Synthesis and Physicochemical Properties of Alternating α,β-Oligodeoxyribonucleotides with Alternating (3′→3′)- and (5′→5′)-Internucleotidic Phosphodiester Linkages

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A simple and straightforward synthesis of α-2′-deoxycytidine and α-2′-deoxyadenosine derivatives **6a,b** has been achieved from commercial N^4-benzoyl-β-2′-deoxycytidine and N^6-benzoyl-β-2′-deoxyadenosine, respectively. Properly protected α-2′-deoxyribonucleosides **8a-d** were converted to the corresponding 5′-phosphoramidite derivatives **9a-d**. These and commercial β-2′-deoxyribonucleoside 3′-phosphoramidites were readily incorporated into alternating α,β-oligodeoxyribonucleotides with alternating (3′→3′)- and (5′→5′)-internucleotidic phosphodiester linkages by standard solid-phase synthesis methods. The α,β-oligodeoxyribonucleotide **12** (Scheme 3) was considerably more resistant to hydrolysis catalyzed by snake venom phosphodiesterase, calf spleen phosphodiesterase, and S1 nuclease than the parent unmodified oligonucleotide **17** (Table 2). Thermal stability of the complex composed of **12** and complementary unmodified β-oligomer **18** was comparable to that measured for the hybrid composed of the β-oligodeoxyribonucleoside phosphorothioate **20** and **18** but less than that of the native DNA duplex **17:18** (Table 3). The differences in thermal stability (ΔT_m) and free energy of dissociation ($\Delta\Delta G°_{37}$) observed between the duplex **12:18** and the singly mismatched complex **12:19** (9 °C and −2.5 kcal/mol, respectively) (Tables 3 and 4) suggest that the sequence specificity of **12** toward a complementary unmodified β-DNA oligomer is comparable to that of **17**. In addition, the CD spectrum of **12:18** at 22 °C resembles more closely that of the natural DNA duplex **17:18** than that of the α,β-DNA duplex **12:13** (Figure 3). These findings indicate that the duplex **12:18** exhibits, at least to some extent, a B-type helicity. The α,β-oligodeoxyribonucleotide **12** formed also a complex with the complementary β-oligoribonucleotide **23** but with much reduced affinity (T_m = 35 °C) than that measured with the complementary DNA sequence **18** (T_m = 54 °C). CD spectroscopy indicated that the complex **12:23** adopted a conformation similar to that observed for duplex **17:23** at 22 °C. Unlike the DNA–RNA heteroduplex **17:23**, the complex **12:23** was not a substrate for *E. coli* RNase H.

Antisense oligonucleotides have been applied extensively to the regulation of gene expression in cell cultures.[4] For example, inhibition of gene expression may result from the binding of an oligonucleotide complementary to a specific messenger RNA target. The specificity of such binding is ruled by Watson–Crick base-pairing interactions and, thus, provides an attractive route for the development of selective drugs. In addition to specificity, another critical factor that affects the biological activity of antisense oligonucleotides is the resistance of these biomolecules to various nucleases encountered both in cell culture media and inside cells.

In an effort to develop a novel class of antisense oligodeoxyribonucleotides, achiral at phosphorus, that would display affinity to complementary DNA and RNA sequences and resistance to the nucleolytic activity of nucleases, alternating α,β-oligothymidylates with alternating (3′→3′)- and (5′→5′)-phosphodiester linkages have been proposed as models.[5] It was originally hypothesized that the alternate substitution of a β-monodeoxyribonucleotide for an α-monodeoxyribonucleotide, through the formation of a (3′→3′)-/(5′→5′)-internucleotidic linkage motif, should impart base-pairing properties to these oligonucleotide analogues that would be similar to those of natural oligodeoxyribonucleotides. Furthermore, nucleases might not recognize and hydrolyze unnatural (3′→3′)- and (5′→5′)-phosphodiester linkages as rapidly as the (3′→5′)-phosphodiester functions of unmodified oligonucleotides.

α,β-Oligothymidylates with alternating (3′→3′)- and (5′→5′)-phosphodiester linkages (α,β-dT_{28}) were efficiently prepared by the phosphoramidite approach[6,7] and led to the formation of hybrids with either polydeoxyriboadenylic acid (β-dA_{28}) or polyriboadenylic acid (poly rA).[5] Although these hybrids were, thermally, as stable as those obtained with the "perphosphorothioated"[8] β-oligothymidylate S-dT_{28} under defined conditions, α,β-dT_{28}

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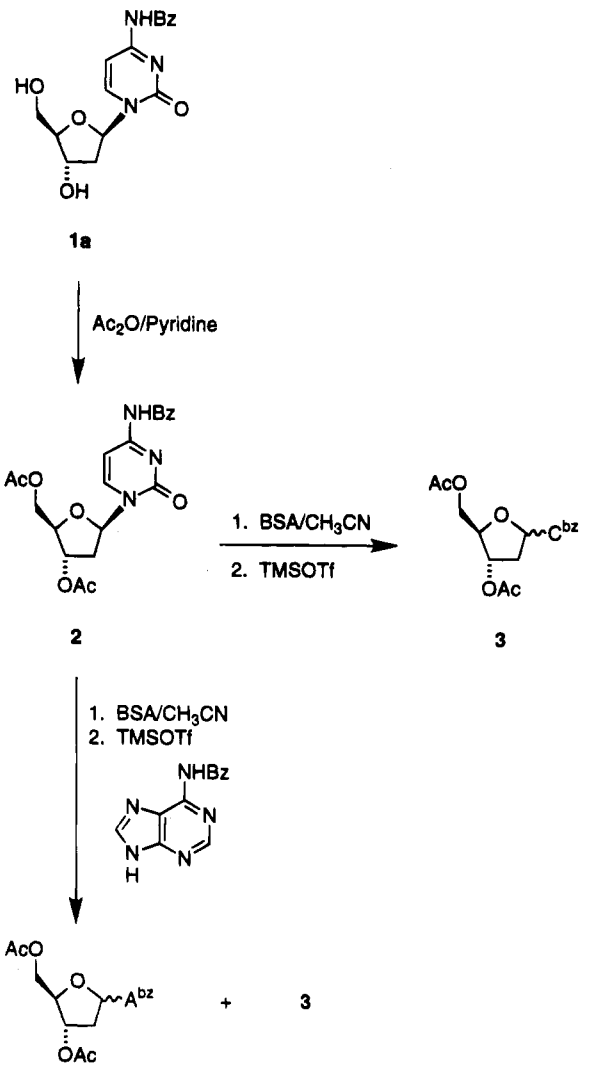
were not as resistant as *S*-dT$_{28}$ to exonucleases, such as snake venom and calf spleen phosphodiesterases. However, α,β-dT$_{28}$ were considerably more resistant than *S*-dT$_{28}$ to the endonucleolytic activity of S1 nuclease.[5]

We now report a shorter synthesis of α-2′-deoxycytidine and α-2′-deoxyadenosine derivatives and the incorporation of these nucleosides into alternating α,β-oligodeoxyribonucleotides with alternating (3′→3′)-/(5′→5′)-internucleotidic linkages to evaluate further the potential of these oligonucleotide analogues as antisense molecules.[9] Oligonucleotides (24-mers) complementary to a sequence (nt. 5347−5370 according to Ratner *et al.*[10]) that includes the splice acceptor site of the second exon encoding the HIV-1 *tat* gene product[11] have been prepared. The physicochemical properties of these oligonucleotide analogues and their resistance to nucleases will also be reported.

Results and Discussion

Over the years, a number of procedures for the synthesis of α-nucleoside derivatives have been developed.[12] The method reported by Yamaguchi and Saneyoshi[12c] (Scheme 1) has attracted our attention because of its potential simplicity. Specifically, the synthetic approach consisted of the acetylation of β-(*N*^{4}-benzoyl)-2′-deoxycytidine (**1a**) to the fully protected deoxyribonucleoside **2** which, upon treatment with *N,O*-bis(trimethylsilyl)acetamide (BSA) and trimethylsilyl triflate (TMSOTf), produced either **3**, as a mixture of α- and β-anomers, or both α- and β-(*N*^{6}-benzoy)-3′-di-*O*-acetyl-2′-deoxyadenosine in the presence of *N*^{6}-benzoyladenine.[12c]

Scheme 1[a]



[a] Key: Bz, benzoyl; Ac, acetyl; BSA, *N,O*-bis(trimethylsilyl)-acetamide; TMSOTf, trimethylsilyl trifluoromethanesulfonate; C^{bz}, *N*^{4}-benzoylcytosin-1-yl; A^{bz}, *N*^{6}-benzoyladenin-9-yl.

In order to reduce the number of synthetic steps involved in this approach, it was rationalized that the acetylation of **1a** was unnecesssary because free hydroxyl groups would be protected by silylation with BSA prior to the anomerization reaction. This rationale was tested by the reaction of **1a** with 3 molar equiv of BSA in acetonitrile and the subsequent addition of 2.6 molar equiv of TMSOTf (Scheme 2). Thin layer chromatography indicated that optimum formation of the α-anomer was achieved within 24 h at ambient temperature. It was, then, necessary to neutralize triflic acid and unreacted TMSOTf with a methanolic suspension of sodium bicarbonate to avoid cleavage of the glycosidic linkage during workup. Regioselective silylation of the anomeric nucleosides, at the 5′-hydroxy function, by incremental addition of *tert*-butyldimethylchlorosilane and imidazole in *N,N*-dimethylformamide led, after silica gel chromatography, to the isolation of the 5′-silylated α-anomer **6a** in 46% yield based on **1a**.

The preparation of anomeric 2′-deoxyadenosine derivatives (**4**) from **2** according to the procedure of Yamaguchi

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(8) The term "perphosphorothioated" means that all phosphodiester functions of a given oligonucleotide are phosphorothioates.

