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# Targeting abasic site-containing DNA with annelated quinolizinium derivatives: The influence of size, shape and substituents

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The interactions of regular DNA and abasic site-containing DNA (AP-DNA) with quinolizinium (**1a**), the linearly fused benzo[*b*]quinolizinium (**2a**), the angularly fused benzo[*a*]quinolizinium (**3a**), benzo[*c*]quinolizinium (**4a**), and dibenzo[*a,f*]quinolizinium (**5a**) as well as derivatives thereof were studied with photometric and viscosimetric titrations (regular DNA), fluorimetric titrations and thermal DNA denaturation experiments (regular DNA and AP-DNA). Whereas the parent quinolizinium ion (**1a**) and the benzo-annelated derivatives **2a**, **3a** and **4a** exhibit no significant affinity to AP-DNA, additional benzo-annelation in **5a** leads to an increased selective stabilization of AP-DNA by this ligand. Hence, the latter compound represents the first example of a ligand that does not require ancillary substituents for efficient AP-DNA stabilization. In addition, studies of derivatives with varied substitution pattern revealed an impact of substituents on the stabilization of the AP-DNA. We discovered that a chloro substituent affects the propensity of a ligand to bind to AP-DNA in a similar way as the methyl substituent and may be employed complementary to the known methyl effect to increase the binding affinity of a ligand.

## Introduction

The damage of the nucleic bases of DNA is an essential process within a cell that may lead to mutation or cell death.[1] Specifically, oxidized, alkylated or protonated nucleic bases may be eliminated from the DNA either by hydrolysis of the *N*-glycosidic bonds of the nucleotides[2] or by enzymatic repair of damaged nucleobases by the base excision repair (BER) mechanism.[3,4] In each case, abasic sites (apurinic or apyrimidinic sites; AP sites) are formed as mutagenic or carcinogenic DNA lesions.[5] Notably, such AP sites add another type of receptor unit to DNA, that offers binding sites for a variety of guest molecules, specifically DNA-intercalating ligands. Hence the AP site may readily accommodate a DNA intercalator as a substitute for the missing nucleic base. However, other than intercalation into the regularly matched DNA, that is accompanied by unwinding, lengthening, and kinking of the double helix, the *insertion* of a ligand into an AP site does not induce significant changes in the DNA structure.[6] Furthermore, it has been proposed that ligands that bind selectively to AP sites may be employed for analytical and therapeutical purposes.[3,7,8] Along these lines, structural features have been identified that establish a selective association of a given ligand with the abasic position in DNA. Thus, DNA intercalators such as proflavine[9] and berberine,[10] or threading DNA intercalators,[11] have been shown to bind to AP sites. High affinities and selectivities of a ligand towards abasic site-containing DNA (AP-DNA) are accomplished by hydrogen bonding between the external ligand and the nucleic base that is opposite to the abasic site, thus mimicking the regular Watson-Crick base pairing. Typical examples for such ligands are naphthyridine derivatives or similar hetarenes that resemble the structures and hydrogen-bond patterns of the nucleic bases.[12,13] To increase the affinity of the ligand to the DNA, it may be connected through an appropriate linker with an intercalator that provides an additional, non-specific binding interaction with the DNA.[14,15] In addition, it has been demonstrated that aminoalkyl-substituted intercalators also bind selectively to abasic sites.[16,17] In this case the intercalator is accommodated within the abasic site, presumably by $\pi$ stacking, while the protonated aminoalkyl substituent provides an additional stabilization of the ligand–DNA complex by the association in the DNA groove. In a different approach, metalloinsertors with sterically expansive ligands have been developed that exhibit a high selectivity to AP-DNA as compared with regular duplex DNA, because other than the latter the AP-DNA is able to compensate sufficiently the unfavorable energetic contribution from the steric interactions with the ligand.[6,18] Overall, the survey of AP-DNA ligands may lead to the conclusion that in principle, any DNA intercalator is – with appropriate substitution pattern – a suitable ligand for AP-sites. Nevertheless, to

consider an AP-DNA ligand for therapeutic or analytical applications it has to bind with high affinity and selectivity into the abasic position. Therefore, the knowledge of the structural features of an intercalator that govern its interaction with abasic sites may be useful for the rational development of AP-DNA targeting ligands. In this regard, however, the choice of intercalator structures seems rather arbitrary, and to the best of our knowledge there are no systematic studies reported on the influence of variations of size and shape of a given intercalator structure on their AP-DNA binding properties. To gain more information about this particular aspect we were searching for a general structural basis for such systematic studies, with the long-term goal to assess the general design principles for efficient functional AP-DNA ligands. For this purpose, we chose the quinolizinium ion (**1a**) as the key structure, because it has been demonstrated already that the form and dimension of annelated quinolizinium derivatives have a significant influence on the strength and selectivity of their binding interaction with duplex, triplex and quadruplex DNA.[19] In addition, we have demonstrated that the aminoalkyl-substituted derivatives of the linearly fused benzo[*b*]quinolizinium (**2a**) have the propensity to bind in abasic positions. Nevertheless, the interactions of the angularly fused derivatives benzo[*a*]quinolizinium (**3a**), benzo[*c*]quinolizinium (**4a**), and dibenzo[*a*,*f*]quinolizinium (**5a**) (Chart 1) with AP-DNA have not been reported, so far. Hence, in an attempt to further identify the influence of the size and the shape of quinolizinium ions on their association with AP-DNA we examined the interactions of derivatives of the annelated quinolizinium ions **3a**–**5a** with regular DNA and with AP-DNA. Furthermore, derivatives of the benzo[*c*]quinolizinium **4a**–**h** were studied to assess the influence of substituents. In particular, we were interested in the chloro substituent, because it is well known that the chloro substituent may replace the methyl group without changing the steric demand of a compound.[20] Especially considering the known methyl-effect on AP-DNA ligands[13d,13e,21] we were interested to explore the effect of the chloro substituent, because in this particular case any difference or similarity is based exclusively on electronic effect. For comparison, selected derivatives of the parent quinolizinium (**1a**) were also included in this study.

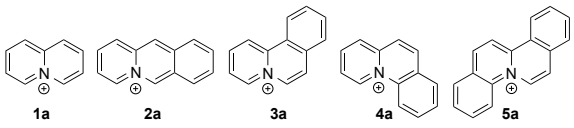
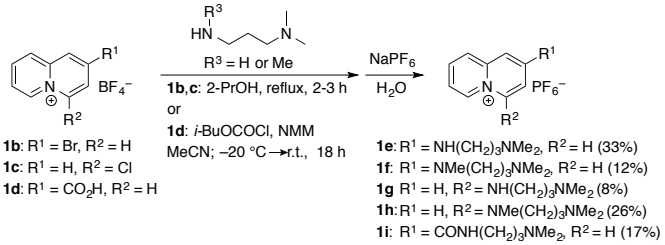



**Chart 1**. Structures of the quinolizinium ion (**1a**) and its benzo- and dibenzo-annelated derivatives **2a**–**5a**.

