*Journal of Radioanalytical and Nuclear Chemistry, Vol. 240, No. 3 (1999) 925–927*

# Preparation of [$^{18}$F]fluoromisonidazole by nucleophilic substitution on THP-protected precursor: Yield dependence on reaction parameters

**M. Patt, M. Kuntzsch, H.-J. Machulla***

*Section Radiopharmacy, PET-Center, Eberhardt-Karls-University, Tübingen, Germany*

(Received May 25, 1998)

The dependence of the radiochemical yield of [$^{18}$F]fluoromisonidazole (1) on different reaction parameters such as reaction time, temperature and amount of precursor was investigated for the nucleophilic substitution of tosylate by [$^{18}$F]fluoride and subsequent hydrolysis of the protecting group on 1-(2'-nitro-1'-imidazolyl)-2-O-tetrahydropyranyl-3-O-toluenesulfonylpropanediol as the precursor molecule (2). Highest yields (86%±6%) were obtained using 10 mg (2) at 100 °C for 10 minutes, whereas both at 80 and 120 °C the yields were lower (46%±11% and 29%±14%, respectively). A rapid decrease of the yield was observed when the reaction time exceeded 15 minutes, i.e., at 100 °C using 5 mg (2) the radiochemical yield decreased from 61%±8% at 15 minutes to 18%±10% at 60 minutes.

## Introduction

1-(2'-nitro-1'-imidazolyl)-3-fluoro-2-propanol, FMISO (1), labeled with the short-lived positron-emitter fluorine-18, was suggested as a tracer for the determination of hypoxic tissue in vivo with PET in 1984.[1] Since that time, several synthetic approaches to FMISO have been investigated with the aim of improving radiochemical yields and simplifying the synthetic procedure in order to allow clinical applications of the tracer.[2–10]

These synthetic strategies can be divided into two main groups: a nucleophilic substitution on a protected precursor with subsequent removal the protection group or epoxide ring-opening and the production of a $^{18}$F-labeled reaction intermediate epifluorohydrin with subsequent coupling to the nitroimidazole moiety under basic conditions. Both approaches have their motivation based on practical considerations. The first method tends to minimise the number of chemical transformations needed, with the aim of a high labeling efficiency and a one-pot synthesis, the second is aimed at utilising off-the-shelf chemical components and has therefore been drawing greatest attention for routine applications.[6]

From a critical review of the literature the most promising direct labeling approach of FMISO seemed to be the nucleophilic substitution of the tosylate leaving group by [$^{18}$F]fluoride on the tetrahydropyranyl-protected precursor 1-(2'-nitro-1'-imidazolyl)-2-O-tetrahydropyranyl-3-O-toluenesulfonylpropanediol (2), NITTP, with subsequent hydrolysis of the protecting group (Fig. 1).[11,12] A major disadvantage that restricted a wider use of the precursor suggested by LIM et al.[11] was the time-consuming low yield synthesis of the starting material. Since recently the starting compound NITTP (2) became commercially available, this labeling approach for [$^{18}$F]FMISO gained additional attractivity.

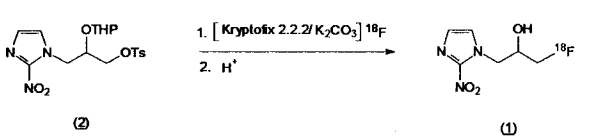
However, since only very few details about the applied reaction parameters were reported so far, for routine applications of [$^{18}$F]FMISO a systematic investigation of the dependence of radiochemical yield on temperature, reaction time and amount of precursor was necessary.

## Experimental

1-(2'-Nitro-1'-imidazolyl)-2-O-tetrahydropyranyl-3-O-toluenesulfonylpropanediol (2), NITTP, was obtained from ABX, Advanced Biomedical Compounds, Schillerstr. 23, D-01326 Dresden, Germany. $CH_3CN$ for DNA synthesis, $K_2CO_3 \cdot 1.5\ H_2O$, suprapur, and 1M HCl were obtained from Merck, Darmstadt, Germany. 4,7,13,16,21,24-hexaoxa-1,10-diazabicyclo [8.8.8]hexacosane (Kryptofix® 222) was obtained from Sigma-Aldrich, Steinheim, Germany. All reagents were used without further purification.

### *Labeling procedure*

[$^{18}$F]Fluoride was produced at the cyclotron of the PET-Center (PETtrace, GE Medical Systems) via the $^{18}$O(p,n)$^{18}$F reaction.




