Electronic Supplementary Material (ESI) for Chemical Communications

Supporting Information

A highly selective fluorescent chemosensor for guanosine-5'-triphosphate via excimer formation in aqueous solution of physiological pH

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1. Experimental techniques:

General consideration: The new compound was fully characterized with standard spectroscopic techniques. Microanalyses were performed on a Carlo 1102 elemental analysis instrument. Electronic absorption (UV-Vis) spectra were recorded using a Shanghi 756 MC UV-Vis spectrometer.^{1}H NMR and ^{13}C NMR spectra were performed on a Bruker Advance DPX500 (500 MHz) spectrometer at 298 K. High resolution mass spectra were obtained on a Micromass Platform II mass spectrometer. Fluorescence studies were performed on Shimadzu RF-5301 PC spectrofluorophotometer at 298 K. Imidazole was purchased from Aldrich and used as such. Sodium salts of $H_2PO_4^-$ (Pi), pyrophosphate (PPi), AMP, ADP, ATP and GTP and *n*-TBA salts of F^- and Cl^- were also purchased from Aldrich and used without further purification. 1,8-Bis(hydroxymethyl)anthracene was purchased from Tokyo Kasei organic Chemicals.

2. Synthesis of cyclophane (1)

To a solution of 1-(1*H*-imidazol-1-ylmethyl)-1*H*imidazole (0.54 mmol, 80mg) and 1,8-bis(bromomethyl) anthracene (0.54 mmol, 200mg) in dry DMF (10 mL) was heated under reflux at 90 ^{o}C for 48 h.The resulting hot solution was filtered and residue was recrystallized from CH_3OH.The obtained product was in 85 % yield. (mp >300ºC (dec.); ^{1}H NMR (500 MHz, $(CD_3)_2SO$, 25º C) δ 6.35 (s, 8H, -N-C**H2**-C-) 6.78 (s, 4H, -N-C**H2**-N-), 7.65−8.85 (m, 24H, imidazolium ring -N-C**H**=C**H**-N- and anthracene ring), 9.80 (S, 4H, imidazolium C2 H) ^{13}CNMR (500 MHz, $(CD_3)_2SO$, 25º C) δ 50.55, 58.03, 117.46, 122.24, 123.60, 125.58,128.56, 128.96, 129.05, 129.51, 129.93, 130.10, 131.40, 137.98 : HRMS (EI, m/z): $[M- Br]^+$ calc.: 1024.480; found: 1024.481; Anal. Calcd for $C_{46}H_{40}Br_4N_8$: C, 53.72; H, 4.31; N, 10.89, Found: C, 53.05; H, 4.54; N, 10.60.

