Tetraphenylethene-based tetracationic cyclophanes and their
selective recognition for amino acids and adenosine derivatives
in water

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General Experimental Details.

Starting materials were purchased from commercial suppliers were used without further purification. \( \text{SI1}^{1} \), \( \text{SI6}^{2} \), and \( 3^{3} \) was prepared according to the published procedure. Melting points were determined using XT-4 apparatus. IR spectra were recorded on a Bruker IFS 120HR spectrometer and were reported in cm\(^{-1}\). \(^{1}\)H and \(^{13}\)C NMR spectra were done on a Bruker ascend spectrometer at 400MHz. UV-Vis spectra were measured using an Agilent Cary-100 spectrophotometer. Fluorescence spectra were recorded on a Horiba Fluorolog-3 spectrometer. Fluorescence decay profiles were recorded on a Flsp920. Electron Spray Ionization (ESI) mass spectra were acquired by using a UltiMate3000 electrospray instrument. Isothermal titration calorimetry (ITC) was carried out using a VP-ITC (Malvern) at 25 °C, and computer fitting of the data were performed using the VP-ITC analyze software. X-ray Crystal Structure was performed on a Bruker D8 Venture photon II.
Compound SI3. To a solution of SI2 (2.02 g, 12 mmol) in dry THF (20 mL) was added 6.2 mL of a 1.6 M solution of n-butyllithium in hexane at 0°C under a N₂ atmosphere. The resulting orange-red solution was stirred for 30 min at that temperature. Next, SI1 was dissolved in appropriate dry THF. This solution was added to the above mixture slowly with a dropping funnel, and the reaction mixture was allowed to warm to room temperature with stirring for 6 h. The reaction was quenched with addition of an aqueous solution of saturated ammonium chloride and the organic layer was extracted with CH₂Cl₂ (3 × 50 mL) and the combined organic layers were washed with saturated brine solution and dried over anhydrous MgSO₄. The solvent was evaporated and the resulting crude alcohol (containing excess SI2) was subjected to acid catalyzed dehydration as follows.

The crude alcohol was dissolved in about 80 mL of toluene in a 100 mL round flask with the 4Å molecular sieve dehydration unit. A catalytic amount of p-toluenesulphonic acid (342 mg, 1.8 mmol) was added and the mixture was refluxed for 5 h and cooled to room temperature. The toluene layer was washed with 10% aqueous NaHCO₃ solution (3 × 25 mL) and dried over anhydrous MgSO₄ and evaporated to afford the crude SI3.
The crude product was purified by a silica gel column chromatography using petroleum ether as eluent, and white solid of pure SI3 was obtained. The $^1$H NMR matches that reported in the reported literature.\(^1\)

**Compound 3.** Under a N\(_2\) atmosphere, compound SI3 (1.08 g, 3.0 mmol), NBS (1.18 g, 8.4 mmol) and dibenzoyl peroxide (36 mg, 0.15 mmol) were added to CCl\(_4\) (20 mL). The solution was heated to reflux for another 12 h. The mixture was cooled to room temperature and filtered to remove suspension. Next, CH\(_2\)Cl\(_2\) (20 mL) was added to the filtrate and then the solution was washed with water and brine, respectively, for three times, it was dried over anhydrous Na\(_2\)SO\(_4\), filtered and evaporated under vacuum to dryness. The residue was purified by a silica gel column chromatography using petroleum/dichloromethane ether as eluent, and white solid of pure compound 3 was obtained. The $^1$H NMR matches that reported in the reported literature.\(^3\)

**Compound 4.** 4,4’-bipyridine (603 mg, 3.86 mmol) was dissolved in 20 mL of CH\(_3\)CN in a 50 mL round flask and the solution was brought to reflux. Next, compound 3 (200 mg, 0.39 mmol) was dissolved in 5 mL of acetonitrile. This solution was added to the bipyridine refluxing solution slowly with a dropping funnel. Then the mixture was refluxed for an additional 48 h. The precipitate formed was filtered and washed with acetonitrile (3 $\times$ 35 mL), and compound 4 (yield: 97%) was obtained by dried in high vacuum. M.p. 170-171 °C. IR (KBr, cm\(^{-1}\)): 3128w, 3059w, 1637s, 1598w, 1404m, 1216w, 1153w, 841s, 700w, 560s. $^1$H NMR (400 MHz, CD\(_3\)CN): 8.85 (d, $J$ = 5.7, 4H), 8.72 (d, $J$ = 6.6, 4H), 8.29 (d, $J$ = 6.6, 4H), 7.77 (d, $J$ = 5.7, 4H), 7.22 (d, $J$ = 8.1, 4H), 7.20-7.10 (m, 10H), 7.05-6.95 (m, 4H), 5.63 (s, 4H). $^{13}$C NMR (100 MHz, CD\(_3\)CN): 155.5, 152.1, 145.8, 145.7, 144.1, 143.8, 142.0, 139.7, 132.9, 132.0, 131.7, 129.6, 128.8, 127.9, 127.1, 122.7, 64.6. MS (ESI): m/z 335.1552 ([4 - 2PF\(_6^{-}\)]\(^2+\), calcd. for 335.1543) and m/z 815.2676 ([4 - PF\(_6^{-}\)]\(^+\), calcd. for 815.2733).

