Intercalating Nucleic Acids with Insertion of 5-[(Pyren-1-yl)methylidene]hydantoin-Substituted Butane-1,2-diol

by **Youssef Lotfy Aly**[1]), **Michael Wamberg**, and **Erik B. Pedersen***

Nucleic Acid Center[2]), Department of Chemistry, University of Southern Denmark, Campusvej 55, DK-5230 Odense M
(phone: +45 65502555; fax: +45 66158780; e-mail: ebp@chem.sdu.dk)

Isopropylidene-protected (*S*)-4-*O*-(methylsulfonyl)butane-1,2,4-triol was used for alkylation of 5-[(pyren-3-yl)methylidene]hydantoin to give the N^3-monoalkylated product **4** in 29% yield together with a dialkylated product **5** in 12% yield. After deprotection, compound **4** was transformed into a dimethoxytrityl (DMT)-protected phosphoramidite building block **9** for standard DNA synthesis. When inserted as a bulge in the triplex-forming oligomer, compound **6** stabilizes a DNA triplex, whereas the corresponding DNA/DNA and DNA/RNA duplexes are slightly destabilized. For the triplex, fluorescence enhancement was observed at 500 nm.

1. Introduction. – In recent years, much effort has been put into the design and synthesis of new oligonucleotides in order to improve the hybridization affinity towards complementary DNA or RNA sequences. By chemical modification of the nucleosides, oligonucleotide analogues with better affinity towards single-stranded RNA (ssRNA) and towards single-stranded DNA (ssDNA) were discovered; for example, locked nucleic acid (LNA) [1], hexitol nucleic acid (HNA) [2], and other derivatives were made with a modified phosphodiester backbone. These include peptide nucleic acid (PNAs) [3], phosphoramidate ($^{3'}$N–$^{5'}$P) oligonucleotides [4], and morpholino-containing analogs [5]. Nucleic acid duplexes are stabilized both by H-bonding interactions and by base stacking [6]. Thus, hydrophobic polyaromatic rings with a large surface area can replace the natural nucleic base for mimicking base stacking in *Watson–Crick/Hoogsteen* base pairing [7].

The design and the synthesis of various oligonucleotides containing planar polycyclic aromatic chromophores such as pyrene, anthracene, and others, which have longer fluorescence lifetimes and are able to form π-stacking interactions in aqueous solution, have been the subject of active research in recent years [8]. Pyrene is an excimer-forming molecule that has been incorporated into oligodeoxynucleotides (ODNs) by several groups. *Ebata et al.* incorporated a pyrene-modified nucleotide in the 5′-end of one ODN and a pyrene-modified nucleotide into the 3′-end of another [9]. *Christensen et al.* [10] [11] reported that a 1-*O*-[(pyren-1-yl)methyl]glycerol can be inserted into oligo-

[1]) On leave from Chemistry Department, Faculty of Education, Kafr El-Sheikh branch Tanta University, Egypt.

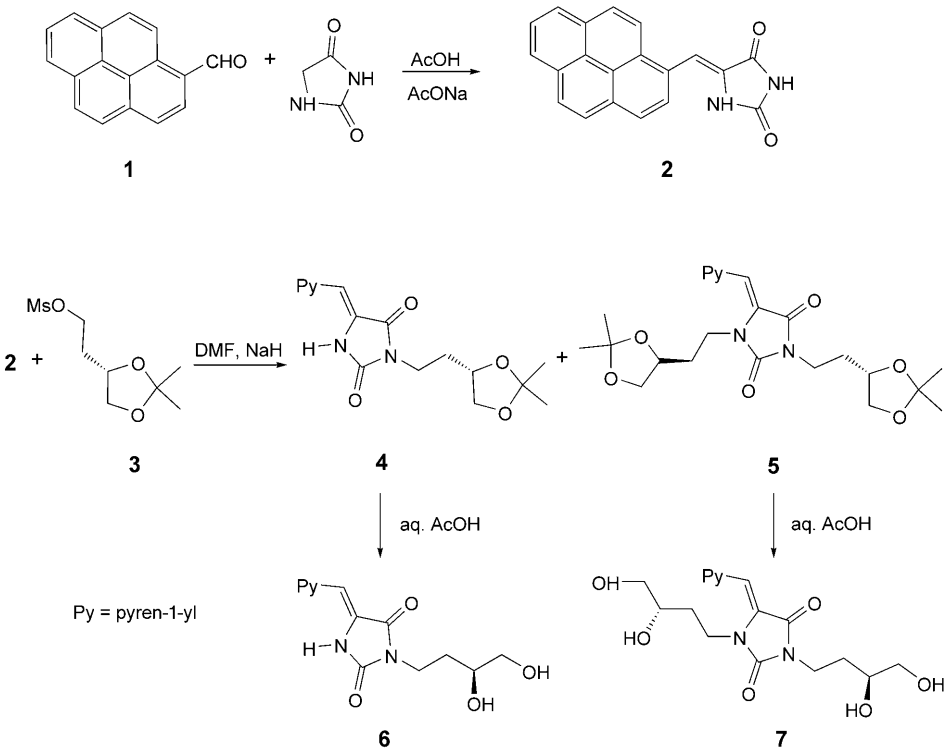
[2]) A research center funded by the *Danish National Research Foundation* for studies on nucleic acid chemical biology.

