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Facile and efficient approach for the synthesis of N^2-dimethylaminomethylene-2′-*O*-methylguanosine

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ABSTRACT

A convenient method for the synthesis of N^2-dimethylaminomethylene-2′-*O*-methylguanosine (**1**), which is a useful intermediate for oligonucleotide construction, was developed. We chose the di-*tert*-butylsilyl group and the triisopropylbenzenesulfonyl group as sugar and base protecting groups, respectively. These protecting groups were stable during the 2′-O-methylation step with MeI and NaH. Our six-step synthesis of **1** is easy to perform using commercially available reagents, and requires only three chromatographic purifications. Compound **1** was obtained in 56% yield from guanosine.

Oligonucleotides containing 2′-*O*-methylribonucleosides have high resistance to nucleases and form hybrids with complementary RNAs, the thermal stability of which is higher than that of unmodified oligoribonucleotide duplexes.[1–4] Because of these characteristics, 2′-*O*-methyloligonucleotides have been employed to regulate specific gene expression in antisense and RNAi methodologies.[4–6] The usefulness of 2′-*O*-methylribonucleosides increases the importance of developing efficient methods for their synthesis. Among the four ribonucleosides, guanosine is the most difficult to methylate selectively at the 2′-hydroxyl group because of the acidic N^1-proton at the base moiety. Methylation procedures for non-protected or partially protected ribonucleosides were developed using diazomethane or trimethylsilyldiazomethane.[7] However, the yields were often very low and the presence of the undesired 3′-*O*-methyl isomer complicated the purification, which was usually achieved by anion-exchange chromatography on Dowex. Robins et al. reported that the use of 1 equiv of diazomethane and stannous chloride as the catalyst gave relatively high yields of 2′-*O*-methylribonucleosides except for guanosine that underwent base methylation.[8] The use of bifunctional sugar protecting groups, such as the tetraisopropyldisiloxane (TIPDS) bridge, has resolved the isomer issue. However, the methylation of 3′,5′-*O*-protected guanosine using MeI/NaH or MeI/Ag_2O reagent was not successful due to the undesired base methylation.[2,9] Therefore, protection of the O^6-position of guanosine prior to the methylation is necessary. Recently, Chow et al. reported that the methylation of 3′,5′-*O*-methylene-bis-diisopropylsilyl (MDPS)-guanosine in the presence of sodium bis(trimethylsilyl)amide as a base proceeded in good yield with high selectivity without the base protection.[9,10] However, MDPS-Cl_2 is not commercially available and the use of $MeCl_{gas}$ as the methylating reagent is inconvenient because it is difficult to handle. Therefore, one general way to avoid the methylation at N^1 of guanosine is to use 2,6-diaminopurine or 2-amino-6-chloropurine analogues[11–13] as the starting material. However, these analogues are much more expensive than guanosine and the transformation into guanine analogues is required after the methylation step. Another way to avoid the methylation at N^1 of guanosine is to use O^6-protected guanosine derivatives, such as O^6-nitrophenyl,[14] O^6-nitrophenylethyl,[15] and O^6-diphenylcarbamoyl (DPC).[14] O^6-Nitrophenylethyl and O^6-nitrophenyl protections have been reported to be useful for the methylation of guanosine, while O^6-DPC protection gave unsatisfactory results. Grotli et al. reported the methylation of O^6-*tert*-butyldiphenylsilyl (TBDPS)-3′,5′-*O*-TIPDS-guanosine with MeI and expensive 2-*tert*-butylimino-2-diethylamino-1,3-dimethylperhydro-1,3,2-diazaphosphorine (BEMP).[14,16] They achieved the five-step synthesis of N^2-dimethylaminomethylene-2′-*O*-methylguanosine (48% from guanosine). However, O^6-silyl protecting groups, such as *tert*-butyldimethylsilyl (TBDMS) and TBDPS, are known to be unstable during chromatography on silica gel.[17,18] Zlatev et al. reported that O^6-trimethylsilylethyl was stable during silica gel column chromatography.[17] However, this eight-step synthesis of N^2-isobutyryl-2′-*O*-methylguanosine gave low yield (28% from guanosine). Meanwhile, Saneyoshi et al. reported a convenient method for cyanoethylation at the 2′-hydroxyl group of O^6-triisopropylbenzenesulfonyl (TPS)-protected guanosine derivative with acrylonitrile and Cs_2CO_3.[19] Hence, we studied

