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Research Article

Exchange radioiodination produces inversion at C-4 of 1-(4-deoxy-4-iodo-β-D-xylopyranosyl)-2-nitroimidazole

R.F. Schneider[1], W.A. Price[2], and J.D. Chapman*[,1]
[1] *Department of Radiation Oncology, Tumor Biology and Biophysics Section, Fox Chase Cancer Center, 7701 Burholme Avenue, Philadelphia, PA 19111, USA*
[2] *Department of Chemistry and Biochemistry, La Salle University, 1900 West Olney Avenue, Philadelphia, PA 19141,USA*

Summary

The title compound, **3a**, when exchange labeled with ^{125}I results in three new labeled products. The major labeled product (84.1%) is 1-(4-deoxy-4-iodo-β-L-arabinopyranosyl)-2-nitroimidazole, **3b**, that could result from inversion of configuration at C-4. Exchange labeling carried out under conditions of kinetic control yielded dramatically different product ratios than thermodynamic equilibrium reactions. Confirmation of these results was established by extensive ^{1}H NMR spectral analyses. A possible mechanism is presented.

Key Words: radioiodination; exchange radiolabeling; inversion; pyranosides; ^{1}H NMR spectroscopy; iodine-125

Introduction

In a previous study reported in this journal,[1] we described the preparation of various radioiodinated azomycin substituted pyranosides

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*Correspondence to: J. D. Chapman, Department of Radiation Oncology, Fox Chase Cancer Center, 7701 Burholme avenue, Philadelphia, PA 19111, USA. E-mail: jd_chapman@fccc.edu

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and furanosides and their evaluation as possible markers of tumor hypoxia. Those data showed that when 4-iodinated azomycin pentose pyranosides were exchange labeled with ^{125}I, more than one product was obtained. We have since identified some of these products and have examined the factors that determine their formation. This information should be valuable to others working in the field.

Results and discussion

While the focus of this paper is centered on understanding the mechanisms for the appearance of multiple products arising from the exchange radioiodination of **3a**, there were sufficient problems in the synthesis of the iodinated sugars used in this study to warrant special discussion. For example, we were unable to prepare **3b** via the triphenylphosphine route shown in Figure 1.

The synthesis of **3c** was included in an effort to gain some information about the effect of precursor geometry, **2c**, on the course of the subsequent iodination reaction. The syntheses of **3a** and **3c** and the attempted synthesis of the 4-iodo epimer of **3a**, compound **3b**, are shown in Figure 1. The preparation of **3a** was carried out as previously reported.[1] We attempted to synthesize **3b** via a route identical to that used for the preparation of **3a**. However, using the standard iodine–triphenylphosphine–pyridine system, we could not form the epimeric 4-iodoarabinoside, **3b**. This compound was later isolated, purified and characterized as a product of NaI exchange with **3a**. The C-3 epimer of **3b**, 1-(4-deoxy-4-iodo-β-L-lyxopyranosyl)-2-nitroimidazole, **3c**, can be prepared by the triphenylphosphine method, although in low yield.[1] As shown in Figure 1, the only difference between the precursor leading to **3b** and that leading to **3a** or **3c** is in the geometry of the hydroxyl groups on C-3 and C-4. In the former, both the C-3 and C-4 hydroxyl groups are equatorial, while in the latter, one is axial and the other equatorial. The initial step of the iodination of **2a** involves the formation of an oxytriphenylphosphonium intermediate with the equatorial C-4 hydroxyl group, followed by its rate-determining backside displacement by attack of iodide at C-4 to yield **3a**.[2,3] It appears that the differences in precursor geometry have a profound effect upon the course of the iodination reaction, and further study of this problem is warranted.

