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Anthraquinones as Artificial DNA Building Blocks

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Synthesis and properties of oligodeoxynucleotides containing anthraquinone-derived building blocks with flexible linkers are described. Starting from the 1,4-, 1,5-, 1,8- and 2,6-dihydroxyanthraquinone isomers, the corresponding phosphoramidites were prepared and incorporated into oligonucleotides. The site of linker attachment was found to be of critical importance for hybrid stability. Whereas the 2,6-isomer led to a significant stabilization, all other isomers had a negative effect on the stability of the duplex. Spectroscopic studies showed that the anthraquinones behave as fluorescence quenchers. Models of anthraquinone-modified double-stranded hybrids are proposed.

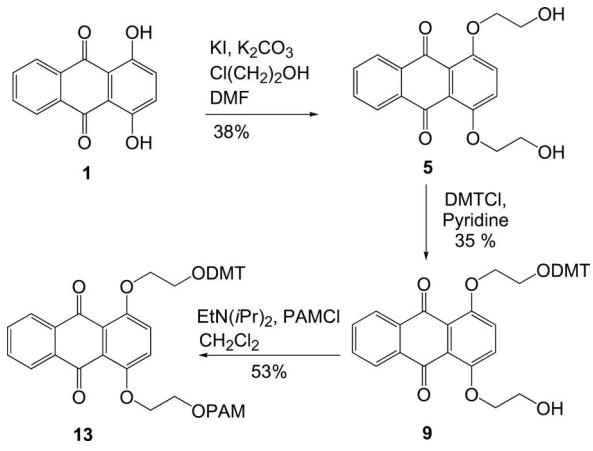
Introduction

The use of chemically modified nucleic acids is a rapidly growing area. Oligonucleotides containing unnatural building blocks are commonly used in the areas of diagnostics, supramolecular chemistry and materials research.[1–5] Among the many modifications, building blocks lacking a sugar or a sugar-like moiety are increasingly used as versatile components. In particular, polyaromatic compounds, which often possess interesting electronic and spectroscopic properties, were found to integrate well into DNA without compromising hybrid stability. Typical modifications of this kind include stilbene,[6,7] phenanthrene,[8–12] pyrene,[13–20] perylene,[21–25] or phenanthroline.[26,27] One of the primary reasons for the positive effect of the modifications on stability is their tendency to develop stacking interactions among themselves or with the nucleobases.[16,17,28,29] Anthraquinone and its derivatives are well-known intercalators.[30,31] They are a frequently found motif in DNA targeting drugs.[30,32–36] Not surprisingly, conjugation of anthraquinone to oligonucleotides has served as a common strategy for the development of high affinity oligonucleotides.[37–45] Furthermore, the low reduction potential of anthraquinone derivatives opens possibilities for charge transport through DNA[46–50] and electrochemical DNA sensing.[49,51–55] In addition, anthraquinone derivatives can act as fluorescence quenchers,[38,54,56] they are investigated as photo-activated nucleases[57,58] and they can serve as molecular entities for supramolecular assemblies.[59] On this background, we investigated the use of anthraquinone as an elemental building block for the construction of DNA-like structures. In particular, we were interested in the influence of the geometrical attachment of the linkers on hybrid formation and in their properties as fluorescence quenchers. Here, we report the synthesis of four isomeric anthraquinone phosphoramidites, their incorporation into oligonucleotides as well as the properties of the resulting oligomers.

Results and Discussion

Synthesis of the Phosphoramidite Building Blocks and Oligonucleotides

Incorporation of anthraquinone derivatives with different geometries into DNA should give an indication of the effects that the attachment sites of the linkers have on the stability of the duplex. Thus, the four dihydroxyanthraquinones **1**–**4** (the 1,4-, 1,5-, 1,8- and 2,6-isomers) were



Scheme 1. Synthesis of the 1,4-substituted anthraquinone phosphoramidite building block **13** (DMT = 4,4′-dimethoxytrityl, PAM = 2-cyanoethyl diisopropylamidochloridophosphite).

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Table 1. The 1,4-, 1,5-, 1,8- and 2,6-substituted anthraquinone intermediates and phosphoramidites.

