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Stereospecific deuteration of α-furanosyl azomycin nucleosides: A model reaction for tritium radiolabeling

Piyush Kumar,[a,*] Saeed Emami,[b,†] Alexander J. B. McEwan[a] and Leonard I. Wiebe[a,b]

[a]*Department of Oncologic Imaging, Cross Cancer Institute, 11560 University Avenue Edmonton, Alta., Canada T6G 1Z2*
[b]*Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, Alta., Canada T6G 2N8*

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Abstract—Stereospecific synthesis of 1-α-D-(2-deuteroribofuranosyl)-2-nitroimidazole (2′-[^{2}H]-α-AZR) is reported. This, deuteration was independent of the configuration of C-2′ –OH group (arabinose or ribose) in sugar moiety of starting molecules. Slightly better yield (>37%) of the deuterated product, **6**, from arabinosyl precursor in comparison to corresponding ribose precursor (29%) was obtained which may reflect better stereochemical availability of C-2′ –OH in arabinose during oxidation.

Advanced imaging techniques such as positron emission tomography (PET), single-photon emission computed tomography (SPECT) and magnetic resonance spectroscopy (MRS) enable the in vivo visualization of biological processes and metabolic pathways of tumors, providing information about metabolism, physiology, and molecular biology at cellular and molecular levels.[1] Hypoxia, a subject of many reports,[2,3] is present in a wide range of clinical disorders including cancer. This hypoxic microenvironment within tumors promotes both local invasion and distant metastasis,[2,3] and is associated with resistance to anticancer therapies, in particular, ionizing radiation.[4,5] Selective uptake of radiotracers in various pathological disorders, including benign tumors and cancers, can be used to identify molecular targets, monitor therapeutic efficacy, and develop new treatment modalities.

Radiolabeled nitroimidazoles[6] play a significant role in hypoxia (virtual absence of O_2) management in a variety of pathological disorders.[6–13] They form adducts with tissue macromolecules as the basis for their selective accumulation, and hence imaging properties, in target hypoxic tissues. The specificity of adduct formation in hypoxic cells only is attributable to the oxygen-reversible one-electron reduction of the nitro substituent.[11] In the absence of oxygen, reductive formation of chemically-reactive species by metabolically-viable, functional reductases leads to adduct formation with a range of tissue components (Fig. 1).

1-α-D-(5-Deoxy-5-[^{18}F]fluoroarabinofuranosyl)-2-nitroimidazole[13,14] ([^{18}F]FAZA) is a PET radiotracer that selectively accumulates in hypoxic cells and is currently used in human cancer patients in several cancer hospitals, to develop improved treatment plans,[15,16] by assessing the level of hypoxia in solid tumors. For example, an in vivo [^{18}F]FAZA PET imaging study reports a

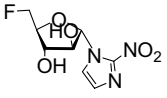

Figure 1. FAZA.

Abbreviations: 2′-[^{2}H]-α-AZR, 1-α-D-(2-deuteroribofuranosyl)-2-nitroimidazole; TIPDS α-AZA, 1-α-D-(3,5-*O*,*O*-tetraisopropyldisilyloxyarabino-furanosyl)-2-nitroimidazole; TIPDS α-AZR, 1-α-D-(3,5-*O*,*O*-tetraisopropyldisilyloxyribofuranosyl)-2-nitroimidazole; TIPDS 2′-[^{2}H]-α-AZR, 1-α-D-(3,5-*O*,*O*-tetraisopropyldisilyloxyribofuranosyl-2′-deueroribofuranosyl)-2-nitroimidazole; PET, positron emission tomography; SPECT, single-photon emission computed tomography; $T_{1/2}$, half-life; MS, metabolic studies; [^{18}F]FAZA, 1-α-D-(5-deoxy-5-[^{18}F]fluoroarabinofuranosyl)-2-nitroimidazole; NMR, nuclear magnetic resonance; TIPDS, tetraisopropyldisilyloxy; EI, exact ionization; s, singlet; d, doublet; dd, doublet of doublet; ddd, doublet of doublet of doublet; D, deuterium; Im, imidazole.

Keywords: Tumor hypoxia; Azomycin nucleosides; PET radiodiagnostics; FAZA; Stereospecific deuteration.

* Corresponding author. E-mail: piyushku@cancerboard.ab.ca
† Present address: Department of Medicinal Chemistry, Faculty of Pharmacy, Mazandaran University of Medical Sciences, Sari, Iran.

