JOURNAL OF LABELLED COMPOUNDS AND RADIOPHARMACEUTICALS
J Label Compd Radiopharm 2004; **47**: 37–45.
Published online in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/jlcr.795

Research Article

Preparation of fluorine-18-labelled fluoromisonidazole using two different synthesis methods

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Summary

^{18}F-labelled fluoromisonidazole [1H-1-(3-[^{18}F]fluoro-2-hydroxypropyl)-2-nitroimidazole; ([^{18}F]FMISO)] is used as an *in vivo* marker of hypoxic cells in tumours and ischaemic areas of the heart and the brain. The compound plays an important role in evaluating the oxygenation status in tumours during radiotherapy. In this paper, we report experiments carried out in our laboratory in synthesizing [^{18}F]FMISO using two different methods. The first method (I) for the [^{18}F]FMISO synthesis was the fluorination of (2R)-(−)-glycidyl tosylate to [^{18}F]epifluorohydrin. The subsequent nucleophilic ring opening, achieved with 2-nitroimidazole, leads to labelled FMISO. The second method (II) was the fluorination of the protected precursor 1-(2′-nitro-1′-imidazolyl)-2-*O*-tetrahydropyranyl-3-*O*-toluenesulphonyl-propanediol, followed by a rapid removal of the protecting group. With the first method, the radiochemical yield was about 10% at the end of the synthesis (EOS), and the radiochemical purity was over 99%. The radiochemical yield in the second method was 21% (EOS) on an average, and the radiochemical purity was over 97%. When an automated commercial synthesis module was used with method II, slightly better and more reproducible yields were achieved. The improvement in the synthesis yield with the automated apparatus will be valuable when working with high activities, and therefore it is under further development. Copyright © 2003 John Wiley & Sons, Ltd.

Key Words: fluorine-18 fluoromisonidazole; FMISO; hypoxia; positron emission tomography

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Contract/grant sponsor: Finnish Government EVO Grant; contract grant number: TXH 0214

Received 17 July 2003
Revised 19 October 2003
Accepted 20 October 2003

Introduction

Misonidazole (MISO) and its derivatives, like fluoroerythronitroimidazole (FETNIM), have been shown to accumulate in hypoxic but viable cells.[1,2] Consequently, their radiolabelled analogues are used as markers of hypoxic tissues.[2–8] Despite intensive studies, the binding mechanism of nitroimidazole compounds to hypoxic cells is not fully understood, thus the validation of the models determining hypoxic cell fraction still requires further investigation.[1] PET and [^{18}F]FMISO (1H-1-(3-[^{18}F]fluoro-2-hydroxypropyl)-2-nitroimidazole) can help estimate the oxygenation status of tumours in any part of the body. The tracer has also been used to study the relative hypoxic volume of tumours during the course of radiation treatment.[9] Recently, improvement in response to treatment with new selective experimental chemotherapy agents has been observed by using [^{18}F]FMISO and PET.[9] Despite some disadvantages, [^{18}F]FMISO is now the most used radiotracer of the misonidazole derivatives. Here, we report on our experiments of the synthesis of [^{18}F]FMISO using two different synthesis methods (methods I and II). Method II is also adapted to an automated synthesis module. Our aim is to produce [^{18}F]FMISO in high radioactivity amounts, and therefore the development and the improvement of method II with the automated synthesis module are important.

Results and discussion

Synthesis of [^{18}F]FMISO via [^{18}F]EPI-F (Figure 1)

The [^{18}F]FMISO was prepared in two steps via [^{18}F]epifluorohydrin ([^{18}F]EPI-F). The radiochemical yield at the end of the first step of the synthesis for the [^{18}F]EPI-F was in general over 70% (EOB). The radiochemical yield of the [^{18}F]FMISO at the end of the second step of the synthesis was about 40%

