JOC Article

A New Route to 2′-*O*-Alkyl-2-thiouridine Derivatives via 4-*O*-Protection of the Uracil Base and Hybridization Properties of Oligonucleotides Incorporating These Modified Nucleoside Derivatives

Itaru Okamoto,[†] Koh-ichiroh Shohda,[†] Kohji Seio,[‡,§] and Mitsuo Sekine*[,†,§]

Department of Life Science and Frontier Collaborative Research Center, Tokyo Institute of Technology, Nagatsuta, Midori-ku, Yokohama 226-8501, Japan, and CREST, JST (Japan Science and Technology Corporation) Nagatsuta, Midori-ku, Yokohama 226-8501, Japan

msekine@bio.titech.ac.jp

Received August 26, 2003

Oligonucleotides containing 2-thiouridine (s^2U) in place of uridine form stable RNA duplexes with complementary RNAs. Particularly, this modified nucleoside has proved to recognize highly selectively adenosine, the genuine partner, without formation of a mismatched base pair with the guanosine counterpart. In this paper, we describe new methods for the synthesis of 2-thiouridine and various 2′-*O*-alkyl-2-thiouridine derivatives. Oligoribonucleotides having these modified nucleoside derivatives were synthesized, and their hybridization and structural properties were studied in detail by the ^{1}H NMR analysis of these modified nucleosides and T_m experiments of RNA duplexes with their complementary RNA strands.

Introduction

2-Thiouridine (s^2U) and its derivatives have been discovered from transfer RNAs.[1] It has been recognized that the 2-thiocarbonyl group of s^2U plays a key role in changing its sugar conformation to a rigid C3′-*endo* form seen in typical RNA duplexes so that a modified s^2U-A base pair is more stabilized than the unmodified one.[2] It was also reported that s^2U derivatives found at the first letter of anticodon (the wobble position) improve the capability of base recognition to distinguish adenosine from guanosine located at the third letter of codon triplets on mRNAs and, moreover, enforce the codon−anticodon minihelix structure.[3] Actually, Testa and colleagues reported that the melting temperatures of RNA duplexes having an s^2U-G wobble base pair were considerably lower than those of RNA duplexes having a matched s^2U-A base pair.[4] This result indicated that 2-thiouridine forms a more stable base pair with adenosine than guanosine and exhibits predominant selectivity for formation of a base pair with adenosine over guanosine. Furthermore, our recent paper also revealed that the 2′-*O*-methyl-2-thiouridine (s^2Um) has a similar base recognition ability.[5]

The exact base pair recognition is due mainly to the weak hydrogen bonding property of the 2-thiocarbonyl group toward the 2-amino group of the guanine base. A G-U wobble base pair requires two hydrogen bonds.[6] One is the hydrogen bond between the 2-carbonyl oxygen of the uracil base and the 1-amido proton of the guanine base. The other is the hydrogen bond between the 6-carbonyl oxygen of the guanine base and the 3-imido proton of the uracil base. Generally, an electrostatic interaction between two components is a key factor for the hydrogen bonding.[7] Since the sulfur atom of the 2-thiocarbonyl group is bigger than the oxygen of the carbonyl group so that the polarized minus charge on the sulfur is dispersed on its surface, the hydrogen bonding ability of the 2-thiocarbonyl group is considerably weakened.

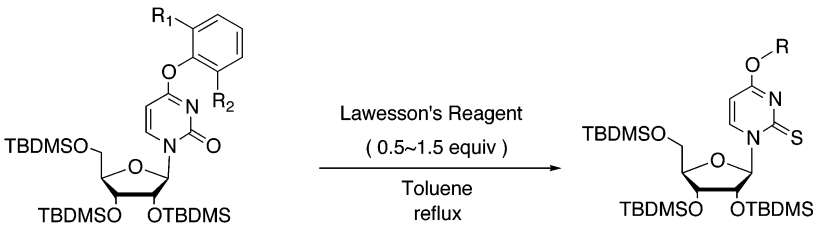
The thermal stability of RNA duplexes containing s^2U is due mainly to the rigid ribose residue, which prefers C3′-*endo* conformation. This C3′-*endo* predominance is entropically favorable for stable formation of A-type RNA duplexes, because all the ribose conformations of A-type

* To whom correspondence should be addressed. Phone (fax): +81-45-924-5706.
† Department of Life Science, Tokyo Institute of Technology.
‡ Frontier Collaborative Research Center, Tokyo Institute of Technology.
§ CREST.

(1) (a) Hall, R. H. In *The Modified Nucleosides in Nucleic Acids*; Columbia University Press: New York, 1971. (b) Chackalaparampil, I.; Cherayil, J. D. *Biochem. Int.* **1981**, *2*, 121. (c) Edmonds, C. G.; Crain, P. F.; Hashizume, T.; Gupta, R.; Stetter, K. O.; McCloskey, J. A. *J. Chem. Soc., Chem. Commun.* **1987**, 909. (d) Kimura-Harada, F.; Saneyoshi, M.; Nishimura, S. *FEBS Lett.* **1971**, *13*, 335.
(2) (a) Yamamoto, Y.; Yokoyama, S.; Miyazawa, T.; Watanabe, K.; Higuchi, S. *FEBS Lett.* **1983**, *157*, 95. (b) Sierzputowska-Gracz, H.; Sochacka, E.; Malkiewicz, A.; Kuo, K.; Gehrke, C. W.; Agris, P. F. *J. Am. Chem. Soc.* **1987**, *109*, 7171.
(3) Yokoyama, S.; Watanabe, T.; Murao, K.; Ishikura, H.; Yamaizumi, Z.; Nishimura, S.; Miyazawa, T. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 4905.
(4) Testa, S. M.; Disney, M. D.; Turner, D. H.; Kierzek, R. *Biochemistry* **1999**, *38*, 16655.
(5) Shohda, K.; Okamoto, I.; Wada, T.; Seio, K.; Sekine, M. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 1795.
(6) Sugimoto, N.; Kierzek, R.; Freier, S. M.; Turner, D. H. *Biochemistry* **1986**, *25*, 5755.
(7) Kawahara, S.; Wada, T.; Kawauchi, S.; Uchimaru,T.; Sekine, M. *J. Phys. Chem. A* **1999**, *103*, 8516.

10.1021/jo035246b CCC: $25.00
Published on Web 11/25/2003

SCHEME 1



1a: R_1=NO_2, R_2=H **1b**: R_1, R_2=H
1c: R_1, R_2=Cl **1d**: R_1,R_2=CH_3

RNA duplexes are C3′-*endo*.[8] In addition, the stacking effect between the 2-thiocarbonyl group and the 1- or 9-nitrogen atom of 3′-downstream nucleoside bases is also important for stabilization of RNA duplexes.[8] These properties of oligoribonucleotides containing 2-thiouridine or its derivatives are favorable for the antisense strategy and SNPs analysis.

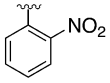
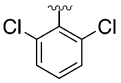
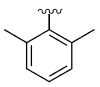
In general, 2-thiouridine has been prepared by glycosylation of a silylated 2-thiouracil base with ribose derivatives.[9] However, this method incurs several problems: It requires the use of expensive glycosyl donors and moisture-sensitive reactions. We have also encountered difficulty in isolating the desired product because of inseparable emulsion during the extraction. Therefore, it seems to us that it is difficult to perform a large-scale synthesis because of these inherent problems.

In this paper, we report a new convenient route to 2-thiouridine or its derivatives via selective conversion of the 2-carbonyl group to a 2-thiocarbonyl group. Moreover, the synthesis of various 2′-*O*-alkyl-2-thiouridine derivatives is described along with the clarified relationship between the 2′-*O*-modification and the duplex stability.

Results and Discussion

Synthesis of 2-Thiouridine (s^2U). Necessity of Protecting Groups for Protection of the 4-Carbonyl Group of Uridine. Lawesson's reagent or phosphorus pentasulfide has been generally used as a reagent for the conversion of the carbonyl group of nucleobases to a thiocarbonyl group.[10] The uracil base residue has 2- and 4-carbonyl groups. In general, the reactivity of the 4-carbonyl group is much higher than that of the 2-carbonyl group. Therefore, when the uracil base residue is allowed to react with thionating reagents without protection, 4-thiouracil derivatives are formed predominantly. This method provides a general route to 4-thiouridine and 2,4-dithiouridine but 2-thiouridine derivatives cannot be obtained.[11] Therefore, protection of the 4-carbonyl group is necessary to selectively thionate the 2-carbonyl group. Reese and colleagues reported the use of phenyl ether-type protecting groups for avoidance of side reactions on the 4-carbonyl group of uracil and thymine residues in their oligonucleotide synthesis.[12] Such a 4-*O*-protection strategy is also available for the 2-thionation of the 2-carbonyl group of the uracil base because Laweeson's reagent and phosphorus pentasulfide do not damage phenyl ether-type functions.

TABLE 1. Thionation of 4-*O*-Aryluridine Derivatives 1a−d with Lawesson's Reagent

Entry	R	L.R. (equiv)	Time	Yield
1		1.5	2 h	21%
2		1.5	2 h	29%
3		1.5	2 h	68%
4		1.5	2 h	94%

To check this possibility, several 4-*O*-protected derivatives **1a**−**d** were synthesized from 2′,3′,5′-*O*-tris(*tert*-butyldimethylsilyl)uridine according to a modified procedure of Reese. It was reported that the reactivity and solubility of Lawesson's reagent were superior to those of phosphorus pentasulfide. Therefore, Lawesson's reagent was chosen for the thionation of 4-*O*-protected uridine derivatives **1a**−**d** (Scheme 1). The use of the 2,6-dimethylphenyl derivative **1d** gave the most successful result, as shown in Table 1.

The 2-thionation of the uracil residue of **1d** resulted in a significant shift of the NMR resonance signal of 1′-H to low magnetic field, as shown in Figure 1.

The low yields of other 4-*O*-protected derivatives are due to partial elimination of the protecting group and formation of 2,4-dithiouridine derivatives. It is likely that the use of phenyl groups having more electron-withdrawing or smaller substituents tends to cause elimination of the protecting group. Therefore, sterically hindered phenol groups with electron-donating substituents such as a methyl group are suitable for the 2-thionation.

(8) Seanger, W. In *Principles of Nucleic Acid Structure*; Springer-Verlag: Berlin, Germany, 1981; Chapter 10.
(9) (a) Vorbrüggen, H.; Strehlke, P. *Chem. Ber.* **1973**, *106*, 3039. (b) Niedballa, U.; Vorbrüggen, H. *J. Org. Chem.* **1974**, *39*, 3654.
(10) (a) Cherkasov, R. A.; Kutyrev, G. A.; Pudovik, A. N. *Tetrahedron* **1985**, *41*, 2567. (b) Cava, M. P.; Levinson, M. I. *Tetrahedron* **1985**, *41*, 5061.
(11) (a) Chien-Hua, N. *Anal. Biochem.* **1984**, *139*, 404. (b) Fox, K.; Praag, V. P.; Wempen, I.; Doerr, I. L.; Cheong, L.; Knoll, J. E.; Eidinoff, M. L.; Bendich, A.; Brown, G. B. *J. Am. Chem. Soc.* **1959**, *81*, 178.
(12) Reese, C. B.; Skone, P. A. *J. Chem. Soc., Perkin Tran. 1* **1984**, 1263.

