VOLUME 118, NUMBER 40
OCTOBER 9, 1996

JOURNAL OF THE AMERICAN CHEMICAL SOCIETY

Unprecedented Mild Acid-Catalyzed Desilylation of the 2′-O-*tert*-Butyldimethylsilyl Group from Chemically Synthesized Oligoribonucleotide Intermediates *via* Neighboring Group Participation of the Internucleotidic Phosphate Residue

Shun-ichi Kawahara, Takeshi Wada, and Mitsuo Sekine*

Contribution from the Department of Life Science, Tokyo Institute of Technology, Nagatsuta, Midoriku, Yokohama 226, Japan

Received June 10, 1996[⊗]

Abstract: Hydrolytic removal of the 2′-*tert*-butyldimethylsilyl (TBDMS) group from a 2′-*O*-TBDMS protected UpU dimer [U(2′-Si)pU] (**1**) (Si = TBDMS) and related derivatives under various acidic conditions was studied in detail. First, desilylation of **1** by use of acetic acid was examined. Consequently, we made the unprecedented discovery that cleavage of the 2′-silyl ether linkage occurred fastest at a very low concentration of acetic acid within the range of 5−10%, depending on the temperature. Formic acid could cleave the silyl ether much faster than acetic acid, but the relationship between the reaction rate and the concentration of acid was different from that of acetic acid. The use of 20−40% formic acid resulted in very effective elimination of the 2′-TBDMS group. Moreover, diluted HCl solution (pH 2.0) could cleave the Si−O bond faster than acetic acid at 30 °C. In contrast, the 2′-silyl group of the corresponding methylphosphonate derivative [U(2′-Si)p(Me)U] (**3**) was much more stable than that of **1**. In the case of a diastereomeric mixture of the phosphorothioate dimer [U(2′-Si)psU] (**2**), a big difference in reaction rate between the Rp- and Sp-diastereomers was observed. These results strongly suggest that neighboring group participation of the 3′-5′ phosphorodiester group is involved in the present acid-catalyzed 2′-desilylation. These conditions were successfully applied to the deprotection of the 2′-TBDMS group of an RNA intermediate which was chemically synthesized by the conventional phosphoramidite approach on a CPG gel.

Introduction

Chemically synthesized oligonucleotides[1] have proved to be useful not only for basic studies of biochemistry[2] and molecular recognition[3] but also for medicinal applications.[4] With the increasing demand for synthetic RNAs in the field of molecular biology,[5] the chemical synthesis of RNA oligomers has been rapidly developed during the past 10 years and is now established by use of the phosphoramidite and *H*-phosphonate approaches.[6−8] In the current RNA synthesis, the *tert*-butyldi-

[⊗] Abstract published in *Advance ACS Abstracts*, September 1, 1996.

(1) (a) Engels, J. W.; Uhlmann, E. *Angew. Chem. Int. Ed. Engl.* **1989**, *28*, 716−734. (b) Englisch, U.; Gauss, D. H. *Angew. Chem. Int. Ed. Engl.* **1991**, *30*, 613−722.

(2) (a) Steitz, T. *Q. Rev. Biophys.* **1990**, *23*, 205. (b) Harrison, S. *Nature* **1991**, *353*, 715−719.

(3) (a) Hélène, C.; Lancelot, G. *Prog. Biophys. Mol. Biol.* **1983**, *41*, 43. (b) Nguyen T.; Hélène, C. *Angew. Chem. Int. Ed. Engl.* **1993**, *32*, 666−690.

(4) Murray, J. A. H., Ed. *Antisense RNA and DNA*; Wiley-Liss, Inc.: New York, 1992; and references cited therein.

(5) Davis, R. H. *Curr. Opin. Biotechnol.* **1995**, *6*, 213−217.

(6) For reviews of RNA synthesis see, Gait, M. J.; Pritchard, C.; Slim, G. In *Oligonucleotides and Analogues, A Practical Approach*; Eckstein, F., Ed.; IRL Press: Oxford, 1991; pp 25−48. (b) Gait, M. J. *Curr. Opin. Biotechnol*. **1991**, 2, 61−8. (c) Damha, M. J.; Ogilvie, K. K. In *Protocols for Oligonucleotides and Analogs, Synthesis and Properties*; Agrawal, S., Ed.; Humana Press: Totowa, 1993; Chapter 5, pp 81−114. (d) Uhlmann, E.; Peyman, A. *Chem. Rev.* **1990**, *90*, 543−584. (e) Damha, M. J.; Ogilvie, K. K. *Methods Mol. Biol.* **1993**, *20*, 81−114.

S0002-7863(96)01959-2 CCC: $12.00

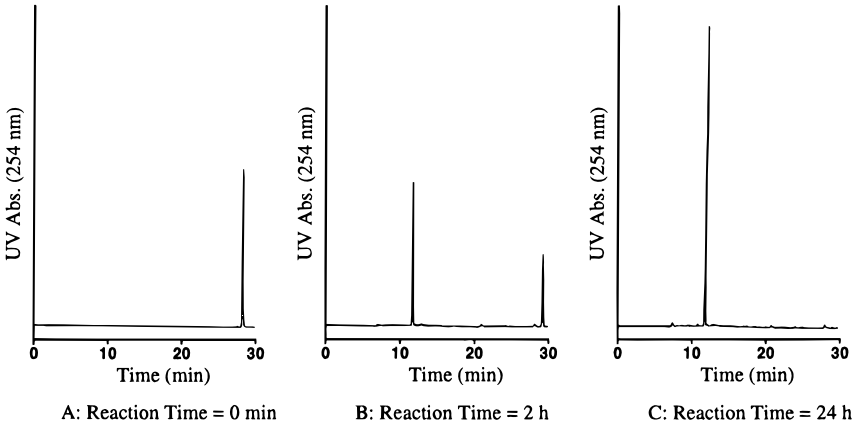


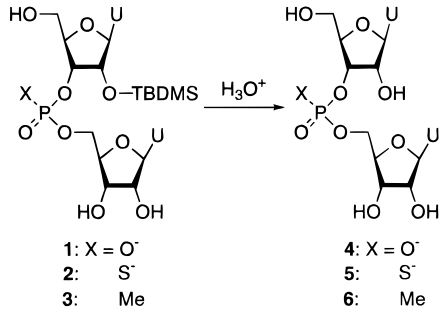
Figure 1. The time dependent HPLC profiles of hydrolysis of U(2′-Si)pU (**1**) in 20% AcOH at 30 °C.

methylsilyl (TBDMS) group has been most widely used as a reliable protecting group,[8a,9] because of the easy preparation of 2′-*O*-TBDMS-ribonucleoside derivatives[10] and facile removal by the action of fluoride ion sources[11] such as tetrabutylammonium fluoride (TBAF)[12] and $Et_3N{\cdot}3HF$.[13]

It has long been realized in nucleic acid chemistry that the TBDMS group attached to the 2′-hydroxyl group of RNA oligomers would be rather resistant to acids, since the acid hydrolysis of secondary TBDMS ethers usually requires strongly acidic conditions[8b] such as aqueous HCl[14,15] or prolonged reaction times of as much as 20−25 h when aqueous acetic acid was employed.[12,16] This fixed idea might also be deduced from the well-known fact that treatment of 2′,3′,5′-*O*-tris(*tert*-butyldimethylsilyl)ribonucleosides with 80% acetic acid gives 2′,3′-*O*-bis(*tert*-butyldimethylsilyl)ribonucleosides selectively in high yields.[10b] Therefore, to date, no papers have been published on the hydrolytic property of the 2′-TBDMS group in protected RNA oligomer intermediates.

