Supporting Information

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

Ping Hu, Gao-Feng Liu, Liang-Nian Ji and Zong-Wan Mao*
MOE Key Laboratory of Bioinorganic and Synthetic Chemistry, School of Chemistry and Chemical Engineering, Sun Yat-Sen University, Guangzhou 510275, China
E-mail: cesmzw@mail.sysu.edu.cn

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 CaH_2 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: ^{1}H NMR, ^{13}C NMR and ^{31}P NMR spectra were recorded on a Varian Mercury plus 300 spectrometers and the ^{13}C NMR and ^{31}P NMR are proton-decoupled. Elemental contents were analyzed by a Perkin-Elemer 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 $HClO_4$ 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 log*β* and the pK_a 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 $NaClO_4$. 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. 8*d-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^{-1} s^{-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} ($M^{-1}s^{-1}$) 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.

$$k_{obs} = \frac{(10^{-3pH-pK_1})k_{H_2L} + (10^{-2pH-pK_1-pK_2})k_{HL} + (10^{-pH-pK_1-pK_2-pK_3})k_{L} + (10^{-pK_1-pK_2-pK_3-pK_4})k_{L^-}}{10^{-4pH} + 10^{-3pH-pK_1} + 10^{-2pH-pK_1-pK_2} + 10^{-pH-pK_1-pK_2-pK_3} + 10^{-pK_1-pK_2-pK_3-pK_4}}$$

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. 9*b* in main article.

Synthesis of L

The synthesis of **L** was modified from the method from ref. 9*d* 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 CaH_2 and redistilled), followed by 2,6-bis(bromomethyl)pyridine (30 mg, 0.113 mmol). This mixture was stirred and heated up to 70 ^oC for 12 hours and diluted by 200 ml acetone. Then light-yellow precipitate was found. The crude product was collected by centrifugation and washed with acetone, EtOH and Et_2O. Sephadex G-25 column chromatogram (mobile phase: 0.1 M $NH_3 \cdot H_2O$) 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* (caculated) = 1186.41 ($L+2H^+$),

m/z (found) = 1186.24 ($L+2H^+$); Elemental analysis calculated for $C_{91}H_{191}N_3O_{90}$ (**L** • $18H_2O$) (%): C 39.49, H 6.96, N 1.52. Found (%): C 39.59, H 6.86, N 1.50; ^{1}H-NMR (d^6-DMSO, 300 MHz) δ_H: 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 $PyCH_2$), 3.47-3.22 (m, 64H, H-2,4 and $18H_2O$), 2.69-2.67 (w, 2H, NH-3). ^{13}C-NMR (d^6-DMSO, 75MHz) δ_C: 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 H_2O, added by $Cu(ClO)_2 \cdot 6H_2O$ (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 Et_2O. Light-blue powder (181 mg, 84.6% yield) was found. (ESI-MS) *m/z* (caculated) = 1217.37 (CuL^{2+}), *m/z* (found) = 1217.00 (CuL^{2+}); Elemental analysis calculated for $C_{91}H_{183}Cl_2CuN_3O_{94}$ (**CuL** •$18H_2O$) (%): C 36.95, H 6.24, N 1.42. Found (%): C 36.87, H 6.15, N 1.45.

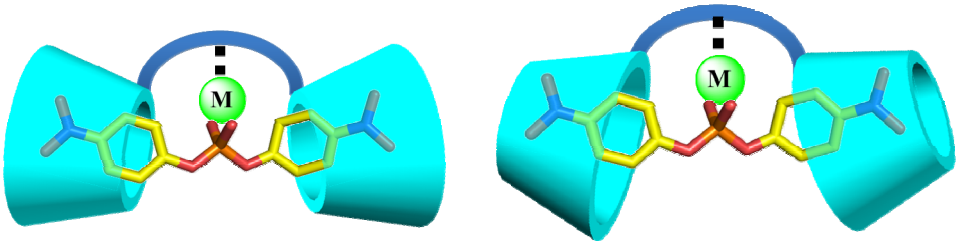
The BNPP cleavage product analysis

Fig. 3 in main article showed that the time dependent ^{31}P-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 $\mathbf{NP}^-$ are released is also confirmed by the detection of $[\mathbf{NP}^-]_{final}$ / $[\mathbf{PL}]_{final}$ as shown in Fig. S3. The concentration of $\mathbf{NP}^-$ 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: $[\mathbf{BNPP}]_{final}$ = 5.7 mM, $[\mathbf{PL}]_{final}$ = 4.3 mM and $[\mathbf{NP}^-]_{final}$ = 8.5 mM. So the ratio of $[\mathbf{NP}^-]_{final}$ / $[\mathbf{PL}]_{final}$ is 1.98 which means 2 mole $\mathbf{NP}^-$ 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. 9*a* 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. 9*a* 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].

