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Methylation of Amide and Thiol Functions with [^{11}C]Methyl Triflate, as Exemplified by [^{11}C]NMSP, [^{11}C]Flumazenil and [^{11}C]Methionine

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Summary

[^{11}C]Methyl triflate was compared with [^{11}C]methyl iodide as a labelled precursor in the synthesis of PET radioligands through ^{11}C-methylation of amide and thiol functions. [^{11}C]NMSP, [^{11}C]flumazenil and [^{11}C]methionine were prepared in 50-75% radiochemical yields using smaller amounts of precursors, shorter reaction times and lower reaction temperatures than previously used when these compounds were prepared from [^{11}C]methyl iodide. By minimizing the amount of base used in the synthesis of [^{11}C]methionine from [^{11}C]methyl triflate and L-homocysteine thiolactone in water a lower amount (1-2%) of D-[^{11}C]methionine was obtained. The results demonstrate that [^{11}C]methyl triflate is compatible with low concentrations of aqueous sodium hydroxide, which enable its use in the preparation of PET radioligands through ^{11}C-methylation of amide and thiol functions.

Key words: [^{11}C]Methyl triflate, [^{11}C]NMSP, [^{11}C]flumazenil, [^{11}C]methionine

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INTRODUCTION

The most commonly used labelled precursor in routine preparation of ^{11}C-radiopharmaceuticals for PET is [^{11}C]methyl iodide ([^{11}C]MI) (1, 2). [^{11}C]Methyl triflate, ([^{11}C]MT), has been introduced as a highly reactive alternative to [^{11}C]MI (3). We have previously performed a comparison with [^{11}C]MT and [^{11}C]MI as labelled reagents for the synthesis of commonly used PET radioligands such as [^{11}C]NNC 756, [^{11}C]deprenyl, [^{11}C]MHED, [^{11}C]β-CFT and [^{11}C]nicotine by ^{11}C-methylation of amine functions (4, 5). It was demonstrated that [^{11}C]MT is compatible with the base pentamethylpiperidine (PMP), which thus can be used to prepare free amines from their corresponding salts *in situ*. The use of [^{11}C]MT generally gives higher yields from smaller amounts of precursor, shorter reaction times and lower reaction temperatures (4-9). These results are important for a reliable routine production, specific radioactivity and automation.

Recently, the use of [^{11}C]MT was extended to the alkylation of phenol and carboxylic acid functions for the preparation of [^{11}C]FLB 457, [^{11}C]MDL 100907 and [^{11}C]β-CIT-FE (10). It was demonstrated that [^{11}C]MT is compatible with low concentrations of sodium hydroxide, which implies that [^{11}C]MT might also be a useful precursor for the synthesis of ^{11}C-labelled radiopharmaceuticals through methylation of other functional groups such as amides and thiols.

In a continuation of our previous studies we have compared [^{11}C]MT with [^{11}C]MI as labelled precursors in the ^{11}C-methylation of amide and thiol functions. The dopamine D-2/ serotonin 5-HT2 receptor antagonist [^{11}C]NMSP (11, 12), the benzodiazepine receptor antagonist [^{11}C]flumazenil (13, 14) and the amino acid [^{11}C]methionine (1, 15, 16) were selected as model substances. The reason for this selection was that these PET radiopharmaceuticals are commonly used around the world. A general feature of the reported preparation of these radiopharmaceuticals is that an ionic form of the precursor is generated *in situ* using organic bases such as tetrabutylammonium hydroxide or inorganic bases such as sodium hydroxide. The aim of this study was to evaluate if [^{11}C]MT is compatible also with these bases during the ^{11}C-methylation of amide and thiol functions. In addition, the formation of D-[^{11}C]methionine as a function of NaOH concentration in the methylation of the anion of homocysteine with [^{11}C]MT in water was studied.