## Results

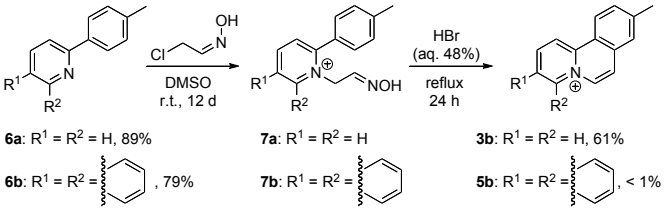
### Synthesis

The parent quinolizinium (**1a**)[22] and the benzo-annelated derivatives **3a**,[23] **4a**,[24] and **5a**[25] were synthesized according to reported protocols.[26] The aminoalkyl-substituted quinolizinium derivatives **1e**–**1g** were obtained by nucleophilic aromatic substitution reactions of the 2-bromoquinolizinium (**1b**) or 4-chloroquinolizinium (**1c**).[27] Thus, the reaction of *N*,*N*-dimethyl-1,3-diaminopropane or *N*,*N*,*N*'-trimethyl-1,3-diaminopropane with **1b** or **1c** in isopropanol gave the quinolizinium derivatives **1e**–**1h**, respectively, that were isolated by ion metathesis with hexafluorophosphate and subsequent crystallization. The yields were rather low (8–33%), as usually observed with nucleophilic substitution reactions at quinolizinium ions.[27,28] The aminoalkyl-substituted quinolizinium-2-carboxamide **1i** was obtained by amidation of quinolizinium-2-carboxylic acid (**1d**)[29] with *N*,*N*-dimethyl-1,3-diaminopropane through a mixed-anhydride that is formed by treatment of the acid with isobutyl chloroformate in acetonitrile in the presence of a mild base (N-methylmorpholine, NMM) (Scheme 1).




**Scheme 1**. Synthesis of aminopropylamino-substituted quinolizinium derivatives **1e**–**1i**.

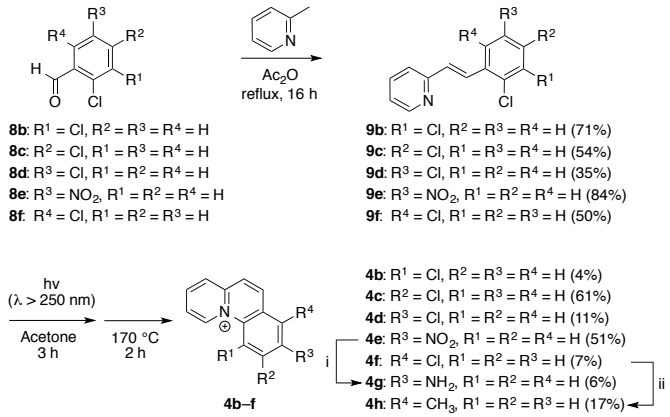
The 9-methylbenzo[*a*]quinolizinium (**3b**) and 9-methyldibenzo[*a*,*f*]quinolizinium (**5b**) were obtained by the cyclodehydration method.[30] For that purpose 2-(4-methylphenyl)pyridine (**6a**) and 2-(4-methylphenyl)quinoline (**6b**) were quaternized by the reaction with chloroacetaldehyde oxime. Without further isolation the resulting pyridinium derivatives **7a** and **7b** were converted to the cyclized products **3b** and **5b** in refluxing aq. HBr (48%) in very low yield.




**Scheme 2**. Synthesis of 9-methylbenzo[*a*]quinolizinium (**3a**) and 9-methyldibenzo[*a*,*f*]quinolizinium (**5b**).

The benzo[*c*]quinolizinium derivatives **4b**–**h** were synthesized according to established protocols (Scheme 3). Thus, the reaction of 2-methylpyridine with the corresponding dichlorobenzaldehyde derivatives **8b**–**f** gave the *trans*-styrylpyridines **9b**–**f** that were irradiated in acetone ($\lambda > 250$ nm) and subsequently heated to 170 °C (except for the case of **9e**) to give the benzo[*c*]quinolizinium derivates **4b**–**f** in low to moderate yields. The products were precipitated and isolated as tetrafluoroborate salts. The 8-nitrobenzo[*c*]quinolizinium (**4e**) was reduced to the 8-aminobenzo[*c*]quinolizinium (**4g**) with $SnCl_2 \times 2H_2O$. The 7-chlorobenzo[*c*]quinolizinium (**4f**) was transformed in low yield to the 7-methyl-substituted derivative

**4h** by a Suzuki coupling with Pd(dppf)$Cl_2$-$CH_2Cl_2$ (dppf = 1,1'-bis(diphenylphosphino)ferrocene) as a catalyst.




**Scheme 3**. Synthesis of benzo[*c*]quinolizinium derivatives **4b–h** (i: $SnCl_2(H_2O)_2$, EtOH, 90 °C, 3.5 h. ii: $MeB(OH)_2$, Pd(dppf)$Cl_2$-$CH_2Cl_2$, KF, DME/$H_2O$/MeOH (2/1/1), 110 °C, 70 h).

All known compounds (**1a–d**, **2a**, **3a**, **4a**, **5a**, **9b–f**) were identified by comparison with literature data. All new compounds (**1e–i**, **3b**, **4b**, **4d**, **4f–h**, **5b**) were characterized by $^1$H-NMR and $^{13}$C-NMR spectroscopy, mass spectrometry and elemental analysis.

### Spectrometric titrations

To examine the general affinity of the quinolizinium derivatives **3–5** to double-stranded DNA, the binding interactions of these ligands with calf thymus DNA (ct DNA) were examined by spectrophotometric titrations. As a general trend, the intensity of the long-wavelength absorption bands decreased significantly upon interaction with DNA, and a red-shifted band developed with increasing DNA concentration, as exemplarily shown for **1f**, **1h**, **3a**, **4a**, and **5b** (Fig. 1). This effect is less pronounced and in some cases even very small with quinolizinium derivatives **1a–i** (Fig. 1A). In most cases, isosbestic points were maintained in the course of the titration. The fitting of the resulting binding isotherms to the neighbor-exclusion model[31] gave the apparent binding constants (Table 1). Among the tested compounds, the dibenzoquinolizinium derivatives **5a** and **5b** have the largest binding constant $K_b$ (**5a**: $1.9 \times 10^5$ $M^{-1}$;[19d] **5b**: $1.5 \times 10^5$ $M^{-1}$), whereas the ones of the monobenzo-annelated quinolizinium derivatives **3a**,**b, 4a–d**, and **4f–h**, are smaller, i.e. in the range of $2.4–6.7 \times 10^4$ $M^{-1}$.

