Stereodefined Synthesis of O3′-Labeled Uracil Nucleosides. 3′-[^{17}O]-2′-Azido-2′-deoxyuridine 5′-Diphosphate as a Probe for the Mechanism of Inactivation of Ribonucleotide Reductases

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Thermolysis of a 2′-[^{16}O]-*O*-benzoyl-[^{17}O]-5′-*O*-(*tert*-butyldimethylsilyl)-O^2,3′-cyclouridine derivative gave the more stable 3′-[^{17}O]-*O*-benzoyl-[^{16}O]- 5′-*O*-(*tert*-butyldimethylsilyl)-O^2,2′-cyclouridine isomer, which was converted into 3′-[^{17}O]-2′-azido-2′-deoxyuridine by deprotection and nucleophilic ring opening at C2′ with lithium azide. The 5′-diphosphate was prepared by nucleophilic displacement of the 5′-*O*-tosyl group with tris(tetrabutylammonium) hydrogen pyrophosphate. Model reactions gave ^{16}O and ^{18}O isotopomers, and base-promoted hydrolysis of an O^2,2′-cyclonucleoside gave stereodefined access to 3′-[^{18}O]-1-(β-D-arabinofuranosyl)uracil. Inactivation of ribonucleoside diphosphate reductase with 2′-azido-2′-deoxynucleotides results in appearance of EPR signals for a nitrogen-centered radical derived from azide, and 3′-[^{17}O]-2′-azido-2′-deoxyuridine 5′-diphosphate provides an isotopomer to perturb EPR spectra in a predictable manner.

Introduction

Ribonucleotide reductases (RNRs) are ubiquitous enzymes that execute the only known conversion of 5′-(di or tri)phosphate esters of ribonucleosides into 2′-deoxynucleotides. Inhibition of RNRs disrupts this primary source of DNA components and is an appealing target for rational design of agents against rapidly proliferating viruses and cancer cells.[1] Ribonucleoside diphosphate reductase (RDPR) from *Escherichia coli* consists of two pairs of nonidentical subunits (R1 and R2) whose X-ray structures have been determined.[2] A thiyl radical at Cys439 initiates nucleotide reduction by abstraction of H3′ from substrate ribonucleotides.[3] Water (O2′) is then lost from C2′ of the resulting hydrogen-bonded C3′ radical. Siegbahn's theoretical analysis correlated amino acid residues identified in X-ray structures with mechanistic processes.[4] Generation of radicals at C3′ of adenosine[5] and C3 of homoribofuranose model compounds has been shown to initiate reaction cascades that simulate processes postulated to occur during enzymatic 2′-deoxygenation of ribonucleotides.[6]

Direct observation of radical intermediates is problematic, but recent reports of detection of substrate-derived radicals during inactivation of RDPR[7] and evidence for radical intermediates with cytidine 5′-diphosphate and R1 mutants of RDPR[8,9] have appeared. Thelander et al. found that 2′-azido-2′-deoxyuridine 5′-diphosphate (**1a**) (Figure 1) was a potent inactivator of RDPR.[10] Sjöberg et al. observed that inactivation of RDPR by **1a** was accompanied by appearance of new EPR signals for a nitrogen-centered radical and concomitant decay of peaks for a tyrosyl radical, which was the first direct evidence for free-radical chemistry with RDPR. The structure of the elusive nitrogen radical has been investigated extensively.[11−15] Inactivation of RDPR with doubly labeled 2′-[$^{15}N_3$]-azido-2′-[^{13}C]-**1a** resulted in formation of a nitrogen-centered radical that had no EPR hyperfine interaction with ^{13}C2′, which was consistent with C2′−N_3 bond cleavage.[13] Figure 1 contains mechanistic rationalization of these results. Abstraction of H_a from **1a** by the Cys439 thiyl radical is followed by loss of azide from the C3′ radical intermediate in **A**. An azido species reacts with the sulfhydryl of Cys225 to generate N_2 and the

