Highly Nuclease-Resistant Phosphodiester-Type Oligodeoxynucleotides Containing 4′α-*C*-Aminoalkylthymidines Form Thermally Stable Duplexes with DNA and RNA. A Candidate for Potent Antisense Molecules†

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Abstract: The properties of phosphodiester oligodeoxynucleotides (ODNs) containing 4′α-*C*-aminomethyl, -ethyl, -propyl, and -*N*-(2-aminoethyl)carbamoylthymidines (**1**, **2**, **4**, and **5**) as potential antisense molecules are investigated in detail. We developed new radical chemistry with a vinylsilyl or an allylsilyl group as a temporary radical acceptor tether to synthesize the required 4′α-branched thymidines. Thus, an intramolecular radical cyclization of 4′-phenylseleno nucleosides **7a** and **7b**, which have a dimethylvinylsilyl and a dimethylallylsilyl group at the 3′-hydroxyl, respectively, with Bu_3SnH/AIBN and subsequent Tamao oxidation provided 5′-*O*-[dimethoxytrityl(DMTr)]-4′α-*C*-(2-hydroxyethyl)thymidine (**8a**) and 5′-*O*-DMTr-4′α-*C*-(3-hydroxypropyl)thymidine (**8b**). Compounds **8a** and **8b** were then converted into 4′α-*C*-(2-trifluoroacetamidoethyl)thymidine **12a** and 4′α-*C*-(3-trifluoroacetamidopropyl)thymidine **12b**, which were phosphitylated to give the phosphoramidite units **14a** and **14b**. The phosphoramidite units of **1** and **5** were prepared by previous methods. The nucleosides **1**, **2**, **4**, and **5** were incorporated into the 18-mer, 5′-d[MTMTMTMTMTMTMT-MTMT]-3′, where M is 5-methyl-2′-deoxycytidine, instead of T at various positions. We also prepared a 21-mer ODN **29** with a mixed sequence containing five residues of **2**. The ODNs containing the modified nucleosides formed more stable duplexes with complementary DNA than the corresponding unmodified ODN. These ODNs also formed stable duplexes with the complimentary RNA. The ODNs containing the modified nucleosides were significantly resistant to nucleolytic hydrolysis by both snake venom phosphodiesterase (a 3′-exonuclease) and DNase I (an endonuclease) and were also very stable in PBS containing 50% human serum. It is worthwhile to note that these ODNs contain natural phosphodiester linkages. Furthermore, the duplexes formed by the ODNs containing the modified nucleosides and their complementary RNAs were good substrates for *Escherichia coli* RNase H and HeLa cell nuclear extracts as a source of human RNase H. Thus, these ODNs were identified as candidates for antisense molecules.

Introduction

Oligodeoxynucleotides (ODNs) and their analogues have been shown to specifically inhibit gene expression.[1] Because of their potential to control diseases of known genetic etiology, development of these compounds as therapeutic agents is of great interest. Antisense ODNs bind to mRNAs by Watson−Crick base-pairing and inhibit translation of mRNAs in a sequence-specific manner. One of the major problems encountered when using naturally occurring phosphodiester ODNs as antisense or antigene molecules is their rapid degradation by nucleases found in cell culture media and inside cells.[1] Therefore, many types of backbone-modified ODNs such as methylphosphonates, phosphoramidates, and phosphorothioates have been synthesized and used for antisense and antigene studies.[1,2] However, the benefits of such stabilization against enzymatic degradations are sometimes counteracted by the loss of other properties that are important for antisense activity. Phosphorothioate ODNs tend to have lower binding affinity for their complementary RNA targets than unmodified phosphodiester ODNs, presumably because they are diastereomeric mixtures at the thiophosphodiester linkages.[3] Although RNase H cleavage is important in antisense strategy, RNA is not a substrate for the enzyme when methylphosphonate ODN is the complementary strand.[4] Furthermore, phosphorothioate ODNs have been reported to exhibit non-sequence-specific activity.[5]

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ODNs having natural phosphodiester linkages have also been studied as antisense molecules. These ODNs form a stable duplex specifically with complementary RNA and also elicit significant RNase H activity in the duplex with their complementary RNA strands but are often rapidly hydrolyzed by nucleases because of their natural phosphodiester linkages.[1] We hypothesized that the natural phosphodiester ODNs carrying basic amino alkyl chains near their phosphodiester moieties might be resistant to nucleases. Nucleases hydrolyze phosphodiester linkages by a general acid−base catalysis mechanism, including acidic and/or basic amino acid residues at their active sites. The presence of a basic amino group very near the phosphodiester moiety of ODNs may prevent nucleolytic hydrolysis by forming an ionic bond with the acidic phosphodiester moiety of ODNs. It is also possible that the amino group attached to ODNs interrupts the catalytic system of nucleases by bonding with an acidic amino acid residue or by repulsing a basic amino acid residue at the enzyme active sites.

On the other hand, naturally occurring polyamines, such as spermidine and spermine, are known to bind strongly to DNAs and to stabilize duplex and triplex formation.[6] The enhanced thermal stability of duplexes and triplexes is explained by the reduction of the anionic electrostatic repulsion between the phosphate moieties by the cationic amino groups. Therefore, attaching amino groups to ODNs should effectively increase the thermal stability of the duplexes and triplexes.[7]

Wang and Seifert reported that ODNs containing 4′-*C*-(aminomethyl)thymidine (**1**) exhibited hybridization to both complementary DNA and RNA strands that were similar to or better than those of unmodified ODNs and were more resistant to nucleolytic hydrolysis by snake venom phosphodiesterase than unmodified ODNs, although endonuclease resistance was not examined (Figure 1).[8]

Based on these findings and considerations, we designed 4′-*C*-[2-[[*N*-(2-aminoethyl)carbamoyl]oxy]ethyl]thymidine (**5**) as a nucleoside unit for introducing into ODNs,[9] since the 4′α-position of nucleosides is very close to the internucleotide phosphodiester linkages in ODNs. As expected, we found that ODNs containing **5** were more resistant to nucleolytic hydrolysis by both snake venom phosphodiesterase (a 3′-exonuclease) and DNase I (an endonuclease) than unmodified parent ODNs, while the modified ODNs only slightly destabilized duplexes with both complementary DNA and RNA strands.[9]

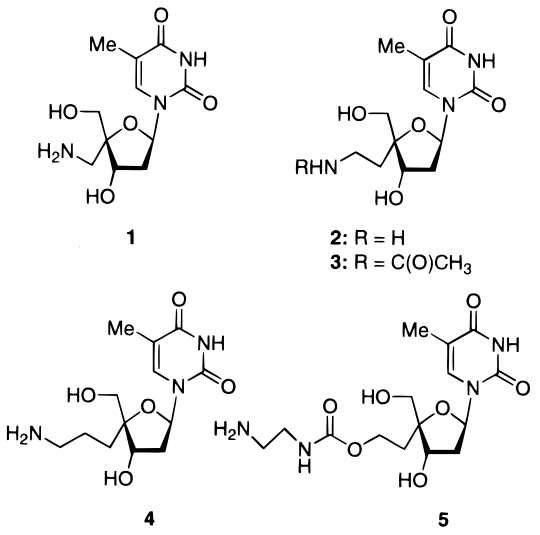

Figure 1. Structures of the modified nucleoside analogues.

These results suggest that ODNs containing nucleosides with an aminoalkyl chain at the 4′α-position, which would be accommodated in the minor groove of B-type DNA, are good candidates for novel antisense molecules and prompted us to further our study. If it is indeed true that the aminoalkyl chain attached to the 4′α-position of ODNs interrupts the hydrolysis of phosphodiester linkage by nucleases and/or stabilizes duplexes, then the three-dimensional positioning of the amino group in ODNs is pivotal to its functioning. Thus, we designed the thymidine analogues **2** and **4** with different lengths of aminoalkyl chains at their 4′α-*C*-positions as possible nucleoside units for our antisense study. We synthesized ODNs containing thymidine analogues **2** and **4**, as well as the previously reported analogues **1** and **5**, with natural phosphodiester linkages. The thermal stability of duplexes containing these ODNs and their complementary DNA and RNA strands was studied as well as the resistance of these ODNs to nucleolytic hydrolysis by snake venom phosphodiesterase and DNase I (an endonuclease). In addition, we also examined whether the duplexes of these ODNs with their complementary RNA strands could elicit RNase H activity. This report describes the detailed results of these studies.

Results and Discussion

Synthesis. A general method for preparing 4′α-aminoalkyl nucleosides was needed for our antisense study, since an efficient method for preparing 4′α-branched nucleoside did not exist.[10] Consequently, we developed new radical chemistry using a vinylsilyl[11] or an allylsily group as a temporary radical acceptor tether.

Thus, an intramolecular radical cyclization reaction of **7a**, which has a dimethylvinylsilyl group at the 3′-hydroxyl, with Bu_3SnH/AIBN and subsequent Tamao oxidation[12] readily provided the 5′-*O*-[dimethoxytrityl(DMTr)]-4′α-*C*-(2-hydroxyethyl)thymidine (**8a**).[9,11] The 5′-*O*-DMTr-4′α-*C*-(3-hydroxypropyl)thymidine (**8b**) was also synthesized by a similar intramo-

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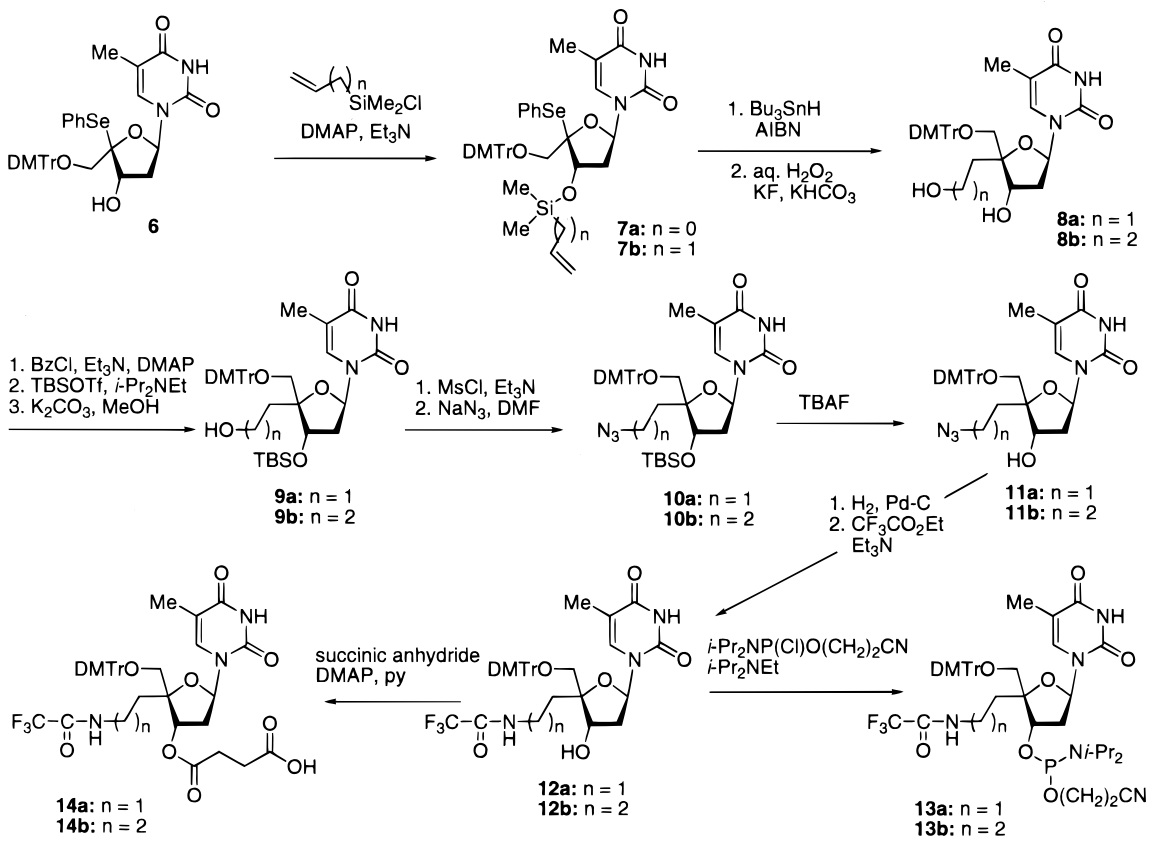
Scheme 1



