Electronic Supplementary Material (ESI) for Analytical Methods.

Supporting Information

Naphthalimide-linked bispyridinium clefts in selective aqueous sensing of triphosphate and triphosphate-based biomolecules

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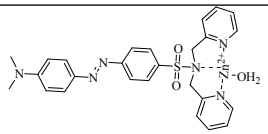
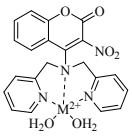
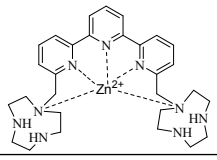
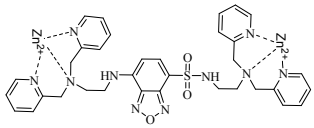
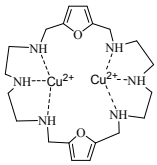
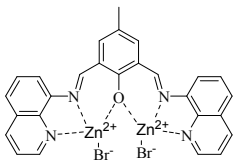
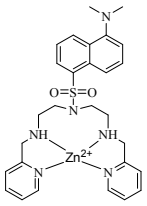
Table S1: Reported receptors on the triphosphate/pyrophosphate anions

Sl. No.	Structure of Compound	Selectivity	Solvent	Ref.
1.		Triphosphate	HEPES buffer solution, pH = 7.4	*Anal. Chem.* 2008**, 80**, 5312
2.	 M = Zn, Cd, Cu	Pyrophosphate	HEPES buffer solution, pH = 7.4	*Inorg. Chem*. 2013, **52**, 11034.
3.		Di and Triphosphate	Tris-HCl buffer, pH = 7	*Chem. Eur.J.* 2016, **22**, 14890.
4.		Triphosphate/ pyrophosphate	HEPES buffer (pH = 7.2), containing 3% DMSO	*Dalton Trans*., 2009, 7888
5.		Triphosphate	Aqueous solution	*Inorg. Chim. Acta,* 1998, **270**, 207
6.		Pyrophosphate	Aqueous medium, pH = 7.4	*Anal. Chem., 2013, **85**, 8369*

7.		Pyrophosphate / Triphosphate	Aqueous solution	*Inorganic Chemistry*, 2008, **47**, 6175

1. Change in emission of receptor 1 with various anions in CH_3CN-H_2O (1:1,v/v, 10 mM HEPES, pH = 6.8).

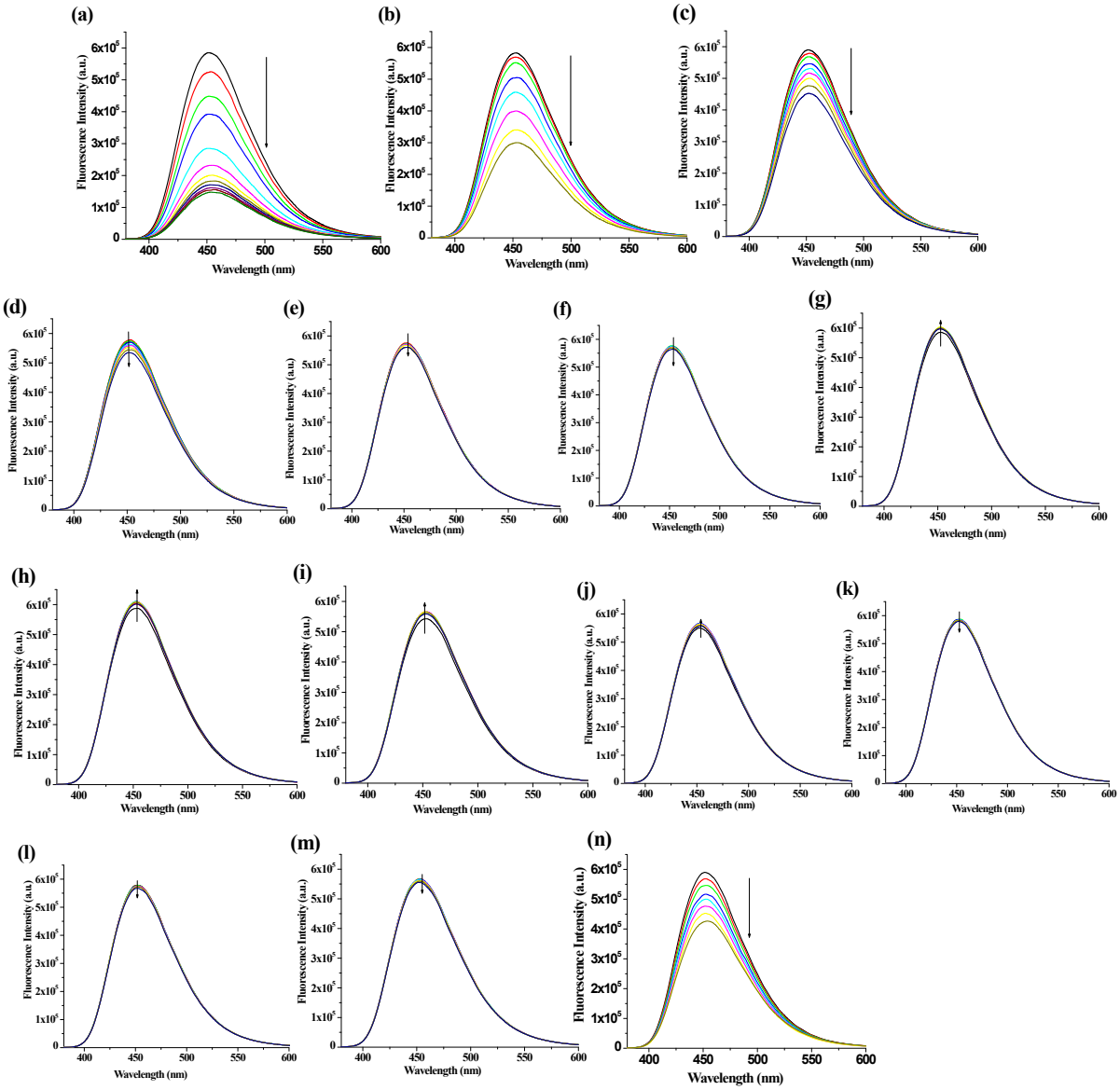


Figure S1. Change in emission of **1** (c = 2.5 x 10^{-5} M) in CH_3CN-H_2O (1:1, v/v, 10 mM HEPES, pH = 6.8) upon addition of 4 equiv. amounts of (a) $P_3O_{10}^{5-}$, (b) ATP, (c) $HP_2O_7^{3-}$ (d) ADP, (e) AMP, (f) $H_2PO_4^-$, (g) F^-, (h) Cl^-, (i) Br^-, (j) I^-, (k) OAc^-, (l) HSO_4^-, (m) NO_3^- and (n) $P_2O_7^{4-}$ [concentration of anions was 1 x 10^{-3} M; all anions were used as tetrabutylammoniun salts except $P_3O_{10}^{5-}$, $P_2O_7^{4-}$, ATP, ADP and AMP which were taken as sodium salt].

