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Synthesis of 1-β-D-(5-Deoxy-5-iodoarabinofuranosyl)-2-nitroimidazole (β-IAZA): A Novel Marker of Tissue Hypoxia

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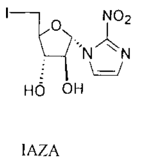
The present work describes the synthesis of the β-isomer of 1-α-D-(5-deoxy-5-iodoarabinofuranosyl)-2-nitroimidazole (IAZA). Radioiodinated IAZA (^{123}I-IAZA) has been extensively studied as a radiopharmaceutical for the diagnosis of regional and/or focal tissue hypoxia in a variety of clinical pathologies. The β-anomer of IAZA, 1-β-D-(5-deoxy-5-iodoarabinofuranosyl)-2-nitroimidazole (β-IAZA, 1), was synthesized *via* an unconventional route starting from 1-β-D-(ribofuranosyl)-2-nitroimidazole (AZR), with a change of configuration at the C-2′-position to afford 1-β-D-(arabinofuranosyl)-2-nitroimidazole (β-AZA, 7). Nucleophilic iodination of the 5′-*O*-toluenesulfonyl-2′,3′-di-*O*-acetyl precursor of β-AZA, 9, followed by deprotection, afforded 1 in satisfactory yield. β-IAZA (1) was also synthesized from 7 using molecular iodine and triphenylphosphine.

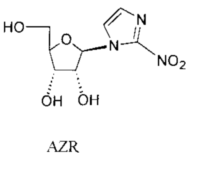
Key words synthesis; hypoxia marker; azomycin nucleoside; 1-β-D-(5-deoxy-5-iodoarabinofuranosyl)-2-nitroimidazole (β-IAZA)

Decreased oxygen levels in tumor cells increases their resistance to the damaging effects of ionizing radiations,1) an effect that is thought to greatly reduce the efficacy of conventional low linear energy transfer (LET) radiation (*e.g.* X-ray) therapies.2) 2-Nitroimidazole (azomycin) nucleosides are highly diffusible radiosensitizers that readily permeate hypoxic tissues, where they are bioreductively activated by single electron transfer and subsequently selectively bound as molecular adducts within viable hypoxic cells. The reversibility of this single electron reduction in the presence of oxygen limits adduct formation to cells that are pathologically hypoxic.3) This oxygen-dependent selectivity forms the basis for non-invasive (imaging) diagnosis of an hypoxic region with radiolabelled nitroimidazoles.4,5) In the past, a number of radioiodinated azomycin α-nucleosides have been synthesized and explored to detect and monitor regional hypoxia.6—8) Of these, 1-α-D-(5-deoxy-5-iodoarabinofuranosyl)-2-nitroimidazole (IAZA) has been widely studied and clinically used in a variety of pathologies involving tissue hypoxia.9—14)

Previous studies on the synthesis of 1-α-D-(5-deoxy-5-iodoarabinofuranosyl)-2-aminoimidazole (iodo aminoimidazole arabinoside; IAIA), a potential nitroreductase reduction metabolite of IAZA,15,16) revealed that IAZA, which had previously been assigned the β-configuration, was actually the α-anomer.17) Furthermore, *in vitro* studies indicated that IAZA was not transported by the NBMPR (nitrobenzylthioinosine)-sensitive equilibrative nucleoside transporter in erythrocytes,18) which was not unexpected given that these transporters handle physiological nucleosides that have the β-nucleoside configuration.19) These findings led to this investigation of the synthesis of 1-β-D-(5-deoxy-5-iodoarabinofuranosyl)-2-nitroimidazole (β-IAZA).



IAZA



AZR

During coupling of the sugar halide with an activated base, the plane of attack by the base (azomycin) at the anomeric centre (C-1′) of the sugar is normally directed by the configuration of the C-1′ leaving group (*e.g.* Br) and the configuration of the protected hydroxyl group at C-2′; when they are *cis*, displacement of the halogen occurs with inversion of configuration at C-1′. In the case of arabinosyl sugar coupling, benzoyl (or acyl) protection at C-2′ with a *trans* C-1′ halide, there is participation by the protecting group. This results in coupling with no inversion of configuration at C-1′, according to the *trans* rule of nucleoside synthesis.20) This problem can often be circumvented by using a protecting group at C-2′ that does not participate in displacement of the halide at C-1′ (*e.g.* benzyl),21) or by C-2′ inversion of configuration of the corresponding β-ribonucleoside.21) In the present work, the utilization of benzyl protection at C-2′ is complicated by the reductive deblocking step, which threatens the integrity of the nitro substituent on the azomycin moiety. Consequently, inversion of configuration at C-2′ of 1-β-D-(ribofuranosyl)-2-nitroimidazole (β-AZR) was the preferred approach to the synthesis of β-IAZA.

Results and Discussion

In coupling azomycin with *trans* arabinosyl bromide, electronic interaction of the commonly used base-labile protective groups (benzoyl and acetyl) with the C-1′ halogen resulted in exclusive formation of α-anomer (IAZA),22) as expected. The introduction of alternate protecting groups, *e.g.* benzyl or substituted silyl (non-acylated),23,24) proceeded sluggishly. In addition, these groups were cleaved during arabinose bromination under the acidic reaction conditions that are generated during halogenation at C-1′.23) The fact that catalytic de-benzylation is also capable of reducing the nitro substituent on the imidazole ring further limits the effectiveness of using benzylation to protect sugar hydroxyl groups in this coupling sequence. *p*-Methoxybenzyl protection of the hydroxyl groups of arabinofuranose, which theoretically could readily be removed by DDQ oxidation25) following

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coupling with 2-nitroimidazole, was also not effective because chlorination or bromination at C-1′ to form the respective arabinose halide led to de-benzylation prior to coupling. The best alternate approach to synthesize **1**, therefore, was to invert the configuration of the 2′-OH group of 1-β-D-(ribofuranosyl)-2-nitroimidazole (AZR).[26,27]

The hydroxyl groups at 3′ and 5′ positions in AZR were protected with 1,1,3,3-tetraisopropyldisiloxane, leaving the 2′-OH group of **2** available for trifluoromethanesulfonylation that, at lower temperatures, afforded the triflate (**3**) in 88% yield.[28] Reaction of **3** with tetrabutylammonium acetate resulted in the formation of 1-β-D-[3,5-*O*,*O*-(1,1,3,3-tetraisopropyldisyliloxy)-2-*O*-acetylarabinofuranosyl]-2-nitroimidazole (**4**), with inversion of configuration at C-2′. Deacetylation of **4**, followed by desilylation in neutral reaction medium, afforded 1-β-D-(arabinofuranosyl)-2-nitroimidazole (β-AZA, **7**) *via* **5**. Alternatively, when **4** was desilylated first, it gave 1-β-D-(2-*O*-acetyl arabinofuranosyl)-2-nitroimidazole (**6**), that upon deacetylation afforded **7** (Chart 1). The change of ribosyl to arabinosyl (configuration at C-2′) led to a significant change in the amplitude of the coupling constants between H-1′–H-2′ and H-3′–H-2′ of AZR and **7**.