lecular radical cyclization reaction at the 4′-position with an allyldimethylsilyl group[13] as a radical acceptor tether. The 4′-phenylselenothymidine derivative **6**[14] was treated with allylchlorodimethylsilane and Et_3N in the presence of DMAP in toluene to afford an allylsilyl derivative **7b**, the substrate for the radical reaction, in 92% yield. When a solution of 1.5 equiv of Bu_3SnH and AIBN (0.05 equiv) in benzene was added slowly over 8 h to a solution of **7b** in benzene (0.01 M) at 80 °C, the desired 4′α-(3-hydroxypropyl)thymidine **8b**, which was derived from the 7-*endo*-cyclized product, was isolated in 66% yield, after treatment of the radical reaction products under Tamao oxidation conditions.

Compounds **8a** and **8b** were converted into the 4′α-*C*-trifluoroacetamidoethyl- and 4′α-*C*-trifluoroacetamidopropyl-thymidine derivatives **12a** and **12b**, respectively. After protection of the primary hydroxyl group of **8a** with a benzoyl (Bz) group, the 3′-hydroxyl group was then protected with a *tert*-butyldimethylsilyl (TBS) group. Subsequent removal of the Bz group gave the properly protected thymidine derivative **9a** in 70% yield from **8a**. Methanesulfonylation of **9a** followed by displacement with azide ion afforded the azidoethyl derivative **10a**, which was then treated with tetrabutylammonium fluoride (TBAF) to give **11a** in high yield. Catalytic hydrogenation of **11a** with Pd−C in MeOH and subsequent protection of the generated amino group with a trifluoroacetyl group afforded the desired 4′-branched thymidine derivative **12a** in 73% yield. In a similar manner, the corresponding trifluoroacetamidopropyl derivative **12b** was synthesized from **8b**.

The 4′α-branched nucleosides **12a** and **12b** were phosphitylated by the standard procedure[15] to give the corresponding phosphoramidites **13a** and **13b** in 91% and 62% yields, respectively, which were used as the nucleotide units for the DNA synthesizer. To incorporate **2** and **4** into the 3′-end of the ODNs, **12a** and **12b** were further modified to produce the corresponding 3′-succinates **14a** and **14b**, which were then reacted with controlled pore glass (CPG) to give a solid support containing **2** (28 μmol/g) and **4** (38 μmol/g), respectively (Scheme 1).

The ODNs used in this study were synthesized on a DNA synthesizer by the phosphoramidite method.[16] The nucleosides **1**, **2**, **4**, and **5** were incorporated into the 18-mer, 5′-d[MTMT-MTMTMTMTMTMTMT]-3′, where M is 5-methyl-2′-deoxycytidine, instead of T at various positions. We also prepared a 21-mer ODN **29** with a mixed sequence [5′-d[ACETGATEG-CAEAAAECTTAE]-3′, where E is **2**]. The sequences of the

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Table 1. Sequences of ODNs[a]

ODNs	
15	5′-MTMTMTMTMTMTMTMTMT-3′
16-Y, -E, -P, or -Z	5′-M$\underline{\text{T}}$MTMTMTMTMTMTMTMT-3′
17-Y, -E, -P, or -Z	5′-M$\underline{\text{T}}$MTMTMTMTMTMTMT$\underline{\text{T}}$−3′
18-Y, -E, -P, or -Z	5′-MTMTMTMTM$\underline{\text{T}}$MTMTMT$\underline{\text{T}}$-3′
19-Y, -E, -P, or -Z	5′-MTMTMTMTM$\underline{\text{T}}$MTMTMTMT-3′
20-Y, -E, -P, or -Z	5′-MTMTM$\underline{\text{T}}$MTMTMTM$\underline{\text{T}}$MTMT−3′
21-Y, -E, -P, or -Z	5′-M$\underline{\text{T}}$MTMTMTMTM$\underline{\text{T}}$MTMTMTM$\underline{\text{T}}$−3′
22-Y, -E, -P, or -Z	5′-M$\underline{\text{T}}$MTM$\underline{\text{T}}$MTMTMTM$\underline{\text{T}}$MTMTM$\underline{\text{T}}$−3′
23	5′-M$\underline{\text{T}}$MTM$\underline{\text{T}}$MTMTM$\underline{\text{T}}$MTM$\underline{\text{T}}$MTM$\underline{\text{T}}$−3′
24	5′-d[$\underline{\text{T}}$G(GA)$_9$GGT]−3′
25	5′-r[(AG)$_9$A]−3′
26	5′-d[ACTTGATTGCATAAATCTTAT]−3′
27	5′-d[ATAAGATTTATGCAATCAAGT]−3′
28	5′-r[AUAAGAUUUAUGCAAUCAAGU]−3′
29	5′-d[AC**E**TGAT**E**GCA**E**AAA**E**CTTA**E**]−3′

[a] **Y** = ($\underline{\text{T}}$ = **1**); **E** = ($\underline{\text{T}}$ = **2**); **P** = ($\underline{\text{T}}$ = **4**); **Z** = ($\underline{\text{T}}$ = **5**); **23** = ($\underline{\text{T}}$ = **3**); M = 5-methyl-2′-deoxycytidine.

ODN analogues synthesized are shown in Table 1. The ODNs containing the nucleoside **1** were synthesized according to the method reported by Wang and Seifert.[8] The ODNs containing the nucleoside **5** were also synthesized by a previously described method.[9] The average coupling yields of **13a** and **13b** were 92% and 93%, respectively, using 0.12 M solutions of the amidites in CH_3CN and a coupling time of 300 s. The fully protected ODNs (1 μmol) linked to the solid supports were treated with concentrated NH_4OH at 55 °C for 16 h, followed by C-18 column chromatography; detritylation gave the ODNs in 10−48 OD_{260} units. The ODN **22-E**, which contains five residues of **2**, was treated with Ac_2O in 0.2 M HEPES buffer to give the ODN **23**. Each ODN analogue obtained showed a single peak on reversed-phase HPLC. Furthermore, these ODNs were analyzed by electrospray ionization (ESI) mass spectrometry, and the observed molecular weights supported their structures (see Experimental Section).

Thermal Stability. Thermal stability of duplexes formed by these ODNs and the complementary DNA, 5′−d[TG(GA)$_9$GGT]-3′ (**24**), was studied by thermal denaturation in a buffer of 0.01 M sodium phosphate (pH 7.0) containing 0.01 M NaCl. Each profile of the thermal denaturation showed a single transition corresponding to a helix-to-coil transition (data not shown). Melting temperatures (T_ms) are listed in Table 2. The T_m of the control ODN **15** was 54.4 °C. All of the ODNs containing **1**, **2**, or **4** stabilized the ODN/DNA duplexes. The stability of the duplexes was dependent on the position, number, and length of the aminoalkyl linkers of the modified nucleosides. The ODNs, which contained one residue of **1**, **2**, or **4** at their center, stabilized the duplexes more than the ODNs containing one residue of **1**, **2**, or **4** at their 3′-ends or near their 5′-ends. The duplexes became more stable as the number of **1**, **2**, or **4** increased. The ΔT_m^1 values [T_m (each ODN) − T_m (the control ODN **15**)] for the ODNs containing five residues of **1**, **2**, or **4** were +4.5, +5.7, or +4.1 °C, respectively. The ODNs containing one or two residues of **5** destabilized the duplexes, whereas the ODNs containing three, four, or five residues of **5** stabilized the duplexes. The ΔT_m^1 value for the ODNs containing five residues of **5** was +2.4 °C. The ΔT_m^1 value for the ODNs containing **2** was greater than those for the ODNs containing the same numbers of **1**, **4**, or **5** except for the ODNs **17-P** and **21-P**. Additionally, the ΔT_m^1 value for the 21-mer ODN **29** with a mixed sequence, which has five residues of **2**, was +6.8 °C. Therefore, analogues containing **2** with an aminoethyl chain at the 4′α-position seemed to efficiently stabilize the ODN/DNA duplexes.

Table 2. Hybridization Data[a]

	ODN/DN[b]				ODN/RN[c]	
ODN	0.01 M NaCl T_m, °C	ΔT_m^1 [d] °C	0.1 M NaCl T_m, °C	ΔT_m^2 [e] °C	0.1 M NaCl T_m, °C	ΔT_m^1 [d] °C
15	54.5		65.6	+11.2	73.9	
16-Y	54.4	+0.0			73.9	+0.0
17-Y	54.4	+0.0			73.7	−0.2
18-Y	54.5	+0.1	65.9	+11.4	73.3	−0.6
19-Y	55.4	+1.0			72.9	−1.0
20-Y	56.8	+2.4	66.9	+10.1	73.0	−0.9
21-Y	57.5	+3.1			72.3	−1.6
22-Y	58.9	+4.5	68.7	+9.8	71.7	−2.2
16-E	55.0	+0.6			73.7	−0.2
17-E	54.5	+0.1			73.8	−0.1
18-E	55.5	+1.1	67.2	+11.7	73.5	−0.4
19-E	55.7	+1.3			72.1	−1.8
20-E	56.9	+2.5	67.7	+10.8	72.3	−1.6
21-E	58.1	+3.7			71.5	−2.4
22-E	60.1	+5.7	70.1	+10.0	71.2	−2.7
16-P	54.4	+0.0			73.3	−0.6
17-P	54.6	+0.2			73.4	−0.5
18-P	55.1	+0.7	65.8	+10.7	73.1	−0.8
19-P	55.6	+1.2			72.5	−1.4
20-P	56.4	+2.0	67.3	+10.9	72.5	−1.4
21-P	58.3	+3.9			72.0	−1.9
22-P	58.5	+4.1	68.5	+10.0	71.2	−2.7
16-Z	52.1	−2.3			73.2	−0.7
17-Z	52.7	−1.7			73.5	−0.4
18-Z	50.8	−3.6	64.9	+14.1	72.8	−1.1
19-Z	53.9	−0.5			72.3	−1.6
20-Z	54.9	+0.5	65.7	+10.8	72.6	−1.3
21-Z	55.6	+1.2			71.8	−2.1
22-Z	56.8	+2.4	68.5	+11.7	71.4	−2.5
23	48.3	−6.1	63.1	+14.8		
26	42.3	−			−49.8	
29	49.1	+6.8			49.4	−0.4

[a] Experimental conditions are described in the Experimental Section. [b] The complementary DNA: **24** for **15**−**23**; **27** for **26** and **29**. [c] The complementary RNA: **25** for **15**−**23**; **28** for **26** and **29**. [d] ΔT_m^1 = [T_m(each ODN) − T_m(the control ODN **15**)]. [e] ΔT_m^2 = [T_m(0.1 M NaCl) − T_m (0.01 M NaCl)].

