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SIMPLE ONE-POT SYNTHESIS OF A
2'-TRITIUM LABELED C-DEOXYNUCLEOSIDE

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Abstract: The deoxygenation and 2'-labeling of a C-ribonucleoside by reductive elimination with tri-*n*-butyltin hydride [^3H] in a one-pot reaction is described. The approach is a safe, simple, efficient, and general method for 2'-labeling of nucleosides. © 1999 Elsevier Science Ltd. All rights reserved.

Recently, work in many laboratories has shown that a wide range of heterocyclic bases can contribute to molecular recognition between two strands of DNA and RNA.^{1–7} Nucleoside analogs bearing these heterocycles and labeled with radioactive isotopes would be valuable tools to expand our understanding of this range. With natural nucleosides, a variety of enzymatic⁸ and chemical^{9,10} procedures have been reported for introducing isotopes of hydrogen, including deuterium^{11–15} and tritium. These procedures are often inconvenient, requiring multiple steps, giving low yields, causing anomerization, or generating tritium gas as a side product.

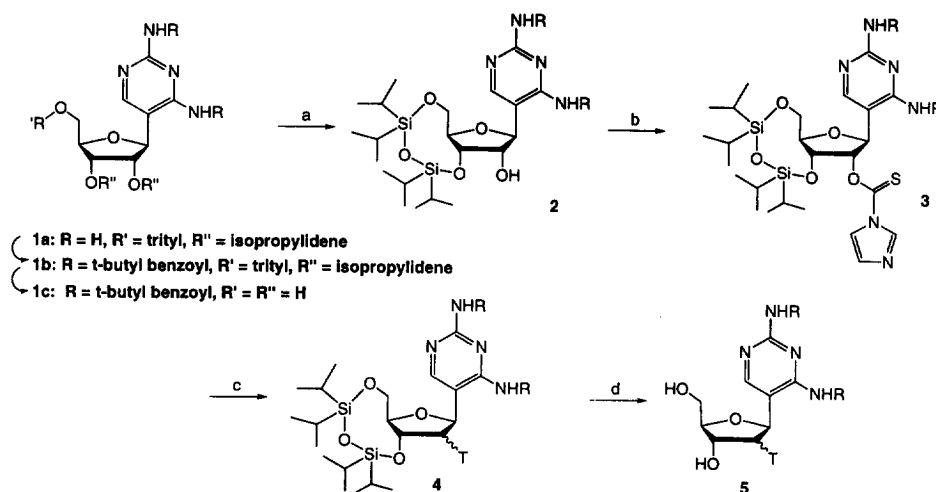


Figure 1 The synthetic scheme for a [$2'\text{-}^3\text{H}$]-2'-deoxy-C-nucleoside from its ribonucleoside is shown

We report here a procedure that exploits the known ability of deuterated tri-*n*-butyltin hydride to transfer a hydrogen specifically to a radical at the C-2' position of a nucleoside.^{15,16} The procedure was adapted first to incorporate a tritium from tri-*n*-butyltin tritiide (Moravek Biochemicals Inc., 577 Mercury Lane, Brea, CA 92821, U.S.A.), which was prepared by reacting tri-*n*-butyltin hydride with MeMgBr, and hydrolyzing the intermediate with tritiated water.^{17,18} The specific activity of the product (22 mCi/mmol) depended solely on the activity of tritiated water. Distillation yielded tri-*n*-butyltin tritiide (>96% pure as determined by HPLC), which was stored neat at –20 °C.

To explore the scope of this reaction with some of the more novel nucleoside analogs, we developed a one-pot procedure applying the reagent to 2,4-diamino-5-(β-D-ribofuranosyl)-pyrimidine (**1a**),^{19–21} a nonstandard pyrimidine that presents a hydrogen bond donor-acceptor-donor pattern when forming a Watson-Crick base pair. The exocyclic amino groups of **1a** were protected as amides via DMAP-catalyzed reaction with *p*-*tert*-butyl benzoyl chloride (pyridine, 0 °C, 2 h). The substitution of the protection group for the exocyclic amino groups from the traditional benzoyl to the more hydrophobic *p*-*tert*-benzoyl simplified the silica-gel purification of the final product. The isopropylidene- and trityl protection groups were removed (10% HCl in MeOH, 2 h), and the ribose derivative **1c** was silylated at the 3'- and 5'-positions with dichlorotetraisopropyl disiloxane (pyridine, 4 h) to yield **2**. Reaction of the 2'-hydroxyl group with 2,2'-thiocarbonyl-diimidazole in DMF yielded the thiocarbonylimidazolide derivative **3**.