In this paper, we wish to report for the first time an unexpectedly facile acid-catalyzed elimination of the 2′-TBDMS group from 2′-*O*-(*tert*-butyldimethylsilyl)uridylyl(3′-5′)uridine [U(2′-Si)pU] (**1**) and related derivatives U(2′-Si)psU (**2**) and U(2′-Si)p(Me)U (**3**) and its successful application to the deprotection of chemically synthesized RNA intermediates. The mechanism of the present desilylation involving neighboring group participation of the internucleotidic phosphodiester group is also proposed.

Scheme 1



Results and Discussion

This study was started after our finding that a 2′-*O*-TBDMS-protected UpU dimer **1**,[9a] which was synthesized as a reference material for the study of the synthesis of antisense nucleic acids, gradually decomposed upon storage in aqueous solution to release the TBDMS group, thus giving rise to a considerable amount of UpU. To elucidate the mechanism of this extremely easy elimination reaction in more detail, we studied the hydrolytic cleavage of **1** under various conditions.

Acidic Hydrolysis of the TBDMS Group of 2′-*O*-TBDMS-Protected UpU Dimer [U(2′-Si)pU)] (1). First, desilylation of **1** under acidic conditions was investigated (Scheme 1). Compound **1** was treated with an aqueous acetic acid solution at 30 and 50 °C, an aqueous formic acid solution at 30 °C, or 0.01 M HCl (pH 2.0) at 30 °C. The reactions were monitored by reverse-phase HPLC. Figure 1 shows the typical HPLC profiles of the reaction mixture obtained by treatment of **1** with 20% acetic acid at 30 °C.

These acid-catalyzed desilylations were found to obey pseudo-first-order kinetics as demonstrated by linear relationships between the log of the amount of the remaining **1** and the time of incubation (Figure 2). Figure 3 shows the relationship between the reaction rate and the concentration of acetic acid or formic acid at 30 and 50 °C. Interestingly, the hydrolysis of the 2′-*O*-TBDMS ether linkage was rather slow in 80% acetic acid which has been used frequently to remove the 5′-*O*-TBDMS group from 5′-*O*-TBDMS-nucleosides.[11] In contrast, the fastest cleavage of the 2′-silyl ether was observed at rather low concentrations of ca. 4% and 8% acetic acid at 50 and 30 °C, respectively (Figure 3).[17] On the other hand, the 2′-silyl ether was cleaved much faster by treatment with formic acid than acetic acid and the fastest desilylation was achieved at a

(7) Scaringe, S. A.; Francklyn, C.; Usman, N. *Nucleic Acids Res*. **1990**, *18*, 5433−5441.
(8) (a) Rozners, E.; Westman, E.; Strömberg, R. *Nucleic Acids Res.* **1994**, *22*, 94−9. (b) Stawinski, J.; Strömberg, R.; Westman, E. *Nucleic Acids Symp. Ser*. **1991**, *24*, 228.
(9) (a) Stawinski, J.; Strömberg, R.; Thelin, M.; Westman, E. *Nucleic Acids Res.* **1988**, *16*, 9285−9298. (b) Wu, T.; Ogilvie, K. K. *J. Org. Chem.* **1990**, *55*, 4717−4724.
(10) (a) Ogilvie, K. K. *Can. J. Chem.* **1973**, *51*, 3799−3807. (b) Ogilvie, K. K.; Beaucage, S. L.; Schifman, A., L.; Sadana, K., L. *Can. J. Chem.* **1978**, *56*, 2768−2780.
(11) (a) Colvin, E. W. *Silicon Reagents in Organic Synthesis*; Academic Press: London, 1988. (b) Greene, T. W.; Wuts, P. G. M. *Protective Groups in Organic Synthesis*; John Wiley and Sons Inc.: New York, 1991.
(12) (a) Corey, E. J.; Venkateswawarlu, A. *J. Am. Chem. Soc.* **1972**, *94*, 6190−6191. (b) Ogilvie, K. K.; Beaucage, S. L.; Entwistle, D. W. *Tetrahedron Lett*. **1976**, *17*, 1255−1256. (c) Lalonde, M; Chan, T. H. *Synthesis* **1985**, 817−845.
(13) Wincott, F.; DiRenzo, A.; Shaffer, C.; Grimm, S.; Tracz, D.; Workman, C.; Sweedler, C.; Gonzalez, C.; Scaringe, S.; Usman, N. *Nucleic Acids Res.* **1995**, *23*, 2677−2684.
(14) Cunico, R. F.; Bedell, L. *J. Org. Chem.* **1980**, *45*, 4797−4798.
(15) Wetter, H.; Oertle, K. *Tetrahedron Lett*. **1985**, *26*, 5515−5518.
(16) Ogilvie, K. K.; Thompson, E. A.; Quillam, E. A.; Westmore, J. B. *Tetrahedron Lett*. **1974**, 2865−2868.

(17) When **1** was incubated in acetic acid at concentrations higher than 40% at 50 °C or in formic acid at concentrations higher than 60% at 30 °C, significant decomposition occurred, so the reaction rate could not be estimated.

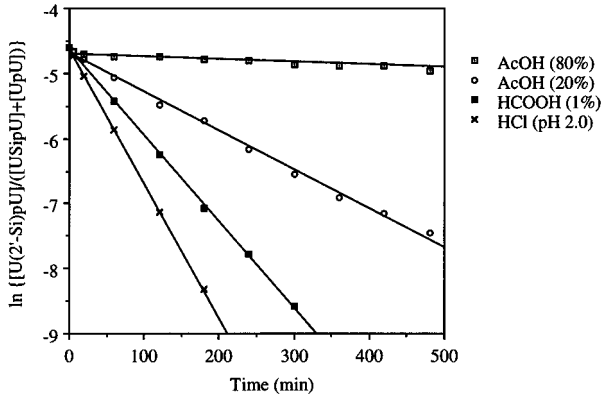


Figure 2. The kinetic data of hydrolytic cleavage of the TBDMS group of U(2′-Si)pU (**1**) under acidic conditions using acetic acid, formic acid, and HCl.