The fluorescence of the benzo- and dibenzo-annelated quinolizinium derivatives was quenched upon addition of ct DNA (cf. ESI). The efficiency of the quenching process was deduced from the plot of the relative emission intensity, $I_0$ / $I$,

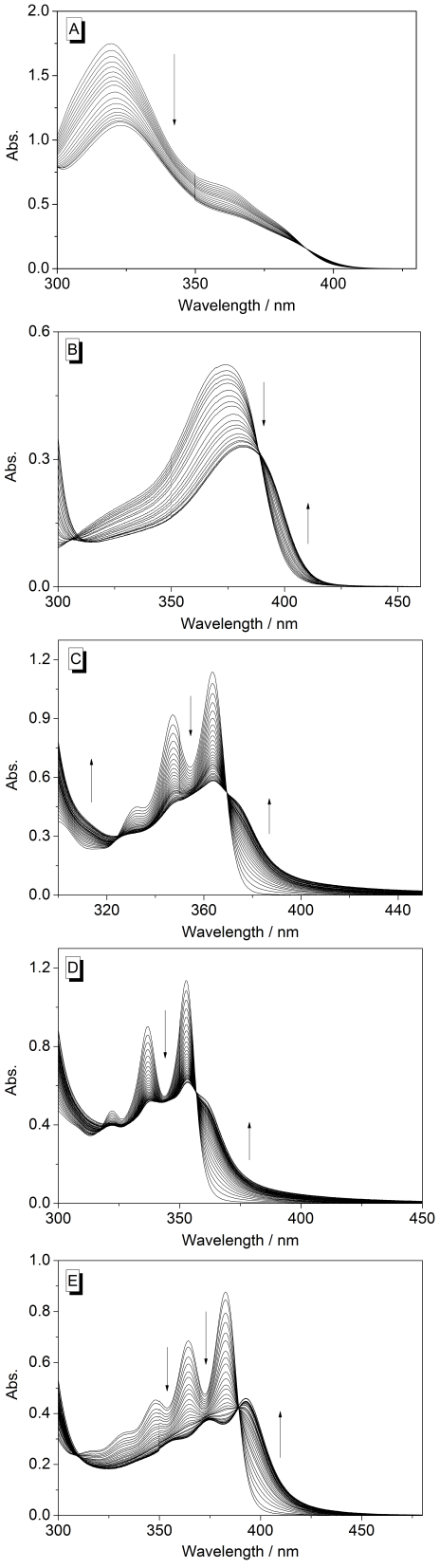



**Fig. 1**. Photometric titration of ct DNA to quinolizinium derivatives **1f** (A), **1h** (B), **3a** (C), **4a** (D), and **5b** (E) in phosphate buffer (**1f**, **3a**, **4a**, **5b**: $c_{Ligand}$ = 0.10 mM, **1h**: $c_{Ligand}$ = 0.05 mM; pH 7).

*versus* the DNA concentration (Fig. 2). According to the resulting Stern-Volmer quenching constants, $K_{SV}$, the quenching of the dibenzoquinolizinium derivatives **5a** and **5b** by ct DNA (**5a**: $1.7 \times 10^5$ $M^{-1}$; **5b**: $2.6 \times 10^5$ $M^{-1}$) is about 10 times more efficient than that of the benzo-annelated quinolizinium derivatives **3** and **4** ($0.3–2.8 \times 10^4$ $M^{-1}$). Notably, the Stern-Volmer constants $K_{SV}$ of the derivatives **3**–**5** are in the same range as the corresponding binding constants with ct DNA, which indicates static quenching, i.e. the radiationless deactivation of the excited quinolizinium mainly takes place within the binding site.

The interactions of the quinolizinium derivatives with AP-DNA were investigated exemplarily by fluorimetric titrations with the double-stranded oligodeoxyribonucleotides **TX** that contains an apurinic site opposite to thymine (Chart 2). To maintain sufficient stability of the AP-DNA strand the regular deoxyribose residue at the AP site was substituted with tetrahydrofuran. The addition of **TX** led to a decrease of emission intensity of all tested compounds **3a**, **3b**, **4a**, **5a** and **5b** (Fig. 3). The data from the fluorimetric titrations were analyzed as binding isotherms that were fitted to the theoretical data of a 1:1 stoichiometry to give the apparent binding constants, $K_b$, according to established protocols (Table 1).[13a] These analyses revealed that the binding constants of the ligands **5a** and **5b** ($1.4 \times 10^5$ $M^{-1}$ and $1.2 \times 10^5$ $M^{-1}$) are larger than the ones of **3a**, **3b**, and **4a** that range from $1.4 \times 10^4$ $M^{-1}$ (**3a**) to $2.5 \times 10^4$ $M^{-1}$ (**3b**).

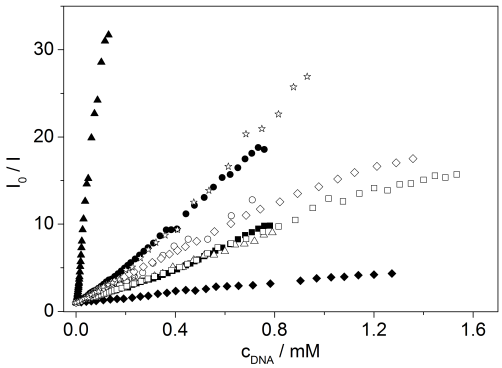



**Fig. 2**. Relative fluorescence intensities of benzoquinolizinium derivatives **3a** (■), **3b** (●), **4a** (△), **4b** (◆), **4c** (○), **4d** (□), **4g** (◇), **4h** (☆), and **5b** (▲) upon addition of ct DNA in phosphate buffer (16 mM, pH = 7.0).

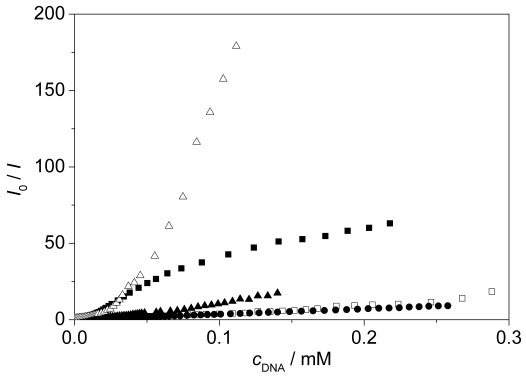
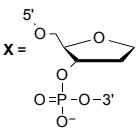



**Fig. 3**. Relative fluorescence intensities of benzoquinolizinium derivatives **3a** (●), **3b** (▲), **4a** (□), **5a** (■), and **5b** (△) upon addition of AP-DNA **TX** in phosphate buffer (38 mM, pH = 7.0).

