

DNA-binding and DNA-photocleaving properties of 12a,14a-diazoniapentaphene

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Abstract

The association of 12a,14a-diazoniapentaphene dibromide (**5**) with DNA and its ability to photoinduce DNA damage was studied. Spectrophotometric and fluorimetric titrations show that the title compound (**5**) binds to DNA ($K \approx 5 \cdot 10^5 \text{ M}^{-1}$) with the highest affinity towards GC-rich regions. The highest affinity was observed for GC base pairs. Compound **5** binds to DNA primarily by intercalation as shown by LD spectroscopy and energy-transfer experiments. Moreover, efficient DNA damage was observed on UVA irradiation in the presence of **5**. Preliminary experiments suggest that singlet oxygen may be involved in the photoinduced DNA damage.

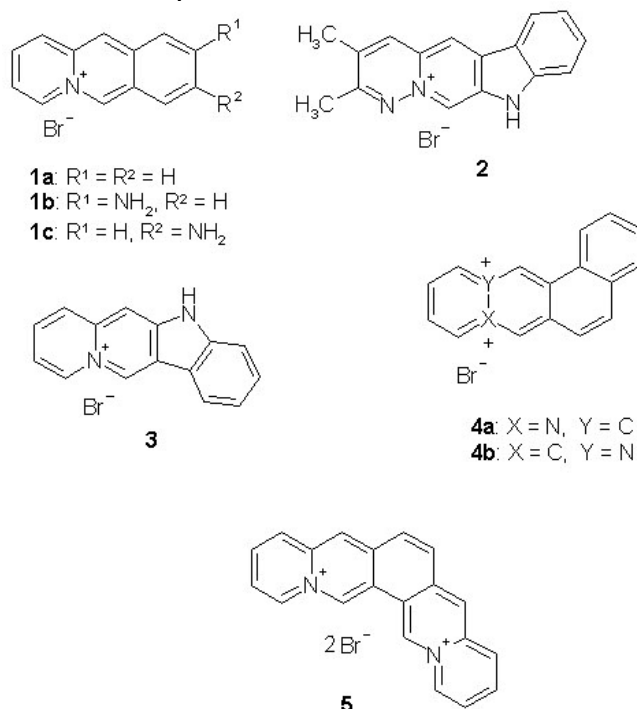
Keywords: DNA binders, DNA photocleavage, linear dichroism, photobiology, quinolizinium salts

Introduction

In recent years much interest has been focused on DNA binders, which may modify the genetic material.¹⁻³ Along these lines, there has been increased interest in the discovery and investigation of compounds that damage DNA upon irradiation. These compounds, also called photonucleases,³ exhibit a large potential for therapeutical applications such as photodynamic chemotherapy,⁴ because they are often inert until activated by light and, thus, the DNA-damage reaction may be controlled both in a spatial and temporal sense.

Photonucleases, like any other small DNA-binding molecules, associate by intercalation or by fitting into the minor groove.^{3,4} Importantly, the type and the efficiency of the photocleavage reaction will depend on the binding affinity and the binding site that the photonuclease occupies. Intercalators usually exhibit a planar structure with at least two annelated aromatic rings. In most

cases, a positive charge is required for an appropriate binding affinity.^{3b,3c} This cationic moiety is usually provided by a quaternary nitrogen atom which may be generated either by simple protonation as for example in the aminoacridine series,⁵ or by *N*-alkylation as found in the well-known phenantridine series.⁶ Alternatively, the cationic nitrogen atom may be introduced as the bridgehead atom between two annelated aromatic rings such as in the benzo[2*b*]quinolizinium salts **1a–c**, and related quinolizinium salts such as **2**, **3** and **4a–b**, whose DNA-binding and DNA-photodamaging properties have already been demonstrated.^{7–12} We now conducted a detailed study on the binding interactions of the known bis-quinolizinium salt **5**, namely 12a,14a diazoniapentaphene dibromide¹³ with DNA along with their propensity for photoinduced DNA-damage. We assumed that the extended π system as well as the additional positive charge may lead to more pronounced binding affinity towards DNA compared with **1–4**. Herein, the photobiological features of **5** will be presented and discussed.



Scheme 1

Results and Discussion

Spectrometric Titrations

The association of **5** to DNA in buffered aqueous solution was monitored by spectrophotometric and spectrofluorimetric titrations with salmon testes DNA (st DNA) (Figures 1 and 2). Along with a significant hypochromism (43%) and a partial loss of the absorption-band fine structure, the absorption maximum of quinolizinium salt **5** exhibits a bathochromic shift of 12 nm in the presence of st DNA relative to the free dye **5** (Figure 1). Moreover, isosbestic points were detected (311, 345 and 398 nm) in each case which indicate that one type of quinolizinium-DNA complex is formed almost exclusively under these conditions.

