Fluorescence-mediated sensing of guanosine derivatives based on multitopic hydrogen bonding

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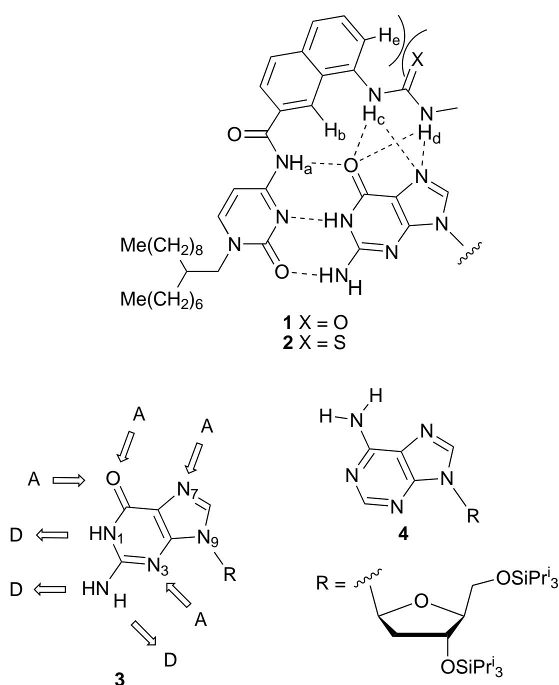
The fluorescence emission of receptor 1 is selectively quenched upon binding of a guanosine derivative by five hydrogen bonds.

Because of their critical role in nearly all biological processes, molecular recognition of nucleotides has attracted much attention. By analogy with the formation of double-stranded DNA or RNA, many artificial receptors have been designed and synthesized to recognize nucleobases by complementary hydrogen bonding.[1] While a few of them have chromogenic or fluorogenic groups for optical signalling,[2–4] the moderate strengths of their complexes, which are stabilized by only three hydrogen bonds, are still unsatisfactory in view of optical sensor applications. Here we demonstrate very selective quenching of the fluorescence emission of receptors **1** and **2** upon binding of a guanosine derivative by five hydrogen bonds in a binary solvent mixture of chloroform and DMSO, and report on an application of receptor **1** in a fluorescence-based optical sensor[5] for guanosine 5′-triphosphate.



In addition to the Watson–Crick type hydrogen bonding site on the N-1 side (two hydrogen bond donor atoms and an acceptor in a DDA array), natural guanosine derivatives have an acceptor–donor (AD) and an acceptor–acceptor (AA) hydrogen bonding site on the side of N-3 and N-7, respectively (for instance, see **3**). On the basis of the secondary interaction model,[6] hydrogen bonded complexes with the AA·DD motif are expected to be more stable than those with the AD·DA motif. We therefore linked a urea or thiourea group as DD hydrogen bonding site to the cytosine group by using 8-amino-naphthalene-2-carboxylic acid as rigid spacer, giving receptors† **1** and **2** with a fairly high level of preorganization.

Complexation of receptor **1** or **2** (1.0 mM) with the lipophilic guanosine derivative **3** (0–25 mM) in the binary solvent mixture of $CDCl_3$ and $[^2H_6]$DMSO (4 : 1, v/v) was confirmed by ^{1}H NMR spectroscopy. In this concentration range, neither the receptors nor guest were observed to self-associate. For receptor **1**, complexation-induced downfield shifts for all NH hydrogens of both **1** and **3** were found, confirming the formation of hydrogen bonds. A ^{1}H NMR titration experiment showed the association constant (K_{ass}) to be 1.7×10^2 dm^3 mol^{-1}.

The occurrence of a downfield shift for all NHs of **1** and **3**, and the similarity of the association constants as determined for H_a, H_b, H_c and H_d, suggest the formation of a 1 : 1 complex based on five hydrogen bonds.‡ To the best of our knowledge this is only the second receptor that forms more than three hydrogen bonds with the guanine base.[7] While improving its preorganization might further increase its binding strength [for a guanine receptor with very high preorganization, $K_{ass} > 1.9 \times 10^5$ dm^3 mol^{-1} in $CDCl_3$–$[^2H_6]$DMSO (4 : 1, v/v)],[7] the most interesting aspect of receptor **1** is its fluorescent property (*vide infra*).

