4,9-Diazapyrenium cations. Synthesis, physico-chemical properties and binding of nucleotides in water

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A series of new mono- and di-cationic 4,9-diazapyrenium derivatives were synthesized, for which *in vitro* anticancer activity has been demonstrated already. Their spectroscopic (NMR, electronic absorption and fluorescence) properties and the influence of positive charges, aromatic surface and substituents on the formation of stacked complexes with major nucleotides in water (this article) and on their interaction with nucleic acids (following article in this issue) was investigated. A reversible pH dependent formation of 5-hydroxy-4,9-diazapyrenium mono-pseudobase (DMOH) was observed for mono- and di-cationic 4,9-diazapyrenium derivatives in aqueous solution. The equilibrium constants, expressed as pK_{DMOH}, were determined. Binding of nucleotides (AMP, ADP, ATP, GMP and CMP) was studied in buffered (pH 5) aqueous solution by fluorescence. The formation of stacked nucleic base–diazapyrenium complexes with 1 : 1 stoichiometry (log K_s 1.6–2.8) was observed. The stability constants were found to be independent of nucleotide charge, showing the dominance of aromatic stacking over coulombic interactions in such complexes. The presence of 6-phenyl and 5,10-diphenyl substituents on the phenanthridinium and 4,9-diazapyrenium systems, respectively, diminish nucleotide binding in both cases. The larger aromatic surface of the 4,9-diazapyrenium system, relative to the phenanthridinium (log K_s <1 to 2.3), does not considerably enhance nucleotide binding by the former. The nucleotide binding is somewhat stronger compared to neutral pyrene (log K_s 1.1–1.7) possessing a comparable aromatic surface. Singly charged 4,9-diazapyrenium derivatives bind nucleotides with a strength comparable to doubly charged 2,7-diazapyrenium derivatives. Some diazapyrenium derivatives exhibit high AMP/CMP selectivity (**13**, 11.7 and **15**, 9.3) and moderate GMP/CMP selectivity (**13**, 6.0 and **12**, 5.5). The observed selectivities are considerably higher than those exhibited by pyrene, ethidium or 2,7-diazapyrenium.

Introduction

Interactions between aromatic π systems play a significant role in molecular recognition events in biology as well as in the structural organization of biomolecules such as proteins and nucleic acids.[1] In chemistry, the same type of interactions are frequently found as dominant or supplemental binding forces determining the packing mode of aromatic molecules in crystalline state[2] or in various types of synthetic supramolecular complexes,[3] especially those formed between receptors containing aromatic units and aromatic guests.[4–7] The well known phenomenon of double stranded (ds)-DNA intercalation, involving the insertion of an aromatic compound between vicinal base pairs may be considered paradigmatic for aromatic π–π interactions.[8] This phenomenon is also of paramount interest for a rational approach to the design of synthetic drugs acting on the DNA level. Numerous synthetic and natural aromatic or heteroaromatic compounds have been shown to strongly bind to ds-DNA, forming π–π complexes with nucleic bases.[9]

For some 4,9-diazapyrenium derivatives a pronounced *in vitro* anticancer activity has been recently reported (see ref. 13). In the context of the observed biological activity and their apparent structural resemblance to classical pyrene, ethidium bromide and 2,7-diazapyrenium nucleic acid intercalators a detailed study of the 4,9-diazapyrenium system seems to be of high interest. The positively charged acridinium and phenanthridinium derivatives proflavine (**PF**) and ethidium bromide † (**EB**) (Chart 1), are among the most extensively studied DNA intercalators.[8,9] Recently, the novel intercalator 2,7-diazapyrenium dication (Chart 1) has been described, possessing a very large aromatic surface and a double positive charge on the aromatic system.[10] This compound, combining molecular features of pyrene, methylviologen and heteroaromatic nucleic acid intercalators, exhibited exciting properties such as strong ds-DNA intercalation and visible light induced DNA cleavage, while in aqueous media the binding of nucleotides by aromatic π–π stacking interactions with nucleic bases was observed.[10,11]

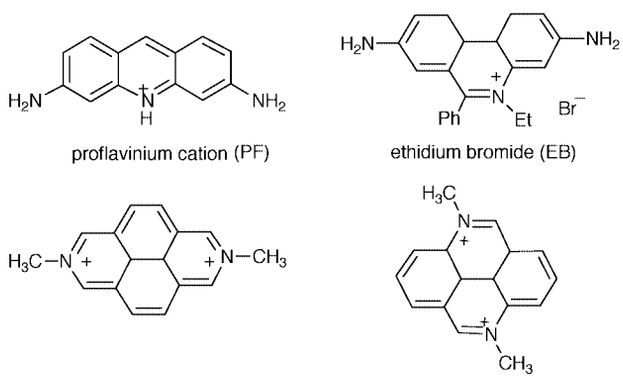


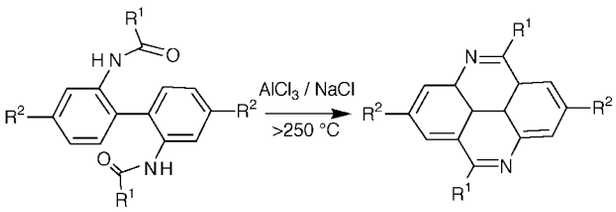
Chart 1

Recent results of Wilson[12] on the discovery of RNA specific antiviral agents, point to the very intriguing property of ethidium, intercalating more strongly to double stranded RNA

† IUPAC name for ethidium bromide is 3,8-diamino-5-ethyl-6-phenylphenanthridin-5-ium bromide.

regions than DNA, which makes phenanthridinium derivatives promising candidates for the development of RNA selective binders. These interesting properties of 2,7-diazapyrenium and phenanthridinium derivatives turned our attention toward the isomeric 4,9-diazapyrenium system which, compared to the former, possesses more structural resemblance to ethidium while possessing a larger aromatic surface and the potential to carry one or two positive charges (Chart 1). Surprisingly, we found that apart from the synthesis of a few representatives some 40 years ago, very little is known about physico-chemical and biological properties of 4,9-diazapyrenium derivatives. A recent study showed pronounced *in vitro* anticancer activity of **6** and **8**; interestingly, the growth inhibitory effects of **6** and **8** were found much less pronounced on normal human fibroblasts.[13]

In this work, we describe the synthesis of a series of mono- and bi-functional 4,9-diazapyrenium derivatives carrying one or two positive charges on the 4,9-diazapyrenium system, together with their spectroscopic (NMR, electronic absorption and fluorescence) properties. We also present studies aiming to uncover the possible influence of positive charges, large aromatic surface and various substituents on the formation of stacked complexes with major nucleotides in water. These results are compared with those related to uncharged pyrene, to doubly positively charged 2,7-diazapyrenium systems with comparable aromatic surface and to singly charged ethidium and other singly charged phenanthridinium derivatives possessing smaller aromatic surface compared to the former. Such comparative study of structurally related aromatic systems may shed more light on the importance of charges and aromatic surface on binding and the recognition of nucleic bases by the formation of stacked complexes.

