

## Binding of Nucleotides in Water by Phenanthridinium Bis(intercaland) Receptor Molecules

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The bis(phenanthridinium) receptor molecules **3–5** bind nucleotides in aqueous media by stacking interactions between *cis*-oriented receptor units and the intercalated nucleic base part of a nucleotide with stability constants in the range  $10^5$ – $10^6$  dm<sup>3</sup> mol<sup>-1</sup>, the highest measured to date for these type of receptors and substrates.

Binding and recognition of lipophilic nucleic base derivatives in apolar media has been realized by various synthetic receptor molecules providing stacking and hydrogen-bonding recognition sites. To date, selective receptors for each of the major nucleic bases have been prepared.<sup>1</sup> In contrast, the development of selective receptors for the binding and recognition of each of the major nucleotides in aqueous media is at an early stage.<sup>2</sup> The main problem of nucleotide recognition lies in the fact that hydrogen bonding, as an obvious recognition mode for nucleic bases, is highly disfavoured in water.<sup>3</sup> The possible solution of the nucleotide recognition problem could be achieved by the construction of water-soluble receptor molecules possessing lipophilic binding and built-in hydrogen-bonding base recognition sites, both protected from water solvation. The obvious first condition that has to be fulfilled on the way to such selective receptors is to design water soluble molecules which are able to bind efficiently the nucleic base part of a nucleotide in their lipophilic cavity. Until now, only two types of receptors satisfying the above conditions have been prepared: the azoniacyclophanes<sup>4</sup> and cyclobis(intercalands),<sup>5</sup> the latter being constructed from two acridinium units of known DNA intercalators<sup>6</sup> or two naphthalene units. Both types of receptors bind purine slightly better than pyrimidine nucleotide monophosphate, with stability constants of  $10^2$ – $10^3$  dm<sup>3</sup> mol<sup>-1</sup> and  $10^3$ – $10^4$  dm<sup>3</sup> mol<sup>-1</sup>, respectively.

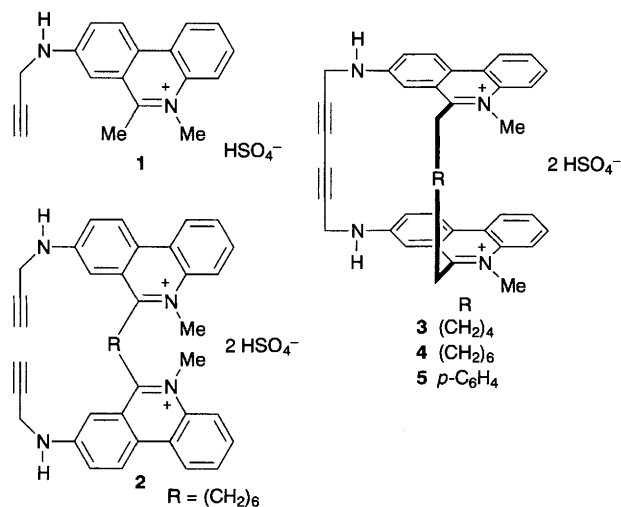
Here we report on the synthesis of water soluble acyclic **2** and cyclic **3–5** bis(intercaland) receptor molecules, constructed from two units of another type of known DNA intercalator, namely phenanthridinium. The highly fluorescent phenanthridinium derivative, ethidium, is widely used for the staining of DNA fragments in gel electrophoretic experiments and may be considered as one of the most useful intercalators for analytical purposes.<sup>7</sup> Our binding studies show that the bis(phenanthridinium) receptors **3–5** are able to bind strongly to nucleotides in water with stability constants ( $K_s$ ) from  $10^5$  to  $10^6$  dm<sup>3</sup> mol<sup>-1</sup>, the highest measured to date for this type of receptor.<sup>4,5</sup>

The bis(phenanthridinium) receptors **2–5** were prepared from the corresponding 8,8'-(benzyloxycarbonylamino)bis(phenanthridine) derivatives described earlier.<sup>8</sup> The synthetic steps comprise of methylation of the phenanthridine nitrogens using methyl trifluoromethylsulfonate (methyl triflate), the removal of benzyloxycarbonyl protection from the formed 5-methylphenanthridinium triflates using trifluoromethylsulfonic acid, and anion exchange of bis(phenanthridinium) triflates into hydrogensulfates by the use of tetrabutylammonium hydrogensulfate. 5,6-Dimethyl-8-(prop-2-ynylamino)phenanthridinium hydrogensulfate **1** was prepared for comparison purposes.<sup>8†</sup>

