Pergamon

Bioorganic & Medicinal Chemistry Letters 9 (1999) 723–726

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

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

Stefan Lutz and Steven A.Benner*

Department of Chemistry, University of Florida, Gainesville, FL 32611, U.S.A.

Received 20 October 1998; accepted 25 January 1999

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

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 enzymatic[8] 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.

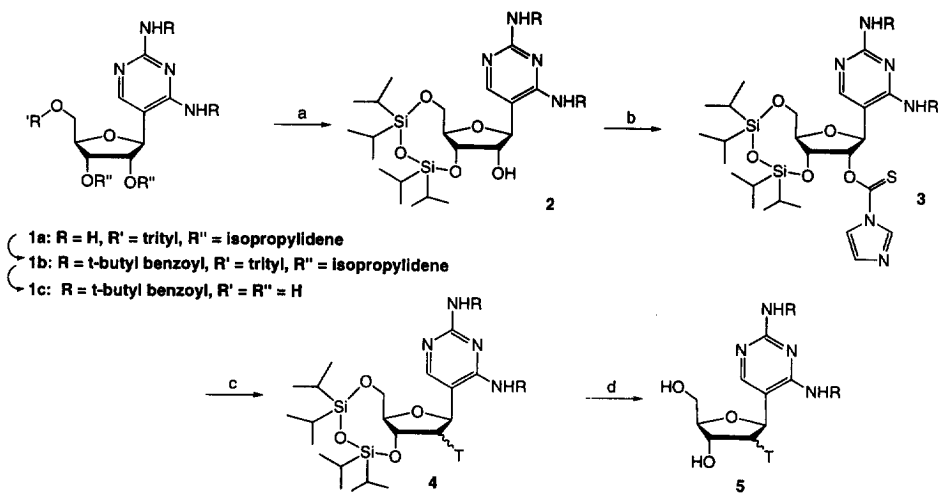

Figure 1 The synthetic scheme for a [2'-^{3}H]-2'-deoxy-C-nucleoside from its ribonucleoside is shown

0960-894X/99/$ - see front matter
PII: S0960-894X(99)00068-2

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/mmole) depended sole 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 dichloro-tetraisopropyl 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'-^{3}H]-2'-deoxy-nucleoside **5**, which was purified by silica-gel chromatography (8% MeOH/$CHCl_3$). 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 [^{3}H]-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 chemical[23] and enzymatic methods.[24,25] Applying this strategy of 2'-radiolabeling to a variety of nucleosides or nucleosides analogs makes these compounds valuable tools for high-throughput screening techniques such as the Scintillation Proximity Assay (SPA™, Amersham Life Science Inc.).[26]

Acknowledgment: The authors are grateful to the Biomolecular Science & Technology Program from the Office of Naval Research (N00025-96-1-0362) for financial support of this work, and Moravek Biochemicals CA for the collaboration in the synthesis of tritiated tri-*n*-butyltin hydride.

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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**: ^{1}H 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 (M^+ + 1), 243 (M^+ - 601), 161 (M^+ - 683).

    Compound **1c**: ^{1}H NMR (300 MHz, 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, 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**: ^{1}H 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**: ^{1}H 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**: ^{1}H 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**: ^{1}H 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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