Ground- and Excited-State Properties of Molecular Complexes between Adenine and 2,7-Diazapyrene and Its *N*-Methylated Cations

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Received: March 27, 1997; In Final Form: September 17, 1997[⊗]

It has recently been found that 2,7-diazapyrenes upon interaction with nucleic acids form stacked ("intercalation") complexes, which for the methylated derivatives exhibit new absorption features assigned as charge-transfer (CT) transitions.[1] To better understand the basis of these interactions and associated optical properties, the geometries and electronic spectra of complexes of adenine (A) with 2,7-diazapyrene (DAP), *N*-methyl-2,7-diazapyrenium ($MDAP^+$), and *N*,*N*′-dimethyl-2,7-diazapyrenium ($DMDAP^{2+}$) have been modeled using semiempirical AM1 and PM3 geometry optimizations, ab initio (vacuum and Onsager model) energy calculations, and ZINDO/S calculations. In addition, absorption spectra, fluorescence quenching, and ^1H NMR spectra for the complexes in aqueous solution have been measured. For the A-DAP complex, a coplanar, hydrogen-bonded complex is predicted by the calculations, while A-$MDAP^+$ and A-$DMDAP^{2+}$ complexes should have edge-to-face geometry. The association is predicted to be of electrostatic nature, mainly between the pyridinium nitrogen ($MDAP^+$, $DMDAP^{2+}$) and N^1/NH_2 of adenine. There seems to be a preference (6 kcal/mol) for the hydrogen-bonded A-DAP complex, and the energetic difference between face-to-face and edge-to-face A-$MDAP^+$ and A-$DMDAP^{2+}$ complexes is 3 and 8 kcal/mol, respectively (Onsager ab initio, $\epsilon = 79.5$). By contrast, the ^1H NMR data and experimental absorption spectra in conjunction with calculated spectra instead indicate that all three adenine−diazapyrene complexes assume face-to-face arrangement in water because of hydrophobic effects. In agreement with the putative CT absorption of diazapyrenium−DNA complexes, absorption tails are also observed for A-$DMDAP^{2+}$ and A-$MDAP^+$, however not for the A-DAP complex. Most satisfactorily, charge-transfer transitions are predicted by the calculations to occur in the correct wavelength region for A-$DMDAP^{2+}$ (strongest) and A-$MDAP^+$, while A-DAP is predicted not to have any CT transitions. Correspondingly, the observation of quenching of fluorescence of $MDAP^+$ and $DMDAP^{2+}$ (but not DAP) by adenine can explained by charge transfer from adenine to the diazapyrenium.

Introduction

Azaaromatic compounds such as 2,7-diazapyrene (DAP) and its *N*-methyl cations *N*-methyl-2,7,-diazapyrenium ($MDAP^+$), and *N*,*N*′-dimethyl-2,7-diazapyrenium ($DMDAP^{2+}$; Figure 1) are of long-standing interest in general because of their photophysical properties, specifically in context of interactions with DNA[2−4] and some of its mononucleotides.[5] In a study dealing with complexes of quaternary azaaromatics with aromatic anions, association of $DMDAP^{2+}$ to adenine (A) was indicated by the occurrence of fluorescence quenching.[5] A face-to-face arrangement of donor and acceptor moieties was proposed on the basis of ^1H NMR shift data, but neither ground-state interactions nor the excited-state properties of such diazapyrene−nucleobase complexes have yet been characterized at any detail. In nucleotide-$DMDAP^{2+}$ electron-transfer experiments, the limiting rate constant was found to be ca. 200 times smaller than expected for a contact charge-transfer complex.[3] This was explained by a donor−acceptor distance of about 10 Å, at variance with the proposed face-to-face arrangement.

The three diazapyrenes DAP, $MDAP^+$, and $DMDAP^{2+}$ provide a series of homologs where charge, electron density, and hydrogen bonding sites vary while the "π surface" remains essentially unchanged. We have recently characterized their interactions with DNA using linear and circular dichroism spectroscopy, and calorimetry.[1] From the spectroscopic results all three compounds were concluded to bind to DNA by intercalation between basepairs. It was suggested that restriction of rotational freedom within the intercalation pocket, when passing from the nonionic DAP to its methyl derivatives, may be a result of the ionic charge of the ligand molecule.[1] Furthermore, it was found that the absorption spectra of the diazapyrene−DNA complexes (Figure 2) differ in a significant way. While the spectrum of DAP−DNA shows only hypochromism and red-shift, the spectra of $MDAP^+$−DNA, and especially $DMDAP^{2+}$−DNA, also tail far into the visible. The tailing was ascribed to charge-transfer absorption. To obtain more information about the interaction of DAP, $MDAP^+$, and $DMDAP^{2+}$ with the nucleobases, we here investigate the formation, and the photophysical and thermodynamic properties, of their molecular complexes with adenine using fluorescence spectroscopy and quantum mechanical calculations. It is concluded that all three diazapyrene−adenine complexes are of face-to-face type in aqueous solution, and that the complexation is mainly driven by hydrophobic effects. The $MDAP^+$−DNA and $DMDAP^{2+}$−DNA absorption tails can be explained by a distribution of charge-transfer transitions.

Experimental Section

Adenine and adenosine were purchased from Sigma-Aldrich Co. and used without further purification. DAP, $MDAP^+$, and $DMDAP^{2+}$ were prepared according to the literature.[5,6] Fluorescence and absorption spectra were recorded in deionized water, and van't Hoff plots constructed from fluorescence quenching data and absorbance measurements. Fluorescence lifetimes were measured both by the phase-modulation technique on a Spex Fluorolog τ-2 using a 370 nm cutoff filter in the emission channel, and by time-correlated single photon counting,

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[⊗] Abstract published in *Advance ACS Abstracts,* November 15, 1997.

