Pyrenemethyl *ara*-Uridine-2′-carbamate: A Strong Interstrand Excimer in the Major Groove of a DNA Duplex

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In memory of Yuri A. Berlin

The synthesis of new nucleoside derivatives, ara*-uridine-2′-carbamates, and their incorporation into synthetic DNA oligomers is described. The modification directs ligands into the major groove of duplex DNA and somewhat destabilizes the duplexes of modified oligonucleotides with complementary DNA or RNA. In the case of pyrenemethyl carbamate modification in DNA – DNA duplexes, the destabilization is considerably reduced. The pyrenemethyl derivative also shows remarkable spectral properties: a 'reversed' absorbance change for pyrene at 350 nm in the course of denaturation of the DNA duplex, as compared to the change seen in the nucleotide absorbance at 260 nm. This derivatization also causes pronounced sequence-dependent excimer formation in the major groove.*

KEYWORDS:

nucleosides · DNA · fluorescence · major groove · pyrene excimer

Introduction

Recently developed fluorescence techniques based on interacting fluorophores, for example, fluorescence resonance energy transfer (FRET),[1] "molecular beacons",[2] and combined fluorophores,[3] have revolutionized many fields of application of labeled oligonucleotides: sequencing, hybridization analysis (including DNA array-based methods and real-time PCR), and studies of nucleic acid – nucleic acid and nucleic acid – protein interactions.

A long lifetime of the excited state and the possibility of easy excimer formation[4] are distinctive features of the pyrene fluorophore that allow its application for detection of nucleic acid interactions both as a single label[5] and in excimer-forming pairs or as multipyrene probes.[6] Moreover, the flat structure of the pyrene residue facilitates its stacking with nucleobases.[7] A pyrene fluorophore attached to the 2′-position of uracil nucleosides in DNA[5i,j,m] or 2′-*O*-Me RNA[5k] oligomers shows a considerable increase in fluorescence intensity upon binding with complementary RNA. To the best of our knowledge, excimer emission from two pyrene fluorophores bound to *different* complementary strands within an *internal* part of a DNA duplex has previously only been observed with pyrene pseudonucleosides[6g] or by modification with (+)-*anti*-benzo[*a*]pyrene diol epoxide of two guanine residues in N^2 positions within a (5′)-CG sequence.[8] In the latter case, a pyrene excimer is formed in the minor groove of the DNA double helix.[8]

We prepared a new pyrene nucleoside, pyrenemethyl *ara*-uridine-2′-carbamate (U^P), which is expected to direct the pyrene residue into the major groove of a DNA duplex and which has a rather rigid carbamate-based linker for pyrene attachment. For comparison and for elucidation of the influence of pyrene on duplex stability, butyl *ara*-uridine-2′-carbamate (U^B) was prepared in a similar manner.

Results and Discussion

Synthesis of phosphoramidites

Modified phosphoramidites were prepared as shown in Scheme 1. The reaction of uracil-1-β-D-arabinofuranoside (**1**) with 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane gave protected derivative **2**. Subsequent reaction of **2** with 1,1′-carbonyldiimidazole in dry DCM afforded 2′-*O*-(imidazol-1-ylcarbonyl)-3′,5′-*O*-

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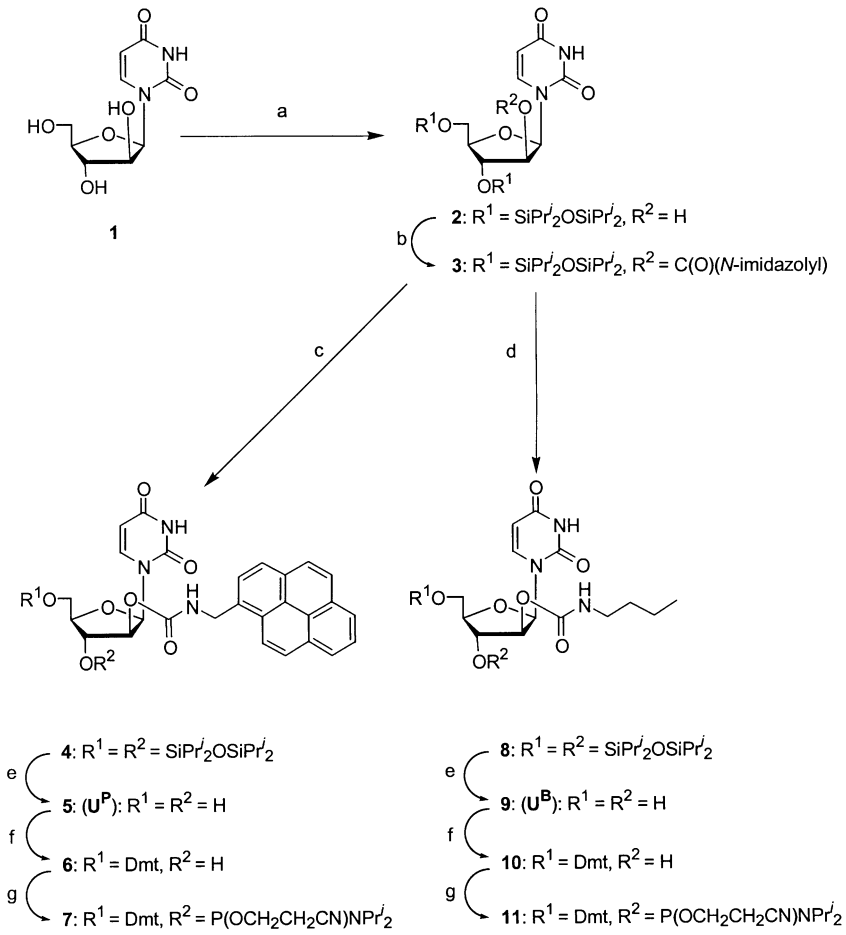
Supporting information for this article is available on the WWW under http://www.chembiochem.org or from the author.