**Compound 1. (Approach 1)** To a solution of compound 4 (200 mg, 0.28 mmol), 5 (55 mg, 0.28 mmol), and tetrabutylammonium iodide (TBAI, 21 mg, 0.20 mmol) in dry
MeCN (100 mL) was heated at 85 °C for 72 h. Then the crude product was obtained by centrifuge and was dried in high vacuum. The crude product was purified by anion conversion in water. Next, pale yellow solid of pure 1•4PF$_6^-$ (yield: 35%) was obtained by addition of an excess of NH$_4$PF$_6$. M.p. 233 -234 °C. IR (KBr, cm$^{-1}$): 3129w, 3058w, 2925w, 2848w, 1636s, 1559w, 1504w, 1441m, 1216w, 1153m, 832vs, 700w, 637w, 559s. $^1$H NMR (100 MHz, CD$_3$CN): 8.92 (d, $J = 7.0$, 4H), 8.84 (d, $J = 7.0$, 4H), 8.24 (d, $J = 7.0$, 4H), 8.23 (d, $J = 7.0$, 4H), 7.63 (s, 4H), 7.20-7.10 (m, 6H), 7.09 (s, 8H), 7.05-7.00 (m, 4H), 5.79 (s, 4H), 5.67 (s, 4H). $^{13}$C NMR (100 MHz, CD$_3$CN): 150.8, 146.5, 146.2, 145.4, 144.3, 139.7, 136.7, 132.5, 132.4, 131.7, 131.5, 129.0, 128.8, 128.5, 128.4, 127.8, 65.4, 65.2. MS (ESI): $m/z$ 532.1521 ([1•4PF$_6^-$ - 2PF$_6^-$]$^{2+}$, calcld. for 532.1498). An excess of TBACl (205.66mg, 0.74mmol) was added to the acetonitrile solution of 1•4Cl$^-$ (100mg, 0.074 mmol) and stirred overnight at room temperature. Then the mixture was centrifuged and washed twice with acetonitrile. Pure 1•4Cl$^-$(61 mg, yield: 90%) was obtained by dried in high vacuum. M.p. 221-222 °C. IR (KBr, cm$^{-1}$): 3118w, 3041m, 1629s, 1551w, 1497w, 1443m, 1217w, 1156m, 791m, 759w, 691w, 628w. $^1$H NMR (400 MHz, D$_2$O): 9.14 (d, $J = 6.3$, 4H), 9.00 (d, $J = 6.3$, 4H), 8.37 (d, $J = 6.3$, 4H), 8.35 (d, $J = 6.3$, 4H), 7.67 (s, 4H), 7.25-7.05 (m, 18H), 5.92 (s, 4H), 5.78 (s, 4H). $^{13}$C NMR (100 MHz, CD$_3$OD): 151.2, 151.1, 147.2, 146.7, 146.1, 144.7, 143.9, 140.1, 137.3, 133.0, 132.0, 131.9, 129.2, 128.9, 128.8, 128.5, 128.0, 65.5, 65.4 (only 19 of the 20 resonances expected were observed).

**Compound 2.** To a solution of compound 4 (200 mg, 0.21 mmol), compound 3 (109 mg, 0.21 mmol), and tetrabutylammonium iodide (TBAI, 15.5 mg, 0.042 mmol) in dry MeCN (100 mL) was heated at 85 °C for 72 h. Then the crude product was obtained by centrifuge and was dried in high vacuum. The crude product was purified by anion conversion in water. Next, light yellow solid of pure 2•4PF$_6^-$ (yield: 42%) was obtained by addition of an excess of NH$_4$PF$_6$. M.p. 252-253 °C. IR (KBr, cm$^{-1}$): 3121w, 3056w, 2917w, 2856w, 1629s, 1559w, 1497w, 1443m, 1216w, 1153w, 838vs, 700m, 629w, 559s. $^1$H NMR (400 MHz, CD$_3$CN): 8.86 (d, $J = 6.6$, 8H), 8.29 (d, $J = 6.6$, 8H), 7.25-7.10 (m, 28H), 7.10-7.00 (m, 8H), 5.69 (s, 8H). $^{13}$C NMR (100 MHz, CD$_3$CN):
151.1, 146.3, 145.8, 144.3, 143.9, 139.7, 132.8, 131.6, 129.5, 128.8, 128.3, 127.9, 65.1 (only 13 of the 14 resonances expected were observed). MS (ESI): m/z 659.2058 ([2•4PF₆⁻ - 2PF₆⁻]²⁺; calcd. for 659.2045). An excess of TBACl (172.31mg, 0.62mmol) was added to the acetonitrile solution of 2•4PF₆⁻ (100mg, 0.062 mmol) and stirred overnight at room temperature. Then the mixture was centrifuged and washed twice with acetonitrile. Pure 2•4Cl⁻ (62mg, yield: 85%) was obtained by dried in high vacuum. M.p. 275-276 °C. IR (KBr, cm⁻¹): 3118w, 3025w, 2917w, 2855w, 1628s, 1559w, 1497w, 1442s, 1210w, 1155w, 790m, 759m, 698s, 628w. ¹H NMR (400 MHz, CD₃OD): 9.01 (d, J = 6.9, 8H), 8.40 (d, J = 6.9, 8H), 7.25-7.15 (m, 28H), 7.15-7.05 (m, 8H), 5.76 (s, 8H). ¹³C NMR (100 MHz, CD₃OD): 151.6, 146.9, 146.6, 144.8, 144.0, 140.0, 133.4, 132.5, 132.1, 129.9, 129.0, 128.6, 128.1, 65.6.