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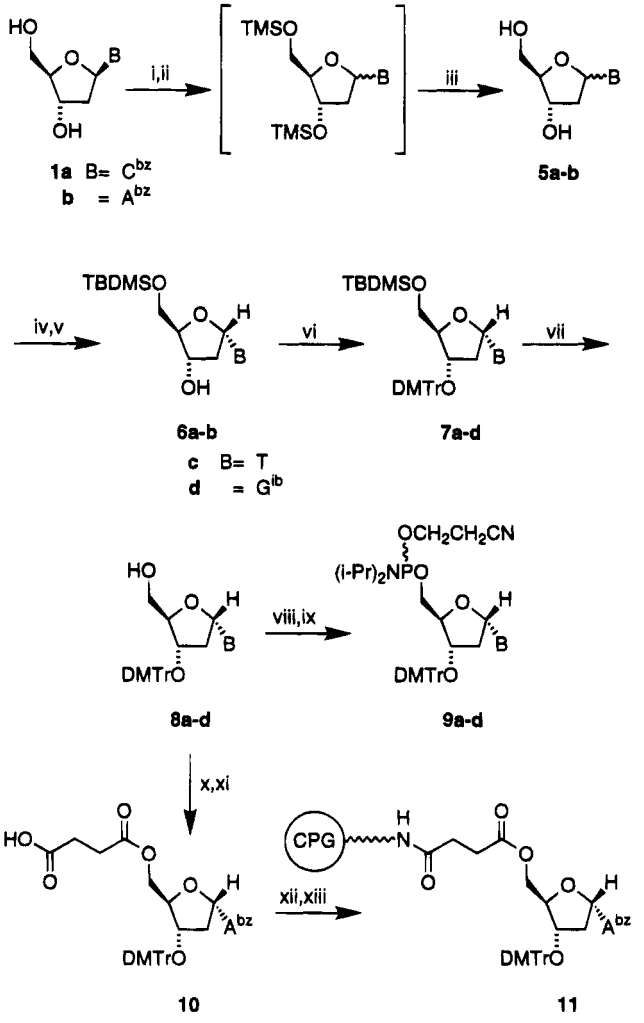
Scheme 2[a,b]



[a] Conditions: (i) BSA in CH_3CN; (ii) TMSOTf; (iii) $NaHCO_3$ in CH_3OH; (iv) TBDMS-Cl/imidazole in DMF; (v) silica gel chromatography; (vi) DMTrCl in pyridine; (vii) *n*-Bu_4NF in THF; (viii) *N,N*-diisopropylammonium tetrazolide in CH_2Cl_2; (ix) $[(i\text{-}Pr)_2N]_2\text{-}POCH_2CH_2CN$; (x) succinic anhydride/DMAP in pyridine; (xi) citric acid extraction; (xii) *p*-nitrophenol/DCC in pyridine; (xiii) LCAA-CPG/$(CH_3CH_2)_3N$ in DMF. [b] Key: TMS, trimethylsilyl; TBDMS, *tert*-butyldimethylsilyl; DMTr, (di-*p*-anisyl)phenylmethyl; DMAP, 4-(dimethylamino)pyridine; DCC, 1,3-dicyclohexylcarbodiimide; LCAA-CPG, long chain alkylamine controlled-pore glass.

and Saneyoshi[12c] (Scheme 1) may produce anomeric 2′-deoxycytidine contaminants (**3**) and complicate the isolation of the desired α-2′-deoxyadenosine derivative. To circumvent this potential problem, β-(N^6-benzoyl)-2′-deoxyadenosine (**1b**) was anomerized under the conditions described for **1a** (Scheme 2). The anomerization rates of **1b** were considerably slower than those observed with **1a**, as it took a reaction time of 87 h to generate optimum yields of α-(N^6-benzoyl)-2′-deoxyadenosine. After workup, 5′-silylation, and purification of the anomeric mixture, the α-2′-deoxyadenosine derivative **6b** was isolated in 29% yield based on **1b**. For reasons that are still unclear, anomerization of either β-(N^2-isobutyryl)-2′-deoxyguanosine or β-2′-deoxyguanosine, under conditions similar to those predicated for **1a**, failed to produce α-2′-deoxyguanosine derivatives in yields comparable to those obtained for **6a** or **6b**. Consequently, the synthesis of α-(N^2-isobutyryl)-2′-deoxyguanosine was accomplished from the reaction of 1-*O*-acetyl-3,5-bis-*O*-(*p*-nitrobenzoyl)-2-deoxy-D-ribofuranose with trimethylsilylated guanine according to the procedure of Morvan *et al.*[12g]

Table 1. 500 MHz ^{1}H-NMR Chemical Shifts and ^3J-Couplings of the Anomeric Proton of Selected α- and β-2′-Deoxyribonucleosides[a]

nucleoside	δ (ppm)	$J_{H1'-H2'}$ (Hz)[b]	$J_{H1'-H2''}$ (Hz)[b]	Σ*J* (Hz)
α-dC	6.24	6.9	2.2	9.1
β-dC	6.66	7.7	6.8	14.5
α-dA	7.00	7.8	2.4	10.2
β-dA	6.48	7.4	6.1	13.5
α-dG	6.49	7.8	3.1	10.9
β-dG	6.36	7.4	6.5	13.9

[a] Spectra were recorded in D_2O. TMS was used as an external standard. [b] H′ and H″ were defined according to ref 15b. In the case of α-nucleosides, *J* couplings were directly measured from $C_{1'}$-H signals, whereas *J* couplings of β-nucleosides were measured from respective $C_{2'}$-H′ and $C_{2'}$-H″ signals. The chemical shift of $C_{2'}$-H′ is downfield relative to that of $C_{2'}$-H″.

α-(N^2-Isobutyryl)-5′-*O*-(*tert*-butyldimethylsilyl)-2′-deoxyguanosine (**6d**) and α-(5′-*O*-*tert*-butyldimethylsilyl)-thymidine (**6c**) were prepared from α-(N^2-isobutyryl)-2′-deoxyguanosine and commercial α-thymidine, respectively, under conditions reported by Ogilvie.[13] The silylated α-nucleoside derivatives **6a-d** were converted to **7a-d** by treatment with di-*p*-anisylphenylmethyl chloride in pyridine. The fully protected α-nucleosides **7a-d** were purified by silica gel chromatography and, without further characterization, desilylated upon reaction with tetra-*n*-butylammonium fluoride in tetrahydrofuran. Precipitation of crude α-nucleosides in ice–water and subsequent purification by silica gel chromatography afforded pure α-(3′-*O*-di-*p*-methoxytrityl)-2′-deoxynucleosides **8a-d** in yields exceeding 90%.

A sample of each of the α-2′-deoxynucleosides **8a**, **8b**, and **8d** was deprotected and the anomeric configuration of each nucleoside determined by ^{1}H-NMR spectroscopy at 500 MHz. When compared to the anomeric proton of β-2′-deoxynucleosides, which displayed overlapping doublets with $J_{H1'-H2'} \approx J_{H1'-H2''}$, the anomeric proton of **8a**, **8b**, and **8d** exhibited two discrete doublets with $J_{H1'-H2'} > J_{H1'-H2''}$.[14] Chemical shifts and *J* couplings of these anomeric protons are listed in Table 1. The three-bond couplings associated with $C_{1'}$-H are indicative of sugar conformation,[15a] and in the case of β-2′-deoxynucleosides, for example, the sum of the couplings of $C_{1'}$-H with $C_{2'}$-H′ and $C_{2'}$-H″ exceeds 13.3 Hz with $J_{H1'-H2'} > J_{H1'-H2''}$ (see Table 1). Although these features characterize $C_{2'}$-*endo* or *S*-type sugar puckers found in B-DNA,[16] the ratios $J_{H1'-H2'}$:$J_{H1'-H2''}$, which are near unity, suggest a significant contribution from $C_{3'}$-*endo* or *N*-type sugar puckers to the overall conformation of β-2′-deoxynucleosides in aqueous solution.[15a] Interestingly, the ratios $J_{H1'-H2'}$:$J_{H1'-H2''}$ associated with α-2′-deoxynucleosides exceed unity but the sum of the *J* couplings is less than 13.3 Hz. These *J* values indicate that the sugar pucker of α-2′-deoxynucleosides is predominantly $C_{2'}$-*endo* with an amplitude lower than that of β-2′-deoxynucleosides.[15a]

Conversion of the α-2′-deoxynucleosides **8a-d** to the corresponding phosphoramidite derivatives **9a-d** was

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Scheme 3[a]

12 5'-d(βT$_p$αC$_p$βG$_p$αA$_p$βC$_p$αA$_p$βC$_p$αC$_p$βC$_p$αA$_p$βA$_p$αT$_p$βT$_p$αC$_p$βT$_p$αG$_p$βA$_p$αA$_p$βA$_p$αA$_p$βT$_p$αG$_p$βG$_p$αA)-5'

13 5'-d(βT$_p$αC$_p$βC$_p$αA$_p$βT$_p$αT$_p$βT$_p$αT$_p$βC$_p$αA$_p$βG$_p$αA$_p$βA$_p$αT$_p$βT$_p$αG$_p$βG$_p$αG$_p$βT$_p$αG$_p$βT$_p$αC$_p$βG$_p$αA)-5' [SENSE]

14 5'-d(βT$_p$αC$_p$β**C**$_p$αA$_p$βC$_p$α**T**$_p$βC$_p$αC$_p$βC$_p$αA$_p$βA$_p$α**A**$_p$βT$_p$αC$_p$βT$_p$α**C**$_p$βA$_p$αA$_p$βA$_p$αA$_p$βT$_p$α**C**$_p$βG$_p$αA)-5'

15 5'-d(βT$_{ps}$αC$_{ps}$βG$_{ps}$αA$_{ps}$βC$_{ps}$αA$_{ps}$βC$_{ps}$αC$_{ps}$βC$_{ps}$αA$_{ps}$βA$_{ps}$αT$_{ps}$βT$_{ps}$αC$_{ps}$βT$_{ps}$αG$_{ps}$βA$_{ps}$αA$_{ps}$βA$_{ps}$αA$_{ps}$βT$_{ps}$αG$_{ps}$βG$_{ps}$αA)-5'

16 5'-d(βT$_{ps}$αC$_{ps}$βG$_p$αA$_p$βC$_p$αA$_p$βC$_p$αC$_p$βC$_p$αA$_p$βA$_p$αT$_p$βT$_p$αC$_p$βT$_p$αG$_p$βA$_p$αA$_p$βA$_p$αA$_p$βT$_p$αG$_{ps}$βG$_{ps}$αA)-5'

17 5'-β-d(T$_p$C$_p$G$_p$A$_p$C$_p$A$_p$C$_p$C$_p$C$_p$A$_p$A$_p$T$_p$T$_p$C$_p$T$_p$G$_p$A$_p$A$_p$A$_p$A$_p$T$_p$G$_p$G$_p$A)-3'

18 5'-β-d(T$_p$C$_p$C$_p$A$_p$T$_p$T$_p$T$_p$T$_p$C$_p$A$_p$G$_p$A$_p$A$_p$T$_p$T$_p$G$_p$G$_p$G$_p$T$_p$G$_p$T$_p$C$_p$G$_p$A)-3' [SENSE]

19 5'-β-d(T$_p$C$_p$C$_p$A$_p$T$_p$T$_p$T$_p$T$_p$C$_p$A$_p$**C**$_p$A$_p$A$_p$T$_p$T$_p$G$_p$G$_p$G$_p$T$_p$G$_p$T$_p$C$_p$G$_p$A)-3' [SENSE]

20 5'-β-d(T$_{ps}$C$_{ps}$G$_{ps}$A$_{ps}$C$_{ps}$A$_{ps}$C$_{ps}$C$_{ps}$C$_{ps}$A$_{ps}$A$_{ps}$T$_{ps}$T$_{ps}$C$_{ps}$T$_{ps}$G$_{ps}$A$_{ps}$A$_{ps}$A$_{ps}$A$_{ps}$T$_{ps}$G$_{ps}$G$_{ps}$A)-3'

21 5'-β-d(T$_{ps}$C$_{ps}$C$_{ps}$A$_{ps}$T$_{ps}$T$_{ps}$T$_{ps}$T$_{ps}$C$_{ps}$A$_{ps}$G$_{ps}$A$_{ps}$A$_{ps}$T$_{ps}$T$_{ps}$G$_{ps}$G$_{ps}$G$_{ps}$T$_{ps}$G$_{ps}$T$_{ps}$C$_{ps}$G$_{ps}$A)-3' [SENSE]

22 5'-β-d(T$_{ps}$C$_{ps}$G$_p$A$_p$C$_p$A$_p$C$_p$C$_p$C$_p$A$_p$A$_p$T$_p$T$_p$C$_p$T$_p$G$_p$A$_p$A$_p$A$_p$A$_p$T$_p$G$_{ps}$G$_{ps}$A)-3'

23 5'-β-(U$_p$C$_p$C$_p$A$_p$U$_p$U$_p$U$_p$U$_p$C$_p$A$_p$G$_p$A$_p$A$_p$U$_p$U$_p$G$_p$G$_p$G$_p$U$_p$G$_p$U$_p$C$_p$G$_p$A)-3' [SENSE]

