Supplementary Data for

Novel Benzimidazolium-urea-based Macrocyclic Fluorescent Sensors: Synthesis, Ratiometric Sensing of $H_2PO_4^-$ and Improvement of the Anion Binding Performance via Synergistic Binding Strategy

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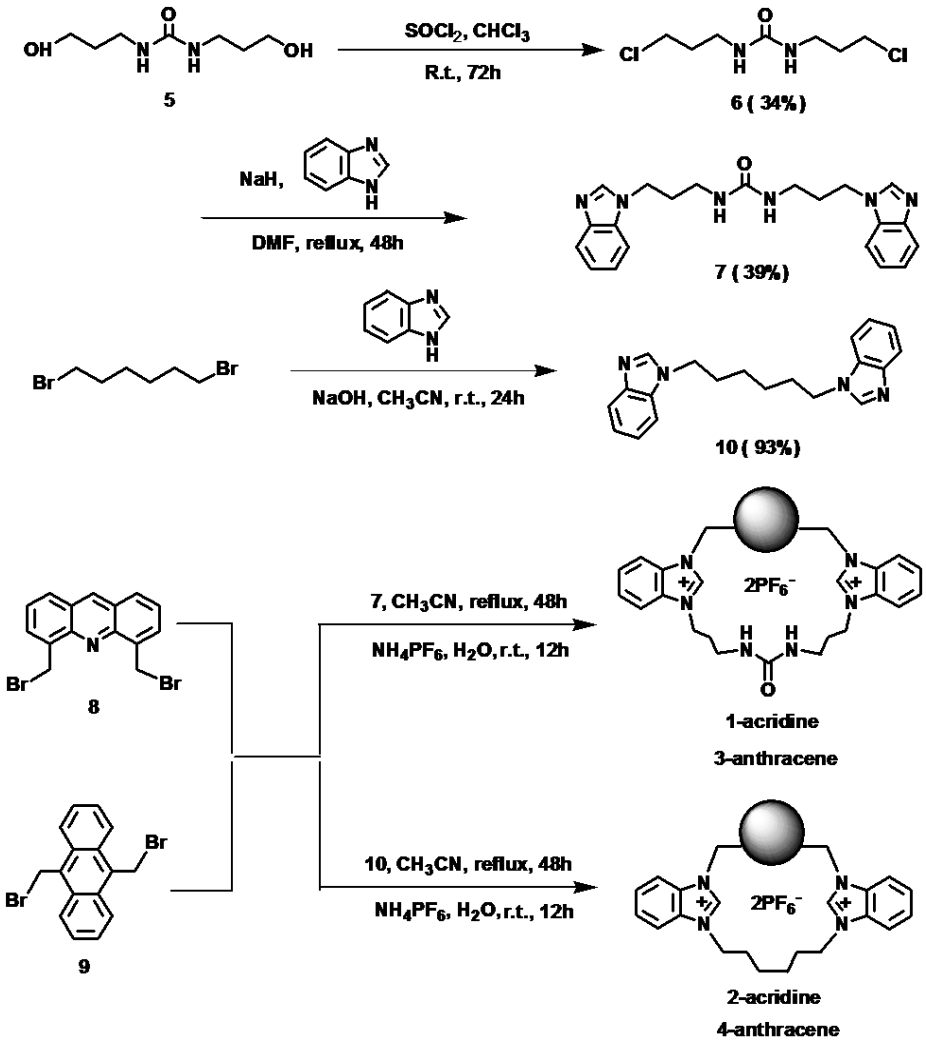
Content

1. Materials and Instruments

All chemicals were purchased from Sinopharm Chemical Reagent Co. (Shanghai, China) otherwise specified below, and were used without further purification. The stock solutions of anions were prepared from TBAX (where X = F^-, Cl^-, Br^-, I^-, AcO^-, SCN^-, HSO_4^-, $H_2PO_4^-$ and ClO_4^-). ^1H NMR and ^{13}C NMR spectra were recorded in $(CD_3)_2SO$ at 293 K on a Bruker Avance DRX 500 FT NMR spectrometer, operating at 500 MHz and 125 MHz for ^1H NMR spectra and ^{13}C NMR spectra respectively. Fluorescence spectra were carried out in a Hitachi F-4500 fluorescence spectrometer. HRMS spectra were acquired using an AB SCIEX 4800 plus MALDI-TOF reflector.

2. Synthesis and Characterization



Scheme S1 Synthesis of fluorescent sensors **1-4**.

1, 3-bis-(3-chloro-propyl)-urea 6. $SOCl_2$ (20 mL) was added dropwise to a solution of 1, 3-bis-(3-hydroxyl-propyl)-urea **5** (5.22 g, 30 mmol) in $CHCl_3$ (50 mL), and the mixture was stirred at r.t. under N_2 for 72 h. After completion of the reaction, add water to the resulting mixture to react with the excess $SOCl_2$. Collect the organic phase and evaporate the $CHCl_3$ under vacuum to achieve the white compound 1, 3-bis-(3-chloro-propyl)-urea **6** in 34% yield (2.15 g). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.96 (t, *J* = 5.8, 4H), 3.31 (d, *J* = 5.2, 4H), 3.60 (t, *J* = 5.2, 4H), 5.17 (s, 2H).

Synthesis of 1, 3-bis-(3-benzoimidazol-1-yl-propyl)-urea 7. A solution of benzimidazole (0.661 g, 5.6 mmol) in anhydrous DMF (15 mL) was added dropwise to 6 mL DMF solution of 60 % NaH (0.288 g, 7.2 mmol) at 0 °C. The mixture was stirred at r.t. for 20 min, and then increased the temperature to 80 °C, followed by adding **6** (0.422 g, 2.0 mmol) dropwise. The resulting mixture was stirred at 80 °C for 24 h, and the solvent was removed in vacuo. The residue was dissolved in water (100 mL) and extracted with $CHCl_3$ (4 × 20 mL). The organic phases were dried by anhydrous Na_2SO_4, and the solvents were removed under reduced pressure to give a mixture. The mixture was purified by chromatography on silica gel (CH_2Cl_2/MeOH = 40/1, v/v) to give the white solid compound **7** in 39% yield (0.290 g). ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.88-1.93 (m, 4H), 2.98 (q, J_1 = 12.5, J_2 = 12.4, 4H), 4.26 (t, *J* = 6.8, 4H), 6.20 (s, *J* = 5.5, 2H), 7.19-7.27 (m, 4H), 7.62 (dd, J_1 = 7.7, J_2 = 7.9, 4H), 8.34 (s, 2H).