The receptors **3–5** contain flexible (CH<sub>2</sub>)<sub>6</sub> (**3**), (CH<sub>2</sub>)<sub>8</sub> (**4**) and rigid *p*-xylylene (**5**) bridges at the phenanthridine 6,6'-positions and rigid bis(acetylenic) bridges at the 8,8'-amino positions. Examination of space-filling models shows that such bridging should allow the existence of conformations with phenanthridinium unit separations of *ca.* 3.5–4 Å, suitable for the

accommodation of the nucleic base part of a nucleotide. In this way, the intercalative binding of nucleotides similar to that previously found for the acridinium cyclobis(intercaland) type of receptor<sup>5</sup> should be realized.

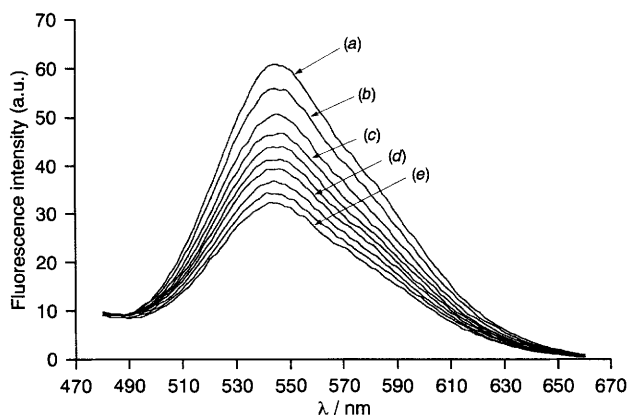
The phenanthridinium derivative **1**, acyclic **2** and cyclic **3–5** bis(phenanthridinium) derivatives showed pronounced differences in their electronic absorption and fluorescence spectra. Comparison of the electronic absorption spectra of **1–5**, taken in an aqueous buffer (sodium cacodylate, pH 6;  $c_1 = 2.4 \times 10^{-5}$  mol dm<sup>-3</sup>,  $c_{2-5} = 1.2 \times 10^{-5}$  mol dm<sup>-3</sup>,  $\lambda_{\text{max}}$  of **1–5** are 273.4, 274.7, 276.0, 272.5 and 276.0 nm, respectively;  $\epsilon_1 = 38\,860$ ,  $\epsilon_2 = 63\,266$ ,  $\epsilon_3 = 36\,781$ ,  $\epsilon_4 = 53\,592$  and  $\epsilon_5 = 46\,233$  dm<sup>3</sup> mol<sup>-1</sup> cm<sup>-1</sup>), shows hypochromicity for **2–5**. The ratios of the molar extinction coefficients of the bis(phenanthridinium) derivatives to monomeric **1** [ $\epsilon_2/\epsilon_1 = 1.62$ ,  $\epsilon_3/\epsilon_1 = 0.95$ ,  $\epsilon_4/\epsilon_1 = 1.37$  and  $\epsilon_5/\epsilon_1 = 1.18$ ] show an exceptionally large hypochromic effect for **3**. The bis(phenanthridinium) derivative **5** with the rigid *p*-xylylene bridge at the phenanthridinium 5,5'-position exhibited a higher molar extinction coefficient than the more flexible **3**. Strong hypochromic effects have been observed for similar charged  $\pi$  systems and explained by  $\pi$ - $\pi$  stacking interactions.<sup>9</sup> The observed hypochromicity for **2–5**, however, cannot be explained solely on the basis of intra- and/or inter-molecular stacking of phenanthridinium units. In the case of intramolecular stacking, the hypochromic effect for the more flexible **2** should be more pronounced than that for the rigid **3** and **5**. On the other hand, intermolecular stacking (dimerization) can be ruled out since each of **1–5** obeyed the Lambert-Beer law in the  $1 \times 10^{-6}$ – $5 \times 10^{-5}$  mol dm<sup>-3</sup> concentration range, as determined experimentally.<sup>10</sup> Nevertheless, some contribution of intramolecular stacking to the overall observed hypochromicity may be assumed on the basis of fluorescence quenching effects observed for the same compounds.