[a] Unless otherwise indicated, oligonucleotides correspond to an antisense sequence overlapping the splice acceptor site of the second exon that encodes the HIV-1 *tat* gene product. Underlined bold letters in sequences **14** and **19** represent mismatches. p = phosphate diester; ps = phosphorothioate diester.

achieved by treatment with 2-cyanoethyl *N,N,N',N'*-tetraisopropylphosphorodiamidite in the presence of a catalytic amount of the *N,N*-diisopropylammonium salt of 1*H*-tetrazole according to the procedure of Barone *et al.*[17] The 5'-phosphoramidites **9a-d** were purified by silica gel chromatography and isolated as white foams in yields greater than 90%. Commercial β-deoxyribonucleoside 3'-phosphoramidites and **9a-d** were applied to the solid-phase synthesis of alternating α,β-oligodeoxyribonucleotides with alternating (3'→3')-/(5'→5')-internucleotidic phosphodiester linkages.[9] In the context of antisense applications, these oligonucleotide analogues presented a 5'-hydroxyl function at each terminus to provide increased resistance to hydrolysis catalyzed by ubiquitous (3'→5')-exonucleases which, for example, are prevalent in cell culture media.[18]

Solid-phase syntheses of alternating α,β-oligodeoxyribonucleotides with terminal 5'-hydroxy functions required the conjugation of aminated controlled-pore glass (LCAA-CPG) with the 5'-arm of an α-deoxyribonucleoside derivative. Thus, α-(*N*^{6}-benzoyl)-3'-*O*-(di-*p*-methoxytrityl)-2'-deoxyadenosine (**8b**) was treated with succinic anhydride, according to the method of Caruthers *et al.*,[19] to give the corresponding hemisuccinate ester **10** which, upon activation, reacted with LCAA-CPG to generate **11**. After inactivation of unreacted amine functions with acetic anhydride, the CPG support **11** was suitable for the synthesis of the α,β-oligomers **12**–**16** (Scheme 3). To ensure optimum coupling yields (>98%), the time allocated to the condensation of α-deoxyribonucleoside phosphoramidites **9a-d** during the synthesis of α,β-oligomers was extended by a factor of *ca.* 2.7 relative to that which was necessary for standard β-deoxyribonucleoside 3'-phosphoramidites. The β-oligodeoxyribonucleotides **17**–**22** were also synthesized to establish meaningful control experiments. Incidentally, the preparation of the phosphorothioated oligonucleotide analogues **15**, **16**, and **20**–**22** was accomplished by the sulfurization of selected phosphite triester functions with 0.05 M 3*H*-1,2-benzodithiol-3-one 1,1-dioxide in acetonitrile.[20]

Oligodeoxyribonucleotides **12**–**22** were purified by reversed-phase HPLC by taking advantage of the hydrophobicity of the 5'-*O*-(di-*p*-methoxytrityl)group, and the use of a programmed buffer gradient composed of aqueous 0.1 M sodium acetate (pH 7.0) and acetonitrile. Given the cytotoxicity of the triethylammonium salt of oligonucleotide phosphodiesters,[21] sodium acetate buffers are recommended over popular triethylammonium acetate buffers for the purification of those oligonucleotides that, ultimately, will be involved in cell culture experiments. The purity of detritylated **12**–**22** was assessed by polyacrylamide gel electrophoresis under denaturing conditions. Figure 1 shows the relative electrophoretic mobility of selected oligomers. As expected, phosphorothioated oligomers (**15**, **16**, **20**, and **22**) moved slower than the parent phosphodiesters **12** and **17**.[20a]

Representative oligonucleotide analogues were additionally characterized by ^{31}P-NMR spectroscopy.[22] Resonances corresponding to (5'→5')-phosphodiester linkages had different chemical shifts than those corresponding to (3'→3')-phosphodiester functions. Consequently, ^{31}P-NMR resonances generated by the α,β-oligodeoxyribonucleotide **12** appeared as two distinct groups centered at δ ~1.5 ppm, whereas resonances produced by the (3'→5')-β-oligodeoxyribonucleotide **17** showed as a single group at about the same chemical shift (see Figure 2). Phosphorothioated oligonucleotide analogues displayed a similar behavior, and proper P(S) resonances (δ ~57 ppm) were found in correct ratios with respect to P(O) resonances (δ ~0 ppm).[22]

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(22) 121 MHz ^{31}P-NMR spectra of oligodeoxyribonucleotides **15**, **16**, **20**, and **22** are shown in the supplementary material.

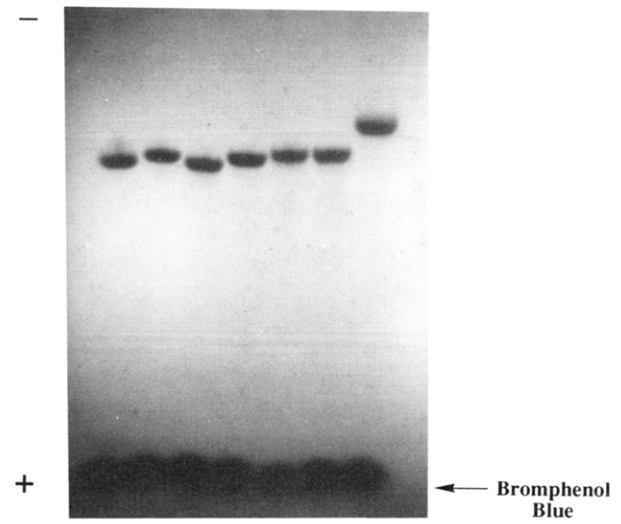

Figure 1. Relative electrophoretic mobility of selected oligonucleotides and their analogues (24-mers) on a 20% polyacrylamide−7 M urea gel at pH 8.3 (1X TBE buffer). From left to right: **17**; **22**; **12**; **16**; **20**; **15**; **23**.

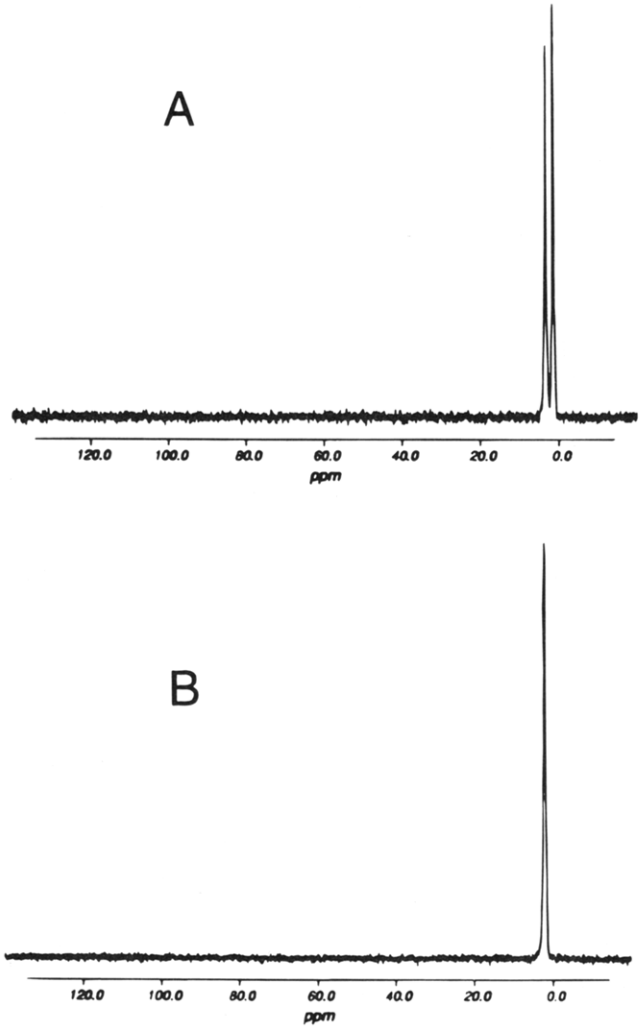

Figure 2. Comparison of the 121 MHz ^{31}P-NMR spectrum of the α,β-oligodeoxyribonucleotide **12** (panel A) with that of the unmodifided β-oligomer **17** (panel B) in D_2O.

A unique property of the α,β-oligonucleotide **12** is its low hypochromicity (10%) at 260 nm and 22 °C when compared to that measured for the native β-oligomer **17** (23%) under identical conditions. These data suggest that the nucleobases of **12** induced less stacking than those of **17**, presumably because of structural differences. The oligonucleotide analogue **12** displayed, however, enhanced resistance to hydrolysis by snake venom phosphodiesterase (SVP is predominantly a 3′-exonuclease), calf spleen phosphodiesterase (CSP is a 5′-exonuclease), and S1 nuclease (an endonuclease) relative to the unmodified oligonucleotide **17** (see Table 2). For example, the α,β-oligomer **12** was hydrolyzed to the extent of 6% by S1 nuclease and 69% by CSP after an incubation time of 24 h and 48 h, respectively, at 37 °C. Under these conditions, the natural oligomer **17** was totally hydrolyzed by each of these nucleases within 15 min. The inherent resistance of oligonucleotides structurally related to **12** has also been demonstrated by Debart *et al.* who reported that the half-life of the α,β-oligodeoxyribonucleotide 5′-d($\alpha T_p\beta C_p\alpha T_p\beta T_p\alpha A_p\beta A_p\alpha C_p\beta C_p\alpha C_p\beta A_p\alpha C_p\beta A$)-5′ in RPMI 1640 medium, supplemented with 10% inactivated fetal calf serum, was 5.5 d at 37 °C. Under similar conditions, the corresponding unmodified oligonucleotide had a half-life of only 11 min.[23]

Table 2. Relative Stability of Oligonucleotide Analogues to the Nucleolytic Activity of Snake Venom (SVP) and Calf Spleen (CSP) Phosphodiesterases, and Nuclease S1[a]

	estimated percentage of hydrolysis *vs* time (h) at 37 °C		
oligomer	SVP	CSP	S1
12	81 (1)	69 (48)[b]	6 (24)
17	100 (<0.25)	100 (<0.25)	100 (<0.25)
16	89 (3)	0 (48)	6 (24)
22	88 (0.5)	76 (48)	100 (<0.25)
15	0 (24)	0 (48)	0 (48)
20	15 (24)	5 (48)	90 (0.5)

[a] Detailed protocols pertaining to the enzymatic hydrolysis of oligodeoxyribonucleotide analogues are provided in the Experimental Section. [b]Most of the hydrolysis products (87%) consist of shorter oligomers tentatively ascribed to 22- and 23-mers.

