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Synthesis of DNA with Phenanthridinium as an Artificial DNA Base

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A phenanthridinium-containing DNA building block was synthesized as an ethidium nucleoside analogue starting from 3,8-diamino-6-phenyl-phenanthridine. Using this building block, oligonucleotides bearing the phenanthridinium moiety as an artificial DNA base were prepared via automated solid-phase phosphoramidite chemistry. The modified phenanthridinium-containing DNA duplexes were characterized by UV/vis absorption spectroscopy (including the melting behavior), CD spectroscopy, and steady-state fluorescence spectroscopy. These experiments reveal the expected similarity of the synthetic phenanthridinium moiety with noncovalently bound ethidium. More importantly, the results show clearly that the artificial phenanthridinium base is intercalated within the DNA base stack. The counterbase as part of the complementary strand seems to have only a minor influence on the intercalation properties of the phenanthridinium moiety.

Introduction

One of the interesting properties of planar polycyclic aromatic molecules is their ability to intercalate between two adjacent base pairs in duplex DNA, which was first proposed by Lerman.[1] Among such intercalators, the highly polar or even charged systems represent the most potent ones with respect to noncovalent stacking interactions. Thus, it is believed that the electrostatic energy plays an important role in the intercalation process.[2] 3,8-Diamino-5-ethyl-6-phenylphenanthridinium ("ethidium") is one of the well-known positively charged intercalators for duplex DNA and has been widely used as a fluorescent staining agent due to the significant fluorescence enhancement that it exhibits upon intercalation.[3] Ethidium and its derivatives are also well-known as potent trypanocidal drugs.[4] To evaluate the specific binding features of ethidium such as affinity, base sequence specifity, and fluorescence properties, different experimental approaches were tried in the past. Among these are the synthesis and application of mono- or bifunctional DNA intercalators bearing ethidium derivatives,[5] theoretical calculations,[2] and the application of the photochemical reactivity beyond the absorption of the nucleic acids (>300 nm).[6,7] Ethidium plays also an important role with respect to the studies of photoinduced processes in DNA. In most of these experiments, noncovalent bound ethidium was used to photoinitiate charge transfer[8] or energy transfer processes.[9] To investigate the distance dependence of charge transfer in DNA, Barton and coworkers have used modified oligonucleotides containing ethidium covalently attached to the 5′-end via an alkyl linker.[10]

We chose a new approach in order to start the study of the stacking interactions of ethidium and the corre-

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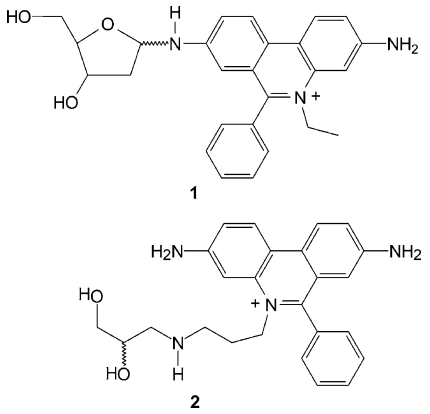
SCHEME 1. Ethidium Nucleoside 1 and Ethidium Nucleoside Analogue 2



sponding photoinitiated charge transfer processes. In our DNA system, the charged phenanthridinium heterocycle of ethidium is incorporated as an artificial DNA base at specific sites in duplex DNA. Herein, we want to present the synthetic work that is necessary for this DNA system, including the synthesis of the corresponding DNA building block as well as the phenanthridinium-modified oligonucleotides. Furthermore, we examined the spectroscopic properties of two different sets of modified DNA duplexes in order to elucidate that the phenanthridinium heterocycle is in an intercalated position similar to noncovalently bound ethidium.

Results and Discussion

Design of the DNA Building Block. Early steps in the synthesis of ethidium and its derivatives were made by Walls et al.,[11] Watkins et al.,[12] and Berg et al.[13] More recently, work by Schacht et al. showed clearly that the amino group in position C-3 of ethidium is deactivated in comparison to the C-8 amino group due to the mesomeric stabilization of the positive charge (Scheme 1).[14] Accordingly, we synthesized the peracetylated form of the ethidium nucleoside 8-(2′-deoxy-D-ribofuranosyl)-3-acetamido-5-ethyl-6-phenyl-phenanthridinium (**1**) via a regioselective modification of the C-8 amino group.[15] The nucleoside **1** contains the ethidium moiety glycosidically linked to 2′-deoxyribofuranose. Glycosides with ethidium as an aglycon, e.g., 3- and 8-*N*-glucuronosyl-ethidium, have been identified previously as the major metabolites of ethidium.[16] Although a significant mesomeric stabilization exists within the ethidium aromatic system, the glycosylamine **1** does not exhibit a stability toward basic hydrolysis that is high enough to guarantee the success of subsequent incorporation into oligonucleotides. Thus, we decided to replace the 2′-deoxyribofuranoside moiety with an acyclic linker system that is tethered to the N-5 position of the phenanthridinium system. Avoiding the labile glycosidic bond, the ethidium nucleoside analogue **2** should be suitable for the preparation of DNA building blocks and phenanthridinium−DNA conjugates via automated phosphoramidite chemistry. To our knowledge, there was only one attempt in the past to incorporate the phenanthridinium heterocycle into oligonucleotides via phosphoramidite chemistry.[17]

Synthesis of Phenanthridinium-Containing Oligonucleotides. The synthetic procedure begins with the protection of the two exocyclic amino functions in position C-3 and C-8 of 3,8-diamino-6-phenyl-phenanthridine (**3**) which is the commercially available starting material. The DNA base heterocycles are typically protected by amide groups.[18] Ethidium or phenanthridinium derivatives carrying either acetyl or isobutyroyl amide groups on the exocyclic amino functions cannot be deprotected by aqueous base hydrolysis as applied during the typical DNA workup conditions.[19] On the other hand, phenanthridine derivatives bearing more labile amide groups such as the trifluoroacetyl group do not exhibit sufficient stability during the subsequent alkylation step at position N-5.[19] Hence, the allyloxycarbonyl (alloc) group[19] was chosen as a suitable protecting group for following reasons. (i) Alloc groups are orthogonal to all other applied protecting groups during the phosphoramidite and subsequent oligonucleotide synthesis. (ii) Alloc groups can be cleaved selectively using Pd(0) catalysis after oligonucleotide synthesis on solid phase (CPG).[20] Accordingly, the preparation of the ethidium nucleoside analogue **2** started by the treatment of the phenanthridine **3** with chloroallylformiate (Scheme 2). Subsequently, the alkylation of the bis-alloc-protected phenanthridine derivative **4** was tried with either 1,3-dibromopropane, 1,3-diiodopropane, or 3-iodopropyl triflate and analyzed by HPLC-MS (Table 1). Among the various conditions, THF represents the best solvent for this reaction due to the observation that the alkylation product **5** is insoluble in contrast to the starting material **4**. Hence, the product **5** can be collected in good purity simply by filtration. Using the typical conditions for a nucleophilic substitution, the phenanthridine **5** was linked to the amino group of **7**. The DMT-protected 3-amino-1,3-propanediol **7** can be synthesized according to the literature[21] and carries the DMT-protecting group at the primary hydroxy function, which is necessary for the subsequent oligonucleotide coupling by the DNA synthesizer.

