Supporting Information

for

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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Synthesis of Na₄(S,S)-12 (Na₄(S,S)-1,2-CDP₂), Na₄(R,R)-12 (Na₄(R,R)-1,2-CDP₂), Na₄-13 (Na₄cis-1,3-CDP₂) and Na₄-14 (Na₄trans-1,4-CDP₂). (S,S)- and (R,R)-12 were synthesized from (S,S)- and (R,R)-22 (>99% ee) prepared by the kinetic resolution of trans-1,2-cyclohexanediol diacetate using an enzyme (Lipase Amano P30) according to a literature procedure¹ (Scheme S1). The phosphorylation of 22 followed by oxidation with m-CPBA afforded (S,S)-23 in 68% yield and (R,R)-23 in 78% yield, respectively. (S,S)- and (R,R)-12 were obtained as sodium salts by the catalytic deprotection of the benzyl groups with H₂–Pd/C and basification with aqueous sodium hydroxide. Na₄-13 (Na₄cis-1,3-CDP₂) and Na₄-14 (Na₄trans-1,4-CDP₂) were prepared in a similar manner as that for (S,S)-12 ((S,S)-1,2-CDP₂) from 24² and 26³ (Scheme S1).
Scheme S1

**O,O’-Bis(dibenzyloxyphosphoryl)-(S,S)-1,2-cyclohexanediol.** ((S,S)-23). To a CH₂Cl₂ solution (10 mL) of (S,S)-1,2-cyclohexanediol ((S,S)-22, >99% ee)¹ (154 mg, 1.32 mmol) and 1H-tetrazole (556 mg, 7.93 mmol) was added iPr₂NP(OBn)₂ (1.23 g, 3.57 mmol) at 0 °C. After stirring for 2 h at rt, 3-chloroperbenzoic acid (m-CPBA) (1.37 g, 7.93 mmol) was added to the reaction mixture at −40 °C, and the resulting mixture was stirred overnight at rt, and then filtered to remove insoluble solids. The filtrate was washed with sat. Na₂S₂O₃ and the aqueous layer was extracted with CH₂Cl₂. The combined organic layer was washed with sat. NaHCO₃ and brine, dried over Na₂SO₄. After filtration and evaporation, the resulting residue was purified by silica gel column chromatography (hexane/AcOEt =
5:1 to AcOEt only) to afford \((S,S)-\text{23}\) as a colorless liquid (842 mg, quant.). \([\alpha]^0_{D} = 19.9\) (c = 1.0 in CHCl₃). IR (neat): \(\nu = 3064, 3033, 2943, 2867, 1497, 1455, 1381, 1276, 1214, 1037, 1013, 920, 897, 881, 844, 737, 697\) cm\(^{-1}\). \(^1\)H NMR (300 MHz, CDCl₃/TMS): \(\delta = 1.21–1.25\) (2H, br), 1.40–1.52 (2H, br), 1.60–1.70 (2H, br), 2.15–2.19 (2H, \(d, J = 13.2\) Hz), 4.26–4.36 (2H, m), 4.99 (4H, \(d, J = 7.7\) Hz), 5.01 (4H, \(d, J = 7.5\) Hz), 7.24–7.33 ppm (20H, m). \(^13\)C NMR (100 MHz, CDCl₃/TMS): \(\delta = 22.76, 31.04, 69.10\) (d, \(J_{C-P} = 5.0\) Hz), 69.25 (d, \(J_{C-P} = 5.8\) Hz), 127.82, 127.85, 128.31, 128.38, 128.46, 128.49, 135.89 (d, \(J_{C-P} = 7.4\) Hz), 135.91 ppm (d, \(J_{C-P} = 7.4\) Hz). \(^31\)P NMR (162 MHz, CDCl₃/external 85\% H₃PO₄): \(\delta = -1.21\) ppm. HRMS (FAB\(^+\)): calcd for [M+H]\(^+\), 637.2115; found, 637.2120.

