Chemical Synthesis of a 5′-Terminal TMG-Capped Triribonucleotide $m_3^{2,2,7}G^{5'}$pppAmpUmpA of U1 RNA

Mitsuo Sekine,* Michinori Kadokura, Takahiko Satoh, Kohji Seio, and Takeshi Wada

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

Utz Fischer, Vicki Sumpter, and Reinhard Lührmann

Institut für Molekularbiologie und Tumorforschung, Philipps-Universität Marburg, Emil-Mannkopff-Strasse 2, D-3550 Marburg, Germany

Received December 27, 1995[⊗]

The 5′-terminal TMG-capped triribonucleotide, $m_3^{2,2,7}G^{5'}$pppAmpUmpA, has been synthesized by condensation of an appropriately protected triribonucleotide derivative of ppAmpUmpA with a new TMG-capping reagent. During this total synthesis, it was found that the regioselective 2′-*O*-methylation of 3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-*N*-(4-monomethoxytrityl)adenosine was achieved by use of MeI/Ag_2O without affecting the base moiety. A new route to 2-*N*,2-*N*-dimethylguanosine from guanosine *via* a three-step reaction has also been developed by reductive methylation using paraformaldehyde and sodium cyanoborohydride. These key intermediates were used as starting materials for the construction of a fully protected derivative of pAmpUmpA and a TMG-capping reagent of Im-p$m_3^{2,2,7}$G. The target TMG-capped tetramer, $m_3^{2,2,7}G^{5'}$pppAmpUmpA, was synthesized by condensation of a partially protected triribonucleotide 5′-terminal diphosphate species, ppA^{MMTr}mpUmpA, with Im-p$m_3^{2,2,7}$G followed by treatment with 80% acetic acid. The structure of $m_3^{2,2,7}G^{5'}$pppAmpUmpA was characterized by ^{1}H and ^{31}P NMR spectroscopy as well as enzymatic assay using snake venom phosphodiesterase, calf intestinal phosphatase, and nuclease P1.

Introduction

U1 RNA is a small nuclear RNA which was found in the nuclei of eukaryotic cells[1] and plays an important role in splicing of pre-mRNAs.[2] One unique feature of U1 RNA is the cap structure containing 2,2,7-trimethylguanosine (TMG) at its 5′-end. Mattaj[3] and Lührmann[4] have recently reported the interesting finding that TMG-capped RNAs can transport from the cytoplasm to the nucleus but 7-monomethylguanosine (MMG)-capped RNAs cannot. Their studies revealed that the nucleomembrane transport of capped RNAs is strictly regulated by the number of methyl groups on the guanosine base of the 5′-terminal cap structure. TMG-capped RNAs have also proved to have potential translational activity compared with MMG-capped RNAs.[5] The 5′-terminal hypermethylated structure of U1 RNA is thought to be essential for transport and splicing of RNAs.[6] Because of these exciting results, much attention has been paid to the structure−function relationships of U1 RNA having a TMG cap.[7] Therefore, chemical approaches to the synthesis of such unique terminal sequences should be developed to clarify the biochemical meaning of the hypermethylated structure in more detail. In connection with this study, Hata and his co-workers have reported extensive studies of the chemical and enzymatic synthesis of MMG-capped oligoribonucleotides.[8] The minimum-sized TMG caps $m_3^{2,2,7}G^{5'}$pppG and $m_3^{2,2,7}G^{5'}$pppA were first synthesized by Darzynkiewicz *et al.*[5a] Later, Iwase *et al.* reported these TMG caps in a different way.[9] Quite recently, Stepinski *et al.* have reported the synthesis of DMG- and TMG-caps as well as MMG cap analogs.[10] Lönnberg extensively studied the fluorescence and absorption spectroscopic properties of these caps.[11] However, these previous syntheses involved a tedious route to a key

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

(1) (a) Busch, H.; Reddy, R.; Rothblum, L.; Choi, C. Y. *Annu. Rev. Biochem.* **1982**, *51*, 617−654. (b) Reddy, R.; Busch, H. Prog. *Nucleic Acid Res. Mol. Biol.* **1983**, *30*, 127−162.

(2) (a) Padgett, R. A.; Mount, S. M.; Steitz, J. A.; Sharp, P. A. *Cell* **1983**, *35*, 101−107. (b) Krämer, A.; Keller, W.; Appel, B.; Sharp, P. A. *Cell* **1984**, *38*, 299−307. (c) Krainer, A. R.; Maniatis, T. *Cell* **1985**, *42*, 725−736. (d) Birnstiel, M. L., Ed. *Structure and function of major and minor small nuclear ribonucleoprotein particles*; Springer-Verlag Press: Berlin, 1988. (e) Maniats, T.; Reed, R. *Nature* **1987**, *325*, 673−678. (f) Krol, A.; Westhof, E.; Bach, M.; Lührmann, Ebel, J.-P.; Carbon, P. *Nucleic Acids Res.* **1990**, *18*, 3803−3811.

(3) Hamm, J.; Darzynkiewicz, E.; Tahara, S. M.; Mattaj, I. W. *Cell* **1990**, *62*, 569−577. (b) Hamm, J.; Mattaj, I. W. *Cell* **1990**, *62*, 109−118. (c) Mattaj, I. W. *Cell* **1986**, *46*, 905−911.

(4) Fischer, U.; Lührmann, R. *Science* **1990**, 786−790.

(5) (a) Darzynkiewicz, E.; Stepinski, J.; Ekiel, I.; Jin, Y.; Haber, D.; Sijuwade, T.; Tahara, S. M. *Nucleic Acids Res.* **1988**, *16*, 8953−8962. (b) Darzynkiewicz, E.; Stepinski, J.; Ekiel, I.; Goyer, C.; Sonneberg, N.; Temeriusz, A.; Jin, Y.; Sijuwade, T.; Haber, D. *Biochemistry* **1989**, *28*, 4771−4778. (c) Tokumoto, Y.; Iwase, R.; Sekine, M.; Hata, Y.; Ohshima, Y. *Biochim. Biophys. Res. Commun.* **1989**, *162*, 977. (d) E. Darzynkiewicz, E.; Stepinski, J.; Tahara, S. M.; Stolarski, R.; Ekiel, I.; Haber, D.; Neuvonen, K.; Lehikoinen, P.; Labadi, I.; Lönnberg, H. *Nucleosides Nucleotides* **1990**, *9*, 599−618.

(6) Fischer, U.; Sumpter, V.; Sekine, M.; Satoh, T.; Lührmann, R. *EMBO J.* **1993**, *12*, 573−583.

(7) Plessel, G.; Fischer, U.; Lührmann, R. *Mol. Cell. Biol.* **1994**, *14*, 4160−72; Fischer, U.; Heinrich, J.; van Zee, K.; Fanning, E.; Lührmann, R. *J. Cell. Biol.* **1994**, *125*, 971−980; Yang, H.; Moss. M. L.; Lund, E.; Dahlberg, J. E. *Mol. Cell Biol.* **1992**, *12*, 1553−60.

(8) (a) Hata, T; Nakagawa, I.; Shimotohno, K.; Miura, K. *Chem. Lett.* **1976**, 987−990. (b) Nakagawa, I.; Konya, S.; Ohtani, S.; Hata, T. *Synthesis* **1980**, 556- 557. (c) Yamaguchi, K.; Nakagawa, I; Sekine, M.; Hata, T.; Shimotohno, K.; Hiruta, H.; Miura, K. *Nucleic Acids Res.* **1984**, *12*, 2939−2954. (d) Kamimura, K.; Osaki, Y.; Sekine, M.; Hata, T. *Tetrahedron Lett.* **1984**, *25*, 2683−2686. (e) Sekine, M.; Nishiyama, S.; Kamimura, K.; Osaki, Y.; Hata, T. *Bull. Chem. Soc. Jpn.* **1985**, *58*, 850−860. (f) Sekine, M.; Kamimura, K.; Hata, T. *J. Chem. Soc., Perkin Trans 1* **1985**, 997−1000. (g) Hata, T.; Sekine, M.; Miura, K. *Nucleosides Nucleotides* **1987**, *6*, 131−136. (h) Iwase, R.; Sekine, M. Hata, T.; Miura, K. *Tetrahedron Lett.* **1988**, *29*, 2969−2972. (i) Sekine, M.; Iwase, R.;. Hata, T.; Miura, K. *J. Chem. Soc., Perkin Trans 1* **1989**, 969−978. (j) Fukuoka, K.; Suda, F.; Suzuki, R.; Ishikawa, M.; Hata, T. *Chem. Lett.* **1994**, 499−502. (k) Fukuoka, K.; Suda, F.; Suzuki, R.; Takaku, H.; Ishikawa, M.; Hata, T. *Tetrahedron. Lett.* **1994**, *35*, 1063−1066.

(9) Iwase, R.; Sekine, M.; Tokumoto, Y.; Ohshima, Y.; Hata, T. *Nucleic Acids Res.* **1989**, *17*, 8979−8989.

S0022-3263(95)02263-8 CCC: $12.00

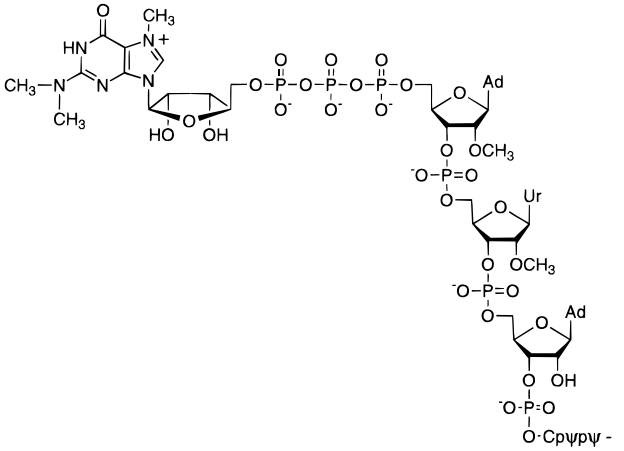


Figure 1. The 5′-terminal structure of U1 RNA from Hela cells.

intermediate, 2-*N*,2-*N*-dimethylguanosine,[12] and its conversion into a TMG-capping reagent via a multi-step reaction. If $m_3^{2,2,7}G^{5'}$pppAmpUmpA, the smallest acceptor in RNA ligation, could be chemically prepared, TMG-capped U1 RNA fragments with appropriate sequences could be enzymatically synthesized by ligation of the TMG-capped trimer with 3′-downstream RNA fragments (pCpψpψ.....) that can be easily synthesized by an RNA synthesizer. It should be noted that the combination of the purine nucleoside A at the 3′-end of $m_3^{2,2,7}G^{5'}$ppp-AmpUmpA and the pyrimidine nucleoside C at the 5′-end of the 3′-downstream RNA fragments is the best one in RNA ligation according to the previous results reported by Uhlenbeck and other groups.[13]

In this paper, we report a markedly improved method for the synthesis of 2-*N*,2-*N*-dimethylguanosine as well as a new procedure for the regioselective 2′-*O*-methylation of adenosine derivatives and an application of these procedures to the chemical synthesis of $m_3^{2,2,7}G^{5'}$-pppAmpUmpA.

Results and Discussion

Synthesis of a Diphosphorylated Triribonucleotide Block. For the synthesis of a triribonucleotide block, appropriately protected 2′-*O*-methylated adenosine units have been prepared usually by several-step reactions from 2′-*O*-methyladenosine, which has been prepared by reaction of adenosine with diazomethane or methyl iodide.[14] These procedures involved the use of a hazardous reagent and tedious separation from its 3′-regioisomer using ion exchange column chromatography followed by careful recrystallization. In fact, several indirect methods suitable for the large-scale synthesis of 2′-*O*-methylated adenosine derivatives have recently been employed although they required more reaction steps.[15]

If the selective 2′-*O*-methylation could be achieved by using more readily available 3′,5′,*N*-protected ribonucleosides, a bothersome process to separate the 2′- and 3′-*O*-methylated regioisomers could be avoided. To our knowledge, however, all attempts to obtain 2′-*O*-methyl derivatives from 3′,5′,*N*-protected ribonucleosides have failed. For example, the reaction of a typical *N*-benzoylated adenosine derivative **1**[16] with methyl iodide in the presence of silver oxide in benzene gave predominantly the 2′,N^1-dimethylated product **2** over the desired product.

