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Novel Synthesis of 2′-*O*-methylguanosine

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Abstract—An efficient and chemoselective synthesis of 2′-*O*-methylguanosine (**6**) has been accomplished in high yield without protection of the guanine base. The salient feature of the synthesis of **6** lies in the application of methylene-bis-(diisopropylsilyl chloride), ($MDPSCl_2$, **2**) as a new 3′,5′-*O*-protecting group for nucleosides. Use of CH_3Cl as a weak electrophile and NaHMDS as a mild base was crucial to the success of the 2′-*O*-methylation of 3′,5′-*O*-protected guanosine.

In the last decade, several oligonucleotide-based drugs have entered human clinical trials for the treatment of a variety of viral, infectious or cancer-related diseases.[1] Among these, 2′-*O*-methyl ribonucleotides (Me-RN) have been extensively utilized as a second-generation oligonucleotide constructs due to their favorable in-vitro and in-vivo properties.[2,3] Additionally, Me-RN have found recent applications in studying pre-mRNA splicing, examining the structures of spliceosomes and preparing nuclease-resistant hammerhead ribozymes.[4] Me-RN have been found as minor components in RNA of various origins.

The exceptional value of Me-RN has stimulated a considerable effort towards synthesis of 2′-*O*-methylated nucleosides as key building blocks using a variety of synthetic approaches. Although some of these strategies have been successfully implemented to the production of pyrimidine containing 2′-*O*-methyl nucleosides, they are much less efficient in the case of the purine analogues.[5] In the latter case, the problems derive from the inherent reactivity of the purine bases towards alkylation (chemoselectivity) and the need for selective protection of the 3′- and 5′-OH groups (regioselectivity).[6,7]

Initially methylation procedures were developed using diazomethane or trimethylsilyldiazomethane on partially protected nucleosides.[8] However, these approaches suffer from the toxicity of the methylating reagents and their incompatibility with any large-scale applications. Moreover, the low yields of product obtained under these conditions and the concomitant formation of the undesired 3′-*O*-methyl isomer further complicates purification.[5] Alternatively, the use methyl iodide in combination with sodium hydride[9] or silver oxide[10] has been explored on nucleobase-protected ribonucleosides. The problems of regioselectivity of methylation have led to efforts to simultaneously protect the 3′- and 5′-OH groups using tetraisopropyl dichlorodisilyloxane ($TIPDSCl_2$, **1**),[11] or $TBSCl_2$.[7,12] Since these protecting groups were found to be labile under strong alkaline conditions they require the use of sterically hindered organic bases, such as BDDDP or BEMP,[13] whose high cost renders this method unsuitable for any large-scale nucleoside synthesis.[14]

We have recently reported on the synthesis of a novel silicon-based protecting group, referred to as methylene-bis-(diisopropylsilyl chloride), ($MDPSCl_2$, **2**) (Fig. 1).[15] The design of this reagent was based on the hypothesis that the fragility of $TIPDSCl_2$, (**1**) was due to the inductive effect of the oxygen atom that interconnects the two silicon groups. We envisioned that this effect could favor the formation of a pentacoordinated silicon intermediate during the basic conditions required for alkylation, thereby accelerating its deprotection and giving rise to side products formed by a non-selective alkylation.[16] Based on this hypothesis, we developed reagent **2** in which a methylene unit is used to bridge the two silicon atoms. Being isosteric to **1**, we predicted that **2** would exhibit a similar reactivity and selectivity profile as **1**, but display an extended stability under basic

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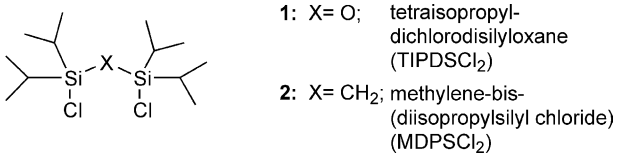
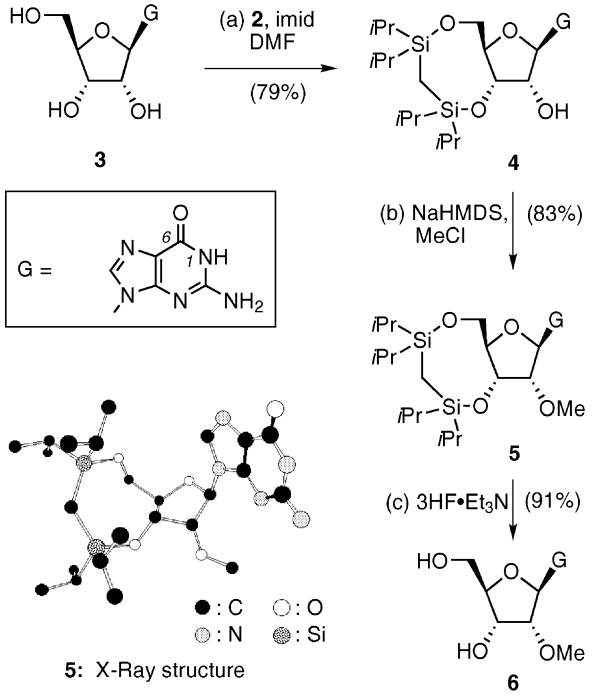


