JOC*Article*

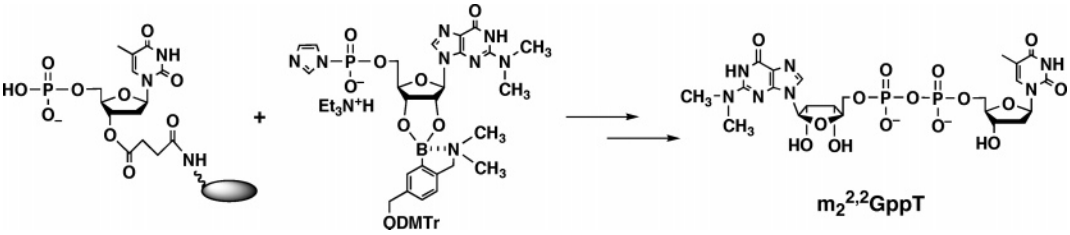
Chemically Stabilized Phenylboranylidene Groups Having a Dimethoxytrityl Group as a Colorimetrically Detectable Protecting Group Designed for *cis*-1,2-Diol Functions of Ribonucleosides in the Solid-Phase Synthesis of $m_2^{2,2}G^{5'}ppT$

Mitsuo Sekine,*[,†,‡] Morihiro Aoyagi,[†] Masatoshi Ushioda,[†] Akihiro Ohkubo,[†] and Kohji Seio[‡,§]

Department of Life Science, Tokyo Institute of Technology, Division of Collaborative Research for Bioscience and Biotechnology, Frontier Collaborative Research Center, and CREST, JST (Japan Science and Technology Agency), Nagatsuta, Midoriku, Yokohama 226-8501, Japan

msekine@bio.titech.ac.jp

Received June 14, 2005



To not only improve the inherently poor stability of the phenylboranylidene group as a protecting group of the 2′,3′-*cis*-diol function of ribonucleosides but also introduce a colorimetrically detectable function into its mother structure, various 2-[(dialkylamino)methyl]phenylboronic acid derivatives having a [(4,4′-dimethoxytrityl)oxy]methyl group were synthesized. The reaction of uridine with these substituted phenylboronic acid derivatives gave the corresponding 2′,3′-*O*-phenylboranylideneuridine derivatives. The stability of these phenylboranylidene groups was examined. As a result, it was shown that the steric hindrance around the amino group greatly influenced the stability of the 2-substituted phenylboranylidene groups. The 2-aminomethyl-5-[[(4,4′-dimethoxytrityl)oxy]-methyl]phenylboranylidene group was the most stable. Its 2-dimethylamino counterpart, the 2-[(dimethylamino)methyl]-5-[[(4,4′-dimethoxytrityl)oxy]methyl]phenylboranylidene group, was the second most stable. When the most and second stable protecting groups were applied to the synthesis of $m_2^{2,2}G^{5'}ppT$ on controlled pore glass, the second stable protecting group showed the best result. The use of this DMTr-containing protecting group enabled us to estimate colorimetrically the amount of the $m_2^{2,2}G$ residue that was incorporated into the reactive site of the pT-loaded CPG resin.

Introduction

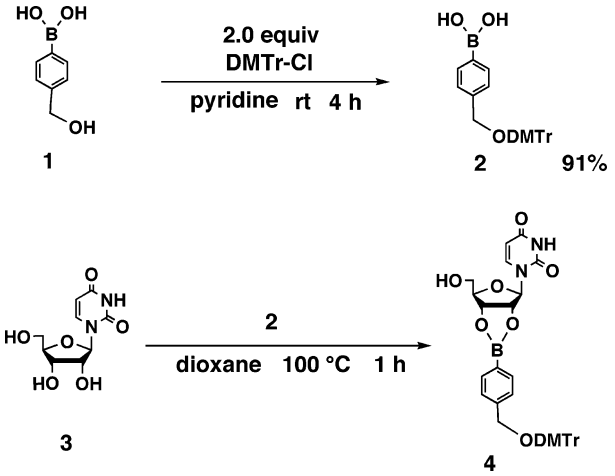
In our continuous studies[1−3] on the chemical synthesis of 2,2-dimethylguanosine- ($m_2^{2,2}G$) and 2,2,7-trimethylguanosine-cap ($m_2^{2,2,7}G$) structures which play an important role in transport of these capped RNAs between the cytoplasm and the nucleus in cells,[1,2] a variety of capping reagents to construct the cap structures at the 5′-terminal site of oligoribonucleotides have been reported.[3−7] In an attempt to improve the solubility of capping reagents in organic solvents, we have used the lipophilic phenylboranylidene group as the protecting group for the 2′,3′-*cis*-diol function of $m_2^{2,2}G$ in the solid-phase synthesis of $m_2^{2,2}G$-capped RNAs.[8] However, this protecting group is so labile that in aqueous solution the cyclic phenylboronate ester linkages are easily hydrolyzed. If the phenylboranylidene group can not only be stabilized but also substituted with a substituent

† Tokyo Institute of Technology.
‡ CREST, JST (Japan Science and Technology Agency).
§ Frontier Collaborative Research Center.

(1) Huber, J.; Cronshagen, U.; Kadokura, M.; Marshallsay, C.; Wada, T.; Sekine, M.; Lührmann, R. *EMBO J.* **1998**, *17*, 4114−26.
(2) Fisher, U.; Sumpter, V.; Sekine, M.; Satoh, T.; Lührmann, R. *EMBO J.* **1993**, *12*, 573−583.
(3) Kadokura, M.; Wada, T.; Seio, K.; Moriguchi, T.; Huber, J.; Lührmann, R.; Sekine, M. *Tetrahedron Lett.* **2001**, *42*, 8853−8856.
(4) Sekine, M.; Kadokura, M.; Satoh, T.; Seio, K.; Wada, T. *J. Org. Chem.* **1996**, *61*, 4412−4422.
(5) Sekine, M.; Ushioda, M.; Wada, T.; Seio, K. *Tetrahedron Lett.* **2003**, *44*, 1703−1707.
(6) Uahioda, M.; Kadokura, M.; Moriguchi, T.; Kobori, A.; Aoyagi, M.; Seio, K.; Sekine, M. *Helv. Chim. Acta* **2002**, *85*, 2930−2945.
(7) Aoyagi, M.; Ushioda, M.; Seio, K.; Sekine, M. *Nucleic Acids Res. Supplement* **2003**, *3*, 149−150.

10.1021/jo051202m CCC: $30.25
Published on Web 09/10/2005

SCHEME 1. Synthesis of 2′-3′-*O*-[4-(4,4′-Dimethoxytrityl)oxyphenyl]boranylideneuridine (4)



having a dye precursor such as 4,4′-dimethoxytrityl (DMTr) by chemical modification, such a modified phenylboranylidene group would provide new insight into the solid-phase synthesis of capped RNAs. In particular, if the efficiency of a reaction carried out on polymer supports can be easily estimated by a colorimetric method, the solid-phase synthesis becomes more practical. With this in mind, we studied modified phenylboranylidene groups having the DMTr group to realize this idea.

In this paper, we report comprehensive studies of modified phenylboranylidene groups with a DMTr group and a dialkylamino substituent that controls their stability.

Results and Discussion

Synthesis of 4-[(4,4′-Dimethoxytrityl)oxymethyl]-phenylboronic Acid. To examine the stability of a variety of 2′,3′-*O*-phenylboranylidene ribonucleoside derivatives, the synthesis of a phenylboronic acid derivative **2** having a DMTr group was carried out by the reaction of 4-(hydroxylmethyl)phenylboronic acid (**1**) with 4,4′-dimethoxytrityl chloride (DMTrCl) in pyridine, as shown in Scheme 1.

It turned out that this reaction required at least 2 equiv of DMTrCl. This result suggested that DMTrCl reacted competitively with both hydroxyl groups of the hydroxylmethyl substituent and the boronic acid. One of the two hydroxyl groups on the boronic acid residue of **1** was reactive under the conditions used so that 2 equiv of DMTrCl was consumed. During the workup involving addition of methanol followed by extraction, the DMTr ester was automatically hydrolyzed to give the desired compound **2** in 91% yield after silica gel column chromatography. It is known that phenylboronic acid exhibits a only weakly acidic property of pK_a 8.8.[10] As suggested by this property, it was confirmed that the DMTr group of **2** was sufficiently stable on storage or in organic solvents such as CH_2Cl_2 and dioxane.

