Jean Lhomme
Jean-François Constant
Martine Demeunynck
LEDSS, Chimie Bioorganique,
UMR CNRS 5616,
Université Joseph Fourier,
BP 53,38041 Grenoble
Cedex 9, France

Abasic DNA Structure, Reactivity, and Recognition

Abstract: *Loss of a base in DNA, i.e., creation of an abasic site leaving a deoxyribose residue in the strand, is a frequent lesion that may occur spontaneously, or under the action of radiations and alkylating agents, or enzymatically as an intermediate in the repair of modified or abnormal bases. The abasic site lesion is mutagenic or lethal if not repaired. From a chemical point of view,the abasic site is an alkali-labile residue that leads to strand breakage through β- and δ- elimination. Progress in the understanding of the chemistry and enzymology of abasic DNA largely relies upon the study of synthetic abasic duplexes. Several efficient synthetic methods have thus been developed to introduce the lesion (or a stable analogue) at defined position in the sequence. Physicochemical and spectroscopic examination of such duplexes, including calorimetry, melting temperature, high-field nmr and molecular modeling indicate that the lesion strongly destabilizes the duplex, although remaining in the canonical B-form with structural modifications strictly located at the site of the lesion. Probes have been developed to titrate the damage in DNA in vitro. Series of molecules have been devised to recognize specifically the abasic site, exhibiting a cleavage activity and mimicking the AP nucleases. Others have been prepared that bind strongly to the abasic site and show promise in potentiating the cytotoxic and antitumor activity of the clinically used nitrosourea (bis-chloroethylnitrosurea).* Biopoly 52: 65–83, 1999

Keywords: *DNA; abasic; apurinic; apyrimidinic; oligonucleotide synthesis; reactivity; structure; enzymology; cleaving agent; repair inhibitors*

INTRODUCTION

The genome integrity is permanently challenged by the agression by endogenous or exogenous agents. Abasic sites (apurinic or apyrimidinic or AP-sites) are probably the most common lesions. They result from the hydrolytic cleavage of the glycosidic bond leaving deoxyribose residues in the DNA strand. This process can occur spontaneously.[1] Abasic site formation is markedly increased during the repair processes of damaged nucleic bases. The base excision repair pathway (BER) involves specific DNA glycosylases that remove modified or abnormal nucleic bases, and generate abasic sites as intermediates. These miscoding and noninformative damages have to be repaired prior to replication and transcription, and several DNA repair pathways have evolved to counteract their threat.[2–4] AP-sites also appear to be able to interfere with other vital enzymes such as topoisomerases. The diversity of the biological implications of AP-sites induced a great interest during the past two decades. In this review, we report on some aspects of the

Correspondence to: J. Lhomme; e-mail: Jean.Lhomme@ujf-grenoble.fr

Contract grant sponsor: Ligue Nationale contre le Cancer and the Association pour la recherche sur le Cancer

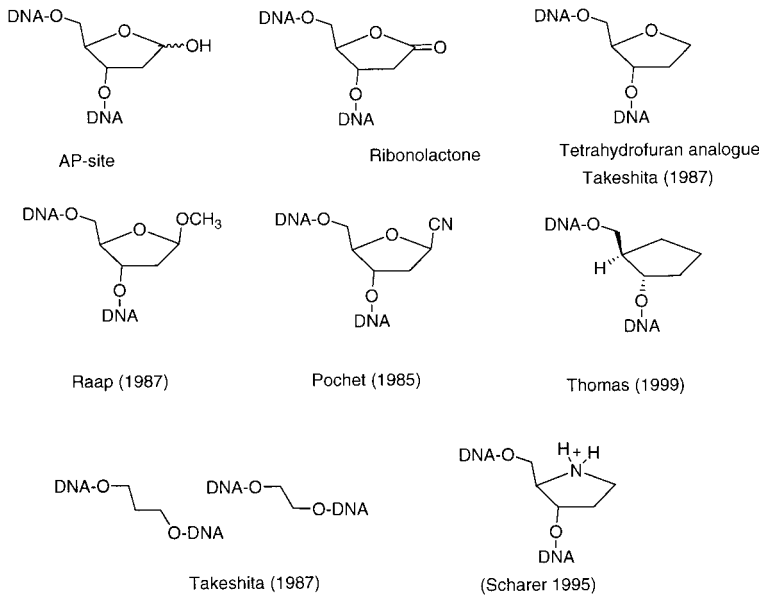


FIGURE 1 Abasic sites structures and their analogues.

chemistry and structural effects of AP-sites. We focus on the recognition of abasic sites by small molecules and their effects on the repair process. We report briefly on deoxyribonolactone, a baseless lesion that can be considered as an oxidized form of the AP-site (Figure 1).

CHEMISTRY OF THE ABASIC SITE

Abasic Sites Generation—Synthesis of Abasic DNA

Abasic sites result from hydrolytic cleavage of the N-glycosylic bond. This process is increased by any factor or chemical modification that develops a positive charge on the nucleic base and labilizes the glycosylic bond.[1] Most methods used to generate abasic sites in DNA or in synthetic oligonucleotides are based on this strategy. Glycosyl bond cleavage is thus highly pH dependent, and the rates of cleavage are higher for deoxypurine nucleosides than for deoxypyrimidine ones, deoxyguanosine being the most labile nucleoside. Treatment of DNA in acidic conditions generates abasic sites in a controlled fashion. For example, warming pBR 322 plasmid DNA in 0.1*M* NaCl, 0.01*M* sodium citrate, pH 5 at 70°C for 8 min generates 2 apurinic sites per molecule.[7] Alkylation of DNA by dimethylsulfate and other alkylating agents occurs preferentially at position 7 of the guanine residues, introducing a positive charge and therefore increasing the rate of depurination by several orders of magnitude. The formation of abasic sites in alkylated DNA is accelerated at elevated temperatures.[8,9]

Various methods have been reported to prepare oligonucleotides containing abasic sites at predetermined positions. Most strategies involve synthesis of unnatural or modified deoxynucleoside precursors and their incorporation into oligomers by automated phosphoramidite solid phase synthesis. Stuart and Chambers[10] took advantage of the property of uracil-DNA glycosylase to develop an efficient preparation of abasic oligonucleotides. Uracil-DNA glycosylase removes uracil residues formed in DNA by deamination of cytosines. The authors introduced the deoxyuridine residue at selected positions of oligomers by automated solid phase synthesis and generated the abasic site by treatment of the resulting oligonucleotide with uracil DNA-glycosylase. The greater lability of purine residues in acidic conditions has been used in early studies by Vasseur et al.[11,12] to prepare oligodeoxypyrimidines containing abasic sites by hydrolysis of N-benzoyl-adenosine residues.[11] Other nucleotide analogues, such as 2-pyrimidinone-deoxynucleotide,[13] are transformed into AP-sites under mild acidic conditions (pH 3, room temperature). Studying the chemical stability of 8-substituted purine nucleosides, we noticed that the presence of electron-withdrawing groups at C-8 facilitates the N-glycosidic bond cleavage. The effect is a 10^4 rate acceleration with a sulfone group as a substituent.[14] We therefore designed a new route to AP-site formation in which 8-propyl-

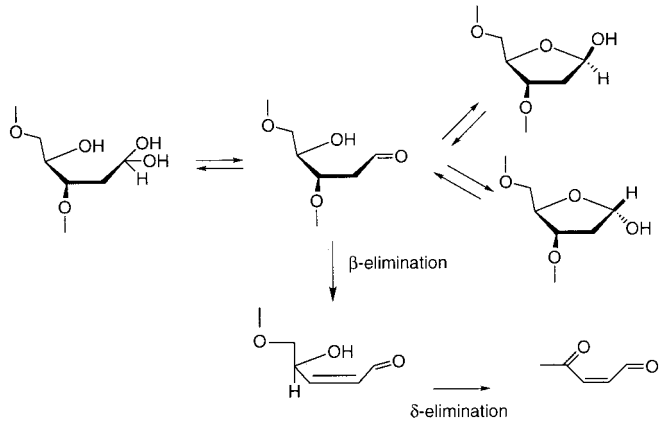


FIGURE 2 Isomeric forms of AP-site and mechanisms of hydrogen abstraction.

thio-deoxyadenosine was incorporated in oligomers.[15] Treatment of the resulting oligonucleotide with peroxymono-sulfate selectively oxidizes the sulfide into sulfone, which triggers depurination in mild acidic conditions. Direct protection of the anomeric hydroxyl group of 2′-deoxyribose was also used. Different protecting groups have been designed, such as the *tert*-butyl-dimethyl silyl ether, which is cleaved by mild acidic treatment,[16] and the photolabile *o*-nitrobenzyl group,[17] which allows synthesis on a preparative scale.

Site-specific introduction of abasic site in duplex DNA has been recently reported, based on the formation of a covalent cross-link between an alkylating antisense oligonucleotide probe and the target DNA, followed by thermal depurination of the alkylated nucleotide.[18]

Several chemically stable analogues of abasic sites, mimicking both the cyclic[19–22] and the open-chain[19] forms of the deoxyribose moiety, have been designed (Figure 1). A pyrrolidine analogue has also been prepared by Verdine and colleagues as inhibitor of the DNA repair enzyme AlkA, the enzyme that creates abasic sites by cleavage of the N3-methyl-deoxyadenosine glycosylic bond.[23]

The Reactivity of the Abasic Site

The detailed structure of abasic sites has been characterized by nmr spectroscopy analysis of ^{17}O- and ^{13}C-labeled abasic site containing oligodeoxynucleotides.[24–26] The abasic site might exist in three different forms in equilibrium, the open-chain aldehyde, the hemiacetals, and hydrate (Figure 2). The nmr studies of this equilibrium in solution indicate that the mixture of cyclic hemiacetals is the predominant form in the duplex, (nearly 1:1 α and β forms), the aldehyde only represents 1% of the total. The existence of the aldehyde form confers to the abasic site high sensitivity to alkaline conditions. Bailly and Verly[27,28] showed that in mild alkaline conditions, abasic sites undergo a β-elimination reaction with formation of an α,β-unsaturated aldehyde. In a more drastic alkaline medium, a second elimination reaction (designed as δ-elimination) occurs (Figure 2). The same reactivity was observed in the presence of polyamines.[29] Spermine induces β-elimination in quantitative yield; by contrast the δ-elimination reaction is a much slower process.[27] The δ-elimination has also been proposed to follow β-elimination cleavage of AP sites during enzymatic repair of damaged DNA.[27,28,30] The presence of thiols, such as β-mercaptoethanol, in the reaction medium totally prevents the δ-elimination process,[31] probably through Michael addition on the intermediate α,β-unsaturated aldehyde.