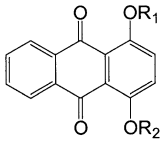
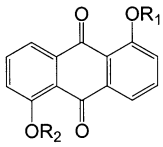
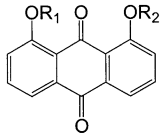
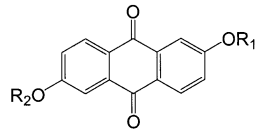
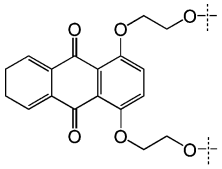
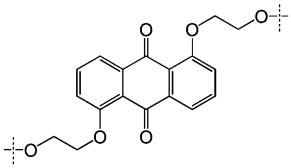
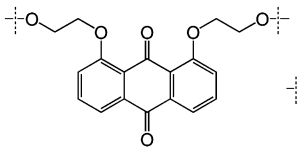
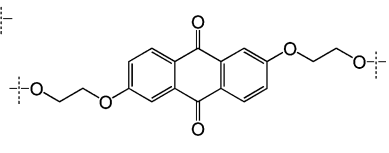
1,4-Isomer	1,5-Isomer	1,8-Isomer	2,6-Isomer
1 $R^1 = R^2 = H$	**2** $R^1 = R^2 = H$	**3** $R^1 = R^2 = H$	**4** $R^1 = R^2 = H$
5 $R^1 = R^2 = (CH_2)_2OH$	**6** $R^1 = R^2 = (CH_2)_2OH$	**7** $R^1 = R^2 = (CH_2)_2OH$	**8** $R^1 = R^2 = (CH_2)_2OH$
9 $R^1 = (CH_2)_2ODMT$ $R^2 = (CH_2)_2OH$	**10** $R^1 = (CH_2)_2ODMT$ $R^2 = (CH_2)_2OH$	**11** $R^1 = (CH_2)_2ODMT$ $R^2 = (CH_2)_2OH$	**12** $R^1 = (CH_2)_2ODMT$ $R^2 = (CH_2)_2OH$
13 $R^1 = (CH_2)_2ODMT$ $R^2 = (CH_2)_2OPAM$	**14** $R^1 = (CH_2)_2ODMT$ $R^2 = (CH_2)_2OPAM$	**15** $R^1 = (CH_2)_2ODMT$ $R^2 = (CH_2)_2OPAM$	**16** $R^1 = (CH_2)_2ODMT$ $R^2 = (CH_2)_2OPAM$
 1,4-isomer	 1,5-isomer	 1,8-isomer	 2,6-isomer

Table 2. Melting temperatures of hybrids containing pairs of identical anthraquinone building blocks.

	Oligonucleotide	T_m[a] [°C]	ΔT_m[b] [°C]
17 **18**	(5′) AGC TCG GTC ATC GAG AGT GCA (3′) TCG AGC CAG TAG CTC TCA CGT	71.4	–
19 **20**	(5′) AGC TCG GTC A**H$_{14}$**C GAG AGT GCA (3′) TCG AGC CAG T**H$_{14}$**G CTC TCA CGT	66.0	–5.4
21 **22**	(5′) AGC TCG GTC A**H$_{15}$**C GAG AGT GCA (3′) TCG AGC CAG T**H$_{15}$**G CTC TCA CGT	66.2	–5.2
23 **24**	(5′) AGC TCG GTC A**H$_{18}$**C GAG AGT GCA (3′) TCG AGC CAG T**H$_{18}$**G CTC TCA CGT	65.9	–5.5
25 **26**	(5′) AGC TCG GTC A**H$_{26}$**C GAG AGT GCA (3′) TCG AGC CAG T**H$_{26}$**G CTC TCA CGT	76.6	+5.2
 **H14**	 **H15**	 **H18**	 **H26**	

[a] Conditions: 1.0 μM oligonucleotide concentration (each strand), 10 mM phosphate buffer (pH 7.4) and 100 mM NaCl. [b] Difference in T_m relative to reference duplex **17*18**.

