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Trimethylsilyl derivatives of N^4-Boc-cytosine and their effect on I_2-mediated nucleosidation of *O*-MTM ethers

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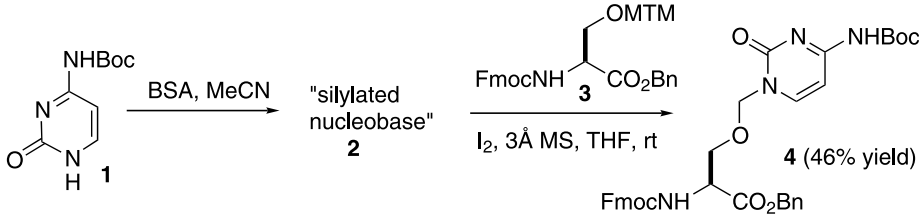
Abstract—Mono- and bis(trimethylsilyl) derivatives of N^4-Boc-cytosine were synthesized and characterized by ^{1}H NMR. Only the mono(trimethylsilyl)-N^4-Boc-cytosine participates in the iodine-mediated nucleosidation of *N*-Fmoc-*O*-methylthiomethyl serine benzyl ester to produce the cytosine nucleoamino acid, while the bis(trimethylsilyl) derivative failed to give any product. A tentative mechanistic explanation is proposed.

The silylation of nucleobases and their subsequent reaction with sugar derivatives is a general route to nucleosides and nucleoside analogues.[1] Trimethylsilyl derivatives of *N*-protected or parent nucleobases are quite soluble in non-polar solvents but are rapidly decomposed by alcohols or water to regenerate the starting nucleobases. Nishimura et al. reported the physical characterization and IR spectral data for trimethylsilyl derivatives of several parent and acyl-protected nucleobases.[2] The number of trimethylsilyl residues attached to the bases was calculated from the loss in weight upon decomposition by aqueous ethanol. Only the bis-silylated products were reported for cytosine and N^4-acetylcytosine. These workers claimed that it was not evident from the IR spectra whether the *N*- or *O*-trimethylsilyl form of bis(trimethylsilyl)-N^4-acetylcytosine was involved.[2b] Subsequently, researchers usually performed the glycosylation reaction without isolating the silylated nucleobases or any intermediate species. The ambiguous terminology 'silylated nucleobase' has been frequently used in the literature without any supporting data as to the actual chemical species involved. A bis-silylated intermediate was presumed to be involved in the glycosylation of N^4-benzoylcytosine[3] but mono-silylated N^4-acetylcytosine and N^4-benzoylcytosine have also been reported as reactive species in similar nucleosidation reactions.[4] After the introduction of N^4-isobutyryl cytosine by Sugiura et al.,[5] participation of a mono-silylated N^4-isobutyrylcytosine species was assumed by other researchers.[6] However, no definitive characterization data supporting the chemical constitution of the silylated intermediates were reported for N^4-benzoyl, acetyl, or isobutyrylcytosine.

The synthesis of a new cytosine derivative, N^4-Boc-cytosine, was reported in two European patents[7] and in our previous work.[8] We had described the synthesis of a cytosine-containing nucleoamino acid **4** (Eq. (1)) from *N*-Fmoc-*O*-methylthiomethyl serine benzyl ester (MTM) ether **3**, adopting a procedure that had been used to synthesize acyclic nucleoside derivatives.[9] The



Equation 1. Cytosine nucleoamino acid synthesis.

Keywords: silylation; N^4-Boc-cytosine; NMR; nucleosidation.

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silylated product **2** was not characterized during our initial studies. Instead, the solubility of the silylated nucleobase in non-polar solvents was noted as an indication of its formation. The final product **4** was characterized as the N^1-regioisomer by NMR analysis (HMBC and HMQC) of the fully deprotected nucleoamino acid.[10] A bis(trimethylsilyl) derivative was presumed to be the silylated species involved in the reaction. However, the procedure developed for silylation of N^4-Boc-cytosine **1** was capricious. The frequent failure of the subsequent nucleosidation reaction prompted us to investigate the nature of the silylated species involved in this reaction. Herein, we report the synthesis and characterization of silylated intermediates of N^4-Boc-cytosine and their roles in the subsequent iodine-mediated nucleosidation reaction.

