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Interactions of novel phenanthridinium–nucleobase conjugates with complementary and non-complementary nucleotides in aqueous media†

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epoc ABSTRACT: A series of novel phenanthridine–nucleobase conjugates were prepared and studied by spectroscopic methods. An analysis of ^{1}H NMR, UV–Vis and fluorescence spectra in aqueous media revealed intramolecular aromatic stacking interactions between the phenanthridinium unit and the nucleobase, due to the conformation of the molecules. Fluorimetric titrations showed that phenanthridinium–nucleobase conjugates **8, 10** and reference phenanthridine compound **12** form 1:1 non-covalent complexes with nucleotides in water with binding constants ranging from 10 to 100 mol^{-1} dm^3. Interestingly, compounds **9** and **11** form intercalative-type 1:1 complexes with the nucleotide aromatic unit inserted between phenanthridinium and covalently attached nucleobase, yielding binding constants of 10^3–10^4 mol^{-1} dm^3. Aromatic $\pi\cdots\pi$ stacking interactions were found to be dominant in complexes with nucleotides of all compounds studied. Copyright © 2002 John Wiley & Sons, Ltd.
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KEYWORDS: phenanthridine–nucleobase conjugates; nucleotides; stacking interactions

INTRODUCTION

Besides the major structure types DNA and RNA, there are some specific structural motifs which, although less frequent, also have an important role in life.[1] Especially RNA, which occurs in biological systems mainly in single-stranded form, tends to form a variety of perturbed double-stranded regions. In both double-stranded DNA and RNA, single-stranded regions can be found that form loops, hairpins, abasic sites, etc.[2] For example, two such single-stranded regions, namely TAR and RRE, control the replication cycle of HIV-1 virus.[3] It is also known that abasic site formation and lesion have important roles in repair mechanisms of DNA.[4] Therefore, the design and synthesis of small synthetic molecules that can specifically interact with single-stranded regions of RNA and DNA are of great importance for understanding and influencing biological functions of nucleic acids.

In the last decade, a few groups have reported on small synthetic molecules consisting of a nucleobase covalently attached to an intercalator. Such compounds were supposed to act as multifunctional receptors, providing hydrogen-bonding recognition between attached nucleobase and complementary nucleotide, additionally stabilized by aromatic stacking interactions with the intercalator unit. So far, such conjugates have been constructed from acridine,[5] phenanthridine,[6] naphthalene diimide[7] or porphyrin[8] intercalator units and mostly adenine or thymine as tethered nucleobase. Surprisingly, with most of the conjugates prepared, only the interactions with complementary polynucleotides were studied. Although significant specific interactions were reported, it should be taken into account that in such rather complicated systems not only is base-pair hydrogen bonding involved, but also electrostatic interactions of positively charged linkers with phosphates and hydrophobic interactions may have an important role.[9] To our knowledge, only one publication has described the study of the simple system consisting of the interaction of acridine–nucleobase conjugate with different nucleobase derivatives, and according to the binding constants, a threefold preference for complementary over non-complementary combination was reported.[5c] However, the authors pointed out that there is no direct evidence of hydrogen bonding between complementary nucleobases.

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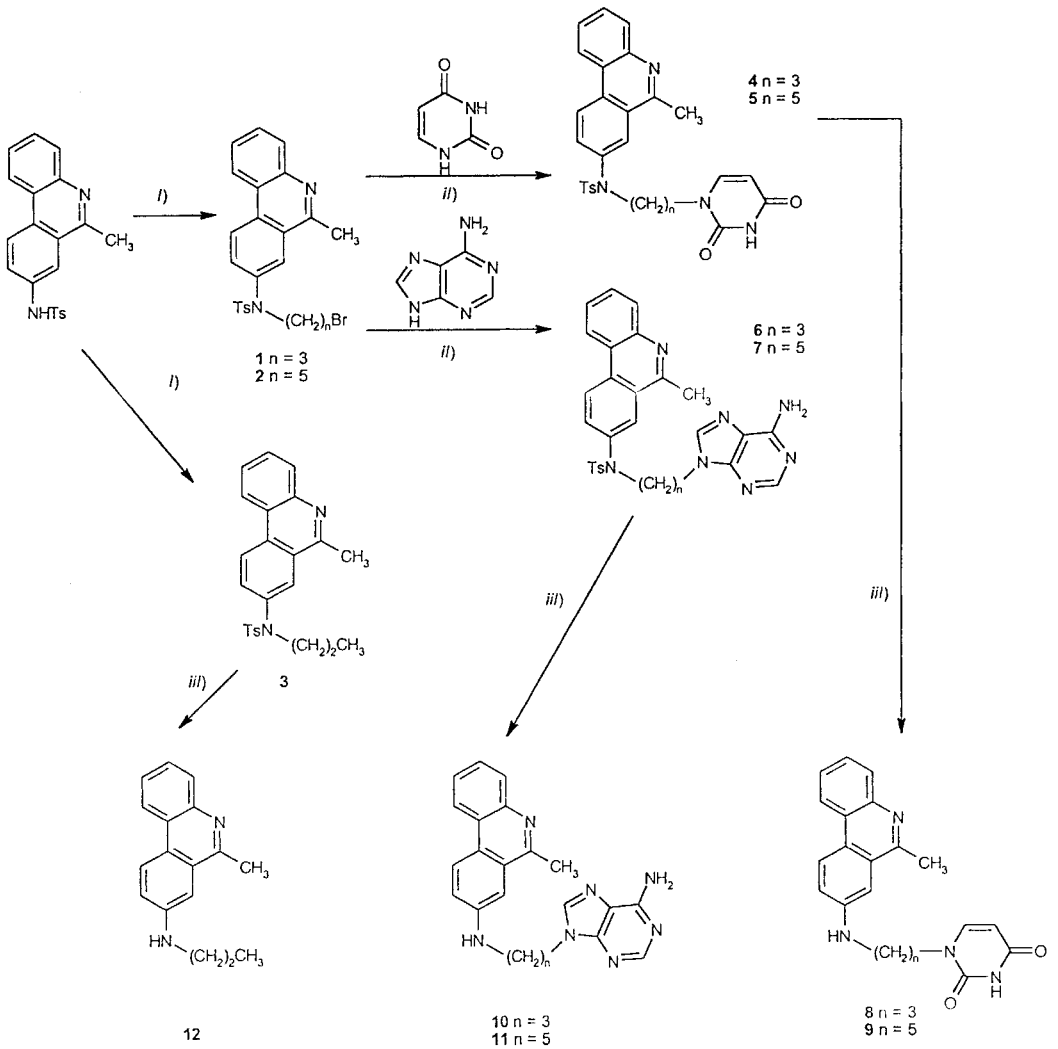