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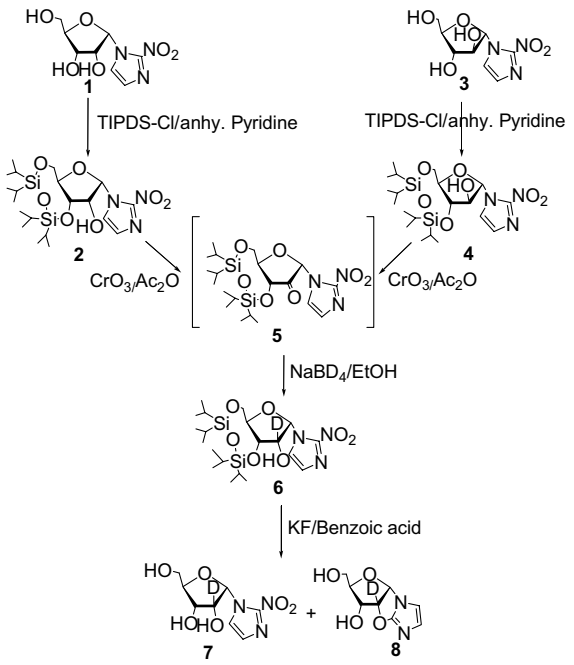
correlation between the hypoxia levels and treatment response of gefitinib in A431 xenografts in comparison with untreated control tumors.[17]

Although the role of ^{18}F labeled azomycin nucleosides in PET diagnosis of hypoxia is well established, the relatively short half-life of radiofluorine (~109 min) limits certain types of metabolic investigation, including determination of pharmacokinetic and biodistribution patterns at desirable intermediate times (nominally >6 h) after administration of these compounds to animals and humans. Incorporation of a longer lived radioisotope, for example, tritium (^{3}H; $T_{1/2} \sim 12.3$ years) can help resolve these limitations, especially since ^{3}H is an isotopic label that will not alter the molecules' properties as long as it is introduced as a substitute for hydrogen. In addition, site specific isotopic labeling of these compounds is essential to explore detailed metabolic pathways, especially in cases where the labeled molecule is theoretically subject to metabolic fragmentation. In azomycin nucleosides, enzymatic cleavage of the nucleosidic bond is potentially a major metabolic pathway. It is therefore necessary to incorporate the long $T_{1/2}$ radionuclide (e.g., ^{3}H) to the fragment that would normally carry the radioimaging radionuclide (e.g., ^{18}F), that is, in the sugar moiety and is generated by hydrolysis of the nucleoside bond giving rise to two metabolites, the fluorosugar and 2-nitroimidazole. ^{3}H labeling in the nitroimidazole moiety would not reveal any information related to the metabolism of the sugar component, and vice-versa.

The current work describes a one pot stereospecific deuterium (^{2}H) labeling of the sugar moiety of azomycin nucleosides, which can be easily extended to the incorporation of tritium radioisotope in these molecules and would be helpful in metabolic studies and in vivo in situ NMR characterization of metabolites. Importantly, this deuteration labeling methodology serves as a model for developing tritiated azomycin nucleosides and would enable biological evaluations where extended time period (>24 h) is required which is not possible with ^{18}F labeled analogs. The site specific stereoselective synthesis of deuterated azomycin nucleoside, 1-α-D-(2′-deutereoribofuranosyl)-2-nitroimidazole (2′-[^{2}H]-α-AZR), is now reported.

Overall, the formation of 2′-[^{2}H]-α-AZR; **7** (Scheme 1), was independent of the configuration of the –OH group at C-2′ (arabinose or ribose). Oxidation of 1-α-D-(3′,5′-*O*,*O*-tetraisopropyldisyloxyribofuranosyl)-2-nitroimidazole (TIPDS α-AZR, **2**),[18] or 1-α-D-(3′,5′-*O*,*O*-tetraisopropyldisyloxyarabinofuranosyl)-2- nitroimidazole (TIPDS α-AZA, **4**),[19] yielded a common C-2′ keto intermediate **5**, which underwent stereospecific deuteration at C-2′ carbonyl to afford the deuterium labeled product **6**.

Thus, CrO_3 assisted oxidation of the 2′-OH group in **2** and **4** at 0 °C led to the formation of a planar C-2′ keto product **5**,[20] which was not isolated but used for deuteration after removal of the solvent under vacuum. This keto product **5** is sp^2 hybridized with a trigonal planar conformation that can theoretically be attacked at C-2′ carbon by a nucleophile (other than H) from either the *endo* or *exo* plane, thereby forming stereomers. However, the 2-nitroimidazole substituent, placed in the α-configuration of the sugar moiety, offers a large stereochemical impediment to approach the nucleophile from the *endo* plane, thereby enhancing the probability of nucleophilic attack from the *exo* plane (Fig. 2).



