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# An efficient preparation of protected ribonucleosides for phosphoramidite RNA synthesis

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**Abstract**—An efficient synthesis of protected ribonucleosides useful for phosphoramidite RNA synthesis is described. Di-*t*-butylsilylene group was employed for simultaneous protection of 3′- and 5′-hydroxyl functions of nucleoside. Subsequent silylation of free 2′-OH group followed by introduction of suitable protection on the base moiety, removal of cyclic silyl protection and tritylation of 5′-OH gave target compounds in 60–66% overall yield. 

Increasing demand for synthetic RNA-based therapeutics stimulated a search for efficient and practical routes toward protected ribonucleosides utilized in solid-phase RNA synthesis. Two approaches are currently used for the preparation of 5′-*O*-DMT-*N*-acyl-2′-*O*-TBDMS precursors: (a) the original synthesis[1] that starts with preparation of *N*-acyl-protected nucleosides using 'transient protection'[2] as the first step, followed by protection of 5′-hydroxy group as 4,4′-dimethoxytrityl ether and silylation of 2′-OH; (b) an elegant synthesis by Jones and co-workers that relies on regioselective phosphonylation–silylation of 5′-*O*-DMT-*N*-acyl-protected ribonucleosides followed by removal of the 3′-phosphonate group without migration of 2′-*O*-TBDMS-protection.[3]

The main drawback of the first methodology is the lack of silylation regioselectivity that results in formation of the mixture of 2′-*O*-, 3′-*O*- and 2′,3′-*O*-bis silyl ethers. It was shown that the use of silver nitrate or silver perchlorate in the silylation reaction can increase the 2′-*O*-regioselectivity.[4] However, in the case of guanosine derivatives, approximately 1:1 mixture of 2′- and 3′-silyl ethers is obtained even if silver nitrate or perchlorate additives are used. To the best of our knowledge, only column chromatography allows separation of pure 2′-*O*-silyl protected nucleosides. Although the yield in this step can be increased by subsequent equilibration of undesirable 3′-silyl compound to a mixture of 2′- and 3′-isomers, this step is very time and raw material consuming. Jones's methodology represents significant improvement but still requires chromatographic separation of 2(3′) phosphonate intermediates. In addition, high 2′-regioselectivity (8–9:1) in the phosphonylation–silylation reaction is observed only for purine ribonucleosides.[3]

We challenged ourselves with the task of developing general methodology for the preparation of 5′-*O*-DMT-*N*-acyl-2′-*O*-TBDMS precursors that would address the above shortcomings and would be amenable to scale-up. We argued that this objective can be achieved if one identifies suitable protecting group for simultaneous protection of 3′- and 5′-hydroxyl groups of nucleoside that would be stable during introduction of the 2′-*O*-TBDMS group and can then be removed selectively and without undesirable 2′→3′silyl migration.

Initially, we turned our attention to tetraisopropyldisiloxane protection (TIPDS)[5] that is widely used for simultaneous protection of 3′- and 5′-hydroxyls of ribonucleosides. Unfortunately, in our hands 2′-*O*-*tert*-butyldimethylsilyl ether did not withstand the conditions commonly used for removal of the TIPDS group ($n$-$Bu_4NF$/THF or $Et_3N{\cdot}3HF$/THF). As an alternative, the di-*tert*-butylsilylene group, first introduced by Trost[6] and Corey,[7] and later employed by Furusawa et al.[8] for simultaneous protection of 3′- and 5′-hydroxyls of nucleosides was investigated. This group appeared to be more attractive since it could be cleaved with fluoride ion under very mild conditions.[6,8,9]

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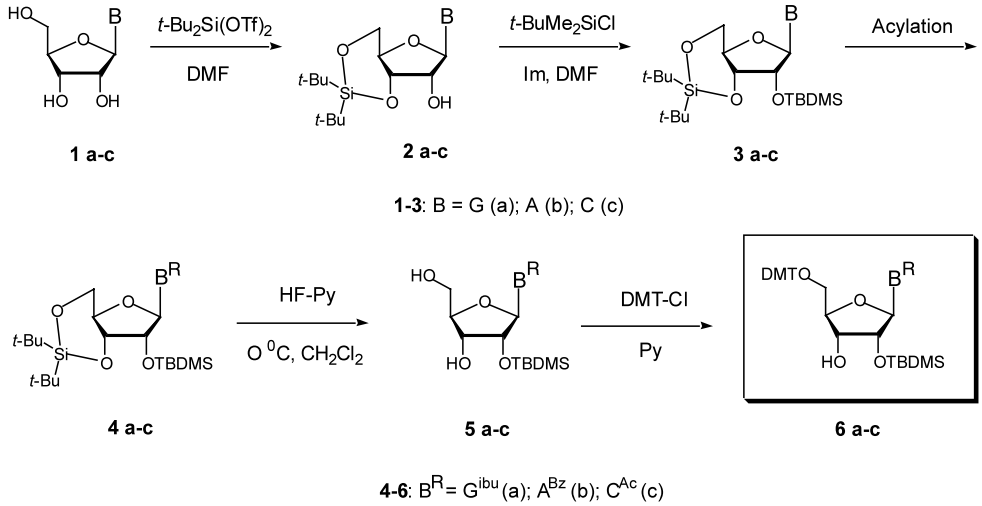