It must be noted that while the perphosphorothioated β-oligomer **20** was slowly hydrolyzed by CSP (only 5% hydrolysis was observed after 48 h at 37 °C), S1 nuclease digested 90% of the oligonucleotide analogue within 30 min at the same incubation temperature. Nonetheless, **20** was considerably more stable than **12** to hydrolysis by SVP. Typically, the incubation of **12** with SVP for 1 h at 37 °C led to 81% hydrolysis of the oligomer. Under these conditions, **20** was hydrolyzed to the extent of only 15% after 24 h of incubation. The incorporation of two phosphorothioate functions at each terminus of **12** produced the oligonucleotide **16** which was significantly more resistant to SVP and CSP. Specifically, **16** was not hydrolyzed upon incubation with CSP for 48 h at 37 °C, whereas incubation with SVP caused 89% hydrolysis after 3 h. Others have also demonstrated that end-capping of oligonucleotides with modified phosphodiester functions improved resistance to exonucleases while maintaining hybridization abilities.[18,24] The conversion of **12** to the oligonucleoside phosphorothioate **15** provided complete resistance to SVP, CSP, and S1 nuclease. No

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Table 3. Thermal Denaturation Temperatures of DNA–DNA and DNA–RNA Complexes

hybrid	T_m[a] (°C)	ΔT_m[b] (°C)	hypochromicity[c] (%)
17:18	67		22
17:19	58	9	21
20:18	57	10	20
21:17	57	10	19
20:19	51	16	18
12:18	54	13	22
13:17	53	14	16
12:19	45	22	21
14:18	*[d]	N/A	N/A
12:13	61	6	16
15:18	43	24	16
16:18	54	13	22
17:23	62 (70)		19 (19)
20:23	52 (62)	10 (8)	17 (19)
12:23	35 (41)	27 (29)	15 (16)
14:23	*	N/A	N/A
16:23	34 (40)	28 (30)	14 (17)
15:23	* (*)	N/A	N/A

[a] Thermal denaturation of DNA–DNA and DNA–RNA complexes was effected in 1X PBS (pH 7.2) and monitored at 260 nm. Values in parentheses were obtained in 1.0 M NaCl PBS buffer pH 7.2. The concentration of each oligomer was *ca.* 2.5 μM. [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 unmodified duplex **17:18** or **17:23**, respectively. [c] Thermal hypochromicity is the increase of absorbance in percent at 260 nm, relative to the initial absorbance of each complex, calculated according to the following formula: $[(A_{coil} - A_{duplex})/A_{coil}]100$. [d] The symbol * indicates that no thermal transition was observed for the complex. The variability between repeated T_m measurements was *ca.* 1 °C.

hydrolysis was observed after an incubation time of 24 h (SVP) or 48 h (CSP and S1 nuclease) at 37 °C (Table 2).

Like α,β-oligothymidylates,[5] the α,β-oligodeoxyribonucleotides **12** and **13** formed hybrids with complementary unmodified DNA oligomers (**18** and **17**, respectively). The stoichiometry of the hybrid **12:18** was determined according to the method of continuous variation,[25] at a fixed total strand concentration (*ca.* 5 μM), in 1X PBS buffer (pH 7.2) at 260 nm and 22 °C. Under these conditions, the UV mixing curve exhibited a break point at a molar fraction of *ca.* 0.5 which revealed the formation of a 1:1 complex.[26]

The thermal stability of the duplex **12:18** was, however, lower (T_m = 54 °C) than that of the unmodified DNA duplex **17:18** (T_m = 67 °C) (Table 3). The affinity of **12** for **18** was assessed by the substitution of oligomer **19** for **18** before hybrid formation. The singly mismatched complex **12:19** was, expectedly, less stable (T_m = 45 °C) than **12:18** (T_m = 54 °C). The difference in thermal stability of these two hybrids (ΔT_m = 9 °C) correlated well with that observed for unmodified **17:19** and **17:18** (ΔT_m = 9 °C) (Table 3). The difference in free energy of dissociation computed for **12:18** and **12:19** ($\Delta\Delta G°_{37}$ = −2.5 kcal/mol) also correlated well with that calculated for **17:18** and **17:19** ($\Delta\Delta G°_{37}$ = −3.2 kcal/mol) (Table 4) and indicated that the sequence specificity of **12** toward **18** was comparable to that of the native β-oligonucleotide **17**. The incorporation of five mismatches into the duplex **12:18** by the substitution of the α,β-oligonucleotide **14** for **12** led to an unstable complex with no observable thermal helix-to-coil transition. These results further support the sequence specific recognition of **18** by **12**. By comparison, hybrids composed of either the perphosphorothioated oligomer **20** or **21** with a complementary unmodified DNA strand (**18** or **17**) exhibited a thermal stability (T_m = 57 °C) comparable, albeit slightly higher, to that observed with α,β-oligonucleotides under similar conditions. Interestingly, the difference in thermal stability between the complex **20:18** and the mismatched complex resulting from the interaction of **20** with **19** was not as large (ΔT_m = 6 °C) as that measured between **17:18** and **17:19** (ΔT_m = 9 °C) or **12:18** and **12:19** (ΔT_m = 9 °C) (Table 3). These findings suggest that while the affinity of the α,β-oligonucleotides structurally related to **12** for complementary β-DNA oligomers is marginally less than that of perphosphorothioated β-oligodeoxyribonucleotides, the specificity of α,β-oligonucleotides toward β-DNA targets appears to be superior to that of these thioated β-oligomers.

Table 4. Thermodynamic Parameters Calculated from Experimental Thermal Denaturation Curves of Selected DNA–DNA and DNA–RNA Complexes[a]

hybrid	$\Delta G°_{37}$ (kcal/mol)	$\Delta H°$ (kcal/mol)	$\Delta S°$ (cal/mol·K)
17:18	−19.5	−140	−390
17:19	−16.3	−141	−416
12:18	−12.0	−118	−337
12:19	−10.5	−122	−359
12:13	−15.0	−115	−322
17:23	−16.1	−121	−339
12:23	−6.8	−70	−220

[a] Thermodynamic parameters were derived from a six-parameter fit of experimental thermal denaturation curves to a two-state model.[32] Error in the approximation of $\Delta H°$ and $\Delta S°$ from curve-fitting was *ca.* 5%.

The lower thermal stability of the duplex **12:18** with respect to that of the duplex **17:18** may be attributed, in part, to the lengths of the (3′→3′)- and (5′→5′)-phosphodiester linkages which are shorter and longer, respectively, than natural (3′→5′)-phosphodiester linkages. Such disparity in internucleotidic distances may detrimentally affect the stacking of the nucleobases and impair hybridization with unmodified oligonucleotides. Additional conformational factors may also contribute to the lower thermal stability of **12:18**. For example, the duplex composed of the α,β-oligodeoxyribonucleotides **12** and **13** exhibited a lower thermal stability (T_m = 61 °C) than that of the native DNA duplex **17:18** (T_m = 67 °C) (Table 3) in spite of the similarity between the internucleotidic motifs of each DNA strand. Comparison of the circular dichroism (CD) spectra of the duplexes **12:18**, **12:13**, and **17:18** in 1X PBS buffer, at 22 °C, suggests that the conformation adopted by the duplex **12:13** is essentially of opposite handedness relative to that of the natural DNA duplex **17:18** (see Figure 3). It is interesting to note that the CD spectrum of **12:18** resembles that of the natural duplex **17:18** rather than that of the α,β-duplex **12:13**. In this context, it would appear that the duplex **12:18** exhibits, at least to some extent, a B-type helicity. The considerable reduction in amplitude and slight bathochromic shift (*ca.* 4 nm) of the B_{2u} transition to 278 nm, with respect to that observed for **17:18** (274 nm) (Figure 3) may, nonetheless, be indicative of the conformational differences responsible for the lower thermal stability of **12:18** relative to **17:18**.

While α,β-oligodeoxyribonucleotides **12**, **15**, and **16** formed fairly stable complexes with the complementary DNA sequence **18**, the substitution of the RNA sequence **23** for **18** led to the formation of relatively unstable hybrids in 1X PBS buffer (see Table 3). Particularly, the complexes **12:23** and **16:23** exhibited thermal denatur-

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(26) UV mixing curves of the complexes **12:18** and **12:13** are shown in the supplementary material.

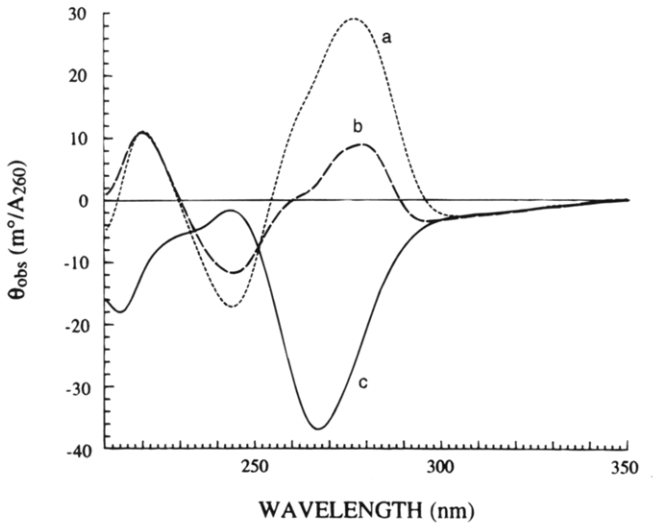

Figure 3. Circular dichroism spectra, normalized to 1.0 absorbance unit at 260 nm, of the duplexes **17:18** (curve a), **12:18** (curve b), and **12:13** (curve c) in 1X phosphate-buffered saline (pH 7.2) at 22 °C.

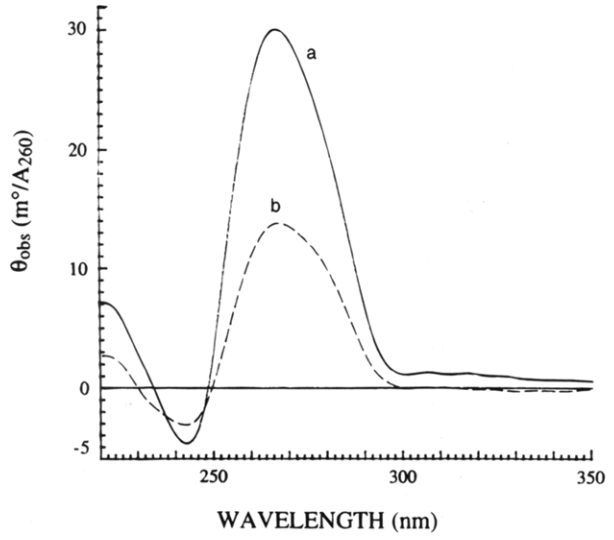

Figure 4. Circular dichroism spectra, normalized to 1.0 absorbance unit at 260 nm, of the complexes **17:23** (curve a), and **12:23** (curve b) in 1X phosphate-buffered saline (pH 7.2) at 22 °C.

ation temperatures of 35 and 34 °C, respectively. These hybrids were considerably less stable than either the unmodified DNA–RNA duplex **17:23** (T_m = 62 °C) or the complex composed of the perphosphorothioated oligomer **20** and **23** (T_m = 52 °C) under similar conditions. The low affinity of the α,β-oligodeoxyribonucleotide **12** for the complementary RNA oligomer **23** is surprising considering the affinity of β- and, especially, α-oligomers for RNA.[27]

Circular dichroism spectra of the complexes **17:23** and **12:23** in 1X PBS buffer at 22 °C showed that the conformation adopted by the complex **12:23**, under these conditions, resembled that of the native duplex **17:23** (see Figure 4). Similar to the CD spectra of the duplexes **12:18** and **17:18**, the CD spectrum of the complex **12:23** showed a significant reduction in amplitude along with