Synthesis of 1, 6-bis-(benzoimidazol-hexane) 10. To a solution of benzimidazole (1.18 g, 10 mmol) in CH_3CN (30 mL), NaOH (25%, 8 mL) aqueous solution was added and the mixture was stirred under N_2 for 4 h. Then the dibromohexane (0.986 g, 4.0 mmol) was added in one portion and the reaction was stirred at r.t. for 24 h. After completion of the reaction, adding water to the reaction solution to make the product precipitated. The precipitate was filtered off, washed thoroughly with CH_3CN (3 × 50 mL) to give the white compound **10** (1.18 g, 93%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.28-1.41 (m, 4H), 1.82-1.92 (m, 4H), 4.13 (t, *J* = 6.8, 4H), 7.29-7.34 (m, 6H), 7.81-7.86 (m, 4H).

Synthesis of sensors 1 and 3. To 150 mL acetonitrile, the compound **7** (0.370 g, 1.0 mmol) in 200 mL acetonitrile and the 200 mL acetonitrile solution of **8/9** (1.0 mmol) were added slowly at the same rate for 9 h under reflux. The resulting mixture was stirred and refluxed for another 48 h, and then the solution was concentrated. The crude bromide

salt was purified by column chromatography on silica gel using $CH_3OH/CHCl_3$ (5:1, v/v) as eluent to give the pure bromide salt of sensor **1/3** (85%/75%). On treatment of the aqueous solution of the bromide salt of **1/3** (1.0 mmol) with a saturated aqueous solution of NH_4PF_6, the precipitate formed. The precipitate was collected, washed thoroughly with water and dried to give the hexafluorophosphate salt of sensor **1/3** (90%/93%) as faint yellow solid. Sensor **1**: ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.10 (t, J = 5.8, 4H), 3.01 (t, J = 5.7, 4H), 4.56 (t, J = 6.3, 4H), 6.02 (t, J = 5.8, 2H), 6.56 (s, 4H), 7.59 (t, J = 7.8, 2H), 7.65-7.71 (m, 4H), 7.97 (d, J = 8.0, 4H), 8.13 (d, J = 8.3, 2H), 8.29 (d, J = 8.4, 2H), 9.33 (s, 1H), 9.93 (s, 2H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 28.05, 36.41, 45.05, 48.06, 114.00, 125.70, 126.27, 126.44, 126.56, 129.77, 130.96, 131.21, 131.27, 131.34, 132.66, 138.24, 143.27, 145.57, 158.48. HRMS: $C_{36}H_{35}ON_7PF_6^+$, (M-PF_6) requires 726.2539, found 726.2534. Sensor **3**: ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.64 (s, 2H), 2.50 (s, 2H), 4.22 (s, 2H), 5.93 (t, J = 6.3, 1H), 6.88 (s, 2H), 7.69 (q, J_1 = 6.9, J_2 = 6.9, 2H), 7.86 (t, J = 7.8, 1H), 7.96 (t, J = 7.8, 1H), 8.15 (d, J = 8.3, 1H)), 8.37 (s, 1H), 8.52 (t, J_1 = 6.8, J_2 = 6.8, 2H), 8.61 (d, J = 8.3, 1H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 29.78, 33.87, 42.86, 43.29, 113.57, 114.64, 124.54, 125.39, 127.05, 127.36, 128.04, 130.93, 131.32, 132.05, 140.61, 158.92. HRMS: $C_{37}H_{36}ON_6PF_6^+$, (M-PF_6) requires 725.2587, found 725.2596.

Synthesis of sensor 2. The synthetic procedure and characterization data of sensor **2** are in accord with our previously reported literature.[1] ^{1}H NMR (500 MHz, DMSO-d_6) δ 0.80 (s, 4H), 1.58 (s, 4H), 4.23 (t, J = 5.8, 4H) 6.30 (s, 4H) 7.47 (d, J = 7.7, 2H) 7.56 (t, J = 7.8, 2H), 7.83 (t, J = 7.6, 4H) 8.22 (d, J = 7.7, 4H), 8.45 (d, J = 8.5, 2H), 9.11 (s, 2H), 9.46 (s, 1H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 25.62, 27.54, 46.43, 47.63, 113.60, 113.95, 126.69, 127.02, 127.20, 127.28, 129.72, 131.23, 131.49, 131.85, 135.61, 139.06, 141.71, 146.76. HRMS: $C_{35}H_{33}N_5PF_6^+$, (M-PF_6) requires 668.2372, found 668.2369.

Synthesis of sensor 4. To 150 mL acetonitrile, the bis-benzimidazole **10** (0.317 g, 1.0 mmol) in 200 mL acetonitrile and the 200 mL acetonitrile solution of the 9,10-bis(bromomethyl)anthracene **9** (0.364 g, 1.0 mmol) were added slowly at the same rate for 9 h under reflux. The resulting mixture was stirred and refluxed for another 48 h, and then the solution was concentrated. The crude bromide salt was purified by column chromatography on silica gel using MeOH/$CHCl_3$ (5:1, v/v) as eluent to give the pure bromide salt of sensor **4** (83%). On treatment of the aqueous solution of the bromide salt

of **4** (1.0 mmol) with a saturated aqueous solution of NH_4PF_6, the precipitate formed. The precipitate was collected, washed thoroughly with water and dried to give the hexafluorophosphate salt of sensor **4** (88%) as faint yellow solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ -0.11 (s, 2H), 1.41 (s, 2H), 4.26 (t, J = 5.9, 2H), 6.98 (s, 2H), 7.59-7.61 (m, 2H), 7.78 (t, J = 7.9, 1H), 7.93 (t, J = 7.9, 1H), 8.09 (d, J = 8.3, 1H), 8.35-8.37(m, 2H), 8.40 (s, 1H), 8.69 (d, J = 8.4, 1H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 19.61, 23.26, 42.77, 45.31, 113.78, 113.95, 124.14, 127.18, 127.40, 127.81, 128.05, 129.96, 130.28, 132.04, 141.76. HRMS: $C_{36}H_{34}N_4PF_6^+$, (M-PF_6) requires 667.2420, found 667.2420.

