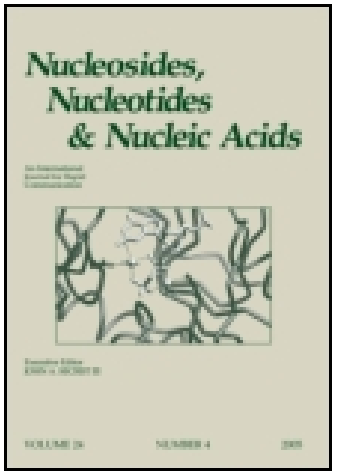


Nucleosides, Nucleotides and Nucleic Acids

Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/lncn20

PYRENE INTERCALATING NUCLEIC ACIDS WITH A CARBON LINKER

Michael E. Østergaard [a], Michael C. Wamberg [a] & Erik B. Pedersen [a]

[a] Nucleic Acid Center, Department of Physics and Chemistry, University of Southern Denmark, Odense M, Denmark

Published online: 11 Apr 2011.

To cite this article: Michael E. Østergaard, Michael C. Wamberg & Erik B. Pedersen (2011) PYRENE INTERCALATING NUCLEIC ACIDS WITH A CARBON LINKER, Nucleosides, Nucleotides and Nucleic Acids, 30:3, 210-226, DOI: 10.1080/15257770.2011.567202

To link to this article: http://dx.doi.org/10.1080/15257770.2011.567202

Nucleosides, Nucleotides and Nucleic Acids, 30:210–226, 2011

ISSN: 1525-7770 print / 1532-2335 online
DOI: 10.1080/15257770.2011.567202

PYRENE INTERCALATING NUCLEIC ACIDS WITH A CARBON LINKER

Michael E. Østergaard, Michael C. Wamberg, and Erik B. Pedersen
Nucleic Acid Center, Department of Physics and Chemistry, University of Southern Denmark, Odense M, Denmark

□ *We have synthesized a carbon linker analogue of INA (oligonucleotides containing insertions of 1-O-(1-pyrenylmethyl)glycerol). Thermal stability studies showed an increase in melting temperature in favor of the carbon linker analogue. We also synthesized a carbon linker analogue with two pyrenes geminally attached. Fluorescence studies of this intercalating nucleic acid with the pyrene moieties inserted as a bulge showed formation of an excimer band. When a mismatch was introduced at the site of the intercalator, an excimer band was formed for the destabilized duplexes whereas an exciplex band was observed when the stability of the duplex was retained.*

Keywords Intercalating nucleic acids; pyrene intercalators; INA

INTRODUCTION

The use of intercalating nucleic acids to increase the stability of duplexes has recently gained attention.[1] One of the most promising candidates for the intercalating moiety is pyrene because it has almost the same surface area as a regular Watson-Crick base pair, which makes a large stabilization through base stacking with neighboring base pairs.[2] Another advantage is that pyrene is a fluorescent molecule and if two pyrene residues come close together (3–5 Å) the formation of an excimer band is possible.[3]

When 1-*O*-(1-pyrenylmethyl)glycerol is inserted as a bulge into DNA oligonucleotides (INA), a significant discrimination between DNA and RNA is achieved,[4] as the DNA duplex is stabilized while the RNA duplex is destabilized. Previously we have examined the stereochemistry of INA, showing the *R*-isomer being better than the *S*-isomer.[5] We have also investigated

Received 30 September 2010; accepted 25 February 2011.
Dr. Vyacheslav Filichev is acknowledged for the preparation of ODN7.
The Nucleic Acid Center is a research center funded by The Danish National Research Foundation for studies on nucleic acid chemical biology.
Address correspondence to Erik B. Pedersen, Nucleic Acid Center, Department of Physics and Chemistry, University of Southern Denmark, Campusvej 55, DK-5230, Odense M, Denmark. E-mail: ebp@ifk.sdu.dk

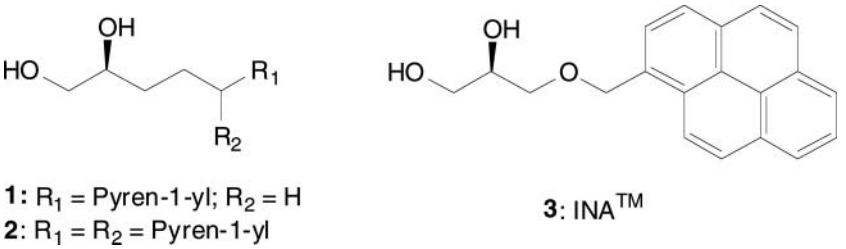


FIGURE 1 Carbon linker analogues **1** and **2** of INA **3**.

the influence of the linker length, showing that a longer linker will decrease the stabilization of the duplex.[6]

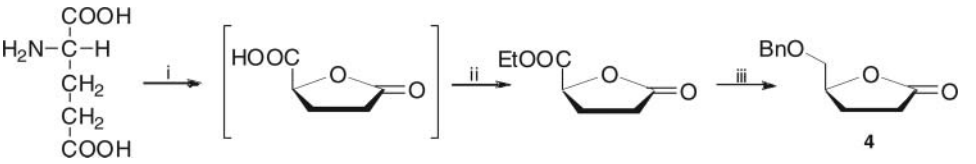
In this article, we present a carbon linker analogue **1** to INA (**3**) and an intercalating nucleic acid **2** having two pyrene residues attached via the same carbon linker (Figure 1). The only difference between **1** and **3** is the substitution of an oxygen atom with a methylene group. The methylene group is often considered a bioisoster for oxygen, and we want to examine how this substitution can affect duplex stability of the intercalating nucleic acid.

Furthermore, we have investigated the behavior of **2** with two pyrenes in close proximity of the intercalating site. There have been other reports about synthetic oligonucleotides possessing two pyrenes,[7–9] but none with bonding of two pyrenes to the same carbon atom where this has been reported for masked polyols.[10, 11] The use of two fluorophores can be used to detect single nucleotide polymorhism (SNP). Yamane et al.[12] used pyrene as a quencer and fluorescein as a fluorophore to detect a mismatched base pair, while Dioubankova et al.[13] and Christensen and Pedersen[14] have used two pyrenes to detect point mutations. In this study we have investigated the capability of bispyrene-labeled compound **2** to detect mutations.

RESULTS AND DISCUSSION

Enantiomerically pure (*S*)-γ-benzyloxymethyl-γ-butyrolactone (**4**) was obtained from L-glutamic acid in four steps according to Taniguchi et al.[15] L-glutamic acid was converted to (*S*)-γ-carboxy-γ-butyrolactone, which was esterificated by reaction with EtOH using TsOH in catalytic amount. The ester was reduced with $NaBH_4$ in EtOH to (*S*)-γ-hydroxymethyl-γ-butyrolactone which was *O*-benzyl protected using benzyl bromide and Ag_2O in DMF to obtain **4**. The yield for the last reaction was raised slightly in our hands by using NaH and microwave conditions instead of Ag_2O (Scheme 1).