**Na₄(S,S)-12·3H₂O (Na₄(S,S)-1,2-CDP₂·3H₂O).** A mixture of \((S,S)-\text{23}\) (842 mg, 1.32 mmol) and Pd/C (5 wt. %, 100 mg) in degassed MeOH (10 mL) was stirred for 10 h at rt under a H\(_2\) atmosphere (1 atm). The mixture was filtered through a celite pad to remove the catalyst and the resulting solution evaporated. To the resulting residue, 1N NaOH\(_{aq}\) (5.3 mL, 5.3 mmol) was added at rt, and the mixture was filtered through a pad of cotton to remove the insoluble solids. After evaporation of the filtrate, precipitation with MeOH afforded Na₄(S,S)-1,2-CDP₂ as a colorless solid (410 mg, 85% yield). Mp >300 °C (dec.). \([\alpha]^0_{D} = 12.1\) (c = 0.5 in H₂O). Anal. Calcd (%) for C\(_6\)H\(_{16}\)Na₄O\(_{11}\)P\(_2\): C, 17.24; H, 3.86. Found: C, 17.17; H, 4.00. IR (KBr pellet): \(\nu = 3374, 2937, 2863, 1654, 1097, 990, 971, 881, 794\) cm\(^{-1}\). \(^1\)H NMR (300 MHz, D\(_2\)O/TSP): \(\delta = 1.23–1.37\) (2H, br), 1.37–1.54 (2H, br), 1.56–1.70 (2H, br), 1.99–2.15 (2H, br), 3.87–4.00 (2H, br). \(^13\)C NMR (75 MHz, D\(_2\)O/1,4-dioxane): \(\delta = 23.03, 31.32, 75.91\) ppm (m). \(^31\)P NMR (162 MHz, D\(_2\)O/external 85\% H₃PO₄): \(\delta = 3.70\) ppm. HRMS (FAB\(^-\)): calcd for [M–Na\(^+\)], 340.9544; found, 340.9543.

**\(O,O'\)-Bis(dibenzyloxyphosphoryl)-cis-1,3-cyclohexanediol (25).** The 25 was synthesized from 24 in a similar manner as that for \((S,S)-\text{23}\). colorless oil (quant). IR (neat): \(\nu = 3479, 3064, 3033, 2949, 2868, 1498, 1456, 1381, 1273, 1215, 1022, 903, 789, 737, 698, 604, 451\) cm\(^{-1}\). \(^1\)H NMR (300 MHz, CDCl₃/TMS): \(\delta = 1.01–1.34\) (3H, m), 1.56 (1H, \(q, J = 11.2\) Hz), 1.68–1.75 (1H, m), 1.91–2.00 (2H, m), 2.35–2.40 (1H, m), 4.13–4.22 (2H, m), 4.99 (8H, \(d, J = 8.3\) Hz), 7.25–7.35 ppm (20H, m). \(^13\)C NMR
Na₄cis-1,3-CDP₂·4.5H₂O (Na₄13·4.5H₂O). A mixture of 25 (250 mg, 0.393 mmol) and Pd/C (5 wt. %, 60 mg) in degassed MeOH (6 mL) was stirred for 9 h at rt under a H₂ atmosphere (1 atm). The mixture was filtered through a celite pad to remove the catalyst and evaporated. The resulting solid was passed through a column of ion exchange resin (Dowex 50WX8 as H⁺ form) and water was removed under a vacuum. To the resulting residue, 1N NaOHaq (1.57 mL, 1.57 mmol) was added at rt. After evaporation, precipitation with MeOH/EtOH afforded Na₄cis-1,3-CDP₂ as a colorless solid (148 mg, 85% yield). Anal. Calcd (%) for C₆H₁₉Na₄O₁₂.₅P₂: C, 16.19; H, 4.30. Found: C, 16.15; H, 4.08. IR (KBr pellet): ν = 3330, 2937, 1668, 1466, 1390, 1039, 968, 872, 816, 667, 594 cm⁻¹. ¹H NMR (300 MHz, CDCl₃/TSP): δ = 1.19–1.31 (d, Jₐ-C = 5.0 Hz), 3.99 (t, Jₐ-C = 5.6 Hz), 6.93 (d, Jₐ-C = 5.6 Hz), 7.45 (d, Jₐ-C = 6.2 Hz), 127.64 (d, Jₐ-C = 2.5 Hz), 128.24 (d, Jₐ-C = 1.9 Hz), 128.29 (d, Jₐ-C = 1.2 Hz), 135.53 (d, Jₐ-C = 7.4 Hz). ³¹P NMR (162 MHz, CDCl₃/external 85% H₃PO₄): δ = –1.24 ppm. HRMS (FAB⁺): calcd for [M+H]⁺, 637.2115; found, 637.2119.

