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Automated synthesis and purification of [^{18}F]fluoro-[*di-deutero*]methyl tosylate

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Automated synthetic procedures of [^{18}F]fluoro-[*di-deutero*]methyl tosylate on a GE TRACERlab FX F-N module and a non-commercial synthesis module have been developed. The syntheses included azeotropic drying of the [^{18}F]fluoride, nucleophilic ^{18}F-fluorination of bis(tosyloxy)-[*di-deutero*]methane, HPLC purification and subsequent formulation of the synthesized [^{18}F]fluoro-[*di-deutero*]methyl tosylate (d_2-[^{18}F]FMT) in organic solvents. Automation shortened the total synthesis time to 50 min, resulting in an average radiochemical yield of about 50% and high radiochemical purity (>98%). The possible application of this procedure to commercially available synthesis modules might be of significance for the production of deuterated ^{18}F-fluoromethylated imaging probes in the future. Copyright © 2013 John Wiley & Sons, Ltd.

Keywords: automated synthesis; ^{18}F-fluoromethylation; [^{18}F]fluoromethyl tosylate; [^{18}F]fluorine; PET

Introduction

^{11}C-Methylation is a well-developed labeling strategy toward tracers for positron emission tomography (PET). The short half-life of carbon-11 (20.1 min) limits its use to centers with an on-site cyclotron. In this regard, [^{18}F]fluorine, offering a half-life of 109.8 min, is the PET nuclide of choice for routine clinical application. In particular, ^{18}F-fluoroalkylation is a useful way of inserting [^{18}F]fluorine into molecules, thus avoiding the harsh reaction conditions of ^{18}F-direct fluorinations. The most common ^{18}F-fluoroalkylation agent is [^{18}F]fluoroethyl tosylate, which has been used for labeling a large number of radiopharmaceuticals for oncological, neurological or cardiological studies.

However, for CNS ligands, replacement of the ^{11}C-methyl group by an ^{18}F-fluoroethyl moiety can cause a decrease in affinity, selectivity and brain uptake and, in some cases, different metabolites. Therefore, ^{18}F-fluoromethylation seems to be an interesting and structurally sensible alternative method of inserting ^{18}F-fluorine into biomolecules, which are known candidates for ^{11}C-methylation. For example, the insertion of an ^{18}F-fluoromethyl group in the SERT ligand (+)-McN5652 led to an improvement of biological characteristics compared with the ^{18}F-fluoroethyl analog.[1]

For ^{18}F-fluoromethylation, diverse precursors such as [^{18}F]bromofluoromethane, [^{18}F]fluoromethyl iodide, [^{18}F]fluoromethyl triflate and [^{18}F]fluoromethyl tosylate have been reported.[2] The most commonly used labeling precursor is [^{18}F]bromofluoromethane, which is synthesized from dibromomethane, [^{18}F]F^- and Kryptofix® 2.2.2./K_2CO_3 with radiochemical yields (RCY) of up to 62%.

[^{18}F]Fluoromethyl tosylate was first reported by Block and Coenen in 1987.[3] The reaction of bis(tosyloxy)methane, which was synthesized from diiodomethane and silver tosylate in acetonitrile[4], with [^{18}F]F^- led to approximately 40% radiochemical yield. Neal and Berridge re-examined the synthesis in 2005.[5] They found out that the addition of small amounts of water to the reaction mixture significantly increased the formation of [^{18}F]fluoromethyl tosylate, resulting in average yields of about 70%. However, reproducing these results seemed to be difficult because Smith *et al*. reported significantly lower radiochemical yields ($28 \pm 7\%$, $n = 4$) under the same reaction conditions. By using 18-crown-6 as phase transfer catalyst, instead of Kryptofix® 2.2.2, the RCYs could be increased ($57 \pm 8\%$, $n = 12$), as the bis(tosyloxy)methane precursor seems to decompose under the typical Kryptofix® 2.2.2./K_2CO_3 reaction conditions.[6]

Although it has been reported that ^{18}F-fluoromethylated compounds tend to decompose under release of [^{18}F]F^- *in vivo*, Zhang *et al*. showed that substitution of the hydrogen atoms in the [^{18}F]fluoromethyl moiety for deuterium led to a higher stability. This kinetic isotope effect is a result of the carbon–deuterium bond being 6 to 10 times stronger than the carbon–hydrogen bond.[7,8] Hence, in addition to [^{18}F]fluoroiodo-d_2-methane, which was used for the ^{18}F-fluoro-d_2-methylation in the above-mentioned study, [^{18}F]bromofluoro-d_2-methane was also examined as labeling precursor.[9,10] The major disadvantage of using these precursors relates to the handling the volatile radioactive intermediates ([^{18}F]$FICD_2$ or [^{18}F]$BrFCD_2$) and their purification from the corresponding precursor (I_2D_2C or Br_2D_2C).