To study the stability of the 4-[[(4,4′-dimethoxytrityl)-oxy]methyl]phenylboranylidene group when it was introduced into the 2′,3′-*cis*-diol function of nucleosides, the reaction of uridine (**3**) with **2** was carried out. As a result, the reaction of uridine with 1 equiv of **2** in dioxane at 100 °C for 1 h gave quantitatively the 2′,3′-*O*-boronated product **4**, which was determined by the ^{1}H NMR analysis of the reaction mixture. However, this product proved to be unstable like the usual unsubstituted 2′,3′-*O*-phenylboronated species. To evaluate quantitatively the stability of the product **4** compared with those of the other derivatives described later, compound **4** was dissolved in DMSO-d_6 and 200 equiv of water was added. The time course of the hydrolysis of **4** was analyzed by ^{1}H NMR. The 1′-proton of **4** appeared at 5.9 ppm while that of uridine (**3**) was observed at 5.75 ppm. As shown in Figure 1, 40% of **4** was hydrolyzed after 5 min and the hydrolysis was completed after 1 h.

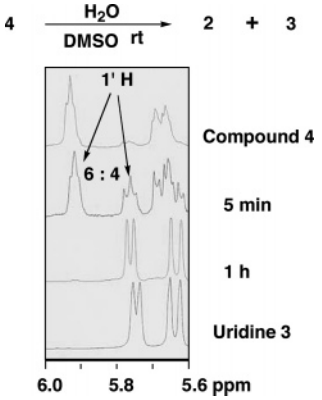


FIGURE 1. Time course of the hydrolysis of **4** by addition of water in DMSO.

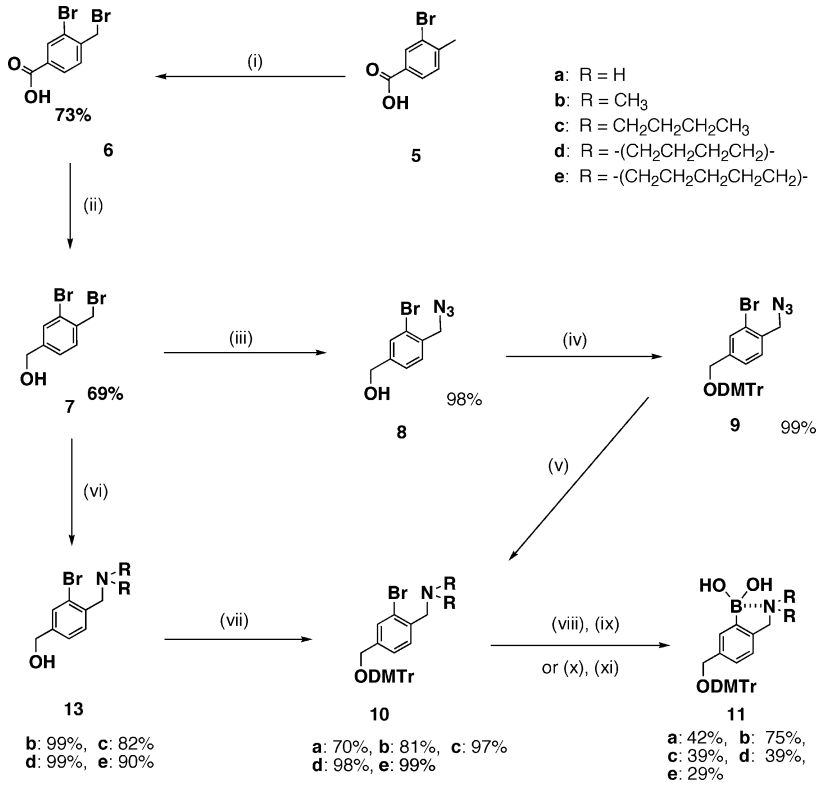
Although the stability of **4** was very poor, it was confirmed that the solubility of **4** increased dramatically in organic solvents so that **4** was freely soluble even in $CHCl_3$.

Synthesis and Properties of Modified Phenylboronic Acid Derivatives. It is known that phenyboronic acid diesters can be stabilized by addition of an alkylamino group at the ortho position due to intramolecular coordination of the amino group with the boron atom.[11−14] Therefore, we designed several phenylboronic acid derivatives substituted with an amino group and a DMTr-oxymethyl group at the ortho and meta positions, respectively, as the precursors of protecting groups. We chose 3-bromo-4-methylbenzoic acid (**5**) as a common starting material because of its easy accessibility to the synthesis of these modified phenylboronic acid derivatives. To examine the substituent effect of alkylamino groups at the ortho position, various [2-alkylamino-5-[(4,4′-dimethoxytrityl)oxy]methyl]phenylboronic acid derivatives (**11a**−**e**) were synthesized, as shown in Scheme 2.

Reaction of **5** with NBS in the presence of AIBN under reflux in CCl_4 gave the dibromo derivative **6** in 73% yield. For the synthesis of **11a**, compound **6** was treated with

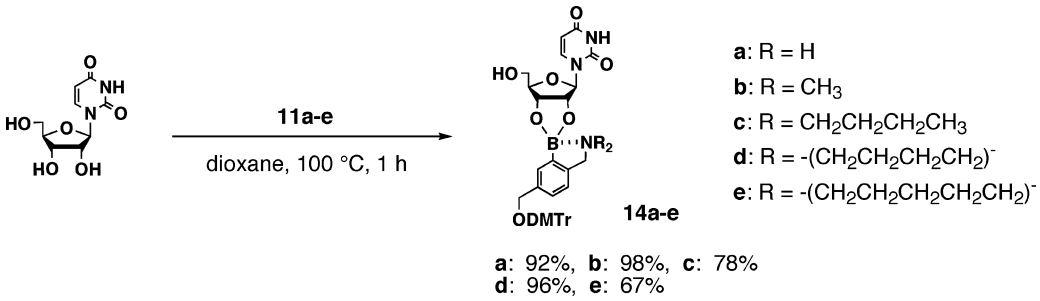
(8) Iwase, R.; Sekine, M.; Tokumoto, Y.; Ohshima, Y.; Hata, T. *Nucleic Acids Res.* **1989**, *17*, 8979−8989.
(9) Sekine, M.; Hata, T. *Curr. Org. Chem.* **1999**, *3*, 25−66.
(10) Yan, J.; Springsteen, G.; Deeter, S.; Wang, B. *Tetrahedron* **2004**, *60*, 11205−11209.
(11) Burgemeister, T.; Grobe-Einsler, R.; Grotstollen, R.; Mannschreck, A.; Wulff, G. *Chem. Ber.* **1981**, *114*, 3403−3441.
(12) Lauer, M.; Wulff, G. *J. Organomet. Chem.* **1983**, *256*, 1−9.
(13) Wulff, G.; Stellbrink, H. *Recl. Trav. Chim. Pays-Bas* **1990**, *109*, 216−221.
(14) Wulff, von G.; Lauer, M.; Böhnke, H. *Angew. Chem.* **1984**, *96*, 714−716.

SCHEME 2. Synthesis of 2-(Dialkylaminomethyl)-5-[[(4,4′-dimethoxytrityl)oxy]methyl]phenylboronic Acid Derivatives[a]



[a] Reagents and conditions: (i) NBS (1.05 equiv), AIBN (cat.), CCl_4, reflux, 1 h; (ii) 1 M BH_3 THF (2 equiv), THF, rt, 3 h; (iii) NaN_3 (5.0 equiv), DMF, 90 °C, 12 h; (iv) DMTrCl (1.2 equiv), pyridine, rt, 1.5 h; (v) PPh_3 (1.5 equiv), THF, rt, 1 h; (vi) HNR_2 **12** (4.0 equiv), THF, rt, 2 h; (vii) DMTrCl, pyridine, rt, 4 h, (viii) *n*-BuLi (2.1 equiv), THF, −78 °C, 1 h; (ix) $B(OCH_3)_3$ (5.0 equiv), THF, −78 °C, 1.5 h then H_2O; (x) *n*-BuLi (1.1 equiv), THF, −78 °C, 30 min; (xi) $B(OCH_3)_3$ (5.0 equiv), THF, −78 °C, 1 h, then H_2O.

SCHEME 3. Synthesis of Substituted Phenylboronate Esters of Uridine



BH_3 THF complex to give the alcohol **7** in 69% yield. This alcohol was further converted to the azide **8** in 98% yield, which, in turn, was allowed to react with DMTrCl to give the ether **9** in 99% yield. Treatment of **9** with triphenylphosphine followed by hydrolysis of the resulting iminophosphorane intermediate[15] gave **10a** in 70% yield.

