

Intercalating nucleic acids (INAs) with insertion of *N*-(pyren-1-ylmethyl)-(3*R*,4*R*)-4-(hydroxymethyl)pyrrolidin-3-ol. DNA (RNA) duplex and DNA three-way junction stabilities

Vyacheslav V. Filichev and Erik B. Pedersen*

Nucleic Acid Center †, Department of Chemistry, University of Southern Denmark, DK-5230 Odense M, Denmark. E-mail: EBP@chem.sdu.dk; Fax: +45 6615 8780; Tel: +45 6550 2555

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N-(Pyren-1-ylmethyl)-(3*R*,4*R*)-4-(hydroxymethyl)pyrrolidin-3-ol was synthesised from (3*R*,4*R*)-4-(hydroxymethyl)pyrrolidin-3-ol and (3*R*,4*S*)-4-[(1*S*)-1,2-dihydroxyethyl]pyrrolidin-3-ol using alkylation with 1-(chloromethyl)pyrene or reductive amination with pyrene-1-carbaldehyde and NaCNBH₃. The incorporation of *N*-(pyren-1-ylmethyl)azasugar moiety into oligodeoxynucleotides (ODN) as a bulge to form an intercalating nucleic acid (INA) induced a slight destabilization of INA–DNA duplex, whereas the INA–RNA duplex was strongly destabilized and 9 °C difference per modification in thermal stability between INA–DNA over INA–RNA duplexes was observed. The stabilization of a DNA three way junction (TWJ) was improved when the intercalator moiety was inserted into the junction region as a bulge.

1. Introduction

The stabilized formation of the secondary structures of nucleic acids with chemically modified nucleosidic units is the main goal at the transcriptional (antigene) and translational (antisense) level of the gene expression.¹ The increased affinity and sequence selectivity are desirable properties to achieve a good recognition for therapeutic targets. It is known that there are some differences in the three-dimensional structures of DNA–DNA and DNA–RNA duplexes. By the chemical modification of nucleosidic glycons some oligonucleotide analogues having better increased affinity towards single stranded RNA (ssRNA) and towards single stranded DNA (ssDNA) were discovered, for example locked nucleic acids (LNA),² hexitol nucleic acids (HNA)³ and others.⁴ On the other hand all nucleic acids are stabilized both by hydrogen bonding interactions and by base stacking.¹ Therefore polyaromatic units with hydrophobicity in conjunction with a large surface area can be used in place of the natural nucleic bases for mimicking base stacking in Watson–Crick/Hoogsteen base pairing.⁵ The design and the synthesis of various oligonucleotides containing planar polycyclic aromatic chromophores such as pyrene, anthracene, dansyl and others, which have a long fluorescence lifetime and possibilities to form π -stacking interactions in aqueous solutions, have been the subject of active research in recent years.⁶ However there are only a few articles focused on the possible discrimination between ssDNA and ssRNA with the modification or substitution of nucleic bases in the complementary sequences. Bleczynski *et al.* observed the DNA/RNA discrimination by insertion of cholic acid and its deoxy derivatives into the 5'-termini of short oligonucleotides.⁷ Recently we have found that intercalating nucleic acids (INAs) containing 1-*O*-(pyrenylmethyl)glycerol insertions have a strong preference for hybridization with DNA over RNA,⁸ but little is known about the requirements of the backbone structure for the selectivity between DNA and RNA.

Single stranded oligonucleotides are often written as single stretches, but because of self pairing, branched structures (hairpins, internal loops, junctions) have been observed.⁹ For this reason, the number of possible targets is lower than it is

often believed. It has actually been shown that recognising the structure of nucleic acids could be achieved by forming a three way junction (TWJ) without disruption of the stem.¹⁰ Formation of the TWJ has been confirmed in NMR studies on complexes with two unpaired bases at the branch point and a preferred coaxial base stacking interaction was indicated.¹¹ Based on these findings we started out investigations devoted to the synthesis of pyren-1-yl units linked to different sugar-mimicking fragments which were used for insertions into ODNs for hybridization studies.¹²

