Synthesis of (2-Deoxy-α- and -β-D-*erythro*-pentofuranosyl)(thymin-1-yl)alkanes and Their Incorporation into Oligodeoxyribonucleotides. Effect of Nucleobase−Sugar Linker Flexibility on the Formation of DNA−DNA and DNA−RNA Hybrids

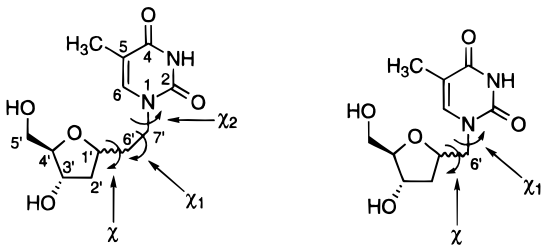
Jila H. Boal, Andrzej Wilk, Carlo L. Scremin, Glenn N. Gray,[1] Lawrence R. Phillips,[1] and Serge L. Beaucage*

Division of Hematologic Products, Center for Biologics Evaluation and Research, Food and Drug Administration, 8800 Rockville Pike, Bethesda, Maryland 20892, and Laboratory of Drug Discovery Research and Development, Developmental Therapeutics Program, National Cancer Institute, Frederick, Maryland 21701

Received August 8, 1996[⊗]

On the basis of modeling studies, the (2-deoxy-α- and β-D-*erythro*-pentofuranosyl) (thymin-1-yl) alkanes **1a,b** and **2a,b** were selected as potential conformational probes for *alt*DNA oligonucleotides. A straightforward approach to the synthesis of **1a,b** and **2a,b** from commercial 2-deoxy-D-ribose (**3**) and 1-*O*-methyl-2-deoxy-3,5-di-*O*-*p*-toluoyl-D-*erythro*-pentofuranose (**13**), respectively, was developed. These nucleoside analogues were converted to the phosphoramidite derivatives **27a,b**−**30a,b** and incorporated into oligonucleotide **31** at predetermined sites and defined internucleotidic motifs. The insertion of **1a,b** according to either a (3′ → 5′)- or a (3′ → 3′)-internucleotidic polarity produced oligonucleotides exhibiting a slightly higher affinity for their complementary unmodified DNA sequence than for the corresponding RNA sequence (Table 3). Conversely, the incorporation of **2a** into **31** according to a (3′ → 3′)-orientation generated, for the first time, an *alt*DNA oligonucleotide displaying a greater affinity for its complementary unmodified RNA sequence (ΔT_m = 6 °C) than for the corresponding DNA sequence (ΔT_m = 10 °C). This hybrid was, however, thermodynamically less stable than the duplex having unmodified α-2′-deoxythymidine similarly incorporated into **31** ($\Delta\Delta T_m$ = 3 °C).

Our efforts toward designing oligonucleotide analogues that possess achiral phosphorus centers and offer substantial resistance to nucleases have led to the development of α,β-oligodeoxyribonucleotides with alternating (3′ → 3′)- and (5′ → 5′)-internucleotidic phosphodiester linkages (*alt*DNA).[2] These oligonucleotide analogues have not only shown superior resistance to nucleases but also have generated relatively stable complexes with complementary DNA or RNA sequences.[2,3] Thermodynamically, *alt*DNA−DNA hybrids are only slightly less stable than unmodified DNA−DNA duplexes (ΔT_m/modification = 1.1 °C).[2a] By comparison, *alt*DNA−RNA complexes are considerably less stable than unmodified DNA−RNA duplexes (ΔT_m/modification = 2.2 °C).[2a] The reduced thermal stability of *alt*DNA−RNA complexes is thought to result from the unnatural arrangement of the internucleotidic phosphodiester motifs of *alt*DNA oligomers that could affect base-pair formation more extensively in A-type than in B-type helices.[4] In this context, it can be argued that providing additional flexibility to *alt*DNA nucleobases by the insertion of a methylene or ethylene tether between each carbohydrate and nucleobase of α-nucleotidic residues would allow complementary nucleobases to better align and form stable Watson−Crick base-pairs. We modeled the modified nucleosides **1a** and **1b** in either the parallel or the antiparallel orientation into an A-type DNA−RNA helix and a B-type DNA−DNA duplex and discovered two energetically preferred B-type helices (see Figure 1, top and middle sections).



The torsion angle χ_1 ($C_{1'}-C_{6'}-C_{7'}-N_1$) of the ethylene tether for either **1a** or **1b**, which allowed for optimal base-pairing within B-helices, was 180°. Similarly modeled, the less flexible nucleoside analogue **2a** led to an A-type helix that was energetically comparable to that of an unmodified DNA−RNA duplex (see Figure 1, bottom section). Given the favorable structural features of these models, we now report the detailed synthesis and characterization of the nucleoside analogues **1a,b** and **2a,b**[5] and their incorporation into oligonucleotides according to defined internucleotidic motifs. Thermal stability of hybrids generated upon annealing these oligodeoxyribonucleotide analogues with complementary unmodified DNA and RNA oligomers will also be reported.

* To whom correspondence should be addressed. Tel.: (301) 496-3378. Fax: (301) 480-3256. E-mail: beaucage@helix.nih.gov.

⊗ Abstract published in *Advance ACS Abstracts,* November 15, 1996.

(1) National Cancer Institute at Frederick.

(2) (a) Koga, M.; Wilk, A.; Moore, M. F.; Scremin, C. L.; Zhou, L.; Beaucage, S. L. *J. Org. Chem.* **1995**, *60*, 1520−1530. (b) Koga, M.; Moore, M. F.; Beaucage, S. L. *J. Org. Chem.* **1991**, *56*, 3757−3759.

(3) Debart, F.; Tosquellas, G.; Rayner, B.; Imbach, J.-L. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 1041−1046.

(4) (a) Scremin, C. L.; Boal, J. H.; Wilk, A.; Phillips, L. R.; Zhou, L.; Beaucage, S. L. *Tetrahedron Lett.* **1995**, *36*, 8953−8956. (b) Scremin, C. L.; Boal, J. H.; Wilk, A.; Phillips, L. R.; Beaucage, S. L. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 207−212.

S0022-3263(96)01548-4

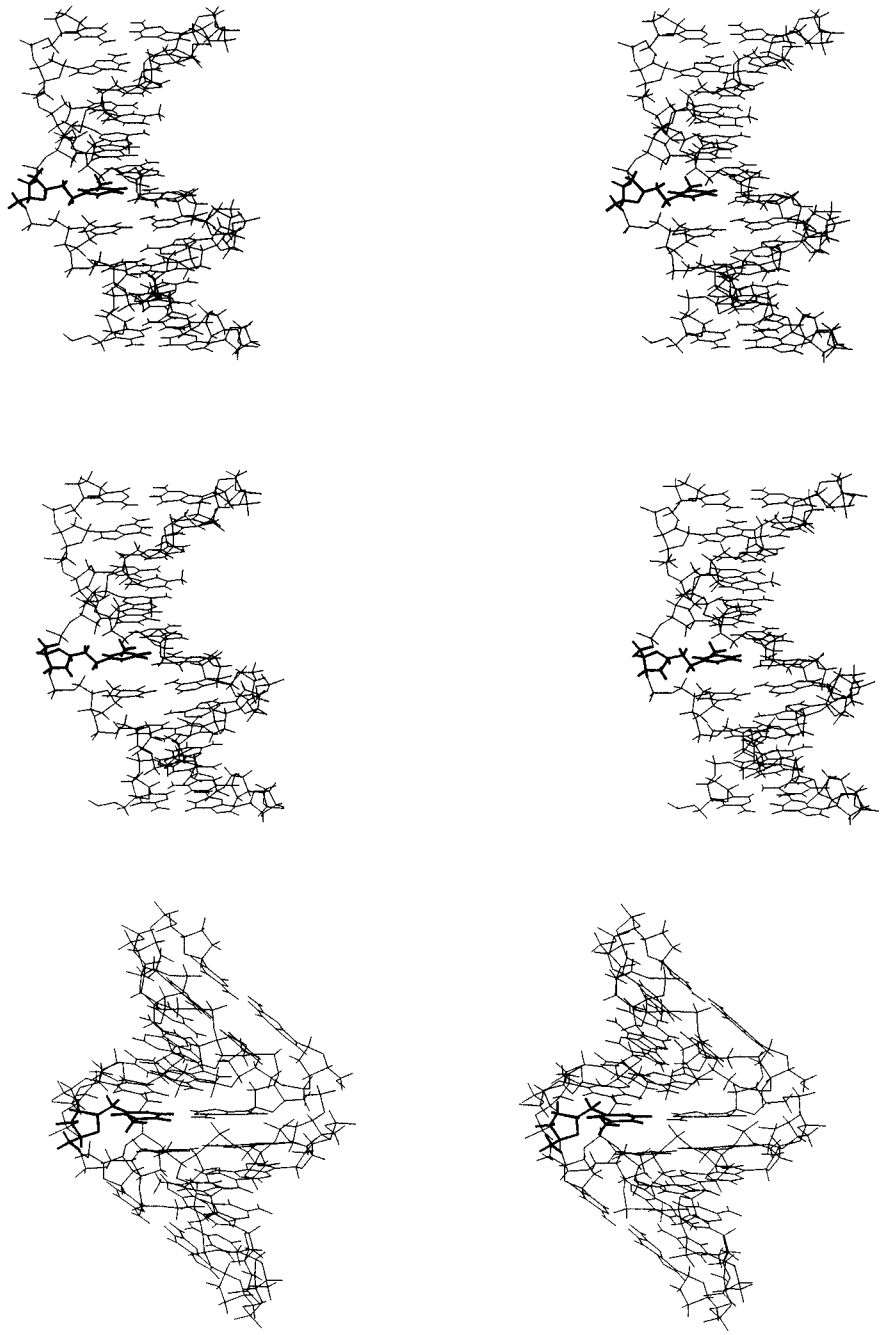


Figure 1. Top: Stereoview of an energy-minimized B-type DNA−DNA helix composed of the oligodeoxyribonucleotide 5′-d(GCGTTN*TTGCG) and its complementary unmodified DNA sequence. N* is **1a** linked through (3′ → 3′)- and (5′ → 5′)-phosphodiester linkages (shown as broader lines). Middle: Same as above but boldface N* is **1b** inserted according to natural (3′ → 5′)-internucleotidic polarity. Bottom: Stereoview of an energy-minimized A-type DNA−RNA helix composed of the above oligomer and its complementary unmodified RNA sequence. Boldface N* is **2a** linked through (3′ → 3′)- and (5′ → 5′)-phosphodiester linkages.