The final step of the synthesis of the phosphoramidite **9** was accomplished by standard procedures. Using automated solid-phase synthesis, the DNA building block **9** could not be incorporated into the oligonucleotide **ODN1** in a reproducible way. The secondary amino group as part of the acyclic linker system of **8** interferes probably with the phoshoramidite synthesis and thus with the efficiency during the oligonucleotide coupling. Hence, the amino group of **8** needs to be protected by the trifluoroacetyl group, which can be cleaved under the

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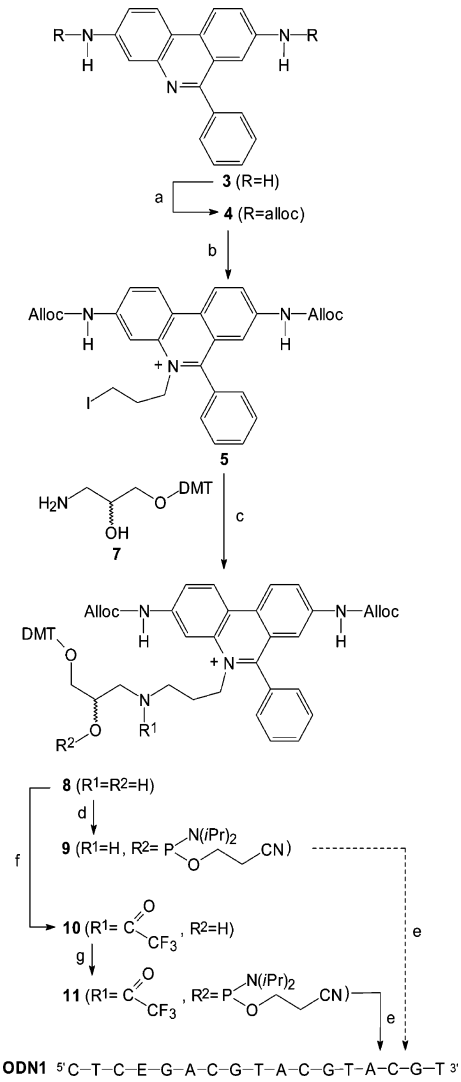
SCHEME 2. Synthesis of the DNA Building Block 11[a]

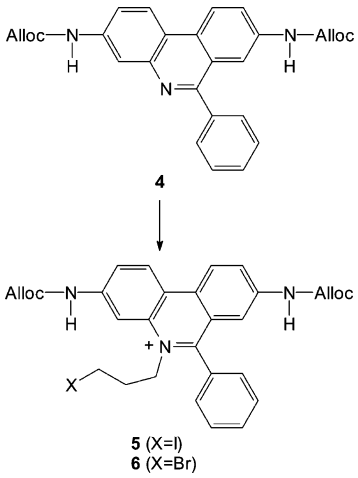


[a] Conditions: (a) allyl chloroformiate (10 equiv), CH_2Cl_2, rt, 24 h, 98%; (b) 1,3-diiodopropane, THF, 65 °C, 9 days, 82%; (c) **7** (1.5 equiv), DIPEA (3 equiv), DMF, rt, 55 °C, 91%; (d) 2-cyanoethyl-*N*,*N*-di*iso*propylchloro-phosphoramidite (1.4 equiv), DIPEA (3 equiv), CH_2Cl_2, rt, 2 h; (e) DNA synthesizer; (f) $(CF_3CO)_2O$ (3 equiv), CH_2Cl_2/pyridine 10:1 v/v, 0 °C, 30 min, rt, 30 min, 94%; (g) 2-cyanoethyl-*N*,*N*-di*iso*propylchloro-phosphoramidite (1.4 equiv), DIPEA (3 equiv), CH_2Cl_2, rt, 2 h, 85%. Counterions have not been identified.

typical basic conditions during DNA workup. The phenanthridinium derivative **8** was treated with trifluoroacetic anhydride in pyridine and converted subsequently to the phosphoramidite **11**. According to the trityl data that was collected by the synthesizer during the preparation of oligonucleotide **ODN1**, the corresponding phosphoramidite **11** showed a coupling efficiency of ~60% using a coupling time of 1 h (in comparison to 1.5 min for standard couplings) and using a concentration of 0.2 M (in MeCN, instead of 0.067 M for standard phosphoramidites). Furthermore, the coupling efficiency of **11** could be increased to ~80% by repeating the coupling cycle three times each followed by a washing step to remove the unreacted or decomposed phosphoramidite **11**.

The cleavage protocol for the alloc protecting groups was worked out using the phenanthridinium derivative

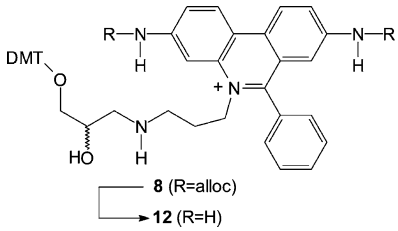
TABLE 1. Alkylation of the Bis-alloc-Protected Phenanthridine 4[a]



reagent	solvent	conditions	yield
$Br-(CH_2)_3-Br$	$Br-(CH_2)_3-Br$	8 h, 150 °C	traces (**6**)[b]
$Br-(CH_2)_3-Br$	THF	9 days, 65 °C	45% (**6**)[c]
$I-(CH_2)_3-I$	$I-(CH_2)_3-I$	8 h, 150 °C	30% (**5**)[c]
$I-(CH_2)_3-I$	THF	9 days, 65 °C	82% (**5**)[c]
$TfO-(CH_2)_3-I$	$Ph-NO_2$	4 h, rt	41% (**5**)[b]

[a] Counterions have not been identified. [b] Determined by HPLC-MS. [c] Isolated yield.

TABLE 2. Removal of the Alloc Groups from the Phenanthridinium Derivative 8[a]

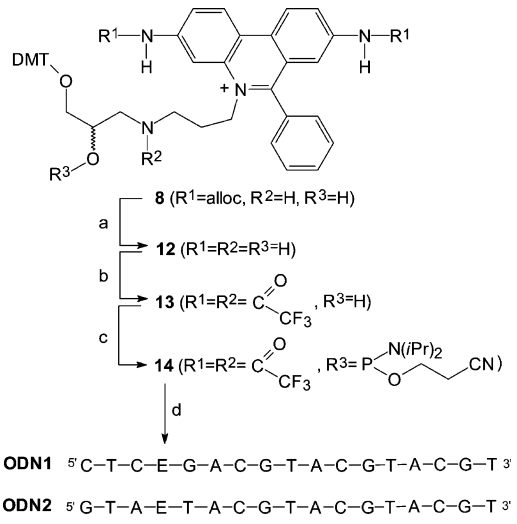


allyl acceptor	main product[b]	side products[b]
dimedone	86%	14%
aniline	80%	20%
3-amino-propan-1-ol	quant	traces
benzylamine	82%	18%
morpholine	85%	14%[c]
Bu_3SnH/H_2O	quant	not detectable

[a] Counterions have not been identified. [b] Determined by HPLC-MS. [c] Including 13% monodeprotected derivative of **8**.