**Compound SI6.** 4,4'-bipyridine (592 mg, 3.79 mmol) was dissolved in 10 mL of CH₃CN in a 50 mL round flask and the solution was brought to reflux. Next, p-bis-(bromo-methyl) benzene 5 (100 mg, 0.38 mmol) was dissolved in 5 mL of acetonitrile. This solution was added to the bipyridine refluxing solution slowly with a dropping funnel. Then the mixture was refluxed for an additional 24 h. The precipitate formed was filtered and washed with acetonitrile (3 × 35 mL), and dried in high vacuum. The ¹H NMR matches that reported in the literature.²

**Compound 1. (Approach 2)** To a solution of SI6 (200 mg, 0.28 mmol), 3 (55 mg, 0.28 mmol), and tetrabutylammonium iodide (TBAI, 21 mg, 0.20 mmol) in dry MeCN (100 mL) was heated at 85 °C for 72 h. Then the crude product was obtained by centrifuge and was dried in high vacuum. The crude product was purified by anion conversion in water. Next, yellow solid of pure 1•4PF₆⁻ (yield: 30%) was obtained by a
silica gel column chromatography using CH$_2$Cl$_2$/saturated NH$_4$PF$_6$ solution (5:1) as eluent.

**X-ray Crystal Structure Determination.** Diffraction data for the complexes 1•4PF$_6^-$ and 2•4PF$_6^-$ were performed on a Bruker D8 Venture photon II, at low temperature (153 K) with graphite-monochromated Mo Kα radiation (λ = 0.71073 Å). An empirical absorption correction using SADABS was applied for all data. Both structures were solved and refined to convergence on $F^2$ for all independent reflections by the full-matrix least squares method using SHELXL-2018/3 in OLEX2 1.2. All non-hydrogen atoms, including those in disordered parts, were refined anisotropically. All H-atoms were also included at calculated positions and refined as riders, with $U_{	ext{iso}}$(H) = 1.2 $U_{	ext{eq}}$.

In 1•4PF$_6^-$, two PF$_6^-$ anions were found to be disordered and were modelled with two orientations having relative occupancies of 0.54:0.46 and 0.57:0.43 for the two parts, separately. The geometries of the disordered parts were restrained to be similar. The anisotropic displacement parameters of the disordered molecules in the direction of the bonds were restrained to be equal with a standard uncertainty of 0.01 Å$^2$. They were also restrained to have the same $U_{ij}$ components, with a standard uncertainty of 0.04 Å$^2$. In 1•4PF$_6^-$, four CH$_2$Cl$_2$ and two CHCl$_3$ molecules co-crystallized in each unit cell of complex 1•4PF$_6^-$, with the corresponding electron densities removed using the SQUEEZE subroutine implemented within the software program PLATON (Ver. 201118), and the resulting .fab file was processed with OLEX2 1.2, using the ABIN instruction. Approximately 280 electron equivalents were removed from the unit cell. The total void volume was 1804 Å$^3$ indicated by PLATON, equivalent to 25.38 % of the unit cell’s total volume. In 2•4PF$_6^-$, the PF$_6^-$ anion was found to be disordered and was modelled with two orientations having relative occupancy of 0.55:0.45. The geometries of the disordered parts were restrained to be similar. The anisotropic displacement parameters of the disordered molecules in the direction of the bonds were restrained to be equal with a standard uncertainty of 0.01 Å$^2$. They were also restrained to have the same $U_{ij}$ components, with a standard uncertainty of 0.04 Å$^2$. There are 32 severely disordered Et$_2$O molecules in the unit cell of 2•4PF$_6^-$ and compound 2 has
crystallographically imposed 2/m symmetry. They were also removed by the SQUEEZE subroutine in PLATON (Ver. 201118) and the resulting .fab file was processed with OLEX2 1.2. using the ABIN instruction. Approximately 1324 electron equivalents were removed from each unit cell. The total void volume was 4070 Å³ indicated by PLATON, equivalent to 39.81 % of the unit cell’s total volume. Crystallographic data and refinement details for 1•4PF₆⁻ and 2•4PF₆⁻ are given in Table S1. CCDC 1870620 (1•4PF₆⁻) and 1870621 (2•4PF₆⁻). These data can be obtained free of charge from the Cambridge Crystallographic Data Centre www.ccdc.cam.ac.uk/data_request/cif.

References

Figure S1. $^1$H NMR spectrum recorded (400 MHz, D$_2$O, RT) for 1•4Cl$^-$. 

Figure S2. $^{13}$C NMR spectrum recorded (400 MHz, CD$_3$OD, RT) for 1•4Cl$^-$. 
Figure S3. a) COSY and b) NOESY $^1$H NMR spectrum recorded (400 MHz, D$_2$O, 298 K) for 1$\cdot$4Cl$^-$ (2.0 mM).

Figure S4. $^1$H NMR spectrum recorded (400 MHz, CD$_3$CN, RT) for 1$\cdot$4PF$_6^-$.
**Figure S5.** $^{13}$C NMR spectrum recorded (100 MHz, CD$_3$CN, RT) for 1•4PF$_6^−$.

**Figure S6.** $^1$H NMR spectrum recorded (400 MHz, CD$_3$CN, RT) for 4•2PF$_6^−$. 
Figure S7. $^{13}$C NMR spectrum recorded (100 MHz, CD$_3$CN, RT) for 4•2PF$_6$.

Figure S8. $^1$H NMR spectrum recorded (400 MHz, D$_2$O, RT) for 2•4Cl.
Figure S9. $^{13}$C NMR spectrum recorded (100 MHz, CD$_3$OD, RT) for 2•4Cl$^-$. 

