This article was downloaded by: [University of California, San Diego] On: 08 June 2015, At: 03:17 Publisher: Taylor & Francis Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



# Nucleosides, Nucleotides and Nucleic Acids

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

## Synthesis and DNA Binding Properties of a New Benzo[f]Imidazo[1,5b]-Isoquinoline-Butan-1,2-Diol Derivative

Youssef L. Aly <sup>a</sup> & Erik B. Pedersen <sup>a</sup>

<sup>a</sup> Nucleic Acid Center, Department of Chemistry , University of Southern Denmark , Odense M, Denmark Published online: 22 Dec 2010.

To cite this article: Youssef L. Aly & Erik B. Pedersen (2006) Synthesis and DNA Binding Properties of a New Benzo[f]Imidazo[1,5b]-Isoquinoline-Butan-1,2-Diol Derivative, Nucleosides, Nucleotides and Nucleic Acids, 25:12, 1335-1344, DOI: <u>10.1080/15257770600918524</u>

To link to this article: http://dx.doi.org/10.1080/15257770600918524

### PLEASE SCROLL DOWN FOR ARTICLE

Taylor & Francis makes every effort to ensure the accuracy of all the information (the "Content") contained in the publications on our platform. However, Taylor & Francis, our agents, and our licensors make no representations or warranties whatsoever as to the accuracy, completeness, or suitability for any purpose of the Content. Any opinions and views expressed in this publication are the opinions and views of the authors, and are not the views of or endorsed by Taylor & Francis. The accuracy of the Content should not be relied upon and should be independently verified with primary sources of information. Taylor and Francis shall not be liable for any losses, actions, claims, proceedings, demands, costs, expenses, damages, and other liabilities whatsoever or howsoever caused arising directly or indirectly in connection with, in relation to or arising out of the use of the Content.

This article may be used for research, teaching, and private study purposes. Any substantial or systematic reproduction, redistribution, reselling, loan, sub-licensing, systematic supply, or distribution in any form to anyone is expressly forbidden. Terms & Conditions of access and use can be found at <a href="http://www.tandfonline.com/page/terms-and-conditions">http://www.tandfonline.com/page/terms-and-conditions</a>

Nucleosides, Nucleotides, and Nucleic Acids, 25:1335–1344, 2006 Copyright © Taylor & Francis Group, LLC ISSN: 1525-7770 print / 1532-2335 online DOI: 10.1080/15257770600918524



#### 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** D 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-glyceropentafuranoside 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-1,2diol; 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,<sup>[1-3]</sup> using this strategy, workers have examined the importance of hydrogen bonding in stabilizing DNA and RNA structure,<sup>[1]</sup> in protein-DNA interaction,<sup>[2]</sup> and in the fidelity of enzymatic DNA and RNA synthesis.<sup>[3]</sup>

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

Received 20 December 2005; accepted 5 May 2006.

The Nucleic Acid Center is a research center funded by the Danish National Research Foundation for studies on nucleic acid chemical biology.

Youssef L. Aly is on leave from Chemistry Department, Faculty of Education Kafr El-Sheikh Branch, Tanta University, Kafr El-Sheikh, Egypt.

Mr. Michael Wamberg is gratefully acknowledged for his help on oligo synthesis.

Address correspondence to Erik B. Pedersen, Nucleic Acid Center, Department of Chemistry, University of Southern Denmark, Campusvej 55, DK-5230 Odense M, Denmark. E-mail: ebp@chem.sdu.dk.

INA (intercalating nucleic acids)<sup>[4]</sup> and TINA (twisted intercalating nucleic acids),<sup>[5]</sup> 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.<sup>[6]</sup> 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<sup>[7]</sup> 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.<sup>[8,9]</sup> 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 <sup>1</sup> H, <sup>13</sup> 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.<sup>[6]</sup>

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  $-1.5^{\circ}$ C to  $-7^{\circ}$ 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  $\Delta T_m$  –11.5°C to –13°C (Table 2, entries 4–6).



SCHEME 2 Synthesis of DMT-protected amidite 7.