Recently we synthesised the enantiomerically pure 1-aza analogue of 2-deoxy-D-ribofuranose having a nitrogen instead of the anomeric carbon and a methylene group instead of the ring oxygen.¹³ The corresponding 1'-aza-C-pseudonucleosides in which a carbon in the heterocyclic base is linked to the nitrogen of (3*R*,4*R*)-4-(hydroxymethyl)pyrrolidin-3-ol were also obtained.¹⁴ In continuation of this we now investigate the synthesis of *N*-pyrenemethyl type pseudonucleoside of the enantiomerically pure 1-aza analogue of 2-deoxy-D-ribofuranose and its insertion into several ODNs to form INAs in order to investigate their thermal stability when forming duplexes with complementary ssDNA and ssRNA or when forming DNA TWJs.

2. Results and discussion

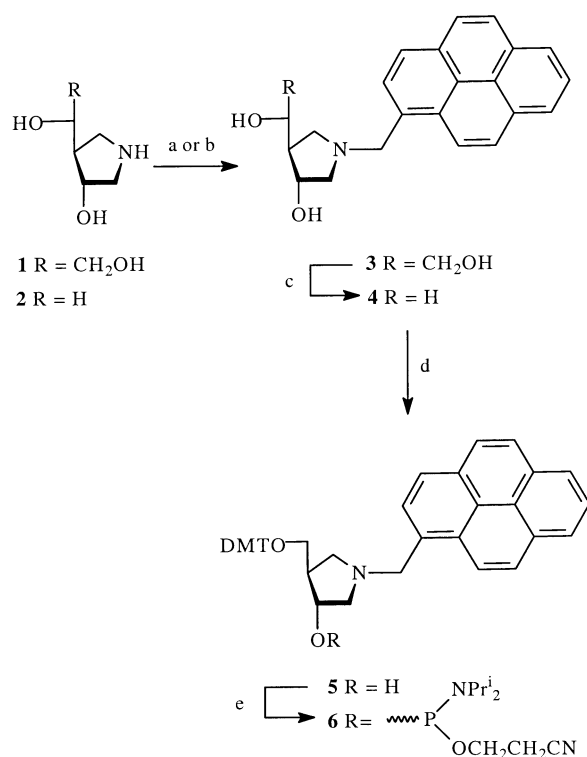
The synthesis of 1'-aza pyrenemethyl pseudonucleoside **4** started from the previously published enantiomerically pure 1-aza analogues of 2-deoxy-D-hexofuranose **1** or 2-deoxy-D-ribofuranose **2**.¹³ To find the most efficient synthesis of the target compound **4**, we used pyrene substrates having chloromethyl and carbaldehyde functionalities that could be coupled with the secondary amines **1** and **2**. We found that alkylation by 1-(chloromethyl)pyrene in the presence of Et₃N led to a lower yield (57% and 46% for **3** and **4**, respectively) compared to reductive amination with pyrene-1-carbaldehyde and NaCNBH₃ (63% and 57%, respectively, Scheme 1). Surprisingly low overall yield 40% was observed in the synthesis of **4** using oxidative cleavage of the diol **3** followed by reduction of the obtained aldehyde. This may be due to low stability of the pyren-1-ylmethyl azasugars **3** and **4**, which should be stored in a refrigerator under nitrogen or used immediately to avoid low yields in subsequent reactions.

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Table 1 Sequences and hybridization data of synthesised ODNs in DNA–DNA (RNA) duplexes

No.	X	Y	$T_m/^\circ\text{C}$ (DNA–DNA)	$\Delta T_m/^\circ\text{C}$	$T_m/^\circ\text{C}$ (DNA–RNA)	$\Delta T_m/^\circ\text{C}$	$\Delta\Delta T_m$ DNA–RNA
A	—	—	43.0		42.2		
B	G	—	32.2	–10.8	32.6	–9.6	1.2
C	I	—	41.8	–1.2	32.2	–10.0	8.8
D	I	I	39.4	–1.2	21.8	–10.2	9.0

I = inserted nucleoside analogue **4**, ΔT_m = decrease in T_m per modification, $\Delta\Delta T_m$ DNA–RNA = discrimination in T_m between DNA–DNA and DNA–RNA duplexes per modification

**Scheme 1** (a) 1-(chloromethyl)pyrene, Et_3N , DMF; (b) pyrene-1-carbaldehyde, NaCNBH_3 , DMF–EtOH (3 : 1); (c) 1) NaIO_4 , 2) NaBH_4 ; (d) DMTCl, pyridine; (e) $\text{NC}(\text{CH}_2)_2\text{OP}(\text{NPr}_2)_2$, N,N -diisopropylammonium tetrazolide, CH_2Cl_2 .