In this study we found that, when the MMTr group was used as the 6-*N* protecting group,[17] the selective 2′-*O*-methylation was successfully carried out without the N^1-methylation on the masked adenine moiety: Reaction of 6-*N*-(monomethoxytrityl)-3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)adenosine[18] (**3**) with methyl iodide as the solvent in the presence of silver oxide[19] under reflux for 4 h afforded selectively the 2′-*O*-methylated product **4** in 83% yield. These conditions are essential for the high yield synthesis of **4**, since the yield of **4** was reduced drastically to 27% due to the overmethylation on the base residue and the competitive decomposition of the methylated products when the reaction was prolonged for 15 h.

Two appropriately protected 3′-phosphoramidite units **7** and **11** of Am and Um were synthesized as follows: Removal of the silyl group from **4** by the action of KF/Et_4NBr/H_2O[20] in acetonitrile gave quantitatively the 3′,5′-diol **5**. The tritylation of **5** with dimethoxytrityl chloride gave compound **6** in 91% yield. The phosphitylation of **6** with chloro(2-cyanoethoxy)(diisopropylamino)phosphine[21] gave the amidite unit **7** in 85% yield. In a similar manner, the N^3-benzoyl-2′-*O*-methyluridine 3′-phosphoramidite **11** was synthesized in 79% yield from the 5′-*O*-dimethoxytrityl ether derivative **10**, which was prepared *via* compound **9** from compound **8** as described previously.[19,22]

(10) (a) Stepinski, J; Bretner, M.; Jankowska, M.; Felczak, K.; Stolarrski, R.; Wieczorek, Z.; Cai, A. L.; Rhoads, R. E. *Nucleosides Nucleotides* **1995**, 14, 717−721. (b) Chlebicka, L.; Wieczovek, Z.; Stolarski, R.; Stepinski, J.; Darezynkiewicz, E.; Sugar, D. *Nucleosides Nucleotides* **1995**, 14, 771−775.

(11) Wieczorek, Z.; Stepinski, J.; Jankowska, M.; Lonnberg, H. *J. Photochem. Photobiol.* **1995**, 57−63.

(12) (a) Gerster, J. F.; Robins, R. K. *J. Am. Chem. Soc.* **1965**, *87*, 3752−3759. (b) Gerster, J. F.; Robins, R. K.; *J. Org. Chem.* **1966**, *31*, 3258−3262. (c) Yamazaki, A.; Kumashiro, I.; Takenishi, T. *J. Org. Chem.* **1967**, *32*, 3032−3038. (d) Okutsu, M.; Yamazaki, A. *Nucleic Acids Res.* **1976**, *3*, 237−250. (e) Bridson, P. K.; Reese, C. B. *Bioorg. Chem.* **1979**, *8*, 339. (f) Sekine, M.; Satoh, T. *J. Org. Chem.* **1991**, *56*, 1224−1227. (g) Avino, A. M.; Mayordomo, A.; Espuny, R.; Bach, M.; Eritja, R. *Nuclesides Nucleotides* **1995**, *14*, 1613−1617.

(13) England, T. E.; Uhlenbeck, O. C. *Biochemistry* **1978**, *17*, 2069−2076. (b) Ohtsuka, E.; Nishikawa, S.; Fukumoto, R.; Tanaka, S.; Markham, A. F.; Ikehara, M.; Sugiura, M. *Eur. J. Biochem.* **1977**, *81*, 285−291. (c) Kikuchi, Y.; Hishinuma, F.; Sakaguchi, K. Proc. *Natl. Acad. Sci. U.S.A.* **1978**, *75*, 1270−1273.

(14) (a) Broom, A. D.; Robins, R. K. *J. Am. Chem. Soc.* **1965**, *87*, 1145−1146. (b) Khwaja, T. A.; Robins, R. K. *J. Am. Chem. Soc.* **1966**, *88*, 3640−3643. (c) Martin, D. M. G.; Reese, C. B.; Stephenson, G. F. *Biochemistry* **1968**, *7*, 1406−1411. (d) Gin, J. B.; Dekker, C. A. *Biochemistry* **1968**, *7*, 1413−1420. (e) Robins, M. J.; Naik, S. R. *Biochim. Biophys. Acta* **1971**, *246*, 341−343. (f) Robins, M. J.; Naik, S. R.; Lee, A. S. K. *J. Org. Chem.* **1974**, *39*, 1891−1899. (g) Robins, M. J.; Lee, A. S. K.; Norris, F. A. *Carbohydr. Res.* **1975**, *41*, 304−307. (h) Kazimierczuk, Z.; Darzynkiewicz, E.; Shugar, D. *Biochemistry* **1976**, *15*, 2735−2740. (i) Yano, J.; Kan, L. S.; Ts'O, P. O. P. *Biochim. Biophys. Acta* **1980**, *629*, 178−183. (j) Yamauchi,K.; Nakagima, T.; Kinoshita, M. *J. Org. Chem.* **1980**, *45*, 3865−3868. (k) Inoue, H.; Sakata, T.; Fujimoto, K.; Ikehara, M. *Nucleic Acids Res.* **1987**, 6131−6148.

(15) (a) Inoue, H.; Hayase, Y.; Imura, A.; Iwai, S.; Miura, K.; Ohtsuka, E. *Nucleic Acids Res.* **1987**, *15*, 6131−6148. (b) Sproat, B. S.; Beijer, B.; Iribarren, A. *Nucleic Acids Res.* **1990**, *18*, 41−49.

(16) Markiewicz, W. T. *J. Chem. Res. Miniprint* **1979**, 173.

(17) Hata, T.; Gokita, N.; Sakairi, N.; Yamaguchi, K.; Sekine, M.; Ishido, Y. *Bull. Chem. Soc. Jpn.* **1982**, *55*, 2949−2955.

(18) Honda, S.; Urakami, K.; Koura, K.; Terada, K.; Sato, Y.; Kohno, K.; Sekine, M.; Hata. T. *Tetrahedron* **1984**, *40*, 153−163.

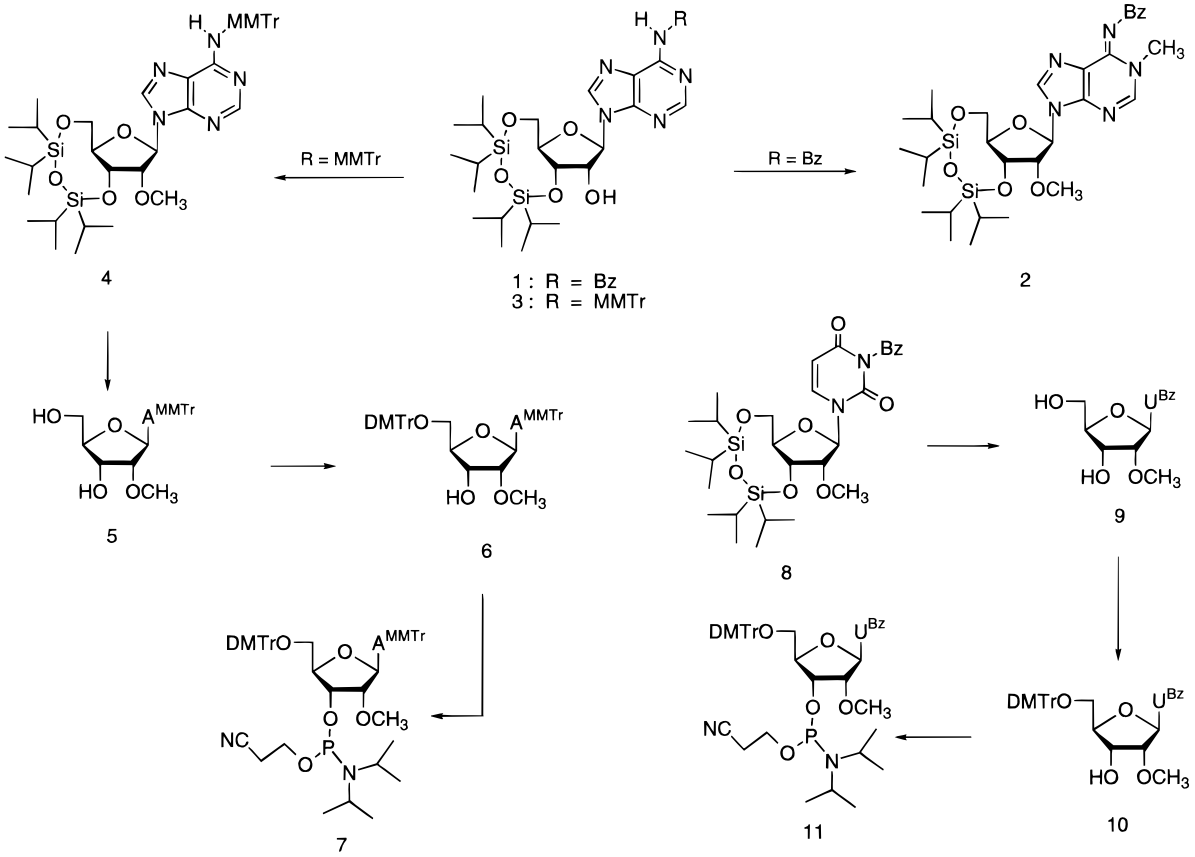
(19) Kamimura, T.; Masegi, T.; Hata, T. *Chem. Lett.* **1982**, 965−968.

(20) Kamimura, T.; Tsuchiya, M.; Urakami, K.; Koura, K.; Sekine, M.; Shinozuka, K.; Miura, K.; Hata, T. *J. Am. Chem. Soc.* **1984**, *106*, 4552−4557.

(21) Tanimura, H.; Maeda, M.; Fukazawa, T.; Sekine, M.; Hata, T. *Nucleic Acids Res.* **1989**, *17*, 8135−8147.

(22) Sekine, M. *J. Org. Chem.* 1989, 54, 2321−2326.

