Supporting Information for

Preassembly-driven ratiometric sensing of $H_2PO_4^-$ anions in organic and aqueous environments

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General information

Experimental Section:

(1) Reagent and apparatus:

All chemicals were of reagent grade quality obtained from commercial sources and the solvents used were purified by standard procedures. All solvents used for spectroscopic test are spectrostropic grade. A JASCO FP-6300 spectrofluorimeter was used for fluorescence measurements. ^{1}H NMR and ^{13}C NMR spectra were obtained on a Bruker AVANCE-400 spectrometer. Mass spectra were measured on an Agilent 6310 MS spectrometer and a Q-TOF MS spectrometer. The solution lifetime fluorescent spectra were measured on EDINBURGH FS920.

(2)General procedure for fluorescence titrations:

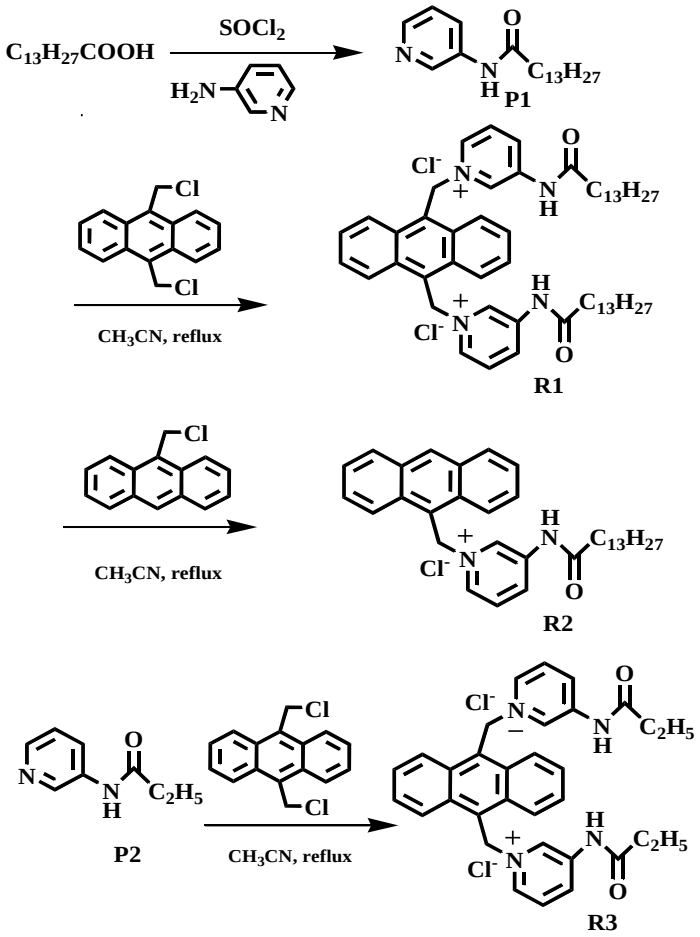
In this fluorescence titration experiment, the concentration of the receptor R1 was fixed at (5.0 μM) in $CHCl_3$. A 3 mL mixture solution of the R1 and Pi was used for the fluorescence measurement every time. Stock solutions of Pi (as the corresponding TBA salt) in the concentration range 10^{-3} M in $CHCl_3$ were individually added in different amounts to the receptor solution until the fluorescence spectra did not change.

(3) Calculation of detection limit:

The fluorescence titration data was used to calculate the detection limit based on a reported method. According to the result of the titration experiment, the fluorescent intensity data at 500 nm were normalized between the minimum intensity and the maximum intensity. A linear regression curve of $I_{min} - I_F/I_{min} - I_{max}$ against log[Pi] was created based on the titration experiment date , and the point at which this line crossed the horizontal axis was taken as the detection limit.

(4) $H_2PO_4^-$ ion complexation in water–$CHCl_3$ biphasic mixture

In a typical experiment, a solution of R1 in $CHCl_3$ (3.0 mL, 3.0×10^{-4} M) was layered with an aqueous solution (3mL). In two separate experiments, 10 equiv. of a concentrated NaH_2PO_4 solution (3.0×10^{-3} M) were added to the aqueous layer. After shaking these mixtures for 5 minutes, the colors of the organic layer changed from yellow to indigo. The fluorescence spectra were also recorded and showed an increase at 439 nm. Analogous experiments with F^-,Cl^-,Br^-,I^-, NO_3^-,AcO^-,CN^-, ADP, AMP, ATP, CDP, CTP, GTP, UTP, HSO_4^-, PO_4^{3-}, HPO_4^{2-}, SO_4^{2-}, $P_4O_7^{4-}$ did not result in a color change.