Entry	Sequence	Expected mass	MALDI TOF analysis
1	5'-CTCAAG5CAAGTC-3'	4012.3	4010.0
2	5'-AGCTTG5CTTGAG-3'	4074.3	4076.3
3	5'-CTCAA5G5CAAGCT-3'	4411.3	4415.5
4	5'-CTCA5AG5CAAGCT-3'	4411.3	4415.0
5	5'-CTCA5AGCA5AGCT-3'	4411.3	4414.3

TABLE 1 Masses of the oligos

For RNA a similar destabilization and sequence dependency was observed (Table 3). A destabilization of the DNA/RNA duplex was only  $-1^{\circ}$ C when **5** was inserted as a bulge in a purine rich region (Table 3,entry 2), while large destabilizations ( $-10^{\circ}$ C to  $-14^{\circ}$ 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.

Entry	Sequence	T <sub>m</sub> (°C)	$\Delta$ T <sub>m</sub> (°C)
1	5'-AGCTTG CTTGAG-3'	46	_
	3'-TCGAAC GAACTC-5'		
2	5'-AGCTTG CTTGAG-3'	44.5	-1.5
	3'-TCGAAC5GAACTC-5'		
3	5'-AGCTTG5CTTGAG-3'	39	-7
	3'-TCGAAC GAACTC-5'		
4	5'-AGCTTG C TTGAG-3'	34.5	-11.5
	3'-TCGAAC 5 G 5AACTC-5'		
5	5'-AGCT TGCT TGAG-3'	33.5	-12.5
	3'-TCGA5ACGA5ACTC-5'		
6	5'-AGCT TGCT TGAG-3'	33.0	-13
	3'-TCGA5ACGA5ACTC-5'		

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

1339

Entry	Sequence	T <sub>m</sub> (°C)	$\Delta T_m$ (°C)
1	5'-AGCUUG CUUGAG-3' 8'-TCCAAC CAACTC-5'	40	_
2	5'-AGCUUG CUUGAG-3' 8'-TCGAAC5GAACTC-5'	39	-1
3	5'-AGCUUG C UUGAG-3' 8'-TCGAAC5C5AACTC-5'	29.5	-10.5
4	5'-AGCUUG CU UGAG-3' 3'-TCGAAC5CA5ACTC-5'	29	-11
5	5'-AGCU UGCU UGAG-3' 3'-TCGA5ACGA5ACTC-5'	26	-14

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

#### FLUORESCENCE

The fluorescence emission upon excitation of **5** inserted into single strands was found at  $\approx$ 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.<sup>[10]</sup>

#### CONCLUSION

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

	5'-AGCTTG Y CTTGAG -3' 3'-TCGAAC X GAACTC -5'		
Entry	Y, X	$T_{m}$	$\Delta Tm$
1	—, —	46.0	
	5, 5	47.0	+ 1
2	А, —	31.5	_
	A, <b>5</b>	30.4	- 1.1
3	С, —	33.5	_
	С, 5	32.9	-0.6
4	G, —	33.7	_
	G, <b>5</b>	32.9	-0.8
5	Т, —	33.4	_
	T, <b>5</b>	32.9	-0.5

