Search for DNA Repair Inhibitors: Selective Binding of Nucleic Bases−Acridine Conjugates to a DNA Duplex Containing an Abasic Site†

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Received April 3, 1997⊗

The abasic site is one of the most frequent DNA lesions generated by spontaneous or enzymatic cleavage of the N-glycosidic bond. The abasic site is also an intermediate in the nucleotide and base excision DNA repair. We examined molecules which recognize and cleave DNA at the abasic site with high efficiency. These molecules incorporate in their structure a nucleic base for abasic site recognition, an intercalator for DNA binding, and a polyamino linker for ionic interaction and DNA cleavage. Such compounds, by interfering with abasic sites in DNA, are also inhibitors of DNA repair. In order to better understand the parameters of the interaction, we carried out a UV thermal denaturation study of synthetic oligonucleotides containing the lesion both in the absence and in the presence of the drugs. A similar study was also carried out using the corresponding nonmodified oligonucleotide. The results indicate selective binding of the base−chain−intercalator conjugates to the abasic site containing oligonucleotides.

The removal of a heterocyclic base in DNA resulting from the hydrolysis of the N-glycosidic bond produces an abasic site or AP site (apurinic or apyrimidinic site). Abasic site formation occurs spontaneously under physiological conditions due to depurination,[1] a process that is markedly increased by modification of the purines at N-3 and/or N-7 positions, either chemically[2] (alkylating agents, carcinogens...) or by physical agents[3] (UV, γ-radiations...). Abasic sites are also produced enzymatically in the repair process involving specialized DNA *N*-glycosylases[4−7] which remove damaged or abnormal bases. The action of DNA *N*-glycosylases followed by the action of AP-endonucleases which cleave the phosphodiester bond adjacent to the lesion constitutes the initiation step in the base excision repair pathway of many DNA lesions. If unrepaired, AP sites are bypassed inefficiently in cells.[8,9−11] When bypass occurs, mutation can result since AP sites are noninstructive lesions.[12] Abasic site repair is thus a critical cellular activity. Two major classes of repair enzymes are known to cleave DNA at abasic sites. Class I AP-endonucleases, also known as AP-lyases (3′-AP-endonucleases), cleave the 3′-phosphodiester bond of the AP site by a β-elimination mechanism, leaving an α,β-unsaturated carbonyl derivative at the 3′ terminus[13−15]. Class II AP-endonucleases (5′-AP-endonucleases) cleave hydrolytically the phosphodiester bond 5′ to the AP site producing a 3′-hydroxyl nucleotide and a 5'-phosphate deoxyribose residue.[16] Some small molecules have been shown to mimic AP lyase activity. Polyamines are the simplest ones.[17] A tripeptide (Lys-Trp-Lys)[18−20] and intercalating agents such as 9-aminoellipticine[21,22] or 3-aminocarbazole[23,24] are also able to cleave AP sites with high efficiency.

Due to the biologically significant roles of abasic sites as intermediates in the repair of damaged DNA and as mutagenic and carcinogenic lesions, there has been a great challenge in the last years to design molecules which could be able to selectively recognize this lesion and interfere with the repair process.[25, 26] We have synthesized a family of molecules that recognize and cleave specifically DNA at abasic sites through β-elimination catalysis.[27−32] A schematic representation of the cleavage complex is shown in Figure 1. These molecules are composed of different structural units which have a defined role: (a) an intercalator for targeting the molecule to DNA, (b) a nucleic base for the recognition of the abasic site, and (c) a polyamino linker endowed with two functions, the protonated amino groups are implicated in the ionic binding of the molecule to the phosphate backbone and the unprotonated amino groups catalyze the cleavage of the phosphodiester bond adjacent to the abasic site through a β-elimination mechanism.[27] We successively modified each of the three moieties (i.e., intercalator, chain, base) to obtain molecules possessing the highest cleavage activity. The most efficient ones, i.e., 2,6-diaminopurine−triamine−acridine (DAP-triam-Acr, **1**) and adenine−triamine−acridine (Ade-triam-Acr, **2**) (Figure 1), have been shown to cleave the abasic lesion present in a DNA molecule at a nanomolar concentration (pBR 322 DNA plasmid containing an average of 1.8 apurinic sites in its 4362 base pair sequence).[29] These "synthetic nucleases" exhibit a cleaving efficiency for apurinic sites in DNA much higher than the prototypical tripeptide Lys-Trp-Lys .[29] In both compounds (**1** and **2**), the amino functions of the chain are separated by methylene groups. We recently reported that the length of these polymethylene groups controls the pK_a's of the two amines[32] and adjusts the size of the global molecule to fit the abasic site locus. In a recent study,[33] we have investigated the hypothesis that compounds **1** and **2**, interacting with the abasic site, might inhibit the base excision DNA repair. Such an inhibitory effect might be useful to sensitize tumor cells to the activity of some alkylating agents. Indeed nitrosoureas are compounds whose antitumor activity is related to their ability to generate modified nucleic bases in cellular DNA.[34]

† This work was supported by the Association pour la Recherche sur le Cancer (ARC) and the Ligue Nationale Française Contre le Cancer (LNFCC).

