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Biomimetic Modeling of the Abstraction of H3' by Ribonucleotide Reductases. 1,5-Hydrogen Atom Transfer of H3 to Aminyl and Oxyl, but Not Thiyl, Free Radicals in Homoribofuranose Derivatives[1]

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Abstract: Generation of 6-oxyl radicals from homoribofuranose (5-deoxy-D-*ribo*-hexofuranose) 6-*O*-nitro esters with Bu_3SnD/AIBN/benzene/Δ resulted in abstraction of H3 by a [1,5]-hydrogen atom shift. Transfer of ^2H from the stannane to •C3 effected incorporation of deuterium at C3. Analogous treatment of 6-azido-6-deoxy-D-*ribo*-hexofuranose derivatives gave C3-deuterated aminosugars. In contrast, no deuterium incorporation was detected upon parallel treatment of 6-thio-D-*ribo*-hexofuranose derivatives. Abstraction of H3' by a thiyl radical (•SCys) is the postulated first step in reactions that are utilized by ribonucleotide reductases to convert ribonucleotides into 2'-deoxynucleotides. Results are discussed relative to the enzyme reaction cascade that couples abstraction of H3' with irreversible loss of water from C2'.

Keywords: biomimetic reactions, carbohydrates, enzymes and enzyme reactions, radicals and radical reactions

INTRODUCTION

Ribonucleotide reductases (RNRs) are the essential enzymes that catalyze the conversion of 5'-(di or tri)phosphate esters of ribonucleosides into their 2'-deoxynucleotides to provide the only *de novo* source of monomers for DNA synthesis.[2] The *Escherichia coli* ribonucleoside diphosphate reductase (RDPR) has two nonidentical homodimeric subunits, R1 and R2, whose structures have been investigated recently by X-ray crystallography.[3] R1 contains substrate and allosteric effector binding sites as well as cysteine residues required for catalysis. R2 contains a diiron chelate and an essential tyrosyl free radical. The RDPRs of mammalian cells and certain viruses have similar homodimeric subunit structures and functions.[2]

Stubbe and coworkers have proposed catalytic mechanisms for substrate reduction and mechanism-based inactivation of *E. coli* RDPR. In a recent refinement of the substrate-reduction mechanism,[2c] a proximate thiyl radical in R1 (•SCys439) is generated by long-range electron transfer from the tyrosyl radical (•Tyr122) in R2.[4] Abstraction of H3' from the substrate ribonucleotide (**1**, Scheme 1) by •SCys439 (or an analogous thiyl radical generated by participation of adenosylcobalamin with a RTPR[5]) generates a C3' radical **2**, which undergoes loss of the hydrogen-bonded 2'-hydroxyl group as water. The carboxylate of Glu441 could function as a general base to remove the 3'-hydroxyl proton and assist cleavage of the C2'–O2' bond.[2c,3] Hydrogen and electron

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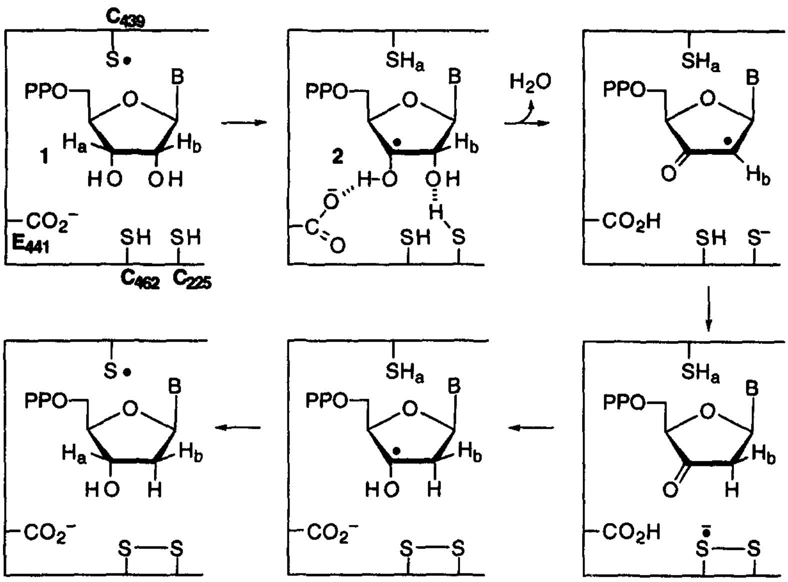
transfers via Cys225/Cys462 in R1 result in completely stereoselective replacement of the 2'-hydroxyl group with hydrogen at the α-face. Return of H3' from HSCys439 to C3' gives the 2'-deoxynucleotide product and regenerates the bioinitiation radical (•SCys439). Thus, the reaction cascade is initiated by abstraction of H3', and the cycle is completed when the C3' radical regains H3' from H–SCys439.[2c,3f]

Scheme 1[a]



[a]Proposed substrate mechanism for ribonucleoside diphosphate reductase.[2c]

We have recently demonstrated that treatment of 6'-*O*-nitrohomo(uridine and adenosine) derivatives with Bu_3SnD/AIBN/benzene/Δ generates 6'-oxyl radicals that abstract H3' via a [1,5]-hydrogen shift to produce C3' radicals.[1,6] Studies with 6'-*O*-nitro esters of 2'-chloro-2'-deoxyhomouridine or 2'-*O*-tosylhomoadenosine were in harmony with radical-relay elimination of a chlorine atom or toluenesulfonic acid, respectively.[1,6] Lenz and Giese generated adenosine C3'-radicals by photolysis of xylo-seleno esters, and showed that loss of the 2'-hydroxyl group was subject to general base catalysis.[7] Sugars linked to benzophenones have been prepared as photoactive models for relay generation of C3 radicals,[8] but a [1,6]-hydrogen shift would be required. It has been demonstrated that a [1,5]-hydrogen shift (6-membered transition state) is strongly favored.[9]

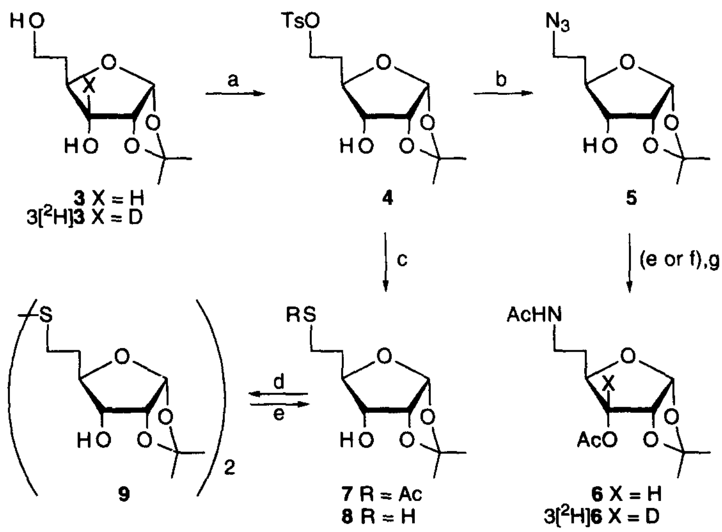
Thiols are excellent hydrogen-atom donors, and it is commonly assumed that thiyl radicals should be poor hydrogen-atom abstractors. However, bond dissociation energies (BDEs) for RS–H and RR'(HO)C–H systems are similar, and molecular associations with protein residues at the active sites of RNRs might alter the BDEs measured with model compounds. The coupling of H3' abstraction by •SCys439 with a [1,2]-electron shift and concerted loss of water from C2' (hydrogen bond linked 3'OH···"XH"···OH2') has intuitive[1,6b] and theoretical[10] support. Small BDE differences would be advantageous overall, since •C3' must regain H3' from Cys439 to complete the reaction cascade and regenerate •SCys439 for the next catalytic cycle. The abstraction of H3' by a thiyl radical is a continuing point of concern,[11] since a close chemical precedent is lacking.

We now report syntheses of 6-(azido, *O*-nitro, and thio) derivatives of homoribofuranose (5-deoxy-D-*ribo*-hexofuranose) and a 6'-azido-6'-deoxyhomouridine derivative. Treatment of these free radical precursors with Bu_3SnD/AIBN/benzene/Δ generated the nitrogen, oxygen, and sulfur radicals with a 1,5-relationship to H3. Radical-relay generation of •C3 was observed by incorporation of deuterium with the aminyl and oxyl radicals, but no incorporation of deuterium at C3 was detected with the 6-thiyl radical.