On the other hand, the ODN **23** containing five residues of **3**, which has an acetamidoethyl chain at the 4′-position, destabilized the ODN/DNA duplex as compared with the control duplex (ΔT_m^1 = −6.1 °C). Thus, the enhanced thermal stability of the duplexes containing **2** was likely due to the effect of the terminal ammonium ion.

To confirm the effects of the ammonium ions at the end of the alkyl chains of **1**, **2**, **4**, and **5** on the thermal stabilities of the duplexes, thermal denaturation was also performed under higher ionic strength (0.1 M NaCl). The ΔT_m^2 values obtained [T_m (0.1 M NaCl) − T_m (0.01 M NaCl)] were compared, as shown in Table 2. The ΔT_m^2 values for the ODNs containing three or five residues of **1**, **2**, or **4** were smaller than those for the control ODN **15** (+11.2 °C), whereas the ΔT_m^2 values for the ODNs containing **5** or **3** were similar to or lower than those for the control ODN **15**. These results suggest that the terminal ammonium ions in **1**, **2**, and **4** effectively neutralize the phosphate negative charges.[17]

(17) Models of ODN/DNA and ODN/RNA duplexes containing **2** were derived from molecular dynamics (MD) simulations. Initial structures of ODN/DNA (B-form) and ODN/RNA (A-form) duplexes were generated using Biopolymer's molecular modeling system (Molecular Simulations Inc., San Diego, CA). In the ODN containing the **2**/DNA duplex, the ammonium ion at the end of the aminoethyl chain of **2** forms an intramolecular ionic bond with the *pro-S*-oxygen atom of the phosphodiester linked to the 3′-position of **2**. In the ODN containing **2**/RNA duplex, the ammonium ion of **2** intramolecularly interacts with the *pro-S*-oxygen atoms of both the 3′- and 5′-phosphate groups of **2**.

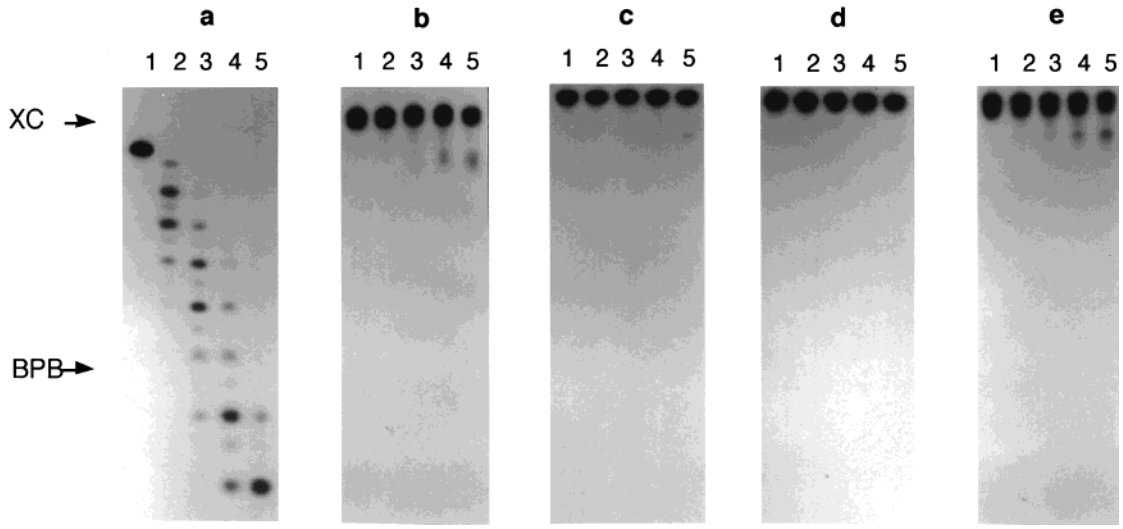

Figure 2. Polyacrylamide gel electrophoresis of 5′-^{32}P-labeled ODNs hydrolyzed by snake venom phosphodiesterase: (a) **15**; (b) **22-Y**; (c) **22-E**; (d) **22-P**; (e) **22-Z**. ODNs were incubated with snake venom phosphodiesterase for 0 min (lane 1), 10 min (lane 2), 30 min (lane 3), 60 min (lane 4), and 120 min (lane 5). Experimental conditions are described in the Experimental Section.

Stable duplex formation with mRNA is one of the most important factors in antisense research. The thermal stability of the duplexes between these ODNs and a complementary RNA, 5′-r[(AG)$_9$A]-3′ (**25**), was next studied by thermal denaturation in a buffer of 0.01 M sodium phosphate containing 0.1 M NaCl. Each profile of the thermal denaturation showed a single transition corresponding to a helix-to-coil transition (data not shown). Melting temperatures (T_ms) are listed in Table 2. The T_m of the control ODN **15** was 73.9 °C. All of the ODNs containing **1**, **2**, **4**, or **5** slightly destabilized the ODN/RNA duplexes. The stability of the duplexes was dependent on the position and number of the modified nucleosides. The ODNs which contained one residue of **1**, **2**, **4**, or **5** at their center destabilized the duplexes more than the ODNs containing one residue of **1**, **2**, **4**, or **5** at their 3′-ends or near their 5′-ends, respectively. The duplexes became less stable as the number of the modified nucleosides increased. However, even when five residues of **1**, **2**, **4**, or **5** were incorporated into the 18-mers, the ΔT_m^1 values for these ODNs were −2.2, −2.7, −2.7, and −2.5 °C, respectively. The ΔT_m^1 value for the ODN **29** with a mixed sequence, which has five residues of **2**, was only −0.4 °C. Therefore, DNA/RNA duplexes formed by the ODNs containing **1**, **2**, **4**, or **5** are stable enough for use in antisense studies.

Circular Dichroism. To study the global conformation of the duplexes, we measured the circular dichroism (CD) spectra of duplexes composed of ODNs containing five residues of **1**, **2**, **4**, or **5** (ODNs **22-Y**, **22-E**, **22-P**, and **22-Z**, respectively) and either the complementary DNA (**24**) or RNA (**25**) at 15 °C.

With DNA (**24**) as a complementary strand, the spectrum of the control duplex (**15**) showed a positive CD band at 281 nm and a negative CD band at 239 nm which were attributable to a B-like DNA conformation (Supporting Information). The shapes of the spectra of the duplexes containing **1**, **2**, **4**, or **5** were similar to those of the control duplex. However, the positive CD bands in the spectra of the duplexes containing **1**, **2**, **4**, or **5** were slightly shifted to longer wavelengths (ca. 1−3 nm) and the intensity of the negative bands at around 240 nm was increased compared with those for the control duplex. Furthermore, the intensity of the positive bands in the spectra of the duplexes containing **1**, **2**, or **4** was slightly reduced and that of the duplex containing **5** was slightly increased, relative to that of the control duplex.

On the other hand, when RNA (**25**) was used as a complementary strand, the spectrum of the control duplex (**15**) showed positive CD bands at 231 and 267 nm and a large negative CD band at 212 nm (Supporting Information). The spectra of the ODNs containing **1**, **2**, **4**, or **5** were slightly different from those of the control duplex (Supporting Information). The spectra of the ODNs containing **1**, **2**, **4**, or **5** showed red shifts (ca. 16 nm) of the positive CD bands to around 283 nm and slight reductions in the intensity of the negative bands at 212 nm, relative to that of the control duplex. Furthermore, the intensity of the positive bands in the spectra of the duplexes containing **1**, **2**, or **4** was slightly reduced while that of the duplex containing **5** was slightly increased, relative to that of the control duplex.

These results suggest that the modified nucleosides only slightly affect the global conformations of both ODN-DNA and ODN−RNA duplexes.

Nuclease Resistance. The susceptibility of the ODNs to nucleolytic digestion was examined. Two kinds of nucleases, snake venom phosphodiesterase and DNase I, were used in this study as models for a 3′-exonuclease and an endonuclease, respectively. The stability of the ODNs in human serum was also investigated.

The ODNs **22-Y**, **22-E**, **22-P**, and **22-Z** containing five residues of each nucleoside analogue were labeled at the 5′-end with ^{32}P and incubated with snake venom phosphodiesterase or DNase I. The reactions were then analyzed by polyacrylamide gel electrophoresis under denaturing conditions.[18]

Figure 2 shows the results with snake venom phosphodiesterase. Although the control **15** was hydrolyzed randomly within 10 min, the phosphodiester linkages at the 5′-sides of the modified nucleosides were resistant to the nuclease. After 1 h, no enzymatic degradation of the ODNs containing **2** or **4** was observed at all. The half-lives of ODNs **15**, **22-Y**, **22-E**, **22-P**, and **22-Z** were about 2 min, 2.9 h, 14.4 h, 17.8 h, and 5.2 h, respectively. Among the ODNs, the ODNs **22-E** containing **2** and **22-P** containing **4** were highly resistant to snake venom phosphodiesterase.

The phosphodiester linkages around the modified nucleosides were also highly resistant to the endo-hydrolysis by DNase I.