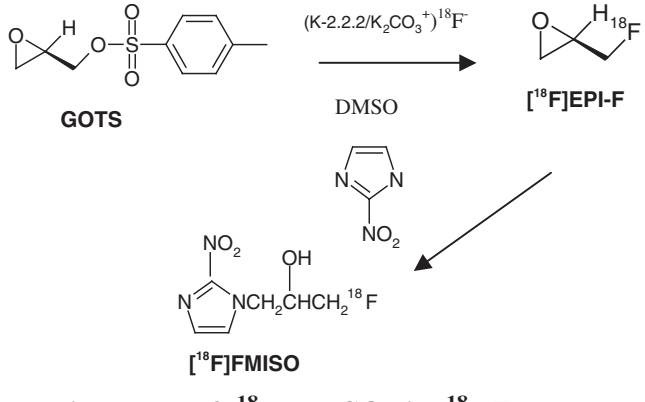

Figure 1. Synthesis scheme of [^{18}F]FMISO via [^{18}F]EPI-F

(EOB) in a total synthesis time of about 180 min.[10] The identity of the product was confirmed by comparing the HPLC and the TLC chromatograms of the [^{18}F]FMISO with those of the unlabelled reference material. The [^{18}F]EPI-F could not be detected on the TLC plate due to its volatility.

In the first step of the synthesis, the yields were very low in the early experiments, mainly due to the volatility of the [^{18}F]EPI-F. However, with minor modifications, the [^{18}F]EPI-F yield could be increased from 40 to about 70% (EOB). The use of reaction vials specially made for this purpose and increasing the reaction temperature from 115 to 125°C improved the radiochemical yield remarkably. Furthermore, adding acetonitrile (CH_3CN) in very small portions several times when distilling the [^{18}F]EPI-F also seemed to increase the yield.

In the second step of the synthesis, the [^{18}F]FMISO yield could be increased from 10 to 23% (EOB) by optimizing the design of the reaction vial. Using an ultrasonic bath for mixing increased the yield further from about 23 to 37% (EOB); see also Table 1.

The uncorrected yield of the [^{18}F]FMISO was at best about 13% (EOS) in a synthesis time of about 180 min. The yield was approximately at the same level as reported by Grierson *et al.*[11] but clearly lower than those reported by McCarthy *et al.*[12] However, our synthesis time was longer. The most problematic issue in the second part of the synthesis was the amount of unreacted 2-nitroimidazole (2-NIM). In this method, 2-NIM was used in excess to achieve a complete reaction between the 2-NIM and the [^{18}F]EPI-F. Normal-phase HPLC failed to separate the 2-NIM and the other impurities (e.g. the rest of the unreacted [^{18}F]EPI-F) from the product. However, reverse-phase HPLC worked well when a silica gel flash column was used before the HPLC purification. The radiochemical purity of the [^{18}F]FMISO after the HPLC purification was over 99%, confirmed by TLC using both autoradiography and scanning, as well as by HPLC. The HPLC UV absorption

Table 1. The distribution of radioactivity in method I during the synthesis. All the results are decay corrected to the initial activity of fluoride-18

	EPI-F yield (%)	FMISO crude yield (%)	FMISO HPLC purified yield (%)	Fluorination vial (%)	Silica gel column (%)	Loss of activity in rotary evaporation and filtration (%)
(1)	70 ± 7	26 ± 7	23 ± 4	6 ± 4	11 ± 5	35 ± 6
(2)	75 ± 9	40 ± 7	37 ± 7	7 ± 5	16 ± 2	19 ± 6

Note: (1) An average of 5 consecutive syntheses, where a reaction vial of an optimized design was used. (2) An average of 5 consecutive syntheses, where both a reaction vial of an optimized design and ultrasonic mixing were used.

detection demonstrated the presence of a small amount of an unknown compound, some 2-NIM, and the mass peak of fluoromisonidazole.

Synthesis of [^{18}F]FMISO via protected [^{18}F]NITTP (Figure 2)

The [^{18}F]FMISO was prepared in one step, starting from 1-(2′-nitro-1′-imidazolyl)-2-*O*-tetrahydropyranyl-3-*O*-toluenesulphonyl-propanediol (NITTP). The radiochemical yield for the [^{18}F]FMISO was 40% (EOB) on an average after a synthesis time of 96 min.

The identity of the intermediates and the final product were confirmed by comparing the chromatograms with unlabelled reference materials. The radiochemical purity of the final product was over 97%, confirmed by TLC and HPLC. The most problematic issues in this synthetic approach were the high losses of activity on silica Sep Paks and on the walls of the reaction vials, consequently decreasing the synthesis yield (Table 2).