 DOI: 10.1002/cbic.200300678



Scheme 1. *Synthesis of U^P: a) O(SiPr$^i_2$Cl)$_2$ (1.1 equiv), pyridine; b) 1,1′-carbonyldiimidazole (2.5 equiv), dichloromethane (DCM); c) 1-pyrenemethylamine (1.1 equiv), MeCN, tetrahydrofuran (THF); d) 1-butylamine, (1.1 equiv), MeCN, THF; e) Et$_3$N·3HF (2.4 equiv), THF; f) 4,4′-dimethoxytrityl chloride (DmtCl; 1.1 equiv), pyridine; g) bis(*N*,*N*-diisopropylamino)-2-cyanoethoxyphosphine, diisopropylammonium tetrazolide, DCM.*

(tetraisopropyldisiloxan-1,3-diyl)uracil-1-β-D-arabinofuranoside (**3**) in quantitative yield. Imidazolide **3** is stable during aqueous extraction, but is prone to hydrolysis under acidic or alkaline conditions. Some decomposition was also observed during silica gel chromatography. The treatment of **3** with 10% excess 1-pyrenylmethylamine in MeCN-THF (1:1 v/v) at 55 °C gave 2′-carbamate product **4** after several days. We used Et$_3$N·3HF in THF to remove the Markiewicz 3′,5′-*O*-silyl protection group from **4**. This gave only the desired 2′-carbamate nucleoside **5** (U^P) after overnight deprotection. Crystalline **5** was treated with DmtCl to give 5′-*O*-protected nucleoside **6**. This compound was then phosphitylated with bis(*N*,*N*-diisopropylamino)-2-cyanoethoxyphosphine in DCM in the presence of diisopropylammonium tetrazolide to give phosphoramidite **7**, which was isolated by column chromatography. Phosphoramidite **11** was synthesized in a similar way; 1-butylamine was used as an aliphatic amine.

Thermal stability of modified duplexes

Phosphoramidite reagents **7** and **11** were used to prepare a series of oligonucleotides (Tables 1, 2), which were used for thermal denaturation studies of their duplexes, formed by annealing to complementary DNA and RNA oligonucleotides. The sequence chosen was a 15-mer complementary to residues 22 – 36 of the *trans*-activation responsive region (TAR) of the human immunodeficiency virus type 1 (HIV-1) RNA.[9]

Table 1 shows that *ara*-2′-carbamate modification is destabilizing although the pyrene residue somewhat neutralizes the negative influence of the carbamate. The effective destabilization, ΔT_m per modification, is -2.2 °C for single and -1.7 °C for double pyrene modification in a DNA – DNA 15-mer duplex. The butyl carbamate, however, contributes a defined level of destabilization, which is more than than additive for double modification. The results correlate with those obtained for *ribo*-2′-carbamates.[5m] In contrast to the results for DNA – DNA duplexes, when hybridized to a DNA – RNA duplex, a single pyrene modification leads to a pronounced decrease in the T_m value, which surprisingly remains almost unchanged upon additional modification with a second pyrene residue.

During ON09 × ON02 duplex melting, the pyrene absorbance at 350 nm undergoes the opposite change to that seen for the nucleotide absorbance at 260 nm (Figure 1). The effect is similar to our previous observations on 2′-*ribo*-carbamates.[5m] The melting temperature values (determined as extremums for the

Table 1. *Thermal stabilities of modified duplexes.*

Oligonucleotide	Sequence, 5′ → 3′	Duplex with complementary DNA (ON02) ATTTGAGCCTGGGAC		Duplex with complementary RNA (ON03) AUUUGAGCCUGGGAC	
		T_m, °C	ΔT_m, °C[a]	T_m, °C	ΔT_m, °C[a]
ON01	CTCCCAGGCTCAAAT	57.6	–	59.2	–
ON04	CTCCCAGGCU^{B}CAAAT	51.4	−6.2	n.d.[b]	n.d.
ON05	CU^{B}CCCAGGCTCAAAT	53.5	−4.1	n.d.	n.d.
ON06	CU^{B}CCCAGGCU^{B}CAAAT	45.8	−11.8	n.d.	n.d.
ON07	CTCCCAGGCU^{P}CAAAT	55.4	−2.2	52.9	−6.3
ON08	CU^{P}CCCAGGCTCAAAT	55.5	−2.1	53.2	−6.0
ON09	CU^{P}CCCAGGCU^{P}CAAAT	54.3	−3.3	52.3	−6.9

[a] The difference in T_m between the modified and corresponding unmodified duplexes; [b] n.d., not determined.

Table 2. *Oligonucleotides used for fluorescence measurements.*

Oligonucleotide	Sequence, 5′ → 3′
ON10	CTCCCAGGCTCAAAU^{P}CTGG
ON11	AU^{P}TTGAGCCTGGGAG
ON12	ATU^{P}TGAGCCTGGGAG
ON13	ATTU^{P}GAGCCTGGGAG
ON14	CCAGAU^{P}TTGAGCCTGGGAG
ON15	CCAGATU^{P}TGAGCCTGGGAG
ON16	CCAGATTU^{P}GAGCCTGGGAG

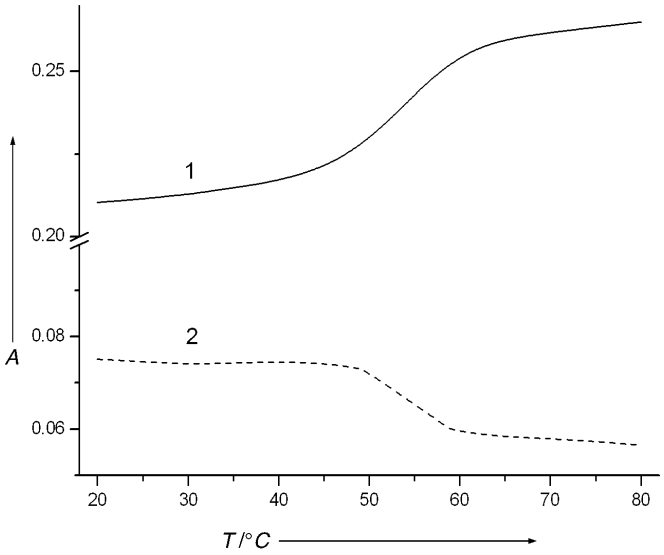


Figure 1. *Thermal denaturation curves of the duplex ON09 × ON02 in hybridization buffer (see the Experimental Section for details) detected at 260 nm (1) and 350 nm (2).*

first-order derivatives of both curves, data not shown) coincide, which indicates that pyrene absorbance is a suitable parameter to be used for T_m measurements. This is the case only for duplexes with oligonucleotide ON09; other duplexes with one pyrene residue or duplexes with two pyrene residues in different strands do not display the S-shaped pyrene hypochromism.[10]