Scheme 1. Synthesis of 1-α-D-(2′-deuteroribofuranosyl)-2-nitroimidazole (2′-[^{2}H]-α-AZR).

This was indeed found to be the case. Reduction of **5** using $NaBD_4$ at 0 °C led to the formation of *exo* deuterated product exclusively, affording only 1-α-D-(3′,5′-*O*,*O*-tetraisopropyldisilyloxy-2′-deuteroribofuranosyl)-2-nitroimidazole (TIPDS 2′-[^{2}H]-α-AZR; **6**).[21] The

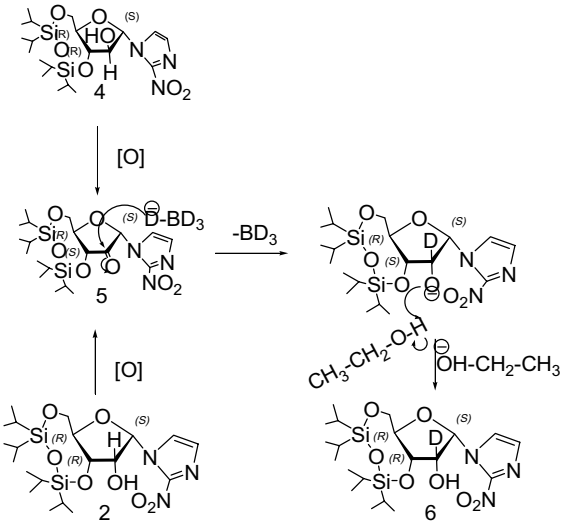


Figure 2. Oxidative reduction mechanism leading to stereospecific deuteration of **2** and **4**.

overall yield of **6** was slightly higher (>37%) when this synthesis initiated from **4**, the synthon in arabinose configuration, in comparison to the ribose synthon **2** (29%). Reduced yield of **6**, when starting from ribose precursor **2**, is possibly a result of restricted access of bulky oxidizing reagent, pyridinium acetochromate, to the C-2′ –OH. Stereochemical crowding by C-1′-nitroimidazolyl and the 3′,5′-TIPDS group, both in the α-plane of TIPDS α-AZR **2**, most likely limit approach by pyridinium acetochromate to the C-2′ –OH, which is in the *endo* configuration, and, thereby, diminish nucleophilic attack by this hydroxyl, as the first step in the oxidation mechanism.[22] Incorporation of deuterium at C-2′ in **6** was confirmed by changes in the coupling patterns of neighboring protons H-1′ and H-3′, and the disappearance of the H-2′ proton in the ^{1}H NMR spectrum of **6**. Appearance of H-3′ as a doublet at δ 4.47 ($J_{4',3'} = 8.2$ Hz) and H-1′ as a singlet at δ 6.63 ppm in the proton spectrum of **6** indicated the incorporation of deuterium and the formation of a ribose configured product. Relatively larger coupling constant for $H_{4'-3'}$ in **6**, the deuterated analog, in comparison to its protonated molecule **4**, may be related to the impact of the slightly larger atomic size of deuterium in comparison to hydrogen.

The ^{13}C NMR spectrum provided additional support to the incorporation of deuterium in *exo* plane in **6**, with the presence of a weak triplet for C-2′ due to ^{2}H–^{13}C coupling with this carbon.[23] Desilylation of **6** was assisted by generating HF using potassium fluoride and benzoic acid at 75 °C to afford 2′-[^{2}H]-α-AZR, **7**,[24] in optimum yield (>77%) and (2*R*,3*S*,3a*S*,8a*S*)-2-hydroxymethyl)-3a-deutero-2,3,8a-trihydrofuro[2,3-*d*]imidazolo[2,1-*b*]oxazol-3-ol, **8**, as a minor product (~15%).[25] It appears that the resonance stabilized nitro group at C-2, being in the same plane as C-2′ –OH, is stereochemically activated, leading to intramolecular elimination of HNO_2 to produce this tricyclic product, **8**. Strong upfield movement in the chemical shifts (δ) of proton (H-1′, +0.45 and imidazole H-4, +0.52 and H-5, +0.80 ppm, respectively) and carbon signals (C-1, +4.40; C-3, +5.07 imidazole C-4, +2.13; imidazolyl C-5, +2.73 ppm, respectively) in **8** were observed in comparison to the same nuclei in **7**. This observation is in accordance with deuterium substitution effects on neighboring nuclei,[26–28] and confirms the formation of this unexpected tricyclic product **8** during the desilylation process. In addition, this also supports the concept that stereochemical crowding in the *endo* plane hinders the approach of CrO_3 during the oxidation process when C-2′ –OH group is placed in ribose configuration (TIPS α-AZR, **2**).