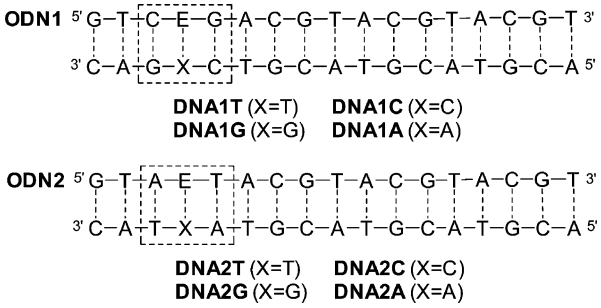
8 (Table 2). A critical side reaction of this procedure is the potential allyl transfer from the intermediate Pd complex to the previously liberated exocyclic amino functions of **12**. The application of a slightly flawed protocol for the deprotection of the phenanthridinium-oligonucleotide conjugates on solid phase would result in an allyl transfer to one of the functional groups of the DNA bases. Hence, it is crucial to find optimal deprotection conditions. Depending on the nature of the applied allyl acceptor, different amounts of side products were detected by HPLC-MS analysis. According to these results, 3-aminopropane-1-ol was used as the allyl acceptor during the deprotection of the oligonucleotide **ODN1** on CPG. Finally, **ODN1** was cleaved off the CPG by treatment with concentrated aqueous NH_3 at 60 °C. MALDI-TOF mass spectra of the crude product clearly

SCHEME 3. Synthesis of the DNA Building Block 14[a]



[a] Conditions: (a) Bu_3SnH (3.2 equiv), $Pd(PPh_3)_4$ (0.02 equiv), PPh_3 (0.2 equiv), CH_2Cl_2/H_2O 300:1 v/v, rt, 90 min, 97%; (b) $(CF_3CO)_2O$ (6 equiv), CH_2Cl_2/pyridine 5:1 v/v, 0 °C, 10 min, rt, 10 min, 59%; (c) 2-cyanoethyl-*N*,*N*-di*iso*propylchloro-phosphoramidite (1.5 equiv), Et_3N (3 equiv), CH_2Cl_2, rt, 2 h; (d) DNA synthesizer. Counterions have not been identified.

SCHEME 4. DNA Duplex Set 1 and 2 (E = phenanthridinium intercalation site)



showed the presence of the correct mass of **ODN1**. Nevertheless, the purification of **ODN1** by semipreparative HPLC failed due to imprecise retention behavior that was probably the result of the presence of numerous side products with similar retention times. Hence, the protecting group strategy for the preparation of the phenanthridinium-DNA building block had to be changed in such a way that the synthesized oligonucleotide does not get into contact with the Pd(0) catalyst at any time. Accordingly, the two alloc protecting groups of the phenanthridinium **8** were cleaved using Bu_3SnH[22] to yield **12** quantitatively. Subsequently, the trifluoroacetylamido groups were introduced by treatment of **12** with trifluoroacetic acid anhydride (Scheme 3). This synthetic procedure has the advantage that the trifluoroacetyl group is incorporated additionally as a protecting group of the secondary amino function of the alkyl linker. The preparation of the phosphoramidite **14** was finished using standard procedures. Finally, the DNA building block **14** was used for the preparation of the oligonucleotides **ODN1** and **ODN2**. The coupling protocols of the DNA

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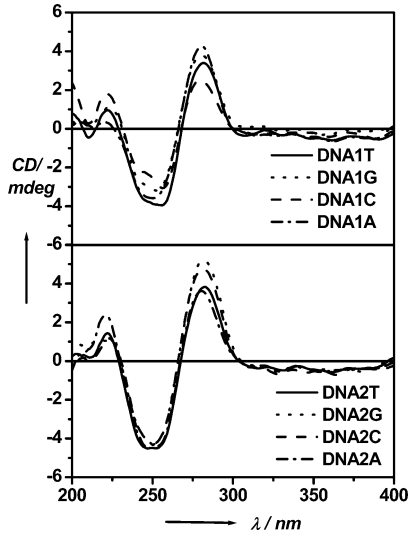

FIGURE 1. CD spectra of duplex sets **DNA1X** (top) and **DNA2X** (bottom) using 2.5 μM duplex, 10 mM Na−P_i-buffer, pH 7, 25 °C.

synthesizer had to be changed as described above for the coupling of the DNA building block **11**. Using the phosphoramidite **14**, quantitative coupling (>95%) comparable to commercially available phosphoramidites was achieved. Both phenanthridinium-containing oligonucleotides, **ODN1** and **ODN2**, were identified by MALDI-TOF mass spectrometry, purified by semipreparative HPLC and quantified by their UV/vis absorption.

Spectroscopic Characterization of Phenanthridinium-Modified DNA Duplexes. Two sets of DNA duplexes, **DNA1X** and **DNA2X**, have been prepared using the previously synthesized oligonucleotides **ODN1** and **ODN2**, respectively (Scheme 4). The sequence of both sets of DNA duplexes is nearly identical except for the bases, which are adjacent to the phenanthridinium intercalation site (E). The phenanthridinium moiety is flanked by G−C base pairs in duplex set **DNA1X** and by A−T base pairs in duplex set **DNA2X**. These different flanking sequences were designed in order to study the phenanthridinium intercalation properties by optical spectroscopy and compare them to ethidium noncovalently bound to poly(dG-dC)-poly(dC-dG) and poly(dA-dT)-poly(dT-dA), respectively.[3,23] The difference within each of the two DNA duplex sets is the counterbase, which is either T, G, C, or A. The DNA duplexes **DNA1X** and **DNA2X** were prepared by slow cooling of the phenanthridinium-modified oligonucleotide **ODN1** or **ODN2**, respectively, with 1.2 equiv of the complementary unmodified oligonucleotide strand to ensure the quantitative formation of the modified duplex.

The DNA duplex sets **DNA1X** and **DNA2X** were characterized by CD spectroscopy (Figure 1), UV/vis absorption spectroscopy (Figure 2) including the melting behavior (Table 3), and steady-state fluorescence spectroscopy (Figure 3). All these measurements were performed to show that the phenanthridinium moiety is intercalated within the DNA base stack and to elucidate

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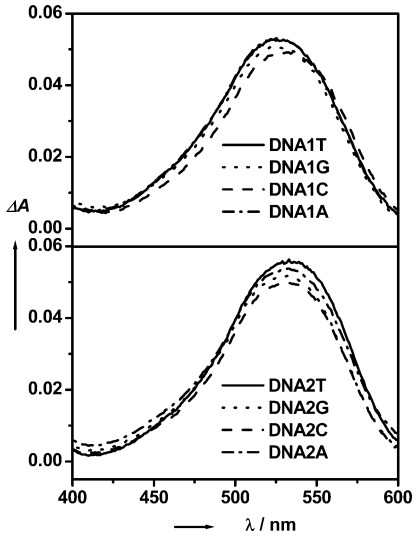

FIGURE 2. UV/vis absorption spectra of duplex sets **DNA1X** (top) and **DNA2X** (bottom) using 12.5 μM duplex, 10 mM Na−P_i-buffer, pH 7, 25 °C.

TABLE 3. Melting Temperatures of DNA Duplex Sets DNA1X and DNA2X (2.5 μM Duplex, 250 mM NaCl, 10 mM Na−P_i-Buffer, pH 7)

DNA1X	T_m	DNA2X	T_m
DNA1T	63 °C	**DNA2T**	55 °C
DNA1G	66 °C	**DNA2G**	55 °C[a]
DNA1C	64 °C	**DNA2C**	53 °C[a]
DNA1A	64 °C	**DNA2A**	54 °C

[a] Additional melting point at ca. 70 °C.