The 4,4'-dimethoxytriphenylmethyl (DMT) protected phosphoramidite **6** is required for the oligonucleotide synthesis. The primary alcohol **4** was treated with an excess of DMTCl in pyridine with further purification on a silica gel column to give compound **5** in 61% yield. The synthesis of the final phosphoramidite by treatment with 2-cyanoethyl- N,N -isopropylchlorophosphoramidite in the presence of the excess of Hunig's base¹² failed. To obtain the required phosphoramidite **6** we used an alternative method with 2-cyanoethyl- N,N,N',N' -tetra-isopropylphosphorodiamidite and N,N -diisopropylammonium tetrazolide.¹⁵ The compound **6** was obtained in 57% yield.

The phosphoramidite **6** was incorporated into different oligonucleotide sequences to give INAs on an automated solid phase DNA synthesizer using an increased coupling time (24 min) and repeating the cycle twice. The coupling efficiencies for the pyrene azasugar derivative **6** were approximately 80–85% compared to approximately 99% for commercial phosphoramidites (2 min coupling).

The synthesised INAs were used in the hybridization studies of INA–DNA and INA–RNA duplexes (Table 1) and INA–DNA three way junction (TWJ) (Table 2).

Table 2 Hybridization data ($T_m/^\circ\text{C}$) for the DNA three-way junction

	F1 (X = 0)	F2 (X = A)	F3 (X = I)
E1	38.6	39.4	48.6
E2	<18.0	20.2	24.2
E3	<18.0	19.4	<18.0

I = inserted nucleoside analogue **4**.

INA with incorporation of the N -(pyren-1-ylmethyl)-azasugar as the bulge resulted in lowering of the melting temperature with 1.2 °C per modification towards ssDNA (Table 1). The corresponding reference duplex in entry **B** containing a bulging deoxynucleotide (dG) had a considerably lower T_m = 32.2 °C (T_m = –10.8 °C). For the INA–RNA duplexes the pyrene containing sequence **C** and the reference **B** decreased the stability of the INA–RNA duplex with 10 °C and 9.6 °C, respectively, compared to the perfectly matched duplex (entry **A**). Consequently, INA with pyrene azasugar incorporated as the bulge has better hybridization affinity towards the complementary ssDNA than towards ssRNA. The differences in melting temperatures for ssDNA and ssRNA seems to be additive with respect to the number of pyrene moieties in the targeting ODN. These results are also in agreement with other investigations where 1- O -(pyren-1-ylmethyl)glycerol was inserted twice as bulges. A larger discrimination up to 25.8 °C between INA–DNA and INA–RNA was then observed.⁸ In that case the INA–DNA structure is stabilized compared to the wild type duplex in contrast to our case where a slight decrease is observed for T_m . The flexibility of the bulge may be an important factor to obtain both duplex stabilization and discrimination. In order to understand the nature of the difference in the thermal stability between DNA and RNA duplexes observed, further experiments are in progress using NMR spectroscopy for structural examination of the duplexes. The synthesis of different linkers and planar aromatic moieties is also in progress. The RNA/DNA discrimination displayed may be applied for purification or detection of DNA targets in a mixture with the very same sequences of RNA.

As a second target in our investigation we chose a DNA three way junction (TWJ) composed of two arms linked to a stem (Table 2). Here we observed a considerable stabilization when the pyrene azasugar intercalator was inserted in the INA (**F3**)

compared to the ODN having dA at the same position (**F2**) or without an insertion in the ODN (entry **F1**). To be sure that hybridization in the arms is important for the stability of the complex; we prepared ODNs with mismatches in either arm of TWJ (entry **E2** and **E3**). In both cases it resulted in a large lowering of the hybridization affinity.