	CuL	
log K_f	pK_{a1}	pK_{a2}
12.40	6.01	9.54

[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.

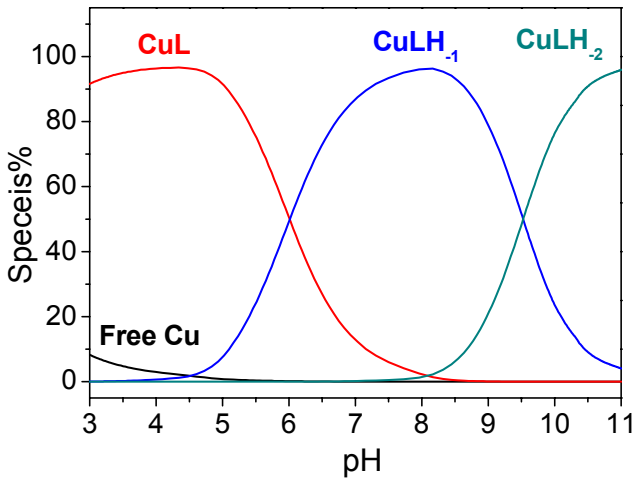


Fig. S1 Species distribution of **CuL** on pH I = 0.1 M ($NaClO_4$) and T = 298 ± 0.1 K

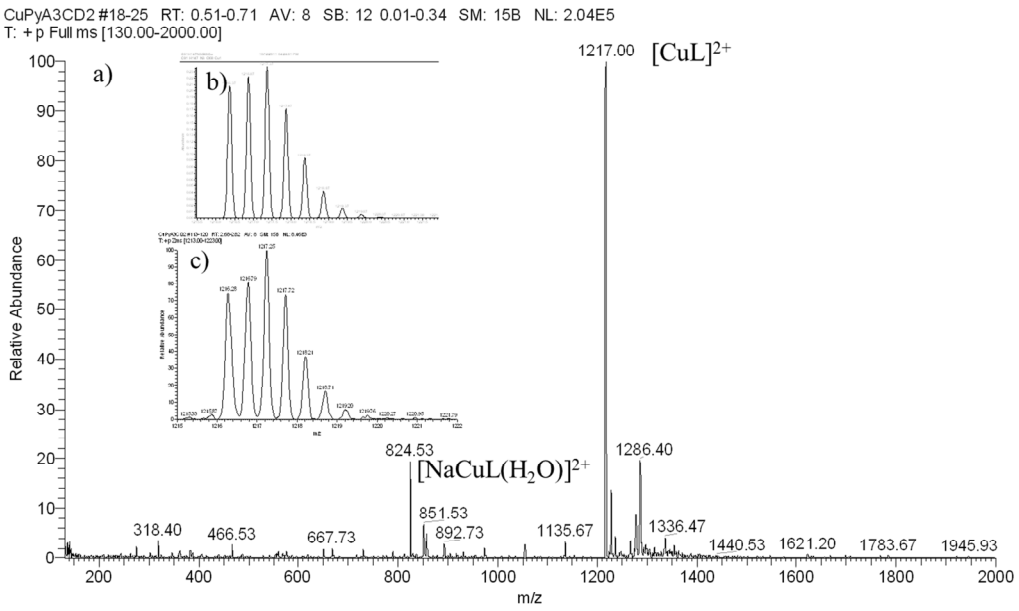


Fig. S2 Positive charge ESI-MS spectra of **CuL** detected in a neutral solution: a) Full spectrum; b) Bivalence ion isotopes spectrum detected; c) Bivalence ion isotopes spectrum of computer simulation.

