A General Synthesis of C2′-Deuteriated Ribonucleosides

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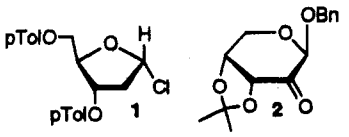
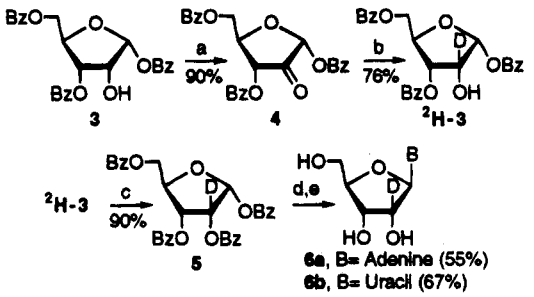
Nucleosides isotopically substituted at specific positions are useful for mechanistic and spectroscopic studies. In particular, isotopic substitution of the hydrogens at the C2′ position of nucleosides is useful for discerning the conformation of monomeric species and those within oligonucleotides.[1] In addition, nucleosides enriched at C2′ with deuterium (or tritium) are valuable mechanistic probes for enzymatic reactions, as well as in studies on the oxidative damage of nucleic acids.[2] Despite their usefulness, a general method for the synthesis of C2′ deuteriated (tritiated) ribonucleosides has not been reported. We wish to report a concise, general method for the synthesis of ribonucleosides substituted at C2′ with deuterium or tritium, in which isotopic incorporation is carried out prior to glycosidic nucleoside bond formation. After appropriate derivatization, the labeled carbohydrate is transformed into β-nucleosides via established methods.[3]



Synthetic methodology exists for the stereoselective isotopic enrichment at the pro-*S* or pro-*R* C2′ hydrogen of substituted deoxyriboses that are precursors for β-2′-deoxyribonucleosides.[4] General approaches that achieve dideuteriation at C2′ of deoxyribonucleosides wherein deuteriation is carried out prior to glycoside bond, formation have also been reported.[5] Recently, dideuteriation at C2′ (82.65% dideuteriation) was achieved via equilibration of 2-deoxyribonolactone in NaOMe/MeOD.[5a] The deuteriated lactone was carried on to isotopically enriched 2′-deoxyribonucleosides via the isotopomer of the known nucleoside precursor, α-D-3,5-di-*O*-*p*-toluoyl-2-deoxyribosyl chloride (**1**). While this methodology is suitable for large-scale preparation of isotopically enriched 2′-deoxynucleosides, synthesis of ribonucleosides deuteriated (tritiated) at C2′ via this method is not feasible. In principle, the general method for the synthesis 2′-deoxy-2′,2″-dideuterionucleosides reported by Chattopadhyaya is adaptable to the synthesis of 2′-deuterioribonucleosides. In this approach, **2** is stereoselectively reduced by attack from the β face.[5b] However, significant subsequent protecting group manipulations must be performed in order to utilize **2** for the synthesis of nucleosides. We utilized a similar approach on a substrate that contains protecting groups compatible with nucleoside synthesis. In addition to providing a general method for the synthesis of 2′-deuterioribonucleosides, the plan outlined in Scheme 1 is less cumbersome than any proceeding from a 2′-ketonucleoside. Reduction of the ketone in these substrates results in highly selective formation of the arabinofuranose, via attack from the α-face.[6] In order to obtain the desired ribose configuration, one must then invert the stereochemistry of the secondary hydroxyl group. Furthermore, since the isotope is introduced using $NaBD_4$, synthesis of tritiated nucleosides from **3** does not require any alterations in the procedure.

Scheme 1[a]



[a] Key: (a) Dess–Martin; (b) $CeCl_3$, $NaBH_4$, THF, 25 °C; (c) BzCl, pyridine; (d) bis(trimethylsilyl)acetamide, adenine (uracil), CH_3CN then **5**, TMSOTf ($SnCl_4$); (e) saturated methanolic ammonia.