Results and Discussion

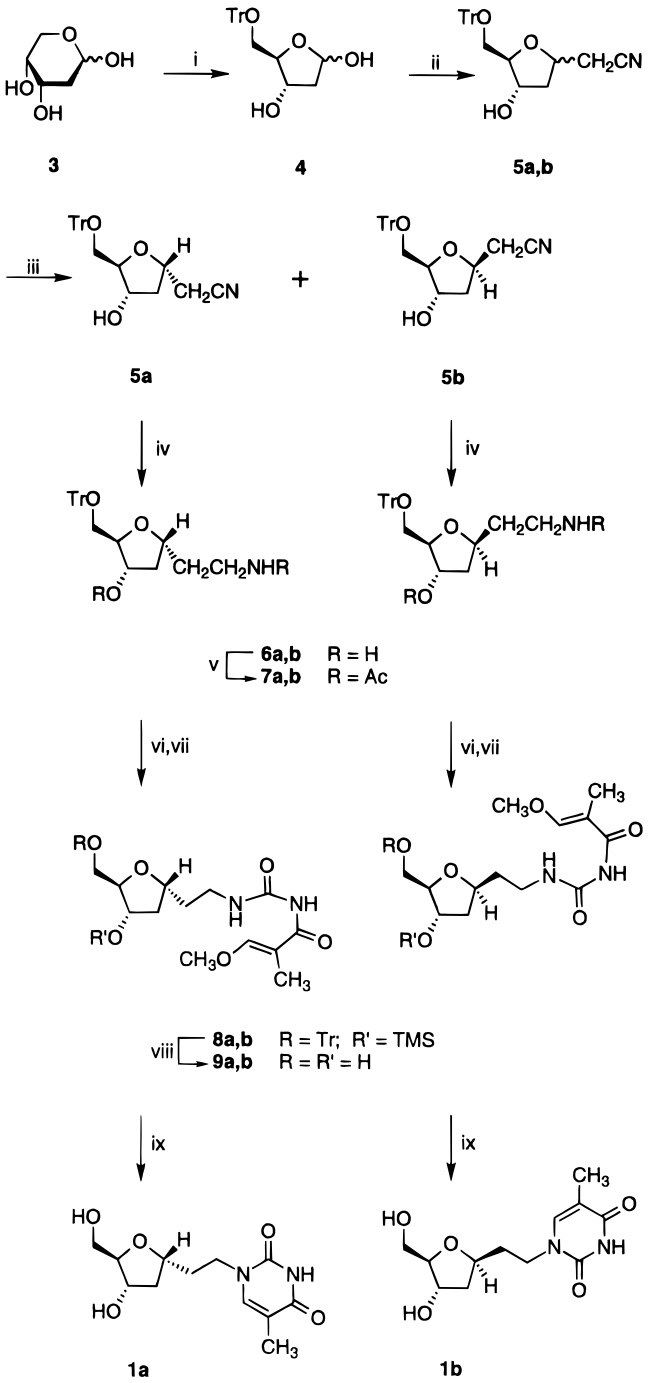
A straightforward approach to the preparation of **1a,b** from 2-deoxy-D-ribose is described in Scheme 1. The 5-*O*-trityl-2-deoxyribofuranoside (**4**) was prepared in 60% yield from the reaction of 2-deoxy-D-ribose (**3**) with triphenylmethyl chloride under conditions adapted from the procedure of Kam and Oppenheimer.[6] A Wittig−Horner condensation of **4** with diethyl (cyanomethyl)-phosphonate produced the (2-deoxyribofuranosyl)acetonitrile derivatives **5a,b** in almost quantitative yields as nearly an equimolar mixture of α- and β-epimers.[7] The facile separation of **5a** and **5b** by silica gel chromatography allowed isolation of each epimer in gram quantities. Reduction of **5a** and **5b** to the corresponding amines, however, presented difficulties. Treatment of either **5a** or **5b** with lithium aluminum hydride (LAH) in THF produced a complex mixture of reaction products.

(5) Preliminary data pertaining to the preparation of **1a,b** and **2a,b** have been reported earlier by us.[4] During the edition of this manuscript, an alternate synthesis of **2b** and its incorporation into oligonucleotides have appeared; see: Hossain, N.; Hendrix, C.; Lescrinier, E.; Van Aerschot, A.; Busson, R.; De Clercq, E.; Herdewijn, P. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 1465−1468.

(6) Kam, B. L.; Oppenheimer, N. J. *Carbohydr. Res.* **1979**, *69*, 308−310.

(7) Syntheses of (ribofuranosyl)acetonitrile derivatives from the corresponding ribofuranoses and either (cyanomethyl)triphenylphosphorane or diethyl (cyanomethyl)phosphonate have been reported.[29]

Scheme 1[a,b]

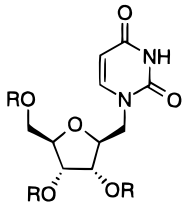


[a] Conditions: (i) TrCl/DMAP/C_5H_5N, 50 °C, 6 h; (ii) $(EtO)_2P(O)CH_2CN$/NaH/THF, 5 °C, 6 h; (iii) silica gel chromatography; (iv) $(CH_3)_2S{\cdot}BH_3$/THF/reflux, 40 min; (v) Ac_2O/C_5H_5N, 25 °C; (vi) TMS-Cl/Et_3N/THF, 25 °C, 2 h; (vii) $CH_3OCHC(CH_3)CONCO/Et_3N/C_6H_6$, 25 °C, 3 h; (viii) 10% TFA/CH_2Cl_2, 25 °C, 2 h; (ix) concd NH_4OH, 50 °C, 12 h. [b]Key: Et, ethyl; Tr, triphenylmethyl; Ac, acetyl; TMS, trimethylsilyl; TFA, trifluoroacetic acid.

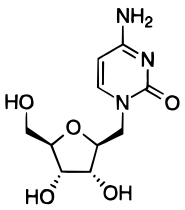
Consistent with these findings is the reduction of β-oxypropionitriles with LAH in THF, which produces significant cleavage at the ether linkage.[8] Moreover, the reduction of aliphatic nitriles with LAH proceeds with evolution of hydrogen resulting from an attack of the reagent on the active hydrogens adjacent to the nitrile function.[8,9] Such hydrogen abstraction has been presumed to be responsible for the decreased yields of amine products obtained in these reduction reactions.[8] However, addition of aluminum chloride to LAH enables complete reduction of nitriles, even those with active α-hydrogens, without release of hydrogen.[10] Incidentally, the reaction of aluminum chloride with LAH is known to generate aluminum hydride,[11] which is also effective in the reduction of nitriles with active α-hydrogens.[9]

Thus, the reduction of **5a,b** with aluminum hydride in THF gave highly pure (2-deoxyribofuranosyl)aminoethanes **6a,b**;[12] thin-layer chromatography (TLC) analysis of the corresponding peracetylated derivatives indicated a nearly quantitative reaction. The preparation of aluminum hydride according to the method of Finholt *et al.*[13] can, however, be hazardous and must be carried out in a drybox under an inert atmosphere. Consequently, the use of other acidic reducing reagents was investigated. As a result, the commercial borane−dimethyl sulfide complex was found to be especially effective in the conversion of **5a,b** to **6a,b**; the reduction proceeded smoothly to provide yields exceeding 95% within 1 h in refluxing THF. The aminoethylated glycosides **6a,b**, were characterized as their diacetate derivatives **7a,b**. Without further purification, **6a,b** were reacted with trimethylsilyl chloride and then condensed with 3-methoxy-2-methylacryloyl isocyanate, a reagent generated *in situ* from the parent acryloyl chloride and silver cyanate.[14] The resulting crude acryloylurea derivatives **8a,b** were treated with 10% trifluoroacetic acid in dichloromethane for 2 h at ambient temperature. After workup and purification of the reaction products by silica gel chromatography, the acryloylurea derivatives **9a,b** were isolated in *ca.* 40% yield (based on **5a,b**). Cyclization of **9a,b** to give **1a,b** in high yield (*ca.* 90%) was accomplished in concentrated ammonium hydroxide at 50 °C for 12 h in a pressure vessel.

A similar approach to the preparation of the α- and β-2′-deoxyribonucleoside analogues **2a,b** is outlined in Scheme 2. Although syntheses of "homouridine" (**10**, **11**), "homocytidine" (**12**), and analogous D-ribitol derivatives have been reported,[15,16] the preparation of **2a,b** has not been described.[5]



10 R = CH_2Ph
11 R = H



12

Thus, commercial 1-*O*-methyl-2-deoxy-3,5-di-*O*-*p*-toluoyl-D-*erythro*-pentofuranoside (**13**) was converted by the

(8) (a) Soffer, L. M.; Parotta, E. W. *J. Am. Chem. Soc.* **1954**, *76*, 3580−3583. (b) Soffer, L. M.; Katz, M. *J. Am. Chem. Soc.* **1956**, *78*, 1705−1709.

(9) Brown, H. C.; Yoon, N.-M. *J. Am. Chem. Soc.* **1966**, *88*, 1464−1472.

(10) Nystrom, R. F. *J. Am. Chem. Soc.* **1955**, *77*, 2544−2545.

(11) (a)Brown, H. C.; Choi, Y.-M.; Narasimhan, S. *Synthesis* **1981**, 605−606. (b) Lane, C. F. *Aldrichim. Acta* **1975**, *8*, 20−23.

(12) Zhou, L.; Beaucage, S. L. Unpublished results.

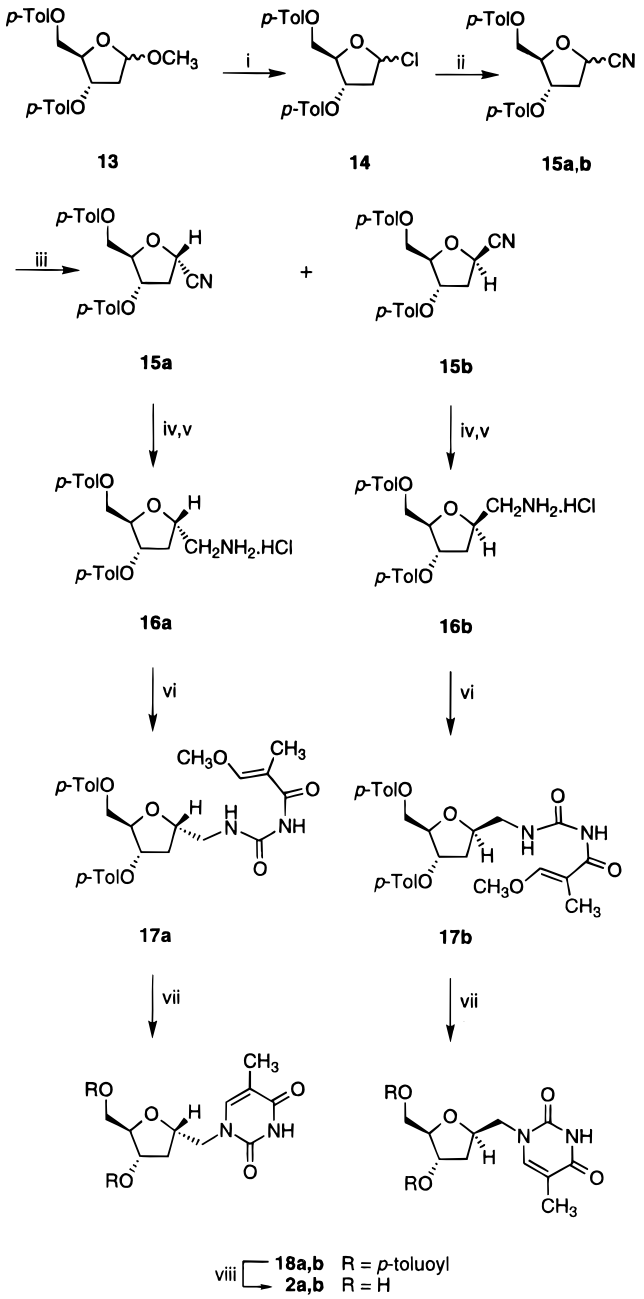
(13) Finholt, A. E.; Bond, Jr., A. C.; Schlesinger, H. I. *J. Am. Chem. Soc.* **1947**, *69*, 1199−1203.

(14) Shealy, Y. F.; O'Dell, C. A.; Thorpe, M. C. *J. Heterocycl. Chem.* **1981**, *18*, 383−389.

(15) Bobek, M.; Farkas, J. *Collect. Czech. Chem. Commun.* **1969**, *34*, 1684−1689.

(16) (a) Cleophax, J.; Defaye, J.; Gero, S. D. *Bull. Soc. Chim. Fr.* **1967**, 104−107. (b) Defaye, J.; Reyners, T. *Bull. Soc. Chim. Biol.* **1968**, *50*, 1625−1635.

Scheme 2[a,b]



[a] Conditions: (i) HCl (g)/AcOH; (ii) Et_2AlCN/toluene/THF, 25 °C, 7 h; (iii) silica gel chromatography; (iv) BH_3/THF 25 °C, 1 h; (v) HCl (g)/CH_3OH; (vi) $CH_3OCHC(CH_3)CONCO/Et_3N/C_6H_6$, 25 °C, 16 h; (vii) AcOH/concd HCl (10:1 v/v), 25 °C, 16 h; (viii) KOH in EtOH−H_2O (2:1) and, then AG 50W-X12 (H^+). [b]Key: *p*-Tol, *p*-toluoyl; Ac, acetyl; Et, ethyl.

method of Hoffer[17] to the halogenated glycoside **14**. Condensation of **14** with diethylaluminum cyanide under the conditions reported by Iyer *et al.*[18a] produced the cyanoglycosides **15a,b** as an almost equimolar mixture of α- and β-anomers in yields greater than 95%. Like **5a** and **5b**, these anomers were easily separated by silica gel chromatography and chemoselectively reduced by diborane in THF to generate the aminomethylated glycosides **16a** and **16b** in isolated yields of *ca.* 85%.

