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A fluorescent cavitand for the recognition of GTP

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Abstract—A new fluorescent cavitand bearing four imidazolium groups as well as four pyrene groups was synthesized for the recognition of GTP through $(C–H)^+–X^-$ hydrogen bond formation.

Even though considerable efforts have been devoted to develop fluorescent chemosensors for various anions during the last few decades,[1] there have been relatively few reports on adenosine 5′-triphosphate (ATP) selective receptors that show the fluorescent changes[2] or color changes.[3] ATP is known to be the universal energy currency in all of the biological systems and has been a significant target for the design of molecular receptors. Even though guanosine 5′-triphosphate (GTP) also plays an important role in biological systems, only one example of a selective fluorescent chemosensor for GTP is reported so far.[4]

Cavitands are synthetic host compounds with open-ended enforced cavities large enough to accommodate organic guest molecules and ions.[5] Compared to the calix[4]arene derivatives, relatively few reports are available for the cavitand derivatives which were utilized as host compounds for anion recognition.[6] Noteworthy and most related was a paper reported by Diederich and co-workers in which tetrakis(phenylamidnium)-cavitands were used as receptors for the selective binding with ATP.[6c]

We report herein, on a new fluorescent cavitand derivative bearing four imidazolium groups as well as four pyrene groups as a fluorescent receptor for GTP. The binding properties toward various anions were investigated based on the fluorescence experiments. To our knowledge, host **1** is the first fluorescent cavitand, which displays a selective fluorescent change with GTP.

Our synthesis began with tetrahydroxy-cavitand, which was prepared following the published procedure.[7,8] However, it is worth to mention the recent publication by Kaifer and co-workers in which the yield of tetrabromocavitand, the precursor of tetrahydroxycavitand, was improved from 52% to 94%.[8] Tetrachloride **2** was synthesized by following the published procedure.[9] 1-Bromomethylpyrene[10] was reacted with imidazole using sodium hydride in THF giving 1-imidazolylmethylpyrene **3**[11] in 85% yield. This intermediate was reacted with tetrachloride **2** and NaI in acetonitrile refluxing for 24 h followed by anion exchange with NH_4PF_6, which gave the tetra(imidazolium–pyrene)-cavitand **1**[12] in 43% yield (Scheme 1).

Figure 1 explains the fluorescent changes of compound **1** (6 μM) upon the addition of pyrophosphate, $H_2PO_4^-$, ATP, ADP, CTP, and GTP in DMSO-20 mM HEPES (6:4, v/v). Compound **1** displayed a large CHEQ (chelation enhanced fluorescence quenching) effect with GTP, even though **1** also displayed relatively small CHEF effects for ATP, CTP, and ADP. There were almost no fluorescent changes even when 100 equiv of pyrophosphate and $H_2PO_4^-$ were added. Figure 2 clearly shows the CHEQ effects with increasing GTP concentration. From the fluorescent titrations the association constants for GTP (Fig. 2), ATP (Fig. 3), and CTP are calculated