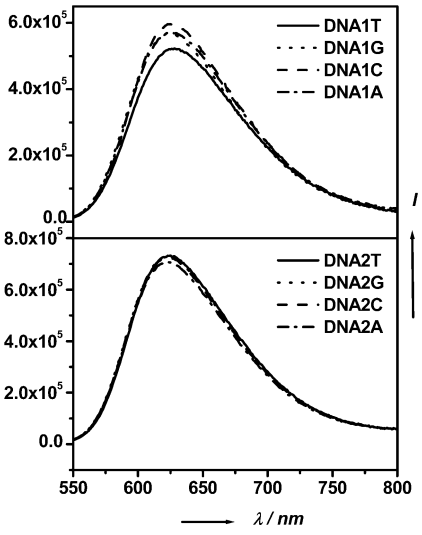

FIGURE 3. Steady-state fluorescence spectra of duplex sets **DNA1X** (top) and **DNA2X** (bottom) using 12.5 μM duplex, 10 mM Na−P_i-buffer, pH 7, at 25 °C.

the influence of the counterbase in the complementary strands. The overall B-DNA conformation is confirmed by CD spectroscopy (Figure 1). Additional to the signals typical for DNA between 200 and 300 nm, a broad negative band was detected between 320 and 400 nm that is typical for intercalated ethidium.[24] CD spectra of ethidium bound to macromolecules in a nonintercalative fashion do not exhibit this negative band.[25]

Interestingly, all UV/vis absorption spectra of the duplex sets **DNA1X** and **DNA2X** show a maximum in the range of 521−533 nm, which is typical for intercalated ethidium.[3,23] In comparison, the absorption spectrum of "free" ethidium in aqueous solution has its maximum at ∼480 nm.[26] Remarkably, there is no significant difference of the absorption maximum between duplex set **DNA1X** (with adjacent A−T base pairs) and duplex set **DNA2X** (with adjacent G−C base pairs). Furthermore, the absorption properties of the chromophore do not vary significantly within each of the duplex sets. These results indicate that the intercalation properties of the phenanthridinium moiety do not depend significantly on the duplex environment, including adjacent base pairs and counterbase.

This interpretation is supported by the melting properties of the modified DNA duplexes (Table 3). The duplex set **DNA1X** exhibits very similar melting temperatures between 63 and 66 °C, and duplex set **DNA2X** exhibits melting temperatures between 53 and 56 °C. The difference between the melting temperature ranges of both duplex sets is due to the fact that set **DNA1X** contains nine G−C pairs whereas set **DNA2X** contains only seven G−C pairs. It is important to note that the protonated secondary amino function of the phenanthridinium linker represents a second artificial positive charge in addition to the positive charge of the phenanthridinium heterocycle, which influences the thermal stability of the modified DNA duplexes. The intercalation of the artificial phenanthridinium base seems not to interfere with the presence of the different counterbases T, G, C, or A. This result is quite surprising since the steric demand of the phenanthridinium group is expected to be at least as high as that of two complementary bases together. One explanation for the similarity of the absorption spectra and the melting temperatures could be an extrahelical position of the counterbase to allow the best intercalation of the phenanthridinium group.

Finally, the steady-state fluorescence spectra of the modified DNA duplexes were recorded using an excitation wavelength of 520 nm. The emission maxima can be found in the range of 622 and 625 nm, which is typical for intercalated ethidium.[3,23] The emission maximum of "free" ethidium in water can be found at ∼635 nm.[26] Neither the different counterbases T, G, C, or A nor the different base pairs in the duplex environment (A−T in duplex set **DNA1X** or G−C in duplex set **DNA2X**) influence the emission properties significantly, as indicated by the similar emission maxima and nearly the same quantum yield.

In conclusion, the characterization of duplexes **DNA1X** and **DNA2X** by different methods of optical spectroscopy shows clearly that the phenanthridinium moiety is intercalated as an artificial DNA base and stabilized by the $\pi-\pi$ interactions within the base stack. Neither the adjacent base pairs nor the counterbase of the complementary oligonucleotide strand interfere significantly with these intercalation properties.

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Conclusion

The phenanthridinium heterocycle of ethidium represents an important and potent positively charged intercalator for DNA analytics. To study the corresponding intercalation properties in a site-selective manner, a DNA system was designed bearing the phenanthridinium heterocycle as an artificial DNA base. The phosphoramidite **14** was prepared as an ethidium nucleoside analogue starting from 3,8-diamino-6-phenyl-phenanthridine (**3**) and incorporated into oligonucleotides via automated synthesis on solid phase. The main synthetic procedures for this DNA system were optimized to allow an efficient and facile synthesis of phenanthridinium-modified DNA. The characterization of the phenanthridinium-containing DNA duplexes by UV/vis absorption spectroscopy (including the melting behavior), CD spectroscopy, and steady-state fluorescence spectroscopy clearly shows that the artificial phenanthridinium base is intercalated within the DNA base stack. The counterbase as part of the complementary strand as well as the adjacent duplex environment seem to have only a minor influence on the intercalation properties of the phenanthridinium moiety.

Experimental Section

Materials and Methods. ^{1}H, ^{13}C, and the two-dimensional NMR spectra were recorded at 300 K on a 250 or 500 MHz spectrometer. NMR signals were assigned on the basis of two-dimensional NMR measurements (DQF-COSY, HMQC, TOCSY, HSQC, and ROESY). ESI mass spectra were measured in the analytical facility of the institute. MALDI-TOF was performed in the analytical facility of the institute using 3-hydroxypicolinic acid in aqueous ammonium citrate as the matrix. Analytical chromatography was performed on Merck silica gel 60 F254 plates. Flash chromatography was performed on Merck silica gel (40−63 μm). C18-RP analytical and semipreparative HPLC columns (300 Å) were purchased from Supelco. Solvents were dried according to standard procedures. All reactions were carried out under argon. Commercial chemicals were used without further purification. All spectroscopic measurements were performed in quartz glass cuvettes (1 cm, pump−probe laser spectroscopy: 1 mm) and using Na−P_i-buffer (10 mM). The melting temperatures were recorded using 2.5 μM duplex and 250 mM NaCl at 260 nm and 10−80 °C with intervals of 1 °C. The B-DNA conformation of all duplexes was confirmed by CD spectroscopy (2.5 μM duplex, 185−400 nm). The absorption and fluorescence spectra were recorded using 12.5 μM duplex and corrected for Raman emission from the buffer solution. All emission spectra were recorded with a band-pass of 2 nm for both excitation and emission and are intensity corrected.

3,8-Bis-allyloxycarbonylamino-6-phenyl-phenanthridine (4). 3,8-Diamino-6-phenylphenanthridine (**3**) (5.0 g, 17.5 mmol) was suspended in an argon atmosphere in dry CH_2Cl_2 (150 mL). Allyl chloroformate (18.6 mL, 175 mmol, 10 equiv) was added slowly. After 24 h at rt, the reaction was quenched with aqueous NH_3 (6 N, 100 mL). The brown solid was collected by filtration, washed with water, and dried under reduced pressure to yield 7.76 g (98%) of a pale brown solid. TLC (EE/MeOH/H_2O 6:2:1 v/v) R_f = 0.96. ^{1}H NMR (250 MHz, DMSO-d_6): δ 10.08 (s, 1H, NH), 10.06 (s, 1H, NH), 8.74 (d, ^3J = 8.8 Hz, 1H, H1), 8.64 (d, ^3J = 9.0 Hz, 1H, H10), 8.28 (s, 1H, H4), 8.20 (s, 1H, H7), 7.96 (d, ^3J = 8.7 Hz, 1H, H9), 7.80 (d, ^3J = 8.4 Hz, 1H, H2), 7.68 (m, 2H, arom.), 7.57 (m, 3H, arom.), 6.10−5.90 (m, 2H, CH_2=C*H*), 5.37 (m, 2H, C*H*$_2$=CH), 5.24 (m, 2H, C*H*$_2$=CH), 4.67 (d, ^3J = 5.7 Hz, 2H, CH_2O), 4.60 (d, ^3J = 5.7 Hz, 2H, CH_2O). ^{13}C NMR (126 MHz, DMSO-d_6): δ 161.7, 154.6, 154.5, 144.6, 140.8, 140.5, 139.1, 134.5 (CH_2=*C*H), 134.4 (CH_2=*C*H), 130.8, 129.9, 129.6, 129.5, 125.7, 124.5, 124.1, 120.2, 119.8 (*C*H_2=CH), 119.1 (*C*H_2=CH), 118.9, 117.8, 116.4, 66.2 (OCH_2), 66.1 (OCH_2). MS (ESI): *m*/*z* (%) 454.2 (100) [M + H]$^+$, 396.3 (24) [M + H − C_3H_6O]$^+$, 338.4 (12) [M + H − 2C_3H_6O]$^+$.