**NX (N** = C, T, G, A):
ODN 1: GCG-TG**N**-GTG-CG
ODN 3: CGC-AC**X**-CAC-GC




**TA**:
ODN 1T: GCG-TG**T**-GTG-CG
ODN 2A: CGC-AC**A**-CAC-GC
**CG**:
ODN 1C: GCG-TG**C**-GTG-CG
ODN 2G: CGC-AC**G**-GTG-CG

**Chart 2**. Structures of regular DNA **TA** and **CG** and of AP-DNA **CX**, **TX**, **AX**, **GX**.

**Table 1.** Binding parameters of ligands **3**–**5** with DNA.

| | $K_b^{ctDNA}$ / $M^{-1} \times 10^4$ [a] | $n$ [a] | $K_{SV}^{ctDNA}$ / $M^{-1} \times 10^4$ [b] | slope of plot $(\eta/\eta_0)^{1/3}$ *vs* *LDR* ($r^2$) [c] | $K_b^{TX}$ / $M^{-1} \times 10^4$ [d] |
|---|---|---|---|---|---|
| **3a** | 4.0 | 3 | 1.1 | 0.5 (0.97) | 1.4 |
| **3b** | 13 | 4 | 2.4 | 0.5 (0.96) | 2.5 |
| **4a** | 2.7 | 3 | 1.0 | 0.3 (0.98) | 1.9 |
| **4b** | n.d. | n.d. | 0.3 | 0.2 (0.94) | n.d. |
| **4c** | 2.4 | 2 | 1.6 | 0.7 (0.96) | n.d. |
| **4d** | 2.5 | 4 | 1.0 | 0.3 (0.97) | n.d. |
| **4f** | 2.6 | 3 | 1.2 | 0.5 (0.97) | n.d. |
| **4g** | 2.9 | 3 | 1.3 | 0.4 (0.92) | n.d. |
| **4h** | 6.7 | 4 | 2.8 | 0.5 (0.95) | n.d. |
| **5a** | 19 [f] | 3 [f] | 17 [f] | n.d. | 14 |
| **5b** | 15 | 2 | 26 | 0.9 (0.99) | 12 |

[a] Binding constant, $K_b^{ctDNA}$, and binding-site sizes, $n$, (in base pairs), obtained from photometric titrations with ct DNA ($c_{Ligand}$ = 0.10 mM in BPE buffer). [b] Stern-Volmer quenching constant, $K_{SV}^{ctDNA}$, obtained from fluorimetric titrations with ct DNA ($c_{Ligand}$ = 10 μM in BPE buffer). [c] Slope of the best-fit line of a plot of relative viscosity, $(\eta/\eta_0)^{1/3}$, *vs LDR* ($c_{DNA}$ = 1.0 mM in BPE buffer); $r^2$ of the linear fit is given in brackets. [d] Binding constant, $K_b^{TX}$, obtained from fluorimetric titrations with **TX** ($c_{4a}$ = 1.0 μM; $c_{3a/3b/5a/5b}$ = 10 μM in ODN buffer) considering a 1:1 stoichiometry. [e] n.d. = not determined. [f] Ref. 19d.

### Viscometric titrations

For the determination of the binding mode with double-stranded DNA, viscometric titrations of the ligands **1a**, **3a**,**b**, **4a**–**4d**, **4f**–**4h**, and **5a**,**b** to ct DNA were performed and compared with the data obtained with the known intercalator ethidium bromide under identical conditions. Thus, the change of the specific viscosity $(\eta/\eta_0)^{1/3}$ of the aqueous DNA solution upon addition of the ligands was determined and plotted *versus* the *LDR* as shown exemplarily for the ligands **4b**, **4c** and **4f** (Fig. 4). The data were analyzed by linear regression, and the slope of the best-fit line was determined as a measure of the extent of DNA stiffening upon intercalation (Table 1). The parent quinolizinium (**1a**) has essentially no influence on the viscosity of the DNA solution (slope: –0.1), and the benzo[*c*]quinolizinium derivatives **4a**, **4b**, and **4d** induce only a small enhancement of the viscosity (slope: 0.2–0.3). In contrast, the viscosity of the solution increases to a greater extent upon addition of all other tested ligands (slope = 0.4–0.7). Nevertheless, in the presence of ethidium bromide a much stronger effect was observed (slope = 0.8).

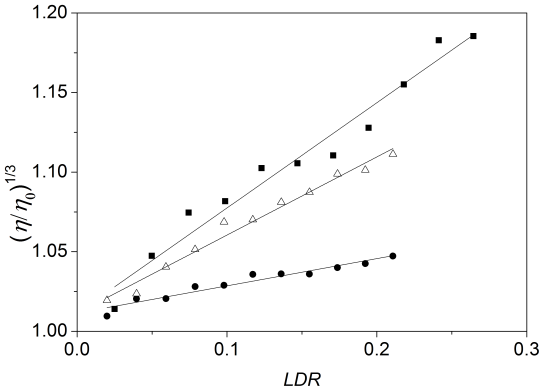



**Fig. 4.** The relative specific viscosity of an aqueous solution of ct DNA in the presence of the quinolizinium derivatives **4b** (●), **4c** (■) and **4f** (△) represented as plot of relative viscosity, $(\eta/\eta_0)^{1/3}$, *vs* ligand-DNA ratio, *LDR*.

### DNA denaturation studies

Because the induced shift of the DNA melting temperature ($\Delta T_m$) in the presence of a ligand is directly related to the stabilization or destabilization of the duplex DNA toward dissociation, thermal DNA denaturation experiments represent a powerful tool to assess ligand-DNA interactions.[32] Thus, the interaction of the quinolizinium derivatives **1–5** with the AP-containing undecamers **CX**, **TX**, **GX**, **AX** (Chart 2) and of the regular oligonucleotides **TA** and **CG** were determined by photometric monitoring of the DNA melting at different *LDR* (Table 2). The parent quinolizinium **1a** does neither stabilize the regular DNA **TA** or **CG**, nor the AP-DNA **CX**, **TX**, **GX**, **AX** ($\Delta T_m$ < 1 °C, resp.); and the same was shown exemplarily for the chloro-substituted derivative **1c** and AP-DNA **TX**. The functionalization with 2- or 4-aminopropylamino substituents resulted in different effects on the DNA melting temperature, depending on the type and position of the substituent and on the particular DNA form. Thus, the substituted derivatives **1e–i** induce no or only a very small shift of the DNA melting temperature, $\Delta T_m$, of regular DNA **TA** (–0.6–0.4 °C) or AP-DNA **TX** (–1.4–0.3 °C), **GX** (0.3–2.9 °C), and **AX** (0.0–2.3 °C), whereas the AP-site containing **CX** was significantly stabilized by the ligands **1e** and **1h**, as indicated by higher melting temperatures (**1e**: $\Delta T_m$ = 6.2 °C; **1h**: $\Delta T_m$ = 6.0 °C). At the same time, the derivatives **1f** and **1g** stabilize **CX** to a significantly lesser extent ($\Delta T_m$ = 3.5 and 1.0 °C).