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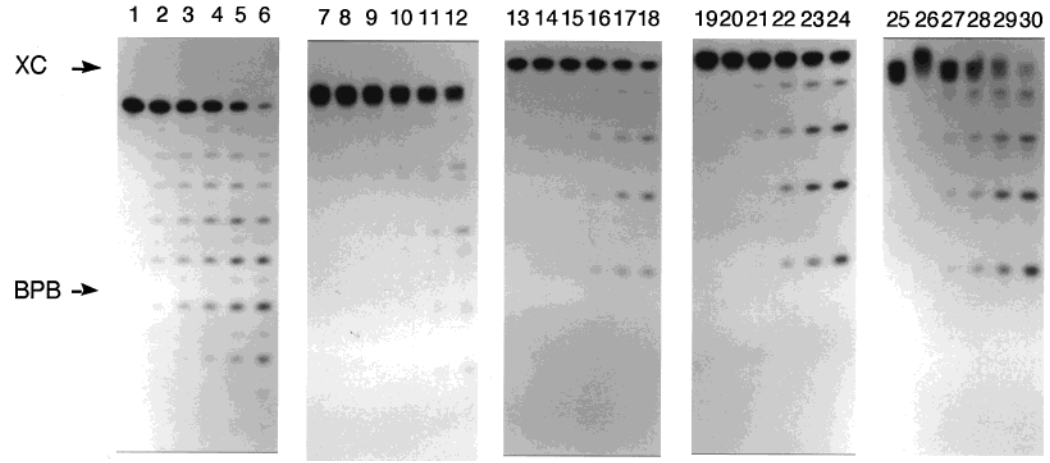

Figure 3. Polyacrylamide gel electrophoresis of 5′-^{32}P-labeled ODNs hydrolyzed by DNase I: **15** (lanes 1−6); **22-Y** (lanes 7−12); **22-E** (lanes 13−18); **22-P** (lanes 19−24); **22-Z** (lanes 25−30). ODNs were incubated with DNase I for 0 min (lanes 1, 7, 13, 19, and 25), 6 min (lane 2), 18 min (lane 3), 30 min (lane 4), 1 h (lane 5), 2 h (lanes 6, 8, 14, 20, and 26), 6 h (lanes 9, 15, 21, and 27), 12 h (lanes 10, 16, 22, and 28), 24 h (lanes 11, 17, 23, and 29), and 48 h (lanes 12, 18, 24, and 30). Experimental conditions are described in the Experimental Section.

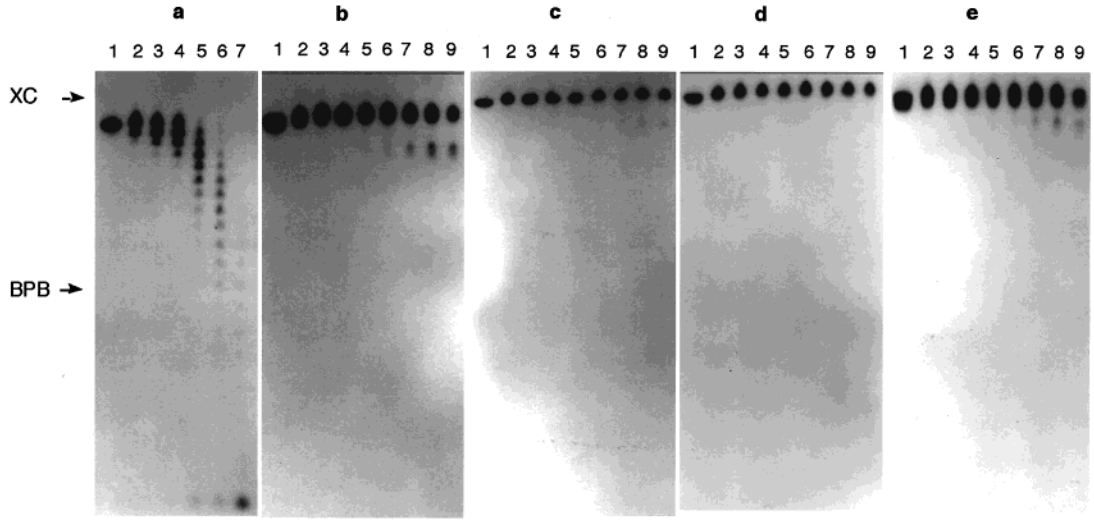

Figure 4. Polyacrylamide gel electrophoresis of 5′-^{32}P-labeled ODNs incubated in PBS containing 50% human serum: (a) **15**; (b) **22-Y**; (c) **22-E**; (d) **22-P**; (e) **22-Z**. ODNs were incubated for 0 min (lane 1), 15 min (lane 2), 30 min (lane 3), 1 h (lane 4), 3 h (lane 5), 6 h (lane 6), 12 h (lane 7), 24 h (lane 8), and 48 h (lane 9). Experimental conditions are described in the Experimental Section.

The half-lives of the ODNs **22-Y**, **22-E**, **22-P**, and **22-Z** were about 27, 29, 28, and 13 h, respectively, while that of the control was 20 min (Figure 3). Interestingly, the ODNs **22-Y**, **22-E**, and **22-P** having relatively shorter aminoalkyl chains were more resistant to the nuclease than the ODN **22-Z** with a longer aminoalkyl chain.

Next, we examined the effects of the terminal ammonium cations of the aminoalkyl chains on the resistance of the ODNs to an endonuclease. We compared the susceptibility of ODN **23** containing **3** having an acetamidoethyl chain at the 4′α-position to nucleolytic digestion by DNase I with that of ODN **22-E** containing **2** having an aminoethyl chain (Supporting Information). The half-lives of the ODN **15**, **22-E**, and **23** were 35 min, 30 h, and 3.4 h, respectively. The ODN **23** containing **3** was much less resistant to the nuclease than the ODN containing **2**. Therefore, this suggested to us that the effects of the terminal ammonium ions of the aminoalkyl linkers played an important role in nuclease resistance of the ODNs.

Antisense ODNs should be stable in blood, which contains various enzymes including nucleases. Therefore, we investigated the stabilities of ODNs **22-Y**, **22-E**, **22-P**, and **22-Z** in human serum. A mixture of ^{32}P-labeled ODN **22-Y**, **22-E**, **22-P**, or **22-Z** (at their 5′-ends) with their unlabeled ODN (25 μM) was incubated separately in PBS containing 50% human serum and analyzed by 20% PAGE under denaturing conditions (Figure 4). While the half-life of the control **15** was 27 min, it was 1.7, 2.5, 7.3, and 2.7 days respectively for ODN **22-Y**, **22-E**, **22-P**, or **22-Z**. This experiment clearly shows that the ODNs containing the modified nucleosides are extremely stable in human serum.

We also investigated the susceptibility of the ODN **29** with a mixed sequence against DNase I and human serum. While the half-life of the control ODN **26** was 7 min, the ODN **29** was not hydrolyzed after 48 h incubation against DNase I under the same conditions described above. The ODN **29** was also about 21-fold more stable than the control ODN **26** during incubation in human serum described above ($t_{1/2}$ of **26** and **29**, 37 min and 13 h, respectively; supporting information).

From these results, the ODNs containing nucleosides with the aminoalkyl chains at the 4′α-position, especially those containing nucleosides with the aminoethyl and aminopropyl chains, were found to be resistant enough to enzymatic degradations to be used as antisense molecules.

Degradation by RNase H. It has been postulated that antisense activity of antisense ODNs is due, at least in part, to cleavage of the RNA strand of a DNA/RNA duplex by RNase

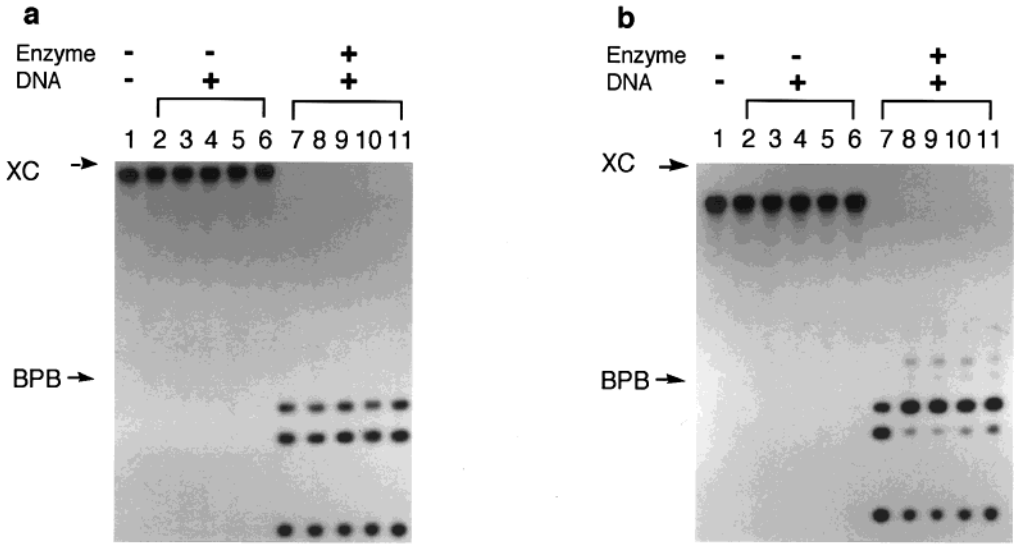

Figure 5. Polyacrylamide gel electrophoresis of 5′-^{32}P-labeled RNA **25** hydrolyzed by *E. coli* RNase H in the presence or in the absence of complementary strands: (a) lane 1, **25**; lane 2, **25** + **15**; lane 3, **25** + **20-Y**; lane 4, **25** + **20-E**; lane 5, **25** + **20-P**; lane 6, **25** + **20-Z**; lane 7, **25** + **15** + enzyme; lane 8, **25** + **20-Y** + enzyme; lane 9, **25** + **20-E** + enzyme; lane 10, **25** + **20-P** + enzyme; lane 11, **25** + **20-Z** + enzyme; (b) lane 1, **25**; lane 2, **25** + **15**; lane 3, **25** + **22-Y**; lane 4, **25** + **22-E**; lane 5, **25** + **22-P**; lane 6, **25** + **22-Z**; lane 7, **25** + **15** + enzyme; lane 8, **25** + **22-Y** + enzyme; lane 9, **25** + **22-E** + enzyme; lane 10, **25** + **22-P** + enzyme; lane 11, **25** + **22-Z** + enzyme. RNAs were incubated with *E. coli* RNase H in the presence or in the absence of the complementary strands at 30 °C for 20 min (a) or 40 min (b). Experimental conditions are described in the Experimental Section.

H.[1c,19] We therefore examined whether the ODN/RNA heteroduplex between an ODN containing three residues (**20-Y**, **20-E**, **20-P**, or **20-Z**, respectively) and five residues (**22-Y**, **22-E**, **22-P**, or **22-Z**, respectively) of **1**, **2**, **4**, or **5** and its complementary RNA **25** could elicit RNase H activity. The duplexes consisting of these ODNs and RNA **25** labeled with ^{32}P at the 5′-end were incubated with *Escherichia coli* RNase H, and the products were analyzed by polyacrylamide gel electrophoresis (Figure 5). The RNAs in the duplexes with the ODNs containing three residues of the modified nucleosides were completely hydrolyzed after 20 min, and the rates were similar to those of a control experiment with **15**. When the ODNs contained five residues of the modified nucleosides, the complementary RNAs in the duplexes were also degraded, although the rates were slightly decreased compared with those in the unmodified duplex. The ODN **29** with a mixed sequence also elicited effective cleavage of the complementary RNA **28** (supporting information).

For oligonucleotide therapeutics, antisense ODNs are often required to elicit RNase H activity in human cells. Therefore, we next examined cleavage of RNAs by HeLa cell nuclear extracts as a source of human RNase H.[20] The experiments were carried out using the same heteroduplexes described above. All the RNA strands in the duplexes were found to be effectively cleaved by HeLa cell nuclear extracts (Figure 6).

