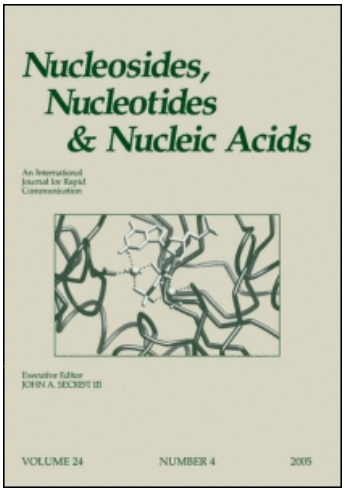

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SYNTHESIS AND DNA BINDING PROPERTIES OF A NEW BENZO[f]IMIDAZO[1,5b]-ISOQUINOLINE-BUTAN-1,2-DIOL DERIVATIVE

Youssef L. Aly and Erik B. Pedersen □ *Nucleic Acid Center, Department of Chemistry, University of Southern Denmark, Odense M, Denmark*

□ *A benzo[f]imidazo[1,5b]-isoquinoline derivative* ***4*** *with a 1,2-butandiol linker was prepared by reaction of a trimethylsilylated 5-naphthylidenehydantoin* ***3*** *with a 2,3-dideoxy-D-glycero-pentafuranoside* ***2*** *in 22% yield. After deprotection, the resulting compound* ***5*** *was converted to a DMT protected phosphoramidite building block* ***7*** *for standard DNA synthesis. DNA/DNA, DNA/RNA duplexes with* ***5*** *inserted as bulges were destabilized, except when the new amidite was used for the synthesis of a zipping duplex.*

Keywords Intercalating nucleic acids; Benzo[f]imidazo[1,5b]-isoquinoline-butan-1,2-diol; DNA duplex destabilization; Fluorescence

INTRODUCTION

A large number of nonnatural analogues of DNA have been synthesized in recent years. Changing the structure of the base moiety has been a useful strategy for improving the structure and the function in DNA. For example, a number of bases have been used to test the importance of specific hydrogen bonding interactions which may be important for function of natural nucleic acid bases,[1–3] using this strategy, workers have examined the importance of hydrogen bonding in stabilizing DNA and RNA structure,[1] in protein-DNA interaction,[2] and in the fidelity of enzymatic DNA and RNA synthesis.[3]

The presence of a large aromatic system can lead to a higher affinity for single stranded DNA and double stranded DNA as it has been shown for

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INA (intercalating nucleic acids)[4] and TINA (twisted intercalating nucleic acids),[5] respectively. Moreover, the presence of the heterocycle may have an effect on the electrostatic properties of the chromophore, which could improve the affinity and sequence selectivity of this kind of intercalator.

Previously, we have designed a route for the synthesis of a condensed isoquinoline derivatives, through a condensation reaction of a naphthylideneimidazoledione with a deoxyribose derivative.[6] We now have developed this class of compounds for the insertion into DNA as a new example of an intercalating nucleic acid.

RESULTS AND DISCUSSION

Methyl 2,3-dideoxy-5-*O*-(4-phenylbenzoyl)-D-glycero-pentafuranoside (**2**) was prepared as described in the literature[7] by treatment of 2-deoxyribose (**1**) with HCl in methanol and subsequently with 4-phenyl benzoyl chloride in pyridine followed by acylation with phenyl chlorothiocarbonate and deoxygenation by tri-*n*-butyltin hydride. The trimethylsilylated naphthylidene-hydantoin derivative **3**[6] was condensed as devised by Vorbrüggen et al.[8,9] with the protected dideoxy sugar **2** in the presence of trimethylsilyl trifluoromethanesulfonate (TMS triflate) at 5°C for 3 days. The reaction resulted in a ring closure reaction and the pseudo nucleoside **4** was isolated in 22% yield as pure stereoisomer, however, the configuration of C-7 is not known. The structure of compound **4** was confirmed by ^{1}H, ^{13}C-NMR, and mass spectroscopy and the spectroscopic data are in agreement with analogous compound synthesized from a 2-deoxyribose derivative by the group of Pedersen et al.[6]