The reaction of nucleosides **1a–c** with 1.1 equiv. of di-*tert*-butylsilyl bis(trifluoromethanesulfonate) in DMF at 0°C proceeded smoothly and in high yield to afford 3′,5′-*O*-protected intermediates **2a–c**.[10] Without isolation from the reaction mixture, compounds **2a–c** were silylated affording derivatives **3a–c** as crystalline solids in 80–87% yield.

Because all sugar hydroxyls of **3a–c** are now protected, base amino functions can be easily derivatized with suitable protecting group. Acylation of $N^2$-position of guanosine derivative **3a** with 2 equiv. of isobutyryl chloride in a dichloromethane–pyridine mixture provided, after short methylamine treatment, crystalline **4a** in nearly quantitative yield. $N^6$-Benzoyladenosine derivative **4b** was prepared using benzoyl chloride and a similar sequence of reactions in 77% yield after crystallization from acetonitrile. Crude cytidine derivative **3c** was acetylated using 1.5 equiv. of acetic anhydride to furnish **4c** in 76% yield (overall from cytidine) after crystallization from ethylacetate.

Removal of di-*tert*-butylsilylene protection using HF–Py in dichloromethane at 0°C furnished compounds **5a–c** in a high yield. The purity of compounds **5a–c** after aqueous work up was high enough to proceed to the next step without additional purification. Reaction with dimethoxytrityl chloride in pyridine gave compounds **6a–c**;[11] no significant 2′→3′-silyl migration was observed under the reaction conditions. Guanosine and cytidine derivatives **6a** and **6c** were isolated by crystallization. For the isolation of adenosine derivative **6b**, silica gel chromatography on a short column was necessary. The overall yield of target compounds **6a–c** from the starting nucleosides **1a–c** was 60–66%. This is comparable to the yields obtained using the procedure reported by Jones.[3] Another advantage of this methodology is that all key intermediates as well as the final compounds **6a** and **6c** are crystalline, thus, eliminating chromatographic purification steps. In the synthesis of **6b** only one simple chromatography step is necessary. When the above procedure (without acylation step) was applied to the preparation of 5′-*O*-DMT-2′-*O*-TBDMS uridine the target compound was obtained in 71%.[11]

In conclusion, we have developed a general and efficient methodology for the preparation of 5′-*O*-DMT-*N*-acyl-2′-*O*-TBDMS-protected nucleoside precursors for phosphoramidite RNA synthesis. The utilization of this methodology for the synthesis of 2′-*O*-alkylribonucleosides, as well as 2′-*O*-*tri*-isopropylsyliloxymetyl[12] protected precursors for phosphoramidite RNA synthesis will be reported shortly.

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10. In the case of cytidine the addition of trifluoromethanesulfonic acid (1 equiv.) was necessary to improve the selectivity of the reaction.
11. The typical procedure is as follows: Guanosine (11.33 g, 40 mmol) was suspended in DMF (80 mL) and di-*tert*-butylsilyl ditriflate (14.3 mL, 44 mmol) was added drop-