E. coli RNase H requires at least four contiguous unmodified 2′-deoxyribonucleotide residues to elicit cleavage of the RNA strand,[21] and a minimum of five contiguous unmodified 2′-deoxyribonucleotides was required for efficient activation of HeLa RNase H.[20b] Therefore, it is noteworthy that ODNs **22** elicit efficient cleavage of the RNA strand by RNase H, although they have four regions of three contiguous unmodified 2′-deoxyribonucleotide residues in the antisense strand.

Conclusion

To develop the nuclease-resistant antisense and antigene ODNs with natural phosphodiester linkages, we designed and synthesized ODNs containing 4′α-*C*-aminoalkylthymidines. The nucleoside units **2**, **4**, and **5** were efficiently synthesized, using novel radical chemistry as the key step.

The ODNs containing these nucleosides increased the thermal stability of the duplexes with their complementary DNAs. Among the ODNs, the one containing **2** with the aminoethyl chain thermally stabilized the ODN/DNA duplex the most. The ΔT_m[1] value [T_m (the duplex containing the modified nucleoside) − T_m (the control duplex)] for the ODN/DNA duplex **22-E** and **29** containing five residues of **2** was +5.7 and +6.8 °C, respectively.

The ODNs containing these nucleosides formed stable duplexes with the complimentary RNA, although the thermal stability was slightly decreased compared with that of the control duplex. The ΔT_m[1] values for the ODN/RNA duplexes containing five residues of **1**, **2**, **4**, or **5** were about −2 °C. However, the ΔT_m[1] value for the 21 mer duplex between mixed sequence ODN **29** and RNA **28** was reduced to −0.4 °C. Therefore, the DNA/RNA duplexes formed by the ODNs would be stable enough for exhibiting antisense activities.

The ODNs containing **1**, **2**, **4**, or **5** were significantly resistant to both snake venom phosphodiesterase and DNase I, and were also very stable in human serum. The stability of these ODNs against enzymatic hydrolyses is noteworthy, since they have natural phosphodiester linkages. Furthermore, the duplexes formed by the ODNs containing **1**, **2**, **4**, or **5** and their complementary RNAs were good substrates for *E. coli* and human RNase H.

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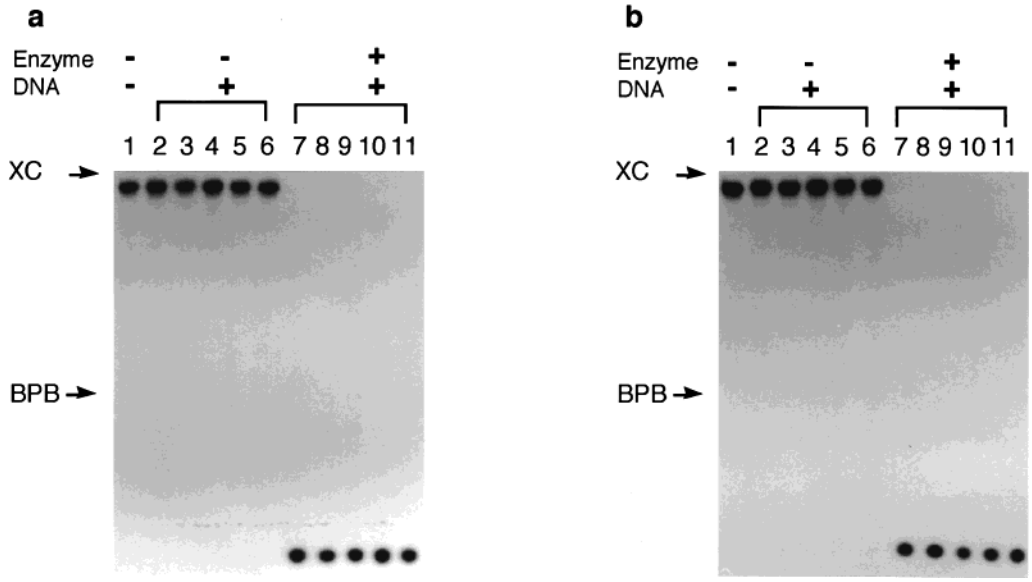

Figure 6. Polyacrylamide gel electrophoresis of 5′-^{32}P-labeled RNA **25** hydrolyzed by HeLa RNase H in the presence or in the absence of complementary strands: (a) lane 1, **25**; lane 2, **25** + **15**; lane 3, **25** + **20-Y**; lane 4, **25** + **20-E**; lane 5, **25** + **20-P**; lane 6, **25** + **20-Z**; lane 7, **25** + **15** + enzyme; lane 8, **25** + **20-Y** + enzyme; lane 9, **25** + **20-E** + enzyme; lane 10, **25** + **20-P** + enzyme; lane 11, **25** + **20-Z** + enzyme; (b) lane 1, **25**; lane 2, **25** + **15**; lane 3, **25** + **22-Y**; lane 4, **25** + **22-E**; lane 5, **25** + **22-P**; lane 6, **25** + **22-Z**; lane 7, **25** + **15** + enzyme; lane 8, **25** + **22-Y** + enzyme; lane 9, **25** + **22-E** + enzyme; lane 10, **25** + **22-P** + enzyme; lane 11, **25** + **22-Z** + enzyme. RNAs were incubated with HeLa cell nucleoar extracts in the presence or in the absence of the complementary strands at 30 °C for 20 min. Experimental conditions are described in the Experimental Section.

These results demonstrated that the natural phosphodiester ODNs containing the 4′α-aminoalkyl nucleosides, especially the nucleoside **2** with the aminoethyl chain, possess the desired properties for an antisense and/or antigene molecule: (1) formation of the stable duplex with complimentary DNA and RNA, (2) resistance to nucleases, and (3) RNase H activity in the duplex with RNA. Thus, the ODNs are good candidates for antisense and antigene molecules. Applications of the ODN containing **2** as an antisense ODN are currently being studied.

Experimental Section

NMR spectra were recorded at 270 or 500 (^{1}H), at 100 or 125 (^{13}C), and at 202 MHz (^{31}P) and are reported in ppm downfield from TMS or 85% H_3PO_4. *J* values are given in hertz. Mass spectra were obtained by the fast atom bombardment (FAB) method. Thin-layer chromatography was done on Merck coated plates $60F_{254}$. The silica gel or the neutralized silica gel used for column chromatography were Merck silica gel 5715 or ICN silica 60A, respectively.

1-[3-*O*-(Allyldimethylsilyl)-2-deoxy-5-*O*-(dimethoxytrityl)-4-*C*-(phenylseleno)-α-L-*threo*-pento-1,4-furanosyl]thymine (7b). A mixture of **6** (2.8 g, 4.0 mmol), allylchlorodimethylsilane (1.2 mL, 8.0 mmol), DMAP (100 mg, 0.8 mmol), and Et_3N (1.1 mL, 8.0 mmol) in toluene (100 mL) was stirred at room temperature for 30 min. After insoluble materials were filtered off, the filtrate was partitioned between EtOAc and H_2O. The organic layer was washed with brine, dried (Na_2SO_4), and evaporated under reduced pressure. The residue was purified by column chromatography (SiO_2, 50% EtOAc in hexane) to give **7b** (2.98 g, 92% as a white foam): FAB-MS *m*/*z* 799 (MH^+); ^{1}H NMR (270 MHz, $CDCl_3$) δ 8.55 (br s, 1 H), 7.54−6.79 (m, 19 H), 6.54 (dd, 1 H, J = 7.4, 6.2), 5.53 (ddd, 1 H, J = 7.9, 11.2, 15.9), 4.76 (dd, 2 H, J = 11.2, 15.9), 4.44 (dd, 1 H, J = 5.5, 3.3), 3.78 (s, 6 H), 3.60 (d, 1 H, J = 10.5), 3.00 (d, 1 H, J = 10.5), 2.48 (ddd, 1 H, J = 7.4, 5.5, 13.2), 2.35 (ddd, 1 H, J = 6.2, 3.3, 13.2), 1.96 (s, 3 H), 1.35 (d, 2 H, J = 7.9), −0.16, −0.17 (each s, each 3 H). HRMS (FAB) calcd for $C_{42}H_{47}N_2O_7SeSi$: 799.2314. Found: 799.2302. Anal. Calcd for $C_{42}H_{46}N_2O_7SeSi\cdot{}^1/_4H_2O$: C, 62.87; H, 5.84; N, 3.49. Found: C, 62.95; H, 6.01; N, 3.46.

5′-*O*-(Dimethoxytrityl)-4′-*C*-(3-hydroxypropyl)thymidine (8b). A solution of Bu_3SnH (1.0 mL, 3.75 mmol) and AIBN (20 mg, 0.12 mmol) in benzene (20 mL) was added slowly over 8 h to a solution of **7b** (2.0 g, 2.5 mmol) in benzene (250 mL) at 80 °C. The solvent was evaporated under reduced pressure, and the residue was partitioned between MeCN and hexane. The MeCN layer was evaporated under reduced pressure. The residue was dissolved in MeOH/THF (1:1, 50 mL), and aqueous H_2O_2 (30%, 1.4 mL, 12.5 mmol), KF (750 mg, 12.5 mmol), and $KHCO_3$ (400 mg, 0.4 mmol) were added to the above solution. The resulting mixture was stirred at room temperature overnight. Aqueous $Na_2S_2O_3$ (1 M, 60 mL) was added, and the resulting insoluble materials were filtered off. The filtrate was evaporated under reduced pressure, and the residue was purified by column chromatography (SiO_2, 4% MeOH in $CHCl_3$) to give **8b** (995 mg, 66% as a white foam): FAB-MS *m*/*z* 603 (MH^+); ^{1}H NMR (270 MHz, $CDCl_3$) δ 8.77 (br s, 1 H), 7.51 (s, 1 H), 7.41−6.82 (m, 13 H), 6.35 (t, 1 H, J = 6.3), 4.53 (m, 1 H), 3.79 (s, 6 H), 3.58 (m, 2 H), 3.28 (s, 2 H), 2.41 (br d, 2 H, J = 6.3), 1.79−1.38 (m, 4 H), 1.49 (s, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.70, 158.60, 150.53, 144.15, 135.60, 135.25, 130.04, 128.09, 127.92, 127.09, 113.22, 111.14, 88.60, 87.15, 83.70, 77.21, 73.58, 66.64, 62.74, 55.27, 40.76, 28.39, 26.67, 11.94. HRMS (FAB) calcd for $C_{34}H_{39}N_2O_8$: 603.2704. Found: 603.2715. Anal. Calcd for $C_{34}H_{38}N_2O_8\cdot{}^7/_4H_2O$: C, 64.39; H, 6.60; N, 4.42. Found: C, 64.51; H, 6.43; N, 4.43.