N-pyridin-3-yl- Tetradecanamide (P1) was synthesized according to reference S1.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.81 (s, 1H), 8.74 (s, 1H), 8.41 (d, *J* = 8.3 Hz, 1H), 8.29 (d, *J* = 4.3 Hz, 1H), 7.33 (m, 1H), 3.14 (m, 2H), 1.81 – 1.63 (m, 2H), 1.33 – 1.16 (m, 20H), 0.88 (t, *J* = 6.8 Hz, 3H).

N-pyridin-3-yl-propionamide (P2) was synthesized according to reference Crystal Growth & Design, 11(12), 5649-5658; 2011

^{1}H NMR (400 MHz, $CDCl_3$) δ[ppm]: 11.38(s, 1H, NH), 9.56 (s, 1H, CH of C_2 of pyridine), 8.91(d, 1H, CH of C_6 of pyridine), 8.54 (d, 1H, CH of C_4 of pyridine), 7.83–7.87 (m, 1H, CH of C_5 of pyridine), 2.09 (t, 2H) , 0.80 (t, 3H).

Synthesis of compound **R1**

A mixture of N-pyridin-3-yl- Tetradecanamide (P1) (3 mmol) and 9,10-bis(chloromethyl)anthracene(1.5 mmol) in dry CH_3CN was refluxed for 40 h, and gradually yellow precipitate was formed. When the precipitate was no longer increased, filtered off at a high temperature and washed several times with hot CH_3CN. The product was purified by silica gel column chromatography to give yellow solid.Yield:68%

^{1}H NMR (400 MHz, $CDCl_3$) δ 10.98 (s, 1H), 10.38 (s, 1H), 9.35 (d, *J*= 8.1 Hz, 1H), 8.35 (s, 1H), 7.96 (d, *J*= 3.3 Hz, 3H), 7.38 – 7.24 (m, 2H), 7.16 (s, 2H), 2.06 – 1.65 (m, 2H), 1.47 (dd, *J*= 14.4, 7.2 Hz, 2H), 1.28 – 1.05 (m, 20H), 0.86 (t, *J*= 7.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.94, 141.46, 137.97, 133.48, 132.48, 128.31, 123.87, 123.33, 55.87, 45.69, 36.97, 32.30, 29.60, 29.09, 24.61, 22.88, 14.44, 8.72.HRMS: calcd for $C_{54}H_{76}N_4O_2^{2+}$, 812.5968; Found 812.5950.

Synthesis of compound **R2**

This compound was prepared by using the same conditions as those used for the preparation of **R1** except that 9-(chloromethyl)anthracene was used in place of 9,10-bis(chloromethyl)anthracene.Yield:73%

^{1}H NMR (400 MHz, $CDCl_3$) δ 12.35 (s, 1H), 10.37 (s, 1H), 9.26 (s, 1H), 8.70 (s, 1H), 8.13 (d, *J* = 8.6 Hz, 4H), 7.58 (t, *J* = 36.6 Hz, 6H), 6.83 (s, 2H), 3.11 (s, 2H), 1.71 (s, 2H), 1.25 (s, 20H), 0.87 (d, *J* = 6.6 Hz, 3H);^{13}C NMR (100 MHz, $CDCl_3$) δ 173.99, 141.52, 138.29, 133.24, 132.88, 131.27, 128.92, 123.92, 123.46, 55.38, 36.60, 31.89, 25.25, 22.12, 13.92. HRMS: calcd for $C_{34}H_{43}N_2O^+$, 495.3375; Found 495.3368.

Synthesis of compound **R3**

This compound was prepared by using the same conditions as those used for the preparation of R1 .Yield:71%

^{1}H NMR (400 MHz, DMSO) δ 11.26 (s, 1H), 9.17 (s, 1H), 8.74 (d, 2H), 8.55 (d, 2H), 8.07 (1H), 7.76 (dd, *J* = 6.9, 3.1 Hz, 2H), 7.09 (s, 2H), 2.35 (q, *J* = 7.5 Hz, 2H), 1.02 (t, *J* = 7.5 Hz, 3H); ^{13}C NMR (100 MHz, DMSO) δ 173.46, 139.67, 138.27, 133.61, 133.12, 131.43, 128.40, 128.19, 125.86, 124.54, 56.00, 29.36, 9.02.

HRMS: calcd for $C_{32}H_{32}N_4O_2^{2+}$, 504.2525; Found 504.2520.

