Nucleosides and Nucleotides. 174. Synthesis of Oligodeoxynucleotides Containing 4′-*C*-[2-[[*N*-(2-Aminoethyl)carbamoyl]oxy]ethyl]thymidine and Their Thermal Stability and Nuclease-Resistance Properties[1]

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The synthesis and properties of oligodeoxynucleotides (ODNs) containing 4′-*C*-[2-[[*N*-(2-aminoethyl)-carbamoyl]oxy]ethyl]thymidine (**3**) are described. 4′α-(2-Hydroxyethyl)thymidine (**4**), which is a precursor for phosphoramidite **5**, was synthesized using a newly developed intramolecular radical cyclization reaction at the 4′-position of thymidine derivative **7**. The radical reaction of 4′*β*-(phenylseleno)-3′-*O*-(dimethylvinylsilyl)thymidine derivative **7**, which was prepared from thymidine in several steps, with Bu_3SnH and AIBN, followed by Tamao oxidation, gave either 4′α-(2-hydroxyethyl) derivative **6** or 4′α-(1-hydroxyethyl) derivative **13**, respectively. With a low Bu_3-SnH concentration, the reaction gave **6**, via 6-*endo*-radical-cyclized product **11**, as a sole product in 87% yield. The reaction of **7** in the presence of excess Bu_3SnH gave **13** in 75% yield, via 5-*exo*-cyclized product **12**, as a diastereomeric mixture. The 4′α-(2-hydroxyethyl) derivative **6** was then converted into a 4′-*C*-[2-[[*N*-(2-aminoethyl)carbamoyl]oxy]ethyl]thymidine derivative **14**, which was phosphitylated to give phosphoramidite **5** in 72% yield. In this study, **3** was incorporated into a nonadecamer, d[CTGGCTCAGCTCGTCTCAT]-3′, and a heptadecamer, d[CTCGTACCATTCCGCTC]-3′, instead of T at various positions. ODNs containing **3** were more resistant to nucleolytic hydrolysis by both snake venom phosphodiesterase (a 3′-exonuclease) and DNase I (an endonuclease) than unmodified parent ODNs, although ODNs containing **3** only slightly destabilized duplex formation with both complementary DNA and RNA strands. Furthermore, the duplex formed by an ODN containing **3** and its complementary RNA was a good substrate for *Escherichia coli* RNase H.

Introduction

Antisense oligodeoxynucleotides (ODNs) have been applied extensively to the regulation of cellular and viral gene expression.[2] They hybridize to mRNA targets by Watson−Crick base-pairing and inhibit translation of mRNA in a sequence-specific manner. One of the major problems encountered when using naturally occurring phosphodiester ODNs as antisense molecules is their rapid degradation by nucleases found in cell culture media and inside cells.[2] Therefore, several types of backbone-modified ODNs such as methylphosphonates, phosphoramidates, and phosphorothioates have been synthesized and used for antisense studies.[2,3]

However, the benefits of such enzymatic stabilizations 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, possibly because they occur in diastereomeric mixtures.[4] Although the role of RNase H cleavage in the antisense strategy is not clearly understood, it has been reported that RNA is not a substrate for the enzyme when methylphosphonate ODN is a complementary strand.[5] Furthermore, phosphorothioate ODNs have been reported to exhibit nonsequence-specific activity.[6]

On the other hand, several studies have demonstrated that ODNs that have been modified at the 3′-end are more resistant to 3′-exonucleases than unmodified ODNs.[7] These 3′-modified ODNs form stable duplexes with complementary DNA and RNA strands, and these duplexes are substrates for RNase H. Therefore, 3′-modified ODNs have the properties of antisense molecules, except that they can be degraded by endonucleases inside cells.

We recently reported the synthesis of ODNs with natural phosphodiester linkages containing a 2′-deoxyuridine analogue **1** that carries an aminoalkyl linker at the 1′-position (Figure 1).[8] We found that ODNs containing **1** slightly destabilized duplex formation with a complementary RNA, while these ODNs were more resistant to nucleolytic hydrolysis by both snake venom

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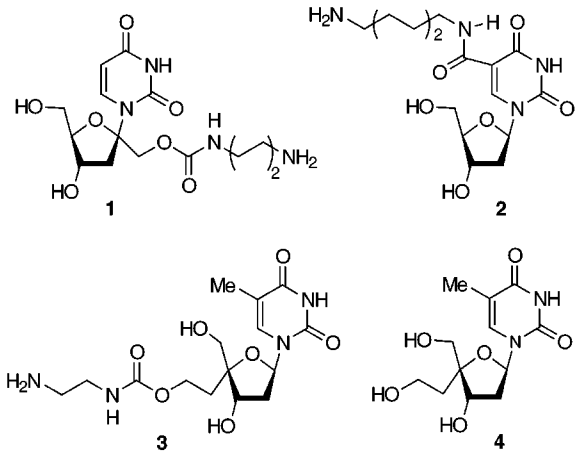


Figure 1. Structures of the modified nucleoside analogues.

phosphodiesterase (a 3′-exonuclease) and nuclease P1 (an endonuclease) than unmodified ODNs. More recently, we also synthesized ODNs containing a 2′-deoxyuridine analogue **2**, which carries an aminohexyl linker at the 5-position of uracil.[9] These ODNs greatly stabilized duplex formation with both complementary DNA and RNA strands. Furthermore, these ODNs were more resistant to nucleolytic hydrolysis by both snake venom phosphodiesterase and nuclease S1 (an endonuclease) than unmodified ODNs and were stable in medium containing 10% fetal calf serum. These results indicate that ODNs that have been suitably modified by aminoalkyl linkers are resistant not only to exonucleases but also to endonucleases, even when they have natural phosphodiester linkages.