3′-*O*-(*tert*-Butyldimethylsilyl)-5′-*O*-(dimethoxytrityl)-4′-*C*-(2-hydroxyethyl)thymidine (9a). To a solution of **8a** (588 mg, 1.0 mmol) in CH_2Cl_2 (20 mL) at −78 °C was added a solution of DMAP (18 mg, 0.15 mmol), Et_3N (181 μL, 1.3 mmol), and BzCl (151 μL, 1.3 mmol) in CH_2Cl_2 (5 mL) slowly, and the resulting mixture was stirred at −78 °C for 10 min. After H_2O was added, the resulting mixture was diluted with $CHCl_3$. The organic layer was washed with H_2O and brine, dried (Na_2SO_4), and evaporated under reduced pressure. The residue was purified by column chromatography (SiO_2, 50% EtOAc in hexane) to give the 4′-*C*-(benzoyloxyethyl)thymidine derivative. To a solution of the above compound in CH_2Cl_2 (10 mL) at −18 °C was added *N*,*N*-diisopropylethylamine (410 μL, 2.4 mmol), and TBSOTf (550 μL, 2.4 mmol, 3.0), and the resulting mixture was stirred at room temperature for 1 h. After MeOH was added, the resulting mixture was evaporated under reduced pressure, and the residue was partitioned between EtOAc and H_2O. The organic layer was washed with brine, dried (Na_2SO_4), and evaporated under reduced pressure. A mixture of the residue and K_2CO_3 (166 mg, 1.2 mmol) in MeOH (30 mL) was stirred at room

temperature overnight. The solvent was evaporated under reduced pressure, and the residue was purified by column chromatography (SiO_2, 50% EtOAc in hexane) to give **9a** (494 mg, 70% as a white foam): FAB-MS *m*/*z* 703 (MH^+); ^{1}H NMR (270 MHz, $CDCl_3$) δ 8.30 (br s, 1 H), 57.54 (s, 1 H), 7.41−6.82 (m, 13 H), 6.29 (t, 1 H, J = 6.6), 4.61 (dd, 1 H, J = 4.9, 6.6), 3.79 (s, 6 H), 3.70 (m, 2 H), 3.38 (d, 1 H, J = 10.3), 3.16 (d, 1 H, J = 10.3), 2.32 (ddd, 1 H, J = 6.6, 4.9, 10.5), 2.21 (ddd, 1 H, J = 6.6, 6.6, 10.5), 1.70−1.59 (m, 2 H), 1.47 (s, 3 H), 0.85 (s, 9 H), 0.05, −0.02 (each s, each 3 H). HRMS (FAB) calcd for $C_{39}H_{51}N_2O_8Si$: 703.3411. Found: 703.3438. Anal. Calcd for $C_{39}H_{50}N_2O_8Si$: C, 66.64; H, 7.17; N, 3.99. Found: C, 66.71; H, 7.23; N, 3.89.

4′-*C*-(Azidoethyl)-3′-*O*-(*tert*-butyldimethylsilyl)-5′-*O*-(dimethoxytrityl)thymidine (10a). To a solution of **9a** (562 mg, 0.8 mmol) in CH_2Cl_2 (15 mL) at 0 °C was added Et_3N (223 μL, 1.6 mmol) and MsCl (124 μL, 1.6 mmol), and the resulting mixture was stirred at room temperature for 1.5 h. After H_2O was added, the resulting mixture was diluted with $CHCl_3$. The organic layer was washed with H_2O and brine, dried (Na_2SO_4), and evaporated under reduced pressure. A solution of the residue and NaN_3 (520 mg, 8.0 mmol) in DMF (10 mL) was stirred at room temperature overnight. After H_2O was added, the resulting mixture was diluted with EtOAc. The organic layer was washed with H_2O and brine, dried (Na_2SO_4), and evaporated under reduced pressure. The residue was purified by column chromatography (SiO_2, 30% EtOAc in hexane) to give **10a** (488 mg, 83% as a white foam): FAB-MS *m*/*z* 728 (MH); ^{1}H NMR (270 MHz, $CDCl_3$) δ 8.08 (br s, 1 H), 7.54 (s, 1 H), 7.42−6.82 (m, 13 H), 6.23 (t, 1 H, J = 6.4), 4.61 (dd, 1 H, J = 4.5, 6.5), 3.80 (s, 6 H), 3.39 (ddd, 1 H, J = 6.6, 9.3, 12.1), 3.33 (d, 1 H, J = 10.2), 3.24 (ddd, 1 H, J = 5.7, 9.3, 12.1), 3.11 (d, 1 H, J = 10.2), 2.29 (dd × 2, 2 H, J = 6.4, 4.5, 6.5), 2.00 (ddd, 1 H, J = 9.3, 5.7, 14.6), 1.68 (ddd, 1 H, J = 6.6, 9.3, 14.6), 1.48 (s, 3 H), 0.87 (s, 9 H), 0.06, 0.008 (each s, each 3 H); ^{13}C NMR (125 Mz, $CDCl_3$) δ 163.95, 159.05, 150.50, 144.34, 135.61, 135.41, 135.38, 130.35, 130.33, 128.40, 128.25, 127.48, 113.55, 111.34, 87.83, 87.32, 84.17, 73.26, 65.86, 55.50, 47.20, 41.23, 31.56, 25.56, 18.20, 12.12, 11.69, −4.42, −4.83; IR (Nujol) 2095 cm^{-1} (−N_3). HRMS (FAB) calcd for $C_{39}H_{50}N_5O_7Si$: 728.3479. Found: 728.3455. Anal. Calcd for $C_{39}H_{49}N_5O_7Si\cdot{}^1/_4H_2O$: C, 63.95; H, 6.81; N, 9.56. Found: C, 63.95; H, 6.84; N, 9.57.

4′-*C*-(Azidoethyl)-5′-*O*-(dimethoxytrityl)thymidine (11a). A mixture of **10a** (73 mg, 0.1 mmol) and TBAF (1 M in THF, 200 μL, 0.2 mmol) in THF (5 mL) was stirred at room temperature for 3 h. The resulting mixture was evaporated under reduced pressure, and the residue was purified by column chromatography (SiO_2, 2% MeOH in $CHCl_3$) to give **11a** (53 mg, 86% as a white foam): FAB-MS *m*/*z* 613 (M^+); ^{1}H NMR (270 MHz, $CDCl_3$) δ 8.20 (br s, 1 H), 7.43 (s, 1 H), 7.39−6.85 (m, 13 H), 6.31 (t, 1 H, J = 6.6), 4.45 (dd, 1 H, J = 9.9, 4.2), 3.81 (s, 6 H), 3.44 (dd, 1 H, J = 6.2, 12.6), 3.30 (d, 1 H, J = 10.0), 3.26 (d, 1 H, J = 10.0), 3.21 (dd, 1 H, J = 6.2, 12.6), 2.47 (d, 1 H, J = 4.2), 2.43 (dd, 2 H, J = 6.6, 9.9), 1.98 (t, 2 H, J = 6.2), 1.56 (s, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.66, 144.00, 135.04, 135.04, 134.94, 130.00, 128.01, 127.95, 127.15, 113.26, 111.12, 87.59, 87.20, 84.10, 73.68, 66.55, 47.03, 140.49, 31.07, 12.04; IR (Nujol) 2093 cm^{-1} (−N_3). HRMS (FAB) calcd for $C_{33}H_{35}N_5O_7$: 613.2534. Found: 613.2528. Anal. Calcd for $C_{33}H_{35}N_5O_7\cdot{}^3/_4H_2O$: C, 63.89; H, 5.76; N, 10.96. Found: C, 63.78; H, 5.93; N, 11.22.

5′-*O*-(Dimethoxytrityl)-4′-*C*-[(2-*N*-trifluoroacetyl)aminoethyl]-thymidine (12a). A mixture of **11a** (44 mg, 72 mmol) and Pd−C (10%, 10 mg) in MeOH (5 mL) was stirred under atmospheric pressure of H_2 at room temperature overnight. The catalyst was filtered off with Celite, and the filtrate was evaporated under reduced pressure. A solution of the residue, Et_3N (50 μL, 0.36 mmol), and ethyl trifluoroacetate (43 μL, 0.16 mmol) in MeOH (10 mL) was stirred at room temperature for 2 h. The solvent was evaporated under reduced pressure, and the residue was purified by column chromatography (SiO_2, 2% MeOH in $CHCl_3$) to give **12a** (36 mg, 73% as a white foam): FAB-MS *m*/*z* 684 (MH^+); ^{1}H NMR (270 MHz, $CDCl_3$) δ 8.23 (br s, 1 H), 7.40 (s, 1 H), 7.38−6.84 (m, 13 H), 7.12 (br s, 1 H), 6.34 (t, 1 H, J = 7.1), 4.52 (t, 1 H, J = 4.5), 3.81 (s, 6 H), 3.47−3.34 (m, 2 H), 3.26 (m, 2 H), 2.41 (dd × 2, 2 H, J = 7.1, 4.5), 2.00 (m, 2 H), 1.58 (s, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.87, 158.65, 150.75, 143.94, 135.44, 134.94, 134.85, 129.98, 127.98, 127.94, 127.15, 113.26, 111.43, 88.24, 87.33, 84.26, 77.21, 73.77, 66.63, 55.22, 40.52, 35.79, 30.76, 11.91. HRMS (FAB) calcd for $C_{35}H_{37}N_3O_8F_3$: 684.253. Found: 684.2544. Anal. Calcd for $C_{35}H_{36}N_3O_8F_3\cdot{}^8/_5H_2O$: C, 59.00; H, 5.54; N, 5.89. Found: C, 58.84; H, 5.28; N, 5.79.

3′-*O*-(*tert*-Butyldimethylsilyl)-5′-*O*-(dimethoxytrityl)-4′-*C*-(3-hydroxypropyl)thymidine (9b). To a solution of **8b** (1.28 g, 2.1 mmol) in CH_2Cl_2 (80 mL) at −78 °C was added a solution of DMAP (30 mg, 0.25 mmol), Et_3N (380 μL, 2.73 mmol), and BzCl (316 μL, 2.73 mmol) in CH_2Cl_2 (10 mL) slowly, and the resulting mixture was stirred at −78 °C for 1 h and then at 0 °C for 1 h. After H_2O was added, the resulting mixture was diluted with $CHCl_3$. The organic layer was washed with H_2O, aqueous $NaHCO_3$ (saturated), and brine, dried (Na_2SO_4), and evaporated under reduced pressure. The residue was purified by column chromatography (SiO_2, 1% MeOH in $CHCl_3$) to give the 4′-*C*-(benzoyloxypropyl)thymidine derivative. A mixture of the above compound, imidazole (849 mg, 12.5 mmol), and TBSCl (468 mg, 3.12 mmol) in DMF (20 mL) was stirred at room temperature overnight. After H_2O was added, the resulting mixture was evaporated under reduced pressure, and the residue was partitioned between EtOAc and H_2O. The organic layer was washed with brine, dried (Na_2SO_4), and evaporated under reduced pressure. A mixture of the residue and K_2CO_3 (216 mg, 1.56 mmol) in MeOH (30 mL) was stirred at room temperature overnight. The solvent was evaporated under reduced pressure, and the residue was purified by column chromatography (SiO_2, 1% MeOH in $CHCl_3$) to give **9b** (845 mg, 54% as a white foam): FAB-MS *m*/*z* 716 (M^+); ^{1}H NMR (270 MHz, $CDCl_3$) δ 8.40 (br s, 1 H), 7.66 (s, 1 H), 7.41−9.82 (m, 13 H), 6.24 (t, 1 H, J = 6.3), 4.68 (dd, 1 H, J = 5.3, 6.9), 3.79 (s, 6 H), 3.58 (m, 2 H), 3.20 (d, 1 H, J = 10.0), 3.08 (d, 1 H, J = 10.0), 2.35 (ddd, 1 H, J = 6.3, 5.3, 13.5), 2.24 (ddd, 1 H, J = 6.3, 6.9, 13.5), 1.75−1.45 (m, 4 H), 1.42 (s, 3 H), 0.84 (s, 9 H), 0.04, −0.04 (each s, each 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.73, 158.55, 150.23, 144.11, 135.48, 135.22, 130.02, 130.01, 128.11, 127.84, 127.06, 113.15, 113.11, 110.91, 88.49, 86.79, 83.43, 72.17, 65.23, 63.21, 55.24, 41.22, 28.45, 26.86, 25.73, 17.99, 11.83, −4.55, −4.98. HRMS (FAB) calcd for $C_{40}H_{52}N_2O_8Si$: 716.3489. Found: 716.3464. Anal. Calcd for $C_{40}H_{52}N_2O_8Si\cdot{}^3/_4H_2O$: C, 65.77; H, 7.38; N, 3.84. Found: C, 65.75; H, 7.25; N, 3.92.

