A highly selective fluorescent probe for pyrophosphate in aqueous solution†

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A new 4-amino-1,8-naphthalimide-based fluorescent chemosensor bearing a guanidiniocarbonyl pyrrole moiety has been synthesized. The sensor displays a selective fluorescent enhancement with pyrophosphate over ATP, ADP, AMP and other inorganic anions in aqueous solution.

The recognition and sensing of anions have received considerable attention for their important roles in biological, industrial and environmental processes.[1] However, there are only a few artificial receptors that allow the recognition of anions in aqueous solution.[2] The reason is that the hydrogen bond and ion pairing interaction between host and guest molecules for molecular recognition would be weakened significantly by the competitive influence of protic solvents. Therefore, the receptors reported so far for the effective recognition of target molecules in water all need a combination of multiple nocovalent interactions, such as multiple hydrogen bonds,[3] ion pairing interaction[4] or metal coordination,[5] to overcome the competitive influence of water. Among them, the guanidiniocarbonyl pyrroles introduced by Schmuck can strongly bind carboxylate or phosphate anions even in aqueous solvents through a combination of ion pairing and multiple hydrogen bonds.[6] Excellent work has been done in studying the complexation between guanidiniocarbonyl pyrroles and amino acid carboxylates by NMR spectroscopy,[7] but there are few reports about fluorescent sensors based on guanidiniocarbonyl pyrroles.[8] Compared with other chemosensors, fluorescent sensors appear to be particularly attractive due to the simplicity and sensitivity of fluorescence, as well as providing on-line and real-time analysis,[9,10] so we are interested in developing luminescent chemosensors for the detection of anions based on the guanidiniocarbonyl pyrrole moiety.

Among the various anionic analytes, pyrophosphate (PPi) is a biologically important target because it is the product of ATP hydrolysis under cellular conditions.[11] PPi also plays an important role in energy transduction in organisms and could control metabolic processes by participating in enzymatic reactions.[12] The recognition effect of guanidiniocarbonyl pyrrole receptors with sugar phosphates in aqueous solution[13] led us to expand our approach to the detection of PPi by guanidiniocarbonyl pyrroles. In this communication, we report the synthesis of a new long-wavelength intramolecular charge-transfer (ICT) fluorescent receptor **1**, in which the guanidiniocarbonyl pyrrole cation is covalently attached to a *N*-butyl-4-hydrazino-1,8-naphthalimide moiety. The employment of 4-amino-1,8-naphthlimide[14] as a fluorophore is ascribed to its high fluorescence quantum yield and long wavelength emission.[15] On the other hand, Gunnlaugsson and Pfeffer have shown that the 4-amino proton in the 4-amino-1,8-naphthalimide structure, which is quite acidic, can be used to enhance both the strength and selectivity for the binding of phosphate anions.[16] Therefore, we designed this naphthalimide–hydrazino–guanidiniocarbonyl pyrrole structure (a fluorophore–spacer–receptor structure[17]) to satisfy the geometrical requirements of PPi and enable effective fluorescent sensing. It is expected that the electrostatic interactions and multiple hydrogen bonds of guanidiniocarbonyl pyrrole, combined with the increased acidity of the 4-amino proton, would favor the receptor–anion interaction and geometrical complementarity, thus producing effective fluorescent sensing of the recognition event. Investigation into the fluorescent sensing property of the receptor **1** has revealed that receptor **1** is a highly selective fluorescent probe for PPi over other anions, including its analogues ATP, AMP, ADP and Pi in aqueous solution.

Receptor **1** was synthesized according to Scheme 1 (see ESI†). *N*-Butyl-4-bromo-1,8-naphthalimde **2** reacted with hydrazine monohydrate to give **3** as a yellow solid in 90% yield. 5-(Methoxycarbonyl)-1*H*-pyrrole-2-carboxylic acid **4** was converted into the acyl chloride **5** by reaction with oxalyl chloride in CH_2Cl_2 in the presence of a catalytic amount of DMF. Without further purification, the crude acyl chloride was then treated with **3** in CH_2Cl_2 to give **6** in 55% yield. The guanidinylation of **6** was achieved by heating the ester and an excess of guanidine in DMF under nitrogen. Upon acidification with hydrochloric acid, the crude product (hydrochloric salt) precipitated in 40% yield. After reaction with picric acid, host **1** was obtained as the picrate salt.