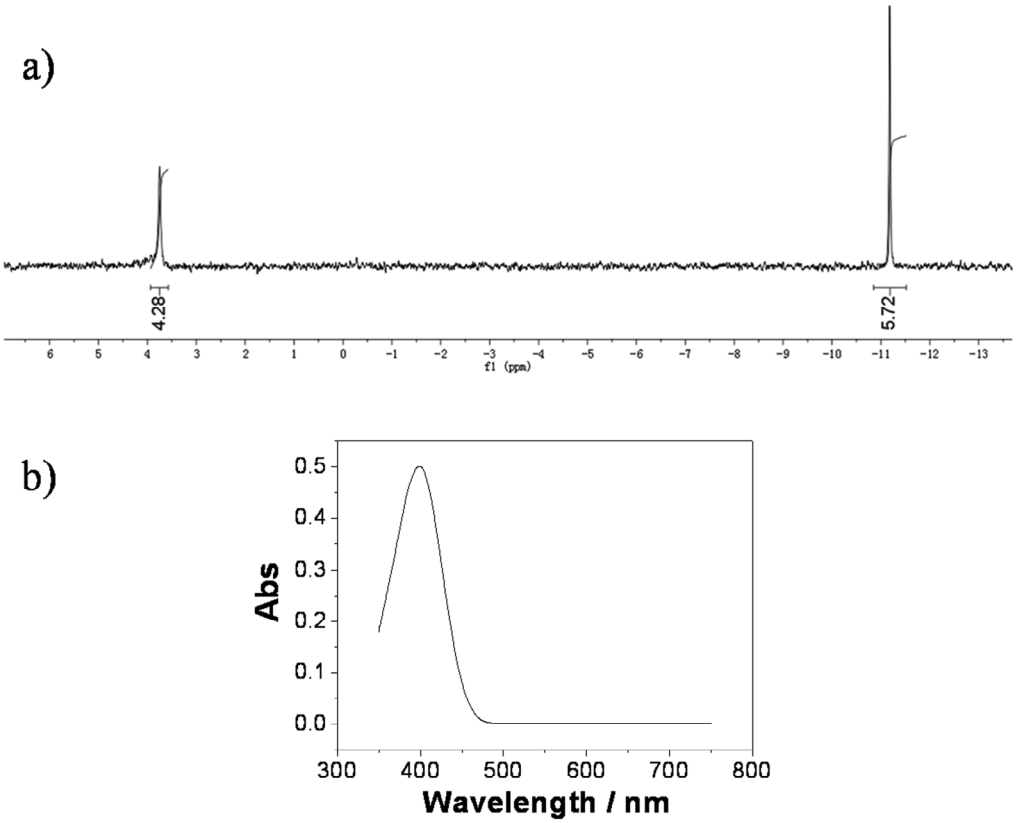


Fig. S3 a) ^{31}P-NMR spectrum of **BNPP** cleavage promoted by **L** in the solution of 10% D_2O in 50 mM CHES (I = 0.1 M with $NaClO_4$) at pH 9.0 and 308 ± 0.1K after 2 weeks; b) uv-vis spectrum of this sample after diluted 300 times with buffer.

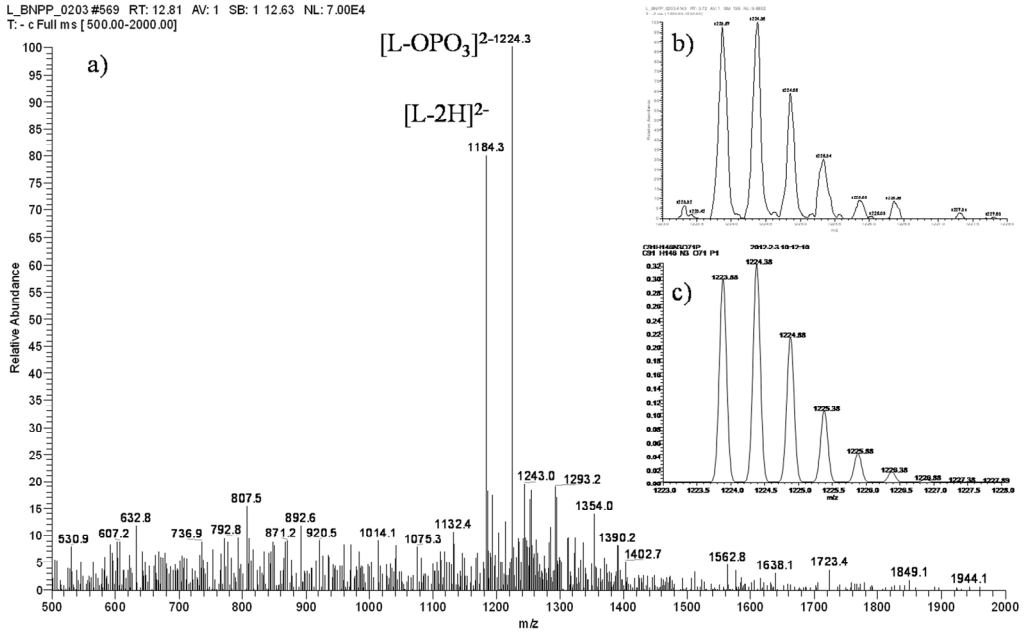


Fig. S4 Negative charge ESI-MS spectra of mixed solution of **BNPP** and **L** for 2 week detected in water: a) Full spectrum; b) Bivalence ion isotopes spectrum detected; c) Bivalence ion isotopes spectrum of computer simulation.