For the synthesis of **11b**−**e**, the reactions of **7** with alkylamines **12b**−**e** were carried out to give benzylamine derivatives **13b**−**e** in high yields (82%−99%). Further dimethoxytritylation of **13b**−**e** gave the DMTr ether derivatives **10b**−**e** in 81−99% yields. Transmetalation of **10a**−**e** with *n*-butyllithium followed by treatment with trimethyl boronate gave the desired products **11a**−**e** in 29−75% yields.

Synthesis and Stability of Boronated Uridine Derivatives. For the 2′,3′-*O*-boronation of uridine with substituted phenylboronic acids **11a**−**e**, we employed conditions similar to that described for the synthesis of **4**. In all reactions, the 2′,3′-*O*-cyclic boronate esters **14a**−**e** were obtained as white powders by precipitation into diisopropyl ether−ethyl acetate (9:1, v/v) (Scheme 3). To examine the stability of these boronated ester derivatives in DMSO in the presence of 200 equiv of water, they were dissolved in DMSO-d_6 and their ^1H NMR spectra were measured in NMR tubes. The chemical shift of the 1′-proton of **14a**−**e** changed downfield to a degree of 0.15 ppm upon hydrolysis of the boronate esters, as observed in the previous experiment using **4**. The results of the ratio of hydrolysis are summarized in Figure 2.

It was found that the 2-unsbstituted phenylboranylidene group of **14c** was the most unstable. Interestingly,

(15) Gryaznov, S.; Letsinger, R. *Tetrahedron Lett.* **1992**, *33*, 4127−4128.

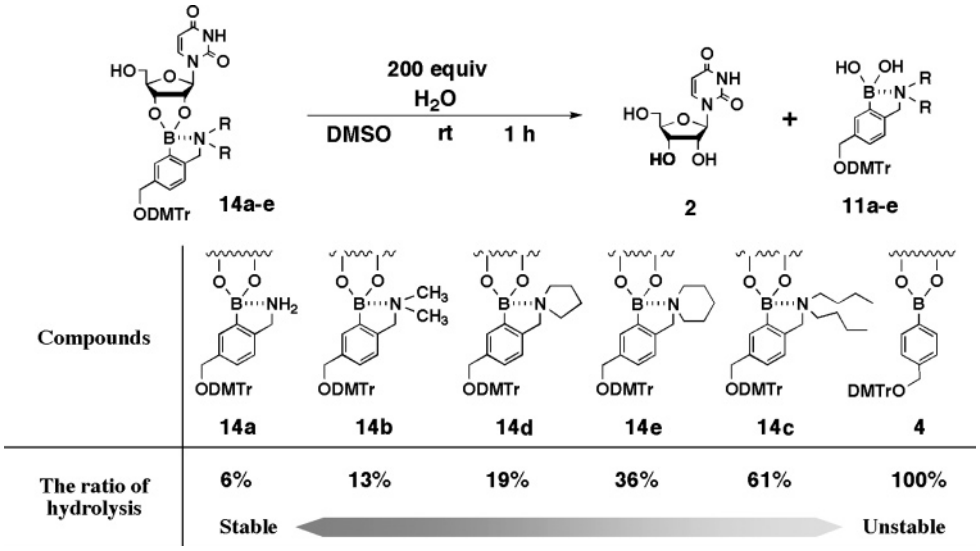
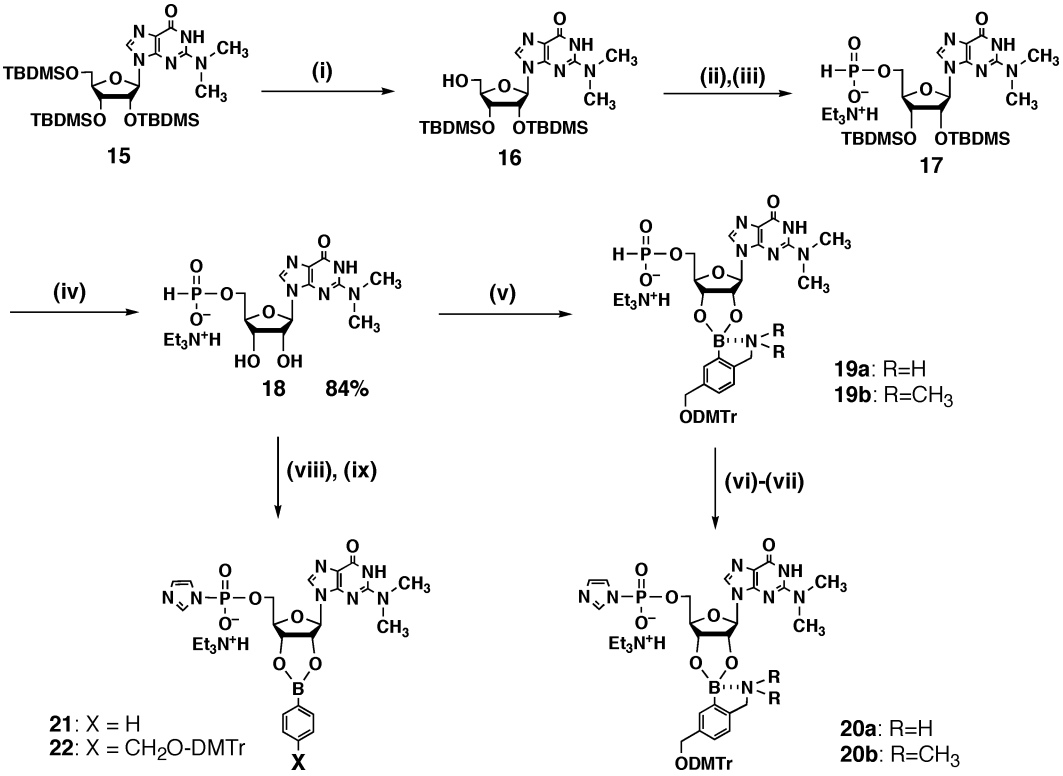


FIGURE 2. Comparative stability of substituted phenylboronic esters of uridine in DMSO in the presence of water.

SCHEME 4. Synthesis of *N,N*-Dimethylguanosine 5′-Phosphorimidazolidate Derivative Having Substituted Phenylboronate Functions via *N,N*-Dimethylguanosine 5′-Phosphonate 18[a]