Like other amino-containing ICT systems, the fluorescence of **1** is pH-sensitive (Fig. 1). When $6.97 < pH < 11.08$, the hydrogen bond interaction between the 4-amino proton and hydroxide enhances the intramolecular charge transfer (ICT) of receptor **1**, which leads to the fluorescence intensity increase significantly. When $pH > 11.08$, the deprontonation of the 4-amino-1,8-naphthalimide quenches the fluorescence of receptor **1**, which is the same as that described by Gunnlaugsson and Pfeffer.[15,16] The mechanism is supported by the UV-vis spectrum of receptor **1** at different pH values (Fig. S5, S6†).

Knowing that changes in solvent property can have a dramatic effect on molecular recognition, fluorescence titrations were performed in H_2O–DMSO (in the range 60–99% water (10 mM HEPES buffer, pH = 7.4)) in an effort to gauge the effect of solvent composition on spectroscopy and association. The spectroscopic properties and binding constants are shown in Table 1. As DMSO is an extremely good H-bond acceptor, its electron donor capacity is even higher than that of water,[18] so the fluorescence maxima of **1** undergoes a red shift with increasing DMSO content. Such behavior is also indicative of the ICT character of receptor **1** upon

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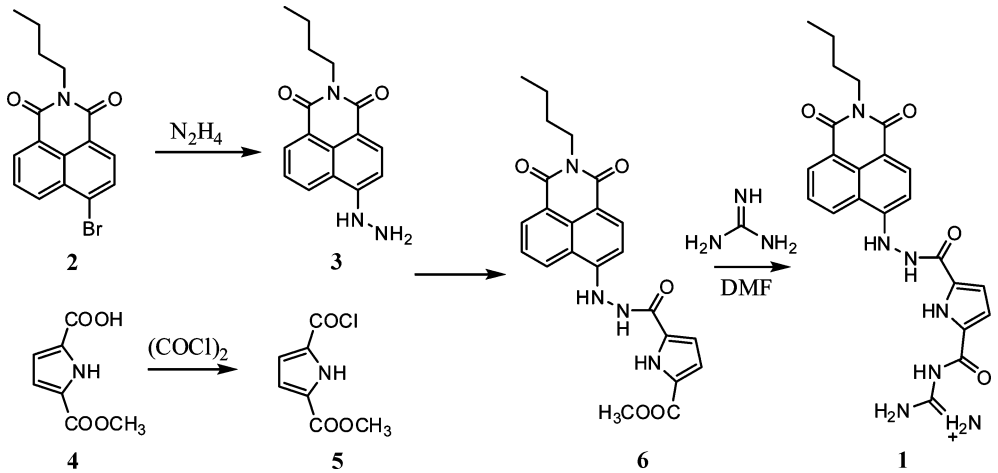

Scheme 1 Synthesis of guanidiniocarbonyl pyrrole receptor **1**.

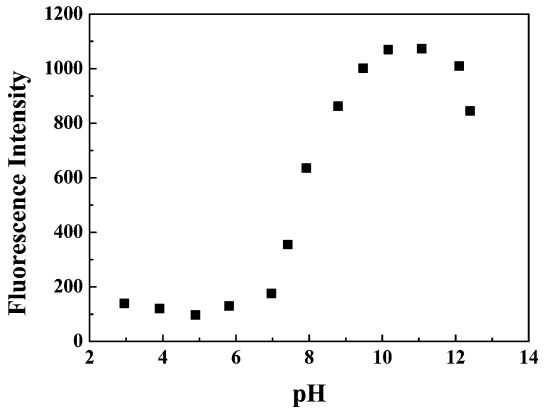

Fig. 1 pH dependence of the fluorescence emission peak of receptor **1**.

Table 1 Spectrum characters and association constants for receptor **1** with PPi (sodium salt) in water–DMSO

$H_2O/(H_2O + DMSO)$	λ_{ex}/nm	λ_{em}/nm	K_{ass}/M^{-1} [a]	F/F_0 [b]
99%	456	505	24	4.55
90%	460	510	78	2.48
80%	466	518	216	1.55
70%	472	522	nd[c]	1.17
60%	480	525	nd[c]	1.06

[a] Association constants K_{ass} were calculated by nonlinear least-squares fitting with a 1 : 1 association model; error limits in K_{ass} were estimated to be ±10%. [b] Fluorescence intensities are corrected. [c] Not determined; the changes in the spectra were too small to calculate the association constants precisely.