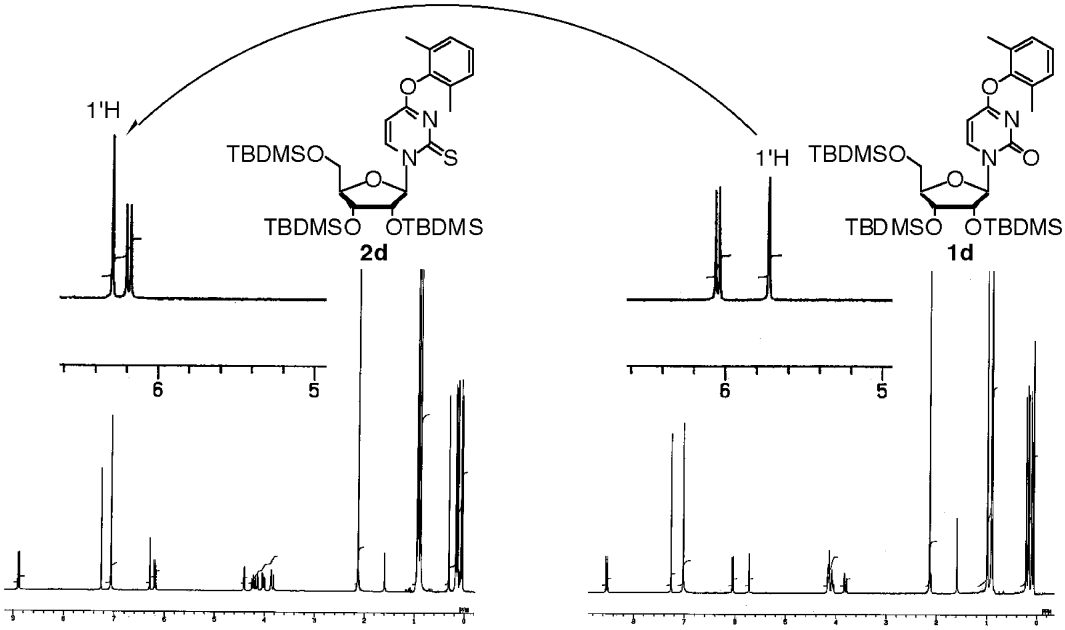


FIGURE 1. ^{1}H NMR spectra of compounds **1d** and **2d**.

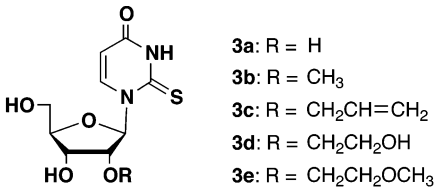


FIGURE 2. Uridine derivatives **3a**−**e** protected with various aryl groups.

Next, to demonstrate the utility of our method, we synthesized various 2′-*O*-alkylated-2-thiouridine derivatives (Figure 2).

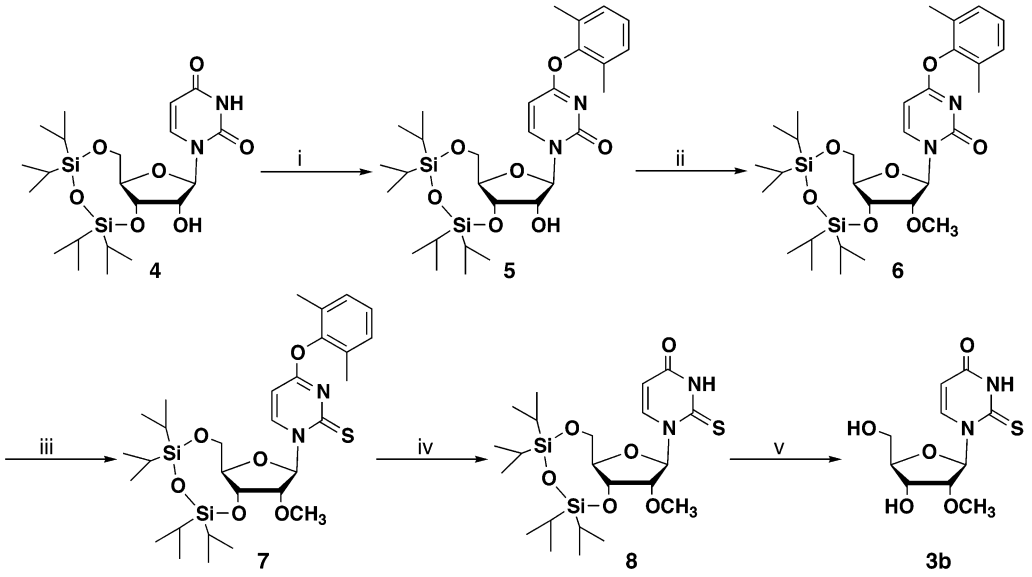
Synthesis of 2′-*O*-Alkyl-2-thiouridine Derivatives (3b−d). We previously reported the synthesis of 2′-*O*-methyl-2-thiouridine **3b** via selective 2′-*O*-methylation of 2-thiouridine.[5] In this method, we used 2-thiouridine **3a** as the starting material. Our new method gave **3b** via 3′,5′-*O*-(1,1,3,3-tetraisopropylsiloxane-1,3-diyl)uridine (**4**) more easily from commercially available uridine as the starting material. The synthetic intermediate **5** can be used as the common precursor for the synthesis of various 2′-*O*-alkylated 2-thiouridine derivatives. We thought the 2′-alkylation of intermediate **5** was better than that of the 4-*O*-(2,6-dimethylphenyl)-2′,3′-*O*-(1,1,3,3-tetraisopropyldisilane-1,3-diyl)-2-thiouridine intermediate, since the synthesis of this compound requires an additional two reactions for introduction and removal of a protecting group at the 2′-position to avoid side reactions during the 2-thionation. The 2-thiocarbonyl group of the 2-thoiuracil base moiety might be more reactive to electrophiles such as alkyl halides than the 2-carbonyl group of the uracil base moiety. Therefore, in this study, we selected the intermediate **5** as the common precursor and the 2′-*O*-alkylation reactions were carried out before the 2-thionation.

Synthesis of 2′-*O*-Methyl-2-thiouridine (3b: s^{2}Um). First, the 3′- and 5′-hydroxyl groups of uridine were protected by use of the 1,1,3,3-tetraisopropyldisiloxane-1,3-diyl (TIPDS) group. Subsequently, the base moiety of the resulting product **4** was protected with the 2,6-dimethylphenyl group under conditions similar to those described by Reese via transient protection of the 2′-hydroxyl group with a trimethylsilyl group. However, the yield of the desired product **5** was significantly low. The reason for the low yield is explained on the assumption that the base moiety was also masked by the trimethylsilyl group so that the base modification was impossible under these conditions. Accordingly, protection of the 4-carbonyl group was performed by use of phase-transfer conditions.[13] These conditions gave the desired product **5** in good yield. The 2′-*O*-methylation of **6** with CH_3I−NaH in DMF gave the 2′-*O*-methylated product **6**. The 2-thionation of **6** was successively done by using Lawesson's reagent. However, separation of the desired product **7** from byproducts derived from Lawesson's reagent was very difficult. Therefore, phosphorus pentasulfide was used as an alternative thionating reagent that allows much easier workup. Thus, the product **7** was synthesized in good yield (77%). Finally, both the TIPDS and 2,6-dimethylphenyl groups of **7** were removed in the usual manner. Thus, the desired product **3b** could be successfully obtained in an overall yield of 38% from uridine (Scheme 2).

Synthesis of 2′-*O*-Allyl-2-thiouridine (3c: s^{2}Uallyl). Sproat and colleagues reported the synthesis of 2′-*O*-allylated nucleosides.[14] 2′-*O*-Allyl nucleosides are potent antisense molecules because of their high nuclease resistance. Moreover, the allyl intermediate **9** could be converted into other 2′-*O*-alkyl compounds.[15] Therefore, we synthesized 2′-*O*-allyl-2-thiouridine. The fully protected uridine derivative **9** was synthesized by treatment of **5** with allyl ethyl carbonate in the presence of a tris-

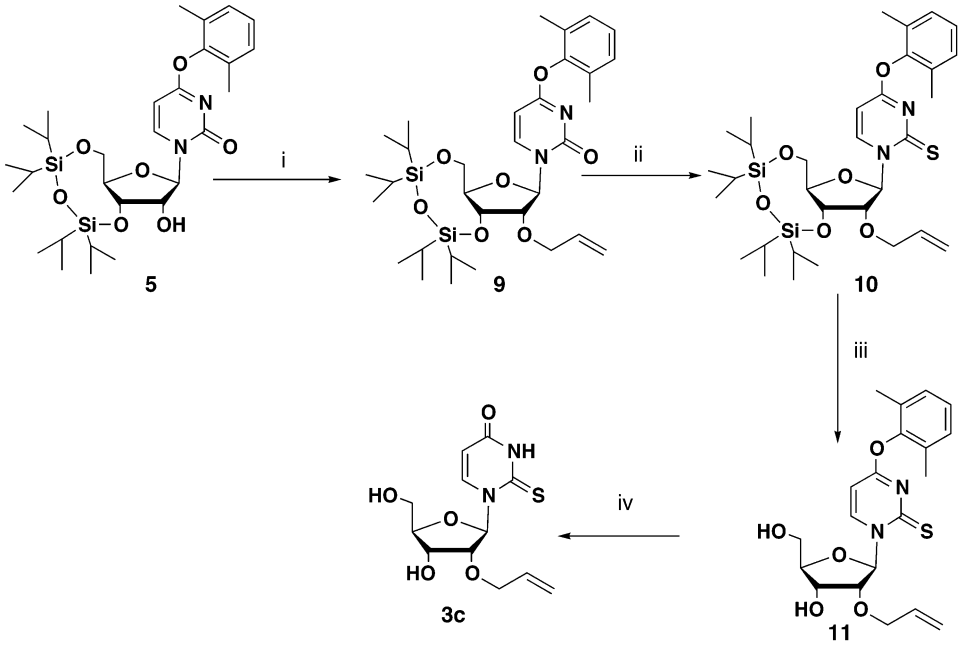
(13) Sekine, M. *J. Org. Chem.* **1989**, *54*, 2321.
(14) (a) Sproat, B. S.; Iribarren, A.; Beijer, B.; Pieles, U.; Lamond, A. I. *Nucleosides Nucleotides* **1991**, *10*, 25. (b) Sproat B. S.; Iribarren, A. M.; Garcia, R. G.; Beijer, B. *Nucleic Acids Res.* **1991**, *19*, 733.
(15) Maglott, E.; Glick, G. D. *Nucleic Acids Res.* **1998**, *26*, 1301.

SCHEME 2. Synthesis of s²Um[a]



[a] Reagents and conditions: (i) (a) TPSCl, Bu_4NBr in CH_2Cl_2/Na_2CO_3 aq, (b) 2,6-dimethylphenol, Et_3N, 1,4-diazabicyclo-2,2,2-octane in CH_3CN, 92%; (ii) NaH, CH_3I in DMF, 84%; (iii) P_2S_5, K_2CO_3 in toluene, 77%; (iv) *syn*-*o*-nitrobenzaldoxime, TMG in CH_3CN, 70%; (v) Bu_4NF in THF, 99%.

