A New Class of Artificial Nucleases That Recognize and Cleave Apurinic Sites in DNA with Great Selectivity and Efficiency

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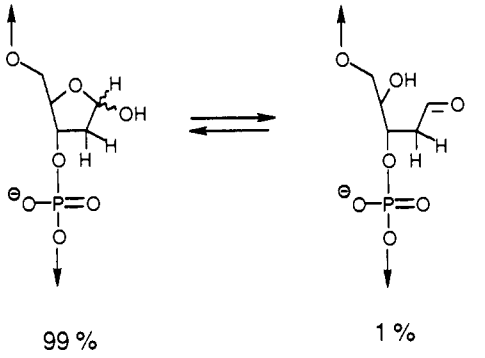
Abstract: A series of tailor-made molecules, **1** and **4–7**, have been prepared to recognize and cleave DNA at apurinic sites. These molecules incorporate in their structure different units designed for specific functions: (1) an intercalator for DNA binding, (2) a nucleic base for abasic site recognition, and (3) a linker endowed with both a binding function and a cleavage function (Scheme II). The constituent units were varied successively in the series of molecules to get insight into their mode of action and prepare more active compounds. ^{1}H NMR spectroscopy reveals the absence of intramolecular ring–ring stacking interactions in water between the base and the intercalator in all molecules **1** and **4–7**. All bind to calf thymus DNA with binding constants ranging from 10^4 to 10^6 M^{-1}. Their nuclease activity was estimated by measuring their ability to induce single strand breaks in depurinated pBR 322 plasmid DNA. The most efficient molecule, **5**, exhibits high recognition selectivity and cleavage efficiency: at nanomolar concentrations, **5** recognizes and cleaves the abasic lesion present in a DNA molecule containing an average of 1.8 apurinic sites in its 4 362 base pairs sequence. Molecule **5** exhibits higher cleaving efficiency than the reported tripeptide Lys-Trp-Lys: 10^{-8} M concentrations of the former (**5**) lead to cleavage ratios comparable to those observed for the latter used as 10^{-3} M concentration. This enzyme mimic **5** can be used advantageously as a substitute to the natural nuclease for *in vitro* cleavage of depurinated DNA.

Apurinic/apyrimidinic sites (AP sites) in DNA are generated by cleavage of the *N*-glycosyl linkage between a base and its deoxyribose moiety. Due to the relative instability of the *N*-glycosidic bond, such baseless sites in DNA may occur spontaneously with a relatively high frequency (estimated to be 10^4 lesions per cellular cycle).[1] This reaction is much easier for purines (depurination) than for pyrimidines since protonation of the N7 atom of guanine and of N3 of adenine labilizes the glycosidic bond to give a highly unstable cyclic carboxonium ion which leads to 2-deoxyribose. This hydrolytic process is markedly accelerated by chemical modifications of the nucleic bases[2] (alkylating agents, carcinogens...) and by physical agents[3,4] (UV, γ radiations...). In cells, abasic sites are also produced enzymatically during the repair process of abnormal or modified bases. The alterations in the base are specifically recognized and removed by highly specialized *N*-glycosylases,[5,6–8] generating abasic sites as intermediates in the repair system.

From the chemical point of view, the abasic site corresponds to a ca. 40:60 mixture of α- and β-hemiacetals[9] in tautomeric equilibrium with the ring-opened aldehydic form which represents less than 1% of the total[10] (Scheme I).

Scheme I



If not repaired, the abasic site, which is a noninformative lesion,[11] may promote misincorporation of nucleotides *in vitro*[12] and can be cytotoxic and mutagenic.[3,13] Abasic site repair is thus a critical cellular activity, and this lesion should be short lived *in vivo*: in procaryotic cells, the abnormal bases are repaired by either of two pathways involving specific glycosylases to yield an abasic site in all cases.[8–14] Along the first pathway, the abasic site produced is enzymatically excised as deoxyribose 5-phosphate and the resulting gap is repaired (i.e. uracyl-DNA *N*-glycosylase). The other pathway involves polyfunctional enzymes that excise the damaged base and then catalyze strand scission on the 3′ side of the abasic site. The breakage results from β-elimination of the 3′-phosphate catalyzed by a basic function of the enzyme which abstracts the acidic proton α to the aldehydic group of the ring-opened abasic site form (i.e. *E. coli* endonuclease III, UV endonuclease V from bacteriophage T_4, and *E. coli* FPG protein).[15–18] The α,β-unsaturated aldose 5-phosphate generated

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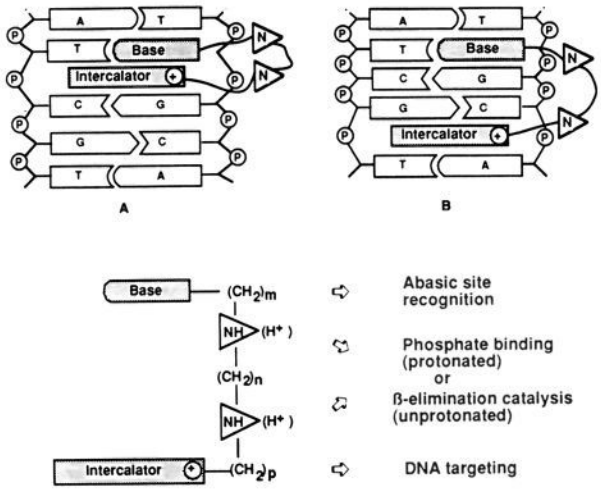
Scheme II



at the 3′ end of DNA is then excised and the resulting gap repaired by other specific enzymes. Because the strand scission is not due to phosphodiester hydrolysis, Verly suggested that this class of enzymes should be referred to as AP lyases.[15b] The mechanism of β-elimination and its stereochemistry have been demonstrated on synthetic depurinated oligonucleotides by high-field NMR spectroscopy.[15a]

Some chemical agents which mimic DNA AP endonuclease activity have been reported. Polyamines are the simplest ones.[19] A tryptophane-containing tripeptide (lysyltryptophyllysine, Lys-Trp-Lys) recognizes and cleaves DNA specifically at abasic sites.[20] The intercalating agent 9-aminoellipticine also cleaves DNA at AP sites at low doses.[21] These artificial nucleases belong to the AP lyase class, and formation of a Schiff base between their primary amines and the aldehydic form of the abasic site has been suggested to be the activated intermediate for the β-elimination catalysis.[22]

We have developed a program with the ultimate goal of engineering molecules which can selectively recognize and cleave apurinic sites in DNA and function like enzyme mimics to be used as substituents for mild and specific abasic site cleavage in molecular biology experiments. These "hybrid" molecules incorporate in their structure different units designed for specific functions: an intercalator for DNA binding, a nucleic base for apurinic site recognition, and a linker endowed with both a binding function and a cleavage function. Schematic representations for the envisioned cleavage complex are indicated (Scheme II), in which the base and the intercalator can either stack intramolecularly in the abasic pocket or "bisintercalate" with the two aromatic units separated by one or two base pairs. In 1988, we reported on the properties of the first member of the series, the hybrid molecule **1**.[23] This molecule is constituted of a base (adenine) linked to the intercalator 9-amino-6-chloro-2-methoxyacridine by a polyamino chain. This compound appeared to be one of the most efficient apurinic site cleaving agents known. It is active at nanomolar concentrations. We also showed[23] that the three components of the molecule are necessary to the activity, the two "half-molecules" **2** and **3** being almost totally inactive. A mechanism of β-elimination has been suggested for the cleavage on the basis of enzymatic experiments.[23]

