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Transient Silylation of the Guanosine O6 and Amino Groups Facilitates *N*-Acylation

Yupeng Fan, Barbara L. Gaffney, and Roger A. Jones*

*Department of Chemistry and Chemical Biology, 610 Taylor Road, Rutgers,
The State University of New Jersey, Piscataway, New Jersey 08854*

jones@rutchem.rutgers.edu

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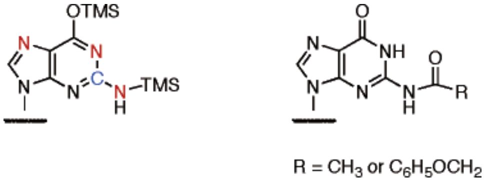
ABSTRACT



The formation of a guanosine derivative silylated at both the O6 and amino groups was identified by ^{15}N NMR. This intermediate allows facile reaction with acetyl chloride or phenoxyacetyl chloride to give in high yield the corresponding *N*-protected guanosine derivatives, suitable for use in RNA synthesis. The acetyl and phenoxyacetyl amino protecting groups are, respectively, 4 and 230 times more labile than the isobutyryl group to methylamine/ethanol deprotection.

Synthetic methods for the preparation of fully protected ribonucleosides with the labile *N*-acyl groups[1] that are preferred for RNA synthesis are not yet optimal, particularly for guanosine. Traditionally, the first step in protection of guanosine, adenosine, and cytidine has been *N*-acylation,[2] and prior transient protection of the sugar hydroxyls using the trimethylsilyl (TMS) group has become standard practice.[3] The subsequent challenge of protection of the 2′ hydroxyl has led to a variety of approaches and protecting groups.[4] At the present time, the *tert*-butyldimethylsilyl group (TBS) is probably the most widely used 2′ protecting group.[5] The approaches to introduction of the TBS group at the 2′ position vary widely in selectivity,[6] with the exception of a procedure reported recently by Beigelman[7] that affords complete specificity by simultaneous protection of the 3′ and 5′ hydroxyls with a di-*tert*-butylsilylene group.

We have used the Beigelman procedure to protect the guanosine amino group with an isobutyryl group in excellent yield, just as reported.[7] In contrast, when we attempted to introduce more labile amino protecting groups, such as acetyl (which we find to be 4 times more labile than isobutyryl) or phenoxyacetyl (which we find to be 230 times more labile than isobutyryl), the reaction mixtures were dark and the yields were unsatisfactory. Yet acylation of guanosine with these labile groups, using TMS transient protection, is known to proceed smoothly.[1b−e] When we then explored treatment

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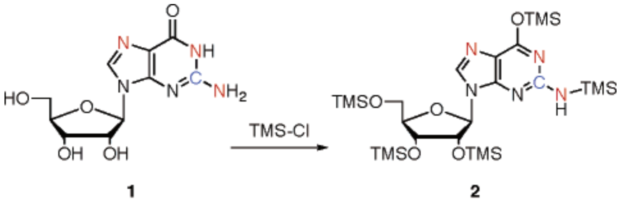
of the 3′,5′-di-*tert*-butylsilylene derivative **5** with TMSCl before acylation, we found that these acylations also proceeded smoothly, without generation of dark mixtures. Unlike acetylation of guanosine, which is problematic in part for reasons of solubility of the starting material and product, **5** is more soluble in a variety of solvents, so that reaction with TMSCl must be doing something beyond aiding solubility. One likely effect of TMSCl treatment is silylation of the O6, which is known to be susceptible to a variety of acylation, silylation, phosphorylation, and sulfonylation reactions.[8]

To determine whether O6 silylation was taking place upon TMSCl treatment, we monitored by ^{15}N NMR the reaction of [2-^{13}C-1,7,NH$_2$-^{15}N$_3$]-guanosine[9] (**1**) with TMSCl (Scheme 1). Upon addition of approximately 10 equiv[10] of TMSCl, the insoluble **1** quickly dissolved and ^{15}N NMR showed, in addition to the expected protonated N1 resonance at 153 ppm,[11] a new peak of approximately equal size at 217 ppm, both of which were broad. A chemical shift greater than 200 ppm would be expected for the unprotonated sp^2 N1 that would be formed upon O6 derivatization.[11] With the addition of approximately 10 more equiv of TMSCl to ensure complete reaction, the peak at 153 ppm disappeared, while the broad peak at 217 ppm increased. These results are consistent with silylation of O6.