Fluorescense measurements

The above-described vivid effect is in agreement with the fluorescence spectra of conjugate ON09 and its DNA duplex (Figure 2). The modified oligonucleotide shows a strong broad fluorescence around 490 nm (an excitation wavelength, λ_{ex}, of 330 nm, close to the second absorbance maximum, was used). This result indicates the feasibility of bringing pyrene residues close to each other to permit excimer formation to occur. The excimer-to-monomer fluorescence intensity ratio for ON09 is approximately one order of magnitude higher than that for the corresponding oligonucleotide containing a pyrene *ribo*-2′-carbamate.[5m] In a duplex, the two pyrene residues are attached to essentially a rigid, rod-like DNA double helix and are held at a maximum distance from each other. This arrangement completely excludes the possibility of excimer formation and indeed

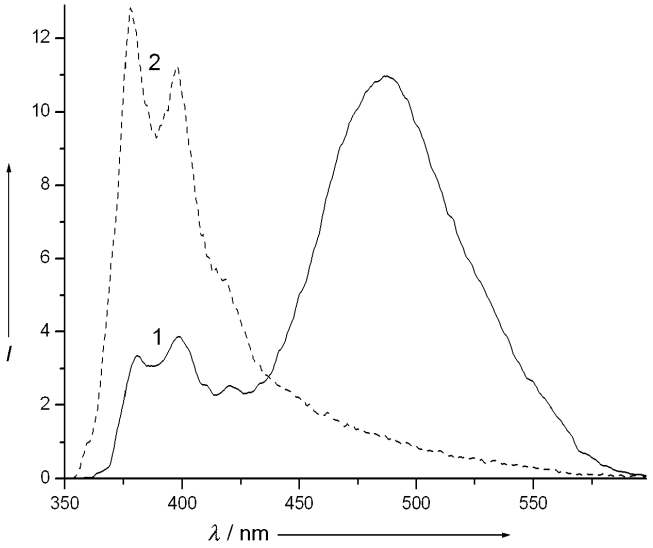


Figure 2. *Fluorescence spectra of single-stranded doubly pyrene-modified oligonucleotide ON09 (1) and its duplex with complementary DNA ON09 × ON02 (2); $\lambda_{ex} = 330$ nm.*

might increase the pyrene absorbance (see ref. [5m]). Thus, conjugate ON09 represents a "molecular-beacon-like" bis-pyrene oligonucleotide probe with fluorescence at 490 nm and switching to 380 nm after hybridization. In sharp contrast to *ribo*-analogues,[5m] conjugates ON07 – ON09 do not show any fluorescence enhancement upon binding with complementary RNA,[10] which indicates that pyrene interacts effectively and is also quenched in the major groove of the DNA – RNA duplex.

To find the most effective pyrene-pair layout for excimer formation, several duplexes containing two pyrene residues attached to different DNA strands were prepared and their fluorescence spectra were recorded. Qualitative results are given in Table 3. Only one structure shows strong excimer emission accompanied by a nearly complete absence of "monomer" fluorescence: the structure with the pyrene nucleosides positioned 3′ to each other and separated by one base pair (duplexes ON07 × ON13 and ON07 × ON16). When U^{P} residues are separated by two or three base pairs, the broad band of long-wavelength fluorescence disappears (Figure 3). The overlap of the two pyrene residues in the favorable case is likely to be somewhat preorganized. The "static" character of the excimer[4] (high value of excimer-to-monomer fluorescence intensity ratio) indirectly supports this assumption. The location of the two stacked pyrene residues parallel to the major groove might also be stabilized by hydrogen bonding of the linker carbamate NH groups with the 5′-phosphate group of one U^{P} residue (Hb1) and with the *N*7-position of the dG residue of the internal base pair (Hb2).

Molecular dynamics studies

Molecular dynamics (MD) computational studies on duplexes ON07 × ON13 (Model 1), ON07 × ON12 (Model 2), and ON07 ×

Table 3. *Fluorescence of pyrene-modified oligonucleotides and duplexes.*

Oligonucleotide or duplex	Schematic structure	Pyrene fluorescence monomer	excimer
ON07		+	−
ON09		+	+
ON09 × ON02		+	−
ON07 × ON11		+	−
ON07 × ON12		+	−
ON07 × ON13		−	+
ON07 × ON14		+	−
ON07 × ON15		+	−
ON07 × ON16		−	+
ON09 × ON11		+	−
ON09 × ON12		+	−
ON09 × ON13		+	+
ON10 × ON13		+	−
ON10 × ON12		+	−
ON10 × ON11		+	+
ON10 × ON16		+	−
ON10 × ON15		+	−
ON10 × ON14		+	−

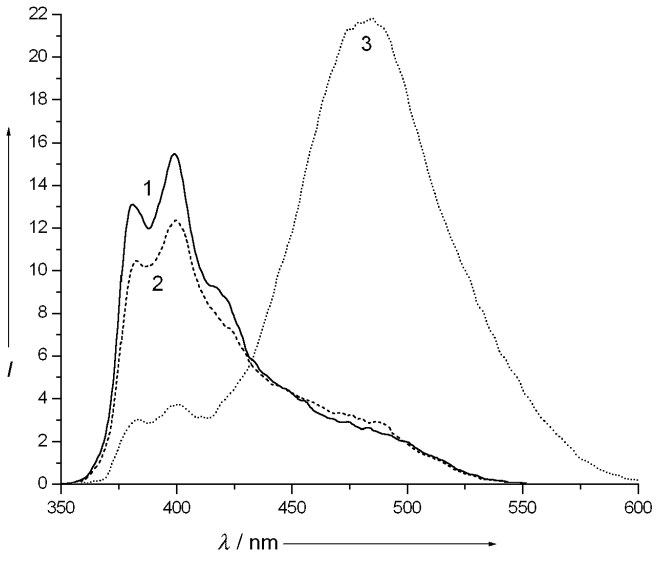


Figure 3. *Fluorescence spectra of duplexes ON07 × ON11 (1), ON07 × ON12 (2), and ON07 × ON13 (3); $\lambda_{ex} = 330$ nm.*

ON11(Model 3)[10] show that the degree of interaction between chromophores depends critically on their sequential position. Pyrene residues are in contact practically all the time during MD simulation (> 80%) in Model 1, approximately half the MD time in Model 2, and less then 5% of the MD time in Model 3. No remarkable differences in interaction of pyrene residues for structures with *cis* and *trans* amide bond conformation of the linker were detected. The addition of constraints fixing the hydrogen bond Hb2 has no significant effect on stabilization of the state with chomophores in contact. Fixation of Hb1 leads to a considerable decrease in the distance between chromophores in Model 1 with a *cis* conformation of the amide bond.