nuleotides to give higher affinity for complementary ssDNA than unmodified DNA. They found an optimal length of the linker to the intercalator to obtain a large increase in affinity between the intercalating nucleic acid and the target DNA sequences. It could be an advantage to extend this approach to intercalators showing fluorescence at longer wavelength than pyrene. In this work, we have, therefore, synthesized a (pyren-1-yl)hydantoin DNA building block to investigate this possibility.

2. Results and Discussion. – 2.1. *Chemistry.* The (pyren-1-yl)hydantoin derivative **2** was prepared in 27% yield by reacting pyrene-1-carbaldehyde (**1**) and hydantoin (=imidazolidine-2,4-dione) in glacial AcOH and freshly fused AcONa. Reaction of the sodium salt of **2** with 2-[(*S*)-2,2-dimethyl[1,3]dioxolan-4-yl]ethyl methanesulfonate (**3**) [12] in dry DMF under N_2 afforded the monoalkylated imidazolidine-2,4-dione **4** as the major product and the bisalkylated derivative **5** as a minor product. Deprotections of **4** and **5** were performed by treatment with 80% aqueous AcOH at room temperature to afford the hydroxy derivatives **6** and **7**, respectively (*Scheme 1*). As expected, no NOE effect was obtained in *N*-CH_2 upon irradiation of the vinylic H-atom of compound **6** which is in conformity with N^3-monoalkylation.

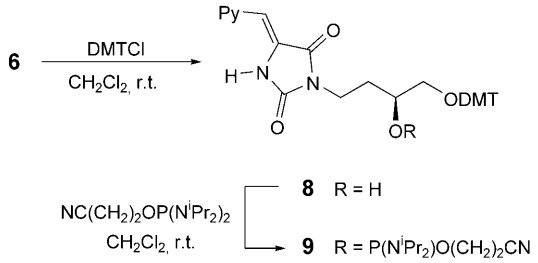
The primary OH group of compound **6** was protected by reaction with 4,4′-dimethoxytrityl chloride (DMTCl) in CH_2Cl_2 at room temperature under N_2 to give the DMT-protected compound **8**, which was converted to the phosphoramidite **9** with (2-

Scheme 1



cyanoethyl)-*N,N,N′,N′*-tetraisopropylphosphordiamidite (S*cheme 2*). This amidite was used for the synthesis of oligonucleotides, which all showed correct masses in MALDI-TOF-MS analysis (*Table 1*).

Scheme 2



Py = pyren-1-yl; DMT = 4,4'-dimethoxytrityl

Table 1. *Masses of the Oligonucleotides.* **X** = Compound **6**.

Oligonucleotide	Sequence	Expected mass	MALDI-TOF Analysis
Phy 1	3′-TCGAAC **X** GAACTC-5′	4074	4073
Phy 2	5′-AGCTTG **X** CTTGAG-3′	4136	4136
Phy 3	5′-CCCCTT **X** TCTTTTTT-3′	4581	4580
Phy 4	5′-**X**CCCCTTTCTTTTTT-3′	4581	4580
Phy 5	5′-CCCCTT **X**CTTTTTT-3′	4277	4276

The amidite **9** was used for insertion of the monomer **6** into DNA, and the hybridization properties of the modified oligonucleotides towards complementary DNA and RNA were evaluated by thermal denaturation studies. The melting temperatures (T_m), determined as the maxima of the first derivatives of the melting curve, are listed in *Tables 2–4* together with the differences in the melting temperature (ΔT_m) between modified and unmodified oligomers in the melting temperatures.

The (pyren-1-yl)hydantoin derivative **6** was found to destabilize the DNA/DNA duplex by $-2°$ to $-6°$ when inserted as a bulge into the central region of the sequence (*Table 2*, *Entries 2* and *3*). However, contrary to the corresponding 1-*O*-[(pyren-1-yl)methyl]glycerol, two opposing zipping moieties of **6** (*Entry 4*) were found to stabilize the duplex by 4.5° like what has been found by *Brotschi* and *Leumann* for zipping of biphenyl units [13].