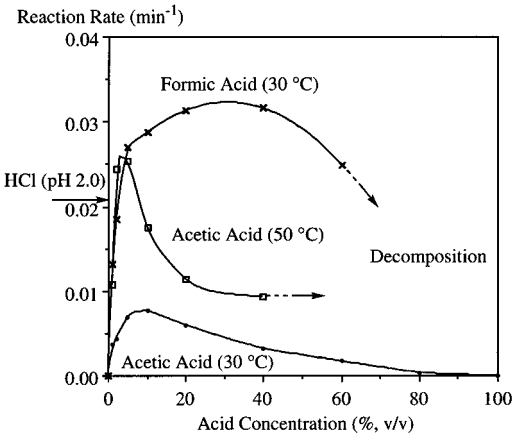


Figure 3. The relationship between the reaction rates and the acid concentration in acid-catalyzed desilylation of U(2′-Si)pU (**1**).

concentration of ca. 30% formic acid. The 2′-TBDMS group of **1** can be cleaved fast by treatment with 0.01 M HCl (pH 2.0, 30 °C), which usually is employed for removal of acid-labile 2′-protecting groups in oligoribonucleotide synthesis,[18] so that 4 h was required for completion. Moreover, the reaction proceeded without any side reactions even after 24 h as evidenced by HPLC.[19]

The difference in the reaction rate of hydrolytic desilylation between acetic acid and formic acid might be due to not only the difference in p*K*a between formic acid (p*K*a 3.75) and acetic acid (p*K*a 4.74) but also the solvation effect of the acids. For example, the proton concentration of 5% formic acid (1.33 M, pH 1.8) is almost twice that of 10% acetic acid (1.75 M, pH 2.3), but the rate constants of the desilylations using 5% formic acid and 10% acetic acid were determined to be 2.7×10^{-2} and 7.7×10^{-3} min^{-1}, respectively, showing that the desilylation of **1** occurred not 2 but 3.5 times faster in the former than in the latter. The profile of the reaction rates in acetic acid seems to obey fourth order kinetics regarding [H_2O] (Figure 4A), but the reaction rate profile in formic acid seems first order to [H_2O] (Figure 4B), from the mathematical consideration.[19] These results indicate that the solvation network in this reaction is important. Therefore, the solvation effect is discussed as follows.

It is known that hydrolysis of TBDMS ethers derived from secondary alcohols requires stronger conditions than that of TBDMS ethers from primary alcohols.[10] In addition, to date no papers have appeared concerning desilylation of the TBDMS group in secondary TBDMS ethers by the use of weak acids at low concentrations such as 4% acetic acid.[11] Since the acid hydrolysis of the 2′-TBDMS group of **1** proceeded unexpectedly fast even in such a weak acid, neighboring group participation of the vicinal internucleotidic phosphate residue[20] seems to be important (Scheme 2). Similar rate enhancements have been reported in the deprotection of 2′-tetrahydropyranylated and 2′-Ftmp-protected UpU derivatives.[21,22] In such reactions involving the neighboring group participation, the charge distribution of the reaction species changes from "A−B + C" to "$A^- +$ B−C^+" through "[$A^{\delta-}$···B···$C^{\delta+}$]" where A−B and C refer to RO−$SiMe_2$tBu and H_2O. Thus, it is likely that the reaction should proceed faster in polar solvents owing to the solvation effect than in nonpolar solvents if the attack of water on the silyl ether is a rate-determining step. Since the specific permittivities of acetic acid, formic acid, and water are 58.5, 6.15, and 78.5, respectively, the reaction in acetic acid is more affected by [H_2O] than in formic acid.

Acidic Hydrolysis of the TBDMS Group of 2′-*O*-TBDMS-Protected UpU Dimer Derivatives [U(2′-Si)psU) (2) and U(2′-Si)p(Me)U (3)]. To ascertain whether the participation of the internucleotidic phosphodiester residue is involved in the present desilyalation, the phosphorothioate derivative [U(2′-Si)psU, **2**][23] was synthesized. If hydrolysis of the 2′-TBDMS group of **1** does not involve neighboring group participation, no difference in the reaction rates between the diastereomers of **2** should be observed. This is because the elimination abilities of the TBDMS groups in the diastereomers are not so different from each other. In contrast, if the reaction proceeds with neighboring group participation, the rates of desilylation of the diastereomers should be significantly different from each other because the hydrogen bonding between the oxygen of the 2′-silyl ether and the hydrogen of the protonated internucleotidic phosphodiester [(RO)(R′O)P(O)(OH)] can be formed favorably for only one of the diastereomers.

Since the ^{31}P NMR resonance signals of the diastereomeric phosphorothioate dimers R_p-[U(2′-Si)psU] (**2b**) and S_p-[U(2′-Si)psU] (**2a**) have been assigned by Stawinski *et al.*,[23] the desilylations of **2** were monitored by ^{31}P-NMR. Figure 5B shows the HPLC profile of the mixture obtained by the reaction of **2** with 0.01 M HCl−D_2O (9:1, v/v) at 23 °C for 8 h. As described in the hydrolysis of **1**, these diasteroisomers have been shown to undergo pseudo-first-order hydrolysis. On the other hand, a marked difference in hydrolytic rate between these diastereomers was observed as shown in Figure 6: The *R*p isomer **2b**, -2.7×10^{-3} min^{-1}; the *S*p isomer **2a**, -5.3×10^{-3} min^{-1}.

Next, desilylation of the methylphosphonate derivative [U(2′-Si)p(Me)U] (**3**)[24] was carried out to compare with that of **1**. Since the methylphosphonate diester bond is neutral, the possibility of neighboring group participation is ruled out and simple hydrolysis of the 2′-TBDMS ether linkage should be expected. When the methylphosphonate dimer **3** was treated with 0.01 M HCl (pH 2.0) at 30 °C under the same conditions as prescribed for the hydrolysis of the TBDMS ether of **1**, the desilylation proceeded much more slowly ($t_{1/2}$ = 7 h) than for

(18) Griffin, B. E.; Jarman, M.; Reese, C. B. *Tetrahedron* **1968**, *24*, 639−622.

(19) See supporting information.

(20) Tsuruoka, H.; Shohda, K.; Wada, T.; Sekine, M. submitted for publication.

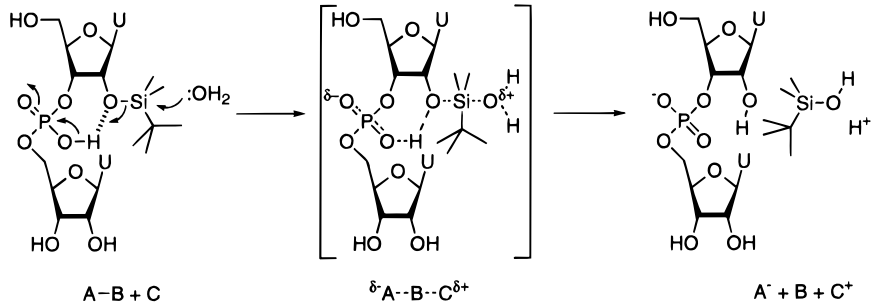
(21) Rao, T. S.; Reese, C. B.; Serafinowska, H. T.; Takaku, H.; Zappia, G. *Tetrahedron Lett.* **1984**, *28*, 4897−4900.

(22) Reese, C. B. *Nucleosides Nucleotides* **1991**, *10*, 81−97.

(23) Stawinski, H. A. J.; Strömberg, R.; Thelin, M. *J. Org. Chem.* **1992**, *57*, 6163−6169.

(24) (a) Jager, A.; Engels, J. *Tetrahedron Lett.* **1984**, *25*, 1437−1440. (b) Helinski, J.; Dabkowski, W.; Michalski, J. *Tetrahedron Lett.* **1991**, *32*, 498l-4984.

