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Photocleavage of DNA by Tetracationic Intercalands Containing Phenazine and Viologen Subunits

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Abstract

The synthesis of tetracationic intercalands **1** and **5** containing phenazine and viologen subunits is described. DNA photocleavage by **1** and **5** is reported.

Keywords: Polycyclic heterocyclic compounds; pyridinium salts; nucleic acids; photochemistry.

DNA can be cleaved photochemically [1] either *in vitro* or *in vivo* with ultraviolet light. Many photosensitizers have been shown to cleave DNA, most of them *in vitro*, the sites of cleavage depending on the sensitizers used. Non sequence-specific DNA photocleaving agents are useful for DNA photo-footprinting and photo-sequencing. In addition new DNA cleaving agents are of practical interest as potential antitumor agents. In previous papers the photochemical cleavage of DNA with a bis-dimethyldiazapyrenium tetracation [2] and the photocleavage of oligonucleotides at guanine sites with a dimethyldiazaperopyrenium dication [3] were described. A photoexcited intercalating dye [4] (ethidium bromide) can transfer an electron to a viologen unit which by reaction with O_2 produces $O_2^{\cdot-}$. This in turn can be converted [5] to ·OH, which is probably the species responsible for DNA strand scission. Visible light-induced DNA cleavage by a bis-9-acridinyl derivative containing a viologen linker chain has also been reported [6].

Taking these considerations into account we have designed the cyclo-bis-intercaland **1** with the following structural features: a) a rigid cavity suitable for inclusion of aromatic substrates or intercalation with DNA, b) photosensitizing intercalating phenazine subunits, c) viologen subunits with electron-acceptor character to act as efficient electron mediators and

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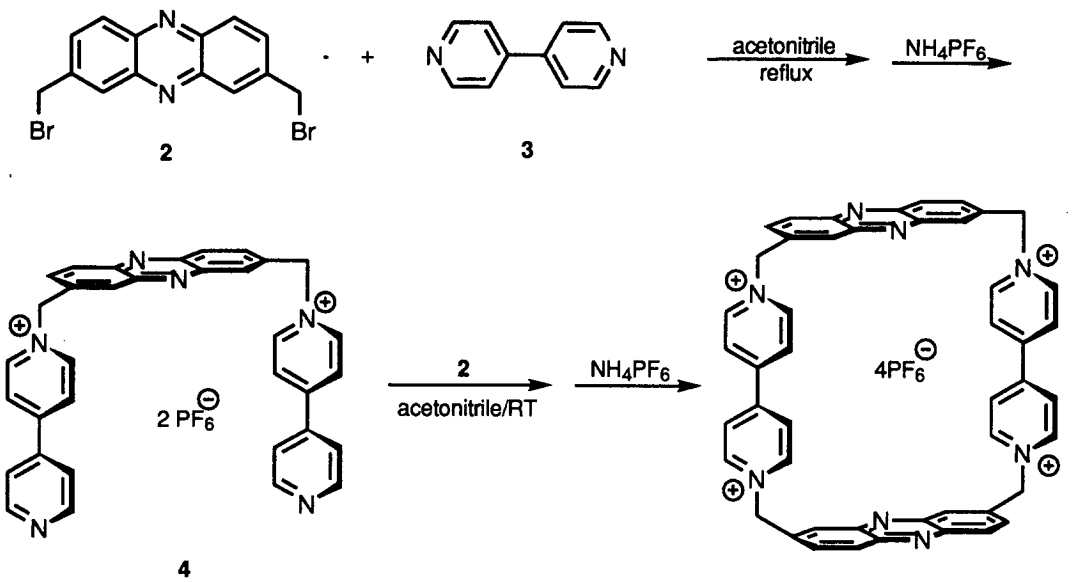
d) a tetracationic nature to allow control of solubility by appropiate selection of the counterions. These characteristics in conjunction with the close proximity of the phenazine and viologen groups should endow **1** with the ability to bis-intercalate with DNA and effect photocleavage of the DNA strands ("photonuclease"-type compound). Moreover **1** could bind electron-rich aromatic substrates in organic or aqueous solvents [7-10]. The synthesis of the cyclo-bis-intercaland **1** (Scheme 1) was performed by starting from 2,8-bis(bromomethyl)phenazine **2** prepared according to the reported procedure [11]. The first step involves the synthesis[1] of **4** from 2,8-bis(bromomethyl)phenazine **2** and 4,4'-bipyridine **3**.