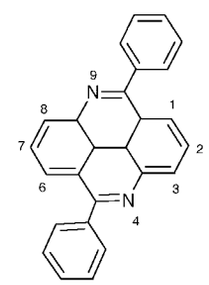


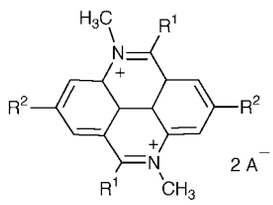
1 $R^1 = R^2 = H$

2 $R^1 = H$; $R^2 = CH_3$

3 $R^1 = CH_3$; $R^2 = H$

4 $R^1 = Ph$; $R^2 = H$





5 $R^1 = R^2 = H$; $A = CF_3SO_3^-$

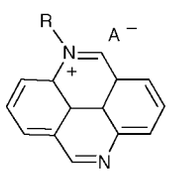
6 $R^1 = R^2 = H$; $A = HSO_4^-$

7 $R^1 = H$; $R^2 = CH_3$; $A = CF_3SO_3^-$

8 $R^1 = H$; $R^2 = CH_3$; $A = HSO_4^-$

9 $R^1 = Ph$; $R^2 = H$; $A = CF_3SO_3^-$

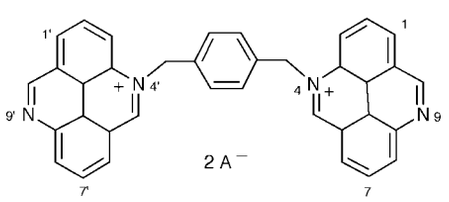
10 $R^1 = Ph$; $R^2 = H$; $A = HSO_4^-$



11 $R = CH_3$; $A = CF_3SO_3^-$

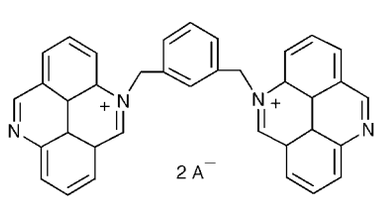
12 $R = CH_3$; $A = HSO_4^-$

13 $R = CH_2Ph$; $A = Br^-$



14 $A = Br^-$

15 $A = Cl^-$



16 $A = Br^-$

Chart 2

Results and discussion

Synthesis

Until now, the synthesis of very few 4,9-diazapyrenes had been reported in only three papers published 40 years ago.[14] In the present work, the 4,9-diazapyrenes **1**–**3** have been prepared using the modified Mosby procedure[14c] based on double cyclization of the respective biphenyl 2,2′-diamides in $AlCl_3$–NaCl melt at 250–270 °C. Diazapyrene **4** was obtained by a stepwise procedure *via* phenanthridine 9-benzamide intermediate, as reported previously[14a] (Chart 2).

The first 4,9-dimethyl-4,9-diazapyrenium dications were prepared by heating the respective 4,9-diazapyrene derivatives with dimethyl sulfate in nitrobenzene at 200–210 °C.[14a] In order to prepare the dications of **1**–**4** (Chart 2), we examined their reaction with highly reactive methyltriflate in bromobenzene, which had been previously established as very efficient in the methylation of acridine[15] and phenanthridine[16] ring nitrogens. Such reaction conditions appeared to have advantage over the dimethyl sulfate–nitrobenzene system, as the removal of nitrobenzene is difficult and the formation of more lipophilic 4,9-diazapyrenium triflate salts (compared to methyl sulfates) allowed purification by conventional methods (chromatography). Heating of diazapyrenes **1**, **2** and **4** with methyl triflate at 140 °C in bromobenzene for 30 min (**1**, **2**) or 2 hours (**4**) gave 4,9-dimethyl-4,9-diazapyrene-4,9-diium triflates **5**, **7** and **9** in 60–80% yield. However, the same reaction with **3** having 5,10-dimethyl substituents failed, apparently due to the steric hindrance imposed by these substituents which prevented vicinal *N*-methylation.

The preparation of 4,9-diazapyrenium mono-cations by single *N*-methylation proceeds under milder conditions than double *N*-methylation. The 4,9-diazapyrene (**1**) for example, was *N*-methylated simply by addition of one equivalent of methyl triflate to its dichloroethane solution at room temperature, which resulted in instantaneous precipitation of 4-methyl-4,9-diazapyrenium triflate. However, mono-*N*-alkylations with benzylic halogenides or *p*- and *m*-xylylene dihalogenides needed prolonged heating (4–24 hours) of reactants in acetonitrile under reflux. In this way 4,9-diazapyrenium monocation **13** and *p*- or *m*-xylylene bridged bis(4,9-diazapyrenium) cations **14**–**16** were obtained in 60–86% yield.

The 4,9-diazapyrenium mono- and di-chloride or di-bromide salts (**13**, **15** and **16**) are sufficiently soluble in water to enable studies of their interactions with nucleotides to be performed in aqueous media. However, the dibromide **14** is only slightly water soluble, so it must be converted to the dichloride, which exhibits better water solubility. The 4,9-diazapyrenium mono- (**11**) and di-(**5**, **7**, **9**) triflate salts are lipophilic compounds and,

in order to obtain the water soluble salts, triflate anions were exchanged for highly hydrophilic hydrogensulfates by treating the former with tetrabutylammonium hydrogensulfate in acetonitrile.

^{1}H-NMR spectra of 4,9-diazapyrenes 1–4, 4,9-diazapyrenium mono-(11–15) and dications (5, 7, 9)

The assignment of aromatic protons in the ^{1}H-NMR spectra of prepared 4,9-diazapyrenes and diazapyrenium cations (Tables 1 and 2) was accomplished from COSY and NOESY data. In the spectra of diazapyrenes **1**–**4** taken in $CDCl_3$ the aromatic protons appear in the δ 8.1–9.7 region (Table 1). The singlets of the magnetically equivalent H5,H10 protons of **1** and **2** appear in the lowest field (δ 9.64) due to the electron-withdrawing effect of the N4,N9 nitrogens. In the spectra of **1**, **3** and **4** the equivalent H2,H7 protons have the lowest chemical shift. They appear as a pseudotriplet formed from the overlapping doublet of doublets as a consequence of *ortho*-couplings with H1,H3 and H6,H8 protons.

In the proton NMR spectra of the dicationic 4,9-dimethyldiazapyrenium triflates **5** and **7**, taken in CD_3CN, all diazapyrenium resonances are strongly shifted downfield relative to their position in the spectra of uncharged **1** and **2** (Table 1). The strongest downfield shifts ($\Delta\delta_{Hi} = \delta_{Hi}$**5** $- \delta_{Hi}$**1** or δ_{Hi}**7** $- \delta_{Hi}$**2** in ppm) in charged **5** and **7**, relative to neutral diazapyrenes **1** and **2**, respectively, are observed for protons being in *para* (**5**,**1**; $\Delta\delta_{H1,H6} = 1.05$; **7**,**2**; $\Delta\delta_{H1,H6} = 0.95$) and vicinal (**5**,**1**; $\delta_{H5,H10} = 0.88$; **7**,**2**; $\delta_{H5,H10} = 0.88$) positions to positively charged N4,N9. The downfield shifts of H3,H8 and H2,H7 of **5** and **7** relative to **1** and **2**, being *ortho* and *meta* to positively charged nitrogens, are somewhat less pronounced. However, comparison of chemical shifts ($\Delta\delta_{Hi} = \delta_{Hi}$**9** $- \delta_{Hi}$**4**) in the spectra of charged **9** and uncharged **4**, both with two C5,C10 phenyl substituents, revealed the highest downfield shift for the H3,H8 doublet ($\Delta\delta_{H3,H8} = 0.68$). This may be explained by partial cancellation of the *para*- and vicinal N^+ deshielding effects through strong shielding of H1,H6 protons, being close to the π system of orthogonally positioned C5,C10 phenyl substituents.

Table 1 Chemical shifts (δ/ppm) of 4,9-diazapyrene and dicationic 4,9-dimethyl-4,9-diazapyrene-4,9-diium protons in ^{1}H-NMR spectra of **1**–**4**[a] and **5**, **7** and **9**[b]

Compound	H1,H6	H3,H8	H2,H7	H5,H10
1	8.34, d	8.61, d	8.22, pt[c]	9.64, s
2	8.22, s	8.49, s	—	9.64, s
3	8.40, d	8.51, d	8.17, pt	—
4	8.48, d	8.78, d	8.23, pt	—
5	9.39, d	9.43, d	8.94, pt	10.52, s
7	9.17, s	9.25, s	—	10.38, s
9	8.63, d	9.46, d	8.79, pt	—

[a] Taken in $CDCl_3$. [b] Taken in CD_3CN. [c] The signal appears as pseudotriplet (pt) formed by overlap of doublet of doublets due to two *ortho*-couplings.