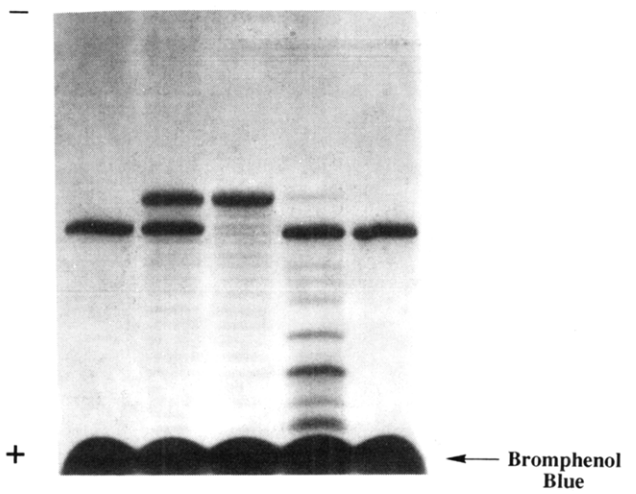

Figure 5. Relative sensitivity of modified and unmodified DNA–RNA complexes to *E. coli* RNase H. Hydrolysis products were analyzed on a 20% polyacrylamide–7 M urea gel at pH 8.3 (1X TBE buffer). From left to right: **12** + RNase H; complex **12:23** + RNase H; **23** + RNase H; duplex **17:23** + RNase H; **17** + RNase H. See the Experimental Section for the details of these assays.

a small bathochromic shift (*ca.* 4 nm) of the B_{2u} transition to 264 nm relative to that recorded for the natural DNA–RNA heteroduplex **17:23** (Figure 4). Given that RNA adopts an A-type helical geometry,[28] it is conceivable that the α,β-oligonucleotide **12** does not accommodate the A-type helicity, dictated by the oligonucleotide **23** during complex formation, as readily as the B-type helicity conferred by the oligomer **18**. A conformational incompatibility between **12** and **23** may be responsible for the lower thermal stability of the complex **12:23** compared to that of the duplexes **12:18** and **17:23**. In agreement with this argument, the free energy of dissociation of the complex **12:23** ($\Delta G°_{37}$ = −6.8 kcal/mol) was lower than that of the duplexes **12:18** and **17:23** by factors of 1.8 and 2.4, respectively (Table 4).

Although the ability of oligonucleotide analogues to form stable complexes with complementary RNA targets is of major importance in the development of potent antisense biomolecules, the biological recognition of DNA–RNA hybrids deserves consideration. It has even been postulated that the antisense activity of DNA oligonucleotides was due, at least in part, to the cleavage of the RNA strand of a DNA–RNA heteroduplex by RNase H.[18] It is, therefore, of interest to test the modified complex **12:23** for its ability to elicit RNase H-mediated hydrolysis of the RNA strand. Our findings revealed that like hybrids composed of α-anomeric oligodeoxyribonucleotides and complementary β-oligoribonucleotides,[27] the complex **12:23** was not a substrate for *E. coli* RNase H even after an incubation of 22 h at 22 °C. Under identical conditions, the RNA strand of the natural heteroduplex **17:23** was almost completely hydrolyzed (>95%) by *E. coli* RNase H (see Figure 5). To ensure that the complex **12:23** was formed in the buffer used for the RNase H assay, a thermal denaturation profile of the complex was recorded in such a buffer. The hybrid **12:23** exhibited a sharp melting transition (T_m = 37 °C) and, thereby, confirmed that the complex was not a substate for *E. coli* RNase H.

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(28) Bush, C. A.; Brahms, J. In *Physico-Chemical Properties of Nucleic Acids;* Duchesne, J., Ed.; Academic Press: London and New York, 1973; Vol. 2, pp 147–186.

To summarize, a simpler synthesis of α-2′-deoxycytidine and α-2′-deoxyadenosine derivatives **6a,b** has been achieved from commercial β-deoxyribonucleosides. The incorporation of these nucleoside analogues into alternating α,β-oligodeoxyribonucleotides with alternating (3′→3′)- and (5′→5′)-internucleotidic linkages led to a class of oligonucleotide analogues with increased resistance to nucleases relative to unmodified oligodeoxyribonucleotides. The interaction of the α,β-oligonucleotide **12** with a complementary unmodified DNA oligomer resulted in the formation of a 1:1 complex with a helicity that resembled more that of the native DNA duplex **17:18** than that of an α,β-DNA complex, as judged by CD spectroscopy. The thermal stability of the duplex **12:18** was lower than that measured for **17:18** but the sequence specificity with which **12** recognized **18** was similar to that of the β-oligomer **17**. However, the affinity of the α,β-oligonucleotide **12** for a complementary β-oligoribonucleotide was considerably lower than that observed for a complementary β-DNA oligomer, presumably because of conformational incompatibility between **12** and **23** upon hybrid formation.

It is known that one of the major structural differences between B-helical DNA and the A-form of RNA is the tilting of the nucleobases. Typically, the bases are perpendicular to the helix axis in B-type DNA, whereas the nucleobases of A-type RNA display a tilting of *ca.* 14–20° with respect to the helical axis.[28] Thus, it seems reasonable to speculate that the affinity of the α,β-oligonucleotide **12** for the β-oligoribonucleotide **23** may improve if a greater flexibility of the nucleobases could be imparted to α-nucleotidic residues in order to better accommodate the A-type helicity of **23**. Syntheses of α-nucleosides with nucleobases linked to the carbohydrate entities through flexible linker arms are in progress in the laboratory and will be reported in due course.

Experimental Section

Materials and Methods. Chemicals and common solvents including those that are anhydrous were purchased from commercial sources and used without further purification. α-Thymidine, N^4-benzoyl-2′-deoxycytidine, N^6-benzoyl-2′-deoxyadenosine, guanine, 2-deoxy-D-ribose, and long chain alkylamine controlled-pore glass (LCAA-CPG) were obtained from Sigma and used as received.

E. coli RNase H, S1 nuclease from *Aspergillus oryzae*, snake venom (*Crotallus durissus*), and calf spleen phosphodiesterases were purchased from Boehringer and Mannheim and stored as recommended by the manufacturer. Sterile 1X phosphate buffered saline (PBS) pH 7.2 was obtained from S. and S. Media, Inc.; the ingredients of the buffer are sodium chloride (8.5 g/L), sodium dihydrogen phosphate (0.73 g/L), and potassium dihydrogen phosphate (0.21 g/L). 10X TBE buffer was purchased from Bio-Rad Laboratories; a 1X solution of the buffer is composed of 89 mM Tris, 89 mM boric acid, and 2mM EDTA (pH 8.3).

Chromatography on silica gel columns was performed at 30 psi with high-performance silica gel H (10 ± 4 μm) (Analtech), whereas analytical thin-layer chromatography (TLC) was carried out on glass plates coated with a 0.20 mm thick layer of silica gel 60 F_{254} (Sigma). Melting points were reported uncorrected.

NMR spectra were recorded with spectrometers operating 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 run in deuterated solvents. 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. ^{13}C-NMR spectra were recorded in $CDCl_3$ from which the middle line of the resonances observed (77.00 ppm) was used as internal reference. Chemical shifts δ are reported in parts per million (ppm).

Fast-atom bombardment mass spectra were recorded in both negative and positive ion modes. Six KeV Xe atoms were used to ionize samples dissolved in 3-nitrobenzyl alcohol:glycerol (9:1).

α-(5′-*O*-*tert*-Butyldimethylsilyl)thymidine (6c). The regioselective silylation of the 5′-hydroxy function of α-thymidine was achieved according to the procedure reported by Ogilvie.[13] To α-thymidine (1.00 g, 4.13 mmol) and imidazole (685 mg, 10.01 mmol) in *N,N*-dimethylformamide (4 mL) was added *tert*-butyldimethylchlorosilane (690 mg, 4.58 mmol). The solution was stirred at ambient temperature under an atmosphere of argon for 1 h and, then, added to crushed ice (300 mL). The precipitate was filtered, washed with water, and dissolved in chloroform (100 mL). The solution was extracted twice with water (100 mL). The organic layer was dried over anhydrous magnesium sulfate and evaporated to dryness under vacuo. The product was dissolved in chloroform (10 mL) and precipitated upon addition of hexane (150 mL). The white solid was filtered, washed with hexane, and dried to afford **6c** (mp 129–130 °C) in 75% yield (1.101 g, 3.09 mmol). FAB-MS: *m/e* 357 $(M + H)^+$. ^{1}H-NMR ($CDCl_3$): δ 0.16 (s, 6H), 0.99 (s, 9H), 1.91 (s, 3H), 2.50 (m, $^2J_{2'',2'}$ = 14.7 Hz, 1H), 2.72 (m, $^2J_{2',2''}$ = 14.7 Hz, $J_{2'}$ = 7.2 Hz, $J_{2'}$ = 5.6 Hz, 1H), 3.70 (dd, $^2J_{5'',5'}$ = 11.0 Hz, $J_{5'',4'}$ = 4.8 Hz, 1H), 3.78 (dd, $^2J_{5',5''}$ = 11.0 Hz, $J_{5'}$ = 3.4 Hz, 1H), 4.49 (m, 1H), 4.56 (m, 1H), 6.20 (dd, $J_{1',2''}$ = 1.4 Hz, $J_{1'}$ = 7.2 Hz, 1H), 7.59 (s, 1H). ^{13}C-NMR ($CDCl_3$): δ 164.4, 150.9, 137.6, 109.1, 90.1, 88.6, 72.4, 64.0, 41.0, 25.9, 18.2, 12.4, −5.4, −5.5. Anal. Calcd for $C_{16}H_{28}N_2O_5Si$: C, 53.91; H, 7.92; N, 7.86; O, 22.44. Found: C, 53.76; H, 7.93; N, 7.94; O, 22.51.

α-(N^2-Isobutyryl)-5′-*O*-(*tert*-butyldimethylsilyl)-2′-deoxyguanosine (6d). α-(N^2-Isobutyryl)-2′-deoxyguanosine was prepared according to the procedure described by Morvan *et al.*[12g] The regioselective silylation of the 5′-hydroxy function of α-(N^2-isobutyryl)-2′-deoxyguanosine (1.303 g, 3.86 mmol) was accomplished with a slight modification of the procedure employed for **6c**. An incremental addition of *tert*-butyldimethylchlorosilane (2.2 equiv) to the nucleoside was necessary for the completion of the reaction within 6.5 h. The crude material obtained from the addition of the reaction mixture to cracked ice was filtered, washed with water, and dissolved in chloroform (400 mL). The solution was extracted once with water (100 mL). The chloroform layer was collected, dried over anhydrous magnesium sulfate, and evaporated to dryness under reduced pressure. The material left was recrystallized from dichloromethane–ethyl ether. The white crystals were filtered, washed with ethyl ether, and dried to give pure **6d** (167–168 °C) in 85% yield. FAB-MS: *m/e* 452 $(M + H)^+$. ^{1}H-NMR ($CDCl_3$): δ 0.07 (s, 6H), 0.89 (s, 9H), 1.23 (d, *J* = 6.8 Hz, 6H), 2.65 (m, 2H), 2.78 (m, *J* = 6.8 Hz, 1H), 3.73 (m, 2H), 4.44 (m, 1H), 4.54 (m, 1H), 6.18 (dd, $J_{1'}$ = 7.3 Hz, $J_{1',2''}$ = 1.8 Hz, 1H), 8.12 (s, 1H). ^{13}C-NMR ($CDCl_3$): δ 179.3, 155.5, 147.5, 139.3, 120.7, 89.5, 86.1, 72.4, 64.1, 41.1, 36.2, 25.9, 19.0, 18.9, 18.3, −5.4, −5.5. Anal. Calcd for $C_{20}H_{33}N_5O_5Si$: C, 53.19; H, 7.37; N, 15.51; O, 17.71. Found: C, 53.16; H, 7.40; N, 15.57; O, 17.68.