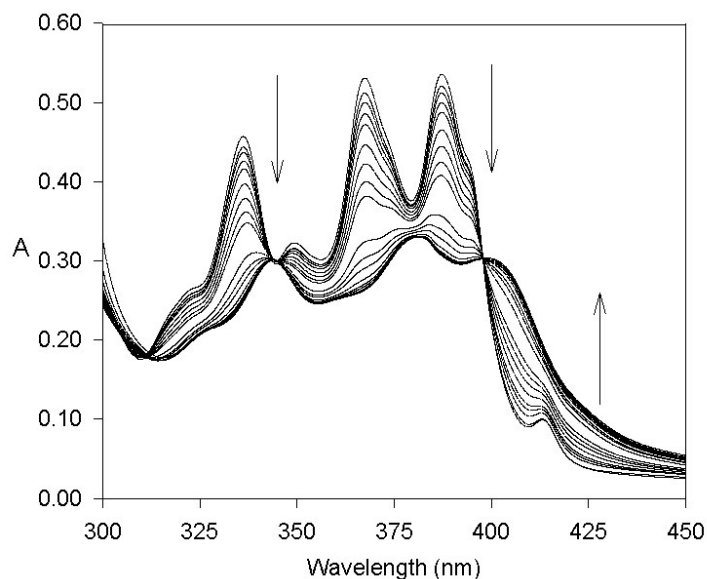


Figure 1. Spectrophotometric titration of **5** with st-DNA in ETN buffer (0.01 M, pH = 7.0, T = 25 °C), arrows indicate the change of the absorption band with successive DNA addition.

The emission intensity of the free dye **5** was quenched upon addition of st-DNA and synthetic polynucleotides, (poly[dG-dC]-poly[dG-dC] and poly[dA-dT]-poly[dA-dT]). Especially the addition of poly[dG-dC]-poly[dG-dC] led to a significant decrease of emission quantum yield (Figure 3); however, a shift of the band maximum was not observed. It may be concluded that the emission of the DNA-bound quinolizinium molecule is totally quenched and the observed reduced fluorescence results solely from the non-associated dye molecule. Such an efficient quenching of the excited state usually requires a close proximity between the fluorophore and the quencher, which is realized in an intercalative binding mode.

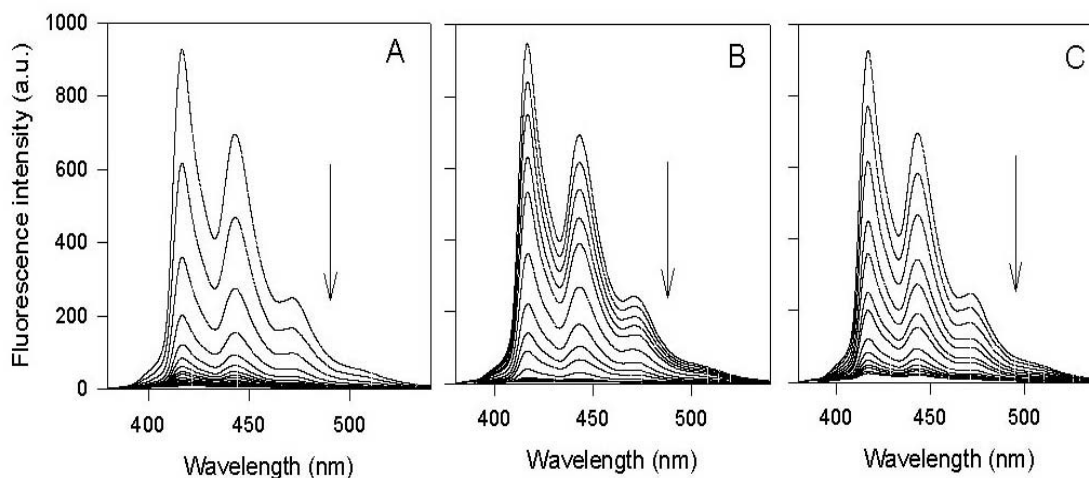


Figure 2. Fluorimetric titrations of st-DNA (A), Poly[dG-dC]-poly[dG-dC] (B), and Poly[dA-dT]-poly[dA-dT] to dye **5** ($[5] = 5 \times 10^{-6}$ M, in 0.01 M ETN buffer, pH = 7.0, T = 25 °C). The dye was excited at the isosbestic point (345 nm) found in the spectrophotometric titrations.

The fluorescence-titration data were used to derive a Scatchard plot,¹⁴ which was analyzed according to the model of McGhee and von Hippel,¹⁵ to estimate the association constant (K_i) and the number of base pairs, n , covered by one ligand (Table 1). The quinolizinium derivative **5** has an affinity to DNA which is approximately five times larger than the one of the naphthoquinolizinium derivatives **4a** and **4b**.