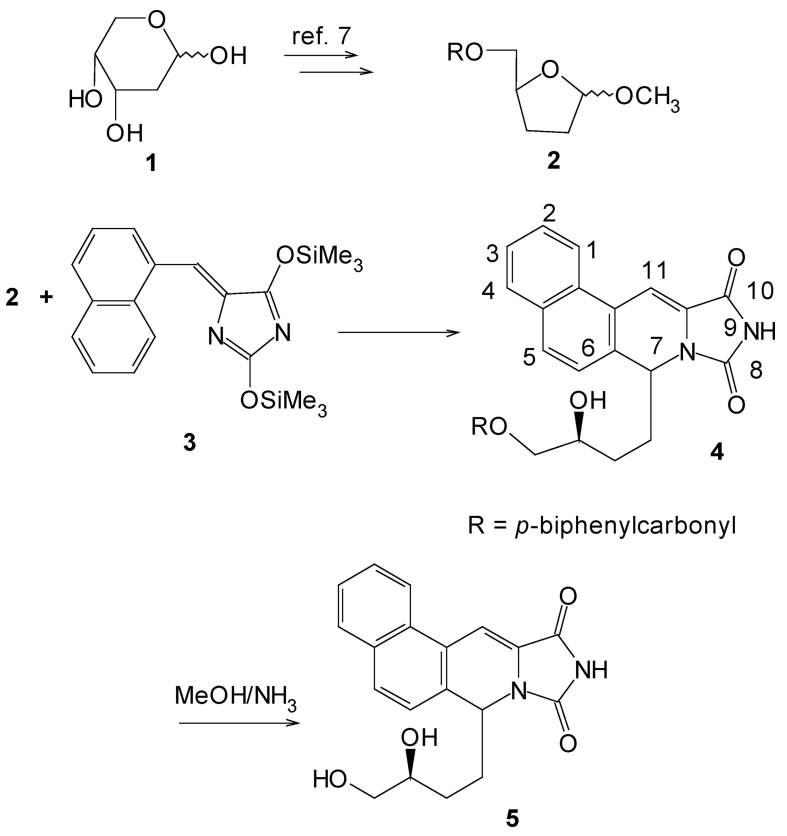
Removal of the protecting biphenylcarbonyl group from the glycon moiety in **4** with methanolic ammonia at room temperature furnished the diol derivative **5** (Scheme 1).

The primary hydroxy group of compound **5** was protected by reaction with 4,4′-dimethoxytrityl chloride (DMTCl) in CH_2Cl_2 at room temperature to give the DMT-protected compound **6**, which was converted to the phosphoramidite **7** with 2-cyanoethyl-*N,N,N',N'*-tetraisopropylphosphordiamidite (Scheme 2).

The amidite **7** was used for the synthesis of oligos, which all showed correct masses in MALDI-TOF-MS analysis (Table 1).

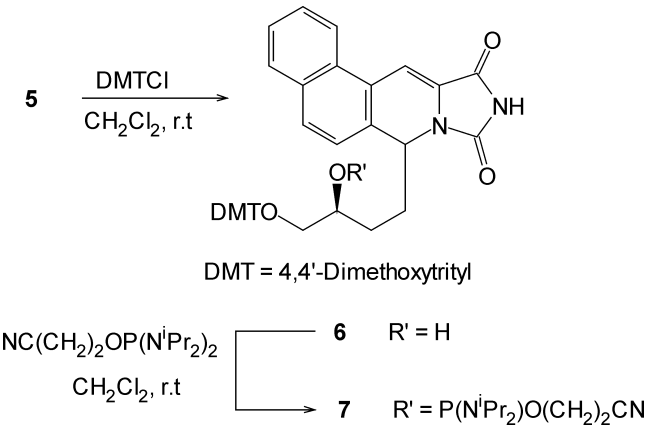
The new modified oligos were hybridized toward complementary DNA and RNA in such a way that the pseudonucleoside **5** formed bulges and the duplexes were evaluated by thermal denaturation studies. The melting temperatures (T_m) were determined as the maxima of the first derivatives of the melting curve and they are listed in Tables 2 and 3.

