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Photoreactive threading agent that specifically binds to abasic sites in DNA

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Abstract—We report the synthesis and study of a photoreactive nitrobenzamide containing acridine that specifically interacts at abasic site in DNA by threading intercalation and introduces under irradiation a lesion on the opposite strand at the unpaired pyrimidine.

The abasic site, that is, the loss of a base, is one of the most frequent lesions in DNA.[1] Although it can occur spontaneously,[2] this lesion results from the action of various chemical and physical agents used in chemotherapy. Abasic sites are also produced during the base excision repair pathway following the excision of modified or non-natural bases by glycosylases.[3] Previous works in our laboratory focused on the design of molecules for the specific recognition of abasic sites and capable of interfering with the repair of this lesion.[4] The first series of acridine–purine heterodimers was shown to specifically interact at abasic sites and cleave the DNA strand.[5] These molecules behave as 'artificial nucleases', and cleavage is triggered in the pre-formed drug–DNA complex by a non-protonated secondary amine of the linking chain of the drug acting as a β-elimination catalyst. NMR studies of the interaction of the acridine–purine heterodimer with a duplex DNA undecamer containing a stable analogue of the abasic site have revealed that the drug fits perfectly the abasic site. The purine moiety is docked in the abasic pocket and the acridine moiety is intercalated at a two base pair distance on the 5′ side of the lesion.[6] Starting from this optimized structure, we have modified the acridine nucleus to endow the heterodimer with a photodamaging or cleavage activity. The goal for creating multiply damaged sites (MDS) upon activation of the drug is to interfere with DNA repair as MDS are known to be a challenge for the repair machinery.[7] Two conjugates in which a photoactive nitrobenzamide was tethered to the 2-position of the acridine were therefore successfully tested as photodamaging agents.[8] Upon illumination they were shown to cleave DNA on both strands in close proximity of the abasic site after alcaline treatment. We report here on the synthesis and DNA interaction of a new threading molecule which contains a photoactive nitrobenzamide group linked at the 4-position of the acridine nucleus, and a cationic amino group at the 9-position. The novelty is based on the polyammonium substitution of the acridine ring, which favours threading interaction of the drug specifically and more tightly at abasic site.

The new molecule was prepared in two steps according to Denny's procedure[9] (Scheme 1). Reaction of **3**, prepared as previously described[8] from acyl chloride **1** and amine **2**, with dimethylaminopropylamine in phenol produced the acridine conjugate **4**.[†] It was isolated as the bis-hydrochloride in 44% yield.

We investigated the photoactivity of compound **4** on a 23 mer oligonucleotide duplex containing the tetrahydrofuran analogue of abasic site[6] (noted X) facing the unpaired

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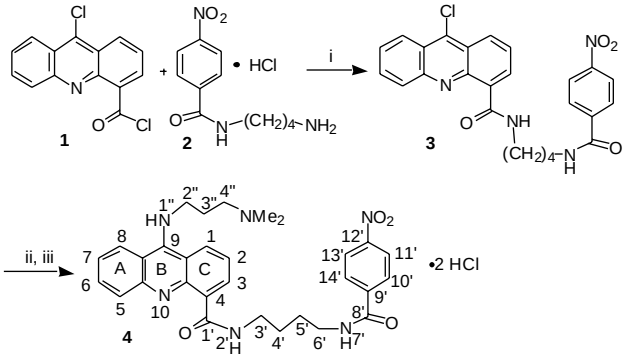
† Compound **4.** Mp 240–245 °C; ms (FAB, NBA) *m/z* 543 $(M+1)^+$; UV (free base, H_2O) λ_{max} (ε) 423.1 (7900), 263.7 (47,000).