SCHEME 3. Synthesis of s²Uallyl[a]



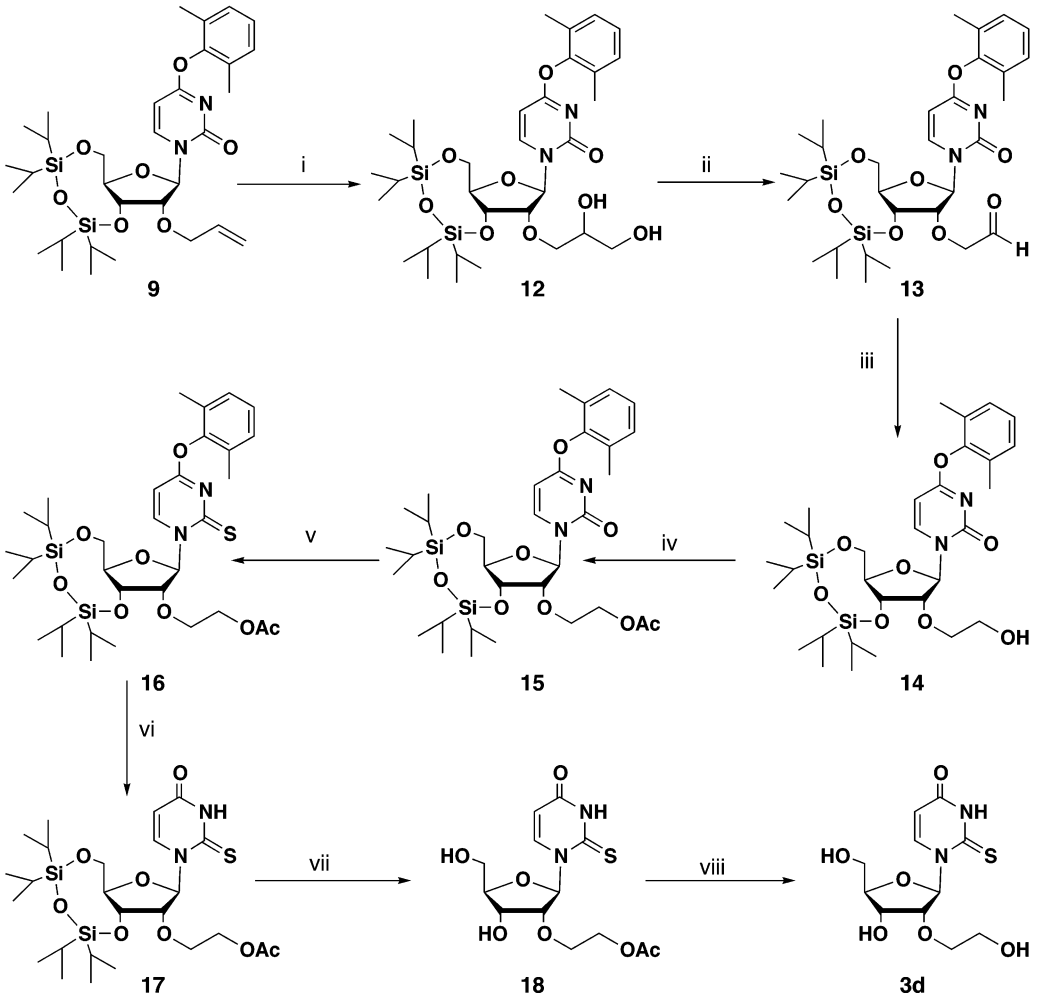
[a] Reagents and conditions: (i) allyl ethyl carbonate, PPh_3, tris(dibenzylideneacetone)-dipalladium(0) in THF, 97%; (ii) Lawesson's reagent in toluene, 77%; (iii) AcOH, Bu_4NF in THF, 100%; (iv) *syn*-*o*-nitrobenzaldoxime, TMG in CH_3CN, 77%.

(dibenzylideneacetone)-dipalladium(0) complex.[16] The 2-thionation of the uridine derivative **9** was carried out by using Lawesson's reagent to give the 2′-*O*-allyl product **10** in good yield. The usual deprotection of **10** gave the desired product **3c** in an overall yield of 53% from the 4-*O*-protected uridine derivative **5** via intermediate **11**, as shown in Scheme 3.

Synthesis of 2′-*O*-Hydroxyethyl-2-thiouridine (3d: s²Uhethyl). Next, we synthesized the 2′-*O*-hydroxyethyl-2-thiouridine. Oxidation of the 2′-*O*-allyl product **9** with OsO_4 in the presence of *N*-methylmorpholine *N*-oxide gave the diol **12**, which, in turn, was allowed to react with $NaIO_4$. The resulting aldehyde **13** was reduced by treatment with $NaBH_4$ to afford the saturated alcohol **14**. Treatment of **14** with Ac_2O in pyridine gave the acetate **15**. A similar 2-thionation of **15** gave the product **16** in good yield. Thus, the desired product **3d** could be

(16) (a) Lakhmiri, R.; Lhoste, P.; Sinou, D. *Tetrahedron Lett.* **1989**, *30*, 4669. (b) Kumar, V.; Gopalakrishnan, V.; Ganesh, K. N. *Bull. Chem. Soc. Jpn.* **1992**, *65*, 1665.

SCHEME 4. Synthesis of s²Uhethyl[a]



[a] Reagents and conditions: (i) OsO_4, NMO in acetone/water, 100%; (ii) $NaIO_4$ in dioxane/water, 98%; (iii) $NaBH_4$ in MeOH, 86%; (iv) Ac_2O in pyridine, 91%; (v) Lawesson's reagent in toluene, 86%; (vi) *syn-o*-nitrobenzaldoxime, TMG in CH_3CN, 76%; (vii) AcOH, Bu_4NF in THF, 99%; (viii) conc NH_3, 99%.

successfully synthesized after deprotection in an overall yield of 44% from uridine derivative **5** via intermediates **17** and **18** (Scheme 4).

Synthesis of 2′-*O*-Methoxyethyl-2-thiouridine (3e: s²Umoe). It was reported that oligoribonucleotides containing 2′-*O*-methoxyethylated nucleosides form stable RNA duplexes and have nuclease resistance.[17] Therefore, one can expect that more favorable antisense oligonucleotides could be created by a combination of the 2′-*O*-methoxyethyl group and the 2-thiouracil residue. Our interest was focused on this possibility.

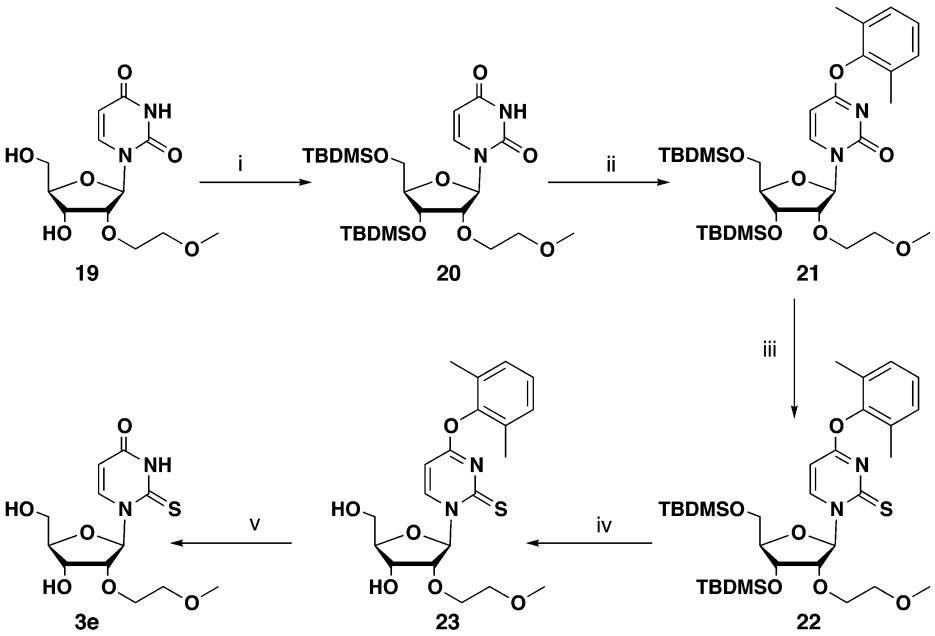
Reese and colleagues reported a convenient method for the synthesis of 2′-*O*-(methoxyethyl)uridine **19** via a ring-opening substitution of 2,2′-anhydrouridine with metal methoxyethoxides.[17] Therefore, 2′-*O*-methoxyethyl-2-thiouridine was synthesized by a series of reactions from **19** as follows: Silylation of 2′-*O*-(methoxyethyl)uridine **19** followed by 4-*O*-protection of the intermediate **20** with the 2,4-dimethylphenyl group gave the fully protected uridine derivative **21**. The successive 2-thionation of **21** was achieved in the same manner as described for compound **10**. Finally, both the TBDMS and 2,6-dimethylphenyl groups were removed from **22** via a two-step procedure in the usual manner. Thus, the desired product **3e** could be synthesized in an overall yield of 48% from 2′-*O*-(methoxyethyl)uridine **19** (Scheme 5).

¹H NMR Analysis of 2′-*O*-Alkyl-2-thiouridines. To study the conformational properties of 2′-*O*-alkyl-2-thiouridine derivatives, the ¹H NMR spectra of these nucleosides were measured in sodium phosphate buffer (pH 7.0). The ratios of the C3′-*endo* and C2′-*endo* conformers of these nucleosides were calculated by using the coupling constants $J_{1'2'}$ and $J_{3'4'}$. These results are shown in Table 2.

The % N (C3′-*endo*) values of **3d** and **3e** were calculated to be 76% and 72%, respectively, while those of s²U and s²Um reported previously were 70% each.[2b,5] These results indicated that the gauche effect of the 2′-*O*-hydroxyethyl group and 2′-*O*-methoxyethyl group leads to predominance of the C3′-*endo* conformer of **3d** and **3e**. On the other hand, the 2′-*O*-allylation of s²U slightly reduced the % N value.

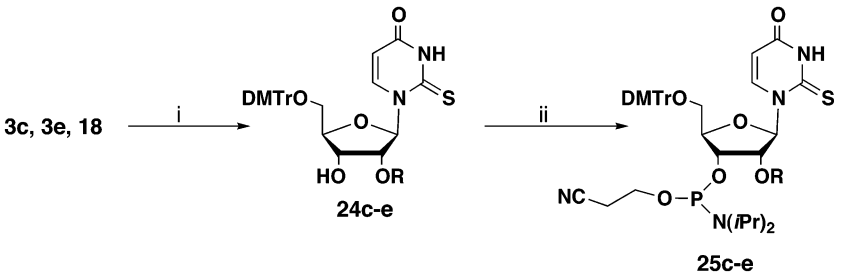
(17) (a) Martin, P. *Helv. Chim. Acta* **1995**, *78*, 486. (b) Altman, K.-H.; Dean, N. M.; Fabbro, D.; Freier, S. M.; Geiger, T.; Häner, R.; Hüsken, D.; Martin, P.; Monia, B. P.; Müller, M.; Natt, F.; Nicklin, P.; Phillips, J.; Pieles, U.; Sasmor, H.; Moser, H. E. *Chimia* **1996**, *50*, 168.

SCHEME 5. Synthesis of s²Umoe[a]



[a] Reagents and conditions: (i) TBDMSCl, imidazole in DMF, 86%; (ii) (a) TPSCl, Bu_4NBr in CH_2Cl_2/Na_2CO_3 aq, (b) 2,6-dimethylphenol, Et_3N, 1,4-diazabicyclo-2,2,2-octane in CH_3CN, 89%; (iii) Lawesson's reagent in toluene; (iv) AcOH, Bu_4NF in THF, 78% (2 steps); (v) *syn*-*o*-nitrobenzaldoxime, TMG in CH_3CN, 80%.

SCHEME 6. Synthesis of Phosphoramidites[a]



[a] Reagents and conditions: (i) DMTrCl in pyridine; (ii) 2-cyanoethoxy-bis(diisopropylamino)-phosphine, diisopropylethylammonium 1*H*-tetrazolide in CH_2Cl_2.

TABLE 2. Coupling Constants (Hz) and % N

	U[a]	Um[b]	s²U[a]	s²Um[c]	s²Uallyl	s²Uhethyl	s²Umoe
$J_{1'2'}$	4.6	4.0	2.5	3.1	3.4	2.4	2.9
$J_{3'4'}$	5.2	5.9	6.0	7.0	6.7	7.6	7.3
$J_{1'2'} + J_{3'4'}$	9.8	9.9	8.5	10.1	10.1	10.0	10.2
% N[d] (C3′-*endo*)	53	60	70	70	67	76	72

[a] These coupling constants were reported in ref 2b. [b] These coupling constants were reported in ref 19. [c] These coupling constants were reported in ref 5. [d] These values were calculated according to the equation % N (C3′-*endo*) = $\{J_{1'H-2'H}$ (Hz)/($J_{1'H-2'H}$ (Hz) + $J_{3'H-4'H}$ (Hz)$\}$ × 100.[20]

The C3′-*endo* predominance is a favorable property for stable RNA duplex formation, because all ribose puckering modes of RNA duplexes are fixed in the C3′-*endo* conformation and pre-fixation of ribose conformation in this manner is entropically favorable. Next, we examined the stability of RNA duplexes containing modified nucleosides.