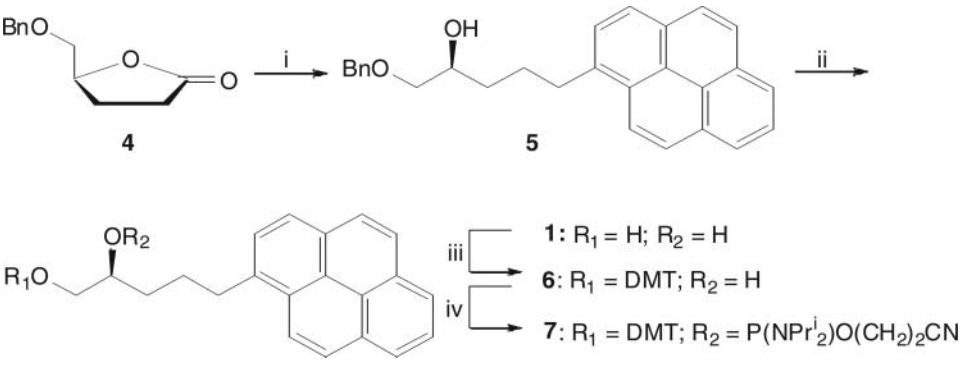
1-Bromopyrene was synthesized from pyrene according to Lock[16] and converted to pyren-1-yllithium using *n*-BuLi. Reaction of pyrenyllithium



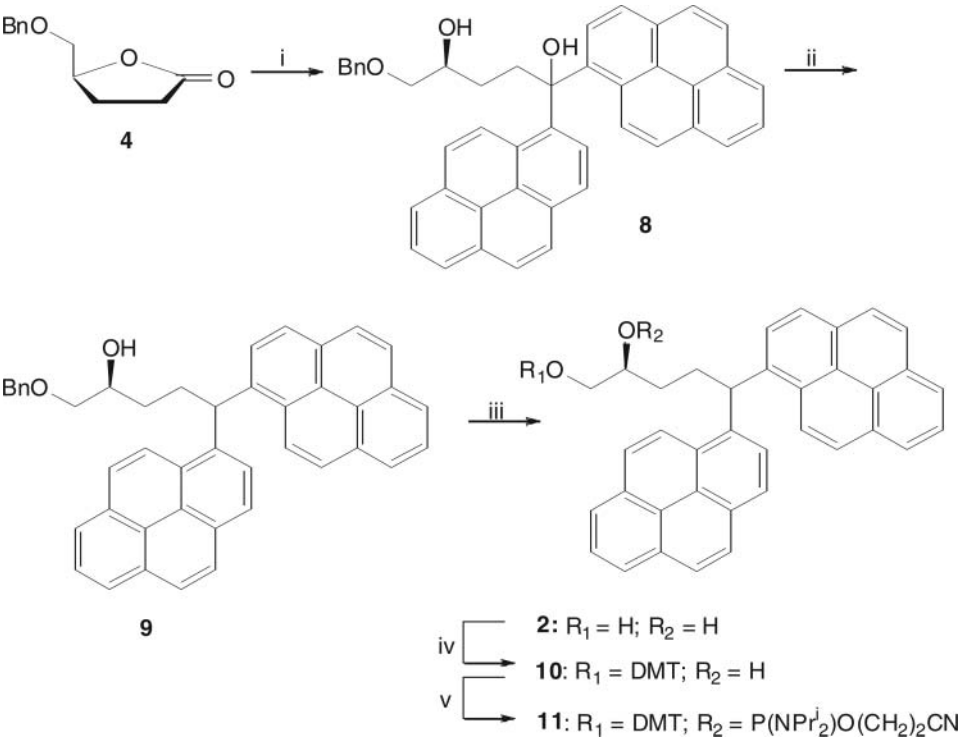
SCHEME 1 Reagents and conditions: i: a) $HCl_{(Conc.)}$, $NaNO_2$, H_2O; ii: EtOH, TsOH, 57%; iii: a) $NaBH_4$, EtOH, 64%, b) NaH, DMF, MW, 62%.

reagent with (*S*)-γ-benzyloxymethyl-γ-butyrolactone (**4**) gave a lactole, which, without further purification, was reduced using boron trifluoride etherate and triethylsilane to (*S*)-1-benzyloxy-5-pyren-1-ylpentan-2-ol (**5**) in 35% overall yield (Scheme 2). To prevent pyren-1-yllithium from reacting twice with the same molecule, it was added slowly to (*S*)-γ-benzyloxymethyl-γ-butyrolactone (**4**). The *O*-benzyl protection group at (*S*)-1-benzyloxy-5-pyren-1-ylpentan-2-ol (**5**) was removed by catalytic hydrogenation using palladium on charcoal to give (*S*)-5-pyren-1-ylpentan-1,2-diol (**1**) in 22% yield. Unfortunately, it could also partly reduce the pyrene which explains the low yield. The diol was made ready for oligonucleotide synthesis using standard chemistry. The primary alcohol was protected using 4,4′-dimethoxytrityl chloride in dry pyridine to give the *O*-5-(4,4′-dimethoxytrityl) protected compound **6** in 63% yield. Finally, the secondary alcohol was phosphitylated using 2-cyanoethyl-*N*,*N*,*N*′,*N*′-tetraisopropylphosphordiamidite to give **7** in 82% yield.

To add two pyrene residues to the same carbon atom, pyren-1-yllithium was prepared as described above and then (*S*)-γ-benzyloxymethyl-γ-butyrolactone (**4**) was added to give (*S*)-5-benzyloxy-1,1-dipyren-1-ylpentan-1,4-diol (**8**) in 56% yield. This compound was reduced with boron trifluoride etherate and triethylsilane to give **9** in 55% yield and deprotected with catalytic hydrogenation giving (*S*)-5,5-dipyren-1-ylpentan-1,2-diol (**2**) in 56%



SCHEME 2 Reagents and conditions: i: a) 1-Bromopyrene, *n*-BuLi, THF, added slowly to **4**, b) $BF_3{\cdot}Et_2O$, Et_3SiH, CH_2Cl_2, 35%; ii: H_2, Pd on charcoal, CH_2Cl_2:MeOH (1:9), 22%; iii: DMT-Cl, pyridine, 63%; iv: $P(NPr^i_2)_2O(CH_2)_2CN$, diisopropylamine tetrazolide, CH_2Cl_2, 82%.



SCHEME 3 Reagents and conditions: i: 1-Bromopyrene, *n*-BuLi, THF, 56%; ii. $BF_3{\cdot}Et_2O$, Et_3SiH, CH_2Cl_2, 55%; iii: H_2, Pd on charcoal, CH_2Cl_2:MeOH (1:9), 22%; iv: DMT-Cl, pyridine, 60%; v: $P(NPr^i_2)_2O(CH_2)_2CN$, diisopropylamine tetrazolide, CH_2Cl_2, 80%.

yield. This diol was protected with 4,4′-dimethoxytrityl chloride in 60% yield and phosphitylated to give **11** in 80% yield (Scheme 3).

THERMAL MELTING STUDIES

Seven different oligonucleotides were synthesized, four incorporating **1** and three incorporating **2**. The sequence was chosen in order to compare the properties of **1** with **3**. The latter has earlier been incorporated into a highly conserved HIV-1 long terminal repeat region which is the same sequence as used herein.[4,17]

DNA duplex stability was increased by 3–4°C when **1** was incorporated as a bulge (Tables 1 and 2), and when two incorporations were inserted four nucleotides apart, a stabilization of 15.3°C was observed. This means that there is a cooperative stabilization because otherwise only a stabilization of 6–8°C would be observed. However, if two incorporations were made too close to its other, this cooperative stabilization was lost (Table 1, ODN 3).