The benzo[*a*]quinolizinium derivatives **3a** and **3b** have essentially no effect on the melting temperature of regular DNA ($\Delta T_m$ < 1 °C, resp.), and also the AP-DNA is only slightly stabilized in the presence of these ligands. Specifically, the largest value of $\Delta T_m$ induced by derivative **3a** is just 3.6 °C for **TX**, while the shifts of $T_m$ of the other AP-DNA forms is even smaller. Nevertheless, it should be noted that the introduction of a methyl group in **3b** induces a significantly larger increase of the melting temperature of the AP-DNA as compared to **3a**, which is especially pronounced in the case of the apurinic DNA (**3a**: $\Delta T_m$ = 2.2–3.6 °C; **3b**: $\Delta T_m$ = 6.2–6.5 °C). The benzo[*b*]quinolizinium ion (**2a**) does not have a stabilizing effect on regular DNA **TA** and **CG** or apyrimidinic DNA **AX** and **GX**; however, the association of **2a** with the AP-DNA **CX** and **TX** resulted in a stabilization ($\Delta T_m$ = 4.4–5.1 °C; *LDR* = 2). The parent benzo[*c*]quinolizinium **4a** does not stabilize regular DNA or AP-DNA ($\Delta T_m$ < 1.6 °C). Whereas the introduction of a chloro-substituent in 10-position in derivative **4b** did not change the DNA-stabilizing properties of the ligand, the addition of 7-, 8-, or 9-chlorobenzo[*c*]quinolizinium **4c**, **4d** or **4f** to the AP-DNA **CX** or **TX** induced a small, but significant shift of the melting temperatures ($\Delta T_m$ = 5.1–5.7 °C; *LDR* = 2).

**Table 2**. Stabilization of regular and AP site-containing duplex DNA by quinolizinium derivatives **1–5** as determined from the thermal DNA denaturation studies.

| | Induced $\Delta T_m$ / °C at *LDR* = 0.5 and 2 [a] | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **CX** | | **TX** | | **AX** | | **GX** | | **TA** | | **CG** | |
| *LDR*[b] | 0.5 | 2 | 0.5 | 2 | 0.5 | 2 | 0.5 | 2 | 0.5 | 2 | 0.5 | 2 |
| **1a** | –1.6 | 0.5 | –0.5 | 0.0 | 0.0 | –0.9 | –0.3 | –0.1 | n.d. | –0,9 | 0.0 | –0,1 |
| **1c** | n.d. | n.d. | n.d. | 0.4 | n.d. | n.d. | n.d. | n.d. | n.d. | 0.4 | n.d. | n.d. |
| **1e** | n.d. | 6.2 | n.d. | –1.4 | n.d. | 2.3 | n.d. | 2.9 | n.d. | 0.3 | n.d. | n.d. |
| **1f** | n.d. | 3.5 | n.d. | 0.2 | n.d. | 0.5 | n.d. | 2.4 | n.d. | 0.4 | n.d. | n.d. |
| **1g** | n.d. | 1.0 | n.d. | –0.2 | n.d. | 0.0 | n.d. | 0.1 | n.d. | 0.3 | n.d. | n.d. |
| **1h** | n.d. | 6.0 | n.d. | 0.3 | n.d. | 2.0 | n.d. | 0.3 | n.d. | –0.6 | n.d. | n.d. |
| **1i** | n.d. | 2.4 | n.d. | –1.0 | –0.9 | 0.3 | –0.5 | 1.1 | –0.6 | 1.2 | 1.4 | 1.9 |
| **2a** | 2.4 | 5.1 | 2.1 | 4.4 | 0.0 | –0.1 | 0.6 | –0.9 | 0.1 | 0.3 | 0.2 | 0.4 |
| **3a** | 1.2 | 2.2 | 1.4 | 3.6 | –0.7 | 1.2 | 0.6 | 1.8 | –0.3 | 0.7 | 0.0 | 0.1 |
| **3b** | 3.6 | 6.5 | 3.8 | 6.2 | –2.1 | –0.5 | 1.4 | 3.1 | 0.0 | 0.4 | 0.3 | 1.8 |
| **4a** | 0.8 | 1.5 | 0.2 | 1.1 | –0.3 | 1.5 | 0.4 | 1.0 | –0.1 | 0.1 | 0.1 | 0.7 |
| **4b** | –0.5 | 0.4 | –0.3 | –0.1 | 0.3 | 0.2 | –0.1 | 1.5 | 0.3 | 0.6 | 0.1 | 0.2 |
| **4c** | 2.6 | 5.5 | 3.1 | 5.4 | 0.6 | 0.1 | 0.4 | 1.1 | 0.1 | 0.5 | 0.0 | 0.2 |
| **4d** | 2.8 | 5.6 | 2.1 | 5.7 | –0.3 | 1.1 | 0.6 | 3.1 | 1.0 | 1.7 | 0.1 | 0.4 |
| **4g** | 3.5 | 6.9 | 4.1 | 7.0 | –0.9 | 2.8 | 2.0 | 4.1 | 0.0 | 1.2 | 0.2 | 0.6 |
| **4f** | 2.7 | 5.5 | 2.6 | 5.1 | –0.7 | –0.7 | 0.7 | 0.8 | 0.1 | 0.3 | 0.3 | 1.4 |
| **4h** | 4.2 | 7.5 | 3.1 | 6.9 | –0.3 | 3.3 | 1.6 | 4.8 | 0.1 | 0.6 | 0.0 | 0.6 |
| **5a** | 6.2 | 11.8 | 8.2 | 11.3 | 3.9 | 7.6 | 3.7 | 7.9 | 0.6 | 2.5 | 0.9 | 2.4 |
| **5b** | 10.3 | 16.4 | 13.1 | 15.6 | 3.9 | 9.1 | 4.3 | 9.6 | 1.0 | 2.7 | 0.9 | 2.8 |

[a] $\Delta T_m$: Shift of the DNA melting temperature in the presence of the ligand; *LDR* = ligand-DNA ratio; $c_{DNA}$ = 5.0 μM in aqueous phosphate buffer, $[Na^+]$ = 38.1 mM; estimated error ± 0.5 °C, n.d. = not determined. [b] *LDR* values refer to the molar concentration of the oligonucleotide duplexes.