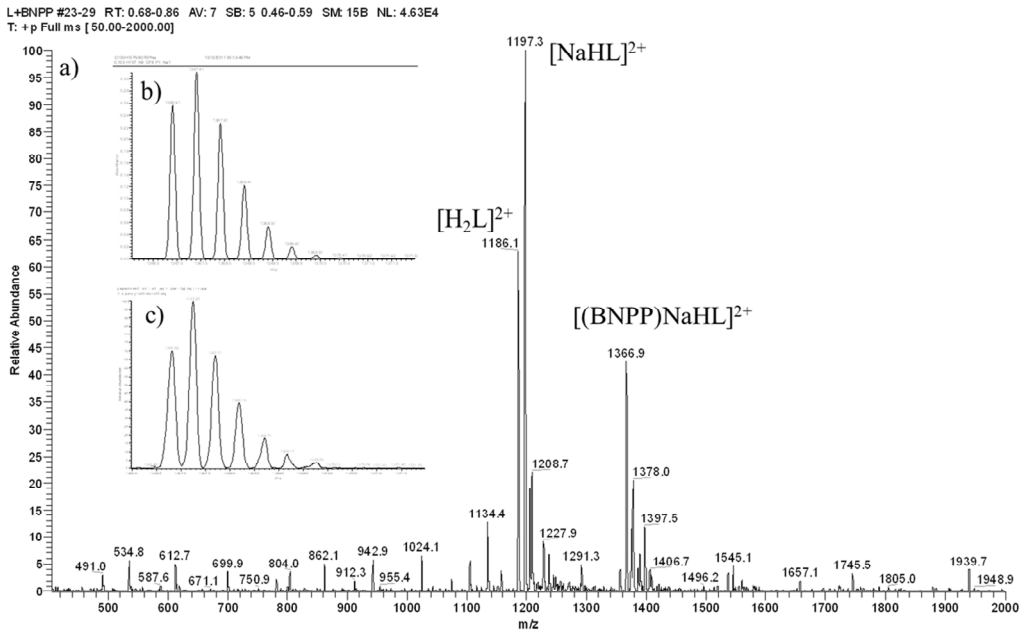


Fig. S5 Positive charge ESI-MS spectra of mixed solution of **BNPP** and **L** in water and this sample were fresh prepared. [**L**] = [**BNPP**] = 10 mM a) Full spectrum; b) Bivalence ion isotopes spectrum detected; c) Bivalence ion isotopes spectrum of computer simulation.

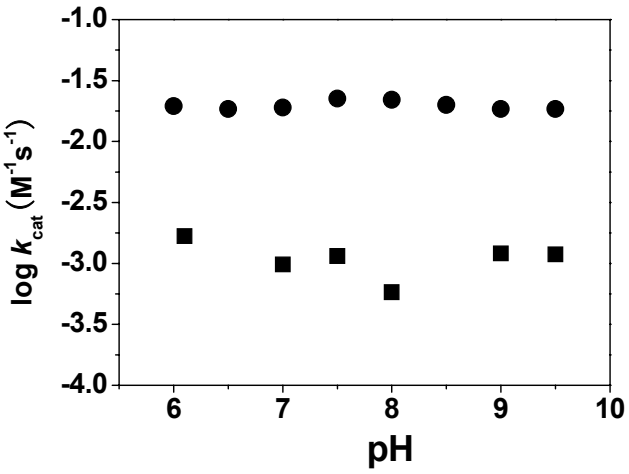


Fig. S6 The second-order rate constants (k_{obs}) of **BNPP** cleavage in the present of **L** (●) and **CuL** (■) in different pH conditions. I = 0.1 M ($NaClO_4$), T = 308 ± 0.1 K and [**BNPP**] = 1mM.

