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Naphthoquinolizinium derivatives as a novel platform for DNA-binding and DNA-photodamaging chromophores †

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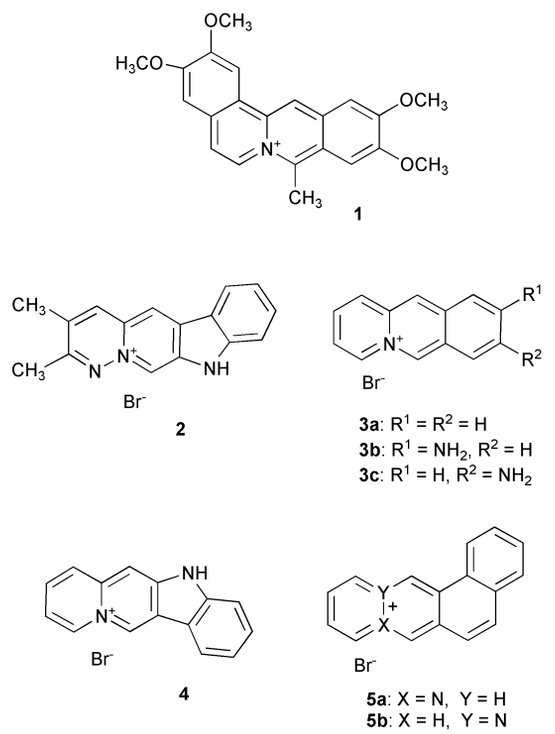
The association of the naphtho[1,2-*b*]quinolizinium bromide (**5a**) and naphtho[2,1-*b*]quinolizinium bromide (**5b**) with DNA and the propensity of these cationic arenes to damage DNA after UV-A irradiation have been studied. Spectrophotometric and fluorimetric titrations show that the two isomers **5a** and **5b** bind to DNA ($K \approx 10^5$ M^{-1}). The highest affinity was observed for GC base pairs. The mode of binding was investigated by CD and LD spectroscopy. Whereas quinolizinium **5a** exclusively intercalates in DNA, the isomer **5b** exhibits a deviation from perfect intercalation into the double helix. Moreover, efficient DNA damage was observed on UV-A irradiation in the presence of the quinolizinium salts. Primer extension analysis indicates that the photocleavage takes place preferentially at guanine-rich regions.

Introduction

The association of cationic aromatic dyes to DNA has attracted considerable interest.[1–4] Such a complex formation results in a significant modification of the DNA structure which may have a profound influence on the gene expression.[5,6] In most cases, the binding interactions are easily detected by a change of the absorption and emission spectra of the dye upon DNA addition. This modification of the absorption and emission may also be used to mark, to probe or to characterize the nucleic-acid structure.

While the association of cationic dyes to DNA is a reversible process, the DNA damage, which frequently occurs on irradiation of dye-DNA complexes, is often irreversible.[7–10] The latter DNA damage may lead to cell death or mutations, and must be avoided in healthy systems. However, this photoinduced DNA-damage may be applied in photochemotherapy to remove unwanted cells.[11,12]

Among the compounds investigated along these lines are quinolizinium derivatives such as coralyne (**1**)[13,14] and the related quinolinium derivative **2**.[15] Moreover, we have observed recently that the benzoquinolizinium derivatives (Scheme 1) (acridizinium) **3a**–**c** and the indoloquinolizinium **4** exhibit DNA-binding and DNA-photodamaging properties.[16–18] However, other examples for DNA-binding quinolizinium derivatives with photonuclease activity are still rare.[19] During our studies of the influence of the substitution pattern of quinolizinium derivatives on their interaction with DNA, we became interested in the known naphtho[1,2-*b*]quinolizinium bromide (**5a**) and naphtho[2,1-*b*]quinolizinium bromide (**5b**).[20] The investigation of these compounds may allow to evaluate the influence of the position of the positive charge within the benzo[*a*]anthracene framework on the DNA interactions. Moreover, we anticipated that the presence of a fourth aromatic ring in quinolizinium derivatives **5**, in comparison to the benzo-annellated quinolizinium salts **3**, may enhance the interaction between DNA base pairs and the π system, since it was shown that the extension of the π surface by one benzene moiety increases the binding energy by about 0.4 kcal mol^{-1}.[21]



Scheme 1

Experimental section

Materials

The naphthoquinolizinium derivatives **5a** and **5b** were synthesized according to the published procedure.[20] Calf thymus DNA (ct DNA), salmon testes DNA (st DNA) sodium salt and

† Electronic supplementary information (ESI) available: fluorometric titrations and CD spectra. See http://www.rsc.org/suppdata/pp/b2/b204275d/

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polydeoxyadenylic-thymidylic acid sodium salt, (poly[dA-dT]–poly[dA-dT]), were purchased from Sigma (St Louis, MO, USA); and polydeoxyguanylic-deoxycytidylic acid sodium salt, (poly[dG-dC]–poly[dG-dC]), polydeoxyinosylic-deoxycytidylic, (poly[dI-dC]–poly[dI-dC]), were purchased from Amersham Pharmacia Biotech (Piscataway, NJ, USA) and used without further purification. The actual concentrations (in nucleotides) were determined by UV spectroscopy (ct DNA, st DNA and (poly[dA-dT]–poly[dA-dT]): ε_{260} = 6600 M^{-1} cm^{-1}, (poly-[dG-dC]–poly[dG-dC]): ε_{254} = 8400 M^{-1} cm^{-1}, (poly[dI-dC]–poly[dI-dC]): ε_{251} = 6900 M^{-1} cm^{-1}). 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 emission spectra were recorded on a Perkin-Elmer LS-50B luminescence spectrophotometer. All measurements were carried out in ETN buffer (10 mM TRIS, 1 mM EDTA, 10 mM NaCl) at pH = 7.0 and 25 °C. The titration experiments were performed according to published procedures[17] and the resulting Scatchard plots[22] were analyzed by the method of McGhee and von Hippel[23] to obtain the intrinsic binding constant (K) and the binding site size (n).