Silylation of N^4-Boc-cytosine **1** was achieved using bis(trimethylsilyl)acetamide (BSA). This silylating reagent was first introduced in 1963,[11] and its properties were extensively studied by Klebe et al.[12] The exchange of one trimethylsilyl group from BSA to the acceptor molecule generates mono(trimethylsilyl)acetamide, which can be easily removed under vacuum. While exploring the BSA-mediated silylation, it was observed that after the addition of BSA (1.5–2.0 equiv.) to a suspension of N^4-Boc-cytosine **1** in CH_3CN over a period of 10 min, a clear solution was obtained. The reaction mixture was then allowed to stir at room temperature for another 4 h, at which point solvent removal under reduced pressure furnished a yellow oil. The oil was then dried under vacuum for several hours at 40 to 50°C (0.5–1.0 mm Hg) to afford a white solid that was soluble in THF. However, the formation of a white solid did not always accompany every reaction performed under similar conditions. In some runs, the yellow oil did not solidify even after drying overnight under vacuum and did not participate in the desired nucleosidation reaction. It was also observed that even the presence of residual oil along with the white solid leads to the formation of side products and a lower yield of product **4**. An alternative silylation method using the silylating reagent *N*,*N*′-hexamethyldisilazane (HMDS) along with a catalytic amount of $(NH_4)_2SO_4$ was also investigated.[13] However, this reaction requires a higher temperature (90–95°C) and longer time, especially if the reaction scale is large (>6 g of **1**). Under such conditions, the Boc group of N^4-Boc-cytosine **1** was cleaved, as indicated by NMR analysis of the decomposed product. In most cases, a white solid was obtained which was insoluble in THF, indicating the formation of cytosine. Vorbrüggen and Bennua also observed that *N*-acyl groups could be lost during the silylation of N^4-acetylcytosine with HMDS in pyridine or in the presence of catalytic amounts of trimethylsilyl chloride.[14]

It was crucial at this point to characterize the white solid and yellow oil obtained from the BSA reactions. The ^{1}H NMR spectra of silylated products were obtained in dry benzene-d_6 (freshly distilled from sodium) under Ar atmosphere (Fig. 1). Contrary to our assumption, NMR analysis showed the white solid that does produce **4** to be the mono-silylated product, 2-trimethylsilyl-N^4-Boc-cytosine **2** (Fig. 1, Panel A). Upon increasing the amount of BSA (4 equiv.) during silylation, pure bis-silylated product **5** was obtained as yellow oil (Fig. 1, Panel C). Although bis-silylated N^4-Boc-cytosine can be formulated as being either *N*-silylated **6** or *O*-silylated **5**, it is more likely that the compound exists as the *O*-silyl form because of the thermodynamic stability of the Si–O bond over the Si–N bond and resonance stabilization with the aromatic heterocycle (Scheme 1).[15] In addition, the NMR data shows only one signal from the protons of the trimethylsilyl groups. Two close signals were reported for the *N*,*O*-isomer of bis(trimethylsilyl)-benzamide.[16]

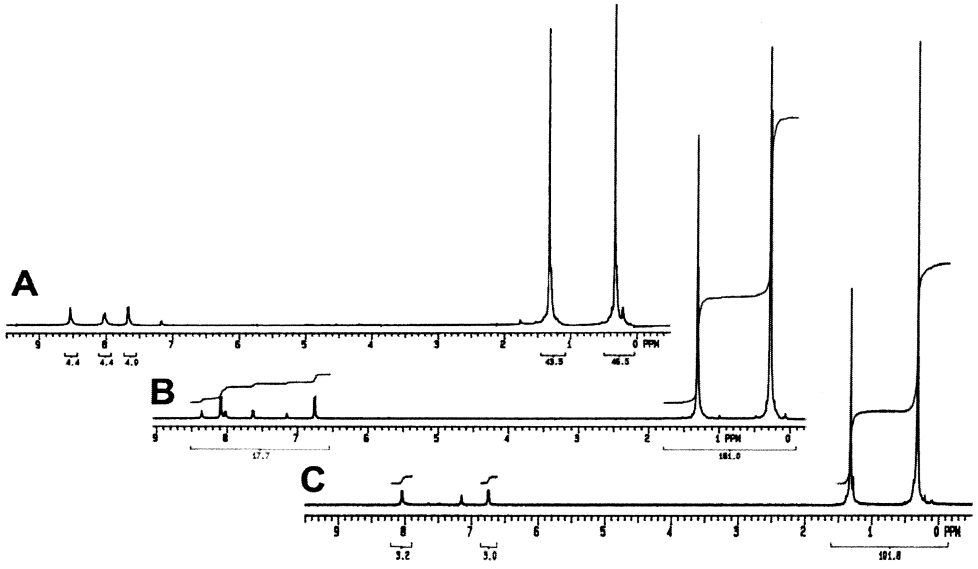
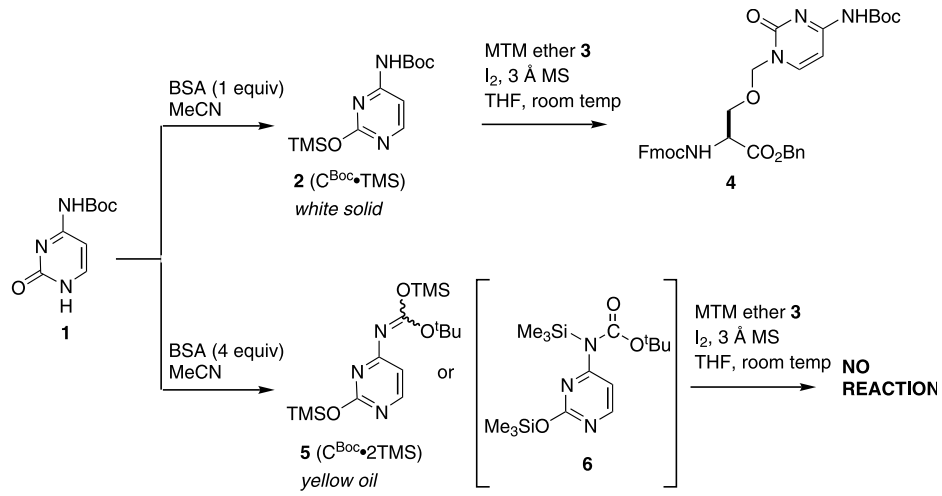