Although the [^{18}F]FMISO was prepared in one labelling step, the synthesis was quite complicated to be performed routinely. The synthetic route requires two separate Sep Pak purifications and two evaporations. These operations lasted approximately 30 min and were quite inconvenient in manual/remote control work. The large variations observed in the yields of the labelled

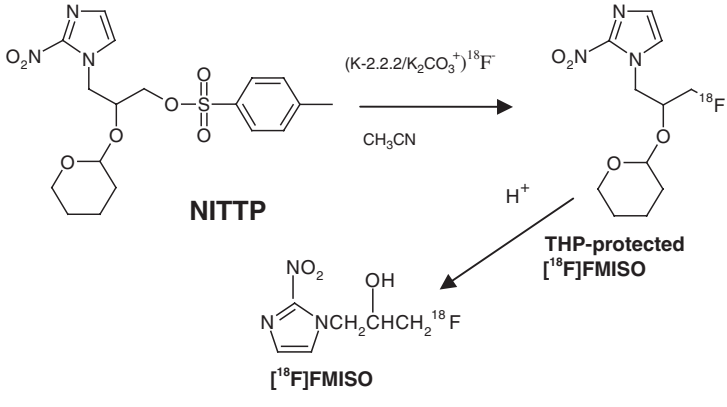

Figure 2. Synthesis scheme of [^{18}F]FMISO via NITTP

FMISO (Table 2) are largely due to the above-mentioned complicated

Table 2. The distribution of activity in method II during the synthesis. All the results are decay corrected to the initial activity of fluoride-18 ($n = 5$)

FMISO final product (%)	Silica Sep Paks (%)	Fluorination vial (%)	Synthesis vial after synthesis (%)	Activity not accounted for (%)
40 ± 12	22 ± 2	13 ± 8	10 ± 11	16 ± 7

Table 3. The distribution of activity in Method II using automated FDG synthesis module. All the results are decay corrected to the initial activity of fluoride-18 ($n = 7$)

FMISO final product (%)	Silica Sep Paks (%)	Fluorination vial (V1) (%)	Second synthesis vial (V2) (%)	Activity in C-18 and alumina Sep Paks and filter (%)	Activity not accounted for (%)
34 ± 6	30 ± 7	15 ± 7	4 ± 2	3 ± 2	14 ± 5

operations. The advantage of this process is that no HPLC is needed for the purification of the labelled [^{18}F]FMISO. In our experiments, the synthesis time was in general about 100 min, and at best 86 min. In comparison with the yield of the [^{18}F]FMISO reported by Lim and Berridge[13] and Patt *et al.*,[14] our results yields were lower, partly due to the longer synthesis time used.

Synthesis of [^{18}F]FMISO via protected [^{18}F]NITTP using automated synthesis module

The [^{18}F]FMISO was prepared in one step as described above in method II using an automatic FDG synthesis module made by IBA (Ion Beam Applications, Belgium).[15] The radiochemical yield for the final product was 34% (EOB) on an average after a synthesis time of about 50 min (Table 3). The identities of the [^{18}F]FMISO and the intermediates were confirmed by TLC using autoradiography and scanning, as well as by HPLC, by comparing the chromatograms with the unlabelled reference materials. In addition, liquid chromatography mass spectrometry (LC–MS) was used for analyzing the end product. The radiochemical purity of the product was over 97%. Besides the [^{18}F]FMISO (R_f value of 0.41 ± 0.03), the only radioactive peak observed on the TLC plate was a very small amount of free $^{18}F^-$. The LC–MS analysis showed that the product contained a just detectable mass of FMISO, and, in addition, three impurities in very low amounts, confirming the high chemical purity of the [^{18}F]FMISO.

The synthesis time could be reduced to 50 min, compared to the 96 min used for the manual synthesis. However, the yield did not increase. The majority of the yield loss occurred in the silica Sep Paks (mostly $^{18}F^-$), which indicates an unsatisfactory labelling reaction, and in the fluorination vial (V1) (Table 3). Similar behaviour was found in the manual synthesis using the same method (see also Table 2). The activity in the fluorination vial was mainly a labelled unhydrolyzed [^{18}F]FMISO intermediate. The yield of the [^{18}F]FMISO was lower than reported by Lim and Berridge[13] and Patt *et al.*[14] However, the synthesis with the module was more reliable than in manual work. The yields

were also reproducible, which is important in routine PET-imaging. Further improvements in order to increase the yield are under study.