Scheme 1



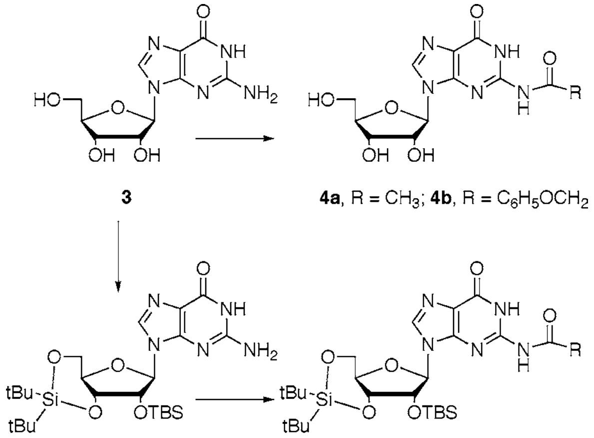
1 **2**

N = ^{15}N; C = ^{13}C

In addition to the expected changes in the N1 resonance, we also observed formation of a new moderately broad signal at 85 ppm, with a corresponding decrease in intensity of the amino resonance at 75 ppm.[11] After addition of the first portion of TMSCl, both were present; with the addition of the second portion of TMSCl, the original peak at 75 ppm was greatly reduced, while the one now at 86 ppm became much sharper. The 86 ppm resonance could in principle result from the O6 derivatization, although amino derivatization with one or two TMS groups would be more likely. The issue was resolved by an undecoupled spectrum, in which the 86 ppm resonance appeared as a doublet of doublets, due to coupling to ^{13}C2 and the remaining amino proton (J_{NH} = 76 Hz, J_{NC} = 17 Hz), establishing that the chemical shift change is due to a single TMS group on the amino group. These data thus demonstrate formation of an intermediate (**2**) that is silylated at both the O6 and amino groups.

Silylation of the O6 and amino groups appears both to prevent O6 acylation, which can lead to degradation to polar impurities and dark colors, and to accelerate amino acylation, as the reaction requires only 1.1 equiv of reagent with no need for hydroxybenzotriazole.[1e] Our acylation procedure (Scheme 2) for either guanosine (**3**) or 3′,5′-*O*-di-*tert*-butylsilylene-2′-*O*-TBS-guanosine (**5**) takes place in a cold mixture of pyridine and methylene chloride. Sequential addition of either 9 equiv of TMSCl for **3** or 6 equiv of TMSCl for **5**, as well as 1.1 equiv of acid chloride, is followed by quenching with methanol (for **4a**,**b**) or aqueous sodium bicarbonate (for **6a**,**b**) to give crystalline products in yields of 98% for *N*-acetylguanosine (**4a**) and *N*-phenoxyacetylguanosine (**4b**), 82% for *N*-acetyl-3′,5′-*O*-di-*tert*-butylsilylene-2′-*O*-TBS-guanosine (**6a**), and 90% for *N*-phenoxyacetyl-3′,5′-*O*-di-*tert*-butylsilylene-2′-*O*-TBS-guanosine (**6b**).

Scheme 2



3

4a, R = CH$_3$; **4b**, R = C$_6$H$_5$OCH$_2$

5

6a, R = CH$_3$; **6b**, R = C$_6$H$_5$OCH$_2$

To compare deprotection rates of the guanine *N*-isobutyryl, acetyl, and phenoxyacetyl groups, 9 μmol samples of **6a**, **6b**, and the corresponding isobutyryl derivative[7] were dissolved in 1.4 mL of a solution that was 1.3% methylamine, 2.6% ethanol, and 96% methylene chloride by weight. The

Table 1. Deprotection of *N*-Acylguanosine Derivatives

N-acyl group	time required for 50% deprotection
isobutyryl	18 h
acetyl	4.5 h
phenoxyacetyl	4.7 min

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[9] Zhao, H.; Pagano, A. R.; Wang, W.; Shallop, A.; Gaffney, B. L.; Jones, R. A. *J. Org. Chem.* **1997**, *62*, 7832–7835.

[10] The very small amount of TMSCl used was difficult to measure accurately; therefore, 10 equiv is only approximate and should be regarded as an upper limit.

[11] Levy, G. C. *Nitrogen-15 Nuclear Magnetic Resonance Spectroscopy*; John Wiley & Sons: New York, 1979.

samples were monitored over 20 h by reversed-phase HPLC. The time required for 50% completion was determined by integration at 254 nm giving the data shown below in Table 1.