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⊗ Abstract published in *Advance ACS Abstracts,* August 1, 1997.

S0022-2623(97)00225-2 CCC: $14.00

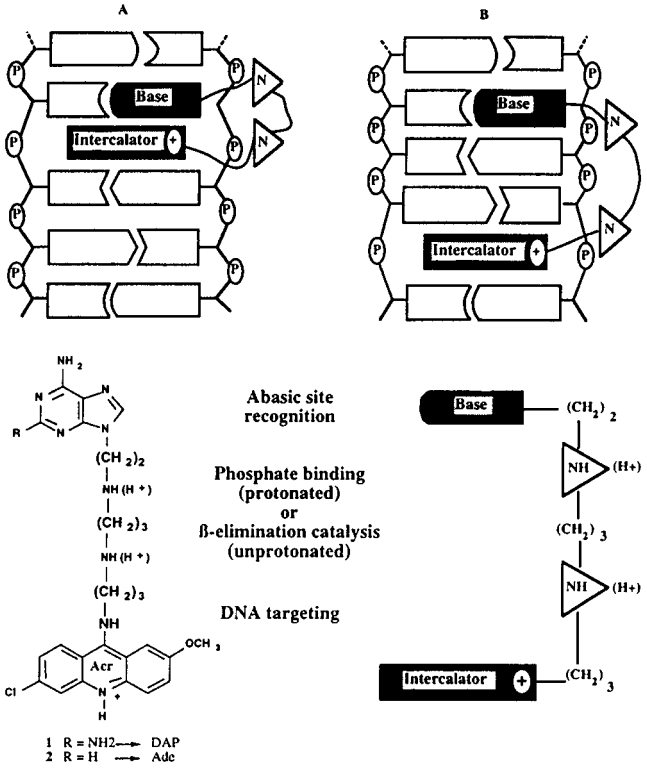
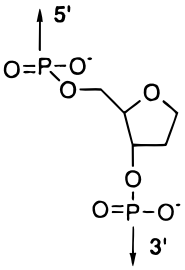


Figure 1. Structures of the synthetic molecules and schematic representation of their interaction at the abasic site.

Some of these damages are repaired by the nucleotide and base excision repair process.[35] By using a new *in vitro* assay based on the quantification of nucleotide and base excision repair, it was shown that compounds **1** and **2** act as inhibitors of the repair pathway. Furthermore, these drugs potentiate the toxic effect of the alkylating anticancer drug *N*, *N*′-bis(2-chloroethyl)-*N*-nitrosourea (BCNU) on L1210 cells in culture. The toxic effect of BCNU (25% cell proliferation at 10^{-5} M) was increased 100 times in the presence of a noncytotoxic concentration (10^{-6} M) of **2** (25% cell proliferation at 10^{-7} M).[33, 36]

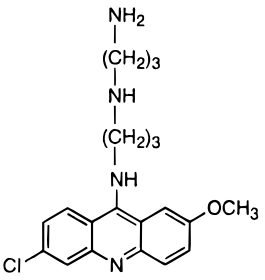
In order to better understand the parameters necessary to the interaction at the abasic site and to design a system able to interfere efficiently with the repair of the abasic lesion in DNA, we undertook a thermal denaturation study (T_m measurement) of synthetic oligonucleotides containing the lesion both in the absence and in the presence of drugs **1** and **2**. For the initial tests of relative binding affinities, we used ΔT_m measurements (where $\Delta T_m = T_m$(duplex in presence of drug) $- T_m$(duplex alone)) under conditions where the ΔT_m values are directly related to the thermodynamic binding constants.[37] The advantage of this method is that it provides direct comparison of relative affinities. This method is quite generally used to determine the T_m and the thermodynamic data although it implicates several approximations.[38] Due to the instability of the abasic site and the efficiency of the artificial nucleases **1** and **2**, we replaced the deoxyribose moiety with a chemically stable analog, 3-hydroxy-2-(hydroxymethyl)-tetrahydrofuran[39,40] (Chart 1). This analog (designated as X) was incorporated in strand 2 using phosphoramidite chemistry. A 1:1 mixture of strand 1 (T strand) and the modified strand 2 (X strand) produces a duplex designated as the "TX duplex" containing an apurinic site analog, in which the base opposite the lesion, i.e., a thymine, is susceptible to pair with the base (adenine or diaminopurine) of synthetic molecules **1** and **2**. Note

Chart 1



Abasic site analog (X)

