

addition of (*S*,*S*)-**11** also indicated strong 1:1 complexation between (*S*,*S*)-**11** and (*S*,*S*)-1,2-CDP$_2$ at pD 7.4 (1 M HEPES buffer) and 25 °C (Figure 4) (The phosphorus signal of (*S*,*S*)-1,2-CDP$_2$ was shifted from 1.810 ppm to 3.334 ppm upon the addition of (*S*,*S*)-**11**). Similar ^{1}H NMR and ^{31}P NMR titration results were obtained for (*S*,*S*)-**11**−(*R*,*R*)-1,2-CDP$_2$ (log K_{app} value >6 for 1:1 complexation; open circles in Figure 3 and 4), (*R*,*R*)-**11**−(*S*,*S*)-1,2-CDP$_2$, and (*R*,*R*)-**11**−(*R*,*R*)-1,2-CDP$_2$ (log K_{app} value >6 for 1:1 complexation, see Table 2).

It should be mentioned that ^{1}H NMR and ^{31}P NMR spectra of a mixture of **11** and 1,2-CDP$_2$ in D$_2$O under these conditions exhibited averaged signals of uncomplexed and complexed species of **11**, meaning that complexes of **11** with 1,2-CDP$_2$ are thermodynamically stable but kinetically labile on the NMR time scale.[11,18]

^{1}H NMR titrations of (*S*,*S*)-**11** with *trans*-1,4-CDP$_2$ showed inconsistent changes in the chemical shifts (data not shown). An irregular change in chemical shifts for the **7**−(*S*,*S*)-1,2-CDP$_2$ complex was also observed in a ^{1}H NMR titration experiment (data not shown). These results indicate that these host−guest complexations

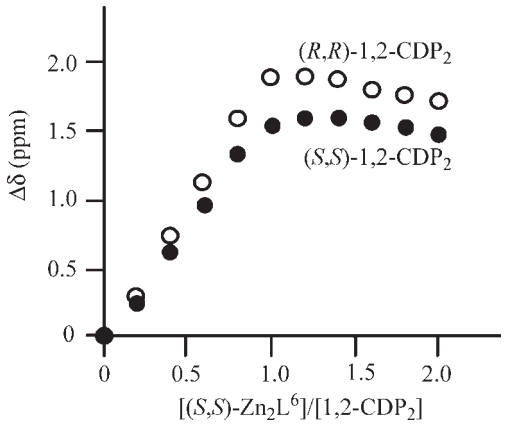


Figure 4. ^{31}P NMR titration curves for (*S*,*S*)-**12** ((*S*,*S*)-1,2-CDP$_2$) (closed circles) and (*R*,*R*)-**12** ((*R*,*R*)-1,2-CDP$_2$) (open circles) with (*S*,*S*)-**11** ((*S*,*S*)-Zn$_2$L^6) at pD 7.4 (1 M HEPES). [(*S*,*S*)-1,2-CDP$_2$] = [(*R*,*R*)-1,2-CDP$_2$] = 5 mM.

occur not only in a 1:1 ratio but that several other ratios are also involved, as supported by the electrospray ionization-mass spectrometry (ESI-MS), as described below. The results of a ^{1}H NMR titration of **7** (Zn$_2$L^4) with (*S*,*S*)-1,2-CDP$_2$ and other CDP$_2$ suggest that several complexes are formed.

