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Synthesis of 3'-Deuterated Pyrimidine Nucleosides Via Stereoselective Reduction of a Protected 3-Oxoribose

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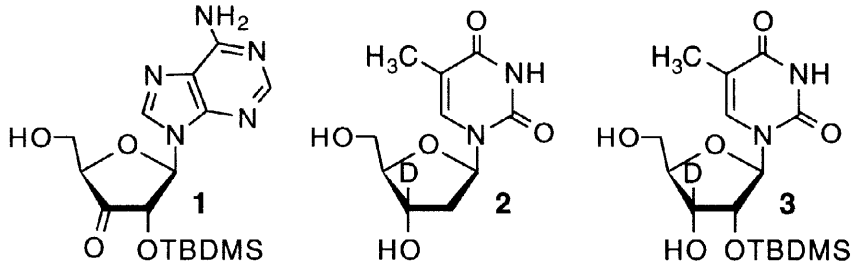
Abstract: Thymidine (**2**) and 5-methyl-2'-*O*-(*t*-butyldimethylsilyl)uridine (**3**) deuterated at the C3'-position were prepared with complete stereocontrol via NaB^2H_4 reduction of a 3-oxoribose derivative (**5**). Utilization of benzyl protecting groups in the deuterated glycosidation substrate facilitates the synthesis of ribonucleosides and 2'-deoxyribonucleosides with minimal protecting group manipulations.

Deuterated nucleosides incorporated in nucleic acids are useful as mechanistic probes and tools for structural (NMR) analysis.[1,2] General methods for the synthesis of nucleosides containing deuterium at the C1', C2', C4', or C5'-positions have recently been reported.[3-6] In addition, methods have been developed for selective isotopic incorporation at the *pro-R* or *pro-S* positions of the C2'- or C5'-methylene groups of the nucleosides. In particular, stereoselective reduction of a C3'-ketoadenosine derivative (**1**) was achieved by taking advantage of oxygen chelation.[7] Coordination of triacetoxyborohydride to the C5'-hydroxyl group of **1** enabled Robins et al. to overcome the steric bias towards preferential nucleophilic attack from the α-face of the nucleoside.[7,8] In this Letter we report an alternative method for the stereoselective synthesis of C3'-deuterated nucleosides in which the isotopic label is incorporated prior to glycosidic bond formation. Deuterated 2'-deoxyribonucleosides and ribonucleosides which can be transformed into phosphoramidites via standard procedures are prepared with minimal protecting group manipulations using the method described.



When devising the strategy for the synthesis of **2** and **3**, we sought to develop a route that met the following criteria:

1. Introduction of the isotopic label should proceed with high facial selectivity.
2. Glycosidation should be effected under Lewis acid mediated conditions with high selectivity.

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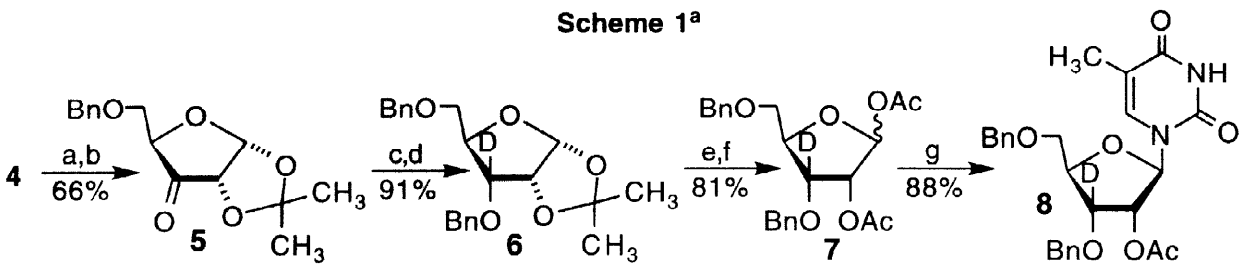
3. A single nucleoside glycosidation product would be carried on to monomers amenable to chemical DNA or RNA synthesis without deprotecting and reprotecting the hydroxyl groups.

Consequently, we developed a method (Scheme 1) in which attack by a nucleophile on the α-face of a readily available substrate (**5**) was blocked (criterion 1). The protecting groups for the C3- and C5-hydroxyls of the resulting ribose (**6**) were chosen so as to be stable to the conditions employed for deprotecting the C2-hydroxyl group in **8** (criterion 3); whereas the C2-hydroxyl protecting group (**7**) was chosen so as to ensure stereocontrol via neighboring group participation during the Lewis acid mediated glycosidation (criterion 2).