3. Conclusion

Thus we prepared and used *N*-(pyren-1-ylmethyl)-(3*R*,4*R*)-4-(hydroxymethyl)pyrrolidin-3-ol (**4**) in the synthesis of several INAs and investigated the hybridization affinity of INA–DNA, INA–RNA duplexes and DNA TWJ region. When the *N*-(pyren-1-ylmethyl)azasugar was inserted as a bulge we observed good discrimination between stabilities of INA–DNA and INA–RNA duplexes and the increased stability of a DNA three-way junction. We believe that these findings will be helpful in molecular design of targets and probes for DNA diagnostics.

4. Experimental

4.1 General

NMR spectra were recorded on a Bruker AC-300 FT NMR spectrometer at 300 MHz for ¹H NMR and at 75.5 MHz for ¹³C NMR. Internal standards used in ¹H NMR spectra were TMS (δ : 0.00) for CDCl₃, CD₃OD; in ¹³C NMR were CDCl₃ (δ : 77.0), CD₃OD (δ : 49.0). Accurate ion mass determination was performed on a Kraton MS-50-RF equipped with FAB source. The [M + H]⁺ ions were peakmatched using ions derived from the glycerol matrix. Thin layer chromatography (TLC) analyses were carried out with use of TLC plates 60 F₂₅₄ purchased from Merck and were visualized in UV light (254 and/or 343 nm) and/or with a ninhydrin spray reagent (0.3 g ninhydrin in 100 cm³ butan-1-ol and 3 cm³ HOAc) for azasugars and their derivatives. The silica gel (0.063–0.200) used for column chromatography was purchased from Merck. ODNs were synthesised on an Assembler Gene Special DNA-Synthesizer (Pharmacia Biotech). Purification of 5'-*O*-DMT-on and 5'-*O*-DMT-off ODNs were accomplished using a Waters Delta Prep 4000 Preparative Chromatography System. The modified ODNs were confirmed by MALDI-TOF analysis on a Voyager Elite Biospectrometry Research Station from PerSeptive Biosystems. All solvents were distilled before use. The reagents used were purchased from Aldrich, Sigma or Fluka. The reagents for Gene Assembler were purchased from Cruachem (UK).

4.1.1 *N*-(Pyren-1-ylmethyl)-(3*R*,4*S*)-4-[(1*S*)-1,2-dihydroxyethyl]pyrrolidin-3-ol (3**).** *Method A.* Azasugar **1** (50 mg, 0.34 mmol) was dissolved in DMF (5 cm³); 1-(chloromethyl)pyrene (103 mg, 0.41 mmol) and Et₃N (0.057 cm³, 0.41 mmol) were added. The reaction mixture was stirred at room temp. under nitrogen overnight. The solvent was evaporated under reduced pressure and co-evaporated with toluene (2 × 5 cm³). The residue was chromatographed on a silica gel column with CH₂Cl₂–MeOH (0–20%, v/v) as eluent affording the pure product **3** (70 mg, 57%): *R*_f 0.20 (10% MeOH–CH₂Cl₂); δ_{H} (CD₃OD) 2.36 (1 H, m, H-4), 2.95 (1 H, m, H-5), 3.08 (1 H, dd, *J* 2.8 and 10.5, H-2), 3.22 (1 H, dd, *J* 5.4 and 12.0, H-5), 3.34 (1 H, m, H-2), 3.50–3.65 (3 H, m, CH[OH]CH₂OH), 4.42 (1 H, m, H-3), 4.65 (2 H, s, CH₂pyren-1-yl), 4.88 (3 H, br s, 3 × OH), 7.90–8.40 (9 H, m, H_{arom}); δ_{C} (CD₃OD) 50.7 (C-4), 56.7 (C-5), 57.2 (C-2), 62.7 (CH₂pyren-1-yl), 65.7 (CH₂OH), 71.8 (CH[OH]), 72.7 (C-3), 123.8, 125.5, 125.8, 125.9, 126.6, 126.8, 127.3, 128.0, 128.2, 129.1, 129.4, 129.9, 131.2, 131.9, 132.5, 133.2 (pyren-1-yl); *m/z* (FAB) 362.1748 [M + H]⁺, C₂₃H₂₄NO₃ requires 362.1756.