α-(N^4-Benzoyl)-5′-*O*-(*tert*-butyldimethylsilyl)-2′-deoxycytidine (6a). To a stirred suspension of β-(N^4-benzoyl)-2′-deoxycytidine (**1a**, 7.00 g, 21.13 mmol) in dry acetonitrile (30 mL) was added 15.7 mL of *N,O*-bis(trimethylsilyl)acetamide (12.90 g, 63.39 mmol), dropwise, under an atmosphere of argon at 22 °C. The mixture was allowed to stir under these conditions for 1 h, and 10.6 mL of trimethylsilyl trifluoromethanesulfonate (12.21 g, 54.95 mmol) was added, dropwise, to the solution. The rate of the reaction was monitored by TLC and, after 24 h, the reaction mixture was evaporated to dryness under reduced pressure. The residue was repeatedly dissolved in dry acetonitrile (3 × 20 mL) and concentrated to dryness under vacuum. The material left was cooled in an ice bath, and a suspension of sodium bicarbonate (70 g) in methanol (350 mL) was added, dropwise, until neutrality of the vigorously stirred reaction mixture was obtained. Solid sodium bicarbonate was removed by filtration and washed with methanol (150 mL). The filtrates were combined and evapo-

rated to dryness under reduced pressure. The crude material (21.97 g) was left under vacuum overnight and, then, dissolved in dry *N,N*-dimethylformamide (30 mL). Imidazole (3.165 g, 46.49 mmol) was added to the solution followed by *tert*-butyldimethylchlorosilane at the rate of 0.33 molar equiv per hour until 2 molar equiv were added. A second addition of imidazole (3.165 g, 46.49 mmol) was performed along with that of 1 molar equiv of *tert*-butyldimethylchlorosilane at the rate of 0.33 molar equiv per hour. Upon completion of the reaction (TLC), the reaction mixture was poured over crushed ice (1.2 L). The precipitate was isolated by filtration and washed with water (50 mL). The solid was dissolved in dichloromethane (500 mL) and successively washed with brine (200 mL) and water (300 mL). The organic phase was collected, dried over anhydrous magnesium sulfate, and evaporated to dryness. The mixture of silylated nucleosides (8.25 g) was separated by silica gel chromatography. The desired α-nucleoside eluted from the column with 55% ethyl acetate in dichloromethane and was isolated as a white solid (mp 191−192 °C) in 46% yield (4.303 g, 9.66 mmol) based on **1a**. FAB-MS: *m/e* 446 $(M + H)^+$. ^{1}H-NMR ($CDCl_3$): δ 0.08 (s, 6H), 0.91 (s, 9H), 2.68 (m, 2H), 3.69 (dd, $^2J_{5'',5'}$ = 11.2 Hz, $J_{5'',4'}$ = 4.2 Hz, 1H), 3.75 (dd, $^2J_{5',5''}$ = 11.2 Hz, $J_{5'}$ = 3.3 Hz, 1H), 4.49 (m, 1H), 4.53 (m, 1H), 6.06 (dd, $J_{1'} \approx J_{1'} \approx$ 3.8 Hz, 1H), 7.40 (d, $J_{5,6}$ = 7.3 Hz, 1H), 7.48 (t, *J* = 7.6 Hz, 2H), 7.59 (t, *J* = 7.6 Hz, 1H), 7.88 (d, *J* = 7.6 Hz, 2H), 8.04 (d, $J_{6,5}$ = 7.3 Hz, 1H). ^{13}C-NMR ($CDCl_3$): δ 166.3, 162.2, 155.1, 145.5, 133.0, 128.8, 127.7, 95.5, 90.8, 90.5, 72.4, 63.9, 41.4, 25.9, 18.3, −5.4, −5.5. Anal. Calcd for $C_{22}H_{31}N_3O_5Si$: C, 59.30; H, 7.01; N, 9.43; O, 17.95. Found: C, 59.36; H, 7.05; N, 9.43; O, 17.89.

α-(N^6-Benzoyl)-5′-*O*-(*tert*-butyldimethylsilyl)-2′-deoxyadenosine (6b). Anomerization of β-(N^6-benzoyl)-2′-deoxyadenosine (**1b**, 21.00 g, 59.09 mmol) was achieved according to the procedure described for the preparation of **6a**. Optimum formation of the α-nucleoside occurred after a reaction time of 87 h according to TLC. After a workup similar to that performed for **6a**, the silylated α-nucleoside **6b** was eluted from a silica gel column with 45% ethyl acetate in dichloromethane and isolated as a white solid (mp 148−149 °C) in 29% yield (7.959 g, 16.95 mmol) based on **1b**. FAB-MS: *m/e* 470 $(M + H)^+$. ^{1}H-NMR ($CDCl_3$): δ 0.09 (s, 6H), 0.93 (s, 9H), 2.50 (m, $^2J_{2'',2'}$ = 14.4 Hz, 1H), 3.00 (m, $^2J_{2',2''}$ = 14.4 Hz, $J_{2'}$ = 8.6 Hz, $J_{2'}$ = 7.0 Hz, 2H), 3.73 (m, 2H), 4.43 (m, 1H), 4.52 (m, $J_{3',2''}$ = 7.0 Hz, 1H), 6.34 (dd, $J_{1'}$ = 8.6 Hz, $J_{1',2''}$ = 1.8 Hz, 1H), 7.47 (t, *J* = 7.5 Hz, 2H), 7.56 (t, *J* = 7.5 Hz, 1H), 8.01 (d, *J* = 7.5 Hz, 2H), 8.25 (s, 1H), 8.73 (s, 1H). ^{13}C-NMR ($CDCl_3$): δ 164.8, 151.7, 150.1 149.8, 143.2, 133.4, 132.7, 128.7, 127.9, 123.8, 90.4, 86.8, 72.9, 64.4, 41.3, 25.8, 18.1, −5.5, −5.6. Anal. Calcd for $C_{23}H_{31}N_5O_4Si$: C, 58.83; H, 6.65; N, 14.91; O, 13.63. Found: C, 58.79 H, 6.67; N, 14.96; O, 13.59.

General Procedure for the Preparation of the α-(3′-*O*-Di-*p*-methoxytrityl)-2′-deoxyribonucleosides 8a-d from 6a-d.[29] Typically, 1.00 g of α-(N^6-benzoyl)-5′-*O*-(*tert*-butyldimethylsilyl)-2′-deoxyadenosine (**6b**, 2.13 mmol) was dissolved in dry pyridine (15 mL) under an inert atmosphere. 4,4′-Dimethoxytrityl chloride (2.20 g, 6.49 mmol) was added to the solution which was stirred at ambient temperature for 12 h. TLC indicated incomplete reaction. Addition of 4,4′-dimethoxytrityl chloride (1.5 molar equiv) was then performed every 4 h. A total of 6 molar equiv of tritylating reagent was required for a complete reaction. Methanol (20 mL) was added and the reaction mixture evaporated to dryness under reduced pressure. The material left was dissolved in dichloromethane (100 mL) and extracted with water (30 mL). The organic layer was collected, dried over anhydrous magnesium sulfate, and concentrated under vacuum and the residue purified by silica gel chromatography. α-(N^6-Benzoyl)-5′-*O*-(*tert*-butyldimethylsilyl)-3′-*O*-(di-*p*-methoxytrityl)-2′-deoxyadenosine (**7b**) was eluted from the column with dichloromethane:methanol (99:1) and isolated in 94% yield (1.543 g, 2.00 mmol). Without further characterization, a major portion of the product (1.2 g, 1.56 mmol) was dissolved in tetrahydrofuran (1.5 mL) and stirred with 1 M tetra-*n*-butylammonium fluoride (4 mL, 4 mmol) for 2 h, at 22 °C, under an atmosphere of argon. The reaction mixture was then added to ice and water (300 mL). The precipitate was filtered, washed with water, dissolved in dichloromethane (200 mL) containing pyridine (2 mL), and successively washed with a saturated solution of sodium bicarbonate (50 mL) and water (50 mL). The organic layer was dried over anhydrous magnesium sulfate and evaporated to dryness and the residue purified by silica gel chromatography. α-(N^6-Benzoyl)-3′-*O*-(di-*p*-methoxytrityl)-2′-deoxyadenosine (**8b**) eluted from the column with dichloromethane:methanol (97:3) and was isolated in 92% yield (939 mg, 1.43 mmol) as a white powder. FAB-MS: *m/e* 658 $(M + H)^+$. ^{1}H-NMR ($CDCl_3$): δ 1.86 (m, $^2J_{2'',2'}$ = 14.9 Hz, 1H), 2.30 (m, $^2J_{2',2''}$ = 14.9 Hz, $J_{2'}$ = 7.9 Hz, $J_{2'}$ = 6.9 Hz, 1H), 3.34 (dd, $^2J_{5'',5'}$ = 11.4 Hz, $J_{5'',4'}$ = 4.6 Hz, 1H), 3.62 (dd, $^2J_{5',5''}$ = 11.4 Hz, 1H), 3.73 (s, 6H), 4.33 (m, 1H), 4.40 (m, 1H), 6.40 (dd, $J_{1'}$ = 7.0 Hz, 1H), 6.78 (d, *J* = 8.6 Hz, 4H), 7.19 (d, *J* = 8.6 Hz, 4H), 7.22 (m, 5H), 7.45 (t, *J* = 7.4 Hz, 2H), 7.56 (t, *J* = 7.4 Hz, 1H), 8.04 (d, *J* = 7.4 Hz, 2H), 8.57 (s, 1H), 8.75 (s, 1H). ^{13}C-NMR ($CDCl_3$): δ 164.8, 158.6, 152.4, 151.4, 149.4, 144.5, 141.9, 135.8, 135.7, 133.6, 132.6, 129.8, 129.7, 128.6, 128.1, 127.9, 127.1, 122.9, 113.4, 88.5, 87.8, 85.5, 74.6, 62.6, 55.1, 39.7. Anal. Calcd for $C_{38}H_{35}N_5O_6$: C, 69.39; H, 5.36; N, 10.65. Found: C, 69.46; H, 5.34; N, 10.62.

The tritylated α-deoxynucleosides **8a** and **8c-d** were similarly prepared and isolated in similar yields. **α-(N^4-Benzoyl)-3′-*O*-(di-*p*-methoxytrityl)-2′-deoxycytidine (8a).** FAB-MS: *m/e* 634 $(M + H)^+$. ^{1}H-NMR ($CDCl_3$): δ 2.17 (m, $^2J_{2'',2'}$ = 14.8 Hz, 1H), 2.52 (m, $^2J_{2',2''}$ = 14.8 Hz, $J_{2'}$ = 7.9 Hz, $J_{2'}$ = 6.9 Hz, 1H), 3.07 (dd, $^2J_{5'',5'}$ = 12.1 Hz, $J_{5'',4'}$ = 4.1 Hz, 1H), 3.34 (dd, $^2J_{5',5''}$ = 12.1 Hz, $J_{5'}$ = 2.5 Hz, 1H), 3.72 (s, 6H), 3.85 (m, 1H), 4.28 (m, 1H), 6.22 (dd, $J_{1'}$ = 7.9 Hz, 1H), 6.78 (d, *J* = 8.9 Hz, 4H), 7.20 (d, *J* = 8.9 Hz, 4H), 7.21 (d, *J* = 7.5 Hz, 2H), 7.28 (t, *J* = 7.5 Hz, 2H), 7.29 (t, *J* = 7.5 Hz, 1H), 7.48 (t, *J* = 7.4 Hz, 2H), 7.57 (d, $J_{5,6}$ = 7.4 Hz, 1H), 7.58 (t, *J* = 7.4 Hz, 1H), 7.93 (d, *J* = 7.4 Hz, 2H), 8.12 (d, $J_{6,5}$ = 7.3Hz, 1H). ^{13}C-NMR ($CDCl_3$): δ 166.5, 162.2, 158.6, 155.2, 145.4, 144.6, 136.0, 135.9, 133.0, 129.9, 128.9, 128.0, 127.9, 127.6, 127.0, 113.3, 96.0, 89.8, 89.5, 87.9, 74.4, 62.8, 55.1, 40.9. Anal. Calcd for $C_{37}H_{35}N_3O_7$: C, 70.13; H, 5.57; N, 6.63. Found: C, 69.99; H, 5.60; N, 6.65.

