



# Synthesis of $\beta$ -azomycin nucleosides: 1-( $\beta$ -D-2-iodo-2-deoxyarabinofuranosyl)-2-nitroimidazole ( $\beta$ -2-IAZA), a novel marker of tissue hypoxia

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**Abstract**—This study describes a non-conventional approach to the synthesis of 1- $\beta$ -D-[2-deoxy-2-iodoarabinofuranosyl]-2-nitroimidazole ( $\beta$ -2-IAZA), a positional and configurational isomer of 1- $\alpha$ -D-[5-deoxy-5-iodoarabinofuranosyl]-2-nitroimidazole (IAZA). [<sup>123</sup>I]IAZA, a radiopharmaceutical used clinically to image regional tissue hypoxia in a number of pathologies, is synthesized by coupling the appropriately protected 1- $\alpha$ -halo arabinofuranoside, with azomycin, with retention of configuration. To circumvent participation of the C-2' protecting group, which prevents  $\beta$ -anomer formation during coupling, the riboside 1- $\beta$ -D-(ribofuranosyl)-2-nitroimidazole (AZR) was elaborated to 1- $\beta$ -D-(3,5-tetraisopropylidisilyloxy-2-*O*-trifluoromethanesulfonylribofuranosyl)-2-nitroimidazole. Nucleophilic displacement of the 2-*O*-trifluoromethanesulfonyl leaving group by iodide results in inversion of configuration at the ribosyl C-2-position, thereby affording silylated arabinofuranosyl-2'-IAZA, which was desilylated under neutral conditions to afford  $\beta$ -2-IAZA. © 2002 Elsevier Science Ltd. All rights reserved.

The effectiveness of conventional low linear energy transfer (LET) radiation (e.g. X-rays) therapies is seriously reduced by hypoxia in tumor tissue.<sup>1</sup> Appropriate compensation for the presence of hypoxic tissue is considered to be important in improving therapeutic outcomes.<sup>2–4</sup> Assessment of tumor hypoxia has been achieved by non-invasive imaging using [<sup>123</sup>I]iodoazomycin arabinoside ([<sup>123</sup>I]IAZA) and other nitroimidazole-based radiosensitizers which, due to their unique reduction potential, are selectively trapped in hypoxic cells.<sup>5–8</sup> Hypoxia-selective uptake of [<sup>123</sup>I]IAZA has been demonstrated in a number of patients with varying pathological disorders including cancer,<sup>9,10</sup> diabetes,<sup>11</sup> arthritis<sup>12</sup> and peripheral vascular disease,<sup>13</sup> making it the most widely studied hypoxia marker in clinical research.

Iodine in IAZA is covalently bound to a primary carbon atom. Primary iodides are much more susceptible to deiodination than secondary iodides. In pre-clinical and clinical studies, [<sup>123</sup>I]IAZA undergoes modest deiodination, which unnecessarily increases the radiation dose to the thyroid gland and also contributes to undesired background radioactivity in later-time images.<sup>13</sup> It is also known that IAZA, an  $\alpha$ -arabinofuranosyl nucleoside, is not transported by cellular nucleoside transporters,<sup>14</sup> whereas many  $\beta$ -arabinosyl nucleosides are efficiently internalized by these transporters.<sup>15</sup>

The present study reports the synthesis of a novel iodoazomycin nucleoside, which is designed to be chemically more stable than IAZA and to be a substrate for cellular *trans*-membrane transport. Initial attempts to synthesize  $\beta$ -analogs of IAZA, by coupling 1- $\alpha$ -bromo-2,3,5-tri-*O*-benzoyl arabinofuranose with 1-trimethylsilyl-2-nitroimidazole, resulted in the exclusive formation of  $\alpha$ -coupled product.<sup>16</sup> The formation of  $\alpha$ -isomer is attributed to the electronic involvement of carbonyl function of the benzoyl/acetyl protecting group located at the arabinosyl C-2- $\beta$  position, with bromine located at the arabinosyl C-1- $\alpha$ -position.<sup>17</sup> Intramolecular interaction of the benzoyl carbonyl oxygen with the electropositive C-1 generates stereochemi-

**Abbreviations:**  $\beta$ -2-IAZA, 1- $\beta$ -D-[2-deoxy-2-iodoarabinofuranosyl]-2-nitroimidazole; IAZA, 1- $\alpha$ -D-[5-deoxy-5-iodoarabinofuranosyl]-2-nitroimidazole; AZR, 1- $\beta$ -D-ribofuranosyl-2-nitroimidazole;  $\alpha$ -IAZP, 1- $\alpha$ -D-[4-deoxy-4-iodoxylopyranosyl]-2-nitroimidazole; NOE, nuclear Overhauser effect.