2. Change in emission of receptor 2 with various anions in CH_3CN-H_2O (1:1,v/v, using 10 mM HEPES, pH-6.8).

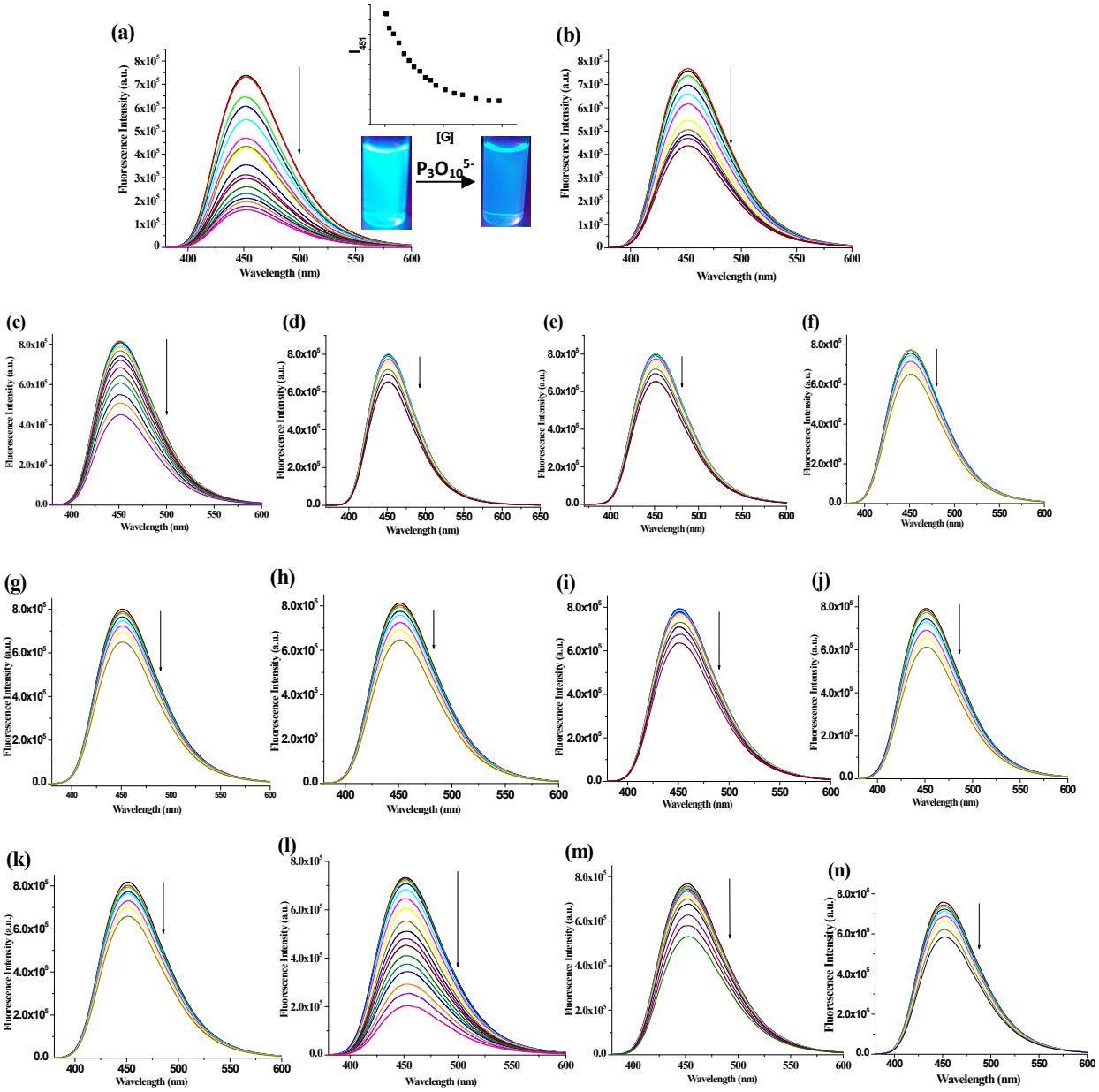


Figure S2. Change in emission of **2** (c = 2.5 x 10^{-5} M) in CH_3CN-H_2O (1:1, v/v, using 10 mM HEPES, pH = 6.8) upon addition of 25 equiv. amounts of (a) $P_3O_{10}^{5-}$, (b) $P_2O_7^{4-}$, (c)$HP_2O_7^{3-}$ (d) $H_2PO_4^-$, (e) F^-, (f) Cl^-, (g) Br^-, (h) I^-, (i) OAc^-, (j) HSO_4^-, (k) NO_3^-, (l) ATP, (m) ADP and (n) AMP [concentration of anions was 1 x 10^{-3} M; all anions were used as tetrabutylammoniun salts except $P_3O_{10}^{5-}$, $P_2O_7^{4-}$, ATP, ADP and AMP which were taken as sodium salt].