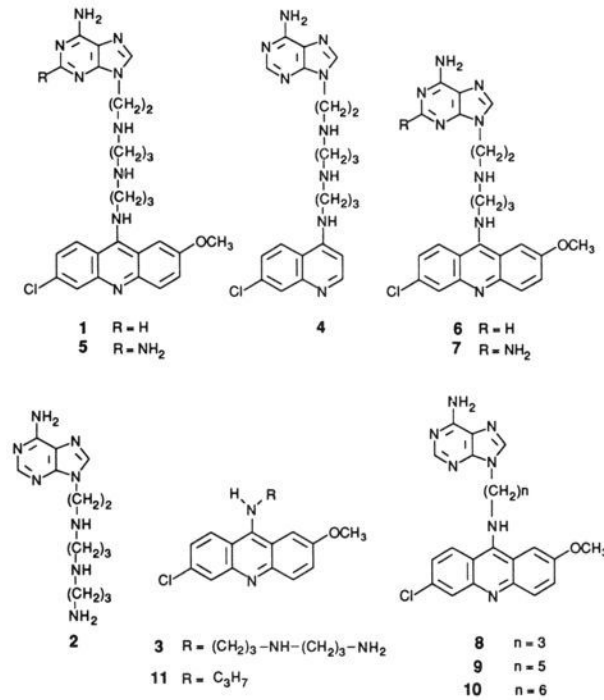


Figure 1.

To understand how this molecule, **1**, recognizes and cuts depurinated DNA and to develop more active compounds, we have prepared a series of new molecules, **4–7**, in which we have varied successively all components (Figure 1). The aminoacridine moiety was replaced by a weaker DNA-binding agent, aminoquinoline.[24] The adenine ring was replaced by a nonnatural nucleic base, diaminopurine, which can pair both with thymine through three hydrogen bonds and with cytosine through one hydrogen bond. The linking chains are of two types: long with two aliphatic secondary amines that could play different roles depending on their protonation state (phosphate binding and β-elimination catalysis) and short chains including only one nitrogen atom to fulfill the two activities (Scheme II).[25,26] We also examined for comparison the series in which the chain does not possess any secondary amine, i.e. adenine and aminoacridine are tethered by a polymethylene bridge (these molecules, **8–10**, had been prepared and examined in the past to investigate the stacking interactions between heteroaromatics and nucleic acid bases as involved in

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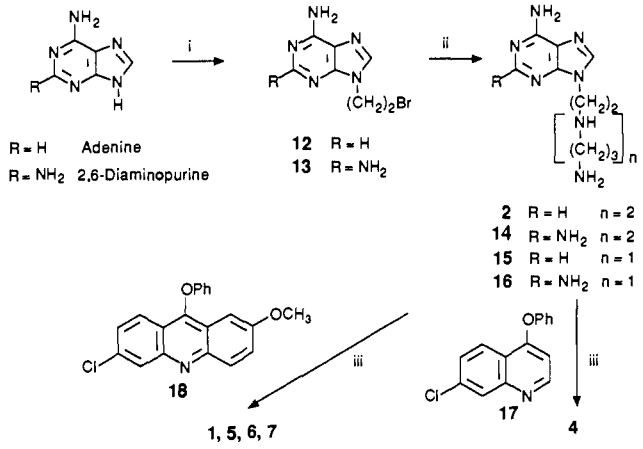
(25) The amino functions at positions 4 and 9, respectively, of the quinoline and acridine rings are not considered as basic entities of the linking chain. Due to electron delocalization on the ring, they loose their basic character and induce a significant increase of the basicity of the heterocyclic nitrogens which are protonated at neutral pH (4-amino-7-chloroquinoline, $pK = 9.17$; 9-amino-6-chloro-2-methoxyacridine, $pK = 9.89$).[26]

(26) (a) Clarck, J.; Perrin, D. D. *Q. Rev., Chem. Soc.* **1964**, *295*–298. (b) Albert, A. *Physical Methods in Heterocyclic Chemistry*; Katritzky, A. R., Ed.; Academic Press: New York, 1963; Vol. I, p 74.

Scheme III[a]



[a] (i) $Br(CH_2)_2Br$, DMF, K_2CO_3, 80 °C. (ii) $H_2N(CH_2)_3NH_2$ or $H_2N(CH_2)_3NH(CH_2)_3NH_2$, MeOH, 60 °C. (iii) PhOH, 80 °C.

the intercalation process[27,28]). We report here on the synthesis and conformation of the hybrid molecules **1** and **4–7**. We describe their interaction with calf thymus DNA and compare their ability to cleave apurinic site containing pBR 322 DNA as a function of the moieties present in their structure. We show that the most efficient molecule, **5**, in which diaminopurine is linked to aminoacridine by a long diamino chain, exhibits high recognition selectivity and cleavage efficiency: at nanomolecular concentrations, this molecule recognizes and cleaves the abasic lesion present in a DNA molecule containing an average of 1.8 apurinic sites in its 4 362 base pairs sequence.

Synthesis

The general strategy used for the synthesis of the "hybrid" molecules **1** and **4–7** implies as a first step the alkylation of the purine base and subsequent introduction of the chain. The intercalating moiety was introduced at the final stage.

As described in Scheme III, the purine (adenine or 2,6-diaminopurine) was first alkylated with dibromoethane in DMF in the presence of potassium carbonate. Under these conditions, the alkylation selectively occurred at the N9 position (no alkylation was observed at the N7 position). To avoid dimerization of the purine, we used a large excess of the alkylating agent and dilute solutions. Compounds **12** and **13** were obtained in 77 and 63% yields, respectively. The reaction of the bromo derivatives **12** and **13** with the polyamines [1,3-diaminopropane or bis(3-aminopropyl)amine] was again performed in highly dilute solutions with a 30-fold excess of the polyamine in methanol and led to compounds **2** and **14–16**. All traces of the free polyamines had to be removed before introduction of the intercalating moiety. Due to the high polarity of compounds **2** and **14–16**, their purification turned out to be a major problem. We chose two different techniques: preparative reverse-phase HPLC or evaporation of the polyamine by heating under reduced pressure and successive precipitations of the residue from an ethanol–diethyl ether mixture. The products were then crystallized as the hydrochlorides from methanol–hydrochloric acid mixtures. Reaction of the hydrochlorides with the phenoxy derivatives **17** and **18** (prepared from the commercial chloro compounds) in phenol yielded the desired "hybrid" molecules **4** and **1** and **5–7**, respectively (yields from 20 to 70%). These were purified by crystallization from acidic methanol.

Conformational Study of the Hybrid Molecules

In the past we have prepared and examined a series of comparable heterodimeric molecules in which the same 9-amino-

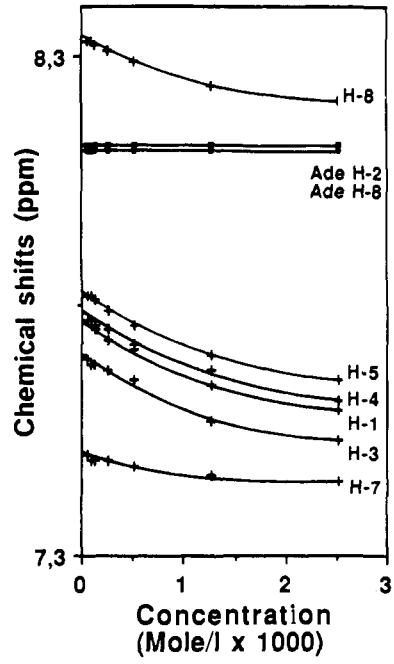


Figure 2. Concentration dependency (6×10^{-5} to 2.5×10^{-3} M) for the proton chemical shifts of Ade–C_2–NH–C_3–NH–C_3–NHAcr (**1**) in D_2O, 0.04 M sodium deuterioacetate buffer, pD 5.5, T = 21 °C.