Circular dichroism

Circular dichroism spectra were recorded in 0.01 M ETN buffer solution at different dye-DNA ratios on a Jasco J500A spectropolarimeter. The DNA concentration was 0.15 mM, and the measurements were performed at [DNA]/[dye] = 0.08, 0.20, and 0.40.

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 as previously described.[17,24] DNA concentration was 2.27 mM and the measurements were performed at [DNA]/[dye] = 0.04 and 0.08.

DNA-photocleavage experiments

Solutions (10 μL) of 20 μg mL^{-1} of pBR322 plasmid DNA and 0.1 mM of the respective salts **5a** or **5b** were irradiated with a Rayonet photoreactor at λ = 350 nm for 5, 10 and 15 min. The experiments were carried out under aerobic (atmospheric) or anaerobic conditions. For the experiments under anaerobic conditions, argon gas was bubbled through each solution for at least 5 min prior to the irradiation. For comparison, plasmid DNA was irradiated under the same conditions for 15 min in the absence of the dye. This DNA sample did not show significant damage under these conditions.

DNA-strand breaks were determined directly from the photolysate by agarose-gel electrophoresis with ethidium bromide as indicator. The spots were detected by exposure to an UV transilluminator and recorded with a Herolab EASY 429K camera. The amounts of damaged open-circular (relaxed) and remaining supercoiled DNA were determined from the light intensities of the spots.

Irradiation of 5a or 5b in the presence of ct DNA, search for oxidation products of guanine[25]

Each reaction mixture contained 30 μL (62.5 μM in guanine) of *calf-thymus* DNA (from a 1.0 μg mL^{-1} stock solution in water) and 37.5 μL (625 μM) of **5a** or **5b** (from a 5 × 10^{-3} M stock solution in water) in 5 mM phosphate buffer, pH 7.0 (7.5 μL of a 200 mM buffer solution) and was filled with water to a total volume of 300 μL. For comparison a solution without **5a** or **5b** was used as a reference. The solutions were irradiated in glass vials at 350 nm in a Rayonet photoreactor at 10 °C for 15 min. After the photolysis, the samples were taken for analysis. Aliquots of 80 μL were used for the determination and quantification of 8-oxo-7,8-dihydroguanine (8-oxoGua). For each solution two separate aliquots were used to check the reproducibility of the analysis. The solutions were extracted with ethyl acetate (2 × 160 μL) and the DNA was precipitated by addition of 11.0 μL of aqueous sodium acetate (3 M, pH 5.0) and three volumes of ethanol (−50 °C). The samples were stored for 12 h at −50 °C. After centrifugation (5 min, 15000 rev min^{-1}), the liquid phase was removed by means of a pipette and the precipitated DNA was dried for 40 min at 20 °C/10^{-2} Torr. For the DNA hydrolysis,[25] the pellet was diluted in 13.0 μl HF–pyridine (70% HF) and stored at 37 °C for 40 min. After the addition of 200 μl of a $CaCO_3$ suspension, the sample was shaken for 30 min and subsequently centrifuged. The aqueous solution was decanted, and to the $CaCO_3$ residue was added another 200-μl aliquot of water and the extraction was repeated once. The combined aqueous solutions were lyophilised, and the residue was diluted with 80 μl of water. The amount of 8-oxo-7,8-dihydroguanine, 8-oxoGua, was quantified by HPLC with UV and electrochemical detection. The HPLC analytical system consisted of Bischoff HPLC pumps, model 2200 (Bischoff GmbH, Leonberg, Germany), equipped with a Rheodyne loop injector, model 7125 (Berkly, CA, USA). For the detection of the DNA bases, a Waters 994 photodiode array detector (Waters GmbH, Eschborn, Germany) was used, connected in series with an ESA coulochem model 5100A electrochemical detector, the latter supplied with a model 5011 high sensitive analytical cell (ESA; Inc. Bedford, MA, USA) for the detection of 8-oxoGua. All HPLC solvents were passed through a 0.45-μM Sartorius cellulose filter before use. The separation of the DNA bases was achieved on a 250 × 4.6 mm (i. d.) Eurospher 100-C18 7 μm column (Knaur GmbH, Berlin, Germany) by using a 90 : 10 mixture of 50 mM sodium citrate buffer (pH 5.0) and methanol at a flow rate of 1 mL min^{-1}. For the electrochemical detection of 8-oxoGua the oxidation potential was set at 350 mV.