ESI-MS Study of Complexes of (*S*,*S*)-11 ((*S*,*S*)-Zn$_2$L^6) and 7 (Zn$_2$L^4) with CDP$_2$. Figure 5 shows ESI (positive) mass spectra for mixtures of (*S*,*S*)-**11**·(NO$_3$)$_4$ with (a) Na$_4$·(*S*,*S*)-1,2-CDP$_2$, (b) Na$_4$·*cis*-1,3-CDP$_2$, and (c) Na$_4$·*trans*-1,4-CDP$_2$ in H$_2$O at pH 7.4 ([(*S*,*S*)-**11**·(NO$_3$)$_4$] = [Na$_4$·CDP$_2$] = 0.2 mM) (See the Supporting Information, Figures S4−S6 for assignments of the ESI mass spectra). Various ESI-MS conditions (ion source temperature: rt or 250 °C, orifice voltage: 40, 80, or 120 V) gave similar results. Signals at m/z = 447−453 (peak i), 458−464 (peak ii), 871−883 (peak iii), and 893−905 (peak iv) in Figure 5a were assigned as [((*S*,*S*)-**11**)$^{4+}$ + (Na·H·(*S*,*S*)-1,2-CDP$_2$)$^{2-}$]$^{2+}$, [((*S*,*S*)-**11**)$^{4+}$ + (Na$_2$·(*S*,*S*)-1,2-CDP$_2$)$^{2-}$]$^{2+}$, [((*S*,*S*)-**11**)$^{4+}$ + (H·(*S*,*S*)-1,2-CDP$_2$)$^{3-}$]$^{+}$, and [((*S*,*S*)-**11**)$^{4+}$ + (Na·(*S*,*S*)-1,2-CDP$_2$)$^{3-}$]$^{+}$, respectively, which fit to the theoretical mass distribution spectra for a 1:1 (*S*,*S*)-**11**−(*S*,*S*)-1,2-CDP$_2$ complex (For a representative example, see the Supporting Information, Figure S7). In the case of *cis*-1,3-CDP$_2$ and *trans*-1,4-CDP$_2$ (Figure 5b and 5c), peak v was observed at m/z = 796−804, which indicates the presence of 2:1 (*S*,*S*)-**11**−CDP$_2$ complexes ([2((*S*,*S*)-**11**)$^{4+}$ + (CDP$_2$)$^{4-}$·2(NO$_3^-$)]$^{2+}$). These results imply that (*S*,*S*)-**11** forms a 1:1 complex with (*S*,*S*)-1,2-CDP$_2$, while mixtures of (*S*,*S*)-**11** with *cis*-1,3-CDP$_2$ and *trans*-1,4-CDP$_2$ afford several complexes including 1:1, 2:1 and other complexes.

For comparison, we also performed ESI-MS experiments (positive) for mixtures of **7**·(NO$_3$)$_4$ with (a) Na$_4$·(*S*,*S*)-1,2-CDP$_2$, (b) Na$_4$·*cis*-1,3-CDP$_2$, and (c) Na$_4$·*trans*-1,4-CDP$_2$ in H$_2$O at pH 7.4 ([**7**·(NO$_3$)$_4$] = [Na$_4$·CDP$_2$] = 0.2 mM) (Supporting Information, Figures S8−S10), indicates the formation of 1:1, 2:1 and other complexes for mixtures of **7**−*cis*-1,3-CDP$_2$ and **7**−*trans*-1,4-CDP$_2$.

Complexation Properties of (*S*,*S*)-11 ((*S*,*S*)-Zn$_2$L^6) with *trans*-1,2-CDP$_2$, as Examined by Potentiometric pH Titrations. An analysis of a potentiometric pH titration curve for a mixture of 0.5 mM (*S*,*S*)-**11** ((*S*,*S*)-Zn$_2$L^6) and

Table 2. Complexation Constants (log K_{app}) for Ditopic Zinc(II)-cyclen Complexes with CDP_2 at pH 7.4 and 25 °C Determined by Potentiometric pH Titration, ITC, ^{1}H NMR Titration, and ^{31}P NMR Titration

	titration methods	(*S*,*S*)-**11** ((*S*,*S*)-Zn_2L^6)	(*R*,*R*)-**11** ((*R*,*R*)-Zn_2L^6)	**7** (Zn_2L^4)
(*S*,*S*)-**12** ((*S*,*S*)-1,2-CDP_2)	potentiometric pH	7.6[a] (9.3[b])	7.6[a] (9.4[b])	
	ITC	6.9[a]	7.1[a]	complicated complexation
	^{1}H NMR	> 6[a]	> 6[a]	complicated complexation
	^{31}P NMR	> 6[a]		
(*R*,*R*)-**12** ((*R*,*R*)-1,2-CDP_2)	potentiometric pH	7.3[a] (9.2[b])	7.5[a] (9.2[b])	
	ITC	7.0[a]	6.7[a]	
	^{1}H NMR	> 6[a]	> 6[a]	
	^{31}P NMR	> 6[a]		
13 (*cis*-1,3-CDP_2)	ITC	6.3[a]		
14 (*trans*-1,4-CDP_2)	ITC	complicated complexation		

[a] The log K_{app} value determined by eqs 5−7 in the text. [b] The log K_s value defined by eq 4 in the text.