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



**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 (<sup>1</sup>H 300 MHz, <sup>13</sup>C 75.5 MHz, <sup>31</sup>P 121.5 MHz);  $\delta$  values in ppm relative to Me<sub>4</sub>Si as internal standard (<sup>1</sup>H NMR); for <sup>13</sup>C NMR: CDCl<sub>3</sub> ( $\delta$  77.0), DMSO ( $\delta$  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)<sup>+</sup> and (M+Na)<sup>+</sup> 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-naphthylidenehydantoin (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]-7H-7a, 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 silvlated 5-naphthylidenehydantoin 3 in dry MeCN (30 mL) and the mixture was cooled to  $-50^{\circ}$ C.A solution of trimethylsilyl trifluoromethanesulfonate (1.08 mL, 6 mmol) in dry MeCN (10 mL) was added dropwise during 5 min at  $-50^{\circ}$ C and the mixture was stirred at  $-30^{\circ}$ C for 4 hours and then at  $5^{\circ}$ C for 2 days. The reaction mixture was diluted with CH<sub>2</sub>Cl<sub>2</sub> (200 mL), washed with cold saturated aqueous NaHCO<sub>3</sub> (200 mL) and water (2  $\times$ 100 mL), and dried over anhydrous MgSO<sub>4</sub>. 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. <sup>1</sup>H-NMR (DMSO-d<sub>6</sub>)  $\delta$  1.25–1.14 (m, 2H, H-2'), 1.42–1.44 (m, 1H, 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<sub>arom</sub>), 9.35 (s, 1 H, NH); <sup>13</sup> C-NMR (DMSO-d<sub>6</sub>) 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]-7H-7 a, 9-diazacyclopenta[b]phenanthrene-8,10dione (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<sub>3</sub>/MeOH (95:5, v/v) to afford compound 5. Yield: 0.2 g (44%); yellow foam; <sup>1</sup>H-NMR (DMSO-d<sub>6</sub>)  $\delta$  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<sub>arom</sub>), 11.57 (s, 1 H, NH); <sup>13</sup>C-NMR (DMSO-d<sub>6</sub>)  $\delta$  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<sub>arom</sub>), 154.41, 163.63 (2 × C = O). HRMS (MALDI) *m*/z Calcd for C<sub>19</sub>H<sub>18</sub>N<sub>2</sub>O<sub>4</sub> [M<sup>+</sup> + 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<sub>2</sub>Cl<sub>2</sub> (40 mL) under nitrogen was added dry Et<sub>3</sub> N (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<sub>3</sub> N to give compound **6**. Yield 1.18 g (67%); yellow foam; <sup>1</sup>H-NMR (CDCl<sub>3</sub>)  $\delta$  1.22–1.28 (m, 2H, H-2'), 2.74–2.77 (m, 1H, H-1'), 2.83–2.85 (m, 1H, H-1'), 3.52 (s,1H, OH), 3.71, 3.72 (2 s, 6 H, 2 × OCH<sub>3</sub>), 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<sub>arom</sub>), 7.82 (s, 1 H, NH); <sup>13</sup> C-NMR (CDCl<sub>3</sub>)  $\delta$  29.66 (C-1'), 30.34 (C-2'), 55.65 (OCH<sub>3</sub>), 59.13 (C-7), 69.42 (C-4'), 74.98 (C-3'), 85.91 (*C*-Ar<sub>3</sub>), 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<sub>arom</sub>), 158.51, 163.35 (2 × C = O). HRMS (MALDI) *m/z* Calcd for C<sub>40</sub>H<sub>36</sub>N<sub>2</sub>O<sub>6</sub> [M<sup>+</sup> + Na] 663.2466, found 663.2463.

7-[(S)-3-O-(2-Cyanoethoxy(diisopropylamino)phosphino)-4-(4,4'-dimethoxytrityloxy)butyl]-7H-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<sub>2</sub>Cl<sub>2</sub> (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<sub>2</sub>O (1 mL), followed by addition of CH<sub>2</sub>Cl<sub>2</sub> (10 mL). The mixture was washed with saturated. aqueous. NaHCO<sub>3</sub> (2 × 10 mL). The organic phase was dried over MgSO<sub>4</sub>, 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<sub>3</sub> N to afford compound 7.