^{1}H-NMR spectra of mono- (**12**, **13**) and bis(4,9-diazapyrenium) (**15**, **16**) mono-cations (Table 2) are more complicated than the spectra of dicationic species **5**, **7** and **9**, due to the loss of C_2 symmetry in the former. The chemical shift of the H10 singlet varies in the spectra of **12**, **13**, **15** and **16** depending on the acidity of their D_2O solutions and hence the extent of N9 protonation. In the spectrum of **12**, extensive overlap of the H1,H3 and H6,H8 resonances prevented a more precise determination of their chemical shifts. Comparison of chemical shifts in the bifunctional **15** and **16** with the monofunctional **12** and **13** diazapyrenium derivatives shows a similar range of aromatic proton chemical shifts in both cases. Lack of appreciable upfield shifts of diazapyrenium protons in bifunctional derivatives in comparison with monofunctional ones, suggests the absence of any considerable intramolecular self-stacking of diazapyrenium units.

Dicationic 4,9-diazapyrenium derivatives showed no evidence of self-association in NMR and UV concentration ranges of 10^{-2}–10^{-3} and 10^{-4}–10^{-6} mol dm^{-3}, respectively. In the specified concentration ranges, the NMR shifts changed by <0.002 ppm and UV spectra were found to be concentration independent. In contrast, mono-cationic **12** exhibited strong upfield shifts of up to 0.10 ppm on going from 2×10^{-4} to 1×10^{-3} mol dm^{-3} concentration, indicating self-association in this concentration range. Unfortunately, the shift-concentration profiles were almost linear due to sensitivity and solubility problems, so no limiting shifts for the monomer or the dimer could be obtained. The self association constant of **12** can be expected to be close to that of ethidium bromide (K_{ass} = 350–400 mol^{-1} dm^3).[17]

Electronic absorption and fluorescence spectra

The electronic absorption spectra of mono-cationic and dicationic 4,9-diazapyrenium derivatives (Table 3) taken in buffered aqueous solution (pH 4–5) were found to be concentration independent in the 10^{-6} to 3×10^{-5} mol dm^{-3} range, showing an absence of self-association in this concentration range. Thus, the electronic absorption spectra presented in Table 3 are taken at 2×10^{-5} M concentration and correspond to monomeric species.

The mono-cationic and di-cationic 4,9-diazapyrenes show strong absorptions in the visible domain. Comparison of the spectra of mono-cationic **12** and dicationic **6** diazapyrenes shows considerable differences in the positions of maxima and absorbance intensities. In the 300–400 nm region, the dicationic **6** shows three maxima (λ_{max}/nm (ε/dm^3 mol^{-1} cm^{-1}) 344 (13400), 369 (9400) and 389 (13800)) together with a shoulder at 330 nm while for mono-cationic **12** only two bands (λ_{max} (ε) 351 (9000) and 389 (10000)) could be observed. Fig. 1 shows the electronic absorption spectra of the mono-functional (**13**) and the di-functional diazapyrenium derivative **16** with *m*-xylylene bridge. At λ_{max} 393 nm, being one of the electronic absorptions of the 4,9-diazapyrenium system, the molar extinction coefficient ε of **16** is almost twice that of **13**, and the same also holds for the bis-derivative **15** with *p*-xylylene bridge. At λ_{max}

Table 2 Chemical shifts (δ/ppm) of monocationic 4,9-diazapyrenium protons in ^{1}H-NMR spectra of **12**, **13**, **15** and **16**[a]

Compound	H1	H2	H3	H5	H6	H7	H8	H10
12[b]		9.01–9.13, m[c] (H1, H2, H3)		10.73, s		8.64–8.74, m[c] (H6, H7, H8)		10.05, s
13	7.22, d	7.97–8.08, m (H2, ArH)	8.54, m (H3, H10)	9.83, s	8.34, d	7.36, pt[d]	7.15, d	8.45, s
15[b]	7.06, d	7.76–7.75, m[c] (H2, H7, ArH)	8.85, d	10.51, s	8.61, d	7.76–7.95, m[c] (H7, H2, ArH)	7.51, d	7.12, s
16	7.70–7.76, m (H1, ArH)	8.24–8.29, m[c] (H2, H7)	8.80, d	9.98, s	8.39, d	8.24–8.29, m[c] (H7, H2)	7.94, d	9.49, s

[a] Taken in D_2O. [b] Compounds **11** and **14** having different anions gave very similar spectra. [c] Positions of H1/H3 and H6/H8 protons exchangeable. [d] Pseudotriplet.

Table 3 Electronic absorption spectra of mono- and dicationic 4,9-diazapyrenium derivatives **6**, **8**, **10**, **12**, **13**, **15** and **16**[a]

Compound	λ_{max}/nm (ε/10^3 dm^3 mol^{-1} cm^{-1})
6	216 (33.3); 235 (45.3); 258 (12.7); 267 (13.6); 344 (13.4); 369 (9.4); 389 (13.8)
8	217 (49.4); 239 (67.8); 248 (33.2); 258 (22.8); 267 (23.2); 344 (21.1); 369 (9.9); 388 (19.8); 412 (7.2)
10	218 (40.4); 243 (45.4); 263 (24.2); 272 (25.3); 335 (12.8); 351 (14.2); 380 (12.9); 400 (24.1)
12	205 (20.7); 236 (55.8): 279 (9.3); 351 (9.0); 389 (10.0)
13	238 (74.6); 266 (19.4); 281 (10.4); 317 (11.0); 333 (12.7); 353 (9.9); 371 (11.0); 392 (8.1)
15	236 (93.1); 282 (18.1); 356 (15.2); 393 (15.4)
16	232 (94.4); 282 (18.3); 356 (14.9); 371 (14.5); 393 (15.2)

[a] Aqueous solution; pH 4–5; $c = 2.0 \times 10^{-5}$ M.

Table 4 Fluorescence emission intensities (EI_i) of mono- and dicationic 4,9-diazapyrenium derivatives **6**, **8**, **10** and **15** relative to ethidium bromide (EI_{EB})[a]

Compound	λ_{em}/nm	EI_i/EI_{EB}
6	397	667
8	418	2014
10	415	6
15	439	343

[a] Taken in buffered aqueous solution at pH 5.

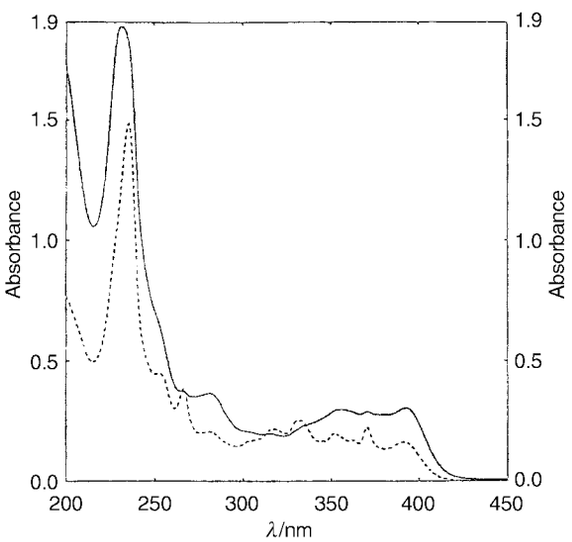


Fig. 1 Electronic absorption spectra of *m*-xylylene bridged bifunctional 4,9-diazapyrenium derivative **16** (—) and mono-functional 4-benzyl-4,9-diazapyrenium derivative **13** (----) taken in aqueous buffer (citric acid–Na_2HPO_4, pH 5), $c = 2 \times 10^{-5}$ mol dm^{-3}.

235 nm however, the $\varepsilon_{16}/\varepsilon_{13}$ is only 1.3, which can be explained by the considerable contribution of xylylene bridge absorption at this wavelength. The absence of any appreciable hypochromicity leads to the conclusion that diazapyrenium units **15** and **16** are in an *anti*-orientation, in agreement also with the ^{1}H-NMR results.