The excimer fluorescence observed from 5′-to-5′-facing adjacent fluorescent nucleosides (duplex ON10 × ON11) appears to be due to some near-terminal duplex distortion. Four-base duplex extension (duplexes ON10 × ON14), with the placement of the two modified nucleosides maintained, leads to complete elimination of excimer emission (Figure 4).

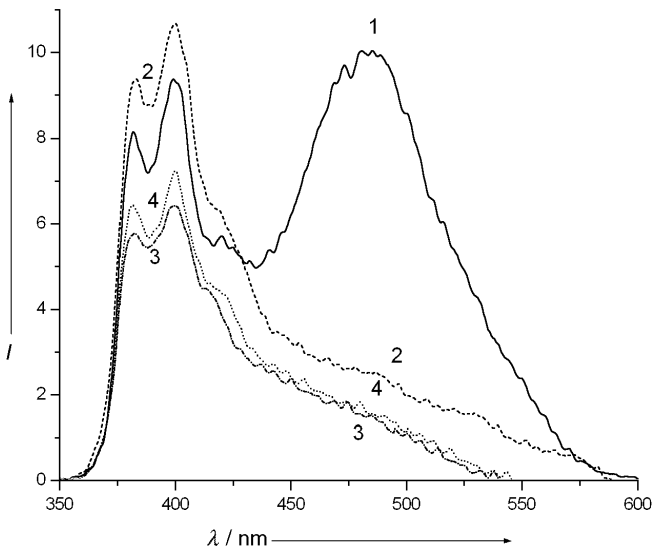


Figure 4. *Fluorescence spectra of duplexes ON10 × ON11 (1), ON10 × ON12 (2), ON10 × ON13 (3), ON10 × ON14 (4); $\lambda_{ex} = 330$ nm.*

Conclusion

The new pyrenemethyl carbamate nucleoside U^P derived from *ara*-uridine shows remarkable fluorescence properties and is able to form excimers within the major groove of a DNA duplex when two U^P residues in different strands are positioned 3′ to each other and are separated by one base pair.

Experimental Section

Instrumentation: 500 MHz ^{1}H and 202.4 MHz ^{31}P NMR spectra were measured on a Bruker DRX 500 spectrometer at 303 K. 600 MHz ^{1}H NMR spectra were obtained on a Varian Unity 600 NMR spectrometer at 303 K. Chemical shifts (δ) for ^{1}H and ^{31}P are referenced to internal solvent resonances and reported relative to $SiMe_4$ and 85% aq H_3PO_4, respectively. ^{1}H NMR coupling constants are refer to apparent multiplicities. MALDI-TOF mass spectra were

measured on a Voyager-DE BioSpectrometry Workstation (PerSeptive Biosystems) in positive ion mode. Elemental analysis was performed on a CHNS-analyzer/EA1112 "Thermosinigan".

Oligonucleotide synthesis was carried out on an ABI 380B DNA/RNA synthesizer on a 1-μmol scale with 2′-deoxynucleoside phosphoramidite reagents obtained from Cruachem (Scotland) and standard synthetic procedures. For coupling of normal deoxyribonucleoside phosphoramidites, the standard protocol (0.1 M amidite in acetonitrile, 5 min) was used. For 2′-modified nucleoside phosphoramidites **7** and **11**, amidite (0.1 M) in acetonitrile and a coupling time 10 min were used. Under these conditions, the coupling efficiency based on the Dmt cation assay was higher then 99% for every step involving modified phosphoramidites.

Thermal denaturation experiments with oligonucleotide duplexes were carried out on a Perkin Elmer Lambda 40 UV/Vis Spectrometer with a PTP 6 (Peltier Temperature Programmer) device in hybridization buffer (100 mM NaCl, 10 mM Na-phosphate, 0.1 mM ethylenediaminetetraacetate (EDTA), adjusted to pH 7.0) with oligonucleotides at a concentration of 3×10^{-6} M. Duplex stability studies were performed with an increase in temperature from 20 to 80 °C at a rate of 1 °C min^{-1}. The solutions were heated to 96 °C, kept for 5 min, and then gradually cooled before melting experiments.

Fluorescence spectra were obtained on a Perkin Elmer LS 50B luminescence spectrometer with an excitation wavelength of 330 nm in hybridization buffer, and oligonucleotides at a concentration of 3×10^{-6} M. No special efforts were made to remove oxygen from the solution.

Molecular dynamics studies: The starting models of DNA duplexes for MD studies were constructed in the program InsightII[11] as B-form duplexes. Both *cis* and *trans* conformations of the amide bond in the carbamate linker were considered. MD simulation in a vacuum was carried out for 100 ps at constant temperature (300 K) with the Discover[11] program and cvff[12] force field. DNA strands were fixed to prevent destruction of the double-helix structure during the MD simulations because of the influence of the uncompensated charges of phosphate groups and the absence of solvent.

Syntheses:

3′,5′-*O*-(tetraisopropyldisiloxan-1,3-diyl)uracil-1-β-D-arabinofuranoside (2): Uracil-1-β-D-arabinofuranoside (3.67 g, 15 mmol) was coevaporated with pyridine (3 × 20 mL), dissolved in dry pyridine (15 mL), cooled in an ice bath, and Markiewicz reagent (5.0 g, 15.9 mmol) was added in one portion with stirring. After 2 h, the cooling system was removed and the mixture was kept overnight at ambient temperature. The mixture was evaporated, and the residue was chromatographed on a silica gel column (step gradient 0 →50% EtOAc in $CHCl_3$). Fractions containing product were combined, evaporated, and the residue was dried in vacuo to afford pure **2** (6.70 g, 92%) as a white foam. R_f: 0.65 ($CHCl_3$/EtOAc 1:1 (v/v)). ^{1}H NMR (600 MHz, [D$_6$]DMSO): $\delta = 11.29$ (s, 1H, N*H*, exchangeable with D_2O), 7.45 (d, 1H, $J_{5,6} = 7.9$ Hz, H-6), 6.01 (d, 1H, $J_{1',2'} = 6.4$ Hz, H-1′), 5.81 (d, 1H, $J_{2',OH} = 5.8$, O*H*, exchangeable with D_2O), 5.53 (d, 1H, $J_{5,6} = 7.9$, H-5), 4.31 (m, 1H, H-2′), 4.10 (apparent t, 1H, $J_{2',3'} = J_{3',4'} = 7.6$ Hz, H-3′), 4.01 (dd, 1H, $J_{5'a,5'b} = 12.8$, $J_{5'a,4'} = 3.4$, H-5′a), 3.93 (dd, 1H, $J_{5'a,5'b} = 12.8$, $J_{5'b,4'} = 2.7$, H-5′b), 3.70 (m, 1H, H-4′), 1.08 – 0.98 (m, 28H, Pr^i) ppm. MS (matrix 2,4,6-trihydroxyacetophenone (2,4,6-THAP)): calcd: 487.83; found: 487.71 $[M+H]^+$.

