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DNA photocleavage by dicationic bisintercalants

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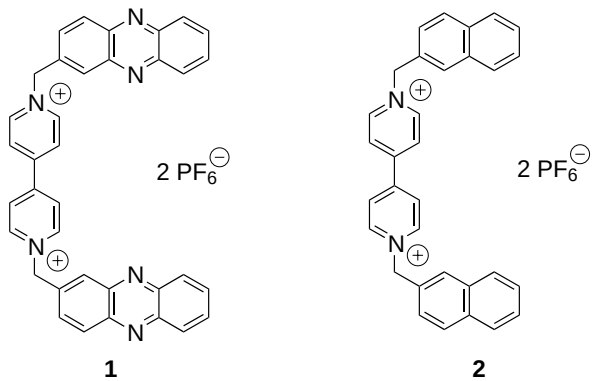
Abstract—The synthesis of new dicationic bisintercalants containing phenazine or naphthalene as photosensitizers and 4,4′-bipyridinium as a linker is reported. Irradiation in the presence of 0.5 μM concentrations of the phenazine intercalants affords cleavage of plasmid DNA (at 22°C, pH 7.0) in good to excellent yields. © 2001 Published by Elsevier Science Ltd.

Synthetic reagents which cleave nucleic acids[1,2] (nucleic acid photocleavers) are useful tools in molecular biology and medicine. Photocleavage of nucleic acids allows the use of light to trigger nuclease activity. Nucleases that are activated by visible or near-UV light can be used for examination of processes such as transcription and to probe nucleic acid structure as photofootprinting and photo-sequencing agents. On the other hand, photosensitization of DNA by drugs may be useful as a potential anti-tumor therapy. DNA photocleavage can occur by a wide variety of mechanisms such as (1) hydrogen atom abstraction from the sugar ring by photochemically generated radicals, (2) direct electron transfer from the base (usually guanine) to the photoexcited cleaver, (3) singlet oxygen production by transfer of energy from the excited photocleaver, and (4) formation of base adducts.

We have previously[3] synthesized tetracationic monointercalant and *cyclo*-bisintercalant photonucleases containing phenazine and viologen subunits. Upon 45 minutes of UV irradiation, the monointercalant afforded DNA cleavage in 17% yield, whereas cleavage efficiency produced by the *cyclo*-bisintercalant was 50%. It is conceivable that these relatively low levels of photocleavage were the result of a reduction in DNA binding arising from destabilizing steric interactions between the viologen linkers and the double-helical framework.[2d] With the aim of enhancing cleavage efficiencies, we designed water soluble dicationic intercalants which are endowed with a greater potential ability to intercalate and effect photocleavage of duplex DNA. In this paper, we describe the preparation of compounds **1**–**4**, and demonstrate that irradiation of the new phenazine intercalants produces cleavage of plasmid DNA in good to excellent yields.

Bisintercalants **1** and **2** have photosensitizing subunits joined by a rigid 4,4′-bipyridinium linker (Fig. 1). The viologen linker can facilitate DNA-binding by electrostatic interactions with negatively charged phosphate groups and can act as an efficient cosensitizer by accepting an electron from a photoexcited intercalator in a DNA matrix.[4]

DNA intercalants linked to crown ethers show an enhanced binding affinity to DNA in the presence of some (K^+ or Na^+) alkaline metals. With the purpose of increasing DNA binding affinity, we have synthesized



1 **2**

Figure 1.

Keywords: DNA; photocleavage; bisintercalants; phenazine; naphthalene; viologen; benzo-15-crown-5.

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the phenazine derivative **3** containing 4,4′-bipyridinium and benzo-15-crown-5 subunits (Fig. 2). Intercalant **4** lacking the 15-crown-5 moiety was synthesized to study the effect of this subunit on DNA photocleavage.

Synthesis of bisintercalant **1** (Scheme 1) was carried out in two steps from 2-(bromomethyl)phenazine **5**.[5] Reaction of 4,4′-bipyridine (3 equivalents) with 2-(bromomethyl)phenazine **5** (1 equivalent) in dry acetonitrile at reflux for 4 hours, afforded **6**[6] in 30% yield. In the second step of the synthetic procedure, a solution of an equimolar mixture of **6** and 2-(bromomethyl)phenazine in dry acetonitrile was refluxed for 4 days affording **1**[7] in 57% yield.