Scheme 1



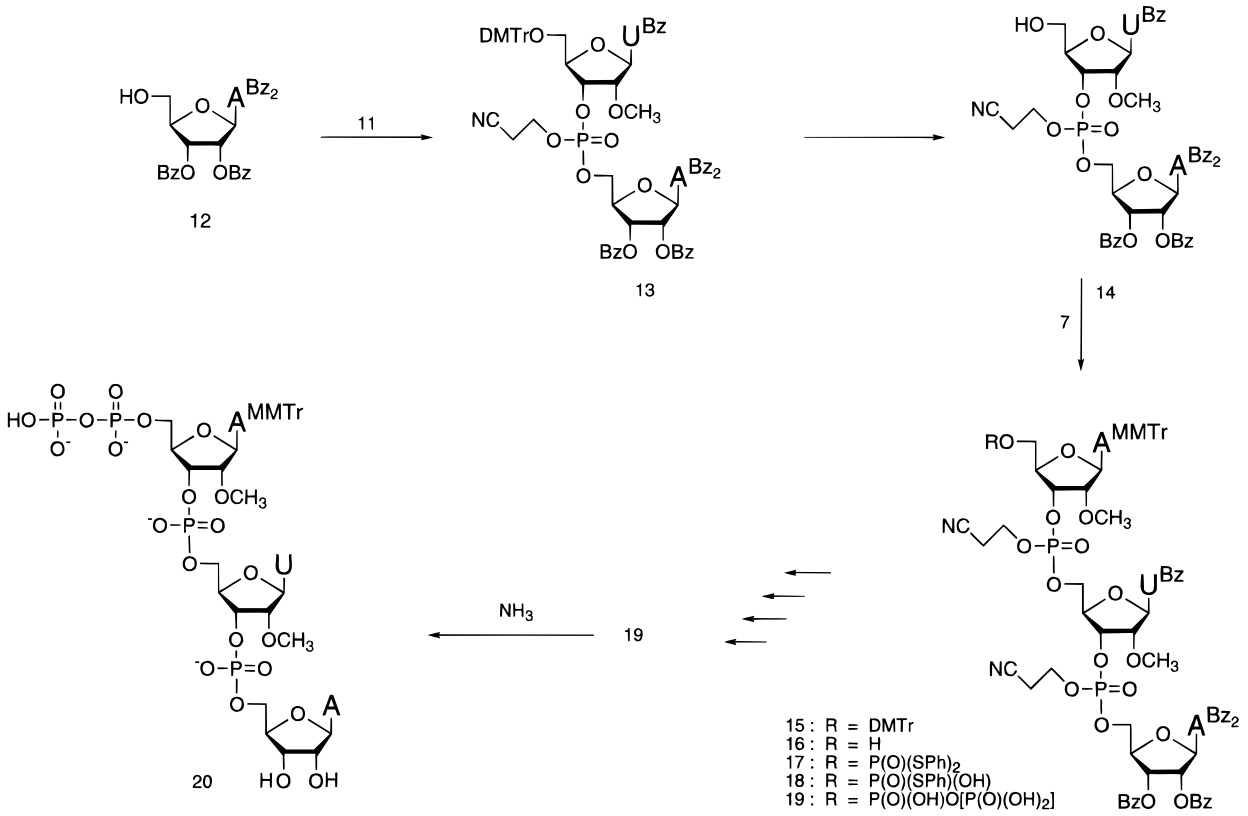
Condensation of **11** with the 3′-terminal adenosine unit **12** in acetonitrile in the presence of 1*H*-tetrazole gave the fully protected dimer **13** in 86% yield. Removal of the DMTr group from **13** was carried out by the use of 1% trifluoroacetic acid in CH_2Cl_2 to give the 5′-hydroxyl compound **14** in 97% yield. This product was further condensed with 2.0 equiv of **7** in a similar manner to give the fully protected trimer block **15** in 86% yield. The selective deprotection (84%) of **15** with 1 M $ZnBr_2$ in CH_2Cl_2−*i*PrOH (85:15, v/v)[18,20,23] followed by phosphorylation of the resulting 5′-hydroxyl product **16** with cyclohexylammonium *S*,*S*-diphenyl phosphorodithioate[24] in the presence of isodurenedisulfonyl dichloride (DDS)[25] and 1*H*-tetrazole afforded the 5′-phosphorylated species **17** in 84% yield.

Treatment of **17** with pyridinium phosphinate[26] in pyridine gave quantitatively the partially hydrolyzed product **18**. This compound was easily extracted with CH_2Cl_2 with the help of the liphophilic MMTr group and allowed to react with monotributylammonium phosphate in the presence of iodine. After removal of excess iodine, the resulting 5′-terminal diphosphate derivative **19** was *in situ* treated with concentrated ammonia−pyridine to give the 6-*N*-monomethoxytritylated trimer block, ppAm^{MMTr}pUmpA (**20**), in 73% yield. This compound was easily monitored by TLC analysis because of the presence of the MMTr group. The purity of **20** was more than 95%.

Synthesis of 2-*N*,2-*N*-Dimethylguanosine (DMG). For the synthesis of a TMG-capping reagent **32**, we have explored a convenient route to 2-*N*,2-*N*-dimethylguanosine (**28**: DMG) from guanosine by *only a three-step reaction*. Borch and Hassid first reported a convenient method for conversion of primary amines to *N*,*N*-dimethylamines by reductive methylation.[27a] Later, other groups successfully applied this method to the synthesis of other amino compounds.[27b,c] Therefore, we have studied in detail this procedure for the synthesis of DMG. Consequently, we found that reaction of the easily obtained 2′,3′,5′-tri-*O*-acetylguanosine (**21**)[28] with paraformaldehyde in the presence of sodium cyanoborohydride in acetic acid at 40 °C for 4 h gave exclusively the *N*,*N*-dimethylated product **23** in 95% yield. Five stepwise additions of 3 equiv each of sodium cyanoborohydride and paraformaldehyde was required for optimization of the yield of **23**. DMG was prepared by hydrolysis of **23**. This simple method for the synthesis of DMG is a really remarkable improvement, since the previous methods developed by a number of laboratories[12] required a series of reactions involving *more than six steps*. Quite recently, Eritja and co-workers[12g] reported an improved method for the synthesis of DMG using (4-nitrophenyl)-ethyl as the O^6 protecting group of guanosine but this approach still required a six-step reaction. Therefore, the present reductive methylation provided the hitherto shortest route to DMG. The results of the 2-*N*,2-*N*-dimethylation of **21** and 2′,3′,5′-*O*-tris(*tert*-butyldimethylsilyl)guanosine (**24**) under various conditions are summarized in Table 1.

(23) Ito, H.; Ike, Y.; Ikuta, S.; Itakura, K. *Nucleic Acids Res.* **1982**, *10*, 1755−1769.
(24) (a) Sekine, M.; Hamaoki, K.; Hata, T. *J. Org. Chem.* **1979**, *44*, 2325−2326. (b) Sekine, M.; Hamaoki, K.; Hata, T. *Bull. Chem. Soc. Jpn.* **1981**, *54*, 3815−3927. (c) Yamaguchi, K.; Honda, S.; Nakagawa, I.; Hata, T. *Chem. Lett.* **1978**, 507−508.
(25) Sekine, M.; Matsuzaki, J.; Hata, T. *Tetrahedron* **1985**, *41*, 5279−5288.
(26) Sekine, M.; Matsuzaki, J.; Hata, T. *Tetrahedron Lett.* **1981**, *22*, 3209−3212.
(27) (a) Borch, R. F.; Hassid, A. I. *J. Org. Chem.* **1972**, *37*, 1673−1674. (b) Gribble, G. W.; Nutaitis, C. F. *Synthesis* **1987**, 709−711. (c) Kim, S.; Oh, C. H.; Ko, J. S.; Ahn, K. H.; Kim, Y. J. *J. Org. Chem.* **1985**, *50*, 1927−1932.
(28) Bredereck, H. *Chem. Ber.* **1947**, *80*, 401.

Scheme 2



Scheme 3

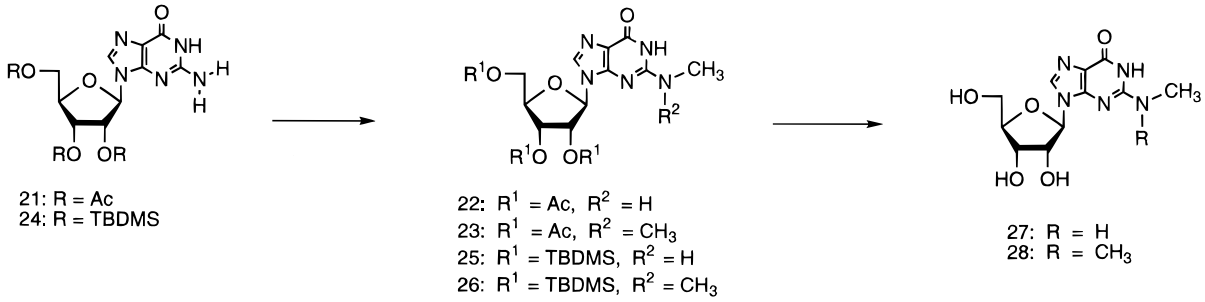


Table 1. Methylation of *O*-Protected Guanosine Derivatives 21 and 24

compd	$(CH_2O)_n$, equiv	$NaBH_3CN$,equiv	temp, °C	time, h	monomethylated product (yield, %)	dimethylated product (yield, %)
21	10	5	20	50	**22** (52)	**23** (52)
21	10	5	40	8		**23** (54)
21	10	5	60	7		**23** (63)
21	3 × 2	3 × 2	40	12	**22** (26)	**23** (36)
21	3 × 5	3 × 5	40	8		**23** (95)
24	10	10	40	20		**26** (9)
24	5 × 5	5 × 5	40	10		**26** (65)

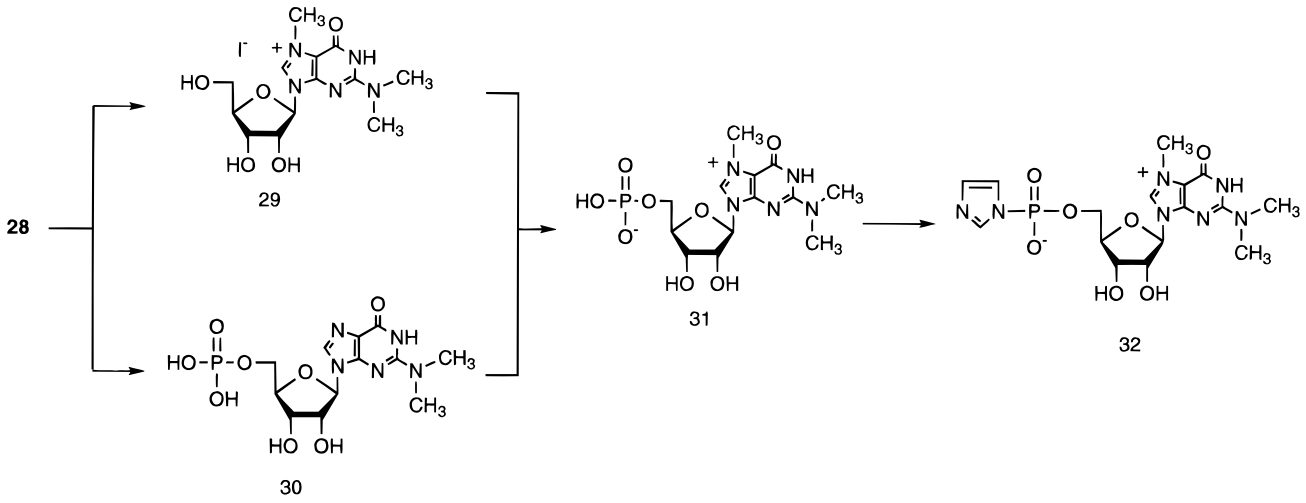
The acetyl group was superior to the *tert*-butyldimethylsilyl group which would cause steric hindrance around the amino group of **24**. The use of similar conditions ($ZnCl_2$, $NaCNBH_3$)[27c] for the reductive methylation of **21** resulted in a poorer yield of **23**.

When a lesser amount of sodium cyanoborohydride was employed, a mixture of a monomethylated product **22** and **23** was obtained as shown in Table 1. The former could be easily isolated. As far as the synthesis of **22** is concerned, 5 equiv of sodium cyanoborohydride should be used. Compound **22** was quantitatively converted to MMG by deacetylation. Although the photoirradiated one-step synthesis of MMG from guanosine by use of *tert*-butyl peracetate was reported, this procedure resulted in competitive formation of other byproducts and did not allow a large-scale synthesis of MMG.[29] On the basis of these criteria, our new route to MMG *via* the three-step reaction is practical and superior to the previous photomediated method.

Synthesis of a TMG-Capped Triribonucleotide, $m_3^{2,2,7}G^{5'}pppAmpUmpA$. In our previous paper, we prepared a TMG-capping reagent from DMG *via* a five-step reaction (2′,3′-*O*-methoxylmethylation, 5′-bis(phenylthio)phosphorylation, demethoxymethylation, dephen-

(29) (a) Bridson, P. K.; Markiewicz, W.; Reese, C. B.; *Synthesis* **1980**, 1025 (b) Kemal, A.; Reese, C. B. *Synthesis* **1980**, 1025−1028. (c) Zady, M. F.; Wong, J. L. *J. Org. Chem.* **1980**, *45*, 2373−2377. (d) Boryski, J.; Ueda, T. *Nucleosides Nucleotides* **1985**, *4*, 595- 606.