In practice, α-D-1,3,5-tri-*O*-benzoylribofuranose (**3**) was oxidized to the respective ketone (**4**). While a variety of common oxidation procedures (Swern, PDC) were examined, we found that the Dess–Martin periodinane was the most convenient, reliable reagent for large-scale preparation of **4**.[7] The workup procedure employed in this reaction was critical for obtaining good yields of **4** that could be purified via crystallization. Prolonged exposure of the crude reaction to a sodium thiosulfate solution saturated with sodium bicarbonate results in a complex, inseparable mixture of **4** and partially debenzoylated products. Subjection of the crude mixture to benzoylation conditions does not yield clean **4**. We suggest that the sodium thiosulfate solution used to destroy the excess Dess–Martin reagent is sufficiently basic so as to induce debenzoylation (e.g., **7**) directly and/or indirectly via benzoyl migration in the hydrate of **4**. Subsequent tautomerization (e.g., **7** to **8**) may explain why rebenzoylation does not produce **4** cleanly. Exposure to alkaline sodium thiosulfate was minimized by removing the solvent in vacuo. The crude mixture was redissolved in diethyl ether, a solvent in which the reagent is sparsely soluble, and filtered. This slight modification enabled us to carry out the subsequent workup in the prescribed manner, yielding a mixture of **4** and its hydrate in 90% yield. The ketone and its hydrate are distinguishable by ^1H NMR. The resonance attributable

(1) (a) Yamakage, S.-I.; Maltseva, T. V.; Nilson, F. P.; Földesi, A.; Chattopadhyaya, J. *Nucleic Acids Res.* **1993**, *21*, 5005. (b) Földesi, A.; Nilson, F. P. R.; Glemarec, C.; Gioeli, C.; Chattopadhyaya, J. *Tetrahedron* **1992**, *48*, 9033.

(2) (a) McGall, G. H.; Rabow, L. E.; Ashley, G. W.; Wu, S. H.; Kozarich, J. W.; Stubbe, J. *J. Am. Chem. Soc.* **1992**, *114*, 4958. (b) Saito, I. *Pure Appl. Chem.* **1992**, *64*, 1305. (c) Stubbe, J. *J. Biol. Chem.* **1985**, *260*, 9972.

(3) For an overview of nucleoside synthesis see: Blackburn, G. M.; Gait, M. J. *Nucleic Acids in Chemistry and Biology*; IRL Press: Oxford, 1990.

(4) (a) Fraser-Reid, B.; Radatus, B. *J. Am. Chem. Soc.* **1971**, *93*, 6342. (b) Pathak, T.; Bazin, H.; Chattopadhyaya, J. *Tetrahedron* **1986**, *42*, 5427.

(5) (a) Hodge, R. P.; Brush, C. K.; Harris, C. M.; Harris, T. M. *J. Org. Chem.* **1991**, *56*, 1553. (b) Wu, J-C.; Bazin, H.; Chattopadhyaya, J. *Tetrahedron* **1987**, *43*, 2355.

(6) (a) Robins, M. J.; Samano, V.; Johnson, M. D. *J. Org. Chem.* **1990**, *55*, 410. (b) Hansske, F.; Madej, D.; Robins, M. J. *Tetrahedron* **1984**, *40*, 125.

(7) (a) Dess, D. B.; Martin, J. C. *J. Am. Chem. Soc.* **1991**, *113*, 7277. (b) Ireland, R. E.; Liu, L. *J. Org. Chem.* **1993**, *58*, 2899.

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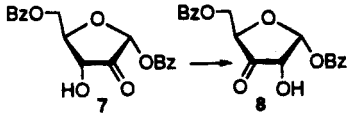
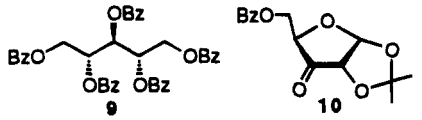
to the proton at the anomeric position of **4** appears 0.21 ppm upfield from the respective signal in the hydrate, while the proton at C3 appears more than 0.6 ppm further downfield in the ketone (**4**).



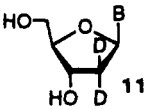
Reduction of **4** by $NaBH_4$ in protic solvents resulted in poor recovery of a complex mixture of benzoylated carbohydrates. Under these conditions, the major product obtained following perbenzoylation was often the ribitol (**9**), which presumably arises from loss of the benzoyl group from C1 and reduction of the ring-opened lactol. In order to circumvent this problem, reduction of the carbonyl group was pursued under aprotic conditions employing either heterogeneous catalysis or hydride reagents. Although hydrogenation of **10** over PtO_2 proceeds smoothly, **4** resisted all attempts at reduction by this method.[8a] A greater degree of success was enjoyed using $LiAlD_4$, followed by benzoylation of the crude mixture. However, the extent of reaction was difficult to monitor. Consequently, yields of [^{2}H]-**3** obtained from this procedure varied widely, and the reaction was not amenable to large-scale preparations of the deuteriated carbohydrate.