Scheme 1. Chemical synthesis of acridine–benzamide heterodimer **4**. Reagents and conditions: (i) DMF, NEt_3, 0 °C 30 min then rt 1 h; (ii) Me_2N-$(CH_2)_3$-NH_2, PhOH, 70 °C, 5 h; (iii) MeOH, HCl.

base thymine, adenine, guanine or cytosine (XT, XA, XG and XC duplexes, respectively). The oligonucleotides used for this study were synthesized on a Perseptive/Biosystem 8700 synthesizer. The phosphoramidite of the tetrahydrofuran analogue was synthesized as previously described.[10] The duplex 23 mer sequences were numbered as follows: d($C_1G_2C_3G_4T_5A_6C_7G_8C_9A_{10}C_{11}X_{12}C_{13}$ $A_{14}C_{15}G_{16}C_{17}A_{18}$ $T_{19}G_{20}C_{21}G_{22}C_{23}$) for the abasic strand and d($G_{24}C_{25}G_{26}C_{27}A_{28}T_{29}G_{30}C_{31}G_{32}T_{33}G_{34}Z_{35}G_{36}$ $T_{37}G_{38}C_{39}$ $G_{40}T_{41}A_{42}C_{43}G_{44}C_{45}G_{46}$) with Z_{35} = A, G, T or C for the complementary strand.

Compound **4** was incubated with all four duplexes, 5′-^{32}P successively labelled on both strands and irradiated at 4 °C for 2 h (at $\lambda > 320$ nm). Alkali-labile induced lesions were visualized by polyacrylamide gel electrophoresis after piperidine treatment. In the absence of UV illumination, no cleavage of DNA was observed. Under illumination, as shown in Figure 1, very few non-specific damages were observed on abasic strand, regardless of the nature of the unpaired base. However, on the complementary strand, strong specific cleavage was observed after piperidine treatment only for sequences containing a thymine or a cytosine opposite to the lesion (XT and XC duplexes). This cleavage is located at the unpaired base as shown in Figure 2 for XT duplex. This first result clearly indicates that compound **4** shows a very specific photodamaging activity towards the unpaired base facing the abasic site when this base is a pyrimidine. No alkali-labile lesion was produced when the unpaired base was a purine. As proposed by Nielsen et al.[11] the mechanism of nitrobenzamide induced photocleavage partly involves hydrogen abstraction from the 2′-deoxyribose of the DNA backbone, elimination of the base and cleavage of the phosphate backbone by a β-elimination process catalyzed by hot piperidine treatment. Nevertheless, this process cannot explain the base specificity. Indeed, the specificity of cleavage may also reflect the higher binding affinity of the reagent for apurinic sites (unpaired T, C) rather than for apyrimidinic sites (unpaired G, A). This would not be so surprising since the latter should be less accessible due to the stacking of the purine bases.

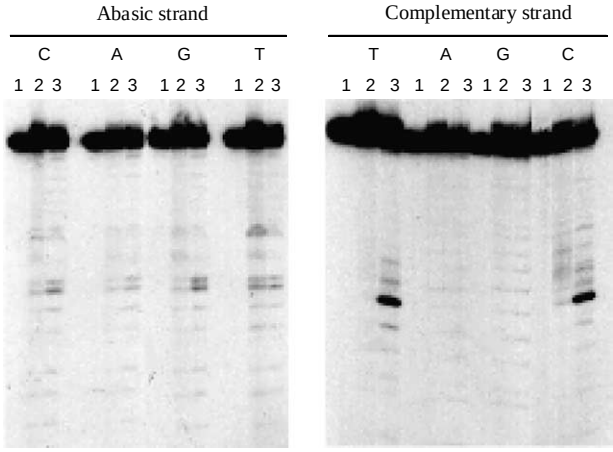


Figure 1. Autoradiogram of 20% denaturing polyacrylamide gel showing the photocleavage activity of compound **4** as a function of the nature of the unpaired base Z. Oligonucleotide (0.5 mM) was incubated with compound **4** (2 mM) in buffered solution (10 mM sodium phosphate buffer, pH 7, 20 mM NaCl and 1 mM EDTA) and then irradiated (with an ORIEL Xe/Hg 200 W lamp filtered with a 2 M KNO_3 solution) for 2 h at 4 °C. The resultant solution was treated with piperidine (1 M) at 90 °C for 10 min, followed by BuOH precipitation. Lane 1, oligomer XZ in the presence of the drug; lane 2, oligomer XZ + **4** illuminated; lane 3, oligomer XZ + **4** illuminated after piperidine treatment. Z_{35} = C, A, G and T.