Next, it was investigated how the (pyren-1-yl)hydantoin derivative behaves as a general base (*Table 2*, *Entries 5–8*). Indeed, rather a low fluctuation was observed in the melting temperature, when **6** was opposing the natural bases A, C, G, and T. This is considered an interesting finding because this was combined with high thermal stabilities of the duplexes.

In *Table 3,* the ability of the (pyren-1-yl)hydantoin derivative **6** to stabilizes triplexes is reported. Compound **6** stabilizes a triplex at pH 6 by 6.5° when inserted as bulge (*Entry 3*), probably by stacking to all three strands due to the size of the interca-

Table 2. *Melting Temperatures of DNA/DNA Duplexes with Compound* **6** *Inserted as a Bulge or as a Universal Base at pH 7.0*

		5′-AGCTTG**Y**CTTGAG-3′ 3′-TCGAAC**X**GAACTC-5′		
Entry	**Y**	**X**	T_m [°]	ΔT_m [°]
1	–	–	48	–
2	–	6	46	−2
3	6	–	42	−6
4	6	6	52.5	4.5
5	A	6	52	
6	C	6	55	
7	G	6	51	
8	T	6	54	

Table 3. *Melting Temperatures of DNA Triplexes with Compound* **6** *Inserted as a Bulge, a Dangling End, or a Universal Base.* **X** = Compound **6**.

Entry		Buffer 1 (pH 6.0) T_m [°]	Buffer 2 (pH 7.2) T_m [°]	Parallel duplex[a]) (pH 6.0) T_m [°]
1	Target DNA 3′-CTG CCC CTT TCT TTT TT-5′ 5′-GAC GGG GAA AGA AAA AA-3′ TFO[b]):	55.5	55.6	
2	5′-CCC CTT TCT TTT TT-3′	25.5	< 5.0	16
3	5′-CCC CTT **X**TCT TTT TT-3′	32	23	27
4	5′-**X**CCC CTT TCT TTT TT-3′	38.5	15	28
5	5′-CCC CTT **X**CT TTT TT-3′	< 5.0	< 5.0	14

[a]) Formation of a duplex with 5′-GAC GGG GAA AGA AAA AA-3′. [b]) Triplex-forming oligodeoxynucleotide.

lator. The size of the intercalator is considered important as the smaller 1-*O*-[(pyren-1-yl)methyl]glycerol derivative was destabilizing a triplex when inserted as a bulge. A further advantage of **6** could be that it is a conjugated system where twisting can be tolerated at the single bond linking the pyrene and hydantoin moieties. This may allow the intercalator to be adopted for intercalation in a non-planar fashion. In NMR studies, a similar propeller twisting has been observed for intercalation of TOTO into duplexes [14]. When **6** is added to the 5′-end, the lid effect (*Entry 4*) raises the melting temperature by 13°. On the other hand, compound **6** destabilizes the triplex when inserted as a universal base in the TFO (*Entry 5*). At pH 7.2, compound **6** stabilizes the triplex when inserted as bulge in the central part of the sequence (*Entry 3*) and at the 5′-end (*Entry 4*) when compared with unmodified oligonucleotides. In case of parallel duplexes, **6** also stabilizes the duplexes by 11–12° when inserted as a bulge or at the end of the triplex at pH 6.0 (*Table 3*, *Entries 3* and *4*), whereas it destabilizes the parallel duplex by −2° when inserted as a universal base (*Table 3*, *Entry 5*).

A destabilization of the DNA/RNA duplex by −5° at pH 7.0 was observed when compound **6** was inserted as a bulge (*Table 4*).

Table 4. *Melting Temperatures of DNA/RNA Duplexes with Compound* **6** *Inserted as a Bulge at pH 7.0*