6-chloro-2-methoxyacridine ring is linked to the nucleic bases adenine, thymine, and guanine by polymethylenic chains ($(CH_2)_n$) of varying length (n = 3, 5, 6).[27-28] A detailed analysis was described, including hypochromism measurement in the UV, chemical shift variations by Fourier transform proton magnetic resonance, and fluorescence emission. These techniques had shown that all molecules exist mainly in folded conformations in water in the temperature range 0–90 °C, with the acridine and the base rings being stacked one on top of the other. In a given series (see for example compounds **8–10**, in which the base is adenine), the degree of folding is dependent upon the length of the linking chain. The molecules possessing the shortest link exhibit the highest degree of intramolecular stacking of folding.[28]

Due to the presence of acridine, these molecules bind to DNA. However, the binding turned out to be modulated by the intramolecular stacking phenomenon, as the molecules must unfold for the acridine ring to intercalate in DNA, leaving the attached base outside the double helix. For example, the short chain containing molecule **8** (n = 3), that adopts the most stable folded conformation, elicits lower DNA-binding affinity (K_a = 4.5×10^3 M^{-1}) than the analog **10** (K_a = 6.8×10^4 M^{-1}), which possesses a longer chain (n = 6) and shows less tendancy to fold intramolecularly.[28] Consequently, *it appeared necessary, as a prerequisite, to study possible intramolecular interactions* in the new molecules **1** and **4–7** to *detect any influence on their DNA-binding* parameters and thus on their cleavage efficiency at apurinic sites.

^1H NMR spectroscopy had proved very useful to detect the intramolecular stacking interactions in molecules base–C_n–NHAcr, since both nucleic bases and acridine exhibit strong shielding effects. Intramolecular stacking gives rise to upfield shifts of the proton signals with respect to the corresponding chemical shifts in the bases and the acridine residues alone. The experimental shielding effects reflect the proportion of folded molecules. However, self-association of nucleic bases and acridine moieties induces upfield shifts also, so it was necessary to investigate the self-association phenomenon exhibited by all moieties involved in the folding process.

Self-Association of Hybrid Molecule 1. We examined in detail molecule **1** (which is representative of the series) by recording its ^1H NMR spectrum at 400 MHz in water (D_2O, pD 5.5) at

(27) Constant, J. F.; Carden, B. M.; Lhomme, J. *J. Heterocycl. Chem.* **1985**, *22*, 1035–1040.
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Scheme IV

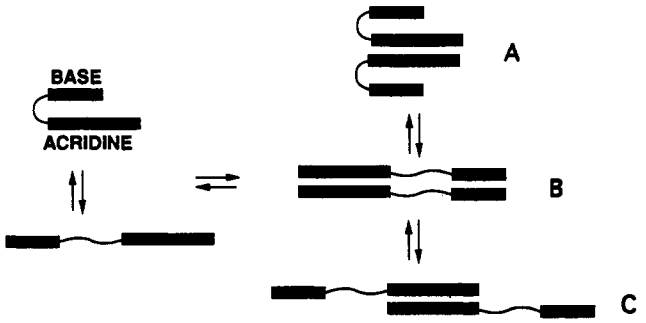

Table I. Comparison of the ^1H NMR Data[a] for the "Hybrid" Molecules and the Corresponding References (Half-Molecules)

	compound	acridine H_1	acridine H_8	purine H_2	purine H_8
δ_0[b]	Ade–C_2–NH–C_3–NH–C_3–NHAcr (**1**)	7.77	8.34	8.12	8.11
	Ade–C_2–NH–C_3–NH–C_3–NH_2 (**2**)			8.24	8.13
	AcrNH–C_3 (**11**)	7.69	8.39		
$\Delta\delta_0$[c]	Ade–C_2–NH–C_3–NH–C_3–NHAcr (**1**)	0.08	0.05	0.12	0.02
	Ade–C_6–NHAcr (**10**)	0.29	0.16	0.56	0.30
	Ade–C_5–NHAcr (**9**)	0.40	0.15	0.80	0.30
	Ade–C_3–NHAcr (**8**)	0.48	0.22	0.75	0.21
δ[d]	Ade–C_2–NH–C_3–NH–C_3–NHAcr (**1**)	7.75	8.33	8.12	8.11
	DAP–C_2–NH–C_3–NH–C_3–NHAcr (**5**)	7.76	8.32		7.80
	Ade–C_2–NH–C_3–NHAcr (**6**)	7.67	8.25	7.75	7.75
	DAP–C_2–NH–C_3–NH–C_3–NH_2 (**14**)				7.85

[a] D_2O, sodium deuterioacetate buffer, pD 5.6, 20 °C. [b] Chemical shifts, δ_0, extrapolated to zero concentration. [c] Differences between δ_0 of the hybrid molecule and δ_0 of the corresponding half-molecule. [d] Chemical shifts measured at 10^{-4} M concentration.

different concentrations ranging from 6×10^{-5} to 2.5×10^{-3} M. The chemical shifts of all acridine protons are strongly dependent on concentration (Figure 2), while H2 and H8 adenine protons are not modified by dilution (δ = 8.11 and 8.12 ppm, respectively). The deshielding of the acridine signals alone when dilution is increased is clear evidence of the presence of intermolecular stacking interactions involving the acridine rings only.[29]

Intramolecular Stacking in Hybrid Molecule 1. Extrapolation of curves $\delta = f$(conc) to infinite dilution gives the chemical shifts δ_0 (hybrid) for the free molecule **1**, as quoted in Table I. Also indicated are the chemical shifts calculated for the "half-molecules" **2** and **11**.[28] The differences $\Delta\delta_0$ for the corresponding protons in the "hybrid" molecules and in the "half molecules" are small, indicating a low degree of intramolecular stacking. This is clearly apparent when these values are compared to those measured for molecules **8**, **9**, and **10** ($(CH_2)_n$ with n = 3, 5, and 6), which have been shown to exist totally in folded conformations under the same conditions (with overlap geometries between adenine and acridine differing in the three molecules).[28]

Study of Hybrid Molecules 1 and 4–7. A study of the concentration dependency of the chemical shifts in the representative molecule **1** (Figure 2) indicates that the variations become negligible for concentrations lower than 1×10^{-4} M. We thus recorded the ^1H NMR spectra at this concentration to evaluate the importance of the intramolecular stacking phenomenon for all other hybrid molecules (Table I). In all cases, the chemical shift differences observed for the hybrid molecules and the constituent aromatic units are small or negligible.

(29) These results are in accordance with the presence of aggregates involving acridine–acridine contacts, while the purine moieties are not involved, suggesting type C aggregates (Scheme IV). The previous molecules such as **8–10**, in which the link between the base and acridine is a hydrophobic polymethylene chain, form aggregates to which type A and B structures had been ascribed, in which adenine, acridine rings, and hydrophobic chains are involved.[28] In the present case, type A aggregates are unfavored due to the length of the link and type B aggregates are probably destabilized in favor of type C, due to repulsion between the protonated amino linking chains.

Table II. Complexation Constants for Native Calf Thymus DNA, Measured by BET Displacement and Cleavage Activity on pBR322 DNA Plasmid Containing ≈1.8 Apurinic Sites[a]

compound	affinity (M^{-1}) $K \times 10^{-4}$	cleavage activity b	cleavage activity c (%)
Ade–C_2–NH–C_3–NH–C_3–NHAcr (**1**)	20	5×10^{-9}	100
Ade–C_2–NH–C_3–NH–C_3–NHQ (**4**)	1	8×10^{-8}	90
DAP–C_2–NH–C_3–NH–C_3–NHAcr (**5**)	110	1×10^{-9}	100
Ade–C_2–NH–C_3–NHAcr (**6**)	7	$>10^{-5}$	23
Ade–C_6–NHAcr (**10**)	6.8		0
DAP–C_2–NH–C_3–NHAcr (**7**)	20	$>10^{-5}$	40
AcrNH–C_3 (**11**)	6.4		0

[a] Plasmid concentration = 2×10^{-9} M, incubation time = 20 min, pH 7.4, T = 37 °C. [b] Drug concentration (M) inducing 50% cleavage. [c] Observed cleavage ratio for 1×10^{-5} M drug concentration.