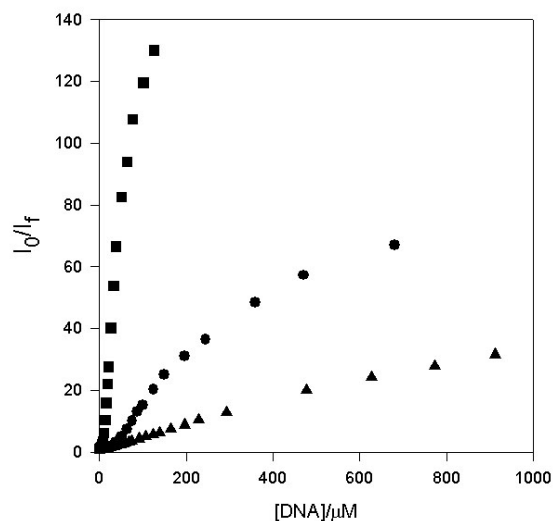


Figure 3. Fluorescence quenching of salt **5** on addition of st DNA (●), poly[dG-dC]-poly[dG-dC] (■) and poly[dA-dT]-poly[dA-dT] (▲), in 0.01 M ETN buffer, pH = 7.0, $T = 25$ °C.

The bis-quinolizinium salt **5** exhibits the highest binding affinity towards poly[dG-dC]-poly[dG-dC] ($K_i = 3.0 \times 10^6 \text{ M}^{-1}$). The comparison with the binding constant with poly[dA-dT]-poly[dA-dT] ($6 \times 10^4 \text{ M}^{-1}$) reveals that there is a preferential binding for **5** at GC-rich regions of the DNA. Moreover, the exclusion parameter n , that is the binding site size, is ca. 2 base pairs for DNA and Poly[dG-dC]-poly[dG-dC], which is in agreement with the intercalation of the dye into the double strand of the polynucleotide. By contrast, this value is larger for Poly[dA-dT]-poly[dA-dT] which is not consistent with an intercalative binding mode.

Table 1. Binding constants, K_i , and exclusion parameter, n , obtained from fluorimetric titrations^a

	$K_i \text{ } 10^5 \text{ M}^{-1}$	n^b
st-DNA	5.7 ± 0.2	2.8 ± 0.2
Poly[dG-dC]-poly[dG-dC]	30.0 ± 0.5	1.51 ± 0.02
Poly[dA-dT]-poly[dA-dT]	0.61 ± 0.007	4.5 ± 0.1

^a In ETN buffer (0.01 M, pH=7.0). ^b In base pairs pairs.

Flow linear dichroism

The LD spectra of **5** in the presence of DNA were also determined (Figure 4), since this methodology has been shown to be an efficient tool to evaluate the distinct binding mode between a dye and nucleic acids.^{16,17} The LD signal of **5** at different dye-DNA ratios is negative at all mixing ratios both in the UV region where the DNA and the dye absorb and at wavelengths where only the dye absorbs (Figure 4, panel A). The LD signals in the long-wavelength absorption regions of the dye (300–450 nm) are negative in the presence of DNA, which indicates that the transition dipole and thus the π system of **5** is coplanar to the one of the nucleic bases upon binding to the DNA.^{16,17} A significant increase of the LD signal intensities in the absorption band of the DNA bases (260 nm) was also observed upon addition of **5**, which reveals stiffening of the DNA molecule and a resulting better orientation of the DNA molecules along the flow lines.

The reduced LD spectrum (LD_r) provides further information on the average orientation of the transition moment of the dye relative to those of the DNA bases and allows to distinguish between homogeneous and heterogeneous binding. A nearly constant value of LD_r over the range 310–400 nm was observed (Figure 4, panel B), which proves an almost exclusive intercalation of quinolizinium derivative **1** into the DNA.¹⁶

