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Synthesis of 2′-*O*-Methoxyethylguanosine Using a Novel Silicon-Based Protecting Group

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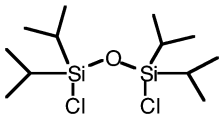
Abstract: A short and efficient synthesis of 2′-*O*-methoxyethylguanosine (**8**) is described. Central to this strategy is the development of a novel silicon-based protecting group ($MDPSCl_2$, **2**) used to protect the 3′,5′-hydroxyl groups of the ribose. Silylation of guanosine with **2** proceeded with excellent regioselectivity and in 79% yield. Alkylation of the 2′-hydroxyl group of **6** proceeded with methoxyethyl bromide and NaHMDS and afforded compound **7** in 85% yield, without any noticeable cleavage of the silyl protecting group and without the need to protect the guanine base moiety. Finally, deprotection of **7** was achieved using TBAF and produced **8** in 97% yield.

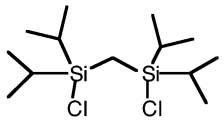
Modified oligonucleotides are currently under evaluation for their medicinal potential against a variety of viral, infectious, or cancer-related diseases and have marked the beginning of antisense strategies as effective therapeutic approaches.[1,2] Some of the common chemical modifications[3] include alkylations of the 2′-hydroxyl group of the ribose unit, giving rise to 2′-*O*-methyl[4] or 2′-*O*-*m*ethoxy*e*thyl (MOE) nucleotides.[5] Among them, the MOE nucleotides were found to exhibit high resistance to degradation by various nucleases and form hybrids of high thermal stability with complimentary RNA, rendering them suitable for a second generation of antisense constructs.[6,7]

Given the medicinal importance of 2′-*O*-MOE oligoribonucleotides, a considerable effort has been devoted toward their synthesis using a combination of chemical and enzymatic approaches. Although these strategies have been successfully implemented to the production of pyrimidine-containing 2′-*O*-MOE nucleosides,[8] they are much less efficient in the case of the purine analogues. In the latter case, the problems derive from the inherent reactivity of the purine bases, requiring the use of appropriate protecting groups and the need for selective protection of the C3′ and C5′ hydroxyl groups.[9] Efforts to overcome these problems have led to attempts to simultaneously protect the 3′- and 5′-hydroxyl groups, using tetraisopropyldichlorodisilyloxane ($TIPDSCl_2$, **1**)[10] or $TBSCl_2$.[11] However, both of these reagents proved to be labile during the strongly basic conditions required for methoxyethylation of the adjacent 2′-hydroxyl group. Sterically hindered organic bases, such as BEMP,[12] have also been tested, but their cost renders them unsuitable for large-scale application of the derived nucleosides.

Recognizing the significance of the above problem and the current limitations in the choice of protecting groups, we sought to develop a novel silicon-based protecting group for a 3′- and 5′-selective protection of the ribose scaffold. We theorized that the fragility of $TIPDSCl_2$ under basic conditions may be due to the inductive effect of the oxygen atom that interconnects the two silicon groups. Based on this hypothesis, we decided to synthesize and study compound **2**, in which a methylene unit is used to bridge the two silicon groups. Being isosteric to **1**, we expected that **2** would exhibit a similar reactivity and selectivity profile with **1** but display an extended stability under basic conditions.



1: tetraisopropyldichlorodisilyloxane ($TIPDSCl_2$)



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

The synthesis of compound **2** is shown in Scheme 1. Commercially available disilane **3** was converted to tetraalkylated silane **4** upon treatment with the ap-

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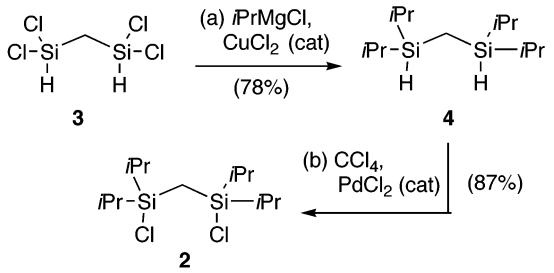
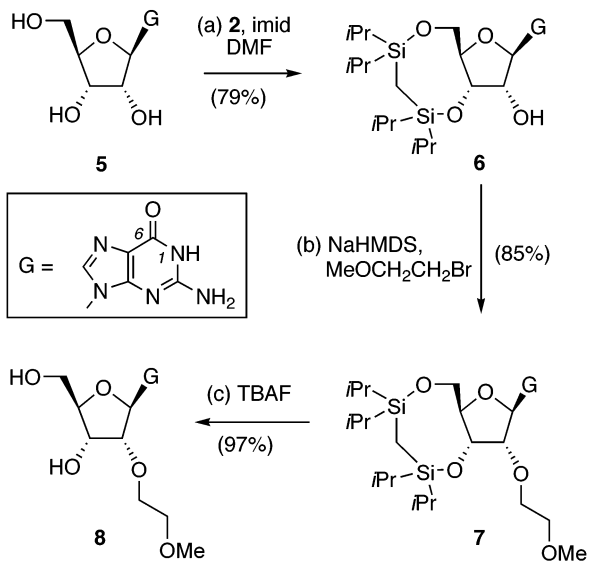
SCHEME 1[a]