These problems were overcome by using $NaBH_4(D_4)$ and $CeCl_3$ in THF.[8b,c] Again, the workup was crucial for obtaining satisfactory results. Aqueous or saturated NH_4Cl quench yielded a complex mixture of products indicative of benzoyl migration. However, quenching the reaction with acetic acid reproducibly yielded **3** in 90% yield. No evidence of attack from the α-face was evident by ^{1}H NMR, or mixed melting point. Interestingly, when the reaction was carried out using deuteriated hydride, quenching with acetic acid resulted in only 87% deuteriation (^{1}H NMR). The percent deuterium incorporation was determined on this compound because the C2 hydrogen in **3** is occluded by the hydrogens on C4 and C5 in the ^{1}H NMR. The residual protio atom at the newly formed secondary hydroxyl carbon was determined to be 6−8% via integration of the respective base-line resolved resonances in the ^{1}H NMR of **5** and **6a**.

Following benzoylation of [^{2}H]-**3**, Lewis acids were used to mediate glycosidation of **5** with silylated nucleobases.[9] Although such reactions are commonly carried out using the β-anomer of the appropriately acylated sugar, the proposed mechanism for the stereoselective bond formation involves the intervention of an 1,2-α-acyloxonium ion.[9a] The stereochemistry of the glycosidic bond forming reaction should be independent of whether the carbohydrate substrate is α or β. Glycosidation of **5** by either the bis(trimethylsilyl) ether of uracil or N_6-benzoyladenine, followed by ammonolysis and purification, produced the desired C2′-deuteriated ribonucleosides in 75% and 55% yield, respectively. Ribonucleosides (**6**) can be carried on to the respective phosphoramidites, or nucleoside triphosphates via known methodology.[3,10,11]

Summary. The reaction sequence described above provides a concise, general synthesis of ribonucleosides that are isotopically enriched at C2′. Furthermore, 2′-deoxy-2′,2″-dideuteribonucleosides **11** are obtainable from



6 via known methods which do not require separation of diastereomers following glycosidation.[5,10a]

Experimental Section

General Methods. ^{1}H NMR spectra were recorded at 300 MHz. All reactions were run under nitrogen atmosphere in oven-dried glassware, unless specified otherwise. Pyridine and CH_2Cl_2 were freshly distilled from CaH_2. THF was freshly distilled from Na benzophenone ketyl. MeCN was freshly distilled from CaH_2 after passing through anhydrous $CuSO_4$. Benzoyl chloride was freshly distilled from itself. $CeCl_3 \cdot 7H_2O$, N^6-adenine, and carbohydrate substrates were dried in vacuo over P_2O_5. α-D-1,3,5-tri-*O*-benzoylribofuranose[12] was obtained from Pfanstiehl. $NaBD_4$ (98% isotopic enrichment) was obtained from Cambridge Isotope Laboratories. Dess−Martin periodinane was prepared by the method reported by Ireland.[7b]

α-D-1,3,5-Tri-*O*-benzoyl-2-ketoribofuranose (4). α-D-1,3,5-Tri-*O*-benzoylribofuranose (**3**) was added to a solution of Dess−Martin periodinane (7.73 g, 16.2 mmol) in 50 mL of CH_2Cl_2 at 0 °C. The mixture was allowed to warm to room temperature and stirred for 12 h. The solvent was removed in vacuo and the residue triturated with diethyl ether (100 mL). Following filtration through a pad of $MgSO_4$, the organic solvent was stirred with an equal volume of $Na_2S_2O_3 \cdot 5H_2O$ (12.5 g) in 100 mL of saturated $NaHCO_3$ until the organic layer became clear (~10 min). The organic layer was separated, washed with brine, and dried over $MgSO_4$ prior to removing the solvent in vacuo. Ketone **4** (4.47 g, 90%) was precipitated from a mixture of ether and hexanes: mp (softens) 50−55 °C; IR (KBr) 3437 (bd), 3064 (w), 2950 (w), 1795 (m), 1727 (s), 1601 (m), 1584 (m), 1452 (m), 1316 (m), 1271 (s) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.12−7.99 (6H, m), 7.64−7.34 (9H, m), 6.39 (1H, s), 6.18 (1H, s), 5.86 (1H, d, J = 8.8 Hz), 5.23 (1H, d, J = 5.4 Hz), 5.03 (1H, dd, J = 4.3, 8.7 Hz), 5.00 (1H, bd s), 4.82 (1H, dd, J = 3.5, 12.5 Hz), 4.61 (1H, dd, J = 4.5, 12.5 Hz), 4.08 (1H, bd s); ^{13}C NMR ($CDCl_3$) δ 201.3, 165.9, 165.4, 165.2, 134.0, 133.9, 133.3, 130.3, 130.2, 130.0, 129.7, 129.3, 128.5, 128.2, 91.4, 78.1, 72.1, 63.2; HRMS (FAB) calcd 483.1056 (M + Na), found 483.1051.