3. Change in absorbance of receptor 1 with various anions in CH_3CN-H_2O (1:1, v/v, 10 mM HEPES, pH = 6.8).

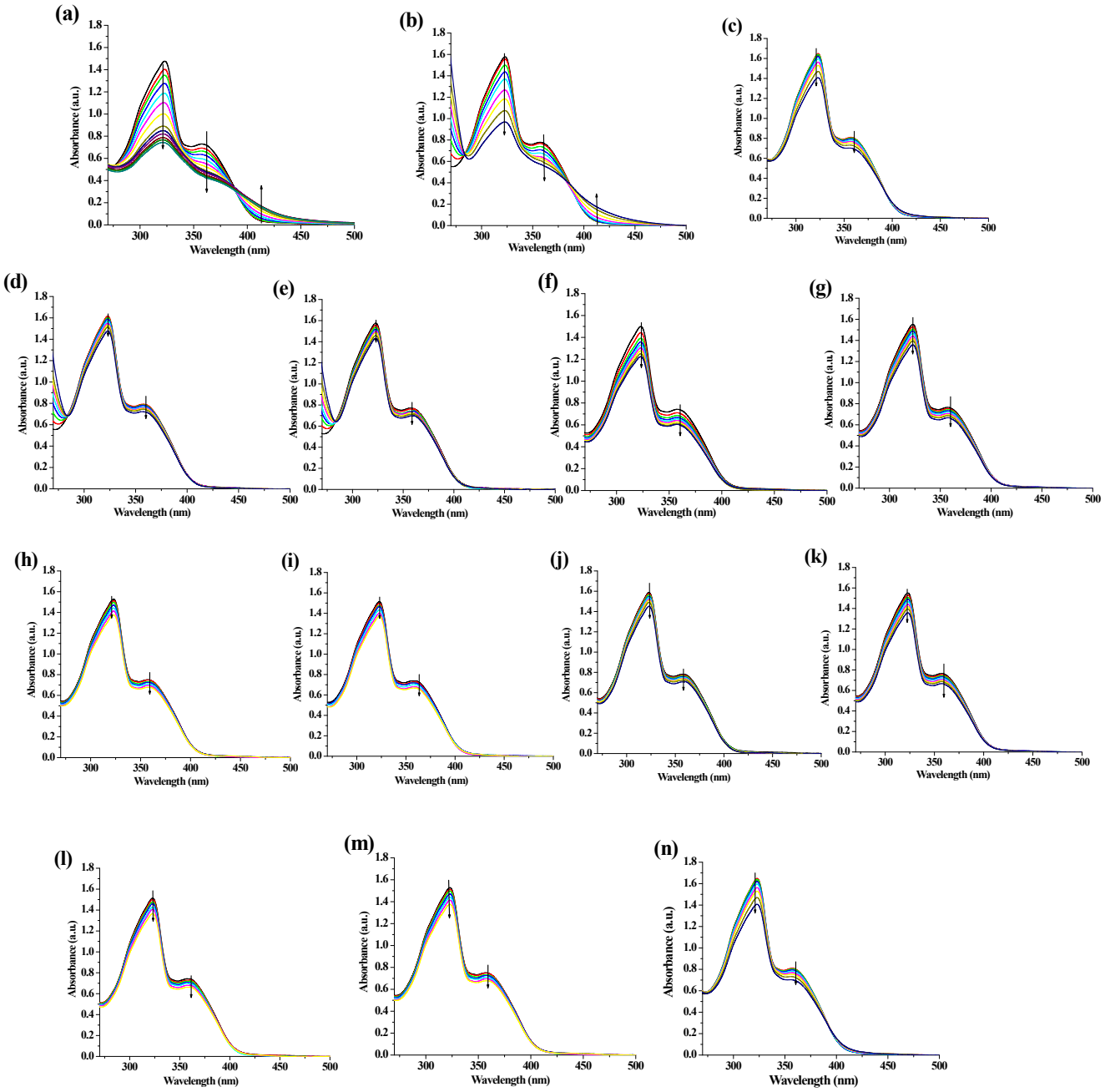


Figure S3. Change in absorbance of **1** (c = 2.5 x 10^{-5} M) in CH_3CN-H_2O (1:1, v/v, 10 mM HEPES, pH = 6.8) upon addition of 4 equiv. amounts of (a) $P_3O_{10}^{5-}$, (b) ATP , (c)$HP_2O_7^{3-}$ (d) ADP, (e) AMP, (f) $H_2PO_4^-$, (g) F^-, (h) Cl^-, (i) Br^- , (j) I^- , (k) OAc^-, (l) HSO_4^-, (m) NO_3^- and (n) $P_2O_7^{4-}$ [concentration of anions was 1 x 10^{-3} M; all anions were used as tetrabutylammoniun salts except $P_3O_{10}^{5-}$, $P_2O_7^{4-}$, ATP, ADP and AMP which were taken as sodium salt].

4. Change in absorbance of receptor 2 with various anions of sodium salt in CH_3CN-H_2O (1:1, v/v, using 10 mM HEPES, pH = 6.8).

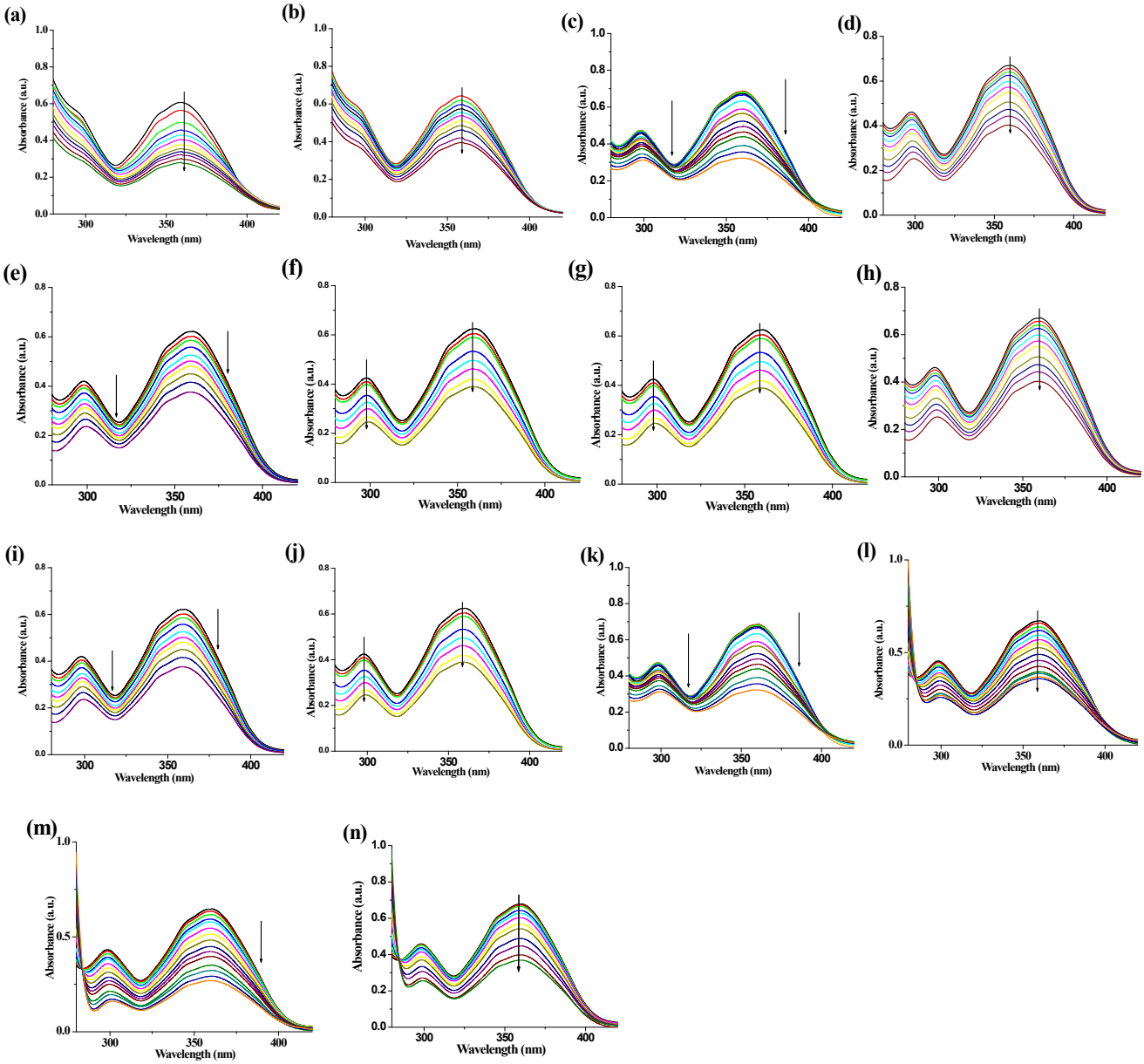


Figure S4. Change in absorbance of **2** (c = 2.5 x 10^{-5} M) in CH_3CN-H_2O (1:1, v/v, using 10 mM HEPES, pH = 6.8) upon addition of 25 equiv. amounts of (a) $P_3O_{10}^{5-}$, (b) $P_2O_7^{3-}$, (c)$HP_2O_7^{3-}$ (d) $H_2PO_4^-$, (e) F^-, (f) Cl^-, (g) Br^-, (h) I^-, (i) OAc^-, (j) HSO_4^-, (k) NO_3^- (l) ATP, (m) ADP and (n) AMP [concentration of anions was 1 x 10^{-3} M; all anions were used as tetrabutylammoniun salts except $P_3O_{10}^{5-}$, $P_2O_7^{4-}$, ATP, ADP and AMP which were taken as sodium salt].

5. Job and Non-linear binding constant plots for 2

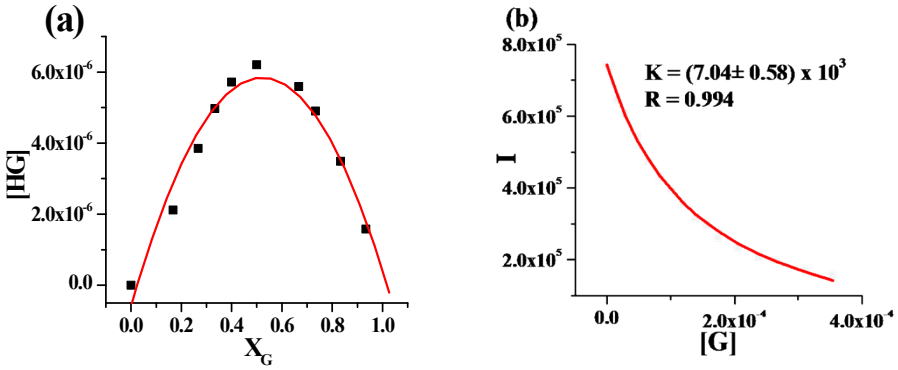


Figure S5. (a) UV-Vis Job's plot of receptor **2** with $P_3O_{10}^{5-}$ at 360 nm in $CH_3CN–H_2O$ (1: 1, v/v, pH = 6.8, 10 mM HEPES buffer) where [H] = [G] = $2.5x10^{-5}$ M; (b) Binding constant curve from non-linear fitting of fluorescence titration data.