The pyrene intercalator showed discrimination between DNA and RNA, as **1** in all cases destabilized the RNA duplexes (Table 1). These results

TABLE 1 Melting temperatures of duplexes with monomers **1–3** inserted as a bulge and theoretical and experimental masses

		DNA 5′-AGCTTGCTTGAG-3′		RNA 5′-AGCUUGCUUGAG-3′		Mass	
ODN	Sequence	T_m/°C	ΔT_m	T_m/°C	ΔT_m	Calc.	Found
1	5′-CTCAAGCAAGCT-3′	47.4	—	42.4	—	—	—
2	5′-CTCAAG**1**CAAGCT-3′	51.7	+4.3	37.9	−4.5	3979	3979
3	5′-CTCAA**1**G**1**CAAGCT-3′	53.6	+6.2	35.5	−6.9	4344	4345
4	5′-CTCA**1**AGCA**1**AGCT-3′	62.7	+15.3	34.6	−7.8	4344	4345
5	5′-CTCAAG**2**CAAGCT-3′	39.7	−7.7	30.6	−11.8	4179	4178
6	5′-**2**CTCAAGCAAGCT-3′	56.3	+8.9	45.9	+3.5	4179	4180
7[4]	5′-CTCAAG**3**CAAGCT-3′	50.1	+2.7	37.8	−4.6		

show that **1** can only make optimal base stacking in a B-type helix. Earlier reported results show that **3** (INA) has the same properties as **1** and destabilizes a RNA duplex to the same extent,[4] but the stabilization of a DNA duplex is lower (+2.7°C) compared to **1** (+4.3°C). The reason for this difference could be the slight change in the dihedral angle from a C-O group to a methylene group and/or because of a conformational preference of the methylene group. It is known that 5-alkyl-1,3-dioxanes have lower conformational preferences than the corresponding cycloalkanes.[18] Apparently, the substitution of the oxygen atom with a less electronegative methylene group does not give a negative stabilization effect in the aqueous surroundings of the DNA strand.

Shifting the intercalator to the complementary strand (Table 2, ODNs 8–9) gave a slightly lower stabilization for **1** and a higher destabilization for **2**. For **2** the change in ΔT_m was from −7.7°C to −10.3°C.

Furthermore we have investigated the pyrene intercalator as a universal base. Results are presented in Table 3. Positions 7 in both strands were varied with all four natural bases, **1**, and **2**. INA (**3**) was inserted in one of the strands for comparison. Incorporation of the pyrene intercalators **1–3** in the 13-mer duplex resulted in a lowering of the melting temperature (T_m) no matter the opposite base compared to the matched base pair. However, the

TABLE 2 Melting temperatures of duplexes with the intercalator shifted to the complementary strand[a]

				Mass	
ODN	Sequence	T_m/°C	ΔT_m	Calc.	Found
8	5′-AGCTTG**1**CTTGAG-3′	50.8	+3.4	4040	4040
9	5′-AGCTTG**2**CTTGAG-3′	37.1	−10.3	4241	4241

[a]The target sequence was 3′-TCGAACGAACTC-5′ and the reference melting temperature for wildtype duplex without insertions was 47.4°C.

TABLE 3 Melting temperatures of duplexes where a natural nucleotide was replaced either with another natural nucleotide or with monomers **1–3**. The melting temperature of **3** versus **3** was taken from the literature[5]

	5′-AGCTTG**X**CTTGAG-3′ 3′-TCGAAC**Y**GAACTC-5′						
X\Y	A	C	G	T	1	2	3
A	39.4	37.4	45.8	50.5	46.8	41.0	43.1
C	35.5	23.6	54.3	35.1	47.3	41.1	44.1
G	45.9	52.4	46.7	43.1	44.1	37.4	41.5
T	48.9	32.5	36.1	35.8	46.4	40.4	43.1
1	42.0	44.2	41.4	42.9	44.0	37.0	
2	34.8	36.1	33.7	33.8	36.4	34.8	
3							43.6

intercalators have a universal base effect as the T_m varied only 3.2°C for **1** and 3.6°C for **2**. For INA (**3**), the narrow range is 2.6°C. Shifting the intercalator to the opposite strand resulted in a more narrow range. Not surprisingly, the highest T_m is achieved when the intercalator is placed opposite a cytosine and the lowest T_m when the intercalator is facing a guanosine.

Previously we have shown that two intercalating pyrene moieties inserted in complementary strands directly opposite each other will lead to destabilization of the duplex.[5,19] This was also the case in this study. While destabilizing, the carbon analogue **1** gave a marginally higher thermal melting than is reported for INA.[5] When **2** was inserted opposite either **1** or **2**, it lead to a further destabilization.

From Table 1, can be seen that **2** is actually destabilizing the DNA duplex by −7.7°C, when inserted as a bulge in the middle of the sequence. Nearly the same value was obtained when ethylene glycol was inserted instead of an intercalator.[4] This might indicate that the two pyrene moieties do not intercalate, probably due to steric crowding of the geminal bonded pyrenes. For the RNA duplex, the destabilization is −11.8°C, which implies a further disturbance of the duplex structure than when ethylene glycol was inserted as a bulge.[4] When **2** was inserted as an end-positioned intercalating pseudonucleotide (ODN 6), a stabilization of both DNA and RNA duplexes was achieved due to the lid effect. For further examination on whether **2** is intercalating or not, we carried out fluorescence experiments on ODN5.

FLUORESCENCE STUDIES

While **1** gave standard pyrene fluorescence emission spectra, **2** gave rise to an excimer band with a maximum at 480 nm, when ODN5 was hybridized to the complementary strand, upon excitation at 340 nm. The single strand (Figure 2A, ODN5) led to the formation of a band with a maximum at 445 nm. Yamana et al.[20] have made similar observations of band formation

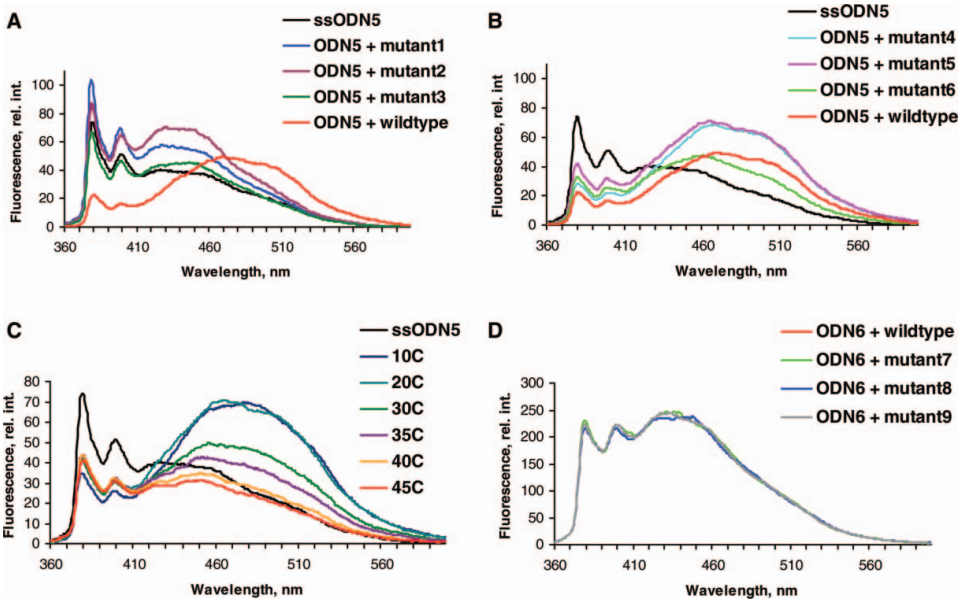


FIGURE 2 Fluorescence emission spectra of (A and B) ODN5 hybridized with mutant 1–6 and wildtype strands; (C) single stranded ODN5 and hybridized with mutant 5 at increasing temperatures; and (D) ODN6 hybridized with mutant 7–9 and with wildtype strands.

at 450 nm, when studying fluorescence of oligonucleotides containing 2′-*O*-(1-pyrenylmethyl)uridine. They ascribe this fluorescence emission to an exciplex of the pyrene and an adjacent base in the duplex. In our case, the greater flexibility of the nucleobases in the single strand might allow these to come in close proximity of one of the pyrenes, which leads to the formation of the exciplex band. For the hybridized ODN5, the destabilization implies that the pyrenes do not intercalate. However, base paring still occurs, which might leave the pyrenes outside the duplex and unable to interact with the nucleobases. Instead the excimer band formation is seen.