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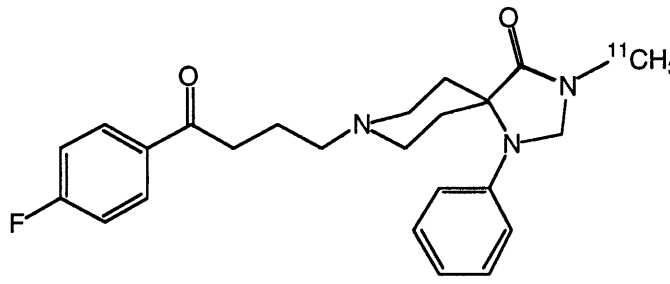
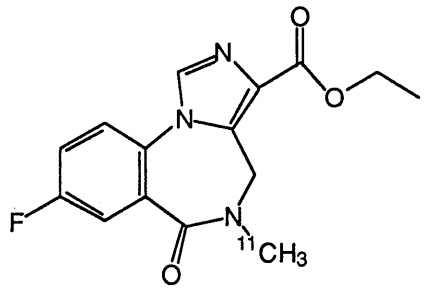
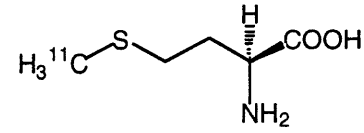


[^{11}C]NMSP



[^{11}C]Flumazenil



[^{11}C]Methionine

Figure 1. Structures of the ^{11}C-labelled radioligands synthesized by methylation of amide and thiol functions from [^{11}C]MT

EXPERIMENTAL

Materials & General Methods

Materials

Spiperone and L-homocysteine thiolactone were obtained from SIGMA and N-methylspiperone was obtained from RBI, Natick, USA. Ro 15-5528 and Ro 15-1788 were kindly supplied by Prof. Hunkeler, Roche, Basel. Other chemicals were obtained from commercial sources and were of analytical grade.

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Production of [^{11}C]MT

The [^{11}C]carbon dioxide was produced at the Karolinska Hospital with a Scanditronix RNP 16 cyclotron and at the Accelerator Laboratory of Åbo Akademi with a 103 cm isochronous Efremov cyclotron using the $^{14}N(p,\alpha)^{11}C$ reaction. [^{11}C]MT was prepared on line from [^{11}C]MI according to the procedures described in details given previously (4, 10).

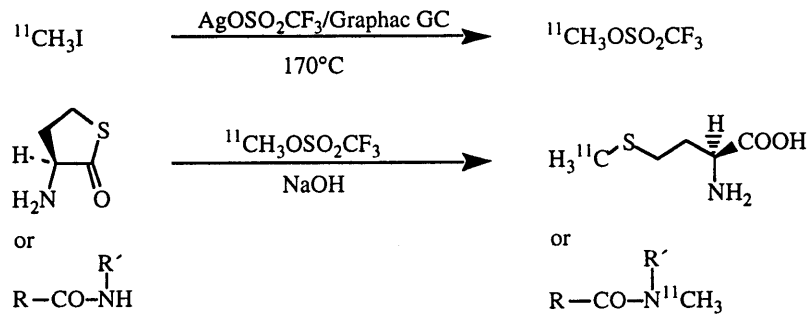

Figure 2. Preparation of [^{11}C]MT from [^{11}C]MI and use in ^{11}C-methylation of amide and thiol functions

HPLC analysis and purification of radioligands

Semipreparative HPLC was performed using either a reversed-phase Waters μ-Bondapak-C18 column (300 x 7.8 mm, 10 μm) or, in the case of [^{11}C]methionine, an analytical strong cation exchange Phenomenex Optisil SCX column (250 x 4.6 mm, 10 μm) in series with a reversed-phase Waters μ-Bondapak-C18 column (300 x 7.8 mm, 10 μm). A UV-detector (wavelength = 254 nm) was used in series with a radioactivity detector. Filters used in the sterile filtration of the final products were obtained from Millipore (Millex-GV, 0.22 μm) or Gelman Ltd (Acrodisc, 0.22 μm). The radiochemical purity was analysed by reversed-phase HPLC using a Waters μ-Bondapak-C18 column (300 x 3.9 mm, 10 μm) or, in the case of [^{11}C]methionine, a Waters μ-Bondapak NH_2 column (300 x 3.9 mm, 10 μm). The optical purity of [^{11}C]methionine was analyzed by HPLC using a Daicel Crownpak CR(+) column (150 x 4 mm, 5 μm) as earlier described (17).