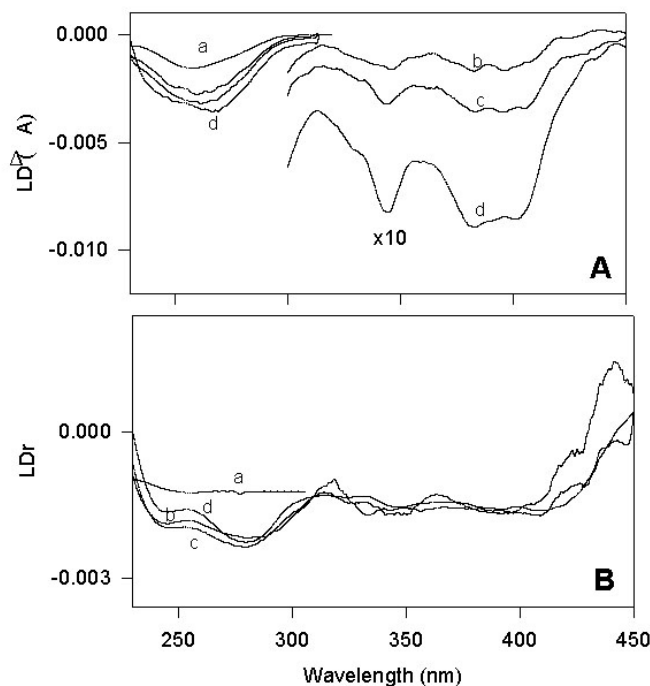


Figure 4. Linear dichroism LD (panel A), and reduced linear dichroism LD_r (panel B) spectra of mixtures of st-DNA and **1** at different dye-DNA ratios (a = 0.00, b = 0.04, c = 0.08, d = 0.2,) recorded in 0.01 M ETN buffer pH = 7.0).

Fluorescence energy transfer

Fluorescence-energy transfer experiments, in which the energy transfer from the excited DNA bases to a fluorescent ligand is monitored by fluorescence excitation spectroscopy, provide a

complementary addition to linear dichroism spectroscopy to analyze the binding mode of a dye with DNA.¹⁸ Excitation energy transfer exhibits a distance dependence of R^{-6} where R is the distance between the species involved in the transfer of energy. This distance dependence, coupled with the relatively low fluorescence quantum yield of the DNA bases results in a short (4-7 Å) Förster critical distance R_0 at which half of the energy of the excited DNA bases is transferred to the bound molecule.¹⁹ Intercalation of the molecule between stacked bases within the DNA host duplex is associated with small host-guest distances (about 2-4 Å). Thus, the observation of efficient energy transfer from a host DNA to a bound molecule, as indicated by a high fluorescence quantum yield, is consistent with an intercalative mode of binding. By contrast binding of a molecule to the minor groove and/or dye stacking along the surface of the helix are associated with larger separations between the bases and the DNA-binder. Consequently, the latter binding modes are consistent with the absence of energy transfer from the host DNA to a bound dye; however, it should be noted that fluorescence-energy transfer has been observed for some selected groove-bound molecules.²⁰

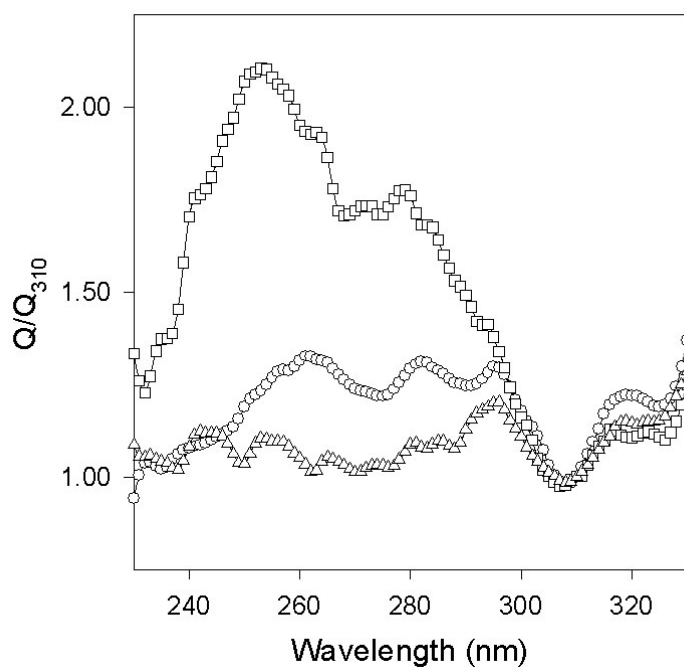


Figure 5. Fluorescence-energy transfer in complexes of DNA and **5**. Plots of Q/Q_{310} versus λ ; st-DNA (\circ), Poly[dG-dC]-poly[dG-dC] (\square), and poly[dA-dT]-poly[dA-dT] (\triangle), $[\text{dye}]/[\text{DNA}] = 0.5$; in ETN buffer; (0.01 M, pH = 7.0; $T = 25^\circ\text{C}$, the spectra were monitored at the emission wavelength of 452 nm).

Figure 5 shows the fluorescence excitation spectra of **5** bound to st-DNA, poly[dA-dT]-poly[dA-dT] or poly[dG-dC]-poly[dG-dC] given as the wavelength dependence of the relative quantum yield Q_λ divided by the fluorescence quantum yield of the free dye Q_{310} . The data were normalised with respect to 310 nm where the DNA bases do not absorb. These plots show the wavelength of the absorbed photons which result in dye emission at the longest wavelength

maximum and match very well the corresponding absorption maxima of the corresponding DNA bases (255-260 nm).