A site specific (at C-2′) stereoselective one pot synthesis of deuterated azomycin nucleoside, 1-α-D-(2′-deutereoribofuranosyl)-2-nitroimidazole (2′-[^{2}H]-α-AZR) has been described. Both ribose and arabinose precursors, **2** and **4**, upon oxidative-deuteration, led to the formation of ribose configured deuterated product exclusively. Although isolated deuteration yields were moderate in case of these α-azomycin nucleosides in comparison to basic sugar molecules,[29,30] it could be due to stereochemical crowding by the substituents in the *endo* plane that hindered the approach of pyridinium acetochromate during the oxidation process of TIPDS α-AZR, **2** and TIPDS α-AZA, **4**.

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18. **1-α-D-(3′,5′ -*O*,*O*-tetraisopropyldisyloxyribofuranosyl)-2-nitroimidazole (2)**. Tetraisopropyldisilyloxy-dichloride (76 mg, 0.24 mmol) was added to a stirred solution of 1-α-D-(ribofuranosyl)-2-nitroimidazole **1** (50 mg, 0.2 mmol)

in anhydrous pyridine (3 mL) and the reaction was continued at 22 °C for 16 h. The TLC chromatographic examination (system A) of these reaction mixtures showed complete disappearance of **1** and the formation of a new spot at higher Rf. The reaction was quenched by addition of few pieces of ice and the solvent was removed from the reaction mixture by rotary evacuation. The residual viscous mass of impure **2** was chromatographed individually on a silica gel column using hexanes/ethyl acetate (3:1; v/v) to afford corresponding pure silylated product, **2**. Yield, 76.3%; mp 106–108 °C; ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C, TMS) δ 0.90–1.10 (multiple peaks, 32 H, 8× CH_3s and 4 CH of isopropyl moiety), 4.08 (dd, J_{gem} = 14.9 Hz, $J_{4',5'} = 2.4$ Hz, 1H, H-5′), 4.12 (dd, J_{gem} = 14.9 Hz, $J_{4',5''} = 3.2$ Hz, 1H, H-5″), 4.18 (ddd, $J_{3',4'} = 5.5$ Hz, $J_{5',4'} = 2.4$ Hz, $J_{5'',4'} = 3.2$ Hz, 1H, H-4′), 4.47 (dd, $J_{4',3'} = 5.5$ Hz, $J_{2'3'} = 4.7$ Hz, 1H, H-3′), 4.67 (dd, d, $J_{1',2'} = 4.3$ Hz, $J_{3',2'} = 4.7$ Hz, 1H, H-2′), 6.64 (d, $J_{2',1'} = 4.3$ Hz, 1H, H-1′), 7.18 (d, $J_{5,4} = 1.2$ Hz, 1H, Im H-4), 7.38 (d, $J_{4,5} = 1.2$ Hz, 1H, Im H-5); ^{13}C NMR (75.46 MHz, $CDCl_3$, 25 °C, TMS) δ 12.39–17.41 (isopropyl CH_3s and CHs), 61.28 (C-5′), 71.07 (C-2′), 72.06 (C-4′), 82.20 (C-3′), 90.38 (C-1′), 122.31 (C-4), 127.94 (C-5), 152.9 (C-2), respectively.