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Preparation of [^{11}C]NMSP

[^{11}C]MT was trapped at 0°C in a sealed reaction vessel containing spiperone (0.3 mg), sodium hydroxide (0.5 M (aq), 1 μl) and acetone (100 μL). The vessel was heated at 60°C for 1 min. Mobile phase (500 μL) was added before injection onto the semi-preparative reversed-phase HPLC column. [^{11}C]NMSP eluted after 8-9 min with acetonitrile/0.01 M phosphoric acid (35/65) and a flow rate of 6 mL/min with a retention time identical to a standard reference sample (Fig. 3**A**). Propylene glycol/ethanol (7/3, 800 μL) was added to the eluent containing the purified product. After evaporation of the mobile phase, the residue was dissolved in 8 mL physiological phosphate buffer (pH=7.4) and filtered through a 0.22 μm sterile filter, giving a solution which was sterile and free from pyrogens.

Preparation of [^{11}C]flumazenil

[^{11}C]MT was trapped at 0°C in a sealed reaction vessel containing Ro 15-5528 (0.5 mg), sodium hydroxide (0.5 M (aq), 3 μl) and acetone (100 μL). The vessel was heated at 60°C for 1 min. Mobile phase (500 μL) was added before injection onto the semi-preparative reversed-phase HPLC column. [^{11}C]Flumazenil eluted after 13-14 min with acetonitrile/0.01 M phosphoric acid (22/78) and a flow rate of 6 mL/min with a retention time identical to a standard reference sample (Fig. 3**B**). Propylene glycol/ethanol (7/3, 800 μL) was added to the eluent

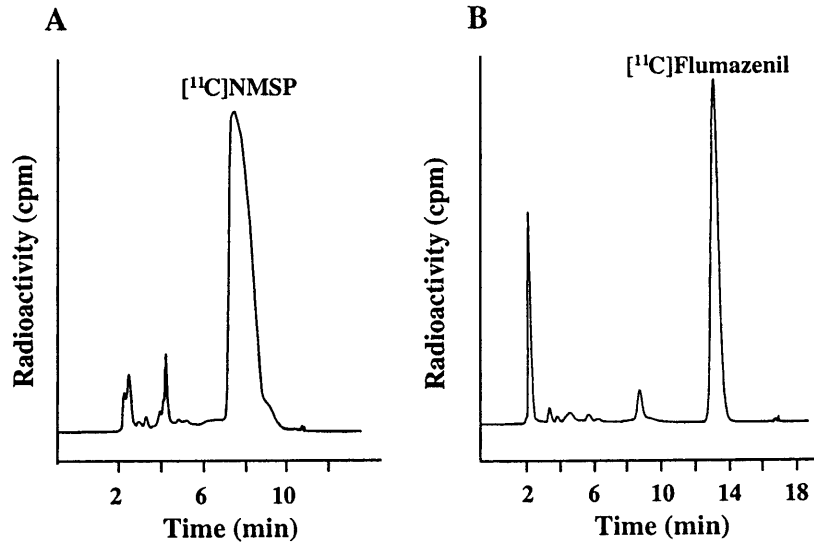

Figure 3. Semi-preparative HPLC chromatograms (radioactivity) from the purification of **A**: [^{11}C]NMSP and **B**: [^{11}C]flumazenil.