Target DNA/RNA	**X** = – T_m [°]	**X** = **6** T_m [°]	ΔT_m [°]
5′-AGCUUG CUUGAG-3′ 3′-TCGAAC **X** GAACTC-5′	41	36	–5

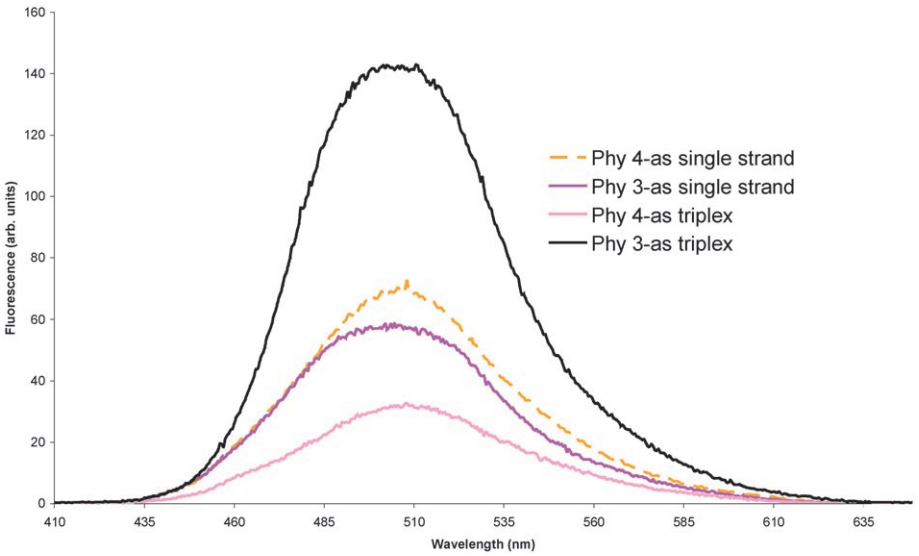


Figure. *Fluorescence measurement of compound* **6** *as single strand and triplex*

2.2. *Fluorescence.* The fluorescence emission upon excitation of **6** inserted into single strands and into triplexes was found at *ca.* 500 nm (*Figure*). The highest fluorescence intensity was recorded for triplexes when **6** was inserted as a bulge in the central part of the sequence, and the lowest fluorescence intensity was recorded when **6** was added to the end of the sequence, whereas intermediate intensities were observed for the single strands. Although fluorescence quenching is expected due to a charge-transfer process upon intercalation into a DNA duplex, the opposite has been observed for TOTO which is nearly non-fluorescent when it is not intercalated into a duplex [14]. We assume that **6** is the first example of a propeller twist molecule which shows fluorescence enhancement upon intercalation into a triplex in analogy with the fluorescence enhancement observed for the dye TOTO, which forms a propeller twist when intercalated into a duplex.

3. Conclusion. – Through melting-temperature studies, we have shown that compound **6** upon insertion as a bulge stabilizes a DNA triplex at pH 6.0 and 7.2 as well as a DNA/DNA parallel duplex at pH 6.0, while it destabilizes an antiparallel DNA/DNA duplex when inserted as a bulge in the central region of the sequence.

Experimental Part

General. TLC: TLC Plates *60 F 254 (Merck)*, visualized by UV light (254 nm). Column chromatography (CC): silica-gel-packed column (silica gel *60*, 0.040 ± 0.063 mm, *Merck*). Solvents used for CC were distilled prior to use, while reagents were used as purchased. NMR Spectra: *Varian Gemini 2000* spectrometer (^{1}H: 300 MHz, ^{13}C: 75.5 MHz, ^{31}P: 121.5 MHz); δ values in ppm relative to Me_4Si as internal standard for ^{1}H-NMR); for ^{13}C-NMR: $CDCl_3$ (δ 77.0) and DMSO (δ 39.44). Accurate ion-mass determinations were performed using the *4.7 T Ultima Fourier Transform* (FT) mass spectrometer (*IonSpec*, Irvine, CA). The $[M+H]^+$ and $[M+Na]^+$ ions were peak-matched using ions derived from the 2,5-dihydroxybenzoic acid matrix. All modified oligonucleotides were confirmed by MALDI-TOF MS analysis on a *Voyager Elite Biospectrometry Research Station* from *PerSeptive Biosystems*.

5-[(Pyren-1-yl)methylidene]imidazolidine-2,4-dione (**2**). *Pyrene-1-carbaldehyde* (**1**; 2.3 g, 0.01 mol), imidazolidine-2,4-dione (1.1 g, 0.011 mol), and fused AcONa (3 g, 0.36 mol) were dissolved in glacial AcOH (50 ml). The mixture was refluxed for 24 h. The solid formed in the hot mixture was filtered off after cooling, washed with EtOH, and dried. The mother layer was refluxed with excess of imidazolidine-2,4-dione (0.55 g, 0.005 mol) and fused AcONa (2 g, 0.24 mol) for another 15 h, and the solid formed was cooled and likewise isolated to yield **2** (0.85 g, 27%). Yellow solid. M.p. 320°–322°. ^{1}H-NMR (DMSO): 7.13 (*s*, CH=C), 8.10–8.39 (*m*, 9 arom. H), 11.01–11.08 (br. *s*, 2 NH). ^{13}C-NMR (DMSO): 104.77 (C(5)); 123.03 (*C*H=C); 123.74, 123.96, 124.98, 125.54, 125.71, 126.46, 126.70, 127.10, 127.27, 127.87, 128.19, 128.63, 130.21, 130.28, 130.69, 130.78 (C(arom)); 155.70, 165.29 (2 CO); HR-MALDI-MS: 335.0794 ($C_{20}H_{12}N_2O_2^+$, $[M+Na]^+$; calc. 335.0791).