19. **1-α-D-(3′,5′ -*O*,*O*-tetraisopropyldisyloxyarabinofuranosyl)-2-nitroimidazole (4).** The synthesis of this compound was done by reacting 1-α-D-(arabinofuranosyl)-2-nitroimidazole **3** (50 mg, 0.2 mmol) and $TIPDSCl_2$ (76 mg, 0.24 mmol) following similar conditions as described for compound **2**. Yield, 26.5%; mp 83–84 °C; ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C, TMS) δ 0.90–1.13 (m, 28 H, 4× isopropyl H), 2.87 (d, $J_{2'-OH} = 3.6$ Hz, 1H, OH), 4.03 (dd, $J_{4',5'} = 6.4$ Hz, J_{gem} = 12.2 Hz, 1H, H-5′), 4.12 (d, $J_{4',5''} = 3.2$ Hz, J_{gem} = 12.2 Hz, 1H, H-5″), 4.19 (ddd, $J_{3'4'} = 7.0$ Hz, $J_{5',4'} = 6.4$ Hz, $J_{5'',4'} = 3.2$ Hz, 1H, H-4′), 4.43 (dd, $J_{1',2'} = 2.4$ Hz, $J_{3',2'} = 4.3$ Hz, 1H, H-2′), 4.47 (dd, $J_{2',3'} = 4.3$ Hz, $J_{4',3'} = 7.0$ Hz, 1H, H-3′), 6.35 (d, $J_{2',1'} = 2.4$ Hz, 1H, H-1′), 7.23 (d, $J_{5,4} = 0.6$ Hz, 1H, Im H-4), 7.41 (d, $J_{4,5} = 0.6$ Hz, 1H, H-5); ^{13}C NMR (75.46 MHz, $CDCl_3$, 25 °C, TMS) δ 12.52–13.43 (isopropyl CHs), 16.95–17.51 (isopropyl CH_3s), 62.70 (C-5′), 78.76 (C-4′), 84.93 (C-3′), 85.65 (C-2′), 94.75 (C-1′), 121.90 (C-4), 128.79 (C-5); HRMS (ES^+) for $C_{20}H_{37}N_3O_7NaSi_2$ calcd 510.20623; found 510.20664 (100%).
20. **1-α-D-(3′,5′-*O*,*O*-tetraisopropyldisilyloxy-2′ -keto-ribofuranosyl)-2-nitroimidazole (5)**. A stirred suspension of chromium trioxide (15 mg, 0.15 mmol) in CH_2Cl_2 (1 mL) was cooled to 0 °C. Once the temperature was maintained, acetic anhydride (15 μL; 0.15 mmol) in pyridine (25 μL) was added to this solution. Depending on the reaction method, **2** or **4** (24 mg, 0.05 mmol) was added to this solution after 3 min and the reaction was stirred for the required period (3 h in case of **2** and 2 h when **4** was used as a precursor). Once the TLC examination (system B) showed complete disappearance of the starting material, the solvents were removed by vacuum-assisted evaporation. This intermediate product **5** was not isolated and used as such in deuteration reaction.
21. **1-α-D-(3′,5′-*O*,*O*-tetraisopropyldisilyloxy-2′-deuteroribofuranosyl)-2-nitroimidazole (TIPDS-2′-[^{2}H]-α-AZR; 6).** The intermediate product **5**, obtained after the oxidation, as described above, was cooled to 0 °C, dissolved in anhydrous ethanol (1 mL) and stirred. This suspension was treated with sodium borodeuteride (3 mg) and the stirring was continued under preset cold conditions. Another portion of $NaBD_4$ (3 mg) was added to the reaction mixture after 30 min and the stirring was extended for an additional 1 h. TLC chromatographic examination (system B) of the reaction mixture at this time showed complete disappearance of the keto product **5**. The solvent was removed under vacuum and the residue was purified on a silica gel column using hexanes/ethyl acetate (3:1, v/v) as an eluent, and afforded **6** in 37.3% yield when **4** was used as a starting material, and in 29% yield when **2** was the reaction precursor. Mp 83–84 °C; ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C, TMS) δ 1.00–1.12, 4.06 (dd, J_{gem} = 13.4 Hz, $J_{4',5'} = 3.7$ Hz, 1H, H-5′), 4.11 (dd, J_{gem} = 13.4 Hz, $J_{4',5''} = 3.4$ Hz, 1H, H-5″), 4.17 (ddd, $J_{3',4'} = 