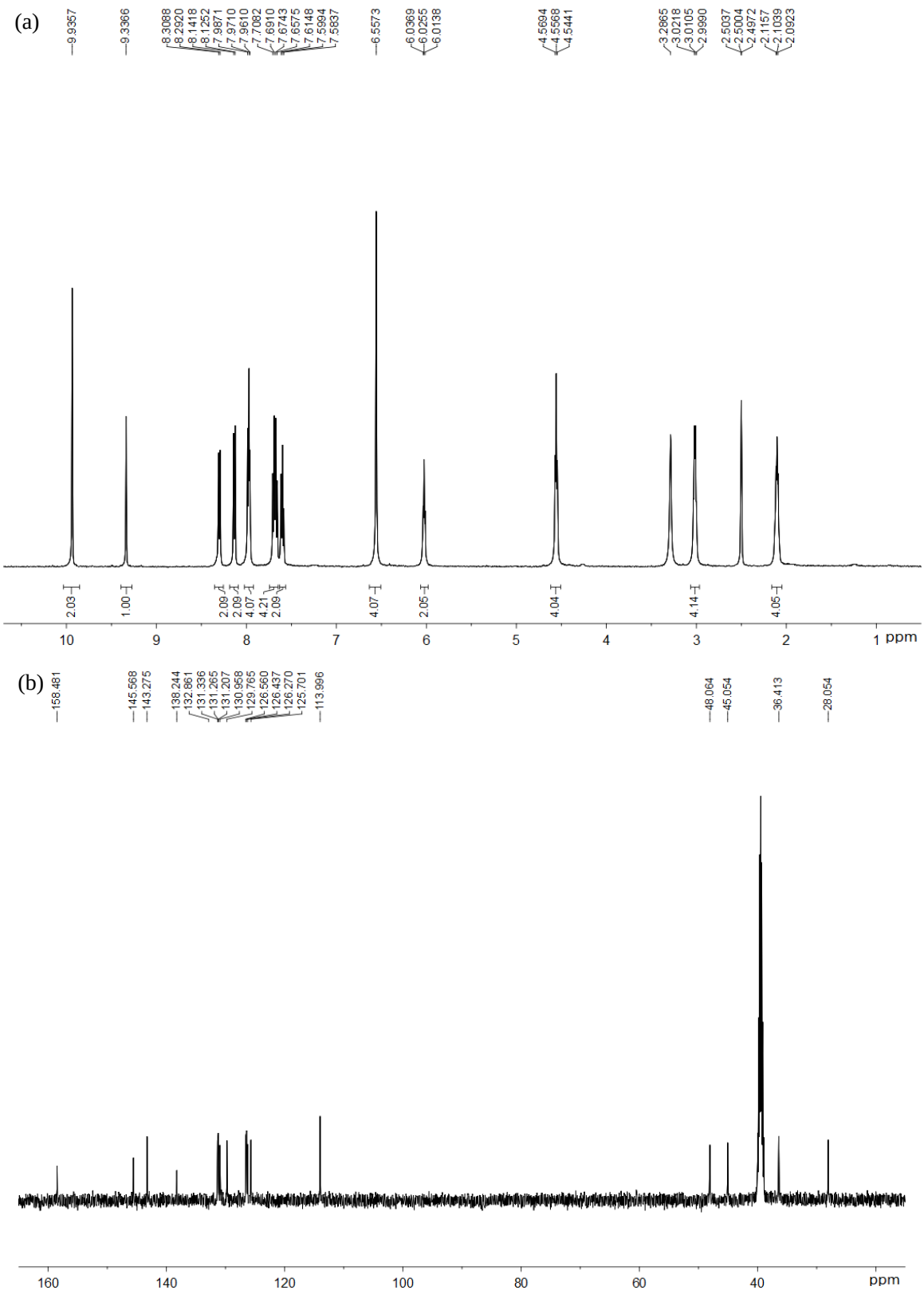
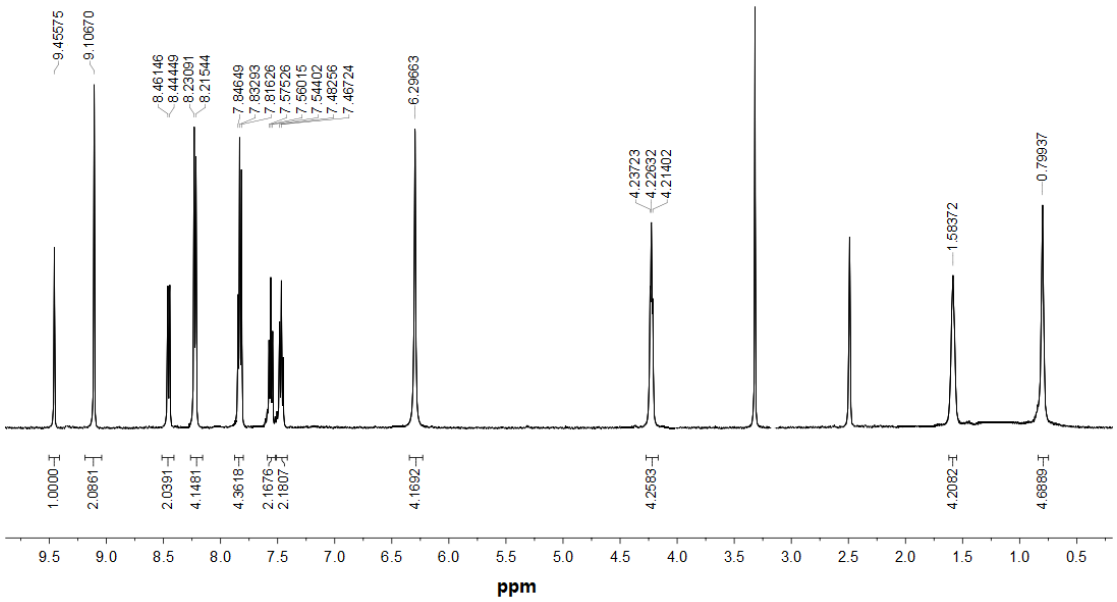


Fig. S1 ^{1}H NMR (a) and ^{13}C NMR (b) of sensor **1** in DMSO-d_6.