chosen for the study. Representative for all four isomers, the synthesis of the 1,4-substituted phosphoramidite building block is shown in Scheme 1. Introduction of the linkers was done by treatment with 2-chloroethanol in the presence of potassium carbonate and potassium iodide following a similar method described in the literature.[60] The obtained bis-(hydroxyethylated) compounds **5**–**8** were converted into the monoprotected derivatives **9**–**12** by reaction with 4,4′-dimethoxytrityl chloride. Phosphitylation under conventional conditions yielded the phosphoramidite derivatives **13**–**16**. The structures of all anthraquinone derivatives are shown in Table 1. The phosphoramidites were subsequently incorporated into oligonucleotides by using the phosphoramidite procedure.[61,62] Deprotection (conc. NH_3, 50 °C), followed by HPLC purification yielded oligonucleotides **17**–**26** (Table 2). The correct molecular weights of all oligomers were verified by mass spectrometry (Supporting Information).

Influence of Anthraquinone Building Blocks on Hybrid Stability

The effect of the four non-nucleosidic building blocks on the stability of the duplex was analyzed by thermal denatur-

ation experiments. As shown in Table 2, incorporation of a pair of 1,4-, 1,5- or 1,8-derivatives in each strand (**19*20**, **21*22** and **23*24**) results in a considerable decrease in hybrid stability. All three regioisomeric modifications reduce the melting temperature (T_m) by approximately 5 °C (ΔT_m = –5.4, –5.2 and –5.5 °C, respectively). In contrast, incorporation of a pair of the 2,6-isomer in opposite positions results in a significant increase in stability (ΔT_m = +5.2 °C). Circular dichroism (CD) spectral analysis of anthraquinone hybrids **19*20**, **21*22**, **23*24** and **25*26** are consistent with a DNA *B*-conformation (Figure 1).

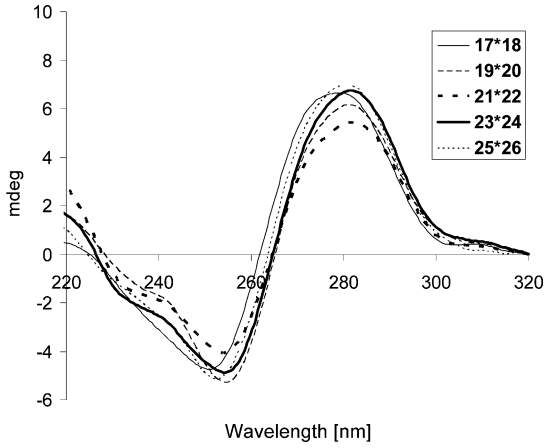


Figure 1. CD spectra of hybrids **19*20**, **21*22**, **23*24** and **25*26**. Conditions: see Table 2.

Because of the well-known property of anthraquinones to intercalate into DNA, we calculated possible models containing the building blocks in an interstrand stacked arrangement. The minimization process was done with amber force field[63] by considering the two anthraquinone moieties and one base pair on each side. After minimization, the remaining natural bases were attached by using B-DNA parameters. The hybrid models thus obtained are depicted in Figure 2. As can be seen, only the 2,6-linked anthraquinone units are arranged in a face-to-face stacking mode. Stacking of the anthraquinone moieties in the hybrids containing the other isomeric building blocks seems much less favourable. Although this would be in agreement with the large differences observed in the stabilities of the duplex, alternative structures can, of course, not be excluded. In fact, a recent crystal structure of a bis(alkoxy)anthraquinone revealed that crystal packing is stabilized by intermolecular C–H···O nonclassical hydrogen bonds, whereas no π–π stacking interactions were observed.[64]

We subsequently studied the effect of mixed pairs, in which the most stable 2,6-isomer was placed opposite to one of the other isomeric building blocks. The T_m values are shown in Table 3. For all hybrids (**25*20**, **25*22** and **25*24**) a decrease in stability (ΔT_m = –2.8, –0.8, –5.3 °C, respectively) relative to the unmodified duplex was observed. The decrease in stability, however, was relatively small for hybrids **25*20** and **25*22** (cf. Table 2, ΔT_m values of –5.4 and –5.2 °C, for the respective hybrids with identical anthraquinone modifications), which further illustrates the