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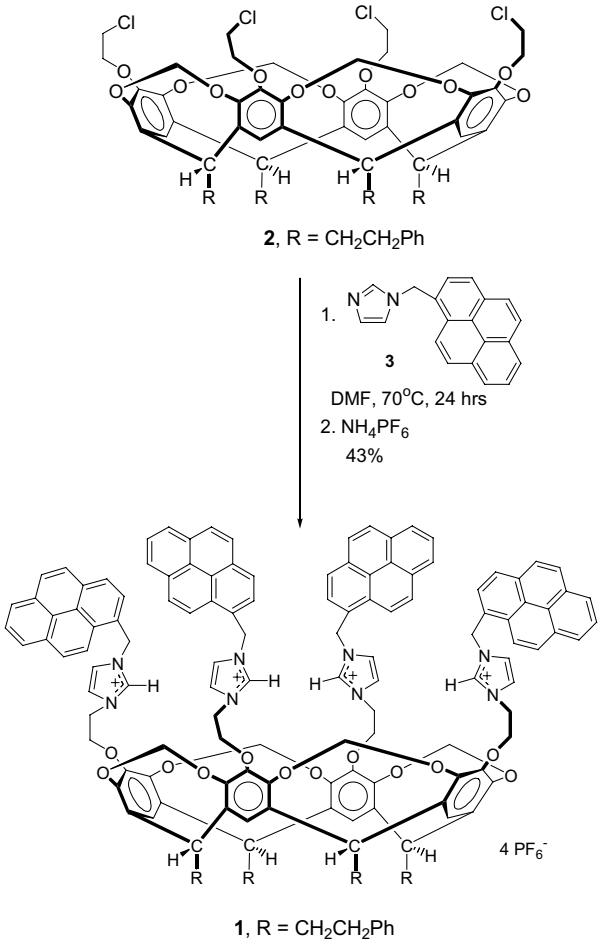

Scheme 1. Synthesis of compound **1**.

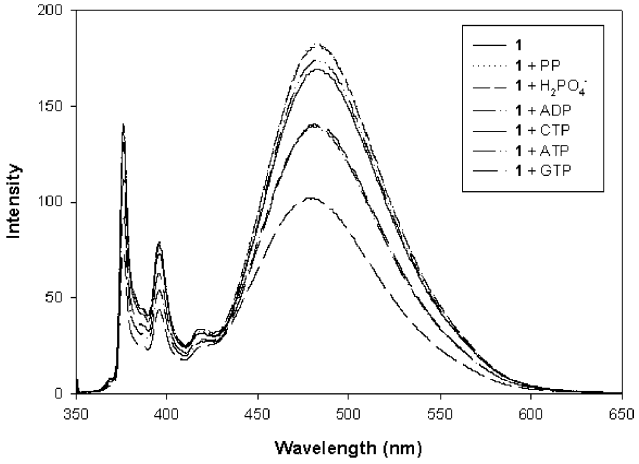

Figure 1. Fluorescent emission changes of **1** (6 μM) upon addition of tetrabutylammonium salts of $H_2PO_4^-$, and pyrophosphate and sodium salts of ADP, ATP, CTP, and GTP (100 equiv) in DMSO/20 mM aqueous HEPES buffer at pH 7.4 (6:4, v/v) (excitation at 367 nm).

as 73,800, 14,040, and 7700 M^{-1} (errors <10%), respectively (Fig. 4).[13] The selectivity for GTP over ATP and CTP was more than over 5 times and 10 times, respectively. In addition, the Job plot analysis indicates the formation of 1:1 complexes.

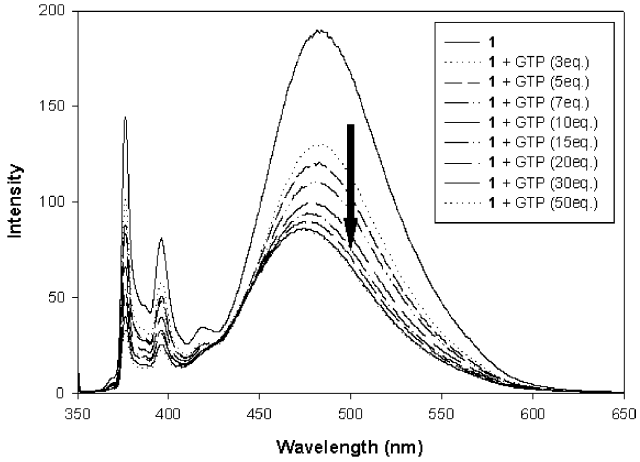

Figure 2. Fluorescence spectra of **1** (6 μM) upon the addition of GTP in DMSO/20 mM aqueous HEPES buffer at pH 7.4 (6:4, v/v) (excitation at 367 nm).