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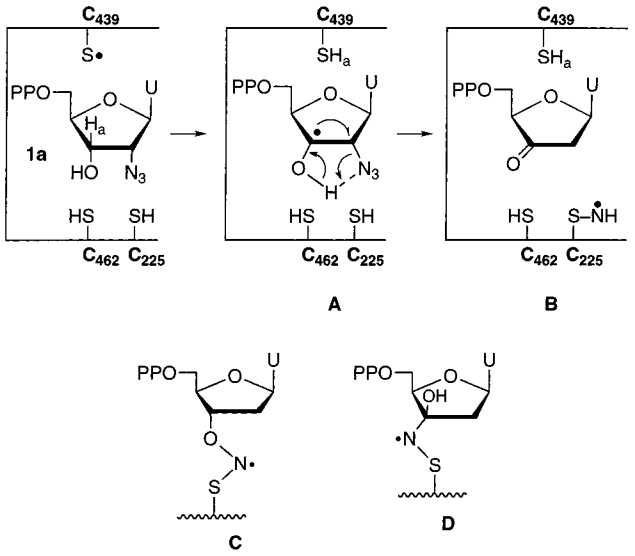


Figure 1. Proposed formation of the nitrogen-centered radical (**C** or **D**) during mechanism-based inhibition of RDPR with 2′-azido-2′-deoxyuridine 5′-diphosphate.

nitrogen-centered radical in **B**. Abstraction of hydrogen by C2′ generates the 2′-deoxy-3′-ketonucleoside in **B**.[13,14]

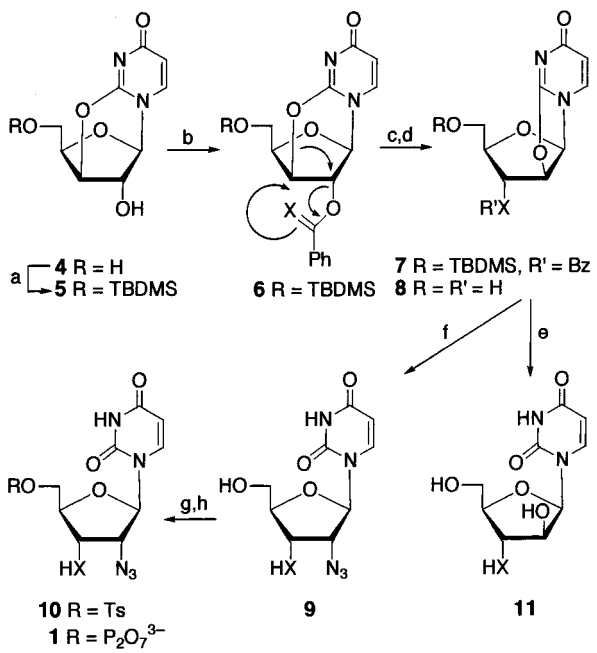
EPR studies with 3-[^{2}H]-cysteine-RDPR indicated that the nitrogen radical hyperfine interaction results from a β-hydrogen of Cys225.[15] An initial nitrogen-centered radical (undetected by EPR) might react with O3′ or C3′ of the intermediate in **B** to generate **C** or **D**, respectively.[15] Chemical arguments favor **D** because precedents exist for addition of aminyl radicals to carbonyl or imino groups.[16] Spectroscopic data[15] and molecular modeling favor **C**, but precedents are lacking. Calculations for model radicals [R−S−N−(H)•] are in harmony[17] with the hypothesis.[15] We targeted 3′-[^{17}O]-2′-azido-2′-deoxyuridine 5′-diphosphate (**1c**) (Scheme 1) to probe this hypothesis. EPR spectra of 3′-[^{17}O]-**C** should have one-bond hyperfine interactions with ^{17}O, whereas two-bond spectral distortions with 3′-[^{17}O]-**D** should be much smaller.

Results and Discussion

Methods for synthesis of nucleosides with oxygen isotopes are limited, and especially for sugar isotopomers.[18−20] Reversible hydration (HCl/$H_2{}^{18}O$) of a 3′-ketouridine derivative followed by reduction ($NaBH_4$) and separation from the xylo epimer (major) gave 3′-[^{18}O]-uridine in low yield.[19a] Other [^{18}O][19b,c] and [^{17}O][20] isotopomers were prepared from cyclonucleosides.

The key step in our synthesis of 3′-[^{17}O]-2′-azido-2′-deoxyuridine (**9c**) (Scheme 1) was the Fox thermal rearrangement[21] of 2′-*O*-benzoyl-O^2,3′-anhydroxylo nucleoside **6** into the 3′-*O*-benzoyl-O^2,2′-anhydroarabino isomer **7**. Protection (O5′) of O^2,3′-anhydro-1-(β-D-xylofuranosyl)uracil[21] (**4**) with TBDMS chloride gave **5** (80%). Treatment of **5** with [^{16}O, ^{18}O, or ^{17}O]-benzoyl chloride (**3**) gave the 2′-*O*-benzoyl derivatives **6a**−**c**, respectively.