We can safely conclude that the *intramolecular interaction is weak or absent and can in no way diminish the binding to DNA.* This is supported by the observation that even in the Ade–C_n–NHAcr series **8–10**, which exhibits strong intramolecular interactions, the affinity for DNA of Ade–C_6–NHAcr (**10**) becomes close to that of AcrNH–C_3 (**11**) despite the strong shielding effects measured by ^1H NMR.[28] This implies that, when the linking chain is long enough, the intramolecular stacking, even if measurable, does not influence the binding of the acridine ring to DNA.[28]

Interaction with DNA

Binding to DNA is a prerequisite for AP site recognition and cleavage. For this reason, we measured the affinity constants for DNA for all molecules using competition experiments with ethidium bromide as previously described.[28] The experiments were performed using native calf thymus DNA and not depurinated DNA. Indeed, to be reliable, a study with depurinated DNA would necessitate a highly transformed DNA with the consequence of instability (strand scission) and important conformational changes.

Results are summarized in Table II. Varying successively the three components of the molecules causes clear changes in the properties of binding to DNA. When the intercalator (the vector) is an acridine ring, the affinity is 20-fold higher than for the corresponding quinoline-containing molecule (comparison of **1** versus **4**). This is consistent with the well-known stronger interaction exhibited by aminoacridines compared to aminoquinolines.[24] The molecules containing chains with two aliphatic secondary amines (**1** and **5**) bind respectively 3 and 5 times more efficiently than the corresponding compounds possessing only one secondary amine in the linker (**6** and **7**). This indicates the importance of the ionic interactions due to protonated amino groups, as expected. When adenine is replaced by diaminopurine, the affinity is also improved by a factor of 3 or 5 (**5** vs **1**; **7** vs **6**). Since this was measured on native DNA, one may suspect a groove binding mode for the diaminopurine ring that is not observed for the adenine ring (i.e. **11** and **10**; $K_{aff} \approx 6.5 \times 10^4$ M^{-1}).

As expected, all of these compounds interact with native DNA as monointercalators. The number of covered sites is two, and the rule of the excluded site is obeyed.[30]

Activity at AP Sites in DNA

The nuclease activity of the different compounds was estimated by measuring their ability to induce single strand breaks in depurinated pBR 322 plasmid DNA. Depurination was effected under controlled conditions (sodium acetate buffer, 25 mM, pH 4.9, 70 °C, 20 min) to produce an average of 1.8 apurinic sites per DNA molecule as reported previously.[23a] The cleavage activity

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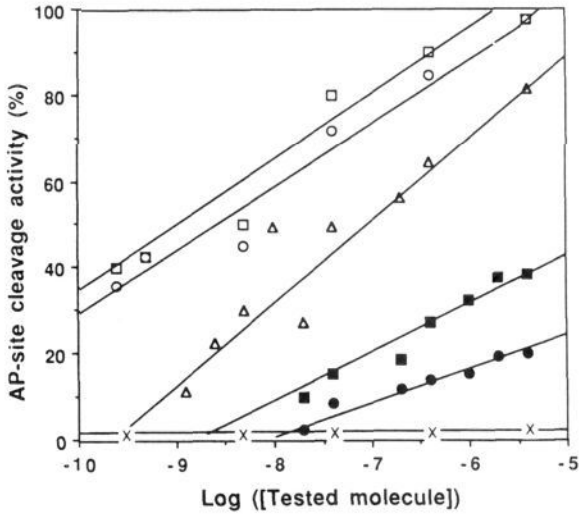

Figure 3. Percentage of AP site cleavage of pBR322 DNA plasmid containing 1.8 apurinic sites expressed as a function of drug concentration: □, DAP–C_2–NH–C_3–NH–C_3–NHAcr (**5**); ○, Ade–C_2–NH–C_3–NH–C_3–NHAcr (**1**); △, Ade–C_2–NH–C_3–NH–C_3–NHQ; ■, DAP–C_2–NH–C_3–NHAcr (**7**); ●, Ade–C_2–NH–C_3–NHAcr (**6**); ×, Ade–C_6–NHAcr (**10**), AcrNH–C_3 (**11**) (plasmid concentration = 2×10^{-9} M, incubation time = 20 min, pH 7.4, T = 37 °C).

at apurinic sites was determined by incubating the depurinated DNA at 37 °C, pH 7.4, for 20 min in the presence of varying concentrations of the synthetic molecules. Cleavage induces single strand breaks that convert the circular covalently closed form (supercoiled or form I) into the open circular form (relaxed or form II). These two conformations of the plasmid were separated by agarose gel electrophoresis, and their relative percentages were estimated after ethidium bromide staining, photography under UV illumination, and densitometry of the negative film. The percentage of cleavage was calculated as follows:[31]

$$\text{cleavage ratio} = (\text{amount of from II}/(\text{amount of form II} + \text{amount of form I})) \times 100$$

The values thus obtained are reported in Figure 3, where the cleavage ratio is expressed as a function of the log(drug concentration). In Table II, for an easier comparison of the activities, we indicate for each compound (a) the concentrations of the molecules leading to 50% DNA cleavage and (b) the percentage of DNA cleavage induced by the molecules present at 10^{-5} M concentrations.

It is noteworthy that compounds without secondary amines in the linking chain are totally inactive. Increasing the polyamino chain length enhances cleavage activity (**1** > **6** and **5** > **7**). Compounds in which an intercalating agent with higher affinity for DNA is incorporated are more efficient (**1** > **4**).

The activity of compound **5** was compared with that of the tripeptide Lys-Trp-Lys (LTL), which is regarded as the prototypical cleaving agent at AP sites.[20] Because LTL is much less efficient than **5**, incubation with depurinated DNA had to be run for at least 1 h to observe significant cleavage and to make a comparison with compound **5** (Figure 4). The concentrations used for the drugs range from 10^{-3} to 10^{-5} M for LTL whereas they range from 10^{-5} to 10^{-8} M for **5**. As apparent from the gel, the synthetic molecule **5** used at 10^{-8} M concentration (lane 8) is at least as efficient as LTL at 10^{-3} M (lane 2).

Discussion

We have prepared a series of tailor-made molecules that bind to DNA and cleave DNA at apurinic sites *in vitro*. These molecules incorporate in their structure different units for different specific functions: an intercalator for DNA binding, a nucleic base for abasic site recognition, and a linking chain carrying amino functions for both abasic site cleavage and phosphate binding.

(31) In our experimental conditions, we could not detect any difference in the intensity of fluorescence emitted by ethidium bromide bound to form I or II of the plasmid pBR 322.