Figure S10. $^1$H NMR spectrum recorded (400 MHz, CD$_3$CN, RT) for 2•4PF$_6^-$. 
**Figure S11.** $^{13}$C NMR spectrum recorded (100 MHz, CD$_3$CN, RT) for 2•4PF$_6$.

**Figure S12.** ESI-MS spectrum of 1•4PF$_6$ in CH$_3$CN. Expansions confirm the expected m/z spacing of 0.5 for the 2$^+$ ion.
**Figure S13.** ESI-MS spectrum of $4 \cdot 2\text{PF}_6^-$ in CH$_3$CN. Expansions confirm the expect m/z spacing of 0.5 for the $2^+$ ion and 1 for the $1^+$ ion.

**Figure S14.** ESI-MS spectrum of $2 \cdot 4\text{PF}_6^-$ in CH$_3$CN. Expansions confirm the expect m/z spacing of 0.5 for the $2^+$ ion.
Figure S15. $^1$H NMR of $1\cdot4$Cl$^-$ (400 MHz, DMSO-$d_6$), 20 mM (20 μL) 1,3,5-trimethoxy-benzen as internal reference. The solubility of $1\cdot4$Cl$^-$ (20 μL D$_2$O solution was dried in high vacuum) was calculated to be 48.98 mM (an average value of two sets of data).

Figure S16. $^1$H NMR of $2\cdot4$Cl$^-$ (400 MHz, DMSO-$d_6$), 20 mM (20 μL) 1,3,5-trimethoxy-benzen as internal reference. The solubility of $2\cdot4$Cl$^-$ (40 μL D$_2$O solution
was dried in high vacuum) was calculated to be 3.36 mM (an average value of two sets of data).

Table S1. Crystal Data and Structure Refinement for Cyclophane 1•4PF₆⁻ and 2•4PF₆⁻

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¹R1 = Σ||Fo|−|Fc||/Σ|Fo|, ²wR2 = [Σ[w(Fo²−Fc²)²]/Σ[w(Fo²)]²].
³Quality-of-fit = [Σ[w(Fo²−Fc²)]²/(Nobs−Nparams)]½, based on all data.
**Figure S17.** Solid-state (super)structures of cyclophane 1•4PF$_6^-$ obtained from single-crystal X-ray crystallography. a) top view, b) front view and c) side view of cyclophane 1•4PF$_6^-$. d) top view, e) side view and f) front view of molecular packing of cyclophane 1•4PF$_6^-$. Color code: N, blue; C, gray; H, white. (The solvent molecules and PF$_6^-$ pairs are omitted.)
Figure S18. Solid-state (super)structures of cyclophane 2•4PF$_6^-$ obtained from single-crystal X-ray crystallography. a) front view, b) top view and c) side view of cyclophane 2•4PF$_6^-$. d) neighboring 2•4PF$_6^-$ molecules contact with each other through C−H⋯π interactions. e) side view, f) front view and g) top view of molecular packing of cyclophane 2•4PF$_6^-$. Color code: N, blue; C, gray; H, white. (The solvent molecules and PF$_6^-$ pairs are omitted.)

Table S2 Summary of the binding constants corresponding to host-guest as deduced from ITC and $^1$H NMR spectroscopic titrations studies carried out in phosphate buffer.

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<td>1•4Cl$^-$</td>
<td>HPTS</td>
<td>$(4.88 \pm 1.95) \times 10^8$</td>
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<td>ATP</td>
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<td>ADP</td>
<td>$(5.07 \pm 0.28) \times 10^3$</td>
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<td>AMP</td>
<td>$(1.09 \pm 0.16) \times 10^3$</td>
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<td>tryptophan</td>
<td>$(1.21\pm0.04) \times 10^3$/ $(2.67 \pm 0.2) \times 10^3$(NMR)</td>
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<tr>
<td>2•4Cl$^-$</td>
<td>HPTS</td>
<td>$(1.42 \pm 0.98) \times 10^9$</td>
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Figure S19. $^1$H NMR spectra (400MHz, D$_2$O, phosphate buffer, pH = 7.4): a) HPTS (0.4 mM); b) HPTS (0.4 mM) and 1•4Cl$^-$ (0.4 mM); c) HPTS (0.4 mM) and 1•4Cl$^-$ (0.8 mM); d) 1•4Cl$^-$ (0.4 mM) at 298K.

Figure S20. a) COSY and b) NOSEY $^1$H NMR spectrum recorded (400 MHz, D$_2$O, 298 K) for 1•4Cl$^-$ (0.4 mM) with HPTS (0.4 mM).
Figure S21. $^1$H NMR spectra (400MHz, DMSO-$d_6$): a) $1\cdot4\text{Cl}^-$ (0.4 mM); b) $1\cdot4\text{Cl}^-$ (0.4 mM) and HPTS (0.4 mM); c) $1\cdot4\text{Cl}^-$ (0.4 mM) and HPTS (0.8 mM); d) HPTS (0.4 mM).

Figure S22. $^1$H NMR spectra (400MHz, D$_2$O, phosphate buffer, pH=7.4): a) $2\cdot4\text{Cl}^-$ (0.4 mM); b) $2\cdot4\text{Cl}^-$ (0.4 mM) and HPTS (0.4 mM); c) HPTS (0.4 mM) at 298K.
Figure S23. $^1$H NMR spectra (400 MHz, DMSO-$d_6$): a) $2\cdot4\text{Cl}^-$ (0.4 mM); b) $2\cdot4\text{Cl}^-$ (0.4 mM) and HPTS (0.4 mM); c) $2\cdot4\text{Cl}^-$ (0.4 mM) and HPTS (0.8 mM); d) HPTS (0.4 mM).