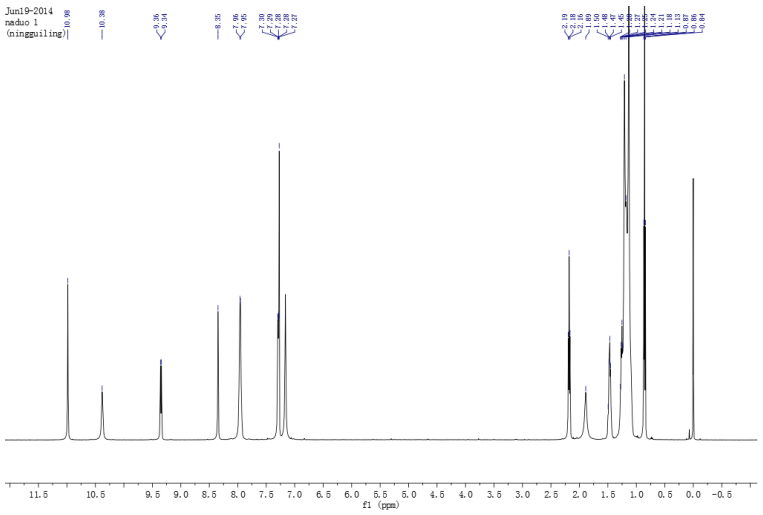


Figure S1 ^{1}H-NMR of R1

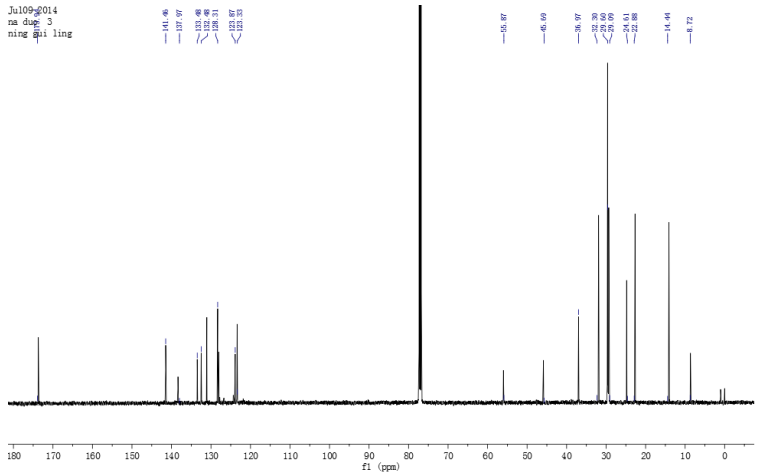


Figure S2 ^{13}C-NMR of R1

ND-3

14071012 13 (0.241) AM (Cen,2, 80.00, Ht,5000.0,0.00,1.00); Sm (SG, 2x3.00); Cm (13:16)