In the presence of 2,2'-azo-bisisobutyronitrile and toluene, **3** was refluxed with the radiolabeled tri-*n*-butyltin hydride for 1 h, yielding **4**. Treatment with tetrabutylammonium fluoride (1 M, THF) gave the [2'-³H]-2'-deoxy-nucleoside **5**, which was purified by silica-gel chromatography (8% MeOH/CHCl₃). In preliminary studies, all intermediates were isolated and characterized.²² In its one-pot version, the yield of **5** was 75% (based on **1c**); the radioactive yield based on the specific activity of the [³H]-tri-*n*-butyltin hydride was 50%. Radiolabeled product was analyzed by thin-layer chromatography (TLC) and was identical to the reference material. In addition, the TLC plates were exposed to a tritium-sensitive imager screen. Radioactivity was detected only in the product spot.

This efficient one-pot synthesis of 2'-radioisotopic labeled deoxynucleoside is expected to be applicable to a wide range of nucleoside analogs, given the known tolerance of tri-*n*-butyltin hydride deoxygenation to a variety of heterocycles. These compounds can be readily converted into the corresponding triphosphates by chemical²³ and enzymatic methods.^{24,25} Applying this strategy of 2'-radiolabeling to a variety of nucleosides or nucleoside analogs makes these compounds valuable tools for high-throughput screening techniques such as the Scintillation Proximity Assay (SPATM, Amersham Life Science Inc.).²⁶