Figure S24. a) COSY and b) NOSEY $^1$H NMR spectrum recorded (400 MHz, DMSO-$d_6$, 298 K) for $2\cdot4\text{Cl}^-$ (1.0 mM) with HPTS (1.0 mM).
Figure S25. ESI-MS spectrum of 1•HPTS.

Figure S26. ESI-MS spectrum of 2•HPTS.
Figure S27. UV-vis absorption of HPTS (5 μmol) in phosphate buffer (pH = 7.4) upon addition of a) 1•4Cl⁻ and b) 2•4Cl⁻.

Figure S28. Job’s plots obtained by recording the absorption a) at 349 nm for the solution of 1•4Cl⁻ and HPTS ([1•4Cl⁻]+[HPTS] = 10 μmol), b) at 403 nm for the solution of 2•4Cl⁻ and HPTS ([2•4Cl⁻]+[HPTS] = 10 μmol) in phosphate buffer (pH = 7.4) at RT, confirming the 1:1 stoichiometry of both complexes.
Figure S29. a) ITC titration of \(1\cdot4\text{Cl}^-\) (0.1 mM) with HPTS (0.01 mM) in phosphate buffer (pH = 7.4); b) ITC titration of \(2\cdot4\text{Cl}^-\) (0.1 mM) with HPTS (0.01 mM) in phosphate buffer (pH = 7.4).

Figure S30. \(^{1}\text{H}\) NMR spectra (400MHz, D\(_2\)O, phosphate buffer, pH = 7.4): a) \(1\cdot4\text{Cl}^-\) (0.4 mM); b) \(1\cdot4\text{Cl}^-\) and tryptophan (0.4 mM); c) \(1\cdot4\text{Cl}^-\) and tryptophan (0.8 mM); d) tryptophan (0.4 mM) at 298K.
Figure S31. $^1$H NMR spectra (400 MHz, D$_2$O, phosphate buffer, pH = 7.4, 298 K) of 1•4Cl$^-$ at a concentration of 0.40 mM upon the addition of tryptophan: a) 0.00 mM, b) 0.08 mM, c) 0.16 mM, d) 0.24 mM, e) 0.32 mM, f) 0.40 mM, g) 0.48 mM, h) 0.60 mM, i) 0.72 mM, j) 0.84 mM, k) 0.96 mM, l) 1.08 mM, m) 1.20 mM, n) 1.40 mM, o) 1.60 mM, p) 1.80 mM, q) 2.00 mM, r) 2.20 mM, s) 2.40 mM, t) 2.80 mM, u) 3.20 mM, v) 3.60 mM, w) 4.00 mM.
**Figure S32.** Non-linear fitting curve of the chemical shift changes of H$_f$ of 1•4Cl$^-$ versus the concentration of tryptophan.

**Figure S33.** a) COSY and b) NOESY 1H NMR spectrum recorded (400 MHz, D$_2$O, 298 K) for 1•4Cl$^-$ (0.4mM) with tryptophan (0.4 mM).

**Figure S34.** 1H-NMR spectral Job-plots (400MHz, D$_2$O, phosphate buffer, pH = 7.4, 10 mM) corresponding to the binding between 1•4Cl$^-$ and tryptophan ([1•4Cl$^-] + [tryptophan] = 5 \times 10^{-4}$ mM). The maximum values are determined to be 0.5 at such an overall concentration and this finding is consistent a 1:1 (host:guest) binding stoichiometry.
Figure S35. ESI-MS spectrum of 1•Trp.

The method of the recognition sensitivity:

The recognition sensitivity (i.e. the detection limits) of host for guests were determined by UV-vis experiments in phosphate buffer (10 mmol, pH = 7.4). With different concentrations of 2 μL guests (Trp and ATP) solution (0, 2, 4, 6, 8, 10 μmol) were added into 2.4 mL host (1•4Cl-) while keeping the host concentration constant (10 μmol) in all the samples. And UV-vis absorption of host-guest solution was measured after mixing homogeneously. Next, the difference ΔA between the different host-guest mixtures and host of absorption values in a certain wavelength was taken, and the linear fitting between ΔA and the corresponding guest concentration can be conducted ([guest] as the x-axis, ΔA as the y-axis). When y = 0, the obtained x value is the detection limits of guest that the lowest concentration of the guest can be detected by host.
**Figure S36.** (a) UV-vis absorption of the $1\cdot4\text{Cl}^-$ and $1\cdot\text{Trp}$ in phosphate buffer (10 mmol, pH = 7.4) at 298K; (b) A plot of the difference of the absorption intensity $\Delta A$ at 280 nm versus the concentration of Trp. Linear Equation: $y = 0.00447x - 0.0016$, $R^2 = 0.99941$; $y = 0$, $x = 0.36$ μmol.
**Figure S37.** $^1$H NMR spectra (400MHz, D$_2$O, phosphate buffer, pH=7.4): a) 1•4Cl$^-$ (0.4 mM); b) 1•4Cl$^-$ and tyrosine (0.4 mM); c) 1•4Cl$^-$ and tyrosine (0.8 mM); d) tyrosine (0.4 mM) at 298K.