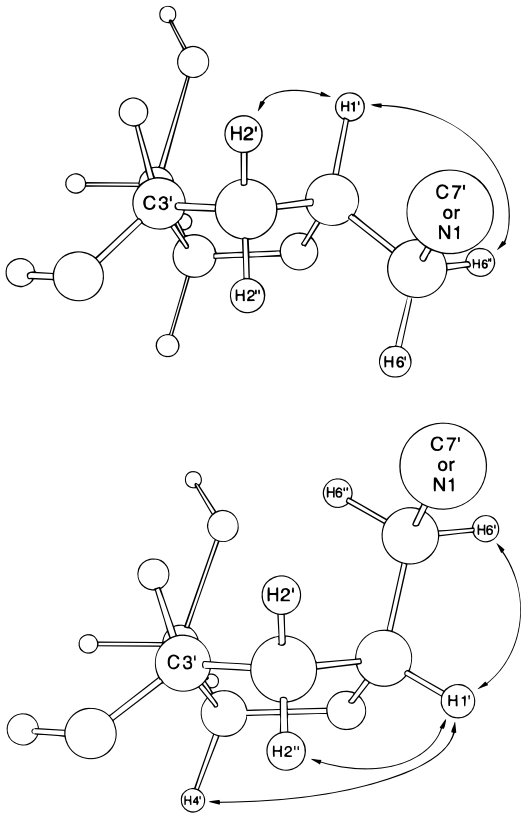


Figure 2. Structure displaying the proximity of H-1′ to H-2′and H-6″ in the case of **1a** and **2a** (top) and H-1′ to H-2″, H-4′, and H-6′ in the case of **1b** and **2b** (bottom) according to NOESY experiments performed at 300 MHz in D_2O.

Treatment of **16a,b** with 3-methoxy-2-methylacryloyl isocyanate gave the acryloylurea derivatives **17a,b** in *ca.* 80% yield. Acid-catalyzed cyclization[15] of purified **17a,b** produced the thymine nucleoside analogues **18a,b** in *ca.* 90% yield. Saponification of **18a,b** with aqueous potassium hydroxide quantitatively produced **2a,b**. The nucleoside analogues **1a,b** and **2a,b** were characterized by high-resolution ^{1}H- and ^{13}C-NMR spectroscopies; spectral data are presented in Tables 1 and 2, respectively. ^{1}H-NMR chemical shift assignments were based on the results of COSY experiments,[19] and coupling constants were determined from fine splitting patterns of resonances at 500 MHz. The structure of the α-deoxyribonucleosides **1a** and **2a** is clearly differentiated from that of the β-deoxyribonucleosides **1b** and **2b** on the basis of their respective NOESY spectra at 300 MHz. The presence of the corresponding crosspeaks confirms the proximity of H-1′ to H-2′ and H-6″ in the case of **1a** and **2a** and the proximity of H-1′ to H-2″, H-4′, and H-6′ in the case of **1b** and **2b** (see Figure 2).[20]

Table 1 shows the similarities between the ^{1}H-NMR chemical shifts of H-5′ and H-5″ and those reported for unmodified α- and β-thymidine.[21] On the basis of previously reported chemical shifts for H-5′ and H-5″ of unmodified β-deoxyribonucleosides,[21,22] H-5′ signals of

(17) Hoffer, M. *Chem. Ber.* **1960**, *93*, 2777−2781.

(18) (a) Iyer, R. P.; Phillips, L. R.; Egan, W. *Synth. Commun.* **1991**, *21*, 2053−2063. The synthesis of **15a**,**b** has also been achieved by treatment of **14** with sodium cyanide in 1,2-dimethoxyethane.[18b] (b) Kolb, A.; Huynh Dinh, T.; Igolen, J. *Bull. Soc. Chim. Fr.* **1973**, 3447−3448.

(19) ^{1}H-COSY spectra of **1a,b** and **2a,b** are available as Supporting Information.

(20) ^{1}H-NOESY spectra of **1a,b** and **2a,b** are shown as Supporting Information. H-2′ and H-2″ are defined according to: Wood, D. J.; Hruska, F. E.; Ogilvie, K. K. *Can. J. Chem.* **1974**, *52*, 3353−3366.

(21) Cadet, J.; Taïeb, C.; Remin, M.; Niemczura, W. P.; Hruska, F. E. *Biochim. Biophys. Acta* **1980**, *608*, 435−445.

(22) Ritchie, R. G. S.; Perlin, A. S. *Carbohydr. Res.* **1977**, *55*, 121−128.

Table 1. 500 MHz ^{1}H-NMR Spectral Data for the Deoxyribonucleoside Analogues 1a,b and 2a,b in D_2O[a,b]

	H-1′ ($J_{1'-2'}$) ($J_{1'-2''}$) ($J_{1'-6'}$) ($J_{1'-6''}$)	H-2′ ($J_{2'-1'}$) ($J_{2'-2''}$) ($J_{2'-3'}$)	H-2″ ($J_{2''-1'}$) ($J_{2''-2'}$) ($J_{2''-3'}$)	H-3′ ($J_{3'-2'}$) ($J_{3'-2''}$) ($J_{3'-4'}$)	H-4′ ($J_{4'-3'}$) ($J_{4'-5'}$) ($J_{4'-5''}$)	H-5′ ($J_{5-4'}$) ($J_{5'-5''}$)	H-5″ ($J_{5''-4'}$) ($J_{5''-5'}$)	H-6′ ($J_{6'-1'}$) ($J_{6'-6''}$) ($J_{6'-7'}$) ($J_{6'-7''}$)	H-6″ ($J_{6''-1'}$) ($J_{6''-6'}$) ($J_{6''-7'}$) ($J_{6''-7''}$)	H-7′ ($J_{7'-6'}$) ($J_{7'-6''}$) ($J_{7'-7''}$)	H-7″ ($J_{7''-6'}$) ($J_{7''-6''}$) ($J_{7''-7'}$)	H-6 (J_{6-CH_3})	CH_3 (J_{CH_3-6})
1a	4.16 (6.9) (7.5) (4.8) (7.8)	2.47 (6.9) (13.3) (6.9)	1.70 (7.5) (13.3) (6.0)	4.30 (6.9) (6.0) (5.0)	3.93 (5.0) (3.7) (6.3)	3.70 (3.7) (12.1)	3.61 (6.3) (12.1)	1.99 (4.8) (14.1) (7.1) (7.8)	2.05 (7.8) (14.1) (7.8) (6.1)	3.83 (7.1) (7.8) (14.8)	3.90 (7.8) (6.1) (14.8)	7.54 (1.0)	1.89 (1.0)
1b	4.17 (10.2) (5.6) (2.3) (7.9)	1.81 (10.2) (13.5) (6.3)	2.00 (5.6) (13.5) (2.0)	4.25 (6.3) (2.0) (2.7)	3.83 (2.7) (4.3) (5.8)	3.62 (4.3) (12.0)	3.55 (5.8) (12.0)	1.95 (2.3) (13.8) (6.7) (8.2)	1.89 (7.9) (13.8) (8.1) (5.9)	3.77 (6.7) (8.1) (14.0)	3.86 (8.2) (5.9) (14.0)	7.48 (1.2)	1.84 (1.2)
2a	4.36 (7.8) (5.5) (3.5) (7.9)	2.36 (7.6) (13.9) (6.9)	1.69 (5.5) (13.6) (4.7)	4.24 (6.9) (4.7) (4.1)	3.90 (4.1) (4.0) (5.5)	3.58 (4.0) (12.2)	3.51 (5.5) (12.2)	3.84 (3.4) (14.5)	3.89 (7.8) (14.5)			7.43 (1.1)	1.81 (1.1)
2b	4.36 (9.7) (5.9) (2.9) (7.3)	1.81 (9.8) (13.6) (5.8)	1.99 (5.9) (13.7) (2.2)	4.24 (5.6) (2.3) (2.6)	3.84 (2.4) (4.6) (5.5)	3.53 (4.6) (12.1)	3.47 (5.6) (12.1)	3.95 (2.9) (14.6)	3.79 (7.3) (14.6)			7.41 (1.1)	1.81 (1.1)

[a] Chemical shifts are given in parts per million relative to the sodium salt of 3-(trimethylsilyl)propionic acid as an internal standard (0 ppm). [b] *J* couplings are in parentheses and expressed in Hz.

Table 2. 125 MHz ^{13}C-NMR Chemical Shifts of the Deoxyribonucleoside Analogues 1a,b and 2a,b in D_2O[a]

	C-2	C-4	C-5	C-6	C-1′	C-2′	C-3′	C-4′	C-5′	C-6′	C-7′	CH_3
1a	154.7	169.3	113.1	145.7	78.2	41.8	74.3	87.2	63.7	36.5	48.4	13.7
1b	154.7	169.4	113.2	145.6	78.7	42.1	75.0	89.1	64.6	36.2	48.4	13.8
2a	154.8	169.2	112.8	146.2	79.0	39.0	74.2	87.9	63.9	54.3		13.8
2b	155.0	169.4	113.0	146.2	79.2	39.1	74.8	89.4	64.5	53.9		13.7

[a] Proton-decoupled chemical shifts are listed in parts per million relative to the resonance of the middle line of $CDCl_3$ (77.0 ppm) as an external standard.

1a,b and **2a,b** are tentatively assigned downfield relative to those of H-5″. According to coupling constant arguments, chemical shifts for H-6′, H-6″, H-7′, and H-7″ listed in Table 1 are also tentatively assigned. Specifically, H-6′ signals for **1b** and **2b** are downfield to those of H-6″. Conversely, H-6′ signals for **1a** and **2a** are upfield of those of H-6″, and signals for H-7′ are also assigned upfield of those of H-7″ for both **1a** and **1b**. Incidentally, ^{13}C-NMR chemical shift assignments for **1a,b** and **2a,b** (Table 2) are supported by ^{1}H−^{13}C-COSY data.[23]

The incorporation of **1a,b** and **2a,b** into oligodeoxyribonucleotides according to defined internucleotidic motifs was achieved by standard phosphoramidite chemistry.[24] Scheme 3 illustrates the conversion of **1a,b** and **2a,b** to the phosphoramidites **27**−**30a,b**. Specifically, the condensation of **1a,b** and **2a,b** with 4,4′-dimethoxytrityl chloride (DMTr-Cl) in pyridine generated the 5′-*O*-protected nucleosides **19a,b** and **20a,b**, in 75−85% yield. Unlike the synthesis of **19a,b** and **20a,b** the preparation of the 3′-*O*-protected nucleosides **25a,b** and **26a,b** required additional steps. Typically, **1a,b** and **2a,b** were silylated, regioselectively, at the 5′-hydroxy function upon reaction with *tert*-butyldimethylchlorosilane in the presence of imidazole in dry DMF to produce **21a,b** and **22a,b** in isolated yields ranging from 90 to 95%. These 5′-*O*-silylated nucleoside analogues were reacted with DMTr-Cl in pyridine to give the fully protected nucleosides **23a,b** and **24a,b**. Without further purification, **23a,b** and **24a,b** were desilylated by treatment with tetra-*n*-butylammonium fluoride in THF to provide **25a,b** and **26a,b** in isolated yields of 68−80%. Phosphitylation of **19a,b**, **20a,b**, **25a,b**, and **26a,b** by 2-cyanoethyl *N*,*N*,*N*′,*N*′-tetraisopropylphosphorodiamidite and catalytic amounts of *N*,*N*-diisopropylammonium tetrazolide in anhydrous dichloromethane[25] generated the deoxyribonucleoside phosphoramidites **27**−**30a,b** in yields exceeding 88%. These phosphoramidites were characterized by ^{31}P-NMR spectroscopy (see Experimental Section).