Scheme 4

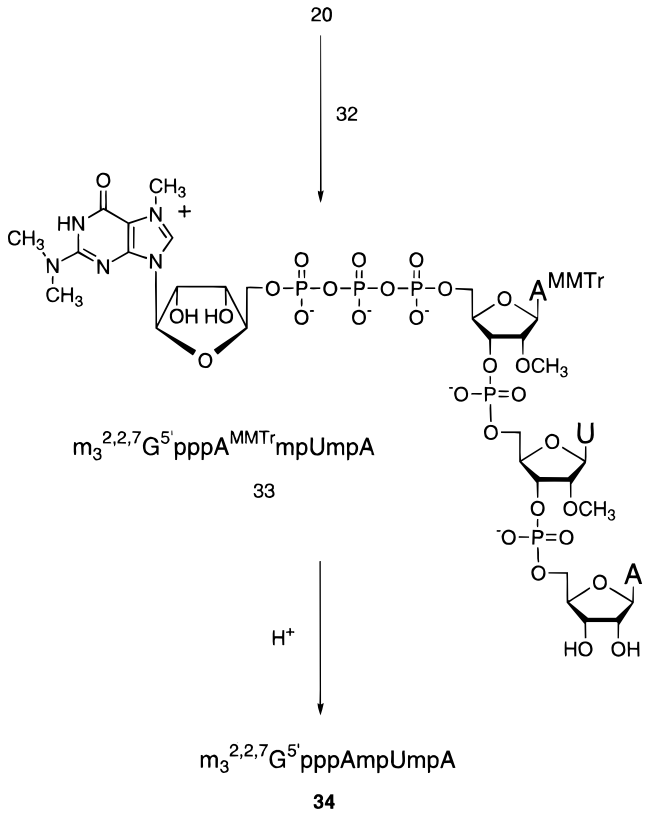


ylthiolation, and *N*-methylation).[8] Since this procedure was inconvenient, we have explored a more straightforward route to a TMG-capping reagent **32** as follows: Reaction of DMG with an excess amount of methyl iodide in DMF at 40 °C for 4 h followed by the successive treatment of the 7-methylated DMG derivative **29** with phosphorus oxychloride in trimethyl phosphate[30] gave the 5′-phosphorylated product (**31**: $pm_3^{2,2,7}G$) in 65% yield. Reaction of **31** with imidazole in the presence of triphenylphosphine and 2,2′-dipyridyl disulfide[31] in DMF gave ImpTMG (**32**) as sodium salt in 73% yield (method A). The TMG-capping reagent **32** was also prepared as an inner salt in a reverse way by phosphorylation of DMG followed by methylation of the product **30** with methyl iodide (method B). Since this inner salt was soluble in DMF, it was used *in situ* without salt exchange for the triphosphate bond formation.

First, condensation of the pyridinium salt of **20** with the inner salt **32**, *in situ* prepared by method B, in DMF afforded $m_3^{2,2,7}G^{5'}pppA^{MMTr}mpUmpA$, which was easily detected with the help of the MMTr group. The successive treatment of $m_3^{2,2,7}G^{5'}pppA^{MMTr}mpUmpA$ with 80% acetic acid gave $m_3^{2,2,7}G^{5'}pppAmpUmpA$ in an overall yield of 40%. When the sodium salt of **32** obtained as a white powder by method A was passed through a column of Dowex 50 W X8 (pyridinium form), the resulting inner salt was hardly soluble in DMF. It is not clear why the different solubility was observed in two inner salt materials obtained via method A and directly from method B. The capping reaction using the above insoluble inner salt resulted in a considerably reduced yield of $m_3^{2,2,7}G^{5'}$-pppAmpUmpA. It should be noted that the inner salt **32** in situ generated by method B was sufficiently soluble in DMF so that the capping reaction could be carried out in an almost homogeneous solution giving a reasonable yield of $m_3^{2,2,7}G^{5'}pppAmpUmpA$.

Sawai and co-workers[32] have recently reported that the diphosphate and triphosphate bridge formation could be done in aqueous media. Therefore, we tried the capping reaction of **20** in DMF containing some amount of water which enabled us to use the sodium salt of **32** without salt exchange. Consequently, addition of water to the

Scheme 5



mixture of **20** and the sodium salt **32** accelerated the capping reaction to give $m_3^{2,2,7}G^{5'}pppAmpUmpA$ in 32% yield from **20** after acid treatment.

The isolation of $m_3^{2,2,7}G^{5'}pppAmpUmpA$ was performed by paper electrophoresis on a relatively large scale followed by purification using reverse phase HPLC. The paper electrophoresis using Whatman 3MM papers made it possible to separate ca. 100 A_{260} unit of the TMG-capped trimer in one experiment. The electrophoretic chromatogram of the deprotected mixture derived from the condensation of **20** with **32** is shown in Figure 2. This electrophoresis was quite effective for separation of the desired TMG-capped tetramer from the unreacted capping reagent and other byproducts, but the material separated by paper electrophoresis contained a considerable amount of unreacted diphosphate **20**, as evidenced

(30) Yoshikawa, M.; Kato, T.; Takenishi, T. *Bull. Chem. Soc. Jpn.* **1969**, *42*, 3505−3508.
(31) (a) Mukaiyama, T.; Hashimoto, M. *J. Am. Chem. Soc.* **1972**, *94*, 8528−8532.
(32) Shimazu, M.; Shinozuka, K.; Sawai, H. *Tetrahedron Lett.* **1990**, *31*, 235−238.

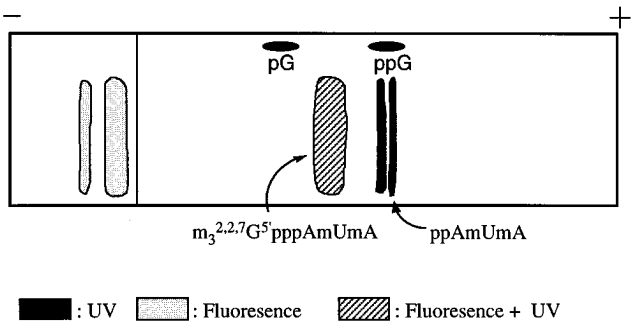


Figure 2. Paper electrophoresis of the mixture obtained by the reaction of the pyridinium salt of **20** with **32** in situ prepared by method B.

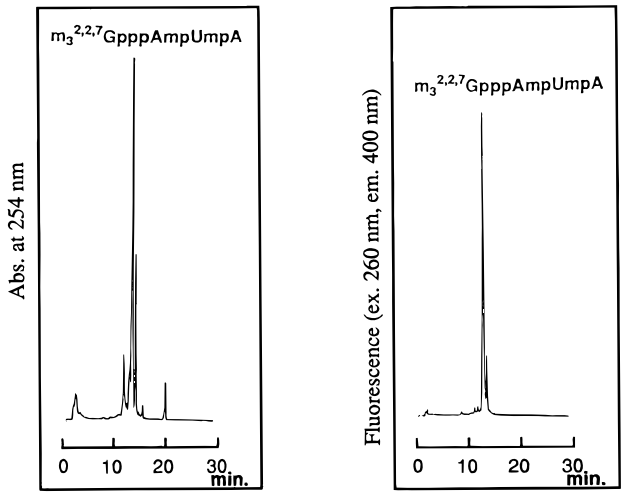


Figure 3. The HPLC profile of the material separated by paper electrophoresis as shown in Figure 2. The main peak is $m_3^{2,2,7}G^{5'}$pppAmpUmpA.

by reverse phase HPLC (Figure 3). Finally, $m_3^{2,2,7}G^{5'}$-pppAmpUmpA was purified by reverse phase HPLC.

The structure of $m_3^{2,2,7}G^{5'}$pppAmpUmpA thus obtained was confirmed by ^{1}H NMR, which exhibited four sharp resonance signals at the aromatic region and four sets of 1′-H resonance doublet signals around 6 ppm, as shown in Figure 4A. The ^{31}P NMR spectrum of $m_3^{2,2,7}G^{5'}$-pppAmpUmpA (Figure 4B) clearly shows three types of phosphorus resonance signals. In the high field of −24.66 ppm, a typical resonance of the β-phosphate of the triphosphate bridge appeared, while the α- and γ-phosphate resonance signals were seen at −13.35 and −13.18 ppm, respectively. The internucleotidic phosphate groups showed two peaks at −3.12 and −2.96 ppm.

Characterization of the final product was also performed by enzyme analysis using venom phosphodiesterase and calf intestinal phosphatase. The former is known to digest RNA form the 3′-side as an exonuclease to give 5′-nucleotides. The latter is an enzyme used for dephosphophoylation of nucleotides. The successive treatments of $m_3^{2,2,7}G^{5'}$pppAmpUmpA with two enzymes gave a mixture of TMG, Am, Um, and A in a ratio of nearly 1:1:1:1 as shown in panels B1 and B2 of Figure 5B. Digestion of the synthetic TMG-capped trimer with nuclease P1, which has 3′-exonuclease and endonuclease acitivities to RNA, gave an equimolar mixture of $m_3^{2,2,7}G^{5'}$pppAm, pUm and pA as shown in panels C1 and C2 of Figure 5C. The TMG cap $m_3^{2,2,7}$GpppAm was further digested with snake venom phosphodiesterase followed by calf intestinal phosphatase to give a nearly 1:1 mixture of $m_3^{2,2,7}$G and Am as evidenced by Figure 5D. All digestion products containing $m_3^{2,2,7}$G were clearly detected by a fluorescence detector at ex 260 nm, em 400 nm as depicted in panels A2, B2, C2, and D2 of Figure 5.

Conclusions

The present method provided an efficient route to the 5′-terminal U1 RNA tetramer fragment, which would be used as a powerful tool for elucidation of the mechanism of mRNA splicing as well as transportation of RNAs since the TMG capped structure can be used as a minimum substrate capable of the 3′-selective ligation with pCp and other labeled pCpX derivatives. Our preliminary result showed that the synthetic 5′-terminal oligoribonucleotide, $m_3^{2,2,7}G^{5'}$pppAmpUmpA, could serve as a good substrate in the 3′-end ^{32}P labeling with ^{32}pCp in the presence of RNA ligase, as expected. Further application of $m_3^{2,2,7}G^{5'}$pppAmpUmpA to the study of interaction between snRNPs-U1RNA is now in progress.

Experimental Section

Melting points are uncorrected. ^{1}H and ^{13}C NMR spectra were measured at 270 and 67.8 MHz, respectively, with TMS as the internal standard. ^{31}P NMR spectra were recoreded at 109.25 MHz in D_2O with 80% H_3PO_4 as the external reference. In the case of **20** and **34**, the lyophilized materials were dissolved in D_2O for these NMR analyses. Paper chromatography was performed by use of a descending technique with Whatman 3MM papers and Toyo Roshi 51 papers using the following solvent system: 2-propanol−concd aqueous ammonia−water, 7:1:2, v/v/v. Column chromatography was performed with silica gel C-200 purchased from Wako Co., Ltd., and a minipump for a goldfish bowl was conveniently used to attain sufficient pressure for rapid chromatographic separation. Reverse-phase column chromatography was performed by the use of μBondapak C-18 silica gel (Prep S-500, Waters). TLC was performed on precoated TLC plates of silica gel 60 F-254 (Merck). Reverse-phase HPLC was performed on a Waters Model A25 using a μBondasphere 5 μm C18−100 Å, 3.9 mm × 15 cm (Waters) with a linear gradient starting from 0.1 M NH_4OAc, pH 7.0, and applying CH_3CN at a flow rate of 1.0 mL/min for 30 min. Ribonucleosides were purchased from Yamasa Co., Ltd. Pyridine was distilled two times from *p*-toluenesulfonyl chloride and from calcium hydride and then stored over molecular sieves 4A. Elemental analyses were performed by the Microanalytical Laboratory, Tokyo Institute of Technology, at Nagatsuta.