Vield: 120 mg (53%); yellow foam; <sup>1</sup>H-NMR (CDCl<sub>3</sub>) δ 0.91–0.93 (m, 2 H, H-2'), 1.06–1.08 (m, 12 H, 4 × CH<sub>3</sub>[Pr<sup>i</sup>]), 1.23–1.25 (m, 2 H, H-1'), 2.03–2.04 (m, 2 H, CH<sub>2</sub>CN), 3.36–3.41 (m, 2 H, OCH<sub>2</sub>CH<sub>2</sub>CN), 3.47–3.54 (m, 2 H, 2 × CH(CH<sub>3</sub>)<sub>2</sub>), 3.72 (s, 6 H, 2 × OCH<sub>3</sub>), 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<sub>arom</sub>), 8.68 (s, 1 H, NH); <sup>13</sup>C-NMR (CDCl<sub>3</sub>) δ 14.15 (CH<sub>2</sub>CN), 19.97 (CH<sub>2</sub>CH<sub>2</sub>), 24.31, 24.40, 24.49, 24.55 (4 × CH<sub>3</sub>), 29.59, 29.85 (C-1', C-2'), 55.10 (OCH<sub>3</sub>), 59.51 (C-7), 65.24 (C-4'), 73.51(C-3'), 86.08 (C-Ar<sub>3</sub>), 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<sub>arom</sub>), 158.24, 163.24 (2 × C = O); <sup>31</sup>P-NMR (CDCl<sub>3</sub>) δ 148.65 (s), 149.03 (s) in ratio 5:4, respectively. HRMS (MALDI) m/z Calcd for C<sub>49</sub>H<sub>52</sub>N<sub>4</sub>O<sub>7</sub>P [M<sup>+</sup> + 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<sub>18</sub> column. Buffer A, 0.05 M triethyl ammonium acetate in H<sub>2</sub> O, pH 7.4; buffer B, 75% CH<sub>3</sub>CN in H<sub>2</sub> O; 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  $\mu$ l 80% aq. AcOH (20 minutes), diluted with 1 M aq. NaOAc (150  $\mu$ l) and precipitated from ethanol (600  $\mu$ l).

#### **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<sub>2</sub>HPO<sub>4</sub>·2H<sub>2</sub>O, 140 mM NaCl buffer at pH 7.0 for 1.0  $\mu$ M 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.

#### REFERENCES

- 1. SantaLucia, J. Jr.; Kierzek, R.; Turner, D.H. Context dependence of hydrogen bond free energy revealed by substitutions in an RNA hairpin. *Science* **1992**, 256, 217–219.
- (a) Smith, S.A.; Rajur, S.B.; McLaughlin, L.W. Specific purine N7-nitrogens are critical for high affinity binding by the Trp repressor. *Nature Struct. Biol.* **1994**, 1, 198. (b) Lesser, D.R.; Kurpiewski, M.R.; Jen-Jacobson, L. The energetic basis of specificity in the Eco RI endonuclease-DNA interaction. *Science* **1990**, 250, 776–786.
- (a) Kornberg, A.; Baker, T.A. *DNA Replication*, (W.H. Freeman, New York, 1992), 2nd ed. (b) Echols, H.; Goodman, M.F. Fidelity mechanisms in DNA replication. *Annu. Rev. Biochem.* 1991, 60, 477–511.
  (c) Strazewski, P.; Tamm, C. Replication experiments with nucleotide base analogs. *Angew. Chem.* 1990, 102, 37–59.
- Christensen, U.B.; Pedersen, E.B. Intercalating nucleic acids containing insertions of 1-O-(1pyrenylmethyl)glycerol: stabilisation of dsDNA and discrimination of DNA over RNA. *Nucl. Acids Res.* 2002, 30, 4918–4925.

- Filichev, V.V.; Pedersen, E.B. Stable and selective formation of Hoogsteen-type triplexes and duplexes using Twisted Intercalating Nucleic Acids (TINA) prepared via postsynthetic Sonogashira solid-phase coupling reactions. J. Am. Chem. Soc. 2005, 127, 14849–14858.
- El-Barbary, A.A.; Khodair, A.I.; Pedersen, E.B. Hydantoin analogs of thymidine. J. Org. Chem. 1993, 58, 5994–5999.
- Motawia, M.S.; Pedersen, E.B. A new route to 2',3'-dideoxycytidine. Liebigs Ann. Chem. 1990, 6, 599–602.
- Vorbrüggen, H.; Krolikiewicz, K.; Bennua, B. Nucleosides syntheses 22. Nucleosides syntheses with trimethylsilyl triflate and perchlorate as catalysts. *Chem. Ber.* 1981, 114, 1234–1235.
- Vorbrüggen, H.; Bennua, B. Nucleosides syntheses 25. a new simplified nucleosides syntheses. *Chem* Ber. 1981, 114, 1279–1286.
- Lewis, F.; Zhang, Y.; Liu, Xiaoyang.; Xu, Ning.; Letsinger, R. Naphthalenedicarboxamides as fluorescent probes of inter- and intramolecular electron transfer in single strand, hairpin, and duplex DNA. J. Phys. Chem. B 1999, 103, 2570–2578.