2′-*O*-(Imidazol-1-ylcarbonyl)-3′,5′-*O*-(tetraisopropyldisiloxan-1,3-diyl)uracil-1-β-D-arabinofuranoside (3): 1,1′-Carbonyldiimidazole (2.50 g, 15.5 mmol) was added in one portion to a solution of 3′,5′-*O*-(tetraisopropyldisiloxan-1,3-diyl)uracil-1-β-D-arabinofuranoside (3.00 g, 6.2 mmol) in dry DCM (50 mL). TLC ($CHCl_3$-EtOAc 1:1 v/v) showed that the conversion of the starting nucleoside ($R_f = 0.65$) into an imidazolide with lower mobility on TLC plates is complete after 0.5 – 2 h. The solution was washed with water (2 × 50 mL), dried (Na_2SO_4), evaporated, coevaporated with dry DCM (50 mL), and the residue was dried in vacuo to give the imidazole derivative **3** (3.58 g, 100%) as a white foam, pure according to TLC and NMR spectroscopy. R_f: 0.5 ($CHCl_3$/EtOAc 1:1 (v/v)). ^{1}H NMR (600 MHz, [D$_6$]DMSO): $\delta = 11.38$ (s, 1H, H-3, exchangeable with D_2O), 8.17 (s, 1H, imidazole), 7.67 (d, 1H, $J_{5,6} = 8.2$ Hz, H-6), 7.45 (s, 1H, imidazole), 7.11 (s, 1H, imidazole), 6.26 (d, 1H, $J_{1',2'} = 6.4$ Hz, H-1′), 5.85 (apparent t, 1H, $J_{1',2'} = J_{2',3'} = 7.0$ Hz, H-2′), 5.63 (d, 1H, $J_{5,6} = 8.2$ Hz, H-5), 4.58 (m, 1H, H-3′), 4.15 – 4.10 (m, 1H, H-5′a), 4.03 – 3.96 (m, 2H, H-4′, H-5′b), 1.11 – 0.81 (m, 28H, Pr^i) ppm. MS (matrix 2,4,6-THAP): calcd: 582.29; found: 581.79 $[M+H]^+$.

2′-*O*-(Pyren-1-ylmethylaminocarbonyl)-3′,5′-*O*-(tetraisopropyldisiloxan-1,3-diyl)uracil-1-β-D-arabinofuranoside (4): The 1-pyrenemethylamine, prepared from hydrochloride (0.50 g, 1.8 mmol), was added to a solution of 2′-*O*-(imidazol-1-ylcarbonyl)-3′,5′-*O*-(tetraisopropyldisiloxan-1,3-diyl)uracil-1-β-D-arabinofuranoside (0.87 g, 1.5 mmol) in MeCN (20 mL) and the mixture was kept for one week at ambient temperature. The conversion of the starting amine was monitored by TLC (EtOAc). The reaction mixture was diluted with DCM (50 mL), washed with water (100 mL), 5% citric acid (100 mL), and water (100 mL), then dried (Na_2SO_4), evaporated, and the residue was chromatographed on a silica gel column (step gradient 0 →10% EtOAc in $CHCl_3$). Fractions containing product were combined, evaporated, and the residue was dried in vacuo to afford pure **4** (0.98 g, 88%) as a yellow foam. R_f: 0.51 ($CHCl_3$/EtOAc 2:1 (v/v)). ^{1}H NMR (600 MHz, [D$_6$]DMSO): $\delta = 11.41$ (s, 1H, H-3, exchangeable with D_2O), 8.36 (d, 1H, $J_{9'',10''} = 9.5$ Hz, pyrene H-10), 8.32 – 8.05 (m, 8H, Ar*H* (pyrene), OCON*H*), 7.88 (d, 1H, $J = 7.9$ Hz, Ar*H* (pyrene H-2)), 7.49 (d, 1H, $J_{5,6} = 8.3$ Hz, H-6), 6.18 (s, 1H, H-1′), 5.61 (d, 1H, $J_{5,6} = 8.3$ Hz, H-5), 5.46 (m, 1H, H-2′), 4.89 (m, 2H, C*H*$_2$Pyr), 4.09 – 4.06 (m, 1H, $^2J_{5'a,5'b} = 13.4$ Hz, $J_{4',5'a} = 3.7$ Hz, H-5′a), 3.95 – 3.92 (m, 1H, $^2J_{5'a,5'b} = 13.4$ Hz, $J_{4',5'b} = 2.5$ Hz, H-5′b), 3.87 (m, 1H, H-3′), 3.60 (m, 1H, H-4′), 1.08 – 0.83 (m, 28H, Pr^i) ppm. MS (matrix 2,4,6-THAP): calcd: 745.70; found: 745.00 $[M+H]^+$. Elemental analysis: calcd (%) for $C_{39}H_{49}N_3O_8Si_2$: C 62.96, H 6.64, N 5.65; found: C 62.57, H 6.62, N 5.77.