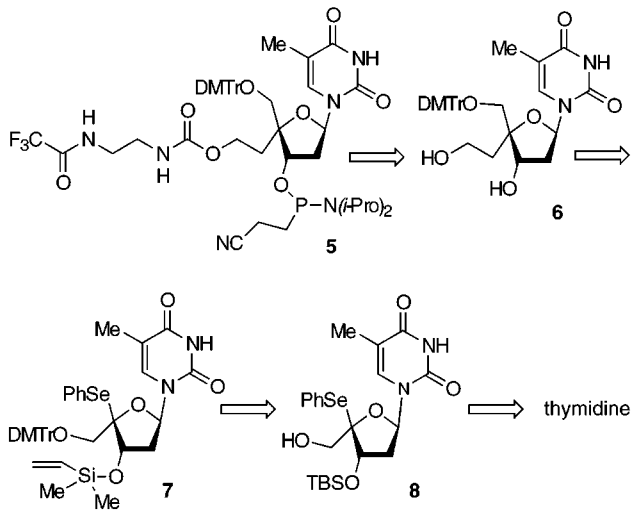
The 4′α-position of nucleosides is much closer to the internucleotide phosphodiester linkages in ODNs than the 1′- and 5-positions. Therefore, we envisioned that ODNs containing a nucleoside analogue with an aminoalkyl-linker at the 4′α-position would be more resistant to endonucleases than ODNs containing a nucleoside analogue with an aminoalkyl linker at the 1′- or 5-position.

In this paper, we report the synthesis of ODNs containing 4′-*C*-[2-[[*N*-(2-aminoethyl)carbamoyl]oxy]ethyl]thymidine (**3**). The thermal stability of duplexes consisting of these ODNs and their complementary DNA or RNA strands, the resistance of these ODNs to nucleolytic hydrolysis by snake venom phosphodiesterase and DNase I (an endonuclease), and whether the duplex of an ODN containing **3** with a complementary RNA strand can elicit RNase H activity were also studied.

Results and Discussion

Synthesis. To synthesize ODNs containing **3**, a 4′α-branched nucleoside phosphoramidite unit **5** is needed. However, efficient general synthetic methods for preparing 4′-branched nucleoside analogues have not been developed.[10] Although only a few examples of 4′-branched nucleoside analogues have been reported,[10] the necessary 2′-deoxy-4′α-(2-hydroxyethyl)nucleoside **4** (Figure 1), which can be a precursor for the synthesis of **5**, is unknown.

Scheme 1



Recently, we developed a regio- and stereoselective method for introducing a 1-hydroxyethyl or 2-hydroxyethyl group at the β-position of a hydroxyl of halohydrins and selenoalkanols with a dimethyl or diphenylvinylsilyl group as a radical-acceptor tether via intramolecular radical cyclization reaction and subsequent Tamao oxidation reaction.[11] Therefore, we planned to synthesize the desired 4′α-branched nucleoside using this intramolecular radical cyclization reaction at the 4′-position of a thymidine derivative with a dimethylvinylsilyl group as a radical acceptor tether. Our synthetic strategy is outlined in Scheme 1. A substrate **7** for the radical reaction can be prepared from a known 4′-(phenylseleno)thymidine derivative **8**.[12] Radical reaction of **7** with low concentrations of Bu_3SnH and subsequent Tamao oxidation[13] will give the desired 4′α-branched nucleoside **6**, which can be further derivatized to the corresponding phosphoramidite unit **5**.

The 4′-(phenylseleno)thymidine derivative **8** was prepared starting from thymidine in six steps via a 5′-formyl derivative of thymidine according to a method recently

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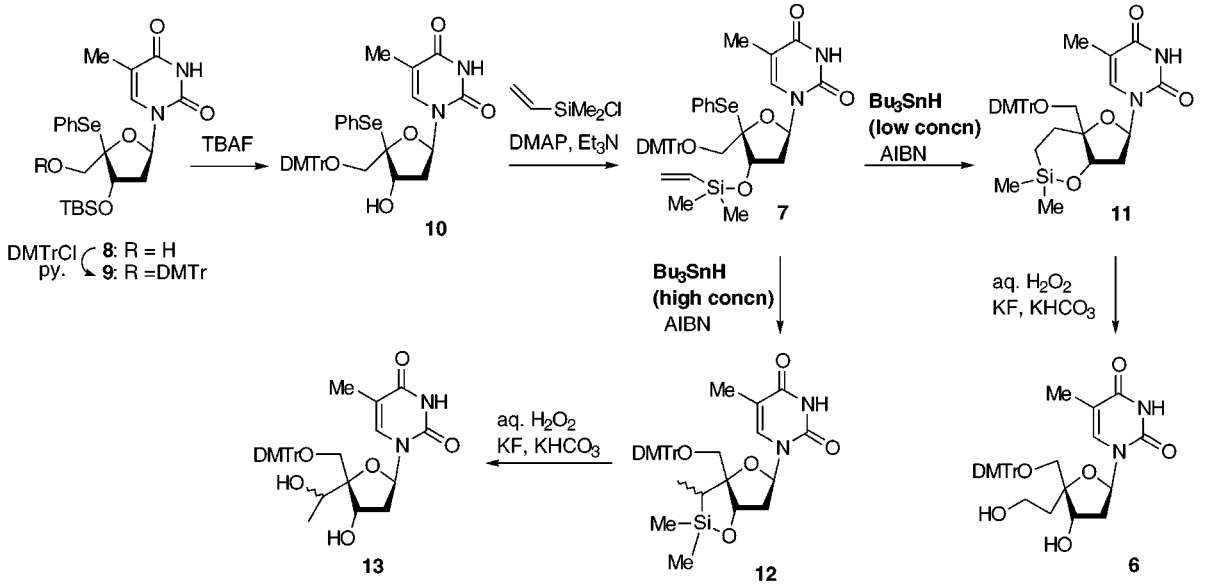
Scheme 2



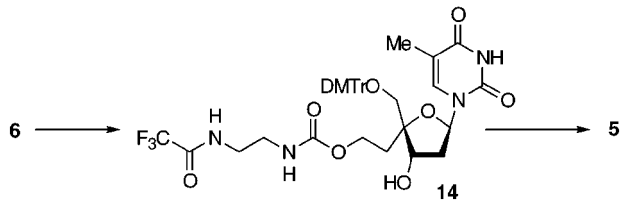
developed by Giese and co-workers.[12] Introduction of a dimethylvinylsilyl group as a radical acceptor tether was performed by treating **10** with dimethylvinylchlorosilane and Et_3N in the presence of DMAP in toluene[14] to afford a vinylsilyl derivative **7**, a substrate for the radical reaction, in 95% yield (Scheme 2).