Synthesis of 2′-*O*-Alkyl-2-thiouridine Containing Oligoribonucleotides. To evaluate the duplex stability of oligoribonucleotides containing these modified nucleosides, we synthesized the phosphoramidite building block **25c**−**e**.

2′-*O*-Alkyl-2-thiouridine phosphoramidite building block **25c**−**e** was synthesized in the usual manner (Scheme 6). The 2′-*O*-methyl-2-thiouridne phosphoramidite **25b** was prepared by the method reported previously.[5] Oligoribonucleotides containing 2′-*O*-alkyl-2-thiouridine were synthesized with use of commercially available PAC-protected phosphoramidites and standard coupling conditions except for the use of *tert*-butyl hydroperoxide oxidation on a commercial DNA synthesizer.[21] Release, deprotection, and desilylation of each oligonucleotide were achieved with aqueous ammonia-EtOH and 1 M tetrabutylammonium fluoride in THF. The fully deprotected RNA was purified by anion-exchange HPLC. The

(18) Legorburu, U.; Reese, C. B.; Song, Q. *Tetrahedron* **1999**, *55*, 5635.
(19) Davies, D. B. *Prog. NMR Spectrosc.* **1978**, *12*, 135.
(20) Davies, D. B.; Danyluk, S. S. *Biochemistry* **1974**, *13*, 4417.
(21) (a) Kumar, R. K.; Davis, D. R. *J. Org. Chem.* **1995**, *60*, 7726. (b) Kumar, R. K.; Davis, D. R. *Nucleosides Nucleotides* **1997**, *16*, 1469. (c) Kumar, R. K.; Davis, D. R. *Nucleic Acids Res.* **1997**, *25*, 1272.

TABLE 3. Melting Temperature Analysis of RNA−RNA Duplex Containing 2-Thiouridine Derivatives

Sequence rCGUU<u>U*</u>UUGC/rGCAAAAACG

U*	U[a]	s^2U[a]	s^2Um[a]	$s^2Uallyl$	$s^2Uhethyl$	s^2Umoe
T_m (°C)	36.7	43.6	42.2	40.1	40.6	41.4
ΔT_m (°C)[b]		+6.9	+5.5	+3.4	+3.9	+4.7

[a] These values were reported in ref 5. [b] ΔT_m = (T_m value of a modified duplex) − (T_m value of a wild-type duplex).

oligoribonucleotides obtained were characterized by MALDI-TOF mass spectrometry.

Study of RNA Duplex Stability. The hybridization properties of the modified oligoribonucleotides toward their complementary strands were evaluated by melting temperature (T_m) experiment. These results are shown in Table 3.

These data showed that all 2′-*O*-alkyl-2-thiouridine derivatives enhanced the hybridization affinity for the complementary RNA strand. The RNA oligomer containing 2-thiouridine **3a** formed the most stable RNA duplex. The RNA oligomer containing 2′-*O*-methyl-2-thiuridine **3b** formed the second most stable RNA duplex. The order of the stability of the modified RNA oligomers tested was 2′-hydroxyl > 2′-*O*-methyl> 2′-*O*-methoxyethyl > 2′-*O*-hydroxyethyl > 2′-*O*-allyl.

Although the C3′-*endo* predominance of **3d** and **3e** was much larger than that of **3a** and **3b**, the stability of RNA duplexes was slightly decreased. It can be assumed that increase of the hydrophobicity of the alkyl group results in disturbance of the hydrogen bonding network around the 2′-position so that RNA duplexes are significantly destabilized. Despite the loss of the hydrogen bonding network, the RNA oligomers containing these modified nucleosides were still capable of formation of more stable RNA−RNA duplexes than the unmodified RNA oligomer. These results indicated that the 2-thiocarbonyl group potentially affects stabilization of RNA duplexes.

Conclusion

We have developed a new method for the synthesis of 2-thiouridine derivatives **3b**−**e** via 4-*O*-protection. 2′-*O*-Methyl, 2′-*O*-allyl, 2′-*O*-hydroxyethyl, and 2′-*O*-methoxyethyl 2-thiouridine derivatives were successfully synthesized with our method. The thermal stabilities of oligoribonucleotides containing 2′-*O*-alkyl-2-thiouridine derivatives were also studied. The melting temperature analysis of oligoribonucleotides containing 2-thiouridine derivatives suggested that the 2-thiocarbonyl group is an essential factor for the stabilization of RNA−RNA duplexes. Even if unfavorable hydrophobic substituents are introduced into the 2′-hydroxyl position, the 2-thionation can compensate the destabilization arising from this substitution effect. Although we did not analyze the base pair selectivity of oligoribonucleotides having 2′-*O*-alkyl-2-thiouridine derivatives in this study, our previous paper[5] on melting temperature studies of RNA duplexes containing s^2Um strongly indicated that the 2′-*O*-methylation of 2-thiouridine does not affect the original base recognition ability of 2-thiouridine that can form precisely a W−C base pair only with the adenine base. Further more detailed studies of the base recognition ability of other 2′-*O*-alkylated thiouridine derivatives are now under way in this lab. In conclusion, oligoribonucleotides incorporating 2-thiouridine derivatives would provide a potential tool for the design of new antisense molecules.

Experimental Section

4-*O*-(2-Nitrophenyl)-2′,3′,5′-*O*-tris(*tert*-butyldimethylsilyl)uridine (1a). 2′,3′,5′-*O*-Tris(*tert*-butyldimethylsilyl)uridine (2.00 g, 3.41 mmol) was dissolved in dry CH_2Cl_2 (70 mL), and Et_3N (542 μL, 3.75 mmol), 2,4,6-triisopropylbenzensulfonyl chloride (820 mg, 3.75 mmol), and 4-(dimethylamino)pyridine (104 mg, 0.85 mmol) were added. After being stirred at room temperature for 18 h, the mixture was diluted with $CHCl_3$. The $CHCl_3$ solution was washed once with saturated $NaHCO_3$ (aqueous) and twice with brine. The combined organic extracts were dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was rendered anhydrous by repeated coevaporation with dry pyridine. The residue was further coevaporated with dry toluene and finally dissolved in dry CH_3CN (35 mL). A solution of 2-nitrophenol (474 mg, 3.41 mmol), Et_3N (3.45 mL, 25 mmol), and 1,4-diazabicyclo[2,2,2]octane (38 mg, 0.34 mmol) in dry CH_3CN (35 mL) was added. After being stirred at room temperature for 30 min, the mixture was concentrated in vacuo. The residue was dissolved in $CHCl_3$. The $CHCl_3$ solution was washed once with saturated $NaHCO_3$ (aqueous) and twice with brine. The combined organic extracts were dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by column chromatography with hexanes−Et_2O (hexanes−ethyl acetate in the case of **1b**−**d**) to afford **1a** as a foam (1.54 g, 64% yield): ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.02−0.20 (18H, m), 0.87−0.97 (27H, m), 3.77−4.15 (5H, m), 5.64 (1H, s), 6.12 (1H, d, J = 7.26 Hz), 7.22−8.12 (4H, m), 8.62 (1H, d, J = 7.26 Hz); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ −5.4, −5.1, −4.9, −4.0, −3.8, 18.2, 18.7, 26.0, 26.0, 26.2, 60.5, 68.6, 76.0, 82.8, 91.4, 94.0, 125.6, 125.9, 126.5, 134.7, 141.6, 145.0, 145.3, 154.8, 170.6. HRMS (ESI) m/z (M + H) calcd for $C_{33}H_{58}N_3O_8Si_3$ 708.3536, foun: 708.3580.

4-*O*-Phenyl-2′,3′,5′-*O*-tris(*tert*-butyldimethylsilyl)uridine (1b): ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.03−0.23 (18H, m), 0.86−0.95 (27H, m), 3.77−4.15 (5H, m), 5.65 (1H, s), 6.00 (1H, d, J = 7.25 Hz), 7.11−7.37 (5H, m), 8.55 (1H, d, J = 7.25 Hz); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ −5.5, −5.1, −5.1, −5.0, −4.0, −3.9, 18.2, 18.7, 25.9, 26.0, 26.2, 60.4, 68.5, 76.0, 82.6, 91.3, 94.4, 121.7, 125.6, 129.4, 144.4, 151.5, 155.2, 171.2. HRMS (ESI) m/z (M + H) calcd for $C_{33}H_{59}N_2O_6Si_3$ 663.3678, found 663.3679.

4-*O*-(2,6-Dichlorophneyl)-2′,3′,5′-*O*-tris(*tert*-butyldimethylsilyl)uridine (1c): ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.05−0.19 (18H, m), 0.87−0.98 (27H, m), 3.77−4.15 (5H, m), 5.69 (1H, s), 6.13 (1H, d, J = 7.25 Hz), 7.07−7.32 (3H, m), 8.58 (1H, d, J = 7.25 Hz,); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ −5.4, −5.1, −5.0, −4.9, −4.1, −3.9, 18.2, 18.2, 18.7, 26.0, 26.2, 60.6, 68.8, 76.1, 82.9, 91.3, 91.31, 93.6, 127.0, 128.6, 128.9, 144.7, 145.2, 154.9, 169.4. HRMS (ESI) m/z (M + H) calcd for $C_{33}H_{57}Cl_2N_2O_6Si_3$ 731.2901, found 731.2917.

4-*O*-(2,6-Dimethylphneyl)-2′,3′,5′-*O*-tris(*tert*-butyldimethylsilyl)uridine (1d): ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.06−0.21 (18H, m), 0.89−0.98 (27H, m), 2.13 (6H, s), 3.79−4.16 (5H, m), 5.72 (1H, s), 6.03 (1H, d, J = 7.26 Hz), 7.02 (3H, m), 8.62 (1H, d, J = 7.26 Hz); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ −5.4, −5.1, −5.0, −4.9, −4.1, −3.9, 16.6, 16.6, 18.1, 18.2, 18.7, 25.9, 26.2, 60.7, 68.7, 76.1, 82.9, 91.1, 91.1, 93.7, 125.6, 128.5, 130.1, 144.5, 149.0, 155.4, 170.4. HRMS (ESI) m/z (M + H) calcd for $C_{35}H_{63}N_2O_6Si_3$ 691.3994, found 691.3952.

General Procedure for the 2-Thionation of 4-*O*-Protected Uridine Derivatives (2a−d). An appropriate 4-*O*-protected uridine derivative **1a**−**d** (1 mmol) was dissolved in anhydrous toluene (10 mL). Lawesson's reagent (607 mg, 1.5 mmol) was added. After being stirred under reflux for 2 h, the mixture was cooled to room temperature. The insoluble materials were filtered off, and the filtrate was concentrated

under reduced pressure. The residue was dissolved in $CHCl_3$ and washed twice with saturated $NaHCO_3$ (aqueous). The combined organic extracts were dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by chromatography with hexanes−Et_2O to give the compound **2a**−**d** as listed in Table 1.

4-*O*-(2-Nitrophenyl)-2′,3′,5′-*O*-tris(*tert*-butyldimethylsilyl)-2-thiouridine (2a): ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.03−0.18 (18H, m), 0.87−0.98 (27H, m), 3.82−4.35 (5H, m), 6.21 (1H, s), 6.40 (1H, d, J = 7.58 Hz), 7.32−7.43 (2H, m), 7.63−7.68 (1H, m), 8.09 (1H, m), 8.98 (1H, d, J = 7.58 Hz); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ −5.4, −4.9, −4.9, −4.6, −3.7, −2.9, 18.3, 18.4, 18.8, 26.1, 26.3, 26.3, 60.2, 68.4, 76.0, 82.7, 95.1, 98.8, 125.4, 126.0, 126.7, 144.7, 164.8, 180.9. HRMS (ESI) m/z (M + H) calcd for $C_{33}H_{58}N_3O_7SSi_3$ 724.3303, found 724.3379.