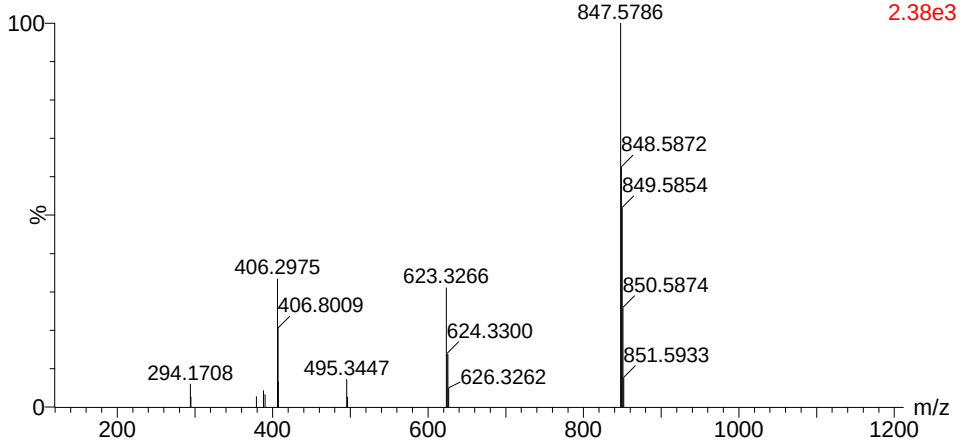


Figure S3 HRMS of R1

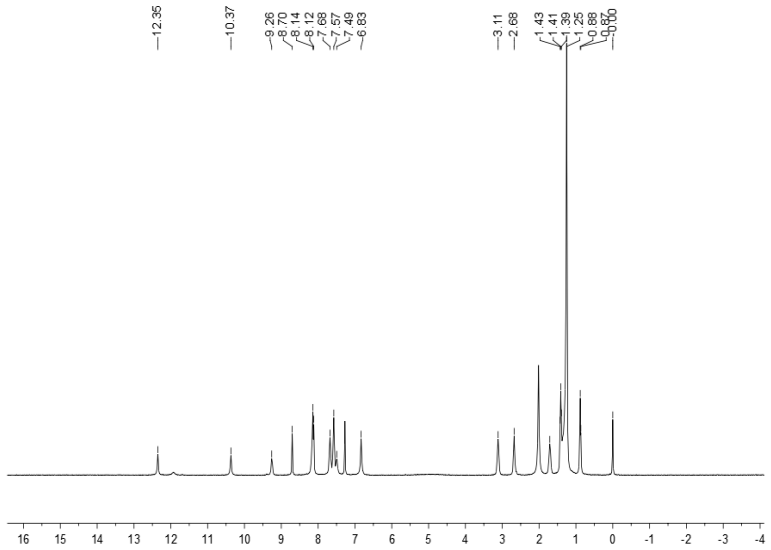


Figure S4 ^{1}H-NMR of R2

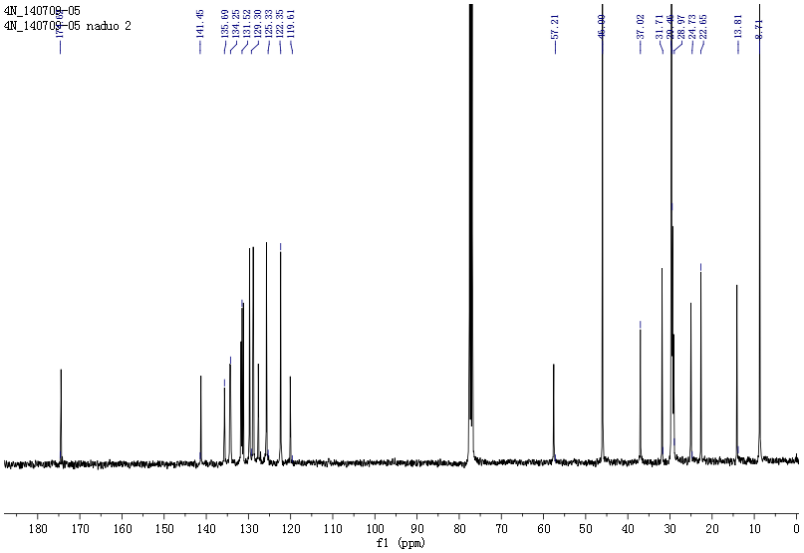


Figure S5 ^{13}C-NMR of R2

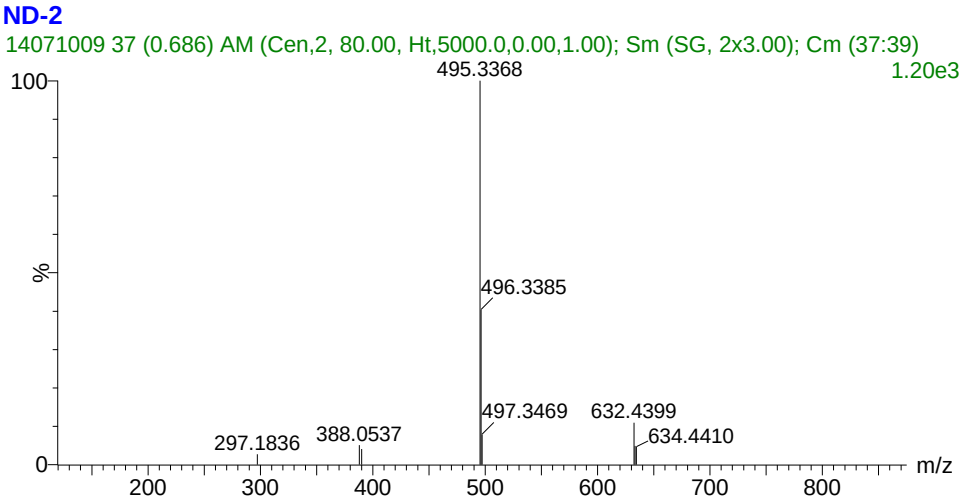


Figure S6 HRMS of R2

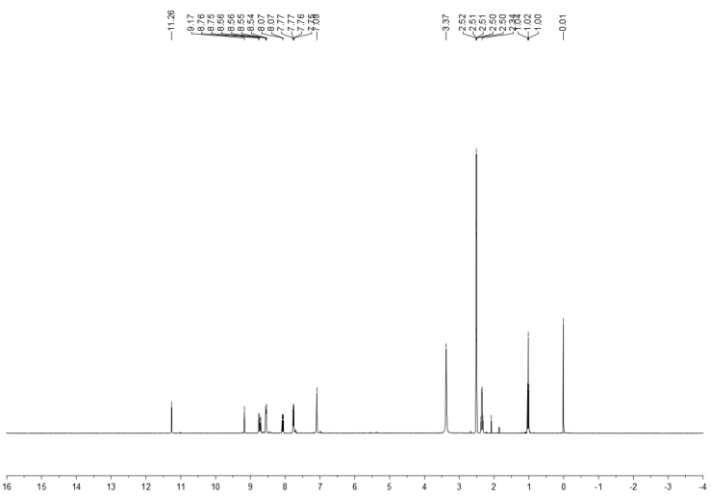


Figure S7 ^{1}H-NMR of R3

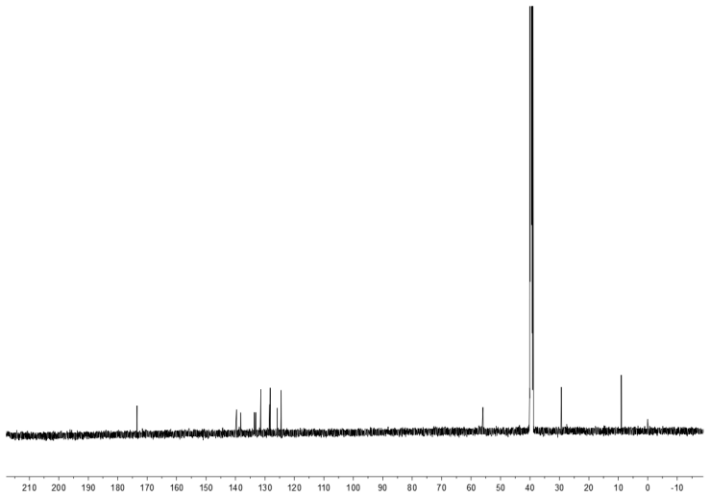


Figure S8 ^{13}C-NMR of R3