6. Detection Limit

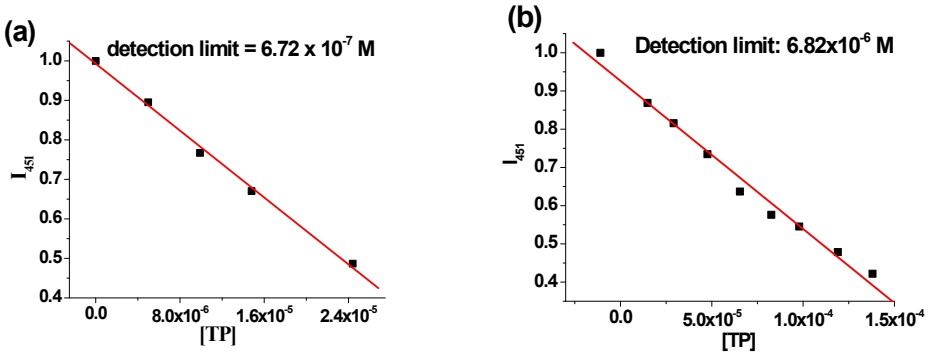


Figure S6. Detection limits for receptor (a) **1** and (b) **2** (c = 2.5 x 10^{-5} M) with $P_3O_{10}^{5-}$ ($[P_3O_{10}^{5-}]$ = 1 x 10^{-3} M) at 451nm in CH_3CN-H_2O (1:1, v/v, 10 mM HEPES, pH = 6.8).

7. Interaction with triphosphate-based nucleotides

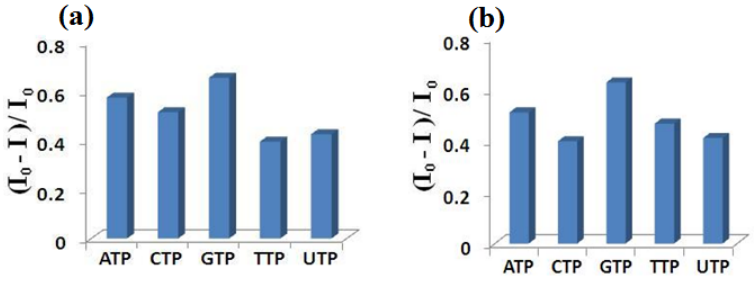


Figure S7. Change in fluorescence ratio of (a) **1** (*c* = 2.5 x 10^{-5} M) in presence of 4 equiv. and (b) **2** (*c* = 2.5 x 10^{-5} M) in presence of 25 equiv. amounts of sodium salts of triphosphate-based nucleotides in $CH_3CN–H_2O$ (1: 1, v/v, pH = 6.8, 10 mM HEPES buffer).

Table S2. Binding constants for compounds 1 and 2 with triphosphate-based nucleotides.

Triphosphate-based nucleotides	Binding constants for compound 1 (M^{-1})	Binding constants for compound 2 (M^{-1})
ATP	$1.90 \pm 0.26 \times 10^4$	$4.05 \pm 0.62 \times 10^3$
CTP	$2.19 \pm 0.41 \times 10^4$	$2.57 \pm 0.46 \times 10^3$
GTP	$2.54 \pm 0.51 \times 10^4$	$3.98 \pm 0.67 \times 10^3$
TTP	$1.08 \pm 0.12 \times 10^4$	$3.53 \pm 0.45 \times 10^3$
UTP	$(8.47 \pm 2.0) \times 10^4$	$4.42 \pm 0.25 \times 10^3$

8. Interference study in the binding of $P_3O_{10}^{5-}$ (PPPi)

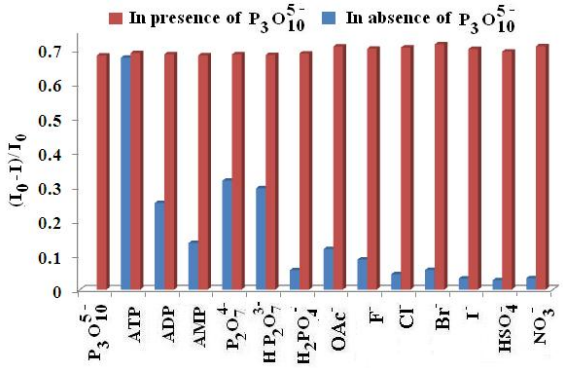


Figure S8. Change in fluorescence ratio of **2** (c = 2.5 x 10^{-5} M) upon addition of 25 equiv. amounts of $P_3O_{10}^{5-}$ to the receptor solution containing other anions in 25 equiv. amounts in CH_3CN–H_2O (1: 1, v/v, pH = 6.8, 10 mM HEPES buffer).

9. ^{1}H NMR titration experiment

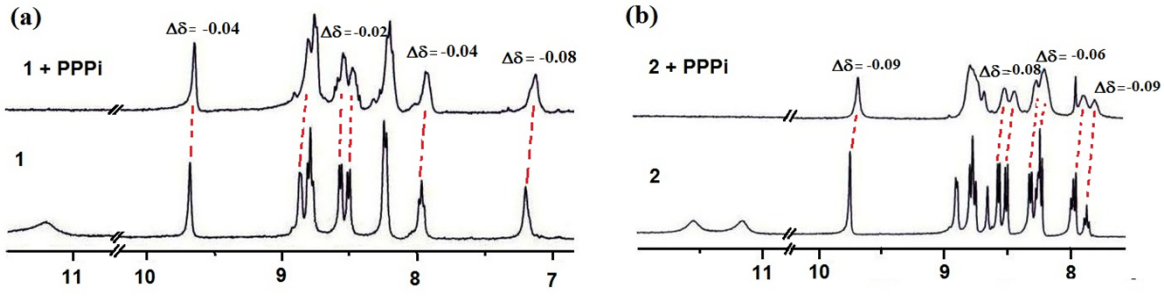


Figure S9. Partial ^{1}H NMR (400 MHz) spectra of (a) **1** (c = 1.64 x 10^{-3} M) and (b) **2** (c = 1.63 x 10^{-3} M) itself and in presence of 1 equiv. amount of sodium triphosphate in d_6-DMSO/D_2O mixture.

10. Enzymatic hydrolysis

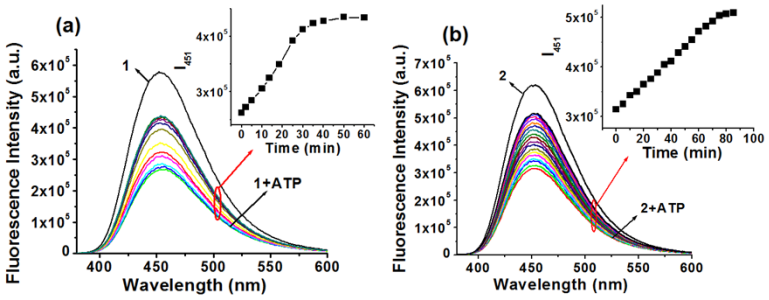


Figure S10. Change in emission of (a) the ensemble of **1** (c = 2.5 x 10^{-5} M) with 4 eq. of ATP upon addition of 0.4 ml alkaline phosphatase (c = 1 mg/ml, prepared in water); (b) the ensemble of **2** (c = 2.5 x 10^{-5} M) with 25 eq. of ATP upon addition of 0.4 ml alkaline phosphatase (c = 1 mg/ml, prepared in water) with time. Insets show the change in fluorescence intensity at 451 nm with time.