α-D-1,3,5-Tri-*O*-benzoyl-2-deuterioribofuranose ([^{2}H]-3). Dried $CeCl_3 \cdot 7H_2O$ (2.25 g, 9.1 mmol) and **4** (4.2 g, 9.1 mmol) in THF (50 mL) were stirred for 30 min at room temperature. $NaBD_4$ (764 mg, 18.2 mmol) was added in one portion, and the mixture was stirred for 1 h at room temperature. The reaction was quenched by the slow addition (via syringe pump) of AcOH-*d* (4.4 g, 72 mmol). After being stirred for 1 h, the reaction was diluted with diethyl ether (200 mL) and washed with H_2O. Drying and concentrating the organic layer as described above gave crude [^{2}H]-3 (3.85 g, 91.4%). This crude material worked as well as the recrystallized material in the subsequent reaction. Further purification by crystallization from EtOH:dichloroethane (1:1, 24 mL) yielded [^{2}H]-**3** (3.19, 75.6%): mp 144−145 °C (commercial protio material 146−147 °C); IR (film) 3061 (bd), 2361 (w), 1721 (s), 1602 (m), 1451 (m), 1316 (m), 1270 (s) cm^{-1};

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(9) (a) Vorbrüggen, H.; Bennua, B. *Chem. Ber.* **1981**, *114*, 1279. (b) Dudycz, L. W.; Wright, G. E. *Nucleosides Nucleotides* **1984**, *3*, 33.

(10) (a) Robins, M. J.; Wilson, J. S. *J. Am. Chem. Soc.* **1981**, *103*, 932. (b) Usman, N.; Ogilvie, K. K.; Jiang, M.-Y.; Cedergren, R. J. *J. Am. Chem. Soc.* **1987**, *109*, 7845.

(11) (a) Davisson, V. J.; Davis, D. R.; Dixit, V. M.; Poulter, C. D. *J. Org. Chem.* **1987**, *52*, 1794. (b) Hoard, D. E.; Ott, P. G. *J. Am. Chem. Soc.* **1965**, *87*, 1785.

(12) Brodfuehrer, P. R.; Sapino, C.; Howell, H. G. *J. Org. Chem.* **1985**, *50*, 2597.

^{1}H NMR ($CDCl_3$) δ 8.12–8.01 (6H, m), 7.62–7.35 (9H, m), 6.66 (1H, s), 5.56 (1H, d, J = 1.9 Hz), 4.73 (1H, ddd, J = 3.3, 1.9, 1.2 Hz), 4.62 (2H, dd, J = 1.4, 1.9 Hz), 2.73 (1H, s).

α-D-1,2,3,5-Tetra-*O*-benzoyl-2-deuterioribofuranose ([^{2}H]-5). Freshly distilled benzoyl chloride (7.0 g, 50.1 mmol) was added via syringe to [^{2}H]-**3** (2.32 g, 5.0 mmol) in pyridine (25 mL). After being stirred for 12 h, the reaction was quenched via addition of H_2O (25 mL), immediately followed by portionwise addition of $NaHCO_3$ (8.4 g, 100 mmol). The mixture was extracted with diethyl ether (400 mL) and the organic layer dried and concentrated as described above. Flash chromatography[13] of the residue (EtOAc:hexanes 3:7) yielded [^{2}H]-**5** (2.56 g, 90%) as a white foam: IR (film) 3063 (w), 3034 (w), 1913 (w), 1728, (s), 1602 (m), 1584, (w), 1492 (w), 1451, (m), 1316, (m), 1270 (s), 1177 (m), 1160 (m), 1112 (m), 1094 (m), 1068 (m), 1025 (m), 1008 (m) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.12–8.02 (6H, m), 7.86–7.83 (2H, m), 7.59–7.24 (12H, m), 6.94 (1H, s), 5.90–5.89 (1H, d, J = 2.2 Hz), 4.92–4.90 (1H, m), 4.79–4.64 (2H, dd × dd, J = 3.1, 12.2, 3.6, 12.2 Hz).