Experimental

General

Materials. For method I the (2R)-(−)-glycidyl tosylate (GOTS), the 2-NIM and the dry CH_3CN were purchased from Aldrich, and the dimethylformamide (DMF) from Fluka. The aminopolyether, 4,7,13,16,21,24-hexaoxa-1,10-diazabicyclo[8,8,8]hexacosane (Kryptofix [2.2.2]), other chemicals (analytical grade) and the silica gel 60_{254} TLC plates were obtained from Merck. Fluoromisonidazole was a gift from Roche (Nutley, NJ, USA). Films (X-omat) for autoradiography were purchased from Kodak.

For method II, NITTP, FMISO and the reference standard 1-(2,3-dihydroxypropyl)-2-nitroimidazole were purchased from ABX (Dresden, Germany). All other reagents and solvents were obtained from Aldrich. The silica gel 60_{254} TLC plates were obtained from Merck.

Production of [^{18}F] fluoride. The [^{18}F]fluoride was produced with a tandem van de Graaf accelerator (Department of Physics, University of Helsinki) with 9.6 MeV protons or with a cyclotron, IBA Cyclone 10/5 (Laboratory of Radiochemistry, University of Helsinki) with 10 MeV protons, using the nuclear reaction $^{18}O(p,n)^{18}F$.

Synthetic method I

Synthesis of [^{18}F]FMISO (Figure 1). The synthesis was carried out according to the method described by Grierson *et al.*[11,16] with some modifications of our own.[10] The [^{18}F]FMISO was produced by displacement of the tosyl group from GOTS with ^{18}F-fluoride to afford ^{8}F]EPI-F. The subsequent nucleophilic ring opening of the [^{18}F]EPI-F with 2-NIM then afforded the [^{18}F]FMISO. First, N.C.A. aqueous ^{18}F-fluoride was added to a mixture of Kryptofix [2.2.2] (23 mg) and K_2CO_3 (4.6 mg) in CH_3CN/water (86:14). Next, azeotropic distillation at 130°C under a N_2-flow was carried out by adding CH_3CN in three 1.5-ml-portions. GOTS (40 mg) in 0.6 ml dimethylsulphoxide (DMSO) was then added to the dried residue. After that, labelled EPI-F was distilled with CH_3CN to a vial containing 0.6 ml of DMF held at 0°C. The CH_3CN was added in small portions of 0.1–0.2 ml during 25 min. In the second step of the synthesis, the EPI-F was reacted with 2-NIM (40 mg) and KOH in a borosilicate vial held at 120°C for 45 min, and subsequently the crude product was purified.

Purification of [^{18}F]FMISO. The labelled crude [^{18}F]FMISO was purified with column chromatography (Silica gel 60; mesh 0.060–0.200 mm, 30%-

CH_3CN-chloroform, 2 ml/min) before HPLC. Subsequently collected fractions were evaporated and the residue dissolved in 5%-ETOH and filtered through a 0.22 μm membrane filter. Finally the compound was purified by HPLC to a radiochemical purity higher than 99%, using a system consisting of a pump, an automatic sample injector, a UV absorption detector and an analytical reversed-phase Spherisorb 10 ODS C-18 column (250 × 4.6 mm, 10 μm, HPLC Tech. LTD.). The mobile phase used was 5%-ethanol–water solution at a flow rate of 2 ml/min.

Analyses of [^{18}F]FMISO. The analyses of the final product and the reference compounds were carried out with the same HPLC system as mentioned above. TLC was used in determining the compounds during various synthesis steps. The masses on the TLC plates were visualized at 254 nm by using a UV lamp. The plates were developed with 30%-CH_3CN-chloroform solution. The radioactivity on the plates was detected by means of film autoradiography and/or a scanning system.

Synthetic method II

Synthesis of [^{18}F]FMISO (Figure 2). In this approach, the [^{18}F]FMISO was prepared in one step, where the ^{18}F is directly incorporated into the product structure. The protected precursor NITTP is fluorinated, and, subsequently, the protecting group is removed. The synthesis was carried out according to the method of Lim and Berridge,[13] with some minor modifications of our own. First, N.C.A. aqueous ^{18}F-fluoride was added to a vial containing Kryptofix [2.2.2] (13.5 mg) and K_2CO_3 (1.7 mg) in CH_3CN/water (5:1). This mixture was dried as described in Method I, but at a temperature of only 110 °C, instead of 130°C. Then, the tosylate precursor (5 mg) was added to the dried residue in 2 ml of CH_3CN, and refluxed at 100°C for about 10 min. After that, the mixture was cooled and concentrated to about half the volume under a N_2-flow. Next, 2 ml of diethyl ether was added, whereupon the mixture was loaded onto two silica Sep Paks connected in series. The Sep Paks were then eluted with 5–6 ml of diethyl ether, and the eluate was evaporated with a rotary evaporator. Subsequently, the residue was hydrolyzed for 3 min at 100°C, using 2 ml of 1 N HCl. After that, the mixture was neutralized with 1 ml of 2 N NaOH, and buffered with 1 ml of 1 N $NaHCO_3$. The solution was then eluted through a C-18 Sep Pak, an Alumina Sep Pak and a Millipore filter connected in series. The Sep Paks were further eluted with 4 ml of 10%-ethanol, and finally, the eluates were combined.