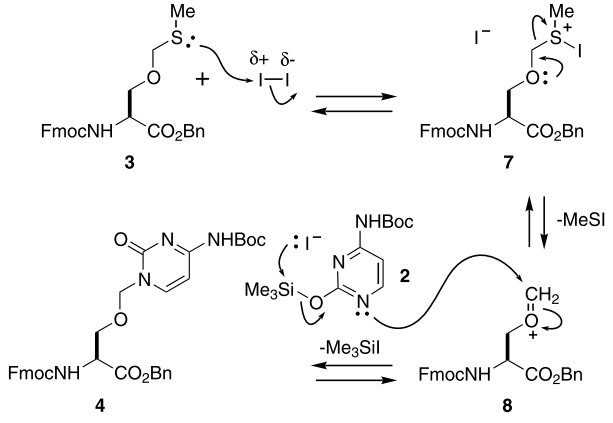
Figure 1. ^{1}H NMR (300 MHz, benzene-d_6) spectra of silylated N^4-Boc-cytosine products. Panel A: 2-(trimethylsilyl)-N^4-Boc-cytosine **2**. Panel B: mixture of 2,4-bis(trimethylsilyl)-N^4-Boc-cytosine **2** and 2-(trimethylsilyl)-N^4-Boc-cytosine **5**. Panel C: 2,4-bis(trimethylsilyl)-N^4-Boc-cytosine **5**; δ 8.05 (d, H-6), 6.74 (d, H-5), 1.35 (s, 9H), 0.32 (s, 18H).



Scheme 1. Synthesis of mono- and bis(trimethylsilyl)-N^4-Boc-cytosine.

If 2 equiv. of BSA were used, a mixture of mono- and bis-silylated products was evident from the sample's NMR spectrum (Fig. 1, Panel B). Unlike mono-silylated product **2**, the bis-silylated product **5** is a distillable liquid that could be transformed to mono-silylated product upon prolonged heating. We thus established that successful nucleosidation depends on the presence of pure mono-silylated nucleobase **2**. In order to obtain the mono-silylated product **2** in maximum yield and prevent the formation of the bis-silylated product **5**, a new protocol for the silylation reaction was developed.[17] Controlled addition of BSA was the key to achieving the selective formation of mono-silylated product. This silylation procedure was reproduced several times on a large-scale and was successfully applied to the subsequent nucleosidation reactions.[18]

Drawing on what is known about the glycosylation of silylated nucleobases, a plausible mechanism explaining why the mono-silylated product **2** participates in the nucleosidation (and why **5** does not) can be forwarded (Scheme 2). In the first step, the thiophilic iodine converts MTM ether **3** to an oxonium species **8**. Possibly assisted by iodide, the mono-silylated cytosine **2** can attack **8** to form the N^1-nucleoamino acid **4** and trimethylsilyl iodide. The overall reaction is slow and favors formation of N^1 regioisomer. The production of the N^1 isomer **4** plateaus after 48 h and approximately half of the starting material **3** can be recovered. Prolongation of reaction begins to produce N^3 isomer in detectable amounts (data not shown). Although it seems that the iodine must react with MTM ether **3** first to form the cation, it is imperative that this step occurs in the presence of **2**. Once the cation **8** forms, it reacts immediately with the monosilylated base to form the product. The signature of a successful nucleosidation is the persistence of a dark brown color during the reaction, which is indicative of molecular iodine. However, if iodine is added first, MTM ether **3** is converted to other unidentified by-products and **4** is not formed. It was also observed that the color of the reaction faded to yellowish brown, which indicates the consumption of



