9-(4-Nitrobenzamidopolymethylene)aminoacridines and their Photochemical Cleavage of DNA

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9-(4-Nitrobenzamidopolymethylene)aminoacridines with linkers containing four, six, and eight methylene groups were prepared and shown to be efficient photochemical single-strand cleavers of DNA.

A number of attempts have been made to obtain photochemical DNA cleavers.[1] In most cases heavy metal complexes, *e.g.*, iron porphyrin complexes,[2] have been shown to cleave DNA *via* singlet oxygen, whereas HO-radical mechanisms have been implied for complexes of lighter metals, *e.g.* trisphenanthroline cobalt(III) complexes.[3] Most recently a photo-redox mechanism has been suggested for 2,7-diazapyrenium cation DNA-cleavage.[4]

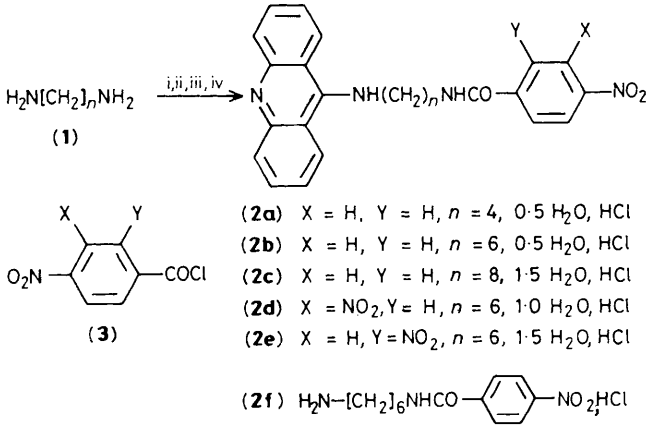
The present approach towards obtaining photochemical DNA cleavers (photonucleases) is based on designing compounds with two ligands bound to the ends of a linker, one ligand to obtain strong, preferentially well-defined binding to DNA, the other ligand to serve as the photochemical DNA cleaver.

We chose as the DNA-binding ligand a well known DNA-intercalator, namely 9-aminoacridine;[5] the linker was polymethylene and the photocleaving ligand was a 4-nitrophenyl group. Model building showed that the linker should contain four or more methylene groups to enable the nitrophenyl group to reach the sugar phosphate DNA backbone when the acridine was intercalated, and the 9-amino group, as predicted, extruded into the minor groove.[6] Thus compounds (**2a—f**) were synthesized as outlined in Scheme 1, and their photonuclease activity examined (see Table 1 and Figure 1).

In a typical experiment, circular supercoiled DNA [pUC 19; 0.3 μg in 10 μl, 10 mM $(CH_2OH)_3NMe$ (Tris), HCl, 1 mM ethylenediaminetetra-acetic acid (EDTA), pH 7.4] to which was added the reagent dissolved in 1 μl dimethylsulphoxide, was irradiated (λ ~300 nm) for 30 min. The photocleavage efficiency was determined as the degree of relaxation of the supercoiled DNA (Figure 1).

From these experiments it was found that photocleavage by (**2b**) is linearly dependent on log reagent concentration (0.3—10 μM) and time of irradiation (0—30 min), and independent of pH (4.5—7.5). Furthermore, the efficiency as a function of wavelength indicates that only light corresponding to the 4-nitrophenylcarbonyl chromophore results in photocleavage. The addition of sodium azide,[9] up to a concentration of 10 mM, had no influence on the cleaving efficiency, which almost certainly eliminates the possibility of singlet oxygen as an intermediate. The quantum efficiency of (**2b**) for DNA photocleavage at λ ~300 nm was estimated to be 10^{-5}.

The equilibrium constants were determined by phase distribution[10] and are typical of related 9-aminoacridines which intercalate with DNA. The changes in the u.v.-absorption spectrum upon addition of DNA to (**2b**) also indicate intercalation. The viscosity of sonicated DNA as a function of added (**2b**) was measured giving a viscosity index of 1.3. This



Scheme 1. *Reagents and conditions:* i, Boc–N_3, *cf.* ref. 7; ii, (**3**), EtOAc–H_2O, solid $NaHCO_3$ (4 equiv.), room temp., 24 h; iii, HCl–HOAc, *cf.* ref. 8; iv, 9-phenoxyacridine, *cf.* ref. 8. All compounds showed satisfactory elemental analyses, and i.r. and ^1H n.m.r. spectra.