The stereospecificity of the enzymatic and of the base-catalyzed β-eliminations were determined using stereospecifically tritiated abasic sites at the 2′ position in the DNA polymer.[32] The β-elimination reaction that occurs in 0.1N NaOH proceeds by an *anti* β-elimination process with abstraction of the 2′-pro-R hydrogen and formation of the *trans* α,β-unsaturated aldehyde. A different stereospecificity is observed for the enzymatic cleavage with endonuclease III and uv endonuclease V,[32,33] which catalyze the abstraction of the 2′-pro-S hydrogen during the *syn* β-elimination reaction (Figure 3). Interestingly, the thermal degradation of abasic sites at neutral pH follows a syn β-elimination with formation of the *trans* α,β-unsaturated aldehyde, which slowly isomerizes to give the *cis* α,β-isomer.[34]

The reactivity of the abasic site has been used by several groups to derivatize oligonucleotides. Most

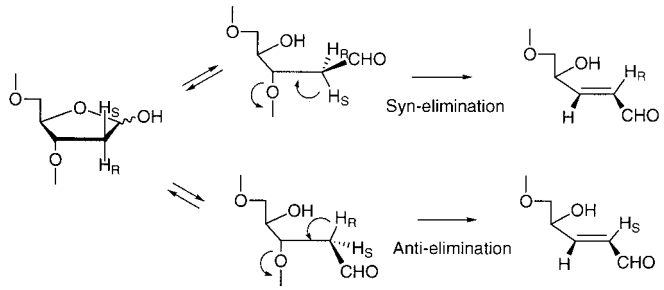


FIGURE 3 Stereoselectivity of the β-elimination reaction.

methods involve formation of a Schiff base between the electrophilic aldehydic abasic site and amino group containing ligands, followed by reduction (reductive amination). Drugs such as 9-aminoellipticine and 3-aminocarbazole[12] (Figure 4), psoralen derivatives, DNA-cleaving agents (aminophenanthroline), polyamines, and fluorophores have thus been grafted to oligonucleotides. Duplex cross-linking also involving reductive amination between one oligonucleotide carrying an amino group tethered to the 2′-hydroxyl of a nucleoside residue and the complementary sequence containing an abasic site has been recently reported.[35] Derivatization by reductive amination is a two-step process. However, a rapid and quantitative one-step derivatization can be obtained by reaction with alkoxyamines leading to stable oxime ethers. This reaction has been largely used for detection and titration of abasic sites (see below). Stable analogues of the abasic site have been used equally to derivatize oligonucleotides.[36]

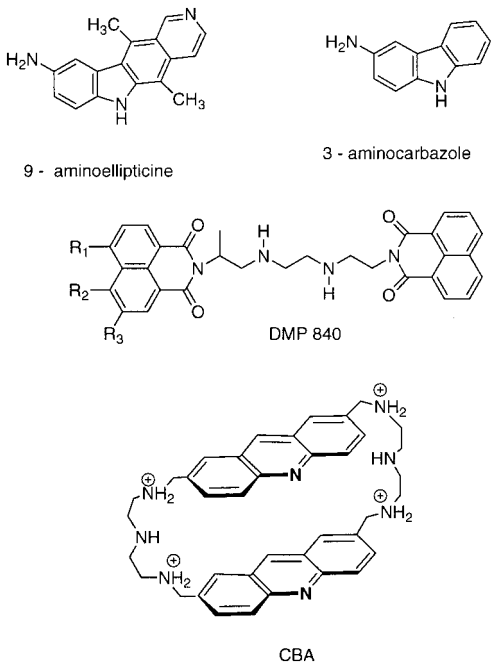


FIGURE 4 Structure of some drugs specific for AP-sites.

Structure of Abasic DNA

Several groups have studied the influence of the presence of an abasic site upon the conformation of DNA. The general approach has been to examine synthetic oligonucleotides containing an abasic site,[37–40] or most frequently, the stable tetrahydrofuran analogue of the abasic site[20,41–44] or the open analogue[45] (Figure 1), by high field nmr spectroscopy and restrained molecular dynamics. Different types of abasic duplexes have been examined, according to the nature of the base opposite the abasic site, a purine or a pyrimidine (i.e., respectively an apyrimidinic or an apurinic site) and according to the nature of the flanking bases. The general observations are that the backbones of these duplex DNAs are regular, as shown by the ^{31}P-nmr data.[38,40] One- and two-dimensional ^{1}H-nmr data indicate that the duplexes are right-handed and B-form. Conformational changes due to the presence of the abasic site extend to the base pairs adjacent to the lesion site, being dependent on the nature of the flanking and opposite bases. All studies on duplexes containing unpaired purines (apyrimidinic sites), flanked by a variety of neighboring base pairs, show that the purine remains stacked within the helix in an intrahelical conformation. This has been observed in duplexes in which the apyrimidinic site is the deoxyribose residue[38,40] or the stable tetrahydrofuran analogue.[42,44,46,47] The situation is more complex for apurinic sites. The few available studies tend to indicate that the pyrimidine opposite the abasic site may be either stacked inside the helix or expelled outside, or in equilibrium between the two situations, depending on the nature of the flanking bases. Cuniasse et al.[47] observed that when the apurinic site (X) is flanked by purine residues on both sides, e.g., G X G, an opposite cytosine becomes extrahelical, while an opposite thymine is in equilibrium between intra-

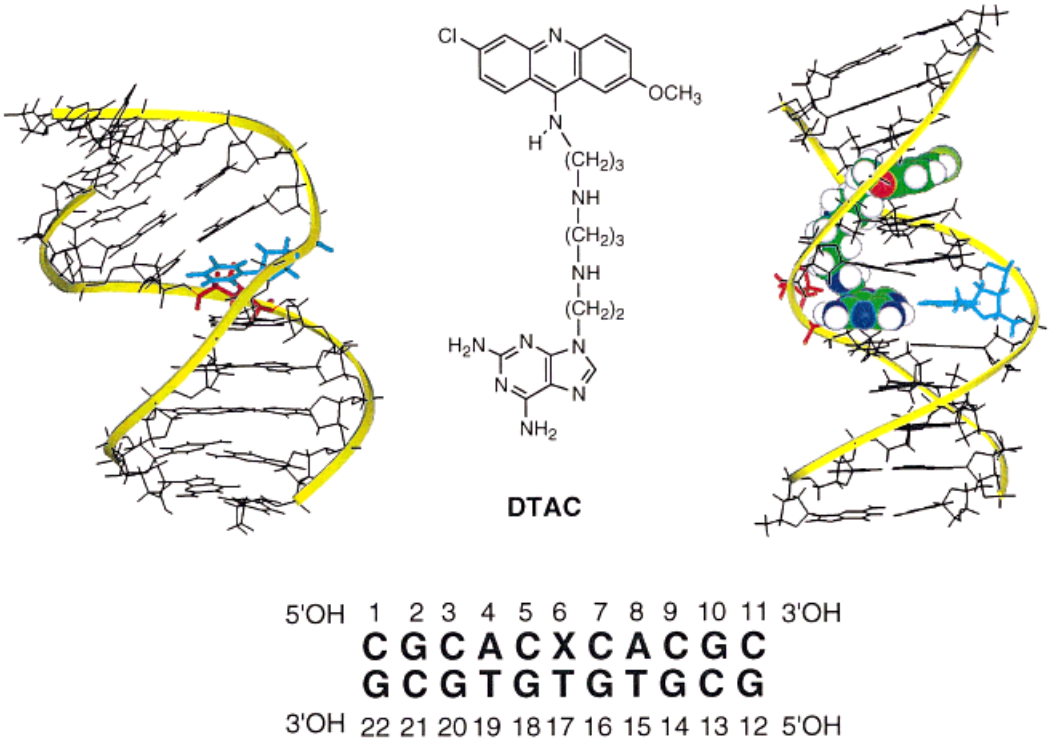


FIGURE 5 Molecular modeling based structure of an AP site containing undecamer (left) and the major complex DTAC-abasic undecamer (right). The drug is drawn in CPK, the AP-site is drawn in red, and the unpaired thymine in blue.

and extrahelical conformations. Singh et al. observed qualitatively the same extrahelical form for a thymine residue opposite the aldehydic abasic site.[39] In studying a duplex in which the apurinic site (X) is flanked by two pyrimidine residues, i.e., C X C, we observed that thymine opposite X stacks inside the helix[41] (Figure 5). These differences were interpreted in terms of base–base stacking interactions between the flanking bases.

Some differences were also observed between the "true" abasic site (also called "aldehydic" abasic site) and the stable tetrahydrofuran analogue. In the case of the true abasic site, which exists predominantly as a mixture of α- and β-anomers, Goljer et al. reported that the deoxyribose residue is within the helix for the β-anomer, while the sugar in the α-configuration is out of the helix.[38] Another feature that distinguishes the structure of an abasic duplex from that of the parent unmodified oligonucleotide, which was more recently evidenced, is formation of a kink at the site of the lesion. The bending value into the major groove was estimated to be $\approx$30° in an apyrimidinic undecamer[41] (Figure 5). A bend angle of $\approx$10° was reported quite recently[46] for an apurinic dodecamer designed as a model to study interactions with topoisomerase II. All most recent studies point to the importance of the abasic lesion to considerably increase the flexibility of duplex DNA. The diffusion rate of the abasic duplex has been found to be significantly faster than that of the corresponding undamaged duplex.[48]

The thermodynamic consequences of the abasic lesion have been examined calorimetrically and by CD by monitoring thermally induced dissociation of duplexes containing the tetrahydrofuranyl abasic site in which the base opposite the lesion and the base pairs neighboring the lesion were systematically varied.[49,50] The conclusions correlate those obtained by nmr and precise the thermodynamic consequences of the modification: the presence of the lesion in all sequence contexts studied does not alter the global B-form conformation—it induces significant enthalpy destabilization of the duplex, with a magnitude depending on the sequence context. The thermodynamic impact of the lesion is dominated by the nature of the neighboring base pairs.[49,50]

All structural informations acquired experimentally from the nmr data and to a lesser extent from calorimetric studies point to the major influence of the nature of the base opposite the abasic site and of the

flanking bases. Due to the inherent impossibility to study experimentally all possible combinations, the theoretical approach is attractive. An early report by Cognet et al.[51] describes molecular mechanics and molecular dynamics study of an abasic duplex in an effort to correlate with nmr data. More recently a comprehensive study of sequence effects has been developed in our laboratory.[52–55] Calculations were carried out on a series of DNA 11-mers containing all possible unpaired bases (N), adenine, guanine, cytosine, or thymine, present within two distinct sequence contexts, either with pyrimidine flanking bases (C N C), or purine flanking bases (G N G). The abasic site X is the tetrahydrofuran analogue, thus allowing comparison with experimental data. Molecular modeling calculations were carried out with the JUMNA algorithm (Junction Minimization of Nucleic Acids), using extensive conformational search techniques to locate the most stable abasic conformations and using Poisson–Boltzmann-corrected electrostatics to account for solvation effects. It was first shown that this JUMNA procedure is capable of reproducing the overall structural features of the natural and damaged duplexes without the use of experimental constraints.[55] The results obtained for the whole series of abasic duplexes are in very good agreement with the experimental data when available. They point to strong sequence effects on both the position of the unpaired base (intra- or extrahelical) and on the overall curvature induced by the abasic lesion. For C N C, unpaired purines are found to lie within the helix, while unpaired pyrimidines are either extrahelical or in equilibrium between the intra- and extrahelical forms. For G N G, all unpaired bases lead to intrahelical forms, but with marked sequence-dependent differences in induced curvature. Calculations also indicate that the abasic lesions modify the deformability of the duplex, generally increasing its flexibility, but again to an extent that depends on the nature of the abasic site and on the surrounding base sequence.[56]

Abasic Site Detection and Quantification

As abasic site formation can occur spontaneously or indirectly under the action of DNA damaging agents, the measurement of abasic sites is of great interest. A quantitative assay for this lesion may provide a measure of the exposure of DNA to various mutagens or genotoxic compounds such as environmental or chemotherapeutic agents. Most of the described methods are based on the reactivity of deoxyribose. In its opened aldehydic form, deoxyribose may undergo two types of reaction: (1) strand breakage in alkaline conditions and (2) condensation with amino nucleophiles.

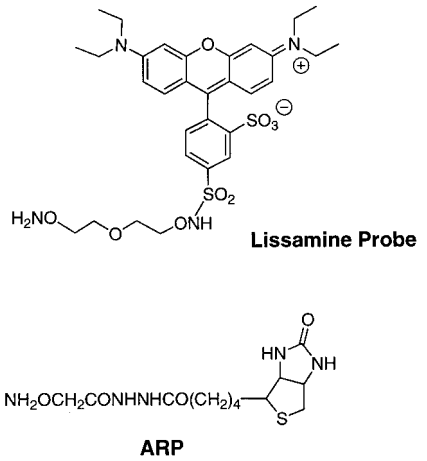


FIGURE 6 Structure of some oxy-amino reporter groups for AP-site assay.

