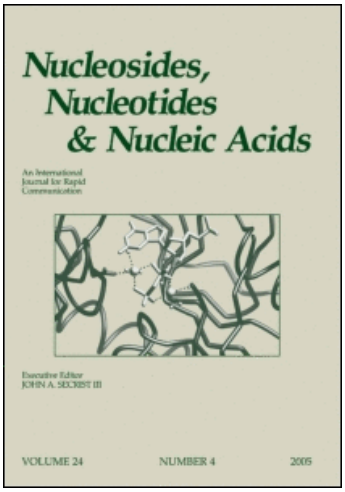

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A One-Pot Synthesis of 1-α- And 1-β-D-Arabinofuranosyl-2-Nitroimidazoles: Synthons to the Markers of Tumor Hypoxia

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A ONE-POT SYNTHESIS OF 1-α- AND 1-β-D-ARABINOFURANOSYL-2-NITROIMIDAZOLES: SYNTHONS TO THE MARKERS OF TUMOR HYPOXIA

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▫ *1-α- and 1-β-D-Arabinofuranosyl-2-nitroimidazole (α-AZA and β-AZ A) are synthons for a number of potential markers of tissue hypoxia. A one pot synthesis in which 2-nitroimidazole is coupled with a mixture of α-and β-1-O-acetyl-2,3,5-tri-O-benzoyl-D-arabinofuranose in the presence of stannic chloride, followed by deprotection using ammonia/methanol, is described. Previously reported conditions for coupling 2-nitroimidazole to 1-α-bromoarabinofuranose protected by base-hydrolyzable groups afforded α-AZA almost exclusively.*

Keywords α-AZA, β-AZA, One Pot Synthesis, Hypoxia

INTRODUCTION

Many human solid tumors are less well oxygenated than normal tissues. This leads to resistance to radiotherapy and anticancer chemotherapy, as well as a predisposition to increased tumor metastases.[1] An important class of compounds studied as radiosensitizers are substituted 2-nitroimidazoles and, of these, a number of iodine-labeled 2-nitroimidazole nucleosides coupled to a radiolabeled sugar group have been shown to undergo reductive trapping in cells with low oxygen pressures.[2] Both α-and β-1-D-(5-deoxy-5-iodoarabinofuranosyl)-2-nitroimidazole

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(α-IAZA; β-IAZA) have been developed as diagnostic radiopharmaceuticals to detect and monitor regional hypoxia in disease.[3–6]

The present study reports the synthesis of β-AZA, the precursor for β-IAZA, by simple coupling of azomycin (2-nitroimidazole) with arabinofuranose. Initial attempts to synthesize β-azomycin arabinofuranoside (β-AZA) by coupling 1-α-bromo-2,3,5-tri-*O*-benzoyl arabinofuranose with 1-trimethylsilyl-2-nitroimidazole resulted in the exclusive formation of α-coupled product,[3,7] as expected according to the *trans* rule, whereby the benzoyl carbonyl group in the 2′-ara configuration facilitates this mechanism.[8]

Intramolecular involvement of the β-plane electrons with electropositive C-1 creates steric hindrance to nucleophilic (2-nitroimidazole) approach from the β-face and results in the exclusive formation of α-azomycin arabinofuranoside (α-AZA). Efforts to avoid this retention of configuration have included replacing the benzoyl protective groups with benzyl-or substituted silyl-(non-carbonylated) groups.[9] An alternative approach to synthesize β-AZA via inversion of configuration at C-2′ of the corresponding β-azomycin riboside has been reported.[6]

To date there have been no reports of a one pot synthesis to provide useful yields of both α-and β-arabinofuranosyl azomycin nucleosides in reasonable yields. We now report a procedure where both α-and β-anomers are synthesized in good yield in a single reaction.