The isoquinoline derivative **5** was found to destabilize the DNA/DNA duplexes by −1.5°C to −7°C when inserted as a bulge into the central region of the sequence (Table 2, entries 2 and 3). The stability was strongly dependent on the sequence so that a pyrimidine rich neigborhood of the



SCHEME 1 Synthesis of compound 5.

pseude nucleoside resulted in a substantially higher destabilization. The destabilization was further enhanced for intercalating nucleic acid having double insertions of **5** separated by 1–4 nucleobases as deduced from ΔT_m −11.5°C to –13°C (Table 2, entries 4–6).



SCHEME 2 Synthesis of DMT-protected amidite 7.

TABLE 1 Masses of the oligos

Entry	Sequence	Expected mass	MALDI TOF analysis
1	5′-CTCAAG**5**CAAGTC-3′	4012.3	4010.0
2	5′-AGCTTG**5**CTTGAG-3′	4074.3	4076.3
3	5′-CTCAA**5**G**5**CAAGCT-3′	4411.3	4415.5
4	5′-CTCA**5**AG**5**CAAGCT-3′	4411.3	4415.0
5	5′-CTCA**5**AGCA**5**AGCT-3′	4411.3	4414.3

For RNA a similar destabilization and sequence dependency was observed (Table 3). A destabilization of the DNA/RNA duplex was only –1°C when **5** was inserted as a bulge in a purine rich region (Table 3,entry 2), while large destabilizations (−10°C to −14°C) were observed in all cases for double insertion (Table 3, entries 3–5).

In Table 4, it is investigated whether insertion of **5** opposite to a bulge can compensate for the missing natural nucleobase. This often is observed for pseudonucleotides with intercalating properties. However, in this case absolutely no extra stabilization was achieved by inserting **5** opposite to bulging nucleobases. It seems that the structure of **5** is too large and, therefore, causes steric interactions for intercalation. However, it is interesting to note that the stabilization is maintained when 2 modified nucleotides **5** are opposing each other in a zipping manner. In fact the melting temperature was increased by 1°C in this case. Besides this case, the steric requirement of **5** only seems to allow intercalation in case of purine rich oligos as shown for entries 2 in Table 2 and 3 as deduced from marginal decreases in melting temperature.

TABLE 2 Melting temperatures of DNA/DNA duplexes with compound **5** inserted as a bulge at pH = 7

Entry	Sequence	T_m (°C)	ΔT_m (°C)
1	5′-AGCTTG CTTGAG-3′ 3′-TCGAAC GAACTC-5′	46	—
2	5′-AGCTTG CTTGAG-3′ 3′-TCGAAC**5**GAACTC-5′	44.5	−1.5
3	5′-AGCTTG**5**CTTGAG-3′ 3′-TCGAAC GAACTC-5′	39	−7
4	5′-AGCTTG C TTGAG-3′ 3′-TCGAAC **5** G **5**AACTC-5′	34.5	−11.5
5	5′-AGCT TGCT TGAG-3′ 3′-TCGA**5**ACGA**5**ACTC-5′	33.5	−12.5
6	5′-AGCT TGCT TGAG-3′ 3′-TCGA**5**ACGA**5**ACTC-5′	33.0	−13

TABLE 3 Melting temperatures of DNA/RNA duplexes with **5** inserted as a bulge at pH = 7