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containing the purified product. After evaporation of the mobile phase, the residue was dissolved in 3 mL propylene glycol/ethanol (7/3) and 5 mL physiological phosphate buffer (pH=7.4) and filtered through a 0.22 μm sterile filter, giving a solution which was sterile and free from pyrogens.

Preparation of [^{11}C]methionine

[^{11}C]MT was trapped at room temperature in a sealed reaction vessel containing L-homocysteine thiolactone (1.0 mg), sodium hydroxide (1.0 M (aq), 9 μL) and distilled and deionized water (100 μL). The vessel was heated at 60°C for 1 min. Mobile phase (300 μL) was added before injection onto the analytical cation exchange column connected in series with the semi-preparative reversed-phase HPLC column. [^{11}C]Methionine eluted after 6-7 min with 0.05 M sodium dihydrogenphosphate and a flow rate of 3.5 mL/min with a retention time identical to a standard reference sample (Fig. 4). Propylene glycol/ethanol (7/3, 400 μL) and 4 mL

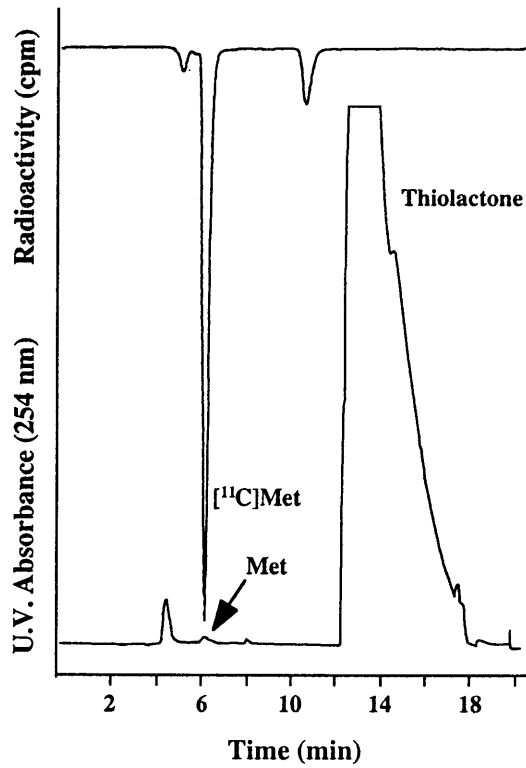

Figure 4. Semi-preparative HPLC chromatogram (radioactivity and UV) from the purification of [^{11}C]methionine.

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physiological phosphate buffer (pH=7.4) was added to the eluent containing the purified product. Filtration through a 0.22 μm sterile filter gave a solution which was sterile and free from pyrogens.

RESULTS AND DISCUSSION

The reactivity of [^{11}C]MI is in most cases sufficient for the routine production of PET radiopharmaceuticals. When considering PET radioligands where the yield is relatively low from [^{11}C]MI, the use of [^{11}C]MT may result in higher yields, shorter reaction times and lower reaction temperatures (4, 5). In addition, the precursor (nor-compound) amount can often be reduced, which is important both when considering the final purification of a PET radioligand and the commercial availability and cost of the precursor.

These two reagents have also been compared in a few other cases, *i.e.* for the synthesis of [^{11}C]epinephrine (6) and [^{11}C]clozapine (7). [^{11}C]MI has been proposed as a reagent for the synthesis of [^{11}C]methionine and [^{11}C]raclopride (8, 9), however no details such as radiochemical yields were presented.

Preparation of PET radioligands by *N*-methylation of amines is performed using mild reaction conditions *i.e.* methylation of the free base dissolved directly or generated *in situ* from a salt by the use of a mild base such as PMP. The methylation of amide anions require stronger bases such as tetrabutylammonium or sodium hydroxide as commonly used for the preparation of [^{11}C]NMSP (11, 12) or [^{11}C]flumazenil (13, 14). We have used [^{11}C]methyl triflate for the synthesis of [^{11}C]NMSP and [^{11}C]flumazenil and found that a small amount of aqueous sodium hydroxide (1.5 eq.) give high and consistent yields with 1 min heating at 60^{o}C and 0.3-0.5 mg of precursors in 100-300 μL of acetone (Table 1).