0.5 mM (*S*,*S*)-**12** ((*S*,*S*)-1,2-CDP_2) (curve b in Figure 2) using the "BEST" program software[30] gave a value for the intrinsic complexation constant, log K_s defined by eq 4, of 9.3 at 25 °C with $I = 0.1$ ($NaNO_3$). From this value, an apparent complex formation constant at pH 7.4 defined by eqs 5−7, log K_{app}, was calculated to be 7.6 (Table 2). The pH-dependent speciation diagram (Figure 6) for a mixture of (*S*,*S*)-**11** (0.5 mM) and (*S*,*S*)-1,2-CDP_2 (0.5 mM), obtained using the "SPE" program software,[30] indicates that the complexation was quantitative at physiological pH 6.2−8.6. Similar results were observed for mixtures of (*S*,*S*)-**11**−(*R*,*R*)-1,2-CDP_2, (*R*,*R*)-**11**−(*S*,*S*)-1,2-CDP_2, and (*R*,*R*)-**11**−(*R*,*R*)-1,2-CDP_2 (Table 2). On the basis of the above finding, it can be concluded that the affinities of (*S*,*S*)- and (*R*,*R*)-**11** for both enantiomers of *trans*-1,2-CDP_2 (four combinations in total) are almost identical.

$$Zn_2L^6(H_2O)_2 + (CDP_2)^{4-} \rightleftarrows Zn_2L^6-(CDP_2)^{4-} \text{ complex :}$$

$$K_s = [Zn_2L^6-(CDP_2)^{4-}]/[Zn_2L^6(H_2O)_2]\cdot[(CDP_2)^{4-}] \quad (M^{-1}) \tag{4}$$

$$K_{app} = [Zn_2L^6-(CDP_2)^{4-}]/[Zn_2L^6]_{free}\cdot[(CDP_2]_{free} \quad \text{(at designated pH)} \quad (M^{-1}) \tag{5}$$

$$[Zn_2L^6]_{free} = [Zn_2L^6(H_2O)_2] + [Zn_2L^6(H_2O)(HO^-)] + [Zn_2L^6(HO^-)_2] \tag{6}$$

$$[CDP_2]_{free} = [(CDP_2)] + [(CDP_2)^{1-}] + [(CDP_2)^{2-}] + [(CDP_2)^{3-}] + [(CDP_2)^{4-}] \tag{7}$$

ITC for the Complexation of (*S*,*S*)-11 ((*S*,*S*)-Zn_2L^6) with CDP_2. We studied the complexation of the ditopic Zn^{2+} receptor (*S*,*S*)-**11** ((*S*,*S*)-Zn_2L^6) with (*S*,*S*)-**12** ((*S*,*S*)-1,2-CDP_2), (*R*,*R*)-**12** ((*R*,*R*)-1,2-CDP_2), **13** (*cis*-1,3-CDP_2), and **14** (*trans*-1,4-CDP_2) by ITC at pH 7.4 (50 mM HEPES with $I = 0.1$ ($NaNO_3$)) and 25 °C (Figure 7 and in the Supporting Information, Figures S11−S12). In typical experiments, aqueous solutions of (*S*,*S*)-**11**, in a syringe, were titrated into a cell containing aqueous solutions of CDP_2. A typical curve for the titration of (*S*,*S*)-1,2-CDP_2 (0.1 mM) with (*S*,*S*)-**11** (1 mM), shown in Figure 7 (the reaction was exothermic), was analyzed by means of nonlinear curve fitting. The log K_{app} values defined by eq 5 (6.9 ± 0.1 for a 1:1 complex of (*S*,*S*)-**11**−(*S*,*S*)-1,2-CDP_2 and 7.0 ± 0.1 for (*S*,*S*)-**11**−(*R*,*R*)-1,2-CDP_2) were in reasonably good agreement with the corresponding log K_{app} values obtained by potentiometric pH titration (Table 2), indicating negligible chiral discrimination of (*S*,*S*)- and (*R*,*R*)-1,2-CDP_2 by (*S*,*S*)-**11**. Though a small amount of 2:1 (*S*,*S*)-**11**−*cis*-1,3-CDP_2 was observed in ESI-MS (peak (v) in Figure 5b), a nonlinear curve fitting analysis of an ITC curve for (*S*,*S*)-**11** with *cis*-1,3-CDP_2 suggested a 1:1 complexation with a log K_{app} value of 6.3 (Supporting Information, Figure S11 and Table 2), which is smaller than that for *trans*-1,2-CDP_2. The titration curve for (*S*,*S*)-**11** and *trans*-1,4-CDP_2 (**14**) was complicated (Supporting Information, Figure S12).