The radical reactions were performed with Bu_3SnH using AIBN as a radical initiator, and the products were isolated after Tamao oxidation. When a solution of 1.8 equiv of Bu_3SnH and AIBN (0.5 equiv) in benzene was added slowly over 4 h to a solution of **7** in benzene (0.01 M) at 80 °C, the desired 4′α-(2-hydroxyethyl)thymidine derivative **6**, which was derived from a 6-*endo*-cyclized product **11**, was isolated in 87% yield.[15] The regioselectivity could be completely reversed depending on the reaction conditions used. When **7** was treated with 3.0 equiv of Bu_3SnH at 60 °C in benzene in the presence of AIBN, a diastereomeric mixture of 4′α-(1-hydroxyethyl) derivatives **13**, which was derived from a 5-*exo*-cyclized product **12**, was obtained in 75% yield.[16] Therefore, the radical reaction with the vinylsilyl tether may be useful for the regio- and stereoselective introduction of a 1- or 2-hydroxyethyl group to various systems.

To synthesize the phosphoramidite unit **5**, **6** was then converted into carbonylimidazolide, which was reacted with 1,2-diaminoethane. Without purifying the product, the amino group was protected with a trifluoroacetyl group to give **14**, which was then phosphitylated by a standard procedure[17] to give the corresponding nucleoside 3′-phosphoramidite **5** (Scheme 3).

ODNs containing **3** were synthesized on a DNA synthesizer by the phosphoramidite method.[18] In this study, **3** was incorporated into a nonadecamer, d[CTGGCTCAGCTCGTCTCAT]-3′, and a heptadecamer, d[CTCGTACCATTCCGCTC]-3′, instead of T. The sequences of the ODN analogues synthesized are shown in Tables 1

Scheme 3



and 2. The average coupling yield of **5** was 96% using a 1.2 M solution of the amidite in CH_3CN and a coupling time of 360 s.[17] The fully protected ODNs (1 μmol) linked to the solid supports were treated with concentrated NH_4-OH at 55 °C for 16 h, followed by C-18 column chromatography; detritylation gave ODNs (**16**−**20** and **22**−**24**) in 32−95 OD_{260} units. Each ODN analogue obtained showed a single peak on reversed-phase HPLC. Furthermore, ODNs **16** and **22** were analyzed by electrospray ionization (ESI) mass spectrometry, and the observed molecular weights supported their structures (see Experimental Section).

Thermal Stability. The stability of the duplexes formed by these ODNs and a complementary DNA, 5′-d[ATGAGACGAGCTGAGCCAG]-3′ (**25**), or RNA, 5′-r[GAGCGGAAUGGUACGAG]-3′ (**26**), was studied by thermal denaturation. To confirm the effect of the terminal ammonium ion of the aminoalkyl linker of **3** on the thermal stability of the duplexes, thermal denaturation was performed under both low ionic strength (0.05 M NaCl) and high ionic strength (0.5 M NaCl) in a buffer of 0.01 M sodium phosphate (pH 7.0). One transition was observed in the melting profile of each duplex. Melting temperatures (T_m's) are listed in Tables 1 and 2.

With DNA (**25**) as a complementary strand, the T_m's for duplexes containing **3** were the same as or only slightly smaller than that of the control duplex [64.5 °C (0.05 M NaCl) and 76.5 °C (0.5 M NaCl), respectively] (Table 1). The modified nucleoside **3** did not greatly destabilize duplex formation. Even when four molecules of **3** were incorporated into the parent ODN instead of

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(15) The ratio of **11**:**12** was 14:1 from the ^{1}H NMR spectra of the radical reaction product.

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Table 1. Synthesized ODNs and ODN−DNA Hybridization Data[a]

	ODNs[b]	0.05 M NaCl T_m (°C)	0.5 M NaCl T_m (°C)	ΔT_m (°C)
15	5′-CTG GCT CAG CTC GTC TCA T-3′	64.5	76.5	12.0
16	5′-CTG GCT CAG CTC GTC **3**CA T-3′	64.0	76.5	12.5
17	5′-C**3**G GCT CAG CTC GTC TCA T-3′	63.5	76.5	13.0
18	5′-C**3**G GCT CAG CTC GTC **3**CA T-3′	64.5	76.0	11.5
19	5′-C**3**G GCT CAG C**3**C GTC **3**CA T-3′	64.0	76.0	12.0
20	5′-C**3**G GC**3** CAG C**3**C GTC **3**CA T-3′	64.0	75.5	11.5

[a] Experimental conditions are described in the Experimental Section. [b] **3**, see Figure 1.

Table 2. Synthesized ODNs and ODN−RNA Hybridization Data[a]

	ODNs[b]	0.05 M NaCl T_m (°C)	0.5 M NaCl T_m (°C)	ΔT_m (°C)
21	5′-CTC GTA CCA TTC CGC TC-3′	61.0	72.5	11.5
22	5′-CTC GTA CCA **3**TC CGC TC-3′	60.0	71.5	11.5
23	5′-CTC G**3**A CCA T**3**C CGC TC-3′	60.5	71.5	11.0
24	5′-C**3**C G**3**A CCA **3**TC CGC **3**C-3′	60.0	70.5	10.5

[a] Experimental conditions are described in the Experimental Section. [b] **3**, see Figure 1.