2′-*O*-(Butylaminocarbonyl)-3′,5′-*O*-(tetraisopropyldisiloxan-1,3-diyl)uracil-1-β-D-arabinofuranoside (8): Butylamine (0.9 mL, 8.5 mmol) was added to a solution of 2′-*O*-(imidazol-1-ylcarbonyl)-3′,5′-*O*-(tetraisopropyl-disiloxan-1,3-diyl)uracil-1-β-D-arabinofuranoside (0.35 g, 0.6 mmol) in MeCN (4 mL) and the mixture was kept for 48 h (monitoring by TLC in $CHCl_3$-EtOAc, 1:1 v/v). The reaction mixture was evaporated, diluted with DCM (50 mL), washed with water (40 mL), 5% citric acid (40 mL), and water (40 mL), then dried (Na_2SO_4), evaporated, and the residue was chromatographed on a silica gel column (step gradient 0 →15% EtOAc in $CHCl_3$). Fractions containing product were combined, evaporated, and the residue was dried in vacuo to afford **8** (0.32 g, 91%) as a white foam. R_f: 0.37 ($CHCl_3$/EtOAc 1:1 (v/v)). ^{1}H NMR (600 MHz, [D$_6$]DMSO): $\delta = 11.37$ (s, 0.2H), 11.29 (s, 0.8H) (H-3), 7.48 (d, 0.2H), 7.41 (d, 0.8H) ($J = 7.9$ Hz, H-6), 7.22 (t, 0.8H), 7.03 (t, 0.2H) ($J = 5.8$ Hz, OCON*H*), 6.10 (br s, 1H, H-1′), 5.59 (d, 0.2H), 5.55 (d, 0.8H) ($J = 7.9$ Hz, H-5), 5.43 (apparent t, 0.2H), 5.33 (apparent t, 0.8H) ($J = 7.0$ Hz, H-2′), 4.50 – 4.30 (m, 1H, H-3′), 4.06 (dd, 1H, $^2J_{5'a,5'b} = 12.8$ Hz, $J_{4',5'a} = 3.7$ Hz, H-5′a), 3.94 (dd, 1H, $^2J_{5'a,5'b} = 12.8$ Hz, $J_{4',5'b} = 2.7$ Hz, H-5′b), 3.88 – 3.82 (m, 1H, H-4′), 2.89 (m, 1.6H), 2.80 (m, 0.4H) (NCH_2), 1.35 – 1.13 (m, 4H, NCH$_2$CH_2CH_2), 1.09 – 0.78 (m, 33H, Pr^i, NCH$_2$CH$_2$CH$_2$CH_3) ppm. MS (matrix 2,4,6-THAP): calcd: 589.40, 611.52, 627.41; found: 586.85 $[M+H]^+$, 608.83 $[M+Na]^+$, 624.94 $[M+K]^+$. Elemental analysis: calcd (%) for $C_{26}H_{47}N_3O_8Si_2$: C 53.30, H 8.09, N 7.38; found: C 53.74, H 8.27, N 7.38.

2′-*O*-(Pyren-1-ylmethylaminocarbonyl)uracil-1-β-D-arabinofuranoside (5, U^{P}): Triethylamine trihydrofluoride (0.48 mL, 2.9 mmol) was added to a solution of 2′-*O*-(pyren-1-ylmethylaminocarbonyl)-3′,5′-*O*-(tetraisopropyldisiloxan-1,3-diyl)uracil-1-β-D-arabinofuranoside (0.87 g, 1.2 mmol) in THF (5 mL) in a teflon flask (Nalgene, screw-top) and the mixture was left for 6 h or overnight at room temperature (the completion of deprotection was checked by TLC (10% MeOH in $CHCl_3$, v/v) then diluted with hexane (25 mL). The upper layer was discarded and the residue (oil) was washed with toluene/hexane (1:1 (v/v), 3 × 25 mL) by decantation, triturated in absolute ethanol (5 mL), and the crystalline product was filtered off, washed with ethanol (5 mL), diethyl ether (10 mL), and dried in vacuo to afford nucleoside **5** (0.57 g, 97%) as yellow crystals. R_f: 0.17 (10% MeOH in $CHCl_3$ (v/v)). M.p.: 225–226 °C (ethanol). ^{1}H NMR (600 MHz, [D$_6$]DMSO): δ = 11.36 (br s, 1H, H-3, exchangeable with D_2O), 8.33–8.06 (m, 9H, Ar*H* (pyrene) OCONH), 7.89 (d, 1H, J = 7.6 Hz, Ar*H* (pyrene H-2)), 7.66 (d, 1H, $J_{5,6}$ = 7.9 Hz, H-6), 6.20 (d, 1H, $J_{1',2'}$ = 4.5 Hz, H-1′), 5.74 (d, 1H, $J_{3',OH}$ = 4.6 Hz, CHO*H*, exchangeable with D_2O), 5.62 (d, 1H, $J_{5,6}$ = 7.9 Hz, H-5), 5.12 (t, 1H, $J_{5',OH}$ = 4.3 Hz, CH_2O*H*, exchangeable with D_2O), 4.98 (m, 1H, H-2′), 4.88 (m, 2H, CH_2Pyr), 4.13 (m, 1H, H-3′), 3.79 (m, 1H, H-4′), 3.69–3.58 (m, 2H, H-5′) ppm. MS (matrix 2,4,6-THAP): calcd: 503.73, 526.43; found: 502.50 [M+H]$^+$, 524.48 [M + Na]$^+$.

2′-*O*-(Butylaminocarbonyl)uracil-1-β-D-arabinofuranoside (9, U^{B}): Triethylamine trihydrofluoride (0.14 mL, 0.85 mmol) was added to a solution of 2′-*O*-(butylaminocarbonyl)-3′,5′-*O*-(tetraisopropyldisiloxan-1,3-diyl)uracil-1-β-D-arabinofuranoside (0.19 g, 0.32 mmol) in THF (3 mL) in a teflon flask (Nalgene, screw-top) and the mixture was left for 6 h or overnight at room temperature (the completion of deprotection was checked by TLC (10% MeOH in $CHCl_3$, v/v), then diluted with hexane (25 mL). The upper layer was discarded, and the residue (oil) was washed with toluene/hexane (1:1 (v/v), 3 × 25 mL) by decantation to afford a viscous oil, which was used in the next step without further purification. R_f: 0.08 (10% MeOH in $CHCl_3$ (v/v)). MS (matrix 2,4,6-THAP): calcd: 346.10; found: 344.34 [M+H]$^+$.