Chart 2



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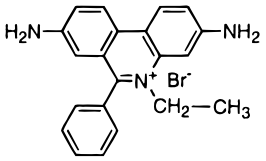

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Strand 1 : GCGTGTGTGCG	Strand 1 : GCGTGTGTGCG
Strand 2 : CGCACXCACGC	Strand 3 : CGCACACACGC
TX duplex	TA duplex

that when X is replaced by A, strand 3 (referred to as the A strand) can pair with strand 1 (referred to as the T strand) to form a fully paired Watson−Crick double helix designated as the "TA duplex". The thermal denaturation studies were investigated on the Watson−Crick TA duplex and the modified abasic TX duplex. We measured the effect on the duplex stability of drugs **1** and **2** and for comparison, of molecules devoid of the recognition moiety, the acridine−triamine (Acr-triam, **3**) and a classical intercalator, ethidium bromide (EB, **4**) (Chart 2).

Results and Discussion

Influence of the Apurinic Lesion on the Stability of the DNA Duplex. We used UV spectrophotometry to study the thermal denaturation and to determine T_ms for the abasic site containing duplex (TX) and for the conventional TA duplex. Figure 2 shows the curves $\alpha = f(T)$ obtained from the standard UV melting curves (as described in the Experimental Section) for the two duplexes. The sigmoidal curves exhibit melting cooperativity and were analyzed to derive the T_m values and the Van't Hoff transition enthalpy ΔH_{VH}, entropy ΔS_{VH}, and free energy $\Delta G°_{VH}$. The resulting data are listed in Table 1. Very few studies of the effect of abasic sites on duplex stability are described in the literature. In 1984, Millican and co-workers[39] investigated for the first time the consequences of this lesion from the thermodynamic point of view. They determined the T_ms (by UV spectrophotometry) of two 15-mer duplexes contain-

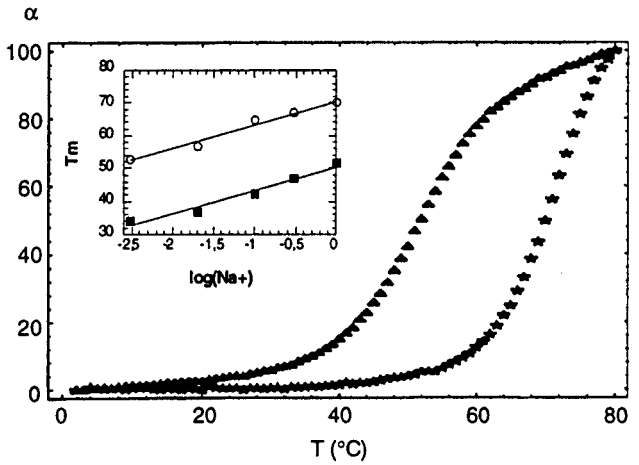


Figure 2. T_m curves for TX (▲) and TA (★) in phosphate buffer (0.01 M sodium phosphate, 0.001 M EDTA, 1 M NaCl, pH 7). For salt concentration dependence, the T_m studies were conducted in a range of salt concentrations (top, insert): TA duplex (○) and TX duplex (■).

Table 1. Spectroscopically Measured T_m,[a] ΔT_m, and Van't Hoff Transition Enthalpies ($\Delta H°$), Entropies ($\Delta S°$), and Free Energies ($\Delta G°$) for the Disruption of the Fully Paired TA Duplex and the Duplex Containing the Model Abasic Site (TX)

duplex	$\Delta H°$	$\Delta S°$	$\Delta G°$ [b]	T_m (°C)	ΔT_m (°C)
TA	89.2	235	19.2	70	
TX	56.4	149	12	51.5	18.5

[a] T_m was measured at a total strand concentration of 7.69 μM in 10 mM phosphate buffer (pH 7.0) containing 1 mM EDTA and 1 M NaCl. $\Delta T_m = T_m$(TA) − T_m(TX). The free energy and enthalpy data are in kcal/mol; the entropy data are in cal/mol/K. [b] $\Delta G°$ was calculated at 25 °C.

ing an apyrimidinic site analog and the corresponding fully paired duplexes. The T_m decrease corresponding to the loss of a thymine and a cytosine was respectively 16.5 and 15 °C. In 1989, Grollman and Breslauer[41] reported a similar study on the effect of an apyrimidinic site analog (the missing base being a thymine) on the stability and melting behavior of a DNA duplex of 13 bp. They showed that the abasic site led to a decrease in duplex stability equal to 13.9 °C and a loss in free energy of 6.5 kcal/mol. All these thermodynamic studies relate to oligonucleotides containing apyrimidinic site analogs. The duplex that we have studied is different in terms of length and sequence (11-mer and GC rich), and the missing base is a purine. Inspection of the melting profiles and T_m values reveals that the apurinic site analog X decreases the duplex melting temperature (ΔT_m) by almost 18.5 °C relative to the conventional TA duplex and leads to a reduction in free energy ($\Delta\Delta G°$) by 7.2 kcal/mol (at 25 °C). These effects are much more important than those reported for the apyrimidinic site analogs. The greatest decrease in stability provoked by the abasic site in our 11-mer duplex compared to the 13-mer duplex reported by Grollman[41] can be explained by the fact that a purine ring contributes more to the base stacking than a pyrimidine, and thus loss of a purine is more destabilizing than loss of a pyrimidine.