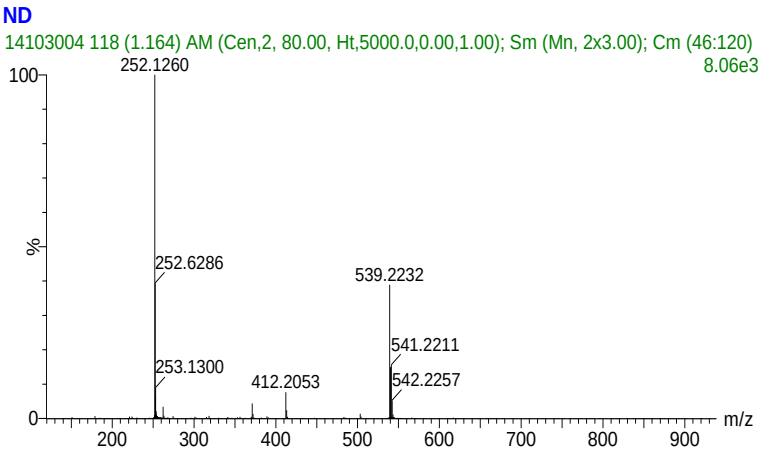


Figure S9 HRMS of R3

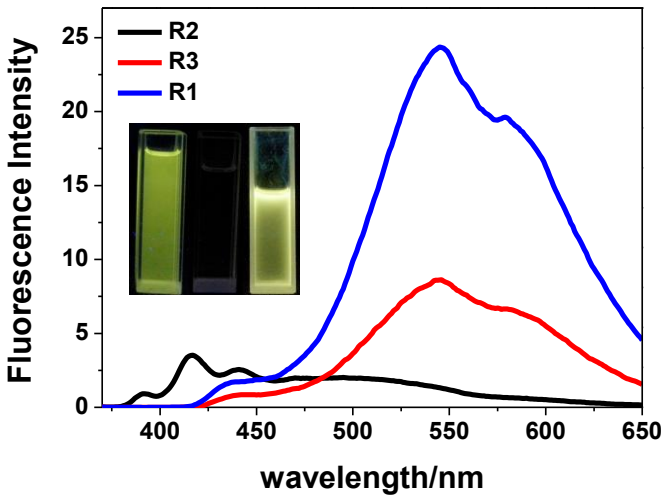


Figure S10. Fluorescence emission spectra of **R1, R2** and **R3** in $CHCl_3$.(1 μM).. Inset **R1, R2** and **R3** at a concentration of 1 μM taken photos under 365 nm UV illumination

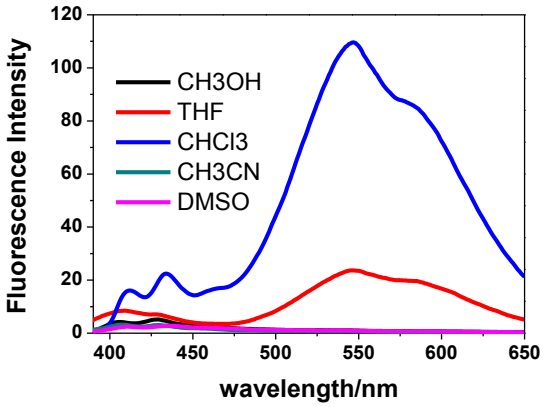


Figure S11. Fluorescence emission of **R3** in different solvents (10 μM) at an excitation of 360 nm.