4,9-Diazapyrenium derivatives show strong fluorescence in aqueous solution. The intensities of their fluorescence emission (EI) are compared with that shown by ethidium bromide under the same experimental conditions (Table 4). In general, dicationic 4,9-diazapyrenium derivatives showed enhanced emission intensities (EI) compared to ethidium. However, their emission is strongly substitution dependent. For example, compound **8**, having 2,7-dimethyl substituents, exhibited 2000 times stronger fluorescence emission than ethidium, three times stronger than unsubstituted derivative **6**, and some 300 times stronger than derivative **10** having 5,10-diphenyl substituents. The *p*-xylylene bridged bifunctional derivative **15**, having two singly charged diazapyrenium units, exhibited about 50% lower fluorescence emission intensity than doubly charged **6**.

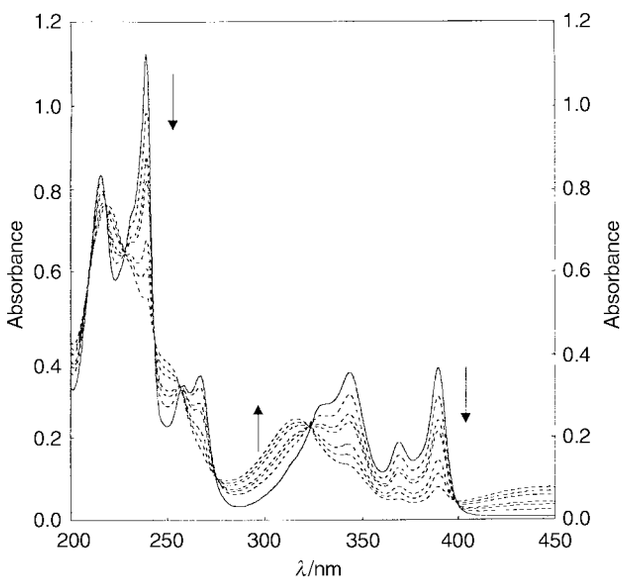


Fig. 2 Changes of the electronic absorption spectra of **6** ($c = 2 \times 10^{-5}$ mol dm^{-3}) produced by pH variation from 3 (—) to 8 (----).

Reversible pH dependent formation of 4,9-diazapyrenium monopseudobase (DMOH) in water

The electronic absorption, ^{1}H-NMR and fluorescence spectra of mono- and di-cationic 4,9-diazapyrenium salts taken in water showed strong pH dependence. For example, the electronic absorption spectra of dicationic salt **6** obtained by variation of pH from 3 to 8 exhibited pronounced changes in the position and intensity of the absorption maxima, together with appearance of a set of isosbestic points in the 220–400 nm region (Fig. 2). This observation is in agreement with a two-species equilibria. The most prominent changes upon pH variation appear in the region of electronic absorptions of the 4,9-diazapyrenium system. On going from pH 3 to 8, the intensities of the absorbances at λ_{max} 389, 369 and 344 nm decreased gradually, reaching a minimum at pH 8. These observations suggest diminished conjugation and charge in the product formed at higher pH. Reacidification to pH 3 produced the starting spectrum, however with considerably lower absorption intensities. Such behavior was found for all prepared diazapyrenium salts, except the diphenyl derivative **10**, which upon reacidification showed a fully restored UV spectrum and hence fully reversible pH dependent transformation. Similar behavior was observed for acridinium and phenanthridinium derivatives and is ascribed to the reversible formation of the corresponding pseudobase ROH (Scheme 1).[18] In order to find out if a pseudobase also forms from diazapyrenium derivatives, the ^{1}H-NMR spectra of **10** at pH 2.3 and 7.5 were analyzed. In weakly basic conditions, a single product, in addition to a small amount of unchanged **10**, could be observed (Fig. 3). The proton resonances of the product in the aromatic region appear upfield (δ 7.0–8.5) compared to those in the starting dicationic diazapyrenium derivative (δ 7.6–9.4), pointing to a decrease in

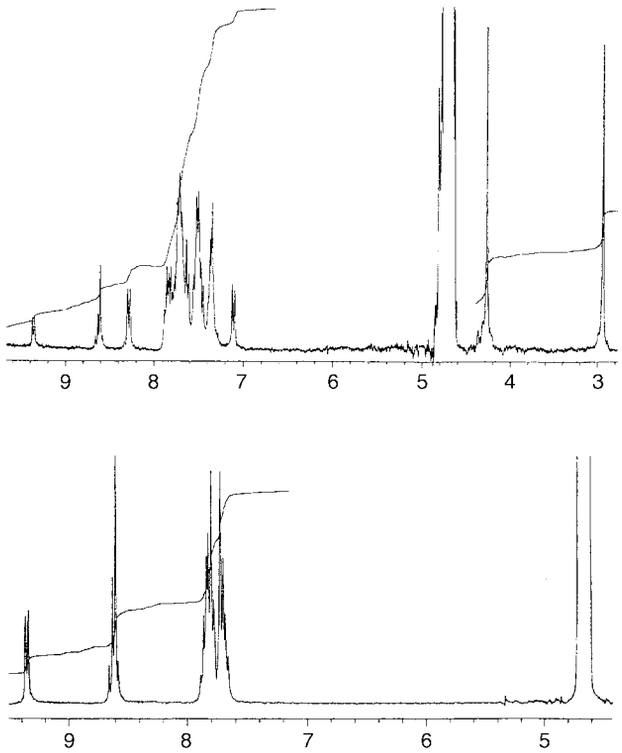
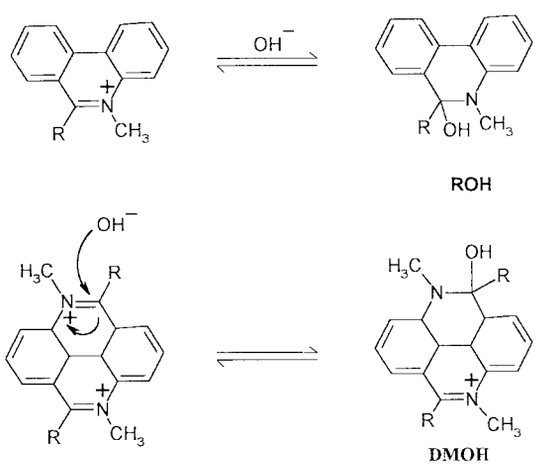


Fig. 3 ^{1}H-NMR spectra of **10** at pH 2.3 (lower) and 7.5 (upper).



Scheme 1

positive charge in the product formed in basic conditions. In addition, two CH_3 singlets appear at δ 4.26 and 2.93, the first assignable to phenanthridinium[16] N5–CH_3 and the second to Ar–NH–CH_3 protons, respectively. Thus, the spectral data for the product formed from **10** in weakly basic conditions are fully in agreement with the diazapyrenium mono-pseudobase (DMOH) structure still containing phenanthridinium moiety (Scheme 1). Upon reacidification, the spectrum of **10** is fully restored. Similar changes in ^{1}H-NMR spectra could be found also for **6** during pH variation from 2 to 8, although in this case a mixture of several products forms in weakly basic conditions. Upon reacidification, the spectrum is not fully restored, showing in addition to **6** the presence of other unidentified products. Apparently, formation of the pseudobase from **6** (and other diazapyrenium derivatives except **10**) is followed by additional subsequent transformations, most probably the pseudobase ring opening giving an amino aldehyde and its condensation products, which can not be reconverted to diazapyrenium structure by acidification.

To assess the tendencies of diazapyrene mono-pseudobase (DMOH) formation from **6**, **10**, **12**, **13**, **15** and **16**, the equilibrium constants (expressed as pK_{DMOH}) were calculated from pH dependent changes in their electronic absorption spectra (Table 5). Compared with pK_{ROH} values for the pseudobase (ROH) formed from 5-methylphenanthridinium (pK_{ROH} 11.9) and 5-methyl-6-phenylphenanthridinium (pK_{ROH} 13.5),[18] the pK's for diazapyrenium compounds, both mono- and doubly-charged are much lower. The lowest pK_{DMOH} was measured for **6**, with a double positive charge on the diazapyrenium system, which makes it especially susceptible to hydroxide ion attack at C5 or C10 positions. Doubly charged **10** has an order of magnitude higher pK_{DMOH} value than **6**, apparently as a consequence of the electron donating effects of the 5-phenyl substituents. For mono-cationic **12** and **13**, considerably higher pK_{DMOH} values were measured than for dicationic **6** and **10**, being, however, still much lower than that measured for 5-methylphenanthridinium.[18] Bifunctional diazapyrenium compounds **15** and **16**, with one positive charge on each diazapyrenium unit, showed close to one order of magnitude lower pK_{DMOH} values than singly charged mono-derivatives **12** and **13**, and about two orders of magnitude higher values than the doubly charged mono-diazapyrenium derivative **6**. These observations indicate that the reactivity of such systems toward pseudobase formation largely depends on the amount of positive charge present in the diazapyrenium system and also on the electronic properties of the substituents at the C5, C10 positions. Compared to the phenanthridinium system, both mono-cationic and especially dicationic diazapyrenium derivatives are much less stable in weakly basic conditions, giving the corresponding pseudobases at a considerably lower pH.