**Figure S38.** $^1$H NMR spectra (400MHz, D$_2$O, phosphate buffer, pH=7.4): a) 1•4Cl$^-$ (0.4 mM); b) 1•4Cl$^-$ and phenylalanine (0.4 mM); c) 1•4Cl$^-$ (0.4 mM) and phenylalanine (0.8 mM); d) phenylalanine (0.4 mM) at 298K.
**Figure S39.** $^1$H NMR spectra (400MHz, D$_2$O, phosphate buffer, pH=7.4): a) $1^{1}\cdot4$Cl$^-$ (0.4 mM); b) $1^{1}\cdot4$Cl$^-$ and histidine (0.4 mM); c) $1^{1}\cdot4$Cl$^-$ and histidine (0.8 mM); d) histidine (0.4 mM) at 298K.

**Figure S40.** $^1$H NMR spectra (400MHz, D$_2$O, phosphate buffer, pH=7.4): a) $1^{1}\cdot4$Cl$^-$ (0.4 mM); b) $1^{1}\cdot4$Cl$^-$ and cysteine (0.4 mM); c) $1^{1}\cdot4$Cl$^-$ and cysteine (0.8 mM); d) cysteine (0.4 mM) at 298K.
Figure S41. $^1$H NMR spectra (400MHz, D$_2$O, phosphate buffer, pH=7.4): a) 1•4Cl$^-$ (0.4 mM); b) 1•4Cl$^-$ and aspartic acid (0.4 mM); c) 1•4Cl$^-$ and aspartic acid (0.8 mM); d) aspartic acid (0.4 mM) at 298K.

Figure S42. $^1$H NMR spectra (400MHz, D$_2$O, phosphate buffer, pH=7.4): a) 1•4Cl$^-$ (0.4 mM); b) 1•4Cl$^-$ and arginine (0.4 mM); c) 1•4Cl$^-$ and arginine (0.8 mM); d) arginine (0.4 mM) at 298K.
**Figure S43.** $^1$H NMR spectra (400MHz, D$_2$O, phosphate buffer, pH=7.4): a) 1•4Cl$^-$ (0.4 mM); b) 1•4Cl$^-$ and lysine (0.4 mM); c) 1•4Cl$^-$ and lysine (0.8 mM); d) lysine (0.4 mM) at 298K.

**Figure S44.** $^1$H NMR spectra (400MHz, D$_2$O, phosphate buffer, pH=7.4): a) 1•4Cl$^-$ (0.4 mM); b) 1•4Cl$^-$ and glycine (0.4 mM); c) 1•4Cl$^-$ (0.4 mM) at 298K.

**Figure S45.** $^1$H NMR spectra (400MHz, D$_2$O, phosphate buffer, pH=7.4): a) 1•4Cl$^-$ (0.4 mM); b) 1•4Cl$^-$ and glutamate (0.4 mM); c) glutamate (0.4 mM) at 298K.
Figure S46. $^1$H NMR spectra (400MHz, D$_2$O, phosphate buffer, pH=7.4): a) 1•4Cl$^-$ (0.4 mM); b) 1•4Cl$^-$ and leucine (0.4 mM); c) leucine (0.4 mM) at 298K.

Figure S47. $^1$H NMR spectra (400MHz, D$_2$O, phosphate buffer, pH=7.4): a) 1•4Cl$^-$ (0.4 mM); b) 1•4Cl$^-$ and proline (0.4 mM); c) proline (0.4 mM) at 298K.
Figure S48. $^1$H NMR spectra (400MHz, D$_2$O, phosphate buffer, pH=7.4): a) 1•4Cl$^-$ (0.4 mM); b) 1•4Cl$^-$ and threonine (0.4 mM); c) threonine (0.4 mM) at 298K.

Figure S49. $^1$H NMR spectra (400MHz, D$_2$O, phosphate buffer, pH=7.4): a) 1•4Cl$^-$ (0.4 mM); b) 1•4Cl$^-$ and asparaginate (0.4 mM); c) asparaginate (0.4 mM) at 298K.
Figure S50. $^1$H NMR spectra (400MHz, D$_2$O, phosphate buffer, pH=7.4): a) 1•4Cl$^-$ (0.4 mM); b) 1•4Cl$^-$ and isoleucine (0.4 mM); c) isoleucine (0.4 mM) at 298K.

Figure S51. $^1$H NMR spectra (400MHz, D$_2$O, phosphate buffer, pH=7.4): a) 1•4Cl$^-$ (0.4 mM); b) 1•4Cl$^-$ and alanine (0.4 mM); c) alanine (0.4 mM) at 298K.
Figure S52. $^1$H NMR spectra (400MHz, D$_2$O, phosphate buffer, pH=7.4): a) 1•4Cl$^-$ (0.4 mM); b) 1•4Cl$^-$ and serine (0.4 mM); c) serine (0.4 mM) at 298K.

Figure S53. $^1$H NMR spectra (400MHz, D$_2$O, phosphate buffer, pH=7.4): a) 1•4Cl$^-$ (0.4 mM); b) 1•4Cl$^-$ and methionine (0.4 mM); c) methionine (0.4 mM) at 298K.
Figure S54. $^1$H NMR spectra (400MHz, D$_2$O, phosphate buffer, pH=7.4): a) $\text{1}\cdot\text{4Cl}^-$ (0.4 mM); b) $\text{1}\cdot\text{4Cl}^-$ and glutamine (0.4 mM); c) glutamine (0.4 mM) at 298K.

