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Note

A new synthesis of the labeling precursor for [^{18}F]-fluoromisonidazole

Tom C. H. Adamsen*, John R. Grierson and Kenneth A. Krohn
Molecular Imaging Research Center, Department of Radiology, University of Washington, Seattle, WA 98195-6004, USA

Summary

[^{18}F]-Fluoromisonidazole is the most widely used radiopharmaceutical for imaging hypoxia in tumors.

The precursor for [^{18}F]-fluoromisonidazole was prepared from 1,3-dibromo-2-propanol in 5 steps from available materials and straightforward purification steps. The overall yield for this synthesis was 18%. Copyright © 2005 John Wiley & Sons, Ltd.

Key Words: hypoxia; misonidazole; fluorine-18; positron emission tomography

Introduction

Several imaging agents have been developed for non-invasive imaging of hypoxia in tumors. The bioreductive alkylating agent [^{18}F]-fluoromisonidazole (FMISO), an azomycin-based hypoxic cell sensitizer, is the most widely used radiopharmaceutical for quantifying hypoxic tissue using PET.[1] Activation of FMISO occurs via bio-reduction of its nitro structure. It is believed that successive reductions produce a hydroxylamine, which covalently conjugates to intracellular macromolecules. On the other hand, sufficient oxygen can intercept the initially reduced species to regenerate FMISO, which provides an escape mechanism to cellular retention and make it freely diffusible between blood and tissues.

Due to the increased demand for FMISO we developed an in-house source for the precursor (Figure 1). The most convenient radiosynthesis of FMISO uses the tosylate labeling precursor (**5**) introduced by Lim and Berridge.[2]

*Correspondence to: Tom C. H. Adamsen, Molecular Imaging Research Center, Department of Radiology, University of Washington, Room NW042, Box 356004, Seattle, WA 98195-6004, USA. E-mail: tomad@u.washington.edu

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However, the commercial product is expensive and its alternative chemical synthesis is cumbersome, low yielding and requires handling of anhydrous ammonia and elemental sodium. This led us to develop a new source of this labeling precursor: We report a gram-scale, 5-step synthesis of the labeling precursor (**5**), as a mixture of diastereomers, starting from 1,3-dibromopropane-2-ol.

Results and discussion

Synthesis of the precursor for [^{18}F]-fluoromisonidazole started with an alcohol protection of 1,3-dibromopropane-2-ol using dihydropyran in the presence of pyridinum-p-toluenesulfonate and gave (**1**) in 80% yield. Compound (**1**) was converted to the di-acetate (**2**) by reaction with tetrabutylammonium acetate, catalyzed by tetrabutylammonium iodide in a 77% yield. Diacetate cleavage to the diol was accomplished with ammonia in methanol to yield 88% of (**3**). Dioltosylation was achieved according to the method of Lim and Berridge,[2] yielding the ditolsylate (**4**) in 84%. The final precursor (**5**) was prepared by the condensation of 2-nitroimidazole with the tosylate in a 40% yield.

The overall yield for this synthesis was 18% and was carried out with readily available materials and did not require handling of refluxing anhydrous ammonia and elemental sodium, thus reducing some potentially hazardous steps.

Our new method of FMISO precursor synthesis produces an enriched mixture of diastereomers, that are racemic, evident from the obtained NMR spectra. The diastereomers result from the presence of two racemic stereocenters present in compound (**5**). The consequence of using the mixture we described is that racemic FMISO is produced during the radiosynthesis rather than a single enantiomer, when the commercially product (a single diastereomer) is used. Since the nitro in FMISO is at a distance from its side chain stereocenter, the enantiomeric nature of FMISO is not believed, or proven, to be of any concern.

Experimental

Materials: All materials were purchased from Aldrich (Milwaukee, WI) and were used as provided.

Instruments: Melting point determinations were carried out using an Electrothermal® melting point apparatus. Column chromatography was carried out with either 230–400 mesh silica gel (Merck) or 150 mesh neutral aluminum oxide (Aldrich) and TLC with 60 F_{254} silica gel analytical plates (Merck). ^{1}H-NMR (300 MHz) spectra were recorded using either a Varian Gemini 300 or a Bruker Avance-300.