Table 5 pK_{DMOH} values for mono-pseudobase (DMOH) formation from 4,9-diazapyrenium compounds **6**, **10**, **12**, **13**, **15** and **16**

Compound	λ_{max}/nm	pK_{DMOH}
6	239	5.39
10	440	6.54
12	236	9.63
13	235	8.28
15	393	7.28
16	393	7.58

[a] Concentration of each 4,9-diazapyrenium salt was 2×10^{-5} M. Changes of absorbance at specified λ_{max} occurring upon titration with 0.1 M HCl or NaOH (pH range 2 to 10) were used for calculations.

Binding of nucleotides in water

Several polycyclic aromatic and heteroaromatic DNA intercalators, including pyrene,[19] mono-cationic acridinium,[6a,20] phenanthridinium,[6d] and dicationic 2,7-diazapyrenium derivatives,[10,11] also exhibited moderate binding of nucleotides in aqueous media by the formation of stacked complexes with nucleic bases of 1 : 1 stoichiometry. The stability of such complexes, as well as aromatic π–π complexes in general, is believed to depend mostly on the extent of overlap of the π–surfaces in contact, as well as on the charges of interacting aromatic systems.[1b,21] It has also been shown recently that in such complexes formed in water the stacking interaction between aromatic parts is much stronger than the hydrophobic forces.[21] The prepared 4,9-diazapyrenium derivatives (Chart 2) possess a large aromatic surface, comparable to pyrene. However, in contrast to the latter, these compounds have two nitrogens within the aromatic system, which upon methylation can provide singly or doubly-positively charged derivatives. Compared to phenanthridinium derivatives, 4,9-diazapyrenium derivatives possess a larger aromatic surface, which can have a single positive charge, like phenanthridinium, or be doubly-charged. On the other hand, different positions of positive charges on isomeric 2,7- and 4,9-diazapyrenium systems, and hence directions of the permanent dipoles present, could result in different binding and/or recognition of nucleotide bases by these systems. We

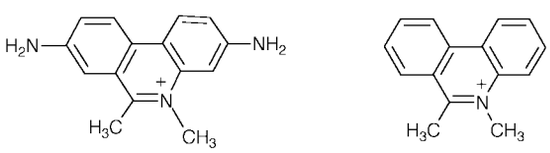
Table 6 Stability constants (log K_s) and purine/pyrimidine nucleotide selectivity factors (K_s(Pu)/K_s(Py)) for complexes of 4,9-diazapyrenium and phenanthridinium derivatives with nucleotides[a]

Compound	AMP	ADP	ATP	GMP	CMP	K_s(AMP)/ K_s(CMP)	K_s(GMP)/ K_s(CMP)
10	1.67	1.74	1.78	1.66	<1		
12	2.21	2.23	2.24	2.11	1.37	6.9	5.5
13	2.40	2.31	2.37	2.11	1.33	11.7	6.0
15	2.60	2.66	2.85	2.23	1.63	9.3	3.8
16	2.25	2.11	2.21	2.10	1.63	4.2	2.9
EB[b]	1.87	1.74	2.02	1.77	<1		
17	2.12	2.00	2.21	1.95	<1		
18	2.26	2.24	2.32	1.95	1.57	4.8	2.3

[a] Compound, λ_{ex}, λ_e: **10**, 400, 432; **12**, 350, 420; **13**, 353, 391; **15**, 393, 439; **16**, 393, 445; **EB**, 480, 595; **17**, 460, 582; **18**, 372, 414 nm. [b] **EB** denotes ethidium bromide.

undertook nucleotide binding studies with selected 4,9-diazapyrenium and phenanthridinium derivatives in order to find out if these differences in the aromatic surface and charge of the compared aromatic systems are reflected in their binding and potential recognition of nucleotides.

Interaction of 4,9-diazapyrenium derivatives **10**, **12**, **13**, **15** and **16** (Chart 2) and phenanthridinium derivatives **EB** (Chart 1) and **17**, **18** (Chart 3) with nucleotides in buffered aqueous



17 **18**

Chart 3

solution (pH 5; citric acid–Na_2HPO_4) at constant ionic strength (NaCl or Na_2SO_4, 0.01 M) was studied by fluorescence. Such studies were not possible with **6** and **8** due to their instability at this pH (see preceding paragraph and Table 5). Titrations of aqueous solutions of the 4,9-diazapyrenium derivatives and the phenanthridinium derivative **18** with selected nucleotides produced quenching of their fluorescence emission. However, in the case of ethidium and **17**, both having 3,8-diamino substituents, strong enhancements of emission were observed. The observed quenching and enhancements are in agreement with π–π stacking interactions between diazapyrenium or phenanthridinium systems and nucleic bases. The emission enhancements of ethidium and **17** upon binding to nucleotides can be interpreted according to Kearns.[22] He presents evidence for **EB** self-quenching in water, which occurs by amino proton transfer to water molecules in the excited state. It seems plausible that in the π–π stacked complex between **EB** or **17** and an electron rich nucleic base, the positive charge of the phenanthridinium system is partially neutralized. This should lower the amino proton acidity and hence the rate of its transfer to water molecules. Consequently, the quenching of ethidium emission in a complex with a nucleic base should be prevented and emission enhancements should be expected with increase of nucleotide concentration.

The stability constants (Table 6) were calculated from titration data by the non-linear least squares fitting using the SPECFIT[23] program. In each case, the best fit was obtained for 1 : 1 complex stoichiometry (see, for example, Fig. 4). The stability constants determined for dicationic diazapyrene derivative **10** are close to those obtained for ethidium and somewhat lower than those for the phenanthridinium derivatives **17** and **18** and the same nucleotides. This would imply that the larger aromatic surface and double positive charge of **10**, as compared to **EB**, **17** and **18**, does not result in an enhanced binding of nucleotides. It should be noted, however, that **17** and **18**, lacking a

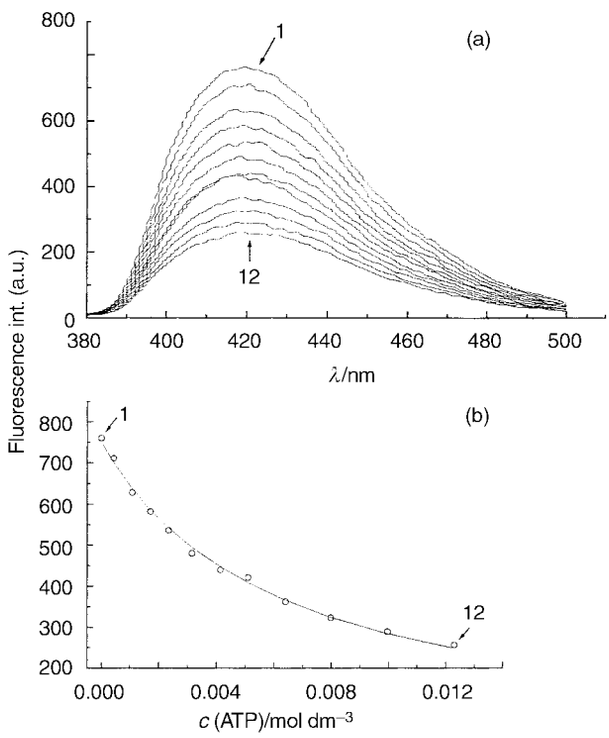


Fig. 4 Fluorimetric titration of **12** ($c = 1.5 \times 10^{-6}$ mol dm^{-3}) with ATP ($c_{ATP} = 0$ (1)–1.23×10^{-2} mol dm^{-3} (12)) (a). Experimental (○) and calculated (—) fluorescence intensities for 1 : 1 complex stoichiometry as a function of ATP concentration (b).