Complexation of (*S*,*S*)- and (*R*,*R*)-11 ((*S*,*S*)- and (*R*,*R*)-Zn_2L^6) with Ins(1,4,5)P_3, as Studied by ESI-MS and ITC. Finally, the complexation of (*S*,*S*)- and (*R*,*R*)-**11** with Ins(1,4,5)P_3 was examined. In ESI-MS, several peaks corresponding to a 2:1 complex of **11** and Ins(1,4,5)P_3 were observed (Supporting Information, Figure S13). ITC experiments for Ins(1,4,5)P_3 with (*S*,*S*)- and (*R*,*R*)-**11** at 25 °C and pH 7.4 (50 mM HEPES with $I = 0.1$ ($NaNO_3$)) indicated two step complexations in both cases, while the titration curves were different (Figure 8). Thermal parameters (binding constants, enthalpy and entropy changes) for each complexations are summarized in Table 3, and the proposed binding modes are shown in Scheme 7. The log K_{app1} values for the first binding of Ins(1,4,5)P_3 with (*S*,*S*)-**11** (8.0) and with (*R*,*R*)-**11** (7.6) are somehow greater than the log K_{app} values for **11** and *trans*-1,2-CDP_2 (7.0−6.7) obtained by ITC (Table 3). We therefore concluded that Ins(1,4,5)P_3 binds to the first molecule of (*S*,*S*)-**11** at the P4 and P5 to form 1:1 complex **19**, as shown in Scheme 7. The more negative ΔH value for the complexation with (*S*,*S*)-**11** than that with (*R*,*R*)-**11** indicates somewhat more favorable interaction with (*S*,*S*)-**11**, possibly because of additional interactions such as hydrogen bonding and/or hydrophobic interaction in a complex involving (*S*,*S*)-**11**. However, the enthalpic advantage for the complexation with (*S*,*S*)-**11** ($\Delta\Delta H = -2.6$ kcal/mol) is canceled by the less negative $-T\Delta S$ ($\Delta(-T\Delta S) = 2.1$ kcal/mol), which results in a similar log K_{app1} value (ΔG value) for complexation to that with (*R*,*R*)-**11**. These results suggest that this complexation system obeys the empirical rule of enthalpy−entropy compensation.[37,38]

The second log K_{app2} determined from data shown in Figure 8 were 5.9 for (*S*,*S*)-**11** and 5.5 for (*R*,*R*)-**11**, which