Primer Extension and DNA Sequencing

Plasmid pUC18 was purified from transformed *E. coli* strain HB101 by the GeneElute™ plasmid miniprep kit (Sigma Saint Louis, MO USA) and stored at 4 °C in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH = 8.0). Plasmid DNA (50 μg ml^{-1}) was treated with **5a** or **5b** at a molar dye-DNA ratio of 0.4 in 50 μl of 0.01 M ETN buffer and subsequently irradiated with an UV-A lamp (Philips HPW 125 W, λ = 365 nm; energy emission 0.2848 J cm^{-2} min^{-1}). Aliquots were taken after 0, 30, and 60 min and stored at 0 °C. Primer extension analysis was performed on the irradiated DNA (2.5 μl) in a 10 μl reaction volume containing the four deoxynucleotides triphosphates (dNTP, 200 μM each, Amersham Pharmacia Biotech, Little Chaffold, UK), Taq polymerase (2 U) and the supplied buffer with magnesium chloride (Roche Diagnostic, Mannheim, Germany), and the lacZ reverse primer M13, that was end labeled with [γ-^{33}P] adenosine triphosphate following the instruction of the fmol-DNA sequencing kit (Promega Corp. Madison WI, USA). The same labeled primer was used for sequencing the pUC18 plasmid to determine the position at which DNA photocleavage occurred. Primer extension and cycle sequencing, were run in parallel reactions using thirty PCR cycles programmed as follows: denaturation at 94 °C for 30 s, annealing at 56 °C for 30 s and extension for 1 min at 72 °C. Aliquots (1 μl) were loaded on a denaturing 6% acrylamide, 50% urea gel and separated by gel electrophoresis; the gel was subsequently dried and exposed to Kodak X-OMAT UV films.

Results

Spectrophotometric and fluorimetric titration

To evaluate the interaction of quinolizinium derivatives **5a** and **5b** the addition of salmon testes (st) and calf thymus (ct) DNA to a buffered aqueous solution of salts **5a** and **5b** was monitored by absorption and emission spectroscopy. With an increasing DNA concentration a decrease of the absorbance was observed, along with a significant red shift of 11 nm (**5a**) and 12 nm (**5b**) and a partial loss of the fine structure (Fig. 1). Moreover, isosbestic points were detected (**5a**: 411 and 345 nm; **5b**: 398 and 340 nm) in each case which indicate that one type of quinolizinium-DNA complex is formed almost exclusively under these conditions.

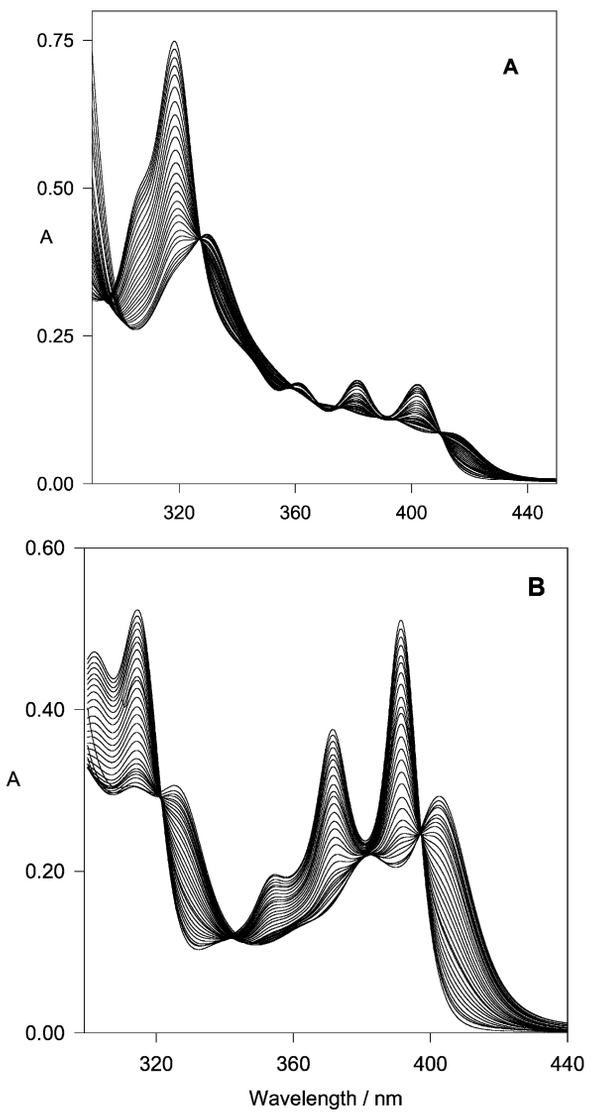

Fig. 1 Spectrophotometric titration of naphthoquinolizinium **5a** (**A**) and **5b** (**B**) with st DNA in ETN buffer (0.01 M, pH = 7.0, T = 25 °C).

The fluorescence intensity of naphthoquinolizinium salts **5a** and **5b** is significantly quenched on addition of st or ct DNA and synthetic polynucleotides (Fig. 2; *cf.* ESI†: Fig. S1 and S2), and in most cases essentially no shift of the emission maximum was observed. By contrast, a red shift of the emission maximum from 418 to 439 nm was observed on addition of (poly[dA-dT]–poly[dA-dT]) for compound **5a**. A Stern–Volmer plot derived from the fluorimetric titration of ct DNA exhibits linear behavior of the titration curve of salt **5b**, whereas the one of the isomers **5a** is linear at DNA concentrations up to 60 mM (Fig. 2). From these data, quenching efficiencies were estimated by the Stern–Volmer constant K_{SV} (**5a**: K_{SV} = 8600 M^{-1}; **5b**: 5700 M^{-1}), which are comparable to reported data.[26,27]

The fluorescence-titration data for compounds **5a** and **5b** have been used to determine the binding constants between the quinolizinium salts **5a** or **5b** and DNA. From the binding isotherms the association constant (K) and the number of base pairs covered by one ligand (n) have been estimated (Table 1). The naphthoquinolizinium derivatives **5a** and **5b** have an affinity to DNA which is approximately ten times larger than the one of the benzoquinolizinium (**3a**).[17] Both compounds exhibit a significantly higher affinity for (poly[dG-dC]–poly[dG-dC]) than for (poly[dA-dT]–poly[dA-dT]) and (poly[dI-dC]–poly[dI-dC]) (Table 1).