2′,3′,5′-Tri-*O*-acetyl-2-*N*,2-*N*-dimethylguanosine (23).[12f] To a solution of compound **21** (219 mg, 0.5 mmol) in acetic acid (5 mL) were added paraformaldehyde (45 mg, 1.5 mmol) and sodium cyanoborohydride (314 mg, 1.5 mmol). The mixture was vigorously stirred at 40 °C. At 8-h intervals (four times), paraformaldehyde (45 mg, 1.5 mmol), and sodium cyanoborohydoride (314 mg, 1.5 mmol) were stepwise added to the mixture. The solvent was removed under reduced pressure, and the residue was dissolved in CH_2Cl_2−pyridine (1:1, v/v). The solution was washed three times with saturated $NaHCO_3$. Every aqueous layer was back-extracted with CH_2Cl_2−pyridine (2:1, v/v). After extraction was performed, the organic phase was collected, dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The residue was chromatographed on a column of silica gel (10 g) eluted with CH_2Cl_2−MeOH (98.5:1.5−98.2:1.8, v/v) to give **23** (208 mg, 95%): ^{1}H NMR (270 MHz, $CDCl_3$) δ 2.08, 2.11, and 2.12 (9H, s), 3.24 (6H, s), 4.25 (1H, dd, J = 4.6 Hz, J = 11.2 Hz), 4.37 (1H, m), 4.44 (1H, dd, J = 3.3 Hz, J = 11.2 Hz), 5.69 (1H, t, J = 5.6 Hz), 5.90 (1H, d, J = 4.1 Hz), 6.07 (1H, dd, J = 4.1 Hz, J = 5.6 Hz), 7.59 (1H, s), 10.34 (1H, br).

2′,3′,5′-Tri-*O*-acetyl-2-*N*-methylguanosine (22).[12f] To a solution of compound **21** (4.09 mg, 10 mmol) in acetic acid (100

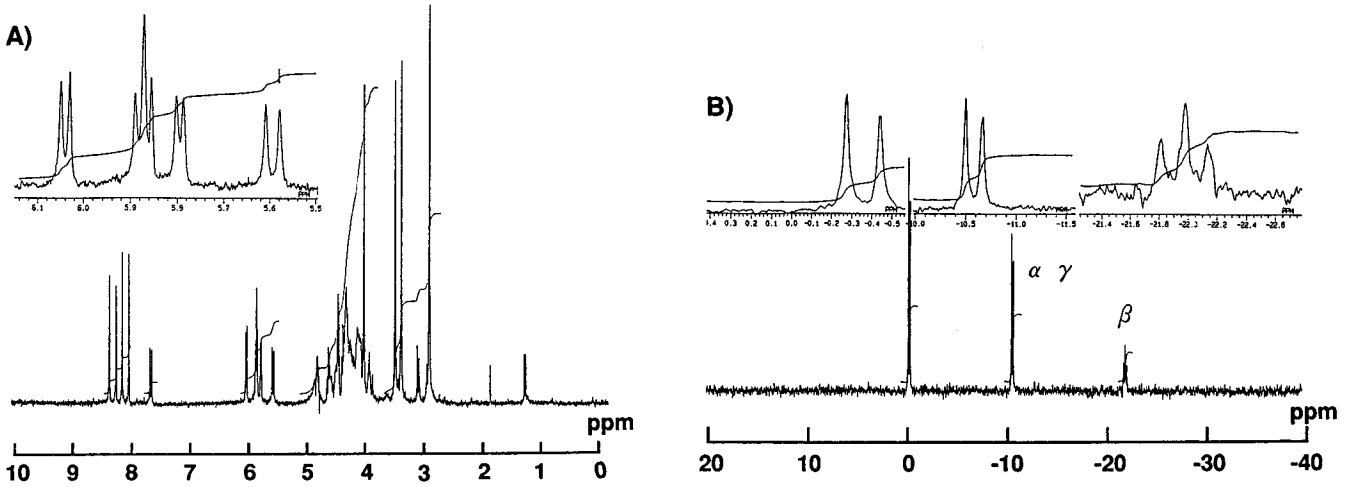


Figure 4. Panel A: ^{1}H NMR spectrum of $m_3^{2,2,7}G^{5'}$pppAmpUmpA in D_2O. Panel B: ^{31}P NMR spectrum of $m_3^{2,2,7}G^{5'}$pppAmpUmpA in D_2O.

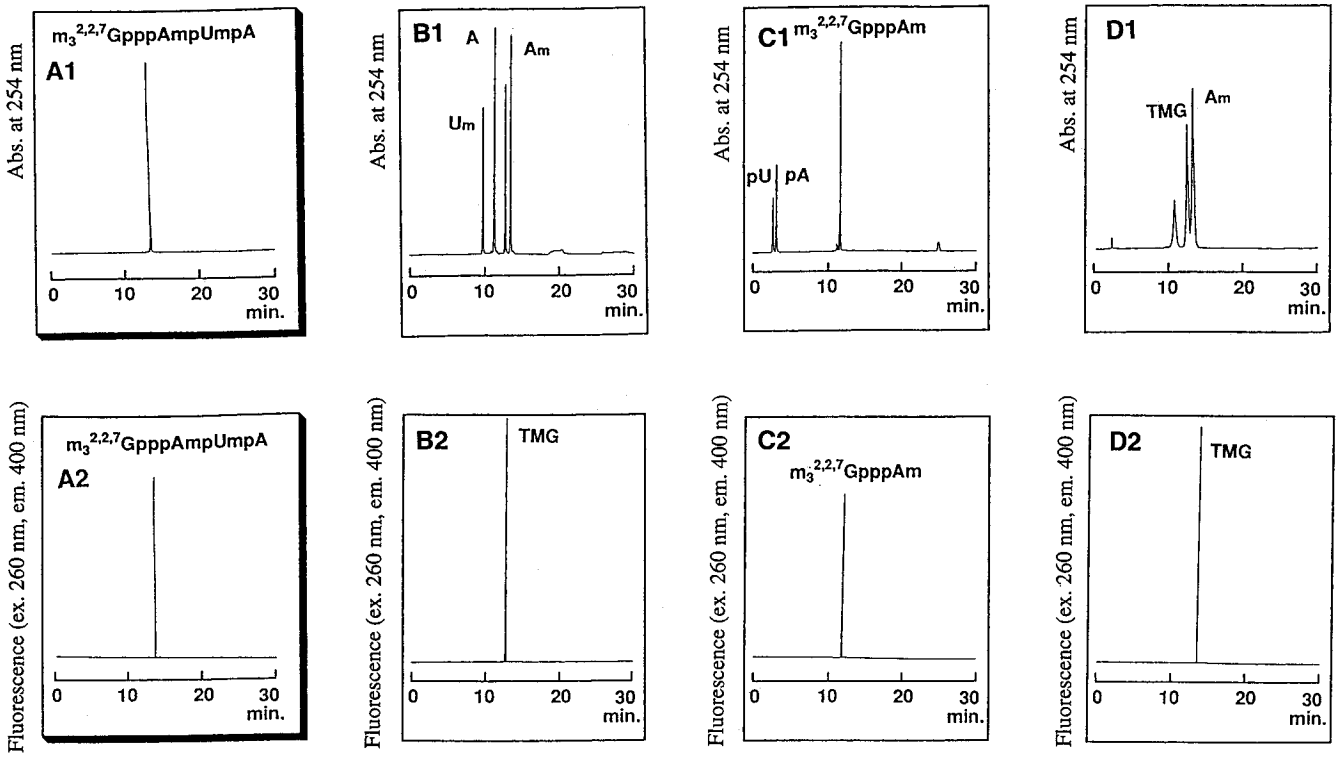


Figure 5. Panel A: The HPLC profiles of purified $m_3^{2,2,7}G^{5'}$pppAmpUmpA. Panel B: The HPLC profiles of the mixture of $m_3^{2,2,7}G$ (TMG), Am, Um, and A which was obtained after treatment of $m_3^{2,2,7}G^{5'}$pppAmpUmpA with SVPD and AP. Panel C: The HPLC profiles of the mixture of $^{m}3^{2,2,7}G^{5'}$pppAm, pUm and pA which was obtained after treatment of $m_3^{2,2,7}G^{5'}$pppAmpUmpA with nuclease P1. Panel D: The HPLC profiles of the mixture of TMG and Am which was obtained after treatment of $m_3^{2,2,7}G^{5'}$pppAm with SVPD and AP.

mL) were added paraformaldehyde (310 mg, 30 mmol) and sodium cyanoborohydride (1.13 g, 30 mmol). The mixture was vigorously stirred at 40 °C. After 8 h paraformaldehyde (310 mg, 10 mmol) and sodium cyanoborohydoride (1.13 g, 10 mmol) were added to the mixture. The solvent was removed under reduced pressure, and the residue was dissolved in CH_2Cl_2−pyridine (1:1, v/v). The solution was washed three times with saturated $NaHCO_3$. Every aqueous layer was back-extracted with CH_2Cl_2−pyridine (2:1, v/v). After extraction was performed, the organic phase was collected, dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The residue was chromatographed on a column of silica gel (50 g) eluted with CH_2Cl_2−MeOH (98.5:1.5−98.2:1.8, v/v) to give **23** (1.58 g, 36%) and CH_2Cl_2−MeOH (97:3, v/v) to give **22** (1.10 g, 26%): ^{1}H NMR (270 MHz, $CDCl_3$) δ 2.08, 2.120, and 2.123 (9H, s), 3.03 (3H, d, J = 4.6 Hz), 4.29 (1H, dd, J = 5.3 Hz, J = 11.7 Hz), 5.84 (1H, m), 5.93 (1H, d, J = 4.0 Hz), 6.05 (1H, dd, J= 4.0 Hz, J = 5.6 Hz), 7.60 (1H, s), 12.00 (1H, br).

2′,3′,5′-*O*-Tris(*tert*-butyldimethylsilyl)-2-*N*,2-*N*-dimethylguanosine (26). To a solution of compound **24** (313 mg, 0.5 mmol) in acetic acid (5 mL) were added paraformaldehyde (45 mg, 1.5 mmol) and sodium cyanoborohydride (314 mg, 1.5 mmol). The mixture was vigorously stirred at 40 °C. At 8-h intervals (four times), paraformaldehyde (45 mg, 1.5 mmol) and sodium cyanoborohydoride (314 mg, 1.5 mmol) were stepwise added to the mixture. The solvent was removed under reduced pressure, and the residue was dissolved in CH_2Cl_2. The solution was washed three times with saturated $NaHCO_3$. Every aqueous layer was back-extracted with CH_2Cl_2−pyridine (2:1, v/v). After extraction was performed, the organic phase was collected, dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The residue was

chromatographed on a column of silica gel (10 g) eluted with CH_2Cl_2−MeOH (98.5:1.5−98.2:1.8, v/v) to give **26** (214 mg, 65%): ^{1}H NMR (270 MHz, $CDCl_3$) δ −0.17, −0.04, 0.09, 0.10. 0.11 (18H, s), 0.81, 0.92, 0.93 (27H, s), 3.24 (6H, s), 3.78 (1H, dd, J = 2.6 Hz, J = 11.4 Hz), 3.92 (1H, dd, J = 4.3 Hz, J = 11.4 Hz), 4.06 (1H, m), 4.28 (1H, t, J = 4.0 Hz), 4.49 (1H, t, J' = 4.6 Hz), 5.88 (1H, d, J = 5.0 Hz), 7.82 (1H, s), 11.30 (1H, br); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ −5.50, −5.46, −4.99, −4.76, −4.53, 17.77, 17.99, 18.40, 25.59, 25.73, 25.97, 26.13, 38.26, 62.54, 71.83, 75.55, 84.71, 87.35, 116.39, 136.46, 151.55, 152.85, 159.21. Anal. Calcd for $C_{30}H_{59}N_5O_5Si_3{\cdot}^1/_2H_2O$: C, 54.34; H, 9.12; N, 10.56. Found: C, 54.46; H, 9.10; N, 10.58.

2-*N*,2-*N*-Dimethylguanosine (28).[12f] To a solution of compound **22** (5.8 g, 13.3 mmol) in pyridine (400 mL) was added 0.5 M NaOH (400 mL, 0.02 mol). After being stirred at room temperature for 20 min, the mixture was applied to a resin of Dowex 50WX8 (pyridinium form, 200 mL) and elution was performed with pyridine−water (4000 mL, 9:1, v/v). The eluate was evaporated under reduced pressure. Crystallization of the residue from water (300 mL) gave **28** (3.04 g, 73%): mp 218 °C (lit.[12f] 242 °C); ^{1}H NMR (270 MHz, DMSO-d_6-D_2O, at 70 °C) δ 3.06 (6H, s), 3.53 (1H, dd, J = 4.6 Hz, J = 11.6 Hz) , 3.63 (1H, dd, J = 4.0 Hz, J = 11.6 Hz), 3.96 (1H, m), 4.14 (1H, t, J = 4.6 Hz), 4.51 (1H, t, J = 5.5 Hz), 5.72 (1H, d, J = 5.5 Hz), 7.84 (1H, s).