(a)



(b)

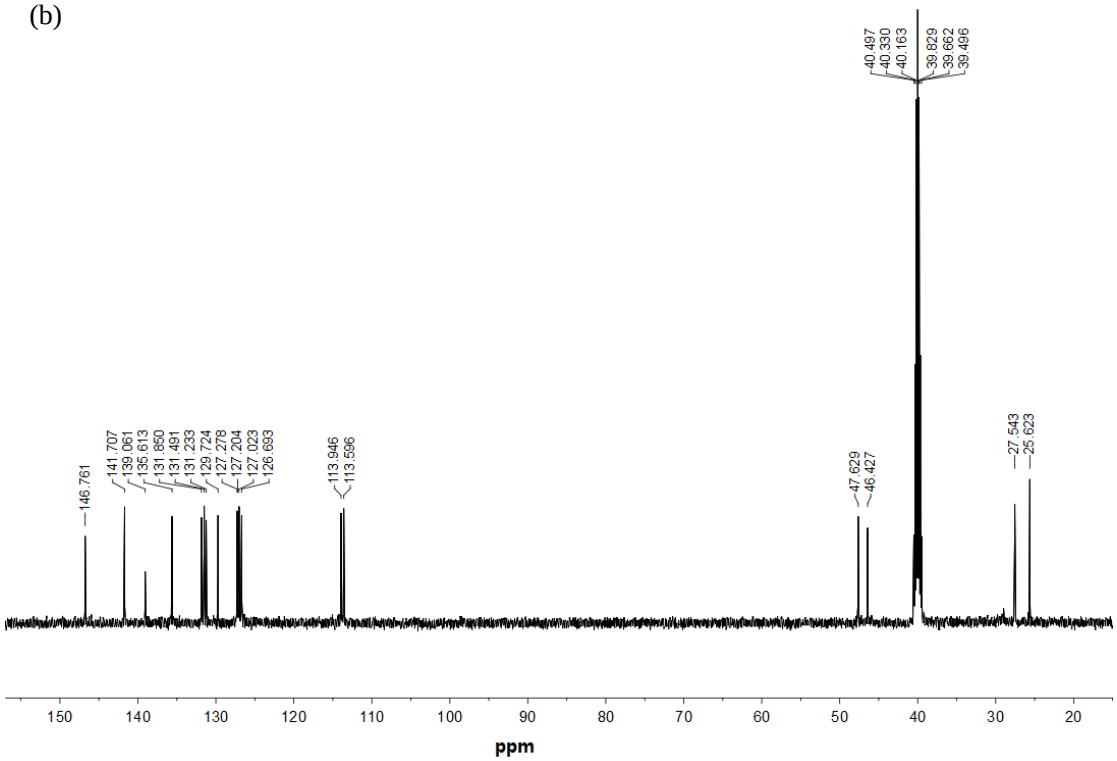


Fig. S2 ^{1}H NMR (a) and ^{13}C NMR (b) of sensor **2** in DMSO-d_6.

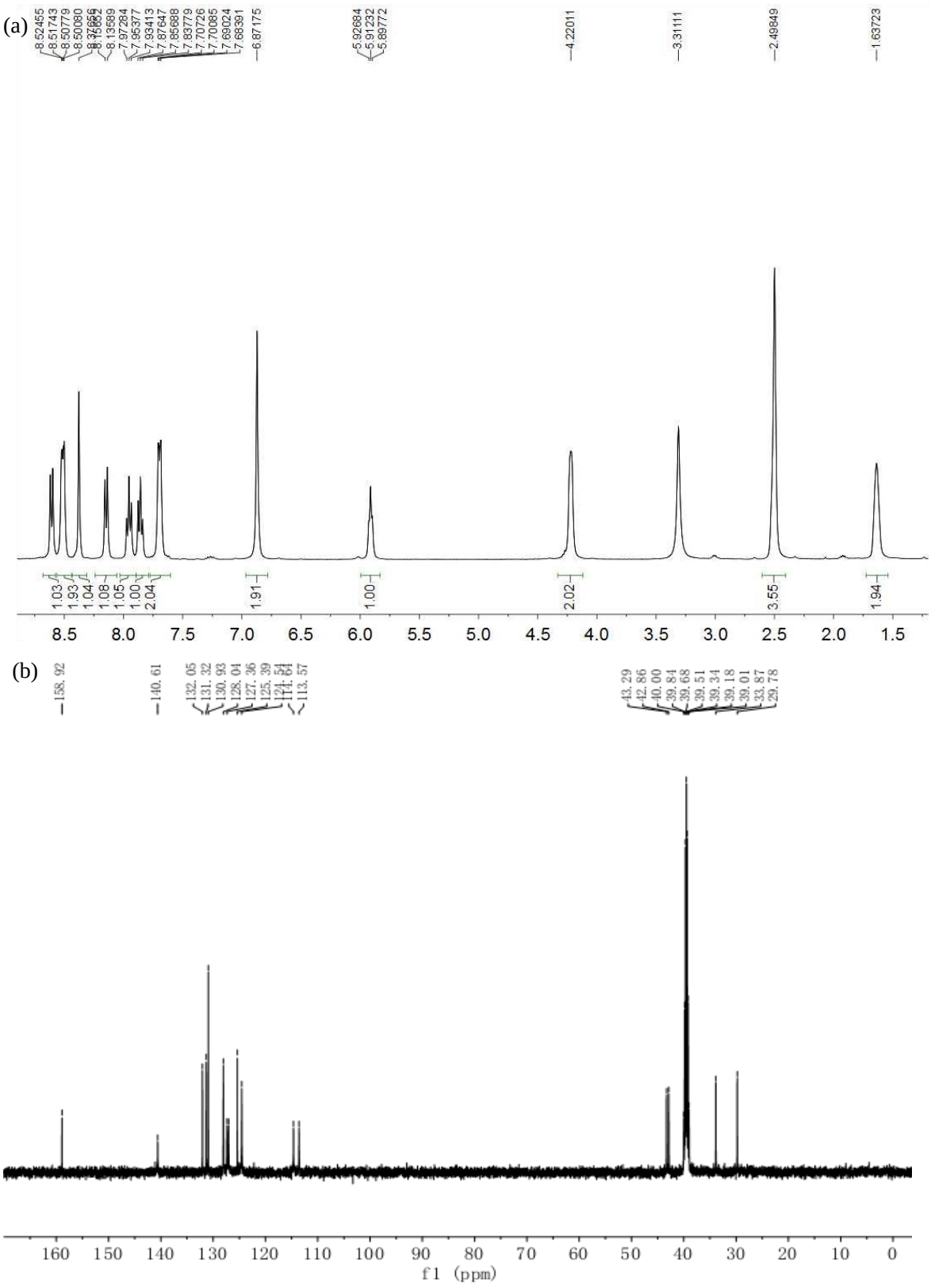


Fig. S3 ^{1}H NMR (a) and ^{13}C NMR (b) of sensor **3** in DMSO-d_6.