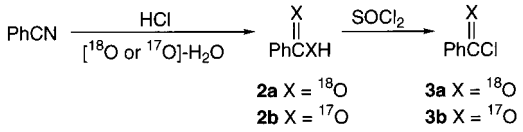
Scheme 1[a]



Series: a, X = ^{16}O; **b**, X = ^{18}O; **c**, X = ^{17}O

[a] Key: (a) TBDMSCl/imidazole/DMF; (b) PhC[O]Cl/pyridine; (c) △; (d) (i) NH_3/MeOH, (ii) NH_4F/MeOH; (e) NaOH/H_2O; (f) LiF/Me_3SiN_3/TMEDA/DMF; (g) TsCl/pyridine; (h) $(Bu_4N)_3HP_2O_7$/CH_3CN.

Scheme 2



The [^{18}O or ^{17}O]-benzoyl chlorides **3** were prepared by acid-catalyzed hydrolysis (HCl/[^{18}O or ^{17}O]H_2O) of benzonitrile and treatment of the benzoic acids **2** with thionyl chloride (Scheme 2). Isotopic incorporation (MS) was calculated[22] to be 90% for [2 × ^{18}O]-**2a** or 53% for [2 × ^{17}O]-**2b** beginning with 97.4% (^{18}O) or 74.5% (^{17}O) enriched water, respectively. [^{17}O]-Benzoic acid and its derivatives had been prepared by alkaline hydrolysis of methyl benzoate[23a] or by acid-catalyzed exchange (HCl/BzOH/$H_2{}^{17}O$).[23b] The mass spectrum of **6c** had peaks at *m*/*z* 446 (100%) for [^{17}O]MH^+ and *m*/*z* 445 (78%) for [^{16}O]-MH^+, which is consistent with 50% ^{17}O enrichment.[22]

Rearrangement of **6c** gave **7c** (48% ^{17}O enrichment), and parallel treatment of **6b** gave **7b** (81% ^{18}O). Benzoyl

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(NH_3/MeOH) and TBDMS (NH_4F/MeOH)[24] groups were removed, and RP-HPLC gave [^{17}O]-**8c** (65%). Base-promoted hydrolysis of **8b** gave 3′-[^{18}O]-1-(β-D-arabinofuranosyl)uracil (**11b**). Treatment of **8** with LiF/TMEDA/Me_3SiN_3[25a] gave **9** in higher yields than with Moffatt's LiN_3/HMPA procedure.[25b] The 3′-[^{17}O]-2′-azido-2′-deoxyuridine (**9c**) had 47% ^{17}O. Selective tosylation of **9c** gave **10c** (63%) and small quantities of the 3′,5′-di-*O*-tosyl byproduct. The Poulter[26] displacement of tosylate with tris(tetrabutylammonium) hydrogen pyrophosphate, purification, and conversion to the sodium salt gave **1c** (^{1}H and ^{31}P NMR). No ^{1}H NMR signals for ammonium salts were observed, but ^{31}P NMR indicated the presence of inorganic pyrophosphate. The ^{17}O NMR[27] spectrum of our doubly labeled [^{17}O]-benzoic acid (**2b**) had one signal at δ 205 (line width 308 Hz) in harmony with reported data,[23a,28] and that of the 3′-[^{17}O]-2,2′-anhydrouridine **8c** had a peak at δ −66 (line width 85 Hz). The ^{17}O shift in **8c** is upfield from that of the secondary OH of 2-propanol (δ 40[29]) and O2′ of 2′-[^{17}O]-1-(β-D-arabinofuranosyl)uracil (δ −11[20b]).

In summary, Fox thermolysis of *O*^{2},3′-cyclouridine derivative **6c** gave the more stable *O*^{2},2′-cyclouridine isomer with rearrangement of the 2′-*O*-benzoyl[^{17}O] group to give the 3′-[^{17}O]-*O*-benzoyl product **7c**. This stereodefined rearrangement can be applied to ^{17}O or ^{18}O labeling of a variety of pyrimidine nucleosides. Deprotection and anhydro ring opening (LiN_3) gave 3′-[^{17}O]-**9c**, which was converted into 3′-[^{17}O]-2′-azido-2′-deoxyuridine 5′-diphosphate (**1c**). Studies on inhibition of ribonucleoside diphosphate reductase with **1c** are in progress.[30]