We also investigated the influence of the salt concentration on the duplex stabilities. Curves expressing the evolution of T_ms as a function of log(sodium ion activity) are reported in Figure 2 (top, insert). As expected, increasing the salt concentration increases the duplex stability. T_m vs log(sodium ion activity) plots are linear

Table 2. T_m Values Measured for the TX Duplex in the Presence of Increasing Amounts of DAP-triam-Acr (**1**) at Various Salt Concentrations[a]

	r[b]				
[Na^+]	0	0.5	1	1.5	2
3 mM	34.2	53	57.7	60	61.6
20 mM	36.9	52.6	57.3	60.5	61.9
0.1 M	42.7	56	59.3	60.3	61.1
0.3 M	47.1	54	55.8	56.5	57
1 M	51.5	54.3	55.2	55.7	55.5

[a] T_m was measured at a total strand concentration of 7.69 μM and various ratios of the drug **1** in 10 mM phosphate buffer (pH 7.0) containing 1 mM EDTA and various concentrations of NaCl. [b] r = [drug]/[duplex].

in both cases with slopes for TA and TX of 6.8 and 7.2, respectively. This order of magnitude is expected for short oligonucleotides having low charge density compared to DNA or RNA polymers. Wilson and coworkers[42] reported an 8.4 slope value for an octamer duplex. The presence of an abasic site does not seem to significantly affect the slope value, and ΔT_m (ΔT_m = T_m(duplex TA) − T_m(duplex TX)) appeared to be insensitive to salt concentration. Whatever the sodium ion activity, the T_m measured for the TX duplex is lower than the T_m measured for the TA duplex. The linearity of the plots indicates that it is possible to compare the two duplexes at any salt concentration.

Influence of Salt Concentration on Duplex Stability in the Presence of Drugs. Since the nucleic acid backbone is highly charged,[43] its structure and interaction with small cationic ligands are strongly dependent on the ionic content of the medium. Binding of counterions reduces the effective charge of the nucleic acid and strongly stabilizes the double-stranded form. On the other hand, this increase of the ionic strength strongly affects the interaction properties of the cationic ligands with the polymer in solution. In order to study the salt effect on the duplex stabilization by specific drugs, we measured the T_ms of the TX duplex in interaction with DAP-triam-Acr (**1**), one of the drugs that exhibited the best abasic site recognition and cleavage.[29] The r ratio (r = [drug]/[duplex]) and the sodium concentration were varied. T_m values are listed in Table 2 and are shown as a function of log(sodium ion activity) in Figure 3. The drug stabilizes the duplex in all conditions of ionic strength and concentration. First observations of the plots indicate the dramatic influence of salt concentration on the variations of T_m values in the presence of increasing amounts of the drug. Very large variations are observed at low ionic strengths, while higher ionic strengths seem to level out the influence of the drug on the T_m value of the duplex. This is illustrated by the 27.4 °C T_m variation measured in 3 mM NaCl solution when the drug to DNA ratio is varied from r = 0 (T_m 34.2 °C) to r = 2 (T_m 61.6 °C). The corresponding value falls to 4 °C in 1 M NaCl (T_m 51.5 and 55.5 °C, respectively, for r = 0 and 2). It is interesting to examine graphically the evolution of T_m as a function of ionic strength. It appears that the difference in the T_m variations at different ionic strengths results from the combination of at least two effects. (1) In the absence of drug (r = 0) high ionic strength, as already stated, stabilizes the duplex which is reflected by the 17.3 °C increase of the T_m measured in 1 M NaCl (T_m 51.5 °C) compared to the value (T_m 34.2 °C) monitored in 3 mM NaCl. (2) In the presence of 2 equiv

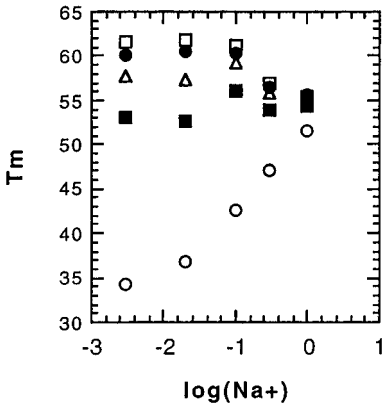


Figure 3. Effect of sodium ion activity on the T_m for the TX duplex in the absence and presence of different amounts of DAP-triam-Acr (**1**). T_m values were measured for different ratios (r) of drug: r = [drug]/[TXduplex] = (○) 0, (■) 0.5, (△) 1, (●) and 1.5, (□) 2 at pH 7; buffer, 0.01 M sodium phosphate, 0.001 M EDTA, and various concentrations of NaCl (0.003, 0.02, 0.1, 0.3, 1 M).