This report describes the first automated synthesis of [^{18}F]fluoro-[*di-deutero*]methyl tosylate ([^{18}F]fluoro-d_2-methyl tosylate) from bis(tosyloxy)-[*di-deutero*]methane using a GE TRACERlab FX F-N synthesis module and a non-commercial synthesis module, with an integrated preparative HPLC system.

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Abbreviations: *d_2-[^{18}F]FMT [^{18}F]Fluoro-[di-deutero]methyl tosylate; TBABC Tetrabutylammonium bicarbonate.*

Results and discussion

In first experiments, labeling was performed by using Kryptofix® 2.2.2., potassium carbonate and acetonitrile or dimethylformamide as solvents at 85 and 100 °C. This approach gave only poor yields of [^{18}F]fluoro-d_2-methyl tosylate because of decomposition of the precursor caused by the strong basicity of the system, which is consistent with the experience of Smith *et al.*[6] Hence, tetraalkylammonium salts, instead of Kryptofix® 2.2.2./K_2CO_3, were investigated because they permit ^{18}F-fluorinations under milder conditions by forming a tetrabutylammonium [^{18}F]fluoride ([^{18}F]TBAF) complex after addition of [^{18}F]fluoride. Of these ammonium salts, tetrabutylammonium bicarbonate (TBABC) in particular gives rise to very mildly basic conditions and was therefore selected for the labeling. After optimization of the amount of phase transfer catalyst used in the synthesis, the highest radiochemical yields of about 50% were obtained by using 1.75 equivalents of TBABC and 1 equivalent of bis(tosyloxy)-[*di-deutero*]methane. Under these reaction conditions, the radiochemical yield of the undesired by-product tosyl [^{18}F]fluoride decreased to 20–25%. The addition of small amounts of water, as described in the literature[5], led to reduced formation of the undesired by-product tosyl [^{18}F]fluoride, but the radiochemical yields of [^{18}F]fluoro-d_2-methyl tosylate significantly decreased too. As successful radiosyntheses in tertiary alcohols are well-known[11], diverse mixtures of acetonitrile and *tert*-butanol as a protic replacement for water were analyzed. A general increase in RCYs was observed using these mixtures, although a formation of tosyl [^{18}F]fluoride was also detected. The highest radiochemical yields (~50%) of d_2-[^{18}F]FMT were achieved using a solvent mixture of 75% acetonitrile and 25% *tert*-butanol (Figure 1).

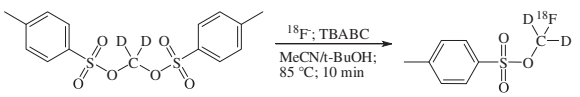


Figure 1. Reaction conditions for the radiosynthesis of [^{18}F]fluoro-d_2-methyl tosylate.

Optimization of the reaction conditions for the formation of [^{18}F]fluoro-d_2-methyl tosylate also included the variation of the reaction times. It was found that heating for 10 min gave the highest radiochemical yields (55–60%), whereas shorter reaction times led to significantly lower radiochemical yields. Increasing the reaction times further did not increase the product formation. Optimal yield for the manual synthesis of d_2-[^{18}F]FMT was achieved by heating the bis(tosyloxy)-[*di-deutero*]methane with the [^{18}F]TBAF complex in a mixture of acetonitrile and *tert*-butanol at 85 °C for 10 min. A radiochemical yield of 46 ± 6% (n = 10) of the desired product was achieved within 60 min, including HPLC purification.