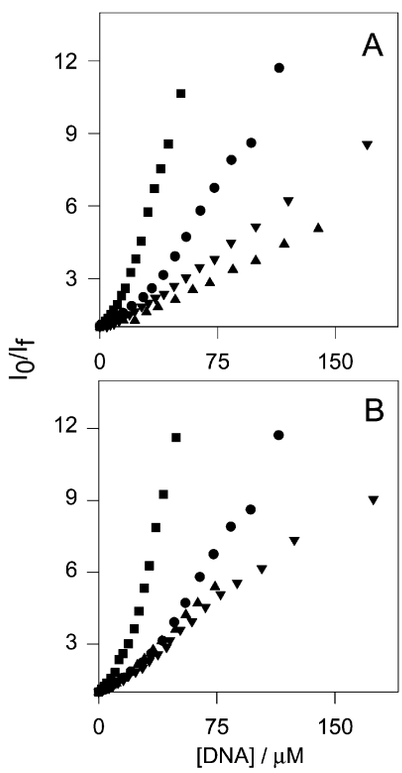

Fig. 2 Fluorescence quenching of salts **5a** (**A**) and **5b** (**B**) on addition of st DNA (●), (poly[dG-dC]–poly[dG-dC]) (■), (poly[dA-dT]–poly[dA-dT]) (▲), and (poly[dI-dC]–poly[dI-dC]) (▼) in ETN buffer (0.01 M, pH = 7.0, T = 25 °C).

Table 1 Binding constants (K) and binding site size (n) of salts **5a** and **5b** determined from spectrofluorimetric titrations with st DNA, (poly[dA-dT]–poly[dA-dT]), (poly[dG-dC]–poly[dG-dC]) and (poly[dI-dC]–poly[dI-dC])[a]

		st DNA	(poly[dA-dT]-poly[dA-dT])	(poly[dG-dC]-poly[dG-dC])	(poly[dI-dC]-poly[dI-dC])
5a:	K [M^{-1}] × 10^5	1.9 ± 0.1	1.3 ± 0.1	5.6 ± 0.3	1.1 ± 0.1
	n	2.6 ± 0.1	3.2 ± 1.2	1.4 ± 0.1	1.4 ± 0.2
5b:	K [M^{-1}] × 10^5	2.1 ± 0.3	0.9 ± 0.1	5.4 ± 0.9	1.9 ± 0.1
	n	2.6 ± 0.2	3.2 ± 1.2	1.3 ± 0.1	2.3 ± 0.3

[a] K is the binding constant and n is the binding-site size (in base pairs), determined in ETN buffer solution (pH 7.0, 0.01 M); data represent three independent experiments.

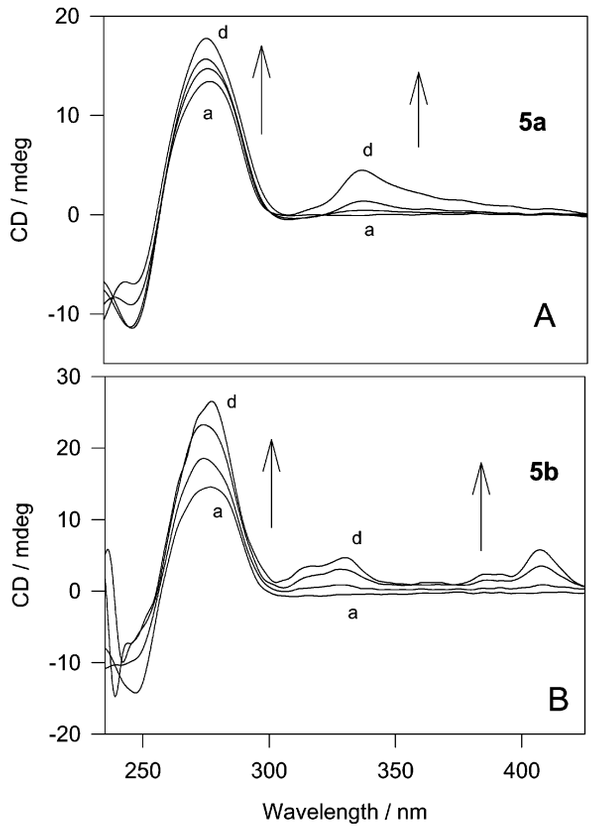

Fig. 3 CD spectra of **5a**, (**A**) and **5b** (**B**) in the presence of st DNA at different [Dye]/[DNA] ratios ($a = 0.00$, $b = 0.08$, $c = 0.20$, $d = 0.40$).

Circular dichroism

The CD spectra of DNA in the presence of the salts **5a** and **5b** reveal a significant perturbation of the DNA signal since the positive CD band of the DNA at 275 nm increases upon successive addition of the salts **5a** and **5b** (Fig. 3). Also, induced CD (ICD) signals were observed in the chromophore absorption region of **5a** and **5b** (300–440 nm).

Linear dichroism

Linear dichroism (LD) spectra of quinolizinium salts **5a** and **5b** were recorded in the presence of st DNA at different dye-DNA ratios (Fig. 4). In both cases, negative LD signals were observed in the UV region where the DNA and the dyes **5a** or **5b** contribute to the absorbance and also in the absorption region where only the dyes absorb. Most notably, a significant increase of the LD-signal intensity in the DNA absorption band (260 nm) was observed in the presence of **5a** and **5b** (Fig. 4, B1 and B2).