Experimental Section

Uncorrected melting points were determined with a capillary tube apparatus. UV spectra were recorded with solutions in MeOH. ^{1}H (Me_4Si) NMR spectra were determined with solutions in $CDCl_3$ at 400 MHz, ^{13}C (Me_4Si) at 100.6 MHz, and ^{31}P (H_3PO_4) at 161.9 MHz unless otherwise noted, and ^{17}O spectra ($H_2{}^{17}O$ external) were recorded at 54.245 MHz and a 90° pulse angle with a Brüker DPX-400 spectrometer equipped with a 5-mm broad-band probe at ambient temperature. Mass spectra (MS) were obtained with atmospheric pressure chemical ionization (APCI) except when CI (CH_4) is noted. Labeled water (97.4% ^{18}O or 74.5% ^{17}O enrichment) was purchased from Isotec. Reagent grade chemicals were used and solvents were dried by reflux over and distillation from CaH_2 under an argon atmosphere. TLC was performed on silica plates with MeOH/$CHCl_3$ (1:9), MeOH/EtOAc (1:19), or EtOAc/*i*-PrOH/H_2O (4:1:2, upper layer; S1). Merck kieselgel 60 (230−400 mesh) was used for column chromatography. Preparative reversed-phase (RP)-HPLC was performed with a Supelcosil LC-18S column.

Concentrated HCl in [^{18}O or ^{17}O]-H_2O. H_2O (97.4% ^{18}O, 1.05 g, 52.5 mmol; or 74.5% ^{17}O, 1.08 g, 57.7 mmol) was placed in long pressure tube (Ace glass) and cooled in an ice bath. HCl (anhydrous) was bubbled in for 3 min, and the HCl content (∼37%) was calculated from the increased mass.

[2 × ^{18}O]-Benzoic Acid (2a). PhCN (1.78 mL, 1.8 g, 17.5 mmol) was suspended in concentrated HCl/$H_2{}^{18}O$ in a long pressure tube, and the mixture was heated for 20 h at 100 °C with vigorous stirring. The thick suspension was partitioned between ice-cold $NaHCO_3/H_2O$//CH_2Cl_2, and the organic layer was quickly extracted with $NaHCO_3/H_2O$. The combined aqueous phase was washed with CH_2Cl_2 to remove traces of PhCN and $BzNH_2$. CH_2Cl_2 was added to the aqueous layer and the mixture was acidified (dilute HCl/H_2O, pH ∼3). The organic layer was washed (brine) and dried (Na_2SO_4), and volatiles were evaporated to give **2a** (1.55 g, 70%) as a white solid: ^{1}H NMR δ 7.49 (t, *J* = 7.4 Hz, 2H), 7.65 (t, *J* = 7.4 Hz, 1H), 8.15 (d, *J* = 7.0 Hz, 2H), 13.00 (br s, 1H); ^{13}C NMR δ 129.0, 129.8, 130.7, 134.3, 173.1; MS *m*/*z* 127 (100, [2 × ^{18}O]-MH$^+$), 125 (9.7, [$^{18/16}$O]MH$^+$), 123 (1.8, [2 × ^{16}O]MH$^+$).

[2 × ^{17}O]-Benzoic Acid (2b). Treatment of PhCN (2.06 mL, 2.08 g, 20.2 mmol) with HCl/$H_2{}^{17}O$ (as described for **2a**) gave **2b** (1.54 g, 62%): ^{1}H NMR and ^{13}C NMR peaks were the same as for **2a** except δ 173.0−173.3 (m); MS *m*/*z* 125 (100, [2 × ^{17}O]MH$^+$), 124 (59, [$^{17/16}$O]MH$^+$), 123 (25, [2 × ^{16}O]MH$^+$).

[^{18}O]-Benzoyl Chloride (3a). [^{18}O]-Benzoic acid (**2a**, 0.88 g, 6.98 mmol) was placed in a 15-mL round-bottomed flask and $SOCl_2$ (0.65 mL, 1.05 g, 8.8 mmol) was added dropwise with stirring. The mixture was heated gently for 1 h at 100 °C (1 mL of $SOCl_2$ was added after 20 min to make a clear solution), cooled, and then distilled (∼70 °C) at atmospheric pressure to remove excess $SOCl_2$. A vacuum was applied, and the bath temperature was slowly increased to ∼130 °C to give one fraction of **3a** (0.85 g, 85%) as a colorless liquid.