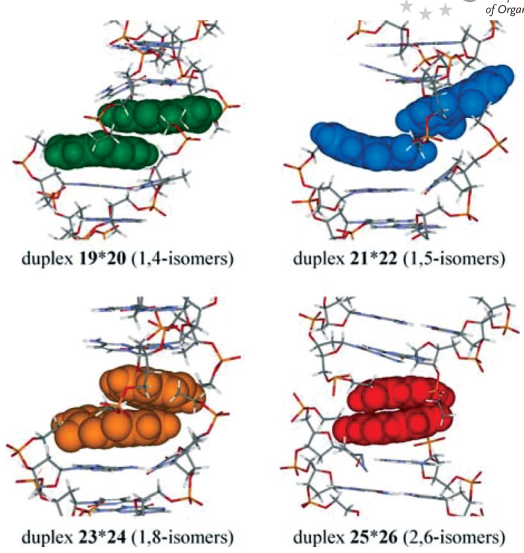


Figure 2. Amber-minimized models of DNA hybrids containing isomeric anthraquinone building blocks as indicated. Anthraquinone units are shown in space-filling representation.

stabilizing effects of the 2,6-isomer. In agreement with previous observations with non-nucleosidic phenanthrene building blocks,[11] a significant destabilization was observed with all regioisomeric anthraquinone moieties if placed opposite to a thymidine residue. Anthraquinone groups had no stabilizing effect on DNA with an abasic site (see Supporting Information). This is surprising, as several reports exist in the literature describing a significant structural stabilization of abasic site-containing DNA.[26,65–67]

Table 3. Melting temperatures in hybrids containing mixed pairs of anthraquinone units.

	Oligonucleotide	T_m[a] [°C]	ΔT_m[b] [°C]
17	(5′) AGC TCG GTC ATC GAG AGT GCA	71.4	–
18	(3′) TCG AGC CAG TAG CTC TCA CGT		
25	(5′) AGC TCG GTC A**H$_{26}$**C GAG AGT GCA	68.6	–2.8
20	(3′) TCG AGC CAG T**H$_{14}$**G CTC TCA CGT		
25	(5′) AGC TCG GTC A**H$_{26}$**C GAG AGT GCA	70.6	–0.8
22	(3′) TCG AGC CAG T**H$_{15}$**G CTC TCA CGT		
25	(5′) AGC TCG GTC A**H$_{26}$**C GAG AGT GCA	66.3	–5.3
24	(3′) TCG AGC CAG T**H$_{18}$**G CTC TCA CGT		

[a] Conditions: 1.0 μM oligonucleotide concentration (each strand), 10 mM phosphate buffer (pH 7.4) and 100 mM NaCl. [b] Difference in T_m relative to reference duplex **17*18**.

Fluorescence Quenching by Anthraquinone Building Blocks

Anthraquinones have been described as nonfluorescent quenchers and are, therefore, of interest for applications in diagnostic tools.[38,56] The four different anthraquinone building blocks were investigated for their influence on

pyrene excimer fluorescence. As shown in Figure 3, all four isomers have a significant quenching effect if placed opposite to two pyrene building blocks. Single strand **27** containing two pyrene moieties next to each other shows pyrene excimer fluorescence with a maximum at 505 nm.[68] Complementary single strands **20**, **22**, **24** and **26** with the different anthraquinone moieties opposite the two pyrene units led to significant reduction (60 to 70%) in the fluorescence signal. The values are given in Table 4 along with the T_m data of the different hybrids. The combination of a fluorophore with a quencher is used in many types of molecular probes. Efficient quenching of excimer fluorescence is difficult to achieve.[69,70] Excimer quenching upon hybrid formation with interstrand stacking modifications opens a new way for the design of DNA-based probes.

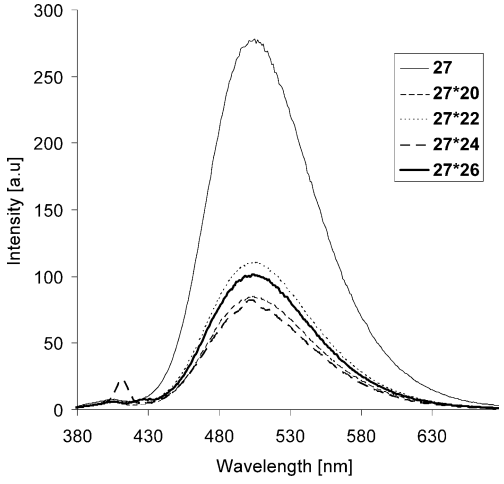
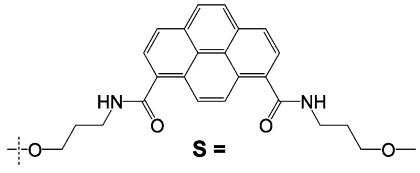


Figure 3. Fluorescence spectra of single strand **27** containing two pyrene units and that of hybrids between **27** and complementary strands containing the different anthraquinone building blocks. Conditions: see Table 2.