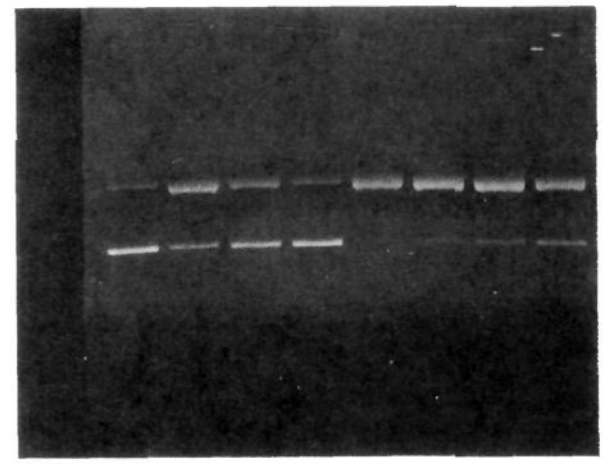

Figure 4. Relaxation of supercoiled depurinated pBR322 DNA (≈1.8 AP sites per molecule, 8.5×10^{-6} M nucleotide phosphate unit) incubated for 1 h at 37 °C in 10 μL of a 10 mM Tris-HCl buffer (pH 7.4) containing 1 mM EDTA in the presence of various amounts of reagent **5** and Lys-Trp-Lys (LTL): lane 1, depurinated DNA; lanes 2–4, LTL at 10^{-3}, 10^{-4}, and 10^{-5} M, respectively; lanes 5–8, compound **5** at 10^{-5}, 10^{-6}, 10^{-7}, and 10^{-8} M, respectively.

The conformational study of these molecules indicates the absence of intramolecular ring–ring stacking interactions that could modify significantly the binding to DNA.

(1) All molecules prepared bind to calf thymus DNA with fair to good affinity with binding constants ranging from 10^4 to 10^6 M^{-1}, i.e. the variations are within 2 orders of magnitude. In the acridine series, all the heterodimers have a better affinity than the reference compound AcrNH–C_3 (**11**), confirming the hypothesis that the secondary amines of the linking chain play an important role in the binding process. The apurinic site cleavage activities on the other hand show extremely large variations. The molecules in which the link is a polymethylene chain exhibit zero activity, while the most active cleaves at nanomolar concentrations.

(2) Table II shows that, in all series of analogous molecules, *i.e. molecules which possess the same linking chain, cleavage* efficiency parallels DNA affinity. In molecules **1** and **4**, in which the base and the chain are identical, replacement of aminoquinoline by aminoacridine increases the association constant by a factor of 20 and the cleavage activity increases similarly, with a "concentration factor" of 16 (corresponding to the concentrations required for an identical DNA cleavage ratio). The same holds for molecules **1** and **5**, in which replacement of adenine by diamino purine increases both affinity and cleavage activity. The same observation arises from comparison of molecules **6** and **7**, which contain a monoamino link.

(3) The length and/or nature of the linking chain is extremely determinant for cleavage, while affecting binding to a smaller extent. The pairs of molecules **1**–**6** and **5**–**7** can be compared, in which the same chain modification exists, changing from the "short" monoamino link –$(CH_2)_2$–NH–$(CH_2)_3$ in **6** and **7** to the "long" diamino bridge –$(CH_2)_2$–NH–$(CH_2)_3$–NH$(CH_2)_3$– in **1** and **5**. For both pairs the "long" diamino link increases DNA binding (respectively by such factors as 3 and 5), while the cleavage *activity increases dramatically (concentration factors larger than* 4 orders of magnitude).

(4) The most active molecules exhibit a cleaving efficiency for apurinic sites in DNA which is much higher than the tripeptide Lys-Trp-Lys.

All of these results are consistent with the hypothesis that the mode of action is β-elimination. Each constituent of the molecule

exerts a specific function as indicated in Scheme II: (1) DNA binding through a $\pi-\pi$ stacking interaction involving the intercalating moiety, (2) abasic site recognition by the nucleic base, (3) additional DNA binding through ionic interactions between the protonated nitrogen(s) of the chain and the biopolymer phosphates, and (4) Ap site cleavage by the free amino base of the linking chain. Indeed a possible explanation for the increased cleavage activities and binding constants observed for the "longest" chain containing molecules could reside in the local p*K*'s exhibited by the two secondary amines. As a consequence of the number of methylene units separating the nitrogen atoms, the nucleic base, and the protonated aminoacridine, it seems reasonable to assume that the two secondary amines of the linking chain exhibit a significant basicity decrease, with a fairly large p*K* difference for the two amines. Most likely, at neutral pH, an important fraction of the most basic nitrogen is protonated while the second amine is mainly unprotonated. In the literature, large differences in p*K* values for full protonation are reported for "diethylenetriamine" (3-azapentane-1,5-diamine (p*K* = 4.25))[32] and for "di-1,3-propanetriamine" (4-azaheptane-1,7-diamine (p*K* = 7.72)).[33] Comparable distance effects on p*K*'s have been reported for polyazamacrocycles.[34] This leads to a situation in which the protonated nitrogen constitutes an additional DNA-binding site involving electrostatic interaction with the phosphates, while leaving a fraction of unprotonated amino groups which can function as basic catalysts to abstract a proton and induce β-elimination. The general conformation of the complex would be such that this basic site is located in close proximity to the hydrogen to be abstracted.

This general scheme still needs to be demonstrated. For example, the present results do not give any information as to whether the mode of interaction corresponds to a type A or to a type B complex (Scheme II). However, the scheme does explain the absence of cleaving properties exhibited by the "half-molecules" **2** and **3** and excludes the involvement of a Schiff base as reported for the tripeptide Lys-Trp-Lys[20] and aminoellipticine.[21]

In conclusion, it appears that such molecules as **1** and **5** are very close in character to enzyme mimics, possessing both the recognition and the reactivity properties of a nuclease enzyme. They cleave DNA selectively at abasic sites and are active at nanomolar concentrations at neutral pH, 37 °C. For comparison, the normal chemical cleavage conditions at abasic sites require warming to 65 °C in the presence of 0.4 M NaOH or 1 M piperidine at 90 °C. Further studies are in progress to pinpoint the exact mode of action of these types of molecules, and the generality of their use as specific cleavage catalysts for abasic sites in molecular biology experiments. In particular, oligonucleotides containing specifically located abasic sites ("true" abasic sites and "stable" analogs of abasic sites) are currently being prepared to get detailed information concerning both the cleavage mechanism and the recognition mode through molecular modeling coupled with NMR experiments.

Our synthetic "nucleases" are chemically stable and are totally inactive on native DNA whereas the natural enzymes are fragile and have secondary endonuclease activity that can be misleading. Thus, one application of obvious interest would be their use as replacements of natural AP endonucleases for both specific apurinic sites detection and assay in DNA.

Experimental Section

General Procedures. All solvents or reagents were of reagent grade quality and were used without further purification. Analytical TLC was performed on 0.2-mm silica 60 coated aluminum foils with F-254 indicator (Merck). Analytical HPLC was performed on Millipore-Waters equipment (two M-510 pumps, solvent gradient M680) with UV detection (M490 and diode array 990). A reverse-phase μ-Bondapak C-18 column (Millipore-Waters, 3.9 × 300 mm) was used with a linear methanol–water pH 2.5 gradient, flow 2 mL/min for 10 min. For preparative HPLC, a Chromatospac Jobin-Yvon was used (reverse-phase C_8-Merck). Melting points were measured on a Totoli apparatus and are uncorrected. ^{1}H NMR spectra were recorded on Bruker WP80, AM200, and AM400 spectrometers. Spectra were referenced to the residual proton solvent peaks. Infrared spectra were recorded on Perkin-Elmer 298 and 1320 spectrometers. Mass spectra were recorded on Varian MAT311 and AET MS30. Elemental analyses were performed by the "Service Central de Microanalyse du CNRS". For all products, the purity was ascertained by ^{1}H NMR and HPLC analyses. In several cases, correct elemental analysis could not be obtained due to the polar and hygroscopic character of the compounds.