Alkaline degradation of abasic sites induces strand breakage that can be estimated either by sedimentation in a sucrose gradient[57] or alkaline elution.[58] AP sites were also measured by using a ^{32}P-postlabeling assay.[59]

Most reported methods for AP-site quantification are based on condensation between the aldehydic function of the deoxyribose residues and amino reporter groups (Figure 6). 9-Aminoellipticine (9-AE) was shown to react with AP-sites to form a fluorescent Schiff base that was quantified by measuring the luminescence emission.[9] The reactivity of other nucleophilic reagents has been investigated more than 30 years ago by Livingstone.[60,61] It was shown that hydrazino and oxyamino functions react with aldehydic abasic sites to yield stable Schiff base analogues. This reaction was used to titrate abasic sites with ^{14}C-labeled methoxyamine. This compound reacts with the aldehydic form of the abasic site to give a stable oxime ether. Since methoxyamine has no degradation action on DNA, AP-sites can be estimated from counting the acid-insoluble radioactivity.[62,63] Unlabeled methoxyamine was also used successfully to monitor in cultured cells the repair of various lesions such as alkyl DNA adducts known to generate AP-sites as intermediates.[64,65]

In order to design more sensitive assays, new oxyamino carrying probes were synthesized. A nitrobenzyl group could be detected by a direct Elisa assay using specific antibodies.[66] A biotine derivative named ARP (for Aldehyde Reactive Probe) allowed the sensitive detection of 1 AP-site per 10^4 nucleotides in 70–100 ng of DNA. ARP was used to monitor spontaneous and chemically induced AP-sites in

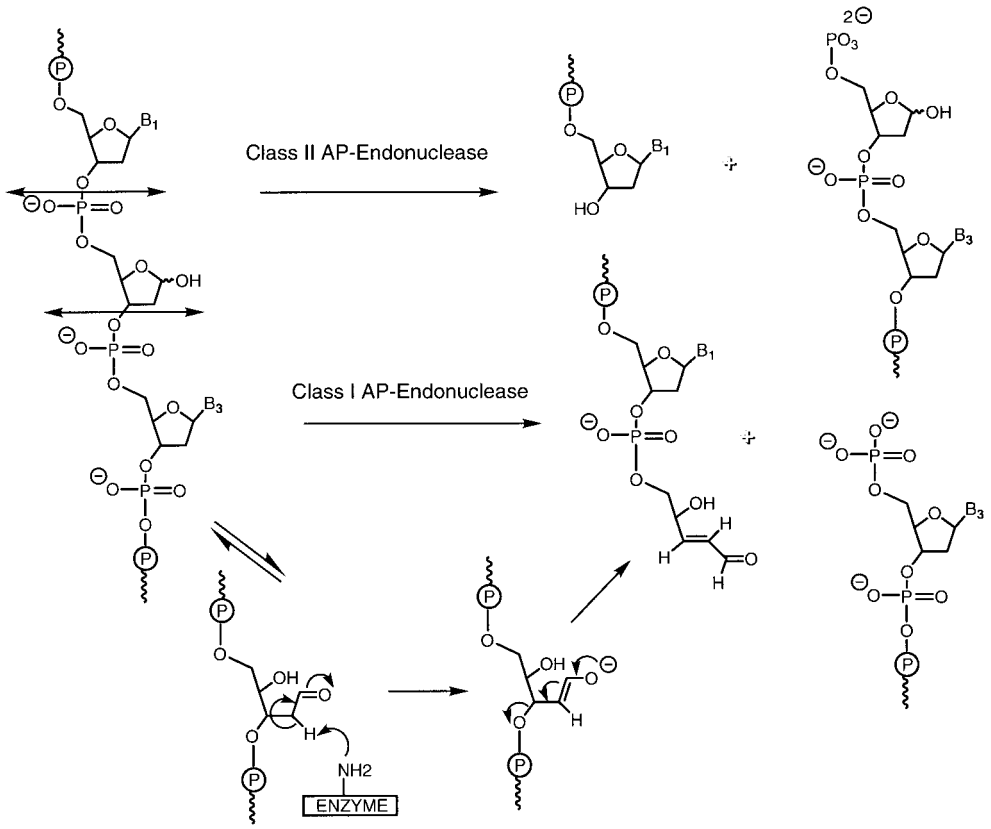


FIGURE 7 DNA strand-breakage mechanism of class I and class II AP-endonucleases.

vitro[67] and in mammalian tissues,[68] and to study the repair of alkylated nucleic bases in cultured cells.[69,70] Although the ARP assay is considered to be very sensitive to detect AP-sites, it cannot constitute a quantitative method since DNA is adsorbed on a surface and ARP is revealed through an ELISA-like assay.

In this laboratory, we designed a sensitive and quantitative fluorescence assay using an oxyamino fluorescent probe (Lissamine-Rhodamine B)[71] that reacts in solution with depurinated DNA.The method requires simple experimental settings and allows the precise measurement of 1 AP-site per 10^5 nucleotides in 40 μg of DNA.[72] Comparable fluorescent reagents have been reported using fluorescein[73,74] or acridinium salts[75] as reporter groups. Fluorescein labeling presents several advantages. Fluorescence energy transfer (FRET) between two closely spaced fluorescein moieties provokes a luminescence intensity decrease. This phenomenon allows detection of AP-sites generated in close proximity (within 10–20 base pairs or less).[76] The sensitivity of the AP-site fluorescein labeling could be increased significantly by using specific antibodies in an Elisa-like assay. This technique was used to study mismatch repair by the MutY enzyme.[77]

ENZYMOLOGY OF THE ABASIC SITE

AP-sites are intermediates in the BER pathway. The BER process removes damaged or inappropriate nucleic bases by enzymatic cleavage of the C1′—N glycosidic bond, generating an AP-site.[78] Many BER glycosylases have been isolated and characterized.[79,80] We already mentioned uracil-DNA glycosylase, which removes uracil moieties formed by spontaneous deamination of cytosine. Other common glycosylases include fpg, which removes 8-oxo guanine residues, or alkA, which removes alkylated adenine moieties**.** Some of the glycosylases have an associated AP-lyase (class I) activity that cleaves DNA at the 3'side of AP-site and initiates their repair (Figure 7). In the other cases, the AP-site is left as an intermediate that is processed by other repair enzymes (Figure 8). The first step is the cleavage of the DNA backbone at the 5′ side of the AP-site by specific class II (hydrolytic) AP-endonucleases.[81] These enzymes generate a 3′-OH group and a 5′-deoxyribose 5′-phosphate residue that is removed by a DNA deoxyribose phosphodiesterase. The gap is then restored by a DNA polymerase and a DNA ligase.[82] Class I

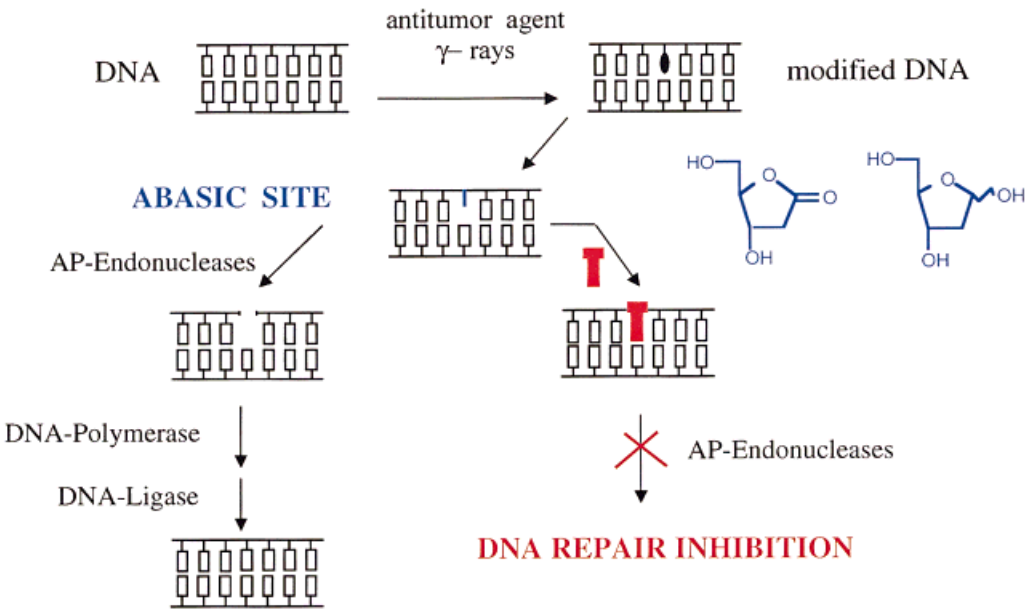


FIGURE 8 Schematic representation of AP-site generation and repair.

AP-endonucleases or AP-lyases are associated with glycosylase activities and proceed via a β-elimination mechanism,[83] leaving a 3′-α,β–unsaturated aldehyde residue and a 5′-phosphate group. The β-elimination process occurs on the open aldehydic form of the deoxyribose residue and involves a

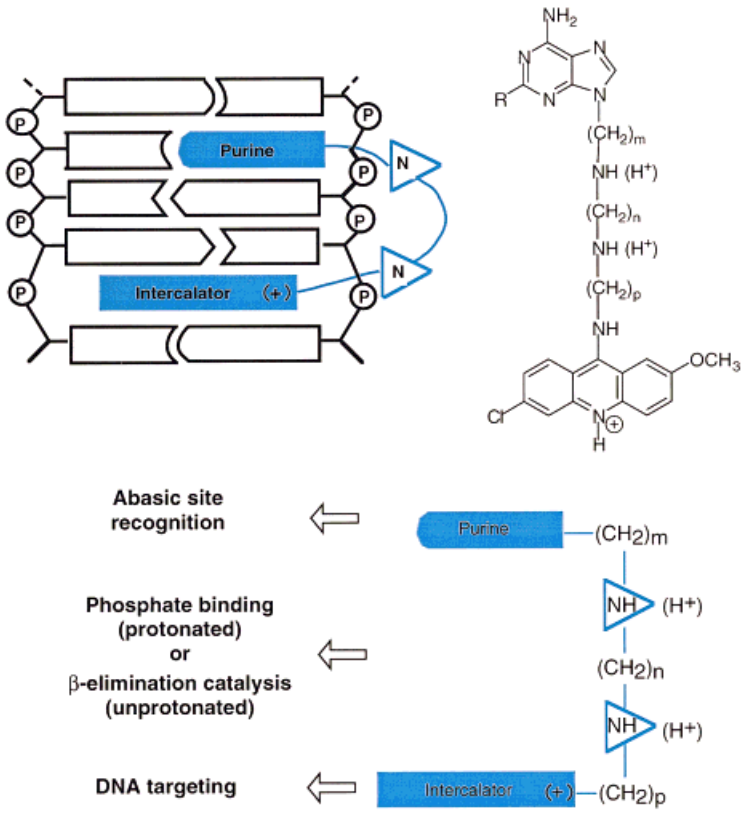


FIGURE 9 Schematic representation of the specific recognition of AP-site by base–chain–aminoacridine conjugates.

stereospecific abstraction of the 2′-pro-S hydrogen atom (Figure 3).