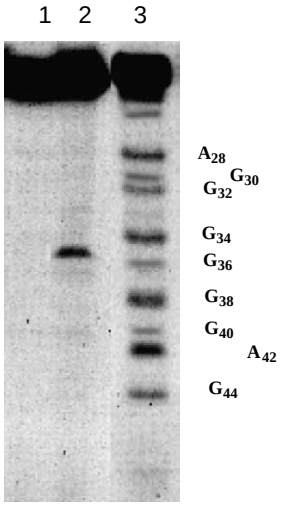


Figure 2. Autoradiogram of 20% denaturing polyacrylamide gel showing the photocleavage activity of compound **4** on the oligomer containing XT. Lane 1, oligomer + **4**; lane 2, oligomer + **4** illuminated after treatment with piperidine; lane 3, G + A Maxam–Gilbert sequencing.

Following these interesting results, high-field NMR studies (Varian Unity + 500) were undertaken to get an insight into the mode of binding of **4** with DNA. The interaction of **4** with an 11 mer oligonucleotide d($C_1G_2C_3A_4C_5X_6C_7A_8C_9G_{10}C_{11}$) d($G_{12}C_{13}G_{14}T_{15}G_{16}$ $T_{17}G_{18}T_{19}G_{20}C_{21}G_{22}$) was studied similarly to previous studies.[6,12,13] Known amounts of **4** were added to a duplex DNA sample and the complex formation was monitored by ^{1}H NMR. Original undecamer resonances completely disappeared when a 1:1 ratio was reached while new resonances appeared, indicating that 1:1

complexes were formed. Chemical exchange peaks were detected in 2D NOESY and TOCSY spectra, and revealed the formation of a major complex (around 70%) along with several minor complexes.

The non-exchangeable proton resonances of the duplex DNA in the major complex were unambiguously assigned using 2D NOESY and TOCSY spectra. Sequential connectivities along the duplex could be followed from $C_1{\cdot}G_{22}$ to $C_{11}{\cdot}G_{12}$ indicating that no intercalation of the acridine or nitrobenzamide ring occurs between the base pairs flanking the abasic site. However, significant variations of chemical shifts (when compared to the free duplex resonance chemical shift)[6] are observed at the central part of the sequence, that is, around the abasic site. The greatest changes occur for C_5–C_7 residues suggesting a position of the drug close to these bases.

The chemical shifts of **4** assigned by analysis of 2D NMR data set are listed in Table 1. We notice a significant upfield shift (by up to 1.68 ppm) for the acridine ring protons with respect to the free drug, whereas no dramatic changes are detected for the nitrobenzamide ring. This suggests the insertion of the acridine moiety only within the duplex, the nitrobenzamide ring being most likely located in one of the grooves.

Identification of intermolecular NOEs allowed us to further confirm the position of the drug. Significant drug–DNA cross-peaks were indeed found between aromatic protons of the drug and X_6–H1′, X_6–H1″, C_7–H1′ and C_5–H1′ sugar protons of the DNA duplex. This indicates that the acridine is inserted in the abasic pocket, which is consistent with a 'non-intercalative' binding mode and a location of the drug close to C_5 and C_7 bases as suggested above. But due to ambiguous assignment of H5, H6, H7 and H8 protons of **4**, it was not possible to determine precisely the orientation of the acridine moiety. The detected NOE could indeed either be **4**-H6/C_7–H1′, **4**-H6/X_6–H1′, **4**-H7/X_6–H1′, **4**-H7/X_6–H1″ and **4**-H8/X_6–H1′, or **4**-H7/C_7–H1′, **4**-H7/X_6–H1′, **4**-H6/X_6–H1′, **4**-H6/X_6–H1″ and **4**-H5/X_6–H1′. The difference between these two data sets is only due to a rotation of 180° of the acridine ring around the main axis of the aromatic chromophore.

In any case, the NOE described above are in favour of the acridine orientation where their A cycle is directed towards the minor groove, constraining the bis-methylated chain to be located in this groove. However, probably due to the high flexibility of the linker, we could not evidence NOE between the chain and the DNA duplex and thus could not position it in the complex. In contrast, NOE observed between the nitrobenzamide ring protons and T_{17}-CH_3 demonstrate its close proximity to T_{17}.