[^{11}C]Methionine is commonly prepared by methylation of L-homocysteine sulphide anion gererated either from L-homocysteine thiolactone (15) or L-*S*-benzyl-homocysteine (1, 16) with [^{11}C]methyl iodide. Ichiwata *et. al.* has demonstrated that racemization in the thiolactone synthesis method increased as the concentration of sodium hydroxide is increased (18). We have found that the radiochemical yield of this method is also influenced by the sodium hydroxide concentration, which means that low racemization is obtained with relatively low radiochemical yield.

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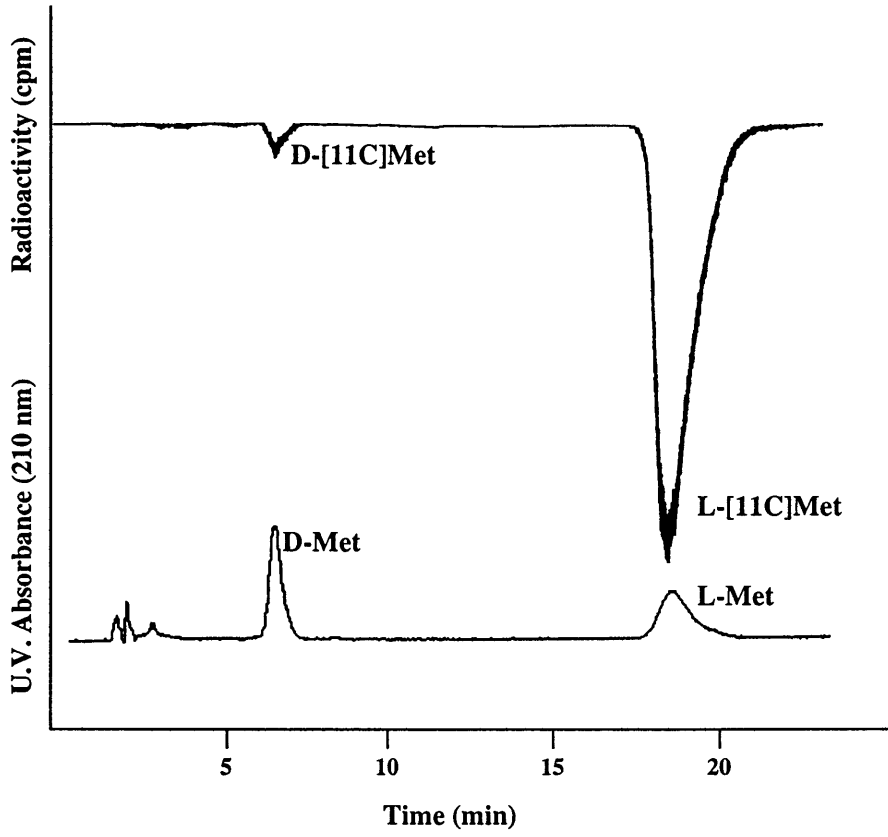

Figure 5. Analytical chiral HPLC chromatogram (radioactivity and UV) after injection of D/L-[^{11}C]methionine together with unlabelled standards of the pure enantiomers.

As we found that [^{11}C]methyl triflate is compatible with low concentrations of sodium hydroxide, and it has higher reactivity than [^{11}C]methyl iodide we postulated that high radiochemical yields and low racemization can be obtained using [^{11}C]methyl triflate in the thiolactone method. The dependence of racemization on sodium hydroxide concentration is shown in Figures 5 and 6. As can be seen in Figure 6 the amount of D-[^{11}C]methionine is 1-2% when the ratio of NaOH to L-homocysteine thiolactone is around 1.5. At ratios of 2 and higher the amount of D-[^{11}C]methionine increases rapidly. The radiochemical yield is high using 1.5 eq. of sodium hydroxide and 1 min heating at 60^{o}C in water (Table 1). Interestingly, when performing the same reaction in 50% aqueous ethanol, a low radiochemical yield (20%) was obtained, the main reaction product being a so far unidentified lipophilic compound, which may be the *N*-methylated form of the thiolactone.