Entry	Sequence	T_m (°C)	ΔT_m (°C)
1	5′-AGCUUG CUUGAG-3′ 3′-TCGAAC GAACTC-5′	40	—
2	5′-AGCUUG CUUGAG-3′ 3′-TCGAAC**5**GAACTC-5′	39	−1
3	5′-AGCUUG C UUGAG-3′ 3′-TCGAAC**5**G**5**AACTC-5′	29.5	−10.5
4	5′-AGCUUG CU UGAG-3′ 3′-TCGAAC**5**GA**5**ACTC-5′	29	−11
5	5′-AGCU UGCU UGAG-3′ 3′-TCGA**5**ACGA**5**ACTC-5′	26	−14

FLUORESCENCE

The fluorescence emission upon excitation of **5** inserted into single strands was found at ≈460 nm. The highest fluorescence intensity was recorded for single strands when **5** was inserted 2 times as bulges, whereas the lowest fluorescence intensity was observed for the corresponding duplexes (Figure 1). This can be taken as an evidence of intercalation of **5** on duplex formation. No eximer band was observed for **5** when inserted 1 or 2 times in the sequence as it was founded by Lewis and coworkers for naphthalene base analogue in 1996.[10]

CONCLUSION

Through thermal melting temperature studies we have shown that insertion of **5** in both strands of a DNA duplex in a zipping manner

TABLE 4 Melting temperature of DNA/DNA duplexes with compound **5** as a universal base or with zipping of **5** at pH = 7

		5′−AGCTTG **Y** CTTGAG −3′ 3′−TCGAAC **X** GAACTC −5′	
Entry	**Y**, **X**	T_m	ΔTm
1	—, —	46.0	—
	5, **5**	47.0	+ 1
2	A, —	31.5	—
	A, **5**	30.4	− 1.1
3	C, —	33.5	—
	C, **5**	32.9	− 0.6
4	G, —	33.7	—
	G, **5**	32.9	− 0.8
5	T, —	33.4	—
	T, **5**	32.9	− 0.5

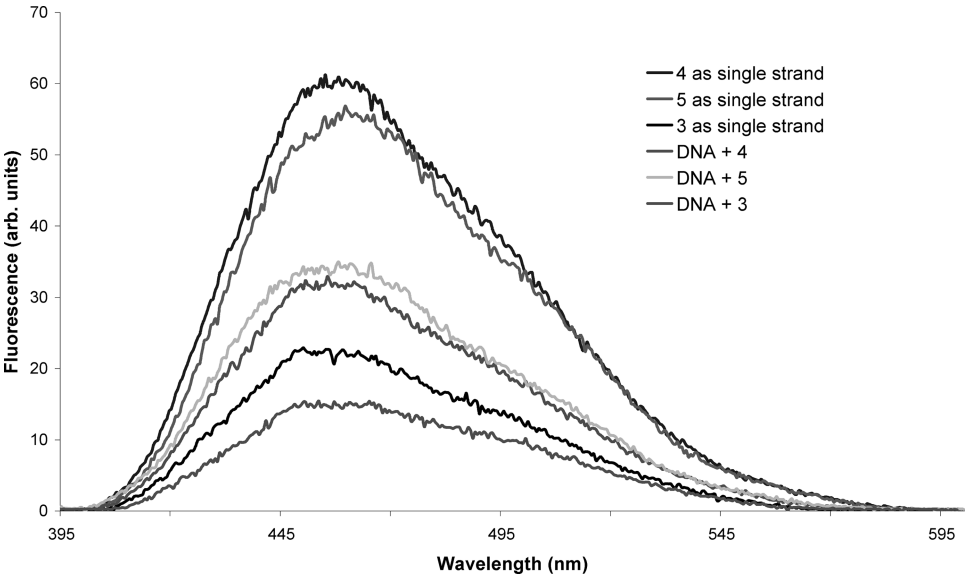


FIGURE 1 Fluorescence measurement of single strand DNA and its duplexes with complementary DNA. Numbering refers to entries in Table 1. The duplexes are found in entries 4 and 5 in Table 2.

stabilized the duplex whereas this was not the case on insertion of **5** as bulges in DNA/DNA or DNA/RNA duplexes.