5-phenyl substituent, bind nucleotides more strongly than **EB**. Apparently, there is an unfavorable influence of 5-phenyl substituents on the binding strength. This unfavorable effect can be explained both by the electron donating effect, which partly compensates for the polarization of the aromatic system due to the positive charge on nitrogen, and by steric effects preventing the optimal nucleic base stack. These effects are particularly pronounced for **10**, which showed an unexpectedly weak binding of nucleotides compared to the other examined 4,9-diazapyrenium derivatives. The stability constants for monocationic **12**, **13** and bifunctional diazapyrenium derivatives **15**, **16** are comparable to those of phenanthridinium derivatives **17**, **18** despite the larger aromatic surface of the former and the presence of two diazapyrenium units in the latter. No cooperative binding could be observed for the bifunctional diazapyrenium derivatives **15** and **16**, as appears from the comparison of their K_s values with those of monofunctional **13**. The important general observation for nucleotide binding with different 4,9-diazapyrenium derivatives is that the measured stability constants were independent of nucleotide charge. For all the compounds studied, the K_s values are similar for ATP, ADP and AMP, carrying 4, 3 and 2 negative charges, respect-

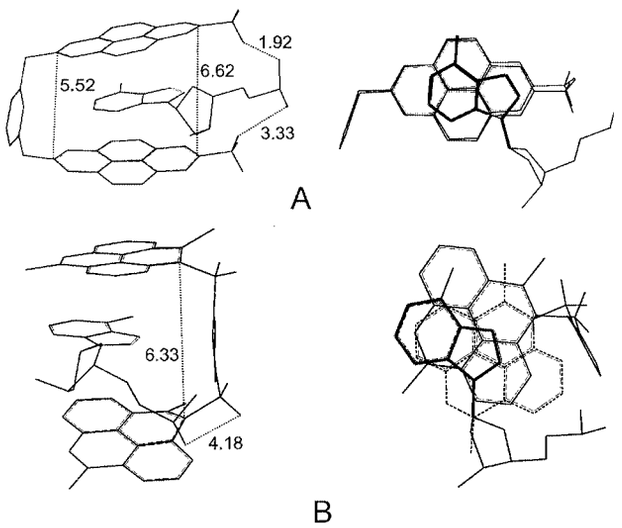


Fig. 5 Side and top views of minimized structures of bifunctional 2,7- (A) and 4,9- (B) diazapyrenium cleft complexes with AMP produced by molecular modelling. The distances in Å between quaternized nitrogens and between negatively charged phosphate oxygens and positively charged N2 methyl hydrogens (A) and N4 methylene hydrogens (B) are indicated.

ively. This result reveals that the aromatic stacking interactions between 4,9-diazapyrenium or phenanthridinium units and nucleic bases are exclusive binding forces stabilizing these complexes. Apparently, these interactions are much stronger than the coulombic interaction between the positively charged diazapyrenium system and the negatively charged nucleotide phosphate.

Comparison of our results on nucleotide binding by the bifunctional derivatives **15** and **16** with those obtained with isomeric 2,7-diazapyrenium derivatives[11b] under similar conditions reveals some striking differences. For example, the bis(7-methyl-2,7-diazapyrenium) derivative with *m*-xylylene bridge connecting 2,2′-nitrogens showed positive cooperativity in the binding of nucleotides.[11b] This gives complexes with the nucleic base inserted in the cleft formed by two 2,7-diazapyrenium units. The measured K_s for the bifunctional 2,7-diazapyrenium derivative is six times larger than the monofunctional one with the same nucleotide, which contrasts the same comparison for isomeric bifunctional **16** and monofunctional **13**. Besides cooperative binding, the bifunctional 2,7-diazapyrenium derivative also showed clear nucleotide charge dependence,[11b] with ATP being more strongly bound than ADP or AMP. To account for the observed differences, we estimated the possible adenosine binding conformations of both types of bifunctional receptors by molecular modelling[24] (Fig. 5). This revealed that the observed differences are likely to be a consequence of a much deeper cleft that might be formed by the two *syn*-oriented 2,7-diazapyrenium units bridged at 2,2′-nitrogens, than by the two 4,9-diazapyrenium units connected by the same bridge at 4,4′-nitrogens. Such a deep cleft may allow stacking of the nucleic base and, simultaneously, hydrogen bonding interactions between negatively charged phosphate(s) and positively charged 7,7′-N–CH_3 hydrogens located at the other end of diazapyrene long axes (see Fig. 5A). For **16**, in contrast, the modelled cleft formed by two *syn* 4,9-diazapyrenium units is much shallower due to bridging at nitrogens located on the diazapyrene shorter axis (Fig. 5B). In the complex the two 4,9-diazapyrenium units are located a considerable way from the superpositioned arrangement. Further, the positive charges on 4,4′-N–CH_2 hydrogens in such a complex are located on the opposite side of the cleft, so that simultaneous base stacking and hydrogen bonding interactions with phosphates (4-N–$CH_2 \cdots {}^{-}O$–P–distance 4.18 Å, Fig. 5B) are not possible. In addition, the model shows that no deep insertion of nucleic base is possible in the shallow cleft of **16** due to steric hindrance between the orthogonal deoxyribose unit and 4,9-diazapyrenium units. Consequently, due to the small overlap of aromatic surfaces in such a complex, the adenine single stacking with one diazapyrenium unit of **16** seems to be thermodynamically more favorable, as compared to cleft binding. These conclusions from model examination are fully supported by the experimental results with **16**, showing that binding constants are independent of the charge present in nucleotides. In addition, with **15** and **16**, the best fit of fluorescence titration data was obtained for fluorescence active complexes of 1 : 1 stoichiometry, being at variance with monofunctional 4,9-diazapyrenium derivatives where the best fits for inactive 1 : 1 complexes were obtained.

Table 6 presents the selectivity factors for tested purine–pyrimidine nucleotides expressed as the ratio of their stability constants. For **10**, **EB** and **17**, the stability constants for CMP are too small (<1) to be precisely determined by fluorescence, so selectivity factors can not be calculated for these compounds. In general, all the examined 4,9-diazapyrenium and phenanthridinium derivatives bind AMP and GMP considerably better than CMP. The AMP/CMP selectivity factors are between 4 and 12, and those of GMP/CMP between 2 and 6. The highest AMP/CMP selectivity was exhibited by the mono-functional **13** (selectivity factor 11.7) with N4-benzyl substituent and bifunctional **15** (selectivity factor 9.3) with 4,4′-*p*-xylylene bridge. The GMP/CMP selectivities are lower.

Conclusions

The following are the general conclusions formulated on the basis of the presented nucleotide binding studies: (*a*) mono- and bi-functional derivatives with one positive charge on the 4,9-diazapyrenium unit bind nucleotides with a strength comparable to phenanthridinium derivatives (**17**, **18**) and slightly better than ethidium. The larger aromatic surface of these compounds, compared to the phenanthridinum system, does not considerably enhance nucleotide binding; (*b*) the observed GMP/CMP, and particularly AMP/CMP selectivities, however, are considerably higher for 4,9-diazapyrenium than for phenanthridinium derivatives; (*c*) compared to pyrene (K_s for AMP, GMP and CMP is 52, 45 and 13, respectively)[19] both stronger binding and higher purine–pyrimidine selectivities were obtained for 4,9-diazapyrenium derivatives; (*d*) monofunctional 4,9-diazapyrenium derivatives carrying one positive charge bind nucleotides with a strength comparable to a monofunctional 2,7-dimethyldiazapyrenium[11] derivative with two positive charges. These conclusions show that 4,9-diazapyrenium derivatives exhibited very different properties in the binding of nucleotides in comparison with pyrene, phenanthridinium and 2,7-diazapyrenium intercalators. This, together with the observed anticancer activity of **6** and **8**,[13] makes the investigation of their interactions with DNA and RNA most intriguing. Such studies may reveal some characteristic properties of these compounds, which could possibly be related to their biological activity.