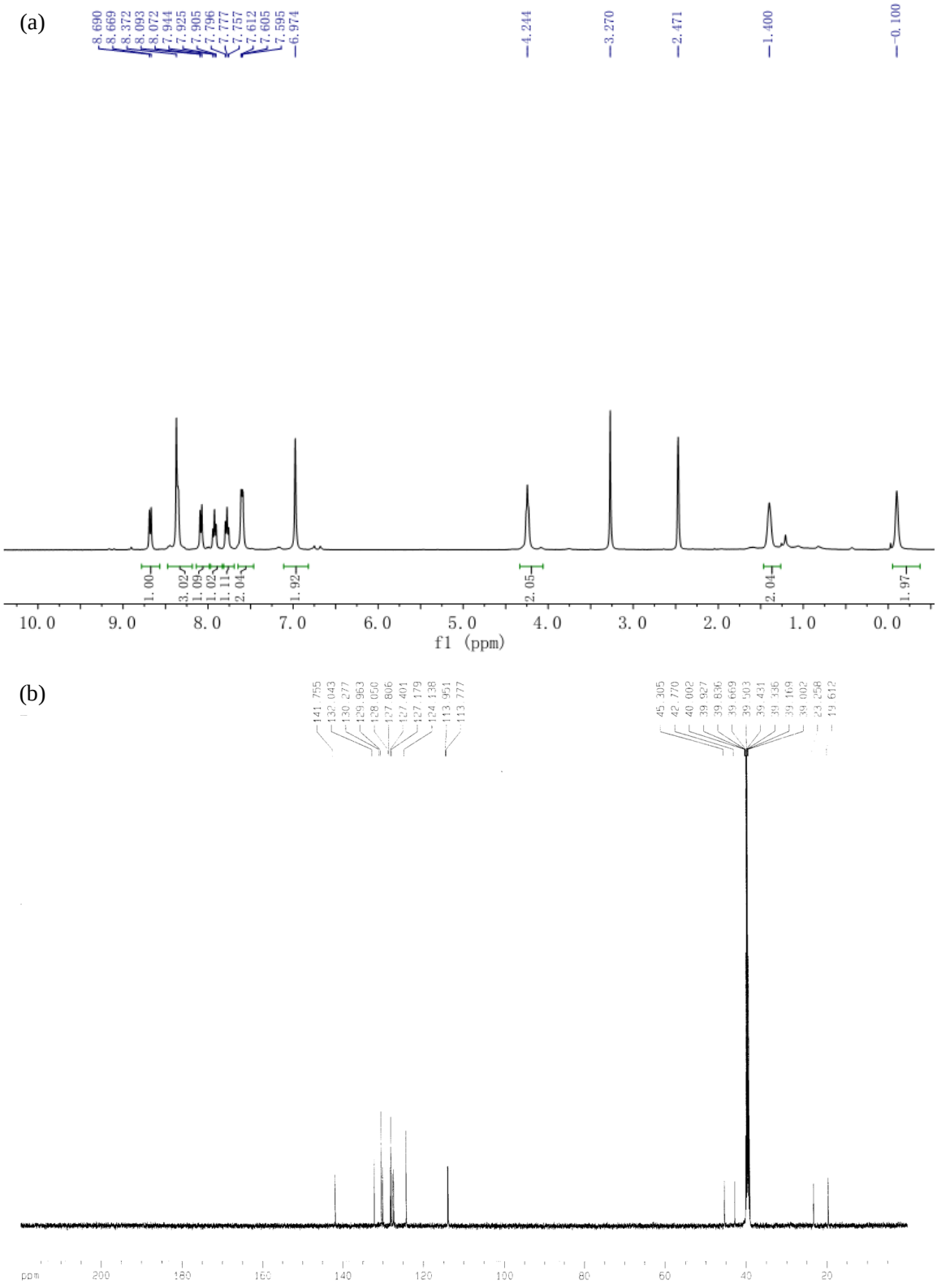


Fig. S4 ^{1}H NMR (a) and ^{13}C NMR (b) of sensor **4** in DMSO-d_6.

3. X-ray structure determination

Crystal of sensor **2** suitable for X-ray diffraction studies was obtained from slow evaporation of concentrated solutions of the sensor **2** in CH_3OH/CH_3COCH_3. X-ray diffraction data from suitable crystals were collected on a Bruker Apex-II CCD area detector equipped with graphite-monochromated Mo Kα radiation (λ = 0.71073 Å). Empirical absorption corrections were applied using SADABS program.[2] The structures were solved by the direct method and refined by the full-matrix least-squares method on F^2, with all non-hydrogen atoms refined with the anisotropic thermal parameters.[3] All the hydrogen atoms attached to carbon atoms were placed in calculated positions and refined using the riding model, and the water hydrogen atoms were located from the different maps. The -$(CH_2)_6$- tether between two benzimidazolium rings was refined with a disordered model: two sets of half-occupied positions were assumed and the C-N and C-C distances were restrained using the SHELXL DFIX instructions. One of the two PF_6^- anions was refined with disordered F atoms. All calculations were carried out with the SHELXTL software. A summary of the crystallographic data and refinement parameters of sensor **2** are shown in Table S1.