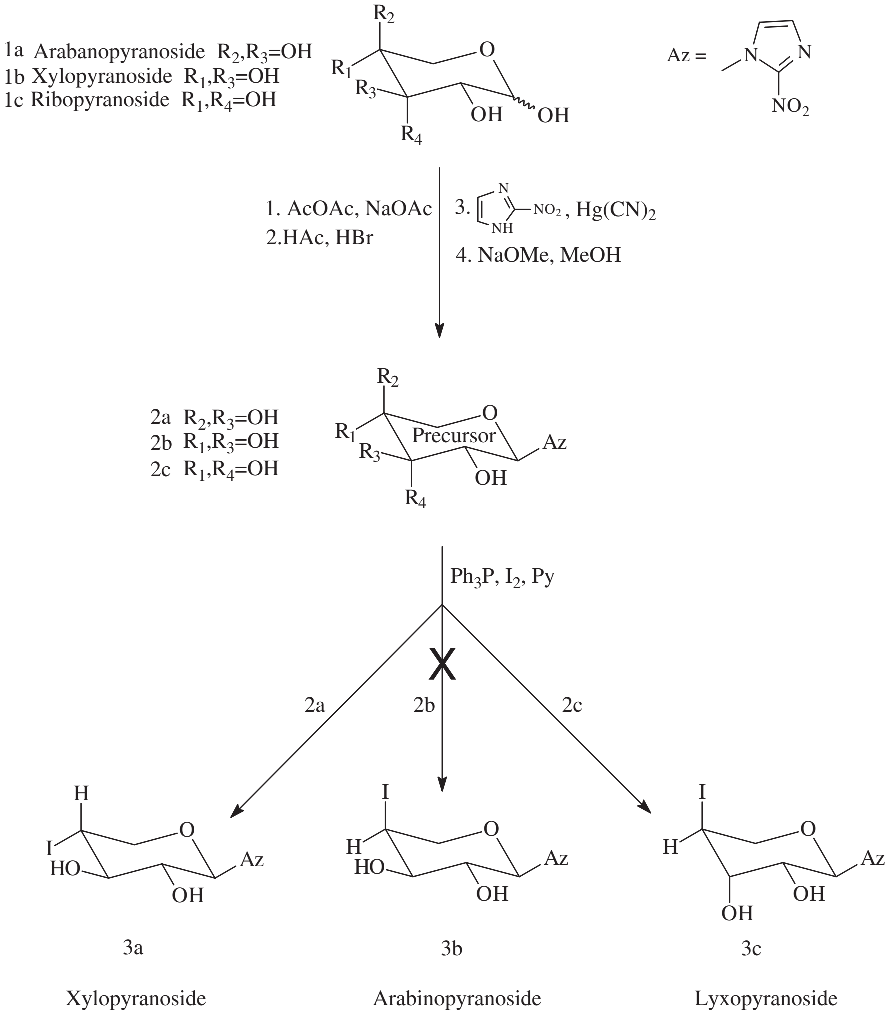


Figure 1. Synthetic routes to 3a, 3b, 3c

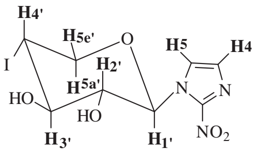
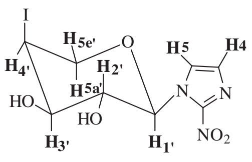
The data presented in Table 1 show the results of the HPLC analysis of the products obtained when **3a** was exchange radiolabeled with ^{125}I. The total amount of radiolabeled compounds represents only a small fraction (approximately 10^{-4}) of the material. In an effort to obtain amounts of material sufficient for structural analysis, a large-scale exchange reaction was carried out with equimolar amounts of **3a** and sodium iodide. A complimentary reaction involving the exchange of **3b** with NaI was also studied, and the results of these experiments are also shown in Table 1. After 23 h, the ratio of products, **3a** versus **3b**, is

Table 1. Reaction products from the exchange radioiodination of compound 3a, and the non-radioactive exchange iodination of substrates 3a or 3b

Expt	Reaction	Time (h)	3a (%)[a]	3b (%)[b]	4 (%)[c]
1.	Radioiodination[d,e] [**3a** > > > > [$^{125}I^-$]	8	2.3	84.1	13.6
2.	Starting with **3a** [**3a**] = [I^-]	2	91.2	8.8	—
	Starting with **3a** [**3a**] = [I^-]	4	87.7	12.3	—
	Starting with **3a** [**3a**] = [I^-]	7	82.7	17.3	—
	Starting with **3a** [**3a**] = [I^-]	23	82.2	17.8	Trace
3.	Starting with **3b**[e] [**3a**] = [I^-]	23	81.9	17.1	Trace

[a] Xylopyranoside (HPLC retention time = 52.2 min).
[b] Arabinopyranoside (RT = 26.5).
[c] Unknown (3-iodolyxopyranoside ?, RT = 45.6).
[d] Only about 0.32 nmol out of ~2.8 μmol of **3a** becomes radioiodinated. Approximately 70% of the applied radioactivity was recovered in labeled products.
[e] Average of two runs.