[a] Reagents and conditions: (a) 4.4 equiv of *i*-PrMgCl (2.0 M in THF), 0.01 equiv **of** $CuCl_2$, THF, 65 °C, 5 h, 78%; (b) 1.0 equiv of CCl_4, 0.02 equiv of $PdCl_2$, 60 °C, 2 h, 87%.

SCHEME 2[a]



[a] Reagents and conditions: (a) 1.15 equiv of **2**, 5.0 equiv of imid, DMF, 0−25 °C, 5 h, 79%; (b) 3.0 equiv of NaHMDS, 0.3 equiv of TBAI, 3.0 equiv of $MeOCH_2CH_2Br$, DMF, −20 °C, 3 h, 85%; (c) 1.0 equiv of TBAF (1.0 M in THF), THF, 35 °C, 5 h, 97%.

propriate Grignard reagent in the presence of catalytic $CuCl_2$. Chlorination of silane **4** was achieved in refluxing carbon tetrachloride using catalytic $PdCl_2$.[13] Following filtration of the residual $PdCl_2$ under inert atmosphere, compound **2** was isolated after vacuum distillation (112−114 °C at 0.45 mmHg) in 68% combined yield.

The application of disilane **2** to the synthesis of 2′-*O*-MOE-guanosine is summarized in Scheme 2. Treatment of guanosine (**5**) with a slight excess of disilane **2** in the presence of imidazole produced the desired 3′,5′-protected nucleoside **6** in 79% yield.[14,15] As compared to similar protection with disilane **1**, the reaction with **2** was somewhat slower, presumably due to the decreased reactivity of the silyl chloride functionality. Nonetheless, the regioselectivity of the reaction was excellent and comparable to that observed for **1**. This is attributed to the steric effects of the isopropyl groups in **1** and **2** that propel the silicon chloride to react initially with the primary 5′-hydroxyl group and subsequently with the neighboring 3′-hydroxyl unit.[10] It is also worth mentioning that compound **6** can be easily crystallized, and its structure was further confirmed via a single-crystal X-ray analysis.[16]

TABLE 1. Effect of Base during Alkylation of 6

base (3.0 equiv)	*T* (°C)	TBAI (equiv)	results compd ratio **5**/**6**/**7**
NaH	25	0.1	3:7:0
NaH	0	N/A	2:8:0
t-BuOK	25	0.1	3:7:0
BEMP	25		0:0:others[a]
CsOH	0		9:1:0
LiHMDS	−20	0.3	no reaction
KHMDS	−20	0.3	1:8:0
NaHMDS	−10		1:8:1:others[b]
NaHMDS	−20	0.3	0:1:9[c]

[a] N alkylation(90%) together with dialkylation(10%) were observed. [b] 2′-MOE-alkylated product (10%) and some (<10%) cleaved material were formed. [c] A ratio of 1:9 of starting material/desired product was observed.

The conversion of **6** to compound **7** was examined under a variety of alkylation conditions summarized in Table 1. Use of sodium hydride, potassium *tert*-butoxide, and CsOH as base resulted in partial desilylation when the reaction was performed at 0−25 °C. In contrast to the above conditions, use of BEMP as the base did not result in desilylation but yielded N^1-alkylated product. Promising results were obtained when NaHMDS was examined as the base, leading predominantly to the desired alkylated product **7**. This reaction was further improved by adding a catalytic amount of TBAI and performing the alkylation at −20 °C. Under these optimized conditions, purified compound **7** was isolated in 85% yield. Interestingly, use of NaHMDS under the above conditions did not produce any N-alkylated adduct, thus eliminating the need to protect the nucleobase during alkylation. The excellent regioselectivity of alkylation under these conditions may be explained if we consider that HMDS reacts transiently with the guanine nucleus to produce the corresponding O^6-TMS ether.[17] Although this group is known to be unstable during isolation,[9,12] it could lead to a temporarily protected nucleobase, hence allowing alkylation exclusively at the 2′-hydroxyl group.[18] It is also worth mentioning that guanosine protected with disilane **1** underwent complete deprotection under the above conditions, supporting our hypothesis that the fragility of **1** derives from the

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(14) The effects of different bases (pyridine, imidazole, triethylamine, and lutidine) and temperature (0−70 °C) for the protection of **5** were investigated. Among them, imidazole was found to produce the best results.