S1089-5639(97)01082-7 CCC: $14.00 © 1997 American Chemical Society

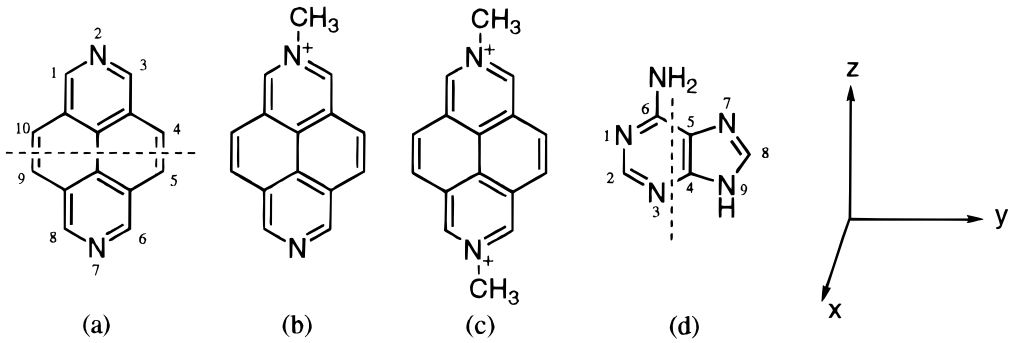


Figure 1. (a) 2,7-Diazapyrene, (b) *N*-methyl-2,7-diazapyrenium, (c) *N*,*N*′-dimethyl-2,7-diazapyrenium, and (d) adenine. The dashed lines indicate the division of the molecules for the charge separation calculation. The coordinate system shows the axes used in the transition moment assignments.

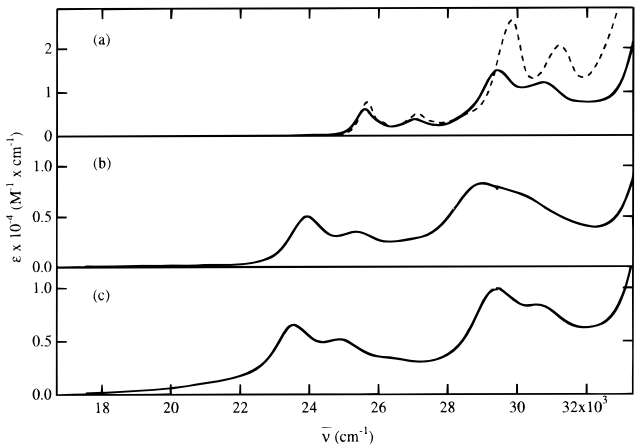


Figure 2. Absorption spectra of (a) DNA-DAP (solid), and A-DAP (dashed), (b) DNA-MDAP$^+$, (c) DNA-DMDAP^{2+} at pH 7. Note the absorption tails in DNA-MDAP$^+$ and DNA-DMDAP^{2+}. The increase in absorption at the blue edge of the spectra is due to absorption of the nucleobases.

using a nitrogen filled Edinburgh Instruments flashlamp for excitation at 337 nm, and a Jobin−Yvon monochromator for the emission. Absorption spectra were recorded on a Varian Cary 4B spectrometer. ^{1}H NMR spectra were recorded in D_2O and DMF-d_7 on a 400 MHz Varian instrument. The residual HOD resonance was, when necessary, suppressed by decoupler presaturation.

Quantum Mechanical Calculations

Geometry Optimizations. AM1 and PM3 calculations were performed using MOPAC 6.0[7] on an IBM RS6000 workstation. Optimizations were performed using tight SCF and gradient criteria (MOPAC keyword PRECISE). The methodology of the optimizations involved the following steps. (1) The adenine was placed 3.5 Å from and parallel to the diazapyrene plane, shifted toward one of the diazapyrene nitrogens. (2) The adenine was rotated around the normal axis of the adenine plane through 360° in 10° steps, and the energy was calculated for each arrangement. (3) From the deepest energy minimum, the adenine was moved both toward and away from the diazapyrene, and the energy was calculated for each interplanar separation. (4) From the lowest energy arrangement, a full geometry optimization was started. (5) The vibrational spectrum was calculated for the optimized geometry and checked for imaginary frequencies.

To examine the possible formation of diazapyrene−water complexes, a water molecule was placed in the vicinity of the pyridine nitrogen (DAP) or pyridinium nitrogen (MDAP$^+$, DMDAP^{2+}), and a full geometry optimization was started.

Hartree−Fock Energy Calculations. As the size of the studied systems precluded full ab initio geometry optimizations, only single-point calculations at the HF/6-31G*//PM3 level were performed using the Gaussian 94 suite of programs[8] on Silicon Graphics and IBM RS/6000 workstations. The relative energies of the complexes were calculated as

$$\Delta E = E(\text{complex}) - E(\text{adenine}) - E(\text{diazapyrene}) + \text{BSSE}$$

where BSSE denotes the basis set superposition error. In the case of the fully optimized complexes, the BSSE was calculated as the energy difference between the monomers as taken from the semiempirically optimized complex, with and without the basis set of the neighboring molecule (Gaussian keyword MASSAGE). This was done in order to take into account the possible geometry changes during the optimization.

To take into account the effects of water, the Onsager model self-consistent reaction field (Gaussian keyword SCRF=DIPOLE) was used. Solute cavity radii were determined in separate calculations using the keyword VOLUME, and the dielectric constant was set to 79.5.

Since the individual atomic charges are strongly affected by both the choice of Hamiltonian and by the method of population analysis, we generally have avoided drawing any conclusions based on the comparison of atomic charges. To estimate the charge shift (induction) in the complexes, the molecules were divided into two parts each (Figure 1, dashed lines). The individual atomic charges obtained from the ab initio Mulliken population analysis were summed up for estimation of the charge of the molecular fragments. For the three diazapyrenes, the division is done along the molecular short-axis. Due to the lack of symmetry in adenine, the molecule was arbitrarily divided into two parts, one consisting of the five-membered ring, and the other containing the ring atoms 1, 2, 3, and 6, as well as the exocyclic amino group.

Calculation of Electronic Absorption Spectra. Electronic absorption spectra of both face-to-face oriented and fully optimized complexes were calculated using the ZINDO/S program.[9,10] For the singly excited configuration interaction, a CI space including all single excitations among the n, π, and π* molecular orbitals was used.