**Keywords:**  $\beta$ -2-IAZA; IAZA; iodoazomycin arabinoside; selective iodination; hypoxia markers.

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cal hindrance for a nucleophilic (2-nitroimidazole) approach from the  $\beta$ -plane, and consequently results in the exclusive formation of  $\alpha$ -azomycin arabinofuranoside.<sup>18</sup> The introduction of alternative protecting groups such as benzyl<sup>19</sup> or substituted silyl (non-carbonylated) groups proceeded sluggishly, and these groups were subject to cleavage during bromination under acidic reaction conditions,<sup>20</sup> and the removal of the benzyl ether protecting groups by catalytic reduction also reduces the nitro substituent on the imidazole ring. It was therefore decided to proceed via the synthesis of AZR,<sup>21</sup> **1**, and to invert the configuration, from ribose to arabinose, during iodination. The synthesis and complete characterization of  $\beta$ -2-IAZA are now reported (Scheme 1).

1- $\beta$ -D-Ribofuranosyl-2-nitroimidazole (AZR) **1** (2.2 g, 8.97 mmol), on treatment with 1,1,3,3-tetraisopropyl-disiloxane (3.35 mL, 10.47 mmol) in anhydrous pyridine (30 mL) at 22°C for 16 h, was converted to the corresponding tetraisopropylsilyloxyribofuranoside **2** in 78% yield (3.4 g). Compound **2** (3.04 g, 6.23 mmol) was treated with trifluoromethanesulfonyl chloride (1.26 mL, 11.84 mmol) and 4-*N,N*-dimethylaminopyridine (2.286 g, 18.71 mmol) in anhydrous  $\text{CH}_2\text{Cl}_2$  (90 mL) for 1.5 h at 0°C to afford corresponding triflate **3** in 88% (3.4 g) yield. Iodination of **3** (63.3 mg, 0.1 mmol) in anhydrous 2-pentanone (12 mL) under reflux at 110°C for 1.5 h gave the corresponding tetraisopropylsilyloxy-2-deoxy-2-iodoarabinofuranoside **4** in 95% yield (58 mg). Deprotection of protective groups in **4** (0.439 g, 0.825 mmol) using ammonium fluoride (87.2 mg, 2.3 mmol) in methanol (40 mL) under reflux for 2.5 h afforded intended  $\beta$ -2-IAZA **5** (0.213 g, 73%).<sup>22</sup>

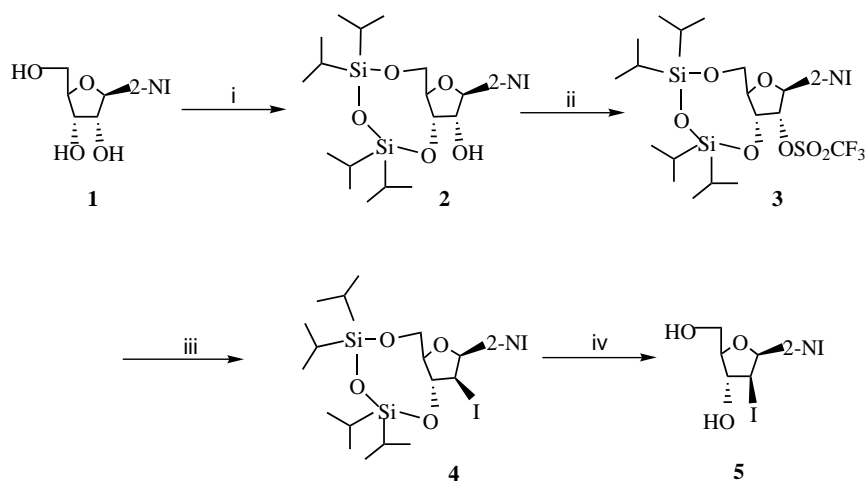
The stereochemistry of **5** was determined on the basis of nuclear Overhauser effect (NOE) experiments. Irradiation at C-5 imidazole proton affected C-4 imidazole H (6.9%), C-3' H of the arabinofuranosyl moiety (6.8%)