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22. Protons on the ribose were assigned based on NOE experiments and homonuclear COSY. The stereochemistry at the anomeric center was determined by NOE diff. experiments.
 Compound **1b**: ^1H NMR (500 MHz, CDCl_3) δ : 11.8 (s, 1H, NH), 10.2 (s, 1H, NH), 8.52 (s, 1H, H-6), 8.08 (d, 2H, arom.H), 7.85 (d, 2H, arom.H), 7.51 (d, 2H, arom.H), 7.49 (d, 6H, arom.H), 7.44 (d, 2H, arom.H), 7.29 (t, 6H, arom.H), 7.22 (q, 3H, arom.H), 5.58 (d, 1H, H-1', $J = 3.3$ Hz), 4.87 (q, 1H, H-2', $J = 3.3, 6.3$ Hz), 4.57 (t, 1H, H-3', $J = 6.3, 5.6$ Hz), 4.29 (q, 1H, H-4', $J = 5.6, 2.5$ Hz), 3.37 (m, 2H, H-5'B/A), 1.70 (s, 3H, isoprop. CH_3), 1.38 (s, 3H, isoprop. CH_3), 1.34 (s, 9H, *t*-bu), 1.32 (s, 9H, *t*-bu), ppm; ms: m/z 845 ($\text{M}^+ + 1$), 243 ($\text{M}^+ - 601$), 161 ($\text{M}^+ - 683$).
 Compound **1c**: ^1H NMR (300 MHz, $\text{MeOH}-d$) δ : 8.67 (s, 1H, H-6), 7.92 (dd, 4H, arom.H), 7.52 (q, 4H, arom.H), 4.91 (m, 1H, H-1), 4.12 (m, 3H, H-2', H-3', H-4'), 3.79 (dd, 1H, H-5'B, $J = 2.7, 12.2$ Hz), 3.70 (dd, 1H, H-5'A, $J = 4.2, 12.2$ Hz), 1.39 (s, 9H, *t*-bu), 1.32 (s, 9H, *t*-bu), ppm; ^{13}C -NMR (300 MHz, $\text{MeOH}-d$) δ : 167.1; 167.0; 158.0; 157.9; 157.8; 157.4; 157.1; 132.2; 132.2; 129.0; 128.9; 126.8; 126.7; 118.1; 88.4; 79.3; 77.6; 73.2; 63.3; 35.9; 31.5 ppm.
 Compound **2**: ^1H NMR (500MHz, CDCl_3) δ : 9.95 (s, br, 1H, NH), 9.00 (s, 1H, NH), 8.71 (s, 1H, H-6), 7.88 (dd, 4H, arom.H), 7.47 (d, 4H, arom.H), 4.84 (d, 1H, H-1' $J = 5.5$ Hz), 4.35 (t, 1H, H-3', $J = 6.5$ Hz), 4.16 (m, 1H, H-2'), 4.14 (dd, 1H, H-5'B, $J = 3.2, 11.6$ Hz), 3.99 (hex, 1H, H-4', $J = 3.2, 7.0, 6.5$ Hz), 3.93 (q, 1H, H-5'A, $J = 7.0, 11.6$ Hz), 1.34 (s, 18H, *t*-bu), 1.11-0.96 (m, 28H, isopropyl.), ppm.
 Compound **3**: ^1H NMR (300 MHz, CDCl_3) δ : 8.56 (s, br, 1H, NH), 8.32 (s, 1H, NH), 8.12 (s, 1H, H-6), 7.90 (m, 4H, arom.H), 7.69 (s, 1H, imid.), 7.60 (s, 1H, imid.), 7.48 (m, 4H, arom.H), 7.03 (s, 1H, imid.), 6.14 (d, 1H, H-2', $J = 5.0$ Hz), 5.55 (s, 1H, H-1'), 4.45 (q, 1H, H-3' $J = 5.0, 8.7$ Hz), 4.18 (m, 1H, H-5'B, $J = 12.3$ Hz), 4.02 (m, 2H, H-4'/H-5'A), 1.31 (s, 9H, *t*-bu), 1.25 (s, 9H, *t*-bu), 1.06 - 0.8 (m, 28H, isopropyl.), ppm.
 Compound **4**: ^1H NMR (300 MHz, CDCl_3) δ : 10.0 (s, br, 1H, NH), 8.90 (s, br, 1H, NH), 8.44 (s, 1H, H-6), 7.84 (dd, 4H, arom.H), 7.47 (t, 4H, arom.H), 5.17 (q, 1H, H-1', $J = 8.4, 6.3$ Hz), 4.50 (m, 1H, H-3'), 4.13 (dd, 1H, H-5'B, $J = 3.6, 11.5$ Hz), 3.97 (m, 1H, H-4'), 3.75 (m, 1H, H-5'A, $J = 8.7, 11.5$ Hz),

2.39 (m, 1H, H-2'B), 2.30 (m, 1H, H-2'A), 1.33 (s, 9H, *t*-bu), 1.32 (s, 9H, *t*-bu), 1.10 - 0.97 (m, 28H, isopropyl.), ppm; ms: m/z 811 ($M^+ + 23$), 789 ($M^+ + 1$), 161 ($M^+ - 627$).

Compound 5: ^1H NMR (300 MHz, MeOH-*d*) δ : 8.48 (s, 1H, H-6), 7.95 (dd, 4H, arom.H), 7.57 (dd, 4H, arom.H), 5.29 (q, 1H, H-1', $J = 5.1, 10.5$ Hz), 4.32 (m, 1H, H-3'), 4.06 (m, 1H, H-4'), 3.73 (dd, 1H, H-5'B, $J = 3.9, 12.0$ Hz), 3.64 (dd, 1H, H-5'A, $J = 5.4, 12.0$ Hz), 2.38 (dd, 1H, H-2'B, $J = 5.1, 13.0$ Hz), 2.20 (hex, 1H, H-2'A, $J = 6.0, 13.0, 10.5$ Hz), 1.36 (s, 18H, *t*-bu), ppm; ^{13}C NMR (300 MHz, MeOH-*d*) δ : 167.2; 167.1; 158.5; 158.2; 158.0; 157.7; 157.5; 132.4; 132.0; 128.9; 126.9; 126.7; 119.4; 89.9; 77.1; 73.5; 63.6; 54.2; 41.8; 36.0; 35.9; 31.5 ppm; ms: m/z 569 ($M^+ + 23$), 547 ($M^+ + 1$), 161 ($M^+ - 385$).

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