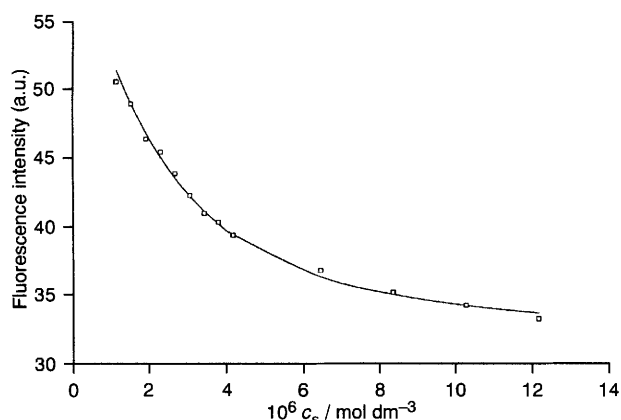
**Table 1** Stability constants  $K_s$  for various 1 : 1 receptor–substrate complexes<sup>a,b</sup>

| Receptor | log $K_s$         |                   |                   |                   |                   |                   |                   |      |
|----------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|------|
|          | ATP <sup>4-</sup> | ADP <sup>3-</sup> | AMP <sup>2-</sup> | GMP <sup>2-</sup> | CMP <sup>2-</sup> | UMP <sup>2-</sup> | TMP <sup>2-</sup> | A    |
| <b>3</b> | 5.80              | 5.65              | 5.38              | 5.52              | 5.84              | 5.52              | 5.53              | 5.62 |
| <b>4</b> | 5.98              | 5.68              | 5.73              | 5.42              | 5.41              | 5.41              | 5.47              | 5.12 |
| <b>5</b> | 5.95              | 5.78              | 5.98              | 5.85              | 6.00              | 5.94              | 5.97              | 5.35 |
| <b>1</b> | 4.94              | 4.46              | 4.63              | 4.60              | 4.73              | 4.45              | 4.21              | 4.76 |
| <b>2</b> | 5.32              | 5.47              | 5.51              | 5.33              | 5.43              | 5.08              | 4.83              | 5.19 |

<sup>a</sup> For experimental conditions see footnote †. <sup>b</sup> A = adenosine; ATP<sup>4-</sup> = adenosine triphosphate; ADP<sup>3-</sup> = adenosine diphosphate; AMP<sup>2-</sup> = adenosine monophosphate; GMP<sup>2-</sup> = guanosine monophosphate; CMP<sup>2-</sup> = cytidine monophosphate; UMP<sup>2-</sup> = uridine monophosphate; TMP<sup>2-</sup> = thymidine monophosphate.



**Fig. 1** Fluorimetric titration of **4** ( $c_4 = 2.22 \times 10^{-6}$  mol dm<sup>-3</sup>) with AMP<sup>2-</sup>.  $c_{AMP}$ : (a) 0; (b)  $3.97 \times 10^{-7}$ ; (c)  $1.96 \times 10^{-6}$ ; (d)  $4.22 \times 10^{-6}$ ; (e)  $1.41 \times 10^{-5}$  mol dm<sup>-3</sup>.



**Fig. 2** Experimental (□) and calculated (—) fluorescence intensities at 544 nm (excitation at 276 nm) of the solution of **4** as a function of AMP<sup>2-</sup> concentration

In the fluorescence emission spectra of **1–5**, taken in an aqueous buffer solution (sodium cacodylate, pH 6;  $c_1 = 2.4 \times 10^{-6}$  mol dm<sup>-3</sup>,  $\lambda_{excit} = 276$  nm,  $\lambda_{emiss} = 536$  nm,  $I_{max} = 600$ ;  $c_{2-5} = 1.2 \times 10^{-6}$  mol dm<sup>-3</sup>; **2**,  $\lambda_{excit} = 276$  nm,  $\lambda_{emiss} = 536$  nm,  $I_{max} = 30$ ; **3**,  $\lambda_{excit} = 277$  nm,  $\lambda_{emiss} = 529$  nm,  $I_{max} = 150$ ; **4**,  $\lambda_{excit} = 281$  nm,  $\lambda_{emiss} = 531$  nm,  $I_{max} = 786$ ; **5**,  $\lambda_{excit} = 281$  nm,  $\lambda_{emiss} = 536$  nm,  $I_{max} = 241$ ), the emission intensity ( $I_{max}$ ) observed for **1** was about 4 times that of **3** and 20 times that of **2**, as a consequence of fluorescence quenching. Since in the  $5 \times 10^{-7}$ – $5 \times 10^{-6}$  mol dm<sup>-3</sup> concentration range the emission intensity for each of **1–5** was found to depend linearly on its concentration, intermolecular stacking (dimerization) as the prime cause of the observed quenching may be excluded. Consequently, the quenching observed should result from intramolecular stacking of the phenanthridinium units in