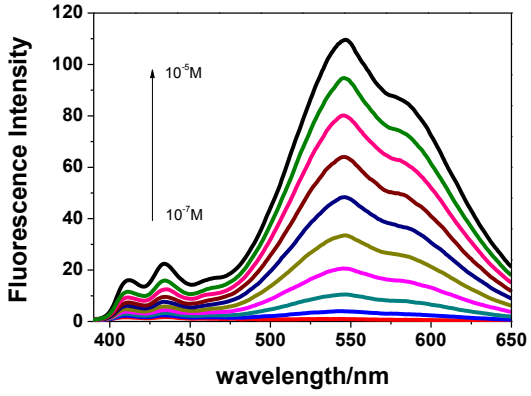


Figure S12. Fluorescence emission spectra of various concentrations (10^{-7} to 10^{-5} M) of **R3** in $CHCl_3$.

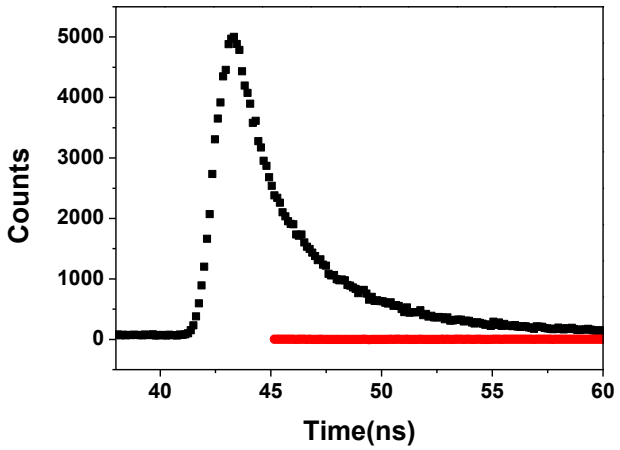


Figure S13. Life-time decay profiles of R1 in CHCl3 (l_{exc}=470 nm; $l_{monitored}$=546 nm). Solution-state studies were carried out at a concentration of 10^{-3}M.

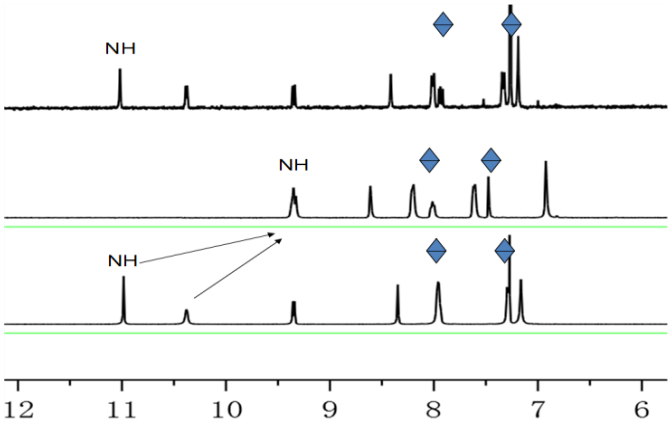


Figure S14. Partial ^{1}H NMR spectra of **R1** in the absence (lower) , the presence (middle) of 5% CD_3CN in $CDCl_3$ and the presence(upper) of 0.5 equiv $H_2PO_4^-$ in $CDCl_3$. Black parallelograms denote the all protons of anthracene moieties.

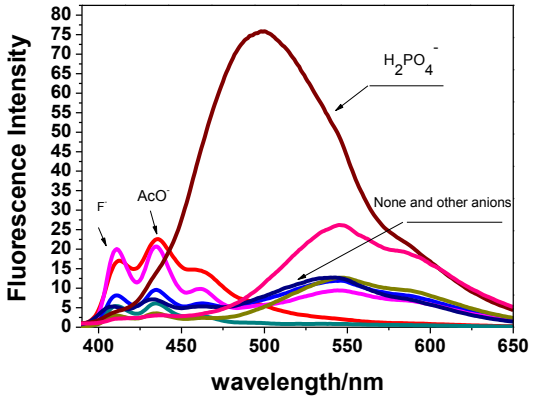


Figure S15. Fluorescence emission spectra of receptor R1 (5.0 μM) upon addition of 5 equiv. of various anions in $CHCl_3$

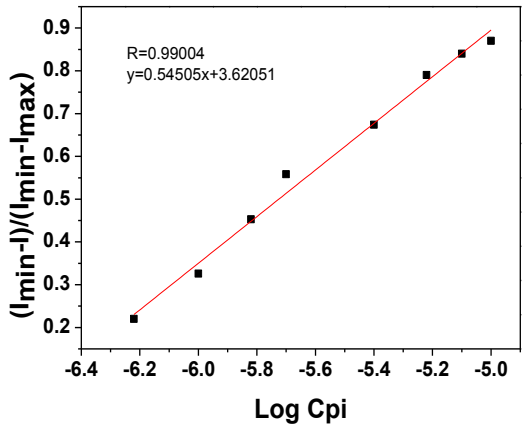


Figure S16. Normalized response of fluorescence emission spectra of **R1** to the change of Pi concentrations in $CHCl_3$ (5 μM) $I=I_{excimer\ 498}/I_{excimer546}$