2-*N*,2-*N*,7-Trimethylguanosine 5′-*O*-Phosphate (31). To a suspension of **28** (311 mg, 1 mmol) in dry DMF (17 mL) was added methyl iodine (1.25 mL, 20 mmol). After the mixture was stirred at 40 °C for 4 h, the solvent was removed under reduced pressure. Compound **29** was precipitated from ether (100 mL) and was dissolved in trimethyl phosphate (6 mL). The resulting solution was stirred at 0 °C for 10 min, and phosphorus oxychloride (280 μL, 1.3 mmol) was added. After being stirred at 0 °C for 4 h, the mixture was diluted with water and washed three times with CH_2Cl_2. The aqueous phase was evaporated under reduced pressure. Compound **31** was purified by the following methods: **Method A**. The residue was applied to a column of Dowex 50WX8 (H^+ form, 60 mL) and eluted with water to give **31** (249 mg, 61%). **Method B**. The residue was chromatographed on Whatman 3MM papers developed with butanol−acetic acid−H_2O (5:2:3, v/v/v). The band of R_f 0.7 was cut and eluted with water. The eluate was condensed and applied to a column of Dowex1X8 (HCO_3^- form, 60 mL). Elution with water followed by lyophilization gave **31** (260 mg, 65%): ^{1}H NMR (270 MHz, D_2O) δ 3.02 (6H, s), 3.92 (3H, s), 3.92−4.08 (2H, m), 4.21 (1H, m), 4.29 (1H, m), 4.53 (1H, m), 5.93 (1H, d, J = 3.3 Hz), 8.94 (1H, s); ^{13}C NMR (67.8 MHz, D_2O) δ 31.35, 36.91, 38.65, 64.36, 70.04, 75.29, 84.72, 90.51, 107.80, 137.37, 150.45, 154.90, 156.29; ^{31}P NMR (109.25 MHz, D_2O) δ 0.65: FAB Calcd for m/z 404.0971. Observed for m/z 404.0964.

5′-*O*-(Imidolylphospho)-2-*N*,2-*N*,7-trimethylguanosine (32). Method A. A mixture of **31** (81 mg, 0.2 mmol) and imidazole (136 mg, 2 mmol) was rendered anhydrous by successive coevaporations with dry pyridine, dry toluene, and dry DMF, and finally suspended in dry DMF (2 mL). To the mixture was added triethylamine (200 μL), tri-*n*-octylamine (100 μL), triphenylphosphine (157 mg, 0.6 mmol), and 2,2′-dipyridyl disulfide (132 mg, 0.6 mmol), and the mixture was stirred at room temperature for 2 h. Precipitation of the mixture from acetone−ether−triethylamine (61 mL, 40:20:1, v/v/v) gave **32** (65 mg, 73%). **Method B.** Compound **28** (311 mg, 1 mmol) was suspended in trimethyl phosphate (6 mL), and the mixture was stirred at 0 °C for 10 min. Phosphorus oxychloride (280 μL, 3 mmol) was added to the resulting solution. After being stirred at 0 °C for 4 h, the mixture was diluted with water and washed three times with CH_2Cl_2. The aqueous phase was evaporated under reduced pressure. The residue was chromatographed on Whatman 3MM papers developed with butanol−acetic acid−H_2O (5:2:3, v/v/v) to give **30** (350 mg, 90%). Part of this compound (200 mg, 0.5 mmol) was suspended in DMF (10 mL), and methyl iodide (625 μL, 10 mmol) was added. After the mixture was stirred at 40 °C for 16 h, the solvent was removed under reduced pressure. The residue was dissolved in dry DMF, and 1,1′-carbonyldiimidazole (811 mg, 5 mmol) was added. After being stirred at room temperature for 2 h, the mixture was treated with methanol (320 μL). The solvent was removed under reduced pressure. The residue was dissolved in dry DMF (5 mL), and **32** was stored as a 0.01 mM solution in dry DMF at 0 °C. **32**: ^{1}H NMR (270 MHz, D_2O) δ 3.07 (6H, s), 4.04 (3H, s), 4.04−4.07 (2H, m), 4.19 (1H, m), 4.28 (1H, m), 4.42 (1H, m), 5.95 (1H, d, J = 4.0 Hz), 6.92, 7.10, 7.77 (3H, s); ^{31}P NMR (109.25 MHz, D_2O) δ -7.27.

3′,5′-*O*-(1,1,3,3-Tetraisopropyldisiloxane-1,3-diyl)-2′-*O*-methyl-6-*N*-(monomethoxytrityl)adenosine (4). Compound **3** (7.82 g, 10 mmol) was dissolved in methyl iodide (94 mL, 1.5 mol), and silver oxide (11.5 g, 50 mmol) was added. The mixture was refluxed for 4 h. The resulting precipitate was filtered and washed with CH_2Cl_2. The filtrate and the washing were combined and evaporated under reduced pressure. The residue was chromatographed on a column of silica gel (400 g) eluted with hexane−EtOAc (5:1, v/v) to give **4** (6.57 g, 83%): ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.06 (14 H, m), 3.66 (3 H, s), 3.78 (3 H, s), 3.94 (1H, dd, J = 13.4 Hz), 4.01−4.15 (2 H, m), 4.14 (1H, dd, J = 13.4 Hz), 4.85 (1 H, m), 5.94 (1H, s), 6.79 (2 H, d, J = 8.6 Hz), 6.91 (1 H, s), 7.23−7.36 (14 H, m), 7.99 (1 H, s), 8.01 (1 H, s); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 12.49, 12.81, 12.90, 13.37, 14.14, 16.86, 17.00, 17.05, 17.15, 17.24, 17.27, 17.40, 55.15, 59.52, 59.75, 69.71, 70.91, 81.13, 83.40, 88.43, 113.06, 121.42, 126.76, 127.78, 128.86, 130.17, 137.20, 138.49, 145.14, 148.00, 152.24, 153.98, 158.24. Anal. Calcd for $C_{42}H_{57}N_5O_6Si_2$: C, 64.34; H, 7.33; N, 8.93. Found: C, 64.21; H, 7.16; N, 8.45.

2′-*O*-Methyl-6-*N*-(monomethoxytrityl)adenosine (5). To a solution of **4** (6.0 g, 7.65 mmol) in acetonitrile−water (77 mL, 99:1, v/v) were added KF (3.6 g, 61 mmol) and Et_4NBr (12.86 g, 61 mmol). The mixture was stirred at room temperature for 6 h. The solvent was removed under reduced pressure, and the residue was partitioned between CH_2Cl_2 (200 mL) and water (200 mL) in a separatory funnel A. The aqueous layer was transferred to another separatory funnel B and extracted with CH_2Cl_2.(200 mL), and then the aqueous layer was discarded. The CH_2Cl_2 layer in the first separatory funnel A was washed five times with water (200 mL), which was transferred to separatory funnel B, extracted with the same CH_2Cl_2, and discarded each time. The two CH_2Cl_2 layers were combined, dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The residue was chromatographed on a column of silica gel (200 g) eluted with CH_2Cl_2−MeOH (100:0−98.5:1.5, v/v) to give **5** (4.0 g, 97%): ^{1}H NMR (270 MHz, $CDCl_3$) δ 3.36 (3H, s), 3.69 (1H, dd, J = 12.2 Hz), 3.78 (3H, s), 3.91 (1H, dd, J = 12.2 Hz), 4.33 (1H, br s), 4.56 (1H, m), 4.71 (1H, dd, J = 7.6 Hz, J = 4.6 Hz), 5.83 (1H, d, J = 7.6 Hz), 6.80 (2H, d, J = 8.9), 7.06 (1H, s), 7.23−7.36 (14H, m), 7.78 (1H, s), 8.01 (1H, s); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 55.11, 58.64, 63.16, 70.46, 71.03, 82.46, 88.23, 89.51, 113.08, 122.34, 126.85, 127.82, 128.77, 130.12, 136.84, 140.13, 144.83, 147.21, 151.61, 154.50, 158.27. Anal. Calcd for $C_{31}H_{31}N_5O_5{\cdot}^3/_4H_2O$: C, 65.64; H , 5.77; N, 12.35. Found: C, 65.82; H, 5.63; N, 12.00.

2′-*O*-Methyl-5′-*O*-(dimethoxytrityl)-6-*N*-(monomethoxytrityl)adenosine (6). Compound **5** (1.107 g, 2.0 mmol) was rendered anhydrous by repeated coevaporations with dry pyridine and dissolved in dry pyridine (20 mL). Dimethoxytrityl chloride (813 mg, 2.4 mmol) was added, and the mixture was stirred at room temperature for 12 h. The mixture was diluted with CH_2Cl_2, and the CH_2Cl_2 solution was washed two times each with saturated $NaHCO_3$ (150 mL) and with water. The organic phase was collected, dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The residue was chromatographed on a column of silica gel (100 g) eluted with CH_2Cl_2−MeOH (100:0−99:1, v/v) to give **6** (1.67 g, 98%): ^{1}H NMR (270 MHz, $CDCl_3$) δ 3.38 (1H, dd, J = 4.4 Hz, J = 11.6 Hz), 3.49 (1H, dd, J = 3.3 Hz, J = 11.6 Hz), 3.52 (3 H, s), 3.76 (9 H, s), 4.08 (1 H, m), 4.30−4.35 (2 H, m), 6.01 (1 H, d, J = 3.6 Hz), 6.69 (2 H, d, J= 8.9), 6.71 (2 H, d, J = 8.9 Hz), 6.81 (1 H, s), 7.09−7.33 (21 H, m), 7.87 (1 H, s), 7.90 (1 H, s); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 21.37, 55.10, 58.71, 62.98, 69.69, 70.89, 83.06, 83.67, 86.33, 86.45, 113.03, 113.08, 121.19, 125.19, 126.74, 126.83, 127.76, 128.07, 128.12, 128.81, 128.93, 129.96, 130.03, 130.12, 135.53, 135.67, 137.11, 138.20, 144.42, 145.10, 148.37, 152.27, 153.98, 158.18, 158.44. Anal Calcd for

$C_{52}H_{49}N_5O_7$; C, 72.97; H, 5.77; N, 8.18. Found: C, 73.53; H, 5.61; N, 7.79.

2′-*O*-Methyl-5′-*O*-(dimethoxytrityl)-6-*N*-(monomethoxytrityl)adenosine 3′-*O*-(2-cyanoethyl *N*,*N*-diisopropylphosphoramidite) (7). Compound **6** (428 mg, 0.5 mmol) was rendered anhydrous by repeated coevaporations with dry pyridine and then with dry toluene and dissolved in CH_2Cl_2 (5 mL). To the solution were added ethyldiisopropylamine (0.357 mL, 1.2 mmol) and (2-cyanoethoxy)(*N*,*N*-diisopropylamino)phosphine (0.163 mL, 0.75 mmol). The resulting mixture was stirred under argon atmosphere at room temperature for 1 h. The mixture was diluted with CH_2Cl_2, and the CH_2Cl_2 solution was washed two times each with saturated $NaHCO_3$ (100 mL) and with water. The organic phase was collected, dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The residue was chromatographed on a column of silica gel (25 g) eluted with hexane−EtOAc−pyridine (70:30:0.5, v/v/v) to give **7** (449 mg, 85%): ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.05−1.17 (12 H, d, J = 6.6 Hz), 2.36 and 2.63 (2 H, t, J = 6.6 and 6.3 Hz), 3.45 and 3.46 (3 H, s), 3.28−3.65 (4 H, m), 3.76 and 3.77 (9 H, s), 3.84−3.94 (2 H, m), 4.31 and 4.36 (1H, m), 4.55−4.68 (2 H, m), 6.06 (1H, m), 6.77−6.82 (6H, m), 6.92 (1H, bs), 7.13−7.42 (27H, m), 7.72, 7.96, 7.97, 7.98 (2 H, s, 2H, 8H); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 19.97, 20.07, 20.20, 20.29, 24.46, 24.57, 29.60, 43.00, 43.18, 55.11, 57.81, 58.10, 58.24, 58.71, 58.96, 62.61, 63.09, 70.48, 70.73, 70.87, 81.62, 81.94, 83.49, 86.31, 86.42, 86.51, 113.05, 117.32, 117.63, 121.33, 126.74, 126.81, 127.77, 128.09, 128.18, 128.81, 129.97, 130.05, 130.12, 135.49, 135.60, 137.16, 138.78, 144.35, 145.12, 148.64, 148.73, 152.26, 154.00, 158.20, 158.45; ^{31}P NMR (109.25 MHz, $CDCl_3$) δ 151.63, 150.84 (40; 60). Anal. Calcd for $C_{61}H_{66}N_7O_8P$; C, 69.36; H, 6.30; N, 9.29. Found: C, 69.21; H, 6.42; N, 9.04.