β-AZA (**7**) underwent iodination with triphenylphosphine and molecular iodine *via* the alkoxytriphenylphosphonium intermediate[28] (Chart 2), or through the sulfonate intermediate (Chart 3) to afford **1**. Synthesis of **1** mediated by triphenylphosphine and molecular iodine resulted in the formation of **1**; however, the chemical yield *via* this route was quite low (38%), largely due to the formation of several side products and incomplete consumption of β-AZA (**7**).

Synthesis of **1** started by placing a suitable leaving group at C-5′- that could be easily substituted by iodide. Tosylation of 2′-*O*-acetyl β-AZA (**6**) in anhydrous pyridine gave 5′-*O*-tosyl-2′-*O*-acetyl-β-AZA (**8**) in 52% yield. Acetylation of **8** using acetic anhydride, afforded 5′-*O*-tosyl-2′,3′-di-*O*-acetyl-β-AZA, (**9**) (88%). Reaction of this compound with pulverized sodium iodide in anhydrous 2-pentanone afforded 5′-deoxy-5′-iodo-2′,3′-di-*O*-acetyl-β-AZA (**10**, 80%) (Chart 3, Method A). An attempt to prepare 5′-*O*-trifluoromethanesulfonyl-2′,3′-di-*O*-acetyl β-AZA (**9**) to exploit the advantages of the trifluoromethane sulfonyl leaving group for radiochemical synthesis, from 2′,3′-di-*O*-acetyl-β-AZA (**12**), led to the unexpected synthesis of 5′-deoxy-5′-chloro-2′,3′-di-*O*-acetyl-β-AZA (**13**).

The synthesis of 2′,3′-di-*O*-acetyl-β-AZA (**12**) started with reaction of 2′-*O*-acetyl-β-AZA (**6**) with *t*-butyldiphenylchlorosilane (TBDPS-chloride), followed by reaction with acetic anhydride in anhydrous pyridine, to provide 5′-*O*-TBDPS-2′,3′-di-*O*-acetyl-β-AZA (**11**) in 88% yield. Selective desilylation of **11** in neutral medium using potassium fluoride and benzoic acid afforded **12** (92%). Reaction of **12** with trifluoromethane sulfonyl chloride resulted in the formation of 5′-deoxy-5′-chloro-2′,3′-di-*O*-acetyl β-AZA (**13**) in nearly quantitative yield (96%) in place of the expected 5′-*O*-trifluoromethane sulfonyl derivative of **12**. Fortunately for the objectives of this work, **13**, on reaction with sodium iodide, was readily converted to the desired 5′-deoxy-5′-iodo-2′,3′-di-*O*-acetyl-β-AZA (**10**) in 87% yield

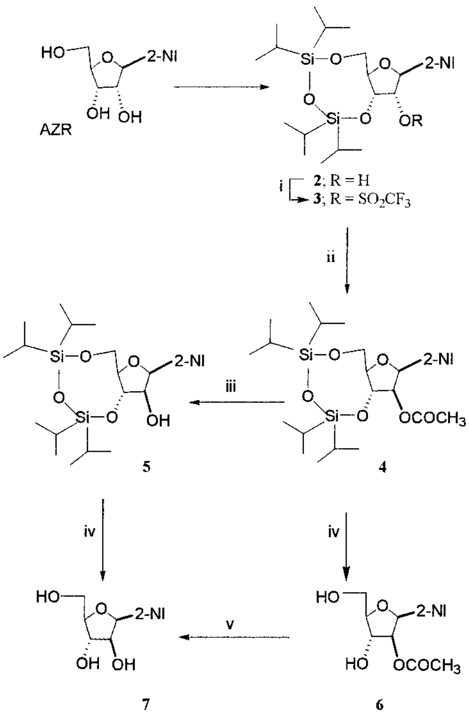

Chart 1. Synthetic Route to β-AZA (**7**)

2-NI=2-nitroimidazole, i=CF$_3$SO$_2$Cl; ii=NH$_4$OAc; iii=1 N NaOH; iv=KF/C$_6$H$_5$COOH, and v=2 M NH$_3$MeOH.

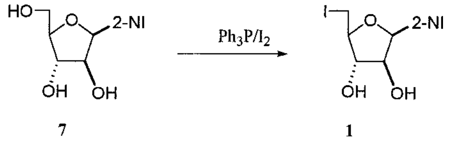

Chart 2. Synthetic Route to β-IAZA (**1**)

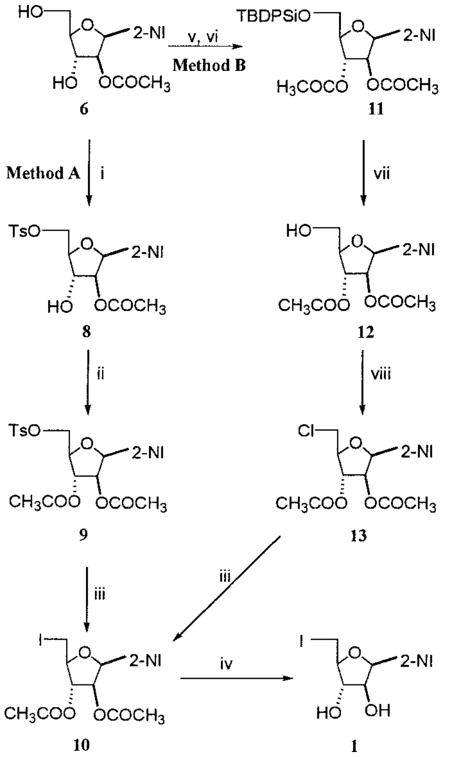

Chart 3. Alternate Synthetic Route to β-IAZA (**1**)

2-NI=2-nitroimidazole; i=tosyl chloride/pyridine; ii=Ac$_2$O/pyridine; iii=NaI/2-pentanone; iv=2 M NH$_3$/MeOH; v=TBDPS chloride/pyridine; vi=Ac$_2$O; vii=KF/benzoic acid and viii=CF$_3$SO$_2$Cl/DMAP/CH$_2$Cl$_2$.