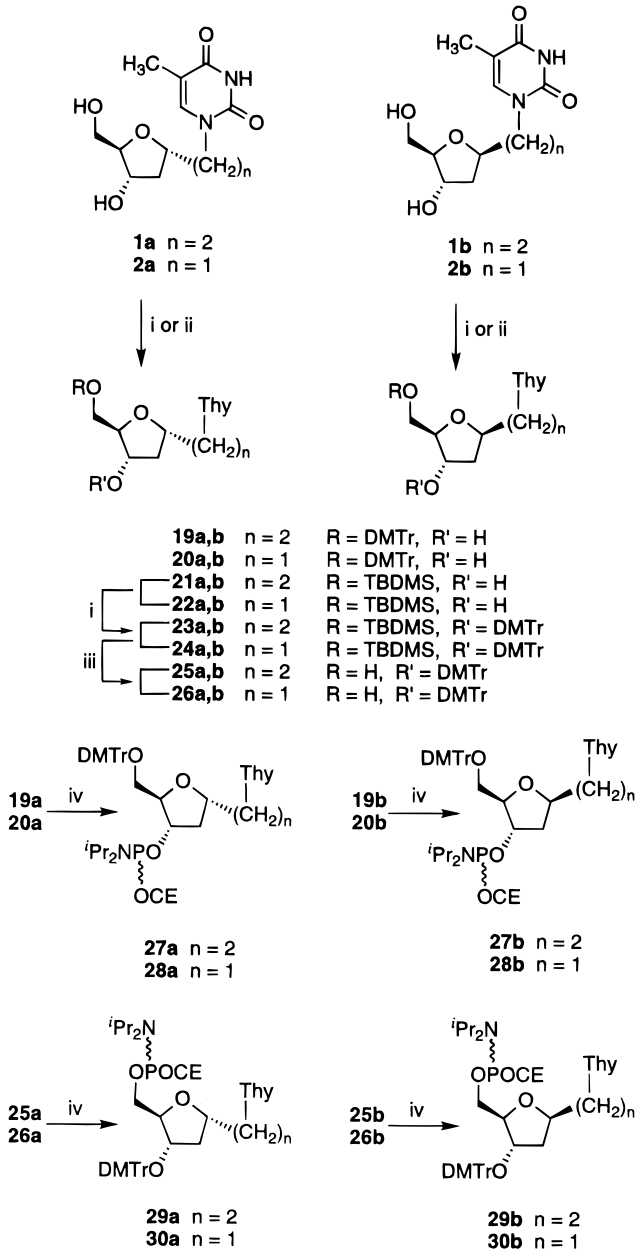
Each of the modified phosphoramidites **27**−**28a,b** and **29**−**30a,b** was incorporated by routine solid-phase oligonucleotide synthesis into 5′-d(TCGACACCCAATN*C-N*GAAAATGGA) (**31**) at selected sites (N*) according to native (3′ → 5′)- or inverted (3′ → 3′)-internucleotidic polarity, respectively (Table 3). Additional DNA sequences carrying unmodified β-2′-deoxythymidine (β-dT) and α-2′-deoxythymidine (α-dT) inserted in both internucleotidic orientations at the indicated sites within **31** have been synthesized to generate meaningful control experiments. These modified oligonucleotides were annealed to either a complementary unmodified DNA or an RNA oligomer. The stability of each complex was determined and its thermal denaturation temperature (T_m) reported in Table 3. To better assess the thermodynamic stability of the hybrids, mismatches were created

(23) ^{1}H−^{13}C-COSY spectra of of **1a,b** and **2a,b** are provided as Supporting Information.

(24) (a) Beaucage, S. L. In *Methods in Molecular Biology, Vol. 20: Protocols for Oligonucleotides and Analogs*; Agrawal, S., Ed.; Humana Press: Totowa, 1993; pp 33−61. (b) Beaucage, S. L.; Caruthers, M. H. In *Bioorganic Chemistry: Nucleic Acids*; Hecht, S. M., Ed.; Oxford University Press: New York, 1996; pp 36−74. (c) Beaucage, S. L.; Iyer, R. P. *Tetrahedron* **1992**, *48*, 2223−2311.

(25) Barone, A. D.; Tang, J.-Y; Caruthers, M. H. *Nucl. Acids Res.* **1984**, *12*, 4051−4061.

Scheme 3[a,b]



[a] Conditions: (i) DMTrCl/DMAP/C_5H_5N, 25 °C; (ii) TBDMSCl/imidazole/DMF, 25 °C, 1 h; (iii) 1.0 M *n*-Bu_4NF in THF, 25 °C, 2 h; (iv) $(^iPr_2N)_2POCH_2CH_2CN$/cat. DIAT/CH_2Cl_2, 25 °C, 4 h. [b] Key: DMTr, 4,4′-dimethoxytrityl; DMAP, 4-(dimethylamino)pyridine; TBDMS, *tert*-butyldimethylsilyl; Bu, butyl; DIAT, *N,N*-diisopropylammonium salt of 1*H*-tetrazole; CE, 2-cyanoethyl; ^{i}Pr, (1-methyl)ethyl; Thy, thymin-1-yl.

at the indicated sites within **31** (Table 3) by insertion of *β*-2′-deoxyadenosine (*β*-dA) through natural (3′ → 5′)-internucleotidic linkages.

Modeling studies predicted that the incorporation of **1a,b** into oligodeoxyribonucleotides would produce energetically preferred B-type helices (*vide supra*). Experiments showed that insertion of **1b** in **31**, according to natural (3′ → 5′)-internucleotidic orientation, led to the formation of a DNA−DNA complex (T_m = 63 °C) that is thermodynamically more stable than the corresponding DNA−RNA hybrid (T_m = 54 °C, Table 3) but less stable than the parent unmodified DNA−DNA or DNA−RNA duplex (T_m = 68 or 61 °C, respectively). Despite the fact that the preferred torsion angle χ_1 ($C_{1'}-C_{6'}-C_{7'}-N_1$) of the ethylene tether in **1b** is 180° according to modeling

Table 3. Thermal Denaturation Temperatures of DNA−DNA and DNA−RNA Hybrids Composed of 5′-d(TCGACACCCAATN*CN*GAAAATGGA) (31) and 5′-d(TCCATTTTCAGAATTGGGTGTCGA) or 5′-r(UCCAUUUUCAGAAUUGGGUGUCGA)

		DNA−DNA hybrids		DNA−RNA hybrids	
N*	orientation	T_m[a] (°C)	ΔT_m[b] (°C)	T_m (°C)	ΔT_m (°C)
β-**dT**	(3′ → 5′)	68		61	
α-**dT**	(3′ → 5′)	60	8	60	1
β-**dA**	(3′ → 5′)	57	11	50	11
1a	(3′ → 5′)	59	9	52	9
1b	(3′ → 5′)	63	5	54	7
2a	(3′ → 5′)	57	11	49	12
2b	(3′ → 5′)	58	10	52	9
β-**dT**	(3′ → 3′)	55	13	50	11
α-**dT**	(3′ → 3′)	66	2	58	3
1a	(3′ → 3′)	61	7	52	9
1b	(3′ → 3′)	58	10	50	11
2a	(3′ → 3′)	58	10	55	6
2b	(3′ → 3′)	56	12	50	11

[a] Thermal denaturation of DNA−DNA and DNA−RNA hybrids was performed in PBS buffer (pH 7.2) and monitored at 260 nm. The concentration of each oligomer was *ca.* 2.5 *μ*M. [b] ΔT_m is the difference between the T_m of either modified DNA−DNA or DNA−RNA complexes and the T_m of the corresponding unmodified duplex. The variability between repeated T_m measurements was *ca.* 1 °C.

studies (*vide supra*), it is reasonable to assume that the increased number of conformers causes an entropic destabilization of the hybrid.

As expected, the (3′ → 3′)-incorporation of **1b** into **31** produced DNA−DNA and DNA−RNA hybrids that were less stable than the parent unmodified duplexes (ΔT_m = 10 and 11 °C, respectively). The thermal stability of these complexes is comparable to either those resulting from the (3′ → 3′)-incorporation of *β*-dT into **31** (ΔT_m = 13 °C for the DNA−DNA complex and 11 °C for the DNA−RNA hybrid, Table 3) or those having mismatches created by the (3′ → 5′)-insertion of *β*-dA in **31** (ΔT_m = 11 °C for both DNA−DNA and DNA−RNA complexes).

The (3′ → 5′)-incorporation of **1a** or an unmodified α-dT into **31** led to the formation of DNA−DNA hybrids displaying similar thermal stabilities (T_m = 59 or 60 °C, respectively). Surprisingly, the (3′ → 3′)-incorporation of **1a** into **31** only slightly improves the stability of the DNA−DNA hybrid (T_m = 61 °C) relative to that obtained with the (3′ → 5′)-insertion of **1a** (T_m = 59 °C). This stabilization (ΔT_m = 2 °C) is small when compared to that generated by the (3′ → 3′)-incorporation of unmodified α-dT into **31** (ΔT_m = 6 °C) under similar conditions. It must also be noted that the incorporation of **1a** and **1b** into **31** in either orientation did not provide a higher affinity for the complementary RNA oligomer. For example, the (3′ → 3′)-incorporation of **1a** into **31** generated an *alt*-motif that showed a higher affinity for its DNA complement (ΔT_m = 7 °C) than for its RNA complementary sequence (ΔT_m = 9 °C, Table 3). These data are consistent with the physicochemical properties of *alt*DNA oligomers studied earlier.[2]

The incorporation of **2a** and **2b** into **31** according to either orientation, and *β*-dA in the native (3′ → 5′)-internucleotidic polarity, produced DNA−DNA hybrids of similar thermal stabilities (T_m = 56−58 °C). Thus, the incorporation of **2a** or **2b** into a DNA−DNA hybrid is equivalent to the incorporation of a mismatch into an unmodified DNA−DNA duplex. However, the (3′ → 3′)-incorporation of **2a** into **31** generated a significantly greater affinity for the complementary RNA oligomer

(ΔT_m = 6 °C) than for the corresponding DNA complement (ΔT_m = 10 °C). This is the first time that an oligodeoxyribonucleotide with an *alt*-motif demonstrates a higher affinity for RNA than for DNA complementary sequences. These data are also in agreement with the predictions that emerged from modeling studies (see Figure 1, bottom section). It must, however, be pointed out that the (3′ → 3′)-incorporation of α-dT into **31** still leads to a DNA−RNA hybrid that is more stable (T_m = 58 °C) than that obtained from the (3′ → 3′)-integration of **2a** into **31** (T_m = 55 °C). Thus, the nucleobase flexibility of **2a** presumably contributed to entropic factors that adversely affected the thermostability of the modified DNA−RNA hybrid to a greater extent than that observed with the parent unmodified α-nucleoside under identical conditions.

Experimental Section

Materials and Methods. Common chemicals and solvents including those that are anhydrous were purchased from commercial sources and used without further purification. 2-Deoxy-D-ribose (**3**) and 1-*O*-methyl-2-deoxy-3,5-di-*O*-*p*-toluoyl-D-*erythro*-pentofuranoside (**13**) were obtained from Sigma and used as received. Triphenylmethyl chloride, 4,4′-dimethoxytrityl chloride, 4-(dimethylamino)pyridine, diethyl (cyanomethyl)phosphonate, sodium hydride, 1.0 M borane−tetrahydrofuran complex in THF, 1.0 M diethylaluminum cyanide in toluene, 2.0 M borane−methyl sulfide complex in THF, trimethylsilyl chloride, methyl methacrylate, sodium hydrogen sulfate, silver cyanate, *tert*-butyldimethylchlorosilane, imidazole, 1.0 M tetra-*n*-butylammonium fluoride in THF, (2-cyanoethyl)-*N*,*N*,*N*′,*N*′-tetraisopropyl phosphordiamidite, diisopropylamine, and 1*H*-tetrazole were purchased from Aldrich. Trifluoroacetic acid (peptide synthesis grade) and basic ion-exchange resin Amberlite IRA-93 (free base) were obtained from Applied Biosystems and Sigma, respectively. 3-Methoxy-2-methylacryloyl chloride was prepared according to the method of Shaw and Warrener.[26]

Chromatography on silica gel columns was performed using Merck silica gel 60 (230−400 mesh), whereas analytical thin-layer chromatography (TLC) was conducted on 2.5 cm × 7.5 cm glass plates coated with a 0.25 mm thick layer of silica gel 60 F_{254} (Whatman). Reported melting points are uncorrected. Optical rotations (α) of **1a** and **1b** were measured at 589 nm in methanol at 25 °C. Specific rotations ([α]) were calculated according to the formula $[\alpha] = \alpha/(lc)$, where α is the observed rotation, l is the sample cell length in dm, and c is the sample concentration in g/mL.