Scheme 1. Synthesis of compounds **8**–**12**. (*i*) $Br(CH_2)_3Br$ or $Br(CH_2)_5Br$ or $Br(CH_2)_2CH_3$, K_2CO_3, dry DMF, Ar, r.t.; (*ii*) NaH, dry DMF, Ar, 40–50 °C; (*iii*) (1) 2:1 CH_3COOH–H_2SO_4, 80–100 °C, (2) NaOH–H_2O

To shed more light on the importance of hydrogen bonding recognition vs aromatic $\pi\cdots\pi$ stacking, we prepared a series of novel phenanthridinium–adenine and uracil conjugates differing in the length of the aliphatic linker and studied their interactions with nucleotides in aqueous media.

RESULTS

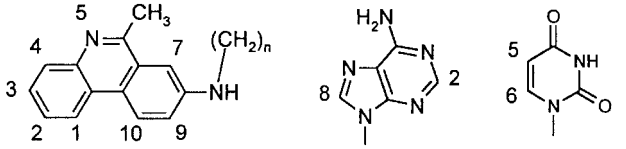
Synthesis

The general strategy used for the synthesis of the conjugate molecules 8–11 comprises the alkylation of the phenanthridine derivative, by dibromoalkanes of appropriate lengths, followed by the introduction of nucleobase at the other end of the alkyl tether. Compound 12 and also **Ade-C_3** [9-(*n*-propyl)adenine] and **Ura-C_3** [1-(*n*-propyl)uracil][10] were prepared for comparison purposes.

8-Tosylamino-6-methylphenanthridine[11] was prepared in four steps, starting from commercially available 2-aminobiphenyl, by the Morgan–Walls reaction[12] based on the middle pyridine ring formation by intramolecular electrophilic cyclization of the 2-amidobiphenyl derivative using $POCl_3$.

As outlined in Scheme 1, 8-tosylamino-6-methylphenanthridine was alkylated using a large excess of mono- or dibromoalkane in dry DMF in the presence of potassium carbonate, to afford protected alkylaminophenanthridines **1**–**3** (60–88% yields). The reaction of bromo derivatives **1** and **2** with a large excess of adenine or uracil was performed under an argon atmosphere at 40–50 °C in dry DMF in the presence of NaH giving compounds **4**–**7** (40–90% yields). Under these conditions, the alkylation of uracil selectively occurred at the

Table 1. Chemical shifts[a] δ_0 (ppm) of aromatic protons extrapolated to zero concentration for the conjugates **8–11** and reference compounds **12**, **Ade-C$_3$** and **Ura-C$_3$**



	8	9	10	11	12	Ade-C$_3$	Ura-C$_3$
H-1	8.52	8.59	8.55	8.6	8.66		
H-2	7.75	7.81	7.82	7.85	7.81		
H-3	7.71	7.76	7.77	7.79	7.79		
H-4	7.91	7.95	8.01	8.01	7.96		
H-7	7.16	7.34	6.94	7.12	7.68		
H-9	7.48	7.6	7.26	7.43	7.53		
H-10	8.5	8.58	8.38	8.49	8.66		
Ade-H2			8.29	8.18		8.33	
Ade-H8			7.95	8.0		8.27	
Ura-H5	5.56	5.42					5.73
Ura-H6	7.52	7.44					7.55

[a] Calculated using concentration dependence data (2.0×10^{-4}–1.0×10^{-3} mol dm^{-3} for all compounds); 1.0×10^{-3} mol dm^{-3} DCl in D_2O, pD = 3, T = 21 °C.

N-1 position and adenine at the N-9 position. Removal of tosyl protection from **3–7** was achieved by heating at 100 °C under acidic conditions. Compounds **8–12** were obtained in 40–93% yields. They were found to be sufficiently soluble in water under acidic conditions to enable studies of their interactions with nucleotides in aqueous media.