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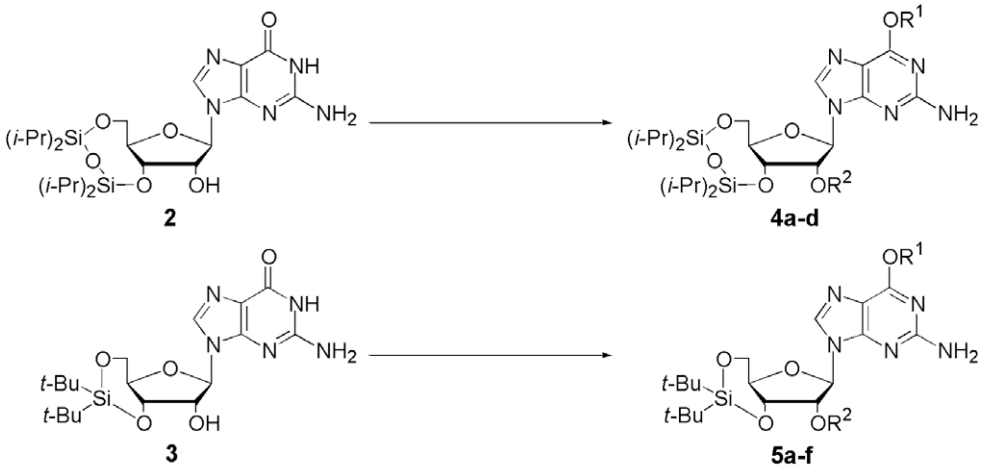
Table 1
O^6-Arenesulfonylation of 3′,5′-*O*-protected guanosines[a]



a: R^1 = Ts, R^2 = H, **b**: R^1 = R^2 = Ts, **c**: R^1 = Mes, R^2 = H, **d**: R^1 = R^2 = Mes, **e**: R^1 = TPS, R^2 = H, **f**: R^1 = R^2 = TPS

Entry	Starting material	Reagent	Reaction time (min)	Product	Yield (%)	Product ratio
1	**2**	Ts-Cl (3.0 equiv)	60	**4a**, **4b**	82.1	**4a**:**4b** = 70:30
2	**2**	Mes-Cl (3.0 equiv)	75	**4c**, **4d**	99.8	**4c**:**4d** = 78:22
3	**3**	Ts-Cl (3.0 equiv)	60	**5a**, **5b**	Quant.	**5a**:**5b** = 90:10
4	**3**	Mes-Cl (3.0 equiv)	75	**5c**, **5d**	Quant.	**5c**:**5d** = 97:3
5	**3**	TPS-Cl (3.0 equiv)	120	**5e**, **5f**	Quant.	**5e**:**5f** = 100:0

[a] Reactions were carried out using recrystallized starting materials in the presence of *p*-toluenesulfonyl chloride (Ts-Cl), 2-mesitylenesulfonyl chloride (Mes-Cl) or TPS-Cl, triethylamine (6 equiv) and DMAP (0.2 equiv) in CH_2Cl_2 at room temperature.

whether or not arenesulfonyl groups could be used as an O^6-protecting group under conventional MeI/NaH methylation conditions. Herein we describe an easy and efficient method to prepare N^2-dimethylaminomethylene-2′-*O*-methylguanosine (**1**).

First, we studied the selectivity of the arenesulfonylation at the O^6-position of 3′,5′-*O*-protected guanosine without 2′-*O*-protection by using 3′,5′-*O*-TIPDS-guanosine (**2**) and 3′,5′-*O*-di-*tert*-butylsilanediyl (DTBS)-guanosine (**3**). The results are shown in Table 1. The arenesulfonylation of **2** showed moderate selectivity for the O^6-position, while that of **3** showed high selectivity. Moreover, the selectivity increased as the arenesulfonylating reagents became bulkier. Indeed, the arenesulfonylation of **3** using TPS-Cl was specific for the O^6-position. A conformational search suggested that **3** has more rigid conformational features than **2**, and the distance between the 2′-hydroxyl group and the silicon alkyl side chain is longer in **2** than in **3** (Fig. 1).[20] This suggests that the selectivity is based on the steric effect of the alkyl chain of silyl protecting groups and the rigidity of the sugar moiety. As a result, we chose to use DTBS and TPS groups as sugar and base protecting groups, as shown in Scheme 1.