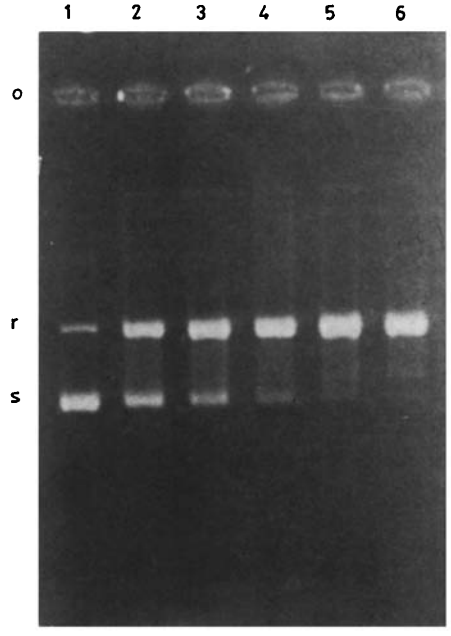


Figure 1. Photocleavage of pUC 19 DNA by (**2b**). The reaction conditions were as described in the text and in Table 1 with the following concentrations of (**2b**): lane 1, 0; 2, 2×10^{-6}; 3, 5×10^{-6}; 4, 10^{-5}; 5, 2×10^{-5}; 6, 4×10^{-5} M. The samples were analysed by gel electrophoresis in 1% agarose (Tris-borate buffer, pH 8.3). 0 Denotes the origin, r, the position of relaxed circular plasmid, and s, the position of supercoiled circular plasmid.

Table 1. Relative cleavage efficiency and equilibrium constants.[a]

Compound	Relative cleavage[b]	K_{eq}/M^{-1}
(**2a**)	90	7×10^5
(**2b**)	100[c]	6×10^5
(**2c**)	80	4×10^5
(**2d**)	10	
(**2e**)	75	
(**2f**)	10	

[a] The samples were irradiated with a Phillips fluorescence tube TL 20/12 emitting at λ 300 nm for 30 min in 10 mM Tris, HCl, 1 mM EDTA, pH 7.4. The DNA concentration was 30 μg ml^{-1} and the total volume of each sample was 10 μl. [b] The relative cleavage was determined as the ratio of concentrations which gave the same degree of cleavage. All set relative to (**2b**). Reagents having an OH group in place of the NO_2 group did not photocleave DNA. [c] 2.0×10^{-6} M (**2b**) gave 50% cleavage at the conditions specified in note a.

compares well with 9-aminoacridine which has a viscosity index of 1.5, and is known to bind to DNA by intercalation.[6]

Photocleavage by (**2b**) occurred at all bases but with some preference for G > C > T ≫ A, as judged from analyses on Maxam–Gilbert sequencing gels. No formation of 3′-OH,5′-phosphate nicks as evidenced by nick-translation was detected.[11]

Finally, the two analogues with an additional 2- or 3-nitro group, *i.e.* (**2d**) and (**2e**), were examined. As seen in Table 1, (**2e**) is nearly as efficient as (**2b**), whereas (**2d**) is much less efficient. Compound (**2f**) cleaved DNA with about one tenth of the efficiency of (**2b**).

The mechanism whereby these compounds cleave DNA is still unknown. However, at the present time we believe it involves initial 3′- and/or 4′-hydrogen abstraction from the deoxyribose unit, followed by oxygen transfer. An attack at the 3′ position would immediately lead to degradation *via* the monophosphorylated hydrate. An attack at the 4′-position would be analogous to the proposed mechanism of DNA cleavage by bleomycin,[12] where it is suggested that a free base is released *via* a 4′-keto intermediate with a free 5′-phosphate as the terminal product.

3-Nitrobenzamide derivatives have also been investigated and were found to be much less efficient.[13]

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