Table 4. Quenching of pyrene excimer fluorescence by the different anthraquinone building blocks.

	Oligonucleotide	T_m[a] [°C]	ΔT_m[b] [°C]	Q[c] [%]
27	(5′) AGC TCG GTC **S S** C GAG AGT GCA	64.0	–7.4	60
20	(3′) TCG AGC CAG T**H$_{14}$**G CTC TCA CGT			
27	(5′) AGC TCG GTC **S S** C GAG AGT GCA	64.6	–6.8	64
22	(3′) TCG AGC CAG T**H$_{15}$**G CTC TCA CGT			
27	(5′) AGC TCG GTC **S S** C GAG AGT GCA	64.9	–6.5	70
24	(3′) TCG AGC CAG T**H$_{18}$**G CTC TCA CGT			
27	(5′) AGC TCG GTC **S S** C GAG AGT GCA	66.3	–5.1	71
26	(3′) TCG AGC CAG T**H$_{26}$**G CTC TCA CGT			



[a] Conditions: 1.0 μM oligonucleotide concentration (each strand), 10 mM phosphate buffer (pH 7.4) and 100 mM NaCl. [b] Difference in T_m relative to reference duplex **17*18**. [c] Reduction of excimer fluorescence relative to single-strand **27**.

Conclusions

Four isomeric anthraquinone building blocks differing in the attachment site of the linker were synthesized and incorporated into oligodeoxynucleotides. The site of linker attachment was found to have a strong influence on duplex stability. Hybrids containing a pair of the 1,4-, 1,5- and 1,8-isomers led to substantial reduction in the T_m value (ΔT_m = –5.4, –5.2 and –5.5 °C, respectively). In contrast, the 2,6-isomer resulted in a considerable increase in stability (ΔT_m = +5.2 °C). Hybrids containing mixed pairs of isomeric anthraquinone moieties show moderate-to-significant destabilization. Molecular models suggest that the positive effect of the 2,6-isomer is a result of interstrand stacking interactions between the two anthraquinone units. In the case of the other isomers, stacking interactions seem much less favourable. All anthraquinone building blocks act as fluorescence quenchers. If placed opposite to two pyrene building blocks, excimer fluorescence is quenched by 60–70%. The anthraquinone derivatives described here extend the set of artificial building blocks with potential application in diagnostics or in DNA-based nanomaterials.

Experimental Section

General: Reactions were carried out under a nitrogen atmosphere by using distilled, anhydrous solvents. Flash column chromatography (CC) was performed by using silica gel 60 (63–32 μM, Chemie Brunschwig AG). If compounds were sensitive to acid, the silica was pretreated with solvent containing 1% Et_3N. All NMR spectra were measured at room temperature with a Bruker AC-300 spectrometer. ^{1}H NMR chemical shifts (δ) are reported in ppm relative to the residual undeuterated solvent ($CDCl_3$: 7.27 ppm). Multiplicities are abbreviated as follows: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet. ^{13}C NMR chemical shifts are reported in ppm relative to the residual nondeuterated solvent ($CDCl_3$: 77.00 ppm). ^{31}P NMR chemical shifts are reported in ppm relative to 85% H_3PO_4 as an external standard. Electrospray ionization (ESI) mass spectra were recorded with VG platform Fisons instruments.