This mechanism has been extensively studied by nmr spectroscopy.[32,33,84] The formation of a Schiff base between an amino residue of the enzyme and the aldehyde group of the abasic site has been postulated.[85]

Class II enzymes are considered as the most abundant AP-endonuclease activities in most cells.[86] The x-ray structure of the main representative of the two conserved AP-endonuclease families have been solved : Exonuclease III from *Escherichia coli*[87] and APE-I, its human homolog,[88] belong to the first family . Endonuclease IV from *E. coli* belongs to the second family, and recently, Tainer et al.[89] established the structure of its complex with an abasic site containing DNA. It was shown that the enzyme induces a strong DNA bending and promotes a double-nucleotide flipping in order to sequester the AP-site in its active pocket. The phosphodiester bond cleavage is catalyzed by three Zn^{2+} ions. It is interesting to note that the stable tetrahydrofuran analogue of the abasic site is normally processed by these class II enzymes.

If not repaired, the abasic site can be mutagenic[90–93] or lethal.[94] A major deleterious effect of the persistance of the noncoding AP-site effect in the cell is the blockage of DNA replication.[95] From in vitro experiments with DNA polymerases from various organisms, it was shown that dAMP is preferentially incorporated ("A rule"), but not exclusively, opposite abasic lesions.[19,94,96–98] Precise kinetic studies were made with synthetic gapped matrix containing a site specific abasic analogue (tetrahydrofuran). By varying the position of the 3′- and 5′- terminus of the primer relative to the abasic site, Livneh et al.[99] showed that bypass synthesis by DNA polymerase I was not affected when the 3′ boundary is in the range of -1 to -5. However, bypass synthesis increases with the distance of the 5′ end of the gap from the lesion. Grollman et al.[100] studied the translesional synthesis by calf thymus DNA polymerase α and by the Klenow fragment of *Escherichia coli* DNA polymerase I in DNA templates containing a single natural abasic site or its tetrahydrofuran analogue. In all cases the A rule was obeyed. All these studies show that the replication process is dramatically affected by the presence of abasic sites on the coding strand. The preservation of the miscoding potential of this lesion might be of interest for the design of new cancer therapy strategies. Indeed, antitumor alkylating agents represent a class of therapeutic drugs widely used in clinical treatments. The cytotoxic effects of these drugs on tumor cells is thought to be due to the massive production of alkylated nucleic bases, the repair of which generates a great number of AP-sites. The design of specific AP-endonucleases inhibitors might be a seducing way to potentiate the cytotoxic effect of antitumor alkylating agents (Figure 8).

Recent work showed that abasic sites may interfere with other vital enzymes in cells such as topoisomerases. It appeared notably that apurinic sites and other DNA damages act as position specific topoisomerase II poisons.[6] Apurinic sites appear to stimulate DNA breakage up to 20-fold when located within the 4-base overhang produced by topoisomerase II mediated cleavage.[6,101–103] This lesion appears to be 1000 times more potent than specific topoisomerase II inhibitors such as etoposide.[104] Topoisomerase I activity is also affected by the presence of abasic sites; depending on its position, a single abasic site is able to induce new topoisomerase I cleavage sites or to trap the topoisomerase I cleavable complex.[5]

DNA CLEAVING AGENTS— NUCLEASE MIMICS

Tripeptide Lys–Trp–Lys

Oligopeptides containing aromatic and basic amino acids have been shown by various spectroscopic methods to bind to DNA containing abasic sites and to catalyze the cleavage of the phosphodiester bond adjacent to the lesion. The tripeptide Lys–Trp–Lys has been most thoroughly studied. Binding to apurinic sites is specific. It has been estimated that the asssociation constant for abasic DNA is two orders of magnitude higher than for native DNA.[105] A two-step process was proposed in which the initial complex involving electrostatic interactions between the protonated lysine residues and the phosphates on the DNA backbone is converted into a second one in which the tryptophan residue stacks with the nucleic bases and docks inside the abasic cavity. The peptide Lys–Trp–Lys therefore can recognize apurinic sites in a double-stranded structure through stacking interactions.

Further studies showed that the tripeptide could catalyze the cleavage of the phosphodiester bond adjacent to the AP-site.[106–108] Nicking of supercoiled PM2 DNA containing AP-sites was shown to be ionic strength-dependent and to generate 3′-hydroxyl and 5′-phosphoryl termini. Comparable cleaving efficiency was observed for the tripeptide Lys–Tyr–Lys. Nicking by tetrapeptides such as Lys–Gly–Trp–Lys and Lys–Trp–Gly–Lys was also reported.[105] Peptides without aromatic residues such as Lys–Ala–Lys or Lys–Lys–Lys can also promote nicking, but at 400 and 200 times higher concentrations, respectively,

than Lys–Trp–Lys. At a similar concentration, spermine and spermidine were 100- and 2000-fold, respectively, less active than Lys–Trp–Lys.[107]

All data are consistent with a β-elimination reaction catalyzed by the tripeptide. Specific binding of the tripeptide at AP-sites places the amino group of a lysine residue in close proximity to the aldehydic function of the ring-opened deoxyribose residue. Proton abstraction at the C-2 carbon, occurring either on the free aldehyde or/and on the imine formed with an amino group of the tripeptide, results in β-elimination of the 3′-phosphate.[108] Detailed inspection of the reaction using oligonucleotides specifically ^{3}H- and ^{13}C-labeled at the AP site indicated that the peptide catalyzed reaction proceeds by abstraction of the 2′-pro-R hydrogen, as also occurs under alkaline conditions, to give the *trans* α,β-unsaturated aldose residue[32] (Figure 3).

Aminoellipticine and Related Drugs

In a series of papers, Imbach et al. and Malvy et al. have shown that the intercalating agent 9-AE and structurally related aminocarbazole (Figure 4) interact with AP-sites and induce DNA cleavage at these sites at low doses.[109–111] The mechanism of cleavage by 9-AE was most thoroughly examined. It was proposed to involve formation of a Schiff base following interaction of the drug at the AP-site. The β-elimination then occurs. This results in the formation of a 2′,3′-unsaturated deoxyribose and cleavage of the DNA strand at the 3′ side of the site. The Schiff base may lead via unstable intermediates to a 9-AE adduct on the 3′ position of the deoxyribose.[112,113] Malvy and co-workers also made the interesting observation that 9-AE and related compounds that interact with AP sites without inducing DNA cleavage potentiate the cytotoxicity of the alkylating agent dimethylsulfate in *E. coli* via an apurinic site-dependent mechanism. 9-AE thus appeared to be a candidate for the inhibition of DNA repair.[114]

Nucleic Base–Acridine Conjugates

With the ultimate goal of developing molecules capable of interfering with the repair process in the cell and possibly show cytotoxic activity, a series of hybrid molecules have been designed in this laboratory to bind specifically to abasic sites in DNA.[115–117] These tailor-made molecules include (1) a recognition unit, i.e., a nucleic base to insert into the abasic cavity and bind to the complementary base in the opposite strand; (2) an intercalator for targeting the hybrid to DNA through strong but nonsequence-specific binding to DNA, and (3) a linking chain endowed with a binding and/or a cleavage activity (See Figure 9 on p. 72).

The first molecules prepared were shown to bind to calf thymus DNA and to incise plasmid DNA at the abasic sites.[118,119] In an effort to precise the mode of interaction and cleavage, and to devise more efficient molecules, a series of drugs were prepared in which the three modular constituents were successively varied (Figure 10). The strong amino–acridine intercalator was replaced by the poor DNA–binder aminoquinoline, adenine was substituted by 2,6-diaminopurine that presents larger H-bonding capacities or by a tricyclic analogue,[120] and the linking chain was varied in length and nature, including polymethylenes, amino and amido functions. The reference compounds including only two modules, i.e., the intercalator and the linker and the base plus linker also were prepared for comparison. The association constants with calf thymus DNA were determined and the cleavage efficiency was evaluated using pBR 322 plasmids containing abasic sites[117](Table I).

All molecules bind to DNA and for molecules possessing identical linker a relationship is observed between binding and cleavage. The three parts of the molecules are necessary for cleavage, the "half molecules" (intercalator–chain and nucleic base–chain) being almost totally inactive in the conditions of the experiments. Presence of at least one secondary aliphatic amine in the linking chain is necessary for cleavage activity. The most efficient molecule DTAC possesses 2,6-diaminopurine as the nucleic base, 9-aminoacridine nucleus as intercalator, and two secondary amines in the linker. This molecule is exceptionally active, being able to cleave plasmid DNA at nanomolar concentrations, i.e., it is more efficient than Lys–Trp–Lys by several orders of magnitude. All data can be interpreted by formation of a specific complex to which all three modules participate, prior to cleavage. Thus the acridine containing DTAC hybrid is more efficient than the quinoline containing analogue **1**. The adenine and diaminopurine containing molecules (ATAC and DTAC), which can pair with thymine in the complementary strand, cleave more efficiently the labeled synthetic 23-mer duplex containing thymine at position opposite the abasic site than the three other duplexes containing respectively the A, G, and C residues at this same critical position (unpublished results). For the ATAC and DTAC molecules, which possess the same linking chain, one of the amino groups of the linker that is essentially protonated at neutrality, participates to formation of the specific complex by interacting ionically with the DNA phosphates, while the second amino group of

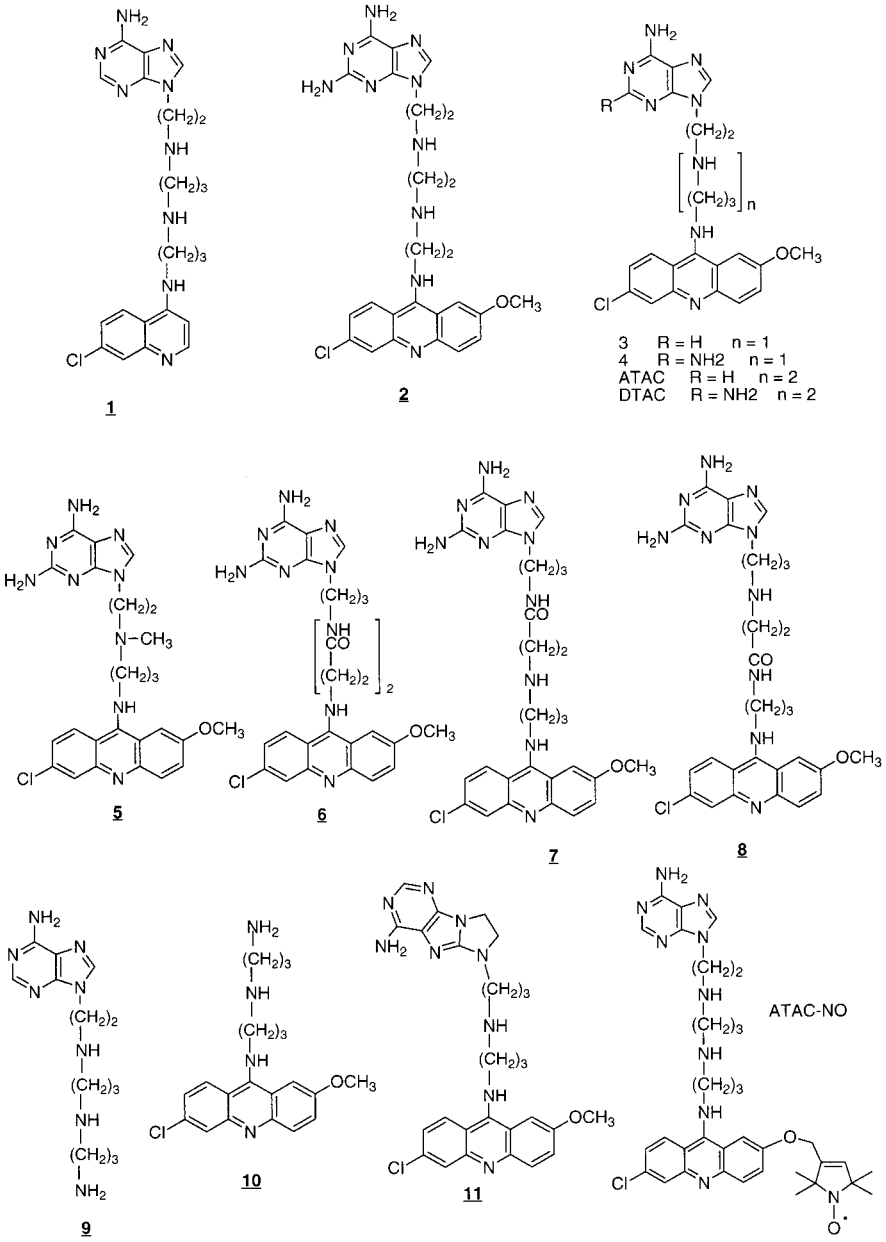


FIGURE 10 Structure of base–chain–aminoacridine conjugates.

the linker, being essentially unprotonated, catalyzes the cleavage (see Figure 11 for pKa's of the molecule).[121] It is interesting to note the critical effect of the degree of protonation of the amines in the linker. Changing the number of methylenes in the bridge as in molecule **2** dramatically modifies the pKa's of the amines (Figure 11) and the resulting molecule exhibits very different pH/activity profile with the highest cleaving activity observed at acidic pHs.