NMR spectra were recorded at either 7.05 T (300 MHz for ^{1}H) or 11.75 T (500 MHz for ^{1}H). Proton-decoupled ^{31}P- and ^{1}H-NMR spectra were obtained using deuterated solvents; unless otherwise indicated, tetramethylsilane (TMS) was used as internal reference for ^{1}H-NMR spectra and 85% phosphoric acid in deuterium oxide as an external reference for ^{31}P-NMR spectra. Proton-decoupled ^{13}C-NMR spectra were recorded in either $CDCl_3$ or D_2O. When indicated, the middle line of the resonances observed for $CDCl_3$ (77.00 ppm) was used as an internal or external reference. Chemical shifts δ are reported in parts per million (ppm). ^{1}H-NOESY (mixing time τ_m = 1 s) and ^{1}H-COSY experiments were performed at 300 MHz in D_2O.

Low-resolution FAB mass spectra were acquired from samples dissolved in a 3-nitrobenzyl alcohol matrix and bombarded with 8 keV fast xenon atoms. Accurate mass scans covering a spectral region that included the molecular species was performed at a resolution of *ca.* 10 000; a mass calibration standard consisting of cesium iodide:rubidium iodide:sodium iodide with molar ratios of 1:1:1 was added to the sample prior to analysis.

Modeling studies were performed in the CHARMm force field. The test sequence 5′-d(GCGTTN*TTGCG) where N* is **1a,b** or **2a,b** inserted according to defined internucleotidic motifs, served in the construction of B- or A-type helices for DNA−DNA or DNA−RNA duplexes, respectively. Sixteen preliminary structures were relaxed by initial minimization, and short simulations (10 ps) were performed in vacuum on constrained helices. Trajectories of lowest energy were selected and subjected to the Adopted Basis Newton−Raphson minimization method to achieve a 10^{-4} kcal/mol energy gradient. Stereoviews of the most energetically favored structures are shown in Figure 1.

2-Deoxy-5-*O*-trityl-D-*erythro*-pentofuranose (4). The synthesis of **4** was achieved by modification of a previous procedure.[6] Typically, 2-deoxy-D-ribose (**3**) (30.0 g, 0.22 mol) was dried by coevaporation with dry pyridine (3 × 30 mL) and then dissolved in the same anhydrous solvent (250 mL). Triphenylmethyl chloride (73.6 g, 26.4 mol) and a catalytic amount of 4-(dimethylamino)pyridine (200 mg) were added to the solution. After the solution was stirred at 50 °C for 6 h, methanol (10 mL) was added. The resulting dark orange solution was stirred for 30 min at room temperature prior to evaporation of the solvents under reduced pressure. To ensure the removal of any remaining pyridine, the gumlike material was coevaporated with ethanol−toluene (1:4) (2 × 50 mL). The residue was then dissolved in methylene chloride (600 mL) and washed with water (3 × 300 mL). The collected organic phases were dried over anhydrous sodium sulfate and evaporated to dryness under vacuum. The crude product **4** was purified by silica gel chromatography, first using methylene chloride and, then, an increasing gradient of methanol (1−4%) in methylene chloride as eluents. 2-Deoxy-5-*O*-trityl-D-*erythro*-pentofuranose was isolated as a colorless amorphous material in 60% yield (49.6 g, 0.13 mol). ^{1}H-NMR spectral data agreed with those reported by Kam and Oppenheimer.[6]

(2-Deoxy-5-*O*-trityl-α- and -β-D-*erythro*-pentofuranosyl)acetonitrile (5a and 5b). Diethyl (cyanomethyl)phosphonate (66.3 mL, 72.6 g, 0.41 mol) was added dropwise over 50 min to a stirred ice-cold suspension of sodium hydride (8.6 g, 0.36 mol) in dry THF (280 mL) under a stream of argon. After the evolution of hydrogen subsided, a solution of **4** (44.0 g, 0.12 mol) in dry THF (300 mL) was added dropwise over 30 min. The reaction mixture was stirred at 0 °C under an inert atmosphere, and reaction progress was monitored by TLC until **5a** and **5b** were formed in a nearly equimolar ratio (6 h). Volatiles were then removed by the use of a rotary evaporator. The residue was dissolved in ether, washed with water, dried over magnesium sulfate, and finally evaporated to dryness. The mixture of epimers (**5a** and **5b**) was separated by silica gel chromatography using a gradient of ethyl acetate in hexane. The epimer **5b** eluted from the silica gel column with 20% ethyl acetate in hexane, whereas elution of **5a** required 25−30% ethyl acetate in hexane. The glycoside **5a** (12.01 g, 30.1 mmol) was isolated as a white solid. ^{1}H- and ^{13}C-NMR data pertaining to **5a** have been reported (ref 4a). FAB-HRMS: calcd for $C_{26}H_{25}NO_3$ ($M^{\bullet+}$) 399.1834, found 399.1833. The glycosylated acetonitrile derivative **5b** (8.08 g, 20.2 mmol) was also isolated as a white solid. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 1.95 (ddd, J = 13.1, 9.2, 6.2 Hz, 1H), 2.08 (ddd, J = 13.1, 5.7, 2.6 Hz, 1H), 2.62 (dd, J = 16.7, 5.4 Hz, 1H), 2.66 (dd, J = 16.7, 5.5 Hz, 1H), 3.17 (dd, J = 9.9, 5.9 Hz, 1H), 3.30 (dd, J = 9.9, 4.7 Hz, 1H), 3.92 (ddd, J = 5.9, 4.7, 3.0 Hz, 1H), 4.38 (m, 1H), 4.40 (m, 1H), 7.25 (t, J = 7.8 Hz, 3H), 7.31 (t, J = 7.8 Hz, 6H), 7.44 (d, J = 7.8 Hz, 6H). ^{13}C-NMR (75 MHz, $CDCl_3$): δ 23.9, 40.0, 64.3, 73.4, 74.1, 86.3, 86.9, 116.9, 127.2, 127.9, 128.6, 143.7. FAB-HRMS: calcd for $C_{26}H_{25}NO_3$ ($M^{\bullet+}$) 399.1834, found 399.1835.

A mixture of both epimers (9.26 g, 23.2 mmol) was also isolated along with pure **5a** and **5b**. Thus, the overall yield for the preparation of **5a** and **5b** was 61%.

1-(2-Deoxy-5-*O*-trityl-α- and -β-D-*erythro*-pentofuranosyl)-2-aminoethane (6a and 6b). (2-Deoxy-5-*O*-trityl-α-D-*erythro*-pentofuranosyl)acetonitrile (**5a**, 9.5 g, 23.8 mmol) was dried by coevaporation with anhydrous toluene (3 × 20 mL) under reduced pressure. Dry **5a** was then dissolved in anhydrous THF (50 mL), and 100 mL of 2.0 M borane−methyl sulfide complex in THF (200 mmol) was added to the solution by use of a syringe under an inert atmosphere. The reaction

(26) Shaw, G.; Warrener, R. N. *J. Chem. Soc.* **1958**, 153−156.

mixture was heated to reflux for at least 40 min. Dimethyl sulfide that escaped condensation was trapped with a 5% aqueous solution of sodium hypochlorite. The solution was then cooled to 0 °C, and methanol was carefully added until the evolution of hydrogen had subsided. Volatiles were removed by evaporation under reduced pressure, and the residue was dissolved in dichloromethane (400 mL), which was extracted with 5% aqueous sodium bicarbonate saturated with sodium chloride (200 mL). The aqueous phase was back-extracted twice with ethyl ether (200 mL) to ensure complete extraction of the reaction product. The combined organic extracts were dried over anhydrous sodium sulfate and evaporated to dryness in vacuo. Crude **6a** (9.02 g, 22.4 mmol, 94%) was used in the next step without further purification. The β-epimer **6b** was prepared under similar conditions and yield. The aminoethylated glycoside derivatives **6a** and **6b** were characterized after conversion to their corresponding diacetates **7a** and **7b** by treatment with acetic anhydride and pyridine (1:1, v/v). **7a**: ^{1}H- and ^{13}C-NMR data have been reported (ref 4a). FAB-HRMS: calcd for $C_{30}H_{34}NO_5$ (MH^+) 488.2437, found 488.2438. **7b**: ^{1}H-NMR (300 MHz, $CDCl_3$), δ 1.71 (m, 2H), 1.89 (s, 3H), 1.92 (m, 1H), 2.05 (s, 3H), 2.10 (m, 1H), 3.18 (dd, J = 9.9, 4.8 Hz, 1H), 3.26 (dd, J = 9.9, 3.8 Hz, 1H), 3.30 (ddd, J = 10.5, 3.8, 3.4 Hz, 1H), 3.59 (m, 1H), 4.06 (ddd, J = 4.2, 4.0, 2.0 Hz, 1H), 4.18 (m, 1H), 5.17 (m, 1H), 6.30 (t, J = 5.2 Hz, 1H), 7.24 (t, J = 6.8 Hz, 3H), 7.30 (t, J = 7.7 Hz, 6H), 7.45 (d, J = 7.0 Hz, 6H). ^{13}C-NMR (75 MHz, $CDCl_3$): δ 21.1, 23.4, 34.0, 38.0, 38.9, 64.5, 76.4, 78.7, 84.1, 86.7, 127.1, 127.8, 128.7, 143.8, 170.0, 170.5. FAB-HRMS: calcd for $C_{30}H_{34}NO_5$ (MH^+) 488.2437, found 488.2440.

N-[(2-Deoxy-α- and *β*-D-*erythro*-pentofuranosyl)ethyl]-N′-(3-methoxy-2-methylacryloyl)urea (9a and 9b). The preparation of **9a** from **6a** and its characterization by ^{1}H- and ^{13}C-NMR spectroscopies have been reported (ref 4a). FAB-HRMS: calcd for $C_{13}H_{23}N_2O_6$ (MH^+) 303.1556, found 303.1552. The β-epimer **9b** was prepared (35%) under conditions similar to those used for the preparation of **9a**. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 1.67 (m, 1H), 1.75 (s, 3H), 1.80 (m, 1H), 1.93 (m, 1H), 1.98 (m, 1H), 3.30 (m, 1H), 3.63 (m, 2H), 3.76 (m, 1H), 3.85 (s, 3H), 3.95 (m, 1H), 4.15 (m, 1H), 4.26 (m, 1H), 7.35 (s, 1H), 8.3 (bs, 1H), 9.21 (dd, J = 6.5, 3.2 Hz, 1H). ^{13}C-NMR (75 MHz, $CDCl_3$): δ 8.7, 34.2, 38.0, 41.6, 61.5, 63.7, 72.4, 78.5, 87.9, 106.9, 153.8, 158.9, 169.5. FAB-HRMS: calcd for $C_{13}H_{23}N_2O_6$ (MH^+) 303.1556, found 303.1559.

1-(2-Deoxy-α- and *β*-D-*erythro*-pentofuranosyl)-2-(thymin-1-yl)ethane (1a and 1b). The acryloylurea derivative **9a** (1.1 g, 3.6 mmol) was dissolved in concentrated ammonium hydroxide (80 mL) and heated in a glass-lined stainless steel pressure vessel at 50 °C for 12 h. The solution was then evaporated to a gum under reduced pressure. The crude product was dissolved in a minimum amount of ethanol (5 mL), to which ethyl ether (150 mL) was added. The resulting suspension was cooled to 0 °C until complete precipitation of the nucleoside was obtained. The precipitate was filtered and dried under vacuum. The nucleoside analogue **1a** (0.9 g, 3.3 mmol) was isolated in 92% yield. UV (H_2O, pH 7): λ_{max} 272 nm (ϵ 8900), λ_{min} 238 nm (ϵ 1600). $[\alpha]^{25}$: +50.2 (c 1.0, CH_3OH). FAB-HRMS: calcd for $C_{12}H_{19}N_2O_5$ (MH^+) 271.1294, found 271.1293. The β-nucleoside **1b** was similarly prepared and isolated with a similar yield. UV (H_2O, pH 7): λ_{max} 272 nm (ϵ 8700), λ_{min} 238 nm (ϵ 2400). $[\alpha]^{25}$: −14.3 (c 1.8, CH_3OH). FAB-HRMS: calcd for $C_{12}H_{19}N_2O_5$ (MH^+) 271.1294, found 271.1294.

^{1}H- and ^{13}C-NMR peak assignments for **1a** and **1b** are listed in Tables 1 and 2.