11. Detection of Ca^{2+}/Mg^{2+}

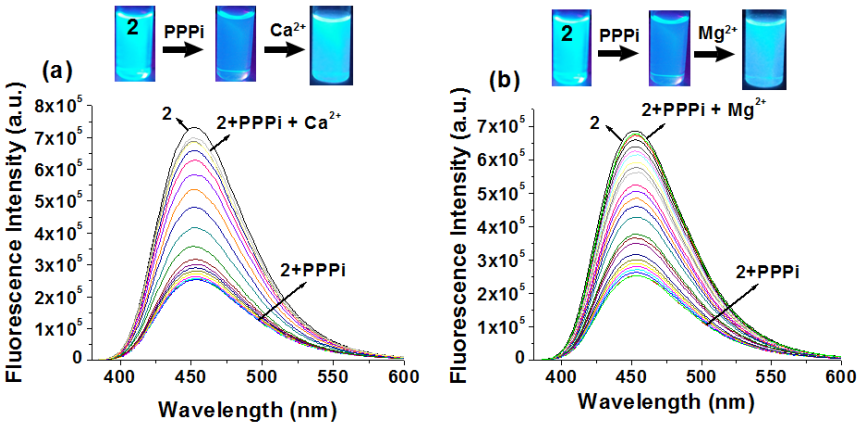


Figure S11. Change in emission of the ensemble **2**-PPPi [prepared by mixing **2** (c = 2.5 x 10^{-5} M) with 25 equiv. amounts of PPPi (c = 1 x 10^{-3} M)] upon addition of (a) Ca^{2+} (c = 5 x 10^{-3} M) and (b) Mg^{2+} ions (c = 5 x 10^{-3} M) in CH_3CN/H_2O (1: 1, v/v, pH = 6.8, 10 mM HEPES buffer) [Insets display the change in color of ensemble **2**-PPPi with the addition of Ca^{2+}/Mg^{2+} as their perchlorate salts].

12. Response to Ca^{2+}, Mg^{2+}, Al^{3+} and Zn^{2+} and a comparative view

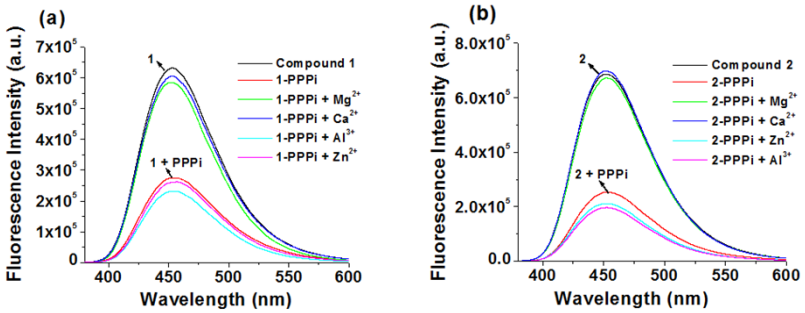


Figure S12. Change in emission of ensemble of (a) **1**-PPPi [prepared by mixing **1** (c = 2.5 x 10^{-5} M) with 4 equiv. amounts of PPPi (c = 1 x 10^{-3} M)] and (b) **2**-PPPi [prepared by mixing **2** (c = 2.5 x 10^{-5} M) with 25 equiv. amounts of PPPi (c = 1 x 10^{-3} M)] (c = 2.5 x 10^{-5} M) in the presence of different metal ions (c = 5 x 10^{-3} M) in CH_3CN/H_2O (1: 1, v/v, pH = 6.8, 10 mM HEPES buffer).

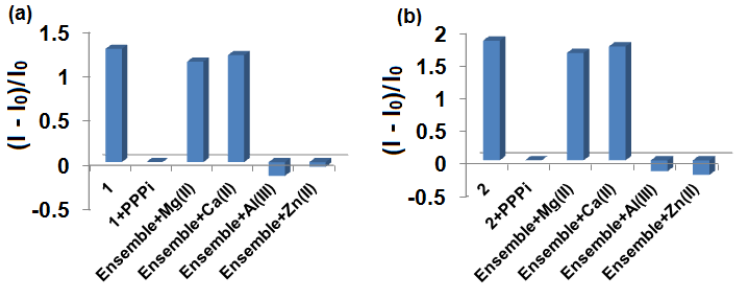


Figure S13. Bar plot indicating the retrieval of fluorescence intensity of (a) **1** and (b) **2** upon addition of metal ions (c = 5 x 10^{-3} M) to their respective ensembles in CH_3CN/H_2O (1: 1, v/v, pH = 6.8, 10 mM HEPES buffer) [Ensemble preparation: **1**.PPPi was obtained by mixing **1** (c = 2.5 x 10^{-5} M) with 4 equiv. amounts of PPPi (c = 1 x 10^{-3} M) and **2**.PPPi was obtained by mixing **2** (c = 2.5 x 10^{-5} M) with 25 equiv. amounts of PPPi (c = 1 x 10^{-3} M)].