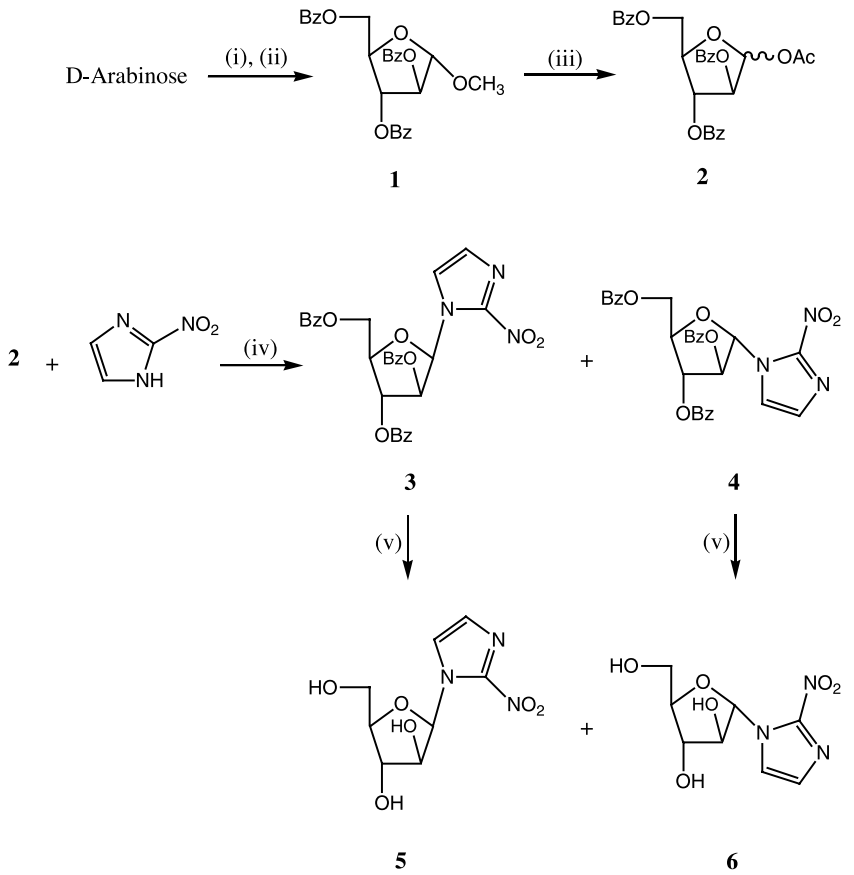
DISCUSSION

Interaction of the protective group at C-2′ hydroxyl plays a significant role in determining the final conformation of the coupled base. The commonly used base-labile (benzoyl and acetyl) protective groups interact electronically with the C-1′ halogen during coupling at C-1′, resulting in exclusive formation of α-AZA,[7] even when azomycin is coupled with the *trans* (α-)arabinofuranosyl bromide. The introduction of alternate, non-carbonyl protecting groups such as benzyl-or substituted silyl-moieties either proceeded sluggishly or these groups were cleaved under the acidic reaction conditions that develop during displacement of the C-1′ halogen. It was observed that nucleosidic bond in azomycin nucleosides cleaves very rapidly under acidic conditions (pH = 4 and below) to generate free sugar and 2-nitroimidazole. The use of HBr gas for bromination generated strong acidic conditions that caused deprotection when tert-butyldiphenylsilyl or benzyl groups were used as protective functions.[10] The fact that catalytic de-benzylation is also capable of reducing the nitro substituent on the imidazole ring further limits the effectiveness of protection by benzylation. *p*-Methoxybenzyl protection of the arabinofuranose hydroxyl groups, which can theoretically be readily removed by DDQ oxidation[11] was also not effective because chlorination or bromination at C-1′ to form the respective arabinose halide led to de-benzylation prior to coupling.