4-*O*-Phenyl-2′,3′,5′-*O*-tris(*tert*-butyldimethylsilyl)-2-thiouridine (2b): ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.03−0.23 (18H, m), 0.92−1.01 (27H, m), 3.86−4.43 (5H, m), 6.29 (1H, s), 6.31 (1H, d, J = 7.58 Hz), 7.19−7.45 (5H, m), 8.96 (1H, d, J = 7.58 Hz); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ −5.5, −5.0, −5.0, −4.7, −3.7, −2.9, 18.2, 18.4, 18.7, 26.1, 26.1, 26.2, 60.1, 68.3, 75.9, 82.6, 95.0, 99.0, 121.4, 125.9, 129.5, 145.9, 151.5, 165.4, 181.1. HRMS (ESI) m/z (M + H) calcd for $C_{33}H_{59}N_2O_5SSi_3$ 679.3452, found 679.3387.

4-*O*-(2,6-Dichlorophneyl)-2′,3′,5′-*O*-tris(*tert*-butyldimethylsilyl)-2-thiouridine (2c): ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.05−0.19 (18H, m), 0.82−0.98 (27H, m), 3.82−4.38 (5H, m), 6.26 (1H, s), 6.40 (1H, d, J = 7.26 Hz), 7.11−7.36 (3H, m), 8.94 (1H, d, J = 7.26 Hz); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ −5.4, −4.9, −4.6, −3.7, −2.9, 18.3, 18.5, 18.8, 26.0, 26.1, 26.2, 26.3, 60.3, 68.6, 76.1, 77.5, 82.8, 95.0, 98.1, 127.2, 128.8, 128.8, 144.6, 146.6, 163.7, 181.2. HRMS (ESI) m/z (M + H) calcd for $C_{33}H_{57}Cl_2N_2O_5SSi_3$ 747.2673, found 747.2776.

4-*O*-(2,6-Dimethylphenyl)-2′,3′,5′-*O*-tris(*tert*-butyldimethylsilyl)-2-thiouridine (2d): ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.03−0.17 (18H, m), 0.82−1.07 (27H, m), 2.12 (6H, s), 3.81−4.31 (5H, m), 6.17 (1H, d, J = 7.59 Hz), 6.29 (1H, s), 7.05 (3H, s), 8.87 (1H, d, J = 7.59 Hz); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ −5.5, −5.1, −5.0, −4.7, −3.8, −3.0, 16.6, 16.7, 18.2, 18.4, 18.6, 26.0, 26.1, 60.3, 68.5, 76.0, 82.7, 94.8, 94.9, 97.5, 126.0, 128.7, 130.0, 146.1, 148.9, 164.9, 181.5. HRMS (ESI) m/z (M + H) calcd for $C_{35}H_{63}N_2O_5SSi_3$ 707.3765, found 707.3782.

4-*O*-(2,6-Dimethylphenyl)-3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)uridine (5). To a solution of 3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)uridine **4** (4.86 g, 10 mmol) in CH_2Cl_2 (200 mL) were added Na_2CO_3 (0.2 M solution 400 mL), tetrabutylammnium bromide (1.29 g, 4 mmol), and 2,4,6-triisopropylbenzenesulfonyl chloride (3.94 g, 13 mmol). The resulting two-phase solution was stirred vigorously at room temperature for 15 h. The organic phase was collected, and the aqueous phase was washed twice with CH_2Cl_2. The combined organic extracts were dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was rendered anhydrous by repeated coevaporation with dry pyridine. The residue was further coevaporated with dry toluene and finally dissolved in dry CH_3CN (100 mL). A solution of 2,6-dimethylphenol (1.2 g, 10 mmol), Et_3N (4.1 mL, 30 mmol), and 1,4-diazabicyclo[2,2,2]octane (112 mg, 1 mmol) in dry CH_3CN (100 mL) was added. After being stirred at room temperature for 30 min, the mixture was concentrated in vacuo. The residue was dissolved in $CHCl_3$. The $CHCl_3$ solution was washed once with saturated $NaHCO_3$ (aqueous) and twice with brine. The combined organic extracts were dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by chromatography with hexanes−ethyl acetate to afford **5** as a white foam (5.4 g, 92% yield): ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.97−1.07 (28H, m), 2.09 (6H, s), 3.31 (1H, br s), 3.95−4.35 (5H, m), 5.71 (1H, s), 6.02 (1H, d, J = 7.26 Hz), 7.01 (3H, s), 8.10 (1H, d, J = 7.26 Hz); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 12.4, 12.5, 12.7, 12.9, 13.3, 16.4, 16.8, 16.8, 16.9, 17.2, 17.3, 17.4, 60.3, 68.8, 74.8, 82.0, 92.2, 94.0, 125.9, 128.7, 130.1, 144.5, 149.1, 155.3, 171.0. Anal. Calcd for $C_{29}H_{46}N_2O_7Si_2$: C, 58.95; H, 7.85; N, 4.74. Found: C, 58.98; H, 7.74; N, 4.83.

2′-*O*-Methyl-4-*O*-(2,6-dimethylphenyl)-3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)uridine (6). Compound **5** (591 mg, 1 mmol) was rendered anhydrous by repeated coevaporation with dry pyridine. The residue was further coevaporated with dry toluene and finally dissolved in dry DMF (10 mL). CH_3I (311 μL, 5 mmol) and NaH (80 mg, 2 mmol) were added, and the mixture was stirred at room temperature for 10 min. The mixture was quenched by addition of acetic acid and diluted with ethyl acetate. The ethyl acetate solution was washed once with saturated $NaHCO_3$ (aqueous) and twice with brine. The combined organic extracts were dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by chromatography with hexanes−ethyl acetate to afford **6** as a white foam (507 mg, 84% yield): ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.99−1.12 (28H, m), 2.12 (6H, s), 3.69 (3H, s), 3.83−4.30 (5H, m), 5.80 (1H, s), 6.03 (1H, d, J = 7.26 Hz), 7.05 (3H, s), 8.30 (1H, d, J = 7.26 Hz); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 12.3, 12.9, 13.0, 13.4, 16.4, 16.8, 16.9, 17.0, 17.3, 17.4, 17.5, 59.1, 59.5, 67.8, 81.7, 83.2, 89.4, 93.6, 125.9, 128.7, 130.2, 144.1, 149.2, 155.3, 171.0. Anal. Calcd for $C_{30}H_{48}N_2O_7Si_2$: C, 59.57; H, 8.00; N, 4.63. Found: C, 59.64; H, 8.10; N, 4.84.

2′-*O*-Methyl-4-*O*-(2,6-dimethylphenyl)-3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-2-thiouridine (7). Compound **6** (302 mg, 0.5 mmol) was rendered anhydrous by repeated coevaporation with dry pyridine. The residue was further coevaporated with dry toluene and finally dissolved in dry toluene (10 mL). Phosphorus pentasulfide (144 mg, 0.65 mmol) and K_2CO_3 (415 mg, 3 mmol) were added. The resulting mixture was refluxed for 3 h and then cooled to room temperature. The insoluble materials were filtered. The filtrate was concentrated in vacuo, and the residue was dissolved in $CHCl_3$. The $CHCl_3$ solution was washed once with saturated $NaHCO_3$ (aqueous) and twice with brine. The combined organic extracts were dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by chromatography with hexanes−ethyl acetate to afford **6** as a pale yellow foam (239 mg, 77% yield): ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.00−1.11 (28H, m), 2.13 (6H, s), 3.77 (3H, s), 3.96−4.30 (5H, m), 6.21 (1H, d, J = 7.26 Hz), 6.34 (1H, s), 7.07 (3H, s), 8.54 (1H, d, J = 7.26 Hz); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 12.4, 12.8, 13.0, 13.4, 16.4, 16.8, 16.9, 17.0, 17.2, 17.4, 59.4, 60.3, 68.1, 82.2, 82.7, 93.8, 97.7, 126.2, 128.9, 130.1, 145.5, 149.0, 165.5, 181.1. Anal. Calcd for $C_{30}H_{48}N_2O_6SSi_2$: C, 58.03; H, 7.79; N, 4.51; S, 5.16. Found: C, 57.94; H, 7.97; N, 4.61; S, 4.97.

2′-*O*-Methyl-3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-2-thiouridine (8). Compound **7** (700 mg, 1.13 mmol) was rendered anhydrous by repeated coevaporation with dry pyridine. The residue was further coevaporated with dry toluene and finally dissolved in dry CH_3CN (5 mL). A solution of 1,1,3,3-tetramethylguanidine (428 μL, 3.4 mmol) and *syn*-*o*-nitrobenzaldoxime (565 mg, 3.4 mmol) in CH_3CN (5 mL) was added. After being stirred at room temperature for 3 h, the mixture was concentrated in vacuo, and the residue was dissolved in $CHCl_3$. The $CHCl_3$ solution was washed once with saturated $NaHCO_3$ (aqueous) and twice with brine. The combined organic extracts were dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by chromatography with hexanes−Et_2O to afford **8** as a white foam (426 mg, 70% yield): ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.93−1.11 (28H, m), 3.72 (3H, s), 3.88−4.30 (5H, m), 6.00 (1H, d, J = 8.25 Hz), 6.25 (1H, s), 8.10 (1H, d, J = 8.25 Hz), 10.82 (1H, br s); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 12.4, 12.8, 13.2, 13.4, 17.0, 17.2, 17.5, 59.3, 60.2, 68.5, 82.1, 83.3, 92.6, 92.7, 106.1, 106.2, 140.4, 160.5, 174.6. HRMS (ESI) m/z (M + H) calcd for $C_{22}H_{41}N_2O_6SSi_2$ 517.2224, found 275.0701.

2′-*O*-Methyl-2-thiouridine (3b). To a solution of compound **8** (168 mg, 0.33 mmol) in THF (3 mL) was added tetrabutylammonium fluoride (172 mg, 0.66 mmol). After being stirred at room temperature for 15 min, the mixture was diluted with

$CHCl_3$. The $CHCl_3$ solution was washed with H_2O. The aqueous phase was collected and concentrated in vacuo. The residue was purified by column chromatography on C-18 gel and eluted with water−acetonitrile. The pure product **3b** was obtained as a white solid (90 mg, 99%): ^{1}H NMR (400 MHz, D_2O) δ 3.61 (3H, s), 3.82−4.01 (2H, m), 4.06−4.17 (2H, m), 4.29−4.33 (1H, m), 6.17 (1H, d, J = 8.25 Hz), 6.75 (1H, d, J = 3.0 Hz), 8.10 (1H, d, J = 8.08 Hz); ^{13}C NMR (67.8 MHz, DMSO) δ 58.4, 59.4, 67.8, 83.5, 84.6, 90.8, 106.5, 140.6, 159.3, 175.6. HRMS (ESI) m/z (M + H) calcd for $C_{10}H_{15}N_2O_5S$ 275.0701, found 275.0691.