13. Detection Limits for ensembles with Ca^{2+}/Mg^{2+}

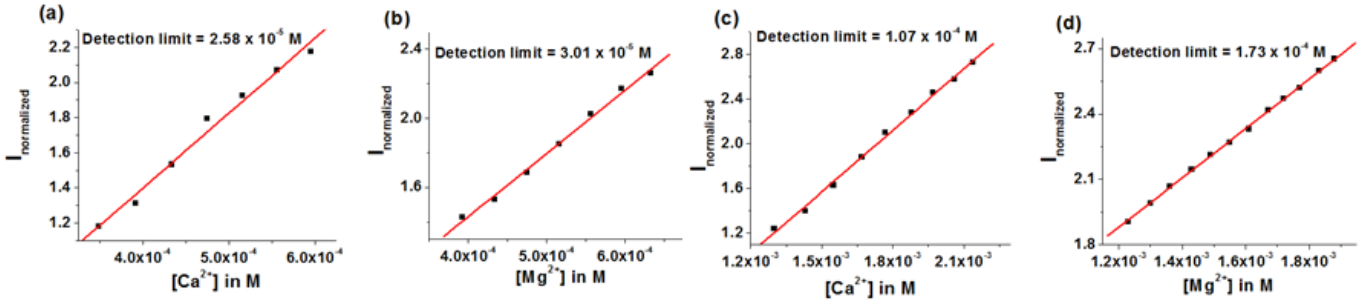
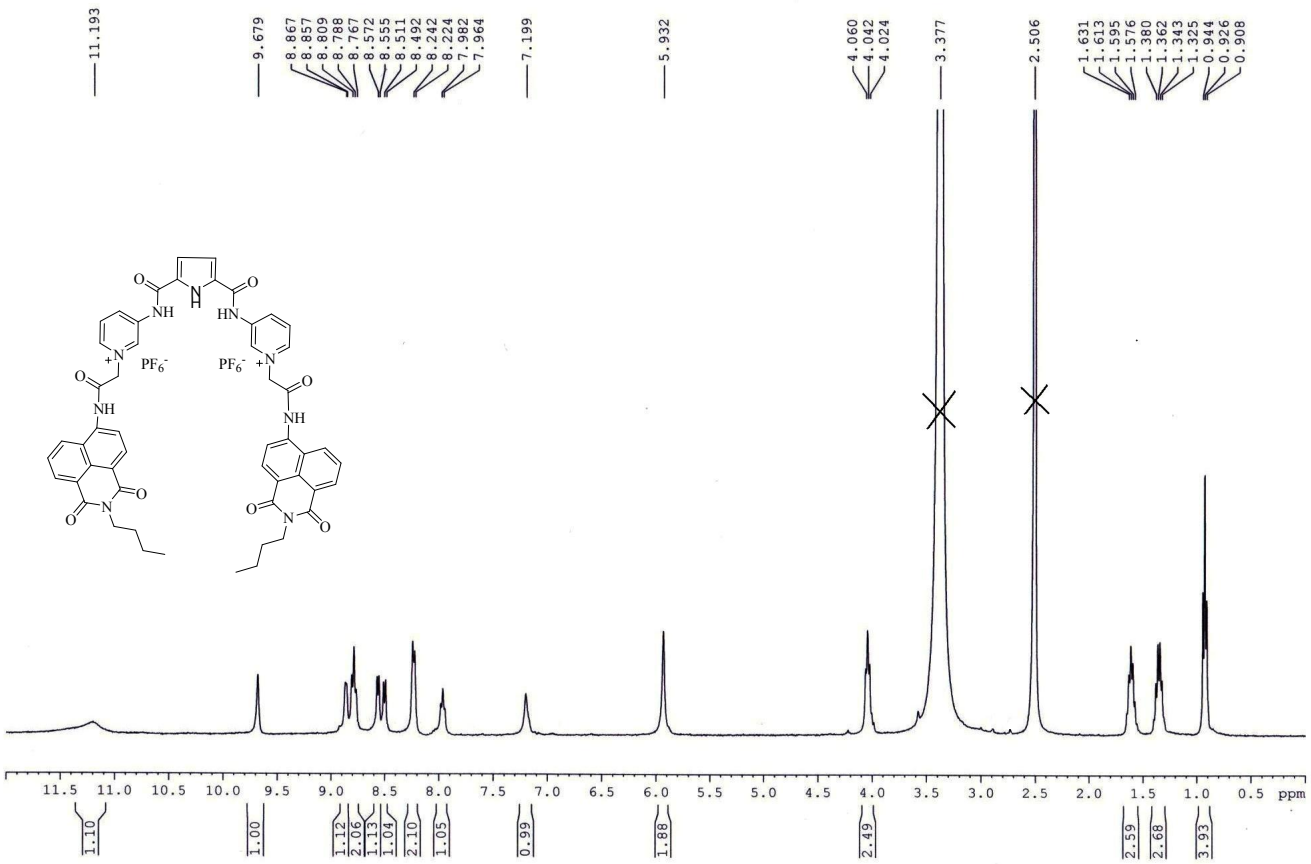
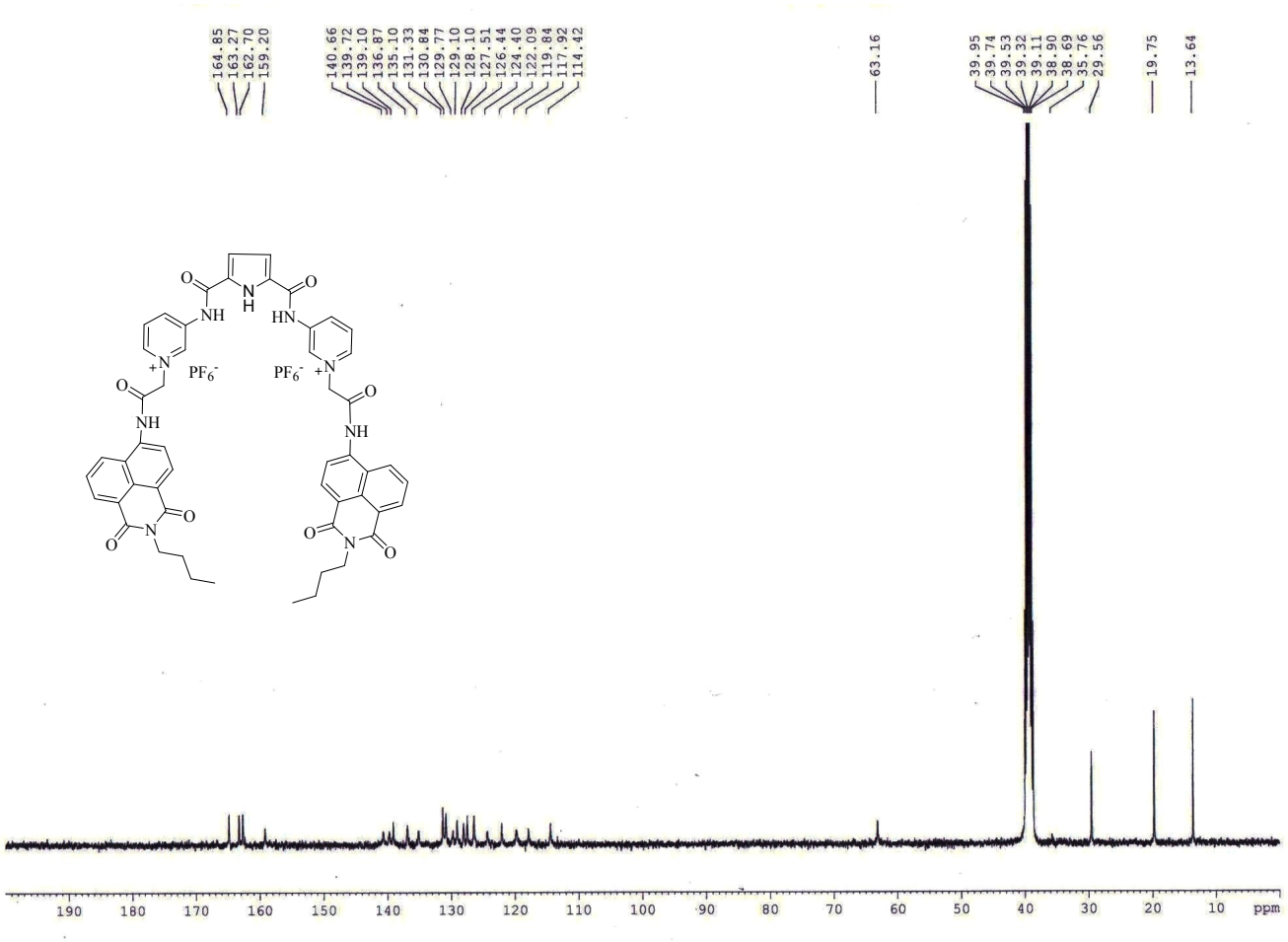


Figure S14. Detection limits for compound **1**-PPPi (c = 2.5 x 10^{-5} M) with of (a) Ca^{2+} (c = 5 x 10^{-3} M) and (b) Mg^{2+} (c = 5 x 10^{-3} M) and compound **2**-PPPi (c = 2.5 x 10^{-5} M) with of (a) Ca^{2+} (c = 5 x 10^{-3} M) and (b) Mg^{2+} (c = 5 x 10^{-3} M) in CH_3CN/H_2O (1: 1, v/v, pH = 6.8, 10 mM HEPES buffer).