To investigate this further, a study of mismatched sequences was performed. The thermal melting studies of these sequences can be seen in Table 4. When the mismatch was introduced at the 3′ position of the intercalator, the duplex stability was not altered much, while a destabilization of 4–6°C was seen, when the mismatch was at the 5′ position of the intercalator. Fluorescence spectra of these sequences revealed an exciplex band with maximum at 445 nm for mismatch at the 3′–site of the intercalator (mutant 1–3) and an excimer band maximum at 480 nm for the sequences with the mismatch at the 5′–site of the intercalator (mutant 4–6) (Figures 2A and 4B).

As no destabilization was observed for the mutant 1–3 even though a mismatch was introduced, the pyrene must interact with the duplex and it is likely to believe it occurs through intercalation. Hereby one of the pyrene

TABLE 4 T_m measurements on mismatches toward ODN5

	5′-AGCTTX–YTTGAG-3′ 3′-TCGAAC2GAACTC-5′			
Target	X	Y	T_m/°C	ΔT_m
Wildtype	G	C	39.7	–
Mutant1	C	C	43.6	+3.9
Mutant2	A	C	38.3	−1.4
Mutant3	T	C	40.2	+0.5
Mutant4	G	G	35.4	−4.3
Mutant5	G	A	33.5	−6.2
Mutant6	G	T	32.9	−6.8

moieties would be close to a nucleobase, and exciplex band formation is seen. This might not be the case, when the mismatch is on the 5′-site of the intercalator. The stability of the duplex is lower, which implies less interaction from/of the pyrenes with the nucleobases. The pyrenes are more free and therefore in positions to form excimer band, which we expect to happen outside the duplex. As no interaction with the nucleobases is possible from there, the possibility of exciplex band formation is ruled out. We have made examples with other sequences for mismatch studies which all resulted in lower duplex stabilities and in all those cases an excimer band was observed in the fluorescence spectrum (data not shown).

A molecular modelling study confirms the possibility of these duplexes (Figure 3). Despite the bulkiness of the intercalating pyrene moieties, there

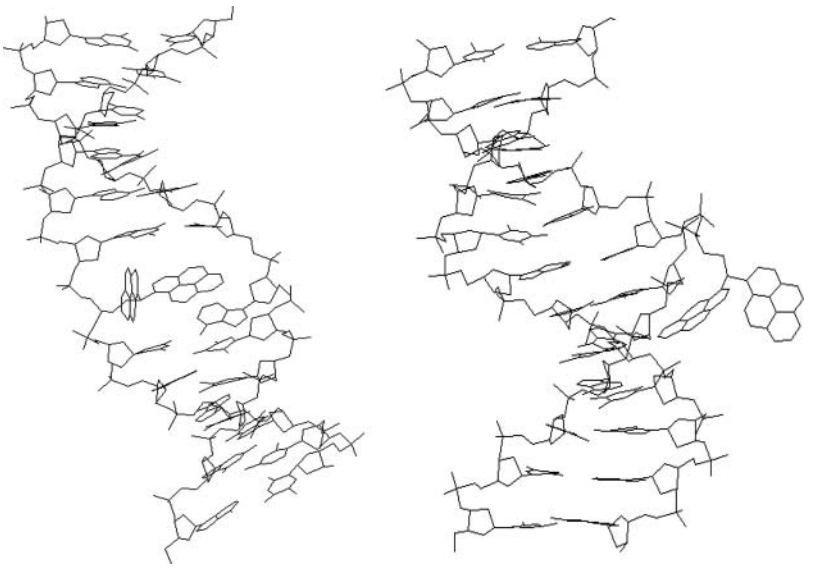


FIGURE 3 Molecular modelling of ODN5 with mutant 2 (left) showing the possible structure of intercalation and ODN5 with wildtype (right) showing the possible structure of minor groove binding.

TABLE 5 Melting temperatures of mismatched duplexes with **2** inserted as an end-positioned intercalating pseudonucleotide (ODN6); the reference melting temperature for the wildtype duplex without insertions was 46.6°C

5′-AGCTTGCTTGAXTTTT-3′ 3′-TCGAACGAACTC2–5′			
Target	X	T_m	ΔT_m
Wildtype	G	60.0	+13.4
Mutant7	C	53.2	+6.6
Mutant8	A	52.3	+5.7
Mutant9	T	52.9	+6.3

is room for it inside the duplex with no major disturbance of the base pairing. The right side of Figure 3 shows the duplex with two pyrenes in the minor groove.

Fluorescence experiments were carried out at 20°C. Control experiments at 10°C and 30°C, respectively, did not show any difference. First, when the T_m was exceeded, did the excimer band profile shift toward the profile of the single strand (Figure 2C).

Finally, a mismatch study with ODN6 was performed. As described above, stabilization was achieved when the intercalator inserted as an end-positioned pseudonucleotide. The target sequence was extended with four thymine bases and a mismatch was introduced at the 3′–site of the intercalator (Table 5), and this resulted in a lower stabilization than observed for the matched oligonucleotide.

Fluorescence studies of ODN6 revealed exciplex band formation (Figure 2D). As the intensity is the same in all four cases, the intercalator **2** must be placed in the same position in the duplex in all four cases. Hybridized to the complementary DNA or RNA, ODN6 gives the same fluorescence spectre.

CONCLUSION

We have synthesized a carbon linker analogue to INA and evaluated the duplex thermal stability which showed an improvement in favor of the carbon linker analogue toward DNA duplexes, while no significant change was observed for RNA duplexes. For an intercalating nucleic acid containing a bulge insertion of two pyrene moieties in a geminal position, we observed in the fluorescence spectra an excimer band whereas for a mismatched duplex an exciplex band was observed when the duplex was not substantially destabilized.

EXPERIMENTAL

NMR spectra were recorded on a Varian Gemini 2000 NMR spectrometer at 300 MHz for ^{1}H, 75 MHz for ^{13}C and 121.5 MHz for ^{31}P. δ values are in ppm

with tetramethylsilane as internal standard for ^{1}H NMR, deuterated solvents ($CDCl_3$ (δ77.00), DMSO (δ39.44)) for ^{13}C NMR, and 85% H_3PO_4 as external standard for ^{31}P NMR. Accurate ion mass determinations were performed using an Ionspec 4.7 T Ultima Fourier transform mass spectrometer (Irvine, CA, USA). The M^+ or $[M+Na]^+$ ions were peak matched using ions derived from the 2,5-dihydroxybenzoic acid matrix. Silica gel (0.040–0.063 mm) used for column chromatography and analytical silica gel TLC plates 60 F_{254} were purchased from Merck (Darmstadt, Germany). Ultraviolet (UV)-light or a stain of $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O/Ce_2(SO_4)_3$ (50:1) in 5% sulfuric acid were used for visualization. Reagents were used as purchased.