EXPERIMENTAL

NMR Spectra were recorded on a 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 (^{1}H NMR); for ^{13}C NMR: $CDCl_3$ (δ 77.0), DMSO (δ 39.44). Accurate-ion mass determinations were performed using the 4.7 T Ultima Fourier transform (FT) mass spectrometer (IonSpec, Irvine, CA, USA). All modified oligos were confirmed by MALDI-TOF MS analysis on a Voyager Elite Biospectrometry Research Station from PerSeptive Biosystems. The $(M+H)^+$ and $(M+Na)^+$ ions were peak matched using ions derived from the 2,5-dihydroxybenzoic acid matrix. The progresses of reactions were monitored by TLC (analytical silica gel 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, Darmstacht, Germany). Solvents used for column chromatography were distilled prior to use, while reagents were used as purchased.

*Silylated of 5-naphthylidenehydantoin (**3**).* A mixture of the 5-naphthylidene-hydantoin (1.9 g, 8.0 mmol), anhydrous $(NH_4)_2SO_4$ (0.005 g, 0.04 mmol), and 1,1,1,3,3,3-hexamethyldisilazane (60 mL) was refluxed overnight. The

clear solution obtained was cooled and the excess of solvent was evaporated in vacuo to give the silylated compound as pale yellow oil.

*7-[(S)-4-(p-Biphenylcarbonyloxy)-3-hydroxybutyl]-7 H-7 a, 9-diazacyclopenta-[b]-phenanthrene-8,10-dione (**4**).* A solution of the sugar **2** (1.68 g, 5.4 mmol) in dry MeCN (20 mL) was added to a stirred solution of the silylated 5-naphthylidenehydantoin **3** in dry MeCN (30 mL) and the mixture was cooled to −50°C. A solution of trimethylsilyl trifluoromethanesulfonate (1.08 mL, 6 mmol) in dry MeCN (10 mL) was added dropwise during 5 min at −50°C and the mixture was stirred at −30°C for 4 hours and then at 5°C for 2 days. The reaction mixture was diluted with CH_2Cl_2 (200 mL), washed with cold saturated aqueous $NaHCO_3$ (200 mL) and water (2 × 100 mL), and dried over anhydrous $MgSO_4$. The mixture was concentrated in vacuo and the residue was chromatographed on a silica gel column with $CHCl_3$/MeOH (95:5, v/v) to afford compound **4** (0.8 g, 22%) as a yellow foam. ^{1}H-NMR (DMSO-d_6) δ 1.25−1.14 (m, 2 H, H-2′), 1.42−1.44 (m, 1 H, H-1′), 1.97−2.01 (m, 1 H, H-1′), 4.01−4.02 (br, s, 1 H, OH), 4.22−4.42 (m, 3 H, H-3′, H-4′), 6.75−6.79 (m, 1 H, H-7), 7.17 (s, 1 H, H-11), 7.45−8.09 (m, 15 H, H_{arom}), 9.35 (s, 1 H, NH); ^{13}C-NMR (DMSO-d_6) δ 16.33 (C-1′), 21.97 (C-2′), 58.22 (C-7), 68.70 (C-4′), 74.14 (C-3′), 115.20 (C-11), 118.75, 123.53, 125.68, 126.17, 126.54, 126.95, 127.17, 127.38, 127.84, 128.39, 128.49, 128.64, 128.82, 129.06, 129.32, 129.53, 129.49, 130.216, 131.02, 138.71, 138.79, 144.63 (C_{arom}), 157.26, 163.53, 165.17 (3 × C = O).