Scheme 2



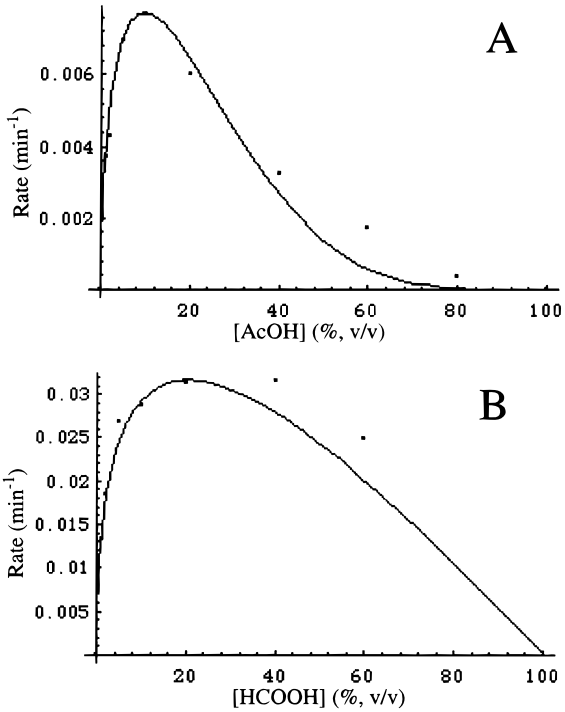


Figure 4. Plots of the calculated (line) and observed (dot) reaction rates.

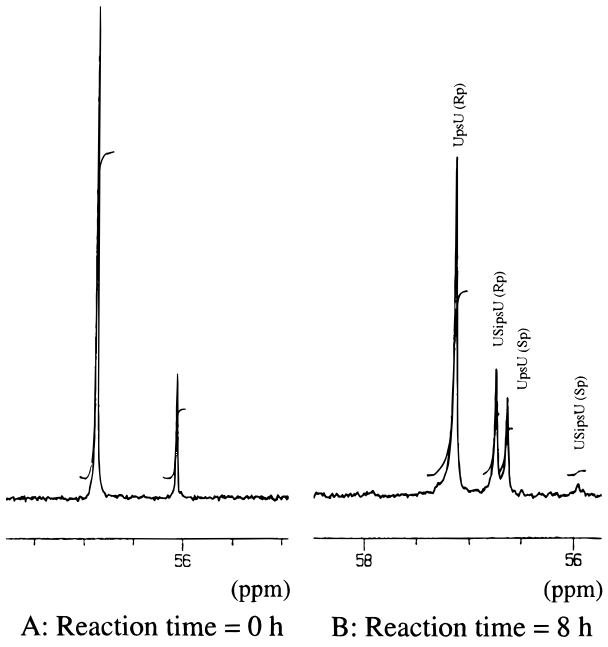


Figure 5. The ^{31}P NMR analysis of the desilyation of a mixture of *R*p- and *S*p-isomers of U(2′-Si)psU (**2**) in 0.01 M HCl−D_2O (9:1, v/v) at 23 °C.

1 ($t_{1/2}$ = 32 min), as clearly shown in Figure 7. In this case, a diastereomeric mixture of **3** was used for the estimation of the reaction rate. As a result, a linear relationship between the time and logarithm of the progress of the reaction was observed, as shown in Figure 7. It turned out that the rate of hydrolysis of the 2′-TBDMS ether in the *R*p isomer was similar to that of the *S*p isomer. On the basis of the above results, a plausible mechanism is depicted in Scheme 2.

Monte Carlo Simulation[25] of a Diastereomeric Pair of the Protonated Form of U(2′-Si)pU. Provided that the hydrolysis of the 2′-TBDMS ether in **1** is assisted by the participation of the internucleotidic phosphodiester group in the protonation process, a favorable conformer of **1** for hydrogen bonding between the acidic hydrogen of the 3′-5′-phosphodiester group and the oxygen of the 2′-silyl ether group must exist. Therefore, conformational analysis using Monte Carlo simulations of a diastereomeric pair of protonated U(2′-Si)pU (**1a** and **1b**, Figure 8) was investigated.

If the neighboring group participation exists, the hydrogen-bonding ability of **1a** is different from that of **1b**, and the reaction pathway is limited to be *via* conformers which are suitable for the hydrogen bonding. Figure 8 shows the relationship between the energy (vertical axis) and the atomic distance between the oxygen atom of the 2′-TBDMS ether and the acidic hydrogen atom of the protonated internucleotidic phosphorodiester (horizontal axis) of the 100 conformers which have lower energy structures of **1a** and **1b**. Obviously, **1a** was favorable to form the hydrogen bond, because the distances between the oxygen atom and the hydrogen atom of most of

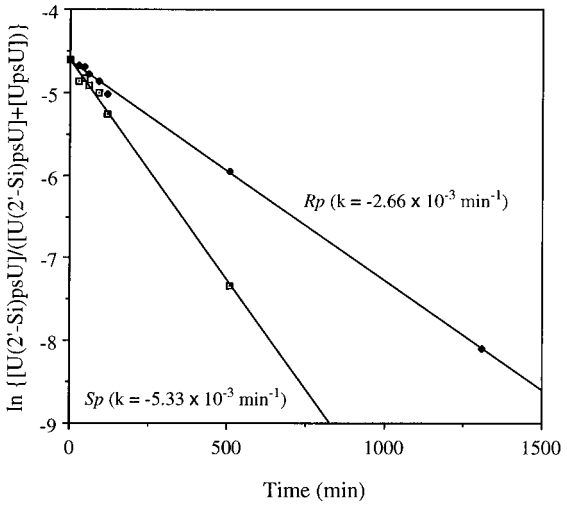


Figure 6. The kinetic data of hydrolytic cleavage of the TBDMS group of the *S*p and *R*p isomers of U(2′-Si)psU (**2**) in 0.01 M HCl−D_2O (9:1, v/v) at 23 °C.

(25) Allen, M. P., Tildesley, D. J. *Computer Simulation of Liquids*, Clarendon Press: Oxford, 1987.

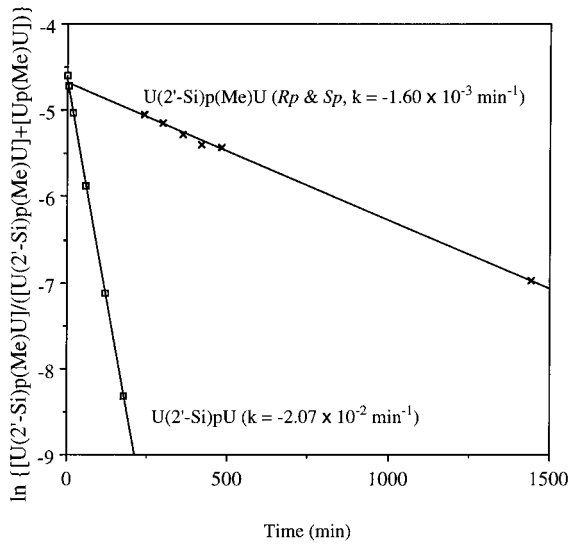


Figure 7. Comparison of the hydrolytic rate of the TBDMS group of U(2′-Si)pU (**1**) with that of U(2′-Si)p(Me)U (**3**).