O,O'-Bis(dibenzyloxyphosphoryl)-trans-1,4-cyclohexanediol (27). The 27 was synthesized in a similar manner as that for (S,S)-23. A colorless solid (287 mg, 52% yield). IR (KBr pellet): ν = 3032, 2952, 2926, 1541, 1496, 1454, 1381, 1257, 1217, 995, 904, 876, 839, 744, 696, 665, 600, 492 cm⁻¹. ¹H NMR (300 MHz, D₂O/TSP): δ = 1.50–1.54 (m), 1.83 (d, J = 8.4 Hz), 4.40 (2H, br), 5.01 (4H, d, J = 8.3 Hz), 5.02 (4H, d, J = 8.4 Hz), 7.31–7.37 (20H, m). ¹³C NMR (75 MHz, D₂O/1,4-dioxane): δ = 20.15, 32.72, 41.98, 71.47 (d, Jₐ-C = 5.0 Hz) ppm (m). ³¹P NMR (162 MHz, D₂O/external 85% H₃PO₄): δ = 4.19 ppm.

Na₄trans-1,4-CDP₂·1.5H₂O (Na₄14·1.5H₂O). The trans-1,4-CDP₂ was synthesized in a similar manner as that for (S,S)-12. A colorless solid (180 mg, 80% yield). IR (KBr pellet): ν = 3332, 2968,
1375, 1128, 1063, 1018, 974, 879, 675, 538 cm\(^{-1}\).  \(^{1}\)H NMR (300 MHz, D\(_2\)O/TSP): \(\delta = 1.37\) (4H, t, \(J = 9.5\) Hz), 2.05 (4H, d, \(J = 5.3\) Hz), 3.92–3.94 ppm (m, 2H).  \(^{13}\)C NMR (75 MHz, CDCl\(_3\)/TMS): \(\delta = 32.20\) (d, \(J_{C-P} = 3.7\) Hz) 73.05 (d, \(J_{C-P} = 5.0\) Hz).  \(^{31}\)P NMR (162 MHz, D\(_2\)O/external 85% H\(_3\)PO\(_4\)): \(\delta = 4.19\) ppm.  Anal. Calcd (%) for C\(_6\)H\(_{11.5}\)Na\(_4\)O\(_9.5\)P\(_2\): C: 18.43, H: 3.35.  Found: C: 18.18, H: 3.51.
Figure S1. The result of ITC for 3 (Zn3L2) with Ins(1,4,5)P3. An aqueous solution of 1 mM Zn3L2 was titrated into 1.4 mL of 50 μM Ins(1,4,5)P3 in a 50 mM HEPES buffered aqueous solution (pH 7.4, I = 0.1 (NaNO3)) at 25 °C.
Figure S2. Potentiometric pH titration curves for 0.5 mM 4Na·(S,S)-1,2-CDP$_2$ + 2.0 mM HCl (plain curve (a)), 0.5 mM 4Na·cis-1,3-CDP$_2$ + 2.0 mM HCl (bold curve (b)), and 0.5 mM 4Na·trans-1,4-CDP$_2$ + 2.0 mM HCl (dashed curve (c)) with $I = 0.1$ (NaNO$_3$) at 25 °C. $Eq$ (HO$^-$) is the number of equivalents of base (NaOH) added.
Figure S3. $^{1}$H NMR spectral changes in (S,S)-11 (1 mM) in D$_2$O at pD 7.4 ± 0.2 and 25 °C with increasing concentration of (S,S)-1,2-CDP$_2$. (a) 0 eq, (b) 0.4 eq, (c) 0.8 eq, (d) 1.0 eq, (e) 1.2 eq, and (e) 1.6 eq of (S,S)-1,2-CDP$_2$ were added.
Figure S4. ESI (positive) mass spectral assignments for (S,S)-11 ([S,S]-Zn$_2$L$_6$(NO$_3$)$_4$) with Na$_4$(S,S)-1,2-CDP$_2$ in H$_2$O at pH 7.4. [([S,S]-Zn$_2$L$_6$(NO$_3$)$_4$) = [Na$_4$(S,S)-1,2-CDP$_2$] = 0.2 mM. Ion source temperature: rt, orifice voltage: 80 V.
Figure S5.  ESI (positive) mass spectral assignments for (S,S)-**11** ((S,S)-Zn$_2$L$^6$. (NO$_3$)$_4$) with Na$_4$cis-1,3-CDP$_2$ in H$_2$O at pH 7.4.  [(S,S)-Zn$_2$L$^6$. (NO$_3$)$_4$] = [Na$_4$cis-1,3-CDP$_2$] = 0.2 mM.  Ion source temperature: rt, orifice voltage: 80 V.