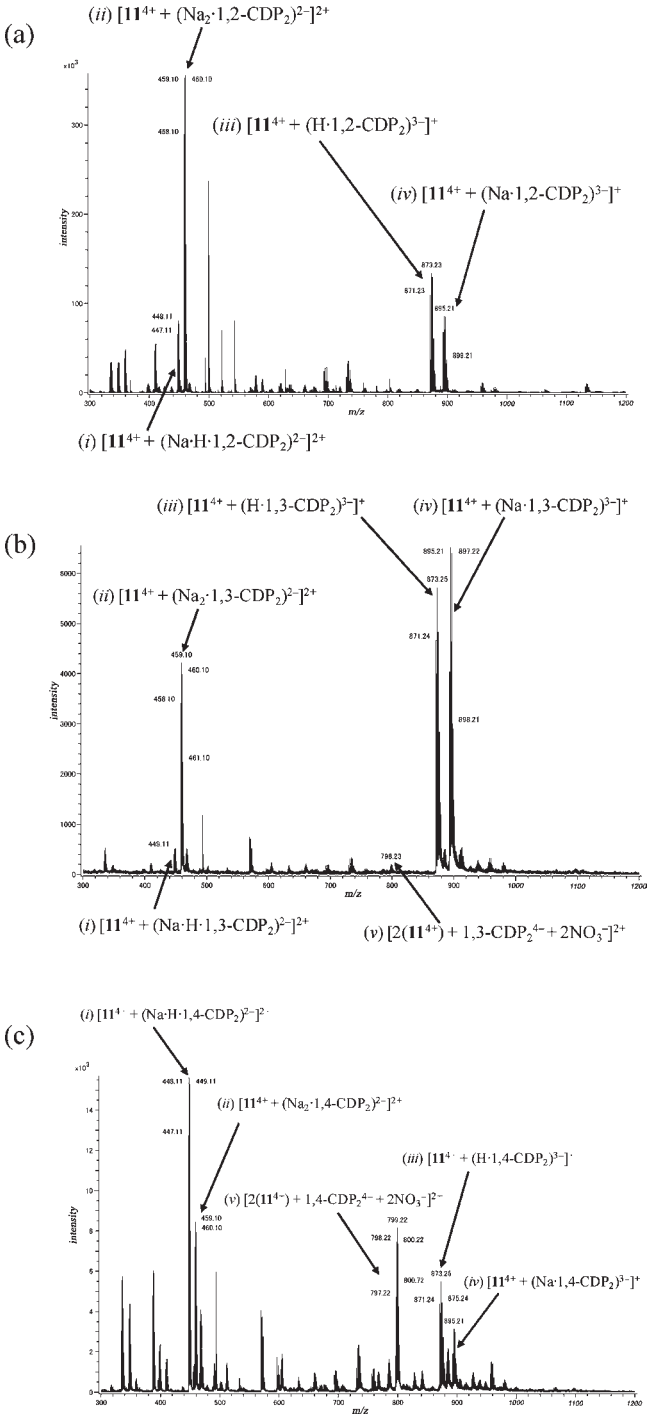


Figure 5. ESI (positive) mass spectra of (*S*,*S*)-**11**·$(NO_3)_4$ with (a) Na_4·(*S*,*S*)-1,2-CDP_2, (b) Na_4·*cis*-1,3-CDP_2, and (c) Na_4·*trans*-1,4-CDP_2 in H_2O at pH 7.4 (ion source temperature: rt, orifice voltage: 80 V). [(*S*,*S*)-**11**·$(NO_3)_4$] = [CDP_2] = 0.2 mM.

are much larger than the log K_{app} value for 1:1 complexations between a monomeric Zn^{2+}−cyclen complex and

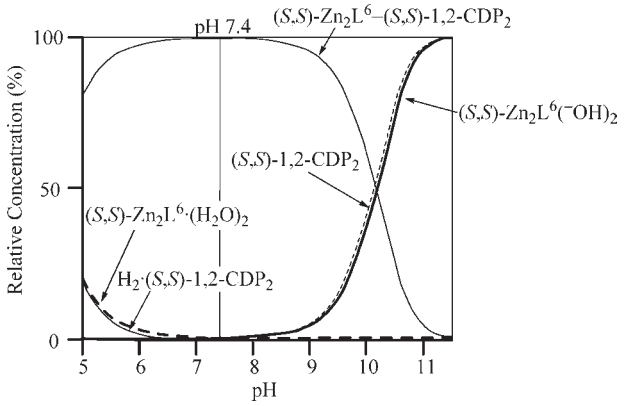


Figure 6. Distribution diagram for an aqueous solution of 0.5 mM (*S*,*S*)-**11** and 0.5 mM (*S*,*S*)-1,2-CDP_2 at 25 °C with I = 0.1 ($NaNO_3$). Species that are present in concentrations of less than 5% are omitted for clarity.