Results

Absorption and Fluorescence Spectra of Adenine−Diazapyrene Complexes. Diazapyrenes DAP, MDAP$^+$, and DMDAP^{2+} have very similar absorption spectra characterized by a first transition (L_b) around 400 nm, and a second transition (L_a) around 330 nm (Figure 3a−c). The transitions are known to be polarized along the molecular short- and long-axes, respectively.[11] Around 230 nm there are two more transitions,

TABLE 1: Calculated (ZINDO/S; PM3 Structures) and Observed Transitions of the Diazapyrenes and Their Complexes with Adenine. Experimental Values in Parentheses

species	energy (cm^{-1})	oscillator strength	transition character	polarization[a]
DAP	27610 (25920)	0.12 (0.05)	π-π*	y (y)
	30930 (30180)	0.66 (0.33)	π-π*	z (z)
	33220	0.015	n-π*	x
A-DAP face-to-face	27570 (25706)	0.11	π-π*	y
	30750 (29895)	0.56	π-π*	z
	32820	0.017	n-π* (DAP)	x
	33070	1.7×10^{-4}	n-π* (DAP)	x
A-DAP hydrogen-bonded	27520	0.12	π-π*	y
	30780	0.71	π-π*	z
	32820	0.014	n-π* (DAP)	x
	33190	0.0026	n-π* (DAP)	x
$MDAP^+$	23500 (24410)	0.19 (0.07)	π-π*	y (y)
	28030 (29670)	0.36 (0.24)	π-π*	z (z)
	31770	0.0047	n-π*	x
A-$MDAP^+$ face-to-face	23500	0.17	π-π* (MDAP)	y
	25890	8.5×10^{-4}	CT (A→MDAP)	10° out of plane
	27970	0.33	π-π* (MDAP)	z
	30360	9.6×10^{-4}	CT (A→MDAP)	10° out of plane
	30850	0.0068	n-π* (MDAP)	x
A-$MDAP^+$ edge-to-face	23700	0.19	π-π* (MDAP)	x, 2° out of plane
	28180	0.37	π-π* (MDAP)	z, 3° out of plane
	30720	8.8×10^{-4}	CT (A→MDAP)	70° out of plane
	30980	0.0069	n-π* (MDAP)	x
	33150	4.1×10^{-4}	CT (A→MDAP)	70° out of plane
$DMDAP^{2+}$	23990 (23920)	0.31 (0.09)	π-π*	y (y)
	30130 (29900)	0.42 (0.26)	π-π*	z (z)
	32870	10^{-4}	π-π*	x
A-$DMDAP^{2+}$ face-to-face	16760	0.0019	CT (A→DMDAP)	x
	21130	10^{-4}	CT (A→DMDAP)	80° out of plane
	24000	0.27	π-π*	y, 5° out of plane
	25990	4.1×10^{-4}	CT (A→DMDAP)	5° out of plane
	27700	2×10^{-5}	CT (A→DMDAP)	80° out of plane
	28000	10^{-4}	CT (A→DMDAP)	30° out of plane
	30160	0.32	π-π*, CT	z
	30330	0.057	π-π*	z, 6° out of plane
	32140	2×10^{-5}	CT (A→DMDAP)	x
	32770	3.3×10^{-4}	π-π*	5° out of plane
A-$DMDAP^{2+}$ edge-to-face	16740	1.7×10^{-4}	CT (A→DMDAP)	70° out of plane
	21410	3×10^{-5}	CT (A→DMDAP)	60° out of plane
	24420	0.30	π-π*, CT	y, 5° out of plane
	25180	0.021	π-π*, CT	x, 10° out of plane
	27390	0.0039	CT (A→DMDAP)	30° out of plane
	28570	3×10^{-5}	CT (A→DMDAP)	x
	30020	0.11	π-π*, CT	z, 5° out of plane
	30470	0.35	π-π*, CT	z, 10° out of plane
	32390	0.0025	CT (A→DMDAP)	10° out of plane
	33060	4.1×10^{-4}	π-π*	10° out of plane

[a] See Figure 1 for coordinate system. In the complexes, "out of plane" means the transition is directed out of the plane containing the diazapyrene.

polarized perpendicularly to each other. The results of the ZINDO/S calculations are in good agreement with the observed transition energies and polarizations (Table 1). The absorption and fluorescence spectra of DAP, $MDAP^+$, and $DMDAP^{2+}$ are related by mirror symmetry, and characterized by small Stokes shifts (ca. 100 cm^{-1}) for DAP and $DMDAP^{2+}$, and a somewhat larger shift (ca. 470 cm^{-1}) for $MDAP^+$. Fluorescence quantum yields are around 0.5, lifetimes of fluorescence being about 10 ns.

For all three diazapyrenes the absorption intensity of the first two transitions is lowered by the addition of adenine, indicating the formation of ground-state complexes (Figure 4). Addition of adenine to an aqueous solution of DAP results in a slightly red-shifted emission of enhanced intensity (Figure 4a). The emission intensity of 7 μM DAP is doubled at an adenine concentration of 3.5 mM. With one lifetime held fixed to the lifetime of the uncomplexed DAP (14.4 ns), the lifetime of the A-DAP complex could be estimated to ca. 20 ns, which is comparable with the lifetime of DAP intercalated into poly-$(dA\text{-}dT)_2$ (24 ns).[1] From absorption titration experiments the

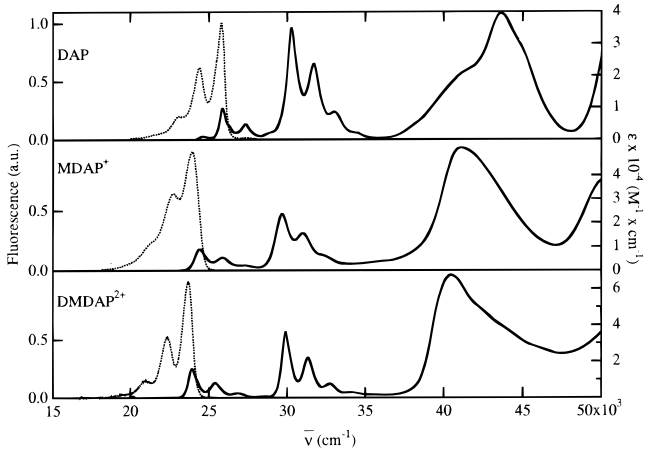


Figure 3. Absorption and emission spectra of (a) DAP, (b) $MDAP^+$, and (c) $DMDAP^{2+}$ in water.

association constant of A-DAP was determined to be ca. 140 M^{-1}. Since the pure spectrum of A-DAP was unknown, an

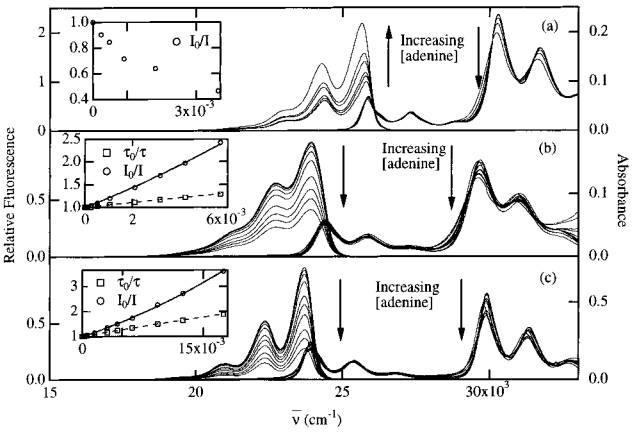


Figure 4. Absorption and emission spectra at 20 °C of (a) A-DAP, (b) A-MDAP$^+$, and (c) A-DMDAP^{2+} at varying adenine concentration. Insets: I_0/I and τ_0/τ, the dashed lines being the best fit ($1+k_q\tau$[A]) to the lifetime data, and the solid lines the best fits of I_0/I to $(1+K_S[A])\times(1+k_q\tau[A])$.

