Synthesis of 1-β-D-(5-Deoxy-5-idoarabinofuranosyl)-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-idoarabinofuranosyl)-2-nitroimidazole (IAZA). Radioiodinated IAZA (123I-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-idoarabinofuranosyl)-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-IAZA, 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-idoarabinofuranosyl)-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-Nitroimidazoles (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-idoarabinofuranosyl)-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-idoarabinofuranosyl)-2-aminoimidazole (iodo aminoimidazole arabinoside; IAI); 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 (nitrobenzylthionosine)-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-idoarabinofuranosyl)-2-nitroimidazole (β-IAZA).

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., benzy],21 or by C-2′ inversion of configuration of the corresponding β-ribo nucleoside.22 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),23 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′.25 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
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-(1,1,3,3-tetraisopropyl disiloxiloxy)-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 alkoxytriphosphorphenium intermediate28) (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’-ido-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-butyl diphenylchlorosilane (TBDDS chloride), followed by reaction with acetic anhydride in anhydrous pyridine, to provide 5’-O-TBDDS-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.

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

2-Ni=2-nitroimidazole; i=CF3SO2Cl; ii=NH4OAc; iii=1M NaOH; iv=KF/C6H5COOH, and v=2M NH4MeOH.

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

2-Ni=2-nitroimidazole; i=tosyl chloride/pyridine; ii=Ac2O/pyridine; iii=NaI/2-pentanone; iv=2M NH4MeOH; v=TBDDS chloride/pyridine; vi=Ac2O; vii=KF/benzotriazole acid and viii=CF3SO2Cl/DMAP/CH2Cl2.

**Chart 3. Alternate Synthetic Route to β-IAZA (1)**
Deprotection of acetyl groups afforded the targeted 1 in 96% yield. The confirmation of halogenation of 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-ILAZA), and to δ: 43.16 in 13 (2’3’-di-O-acetyl-β-chloro-ILAZA). These changes in chemical shift are in accordance with the values reported for similar chemicals.

The structural assignment for 1 was made on the basis of its nuclear Overhauser effect (NOE) (Fig. 1), with further support from 1H- and 13C-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 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 β-H aromatic distances obtained from the optimized structure. 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-L-[arabinofuranosyl]-2-aminoimidazole (AILA) and 5’-iodinated-AILA (IAIA), 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) indicates that inversion of configuration at C-2’, from ribose to arabinose, occurred during the synthesis of 4. The 1H-NMR spectrum of 1 showed a larger H-1’-H-2’ coupling constant (J1,2 = 5.2 Hz), that is in contrast to that of ILAZA (ca.0.6 Hz), and supports the β-stereochemistry 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. 1H- and 13C-NMR spectra were recorded on a Bruker AM 300 spectrometer in deuterated chloroform (CDCl3) or methanol (CD3OD), 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-[3,5-O-(1,1,3,3-Tetraisopropylidioxolanyloxy)-2-acetylribofuranosyl]-2-nitroimidazole (5) A methanolic solution (10 ml) of 1-β-D-[arabinofuranosyl]-2-nitroimidazole (IAZA) (22), and supports the

**Fig. 1.** Optimized Structure and the H–H Inter Atomic Distances (Å) (A) and NOE Correlations (B) for 5-β-ILAZA
7.13 (d, $J_{1,2} = 1.2$ Hz, 1H, H-4) and 7.97 (d, $J_{1,2} = 1.2$ Hz, 1H, H-5). $^{13}$C-NMR (CD$_3$OD) 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$^+$).

**Analysis**

For C$_{3}$H$_{6}$N$_{3}$O$_{8}$: C, 43.77; H, 4.59; N, 12.76.

**Conclusion**

The compound was successfully synthesized and characterized by spectroscopic methods.

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**References**


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**Note**

The above text represents a portion of a research paper or article focused on the synthesis and characterization of the compound described. The full text would include additional details, experimental procedures, and discussion of results.
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_{8}H_{10}IN_{3}O_{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
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 Pro/ID.”