3,8-Bis-allyloxycarbonylamino-5-(3-iodopropyl)-6-phenyl-phenanthridinium Iodide (5). 1,3-Diiodopropane (13 mL) was added to a solution of **4** (5.0 g, 11.0 mmol) in dry THF (40 mL). The solution was refluxed for 9 days (65 °C). The solid product was collected by filtration, washed with THF, and dried under reduced pressure to yield 6.75 g (82%) of a dark yellow solid. ^{1}H NMR (500 MHz, DMSO-d_6): δ 10.53 (s, 1H, NH, 3-Alloc), 10.32 (s, 1H, NH, 8-Alloc), 9.05 (d, ^3J = 9.4 Hz, 1H, H1), 8.99 (d, ^3J = 9.1 Hz, 1H, H10), 8.54 (s, 1H, H4), 8.24 (dd, ^3J = 9.0 Hz, ^4J = 1.8 Hz, 1H, H9), 8.14 (dd, ^3J = 9.0 Hz, ^4J = 1.3 Hz, 1H, H2), 7.78 (s, 1H, H7), 7.85−7.72 (m, 5H, 6-Ph), 6.03 (m, 1H, CH_2=C*H*, 3-Alloc), 5.91 (m, 1H, CH_2=C*H*, 8-Alloc), 5.43 (dd, ^2J = 1.7 Hz, ^3J = 17.4 Hz, 1H, C*H*$_2$=CH, trans, 3-Alloc), 5.32 (dd, ^2J = 1.7 Hz, ^3J = 17.8 Hz, 1H, C*H*$_2$=CH, trans, 8-Alloc), 5.29 (dd, ^2J = 1.1 Hz, ^3J = 10.5 Hz, 1H, C*H*$_2$=CH, cis, 3-Alloc), 5.22 (dd, ^2J = 1.3 Hz, ^3J = 10.4 Hz, 1H, C*H*$_2$=CH, cis, 8-Alloc), 4.71 (m, 2H, H1′), 4.68 (m, 2H, OCH_2, 3-Alloc), 4.55 (m, 2H, OCH_2, 8-Alloc), 3.27 (t, ^3J = 6.2 Hz, 2H, H3′), 2.41 (m, 2H, H2′). ^{13}C NMR (126 MHz, DMSO-d_6): δ 163.4, 153.3, 153.0, 142.1, 139.8, 134.2, 132.9 (CH_2=*C*H, 3-Alloc), 132.8 (CH_2=*C*H, 8-Alloc), 131.5, 131.2, 130.2, 129.6, 128.8, 128.4, 125.7, 125.5, 123.7, 121.4, 121.2, 118.3 (*C*H_2=CH, 3-Alloc), 118.0 (*C*H_2=CH, 8-Alloc), 117.7, 106.7, 65.5 (OCH_2, 3-Alloc), 65.2 (OCH_2, 8-Alloc), 55.7 (C1′), 31.2 (C2′), 2.1 (C3′). MS (ESI): *m*/*z* (%) 622.1 (100) [M]$^+$, 564.2 (10) [M − C_3H_6O]$^+$, 466.5 (15). HRMS (ESI-FTICR): [M]$^+$ calcd for $C_{30}H_{29}IN_3O_4^+$ 622.11973, found 622.11918.

3,8-Bis-allyloxycarbonylamino-5-(3-bromopropyl)-6-phenyl-phenanthridinium Bromide (6). 1,3-Dibromopropane (1.8 mL) was added to a solution of **4** (200 mg, 0.44 mmol) in dry THF (10 mL). The solution was refluxed for 9 days (65 °C). The solid product was collected by filtration and dried under reduced pressure to yield 130 mg (45%) of a dark yellow solid. ^{1}H NMR (500 MHz, DMSO-d_6): δ 10.62 (s, 1H, NH, 3-Alloc), 10.38 (s, 1H, NH, 8-Alloc), 9.10 (d, ^3J = 9.4 Hz, 1H, H1), 9.04 (d, ^3J = 9.2 Hz, 1H, H10), 8.61 (s, 1H, H4), 8.28 (dd, ^3J = 9.0 Hz, ^4J = 1.9 Hz, 1H, H9), 8.17 (dd, ^3J = 9.0 Hz, ^4J = 1.4 Hz, 1H, H2), 7.79 (s, 1H, H7), 7.82−7.73 (m, 5H, 6-Ph), 6.03 (m, 1H, CH_2=C*H*, 3-Alloc), 5.81 (m, 1H, CH_2=C*H*, 8-Alloc), 5.42 (dd, ^2J = 1.6 Hz, ^3J = 17.2 Hz, 1H, C*H*$_2$=CH, trans, 3-Alloc), 5.32 (dd, ^2J = 1.5 Hz, ^3J = 17.7 Hz, 1H, C*H*$_2$=CH, trans, 8-Alloc), 5.29 (dd, ^2J = 1.1 Hz, ^3J = 10.9 Hz, 1H, C*H*$_2$=CH, cis, 3-Alloc), 5.21 (dd, ^2J = 1.3 Hz, ^3J = 10.4 Hz, 1H, C*H*$_2$=CH, cis, 8-Alloc), 4.73 (m, 2H, H1′), 4.72 (m, 2H, OCH_2, 3-Alloc), 4.56 (m, 2H, OCH_2, 8-Alloc), 3.55 (t, ^3J = 6.2 Hz, 2H, H3′), 2.46 (m, 2H, H2′). ^{13}C NMR (126 MHz, DMSO-d_6, 300 K): δ 163.8, 153.5, 153.2, 142.2, 139.9, 134.4, 133.0 (CH_2=*C*H, 3-Alloc), 132.9 (CH_2=*C*H, 8-Alloc), 131.5, 131.3, 130.3, 129.6, 128.9, 128.4, 125.9, 125.6, 123.8, 121.7, 121.3, 118.4 (*C*H_2=CH, 3-Alloc), 118.0 (*C*H_2=CH, 8-Alloc), 117.8, 106.9, 65.6 (OCH_2, 3-Alloc), 65.3 (OCH_2, 8-Alloc), 53.7 (C1′), 30.8 (C3′), 30.6 (C2′). MS (ESI): *m*/*z* (%) 574.2 (100) [M]$^+$, 516.3 (18) [M − C_3H_6O]$^+$.