6-Amino-9-(2-bromoethyl)-9*H*-purine (12). A mixture of adenine (2.5 g, 18.5 mmol), 1,2-dibromoethane (15 g, 79.8 mmol), and potassium carbonate (6 g, 43.4 mmol) in dimethylformamide (100 ml) was stirred at room temperature under nitrogen for 48 h. After filtration, the solvent was evaporated to dryness. The yellow solid was washed with water and dried. Compound **12** was obtained in a pure state as indicated by HPLC and did not require any further purification (3.46 g, 77% yield): mp 195–200 °C dec, (lit.[35] mp = 204–205 °C); IR (KBr) 3300, 3100, 1655, 1585, 1475, 1415, 1360, 1320, 1300, 1225, 1065, 940, 870, 790 cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6) δ 8.15 and 8.10 (2H, 2s, Ade-C$_2$*H* and Ade-C$_8$*H*), 7.25 (2H, s, Ade-N*H*$_2$), 4.50 (2H, t, Ade-C*H*$_2$), 3.90 (2H, t, C*H*$_2$Br); MS (EI) M = 242, *m/z* 243 ((M + 1)$^+$, ^{81}Br, 15), 241 ((M − 1)$^+$, ^{79}Br, 16), 162 (M$^+$ − Br, 2), 148 (M$^+$ − CH$_2$Br, 1), 135 (Ade$^+$, 100); UV (EtOH) λ_{max} (ϵ) 259 (13 850), 210 (14 300) nm.

9-(2-Bromoethyl)-2,6-diamino-9*H*-purine (13). Compound **13** was prepared by the method used for compound **12**, starting from 2,6-diaminopurine sulfate and 1,2-dibromoethane. The mixture was stirred at room temperature for 1 week. Compound **13** was obtained as a white powder in 63% yield: mp 270 °C dec; IR (KBr) 3320, 3140, 1730, 1660, 1590, 1510, 1470, 1450, 1420, 1400, 1350, 1250, 1220 cm^{-1}; ^{1}H NMR (80 MHz, DMSO-d_6) δ 7.70 (1H, s, DAP-C$_8$*H*), 6.75 and 5.65 (4H, 2s, 2N*H*$_2$), 4.35 (2H, t, DAP-C*H*$_2$), 3.80 (2H, t, C*H*$_2$Br); MS (EI) M = 257, *m/z* 258 ((M + 1)$^+$, ^{81}Br, 23), 256 ((M − 1)$^+$, ^{79}Br, 23), 176 (M$^+$ − Br, 4), 150 (DAP$^+$, 100); UV (EtOH) λ_{max} (ϵ) 281 (18 140), 265 (15 780), 210 (33 860) nm. Anal. Calcd for $C_7H_9N_6Br$: C, 32.70; H, 3.53; N, 32.69. Found: C, 33.04; H, 3.51; N, 31.92.

6-Amino-9-(10-amino-3,7-diazadecyl)-9*H*-purine Hydrochloride (2). Compound **12** (3.0 g, 12 mmol) was added to a solution of bis(3-propylamino)amine (48.8 g, 370 mmol) in methanol (190 mL). The mixture was heated under reflux for 24 h. After cooling, the solution was concentrated in vacuo to about 60 mL, then diluted with distilled water (180 mL). Purification was achieved by preparative HPLC. Aliquots of 40 mL were chromatographed on silica RP8 reverse-phase (200 g). A first elution with water (8000 mL) removed excess starting amine. Compound **2** was eluted with methanol (6000 mL). After evaporation of the solvent, the light brown residue was dissolved in acidic methanol, and the solution was kept at −20 °C overnight. Tetrahydrochloride **2** was obtained as a white powder with 26% yield: mp 262–265 °C dec; IR (KBr) 2980, 2765, 1685, 1585, 1415, 1230, 1030, 885, 770 cm^{-1}; ^{1}H NMR (200 MHz, D$_2$O) δ 8.35 and 8.30 (2H, 2s, Ade-C$_2$*H* and Ade-C$_8$*H*), 4.70 (2H, t, Ade-C*H*$_2$), 3.70 (2H, t, C*H*$_2$NH), 3.25–3.10 (8H, m, 4C*H*$_2$), 2.20–2.05 (4H, m, 2C*H*$_2$); MS (CI, CH$_4$) M = 292, *m/z* 293 ((M + 1)$^+$, 100), 276 (M$^+$ − NH$_2$, 10), 262 (M$^+$ − CH$_2$NH$_2$, 14), 248 (M$^+$ − (CH$_2$)$_2$NH$_2$, 15), 233 (M$^+$ − (CH$_2$)$_3$NH$_2$, 4), 219 (M$^+$ − NH(CH$_2$)$_3$NH$_2$, 14), 205 (M$^+$ − CH$_2$NH(CH$_2$)$_3$NH$_2$, 28), 191 (M$^+$ − (CH$_2$)$_2$NH(CH$_2$)$_3$NH$_2$, 39), 142 (M$^+$ − Ade, NH$_2$), 16), 136 ((M + H)$^+$, 40); UV (H$_2$O, pH = 5.5, acetate buffer) λ_{max} (ϵ) 260 (13 150) nm. The compound is extremely polar and hydroscopic and was used without further purification.

9-(10-Amino-3,7-diazadecyl)-2,6-diamino-9*H*-purine (14). A mixture of compound **13** (0.4 g, 1.55 mmol) and bis(3-aminopropyl)amine (4.5 mL, 31 mmol) in methanol (70 mL) was heated under reflux for 3 days. After evaporation of the solvent to dryness, 1 N aqueous sodium hydroxide (5 mL) was added, and the solution was heated at 80 °C and evaporated again under reduced pressure. The oily residue was poured into a large volume of diethyl ether. The gum thus obtained was solubilized in absolute alcohol and precipitated again with diethyl ether. The same procedure was repeated three times to remove any trace of starting amine. The

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final gum was solubilized in methanol, and the desired product was precipitated as the pentahydrochloride by adding 12 N hydrochloric acid in 23% yield: mp 263–266 °C; IR (KBr) 3320, 2940, 2740, 2000, 1680, 1640, 1520, 1450, 1420, 1360, 1310, 1260, 1220, 1140, 1050, 970, 850 cm^{-1}; ^1H NMR, (80 MHz, D_2O) δ 7.85 (1H, s, DAP-C_8H), 4.20 (2H, t, DAP-CH_2), 3.00 (2H, t, CH_2), 2.50 (8H, m, $4CH_2$), 1.55 (4H, quint, $2CH_2CH_2CH_2$); MS (CI, ammoniac, isobutane) M = 307, m/z 308 ((M + H)$^+$, 100), 279 ($M^+ - CH_2NH_2$, 23), 265 ($M^+ - (CH_2)_2NH_2$, 18), 251 ($M^+ - (CH_2)_3NH_2$, 21), 194 ($M^+ - (CH_2)_3NH(CH_2)_3NH_2$, 83); UV ($H_2O$) λ_{max} (ϵ) 280 (7990), 254 (7010) nm. This highly hygroscopic compound was used without further purification.