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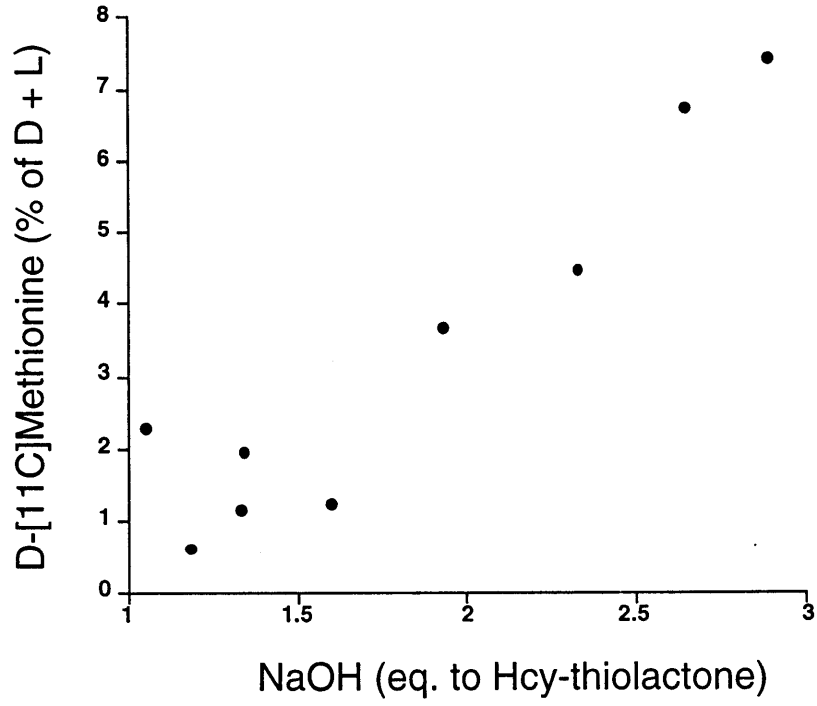

Figure 6. Influence of NaOH concentration on the racemization of [^{11}C]methionine.

As one equivalent of sodium hydroxide is consumed to liberate the free amine of the thiolactone from its hydrochloride, the use of 1.5 equivalents imply that maximally 50% of the thiolactone is hydrolyzed to L-homocysteine sulphide anion. The remaining thiolactone is however efficiently separated from the product using the analytical strong cation exchange

Table 1. Preparation of some PET radioligands by methylation of anions with [^{11}C]methyl triflate.

Radioligand	Precursor (mg, solvent)	Yield (a)
[^{11}C]NMSP	spiperone (0.3, acetone)	50-65
[^{11}C]Flumazenil	Ro 15-5528 (0.5, acetone)	65-75
[^{11}C]Methionine	thiolactone (1.0, water)	60-70

[a]Decay corrected radiochemical yield (%) from [^{11}C]methyl triflate to final sterile filtered product using 1 minute heating at 60°C.

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column in series with the reversed-phase semipreparative column (Figure 4). We observed only minor amounts (1-3%) of oxidized products (17) which also were removed in the purification step. As we have previously noticed that [^{11}C]methionine is susceptible to radiolysis some sterile propylene glycol/ethanol was added to the eluent containing the purified product as a scavenge for radiolysis.

CONCLUSION

[^{11}C]Methyl triflate was found to be compatible with low concentrations of sodium hydroxide in the preparation of PET radioligands by ^{11}C-methylation of amide and thiol anions.

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