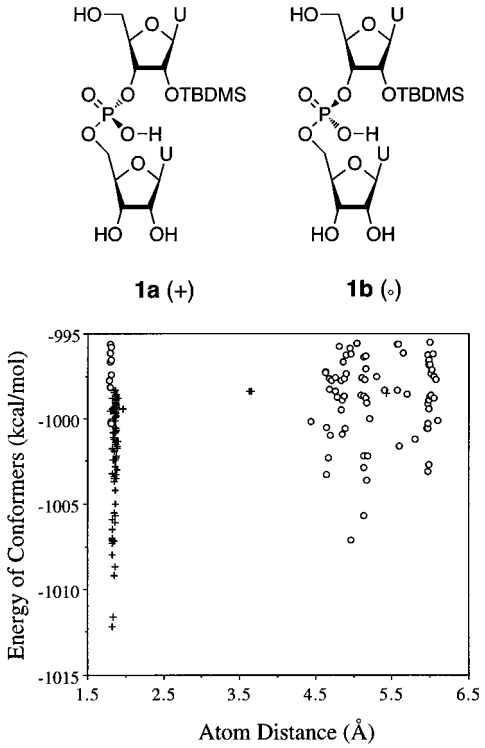


Figure 8. Relationship between the O(2′)−H(phosphate) atom distance and the energy of the conformers of the protonated diastereomers **1a** and **1b** generated by Monte Carlo calculations.

the conformers are short enough to form the hydrogen bond, and the energies of the conformers are relatively low. On the other hand, the distance between the oxygen atom and the hydrogen atom of the conformer of **1b** is too long to form the hydrogen bond, and the energy of each conformer is too high. Therefore, the present hydrolysis seems to occur in favor of **1a**.

Molecular Orbital (MO) Calculation of Phosphorothioate Diesters. In the case of the phosphorothioate derivative **2**, it is important to see which atom is protonated, sulfur or oxygen. In neutral water, most of the phosphorothioate is dissociated and its sulfur atom is negatively charged (Scheme 3, **7a** and **7b**).[26] However, in acidic solution, it is unknown which is predominant, the sulfur protonated form or the oxygen protonated form. The MO calculation of a phosphorothioate triester

Table 1. Atomic Distances, Bond Angles, and Diheadral Angles of Each Molecule[a]

atom distance (Å)		bond angle (deg)		diheadral angle (deg)	
		$(MeO)_2POS^-$ (**7**)			
S^1-P^2	1.9980	$S^1-P^2-O^3$	118.4173		
P^2-O^3	1.4677	$S^1-P^2-O^4$	109.8489		
P^2-O^4	1.6100	$P^2-O^4-C^5$	119.7189		
O^4-C^5	1.3993	$O^4-C^5-H^6$	110.9188	$S^1-P^2-O^4-C^5$	−74.9066
C^5-H^6	1.0857	$O^4-C^5-H^7$	107.2660	$P^2-O^4-C^5-H^6$	67.1413
C^5-H^7	1.0839	$O^4-P^2-O^9$	94.9998	$P^2-O^4-C^5-H^7$	186.2137
C^5-H^8	1.0851	$O^4-C^5-H^8$	110.9382	$P^2-O^4-C^5-H^8$	−54.4184
		$(MeO)_2POSH$ (**8**)			
S^1-P^2	2.0931	$S^1-P^2-O^3$	109.7857		
P^2-O^3	1.4455	$S^1-P^2-O^4$	106.3407		
P^2-O^4	1.5635	$P^2-O^4-C^5$	122.2591	$S^1-P^2-O^4-C^5$	−86.4018
O^4-C^5	1.4246	$O^4-C^5-H^6$	110.2105	$P^2-O^4-C^5-H^6$	67.3490
C^5-H^6	1.0831	$O^4-C^5-H^7$	106.1714	$P^2-O^4-C^5-H^7$	186.2303
C^5-H^7	1.0783	$O^4-C^5-H^8$	110.1343	$P^2-O^4-C^5-H^8$	−54.1737
C^5-H^8	1.0806	$O^4-P^2-O^9$	99.6096	$H^{14}-S^1-P^2-O^4$	−52.7855
S^1-H^{14}	1.3321	$P^2-S^1-H^{14}$	95.8045	$O^3-P^2-S^1-H^{14}$	179.9622
		$(MeO)_2PSOH$ (**9**)			
S^1-P^2	1.9190	$S^1-P^2-O^3$	111.9424		
P^2-O^3	1.5860	$S^1-P^2-O^4$	117.6054		
P^2-O^4	1.5678	$P^2-O^4-C^5$	124.3526	$S^1-P^2-O^4-C^5$	−35.8750
O^4-C^5	1.4234	$O^4-C^5-H^6$	110.0554	$P^2-O^4-C^5-H^6$	66.3792
C^5-H^6	1.0803	$O^4-C^5-H^7$	106.0366	$P^2-O^4-C^5-H^7$	185.7560
C^5-H^7	1.0785	$O^4-C^5-H^8$	110.5645	$P^2-O^4-C^5-H^8$	−55.0603
C^5-H^8	1.0827	$O^4-P^2-O^9$	98.8210	$H^{14}-O^3-P^2-O^4$	51.6633
O^3-H^{14}	0.9449	$P^2-O^3-H^{14}$	114.6540	$S^1-P^2-O^3-H^{14}$	179.9975

[a] Numerical labels used in this table are shown in Figure 9.

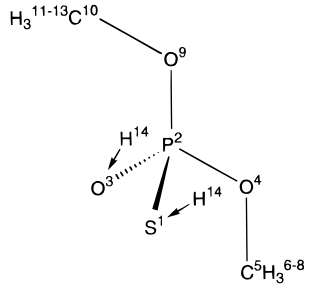
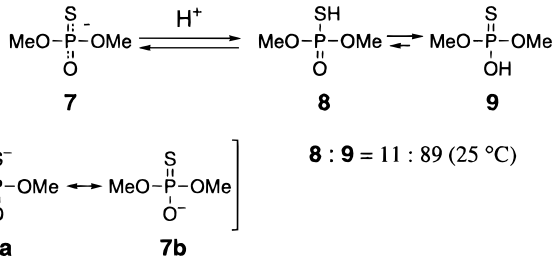


Figure 9. The numerical labels of **7**, **8**, and **9** used in MO calculation.

Scheme 3



was reported by Katagi,[27] but there are no papers regarding the phosphorothioate diester. Therefore, MO calculations (structure optimization, HF/6−311G**; single point energy calculation, MP2/6−311G**) of dimethyl phosphorothioate anion (**7**) and two equilibrium forms of dimethyl phosphorothioate (**8** and **9**) were carried out. Table 1 shows the calculated values of the atomic distances, bond angles, and dihedral angles of each structure.[28] Table 2 shows the total energy of each structure, atomic charge of each atom, energy levels of HOMO and LUMO, and the atomic orbitals having large coefficients at HOMO and LUMO. In the case of **7**, the sulfur atom (S^1) has a larger atomic charge than that of the oxygen atom (O^3), and

(26) (a) Frey, P. A.; Sammons, R. D. *Science* **1985**, *228*, 541−545. (b) Frey, P. A.; Reimschussel, W.; Paneth, P. *J. Am. Chem. Soc.* **1986**, *108*, 1720−1722.

the p-orbital of S^1 has a large coefficient of HOMO. This result suggests that the oxygen-protonated form **7a** is predominant (Scheme 3), being in good agreement with the experimental result.[26] On the other hand, from the total energy values of **8** and **9**, the oxygen-protonated form **9** is 1.23 kcal/mol more stable (1 au = 627.5 kcal/mol) than the sulfur-protonated form **8**. According to the energy difference between **8** and **9**, the ratio of **8** and **9** is calculated to be 11:89 at 25 °C on the basis of the Boltzmann distribution.