Similar results were obtained with thiourea **2**.‡ Upon complexation, downfield shifts were observed for all NH hydrogens of both **2** and **3**. Only the H_c hydrogen of **2** underwent an opposite (upfield) shift. Because the NH hydrogens of thioureas are more acidic than those of ureas, stronger binding was expected for **2** than for **1**.[8] However, the association constant for the complex of **2** and **3** was found to be only 1.2×10^2 dm^3 mol^{-1}, which is slightly smaller than for the complex of receptor **1**. A possible explanation for the unexpectedly weak stability of the complex of **2** may be that steric hindrance between the sulfur and the aromatic hydrogen H_e of **2** leads to a loss of coplanarity between the thiourea group and the naphthyl ring, which is unfavorable for the recognition of the planar guanine base. Indeed, crystal structures as found in the Cambridge Structural Database show that arylthioureas are in general less planar than arylureas.§

With a view to applying these receptors in optical sensors, their UV–VIS and fluorescent spectra were investigated in the binary mixture of $CHCl_3$ and DMSO (4 : 1, v/v). While the UV–VIS spectra of **1** or **2** did not change significantly in the presence and absence of **3**, complexation-induced quenching of their fluorescence emission was observed. Solutions of receptor **1** exhibit strong fluorescence emission in the range 420–530 nm. Upon addition of **3**, the fluorescence emission is strongly quenched. A plot of the ratio of the fluorescence intensity in the absence (I_0) and in the presence (I) of **3** *versus* the concentration of **3** is linear (Fig.1) and allows the determination of the association constant, K_{ass}, of the 1 : 1 complex of **1** and **3** according to eqn. (1),

$$I_0/I = 1 + K_{ass}[C] \quad (1)$$

where [C] is the concentration of **3**. The resulting K_{ass} is 1.8×10^2 dm^3 mol^{-1}, which is in excellent agreement with K_{ass} as obtained by ^{1}H NMR spectroscopy. This shows that the decrease in fluorescence emission is not due to dynamic quenching based on collision of the receptor and the guest in its

excited state, but to static quenching based on complexation. On the other hand, the fluorescence spectrum of receptor **1** was not perturbed at all by addition of the lipophilic adenosine derivative **4** (Fig.1). Increasing the number of hydrogen bonding sites gives a very selective fluorogenic receptor with a significantly larger binding strength than previously reported for chromogenic or fluorogenic nucleobase receptors with only three hydrogen bonding sites ($K_{ass} = 2.8 \times 10^2$ or 9×10^2 $dm^3 mol^{-1}$ in $CDCl_3$ or CD_2Cl_2, respectively[2,3]). A highly selective quenching by **3** but not by **4** was also observed for receptor **2** (data not shown). However, the fluorescence intensity of receptor **2** was twenty times smaller than that of **1**, and receptor **2** does not appear to be photostable because the fluorescence intensity increased monotonously in repetitive emission scans of a dilute solution.

The fluorescent responses of an optical sensor based on a plasticized poly(vinyl chloride) membrane containing receptor **1** is shown in Fig. 2.¶ The response mechanism of this sensor (a bulk optode) is based on an anion-exchange equilibrium between the sample solution and the optode membrane.[9] An increase in the nucleotide concentration in the sample solution leads to receptor-assisted uptake of the nucleotide into the membrane in exchange for Cl^-, thereby changing the intensity of the receptor fluorescence. As expected, stronger quenching of the emission was observed for 5′-GTP than for 5′-ATP, which is due to preferential complexation of **1** with the guanine moiety of 5′-GTP. This is the first example of an optode based on multitopic hydrogen-bond mediated complexation of an analyte and a synthetic receptor. While this optode is very sensitive for 5′-GTP (detection limit < 10 μM), high background concentrations of Cl^- as occurring in biological samples may make a higher discrimination of Cl^- desirable. To improve the selectivity over Cl^-, nucleotide-selective optodes containing two receptor types[10] (receptor **1** and bis-thiourea receptors[8] for binding the nucleobase and phosphate groups, respectively) are conceivable.