3. NMR spectral analysis:

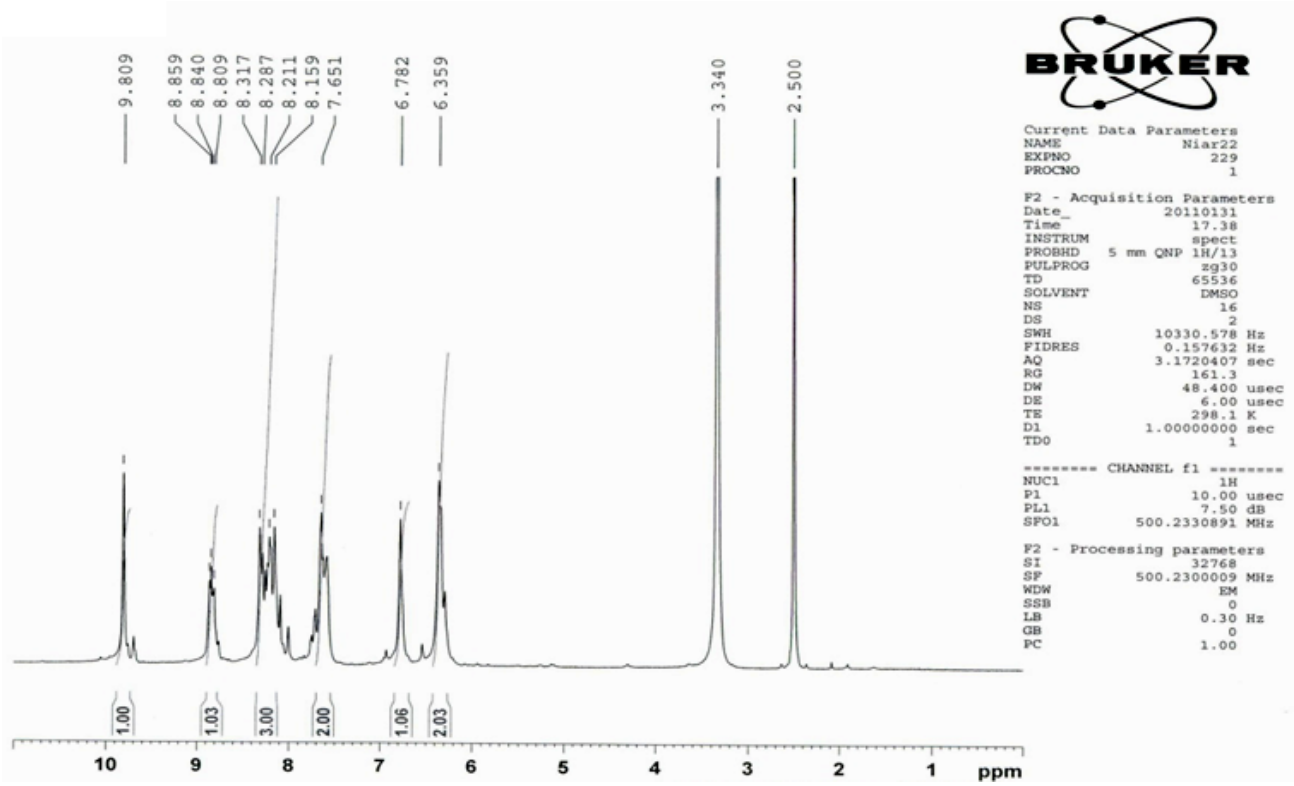

Figure S1. ^{1}H NMR spectrum of compound (**1**) in DMSO-d_6

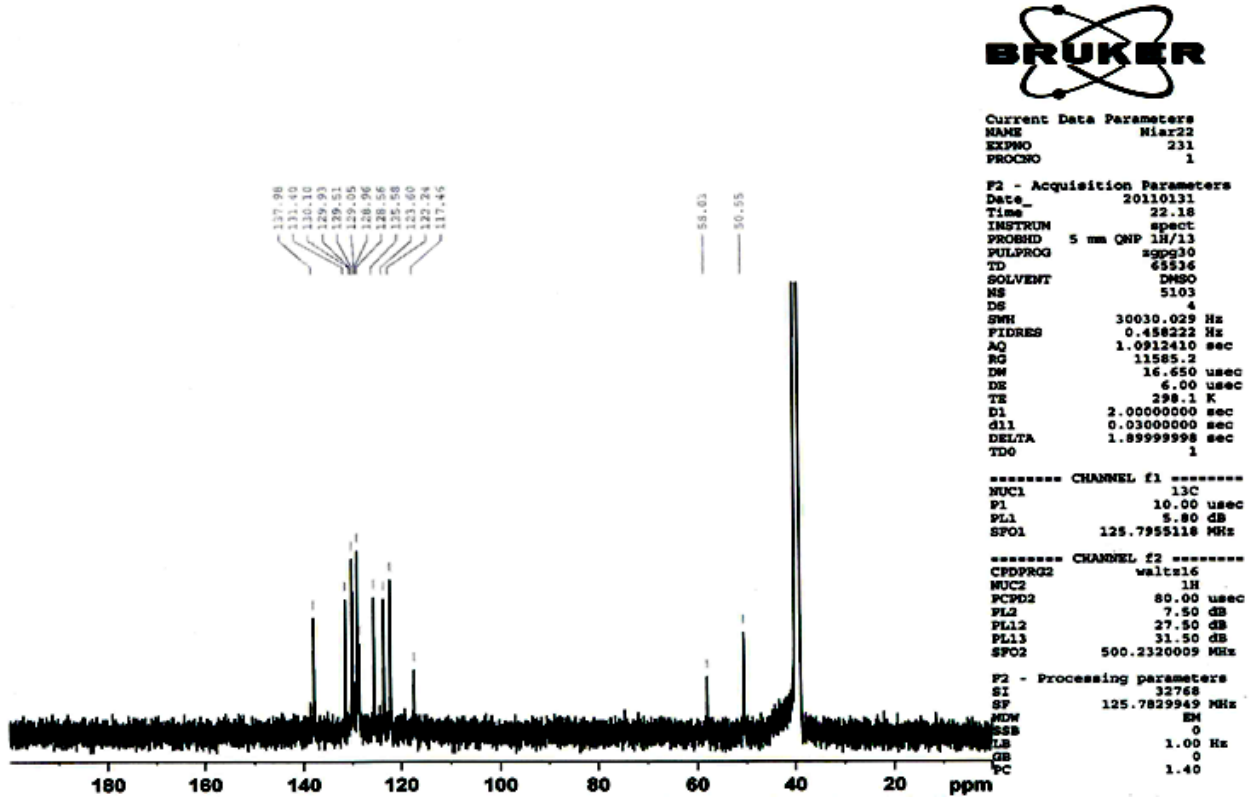

Figure S2. ^{13}C NMR spectrum of compound (**1**) in DMSO-d_6

4. UV-visible Absorbance and Fluorometric Analysis:

All spectrofluorimetric titrations were performed as follows: stock solution of compound (**1**) (1mM) was prepared at pH 7.4 in 10 mM phosphate buffer water mixture and used in the preparation of titration solution by appropriate dilution up to 10 μM. Aliquots of the anions under investigation, GTP and ATP (as the corresponding sodium salt) in 10 mM phosphate buffer water mixture were then injected into the sample solution through a rubber septum in the cap. To account for dilution effects, these stock anion solutions also contained the receptor at its initial concentration. The sample solutions were magnetically stirred for 1 minute after each addition, and then were scanned again. This process was repeated until the change in fluorescence intensity became insignificant. Binding constants (K_a) for anions were derived from plots of F/F_0 vs [anion].[1] Results reported in the main text are the average of at least two independent titrations. The slit width = 1.5 nm; λ_{exc}= 367 nm were used for emission.