Preparation of (*S*)-1-(benzyloxy)-5-(pyren-1-yl)pentan-2-ol (5)

1-Bromopyrene (3.05 g, 10.7 mmol) was dissolved in anhydrous THF (70 mL) under N_2 and the temperature was lowered to −78°C. 2.4 M *n*-BuLi in hexane (5.13 mL, 12.3 mmol) was added within 20 minutes. In another flask (*S*)-γ-benzyloxymethyl-γ-butyrolactone[15] (**4**) (2.00 g, 9.70 mmol) was dissolved in anhydrous THF (20 mL) under N_2 and the temperature is lowered to −78°C. After 30 minutes, the lithium reagent was cannulated slowly to the lactone over 2 hours and the temperature was raised to −30°C over 2 hours. The reaction was quenched by addition of water (70 mL), the mixture was extracted with Et_2O (5 × 50 mL), and the organic phase dried ($MgSO_4$) and evaporated in vacuo to afford an oil. The residue was dissolved in anhydrous CH_2Cl_2 (70 mL) and treated at −78°C with $BF_3{\cdot}Et_2O$ (2.54 mL, 24.3 mmol) and Et_3SiH (3.36 mL, 24.3 mmol) and the mixture was stirred overnight while the temperature was allowed to reach room temperature. The reaction was quenched by addition of saturated aqueous $NaHCO_3$ solution (80 mL), the mixture was extracted with CH_2Cl_2 (3 × 30 mL), and the organic phase dried ($MgSO_4$) and evaporated in vacuo. The residue was purified by silica gel column chromatography (petroleum ether/EtOAc, 4:1). The product was obtained as a yellow foam (1.35 g, 35%). R_f = 0.24 (petroleum ether/EtOAc, 3:1). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.44–1.64 (2H, m, H3), 1.76–2.03 (2H, m, H4), 2.43 (1H, brs, OH), 3.20–3.40 (4H, m, H1, H5), 3.78–3.83 (1H, m, H2), 4.43 (2H, s, OCH_2Ph), 7.20–7.28 (5H, m, phenyl), 7.75–8.21 (m, 9H, pyrene). ^{13}C NMR (75 MHz, $CDCl_3$) δ 27.63 (C4), 32.94 (C5), 33.29 (C3), 70.21 (C1), 73.21 (OCH$_2$Ph), 74.48 (C2), 122.85, 123.33, 124.58, 124.69, 124.73, 124.94, 125.69, 126.47, 127.12, 127.15, 127.43, 127.63, 127.69, 128.37, 128.54, 129.70, 130.82, 131.33, 136.52, 137.86 (Ar). HRMS (MALDI): *m/z* calcd for $C_{28}H_{26}O_2Na^+$ (MNa^+): 417.1825, found 417.1818.

Preparation of (*S*)-5-(benzyloxy)-1,1-(dipyren-1-yl)pentan-1,4-diol (8)

1-Bromopyrene (3.05 g, 10.7 mmol) was dissolved in anhydrous THF (25 mL) under N_2 and the temperature was lowered to −78°C. 2.4 M *n*-BuLi

in hexane (5.13 mL, 12.3 mmol) was added within 20 minutes. After 20 minutes, a solution of (*S*)-γ-benzyloxymethyl-γ-butyrolactone (**4**) (1.10 g, 5.33 mmol) dissolved in anhydrous THF (15 mL) under N_2, was added over 30 minutes and the mixture was stirred for an additional hour before the temperature was raised −30°C within 2 hours. The reaction was quenched by addition of H_2O (50 mL), the mixture was extracted with Et_2O (3 × 40 mL), and the organic phase was dried ($MgSO_4$) and evaporated in vacuo. The residue was purified by silica gel column chromatography (petroleum ether/EtOAc, 2:1) and the product was obtained as a yellow foam (1.81 g, 56%). R_f = 0.47 (petroleum ether/EtOAc, 1:1). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.25–1.55 (2H, m, H3), 2.77 (1H, brs, OH), 2.96–3.20 (4H, m, H2, H5), 3.70–3.75 (1H, m, H4), 3.81 (1H, brs, OH), 4.25 (2H, s, OC*H*$_2$Ph), 7.08–7.20 (5H, m, phenyl), 7.49–7.52, 7.73–7.82, 7.91–8.00, 8.14–8.35, 8.58–8.65 (18H, m, pyrene). ^{13}C NMR (75 MHz, $CDCl_3$) δ 27.88 (C3), 39.41 (C2), 70.67 (C5), 73.04 (O*C*H$_2$Ph), 74.29 (C4), 79.86 (C1), 124.30, 124.59, 124.68, 124.70, 124.77, 124.79, 124.92, 125.11, 125.31, 125.68, 125.80, 125.85, 126.52, 127.24, 127.28, 127.32, 127.38, 127.63, 128.30, 130.11, 130.15, 130.80, 130.87, 131.14, 137.73, 140.96, 141.06 (Ar). HRMS (MALDI): *m/z* calcd for $C_{44}H_{34}O_3Na^+$ (MNa^+): 633.2400, found 633.2411.

Preparation of (*S*)-1-(benzyloxy)-5,5-(dipyren-1-yl)pentan-2-ol (9)

To a solution of (*S*)-5-(benzyloxy)-1,1-di(pyren-1-yl)pentan-1,4-diol (**8**) (1.50 g, 2.45 mmol) in CH_2Cl_2 (20 mL) at −78°C under N_2 was added $BF_3{\cdot}Et_2O$ (0.27 mL, 2.70 mmol) and Et_3SiH (0.35 mL, 2.70 mmol). The mixture was stirred overnight while the temperature was allowed to reach room temperature. The reaction was quenched by addition of saturated aqueous $NaHCO_3$ solution (30 mL), the mixture was extracted with CH_2Cl_2 (3 × 15 mL), and the organic phase dried ($MgSO_4$) and evaporated in vacuo. The residue was purified by silica gel column chromatography (petroleum ether/EtOAc, 3:1) and the product was obtained as a yellow foam (0.801 g, 55%). R_f = 0.22 (petroleum ether/EtOAc, 3:1). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.22–1.26 (2H, m, H3), 2.44–2.57 (1H, m, H4), 2.73–2.76 (1H, m, H4), 3.19–3.25 (1H, m, H1), 3.34–3.37 (1H, m, H1), 3.89 (1H, brs, H2), 4.40 (2H, s, OC*H*$_2$Ph), 6.13 (1H, t, *J* 7.4, H5), 7.20–7.23 (5H, m, phenyl), 7.86–8.09, 8.39–8.43, 8.50–8.53 (18H, m, pyrene). ^{13}C NMR (75 MHz, $CDCl_3$) δ 31.69 (C3), 32.78 (C4), 41.88 (C5), 70.43 (C2), 73.22 (O*C*H$_2$Ph), 74.46 (C1), 122.87, 124.80, 124.90, 124.94, 125.04, 125.17, 125.21, 125.36, 125.39, 125.77, 126.84, 127.41, 127.64, 127.68, 127.84, 127.89, 128.35, 128.69, 128.87, 129.76, 129.81, 130.64, 130.69, 131.31, 131.34, 137.80, 138.60, 139.17 (Ar). HRMS (MALDI): *m/z* calcd for $C_{44}H_{34}O_2Na^+$ (MNa^+): 617.2451, found 617.2443.

General Procedure for the Deprotection of a Benzyl Ether

The benzyl ether was dissolved in a mixture of CH_2Cl_2 and methanol (1:9 v/v) and Pd on charcoal (100 mg pr. 1 mmol) was added. N_2 was let

through the solution for 30 minutes, and then the reaction was evacuated twice and a hydrogen atmosphere at 1 atm was established. The catalyst was filtered off after 48 hours and washed with EtOAc. The organic phase was evaporated and the residue purified by silica gel column chromatography.