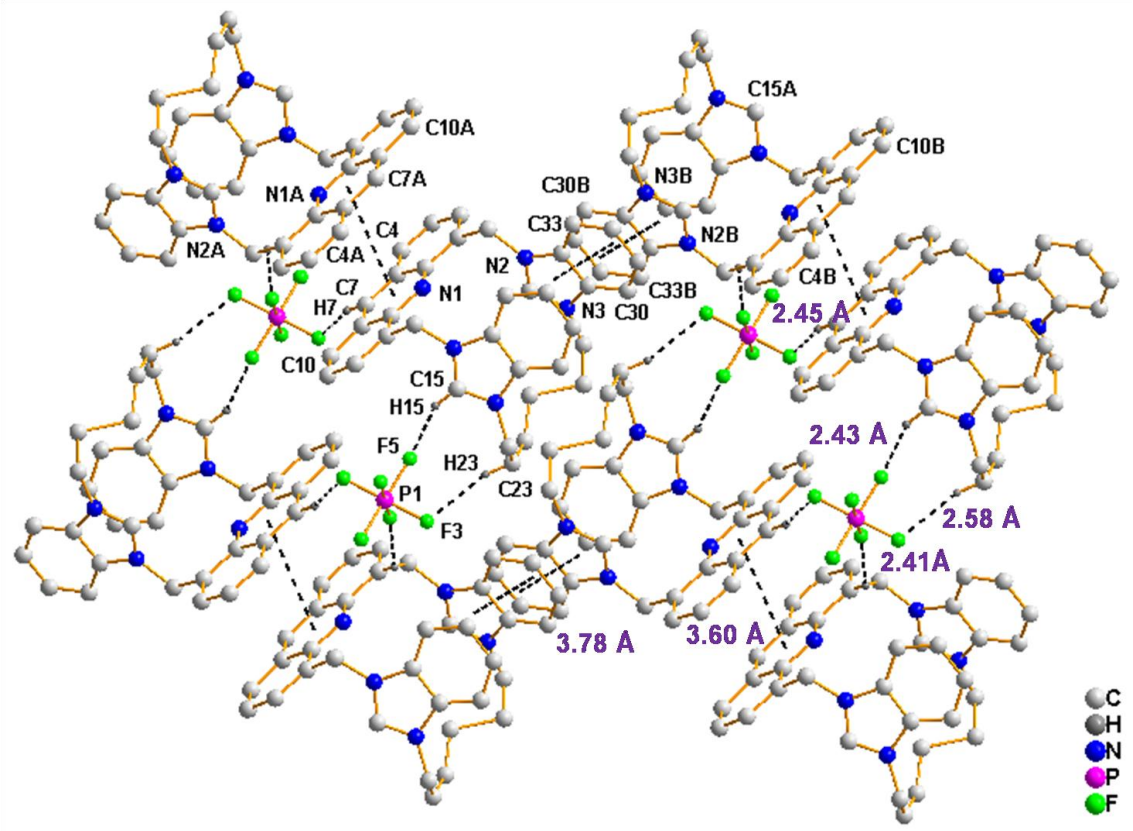


Fig. S5 The self-assembly network of **2**·2(PF_6^-)·(CH_3COCH_3). Nonacidic hydrogen atoms, some PF_6^- counterions, CH_3COCH_3 solvent molecules and one of the two sets of disorder in the sensor molecule are omitted for clarity. Dashed lines represent hydrogen bonds and π-π interactions. Symmetry codes: A = 2-x, 1-y, -z; B = 2-x, 1-y, 1-z.

Table S1 Crystal data and refinement of sensor **2**

Compound	**2**
CCDC	914122
Empirical formula	$C_{38}H_{39}F_{12}N_5OP_2$
M_r	871.68
Temperature (K)	296(2)
Crystal system	Triclinic
Space group	$P\bar{1}$
a/Å	11.3419(14)
b/Å	12.6176(16)
c/Å	15.0660(19)
α/°	71.396(2)
β/°	80.156(2)
γ/°	86.887(2)
V/Å^3	2013.3(4)
Z	2
Crystal size (mm^3)	0.10 × 0.07 × 0.03
D_c/g cm^{-3}	1.438
μ/mm^{-1}	0.202
F(000)	896
θ range (°)	1.82 – 26.00
Reflections collected	11370
Unique reflections	7730
$R_{(int)}$	0.0195
GOF on F^2	0.942
R_1 [I >2σ(I)]	0.0808
wR_2 (all data)	0.2946

4. Fluorescence Titration

Fluorescence titrations of the sensors with $H_2PO_4^-$ were performed as follows: 2 mL of sensor solution (5 μM) was taken into the cuvette, and then certain equivalents of anions were added stepwise to the sensor solution by a micro-injector. As a very small amount of anion solutions were added, the final volume of the solution was nearly unchanged (2 mL). The mixed solution was incubated for 30 s, and then irradiated at a selected excitation wavelength. The corresponding emission values during titration were recorded.

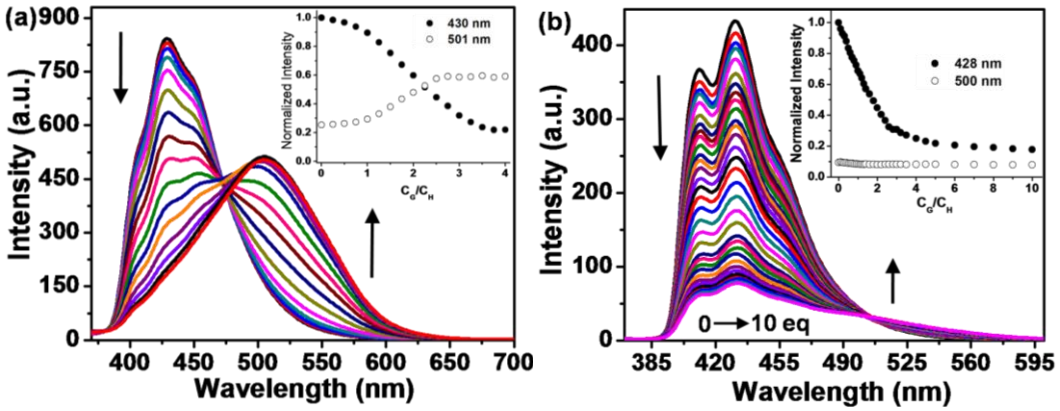


Fig. S6 Fluorescence titrations of 5 μM sensor **2** excited at 358 nm (excitation and emission slit: 5 nm) (a) and sensor **4** excited at 362 nm (excitation and emission slit: 2.5 nm) (b) with $H_2PO_4^-$ in CH_3CN. Insets: normalized emission developments at monomer and excimer peaks as a function of added $H_2PO_4^-$ equivalents.

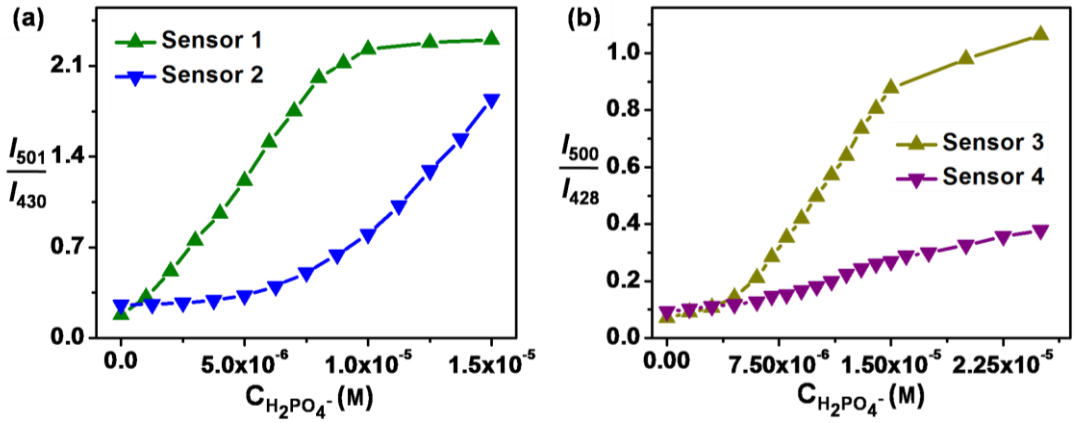


Fig. S7 (a) Intensity ratio (I_{501}/I_{430}) of sensors **1** and **2** as a function of $H_2PO_4^-$ concentration. (b) Intensity ratio (I_{500}/I_{428}) of sensors **3** and **4** as a function of $H_2PO_4^-$ concentration.