Photoinduced DNA cleavage

In order to investigate the DNA photocleavage activity of **5**, it was irradiated under aerobic conditions in buffered aqueous solutions in the presence of supercoiled pBR322 plasmid DNA, which it is a very sensitive tool for damage detection.²¹ The photosensitized DNA-cleavage products were analyzed by agarose-gel electrophoresis and quantified by densitometry. In the dark **5** does not promote DNA strand breaks (not shown). When the plasmid was irradiated in the presence of **5**, an efficient photoinduced DNA-strand cleavage occurs. The correlation of the DNA-strand breaks in the presence of **5** at different dye-DNA ratios with the irradiation time is presented in Figure 6. At low dye-DNA ratios (0.25 and 0.5), at which the dye is intercalated in DNA, no significant cleavage of the plasmid DNA was observed. As the dye-DNA ratio increase (0.1 and 2), i.e. the dye is also externally bound to the macromolecule, a significant nicking of the supercoiled plasmid form took place.

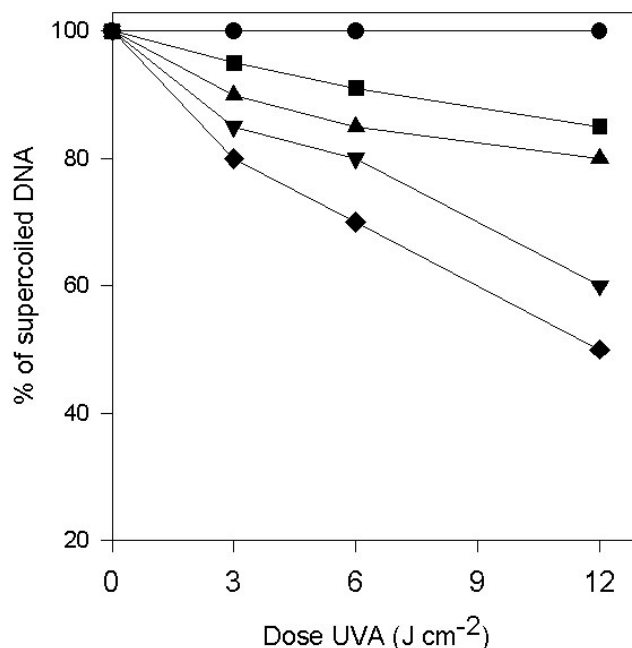


Figure 6. Strand breaks of plasmid DNA (pBR322) expressed as percentage of form I after irradiation, in the presence of dye **5**, at different dye-DNA ratios; (●=0; ■=0.25; ▲=0.5; ▼=1.0; ◆=2.0).

Photocleavage of nucleic acids typically involves an initial oxidative reaction of either a nucleobase or a sugar residue. The damaged nucleotide then degrades either spontaneously (frank strand break) or after treatment of the DNA with hot piperidine (alkali labile sites) or with base excision repair enzymes Fpg (Formamido pyrimidin glycosilase) or Endo III (Endonuclease III). The latter reactions yield shorter DNA strands and small molecule byproducts such as sugar fragments or nucleobase.

After irradiation of DNA in the presence of **5**, we determined the number of DNA modifications sensitive to the following repair enzymes (Figure 7): a) Fpg protein which recognizes 8-hydroxyguanine, purines whose imidazole ring is open (Fapy residues) and sites of base loss (apurinic sites); b) Endonuclease III, which recognizes 5,6 dihydropyrimidines derivatives in addition to apurinic sites. Damaged base release is followed by a β - δ reaction or a β -elimination step, resulting in DNA cleavage.²² Notably, the treatment of pBR322 with the two base-excision repair enzymes after irradiation in the presence of **5** does not significantly increase the number of single-strand breaks, indicating that frank strand breaks are induced upon irradiation of DNA in the presence of dye **5**. Nevertheless, the identity of the reactive intermediate in this cleavage reaction is not clear and needs further detailed investigations.