T, T_m values were 64.0 °C (0.05 M NaCl) and 75.5 °C (0.5 M NaCl), respectively. Furthermore, ΔT_m's [T_m's (0.5 M NaCl) − T_m's (0.05 M NaCl)] for duplexes containing **3** were similar to that of the control duplex (12.0 °C) and were almost independent of the number of **3**. Therefore, the terminal ammonium ion of the amino linker of **3** did not seem to affect the thermal stability of the ODN−DNA duplex.

When RNA (**26**) was used as a complementary strand, the T_m's for ODNs containing **3** followed a trend similar to those for ODN−DNA duplexes, although the T_m values were smaller than those of ODN−DNA duplexes (Table 2). Although ΔT_m's [T_m (0.5 M NaCl) − T_m (0.05 M NaCl)] for duplexes containing **3** became slightly smaller than that of the control duplex (11.5 °C) as the number of **3** increased, the differences were small. Therefore, the terminal ammonium ion of the amino linker of **3** seemed to hardly affect the thermal stability of the ODN−RNA duplex.

In previous papers,[8,9] we described the thermal stability of duplexes constructed from ODNs containing 1′-[[[*N*-(4-aminobutyl)carbamoyl]oxy]methyl]-2′-deoxyuridine (**1**) or 5-[*N*-(6-aminohexyl)carbamoyl]-2′-deoxyuridine (**2**). ODNs containing **2** stabilized duplex formation with a complementary DNA, while those containing **1** greatly destabilized duplex formation with a complementary DNA. When an ODN analogue containing **2** forms a duplex with a complementary DNA, the aminohexyl linker of **2** should be accommodated in the major groove, since the 5-position of the deoxyuridine is in the major groove. On the other hand, when an ODN analogue containing **1** forms a duplex with a complementary DNA, the aminobutyl-linker of **1** should be accommodated in the minor groove, since the 1′-position of the sugar moiety is located in the minor groove. The minor groove of a DNA−DNA duplex is narrower and deeper than its major groove.[19] Therefore, a bulky amino linker may not fit into the narrow minor groove as compared with the wide major groove. The 4′-position of the nucleoside is located in a minor groove in a duplex. However, ODNs containing **3** did not greatly destabilize duplex formation with a complementary DNA. The 4′-position of the nucleoside is closer to the phosphate backbone than the 1′-position in a duplex and is located at the edge of the minor groove. Therefore, the modified nucleoside **3** might not greatly destabilize ODN−DNA duplex formation.

(19) Saenger, W. *Principle of nucleic acid structure;* Springer-Verlag: New York, 1984.

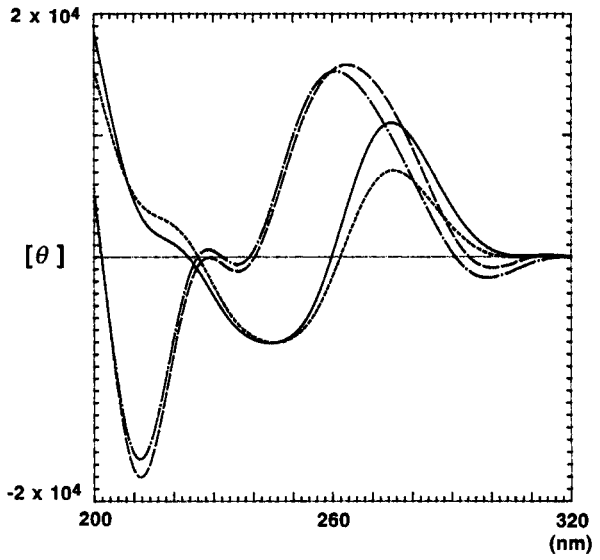

Figure 2. CD spectra of ODN−DNA and ODN−RNA duplexes at 15 °C in 0.01 M sodium phosphate (pH 7.0) containing 0.05 M NaCl: (—) **15-25**; (---) **20-25**; (— —) **21-26**; (—·—) **24-26**.

On the other hand, we previously reported that ODNs containing **1** only slightly destabilized ODN−RNA duplex formation.[8] The result obtained for ODNs containing **3** is similar to that for ODNs containing **1**. Since the minor groove of A-type duplexes (DNA−RNA and RNA−RNA duplexes) is shallower and flatter than that of DNA−DNA duplexes, the amino linker of **1** or **3** could be well accommodated in its minor groove.

Circular Dichroism. To study the global conformation of duplexes, CD spectra of duplexes composed of ODNs containing **3** and either the complementary DNA (**25**) or RNA (**26**) were measured in a buffer of 0.01 M sodium phosphate (pH 7.0) containing 0.05 M NaCl at 15 °C. With DNA (**25**) as a complementary strand, the spectra of the duplexes (**15**−**25** and **20**−**25**) showed a positive CD band at around 275 nm and a negative CD band at around 245 nm that were attributable to B-like DNA conformation (Figure 2). The shapes of the spectra were quite similar to each other, although the intensity of the positive band in the spectrum of the duplex (**20**−**25**) containing **3** was clearly less than that for the unmodified duplex (**15**−**25**).

On the other hand, when RNA (**26**) was used as a complementary strand, the spectra of duplexes (**21**−**26**

and **24**−**26**) showed a positive CD band at around 260 nm and a large negative CD band at around 210 nm (Figure 2). Although the shapes of the spectra were similar to each other, the positive band in the spectrum of the duplex (**24**−**26**) containing **3** was shifted to a shorter wavelength by about 3 nm relative to that in the spectrum of the unmodified duplex (**21**−**26**), and the intensity of the negative band at around 210 nm was slightly reduced compared with that for the unmodified duplex (**21**−**26**). These results suggest that the modified nucleoside **3** slightly affects the global conformation of both ODN−DNA and ODN−RNA duplexes. The T_m values for duplexes containing **3** may reflect these conformational changes of the duplexes.