NMR of specifically labeled compounds is a straightforward method for monitoring formation of unstable intermediates such as **2**. The ^{15}N NMR experiments reported here are the first uses of this approach to obtain direct evidence for transient derivatization of the guanine O6 and amino groups. The O6 derivatization is consistent with known reactivity of the O6 position, including reaction with other silyl compounds.[8e] We are not aware of previous reports of silylation of the guanine amino group, at least under mild conditions analogous to those employed here. These NMR results provide new insight into guanine reactivity, and the experimental procedures for *N*-acylation provide a new, high-yield route for preparation of these important compounds.[12,13]

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Supporting Information Available: Spectra (^{1}H NMR, ^{13}C NMR, ^{15}N NMR, MS, and UV). This material is available free of charge via the Internet at http://pubs.acs.org.

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(12) **Preparation of 4a and 4b.** Guanosine hydrate (**3**, 1.5 g, 5 mmol) was dried three times by azeotropic evaporation of pyridine, the last time leaving 25 mL. Dry dichloromethane (100 mL) was added under N_2, and the mixture was cooled in an ice bath with stirring. TMSCl (5.7 mL, 45 mmol, 9 equiv) was added over 2 min, and the flask was removed from the ice bath and allowed to stir for 2 h, by which time it had become homogeneous. The flask was then cooled again in an ice bath, and acetyl chloride (0.39 mL, 5.5 mmol, 1.1 equiv) or phenoxyacetyl chloride (0.76 mL, 5.5 mmol, 1.1 equiv) was added over 10 min. The mixture was stirred at 0 °C for 1.5 h with acetyl chloride or for 3 h with phenoxyacetyl chloride. Methanol (20 mL) was then added, and the solution was stirred at room temperature for 12 h to complete the desilylation. The mixture was evaporated to an oil, and the product was crystallized. For *N*-acetylguanosine (**4a**), 100 mL of methanol was added and the mixture was stirred at 0 °C for 1 h and filtered to give pure product in 98% yield: mp 157−159 °C. UV $\lambda_{\max}$ 259 nm with shoulder at 279 nm. ^{1}H NMR (DMSO, 300 MHz): δ 12.04 (s, 1H), 11.71 (s, 1H), 8.26 (s, 1H), 5.80 (d, J = 5.7 Hz, 1H), 5.70−4.60 (br, 3H), 4.43 (t, J = 5.4 Hz, 1H), 4.13 (t, J = 4.2 Hz, 1H), 3.90 (q, J = 3.7 Hz, 1H), 3.64 (dd, J = 12 Hz, J = 4.0 Hz, 1H), 3.54 (dd, J = 12 Hz, J = 3.9 Hz, 1H), 2.18 (s, 3H). ^{13}C NMR (DMSO, 75.4 MHz): δ 173.4, 154.8, 148.7, 148.1, 137.4, 120.0, 86.6, 85.2, 73.8, 70.2, 61.1, 23.9. Anal. Calcd for $C_{12}H_{15}N_5O_6 \cdot 1.25CH_3OH$: C, 43.56; H, 5.52; N, 19.17. Found: C, 43.67; H, 5.36; N, 19.13. For *N*-phenoxyacetylguanosine (**4b**), residual pyridine was removed by partial evaporation three times with water, and the mixture was then stirred in about 50 mL of water at 0 °C for 1 h and filtered to give pure product in 98% yield: mp 159−160 °C. UV $\lambda_{\max}$ 260 nm with shoulder at 276 nm. ^{1}H NMR (DMSO, 300 MHz): δ 11.83 (s, 1H), 11.78 (s, 1H), 8.29 (s, 1H), 7.35−7.28 (m, 2H), 7.02−6.95 (m, 3H), 5.82 (d, J = 5.7 Hz, 1H), 5.70−4.90 (br, 3H), 4.87 (s, 2H), 4.45 (t, J = 5.3 Hz, 1H), 4.14 (t, J = 4.2 Hz, 1H), 3.92 (q, J = 3.9 Hz, 1H), 3.65 (dd, J = 12 Hz, J = 4.2 Hz, 1H), 3.55 (dd, J = 12 Hz, J = 4.1 Hz, 1H). ^{13}C NMR (DMSO, 75.4 MHz): δ 170.8, 157.4, 154.8, 148.5, 147.2, 137.7, 129.4, 121.2, 120.3, 114.4, 86.7, 85.3, 74.0, 70.2, 66.2, 61.1. Anal. Calcd for $C_{18}H_{19}N_5O_7$: C, 51.80; H, 4.59; N, 16.78. Found: C, 51.62; H, 4.42; N, 16.42.