2′-*O*-Allyl-4-*O*-(2,6-dimethylphenyl)-3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)uridine (9). Compound **5** (590 mg, 1 mmol) was rendered anhydrous by repeated coevaporation with dry pyridine. The residue was further coevaporated with dry toluene and finally dissolved in dry THF (10 mL). Triphenylphosphine (52 mg, 0.2 mmol) and tris-(dibenzylideneacetone)-dipalladium(0) (18 mg, 0.02 mmol) were added. Fianlly, allyl ethyl carbonate (263 μL, 2 mmol) was added dropwise with stirring under argon, and the reaction mixture was refluxed for 30 min. The reaction mixture was cooled to room temperature and concentrated in vacuo. The residue was purified by column chromatography on silica gel with hexanes−ethyl acetate. The pure product **9** was obtained as a white foam (614 mg, 97%): ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.91−1.12 (28H, m), 2.12 (6H, s), 3.95−4.01 (2H, m), 4.12−4.45 (5H, m), 5.14−5.44 (2H, m), 5.78 (1H, s), 5.88−5.98 (1H, m), 6.00 (1H, d, J = 7.58 Hz), 7.04 (3H, s), 8.10 (1H, d, J = 7.58 Hz); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 12.5, 12.9, 13.0, 13.4, 16.4, 16.8, 16.9, 17.0, 17.3, 17.3, 17.4, 17.5, 59.5, 67.6, 71.0, 80.6, 81.7, 89.9, 93.4, 116.9, 125.7, 128.5, 130.0, 134.3, 143.8, 149.0, 155.1, 170.7. HRMS (ESI) m/z (M + H) calcd for $C_{32}H_{51}N_2O_7Si_3$ 631.3235, found 631.3329.

2′-*O*-Allyl-4-*O*-(2,6-dimethylphenyl)-3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-2-thiouridine (10). Compound **9** (2.47 g, 3.97 mmol) was rendered anhydrous by repeated coevaporation with dry pyridine. The residue was further coevaporated with dry toluene and finally dissolved in dry toluene (40 mL). Lawesson's reagent (2.37 g, 5.87 mmol) was added, and the mixture was refluxed for 1 h. The mixture was cooled to room temperature. The insoluble materials were filtered, and the materials were washed twice with cooled EtOH. The organic phase was concentrated in vacuo. The residue was dissolved in hexane and washed three times with brine. The combined organic extracts were dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by chromatography with hexanes−ethyl acetate to afford **10** as a pale yellow foam (1.95 g, 77% yield): ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.91−1.12 (28H, m), 2.12 (6H, s), 3.95−4.01 (2H, m), 4.12−4.45 (5H, m), 5.14−5.44 (2H, m), 5.78 (1H, s), 5.88−5.98 (1H, m), 6.00 (1H, d, J = 7.58 Hz), 7.04 (3H, s), 8.10 (1H, d, J = 7.58 Hz); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 12.6, 12.8, 13.1, 13.4, 16.5, 16.8, 16.9, 17.0, 17.0, 17.2, 17.3, 17.4, 17.5, 59.4, 67.8, 72.3, 80.4, 82.2, 94.0, 97.4, 117.0, 126.0, 128.8, 130.0, 134.7, 145.4, 148.8, 165.3. HRMS (ESI) m/z (M + H) calcd for $C_{32}H_{51}N_2O_6SSi_3$ 647.3006, found 647.3004.

2′-*O*-Allyl-4-*O*-(2,6-dimethylphenyl)-2-thiouridine (11). To a solution of compound **10** (1.95 g, 3.01 mmol) in THF were added tetrabutylammonium fluoride (1.97 g, 7.53 mmol) and acetic acid (431 μL, 7.53 mmol). After being stirred at room temperature for 10 min, the mixture was diluted with $CHCl_3$. The $CHCl_3$ solution was washed twice with saturated $NaHCO_3$. The combined organic extracts were dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by chromatography with hexanes−ethyl acetate to afford **11** as a pale yellow foam (808 mg, 100% yield):^{1}H NMR (270 MHz, $CDCl_3$) δ 2.12 (6H, s), 3.90−4.24 (5H, m), 4.47−4.83 (2H, dd), 5.20−5.39 (2H, m), 5.88−6.00 (1H, m), 6.24 (1H, d, J = 7.58 Hz), 6.63 (1H, s), 7.06 (3H, s), 8.99 (1H, d, J = 7.58 Hz); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 16.6, 59.0, 66.6, 72.4, 81.2, 84.1, 93.1, 98.0, 118.1, 126.2, 128.9, 130.1, 133.8, 146.7, 148.8, 165.4, 180.8. HRMS (ESI) m/z (M + H) calcd for $C_{20}H_{25}N_2O_5S$ 405.1484, found 405.1473.

2′-*O*-Allyl-2-thiouridine (3c). Compound **11** (1.27 g, 3 mmol) was rendered anhydrous by repeated coevaporation with dry pyridine. The residue was further coevaporated with dry toluene and finally dissolved in dry CH_3CN (20 mL). A solution of 1,1,3,3-tetramethylguanidine (392 μL, 3.1 mmol) and *syn*-*o*-nitrobenzaldoxime (518 mg, 3.1 mmol) in CH_3CN (20 mL) was added. After being stirred at room temperature for 3 h, the mixture was diluted with Et_2O. The Et_2O solution was washed twice with H_2O, and the combined aqueous extracts were concentrated in vacuo. The residue was purified by chromatography with $CHCl_3$−MeOH to afford **3c** as a white powder (690 mg, 77% yield): ^{1}H NMR (400 MHz, D_2O) δ 3.61 (1H, s), 3.82−3.98 (2H, m), 4.14−4.25 (2H, m), 4.29−4.33 (1H, m), 6.17 (1H, d, J = 8.25 Hz), 6.75 (1H, d, J = 3.0 Hz), 8.10 (1H, d, J = 8.08 Hz); ^{13}C NMR (67.8 MHz, DMSO) δ 60.4, 68.7, 71.8, 82.0, 85.4, 91.8, 107.3, 117.7, 135.3, 141.8, 160.8, 176.3. HRMS (ESI) m/z (M + H) calcd for $C_{12}H_{17}N_2O_5S$ 301.0858, found 301.0901.

2′-*O*-(2,3-Dihydroxypropyl)-4-*O*-(2,6-dimethylphenyl)-3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)uridine (12). Compound **9** (2.67 g, 4.23 mmol) was dissolved in a mixture of acetone−H_2O (40 mL, 6:1, v/v). *N*-Methylmorpholine *N*-oxide (1.09 g, 9.31 mmol) and OsO_4 (21 mg, 0.085 mmol) were added. After the mixture was stirred in the dark at room temperature for 20 h, saturated sodium bisulfate (1 mL) was added. The resulting osmium salts were filtered off. The filtrate was diluted with Et_2O. The ethereal solution was washed twice with saturated $NaHCO_3$ (aqueous). The combined organic extracts were dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by chromatography with $CHCl_3$−MeOH to afford **12** as a white foam (2.81 g, 100% yield): ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.92−1.11 (28H, m), 2.12 (6H, s), 3.49−4.30 (9H, m), 5.76 (1H, s), 6.06 (1H, d, J = 7.58 Hz), 7.04 (3H, s), 8.22 (1H, d, J = 7.58 Hz); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 12.6, 12.7, 12.9, 12.9, 13.0, 13.5, 16.5, 16.9, 17.0, 17.1, 17.3, 17.3, 17.4, 17.5, 59.3, 63.5, 63.9, 67.8, 67.9, 70.3, 70.3, 73.2, 74.2, 81.8, 81.8, 82.8, 83.1, 89.9, 90.2, 94.0, 125.9, 128.6, 130.0, 143.5, 149.0, 155.3, 171.0. HRMS (ESI) m/z (M + H) calcd for $C_{32}H_{53}N_2O_9Si_2$ 665.3289, found 665.3234.

2′-*O*-(2-Oxoethyl)-4-*O*-(2,6-dimethylphenyl)-3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)uridine (13). To a solution of compound **12** (3.7 g, 5.56 mmol) in 1,4-dioxane:H_2O (60 mL, 3:1) was added $NaIO_4$ (1.43 g, 6.67 mmol). After being stirred in the dark at room temperature overnight, the mixture was diluted with Et_2O. The ethereal solution was washed twice with saturated $NaHCO_3$. The combined organic extracts were dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by chromatography with $CHCl_3$−MeOH to afford **13** as a white foam (3.44 g, 98% yield): ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.86−1.11 (28H, m), 2.12 (6H, s), 3.95−4.58 (7H, m), 5.78 (1H, s), 6.05 (1H, d, J = 7.58 Hz), 7.04 (3H, s), 8.25 (1H, d, J = 7.25 Hz), 9.78 (1H, s); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 12.4, 13.0, 13.5, 16.5, 16.5, 16.9, 16.9, 17.0, 17.0, 17.1, 17.4, 17.5, 17.5, 59.4, 68.0, 76.2, 81.8, 82.9, 89.8, 93.9, 125.9, 128.6, 130.0, 130.0, 143.7, 149.0, 155.2, 171.0, 200.4. HRMS (ESI) m/z (M + H) calcd for $C_{31}H_{49}N_2O_8Si_2$ 633.3027, found 633.3018.

2′-*O*-Hydroxyethyl-4-*O*-(2,6-dimethylphenyl)-3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)uridine (14). To a solution of compound **13** (1.88 g, 2.97 mmol) in CH_3OH (30 mL) was added $NaBH_4$ (34 mg, 0.89 mmol). After being stirred at room temperature overnight, the mixture was diluted with Et_2O and washed once with saturated $NaHCO_3$ and twice with brine. The combined organic extracts were dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by chromatography with hexanes−ethyl acetate to afford **14** as a white foam (1.62 g, 86% yield):^{1}H NMR (270 MHz, $CDCl_3$) δ 0.86−1.11 (28H, m), 2.12 (6H, s), 3.69−4.31 (9H, m), 5.77 (1H, s), 6.05 (1H, d, J = 7.26 Hz), 7.04 (3H, s), 8.24 (1H, d, J

= 7.25 Hz); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 12.3, 13.0, 13.1, 13.6, 16.5, 16.8, 17.0, 17.0, 17.1, 17.4, 17.5, 17.5, 51.8, 59.5, 67.3, 67.7, 81.6, 82.2, 89.4, 93.7, 125.8, 128.6, 130.0, 143.9, 149.0, 155.2, 170.4, 170.9. HRMS (ESI) *m/z* (M + H) calcd for $C_{31}H_{51}N_2O_8Si_2$ 635.3184, found 635.3239.

2′-*O*-Acetoxylethyl-4-*O*-(2,6-dimethylphenyl)-3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)uridine (15). Compound **14** (1.62 g, 2.55 mmol) was rendered anhydrous by repeated coevaporation with dry pyridine and finally dissolved in dry pyridine (25 mL). Ac_2O (264 μL, 2.81 mmol) was added. After being stirred at room temperature overnight, the resulting mixture was concentrated in vacuo. The residue was dissolved in Et_2O and washed once with brine and twice with saturated $NaHCO_3$. The combined organic extracts were dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by chromatography with hexanes−ethyl acetate to afford **15** as a white foam (1.57 g, 91% yield): ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.80−1.12 (28H, m), 2.01 (3H, s), 2.09 (6H, s), 3.94−4.30 (9H, m), 5.73 (1H, s), 5.99 (1H, d, J = 7.58 Hz), 7.01 (3H, s), 8.24 (1H, d, J = 7.25 Hz); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 12.5, 12.9, 13.0, 13.4, 16.4, 16.9, 17.0, 17.0, 17.1, 17.3, 17.32, 17.4, 17.5, 20.9, 59.4, 63.4, 67.7, 69.0, 81.6, 81.9, 89.8, 93.5, 125.7, 128.5, 130.0, 143.8, 149.0, 155.1, 170.75, 170.8. HRMS (ESI) *m/z* (M + H) calcd for $C_{33}H_{53}N_2O_9Si_2$ 677.3289, found 677.3263.