3,8-Bis-allyloxycarbonylamino-5-{3-[3-(bis-(4-methoxy-phenyl)-phenyl-methoxy)-2-hydroxy-propylamino]-propyl}-6-phenyl-phenanthridinium Iodide (8). 3-Amino-1-[bis-(4-methoxy-phenyl)-phenyl-methoxy]-propan-2-ol (**7**)[21] (649 mg, 1.65 mmol, 1.1 equiv) was added to a solution of **5** (1.124 g, 1.50 mmol) in dry DMF (60 mL). DIPEA (0.45 mL, 3 mmol, 2 equiv) was added, and the solution was stirred at rt for 40 h. Subsequently, 3-Amino-1-[bis-(4-methoxy-phenyl)-phenyl-methoxy]-propan-2-ol (236 mg, 0.60 mmol, 0.4 equiv) and DIPEA (0.22 mL, 1.5 mmol, 1.0 equiv) were added and the reaction was kept at rt for another 15 h. The solution was concentrated to dryness under reduced pressure. The crude product was purified by flash chromatography (SiO_2, CH_2Cl_2/MeOH 100:3 + 0.1% pyridine v/v, eluent = CH_2Cl_2/MeOH 10:3

+ 0.1% pyridine v/v) to give 1.38 g (91%) of a pale yellow solid. TLC (DCM/MeOH 10:3 v/v) R_f = 0.82. ^{1}H NMR (500 MHz, DMSO-d_6): δ 10.66 (s, 1H, NH, 3-Alloc), 10.38 (s, 1H, NH, 8-Alloc), 9.11 (d, ^3J = 9.1 Hz, 1H, H1), 9.05 (d, ^3J = 9.3 Hz, 1H, H10), 8.65 (s, 1H, H4), 8.28 (dd, ^3J = 9.0 Hz, ^4J = 2.0 Hz, 1H, H9), 8.11 (dd, ^3J = 9.0 Hz, ^4J = 1.4 Hz, 1H, H2), 7.78 (s, 1H, H7), 7.83−7.73 (m, 5H, 6-Ph), 7.38 (m, 2H, arom., DMT), 7.32−7.20 (m, 7H, arom., DMT), 6.88 (m, 4H, arom., DMT), 5.99 (m, 1H, CH_2=C*H*, 3-Alloc), 5.92 (m, 1H, CH_2=C*H*, 8-Alloc), 5.38 (dd, ^2J = 1.7 Hz, ^3J = 17.1 Hz, 1H, CH_2=CH, trans, 3-Alloc), 5.32 (dd, ^2J = 1.7 Hz, ^3J = 17.0 Hz, 1H, CH_2=CH, trans, 8-Alloc), 5.27 (dd, ^2J = 1.3 Hz, ^3J = 10.4 Hz, 1H, CH_2=CH, cis, 3-Alloc), 5.22 (dd, ^2J = 1.3 Hz, ^3J = 10.4 Hz, 1H, CH_2=CH, cis, 8-Alloc), 4.68 (d, ^3J = 5.7 Hz, 2H, OCH_2, 3-Alloc), 4.63 (m, 2H, H1′), 4.57 (d, ^3J = 5.7 Hz, 2H, OCH_2, 8-Alloc), 3.89 (m, 1H, C*H*OH), 3.72 (s, 6H, OCH_3), 3.01 (m, 1H, CH_2ODMT), 3.00 (m, 2H, H3′), 2.95 (m, 1H, NHCH_2-CHOH), 2.84 (m, 1H, CH_2ODMT), 2.73 (m, 1H, NHCH_2-CHOH), 2.26 (m, 2H, H2′). ^{13}C NMR (126 MHz, DMSO-d_6): δ 163.5, 158.2 (arom., DMT), 153.5, 153.1, 144.9 (arom., DMT), 142.1, 139.9, 136.2 (arom., DMT), 134.0, 132.9 (CH_2=*C*H, 3-Alloc), 132.8 (CH_2=*C*H, 8-Alloc), 131.4, 131.2, 130.3, 129.9 (arom., DMT), 129.5, 128.9, 128.5, 128.0 (arom., DMT), 126.8 (arom., DMT), 125.9, 125.7, 123.8, 121.6, 121.4, 118.4 (*C*H_2=CH, 3-Alloc), 118.0 (*C*H_2=CH, 8-Alloc), 117.8, 113.3 (arom., DMT), 106.6, 85.6 (O*C*Ph_3), 66.1 (*C*HOH), 65.8 (CH_2ODMT), 65.6 (OCH_2, 3-Alloc), 65.3 (OCH_2, 8-Alloc), 55.3 (OCH_3), 52.2 (C1′), 50.3 (NH*C*H_2CHOH), 44.8 (C3′), 25.3 (C2′). MS (ESI): *m*/*z* (%) 887.4 (100) [M]$^+$, 585.4 (28) [M + H − DMT]$^+$, 303.4 (40) [DMT]$^+$, 468.4 (22). HRMS (ESI−FTICR): *m*/*z* [M]$^+$ calcd for $C_{54}H_{55}N_4O_8^+$ 887.40144, found 887.40129.