Method B. Azasugar **1** (70 mg, 0.48 mmol) was dissolved in DMF–EtOH (3 : 1, 10 cm³) and pyrene-1-carbaldehyde (270

mg, 1.18 mmol) and NaCNBH₃ (74 mg, 1.18 mmol) were added. The reaction mixture was stirred at room temp. under nitrogen overnight. Concentrated HCl was added until pH < 2. Solvent was evaporated under reduced pressure and co-evaporated with toluene (2 × 5 cm³). The residue was purified using silica gel column chromatography with CH₂Cl₂–MeOH (0–20%, v/v) affording the compound **3** (110 mg, 63%).

4.1.2 *N*-(Pyren-1-ylmethyl)-(3*R*,4*R*)-4-(hydroxymethyl)pyrrolidin-3-ol (4**).** *Method A.* Azasugar **2** (100 mg, 0.86 mmol) was dissolved in DMF (10 cm³) and 1-(chloromethyl)pyrene (257 mg, 1.03 mmol) and Et₃N (0.140 cm³, 1.03 mmol) were added. The reaction mixture was stirred at room temp. under nitrogen overnight. The solvent was evaporated under reduced pressure and co-evaporated with toluene (2 × 5 cm³). The residue was dissolved in H₂O–CH₂Cl₂ (1 : 1, 40 cm³) and the water layer was extracted with CH₂Cl₂. The combined organic fractions were dried (Na₂SO₄), evaporated *in vacuo* and chromatographed on a silica gel column with CH₂Cl₂–MeOH (0–20%, v/v) affording the title compound **4** (130 mg, 46%): *R*_f 0.17 (10% MeOH–CH₂Cl₂); δ_{H} (CDCl₃) 2.08 (1 H, m, H-4), 2.29 (1 H, m, H-5), 2.57 (1 H, dd, *J* 2.8 and 10.2, H-2), 2.85 (2 H, m, H-2 and H-5), 3.43 (2 H, s, CH₂OH), 3.47 (2 H, br s, 2(OH)), 4.08 (1 H, m, H-3), 4.19 (2 H, s, CH₂pyren-1-yl), 7.90–8.40 (9 H, m, H_{arom}); δ_{C} (CDCl₃) 49.8 (C-4), 55.9 (C-5), 57.5 (C-2), 62.3 (CH₂pyren-1-yl), 64.2 (CH₂OH), 73.9 (C-3), 123.3, 124.4, 124.6, 124.8, 125.1, 125.9, 127.3, 127.6, 127.8, 129.5, 130.7, 130.9, 131.1 (pyren-1-yl); *m/z* (FAB) 332.1631 [M + H]⁺, C₂₃H₂₄NO₃ requires 332.1651.

Method B. Azasugar **2** (1.18 g, 10.1 mmol) was dissolved in DMF–EtOH (3 : 1, 150 cm³) and pyrene-1-carbaldehyde (3.47 g, 15.1 mmol) and NaCNBH₃ (950 mg, 15.1 mmol) were added. The reaction mixture was stirred at room temp. under nitrogen overnight. Concentrated HCl was added until pH < 2. The solvent was evaporated under reduced pressure and co-evaporated with toluene (2 × 5 cm³). The residue was dissolved in H₂O–CH₂Cl₂ (1 : 1, v/v, 150 cm³) and the water layer was extracted with CH₂Cl₂ (3 × 75 cm³). The combined organic fractions were dried (Na₂SO₄) and evaporated under diminished pressure. The residue was purified using silica gel column chromatography with CH₂Cl₂–MeOH (0–20%, v/v) affording the compound **4** as an oil which crystallised on standing (1.9 g, 57%), mp 104–105 °C.