The reduced linear dichroism (LD_r = LD/A) of the quinolizinium **5a** in the presence of DNA is nearly constant between 300 and 375 nm (Fig. 4, C1) whereas the LD_r of **5b** exhibits a pronounced variation of signal intensity at the different dye-DNA ratios (Fig. 4, C2). Furthermore in the latter case, the intensity of the signal in the dye absorption region is lower compared to the one of the DNA.

DNA photocleavage and sequence selectivity

The naphthoquinolizinium salts **5a** and **5b** were irradiated in the presence of supercoiled plasmid DNA pBR322 in buffered aqueous solutions, and the photolysate was analyzed without further work up (Fig. 5). The single-strand (ss) cleavage of the

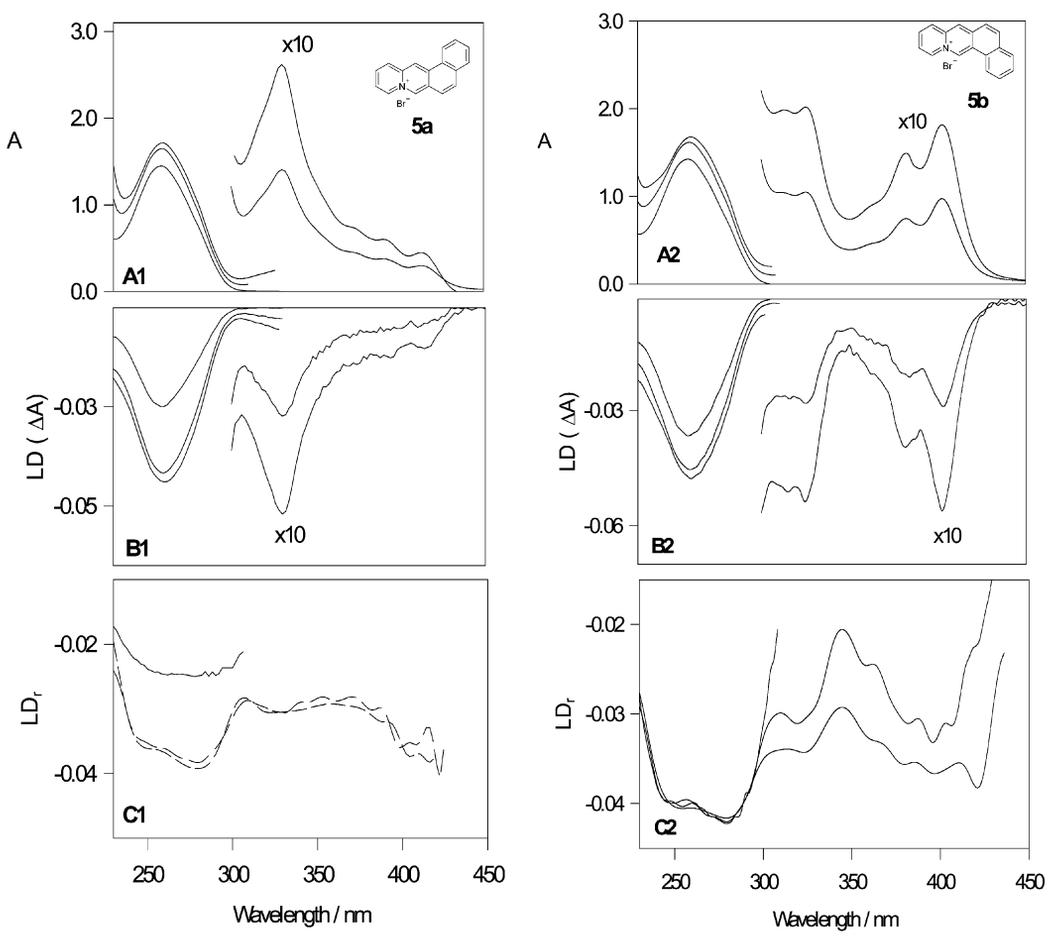

Fig. 4 Absorbance A (**A**), linear dichroism LD (**B**), and reduced linear dichroism LD_r (**C**) spectra of mixtures of st DNA and naphthoquinolizinium derivatives **5a** and **5b** at different [Dye]/[DNA] ratios, ($a = 0.00$, $b = 0.04$, and $c = 0.08$).

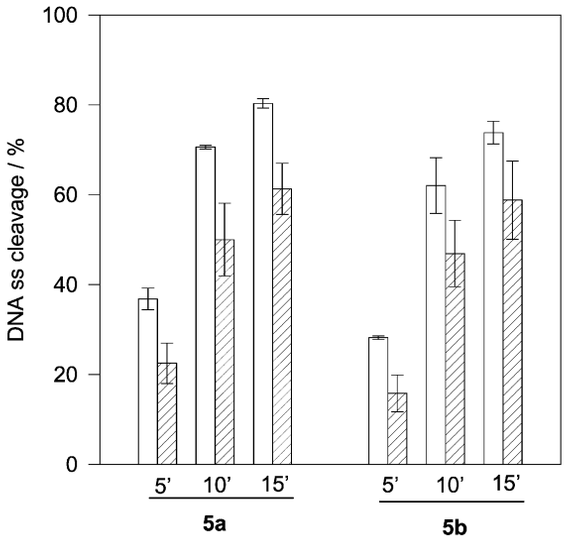

Fig. 5 DNA single-strand (ss) cleavage of plasmid DNA pBR322 upon irradiation in the presence of naphthoquinolizinium salts **5a** and **5b** for 5, 10 and 15 min under aerobic (white column) and anaerobic conditions (gray column); data represent the average of two different runs and are derived from gel-electrophoretic analysis of the photolysate (λ_{max} = 350 nm, *c* (pBR 322) = 10 μg mL^{-1}; *c* (dye) = 0.1 mM).

