NUCLEOSIDES, NUCLEOTIDES & NUCLEIC ACIDS
Vol. 22, Nos. 5–8, pp. 583–587, 2003

$MDPSCL_2$: A New Protecting Group for Chemoselective Synthesis of 2′-*O*-Alkylated Guanosines

Suetying Chow,[1] Ke Wen,[1] Yogesh S. Sanghvi,[2] and Emmanuel A. Theodorakis[1,*]

[1]Department of Chemistry and Biochemistry, University of California, San Diego, La Jolla, California, USA
[2]ISIS Pharmaceuticals Inc., Carlsbad Research Center, Carlsbad, California, USA

ABSTRACT

An improved strategy for the synthesis of 2′-*O*-methyl-guanosine (**6**) and 2′-MOE-guanosine (**8**) is reported. The regioselectivity of the alkylation was attained using a novel silicon-based protecting group, methylene-bis (diisopropyl-silylchloride) ($MDPSCl_2$, **2**). The alkylation proceeded in a chemoselective manner using NaHMDS as the base and MeCl or MOE-Br as the appropriate electrophiles.

INTRODUCTION

Antisense oligonucleotides constitute a promising class of therapeutic agents with potential applications against a variety of inflammatory, infectious, cardiovascular and malignant diseases. (For selected recent reviews in this area, see Ref.[1].) These

*Correspondence: Emmanuel A. Theodorakis, Department of Chemistry and Biochemistry, University of California, San Diego, 9500 Gilman Drive, La Jolla, CA 92093-0358, USA; E-mail: etheodor@ucsd.edu.

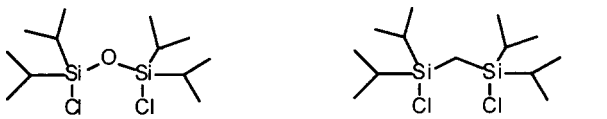
DOI: 10.1081/NCN-120021959
1525-7770 (Print); 1532-2335 (Online)

compounds consist of short sequences of partially modified nucleic acids that are designed to bind in a selective way to their target mRNA, thereby modulating the expression of pathogenic proteins. Among the different strategies pursued for the structural modifications of nucleic acids,[2] alkylations of the 2′-OH group offer several advantages including an improved binding affinity to the target RNA, enhanced nuclease resistance and chemical stability against depurination, improved pharmacokinetics and decreased toxicity. (For a recent monograph on this topic, see Ref.[3].) In addition, 2′-*O*-alkylated nucleosides maintain unaltered the basic scaffold of the parent nucleic acid, ensuring the overall retention of base-pair recognition, which in turn is required for target selectivity.

RESULTS AND DISCUSSION

The remarkable value of 2′-*O*-alkylated nucleosides has provoked a considerable effort directed toward the development of efficient synthetic approaches. (For selected synthetic routes to 2′-*O*-methylated nucleosides, see Ref.[4]. For selected synthetic routes to 2′-*O*-MOE nucleosides, see Ref.[5].) Although some of these strategies have been successfully applied to the synthesis of pyrimidine containing 2′-*O*-modified nucleic acids, they are much less efficient for purine analogs. (For a recent account on this topic, see Ref.[6].) In the latter case, the problems stem from concurrent side-alkylations at both the ribose unit and the purine nucleobase, often requiring the use of multiple protecting groups. The most popular strategies for protection of the ribose unit involve the use of 1,1,3,3-tetraisopropyl-1,3-dichlorodisilyloxane ($TIPDSCl_2$, **1**),[7] or $TBSCl_2$.[8] Although such silanes can protect in a selective manner the 3′- and 5′-hydroxyl groups, they are labile under strong alkaline conditions that are required for the subsequent 2′-*O*-alkylation.

To overcome the problems stemming from the fragility of **1** under basic conditions we developed a new silicon-based protecting group, referred to herein as methylene-bis-(diisopropylsilyl chloride), ($MDPSCl_2$, **2**).[9] 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 bridges the two silicon groups. This effect could be overcome using a methylene unit, instead of oxygen, to bridge the two silicon atoms. Being isosteric to **1**, we theorized that **2** would exhibit a similar reactivity and selectivity profile as **1** but also display an extended stability under basic conditions. Herein we describe an application of **2** to a chemoselective synthesis of 2′-*O*-methylguanosine (**6**) and 2′-*O*-methoxyethyl (MOE) guanosine (**8**) (Sch. 1).



1: tetraisopropyl-dichlorodisilyloxane ($TIPDSCl_2$)

2: methylene-bis-(diisopropylsilyl chloride) ($MDPSCl_2$)

Figure 1.