The melting temperatures of **GX**, **AX**, **TA**, and **CG**, however, remain essentially unchanged in the presence of these ligands. The $\Delta T_m$ shifts induced by the 7-methyl-substituted benzo[*c*]quinolizinium are just a bit larger than the ones of the 7-chlorobenzo[*c*]quinolizinium (**4f**). Also, the 8-aminoquinolizinium (**4g**) induces only slightly larger shifts of the DNA melting temperatures than the 8-chloroquinolizinium (**4d**). As compared to the benzo-annelated quinolizinium derivatives **2**–**4** the addition of the dibenzo[*a*,*f*]quinolizinium ions **5a** and **5b** to AP-DNA leads to a more effective stabilization of the duplex structure. Hence, the melting temperatures, $T_m$, of **TX** and **AX** increase by more than 10 °C, whereas the ones of the apyrimidinic AP-DNA increase to a slightly lesser extent (e.g.: **5a**: $\Delta T_m^{CX}$ = 11.8 °C; $\Delta T_m^{GX}$ = 7.6 °C; *LDR* = 2). Again, the introduction of one additional methyl group into the ligand leads to a more pronounced shift of the DNA melting temperature as shown with the methyl-substituted derivative **5b** ($\Delta T_m^{CX}$ = 16.4 °C; $\Delta T_m^{GX}$ = 9.1 °C; *LDR* = 2) . However, in the case of **5a** and **5b** even the regular duplex **TA** and **CG** is stabilized at *LDR* = 2 ($\Delta T_m$ = 2.4–2.8 °C).

## Discussion

### Association with regularly paired DNA

It was demonstrated that the benzo-annelated quinolizinium derivatives bind to double-stranded DNA, which is in agreement with the known DNA-binding properties of quinolizinium derivatives.[17] Specifically, the hypochromic effect and the red shift during photometric titrations are characteristic of DNA-binding ligands.[33] In addition, the isosbestic points indicate that the complexation proceeds with one main binding mode. At the same time, the lack of isosbestic points or the slight fading thereof during titration reveals a marginal extent of heteregeneous binding of the respective ligand in few cases. Along these lines, it was shown by viscometric titrations that most of the tested quinolizinium derivatives intercalate into DNA as indicated by the increasing viscosity of the DNA solution upon ligand-DNA association (Figure 4). Such a behavior usually indicates an intercalative binding mode, because this interaction leads to a stiffening and lengthening of the DNA biomacromolecule and in turn to an increased viscosity of the solution;[34] as supported by the direct comparison with the known intercalator ethidium bromide.

The association of the quinolizinium derivatives with DNA was further demonstrated by fluorimetric titrations that revealed a quenching of the DNA-bound ligand, most likely caused by a photoinduced electron-transfer reaction between the excited quinolizinium and the nucleic bases.[35]

Overall, the DNA-binding constants of the derivatives **3**–**5** (Table 1) are comparable to those of known organic intercalators with resembling size and substitution pattern.[36] Most notably, the dibenzo-annelated derivatives **5a** and **5b** bind significantly stronger to ct DNA than the monobenzo-annelated derivatives. This observation is in agreement with previous studies on ligand **5a**[19d] and resembling tetracyclic naphthoquinolizinium derivatives[37] that have revealed a significant gain of DNA-affinity of a given quinolizinium-type ligand with an increasing size of the π system of the ligand, as long as the shape of the ligand fits the binding site appropriately.

### Interaction with abasic sites

The main objective of this work was to assess the propensity of quinolizinium derivatives to bind to abasic sites and to identify structural parameters that govern the strength and selectivity of this association.

Firstly, it is obvious from the collected data that the parent quinolizinium (**1a**) does not bind measurably to abasic sites as shown exemplarily with the compounds **1a** and **1c**. This result was somewhat surprising because several bicyclic hetarenes are known that bind to and stabilize abasic site-containing DNA.[13,21] Moreover, it has been proposed that the bicyclic indole fragement of the AP-DNA damaging tripeptide Lys-Trp-Lys acts as binding unit in the abasic site.[38] However, in those cases additional binding interactions assist the accommodation of the ligand in the abasic position; namely hydrogen bonding of the ligand with the nucleic base opposite to the abasic site, or groove binding by additional amino-alkyl substituents. Therefore, we tested whether the affinity of the quinolizinium to AP-DNA may be increased by functionalization with amino-alkyl substituents as in **1e**–**i**. Although a tendency of increasing stabilization of AP-DNA with aminoalkyl-substitution of the quinolizinium ligand may be deduced from the corresponding $\Delta T_m$ values, it should be noted that only the AP-DNA **CX** is considerably stabilized by the derivatives **1e**, **1f**, **1h** and **1i** ($\Delta T_m$ = 2.4–6.2 °C), and **GX** only to some extent by **1e** and **1f** ($\Delta T_m$ = 2.4–2.9 °C). At the same time, the other AP-DNA is not significantly more stabilized as compared to **1a**. Apparently, the position of the aminoalkyl group at the quinolizinium core as well as the substituents at the "internal" amino funtionality (secondary *vs* tertiary) have an impact on the stabilizing effect of the ligand on the particular DNA form, but the present data do not allow a structure-based explanation of this observation.