Mechanism and Kinetics of Desilylation of Phosphorothioate Diastereomers. From consideration of the result of the Monte Carlo simulations and MO calculations, the difference in the reaction rates between the *S*p and *R*p isomers of **2** is explained by the participation of the phosphorothioate diester group in the protonation process of the 2′-silyl ether group. The 2′-TBDMS group in the *S*p isomer of **2**, which is hydrolyzed faster, is protonated in the same direction as **1a**, which is suitable for neighboring group participation based on the Monte Carlo simulation. On the other hand, the 2′-TBDMS group of the *R*p isomer, which is hydrolyzed more slowly, is protonated in the same direction for **1b**, which is unfavorable for neighboring group participation.

Application to Deprotection of the 2′-TBDMS Group of a Chemically Synthesized Oligoribonucleotide Intermediate. The present reaction conditions were applied to the deprotection of a chemically synthesized RNA intermediate. A 2′-*O*-TBDMS-protected 12mer having the sequence of UACGUACGUACG was synthesized on a highly cross-linked polystyrene resin[29] by the standard phosphoramidite method developed by Andrus et al.[30] The base protecting groups of A, C, and G used were phenoxyacetyl, isobutyryl, and dimethylformamidine, respectively.[31] A commercially available polystyrene resin carrying an appropriately protected G unit *via* the usual succinate linker was chosen, and the chain elongation was carried out as usual. Release of the protected oligomer from the resin and deprotection of the N-protecting groups were done by treatment with concentrated ammonia−ethanol (3:1, v/v). The 2′-*O*-TBDMS-protected 12mer having the DMTr group at the 5′-end was treated with 0.01 M HCl (pH 2.0)−dioxane (1:1, v/v) at 35 °C for 12 h. The ion-exchange HPLC profile of the deprotected product thus obtained is shown in Figure 10A. Further purification using ion-exchange HPLC followed by desalting using gel filtration gave the pure oligomer in an overall yield of 54% (Figure 10B,C).

Quite recently, Reese[32] and Hecht[33] have independently reported detailed studies of cleavage and migration of internucleotidic linkages of oligoribonucleotides to acids to check the optimum conditions for removal of the acid-labile Ftmp group. They recommended the use of pH higher than 3 to circumvent such side reactions but also suggested that the phosphoryl migration is highly sequence dependent. Therefore, we carefully checked if such byproducts could be observed by using a high range scale in HPLC for detection of four kinds of 2′-5′ linked dimers (UA, AC, CG, and GU) which would be theoretically formed as nuclease P1 resistant fragments by random phosphoryl migration. In this case, however, we could not observe these byproducts clearly. Digestion of the isolated 12mer with nuclease P1 followed by alkaline phosphatase gave an equimolar mixture of A, G, U, and C (see supporting information). The pH of the mixture of 0.01 M HCl−dioxane (1:1, v/v) used in our study was ca. 2.3, which is a little milder than the pH 2.0 usually employed in RNA synthesis. Accordingly, the cleavage and phosphoryl migration of internucleotidic linkages would be better avoided than when pH 2.0 was used.

Table 2. Total Energy, Atomic Charge, and HOMO and LUMO Energies Representative Orbital Coefficients

total energy (Hartree)			atomic charge (*e*)	orbital energy (Hartree)	
			$(MeO)_2POS^-$ (**7**)		
−1043.4293	1	S	−0.814820	HOMO	−0.15128
	2	P	1.351960	orbital coefficient	
	3	O	−0.804731	0.5580	(S1 10PZ)
	4	O	−0.640237	0.3359	(S1 11PZ)
	5	C	0.032888		
	6	H	0.096339		
	7	H	0.057313		
	8	H	0.087505	1.4482	(C5 4S)
	9	O	−0.640209	1.4458	(C10 4S)
	10	C	0.032849	−1.0774	(H7 3S)
	11	H	0.087569	−1.0755	(H12 3S)
	12	H	0.057299		
	13	H	0.096274		
			$(MeO)_2POSH$ (**8**)		
−1043.9608	1	S	−0.188419	HOMO	−0.39848
	2	P	1.370418	orbital coefficient	
	3	O	−0.725247	0.6010	(S1 10PX)
	4	O	−0.630023	0.2714	(S1 11PX)
	5	C	0.017687		
	6	H	0.110636	LUMO	0.13435
	7	H	0.113376	orbital coefficient	
	8	H	0.125895	−0.8934	(S1 6S)
	9	O	−0.630009	0.9939	(S1 11PZ)
	10	C	0.017645	−0.6813	(C5 4S)
	11	H	0.125913	−0.6811	(C10 4S)
	12	H	0.113375		
	13	H	0.110629		
	14	H	0.068125		
			$(MeO)_2PSOH$ (**9**)		
−1043.9628	1	S	−0.551246	HOMO	−0.35127
	2	P	1.371840	orbital coefficient	
	3	O	−0.602344	0.5753	(S1 10PZ)
	4	O	−0.635231	0.2716	(S1 11PZ)
	5	C	0.015077		
	6	H	0.134919	LUMO	0.13590
	7	H	0.111782	orbital coefficient	
	8	H	0.107469	1.1747	(H14 3S)
	9	O	−0.635266	−0.5188	(O3 4S)
	10	C	0.015108	0.5621	(H11 3S)
	11	H	0.107486		
	12	H	0.111770		
	13	H	0.134901		
	14	H	0.313736		

Conclusion

The TBDMS group used as the 2′-protecting group of chemically synthesized RNA intermediates is easily hydrolyzed in diluted acidic solutions. It was found that there is a characteristic relation between the reaction rate and the acid concentration. From the result of the extended studies of acid hydrolysis of the 2′-TBDMS group of the phosphorothioate and methylphosphonate derivatives **2** and **3**, it was concluded that the neighboring group participation of the internucleotidic

(27) Katagi, T. *Theochem* **1990**, *68*, 61−67.

(28) The structural parameters of O^9 to H^{13} are not shown in Table 1 because of the symmetry of these molecules (O^9 to H^{13} are equal to O^4 to H^8, respectively).

(29) McCollum, C.; Andrus, A. *Tetrahedron Lett.* **1991**, *32*, 4069−4072.

(30) Vu, H.; McCollum, C.; Jacobson, K.; Theisen, P.; Vinayak, R.; Spiess, E.; Andrus, A. *Tetrahedron Lett.* **1990**, *31*, 7269−7272.

(31) (a) Schulhof, J. C.; Molko, D.; Teoule, R. *Tetrahedron Lett.* **1987**, 28, 51−54. (b) Schulhof, J. C.; Molko, D.; Teoule, R. *Nucleic Acids Res.* **1987**, *17*, 397−416. (c) Sinha, N. D.; Davis, P. Usman, N.; Perez, J.; Hodge, R.; Kremsky, J. Casale, R. *Biochemie* 1993, 75, 13−23. (d) Vhaix, C.; Molko, D.; Teoule, R. *Tetrahedron Lett.* **1989**, *30*, 71−74. (e) Wu, T. Ogilvie, K. K.; Pon, R. T. *Nucleic Acids Res.* **1989**, *17*, 3501−3517.