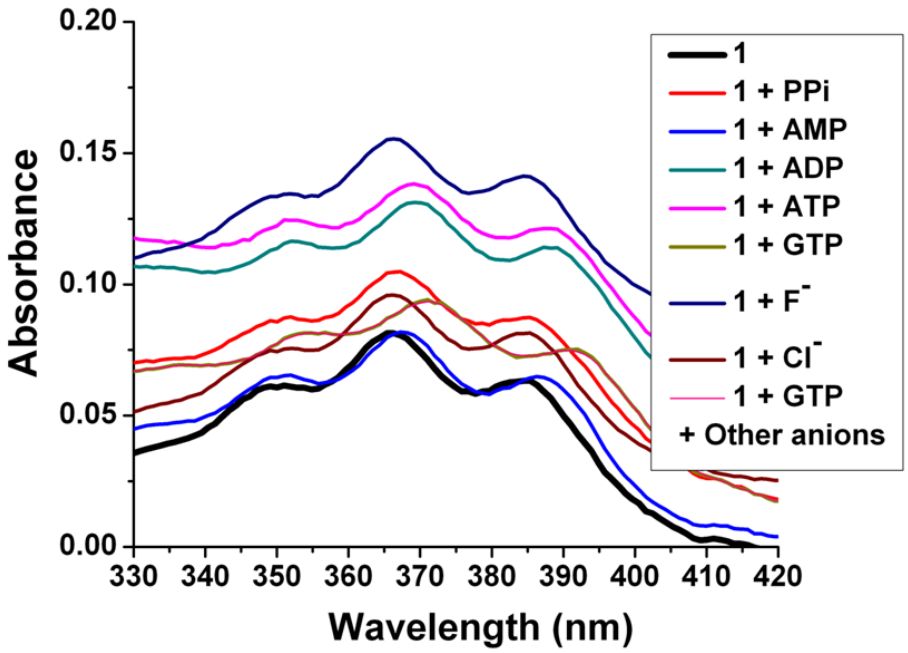

Figure S3. Absorption spectra of **1** (10 μM) upon the addition of sodium salts of pyrophosphate (PPi), AMP, ADP,ATP, GTP and TBA salts of F^- and Cl^- (100 equiv) at pH 7.4 (10 mM phosphate buffer).

5. Representative fluorescence titrations of GTP and ATP and corresponding binding isotherms:

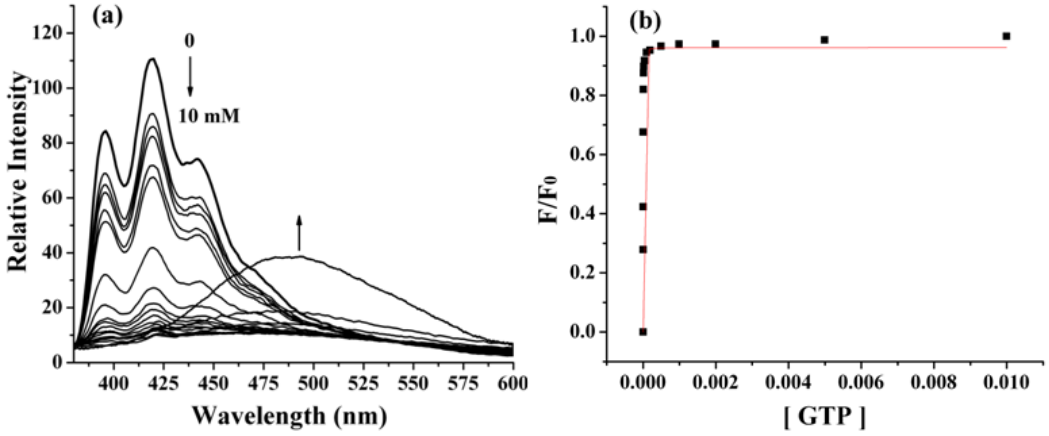

Figure S4. Emission spectra (excitation at 367 nm) of receptor **1** (10 μM) upon addition of Na salt of GTP at pH 7.4 (10 mM phosphate buffer) and the corresponding binding isotherm.