2-Deoxy-3,5-di-*O*-*p*-toluoyl-α- and -*β*-D-*erythro*-pentofuranosyl cyanide (15a and 15b). The cyanoglycosides **15a** and **15b** were prepared by slight modification of a procedure published by Iyer *et al.*[18a] To a cold solution (0 °C) of 2-deoxy-3,5-di-*o*-toluoyl-D-*erythro*-pentofuranosyl chloride (**7**, 10.39 g, 26.2 mmol) in dry THF (35 mL) was added, dropwise, 40 mL of 1.0 M diethylaluminum cyanide in toluene (40.0 mmol) over a period of 30 min under an inert atmosphere. The solution was then allowed to stir at ambient temperature for 7 h. Excess diethylaluminum cyanide was destroyed upon slow addition of methanol (15 mL) at 0 °C in a well-ventilated fume hood (*Caution! Hydrogen cyanide is generated*). After removal of the solvents under reduced pressure, the residue was dissolved in dichloromethane (200 mL), and the solution filtered through silica gel (100 mL) covered with a layer of anhydrous sodium sulfate. The filtering pad was washed with dichloromethane (*ca.* 1 L). The filtrates were evaporated to dryness in vacuo to give an anomeric mixture of **15a** and **15b** (9.58 g). The mixture was loaded onto a column of silica gel and eluted with a gradient of ethyl acetate (3−14%) in hexane. The β-anomer **15b** (4.06 g, 10.7 mmol) emerged first and was recrystallized from ethanol. Mp: 109−110 °C (lit.[18b] mp 110−111 °C). FAB-HRMS: calcd for $C_{22}H_{22}NO_5$ (MH^+) 380.1498, found 380.1504. Further elution of the column with an increasing gradient of ethyl acetate (14−50%) afforded the α-anomer **15a** (4.66 g, 12.3 mmol) that was also recrystallized from ethanol. Mp: 143−144 °C (lit.[18b] mp 145−146 °C). FAB-HRMS: calcd for $C_{22}H_{22}NO_5$ (MH^+) 380.1498, found 380.1497. ^{1}H- and ^{13}C-NMR data for **15a** and **15b** are in agreement with reported values.[18a]

(2-Deoxy-3,5-di-*O*-*p*-toluoyl-α- and -*β*-D-*erythro*-pentofuranosyl)aminomethane (16a and 16b). The preparation of the hydrochloride salt of the aminomethylated α-glycoside **16a** from **15a**, and its characterization by ^{1}H- and ^{13}C-NMR spectroscopies have been described in detail (reference 4b). The aminomethylated β-glycoside **16b** was obtained from **15b** and isolated in a manner similar to that described for **16a**. ^{1}H-NMR (300 MHz, $CDCl_3$) (free amine): δ 1.73 (b, 2H), 2.13 (m, 2H), 2.40 (s, 3H), 2.41 (s, 3H), 2.80 (m, 1H), 3.01 (m, 1H), 4.30 (m, 1H), 4.39 (m, 1H), 4.51 (m, 2H), 5.49 (m, 1H), 7.22 (d, J = 8.0 Hz, 2H), 7.23 (d, J = 8.0 Hz, 2H), 7.86 (d, J = 8.0 Hz, 2H), 7.87 (d, J = 8.0 Hz, 2H). ^{13}C-NMR ($CDCl_3$): δ 21.6, 35.3, 45.4, 64.6, 76.6, 80.4, 82.5, 127.0, 127.1, 129.1, 129.4, 129.6, 130.0, 143.7, 143.9, 166.0, 166.2. Both **16a** and **16b** were sufficiently pure to be used as is.

***N*-[(2-Deoxy-3,5-di-*O*-*p*-toluoyl-α- and -*β*-D-*erythro*-pentofuranosyl)methyl]-*N*′-(3-methoxy-2-methylacryloyl)-urea (17a and 17b).** Under an inert atmosphere, dry silver cyanate (7.19 g, 48 mmol) was added to a solution of 3-methoxy-2-methylacryloyl chloride (2.96 g, 22 mmol) in dry benzene (31 mL). The suspension was stirred under reflux for 30 min and then allowed to cool to room temperature. The supernatant was cannulated into an addition funnel and added dropwise over a period of 30 min to a cold solution (0 °C) of **16b** (3.5 g, 8.4 mmol) and triethylamine (1.96 g, 2.7 mL, 19.4 mmol) in anhydrous benzene (50 mL). Upon completion of the addition, the stirred reaction mixture was allowed to warm to ambient temperature and further stirred overnight under an argon atmosphere. Volatile components were then removed by use of a rotary evaporator. The residue was dissolved in chloroform (200 mL) and extracted, successively, with aqueous 5% sodium bicarbonate (200 mL) and water (200 mL). The organic phase was dried over anhydrous sodium sulfate and evaporated to dryness under reduced pressure. The crude product was purified by silica gel chromatography using a gradient of methanol (0.5−1.5%) in chloroform as an eluent. Pure **17b** was isolated as a white foam in 80% yield (3.5 g, 6.7 mmol). ^{1}H-NMR (300 MHz, $CDCl_3$): δ 1.70 (s, 3H), 2.14 (m, J = 13.7, 10.5, 5.8 Hz, 1H), 2.25 (m, J = 13.6, 5.3 Hz, 1H), 2.40 (s, 3H), 2.42 (s, 3H), 3.61 (m, 2H), 3.83 (s, 3H), 4.45 (m, 1H), 4.52 (d, J = 4.0 Hz, 2H), 5.54 (m, 1H), 7.22 (d, J = 8.0 Hz, 2H), 7.26 (d, J = 8.0 Hz, 2H), 7.27 (m, 1H), 7.94 (d, J = 8.0 Hz, 4H), 8.28 (b, 1H), 9.07 (bt, 1H). ^{13}C-NMR (75 MHz, $CDCl_3$): δ 8.6, 21.6, 35.6, 42.4, 61.4, 64.8, 76.9, 77.8, 82.6, 106.8, 126.9, 127.0, 129.0, 129.1, 129.6, 143.5, 144.0, 154.6, 158.5, 165.9, 166.0, 169.0. FAB-HRMS: calcd for $C_{28}H_{33}N_2O_8$ (MH^+) 525.2237, found 525.2241. The α-anomer **17a** was prepared and isolated as described for **17b**. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 1.75 (s, 3H), 2.05 (m, J = 14.1, 5.1, 3.3 Hz, 1H), 2.41 (s, 6H), 2.65 (m, J = 14.1, 7.4 Hz, 1H), 3.59 (m, 2H), 3.83 (s, 3H), 4.48 (m, 2H), 4.56 (m, 1H), 5.51 (m, 1H), 7.22 (d, J = 7.9 Hz, 4H), 7.91 (d, J = 7.9 Hz, 2H), 7.94 (d, J = 7.9 Hz, 2H), 8.28 (b, 1H), 9.04 (bt, 1H). ^{13}C-NMR (75 MHz, $CDCl_3$): δ 8.7, 21.7, 35.2, 43.7, 61.4, 64.6, 76.4, 77.9, 81.9, 107.0, 126.8, 127.0, 129.1, 129.6, 143.7, 143.9, 154.5, 158.5, 166.1, 166.2, 169.1. FAB-HRMS: calcd for $C_{28}H_{33}N_2O_8$ (MH^+) 525.2237, found 525.2246.

(2-Deoxy-3,5-di-*O*-*p*-toluoyl-α- and *β*-D-*erythro*-pentofuranosyl)(thymin-1-yl) methane (18a and 18b). The preparation of **18a** from **17a** and its characterization by NMR spectroscopy and high-resolution mass spectrometry have been reported (ref 4b). The preparation of **18b** from **17b** was achieved under conditions similar to those reported for **18a**, and pure **18b** was isolated in a similar yield. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 1.75 (s, 3H), 1.98 (ddd, J = 13.9, 11.0, 6.0 Hz, 1H), 2.33 (dd, J = 13.9, 4.8 Hz, 1H), 2.42 (s, 6H), 3.55 (dd, J = 14.2, 7.0 Hz, 1H), 4.20 (dd, J = 14.2, 1.5 Hz, 1H), 4.40 (m, 1H), 4.50 (dd, J = 11.7, 4.5 Hz, 1H), 4.55 (m, 1H), 4.62 (dd, J = 11.7, 3.1 Hz, 1H), 5.52 (m, 1H), 7.10 (s, 1H), 7.25 (d, J = 7.9 Hz, 4H), 7.90 (d, J = 7.9 Hz, 4H), 8.85 (s, 1H). ^{13}C-NMR (75 MHz, $CDCl_3$): δ 12.1, 21.7, 35.6, 50.6, 64.3, 76.4, 77.6, 83.3, 110.2, 126.6, 126.8, 129.2, 129.3, 129.5, 129.7, 141.6, 144.3, 151.1, 163.9, 166.1, 166.2. FAB-HRMS: calcd for $C_{27}H_{29}N_2O_7$ (MH^+) 493.1975, found 493.1979.

(2-Deoxy-α- and -*β*-D-*erythro*-pentofuranosyl)thymin-1-yl)methane (2a and 2b). The saponification of **18a** and **18b** by potassium hydroxide in aqueous ethanol has been described in ref 4b. The characterization of **2a** and **2b** was also reported. ^{1}H- and ^{13}C-NMR peak assignments for **2a** and **2b** are listed in Tables 1 and 2.