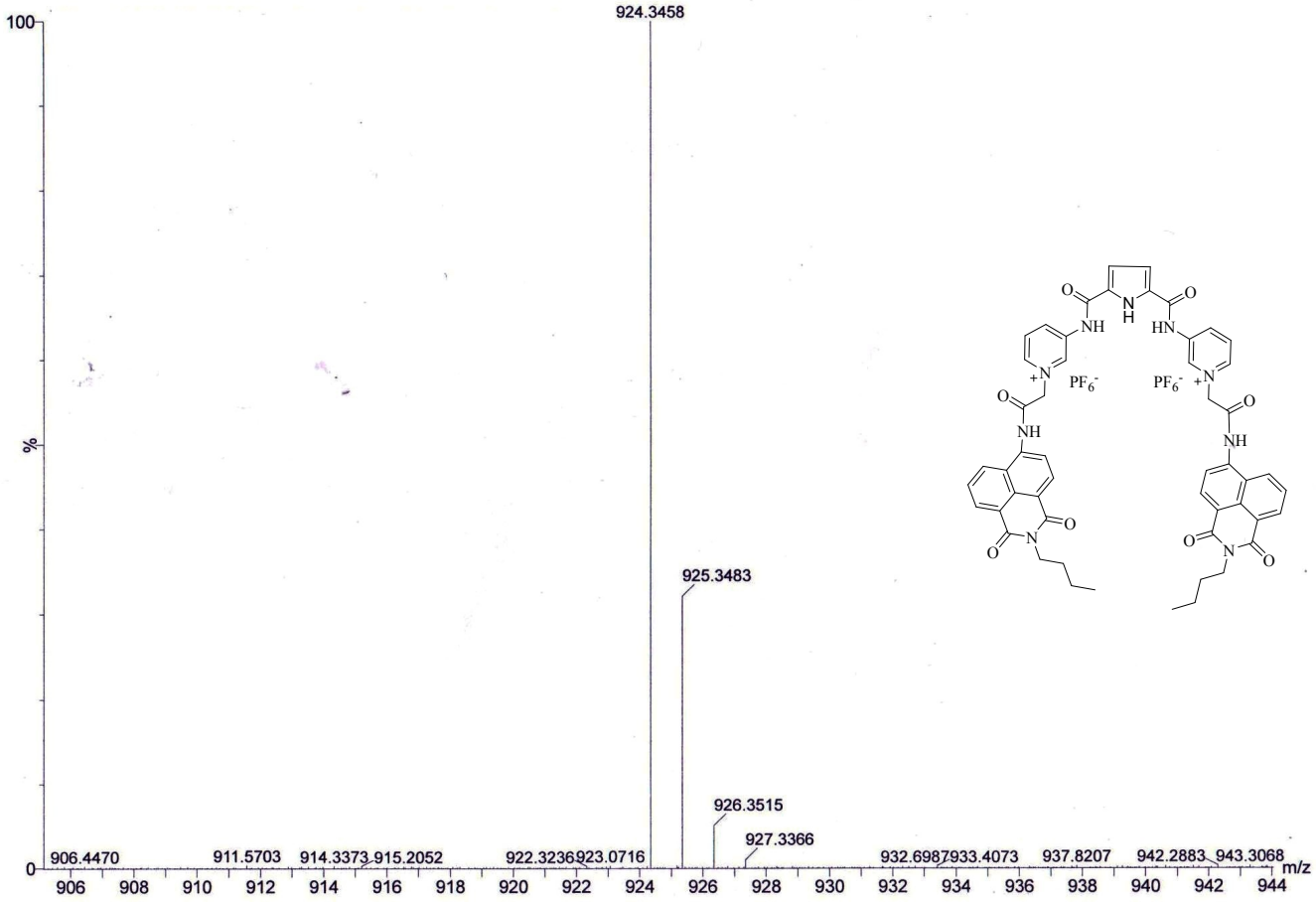
^{1}H NMR (d_6-DMSO, 400 MHz) of 1



^{13}C NMR (d_6-DMSO, 100 MHz) of 1



HRMS of 1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940
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944
m/z
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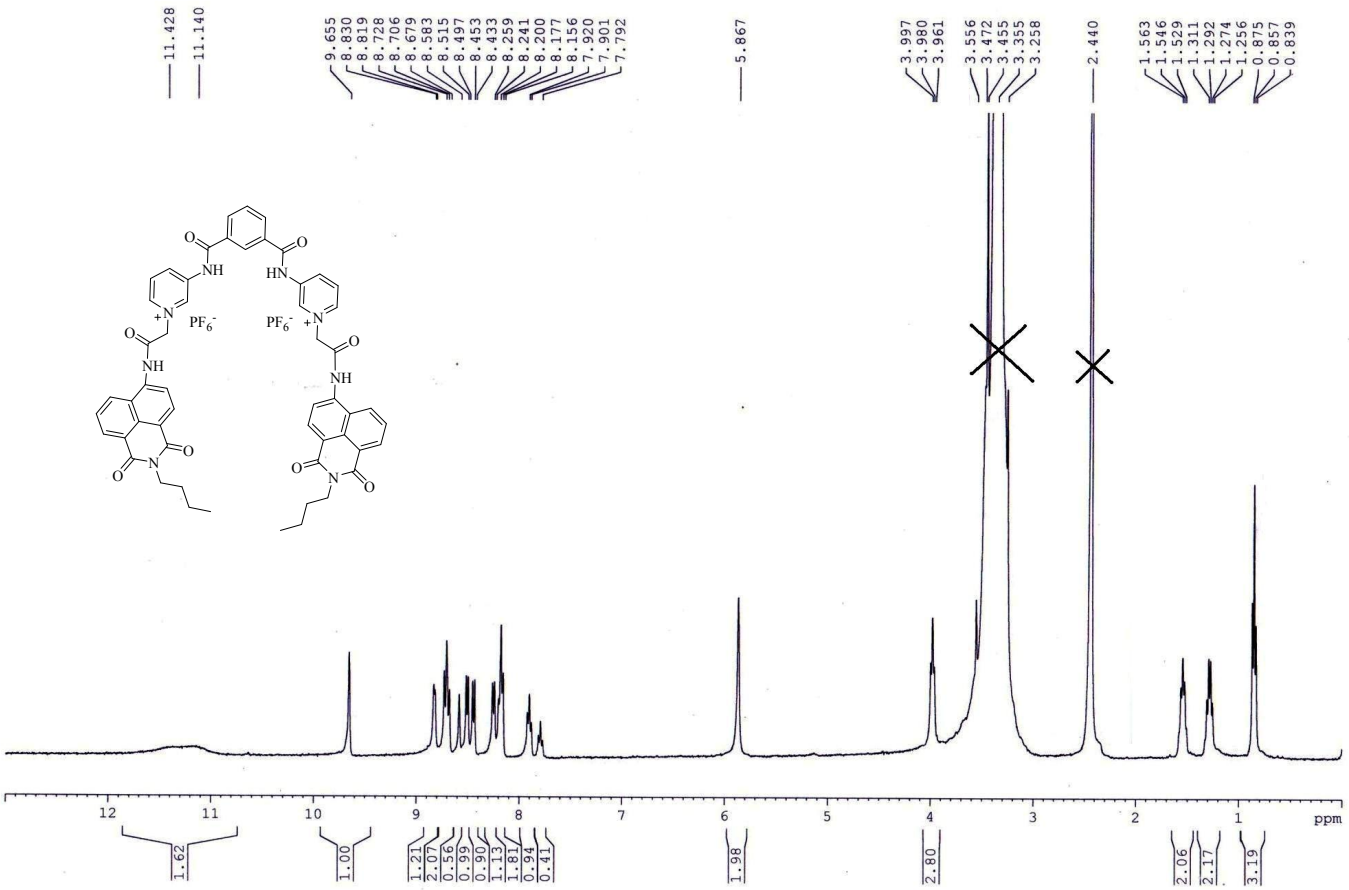