[^{17}O]-Benzoyl Chloride (3b). Treatment of [^{17}O]-benzoic acid (**2b**, 1.4 g, 11.3 mmol) with $SOCl_2$ (1.03 mL, 1.67 g, 14.1 mmol) (as described for **3a**) gave [^{17}O]-benzoyl chloride (**3b**, 1.3 g, 80%).

1-[2,3′-Anhydro-5′-*O*-(*tert*-butyldimethylsilyl)-β-D-xylofuranosyl]uracil (5). TBDMSCl (0.9 g, 6.0 mmol) was added to a stirred suspension of imidazole (0.725 g, 12.5 mmol) and 1-(2,3′-anhydro-β-D-xylofuranosyl)uracil[21] (**4**, 1.13 g, 5 mmol) in dried DMF (30 mL) at ambient temperature under N_2. After 18 h, volatiles were evaporated, and the residue was chromatographed (EtOAc → 60% S1/EtOAc) and recrystallized (EtOH/EtOAc) to give **5** (1.36 g, 80%) as needles: mp 246−247 °C; UV max 250 (sh), 232 nm (ε 7400, 9600), min 241 (sh), 216 nm (ε 7900, 6300); ^{1}H NMR (DMSO-d_6) δ 0.02 (s, 6H), 0.85 (s, 9H), 3.71 (dd, *J* = 11.0, 6.4 Hz, 1H), 3.81 (dd, *J* = 11.0, 6.0 Hz, 1H), 4.41 ("dt", *J* = 6.2, 2.5 Hz, 1H), 4.74 (s, 1H), 4.92 (s, 1H), 5.64 (s, 1H), 5.80 (d, *J* = 7.4 Hz, 1H), 6.45 (br s, 1H), 7.68 (d, *J* = 7.4 Hz, 1H); ^{13}C NMR (DMSO-d_6) δ −5.00, 18.3, 26.0, 61.4, 69.7, 79.5, 83.6, 89.6, 108.2, 141.3, 153.7, 170.6; MS (CI) *m*/*z* 341 (100, MH$^+$), 283 (30, M$^+$ − *t*-Bu); HRMS (CI) *m*/*z* 341.1538 (MH$^+$ = 341.1532). Anal. Calcd for $C_{15}H_{24}N_2O_5Si$ (340.45): C, 52.92; H, 7.11; N, 8.23. Found: C, 52.43; H, 7.26; N, 8.11.

1-[2,3′-Anhydro-2′-*O*-benzoyl-5′-*O*-(*tert*-butyldimethylsilyl)-β-D-xylofuranosyl]uracil (6a). BzCl (50 μL, 60.6 mg, 0.43 mmol) was added to **5** (20 mg, 0.05 mmol) in dried pyridine (1 mL), and the solution was stirred overnight at ambient temperature and then for 2 h at 40 °C. Volatiles were evaporated in vacuo, and toluene was added. Volatiles were evaporated, and the residue was chromatographed (90% EtOAc/hexanes → EtOAc → 25% S1/EtOAc) to give **6a** (10 mg, 50%) as a white solid: mp 238−240 °C; UV max 233 nm (ε 25 800), min 212 nm (ε 9400); ^{1}H NMR δ 0.02 (s, 6H), 0.85 (s, 9H), 3.89 (dd, *J* = 10.7, 7.3 Hz, 1H), 3.93 (dd, *J* = 10.8, 6.0 Hz, 1H), 4.70 ("dt", *J* = 7.4, 2.5 Hz, 1H), 5.22 (s, 1H), 5.55 (s, 1H), 5.70 (s, 1H), 6.13 (d, *J* = 7.3 Hz, 1H), 7.21 (d, *J* = 7.4 Hz, 1H), 7.52 (t, *J* = 7.9 Hz, 2H), 7.70 (t, *J* = 7.4 Hz, 1H), 8.06 (d, *J* = 7.1 Hz, 2H); ^{13}C NMR δ −0.5, 18.8, 26.2, 61.1, 72.6, 77.6, 85.2, 88.8, 110.5, 128.1, 129.3, 130.5, 134.9, 139.8, 153.5, 165.3, 171.4; MS *m*/*z* 445 (100, MH$^+$), 446 (29.6, MH$^+$ + 1), 447 (8.7, MH$^+$ + 2). Anal. Calcd for $C_{22}H_{28}N_2O_6Si$ (444.56): C, 59.44; H, 6.35; N, 6.30. Found: C, 59.03; H, 6.57; N, 6.30.