***N*^{3}-Benzoyl-2′-*O*-methyluridine (9)**. To a solution of **8** (1.81 g, 3 mmol) in dry THF (30 mL) were added acetic acid (0.38 mL, 6.6 mmol) and $Bu_4NF{\cdot}H_2O$ (1.73 mg, 6.6 mmol). The mixture was stirred at room temperature for 30 min. The solvent was removed under reduced pressure, and the residue was dissolved in CH_2Cl_2−pyridine (3:1, v/v). The solution was washed two times each with saturated $NaHCO_3$ and water. The organic phase was collected, dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The residue was dissolved in pyridine−water (300 mL, 1:2, v/v) and passed through a Dowex 50WX8 resin (H^+ form, 80 mL). Further elution was performed with water (600 mL). The eluate was evaporated under reduced pressure and chromatographed on a column of silica gel (60 g) eluted with CH_2Cl_2−MeOH (100:0−98.2:1.8, v/v) to give **9** (1.02 g, 94%): ^{1}H NMR (270 MHz, $CDCl_3$) δ 3.58 (3H, s), 3.87−4.10 (4H, m), 4.34 (1H, dd, J = 5.6 Hz, J = 7.6 Hz), 7.64−7.70 (2H, m), 7.51 (2H, m), 7.67 (1H, m), 7.93−8.00 (3H, m); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 58.65, 60.58, 68.00, 83.22, 84.55, 88.70, 102.09, 129.22, 130.49, 131.20, 135.31, 140.59, 149.20, 162.16, 168.55. Anal. Calcd for $C_{17}H_{18}N_2O_7$: C, 56.35; H, 5.01; N, 7.73. Found: C, 56.35; H, 4.57; N, 7.58.

***N*^{3}-Benzoyl-2′-*O*-methyl-5′-*O*-(dimethoxytrityl)uridine (10).** Compound **9** (0.63 g, 1.7 mmol) was rendered anhydrous by repeated coevaporations with dry pyridine and finally dissolved in dry pyridine (17 mL). To the solution was added dimethoxytrityl chloride (0.65 g, 1.9 mmol). The mixture was stirred at room temperature for 12 h. The mixture was diluted with CH_2Cl_2, and the CH_2Cl_2 solution was washed two times each with saturated $NaHCO_3$ (150 mL) and with water. The organic phase was collected, dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The residue was chromatographed on a column of silica gel (50 g) eluted with hexane−EtOAc−pyridine (65:35:1, v/v/v) to give **10** (1.12 g, 99%): ^{1}H NMR (270 MHz, $CDCl_3$) δ 3.56−3.63 (2H, m), 3.61 (3H, s), 3.81 (6H, s), 3.85 (1H, m), 4.02 (1H, d, J = 8.3 Hz), 4.53 (1 H, br), 5.36 (1H, d, J = 8.3 Hz), 5.94 (1H, s), 6.88 (4H, d, J = 8.6 Hz), 7.14−7.44 (9H, m), 7.50 (2H, m), 7.66 (2H, m), 7.95 (2H, d, J = 7.3 Hz), 8.22 (1H, d, J = 8.3 Hz); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 55.22, 58.64, 60.81, 68.11, 83.24, 83.90, 87.12, 87.30, 101.94, 113.30, 125.25, 127.17, 128.01, 128.12, 128.99, 129.15, 130.08, 130.15, 130.49, 131.30, 134.95, 135.17, 135.22, 137.81, 139.73, 144.24, 149.09, 158.72, 161.99, 168.66. Anal. Calcd for $C_{38}H_{36}N_2O_9$: C, 68.66; H, 5.46; N, 4.22. Found: C, 68.59; H, 5.67; N, 4.13.

***N*^{3}-Benzoyl-2′-*O*-methyl-5′-*O*-(dimethoxytrityl)uridine 3′-*O*-(2-cyanoethyl *N*,*N*-diisopropylphosphoramidite) (11).** Compound **10** (2.00 g, 3 mmol) was rendered anhydrous by coevaporations three times each with dry pyridine, dry toluene, and dry CH_2Cl_2, and finally dissolved in dry CH_2Cl_2 (30 mL). To the solution were added diisopropylethylamine (2.1 mL, 12 mmol) and (2-cyanoethoxy)(*N*,*N*-diisopropylamino)phosphine (0.98 mL, 4.5 mmol). The mixture was stirred at room temperature for 1 h and then diluted with CH_2Cl_2. The CH_2Cl_2 solution was washed two times each with saturated $NaHCO_3$ (150 mL) and water. The organic phase was collected, dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The residue was chromatographed on a column of silica gel (60 g) eluted with hexane−EtOAc−pyridine (70:30:1, v/v/v) to give **11** (2.22 g, 85%): ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.21 (12H, m), 2.47 and 2.65 (2H, t, J = 5.9 and 6.3 Hz), 3.55 and 3.56 (3H, s), 3.81 and 3.82 (6H, s), 3.45−3.95 (6H, m), 4.00 (1H, m), 4.23 (1H, m), 4.57 and 4.76 (1H, m), 5.27 and 5.32 (1H, d, J = 8.3 Hz), 5.92 and 5.97 (1H, d, J = 2.3 Hz), 6.83−6.90 (4H, m), 7.25−8.63 (14H, m), 8.17 and 8.26 (1H, d, J = 8.3 Hz); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 20.20, 20.11, 20.13, 24.19, 24.30, 24.37, 24.46, 42.84, 43.04, 54.90, 57.38, 57.70, 57.86, 58.01, 58.13, 58.40, 60.00, 60.54, 68.93, 69.22, 69.44, 81.85, 82.43, 83.49, 86.63, 86.83, 87.12, 101.49, 101.60, 112.99, 117.50, 117.59, 123.43, 124.99, 126.95, 127.73, 128.01, 128.72, 128.82, 130.01, 130.31, 131.18, 134.61, 134.72, 134.81, 134.93, 135.67, 137.47, 139.69, 143.90,144.13, 148.95, 149.45, 158.49, 161.92, 161.99, 168.73; ^{31}P NMR (109.25 MHz, $CDCl_3$) δ 151.10, 150.98 (43:57). Anal. Calcd for $C_{47}H_{53}N_4O_{10}P{\cdot}^1/_4H_2O$: C, 64.92; H, 6.20; N, 6.45. Found: C, 65.05; H, 6.38; N, 6.49.

Synthesis of Fully Protected Diribonucleotide Derivative 13. A mixture of **12** (205 mg, 0.3 mmol) and 1*H*-tetrazole (95 mg, 1.35 mmol) was rendered anhydrous by coevaporations three times each with dry toluene and dry pyridine and with dry acetonitrile. The mixture was mixed with **11** (390 mg, 0.45 mmol) and dissolved in dry acetonitrile (3 mL). After being stirred under argon atmosphere at room temperature for 2.5 h, the mixture was treated with a solution of iodine (343 mg, 1.35 mmol) in pyridine−water (3 mL, 98:2, v/v). The resulting mixture was stirred at room temperature for 40 min, and then saturated Na_2SO_3 was added to reduce the excess iodine. The mixture was diluted with CH_2Cl_2, and the CH_2Cl_2 solution was washed two times each with saturated $NaHCO_3$ (100 mL) and with water. The organic phase was collected, dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The residue was chromatographed on a column of silica gel (20 g) eluted with hexane−EtOAc−pyridine (45:55:0.5, v/v/v) to give **13** (449 mg, 85%): ^{31}P NMR (109.25 MHz, $CDCl_3$) δ −1.92, −1.99 (61:39). Anal. Calcd for $C_{79}H_{67}N_8O_{19}P$: C, 64.84; H, 4.62; N, 7.66. Found: C, 65.27; H, 4.86; N, 6.87.

Synthesis of 5′-Unprotected Diribonucleotide Derivative 14. To a solution of **13** (283 mg, 0.19 mmol) in CH_2Cl_2 was added trifluoroacetic acid (95 μL). After being stirred at room temperature for 20 min, the mixture was diluted with CH_2Cl_2 and the CH_2Cl_2 solution was washed two times with saturated $NaHCO_3$ (150 mL) and with water. The organic phase was collected, dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The residue was chromatographed on a column of silica gel (25 g) eluted with CH_2Cl_2−MeOH (100:0−98:2, v/v) to give **14** (214 mg, 97%): ^{1}H NMR (270 MHz, $CDCl_3$) δ 2.71−2.79 (2H, m), 3.43 and 3.51 (3H, s), 3.76−3.82 (2H, m), 4.19−4.36 (4H, m), 4.58 (2H, m), 4.72 (1H, m), 5.00−5.02 (1H, m), 5.75−5.85 (1H, m), 5.86−5.89 (1H, m), 6.08−6.12 (1H, m), 6.20 and 6.28 (1H, t, J = 5.3 Hz), 6.58 (1H, d, J = 5.0 Hz), 7.34−8.03 (26H, m), 8.25 and 8.55 (1H, s), 8.67 and 8.69 (1H, s); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 19.39, 19.50, 19.60, 29.60, 53.39, 58.60, 59.73, 62.64, 62.72, 70.84, 71.02, 73.93, 74.25, 81.38, 81.51, 81.80, 83.58, 86.58, 86.74, 88.18, 102.37, 102.43, 116.48, 127.35, 128.09, 128.30, 128.54, 128.57, 128.79, 129.13, 129.38, 129.72, 129.76, 129.80, 130.42, 130.48, 131.21, 131.25, 133.19, 133.78, 133.93, 135.11, 140.45, 141.20, 143.22, 143.56, 149.31, 151.95, 152.56, 152.69, 152.83, 161.98,

165.05, 168.50, 168.59, 172.31, 172.36; ^{31}P NMR (109.25 MHz, $CDCl_3$) δ −1.74, −1.80 (50:44). Anal. Calcd for $C_{58}H_{49}N_8O_{17}P$: C, 60.00; H, 4.25; N, 9.65. Found: C, 60.05; H, 4.58; N, 9.30.

Synthesis of Fully Protected Triribonucleotide Derivative 15. A mixture of **14** (407 mg, 0.35 mmol) and 1*H*-tetrazole (245 mg, 3.5 mmol) was rendered anhydrous by coevaporations three times each with dry toluene and dry pyridine and with dry acetonitrile. The mixture was mixed with **7** (741 mg, 0.7 mmol) and dissolved in dry acetonitrile (3.5 mL). After being stirred under argon atmosphere at room temperature for 1 h, the mixture was treated with a solution of iodine (533 mg, 2.1 mmol) in pyridine−water (3.5 mL, 98:2, v/v). The resulting mixture was stirred at room temperature for 30 min, and then saturated Na_2SO_3 was added to reduce the excess iodine. The mixture was diluted with CH_2Cl_2, and the CH_2Cl_2 solution was washed two times each with saturated $NaHCO_3$ (100 mL) and water. The organic phase was collected, dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The residue was chromatographed on a column of silica gel (38 g) eluted with hexane−EtOAc−pyridine (10:90:0.5, v/v/v) to give **15** (639 mg, 86%): ^{31}P NMR (109.25 MHz, $CDCl_3$) δ -1.39, −1.47, −1.56, −1.77, −1.97, −2.08, −2.27. Anal. Calcd for $C_{113}H_{100}N_{14}O_{26}P_2$: C, 63.65; H, 4.73; N, 9.22. Found: C, 63.36; H, 4.63; N, 8.99.