4′-*C*-(Azidopropyl)-3′-*O*-(*tert*-butyldimethylsilyl)-5′-*O*-(dimethoxytrityl)thymidine (10b). To a solution of **9b** (716 mg, 1.0 mmol) in CH_2Cl_2 (20 mL) at 0 °C was added Et_3N (279 μL, 2.0 mmol) and MsCl (155 μL, 2.0 mmol), and the resulting mixture was stirred at room temperature for 2 h. After H_2O was added, the resulting mixture was diluted with $CHCl_3$. The organic layer was washed with H_2O and brine, dried (Na_2SO_4), and evaporated under reduced pressure. A solution of the residue and NaN_3 (650 mg, 10 mmol) in DMF (10 mL) was stirred at room temperature overnight. After MeOH was added, the resulting mixture was evaporated under reduced pressure, and the residue was partitioned between EtOAc and H_2O. The organic layer was washed with brine, dried (Na_2SO_4), and evaporated under reduced pressure. The residue was purified by column chromatography (SiO_2, 30% EtOAc in hexane) to give **10b** (669 mg, 90% as a white foam): FAB-MS *m*/*z* 741 (M^+); ^{1}H NMR (270 MHz, $CDCl_3$) δ 8.82 (br s, 1 H), 7.59 (s, 1 H), 7.58−6.82 (m, 13 H), 6.23 (dd, 1 H, J = 6.3, 6.6), 4.63 (dd, 1 H, J = 4.6, 6.6), 3.80 (s, 6 H), 3.32 (d, 1 H, J = 10.2), 3.22 (m, 2 H), 3.08 (d, 1 H, J = 10.2), 2.33 (ddd, 1 H, J = 6.3, 4.6, 13.5), 2.22 (ddd, 1 H, J = 6.6, 6.6, 13.5), 1.76−1.47 (m, 4 H), 1.46 (s, 3 H), 0.85 (s, 9 H), 0.063, −0.02 (each s, each 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.67, 158.61, 150.19, 144.07, 135.37, 135.15, 130.01, 128.08, 127.87, 127.09, 113.19, 113.17, 110.94, 88.38, 86.87, 83.63, 72.62, 65.33, 55.25, 52.00, 41.26, 29.48, 25.74, 23.46, 18.01, 11.90, −4.56, −4.98; IR (Nujol) 2095 cm^{-1} (−N_3). HRMS (FAB) calcd for $C_{40}H_{51}N_5O_7Si$: 741.3555. Found: 741.3530. Anal. Calcd for $C_{40}H_{51}N_5O_7Si$: C, 64.75; H, 6.92; N, 9.44. Found: C, 64.84; H, 7.04; N, 9.21.

4′-*C*-(Azidopropyl)-5′-*O*-(dimethoxytrityl)thymidine (11b). A mixture of **10b** (532 mg, 0.72 mmol) and TBAF (1 M in THF, 1.4 mL, 1.4 mmol) in THF (20 mL) was stirred at room temperature for 3 h. The resulting mixture was evaporated under reduced pressure, and the residue was purified by column chromatography (SiO_2, 60% EtOAc in hexane) to give **11b** (390 mg, 86% as a white foam): FAB-MS *m*/*z* 627 (M^+); ^{1}H NMR (270 MHz, $CDCl_3$) δ 8.33 (br s, 1 H), 7.38 (s, 1 H), 7.41−6.83 (m, 13 H), 6.26 (t, 1 H, J = 6.8), 4.53 (dd, 1 H, J =

10.6, 4.3), 3.80 (s, 6 H), 3.24 (m, 4 H), 2.37 (ddd, 2 H, J = 6.8, 10.6, 13.8), 2.03 (d, 1 H, J = 4.3), 1.74−1.45 (m, 4 H), 1.55 (s, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.57, 158.63, 150.28, 144.09, 135.38, 135.13, 135.07, 129.99, 128.02, 127.95, 127.12, 113.25, 111.09, 87.91, 87.10, 83.69, 73.63, 66.20, 55.26, 51.85, 40.60, 28.96, 23.32, 12.06; IR (Nujor) 2093 cm^{-1} (−N_3). HRMS (FAB) calcd for $C_{34}H_{37}N_5O_7$: 627.2690. Found: 627.2712. Anal. Calcd for $C_{34}H_{37}N_5O_7$: C, 65.06; H, 5.94; N, 11.16. Found: C, 65.04; H, 6.05; N, 10.99.

5′-*O*-(Dimethoxytrityl)-4′-*C*-[(2-*N*-trifluoroacetyl)aminopropyl]-thymidine (12b). A mixture of **11b** (390 mg, 0.62 mmol) and Pd−C (10%, 40 mg) in MeOH (15 mL) was stirred under atmospheric pressure of H_2 at room temperature overnight. The catalyst was filtered off with Celite, and the filtrate was evaporated under reduced pressure. A solution of the residue, Et_3N (431 μL, 3.1 mmol), and ethyl trifluoroacetate (370 μL, 3.1 mmol) in MeOH (10 mL) was stirred at room temperature overnight. The solvent was evaporated under reduced pressure, and the residue was purified by column chromatography (SiO_2, 1% MeOH in $CHCl_3$) to give **12b** (343 mg, 79% as a white foam): FAB-MS m/z 697 (M^+); ^{1}H NMR (270 MHz, $CDCl_3$) δ 8.33 (br s, 1 H), 7.36 (s, 1 H), 7.39−6.83 (m, 13 H), 6.70 (br s, 1 H), 6.31 (t, 1 H, J = 6.7), 4.54 (dd, 1 H, J = 5.0, 3.7), 3.80 (s, 6 H), 3.29 (m, 2 H), 3.24 (s, 2 H), 2.39 (m, 2 H, J = 6.7, 5.0), 2.22 (d, 1 H, J = 3.7), 1.74−1.57 (m, 4 H), 1.54 (s, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.80, 158.63, 150.73, 144.06, 135.47, 135.07, 135.02, 130.01, 129.99, 128.01, 127.92, 127.12, 113.35, 88.47, 87.18, 83.93, 77.21, 73.77, 66.47, 55.24, 40.78, 40.33, 29.25, 23.06, 11.90. HRMS (FAB) calcd for $C_{36}H_{38}F_3N_3O_8$: 697.2608. Found: 697.2617. Anal. Calcd for $C_{36}H_{38}F_3N_3O_8 \cdot {}^3/_2H_2O$: C, 59.66; H, 5.70; N, 5.80. Found: C, 59.64; H, 5.46; N, 5.71.

5′-*O*-(Dimethoxytrityl)-4′-*C*-[(2-*N*-trifluoroacetyl)aminoethyl]-thymidine 3′-*O*-(2-Cyanoethyl) *N*,*N*-Diisopropylphosphoramidite (13a). After successive coevaporation with pyridine, **12a** (410 mg, 0.60 mmol) was dissolved in CH_2Cl_2 (10 mL) containing *N*,*N*-diisopropylethylamine (210 μL, 1.2 mmol). Chloro(2-cyanoethoxy)(*N*,*N*-diisopropylamino)phosphine (200 μL, 0.90 mmol) was added to the solution, and the reaction mixture was stirred at room temperature for 1 h. Aqueous $NaHCO_3$ (saturated) and $CHCl_3$ were added to the mixture, and the separated organic layer was washed with aqueous $NaHCO_3$ (saturated) and brine, dried (Na_2SO_4), and concentrated. The residue was purified by column chromatography (a neutralized SiO_2, 40% EtOAc in hexane) to give **13a** (481 mg, 91% as a white foam): FAB-MS m/z 884 (MH^+); ^{31}P NMR δ 150.39, 149.86. HRMS (FAB) calcd for $C_{44}H_{56}F_3N_5O_9P$: 884.3608. Found: 884.3605.

5′-*O*-(Dimethoxytrityl)-4′-*C*-[(2-*N*-trifluoroacetyl)aminoethyl]-3′-*O*-(succinyl)thymidine (14a). After successive coevaporation with pyridine, **12a** (136 mg, 200 μmol) was dissolved in pyridine (3 mL). Succinic anhydride (40 mg, 400 μmol) and DMAP (4.0 mg, 33 μmol) were added to the solution, and the mixture was stirred at room temperature for 3 days. The mixture was diluted with EtOAc and washed with H_2O, aqueous KH_2PO_4 (saturated), and then brine. The separated organic phase was dried (Na_2SO_4) and evaporated. The residue was purified by column chromatography (SiO_2, 5% MeOH in $CHCl_3$) to give **14a** (115 mg, 73% as a white powder): FAB-MS m/z 783 (M^+); ^{1}H NMR (270 MHz, $CDCl_3$) δ 7.36 (s, 1 H), 7.41−6.80 (m, 13 H), 6.27 (dd, 1 H, J = 6.4, 7.6), 5.50 (m, 1 H), 3.77 (s, 6 H), 3.37−3.23 (m, 4 H), 2.62 (m, 4 H), 2.43 (br dd, 2 H, J = 6.4, 7.6), 2.01 (m, 1 H), 1.81 (m, 1 H), 1.47 (s, 3 H). HRMS (FAB) calcd for $C_{39}H_{40}F_3N_3O_{11}$: 783.2612. Found: 783.2613.

5′-*O*-(Dimethoxytrityl)-4′-*C*-[(2-*N*-trifluoroacetyl)aminopropyl]-thymidine 3′-*O*-(2-Cyanoethyl) *N*,*N*-Diisopropylphosphoramidite (13b). Compound **12b** (343 mg, 0.49 mmol) was phosphitylated as described in the preparation of **13a** to give **13b** (273 mg, 62% as a white foam): FAB-MS m/z 898 (MH^+); ^{31}P NMR δ 149.76, 149.24. HRMS (FAB) calcd for $C_{45}H_{56}F_3N_5O_9P$: 898.3764. Found: 898.3780.