2′-*O*-Acetoxylethyl-4-*O*-(2,6-dimethylphenyl)-3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-2-thiouridine (16). Compound **15** (1.57 g, 2.32 mmol) was rendered anhydrous by repeated coevaporation with dry pyridine. The residue was further coevaporated with dry toluene and finally dissolved in dry toluene (50 mL). Lawesson's reagent (1.41 g, 3.48 mmol) was added, and the mixture was refluxed for 1 h. The mixture was cooled to room temperature. The insoluble materials were filtered, and the filtration was twice washed with cooled EtOH. The organic phase was concentrated in vacuo. The residue was dissolved in hexane and washed three times with brine. The combined organic extracts were dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by chromatography with hexane−$CHCl_3$ to afford **16** as a pale yellow foam (1.39 g, 86% yield): ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.91−1.12 (28H, m), 2.12 (6H, s), 3.95−4.01 (2H, m), 4.12−4.45 (5H, m), 5.14−5.44 (2H, m), 5.78 (1H, s), 5.88−5.98 (1H, m), 6.00 (1H, d, J = 7.58 Hz), 7.04 (3H, s), 8.10 (1H, d, J = 7.58 Hz); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 12.6, 12.8, 13.1, 13.4, 16.5, 16.8, 16.9, 16.96, 17.0, 17.2, 17.3, 17.4, 17.5, 59.4, 67.8, 72.3, 80.4, 82.2, 94.0, 97.4, 117.0, 126.0, 128.8, 130.0, 134.7, 145.4, 148.8, 165.3. HRMS (ESI) *m/z* (M + H) calcd for $C_{33}H_{53}N_2O_8SSi_2$ 693.3061, found 693.3028

2′-*O*-Acetoxylethyl-3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-2-thiouridine (17). Compound **16** (1.39 g, 2 mmol) was rendered anhydrous by repeated coevaporation with dry pyridine. The residue was further coevaporated with dry toluene and finally dissolved in dry CH_3CN (20 mL). The solution of 1,1,3,3-tetramethylguanidine (754 μL, 6 mmol) and *syn*-*o*-nitrobenzealdoxime (997 mg, 6 mmol) in CH_3CN (20 mL) was added. After being stirred at room temperature for 5 h, the mixture was diluted with Et_2O. The Et_2O solution was washed twice with H_2O, and the combined aqueous extracts were concentrated in vacuo. The residue was purified by chromatography with $CHCl_3$−MeOH to afford **17** as a white powder (900 mg, 76% yield): ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.76−1.03 (28H, m), 2.01 (3H, s), 3.88−4.24 (9H, m), 5.88 (1H, d, J = 8.24 Hz), 6.16 (1H, s), 8.00 (1H, d, J = 8.24 Hz), 10.28 (1H, s); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 12.7, 12.9, 13.18, 13.2, 13.5, 16.9, 17.0, 17.1, 17.14, 17.2, 17.3, 17.4, 17.5, 17.6, 59.3, 63.7, 68.6, 70.1, 82.2, 82.3, 92.8, 92.9, 106.1, 140.1, 159.9, 170.9, 174.4. HRMS (ESI) *m/z* (M + H) calcd for $C_{25}H_{45}N_2O_8SSi_2$ 589.2435, found 589.2420.

2′-*O*-Acetoxylethyl-2-thiouridine (18). Compound **17** (900 mg, 1.53 mmol) was dissolved in THF (15 mL) and tetrabutylammonium fluoride (1.0 g, 3.83 mmol) and acetic acid (219 μL, 3.83 mmol) were added. After being stirred at room temperature for 15 min, the mixture was diluted with $CHCl_3$. The $CHCl_3$ solution was washed once with pyridine−H_2O (1:1, v/v). The organic phase were collected and concentrated in vacuo. The residue was dissolved in ethyl acetate. The desire product **19** was precipitated from the ethyl acetate solution by addition of hexanes−Et_2O (100 mL, 1:1, v/v). Then precipitates were collected and dried to give the pure product **18** as a white powder (529 mg, 99%): ^{1}H NMR (270 MHz, $CDCl_3$) δ 2.07 (3H, s), 3.94−4.37 (9H, m), 5.98 (1H, d, J = 8.24 Hz), 6.45 (1H, s), 8.40 (1H, d, J = 8.24 Hz); ^{13}C NMR (67.8 MHz, DMSO) δ 21.6, 60.1, 64.0, 68.6, 69.4, 83.2, 85.2, 92.0, 107.2, 141.3, 160.6, 171.0, 176.5. HRMS (ESI) *m/z* (M + H) calcd for $C_{13}H_{19}N_2O_7S$ 347.0913, found 347.0859.

2′-*O*-Hydroxyethyl-2-thiouridine (3d). Compound **18** (52 mg, 0.15 mmol) was dissolved in CH_3OH:28% aqueous NH_3 (2 mL, 3:1, v/v). After being stirred at room temperature for 24 h, the mixture was diluted with Et_2O and washed repeatedly with H_2O. The aqueous phase was collected and concentrated in vacuo. The residue was purified by column chromatography on C-18 gel with water−acetonitrile to afford **3d** as a white powder (45 mg, 99% yield): ^{1}H NMR (400 MHz, D_2O) δ 3.76−3.88 (4H, m), 3.98−4.08 (2H, m), 4.16−4.22 (2H, m), 4.27−4.30 (1H, dd), 6.16 (1H, d, J = 8.09 Hz), 6.75 (1H, d, J = 2.44 Hz), 8.12 (1H, d, J = 8.08 Hz); ^{13}C NMR (67.8 MHz, D_2O) δ 59.8, 60.8, 68.2, 72.7, 82.3, 84.0, 92.0, 106.8, 141.8, 162.8, 175.9. HRMS (ESI) *m/z* (M + H) calcd for $C_{11}H_{16}N_2O_6S$ 305.0807, found 305.0807.

2′-*O*-Methoxyethyl-3′,5′-*O*-bis(*tert*-butyldimethylsilyl)-uridine (20). 2′-*O*-Methoxyethyluridine **19** was prepared by Reese's method.[17] To a solution of compound **19** (1.51 g, 5 mmol) in dry DMF were added imidazole (851 mg, 12.5 mmol) and *tert*-butyldimethylsilyl chloride (1.72 g, 11 mmol). After being stirred at room temperature for 12 h, the reaction mixture was diluted with ethyl acetate. The ethyl acetate solution was washed once with brine and twice with saturated $NaHCO_3$. The combined organic extracts were dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by chromatography with hexanes−ethyl acetate to afford **20** as a white solid (2.27 g, 86% yield): ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.01−0.12 (12H, m), 0.90−0.95 (18H, m), 3.35 (3H, s), 3.55−4.24 (9H, m), 5.67 (1H, d, J = 8.24 Hz), 5.94 (1H, d, J = 1.98 Hz), 8.04 (1H, d, J = 8.24 Hz), 9.69 (1H, br s); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ −5.6, −5.4, −5.0, −4.6, 18.1, 18.4, 25.7, 25.9, 58.9, 60.7, 68.5, 69.7, 71.9, 83.1, 83.7, 87.7, 101.7, 140.0, 150.0, 163.5. HRMS (ESI) *m/z* (M + H) calcd for $C_{24}H_{47}N_2O_7Si_2$ 531.2922, found 531.2887.

2′-*O*-Methoxyethyl-4-*O*-(2,6-dimethylphenyl)-3′,5′-*O*-bis(*tert*-butyldimethylsilyl)uridine (21). To a solution of compound **20** (10.2 g, 19.2 mmol) in CH_2Cl_2 (200 mL) were added Na_2CO_3 (0.2 M solution 400 mL), tetrabutylammnium bromide (2.48 g, 7.68 mmol), and 2,4,6-triisopropylbenzenesulfonyl chloride (7.56 g, 24.96 mmol). The resulting two-phase solution was stirred vigorously at room temperature overnight. The organic phase was collected, and the aqueous phase was washed twice with CH_2Cl_2. The combined organic extracts were dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was rendered anhydrous by repeated coevaporation with dry pyridine. The residue was further coevaporated with dry toluene and finally dissolved in dry CH_3CN (100 mL). A solution of 2,6-dimethylphenol (2.55 g, 19.2 mmol), Et_3N (8 mL, 57.6 mmol), and 1,4-diazabicyclo[2,2,2]octane (646 mg, 5.76 mmol) in dry CH_3CN (100 mL) was added. After being stirred at room temperature for 3 h, the mixture was concentrated in vacuo. The residue was dissolved in $CHCl_3$. The $CHCl_3$ solution was washed once with saturated $NaHCO_3$ and twice with brine. The combined organic extracts were dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by chromatography with hexanes−ethyl acetate to afford **21** as a white foam (10.8 g, 89% yield): ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.04−0.14 (12H, m), 0.83−0.99 (18H, m), 2.11 (6H, s), 3.33 (3H, s), 3.54−3.58 (2H, m), 3.76−3.81 (3H, m), 4.06−4.18 (4H, m), 5.88 (1H, s), 6.00 (1H, d, J = 7.25 Hz),

7.00 (3H, s), 8.48 (1H, d, J = 7.25 Hz); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ −5.4, −5.3, −4.9, −4.5, 16.6, 18.1, 18.5, 25.7, 26.1, 58.8, 60.2, 67.7, 69.7, 71.7, 77.5, 82.8, 83.0, 89.3, 93.7, 125.6, 128.5, 130.0, 144.3, 149.0, 155.2, 170.5. HRMS (ESI) m/z (M + H) calcd for $C_{32}H_{55}N_2O_7Si_2$ 635.3548, found 635.3458.

2′-*O*-Methoxyethyl-4-*O*-(2,6-dimethylphenyl)-3′,5′-*O*-bis(*tert*-butyldimethylsilyl)-2-thiouridine (22). Compound **21** (8.25 g, 13 mmol) was rendered anhydrous by repeated coevaporation with dry pyridine. The residue was further coevaporated with dry toluene and finally dissolved in dry toluene (200 mL). Lawesson's reagent (7.89 g, 19.5 mmol) was added, and the mixture was refluxed for 2 h. The mixture was cooled to room temperature. The insoluble materials were filtered, and the filtrate was twice washed with cooled EtOH. The organic phase was concentrated in vacuo. The residue was dissolved in hexane and washed three times with brine. The combined organic extracts were dried over Na_2SO_4, filtered, and concentrated in vacuo. The crude product was used in the next reaction without further purification.

2′-*O*-Methoxyethyl-4-*O*-(2,6-dimethylphenyl)-2-thiouridine (23). To a solution of the crude compound **22** in THF (140 mL) were added tetrabutylammonium fluoride (7.32 g, 28 mmol) and acetic acid (1.6 mL, 28 mmol). After being stirred at room temperature for 20 h, the mixture was diluted with $CHCl_3$. The $CHCl_3$ solution was washed once with H_2O. The aqueous phase was collected and concentrated in vacuo. The residue was purified by chromatography with $CHCl_3$−MeOH to afford **23** as a white powder (4.64 g, 78% yield): ^{1}H NMR (270 MHz, $CDCl_3$) δ 2.03 (6H, s), 3.32 (3H, s), 3.46−3.59 (2H, m), 3.88−4.36 (7H, m), 6.19 (1H, d, J = 8.25 Hz), 6.43 (1H, s), 6.99 (3H, s), 8.74 (1H, d, J = 8.25 Hz); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 16.6, 58.8, 59.4, 67.1, 71.4, 71.6, 82.9, 84.0, 93.9, 98.1, 126.1, 128.8, 130.1, 145.9, 148.9, 165.3, 180.8. (two-step yield). HRMS (ESI) m/z (M + H) calcd for $C_{20}H_{27}N_2O_6S$ 423.1590, found 423.1563.