Scheme 1[a]



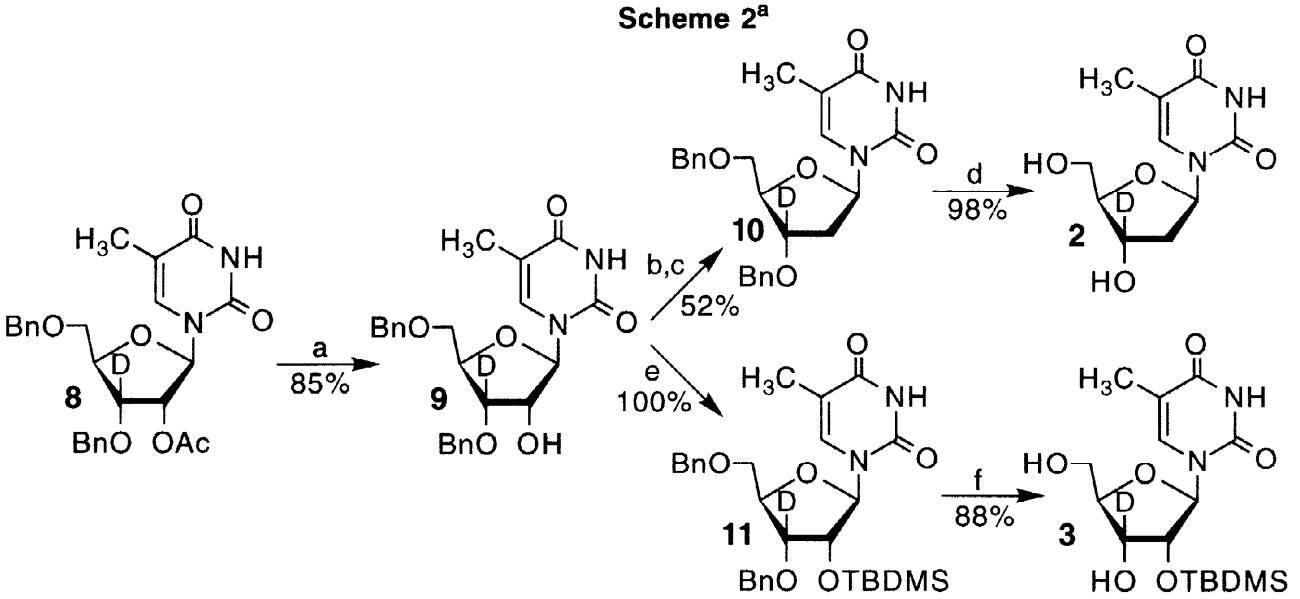
[a]Key: a) $(Bu_3Sn)_2O$, toluene, then BnBr, Bu_4NI (cat.) b) Triacetoxy periodinane c) NaB^2H_4, EtOH d) NaH, DMF, then BnBr e) TsOH (cat.), dioxane/H_2O f) Ac_2O, pyridine, CH_3CN g) Bis-(trimethylsilyl-acetamide, thymine, CH_3CN, then $SnCl_4$

In practice, ketone substrate **5** was obtained from commercially available 1,2-*O*-isopropylidene xylofuranose (**4**) via selective protection of the primary hydroxyl group utilizing the ring opening of the C3,C5-*O*-dibutylstannylene, followed by oxidation of the secondary alcohol.[9] Exclusive attack from the β-face by NaB^2H_4 yielded the crude reduction product, which upon benzylation yielded **6**. The extent of deuterium incorporation in **6**, as determined by ^1H NMR, was greater than 95%. The isopropylidene was converted into the glycosidation precursor (**7**) as a mixture of anomers via a two step procedure in which the intermediate diol was acetylated without purification.[8b] In our hands, this method yielded more consistent results than a previously reported one pot procedure.[10] Glycosidation was effected via standard Lewis acid mediated conditions using *in situ* silylated thymine.[11] The β-anomer of the protected nucleoside (**8**) was obtained as the exclusive product, demonstrating that the stereochemistry at the anomeric center in the substrate (**7**) is inconsequential, and that the directing effect of the acylated C2-hydroxyl group is responsible for the stereocontrol.

The orthogonal nature of the C2'-protecting group in **8** with respect to the benzyl groups used to mask the other nucleoside hydroxyl groups, greatly facilitate its transformation into **2** and **3**. Protected ribonucleosides obtained under Lewis acid glycosidation conditions are typically carried on to deoxyribonucleosides via a reliable five step procedure that involves cleavage of the three ester protecting groups and selective protection of the C3'- and C5'-hydroxyl groups as the disiloxane prior to deoxygenation.[12] An encouraging report recently appeared which streamlines the stereoselective synthesis of 2'-deoxyribonucleosides by using a photolabile acyl protecting group for the C2'-hydroxyl.[13] However, if one wishes to synthesize the respective ribonucleoside precursor suitable for transformation into the phosphoramidite substrate used in chemical synthesis, the fully deprotected nucleoside obtained from the initial glycosidation product must be carried through a series of protecting group