Preparation of (*S*)-5-(pyren-1-yl)pentan-1,2-diol (1)

From (*S*)-1-(benzyloxy)-5-(pyren-1-yl)pentan-2-ol (**5**) (1.03 g, 2.61 mmol) the product was obtained as a light yellow foam (178 mg, 22%). $R_f = 0.14$ (petroleum ether/EtOAc, 2:1). ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.42–1.45, 1.63–1.65 (2H, m, H3), 1.83–1.97 (2H, m, H4), 2.52 (1H, brs, OH), 3.22 (1H, brs, OH), 3.31–3.34 (4H, m, H1, H5), 3.54 (1H, m, H2), 7.93–8.39 (9H, m, pyrene). ^{13}C NMR (75 MHz, DMSO-d_6) δ 27.77 (C4), 32.79, 33.32 (C3, C5), 65.99 (C1), 70.97 (C2), 123.47, 124.12, 124.17, 124.63, 124.78, 124.80, 125.99, 126.31, 127.03, 127.37, 127.39, 127.99, 129.09, 130.35, 130.82, 137.13 (Ar). HRMS (MALDI): *m/z* calcd for $C_{21}H_{20}O_2$ (M^+): 304.1458, found 304.1461.

Preparation of (*S*)-5,5-di(pyren-1-yl)pentan-1,2-diol (2)

From (*S*)-1-(benzyloxy)-5,5-di(pyren-1-yl)pentan-2-ol (**8**) (1.83 g, 3.07 mmol) the product was obtained as a light yellow foam (345 mg, 22%). $R_f =$ 0.18 (petroleum ether/EtOAc, 1:1). ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.53–1.63, 1.74–1.84 (2H, m, H3), 2.52 (1H, brs, OH), 2.52–2.78 (2H, m, H4), 3.20 (1H, brs, OH), 3.23–3.35 (2H, m, H1), 3.58–3.63 (1H, m, H2), 6.28 (1H, t, $J = 6.8$ Hz, H5), 8.00–8.27, 8.62–8.73 (18H, m, pyrene). ^{13}C NMR (75 MHz, DMSO-d_6) δ 31.99 (C3), 32.88 (C4), 41.21 (C5), 65.95 (C1), 71.29 (C2), 122.98, 124.03, 124.27, 124.84, 125.00, 125.13, 125.49, 125.58, 126.12, 126.75, 127.31, 127.68, 128.10, 128.21, 129.12, 129.15, 130.14, 130.17, 130.81, 139.28, 139.67 (Ar). HRMS (MALDI): *m/z* calcd for $C_{37}H_{28}O_2Na^+$ (MNa^+): 527.1982, found 527.2004.

General Procedure for the DMT Protection of Alcohols

The alcohol was dissolved in anhydrous pyridine under N_2. DMT-Cl was added and the mixture was stirred for 4 hours. The solvent was evaporated in vacuo and co-evaporated with anhydrous xylene and dried on a desiccator overnight. The residue was purified by silica gel column chromatography.

Preparation of (*S*)-1-[bis(4-methoxyphenyl)phenylmethoxy]-5-(pyren-1-yl)pentan-2-ol (6)

From (*S*)-5-(pyren-1-yl)pentan-1,2-diol (**1**) (178 mg, 0.58 mmol) the product was obtained as a light yellow foam (170 mg, 63%). $R_f = 0.44$ (petroleum ether/EtOAc, 2:1). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.53–1.63,

1.77–1.87, 1.93–2.03 (4H, m, H3, H4), 2.51 (1H, brs, OH), 2.97–3,04, 3.13–3.16 (2H, m, H1), 3.30 (2H, t, J = 7.7 Hz, H5), 3.72 (6H, s, 2 × OCH_3), 3.83 (1H, m, H2), 6.75–6.78, 7.15–7.29, 7.39–7.41 (13H, m, phenyl), 7.78–8.07, 7.92–8.23 (9H, m, pyrene). ^{13}C NMR (75 MHz, $CDCl_3$) δ 27.68 (C4), 33.25, 33.33 (C3, C5), 55.13, 55.14 (2 × OCH_3), 67.44 (C1), 70.83 (C2), 86.03 (CPh_3), 113.08, 123.40, 124.62, 124.72, 124.76, 124.99, 125.02, 125.73, 126.51, 126.76, 127.15, 127.24, 127.47, 127.79, 128.07, 128.59, 129.73, 129.99, 130.88, 131.39, 135.91, 135.98, 136.64, 144.80 158.43 (Ar). HRMS (MALDI): m/z calcd for $C_{42}H_{38}O_4Na^+$ (MNa^+): 629.2662, found 629.2675.

Preparation of (*S*)-1-[bis(4-methoxyphenyl)phenylmethoxy]-5,5-di (pyren-1-yl)pentan-2-ol (10)

From (*S*)-5,5-di(pyren-1-yl)pentan-1,2-diol (**2**) (315 mg, 0.62 mmol) the product was obtained as a light yellow foam (304 mg, 60%). R_f = 0.44 (petroleum ether/EtOAc, 2:1). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.72–1.77 (2H, m, H3), 2.37 (1H, brs, OH), 2.46–2.74 (2H, m, H4), 2.97–3.14 (2H, m, H1), 3.65 (6H, s, 2 × OCH_3), 3.87–3.94 (1H, m, H2), 6.13 (1H, t, J = 7.5 Hz, H5), 6.67–6.70, 7.14–7.37 (13H, m, phenyl), 7.85–8.12, 8.39–8.53 (18H, m, pyrene). ^{13}C NMR (75 MHz, $CDCl_3$) δ 32.14 (C3), 32.78 (C4), 41.92 (C5), 55.06, 55.08 (2 × OCH_3), 67.25 (C2), 71.07 (C1), 85.98 (CPh_3), 113.03, 122.90, 124.83, 124.92, 125.04, 125.17, 125.21, 125.42, 125.80, 126.73, 126.85, 127.44, 127.77, 127.85, 127.90, 128.04, 128.69, 128.86, 129.75, 129.81, 129.96, 130.67, 130.72, 131.34, 131.36, 135.80, 135.88, 138.68, 139.20, 144.75, 158.38 (Ar). HRMS (MALDI): m/z calcd for $C_{58}H_{46}O_4Na^+$ (MNa^+): 829.3288, found 829.3301.

General Procedure for the Synthesis of a Phosphoramidite

The DMT protected alcohol was dissolved in anhydrous CH_2Cl_2 under N_2 and *N*,*N*-diisopropylammoniumtetrazolide (2.1 eqiv.) was added. Then 2-cyanoethyl-*N*,*N*,*N*′,*N*′-tetraisopropylphosphoramidite (2.2 eqiv.) was added dropwise and the mixture was stirred for 24 hours. The reaction was quenched by addition of water (1 mL). The amount of CH_2Cl_2 solvent was doubled and washed twice with saturated $NaHCO_3$ solution, dried ($MgSO_4$) and the solvent evaporated in vacuo. The product was purified by silica gel column chromatography.