TABLE 2: Dynamic Quenching Constants, Rate Constants for Dynamic Quenching, Association Constants, and Thermodynamic Parameters for A-DAP, A-MDAP$^+$, and A-DMDAP^{2+}

complex	K_D[a]	k_q[b]	K_S[a]	$\Delta H°$[c]	$\Delta S°$[d]
A-DAP	n/a	n/a	140	−9	−20
A-MDAP$^+$	50	5×10^9	150	−5	−8
A-DMDAP^{2+}	50	5×10^9	50	*ca.* −6	*ca.* −10

[a] In M^{-1} at 20 °C. [b] In M^{-1} s^{-1} at 20 °C, calculated for $\tau_{MDAP^+} = 10.6$ ns and $\tau_{DMDAP^{2+}} = 10.5$ ns. [c] In kcal/mol. [d] In cal/mol K.

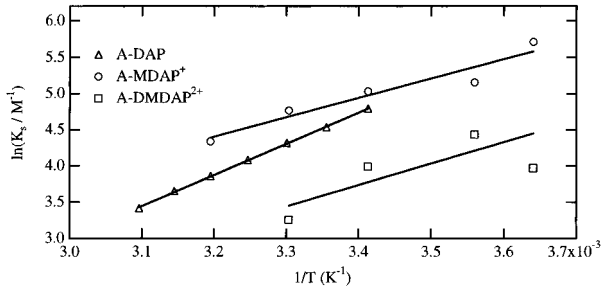
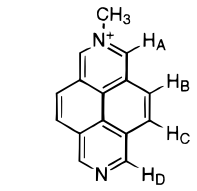


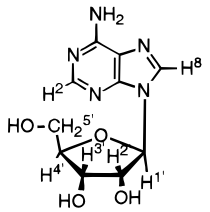
Figure 5. van't Hoff plots for A-DAP, A-MDAP$^+$, and A-DMDAP^{2+}.

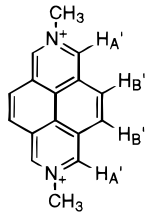
iterative procedure was used for determining the association constant. The final A-DAP spectrum (Figure 2a, dashed line) shows hypochromicity (10−30%) and a red shift of ca. 200 cm^{-1} for the L_b transition, and ca. 400 cm^{-1} for the L_a transition. Contrasting the enhanced fluorescence in A-DAP, the fluorescence of aqueous solutions of MDAP$^+$ or DMDAP^{2+} is quenched by the presence of adenine (Figure 4b,c). Notable is a broadening of the MDAP$^+$ and DMDAP^{2+} L_b absorption accompanying the hypochromicity. Stern−Volmer plots (Figure 4b,c, insets), taking into account both static and dynamic quenching, yield association constants of 150 M^{-1} and 50 M^{-1} at 20 °C for A-MDAP$^+$ and A-DMDAP^{2+}, respectively. The association constant found for A-DMDAP^{2+} is in agreement with a previous report.[5] The dynamic quenching rate constant was found to be 5×10^9 s^{-1} M^{-1} for both systems. Quenching data for complex formation are summarized in Table 2.

All three complexes were found to be formed in exothermic reactions (Table 2, Figure 5). The data obtained for the enthalpy of formation of A-DMDAP^{2+} are too uncertain to allow any conclusions about magnitude and sign of $\Delta S°$, but $\Delta H°$ appears to be more negative than found for A-MDAP$^+$. The experimental uncertainty may be due to oxidation of adenine by DMDAP^{2+}, resulting in too high I_0/I ratios while leaving the τ_0/τ ratios unaffected.

TABLE 3: NMR Chemical Shifts in Parts Per Million (in D_2O) for Adenosine (ca. 25 mM), MDAP$^+$, DMDAP^{2+} and Adenosine-MDAP$^+$/DMDAP^{2+} Mixtures. See Figure Next to Table for Proton Designation. Chemical Shifts Are Relative to HOD (4.80 ppm), and Ratios Are Given as Adenosine:Diazapyrenium







Proton	Pure[a]	1:0.9
H_A	9.58	9.49
H_B	8.38	8.14
H_C	9.42	9.19
H_D	8.28	8.08
H_{Me}	4.85	4.83
H^2	8.25[b]	7.96
H^8	8.12[b]	7.58
$H^{1'}$	5.99	5.68
$H^{2'}$	4.73	4.50
$H^{3'}$	4.37	4.29
$H^{4'}$	4.25	4.21
$H^{5'}$	3.84	3.82

Proton	Pure	1:0.4
H_A'	9.95	9.96
H_B'	8.70	8.72
H_{Me}'	4.94	4.94
H^2	8.25[b]	8.15
H^8	8.12[b]	7.92
$H^{1'}$	5.99	5.87
$H^{2'}$	4.73	4.64
$H^{3'}$	4.37	4.35
$H^{4'}$	4.25	4.24
$H^{5'}$	3.84	3.84

[a] In dilute solution. [b] Distinguished by selective deuteration of H.[8]