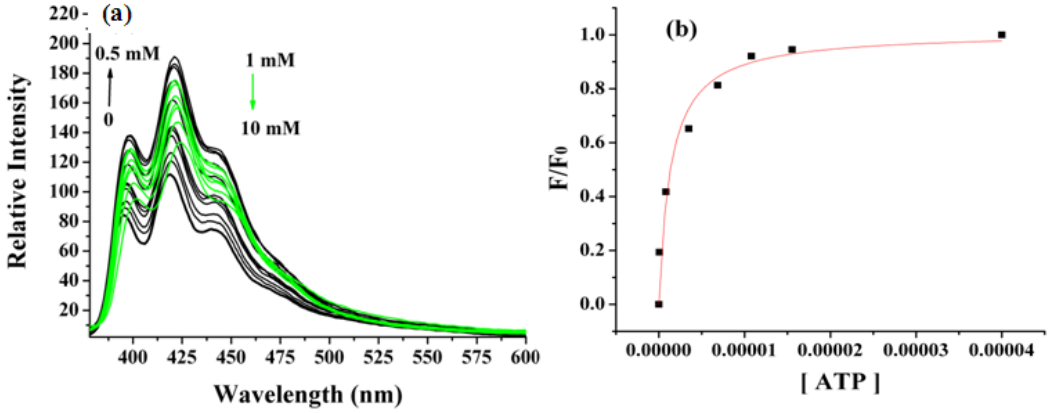

Figure S5 . Emission spectra (excitation at 367 nm) of receptor **1** (10 μM) upon addition of Na salt of ATP at pH 7.4 (10 mM phosphate buffer) and the corresponding binding isotherm.

6. Studies of receptor 1-anion complex stoichiometry (Job plot)

Job plot was performed using fluorescence emission spectroscopy. Job plot was constructed in the usual way and was found to exhibit maxima at 0.5. Such findings support the proposal that receptor **1** forms a 1:1 complex with the anions in question.

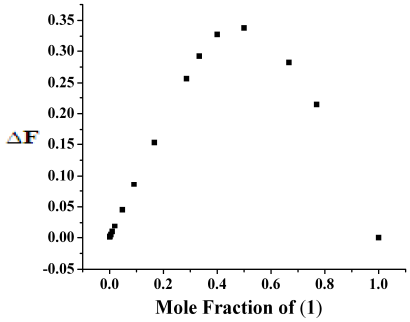

Figure S6. Assessment of the stoichometry of the GTP complex of **1** via Job plot analysis; [**1**] + [GTP] = 10 μM, pH 7.4 (10 mM phosphate buffer), 25ºC.

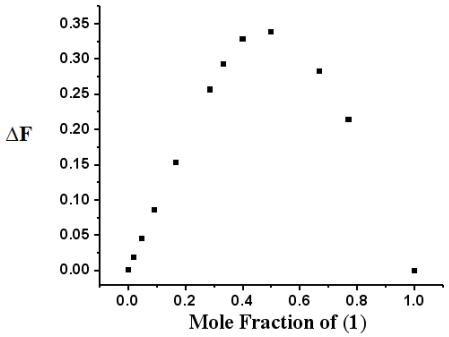

Figure S7. Assessment of the stoichometry of the ATP complex of **1** via Job plot analysis; [**1**] + [ATP] = 10 μM, pH 7.4 (10 mM phosphate buffer), 25ºC.

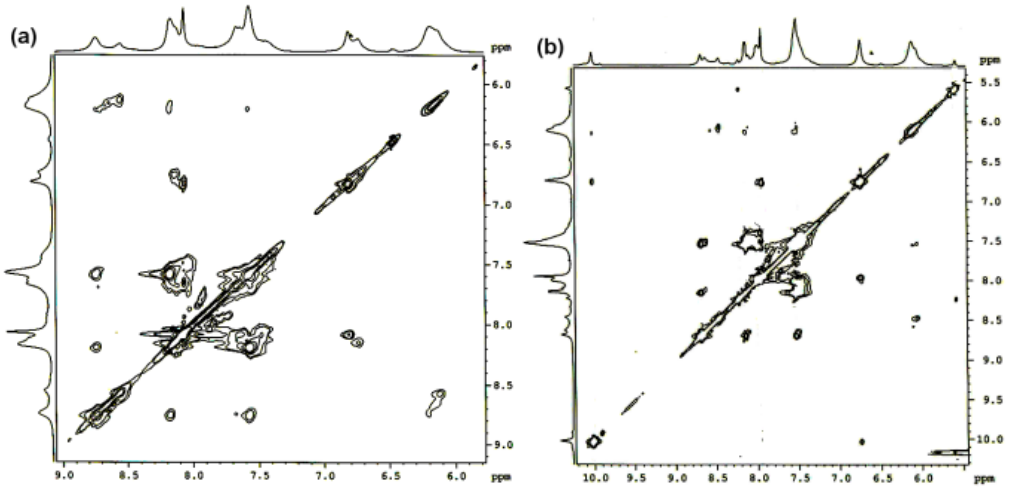

Figure S8. 500 MHz NOESY spectrum of **1** with 1 eq. of (a) GTP (b) ATP in DMSO-d6.