Spectroscopic properties of 8–12 in aqueous media

^{1}H NMR spectroscopy was used to study possible intramolecular stacking interactions in phenanthridinium–nucleobase conjugates **8–11** in aqueous media. Folded conformations of conjugates were detected by comparing the chemical shifts of conjugates with those of reference compounds (**12, Ura-C3** and **Ade-C3**), resulting in upfield-shifted proton signals of the intramolecularly stacked molecules.

Table 2. Chemical shift differences $\Delta\delta_0$ (ppm) for the conjugates **8–11**[a]

	8	9	10	11
H-1	0.14	0.07	0.11	0.06
H-2	0.06	0	−0.01	−0.04
H-3	0.08	0.03	0.02	0
H-4	0.05	0.01	−0.05	−0.05
H-7	0.52	0.34	0.74	0.56
H-9	0.05	−0.07	0.27	0.1
H-10	0.16	0.08	0.28	0.17
Ade-H2			0.04	0.15
Ade-H8			0.32	0.27
Ura-H5	0.17	0.31		
Ura-H6	0.03	0.11		

[a] $\Delta\delta_0 = \delta_0$ (reference compound **12**, **Ade-C$_3$** or **Ura-C$_3$**) $-\delta_0$ (conjugate; values extrapolated to zero concentration, Table 1); T = 21 °C.

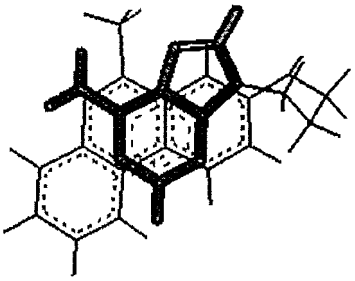


Figure 1. Intramolecularly stacked conformation of **10** obtained by molecular modelling

^{1}H NMR spectra of compounds **8–12** (for numbering, see Table 1) and references **Ade-C$_3$** and **Ura-C$_3$** were recorded at 500 MHz in water (D_2O, DCl, pD 3) under acidic conditions to increase the solubility. Chemical shifts (δ ppm) of aromatic protons of **8–12** were found to be concentration dependent ($c = 2.0 \times 10^{-4}$–1.0×10^{-3} mol dm^{-3}) due to self-stacking interactions. Insolubility of the compounds at higher concentrations prevented the accumulation of enough NMR data to allow exact calculation of K_a. However, according to significant changes in chemical shifts at c(compounds) = 10^{-4}–10^{-3} mol dm^{-3} it was possible to estimate the order of magnitude for $K_a < 10^3$, similar to previously reported data for ethidium bromide (K_a = 180 M^{-1}).[13] This estimate is in accordance with UV–Vis experiments (see below).

By extrapolating the dependence of δ on concentration (**8–12**) to infinite dilution, proton shifts δ_0 were obtained where no intermolecular self-association is present[14] (Table 1). These chemical shifts δ_0 of **8–11** were compared with those of reference compounds **12, Ade-C$_3$** and **Ura-C$_3$**. The experimental shielding effects [$\Delta\delta_0 = \delta_0$ (reference) $-\delta_0$ (conjugate)] obtained in this way can be related to the degree of self-stacking[5e] (Table 2).

The strongest shielding effects were observed for phenanthridine proton H-7 of phenanthridine–adenine conjugates **10** and **11**. Similar but less pronounced effects were observed for some other proton signals also, especially protons H-9 and H-10 and for the Ade-H8 proton. This effect is more noticeable for compound **10** possessing a shorter aliphatic chain between intercalator and nucleobase. Similar effects can be observed also for phenanthridine–uracil conjugates **8** and **9**. Such shielding effects can be explained by intramolecular aromatic $\pi\cdots\pi$ interactions between the phenanthridinium unit and covalently attached nucleobase. This is also supported by

Table 3. Electronic absorption spectra and fluorescence emission intensities of compounds **8–12** and reference compounds[a]

Compound	UV–Vis		Fluorescence ($\lambda_{exc} = 275 \pm 2$ nm)	
	λ_{max} (nm)	ε *(mol^{-1} cm^2)	λ_{em}(nm)	EI_i/EI_{12}
8	275	34.2	535	1.8
9	275	34.6	541	1.2
10	270	13.4	538	1.9
11	268	20.1	545	1.3
12	277	29.3	547	1
Ura-C_3	268	9.8		
Ade-C_3	262	13.7		

[a] Sodium cacodylate buffer ($c = 0.05$ mol dm^{-3}, pH = 5), $c = 2.0 \times 10^{-5}$ mol dm^{-3} for all compounds.

molecular modelling results (Fig. 1). Detailed two-dimensional ^{1}H NMR analysis, which could prove the stacking interactions, was hampered by the low solubility of the compounds which aggregated at concentrations higher than 10^{-3} mol dm^{-3}.

UV–Vis spectra of **8–12** are strongly pH dependent, exhibiting a one-step change at p$K \approx 6$, attributed to protonation of the phenanthridine nitrogen. Owing to the low solubility of phenanthridine at pH 7, UV–Vis experiments were carried out at pH 5, all of the compounds being in a phenanthridinium form (Table 3). The absorbances of compounds **8–12** obey the Lambert–Beer law in the concentration range 1×10^{-6}–4×10^{-5} mol dm^{-3}.