We have also investigated by ^{1}H NMR the complexation of adenosine (chosen for solubility reasons) with MDAP$^+$ and DMDAP^{2+}. The association of adenine with DMDAP^{2+} had been studied by NMR previously,[5] but no chemical shift data have been reported. The A-DMDAP^{2+} data should be interpreted with some care, since DMDAP^{2+} seems to oxidize adenosine at an appreciable rate at NMR concentrations (solutions turned greenish-brown). In fluorescence quenching experiments, adenosine and adenine behave identically upon addition of the diazapyrenes. The addition of MDAP$^+$ to solutions of adenosine in D_2O affects the chemical shifts of both compounds significantly (see Table 3). The $\Delta\delta$ of the MDAP$^+$ protons appears mainly to be due to self-association, as the chemical shift is concentration-dependent. Of the adenosine protons, H^8 shows the largest shift difference (−0.54 ppm) upon addition of MDAP$^+$. H^2 is also shifted markedly upfield (0.29 ppm). As for the sugar protons, the $\Delta\delta$ decreases with increasing distance from the adenyl system. The behavior of the adenosine resonances upon addition of DMDAP^{2+} is essentially the same as in the MDAP$^+$ case, although the effects are smaller, probably due to the lower association constant. Self-association of DMDAP^{2+} seems to be negligible. Water, apparently, is a prerequisite for significant complexation, as the ^{1}H NMR spectra of mixtures of adenosine and MDAP$^+$ in DMF-d_7, recorded at 20 °C and at −40 °C, were merely superpositions

TABLE 4: Heats of Formation and Complex Stabilization Energies Calculated with PM3 and AM1

	PM3			AM1		
species	ΔH_f (kcal/mol)	$\Delta(\Delta H_f)$ (kcal/mol)	interplanar spacing (Å)	ΔH_f (kcal/mol)	$\Delta(\Delta H_f)$ (kcal/mol)	interplanar spacing (Å)
adenine	55.97			86.80		
DAP	78.13			87.16		
$MDAP^+$	226.52			238.84		
$DMDAP^{2+}$	440.06			454.76		
H_2O	−53.43			−59.24		
A-DAP (face-to-face)	133.70	−0.4	4.8	173.78	−0.2	5.1
A-DAP (hydrogen-bonded)	131.44	−2.7		170.92	−3.0	
H_2O−DAP (optimized)	23.27	−1.4		25.24	−2.7	
A-$MDAP^+$ (face-to-face)	279.99	−2.5	4.4	323.50	−2.1	4.6
A-$MDAP^+$ (edge-to-face)	275.75	−6.7		319.16	−6.5	
H_2O−$MDAP^+$ (optimized)	164.80	−8.3		171.30	−8.3	
A-$DMDAP^{2+}$ (face-to-face)	490.87	−5.2	4.3	537.11	−4.4	4.2
A-$DMDAP^{2+}$ (edge-to-face)	484.51	−12		531.04	−10.5	
H_2O−$DMDAP^{2+}$ (edge-to-face)	374.89	−12		383.71	−11.8	

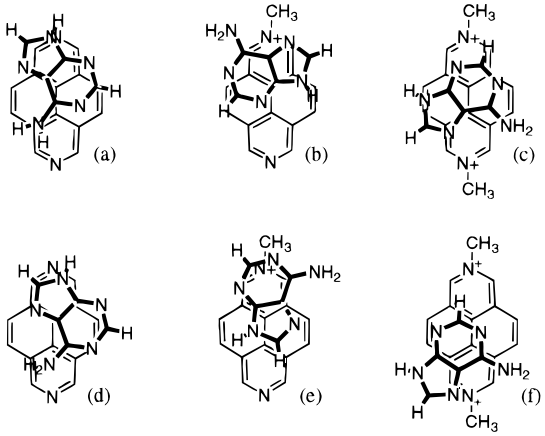


Figure 6. Optimum face-to-face geometries for (a), (d) A-DAP; (b), (e) A-$MDAP^+$; and (c), (f) A-$DMDAP^{2+}$. (a)−(c) are PM3 calculations; (d)−(f) are AM1 calculations. The diazapyrene hydrogens have been omitted for clarity.

of the component spectra. The upfield shifts of the adenosine resonances are consistent with a face-to-face arrangement, and the need for water as solvent shows that hydrophobic effects are important.

Quantum Chemical Calculations: Geometries. Both of the semiempirical methods (AM1 and PM3) predict that face-to-face arrangements be stabilized (Table 4; Figure 6). However, full geometry optimizations give the structures shown in Figure 7. The vacuum condition ab initio calculations (Table 5) qualitatively agree with the semiempirical results, although the semiempirical methods seem to underestimate the strength of the hydrogen bonds in A-DAP, and the face-to-face stabilization in A-$MDAP^+$ and A-$DMDAP^{2+}$. Inclusion of the solvent reaction field gave the results summarized in Table 5.

The optimal face-to-face distance (according to AM1 and PM3) was found to decrease with the charge of the complex. For the uncharged A-DAP complex the optimum was found at about 5 Å, while the optima for A-$MDAP^+$ and A-$DMDAP^{2+}$ were found at 4.5 and 4.2 Å, respectively.

The ground-state perturbation of the monomers, as judged from the *intra*molecular charge shift, varies significantly between the face-to-face and optimized arrangements. All face-to-face complexes show little or no charge transfer character. There is some degree of charge shift in the adenine moiety, but it is only on the order of 0.01 electron units. The common geometric feature seems to be a short (diazapyrene N)−(adenine N) distance (Figure 6).

The optimized A-DAP complex is characterized by coplanar side-by-side arrangement of adenine and DAP, involving association by hydrogen bonds from H(1) of the diazapyrene to N(3) on adenine, and from H(9) on adenine to N(2) on DAP (Figure 7a and d). Hydrogen bonding is evident both from the charge distribution and the bond lengths: in adenine, the five-membered ring is donating electrons (net 0.02 charges) to N(2) of DAP, while the 6-membered ring fragment is accepting 0.03 charges from H(1) of DAP. The carbon−hydrogen and nitrogen−hydrogen bonds involved in hydrogen bonding are elongated by 0.3 pm, compared to the corresponding bond lengths in adenine and 2,7-diazapyrene. However, there are several possible hydrogen-bonded arrangements, most of which are energetically equivalent to the ones shown in Figures 7a and 7d.

In contrast to the coplanar, hydrogen-bonded geometry predicted for A-DAP, the geometries calculated for A-$MDAP^+$ and A-$DMDAP^{2+}$ by the AM1 and PM3 methods reveal an intermolecular edge-to-face association, as shown in Figures 7b,c and 7e,f, and indicate considerable polarization of the adenine moiety. Thus, for A-$MDAP^+$, PM3 predicts the adenine N(1) to be pointing toward N(2) of $MDAP^+$. AM1 calculations lead to a similar structure, with adenine N(1) pointing toward N(2) and the methyl group in $MDAP^+$. There exist other, geometrically similar, arrangements of almost identical energies as those shown in Figures 7b and 7e, all of which are characterized by edge-to-face association, and a broad potential energy minimum.