α-AZA (**6**) has previously been obtained by coupling either 1-α-or 1-β-bromo-2,3,5-tri-*O*-benzoylarabinofuranose and 1-trimethysilyl-2-nitroimidazole in the

presence of $Hg(CN)_2$.[3,7,12] β-AZA (**5**) was prepared via an unconventional route starting from 1-β-D-(ribofuranosyl)-2-nitroimidazole (AZR), with a change of configuration at the C-2′-position.[6] Trace amounts of β-AZA were reported as a side-product in the α-AZA synthesis from 1-α-bromo-2,3,5-tri-*O*-benzoylarabinofuranose, but no chemical characterization was provided.[12]

In the present study, both α-AZA and β-AZA were obtained by reacting 2-nitroimidazole with a mixture of α-and β-1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-D-arabinofuranose (**2**) in the presence of stannic chloride, followed by deprotection in the presence of ammonia/methanol (Scheme 1). The acetoxy group located at the anomeric C-1′ does not create as strong a nucleophilic center at this carbon as would bromine, and therefore the inductive effect of the benzoyl carbonyl function at C-2′ is not strong enough to participate in the orientation of the incoming nucleophile (nitroimidazole). Consequently, when the coupling is performed



SCHEME 1 Reagents and conditions: (i) AcCl, MeOH, 0°C→25°C, 3 h; (ii) pyridine, C_6H_5COCl, 0°C→25°C, overnight; (iii) AcOH, Ac_2O, H_2SO_4, 0°C→25°C, 3 h; (iv) CH_3CN, $Hg(CN)_2$, $SnCl_4$, 60°C, 1.5 h; (v) NH_3/MeOH, 0°C (10 h), 25°C (6 h).

starting from the anomeric mixture of 1-*O*-acetyl substituted precursors **2** (the **2**α:**2**β isomeric ratio is 4:1 as observed by their ^{1}H and ^{13}C NMR spectra), the process affords both α-and β-azomycin coupled nucleosides in satisfactory yields.

In theory, SN_1 nucleophilc substitutions occur with the inversion of configuration and, therefore, the expected isomeric ratio of coupled product **4** according to this concept would be 4:1 for **4**β:**4**α, respectively. Nonetheless, the coupling reaction yielded an isomeric preference for α-product **4** (β:α = 2:1). This implies that the *trans* rule, although suppressed due to the weaker electronic effect of the acetyl C-1′ carbonyl, still applies under these conditions. The precise function of stannic chloride remains obscure but the formation of **4**β can be loosely based upon a proposal by Lemieux and Morgan[13] that involves reduced formation of 1,2-benzoxonium ion due to *trans* location of 1-*O*-acetyl and 2-*O*-benzoyl groups in **2**α in comparison to **2**β. Therefore, approach of a possible tautomer of the intermediate imidazole nitronate ester[14] at 1′-β-position progresses, albeit not in the same isomeric proportions. It is also reported that strong Lewis acids such as stannic chloride may play significant role in partial anomerization leading to the mixture of the isomers.[15]

EXPERIMENTAL

α- and β-1-*O*-Acetyl-2,3,5-tri-*O*-benzoyl-D-arabinofuranosides (2). D-arabinose (16 g, 32.8 mmol) upon treatment with acetyl chloride followed by methanol was converted to 1-*O*-methyl arabinofuranose and benzoylated to afford 1-α-*O*-methyl-2,3,5-tri-*O*-benzoyl arabinofuranose **1** in satisfactory yield (∼52%). Upon acetylation using a mixture of glacial acetic acid (169 mL) and acetic anhydride (34 mL), and conc. H_2SO_4 (10 mL) as a Lewis acid, at 0°C, **1** was converted to a mixture of α-and β-1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-D-arabinofuranoses, **2**. After completion of the reaction (3 h at 22°C) the mixture was poured into ice water (300 mL) and extracted with dichloromethane (2 × 300 mL). The extracts were combined, washed successively with saturated aqueous sodium bicarbonate (2 × 100 mL) and water (2 × 100 mL), dried (anhydrous sodium sulfate), filtered and evaporated under reduced pressure to give an isomeric mixture of **2** as a white foam (16.9 g, 99.76%). The isomeric ratio for **2**-α:**2**-β at this stage was found to be 79:21. The ^{1}H and ^{13}C NMR spectra of the **2**-α-and **2**-β-anomers (1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-D-arabinofuranoses) confirmed their chemical identities.[7,12,16]