Synthesis of [^{18}F]FMISO with an automated synthesis module. The synthesis procedure was based on the method of Lim and Berridge.[13] N.C.A. aqueous ^{18}F-fluoride was transferred to the synthesis vessel containing Kryptofix [2.2.2]

(13.5 mg) and K_2CO_3 (1.7 mg) in CH_3CN/water (8:1). The solvents were evaporated under an argon flow by adding 1 ml of CH_3CN two times. Next, NITTP in 2 ml of CH_3CN was added, and the reaction was performed at 100°C during 10 min. Subsequently, 10 ml of diethyl ether was added, and the product was transferred through two silica Sep Paks to a second vessel (in two 5-ml-portions). The ether was evaporated, and 2 ml of 1 N HCl was added to the residue for hydrolysis at 100°C for 3 min. Then, 1 ml of 2 N NaOH was added to neutralize the solution. The solution was then transferred, through a C-18 Sep Pak, an Alumina Sep Pak and a Millipore filter connected in series, to the product vial containing 1 ml of 1 N $NaHCO_3$. Finally, the column was rinsed with 4 ml of 10%-ethanol. The synthesis time was about 50 min.

Analysis of the compounds. No HPLC system was needed for the purification of the product. The analysis of the intermediate compounds and the quality control were carried out by using HPLC, TLC and LC-MS. The HPLC system was the same as in method I. In this case, however, the column was semipreparative μBondapack C-18 (7.8 × 300 mm, 10 μm; Waters) and the product was eluted using 20%-CH_3CN/H_2O at 2 ml/min. The mobile phase used for the TLC plates was ethyl acetate. The plates were analyzed using a UV lamp at 254 nm and a TLC scanner (Raytest, Germany) for determining the distribution of radioactivity. The APCI-MS spectra were acquired in the centroid mode using a Micromass Quattro II mass spectrometer (Altrincham, UK) connected with a Hewlett-Packard 1100 liquid chromatograph. Nitrogen was used as a drying and an APCI sheath gas at flow-rates of 300 and 50 l/h, respectively. A probe temperature of 450°C, a source temperature of 150°C, a corona voltage of 3.5 kV, and a cone voltage of 20 V were applied to produce the spectra at a mass range of 70–600 m/z using a scanning speed of 2 s. The LC analyses were carried out on a Waters XTerra MS C18 column (4.6 × 150 mm, 5 μm) using methanol, water and formic acid of 0.1% concentration as eluent components. The following programming was used: first, the eluent composition was changed linearly in 5 min from 5%-methanol to 100%-methanol, and then the elution was continued iso-cratically for 5 min. The injection volume was of 20 μl, and the eluent flow rate was 1 ml/min.

Conclusions

We have described the synthesis of [^{18}F]FMISO, for which two different synthesis methods were used. In method I, the [^{18}F]FMISO was prepared in two steps via [^{18}F]EPI-F using GOTS as a precursor, whereas in method II, the [^{18}F]FMISO was prepared in one step using NITTP as a precursor. Method II was also adapted to an automated synthesis module. In our experience so far, the preparation of [^{18}F]FMISO using method II and an automated synthesis module has proved reliable and the yields are reasonably high and

reproducible. This is of importance when large amounts of [18F]FMISO are needed for multiple PET studies on hypoxia e.g. in cancer patients.

Acknowledgements

The authors thank Mr Heikki Sepponen in Department of Physics, University of Helsinki and PhD Kerttuli Helariutta in the Radiochemistry Laboratory of the University of Helsinki for the radionuclide production. This work was partly supported by a Finnish government EVO Grant TXH 0214.

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