The absorption maxima of **8–11** are blue shifted compared with **12**. The comparison of the UV–Vis spectra of **10, 11** and the corresponding references **12** and **Ade-C_3** (Table 3) reveals a hypochromic effect, strongly supporting an intramolecular stacking interaction between phenanthridinium and adenine. These effects are more pronounced for **10**, possessing a shorter flexible chain. The molar absorptivities (ε, Table 3) of **8** and **9** are smaller than sum of ε of **12** and **Ura-C_3**, suggesting an influence of intramolecular interactions.

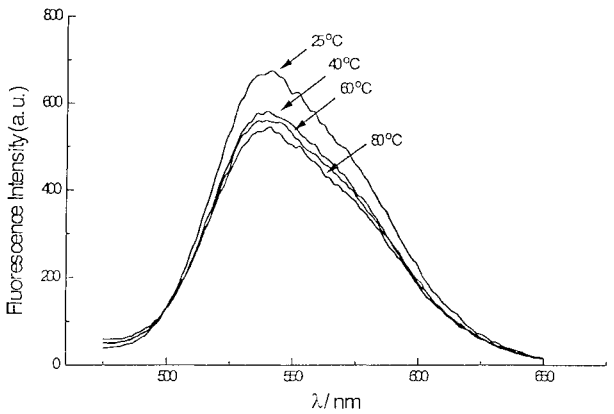


Figure 2. Fluorimetric spectra of **10** ($c = 1.83 \times 10^{-6}$ mol dm^{-3}, Na cacodylate buffer, pH = 5, $\lambda_{exc} = 270$ nm) measured at different temperatures

The assumption that phenanthridinium and nucleobase interact intramolecularly is additionally supported by the fluorimetric study. The fluorescence emissions of compounds **8–11** are significantly higher than for the reference compound **12** (Table 3). Since the complexation of nucleotides by ethidium bromide and also **12** results in an increase in their emission, the former observation suggests intramolecular phenanthridine–nucleobase stacking in **8–11**. In accordance with this, the temperature increase of **8–11** solutions results in quenching of their emission due to the unstacking of intramolecular complexes. However, with the same temperature increase virtually no change in the emission spectra of **12** could be observed. Enhancement of fluorescence was also observed upon addition of D_2O to aqueous solutions of **8–12**, confirming a similar fluorescence quenching mechanism to that described for ethidium bromide[15]. Olmsted and Kearns[15] showed that the emission is quenched by amino proton transfer to water molecules at the excited state of ethidium bromide. Consequently, the observed higher emission of **8–11** compared with that of **12** can be explained by phenanthridinium–nucleobase intramolecular stacking. Apparently, such stacking leads to a decrease in the amino proton transfer rate and therefore has the same effect on the emission intensity of **8–11** as the addition of D_2O.

It is interesting that the spectroscopic effects observed by both UV–Vis and fluorescence measurements, assigned to intramolecular stacking are more pronounced for compounds with shorter a aliphatic linker (**8** and **10**), as also observed in the NMR study. This suggests that the trimethylene linker is better than the pentamethylene linker for intramolecular stacking interactions in these systems.

Interactions with nucleotides

The addition of nucleotides to aqueous solutions of the

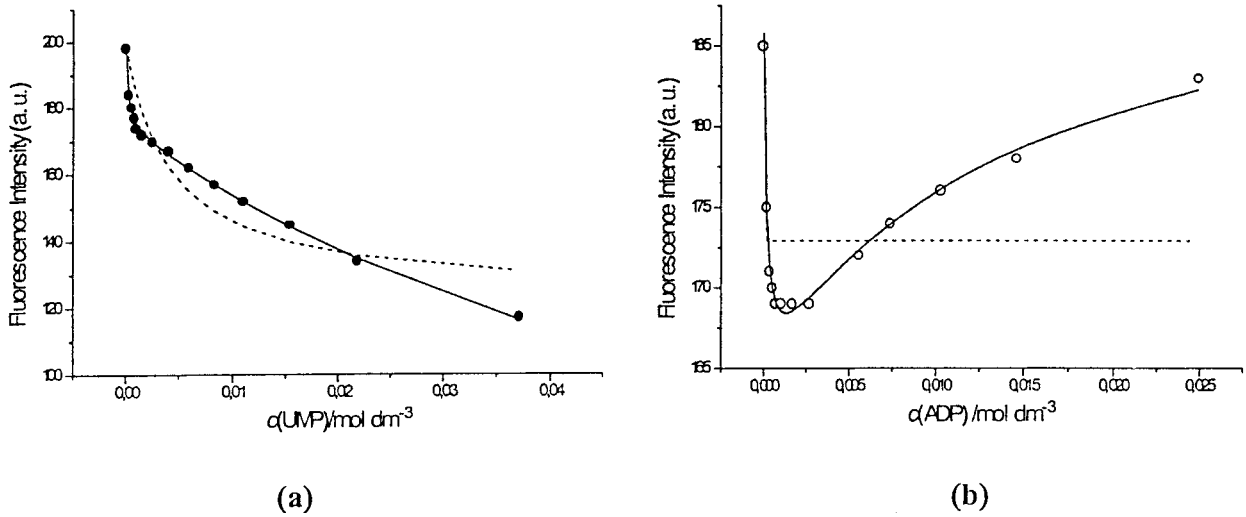


Figure 3. (a) Experimental (●) and calculated data (λ_{exc} = 460 nm, λ_{em} = 553 nm) of **11** obtained for a model of 1:1 (dashed line) and a model of 1:1 + 1:2 (solid line) stoichiometry as a function of UMP^{2-} concentration. (b) Experimental (○) and calculated fluorescence intensities (λ_{exc} = 460 nm, λ_{em} = 548 nm) of **11** obtained for a for a model of 1:1 (dashed line) and a model of 1:1 + 1:2 (solid line) stoichiometry as a function of ADP^{3-} concentration