RESULTS AND DISCUSSION

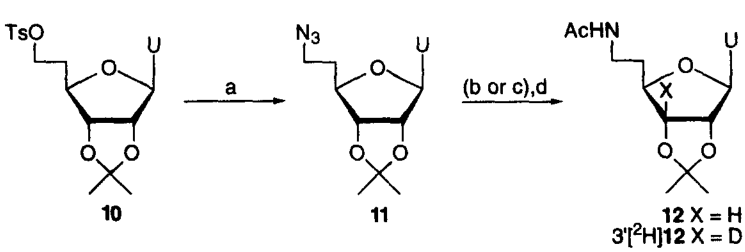
Regioselective tosylation of 5-deoxy-1,2-*O*-isopropylidene-α-D-*ribo*-hexofuranose[1,6b] (**3**) (Scheme 2) gave **4** (80%), which was treated with LiN_3/DMF to give 6-azido compound **5** (85%). Staudinger reduction[12] of **5** (PPh_3/pyridine/NH_3/MeOH, sealed tube) gave a 6-amino intermediate that was acetylated (Ac_2O/DMAP) to give authentic 6-acetamido-3-*O*-acetyl-5,6-dideoxy-2,3-*O*-isopropylidene-α-D-*ribo*-hexofuranose (**6**, 75%).

Scheme 2[a]



[a] (a) TsCl/pyridine. (b) NaN_3/DMF. (c) KSAc/DMF. (d) NH_3/MeOH. (e) Bu_3SnD/AIBN/benzene/Δ. (f) Ph_3P/pyridine/NH_3/MeOH. (g) Ac_2O/DMAP.

Scheme 3[a]

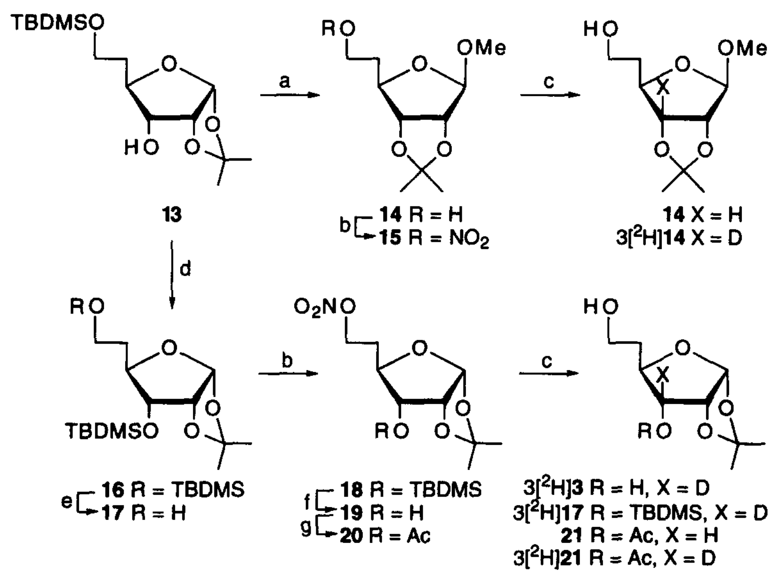


[a] (a) NaN_3/DMF. (b) Bu_3SnD/AIBN/benzene/Δ. (c) Ph_3P/pyridine/NH_3/MeOH. (d) Ac_2O/DMAP.

Nucleoside azides are reduced to amines with Bu_3SnH/AIBN,[13] and treatment of alkyl azides under these conditions is known to generate aminyl radicals that can undergo intramolecular addition[14a,b] and transfer[14c] reactions. Treatment of **5** with Bu_3SnD/AIBN/benzene/Δ followed by acetylation gave **6**/3[^{2}H]**6** [~7:3, 78%; ^{1}H NMR]. These results are compatible with generation of a 6-aminyl radical, [1,5]-transfer[1,6,9] of H3, and quenching of •C3 by deuterium transfer from the stannane.

Tosylation of 2',3'-*O*-isopropylidenehomouridine[1,6a] gave **10** (71%) (Scheme 3), which was treated with NaN_3/DMF to give the 6'-azido derivative **11** (91%). Staudinger reduction and acetylation gave authentic 1-(6-acetamido-5,6-dideoxy-2,3-*O*-isopropylidene-β-D-*ribo*-hexofuranosyl)uracil (**12**, 69%). Treatment of **11** with Bu_3SnD/AIBN/benzene/Δ, followed by acetylation gave **12**/3'[^{2}H]**12** (~3:1, 76%; ^{1}H NMR, HRMS). Thus, aminyl radicals can execute the [1,5]-hydrogen shift abstraction of H3 with a homoribofuranose (or H3' with a homouridine) derivative. However, abstraction occurs with lower overall efficiency (~25%) than in cases with an analogously positioned oxygen radical (~80%).[1,6]

Scheme 4[a]



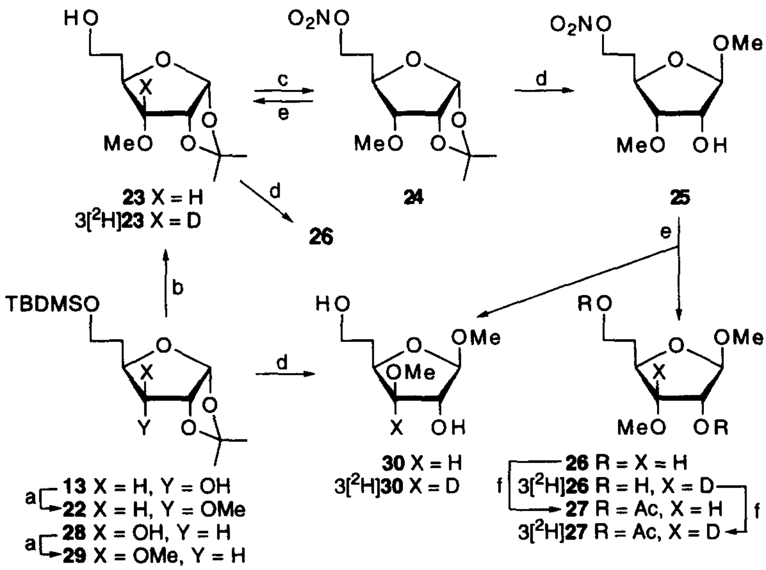
[a] (a) (i) HCl/MeOH; (ii) $Me_2CH(OMe)_2/Me_2CO$/Δ. (b) HNO_3/Ac_2O/–60 °C. (c) Bu_3SnD/AIBN/benzene/Δ. (d) TBDMSCl/imidazole/DMF. (e) HCl/MeOH/H_2O. (f) NH_4F/MeOH/Δ. (g) Ac_2O/DMAP.

Potassium thioacetate displacement with **4** (KSAc/DMF) gave the 6-*S*-acetyl derivative **7** (Scheme 2). Deacetylation of **7** (without exclusion of oxygen) resulted in formation of the disulfide **9**. Treatment of **9** (or its 3-*O*-TBDMS derivative) under our standard conditions with Bu_3SnD/AIBN/benzene/Δ produced the 6-thiol **8**. Generation of 6-thiyl radicals from **9** is confirmed by formation of 6-thiol **8** under the inert atmosphere (argon) conditions.[15] However, this thiyl radical did not effect detected exchange of [^{2}H]3 for H3 (NMR or HRMS). Hydrogen abstraction from activated C–H bonds (α-Hs of alcohols and ethers) by thiyl radicals generated by pulse radiolysis of thiols has been detected,[16,17] but the rate constants for H-atom abstraction by thiyl radical are four orders of magnitude smaller than the rates of H-atom donation by thiols.[16] Substrate reduction by RNRs (Scheme 1) is postulated to involve initial abstraction of H3' by •SCys followed by rapid irreversible loss of H_2O from C2' and subsequent H-atom and electron transfers.[2c,3f] It is possible that thiyl radicals generated from **9** do abstract H3, but that the reverse donation of H3 back to •C3 occurs with a much higher rate (~10^4), and the resulting •S6 radicals abstract deuterium from the stannane to give **8** (after H_2O-exchange workup).

With the goal to develop better models for abstraction of H3 by •S6 followed by loss of a substituent from C2, we examined several furanose systems to investigate overall efficiencies of [^{2}H]3 for H3 exchange. Methanolysis[18] of 6-*O*-(*tert*-butyldimethylsilyl)-5-deoxy-1,2-*O*-isopropylidene-α-D-*ribo*-hexofuranose[1,6b] (**13**) (Scheme 4) followed by 2,3-*O*-isopropylidene formation gave **14**, which was nitrated[19] to give **15**. Silylation of **13** gave the di-*O*-TBDMS derivative **16** (95%), which was selectively deprotected to give **17** (99%). Nitration of **17** followed by desilylation and acetylation gave **20** (79% from **17**).