Dicationic bisintercalant **2**[8] was obtained (39% yield) in one step by dialkylation of 4,4′-bipyridine with two equivalents of 2-(bromomethyl)naphthalene in acetonitrile at room temperature for 18 hours. Intercalant **3**[9] was prepared in 22% yield by heating a solution of **6** and 15-(chloromethyl)benzo-15-crown-5[10] in dry acetonitrile at 50°C for 5 hours. Similarly, synthesis of **4**[11] was performed by refluxing **6** and benzylbromide in dry acetonitrile for 16 hours (65% yield).

In a typical DNA photocleavage experiment, 39.5 μM bp pUC19 plasmid and 0.5 μM concentrations of the bromide salts of intercalants **1**, **2**, **3**, or **4** (in 10 mM potassium phosphate buffer pH 7.0, 22°C) were equilibrated in the dark for 1 hour. The solution was then irradiated at 350 nm (phenazine derivatives **1**, **3**, and **4**) or at 300 nm (naphthalene derivative **2**) under aerobic conditions in a ventilated Rayonet Photochemical Reactor. Aliquots from the reaction were removed at time points from 0 to 50 minutes. The cleaved DNA products were then resolved on a 1% agarose gel, stained with ethidium bromide, and quantified with a Molecular Dynamics FluorImager SI Gel Imaging System.

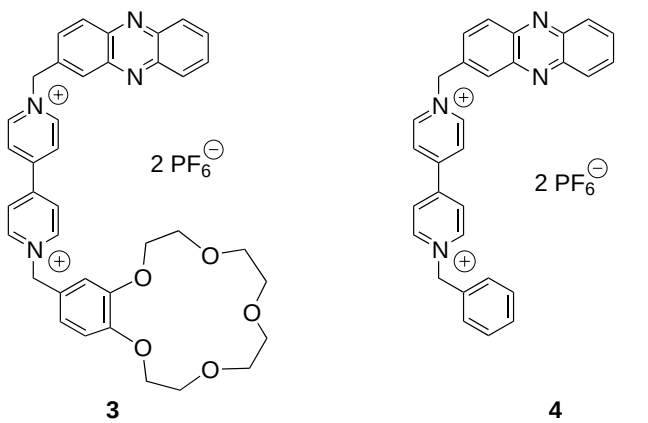
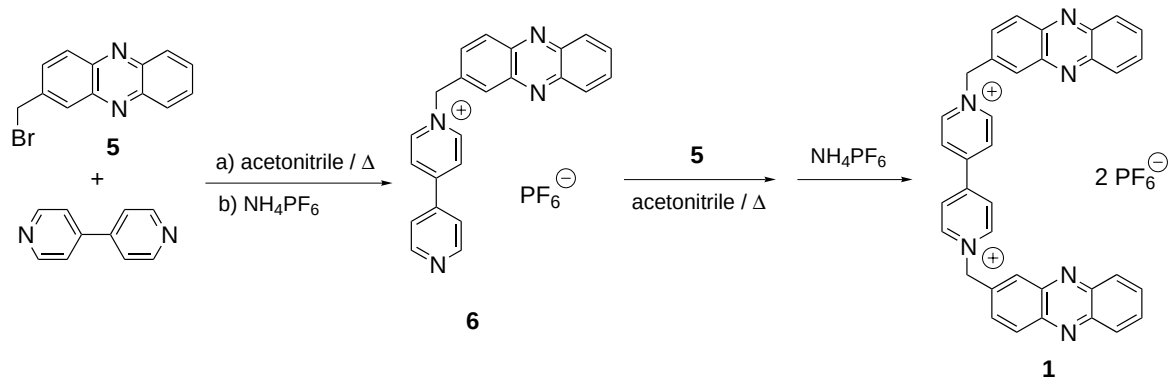


Figure 2.