Table 2. 600 MHz, ^{1}H NMR analysis of 3a and 3b

	
3a	3b
δ 7.69(d, 1H, J=1.1)$\mathbf{H_5}$	δ 7.66(d, 1H, J=1.1)$\mathbf{H_5}$
δ 7.17(d, 1H, J=1.1)$\mathbf{H_4}$	δ 7.21(d, 1H, J=1.1)$\mathbf{H_4}$
δ 6.12(d, 1H, J=9.0)$\mathbf{H_{1'}}$	δ 6.12(d, 1H, J=8.8)$\mathbf{H_{1'}}$
δ 4.25(dd, 1H, ^{2}J=11.6, J=4.8)$\mathbf{H_{5e'}}$	δ 4.56(m, 1H)$\mathbf{H_{4'}}$
δ 4.03(ddd, 1H, J=11.7, J=10.2,J=4.8)$\mathbf{H_{4'}}$	δ 4.24(dd, 1H, ^{2}J=13.5, J=1.5)$\mathbf{H_{5e'}}$
δ 3.95(dd, 1H, J=11.7, 2J=11.6)$\mathbf{H_{5a'}}$	δ 4.13(dd, 1H, ^{2}J=13.5, J=1.5)$\mathbf{H_{5a'}}$
δ 3.68(dd, 1H, J=10.2, J=8.8)$\mathbf{H_{3'}}$	δ 3.80(dd, 1H, J=8.8, J=8.8)$\mathbf{H_{2'}}$
δ 3.55(dd, 1H, J=8.8, J=8.8)$\mathbf{H_{2'}}$	δ 2.90(dd, 1H, J=8.8, J=4.0)$\mathbf{H_{3'}}$

essentially the same in both reactions. The two major products arising from these studies were separated by preparative HPLC.

Proton nuclear magnetic resonance spectroscopic (^{1}H NMR) analysis identified the component with a retention time (RT) = 52.2 min as the starting material, **3a**, and that at 26.5 min as its C-4 iodo epimer, 1-(4-deoxy-4-iodo-*β*-L-arabinopyranosyl)-2-nitroimidazole, **3b**.

A summary of the ^{1}H NMR data leading to these assignments is presented in Table 2. A key feature in the ^{1}H NMR analysis of **3b** can be found at δ4.13 ($H_{5e'}$) and δ4.24 ($H_{5a'}$). Both $H_{5e'}$ and $H_{5a'}$ couple identically to the equatorial $H_{4'}$ due to the very similar dihedral angles. The ^{1}H NMR spectrum for **3a**, however, clearly shows strong coupling (11.7 Hz) between $H_{5a'}$ and $H_{4'}$, whereas $J_{5e'-4'} = 4.8$ Hz. The strong coupling between $H_{1'}$ and $H_{2'}$ in both **3a** and **3b** (9.0 and 8.8 Hz) establishes these protons in a diaxial orientation. Peak assignments were unequivocally verified using correlated spectroscopy (^{1}H,^{1}H COSY, double quantum filtered, 600 MHz).

The conversion of **3a** to a mixture of **3a**/**3b** in the presence of NaI was also studied as a function of time. The data are also presented in Table 1. The reaction appears to reach equilibrium in about 7 h. Also inferred from the data in Table 1 is that **3a** is more stable than its epimer, **3b**, since it is the major equilibration product. It had been reported that the radioiodination of **3a** gave the corresponding radiolabeled version of **3a**, with no inversion at C-4.[4] Unfortunately, the conditions reported for that ‘iodine exchange reaction’ were not detailed enough to allow a direct comparison with our data. Table 1 indicates that the radioiodination of **3a** by standard procedures produces only 2.3% of radiolabeled **3a**, with the majority (84.1%) of the radiolabeled material being a new component, **3b**. A third component, **4** (13.6%), was also detected.