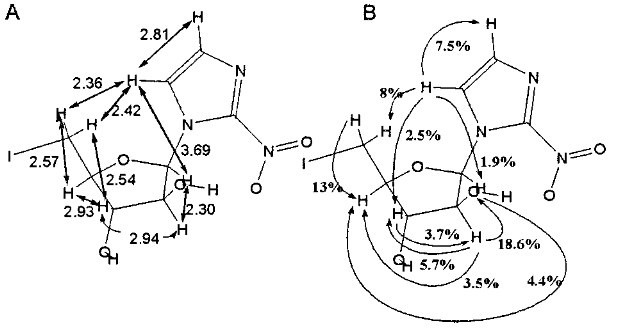

Fig. 1. Optimized Structure and the H–H Inter Atomic Distances (Å) (A) and NOE Correlations (B) for 5-β-IAZA

(Chart 3, Method B). Deprotection of acetyl groups afforded the targeted **1** in 96% yield. The confirmation of halogenation (chloro or iodo) at C-5′ is supported by a strong shielding of C-5′ carbon that moves this resonance to δ: 3.43 in **10** (2′,3′-di-*O*-acetyl-β-IAZA), and to δ: 43.16 in **13** (2′,3′-di-*O*-acetyl-β-chloro-AZA). These changes in chemical shift are in accordance with the values reported for similar chemicals.[29]

The structural assignment for **1** was made on the basis of its nuclear Overhauser effect (NOE) (Fig. 1), with further support from ^{1}H- and ^{13}C-NMR spectroscopy data. As illustrated in Fig. 1, irradiation at H-5 (imidazole proton), showed enhancement of signals for H-4 (7.5%) and H-5′ (8%) protons while the H-3′ (2.5%), and H-1′ (1.9%) protons, not being in the same plane, were not affected so intensely. Further, irradiation at H-1′, which is in the same plane as the H-2′ and H-4′ protons, exerted a strong impact on these protons (11.2% and 4.4%, respectively), but the impact on the H-5 (nitroimidazole) proton was minimal (1.1% enhancement). Irradiation at H-2′ enhanced the H-1′ (18.6%), H-3′ (5.7%), and H-4′ (3.5%) signals, while irradiation at H-3′ did not significantly affect H-2′ (3.7%), H-4′ (2.4%), H-5′ (2.1%), and H-5 (0.7%) proton signals. Irradiation at H-4′ enhanced the signals for H-1′ (9.1%), H-5′ (8.4%), H-2′ (3.9%), and H-3′ (3.9%), while irradiation at H-5′ affected the signals for H-5 (8.3%), H-4′ (13%), and H-3′ (8.5%).

These differential NOE results are in good agreement with what is expected from the inter H–H atomic distances obtained from the optimized structure.[30] This indicates that the configuration of *N*-glycosidic linkage at C-1′ of the arabinoside component is β. The contrasting NOE results are reported for the α-anomers, 1-α-D-[arabinofuranosyl]-2-aminoimidazole (AIA) and 5′-iodinated-AIA (IAIA),[31] where significant NOE enhancements are seen for H-2′ and H-4′ protons when the H-5 proton is irradiated. In addition, the higher impact of irradiation of H-1′ on the signal for H-2′ (and *vice versa*) indicate that inversion of configuration at C-2′, from ribose to arabinose, occurred during the synthesis of **4**. The ^{1}H-NMR spectrum of **1** showed a larger H-1′–H-2′ coupling constant ($J_{1',2'}=5.2$ Hz), that is in contrast to that of IAZA (*ca.*0 Hz),[22] and supports the β-stereo chemistry at C-1′.

Experimental

General Procedure All chemicals used were reagent grade. The solvents were dried over appropriate drying agents and freshly distilled before use. The progress of synthetic reactions was monitored by thin layer chromatography (TLC) using 250 μm Whatman MK6F silica gel micro TLC plates. Column chromatography was performed on Merck silica gel 60 (particle size 70—200 and 230—400 mesh ASTM). Melting points were determined on a Büchi capillary melting point apparatus and are uncorrected. ^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker AM-300 spectrometer in deuterated chloroform ($CDCl_3$) or methanol (CD_3OD), depending on the solubility of the product. Chemical shifts are reported in ppm downfield with respect to tetramethylsilane as an internal standard. The protons and carbons of the sugar moiety and nitroimidazole are represented by a single prime (′) and no prime, respectively. When necessary, Electron spray ionization (ESI) mass spectra were acquired, in lieu of elemental analysis, using a sodium probe on an AEI-MS-12 mass spectrometer.