of drug (r = 2), increasing the NaCl concentration in the same range now leads to a 6.1 °C decrease of the T_m value (T_m 61.6 compared to 55.5 °C, respectively, in 3 mM and 1 M NaCl). This is an interesting observation that cannot be interpreted in simple terms since association of the drug to the duplex involves a number of different types of interactions including notably ionic interactions of the positively charged drug with the DNA phosphates. For our study, we decided to use a salt concentration of 20 mM. At this ionic strength, the value of the T_m is strongly affected by the drug concentration.

Duplex Stabilization and Drug Selectivity. We studied the influence of specific drugs and intercalators on the stability of the duplexes. We tried in particular to demonstrate a specific recognition of the abasic lesion by compounds containing the AP site recognition moiety, i.e., having the base−chain−intercalator structure. A series of T_m measurements were made for the TX duplex and the conventional TA duplex in the presence of various concentrations of different drugs: DAP-triam-Acr **(1)**, Ade-triam-Acr **(2)**, a related derivative devoid of recognition moiety, Acr-triam (**3**), and a classical intercalator such as EB (**4**). The T_m and ΔT_m values (ΔT_m = T_m(duplex in presence of drug) − T_m(duplex alone)) are listed in Table 3. Figures 4 and 5 show the curves expressing the evolution of T_m and ΔT_m as a fonction of the ratio r = [drug]/[duplex].

I. Influence of the Drugs on the Stability of the TA Duplex. All four molecules stabilize the TA duplex quite efficiently. The T_m values exhibit a regular increase when the drug to DNA ratio r is increased. The shapes of the four curves are quite comparable showing no important variations in the slopes, which suggests absence of selectivity and specific stoichiometries for the drug−DNA complexes. However the magnitudes of the T_m variations (ΔT_m) differ significantly for the four molecules, with DAP-triam-Acr (**1**) and Acr-triam (**3**) exhibiting respectively the smallest and highest stabilizations, while the two drugs Ade-triam-Acr (**2**) and DAP-triam-Acr (**1**) behave similarly causing intermediate stabilization. All four molecules possess a chromophore moiety known to intercalate without selectivity but relatively efficiently in DNA. They differ by the presence of a polyamino chain either linked or not linked to a base residue. The data suggest a major contribution of this chain that can bind through ionic interactions between the protonated amino groups and the DNA phosphates.

To better appreciate this contribution we measured the pK_as of drugs **1**−**3** by potentiometric titrations. pK_as are presented in Table 4. The titration curves for **1** and **3** are reported in Figure 6. (The titration curves for **1** and **2** are identical.) From these figures, we can graphically estimate the proportions of the different protonated forms at a given pH. At pH 7, this gives 23% of the biprotonated species and 77% of the triprotonated species for **3**, compared to 4% of the monoprotonated species, 57% of the biprotonated species, and 36% of the triprotonated species for compound **1**. Schematically it can be considered that EB (**4**) carries one permanent positive charge located on the ring and that the major species in solution at pH 7 of the two drugs Ade-triam-Acr (**2**) and DAP-triam-Acr (**1**) possess two positive charges, one on the intercalator and one on the chain, while Acr-triam (**3**) carries three positive charges, two of them being in the chain. All the melting temperature variations (ΔT_m) induced by the drugs could thus be interpreted in terms of stabilization of the duplex by a combination of the well-known $\pi-\pi$ stacking interactions of intercalators with the neighboring base pairs and the ionic interactions with the phosphate backbone. It corresponds to a ΔT_m increase of 7.6 °C at r = 2 for ethidium bromide. For Ade-triam-Acr (**2**) and DAP-triam-Acr (**1**), the presence of an additional charge brought by the linker leads to additional duplex stabilization (ΔT_m 12 °C, r = 2), while the nucleic bases contribute little or zero to stabilization. The presence of two positive charges in the polyamino chain of Acr-triam (**3**) leads to the highest effect (ΔT_m 14.8 °C at r = 2). Such an interpretation, based essentially on stacking and ionic interactions of the polyamino chain, cannot be rigorously demonstrated; however, all data are in accordance with that scheme. In addition it explains the regular increase of T_m observed for all drugs when r is increased. No significant changes in the slopes of the curves are observed since all measures have been performed at relatively low drug to DNA ratios, i.e., much below saturation of all intercalation sites that exist in the duplex.