conjugates **8–12** induces significant changes in their fluorescence emission (Fig. 3), allowing the determination of the binding constants (K_S) and stoichiometries of the conjugate–nucleotide complexes (Table 4). Processing the fluorescence titration data for **8, 10** and **12** with various nucleotides gives the best fit for the 1:1 stoichiometry of complexes and binding constants (K_S) of the same order of magnitude as those found for ethidium bromide.[16,17] No significant differences in affinity were observed for complexes of **8** and **10** with complementary nucleotides. Also, no charge dependence was observed in binding of the AMP^{2-}–ADP^{3-}–ATP^{4-} series, indicating a dominant role of stacking interactions between the phenanthridinium unit of the conjugate and the base of the nucleotide in the complex.

Analysis of the titration data for **9** and **11** with nucleotides (Fig. 3) gives the best fit for the formation of 1:1 and 1:2 stoichiometry complexes (L:N; L = ligand, N = nucleotide). The binding constants of 1:1 complexes (Table 4; K_1) are more than one order of magnitude higher than those of ligands **8, 10** and **12**, and also those measured for ethidium bromide.[16] Since the pentamethylene linker between phenanthridinium and attached base in **9** and **11** allows accommodation of the nucleotide base between them, the significant affinity increase of **9** and **11** can be explained by the formation of an intercalative type of complex [Fig. 4(a)]. This is in line with the observed equal affinity towards pyrimidine and purine nucleotides reported earlier.[18] The complex of 1:2 stoichiometry is formed at higher excess of nucleotides, with a second molecule of nucleotide stacking on the 1:1 complex, probably on the 'free' side of the phenanthridinium unit

Table 4. Stability constants ($\log K_i$) for various ligand–substrate complexes[a,b]

	$\log K_1$, $\log K_2$[c]				
	8	**9**	**10**	**11**	**12**
AMP^{2-}	1.84,–[d]	4.18, 1.99	1.66,–[d]	3.78, 2.02	1.73,–[d]
ADP^{3-}	1.87,–[d]	–[d]	2.58,–[d]	3.79, 1.84	1.78,–[d]
ATP^{4-}	–[d]	–[d]	2.79,–[d]	2.29,–[d]	2.29,–[d]
GMP^{2-}	2.05,–[d]	3.82, 1.66	1.72,–[d]	3.91, 1.03	1.72,–[d]
CMP^{2-}	1.45,–[d]	3.63, 1.36	1.58,–[d]	4.25, 1.06	1.93,–[d]
UMP^{2-}	1.50,–[d]	3.84, 1.32	1.47,–[d]	3.7, 1.1	1.59,–[d]
TMP^{2-}	1.29,–[d]	3.91, 1.3	1.54,–[d]	4.01,<1	1.34,–[d]

[a] For experimental conditions, see Spectroscopic measurements.
[b] AMP^{2-} = adenosine monophosphate; ADP^{3-} = adenosine diphosphate; ATP^{4-} = adenosine triphosphate; GMP^{2-} = guanosine monophosphate; CMP^{2-} = cytidine monophosphate; UMP^{2-} = uridine monophosphate; TMP^{2-} = thymidine monophosphate.
[c] K_1 and K_2 refer to the equilibria $L + N \rightleftharpoons LN$ and $LN + N \rightleftharpoons LN_2$ (L = ligand, N = nucleotide), respectively.
[d] Small spectroscopic changes hampered the determination of K_S.

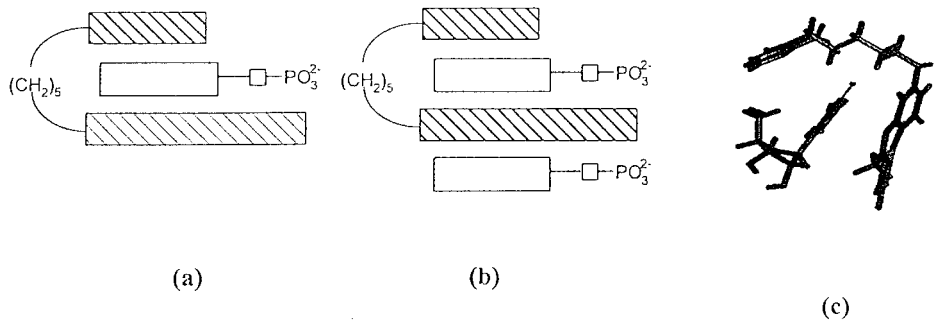


Figure 4. Schematic representation of (a) 1:1 stoichiometry complex and (b) 1:2 stoichiometry complex of compounds **9** and **11** with nucleotides. (c) Side view of minimized structure of **11**–uridine complex obtained by molecular modelling

[Fig. 4(b)]. Binding constant K_2 (equilibrium 1:1 complex of **9** and **11** + nucleotide $\rightleftharpoons$ 1:2 complex) is of the same order of magnitude as found for binding constants K_1 for **12**:nucleotide complexes of 1:1 stoichiometry. The proposed intercalative complex of 1:1 stoichiometry (for **9** and **11**) is supported by computer molecular modelling[19] [Fig. 4(c)]. Again, for **9** and **11** no preferential binding to complementary nucleotides was observed.