and 5'- $\text{CH}_2$  (1.8%), and significantly, also had minimal impact on C-1'-H (0.7%) of the sugar moiety. This confirms the location of iodine insertion in the arabinose configuration at the C-2 position of the sugar moiety, and the  $\beta$ -configuration of the nitroimidazole moiety in relation to the plane of arabinofuranosyl framework. Additional NOE effects were observed for arabinofuranosyl protons at C-2' (14.8%), C-4' (4%), C-5 (2.6%) when the C-1'-H was irradiated. The irradiation at C-2'-H enhanced the signals for C-1'-H (16.9%), C-3'-H (5.3%) and C-4'-H (3.2%), while related enhancements were observed for C-2'-H (1.9%), C-4'-H (1.9%), 5'- $\text{CH}_2$  (2.5%) and C-5-H (6.8%) when C-3'-H was irradiated.

In conclusion,  $\beta$ -2-IAZA, **5**, has been synthesized by nucleophilic substitution at C-2' of the riboside **2**, with inversion of configuration to form the corresponding arabinoside. This synthesis is not possible by coupling the tri-*O*-benzoylated arabinofuranosyl bromide with 2-nitroimidazole because of the intramolecular participation of these protective groups (during bromination) with the substituent at C-1'.

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**Scheme 1.** Synthesis of  $\beta$ -2-IAZA from AZA. *Reagents and conditions:* (i) 1,1,3,3-Tetraisopropylsilyloxy 1,3-dichloride/pyridine; (ii) trifluoromethanesulfonyl chloride/DMAP; (iii) NaI/2-pentanone, 110°C; (iv)  $\text{NH}_4\text{F}/\text{MeOH}$  and 2-NI = 2-nitroimidazole.