7. Theoretical Calculations

The resolution of identity approximation of density functional theory (RI-DFT) using the PBE functional with dispersion (PBE-D) was used to optimize the geometries of **1**-4Br$^-$, **1**-GTP and **1**-ATP complexes. The basis sets used was TZV2P. Energies in the aqueous phase were determined from single point calculations on the gas phase-optimized geometries using the conductor-like screening model (COSMO). Calculations were performed using Turbomole 6.0.2[2] and the most stable structures are shown in Figure S9. The energetic parameters are summarized in Table S1. **N-III** is the most stable geometry for receptor **1**, where Br$^-$ atoms are outside the cavity. However, it is nearly isoenergetic with **C-II** in gas phase, in which three of Br$^-$ counteranions are situated outside the cavity and the imidazolium protons are consequently directed outward. **N-III** has, however, partial π-π interaction between anthracene moieties, which opposes to experimental results which show no π-π stacking. In addition, even if **N-III** is more stable in gas phase for 8.3 kcal/mol, **C-II** can be more stable in the real system with various intermolecular interactions (explicit solvation, counteranions, etc.) Hence, **C-II** was further considered for GTP and ATP complexes instead of **N-III**.

For the 1-ATP complex, a structure with C-2 proton-π interaction is more stable than one with NH-π interaction by 13.82 kcal/mol. For the former, the distance between anthracene moiety and C-2 proton is 2.3 Å, and for the latter, the distance between anthracene moiety and NH is 2.7 Å. In the most stable structures for GTP and ATP complexes, four imidazoium protons interact with O atoms at a distance of 1.0-1.8 Å. On the other hand, the ATP complex with NH-π interaction has longer distance of 1.8-2.1 Å, and one of O atoms interacts with imidazolium C at a distance of 1.4 Å.

Table S1. Relative energies (kcal/mol) in the gas (E^{gas}) and aqueous phase (E^{sol}) with respect to the most stable structure.

Complex	Symmetry	E^{gas}	E^{sol}
1-4Br$^-$ [**N-I**]	C_{2v}	5.78	10.75
1-4Br$^-$ [**N-II**]	C_s	5.20	9.93
1-4Br$^-$ [N-**III**]	C_1	0.00	0.00
1-4Br$^-$ [**N-IV**]	C_1	6.11	8.95
1-4Br$^-$ [**C-I**]	C_1	4.69	13.96
1-4Br$^-$ [**C-II**]	C_1	0.43	8.28
1-ATP [**C-II**] (NH-π)	C_1	35.87	13.82
1-ATP [**C-II**] (C-2 H-π)	C_1	0.00	0.00
1-GTP [**C-II**]	C_1	0.00	0.00