Molecular modelling

Molecular modelling studies were conducted using Sybyl software.[15] Compounds **8–11** were constructed using the Builder package. Conformational space was examined by simulated annealing as a type of molecular dynamics experiment. The number of cycles to run was 20 and the initial temperature for annealing was 700 K. The system was kept at this temperature for 1000 fs, then the temperature was lowered during 1000 fs until 50 K was reached. The annealing function (temperature vs time) was exponential. The resulting *syn* conformation was selected from 20 low-energy conformations and then energy optimized using 1000 steps of Powel minimization until the energy gradient converged at 0.05 kcal mol^{-1} (1 kcal = 4.184 kJ). Atomic partial charges of **11** and uridine were computed by the Gasteiger–Hückel method. Calculations with uridine-5′-monophosphate were not possible owing to a lack of parameterization for the phosphate group. The complex of **11** and uridine was produced by docking of uridine between phenanthridine unit and adenine unit until an energy minimum was found. The structure of complex was then fully energy minimized.

CONCLUSIONS

A series of novel phenanthridinium–nucleobase conjugates were prepared, differing in the nucleobase and the length of the methylene linker. Spectroscopic studies of their aqueous solutions revealed different intra- and intermolecular interactions. According to ^{1}H NMR and UV–Vis experiments, all compounds form intermolecular dimeric associates with binding constants similar to those reported for other phenanthridinium derivatives. Significant differences observed in the ^{1}H NMR, UV–Vis and fluorescence spectra of the conjugates **8–11** compared with reference compound **12** suggest intramolecular aromatic stacking interactions between the nucleobase and the phenanthridinium, supported also by molecular modelling results. This leads to the conclusion that hydrophobic and aromatic stacking interactions force the flexible conjugates to fold into intramolecularly stacked conformations.

The fluorescence titration results showed that **8–12** bind nucleotides efficiently in aqueous media. The increased affinity of **9** and **11** toward nucleotides compared with **8** and **10** is due to the different linker length, the former conjugates with pentamethylene linker forming more stable intercalative type of complexes than the latter. In contrast with Lhomme's proflavin–nucleobase conjugates,[5] we did not observe any significant preference of conjugates **8–11** towards complementary nucleotides. It should be noted that the relatively high buffer concentration (ionic strength) weakens the ion pairing and that the water molecules compete strongly with hydrogen bonding between nucleobases. Therefore, such conjugates would be more promising as selective ligands in the more lipophilic microenvironment found in the complementary single-stranded regions of DNA and RNA.

EXPERIMENTAL

General procedures

^{1}H NMR spectra were recorded on a Varian Gemini 300 operating at 300 MHz and on a Bruker Avance DRX 500 equipped with a 5 mm diameter inverse detection probe,

operating at 500 MHz. In ^{1}H NMR experiments the spectral width was 0.5 Hz and the number of data points was 65K. The presaturation technique was used for water signal suppression. Tetramethylsilane was used as internal standard for organic solvents and cacodylate buffer as internal standard for aqueous media. Chemical shifts (δ) are expressed in ppm and coupling constants (J) in hertz. Signal multiplicities are denoted s (singlet), d (doublet), t (triplet), q (quartet) and m (multiplet). The electronic absorption spectra were obtained on a Pye Unicam SP 8700 spectrometer. IR spectra were recorded on a Perkin-Elmer Model 297 instrument using KBr pellets. Fluorescence spectra were recorded on a Perkin-Elmer LS 50 fluorimeter. Mass spectra were obtained using an Extrel 2001 DD spectrometer and electrospray mass spectrometry (ESMS) was performed using a Varian MAT 711 spectrometer. Preparative thin-layer chromatography (TLC) was carried out using Kieselgel HF_{254} (Merck). Melting-points were determined on a Kofler apparatus and are uncorrected. 8-Tosylamino-6-methylphenanthridine was prepared starting from 4′-nitro-2-aminobiphenyl, as described previously.[11,20] For all products, purity was ascertained by ^{1}H NMR and elemental analysis. In several cases, correct elemental analyses could not be obtained owing to the polar and hygroscopic character of the compounds. Spectroscopic data (^{1}H NMR, IR, ESMS) are available as supplementary material at the epoc website at http://www.wiley.com/epoc.