Figure 1. Structures of $TIPDSCl_2$ (**1**) and $MDPSCl_2$ (**2**).

conditions. Herein, we describe an application of **2** to a new regio- and chemo-selective synthesis of 2′-*O*-methylguanosine (**6**).

The synthesis of 2′-*O*-methylguanosine (**6**) is described in Scheme 1. Protection of guanosine (**3**) proceeded using a slight excess of disilane **2** in the presence of imidazole and produced the desired 3′,5′-protected nucleoside **4** in 79% yield.[17] The excellent regioselectivity of the protection is attributed to the steric effects of the isopropyl groups that favor initially silylation of the 5′-hydroxyl group and subsequently cyclization at the neighboring 3′-hydroxyl group.

To minimize the synthesis steps and maximize the overall efficiency of our strategy, we decided to examine the methylation of **4** without prior protection of the nucleobase. Some of our efforts to develop such a chemoselective alkylation are summarized in Table 1. Our initial studies, with traditionally used NaH and methyl iodide, resulted in formation of substantial amounts of *N*-alkylated product **7** together with dialkylated adduct **8** (Fig. 2). Although the decrease of temperature slowed the formation of dialkylated adduct **8**, it did not improve upon the formation of desired product **5**. Undesirable *N*-alkylation was also predominant



Scheme 1. Reagents and conditions: (a) 1.15 equiv **2**, 5.0 equiv imid, DMF, 0–25 °C, 5 h, 79%; (b) 3.0 equiv NaHMDS, $MeCl_{(g)}$, DMF, −40 to −27 °C, 5 h, 83%; (c) 1.0 equiv $3HF{\cdot}Et_3N$, THF, 35 °C, 14 h, 91%.

during methylation of **4** with other bases, such as KO*t*Bu and BEMP.

Further search for an ideal base resulted in a promising lead with NaHMDS that in combination with MeI gave rise to a mixture of monoalkylated adducts **5** and **7** in a ratio of 4:6. Use of Li and K as alternative cations did not provide further improvements over NaHMDS. The encouraging initial results obtained with NaHMDS together with its overall advantages versus other bases, including low cost, good solubility and safer handling, led us to attempt further improvements of the alkylation reaction by tuning the nature of the electrophile. To this end, we explored the use of $(CH_3O)_2SO_2$, $(CH_3O)_2POH$, $CF_3SO_3CH_3$ and CH_3Cl as potential electrophiles in combination with NaHMDS. Among them, use of CH_3Cl furnished the desired *O*-alkylated product **5** together with dimethylated material **8** in 9:1 ratio and excellent overall yield.[18] The major adduct **5** was obtained in 83% isolated yield by a simple extraction and precipitation from the crude reaction mixture.[19] This compound was easily crystallized and its structure was further confirmed via a single crystal X-ray analysis (Scheme 1). It is important to note that the methylation was performed at lower temperature (−40 to 25 °C) because CH_3Cl is a gas at higher temperatures. The combination of NaHMDS as the base and CH_3Cl as an electrophile at low temperature was critical to the success of this reaction.[20,21] It is also worth mentioning that guanosine protected with disilane **1** underwent deprotection under the above alkylation conditions, supporting our hypothesis that the fragility of **1** is derived from the presence of the oxygen atom.

The desilylation of **5** was achieved with both TBAF and $3HF{\cdot}Et_3N$ as the fluoride sources. In our hands, use of $3HF{\cdot}Et_3N$ proved to be advantageous, since it produced compound **6**, free of any residual ammonium salts, in 91% yield without column chromatography.