1-β-D-[3,5-*O*,*O*-(1,1,3,3-Tetraisopropyldisiloxanyl)-2-*O*-acetylarabinofuranosyl]-2-nitroimidazole (4) A mixture of tetrabutylammonium acetate (9.88 g, 32.66 mmol) and **3** (3.3 g, 5.32 mmol) in anhydrous toluene (150 ml) was stirred at 22 °C for 1 h. The solvent was removed afterwards and the impure residue was purified on a silica gel column by ethyl acetate/toluene (5 : 95, v/v) to give 2.6 g (92%) of pure **4**. mp 134—136 °C. ^{1}H-NMR ($CDCl_3$) δ: 0.84—1.05 (m, 28H, 4×isopropyl H), 1.80 (s, 3H, $COCH_3$), 3.89 (dd, $J_{3',4'}=9.0$ Hz, $J_{5',4'}=2.4$ Hz, 1H, H-4′), 3.98 (dd, $J_{4',5'}=2.4$ Hz, $J_{gem}=13.4$ Hz, 1H, H-5′), 4.14 (d, $J_{gem}=13.4$ Hz, 1H, H-5″), 4.32 (dd, $J_{2',3'}=9.0$ Hz, $J_{4',3'}=9.0$ Hz, 1H, H-3′), 5.87 (d, $J_{3',2'}=9.0$ Hz of d, $J_{1',2'}=6.1$ Hz, 1H, H-2′), 6.66 (d, $J_{2',1'}=6.1$ Hz, 1H, H-1′), 7.18 (s, 1H, H-4) and 7.86 (s, 1H, H-5). ^{13}C-NMR ($CDCl_3$) δ: 12.47—13.49 (4 isopropyl CH), 16.56—17.50 (8 isopropyl CH_3), 19.96 (acetyl CH_3), 60.18 (C-5′), 69.99 (C-4′), 74.32 (C-2′), 81.18 (C-3′), 84.81 (C-1′), 122.64 (C-5), 128.18 (C-4), 144.5 (C-2) and 168.50 ($\underline{C}OCH_3$) ppm. MS (ESI) *m*/*z*: 552 ($M+Na^+$). MS (HR-ESI) *m*/*z*: 552.21799 (Calcd for $C_{22}H_{39}Si_2N_3O_8Na$: 552.21734). *Anal.* Calcd for $C_{22}H_{39}Si_2N_3O_8$: C, 49.88; H, 7.42; N, 7.932. Found: C, 50.15; H, 7.58; N, 7.58.

1-β-D-[3,5-*O*,*O*-(1,1,3,3-Tetraisopropyldisiloxanyl)arabinofuranosyl]-2-nitroimidazole (5) A methanolic solution (10 ml) of **4** (0.225 g, 0.425 mmol) and triethylamine (2.86 ml) was stirred overnight (16 h) at 25 °C then the solvent was removed by rotary evaporation. The residue was loaded on a silica gel column and purified by eluting with ethyl acetate/toluene (15 : 95, v/v) to give 0.16 g (79%) of pure **5**. mp 152—154 °C. ^{1}H-NMR ($CDCl_3$) δ: 0.92—1.10 (m, 28H, 4×isopropyl H), 3.30 (d, $J_{2',OH}=1H$, OH), 3.84 (d, $J_{3',4'}=8.9$ Hz of d, $J_{5',4'}=2.7$ Hz of d, $J_{5'',4'}=1.5$ Hz, 1H, H-4′), 4.03 (d, $J_{4',5'}=2.7$ Hz of d, $J_{gem}=13.5$ Hz, 1H, H-5′), 4.20 (d, $J_{gem}=13.5$ Hz of d, $J_{4',5''}=1.5$ Hz, 1H, H-5″), 4.21 (dd, $J_{2',3'}=9.0$ Hz, $J_{4',3'}=8.9$ Hz, 1H, H-3′), 4.71 (d, $J_{3',2'}=9.0$ Hz of d, $J_{1',2'}=6.1$ Hz of d, $J_{OH\text{-}2'}=4.9$ Hz, 1H, H-2′), 6.66 (d, $J_{2',1'}=6.1$ Hz, 1H, H-1′), 7.17 (d, $J_{5,4}=0.9$ Hz, 1H, H-4) and 7.84 (d, $J_{4,5}=0.9$ Hz, 1H, H-5). ^{13}C-NMR ($CDCl_3$) δ: 12.46—13.48 (4 isopropyl CH), 16.61—17.51 (8 isopropyl CH_3), 60.13 (C-5′), 71.29 (C-3′), 75.50 (C-2′), 80.93 (C-4′), 86.89 (C-1′), 122.63 (C-5), 128.04 (C-4), 144.80 (C-2) ppm. MS (ESI) *m*/*z*: 510.2 ($M+Na^+$). *Anal.* Calcd for $C_{20}H_{37}Si_2N_3O_7$: C, 49.256; H, 7.646; N, 8.616. Found: C, 49.283; H, 7.806; N, 8.322.

1-β-D-[2-*O*-Acetyl-arabinofuranosyl]-2-nitroimidazole (6) Potassium fluoride (1.05 g, 18 mmol), benzoic acid (2.19 g, 17.9 mmol) and **4** (1.9 g, 3.59 mmol) were taken in acetonitrile (80 ml) and heated at 75 °C for overnight. The mixture was cooled, filtered and the filtrate was evaporated. The impure material so obtained was purified on a silica gel column using ethyl acetate as eluant to give 0.98 g (95%) of pure **6**. mp 159—160 °C. ^{1}H-NMR (CD_3OD) δ: 1.80 (s, 3H, $COCH_3$), 3.81 (d, $J_{4',5''}=4.9$ Hz of d, $J_{gem}=12.2$ Hz, 1H, H-5″), 3.88 (d, $J_{gem}=12.2$ Hz of d, $J_{4',5'}=3.6$ Hz, 1H, H-5′), 4.02 (d, $J_{3',4'}=4.9$ Hz of d, $J_{5',4'}=3.6$ Hz of d, $J_{5'',4'}=4.9$ Hz, 1H, H-4′), 4.27 (dd, $J_{2',3'}=4.5$ Hz, $J_{4',3'}=4.9$ Hz, 1H, H-3′), 5.58 (d, $J_{3',2'}=4.5$ Hz of d, $J_{1',2'}=4.9$ Hz, 1H, H-2′), 6.83 (d, $J_{2',1'}=4.9$ Hz, 1H, H-1′), 7.17 (s, 1H, H-4) and 7.98 (s, 1H, H-5). ^{13}C-NMR ($CDCl_3$) δ: 20.04 ($CO\underline{C}H_3$), 61.54 (C-5′), 73.96 (C-3′), 78.47 (C-2′), 86.04 (C-4′), 88.50 (C-1′), 125.20 (C-5), 128.35 (C-4), 144.5 (C-2) and 170.42 (C=O) ppm. *Anal.* Calcd for $C_{10}H_{13}N_3O_7$: C, 41.82; H, 4.56; N, 14.63. Found: C, 41.97; H, 4.53; N, 14.58.