Treatment of **15** with Bu_3SnD/AIBN/benzene/Δ gave **14**/3[^{2}H]**14** (~40:60, 82%; ^{1}H NMR, HRMS). Parallel reactions with **18** gave **17**/3[^{2}H]**17** (~15:85, 85%), **19** gave **3**/3[^{2}H]**3** (~45:55, 97%), and **20** gave **21**/3[^{2}H]**21** (~80:20, 60%) plus the rearranged 6-*O*-acetyl isomers (H3/[^{2}H]3, ~80:20, 36%). The 3-*O*-benzoyl derivative of **19** underwent similar incorporation of deuterium (H3/[^{2}H]3, ~75:25).

Scheme 5[a]



[a] (a) NaH/MeI/DMF. (b) TBAF/THF. (c) HNO_3/Ac_2O/–60 °C. (d) (i) TFA/H_2O; (ii) HCl/MeOH. (e) Bu_3SnD/AIBN/benzene/Δ. (f) Ac_2O/DMAP.

Methylation of **13** gave **22** (85%) (Scheme 5), which was desilylated to give **23**. Nitration of **23** gave **24** (78% from **22**). Treatment of **24** with Bu_3SnD/AIBN/benzene/Δ gave **23**/3[^{2}H]**23** (~30:70, 82%; ^{1}H NMR, HRMS). Successive deprotection of **24** (TFA/H_2O) and methanolosis (HCl/MeOH) gave the methyl furanoside **25**. Glycoside **25** has a free 2-hydroxyl group, and its furanose ring conformation is not restricted by fusion with a five-membered isopropylidene acetal ring. Treatment of **25** with Bu_3SnD/AIBN/benzene/Δ gave the ribo

(**26**)/xylo (**30**) epimers (64:36, 90%). Complete incorporation of deuterium at C3 of the xylo epimer 3[^{2}H]**30** was observed (^{1}H NMR, HRMS), and ~75% ^{2}H incorporation into the ribo epimer (**26**/3[^{2}H]**26**) (~25:75; ^{1}H NMR, HRMS determined after conversion into the di-*O*-acetyl derivatives **27**/3[^{2}H]**27**). A sample of authentic **30** was synthesized by methylation of 6-*O*-(*tert*-butyldimethylsilyl)-5-deoxy-1,2-*O*-isopropylidene-α-D-*xylo*-hexofuranose[1] (**28**) to give **29**, followed by deprotection (TFA/H_2O) and methanolysis (HCl/MeOH). The ^{1}H NMR signal at δ 3.66 (q, J = 2.7 Hz, 1H) for H3 of epimer **30** was adequately resolved (and absent in spectra of 3[^{2}H]**30**). Acetylation of **26**/3[^{2}H]**26** (after chromatographic separation from 3[^{2}H]**30**) gave **27**/3[^{2}H]**27** with spectral resolution of the ^{1}H NMR peak at δ 3.80 (dd, J = 7.4, 4.4 Hz, ~0.25H) for H3. This Δδ shift upon acetylation circumvented the overlap with the signal for H3 of authentic **26** (prepared from **23**; TFA/H_2O and HCl/MeOH).

SUMMARY AND CONCLUSIONS

We have synthesized 6-*O*-nitro-5-deoxy-D-*ribo*-hexofuranose derivatives, and demonstrated efficient [^{2}H]3 for H3 exchange upon generation of 6-oxyl radicals by treatment with Bu_3SnD/AIBN/benzene/Δ. This [1,5]-hydrogen shift abstraction with generation of a C3' radical mimics the initial substrate reaction of RNRs. Deuterium transfer exclusively at the α-face of a homoadenosine analogue has been observed, whereas complete deuterium exchange at C3' occurred with a homouridine analogue with a β/α (~1.3:1) facial delivery.[1] Thus, subtle differences exist between adenine and uracil homonucleosides, and between either of these and the present methyl homoribofuranosides. Conformational preferences might be driven by steric and/or stereoelectronic effects that might alter energy barriers in the obligate 6-membered transition states for these [1,5]-hydrogen shifts. The presence of ester groups at C3 is detrimental for overall deuterium exchange (~20%), whereas TBDMS ether, methyl ether, isopropylidene acetal, and hydroxyl substituents at C3 allowed abstraction of H3 and deuterium transfer more efficiently (50–85%). Aminyl radicals, generated from a 6-azido sugar or a 6'-azidohomouridine, effected [1,5]-hydrogen abstraction with ~20% overall deuterium exchange. Generation of thiyl radicals in a sugar model resulted in no deuterium exchange at C3. However, consideration of rates of H-atom abstraction by thiyl radicals relative to rates of H-atom donation to carbon radicals does not preclude abstraction of H3' by •SCys followed by rapid loss of H_2O from C2' and electron and H-atom transfers at active sites of RNRs. Studies are in progress with more sophisticated models designed to provide biomimetic support for the first-step relay abstraction of H3' by •SCys439 of RDPR.

EXPERIMENTAL SECTION

A capillary apparatus was used for uncorrected melting points. UV spectra were determined with MeOH solutions. ^{1}H (200, 300, or 500 MHz) and ^{13}C (50 or 125 MHz) NMR spectra were determined with solutions in $Me_4Si/CDCl_3$ unless otherwise specified. Mass spectra (MS and HRMS) were obtained by electron impact (20 eV), chemical ionization (CI, CH_4), or fast atom bombardment (FAB, thioglycerol matrix). Reagent chemicals were used, and solvents were dried by reflux over and distillation from CaH_2 (except acetone/P_2O_5) under an argon atmosphere. $NaHCO_3/H_2O$ was saturated at ambient temperature. NH_3/MeOH was saturated at −10 °C. TLC was performed with Merck kieselgel 60-F_{254} sheets, and products were detected with 254 nm light or by color development (I_2 or 10% H_2SO_4/MeOH). Merck kieselgel 60 (230-400 mesh) was used for column chromatography. Elemental analyses were by M-H-W Laboratories, Phoenix, AZ.

5-Deoxy-1,2-*O*-isopropylidene-α-D-*ribo*-hexofuranose (3). This reference compound had: mp 78–79 °C,[1,6b] 76.5–77.5 °C;[20] ^{1}H NMR δ 1.37, 1.58 (2 × s, 2 × 3H), 1.91 (dd, J = 11.2, 6.1 Hz, 2H), 2.91 (br s, 2H, ex), 3.68 (dd, J = 8.8, 5.0 Hz, 1H), 3.76–3.88 (m, 3H), 4.58 (dd, J = 4.7, 4.1 Hz, 1H), 5.79 (d, J = 4.1 Hz, 1H); ^{13}C NMR δ 26.8, 26.9, 35.3, 60.6, 76.3, 79.0, 79.8, 104.3, 113.1; HRMS (CI) *m/z* 187.0959 (29, M – OH [$C_9H_{15}O_4$] = 187.0970).

5-Deoxy-1,2-*O*-isopropylidene-6-*O*-tosyl-α-D-*ribo*-hexofuranose (4). TsCl (1.80 g, 9.44 mmol) was added to **3** (1.75 g, 8.58 mmol) in pyridine (20 mL) at ~0 °C (ice bath), and stirring was continued for 2 h at 0 °C and 1 h at ambient temperature. $NaHCO_3/H_2O$ (saturated, 2 mL) was added, and the mixture was stirred for 15 min. Volatiles were evaporated, and the residue was paritioned ($H_2O/CHCl_3$). The organic phase was washed (1 M HCl/H_2O, $NaHCO_3/H_2O$, brine) and dried ($MgSO_4$). Volatiles were evaporated, and the residue was chromatographed ($CHCl_3$ → 3% $MeOH/CHCl_3$) to give **4** (2.46 g, 80%) as a syrup: ^{1}H NMR δ 1.37, 1.55 (2 × s, 2 × 3H), 1.82–2.20 (m, 2H), 2.30 (br s, 1H, ex), 2.42 (s, 3H), 3.54–3.65 (m, 1H), 3.73 (td, J = 8.8, 4.0 Hz, 1H), 4.06–4.29 (m, 2H), 4.51 (t, J = 4.0 Hz, 1H), 5.73 (d, J = 4.1 Hz, 1H), 7.38, 7.80 (2 × d, J = 8.2 Hz, 2 × 2H); HRMS (FAB) *m/z* 359.1171 (4, MH$^+$ [$C_{16}H_{23}O_7S$] = 359.1165).