Partial Digestion of ODNs with Snake Venom Phosphodiesterase and DNase I. Since resistance to nucleolytic hydrolysis by nucleases is an important factor in antisense studies, the stability of ODNs containing **3** to nucleolytic digestion was examined. Two kinds of nucleases, snake venom phosphodiesterase (a 3′-exonuclease) and DNase I (an endonuclease), were used in this study. ODNs (**16** and **20**) containing one and four molecules of **3** were labeled at the 5′-end with ^{32}P and incubated with an appropriate nuclease, and the reactions were then analyzed by polyacrylamide gel electrophoresis under denaturing conditions.[20]

When snake venom phosphodiesterase was used in the reactions, the control ODN **15** was hydrolyzed almost completely by the enzyme after 120 min of incubation (Figure 3a). In contrast, the phosphodiester linkage at the 5′-side of **3** was highly resistant to the nuclease, and the phosphodiester linkage at the 3′-side of **3** was also slightly resistant to the enzyme (Figure 3b).

On the other hand, when DNase I was used in the reactions, the phosphodiester linkages around **3** were more resistant to enzymatic hydrolysis than those beside thymidine. The half-lives of ODN **15** and ODN **20** were 9 and 140 min, respectively. The modified ODN **20** was 15 times more stable than the unmodified ODN (Figure 4). In a previous paper,[9d] we reported that the heptadecanucleotide containing four molecules of **2** was four times more stable to hydrolysis by nuclease S1 (an endonuclease) than an unmodified ODN. Also, the ODN containing **2** was as resistant to DNase I as to nuclease S1.[21] Therefore, the ODN containing **3** seemed to be more resistant to nucleolytic hydrolysis by endonucleases than the ODN containing **2**.

Degradation of the Target RNA by RNase H. It has been postulated that the antisense activity of antisense ODNs is due, at least in part, to cleavage of the RNA strand of a DNA−RNA duplex by RNase H. We next examined whether the DNA−RNA heteroduplex of ODN containing **3** and its complementary RNA can elicit RNase H activity. The duplex consisting of an ODN (**21** or **24**) and RNA (**26**) labeled with ^{32}P at the 5′-end was incubated with *Escherichia coli* RNase H, and the products were analyzed by polyacrylamide gel electrophoresis. As shown in Figure 5, RNA in the ODN−RNA duplex (**24**−**26**) containing the modified nucleoside and the control ODN−RNA duplex (**21**−**26**) were similarly hydrolyzed by the enzyme. Therefore, the ODN−RNA duplex containing **3** is a substrate for *E. coli* RNase H.

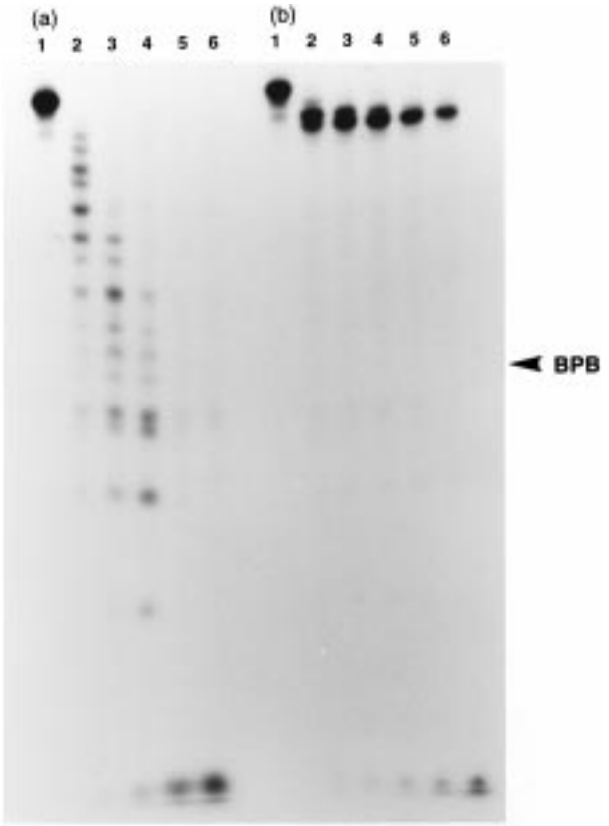

Figure 3. Polyacrylamide gel electrophoresis of 5′-^{32}P-labeled ODNs hydrolyzed by snake venom phosphodiesterase: (a) **15**; (b) **16**. ODNs were incubated with snake venom phosphodiesterase for 0 min (lane 1), 10 min (lane 2), 20 min (lane 3), 30 min (lane 4), 60 min (lane 5), and 120 min (lane 6). Experimental conditions are described in the Experimental Section.

Conclusions

In this paper, we reported the synthesis of ODNs containing 4′-*C*-[2-[[*N*-(2-aminoethyl)carbamoyl]oxy]ethyl]thymidine (**3**). 4′α-(2-Hydroxyethyl)thymidine (**4**) was synthesized using a newly developed intramolecular radical cyclization reaction at the 4′-position of thymidine derivative **7** with a dimethylvinylsilyl group as a radical acceptor tether. ODNs (**16**−**20** and **22**−**24**) containing **3** were synthesized on a DNA synthesizer using **5**. These ODNs containing **3** were more resistant to nucleolytic hydrolysis by both snake venom phosphodiesterase and DNase I than unmodified parent ODNs. ODN **20** was 15 times more stable to nucleolytic hydrolysis by DNase I than an unmodified ODN. The stability of duplexes consisting of ODNs (**15**−**24**) and either their complementary DNA or RNA strands were studied by thermal denaturation. ODNs containing **3** did not greatly destabilize duplex formation with either the complementary DNA or RNA strand. Furthermore, the duplex formed by an ODN containing **3** and the complementary RNA was a substrate for *E. coli* RNase H. These results suggest that an ODN containing **3** is a good candidate as a novel antisense molecule.