DNA was evaluated by quantification of the undamaged supercoiled and the damaged open-circular form of the plasmid DNA by agarose-gel electrophoresis. The analysis of these data revealed an efficient photoinduced DNA-strand cleavage in the presence of the salts **5a** and **5b**. The DNA damage in the presence of **5a** and **5b** is almost equally efficient under aerobic and anaerobic conditions, *i.e.* under aerobic conditions only slightly higher amounts of open-circular plasmid DNA were formed.

The detection of primary base modifications, *i.e.* the initial oxidation of guanine, namely 8-oxo-7,8-dihydroguanine (8-oxoGua), was also attempted. According to an established protocol[25] ct DNA was irradiated in the presence of **5a** and **5b** and in the absence of any dye. After hydrolysis of the DNA, HPLC analysis with an electrochemical detector revealed that the amount of 8-oxoGua is smaller than 1% in each case; precisely, the relative amount of 8-oxo-7,8-dihydroguanine was: **5a**: 0.5 and 0.8%; **5b**: 0.4 and 0.7%; reference sample without dye: 0.7 and 0.3%, indicating that the irradiation of ct DNA in the presence of the quinolizinium salts **5a** and **5b** does not yield 8-oxoGua as a base modification.

To investigate whether the DNA damage proceeds with a sequence selectivity, the sites of DNA cleavage were determined by a primer extension analysis. The plasmid DNA pUC18 was irradiated in the presence of **5a** and **5b** ([dye]/[DNA] = 0.4). The photolysate was used as a template for DNA synthesis by the Taq polymerase initiated from the M13 reverse primer (^{33}P-end labeled). Fig. 6 (panel A), shows the autoradiography of the denaturing gel with the synthesized DNA fragments, which are complementary to the fragments of the photolysate. The inspection of the autoradiogram reveals several spots whose intensity increases with irradiation time (0–60 min). The positions of the complementary polynucleotide fragments in the gel were determined by running the DNA sequencing reactions obtained by the dideoxynucleotide-chain-termination procedure.[28] The position at which the DNA synthesis of the complementary strand stops corresponds directly to the location of the photoinduced DNA damage. The examined sequence of 170 bases indicated 58 cleavage sites, (Fig. 6, Panel B), and the majority of these sites correspond in the template strand to guanine (70.7%) and adenine residues (13.8%). Damage at cytosine (8.6%) and thymine (6.9%) only

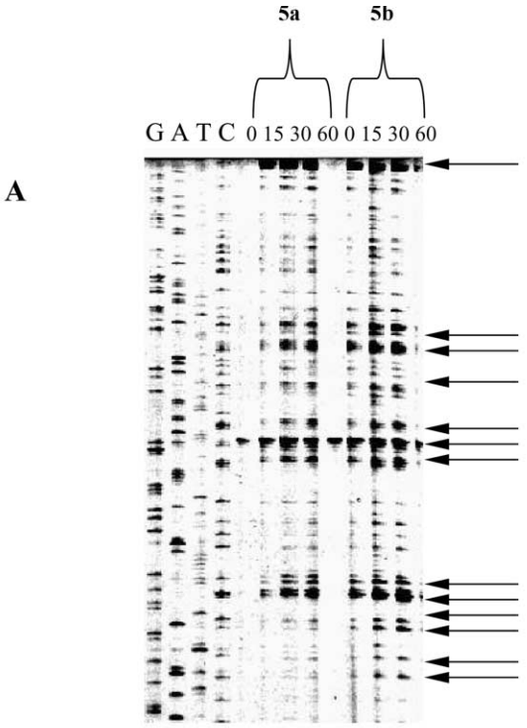

Fig. 6 (A) Autoradiogram of acrylamide-urea gel showing the DNA fragments synthesized from the M13 labeled primer by Taq DNA polymerase. The pUC18 template was irradiated in the presence of the quinolizinium salts **5a** and **5b** 0, 30, and 60 minutes. The pUC18 sequencing reactions were run site by site as a reference (GATC in the autoradiogram). The major termination sites are indicated by arrows. (B) The determined sequence of the synthesized DNA strand of pUC18 plasmid from position 269 to 439 is also shown. All the termination sites are bold. The nucleotides at which the DNA synthesis stops more frequently are bold and shaded.

contributes marginally to the overall DNA damage. The most frequent locations of termination occurred at successive guanine sites.

Discussion

We have recently demonstrated that the benzo-annellated quinolizinium (acridizinium) salts **3a**–**c** bind to DNA and induce DNA photocleavage.[16,17] We proposed that naphthoquinolizinium derivatives **5a** and **5b** may exhibit a stronger interaction with the nucleic acid due to an additional benzene moiety which extends the surface of the planar chromophore and, thus, may increase the π stacking between the dye and the DNA bases. Indeed, our results show that the extension of the π system results in higher binding constants and also higher cleavage activity in comparison to the acridizinium salts **3a**–**c**. Moreover, it is noteworthy that the binding modes of the two dyes differ slightly as shown by LD spectroscopy. Thus, the position of the positive charge in quinolizinium isomers has a significant influence on the DNA-binding properties.