Preparation of Compounds **4** *and* **5**. Compound **2** (1.6 g, 5.2 mmol) was dissolved in anh. DMF (20 ml) under N_2 and heated to 60°. NaH (0.21 g, 5.25 mmol, 60% in mineral oil) was added, and the mixture was stirred for 1 h at 60°. NaI (0.78 g, 5.2 mmol) in hot dry DMF (10 ml) was added to the mixture, and *2-[(*S*)-2,2-dimethyl[1,3]dioxolan-4-yl]ethyl methanesulfonate* (**3**; 1.23 g, 5.5 mmol) was added. The mixture was stirred at 80° for 48 h, and the reaction was quenched with 1 ml of MeOH. The solvent was evaporated under reduced pressure, until complete dryness. The residue was extracted with AcOEt (200 ml) and filtered. The filtrate was evaporated under reduced pressure, and the residual material was chromatographed on a silica-gel column with $CHCl_3$/MeOH 9 : 1 (*v*/*v*) to give **4** and **5**.

*3-[2-[(*S*)-2,2-Dimethyl[1,3]dioxolan-4-yl]ethyl]-5-[(pyren-1-yl)methylidene]imidazolidine-2,4-dione* (**4**). Yield: 0.80 g (29%). White solid. M.p. 85°–87°. ^{1}H-NMR ($CDCl_3$): 1.32, 1.39 (2*s*, 2 Me); 3.48–3.53 (*m*, CH_2); 3.65–3.99 (*m*, NCH_2); 3.98–4.02 (*m*, CH_2O); 4.07–4.12 (*m*, OCH); 7.53 (*s*, CH=C); 7.97–8.21 (*m*, 9 arom. H); 8.62 (*s*, NH). ^{13}C-NMR ($CDCl_3$): 25.54, 26.90 (2 Me); 31.90 (CH_2); 35.85 (NCH_2); 69.07 (CH_2O); 73.75 (OCH); 109.11, (Me_2C); 109.44 (C(5)); 122.96 (*C*H=C); 124.36, 124.84, 124.88, 125.59, 125.81, 125.91, 126.35, 126.65, 127.11, 127.68, 128.44, 128.57, 129.49, 130.66, 131.16, 131.73 (C(arom)); 155.22, 163.58 (2 C=O). HR-MALDI-MS: 463.1613 ($C_{21}H_{22}N_2O_4^+$, $[M+Na]^+$; calc. 463.1628).

*1,3-Bis[2-[(*S*)-2,2-dimethyl-[1,3]dioxolan-4-yl]ethyl]-5-[(pyren-1-yl)methylidene]imidazolidine-2,4-dione* (**5**). Yield: 350 mg (12%). Semisolid. ^{1}H-NMR ($CDCl_3$): 1.35, 1.38, 1.43, 1.46 (4*s*, 4 Me); 3.27–3.29 (*m*, 2 CH_2); 3.59–3.64 (*m*, 2 NCH_2); 3.81–3.84 (*m*, 2 CH_2O); 4.10–4.15 (*m*, 2 OCH); 7.54 (*s,* CH=C); 7.86–8.22 (*m*, 9 arom. H). ^{13}C-NMR ($CDCl_3$): 25.21, 25.64, 26.18, 26.99 (4 Me); 31.21, 31.18 (2 CH_2); 36.35, 39.18 (2 NCH_2); 68.9, 69.13 (2 CH_2O); 73.35, 73.92 (2 OCH); 108.92, 109.15 (Me_2C); 110.50 (C(5)); 123.90 (*C*H=C); 124.04, 124.40, 124.48, 125.75, 125.85, 126.39, 127.05, 127.13, 127.18, 128.22, 128.57, 129.47, 129.82, 130.73, 131.21, 131.49 (C(arom)); 155.47, 163.65 (2 C=O). MALDI-MS*:* 591 ($C_{34}H_{36}N_2O_6^+$, $[M+Na]^+$).

Deprotection of Compounds **4** *and* **5** (*Formation of* **6** *and* **7***, Resp.*). Compound **4** or **5** (1.00 mmol) was dissolved in 80% AcOH (10 ml), and the soln. was stirred overnight at r.t. The solvent was evaporated under reduced pressure, and the residue was co-evaporated with H_2O (3 × 5 ml), and finally with EtOH (3 × 5 ml). The products were purified by CC (silica gel; $CHCl_3$/MeOH 9 : 1 (*v*/*v*)) to give **6** and **7**.