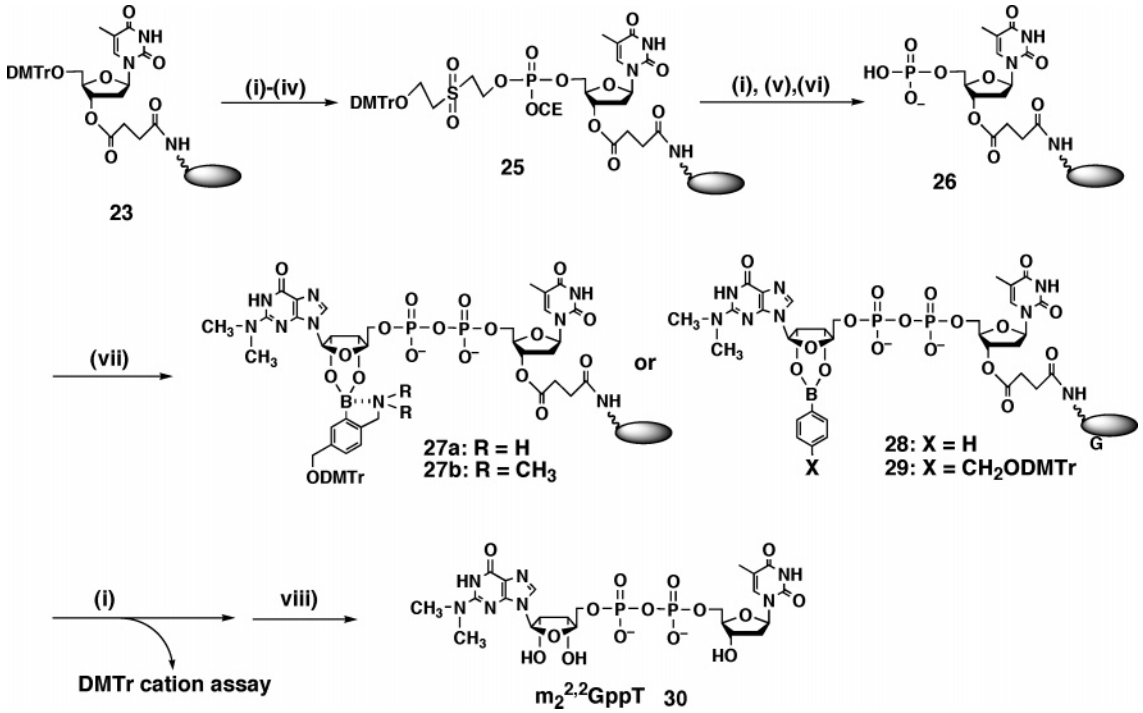


[a] Reagents and conditions: (i) AcOH−THF (3:1:1, v/v/v), 80 °C, 28 h, 73%; (ii) $(PhO)_2P(O)H$ (7 equiv), pyridine, rt, 4 h; (iii) Et_3N-H_2O (1:1, v/v), rt, 20 min, 87% from **16**; (iv) 80% formic acid, rt, 46 h, 84%; (v) **11a** or **11b** (1.2 equiv), dioxane, 100 °C, 1 h, 81% (**19a**) and 98% (**19b**); (vi) TMS-Im (4.0 equiv), Et_3N (4.0 equiv), $MeCN-CCl_4$ (1:1, v/v), rt, 30 min; (vii) MeOH, rt, 10 min, 91% (**20a** from **19a**) and 99% (**20b** from **19b**); (viii) **2** (1.0 equiv) or **4** (1.2 equiv), dioxane, 90 °C, 1 h; (ix) TMS-Im (4.0 equiv), Et_3N (4.0 equiv), $MeCN-CCl_4$ (1:1, v/v), rt, 30 min.

the ratios of the boronate ester and the hydrolyzed product, uridine, were unchanged 5 min and 1 h after water was added to a DMSO solution of **14a**−**e**. The order of the stability of these boronated esters is **14a** > **14b** > **14d** > **14e** > **14c** > **4**. The steric factor of the 2-substituent seems to be essential for stabilization of the boronate ester. The primary amino group was found to be the most effective for the stabilization of the boronate ring, but the hydrolysis occurred to a degree of 6% in the case of **14a**.

Synthesis of 2′,3′-*O*-Boronated 2,2-Dimethylguanosine Capping Units. As part of our continuing studies on the synthesis of capped RNAs, we needed 2,2-dimethylguanosine ($m_2^{2,2}G$)-capped DNAs ($m_2^{2,2}G^{5'}ppDNA$),

SCHEME 5. Soli-Phase Synthesis of 2,2-Dimethylguanosine-5′-yl Thymidine P¹,P²-Diphosphate



[a] Reagents and conditions: (i) 1% TFA, CH_2Cl_2, 15 s × 3; (ii) $DMrTr(CH_2)_2SO_2OP(OCH_2CH_2CN)(NiPr_2)$ (**24**) (20 equiv), 1*H*-tetrazole (80 equiv), 5 min; (iii) 0.15 M I_2 solution in THF/pyridine/H_2O (10:10:1, v/v/v), 15 s × 3; (iv) 0.12 M DMAP in pyridine−Ac_2O (1 mL, 1:9, v/v), 2 min; (v) BSA−pyridine (400 μL, 1:1, v/v), 20 min; (vi) addition of DBU (80 μL), 10 min: (vii) **20a**, **20b**, **21**, or **22** (20 μmol), pyridine, rt, 24 h; (viii) 29% aq NH_3, rt, 1 h.

i.e., one phosphate group-lacking DNA derivative of the precursor of 2,2,7-trimethylguanosine-capped RNA for various studies to clarify the mechanism of membrane transport of capped RNAs between the cytoplasm and the nucleus.[1,2] Therefore, we decided to synthesize $m_2^{2,2}G$-capping reagents **20a** and **20b** for these studies. The synthesis of **20a** and **20b** is shown in Scheme 4. The key intermediate **15** was synthesized by reductive methylation of 2′,3′,5′-*O*-tris(*tert*-butyldimethylsilyl)guanosine with paraformaldehyde and $NaBH_3CN$ in the presence of acetic acid.[3] Selective desilylation of **15** with acetic acid−THF−H_2O gave the 5′-unprotected derivative **16** in 73% yield. Treatment of this product with diphenyl phosphonate[16] followed by hydrolysis gave the 5′-*H*-phohonate derivative **17** in 87% yield. Treatment of **17** with 80% formic acid afforded the 5′-*H*-phosphonate diester **18** in 84% yield.

This compound was further converted to the boronated species **19a** and **19b** by reaction of **11a** and **11b** in dioxane at 100 °C for 1 h in 81% and 98% yields, respectively. Treatment of the products **19a** and **19b** with trimethylsilylimidazole in the presence of CCl_4 gave the phosphorimidazolidates **20a** and **20b** in 91% and 99% yields, respectively.

To see if these new capping reagents are really useful, we also synthesized the capping reagents **21** and **22** which do not have 2-substituents.

Synthesis of 2,2-Dimethylguanosine-5′-yl Thymidine-5′-yl P¹,P²-Diphosphate. As part of our continuous studies on capped oligonucleotides, we required 2,2-dimethylguanosine-capped oligodeoxyribonucleotides ($m^{2,2}G^{5'}$pp-DNA) lacking a phosphate group as new medicinal drugs that are expected to remain in the cytoplasm and bind to mRNA. It is apparently desirable that such molecules should be synthesized by solid-phase synthesis. Therefore, we chose 2,2-dimethylguanosine-5′-yl thymidine-5′-yl P¹,P²-diphosphate as the simplest model of $m^{2,2}G^{5'}$pp-DNA to evaluate the efficiency of modified phenylboronate functions in the solid-phase synthesis. The outline of this strategy is shown in Scheme 5. For the diphosphate bond formation, the typical phosphorimidazolidate coupling[17] of 2′,3′-*O*-masked 2,2-dimethylguanosine 5′-phosphorimidazolidate derivatives **20a**, **20b**, **21**, and **22** with a thymidine 5′-phosphate derivative **26** attached to controlled pore glass (CPG)[18] was used.

Compound **26** was synthesized by a series of reactions involving removal of the DMTr group from DMTrT-succinate-CPG **23** followed by phosphorylation using a phosphitylating reagent **24**[19] and the successive DBU/BSA-mediated deprotection[20] of the phosphate protecting groups of the fully protected product **25**. The amount of the pT residue on the polymer support could be estimated by colorimetric assay of the released DMT cation[21] before the selective deprotection. The diphosphate bond formation was carried out at room temperature for 24 h. The coupling efficiency of this reaction was evaluated

(16) Wada, T.; Sato, Y.; Honda, F.; Kawahara, S.; Sekine, M. *J. Am. Chem. Soc.* **1997**, *119*, 12710−12721.
(17) Maeda, M.; Patel, A. D.; Hampton, A. *Nucleic Acids Res.* **1977**, *4*, 2843−2853.
(18) Sproat, B. S.; Bannwarth, W. *Tetrahedron Lett.* **1983**, *24*, 5771−5774.
(19) Horn, T.; Urdea, M. *Tetrahedron Lett.* **1986**, 27, 4705.
(20) Sekine, M.; Tsuruoka, H.; Iimura, S.; Wada, T. *Nat. Prod. Lett.* **1994**, *5*, 41.
(21) Gait, M. J.; Matthes, H. W. D.; Singh, M.; Sproat, B. S.; Titmas, R. C. *Nucleic Acids Res.* **1982**, *10*, 6243−6254.