The fluorimetric titrations and thermal DNA-denaturation experiments clearly indicate the association of the parent benzo-annelated quinolizinium derivatives **2a**, **3a** and **4a** with AP-DNA. In almost all cases the AP-DNA with apurinic sites is more stabilized by the ligands **2a**, **3a** and **4a** than the apyrimidinic DNA, presumably because the ligands experience more steric repulsion in the smaller apyrimidinic binding site. The exemplary binding constants are in a typical range ($10^4$ $M^{-1}$) of DNA intercalators whose association is mainly supported by dispersion interactions,[39] i.e. with no further specific attractive interactions with the DNA. For comparison, binding constants of AP-DNA ligands that bind also by hydrogen bonding are usually two orders of magnitude larger.[13]

As a general trend, the angularly annelated compound **2a** stabilizes the AP-DNA slightly more than the angular derivatives **3a** and **4a**. This result may be explained by a better

fit of the linear ligand into the abasic site as opposed to the angular ligands, that may interfere with the DNA backbone because of their larger lateral steric demand. At the same time, the introduction of an additional annelated benzene ring in the S-shaped azoniachrysene **5a** results in a higher affinity to AP-DNA, as shown by larger binding constants ($10^5$ $M^{-1}$) and significantly larger induced shifts of the DNA melting temperatures ($\Delta T_m$ = 11–12 °C). These observations are in agreement with preceding experimental and theoretical data, which have provided evidence that the ligand **5a** binds to regular DNA in such a way that one naphthalene part of the ligand intercalates between two base pairs, whereas the other naphthalene unit points inside the groove to gain additional attractive dispersive van-der-Waals interactions.[19d] Most likely, the ligands **5a** and **5b** bind to AP-DNA with a similar binding mode; however, with a slightly different orientation of the ligand within the abasic site, because the ligand occupies the place of the missing base instead of being embedded between the two base pairs in regular DNA. It should be noted, however, that the ligands **5a** and **5b** should experience the same steric interactions within the abasic position as the angular benzoquinolizinium derivatives **3a** and **4a**. Presumably, this latter unfavorable interaction is compensated by the additional accommodation of one part of the ligand in the groove area.

In previous work it has been shown that the introduction of a methyl substituent increases the binding affinity of a ligand toward AP-DNA,[21] which corresponds with the well-established methyl effect of host-guest systems in pharmaceutical chemistry[40] and medicinal chemistry.[41] Upon association of a ligand with AP-DNA the methyl group introduces a favorable hydrophobic effect. Furthermore, the methyl effect operates by a weak, but significant electron donation into the π system that leads to increased stacking interactions. Notably, our studies on quinolizinium derivatives confirmed the methyl effect, because the methyl-substituted derivatives **3b**, **4h**, and **5b** induce a larger shift of DNA melting temperature than the parent compounds **3a**, **4a**, and **5a** (Table 2). To be emphasized is the observation that the combination of benzo-annelation and methyl effect in derivative **5b** induces $\Delta T_m$ shifts for AP-DNA of up to 16 °C, that are unusually large for otherwise non-functionalized hetarene ligands.

As quinolizinium derivatives bind already to regularly paired duplex DNA, it needs to be evaluated whether the ligands exhibit selective interactions with either the regular DNA or the AP-DNA. For that purpose, the selectivity of the ligands for the abasic site may be expressed by their different effect on the melting temperatures of the two DNA forms, as quantified by the $\Delta\Delta T_m$ values that are defined as $\Delta\Delta T_m = \Delta T_m$(AP-DNA) − $\Delta T_m$(regularly matched DNA)[42] (Table 3). These data revealed no or only a small selectivity of the parent benzo[*c*]quinolizinium derivatives **3a** ($\Delta\Delta T_m$ < 3.0 °C) and **4a** ($\Delta\Delta T_m$ < 1.5 °C). From the parent compounds the linear benzo[*b*]quinolizinium **2a** displays the most pronounced, but still marginal differentiation between regular DNA and AP-DNA, especially toward apurinic sites ($\Delta\Delta T_m^{CX}$ = 4.7 °C; $\Delta\Delta T_m^{TX}$ = 4.1 °C, at *LDR* = 2). Thus, the latter has properties that are similar to other cationic DNA-intercalators, such as ethidium bromide,[42] that also exhibit small, but significant selectivity toward AP-DNA. It should be noted, however, that no additional substituent effects contribute to the overall binding of the parent compounds **2a**, **3a** and **4a**. Thus, the data presented herein illustrate the intrinsic ligand properties that govern the affinity and selectivity of a ligand toward AP-DNA.

**Table 3.** Selectivity of DNA stabilization of the ligands **1**–**5**.

| | $\Delta\Delta T_m$ [a] / °C at *LDR* = 0.5 and 2 | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **CX** | | **TX** | | **AX** | | **GX** | |
| *LDR* | 0.5 | 2 | 0.5 | 2 | 0.5 | 2 | 0.5 | 2 |
| **1a** | –1.6 | 0.6 | –0.3 | 0.9 | 0.2 | 0.0 | –0.3 | 0.0 |
| **2a** | 2.2 | 4.7 | 2.0 | 4.1 | -0.1 | -0.2 | 0.4 | 0.5 |
| **3a** | 1.2 | 2.1 | 1.7 | 2.9 | -0.4 | 0.5 | 0.6 | 1.7 |
| **3b** | 3.3 | 4.7 | 3.8 | 5.8 | -2.1 | –0.1 | 1.1 | 1.3 |
| **4a** | 0.7 | 0.8 | 0.3 | 1.0 | –0.2 | 1.4 | 0.3 | 0.3 |
| **4b** | -0.6 | 0.2 | –0.6 | –0.7 | 0.0 | –0.4 | –0.2 | 1.3 |
| **4c** | 2.5 | 5.0 | 3.0 | 4.9 | 0.5 | –0.4 | 0.4 | 0.9 |
| **4d** | 2.7 | 5.3 | 1.1 | 4.0 | -1.3 | –0.6 | 0.5 | 2.7 |
| **4f** | 3.3 | 6.3 | 3.8 | 5.8 | -0.9 | 1.6 | 1.8 | 3.5 |
| **4g** | 2.4 | 4.1 | 2.5 | 4.8 | -0.8 | –1.0 | 0.4 | –0.6 |
| **4h** | 4.2 | 6.9 | 3.0 | 6.0 | -0.4 | 2.6 | 1.6 | 4.2 |
| **5a** | 5.3 | 9.4 | 7.6 | 8.8 | 3.3 | 5.1 | 2.8 | 5.5 |
| **5b** | 9.4 | 13.8 | 12.1 | 12.9 | 2.9 | 6.4 | 3.4 | 6.8 |

[a] $\Delta\Delta T_m = \Delta T_m$(NX) − $\Delta T_m$(TA or CG); N = C, T, A, G; conditions cf. Table 2.