II. Influence of the Drugs on the Stability of the TX Duplex. The plots relating the increase of the melting temperature to the amount of drug added are quite different from those measured for the TA duplex. Stabilization brought by the drugs is considerably higher for all molecules. The weakest effect is observed for EB (**4**). At high values of r (r > 1), the most stabilizing drug is Acr-triam (**3**), while compounds **1** and **2** behave similarly. This observation suggests that again ionic binding of the chain of **3** prevails in the interaction with the duplex. Nevertheless, at low drug/AP site ratio ($r \leq 0.5$), the behavior of the three molecules **1**−**3** is comparable. The superimposition of the curves for **1**−**3** at $r \leq 0.5$ can be explained by the fact that the base moieties adenine and diaminopurine now contribute to the stabilization of the duplex by docking into the abasic site. The resulting stacking interactions between the base of the drug and the bases flanking the abasic lesion participate in the binding to the duplex to an extent comparable to the contribution

Table 3. ΔT_m Values Calculated for the TA/TX Duplexes in the Presence of Various Ratios of the Indicated Drugs in 20 mM NaCl

	r^a						
drugs	0.2	0.4	0.5	0.6	1	1.5	2
1	−/7.8	−/12.6	4.1/15.7	−/17.5	8.3/20.4	10.7/23.6	11.8/25
2	3.1/−	4.6/−	5.2/15.7	6.1/−	8.8/21.6	11.1/24.1	12.1/−
3	−/−	−/−	6.3/15.2	−/−	10.5/21.8	12.9/25.8	14.8/28.9
4	−/−	−/−	2.1/5.8	−/−	4.6/9.9	6.2/13.6	7.6/15.6

[a] r = [drug]/[duplex]. A slash separates T_m values obtained for the TA duplex from those obtained for the TX duplex. $\Delta T_m = T_m$(duplex in the presence of drug) − T_m(duplex alone).

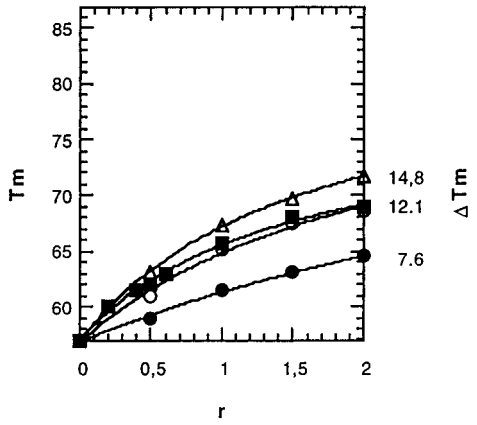


Figure 4. Influence of drugs on the T_m of the fully paired TA duplex. Experiments were conducted at pH 7.0 in a buffer containing 0.01 M sodium phosphate/0.001 M EDTA/0.02 M NaCl. The different drugs tested were (○) DAP-triam-Acr (**1**), (■) Ade-triam-Acr (**2**), (△) Acr-triam (**3**), and (●) EB (**4**). T_m values were measured for various ratios (r) of drugs (r = [drug]/[duplex] = 0.2, 0.4, 0.5, 0.6, 1, 1.5, 2). ΔT_m is defined as the difference between the T_m of the duplex in the presence of drug and the T_m of the duplex alone.

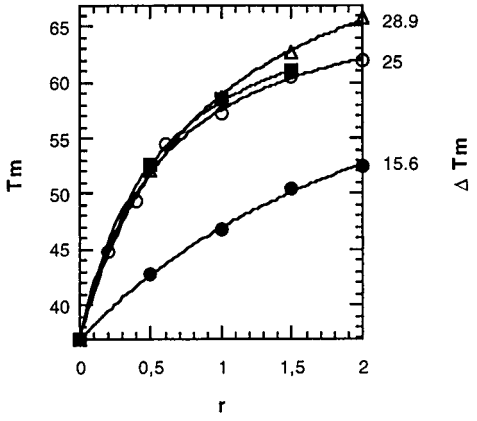


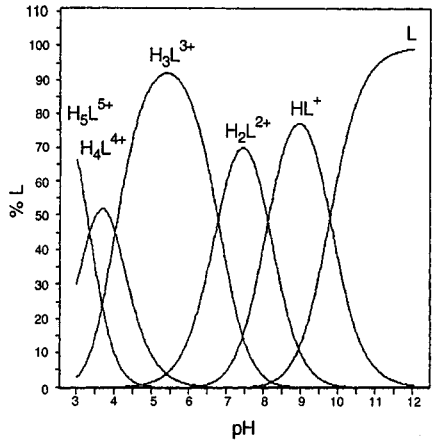
Figure 5. Influence of the drugs on the T_m of the TX duplex (containing the analog abasic site). Experiments were conducted at pH 7.0 in a buffer containing 0.01 M sodium phosphate/0.001 M EDTA/0.02 M NaCl. The different drugs tested were: (○) DAP-triam-Acr (**1**), (■) Ade-triam-Acr (**2**), (△) Acr-triam (**3**), and (●), EB (**4**). T_m values were measured for various ratios r of drugs (r = [drug]/[duplex] = 0.2, 0.4, 0.5, 0.6, 1, 1.5, 2). ΔT_m is defined as the difference between the T_m of the duplex in the presence of drug and the T_m of the duplex alone.