*7-[(S)-3,4-dihydroxybutyl]-7 H-7 a, 9-diazacyclopenta[b]phenanthrene-8,10-dione (**5**).* Compound **4** (0.67 g, 1.3 mmol) was stirred with saturated ammonia in methanol (100 mL) for 3 days at room temperature. The solvent was evaporated in vacuo and the residue was chromatographed on a silica gel with $CHCl_3$/MeOH (95:5, v/v) to afford compound **5**. Yield: 0.2 g (44%); yellow foam; ^{1}H-NMR (DMSO-d_6) δ 1.06−1.14 (m, 2 H, H-2′), 1.44−1.44 (m, 1 H, H-1′), 1.99−2.03 (m, 2 H, H-1′), 3.04−3.16 (m, 3 H, H-3′, H-4′), 4.42−4.52 (br, s, 2 H, 2 OH), 5.46−5.51 (m, 1 H, H-7), 6.88 (s, 1 H, H-11), 7.56−8.01 (m, 6 H, H_{arom}), 11.57 (s, 1 H, NH); ^{13}C-NMR (DMSO-d_6) δ 30.07 (C-1′), 31.11 (C-2′), 58.43 (C-7), 65.60 (C-4′), 70.42 (C-3′), 112.20 (C-11), 125.88, 125.98, 127.82, 128.52, 129.41, 129.80, 130.45, 133.66, 135.16, 135.25 (C_{arom}), 154.41, 163.63 (2 × C = O). HRMS (MALDI) *m/z* Calcd for $C_{19}H_{18}N_2O_4$ [M^+ + Na] 361.1162, found 361.1159.

*7-[(S)-4-(4,4′-Dimethoxytrityloxy) 3-hydroxybutyl]-7 H-7 a, 9-diazacyclopenta[b]-phenanthrene-8,10-dione (**6**).* To compound **5** (0.915 g, 2.71 mmol) in dry CH_2Cl_2 (40 mL) under nitrogen was added dry Et_3N (0.35 mL, 3.26 mmol), followed by addition of 4,4′-dimethoxytrityl chloride (1.1 g, 3.26 mmol). The reaction mixture was stirred at room temperature under nitrogen for 48 hours. The solvent was removed in vacuo, and the residue was purified by column chromatography using 4:5:1 EtOAc-petroleum ether (60–80°C)-Et_3N to give compound **6**. Yield 1.18 g (67%); yellow foam; ^{1}H-NMR

($CDCl_3$) δ 1.22–1.28 (m, 2 H, H-2′), 2.74–2.77 (m, 1 H, H-1′), 2.83–2.85 (m, 1 H, H-1′), 3.52 (s,1 H, OH), 3.71, 3.72 (2 s, 6 H, 2 × OCH_3), 3.77–3.78 (m, 2 H, H-4′), 4.10–4.13 (m, 1 H, H-3′), 5.48 (m, 1 H, H-7), 7.18–7.92 (m, 20 H, H_{arom}), 7.82 (s, 1 H, NH); ^{13}C-NMR ($CDCl_3$) δ 29.66 (C-1′), 30.34 (C-2′), 55.65 (OCH_3), 59.13 (C-7), 69.42 (C-4′), 74.98 (C-3′), 85.91 (*C*-Ar_3), 112.81 (C-11), 113.08, 126.72, 127.80, 128.17, 129.77, 135.80, 144.69, 158.01 (DMT), 125.78, 126.42, 127.47, 127.96, 129.15, 129.86, 129.93, 130.62, 134.51, 142.69 (C_{arom}), 158.51, 163.35 (2 × C = O). HRMS (MALDI) *m/z* Calcd for $C_{40}H_{36}N_2O_6$ [M^+ + Na] 663.2466, found 663.2463.