The production of **3b** from **3a** can be explained by an S_N2-type reaction involving the backside attack of iodide at C-4. This is illustrated in Figure 2. In this case iodide would also be the leaving

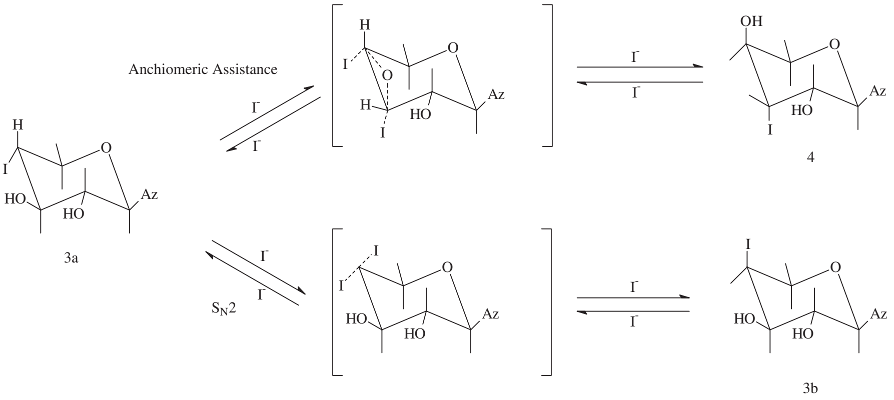


Figure 2. Proposed mechanism for iodine exchange reactions

group. The resulting Walden inversion generates the C-4 iodo epimer of **3a**, the arabinoside **3b**.[5] In the case where the leaving group is identical to the attacking nucleophile, the ratio of the products formed is determined by their relative stability in the ground state, which leads to the conclusion that **3a** is more stable than **3b**.[5] Comparison of the data presented in Table 1 reveals that in the radiolabeling procedure, when there is a 10^4-fold excess of **3a** over radioiodide, radioiodinated **3b** is the major product. In the non-radioactive exchange reaction where equimolar amounts of **3a** and iodide are reacted, **3a** is the major product.

In the radiolabeling procedure that was performed under non-equilibrating conditions, the product of kinetic control, radioiodinated **3b**, predominates. The reaction involving equivalent amounts of **3a** and iodide results in an equilibrium being established and represents thermodynamic control of the product. A significant amount of product **4** resulted only from the radiolabeling procedure carried out under kinetic control. This is a classic example of product distribution being determined by conditions of kinetic versus thermodynamic control.[6] In Figure 2, we present a possible structure for **4**, as well as a mechanism for its formation. The synthetic procedures described in this paper did not yield amounts of **4** sufficient for its definitive characterization.

Experimental

Chemicals and reagents were purchased from Aldrich Chemical Co., Milwaukee, Wisconsin or Sigma Chemical Co., St Louis, Missouri and used as received. Preparative flash chromatography was carried out using E. Merck silica gel 60 (200–400 mesh). TLC was performed on E. Merck silica gel 60 fluorescent plates using a $CHCl_3$/MeOH (77.5:22.5) elution system. ^{125}I (NaI, 1 mCi, carrier free, nominally 2180 Ci/mmol) was obtained in a pH 8.0–9.0 solution from NEN, Boston, MA. Melting points were determined with a Laboratory Devices Mel-Temp II and are uncorrected. Infrared spectra were obtained using KBr pellets with a Nicolet 205 FTIR spectrometer. HPLC analyses were carried out on a Beckman System Gold equipped with a 3.9 × 300 mm Millipore Delta Pak 15 μm C-18 column and a BioScan Flow-Count γ detection system. The solvent system consisted of methanol/water (25:75) at 0.7 ml/min. The uv/vis detector was set at 310 nm. Proton NMR and $^1H,^1H$ correlated spectroscopic studies (COSY) were carried out on a Bruker

600 DMX spectrometer using $CDCl_3$ (99.8% deuterated) as solvent and internal standard ($\delta 7.24$). Gamma counting was carried out with a Packard Cobra II Auto Gamma spectrometer. For ^{125}I, a 15–75 KeV window was used with an efficiency of 81.5% and 4π geometry.