Experimental

^{1}H-NMR spectra were recorded on a Varian-Gemini 300 instrument with tetramethylsilane as internal standard. Chemical shifts (δ) are expressed in ppm and J values in Hz. Signal multiplicities are denoted as s (singlet), d (doublet), t (triplet) pt (pseudotriplet) and q (quartet). The electronic absorption spectra were obtained on a Pye Unicam SP 8-100 spectrometer. IR spectra were recorded on a Perkin-Elmer 297 instrument from KBr pelleted or film samples. Fluorescence spectra were recorded on a Perkin-Elmer LS 50 fluorimeter. High resolution mass spectra were obtained using a Varian MAT 711 spectrometer. For chromatographic purifications of the prepared

compounds, Merck Kieselgel 0.005–0.02 mm for column and Kieselgel HF_{254} for preparative thin layer chromatography were used. 2,2′-Diformamido-4,4′-biphenyl,[14c] 4,9-diazapyrene (**1**),[14c] 5,10-dimethyl-4,9-diazapyrene (**3**)[14c] and 5,10-diphenyl-4,9-diazapyrene (**4**)[14a] were prepared by published procedures. 3,8-Diamino-5,6-dimethyl- (**17**) and 5,6-dimethyl- (**18**) phenanthridin-5-ium hydrogen sulfates were prepared as previously described.[16] Melting points for 4,9-diazapyrene-4,9-diium salts could not be determined due to the thermal decomposition of these compounds.

2,7-Dimethyl-4,9-diazapyrene 2

2,2′-Diformamido-4,4′-dimethylbiphenyl (0.80 g, 3 mmol), anhydrous $AlCl_3$ (15.00 g, 113 mmol) and NaCl (3.50 g, 60 mmol) were mixed and heated until melted (250–270 °C). The melt was stirred for 2 hours, then cooled to 150 °C and poured on ice (100 g) and made alkaline (pH 10) by addition of 5 M aqueous NaOH. The precipitate was collected, dried and subjected to continous extraction with chloroform for 20 hours. The extract was evaporated and the obtained solid purified by column chromatography on silica gel using 5% MeOH in dichloromethane for elution. Evaporation of solvents and recrystallization from chloroform gave **2** (176 mg; 25% yield); mp 250–252 °C (Found: C, 82.89; H, 5.33; N, 12.23. $C_{16}H_{12}N_2$ requires: C, 82.73; H, 5.21; N, 12.06%); δ_H ($CDCl_3$) 2.89 (6H, s, CH_3), 8.22 (2H, s, H1,H6), 8.49 (2H, s, H3,H8), 9.64 (2H, s, H5,H10); ν_{max}/cm^{-1} 3000, 1570, 1460, 1410, 1305, 1268, 930; HRMS (*m*/*z*) 232.09898 (M^+).

4,9-Dimethyl-4,9-diazapyrene-4,9-diium triflates 5, 7 and 9

The bromobenzene solutions of each of 4,9-diazapyrenes **1**, **2** and **4** and methyl triflate in 1 : 2 molar ratio, respectively were heated for 0.5 (**1**, **2**) or 2 hours (**4**) at 140 °C. After cooling to room temperature the precipitate was collected, washed with dry ether and recrystallized from acetonitrile–ether giving **5**, **7** and **9** in 80, 66 and 67% yield, respectively. *4,9-Dimethyl-4,9-diazapyrene-4,9-diium triflate* **5**: (Found: C, 40.78; H, 2.90; N, 5.45. $C_{18}H_{14}F_6N_2O_6S_2$ requires: C, 40.61; H, 2.65; N, 5.26%); δ_H (CD_3CN) 4.98 (6H, s, $2CH_3$), 8.96 (2H, pt, H2,H7), 9.39–9.43 (4H, 2d, H1,H3,H6,H8), 10.52 (2H, s, H5,H10); ν_{max}/cm^{-1} 3420, 3070, 1618, 1525, 1270, 1160, 1030; *2,4,7,9-Tetramethyl-4,9-diazapyrene-4,9-diium triflate* **7**: (Found: C, 42.80; H, 3.49; N, 5.06. $C_{20}H_{18}F_6N_2O_6S_2$ requires: C, 42.86; H, 3.24; N, 5.00%); δ_H (CD_3CN) 3.12 (6H, s, C2– and C7–CH_3), 4.93 (6H, s, N4– and N9–CH_3), 9.17, 9.25 (4H, 2s, H1,H3,H6,H8), 10.38 (2H, s, H5,H10). MS (*m*/*z*) 411.1 ($M^+ - CF_3SO_3^-$), 262.3 ($M^+ - 2\ CF_3SO_3^-$). *5,10-Diphenyl-4,9-dimethyl-4,9-diazapyrene-4,9-diium triflate* **9**: (Found: C, 52.54; H, 3.70; N, 4.18. $C_{30}H_{24}F_6N_2O_6S_2$ requires: C, 52.48; H, 3.52; N, 4.08%); δ_H (CD_3CN) 4.68 (6H, s, $2CH_3$), 7.89–8.04 (10H, m, Ph–H), 8.63 (2H, d, *J* 8,06, H1,H6), 8.79 (2H, pt, *J* 8.06 and 8.44, H2,H7), 9.46 (2H, d, *J* 8.44, H3,H8); ν_{max}/cm^{-1} 3100, 1602, 1590, 1508, 1490, 1462, 1450, 1375, 1265, 1225, 1155, 1030, 1000.

4,9-Dimethyl-4,9-diazapyrene-4,9-diium hydrogen sulfates 6, 8 and 10

To each solution of triflates **5**, **7**, and **9** (0.1 mmol) in acetonitrile (5 ml), the acetonitrile solution of tetrabutylammonium hydrogensulfate (2.0 mmol) was added. After standing for 30 min at room temperature, the formed precipitate was collected, washed with dry dichloromethane and recrystallized from DMSO–CH_2Cl_2 solvent mixture, giving **6**, **8**, and **10** in 76, 82 and 80% yield, respectively. *4,9-Dimethyl-4,9-diazapyrene-4,9-diium hydrogen sulfate* **6**: (Found: C, 44.72; H, 3.93; N, 6.45. $C_{16}H_{16}N_2O_8S_2$ requires: C, 44.86; H, 3.76; N, 6.54%); δ_H (DMSO) 4.95 (6H, s, $2CH_3$), 8.94 (2H, m, H2,H7), 9.37, 9.52 (4H, m, H1,H3,H6,H8), 10.96 (2H, s, H5,H10); ν_{max} (KBr)/cm^{-1} 3440, 1620, 1523, 1460, 1180, 1105; HRMS (*m*/*z*) ($M^+ - 2HSO_4^-$) 234.12469. *2,4,7,9-Tetramethyl-4,9-diazapyrene-4,9-diium hydrogen sulfate* **8**: (Found: C, 47.55; H, 4.30; N 6.25. $C_{18}H_{20}N_2O_8S_2$ requires: C, 47.36; H, 4.42; N, 6.14%); δ_H (DMSO) 3.05 (6H, s, C2– and C7–CH_3), 4.91 (6H, s, N4– and N9–CH_3), 9.15, 9.40 (4H, 2s, H1,H3,H6,H8), 10.79 (2H, s, H5,H10); ν_{max} (KBr)/cm^{-1} 3440, 3060, 1618, 1522, 1460, 1325, 1280, 1220, 1180, 1070, 1005; HRMS (*m*/*z*) ($M^+ - 2HSO_4^-$) 262.15161. *5,10-Diphenyl-4,9-dimethyl-4,9-diazapyrene-4,9-diium hydrogen sulfate* **10**: δ_H (DMSO) 4.64 (6H, s, $2CH_3$), 7.89–8.04 (10H, m, Ph–H), 8.47 (2H, d, *J* 8.03, H1,H6), 8.81 (2H, pt, *J* 8.03 and 8.30, H2,H7), 9.66 (2H, d, *J* 8.30, H3,H8); ν_{max}/cm^{-1} 3070, 3020, 1640, 1605, 1585, 1510, 1490, 1460, 1440, 1290, 1250, 1228, 1180, 1155, 1030; HRMS (*m*/*z*) ($M^+ - HSO_4^-$) 483.13133.