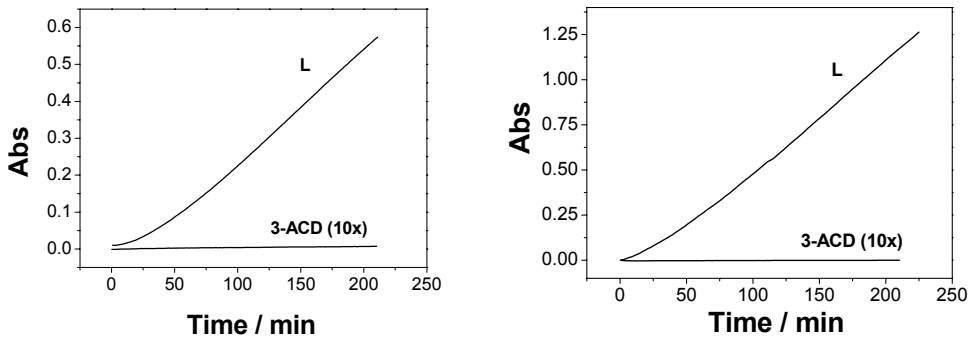


Fig. S7 The initial rate constants (k_{in}) of **BNPP** cleavage in the presence of 0.1 mM **L** or 1 mM 3-ACD (I = 0.1 M with $NaClO_4$) at 308 ± 0.1K, [**BNPP**] = 1mM.(left: pH = 7.0, right: pH = 9.0)

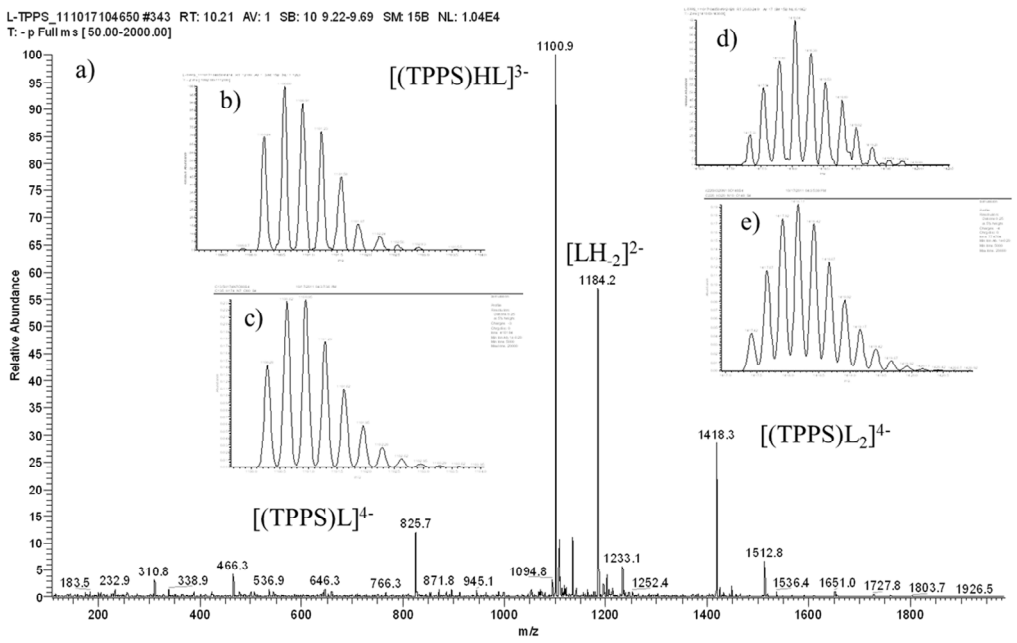


Fig. S8 Negative charge ESI-MS spectra of mixed solution of **TPPS** and **L** detected in a neutral solution: a) Full spectrum; b) Trivalence ion isotopes spectrum detected; c) Trivalence ion isotopes spectrum of computer simulation. d) Tetravalence ion isotopes spectrum detected; e) Tetravalence ion isotopes spectrum of computer simulation. Free **L** and the complexes of (**TPPS)L** and (**TPPS)L$_2$** were found in this ESI-MS spectrum.