1-(4-Deoxy-4-iodo-β-D-xylopyranosyl)-2-nitroimidazole, **3a**

This material was newly synthesized according to a published procedure.[1.] Briefly, L-arabinose was converted into tetra-O-acetyl-α-L-arabinopyranoside by treatment with anhydrous NaOAc and acetic anhydride under reflux. Treatment of the tetra-acetate with 30–32% HBr in acetic acid produced 2,3,4-tri-O-acetyl-α-L-arabinopyranosyl bromide. The bromide was treated with 2-nitroimidazole and $Hg(CN)_2$ in anhydrous CH_3CN at ambient temperature to yield 1–(2,3,4-tri-O-acetyl-β-L-arabinopyranosyl)-2-nitroimidazole. Deacylation in anhydrous methanol in the presence of a catalytic amount of $NaOCH_3$ gave 1-(β-L-arabinopyranosyl)-2-nitroimidazole (**2a** in Figure 1). Treatment of **2a** with triphenylphosphine and iodine in fresh anhydrous pyridine at 60°C for 22 h yielded the title compound, **3a**, 0.235 g.

1-(4-Deoxy-4-iodo-β-L-arabinopyranosyl)-2-nitroimidazole, **3b**

An attempt was made to synthesize this material, the C-4 epimer of **3a**, by the route used to prepare **3a**, but with the substitution of D-xylose for L-arabinose as the starting sugar.. The final iodination of **2b** to **3b** was unsuccessful. This compound was later isolated, purified and characterized as a product of **3a** exchange with NaI (see large-scale conversion experiment, below).

1-(4-Deoxy-4-iodo-β-L-lyxopyranosyl)-2-nitroimidazole, **3c**

This material was synthesized by the route used to prepare **3a**, but with the substitution of tetra-O-acetylribose for L-arabinose.

Exchange radioiodization of **3a**

$Na^{125}I$ (1 mCi, carrier free, nominally 2,180 Ci/mmol) was transferred to a 0.1 ml. reactivial and evaporated to dryness under a stream of dry nitrogen. Anhydrous ethanol (100 µl) was added and re-evaporated to dryness. A solution consisting of 1.0 mg of **3a** dissolved in 20 µl anhydrous DMF was added to the reactivial. The vial was sealed and heated at 100°C for 8.0 h, then cooled and the DMF solvent removed under a stream of dry nitrogen. The labeled **3a** was purified by passing through a mini-column containing (from bottom to top) 40 mg BioRad AG1 × 8 anion resin (200–400 mesh, chloride form) and 50 mg AgCl impregnated Celite (56 mg AgCl/100 mg). The column was washed with 2.5 ml water. The sample was analyzed via HPLC using a Beckman System Gold equipped with a 3.9 × 300 mm Millipore Delta Pak 15 µm C-18 column. The solvent system consisted of methanol/water (25:75) at 0.7 ml/min. The uv–vis detector was set at 310 nm.Radioactivity was detected using a BioScan Flow Count Radiochromatography System set to detect ^{125}I.

*Large-scale conversion of **3a** to **3a**/**3b**. (Alternate synthesis of **3b**)*

A solution of **3a** (99.2 mg, 0.279 mmol) and dry sodium iodide (42 mg, 0.279 mmol) in 1.98 ml of anhydrous dimethylformamide was heated at 100°C. Samples were withdrawn at 2, 4, 7 and 23 h, and analyzed via HPLC. The reaction mixture was cooled to room temperature and the dimethylformamide was removed on a rotary evaporator. The sticky residue, which was dissolved in methanol, was subjected to preparative HPLC using a C-18 Magnum column eluted with methanol/water (25:75) at a flow rate of 0.7 ml/min. The detector was set at 315 nm. Two fractions were collected at RT = 26.5 min (12 mg, 17.8%) and RT = 52.2 min (63 mg, 82.2%).

*Conversion of **3b** to **3a**/**3b***

A solution of **3b** (5.4 mg, 0.015 mmol), and dry sodium iodide (2.27 mg, 0.015 mmol), in 108 µl of anhydrous dimethylformamide was heated at 100°C for 23 h. The cooled reaction mixture was diluted with methanol and analyzed via HPLC, using conditions as previously described. Two peaks were observed at RT = 26.5 min (17.1%) and RT = 52.2 min (81.9%) (see Table 1).

Conclusion

We have shown that the exchange radioiodination of **3a**, when performed under conditions of kinetic control, gives rise to its epimer, radioiodinated **3b**, and not radioiodinated **3a**, as was previously reported.[4] We have also shown that the product mix resulting from exchange iodination is determined by whether the reaction is carried out under kinetic or thermodynamic control. Finally, the direct preparation of **3b** starting with D–xylose failed due to the geometrical arrangement of the C-3 and C-4 hydroxyl groups of its precursor, **2a**.

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