Absorption spectroscopy

The absorption spectra of **5a** and **5b** show significant perturbations in the presence of DNA which usually indicates a strong association of a cationic dye with DNA.[29] Moreover, the presence of clear isosbestic points in the spectrophotometric titrations indicate one major binding mode between the dye and the DNA. Mostly remarkable is the significant red shift on addition of DNA to quinolizinium salts **5**, since these compounds are only slightly solvatochromic.[30] It may be proposed that the significant perturbation of the electronic structure of the chromophore results from efficient π stacking of the aromatic compound with the DNA base pairs due to intercalation. By contrast, the acridizinium bromide (**3a**) does not exhibit such a significant change of the absorption bands on DNA addition.[17] Also, the two salts **5a** and **5b** bind to the nucleic acid with higher affinity than the acridizinium **3a**.

Fluorescence spectroscopy

Since a significant red shift in the absorption spectrum of the salts **5** is induced upon DNA addition, a similar bathochromic shift may be expected in the emission spectrum. By contrast, no such effect has been observed on fluorimetric titration, except for the addition of (poly[dA-dT]–poly[dA-dT]) to **5a**. It may be concluded that the emission of the DNA-bound naphthoquinolizinium 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.

The fluorimetric titration experiments show that the interaction of the naphthoquinolizinium salts **5a** and **5b** with the synthetic polynucleotides has a significant influence on the emission properties of **5a** and **5b**. In particular, a strong quenching effect was observed upon addition of (poly[dG-dC]–poly[dG-dC]) whereas a lower effect is observed with (poly[dA-dT]–poly[dA-dT]) and (poly[dI-dC]–poly[dI-dC]). This behavior has two different origins. Firstly, because of the higher affinity of **5a** and **5b** to (poly[dG-dC]–poly[dG-dC]) fewer unbound, emitting molecules remain in solution, compared to mixtures of **5a** and **5b** with (poly[dA-dT]–poly[dA-dT]) and (poly[dI-dC]–poly[dI-dC]), which exhibit smaller binding constants with the quinolizinium derivatives. Secondly, the quenching of the fluorescence intensity of **5a** and **5b** in the presence of DNA is most likely due to an electron-transfer reaction between the excited dye and the DNA bases in the helix, as has already been shown for other dyes.[26,31–33] Thus, guanine, *i.e.* the DNA base with the lowest one-electron oxidation potential,[34] reacts efficiently with the excited naphthoquinolizinium **5**, whereas the oxidation potential of adenine and hypoxanthine is significantly higher.[35] This assumption is consistent with the observation that the addition of (poly[dA-dT]–poly[dA-dT]) to **5a** resulted in a bathochromic shift. Due to the lower oxidation potential of adenine the photoinduced electron transfer with the excited dye is not as efficient so that the red-shifted emission of the bound dye is not totally quenched.

Binding constants

The binding parameters obtained from the fluorimetric titrations reveal a high binding affinity of **5a** and **5b** to (poly[dG-dC]–poly[dG-dC]) whereas a lower binding constant was determined for (poly[dA-dT]–poly[dA-dT]) and (poly[dI-dC]–poly[dI-dC]). The comparison between hypoxanthine- and guanine-rich polynucleotides is remarkable, because the two corresponding nucleic bases have the same structure except for one amine functionality present in guanine. This leads to the proposal that the presence of this amino group in the minor groove of the GC sequences may additionally favor the interaction between the chromophore and the helix (*e.g.* by hydrogen bonding).

Moreover, the analysis of the fluorimetric titrations according to McGhee and van Hippel yields the exclusion parameter, *i.e.* the binding site size *n*, that reflects the number of binding sites which are unavailable due to the binding of one guest molecule to the DNA host. For the quinolizinium salts **5a** and **5b** the binding site size *n* ranges between one to three base pairs depending on the polynucleotide (Table 1). This observation gives evidence for an intercalation of these compounds according to the neighbor-exclusion model, since groove binding usually results in significantly higher *n* values.[23]

Linear dichroism

The negative LD signals in the long-wavelength absorption of the dyes **5a** and **5b** reveal that both compounds are oriented perpendicular to the flow field which is usually caused by an intercalation into the DNA helix.[36,37] This assumption is further corroborated by the observation that the intensity of the DNA signal increases on addition of the salts **5a** and **5b**. This effect reflects a stiffening of the DNA helix due to intercalation which results in a better orientation of the macromolecule in the hydrodynamic field. According to the LD spectra, this effect is more pronounced for **5a** than for **5b**, which suggests that the interactions of the dyes **5a** and **5b** with DNA are slightly different.

The negative LD_r signal of **5a** is almost independent from the wavelength in the dye-absorption region which confirms an intercalative binding mode. Notably, the amplitude of the signal is significantly larger in the chromophore absorption region compared to the one of the DNA absorption band. Such a behavior has also been observed for ethidium and acridines,[38] ruthenium pyridyl complexes,[39] ellipticine derivatives[40] and cyanine oxazole yellow and methylene blue[41,42] and is rather unexpected, because the magnitude of the LD_r signal should match the one of the DNA band at 260 nm if the orientation of **5a** was coplanar to the average DNA bases. A deviation from this ideal model of the DNA structure may be explained by either static or dynamic tilting of the DNA-base planes from the perpendicular orientation.[36] In fact, the DNA bases may be tilted by as much as 20° from the helix axis.[43] Thus, a higher LD_r amplitude of an intercalator compared to the one of the DNA bases reveals that on average the bound dye molecules are oriented more perpendicular to the helix axis than the DNA bases. Also, a local stiffening of the DNA helix at the intercalation sites may provide a perfect perpendicular orientation of the bound dyes to the DNA helix axis.