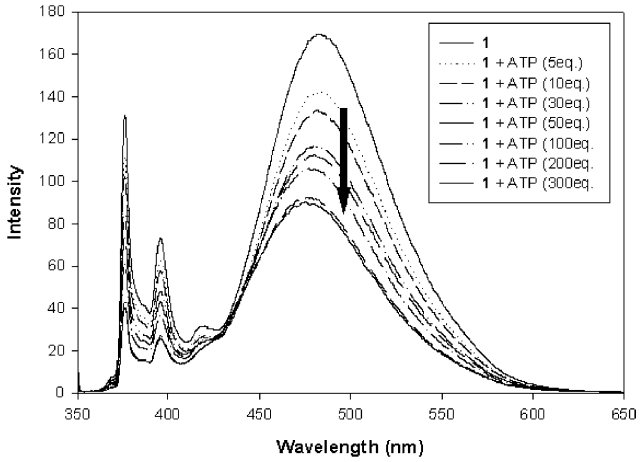

Figure 3. Fluorescence spectra of **1** (6 μM) upon the addition of ATP in DMSO/20 mM aqueous HEPES buffer at pH 7.4 (6:4, v/v) (excitation at 367 nm).

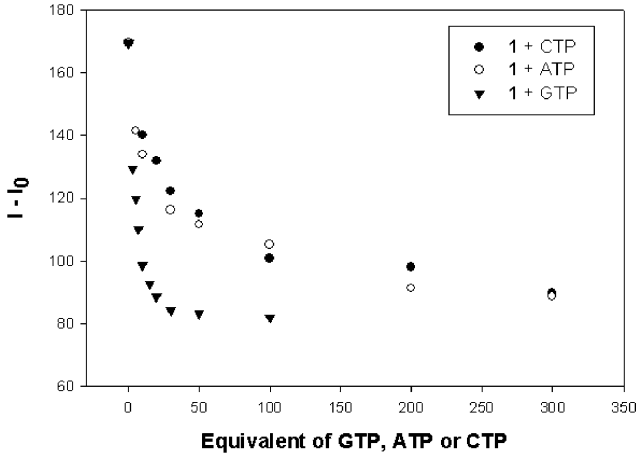

Figure 4. Fluorescence titration curves of **1** (6 μM) in DMSO/20 mM aqueous HEPES buffer at pH 7.4 as a function of. GTP, ATP or CTP concentration (6:4, v/v) (excitation at 367 nm and emission at 482 nm).

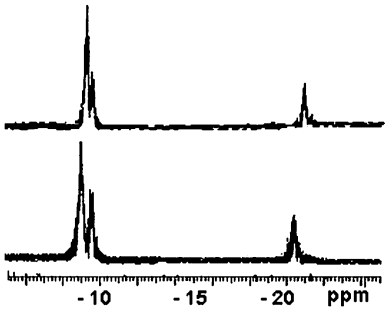

Figure 5. ^{31}P NMR spectra of GTP (2 mM) (a) and the complex of GTP with 1 equiv of **1** (2 mM) (b) in DMSO-d_6–D_2O (95:5, v/v).

The addition of GTP gradually lessens the fluorescent intensity of **1** (the monomer as well as eximer emission peaks) probably due to the electron transfer from the guanine group[14] and/or photo-induced electron transfer (PET). The PET induced CHEQ effects are well described in previous papers,[15] when there is a $(C–H)^+–X^-$ hydrogen bonding between imidazolium moieties and phosphate anions. Due to the solubility problem, ^{31}P NMR was observed in DMSO-d_6–D_2O (95:5, v/v). Upon addition of 1 equiv of **1**, the chemical shifts due to the three different phosphorous groups in GTP moved from −9.41 to −8.86 ppm, −9.68 to −9.37 ppm, and −20.86 to −20.32 ppm, respectively (Fig. 5), which indicates that receptor **1** directly interacts with the phosphate sites.[14b]

In conclusion, a new fluorescent cavitand derivative bearing four imidazolium groups as well as four pyrene groups was synthesized as a fluorescent receptor for GTP. To our knowledge, host **1** is the first fluorescent cavitand, which displays a selective fluorescent change with GTP.