α-(N^2-Isobutyryl)-3′-*O*-(di-*p*-methoxytrityl)-2′-deoxyguanosine (8d). FAB-MS: *m/e* 640 $(M+H)^+$. ^{1}H-NMR ($CDCl_3$): δ 1.18 (d, *J* = 6.7 Hz, 6H), 1.60 (m, 1H), 2.24 (m, 1H), 2.82 (m, *J* = 6.7 Hz), 3.27 (m, 1H), 3.50 (m, 1H), 3.71 (s, 6H), 4.18 (m, 1H), 4.34 (m, 1H), 5.93 (dd, $J_{1'}$ = 7.2 Hz, 1H), 6.76 (d, *J* = 8.6 Hz, 4H), 7.20 (m, 3H), 7.25 (d, *J* = 8.6 Hz, 4H), 7.34 (d, *J* = 6.9 Hz, 2H), 8.24 (s, 1H). ^{13}C-NMR ($CDCl_3$): δ 179.9, 158.6, 156.3, 148.3, 147.9, 144.7, 138.3, 135.9, 135.8, 129.9, 128.0, 127.9, 127.1, 120.4, 113.4, 88.6, 87.9, 84.7, 74.8, 62.6, 55.1, 39.8, 36.0, 19.0, 18.9. Anal. Calcd for $C_{35}H_{37}N_5O_7$: C, 65.72; H, 5.83; N, 10.95. Found: C, 65.64; H, 5.85; N, 10.97.

α-(3′-*O*-Di-*p*-methoxytrityl)thymidine (8c). FAB-MS: *m/e* 545 $(M + H)^+$. ^{1}H-NMR ($CDCl_3$): δ 1.84 (s, 3H), 2.37 (m, 1H), 2.98 (m, 1H), 3.12 (m, 1H), 3.35 (m, 1H), 3.74 (s, 6H), 3.97 (m, 1H), 4.28 (m, 1H), 6.23 (dd, $J_{1'}$ = 7.9 Hz, $J_{1',2''}$ = 0.9 Hz, 1H), 6.81 (d, *J* = 8.9 Hz, 4H), 7.20 (t, *J* = 7.0 Hz, 1H), 7.26 (t, *J* = 7.5 Hz, 2H), 7.29 (d, *J* = 8.6 Hz, 4H), 7.38 (d, *J* = 7.5 Hz, 2H), 7.70 (s, 1H). ^{13}C-NMR ($CDCl_3$): δ 164.3, 158.7, 150.8, 144.7, 137.0, 135.8, 129.9, 128.0, 127.9, 127.1, 113.4, 110.3, 88.8, 88.0, 86.5, 74.6, 63.0, 55.2, 40.0, 12.5. Anal. Calcd for $C_{31}H_{32}N_2O_7$: C, 68.37; H, 5.92; N, 5.14. Found: C, 68.44; H, 5.89; N, 5.12.

General Procedure for the Preparation of the α-deoxyribonucleoside phosphoramidites 9a-d from 8a-d. Dry α-(N^2-isobutyryl)-3′-*O*-(di-*p*-methoxytrityl)-2′-deoxyguanosine (**8d**, 1.65 g, 2.58 mmol) and 221 mg of the *N,N*-diisopropylammonium salt of 1*H*-tetrazole (1.29 mmol) were dissolved in dry dichloromethane (15 mL). 2-Cyanoethyl *N,N,N′*-tetraisopropylphosphorodiamidite (932 mg, 3.1 mmol) in dry dichloromethane (2 mL) was next added to the solution under an inert atmosphere. The reaction mixture was stirred at ambient temperature for 3 h and, then, quenched by the addition of a solution of 0.5% triethylamine in dichloromethane (80 mL) and a saturated aqueous solution of sodium bicarbon-

(29) The α,β-deoxyribonucleoside derivatives **8a-d**, **9a-d**, and **10** did not show sharp melting points because of premature foaming. Consequently, melting points will not be reported for these compounds.

ate (50 mL). The two layers were separated, and the organic phase was washed with brine. The organic layer was dried ($MgSO_4$), filtered, and evaporated to dryness under vacuo and the crude product purified by silica gel chromatography. The α-deoxyribonucleoside phosphoramidite **9d** eluted from the column with dichloromethane:ethyl acetate (3:7) containing triethylamine (0.5%), and was isolated as a white foam. Purified **9d** was triturated with hexane, filtered, washed with hexane, and dried to give 1.981 g of a white powder (2.36 mmol, 91%). FAB-MS: *m/e* 862 $(M + Na)^+$; 840 $(M + H)^+$. ^{31}P-NMR (CD_3CN): δ 149.6 and 149.4 ppm. ^{31}P-NMR ($CDCl_3$): δ 149.6 and 147.8 ppm. The α-deoxyribonucleoside phosphoramidites **9a-c** were also prepared according to this protocol. These were purified by silica gel chromatography that used 30% ethyl acetate in dichloromethane containing triethylamine (0.5%) as eluent. The phosphoramidites **9a-c** were isolated in yields comparable to that of **9d**. **α-[5′-*O*-[(*N,N*-Diisopropylamino)-(2-cyanoethoxy)phosphinyl]-3′-*O*-(di-*p*-methoxytrityl)]-thymidine (9c).** FAB-MS: *m/e* 767 $(M + Na)^+$. ^{31}P-NMR ($CDCl_3$): δ 149.7 and 149.4 ppm. **α-[(*N*⁴-Benzoyl)-5′-*O*-[(*N,N*-diisopropylamino)(2-cyanoethoxy)phosphinyl]-3′-*O*-(di-*p*-methoxytrityl)]-2′-deoxycytidine (9a).** FAB-MS: *m/e* 856 $(M + Na)^+$; 834 $(M + H)^+$. ^{31}P-NMR ($CDCl_3$): δ 149.2 and 149.1 ppm. **α-[(*N*⁶-Benzoyl)-5′-*O*-[(*N,N*-diisopropylamino)(2-cyanoethoxy)phosphinyl]-3′-*O*-(di-*p*-methoxytrityl)]-2′-deoxyadenosine (9b).** FAB-MS: *m/e* 880 $(M + Na)^+$; 858 $(M + H)^+$. ^{31}P-NMR ($CDCl_3$): δ 149.6 and 149.4 ppm.

Derivatization of LCAA-CPG with α-(*N*⁶-Benzoyl)-3′-*O*-(di-*p*-methoxytrityl)-2′-deoxyadenosine (11). To α-(*N*⁶-benzoyl)-3′-*O*-(di-*p*-methoxytrityl)-2′-deoxyadenosine (**8b**, 263 mg, 0.4 mmol) in dry pyridine (800 μL) were added (*N,N*-dimethylamino)pyridine (49 mg, 0.4 mmol) and succinic anhydride (64 mg, 0.64 mmol). The solution was stirred under an inert atmosphere for 20 h at ambient temperature, and then water (100 μL) was added. The solution was stirred for 1 h, and concentrated under reduced pressure, and the residue coevaporated with toluene (3 × 5 mL). The reaction product was dissolved in dichloromethane (40 mL) and extracted with aqueous 10% citric acid (20 mL) followed by water (2 × 20 mL). Pyridine (500 μL) was added to the organic phase which was dried over anhydrous magnesium sulfate and evaporated to dryness under vacuum. The crude product was dissolved in 5 mL of dichloromethane:pyridine (95:5) and added to 200 mL of anhydrous ethyl ether. The precipitate was triturated with ethyl ether, filtered, washed with more ethyl ether, and dried to give 290 mg of the α-deoxyribonucleoside hemisuccinic acid ester **10**. FAB-MS: *m/e* 758 $(M + H)^+$. ^{1}H-NMR ($CDCl_3$): δ 1.86 (m, $^2J_{2'',2'}$ = 14.9 Hz, 1H), 2.44 (m, $^2J_{2',2''}$ = 14.9 Hz, 1H), 2.63 (m, 4H), 3.72 (m, 2H), 3.77 (s, 6H), 4.12 (m, 1H), 4.37 (m, 1H), 6.43 (dd, $J_{1'}$ = 6.7 Hz, 1H), 6.83 (d, *J* = 8.6 Hz, 4H), 7.22 (d, *J* = 8.6 Hz, 4H), 7.30 (m, 5H), 7.51 (t, *J* = 7.4 Hz, 2H), 7.59 (t, *J* = 7.4 Hz, 1H), 8.08 (d, *J* = 7.4 Hz, 2H), 8.59 (s, 1H), 8.76 (s, 1H). ^{13}C-NMR ($CDCl_3$): δ 175.4, 171.7, 165.1, 158.9, 152.1, 151.4, 149.6, 144.4, 141.9, 135.7, 133.6, 132.7, 129.9, 128.7, 128.2, 128.0, 127.2, 123.2, 113.6, 88.2, 85.6, 85.4, 74.6, 64.2, 55.2, 39.7, 29.2, 28.9. Anal. Calcd for $C_{42}H_{39}N_5O_9$: C, 66.57; H, 5.19; N, 9.24. Found: C, 66.64; H, 5.22; N, 9.22.

The α-deoxyribonucleoside derivative **10** (182 mg, 0.24 mmol) and *p*-nitrophenol (34 mg, 0.24 mmol) were dissolved in dry dioxane (800 μL) and anhydrous pyridine (100 μL). *N,N*-Dicyclohexylcarbodiimide (50 mg, 0.24 mmol) in dry dioxane (300 μL) was added to the stirred solution, dropwise, under an inert atmosphere. After a reaction time of 6 h at 22 °C, insoluble *N,N*-dicyclohexylurea was filtered through a plug of glass wool and washed with anhydrous dioxane (2 mL). Filtrates were combined and added to 1 g of LCAA-CPG (500 Å) suspended in dry *N,N*-dimethylformamide (1.4 mL) and triethylamine (300 μL). The yellow suspension was shaken for 20 h at 22 °C by the use of an orbital shaker. The solid support was then filtered and washed with *N,N*-dimethylformamide, dioxane, methanol, and ethyl ether. The concentration of α-(*N*⁶-benzoyl)-3′-*O*-(di-*p*-methoxytrityl)-2′-deoxyadenosine covalently bound to LCAA-CPG (*ca.* 23 μmol/g) was determined spectrophotometrically at 498 nm from the release of the dimethoxytrityl cation. In order to neutralize unreacted amine functions, the CPG support **11** was shaken for 24 h with a large excess of acetic anhydride in the presence of 2,6-lutidine (ABI Capping reagent, 2 mL) and 1-methylimidazole in tetrahydrofuran (ABI Capping reagent, 2 mL). Finally, the support was thoroughly washed with methanol and ethyl ether and vacuum dried.