General Method for Bis(hydroxyethylation) of Dihydroxyanthraquinones (5–8): A solution of dihydroxyanthraquinone (5.0 g, 20.0 mmol) was dissolved in DMF (100 mL). Potassium carbonate (27.7 g, 200 mmol) was added to the mixture. The mixture was stirred for 2 h at 120 °C. 2-Chloroethanol (27 mL, 400 mmol) and potassium iodide (6.65 g, 40.0 mmol) were added to the mixture. The mixture was stirred at 120 °C overnight and then cooled to room temperature and concentrated. Water (100 mL) was added to the residue, and the mixture was extracted with CH_2Cl_2/2-propanol (3:1). The organic phase was washed again with water, dried with magnesium sulfate and evaporated under reduced pressure. The crude product was purified by column chromatography (silica gel;

AcOEt + 10% MeOH). The fractions were combined and concentrated.

1,4-Bis[(hydroxyethyl)oxy]anthraquinone (5): Yield: 2.54 g (38%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 3.99 (t, J = 4.4 Hz, 4 H), 4.29 (t, J = 4.2 Hz, 4 H), 7.37 (s, 2 H), 7.74 (dd, J = 5.9, 3.4 Hz, 2 H), 8.18 (dd, J = 5.8, 3.4 Hz, 2 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 61.16, 73.34, 124.23, 126,90, 127.11, 134.07, 134.37, 154.79, 184.36 ppm. MS (ESI): m/z = 328 $[M]^+$. $C_{18}H_{16}O_6$ M_W = 328.09.

1,5-Bis[(hydroxyethyl)oxy]anthraquinone (6): Yield: 1.82 g (27%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 4.02 (t, J = 4.4 Hz, 4 H), 4.31 (t, J = 4.4 Hz, 4 H), 7.30 (dd, J = 8.3, 1.1 Hz, 2 H), 7.70 (t, J = 8.1 Hz, 2 H), 7.93 (dd, J = 6.6, 1.1 Hz, 2 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 60.77, 72.11, 119.34, 120.17, 120.79, 135.26, 136.96, 159.36, 182.8 ppm. MS (ESI): m/z = 328 $[M]^+$. $C_{18}H_{16}O_6$. M_W = 328.09.

1,8-Bis[(hydroxyethyl)oxy]anthraquinone (7): Yield: 1.93 g (29%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 4.03 (t, J = 4.1 Hz, 4 H), 4.32 (t, J = 4.2 Hz, 4 H), 7.34 (dd, J = 8.4, 1.0 Hz, 2 H), 7.68 (t, J = 7.2 Hz, 2 H), 7.91 (dd, J = 7.7, 1.1 Hz, 2 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 60.77, 72.53, 119.61, 120.17, 121.20, 134.47, 135.02, 159.00, 182.82, 184.15 ppm. MS (ESI): m/z = 328 $[M]^+$. $C_{18}H_{16}O_6$. M_W = 328.09.

2,6-Bis[(hydroxyethyl)oxy]anthraquinone (8): Yield: 1.98 g (30%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 4.04 (t, J = 3.8 Hz, 4 H), 4.29 (t, J = 4.3 Hz, 4 H), 7.29 (m, 2 H), 7.74 (m, 2 H), 8.26 (m, 2 H) ppm. MS (ESI): m/z = 328 $[M]^+$. $C_{18}H_{16}O_6$. M_W = 328.09.

General Method for 4,4′-Dimethoxytrityl (DMT) Protection of Bis[(hydroxyethyl)oxy]anthraquinones (9–12): The diol (1.0 g, 3.0 mmol) was dissolved in absolute pyridine (8 mL). 4,4′-Dimethoxytrityl chloride (1 g, 3.0 mmol) dissolved in absolute pyridine (8 mL) was added dropwise. After stirring at room temperature for 6 h, saturated aqueous sodium hydrogen carbonate solution was added. After extraction with dichloromethane and concentration under reduced pressure, the product was purified by column chromatography (silica gel; AcOEt + 1% TEA). The fractions were combined and concentrated.

1-({[(4,4′-Dimethoxytrityl)oxy]ethyl}oxy)-4-[(hydroxyethyl)oxy]-anthraquinone (9): Yield: 0.66 g (35%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 3.78 (s, 6 H), 3.98 (m, 4 H), 4.28 (m, 4 H), 6.83 (m, 4 H), 7.18 (m, 2 H), 7.28 (m, 3 H), 7.37 (m, 4 H), 7.48 (m, 2 H), 7.73 (m, 2 H), 8.18 (m, 2 H) ppm.