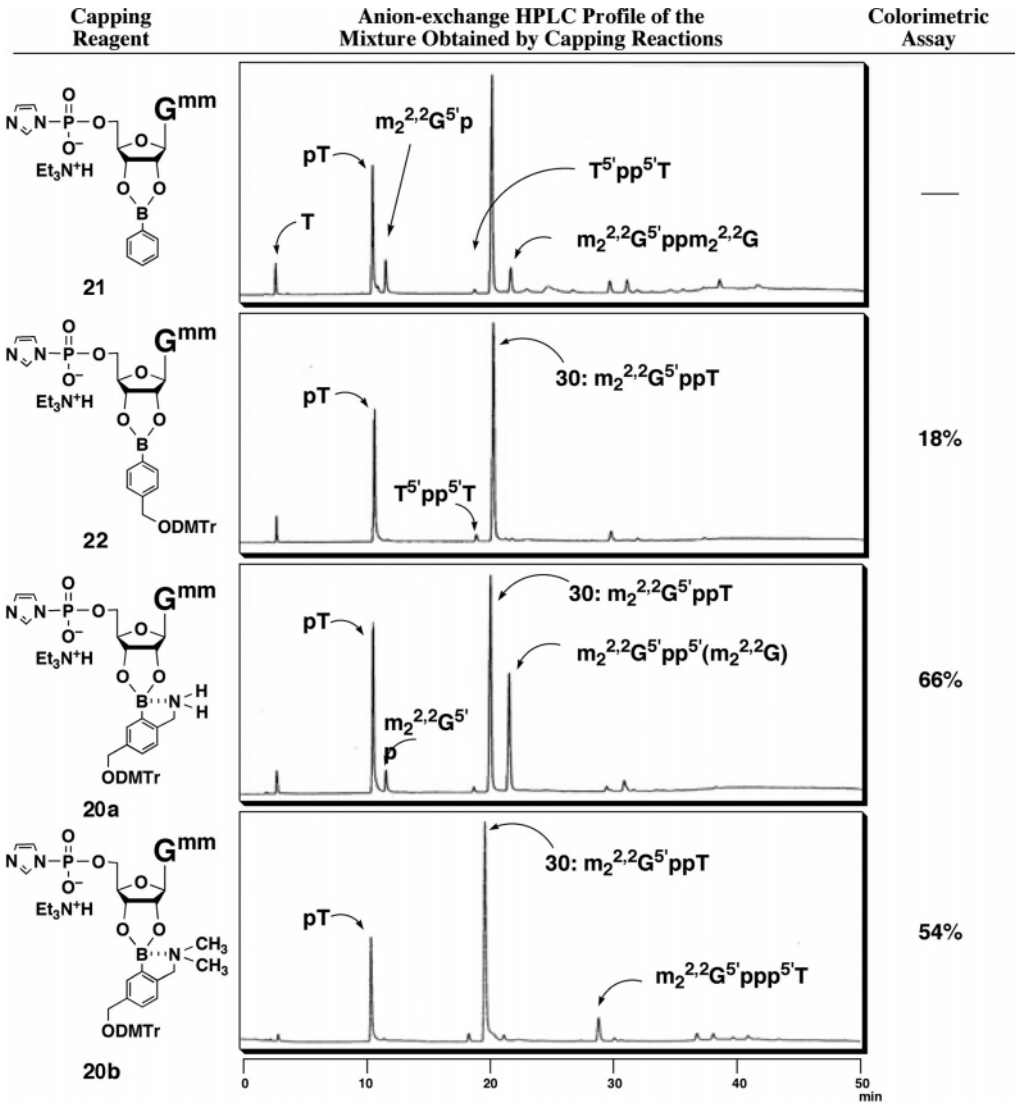


FIGURE 3. Results of diphosphate bond formation between **26** and the $m_2^{2,2}G$ capping reagents **20a**, **20b**, **21**, and **22** by use of the DMTr cation assay and the HPLC analysis.

by the DMTr cation assay. Independently, the final product of $m_2^{2,2}G^{5'}ppT$ was released from the resin by the successive treatments of **27a**, **27b**, **28**, and **29** with 1% trifluoroacetic acid and concd ammonia and quantified by HPLC analysis. These results are summarized in Figure 3.

As shown in Figure 3, the original phenylboronate protection mode gave the most complicated mixture. The detailed analysis of the products involving the desired product **30** disclosed formation of several minor products such as T, pT, $m_2^{2,2}G^{5'}p$, symmetric diphosphate derivatives of $T^{5'}ppT$ and $m_2^{2,2}G^{5'}ppm_2^{2,2}G$, and a triphosphate derivative of $m_2^{2,2}G^{5'}pppT$. Among them, T was formed because of the failure of the phosphitylation.

Since an excess amount of the capping regent was used, it was expected that the once-formed diphosphate derivative **31** reacts with the capping reagent to give a trisubstituted triphosphate derivative **32** so that this intermediate might be fragmentized upon hydrolysis giving rise to $m_2^{2,2}G^{5}p$, $m_2^{2,2}G^{5'}ppm_2^{2,2}G$, and pT depending on the cleavage sites 1, 2 as shown in Figure 4. Formation of $m_2^{2,2}G^{5'}pppT$ is unclear but it is likely that the P−O bond fission of **32** might occur.

Compared with the result of the reaction of **26** with **21**, the reaction of **26** with **22** that has the DMTr group gave a relatively simple HPLC profile. However, the DMTr cation assay (18%) did not exhibit the real result (43%) (Table 1). This result strongly reflects the lability of the DMTr-containing phenylboronate protecting group attached to the 2′,3′-*cis*-diol of the $m_2^{2,2}G$ residue during the reaction and workup.

Among the two reactions tested by use of **20a** and **20b**, the former showed a significant increase of the undesired byproduct $m_2^{2,2}G^{5'}ppm_2^{2,2}G$. On the other hand, the latter showed a very simple HPLC profile indicating that $m_2^{2,2}G^{5'}ppT$ was obtained as the major product. The formation of $m_2^{2,2}G^{5'}ppm_2^{2,2}G$ was considerably suppressed. The integration of the peak area in the HPLC chart indicated that the yield of this product was 50%. In addition to this result, the colorimetric assay showed a reasonable figure of 54%. Actually, $m_2^{2,2}G^{5'}ppT$ could be isolated in 49% yield by HPLC. From these results, it

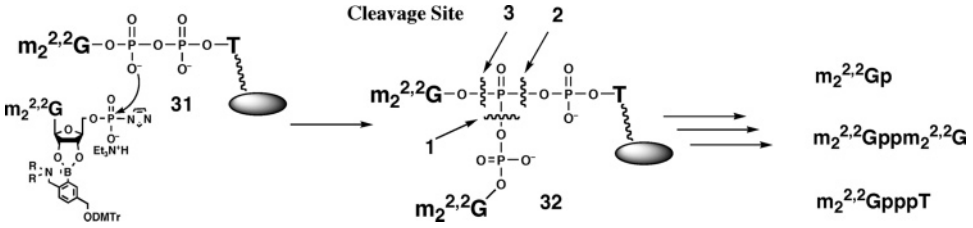


FIGURE 4. Mechanism of formation of minor products.

TABLE 1. Product Distribution of the Capping Reactions Evaluated by HPLC and DMTr Cation Assay

capping unit	product analyzed by HPLC						estd amt of all $m_2^{2,2}$G-containing products (%)	colorimetrical assay (isolated yield, %)
	T-containing products				other products			
	pT	$m_2^{2,2}G^{5'}$ppT	$T^{5'}$ppT	$m_2^{2,2}G^{5'}$pppT	$pm_2^{2,2}G$	$m_2^{2,2}G^{5'}ppm_2^{2,2}G$		
21	56	40	1	3	8	4		
22	55	42	1	2	0	0.5	43	18
20a	62	36	1	1	6	34	72	66
20b	44	50	1	5	1	1	58	54 (49)

was concluded that the [2-dimethylaminomethyl-5-[(4,4′-dimethoxytrityl)oxy]methyl]phenylboranylidene group is the most useful as the 2′,3′-*O*-protecting group capable of estimation of the yield of the capping reaction.

Conclusion

The present new strategy with the help of a colorimetrically detectable transient protecting group has proved to be useful for the convenient estimation of the $m_2^{2,2}$G-capping reaction. In particular, [2-(dimethylamino)methyl-5-[(4,4′-dimethoxytrityl)oxy]methyl]phenylboranylidene as a new protecting group would provide new insight into the development of effective methods for the synthesis of ribonucleoside-capped oligoribonucleotides or oligodeoxyribonucleotides. During this study, it turned out that the DMTr group released from the CPG resin after the capping reaction was derived from not only the desired product but also unstable intermediates such as the trisubstituted triphosphate derivative **32**. This observation is of significance when the polymer-supported synthesis is tried to synthesize oligonucleotide derivatives having a diphosphate linkage. Since large amounts of reagent are required for the solid-phase synthesis, we have to keep such a possibility in mind. Future studies should be necessary to avoid over-phosphorylation on the diphosphate linkage, which is crucial to improve the yield of the diphosphate bond formation on solid supports. Further studies are now under way in our lab.