*3-[(*S*)-3,4-Dihydroxybutyl]-5-[(pyren-1-yl)methylidene]imidazolidine-2,4-dione* (**6**). Yield: 290 mg (68%). White solid. M.p. 231°–233°. ^{1}H-NMR (DMSO): 1.87–1.91 (*m*, CH_2); 1.93, 2.51 (br. *s*, 2 OH); 3.37–3.41 (*m*, NCH_2); 3.56–3.59 (*m*, CH_2OH); 4.60–4.67 (*m*, *C*HOH); 7.40 (*s*, CH=C); 8.07–8.38 (*m*, 9 arom. H); 10.94 (*s*, NH). ^{13}C-NMR (DMSO): 31.90 (CH_2); 35.65 (NCH_2); 65.67 (CH_2OH); 69.25 (CHOH); 105.81 (C(5)); 123.04 (*C*H=C); 123.69, 123.94, 124.96, 125.57, 125.74, 126.45, 126.76, 126.94, 127.24, 127.92, 128.21, 128.67, 128.85, 130.26, 130.75, 130.76 (C(arom)); 155.18, 163.80 (2 C=O). HR-MALDI-MS: 423.1315 ($C_{18}H_{18}N_2O_4^+$, $[M+Na]^+$; calc. 423.1306).

*1,3-Bis[(*S*)-3,4-dihydroxybutyl]-5-[(pyren-1-yl)methylidene]imidazolidine-2,4-dione* (**7**). Yield: 310 mg (63%). Semisolid. ^{1}H-NMR ($CDCl_3$): 1.71–1.76 (*m,* 2 CH_2); 2.79–2.98 (br. *s*, 4 OH); 3.67–3.66 (*m*, 2 NCH_2); 3.77–3.93 (*m*, 2 CH_2OH); 4.09–4.13 (*m*, 2 C*H*OH); 7.43 (*s*, CH=C); 7.91–8.27 (*m*, 9 arom. H). ^{13}C-NMR

($CDCl_3$): 30.06, 31.27 (2 CH_2); 36.12, 38.78 (2 NCH_2); 65.98, 66.31 (2 CH_2OH); 69.44, 69.53 (2 CHOH); 111.89 (C(5)); 123.69 (*C*H=C); 123.85, 123.92, 124.19, 125.76, 126.27, 126.39, 126.56, 126.97, 128.15, 128.49, 128.61, 129.16, 129.26, 130.55, 131.05, 131.38 (C(arom)); 156.39, 163.87 (2 C=O). MALDI-MS: 488 ($C_{28}H_{28}N_2O_6^+$, $[M+Na]^+$).

*3-[(*S*)-4-(4,4′-Dimethoxytrityloxy)-3-hydroxybutyl]-5-[(pyren-1-yl)methylidene]imidazolidine-2,4-dione* (**8**). To compound **6** (1.04 g, 2.71 mmol) in dry CH_2Cl_2 (40 ml), dry Et_3N (0.35 ml, 3.26 mmol) was added under N_2, followed by addition of DMTCl (1.1 g, 3.26 mmol). The mixture was stirred at r.t. under N_2 for 48 h. The solvent was removed under reduced pressure, and the residue was purified by CC (silica gel; AcOEt/petroleum ether/Et_3N 4 : 5 : 1 (*v/v/v*)) to give **8**. Yield: 0.92 g (52%). White foam. ^{1}H-NMR ($CDCl_3$): 1.22–1.26 (*m*, CH_2); 2.03 (br. *s*, OH); 3.12–3.14 (*m*, NCH_2); 3.69–3.72 (*m*, CH_2O), 3.76 (*s*, 2 MeO); 4.01–4.12 (*m*, C*H*OH); 6.76 (*s*, CH=C); 7.23–8.02 (*m*, 22 arom. H); 8.23 (*s*, NH). ^{13}C-NMR ($CDCl_3$): 32.19 (CH_2); 35.59 (NCH_2); 55.45 (MeO); 67.36 (CH_2O); 68.26 (CHOH); 86.09 (Ar_3C); 112.74 (C(5)); 123.02 (*C*H=C); 113.11, 126.76, 127.81, 128.13, 129.57, 135.98, 144.80, 158.44 (DMT); 124.41, 124.97, 125.52, 125.85, 125.89, 126.02, 126.42, 126.67, 127.21, 127.66, 128.54, 128.71, 130.04, 130.74, 131.24, 131.85 (C(arom)); 155.24, 163.88 (2 C=O). HR-MALDI-MS: 725.2622 ($C_{45}H_{38}N_2O_6^+$, $[M+Na]^+$; calc. 725.2639).