1-({[(4,4′-Dimethoxytrityl)oxy]ethyl}oxy)-5-[(hydroxyethyl)oxy]-anthraquinone (10): Yield: 0.59 g (31%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 3.79 (s, 6 H), 4.01 (m, 4 H), 4.31 (m, 4 H), 6.84 (m, 4 H), 7.15 (m, 1 H), 7.30 (m, 4 H), 7.42 (m, 4 H), 7.50 (m, 2 H), 7.70 (m, 2 H), 7.95 (m, 2 H) ppm.

1-({[(4,4′-Dimethoxytrityl)oxy]ethyl}oxy)-8-[(hydroxyethyl)oxy]-anthraquinone (11): Yield: 0.70 g (37%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 3.78 (s, 6 H), 3.82 (m, 4 H), 4.26 (m, 4 H), 6.85 (m, 4 H), 7.15 (m, 1 H), 7.30 (m, 4 H), 7.38 (m, 4 H), 7.48 (m, 2 H), 7.62 (m, 2 H), 7.88 (m, 2 H) ppm.

2-({[(4,4′-Dimethoxytrityl)oxy]ethyl}oxy)-6-[(hydroxyethyl)oxy]-anthraquinone (12): Yield: 0.49 g (26%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 3.78 (s, 6 H), 4.05 (m, 4 H), 4.28 (m, 4 H), 6.82 (m, 4 H), 7.20 (m, 1 H), 7.28 (m, 4 H), 7.35 (m, 4 H), 7.46 (m, 2 H), 7.74 (m, 2 H), 8.25 (m, 2 H) ppm.

General Method for the Phosphitylation of Monoprotected Anthraquinones (13–16): The alcohol (0.50 g, 0.79 mmol) and ethyldiisopropylamine (0.25 g, 2.0 mmol) were dissolved in dichloromethane (10 mL). 2-Cyanoethyl diisopropylamidochloridophosphite (0.225 g, 0.95 mmol) dissolved in dichloromethane (5 mL) was added dropwise. The reaction mixture was stirred at room temperature for 2 h. The resulting mixture was directly applied on a silica-gel column for purification (AcOEt + 1% TEA).

1-[({[(2-Cyanoethoxy)(diisopropylamino)phosphanyl]oxy}ethyl)oxy]-4-({[(4,4′-dimethoxytrityl)oxy]ethyl}oxy)anthraquinone (13): Yield: 0.35 g (53%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.17 (m, 12 H), 2.64 (m, 2 H), 3.58 (m, 2 H), 3.59 (m, 2 H), 3.69 (m, 2 H), 3.79 (s, 6 H), 3.86 (m, 2 H), 4.35 (m, 4 H), 6.83 (m, 4 H), 7.24 (m, 1 H), 7.28 (m, 4 H), 7.39 (m, 4 H), 7.50 (m, 2 H), 7.65 (m, 2 H), 7.87 (m, 2 H) ppm. ^{31}P NMR (122 MHz, $CDCl_3$): δ = 149.11 ppm.

1-({[(2-Cyanoethoxy)(diisopropylamino)phosphanyl]oxy}ethyl)oxy]-5-({[(4,4′-dimethoxytrityl)oxy]ethyl}oxy)anthraquinone (14): Yield: 0.39 g (60%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.18 (m, 12 H), 2.64 (m, 2 H), 3.59 (m, 4 H), 3.70 (m, 2 H), 3.79 (s, 6 H), 3.89 (m, 2 H), 4.33 (m, 4 H), 6.84 (m, 4 H), 7.24 (m, 1 H), 7.28 (m, 4 H), 7.40 (m, 4 H), 7.51 (m, 2 H), 7.66 (m, 2 H), 7.87 (m, 2 H) ppm. ^{31}P NMR (122 MHz, $CDCl_3$): δ = 149.10 ppm.