Experimental Section

3-Bromo-4-(bromomethyl)benzoic Acid (6). 3-Bromo-4-methylbenzoic acid (**6**) (2.15 g, 10 mmol) was dissolved in dry CCl_4 (12.5 mL). To the solution were added *N*-bromosuccimide (1.87 g, 10.5 mmol) and 2,2′-azobis(isobutyronitrile) (82.1 mg, 0.5 mmol). After being vigorously stirred under reflux at an external temperature of 100 °C for 1 h, the mixture was cooled with ice−water. The resulting precipitates were collected by filtration and washed with ethyl acetate. The filtrate and washings were combined and washed three times with 5% citric acid. The organic layer was collected, dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The residual materials were recrystallized from ether to give compound **6** as crystals (2.15 g, 73%): mp 163.0−165.0 °C; ^{1}H NMR (270 MHz, DMSO) δ 4.75 (2H, s), 7.70 (1H, d, 5-H, $J_{5,6}$ = 8.2 Hz), 7.89 (1H, d), 8.08 (1H, s), 13.33 (1H, br); ^{13}C NMR (67.8 MHz, DMSO) δ 33.3, 123.8, 128.8, 131.8, 132.6, 133.3, 141.4, 165.3. Anal. Calcd for $C_8H_6Br_2O_2$: C, 32.69; H, 2.06; Br, 54.37. Found: C, 32.92; H, 2.06; Br, 54.10.

[3-Bromo-4-(bromomethyl)phenyl]methanol (7). To a solution of **6** (5.88 g, 20 mmol) in dry THF (176 mL) was added a 1 M THF solution of BH_3 (40 mL, 40 mmol). After being stirred under argon at room temperature for 1.5 h, the mixture was treated with water (1 mL). The resulting mixture was evaporated under reduced pressure. The residue was partitioned between ethyl acetate and 5% $NaHCO_3$. The organic layer was collected, washed two times with 5% $NaHCO_3$, dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The residue was crystallized from *i*-PrOH−hexane (1:9, v/v) to give compound **7** as crystals (3.86 g, 69%): mp 120.0−121.5 °C; ^{1}H NMR (270 MHz, DMSO) δ 4.49 (2H, s), 4.71 (2H, s), 7.28 (1H, d, 5-H, $J_{5,6}$ = 7.9 Hz), 7.53 (1H, d), 7.58 (1H, s); ^{13}C NMR (67.8 MHz, DMSO) δ 34.4, 61.7, 123.7, 125.9, 130.4, 131.4, 134.9, 145.5. Anal. Calcd for $C_8H_8Br_2O$: C, 34.32; H, 2.88; Br, 57.08.Found: C, 34.34; H, 2.83; Br, 57.30.

[3-Bromo-4-[(dimethylamino)methyl]phenyl]methanol (13b). To a 2.0 M solution of dimethylamine in THF (1.0 mL) was added dropwise a solution of **7** (140.0 mg, 0.5 mmol) in THF (3.0 mL) by use of a syringe. After being stirred at room temperature for 1.5 h, the mixture was evaporated under reduced pressure. The residue was dissolved in 1 M HCl (10 mL). The solution was washed two times with ether, cooled with ice−water, and treated with NaOH (0.80 g, 20 mmol). The resulting mixture was extracted with ethyl acetate. The organic layer was collected, dried over Na_2SO_4, filtered, and evaporated under reduced pressure to give compound **13b** (122.0 mg, 99%): ^{1}H NMR (270 MHz, $CDCl_3$) δ 2.25 (6H, s), 3.50 (2H, s), 4.59 (2H, s), 7.19 (1H, d, 5-H, $J_{5,6}$ = 7.9 Hz), 7.31 (1H, d), 7.51 (1H, s); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 45.3, 45.4, 62.8, 63.9, 124.6, 125.4, 130.81, 130.84, 136.5, 141.7; ESI-mass *m/z* calcd for $C_{10}H_{15}BrNO$ 244.0337, obsd [M + H] 244.0314.

[2-Bromo-4-[[(4,4′-dimethoxytrityl)oxy]methyl]benzyl]-dimethylamine (10b). Compound **13b** (3.49 g, 14.3 mmol) was rendered anhydrous by coevaporation three times with dry pyridine and finally dissolved in dry pyridine (143 mL). To the solution was added 4,4′-dimethoxytrityl chloride (5.81 g, 17.2 mmol). After being stirred under argon at room temperature for 1 h, the mixture was treated with MeOH (1 mL). The solution was evaporated under reduced pressure, and the residue was dissolved in $CHCl_3$. The $CHCl_3$ solution was washed three times with 5% $NaHCO_3$, and the organic layer was collected, dried over Na_2SO_4, filtered, and evaporated

under reduced pressure. The residue was chromatographed on a column of silica gel with hexanes−ethyl acetate (80:20, v/v) containing 0.5% pyridine to give compound **10b** (6.37 g, 81%): ^{1}H NMR (270 MHz, $CDCl_3$) δ 2.29 (6H, s), 3.51 (2H, s), 3.76 (6H, s), 4.13 (2H, s), 6.81−7.50 (15H, m) 7.55 (1H, s); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 45.3, 45.4, 45.5, 45.6, 55.1, 62.9, 64.6, 86.4, 113.0, 124.3, 125.6, 126.6, 127.7, 127.9, 128.0, 129.8, 130.5, 130.9, 135.8, 136.4, 139.7, 144.6, 158.2; ESI-mass *m/z* calcd for $C_{31}H_{33}BrNO_3$ 546.1644, obsd [M + H] 546.1604.

2-[(*N*,*N*-Dimethylamino)methyl]-5-[[(4,4′-dimethoxytrityl)oxy]methyl]phenylboronic Acid (11b). To a stirred solution of **10b** (2.73 g, 5 mmol) in dry THF (10.0 mL) was added at −78 °C a solution of *n*-BuLi in hexane (2.80 mL, 25.0 mmol). After the mixture was stirred at −78 °C for 30 min, a solution of trimethyl boronate (2.80 mL, 25.0 mmol) in dry THF (2.0 mL) was added. The resulting mixture was stirred at −78 °C for 1.5 h and then quenched by addition of 10% NH_4Cl. The resulting solution was partitioned between ethyl acetate (80 mL) and 10% NH_4Cl. The organic layer was collected, washed three times with 10% NH_4Cl, dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The residue was chromatographed on a column of NH silica gel with $CHCl_3$−hexane (95:5, v/v) to give compound **11b** (1.91 g, 75%): ^{1}H NMR (270 MHz, DMSO) δ 2.37 (6H, s), 3.71 (6H, s), 3.83 (2H, s), 4.08 (2H, s), 6.81−7.43 (15H, m) 7.58 (1H, s); ^{13}C NMR (67.8 MHz, DMSO) δ 44.0, 44.1, 54.7, 62.8, 65.1, 85.4, 112.7, 124.1, 125.1, 126.0, 127.1, 127.2, 129.1, 135.5, 135.8, 139.8, 144.5, 157.6; ESI-mass *m/z* calcd for $C_{31}H_{35}BNO_5$ 512.2608, obsd [M + H] 512.2589.

2′,3′-*O*-[2-(*N*,*N*-Dimethylamino)methyl-5-[[(4,4′-dimethoxytrityl)oxy]methyl]phenylboronylidene]uridine (14b). Uridine (48.8 mg, 0.20 mmol) was rendered anhydrous by coevaporation three times with dry pyridine and with dry dioxane and finally dissolved in dry dioxane (4.0 mL). To the solution was added compound **11b** (122.7 mg, 0.24 mmol). After being stirred under argon at 100 °C for 40 min, the mixture was evaporated under reduced pressure. The residue was dissolved in $CHCl_3$ (0.5 mL), and the solution was poured with vigorous stirring into isopropyl ether−ethyl acetate (200 mL, 9:1, v/v). The resulting precipitates were collected to give compound **14b** (143.9 mg, 98%): ^{1}H NMR (270 MHz, DMSO) δ 2.45 (6H, s), 3.545−3.69 (2H, m), 3.73 (6H, s), 3.90 (2H, s), 3.94−3.96 (1H, m), 4.05 (2H, s), 4.61−4.68 (2H, m), 5.00 (1H, bs), 5.65 (1H, d, 5-H, $J_{5,6}$ = 7.9 Hz), 5.88 (1H, d, $J_{1',2'}$ = 2.3 Hz), 6.90−7.45 (15H, m), 7.76 (1H, d), 11.35 (1H, s); ^{13}C NMR (67.8 MHz, DMSO) δ 13.5, 22.8, 44.4, 55.0, 61.5, 63.6, 66.3, 67.3, 78.6, 82.8, 85.7, 87.8, 91.7, 101.8, 113.1, 122.6, 126.1, 126.5, 127.5, 127.7, 128.5, 129.5, 135.6, 135.6, 136.9, 139.5, 141.7, 144.9, 150.2, 157.9, 162.9. Anal. Calcd for $C_{40}H_{42}BN_3O_9$: C, 66.76; H, 5.88; N, 5.84. Found: C, 65.29; H, 6.21; N, 5.05.