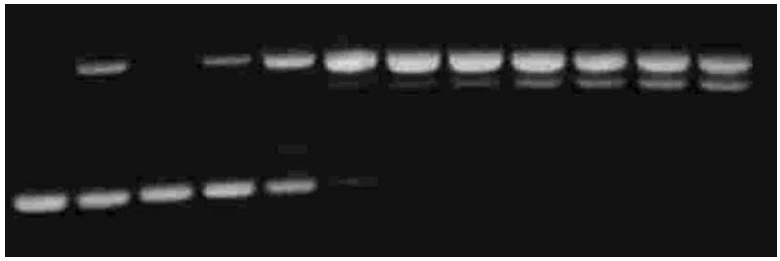
As exemplified by compound **1** (Fig. 3), the four intercalants demonstrated time dependent photocleavage converting the supercoiled plasmid DNA to its nicked and linear forms. In all cases, no cleavage of the plasmid was observed in parallel control reactions run in the dark. (For compound **1**, refer to lane C3 in Fig. 3.) Yields at the 50 minute time point were then averaged over four trials, showing high levels of cleavage for the phenazine intercalants **1**, **3**, and **4** and lower cleavage for the naphthalene bisintercalant **2** (Table 1). The observed order of reactivity was **1**≫**3**>**4**≫**2** for the series of compounds investigated.

It is reasonable to suggest that the high cleavage efficiency of compound **1** may arise from enhanced levels of DNA intercalation conferred by the presence of two highly conjugated phenazine moieties in this compound. The above data also imply that interaction of the crown ether subunit of phenazine intercalant **3** with K^+ ions in the reaction buffer may lead to an increase in DNA binding affinity relative to intercalant **4**. Finally, it is conceivable that the reactivity of compound **2** ($\lambda_{max}=262$ nm) has been affected by the low intensity of the 300 nm light source used to promote specific naphthalene photosensitized DNA cleavage in the absence of excessive levels of non-specific DNA photodegradation (DNA $\lambda_{max}=260$ nm).

In summary, our data indicate that very low concentrations of the phenazine intercalants **1**, **3**, and **4** effect efficient photocleavage of plasmid DNA in aqueous solutions of potassium phosphate buffer pH 7. In particular, 0.5 μM of the phenazine bisintercalant **1** produces complete conversion of plasmid DNA to its nicked and linear forms. Our future studies will focus



Scheme 1.



5	35	4	15	55	86	87	90	84	77	76	71	% Nicked
0	0	0	0	2	6	8	10	16	23	24	29	% Linear
95	65	96	85	43	8	6	0	0	0	0	0	% Supercoiled

Figure 3. Photocleavage of pUC19 plasmid DNA by bisintercalant **1**. C1 and C2 correspond to DNA alone, without and with 50 minutes of 350 nm irradiation, respectively. C3 corresponds to DNA treated for 50 minutes with 0.5 μM bisintercalant **1** (no *hv*). Lanes 1–9 contain DNA in 0.5 μM intercalant **1**, irradiated at 350 nm for 5, 10, 20, 25, 30, 35, 40, 45, and 50 minutes, respectively.

Table 1. Average % yields of DNA photocleavage[a]

Intercalant	_[b]	_[b,c]	**1**[c]	**3**[c]	**4**[c]	_[b,d]	**2**[d]
Irradiation time (min)	0	50	50	50	50	50	50
% Nicked	22±3	33±8	74±4	57±7	67±1	26±2	30±2
% Linear	0±0	0+0	26±4	15±2	4±4	0+0	0+0
% Supercoiled	78±3	67±8	0±0	28±7	29±4	74±8	70±2

[a] The above yields are averaged over four trials. Errors are reported as standard deviation.
[b] DNA cleavage in the absence of intercalant.
[c] Irradiation with eleven 350 nm 24 W Rayonet lamps.
[d] Irradiation with one 300 nm 24 W Rayonet lamp.

on elucidating reaction mechanisms in order to provide insights into the design of more efficient and potent DNA photocleavers.

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6. Compound **6**, mp 231–233°C. ^{1}H NMR (DMSO-d_6): δ 6.21 (s, 2H, CH_2), 7.98–8.04 (m, 4H, Hβ pyridine, H-7, H-8), 8.08 (d, 1H, J=8.8 Hz, H-3), 8.23–8.28 (m, 2H, H-6, H-9), 8.34 (d, 1H, J=8.8 Hz, H-4), 8.38 (s, 1H,

H-1), 8.69 (d, 2H, $J=5.9$ Hz, Hβ pyridinium), 8.86 (d, 2H, $J=5.1$ Hz, Hα pyridine), 9.47 (d, 2H, $J=5.9$ Hz, Hα pyridinium). MS (FAB): m/z 349 (M^+-PF_6) (calcd for $C_{23}H_{17}N_4PF_6$ 494.1).