First experiments on a non-commercial, in-house built synthesis module supported the potential of an automated synthesis, giving a reproducible average radiochemical yield of 47 ± 4% (decay corrected, n = 8) in 74 min. To increase the benefit of [^{18}F]fluoro-d_2-methyl tosylate as a labeling precursor, an automated synthesis was established on a commercially available GE TRACERlab FX F-N synthesis module. The automated synthesis of d_2-[^{18}F]FMT included, separation of [^{18}F]F^- from [^{18}O]H_2O, azeotropic drying, labeling of bis (tosyloxy)-[*di-deutero*]methane with [^{18}F]F^-, purification of the labeled product via HPLC, and formulation of the purified [^{18}F]fluoro-d_2-methyl tosylate in acetonitrile. Using this synthesis module, the whole procedure could be shortened to a total synthesis time of 50 min. [^{18}F]Fluoro-d_2-methyl tosylate was obtained in decay corrected radiochemical yields of 44% (n = 4). The radiochemical purity was determined using HPLC to be >98%. The chromatogram of the semipreparative HPLC purification is presented in Figure 2. Compared with the non-commercial synthesis module, heating, cooling and trapping procedures on the cartridges were about 20 min shorter on the GE TRACERlab synthesis module.

As bis(tosyloxy)-[*di-deutero*]methane, which is easily accessible from diiodo-[*di-deutero*]methane in a one-step synthesis, allows the synthesis of d_2-[^{18}F]FMT in reproducible yields of almost 50% RCY, this labeling precursor offers some benefits compared

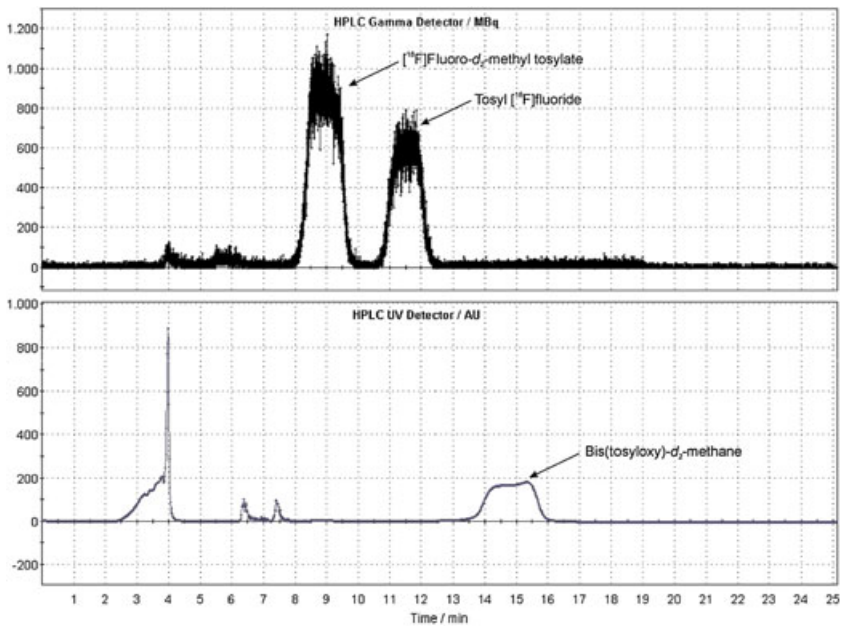


Figure 2. Representative preparative HPLC separation of [^{18}F]fluoro-d_2-methyl tosylate on the GE TRACERlab FX F-N synthesis module.

with other ^{18}F-fluoromethylating agents. In principle, [^{18}F]Fluoro-d_2-methyl tosylate can be synthesized on every module that is able to produce [^{18}F]fluoroethyl tosylate because the reaction steps are quite similar. The total synthesis time of 50 min is comparable to the synthesis time of [^{18}F]FETos. Although the radiochemical yields are somewhat lower, they are within a satisfying range of 45–50%. These results correlate with the RCY published for an automated synthesis of [^{18}F]bromofluoromethane by Bergman *et al.*[12]

The automated synthesis described for d_2-[^{18}F]FMT is a one-step procedure that is characterized by high reproducibility. Such a synthesis is advantageous to other ^{18}F-fluoromethylating compounds that suffer from low reproducibility, such as [^{18}F]fluoromethyl iodide, or are prepared in a two-step synthesis, such as [^{18}F]fluoromethyl triflate. Of further benefit, handling of d_2-[^{18}F]FMT in following synthesis steps may be easier compared with [^{18}F]bromofluoromethane, as this volatile labeling agent may result in higher requirements for the synthesis modules. The automated synthesis of [^{18}F]fluoro-d_2-methyl tosylate also offers the interesting possibility of synthesizing deuterated imaging probes, which are generally more stable *in vivo*.