Compounds

8-(3-Bromopropyltosyl)amino-6-methylphenanthridine (1). 1,3-Dibromopropane (6.8 ml, 66 mmol) and K_2CO_3 (7.6 g, 55 mmol) were suspended in dry DMF (40 ml). To this suspension, a solution of 8-tosylamino-6-methylphenanthridine (2 g, 5.5 mmol) in dry DMF (80 ml) was added dropwise during 30 min and the reaction mixture was stirred for 48 h under an argon atmosphere at room temperature. Water and CH_2Cl_2 were then added to this suspension. The water layer was washed twice with CH_2Cl_2 and the organic extracts were dried over Na_2SO_4 and evaporated, yielding a brown oil to which water was added to give a light grey precipitate of **1** (1.7 g, 64%). The precipitate was filtered, washed with water and used without further purification. Pure compound **1** was obtained by TLC (SiO_2, 2% MeOH in CH_2Cl_2, $R_f = 0.37$) as a white solid, additionally recrystallized from MeOH. Anal. Calcd for $C_{24}H_{23}N_2O_2BrS$ ($M_r = 483.42$): C 59.63, H 4.80, N 5.79. Found: C 59.76, H 4.84, N 5.87%.

8-(5-Bromopentyltosyl)amino-6-methylphenanthridine (2). K_2CO_3 (7.6 g. 55 mmol), 1,5-dibromopentane (9 ml, 66 mmol) and 8-tosylamino-6-methylphenanthridine (2 g, 5.5mmol) in dry DMF (40 + 80 ml) under the same reaction conditions as for **1** gave **2** (1.7 g, 60%) as a white powder. TLC (SiO_2, 2% MeOH in CH_2Cl_2), $R_f = 0.33$. Anal. Calcd for $C_{26}H_{27}N_2O_2SBr$ ($M_r = 511.47$): C 61.05, H 5.32, N 5.48. Found: C 61.21, H 5.05, N 5.30%.

8-(Propyltosyl)amino-6-methylphenanthridine (3). Under the same reaction conditions as for **1** K_2CO_3 (1.53 g, 11.05 mmol), 1-bromopropane (1.2 ml, 11.05 mmol) and 8-tosylamino-6-methylphenanthridine (400 mg, 1.105 mmol) in dry DMF (5 + 15 ml) gave **3** (392 mg, 88%) as a white powder. TLC (SiO_2, 5% MeOH in CH_2Cl_2), $R_f = 0.62$. Anal. Calcd for $C_{24}H_{24}N_2O_2S$ ($M_r = 404.51$): C 71.26, H 5.98, N 6.92. Found: C 71.45, H 6.22, N 6.70%.

8-[3-(Urac-1-yl)propyltosyl)]amino-6-methylphenanthridine (4). Uracil (4.64 g, 41 mmol) that had previously been dried and NaH [1.65 g, 60% (w/w), 41 mmol] were suspended in dry DMF (50 ml) and stirred for 1 h under an argon atmosphere at room temperature. To this suspension, a solution of **1** (2 g, 4.14 mmol) in dry DMF (70 ml) was added dropwise and the reaction mixture was stirred for 48 h under an argon atmosphere at 40–50 °C. Water and CH_2Cl_2 were then added to this suspension. The water layer was washed twice with CH_2Cl_2 and the organic extracts were dried over Na_2SO_4 and evaporated, yielding a brown oil to which water was added to give a light grey precipitate of **4** (1.85 g, 87%). The precipitate was filtered, washed with water and used without further purification. Pure compound **4** was obtained by TLC (SiO_2, 10% MeOH in CH_2Cl_2, $R_f = 0.61$) as a white solid, additionally recrystallized from MeOH. Anal. Calcd for $C_{28}H_{26}N_4O_4S$ ($M_r = 514.60$): C 65.35, H 5.04, N 10.89. Found: C 65.17, H 5.25, N 10.80%.

8-[3-(Urac-1-yl)pentyltosyl)]amino-6-methylphenanthridine (5). Compound **5** was obtained as described for **4**; uracil (1.8 g, 16 mmol), NaH [1.1 g, 60% (w/w), 16 mmol] and **2** (850 mg, 1.6 mmol) in dry DMF (10 + 30 ml) gave **5** as a white powder (350 mg, 39%). TLC (SiO_2, 10% MeOH in CH_2Cl_2), $R_f = 0.65$. Anal. Calcd for $C_{30}H_{30}N_4O_4S$ ($M_r = 542.66$): C 66.40, H 5.57, N 10.32. Found: C 66.63, H 5.40, N 10.29%.

8-[3-(Aden-9-yl)propyltosyl)]amino-6-methylphenanthridine (6). Compound **6** was obtained as described for **4**; adenine (840 mg, 6.2 mmol), NaH [250 mg, 60% (w/w), 6.2 mmol] and **1** (300 mg, 0.62 mmol) in dry DMF (7 + 10 ml) gave **6** as a white powder (300 mg, 90%). TLC (SiO_2, 10% MeOH in CH_2Cl_2), $R_f = 0.39$. Anal. Calcd for $C_{29}H_{26}N_7O_2S$ ($M_r = 536.62$): C 64.90, H 4.88, N 18.27. Found: C 64.55, H 5.04, N 18.09%.

8-[3-(Aden-9-yl)pentyltosyl)]amino-6-methylphenanthridine (7). Compound **7** was obtained as described for **4**; adenine (2.25 g, 16 mmol), NaH [1.1 g, 60% (w/w),

16 mmol] and **2** (850 mg, 1.6 mmol) in dry DMF (10 + 30 ml) gave **7** as a white powder (320 mg, 34%). TLC (SiO_2, 10% MeOH in CH_2Cl_2), $R_f = 0.43$. Anal. Calcd for $C_{31}H_{31}N_7O_2S$ ($M_r = 565.7$): C 65.82, H 5.52, N 17.33. Found: C 66.06, H 5.69, N 17.45%.