Method C. A cooled solution of compound **3** (110 mg, 0.304 mmol) in EtOH (4 cm³) was added to a solution of NaIO₄ (71.6 mg, 0.335 mmol) in H₂O (1.5 cm³) under stirring. After 30 min NaBH₄ (12.3 mg, 0.335 mmol) was added. After 30 min the resulting solution was acidified with 2 M HCl until pH < 2 under vigorous stirring. The solvent was removed *in vacuo*. The residue was dissolved in H₂O–CH₂Cl₂ (1 : 1, v/v, 20 cm³) and extracted with CH₂Cl₂ (4 × 15 cm³). The combined organic layers were dried (Na₂SO₄), evaporated under diminished pressure to dryness affording compound **4** (40 mg, 40%).

4.1.3 *N*-(Pyren-1-ylmethyl)-(3*R*,4*R*)-4-[(4,4'-dimethoxytriphenylmethoxy)methyl]pyrrolidin-3-ol (5**).** Compound **4** (139 mg, 0.42 mmol) was dissolved in anhydrous pyridine (10 cm³) and DMTCl (178 mg, 0.53 mmol) was added. The mixture was stirred for 24 h under nitrogen at room temp. MeOH (1 cm³) was added to quench the reaction and the solvents were evaporated under reduced pressure and co-evaporated with toluene (2 × 5 cm³). The residue was re-dissolved in H₂O–CH₂Cl₂ (1 : 1, v/v, 20 cm³), and the mixture was washed with saturated aqueous NaHCO₃. The organic layer was dried (Na₂SO₄) and concentrated under reduced pressure. Purification using silica gel column chromatography (5–40% EtOAc–cyclohexane, v/v) gave the title compound **5** as a foam (160 mg, 61%) which was used in the next step without further purification: *R*_f 0.45 (49% EtOAc–49% cyclohexane–2% Et₃N, v/v/v); δ_{H} (CDCl₃) 2.20 (1 H, m, H-4), 2.34 (1 H, m, H-5),

2.53 (1 H, br s, OH), 2.62 (1 H, dd, J 5.6 and 9.9, H-2), 2.72 (1 H, dd, J 2.5 and 9.8, H-2), 3.06 (3 H, m, CH_2ODMT and H-5), 3.71 (6 H, s, OCH_3), 4.01 (1 H, m, H-3), 4.21 (2 H, s, $\text{CH}_2\text{pyren-1-yl}$), 6.78 (4 H, m, DMT), 7.10–7.40 (9 H, m, DMT), 7.90–8.40 (9 H, m, H_{arom}); $\delta_{\text{C}}(\text{CDCl}_3)$ 48.8 (C-4), 55.2 (OCH_3), 56.1 (C-5), 58.0 (C-2), 61.9 ($\text{CH}_2\text{pyren-1-yl}$), 64.5 (CH_2OH), 74.9 (C-3), 85.9 (C-Ar₃), 113.0, 123.8–132.3 (DMT and pyren-1-yl), 144.9, 158.4 (DMT); m/z (FAB) 634.2740 $[\text{M} + \text{H}]^+$, $\text{C}_{44}\text{H}_{42}\text{NO}_5$ requires 634.2722.