excitation. Meanwhile, DMSO effectively displaces PPi from the 4-amino moiety, which is why the more DMSO that is present, the less the fluorescence intensity of receptor **1** increases. The association constants for PPi are relatively small, which may due to the 'salt effect' caused by the substrate itself and the buffer.[19]

The recognition properties of **1** (10 μm) were studied in 90% water–DMSO (v/v, 10 mM HEPES buffer, pH = 7.4) with various anions as substrates. Fluorescence titration experiments were recorded on excitation at 460 nm and emission at 510 nm, respectively. As shown in Fig. 2(a), receptor **1** displayed a

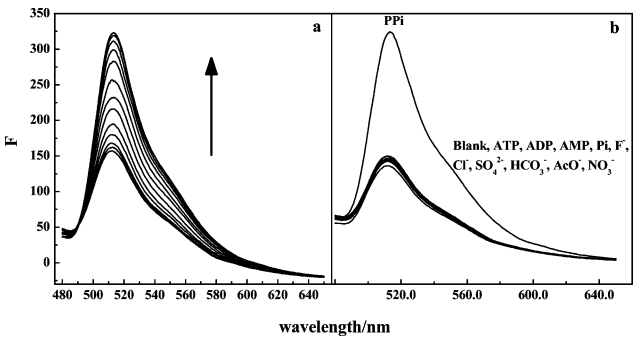

Fig. 2 (a) Fluorescence titration of receptor **1** (10 μm) with PPi (2000 eq.) in 90% water–DMSO (pH = 7.4, 10 mM HEPES buffer). (b) Fluorescence emission changes of receptor **1** (10 μm) upon addition of sodium salts of PPi, ATP, ADP, AMP, Pi, F^-, Cl^-, SO_4^{2-}, HCO_3^-, AcO^- and NO_3^- (0–2000 eq.) in 90% water–DMSO (pH = 7.4, 10 mM HEPES buffer).

chelation-enhanced fluorescence (CHEF) effect with PPi (0–2000 eq.). The emission maximum of **1** showed a bathochromic shift from 510 to 514 nm. The job-plot analysis indicated that receptor **1** formed a 1 : 1 complex with PPi (Fig. S7†); the association constant was 78 M^{-1} as obtained by a nonlinear least-squares fitting method. In contrast, no significant fluorescence changes were observed when ATP, ADP, AMP, Pi, F^-, Cl^-, SO_4^{2-}, HCO_3^-, AcO^- or NO_3^- were added (Fig. 2(b)). These results suggested that receptor **1** has a higher selectivity for PPi over other anions.

In order to obtain a better idea about the nature of the interactions between the guanidiniocarbonyl pyrrole receptor **1** and anionic species considered and the high selectivity of receptor **1** for PPi, the pK_a value of the guanidiniocarbonyl pyrrole receptor **1** was determined by using the Henderson–Hasselbach equation;[20] molecular modeling studies (PM3 of MOPAC 2007 softwware) were also used to obtain the possible interaction models of receptor **1** with either PPi or ATP.

The calculated pK_a value for receptor **1** is 8.18, which is a little higher than that of other guanidiniocarbonyl pyrrole receptors reported by Schmuck (pK_a 6.9–7.9).[21] This means that the acidity (pH 7.4) of the solution that we used for the fluorescence titration of receptor **1** is suitable for the protonation

of guanidiniocarbonyl pyrrole receptor **1**. Under this condition, the guanidinium cation can form an ion pair with the phosphate anion, which is simultaneously hydrogen bonded by the pyrrole NH and another amide NH. Meanwhile, the 4-amino moiety in the 4-amino-1,8-naphthalimide structure is quite acid,[16] favoring the formation of a hydrogen bond between receptor **1** and PPi.

According to the molecular modeling studies (PM3 of MOPAC 2007 software), the rational preorganisation of receptor **1** provides excellent geometric and charge complementarity to PPi (Fig. 3). Due to the appropriate size and geometry of the pyrophosphate anion, it is possible that the proton of the 4-amino moiety could interact with the negative oxygen atom of PPi. The hydrogen bond would promote the ICT of 4-amino-1,8-naphthalimide, resulting in the fluorescence enhancement of receptor **1**. Therefore, the strength and selectivity of receptor **1** for the PPi binding can be ascribed to the combined binding modes, namely, the electrostatic interaction, multiple hydrogen bonds and geometric complementarity between receptor **1** and PPi.