7. Compound **1**, mp 268–269°C. ^{1}H NMR (DMSO-d_6): δ 6.27 (s, 4H, CH_2), 7.98–8.04 (m, 4H, H-7, H-8), 8.08 (d, 2H, $J=9$ Hz, H-3), 8.23–8.28 (m, 4H, H-6, H-9), 8.34 (d, 2H, $J=9$ Hz, H-4), 8.38 (s, 2H, H-1), 8.69 (d, 4H, $J=6.8$ Hz, Hβ pyridinium), 9.58 (d, 4H, $J=6.8$ Hz, Hα pyridinium). MS (FAB): m/z 687 (M^+-PF_6) (calcd for $C_{36}H_{26}N_6P_2F_{12}$ 832.1).
8. Compound **2**, mp 274–276°C. ^{1}H NMR (DMSO-d_6): δ 6.08 (s, 4H, CH_2), 7.58 (dd, 2H, $J=9.9$ Hz, 6.6 Hz, H-6 or H-7), 7.58 (d, 2H, $J=9.5$ Hz, H-7 or H-6), 7.65 (dd, 2H, $J=8.4$ Hz, 1.8 Hz, H-3), 7.91–7.96 (m, 4H, H-5, H-8), 8.0 (d, 2H, $J=8.4$ Hz, H-4), 8.13 (br s, 2H, H-1), 8.70 (d, 4H, $J=7$ Hz, Hβ pyridinium), 9.51 (d, 4H, $J=7$ Hz, Hα pyridinium). MS (FAB): m/z 583 (M^+-PF_6) (calcd for $C_{32}H_{26}N_2P_2F_{12}$ 728.1).
9. Compound **3**, mp 225–227°C. ^{1}H NMR (DMSO-d_6): δ 3.57 (s, 8H, H-5′, H-6′, H-8′, H-9′), 3.71–3.77 (m, 4H, H-3′, H-11′), 4.03–4.06 (m, 4H, H-2′, H-12′), 5.78 (s, 2H, CH_2-benzo-15-crown-5), 6.25 (s, 2H, CH_2-phenazine), 7.0 (d, 1H, $J=8.4$ Hz, H-6″), 7.16 (dd, 1H, $J=8.4$ Hz, 1.8 Hz, H-5″), 7.27 (d, 1H, $J=1.8$ Hz, H-3″), 7.98–8.02 (m, 2H, H-7, H-8), 8.08 (d, 1H, $J=8.8$ Hz, H-3), 8.23–8.28 (m, 2H, H-6, H-9), 8.35 (d, 1H, $J=8.8$ Hz, H-4), 8.37 (s, 1H, H-1), 8.69 (d, 2H, $J=7$ Hz, Hβ′ pyridinium), 8.76 (d, 2H, $J=7$ Hz, Hβ pyridinium), 9.44 (d, 2H, $J=7$ Hz, Hα′ pyridinium), 9.59 (d, 2H, $J=7$ Hz, Hα pyridinium). MS (FAB): m/z 775 (M^+-PF_6) (calcd for $C_{38}H_{38}N_4O_5P_2F_{12}$ 920.2).
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11. Compound **4**, mp 243–245°C. ^{1}H NMR (DMSO-d_6): δ 5.91 (s, 2H, CH_2-phenyl), 6.26 (s, 2H, CH_2-phenazine), 7.44–7.46 (m, 3H, H-3′, H-4′, H-5′), 7.56–7.59 (m, 2H, H-2′, H-6′), 7.98–8.02 (m, 2H, H-7, H-8), 8.1 (d, 1H, $J=8.8$ Hz, H-3), 8.23–8.27 (m, 2H, H-6, H-9), 8.35 (d, 1H, $J=8.8$ Hz, H-4), 8.37 (s, 1H, H-1), 8.73 (d, 2H, $J=7$ Hz, Hβ′ pyridinium), 8.76 (d, 2H, $J=7$ Hz, Hβ pyridinium), 9.47 (d, 2H, $J=7$ Hz, Hα′ pyridinium), 9.59 (d, 2H, $J=7$ Hz, Hα pyridinium). MS (FAB): m/z 585 (M^+-PF_6) (calcd for $C_{38}H_{24}N_4P_2F_{12}$ 730.1).