Detailed information about the mode of recognition of the abasic sites by drugs and about the nature of the drug–DNA complexes that precede cleavage were obtained by the study of the interaction of the drugs with a synthetic DNA duplex containing the stable tetrahydrofuran analogue of the abasic site and a thymine residue opposite the apurinic site in the complementary strand. Ultraviolet thermal denaturation studies demonstrated stabilization of the duplex by the drugs, indicating selective binding to the apurinic site.[122] High field nmr spectroscopy coupled to molecular modeling indicated identical behavior for the two most efficient drugs ATAC and DTAC.[41] Two complexes are formed in which the purine base of the drug inserts into the abasic pocket pairing with

Table I Complexation Constants for Native Calf Thymus DNA, Measured by Ethidium Bromide Displacement and Cleavage Activity on pBR322 DNA Plasmid Containing 1.8 Apurinic Sites[a]

Molecules	Affinity $K \times 10^{-4}$ (M^{-1})	Cleavage Activity	
		[b](%)	[c]
ATAC Ade-C_2-NH-C_3-NH-C_3-NHAcr	20	90	5×10^{-9}
DTAC DAP-C_2-NH-C_3-NH-C_3-NHAcr	110	100	1×10^{-9}
1 Ade-C_2-NH-C_3-NH-C_3-NHClQ	1	90	8×10^{-8}
2 DAP-C_2-NH-C_2-NH-C_2-NHAcr	40	80	7×10^{-7}
3 Ade-C_2-NH-C_3-NHAcr	7	23	$>10^{-5}$
4 DAP-C_2-NH-C_3-NHAcr	20	26	$>10^{-5}$
5 DAP-C_2-N(CH_3)-C_3-NHAcr	4	10	$>10^{-5}$
6 DAP-C_2-NHCO-C_2-NHCO-C_2-NHAcr	1,2	0	
7 DAP-C_2-NHCO-C_2-NH-C_3-NHAcr	50	23	$>10^{-5}$
8 DAP-C_2-NH-C_2-CONH-C_3-NHAcr	35	23	$>10^{-5}$
9 Ade-C_2-NH-C_3-NH-C_3-NH_2		0	
10 AcrNH-C_3-NH-C_3-NH_2		0	

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

thymine in the opposite strand, most probably in the Hoogsteen mode, the acridine intercalates at a two base pairs distance 5′ to the abasic site, and the polyamino chain lies in the minor groove. The two complexes only differ by a 180° rotation of the acridine ring aroud the C9—N bond (Figure 5). Additional data about the kinetics of complex formation were obtained with ATAC-NO,[123] a spin-labeled analogue of ATAC, in which a nitroxide probe was grafted on the acridine nucleus at the position methoxylated in the reference acridine (Figure 10). A characteristic EPR spectrum from which the correlation time was extracted is observed for the specific drug–DNA complex.[124]

The molecules ATAC and DTAC can thus be considered as AP-nuclease mimics, involving formation of a specific complex inside which cleavage occurs, catalyzed by the unprotonated amine of the linker that is favorably located to abstract the α-hydrogen of the aldehydic ring-opened d-ribose. A mechanism involving intermediacy of an iminium ion between the unprotonated amine and the d-ribose·aldehyde cannot be excluded (Figure 12).[121] More generally, the nucleic base–acridine conjugates constitute a family of AP-site cleaving reagents that exhibit the highest efficiency at any ionic strength and that can be worked out to cleave at any desired pH.

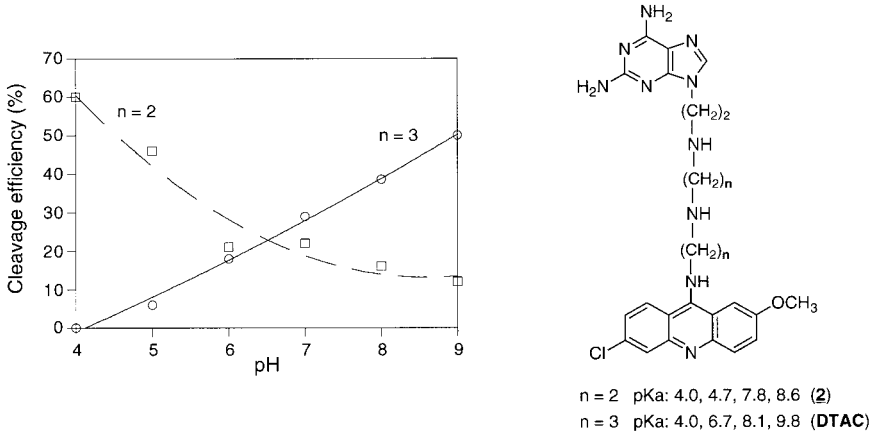


FIGURE 11 AP-site cleavage activity of DTAC (○) and compound **2** (□) as a function of pH. Concentration of ATAC is $10^{-6}M$, concentration of **2** is $10^{-4}M$, plasmid concentration is $10^{-9}M$, incubation time is 30 min at 25°C.

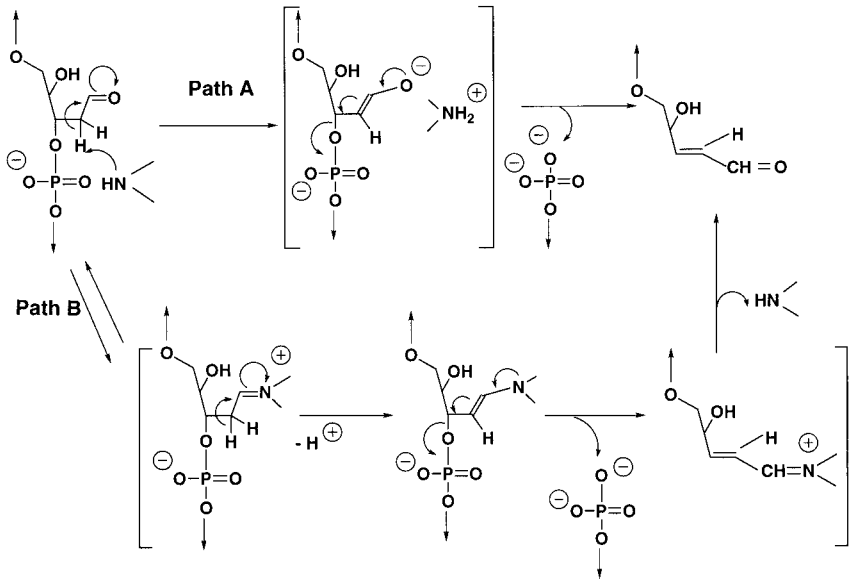


FIGURE 12 Mechanisms of the β-elimination reaction catalyzed by the base–chain–aminoacridine conjugates.

Cyclobisacridine (CBA)

The bisacridine macrocycle CBA that belongs to the cyclobisintercaland family has been prepared[125] and shown by the Lehn's group to associate strongly to nucleosides and monostranded oligonucleotides[126] (Figure 4). Its ability to recognize the abasic lesion in DNA was investigated using the same methodologies as described above for the acridine–nucleic base conjugates. We showed that CBA cleaves the ^{32}P duplex oligomer (23-mer) containing an apurinic site in the middle of the sequence, with lower efficiency, however, than the ATAC and DTAC molecules. Binding to the abasic site is however quite specific. Thermal denaturation experiments indicated that CBA stabilizes the undecamer containing the stable tetrahydrofuran analogue duplex by forming a 1:1 complex, while no interaction was detected with the parent unmodified duplex. CBA displaces the nitroxide abasic site probe ATAC-NO from the abasic duplex. When illuminated in the presence of ^{32}P-labeled model abasic site containing duplex (23-mer), CBA induced selective photocleavage in the vicinity of the abasic lesion on both strands.[127] Combined high field nmr spectroscopy and restrained molecular dynamics calculations indicated formation of a major complex of the threading intercalation mode in which CBA penetrates the double helix at the abasic site sandwiching the base-pair 3′ to the lesion between the two acridine rings, leaving the two protonated linking chains respectively in the major and in the minor groove (See Figure 13 on p. 78).[128]

All these experiments suggest that the abasic lesion represents a site of strong thermodynamic destabilization in DNA and of enhanced flexibility. It thus may represent a site of favoured interaction for drugs such as intercalators.

THE ABASIC SITE AS A TARGET FOR CYTOTOXIC DRUGS

As discussed above, abasic sites are formed in the cell with fairly high frequency either spontaneously or enzymatically as intermediates during the repair of modified bases through the BER mechanism (Figure 8). Molecules that bind specifically and strongly to abasic sites are thus candidates to interfere and possibly inhibit the repair process. Moreover, such an inhibitory effect might be useful to sensitize tumor cells to the activity of alkylating drugs. Indeed, a number of anticancer drugs, mostly of the alkylating family, have been reported to generate abasic sites and are thus susceptible to potentiation by abasic-site specific molecules (Figure 8). A few compounds—aminoellipticine, bisnaphtalimide DMP 840, aminocarbazole (Figure 4), and the nucleic–base conjugates (Figure 10)—have been evaluated along these lines. In addition, more recently it has been reported that genomic damages, such as abasic sites, may influence the activities of topoisomerases. This represents additional impulse for designing abasic site specific drugs. Malvy and colleagues were the first to discuss inhi-

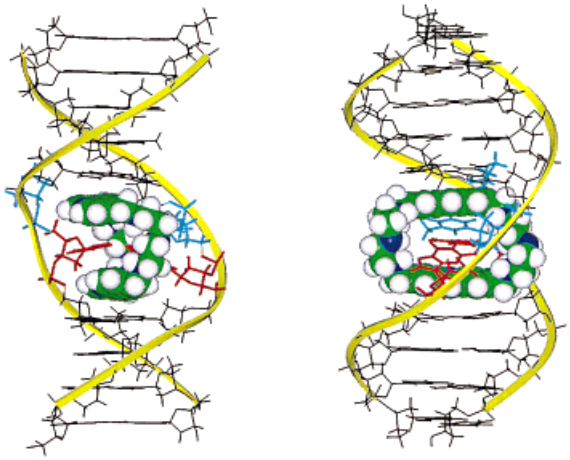
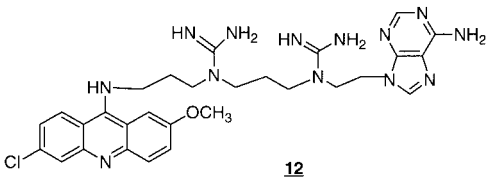


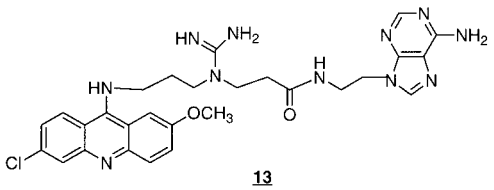
FIGURE 13 Molecular modeling based structure of the CBA–abasic DNA complex. The drug is drawn in CPK. Left view is from the major groove and right view is the same representation after a 90° angle rotation.

bition of BER by drugs acting at pharmacological concentrations. The only inhibitor mentioned before was methoxyamine, a molecule that reacts with abasic sites and inhibits AP-endonucleases in vitro, but at very high doses (50 m*M*).[129] Malvy reported that 9-AE and the structurally related isopropyl-oxazolopyridocarbazole potentiate the cytotoxic effect of alkylating drugs such as dimethyl sulfate in *E. coli* through a mechanism involving apurinic sites.[114] He later showed that, in vitro, 9-AE inhibits 65 % of the endonuclease for apurinic/apyrimidine site activity of *E. coli* exonuclease III.[130] It was suggested that inhibition might result from irreversible adduct formation at the 3′ side of the apurinic site following cleavage.[39,111,112]

More recently, DMP-840,[131] a bisnaphtalimide derivative, was reported as an AP-endonuclease inhibi-



12



13

FIGURE 14 Structure of guanidinium conjugates acting as AP-site repair inhibitors.