Recently, Fensholdt[22] and Wang[23] reported that ODNs containing 4′-*C*-(hydroxymethyl)thymidine, 4′-*C*-(meth-

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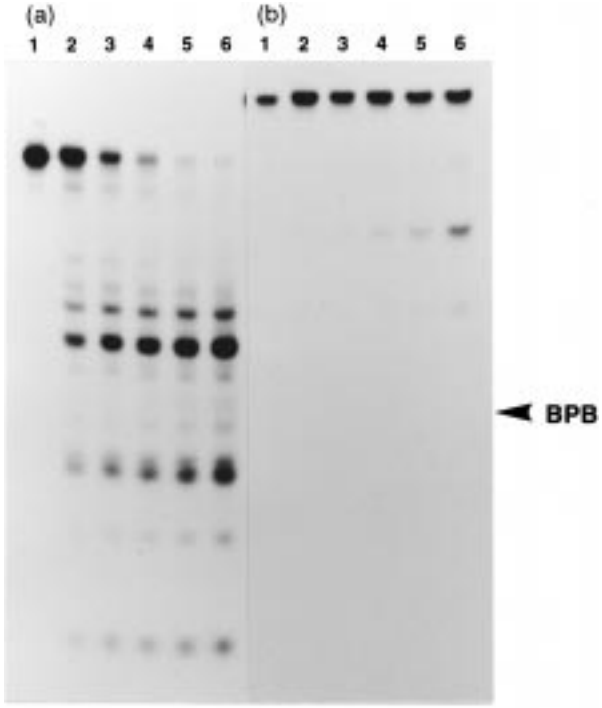

Figure 4. Polyacrylamide gel electrophoresis of 5′-^{32}P-labeled ODNs hydrolyzed by DNase I: (a) **15**; (b) **20**. ODNs were incubated with DNase I for 0 min (lane 1), 10 min (lane 2), 20 min (lane 3), 30 min (lane 4), 60 min (lane 5), and 120 min (lane 6). Experimental conditions are described in the Experimental Section.

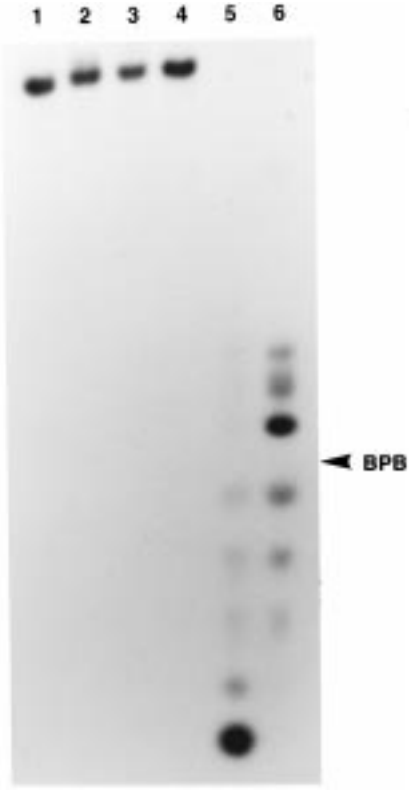

Figure 5. Polyacrylamide gel electrophoresis of 5′-^{32}P-labeled RNA **26** hydrolyzed by *E. coli* RNase H in the presence of complementary strands: lane 1, **26**; lane 2, **26** + **21**; lane 3, **26** + **24**; lane 4, **26** + enzyme; lane 5, **26** + **21** + enzyme; lane 6, **26** + **24** + enzyme. Experimental conditions are described in the Experimental Section.

oxymethyl)thymidine, or 4′-*C*-(aminomethyl)thymidine 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. Various 4′α-branched nucleoside analogues with aminoalkyl-linkers of various lengths can be synthesized using the radical cyclization reaction with a silicon tether described here. The synthesis, thermal stability and nuclease-resistance properties of ODNs containing these 4′α-branched nucleoside analogues are currently under investigation in our laboratory. These results will be reported shortly.

Experimental Section

NMR spectra were recorded at 270 or 500 MHz (^{1}H) 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 fast atom bombardment (FAB). 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-[2-Deoxy-5-*O*-(dimethoxytrityl)-4-*C*-(phenylseleno)-α-L-*threo*-pento-1,4-furanosyl]thymine (10). A mixture of **8**[12] (4.69 g, 9.18 mmol) and DMTrCl (6.22 g, 18.4 mmol) in pyridine (90 mL) was stirred at room temperature. After 3 h, DMTrCl (1.56 g, 4.60 mmol) was added further, and the mixture was stirred at room temperature for 2 h. Ice−water and AcOEt were added, the resulting mixture was partitioned, and the organic layer was washed with H_2O and brine, dried (Na_2SO_4), and evaporated under reduced pressure. The residue was dissolved in THF (80 mL), TBAF (1 M in THF, 14 mL, 14 mmol) was added, and the resulting mixture was purified by column chromatography (SiO_2, 70−100% AcOEt in hexane) to give **10** (4.04 g, 63%) as a foam: ^{1}H NMR (270 MHz, $CDCl_3$) δ 8.12 (br s, 1H), 7.78 (s, 1H), 7.54−6.78 (m, 18H), 6.73 (dd, 1H, J = 8.0, 7.1), 4.65 (m, 1H), 3.80 (s, 6H), 3.64 (d, 1H, J = 10.3), 3.47 (d, 1H, J = 10.3), 3.03 (d, 1H, J = 3.0), 2.52−2.47 (m, 2H), 1.88 (s, 3H); FABMS *m*/*z* 574 (M^+ − base). Anal. Calcd for $C_{37}H_{36}N_2O_7Se$: C, 63.52; H, 5.19; N, 4.00. Found: C, 63.58; H, 5.39; N, 4.09.