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References and notes

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11. Compound **3**: to a reaction mixture of imidazole (57 mg, 8.4 mmol) in THF (20 mL) was added NaH (22 mg, 9.2 mmol) at 0 °C. After stirring 20 min at 0 °C, 1-bromomethylpyrene (200 mg, 0.6 mmol) was added to the reaction mixture. After additional stirring for 1 h at room temperature, the reaction mixture was poured into 50 mL of water, and extracted with $CHCl_3$. The organic layer was then separated, dried over anhydrous magnesium sulfate, and concentrated under reduced pressure. Purification by flash chromatography on silica gel (1:2, hexane–ethyl acetate) afforded **3** (142 mg, 85%) as a yellow solid; ^1H NMR (CD_3CN, 250 MHz) δ 8.16 (dd, 2H, $J = 6.95$, 1.22 Hz), 8.09 (m, 3H), 8.01 (m, 3H), 7.78 (d, 1H, $J = 7.85$ Hz), 7.56 (s, 1H), 7.03 (br s, 1H), 6.91 (br s, 1H), 5.78 (s, 2H); ^{13}C NMR (CD_3CN, 125 MHz) δ 132.2, 130.3, 126.6, 126.2, 125.5, 124.3, 123.9, 123.7, 123.1, 122.9, 122.2, 121.3, 120.8, 119.9, 116.9, 116.5, 114.6, 43.6; HRMS (FAB) m/z 283.1232, calcd for $C_{20}H_{15}N_2$: 283.1235.
12. Compound **1**: a mixture of **2** (120 mg, 0.10 mmol), 1-imidazoylmethylpyrene **3** (140 mg, 0.50 mmol) and NaI (35 mg, 0.23 mmol) in acetonitrile (20 mL) was refluxed for 24 h under N_2. After cooling to room temperature, the reaction mixture was evaporated to dryness under vacuum. The crude product was dissolved into CH_2Cl_2 (10 mL) and washed with distilled water (10 mL × 3

times). After drying the organic layer with $MgSO_4$, the solvent was evaporated to dryness under vacuum. The crude product was purified by Sephadex LH-20 column chromatography ($CHCl_3$–MeOH = 1:1, v/v). The cavitand (iodide salts) was dissolved in 3.24 mL DMF. During the dropwise addition of saturated aqueous NH_4PF_6 solution (1.43 mL), white precipitate was formed. After washing the precipitate several times with water, the desired product was obtained as a white solid (43%); mp 178–180°C, dec; ^{1}H NMR (CD_3CN, 250 MHz) δ 8.58 (s, 4H), 7.89–8.19 (m, 36H), 7.73 (m, 8H), 7.37 (s, 4H), 7.10 (m, 20H), 5.99 (s, 8H), 5.48 (br s, 4H), 4.49 (br s, 4H), 3.95–4.24 (m, 16H), 2.60 (m, 16H); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 147.7, 143.5, 142.2, 139.3, 136.5, 132.6, 131.5, 131.2, 130.6, 129.3, 129.0, 128.8, 128.6, 127.3, 126.9, 126.4, 125.6, 124.9, 124.2, 123.4, 122.7, 121.9, 116.3, 95.0, 68.0, 51.2, 50.2, 46.0, 38.9, 37.3; ESI mass, m/z 1271. 4 [100%, $(\mathbf{1}\text{-}2PF_6^-)^{2+}$], 799.5 [95%, $(\mathbf{1}\text{-}3PF_6^-)^{3+}$].

13. (a) Association constants were obtained using the computer program ENZFITTER, available from Elsevier-BIOSOFT, 68 Hills Road, Cambridge CB2 1LA, U.K.; (b) Conners, K. A. Binding Constants. *The Measurement of Molecular Complex Stability*; Wiley: New York, 1987.
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