A molecular dynamics has been performed in vacuo under experimental restraints to construct a model of the drug binding site. This can only be analyzed from a qualitative point of view due to the reduced number of drug–DNA NOEs and to the presence of minor competing complexes. A representation of the mode of binding in accordance with NMR data is given in Figure 3. A close examination, looking down to the helical axis of the complexation site, is given in Figure 4. NMR data, together with molecular modelling, suggest that the acridine moiety stacks with the bases on one strand (i.e., C_5 and C_7) and not with their complementary counterparts. Similar partial overlap involving stacking of the drug with the two flanking bases on the same strand has only been described previously for

Table 1. ^{1}H chemical shift of compound **4** in the free form and in the major **4**-DNA complex (measured in D_2O at 283 K)

Proton	Free	Bound	$\Delta\delta$[a]
1″ (NH)	na[b]	na	
2″	4.15	3.89	−0.26
3″	2.43	2.31	−0.10
4″	3.40	3.32	
N-Me	2.96	3.00/2.94	−1.02
1	8.35	7.33	+0.18
2	7.50	7.68	−1.68
3	8.15	6.47	−0.64
5	7.50	6.86	−0.55
6	7.50	6.95	−1.37
7	7.90	6.53	−0.69
8	8.20	7.51	
1′ (NH)	na	na	−0.16
3′	3.4/3.3	3.23	−0.26/−0.16
4′	1.81	1.65	−0.15
5′	1.81	1.75	
6′	3.4/3.3	3.14	
7′ (NH)	na	na	
10′/14′	7.70	7.55	
11′/13′	7.50	7.42	

[a] $\Delta\delta = \delta$ (complex) − δ(free) ($\geqslant$0.10 ppm).
[b] na, not assigned.

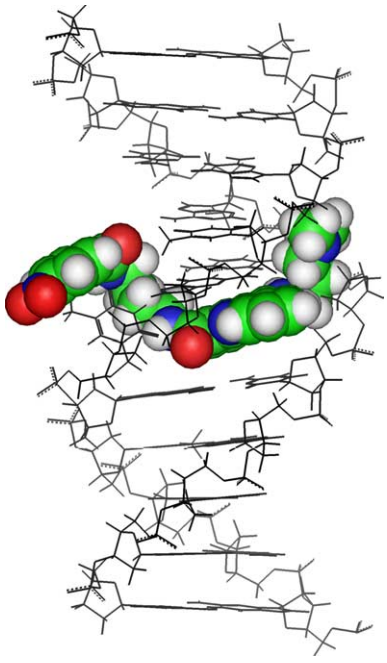


Figure 3. A possible model of **4**/DNA complex displaying the threading of the drug, the acridine positioned into the abasic pocket and the chains located one in each groove.

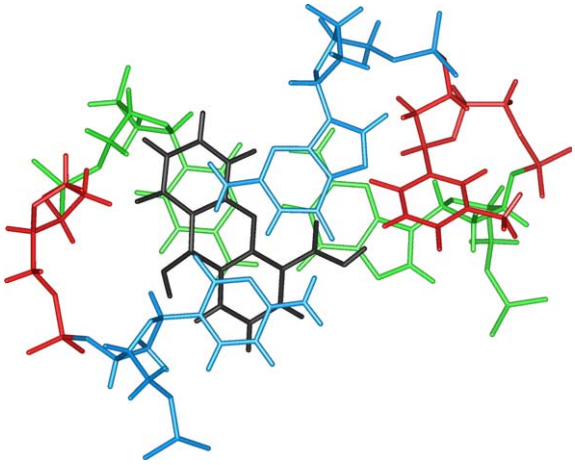


Figure 4. View looking down to the helical axis of the complexation site. Only the acridine (black) and the flanking base pairs C5·G18 (blue), X6·T17 (red) and C7·G16 (green) are represented.

threading intercalators such as nogalamycin[14] and a bisacridine derivative.[12]

In conclusion, compound **4** was shown to specifically induce photodamages at the unpaired base located opposite the abasic site. This specificity suggests a specific interaction of the molecule at the abasic site. NMR data of the major complex are in favour of the threading intercalation of the acridine nucleus in the abasic pocket, positioning the photoreactive nitrobenzamide group in the major groove. In addition to abasic site specificity compound **4** displays a pyrimidine preferred photoreactivity that leads to the formation of alkali-sensitive modifications.

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