## References

- Brown, J. M. *Cancer Res.* **1999**, *59*, 5863–5870.
- Gray, L. H.; Conger, A. D.; Ebert, M.; Hornsey, S.; Scott, O. C. *Br. J. Radiol.* **1953**, *26*, 638.
- Crabtree, H. G.; Cramer, W. *Proc. R. Soc. Ser. B* **1933**, *113*, 238–250.
- Stone, H. B.; Brown, M. J.; Philips, T. L.; Sutherland, R. M. *Radiat. Res.* **1993**, *136*, 422–434.
- Heimbrook, D. C.; Sartorelli, A. C. *Molec. Pharmacol.* **1986**, *29*, 168.
- Grosher, 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.* **1993**, *34*, 885.
- Mannan, R. H.; Somayaji, V. V.; Lee, J.; Mercer, J. R.; Chapman, J. D.; Wiebe, L. I. *J. Nucl. Med.* **1991**, *32*, 1764–1770.
- Mannan, R. H.; Mercer, J. R.; Wiebe, L. I.; Kumar, P.; Somayaji, V. V.; Chapman, J. D. *J. Nucl. Biol. Med.* **1992**, *36*, 60–67.
- Parliament, M. B.; Chapman, J. D.; Urtasun, R. C.; McEwan, A. J.; Goldberg, L.; Mercer, J. R.; Mannan, R. H.; Wiebe, L. I. *Br. J. Cancer* **1991**, *65*, 90–95.
- 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* **1996**, *74*, S209–S212.
- Al-Arafaj, A.; Ryan, E. A.; Hutchinson, K.; Mannan, R. H.; Mercer, J. R.; Wiebe, L. I.; McEwan, A. J. B. *Eur. J. Nucl. Med.* **1994**, *21*, 1338–1342.
- McEwan, A. J. B.; Skeith, K. J.; Mannan, R. H.; Davies, N.; Jamali, F.; Schmidt, R.; Golberg, K.; Wiebe, L. I. *J. Nucl. Med.* **1997**, *38*, 300 abstract.
- Vinjamuri, S.; O'Driscoll, K.; Maltby, P.; McEwan, A. J.; Wiebe, L. I.; Critchley, M. *Clin. Nucl. Med.* **1999**, *24*, 891–892.
- Wang, L.; Wiebe, L. I., unpublished.
- Xu, L. M. Sc. Thesis, University of Alberta, 1988.
- Kumar, P.; Wiebe, L. I.; Atrazheva, E.; Tandon, M. *Tetrahedron Lett.* **2001**, *42*, 2077–2078.
- Lee, H. C.; Kumar, P.; Wiebe, L. I.; McDonald, R.; Mercer, J. R.; Ohkura, K.; Seki, K. *Nucleosides Nucleotides* **1999**, *18*, 1995–2016.
- Reist, E. J.; Benitez, A.; Goodman, L.; Baker, B. R.; Lee, W. W. *J. Org. Chem.* **1962**, *27*, 3274–3279.
- Cheriyian, U. O.; Ogilvie, K. K. *Nucleosides Nucleotides* **1982**, *1*, 233–237.
- Kumar, P., unpublished data.
- Jette, D. C.; Wiebe, L. I.; Flanagan, R. J.; Lee, J.; Chapman, J. D. *Radiation Res.* **1986**, *105*, 169–179.
- All new compounds gave satisfactory  $^1\text{H}$  NMR (300 MHz) spectroscopic and elemental analytical data. Selected data: **2**: 1- $\beta$ -D-(3,5-*O*-Tetraisopropylidisilyloxy-ribofuranosyl)-2-nitroimidazole: mp 59–61°C.  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  0.84–1.02 (m, 28H, 4 $\times$ isopropyl H), 2.45 (br,  $\text{D}_2\text{O}$  exchangeable, 1H, OH), 4.05 (dd, 1H,  $J_{4',5'}=2.7$  Hz,  $J_{\text{gem}}=13.4$  Hz, H-5'), 4.19 (d,  $J_{3',2'}=4.3$  Hz, 1H, H-2'), 4.25 (dd, 1H,  $J_{3',4'}=9.2$  Hz,  $J_{5',4'}=2.8$  Hz, H-4'), 4.32 (d, 1H,  $J_{\text{gem}}=13.4$  Hz, H-5'), 4.43 (dd, 1H,  $J_{2',3'}=4.3$  Hz,  $J_{4',3'}=9.3$  Hz, H-3'), 6.3 (s, 1H, H-1'), 7.09 (d,  $J_{5,4}=1.1$  Hz, 1H, H-4) and 7.74 (d,  $J_{4,5}=1.1$  Hz, 1H, H-5);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  12.60–13.46 (4 isopropyl CH), 16.87–17.50 (8 isopropyl  $\text{CH}_3$ ), 59.86 (C-5'), 68.19 (C-4'), 76.97 (C-3'), 82.28 (C-2'), 92.33 (C-1'), 122.62 (C-5), 128.39 (C-4), 144.5 (C-2) ppm; Chem. Anal. Calcd: C, 49.256; H, 7.646; N, 8.616; found: C, 49.634; H, 7.39; N, 8.484%. **3**: 1- $\beta$ -D-(3,5-*O*-Tetraisopropylidisilyloxy - 2 - *O* - trifluoromethanesulfonylribofuranosyl)-2-nitroimidazole: mp 50–52°C.  