6-Azido-5,6-dideoxy-1,2-*O*-isopropylidene-α-D-*ribo*-hexofuranose (5). NaN_3 (118 mg, 1.8 mmol) and **4** (215 mg, 0.6 mmol) in dried DMF (5 mL) were heated for 5 h at 65 °C, volatiles were evaporated, and the residue was partitioned ($NaHCO_3/H_2O//CHCl_3$). The organic phase was washed (1 M HCl/H_2O, $NaHCO_3/H_2O$, brine), dried ($MgSO_4$), and volatiles were evaporated. The residue was chromatographed (40% EtOAc/hexanes) to give **5** (117 mg, 85%) as an oil: ^{1}H NMR δ 1.36, 1.56 (2 × s, 2 × 3H), 1.74–2.09 (m, 2H), 2.40 (br d, J = 6.0 Hz, 1H), 3.46 (td, J = 7.0, 2.9 Hz, 2H), 3.57–3.70 (m, 1H), 3.78 (td, J = 8.6, 4.2 Hz, 1H), 4.55 (dd, J = 4.8, 4.0 Hz, 1H), 5.79 (d, J = 4.0 Hz, 1H); ^{13}C NMR δ 26.5, 26.7, 31.7, 48.2, 76.0, 77.4, 78.6, 104.0, 112.9; HRMS (FAB) *m/z* 252.0965 (32, MNa$^+$ [$C_9H_{15}N_3O_4Na$] = 252.0960).

6-Acetamido-3-*O*-acetyl-5,6-dideoxy-2,3-*O*-isopropylidene-α-D-*ribo*-hexofuranose (6). A solution of PPh_3 (131 mg, 0.5 mmol) and **5** (46 mg, 0.2 mmol) in pyridine (5 mL) and $NH_3/MeOH$ (5 mL) was stirred in a sealed tube for 16 h at room temperature. Volatiles were evaporated, toluene was added and evaporated (2 ×), and the residue was dissolved (H_2O). The solution was washed (CH_2Cl_2, 3 ×), and volatiles were evaporated. The residue was dried in vacuo, Ac_2O (1 mL) and DMAP (1 crystal) were added, and the solution was stirred for 5 h at ambient temperature. MeOH (5 mL) was added, stirring was continued for 30 min, volatiles were evaporated, and the residue was chromatographed (5% $MeOH/CH_2Cl_2$) to give **6** (43 mg, 75%): ^{1}H NMR δ 1.31, 1.55 (2 × s, 2 × 3H), 1.91–2.06 (m, 5H), 2.13 (s, 3H), 3.24 (dd, J = 8.3, 5.4 Hz, 1H), 3.49–3.60 (m, 1H), 4.13 (td, J = 9.2, 2.9 Hz, 1H), 4.43 (dd, J = 9.2, 4.8 Hz, 1H), 4.79 (dd, J = 4.8, 3.9 Hz, 1H), 5.80 (d, J = 3.9 Hz, 1H), 6.03 (br s, 1H, ex); ^{13}C NMR δ 20.9, 23.6, 26.6, 26.7, 31.5, 37.3, 76.0, 76.6, 77.0, 104.2, 113.2, 170.2, 170.5; HRMS (FAB) *m/z* 288.1436 (100, MH$^+$ [$C_{13}H_{22}NO_6$] = 288.1447).

6-Acetamido-3-*O*-acetyl-3-deuterio-5,6-dideoxy-2,3-*O*-isopropylidene-α-D-*ribo*-hexofuranose (6). A solution of Bu_3SnD (94 µL, 102 mg, 0.35 mmol), AIBN (~2 mg), and **5** (16 mg, 0.07 mmol) in dried benzene (8 mL) was deoxygenated (Ar, 20 min) and heated for 1 h at reflux [AIBN (~2 mg) was added after 30 min]. Treatment of the reaction mixture (as described for **5** → **6**) gave **6**/3[^{2}H]**6** (~70:30; 15 mg, 75%): ^{1}H NMR δ 4.43 (dd, ~0.7H), other peaks like those for **6**; HRMS (CI) *m/z* 288.1432/289.1495 (55:26, MH$^+$ [$C_{13}H_{22}NO_6$]/[$C_{13}H_{21}DNO_6$] = 288.1447/289.1510).

6-*S*-Acetyl-5-deoxy-1,2-*O*-isopropylidene-6-thio-α-D-*ribo*-hexofuranose (7). KSAc (457 mg, 4 mmol) and **4** (358 mg, 1 mmol) in dried DMF (7 mL) were stirred overnight at ambient temperature, volatiles were evaporated, and the residue was partitioned ($NaHCO_3/H_2O//CHCl_3$). The organic phase was washed (1 M HCl/H_2O, $NaHCO_3/H_2O$, and brine) and dried ($MgSO_4$). Volatiles were evaporated, and the residue was chromatographed (40% EtOAc/hexanes) to give **7** (231 mg, 88%) as a white solid: ^{1}H NMR δ 1.38, 1.48 (2 × s, 2 × 3H), 1.72–2.08 (m, 2H), 2.32 (s, 3H), 2.36 (br s, 1H, ex), 2.89–3.17 (m, 2H), 3.63 (br s, 1H), 3.74 (td, J = 8.6, 4.0 Hz, 1H), 4.54 (dd, J = 5.0, 4.0 Hz, 1H), 5.78 (d, J = 4.0 Hz, 1H); ^{13}C NMR δ 25.6, 26.5, 26.7, 30.8, 32.4, 75.9, 78.7, 79.0, 104.0, 112.8, 196.0; HRMS (FAB) *m/z* 263.0949 (100, MH$^+$ [$C_{11}H_{19}O_5S$] = 263.0953).

Bis(5-deoxy-1,2-*O*-isopropylidene-6-thio-α-D-*ribo*-hexofuranose Disulfide (9). A solution of **7** (262 mg, 1 mmol) in NH_3/MeOH (10 mL) was stirred overnight at ambient temperature in a sealed flask. Volatiles were evaporated, and the residue was chromatographed (60% EtOAc/hexanes) to give **9** (209 mg, 95%): TLC ($MeOH/CH_2Cl_2$, 5:95) $R_f \approx 0.1$; ^{1}H NMR δ 1.34, 1.55 (2 × s, 2 × 3H), 1.80–1.96 (m, 1H), 2.08–2.22 (m, 1H), 2.56 (br s, 1H, ex), 2.72–2.90 (m, 2H), 3.62 (dd, J = 8.6, 5.1 Hz, 1H), 3.81 (td, J = 8.6, 4.2 Hz, 1H), 4.53 (t, J = 4.5 Hz, 1H), 5.76 (d, J = 4.0 Hz, 1H); ^{13}C NMR δ 26.5, 26.7, 31.9, 34.8, 75.9, 78.4, 78.7, 103.9, 112.7; HRMS (FAB) *m/z* 461.1268 (MNa$^+$ [$C_{18}H_{30}O_8S_2Na$] = 461.1280).

5-Deoxy-1,2-*O*-isopropylidene-6-thio-α-D-*ribo*-hexofuranose (8). Standard treatment of **9** (21 mg, 0.048 mmol) with Bu_3SnD (153 μL, 165 mg, 0.56 mmol)/AIBN (~1 mg)/benzene/reflux for 3 h (additional AIBN added and reflux continued for 1 h) gave complete conversion to **8** (19 mg, 90%): TLC ($MeOH/CH_2Cl_2$, 5:95) $R_f \approx 0.25$; ^{1}H NMR δ 1.36, 1.56 (2 × s, 2 × 3H), 1.82–2.06 (m, 2H), 2.35 (d, J = 11.0 Hz, 1H), 2.57–2.76 (m, 2H), 3.56–3.65 (m, 1H), 3.80–3.87 (m, 1H), 4.54 (t, J = 3.9 Hz, 1H), 5.76 (d, J = 3.9 Hz, 1H); ^{13}C NMR δ 21.2, 26.6, 26.8, 36.8, 75.8, 78.6, 104.0, 112.7; HRMS (CI) *m/z* 221.0852 (MH$^+$ [$C_9H_{17}O_4S$] = 221.0848). TLC of **8** sometimes showed *trace* amounts of the more slowly migrating disulfide **9**. HRMS (FAB) of **8**, **9**, or **8/9** mixtures had molecular ion peaks for both species.