8.2$ Hz, $J_{5',4'} = 3.7$ Hz, $J_{5'',4'} = 3.4$ Hz, 1H, H-4′), 4.47 (d, $J_{4',3'} = 8.2$ Hz, 1H, H-3′), 6.63 (s, 1H, H-1′), 7.18 (s, 1H, Im H-4), 7.39 (s, 1H, Im H-5); ^{13}C NMR (75.46 MHz, $CDCl_3$, 25 °C, TMS) δ 12.66–13.43 (isopropyl CHs), 16.90–17.41 (isopropyl CH_3s), 61.23 (C-5′), 70.06 (very weak t, C-2′), 71.90 (C-4′), 82.21 (C-3′), 90.37 (C-1′), 122.28 (Im C-4), 127.96 (Im C-5), 154.9 (Im C-2); HRMS (ES^+) for $C_{20}H_{36}N_3O_7NaSi_2D$ calcd 511.21251; found 511.21240 (100%).
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24. **1-α-D-(2′-deutereoribofuranosyl)-2-nitroimidazole (2′-[^{2}H]-α-AZR; 7).** (α-TIPDS 2′-[^{2}H]AZR, **6**, (41 mg, 0.084 mmol) was dissolved in anhydrous acetonitrile (5 mL) and pulverized KF (29 mg, 0.5 mmol) and benzoic acid (61 mg, 0.5 mmol) were added to this solution. The reaction mixture was heated at 75 °C for 6 h and then cooled to 22 °C. The mixture was filtered to remove the inorganic material, washed with an additional aliquot of acetonitrile (2 mL), and the combined filtrate was evaporated to remove the solvent. The viscous residue was purified by silica gel column chromatography using MeOH/CH_2Cl_2 (8:92, v/v) as an eluent to collect pure **7** (yield, 16 mg, 77.7%). (2*R*,3*S*,3a*S*,8a*S*)-2-hydroxymethyl)-3a-deutero-2,3,8a-trihydrofuro [2,3-*d*]imidazolo[2,1-*b*]oxazol-3-ol (**8**), a 2,2′-elimination cyclic product, was also generated along with **7** during the desilylation of **6** (ratio of **7**:**8** is 4:1 as observed by ^{1}H and ^{13}C NMR spectra); ^{1}H NMR (300 MHz, CD_3OD, 25 °C, TMS) δ 3.66 (dd, J_{gem} = 12.2 Hz, $J_{4',5'} = 4.0$ Hz, 1H, H-5′), 3.83 (dd, J_{gem} = 12.2 Hz, $J_{4',5''} = 2.7$ Hz, 1H, H-5″), 4.16 (d, $J_{3',4'} = 5.9$ Hz, 1H, H-3′), 4.30 (ddd, $J_{3',4'} = 5.9$ Hz, $J_{5',4'} = 4.0$ Hz, $J_{5'',4'} = 2.7$ Hz, 1H, H-4′), 6.68 (s, 1H, H-1′), 7.12 (s, 1H, Im H-4), 7.61 (s, 1H, Im H-5); ^{13}C NMR (75.46 MHz, CD_3OD, 25 °C, TMS) δ 62.85 (C-5′), 72.14 (C-4′), 70.05 (C-2′, weak, embedded), 87.44 (C-3′), 91.83 (C-1′), 124.99 (Im C-4), 130.59 (Im C-5), 153.90 (Im C-2); LRES for $C_8H_{10}N_3O_6NaD$ (269.10) M+ present; HRMS (ES+) for $C_8H_{10}N_3O_6NaD$ calcd 269.06028; found 269.06016 (100%).
25. **(2*R*,3*S*,3a*S*,8a*S*)-2-Hydroxymethyl)-3a-deutero-2,3,8a-trihydrofuro[2,3-*d*]imidazolo[2,1-*b*]oxazol-3-ol (8)**. This product appeared along with **7** as a side product during the desilylation of **6**. ^{1}H NMR (300 MHz, CD_3OD, 25 °C, TMS) δ 3.64 (dd, J_{gem} = 12.2 Hz, $J_{4',5'} = 4.0$ Hz, 1H, H-5′), 3.83 (dd, J_{gem} = 12.2 Hz, $J_{4',5''} = 2.7$ Hz, 1H, H-5″), 4.15 (d, $J_{3',4'} = 5.9$ Hz, 1H, H-3′), 4.30 (ddd, $J_{3',4'} = 5.9$ Hz, $J_{5',4'} = 4.0$ Hz, $J_{5'',4'} = 2.7$ Hz, 1H, H-4′), 6.23 (s, 1H, H-1′), 6.60 (d, $J_{5,4} = 1.8$ Hz, 1H, Im H-4), 6.81 (d, $J_{4,5} = 1.8$ Hz, 1H, Im H-5); ^{13}C NMR (75.46 MHz, CD_3OD, 25 °C, TMS) δ 61.89 (C-5′), 71.49 (C-4′), 70.00 (C-2′, weak, embedded), 81.90 (C-3′), 87.43 (C-1′), 122.86 (Im C-4), 127.86 (Im C-5), 153.90 (Im C-2); LRES for $C_8H_{10}N_2O_4DNa$ (222.10) M^+ present; HRMS (ES^+) for $C_8H_{10}N_2O_4D$ calcd 200.07761; found 200.07765 (100%).
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