To prove the feasibility of this method for the synthesis of ^{18}F-fluoro-d_2-methylated tracers, three different compounds, namely [^{18}F]fluoro-d_2-methyl-MH.MZ (d_2-[^{18}F]FM-MH.MZ), [^{18}F]fluoro-d_2-methyl-flumazenil (d_2-[^{18}F]FMF) and [^{18}F]fluoro-d_2-methyl-harmol (d_2-[^{18}F]FMH), were labeled via ^{18}F-fluoro-d_2-methylation. A preliminary optimization for the ^{18}F-fluoro-d_2-methylation was performed on the basis of the reaction conditions for the ^{18}F-fluoroethylation of these compounds, resulting in only slightly different reaction parameters (cf. Table 1). Under these conditions, high radiochemical yields of 65% for d_2-[^{18}F]FM-MH.MZ, 60% for d_2-[^{18}F]FMF and 80% for d_2-[^{18}F]FMH were obtained. It was observed that an efficient drying of the Strata™ X SPE cartridge, on which the labeling precursor is trapped after HPLC purification, is important as the otherwise remaining traces of water cause decomposition of the [^{18}F]fluoro-d_2-methyl tosylate during the subsequent ^{18}F-fluoro-d_2-methylation step.

In summary, with the use of a GE TRACERlab FX F-N synthesis module, a short and very reliable synthesis of [^{18}F]fluoro-d_2-methyl tosylate has been developed, which is suitable for the synthesis of ^{18}F-fluoro-d_2-methylated tracers in high radiochemical yields.

Experimental

General

All chemicals and solvents were purchased from Sigma-Aldrich, Merck or Fisher Scientific and were used without further purification. Bis(tosyloxy)-[*di-deutero*]methane was synthesized from diiodo-[*di-deutero*]methane and silver tosylate following a published procedure.[4] Fluoromethyl tosylate was synthesized from bis(tosyloxy) methane and tetra-*n*-butylammonium fluoride (TBAF) according to a literature method.[13] To obtain the fluoromethyl tosylate in a very high purity, it was isolated via semipreparative HPLC (solid phase: LiChrospher 100 RP-18, 5 μm, 250 × 10 mm, mobile phase: acetonitrile/water (50/50), flow rate: 4 ml/min, t_r: 10.8 min).

No-carrier-added aqueous [^{18}F]fluoride was produced by irradiation of [^{18}O]H_2O via the ^{18}O(p,n)^{18}F nuclear reaction and obtained from DKFZ Heidelberg.

For quality control, a Sykam (Eresing, Germany) HPLC system with an S 1100 HPLC pump, an S 9010 high pressure injection valve, a K-2501 UV–vis detector Knauer (Berlin, Germany) and a Flowstar LB 513 radioactivity detector Berthold (Bad Wildbad, Germany) were used.

Synthesis of [^{18}F]Fluoro-d_2-methyl tosylate

Before starting the synthesis, vials 1 to 4 of the GE TRACERlab FX F-N synthesis module were prepared with TBABC (49 μmol) in methanol (1 ml), bis(tosyloxy)-d_2-methane (28 μmol) in acetonitrile/*tert*-butanol 3/1 (1.2 ml), acetonitrile/water 1/1 (1 ml) and acetonitrile (1 ml). The initial amounts of all reagents are also summarized in Table 2.

The aqueous n.c.a. [^{18}F]fluoride (6–7 GBq) was trapped on a QMA anion exchange cartridge (Waters Corporation, Milford; MA, USA) and eluted with TBABC in anhydrous methanol to the reaction vessel via a helium stream (100 ml/min). Water was removed by azeotropic distillation at 85 °C under vacuum and helium flow for 10 min. After cooling to 40 °C, bis(tosyloxy)-d_2-methane in acetonitrile/*tert*-butanol