4-Methyl-4,9-diazapyrenium triflate 11

Compound **11** was prepared similarly to **5**, **7** and **9** from **1** and methyl triflate in 0.9 : 1 molar ratio at room temperature in dichloroethane. It was converted to *4-methyl-4,9-diazapyrenium hydrogen sulfate* **12** as described for **6**, **8** and **10**. Compound **11**: (Found: C, 52.38; H, 3.18; N, 7.63. $C_{16}H_{11}N_2F_3SO_3$ requires: C, 52.17; H, 3.01; N, 7.61%); δ_H (CD_3CN) 4.86 (3H, s, N–CH_3), 8.65–8.74 and 8.96–9.04 (5H, 2m, H1,H2,H6,H7,H8), 9.16 (1H, d, H3), 9.99 (1H, s, H10), 10.22 (1H, s, H5); ν_{max}/cm^{-1} 3410, 3100, 1605, 1590, 1490, 1462, 1380, 1270, 1225, 1155, 1030. Compound **12**: (Found: C, 54.20; H, 4.38; N, 8.38. $C_{16}H_{12}N_2O_4S{\cdot}x\ H_2O$ requires: C, 53.90; H, 4.22; N, 8.38%); δ_H (DMSO) 4.86 (3H, s, N–CH_3), 8.4–8.74 and 9.01–9.13 (6H, 2m, H1,H2,H3,H6,H7,H8), 10.05 (1H, s, H10); 10.73 (1H, s, H5); ν_{max}/cm^{-1} 3040, 3000, 2460, 1950, 1610, 1513, 1490, 1460, 1265, 1120.

4-Benzyl-4,9-diazapyrenium bromide 13

Benzyl bromide (0.031 g, 0.2 mmol) was added to the solution of **1** (0.041 g, 0.2 mmol) in dry acetonitrile (3 ml) and the mixture was heated under reflux in the dark for 24 hours. The formed precipitate was collected, washed with dry dichloromethane and dried, yielding 76% of **13**. δ_H (D_2O) 6.04 (2H, s, CH_2), 7.15 and 7.22 (2H, 2d, *J* 7.97 and 7.63, H1,H8), 7.36 (1H, pt, H2), 7.45 (3H, s, Ph–H), 7.97–8.08 (3H, m, 2Ph–H and H7), 8.34 (1H, d, *J* 7.64, H6), 8.54 (2H, d and s, H10,H3), 9.83 (1H, s, H5); ν_{max}/cm^{-1} 2960, 1638, 1605, 1580, 1550, 1508, 1490, 1450, 1430, 1360, 1260; HRMS (*m*/*z*) ($M^+ - Br^-$) 295.12427.

4,4′-(1,4-Phenylenedimethyl)bis(4,9-diazapyren-4-ium) dibromide 14, dichloride 15 and 4,4′-(1,4-phenylenedimethyl)-bis(4,9-diazapyren-4-ium) dibromide 16

A solution of diazapyrene **1** (0.05 g, 0.24 mmol) and α,α′-dibromo-1,4-xylene or α,α′-dibromo-1,3-xylene (0.03 g, 0.11 mmol) in dry acetonitrile was refluxed in the dark for 4 hours. The formed precipitate of **14** or **16** was collected, washed with dry dichloromethane and dried under reduced pressure in the dark. Compound **14**, 86% yield; δ_H (D_2O–TFA) 6.34 (s, 4H, $2CH_2$), 7.61 and 7.73 (2 dd, 4H, 2H2 and 2H7), 8.1–8.27 (m, 12H, xyl.-H, 2H1,2H3,2H6,2H8), 9.28 (s, 2H, 2H10), 9.54 (s, 2H, 2H5); ν_{max} (KBr)/cm^{-1} 3410, 2920, 1638, 1605, 1510, 1490, 1460, 1430, 1260.

Compound **16**: 66% yield; δ_H (D_2O) 6.21 (4H, s, $2CH_2$), 7.70–7.76 (6H, m, 2H1,xyl.-H), 7.94 (2H, d, *J* 8.49, 2H8), 8.24–8.29 (4H, m, 2H2,2H7), 8.39 (2H, d, *J* 7,81, 2H6), 8.80 (2H, d, *J* 7.88, 2H3), 9.49 (2H, s, 2H10); 9.98 (2H, s, 2H5); ν_{max}/cm^{-1} 3410, 2960, 1640, 1605, 1585, 1505, 1490, 1460, 1430, 1265; HRMS (*m*/*z*) ($M^+ - 2Br^-$) 512.20168.

Compound (**15**): Dibromide **14** (0.05 g, 0.074 mmol) and freshly prepared AgCl (0.1 g, 0.6 mmol) were suspended in water and heated at 100 °C in the dark for 2 hours under vigorous stirring. After cooling, the formed AgBr and excess of AgCl were separated using a centrifuge. Aqueous solution was

evaporated to dryness, the residue dissolved in a small volume of water and **15** precipitated by addition of acetone in 61% yield. δ_H (D_2O) 6.39 (4H, s, $2CH_2$), 7.06 (2H, d, *J* 7.2, 2H1), 7.12 (2H, s, 2H10), 7.51 (2H, d, *J* 8.01, 2H8), 7.76 and 7.89–7.95 (8H, dd and m, 2H2, 2H7, xyl.-H), 8.61 (2H, d, *J* 7.6, 2H6), 8.85 (2H, d, *J* 8.31, 2H3), 10.51 (2H, s, 2H5); ν_{max}/cm^{-1} 3410, 3060, 1638, 1605, 1510, 1490, 1460, 1430, 1390, 1260; HRMS (*m*/*z*) ($M^+ - Cl^-$) 547.15598.

Binding of nucleotides in aqueous media

Fluorimetric titrations of 4,9-diazapyrenium salts **10**, **12**, **13**, **15**, **16** and phenanthridinium salts **17**, **18** and **EB** with ATP, ADP, AMP, GMP and CMP were performed at room temperature in water at pH 5 and constant ionic strength (0.0243 mol citric acid–0.0514 mol Na_2HPO_4 diluted in 1 dm^3 NaCl or Na_2SO_4, 0.01 mol dm^{-3}). The nucleotides were used as disodium salts. Concentrations of diazapyrenium and phenanthridinium salts were kept constant around 2×10^{-6} mol dm^{-3}. Concentrations of nucleotides were varied from *ca.* 10^{-4} to *ca.* 10^{-2} mol dm^{-3} corresponding to *ca.* 20–80% complexation. Stability constants K_s were calculated by nonlinear analysis performed by the SPECFIT[23] program. In each case, the best fit was obtained for 1 : 1 complex stoichiometry. The results for spectroscopically active complexes were checked by the exclusive 1 : 1 stoichiometry equation (nonlinear fit) according to the literature[6a] and similarly (linear fit) for spectroscopically nonactive complexes.

Molecular modelling[24]

Bifunctional 2,7- and 4,9-diazapyrenium clefts in *syn*-conformations were constructed using Builder package. Gastaiger–Hückel charges were used for clefts (with formal charge +1 for quaternized nitrogens) and adenosine. Calculations with adenosine-5′-mononucleotide were not possible due to lack of parameterization for the phosphate group. Complexes of clefts with adenosine were produced by docking of adenine between diazapyrenium units until energy minimum was found. Generated structures were then fully minimized. At the end the available PO_2^{2-} groups were attached on ribose C5′-oxygens and rotation around C5′O–P bond performed in order to mimic the distance between negatively charged phosphate oxygens and positively charged N7,7′ methyl or N4 methylene hydrogens. For all calculations the TRIPOS force field was used.

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