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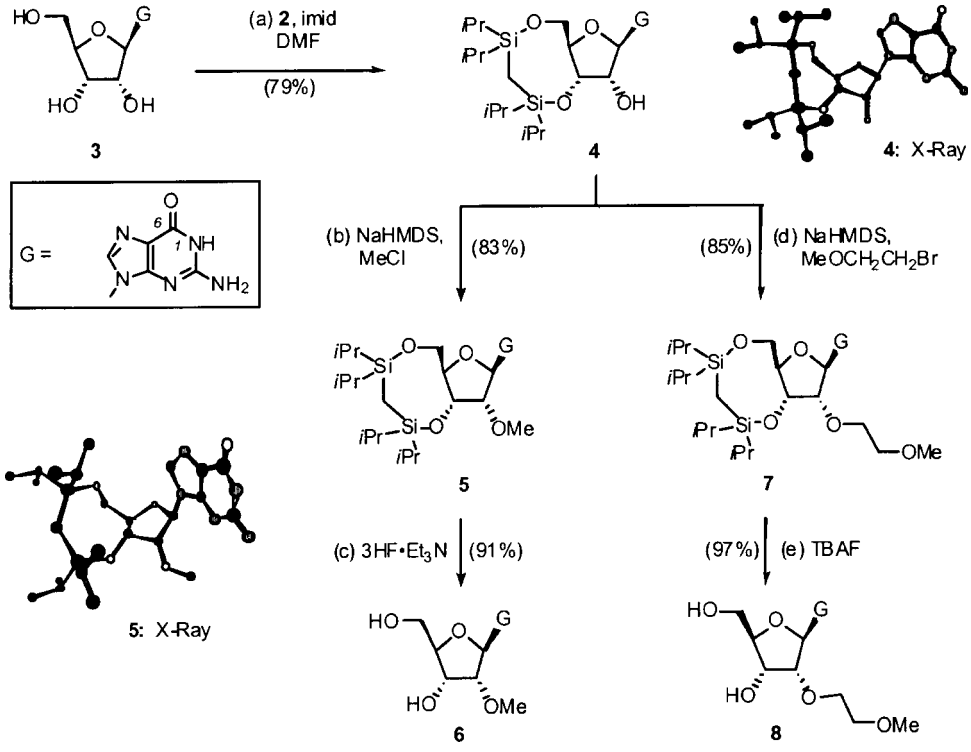
Treatment of guanosine (**3**) with a small excess of disilane **2** in DMF using imidazole as the base afforded compound **4** in 79% yield. The rate of this reaction was slower than the one using disilane **1** as the protecting group, which may be explained by considering that the inductive effect of the oxygen in compound **1** activates the silicon toward nucleophilic substitution. Nonetheless, the regioselectivity during the protection of **3** with **2** was comparable to that obtained with **1**, since in both cases the silicon atoms are hindered in a similar fashion by the presence of the isopropyl groups. Compound **4** was easily crystallized from methanol/dichloromethane and its structure was confirmed by a single crystal X-Ray analysis (Sch. 1).

In order to maximize the overall efficiency of this strategy we decided to study the 2′-*O*-methylation of **4** without protecting the nucleobase. Among the different bases examined, use of NaHMDS in combination with MeI at −20°C was found to produce a mixture of 2′-*O*-methylation along with *N*′-alkylation in a 4:6 ratio. Further optimization of this reaction led us to employ MeCl as a less reactive electrophile. In fact, best results for 2′-*O*-methylation were obtained when this reaction was performed in DMF at −40°C using 3 equivalents of NaHMDS and excess of



Scheme 1. Reagents and Conditions: (a) 1.15 equiv 2, 5.0 equiv imid, DMF, 0°C to 25°C, 5 h, 79%; (b) 3.0 equiv NaHMDS, $MeCl_{(g)}$, DMF, −40°C to −27°C, 5 h, 83%; (c) 1.0 equiv 3HF-Et_3N, THF, 35°C, 14 h, 91%; (d) 3.0 equiv NaHMDS, 0.3 equiv TBAI, 3.0 equiv $MeOCH_2CH_2Br$, DMF, −20°C, 3 h, 85%; (e) 1.0 equiv TBAF (1.0M in THF (1.0 M in THF), THF, 35°C, 5 h, 97%.

MeCl allowing the isolation of **5** in 83% yield. The structure of **5** was unambiguously established by a single crystal X-Ray study (Sch. 1). In a similar manner the synthesis of 2′-*O*-MOE guanosine (**7**) was pursued using NaHMDS in combination with MOE-Br and TBAI. In this case compound **7** was obtained in 85% yield.

The choice of NaHMDS as the base proved to be essential for the chemoselectivity observed during the alkylation. This selectivity could be explained by considering an initial reaction of HMDS with the nucleobase to produce the corresponding O^6-TMS ether. This may serve as a temporary protecting group guarding the nucleobase against alkylation. Such compound is hydrolytically unstable and will regenerate the free guanine upon aqueous extraction.

Deprotection of **5** and **7** was achieved with both TBAF and 3HF•Et_3N as the fluoride sources. This reaction was found to be considerable slower than the one performed in identical substrates protected with $TIPDSCl_2$ (**1**). Nonetheless, treating compound **7** with 1 equivalent of TBAF at 35°C afforded 2′-*O*-MOE guanosine (**8**) in 97% yield. Under similar conditions 2′-*O*-methylguanosine (**6**) was isolated together with a small amount of residual tetrabutyl ammonium salts in 95% overall yield. In our hands use of 3HF•Et_3N was advantageous since it produced compound **6** free of any residual contaminations in 91% yield without column chromatography.

CONCLUSION

In conclusion, we present herein an efficient and chemoselective protocol for the synthesis of 2-*O*-alkylated guanosine. Essential to this strategy is the use of $MDPSCl_2$ (**2**), an isostere of $TIPDSCl_2$ (**1**), that serves to protect the 5′ and 3′ hydroxy groups of the ribose moiety. Being more robust than **1**, silane **2** withstands the basic condition that are required during alkylation, thereby addressing the issues of regioselectivity. Use of NaHMDS as the base is also essential for the chemoselectivity of alkylation. An additional advantage of silane **2** is that it produces compounds that are highly crystalline and can be isolated without the need of column chromatography.

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