1-[2-Deoxy-5-*O*-(4,4′-dimethoxytrityl)-α-D-*erythro*-pentofuranosyl]-2-(thymin-1-yl)ethane (19a). A general procedure for the preparation of [2-deoxy-5-*O*-(4,4′-dimethoxytrityl)-α- and -*β*-D-*erythro*-pentofuranosyl](thymin-1-yl)alkanes (**19a,b** and **20a,b**) is provided as Supporting Information. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 1.66 (ddd, J = 12.7, 7.4, 5.8 Hz, 1H), 1.83 (s, 3H), 1.99 (m, 2H), 2.37 (ddd, J = 12.7, 6.8, 6.4 Hz, 1H), 3.09 (dd, J = 9.5, 6.0 Hz, 1H), 3.24 (dd, J = 9.5, 4.7 Hz, 1H), 3.76 (m, 1H), 3.79 (s, 6H), 3.89 (ddd, J = 13.3, 8.0, 5.0 Hz, 1H), 3.99 (m, 1H), 4.08 (m, 1H), 4.32 (m, 1H), 6.83 (d, J = 8.9 Hz, 4H), 7.06 (s, 1H), 7.22 (t, J = 7.1 Hz, 1H), 7.25 (t, J = 7.1 Hz, 2H), 7.30 (d, J = 8.9 Hz, 4H), 7.42 (d, J = 7.1 Hz, 2H). ^{13}C-NMR (75 MHz, $CDCl_3$): δ 12.2, 35.2, 40.2, 46.1, 55.2, 64.4, 74.5, 75.3, 84.1, 86.3, 110.3, 113.1, 126.8, 127.9, 128.1, 130.0, 135.9, 141.1, 144.7, 150.8, 158.5, 164.2. FAB-HRMS: calcd for $C_{33}H_{36}N_2O_7$ ($M^{\bullet+}$) 572.2523, found 572.2521. **1-[2-Deoxy-5-*O*-(4,4′-dimethoxytrityl)-*β*-D-*erythro*-pentofuranosyl]-2-(thymin-1-yl)ethane (19b).** ^{1}H-NMR (300 MHz, $CDCl_3$): δ 1.77 (m, 2H), 1.87 (s, 3H), 2.02 (m, 2H), 3.10 (dd, J = 9.7, 5.5 Hz, 1H), 3.22 (dd, J = 9.7, 4.5 Hz, 1H), 3.72 (ddd, J = 14.4, 7.7, 7.2 Hz, 1H), 3.79 (s, 6H), 3.91 (m, 1H), 3.97 (m, 1H), 4.13 (dddd, J = 8.9, 8.7, 4.8, 4.5 Hz, 1H), 4.31 (m, 1H), 6.83 (d, J = 8.9 Hz, 4H), 7.03 (s, 1H), 7.22 (t, J = 7.8 Hz, 1H), 7.26 (t, J = 7.8 Hz, 2H), 7.32 (d, J = 8.9 Hz, 4H), 7.43 (d, J = 7.8 Hz, 2H). ^{13}C-NMR (75 MHz, $CDCl_3$): δ 12.2, 34.7, 40.8, 46.2, 55.2, 64.6, 74.4, 75.0, 85.8, 86.2, 110.5, 113.1, 126.9, 127.8, 128.1, 130.1, 136.0, 141.0, 144.8, 150.9, 158.5, 164.2. FAB-HRMS: calcd for $C_{33}H_{36}N_2O_7$ ($M^{\bullet+}$) 572.2523, found 572.2529. **[2-Deoxy-5-*O*-(4,4′-dimethoxytrityl)-α-D-*erythro*-pentofuranosyl]-(thymin-1-yl)methane (20a).** ^{1}H-NMR (500 MHz, $CDCl_3$): δ 1.74 (ddd, J = 13.5, 5.4, 4.3 Hz, 1H), 1.92 (d, J = 1.1 Hz, 3H), 2.38 (ddd, J = 13.5, 7.7, 6.5 Hz, 1H), 3.09 (dd, J = 9.8, 6.0 Hz, 1H), 3.20 (dd, J = 9.8, 4.5 Hz, 1H), 3.73 (dd, J = 14.1, 7.9 Hz, 1H), 3.79 (s, 6H), 4.02 (m, J = 3.3 Hz, 1H), 4.04 (dd, J = 14.1, 3.1 Hz, 1H), 4.35 (m, J = 6.5, 4.1, 3.3 Hz, 1H), 4.40 (dddd, J = 8.3, 7.9, 5.6, 3.2 Hz, 1H), 6.82 (d, J = 8.9 Hz, 4H), 7.17 (q, J = 1.1 Hz, 1H), 7.22 (t, J = 7.3 Hz, 1H), 7.28 (t, J = 7.3 Hz, 2H), 7.29 (d, J = 8.9 Hz, 4H), 7.39 (d, J = 7.3 Hz, 2H), 8.18 (bs, 1H). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 12.3, 37.4, 52.1, 55.2, 64.3, 74.1, 76.9, 85.0, 110.0, 113.1, 126.8, 127.8, 128.0, 129.9, 135.7, 141.8, 144.6, 150.9, 158.5, 163.8. FAB-HRMS: calcd for $C_{32}H_{34}N_2O_7$ ($M^{\bullet+}$) 558.2366, found 558.2380. **[2-Deoxy-5-*O*-(4,4′-dimethoxytrityl)-*β*-D-*erythro*-pentofuranosyl](thymin-1-yl)methane (20b).** ^{1}H-NMR (500 MHz, $CDCl_3$): δ 1.75 (d, J = 1.1 Hz, 3H), 1.82 (ddd, J = 13.3, 9.7, 6.1 Hz, 1H), 2.00 (ddd, J = 13.3, 5.9, 2.5 Hz, 1H), 3.06 (dd, J = 9.9, 5.6 Hz, 1H), 3.25 (dd, J = 9.9, 4.5 Hz, 1H), 3.58 (dd, J = 14.3, 6.8 Hz, 1H), 3.80 (s, 6H), 3.96 (m, J = 5.6, 4.5, 2.9 Hz, 1H), 4.08 (dd, J = 14.3, 2.5 Hz, 1H), 4.32 (m, J = 5.5, 2.5 Hz, 1H), 4.41 (dddd, J = 8.9, 6.8, 6.5, 2.5 Hz, 1H), 6.83 (d, J = 8.9 Hz, 4H), 7.07 (q, J = 1.2 Hz, 1H), 7.23 (t, J = 7.3 Hz, 1H), 7.29 (t, J = 7.3 Hz, 2H), 7.32 (d, J = 8.9 Hz, 4H), 7.41 (d, J = 7.3 Hz, 2H), 8.07 (bs, 1H). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 12.1, 37.6, 51.0, 55.2, 64.0, 73.8, 76.7, 86.0, 111.2, 113.1, 126.9, 127.9, 128.0, 129.9, 135.7, 141.6, 144.5, 150.8, 158.5, 164.0. FAB-HRMS: calcd for $C_{32}H_{34}N_2O_7$ ($M^{\bullet+}$) 558.2366, found 558.2369.

1-[2-Deoxy-5-*O*-(*tert*-butyldimethylsilyl)-α-D-*erythro*-pentofuranosyl]-2-(thymin-1-yl)ethane (21a). A general procedure for the preparation of [2-deoxy-5-*O*-(*tert*-butyldimethylsilyl)-α- and -*β*-D-*erythro*-pentofuranosyl](thymin-1-yl)-alkanes (**21a,b** and **22a,b**) is available as Supporting Information. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 0.26 (s, 6H), 0.88 (s, 9H), 1.63 (ddd, J = 12.7, 6.2, 4.8 Hz, 1H), 1.84 (s, 3H), 1.92 (m, 2H), 2.34 (ddd, J = 12.7, 7.0, 6.4 Hz, 1H), 3.52 (dd, J = 10.6, 5.7 Hz, 1H), 3.67 (dd, J = 10.6, 3.8 Hz, 1H), 3.70 (m, 1H), 3.82 (m, 1H), 3.86 (m, 1H), 4.02 (m, 1H), 4.29 (m, 1H), 7.05 (s, 1H). ^{13}C-NMR (75 MHz, $CDCl_3$): δ −5.5, 12.2, 18.2, 25.8, 35.2, 40.3, 46.2, 64.3, 74.2, 75.5, 85.2, 110.3, 141.2, 151.0, 164.5. FAB-HRMS: calcd for $C_{18}H_{33}N_2O_5Si$ (MH^+) 385.2159, found 385.2152. **1-[2-Deoxy-5-*O*-(*tert*-butyldimethylsilyl)-*β*-D-*erythro*-pentofuranosyl]-2-(thymin-1-yl)ethane (21b).** ^{1}H-NMR (300 MHz, $CDCl_3$): δ 0.26 (s, 6H), 0.88 (s, 9H), 1.70 (m, 2H), 1.85 (s, 3H), 1.95 (m, 2H), 3.47 (dd, J = 10.7, 5.6 Hz, 1H), 3.65 (dd, J = 10.7, 4.0 Hz, 1H), 3.70 (m, 1H), 3.76 (ddd, J = 5.6, 4.0, 3.0 Hz, 1H), 3.85 (m, J = 13.8, 6.5 Hz, 1H), 4.07 (m, 1H), 4.26 (m, 1H), 7.0 (s, 1H). ^{13}C-NMR (75 MHz, $CDCl_3$): δ −5.5, 12.3, 18.3, 25.9, 34.7, 40.9, 46.1, 64.2, 74.2, 75.2, 87.0, 110.5, 140.9, 151.4, 164.7. FAB-HRMS: calcd for $C_{18}H_{33}N_2O_5Si$ (MH^+) 385.2159, found 385.2152. **[2-Deoxy-5-*O*-(*tert*-butyldimethylsilyl)-α-D-*erythro*-pentofuranosyl](thymin-1-yl)methane (22a).** ^{1}H-NMR (500 MHz, $CDCl_3$): δ 0.26 (s, 6H), 0.88 (s, 9H), 1.75 (m, J = 13.5, 4.5 Hz, 1H), 1.91 (s, 3H), 2.39 (m, J = 13.5, 6.9 Hz, 1H), 3.54 (dd, J = 10.5, 5.8 Hz, 1H), 3.70 (dd, J = 10.5, 3.9 Hz, 1H), 3.73 (dd, J = 14.3, 7.9 Hz, 1H), 3.93 (m, 1H), 4.01 (dd, J = 14.3, 2.7 Hz, 1H), 4.38 (m, 2H), 7.16 (m, 1H), 8.55 (bs, 1H). ^{13}C-NMR (125 MHz, $CDCl_3$): δ −5.5, 12.3, 25.8, 37.4, 52.0, 64.1, 73.9, 77.1, 86.1, 110.0, 141.8, 151.1, 164.1. FAB-HRMS: calcd for $C_{17}H_{31}N_2O_5Si$ (MH^+) 371.2002, found 371.2004. **[2-Deoxy-5-*O*-(*tert*-butyldimethylsilyl)-*β*-D-*erythro*-pentofuranosyl](thymin-1-yl)methane (22b).** ^{1}H-NMR (500 MHz, $CDCl_3$): δ 0.10 (s, 6H), 0.90 (s, 9H), 1.80 (m, 1H), 1.90 (s, 3H), 2.00 (m, 1H), 3.52 (dd, J = 14.3, 7.3 Hz, 1H), 3.54 (dd, J = 10.3, 5.4 Hz, 1H), 3.70 (dd, J = 10.5, 3.6 Hz, 1H), 3.88 (m, 1H), 4.13 (d, 14.3 Hz, 1H), 4.32 (m, 1H), 4.41 (m, 1H), 7.17 (m, 1H), 9.03 (bs, 1H). ^{13}C-NMR (125 MHz, $CDCl_3$): δ −5.4, 12.3, 25.9, 37.9, 51.3, 63.8, 73.5, 76.7, 87.2, 109.9, 141.8, 151.1, 164.2. FAB-HRMS: calcd for $C_{17}H_{31}N_2O_5Si$ (MH^+) 371.2002, found 371.2006.

1-[2-Deoxy-3-*O*-(4,4′-dimethoxytrityl)-α-D-*erythro*-pentofuranosyl]-2-(thymin-1-yl)ethane (25a). A general procedure for the preparation of [2-deoxy-3-*O*-(4,4′-dimethoxytrityl)-α- and -*β*-D-*erythro*-pentofuranosyl](thymin-1-yl)alkanes (**25a,b** and **26a,b**) is provided as Supporting Information. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 1.26 (ddd, J = 13.5, 5.7, 4.3 Hz, 1H), 1.68 (ddd, J = 13.5, 6.8, 6.5 Hz, 1H), 1.83 (m, 1H), 1.87 (s, 3H), 2.05 (m, 1H), 3.14 (dd, J = 11.7, 6.4 Hz, 1H), 3.30 (dd, J = 11.7, 2.5 Hz, 1H), 3.73 (m, 1H), 3.77 (s, 6H), 3.88 (m, 1H), 3.98 (m, 1H), 4.05 (m, 1H), 6.83 (d, J = 8.8 Hz, 4H), 7.05 (s, 1H), 7.22 (t, J = 7.6 Hz, 1H), 7.27 (t, J = 7.6 Hz, 2H), 7.32 (d, J = 8.8 Hz, 4H), 7.43 (d, J = 7.6 Hz, 2H). ^{13}C-NMR (75 MHz, $CDCl_3$): δ 12.2, 34.4, 39.8, 46.1, 55.2, 62.4, 75.4, 75.5, 85.4, 87.0, 110.5, 113.2, 126.9, 127.9, 128.3, 130.2, 136.5, 136.6, 140.9, 145.4, 151.1, 158.6, 164.3. FAB-HRMS: calcd for $C_{33}H_{36}N_2O_7$ ($M^{\bullet+}$) 572.2523, found 572.2525. **1-[2-Deoxy-3-*O*-(4,4′-dimethoxytrityl)-*β*-D-*erythro*-pentofuranosyl]-2-(thymin-1-yl)ethane (25b).** ^{1}H-NMR (300 MHz, $CDCl_3$): δ 1.17 (ddd, J = 13.1, 10.7, 6.1 Hz, 1H), 1.33 (ddd, J = 13.1, 4.9, 1.0 Hz, 1H), 1.65 (m, 2H), 1.90 (s, 3H), 3.15 (dd, J = 11.7, 4.7 Hz, 1H), 3.47 (dd, J = 11.8, 2.5 Hz, 1H), 3.72 (ddd, J = 14.5, 7.6, 7.2 Hz, 1H), 3.77 (s, 6H), 3.83 (ddd, J = 14.5, 8.4, 5.3 Hz, 1H), 3.90 (ddd, J = 4.5, 2.9, 2.2 Hz, 1H), 4.11 (m, 2H), 6.82 (d, J = 8.6 Hz, 4H), 7.03 (s, 1H), 7.22 (t, J = 7.4 Hz, 1H), 7.27 (t, J = 7.4 Hz, 2H), 7.32 (d, J = 8.6 Hz, 4H), 7.43 (d, J = 7.4 Hz, 2H). ^{13}C-NMR (75 MHz, $CDCl_3$): δ 12.3, 34.0, 40.7, 46.2, 55.2, 63.0, 75.6, 75.9, 86.6, 86.8, 110.5, 113.2, 126.9, 127.8, 128.3, 130.2, 136.5, 136.6, 140.8, 145.3, 150.8, 158.6, 164.1. FAB-HRMS: calcd for $C_{33}H_{36}N_2O_7$ ($M^{\bullet+}$) 572.2523, found 572.2528. **[2-Deoxy-3-*O*-(4,4′-dimethoxytrityl)-α-D-*erythro*-pentofuranosyl](thymin-1-yl)methane (26a).** ^{1}H-NMR (500 MHz,