2′-*O*-Methoxyethyl-2-thiouridine (3e). Compound **23** (4.07 g, 9.63 mmol) was rendered anhydrous by repeated coevaporation with dry pyridine. The residue was further coevaporated with dry toluene and finally dissolved in dry CH_3CN (90 mL). The solution of 1,1,3,3-tetramethylguanidine (3.63 mL, 28.9 mmol) and *syn*-*o*-nitrobenzaldoxime (4.8 g, 28.9 mmol) in CH_3CN (90 mL) was added. After being stirred at room temperature for 3 h, the mixture was diluted with Et_2O. The Et_2O solution was washed twice with H_2O, and the combined aqueous extracts were concentrated in vacuo. The residue was purified by chromatography with $CHCl_3$−MeOH to afford **3e** as a white powder (2.47 g, 80% yield): ^{1}H NMR (400 MHz, D_2O) δ 3.05 (3H, s), 3.67−3.69 (2H, dd), 3.83−4.29 (7H, m), 6.17 (1H, d, J = 8.24 Hz), 6.75 (1H, d, J = 2.90 Hz), 8.12 (1H, d, J = 8.24 Hz); ^{13}C NMR (67.8 MHz, DMSO) δ 58.1, 59.4, 67.9, 69.8, 71.2, 82.3, 84.5, 91.1, 106.4, 140.7, 159.4, 175.7. HRMS (ESI) m/z (M + H) calcd for $C_{12}H_{18}N_2O_6S$ 319.0964, found 319.0984.

2′-*O*-Allyl-5′-*O*-(4,4′-dimethoxytrityl)-2-thiouridine (24c). 2′-*O*-Allyl-2-thiouridine **3c** (636.7 mg, 2 mmol) was rendered anhydrous by repeated coevaporation with dry pyridine and finally dissolved in dry pyridine (20 mL). 4,4′-Dimethoxytrityl chloride was added. After being stirred at room temperature for 90 min, the mixture was concentrated under reduced pressure. The resulting oily residue was dissolved in $CHCl_3$. The $CHCl_3$ solution was washed twice with brine and twice with saturated $NaHCO_3$ (aqueous). The combined organic extracts were dried over Na_2SO_4, filtered, concentrated in vacuo. The residue was purified by column chromatography with hexanes−ethyl acetate containing 0.5% pyridine to afford **24c** as a white foam (1.16 g, 94% yield): ^{1}H NMR (270 MHz, $CDCl_3$) δ 3.58 (2H, s), 3.78 (6H, s), 4.02−4.06 (2H, m), 4.38−4.63 (3H, m), 5.22−5.40 (3H, m), 5.84−5.98 (1H, m), 6.57 (1H, s), 6.80−6.86 (4H, m), 7.24−7.67 (9H, m), 8.35 (1H, d, J = 8.24 Hz), 10.42 (1H, br s); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 55.3, 55.3, 60.5, 68.0, 72.2, 81.3, 83.4, 87.2, 91.8, 91.9, 106.7, 106.7, 113.2, 118.5, 123.7, 127.1, 128.0, 128.0, 129.9, 130.0, 130.1, 133.4, 134.7, 136.0, 140.9, 144.1, 149.5, 158.5, 158.6, 159.6, 174.7. HRMS (ESI) m/z (M + Na) calcd for $C_{33}H_{34}N_2NaO_7S$ 625.1985, found 625.1902.

2′-*O*-Acetoxylethyl-5′-*O*-(4,4′-dimethoxytrityl)-2-thiouridine (24d): ^{1}H NMR (270 MHz, $CDCl_3$) δ 2.08 (3H, s), 3.78 (6H, s), 3.92−4.54 (9H, m), 5.36 (1H, d, J = 8.24 Hz), 6.46 (1H, s), 6.80−6.85 (4H, m), 7.24−7.37 (9H, m), 8.37 (1H, d, J = 8.24 Hz), 9.76 (1H, br s); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 21.0, 55.2, 60.2, 63.2, 67.8, 70.4, 76.5, 83.0, 83.2, 87.1, 91.8, 91.8, 106.6, 106.6, 113.0, 113.2, 127.1, 127.6, 127.7, 127.9, 128.0, 129.0, 130.0, 130.1, 134.7, 135.0, 140.8, 144.1, 158.5, 158.5, 160.0, 170.9, 174.5. HRMS (ESI) m/z (M + Na) calcd for $C_{34}H_{36}N_2NaO_9S$ 671.2040, found 671.1981.

5′-*O*-(4,4′-Dimethoxytrityl)-2′-*O*-methoxyethyl −2-thiouridine (24e): ^{1}H NMR (270 MHz, $CDCl_3$) δ 3.36 (3H, s), 3.77 (6H, s), 3.50−3.62 (4H, m), 3.84−4.51 (5H, m), 5.39 (1H, d, J = 8.24 Hz), 6.47 (1H, s), 6.80−6.84 (4H, m), 7.21−7.37 (9H, m), 8.33 (1H, d, J = 8.24 Hz), 10.56 (1H, br s); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 55.2, 58.9, 60.5, 68.3, 71.3, 71.6, 83.3, 87.0, 113.2, 127.1, 127.9, 128.0, 129.0, 130.0, 130.1, 134.8, 135.1, 140.8, 144.2, 158.5, 158.5, 159.9, 174.6. HRMS (ESI) m/z (M + Na) calcd for $C_{33}H_{36}N_2NaO_8S$ 643.2090, found 643.2095.

Phosphitylation of 3′-Hydroxyl Group (25c−e). Compound **24c−e** (0.5 mmol) was rendered anhydrous by repeated coevaporation with dry pyridine. The residue was further coevaporated with dry toluene and finally dissolved in dry CH_2Cl_2 (5 mL). To the solution were added diisopropylammonium tetrazolide (0.3 mmol) and 2-cyanoethoxy[bis(diisopropylamino)]phosphine (0.6 mmol) and the solution was stirred at room temperature for 6 h. The mixture was partitioned between CH_2Cl_2 and saturated $NaHCO_3$. The organic layer was collected, dried over Na_2SO_4, filtered, and concentrated in vacuo. The concentrated solution was poured into hexanes−Et_2O. The resulting precipitate was dissolved in CH_2Cl_2 and concentrated in vacuo, then the product **25c−e** was obtained as a white foam.

2′-*O*-Allyl-5′-*O*-(4,4′-dimethoxytrityl)-3′-*O*-(2-cyanoethyl-*N*,*N*-diisopropylphosphoramidite)-2-thiouridine (25c): ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.02−1.22 (14H, m), 2.40−2.61 (2H, m), 3.42−3.93 (13H, m), 4.14−4.64 (4H, m), 5.18−5.36 (3H, m), 5.86−6.02 (1H, m), 6.53−6.50 (1H, m), 6.80−6.85 (4H, m), 7.21−7.37 (9H, m), 8.34 (1H, m); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 15.4, 20.5, 20.6, 24.6, 24.7, 24.7, 24.8, 43.3, 43.3, 43.5, 43.5, 55.3, 55.3, 58.3, 60.3, 65.8, 72.2, 72.2, 77.2, 87.2, 87.3, 92.7, 92.8, 100.5, 106.6, 113.2, 116.9, 117.1, 127.2, 127.9, 128.3, 130.2, 134.3, 134.4, 134.8, 134.9, 135.0, 135.1, 140.8, 140.9, 144.2, 158.7, 158.7, 159.3, 174.8; ^{31}P NMR (109.25 MHz, $CDCl_3$) δ 150.46, 151.21. HRMS (ESI) m/z (M + Na) calcd for $C_{42}H_{51}N_4NaO_8P$: 825.3063, found 825.3144.

2′-*O*-Acetoxylethyl-5′-*O*-(4,4′-dimethoxytrityl)-3′-*O*-(2-cyanoethyl-*N*,*N*-diisopropylphosphoramidite)-2-thiouridine (25d): ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.82−1.23 (14H, m), 2.02 (3H, s), 2.40−2.60 (2H, m), 3.51−4.24 (19H, m), 4.42−4.61 (1H, m), 5.32−5.35 (1H, m), 6.44−6.47 (1H, m), 6.77−6.83 (4H, m), 7.19−7.36 (9H, m), 8.35 (1H, m); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 14.2, 20.4, 20.5, 20.5, 21.0, 21.0, 22.7, 24.5, 24.6, 24.7, 24.8, 24.8, 25.4, 31.6, 34.7, 43.3, 43.4, 55.3, 55.3, 57.9, 58.2, 59.9, 63.6, 63.6, 69.5, 77.1, 81.9, 87.1, 87.2, 92.9, 106.6, 113.2, 113.2, 117.3, 127.2, 127.9, 128.3, 130.2, 134.7, 134.8, 140.7, 140.8, 144.0, 144.1, 158.7, 158.7, 158.7, 159.4, 170.7, 170.7, 174.8; ^{31}P NMR (109.25 MHz, $CDCl_3$) δ 150.25, 151.42. HRMS (ESI) m/z (M + Na) calcd for $C_{43}H_{53}N_4NaO_{10}PS$ 871.3118, found 871.3163.

5′-*O*-(4,4′-Dimethoxytrityl)-3′-*O*-(2-cyanoethyl-*N*,*N*-diisopropylphosphoramidite)-2′-*O*-methoxyethyl-2-thiouridine (25e): ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.01−1.28 (14H, m), 2.40−2.65 (2H, m), 3.34 (3H, s), 3.44−4.60 (18H, m), 5.30−5.35 (1H, m), 6.51−6.57 (1H, m), 6.79−6.84 (4H, m), 7.13−7.39 (9H, m), 8.35−8.38 (1H, m); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 20.1, 20.2, 20.3, 20.4, 23.0, 23.0, 23.0, 23.1, 24.6, 24.7, 24.7, 24.8, 43.2, 43.2, 43.4, 43.4, 45.3, 45.4, 55.3, 55.3, 57.9, 58.1, 58.2, 59.1, 71.0, 72.0, 72.2, 76.5, 77.5, 87.1, 87.2, 92.7, 92.8,

106.5, 113.1, 113.2, 113.2, 117.3, 127.2, 127.7, 127.7, 127.9, 128.2, 128.3, 129.0, 130.2, 134.7, 134.8, 134.9, 135.0, 140.9, 144.0, 144.1, 158.6, 158.7, 159.3, 174.8, 175.0; ^{31}P NMR (109.25 MHz, $CDCl_3$) δ 150.15, 150.97. HRMS (ESI) *m/z* (M + Na) calcd for $C_{42}H_{53}N_4NaO_9PS$ 843.3269, found 843.3257.

Oligonucleotides Synthesis. Oligoribonucleotides were synthesized on an Applied Biosystems 392 oligonucleotide synthesizer on a 1 μmol scale, using PAC phosphoramidites (Pac-A, isopropyl-Pac-G, and acetyl-C) from Glen Research. A 0.1 M solution of each modified nucleoside phosphoramidite was used and the time for coupling was set up to be 10 min. The procedure for oxidation with 10% *tert*-butyl hydroperoxide in acetonitrile was twice repeated by use of the reaction time of 5 min each. The standard PAC RNA phosphoramidite chemistry was used for all the procedures prescribed for deprotection and purification.

Melting Temperature Analysis. A solution of an appropriate oligonucleotide and the complementary strand, both of which were arranged to be 2 μM, was prepared in 10 mM phosphate buffer (pH 7.0) containing 150 mM NaCl and 0.1 mM EDTA. The T_m experiments were done on a BECKMAN DU 650 spectrophotometer. The solution was heated at 60 °C and then cooled to 5 °C within 55 min. Data points were collected every time when the temperature was increased by 1 °C, and the solution was equilibrated at the same temperature for 1 min. Each melting curve was calculated by use of Igor Pro. software (WaveMetrics, Inc.).

^{1}H NMR Spectra Analysis. An appropriate nucleoside derivative (84.6$A_{260\ nm}$) was dissolved in phosphate buffer (600 μL) and lyophilized three times with 99.8% D_2O and finally with 99.95% D_2O (600 μL). The spectra were recorded at 400 MHz.

Acknowledgment. This work was supported by a Grant from "Research for the Future" Program of the Japan Society for the Promotion of Science (JSPS-RFTF97I00301) and a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology, Japan. This work was also supported by CREST of JST (Japan Science and Technology) and partially supported by the COE21 project.

Supporting Information Available: General procedure and ^{1}H, ^{13}C, and ^{31}P NMR spectra of all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

JO035246B