The 3′,5′-hydroxyl groups of guanosine were protected using DTBS-ditriflate and 3′,5′-*O*-DTBS guanosine **3** was given in 89% yield. Then, compound **3** was treated with TPS-Cl, triethylamine, and DMAP to give O^6-TPS-3′,5′-*O*-DTBS-guanosine (**5e**). Undesired 2′-*O*-TPS product and 6,2′-*O*-di-TPS product were not observed on TLC. After column chromatography, compound **5e** was obtained in 97% yield. Subsequently, we attempted to perform the methylation of **5e** with MeI and NaH. However, the yield of the 2′-*O*-methyl derivative was only 58% because of N^2-methylation. Therefore, we considered it necessary to introduce a protecting group of the 2-NH_2 group to avoid the undesired N^2-methylation. Moreover, the amino protection is necessary to synthesize the 3′-*O*-phosphorami-

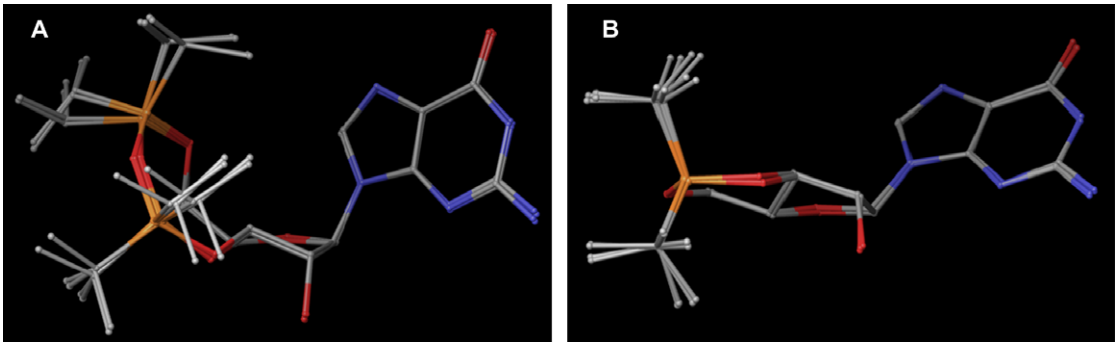
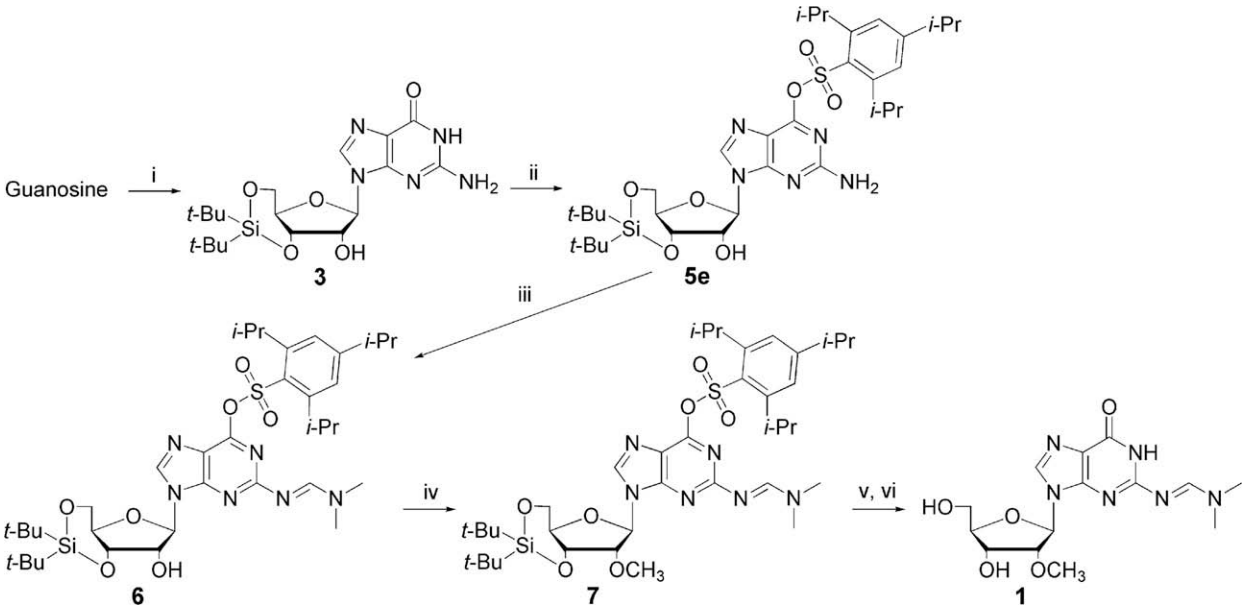