1-[2-Deoxy-5-*O*-(dimethoxytrityl)-3-*O*-(dimethylvinylsilyl)-4-*C*-(phenylseleno)-α-L-*threo*-pento-1,4-furanosyl]thymine (7). A mixture of **10** (1.00 g, 1.43 mmol), DMAP (17 mg, 0.14 mmol), Et_3N (560 μL, 4.0 mmol), and dimethylvinylchlorosilane (500 μL, 4.4 mmol) in toluene (20 mL) was stirred at room temperature for 2 h under argon. Insoluble materials were filtered off, and the filtrate was evaporated under reduced pressure. The residue was partitioned between AcOEt 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, hexane−AcOEt = 3:2) to give **7** (1.23 g, 95%) as a foam: ^{1}H NMR (270 MHz, $CDCl_3$) δ 8.12 (br s, 1H), 7.54−6.72 (m, 29H), 6.61 (dd, 1H, J = 7.0, 7.0), 6.15−5.57 (m, 3H), 4.58 (dd, 1H, J = 5.5, 3.7), 3.84 (d, 1H, J = 10.6), 3.78 (s, 6H), 3.14 (d, 1H, J = 10.6), 2.45 (ddd, 1H, J = 13.3, 7.0, 3.7), 2.33 (ddd, 1H, J = 13.3, 7.0, 5.5), 1.91 (s, 3H): FABMS *m*/*z* 751(M^+ − PhSe). Anal. Calcd for $C_{51}H_{48}N_2O_7SeSi$: C, 67.46; H, 5.33; N, 3.09. Found: C, 67.28; H, 5.35; N, 3.39.

5′-*O*-(Dimethoxytrityl)-4′-*C*-(2-hydroxyethyl)thymidine (6). A solution of Bu_3SnH (1.9 mL, 7.1 mmol) and AIBN (340 mg, 2.07 mmol) in benzene (70 mL) was added slowly over 4 h to a solution of **7** (3.13 g, 4.00 mmol) in benzene (250 mL) at 80 °C. The solvent was evaporated under reduced pressure,

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and the residue was partitioned between MeCN and hexane. The MeCN layer was evaporated under reduced pressure, and the residue was dissolved in MeOH/THF (1:1, 40 mL). Aqueous H_2O_2 (30%, 1.3 mL, 38 mmol), KF (1.05 g, 18.1 mmol), and $KHCO_3$ (400 mg, 4.00 mmol) were added to the above solution, and the resulting mixture was stirred at room temperature for 16 h. Aqueous $Na_2S_2O_3$ solution (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, 1−3% MeOH in $CHCl_3$) to give **6** (2.05 g, 87%) as a white foam: ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.97 (br s, 1H), 7.62 (s, 1H), 7.40−6.83 (m, 13H), 6.48 (t, 1H, J = 7.2), 4.53 (m, 1H), 3.85 (m, 1H), 3.79 (s, 6H), 3.58 (ddd, 1H, J = 10.3, 6.7, 3.5), 3.31 (d, 1H, J = 9.9), 3.24 (d, 1H, J = 9.9), 2.46−2.43 (m, 2H), 2.05 (ddd, 1H, J = 15.0, 8.2, 3.5), 1.81 (ddd, 1H, J = 15.0, 6.7, 3.3), 1.38 (s, 3H); FABMS m/z 589 [M^+ + 1]; HRFABMS calcd for $C_{33}H_{37}N_2O_8$ 589.2549, found 589.2522.

5′-*O*-(Dimethoxytrityl)-4′-*C*-(1-hydroxyethyl)thymidine (13). A solution of **7** (313 mg, 0.400 mmol), Bu_3SnH (320 μL, 1.19 mmol), and AIBN (20 mg, 0.12 mmol) in benzene (4 mL) was stirred at 60 °C for 4 h. 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 treated under Tamao oxidation conditions as described above for **6** to give **13** (177 mg, 75%) as a white foam: ^{1}H NMR (270 MHz, $CDCl_3$) δ 8.30 (br s, 1/3H), 8.23 (br s, 2/3H), 7.41−6.85 (m, 14H), 6.51 (t, 1/3H, J = 7.1), 6.44 (dd, 2/3H, J = 5.9, 8.7), 4.84 (m, 1/3H), 4.74 (m, 2/3H), 4.18−4.09 (m, 1H), 3.81 (s, 6H), 3.58 (d, 2/3H), 3.50 (d, 2/3H, J = 10.0), 3.29 (d, 2/3H, J = 10.0), 3.21 (s, 2/3H), 2.90 (d, 2/3H), 2.71 (d, 1/3H), 2.49−2.36 (m, 2H), 1.54 (s, 2H), 1.46 (s, 1H), 1.15−1.11 (m, 3H); FABMS m/z 588 [M^+]; HRFABMS calcd for $C_{33}H_{36}N_2O_8$ 588.2469, found 588.2488.