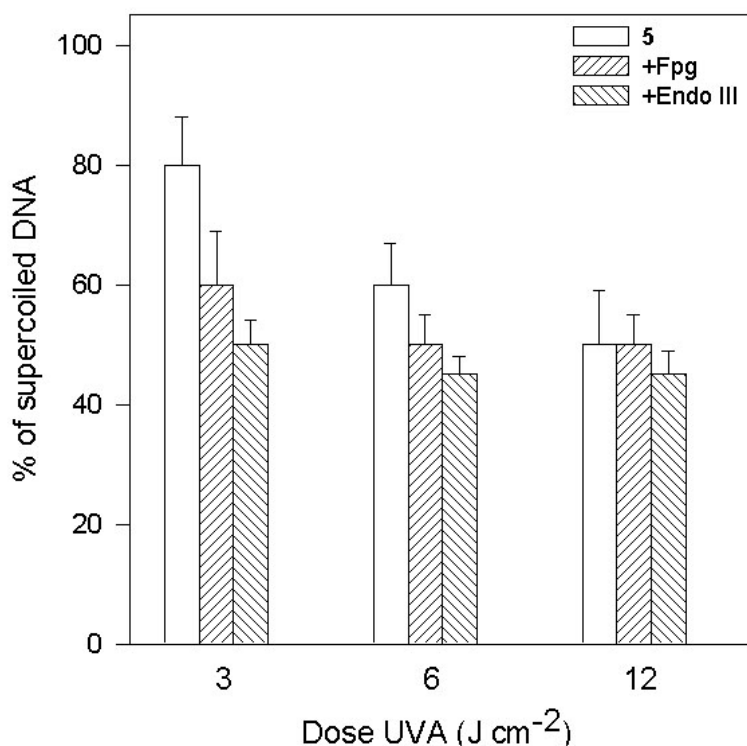


Figure 7. DNA strand breaks photoinduced by the compound **5** expressed as percentage of form I obtained after densitometric analysis of the gel. pBR322 supercoiled circular DNA was irradiated at the indicated doses at a [dye]/[DNA] ratio of **5** and then treated as described in the experimental section.

To further evaluate the influence of reactive oxygen species in the photocleavage of the plasmid DNA, a series of experiments were carried out using appropriate scavengers (Figure 8). The scavengers used were superoxide dismutase (SOD 2000 UI/ml), catalase (2000 UI/ml) which scavenge $O_2^{\bullet-}$ and H_2O_2 respectively; 2,6-diterbutylhydroxy anisole (BHA, 10 μ M) a radical scavenger, N,N'-dimethyl thiourea (DMTU, 1mM) and mannitol (10mM), which scavenge hydroxyl radicals (OH^{\bullet}). Also, the reactions were carried out in deuterated water, in which the lifetime of singlet oxygen is increased by a factor of ca. 14 relative to the one in water.

No significant protection on DNA induced photocleavage was observed with BHA, mannitol and DMTU indicating that free radicals may not involved in the mechanism of DNA cleavage. Notably, a slight increase in the formation of single-strand breaks was observed in the presence of SOD, CAT and with the mixture of the two enzymes.

By contrast, a dramatic increase of frank strand breaks was observed when deuterated phosphate buffer was used, so that it may be concluded that singlet oxygen is involved in the DNA photocleavage induced by **5**.

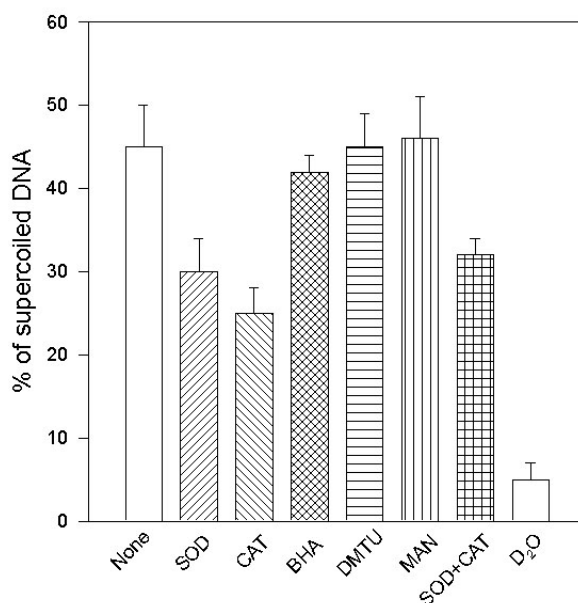


Figure 8. DNA strand breaks photoinduced by the compound **5** at a [dye]/[DNA] ratio of 1. pBR322 supercoiled circular DNA was irradiated at the UVA dose of 12 J cm^{-2} in the presence of different additives. SOD (superoxide dismutase); CAT (catalase); BHA (2,6-diterbutylhydroxy anisole); DMTU (N,N'-dimethyl thiourea); MAN (Mannitol); D₂O (Deuterated water).

In summary, the detailed analysis of the binding properties of **5** with DNA reveals a strong and almost exclusive intercalative association. Bis-quinolizinium salt **5** also exhibits DNA-photodamaging properties, and it was shown that singlet oxygen is the key intermediate in this reaction.