Compared to the electronic indifference of adenine to the $MDAP^+$ cation noted in the face-to-face geometry discussed above, the electronic interactions in the edge-to-face geometry (Figure 7b,e) are 2-fold. Primarily, induced dipoles are formed in both adenine and $MDAP^+$. This effect is most profound in adenine, where the induced charge separation is ca. 0.05 electron units, but also $MDAP^+$ undergoes a charge displacement of ca. 0.015 electron units, thus increasing the dipole moment of

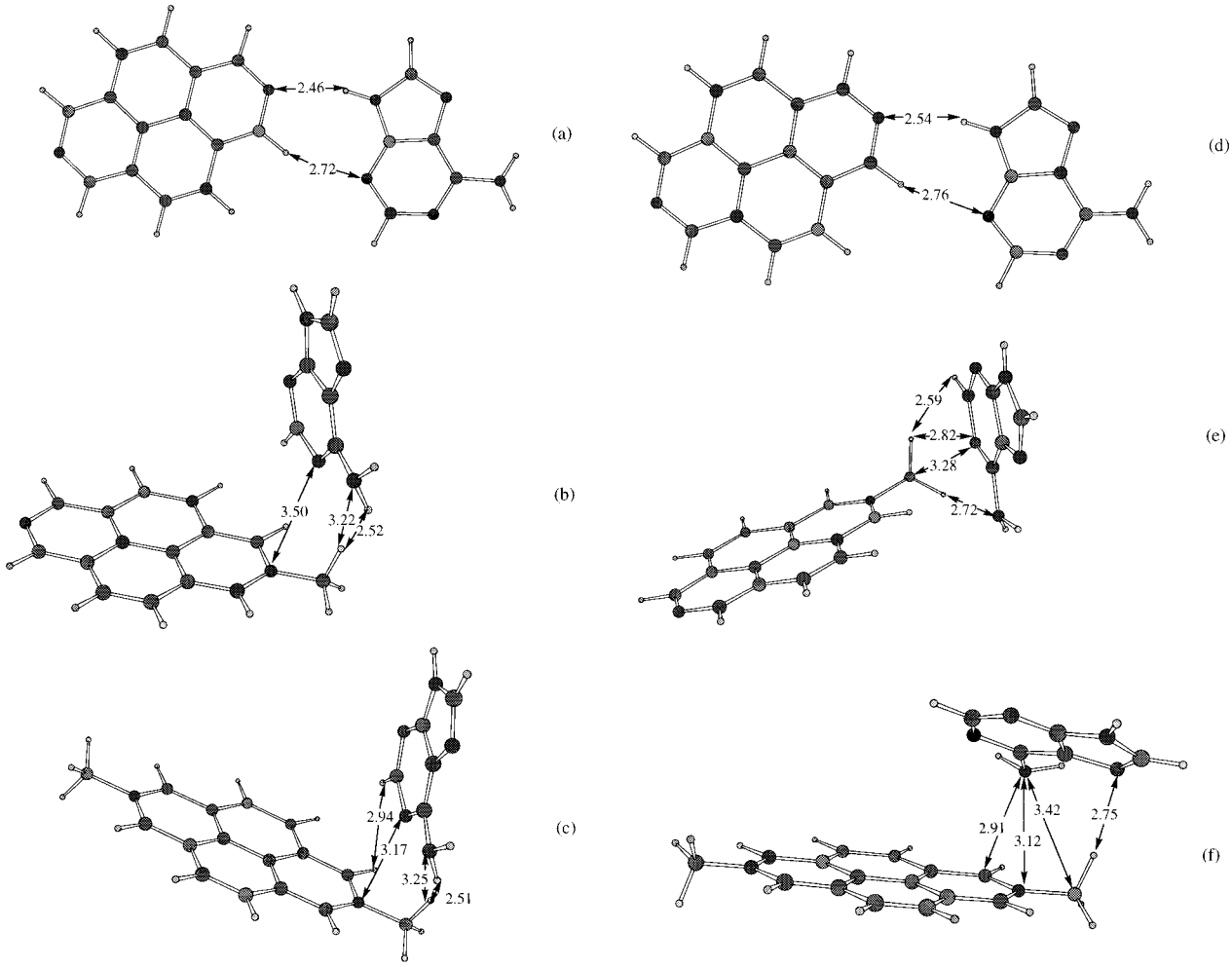


Figure 7. Fully optimized structures of (a), (d) A-DAP; (b), (e) A-MDAP$^+$; and (c), (f) A-DMDAP^{2+} according to PM3 [(a)−(c)], and AM1 [(d)−(f)]. The numbers are predicted interatomic distances in Å.

TABLE 5: Hartree-Fock Energies for PM3 Optimized Monomers, Face-to-face Complexes, and Fully Optimized Complexes

species	E_{HF}[a]	ΔE[b]	BSSE[b]	ΔE[b,c]	E_{SCRF}[a]	ΔE_{SCRF}[b]	BSSE[b]	ΔE_{SCRF}[b,c]
adenine	−464.49274				−464.49418			
DAP	−643.73951				−643.73951			
MDAP$^+$	−683.15032				−683.16112			
DMDAP^{2+}	−722.45676				−722.45676			
H_2O	−76.01056							
A-DAP (face-to-face)	−1108.23253	−0.18	0.01	−0.17	−1108.23305	0.40	0.19	0.59
A-DAP (PM3 opt)	−1108.24417	−7.48	1.11	−6.4	−1108.24489	−7.0	1.10	−5.9
H_2O−DAP (PM3 opt)	−719.75464	−2.87	1.26	−1.6				
A-MDAP$^+$ (face-to-face)	−1147.65052	−4.68	0.63	−4.1	−1147.65869	−2.1	0.63	−1.5
A-MDAP$^+$ (PM3 opt)	−1147.65216	−5.71	1.10	−4.6	−1147.65436	0.59	1.11	1.7
H_2O−MDAP$^+$ (PM3 opt)	−759.17317	−7.71	1.28	−6.4				
A-DMDAP^{2+} (face-to-face)	−1186.96332	−8.67	0.73	−8.0	−1186.97228	−13.4	0.74	−13
A-DMDAP^{2+} (PM3 opt)	−1186.96675	−10.82	1.33	−9.5	−1186.98646	−22.3	1.39	−21
H_2O−DMDAP^{2+} (PM3 opt)	−798.48740	−12.60	1.29	−11				

[a] In Hartrees. [b] In kcal/mol relative to monomers. [c] Including BSSE.