(15) Under similar experimental conditions the selective 3′,5′ protection of other nucleic acids with **2** proceeded in 79−92% yield. See the Supporting Information for more details.

(16) For the X-ray structure of **6**, see the Supporting Information. To the best of our knowledge, this is the first crystal stucture of any 3′,5′-silylated nucleoside.

(17) Our efforts to identify by spectroscopic means the formation of O_6-TMS ether prior to aqueous extraction met with failure. However, a small peak corresponding at the molecular weight of the O_6-TMS protected alkylated nucleoside (MW = 653) was detected by LCMS analysis of the crude reaction mixture. We thank the reviewer for this suggestion.

inductive effect of the oxygen atom. Moreover, exposure of alkylated adduct **7** to excess NaHMDS even at 25 °C did not yield any deprotected material, indicating that compound **7** is stable to any base-induced desilylation.

The TBAF-induced desilylation of **7** was slower than the one performed in the identical substrate protected with **1**. Nonetheless, heating a solution of **7** in THF at 35 °C in the presence of 1 equiv of TBAF produced the desired product **8** in 97% yield. This deprotection was also achieved in the presence of 0.6 equivalents of TBAF in wet THF at 50 °C. In the latter case, however, the reaction was considerably slower (24 h) and produced **8** in 90% yield.[19]

In summary, we describe herein an efficient procedure for the synthesis of 2′-MOE-guanosine (**8**). Essential to our strategy was the development of a novel silicon-based protecting group ($MDPSCl_2$, **2**), which, being isosteric to $TIPDSCl_2$ (**1**), can selectively protect the 3′- and 5′-hydroxyl groups of guanosine and withstands the basic conditions required for alkylation of the 2′-hydroxyl group. This maneuver allowed the synthesis of **8** from guanosine (**5**) in three steps and 65% yield. An additional advantage of silane **2** over **1** is that it produces compounds that are highly crystalline and easily purified without the need of chromatographic techniques. We are currently exploring further applications of disilane **2** as a new and versatile protecting group.

Experimental Section

Bis(diisopropylsilyl)methane (4). A solution of bis(dichlorosilyl)methane (**3**) (10.0 g, 0.05 mol) and $CuCl_2$ (100 mg, 0.74 mmol) in 100 mL of THF was treated with isopropylmagnesium chloride (2.0 M in THF, 103 mL, 0.206 mol) added dropwise at 25 °C over a period of 2 h. After the addition, the mixture was refluxed for 5 h. Water (300 mL) was then added, and the organic phase was separated. The aqueous phase was extracted with hexanes (2 × 50 mL), and the combined organic phases were washed with water and brine and dried over $MgSO_4$. The organic solvents were evaporated under reduced pressure, and the residue was filtered through a 10 cm thick silica gel column using hexane as the eluant to afford product **4** (8.9 g, 0.036 mol, 78% yield): bp 80−82 °C/0.45 mmHg; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.61 (s, 2H), 0.99−1.10 (m, 28H), −0.35 (t, J = 3.9 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 19.0, 18.9, 11.9, −16.2.

Bis(diisopropylchlorosilyl)methane (2). To a mixture of bis(diisopropylsilyl)methane (**4**) (5.0 g, 0.02 mol) and $PdCl_2$ (72.6 mg, 0.41 mmol) was added dry CCl_4 (1.98 mL, 0.021 mol) in one portion. The reaction was kept under argon at 60 °C for 2 h. The $PdCl_2$ was filtered under argon, and the resulting mixture was distilled under reduced pressure to produce compound **2** (5.6 g, 0.02 mol, 87% yield) as a clear liquid. **2**: bp 112−114 °C/0.45 mmHg; ^{1}H NMR (400 MHz, C_6D_6) δ 1.20−1.10 (m, 4 H), 1.05−1.00 (m, 24 H), 0.28 (s, 2H); ^{13}C (100 MHz, C_6D_6) δ 17.6, 16.1, −3.5.