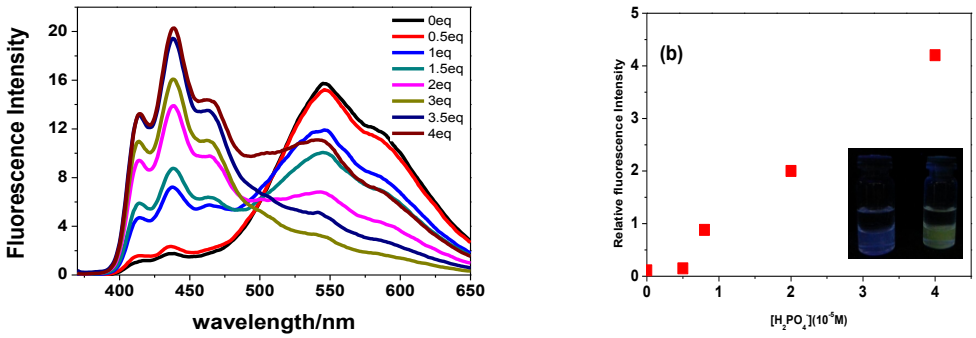


Figure S17 (a) Biphasic extraction experiment of **R1** (1×10^{-5}M) in $CHCl_3$ with increasing concentration of NaH_2PO_4 in water from 0 to 4 eq. (b) The relative fluorescence intensity at 438/548nm plotted against anion concentration Inset: Photograph of vials containing probe **R1** in exposed to aqueous solutions of NaH_2PO_4 after shaking (left) and before shaking (right).

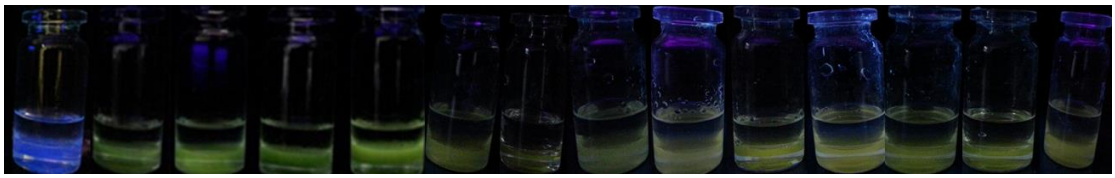


Figure S18. Photograph of vials containing **R1** in exposed to aqueous solutions of NaH_2PO_4 (first) compared to aqueous solutions of F^-,Cl^-,Br^-,AcO^-, ADP, AMP,ATP,CDP,CTP,GTP,UTP,HSO_4^-,SO_4^{2-}

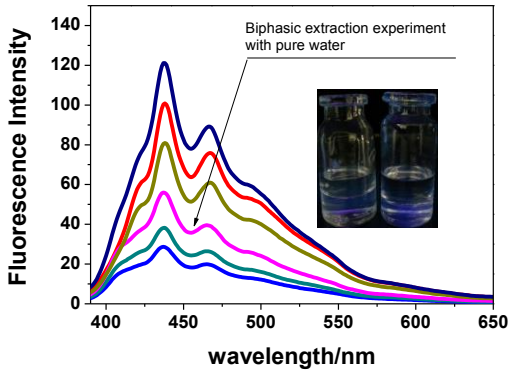


Figure S19. Biphasic extraction experiment of fluorescence spectra of **R3** (3×10^{-4}M) in $CHCl_3$ with increasing concentration of NaH_2PO_4 in water. Inset Photograph of vials containing probe **R3** (3×10^{-4}M) in exposed to aqueous solutions of NaH_2PO_4 (left) and pure water (right)

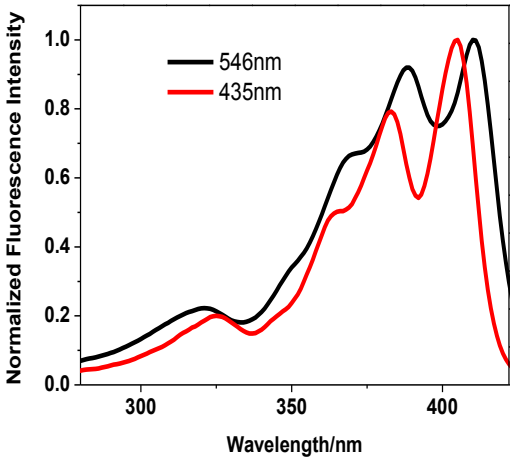


Figure S20. Normalized excitation spectra monitored for the monomer emission at 435 nm and excimer emission at 546 nm .The excitation emission from R1 at the excimer emission (546 nm) is red-shifted (5nm) in comparison with that measured at the monomer emission (435 nm) This proposal can be clarified by characterizing the nature of the static excimer