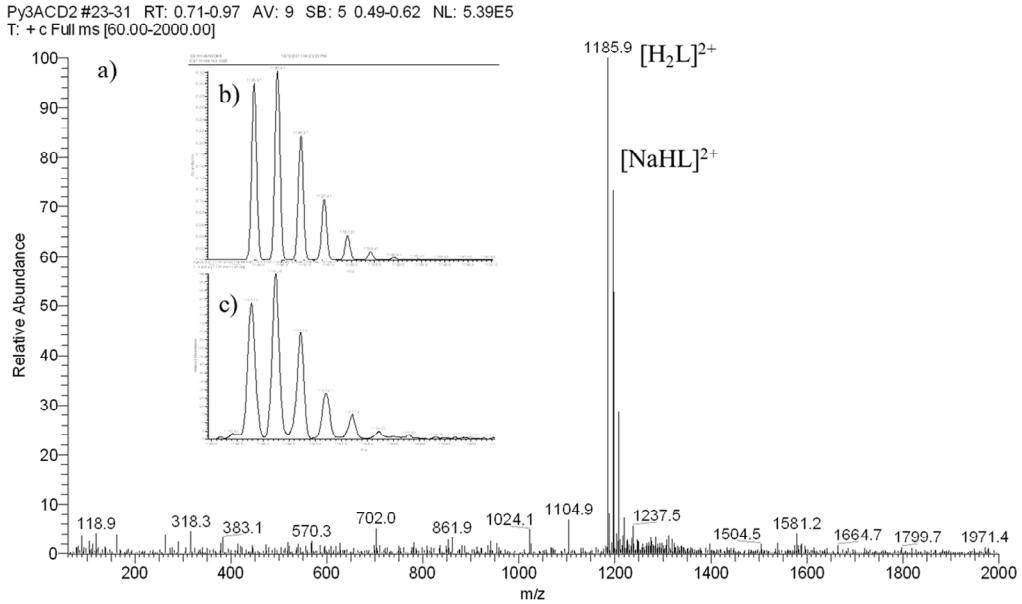


Fig. S9 Positive charge ESI-MS spectra of **L** detected in water: a) Full spectrum; b) Bivalence ion isotopes spectrum detected; c) Bivalence ion isotopes spectrum of computer simulation.

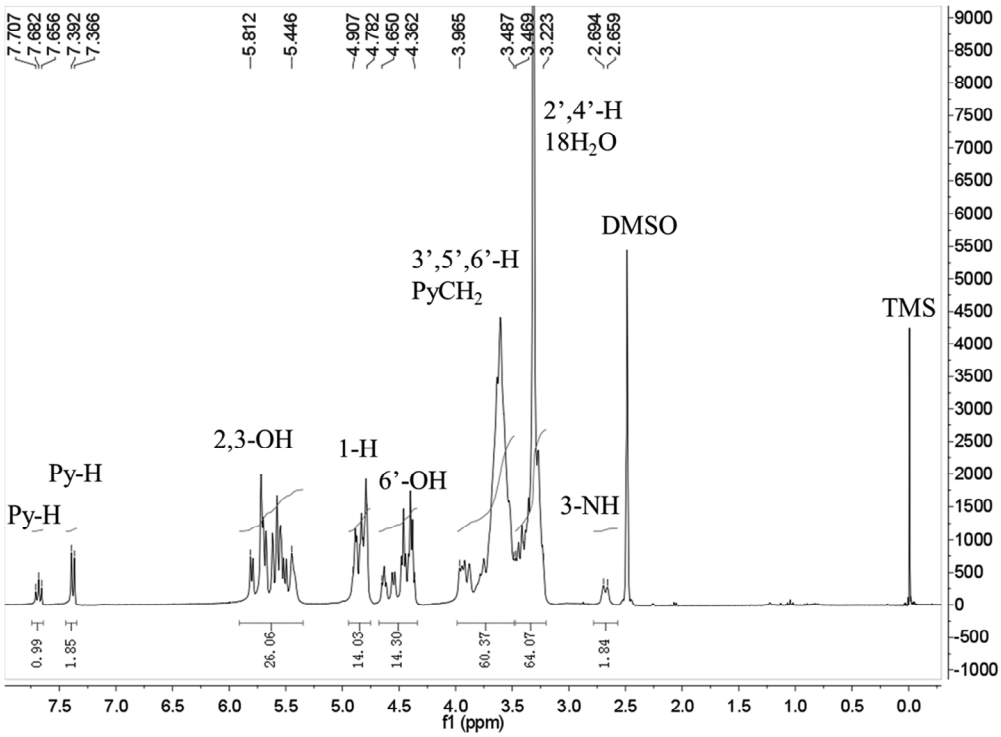


Fig. S10 ^{1}H-NMR spectrum of **L** in *d*$_6$-DMSO.

References

1 G. Gran, *Acta. Chem. Scand.*, 1950, **4**, 559.