Scheme 1: Synthesis of cyclo-bis-intercaland **1**.

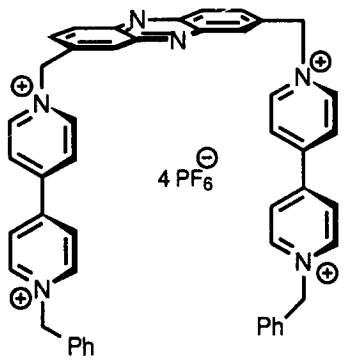
The next step of the synthetic procedure involves the reaction of **4** with another equivalent of 2,8-bis(bromomethyl)phenazine **2**. This was carried out under conditions similar to those used by Hünig et *al.* [12] for the preparation of cyclophanes containing viologen subunits, which was based on the very different solubilities of the starting materials. In our case compound **4** is readily soluble in acetonitrile whereas the 2,8-bis(bromomethyl)phenazine **2** is insoluble in this solvent. Hence a suspension of **2** in a

[1] A suspension of 2,8-bis(bromomethyl)phenazine **2** and 4,4'-bipyridine **3** (2.5 eq) in dry acetonitrile was heated at reflux for 3.5 hours. The resulting precipitate was then collected and the filtrate was concentrated *in vacuo*. The solid was dissolved in water and treated with a saturated aqueous solution of NH_4PF_6 to effect the counterion exchange. The resulting bis-hexafluorophosphate precipitate was purified by flash column chromatography on silica gel using MeOH: H_2O: saturated aqueous NH_4Cl (8:2:2) as eluent. The fractions containing the product were combined and the solvent was removed *in vacuo* affording a solid which was washed several times with ethanol. The solid remaining after evaporation of the ethanol was dissolved in water and treated with a saturated aqueous solution of NH_4PF_6 to afford a yellow precipitate. Filtration of the precipitate yields pure **4** in 54% yield. ^{1}H-NMR (DMSO-d_6, 300 MHz) δ (ppm): 6.22 (s, 4H, CH_2), 8.08-8.13 (m, 6H, Hβ pyridine and H-3(7) phenazine), 8.35 (d, 2H, J= 8.79 Hz, H-4(6) phenazine), 8.41 (d, 2H, J= 1.22 Hz, H-1(9) phenazine), 8.70 (d, 4H, J= 6.84 Hz, Hβ pyridinium), 8.90 (d, 4H, J= 6.1 Hz, Hα pyridine), 9.48 (d, 4H, J= 6.84 Hz, Hα pyridinium).

solution of **4** in acetonitrile provides high dilution conditions, allowing the synthesis of the cyclo-bis-intercaland **1**.[2]

We have also designed the synthesis of compound **5** (Scheme 2) containing phenazine and viologen subunits as a potential threading intercalator and photocleaving agent. Threading intercalators have residence lifetimes in DNA larger than classical intercalators, and the larger lifetimes can be extremely interesting for effects on the photocleavage of nucleic acids.

The synthesis of **5**[3] was performed by reaction of 2,8-bis(bromomethyl)phenazine **2** (1 eq) with 1-benzyl-4-(4-pyridyl)pyridinium bromide [13] (3 eq) in acetonitrile at reflux. Yield: 31%.



5

Scheme 2.

DNA Photocleavage Experiments

Different concentrations of the drugs were used in order to find a balance that avoids precipitation of the complex but is able to cleave the DNA at a reasonable rate. The progression of forms I (supercoiled) DNA) and II (nicked DNA) are easily followed every 5 minutes using 0.5 μM of each compound.