8-[3-(Urac-1-yl)propy)]amino-6-methylphenanthridine (8). Compound **4** (227 mg, 0.44 mmol) was dissolved in a mixture of 2 ml of concentrated H_2SO_4 and 4 ml of concentrated acetic acid and heated under reflux at 80–100 °C for 2 h. The reaction mixture was cooled, poured on ice and made alkaline (pH = 8–9) by addition of 2 M NaOH. Water was evaporated and the residue was extracted for 3 days by Soxlet extraction using ethyl acetate. The solvent was evaporated to give a smaller volume, and 2 g of SiO_2 were suspended in this solution. The suspension was purified by column chromatography (SiO_2, 10% MeOH in CH_2Cl_2). Compound **8** was obtained as a yellow powder (113 mg, 70%), additionally recrystallized from MeOH. TLC (SiO_2, 10% MeOH in CH_2Cl_2), $R_f = 0.53$. Anal. Calcd for $C_{21}H_{20}N_4O_4$ ($M_r = 360.42$): C 69.98, H 5.59, N 15.55. Found: C 69.79, H 5.78, N 15.44%.

8-[3-(Urac-1-yl)pentyl)]amino-6-methylphenanthridine (9). Compound **5** (300 mg, 0.53 mmol) was dissolved in a mixture of 1 ml of concentrated H_2SO_4 and 2 ml of concentrated acetic acid and heated under reflux at 80–100 °C for 2 h. The reaction mixture was cooled, poured on ice and made alkaline (pH = 8–9) by addition of 2 M NaOH. Water was evaporated to gave a smaller volume and the solution was extracted several times with CH_2Cl_2–MeOH (9:1). The organic extracts were dried over Na_2SO_4 and evaporatied to afford **9** as a yellow powder (100 mg, 46%). Pure **9** was obtained by TLC (SiO_2, 10% MeOH in CH_2Cl_2, $R_f = 0.58$) as a yellow powder, additionally recrystallized from MeOH and water. ESMS: *m/z* 389.4.1 ($M^+ + 1$, protonated form).

8-[3-(Aden-9-yl)propyl)]amino-6-methylphenanthridine (10). Compound **6** (210 mg, 0.39 mmol) was dissolved in a mixture of 1.5 ml of concentrated H_2SO_4 and 3 ml of concentrated acetic acid and heated under reflux at 80–100 °C for 2 h. The reaction mixture was cooled, poured on ice and made alkaline (pH = 8–9) by addition of 2 M NaOH. The yellow solid obtained was precipitated, filtered and washed with copious amounts of water to afford pure **10** (138 mg, 93%). Compound **10** was further purified by TLC (SiO_2, 10% MeOH in CH_2Cl_2, $R_f = 0.38$) and additionally recrystallized from MeOH and a small amount of CH_2Cl_2. Anal. Calcd for $C_{22}H_{21}N_7$ ($M_r = 383.46$): C 68.91, H 5.52, N 25.57. Found: C 68.75, H 5.32, N 25.33%.

8-[3-(Aden-9-yl)pentyl)]amino-6-methylphenanthridine (11). Compound **11** was obtained as described for **10; 7** (200 mg, 0.35 mmol) in 1.5 ml of concentrated H_2SO_4 and 3 ml of concentrated acetic acid gave a yellow powder that was purified by TLC (SiO_2, 10% MeOH in CH_2Cl_2, $R_f = 0.34$) to give **11** (60 mg, 41%), which was additionally recrystallized from MeOH and a small amount of water. Anal. Calcd for $C_{24}H_{25}N_7$ ($M_r = 411.51$): C 70.05, H 6.12, N 23.83. Found: C 70.30, H 6.00, N 23.96%.

8-(Propyl)amino-6-methylphenanthridine (12). Compound **12** was obtained as described for **10; 3** (150 mg, 0.37 mmol) in 5 ml of concentrated H_2SO_4 gave pure **12** as a yellow powder (74 mg, 79%). Compound **12** was further purified by TLC (SiO_2, 10% MeOH in CH_2Cl_2, R_f, = 0.71) and recrystallized from MeOH. Anal. Calcd for $C_{17}H_{18}N_2$ ($M_r = 250.33$): C 81.56, H 7.15, N 11.19. Found: C 81.78, H 7.01, N 11.16%.

UV–Vis and fluorescence measurements

The measurements were performed in aqueous buffer solution (sodium cacodylate, 0.05 mol dm^{-3}, pH 5) at constant ionic strength (sodium chloride, 0.1 mol dm^{-3}). At this pH value, the phenanthridinium system is predominantly protonated. Under the experimental conditions used the fluorescence emission intensities of **8–12** were proportional to their concentrations. The concentrations of the compounds were kept constant (around 2.5×10^{-6} mol dm^{-3}) during titration with solutions of the nucleotides. The concentrations of nucleotides varied from 7×10^{-5} to 2×10^{-2} mol dm^{-3}. The excitation wavelength used was >330 nm, where the absorbance of all nucleotides is negligible. Binding constants and stoichiometries of the complexes formed were calculated from the concentration range corresponding to ca 20–80% complexation (for 1:1 stoichiometry) by a non-linear least-squares fitting program.[21]

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