2′,3′,5′-*O*-Tris(*tert*-butyldimethylsilyl)-2-*N*,2-*N*-dimethylguanosine (15). 2′,3′,5′-*O*-Tris(*tert*-butyldimethylsilyl)guanosine (12.52 g, 20 mmol) was dissolved in acetic acid (200 mL). To the solution were added paraformaldehyde (1.80 g, 60 mmol) and $NaBH_3CN$ (3.77 g, 60 mmol). The mixture was vigorously stirred under argon at 45 °C. Six times at 8 h intervals, paraformaldehyde (1.80 g, 60 mmol) and $NaBH_3CN$ (3.77 g, 60 mmol) were added to the suspension and the mixture was vigorously stirred at the same temperature. The reaction was quenched by addition of water (100 mL), and $CHCl_3$ (100 mL) was added. The $CHCl_3$ layer was collected, washed three times with water (50 mL) and five times with 5% $NaHCO_3$ (50 mL), dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The residue was chromatographed on a column of silica gel with hexanes−ethyl acetate (70:30, v/v) to give compound **15** (6.54 g, 50%): ^{1}H NMR (270 MHz, $CDCl_3$) δ −0.18 (3H, s), −0.05 (3H, s), 0.08 (3H, s), 0.09 (6H, s), 0.10 (3H, s), 0.80 (9H, s), 0.91 (9H, s), 0.92 (9H, s), 3.21 (6H, s), 3.74−3.93 (2H, m), 4.02−4.06 (1H, m), 4.25−4.28 (1H, m), 4.45−4.49 (1H, m), 5.86 (1H, d, $J_{1',2'}$ = 5.3 Hz), 7.80 (1H, s), 10.96 (1H, bs); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ −5.4, −4.7, −4.4, −4.0, 17.9, 18.1, 18.2, 18.5, 25.7, 25.8, 26.0, 37.7, 62.6, 71.8, 74.1, 77.1, 84.4, 87.9, 88.0, 115.9, 138.2, 154.3, 153.4, 159.0, 159.3; ESI-mass *m/z* calcd for $C_{30}H_{60}N_5O_5Si_3$ 654.3902, obsd [M + H] 654.3887.

2′,3′-*O*-Bis(*tert*-butyldimethylsilyl)-2-*N*,2-*N*-dimethylguanosine (16). Compound **15** (6.28 g, 9.6 mmol) was dissolved in acetic acid−THF−water (96 mL, 3:1:1, v/v/v). After being stirred at 80 °C for 28 h, the mixture was partitioned between $CHCl_3$ and water. The $CHCl_3$ layer was collected, washed with water (50 mL) and three times with 5% $NaHCO_3$ (50 mL), dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The residue was chromatographed on a column of silica gel with hexanes−ethyl acetate (25:75, v/v) to give compound **16** (3.79 g, 73%): ^{1}H NMR (270 MHz, $CDCl_3$) δ −0.34 (3H, s), −0.10 (3H, s), 0.06 (3H, s), 0.07 (3H, s), 0.75 (9H, s), 0.89 (9H, s), 3.21 (6H, s), 3.63−3.88 (2H, m, 5′-H), 4.06 (1H, m), 4.25−4.27 (1H, m), 4.94−4.98 (1H, m), 5.65 (1H, d, $J_{1',2'}$ = 7.2 Hz), 7.60 (1H, s), 11.51 (1H, bs); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ −5.2, −4.6, −4.5, 17.8, 18.0, 25.7, 25.8, 38.9, 62.4, 73.2, 73.2, 87.2, 89.8, 118.0, 138.6, 150.8, 153.0, 158.7; ESI-mass *m/z* calcd for $C_{24}H_{46}N_5O_5Si_2$ 540.3037, obsd [M + H] 540.3060.

Triethylammonium 2′,3′-*O*-Bis(*tert*-butyldimethylsilyl)-2-*N*,2-*N*-dimethylguanosine 5′-Phosphonate (17). To a solution of diphenyl phosphonate (9.38 mL, 49 mmol) in dry pyridine (12 mL) was added dropwise and slowly a dry pyridine solution (12 mL) of **16** (3.77 g, 7.0 mmol) which, in advance, was dried by coevaporation three times with dry pyridine. After being stirred under argon at room temperature for 4 h, the mixture was treated with triethylamine−water (28 mL, 1:1, v/v). The resulting solution was stirred for 20 min and diluted with $CHCl_3$−pyridine (3:1, v/v). The solution was washed three times with 0.5 M TEAB buffer, and the organic layer was collected, dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The residue was chromatographed on a column of silica gel with $CHCl_3$−MeOH (95:5−70:30, v/v) to give compound **17** (4.29 g, 87%): ^{1}H NMR (270 MHz, DMSO) δ −0.33 (3H, s), −0.08 (3H, s), 0.08 (3H, s), 0.11 (3H, s), 0.72 (9H, s), 0.89 (9H, s), 1.16 (9H, m), 3.00 (6H, m), 3.05 (6H, s), 3.81−3.94 (2H, m), 3.98 (1H, m), 4.30 (1H, m), 4.83−4.87 (1H, m), 5.76 (1H, d, $J_{1',2'}$ = 6.6 Hz), 6.63 (1H, d, J_{PH} = 596.8 Hz), 8.00 (1H, s), 10.76 (1H, bs); ^{13}C NMR (67.8 MHz, DMSO) δ −5.5, −4.7, −4.7, −4.6, 8.4, 17.5, 17.8, 18.8, 25.5, 25.7, 37.8, 45.2, 62.5, 72.7, 73.8, 84.2, 86.4, 116.1, 136.8, 150.6, 152.7, 157.1; ^{31}P NMR (109 MHz, DMSO) δ 2.47; ESI-mass *m/z* calcd for $C_{30}H_{62}N_6O_7PSi_2$ 705.3956, obsd [M + H] 705.3964.

Triethylammonium 2-*N*,2-*N*-Dimethylguanosine 5′-Phosphonate (18). Compound **17** (4.30 g, 6.1 mmol) was dissolved in formic acid−water (4:1, v/v, 60 mL). After being stirred at room temperature for 46 h, the mixture was diluted with $CHCl_3$. The $CHCl_3$ solution was extracted 10 times with 0.1 M TEAB buffer. The aqueous extracts were collected and evaporated under reduced pressure. The residue was chromatographed on a column of DEAE Sephadex A-25 (HCO_3^- form) with 0−1 M NH_4HCO_3 to give **18** (2.4 g, 84%): ^{31}P NMR (109 MHz, DMSO) δ 2.60, 2.74; ESI-mass *m/z* calcd for $C_{18}H_{34}N_6O_7P$ 477.2227, obsd [M + H] 477.2228.