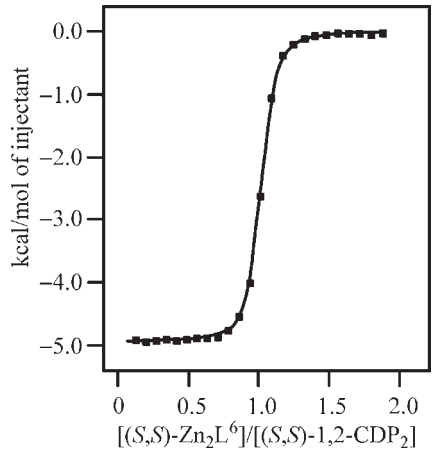


Figure 7. Typical isothermal calorimetric titration curve of (*S*,*S*)-**11** ((*S*,*S*)-Zn_2L^6) with (*S*,*S*)-1,2-CDP_2 (closed square). A solution of (*S*,*S*)-**11** was titrated into 1.4 mL of (*S*,*S*)-1,2-CDP_2 at 25 °C and pH 7.4 (50 mM HEPES with I = 0.1 ($NaNO_3$)). [(*S*,*S*)-**11**] = 1 mM, [(*S*,*S*)-1,2-CDP_2] = 0.1 mM. The solid line represents the best fit using a one binding site model.

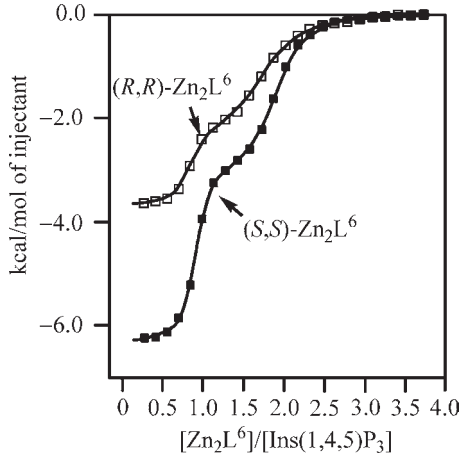


Figure 8. ITC results for complex formation of Ins(1,4,5)P_3 with (*S*,*S*)-**11** ((*S*,*S*)-Zn_2L^6) (closed square) and (*R*,*R*)-**11** ((*R*,*R*)-Zn_2L^6) (open square). A solution of 1 mM **11** in 50 mM HEPES was titrated into 1.4 mL of 0.045 mM Ins(1,4,5)P_3 at 25 °C and pH 7.4 (50 mM HEPES with I = 0.1 ($NaNO_3$)). The solid lines represent the best fit, obtained using a two binding sites model.

(37) (a) Inoue, Y.; Wada, T. *Adv. Supramol. Chem.* **1997**, *4*, 55–96. (b) Rekharsky, M. V.; Inoue, Y. *Chem. Rev.* **1998**, *98*, 1875–1918. (c) Houk, K. N.; Leach, A. G.; Kim, S. P.; Zhang, X. *Angew. Chem., Int. Ed.* **2003**, *42*, 4872–4897.