1-β- and 1-α-D-(2,3,5-Tri-*O*-benzoylarabinofuranosyl)-2-nitroimidazole (3 and **4,** respectively**).** $SnCl_4$ (0.78 mL, 6.21 mmol) was added to a solution of α-and β-1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-D-arabinofuranosides, **2,** in dry acetonitrile (25 mL) in the presence of 2-nitroimidazole (0.42 g, 3.72 mmol) and mercuric cyanide (1.55 g, 6.21 mmol) under argon. After stirring at 60°C for 90 min, the dark reaction solution was cooled and the solvent was removed. The impure mixture was redissolved in dichloromethane (300 mL), filtered, and the filtrate was

washed successively with solutions of saturated aqueous sodium bicarbonate (2 × 100 mL), 40% KI (100 mL), and H_2O (2 × 100 mL). The organic phase was dried (anhydrous sodium sulfate) and filtered. Evaporation of the solvent followed by flash column chromatography on silica gel using benzene–ethyl acetate (10:1, v/v) as eluent afforded **3** (0.74 g, 43%) and **4** (0.35 g, 20%) as white foams, which were crystallized from hexanes-ethyl acetate (2:1, v/v, 20 mL). **3**: mp = 123–124°C, ^{1}H NMR ($CDCl_3$) δ 7.08–8.13 (m, 17 H, H-4, 5, and Ar-*H*), 7.06 (d, *J* = 4.27 Hz, 1 H, H-1′), 6.13 (d, *J* = 4.27 Hz, 1 H, H-2′), 5.67 (s, 1 H, H-3′), 4.98 (dd, *J* = 11.9, 6.41 Hz, 1 H, H-5′a), 4.86 (dd, *J* = 11.9, 3.66 Hz, 1 H, H-5′b), 4.69 (m, 1 H, H-4′). ^{13}C NMR ($CDCl_3$) δ 166.13, 165.23, 164.09, 144.10, 154.76, 134.5, 133.88, 133.41, 129.96, 129.72, 129.60, 129.29, 128.70, 128.61, 128.49, 128.40, 128.27, 127.65, 123.37, 88.42, 82.03, 75.39, 63.06. Anal. calcd. for $C_{29}H_{23}N_3O_9$: C, 62.48; H, 4.16; N, 7.54. Found: C, 61.94; H, 4.04; N, 7.31. The melting point, ^{1}H and ^{13}C NMR spectra for **4** were identical to those reported previously.[12]

1-β- and α-D-Arabinofuranosyl-2-nitroimidazole (*5* and *6*, respectively). To **3** (0.04 g, 0.72 mmol) or **4** (0.03 g, 0.54 mmol) was added a solution of NH_3 in CH_3OH (4 mL of 2M solution) at 0°C under argon and the resulting solution was stirred 10 h at 0°C and 6 h at room temperature. The solvent was then removed under vacuum (rotovap). Purification of the residue via flash column chromatography on silica gel using 15% CH_3OH in CH_2Cl_2 gave **5** (0.16 g, 91%) or **6** (0.12 g, 94%) as white solids, which were recrystallized from ethyl acetate-hexane (1:1, v/v, 5 mL). The melting points, ^{1}H and ^{13}C NMR spectra for **5** and **6** were identical to those reported previously.[6,7]

ABBREVIATIONS

AZA	1-D-arabinofuranosyl-2-nitroimidazole
IAZA	1-D-(5-deoxy-5-iodoarabinofuranosyl)-2-nitroimidazole
DDQ	2,3-Dichloro-5,6-dicyano-1,4-benzoquinone
AZR	1-β-D-(ribofuranosyl)-2-nitroimidazole
$Hg(CN)_2$	mercuric cyanide

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