Triethylammonium 2′,3′-*O*-[2-(*N*,*N*-Dimethylamino)-methyl]-5-[[(4,4′-dimethoxytrityl)oxy]methyl]phenylboronylidene]-2-*N*,2-*N*-guanosine 5′-Phosphonate (19b). Compound **18** (187.6 mg, 0.5 mmol) was rendered by coevaporation three times with dry pyridine and finally dissolved in dry dioxane (10 mL). To the solution was added 2-[(*N*,*N*-dimethylamino)methyl]-5-[(4,4′-dimethoxytrityl)oxy]methyl]-phenylboronic acid (341.1 mg, 0.6 mmol). After being stirred under argon at 100 °C for 1 h, the solution was evaporated under reduced pressure. The residue was dissolved in $CHCl_3$ (500 μL), and the solution was added to a solution of ether−ethyl acetate (300 mL, 9:1, v/v) to give **19b** as a white precipitate (468.4 mg, 98%): ^{1}H NMR (270 MHz, DMSO) δ 1.11 (9H, m), 2.46 (6H, s), 2.91 (6H, s), 2.95−3.01 (6H, m), 3.73 (6H, s), 3.84 (2H, m), 3.90 (2H, s), 4.04 (2H, s), 4.13−4.14 (1H,

m), 4.77 (1H, dd, $J_{3',2'} = 5.9$ Hz, $J_{3',4'} = 3.3$ Hz), 5.05 (1H, dd, $J_{2',1'} = 3.6$ Hz), 5.90 (1H, d), 6.60 (1H, d, $J_{PH} = 595.8$ Hz), 6.90−7.45 (16H, m), 7.97 (1H, s), 10.71 (1H, bs); ^{13}C NMR (67.8 MHz, DMSO) δ 8.36, 8.43, 36.7, 37.6, 44.5, 45.1, 55.0, 63.6, 65.2, 80.0, 82.8, 85.6, 85.7, 86.3, 90.0, 113.1, 115.7, 122.7, 124.9, 126.0, 126.6, 127.5, 127.8, 128.3, 129.5, 135.4, 135.5, 135.6, 136.4, 136.9, 139.6, 144.8, 150.2, 152.7, 157.2, 157.9; ^{31}P NMR (109 MHz, DMSO) δ 2.23. Anal. Calcd for $C_{49}H_{63}BN_7O_{10}P$: C, 61.83; H, 6.67; N, 10.30. Found: C, 57.24; H, 6.24; N, 9.59.

Triethylammonium 2′,3′-*O*-[2-(*N*,*N*-Dimethylamino)-methyl]-5-[[(4,4′-dimethoxytrityl)oxy]methyl]phenylboronylidene]-2-*N*,2-*N*-guanosine 5′-Phosphorimidazolidate (20b). Compound **19b** (142.8 mg, 0.15 mmol) was rendered anhydrous by coevaporation three times with dry pyridine, two times with dry toluene, and with dry CCl_4 and finally dissolved in dry acetonitrile-CCl_4 (1.5 mL, 1:1, v/v). To the mixture were added trimethylsilylimidazole 88.0 μL) and triethylamine (83.9 μL). After being stirred at room temperature for 30 min, the mixture was treated with MeOH (80 μL). The resulting mixture was stirred for an additional 10 min. The solvent was removed by evaporation under reduced pressure, and the residue was dissolved in $CHCl_3$ (500 μL). The $CHCl_3$ solution was poured into a vigorously stirred solution of ether−ethyl acetate (150 mL, 85:15, v/v). The resulting precipitates were collected to give compound **20b** (152.6 mg, 99%): ^{1}H NMR (270 MHz, DMSO) δ 1.14−1.19 (9H, m), 2.44 (6H, s), 2.89 (6H, s), 2.99−3.07 (6H, m), 3.73 (6H, s), 3.65−3.82 (2H, m), 3.90 (2H, s), 4.04 (2H, s), 4.17 (1H, bs), 4.57−4.60 (1H, m), 4.98−5.02 (1H, m), 5.86 (1H, d, $J_{1',2'} = 3.3$ Hz), 6.90−7.45 (18H, m), 7.93 (1H, s), 8.41 (1H, s), 10.71 (1H, bs); ^{13}C NMR (67.8 MHz, DMSO) δ 8.4, 13.5, 37.5, 37.6, 44.4, 45.1, 55.0, 63.6, 64.9, 65.2, 79.8, 82.8, 85.7, 90.0, 113.1, 115.6, 120.4, 122.7, 126.0, 126.6, 127.5, 127.8, 128.3, 129.5, 134.5, 135.5, 135.6, 136.3, 136.9, 139.5, 144.8, 150.2, 152.7, 157.2, 157.9; ^{31}P NMR (109 MHz, DMSO) δ −9.61. Anal. Calcd for $C_{52}H_{65}BN_9O_{10}P$: C, 61.34; H, 6.44; N, 12.38. Found: C, 58.20; H, 6.19; N, 14.23.

Solid-Phase Synthesis of $m_2^{2,2}G^{5'}$ppT 30 by Use of 20a, 20b, 21, and 22. Typical Procedure by Use of 20b. A DMTr-T loaded CPG support (0.5 μmol, 38.7 μmol/g, 12.9 mg) was put in a cylinder-like glass vessel with a glass filter under argon. The following protocol was used for the synthesis of **25**: (1) 1% TFA (1 mL) 15 s × 3, (2) washing with CH_2Cl_2 (1 mL) × 3, (3) washing with CH_3CN (1 mL) × 3, (4) drying under reduced pressure, 10 min, (5) condensation with a 0.1 M solution of the phosphitylating reagent **24** (200 μmol) in dry acetonitrile in the presence of 1-*H*-tetrazole (2.8 mg, 40 μmol), 5 min, (6) washing with dry pyridine (1 mL) ×3, 7) oxidation with a 0.15 M I_2 solution in THF/pyridine/H_2O (10:10:1, v/v/v) (1 mL), 15 s ×3, (8) washing with pyridine (1 mL) × 3, 9) washing with CH_2Cl_2 (1 mL) × 3, 10) drying, (10) capping with a 0.12 M solution of (dimethylamino)pyridine in pyridine-Ac_2O (1 mL, 1:9, v/v), 2 min, (11) washing with CH_2Cl_2 (1 mL) × 3, and (12) drying.

For the synthesis of **26**, the following protocol was used: (1) treatment with 1% TFA (1 mL) 15 s × 3, (2) washing with CH_2Cl_2 (1 mL) × 3, 3) drying, (4) treatment with BSA-pyridine (400 μL, 1:1, v/v), 20 min, (5) addition of DBU (80 mL), 10 min, (6) washing with dry pyridine (1 mL) × 3, (7) treatment with MeOH/Et_3N (1 mL, 4:1, v/v), 5 min, (8) washing with dry pyridine (1 mL) × 3, (9) washing with CH_2Cl_2 (1 mL) × 3.

For the synthesis of **27b**, the following protocol was used: (1) condensation of **26** with a 0.1 M solution of **20b** (20 μmol) in dry pyridine (200 μL) at room temperature for 24 h, (2) washing with dry pyridine (1 mL) × 3, (3) washing with CH_2Cl_2 × 3, (4) drying, (5) treatment with 1% TFA (1 mL) × 3, (6) washing with CH_2Cl_2 (1 mL) × 3, (7) washing with pyridine (1 mL) × 3, (8) washing with H_2O (1 mL) × 3, (9) pyridine (1 mL) × 3, (10) washing with CH_2Cl_2 (1 mL) × 3, (11) drying, and (12) washing with CH_3CN (1 mL) × 3. The CPG support having $m_2^{2,2}G^{5'}$ppT, thus obtained, was treated with 29% aqueous ammonia at room temperature for 1 h. The supernatant was collected, and the CPG was washed three times with acetonitrile. The supernatant and washings were combined and evaporated under reduced pressure. The residue was dissolved in water (1 mL). Purification by use of C_{18} reversed-phase chromatography followed by lyophilization $m_2^{2,2}$GppT **30** (3.58 $A_{254\ nm}$ units, 49% from **20b**): ESI-mass *m/z* calcd for $C_{22}H_{32}N_7O_{15}P_2$ 696.1432, obsd [M + H] 696.3077.

For the HPLC analysis of the products, the following ϵ values at 254 nm were used: pT, 7.4×10^3; T$^{5'}$ppT, 13.3×10^3; $pm_2^{2,2}G$, 12.7×10^3; $m_2^{2,2}G^{5'}$ppT, 18.1×10^3; $m_2^{2,2}G^{5'}$pppT, 18.1×10^3; $m_2^{2,2}G^{5'}ppm_2^{2,2}G$, 22.9×10^3. The ϵ values of these dinucleoside diphosphate or triphosphate derivatives were obtained by addition of the original contributions of both the nucleoside components in consideration of 10% hypochromicity.

DMTr Cation Assay. The 1% TFA solution which was used for removal of the DMTr group was recovered and evaporated under reduced pressure. The residue was dissolved in 60% $HClO_4$−EtOH (3:2, v/v). The amount of the DMTr cation was calculated by use of $\epsilon = 7.17 \times 10^4$ at 498 nm.

Acknowledgment. This work was supported by a Grant from CREST of JST (Japan Science and Technology Agency) and a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology, Japan.

Supporting Information Available: List of experimental procedures for the synthesis of the products other than those described in Experimental Section. This material is available free of charge via the Internet at http://pubs.acs.org.

JO051202M