1-({[(2-Cyanoethoxy)(diisopropylamino)phosphanyl]oxy}ethyl)oxy]-8-({[(4,4′-dimethoxytrityl)oxy]ethyl}oxy)anthraquinone (15): Yield: 0.35 g (53%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.18 (m, 12 H), 2.64 (m, 2 H), 3.59 (m, 4 H), 3.70 (m, 2 H), 3.79 (s, 6 H), 3.89 (m, 2 H), 4.33 (m, 4 H), 6.84 (m, 4 H), 7.24 (m, 1 H), 7.28 (m, 4 H), 7.40 (m, 4 H), 7.51 (m, 2 H), 7.66 (m, 2 H), 7.87 (m, 2 H) ppm. ^{31}P NMR (122 MHz, $CDCl_3$): δ = 148.86 ppm.

2-({[(2-Cyanoethoxy)(diisopropylamino)phosphanyl]oxy}ethyl)oxy]-6-({[(4,4′-dimethoxytrityl)oxy]ethyl}oxy)anthraquinone (16): Yield: 0.25 g (38%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.19 (m, 12 H), 2.65 (m, 2 H), 3.55 (m, 2 H), 3.61 (m, 2 H), 3.78 (s, 6 H), 3.79 (m, 2 H), 4.01 (m, 2 H), 4.33 (m, 4 H), 6.84 (m, 4 H), 7.22 (m, 1 H), 7.28 (m, 4 H), 7.34 (m, 4 H), 7.46 (m, 2 H), 7.73 (m, 2 H), 8.25 (m, 2 H) ppm. ^{31}P NMR (122 MHz, $CDCl_3$): δ = 148.86 ppm.

Synthesis and Analysis of Oligonucleotides: Cyanoethyl phosphoramidites from Transgenomic (Glasgow, UK) were used for oligonucleotide synthesis. Oligonucleotides **17**–**27** were prepared by automated oligonucleotide synthesis by a standard synthetic procedure ("trityl-off" mode) with a 394-DNA/RNA synthesizer (Applied Biosystems). Cleavage from the solid support and final deprotection was done by a treatment with 33% aqueous NH_3 at 55 °C overnight. All oligonucleotides were purified by ion exchange HPLC (Tricorn column SOURCE 15Q 4.6/100 PE 100 15 μm, Merck, L-6250 Intelligent Pump); eluent A = Na_2HPO_4 (20 mM), pH 11.5; eluent B = Na_2HPO_4 (20 mM) + NaCl (2 M), pH 11.5; gradient 0–60% B over 30 min at 25 °C. MS (ESI, negative mode, CH_3CN/H_2O/TEA) of oligonucleotides was performed with a Sciex QSTAR pulsar, (hybrid quadrupole time-of-flight mass spectrometer, Applied Biosystems); data of oligomers **17**–**27** are given in the Supporting Information.

Thermal Denaturation Experiments: Carried out with a Varian Cary-100 Bio-UV/Vis spectrometer equipped with a Varian Cary-block temperature controller. Data were collected with Varian WinUV software at 260 nm (cooling–heating–cooling cycles in the temperature range of 10–90 °C, temperature gradient of 0.5 °C min^{-1}). Experiments were carried out for 1.0-μM oligonucleotide concentration (each strand), 10-mM phosphate buffer and 100-mM NaCl at pH 7.4. Data were analyzed with Kaleidagraph software from Synergy software. Melting temperature (T_m) values were determined as the maximum of the first derivative of the smoothed melting curve.

Fluorescence Data: Collected for 1.0-μM oligonucleotide solutions (1.0 μM of each strand in case of double strands) in phosphate

buffer (10 mM) and NaCl (100 mM) at pH 7.4 with a Varian Cary Eclipse fluorescence spectrophotometer equipped with a Varian Cary-block temperature controller (excitation at 354 nm, excitation and emission slit width 10 and 5 nm, respectively).

CD Spectra: Recorded with a JASCO J-715 spectrophotometer by using quartz cuvettes with an optical path of 1 cm.

Modelling: Structures of DNA hybrids containing anthraquinone units were minimized by using the Amber force field (Hyperchem 7.0). Initial energy minimization was carried out with two stacked anthraquinones. Subsequently, two natural base pairs were added on both ends of the anthraquinone building blocks. After minimization, the remaining natural bases were added by using B-DNA parameters.

Supporting Information (see footnote on the first page of this article): Mass spectrometry data of oligonucleotides **17**–**26** and **28**; absorption spectra of anthraquinone diols **5**–**8**; additional thermal denaturation experiments.

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