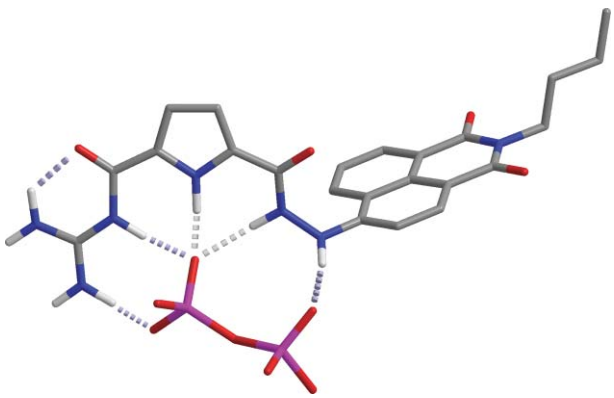

Fig. 3 Calculated energy-minimized structure for the complex between receptor **1** and PPi.

The selectivity of receptor **1** for PPi over ATP can be understood on the basis of the structure of the guest and the charge density of O–P oxygen atoms of the guest involved in complexation. From the energy-minimized structure of the receptor–ATP complex (Fig. 4), we can see that the π–π stacking interaction between the naphthalimide moiety and the adenine fragment, combined with the electrostatic interaction and hydrogen bonds between the guanidiniocarbonyl pyrrole moiety and the phosphate fragment, make the conformation of the receptor–ATP complex relatively

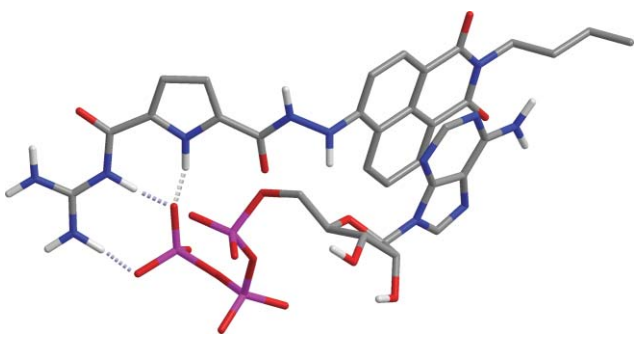

Fig. 4 Calculated energy-minimized structure for the complex between receptor **1** and ATP.

stable. The O–P oxygen atom of ATP cannot form a hydrogen bond effectively with the 4-amino moiety, so the fluorescence of receptor **1** changes by a small amount relative to that when PPi binds. On the other hand, the total anionic charge density of the O–P oxygen atoms involved in the complexation between ATP and guanidiniocarbonyl pyrrole acyl hydrazine sites is smaller than that of the O–P oxygen atoms of PPi.[12] Therefore, the binding affinity of receptor **1** to ATP is relatively weak. We have tried to get the complexation-induced shift of receptor **1** to confirm the interaction models, but unfortunately, the ^{1}H NMR titrations could not be performed because receptor **1** is not soluble enough (millimolar concentrations would be required) in solvent systems with the necessary higher water contents.

In conclusion, we have developed a fluorescent sensor based on the guanidiniocarbonyl pyrrole moiety with high selectivity for PPi in aqueous solution. The receptor shows excellent shape, size and charge complementarity to PPi, which makes it an efficient PPi sensor with the potential for bioanalytical applications.