1-β-D-[Arabinofuranosyl]-2-nitroimidazole (β-AZA, 7) Potassium fluoride (89 mg, 1.53 mmol) and benzoic acid (209 mg, 1.71 mmol) were added to a solution of **5** in CH_3CN (20 ml) and the mixture was heated at 75 °C for 3 h. A TLC check at this time indicated complete disappearance of the starting material **5**. The mixture was cooled down to room temperature (22 °C) and filtered. The filtrate was evaporated and the viscous impure mass was subjected to column chromatography. An elution with ethyl acetate afforded pure **7** (52 mg, 69%). mp 160—162 °C. ^{1}H-NMR (CD_3OD) δ: 3.81 (d, $J_{4',5'}=4.6$ Hz of d, $J_{gem}=11.9$ Hz, 1H, H-5′), 3.89 (d, $J_{gem}=11.9$ Hz of d, $J_{4',5''}=3.0$ Hz, 1H, H-5″), 3.95 (d, $J_{3',4'}=5.2$ Hz of d, $J_{5',4'}=4.6$ Hz of d, $J_{5'',4'}=3.0$ Hz, 1H, H-4′), 4.04 (dd, $J_{2',3'}=5.2$ Hz, $J_{4',3'}=5.2$ Hz, 1H, H-3′), 4.42 (d, $J_{3',2'}=5.2$ Hz of d, $J_{1',2'}=5.2$ Hz, 1H, H-2′), 6.71 (d, $J_{2',1'}=5.2$ Hz, 1H, H-1′), 7.13 (d, $J_{5,4}$=1.2 Hz, 1H, H-4) and 7.97 (d, $J_{4,5}$=1.2 Hz, 1H, H-5). ^{13}C-NMR (CD_3OD) δ: 61.78 (C-5′), 75.79 (C-3′), 77.73 (C-2′), 85.90 (C-4′), 90.77 (C-1′), 125.16 (C-5), 127.87 (C-4), 145.82 (C-2) ppm. MS (ESI) *m/z*: 268 ($M+Na^+$). *Anal.* Calcd for $C_8H_{11}N_3O_6$: C, 39.19; H, 4.52; N, 17.14. Found: C, 39.63; H, 4.73; N, 15.42.

Alternatively, **7** was prepared by deacetylation of **6**. A solution of NH_3/methanol (2 M, 30 ml) was added to **6** (30 mg, 0.51 mmol) and the mixture was stirred at 0 °C for 2 h. A TLC check at this time showed complete deacetylation of **6** and appearance of a new spot at the *Rf* for **7**.

1-*β*-D-[5-*O*-Toluenesulfonyl-2-*O*-acetyl-arabinofuranosyl]-2-nitroimidazole (8) A solution of **6** (0.23 g, 0.8 mmol) in anhydrous pyridine (10 ml) was cooled to −5 °C and tosyl chloride (0.3 g, 1.6 mmol), pre-dissolved in pyridine (5 ml), was added dropwise to this solution. The mixture was stirred at this temperature for 6 h. Additional tosyl chloride (0.1 g, 0.53 mmol) and DMAP (98 mg, 0.8 mmol) were added to the reaction mixture and the stirring was continued for 3 d at 22 °C. The reaction was quenched after tosylation was complete, by adding ice. Pyridine was removed *in vacuo* and the impure product was purified by column chromatography using ethyl acetate/hexanes (40/60, v/v) as eluant to afford 0.18 g (52%) of pure **8**. This compound was subjected to acetylation without any further characterization.

1-*β*-D-[5-*O*-Toluenesulfonyl-2,3-di-*O*-acetylarabinofuranosyl]-2-nitroimidazole (9) Acetic anhydride (200 *μ*l) was added to a solution of **8** (0.18 g, 0.4 mmol) in anhydrous pyridine (10 ml) under stirring. The reaction was continued for 3 h at 22 °C. At that time, a TLC examination of the reaction mixture showed complete conversion of **8** to **9**. The solvent was removed *in vacuo* and the residue was loaded on a silica gel column. Purification using ethyl acetate/hexanes (50 : 50, v/v) afforded pure product **9** (0.17 g, 88%). mp 117—119 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.90 and 2.18 (two s, each for 3H, 2×$COCH_3$), 2.50 (s, 3H, toluyl CH_3), 4.26 (d, $J_{3',4'}$=3.4 Hz of d, $J_{5',4'}$=3.0 Hz of d, $J_{5'',4'}$=4.6 Hz, 1H, H-4′), 4.26 (d, $J_{4',5''}$=4.6 Hz of d, J_{gem}=11.3 Hz, 1H, H-5″), 4.46 (d, J_{gem}=11.3 Hz of d, $J_{4',5'}$=3.0 Hz, 1H, H-5′), 5.14 (dd, $J_{2',3'}$=1.2 Hz, $J_{4',3'}$=3.4 Hz, 1H, H-3′), 5.66 (d, $J_{3',2'}$=1.2 Hz of d, $J_{1',2'}$=3.6 Hz, 1H, H-2′), 6.83 (d, $J_{2',1'}$=3.6, 1H, H-1′), 7.19 (s, 1H, imidazole H-4), 7.42 (d, $J_{2,3}$=$J_{6,5}$=8.3 Hz, 2H, phenyl H-3 and H-5), 7.78 (s, 1H, imidazole H-5) and 7.86 (d, $J_{3,2}$=$J_{5,6}$=8.3 Hz, 2H, phenyl H-2 and H-6). ^{13}C-NMR ($CDCl_3$) δ: 19.76 ($CO\underline{C}H_3$ at C-2′), 19.86 ($CO\underline{C}H_3$ at C-3′), 20.49 (toluyl CH_3), 67.40 (C-5′), 74.27 (C-3′), 75.94 (C-2′), 80.95 (C-4′), 87.76 (C-1′), 123.46 (C-5), 127.77 (phenyl C-2, C-6 and C-4), 129.94 (phenyl C-3 and C-5), 128.11 (C-4), 145.31 (C-2), 132.62 (phenyl C-1), 145.31 (imidazole C-2), 168.22 and 169.47 (two C=O) ppm. *Anal.* Calcd for $C_{19}H_{21}SN_3O_{10}$: C, 47.20; H, 4.38; N, 8.69. Found: C, 47.13; H, 4.21; N, 8.55.