$CDCl_3$): δ 1.23 (ddd, J = 13.9, 3.6, 2.4 Hz, 1H), 1.78 (ddd, J = 13.9, 7.6, 6.6 Hz, 1H), 1.91 (d, J = 1.0 Hz, 3H), 3.18 (dd, J = 11.8, 5.6 Hz, 1H), 3.39 (dd, J = 11.8, 1.5 Hz, 1H), 3.79 (dd, J = 14.0, 9.0 Hz, 1H), 3.80 (s, 6H), 3.94 (dd, J = 14.0, 3.1 Hz, 1H), 4.04 (m, J = 5.6, 2.9, 1.5 Hz, 1H), 4.14 (m, J = 6.6, 2.9, 2.4 Hz, 1H), 4.25 (dddd, J = 9.0, 7.6, 3.6, 3.1 Hz, 1H), 6.85 (d, J = 8.9 Hz, 4H), 7.10 (q, J = 1.0 Hz, 1H), 7.23 (t, J = 7.2 Hz, 1H), 7.31 (t, J = 7.2 Hz, 2H), 7.34 (d, J = 8.9 Hz, 4H), 7.44 (d, J = 7.2 Hz, 2H), 8.17 (bs, 1H). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 12.4, 37.0, 52.2, 55.3, 62.8, 75.4, 77.2, 86.0, 109.9, 113.3, 127.1, 128.0, 128.1, 130.1, 136.2, 136.3, 141.7, 145.1, 150.9, 158.7, 164.1. FAB-HRMS: calcd for $C_{32}H_{34}N_2O_7$ ($M^{\bullet+}$) 558.2366, found 558.2368. **[2-Deoxy-3-*O*-(4,4′-dimethoxytrityl)-*β*-D-*erythro*-pentofuranosyl](thymin-1-yl)methane (26b).** ^{1}H-NMR (500 MHz, $CDCl_3$): δ 1.31 (ddd, J = 13.2, 10.0, 6.1 Hz, 1H), 1.55 (ddd, J = 13.2, 5.5, 1.4 Hz, 1H), 1.90 (d, J = 1.2 Hz, 3H), 3.14 (dd, J = 11.9, 4.4 Hz, 1H), 3.42 (dd, J = 11.9, 3.0 Hz, 1H), 3.63 (dd, J = 14.3, 8.3 Hz, 1H), 3.78 (dd, J = 14.3, 2.7 Hz, 1H), 3.79 (s, 6H), 3.82 (m, 1H), 4.17 (m, 1H), 4.42 (dddd, J = 10.0, 8.3, 5.5, 2.7 Hz, 1H), 6.83 (d, J = 8.9 Hz, 4H), 7.09 (q, J = 1.2 Hz, 1H), 7.22 (t, J = 7.2 Hz, 1H), 7.29 (t, J = 7.2 Hz, 2H), 7.32 (d, J = 8.9 Hz, 4H), 7.43 (d, J = 7.2 Hz, 2H), 8.10 (bs, 1H). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 12.3, 37.3, 52.0, 55.2, 64.3, 74.1, 76.9, 85.1, 110.0, 113.1, 126.9, 127.8, 128.0, 130.0, 135.7, 141.8, 144.6, 150.9, 158.5, 163.9. FAB-HRMS: calcd for $C_{32}H_{35}N_2O_7$ (MH^+) 559.2444, found 559.2446.

General Procedure for the Preparation of the α- and *β*-Deoxyribonucleoside Phosphoramidites 27a,b−30a,b. The preparation of 1-[[2-deoxy-3-*O*-[(*N*,*N*-diisopropylamino)-(2-cyanoethoxy)phosphinyl]-5-*O*-(4,4′-dimethoxytrityl)-*β*-D-*erythro*-pentofuranosyl]-2-(thymin-1-yl)ethane (**27b**) is representative. Dry **19b** (750 mg, 1.31 mmol) and 140 mg of the *N*,*N*-diisopropylammonium salt of 1*H*-tetrazole (0.82 mmol) were dissolved in anhydrous dichloromethane (14 mL) under an inert atmosphere. To the cloudy solution was added 800 mL of 2-cyanoethyl *N*,*N*,*N*′,*N*′-tetraisopropylphosphordiamidite (759 mg, 2.52 mmol) by use of a syringe. The reaction mixture was stirred at ambient temperature for 4 h and then quenched by the addition of a solution of triethylamine (2% v/v) in dichloromethane (80 mL) and brine (80 mL). The two layers were separated, and the organic phase was washed once with brine (50 mL). The organic layer was dried ($MgSO_4$) and evaporated to dryness in vacuo. The crude product was purified by silica gel chromatography; the *β*-deoxyribonucleoside phosphoramidite **27b** was eluted from the column with benzene:triethylamine (90:10) and was isolated as a white foam. Purified **27b** was triturated with hexane, and the resulting suspension was cooled to −20 °C for 2 h. The solid was filtered, washed with cold hexane, and dried to give 840 mg of **27b** as a white powder (1.09 mmol, 83%). FAB-HRMS: calcd for $C_{42}H_{53}N_4O_8P$ ($M^{\bullet+}$) 772.3601, found 772.3600. ^{31}P-NMR (202 MHz, $CDCl_3$): δ 147.2 and 146.9 ppm.[27] The phosphoramidites **27a** and **28a,b**−**30a,b** were similarly prepared and isolated in 80−90% yield. A mixture of dichloromethane:ethyl acetate:triethylamine (65:25:10) was, however, used as eluent for the purification of **28a,b** and **30a,b**. **1-[[2-Deoxy-3-*O*-[(*N*,*N*-diisopropylamino)(2-cyanoethoxy)-phosphinyl]-5-*O*-(4,4′-dimethoxytrityl)-α-D-*erythro*-pentofuranosyl]-2-(thymin-1-yl)ethane (27a).** FAB-HRMS: calcd for $C_{42}H_{53}N_4O_8P$ ($M^{\bullet+}$) 772.3601, found 772.3601. ^{31}P-NMR (202 MHz, $CDCl_3$): δ 147.4 and 147.1 ppm. **[2-Deoxy-3-*O*-[(*N*,*N*-diisopropylamino)(2-cyanoethoxy)phosphinyl]-5-*O*-(4,4′-dimethoxytrityl)-α-D-*erythro*-pentofuranosyl]-(thymin-1-yl)methane (28a).** FAB-HRMS: calcd for $C_{41}H_{52}N_4O_8P$ (MH^+) 759.3523, found 759.3530. ^{31}P-NMR (202 MHz, $CDCl_3$): δ 146.9 and 146.7 ppm. **[2-Deoxy-3-*O*-[(*N*,*N*-diisopropylamino)(2-cyanoethoxy)phosphinyl]-5-*O*-(4,4′-dimethoxytrityl)-*β*-D-*erythro*-pentofuranosyl](thymin-1-yl)methane (28b).** FAB-HRMS: calcd for $C_{41}H_{52}N_4O_8P$ (MH^+) 759.3523, found 759.3528. ^{31}P-NMR (202 MHz, $CDCl_3$): δ 146.7 and 146.4 ppm. **1-[[2-Deoxy-3-*O*-(4,4′-dimethoxytrityl)-5-*O*-[(*N*,*N*-diisopropylamino)(2-cyanoethoxy)phosphinyl]-α-D-*erythro*-pentofuranosyl]-2-(thymin-1-yl)ethane (29a).** FAB-HRMS: calcd for $C_{42}H_{53}N_4O_8P$ ($M^{\bullet+}$) 772.3601, found 772.3600. ^{31}P-NMR (202 MHz, $CDCl_3$): δ 147.9 and 147.6 ppm. **1-[[2-Deoxy-3-*O*-(4,4′-dimethoxytrityl)-5-*O*-[(*N*,*N*-diisopropylamino)(2-cyanoethoxy)phosphinyl]-*β*-D-*erythro*-pentofuranosyl]-2-(thymin-1-yl)ethane (29b).** FAB-HRMS: calcd for $C_{42}H_{53}N_4O_8P$ ($M^{\bullet+}$) 772.3601, found 772.3601. ^{31}P-NMR (202 MHz, $CDCl_3$): δ 147.6 and 147.4 ppm. **[2-Deoxy-3-*O*-(4,4′-dimethoxytrityl)-5-*O*-[(*N*,*N*-diisopropylamino)(2-cyanoethoxy)phosphinyl]-α-D-*erythro*-pentofuranosyl](thymin-1-yl)methane (30a).** FAB-HRMS: calcd for $C_{41}H_{52}N_4O_8P$ (MH^+) 759.3523, found 759.3524. ^{31}P-NMR (202 MHz, $CDCl_3$): δ 147.2 and 147.1 ppm. **[2-Deoxy-3-*O*-(4,4′-dimethoxytrityl)-5-*O*-[(*N*,*N*-diisopropylamino)-(2-cyanoethoxy)phosphinyl]-*β*-D-*erythro*-pentofuranosyl](thymin-1-yl)methane (30b).** FAB-HRMS: calcd for $C_{41}H_{52}N_4O_8P$ (MH^+) 759.3523, found 759.3527. ^{31}P-NMR (202 MHz, $CDCl_3$): δ 146.9 and 146.6 ppm.

Preparation of Oligonucleotides. Standard *β*-cyanoethyl deoxyribonucleoside phosphoramidites and all the reagents required for solid-phase oligodeoxyribonucleotide syntheses were purchased from Applied Biosystems Inc. and used as recommended by the manufacturer. The incorporation of the modified phosphoramidites **27a,b**−**30a,b** into oligonucleotide **31** according to Table 3 was performed under conditions identical to those used for standard phosphoramidites.

Purification of Oligonucleotides. Purification of the oligoribonucleotide 5′-r(UCCAUUUUCAGAAUUGGGUGUC-GA) (Cruachem), along with the modified and unmodified oligodeoxyribonucleotides listed in Table 3, was accomplished by polyacrylamide gel electrophoresis according to literature protocols.[24a,28]

Thermal Denaturation Profiles. Each DNA oligonucleotide was mixed with either a complementary unmodified DNA or RNA oligomer in PBS buffer (pH 7.2); the total strand concentration was kept at *ca.* 5 μM. Thermal denaturation profiles (absorbance *vs* temperature) of the resulting DNA−DNA and DNA−RNA hybrids were measured at 260 nm with a diode array UV/vis spectrophotometer equipped with a Peltier temperature controller that was interfaced to a personal computer. For the temperature range 20−80 °C, 121 measurement points were taken every 0.5 °C, with an equilibration time of 30 s for each measurement point. A heating and a cooling profile were recorded for each complex. The melting temperature (T_m) was determined from the first derivative of each of the heating and cooling curves and reported in Table 3 as the average of these two temperatures. The reproducibility of these measurements was within ±1 °C.

Acknowledgment. This research was supported in part by an appointment to the Postgraduate Research Participation Program at the Center for Biologics Evaluation and Research administered by the Oak Ridge Institute for Science and Education through an interagency agreement between the U.S. Department of Energy and the U.S. Food and Drug Administration.

Supporting Information Available: General procedures for the preparation of **19a,b**−**22a,b**, **25a,b**, and **26a,b** and NMR spectra of new compounds (84 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

JO961548K

(27) ^{31}P-NMR signals reported for **27a**,**b**−**30a**,**b** are downfield relative to that of the external reference (85% phosphoric acid in D_2O).

(28) Damha, M. J.; Ogilvie, K. K. In *Methods in Molecular Biology, Vol 20: Protocols for Oligonucleotides and Analogs;* Agrawal, S., Ed.; Humana Press: Totowa, 1993; pp 81−114.

(29) (a) Ohrui, H.; Jones, G. H.; Moffatt, J. G.; Maddox, M. L.; Christensen, A. T.; Byram, S. K. *J. Am. Chem. Soc.* **1975**, *97*, 4602−4613. (b) De Bernardo, S.; Weigele, M. *J. Org. Chem.* **1977**, *42*, 109−112.