Figure S55. $^1$H NMR spectra (400MHz, D$_2$O, phosphate buffer, pH=7.4): a) $\text{1}\cdot\text{4Cl}^-$ (0.4 mM); b) $\text{1}\cdot\text{4Cl}^-$ and valine (0.4 mM); c) valine (0.4 mM) at 298K.
Figure S56. $^1$H NMR spectra (400MHz, D$_2$O, phosphate buffer, pH=7.4): a) 2•4Cl$^-$ (0.4 mM); b) 2•4Cl$^-$ and tryptophan (0.4 mM); c) 2•4Cl$^-$ and tryptophan (0.8 mM); d) tryptophan (0.4 mM) at 298K.

Figure S57. $^1$H NMR spectra (400MHz, D$_2$O, phosphate buffer, pH=7.4): a) 2•4Cl$^-$ (0.4 mM); b) 2•4Cl$^-$ and phenylalanine (0.4 mM); c) 2•4Cl$^-$ and phenylalanine (0.8 mM); d) phenylalanine (0.4 mM) at 298K.
**Figure S58.** $^1$H NMR spectra (400MHz, D$_2$O, phosphate buffer, pH=7.4): a) $2\cdot 4$Cl$^-$ (0.4 mM); b) $2\cdot 4$Cl$^-$ and tyrosine (0.4 mM); c) $2\cdot 4$Cl$^-$ and tyrosine (0.8 mM); d) tyrosine (0.4 mM) at 298K.

**Figure S59.** $^1$H NMR spectra (400MHz, D$_2$O, phosphate buffer, pH=7.4): a) $2\cdot 4$Cl$^-$ (0.4 mM); b) $2\cdot 4$Cl$^-$ and histidine (0.4 mM); c) $2\cdot 4$Cl$^-$ and histidine (0.8 mM); d) histidine (0.4 mM) at 298K.
Figure S60. $^1$H NMR spectra (400MHz, D$_2$O, phosphate buffer, pH=7.4): a) 2•4Cl$^-$ (0.4 mM); b) 2•4Cl$^-$ and aspartic acid (0.4 mM); c) 2•4Cl$^-$ and aspartic acid (0.8 mM); d) aspartic acid (0.4 mM) at 298K.

Figure S61. $^1$H NMR spectra (400MHz, D$_2$O, phosphate buffer, pH=7.4): a) 2•4Cl$^-$ (0.4 mM); b) 2•4Cl$^-$ and cysteine (0.4 mM); c) 2•4Cl$^-$ and cysteine (0.8 mM); d) cysteine (0.4 mM) at 298K.
Figure S62. $^1$H NMR spectra (400MHz, D$_2$O, phosphate buffer, pH=7.4): a) 2•4Cl$^-$ (0.4 mM); b) 2•4Cl$^-$ and arginine (0.4 mM); c) 2•4Cl$^-$ and arginine (0.8 mM); d) arginine (0.4 mM) at 298K.

Figure S63. $^1$H NMR spectra (400MHz, D$_2$O, phosphate buffer, pH=7.4): a) 2•4Cl$^-$ (0.4 mM); b) 2•4Cl$^-$ and lysine (0.4 mM); c) 2•4Cl$^-$ and lysine (0.8 mM); d) lysine (0.4 mM) at 298K.
**Figure S64.** $^1$H NMR spectra (400MHz, D$_2$O, phosphate buffer, pH=7.4): a) 2$\textsuperscript{•}$4Cl$^-$ (0.4 mM); b) 2$\textsuperscript{•}$4Cl$^-$ and asparaginate (0.4 mM); c) asparaginate (0.4 mM) at 298K.

**Figure S65.** $^1$H NMR spectra (400MHz, D$_2$O, phosphate buffer, pH=7.4): a) 2$\textsuperscript{•}$4Cl$^-$ (0.4 mM); b) 2$\textsuperscript{•}$4Cl$^-$ and alanine (0.4 mM); c) alanine (0.4 mM) at 298K.
Figure S66. $^1$H NMR spectra (400MHz, D$_2$O, phosphate buffer, pH=7.4): a) 2•4Cl$^-$ (0.4 mM); b) 2•4Cl$^-$ and proline (0.4 mM); c) proline (0.4 mM) at 298K.

Figure S67. $^1$H NMR spectra (400MHz, D$_2$O, phosphate buffer, pH=7.4): a) 2•4Cl$^-$ (0.4 mM); b) 2•4Cl$^-$ and glycine (0.4 mM); c) glycine (0.4 mM) at 298K.
Figure S68. $^1$H NMR spectra (400MHz, D$_2$O, phosphate buffer, pH=7.4): a) 2•4Cl$^-$ (0.4 mM); b) 2•4Cl$^-$ and glutamine (0.4 mM); c) glutamine (0.4 mM) at 298K.

Figure S69. $^1$H NMR spectra (400MHz, D$_2$O, phosphate buffer, pH=7.4): a) 2•4Cl$^-$ (0.4 mM); b) 2•4Cl$^-$ and glutamic acid (0.4 mM); c) glutamic acid (0.4 mM) at 298K.
**Figure S70.** $^1$H NMR spectra (400MHz, D$_2$O, phosphate buffer, pH=7.4): a) 2•4Cl$^-$ (0.4 mM); b) 2•4Cl$^-$ and methionine (0.4 mM); c) methionine (0.4 mM) at 298K.

**Figure S71.** $^1$H NMR spectra (400MHz, D$_2$O, phosphate buffer, pH=7.4): a) 2•4Cl$^-$ (0.4 mM); b) 2•4Cl$^-$ and leucine (0.4 mM); c) leucine (0.4 mM) at 298K.
Figure S72. $^1$H NMR spectra (400MHz, D$_2$O, phosphate buffer, pH=7.4): a) 2•4Cl$^-$ (0.4 mM); b) 2•4Cl$^-$ and serine (0.4 mM); c) serine (0.4 mM) at 298K.