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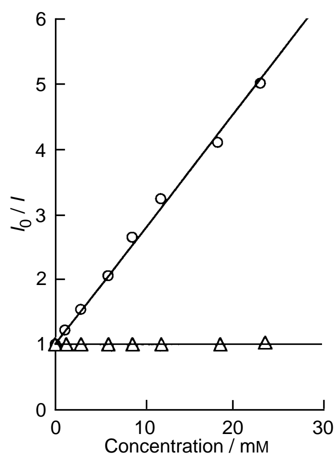

Fig. 1 Plot of the ratios of fluorescence intensities of solutions of receptor **1** (0.11 mM) in $CHCl_3$–DMSO (4 : 1, v/v) in absence (I_0) and in presence (I) of guest **3** (○) or **4** (△). Excitation at 373 nm, emission at 488 nm.

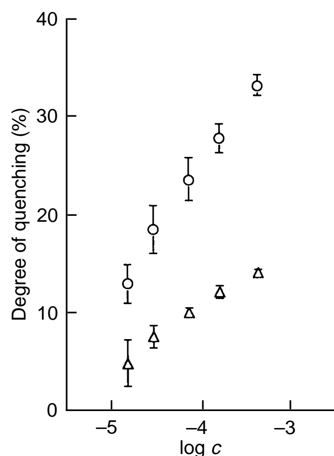

Fig. 2 Fluorescence responses of a membrane¶ based on receptor **1** as a function of the concentration of 5′-GTP (○) and 5′-ATP (△) in an aqueous buffer solution (10 mM tris/HCl at pH 7.4). Excitation at 375 nm, emission at 470 nm.

Footnotes

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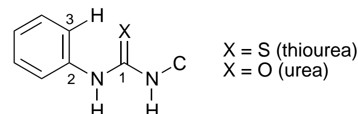
† Both receptors gave satisfactory ^1H NMR, IR and mass spectra and elemental analyses.

‡ Non-linear regression analysis of the ^1H NMR titration data gave the association constants (K_{ass}) and the differences in chemical shifts ($\Delta\delta_{max}$) of complexed and uncomplexed receptor using the residual solvent signal (δ 2.55) for [$^2H_6$]DMSO as reference: receptor **1**, K_{ass} = 173, 171, 153 and 174 $dm^3 mol^{-1}$ and $\Delta\delta_{max}$ = 0.80, 0.39, 0.09 and 1.45 ppm, respectively, for H_{a-d} (δ 10.38, 8.86, 8.60 and 6.09, respectively, in its uncomplexed form); receptor **2**, K_{ass} = 127, 127, 120 and 122 $dm^3 mol^{-1}$ and $\Delta\delta_{max}$ = 0.56, 0.39, −0.12 and 1.70 ppm, respectively, for H_{a-d} (δ 10.70, 8.87, 9.50 and 7.43, respectively, in its uncomplexed form).

§ The torsional angle C(1)–N–C(2)–C(3) in *N*-phenyl and *N,N′*- diphenyl derivatives of urea and thiourea is a good measure of the coplanarity between the aryl ring and urea or thiourea plane. For arylthioureas, the average of the torsional angles in eight cases was found to be 57°, seven of them being in the range from 42 to 87°. For arylureas, the average of the torsional angles in 61 cases was found to be 20°, 50 of them being smaller than 34°.



¶ The membrane was prepared by pouring 200 μl of a THF solution of the membrane components [0.50 mg receptor **1**, 1.94 mg tridodecylmethylammonium chloride (400 mol% relative to receptor **1**), 7.61 mg bis-(ethylhexyl) sebacate and 4.95 mg PVC in 5 ml THF] on a glass plate (12 × 25 × 2.5 mm) and letting the solvent evaporate overnight.

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