Preparation of the Phosphoramidite of (*S*)-1-[bis(4-methoxyphenyl) phenylmethoxy]-5-(pyren-1-yl)pentan-2-ol (7)

From (*S*)-1-[bis(4-methoxyphenyl)phenylmethoxy]-5-(pyren-1-yl)pentan-2-ol (**6**) (170 mg, 0.28 mmol) the product was obtained as a white foam (182 mg, 82%). R_f = 0.47 (petroleum ether/EtOAc, 2:1 and traces of Et_3N). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.99–1.14 (14H, m, 2 × C*H*(CH_3)$_2$), 1.77–1.91

(4H, m, H3, H4), 2.34 (2H, t, J = 6.6 Hz, OCH_2CH_2CN), 2.94–3.18 (2H, m, H1), 3.29–3.36 (2H, m, H5), 3.44–3.55 (2H, m, OCH_2CH_2CN), 3.70 (6H, s, 2 × OCH_3), 3.99–4.06 (1H, m, H2), 6.70–6.75, 7.15–7.29, 7.39–7.43 (13H, m, phenyl), 7.79–8.27 (9H, m, pyrene). ^{13}C NMR (75 MHz, $CDCl_3$) δ 20.11, 20.19 (OCH_2CH_2CN), 24.37, 24.39, 24.46, 24.48, 24.54, 24.57, 24.64, 24.66 (2 × $CH(CH_3)_2$), 26.79, 27.11 (C4), 33.27, 33.34 (C3, C5), 42.86, 43.03, 43.15 (2 × $CH(CH_3)_2$), 55.07 (2 × OCH_3), 57.88, 58.14 (OCH_2CH_2CN), 72.89, 73.09 (C1), 73.77, 74.00 (C2), 85.77, 85.82 (CPh_3), 112.91 (Ar-C), 117.60 (CN), 123.42, 123.56, 124.59, 124.71, 124.96, 125.03, 125.70, 125.73, 126.47, 126.54, 126.60, 127.03, 127.07, 127.22, 127.39, 127.47, 127.63, 128.11, 128.18, 128.57, 129.67, 129.98, 130.02, 130.06, 130.85, 131.36, 136.11, 136.22, 136.63, 136.87, 144.99, 158.27, 158.30 (Ar). ^{31}P NMR (121.5 MHz, $CDCl_3$) δ 149.07, 149.59. HRMS (ESI): *m/z* calcd for $C_{51}H_{55}N_2O_5PK^+$ (MK^+): 845.3480 found 845.3503.

Preparation of the phosphoramidite of (*S*)-1-[bis(4-methoxyphenyl) phenylmethoxy]-5,5-di(pyren-1-yl)pentan-2-ol (11)

From (*S*)-1-[bis(4-methoxyphenyl)phenylmethoxy]-5,5-di(pyren-1-yl)pentan-2-ol (**10**) (253 mg, 0.25 mmol) the product was obtained as a yellow foam (251 mg, 80%). R_f = 0.44 (petroleum ether/EtOAc, 2:1 and traces of Et_3N). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.99–1.05 (14H, m, 2 × $CH(CH_3)_2$), 2.02–2.03 (2H, m, H3), 2.18–2.23 (2H, m, H4), 2.34 (2H, t, J = 6.6 Hz, OCH_2CH_2CN), 2.99–3.23 (2H, m, H1), 3.48–3.56 (2H, m, OCH_2CH_2CN), 3.62 (3H, s, OCH_3), 3.63 (3H, s, OCH_3), 4.05–4.15 (1H, m, H2), 6.10–6.12 (1H, m, H5), 6.62–6.65, 7.15–7.41 (13H, m, phenyl), 7.91–8.12, 8.44–8.52 (18H, m, pyrene). ^{13}C NMR (75 MHz, $CDCl_3$) δ 19.99, 20.06 (OCH_2CH_2CN), 24.44, 24.53, 24.60, 24.69 (2 × $CH(CH_3)_2$), 31.23, 31.82, 31.91, 32.24, 32.40 (C3, C4), 42.19 (C5), 42.96, 43.13 (2 × $CH(CH_3)_2$), 55.02 (2 × OCH_3), 57.93, 58.18 (OCH_2CH_2CN), 73.10, 73.31 (C2), 73.77, 73.99 (C1), 85.83 (CPh_3), 112.91 (Ar-C), 117.57 (CN), 122.93, 122.99, 123.03, 124.85, 124.95, 125.05, 125.18, 125.41, 125.47, 125.52, 125.81, 126.55, 126.60, 126.84, 127.45, 127.67, 127.84, 128.08, 128.14, 128.77, 129.77, 129.97, 130.04, 130.70, 131.36, 136.03, 136.14, 139.00, 139.14, 139.25, 144.96, 145.01, 158.28 (Ar). ^{31}P NMR (121.5 MHz; $CDCl_3$) δ 149.09, 149.46. HRMS (ESI): *m/z* calcd for $C_{67}H_{63}N_2O_5PH^+$ (MH^+): 1007.4547, found 1007.4575.

Molecular Modelling

The molecular modelling was performed with MacroModel 8.1 from Schrödinger Inc. (Cambridge, MA, USA)[21] All calculations were conducted with the AMBER* force field[22,23] and the GB/SA water model.[24] The dynamics simulation was performed with stochastic dynamics,[25] a SHAKE algorithm to constrain bonds to hydrogen,[26] a time step of 1.5 fs, and a simulation temperature of 300 K. Simulation for 1.0 ns generated

250 structures, which were all minimized using a Polak-Ribier conjugated gradient method with a convergence threshold of 0.005 kJ/mol. The minimized structures were examined with XCluster from Schrödinger, and a representative structure for the lowest-energy cluster was selected. The starting structure[19] was taken from the Brookhaven Protein Database and modified in MacroModel. The base pair next to the intercalating site was changed to obtain the desired mismatch.

Oligonucleotide Synthesis and Purification

ODNs were synthesized on a DNA 3400 Synthesizer from Applied Biosystems (Foster City, CA, USA). The phosphoramidites **7** and **11** were dissolved in a 1:1 mixture of dry CH_2Cl_2 and dry MeCN, as a 0.75 M solution and incorporated into the growing oligonucleotide chain using elongated coupling times (10-minute coupling versus 2-minute for normal nucleotide couplings). The yield of the oligo synthesis varied from 61–74% corresponding to 96–98% coupling efficiency pr step. The 5′-*O*-DMT-on oligonucleotides were cleaved off the solid support (room temperature, 2 hours) and deprotected (55°C, overnight) using 32% aqueous ammonia. Purification of the 5′-*O*-DMT-on ODNs was accomplished using a Waters Model 7956 HPLC (Milford, MA, USA) with a Waters 600 controller, a Waters 2996 detector, and a Waters 717 autosampler on a Waters Xterra MS C_{18}; 10 μm, 7.8 × 150 mm column. Buffer A: 0.05M triethyl ammonium acetate in H_2O, pH = 7.4; Buffer B: 75% CH_3CN in H_2O; flow 2.5 mL/min. Gradients: 2 minutes 100% A, linear gradient to 70% B in 38 minutes, linear gradient to 100% B in 7 minutes, 100% B in 3 minutes, linear gradient to 100% A in 1 minutes, and then 100% A in 9 minutes. Product peak at ∼25 minutes. The ODNs were DMT deprotected in 100 μL 80% AcOH (20 minutes), diluted with 1M aqueous NaOAc (150μL) and precipitated from ethanol (600 μL). The ODNs were kept at −18°C for 1 hour followed by centrifugation for 20 minutes upon cooling. The supernatant was decanted off and the ODNs were dried in a vacuum centrifuge. All modified ODNs were confirmed by HiRes-ESI analyses on the Ionspec 4.7 T Ultima Fourier transform mass spectrometer (Irvine, CA, USA) or by MALDI-TOF analysis on a Voyager Elite Bio Spectrometry Research Station from Perceptive Biosystems (Lancashire, UK). The purity of the ODNs was analyzed on an ion exchange LaChrom HPLC (Pleasaton, CA, USA) and found to be over 96%.