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  0.88–1.05 (m, 28H, 4 $\times$ isopropyl H), 3.99 (dd, 1H,  $J_{4',5'}=2.4$  Hz,  $J_{\text{gem}}=13.7$  Hz, H-5'), 4.17 (dd, 1H,  $J_{3',4'}=9.5$  Hz,  $J_{5',4'}=2.4$  Hz, H-4'), 4.28 (d, 1H,  $J_{\text{gem}}=13.7$  Hz, H-5'), 4.53 (dd, 1H,  $J_{2',3'}=3.8$  Hz,  $J_{4',3'}=9.5$  Hz, H-3'), 5.20 (d,  $J_{3',2'}=3.8$  Hz, 1H, H-2'), 6.48 (s, 1H, H-1'), 7.13 (d,  $J_{5,4}=0.9$  Hz, 1H, H-4) and 7.81 (d,  $J_{4,5}=0.9$  Hz, 1H, H-5);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  12.87–13.40 (4 isopropyl CH), 16.56–17.50 (8 isopropyl  $\text{CH}_3$ ), 59.02 (C-5'), 66.60 (C-4'), 82.30 (C-3'), 88.27 (C-2'), 89.58 (C-1'), 118.43 ( $\text{CF}_3$ ), 122.42 (C-5), 128.83 (C-4), 144.5 (C-2) ppm; Chem. Anal. Calcd: C, 40.698; H, 5.855; N, 6.780; Found: C, 40.939; H, 6.235; N, 7.172. **4**: 1- $\beta$ -D-(3,5-*O*-Tetraisopropylidisilyloxy-2-deoxy-2-iodoarabinofuranosyl)-2-nitroimidazole: mp 136–138°C.  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  0.96–1.06 (m, 28H, 4 $\times$ isopropyl H), 3.74 (ddd, 1H,  $J_{3',4'}=8.5$  Hz,  $J_{5',4'}=2.8$  Hz,  $J_{5'',4'}=1.8$  Hz, H-4'), 4.10 (dd, 1H,  $J_{4',5'}=2.8$  Hz,  $J_{\text{gem}}=13.4$  Hz, H-5'), 4.12 (d, 1H,  $J_{\text{gem}}=13.4$  Hz,  $J_{4',5''}=1.8$  Hz, H-5'), 4.53 (dd, 1H,  $J_{2',3'}=9.2$  Hz,  $J_{4',3'}=8.5$  Hz, H-3'), 4.65 (dd,  $J_{3',2'}=9.2$  Hz,  $J_{1',2'}=6.7$  Hz, 1H, H-2'), 6.68 (d,  $J_{2',1'}=6.7$  Hz 1H, H-1'), 7.09 (s, 1H, H-4) and 7.63 (s, 1H, H-5);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  12.49–13.99 (4 isopropyl CH), 16.94–17.31 (8 isopropyl  $\text{CH}_3$ ), 30.27 (C-2'), 60.14 (C-5'), 76.03 (C-4'), 84.04 (C-3'), 86.89 (C-1'), 121.72 (C-5), 128.22 (C-4), 145.5 (C-2) ppm; Chem. Anal. Calcd: C, 40.198; H, 6.072; N, 7.031; found: C, 40.480; H, 6.900; N, 7.170. **5**: 1- $\beta$ -D-[2-Deoxy-2-iodoarabinofuranosyl]-2-nitroimidazole: mp 63–65°C.  $^1\text{H}$  NMR ( $\text{CD}_3\text{OD}$ ): 3.83–3.89 (m, 2H,  $J_{3',4'}=7.3$  Hz,  $J_{5',4'}=3.5$  Hz,  $J_{5'',4'}=3.8$  Hz,  $J_{\text{gem}}=13.2$  Hz, H-4' and H-5'), 3.97 (dd, 1H,  $J_{4',5''}=3.8$  Hz,  $J_{\text{gem}}=13.2$  Hz, H-5), 4.52 (dd, 1H,  $J_{2',3'}=J_{4',3'}=7.3$  Hz, H-3'), 4.78 (dd,  $J_{3',2'}=7.3$  Hz,  $J_{1',2'}=6.4$  Hz, 1H, H-2'), 6.69 (d,  $J_{2',1'}=6.4$  Hz 1H, H-1'), 7.17 (d, 1H,  $J_{5,4}=1.2$  Hz, H-4) and 8.10 (d, 1H,  $J_{4,5}=1.2$  Hz, H-5);  $^{13}\text{C}$  NMR ( $\text{CD}_3\text{OD}$ ):  $\delta$  30.39 (C-2'), 60.07 (C-5'), 78.00 (C-4'), 81.37 (C-3'), 84.44 (C-1'), 112.76 (C-5), 126.56 (C-4), 145.20 (C-2) ppm; LR (ESI) MS  $m/z$ : 378 ( $M^+$ +Na), HR (ESI) MS ( $M^+$ +Na,  $\text{C}_8\text{H}_{10}\text{O}_5\text{N}_3\text{INa}$ ): Calc. mass 377.95629, exact mass 377.95574.