5′-*O*-(Dimethoxytrityl)-4′-*C*-[2-[[*N*-[2-[*N*-(trifluoroacetyl)amino]ethyl]carbamoyl]oxy]ethyl]thymidine (14). *N*,*N*′-Carbonyldiimidazole (80 mg, 0.5 mmol) and DMAP (40 mg, 0.3 mmol) were added to a solution of **6** (966 mg, 1.6 mmol) in DMF (80 mL), and the mixture was stirred at room temperature. After 2, 4, and 6 h, further amounts of *N*,*N*′-carbonyldiimidazole (80 mg, 0.5 mmol) were added to the mixture. After 15 h, 1,2-diaminoethane (0.55 mL, 8.2 mmol) was added to the mixture, which was stirred at room temperature. After 3 h, the solvent was removed by evaporation. The residue was diluted with $CHCl_3$. The organic layer was washed with aqueous 0.1 M NaH_2PO_4, dried (Na_2SO_4), and evaporated under reduced pressure. The residue was dissolved in MeOH (80 mL). Ethyl trifluoroacetate (976 μL, 8.2 mmol) and Et_3N (1.1 mL, 8.2 mmol) were added to the solution with stirring at room temperature. After 14 h, the solvent was removed by evaporation. The residue 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, 1−10% EtOH in $CHCl_3$) to give **14** (818 mg, 65%) as a white foam: ^{1}H NMR (270 MHz, $CDCl_3$) δ 8.92 (br s, 1H), 7.80 (br t, 1H), 7.42−6.84 (m, 14H), 6.42 (t, 1H, J = 6.9), 5.37 (br t, 1H), 4.49 (m, 1H), 4.20 (m, 1H), 4.07 (m, 1H), 3.81 (s, 6H), 3.45 (m, 2H), 3.35 (m, 2H), 3.29−3.28 (m, 2H), 2.98 (d, 1H, J = 5.4), 2.42−2.37 (m, 2H), 2.14−1.92 (m, 2H), 1.57 (s, 3H); FABMS m/z 771 [M^++1]; HRFABMS calcd for $C_{38}H_{42}N_4O_{10}F_3$ 771.2850, found 771.2841.

5′-*O*-(Dimethoxytrityl)-4′-*C*-[2-[[*N*-[2-[*N*-(trifluoroacetyl)amino]ethyl]carbamoyl]oxy]ethyl]thymidine 3′-*O*-(2-Cyanoethyl *N*,*N*-diisopropylphosphoramidite) (5). After successive coevaporation with pyridine, **14** (423 mg, 0.55 mmol) was dissolved in CH_2Cl_2 (10 mL) containing *N*,*N*-diisopropylethylamine (0.192 mL, 1.1 mmol). Chloro(2-cyanoethoxy)(*N*,*N*-diisopropylamino)phosphine (0.184 mL, 0.83 mmol) was added to the solution, and the reaction mixture was stirred for 20 min at room temperature. After 30 min, further amounts of *N*,*N*-diisopropylethylamine (48 μL) and chloro(2-cyanoethoxy)(*N*,*N*-diisopropylamino)phosphine (61 μL) were added to the mixture. After 1.5 h, aqueous saturated $NaHCO_3$ and $CHCl_3$ were added to the mixture, and the separated organic layer was washed with aqueous saturated $NaHCO_3$ and brine, dried (Na_2SO_4), and concentrated. The residue was purified by column chromatography (a neutralized SiO_2, 50−100% AcOEt in hexane) to give **5** (385 mg, 72%) as a foam: ^{31}P NMR (202 MHz, $CDCl_3$) δ 150.28, 149.88; FABMS m/z 971 [M^+ + 1]; HRFABMS calcd for $C_{47}H_{59}F_3N_6O_{11}P$ 971.3931, found 971.3894.

Synthesis of ODNs. ODNs were synthesized on a DNA synthesizer (Applied Biosystem model 381A) 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, the residue was treated with aqueous 80% AcOH at room temperature for 20 min and 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, and then the H_2O layer was concentrated to give deprotected ODN **16** (95), **17** (65), **18** (48), **19** (38), **20** (64), **22** (32), **23** (61), and **24** (33). The yields are indicated in parentheses as OD units at 260 nm on a 1 μmol scale.

Electrospray Ionization Mass Spectrometry. Spectra were obtained on a Quattro II (Micromass, Manchester, U.K.) triple quadrupole mass spectrometer equipped with an ESI source in the negative-ion mode. The HPLC-purified ODN samples were dissolved in aqueous 50% 2-propanol containing 1% triethylamine (10 pmol ODN/μL) and introduced into the ion source through a loop injector with a carrier solvent, 33% aqueous MeOH, flowing at 10 mL/min flow rate. About 15 scans were acquired over approximately 1 min and combined to obtain smoothed spectra. All molecular masses of the ODNs were calculated from the multiple-charge negative-ion spectra. The observed average molecular masses of **16** and **22** were 5860.9 and 5187.5, respectively, and fit the calculated molecular weights (theoretical average molecular masses) for these compounds, i.e., 5860.9 (for **16**, $C_{188}H_{245}N_{65}O_{118}P_{18}$) and 5187.5 (for **22**, $C_{167}H_{220}N_{56}O_{105}P_{16}$) within a commonly accepted error range of ESI MS, 0.01%.

Thermal Denaturation and CD Spectroscopy. Each solution contains ODN (3 μM) and the complementary DNA **25** (3 μM) or RNA **26** (3 μM) in an appropriate buffer. The solution containing each ODN was heated at 90 °C for 10 min and 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 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 (0.4 μg) in the presence of Torula RNA (0.26 OD units at 260 nm) 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 of the reaction mixture were separated and added to a solution of EDTA (5mM, 10 μL), and then the mixture were heated for 3 min at 90 °C. The solutions were analyzed by electrophoresis on 20% polyacrylamide gel containing 7 M urea.

Partial Hydrolysis of ODN with DNase I. Each ODN labeled with ^{32}P at the 5′-end (10 pmol) was incubated with DNase I (1 unit) in the presence of Torula RNA (0.26 OD units at 260 nm) in a buffer containing 100 mM sodium acetate (pH 6.0) and 5 mM $MgCl_2$ (total 20 μL) at 37 °C. At appropriate periods, aliquots of the reaction mixture were separated and added to a solution of EDTA (5mM, 10 μL), and then the mixtures were heated for 3 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) in the presence or in the absence of the complementary strand (50 pmol) in a buffer containing 40 mM Tris−HCl (pH 7.7), 4 mM $MgCl_2$, 1 mM DTT, 4% glycerol, and 0.003% bovine plasma albumin (total 10 μL) at 20 °C. After 20 min, reaction mixtures were heated for 1 min at 90 °C, and then the reactions were analyzed by gel electrophoresis as described above.

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