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(30) In collaboration with Professor J. Stubbe at Massachusetts Institute of Technology.

1-[2,3′-Anhydro-2′-*O*-benzoyl[^{18}O]-5′-*O*-(*tert*-butyldimethylsilyl)-*β*-D-xylofuranosyl]uracil (6b). Treatment of **5** (200 mg, 0.59 mmol) with [^{18}O]-BzCl (**3a**, 140 μL, 170 mg, 1.19 mmol) (as described for **6a**) gave **6b** (180 mg, 68%) with identical physical and spectral properties except MS *m*/*z* 447 (100, [^{18}O]MH$^+$), 445 (15.6, [^{16}O]MH$^+$).

1-[2,3′-Anhydro-2′-*O*-benzoyl[^{17}O]-5′-*O*-(*tert*-butyldimethylsilyl)-*β*-D-xylofuranosyl]uracil (6c). Treatment of **5** (1.15 g, 3.3 mmol) with [^{17}O]-BzCl (**3b**, 500 μL, 606 mg, 4.28 mmol) (as described for **6a**) gave **6c** (0.45 g, 30%) with identical physical and spectral properties except MS *m*/*z* 446 (100, [^{17}O]-MH$^+$), 445 (77.6, [^{16}O]MH$^+$).

1-[2,2′-Anhydro-3′-*O*-benzoyl-5′-*O*-(*tert*-butyldimethylsilyl)-*β*-D-arabinofuranosyl]uracil (7a). Compound **6a** (100 mg, 0.22 mmol) was placed in a 25 mL round-bottomed flask under argon and heated (Bunsen burner) for 1 min until the solid melted and became brown. After cooling, the solid was chromatographed (EtOAc → 15% S1/EtOAc) to give **7a** (65 mg, 65%) as a white solid: mp 224−226 °C; UV max 254 (sh), 230 nm (ϵ 9200, 24 000), min 211 nm (ϵ 9000); ^{1}H NMR δ 0.02 (s, 6H), 0.90 (s, 9H), 3.67 (dd, J = 11.1, 4.4 Hz, 1H), 3.81 (dd, J = 11.2, 5.9 Hz, 1H), 4.50 ("dt", J = 4.4, 2.6 Hz, 1H), 5.60 (d, J = 5.5 Hz, 1H), 5.70 (s, 1H), 6.08 (d, J = 7.4 Hz, 1H), 6.42 (d, J = 5.7 Hz, 1H), 7.42 (d, J = 7.4 Hz, 1H), 7.50 (t, J = 7.9 Hz, 2H), 7.65 (t, J = 7.4 Hz, 1H), 8.05 (d, J = 7.2 Hz, 2H); ^{13}C NMR δ −0.5, 19.0, 26.4, 63.6, 78.3, 87.4, 88.8, 91.2, 110.6, 128.8, 129.1, 130.3, 134.4, 135.4, 160.1, 165.8, 172.4; MS *m*/*z* 445 (100, MH$^+$), 446 (29.6, MH$^+$ + 1), 447 (8.8, MH$^+$ + 2). Anal. Calcd for $C_{22}H_{28}N_2O_6Si$ (444.56): C, 59.44; H, 6.35; N, 6.30. Found: C, 59.80; H, 6.42; N, 6.32.

1-[2,2′-Anhydro-3′-[^{18}O]-*O*-benzoyl-5′-*O*-(*tert*-butyldimethylsilyl)-*β*-D-arabinofuranosyl]uracil (7b). Thermolysis of **6b** (70 mg, 0.15 mmol) (as described for **7a**) gave **7b** (44 mg, 63%) with identical physical and spectral properties except MS *m*/*z* 447 (100, [^{18}O]MH$^+$), 445 (24.0, [^{16}O]MH$^+$).

1-[2,2′-Anhydro-3′-[^{17}O]-*O*-benzoyl-5′-*O*-(*tert*-butyldimethylsilyl)-*β*-D-arabinofuranosyl]uracil (7c). Thermolysis of **6c** (50 mg, 0.11 mmol) (as described for **7a**) gave **7c** (27 mg, 54%) with identical physical and spectral properties except MS *m*/*z* 446 (100, [^{17}O]MH$^+$), 445 (81.9, [^{16}O]MH$^+$).