MDAP$^+$. As a secondary effect, 0.02 electrons are transferred from adenine to MDAP$^+$, thereby forming a weak ground-state charge-transfer complex.

As for the optimized A-DMDAP^{2+} complex (Figure 7c,f), its ground-state molecular geometry closely resembles that described for A-MDAP$^+$. PM3 predicts a pure edge-to-face arrangement, while AM1 leads to a somewhat more overlapping geometry. Just as in A-MDAP$^+$, there exist several edge-on molecular arrangements which are nearly isoenergetic. Both the PM3 and the AM1 calculations suggest that intermolecular

interactions involve the adenine N(1) and $DMDAP^{2+}$ N(2)/methyl region. The adenine polarization noted in A-$MDAP^+$ is enhanced in A-$DMDAP^{2+}$, yielding an adenine charge separation of ca. 0.09 electron units. Contrary to what we intuitively expected, we found the ground-state charge-transfer in A-$DMDAP^{2+}$ to be the same as in A-$MDAP^+$.

Discussion

The discussion will be divided in two parts. First we will discuss the calculated geometries and their properties, and in the second part, we will focus on the spectroscopic properties of the complexes, and compare with experiments.

Complex Geometry. It has been argued that basic face-to-face $\pi-\pi$ interactions, such as in the benzene dimer, are of repulsive nature, and that in order to obtain attractive forces, the π systems must be either laterally offset relative to each other, or assume a T-shaped geometry.[12−14] NMR-spectroscopically deduced interactions in aqueous solution between two adenine moieties tethered together by a flexible linker have been attributed to an intramolecular face-to-face arrangement due to anisotropic charge distribution in adenine.[15] This finding, along with molecular dynamics studies on the benzene dimer,[16] suggests that hydrophobic effects are not necessarily the dominating factor determining the association of aromatic species in aqueous solution.

For the A-DAP, A-$MDAP^+$, and A-$DMDAP^{2+}$ complexes, both AM1 and PM3 calculations lead to *local* energy minima for face-to-face geometries, as could be anticipated because of the presence of inhomogeneous charge distribution in the molecular plane of both adenine and the three diazapyrenes. The calculations show, however, that the face-to-face arrangement itself does not necessarily imply attraction, since it is only when the local dipoles/charges interact favorably that the total energy of the complex is lowered compared to that of the monomers. This is in agreement with earlier investigations in which the presence of charge donating or withdrawing groups was suggested to be important for face-to-face arrangements.[12,17,18]

Interestingly, in the optimal PM3 A-$MDAP^+$ face-to-face geometry, the dipole moments of adenine and $MDAP^+$ are not antiparallel, but form an angle of ca. 70° relative to each other, while AM1 predicts the dipoles of $MDAP^+$ and adenine to oppose each other.

The decrease in optimal distance with increasing charge, and thus decreasing π-electron density, is in agreement with predictions by the previously proposed π-charge model.[12,13] Although the $\pi-\pi$ orbital interaction should be much stronger in a face-to-face geometry than in the edge-to-face geometry, the reason for the weak charge-transfer in the face-to-face systems is that the potential is mainly repulsive, thus resulting in a large interplanar separation of the π systems. In the edge-to-face geometry, the smaller intrinsic interaction is compensated for by the closer proximity of the aromatic systems.

A common feature for the optimized geometries of A-$MDAP^+$ and A-$DMDAP^{2+}$ is the short nitrogen−nitrogen distance between adenine and diazapyrenium moieties (Figure 7). We are inclined to believe that we are mainly seeing non-hydrogen-bond electrostatic interactions in the ionic systems, and possibly weak hydrogen-bonding from the methyl group in the diazapyrenium ion to a nitrogen in adenine.

The PM3 and AM1 geometries of the complexes formed between water and DAP, $MDAP^+$, and $DMDAP^{2+}$ all predict the presence of a hydrogen bond from H(1) of the diazapyrene moiety to the oxygen of a water molecule (not shown). This arrangement does not necessarily affect the formation of the A-$MDAP^+$ and A-$DMDAP^{2+}$ complexes, because water and adenine will probably not be competing for the same association site.

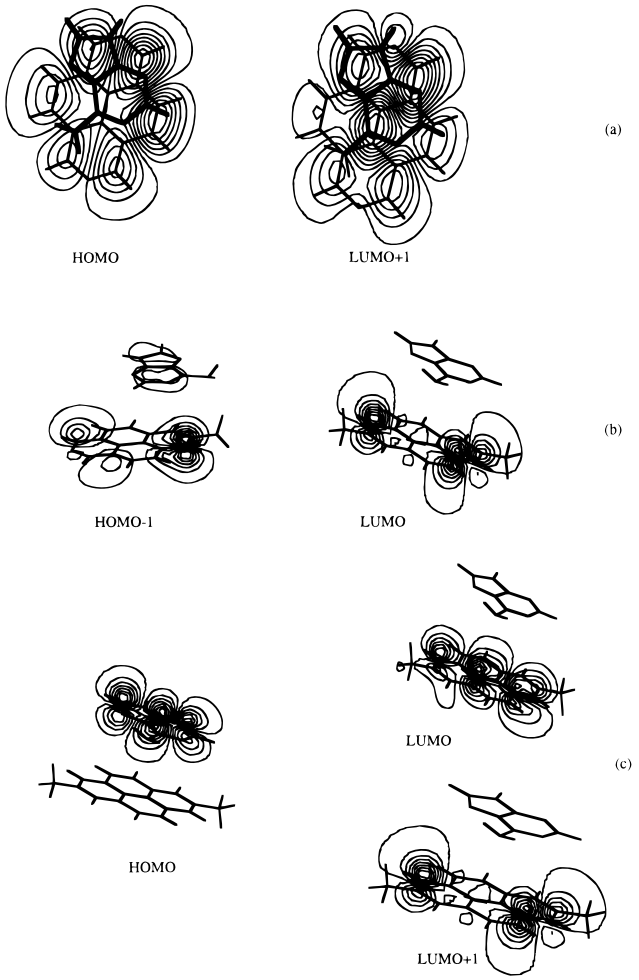


Figure 8. Molecular orbitals as calculated by ZINDO/S. (a) A-DAP, (b) A-$MDAP^+$, and (c) A-$DMDAP^{2+}$. In A-DAP a minor contribution to the first transition is (HOMO-1) → LUMO (not shown). These orbitals are also completely localized on DAP.