brought by the additional positive charge present on the chain of Acr-triam (**3**). At r values below 1, the abasic site is not fully occupied so the stabilizing effect of the base is observed. Beyond this value, the association of the drugs is nonspecific and leads to characteristics similar to those observed for the TA duplex: Acr-triam (**3**) shows the strongest stabilizing effect. The selectivity of drugs **1** and **2** for the abasic site can be expressed in more quantitative terms by examining the $\Delta\Delta T_m$ values ($\Delta\Delta T_m = \Delta T_m$(TX) − ΔT_m(TA), in the presence of drug). These $\Delta\Delta T_m$ values provide a differential comparison of the effects of the presence of an abasic site on the binding of the drugs and reflect the selectivity of the drugs for the abasic pocket. These data are collected in Table 5.

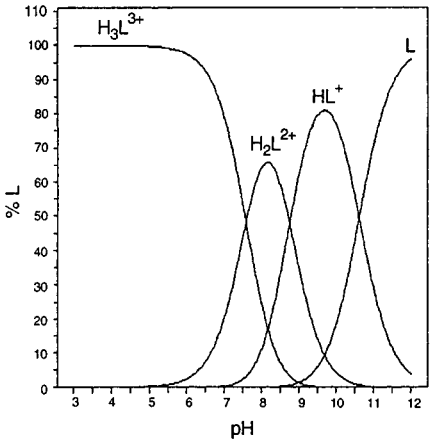
Table 4. Deprotonation Equilibrium Constants for Compounds **1**−**3**

drugs	$-\log K = pK_a$			
1	9.8	8.1	6.7	4.0[a]
2	9.7	8.5	6.5	2.9
3	10.0	8.7	7.6	

[a] A second protonation constant for the purine ring has been measured at pH < 4.



DAP-triam-Acr **1**



Acr-triam **3**

Figure 6. Species distribution obtained by potentiometric titrations for DAP-triam-Acr (**1**) (concentration = 5×10^{-4} M) and Acr-triam (**3**) (concentration = 5×10^{-4} M) over the pH range 2−12. L represents the nonprotonated form of the ligand; HL^+, the monoprotonated ligand; H_2L^{2+}, the biprotonated form; H_3L^{3+}, the triprotonated species; H_4L^{4+}, the tetraprotonated form; and H_5L^{5+}, the pentaprotonated ligand.

Table 5. $\Delta\Delta T_m$[a] Values

	r[b]			
drugs	0.5	1	1.5	2
1	11.6	12.1	12.9	13.2
2	10.5	12.8	13	
3	8.9	11.3	12.9	14.1
4	3.7	5.3	7.4	8

[a] $\Delta\Delta T_m = \Delta T_m$(TX duplex) $- \Delta T_m$(TA duplex). [b] r = [drug]/[duplex].

The r value at which the phenomenon is analyzed is of great importance. Indeed specificity of the interaction could be observed at low ratios (below 1) when the drug concentration does not allow total occupancy of the single specific binding site existing in the duplex (the abasic pocket). At higher ratios ($r > 1$) saturation of the specific site has occurred and the drug can further interact nonspecifically by intercalation. The specificity for compounds **1** and **2** is clearly demonstrated by the high value of $\Delta\Delta T_m$ at $r = 0.5$ (11.6 and 10.5 °C, respectively) compared to the weaker value for Acr-triam (**3**) (8.9 °C) and EB (**4**) (3.7 °C). When the drug concentrations increase, $\Delta\Delta T_m$ values tend to be leveled for **1**−**3** and to rise slowly in similar proportions as a result of nonspecific intercalation.

Conclusion

The thermal denaturation study reported here reveals that the presence of an apurinic site in a 11-mer DNA duplex dramatically reduces the stability of the duplex relative to the corresponding fully paired duplex while not altering its melting cooperativity. The influence of the global charge of the drugs on their interaction with DNA was clearly shown on the natural duplex where the order of stabilization is as follows: At pH 7.0, compound **3** exists mainly as a tricharged species and exerts a more stabilizing effect than **1** and **2** (that are mainly bicharged) and the monocharged EB (**4**).