3,8-Bis-allyloxycarbonylamino-5-{3-[{3-(bis-(4-methoxyphenyl)-phenyl methoxy)-2-hydroxy-propyl}-*N*-(trifluoroacetyl)-amino]-propyl}-6-phenyl-phenanthridinium Iodide (10). In an argon atmosphere **8** (365 mg, 0.36 mmol) was solved in a mixture of dry CH_2Cl_2 (50 mL) and pyridine (5 mL) and cooled to 0 °C. After stirring for 5 min, a solution of trifluoroacetic anhydride (227 mg, 0.152 mL, 1.08 mmol) in dry CH_2Cl_2 (2 mL) was added. The solution was stirred for 30 min at 0 °C, warmed to r. t. and stirred for another 30 min. The solvent was removed under reduced pressure and the crude product solved in CH_2Cl_2 (100 mL), washed with aq. $NaHCO_3$ (2 * 80 mL) and water (80 mL), and dried with Na_2SO_4. The solution was concentrated to dryness under reduced pressure to yield 375 mg of a pale yellow solid (94%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 10.64 (s, 1H, NH, 3-Alloc), 10.38 (s, 1H, NH, 8-Alloc), 9.07 (d, ^3J = 9.0 Hz, 1H, H1), 9.01 (d, ^3J = 9.0 Hz, 1H, H10), 8.69 (s, 1H, H4), 8.29 (dd, ^3J = 9.0 Hz, ^4J = 2.0 Hz, 1H, H9), 8.17 (dd, ^3J = 9.0 Hz, ^4J = 1.4 Hz, 1H, H2), 7.77 (s, 1H, H7), 7.82−7.71 (m, 5H, 6-Ph), 7.37 (m, 2H, arom., DMT), 7.33−7.20 (m, 7H, arom., DMT), 6.87 (m, 4H, arom., DMT), 5.99 (m, 1H, CH_2=C*H*, 3-Alloc), 5.92 (m, 1H, CH_2=C*H*, 8-Alloc), 5.39 (dd, ^2J = 1.7 Hz, ^3J = 17.0 Hz, 1H, CH_2=CH, trans, 3-Alloc), 5.34 (dd, ^2J = 1.7 Hz, ^3J = 17.0 Hz, 1H, CH_2=CH, trans, 8-Alloc), 5.26 (dd, ^2J = 1.3 Hz, ^3J = 10.5 Hz, 1H, CH_2=CH, cis, 3-Alloc), 5.22 (dd, ^2J = 1.3 Hz, ^3J = 10.5 Hz, 1H, CH_2=CH, cis, 8-Alloc), 4.67 (m, 2H, OCH_2, 3-Alloc), 4.58 (m, 2H, H1′), 4.57 (m, 2H, OCH_2, 8-Alloc), 3.95 (m, 1H, C*H*OH), 3.74 (s, 3H, OCH_3), 3.72 (s, 3H, OCH_3), 3.60 (m, 2H, H3′), 3.04 (m, 1H, CH_2ODMT), 3.04 (m, 1H, NCH_2-CHOH), 2.86 (m, 1H, CH_2ODMT), 2.86 (m, 1H, NCH_2CHOH), 2.31 (m, 2H, H2′). ^{13}C NMR (126 MHz, DMSO-d_6): δ 163.3, 158.2 (arom., DMT), 156.8 (q, $^1J_{CF}$ = 36 Hz, *C*O*C*F_3), 153.5, 153.2, 144.9 (arom., DMT), 142.1, 139.9, 136.2 (arom., DMT), 134.0, 132.9 (CH_2=*C*H, 3-Alloc), 132.8 (CH_2=*C*H, 8-Alloc), 131.4, 131.2, 130.3, 129.8 (arom., DMT), 129.4, 129.1 (arom., DMT), 129.0, 128.3, 127.9 (arom., DMT), 126.9 (arom., DMT), 125.8, 125.6, 124.0, 121.6, 121.3, 118.4 (*C*H_2=CH, 3-Alloc), 118.0 (*C*H_2=CH, 8-Alloc), 117.8, 113.3 (arom., DMT), 106.6, 85.6 (O*C*Ph_3), 66.1 (*C*HOH), 50.3 (CH_2ODMT), 65.6 (OCH_2, 3-Alloc), 65.3 (OCH_2, 8-Alloc), 55.2 (OCH_3), 52.7 (C1′), 50.3 (N*C*H_2CHOH), 44.9 (C3′), 26.1 (C2′). MS (ESI): *m*/*z* (%) 983.4 (100) [M]$^+$, 303.4 (22) [DMT]$^+$. HRMS (ESI-FTICR): *m*/*z* [M]$^+$ calcd for $C_{56}H_{54}F_3N_4O_9$ 983.38374, found 983.38381.

3,8-Bis-allyloxycarbonylamino-5-{3-[{3-(bis-(4-methoxyphenyl)-phenyl-methoxy)-2-[(2-cyano-ethoxy)-di*iso*propylamino-phosphanyloxy]-propyl}-*N*-(trifluoroacetyl)-amino]-propyl}-6-phenyl-phenanthridinium Iodide (11): Method A. In an argon atmosphere and protected from light, **8** (241 mg, 0.217 mmol) was dissolved in dry CH_2Cl_2 (10 mL). DIPEA (113 μL, 0.65 mmol, 3 equiv, dried with molecular sieve 4 Å) and 2-cyanoethyl-*N*,*N*-di*iso*propylchlorophosphoramidite (51.4 mg, 48.4 μL, 0.217 mmol, 1.0 equiv) were added. The solution was kept at rt for 30 min. Subsequently, 2-cyanoethyl-*N*,*N*-di*iso*propylchlorophosphoramidite (20.6 mg, 19.4 μL, 0.087 mmol, 0.4 equiv) was added, and the solution was stirred for another 90 min at rt, washed with saturated aqueous $NaHCO_3$, dried with Na_2SO_4, and reduced to dryness in vacuo. The crude product was coevaporated three times with dry Et_2O and dried under reduced pressure to yield **11** (50%, estimated from ESI-MS). **Method B.** In an argon atmosphere and protected from light, **8** (241 mg, 0.217 mmol) was dissolved in dry CH_2Cl_2 (10 mL). Dry DIPAT (18.5 mg, 0.108 mmol, 0.5 equiv) and 2-cyanoethyl-*N*,*N*,*N*′,*N*′-tetra*iso*propyl-phosphoramidite (91.6 mg, 96.5 μL, 0.304 mmol, 1.4 equiv) were added. The mixture was stirred for 8 h at rt, washed with saturated aqueous $NaHCO_3$, dried with Na_2SO_4 and reduced to dryness in vacuo. The residue was coevaporated three times with dry Et_2O and dried under reduced pressure to yield the phosphoramidite (85%, estimated from ESI-MS). MS (ESI): *m*/*z* (%) 1183.6 (100) [M]$^+$, 303.4 (25) [DMT]$^+$.

3,8-Bis-amino-5-{3-[3-(bis-(4-methoxy-phenyl)-phenyl-methoxy)-2-hydroxy-propyl-amino]-propyl}-6-phenyl-phenanthridinium Iodide (12). Pd(PPh_3)$_4$ (20 mg, 0.02 mmol), PPh_3 (52 mg, 0.2 mmol), and Bu_3SnH (0.87 g, 3.0 mmol) were added to a solution of **8** (0.95 g, 0.94 mmol) in dry CH_2Cl_2 (30 mL) and water (0.1 mL). The mixture was stirred at rt for 90 min. The reaction was quenched by addition of water (20 mL), and the solution was concentrated to dryness under reduced pressure. The crude product was purified by flash chromatography (SiO_2, CH_2Cl_2/MeOH 100:5 v/v + 0.1% pyridine, eluent = EE/MeOH/H_2O 6:2:2 v/v + 0.1% pyridine) to give 0.77 g (97%) of a violet solid. ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.69 (d, ^3J = 9.0 Hz, 1H, H1), 8.63 (d, ^3J = 8.9 Hz, 1H, H10), 7.70 (m, 5H, 6-Ph), 7.56 (d, ^3J = 9.5 Hz, 1H, H9), 7.52 (s, 1H, H4), 7.39 (m, 2H, arom., DMT), 7.38 (d, ^3J = 9.5 Hz, 1H, H2), 7.34−7.18 (m, 7H, arom., DMT), 6.89 (m, 4H, arom., DMT), 6.39 (s, 2H, 3-NH_2), 6.27 (s, 1H, H7), 5.51 (s, 1H, OH), 5.35 (s, 1H, 8-NH_2), 4.46 (m, 2H, H1′), 3.88 (m, 1H, CHOH), 3.72 (s, 6H, OCH_3), 3.41 (s, 1H, NH), 3.01 (m, 1H, CH_2ODMT), 2.96 (m, 2H, H3′), 2.95 (m, 1H, NCH_2-CHOH), 2.85 (m, 1H, CH_2ODMT), 2.69 (m, 1H, NCH_2CHOH), 2.18 (m, 2H, H2′). ^{13}C NMR (126 MHz, DMSO-d_6): δ 159.0, 158.2 (arom., DMT), 151.2, 148.1, 144.9 (arom., DMT), 135.7, 135.6, 135.1 (arom., DMT), 134.4, 132.1, 130.9, 129.9 (arom., DMT), 129.5, 128.6, 128.1, 128.0 (arom., DMT), 127.9, 127.7, 126.8 (arom., DMT), 125.2, 124.8, 122.8, 120.1, 117.7, 113.3 (arom., DMT), 108.2, 98.6, 85.6 (O*C*Ph_3), 66.3 (CHOH), 65.8 (CH_2ODMT), 55.3 (OCH_3), 51.2 (C1′), 50.5 (NCH_2CHOH), 45.8 (C3′), 25.2 (C2′). MS (ESI): *m*/*z* (%) 719.3 (100) [M]$^+$, 303.3 (52) [DMT]$^+$.