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 coupling reaction time was extended to 144 s for efficient syntheses of α,β-oligonucleotides and corresponding phosphorothioate analogues. The sulfur transfer reagent 3*H*-1,2-benzodithiol-3-one 1,1-dioxide was prepared, as reported in the literature,[20] and used in the synthesis of oligodeoxyribonucleoside phosphorothioates at a concentration of 0.05 M in acetonitrile.

Purification of Oligonucleotides. Deprotected oligonucleotides bearing only terminal dimethoxytrityl protecting groups were purified by reversed-phase HPLC. A programmed buffer gradient composed of 0.1 M sodium acetate (pH 7.0) and acetonitrile was pumped through a Hamilton PRP-1 column (10 mm O. D × 270 mm), at a flow rate of 2 mL/min, according to the following conditions: A linear gradient of 15% acetonitrile to 25% acetonitrile was established in 10 min and held isocratically for 5 min. Then, a linear gradient of 25% acetonitrile to 45% acetonitrile was programmed for 10 min followed by isocratic pumping for 5 min. Finally, the column was brought to original conditions through a linear gradient of 45% acetonitrile to 15% acetonitrile in 5 min and kept isocratically for 5 more min prior to the next injection.

Fractions containing a purified oligonucleotide obtained from a 1 μmol scale synthesis were collected and evaporated to dryness under reduced pressure by the use of a centrifugal evaporator. The salty oligomeric mixture was dissolved in a minimum of distilled water (*ca.* 1.5 mL) and transferred into two double-sided Biodialysers (1 mL capacity, Sialomed, Inc.) equipped with two membranes (cellulose ester) having molecular weight cutoffs of 500 Da. The dialysis chambers were filled with distilled water to eliminate entrapment of air bubbles, assembled, and immersed in a beaker containing 4 L of magnetically stirred distilled water. After a dialysis time of 3 h, the purified oligonucleotide was retrieved from the dialysis chambers with a syringe. The chambers were carefully washed with water (2 × 250 μL). The washings were combined with the dialysate and evaporated to dryness under low pressure. The residue was treated with aqueous 80% acetic acid (1 mL) for 1 h at ambient temperature, and then the solution was evaporated to dryness under vacuo. The material left was dissolved in water (500 μL) and extracted with ethyl acetate (3 × 500 μL). The aqueous extract was transferred to a 1 mL Biodialyser and dialyzed against 4 L of water for at least 6 h at 22 °C. The dialysate was withdrawn from the chamber and kept frozen at −20 °C.

Purification and analysis of the oligodeoxyribonucleotides **12**, **15**, **16**, **17**, **20**, and **22** and oligoribonucleotide **23** by polyacrylamide gel electrophoresis (Figure 1) were accomplished according to detailed literature protocols.[30]

Enzymatic Digestion of Oligonucleotides and Analysis of the Hydrolysates by Capillary Gel Electrophoresis. Snake Venom Phosphodiesterase. To an oligonucleotide (0.6 O. D_{260}) was added 1 M Tris−HCl pH 9.0 (60 μL), water to a total volume of 597 μL, and snake venom phosphodiesterase (3 μL, 9 × 10^{-3} U). **Calf Spleen Phosphodiesterase.** To an oligonucleotide (0.6 O. D_{260}) was added 1 M ammonium acetate pH 6.5 (60 μL), water to a total volume of 599 μL, and calf spleen phosphodiesterase (1 μL, 4 × 10^{-3} U). **S1 Nuclease**. To an oligonucleotide (0.6 O. D_{260}) was added

(30) (a) Damha, M. J.; Ogilvie, K. K. In *Methods in Molecular Biology, Vol. 20: Protocols for Oligonucleotides and Analogs;* Agrawal, S., Ed.; Humana Press, Inc.: Totowa, 1993; pp 81−114. (b) Beaucage, S. L. In *Methods in Molecular Biology, Vol. 20: Protocols for Oligonucleotides and Analogs;* Agrawal, S., Ed.; Humana Press, Inc.: Totowa, 1993; pp 33−61.

60 μL of 10X S1 buffer (0.33 M sodium acetate pH 4.5, 0.5 M sodium chloride, 0.3 mM zinc sulfate), water to a total volume of 596 μL, and S1 nuclease (4 μL, 40 U). The enzyme digests were incubated at 37 °C. Aliquots (300 μL) were withdrawn at predetermined time points and added to concentrated ammonium hydroxide (900 μL). The samples were subsequently evaporated to dryness under reduced pressure with a centrifugal evaporator. The dry samples were then dissolved in 37 mM Tris–phosphate buffer pH 7.6 (200 μL) and analyzed by capillary electrophoresis. The electrophoretic instrument was equipped with Micro-Gel_{100} capillaries and operated at 300V/cm in 75 mM Tris–phosphate buffer (pH 7.6) containing 10% methanol. Samples were injected electrophoretically at −5 kV for 1–5 s. Unless otherwise indicated (Table 2), the estimated percentage of oligonucleotide hydrolysis is defined as the integrated area under the peaks corresponding to fragments smaller than the full length oligomer and reported as the average of duplicated digestions.

Hypochromicity of Oligodeoxyribonucleotides 12 and 17. Each oligonucleotide (0.4 O. D_{260}) was dissolved in 1 M Tris–HCl pH 9.0 (100 μL) and water to a total volume of 995 μL. The solution was heated in a water bath (100 °C) for 2 min and, then, allowed to cool to ambient temperature (22 °C). The solution was transferred to a UV cuvette (1.0 cm path length) and the absorbance measured at 260 nm relative to a reference cell containing only the buffer. Snake venom phosphodiesterase (5 μL, 15 × 10^{-3} U) was added to the solution which was incubated, in a microcentrifuge tube, at 37 °C for 22 h to ensure complete digestion of the modified oligonucleotide **12**. UV absorbance was measured at 22 °C with respect to a reference cell containing both buffer and snake venom phosphodiesterase in proper concentrations. Percentage of hypochromicity at 260 nmm was derived from the following formula: $[(A_{monomers} - A_{oligomer})/A_{monomers}]100$. Oligodeoxyribonucleotides **12** and **17** exhibited a hypochromicity of 10% and 23%, respectively.

UV Mixing Curves. Oligonucleotides **12**, **13**, and **18** were dissolved in 1X PBS buffer (pH 7.2), and the concentration of each oligomer was determined, spectrophotometrically, at 260 nm and 22 °C. UV mixing curves were constructed according to the method of continuous variation[25] by mixing either **12** and **18** or **12** and **13** in various molar ratios while maintaining a fixed total strand concentration. The curves shown in the supplementary material were obtained by plotting the absorbance at 260 nm of a given complex as a function of its molar ratio. Oligonucleotidic solutions were mixed in a stoppered UV cuvette (1 cm path length) by repeated inversion of the cuvette and allowed to equilibrate at 22 °C for 10 min prior to each absorbance measurement. Complexes **12:18** and **12:13** exhibited maximum hypochromicities at molar ratios interpolated to *ca.* 0.5 which revealed the (1:1) stoichiometry of these complexes.

Thermal Denaturation Profiles. Individual oligonucleotide (*ca.* 0.6 OD_{260}) was mixed with a complementary oligomer in either 1X PBS buffer (pH 7.2) or 1.0 M NaCl 1X PBS buffer (see Table 3). 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 and interfaced with a personal computer. For a temperature range of 20–80 °C, 121 measurement points were taken at 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.

Thermodynamic parameters for selected DNA–DNA and DNA–RNA complexes in 1X PBS buffer (pH 7.2) were derived from individual thermal denaturation curves (Table 4). Extinction coefficients (ϵ_{260}) of α- and β-nucleosides were assumed to be similar at 25 °C.[31] Extinction coefficients of α,β-oligonucleotides and natural β-oligomers were also assumed to be similar and were calculated from those of mononucleotides and dinucleotides according to the nearest-neighbor approximation.[31] These are as follows: 23.1 × 10^4 M^{-1} cm^{-1} for **13** or **18**, 23.8 × 10^4 M^{-1} cm^{-1} for **12** or **17**, and 24.2 × 10^4 M^{-1} cm^{-1} for **23**. Each curve was fitted to a two-state model with sloping base lines. The enthalpy and entropy of dissociation ($\Delta H°$ and $\Delta S°$, respectively) were derived from a six-parameter fit by the use of the MLAB program (Civilized Software, SGI version) according to the methodology described by Marky and Breslauer.[32] Free energy of dissociation ($\Delta G°_{37}$) was calculated acording to $\Delta G°_{37} = \Delta H° - 310.15\Delta S°$. Error associated with the determination of $\Delta H°$ and $\Delta S°$ from curve fitting was *ca.* 5%.

Circular Dichroism Spectroscopy. CD spectra of the hybrids used for thermal denaturation experiments were obtained with a spectropolarimeter equipped with a data processing system for signal averaging. Each spectrum was run at 22 °C in a 1 cm path length cuvette and normalized to 1.0 absorbance unit at 260 nm in 1X PBS buffer (pH 7.2).

RNase H Assay. α,β-Oligodeoxyribonucleotide **12** (0.5 O. D_{260}) and oligoribonucleotide **23** (0.5 O. D_{260}) were dissolved in an incubation buffer (20 mM HEPES–KOH, pH 8.0; 50 mM KCl; 10 mM $MgCl_2$; 1 mM DTT) (48 μL). The complex was heated in a water bath (100 °C) for 2 min and, then, allowed to cool slowly to room temperature (22 °C). *E. coli* RNase H (2 μL, 2U) was added to the solution which was incubated at 22 °C for 40 h. An identical assay was performed with the unmodified DNA–RNA heteroduplex **17:23**. Control experiments involving the treatment of each of the oligomers **12**, **17**, and **23** with *E. coli* RNase H under the same conditions were carried out. At the conclusion of the incubation period, a loading buffer [formamide:10X TBE (4:1) containing 0.2% bromphenol blue] (5 μL) was added to each digest. The samples were heated in a water bath (100 °C) for 2 min, and 25 μL of each sample was loaded on a 20% polyacrylamide–7 M urea gel [40 cm (L) × 20 cm (W) × 0.75 mm] equilibrated with 1X TBE buffer. Electrophoresis was performed at 350 V for 22 h. The gel was then stained with 10 mL of a stock solution of Stains All (1 mg/ mL of formamide) mixed with formamide (10 mL), propan-2-ol (50 mL), 3.0 M Tris pH 8.8 (1 mL), and water (200 mL) for 30 min in the dark. Excess staining solution was discarded, and the gel was washed several times with water prior to photography with Polaroid 55 films through a yellow filter. Figure 5 shows the results of these assays.

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Supplementary Material Available: 500 MHz ^{1}H-NMR spectra of the anomeric proton of α-2′-deoxycytidine, α-2′-deoxyadenosine, and α-2′-deoxyguanosine; 300 MHz ^{1}H- and 75 MHz ^{13}C-NMR spectra of the α-2′-deoxynucleoside derivatives **6a-d**, **8a-d**, and **10**; 121 MHz ^{31}P-NMR spectra of the α-2′-deoxynucleoside 5′-phosphoramidites **9a-d** and oligodeoxyribonucleotides **15**, **16**, **20**, and **22**; FAB-MS of **6a-d**, **8a-d**, **9a-d**, and **10**; CD spectra of **12**, **13**, **17**, and **23**; UV mixing curves of the complexes **12:18** and **12:13**; thermal denaturation profiles of the DNA–DNA and DNA–RNA complexes reported in Table 3 (54 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.

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