(13) **Preparation of 6a and 6b.** 3′,5′-*O*-Di-*tert*-butylsilylene-2′-*O*-TBS-guanosine (**5**, 2.69 g, 5 mmol) was dried three times by azeotropic evaporation of pyridine, the last time leaving 25 mL. Dry dichloromethane (125 mL) was added under N_2, and the mixture was cooled in an ice bath with stirring. TMSCl (3.8 mL, 30 mmol, 6 equiv) was added over 2 min, and the mixture was stirred at 0 °C for 15 min, by which time it had become homogeneous. Acetyl chloride (0.39 mL, 5.5 mmol, 1.1 equiv) or phenoxyacetyl chloride (0.76 mL, 5.5 mmol, 1.1 equiv) was added over 10 min. The mixture was stirred at 0 °C for 1.5 h with acetyl chloride or for 3 h with phenoxyacetyl chloride. The solution was then poured into 150 mL of 5% aq $NaHCO_3$, and the organic layer was isolated. It was washed with an additional 150 mL of 5% aq $NaHCO_3$ and then concentrated to a thick oil. *N*-Acetyl-3′,5′-*O*-di-*tert*-butylsilylene-2′-*O*-TBS-guanosine (**6a**) was crystallized by addition of 100 mL of ethyl ether and stirring in an ice bath for 3 h and filtered to give pure product in 82% yield: mp 174−176 °C, softens 157 °C. UV $\lambda_{\max}$ 259 nm with shoulder at 277 nm. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 11.93 (s, 1H), 8.41 (s, 1H), 7.66 (s, 1H), 5.76 (s, 1H), 4.45 (dd, J = 9.2 Hz, J = 4.8 Hz, 1H), 4.36 (d, J = 4.4 Hz, 1H), 4.25 (dd, J = 9.2 Hz, J = 4.4 Hz, 1H), 4.15 (td, J = 9.8 Hz, J = 4.8 Hz, 1H), 3.96 (t, J = 9.8 Hz, 1H), 2.28 (s, 3H), 1.05 (s, 9H), 1.01 (s, 9H), 0.88 (s, 9H), 0.10 (s, 3H), 0.09 (s, 3H). ^{13}C NMR ($CDCl_3$, 75.4 MHz): δ 171.9, 155.6, 147.7, 147.5, 136.8, 121.8, 91.6, 76.4, 76.1, 74.7, 68.0, 27.7, 27.3, 26.1, 24.8, 23.1, 20.6, 18.6, −4.0, −4.7. Anal. Calcd for $C_{26}H_{45}N_5O_6Si_2$: C, 53.86; H, 7.82; N, 12.08. Found: C, 53.79; H, 7.78; N, 12.02. *N*-Phenoxyacetyl-3′,5′-*O*-di-*tert*-butylsilylene-2′-*O*-TBS-guanosine (**6b**) was crystallized by addition of 30 mL of ethyl ether, followed by 20 mL of hexane, and stirring in an ice bath for 1 h and filtered to give pure product in 90% yield: mp 163−164 °C; UV $\lambda_{\max}$ 260 nm with a shoulder at 276 nm. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 11.74 (s, 1H), 8.91 (s, 1H), 7.70 (s, 1H), 7.36 (m, 2H), 7.10 (m, 1H), 6.96 (m, 2H), 5.81 (s, 1H), 4.69 (s, 2H), 4.50 (dd, J = 9.2 Hz, J = 5.2 Hz, 1H), 4.39 (d, J = 4.4 Hz, 1H), 4.21 (td, J = 10 Hz, J = 5.2 Hz, 1H), 4.12 (dd, J = 9.6 Hz, J = 4.4 Hz, 1H), 4.00 (t, J = 9.8 Hz, 1H), 1.02 (s, 9H), 1.01 (s, 9H), 0.93 (s, 9H), 0.14 (s, 3H), 0.13 (s, 3H). ^{13}C NMR ($CDCl_3$, 75.4 MHz): δ 169.4, 156.3, 155.2, 146.9, 146.2, 136.7, 130.2, 123.4, 122.8, 114.9, 92.2, 76.3, 76.1, 74.8, 68.0, 67.2, 27.7, 27.3, 26.2, 23.1, 20.7, 18.7, −3.8, −4.6. Anal. Calcd for $C_{32}H_{49}N_5O_7Si_2$: C, 57.20; H, 7.35; N, 10.42. Found: C, 56.80; H, 7.30; N, 10.23.