Figure S6. ESI (positive) mass spectral assignments for (S,S)-\(11\) \(((S,S)-\text{Zn}_2L_6\cdot(\text{NO}_3)_4)\) with \(\text{Na}_4\cdot\text{trans}-1,4\text{-CDP}_2\) in H\(_2\)O at pH 7.4. \([(S,S)-\text{Zn}_2L_6\cdot(\text{NO}_3)_4] = [\text{Na}_4\cdot\text{trans}-1,4\text{-CDP}_2] = 0.2\) mM. Ion source temperature: rt, orifice voltage: 80 V.
Figure S7. (a) ESI (positive) mass spectrum of a (S,S)-Zn2L₆–(S,S)-1,2-CDP₂ complex at \( m/z = 871–883 \) (peak (iii)) in Figure 5. (b) Theoretical distribution for \([(S,S)-Zn₂L̄^6 + (H·(S,S)-1,2-CDP₂)^3⁻]^+\) complex \((C_{26}H_{61}N₈O₁₀P₂Zn₂)\).
Figure S8. ESI (positive) mass spectral assignments for 7 (Zn$_2$L$_4$·(NO$_3$)$_4$) with Na$_4$·(S,S)-1,2-CDP$_2$ in H$_2$O at pH 7.4. [Zn$_2$L$_4$·(NO$_3$)$_4$] = [Na$_4$·(S,S)-1,2-CDP$_2$] = 0.2 mM. Ion source temperature: rt, orifice voltage: 80 V.
Figure S9. ESI (positive) mass spectral assignments for 7 (Zn$_2$L$_4$·(NO$_3$)$_4$) with Na$_4$·cis-1,3-CDP$_2$ in H$_2$O at pH 7.4. [Zn$_2$L$_4$·(NO$_3$)$_4$] = [Na$_4$·cis-1,3-CDP$_2$] = 0.2 mM. Ion source temperature: rt, orifice voltage: 80 V.
Figure S10. ESI (positive) mass spectral assignments for 7 (Zn₂L₄(NO₃)₄) with Na₄·trans-1,4-CDP₂ in H₂O at pH 7.4. [Zn₂L₄(NO₃)₄] = [Na₄·trans-1,4-CDP₂] = 0.2 mM. Ion source temperature: rt, orifice voltage: 80 V.
**Figure S11.** An isothermal calorimetric titration curve for (S,S)-\textbf{11} ((S,S)-Zn$^2$L$^6$) with \textit{cis}-1,3-CDP$_2$ (closed squares). A solution of (S,S)-\textbf{11} was titrated into 1.4 mL of \textit{cis}-1,3-CDP$_2$ at 25 °C and pH 7.4 (50 mM HEPES with $I = 0.1$ (NaNO$_3$)). [(S,S)-\textbf{11}] = 1 mM, [\textit{cis}-1,3-CDP$_2$] = 0.088 mM. The solid line represents the best fit using a one binding site model.
Figure S12. Isothermal calorimetric titration result for \((S,S)-\text{11} ((S,S)-\text{Zn}_2\text{L}^6)\) with \textbf{14} (\textit{trans}-1,4-CDP\textsubscript{2}). A solution of 1 mM \((S,S)-\text{Zn}_2\text{L}^6\) was titrated into 1.4 mL of 0.1 mM \textit{trans}-1,4-CDP\textsubscript{2} at 25 °C and pH 7.4 (50 mM HEPES with \(I = 0.1\) (NaNO\textsubscript{3})).
Figure S13. ESI (positive) mass spectrum of (S,S)·11·(NO₃)₄ ((S,S)-Zn₂L₆·(NO₃)₄) with Ins(1,4,5)P₃ in H₂O at pH 7.4. [(S,S)-Zn₂L₆·(NO₃)₄] = 0.08 mM, [Ins(1,4,5)P₃] = 0.04 mM. Ion source temperature: rt, orifice voltage: 100 V.
**Figure S14.** Isothermal calorimetric titration results for (S,S)-**II** ((S,S)-Zn$_2$L$_6^6$) with D-glucose-α-1-phosphate (Glu-1-P). A solution of 10 mM (S,S)-Zn$_2$L$_6^6$ was titrated into 0.2 mL of 0.5 mM Glu-1-P at 25 °C and pH 7.4 (200 mM HEPES with $I = 0.1$ (NaNO$_3$)).
**Table S1.** Parameters for the X-ray Crystal Structure Analysis of (S,S)-11 ((S,S)-Zn$_2$L$_6$).

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The structure was solved by direct method$^4$ and refined using least squares techniques (SHELXL97$^5$).