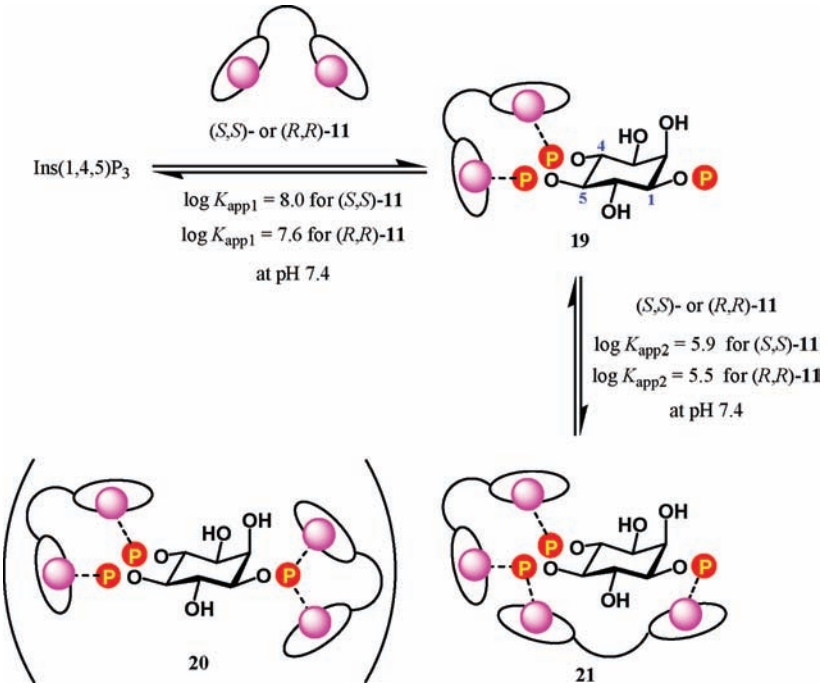
(38) Most disscussion about enthalpy−entropy compensation has been done without metal-containing hosts and/or charged guests (ref 37). As for molecular recognition by metal hosts, Mallik et al. reported the enthalpy−entropy compensation for Cu^{2+}−peptide complexes. Sun, S.; Fazal, M. A.; Roy, B. C.; Chandra, B.; Mallik, S. *Inorg. Chem.* **2002**, *41*, 1584–1590.

Table 3. Thermodynamic Parameters for (*S*,*S*)-**11**−Ins(1,4,5)P_3, (*R*,*R*)-**11**−Ins(1,4,5)P_3, (*S*,*S*)-**11**−(*R*,*R*)-1,2-CDP_2, (*R*,*R*)-**11**−(*R*,*R*)-1,2-CDP_2, and (*S*,*S*)-**11**−*cis*-1,3-CDP_2 Complexes at pH 7.4 and 25 °C. Determined by ITC

host−guest	log K_{app}	ΔH (kcal/mol)	$-T\Delta S$ (kcal/mol)
(*S*,*S*)-**11**−Ins(1,4,5)P_3	8.0[a] (log K_{app1})	−6.3[a]	−4.6[a]
	5.9[b] (log K_{app2})	−3.1[b]	−4.9[b]
(*R*,*R*)-**11**−Ins(1,4,5)P_3[a]	7.6[a] (log K_{app1})	−3.7[a]	−6.7[a]
	5.5[b] (log K_{app2})	−2.4[b]	−5.2[b]
(*S*,*S*)-**11**−(*R*,*R*)-1,2-CDP_2	7.0	−5.2	−4.4
(*R*,*R*)-**11**−(*R*,*R*)-1,2-CDP_2	6.7	−4.9	−4.3
(*S*,*S*)-**11**−*cis*-1,3-CDP_2	6.3	−3.4	−5.2

[a] For first complexation in Scheme 7. [b] For second complexation in Scheme 7.

Scheme 7



phosphate monoester dianions (normally, log K_{app} = 3−4).[13,18] We initially hypothesized that two Zn^{2+}−cyclen units of **11** simultaneously bind to P1 in a sandwich manner,[34] as shown in **20** (Scheme 7). However, ITC curves for (*S*,*S*)-**11** with monophosphates such as Glu-1-P were complicated (see Supporting Information, Figure S14). Accordingly, the second binding of Ins(1,4,5)P_3 with **11** was attributed to ditopic complexation at P1 and P5, as indicated by **21** in Scheme 7, because the log K_{app} value for 1:1 complexation of (*S*,*S*)-**11** with *cis*-1,3-CDP_2 was approximately 6 and the ΔH and $-T\Delta S$ values are almost identical in both cases. It should be noted that the first 1:1 complex of Ins(1,4,5)P_3 with **11** (K_d = 10−25 nM at pH 7.4) is 100-fold more stable than that for the second binding (K_d = 1300−3200 nM at pH 7.4) and is comparable to that with natural $InsP_3R$ despite of only two-phosphate binding sites. These data could be useful for design and synthesis of the potent receptors and sensors for Ins(1,4,5)P_3 and/or inhibitors of Ins(1,4,5)P_3-related intracellular signal transduction pathways.