5. Fluorescence Job's Plot

The continuous variation method (Job's plot)[4] was used for determining the stoichiometric ratio between sensors and $H_2PO_4^-$. In this method, solutions of sensor and $H_2PO_4^-$ of the same concentrations were prepared in CH_3CN. Then sensor and $H_2PO_4^-$ solutions were mixed in different proportions maintaining a total volume of 3 mL and a total concentration of 5 μM of the mixture. Then the emission spectra of the mixed solutions were recorded immediately.

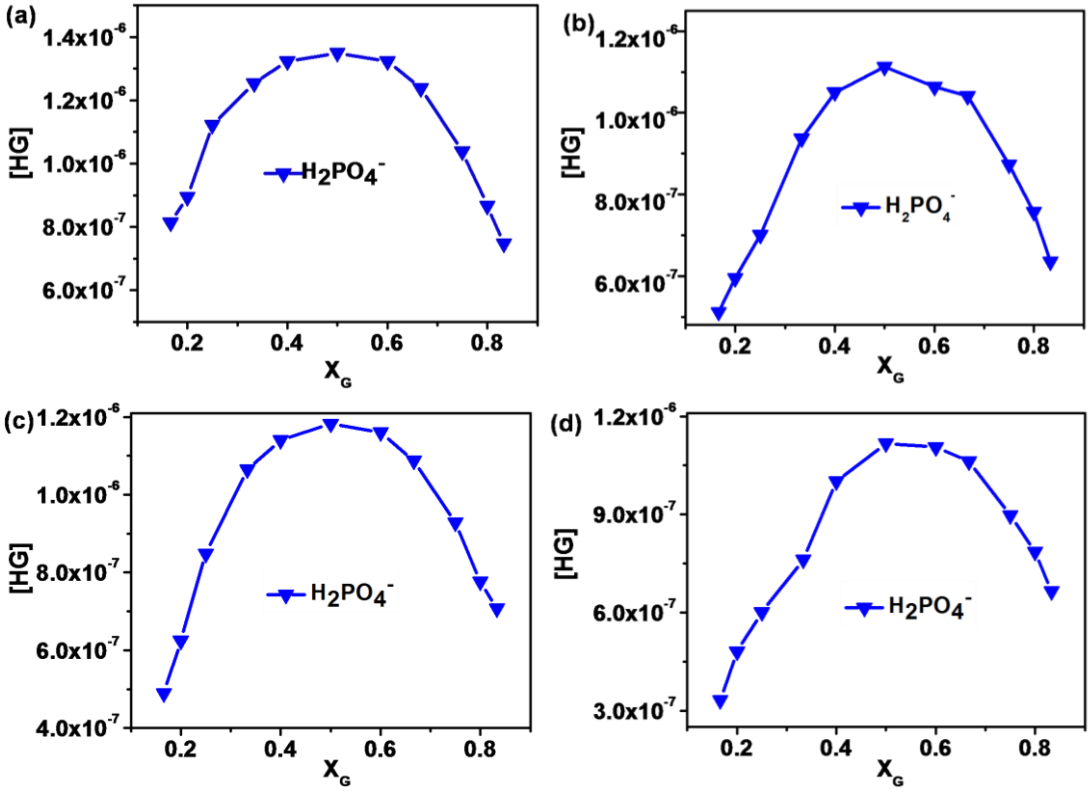


Fig. S8 Fluorescence Job's plots of sensor **1** (a), **2** (b), **3** (c) and **4** (c) with $H_2PO_4^-$.

6. Binding Constant Determination

The change of fluorescence intensity as a function of guest concentration can derive the binging constant. For the complex of 1:1, according to the following relation:

$$I = I_0 + (I_{lim} - I_0) / 2C_H \{C_H + C_G + 1/K - [(C_H + C_G + 1/K)^2 - 4C_H C_G]^{1/2}\}$$

Where I represented the fluorescence intensity, I_0 represented the intensity of pure sensor, C_H and C_G were corresponding concentrations of sensor and guest respectively, K was the binding constant. The binding constant K and correlation coefficient R were obtained by a nonlinear least-square analysis of I versus C_H and C_G using Origin software.

