Supporting Information

Efficient promotion of phosphate diester cleavage by a face-to-face cyclodextrin dimer without metal

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Abbreviations

HPNP 2-hydroxypropyl-4-nitrophenyl phosphate
NPP 4-nitrophenol phosphate

Experimental Procedures

Materials: 2,6-Bis(bromomethyl)-pyridine, Bis(4-nitrophenyl) phosphate (BNPP) were purchased from Sigma-Aldrich; 3-mono-amino-β-cyclodextrin hydrate were purchased from TCI; diisopropylethylamine were purchased from Alfa Aesar. Meso-tetra(4-sulfonatophenyl) porphine (TPPS) was purchased from Frontier Scientific. These reagents were used without further purification. DMF were dried over CaH₂ and then distilled under reduced pressure prior to use. Water used in all physical measurement experiments was purified with Milli-Q Water Purification System and the resistivity of the treated water is more than 18 MegaΩ cm⁻¹.

Physical Measurements: ¹H NMR, ¹³C NMR and ³¹P NMR spectra were recorded on a Varian Mercury plus 300 spectrometers and the ¹³C NMR and ³¹P NMR are proton-decoupled. Elemental contents were analyzed by a Perkin-Elmer 240 elemental analyzer. ESI-MS spectra were performed on a Thremo LCQ-DECA-XP spectrometer. UV-vis spectra were monitored with a Varian Cary 100 UV/Vis spectrophotometer equipped with a temperature controller (± 0.1 K).

Potentiometric Titration: An automatic titrator (Metrohm 702GPD Titrino) coupled to a Metrohm electrode was used and calibrated according to the Gran method.¹ The electrode system was calibrated with buffers and checked by titration of HClO₄ with NaOH solution (0.10 M). The thermostated cell contained 25 mL of 1.0 mM species in aqueous solutions with the ionic strength maintained at 0.10 M by sodium perchlorate. All titrations were carried out in aqueous solutions under nitrogen at 298 ± 0.1K, and initiated by adding fixed volumes of 0.10 M standard NaOH in small increments to the titrated solution. Triplicate measurements were performed, for which the experimental error was below 1%. The titration data were fitted from the raw data with the Hyperquad 2000 program to calculate the logβ
and the pKₐ values of species.
Kinetic Experimental Details: The rate of BNPP cleavage was measured by an initial slope method following the absorption increase at the 400 nm of the released 4-nitrophenoxide (NP) in aqueous solution at 308 ± 0.1 K. At this wavelength, the absorbance of the ester substrate was negligible. MES (pH 5.70-6.80), MOPSO (pH 6.80-7.20), HEPES (pH 7.20-8.10), TAPS (pH 8.10-9.00), and CHES (pH 9.00-9.60) buffers were used (50 mM), and the ionic strength was adjusted to 0.1 M with NaClO4. The pH of the solution was measured after each run, and all kinetic runs with pH variation larger than 0.1 were excluded. The substrate BNPP, buffers, and L and CuL in aqueous solution were freshly prepared. The reactions were initiated by injecting a small amount of BNPP into the buffer solutions of L and CuL and followed by fully mixing at 308 ± 0.1 K. The visible absorption increase was recorded immediately and was followed generally until 5% formation of 4-nitrophenolate, where ε values for 4-nitrophenolate were 1733 (pH 6.0), 4069 (pH 6.5), 9137 (pH 7.0), 13745 (pH 7.5), 16306 (pH 8.0), 17340 (pH 8.5), 17694 (pH 9.0), 17810 (pH 9.5) at 400 nm (ref. 8d-f in main article). When we investigate the inhibition of TPPS, the concentration of TPPS in sample and reference cells were same in order to exclude the influence of the Soret-band absorbance of TPPS near λ = 400 nm.

The initial pseudo-first-order rate constants of L and CuL, $k_{in}$ (s⁻¹), were obtained directly from a plot of the 4-nitrophenolate concentration versus time by the method of initial rates which was linear with R > 0.995. To correct for the spontaneous cleavage of BNPP, each reaction was measured against a reference cell which was identical to the sample cell in composition except for the absence of L and CuL. Errors on $k_{obs}$ values were less than 15%. The $k_{in}$ (s⁻¹) at different L or CuL concentrations were measured. Then, the second-order rate constants $k_{obs}$ (M⁻¹s⁻¹) were determined as the slope of the linear plots of $k_{in}$ versus [L] or [CuL] (release of two NP moles per mole of BNPP was taken into account). The $k_{obs}$ (M⁻¹s⁻¹) at different pH were measured respectively (see Fig. 1 in main article and Fig. S6, ESI). The data of L was fitted to eqn. 1 to give the $k_{cat}$ of each species.