Experimental Section

Materials. 12a,14a-Diazoniapentaphene was synthesized according to literature procedures.¹³ Salmon testes DNA sodium salt and polydeoxyadenylic-thymidylic acid sodium salt, poly(dA-dT)-poly(dA-dT), were purchased from Sigma (Saint Louis, MI USA.); and polydeoxyguanylic-deoxycytidylic acid sodium salt, poly(dGdC)-poly(dG-dC), were purchased from Pharmacia and used without further purification. The actual concentrations (in nucleotides) were determined by UV spectroscopy (st DNA and [poly(dA-dT)-poly(dA-dT)], $\epsilon_{260} = 6600 \text{ M}^{-1}\text{cm}^{-1}$, [poly(dGdC)-

poly(dG-dC)]: $\epsilon_{254} = 8400 \text{ M}^{-1}\text{cm}^{-1}$, The two base excision repair enzymes (BER) formamido-pyrimidine glycosylase (FPG) and Endonuclease III were a generous gift from Dr. S. Boiteux (CEA, Fontenay aux Roses, France). All other reagents were of analytical grade.

DNA binding studies. Nucleic-acid concentrations, expressed with respect to mononucleotides, were determined spectrophotometrically using the reported data for the molar absorption coefficient at the indicated wavelength. UV-Vis absorption spectra were recorded on a Perkin-Elmer Lambda 15 spectrophotometer and fluorescence spectra were recorded on a Perkin-Elmer LS-50B luminescence spectrophotometer. All measurements were carried out in ETN buffer (TRIS 10 mM, EDTA 1 mM and NaCl, 10 mM) at pH = 7.0 and 25°C. The titration experiments were performed according to published procedures⁹⁻¹² and the data, plotted as Scatchard plot,¹⁴ were analyzed by the method of McGhee and von Hippel¹⁵ to obtain the intrinsic binding constant (K_i) and the binding site size (n).

Flow linear dichroism. Linear dichroism (LD) spectra were recorded in a "flow cell" on a Jasco J500A spectropolarimeter equipped with an IBM PC and a Jasco J interface. The determination and interpretation of the data was performed according to a previous publication.^{9,11,12}

Fluorescence contact energy transfer. Contact-energy transfer from DNA bases to the associated dye was determined from the excitation spectra of the DNA-dye complex from 230–330 nm with an interval of 1 nm. Fluorescence was monitored at $\lambda_{em} = 452 \text{ nm}$. The excitation spectra were corrected for the inner filter effect prior to the normalisation.²³ The respective quantum yields Q were determined according to equation 1.

$$Q = q_b/q_f = I_b E_f / I_f E_b \quad (1)$$

The parameters q_b and q_f represent the fluorescent quantum efficiencies of bound and free dye, and I_b and I_f are the fluorescence intensities of the dye in the presence and in the absence of the DNA respectively. E_b and E_f are the corresponding molar extinction coefficients of the dye. The term Q_i/Q_{310} was plotted versus the wavelength. The normalization wavelength of 310 nm was chosen because of the negligible absorbance of DNA at this wavelength.

Irradiation procedure. One HPW 125 Philips lamp, mainly emitting at 365 nm, was used for irradiation experiments. The spectral irradiance of the source was 4.0 mW cm^{-2} as measured by a Cole-Parmer Instrument Company radiometer (Niles, IL), equipped with a 365-CX sensor.

pBR322 DNA strand breaks. Under aerobic conditions, each pBR322 DNA sample (100 ng) dissolved in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 7.5) was irradiated with increasing UVA doses in the presence of the compounds under examination. After irradiation two aliquots of the sample were incubated at 37 °C with Fpg (formamido pyrimidin glycosylase) and Endo III (Endonuclease III) respectively as described by Pflaum.²⁴ The samples were loaded on 1% agarose gel, and the run was carried out in TAE buffer (0.04M Tris-acetate, 1mM EDTA) at 50 V for 4 hrs. After staining in ethidium bromide solution, the gel was washed with water and the DNA bands were detected under UV radiation with a UV transilluminator. Photographs were taken with a digital photcamera Kodak DC256 and the quantification of the bands was achieved by image analyzer software Quantity One (BIO RAD, Milano Italy). The fractions of supercoiled DNA (Form I) and open circular (Form II) were calculated as described.²⁵