6-Amino-9-(6-amino-3-azahexyl)-9*H*-purine (15). A mixture of compound **12** (1.0 g, 4.1 mmol) and 1,3-diaminopropane (10 mL, 120 mmol) in methanol (70 mL) was heated under reflux for 24 h. After evaporation of the methanol and addition of 1 N sodium hydroxide (12 mL), the mixture was evaporated under reduced pressure. The residual oil was stirred with a large volume of diethyl ether for 4 h and filtered through celite, and the filtrate was concentrated under vacuum until formation of a white powder. Filtration afforded **15** as the free base (0.595 g, 62% yield). The trihydrochloride salt was obtained by crystallization from acidic methanol: mp 108–109 °C (free base); IR (KBr) 3260, 3080, 2920, 1660, 1590, 1470, 1410, 1320, 1300, 1240, 1200, 1120, 1070, 1010, 950, 900, 830 cm^{-1}; ^1H NMR (80 MHz, DMSO-d_6) δ 8.10 and 8.05 (2H, 2s, Ade-C_2H and Ade-C_8H), 7.10 (2H, s, Ade-NH_2), 4.10 (2H, t, Ade-CH_2), 2.85 (2H, t, CH_2), 2.50 (4H, m, $2CH_2$), 2.10 (3H, s, NH and NH_2), 1.35 (2H, quint, $CH_2CH_2CH_2$); MS (EI) M = 235, m/z 235 (M^+), 148 ((Ade CH_2)$^+$, 27), 136 ((Ade + H)$^+$, 66); UV (H_2O) λ_{max} (ϵ) 260 (17 880), 205 (27 260) nm. This hygroscopic compound was used without further purification.

9-(6-Amino-3-azahexyl)-2,6-diamino-9*H*-purine (16). Compound **16** was obtained as described before for **15**, starting from **13** (0.5 g, 1.9 mmol) and 1,3-diaminopropane (8 mL, 95 mmol) in 77% yield. The tetrahydrochloride of **16** was formed in acidic ethanol: mp 253–255 °C; IR (Nujol) 3480, 3350, 1670, 1600, 1460, cm^{-1}; ^1H NMR (60 MHz, D_2O) δ 7.80 (1H, s, DAP-C_8H), 4.15 (2H, t, DAP-CH_2), 2.95 (2H, t, CH_2), 2.70 (4H, m, $2CH_2$), 1.65 (2H, quint, $CH_2CH_2CH_2$); MS (EI) M = 250, m/z 250 (M^+, 11), 176 (DAP$(CH_2)_2^+$, 7), 163 (DAP-CH_2, 42), 151 ((DAP + H)$^+$, 100); UV (H_2O) λ_{max} (ϵ) 280 (8590), 254 (6905), 213 (24 110) nm.

7-Chloro-4-phenoxyquinoline (17). A solution of 4,7-dichloroquinoline (5.0 g, 25 mmol) and sodium hydroxide (1.5 g, 38 mmol) in phenol (15 mL) was heated at 100 °C for 7 h. The mixture was then poured in 2 N aqueous sodium hydroxide. Compound **17** was extracted with dichloromethane and purified on silica gel with dichloromethane as the eluent. Evaporation of the solvent afforded **17** as an oil (5.9 g, 92%), and the hydrochloride was obtained by crystallization in acidic methanol: mp 195–198 °C (hydrochloride) (lit.[36] mp = 207.5–210 °C); ^1H NMR (80 MHz, CD_3OD, free base) δ 8.60 (1H, d, QC_2H), 8.35 (1H, d, QC_5H), 7.95 (1H, d, QC_8H), 7.65–7.10 (6H, m, QC_6H and Ph*H*), 6.55 (1H, d, QC_3H).

6-Amino-9-[11-(7-chloroquinolin-4-yl)-3,7,11-triazaundecyl]-9*H*-purine (4). Hydrochloride **2** (0.2 g, 0.45 mmol) and compound **17** (as a free base, 0.3 g, 1 mmol) were heated in phenol (2 mL) at 100 °C under nitrogen for 5 days. After cooling at room temperature, the solution was diluted with methanol and poured in a large volume of diethyl ether. The solid was then filtered off, dissolved in methanol, and precipitated by adding hydrochloric acid. Product **4** was purified by column chromatography (eluted by an methanol–ammonia mixture = 98/2) and finally crystallized as the tetrahydrochloride from acidic methanol in 22% yield: mp 246–248 °C; IR (KBr) 3420, 3240, 3100, 2960, 2790, 2400, 1695, 1670, 1630, 1615, 1590, 1565, 1515, 1450, 1415, 1350, 1210, 1170, 1140, 1090, 900, 870, 815, 760, 675, 630 cm^{-1}; ^1H NMR (80 MHz, D_2O) δ 8.50 and 8.45 (2H, 2s, Ade-C_2H and Ade-C_8H), 8.35 (1H, d, QC_2H), 8.05 (1H, d, QC_5H), 7.70 (1H, s, QC_8H), 7.55 (1H, d, QC_6H), 6.80 (1H, d, QC_3H), 3.70 (4H, m, $2CH_2$), 3.40–3.10 (6H, m, $3CH_2$), 2.50–1.90 (4H, m, $2CH_2$); MS (FAB (+), thioglycerol) M = 454, m/z 455 ((M + 1)$^+$, ^{37}Cl), 453 ((M + 1)$^+$, ^{35}Cl); UV (EtOH/H_2O, 90/10) λ_{max} (ϵ) 343 (15 230), 330 (14 360), 257 (25 510), 237 (18 820) nm. Anal. Calcd for $C_{22}H_{28}N_9Cl \cdot 4HCl \cdot 4H_2O$: C, 39.33; H, 6.00; N, 18.76. Found: C, 39.48; H, 6.04; N, 18.00.

6-Amino-9-[11-(6-chloro-2-methoxyacridin-9-yl)-3,7,11-triazaundecyl]-9*H*-purine Hydrochloride (1). A mixture of tetrahydrochloride **2** (0.2 g, 0.5 mmol) and 6-chloro-2-methoxy-9-phenoxyacridine (**18**)[37] (0.2 g, 0.6 mmol) was stirred in phenol (2 mL) at 80 °C for 5 h under a nitrogen atmosphere. The mixture was then diluted in methanol (50 mL), and the desired compound was separated by chromatography on silica gel (eluted with a methanol and methanol–ammonia mixture = 98/2). Compound **1** was then crystallized from acidic methanol and isolated in 36% yield (0.14 g): mp 203–207 °C dec; IR 3025, 2990, 2615, 1690, 1625, 1590, 1255, 1240, 1165, 1085, 1020, 925, 755 cm^{-1}; ^1H NMR (200 MHz, D_2O) δ 8.45–8.30 (4H, m, Acr-*H* and Ade-*H*), 7.80–7.50 (4H, m, Acr-*H*), 4.80 (2H, m, Ade-CH_2), 4.25 (2H, t, AcrNHCH_2), 4.00 (3H, s, OCH_3), 3.70 (2H, m, Ade-CH_2CH_2), 3.15 (6H, m, $3CH_2$), 2.35 (2H, m, CH_2), 2.10 (2H, m, CH_2); MS (CI, CH_4) M = 533.5, m/z 534 (M^+, 19), 343 (M^+ – Ade$(CH_2)_2$NHCH_2, 88), 329 ((M^+ – Ade$(CH_2)_2$NH-$(CH_2)_2$, 100), 315 (M^+ – Ade$(CH_2)_2$NH$(CH_2)_3$, 60), 272 (M^+ – Ade$(CH_2)_2$NH$(CH_2)_3$NH$(CH_2)_2$, 17), 244 ((AcrH + H$^+$)$^+$, 100); UV (acetate buffer, pH 5.5) λ_{max} (ϵ) 445 (8750), 422 (8700), 345 (4450), 330 (3150), 275 (2500) nm; Anal. Calcd for $C_{27}H_{32}N_9OCl \cdot 7HCl$: 41.08; H, 4.98; N, 15.97. Found: C, 40.99; H, 4.64; N, 15.53.