*3-[(*S*)-3-*O*-[(2-Cyanoethoxy)(diisopropylamino)phosphino]-4-(4,4′-dimethoxytrityloxy)butyl]-5-[(pyren-1-yl)methylidene]imidazolidine-2,4-dione* (**9**). Compound **8** (189 mg, 0.27 mmol) was dissolved in anh. CH_2Cl_2 (10 ml) under N_2. *N,N*-Diisopropylammonium tetrazolide (97 mg, 0.56 mmol) was added, followed by dropwise addition of (2-cyanoethyl)-*N,N,N′,N′*-tetraisopropylphosphordiamidite (190 mg, 0.59 mmol). After 30 h, anal. TLC showed the absence of starting material, and the reaction was quenched with H_2O (1 ml), followed by addition of CH_2Cl_2 (10 ml). The mixture was washed with sat aq. $NaHCO_3$ (2×10 ml). The org. phase was dried ($MgSO_4$), and the solvent was evaporated under reduced pressure. The residue was purified by CC (silica gel; AcOEt/petroleum ether/Et_3N 4 : 5 : 1 (*v/v/v*)) to afford **9**. Yield: 170 mg (70%). Yellow foam. ^{1}H-NMR ($CDCl_3$): 1.09 (*m*); 1.17 (*m*, *Me*$_2$C); 2.02 (*m*, CH_2); 2.38 (*m*, CH_2CN); 2.63 (*m*, OCH_2CH_2CN); 3.21–3.27 (*m*, NCH_2); 3.57–3.59 (*m*, 2 Me_2CH); 3.62–3.71 (*m*, CH_2O); 3.74 (*s*, 2 MeO); 4.07–4.11 (*m*, OCH); 6.81 (*s*, CH=C); 7.33–8.15 (*m*, 22 arom. H); 8.19 (*s*, NH). ^{13}C-NMR ($CDCl_3$): 24.42, 24.57, 24.66, 24.72 (4 Me); 32.89 (CH_2); 35.50 (NCH_2); 43.03(CH_2CN); 43.28 ($POCH_2$); 55.15 (MeO); 60.34 (NCH); 65.78 (CH_2O); 71.27 (POCH); 86.04 (Ar_3C); 108.88 (C(5)); 123.07 (*C*H=C); 117.88 (CN); 113.02, 126.88, 127.72, 128.20, 129.47, 136.05, 144.82, 158.36 (DMT); 124.41, 124.94, 125.82, 125.96, 126.40, 126.64, 126.69, 127.90, 128.27, 128.45, 128.64, 130.04, 130.13, 130.73, 131.23, 131.71 (C(arom)); 154.66, 163.28 (2 C=O). ^{31}P-NMR ($CDCl_3$): 149.17, 149.66 in the ratio 5 : 4. ESI-MS: 851.3534 ($C_{48}H_{53}N_4O_7P^+$, $[M+Na]^+$; calc. 851.3549).

Synthesis and Purification of Oligodeoxynucleotides (ODNs). ODNs were synthesized on an *Expedite*™ *Nucleic Acid Synthesis System* model *8909* from *Applied Biosystems*. The phosphoramidite was dissolved in dry MeCN, as a 0.75M soln. and incorporated into the growing oligonucleotide chain using elongated coupling times (10-min coupling *vs.* 2 min for normal nucleotide couplings). After the completed DNA syntheses, the 5′-*O*-DMT-on oligonucleotides were cleaved off from the solid support (r.t., 2 h) and deprotected (55°, overnight) with 32% aq. NH_3. Purification of the 5′-*O*-DMT-on ODNs was accomplished using a *Waters Model 7956* HPLC with a *Waters 600* controller, and a *Waters 717 Autosampler* on a *Waters Xterra*™ *MS* C_{18} column. Buffer *A*, 0.05M triethyl ammonium acetate in H_2O, pH 7.4; buffer *B*, 75% MeCN in H_2O; flow 2.5 ml/min. Gradients, 2 min 100% *A*, linear gradient to 100% *B* in 28 min, 100% *B* in 10 min, linear gradient to 100% *A* in 1 min, and then 100% *A* in 9 min. The ODNs were DMT-deprotected in 100 µl of 80% aq. AcOH (20 min), diluted with 1M aq. AcONa (150 µl) and precipitated from EtOH (600 µl).