bis(phenanthridinium) derivatives **2**, **3** and **5**.<sup>11</sup> Here, in contrast to UV hypochromicity effects, the expected dependence of the quenching magnitude on the flexibility of the molecule and, hence, more efficient stacking can be clearly observed (compare  $I_{max}$  for flexible acyclic **2** and the more rigid cyclic molecules **3** and **5**).

The addition of increasing amounts of a nucleotide to aqueous solutions of each of **1–5** resulted in the quenching of their fluorescence emissions. This allowed the determination of the stability constants ( $K_s$ ) of the receptor–nucleotide complexes by fluorimetric titrations<sup>‡</sup> (Figs. 1 and 2). The stability constants are collected in Table 1.

The results of binding studies show that stability constants of  $10^5$ – $10^6$  dm<sup>3</sup> mol<sup>-1</sup> for cyclic receptors **3–5** and various nucleotides are the highest measured to date for receptors which bind only the nucleic base part of a nucleotide. The observed 1 : 1 stoichiometry for complexes of **1**, acyclic **2** and cyclic **3–5**<sup>‡</sup> with nucleotides, and the fact that  $K_s$  values for **1** are more than one order of magnitude lower than those of cyclic receptors **3–5**, are in accordance with sandwich or intercalative types of binding for **2** and **3–5**, respectively, characterized by stacking interactions between the two phenanthridinium units and the inserted nucleic base part of a nucleotide. In support of this the binding was found to be virtually charge independent (compare  $K_s$  for ATP<sup>4-</sup>, ADP<sup>3-</sup> and AMP<sup>2-</sup> Table 1) and the  $K_s$  values for electrically neutral adenosine (A) were of the same order of magnitude as those for the highly charged nucleotides. These results clearly show that stacking interactions between the phenanthridinium units and the nucleic base part of a nucleotide are of prime importance for binding of both charged and neutral substrates.

The present results reveal the superiority of phenanthridinium units for the construction of water-soluble receptors capable of strong binding of both purine and pyrimidine nucleotides by stacking interactions. It can be assumed that synthetic modification of **3–5** by the incorporation of a nucleic base in their structure to serve as the recognition site for the complementary nucleotide would result in receptors possessing strong binding and recognition properties. Synthetic studies along these lines are in progress.

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## Footnotes

<sup>†</sup> The spectroscopic and elemental analysis data for all prepared compounds are in agreement with their structures.

<sup>‡</sup> The measurements were performed at room temp. in aqueous buffer solutions (sodium cacodylate, 0.01 mol dm<sup>-3</sup>, pH 6) at constant ionic strength (sodium sulfate 0.01 mol dm<sup>-3</sup>) and at concentrations of **1–5**

around  $2 \times 10^{-6}$  mol dm<sup>-3</sup>. Under these conditions the fluorescence intensities of 1–5 were proportional to their concentrations. The concentrations of nucleotides were varied from ca.  $10^{-7}$  to ca.  $10^{-5}$  mol dm<sup>-3</sup>. For the complexes of 1:1 stoichiometry the following relation can be derived:

$$I = I_0 + (I_{\text{lim}} - I_0)/2c_0\{c_0 + c_s + 1/K_s - [(c_0 + c_s + 1/K_s)^2 - 4c_0c_s]^{1/2}\}$$

where  $I_0$  and  $I_{\text{lim}}$  denote the fluorescence intensities of free and fully complexed receptor, respectively;  $c_0$  is the concentration of receptor;  $c_s$  is the concentration of nucleotide. The stability constants  $K_s$  and values of  $I_{\text{lim}}$  were determined on the basis of the above relation by a nonlinear least-squares analysis performed by SIMPLEX optimization. In all calculations the substrate concentration range corresponding to ca. 20–80% complexation was used. The correlation coefficient was in all cases > 0.99.

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