1-(2,2′-Anhydro-*β*-D-arabinofuranosyl)uracil (8a). NH_3/MeOH (5 mL, saturated at ∼0 °C) was added to **7a** (22 mg, 0.05 mmol) in MeOH (2 mL), and the solution was stirred for 2 h at ∼0 °C. Volatiles were evaporated, NH_4F (28 mg, 0.75 mmol) and dried MeOH (5 mL) were added to the white residue, and the solution was stirred overnight at ambient temperature. Volatiles were evaporated, and the residue was purified by RP-HPLC (8% CH_3CN/H_2O, t_R = 25 min) to give **8a** (8 mg, 71%) as off-white crystals: mp 242−245 °C (lit.[31] mp 238−244 °C); UV max 250, 224 nm (ϵ 7900, 8900), min 237, 212 nm (ϵ 6800, 6300); ^{1}H NMR (DMSO-d_6) δ 3.19 ("dt", J = 11.3, 5.5 Hz, 1H), 3.28 ("dt", J = 11.2, 6.0 Hz, 1H), 4.07 (t, J = 4.5 Hz, 1H), 4.38 (d, J = 3.4 Hz, 1H), 4.99 (t, J = 5.2 Hz, 1H), 5.20 (d, J = 5.6 Hz, 1H), 5.85 (d, J = 7.4 Hz, 1H), 5.90 (d, J = 4.3 Hz, 1H), 6.31 (d, J = 5.7 Hz, 1H), 7.85 (d, J = 7.4 Hz, 1H); ^{13}C NMR (DMSO-d_6) δ 61.7, 75.8, 89.6, 90.1, 90.9, 109.5, 137.7, 160.7, 172.1; MS *m*/*z* 227 (100, MH$^+$), 228 (10.6, MH$^+$ + 1), 229 (1.5, MH$^+$ + 2).

3′-[^{18}O]-1-(2,2′-Anhydro-*β*-D-arabinofuranosyl)uracil (8b). Deprotection of **7b** (8 mg, 0.034 mmol) (as described for **7a**) gave **8b** (3 mg, 73%) with identical physical and spectral properties except MS *m*/*z* 229 (100, [^{18}O]MH$^+$), 227 (27, [^{16}O]-MH$^+$).

3′-[^{17}O]-1-(2,2′-Anhydro-*β*-D-arabinofuranosyl)uracil (8c). Deprotection of **7c** (22 mg, 0.05 mmol) (as described for **8a**) gave **8c** (7.4 mg, 65%) with identical physical and spectral properties except MS *m*/*z* 228 (92.5, [^{17}O]MH$^+$), 227 (100, [^{16}O]-MH$^+$).

2′-Azido-2′-deoxyuridine (9a). LiF (47 mg, 1.8 mmol) was added to dried DMF (2 mL), the stirred suspension was heated (during 10 min) to ∼105 °C, and *N*,*N*,*N*,*N*-tetramethylethylenediamine (2 mL) and TMS−N_3 (240 μL, 210 mg, 1.8 mmol) were added. Stirring was continued for 30 min, **8a** (0.226 g, 1.0 mmol) was added, and the mixture was heated with stirring for 48 h at 110 °C (oil bath). Volatiles were evaporated in vacuo, and MeOH was added to the residue and evaporated (3 ×). The oily residue was dissolved in MeOH (1 mL), and EtOAc (4 mL) was added to precipitate salts and residual starting material. The mixture was filtered and the filtrate was chromatographed (MeOH/EtOAc, 1:9) to give **9a** (0.22 g, 82%) as a slightly yellow foam. This material was purified (RP-HPLC, 15% CH_3CN/H_2O) to give **9a** (140 mg, 52%) as an off-white foam with spectral data[25] as reported: MS *m*/*z* 270 (100, MH$^+$), 271 (12, MH$^+$ + 1).

3′-[^{17}O]-2′-Azido-2′-deoxyuridine (9c). Treatment of **8c** (50 mg, 0.22 mmol) (as described for **9a**) gave **9c** (33 mg, 56%) as a slightly yellow foam that was purified (RP-HPLC) to give **9c** (24 mg, 40%) as an off-white foam with identical spectral data except MS *m*/*z* 271 (98.5, [^{17}O]MH$^+$), 270 (100, [^{16}O]MH$^+$).