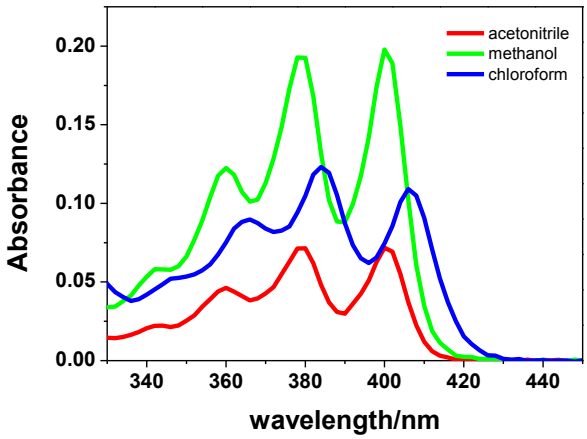


Figure S21. Absorption emission of R1 in different solvent (10 μM).

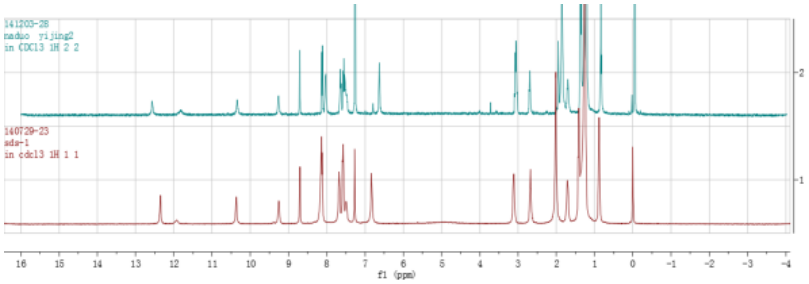


Figure S22. ^{1}H NMR spectra of **R2** in the absence (lower) , the presence (upper) of 5% CD_3CN in $CDCl_3$

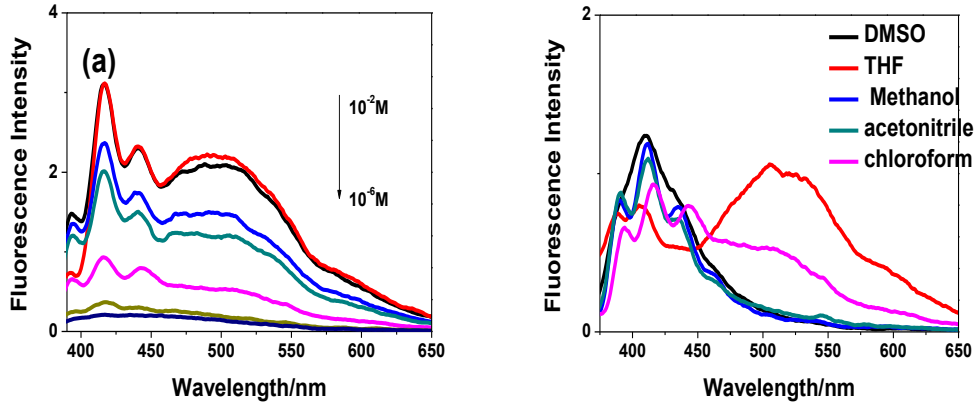


Figure S23. (a) Fluorescence emission spectra of **R2** in various concentrations in $CHCl_3$ (1.0×10^{-6} to 1.0×10^{-2} M). (b) Fluorescence emission of **R2** in different solvent (10 μM).

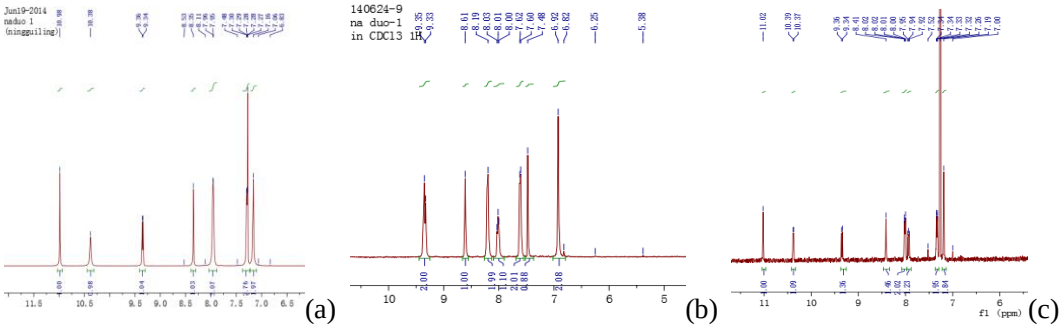


Figure S24. Partial ^{1}H NMR spectra of **R1** in the absence (a) , the presence (b) of 5% CD_3CN in $CDCl_3$ and the presence(c) of 0.5 equiv $H_2PO_4^-$ in $CDCl_3$ with the values of the integrations.

S1.S. Brahmachari, S. Debnath, S. Dutta, and P. K. Das, *Beilstein J. Org. Chem.*, **2010**, 6, 859–868.