1-*β*-D-[5-*O*-*tert*-Butyldiphenylsilyl-2,3-di-*O*-acetyl Arabinofuranosyl]-2-nitroimidazole (11) *t*-Butyldiphenylchlorosilane (0.95 ml, 3.65 mmol) was added to a solution of **6** (0.96 g, 3.34 mmol) in anhydrous pyridine (10 ml) under an inert atmosphere. This mixture was stirred overnight at 30 °C, when analysis by TLC showed complete conversion of **6** to its 5′-*O*-silylated derivative. Acetic anhydride (0.47 ml, 5 mmol) was added *in situ* to this reaction mixture and the stirring was continued for an additional 1.5 h. Afterwards, excess acetic anhydride was decomposed by adding ice to the reaction vessel and the solvent was removed *in vacuo* on a rotary evaporator. Purification of the impure product on a silica gel column, using ethyl acetate/toluene (5 : 95, v/v) as eluant, yielded 1.68 g (88%) of pure **11**. mp 145—146 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.05 (s, 9H, *t*-butyl), 1.68 and 2.05 (two s, each for 3H, 2×$COCH_3$), 3.81 (d, $J_{4',5''}$=4.2 Hz of d, J_{gem}=11.6 Hz, 1H, H-5″), 3.99 (d, J_{gem}=11.6 Hz of d, $J_{4',5'}$=3.4 Hz, 1H, H-5′), 4.09 (m, $J_{3',4'}$=5.3 Hz of d, $J_{5',4'}$=3.4 Hz of d, $J_{5'',4'}$=4.2 Hz, 1H, H-4′), 5.39 (d, $J_{2',3'}$=3.4 Hz of d, $J_{4',3'}$=5.3 Hz, 1H, H-3′), 5.65 (d, $J_{3',2'}$=3.4 Hz of d, $J_{1',2'}$=3.9 Hz, 1H, H-2′), 6.74 (d, $J_{2',1'}$=3.9 Hz, 1H, H-1′), 6.98 (s, 1H, imidazole H-4), 7.30—7.42 (m, 6H, phenyl), 7.52 (s, 1H, imidazole H-5) and 7.60—7.64 (m, 4H, phenyl). ^{13}C-NMR ($CDCl_3$) δ: 19.87 ($CO\underline{C}H_3$ at C-2′), 20.62 ($CO\underline{C}H_3$ at C-3′), 26.77 (*t*-butyl CH_3), 62.12 (C-5′), 74.38 (C-3′), 74.94 (C-2′), 82.32 (C-4′), 86.96 (C-1′), 123.06 (C-5), 127.79 (phenyl C-2, C-6 and C-4), 127.98 (imidazole C-4), 132.67 and 132.35 (C-1 of two phenyls), 135.55 and 135.37 (phenyl C-3 and C-5), 145.31 (imidazole C-2), 168.31 and 169.34 (two C=O) ppm. *Anal.* Calcd for $C_{28}H_{33}SiN_3O_8$: C, 59.25; H, 5.86; N, 7.40. Found: C, 59.79; H, 6.01; N, 7.16.

1-*β*-D-[2,3-di-*O*-Acetyl-arabinofuranosyl]-2-nitroimidazole (12) A solution of **11** (1.60 g, 2.81 mmol) in CH_3CN (80 ml) was heated with potassium fluoride (1.14 g, 19.67 mmol) and benzoic acid (2.40 g, 19.67 mmol) at 75 °C for 16 h. The mixture was then cooled, filtered and the filtrate was subjected to evaporation. Removal of the solvent led to a viscous mass that was purified on a silica gel column. Elution, at first with 40% ethyl acetate/hexane (v/v) and then with 90% ethyl acetate in hexane gave 0.85 g (92%) of pure **12**. mp 105 °C. ^{1}H-NMR (CD_3OD) δ: 1.80 and 2.12 (two s, each for 3H, 2×$COCH_3$), 3.82 (d, $J_{4',5''}$=4.9 Hz of d, J_{gem}=12.5 Hz, 1H, H-5″), 3.94 (d, J_{gem}=12.5 Hz of d, $J_{4',5'}$=3.9 Hz, 1H, H-5′), 4.20 (m, $J_{3',4'}$=4.9 Hz of d, $J_{5',4'}$=3.9 Hz of d, $J_{5'',4'}$=4.9 Hz, 1H, H-4′), 5.28 (d, $J_{2',3'}$=3.4 Hz of d, $J_{4',3'}$=4.9 Hz, 1H, H-3′), 5.71 (d, $J_{3',2'}$=3.4 Hz of d, $J_{1',2'}$=4.6 Hz, 1H, H-2′), 6.85 (d, $J_{2',1'}$=4.6 Hz, 1H, H-1′), 7.19 (s, 1H, imidazole H-4) and 7.98 (s, 1H, imidazole H-5. ^{13}C-NMR ($CDCl_3$) δ: 19.97 ($CO\underline{C}H_3$ at C-2′), 20.60 ($CO\underline{C}H_3$ at C-3′), 61.55 (C-5′), 76.37 (C-3′), 76.47 (C-2′), 84.22 (C-4′), 88.67 (C-1′), 125.13 (C-5), 128.48 (C-4), 145.31 (C-2), 170.18 and 171.43 (two C=O) ppm. *Anal.* Calcd for $C_{12}H_{15}N_3O_8$: C, 43.77; H, 4.59; N, 12.76. Found: C, 43.69; H, 4.55; N, 12.62.