Table 1. Effect of base during methylation of **4**

Base (3.0 equiv)	Temp (°C)	R-X (equiv)	Product ratio **5**:**7**:**8**
NaH	0	MeI (1.4)	1:8:1
NaH	0	MeI (5.0)	1:1:8
NaH	−30	MeI (5.0)	1:6:3
BEMP	0	MeI (5.0)	1:7:2
KO*t*Bu	0	MeI (5.0)	1:8:1
NaHMDS	0	MeI (5.0)	1:7:2
NaHMDS	−20	MeI (5.0)	4:6:0
NaHMDS	−40	MeCl (excess)	9:0:1

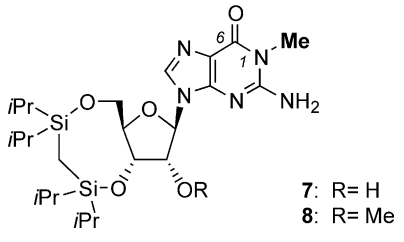


Figure 2. Structures of mono- and di-alkylated adducts **7** and **8**.

In conclusion, we describe herein an efficient chemoselective procedure for the synthesis of 2′-*O*-methylguanosine (**6**). Crucial to the success of our strategy was the recognition that the limitations of the previously reported methods are due to the fragility of the silicon-based protecting groups, such as **1**, under the basic conditions required for alkylation. The isosteric silane **2** was found to possess the necessary stability to withstand the alkylation conditions, thereby addressing the issue of regioselectivity of the methylation. The chemoselectivity of this reaction was achieved in favor of the *O*-alkylation by performing the reaction in presence of NaHMDS as the base and MeCl as the electrophile. This strategy allows the synthesis of compound **6** in three steps and 61% overall yield. To the best of our knowledge, this is the first report describing a chemoselectivity for *O*-alkylation over *N*-alkylation during methylation of guanosine with unprotected base. An additional advantage of silane **2** over **1** is that it produces compounds that are highly crystalline and easily isolated without the need of column chromatography.

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17. The effects of different bases (pyridine, imidazole, triethylamine and lutidine) and temperature (0–70 °C) for the protection of **3** were investigated. Among them, imidazole was found to produce the best results.

18. LC/MS analysis of the crude reaction mixture indicated the presence of starting material **4** in 2% yield but showed complete absence of any mono N^1-alkylated product **7**.

19. To a solution of compound **4** (5.0 g, 9.6 mmol) in 200 mL DMF at −40 °C was added MeCl (bubbled into the reaction mixture for 3–5 min, approximately 5 g), followed by sodium bis(trimethylsilyl)amide (NaHMDS 1.0 M in THF, 28.7 mL, 28.7 mmol). The reaction mixture was stirred for 5 h under argon and subsequently quenched with methanol (5 mL). After evaporation of the THF under reduced pressure the remainder solution was poured into ice to provide compound **5** as a white solid (4.27 g, 7.8 mmol, 83% yield). X-ray quality material was obtained after crystallization from CH_2Cl_2/MeOH. **5**: $R_f = 0.39$ (silica, 10% methanol in dichloromethane); mp: 230–232 °C (dec); $[\alpha]_D^{25}$: +11.8 (*c* 0.13, CH_2Cl_2); IR (film) ν_{max} 1265, 1683, 3054; ^{1}H NMR (400MHz, DMSO-d_6) δ 10.64 (s, 1H), 7.73 (s, 1H), 6.49 (bs, 2H), 5.73 (s, 1H), 4.35 (dd, $J = 4.8$, 9.2 Hz, 1H), 4.05 (t, $J = 6.4$ Hz, 1H), 3.90 (dd, $J = 7.0$, 15.4 Hz, 2H), 3.78 (dd, $J = 2.2$, 13.0 Hz, 1H), 3.53 (s, 3H, OCH_3), 0.97–1.08 (m, 28H), 0.04 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 157.3, 154.5, 151.0, 134.7, 117.4, 87,1, 83.9, 81.2, 71.2, 61.3, 59.5, 18.6, 18.4, 18.3, 18.3, 18.2, 18.2, 14.6, 14.5, 14.5, −9.04; HRMS, calcd for $C_{24}H_{43}N_5O_5Si_2$ ($M + Na^+$) 560.2695, found 560.2688.

20. The excellent regioselectivity of alkylation using HMDS may be explained by considering that this base reacts transiently with the guanine base to produce the corresponding O^6-TMS ether. The latter compound is known to be hydrolytically

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