2′-Azido-2′-deoxy-5′-*O*-(*p*-toluylsufonyl)uridine (10a). TsCl (21 mg, 0.11 mmol) was added to **9a** (20 mg, 0.074 mmol) in dried pyridine (1 mL) and the solution was stirred for 14 h at ambient temperature. Volatiles were evaporated and the residue was partitioned (0.1 M HCl/H_2O//$CHCl_3$). The organic layer was washed ($NaHCO_3/H_2O$, brine), dried ($MgSO_4$), and volatiles were evaporated. The residue was chromatographed ($CHCl_3$ → 5% MeOH/$CHCl_3$) to give a less polar byproduct tentatively assigned (^{1}H NMR, MS) as 2′-azido-2′-deoxy-3′,5′-di-*O*-(*p*-toluylsufonyl)uridine (7 mg, 17%) and then **10a** (21 mg, 67%): ^{1}H NMR δ 4.15−4.20 (m, 2H), 4.30 (d, J = 11.1 Hz, 1H), 4.38 (d, J = 11.4 Hz, 1H), 4.46 (t, J = 5.7 Hz, 1H), 5.75 (d, J = 8.0 Hz, 1H), 5.88 (d, J = 2.8 Hz, 1H), 7.41 (d, J = 7.6 Hz, 2H), 7.51 (d, J = 7.7 Hz, 1H), 7.82 (d, J = 7.4 Hz, 2H), 9.24 (br s, 1H); ^{13}C NMR δ 22.2, 66.3, 67.9, 70.2, 81.7, 88.7, 103.3, 128.3, 130.7, 132.4, 139.7, 145.5, 150.5, 163.5; MS *m*/*z* 424 (100, MH$^+$), 425 (17.5, MH$^+$ + 1). Anal. Calcd for $C_{16}H_{17}N_5O_7S$ (423.40): C, 45.39; H, 4.05; N, 16.54. Found: C, 45.71; H, 3.89; N, 16.78.

3′-[^{17}O]-2′-Azido-2′-deoxy-5′-*O*-(*p*-toluylsufonyl)uridine (10c). Treatment of **9c** (17 mg, 0.063 mmol) with TsCl (as described for **10a**) gave **10c** (17 mg, 64%) with identical spectral data except MS *m*/*z* 425 (100, [^{17}O]MH$^+$), 424 (98.7, [^{16}O]MH$^+$).

3′-[^{17}O]-2′-Azido-2′-deoxyuridine 5′-Diphosphate (1c). $(Bu_4NH)_3HP_2O_7$[26] (54 mg, 0.06 mmol) was added (one portion) to **10c** (17 mg, 0.04 mmol) in dried CH_3CN (0.5 mL), and the solution was stirred for 48 h at ambient temperature. Volatiles were evaporated, and the residue was dissolved (H_2O) and purified by ion-exchange chromatography (DEAE Sephadex A-25, Et_3NHHCO_3/H_2O, 0.05 → 0.50 M). Evaporation of appropriate fractions and coevaporation (H_2O and MeOH, 5 ×) gave **1c** (14 mg, ∼50%) as a triethylammonium salt (∼1 equiv, ^{1}H NMR). This material was dissolved (H_2O) and passed through a column of Dowex 50 × 8(Na$^+$) (H_2O) to give the trisodium salt of **1c** (8 mg, 40%), RP-HPLC (3% H_2O/CH_3CN, t_R 5.6 min), provided **1c** (5.5 mg, 30%) with spectral data as reported[13] for **1a**: ^{1}H NMR (D_2O) δ 4.05−4.17 (m, 2H), 4.26 (t, J = 5.3 Hz, 1H) 4.49 (t, J = 5.0 Hz, 1H), 5.84 (d, J = 8.1 Hz, 1H), 5.95 (d, J = 5.2 Hz, 1H), 7.85 (d, J = 8.0 Hz, 1H); ^{31}P NMR (D_2O) δ −10.17 (d, J = 21.1 Hz, P_α), −9.24 (d, J = 21.1 Hz, P_β) [inorganic pyrophosphate impurity (−9.10 ppm, ∼50%)].

3′-[^{18}O]-1-(*β*-D-Arabinofuranosyl)uracil (11b). NaOH/H_2O (1 M, 1 mL, 1 mmol) was added to **8b** (23 mg, 0.1 mmol) in MeOH/H_2O (1:1, 1 mL), and the solution was stirred overnight and neutralized (AcOH, pH ∼7). Volatiles were evaporated, and the residue was chromatographed (EtOAc → 8% MeOH/EtOAc) to give **11b** (17 mg, 69%) with data as reported[19c,20b] except MS *m*/*z* 247 (100, [^{18}O]MH$^+$), 245 (29, [^{16}O]MH$^+$).

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