5′-*O*-(Dimethoxytrityl)-4′-*C*-[(2-*N*-trifluoroacetyl)aminopropyl]-3′-*O*-(succinyl)thymidine (14b). Compound **12b** (139 mg, 0.2 mmol) was succinylated as described in the preparation of **14a** to give **14b** (52 mg, 33% as a white powder): FAB-MS m/z 797 (M^+); ^{1}H NMR (270 MHz, $CDCl_3$) δ 7.39 (s, 1 H), 7.51−6.73 (m, 13 H), 6.23 (m, 1 H), 5.42 (m, 1 H), 3.79 (m, 6 H), 3.38−3.19 (m, 4 H), 2.70−2.48 (m, 4 H), 2.45−2.34 (m, 2 H), 1.81−1.40 (m, 4 H), 1.50 (s, 3 H). HRMS (FAB) calcd for $C_{40}H_{42}F_3N_3O_{11}$: 797.2769. Found: 797.2763.

Synthesis of the Controlled Pore Glass Support with 14a or 14b. Aminopropyl controlled pore glass (390 mg, 35.0 μmol, 89.8 μmol/g, CPG Inc.) was added to a solution of **14a** (110 mg, 0.14 mmol) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimido hydrochloride (WSCI) (27 mg, 0.14 mmol) in DMF (4 mL), and the mixture was kept at room temperature for 2 days. After the resin was washed with pyridine, 3 mL of a capping solution (0.1 M DMAP in pyridine:Ac_2O = 9:1) was added, and the whole was kept at room temperature for 12 h. The resin was washed with EtOH and acetone and was dried under vacuum. The amount of loaded nucleoside **14a** to the solid support is 28 μmol/g from the calculation of released dimethoxytrityl cation by a solution of 70% $HClO_4$:EtOH (3:2, v/v). In a similar manner, the solid supports with **14b** were obtained in 38 μmol/g of loading amounts.

Synthesis of ODNs. ODNs were synthesized on a DNA synthesizer (Applied Biosystem model 392) by the phosphoramidite method. The fully protected ODNs were then deblocked and purified by the same procedure as for the purification of normal ODNs. That is, each ODN linked to the resin was treated with concentrated NH_4OH at 55 °C for 16 h, and the released ODN protected by a DMTr group at the 5′-end was chromatographed on a C-18 silica gel column (1 × 10 cm, Waters) with a linear gradient of MeCN from 0 to 30% in 0.1 M TEAA buffer (pH 7.0). The fractions were concentrated, and the residue was treated with aqueous 80% AcOH at room temperature for 20 min, then the solution was concentrated, and the residue was coevaporated with H_2O. The residue was dissolved in H_2O and the solution was washed with Et_2O, then the H_2O layer was concentrated to give a deprotected ODN **16-Y** (16), ODN **17-Y** (30), ODN **18-Y** (30), ODN **19-Y** (24), ODN **20-Y** (23), ODN **21-Y** (22), ODN **22-Y** (20), ODN **16-E** (22), ODN **17-E** (48), ODN **18-E** (39), ODN **19-E** (22), ODN **20-E** (20), ODN **21-E** (22), ODN **22-E** (34), ODN **16-P** (14), ODN **17-P** (13), ODN **18-P** (19), ODN **19-P** (40), ODN **20-P** (17), ODN **21-P** (10), ODN **22-P** (12), ODN **16-Z** (19), ODN **17-Z** (26), ODN **18-Z** (26), ODN **19-Z** (20), ODN **20-Z** (27), ODN **21-Z** (23), ODN **22-Z** (20), and ODN **29** (49). The yields are indicated in parentheses as OD units at 260 nm starting from 1-μmol scale.

Acetylation of ODN 22-E. A solution containing ODN **22-E** (1.0 OD unit at 260 nm) and Ac_2O (2 μL) in 0.2 M HEPES buffer (200 μL, pH 7.2) was kept for 1 h at room temperature. Concentrated NH_4OH (400 μL) was added to the mixture, and the whole was kept overnight at 4 °C. The solvent was removed in vacuo, and the residue was purified by HPLC with a C18 column to give ODN **23** (0.7 OD 260 units).

Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry. Spectra were obtained on a Voyager Elite reflection time-of-flight mass spectrometry (PerSeptive Biosystems, Inc., Framingham, MA) equipped with a nitrogen laser (337 nm, 3-ns pulse) in the negative ion mode. 3-Hydroxypicolinic acid (HPA), dissolved in H_2O to give a saturated solution at room temperature, was used as the matrix. Time-to-mass conversion was achieved by calibration by using the peak representing the C^+ cation of the charged derivative to be analyzed. ODN **16-Y**: calculated mass, 5433.7; observed mass, 5430.7. ODN **17-Y**: calculated mass, 5433.7; observed mass, 5431.7. ODN **18-Y**: calculated mass, 5433.7; observed mass, 5429.4. ODN **19-Y**: calculated mass, 5462.8; observed mass, 5460.3. ODN **20-Y**: calculated mass, 5491.8; observed mass, 5488.6. ODN **21-Y**: calculated mass, 5520.9; observed mass, 5519.7. ODN **22-Y**: calculated mass, 5549.9; observed mass, 5546.0. ODN **16-E**: calculated mass, 5447.8; observed mass, 5445.1. ODN **17-E**: calculated mass, 5447.8; observed mass, 5445.8. ODN **18-E**: calculated mass, 5447.8; observed mass, 5445.3. ODN **19-E**: calculated mass, 5490.8; observed mass, 5487.6. ODN **20-E**: calculated mass, 5533.9; observed mass, 5530.5. ODN **21-E**: calculated mass, 5577.0; observed mass, 5568.6. ODN **22-E**: calculated mass, 5620.0; observed mass, 5617.8. ODN **16-P**: calculated mass, 5461.8; observed mass, 5458.8. ODN **17-P**: calculated mass, 5461.8; observed mass, 5457.6. ODN **18-P**: calculated mass, 5461.8; observed mass, 5457.0. ODN **19-P**: calculated mass, 5518.9; observed mass, 5515.5. ODN **20-P**: calculated mass, 5576.0; observed mass, 5575.3. ODN **21-P**: calculated mass, 5633.1; observed mass, 5629.1. ODN **22-P**: calculated mass, 5690.2; observed mass, 5686.1. ODN **16-Z**: calculated mass, 5534.8; observed mass, 5532.0. ODN **17-Z**: calculated mass,

5534.8; observed mass, 5532.0. ODN **18-Z**: calculated mass, 5534.8; observed mass, 5528.4. ODN **19-Z**: calculated mass, 5665.0; observed mass, 5662.1. ODN **20-Z**: calculated mass, 5795.1; observed mass, 5796.3. ODN **21-Z**: calculated mass, 5925.3; observed mass, 5922.4. ODN **22-Z**: calculated mass, 6055.4; observed mass, 6051.6. ODN **23**: calculated mass, 5830.2; observed mass, 5826.1.

Thermal Denaturation and CD Spectroscopy. Each solution contains each ODN (3 μM) and the complementary DNA **24** (3 μM), RNA **25** (3 μM), or target duplex **26** (3 μM) in an appropriate buffer. The solution containing each ODN was heated at 90 °C for 5 min, then cooled gradually to an appropriate temperature, and used for the thermal denaturation studies. Thermal-induced transitions of each mixture were monitored at 260 nm on a Perkin-Elmer Lambda2S. Sample temperature was increased 0.5 °C/min. Samples for CD spectroscopy were prepared by the same procedure used in the thermal denaturation study, and spectra were measured on a JASCO J720 spectropolarimeter at 15 °C. The ellipticities of duplexes were recorded from 200 to 320 nm in a cuvette with a path length of 1 mm. CD data were converted into mdeg·mol of residues^{-1}·cm^{-1}.

Partial Hydrolysis of ODN with Snake Venom Phosphodiesterase. Each ODN labeled with ^{32}P at the 5′-end (10 pmol) was incubated with snake venom phosphodiesterase (20 ng, Boeringer Mannheim) in the presence of Torula RNA (0.15 OD units at 260 nm, Sigma, St. Louis, MO) in a buffer containing 37.5 mM Tris-HCl (pH 8.0) and 7.5 mM $MgCl_2$ (total 20 μL) at 37 °C. At appropriate periods, aliquots (4 μL) of the reaction mixture were separated and added to a solution of EDTA (5 mM, 10 μL); then the mixture was heated for 5 min at 90 °C. The solutions were analyzed by electrophoresis on 20% polyacrylamide gel containing 7 M urea. Densities of radioactivity of the gel were visualized by a Bio-imaging analyzer (Bas 2000, Fuji Co., Ltd).

Stability of ODN in the PBS Containing Human Serum. Each ODN labeled with ^{32}P at the 5′-end (5 pmol) was mixed with the corresponding unlabeled ODN (1 nmol). The mixture was incubated in PBS (40 μL) containing 50% human serum at 37 °C. At appropriate periods, aliquots (4 μL) of the reaction mixture were separated and added to a solution of 10 M urea (16 μL). The mixtures were then analyzed by gel electrophoresis as described above.

Partial Hydrolysis of ODN with DNase I. Each ODN labeled with ^{32}P at the 5′-end (15 pmol) was incubated with DNase I (15 unit, Takara Shuzo) in the presence of Torula RNA (0.39 OD units at 260 nm, Sigma, St. Louis, MO) in a buffer containing 100 mM sodium acetate (pH 6.0) and 5 mM $MgCl_2$ (total 30 μL) at 37 °C. At appropriate periods, aliquots (4 μL) of the reaction mixture were separated and added to a solution of EDTA (5mM, 10 μL), then the mixtures were heated for 5 min at 90 °C. The solutions were analyzed by gel electrophoresis as described above.

Hydrolysis of Duplexes with RNase H. RNA labeled with ^{32}P at the 5′-end (50 pmol) was incubated with *E. coli* RNase H (6 units, Takara Shuzo) in the presence or in the absence of the complementary strand (50 pmol) in a buffer containing 10 mM Tris-HCl (pH 7.9), 10 mM $MgCl_2$, 1 mM DTT, 50 mM NaCl, and 0.01% bovine serum albumin (total 10 μL) at 30 °C. At appropriate periods, the reaction mixtures were heated for 1 min at 90 °C; then the reactions were analyzed by gel electrophoresis as described above.

Hydrolysis of Duplexes with HeLa Cell Nuclear Extracts. RNA labeled with ^{32}P at the 5′-end (50 pmol) was incubated with HeLa nuclear extracts (10 units, Seikagaku Kogyo) in the presence or in the absence of the complementary strand (50 pmol) in a buffer containing 10 mM Tris-HCl (pH 7.9), 10 mM $MgCl_2$, 1 mM DTT, 50 mM NaCl, and 0.01% bovine serum albumin (total 10 μL) at 30 °C. After being incubated for 30 min, the reaction mixtures were heated for 3 min at 90 °C; then the reactions were analyzed by gel electrophoresis as described above.

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Supporting Information Available: CD spectral data, polyacrylamide gel electrophoresis data of ODNs **22-E**, **23**, and **29** with DNase I, ODN **29** with human serum, and ODN **29** with *E. coli* and HeLa RNase H (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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