1-(5-Deoxy-2,3-*O*-isopropylidene-6-*O*-tosyl-β-D-*ribo*-hexofuranosyl)uracil (10). TsCl (71 mg, 0.37 mmol) was added to 1-(5-deoxy-2,3-*O*-isopropylidene-β-D-*ribo*-hexofuranosyl)uracil[1,6a] (90 mg, 0.30 mmol) in dried pyridine (5 mL) at 0 °C, stirring was continued for 14 h at 0 °C, and volatiles were evaporated. The residue was partitioned (2M $HCl/H_2O//CH_2Cl_2$), and the organic phase was washed (saturated $NaHCO_3/H_2O$, brine) and dried (Na_2SO_4). Volatiles were evaporated, and the residue was chromatographed (5% $MeOH/CH_2Cl_2$) to give **10** (118 mg, 87%) as a syrup: UV max 259 nm; ^{1}H NMR δ 1.36, 1.51 (2 × s, 2 × 3H), 2.07 (dd, J = 12.9, 5.8 Hz, 2H), 2.41 (s, 3H), 4.05–4.13 (m, 3H), 4.65 (dd, J = 6.3, 4.8 Hz, 1H), 4.98 (dd, J = 6.3, 1.9 Hz, 1H), 5.46 (d, J = 1.9 Hz, 1H), 5.71 (d, J = 8.0 Hz, 1H), 7.18 (d, J = 8.0 Hz, 1H), 7.30–7.78 (m, 4H), 9.72 (br s, 1H, ex); ^{13}C NMR δ 21.6, 25.4, 27.2, 32.5, 66.9, 83.4, 83.6, 84.2, 94.8, 102.6, 114.8, 127.9, 129.8, 133.5, 142.7, 144.9, 149.5, 162.7; HRMS (FAB) *m/z* 453.1333 (100, MH$^+$ [$C_{20}H_{25}N_2O_8S$] = 453.1332).

1-(6-Azido-5,6-dideoxy-2,3-*O*-isopropylidene-β-D-*ribo*-hexofuranosyl)uracil (11). A solution of NaN_3 (65 mg, 1 mmol) and **10** (90 mg, 0.20 mmol) in dried DMF (3 mL) was stirred for 16 h at ambient temperature, filtered, and volatiles were evaporated. The residue was dissolved (EtOAc), and the solution was washed (H_2O, 2 ×) and dried (Na_2SO_4). Volatiles were evaporated, and the residue was chromatographed (4% $MeOH/CH_2Cl_2$) to give **11** (59 mg, 91%) as a solid foam: UV max 258 nm; ^{1}H NMR δ

1.34, 1.56 (2 × s, 2 × 3H), 2.01 (dd, J = 13.4, 6.8 Hz, 2H), 3.40 (t, J = 6.8 Hz, 2H), 4.07–4.16 (m, 1H), 4.70 (dd, J = 6.6, 5.0 Hz, 1H), 5.03 (dd, J = 6.6, 1.8 Hz, 1H), 5.51 (d, J = 1.8 Hz, 1H), 5.73 (d, J = 8.0 Hz, 1H), 7.20 (d, J = 8.0 Hz, 1H), 9.35 (br s, 1H, ex); ^{13}C NMR δ 25.8, 27.6, 33.0, 48.5, 84.3, 84.8, 85.3, 95.6, 103.1, 115.2, 143.5, 150.4, 164.1; HRMS (CI) *m/z* 324.1298 (100, MH$^+$ [$C_{13}H_{18}N_5O_5$] = 324.1308).

1-(6-Acetamido-5,6-dideoxy-2,3-*O*-isopropylidene-β-D-*ribo*-hexofuranosyl)uracil (12). Reduction and acetylation of **11** (65 mg, 0.2 mmol) (as described for **5** → **6**) (with chromatography, 8% MeOH/CH_2Cl_2) gave **12** (47 mg, 69%) as a solid foam: UV max 259 nm (ε 9800); ^{1}H NMR δ 1.34, 1.56 (2 × s, 2 × 3H), 1.93–2.07 (m, 5H), 3.37 (dd, J = 12.9, 6.7 Hz, 2H), 4.07 (td, J = 6.6, 4.7 Hz, 1H), 4.68 (dd, J = 6.4, 4.7 Hz, 1H), 5.01 (dd, J = 6.4, 1.8, Hz, 1H), 5.56 (d, J = 1.8 Hz, 1H), 5.76 (d, J = 8.0 Hz, 1H), 6.00 (br s, 1H, ex), 7.24 (d, J = 8.0 Hz, 1H), 9.47 (br s, 1H, ex); ^{13}C NMR δ 23.8, 25.8, 27.7, 33.2, 36.9, 84.1, 84.6, 86.1, 95.0, 103.3, 115.4, 143.1, 150.6, 163.6, 170.7; HRMS (CI) *m/z* 340.1513 (100, MH$^+$ [$C_{15}H_{22}N_3O_6$] = 340.1509). Anal. Calcd for $C_{15}H_{21}N_3O_6$ (339.3): C, 53.09; H, 6.24; N, 12.38. Found: C, 52.83; H, 6.21; N, 12.14.

1-(6-Acetamido-5,6-dideoxy-3-deuterio-2,3-*O*-isopropylidene-β-D-*ribo*-hexofuranosyl)uracil (12). Treatment of **11** (16 mg, 0.05 mmol) with Bu_3SnD (67 μL, 73 mg, 0.25 mmol), and acetylation (as for **5** → **6**) (with chromatography, 8% MeOH/CH_2Cl_2) gave **12**/3'[^{2}H]**12** (~75:25; 13 mg, 76%): UV max 259 nm; ^{1}H NMR δ 4.68 (dd, ~0.75H), all other peaks like those for **12**; HRMS (CI) *m/z* 340.1509/341.1569 (100:32; MH$^+$ [$C_{15}H_{22}N_3O_6$]/[$C_{15}H_{21}DN_3O_6$] = 340.1509/341.1571).

Methyl 5-Deoxy-2,3-*O*-isopropylidene-β-D-*ribo*-hexofuranoside (14). This reference compound[1,6b,21] had: ^{1}H NMR δ 1.29, 1.46 (2 × s, 2 × 3H), 1.80 (dt, J = 8.9, 6.1 Hz, 2H), 2.12 (br s, 1H, ex), 3.33 (s, 3H), 3.77 (t, J = 6.1 Hz, 2H), 4.34 (dd, J = 8.9, 6.1 Hz, 1H), 4.58 (s, 2H), 4.94 (s, 1H); ^{13}C NMR δ 25.1, 26.6, 37.5, 55.3, 60.4, 84.5, 85.4, 85.6, 110.0, 112.6; HRMS (CI) *m/z* 219.1230 (100, MH$^+$ [$C_{10}H_{19}O_5$] = 219.1232).

Methyl 5-Deoxy-2,3-*O*-isopropylidene-6-*O*-nitro-β-D-*ribo*-hexofuranoside (15). Fuming HNO_3 (d = 1.5 g/mL; 5 mL) in Ac_2O (10 mL) was added to a cold solution of **14**[1,6b,21] (1.09 g, 5 mmol) in Ac_2O (15 mL), and stirring was continued for 1 h at –60 °C. The solution was poured *carefully* into ice-cold $NaHCO_3/H_2O$ (150 mL), stirring was continued for 30 min at ambient temperature, and the mixture was extracted (EtOAc, 3 ×). The combined organic phase was washed (brine) and dried ($MgSO_4$). Volatiles were evaporated, and the residue was chromatographed (15 → 20% EtOAc/hexanes) to give **15** (1.18 g, 90%): ^{1}H NMR δ 1.30, 1.46 (2 × s, 2 × 3H), 1.95 (dd, J = 13.6, 7.0 Hz, 2H), 3.32 (s, 3H), 4.24 (t, J = 7.6 Hz, 1H), 4.52–4.61 (m, 4H), 4.93 (s, 1H); ^{13}C NMR δ 25.0, 26.5, 32.5, 55.4, 70.4, 83.6, 84.2, 85.5, 110.2, 112.8; HRMS (CI) *m/z* 264.1078 (80, MH$^+$ [$C_{10}H_{18}NO_7$] = 264.1083).

Methyl 5-Deoxy-3-deuterio-2,3-*O*-isopropylidene-β-D-*ribo*-hexofuranoside (14). Bu_3SnD (137 μL, 148 mg, 0.51 mmol), AIBN (5 mg), and **15** (25 mg, 0.095 mmol) in dried benzene (5 mL) were deoxygenated (Ar, 20 min), and the solution was heated for 5 h at reflux [AIBN (~3 mg) added after 2 h]. Volatiles were evaporated, and the residue was chromatographed (30 → 50% EtOAc/hexanes) to give **14**/3[^{2}H]**14** (~40:60; 17 mg, 81%) as an oil: ^{1}H NMR δ 4.58 (s, 1.4H), other peaks like those for **14**; HRMS (CI) *m/z* 219.1230/220.1296 (77/100, MH$^+$ [$C_{10}H_{19}O_5$]/[$C_{10}H_{18}DO_5$] = 219.1232/220.1295).