manipulations, some of which can be less selective than desired.[14] The combination of benzyl and acyl hydroxyl protecting groups eliminated any need for reprotection of the C3'- and C5'-hydroxyl groups. Cleavage with methanolic ammonia, or mild cyanide mediated cleavage of the acetate yields the last common intermediate in the synthesis of **2** and **3**.[15] Synthesis of C3'-^{2}H-thymidine (**2**) was completed via radical deoxygenation, followed by hydrogenolysis of the benzyl protecting groups.[12a] The benzyl protecting groups in **10** proved to be mildly resistant to hydrogenolysis, but were cleaved in good yield using Pd/C and H_2 (50 psi). Hydrogenolysis of the benzyl protecting groups proved to be the most difficult step in the synthesis of **3**, presumably due to the presence of the *t*-butyldimethylsilyl protecting group (**11**), which is commonly used as the 2'-hydroxyl protecting group in the chemical synthesis of RNA. The benzyl groups were untouched by H_2 using Pd/C, and the more reactive Rh/Al_2O_3 resulted in competitive hydrogenation of the thymine double bond. Ultimately, the benzyl groups in **11** were effectively cleaved under transfer hydrogenation conditions using ethanol as solvent.[16] Nucleosides **2** and **3** can be carried on to the requisite phosphoramidites used for chemical DNA and RNA synthesis, respectively, via standard dimethoxytritylation and phosphitylation procedures.

Scheme 2[a]



[a]Key: a) NH_3, MeOH b) Phenyl chlorothionoformate, DMAP, CH_2Cl_2 c) Bu_3SnH, AIBN (cat.), benzene, reflux d) 10% Pd/C, H_2 (50 psi), MeOH e) TBDMSCl, imidazole, DMF f) 10% Pd/C, 1,4-cyclohexadiene (4 eq.), EtOH

The above method provides a stereoselective source of thymidine (**2**) and 5-methyl-2'-*O*-(t-butyldimethylsilyl)uridine (**3**) deuterated at their C3'-positions. The method takes advantage of glycosidation methods that exploit neighboring group participation to control the stereoselectivity of this process, and then utilizes the lability of the same protecting group to transform the initially formed protected nucleoside (**8**) into suitable precursors for chemical DNA and RNA synthesis with fewer protecting group manipulations than required in other methods. Although the lability of purine nucleosides to the hydrogenolysis conditions may pose

a limitation to this method, the chemistry described above should be amenable to the synthesis of other pyrimidine nucleosides containing deuterium or tritium at the C3'-position.[16]

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References

1. Cook, G. P.; Greenberg, M. M. *J. Am. Chem. Soc.* **1996**, *118*, 10025.
2. a) Agrofoglio, L. A.; Jacquinet, J-C.; Lancelot, G. *Tetrahedron Lett.* **1997**, *38*, 1411. b) Tate, S.; Kubo, Y.; Ono, A.; Kainosho, M. *J. Am. Chem. Soc.* **1995**, *117*, 7277.
3. Cook, G. P..; Greenberg, M. M. *J. Org. Chem.* **1994**, *59*, 4704.
4. DeVoss, J. J.; Hangeland, J. J.; Townsend, C. A. *J. Org. Chem.* **1994**, *59*, 2715.
5. Ono, A. (M.); Ono, A.; Kainosho, M. *Tetrahedron Lett.* **1997**, *38*, 395.
6. Kawashima, E.; Aoyama, Y.; Sekine, T.; Miyahara, M.; Radwan, M. F.; Nakamura, E.; Kainosho, M.; Kyogoku, Y.; Ishido, Y. *J. Org. Chem.* **1995**, *60*, 6980.
7. Robins, M. J.; Sarker, S.; Samano, V.; Wnuk, S. F. *Tetrahedron* **1997**, *53*, 447.
8. a) Bender, S. L.; Moffett, K. K. *J. Org. Chem.* **1992**, *57*, 1646. b) Gurjar, M. K.; Kunwar, A. C.; Reddy, D. V.; Islam, A.; Lalitha, S. V. S.; Jagannadh, B.; Rao, A. V. R. *Tetrahedron* **1993**, *49*, 4373.
9. Cruzado, M. C.; Martin-Lomas, M. *Carbohydrate Research* **1988**, *175*, 193.
10. Gosselin, G.; Bergogne, M-C.; Imbach, J-L. in *Nucleic Acid Chemistry Improved and New Synthetic Procedures, Methods and Techniques Part Four*, Editors: Townsend, L. B.; Tipson, R. S.; John-Wiley, New York, 1991.
11. a) Vorbrüggen, H.; Bennua, B. *Chem. Ber.* **1981**, *114*, 1279. b) Dudycz, L. W.; Wright, G. E. *Nucleosides & Nucleotides* **1984**, *3*, 33.
12. a) Robins, M. J.; Wilson, J. S. *J. Am. Chem. Soc.* **1981**, *103*, 932. b) Markiewicz, W. T. *J. Chem. Res.* **1979**, (S), 24.
13. Park, M.; Rizzo, C. J. *J. Org. Chem.* **1996**, *61*, 6092.
14. Hakimelahi, G. H.; Proba, Z. A.; Ogilvie, K. K. *Can. J. Chem.* **1982**, *60*, 1106.
15. Asakura, J.; Tomura, T. *Nucleosides & Nucleotides* **1988**, *7*, 245.
16. Hanessian, S.; Liak, T. J.; Vanasse, B. *Synthesis* **1981**, 396.