9-[11-(6-Chloro-2-methoxyacridin-9-yl)-3,7,11-triazaundecyl]-2,6-diamino-9*H*-purine Hydrochloride (5). Compound **5** was prepared as described for compound **1**, starting from the pentahydrochloride **14** (1 g, 2 mmol) and phenoxyacridine **18** (0.98 g, 3 mmol) in phenol (5 mL). The mixture was heated for 45 min, then the desired product was precipitated by pouring into a large volume of acetone and recrystallized from acidic methanol (0.47 g, 31% yield): mp > 280 °C dec; IR (KBr) 3260, 3080, 2950, 2790, 1690, 1645, 1625, 1590, 1565, 1525, 1465, 1440, 1420, 1380, 1270, 1245, 1235, 1170, 1140, 1120, 1090, 1025, 930, 830, 760 cm^{-1}; ^1H NMR (200 MHz, D_2O) δ 8.20 (1H, d, Acr-C_8H), 7.75–7.45 (7H, m, DAP-C_8H and Acr-*H*), 4.20 (4H, m, $2CH_2$), 4.00 (3H, s, OCH_3), 3.35–3.05 (8H, m, $4CH_2$), 2.30 (2H, quint, $CH_2CH_2CH_2$), 2.05 (2H, quint, $CH_2CH_2CH_2$); MS (FAB (+), glycerol) M = 548, m/z 561 (M + Na)$^+$, 549 (M + H)$^+$; UV (H_2O) λ_{max} (ϵ) 422 (9430), 344 (4890), 278 (64 110) nm; Anal. Calcd for $C_{27}H_{33}N_{10}OCl \cdot 5HCl \cdot H_2O$: C, 41.27; H, 5.26; N, 17.82. Found: C, 41.18; H, 5.16; N, 17.44.

6-Amino-9-[7-(6-chloro-2-methoxyacridin-9-yl)-3,7-diazaheptyl]-9*H*-purine Hydrochloride (6). Hydrochloride **15** (0.25 g, 0.72 mmol) and phenoxyacridine **18** (0.2 g, 0.59 mmol) were heated in phenol (2 mL) at 80 °C for 6 h under a nitrogen atmosphere. After dilution with methanol and precipitation with diethyl ether, the resulting solid was stirred with 1 N aqueous sodium hydroxide cooled in an ice bath. The gum thus formed was solubilized in warm acidic methanol and filtered and the filtrate left overnight at –20 °C to afford yellow crystals of **6** (0.087 g, 21% yield): mp 263–265 °C; IR (KBr) 3300, 3120, 2930, 2840, 1660, 1630, 1600, 1570, 1525, 1480, 1435, 1415, 1335, 1300, 1250, 1235, 1115, 1070, 1030, 930, 860, 825, 795 cm^{-1}; ^1H NMR (80 MHz, D_2O) δ 8.10–7.40 (8H, m, Ade-C_2H, Ade-C_8H and Acr-*H*), 4.50 (2H, m, CH_2), 4.20 (2H, m, CH_2), 3.95 (3H, s, OCH_3), 3.60 (2H, m, CH_2), 3.25 (2H, m, CH_2), 2.30 (2H, m, CH_2); MS (FAB (+) thioglycerol) M = 476.5, m/z 479 ((M + H)$^+$, ^{37}Cl), 477 ((M + H)$^+$, ^{35}Cl); UV (EtOH) λ_{max} (ϵ) 418 (7350), 359 (3450), 342 (2700) nm; Anal. Calcd for $C_{24}H_{25}N_8OCl \cdot 3HCl \cdot 3H_2O$: C, 45.01; H, 5.35; N, 17.50. Found: C, 45.24; H, 5.11; N, 17.52.

9-[7-(6-Chloro-2-methoxyacridin-9-yl)-3,7-diazaheptyl]-2,6-diamino-9*H*-purine (7). Compound **7** was prepared as described for compound **6** in 69% yield: mp 236–238 °C; IR (KBr) 3000, 1680, 1640, 1580, 1530, 1500, 1420, 1390, 1360, 1310, 1270, 1240, 1170, 1090, 1020, 930, 850, 760 cm^{-1}; ^1H NMR (80 MHz, TFA-*d*) δ 8.80 (1H, d, Acr-C_8H), 7.80 (1H, m, Acr-*H*), 7.40 (5H, m, Acr-*H* and DAP-C_8H), 4.50 (2H, t, DAP-CH_2), 4.00–3.10 (9H, m, $3CH_2$ and OCH_3), 2.10 (2H, quint, $CH_2CH_2CH_2$); MS (FAB (+), thioglycerol, acetic acid) M = 491.5, m/z 492 (M + H)$^+$; UV (EtOH) λ_{max} (ϵ) 419 (7420), 359 (3390), 342 (2740) nm; Anal. Calcd for $C_{24}H_{26}N_9OCl \cdot 4HCl \cdot H_2O$: C, 42.78; H, 5.09; N, 18.71. Found: C, 43.32; H, 5.12; N, 18.51.

^1H NMR Studies. ^1H NMR spectra were recorded at 200 MHz (Bruker AM200) and 400 MHz (Bruker AM400), operating in the Fourier transform mode and locked on the deuterium resonance of the solvent D_2O. Stock solutions of the studied compounds were made in a deuterioacetate buffer (0.04 M, pD 5.5). Probe temperature was regulated to ±1 °C by a Bruker BST 100/700 controller.

DNA-Binding Studies. DNA-binding studies were performed with Perkin-Elmer MPF-44A and LS50 spectrofluorimeters in a thermostated quartz cell (25 °C). Excitation and emission were monitored at the ethidium bromide bands (520, 600 nm). All solutions were made in 25

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mM Tris-HCl, pH 7.0, 0.1 M NaCl, and 0.2 mM EDTA buffer. The DNA concentration was 1.6×10^{-5} M (in base pairs), and that of tested compounds, 2×10^{-5} M.

Preparation of DNA Containing Apurinic Sites (AP-pBR322 DNA). pBR322 DNA was dissolved in 25 mM sterilized acetate buffer (pH 4.9) at a concentration of 1 mg/mL and heated at 65 °C for 15 min.[24] This treatment introduced approximately 1.8 apurinic sites per DNA molecule.

Preparation of Organic Molecule Stock Solutions. The compounds were dissolved in a reaction buffer (10 mM Tris-HCl, 1 mM EDTA, pH 7.4) at concentrations ranging from 10^{-2} to 10^{-3} M. The exact concentrations of the compounds in the stock solutions were measured after dilution using molar absorptivities (in buffered water) of the different compounds. The final dilutions of the compounds were made by the addition of reaction buffer.

Incision of AP-pBR322 DNA. A 0.25-μg sample of AP-pBR322 DNA was incubated at 37 °C for 20 min in the presence of the various compounds in 20 μL of reaction buffer (8.5×10^{-6} M in nucleotide phosphate unit). The reaction was stopped by extracting the compounds with butanol saturated with water (100 μL), and the DNA was precipitated by ethanol (75% v/v) in the presence of sodium chloride (0.2 M final concentration).

Quantitation of AP-pBR322 Strand Breakage. The nicked and supercoiled DNA molecules were separated by agarose gel electrophoresis (0.8%) using TBE (Tris–borate (45mM) EDTA (1 mM), pH 8.0) as a migrating buffer and a constant voltage of 100 V for 2 h. After migration, the gel was stained by soaking in TBE containing ethidium bromide (0.5 μg/mL) and examined on a UV transilluminating table (312 nm). The gels were photographed with a Polaroid MP4 camera (orange filter) and Polaroid 55 type films. The relative amounts of the two forms of the plasmid were estimated from the negatives of the films which were densitized on a GS300 scanning densitometer (Hoefer) coupled to a Data Module 730 integrator (Millipore-Waters). Under these conditions, the intensities of the fluorescence emitted by the intercalated ethidium bromide in totally supercoiled (form I) or relaxed (form II) plasmids were identical. The percentage of cleavage was calculated as follows:

$$\% \text{ cleavage} = (\text{amount of form II}/(\text{amount of form II} + \text{amount of form I})) \times 100$$

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