3,6-Di-*O*-(*tert*-butyldimethylsilyl)-5-deoxy-1,2-*O*-isopropylidene-β-D-*ribo*-hexofuranose (16). A solution of dried **13**[1,6b] (960 mg, 3 mmol), TBDMSCl (90 mg, 0.6 mmol), and imidazole (825 mg, 12 mmol) in dried DMF (20 mL) was stirred (under N_2) overnight at ambient temperature. H_2O (2 mL) was added, volatiles were evaporated, and the residue was partitioned (EtOAc//$NaHCO_3/H_2O$). The organic phase was washed (brine) and dried (Na_2SO_4). Volatiles were evaporated, and the residue was chromatographed (10% EtOAc/hexanes) to give **16** (1.24 g, 95%) as an oil: ^1H NMR δ 0.06 (s, 6H), 0.10, 0.12 (2 × s, 2 × 3H), 0.89, 0.91 (2 × s, 2 × 9H), 1.32, 1.53 (2 × s, 2 × 3H), 1.59–2.02 (m, 2H), 3.59 (dd, J = 9.0, 4.5 Hz, 1H), 3.70–3.81 (m, 2H), 3.94 (td, J = 9.0, 3.4 Hz, 1H), 4.39 (dd, J = 4.5, 4.0, 1H), 5.71 (d, J = 4 Hz, 1H); ^{13}C NMR δ –5.3, –4.7, 18.3, 18.7, 25.8, 26.0, 26.5, 26.7, 35.6, 60.2, 76.1, 77.1, 79.2, 103.9, 112.2; HRMS (FAB) *m/z* 433.2789 (12, MH^+ [$C_{21}H_{45}O_5Si_2$] = 433.2806).

3-*O*-(*tert*-Butyldimethylsilyl)-5-deoxy-1,2-*O*-isopropylidene-β-D-*ribo*-hexofuranose (17). HCl/H_2O (1 M, 4 mL) was added to a stirred solution of **16** (1.0 g, 2.3 mmol) in MeOH (20 mL). Stirring was continued for 30 min at ambient temperature, saturated $NaHCO_3/H_2O$ (5 mL) was added, stirring was continued for 15 min, and volatiles were evaporated. EtOAc was added, and the mixture was filtered. Volatiles were evaporated, and the residue was chromatographed (20 → 35% EtOAc/hexanes) to give **17** (730 mg, 99%) as an oil: ^1H NMR δ 0.11, 0.12 (2 × s, 2 × 3H), 0.91 (s, 9H), 1.32, 1.54 (2 × s, 2 × 3H), 1.65–2.01 (m, 2H), 2.02 (br s, 1H, ex), 3.65 (dd, J = 8.6, 4.6 Hz, 1H), 3.79 (t, J = 5.8 Hz, 2H), 4.01 (td, J = 8.6, 3.8 Hz, 1H), 4.41 (t, J = 4.2 Hz, 1H), 5.73 (d, J = 3.8, 1H); ^{13}C NMR δ –4.8, –4.5, 18.2, 25.7, 26.5, 34.4, 60.8, 76.9, 78.6, 78.7, 103.8, 112.5; HRMS (FAB) *m/z* = 341.1764 (22, MNa^+ [$C_{15}H_{30}O_5SiNa$] = 341.1760.

3-*O*-(*tert*-Butyldimethylsilyl)-5-deoxy-1,2-*O*-isopropylidene-6-*O*-nitro-β-D-*ribo*-hexofuranose (18). Nitration (2 h, –60 °C) of **17** (1.6 g, 5 mmol) (as described for **14** → **15**) (with chromatography, 20% EtOAc/hexanes) gave **18** (1.60 g, 88%) as an oil: ^1H NMR δ 0.11, 0.13 (2 × s, 2 × 3H), 0.91 (s, 9H), 1.33, 1.54 (2 × s, 2 × 3H), 1.85 (td, J = 14.4, 7.2 Hz, 1H), 2.11 (tdd, J = 14.4, 7.2, 3.6 Hz, 1H), 3.62 (dd, J = 8.8, 4.6 Hz, 1H), 3.95 (td, J = 8.8, 3.5 Hz, 1H), 4.44 (t, J = 4.2 Hz, 1H), 4.53–4.70 (m, 2H), 5.72 (d, J = 3.8, H2, 1H); ^{13}C NMR δ –4.9, –4.5, 18.1, 25.7, 26.5, 26.5, 29.4, 70.1, 75.5, 76.9, 78.9, 103.8, 112.6; HRMS (FAB) *m/z* = 364.1765 (5, MH^+ [$C_{15}H_{30}NO_7Si$] = 364.1792).

5-Deoxy-1,2-*O*-isopropylidene-6-*O*-nitro-β-D-*ribo*-hexofuranose (19). NH_4F (360 mg, 9.7 mmol) was added to **18** (364 mg, 1 mmol) in MeOH (30 mL), and stirring was continued for 8 h at reflux. Volatiles were evaporated, EtOAc was added, and the suspension was filtered. Volatiles were evaporated, and the residue was chromatographed (20 → 35% EtOAc/hexanes) to give **19** (240 mg, 95%) as an oil: ^1H NMR δ 1.38, 1.57 (2 × s, 2 × 3H), 1.96 (td, J = 14.4, 6.2 Hz, 1H), 2.19 (tdd, J = 14.4, 7.2, 4.0 Hz, 1H), 2.45 (br s, 1H, ex), 3.65 (td, J = 8.7, 5.1 Hz, 1H), 3.80 (td, J = 8.7, 4.0 Hz, 1H), 4.53–4.69 (m, 3H), 5.80 (d, J = 4.0 Hz, 1H); ^{13}C NMR δ 26.3, 26.5, 29.5, 69.8, 75.8, 76.4, 78.3, 103.8, 112.7; HRMS (CI) *m/z* 249.0845 (27, M^+ [$C_9H_{15}NO_7$] = 249.0849).

3-*O*-Acetyl-5-deoxy-1,2-*O*-isopropylidene-6-*O*-nitro-β-D-*ribo*-hexofuranose (20). Ac_2O (10 mL), imidazole (50 mg, 0.73 mmol), and **19** (250 mg, 1 mmol) were stirred for 5 h at 0 °C. MeOH (10 mL) was added, and stirring was continued for 30 min at ambient temperature. Volatiles were evaporated, and the residue was chromatographed (20% EtOAc/hexanes) to give **20** (280 mg, 95%) as an oil: ^1H NMR δ 1.34, 1.56 (2 × s, 2 × 3H), 1.84–2.14 (m, 2H), 2.15 (s, 3H), 4.19 (td, J = 8.8, 3.6 Hz, 1H), 4.47–4.67 (m, 3H), 4.82 (t, J = 3.7 Hz, 1H), 5.80 (d, J = 3.8 Hz, 1H); ^{13}C NMR δ 20.7, 26.4, 29.6, 69.5, 73.6, 75.9, 77.1, 103.9, 113.1, 170.3; HRMS (CI) *m/z* = 291.0959 (19, M^+ [$C_{11}H_{17}NO_8$] = 291.0954).

5-Deoxy-3-deuterio-1,2-*O*-isopropylidene-β-D-*ribo*-hexofuranose (3). Treatment of **19** (25 mg, 0.1 mmol) with Bu_3SnD (137 μL, 148 mg, 0.5 mmol) (as described for **15** → **14**) (with chromatography, 50% EtOAc/hexanes) gave **3**/3[^{2}H]**3** (~45:55; 20 mg, 97%) as an oil: ^{1}H NMR δ 3.68–3.88 (m, 3.45H), other peaks the same as for **3**; ^{13}C NMR δ 76.3 (~0.5C); HRMS (FAB) *m/z* = 206.1145 (4, MH^+ [$C_9H_{16}DO_5$] = 206.1139).