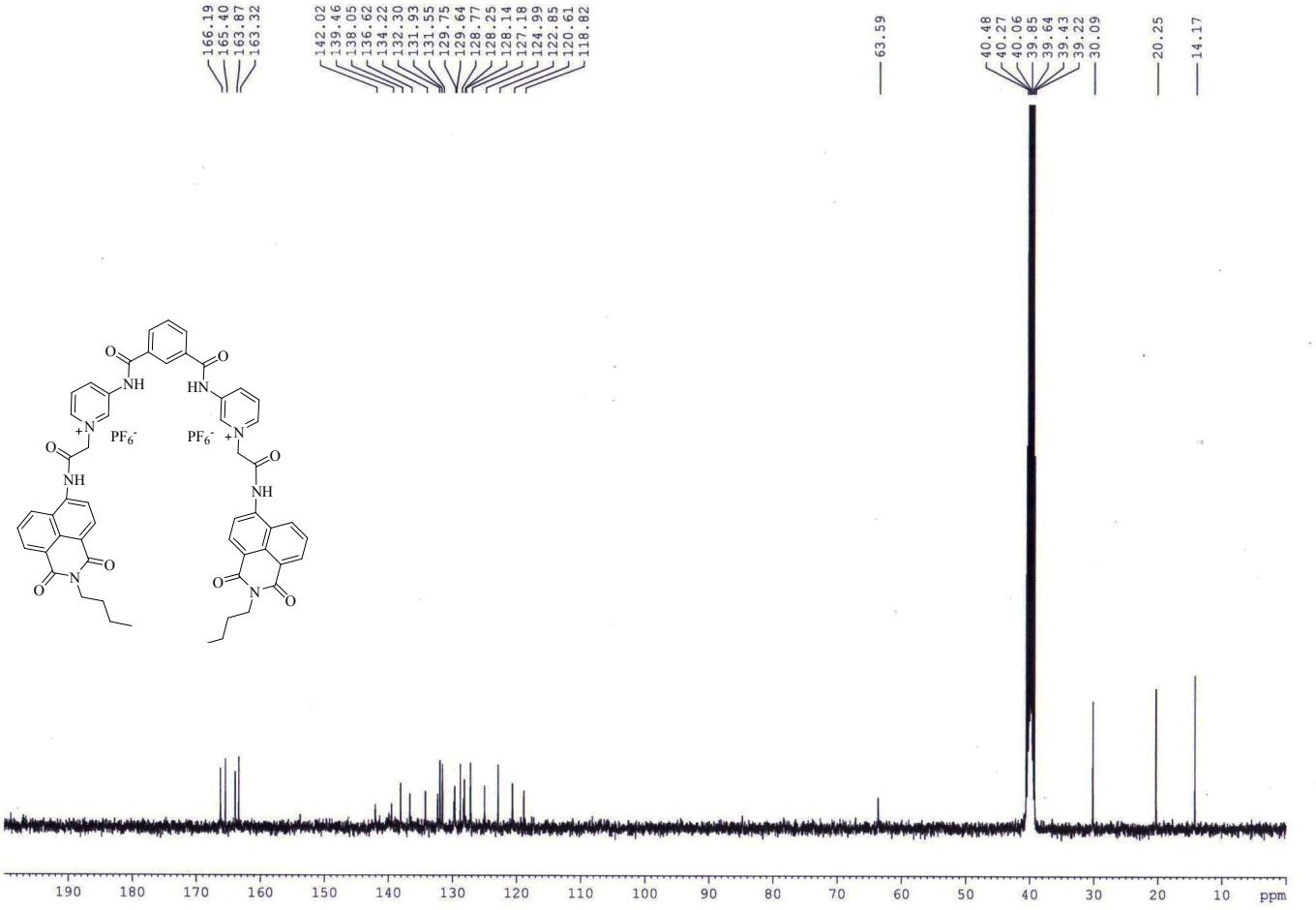
N
H
HN
+N
PF6-
O
NH
O
N
O

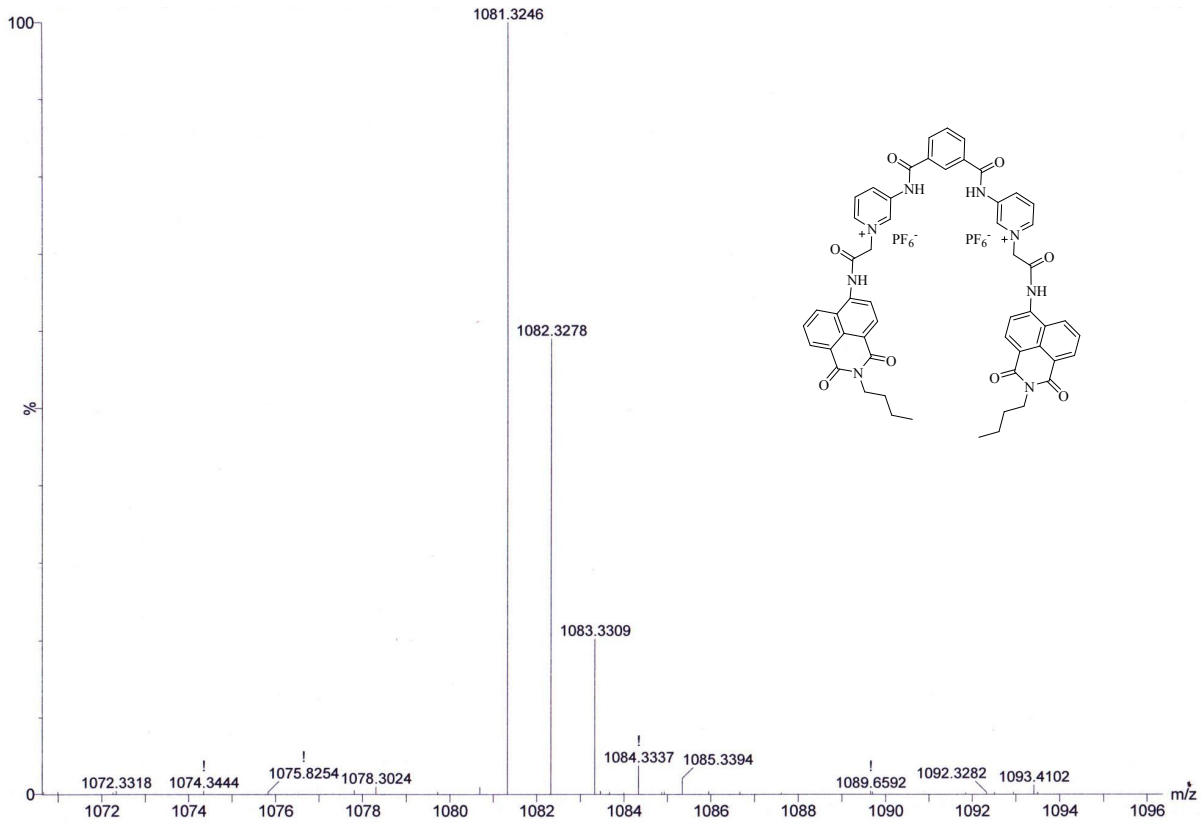
^{1}H NMR (d_6-DMSO, 400 MHz) of 2



^{13}C NMR (d_6-DMSO, 100 MHz) of 2



HRMS of 2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1096
m/z
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NH
HN
N
+
PF6-

^{1}H NMR ($CDCl_3$, 400 MHz) of 3