*7-[(S)-3-O-(2-Cyanoethoxy(diisopropylamino)phosphino)-4-(4,4′-dimethoxytrityloxy)butyl]-7 H-7 a, 9-diazacyclopenta[b]phenanthrene-8,10-dione (***7***).* Compound 6 (0.17 g, 0.27 mmol) was dissolved under nitrogen in dry CH_2Cl_2 (10 mL). *N,N*-Diisopropylammonium tetrazolide (97 mg, 0.56 mmol) was added, followed by dropwise addition of 2-cyanoethyltetraisopropylphosphordiamidite (190 mg, 0.59 mmol). After 30 hours analytical 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 saturated. aqueous. $NaHCO_3$ (2 × 10 mL). The organic phase was dried over $MgSO_4$, and filtered, and the solvent was evaporated under reduced pressure. The residue was purified by column chromatography with 4:5:1 EtOAc-petroleum ether (60–80°C)-Et_3N to afford compound 7.

Yield: 120 mg (53%); yellow foam; ^{1}H-NMR ($CDCl_3$) δ 0.91–0.93 (m, 2 H, H-2′), 1.06–1.08 (m, 12 H, 4 × CH_3[Pr^i]), 1.23–1.25 (m, 2 H, H-1′), 2.03–2.04 (m, 2 H, CH_2CN), 3.36–3.41 (m, 2 H, OCH_2CH_2CN), 3.47–3.54 (m, 2 H, 2 × $CH(CH_3)_2$), 3.72 (s, 6 H, 2 × OCH_3), 3.74–3.75 (m, 2 H, H-4′), 4.10–4.12 (m, 1 H, H-3′), 5.44 (m, 1 H, H-7), 6.72–7.81 (m, 20 H, H_{arom}), 8.68 (s, 1 H, NH); ^{13}C-NMR ($CDCl_3$) δ 14.15 (CH_2CN), 19.97 (CH_2CH_2), 24.31, 24.40, 24.49, 24.55 (4 × CH_3), 29.59, 29.85 (C-1′, C-2′), 55.10 (OCH_3), 59.51 (C-7), 65.24 (C-4′), 73.51(C-3′), 86.08 (*C*-Ar_3), 112.78 (C-11), 117.54 (CN), 112.98, 126.57, 127.87, 128.44, 129.91, 135.88, 144.94, 158.03, (DMT), 125.69, 126.40, 127.45, 127.70, 128.60, 129.85, 130.53, 133.55, 136.04, 142.80 (C_{arom}), 158.24, 163.24 (2 × C = O); ^{31}P-NMR ($CDCl_3$) δ 148.65 (s), 149.03 (s) in ratio 5:4, respectively. HRMS (MALDI) *m/z* Calcd for $C_{49}H_{52}N_4O_7P$ [M^+ + K] 879.3288, found 879.3663.

Synthesis and Purification of Oligos

Oligodeoxynucleotides (ODN) were synthesized on an Expedite Nucleic Acid Synthesis System model 8909 from Applied Biosystems (Foster City, CA, USA). The phosphoramidite was dissolved in dry MeCN, as a 0.75 M solution and incorporated into the growing oligonucleotide chain using elongated coupling times (10 minutes coupling versus 2 minutes for normal nucleotide couplings). After the completed DNA syntheses, the 5′-*O*-DMT-on oligonucleotides were cleaved off from 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, and a Waters 717 autosampler on a Waters Xterra MS C_{18} column. Buffer A, 0.05 M triethyl ammonium acetate in H_2O, pH 7.4; buffer B, 75% CH_3CN in H_2O; flow 2.5 ml/minutes. Gradients, 2 minutes 100% A, linear gradient to 100% B in 28 minutes, 100% B in 10 minutes, linear gradient to 100% A in 1 minute, and then 100% A in 9 minutes. The ODNs were DMT deprotected in 100 μl 80% aq. AcOH (20 minutes), diluted with 1 M aq. NaOAc (150 μl) and precipitated from ethanol (600 μl).

Melting Temperature Measurements

The thermal stability studies were performed on a Perkin-Elmer UV/VIS spectrometer Lambda 20 (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 (1.0°C/minute) and is with an uncertainty +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 389 nm and detection at 395–595 nm. All measurements were conducted at 10°C using the same concentrations and buffer conditions as for the melting studies.

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