By contrast, the LD_r signal of the quinolizinium **5b** is smaller than the one of the DNA bases and the LD_r values are not constant in the long-wavelength absorption band. Thus, the dye **5b** exhibits a slightly different binding mode with DNA than its isomer **5a**, *i.e.* it binds to DNA in a mode which deviates from a perfect intercalation.

Circular dichroism

Achiral molecules exhibit an induced CD (ICD) signal when they form complexes with the DNA.[36,37] The ICD results from the non-degenerative coupling between the transition of the bound ligand and the one of the nucleic-base transitions. Such ICD spectra of the quinolizinium-DNA complexes may provide information about the actual position of a dye in its complex with DNA, since the intensity and the phase of the ICD signal is dependent on the position and the orientation of the chromophore relative to the DNA bases. The ICD bands of the quinolizinium **5a** and **5b** exhibit a shape that is nearly independent of the mixing ratio, indicating one dominant binding geometry at all mixing ratios. In both cases, the ICD signal in the long-wavelength absorption region of **5a** and **5b** is positive, which shows that the transition moments of the two intercalated salts (and thus approximately the long axis of

the chromophore) are aligned parallel to the long axis of the base-pair pocket.[44]

Photoinduced DNA damage

A photoinduced DNA damage was observed in the presence of the naphthoquinolizinium derivatives **5a** and **5b**. Most notably, the extent of DNA damage is not significantly influenced by the presence of oxygen. Thus, depending on the conditions, different mechanisms for the DNA damage may take place, at least one of them oxygen independent.[10,35] In both cases the oxidation of the guanine moiety may be proposed, especially since it is evident from the fluorimetric titrations that the guanine radical cation is formed as an intermediate in the initial photochemical reaction. The latter is known to be oxidized to 8-oxo-7,8-dihydroguanine (8-oxoGua) under aerobic and anaerobic conditions.[35] Nevertheless, since 8-oxoGua could not be detected as a product of the DNA photodamage, it may be concluded that the base modifications only contribute marginally to the overall DNA damage. It may be pointed out that the lack of detection of 8-oxoGua is due to its decomposition during work up (*i.e.* hydrolysis of DNA with HF–pyridine); however, other groups showed that 8-oxoGua can be determined unambiguously by this experimental protocol.[25] Moreover, oxidative transformations of the DNA bases by an intercalated dye usually require alkaline work-up to be detected as DNA-strand breaks in the pBR322 assay.[45] Since no alkaline work up of the reaction mixture was necessary to detect the strand breaks, we propose that the DNA damage is likely to be introduced by hydrogen abstraction at the 2-deoxyribose moiety of the DNA.[10,46,47]

The fluorimetric titrations reveal that the initial photoreaction is an electron transfer from a guanine base to the excited dye. Thus, we suggest that the resulting radical, *i.e.* the reduced quinolizinium, may be the reactive intermediate which abstracts the hydrogen from the 2-deoxyribose moiety.[48,49] Nevertheless, it should be noted that at the present stage it cannot be excluded that the guanine radical cation itself does abstract the hydrogen.[50] The assumption that the photoinduced electron transfer may be an important stage of the DNA damage, is further corroborated by the assessment of the cleavage sites. The primer extension analysis shows that the majority of cleavage sites is located at guanine-rich regions of the DNA. A significant accumulation is observed at GG rich DNA sequences, which have been shown to be more prone to oxidation than a single guanine base.[32] Thus, it seems that the location of the DNA damage is mostly determined by the oxidation potential of the nucleic base at this site, *i.e.* by the thermodynamics of the electron-transfer process. Furthermore, this observation suggests that the electron transfer is an important step in the DNA photodamage. It should be noted that under the conditions of the primer extension analysis labile modifications (true and oxidized abasic sites, base modifications) may be converted also into DNA strand breaks. Also, even base modifications which do not lead to strand breaks may result in stops during the primer extension. In both cases an overestimation of DNA strand breaks by the primer-extension method need to be considered. But although there is no proof at the present stage that the DNA damage detected by the primer extension analysis is mainly due to single-strand breaks, the result of the agarose-gel assay and the absence of 8-oxoGua as DNA degradation product give some evidence for this proposal.

It should be noted, that at the present stage a definite mechanism for the photoinduced DNA damage in the presence of naphthoquinolizinium derivatives **5a** and **5b** cannot be presented, since some experimental results are not consistent. Most importantly, the fate of the guanine radical cation, which is formed in the initial photoreaction, is unclear. Since we did not observe 8-oxoGua as the initial oxidation product, it may be assumed that the radical cation is reverted to guanine in a "back electron transfer" (BET) with an intermediate, that is formed during the multistep photoreaction. Further studies along these lines are necessary and are presently underway to clarify the mechanism.

In summary we have shown that both naphthoquinolizinium derivatives **5a** and **5b** bind to DNA. Moreover, we demonstrated that the position of the positive charge in the benzo[*a*]anthracene framework has an influence on the binding mode. Furthermore, we clearly demonstrated that an efficient DNA damage may be induced on irradiation of complexes of **5a** or **5b** with DNA. Therefore, these cationic aromatic compounds may serve as a promising platform to design even more efficient DNA binders and photonucleases. It should be noted that most cationic aromatic compounds require additional donor substituents such as amino, hydroxy, or alkoxy groups for strong binding to DNA. In the case of the arenes **5a** and **5b** strong binding is achieved even without these substituents, and it may be proposed that the addition of appropriate substituents will increase the binding constants drastically.

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