*1,3-dibromo-2-O-tetrahydropyranylpropanol (**1**)*

A solution of 1,3-dibromopropane-2-ol (25 g, 115 mmol), 3,4-dihydro-2*H*-pyran (20 g, 230 mmol) and a catalytic amount of pyridinum-p-toluenesulfonate (PPTS) in anhydrous THF (50 ml) was stirred at room temperature for 3 h. The reaction progress was followed by TLC using EtOAc/Hexane (silica gel; 30% EtOAc/Hex, $R_f = 0.55$). When the reaction was complete the mixture was partitioned between half saturated brine and EtOAc. Removal of the solvent from the dried ($MgSO_4$) EtOAc extract gave a clear oil that was chromatographed (silica gel 40% EtOAc/Hex) to afford a colorless oil (28 g, 80% yield). ^{1}H-NMR($CDCl_3$, 300 MHz) $\delta = 4.8$ (t, 1H), $\delta = 4.1$–3.9 (m, 2H), $\delta = 3.75$–3.5 (m, 5H), $\delta = 1.9$–1.5 (m, 6H) established the identity of the product.

*1,3-di-acetyl-2-(O-tetrahydropyranyl)-propanol (**2**)*

A mixture of compound (**1**) (10 g, 33 mmol), tetrabutylammonium acetate (30 g, 19 mmol), tetrabutylammonium iodide (1.23 g, 1.9 mmol) and anhydrous potassium carbonate (0.4 g, 2.9 mmol) in anhydrous acetonitrile (80 ml) was stirred at room temperature overnight. The reaction mixture was filtered and the solvent removed to yield an oil that was chromatographed (silica gel 30% EtOAc/Hex) to afford a colorless oil (6.7 g, 77%). ^{1}H-NMR ($CDCl_3$, 300 MHz) $\delta = 4.79$ (s, 1H), $\delta = 4.3$–4.0 (m, 5H), $\delta = 3.9$–3.85 (t, 1H), $\delta = 3.59$–3.45 (d, 1H), $\delta = 2.15$–2.0 (s, 6H), $\delta = 1.9$–1.65 (m, 2H), $\delta = 1.65$–1.45 (m, 4H) established the identity of the product.

2-*(O-tetrahydropyranyl)-propanetriol (**3**)*

Compound (**2**) (5.20 g, 20 mmol) and an anhydrous solution of saturated ammonia in methanol (100 ml) was stirred overnight at room temperature. The solution was then concentrated *in vacuo* to afford a yellow oil (3.10 g, 88% yield), which was used without further purification. ^{1}H-NMR ($CDCl_3$, 300 MHz) $\delta = 4.7$–4.5 (m, 1H), $\delta = 4.1$–3.9 (m, 1H), $\delta = 3.9$–3.4 (3 overlapping m, 6H), $\delta = 1.9$–1.7 (m, 2H), $\delta = 1.7$–1.5 (m, 4H) identified the product.

*2-O-tetrahydropyranyl-1,3-di-O-toluenesulfonylpropanetriol (**4**)*

Following the method of Lim and Berridge[2] this reaction afforded (6.1 g, 84% yield) of compound (**4**): m.p. 108–111°C (108–110°C[2]). ^{1}H-NMR ($CDCl_3$, 300 MHz) $\delta = 7.82$–7.78 (d, 4H), $\delta = 7.40$–7.30 (d, 4H), $\delta = 4.61$ (m, 1H), $\delta = 4.2$–4.0 (m, 6H), $\delta = 3.8$–3.6 (m, 1H), $\delta = 3.5$–3.3 (m, 1H), $\delta = 2.5$ (s, 6H), $\delta = 1.8$–1.4 (2 overlapping m, 6H).