1-*β*-D-[5-Deoxy-5-chloro-2,3-di-*O*-acetyl-arabinofuranosyl]-2-nitroimidazole (13) Trifluoromethanesulfonyl chloride (0.34 ml, 3.19 mmol) was taken in anhydrous dichloromethane (10 ml) and added to a pre-cooled solution of **12** (0.55 g, 1.67 mmol) and DMAP (0.617 g, 5 mmol) in anhydrous CH_2Cl_2 (40 ml) at −78 °C. The reaction mixture was stirred for 2 h at this temperature and then quenched by ice. The mixture was allowed to warm to room temperature (22 °C) to decompose excess triflyl chloride. The organic layer was dried over anhydrous $MgSO_4$, filtered and the filtrate was evaporated by rotary evaporation. The residue was purified on a silica gel column using ethyl acetate/hexanes (40/60, v/v) that afforded pure **13** (0.56 g, 96%). mp 128 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.86 and 2.19 (two s, each for 3H, 2×$COCH_3$), 3.82 (d, $J_{4',5''}$=5.5 Hz of d, J_{gem}=12.2 Hz, 1H, H-5″), 3.94 (d, J_{gem}=12.2 Hz of d, $J_{4',5'}$=3.9 Hz, 1H, H-5′), 4.34 (m, $J_{3',4'}$=3.4 Hz of d, $J_{5',4'}$=3.9 Hz of d, $J_{5'',4'}$=5.5 Hz, 1H, H-4′), 5.24 (d, $J_{2',3'}$=1.8 Hz of d, $J_{4',3'}$=3.4 Hz, 1H, H-3′), 5.69 (d, $J_{3',2'}$=1.8 Hz of d, $J_{1',2'}$=4.3 Hz, 1H, H-2’), 6.85 (d, $J_{2',1'}$=4.3 Hz, 1H, H-1′), 7.19 (s, 1H, H-4) and 7.98 (s, 1H, H-5). ^{13}C-NMR ($CDCl_3$) δ: 19.97 ($CO\underline{C}H_3$ at C-2′), 20.64 ($CO\underline{C}H_3$ at C-3′), 43.16 (C-5′), 74.74 (C-3′), 76.82 (C-2′), 82.26 (C-4′), 87.80 (C-1′), 123.18 (C-5), 128.18 (C-4), 145.36 (C-2), 168.10 and 169.50 (two C=O) ppm. MS (ESI) *m/z*: 370 ($M+Na^+$). *Anal.* Calcd for $C_{12}H_{14}ClN_3O_7$: C, 41.44; H, 4.03; N, 12.09. Found: C, 41.53; H, 4.02; N, 11.78.

1-*β*-D-[5-Deoxy-5-iodo-2,3-di-*O*-acetyl-arabinofuranosyl]-2-nitroimidazole (10)

Method A: **9** (5 mg, 0.01 mmol) was dissolved in anhydrous 2-pentanone (100 *μ*l) in a Reactivial$^{\text{TM}}$ (3 ml), and pulverized sodium iodide (3.7 mg, 0.025 mmol) was added to it. The mixture was heated in a 500 W domestic microwave oven at 'high' for 8 min. Co-chromatography with authentic **10** on HPLC confirmed the formation of **10**. The chemical yield of **10** by this method, without any purification (HPLC yield), was 80%.

Method B: Pulverized anhydrous sodium iodide (0.25 g, 1.63 mmol) was added to a solution of **13** (0.15 g, 0.43 mmol) in anhydrous 2-pentanone (20 ml) and the mixture was refluxed at 110 °C for 16 h. Afterwards, the solvent was evaporated and the recovered residue was chromatographed on a silica gel column. Elution with ethyl acetate/toluene (20 : 80, v/v) gave 0.16 g (87%) pure **10**. mp 161—163 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.85 and 2.18 (two s, each for 3H, 2×$COCH_3$), 3.52 (d, $J_{4',5''}$=5.7 Hz of d, J_{gem}=11.0 Hz, 1H, H-5″), 3.61 (d, J_{gem}=11.0 Hz of d, $J_{4',5'}$=5.2 Hz, 1H, H-5′), 4.19 (m, $J_{3',4'}$=3.4 Hz of d, $J_{5',4'}$=5.2 Hz of d, $J_{5'',4'}$=5.7 Hz, 1H, H-4′), 5.12 (d, $J_{2',3'}$=1.5 Hz of d, $J_{4',3'}$=3.4 Hz, 1H, H-3′), 5.69 (dd, $J_{3',2'}$=1.5, $J_{1',2'}$=3.7 Hz, 1H, H-2′), 6.83 (d, $J_{2',1'}$=3.7 Hz, 1H, H-1′), 7.19 (s, 1H, H-4) and 7.62 (s, 1H, H-5). ^{13}C-NMR ($CDCl_3$) δ: 3.43 (C-5′), 20.01 ($CO\underline{C}H_3$ at C-2′), 20.67 ($CO\underline{C}H_3$ at C-3′), 74.96 (C-3′), 78.70 (C-2′), 82.18 (C-4′), 87.98 (C-1′), 123.52 (C-5), 128.16 (C-4), 145.42 (C-2), 168.00 and 169.41 (two C=O) ppm. *Anal.* Calcd for $C_{12}H_{14}IN_3O_7$: C, 32.82; H, 3.21; N, 9.57. Found: C, 33.24; H, 3.13; N, 9.24.

1-*β*-D-[5-Deoxy-5-iodoarabinofuranosyl]-2-nitroimidazole (*β*-IAZA, 1) Synthesis of **1** (Chart 2) started from *β*-AZA (**7**). Triphenylphosphine (42.9 mg, 0.163 mmol) was added to the solution of **7** (20 mg, 0.08 mmol) in anhydrous pyridine (5 ml). Iodine (41.4 mg, 0.163 mmol) was introduced in the reaction mixture after 5 min and the stirring was continued for 16 h at 70 °C. Afterwards, the reaction was stopped by adding methanol (0.5 ml) to the reaction mixture and the solvent was removed. The residue was chromatographed on a silica gel column using ethyl acetate/hexanes (45 : 55, v/v) that afforded 11 mg (38%) of **1**.