Acknowledgements

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References

1. Kochevar, E.; Dunn, D. D. In *Bioorganic Photochemistry*; Morrison, H., Ed.; John Wiley and Sons: New York, 1990; p 273.
2. Pratviel, G.; Bernadou, J.; Meunier, B. *Angew. Chem., Int. Ed.* **1995**, *34*, 746.
3. Armitage, B. *Chem. Rev.* **1998**, *98*, 1171. (b) Demeunynck, M.; Bailly, C.; Wilson, W. D., Eds.; *DNA and RNA binders*; Wiley-VCH: Weinheim, 2002. (c) Wilson, W.D. In *Nucleic Acids in Chemistry and Biology*; Blackburn, G. M.; Gait, M. J. Eds.; IRL Press: Oxford, U. K. 1996, pp 329. (d) Ihmels, H.; Faulhaber, K.; Viola, G. In *Highlights in Bioorganic Chemistry: Methods and Applications*; Schmuck, C.; Wennemers, H., Eds.; Wiley-VCH: Weinheim, 2004. (e) Da Ros, T.; Spalluto, G.; Boutorine, A. S.; Bensasson, R. V.; Prato, M. *Curr. Pharm. Design* **2001**, *7*, 1781. (f) Pogozelski, W. K.; Tullius, T. D. *Chem. Rev.* **1998**, *98*, 1089. (g) Greenberg, M. M. *Chem. Res. Toxicol.* **1998**, *11*, 1235.
4. Canti, G.; De Simone, A.; Korbelik, M. *Photochem. Photobiol. Sci.* **2002**, *1*, 79. (b) Dougherty, T. J.; Gomer, C. J.; Henderson, B. W.; Jori, G.; Kessel, D.; Korbelik, M.; Moan, J.; Peng, Q. *J. Natl. Cancer Inst.* **1998**, *90*, 889.
5. Lermann, L. S. *J. Mol. Biol.* **1961**, *3*, 18.
6. LePecq, J.-B.; Paoletti, C. *J. Mol. Biol.* **1967**, *27*, 87.
7. Molina, A.; Vaquero, J. J.; Garcia-Navio, J. L.; Alvarez-Builla, J.; de Pascal-Teresa, B.; Gado, F.; Rodrigo, M. M. *J. Org. Chem.* **1999**, *64*, 3907.
8. Ihmels, H.; Engels, B. K.; Faulhaber, K.; Lennartz, C. *Chem. Eur. J.* **2000**, *6*, 2854.
9. Ihmels, H.; Faulhaber, K.; Sturm, C.; Bringmann, G.; Messer, K.; Gabellini, N.; Vedaldi, D.; Viola, G. *Photochem. Photobiol.* **2001**, *74*, 505.
10. Ihmels, H.; Bringmann, G.; Faulhaber, K.; Messer, K.; Sturm, C.; Vedaldi, D.; Viola, G. *Eur. J. Org. Chem.* **2001**, *6*, 1157.
11. Viola, G.; Bressanini, M.; Gabellini, N.; Dall'Acqua, F.; Vedaldi, D.; Ihmels, H. *Photochem. Photobiol. Sci.* **2002**, *11*, 882.
12. Viola, G.; Dall'Acqua, F.; Gabellini, N.; Moro, S.; Vedaldi, D.; Ihmels, H. *ChemBioChem* **2002**, *3*, 101.
13. Bradsher, C. K.; Parham, J. C. *J. Org. Chem.* **1964**, *29*, 856.
14. Schatchard, G. *Ann. N.Y. Acad. Sci.* **1949**, *51*, 660.
15. McGhee, J. D.; von Hippel, P. H. *J. Mol. Biol.* **1974**, *86*, 469.
16. Nordén, B.; Kubista, M.; Kurucsev, T. *Q. Rev. Biophys.* **1992**, *25*, 51.
17. Nordén, B.; Kurucsev, T. *J. Mol. Recognit.* **1994**, *7*, 141.

18. Suh, D. S.; Chaires, J. B. *Biorg. Med. Chem.* **1995**, *3*, 723.
19. Förster, T. *Ann. Phys.* **1948**, *2*, 55.
20. Kyung-Mee, H.; Sang-Doh, C.; Saeyun, L.; Soeg, K. K. *Biochim. Biophys. Acta* **1997**, *1334*, 312.
21. (a) Martinez, L.; Chignell, C. F. *J. Photochem. Photobiol. B: Biol.* **1998**, *45*, 51.(b) Marrot, L.; Belaidi, J. P.; Chaubo, C.; Meunier, J. R.; Perez, P.; Agapakis-Causse, C. *Toxicol. in Vitro* **2001**, *15*, 131.
22. Burrows, J.; Muller, J. G. *Chem. Rev.* **1998**, *98*, 1109.
23. Lakowicz J. R. *Principles of fluorescence spectroscopy*; Plenum Press: New York, 1983, pp 257.
24. Pflaum, M.; Boiteux, S.; Epe, B. *Carcinogenesis* **1994**, *15*, 297.
25. Ciulla, T. A.; Van Camp, J. R.; Rosenfeld, E.; Kochevar, I. E. *Photochem. Photobiol.* **1989**, *49*, 293.