3-*O*-(*tert*-Butyldimethylsilyl)-5-deoxy-3-deuterio-1,2-*O*-isopropylidene-β-D-*ribo*-hexofuranose (17). Treatment of **18** (20 mg, 0.06 mmol) with Bu_3SnD (137 μL, 148 mg, 0.5 mmol) (as described for **15** → **14**) (with chromatography, 25% EtOAc/hexanes) gave **17**/3[^{2}H]**17** (~15:85; 15 mg, 78%) as an oil: ^{1}H NMR δ 3.65 (dd, 0.15H), 4.01 (dd, J = 8.6, 3.8 Hz, 1H), 4.39 (d, J = 3.8 Hz, 1H), other peaks same as for **17**; ^{13}C NMR δ 76.85 (no signal detected), other peaks as for **17**; HRMS (CI) *m/z* 304.1706/305.1769 (10:26, MH^+ – Me [$C_{14}H_{28}O_5Si$]/[$C_{14}H_{27}DO_5Si$] = 304.1698/305.1735; HRMS (FAB) *m/z* 342.1807 (100, MNa^+ [$C_{15}H_{29}DO_5SiNa$] = 342.1823).

3-*O*-Acetyl-5-deoxy-3-deuterio-1,2-*O*-isopropylidene-β-D-*ribo*-hexofuranose (21). Treatment of **20** (20 mg, 0.07 mmol) with Bu_3SnD (137 μL, 148 mg, 0.5 mmol) (as described for **15** → **14**) (with chromatography, 25% EtOAc/hexanes) gave **21**/3[^{2}H]**21** (~80:20; 10 mg, 57%) as an oil: ^{1}H NMR δ 1.34, 1.57 (2 × s, 2 × 3H), 1.61 (br s, 1H, ex), 1.77–2.03 (m, 2H), 2.14 (s, 3H), 3.81 (dd, J = 11.6, 5.9 Hz, 2H), 4.26 (td, J = 8.8, 3.6 Hz, 1H), 4.52 (dd, J = 9.2, 4.6 Hz, 0.8H), 4.82 (t, J = 4.2 Hz, 1H), 5.82 (d, J = 3.8 Hz, 1H); ^{13}C NMR δ 20.7, 26.4, 26.5, 34.3, 60.2, 75.9, 76.0, 76.9, 104.0, 113.0, 171.6; HRMS (FAB) *m/z* 247.1171/248.1251 (7:3, MH^+ [$C_{11}H_{19}O_6$]/[$C_{11}H_{18}DO_6$] = 247.1182/248.1244).

The 6-*O*-acetyl-5-deoxy-3-deuterio-1,2-*O*-isopropylidene-β-D-*ribo*-hexofuranose (H3/[^{2}H]3, ~80:20; 6 mg, 34%) was an oil: ^{1}H NMR δ 1.37, 1.57 (2 × s, 2 × 3H), 1.80–2.19 (m, 2H), 2.06 (s, 3H), 2.33 (br s, 1H, ex), 3.64 (td, J = 8.8, 5.2 Hz, 0.8H), 3.81 (td, J = 8.8, 4.2, 1H), 4.13–4.34 (m, 2H), 4.56 (t, J = 4.6 Hz, 1H), 5.80 (d, J = 4.0 Hz, 1H); ^{13}C NMR δ 21.0, 26.3, 26.5, 31.2, 61.1, 75.8, 78.3, 78.3, 103.8, 112.5, 171.0; HRMS (FAB) *m/z* 247.1188/248.1243 (70:30, MH^+ [$C_{11}H_{19}O_6$]/[$C_{11}H_{18}DO_6$] = 247.1182/248.1244). Deprotection (NH_3/MeOH) of **21**, and the 6-*O*-acetyl isomers, gave **3** with ~20% reduction in the ^{1}H NMR signal for H3.

Analogous treatment (Bu_3SnD) of the 3-*O*-benzoyl ester of **19**, and debenzoylation, gave **3** with ~25% reduction in the ^{1}H NMR signal for H3.

6-*O*-(*tert*-Butyldimethylsilyl)-5-deoxy-1,2-*O*-isopropylidene-3-*O*-methyl-α-D-*ribo*-hexofuranose (22). NaH (60% in oil, 160 mg, 4 mmol) was added to **13**[1,6b] (636 mg, 2 mmol) in dried DMF (10 mL). After 3 min, MeI (250 μL, 568 mg, 4 mmol) was added, and stirring was continued for 3 h at ambient temperature. EtOAc/H_2O was added, the layers were separated, and the organic phase was washed (0.1 M HCl/H_2O, $NaHCO_3$/H_2O, and brine) and dried (Na_2SO_4). Volatiles were evaporated, and the residue was chromatographed (20% EtOAc/hexanes) to give **22** (566 mg, 85%): ^{1}H NMR δ 0.03 (s, 6H), 0.86 (s, 9H), 1.32, 1.55 (2 × s, 2 × 3H), 1.68 (td, J = 13.2, 6.6 Hz, 1H), 1.96 (ddd, J = 13.7, 6.6, 3.5 Hz, 1H), 3.31 (dd, J = 9.0, 4.1 Hz, 1H), 3.46 (s, 3H), 3.65–3.80 (m, 2H), 4.03 (td, J = 9.0, 3.5 Hz, 1H), 4.62 (t, J = 4.0 Hz, 1H), 5.72 (d, J = 3.9 Hz, 1H); ^{13}C NMR δ –5.2, 18.5, 26.1, 26.6, 26.8, 35.9, 58.5, 59.9, 74.9, 76.8, 77.1, 85.1, 104.0, 112.8; HRMS (CI) *m/z* 355.1915 (13, MNa^+ [$C_{16}H_{32}O_5SiNa$] = 355.1917).

5-Deoxy-1,2-*O*-isopropylidene-3-*O*-methyl-α-D-*ribo*-hexofuranose (23). TBAF/THF (1 M; 6 mL, 6 mmol) was added to **22** (498 mg, 1.5 mmol) in THF (10 mL), and stirring was continued for 3 h at ambient temperature. Volatiles were evaporated, and the residue was chromatographed (EtOAc) to give **23** (314 mg, 96%): ^{1}H NMR δ 1.38, 1.59 (2 × s, 2 × 3H), 1.84–1.96 (m, 2H), 2.54 (t, J = 6.0 Hz, 1H, ex), 3.37 (dd, J = 9.0, 4.5 Hz, 1H), 3.50 (s, 3H), 3.78 (q, J = 5.7 Hz, 2H), 4.06–4.12 (m, 1H), 4.62 (t, J = 3.7 Hz, 1H), 5.78 (d, J = 3.9 Hz, 1H); ^{13}C NMR δ 26.4, 26.6, 35.1, 58.1, 60.4, 76.5, 77.3, 84.7, 103.9, 113.0; HRMS (CI) m/z 219.1915 (2, MH$^+$ [$C_{10}H_{19}O_5$] = 219.1249).

5-Deoxy-1,2-*O*-isopropylidene-3-*O*-methyl-6-*O*-nitro-α-D-*ribo*-hexofuranose (24). Nitration of **23** (314 mg, 1.44 mmol) (as described for **14** → **15**) (with chromatography, 25% EtOAc/hexanes) gave **24** (310 mg, 82%): ^{1}H NMR δ 1.34, 1.56 (2 × s, 2 × 3H), 1.88–2.02 (m, 1H), 2.14 (ddd, J = 14.2, 7.0, 4.1 Hz, 1H), 3.32 (dd, J = 8.8, 4.0 Hz, 1H), 3.47 (s, 3H), 4.01 (td, J = 8.8, 4.1 Hz, 1H), 4.53–4.62 (m, 2H), 4.67 (t, J = 3.9 Hz, 1H), 5.75 (d, J = 3.7 Hz, 1H); ^{13}C NMR δ 26.5, 26.8, 30.0, 58.4, 70.0, 74.5, 76.8, 84.8, 104.1, 113.2; HRMS (CI) m/z 264.1083 (100, MH$^+$ [$C_{10}H_{18}NO_7$] = 264.1075).

5-Deoxy-3-deuterio-1,2-*O*-isopropylidene-3-*O*-methyl-α-D-*ribo*-hexofuranose (23). Treatment of **24** (26 mg, 0.1 mmol) with Bu_3SnD (137 μL, 148 mg, 0.5 mmol) (as described for **15** → **14**) (with chromatography, hexanes → 30% EtOAc/hexanes) gave **23**/3[^{2}H]**23** (~30:70; 18 mg, 82%) as an oil: ^{1}H NMR δ 3.37 (dd, 0.3H), other peaks same as for **23**; ^{13}C NMR δ 84.7 (<0.5C); HRMS (FAB) m/z 219.1223/220.1298 (10/35, MH$^+$ [$C_{10}H_{19}O_5$]/[$C_{10}H_{18}DO_5$] = 219.1232/220.1295).