$$k_{obs} = \frac{(10^{-3}pK_{1})k_{H_{2}L} + (10^{-2}pK_{1})k_{HL} + (10^{-1}pK_{1})k_{L} + (10^{-pK_{1}})k_{L}}{10^{-4}pK_{1} + 10^{-3}pK_{1} + 10^{-2}pK_{1} + 10^{-1}pK_{1} + 10^{0}pK_{1} + 10^{+}pK_{1}}$$

eqn. 1

The effect of BNPP concentration (shown as Fig. 2 in main article) was carried out by varying the BNPP concentration at constant concentration of the L. The non-catalyzed reaction constant $k_{uncat} = 4.0 \times 10^{-11}$ s⁻¹ was from ref. 9b in main article.

Synthesis of L

The synthesis of L was modified from the method from ref. 9d in main article. 3-mono-amino-β-cyclodextrin (315 mg, 0.278 mmol) and diisopropylethylamine (90 mg, 0.696 mol) was added to 20 ml of DMF (dried over CaH₂ and redistilled), followed by 2,6-bis(bromomethyl)pyridine (30 mg, 0.113 mmol). This mixture was stirred and heated up to 70 °C for 12 hours and diluted by 200 ml acetone. Then light-yellow precipitate was found. The crude product was collected by centriugation and washed with acetone, EtOH and Et₂O. Sephadex G-25 column chromatogram (mobile phase: 0.1 M NH₃·H₂O) was employed to purify the crude product which was detected by TLC. The product layer was collected, evaporated and dried. White, moisture-sensitive solid was found (180mg, 57.4% yield) (ESI-MS) m/z (calculated) = 1186.41 (L+2H⁺),
m/z (found) = 1186.24 (L+2H+); Elemental analysis calculated for C93H191N3O90 (L • 18H2O) (%): C 39.49, H 6.96, N 1.52. Found (%): C 39.59, H 6.86, N 1.50; 1H-NMR (d6-DMSO, 300 MHz) δH: 7.68 (t, 1H, J = 7.6 Hz, Py-H-4), 7.38 (d, 2H, J = 7.8 Hz, Py-H-3), 5.81-5.45 (m, 26H, OH-2,3), 4.91-4.78 (m, 14H, H-1), 4.65-4.36 (m, 14H, OH-6), 3.96-3.49 (m, 60H, H-3,5,6 and PyCH2), 3.47-3.22 (m, 64H, H-2,4 and 18H 2O), 2.69-2.67 (w, 2H, NH-3). 13C-NMR (d6-DMSO, 75MHz) δC: 159.66, 142.52, 137.64, 124.88, 120.69, 107.43, 104.78, 103.96, 102.56, 82.51, 82.00, 81.06, 76.96, 73.93, 72.90, 72.40, 60.66, 60.16, 52.66.

Synthesis of CuL

L (200 mg, 0.0723 mmol) was dissolved in 1ml H2O, added by Cu(ClO)2·6H2O (200mg, 0.539 mmol) in 1ml EtOH. This mixture was added to 40 ml EtOH and blue precipitated was found and collected by centrifugation and washed with EtOH and Et2O. Light-blue powder (181 mg, 84.6% yield) was found. (ESI-MS) m/z (calculated) = 1217.37 (CuL2+), m/z (found) = 1217.00 (CuL2+); Elemental analysis calculated for C91H183Cl2CuN3O94 (CuL · 18H2O) (%): C 36.95, H 6.24, N 1.42. Found (%): C 36.87, H 6.15, N 1.45.

The BNPP cleavage product analysis

Fig. 3 in main article showed that the time dependent 31P-NMR spectrum of the cleavage process of BNPP in the presence of L at pH 9.0 and 308 ± 0.1K with [L] = 5 mM and [BNPP] = 10 mM. The chemical shift of BNPP was -11.18 ppm and two signals of products were found. The one is at δ = 3.91 is similar to phosphorylated ethanolamine (δ = 3.8 ppm from ref. 12 in main article) so we propose that this peak correspond to phosphorylated L monoester (PL). It’s confirmed by ESI-MS (Fig. S4) that m/z (calculated) = 1224.38, m/z (found) = 1224.38. Furthermore, it is the only final product.