The small series of substituted benzo[*c*]quinolizinium derivatives was examined to explore a possible effect of the chloro substituent. It has been observed already that the introduction of a methyl group into a ligand increases its affinity to AP-DNA (see above), and we demonstrated in this work that this effect also operates in quinolizinium derivatives. Along these lines, we reasoned that a chloro substituent may have a similar effect. The chloro and methyl group have essentially the same steric demand,[43] and like the methyl group the chloro substituent should increase the hydrophobic properties of the ligand. In addition, the chloro atom leads to a slightly changed dipole of the ligand, thus increasing dipole-dipole interactions in the binding site, and it may even operate as hydrogen-bond acceptor. Indeed, the chloro-substituted derivatives **4c**, **4d** and **4f** stabilize especially the apurinic AP-DNA more than the parent compound (e.g. **4a**: $\Delta T_m^{TX}$ = 1.1 °C; **4c**: $\Delta T_m^{TX}$ = 5.4 °C; **4d**: $\Delta T_m^{TX}$ = 5.7 °C **4f**: $\Delta T_m^{TX}$ = 5.1 °C; *LDR* = 2), whereas this effect is smaller with the apyrimidinic AP-DNA. As an exception, the 10-chlorobenzo[*c*]quinolizinium (**4b**) exhibits basically no stabilizing effect toward AP-DNA, most likely because this ligand is significantly twisted out of planarity due to the repulsion between the chloro substituent and the hydrogen atom at C1. Most notably, the 9-chlorobenzo[*c*]quinolizinium (**4f**) has only a slightly smaller effect on the melting temperatures of the employed AP-DNA strands as the 9-methylbenzo[*c*]quinolizinium (**4h**). The same trend was observed for the selectivity of the binding as indicated by resembling $\Delta\Delta T_m$ values (e.g. **4c**: $\Delta\Delta T_m^{TX}$ = 4.9 °C; **4d**: $\Delta\Delta T_m^{TX}$ = 4.0 °C; **4f**: $\Delta\Delta T_m^{TX}$ = 5.8 °C **4h**: $\Delta\Delta T_m^{TX}$ = 6.0 °C;

*LDR* = 2; Table 3). Overall, these results indicate that a chloro substituent affects the ability of a ligand to bind to AP-DNA in a similar way as the methyl substituent.

To determine exemplarily the effect of a strong electron-donating substituent we examined also the amino-substituted derivative **4g**. The electron-donating character of the amino group in **4g** is clearly indicated by the strong red shift of the absorption ($\lambda_{Abs}$ = 375 nm) and emission bands ($\lambda_{Fl}$ = 538 nm) relative to the parent compound **4a** ($\lambda_{Abs}$ = 363 nm, $\lambda_{Fl}$ = 388 nm). The induced shifts $\Delta T_m$ as well as the $\Delta\Delta T_m$ values obtained with this ligand are just somewhat larger (e.g. $\Delta T_m^{\mathbf{TX}}$ = 7.0 °C; $\Delta\Delta T_m^{\mathbf{TX}}$ = 4.8 °C; *LDR* = 2) than the ones recorded with the chloro- or methyl-substituted ligands such as **4d** or **4h**. These results demonstrate that the introduction of donating substituents may also be employed to increase the affinity and selectivity of an AP-DNA ligand. At the same time, it should be noted that the resulting effect is not much stronger than the one observed with chloro- or methyl substituents, so all of these substituents may be employed complementarily as affinity-increasing structural element.

## Conclusions

In summary, we have assessed the structural features that determine the association of ligands with AP-DNA by means of quinolizinium derivatives as the DNA-binding source. As has been demonstrated with studies on triplex and quadruplex DNA[19] the quinolizinium ion serves as a useful reference system to explore the effect of substituents and of the size and shape of the ligand on the DNA-binding properties. As expected the methyl effect and/or the annelation with an additional benzene unit increase the ability of the ligand to stabilize the DNA upon association, respectively, whereupon the annelation effect depends on the shape of the polycyclic system (angular *vs* linear annelation). Most notably, we discovered that the chloro-substituent may be employed complementary to the methyl group to increase the binding affinity. Although we observed increased selectivity of a ligand towards AP-DNA due to the above-given effect, it should be stressed that such as in the case of **5a** these ligands are already very effective binders to regular DNA and even exhibit selectivity towards other DNA forms such as triplex DNA. Therefore, additional variations of the substitution pattern, beyond the ones presented herein, are necessary to accomplish higher selectivities. For future studies in this direction the quinolizinium ion, especially considering the results obtained so far, may represent a promising starting point. Overall these results may be employed for the development of ligands that target abasic site-containing DNA. Specifically, our findings may guide the choice of the size and shape of the intercalating part of the ligand and the strategical selection of substituents that increase the DNA-stabilizing properties.

## Experimental

### Equipment

Absorption spectroscopy: Varian Cary 100 Bio Spectrophotometer. Emission spectroscopy: Varian Cary Eclipse. NMR spectroscopy: Bruker Avance 400, Varian VNMR-S 600.

### Materials

All buffer solutions were prepared from purified water (resistivity 18 MΩ $cm^{-1}$) and biochemistry-grade chemicals. The buffer solutions were filtered through a PVDF membrane filter (pore size 0.45 µm) prior to use. BPE buffer: 6.0 mM $Na_2HPO_4$, 2.0 mM $NaH_2PO_4$, 1.0 mM $Na_2EDTA$; total $Na^+$ concentration 16.0 mM; pH 7.0. ODN buffer: 6.1 mM $Na_2HPO_4$, 3.9 mM $NaH_2PO_4$, 1.0 mM $Na_2EDTA$, 20 mM NaCl; total $Na^+$ concentration 38.1 mM; pH 7.0. Calf thymus DNA (type I; highly polymerized sodium salt; $\varepsilon$ = 12824 $cm^{-1}$ $M^{-1}$) was purchased from Sigma (St. Louis, MO, USA). Oligodeoxyribonucleotides 5´-GCGTGNGTGCG-3´ (N = G, C, A, or T), 5´-CGCACNCACGC-3´ (N = G, A, or tetrahydrofuran spacer); purification: HPLC; quality control: MALDI-TOF; synthesis scale: 1.0 µmol) were purchased from Metabion international (Martinsried, Germany). Melting temperatures of the corresponding duplex DNA ($c_{DNA}$ = 5.0 µM) were determined in ODN buffer. **CX**: $T_m$ = 25.8 °C; **TX**: $T_m$ = 26.4 °C; **AX**: $T_m$ = 32.2 °C, **GX**: $T_m$ = 32.9 °C; **TA:** $T_m$ = 55.1 °C; **CG**: $T_m$ = 58.1 °C. The melting temperatures of the double strands **TA** and **TX** are consistent with the reported ones under identical conditions.[16b]

### Methods

The spectrophotometric and spectrofluorimetric titrations with DNA, and the thermal DNA denaturation studies were performed according to reported protocols.[16b,19c] Binding constants were determined from spectrofluorimetric titrations. The binding constants of the ligands with ct DNA were determined by fitting of the experimental data from the fluorimetric DNA titration to the theoretical model according to the published procedure.[13a,31,44]

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## Notes and references

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† Electronic Supplementary Information (ESI) available: Synthesis of quinolizinium derivatives; photometric and fluorimetric titration spectra, DNA melting curves. See DOI: 10.1039/b000000x/


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