(32) Capaldi, D. C.; Reese, C. B. *Nucleic Acids Res.* **1994**, *22*, 2209−2216.

(33) Morgan, M. A.; Kazakov, S. A.; Hecht, S. M. *Nucleic Acids Res.* **1995**, *23*, 3949−3953.

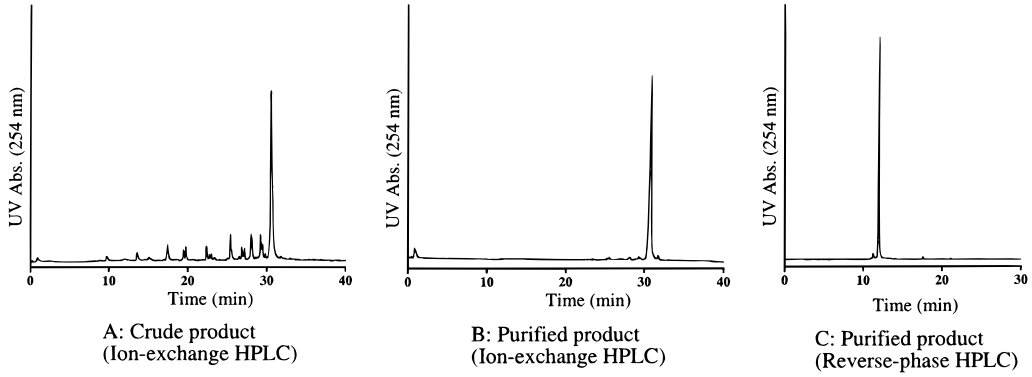
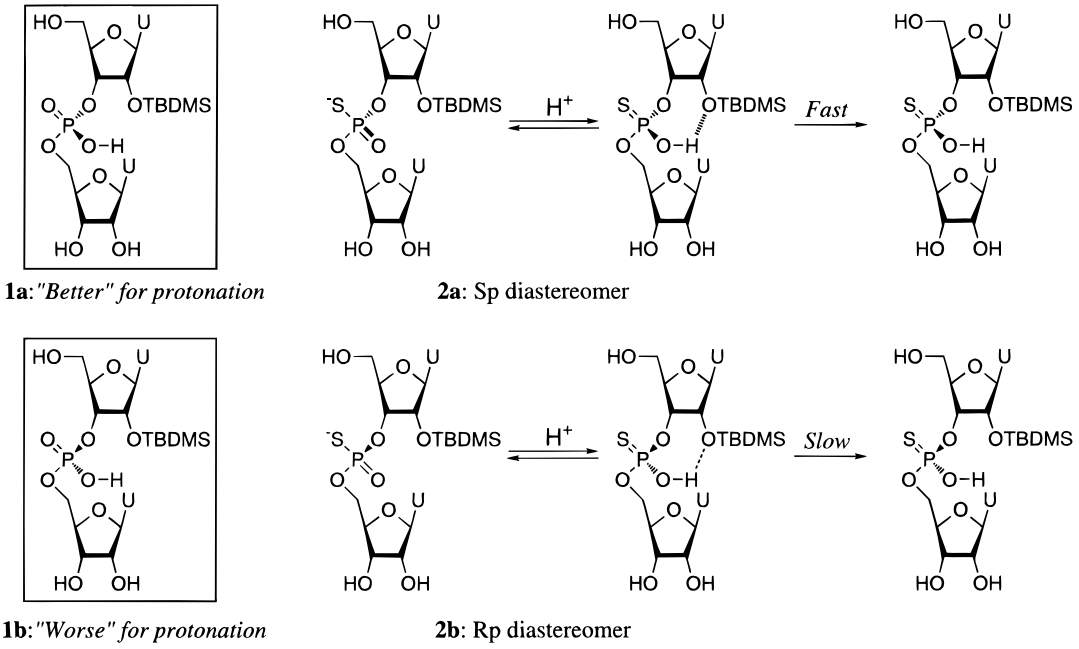


Figure 10. HPLC profiles of the crude and purified RNA 12mer obtained by treatment with HCl (pH 2.0)−dioxane (1:1, v/v).

Scheme 4



phosphorodiester group is highly plausible in this reaction. Finally, the reaction was successfully applied to deprotection of the TBDMS groups of a chemically synthesized RNA oligomer. This method can be used an alternative to the well-known procedure using the fluoride ion.

Experimental Section

General. Reverse-phase HPLC analyses were carried out on a Waters LC module 1 with a μ-Bondasphere C-18 column (3.9 × 150 mm, Waters). Conditions: a linear gradient of 0−30% MeCN in 0.1 M aqueous $AcONH_4$ in 30 min at a flow rate of 1 mL/min at 50 °C. Ion-exchange HPLC analyses were carried out on a Waters LC module 1 with a Gen-Pak FAX column (4.6 × 100 mm, Waters). Conditions: a linear gradient of 10−63% aqueous 1.0 M NaCl containing 25 mM NaH_2PO_4 in 25 mM aqueous NaH_2PO_4 in 40 min at the flow rate of 1 mL/min at 50 °C. ^{1}H-NMR spectra were recorded at 23 °C on a JEOL EX-270 (270.00 MHz) with TMS or $CHCl_3$ (δ = 7.26) as an internal reference. ^{31}P-NMR spectra were measured at 23 °C on a JEOL EX-270 (109.25 MHz) with 85% H_3PO_4 as an external reference. UV spectra were measured at 23 °C on a Hitachi U-2000. Silica gel TLC and column chromatography were performed on Merck Kieselgel 60F-254 and Wakogel C-200, respectively. Reagents were purchased from Tokyo Kasei Co. or Aldrich Chemical Co. unless noted. The organic solvents were purified and dried by the appropriate procedure. Snake venom phosphodiesterase and calf intestine alkaline phosphatase were purchased from Boeringer Mannheim GmbH and Nuclease P1 was purchased from Yamasa Co.

Calculation. Monte Carlo simulations were carried out using Macro Model[34] (version 4.5) software on a Silicon Graphics Inc. Indigo2 workstation. Each structure, obtained on the simulation, was minimized using the Amber* force field.[35] The effect of solvation was included in these simulations by the implicit treatment of solvent water with the GB/SA model.[36] The simulation was continued until the same global minimum structure was found four or five times. The low-energy structures within 20 kcal/mol from a global minimum were sampled. Theoretical calculations were carried out using Gaussian 94[37] on a Cray C-916/12256 super computer. The acidic proton (H^1) was placed outside of the S^1 or O^3 as shown in Figure 9. Although it seems that the structure for which H^{14} was placed between S^1 and O^3 is more stable than the structure used in the calculation, it is not unfavorable to consider neighboring group participation of the internucleotidic phosphodiester group from the result of Monte Carlo simulations. Each structure was optimized using a 6−311G** basis set[38] at the Hartree−Fock level, and single point calculations were carried out at the MP2 level involving electronic correlation to obtain accurate energies and atomic charges.