5′-*O*-(4,4′-Dimethoxytrityl)-2′-*O*-(pyren-1-ylmethylaminocarbonyl)uracil-1-β-D-arabinofuranoside (6): 2′-*O*-(Pyren-1-ylmethylaminocarbonyl)uracil-1-β-D-arabinofuranoside (400 mg, 0.8 mmol) was coevaporated with pyridine (3 × 20 mL), dissolved in dry pyridine (15 mL), cooled in an ice bath, and DmtCl (370 mg, 1.0 mmol) was added in one portion. After completion of the reaction (monitored by TLC), the excess of DmtCl was quenched with MeOH (1 mL), and after 10 min the mixture was diluted with $CHCl_3$ (100 mL), washed with water (100 mL), 5% $NaHCO_3$ (100 mL), and water (100 mL), then dried (Na_2SO_4), evaporated, and coevaporated with toluene (3 × 25 mL). The residue was chromatographed on a silica gel column in a stepwise gradient (0.5 → 1 → 1.5% MeOH in $CHCl_3$/EtOAc (1:1) + 0.5% pyridine (v/v/v/v)). Fractions containing product were combined, evaporated, and the residue was dried in vacuo to afford a white foam (540 mg, 84%). R_f: 0.63 ($CHCl_3$/MeOH 9:1+1% Et_3N (v/v/v)). ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 11.44 (s, 1H, H-3) (exchangeable with D_2O), 8.34–8.06 (m, 8H, Ar*H* (pyrene)), 8.01 (br t, 1H, J = 6.7 Hz, OCONH), 7.87 (d, 1H, $J_{2'',3''}$ = 9.2 Hz, Ar*H* (pyrene H-2)), 7.54 (d, 1H, $J_{5,6}$ = 8.0 Hz, H-6), 7.40–7.17 (m, 9H, Ar*H* (Dmt)), 6.86 (d, 4H, J = 8.2 Hz, Ar*H* (Dmt)), 6.23 (d, 1H, $J_{1',2'}$ = 5.5 Hz, H-1′), 5.80 (br s, 1H, 3′-O*H*, exchangeable with D_2O), 5.43 (d, 1H, $J_{5,6}$ = 8.0 Hz, H-5), 5.12 (m, 1H, H-2′), 4.88 (m, 2H, CH_2Pyr), 4.17 (m, 1H, H-3′), 3.88 (m, 1H, H-4′), 3.72 (s, 6H, CH_3), 3.36–3.10 (m, 2H[*], H-5′) ppm. MS (matrix 2,4,6-THAP): calcd: 805.91, 828.88, 844.78; found: 804.86 [M+H]$^+$, 826.84 [M+Na]$^+$, 842.95 [M+K]$^+$. Elemental analysis: calcd (%) for $C_{48}H_{41}N_3O_9$: C 71.72, H 5.14, N 5.23; found: C 71.35, H 5.17, N 5.49.

5′-*O*-(4,4′-Dimethoxytrityl)-2′-*O*-(butylaminocarbonyl)uracil-1-β-D-arabinofuranoside (10): The crude 2′-*O*-(butylaminocarbonyl)uracil-1-β-D-arabinofuranoside was coevaporated with pyridine (3 × 20 mL), dissolved in dry pyridine (15 mL), cooled in an ice bath, and DmtCl (0.16 mg, 0.48 mmol) was added in one portion. After completion of the reaction (monitored by TLC), the excess DmtCl was quenched with MeOH (1 mL), and after 10 min the mixture was diluted with $CHCl_3$ (100 mL), washed with water (100 mL), 5% $NaHCO_3$ (100 mL), and water (100 mL), then dried (Na_2SO_4), evaporated, and coevaporated with toluene (3 × 25 mL). The residue was chromatographed on silica gel column with a stepwise gradient (0 → 0.5% MeOH in $CHCl_3$/EtOAc (1:1) + 0.5% pyridine (v/v/v/v)). Fractions containing product were combined, evaporated, and the residue was dried in vacuo to afford a white foam (187 mg, 89% yield with reference to the starting material 2′-*O*-(butylaminocarbonyl)-3′,5′-*O*-(tetraisopropyldisiloxan-1,3-diyl)uracil-1-β-D-arabinofuranoside). R_f: 0.44 ($CHCl_3$/MeOH 9:1+1% Et_3N (v/v/v)). ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 11.34 (s, 0.2H), 11.26 (s, 0.8H) (H-3, exchangeable with D_2O), 7.46 (d, 0.2H, $J_{5,6}$ = 8.0 Hz), 7.42 (d, 0.8H, $J_{5,6}$ = 8.0 Hz) (H-6), 7.41–7.22 (m, 9H, Ar*H* (Dmt)), 7.11 (br t, 1H, J = 5.5 Hz, OCONH), 6.89 (d, 4H, J = 8.2 Hz, Ar*H* (Dmt)), 6.15 (d, 1H, $J_{1',2'}$ = 5.5 Hz, H-1′), 5.81 (d, 0.2H, $J_{OH,3'}$ = 4.6 Hz), 5.74 (d, 0.8H, $J_{OH,3'}$ = 5.5 Hz) (3′-O*H*, exchangeable with D_2O), 5.43 (d, 0.2H, $J_{5,6}$ = 8.0 Hz), 5.37 (d, 0.8H, $J_{5,6}$ = 8.0 Hz) (H-5), 4.99 (m, 1H, H-2′), 4.08 (m, 1H, H-3′), 3.86 (m, 1H, H-4′), 3.74 (s, 6H, OCH_3), 3.30 (m, 2H[*], H-5′), 2.97–2.79 (m, 2H, NCH_2), 1.31–1.15 (m, 4H, NCH$_2$CH_2CH_2), 0.82 (t, 2.4H, J = 7.3 Hz), 0.78 (t, 0.6H, J = 7.3 Hz) (CH$_2$CH_3). MS (matrix 2,4,6-THAP): calcd: 671.72, 687.83; found: 668.69 [M+Na]$^+$, 684.80 [M+K]$^+$. Elemental analysis: calcd (%) for $C_{35}H_{39}N_3O_9$: C 65.10, H 6.09, N 6.51; found: C 65.84, H 6.28, N 6.31.