Figure S73. $^1$H NMR spectra (400MHz, D$_2$O, phosphate buffer, pH=7.4): a) 2•4Cl$^-$ (0.4 mM); b) 2•4Cl$^-$ and threonine (0.4 mM); c) threonine (0.4 mM) at 298K.
Figure S74. $^1$H NMR spectra (400MHz, D$_2$O, phosphate buffer, pH=7.4): a) $2\cdot 4\text{Cl}^-$ (0.4 mM); b) $2\cdot 4\text{Cl}^-$ and valine (0.4 mM); c) valine (0.4 mM) at 298K.

Figure S75. $^1$H NMR spectra (400MHz, D$_2$O, phosphate buffer, pH=7.4): a) $2\cdot 4\text{Cl}^-$ (0.4 mM); b) $2\cdot 4\text{Cl}^-$ and isoleucine (0.4 mM); c) isoleucine (0.4 mM) at 298K.
**Figure S76.** $^1$H NMR spectra (400MHz, D$_2$O, phosphate buffer, pH=7.4): a) 1•4Cl$^-$ (0.4 mM); b) 1•4Cl$^-$ and ATP (0.4 mM); c) 1•4Cl$^-$ and ATP (0.8 mM); d) ATP (0.4 mM) at 298K.

**Figure S77.** COSY and NOESY $^1$H NMR spectrum recorded (400 MHz, D$_2$O, phosphate buffer, pH = 7.4, 298 K) for 1•4Cl$^-$ (0.5mM) with ATP (0.5 mM).
**Figure S78.** ITC titration of $1\cdot 4\text{Cl}^-$ (0.1 mM) with ATP (1.5 mM) in phosphate buffer (pH = 7.4).

**Figure S79.** (a) UV-vis absorption of $1\cdot 4\text{Cl}^-$ and $1\cdot \text{ATP}$ in phosphate buffer (10 mmol, pH=7.4) at 298K; (b) A plot of the difference of the absorption intensity $\Delta A$ at 253 nm.
versus the concentration of ATP. Linear Equation: \( y = 0.00257x - 0.00485 \), \( R^2 = 0.99481 \); \( y = 0 \), \( x = 1.89 \ \mu\text{mol} \).

**Figure S80.** $^1$H NMR spectra (400MHz, D$_2$O, phosphate buffer, pH=7.4): a) 1•4Cl$^-$ (0.4 mM); b) 1•4Cl$^-$ and ADP (0.4 mM); c) 1•4Cl$^-$ and ADP (0.8 mM); d) ADP (0.4 mM) at 298K.

**Figure S81.** ITC titration of 1•4Cl$^-$ (0.2 mM) with ADP (2.0 mM) in phosphate buffer (pH = 7.4).
Figure S82. $^1$H NMR spectra (400MHz, D$_2$O, phosphate buffer, pH=7.4): a) 1•4Cl$^-$ (0.4 mM); b) 1•4Cl$^-$ and AMP (0.4 mM); c) 1•4Cl$^-$ and AMP (0.8 mM); d) AMP (0.4 mM) at 298K.

Figure S83. ITC titration of 1•4Cl$^-$ (0.2 mM) with AMP (2.0 mM) in phosphate buffer (pH = 7.4).
Figure S84. ESI-MS spectrum of 1•ATP.

Figure S85. ESI-MS spectrum of 1•ADP.
Figure S86. ESI-MS spectrum of 1•AMP.

Figure S87. $^1$H NMR spectra (400MHz, D$_2$O, phosphate buffer, pH=7.4): a) 2•4Cl$^-$ (0.4 mM); b) 2•4Cl$^-$ and ATP (0.4 mM); c) 2•4Cl$^-$ and ATP (0.8 mM); d) ATP (0.4 mM) at 298K.
Figure S88. UV-vis absorption of 2•4Cl\(^-\) (10μmol) in phosphate buffer (pH = 7.4) upon addition of ATP. The inset shows a plot of absorbance intensity at 315 nm versus the equiv. of ATP.

Figure S89. \(^1\)H NMR spectra (400MHz, D\(_2\)O, phosphate buffer, pH=7.4): a) 2•4Cl\(^-\) (0.1 mM); b) 2•4Cl\(^-\) and ADP (0.1 mM); c) 2•4Cl\(^-\) and ADP (0.2 mM); d) ADP (0.1 mM) at 298K.
Figure S90. UV-vis absorption of $2\cdot4\text{Cl}^-$ (10 μmol) in phosphate buffer (pH = 7.4) upon addition of ADP. The inset shows a plot of absorbance intensity at 315 nm versus the equiv. of ADP.

Figure S91. $^1$H NMR spectra (400MHz, D$_2$O, phosphate buffer, pH=7.4): a) $2\cdot4\text{Cl}^-$ (0.1 mM); b) $2\cdot4\text{Cl}^-$ and AMP (0.1 mM); c) $2\cdot4\text{Cl}^-$ and AMP (0.2 mM); d) AMP (0.1 mM) at 298K.
**Figure S92.** UV-vis absorption of $2\cdot4\text{Cl}^-$ (10 μmol) in phosphate buffer (pH = 7.4) upon addition of AMP. The inset shows a plot of absorbance intensity at 315 nm versus the equiv. of AMP.