Synthesis of 2′-*O*-TBDMS-Protected UpU Derivatives. U(2′-Si)pU,[9a] U(2′-Si)psU,[20] and U(2′-Si)p(Me)U[24] were prepared by the

(34) Mohamadi, F.; Richards, N. G. J.; Guita, W. C.; Likskamp, R.; Caufield, C.; Chang, G.; Hendrickson, T.; Still, W. C. *J. Comput. Chem.* **l990**, *11*, 440−467.

(35) Amber* is the force field involved in Macro Model version 4.5. (a) Kollman, P. A. *Chem. Rev.* **1993**, *93*, 2395−2417. (b) Kollman, P. A. *Tetrahedron Lett.* **1992**, *33*, 7793−7796.

(36) Still, W. C.; Tempczk, A.; Hawley, R. C.; Hendrickson, T. *J. Am. Chem. Soc.* **1990**, *112*, 6127−6129.

literature procedures and stored as 1 mM solution in MeCN at −30 °C. The 2′-silylated RNA 12mer intermediate was prepared by using a Perkin-Elmer model 392 DNA/RNA synthesizer with commercially available reagents (Perkin-Elmer Applied Biosystems) and released from the solid support automatically by treatment four times with 25% NH_3−EtOH (3:1, v/v) at room temperature for 15 min. The 2′-*O*-TBDMS protected dodecaribonucleotide UACGUACGUACG having the DMTr group at the 5′-terminal end was obtained in an overall yield of 95% with the average coupling yield of 99.5%.

Desilylation of 2′-*O*-TBDMS Dimer Derivatives. After 1 mL of 1 mM **1** or **3** solution was evaporated in *vacuo*, 1 mL of acetic acid or formic acid solution [acid concentration: 0, 1, 2, 5, 10, 20, 40, 60, 80 and 100% (v/v)] or 0.01 M HCI (pH 2.0) was added. These solutions were incubated at 30 or 50 °C using a thermal controlled water bath. After 5 and 20 min and 1, 2, 3, 4, 5, 6, 7, 8, and 24 h, an aliquot of 10 μL was sampled from the mixture and poured into 200 μL of 0.1 M ammonium acetate (pH 7.0). When the pH was less than 6, 10 μL of 25% NH_3 was added so as to make the pH 6−7. The mixture was analyzed by reverse-phase HPLC. The reaction rates were calculated from the ratio of **1** to the sum (10 nmol) of **1** and **4**. Compound **6** is not stable and decomposed immediately after the TBDMS group of **3** was removed. The peak corresponding to **6** was not detected so that the reaction rate was calculated from the sum of the decomposed products.

Compound **2** (*R*p:*S*p = 5:1 mixture of the diastereomers, 32 μmol) was dissolved in 0.01 M HCI−D_2O (9:1, 500 μL). ^{31}P-NMR spectra were measured after 8.63, 28.88, 44.50, 59.83, 89.75, 116.5, 509.0, and 1310 min, the midpoints between the starting and end times during the NMR measurement).

Desilylation of 2′-Silylated Dodecaribonucleotide by Use of 0.01 M HCl−Dioxane. A fully protected RNA oligomer (1.0 μmol), which was prepared according to the literature method, was released from the solid support using 25% NH_3−EtOH (3:1, v/v, 15 min × 4) and the phosphate protecting groups were removed at the same time. This solution was incubated at 60 °C for 1 h to remove the base-protecting groups and evaporated in *vacuo*. This 12mer intermediate was dissolved in dioxane (5.0 mL) and 0.01 M HCI (5.0 mL) was added. The mixture was incubated at 35 °C for 12 h. The reaction was quenched by addition of 200 μL of 1.0 M ammonium acetate (pH 7.0). This crude product was analyzed using ion-exchange HPLC. The solution was lyophilized and dissolved in sterilized water (2.0 mL). Part ($^1/_{10}$) of this crude product was purified using anion-exchange HPLC and desalted by using gel filtration to give UACGUACGUACG (5.4 A_{254} unit, 54% based on the assumption of 20% hypochromicity). This product was analyzed by ion-exchange and reverse-phase HPLC.

Enzymatic Studies. The isolated RNA 12mer (1.0 A_{260} unit) was dissolved in 50 mM Tris-HCl buffer (pH 8.0, 80 μL). Snake venom phosphodiesterase (8 unit) and 1 M $MgCl_2$ (4 μl) were added. The reaction mixture was incubated at 37 °C for 3.5 h. After the enzyme was inactivated by heat treatment (100 °C, 1 min), calf intestine alkaline phosphatase (10 unit) was added. After the reaction mixture was incubated at 37 °C for 3.5 h, the enzyme was inactivated by heat treatment (100 °C, 1 min). The nucleoside ratio of the mixture was in good agreement with the stoichiometric one (calcd for A:G:U:C = 1:1:1:1, found A:G:U:C = 1:0.89:0.97:1.13) from the reverse-phase HPLC analysis.

The isolated RNA 12mer (1.0 A_{260} unit) was dissolved in 50 mM sodium acetate buffer (pH 5.4, 50 μL) containing $ZnCl_2$ (0.1 mM). Nuclease P1 (10 unit) was added and the mixture was incubated at 37 °C for 3.5 h. After the enzyme was inactivated by heat treatment (100 °C, 1 min), the mixture was analyzed by the reverse-phase HPLC. There are essentially no phosphoryl-migrated products.

Acknowledgment. This work was supported by a Grant-in-Aid for Scientific Research on Priority Areas from the Ministry of Education, Science, Sports and Culture, Japan. The authors also thank the Toray Scientific Fundation for generous financial support.

Supporting Information Available: Reverse-phase and ion-exchange HPLC profiles of the dimers and oligomer, ^{31}P-NMR profiles of phosphorothioate dimer and the procedure for calculation of the relationship between the reaction rates and acid concentration (8 pages). See any current masthead page for ordering and Internet access instructions.

JA961959I

(37) *Gaussian 94, Revision B. 3*; Frisch, M. J.; Trucks, G. W.; Schlegel. H. B.; Gill, P. M. W.; Johnson, B. G.; Robb, M. A.; Cheeseman, J. R.; Keith, T.; Petersson, G. A.; Montfomery, J. A.; Raghavachari, K.; Al-Laham, M. A.; Zakrzewski, V. G.; Ortiz, J. V.; Foresman, J. B.; Peng, C. Y.; Ayala, P. Y.; Chen, W.; Wong, M. W.; Andres, J. L.; Replogle, E. S.; Gomperts, R.; Martin, R. L.; Fox, D. J.; Binkley, J. S.; Defrees, D. J.; Baker, J.; Stewart, J. P.; Head-Gordon, M.; Gonzales, C.; Pople, J. A. Gaussian, Inc.: Pittsburgh PA, 1995.

(38) (a) Krishnan, R.; Binkley, J. S.; Seeger, R.; Pople, J. A. *J. Chem. Phys.* **1980**, *72*, 650−654. (b) Collins, J. B.; Schleyer, P. von R.; Binkley, J. S.; Pople, J. A. *J. Chem. Phys.* **1976**, *64*, 5142−5151. (c) Pietro, W. J.; Francl, M. M.; Hehre, W. J.; DeFrees, D. J.; Pople, J. A. ; Binkley, J. S. *J. Am. Chem. Soc.* **1982**, *104*, 5039−5048.