Synthesis of 5′-Unprotected Triribonucleotide Derivative 16. Compound **15** (640 mg, 0.3 mmol) was dissolved in a 0.5 M solution of $ZnBr_2$ in CH_2Cl_2 −2-propanol (12 mL, 85:15, v/v) at −5 °C. After being stirred at −5 °C for 2 h, the mixture was diluted with CH_2Cl_2 and the CH_2Cl_2 solution was washed three times with water. The organic phase was collected, dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The residue was chromatographed on a column of silica gel (28 g) eluted with CH_2Cl_2−MeOH (100:0−98:2, v/v) to give **16** (463 mg, 84%): ^{1}H NMR (270 MHz, $CDCl_3$) δ 2.74 (4H, m), 3.32−3.51 (6H, m), 3.77 (3H, m), 4.20−4.43 (10H, m), 4.58 (2H, m), 4.72 (1H, m), 4.78 (1H, m), 5.07 (1H, m), 5.29 (1H, m), 5.81−5.95 (3H, m), 6.15 (1H, m), 6.28 (1H, m), 6.56 (1H, m), 6.78−7.93 (28H, m), 8.48−8.69 (2H, m); ^{31}P NMR (109.25 MHz, $CDCl_3$) δ −1.46, −1.56, −1.63, −1.85, −2.01. Anal. Calcd for $C_{92}H_{82}N_{14}O_{24}P_2 \cdot 2H_2O$: C, 61.05; H, 4.52; N, 10.72. Found: C, 60.54; H, 5.46; N, 9.95.

Synthesis of 5′-Phosphorylated Triribonucleotide Derivative 17. A mixture of **16** (440 mg, 0.24 mmol), cyclohexylammonium *S*,*S*-diphenyl phosphorodithioate (108 mg, 0.29 mmol), and 1*H*-tetrazole (67 mg, 0.96 mmol) was rendered anhydrous by coevaporations three times with dry pyridine and finally dissolved in dry pyridine (2.4 mL). To the mixture was added isodurenedisulfonyl dichloride (DDS, 159 mg, 0.48 mmol), and the mixture was stirred at room temperature for 30 min. The mixture was diluted with CH_2Cl_2, and the CH_2Cl_2 solution was washed two times with saturated $NaHCO_3$ (50 mL) and with water. The organic phase was collected, dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The residue was chromatographed on a column of silica gel (28 g) eluted with CH_2Cl_2−MeOH (100:0−98:2, v/v) to give **17** (423 mg, 84%): ^{1}H NMR (270 MHz, $CDCl_3$) δ 2.56−2.75 (4H, m), 3.36, 3.41, 3.42, 3.43, 3.46, 3.47, 3.49, 3.50 (6H, s), 3.75 (3H, m), 4.20−4.56 (10H, m), 4.71 (1H, m), 4.82 (1H, m), 5,06 (1H, m), 5.25 (1H, m), 5.33 (1H, m), 5.75−5.87 (3H, m), 6.05 (2H, m), 6.14 (1H, m), 6.26 (1H, m), 6.57 (1H, d, J = 5.3 Hz), 6.79, 6.95, 7.21−7.93 (51 H, m), 8.47, 8.50, 8.51, 8.57, 8.67, 8.68, 8.69, 8.693 (2H, s); ^{13}C NMR (60.85 MHz, $CDCl_3$) δ 19.34, 19.63, 53.34, 54.96, 58.58, 58.64, 58.78, 62.61, 64.71, 65.52, 66.17, 66.88, 70.73, 73.66, 73.82, 74.93, 80.23, 80.56, 81.22, 81.62, 86.22, 86.45, 88.64, 89.18, 102.59, 109.60, 111.66, 112.90, 116.50, 116.68, 116.75, 116.86, 121.26, 125.18, 125.30, 125.41, 126.63, 127.40, 127.64, 127.96, 128.19, 128.37, 128.61, 128.99, 129.18, 129.36, 129.60, 129.94, 130.28, 130.96, 131.00, 132.96, 133.62, 135.00, 135.08, 136.86, 139.05, 140.02, 140.25, 140.50, 140.58, 143.49, 143.65, 143.90, 144.87, 147.98, 148.34, 148.39, 148.93, 149.02, 151.81, 151.97, 152.11, 152.28, 152.63, 152.96, 153.91, 158.04, 161.62, 164.89, 165.07, 168.36, 168.41, 169.63, 172.09; ^{31}P NMR (109.25 MHz, $CDCl_3$) δ −1.63, −1.69, −1.73, −1.93, −2.08, −2.16, −2.47, 52.08, 52.33. Anal. Calcd for $C_{104}H_{91}N_{14}O_{27}P_3S_2$: C, 58.75; H, 4.31; N, 9.23; S, 3.02. Found: C, 58.96; H, 4.56; N, 8.96; S, 3.83.

Synthesis of 5′-Pyrophosphorylated Triribonucleotide Derivative 20. Compound **17** (63 mg, 0.03 mmol) was rendered anhydrous by coevaporations three times with dry pyridine and dissolved in a solution of 5 M pyridinium phosphinate (0.30 mL)−triethylamine (0.105 mL, 0.75 mmol). After being stirred at room temperature for 1 h, the mixture was diluted with CH_2Cl_2 (25 mL). The CH_2Cl_2 solution was washed 15 times with 0.5 M triethylammonium bicarbonate (25 mL). Every aqueous layer was back-extracted with CH_2Cl_2−pyridine (25 mL, 4:1, v/v) put in another separatory funnel. After extraction was performed, the two CH_2Cl_2 layers were combined, dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The residue was coevaporated with six times with dry pyridine and then dissolved in a solution of tributylammonium phosphate (1.2 mmol) in dry pyridine (2 mL). The resulting solution was stirred at room temperature for 5 min, and then iodine (152 mg, 0.6 mmol) was added. After being stirred for 2 h, the mixture was diluted with pyridine−water (50 mL, 1:3, v/v) and washed six times with ether (50 mL). Each ethereal extract was back-extracted with pyridine−water (50 mL, 1:2, v/v) in another separatory funnel. The aqueous laylers were combined, dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The residue was treated with 25% ammonia−pyridine (50 mL, 9:1, v/v) at room temperature for 60 h. The solution was diluted with water, washed three times with ether, and evaporated under reduced pressure. The residue was chromatographed on Whatman 3MM papers developed with concd ammonia−2-propanol−H_2O, 7:1:2, v/v/v). The band of R_f 0.2 was cut and eluted with water. Lyophilization of the eluate gave **20** (940 $A_{257.5}$, 73%): ^{31}P NMR (109.25 MHz, D_2O) δ −9.49, −4.65 (5′-pyrophosphate), −0.20 (internucleotidic phosphate); R_f 0.2. UV (H_2O) λ_{max} 257.5 nm.

Synthesis of the 5′-terminal region of U1RNA. Compound **34** (0.022 mmol) obtained in the above experiment was dissolved in water, and the solution was applied to a column of Dowex 50WX8 (pyridinium form, 4 mL), and elution was performed with pyridine−water (50 mL, 1:1, v/v). The eluate was evaporated under reduced pressure. The residue was lyophilized to give a white powder (pyridinium salt of **20**). This compound was dissolved in a 0.01 mM solution of TMG capping unit **32** (2.2 mL, 0.022 mmol, the one obtained by method B) in dry DMF . The mixture was stirred at room temperature for 3 days, and 80% acetic acid (2 mL) was added in this solution. After being stirred at room temperature for 2 h, the mixture was diluted with water and washed three times with ether (20 mL). The aqueous phase was evaporated under reduced pressure. The residue was purified with paper electrophoresis (0.2 M ammonium acetate buffer, 600 V, 2 h) on four sheets of Whatman 3MM papers (14 cm × 46 cm). The band of Mob. 0.7−0.8 (relative to ppG) was cut and eluted with water. The eluate was lyophilized and dissolved in water. Semipreparative reverse phase HPLC gave **34** (373 $A_{258.5}$, 40%): ^{1}H NMR (270 MHz, D_2O) δ 2.94 (6H, s $N(CH_3)_2$), 3.41 (3H, s, 2′-OCH_3 of Am or Um), 3.52 (3H, s, 2′-OCH_3 of Am or Um), 4.04 (3H, s, N^7-CH_3 of TMG), 3.89−4.83 (m, 2′,3′, 4′, 5′$_a$ and 5′$_b$-H of TMG, Am, Um, A), 5.62 (1H, d, $J_{5,6}$ = 8.3 Hz, H-6 of Um), 8.02, 8.13, 8.24, 8.36 (4H, s, 2-H of Am or A, 8-H of Am or A); ^{31}P NMR (109.25 MHz, D_2O) δ −24.66 (β-phosphate of triphosphate, dd, $J_{\alpha,\beta \text{ or } \beta,\gamma}$ = 17.0 or 18.3 Hz), −13.35, −13.18 (α,γ-phosphate of triphosphate bridge), −3.12, −2.96 (internucleotidic phosphate); UV (H_2O) λ_{max} 258.5 nm (hypochromicity 13.3%) $\epsilon_{258.5\ nm} = 42.4 \times 10^3$.

Enzymatic Treatment of $m_3^{2,2,7}G^{5'}$pppAmpUmpA (34) with Snake Venom Phosphodiesterase (SVPD) and Alkaline Phosphatase (AP). Compound **34** (2.0 A at its λ_{max}) was dissolved in 0.05 M Tris-HCl (pH 8.0, 400 μL) containing 0.02 M $ZnCl_2$ and 20 μL of SVPD (20 units, 1.0 unit /μL) in glycerin-water (1:1, v/v) was added. The resulting mixture was incubated at 37 °C for 4 h and heated at 90 °C for 2 min. A 20 μL volume of AP (20 unit, 1.0 unit/μL) was added in the resulting mixture. After being incubated for 3 h, the mixture was heated at 90 °C for 2 min. The mixture was analyzed by reverse-phase HPLC.

Enzymatic Treatment of $m_3^{2,2,7}G^{5'}$pppAmpUmpA (34) with Nuclease P1. Compound **34** (2.0 $A_{258.5}$) was dissolved in 0.05 M AcOH−AcONa (pH 5.3, 400 μL) containing 0.1 mM $ZnCl_2$ and 6 μL of nuclease P1 (6 units, 1.0 unit/μL) in glycerine−water (1:1, v/v) was added. The resulting mixture was incubated at 50 °C for 5 h and then heated at 90 °C for 2 min. The mixture was analyzed by reverse-phase HPLC, and the peak of $m_3^{2,2,7}G^{5'}$pppAm was collected. The fractions collected were freeze-dried two times and used in the next enzyme reaction.

Enzymatic Treatment of $m_3^{2,2,7}G^{5'}$pppAm with Snake Venom Phosphodiesterase and Alkaline Phosphatase. $m_3^{2,2,7}G^{5'}$pppAm (1.0 A_{260}) collected was dissolved in 0.05 M Tris-HCl (pH 8.0, 200 μL) containing 0.02 M $ZnCl_2$, and 10 μL of SVPD (10 units, 1.0 unit/μL) in glycerine−water (1:1, v/v) was added. The resulting mixture was incubated at 37 °C for 4 h and heated at 90 °C for 2 min. To the resulting mixture was added 20 μL of AP (20 unit, 1.0 unit/μL). After being incubated for 3 h, the mixture was heated at 90 °C for 2 min. The mixture was analyzed by reverse-phase HPLC.

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.

Supporting Information Available: Copies of ^{1}H and ^{13}C NMR spectra (40 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, can be ordered from the ACS; see any current masthead page for ordering information.

JO952263V