Alternatively (Chart 3), diacetate (**10**, 0.135 g, 0.31 mmol) was dissolved in NH_3/MeOH (2 M, 8 ml) and the solution was stirred at 0 °C for 2 h, then evaporated to dryness to give the impure **1**. The impure product was purified on a silica gel column using ethyl acetate/hexanes (45 : 55, v/v) to afford 0.105 g (96%) pure **1**. mp 138 °C. ^{1}H-NMR (CD_3OD) δ: 3.55 (dd, $J_{4',5''}$=6.5 Hz, J_{gem}=10.5 Hz, 1H, H-5″), 3.61 (dd, J_{gem}=10.5 Hz, $J_{4',5'}$=6.1 Hz, 1H, H-5′), 4.06—4.10 (m, 2H, H-4′, H-3′), 4.48 (d, $J_{3',2'}$=3.4 Hz of d, $J_{1',2'}$=4.5 Hz, 1H, H-2′), 6.70 (d, $J_{2',1'}$=4.5 Hz, 1H, H-1′), 7.16 (d, $J_{5,4}$=0.9 Hz, 1H, H-4) and 7.76 (d, $J_{4,5}$=0.9 Hz, 1H, H-5). ^{13}C-NMR ($CDCl_3$) δ: 5.22 (C-5′), 77.42 (C-2′), 79.90 (C-3′), 86.33 (C-4′), 92.42 (C-1′), 127.97 (C-5), 128.03 (C-4), 145.40 (C-2) ppm. *Anal.* Calcd for $C_8H_{10}IN_3O_5$: C, 27.06; H, 2.84; N, 11.83. Found: C, 27.38; H, 2.76; N, 11.50.

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References and Notes

1) Tomlinson R. H., Gray L. H., *Br. J. Cancer*, **9**, 539—549 (1955).
2) Brown J. M., *Cancer Res.*, **59**, 5863—5870 (1999).
3) Biaglow J. E., Varnes M. E., Roizen-Towle L., Clark E. P., Epp E. R., Astor M. B., Hall E. J., *Biochem. Pharmac.*, **35**, 77—90 (1986).
4) Adams G. E., Flockhart I. R., Smithen C. E., Stratford I. J., Wardman P., Watts M. E., *Radiat. Res.*, **67**, 9—20 (1976).
5) Chapman J. D., Franko A. J., Sharplin J. A., *Br. J. Cancer*, **43**, 546—550 (1981).
6) Jette D. C., Wiebe L. I., Flanagan R. J., Lee J., Chapman J. D., *Radiat. Res.*, **105**, 169—179 (1986).
7) Wiebe L. I., Jette D. C., Chapman J. D., Flanagan R. J., Meeker B. E., "Nuclear Medicine in Clinical Oncology," ed. by Winkler C., Springer-Verlag, 1986, pp. 402—407.
8) Mannan R. H., "Novel Non-Invasive Markers of Tumor Hypoxia," Ph.D. thesis, University of Alberta, Edmonton, Canada., 1991, pp. 89—90, 145—146.
9) Parliament M. B., Chapman J. D., Urtasun R. C., McEwan A. J., Golberg L., Mercer J. R., Mannan R. H., Wiebe L. I., *Br. J. Radiol.*, **65**, 90—95 (1991).
10) Groshar D., McEwan A. J. B., Parliament M. B., Urtasun R. C., Golberg L. E., Hoskinson M., Mercer J. R., Mannan R. H., Wiebe L. I., Chapman J. D., *J. Nucl. Med.*, **34**, 885—888 (1993).
11) Urtasun R. C., Parliament M. B., McEwan A. J., Mercer J. R., Mannan R. H., Wiebe L. I., Morin C., Chapman J. D., *Br. J. Cancer*, **74**, S209—S212 (1996).
12) Al-Arafaj A., Ryan E. A., Hutchinson K., Mannan R. H., Mercer J., Wiebe L. I., McEwan A. J. B., *Europ. J. Nucl. Med.*, **21**, 1338—1442 (1994).
13) McEwan A. J. B., Skeith K. J., Mannan R. H., Davies N., Jamali F., Schmidt R., Golberg K., Wiebe L. I., *J. Nucl. Med.*, **38** (Suppl.), 300—301 (1997).
14) Vinjamuri S., O'Driscol K., Maltby P., McEwan A. J., Wiebe L. I., Critchley M., *Clin. Nucl. Med.*, **24**, 891—892 (1999).
15) Mannan R. H., Somayaji V. V., Lee J., Mercer J. R., Chapman J. D., Wiebe L. I., *J. Nucl. Med.*, **32**, 1764—1770 (1991).
16) Edwards D. I., *J. Antimicrob. Chemother.*, **31**, 9—20 (1993).
17) Lee H. C., Kumar P., Wiebe L. I., Mercer J. R., Ohkura K., Seki K., *Nucleosides & Nucleotides*, **18**, 1995—2016 (1999).
18) Wang L., Wiebe L. I., Unpublished.
19) Cass C. E., "Drug Transport in Antimicrobial and Anticancer Chemotherapy," ed. by Georgopapadakou N. H., Marcel Deker, New York, 1995, pp. 403—451.
20) Baker R. B., Joseph J. P., Schraub R. E., *J. Am. Chem. Soc.*, **77**, 5905—5910 (1955).
21) Glaudemans C. P. J., Fletcher H. G., Jr., *J. Org. Chem.*, **28**, 3004—3006 (1963).
22) Kumar P., Wiebe L. I., Atrazheva E., Tandon M., *Tetrahedron Lett.*, **42**, 2077—2078 (2001).
23) Kumar P., Wiebe L. I., Unpublished.
24) Cheriyan U. O., Ogilvie K. K., *Nucleosides & Nucleotides*, **1**, 233—237 (1982).
25) Nakajima N., Horita K., Abe R., Yonemitsu O., *Tetrahedron Lett.*, **29**, 4139—4142 (1988).
26) Sugawara T., Ishikura T., Itoh T., Mizuno Y., *Nucleosides & Nucleotides*, **1**, 239—251 (1982).
27) Kumar P., Wiebe L. I., Beiki D., Ohkura K., Seki K., *Tetrahedron Lett.*, **43**, 4427—4429 (2002).
28) Wiley G. A., Rein B. M., Hershkowitz R. L., *Tetrahedron Lett.*, **1964**, 2509—2513 (1964).
29) Silverstein R. M., Bassler G. C., Morrill T. C., "Spectrometric Identification of Organic Compounds," 4th Ed. Pub. John Willey & Sons, New York, 1981, pp. 181—303.
30) Optimized structure was obtained by the AM1 method with MOPAC on CAChe Work system (Release 3.7) on a personal computer and the result was traced by using "CS ChemDraw ProI).
31) Lee H.C., "Novel Radioiodinated IAZA Metabolites for SPECT," Ph. D. thesis. University of Alberta, Edmonton, Canada, 1998, pp. 170—175.