Moreover, it has been established that Zn^{2+}−cyclen complexes bind to dianions of phosphate monoesters, but negligibly to monoanions of phosphate diesters in aqueous solution.[18] Therefore, it can be expected that chiral bis(Zn^{2+}−cyclen) complexes such as **11** could be a potential platform for the design of receptors and sensors for phosphatidylinositol bisphosphate derivatives including phosphatidylinositol 4,5-bisphosphate (PtdIns(4,5)P_2) and stereosiomers, whose P1 makes a phosphodiester moiety that connects the inositol part and the lipid (diacylglycerol) part.

Conclusion

We describe the design and synthesis of some chiral ditopic zinc complexes (*S*,*S*)- and (*R*,*R*)-**11** ((*S*,*S*)- and (*R*,*R*)-Zn_2L^6) containing chiral linkers and examined their complexation with CDP_2 derivatives, which can be assumed to be model compounds for Ins(1,4,5)P_3. The X-ray crystallographic analysis of (*S*,*S*)-**11** showed that the distance between two Zn^{2+} ions in the complex is 6.8 Å, which is consistent with the P4 and P5 of Ins(1,4,5)P_3 or two phosphates of *trans*-1,2-CDP_2. The pK_{a4} value for *trans*-1,2-CDP_2 was 8.4, and greater than those of *cis*-1,3-CDP_2 and *trans*-1,4-CDP_2, suggesting the involvement of hydrogen bonding between these two adjacent phosphates, possibly fixing the molecule in a diequatorial conformation at neutral pH, as previously described by Hiskey. On the basis of ESI-MS, ITC, ^{1}H NMR, and ^{31}P NMR titrations, both enantiomers of **11** formed 1:1 complexes with *trans*-1,2-CDP_2 (log K_{app} = 7−8) in aqueous

solution at physiological pH, although enantioselectivity in the recognition of optically pure *trans*-1,2-CDP_2 by **11** was not so apparent. On the basis of the ITC data for Ins(1,4,5)P_3 with (*S,S*)- and (*R,R*)-**11**, we concluded that (*S,S*)- and (*R,R*)-**11** form 2:1 complexes with Ins(1,4,5)P_3 (**21** in Scheme 7), in which the first molecule of **11** cooperatively recognizes P4 and P5 of Ins(1,4,5)P_3 (in **19**), whose affinity (K_d = 10−25 nM) is comparable to that for $InsP_3R$, and the second molecule of **11** binds to P1 and P5 (**21**). Although the log K_{app} values for first complexations of Ins(1,4,5)P_3 with (*S,S*)- and (*R,R*)-**11** were almost identical, differences in thermodynamic parameters (enthalpy and entropy changes) suggest different binding modes for the two combinations.[39] These results may provide important information for the design and synthesis of chemical receptors, sensors, and inhibitors for Ins(1,4,5)P_3 and related compounds such as PtdIns(4,5)P_2, and for the design of supramolecular complexes using phosphate−metal coordination bonds.

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Supporting Information Available: Synthesis of (*S,S*)- and (*R,R*)-1,2-CDP_2, *cis*-1,3-CDP_2, and *trans*-1,4-CDP_2. ITC data for **3** (Zn_3L^2) with Ins(1,4,5)P_3, for the complexes of (*S,S*)-**11** with *cis*-1,3-CDP_2 and *trans*-1,4-CDP_2, and (*S,S*)-**11** with Glu-1-P. Potentiometric pH titration data for CDP_2 ((*S,S*)- and (*R,R*)-1,2-CDP_2, *cis*-1,3-CDP_2, and *trans*-1,4-CDP_2). ^{1}H NMR spectral changes during the titration of (*S,S*)-**11** with (*S,S*)-1,2-CDP_2. Assignments of the ESI-MS for (*S,S*)-**11** and **7** with (*S,S*)-1,2-CDP_2, *cis*-1,3-CDP_2, and *trans*-1,4-CDP_2. ESI-MS spectrum for the 2:1 complex of (*S,S*)-**11** and Ins(1,4,5)P_3. Crystallographic parameters for (*S,S*)-**11**. This material is available free of charge via the Internet at http://pubs.acs.org.

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