Scheme 2. Plausible mechanism of iodine-mediated nucleosidation.

iodine. In a similar reaction with bis(trimethylsilyl)-N^4-Boc-cytosine **5**, product **4** did not form and the starting material **3** was recovered. In this case, the color of the reaction mixture also fades to yellowish brown. This indicates that iodine may be reacting with **5**, thus preventing the formation of oxonium **8**. When mono- and bis-silylated N^4-Boc-cytosines co-exist in a reaction mixture, hardly any product can be isolated. In the case of nucleosidation with bis-silylated thymine (2,4-bis(trimethylsilyl)thymine, distilled as a colorless liquid), a good yield of thymine nucleoamino acid was obtained following similar reaction conditions.[8] The poisoning effect of **5** on the nucleosidation reaction may be related to the conjugation of an electron-rich isocyanate acetal with the heteroaromatic ring. This would be expected to enhance the system's reactivity toward iodine.

In conclusion, the mono- and bis(trimethylsilyl) derivatives of N^4-Boc-cytosine were synthesized and, for the first time, characterized by NMR spectroscopy. Only the mono-silylated product underwent I_2-mediated nucleosidation with *O*-MTM ether substrate to produce the cytosine-containing nucleoamino acid derivative.

The reaction pattern of these silyl derivatives helps in understanding the mechanism of this nucleosidation reaction.

Acknowledgements

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13. Compound **1** (2.24 g, 10.6 mmol) was heated (90–95°C) with HMDS (50 mL) and $(NH_4)_2SO_4$ (400 mg) under an Ar atmosphere for 4 h whereupon a clear solution was obtained. Upon distillation of excess HMDS under reduced pressure at 95°C, a bright white solid was obtained. The solid was dried under vacuum (0.5–1 mm Hg, 95°C) and used directly in the next reaction.
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17. **Improved procedure for the preparation of N^4-Boc-cytosine·TMS (2)**. A dry 250 mL, one-necked, round-bottom flask, equipped with a magnetic stirring bar and a rubber septum was charged with N^4-Boc-cytosine (**1**, 6.06 g, 28.7 mmol). After flushing this system with Ar, CH_3CN (121 mL) and BSA (7.10 mL, 28.7 mmol) were added. The resulting suspension was stirred at room temperature for 2 h, when another 3.55 mL (14.4 mmol) BSA was added. The reaction mixture became a clear light-yellow solution after 15 min. Stirring was continued at room temperature for another 2 h under an Ar atmosphere, at which point the contents was transferred to a flame-dried short-path distillation assembly (flushed with Ar). The CH_3CN was first distilled under reduced pressure at room temperature and then the residual liquid was heated at 40–50°C under reduced pressure (0.5–1.0 mm Hg) for another 2 h to afford a white solid. This white solid was dried under vacuum (0.5–1.0 mm Hg) at 40–50°C overnight during which time colorless needles formed on the condenser and eventually converted to a white solid after continued heating under vacuum. The white solid (8.13 g, 100 %) in the flask was used directly in the next reaction without further purification. ^{1}H NMR (300 MHz, benzene-d_6) δ 8.54 (s, N–H), 8.30 (d, $J=11.3$ Hz, 1H, H-6), 7.67 (d, $J=11.4$ Hz, 1H, H-5), 1.30 (s, 9H, $(CH_3)_3$C-OC-), 0.34 (s, 9H, $(CH_3)_3$SiO).
18. **Revised procedure for synthesis of Fmoc-S^{CBoc}-OBn (*L*-serine, *O*-[[4-[[(1,1-dimethylethoxy)carbonyl]amino]-2-oxo-1(2*H*)-pyrimidinyl]methyl]-*N*-[(9*H*-fluoren-9-ylmethoxy)-carbonyl]-phenylmethyl ester (4))**. A flask containing 0.63 g of crushed 3 Å MS was flame-dried under vacuum, flushed with Ar, then cooled to room temperature. A solution of Fmoc-Ser^{MTM}-OBn (**3**, 0.628 g, 1.32 mmol in 3 mL dry THF, 0.44 M) was transferred to the flask, followed by the addition of a solution of N^4-Boc-cytosine·TMS (**2**, 0.701 g, 2.47 mmol in 1.4 mL dry THF, 1.25 M) and a solution of iodine (0.334 g, 1.32 mmol in 4 mL of dry THF). The reaction mixture was stirred at room temperature under Ar atmosphere for 48 h, whereupon TLC analysis showed only the presence of the product **4** and some unconverted **3**. Workup and purification was similar to that described in Ref. 8.