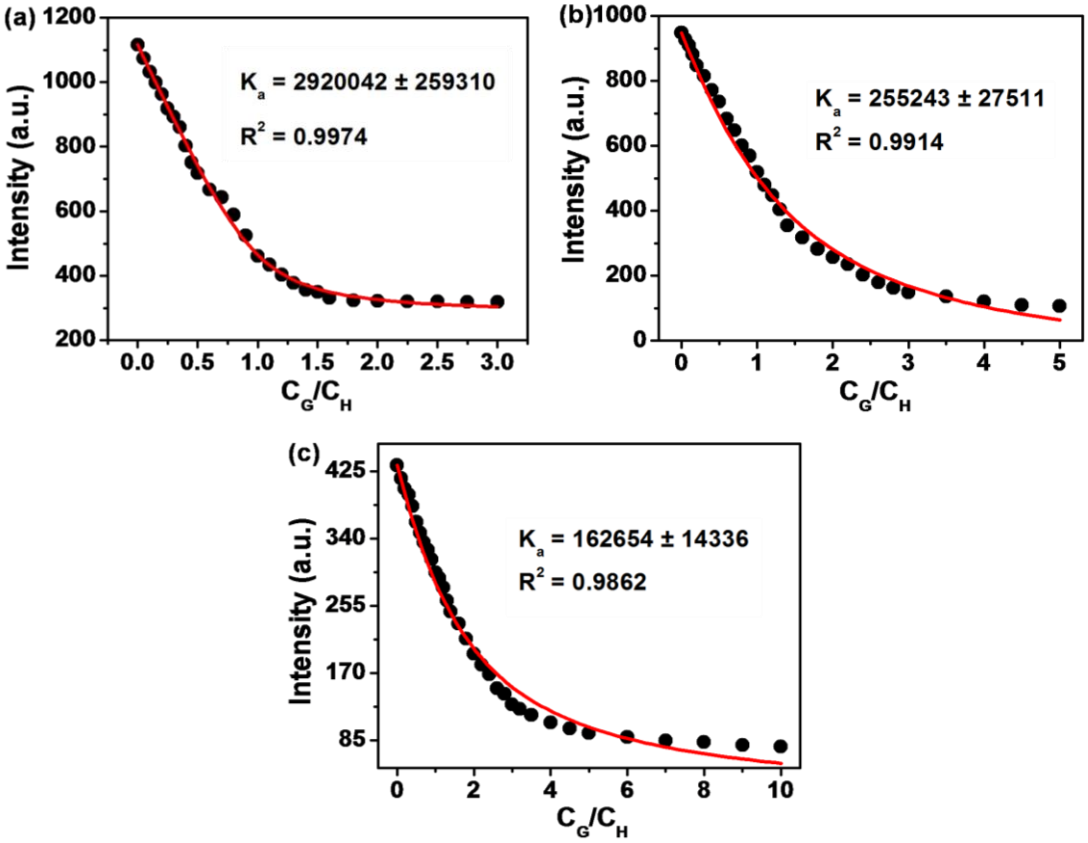


Fig. S9 Best fit of the fluorescence titration profiles of sensors **1** (a), **3** (b) and **4** (c) against $H_2PO_4^-$ for calculating the binding constants.

The binding constant of sensor **2** with $H_2PO_4^-$ was unable to be calculated. As we reported previously,[1] the titration profile exhibited a two-step process during the course of titrations (Fig. S6a), which was most probably due to the $H_2PO_4^-$ complexation with one sensor molecule firstly, and then followed by the $H_2PO_4^-$-induced assembly of sensors forming the acridine dimer at a higher concentration of $H_2PO_4^-$.

7. Sensing Selectivity

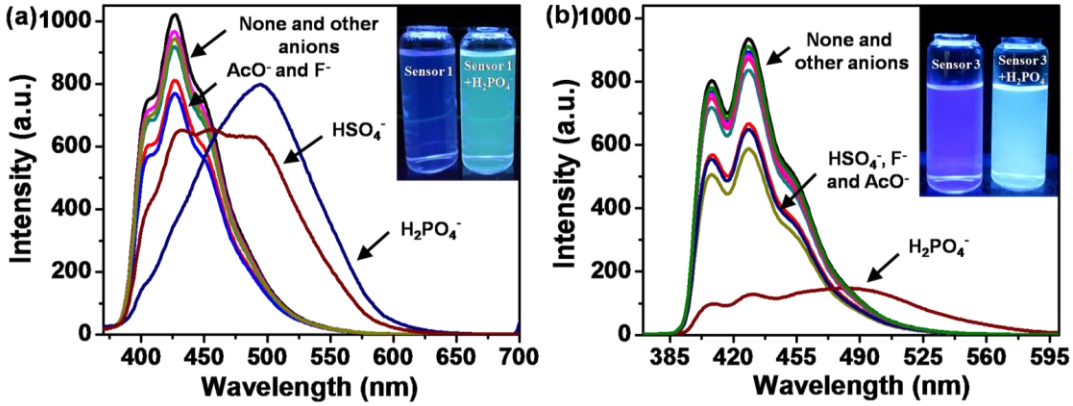


Fig. S10 Fluorescence spectra of 5 μM sensor **1** excited at 357 nm (excitation and emission slit: 5 nm) (a) and sensor **3** excited at 362 nm (excitation and emission slit: 2.5 nm) (b) upon addition of 3 and 5 equiv. of various anions ($H_2PO_4^-$, F^-, Cl^-, Br^-, I^-, AcO^-, SCN^-, HSO_4^- and ClO_4^-) respectively in CH_3CN. Insets: fluorescent color of sensor **1/3** in the absence or presence of $H_2PO_4^-$ under UV lamp excited at 365 nm.

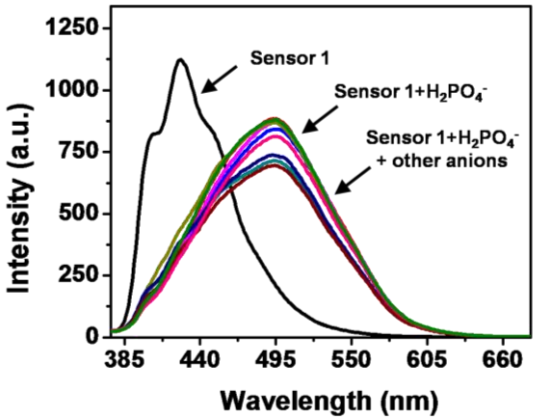


Fig. S11 Fluorescence responses of sensor **1** (5 μM) upon addition of 2 equiv. of $H_2PO_4^-$ and subsequent addition of 20 equiv. of other anions (F^-, Cl^-, Br^-, I^-, AcO^-, SCN^-, HSO_4^- and ClO_4^-).

8. UV-vis Spectra Changes

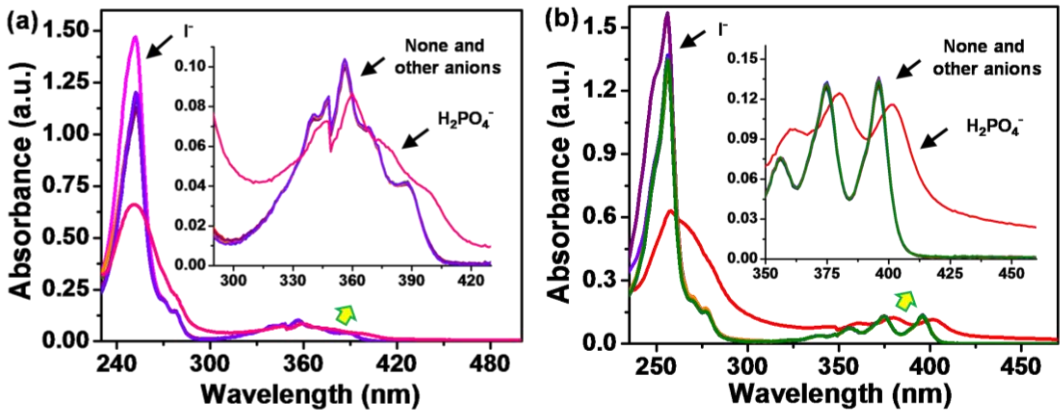


Fig. S12 UV-vis spectra of 10 μM sensors **1** (a) and **3** (b) upon addition of 3 equiv. of various anions ($H_2PO_4^-$, F^-, Cl^-, Br^-, I^-, AcO^-, SCN^-, HSO_4^- and ClO_4^-) in CH_3CN.

9. References

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[4] P. Job, *Ann. Chim.*, 1928, **9**, 113.