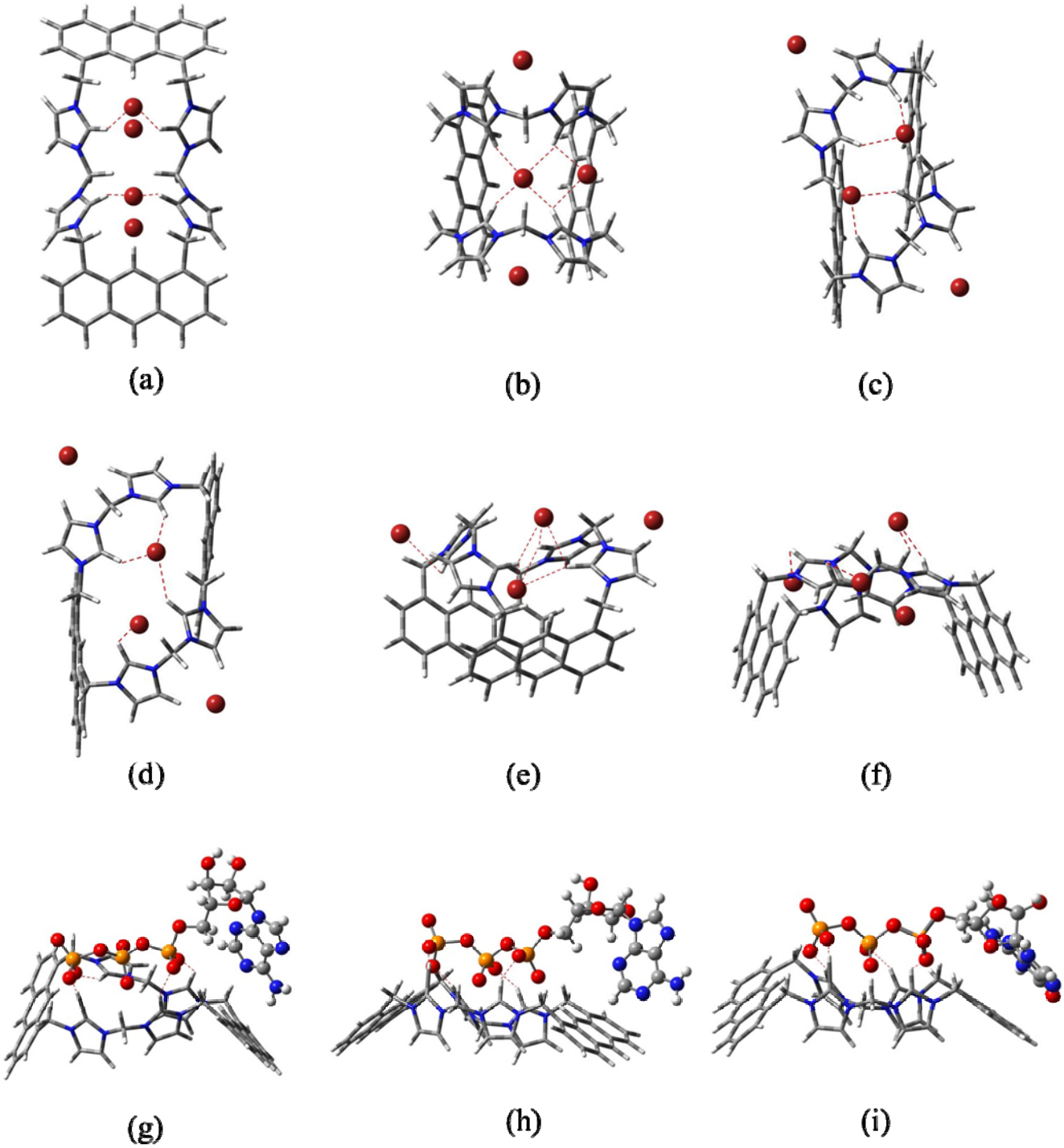

Figure S9. Optimized geometries of **1** complexes at the PBE-D/TZV2P level: Receptor **1**-4Br$^-$ [(a) **N-I**:C_{2v}, (b) **N-II**:C_s, (c) **N-III**:C_1, (d) **N-IV**:C_1, (e) **C-I**:C_1, (f) **C-II**:C_1], **1**-ATP complex [(g) C_1, (h) C_1] and **1**-GTP complex [(i) C_1].

8. References

[1] K. A. Connors, *Binding Constants*, Wiley, New York, **1987**.

[2] *TURBOMOLE V6.0 2009*; a development of University of Karlsruhe and Forschungszen-trum Karlsruhe GmbH: Karlsruhe, Gernany, **2007**. Available from http://www.turbomole.com.