Figure 1. Low-energy conformers of **2** and **3** calculated with OPLS_2005 force field (MACROMODEL ver. 9.1). (A) TIPDS-protected guanosine (**2**), (B) DTBS-protected guanosine (**3**); compound **2** has more flexible sugar and silicon rings than compound **3**. The average distances between the 2′-hydroxyl group and the alkyl chain of the silyl protecting group of **2** and **3** are 4.79 Å and 4.17 Å, respectively.



Scheme 1. Reagents and conditions: (i) di-*tert*-butylsilyl ditriflate/DMF, 0 °C, 1.5 h, 89.3%; (ii) 2,4,6-triisopropylbenzenesulfonyl chloride, triethylamine, DMAP/CH_2Cl_2, 3 h, 96.8%; (iii) *N*,*N*-dimethylformamide dimethyl acetal/DMF, overnight, 99.9%; (iv) MeI, NaH/DMF, molecular sieve 3 Å, 0 °C, 30 min, 84.7%; (v) 2-nitrobenzaldoxime, *N*,*N*,*N*′,*N*′-tetramethyguanidine/MeCN, 1 h; (vi) triethylamine trihydrofluoride, triethylamine/THF, 1 h, 77.0%.

dite derivative, which is a synthetic unit of oligonucleotides. Then, O^6-TPS-3′,5′-*O*-DTBS-guanosine (**5e**) was subjected to formamidination using *N*,*N*-dimethylformamide dimethyl acetal (DMFDA).[21] This reaction proceeded almost quantitatively and the resulting mixture (**6**) was sufficiently pure to be used in the next step. The methylation reaction of **6** was performed with MeI and NaH in dry DMF at 0 °C, and was completed within 30 min. After column chromatography, compound **7** was obtained in 85% yield.[21] Thus, the O^6-TPS protection was proven to be stable under these methylation conditions. Then, compound **7** was subjected to removal of the O^6-TPS group with 2-nitrobenzaldoxime and *N*,*N*,*N*′,*N*′-tetramethylguanidine.[19] Finally removal of the silyl group using triethylamine trihydrofluoride and triethylamine afforded N^2-dimethylaminomethylene-2′-*O*-methylguanosine (**1**).[19] After chromatographic purification, compound **1** was obtained in 77% yield.[22] Compound **1** is a useful intermediate for oligonucleotide construction.

In conclusion, we have developed a novel six-step approach for the synthesis of N^2-dimethylaminomethylene-2′-*O*-methylguanosine (**1**). The use of DTBS protection and NaH is more economical than the use of TIPDS protection and a sterically hindered organic base, such as BEMP. Moreover, the reactions are easy to perform and the overall yield from guanosine is 56%. From the viewpoint of laborsaving, chromatographic isolation in the methylation step is not necessarily required. In this case, **1** was obtained in 62% yield from **5e**, and the overall yield from guanosine was 53% with two chromatographic purifications. The syntheses of N^2-dimethylaminomethylene-2′-*O*-alkylguanosines are currently underway.

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Supplementary data

Supplementary data (general experimental procedures) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.11.016.

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