The study of the duplexes in interaction with the drugs showed that the abasic lesion is specifically recognized by the intercalator−base conjugates **1** and **2**. The contribution of the nucleic base in the stabilization process is comparable to the contribution brought by a positive charge interacting with the phosphate backbone. The results extracted from the fairly straightfoward technique of T_m measurement are of great interest as they parallel the structural data obtained by a detailed high-field NMR and molecular modeling study of the drug−oligonucleotide complex.[44−46] The NMR study provided a clear picture of the complex formed between the TX duplex and compound **1**. It was shown that the acridine moiety was intercalated in the DNA duplex at a 2 bp distance on the 5′-side of the lesion. The recognition moiety (2,6-diaminopurine) inserted into the abasic pocket, pairing with the thymine that faces the apurinic site analog in the opposite strand, is likely in the Hoogsteen mode. The polyamino linker lies in the minor groove.

All these results provide encouraging evidence that derivatives can be designed that interact specifically and strongly with abasic sites in DNA thereby interfering with the repair processes. In addition they show that the T_m measurement could be a fast and straightforward technique to assay the selective recognition of the abasic site by specific drugs.

Experimental Section

Oligonucleotide Synthesis. Conventional or modified (containing the abasic site model: tetrahydrofuran, X) oligodeoxyribonucleotides were synthesized using standard solid phase cyanoethyl phosphoramidite chemistry on a Milligen/Biosearch 8700 DNA synthesizer. The detritylated oligonucleotides were purified by anion exchange chromatography on a Mono Q HR 5/5 column (Pharmacia) and then desalted on Waters Sepack cartridges. Oligonucleotide concentrations were checked spectrophotometrically by measuring the absorbance at 260 nm and using their respective single-stranded molar extinction coefficients (strand 1, $\epsilon = 129\,200\ M^{-1}\ cm^{-1}$; strand 2, $\epsilon = 112\,800\ M^{-1}\ cm^{-1}$; strand 3, $\epsilon = 128\,200\ M^{-1}\ cm^{-1}$).

Preparation of the Solutions. Standard solutions of the DNA oligomers were prepared in a buffer consisting of 10 mM sodium phosphate/1 mM EDTA/various NaCl concentrations, adjusted to pH 7. Except for ethidium bromide obtained from Sigma, the drugs have been synthesized in the laboratory.[29,30] Concentrations of the drugs solutions were determined spectrophotometrically by measuring the absorbance at 420 nm and using the molar extinction coefficient specific to each compound (DAP-triam-Acr (**1**), $\epsilon = 9430\ M^{-1}\ cm^{-1}$; Ade-triam-Acr (**2**), $\epsilon = 9500\ M^{-1}\ cm^{-1}$; Acr-triam (**3**), $\epsilon = 9500\ M^{-1}\ cm^{-1}$).

UV Absorption Spectrophotometry. The absorbance versus temperature melting profiles of the Watson−Crick TA duplex and the modified TX duplex were measured at 260 nm on a Lambda 5 UV/visible spectrophotometer equipped with a Perkin Elmer C570-070 temperature controller and interfaced with a EPSON AX2e computer. All duplex melting curves were measured at a strand concentration of 7.69 μM. Before each melting experiment, samples were heated for 5 min at 80 °C and cooled down slowly to make sure that the starting oligonucleotides were in the duplex state. Samples were heated at a rate of 1 °C/min (starting from 2 to 80 °C) while the absorbance at 260 nm was recorded every 1 min. Melting curves were analyzed with a two-state model.[47] Melting points (T_ms) were determined by using one of the procedures described by Breslauer,[48] in which α is defined as the fraction of single strands in the duplex. Thus any experimental absorbance vs temperature curve can be converted into an α vs temperature profile. From this curve the melting point T_m is defined as the temperature at which $\alpha = 50\%$. The curves expressing the evolution of the optical density (OD) as a function of the temperature were analyzed by using extrapolated base lines and a two-state model to yield Van't Hoff transition enthalpies (ΔH_{VH}) for each melting event. The slope of the transition at the melting temperature T_m (where $\alpha = 50\%$) was measured

$$\left(\frac{\partial\alpha}{\partial T}\right)_{T_m}$$

and the Van't Hoff transition enthalpy $\Delta H°_{VH}$ was calculated using the expressions:

$$\Delta H°_{VH} = 6RT_m{}^2\left(\frac{\partial\alpha}{\partial T}\right)_{T_m}$$

$$\Delta S°_{VH} = \frac{\Delta H°_{VH}}{T_m} + R\ln\frac{C_o}{2}$$

$$\Delta G° = \Delta H° - T\Delta S°$$

where C_o equals the total strand concentration. The details of this calculation have been described.[47]

Errors in the determinations of T_m, $\Delta H°$, $\Delta S°$, and $\Delta G°$ have been estimated respectively as ±0.6 °C, ±2 kcal/mol, ±6 cal/mol/K, and ±8 kcal/mol (average of four independent measurements). It is important to point out that this method is the most generally used to determine the T_m and the thermodynamic data and that it implicates several approximations.[38]

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JM970225T