*1-(2′-nitro-1′-imidazolyl)-2-O-tetrahydropyranyl-3-O-toluenesulfonylpropanediol (**5**)*

Carried out according to Lim and Berridge[2] this reaction afforded a yellow solid (1.28 g, 40% yield): m.p. 105–107°C. ^{1}H-NMR ($CDCl_3$, 300 MHz) $\delta = 7.85$–70 (t, 2H), $\delta = 7.4$–7.3 (d, 2H), $\delta = 7.16$–7.06 (d, 2H), $\delta = 4.8$–4.6 (m, 1H), $\delta = 4.45$–4.25 (m, 1H), $\delta = 4.23$–4.1 (m, 2H), $\delta = 4.1$–3.9 (d, 2H), $\delta = 3.7$–3.5 (m, 1H), $\delta = 3.4$–3.1 (m, 1H), $\delta = 2.5$–2.3 (s, 3H), $\delta = 1.7$–1.5 (m, 1H), $\delta = 1.45$–1.25 (m, 5H). MS (ESI) *m/z*: 426.13 calculated $[M + H]^+$, 426.38 found. This established the identity of the product. By chromatographic analysis (TLC, HPLC) the product was homogeneous and identical to the commercially available (ABX, Radeberg, Germany) material.

*3-[^{18}F]-fluoro-1-(2′-nitro-1′-imidazolyl)-2-propanol ([^{18}F]-fluoromisonidazole) (**6**)*

The [^{18}F]fluoride obtained by proton bombardment of [O-18]water was added to 25 μmol of potassium carbonate and 16 mg Kryptofix 222. Excess water was removed by azeotropic distillation with anhydrous acetonitrile (4×0.8 ml). A solution of dry acetonitrile (2.5 ml) containing 6 mg of compound (**5**) was introduced into the reaction vessel and heated for 7 min. The reaction mixture was refluxed at 100°C for 7 min. In order to remove unreacted [F-18]fluoride, the reaction mixture was eluted through a neutral alumina SepPak (Waters, Millipore) previously conditioned with MeCN. The solvent was evaporated and the residue was hydrolyzed for 3 min in 2 ml of 0.5 N HCl at 100°C. The solution was neutralized with 2.5 ml of 4.2% sodium bicarbonate solution. The product was isolated using an Econosil 10 μ reverse-phase HPLC column that

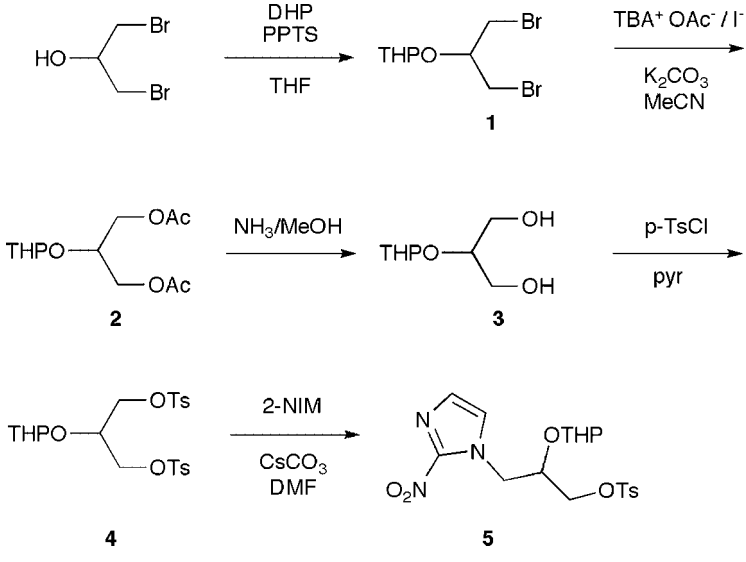

Figure 1. New synthesis of the [^{18}F]-fluoromisonidazole labeling precursor

was eluted with 5% ethanol/sterile water (USP). Decay corrected radiochemical yield (80 min EOB) was 30–40%. Quality control was performed on reverse phase HPLC with radiation and UV detection, and by TLC.

Conclusion

We have improved the fluromisonidazole precursor synthesis with an overall yield of 18%, where Lim and Berridge reported overall yield of 5%. Identity and purity were assayed by ^{1}H-NMR, HPLC, TLC and MS. We have used the precursor to produce [^{18}F]-fluoromisonidazole for PET imaging.

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References

1. Rajendran JG, Krohn KA. *Radiol Clin N Am* 2005; **43**(1): 169–187. DOI: 10.1016/j.rcl.2004.08.004.
2. Lim J-L, Berridge MS. *Appl Radiat Isot* 1993; **44**: 1085–1091.