Melting-Temperature Measurements. The thermal stability studies were performed on a *Perkin-Elmer* UV/VIS spectrometer *Lambda 20* fitted with a *PTP-6* temp. programmer. Melting temperature (T_m) measurements for triplex studies were performed in a 20 mM sodium cacodylate, 100 mM NaCl, 10 mM $MgCl_2$ buffer at pH 6.0 or 7.2. Parallel duplex studies were conducted at pH 6.0. The triplexes were formed by first mixing the two strands of the *Watson–Crick* duplex, each at a concentration of 1.0 µM. The soln. was heated to 80° for 5 min, cooled to r.t., and the third (TFO) strand was added, at a concentration of 1.5 µM, and then kept at 15° for 30 min. T_m Measurements for antiparallel DNA/DNA or DNA/RNA duplex studies were conducted in a 1 mM EDTA, 10 mM $Na_2HPO_4 \cdot 2\,H_2O$, 140 mM NaCl buffer at pH 7.0 for 1.0 µM of each strand. The T_m value was determined as the maximum of the first derivative plots of the melting curves obtained by measuring absorbance at 260 nm against increasing temp. (1.0°/min) and is with an uncertainty ±1.0° as determined by repetitive experiments.

Fluorescence Measurements. The fluorescence measurements were performed on a *Perkin-Elmer* luminescence spectrometer *LS-55* fitted with a *Julabo F25* temp. controller. The triplexes were formed in the same way

as for T_m measurements except that 1.5 μM of the duplex strands and only 1.0 μM of the TFOs were used. Measurements were conducted at 10° in a 20 mM sodium cacodylate, 100 mM NaCl, 10 mM $MgCl_2$ buffer at pH 6.0 with excitation at 400 nm.

REFERENCES

[1] J. Wengel, *Acc. Chem. Res.* **1999**, *32*, 301.
[2] A. Van Aerschot, I. Verheggen, C. Hendrix, P. Herdewijn, *Angew. Chem., Int. Ed.* **1995**, *34*, 1338; C. Hendrix, H. Rosemeyer, I. Verheggen, F. Seela, A. Van Aerschot, P. Herdewijn, *Chem.–Eur. J.* **1997**, *3*, 110.
[3] P. E. Nielsen, *Acc. Chem. Res.* **1999**, *32*, 624.
[4] C. Escude, C. Giovannangeli, J. S. Sun, D. H. Lloyd, J. K. Chen, S. M. Gryaznov, T. Garestier, C. Helene, *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 4365.
[5] J. Summerton, D. Weller, *Antisense Nucleic Acid Drug Dev.* **1997**, *7*, 187.
[6] E. Uhlmann, *Curr. Opin. Drug Discovery Dev.* **2000**, *3*, 203; A. De Mesmaeker, R. Haner, P. Martin, H. E. Moser, *Acc. Chem. Res.* **1995**, *28*, 366; M. Egli, *Angew. Chem., Int. Ed*. **1996**, *35*, 1894; S. Verma, F. Eckstein, *Annu. Rev. Biochem*. **1998**, *67*, 99; D. Praseuth, A. L. Guieysse, C. Helene, *Biochim. Biophys. Acta* **1999**, *1489*, 181; J. Micklefield, *Curr. Med. Chem*. **2001**, *8*, 1157; E. T. Kool, *Chem. Rev.* **1997**, *97*, 1473.
[7] I. Luyten, P. Herdewijn, *Eur. J. Med. Chem*. **1998**, *33*, 515; P. Herdewijn, *Antisense Nucleic Acid Drug Dev.* **2000**, *10*, 293; K. M. Gruckian, B. A. Schweitzer, R. X-F. Ren, C. J. Sheils, D. C. Tahmassebi, E. T. Kool, *J. Am. Chem. Soc*. **2000**, *122*, 2213.
[8] M. J. Davies, A. Shah, I. J. Bruce, *Chem. Soc. Rev*. **2000**, *29*, 97.
[9] K. Ebata, M. Masuko, H. Ohtani, M. Kashiwasake-Jibu, *Photochem. Photobiol*. **1995**, *62*, 836.
[10] U. B. Christensen, E. B. Pedersen, *Nucleic Acids Res.* **2002**, *22*, 4918.
[11] U. B. Christensen, M. Wamberg, F. A. G. El-Essawy, A. E.-H. Ismail, C. B. Nielsen, V. V. Filichev, C. H. Jessen, M. Petersen, E. B. Pedersen, *Nucleosides Nucleotides Nucleic Acid* **2004**, *23*, 207.
[12] H. S. Kim, D. Barak, T. K. Harden, J. L. Boyer, K. A. Jacobson, *J. Med. Chem.* **2001**, *44*, 3092.
[13] C. Brotschi, C. J. Leumann, *Angew. Chem., Int. Ed*. **2003**, *42*, 1655.
[14] D. Stærk, A. A. Hamed, E. B. Pedersen, J. P. Jacobsen, *Bioconjugate Chem*. **1997**, *8*, 869.

Received August 31, 2005