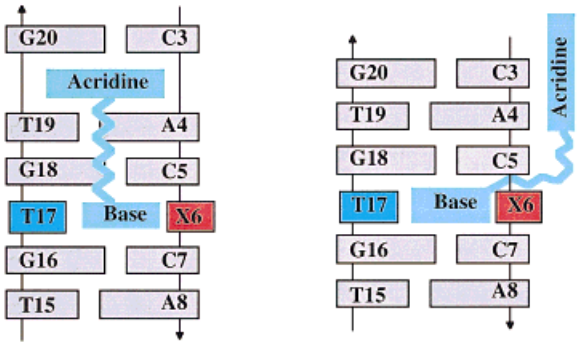


FIGURE 15 Schematic representation of the major (left) and minor (right) **12**–abasic DNA complexes. In both cases, the adenine of the drug is stabilized by Watson–Crick pairing with the unpaired thymine of the abasic oligonucleotide.

tor and went to phase II clinical trials. To date, no potentiation effect of the drug has been identified.[132]

The series of the nucleic base-acridine conjugates that were designed to recognize specifically abasic DNA and that exhibited cleavage activity were tested as potential inhibitors of BER, and their cytotoxicity and possible synergy with anticancer drugs were evaluated in an effort to find some kind of structure–activity relationship.[133] The inhibitory activities on BER were examined, in vitro, with plasmids damaged by methylmethane sulfonate (MMS), indicating that ATAC and DTAC are definite inhibitors with IC_{50} values of 70 and 62 μM, respectively. The other compounds in the series are either less active or inactive. The two compounds also exhibit potentiation of MMS cytotoxicity against L1210 cultured cells. Interestingly sensitization to bis-chloroethylnitrosourea (BCNU), a clinically useful anticancer drug, is also observed. Thus in the presence of BCNU the IC_{50} value shifts from 5.6 μM with BCNU alone to 0.43 or 0.85 μM with a combination of BCNU and ATAC and DTAC, respectively, when the latter were used at a concentration corresponding to 10% cytotoxicity.

Another family of nucleic base–acridine conjugates was also designed in which the amino groups of the linker are replaced by guanidinium and/or amide junctions so as to suppress the cleavage activity while retaining (or increasing) the binding to DNA (Figure 14). The hypothesis is that such compounds, by docking in the abasic site, could mask the lesion to the AP-endonuclease. This might result in inhibition of repair of this damage caused at high frequency by anticancer alkylating agents (Figure 8). Molecule **12** is an analogue of ATAC possessing two guanidinium moieties in the linker.[134] As predicted, **12** binds to DNA with higher affinity than ATAC, it does not

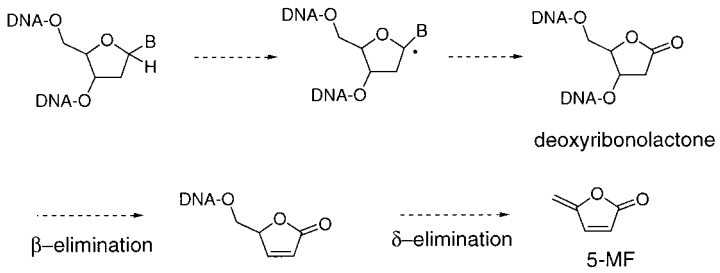


FIGURE 16 Simplified route of deoxyribonolactone formation and structure of some decomposition products.

cleave plasmid abasic DNA, and it forms specific 1:1 complex with the stable model abasic duplex (TX), as indicated by thermal denaturation experiments and high field nmr spectroscopy. Two types of specific complexes were identified by nmr (Figure 15) in which the adenine moiety of the drug is inserted in the abasic cavity. Watson–Crick base pairing of this adenine with thymine in the opposite strand was definitely established by characteristic nuclear Overhauser effects between the complementary bases (T 17-H3 and A-H2 of the drug). This Watson–Crick base pairing was not observed with ATAC and DTAC, which suggests longer lifetime of the complexes and stronger binding of **12**. In the in vitro experiments, **12** shows apparent synergy with BCNU on the murine leukaemia L1210 and human adenocarcinoma A549 cell lines. In vivo, on the murine leukemia P388, the compound shows no antitumor property but definitely potentiates the action of BCNU (treatment with BCNU alone led to 50% of 60 days survival, while association with **12** resulted in 100% survival). Analogue **12** elicits, however, curare-like acute toxicity, possibly due to the presence of the two guanidinium moieties. The analogue **13** that contains one guanidinium and one amido function in the linker was most recently prepared. It also binds to abasic DNA, it inhibits the repair activity of Exonuclease III. However, unlike the bis-guanidinium analogue, **13** shows simple additivity of the toxic effects with BCNU.[135]

All these biological data cannot be directly interpreted in terms of mechanism of action due to the extreme complexity of the system. However, it appears that those compounds that have been shown to bind specifically and strongly to the abasic site equally present the most interesting activities in vitro and even in vivo, most particularly in association with alkylating and/or anticancer drugs that create multiple abasic lesions in the cell. It thus seems that targeting the abasic site by specific drugs may be a pertinent and useful strategy to improve the clinical efficiency of currently available DNA-damaging agents.

OXIDIZED ABASIC SITES: THE 2′-DEOXYRIBONOLACTONE LESION

2′-Deoxyribonolactone is the oxidized form of the abasic site as defined above. It corresponds to a damage in DNA that has been reported to occur under the action of different drugs, notably the enediyne antibiotics[136–139] and by γ-radiolysis.[140] The mode of generation, although the subject of current debate,[141–143] is generally considered to involve radical H-1′ abstraction and oxydation (Figure 16). Different generation modes and properties of this precursor radical have been recently discussed.[144–151] The 2′-deoxyribonolactone lesion has been reported to be mutagenic and resistant to repair enzymes. However, unlike the "common d-ribose abasic site," little is known about the d-ribonolactone lesion, as until very recently, no general method was available to create selectively the lesion in DNA or to synthetize DNA fragments containing the lesion at defined position. In some cases d-ribonolactone has been chemically characterized. Most frequently its presence was detected by its alkali-labile nature, being the subject of successive β- and δ-elimination with ultimate formation of 5-methylene-furanone (5-MF; Figure 16). Quite recently Greenberg and colleagues[152] reported on formation of oligonucleotides containing this lesion via aerobic photolysis of oligonucleotides containing a photolabile ketone. The oligonucleotides were characterized by gel electrophoresis and mass spectrometry. Treatment with secondary amines produces strand breaks via β-elimination. Adduct formation between the β-elimination product and the amine, or added thiol, was also evidenced. The first general and efficient method of preparation of oligonucleotides containing d-ribonolactone at the preselected position was developed in our laboratory.[153,154] The synthesis is based upon illumination ($\lambda > 300$ nm) of a nitroindole nucleoside inserted in a DNA fragment that is quantitatively transformed into d-ribonolactone and nitrosoindole, as a result of H-1′ radical abstraction by one of the nitro group at a 2.4 Å distance.[155] An

undecamer duplex containing the 2′-deoxyribonolactone in the middle of the sequence, opposite a thymine residue in the complementary strand, was prepared and examined by high field nuclear magnetic resonance and molecular modeling.[156] The sequence and location of the d-ribonolactone residue are identical to those in the duplex containing the tetrahydrofuran abasic site, thus allowing direct comparison of the impact of the two lesions on the DNA structure. As observed for the tetrahydrofuran abasic site 2′-deoxyribonolactone induces little change in the general DNA structure that remains in the B-form with only localized perturbations at the site of the lesion. In the sequence context examined, i.e., an apurinic lesion flanked by two cytosines, the thymine residue opposite the lesion adopts an intrahelical location as observed for the "regular" abasic site. No kink could be evidenced at the site of the damage. However, common to the two types of modifications is the considerable destabilization of the duplexes as evidenced by the thermal denaturation experiments (T_m decrease of 20°C). Destabilization and flexibility at the damaged sites may represent the essential features in terms of recognition by repair enzymes.

CONCLUSION

Although it has been more than three decades since the first in vitro studies of abasic sites, a lot more needs to be understood on their influence on the regulation of gene expression. Because they are considered noninformative damage, AP-sites constitute a mutagenic or lethal threat for cells, and have to be repaired prior to replication and transcription. The persistence of AP-sites has been shown to poison other cellular vital enzymes such as topoisomerases. These recent studies show the complexity of the relationships between DNA damage, repair pathways, and gene expression. These findings of great importance suggest also that antitumor drugs of the future should combine potentializing activities such as DNA agression and DNA damage repair inhibition. The finding of synergistic association of drugs (i.e., alkylating agents and AP-sites repair inhibitors) might constitute new and promising strategies for cancer therapy.

I thank all my students and long-time co-workers for their enthusiasm in the study of abasic DNA, including Drs. N. Berthet, C. and Ce. Coulombeau, E. Defrancq, C. Fontaine, J. Garcia, A. Jeunet, M. Kotera, J. and P. Michon, and C. Morat, as well as the Ligue Nationale contre le Cancer (LNC) and the Association pour la recherche sur le Cancer (ARC) for financial support.