(2) (1)

*Fig. 1.* Reaction scheme for the synthesis of [$^{18}$F]FMISO

* E-mail: machulla@uni-tuebingen.de

*0236–5731/99/USD 17.00*


*Elsevier Science B. V., Amsterdam*
*Akadémiai Kiadó, Budapest*

To 32 mg (85 μmol) Kryptofix® 222 in a 5 ml Reactivial equipped with stirring bar, Ar-supply (quality 6.0) and vacuum line were added 150 μl aqueous 0.28M $K_2CO_3$ solution, 1 ml $CH_3CN$ and 100–300 μl [$^{18}$F]fluoride in $H_2{}^{18}O$.

Excess water was removed in vacuum at 100 °C and the resulting complex was dried additionally three times by azeotropic distillation with 1 ml $CH_3CN$ each under an argon stream. A solution of 1 mg, 5 mg or 10 mg NITTP (2) in 2.5 ml $CH_3CN$ was added and the vial was heated for 5, 10, 15, 30 minutes or 60 minutes to 80, 100 or 120 °C. From the resulting reaction mixture 100 μl were added to ice-cold 200 μl 1M HCl. The protection group was removed by heating the resulting mixture to 100 °C for 5 minutes. After cooling to room temperature the solution was diluted with 500 μl of HPLC eluent ($H_2O/C_2H_5OH$ 95/5, v/v) and 100 μl were analysed by HPLC equipped with NaI-radiodetector on a Partisil 10 ODS 3 column, 500 mm×8 mm (CS, Chromatographie Service, D-52374 Langerwehe, Germany), $H_2O/C_2H_5OH$ 95/5, v/v, flow 4 ml/min, UV-detection at 320 nm. Under these conditions the *k'* value for [$^{18}$F]FMISO was 2.3, corresponding to a retention time of 15 minutes. The THP-protected reaction product, 3-[$^{18}$F]fluoro-1-(2'-nitro-1'-imidazolyl)-2-O-tetrahydropyranylpropanol, was not eluted from the column under the applied chromatographic conditions within 1 hour. The peak corresponding to [$^{18}$F]FMISO was collected and the yield was expressed as the amount of radioactivity in the [$^{18}$F]FMISO fraction divided by the total injected activity as well as the area of the [$^{18}$F]FMISO peak in the radioactivity channel divided by the area corresponding to an aliquot of the analysed sample injected behind the HPLC column.

## Results and discussion

The product yield of [$^{18}$F]FMISO was determined at reaction times between 5 and 60 minutes in acetonitrile at 100 °C (Fig. 2). The experiments were performed with 5 mg NITTP (2) in a reaction volume of 2.5 ml $CH_3CN$.

Highest yields of about 60% were obtained after 10 and 15 minutes, whereas surprisingly the yield decreased with longer reaction times resulting in a yield of 38%± 13% ($n$=5) at 30 minutes and 18%±10% ($n$=5) at 60 minutes, indicating a thermal instability of the reaction intermediate 3-[$^{18}$F]fluoro-1-(2'-nitro-1'-imidazolyl)-2-O-tetrahydropyranylpropanol. However, no other radioactive product eluted under the applied chromatographic conditions from the HPLC column within the observed time range.

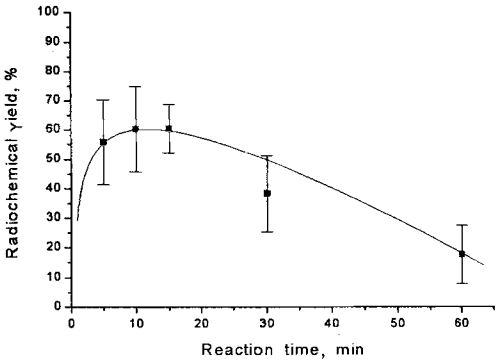



*Fig. 2.* Dependence of radiochemical yield on reaction time (reaction conditions: 100 °C, 5 mg (2) (13.2 μmol), 2.5 ml $CH_3CN$, $n$=5)

Based on these results, a reaction time of 10 minutes was chosen for the optimization of the reaction temperature (Table 1). The experiments were performed with 5 mg NITTP (2) in a reaction volume of 2.5 ml $CH_3CN$ at 80, 100 and 120 °C. The highest yield was obtained at 100 °C, at this reaction temperature 60%± 14% ($n$=5) of the [$^{18}$F]fluoride-activity were converted into [$^{18}$F]FMISO. This result is in good agreement with those reported by LIM et al.[12] who found labeling yields between 43 and 71% under the same conditions. Both at lower and at higher reaction temperatures, i.e., 80 and 120 °C the radiochemical yield was lower, resulting in 46%±11% ($n$=3) and 29%±14% ($n$=3) labeled product, respectively. The stronger decrease of the yield at 120 °C can be interpreted as another hint of the assumed thermal instability of the reaction intermediate.