Methyl 5-Deoxy-3-*O*-methyl-6-*O*-nitro-β-D-*ribo*-hexofuranoside (25). A solution of **24** (265 mg, 1 mmol) in TFA/H_2O (9:1, 2 mL) was stirred at ~0 °C (ice bath) for 1 h. Volatiles were evaporated, and xylene was added and evaporated. The residue was dissolved in MeOH (5 mL), HCl/H_2O (37%, d = 1.2 g/mL; 0.05 mL) was added, and stirring was continued for 3 h. NH_3/H_2O was added (to pH ~7), volatiles were evaporated, the residue was partitioned (EtOAc//$NaHCO_3/H_2O$), and the organic phase was washed (brine) and dried (Na_2SO_4). Volatiles were evaporated, and the residue was chromatographed (60% EtOAc/hexanes) to give **25** (206 mg, 87%) as a yellow oil: ^{1}H NMR δ 1.89–2.20 (m, 2H), 2.74 (br s, 1H, ex), 3.36, 3.44 (2 × s, 2 × 3H), 3.75 (dd, J = 6.9, 4.4 Hz, 1H), 3.99–4.07 (m, 1H), 4.12 (d, J = 4.4 Hz, 1H), 4.52–4.68 (m, 2H), 4.84 (s, 1H); ^{13}C NMR δ 32.7, 55.0, 58.4, 70.2, 72.5, 77.3, 84.5, 108.5; HRMS (CI) m/z 238.0927 (15, MH$^+$ [$C_8H_{16}NO_7$] = 238.0909).

Methyl 5-Deoxy-3-*O*-methyl-β-D-*ribo*-hexofuranoside (26). A sample of **23** (40 mg, 0.18 mmol) was treated with TFA/H_2O and then HCl/MeOH (as described for **24** → **25** to the point of addition of aqueous ammonia). Volatiles were evaporated, the residue was slurried (CH_2Cl_2), and the suspension was filtered (cotton plug). Volatiles were evaporated, and the residue was chromatographed (50 → 95% EtOAc/hexanes) to give **26** (21 mg, 61%): ^{1}H NMR δ 1.87–1.95 (m, 2H), 2.29 (t, J = 4.5 Hz, 1H, ex), 2.58 (d, J = 3.0 Hz, 1H, ex), 3.38, 3.47 (2 × s, 2 × 3H), 3.79–3.85 (m, 3H), 4.08–4.15 (m, 2H), 4.86 (s, 1H); ^{13}C NMR δ 37.6, 55.3, 58.4, 60.9, 72.4, 80.0, 84.7, 108.5; HRMS (CI) m/z 193.1074 (MH$^+$ [$C_8H_{17}O_5$] = 193.1076).

Methyl 3,6-Di-*O*-acetyl-5-deoxy-3-*O*-methyl-β-D-*ribo*-hexofuranoside (27). Acetylation of **26** (41 mg, 0.21 mmol) (as described for **19** → **20**) (with chromatography, 15% EtOAc/hexanes) gave **27** (30 mg, 51%): ^{1}H NMR δ 1.82–1.93 (m, 1H), 1.99–2.11 (m, 1H), 2.06, 2.14 (2 × s, 2 × 3H), 3.35 (s, 3H), 3.36 (s, 3H), 3.80 (dd, J = 7.4, 4.4 Hz, 1H), 4.02 (td, J = 8.1, 4.4 Hz, 1H), 4.15–4.30 (m, 2H), 4.83 (s, 1H), 5.20 (d, J = 4.1 Hz, 1H); ^{13}C NMR δ 16.3, 16.5, 29.8, 50.5, 54.5, 56.8, 69.2, 73.1, 79.0, 101.6, 165.5, 166.5; HRMS (FAB) m/z 299.1121 (24, MNa$^+$ [$C_{12}H_{20}O_7Na$] = 299.1107.

6-*O*-(*tert*-Butyldimethylsilyl)-5-deoxy-1,2-*O*-isopropylidene-3-*O*-methyl-α-D-*xylo*-hexofuranose (29). The methylation of 6-*O*-(*tert*-butyldimethylsilyl)-5-deoxy-1,2-*O*-isopropylidene-α-D-*xylo*-hexofuranose[1] (**28**; 500 mg, 1.57 mmol) (as described above for **13** → **22**) gave **29** (400 mg, 76%): ^{1}H NMR δ 0.05 (s, 6H), 0.89 (s, 9H), 1.31, 1.48 (2 × s, 2 × 3H), 1.88 ("sept", $J \approx 6.5$ Hz, 2H), 3.40 (s, 3H), 3.57 (d, $J = 2.9$ Hz, 1H), 3.72 (dd, $J = 6.8, 5.9$ Hz, 2H), 4.30 (ddd, $J = 7.2, 6.1, 3.0$ Hz, 1H), 4.56 (d, $J =$ 3.9 Hz, 1H), 5.86 (d, $J = 4.1$ Hz, 1H); ^{13}C NMR δ –9.9, –9.9, 13.8, 21.4, 21.7, 22.1, 26.5, 53.2, 55.7, 72.6, 77.1, 80.3, 100.0, 106.6; HRMS (FAB) *m/z* 333.2111 (13, MH$^+$ [$C_{16}H_{33}O_5Si$] = 333.2097).

Methyl 5-Deoxy-3-*O*-methyl-β-D-*xylo*-hexofuranose (30). Treatment of **29** (200 mg, 0.60 mmol) with TFA/H_2O and then HCl/MeOH (as described for **23** → **26**, except chromatography with 3% MeOH/CH_2Cl_2) gave **30** (35 mg, 30%): ^{1}H NMR δ 1.84–1.91 (m, 2H), 3.19 (br s, 2H, ex), 3.38 (s, 3H), 3.41 (s, 3H), 3.66 ("q", $J = 2.7$ Hz, 1H), 3.78 (t, $J = 6$ Hz, 2H), 4.17 (t, $J = 2.1$ Hz, 1H), 4.44 (dt, $J = 8.7$, 5.4 Hz, 1H), 4.77 (d, $J = 1.5$ Hz, 1H); ^{13}C NMR δ 28.1, 51.3, 53.7, 55.9, 74.0, 74.9, 81.6, 105.0; HRMS (CI) *m/z* 193.1076 (30, MH$^+$ [$C_8H_{17}O_5$] = 193.1076).

Methyl 5-Deoxy-3-deuterio-3-*O*-methyl-β-D-*ribo*-hexofuranoside (26) and Methyl 5-Deoxy-3-deuterio-3-*O*-methyl-β-D-*xylo*-hexofuranoside (30). Method A. Treatment of **25** (40 mg, 0.17 mmol) with Bu_3SnD (230 μL, 249 mg, 0.85 mmol) (as described for **15** → **14**) (with chromatography, 20% EtOAc/hexanes → 5% MeOH/CH_2Cl_2) gave **26/30** (64:36; 25 mg, 75%; ^{1}H NMR). A second column chromatography (1 → 2% MeOH/CH_2Cl_2) gave partial separation of the diastereomers [**26** (9 mg) and 3[^{2}H]**30** (3 mg)]. 3[^{2}H]**30**: ^{1}H NMR no peak at δ 3.66 (H3), other peaks were the same as for **30**; HRMS (CI) *m/z* 194.1126 (100, MH$^+$ [$C_8H_{16}DO_5$] = 194.1139). Acetylation of **26** (9 mg, 0.47 mmol) (as described for **19** → **20**) (with chromatography, 5% EtOAc/hexanes) gave **27**/3[^{2}H]**27** (25:75; 10 mg, 77%): ^{1}H NMR δ 3.80 (dd, $J = 7.4, 4.4$ Hz, ~0.25H), other peaks were the same as for **27**; HRMS (FAB) *m/z* 299.1105/300.1184 (21:100, MNa$^+$ [$C_{12}H_{20}O_7Na/C_{12}H_{19}DO_7Na$] = 299.1107/300.1170).

Method B. Treatment of **25** (10 mg, 0.042 mmol) with Bu_3SnD as in the above method A, followed by workup, acetylation, and chromatography (15% EtOAc/hexanes) gave **27**/(2,6-di-*O*-acetyl-3[^{2}H]**30**) (64:36; 10 mg, 90%; ^{1}H NMR); HRMS (FAB) *m/z* 299.1112/300.1186 (5:31, MNa$^+$ [$C_{12}H_{20}O_7Na/C_{12}H_{19}DO_7Na$] = 299.1107/300.1170).

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