REFERENCES

1. Singer, B.; Grunberger, D. Molecular Biology, of Mutagens and Carcinogens; Plenum Press: New York, 1983; pp 16–19.
2. Chaudhry, M. A.; Weinfeld, M. J Biol Chem 1997, 272, 15650–15655.
3. Naegeli, H. Mechanism of DNA Damage Recognition in Mammalian Cells; R. G. Landes Company: Austin, Texas, USA; Springer-Verlag: Heidelberg, Germany, 1997.
4. Wood, R. D. Annu Rev Biochem 1996, 65, 135–167.
5. Pourquier, P.; Ueng, L. M.; Kohlhagen, G.; Mazumder, A.; Gupta, M.; Kohn, K. W.; Pommier, Y. J Biol Chem 1997, 272, 7792–7796.
6. Kingma, P. S.; Osheroff, N. Biochim Biophys Acta 1998, 140, 223–232.
7. Lindahl, T.; Andersson, A. Biochemistry 1972, 11, 3618–3623.
8. Loeb, L. A.; Preston, B. D. Annu Rev Genet 1986, 20, 201–230.
9. Bertrand, J.-R.; Malvy, C.; Paoletti, C. Biochem Biophys Res Commun 1987, 143, 768–774.
10. Stuart, G. R.; Chambers, R. W. Nucleic Acids Res 1987, 15, 7451–7462.
11. Vasseur, J. J.; Rayner, B.; Imbach, J.-L. Biochem Biophys Res Commun 1986, 134, 1204–1208.
12. Vasseur, J.-J.; Peoc'h, D.; Rayner, B.; Imbach, J.-L. Nucleosides Nucleotides 1991, 10, 107–117.
13. Iocono, J. A.; Gildea, B.; McLaughlin, L. W. Tetrahedron Lett 1990, 31, 175–178.
14. Laayoun, A.; Décout, J.-L.; Lhomme, J. Tetrahedron Lett 1994, 35, 4989–4990.
15. Laayoun, A.; Décout, J.-L.; Defrancq, E.; Lhomme, J. Tetrahedron Lett 1994, 35, 4991–4994.
16. Groebke, K.; Leumann, C. Helv Chim Acta 1990, 73, 608–617.
17. Peoc'h, D.; Meyer, A.; Imbach, J.-L.; Rayner, B. Tetrahedron Lett 1991, 32, 207–210.
18. Coleman, R. S.; Pires, R. M. Nucleosides Nucleotides 1999, 18, 2141–2146.
19. Takeshita, M.; Chang, C. N.; Johnson, F.; Will, S.; Grollman, A. P. J Biol Chem 1987, 262, 10171–10179.
20. Raap, J.; Dreef, C. E.; Van der Marel, G. A.; Van Boom, J. H.; Hilbers, C. W. J Biomol Struct Dynam 1987, 5, 219–247.
21. Pochet, S.; Huynh-Dinh, T.; Neumann, J.-M.; Tran-Dinh, S.; Taboury, J. A.; Taillandier, E.; Igolen, J. Tetrahedron Lett 1985, 26, 2085–2088.
22. Thomas, M.; Castaing, B.; Fourrey, J.-L.; Zelwer, C. Nucleosides Nucleotides 1999, 18, 239–243.
23. Schärer, O. D.; Ortholand, J.-Y.; Ganesan, A.; Ezaz-Nikpay, K.; Verdine, G. L. J Am Chem Soc 1995, 117, 6623–6624.
24. Manoharan, M.; Gerlt, J. A. J Am Chem Soc 1987, 109, 7217–7219.

25. Manoharan, M.; Ransom, S. C.; Mazumder, A.; Gerlt, J. A.; Wilde, J. A.; Withka, J. A.; Bolton, P. H. J Am Chem Soc 1988, 110, 1620–1622.
26. Wilde, J. A.; Bolton, P. H.; Mazumder, A.; Manoharan, M.; Gerlt, J. A. J Am Chem Soc 1989, 111, 1894–1896.
27. Bailly, V.; Derydt, M.; Verly, W. G. Biochem J 1989, 261, 707–713.
28. Bailly, V.; Verly, W. G. Biochem J 1988, 253, 553–559.
29. McHugh, P. J.; Knowland, J. Nucleic Acids Res 1995, 23, 1664–1670.
30. Latham, K. A.; Lloyd, R. S. Biochemistry 1995, 34, 8796–8803.
31. Bailly, V.; Verly, W. G. Nucleic Acids Res 1988, 16, 9489–9496.
32. Mazumder, A.; Gerlt, J. A.; Absalon, M. J.; Stubbe, J.; Cunningham, R. P.; Withka, J.; Bolton, P. H. Biochemistry 1991, 30, 1119–1126.
33. Mazumder, A.; Gerlt, J. A.; Rabow, L.; Absalon, M. J.; Stubbe, J.-A.; Bolton, P. H. J Am Chem Soc 1989, 111, 8029–8030.
34. Sugiyama, H.; Fujiwara, T.; Ura, A.; Tashiro, T.; Yamamoto, K.; Kawanishi, S.; Saito, I. Chem Res Toxicol 1994, 7, 673–683.
35. Manoharan, M.; Andrade, L. K.; Cook, P. D. Org Lett 1999, 1, 311–314.
36. Fukui, K.; Morimoto, M.; Segawa, H.; Tanaka, K.; Shimidzu, T. Bioconjugate Chem 1996, 7, 349–355.
37. Goljer, I.; Withka, J. M.; Kao, J. Y.; Bolton, P. H. Biochemistry 1992, 31, 11614–11619.
38. Goljer, I.; Kumar, S.; Bolton, P. H. J Biol Chem 1995, 270, 22980–22987.
39. Singh, M. P.; Hill, G. C.; Peoc'h, D.; Rayner, B.; Imbach, J. L.; Lown, J. W. Biochemistry 1994, 33, 10271–10285.
40. Withka, J. M.; Wilde, J. A.; Bolton, P. H.; Mazumder, A.; Gerlt, J. A. Biochemistry 1991, 30, 9931–9940.
41. Coppel, Y.; Berthet, N.; Coulombeau, C.; Garcia, J.; Lhomme, J. Biochemistry 1997, 36, 4817–4830.
42. Cuniasse, P.; Sowers, L. C.; Eritja, R.; Kaplan, B.; Goodman, M. F.; Cognet, J. A.; LeBret, M.; Guschlbauer, W.; Fazakerley, G. V. Nucleic Acids Res 1987, 15, 8003–8022.
43. Cuniasse, P.; Sowers, L. C.; Eritja, R.; Kaplan, B.; Goodman, M. F.; Cognet, J. A.; Le Bret, M.; Guschlbauer, W.; Fazakerley, G. V. Biochemistry 1989, 28, 2018–2026.
44. Kalnik, M. W.; Chang, C. N.; Grollman, A. P.; Patel, D. J. Biochemistry 1988, 27, 924–931.
45. Kalnik, M. W.; Chang, C. N.; Johnson, F.; Grollman, A. P.; Patel, D. J. Biochemistry 1989, 28, 3373–3383.
46. Cline, S. D.; Jones, W. R.; Stone, M. P.; Osheroff, N. Biochemistry 1999, 38, 15500–15507.
47. Cuniasse, P.; Fazakerley, G. V.; Guschlbauer, W.; Kaplan, B. E.; Sowers, L. C. J Mol Biol 1990, 213, 303–314.
48. Marathias, V. M.; Jerkovic, B.; Bolton, P. H. Nucleic Acids Res 1999, 27, 1854–1858.
49. Gelfand, C. A.; Plum, G. E.; Grollman, A. P.; Johnson, F.; Breslauer, K. J. Biochemistry 1998, 37, 7321–7327.
50. Vesnaver, G.; Chang, C. N.; Eisenberg, M.; Grollman, A. P.; Breslauer, K. J. Proc Natl Acad Sci USA 1989, 86, 3614–3618.
51. Cognet, J. A.; Gabarro-Arpa, J.; Cuniasse, P.; Fazakerley, G. V.; Le Bret, M. J Biomol Struct Dynam 1990, 7, 1095–1115.
52. Ayadi, L.; Coulombeau, C.; Coulombeau, C.; Lavery, R. In Biological Macromolecular Dynamics; Cusak, S., Ed.; Adenine Press: New York, 1997; pp 83–86.
53. Ayadi, L.; Coulombeau, C. Theor Chem Acc 1999, 101, 121–125.
54. Ayadi, L.; Coulombeau, C.; Lavery, R. Biophys J 1999, 77, 318–3226.
55. Ayadi, L.; Jourdan, M.; Coulombeau, C.; Garcia, J.; Lavery, R. J Biomol Struct Dynam 1999, 17, 245–257.
56. Ayadi, L.; Coulombeau, C.; Lavery, R. J Biomol Struct Dynam 1999, in press.
57. Brent, T. P.; Teebor, G. W.; Ducker, N. J. In DNA Repair and Mechanisms; Hanauralt, P. C.; Friedberg, E. C.; Fox, C. F., Eds; Academic Press: New York, 1978; 19–22.
58. Kohn, K. W.; Ewig, R. A. G.; Erickson, L. C.; Zwelling, L. A. In DNA Repair. A Laboratory Manual of Research Procedures; Friedberg, E. C., Hanawalt, P., Ed.; Marcel Dekker: New York: 1981; Vol. 1, pp 379–401.
59. Weinfeld, M.; Liuzzi, M.; Paterson, M. Nucleic Acids Res 1989, 17, 3735–3745.
60. Coombs, M. M.; Livingstone, D. C. Biochem Biophys Acta 1969, 174, 161–173.
61. Livingstone, D. C. Biochem Biophys Acta 1964, 87, 538–540.
62. Talpaert-Borlé, M.; Liuzzi, M. Biochem Biophys Acta 1983, 740, 410–416.
63. Talpaert-Borlé, M.; Liuzzi, M. Eur Patent Application 0122507, 1984. A2.
64. Rosa, S.; Fortini, P.; Karran, P.; Bignami, M.; Dogliotti, E. Nucleic Acids Res 1991, 19, 5569–5574.
65. Fortini, P.; Calcagnile, A.; Vrieling, H.; Van Zeeland, A. A.; Bignami, M.; Dogliotti, E. Cancer Res 1993, 53, 1149–1155.
66. Chen, B. X.; Kubo, K.; Ide, H.; Erlanger, B. F.; Wallace, S. S.; Kow, Y. W. Mutat Res 1992, 273, 253–261.
67. Nakamura, J.; Walker, V. E.; Upton, P. B.; Chiang, S. Y.; Kow, Y. W.; Swenberg, J. A. Cancer Res 1998, 58, 222–225.
68. Nakamura, J.; Swenberg, J. A. Cancer Res 1999, 59, 2522–2526.
69. Asaeda, A.; Ide, H.; Tano, K.; Takamori, Y.; Kubo, K. Nucleosides Nucleotides 1998, 17, 503–513.
70. Asaeda, A.; Hiroshi, T.; Takamori, Y.; Kubo, K. Anal Chim Acta 1998, 365, 35–41.
71. Boturyn, D.; Boudali, A.; Constant, J.-F.; Lhomme, J. Tetrahedron 1997, 53, 5485–5492.