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References

1 See two special issues on anion recognition: *Coord. Chem. Rev.*, 2006, **250**, 2917; *Coord. Chem. Rev.*, 2003, **240**, 1.
2 J. Y. Kwon, N. J. Singh, H. N. Kim, S. K. Kim, K. S. Kim and J. Yoon, *J. Am. Chem. Soc.*, 2004, **126**, 8892; S. L. Tobey, B. D. Jones and E. V. Anslyn, *J. Am. Chem. Soc.*, 2003, **125**, 4026; G. Zong, L. Xian and G. Lu, *Tetrahedron Lett.*, 2007, **48**, 3891; L. Fabbrizzi, F. Foti and A. Taglietti, *Org. Lett.*, 2005, **7**, 2603.
3 P. Anzenbacher, Jr., K. Jursiková and J. L. Sessler, *J. Am. Chem. Soc.*, 2000, **122**, 9350; P. Anzenbacher, Jr., K. Jursiková, V. M. Lynch, P. A. Gale and J. L. Sessler, *J. Am. Chem. Soc.*, 1999, **121**, 11020.
4 D. H. Vance and A. W. Czarnik, *J. Am. Chem. Soc.*, 1994, **116**, 9397; K. Niikura, A. Metzger and E. V. Anslyn, *J. Am. Chem. Soc.*, 1998, **120**, 8533; J. J. Lavigne and E. V. Anslyn, *Angew. Chem., Int. Ed.*, 1999, **38**, 3666.
5 L. Fabbrizzi, N. Marcotte, F. Stomeo and A. Taglietti, *Angew. Chem., Int. Ed.*, 2002, **41**, 3811; S. Mizukami, T. Nagano, Y. Urano, A. Odani and K. Kikuchi, *J. Am. Chem. Soc.*, 2002, **124**, 3920; M. J. McDonough, A. J. Reynolds, W. Y. G. Lee and K. A. Jolliffe, *Chem. Commun.*, 2006, **28**, 2971.
6 C. Schmuck, *Chem. Commun.*, 1999, 843; C. Schmuck and J. Lex, *Org. Lett.*, 1999, **1**, 1779; C. Schmuck and V. Bickert, *Org. Lett.*, 2003, **5**, 4579.
7 C. Schmuck and M. Schwegmann, *J. Am. Chem. Soc.*, 2005, **127**, 3373; C. Schmuck and L. Geiger, *J. Am. Chem. Soc.*, 2005, **127**, 10486; C. Schmuck and J. Dudaczek, *Tetrahedron Lett.*, 2005, **46**, 7101.
8 L. Hernandez-Folgado, C. Schmuck, S. Tomić and I. Piantanida, *Bioorg. Med. Chem. Lett.*, 2008, **18**, 2977.
9 Y. J. Jang, E. J. Jun, Y. J. Lee, Y. S. Kim, J. S. Kim and J. Yoon, *J. Org. Chem.*, 2005, **70**, 9603; K. M. K. Swamy, Y. J. Lee, H. N. Lee, J. Chun, Y. Kim, S.-J. Kim and J. Yoon, *J. Org. Chem.*, 2006, **71**, 8626.
10 T. Gunnlaugsson, T. C. Lee and R. Parkesh, *Org. Lett.*, 2003, **5**, 4065.
11 H. N. Lee, Z. Xu, S. K. Kim, K. M. K. Swamy, Y. Kim, S.-J. Kim and J. Yoon, *J. Am. Chem. Soc.*, 2007, **129**, 3828.
12 D. H. Lee, S. Y. Kim and J.-I. Hong, *Angew. Chem., Int. Ed.*, 2004, **43**, 4777.
13 C. Schmuck and M. Schwegmann, *Org. Lett.*, 2005, **7**, 3517.
14 T. Gunnlaugsson, P. E. Kruger, P. Jensen, J. Tierney, H. D. P. Ali and G. M. Hussey, *J. Org. Chem.*, 2005, **70**, 10875; D. Esteban-Gσmez, L. Fabbrizzi and M. Licchelli, *J. Org. Chem.*, 2005, **70**, 5717; B. Liu and H. Tian, *J. Mater. Chem.*, 2005, **15**, 2681.

15 T. Gunnlaugsson, M. Glynn, G. M. Hussey, P. E. Kruger and F. M. Pfeffer, *Coord. Chem. Rev.*, 2006, **250**, 3094.
16 F. M. Preffer, A. M. Buschgens, N. W. Barnett, T. Gunnlaugsson and P. E. Kruger, *Tetrahedron Lett.*, 2005, **46**, 6579; R. M. Duke and T. Gunnlaugsson, *Tetrahedron Lett.*, 2007, **48**, 8043; T. Gunnlaugsson, P. E. Kruger, T. C. Lee, R. Parkesh, F. M. Pfeffer and G. M. Hussey, *Tetrahedron Lett.*, 2003, **44**, 6575.
17 A. P. de Silva, B. McCaughan, B. O. F. McKinney and M. Querol, *Dalton Trans.*, 2003, **10**, 1902; T. Gunnlaugsson, A. P. Davis, G. M. Hussey, J. Tierney and M. Glynn, *Org. Biomol. Chem.*, 2004, **2**, 1856.
18 C. Schmuck and W. Wienand, *J. Am. Chem. Soc.*, 2003, **125**, 452.
19 C. Schmuck and S. Graupner, *Tetrahedron Lett.*, 2005, **46**, 1295.
20 C. Schmuck and U. Machon, *Eur. J. Org. Chem.*, 2006, **19**, 4385.
21 C. Schmuck, *Chem.–Eur. J.*, 2000, **6**, 709.