Table 1. Reaction conditions for the synthesis of [^{18}F]fluoro-d_2-methyl-MH.MZ (d_2-[^{18}F]FM-MH.MZ), [^{18}F]fluoro-d_2-methyl-flumazenil (d_2-[^{18}F]FMF) and [^{18}F]fluoro-d_2-methyl-harmol (d_2-[^{18}F]FMH) via ^{18}F-fluoro-d_2-methylation in DMSO with a reaction time of 20 min

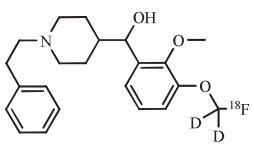
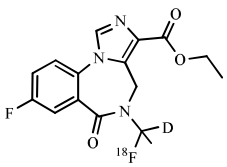
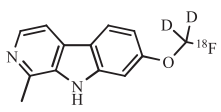
Compound	Structure	Precursor (μmol)	Base	Reaction temperature (°C)	RCY (%)
d_2-[^{18}F]FM-MH.MZ		8	5 N NaOH	120	65
d_2-[^{18}F]FMF		7	NaH	80	60
d_2-[^{18}F]FMH		15	5 N NaOH	110	80

Table 2. Initial amounts for preparation of the TRACERlab synthesis module

	Vial 1	Vial 2	Vial 3	Vial 4
Reagent	TBABC	Bis(tosyloxy)-d_2-methane	None	None
Weight, mg (μmol)	15 (49)	10 (28)	None	None
Solvent	Methanol	Acetonitrile/*tert*-butanol 3/1	Acetonitrile/water 1/1	Acetonitrile
Volume (ml)	1	1.2	1	1

3/1 was added to the reactor, and labeling was achieved by heating for 10 min at 85 °C. After cooling to 55 °C, the reaction mixture was diluted with acetonitrile/water 1/1. The mixture was transferred to a 6-ml loop of an injector valve via helium stream and the product was isolated via semipreparative HPLC (solid phase: Nucleosil® 100-5 C_{18}, 5 μm, 250 × 15 mm (Macherey-Nagel), mobile phase: acetonitrile/water (60/40), flow rate: 8 ml/min, t_r([^{18}F]fluoro-d_2-methyl tosylate): 8 min, t_r(tosyl [^{18}F]fluoride): 11 min, t_r(bis(tosyloxy)-d_2-methane): 14 min). The peak corresponding to [^{18}F]fluoro-d_2-methyl tosylate was collected in a 250-ml flask and was diluted with 40 ml of water. The diluted product was transferred and trapped on a Strata™ X SPE cartridge (Phenomenex), dried with helium and eluted with 1 ml of the desired solvent, to obtain 1.9–2.7 GBq of [^{18}F]fluoro-d_2-methyl tosylate with a radiochemical purity of >98% after a synthesis time of 50 min.

The automated synthesis on the non-commercial synthesis module was accomplished in the same manner, with a slightly different HPLC purification (solid phase: LiChrospher®100 RP 18, 5 μm, 250 × 10 mm (Merck), mobile phase: acetonitrile/water (50/50), flow rate: 4 ml/min, t_r([^{18}F]fluoro-d_2-methyl tosylate): 12 min, t_r(tosyl [^{18}F]fluoride): 16 min, t_r(bis(tosyloxy)-d_2-methane): 32 min).

Conclusion

The first automated radiosynthesis of [^{18}F]fluoro-d_2-methyl tosylate was successfully performed on a GE TRACERlab and a non-commercial synthesis module. The total synthesis time was reduced from 74 min, on a non-commercial module, to 50 min on a GE TRACERlab module. Thus, the synthesis time of d_2-[^{18}F]FMT was comparable with the reported synthesis times of [^{18}F]fluoroethyl tosylate. Furthermore, decay corrected radiochemical yields averaged 47% and were therefore only slightly lower compared with [^{18}F]fluoroethyl tosylate.

In addition, we showed that the automated synthesis of [^{18}F]fluoro-d_2-methyl tosylate is characterized by high reproducibility and is independent from the type of synthesis module that was used. These results may increase the impact of [^{18}F]fluoro-d_2-methyl tosylate as a labeling precursor because this automated synthesis can be adopted to most commercially available synthesis modules that can be used for the production of [^{18}F]fluoroethyl tosylate.

Finally, it has been shown that this labeling precursor allows the synthesis of ^{18}F-fluoro-d_2-methylated compounds in high radiochemical yields.

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Conflict of Interest

The authors did not report any conflict of interest.

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