3′-*O*-(*N*,*N*-Diisopropylamino-2-cyanoethoxyphosphinyl)-5′-*O*-(4,4′-dimethoxytrityl)-2′-*O*-(pyren-1-ylmethylaminocarbonyl)-uracil-1-β-D-arabinofuranoside (7): 5′-*O*-(4,4′-Dimethoxytrityl)-2′-*O*-(pyren-1-ylmethylamino-carbonyl)uracil-1-β-D-arabinofuranoside (262 mg, 0.32 mmol) was coevaporated with dry DCM (2 × 20 mL) and dissolved in dry DCM. Diisopropylammonium tetrazolide (56 mg, 0.32 mmol) and bis(*N*,*N*-diisopropylamino)-2-cyanoethoxyphosphine (0.155 mL, 0.49 mmol) were added and the mixture was stirred under argon for 2 h. After conversion of the starting compound was complete (monitoring by TLC), the mixture was diluted with $CHCl_3$, washed with 5% $NaHCO_3$ (100 mL) and 20% NaCl (100 mL), dried over Na_2SO_4, evaporated to dryness, and the residue was chromatographed on a silica gel column with a stepwise gradient (5 → 10 → 20 → 25% acetone in $CHCl_3$ + 1% Et_3N (v/v/v)). Fractions containing product were combined, evaporated, and the residue was dried in vacuo to afford a white amorphous solid (318 mg, 97%). R_f: 0.24, 0.35 ($CHCl_3$/EtOAC 1:1+1% Et_3N (v/v/v)). ^{1}H NMR (500 MHz, [D$_3$]MeCN): δ = 8.97 (br s, 1H, H-3) (exchangeable with D_2O), 8.30–8.06 (m, 8H, Ar*H* (pyrene)), 7.93 (m, 1H, Ar*H* (pyrene H-2)), 7.51 (d, 0.55H, $J_{5,6}$ = 8.0 Hz), 7.47 (d, 0.45H, $J_{5,6}$ = 8.0 Hz) (H-6, diastereomers), 7.44–7.15 (m, 10H, Ar*H* (Dmt), OCONH), 6.80 (m, 4H, Ar*H* (Dmt)), 6.27 (m, 1H, H-1′) 5.36 (m, 2H, H-2′, H-5), 4.94 (m, 2H, CH_2Pyr), 4.57 (m, 0.55H), 4.47 (m, 0.45H) (H-3′, diastereomers), 4.07 (m, 1H, H-4′), 3.70 (m, 6H, OCH_3), 3.66–3.50 (m, 4H, POCH_2, PNCH), 3.43 (m, 1H), 3.36 (m, 1H), (H-5′, diastereomers), 2.53 (t, 1.1H, J = 5.9 Hz), 2.49 (t, 0.9H, J = 5.9 Hz) (CH_2CN, diastereomers), 1.27–0.94 (m, 12H, CHCH_3) ppm. ^{31}P NMR ([D$_3$]MeCN): δ = 150.71, 150.33 (diastereomers, ≈10:9) ppm. MS (matrix 2,4,6-THAP): calcd.: 1030.59, 1046.49; found: 1027.06 [M+Na]$^+$, 1043.17 [M+K]$^+$.

[*] *Calculated value; the signal of water is also present in the region.*

[*] *Calculated value; the signal of water is also present in the region.*

3′-*O*-(*N*,*N*-Diisopropylamino-2-cyanoethoxyphosphinyl)-5′-*O*-(4,4′-dimethoxytrityl)-2′-*O*-(butylaminocarbonyl)uracil-1-β-D-arabinofuranoside (11): 5′-*O*-(4,4′-Dimethoxytrityl)-2′-*O*-(butylaminocarbonyl)uracil-1-β-D-arabinofuranoside (154 mg, 0.24 mmol) was coevaporated with dry DCM (2×20 mL) and then dissolved in dry DCM (20 mL). Diisopropylammonium tetrazolide (61 mg, 0.36 mmol) and bis(*N*,*N*-diisopropylamino)-2-cyanoethoxyphosphine (0.114 mL, 0.36 mmol) were added and the mixture was stirred under argon for 2 h. After conversion of the starting compound was complete (monitoring by TLC) the mixture was diluted with $CHCl_3$, washed with 5% $NaHCO_3$ (100 mL) and 20% NaCl (100 mL), dried over Na_2SO_4, evaporated to dryness, and the residue was chromatographed on a silica gel column with a stepwise gradient (5 → 10 → 20 → 25% acetone in $CHCl_3$ + 1% Et_3N (v/v/v)). Fractions containing product were combined, evaporated, and the residue was dried in vacuo to afford **11** (184 mg, 91%) as a white amorphous solid. R_f: 0.33, 0.38 ($CHCl_3$/EtOAC 1:1+1% Et_3N (v/v/v)). ^{1}H NMR (500 MHz, [D$_3$]MeCN): $\delta = 8.99$ (br s, 1H, H-3, exchangeable with D_2O), 7.53 – 7.24 (m, 11H, Ar*H* (Dmt), OCON*H*, H-6), 6.89 (m, 4H, Ar*H* (Dmt)), 6.23 (m, 1H, H-1′), 5.40 (m, 1H, H-5), 5.25 (m, 1H, H-2′), 4.53 (m, 0.54H), 4.43 (m, 0.46H) (H-3′, diastereomers), 4.07 (m, 1H, H-4′), 3.81 – 3.35 (m, 12H, OCH_3,POCH_2, PNC*H*, H-5′), 3.10 – 2.92 (m, 2H, NCH_2), 2.64 (t, 1.1H, $J = 5.9$ Hz), 2.53 (t, 0.9H, $J = 5.9$ Hz) (CH_2CN, diastereomers), 1.36 (m, 2H, NCH$_2CH_2$), 1.32 – 1.03 (m 14H, CH_2CH_3, CHCH_3), 0.89 (m, 3H, CH_2CH_3) ppm. ^{31}P NMR ([D$_3$]MeCN): $\delta = 150.74$, 150.19 (diastereomers, ≈7:6) ppm. MS (matrix 2,4,6-THAP): calcd: 873.26, 889.30; found: 868.91 $[M+Na]^+$, 885.02 $[M+K]^+$.

This research was partially supported by Russian Foundation for Basic Research grants (Grant n°s 00-03-32701, 02-03-32376, and 03-03-32196). N.N.D. thanks the Medical Research Council for support during her visit to Cambridge in 2002. We thank Prof. Vladimir A. Efimov and Igor A. Prokhorenko, Moscow (Russia) for their encouraging interest.

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[10] Experimental techniques as well as additional spectral data for oligonucleotides and duplexes are provided in the Supporting Information.

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Received: May 30, 2003 [F678]