Compound 6. Guanosine (**5**) (1.0 g, 3.5 mmol) and imidazole (1.13 g, 17 mmol) were dried by coevaporation with pyridine (2 mL), dissolved in 20 mL of dry DMF, and treated with **2** (1.27 g, 4.1 mmol) added dropwise at 0 °C. The temperature was gradually increased to 25 °C. After 5 h of reaction, TLC showed no further change. The reaction mixture was poured into ice−water, and the precipitated white solid was filtered to afford compound **6** (1.45 g, 2.8 mmol, 79% yield). An analytical sample was obtained by crystallization from MeOH. **6**: R_f = 0.3 (silica, 10% methanol in dichloromethane); mp = 270−271 °C dec; $[\alpha]^{25}_D$ −15 (c = 0.3, CH_2Cl_2); IR (film) ν_{max} 1352, 1530, 1683, 3357; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.61 (s, 1 H), 7.76 (s, 1 H), 6.48 (s, 2 H), 5.67 (s, 1 H), 5.47 (d, J = 4.4 Hz, 1 H), 4.25 (dd, J = 3.6, 8.0 Hz, 1 H), 4.13 (t, J = 4.4 Hz, 1 H), 4.01 (t, J = 3.6 Hz, 1 H), 3.90 (d, J = 8.4 Hz, 1H), 3.81−3.77 (m, 1H), 0.96−1.06 (m. 28H), 0.02 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 157.1, 154.1, 150.8, 134.6, 117.1, 88.7, 81.0, 74.9, 70.5, 61.4, 18.5, 18.3, 18.3, 18.2, 18.1, 18.09, 18.0, 14.5, 14.4, 14.2, 14.1, −9.2; HRMS calcd for $C_{23}H_{41}N_5O_5Si_2$ (M + Na^+) 546.2538, found 546.2522.

Compound 7. To a solution of compound **6** (2.01 g, 3.8 mmol), $BrCH_2CH_2OCH_3$ (1.13 mL, 12.0 mmol), and TBAI (423 mg, 1.2 mmol) in 60 mL of DMF at −20 °C was added sodium bis-(trimethylsilyl)amide (1.0 M in THF, 11.5 mL, 11.5 mmol), and the mixture was stirred for 4 h under argon. After the reaction was quenched with methanol, the THF was evaporated and the residue was precipitated in ice to furnish compound **7** (1.89 g, 3.3 mmol, 85% yield). **7**: R_f = 0.4 (silica, 10% methanol in dichloromethane); $[\alpha]^{25}_D$ −30.3 (c = 0.6, CH_2Cl_2); mp 229−231 °C dec; IR (film) ν_{max} 1265, 1598, 1684, 2867, 2944, 3054; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.67 (s, 1H), 7.74 (s, 1H), 6.48 (s, 2H), 5.73 (s, 1H), 4.35 (dd, J = 4.6, 9.2 Hz, 1H), 4.10 (d, J = 4.8 Hz, 1H), 4.03 (d, J = 11.4 Hz, 1H), 4.00−3.84 (m, 3H), 3.68−3.84 (m, 2H), 3.40−3.52 (m, 1H), 3.45 (s, 3H), 0.96−1.10 (m, 28H), 0.00−0.10 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.6, 153.8, 150.3, 134.1, 116.6, 86.6, 82.0, 80.5, 71.4, 70.4, 70.0, 60.6, 58.1, 18.1, 18.0, 17.9, 17.7, 17.6, 17.6, 17.5, 17.4, 13.9, 13.9, 13.8, 13.8, −9.4; HRMS calcd for $C_{26}H_{47}N_5O_6Si_2$ (M + Na^+) 604.2957, found 604.2983.

2′-O-MOE G (8). To a solution of compound **7** (50 mg, 0.086 mmol) in THF at 25 °C was added TBAF (1 M in THF, 0.09 mL, 0.09 mmol), and the mixture was stirred at 35 °C for 5 h. The solvent was then evaporated under reduced pressure, and the residue was filtered in a short pad of silica gel using 10% methanol in dichloromethane to afford compound **8** (28.5 mg, 0.082 mmol, 97% yield). **8**: R_f = 0.1 (silica, 10% methanol in dichloromethane); $[\alpha]^{25}_D$ −51 (c = 1.4, CH_2Cl_2); mp 247−248 °C dec; IR (film) ν_{max} 1265, 3055, 3223, 3338, 3443; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.64 (s, 1H), 7.95 (s, 1H), 6.46 (s, 2H), 5.78 (d, J = 6.5 Hz, 1H), 5.06 (d, J = 5.0 Hz, 2H), 4.34 (t, J = 5.5 Hz, 1H), 4.24 (t, J = 4.0 Hz, 1H), 3.88 (d, J = 3.0 Hz, 1H), 3.66 (m, 3H), 3.51−3.60 (m, 2H), 3.38−3.40 (m, 1H), 3.32 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 156.8, 153.8, 151.4, 135.5, 116.7, 85.8, 84.4, 81.3, 71.1, 69.0, 68.9, 61.3, 58.0.

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Supporting Information Available: A table for protection of different nucleic acids with **2**, ^{1}H and ^{13}C NMR spectra for compounds **2**, **4**, and **6**−**8,** and X-ray data for compound **6**. This material is available free of charge via the Internet at http://pubs.acs.org.

JO025970E

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(19) At present, the mechanism of the desilylation reaction using subequivalent amounts of TBAF is not clear. Our data indicate that in addition to fluoride anion, water is important for this deprotection protocol.