3,8-Bis-trifluoroacetylamino-5-{3-[3-(bis-(4-methoxyphenyl)-phenyl-methoxy)-2-hydroxy-propyl-*N*-(trifluoroacetyl)-amino]-propyl}-6-phenyl-phenanthridinium Iodide (13). In an argon atmosphere at 0 °C, pyridine (0.9 mL, 11 mmol) and triflouracetic anhydride (0.76 mL, 5.4 mmol) were added to a solution of **12** (0.77 g, 0.9 mmol) in dry CH_2Cl_2 (4.5 mL). The solution was stirred for 10 min at 0 °C and another 10 min at rt. The solution was washed two times with saturated aqueous $NaHCO_3$ (2 × 5 mL), dried with Na_2SO_4, and concentrated to dryness under reduced pressure to yield 0.60 g (59%) of a brown-yellow solid. ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.14 (d, ^3J = 9.0 Hz, 2H, H1 and H10), 9.02,

8.93 (2 s, together 1H, H4), 8.56 (d, ^3J = 9.0 Hz, 1H, H9), 8.31, 8.21 (2 d, ^3J = 9.0 Hz, together 1H, H2), 8.00, 7.98 (2 s, 1H, H7), 7.80−7.65, 7.44−7.36 (m, 5H, 6-Ph), 7.35−7.14 (m, 6H, arom., DMT), 6.93−6.83 (m, 7H, arom., DMT), 4.59 (m, 2H, H1′), 3.94, 3.86 (m, 1H, CHOH), 3.72 (s, 3H, OCH_3), 3.71 (s, 3H, OCH_3), 3.59, 3.51 (m, 2H, H3′), 3.55, 3.43 (m, 1H, CH_2-ODMT), 3.14, 3.06 (m, 1H, NCH_2CHOH), 3.02, 2.97 (m, 1H, CH_2ODMT), 2.83 (m, 1H, NCH_2CHOH), 2.34, 2.31 (m, 2H, H2′). ^{13}C NMR (126 MHz, DMSO-d_6): δ 163.5 (q, $^1J_{CF}$ = 34 Hz, $COCF_3$), 158.5 (q, $^1J_{CF}$ = 33 Hz, $COCF_3$), 158.4 (q, $^1J_{CF}$ = 35 Hz, $COCF_3$), 146.8, 146.7, 145.2, 145.1, 136.0, 135.9, 135.8, 135.7, 135.4, 132.8, 132.4 (C9), 131.2 (arom., DMT), 131.1 (arom., DMT), 131.0, 130.7, 129.7, 129.4 (arom., DMT), 129.3 (arom., DMT), 129.2, 128.9, 128.2 (arom., DMT), 128.0, 127.9, 127.7 (C2), 127.2, 127.1 (C2), 126.9, 126.5, 126.2, 126.1, 126.0, 125.9, 125.8, 125.7, 125.6, 125.4, 125.3 (C1), 125.2 (C10), 123.5, 123.1 (C7), 122.0, 121.7, 117.7 (q, $^2J_{CF}$ = 292 Hz, $COCF_3$), 117.6, 116.6 (q, $^2J_{CF}$ = 291 Hz, $COCF_3$), 116.1 (q, $^2J_{CF}$ = 289 Hz, $COCF_3$), 114.7, 114.6 (arom., DMT), 112.7 (C4), 112.8 (C4), 111.2 (arom., DMT), 110.9 (arom., DMT), 105.4 ($OCPh_3$), 69.7 (CHOH), 68.4 (CHOH), 67.4 (CH_2ODMT), 67.2 (NCH_2CHOH), 67.2 (CH_2ODMT), 56.6 (OCH_3), 54.0 (C1′), 53.2 (C1′), 51.7 (NCH_2CHOH), 51.7 (CH_2ODMT), 51.6 (NCH_2CHOH), 51.5 (CH_2ODMT), 46.8 (C3′), 46.5 (C3′), 28.6 (C2′), 27.5 (C2′). MS (ESI): *m*/*z* (%) 1007.3 (100) $[M]^+$, 303.3 (27) $[DMT]^+$. HRMS (ESI-FTICR): *m*/*z* $[M]^+$ calcd for $C_{52}H_{44}F_9N_4O_7^+$ 1007.30608, found 1007.30553.

3,8-Bis-trifluoroacetylamino-5-{3-[{3-(bis-(4-methoxyphenyl)-phenyl-methoxy)-2-[(2-cyano-ethoxy)-di*iso*propylamino-phosphanyloxy]-propyl}-(2,2,2-trifluoroacetyl)-amino]-propyl}-6-phenyl-phenanthridinium Iodide (14). **13** (0.60 mg, 0.53 mmol) was dissolved in dry CH_2Cl_2 (10 mL) under an argon atmosphere. Et_3N (160 mg, 1.58 mmol, 3 equiv) and 2-cyanoethyl-*N*,*N*-di*iso*propylchloro-phosphoramidite (190 mg, 0.80 mmol, 1.5 equiv) were added, and the solution was stirred for 60 min at rt. At that time, ESI-MS showed the reaction to be complete. The solution was washed with saturated aqueous $NaHCO_3$, dried with Na_2SO_4, and concentrated under reduced pressure. The crude product was solved in CH_2Cl_2 and coevaporated three times with Et_2O (3 × 20 mL) and dried. The brown-yellow solid was used for DNA synthesis without further purification. MS (ESI): *m*/*z* (%) 1207.3 (100) $[M]^+$, 303.3 (64) $[DMT]^+$.

Preparation and Characterization of the Oligonucleotides (General Procedure). The oligonucleotides were prepared via standard phosphoramidite protocols using CPG (1 μmol). After preparation, the trityl-off oligonucleotide was cleaved from the resin and deprotected by treatment with concentrated NH_4OH at 60 °C for 10 h. The oligonucleotide was dried and purified by HPLC on a semipreparative RP-C18 column (300 Å) using the following conditions: A = NH_4-OAc buffer (50 mM), pH = 6.5; B = MeCN; gradient = 0−15% B over 45 min. The oligonucleotides were lyophilized and quantified by their absorbance at 260 nm.[27] Duplexes were formed by heating to 80 °C (10 min), followed by slow cooling.

Solid-Phase Synthesis of the Ethidium-Modified Oligonucleotides ODN 1 and ODN2. The syntheses were performed on a 1 μmol scale (CPG 500 Å) using standard phosphoramidite protocols. Good coupling of the building block **14** was achieved using a coupling time of 1 h and repeating the coupling cycle three times. After preparation, the trityl-off oligonucleotide was cleaved from the resin and deprotected by treatment with concentrated NH_4OH at 60 °C for 5 h. The oligonucleotide was dried and purified by HPLC on a semi-preparative RP-C18 column (300 Å) using the following conditions: A = NH_4OAc buffer (50 mM), pH = 6.5; B = MeCN; gradient = 0−15% B over 45 min. The oligonucleotides were lyophilized, quantified by their absorbance at 260 nm[27] and using ϵ = 45.200 M^{-1} cm^{-1} (260 nm) for **E**.[28] MS (MALDI-TOF): **ODN1** *m*/*z* = 5375 (calcd), 5377 (exp); **ODN2** *m*/*z* = 5374 (calcd), 5374 (exp).

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