The dependence of the radiochemical yield on the amount of precursor was determined in a range between 1 and 10 mg of starting material at a reaction time of 10 minutes and a temperature of 100 °C in $CH_3CN$. As shown in Table 2 radiochemical yields of 60%±14% ($n$=3) were obtained when 5 mg (13.2 μmol) of (2) were used in the reaction. Within the investigated range the highest yield was observed for 10 mg (26.4 μmol) of precursor. Under these conditions 86%±6% ($n$=3) of [$^{18}$F]FMISO were produced. A dramatic decrease in the labeling yield was seen when low amounts of precursor were used for the reaction, i.e., with 1 mg of (2) the radiochemical yield was less than 1%.

*Table 1.* Dependence of radiochemical yield on reaction temperature (reaction conditions: 5 mg (13.2 μmol) NITTP (2), 2.5 ml $CH_3CN$, reaction time 10 minutes, $n$=3, ±sd)

| Temperature, °C | Radiochemical yield, % |
|---|---|
| 80 | 46 ± 11 |
| 100 | 60 ± 14 |
| 120 | 29 ± 14 |

*Table 2.* Dependence of radiochemical yield on amount of precursor (2) (reaction conditions: 100 °C, 2.5 ml $CH_3CN$ reaction volume, 10 minutes reaction time, $n = 3$, ±sd)

| Amount of precursor NITTP (2), mg (μmol) | Radiochemical yield, % |
|---|---|
| 1 (2.6) | 0.8 ± 0.5 |
| 5 (13.2) | 60 ± 14 |
| 10 (26.4) | 86 ± 6 |

In conclusion, the labeling of [$^{18}$F]FMISO by nucleophilic substitution of [$^{18}$F]fluoride for tosylate and subsequent hydrolysis of the protecting group on 1-(2'-nitro-1'-imidazolyl)-2-O-tetrahydropyranyl-3-O-toluenesulfonylpropanediol (2) as suggested by LIM et al. represents an easy and reliable method that should be able to produce high amounts of [$^{18}$F]FMISO since labeling yields of more than 80% can be obtained and the reaction procedure can be performed in any automated system designed for the synthesis of FDG either using the method originally presented by HAMACHER et al.[13] or the heterogenous nucleophilic substitution on a quaternary aminopyridinium resin as implemented in the GE MicroLab.[14,8]

## References


1. P. A. JERABEK, D. D. DISCHINO, M. R. KILBOURN, M. J. WELCH, J. Labelled Compd. Radiopharm., 21 (1984) 1234.
2. P. A. JERABEK, T. B. PATRICK, M. R. KILBOURN, D. D. DISCHINO, M. J. WELCH, Appl. Radiation Isotopes. 37 (1986) 599.
3. A. CHERIF, D. J. YANG, W. TANSEY, E. E. KIM, S. WALLACE, Pharm. Res., 11 (1994) 466.
4. D.-R. HWANG, C. S. DENCE, T. A. BONASERA, M. J. WELCH, Appl. Radiation Isotopes, 40 (1989) 117.
5. J. GRIERSON, C. MATHIS, E. SHANKLAND, J. LINK, Z. GRUNBAUM, J. RASEY, K. KROHN, J. Nucl. Med., 28 (1987) 1081.
6. J. R. GRIERSON, J. M. LINK, C. A. MATHIS, J. S. RASEY, K. A. KROHN, J. Nucl. Med., 30 (1989) 343.
7. D.-R. HWANG, C. S. DENCE, M. J. WELCH, M. E. SHELTON, S. R. BERGMANN, J. Labelled Compd. Radiopharm., 29 (1989) 442.
8. M. SOLBACH, H.-J. MACHULLA, J. Labelled Compd. Radiopharm., 37 (1995) 199.
9. T. J. MCCARTHY, C. S. DENCE, M. J. WELCH, Appl. Radiation Isotopes, 44 (1993) 1129.
10. M. TADA, R. IWATA, H. SUGIYAMA, K. SATO, K. KUBOTA, R. KUBOTA, H. TAKAHASHI, H. FUKUDA, T. IDO, J. Labelled Compd. Radiopharm., 38 (1996) 771.
11. J. L. LIM, M. S. BERRIDGE, J. Labelled Compd. Radiopharm., 32 (1993) 541.
12. J.-L. LIM, M. S. BERRIDGE, Appl. Radiation Isotopes, 44 (1993) 1085.
13. K. HAMACHER, H. H. COENEN, G. STÖCKLIN, J. Nucl. Med., 27 (1986) 235.
14. G. K. MULHOLLAND, T. J. MANGNER, D. M. JEWETT, M. R. KILBOURN, Labelled Compd. Radiopharm., 26 (1989) 378.