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[2] To a solution of **4** in a minimal amount of dry acetonitrile, 2,8-bis(bromomethyl)phenazine **2** (1 eq) was added. The suspension was stirred for 4 weeks at room temperature. The precipitate was then separated, treated with water and submitted to ultrasound for a few minutes. On addition of a saturated aqueous solution of NH_4PF_6 to the filtrate, crude **1** settled out which was filtered and purified by flash column chromatography on silica gel using MeOH: H_2O: saturated aqueous NH_4Cl (8:2:2) as eluent. The product obtained on removing the solvent was washed several times with ethanol. The remaining solid was dissolved in water and extracted (3 times) with ethyl acetate. The combined organic extracts (ethanol + ethyl acetate) were evaporated *in vacuo* and the solid obtained was dissolved in water. **1** was precipitated by adding a saturated aqueous solution of NH_4PF_6. Yield: 18%. ^{1}H-NMR (DMSO-d_6, 300 MHz) δ (ppm): 6.37 (s, 8H, CH_2), 7.10 (br s, 4H, H-1(9), phenazine), 8.23 (dd, 4H, J= 9.16 , J'= 1.83 Hz, H-3(7)), 8.46 (d, 4H, J= 9.16 Hz, H-4(6) phenazine), 8.97 (d, 8H, J= 6.59, Hβ pyridinium), 9.60 (d, 8H, J= 6.59 Hz, Hα pyridinium). MS (FAB) m/e 1159 (M^+-PF_6) (calcd for $C_{48}H_{36}N_8F_{24}P_4$ 1304.7).

[3] ^{1}H-NMR (DMSO-d_6, 300 MHz) δ (ppm): 5.92 (s, 4H, CH_2), 6.26 (s, 4H, CH_2), 7.43-7.48 (m, 6H, phenyl), 7.57-7.60 (m, 4H, phenyl), 8.10 (dd, 2H, J= 8.97, J'= 1.65 Hz, H-3(7) phenazine), 8.35 (d, 2H, J= 8.97 Hz, H-4(6) phenazine), 8.49 (d, 2H, J= 1.65, H-1(9) phenazine), 8.73 (d, 4H, J= 6.78 Hz, Hβ pyridinium), 8.78 (d, 4H, J= 6.78 Hz, Hβ pyridinium), 9.49 (d, 4H, J= 6.78 Hz, Hα pyridinium), 9.60 (d, 4H, J= 6.78 Hz, Hα pyridinium). MS (FAB) m/e 1135 (M^+-PF_6) (calcd for $C_{48}H_{40}N_8F_{24}P_4$ 1280.7).

Figure 1 depicts the photocleavage of supercoiled pBR322 plasmid by 0.5 μM of compound **1** 4Br or compound **5** 4Br on irradiation. When the DNA is irradiated in the presence of the drugs, the intensity of the nicked form band increases, whereas that of form I substantially decreases proportionally to the irradiation time. Preliminary kinetic studies yield a higher kinetic constant (cleavage of form I to II) for compound **1** than for compound **5**. Compound **1** generates 50% cleavage in 45 minutes whereas compound **5** only yields 17% cleavage in the same time. Both compounds produce mainly single strand breaks, however a very light band corresponding to form III (linearized plasmid) is seen after 25 minutes of irradiation. The intensity of this band increases slightly until the end of the irradiation, therefore it is difficult to quantify.

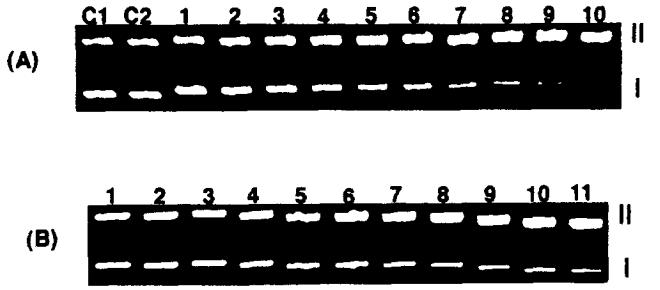

Figure 1. Photocleavage of plasmid DNA pBR322 with (A) compound **1** and (B) compound **5**. C1 and C2 correspond to plasmid DNA (wich contains both forms I and II) without and with 40 minutes of irradiation, respectively. Lanes 1-11, DNA with 0.5 μM of compound **1** (A) or **5** (B), irradiated at 0, 5, 10, 20, 25, 30, 35, 40, 45 or 50 minutes, respectively.

In summary, compounds **1** and **5** generate single strand breaks at very low concentrations. Although the mechanism of the cleavage has not yet been studied, we suppose it will be similar to other viologer derived photonucleases.

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