2′-Deuterioadenosine (6a). Dried [^{2}H]-**5** (2.56 g, 4.51 mmol) was added to N^6-benzoyladenine (1.20 g, 5.0 mmol) which had been persilylated by bis(trimethysilyl)acetamide (2.14 g, 10.5 mmol) in CH_3CN (50 mL). TMSOTf (1.5 g, 6.77 mmol) was added via syringe to the mixture at 0 °C. The mixture was then placed in an oil bath, slowly brought to reflux, and held at this temperature for 12 h. The cooled solution was poured into EtOAc (200 mL), washed with saturated $NaHCO_3$ (3 × 50 mL) and brine (50 mL), and dried over $MgSO_4$. Flash chromatography (EtOAc:Hexanes 1:1–7:3) yielded the tetrabenzoyladenosine (2.67 g, 78%). The tetrabenzoyl nucleoside was deprotected in NH_3-saturated MeOH (30 mL) over the course of 48 h. The MeOH was recharged with NH_3 after 24 h. 2′-Deuterioadenosine (**6a**) was crystallized from 9 mL of H_2O:iPrOH (1:2) which provided 1.92 g (71.0%): mp 231–233 °C (234–236 °C commercially available protio material); ^{1}H $CDCl_3$ (D_2O) δ 8.24 (1H, s), 8.12 (1H, s), 5.90 (1H, s), 4.24 (1H, d, J = 3.8 Hz), 4.09–4.07 (1H, m), 3.76–3.62 (2H, dd × dd, J = 2.8, 12.8, 3.9, 12.8 Hz).

2′-Deuteriouridine (6b). Dried [^{2}H]-**5** (193 mg, 0.34 mmol) was added to uracil (40 mg, 0.36 mmol) which had been silylated as described above. $SnCl_4$ (279 mg, 1.1 mmol) was added via syringe to the solution at −10 °C, which was allowed to stir and warm to room temperature over 12 h, at which time the reaction was worked up as described above. Flash chromatography (EtOAc:hexanes 4:6) yielded the tribenzoyluridine (133 mg, 70%). Ammonolysis was carried out as described above. 2′-Deuteriouridine (57 mg, 96%) was purified by flash chromatography (EtOAc:MeOH 8:2); mp 165–166 °C (166–167 °C commercially available protio material). ^{1}H NMR (D_2O) δ 8.00 (1H, d, J = 8.1 Hz), 5.89 (1H, s), 5.69 (1H, d, J = 8.1 Hz), 4.14 (1H, d, J = 4.7 Hz), 3.99 (1H, ddd, J = 3.0, 1.1, 2.9 Hz), 3.86–3.69 (2H, dd × dd, J = 2.7, 12.3, 3.1, 12.3 Hz).

Acknowledgment. We are grateful for financial support of this research by the National Institutes of Health (GM-46534) and the Elsa U. Pardee Foundation. G.P.C. thanks the U.S. Department of Education for fellowship support under the Graduate Assistance in Areas of National Need Program (Grant No. P200A-10210). Mass spectral determinations were made at the Midwest Center for Mass Spectrometry with partial support by the National Science Foundation, Biology Division (Grant No. DIR9017262).

Supplementary Material Available: ^{1}H NMR spectra of [^{2}H-]**3**, **5**, **6a**, and **6b** (5 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

(13) Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923.

Additions and Corrections

Vol. 59, 1994

Kevin Koch* and Michael S. Biggers. General Preparation of 7-Substituted 4-Chromanones: Synthesis of a Potent Aldose Reductase Inhibitor.

Page 1216, column 2. In Figure 1, cromokalim should be numbered (**1**) and structure **2** should be Ro 25-2636. Reference 3 is incorrect and should be replaced with the following: Cohen, N.; et al. 205th National Meeting of the American Chemical Society, Denver, 1993; MEDI 137. Cohen, N.; Yagaloff, K. *Curr. Opin. Invest. Drugs* **1994**, *3*, 13–22, U.S. Pat. 5 273 999 (Hoffmann-La Roche Inc.)

Jose Marco-Contelles,* Manuel Bernabé, David Ayals, and Belén Sánchez. 6-*endo-dig* Free-Radical Carbocyclizations: A New Strategy for the Synthesis of Cyclitols.

Page 1234. Since publication of our new methodology for the preparation of enantiomerically pure cyclitols, the first example of a 6-endo-digonal closure (applied to the synthesis of racemic *trans*-fused bicyclo[4.3.0]nonanes by atom chain transfer in conveniently functionalized precursors) has come to our attention: Albrecht, U.; Wartchow, R.; Hoffmann, H. M. R. *Angew. Chem., Int. Ed. Engl.* **1992**, *31*, 910.