Two NP- are released is also confirmed by the detection of [NP]final / [PL]final as shown in Fig. S3. The concentration of NP- in this sample is calculated from the absorbance at λ = 400 nm and the concentration of PL and BNPP are calculated from NMR spectra. Before the detection on uv-vis spectroscopy, the original sample was diluted 300 times with same buffer at same pH. After 2 weeks this reaction mixture contain: [BNPP]final = 5.7 mM, [PL]final = 4.3 mM and [NP]final = 8.5 mM. So the ratio of [NP]final / [PL]final is 1.98 which means 2 mole NP- are released when 1 mole BNPP is cleaved. In Fig. 3 in main article, the chemical shift of the other signal (δ = -6.14) is similar with HPNP (δ = -5.48 ppm from ref. 9a in main article) and arylphosphorylethanolamine (δ = -5.5 ppm from ref. 12 in main article) so it correspond to 4-nitrophenol phosphorylated L diester (NPPL) as middle product.

The possibility of the formation of NPP was also excluded because the chemical shift of NPP was near zero (δ = -0.85 ppm from ref. 9a in main article).
Scheme S1 The back-to-back (left) and face-to-face (right) CD dimer complex.

Table S1 The thermodynamic parameters of CuL$^{a}$.

<table>
<thead>
<tr>
<th>CuL</th>
<th>log $K_f$</th>
<th>p$K_{a1}$</th>
<th>p$K_{a2}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>12.40</td>
<td>6.01</td>
<td>9.54</td>
</tr>
</tbody>
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$^{a}$ Ion strength was supported by 0.1 M NaClO$_4$, $T = 298$ K These data allowed the determination of the complex formation constant ($K_f$) and the deprotonation constant ($K_{a}$) of two copper-bound species.

Fig. S1 Species distribution of CuL on pH $I = 0.1$ M (NaClO$_4$) and $T = 298 \pm 0.1$ K
Fig. S2 Positive charge ESI-MS spectra of CuL detected in a neutral solution: a) Full spectrum; b) Bivalence ion isotopes spectrum detected; c) Bivalence ion isotopes spectrum of computer simulation.

Fig. S3 a) $^{31}$P-NMR spectrum of BNPP cleavage promoted by L in the solution of 10% D$_2$O in 50 mM CHES ($I = 0.1$ M with NaClO$_4$) at pH 9.0 and 308 ± 0.1K after 2 weeks; b) uv-vis spectrum of this sample after diluted 300 times with buffer.
Fig. S4  Negative charge ESI-MS spectra of mixed solution of BNPP and L for 2 week detected in water: a) Full spectrum; b) Bivalence ion isotopes spectrum detected; c) Bivalence ion isotopes spectrum of computer simulation.

Fig. S5  Positive charge ESI-MS spectra of mixed solution of BNPP and L in water and this sample were fresh prepared. [L] = [BNPP] = 10 mM a) Full spectrum; b) Bivalence ion isotopes spectrum detected; c) Bivalence ion isotopes spectrum of computer simulation.
**Fig. S6** The second-order rate constants \( (k_{\text{obs}}) \) of BNPP cleavage in the present of L (●) and CuL (■) in different pH conditions. \( I = 0.1 \) M \((\text{NaClO}_4)\), \( T = 308 \pm 0.1 \text{ K} \) and \([\text{BNPP}] = 1\text{mM}\).  

**Fig. S7** The initial rate constants \( (k_{\text{in}}) \) of BNPP cleavage in the presence of 0.1 mM L or 1 mM 3-ACD \( (I = 0.1 \text{ M with } \text{NaClO}_4) \) at 308 ± 0.1K, \([\text{BNPP}] = 1\text{mM} \) (left: pH = 7.0, right: pH = 9.0)
Fig. S8 Negative charge ESI-MS spectra of mixed solution of TPPS and L detected in a neutral solution: a) Full spectrum; b) Trivalence ion isotopes spectrum detected; c) Trivalence ion isotopes spectrum of computer simulation. d) Tetravalence ion isotopes spectrum detected; e) Tetravalence ion isotopes spectrum of computer simulation. Free L and the complexes of (TPPS)L and (TPPS)L₂ were found in this ESI-MS spectrum.

Fig. S9 Positive charge ESI-MS spectra of L detected in water: a) Full spectrum; b) Bivalence ion isotopes spectrum detected; c) Bivalence ion isotopes spectrum of computer simulation.
Fig. S10 $^1$H-NMR spectrum of L in $d_6$-DMSO.

References