Inclusion of a solvent reaction field (using a high solvent dielectric constant to mimic the conditions in aqueous solution) does not substantially affect the energetic ordering of the complexes, except in A-$MDAP^+$, where the face-to-face complex is predicted to be the only (but marginally) stable form. For the A-DAP and A-$DMDAP^{2+}$ complexes, the SCRF energies basically just accentuate the energetic differences between the face-to-face and edge-to-face forms.

From the geometry calculations, the following points emerge. (1) Even when using a polarized continuum model for estimating the dielectric effect of the solvent, the calculations do not predict the correct geometries. (2) The predicted major attractive force in A-$MDAP^+$ and A-$DMDAP^{2+}$ is electrostatic interaction between the adenine nitrogens and the pyridinium nitrogens.

Calculated Spectra. ZINDO/S calculations for A-DAP (both face-to-face and hydrogen-bonded) indicate that the transitions at longer wavelengths than 300 nm are those of DAP alone, with the first transition being HOMO → LUMO+1 (Figure 8a; Table 1). The absence of any A-DAP charge-transfer in the excited state is in agreement with the experimental finding that the fluorescence of DAP is not quenched by adenine. Further, the electronic influence of adenine on the DAP transitions is predicted to be small, which agrees with the only slightly perturbed absorption spectrum of A-DAP.

By contrast, the calculated absorption spectrum of A-MDAP$^+$ contains charge-transfer transitions, albeit the first transition (HOMO-1 → LUMO; Figure 8b; Table 1) is localized on MDAP$^+$. Although they are characterized by very low intensity due to the negligible orbital overlap between adenine and MDAP$^+$, their presence indicates the feasibility of excited-state charge-transfer in A-MDAP$^+$. The strong transitions are almost exclusively localized on MDAP$^+$. For both face-to-face and edge-to-face A-DMDAP^{2+} complexes, the calculated absorption spectra reveal that the first two transitions are pure charge-transfer transitions (HOMO → LUMO; HOMO → LUMO+1) of near zero oscillator-strength (Figure 8c; Table 1). Moreover, even the electric dipole allowed DMDAP^{2+} L_b and L_a transitions have strong charge-transfer character, and the L_a transition at ca. 335 nm is split in two, nearly parallel polarized transitions (Table 1). The ZINDO/S results indicate that MDAP$^+$ and DMDAP^{2+} should differ significantly in their excited-state properties, and that the experimentally observed absorption broadening of A-DMDAP^{2+} and A-MDAP$^+$ could be explained by the presence of a series of low-energy charge-transfer states. Calculated spectra of the face-to-face complexes were found to be remarkably similar to those calculated for complexes of edge-to-face geometry (Table 1).

As intuitively expected, the calculated spectra of the face-to-face complexes show hypochromism (Table 1). By contrast, the diazapyrene transitions of the hydrogen-bonded and edge-to-face structures are predicted *stronger* than those of the diazapyrenes themselves, at variance with the experimental data. In addition, the transitions of the edge-to-face complexes exhibit a hypsochromic shift of as much as 430 cm^{-1} (A-DMDAP^{2+} L_b), while the diazapyrenes rather undergo a bathochromic shift upon complexation with adenine in water. Thus, from our absorption spectra and ^{1}H NMR data, we conclude that face-to-face orientation is dominating in aqueous solutions. This implies the following. (1) Since the face-to-face A-DAP complex is energetically disfavored compared to the hydrogen-bonded one (Table 5), the association in H_2O must be driven by the hydrophobic effect. (2) For A-MDAP$^+$ and A-DMDAP^{2+}, the hydrophobic effect favors the face-to-face arrangement, thus effectively making the edge-to-face arrangement unfavorable. On this basis, the lower association constant for A-DMDAP^{2+} can be explained. Since the "hydrophobic surface" of DMDAP^{2+} is smaller than that of MDAP$^+$ (pyridine vs pyridinium), the entropic gain in the association can be expected to be smaller for DMDAP^{2+} than for MDAP$^+$. This line of reasoning is consistent with results of previous experiments, where the association of adenosine with the considerably more hydrophobic *N,N′*-dimethylanthra[2,1,9-*def*:6,5,10-*d′e′f′*]-diisoquinoline dichloride was found have a binding constant of ca. 300.[3] Further supporting the face-to-face geometry is the fact that intercalation of diazapyrenes between basepairs in DNA produces similar spectral effects as complexation with adenine.[1]

Conclusions

The semiempirical calculations of the electronically excited states can reasonably well account for the experimental shifts and transition moments observed in the isolated diazapyrenes and in their adenine complexes. These assignments are valuable for the interpretation of linear dichroism and circular dichroism spectra of corresponding adducts with nucleic acids. Most interestingly, the recently reported red tail of weak absorption observed in complexes of DMDAP^{2+} with DNA (and to less extent with the MDAP$^+$ complexes) can be explained in detail in terms of emerging new charge-transfer transitions. These are calculated to be noticeably more pronounced in the A-DMDAP^{2+} complex than in A-MDAP$^+$. Even the allowed $\pi-\pi^*$ transitions in A-DMDAP^{2+} were concluded to have considerable charge-transfer character. Neither absorption spectra, nor calculated electronic transitions indicate any ground- or excited-state charge-transfer for A-DAP.

As to the geometry calculations, all theoretical methods failed to predict the correct face-to-face structures, except for A-MDAP$^+$ (which agreement seems a mere coincidence). The failure to predict the correct face-to-face structures can be ascribed to the neglect of entropic stabilization (hydrophobic effects) in the calculations. However, the calculations do give valuable information about the nonentropic parts of the interaction between the diazapyrenes and adenine. Should entropic effects (hydrophobicity) alone have been responsible for the association, one would have expected A-DAP to be more stable than A-MDAP$^+$, which in turn should be more stable than A-DMDAP^{2+}, due to the gradual loss of hydrophobic surface. However, it seems that A-MDAP$^+$ is at least as stable as A-DAP, indicating the importance of the electrostatic interaction between the pyridinium and electron-rich regions of adenine. By contrast, in A-DMDAP^{2+} apparently enough hydrophobicity is lost to lower the association constant compared to A-DAP and A-MDAP$^+$.

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