wise during 15 min under stirring at 0°C. The resulting solution of **2a** was stirred at 0°C for 30 min and treated with imidazole (13.6 g, 200 mmol). The mixture was stirred for 5 min at 0°C and then at room temperature for 25 min. *tert*-Butyldimethylchlorosilane (7.24 g, 48 mmol) was added and the reaction was allowed to proceed at 60°C for 2 h. The precipitate of **3a** was separated by filtration, washed with cold methanol and dried under reduced pressure to give 18.81 g of **3a** (87.4%), mp 375–380°C (dec.). Isobutyryl chloride (10.4 mL, 100 mmol) was added dropwise to a stirred suspension of **3a** (26.89 g, 50 mmol) in dichloromethane (100 mL) and pyridine (30 mL) at 0°C. The reaction was allowed to proceed for 3 h at room temperature, then diluted with methanol (40 mL) and cooled in an ice bath. A solution of methylamine in ethanol (8 M, 25 mL, 200 mmol) was added slowly to the reaction mixture. After 30 min the reaction mixture was concentrated to give a slurry that was diluted with methanol (100 mL) and left for 2 h at 0°C. Precipitate was filtered off, washed with cold methanol and dried to give 29.26 g (96.2%) of **4a**, mp 218–223°C (dec.). Hydrogen fluoride–pyridine (Aldrich, 4 mL, 154 mmol) was carefully diluted with pyridine (25 mL) under cooling. The resulting solution was added slowly to a stirred at 0°C suspension of **4a** (24.36 g, 40 mmol) in dichloromethane (200 mL) and the reaction was allowed to proceed for 2 h at 0°C. The reaction mixture was washed with water followed by saturated sodium bicarbonate solution. The organic layer was dried over magnesium sulfate and evaporated to give crude **5a** as a semi-crystalline material. The latter was dissolved in pyridine (80 mL) and dimethoxytrityl chloride (14.91 g, 44 mmol) was added at 0°C. The reaction mixture was kept at 0°C overnight, quenched with anhydrous methanol (0.5 mL) and concentrated. The residue was partitioned between dichloromethane and water. The organic layer was washed with saturated sodium bicarbonate solution and dried over magnesium sulfate. The solvent was removed under reduced pressure and the residue was crystallized from dichloromethane (20 mL) and ether (200 mL) to provide **6a** as a white fine powder. Yield 24.16 g (78.4%). Mp 141–142°C. $^1$H NMR (400 MHz, DMSO-$d_6$), $\delta$: 12.16 (1H, s, NH), 11.70 (1H, s, NH), 8.19 (1H, s, H8), 7.30 (13H, m, Ph), 5.96 (1H, d, $J_{1',2'}=5.6$ Hz, H1′), 5.15 (1H, d, $J_{OH,3'}=5.2$ Hz, 3′-OH), 4.69 (1H, dd, $J_{2',1'}=5.6$ Hz, $J_{2',3'}=5.2$ Hz, H2′), 4.22 (1H, dd, $J_{3',2'}=5.2$ Hz, $J_{3',OH}=5.2$ Hz, H3′), 4.15 (1H, m, H4′), 3.80 (6H, s, OMe), 3.35 (2H, m, H5′), 2.83 (1H, m, *i*Bu-CH), 1.19 (3H, s, *i*Bu), 1.17 (3H, s, *i*Bu), 0.83 (9H, s, *t*-Bu), 0.06 (3H, s, Me), 0.05 (3H, s, Me).

**6b**: Overall yield 62% from **1b**. $^1$H NMR (400 MHz, DMSO-$d_6$), $\delta$: 11.28 (1H, s, NH), 8.74 (1H, s, H8), 8.67 (1H, s, H2), 8.11 (2H, d, $J=7.6$ Hz, Ph), 7.35 (12H, m, Ph), 6.93 (4H, m, Ph), 6.15 (1H, d, $J_{1',2'}=4.8$ Hz, H1′), 5.27 (1H, d, $J_{OH,3'}=5.6$ Hz, 3′-OH), 4.97 (1H, dd, $J_{2',1'}=4.8$ Hz, $J_{2',3'}=5.2$ Hz, H2′), 4.35 (1H, dd, $J_{3',2'}=5.2$ Hz, $J_{3',OH}=5.6$ Hz, H3′), 4.21 (1H, m, H4′), 3.79 (6H, s, OMe), 3.35 (2H, m, H5′), 0.84 (9H, s, *t*-Bu), 0.05 (3H, s, Me), 0.05 (3H, s, Me).

**6c**: Overall yield 60% from **1c**. Mp 222–223°C (EtOAc–hexanes). $^1$H NMR (400 MHz, DMSO-$d_6$), $\delta$: 10.94 (1H, s, NH), 8.37 (1H, d, $J_{6,5}=7.2$ Hz, H6), 7.40 (9H, m, Ph), 7.07 (1H, d, $J_{5,6}=7.2$ Hz, H5), 6.97 (4H, m, Ph), 5.80 (1H, d, $J_{1',2'}=1.6$ Hz, H1′), 5.19 (1H, d, $J_{OH,3'}=6.4$ Hz, 3′-OH), 4.18 (3H, m, H2′, H3′, H4′), 3.82 (6H, s, OMe), 3.41 (2H, m, H5′), 2.17 (3H, s, Ac), 0.95 (9H, s, *t*-Bu), 0.19 (3H, s, Me), 0.15 (3H, s, Me).

**5′-*O*-DMT-2′-*O*-TBDMS-U**: Overall yield 71% from uridine. NMR (DMSO-$d_6$), $\delta$: 11.45 (1H, s, NH), 7.82 (1H, d, $J_{6,5}=8.2$ Hz, H6), 7.44–7.33 (9H, m, Ph), 6.98 (4H, m, Ph), 5.83 (1H, d, $J_{1',2'}=4.4$ Hz, H1′), 5.37 (1H, d, $J_{5,6}=8.2$ Hz, H5), 5.20 (1H, d, $J_{OH,3'}=6.4$ Hz, 3′-OH), 4.28 (1H, dd, $J_{2',1'}=4.4$ Hz, $J_{2',3'}=4.8$ Hz, H2′), 4.15 (1H, m, H3′), 4.07 (1H, m, H4′), 3.82 (6H, s, $OCH_3$), 3.38 (2H, m, H5′), 0.93 (9H, s, *t*-Bu), 0.14 (3H, s, Me), 0.13 (3H, s, Me).

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