Melting Temperature Measurements

The thermal stability studies were performed on a Perkin-Elmer Lambda 20 UV/VIS spectrometer (Beaconsfield, UK) fitted with a PTP-6 temperature programmer. Melting temperature (T_m) measurements for DNA/DNA and DNA/RNA duplex studies were conducted in a 1 mM EDTA, 10 mM $Na_2HPO_4{\cdot}2H_2O$, 140 mM NaCl buffer at pH 7.0 for 1.0 μM of each strand.

The melting temperature was determined as the maximum of the first derivative plots of the melting curves obtained by measuring absorbance at 260 nm against increasing temperature (1°C/min) and is with an uncertainty of ± 1.0°C as determined by repetitive experiments.

Fluorescence Measurements

The fluorescence measurements were performed on a Perkin-Elmer LS-55 luminescence spectrometer (Beaconsfield, UK) fitted with a Julabo F25 temperature controller (Seelbach, Germany) with excitation at 340 nm and detection at 360–600 nm. All measurements were conducted at 20°C using the same concentrations and buffer conditions as for the melting studies. Control measurements conducted at either 10°C or 30°C did not reveal any difference in emission band profiles.

REFERENCES

1. Filichev, V.V.; Pedersen, E.B. DNA-conjugated organic chromophores in DNA stacking interactions. In *Wiley Encyclopedia of Chemical Biology*, Ed. T. Begley, 2009.
2. Smirnov, S.; Matray, T.J.; Kool, E.T.; Santos, C. Integrity of duplex structures without hydrogen bonding: DNA with pyrene paired at abasic sites. *Nucleic Acids Res.* **2002**, 30, 5561–5569.
3. Winnik, F.M. Photophysics of preassociated pyrenes in aqueous polymer solutions and in other organized media. *Chem. Rev.* **1993**, 93, 587–614.
4. Christensen, U.B.; Pedersen, E.B. Intercalating nucleic acids containing insertions of 1-*O*-(1-pyrenylmethyl)glycerol: Stabilisation of dsDNA and discrimination of DNA over RNA. *Nucleic Acids Res.* **2002**, 30, 4918–4925.
5. Filichev, V.V.; Hilmy, K.M.H.; Christensen, U.B.; Pedersen, E.B. Intercalating nucleic acids: The inversion of the stereocenter in 1-*O*-(pyren-1-ylmethyl)glycerol from *R* to *S*. Thermal stability towards ssDNA, ssRNA and its own type of oligodeoxynucleotides. *Tetrahedron Lett.* **2004**, 45, 4907–4910.
6. Chrisensen, U.B.; Wamberg, M.; El-Essawy, F.A.G.; Ismail, A.E.-H.; Nielsen, C.B.; Filichev, V.V.; Jessen, C.H.; Petersen, M.; Pedersen, E.B. Intercalating nucleic acids: The influence of linker length and intercalator type on their duplex stabilities. *Nucleosides, Nucelotides Nucleic Acids* **2004**, 23, 207–225.
7. Okamoto, A.; Ichiba, T.; Saito, I. Pyrene-labeled oligodeoxynucleotide probe for detecting base insertion by excimer fluorescence emission. *J. Am. Chem. Soc.* **2004**, 126, 8364–8365.
8. Yamana, K.; Iwai, T.; Ohtani, Y.; Sato, S.; Nakamura, M.; Nakano, H. Bis-pyrene-labeled oligonucleotides: Sequence specificity of excimer and monomer fluorescence changes upon hybridization with DNA. *Bioconjugate Chem.* **2002**, 13, 1266–1273.
9. Yamana, K.; Fukunaga, Y.; Ohtani, Y.; Sato, S.; Nakamura, M.; Kim, W.J.; Akaike, T.; Maruyama, A. DNA mismatch detection using a pyrene–excimer-forming probe. *Chem. Commun.* **2005**, 2509–2511.
10. Hainke, S.; Arndt, S.; Seitz, O. Concise synthesis of aryl-C-nucleosides by Friedel-Crafts alkylation. *Org. Biomol. Chem.* **2005**, 3, 4233–4238.
11. Gerard, B.; Sangji, S.; O'Leary, D.J.; Porco Jr., J.A. Enantioselective photocycloaddition mediated by chiral Brønsted acids: Asymmetric synthesis of the rocaglamides. *J. Am. Chem. Soc.* **2006**, 128, 7754–7755.
12. Yamane, A. MagiProbe: A novel fluorescence quenching-based oligonucleotide probe carrying a fluorophore and an intercalator. *Nucleic Acids Res.* **2002**, 30, e97/1–e97/8.
13. Dioubankova, N.N.; Malakhov, A.D.; Stetsenko, D.A.; Korshun, V.A. Detection of point mutations using pyrene-labeled DNA probes. *Russ. Chem. Bull.* **2004**, 53, 463–470.
14. Christensen, U.B.; Pedersen, E.B. Intercalating nucleic acids with pyrene nucleotide analogues as next-nearest neighbors for excimer fluorescence detection of single-point mutations under nonstringent hybridization conditions. *Helv. Chim. Acta* **2003**, 86, 2090–2097.
15. Taniguchi, M.; Koga, K.; Yamada, S. Stereochemical studies—XXX: Stereoselective synthesis of D-ribose from L-glutamic acid. *Tetrahedron* **1974**, 30, 3547–3552.

16. Lock, G. *Ber.*, Über abkömmlinge des pyrens. **1937**, 70B, 926–930.
17. Mujeeb, A.; Kerwin, S.M.; Kenyon, G.L.; James, T.L. Solution structure of a conserved DNA sequence from the HIV-1 genome: restrained molecular dynamics simulation with distance and torsion angle restraints derived from two-dimensional NMR spectra. *Biochemistry* **1993**, 32, 13419–13431.
18. Riddell, F.G.; Robinson, M.J.T. Conformational effects in compounds with six-membered rings—VI: Conformational equilibria in 5-alkyl-1,3-dioxane. *Tetrahedron* **1967**, 23, 3417–3425.
19. Nielsen, C.B.; Petersen, M.; Pedersen, E.B.; Hansen, P.E.; Christensen, U.B. NMR structure determination of a modified DNA oligonucleotide containing a new intercalating nucleic acid. *Bioconjugate Chem.* **2004**, 15, 260–269.
20. Yamana, K.; Iwase, R.; Furutani, S.; Tsuchida, H.; Zako, H.; Yamaoka, T.; Murakami, A. 2′-Pyrene modified oligonucleotide provides a highly sensitive fluorescent probe of RNA. *Nucleic Acids Res.* **1999**, 27, 2387–2392.
21. See http://www.schrodinger.com
22. Weiner, S.J.; Kollman, P.A.; Case, D.A.; Singh, U.C.; Ghio, C.; Alagona, G.; Profeta Jr., S.; Weiner, P. A new force field for molecular mechanical simulation of nucleic acids and proteins. *J. Am. Chem. Soc.* **1984**, 106, 765–784.
23. Weiner, S.J.; Kollman, P.A.; Nguyen, D.T.; Case, D.A. An all atom force field for simulations of proteins and nucleic acid. *J. Comp. Chem.* **1986**, 7, 230–252.
24. Still, W.C.; Tempczyk, A.; Hawley, R.C.; Hendrickson, T. Semianalytical treatment of solvation for molecular mechanics and dynamics. *J. Am. Chem. Soc.* **1990**, 112, 6127–6129.
25. van Gunsteren, W.F.; Berendsen, H.J.C. A leap-frog algorithm for stochastic dynamics. *Mol. Simul.* **1988**, 1, 173–185.
26. Ryckaert, J.-P.; Ciccotti, G.; Berendsen, H.J.C. Numerical integration of the cartesian equation of motion of a system with constraints: molecular dynamics of n-alkanes. *J. Comput. Phys.* **1997**, 23, 327–341.