4.1.4 *N*-(Pyren-1-ylmethyl)-(3*R*,4*R*)-3-*O*-[2-cyanoethoxy-(diisopropylamino)phosphino]-4-[(4,4'-dimethoxytriphenyl-methoxy)methyl]pyrrolidine (6). Compound **5** (140 mg, 0.22 mmol) was dissolved under nitrogen in anhydrous CH_2Cl_2 (5 cm^3). *N,N*-Diisopropylammonium tetrazolide (61 mg, 0.42 mmol) was added followed by dropwise addition of 2-cyanoethyl-*N,N,N',N'*-tetraisopropylphosphorodiamidite (0.140 cm^3 , 0.44 mmol). After 2.0 h analytical TLC showed no more starting material and the reaction was quenched with H_2O (1 cm^3) followed by addition of CH_2Cl_2 (10 cm^3). The mixture was washed with saturated aqueous NaHCO_3 (2 \times 10 cm^3). The organic phase was dried (Na_2SO_4) and the solvents were removed under reduced pressure. The residue was purified using silica gel column chromatography with cyclohexane–EtOAc (0–20%, v/v). Combined UV-active fractions were evaporated *in vacuo* affording **6** (158 mg, 57%) as foam that was co-evaporated with dry acetonitrile (3 \times 30 cm^3) before using it in ODN synthesis. R_f 0.85 (49% EtOAc–49% cyclohexane–2% Et₃N, v/v/v); $\delta_{\text{H}}(\text{CDCl}_3)$ 0.93 (6 H, m, CH_3 [Pr^i]), 1.04 (6 H, m, CH_3 [Pr^i]), 2.30 (2 H, m, H-4 and H-5), 2.48 (2 H, m, CH_2CN), 2.64 (1 H, m, H-2), 2.78 (1 H, m, H-2), 2.98 (2 H, m, $\text{OCH}_2\text{-CH}_2\text{CN}$), 3.08 (1 H, m, H-5), 3.50 (4 H, m, CH [Pr^i] and CH_2ODMT), 3.65 (6 H, s, OCH_3), 4.01 (1 H, m, H-3), 4.20 (2 H, m, $\text{CH}_2\text{pyren-1-yl}$), 6.68 (4 H, m, DMT), 7.05–7.40 (9 H, m, DMT), 7.85–8.40 (9 H, m, H_{arom}); $\delta_{\text{P}}(\text{CDCl}_3)$ 148.2 (s), 149.0 (s) in the ratio 2 : 1.

4.2 Synthesis and purification of modified and unmodified oligodeoxynucleotides

The oligodeoxynucleotides were synthesised on a Pharmacia Gene Assembler[®] Special synthesizer in 0.2 μmol -scale (7.5 μmol embedded per cycle, Pharmacia primer support[™]) using commercially available 2-cyanoethylphosphoramidites and **6**. The synthesis followed the regular protocol for the DNA synthesizer. The coupling time for **6** was increased from 2 to 24 min and the cycle was repeated twice. The 5'-*O*-DMT-on ODNs were removed from the solid support and deprotected with 32% aqueous NH_3 (1 cm^3) at 55 °C for 24 h and then purified on preparative HPLC using a Hamilton PRP-1 column. The solvent systems were buffer A [950 cm^3 0.1 M NH_4HCO_3 and 50 cm^3 CH_3CN (pH = 9.0)] and buffer B [250 cm^3 0.1 M NH_4HCO_3 and 750 cm^3 CH_3CN (pH = 9.0)] which were used in the following order: 5 min A, 30 min linear gradient of 0–70% B in A, 5 min linear gradient of 70–100% B in A. Flow rate was 1 $\text{cm}^3 \text{min}^{-1}$. The purified 5'-*O*-DMT-on ODNs eluted as one peak after approximately 30 min [UV control 254 nm and 343 nm (for pyrene containing ODNs)]. The fractions were concentrated *in vacuo* followed by treatment with 10% aqueous HOAc (1 cm^3) for 20 min and further purification on HPLC under the same conditions to afford detritylated ODNs which eluted at 23–28 min. The purity of oligos synthesised was 99–100% according to the preparative HPLC. The resulted solutions were evaporated *in vacuo* and co-evaporated twice with water to remove volatile salts to afford ODNs, which were used in melting temperature measurements.

All oligonucleotides containing pyrenylmethylazasugar derivative **6** were confirmed by MALDI-TOF analysis (entry **C**: found 4005.65, calcd. 4005.76; entry **D**: 4398.02, calcd. 4398.87; entry **F3**: found 4903.05, calcd. 4904.89).

4.3 Melting experiments

Melting temperature measurements were performed on a Perkin-Elmer UV/VIS spectrometer fitted with a PTP-6 Peltier temperature-programming element. The absorbance at 260 nm was measured from 18 °C to 85 °C in 1 cm cells. The melting temperature was determined as the maximum of the derivative plots of the melting curve. The oligodeoxynucleotides were dissolved in a medium salt buffer (pH = 7.0, 1 mM EDTA, 10 mM $\text{Na}_2\text{HPO}_4 \times 2\text{H}_2\text{O}$, 140 mM NaCl) to a concentration of 1.0 μM for each strand.

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