72. Boturyn, D.; Constant, J. F.; Defrancq, E.; Lhomme, J.; Barbin, A.; Wild, C. P. Chem Res Toxicol 1999, 12, 476–482.
73. Adamczyk, M.; Mattingly, P. G.; Moore, J. A. Bioorg Med Chem Lett 1998, 8, 3599–3602.
74. Makrigiorgos, G. M.; Chakrabarti, S.; Mahmood, A. Int J Radiat Biol 1998, 74, 99–109.
75. Adamczyk, M.; Mattingly, P. G.; Moore, J. A.; Pan, Y. Org Lett 1999, 1, 779–781.
76. Chakrabarti, S.; Mahmood, A.; Makrigiorgos, G. M. Int J Radiat Biol 1999, 75, 1055–1065.
77. Maulik, G.; Botchway, S.; Chakrabarti, S.; Tetradis, S.; Price, B.; Makrigiorgos, G. M. Nucleic Acids Res 1999, 27, 1316–1322.
78. Lindahl, T.; Karran, P.; Wood, R. D. Curr Opin Genet Develop 1997, 7, 158–169.
79. David, S. S.; Williams, S. D. Chem Rev 1998, 98, 1221–1261.
80. Krokan, H. E.; Standal, R.; Slupphaug, G. Biochem J 1997, 325, 1–16.
81. Doetsch, P. W.; Cunningham, R. P. Mutation Res 1990, 236, 173–201.
82. Demple, B.; Harrison, L. Annu Rev Biochem 1994, 63, 915–948.
83. Bailly, V.; Verly, W. G. Nucleic Acids Res 1989, 17, 3617–3618.
84. Manoharan, M.; Mazumder, A.; Ransom, S. C.; Gerlt, J. A.; Bolton, P. H. J Am Chem Soc 1988, 110, 2690–2691.
85. McCullough, A. K.; Dodson, M. L.; Lloyd, R. S. Annu Rev Biochem 1999, 68, 255–285.
86. Demple, B.; Harrison, L.; Wilson, D. M., 3rd; Bennett, R. A.; Takagi, T.; Ascione, A. G. Environ Health Perspect 1997, 105 (Suppl 4), 931–934.
87. Mol, C. D.; Kuo, C. F.; Thayer, M. M.; Cunningham, R. P.; Tainer, J. A. Nature 1995, 374, 381–386.
88. Gorman, M. A.; Morera, S.; Rothwell, D. G.; de La Fortelle, E.; Mol, C. D.; Tainer, J. A.; Hickson, I. D.; Freemont, P. S. EMBO J 1997, 16, 6548–6558.
89. Hosfield, D. J.; Guan, Y.; Haas, B. J.; Cunningham, R. P.; Tainer, J. A. Cell 1999, 98, 397–408.
90. Loeb, L. A.; Preston, B. D.; Snow, E. T.; Schaaper, R. M. Basic Life Sci 1986, 38, 341–347.
91. Schaaper, R. M.; Kunkel, T. A.; Loeb, L. A. Proc Natl Acad Sci USA 1983, 80, 487–491.
92. Kunkel, T. A. Proc Natl Acad Sci USA 1984, 81, 1494–1498.
93. Dogliotti, E.; Fortini, P.; Pasucci, B. In Base Excision Repair of DNA Damage; Hickson, I. D., Ed.; Landes Bioscience: Austin, Texas, USA, 1997; pp 81–101.
94. Gentil, A.; Renault, G.; Madzak, C.; Margot, A.; Cabral-Neto, J. B.; Vasseur, J. J.; Rayner, B.; Imbach, J. L.; Sarasin, A. Biochem Biophys Res Commun 1990, 173, 704–710.
95. Hevroni, D.; Livneh, Z. Proc Natl Acad Sci USA 1988, 85, 5046–5050.
96. Boiteux, S.; Laval, J. Biochemistry 1982, 21, 6746–6751.
97. Cai, H.; Bloom, L. B.; Eritja, R.; Goodman, M. F. J Biol Chem 1993, 268, 23567–23572.
98. Randall, S. K.; Eritja, R.; Kaplan, B. E.; Petruska, J.; Goodman, M. F. J Biol Chem 1987, 262, 6864–6870.
99. Paz-Elizur, T.; Takeshita, M.; Livneh, Z. Biochemistry 1997, 36, 1766–1773.
100. Shibutani, S.; Takeshita, M.; Grollman, A. P. J Biol Chem 1997, 272, 13916–13922.
101. Kingma, P. S.; Osheroff, N. J Biol Chem 1997, 272, 7488–7493.
102. Kingma, P. S.; Osheroff, N. J Biol Chem 1997, 272, 1148–1155.
103. Kingma, P. S.; Osheroff, N. J Biol Chem 1998, 273, 17999–18002.
104. Kingma, P. S.; Corbett, A. H.; Burcham, P. C.; Marnett, L. J.; Osheroff, N. J Biol Chem 1995, 270, 21441–21444.
105. Behmoaras, T.; Toulme, J. J.; Helene, C. Proc Natl Acad Sci USA 1981, 78, 926–930.
106. Duker, N. J.; Hart, D. M. Biochem Biophys Res Commun 1982, 105, 1433–1439.
107. Pierre, J.; Laval, J. J Biol Chem 1981, 256, 10217–10220.
108. Behmoaras, T.; Hélène, C. Nature 1981, 292, 858–859.
109. Malvy, C.; Prevost, P.; Gansser, C.; Viel, C.; Paoletti, C. Chem Biol Interactions 1986, 57, 41–53.
110. Bertrand, J. R.; Vasseur, J. J.; Gouyette, A.; Rayner, B.; Imbach, J. L.; Paoletti, C.; Malvy, C. J Biol Chem 1989, 264, 14172–14178.
111. Bertrand, J. R.; Vasseur, J.-J.; Rayner, B.; Imbach, J.-L.; Paoletti, J.; Paoletti, C.; Malvy, C. Nucleic Acids Res 1989, 17, 10307–10319.
112. Vasseur, J.-J.; Rayner, B.; Imbach, J.-L.; Verma, S.; McCloskey, J. A.; Lee, M.; Chang, D. K.; Lown, J. W. J Org Chem 1987, 52, 4994–4998.
113. Vasseur, J.-J.; Rayner, B.; Imbach, J.-L.; Bertrand, J.-R.; Malvy, C.; Paoletti, C. Nucleosides Nucleotides 1989, 8, 863–866.
114. Malvy, C.; Safraoui, H.; Bloch, E.; Bertrand, J. R. Anticancer Drug Des 1988, 2, 361–370.
115. Berthet, N.; Boudali, A.; Constant, J. F.; Decout, J. L.; Demeunynck, M.; Fkyerat, A.; Garcia, J.; Laayoun, A.; Michon, P.; Lhomme, J. J Mol Recognit 1994, 7, 99–107.
116. Fkyerat, A.; Demeunynck, M.; Constant, J.-F.; Lhomme, J. Tetrahedron 1993, 49, 11237–11252.
117. Fkyerat, A.; Demeunynck, M.; Constant, J.-F.; Michon, P.; Lhomme, J. J Am Chem Soc 1993, 115, 9952–9959.
118. Constant, J. F.; Fkyerat, A.; Demeunynck, M.; Laval, J.; O'Connor, T. R.; Lhomme, J. Anticancer Drug Des 1990, 5, 59–62.
119. Constant, J. F.; O'Connor, T. R.; Lhomme, J.; Laval, J. Nucleic Acids Res 1988, 16, 2691–2703.
120. Belmont, P.; Alarcon, K.; Demeunynck, M.; Lhomme, J. Bioorg Med Chem Lett 1999, 9, 233–236.

121. Belmont, P.; Boudali, A.; Constant, J.-F.; Demeunynck, M.; Fkyerat, A.; Michon, P.; Serratrice, G.; Lhomme, J. New J Chem 1997, 21, 47–54.
122. Berthet, N.; Constant, J. F.; Demeunynck, M.; Michon, P.; Lhomme, J. J Med Chem 1997, 40, 3346–3352.
123. Belmont, P.; Chapelle, C.; Demeunynck, M.; Michon, J.; Michon, P.; Lhomme, J. Bioorg Med Chem Lett 1998, 8, 669–674.
124. Thomas, F.; Michon, J.; Lhomme, J. Biochemistry 1999, 38, 1930–1937.
125. Teulade, M. P.; Vigneron, J. P.; Lehn, J. M. J Supramol Chem 1995, 5, 139–147.
126. Slama-Schwok, A.; Teulade-Fichou, M. P.; Vigneron, J.-P.; Taillandier, E.; Lehn, J.-M. J Am Chem Soc 1995, 117, 6822–6830.
127. Berthet, N.; Michon, J.; Lhomme, J.; Teulade-Fichou, M.-P.; Vigneron, J.-P.; Lehn, J.-M. Chem Eur J 1999, 5, 3625–3630.
128. Jourdan, M.; Garcia, J.; Lhomme, J.; Teulade-Fichou, M.-P.; Vigneron, J.-P.; Lehn, J.-M. Biochemistry 1999, 38, 14205–14213.
129. Liuzzi, M.; Weinfeld, M.; Paterson, M. C. Biochemistry 1987, 26, 3315–3321.
130. Lefrancois, M.; Bertrand, J. R.; Malvy, C. Mutat Res 1990, 236, 9–17.
131. Thompson, J.; Pratt, C. B.; Stewart, C. F.; Avery, L.; Bowman, L.; Zamboni, W. C.; Pappo, A. Invest New Drugs 1998, 16, 45–49.
132. Bobola, M. S.; Berger, M. S.; Stevens, B.; Silber, J. R. In Proceedings of the Annual Meeting of the American Association of Cancer Research; 1997; Vol 38, p 314.
133. Barret, J. M.; Etievant, C.; Fahy, J.; Lhomme, J.; Hill, B. T. Anticancer Drugs 1999, 10, 55–65.
134. Belmont, P.; Jourdan, M.; Demeunynck, M.; Constant, J.-F.; Garcia, J.; Lhomme, J.; Croisy, A.; Carez, D. J Med Chem 1999, 42, 5153–5159.
135. Belmont, P.; Demeunynck, M.; Constant, J.-F.; Lhomme, J. Bioorg Med Chem Lett 2000, 10, 293–295.
136. Kappen, L. S.; Chen, C.; Goldberg, I. H. Biochemistry 1988, 27, 4331–4340.
137. Kappen, K. S.; Goldberg, I. H. Biochemistry 1989, 28, 1027–1032.
138. Povirk, L. F.; Houlgrave, C. W.; Han, Y.-H. J Biol Chem 1988, 263, 19263–19266.
139. Povirk, L. F.; Goldberg, I. H. Proc Natl Acad Sci USA 1985, 82, 3182–3186.
140. Shaw, A. A.; Cadet, J. Int J Radiat Biol 1996, 70, 1–6.
141. Chen, T.; Greenberg, M. M. J Am Chem Soc 1998, 120, 3815–3816.
142. Zelenko, O.; Gallagher, J.; Sigman, D. S. Angew Chem Int Ed Engl 1997, 36, 2776–2778.
143. Chatgilialoglu, C.; Gimisis, T. Chem Commun 1998, 1249–1250.
144. Goyne, T. E.; Sigman, D. S. J Am Chem Soc 1987, 109, 2846–2848.
145. Bose, R. N.; Fonkeng, B. S.; Moghaddas, S.; Stroup, D. Nucleic Acids Res 1998, 27, 1588–1596.
146. Cheng, C.-C.; Goll, J. G.; Neyhart, G. A.; Welch, T. W.; Singh, P.; Thorp, H. H. J Am Chem Soc 1995, 117, 2970–2980.
147. Pitié, M.; Bernardou, J.; Meunier, B. J Am Chem Soc 1995, 117, 2935–2936.
148. Sugiyama, H.; Tsutsumi, Y.; Saito, I. J Am Chem Soc 1990, 112, 6720–6721.
149. Urata, H.; Akagi, M. Nucleic Acids Res 1991, 19, 1773–1778.
150. Buchko, G. W.; Cadet, J. Can J Chem 1992, 70, 1827–1832.
151. Urata, H.; Yamamoto, K.; Akagi, M.; Hiroaki, H.; Uesugi, S. Biochemistry 1989, 28, 9566–9569.
152. Hwang, J. T.; Tallman, K. A.; Greenberg, M. M. Nucleic Acids Res 1999, 27, 3805–3810.
153. Kotera, M.; Bourdat, A.-G.; Defrancq, E.; Lhomme, J. J Am Chem Soc 1998, 120, 11810–11811.
154. Kotera, M.; Bourdat, A.-G.; Defrancq, E.; Jourdan, M.; Garcia, J.; Coulombeau, C.; Lhomme, J. Nucleosides Nucleotides 1999, 18, 1323–1324.
155. Averbuch-Pichot, M. T.; Bourdat, A. G.; Defrancq, E.; Durif, A.; Kotera, M.; Lhomme, J. Zeit Kristallog 1998, 213, 181–182.
156. Jourdan, M.; Garcia, J.; Defrancq, E.; Kotera, M.; Lhomme, J. Biochemistry 1999, 38, 3985–3995.